Analytical Strategies to Solve Problems in Industrial and Official Laboratories

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ANALYTICAL STRATEGIES TO SOLVE PROBLEMS IN INDUSTRIAL AND OFFICIAL LABORATORIES

A dissertation presented in candidacy for the degree of International Doctor of Philosophy.

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“Si vas delante, quizás no te pueda seguir.

Si vas detrás, quizás no te pueda guiar.

Si vas a mi lado, serás mi amigo.”

Anónimo.

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SUMMARY

Scientific discoveries are present in all areas of our life. For this reason, promoting research is necessary since the knowledge acquired in this way is useful for different fields: from designing bridges to slowing climate change, developing new technologies or solving practical problems. A usual way of researching is to apply the concept of research and development (R&D), which involves the presence of an appreciable element of novelty and the resolution of problems and uncertainties using scientific or technological means. Nowadays, companies need R&D to compete internationally in order to succeed. This competence requires to introduce the concept of quality in their processes and products. Quality involves different perceptions and meanings, although its objective is meeting the expectations and needs of customers. The assurance of this parameter is commonly carried out by quality control procedures.

Concretely, along this work, the research carried out has been focus on three tasks related to quality control procedures, being the main objective of this dissertation to employ analytical chemistry as a tool to provide solutions to different problems exposed by official and industrial laboratories, in order to improve the methods used up to now with the consequent economical and environmental benefits.

The main objective has been fulfilled through next sub-objectives: -analytical validation of a method for inorganic anion analysis in different kind of water by capillary ion electrophoresis; -determination of anionic impurities in fluorinated inorganic acids by both capillary electrophoresis and ion chromatography; and -determination of some herbicides in surface water by ion chromatography-electrospray mass spectrometry and post column addition of acetonitrile.

ACRONYMS AND ABBREVIATIONS

ACN	Acetonitrile
ACCase	Acetyl coenzyme A carboxylase
ALS	Acetolactate synthase
AMPA	Amino methyl phosphonic acid
ANOVA	Analysis of variance
AOAC	Association Of Analytical Communities
BGE	Background electrolyte
BIAL	Bialaphos
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CEN	European Committee for Standardization
CGE	Capillary gel electrophoresis
CIE	Capillary ion electrophoresis
CIEF	Capillary isoelectric focusing
CITP	Capillary isotachophoresis
CRM	Certified reference material
CZE	Capillary zone electrophoresis
DAD	Diode array detection
DBV	Divinylbenzene
D/DBP	Disinfectants/disinfection byproducts
DDF	Derivados del Flúor S.A.
EFM	Electroosmotic flow modifier
ELISA	Enzyme-linked inmunosorbent assay
EOF	Electroosmotic flow
EPA	U.S. Environmental Protection Agency
EPSP	Synthase (3-phosphoshikimate 1-carboxyvinyltransferase)
EQS	Environmental quality standards
ESI	Electrospray ionization
ESF	European Social Fund
EU	European Union
FAO	Food and Agriculture Organization
FBA	Tetrafluoroboric acid
FL	Fluorescence
FMOC	Fluorenylmethyloxycarbonyl
FSA	Hexafluorosilicic acid
FTA	Hexafluorotitanic acid
FZA	Hexafluorozirconic acid
GC	Gas chromatography
GLYP	Glyphosate
GLUF	Glufosinate

GV/EJ	Basque Government
HPCE	High performance capillary electrophoresis
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICH	International conference on harmonisation
ICP	Inductively coupled plasma
ICS	Ion chromatography system
IL	Ionic liquids
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MCL	Maximum contaminant level
MEKC	Micellar electrokinetic capillary chromatography
MICINN	Ministry of Science and Innovation
MPPA	3-[hydroxy(methyl)phosphinoyl]propionic acid
MS	Mass spectrometry
m/z	Mass-to-charge ratio
n.a.	Not applicable
n.d.	Not detectable
NMR	Nuclear magnetic resonance
NPDWS	US National Primary Drinking Water Standards
OECD	Organization for Economic Cooperation and Development
OVAT	One variable at the same time
PTFE	Polytetrafluoroethylene
QC	Quality control
RE%	Relative error
RD	Royal decree
R&D	Research and development
RSD	Relative standard deviation
SIM	Selected ion monitoring
SMCL	Secondary maximum contaminant level
SOP	Standard operating procedure
SD	Standard deviation
S/N	Signal to noise ratio
TLC	Thin layer chromatography
TTAB	Tetradecyltrimethylammonium bromide
TTAOH	Tetradecyltrimethylammonium hydroxide
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPV/EHU	University of the Basque Country
USA	United States of America

UV	Ultraviolet
WFD	Water Framework Directive
WHO	World Health Organization

Analytical Strategies to Solve Problems in Industrial and Official Laboratories

Content Page

INTRODUCTION

Chapter I

Chapter I- INTRODUCTION

I.1 SCIENCE AND SOCIETY

Scientific discoveries are present in all areas of our life although society is not fully aware. Most of the time, information about scientific research transcends public opinion only when it implies ethical or moral dilemma. For this reason, it is very important to connect science and society since the more information is transmitted the less is the lack of awareness, so better relationships will be obtained. Only if people understand the relevance of scientific discoveries, these will be appreciated, therefore, research will be taken into account as something valuable in society and worthy to be promoted.

Promoting research is necessary since the knowledge acquired in this way is useful for all sorts of things: from designing bridges to prompting frequent hand washing during flu season, slowing climate change, developing new technologies or solving practical problems.

Countries with high industrial development know the importance of scientific research to keep an efficient industrial network. There are a great number of evidences which show that industry cannot compete successfully if there is not a continuous improvement of its manufacturing processes to reduce costs; of the quality of its products; as well as a development of new products and/or processes that meet not covered needs. Nowadays, companies need to compete internationally if they want to succeed, what is only possible using the last scientific advances. This is the principle of the research and development (R&D) concept.

According to United Nations Educational, Scientific and Cultural Organization (UNESCO) and Organization for Economic Cooperation and Development (OECD), R&D refers to creative work undertaken on a systematic basis in order to increase the stock of knowledge, and the use of this stock of knowledge to create new or improved products, processes, services or other applications. The basic criterion to distinguish R&D from other related activities is the presence of an appreciable element of novelty and the resolution of problems and uncertainties using scientific or technological means [1].

Other evidence of the importance of R&D is identified in current economical situation. Figure 1 shows the economical investment in R&D of different European countries.

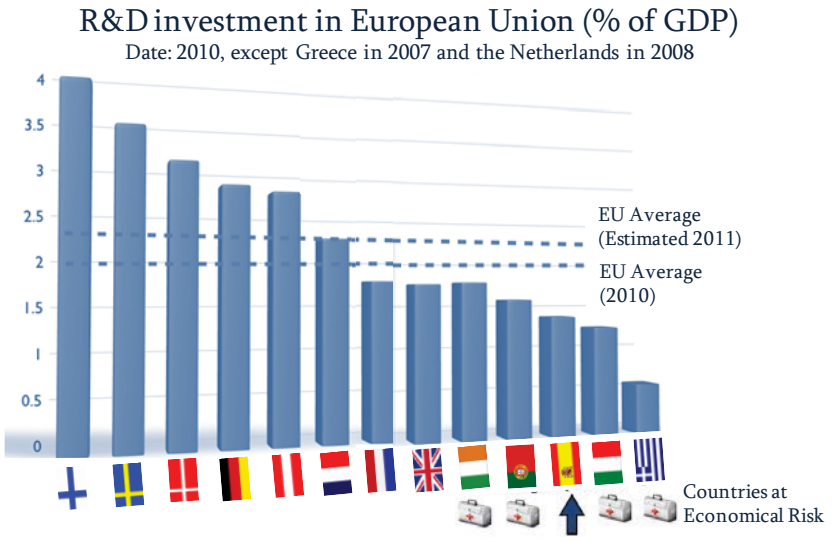

Figure 1 *Economical investment in R&D of different European countries, expressed as Gross Domestic Product percentage (GDP %), with calculated and estimated European Union average of 2010 and 2011 respectively [2].*

Having a look at the economical situation of the European countries it is easy to find a correlation between R&D investments and economical success of countries. These successful countries increase their investment in R&D because they know the benefits of R&D even in crisis times. These benefits are so important that they have promoted the research from very early times, when R&D concept did not exist. Louis Pasteur said:

"*In our century, science is the soul of the prosperity of nations and the living source of all progress. What really lead us forward are a few scientific discoveries and their applications.*"

Even though this sentence was said more than a century ago it is yet applicable. However, nowadays the use of R&D has substituted the earlier way of working

in industry. Each day, empirical procedures play a less important role in the ongoing effort to improve manufacturing methods, making essential a systematic and thorough study. This should be done by those who are specially trained, as scientific staff. This renewal cannot be effectively done by applying only the scientific knowledge, but it is necessary to collaborate with industry to gather as much information as possible about the surroundings of the process to be improved. So understanding the necessity of coexistence and mutual help between staff from both industry and research centres, will give rise to benefits for industry, science and society.

Benefits of the collaboration between scientists and staff from industrial laboratories can be grouped into the following statements:

-problems solving
-productive investments
-costs saving
-increment of the added value of the manufactured products
-better competition in international business through the improvement in technological training of industrial sector companies
-interaction between theoretical and practical knowledge

Within all kinds of industry present in society it is worthwhile to mention the chemical industry due to the crucial role of chemistry in daily life. Looking at our environment, it is easy to realize that there are plenty of substances, materials, objects and even essential living processes related to chemistry. This branch of science is a useful tool to connect pure knowledge with essential "know how". Different specialities of chemistry help us to understand the matter from different points of view providing information about the composition, structure, behaviour, etc... Concretely, Analytical Chemistry allows to confront industrial problems from a scientific point of view to solve them.

Analytical Chemistry can be defined as the science which develops and applies instrument, methods and strategies to obtain information on the composition and chemical nature of matter in space and time, as well as on the value of these measurements, i. e., their uncertainty, validation and/or traceability to

fundamental standards [3]. Application fields of this branch of chemistry are diverse. In industry, the quality control of raw materials and finished products highlights; in trade, certificates of analysis laboratories ensure the quality specifications of the goods; in the clinical field, blood and urine analysis facilitate the diagnosis of several diseases. All these applications can be performed thanks to chemical/instrumental analysis.

Analysis is one part of an analytical process as a way of problem resolution, which involves some more steps, as relevant as analysis one, which can be seen in Figure 2.

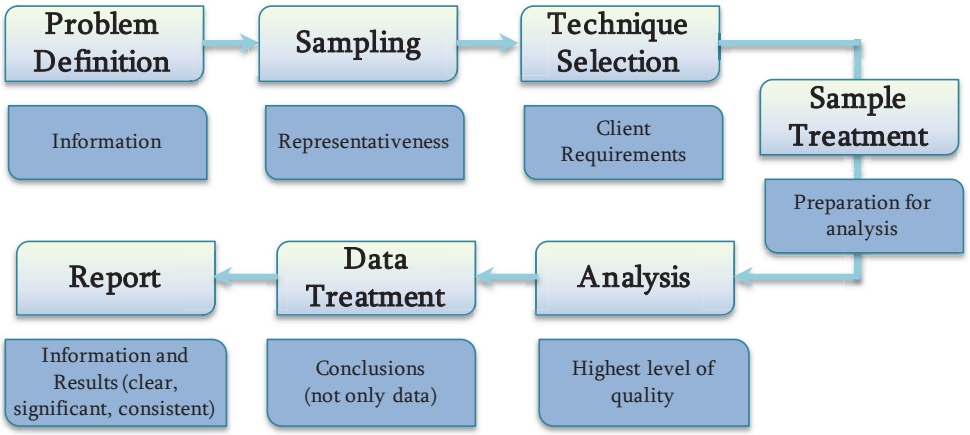

Figure 2 *Representation of the different steps of the analytical process resolution and their main characteristics.*

Analytical problem solving requires three crucial steps to allow the final treatment and interpretation of data acquired through the analysis.

First of all, it is necessary to gather as much information as possible to know the antecedents of the problem. Then sampling has to provide a representative sample to be analyzed. Finally, technique selection needs to cover the requirements of the sample to fulfil the pursue purpose.

I.2. PROBLEMS DEFINITION

Detailed information of the problem is essential in any analytical problem resolution. In this section, a deep explanation of the analytes, matrixes and problems associated with them is given to face the main objective of this scientific memory. This is to provide analytical solutions to problems proposed by official and industrial laboratories.

I.2.1 Analytes

The analytical methodologies developed throughout this research involve inorganic anions, being the analytes under study or, in other cases, the interferences of the matrix. This section will tackle these compounds as analytes under study.

I.2.1.1. Inorganic Anions

Common inorganic anions as fluoride (F^-), bromide (Br^-), chloride (Cl^-), nitrate (NO_3^-), nitrite (NO_2^-), sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}) are studied in this work. The source of these inorganic ions in nature includes natural and anthropogenic processes, last ones are those generated by human activity. The majority of them are present in most natural waters [4]. These anions typically come from industrial and domestic sewage discharges, excessive fertilization of farm lands, fish ponds, etc.

The origin of these anions and some general injurious effects in water are described below, showing the importance of their monitoring and/or determination. Other specific drawbacks caused by these anions in other matrixes will be deeply explained in the introduction of each chapter within this scientific memory. Anions in water at certain concentration are considered inorganic pollutants, whereas when they are contained in industrial products they are considered impurities.

Fluoride

Fluoride is a minor anion in groundwater, usually present at less than 1 mg/L. Natural sources of fluoride are carbonate rocks, which include the mineral

fluorite, while its anthropogenic origin is discharges from fertilizer and aluminium production companies. Fluoride is an anion with high controversy, it is recommended to avoid tooth decay in children at concentration between 0.7-1.2 mg/L [5], whereas it is considered dangerous because it may cause pain and weakness of the bones and staining or mottling of teeth at higher concentrations [6]. In spite of that controversy and taking into account that the main dietary fluoride source is water, not food, nowadays about 40 countries have artificial water fluoridation schemes, although only a small proportion of the population is covered by these schemes. In Spain just 10 % of the population consumes fluoridated water, while in the Netherlands this practice is forbidden since 1973. The U.S. Environmental Protection Agency (EPA) has established a Maximum Contaminant Level (MCL) of 4 mg/L in public drinking water and there is also a Secondary Maximum Contaminant Level (SMCL) of 2.0 mg/L because higher concentrations can cause tooth discoloration [7].

Chloride

Chloride is mainly present in nature in seawater. The presence of this anion in waste water is due to the domestic use of common salt combined with isolated sources as winter road salt addition or water well disinfection operations. Chloride anion at high concentration can cause damages in metallic structures because it increases the electrical conductivity of water and thus its corrosive effect [8]. High content of this anion harms the growing of plants and can react with metallic pipes to form soluble salts [9].This would increase the level of metals in drinking water. EPA has set a secondary maximum contaminant level of 250 mg/L because water containing more than this concentration has a disgusting taste [7].

Sulphate

Sulphate is abundantly present in the Earth's crust. Common natural sources of sulphate are oxidation of iron sulphide minerals in coal or shale and dissolution of the calcium sulphate minerals as gypsum and anhydrite. Other natural source of sulphate ions is sulphur containing organic compounds while an anthropogenic source is industrial water discharge. Sulphate anion in high concentration can cause damages in structures made of concrete and may

contribute to the corrosion of the water distribution systems [10]. Also, if magnesium sulphate is present in water in a high concentration (greater than 500 mg/L) it has laxative effects and disgusting flavour. For these reasons, EPA has set a SMCL of 250 mg/L, although there is no MCL for sulphate [7].

Nitrate

Nitrate is the end product of the aerobic decomposition of organic nitrogenous matter. Highest oxidized form of nitrogen is commonly present in natural water due to the degradation of nitrogenous compounds like proteins or urea. Figure 3 shows the nitrogen cycle [10].

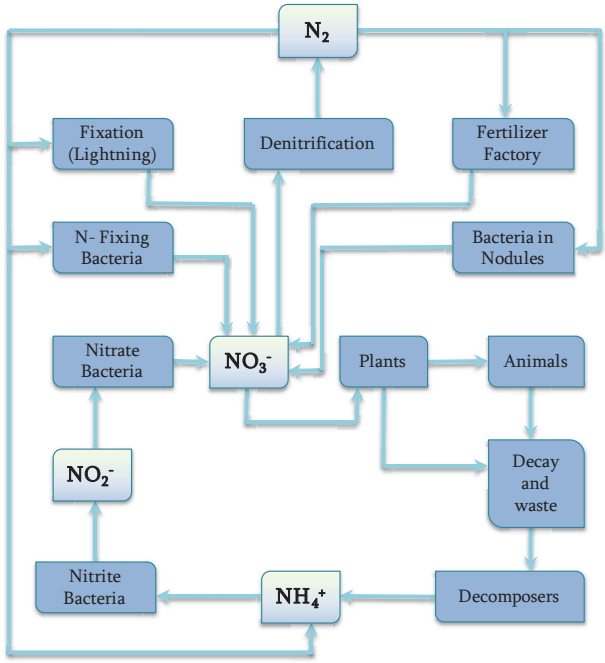

Figure 3 *Nitrogen cycle, summarizing the pathway of nitrogen in nature, the processes in which nitrogen is involved as well as the species in which it is transformed.*

Presence of high contents of nitrate and nitrite is associated with organic matter in decomposition processes, such as animal waste. Known the oxidation process of these anions, the transformation of ammonia to nitrite and from this to nitrate, the presence of one or other anion can give an orientation of the

running time from the pollution moment. In this sense, if certain water has nitrate pollution it can be said that pollution occurred long time before, as there has been enough time for complete oxidation. On the other hand, the presence of nitrite and ammonia would be an index of recent contamination, without enough oxidation time. Therefore, in this last case exits the danger of the presence of pathogenic bacteria, which are the ones that carry out the oxidation processes.

Another natural source of nitrate is storms, electrical discharges generate nitrogen oxides from nitrogen and oxygen in the air. Afterwards, rainwater generates nitric acid, which attacks the carbonate and other basic minerals to form the corresponding nitrate. The most common anthropogenic sources responsible for the high level of nitrate in water are municipal and industrial waste waters, manure lixiviation or fertilized agricultural lands and urban drainage.

Nitrates may act as carcinogens through the formation of N-nitrous compounds [11]. Studies carried out on Chinese populations exposed to high levels of nitrate in drinking water suggested an association between nitrate contamination and stomach, liver [12] and bladder cancer [13, 14]. Besides, nitrate is dangerous because it assists lead solubility with the consequent disadvantages. The MCL for nitrates was set at 10 mg/L according to EPA [7].

Nitrite

Nitrite is another product involved in nitrogen cycle with negative effect in health. The most significant health effect associated with the ingestion of this anion is methemoglobinemia in infants. Methemoglobinemia occurs when hemoglobin, which is the oxygen carrying component of blood, is converted by nitrite to methemoglobin, which does not carry oxygen efficiently [9]. Consequence of this, nitrite can cause respiratory difficulties in children while in adults it is transformed to nitrosamine which is carcinogenic. MCL for nitrites was set at 1 mg/L by EPA [7].

Phosphate

Phosphorus is the eleventh most abundant element in the Earth´s crust. Phosphorus in water is usually found as phosphate [15]. Phosphates enter the water supply from agricultural fertilizer run-off, water treatment and biological wastes and residues. Industrial effluents related to corrosion and scale control, chemical processing, and the use of detergents and surfactants are some anthropogenic sources of this pollutant. High levels of phosphate can cause health problems, such as kidney damage and osteoporosis. Scientific studies have indicated that phosphate mining may be related to higher risk of leukemia, lung cancer and colon cancer due to the radioactivity associated with phosphate is concentrated in clays and sand tailings created by phosphate mining operations. Severe phosphate toxicity can result in hypocalcemia, while moderate phosphate toxicity can result in the deposit of calcium phosphate crystals in various tissues of the body [16]. There is no MCL for phosphate. The recommended maximum concentration in rivers and streams by EPA is a concentration of 0.1 mg/L of total phosphate [7].

Nitrate and phosphate are widely used as inorganic fertilizers so they are anthropogenic pollutants. These anions are responsible for the so-called eutrophication process. Since this process promotes a huge grown of the algae in the surface of water, what avoids the entrance of light into deeper water producing lack of oxygen. For this reason, it is important to keep the control of the concentration of these anions in order not to cause negative effects in the ecosystem.

Bromide

Bromide is usually found in nature with sodium chloride. This is a common component in seawater and volcanic rocks. Concentrations of bromide in fresh water typically range from trace amounts to about 0.5 mg/L while in desalinated waters it may be close to 1 mg/L [7].

Bromide ion has a low degree of toxicity; thus, it has not toxicological concern. However, ozonating source water that contains elevated levels of natural bromide can produce bromated disinfection byproduct (DBP) [17]. World Health Organization (WHO) and EPA have listed bromate as a potential

carcinogen [18]. The EPA Stage 1 disinfectants/disinfection byproducts (D/DBP) rule specifies a MCL of 10 μg/L for bromate [7].

I.2.1.2. Herbicides

According to FAO [19], pesticides are chemicals used for attracting, seducing, destroying or mitigating any pest. These substances are also meant to combat all types of parasites harmful to health. Depending on the nature of its target they receive different names; for weeds they are called herbicides, for fungi fungicides and for insects insecticides. Action mode of pesticides on parasites may be the direct elimination or the control of them, for instance, by interfering with their reproduction process.

Herbicides have widely variable toxicity and can cause a variety of health effects ranging from skin rashes to death.

Herbicides can be grouped by activity, use, chemical family, mode of action, or type of vegetation controlled. Different classifications [20] are summarized in Figure 4.

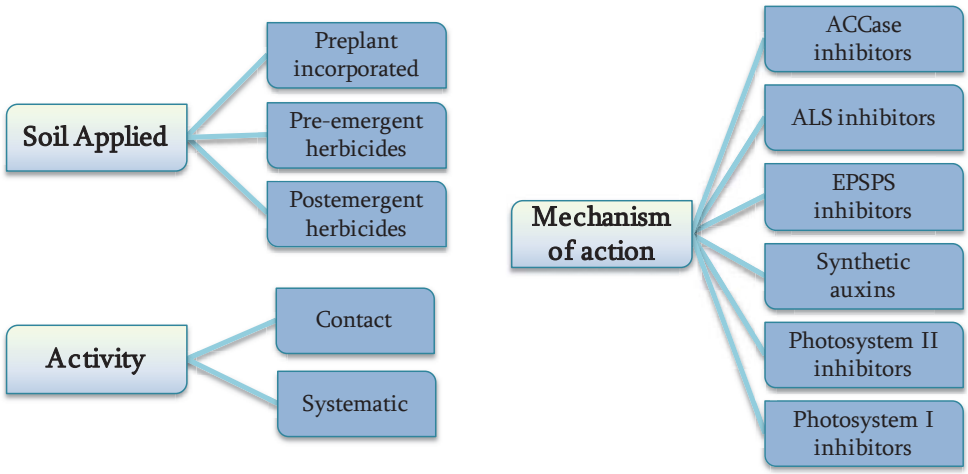

Figure 4 *General classification of herbicides related to the form of soil application, their activity and their mechanism of action.*

Taking into account the classification by activity, there are two types: contact and systemic herbicides. Contact herbicides only destroy the plant tissue in contact with the chemical. Generally, these are the fastest acting herbicides, although they are less effective on perennial plants. On the other hand, the systemic ones are transported through the plant, either from foliar application down to the roots, or from soil application up to the leaves. They are capable of controlling perennial plants and may be slower-acting, but ultimately more effective than contact herbicides. In this WORK, the studied herbicides are systematic.

The most used herbicides are the non-selective ones, since they eliminate all plants which absorb them by green tissue [21]. This kind of herbicides is widely used to clear waste ground, industrial sites and railways, in landscape grass management and in different types of plantation crops.

One of the worldly most used non-selective pesticides is the herbicide, glyphosate (GLYP). Hence, this is one of the compounds studied herein. Other herbicides as glufosinate (GLUF) and bialaphos (BIAL) are also studied in this work.

The use of pesticides has two main drawbacks. One of them is the effect generated on the environment and the other is the human exposition to these toxics during their manipulation. Both aspects are even worse considering the huge amount of these chemicals applied to lands. However, pesticides themselves are not only the problem to deal with but also the degradation products from their partial or total transformation. This process gives new products, in some occasions even more persistent and dangerous than the original pesticides. Therefore, main degradation products from the mentioned herbicides have been also considered as analytes in current research work. Thus the degradation products of GLYP and GLUF, amino methyl phosphonic acid (AMPA) and 3-[hydroxy(methyl)phosphinoyl]propionic acid (MPPA), respectively, were also studied in this work. The degradation product of BIAL is phosphinotricin, which has a half life time of 5 h so it is not included in this research work.

Chemical structures of the analytes studied are shown in Figure 5.

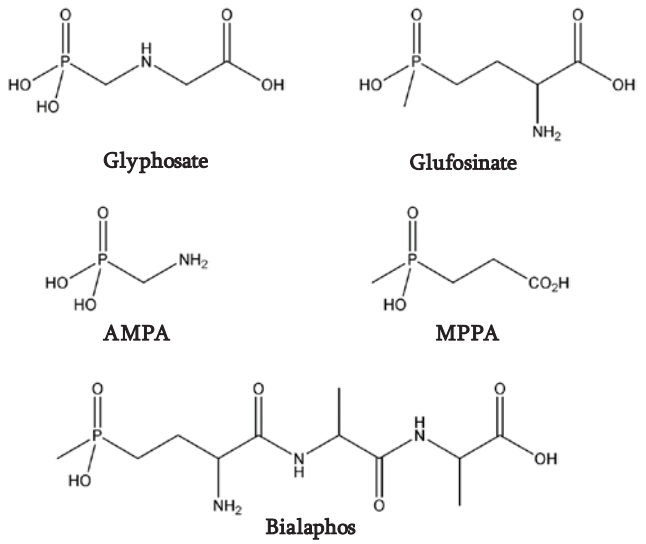

Figure 5 *Chemical structures of the herbicides glyphosate, glufosinate and bialaphos and the main degradation products of GLYP and GLUF, AMPA and MPPA, respectively.*

I.2.2. Matrixes

I.2.2.1. Water

About 71% of earth is covered by water. Drinking water is derived from two basic services, surface water (such as rivers and reservoirs) and ground water. Compounds coming from anthropogenic processes pollute water, provoking that the level and kind of contaminants vary from region to region. Therefore, the contamination of water reflects the degree of contamination of the environment in the course of water from the geological data through which the water flows to the pipes and fixtures of the water distribution system [22]. Pollutants from the air are washed by rain water and they will also be found in the aquifers, streams, rivers, and lakes that supply our drinking water.

As water is needed for all kind of known life, since it makes possible the most diverse chemical and colloid-chemical cell reactions, people need pure, healthy and clean water. Keeping it clean is a task of each individual person living on Earth, while controlling the quality of the water is a priority issue of governments and it is generally carried out by official laboratories.

Good quality water is necessary to promote health and prevent diseases. Governments promote and control the compliance of restrictions to maintain the quality of the water intended for human consumption. The structure of the restriction to be followed is first the European restrictions, then the national ones and, finally, the restrictions set by each state or council. In Spain, the national quality control program for water intended for human consumption is the Royal Decree, RD 140/2003 [23]. All treatment plants or water suppliers must meet the quality criteria established by this restriction.

This Royal Decree establishes that water must be healthy and clean what involves that water cannot contain any type of microorganism, parasite or substance at a quantity or concentration that may cause human health risk. Water requirements specified by RD 140/2003 are listed in Table 1.

Table 1 *Some parameters restricted by the Royal Decree* RD 140/2003 *regarding chemical contaminants in water intended for human consumption [23].*

Parameter	Parametric Value (PV)	Units	Accuracy[a] (% of PV)	Precision[b] (% of PV)	Detection Limit[c] (% of PV)
Bromate	10	µg/L	25	25	25
Chloride	250	mg/L	10	10	10
Fluoride	1.5	mg/L	10	10	10
Nitrate	50	mg/L	10	10	10
Nitrite	0.5	mg/L	10	10	10
Individual Pesticide	0.1	µg/L	25	25	25
Sum of Pesticides	0.5	µg/L	25	25	25
Sulphate	250	mg/L	10	10	10

[a] Accuracy is the systematic error and represents the difference between the mean value of the large number of repeated measurements and the exact value.

[b] Precision is the random error and is usually expressed as the standard deviation (within each batch and between batches) of the dispersion of results about the mean. Acceptable precision is twice the relative standard deviation.

[c] The detection limit is: Whether three times the relative standard deviation within a batch of a natural sample containing a low concentration of the parameter or five times the relative within batch standard deviation of a blank.

Other characteristics that need to be controlled are: smell, colour, taste, pH, conductivity, ammonia, bacteria, metallic content, etc.

Apart from water intended for human consumption, other kind of water is subjected to restrictions, such as surface water. In this sense, all the European Union (EU) member states have to implement management plans in their river basins, including monitoring programs.

European Water Framework Directive (WFD) considers water management from a wide perspective, looking for the protection, the improvement and the prevention of any future deterioration of water bodies, in order to obtain "a good status" of water bodies. According to the WFD, good status of water bodies is obtained when concentrations of the priority substances in water, listed in directive 2008/105/EC [24], are below the established Environmental Quality Standards (EQSs).

I.2.2.2. Fluorinated Compounds

Fluorine is the most electronegative and reactive element of the periodic table. Therefore, it is not found as anion itself. Fluorine is found in nature as the mineral fluorite (CaF_2), fluorapatite ($Ca_5(PO_4)_3F$) and cryolite (Na_3AlF_6). Fluorite contains the greater abundance in fluorine, 30-55% and, therefore, it is used as raw material in fluorine industry [25].

The main deposits of fluorite are located in China (53% of world production), and Mexico. In Europe, there are some economically viable mines, although their number is being gradually reduced. In Spain, the largest deposits are found in Asturias (Caravia and Ribadesella) and there are others smaller in Gipuzkoa, Cantabria and Catalunya.

Fluorite is separated from impurities after being removed from mines or open pits. Then, depending on the degree of purity it is sent to a particular industrial sector. When the purity is at least 97% it is referred to as acid-grade fluorspar, which is used in fluorine industry. After extraction, CaF_2 is mixed with sulphuric acid to produce hydrogen fluoride or hydrofluoric acid in the gaseous form, Eq. 1:

$$CaF_2\,(s) + H_2SO_4\,(aq) \rightarrow CaSO_4\,(s) + 2HF\,(g) \quad \text{(Eq. 1)}$$

From hydrofluoric acid, an extensive range of fluorinated compounds are manufactured, some of these are: fluorides of Na, K, Mg, Al, Li and Ni, bifluorides of NH_4^+ and Na, combined salts like monofluorophosphate and the family of fluorinated acids of B, Ti, Zr, Hf, and Si, and their corresponding salts of Na and K. Among them, the studied compounds herein have been: hexafluorosilicic acid, H_2SiF_6, tetrafluoroboric acid, HBF_4, hexafluorotitanic acid, H_2TiF_6, and hexafluorozirconic acid, H_2ZrF_6.

I.2.3. Analytical Problems to Solve

Each of the analytes previously described represents an analytical problem when it is present in the mentioned matrixes and they will be used for a specific purpose. Therefore, the determination of the analytes of interest in the particular matrixes is crucial to assure the required quality in order to perform an appropriate application.

The word quality involves a lot of different perceptions and meanings, although the main objective of quality is meeting the expectations and needs of customers. The ability to offer customers quality products provides a strong competitive advantage what is translated into higher profits thanks to the higher sales and the reduction of the number of returns to be handled. The assurance of this parameter is commonly carried out by quality control (QC) procedures. QC is a process that is used to ensure a certain level of quality in a product or service [26]. It might include whatever actions a business deems necessary to control and verify certain characteristics of a product. Usually, QC involves thoroughly examining and testing the quality of products. Its basic goal is to ensure that the products provided meet specific requirements and characteristics, such as being dependable, satisfactory, safe and fiscally sound.

Companies engaged in quality control typically test a certain number of products, which are usually chosen randomly [27]. The purpose is to assure the no existence of products or services that do not meet the specified standards of quality fixed by the company. Since, if a problem is identified, it might involve stopping the production until the problem has been corrected with the consequences associated with it.

QC also forms the basis of an efficient business that minimizes waste and operates at high levels of productivity. Therefore, quality control is essential for all business, what includes analysis laboratories, since, as it has been mentioned, QC systems help to reduce levels of waste and rework, cutting costs and improving productivity and production efficiency.

Known its importance from an analytical point of view, QC is the main subject of this section focused on different aspects. Firstly an overview of the situation of different laboratories is given, then, the attention is focused in the antecedent of the issue to, and afterwards, the concrete problem resolution is presented.

I.2.3.1. Official Laboratories

Assurance, control and/or monitoring of the quality of the water are some of the tasks of the official laboratories. There are a lot of different kinds of water analysis laboratories which offer services for several types of water. These services include physical, chemical, and microbiological testing procedures.

In this work, two official analysis laboratories dedicated to the analysis of water samples from two different countries are involved (without any comparative purpose). The first laboratory is the SGIker´s Analytical Research Service (SCAB), which belongs to the University of the Basque Country (UPV/EHU) in Spain. The other one is the Rijkswaterstaat, which is one of the laboratories of the Water Management Centre of the Netherlands. Both laboratories are guided by European restrictions for the type of water they deal with.

SGIker´s Analytical Research Service (SCAB)

SGIker´s Analytical Research Service (SCAB) is a laboratory which analyzes diverse types of analytes in different matrixes. This laboratory can be included in the official laboratories dedicated to the analysis of inorganic anions in different kinds of water, such as drinking water, continental water, wastewater and acid rain.

In 2013, in the SCAB laboratory, approximately 8000 samples were measured, being about 1900 of them water samples. Approximately 35% of these analyses were performed using capillary electrophoresis (CE) with diode array detection (DAD) for inorganic anions determination.

This laboratory is in the process to be accredited under ISO 17025 requirements for inorganic anionic analysis in natural and waste water and acid rain. This accreditation requires the validation and assurance of the quality of the results obtained by the procedure used. In this sense, inorganic anions determination by CE is confronted with that of ion chromatography (IC), which is the analytical technique proposed by EPA as the official one for that purpose.

Although inorganic anions are mostly determined by IC, CE offers several advantages. The most appreciable advantage is the low volume of sample required and the low volume of wastes generated by the technique. This represents an alternative technique to the official one, especially when the sample volume is crucial.

The use of this analytical technique in the routine analysis requires the validation of the electrophoretic method. This validation can be made by using certified reference materials (CRMs) or by comparing results obtained with CE with those calculated using a reference method, as ion chromatographic one, established by EPA, Method 300.1 [28]. Because of the lack of CRM for bromide and nitrite, the validation of the analysis method of these anions in this official laboratory needs to be done through the comparison with the EPA method.

Rijkswaterstaat (RWS)

Rijkswaterstaat is an official laboratory dedicated to the analysis of inorganic anions in surface water, among its different applications. One of the instruments this laboratory is provided with is a capillary ion chromatography system. This equipment was used for the development of new analytical methodologies to cover an unmet analysis in this laboratory up to now.

In the Netherlands, protection of quality water is a topic of great interest and government is extremely implied in its conservation, following the EU normative.

Neither the restrictions mentioned in "Water" section of current scientific memory nor the priority substances list, include GLYP and its main metabolite AMPA. However, it is worthy to mention that both are substances subject to review for possible identification as priority substances or priority hazardous substances by the annex III of the directive 2008/105/EC [24]. For this reason, although it is still not compulsory, these pesticides are also interesting for RWS.

Drinking water in the Netherlands is prepared from different natural water sources such as the rivers Meuse and Rhine, canals, ground water, and dune water. Dutch water companies are responsible for ensuring that reliable drinking water flows from the tap 24 hours per day. This is achieved by purifying the natural water and making sure that it flows to the customer. The purification process of the drinking water is summarized in Figure 6

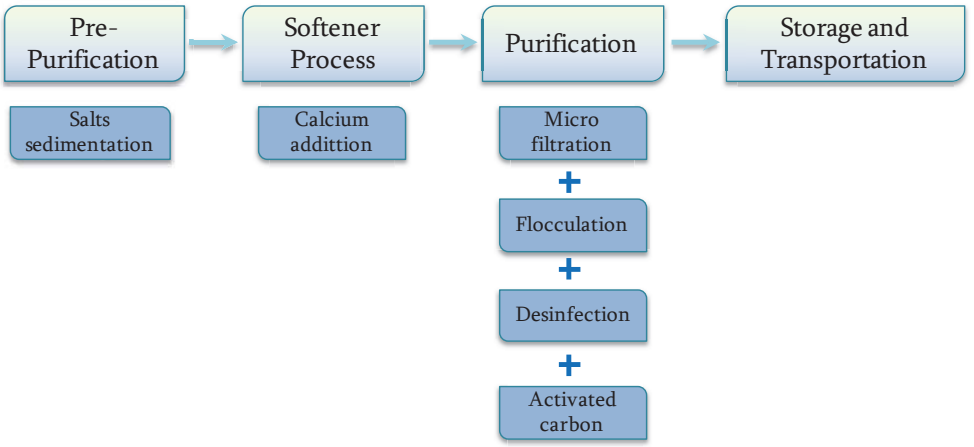

Figure 6 *Water treatment in Dutch water companies for drinking water from its capture to its delivery to consumers.*

As it can be seen in Figure 6, no treatment against strange pollutants is performed, so this kind of pollutants has to be controlled before the entrance to

the water purification plants. Regarding to surface water, RWS is responsible for controlling the presence of pollutants.

Common anions in water are determined by IC while pesticides, in which GLYP is included, are determined by liquid chromatography (LC), with time consuming derivatization steps. For this reason, the development of a faster methodology for the determination of this and other pesticide gains importance to solve the problem associated to this official laboratory.

I.2.3.2. Industrial Laboratories

Derivados del Flúor S.A. (DDF)

Fluorinated compounds production is an important economic and social sector due to the huge amount of applications which depends on it. These applications have great importance because they are involved in the development of essential products for modern life. Besides, this chemical industry sector has a high volume of production and employs a great amount of people. It is estimated that the total number of jobs related to the fluorine industry, including downstream products, is more than 50000. For instance, in 2012, the European production of just hydrofluoric acid was approximately 240000 tonnes with a value of around 320 million EUR.

In Europe the main production centres are placed in 4 different countries: United Kingdom, Spain, Germany and Italy. In Figure 7 the locations of these companies are shown. As it is indicated, Derivados del Flúor S.A. (DDF) [29] is the only fluorinated compounds production company in Spain. They are the manufactures of the majority of fluorinated products studied in this work.

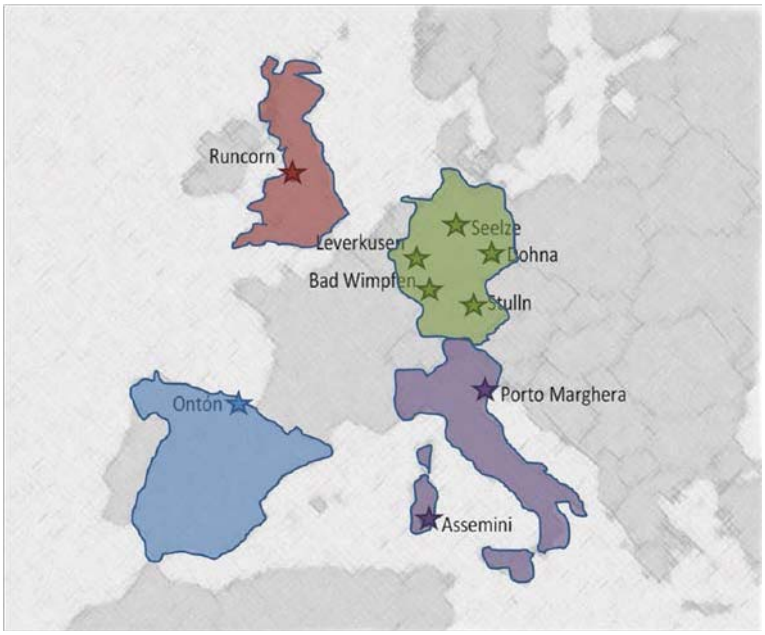

Figure 7 *Location of the production companies of fluorinated compounds in Europe. Lanxess Deutschland GmbH (Germany), Fluorchemie Dohna GmbH (Germany), Fluorchemie Stulln GmbH (Germany), Honeywell Specialty Chemicals Seelze GmbH (Germany), Solvay Fluor GmbH (Germany), Fluorsid S.p.A. (Italy), Solvay Fluor Italy SpA (Italy), Mexichem UK Limited (United Kingdom), Derivados del Flúor SA (Spain).*

Products analyzed in this research work are mainly used in industrial applications. In Figure 8 some of the applications of fluorinated compounds are shown.

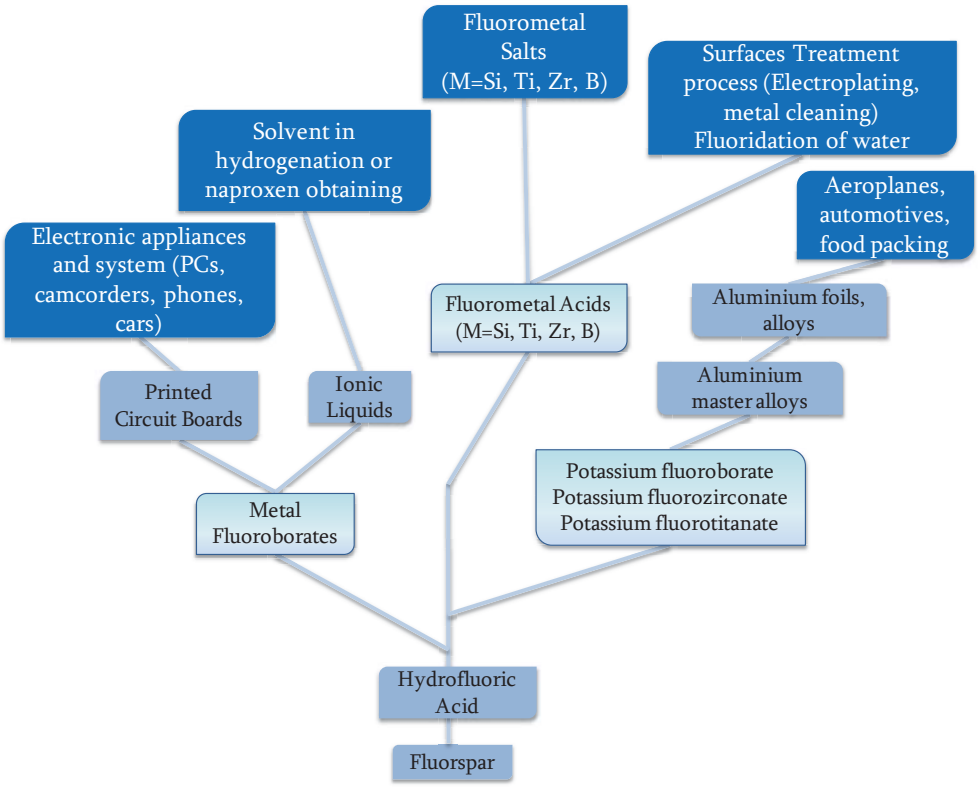

Figure 8 *Summary of applications of different fluorinated compounds, including the analyzed ones in this work.*

All the industrial applications in which the fluorometal acids, object of study, are involved require a quality control management of anionic impurities content due to the drawbacks produced by them. The specific problems which are a consequence of the presence of inorganic anions as impurities in each of the matrixes studied will be widely explained in the different chapters of this scientific memory.

Nowadays, QC of anionic impurities in fluorine industry in Spain is carried out by time consuming chemical methods which are hampered by some interferences, so that, new methodologies for the determination of anions is an important task for problem solving associated to this industrial laboratory.

I.3. ANALYTICAL TECHNIQUES

Techniques to be used in an analytical process depend on the type of analysis to be carried out (qualitative, quantitative), analytes and the specific matrix. For each concrete problem considered in this work, a bibliographic study on analytical techniques previously used to perform the analysis proposed was done. The results in each case will be described in the corresponding chapter.

In this section, a brief description of the analytical techniques used in this work, CE and IC, is provided.

I.3.1. Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) is an analytical technique that separates charged species or ions due to their attraction or repulsion movement under an electric field generated in a conductor electrolyte contained into a capillary.

This technique receives different names as CE, high performance capillary electrophoresis (HPCE) or capillary ion electrophoresis (CIE).

There are six types of capillary electroseparation, five of them based on a continuous system: capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF); and one based on a discontinuous system: capillary isotachophoresis (CITP) [30]. In Figure 9, the most used classification of the different types of capillary electrophoresis is shown.

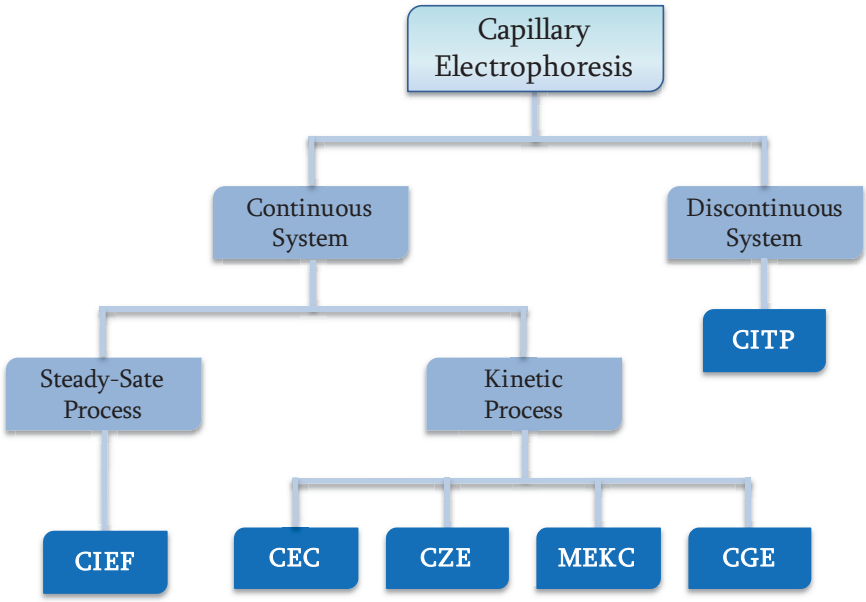

Figure 9 *General classification of the different types of capillary electrophoresis.*

The main difference found in this classification is that a continuous system has a background electrolyte (BGE) along the capillary as buffer whereas a discontinuous system keeps the sample in separate zones by two different electrolytes.

The technique used in this research work was capillary zone electrophoresis.

In CZE mode, anions and cations are separated while neutral molecules migrate together. The separation is promoted by the movement of charged molecules under the action of an electric field generated between the tips of a fused silica capillary filled with a BGE, which is known as electroosmotic flow (EOF). BGE is a conductor solution at a specific pH to maintain the analytes in their ionic state. These ionized analytes interact with the silanol groups of the walls of the capillary and migrate to the detector due to the attraction and repulsion forces generated by the electric field applied. Depending on the composition of the BGE under the action of an electric field, the EOF generated will move to the usual sense in the capillary or to the inverted one generating the called reverse electroosmotic flow. Depending on the charge to mass ratio of the analytes, the

sense of the EOF and the positive or negative electric field applied, they will migrate to the detector at different times in the electropherogram [31]. A scheme of separation basis of the technique and its main components are shown in Figure 10.

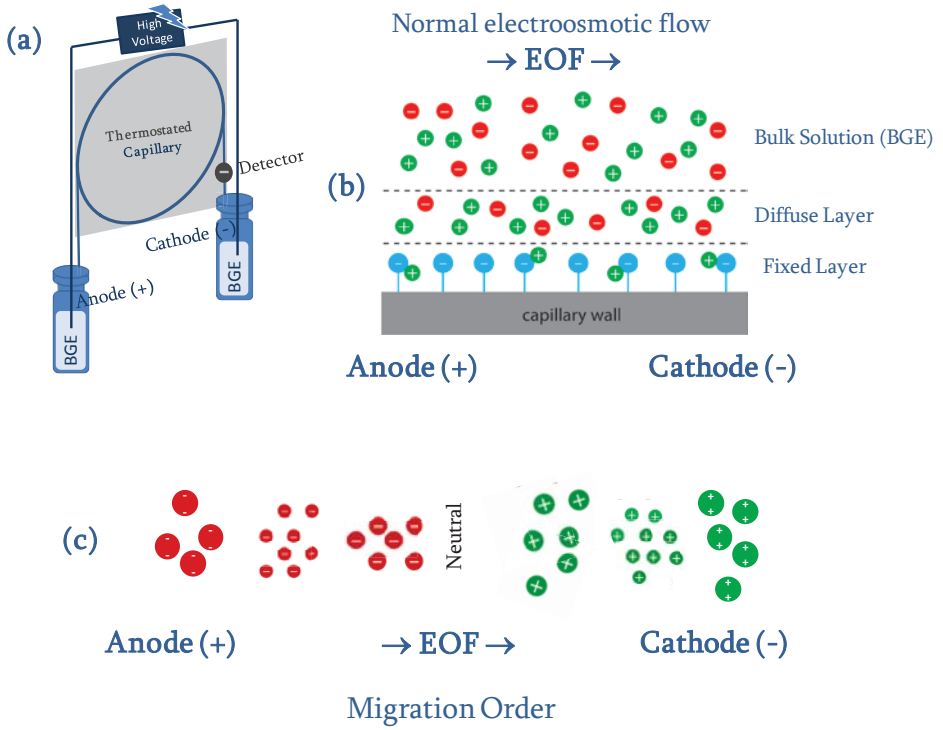

Figure 10 *(a) Basic components of a capillary electrophoresis system. (b) Electroosmotic flow representation in a capillary. EOF is caused by the negatively charged Si-O-groups on the inner wall of the capillary, which attracts the positively charged cations to form the fixed layer. The mobile layer is pulled towards the cathode, dragging the bulk buffer solution with it. (c) Anions and cations migration order [32].*

I.3.2. Ion Chromatography (IC)

Ion chromatography (IC) is an analytical technique used for the separation of ionic and ionizable compounds. IC exploits the different ion exchange affinity of the analytes to develop their separation. Analytes are driven across a column made of a solid support which is called stationary phase. This stationary phase is

provided with functional ligands which promote the separation of the analytes, being the mobile phase, which transports the separated analytes to the detector. The most common detection mode in ion chromatography is the suppressed conductivity, which will be explained later. Figure 11 shows the basic components of this technique and the principles of the suppressed conductivity detection.

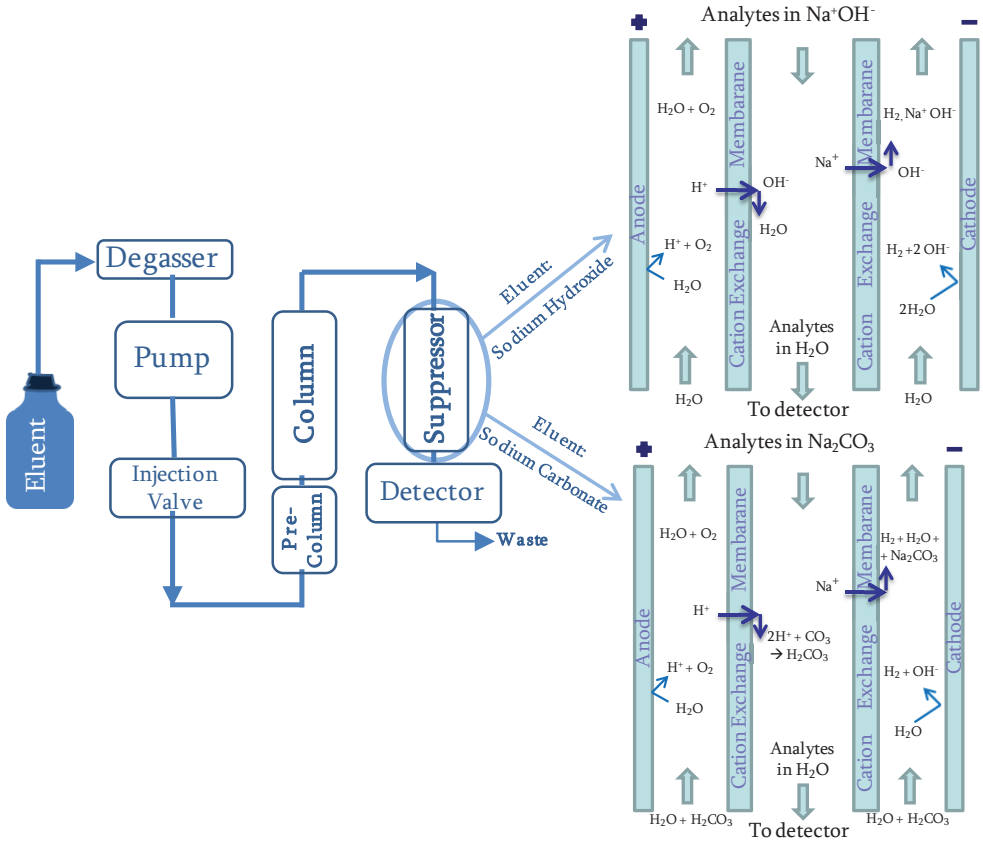

Figure 11 *Basic components of an ion chromatographic system and the process occurred in the suppressor to get the suppressed conductivity detection [33].*

The different interactions of the analytes along the column with those ligands and the strength of the mobile phase determine the final separation of the analytes in the chromatogram. During the elution the analytes with the weakest

ionic interactions elute from the column first while those with a stronger ionic interaction elute later. The elution is promoted by the mobile phase, which is a solution with a low to medium conductivity (for instance, low to medium salt concentration solutions) and a pH value in which analytes are ionized.

I.3.3 Detectors

In this research work, three different detectors were considered, two of them non-specific, such as conductimetric and photometric (diode array detector) ones, and the other, the specific, mass spectrometric one. As these three detection methods are widely known in the scientific field, just a brief summary will be provided in the following sections.

I.3.3.1. Spectrophotometry (Diode Array Detector, DAD)

This is the detection method coupled to CE system for the determination of inorganic anions in water and in hexafluorosilicic acid (chapter II and III of this work, respectively).

Spectrophotometry is a method based on the interaction between electromagnetic radiation and matter. The basic principle is that each compound absorbs or transmits electromagnetic radiation over a certain range of wavelengths. Measurement of the intensity of the radiation as a beam of light passes through sample solution can be used to measure the amount of a known chemical substance.

Depending on the range of wavelengths of the electromagnetic radiation source, spectrophotometric systems can be classified as UV-visible spectrophotometer if it uses radiation over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of the electromagnetic radiation spectrum, or as IR spectrophotometer, if it uses radiation over the infrared range (700 - 15000 nm) of the electromagnetic radiation spectrum.

Within UV-visible spectrophotometer, DAD is an ultraviolet-visible absorption detector [34] which covers from 200 to 600 nm. The representation of its main parts is shown in Figure 12.

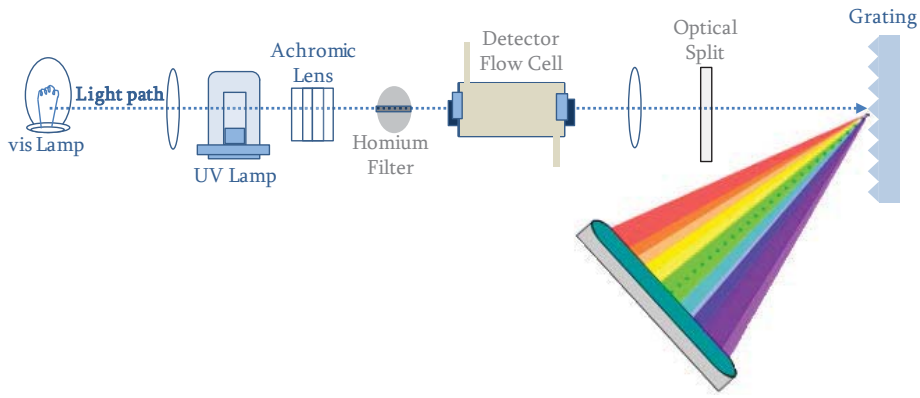

Figure 12 *Schematic representation of a photometric diode array detector (DAD).*

Even though anions generally do not absorb in the UV-vis regions, this technique has been used to their detection by the use of indirect photometric detection. In this mode, an absorbent species or chromophore is added to the background electrolyte (BGE) providing a constant high baseline. When not absorbent ionic analytes are present a drop of the absorbance is observed, what allows their measurement.

I.3.3.2. Suppressed Conductimetric Detector

This is the detection mode used coupled to an IC system for the determination of inorganic anions in fluorinated acids (chapter IV of this research work).

In this case, the detector is a conductivity cell [35]. This is formed by a cavity with two electrodes. The cell scheme and its main components are shown in Figure 13.

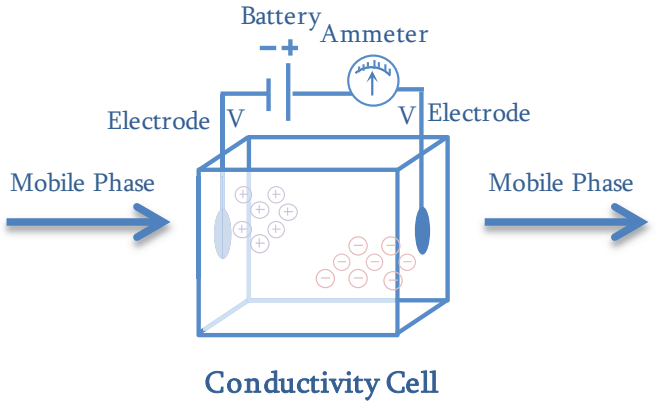

Figure 13 *Schematic representation of the conductivity cell and its main parts.*

Suppressed conductivity is the most used detection mode in IC. A constant voltage applied to the two electrodes contained in the conductivity cell generates a current when the mobile phase flows through the cell. The strength of the mentioned current is proportional to the conductivity of the ionic species in it and their ion conductances. This current provided by mobile phase generates a conductivity background value. When analytes flow into the cell, they provide additional conductivity that generates a peak, proportional to their concentration.

The conductivity of mobile phase solutions is often higher than the conductivities of the analytes. For this reason, suppression is necessary. The suppressor is a device placed between the analytical column and the detector, as can be seen in Figure 11. This device provides hydronium or hydroxyl ions depending on the mobile phase, to neutralize ionized species so that the conductance is lower. This modification of mobile phase produces the reduction of the background of the baseline what implies high sensitivity in the system.

I.3.3.3. Mass Spectrometric Detector

This is the detection system used coupled to ion chromatography system for the determination of organophosporous herbicides and their metabolites in water (chapter V of this work).

This detection technique produces mass spectra of analytes in a sample. The spectrum represents intensity against mass-to-charge ratio (m/z) plotting. It is used to determine the composition of a sample and to elucidate the chemical structures of molecules. Mass spectrometry (MS) works by ionizing chemical compounds to generate charged atoms, molecules or molecule fragments and measuring their m/z. This is achieved by differing the ionized molecules or fragments according to their m/z and detecting by an electron multiplier. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through its characteristic fragmentation pattern [36].

Among all kinds of mass spectrometric analyzers, the one used in this work is a single quadrupole mass spectrometer with electrospray ionization (ESI) source. The scheme of this mass spectrometer is shown in Figure 14.

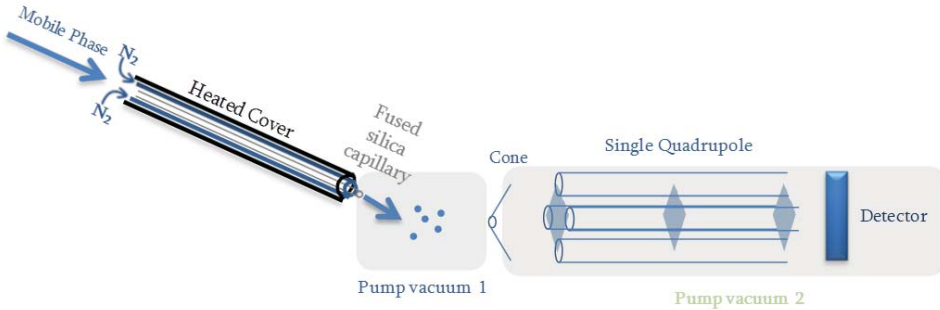

Figure 14 *Schematic representation of electrospray interface together with the single quadrupole analyzer in the mass spectrometric detector.*

This mass spectrometer offers two possibilities of monitoring. First one is full scan, which produces complete mass spectra (chemical fingerprints) for rapid screening of compounds and for analyte confirmation. The second one is

selected ion monitoring (SIM), which monitors only selected ions for target compound analysis and optimized trace level quantification. This mode results in significantly increased sensitivity.

I.4. DETERMINATION OF ENDOGENOUS COMPOUNDS

Endogenous compounds are those which are present in the matrix because of their own nature or their production process. The quantification of these compounds has some drawbacks since there is no blank matrix of them.

There are two options for their quantification; first one is to assure the absence of matrix effect and calculate the concentration, while the second one is to include the matrix in the quantification process. The first option includes the use of external calibration and employs a traditional quantitative process using similar matrix that does not contain the analytes and comparing the results with those obtained in the matrix object of interest. The second one utilizes the standard addition method for compounds quantification and it is shortly explained below.

I.4.1. Standard Addition Method

Standard addition method is usually applied when the matrix of the unknown sample affects the measurement signal and the simulation of the matrix is difficult, time-consuming or impossible to achieve [37].

The use of standard addition method implies two simplifying assumptions: each solution contains exactly the same amount of unknown sample and each solution is diluted up to exactly the same volume. In order not to break these assumptions, solutions should be prepared by pipetting the same volume of the unknown liquid sample or by weighing the same mass of an unknown solid and filling up to the final volume carefully. The development of this method is carried out preparing solutions containing exactly the same amount of unknown sample and different, but well-known, amount of added analyte except for the first solution of the calibration curve. The measurement of the signal is plotted against the volume or concentration of added analyte. The

concentration of the analyte originating from the unknown sample is given directly by the absolute value of the x-intercept of the linear regression. Therefore, standard addition is only applicable if the measurement signals of all solutions fall within the linear working range of the instrument used.

Taking into account the linear regression equation: $Y = mx + b$, where Y is the measured signal or instrument response, m is the slope or instrument sensitivity and b is the Y intercept. The equation of the concentration of the unknown sample (C_x) is:

$$C_x = \frac{bC_S}{mV_x} \qquad \text{Eq. 2}$$

where C_s is the concentration of the added analyte and V_x is the volume of the unknown sample.

Figure 15 shows a graphical scheme of the concentration calculation by this quantification method.

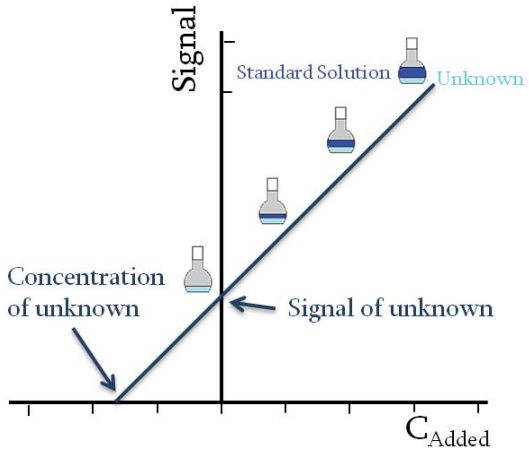

Figure 15 *Graphical representation of the standard addition quantification method.*

The lack of matrixes similar to those studied without the analytes of interest force to use standard addition method although this is a time consuming method to be used as routine analysis. For this reason, a faster and simpler alternative method based on it is proposed as solution in this work.

This alternative method consists of an extrapolation of the data based on the assumption that matrixes of the same type are similar in composition. First, "zero" sample is selected as the sample that provides the lowest chromatographic peak areas for each analyte. The standard addition method is applied to the so called "zero" sample in order to obtain the concentration of anions of interest. Then, external calibrations curves are built using the concentration obtained for "zero" samples and the linear regression data of the additions. These calibration curves are used for the quantitative determination of the rest of samples. Figure 16 shows the representation of the external calibration curves obtained using the alternative method proposed in this work.

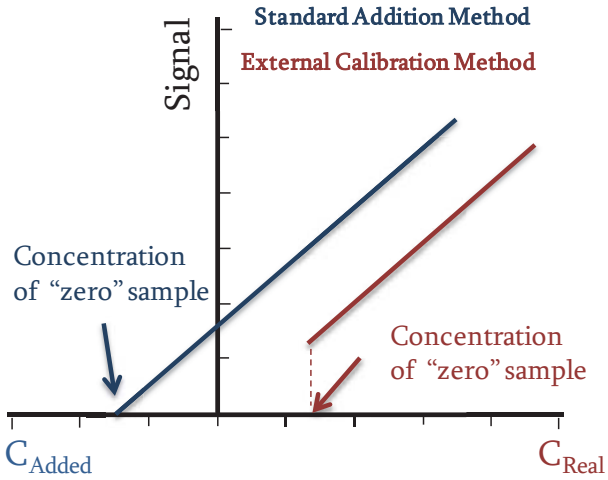

Figure 16 *Graphical representation of the alternative quantification method based on the common standard addition method.*

I.5. VALIDATION OF ANALYTICAL METHODS

Analytical chemistry is a useful tool to solve industrial problems as long as measurements carried out are accurate and reliable. Method validation is a hard task which involves calculation of different parameters and definition of acceptance criteria to ensure the quality of the results obtained with the proposed method.

Method validation means that the developed method is suitable to fulfil the purpose proposed. This procedure involves all steps from the beginning, what is the problem exposition, to the end, what implies to get a reliable solution.

Regarding selected criteria, minimum requirements are generally set by the customer and usually deduced from a previously specified purpose. A formal recognition of this type of quality can be achieved through accreditation or certification based on international quality standards and guidelines, as issued by the International Organization for Standardization (ISO), Organisation for Economic Co-operation and Development (OECD), International Conference on Harmonisation (ICH), and European Committee for Standardization (CEN).

Validation of analytical methods is an essential step in the integral process of quality assurance and quality control of chemical measurements.

There are several guides to validate analytical methods, such as International Union of Pure and Applied Chemistry (IUPAC), Association of Analytical Communities (AOAC) or Eurachem among others. Common parameters for validation of an analytical method are the following: selectivity/specificity, limit of detection, limit of quantification, recovery, working range and linearity, accuracy/trueness, precision (repeatability, reproducibility) and ruggedness/robustness.

These parameters can vary from one validation guide to another and can be also determined in different ways.

Eurachem [38] is the most detailed official guide for theory and practice of method validation. It was developed for ISO accredited laboratories. Therefore, this is the chosen guide to develop the validation of the analytical method for

anions determination by capillary electrophoresis, which is intended to be accredited.

The concept of validation of a method of analysis may be used in, at least, three senses. In the narrow and traditional sense the term denotes validation of a chemical method as described in a standard operating procedure (SOP). In a wider sense validation may be concerned with a method of analysis for instance in an international standard which explicitly leaves freedom to adapt the procedure to the available infrastructure in a specific situation. Finally, in a still wider and perhaps unconventional sense, validation of analytical methods may be considered from the perspective of those who use analytical results for their specifc purposes. The last sense of validation is the one chosen to be applied to validate the developed method for meeting the needs of industrial laboratories.

I.6. OBJECTIVES

Once the analytes, matrixes and problems proposed by official and industrial laboratories have been described, the main objective of this scientific memory is ***to employ analytical chemistry as a tool to provide solutions to different problems exposed by official and industrial laboratories, in order to improve the method used up to now with the consequent economical and environmental benefits.***

The achievement of this ambitious objective needs reaching the three following secondary objectives. The first objective in the field of official laboratories consisted of ***the validation of different quality parameters of the capillary electrophoretic method for the determination of fluoride, bromide, nitrite, nitrate, chloride, sulphate and phosphate in different types of water matrixes such as natural water, waste water and acid rain.***

The second objective in the same field was to ***develop a simple and faster determination method as an alternative to the established one for the determination of some herbicides in surface water by ion chromatography coupled to electrospray mass spectrometry, (IC-ESI-MS).***

Last, regarding fluorine industry, the objective was ***to determine inorganic anionic impurities in complex fluorinated matrixes***. This purpose was affronted using different analytical techniques faster and cleaner than those used up to now in the industrial laboratories. In this point, suitability of CE and IC is tested to overcome the drawbacks associated with the difficulties caused by the complex matrixes.

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USE OF THE EURACHEM (QUAM) GUIDE ON THE ANALYTICAL VALIDATION FOR INORGANIC ANION ANALYSIS BY CAPILLARY ION ELECTROPHORESIS

Chapter II

Chapter II- USE OF THE EURACHEM (QUAM) GUIDE ON THE ANALYTICAL VALIDATION FOR INORGANIC ANION ANALYSIS BY CAPILLARY ION ELECTROPHORESIS

II.1. INTRODUCTION

Inorganic anion pollution of natural waters, such as rivers or lakes, resulting from industrial and domestic sewage discharges and excessive fertilization of farm lands and fish ponds, is increasingly becoming problem in developing countries. Inorganic anionic pollutants include fluoride (*CAS No: 16984-48-8*), bromide (*CAS No: 24959-67-9*), nitrite (*CAS No: 14797-65-0*), and nitrate (*CAS No: 14797-55-8*) defined as primary contaminants by The US National Primary Drinking Water Standards (NPDWS) [1]. Other common inorganic anions, such as chloride (*CAS No: 16887-00-6*) and sulphate (*CAS No: 14808-79-8*), are considered secondary contaminants and are regulated under the *NPDWS*, which are guidelines regarding taste, colour, and certain aesthetic effects [2]. Finally, it is widely reported that phosphate (*CAS No: 14265-44-2*) may be a limiting nutrient for planktonic and attached algae as well as for macrophytes in surface waters [3]. The quality of natural water samples can be seriously affected by the presence of these anionic impurities. As a consequence, they are routinely measured by the Central Service of Analysis Laboratory (University of the Basque Country, UPV/EHU, Spain) to determine their levels of contamination as part of specific quality control programs.

The determination of inorganic anions in environmental waters, such as drinking water, surface water and waste water, is perhaps the most widely used application of capillary ion electrophoresis (*CIE*) and ion chromatography (*IC*) worldwide as described in US Environmental Protection Agency (*EPA*) Method 6500 [4] and Method 300.1 [5], respectively. CIE is increasingly being used in regulated and testing environments that demand validation for quantitative analyses [6 - 8].

Validating an analytical procedure determines whether the procedure meets the requirements of a specific purpose, including that the individual steps reliably fulfil all given specifications and quality characteristics [9]. Since it is materially impossible to guarantee these characteristics for every single test result, international analytical institutions have developed a system that ensures the quality of the results if they have been obtained under certain conditions (International Organization for Standardization [ISO]/IEC Guide 17025:2005,

[10]). According to Bernard King, former chairman of *EURACHEM*, there is evidence to show as much as 30% of all chemical measurements are not fit for their intended purpose [11]. This implies a definite need to prove the integrity of chemical methods in all techniques and application areas. The analytical method described in this paper is useful for the screening analysis of inorganic anions in different aqueous matrixes: drinking water, waste water and acid rain. The basic validation principles of this analytical method have been widely described in terms of its performance criteria or characteristics. In fact, method validation consisted of deriving experimental values for these selected performance criteria. The basic parameters usually refer to the reliability of the analytical method and are commonly derived by using statistical procedures. The trueness, precision, selectivity, sensitivity, linear range, ruggedness, limit of detection, and limit of quantification were the most common [12, 13]. In addition, it is necessary to ensure that the test results will reflect the characteristics of the analyzed sample and not those of the method used to obtain the results. Therefore, the estimated value must not only be traceable but also be associated with an appropriate level of uncertainty so as to ensure whether or not the product complies with regulatory requirements.

Our goal was to validate the *CIE* method described herein, showing that the method meets performance criteria selected in accordance with the Eurachem Guide [14] as useful guide for method validation in analytical chemistry and for uncertainty calculations [15, 16]. Since analytical method validation is not only a process of evaluating characteristics but also of judging suitability for intended use, this report will also demonstrate the performance capabilities of the CIE method for inorganic anion monitoring in natural, acid rain and waste waters.

II.2. EXPERIMENTAL

II.2.1. Reagents and materials

Analytical reagent-grade chemicals were used in all tests without further purification. The calibration standards were daily prepared immediately before use. Certified 1000 mg l^{-1} stock standards were used for Br^-, Cl^-, SO_4^{2-}, NO_2^-,

NO_3^-, F^-, and HPO_4^{2-} anions (Merck, Darmstadt, Germany). Mixed anion solutions typically ranged between 0.5 – 40.0 mg l^{-1}. Multiple procedural blanks consisting of ultrapure water (Milli-Q Element A10, Millipore, Bedford, USA) were analyzed in each series.

Sodium hydroxide solutions were prepared by dissolving different weights of sodium hydroxide pellets (Merck, Darmstadt, Germany). Agilent (Part No 8500-6797) electrolyte buffer, pH = 7.7 (Agilent Technologies, Madrid, Spain) was used and kept at 4 ºC until analysis.

Different Certified Reference Materials (*CRMs*) were used for assessing trueness: *ION 915* (Natural water, National Laboratory for Environmental Testing, Environment Canada, Burlington, Canada), *SPS NUTR WW1*, and *SPS NUTR WW2* (Waste water, SPS, Oslo, Norway), and *AES 05* (Acid Rain, National Laboratory for Environmental Testing, Environment Canada, Burlington, Canada).

II.2.2. Instrumentation and materials

CIE anion analysis was performed on an Agilent Capillary Electrophoresis System (Agilent CE, Agilent Technologies, Madrid, Spain) controlled by HP Chemstation software for instrument control, data acquisition, and analysis. Sensitive detection was based predominantly on indirect ultraviolet (UV) since the majority of the ions lack specific chromophores.

Fused silica capillaries coated with polyimide were used (Agilent G1600-60211, Agilent Technologies, Madrid, Spain): *50 μm ID x 375 μm OD* and an effective length of *40 cm*. Ten standard runs were performed thereby equilibrating the capillary with hydrodynamically injecting, at 400 mbar s, from a 500 μg l^{-1} mixed anion standard solution in water. It was considered as an equilibrated capillary when migration time repeatability of five consecutive runs was RSD < 2.0%.

A Dionex Ion Chromatography System (ICS 1000, Dionex, California, USA) was used for IC analysis. This equipment is an integrated system that, in this case,

consisted of an IonPac precolumn (*AG9-HC, 4 x 50 mm*, Dionex, California, USA), a IonPac column (*AS9-HC, 4 x 250 mm*, Dionex, California, USA), and a ASRS 300 Ultra II (4 mm, Dionex, California, USA) auto suppressed column. A Chromeleon 6.8 Chromatography Data System software to instrument control and data acquisition was used.

The pH was measured using a Crison GLP22 digital pH meter (Crison Instruments, Barcelona, Spain) calibrated with aqueous standards (pH = 4 and pH = 7) immediately prior to use [17, 18].

II.2.3. Analytical procedure

The preservation and handling consideration for the samples were followed in according to ISO 5567-3 [19]. All the samples were analyzed as soon as possible after collection. For nitrite, nitrate, and phosphate, the samples were refrigerated at 4 ºC after collection or preparation and warmed to room temperature before dilution and analysis. In all cases, nitrite and nitrate were determined within 48 hours.

Samples, inorganic anion standards and buffer solutions were filtered through a pre-rinsed 0.22 µm aqueous compatible polytetrafluorostyrene (PTFE) membrane filter (PALL Corporation, Ann Arbor, USA) before transferring the solutions to the analysis vial.

The general analytical conditions listed in Table 1 were used to separate the inorganic anions considered in the CIE and IC methods.

Table 1. *Chromatographic conditions used for analysis of inorganic anions.*

Chromatographic conditions			
Capillary Ion Electrophoresis		*Ion Chromatography*	
Preconditioning:	Buffer flush for 15 min	Flow Rate:	1.0 ml min^{-1}
Capillary:	Fused silica: id = 50 µm, L_{eff} = 40 cm	Columns:	IonPac AG9-HC 4x50 mm IonPac AS9-HC 4x250 mm
Injection:	Pressure: 50 mbar for 8 s.	Injection volume:	25 µl
Applied voltage:	- 30 KV	Eluent:	9.0 mmol l^{-1} Na_2CO_3
Capillary T:	30 ºC	Column T:	30 ºC
Buffer pH:	8.3	Run time:	20 min
Stop time:	10 min		
Detection			
Capillary Ion Electrophoresis		*Ion Chromatography*	
Detection wavelength:	Signal (nm): 350 Reference (nm): 245, 220, 210	Suppressed conductivity detection:	ASRS-ULTRA (4 mm)
Response time:	0.2 s	Mode:	Recycle
Peak width:	0.01 min	Current:	45 mA
Integration:	Peak top type = center of gravity	Backpressure system:	1900 psi
Calibration:	Calculate with corrected areas	Background conductance:	22 µS

The instrumental parameters to be daily tested and controlled were temperature stability, voltage stability, injection precision, detector performance (noise, drift, and wavelength accuracy), and integrator functionality. Additionally, the migration time of each anion was monitored as an indication of the overall function of the CIE instrument. From this experimental migration time, the corrected peak of each peak was used since this could remove minor fluctuations in migration time, due to chemistry, from the analysis of injector

precision. Integrators typically present peak area in dimensions of response to time. This calculation is a simple transformation of the experimental peak area integrated and the corresponding migration time of each analyte ($A_{corr} = A_{peak}$ / *Migration time*).

II.3. RESULTS AND DISCUSSION

II.3.1. Theoretical distribution of phosphate species

The phosphate peak was more efficiently measured when pH of buffer electrolyte was adjusted away from the p*K*a of the couple $H_2PO_4^-/HPO_4^{2-}$ (p*K*a = 7.2) [20]. The Agilent electrolyte buffer used in this work is commercially available at pH = 7.7. The mobility of the inorganic phosphate changes with pH due to different ionization states. In that sense and at a constant total phosphate concentration, an increase in pH led to an increase in the HPO_4^{-2} of inorganic phosphate level (Figure 1).

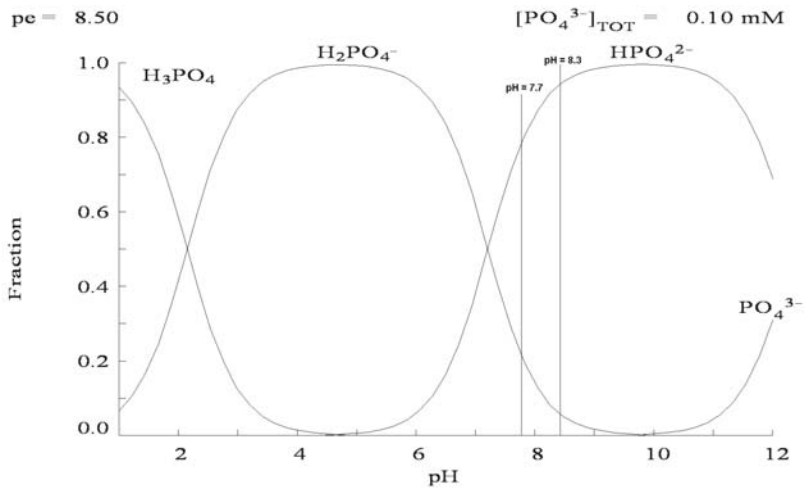

Figure 1. *Distribution of inorganic phosphate (Molar Fraction) species in oxygenated natural waters (0.10 mM = mmol l^{-1} total phosphate concentration and a typical pe = 8.5).*

Approximately, a *1:3* ratio was obtained at pH = 7.7 between $H_2PO_4^-$: HPO_4^{2-} species. From the Figure 1, the more satisfactory pH with respect to the best quality of separation was found to be 8.3, being HPO_4^{2-} the main phosphate species in solution at pH > 8.3. Thus, the electrolyte buffer pH was adjusted to pH = 8.5 ± 0.1 prior to each run by adding NaOH (0.1 mol l^{-1}) drops.

A typical electropherogram of the inorganic anion analysis is shown in Figure 2a resulting in short analysis times of five minutes or less. Under pH = 8.5 conditions (Table I), capillary ion analysis had enough separation efficiency with a very high peak capacity and resolution between all the inorganic anions for accurate quantification.

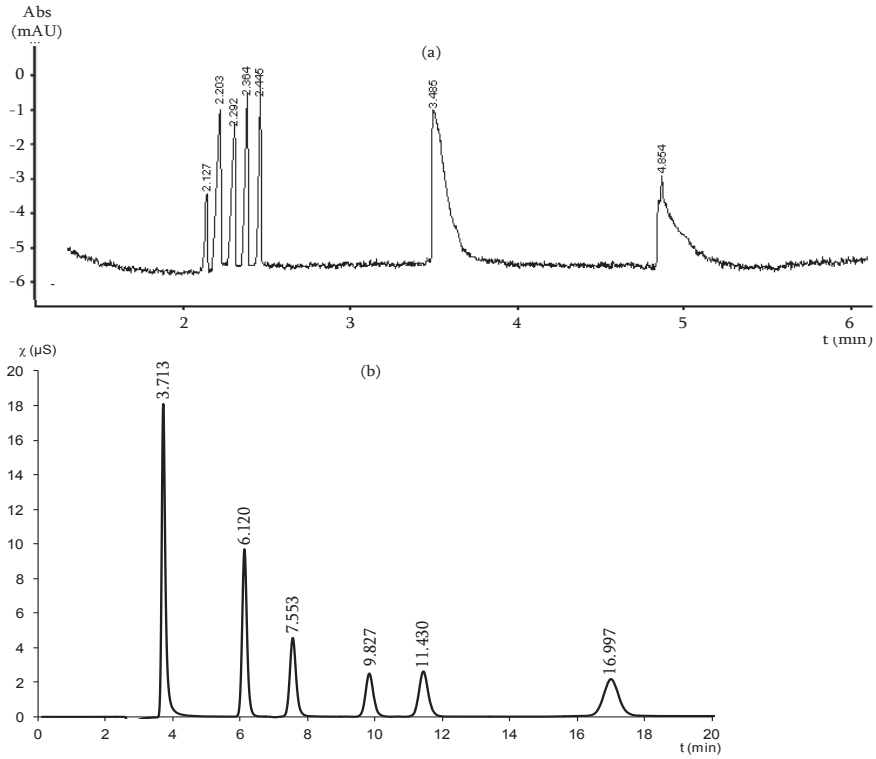

Figure 2. *a) Electropherogram of the 5.0 mg l^{-1} standard mixture of each anion. Peaks are (2.127 min) bromide, (2.203 min) chloride, (2.292 min) sulphate, (2.364 min) nitrite, (2.445 min) nitrate, (3.485 min) fluoride and (4.854 min) phosphate, b) Chromatogram of the 10 mg l^{-1} inorganic anion standard mixture. Peaks are (3.713 min) fluoride, (6.120 min) chloride, (7.553 min) nitrite, (9.827 min) bromide, (11.430 min) nitrate, and (16.997 min) sulphate. Signal 350/80 nm and Reference 245/10 nm.*

II.3.2. Performance criteria

II.3.2.1 Trueness

Trueness is defined as the closeness of agreement between the experimental value obtained from a large set of test results and a true reference value as International Vocabulary of Metrology (VIM) 3 pointed out [21].

CRMs should be used whenever possible because they have the highest level of traceability. Different CRMs were analysed 10 times for trueness assessing in this work (Table 2).

Table 2. *Certified and experimental concentration (mg l^{-1}) values obtained. The uncertainties of the certified values were defined as half width of the 95% confidence interval.*

		Cl^-	SO_4^{2-}	NO_3^-	F^-	HPO_4^{2-}
ION 915 Natural water (Lake Superior)	C_{method}	1.54 ± 0.05	3.50 ± 0.07	1.52 ± 0.09	_	_
	$C_{certfied}$	1.41 ± 0.02	3.41 ± 0.05	1.55 ± 0.06	_	_
	t_{exp}	0.634	0.187	0.243		
SPS NUTR WW1 (Waste water)	C_{method}	5.14 ± 0.09	19.8 ± 0.2	0.95 ± 0.05	0.99 ± 0.02	1.45 ± 0.04
	$C_{certfied}$	5.00 ± 0.05	20.0 ± 0.2	1.00 ± 0.01	1.00 ± 0.01	1.50 ± 0.02
	t_{exp}	1.347	0.629	1.961	0.365	1.192
SPS NUTR WW2 (Waste water)	C_{method}	48.7 ± 0.2	99.1 ± 0.4	4.94 ± 0.08	9.96 ± 0.09	7.41 ± 0.09
	$C_{certfied}$	50.0 ± 0.5	100 ± 1	5.00 ± 0.05	10.0 ± 0.1	7.50 ± 0.08
	t_{exp}	1.317	0.436	0.549	0.222	0.554
AES 05 Acid rain	C_{method}	0.14 ± 0.09	1.23 ± 0.07	1.12 ± 0.03	_	_
	$C_{certfied}$	0.223 ± 0.004	1.28 ± 0.01	1.15 ± 0.08	_	_
	t_{exp}	1.94	0.539	0.16		

From these measurements, a mean concentration ($\overline{C_{method}}$) and a standard deviation (s_{method}) values were obtained for assessing trueness by statistically comparing the mean concentration ($\overline{C_{method}}$) and the certified value (C_{ref}) using a *t* test. Equation 1 was used [20]. Here u_{Cref} is the standard uncertainty of the certified reference concentration value. The t_{exp} value calculated (Table 2) was then compared with the two-sided *t* tabulated value, $t_{tab} = 2.262$, for $\alpha = 0.05$ and the effective degrees of freedom, (ν = number of determinations − 1 = 9). The trueness of the method was assessed for the following analytes: Cl^-, SO_4^{-2}, NO_3^-, F^-, and HPO_4^{-2} since $t_{exp} \leq t_{tab}$ in all cases.

$$t_{exp} = \frac{\left| C_{ref} - \overline{C_{method}} \right|}{\sqrt{u_{Cref}^2 + \frac{s^2}{n}}} \quad (1)$$

However, the trueness for Br^- and NO_2^- could only be assessed by comparison of the CIE experimental data with the corresponding results obtained from IC considered as a reference method. Although spiked (test) samples have the lowest level of traceability for trueness assessing, they were the only alternative in this case because no CRM was found containing these inorganic anions in natural waters. Figure 2a shows the electropherogram for seven anions; this was compared with a typical chromatogram obtained from a conventional anion exchange column (Figure 2b) using the experimental conditions listed in Table 1.

Test samples (spiked water) were analyzed both with the reference method (IC) and the method to be validated (CIE) in different runs (days). Table 3 lists the total concentrations obtained in the different spiked samples using both reference and test methods for nitrite and bromide anions.

Table 3. *Experimental data obtained (mg l^{-1}) for bromide and nitrite in spiked water samples.*

Spiked	Br^-				NO_2^-			
Sample	IC	CIEl	F_{exp}	t_{exp}	IC	CIE	F_{exp}	t_{exp}
1	1.91 ± 0.04	1.92 ± 0.02	4.00	0.548	2.10 ± 0.07	2.09 ± 0.05	1.96	0.285
2	1.94 ± 0.03	1.91 ± 0.02	2.25	2.038	7.48 ± 0.09	7.45 ± 0.09	1.00	0.577
3	4.66 ± 0.07	4.70 ± 0.04	3.06	1.286	4.75 ± 0.06	4.72 ± 0.07	0.73	0.797
4	7.35 ± 0.08	7.30 ± 0.05	2.56	1.298	2.01 ± 0.05	1.96 ± 0.05	1.00	1.732
5	7.33 ± 0.05	7.32 ± 0.05	1.00	0.346	7.55 ± 0.01	7.51 ± 0.08	0.02	1.215

Experimental data were obtained from six replicates in each case using the standard deviation (SD) as the measure of uncertainty. An average concentration $\left(\overline{C_{ref\ method}}\right)$ and a standard deviation $\left(s_{ref\ method}\right)$ values were obtained from the $\left(n_{ref\ method} = 6\right)$ results obtained with the reference method (IC). Subsequently, a *t* test was used to assess the trueness of the test method (CIE) with the $\left(n_{Test\ method} = 6\right)$ data obtained in the same samples: $\left(\overline{C_{Test\ method}}\right)$ and $\left(s_{Test\ method}\right)$. The precision of the analysis showed a relative standard deviation *(RSD)* < 3.0% for the six different measurements of the same sample in all determinations considering both techniques.

Ideally, the results obtained with both methods should be completely correlated (i.e., the correlation coefficient (*r*) should be equal to unity). However, the correlation coefficient cannot be interpreted directly in terms of trueness. Therefore, the *r* values were considered as a preliminary indication and statistical tests (*t*-test, regression line, and comparison of variances) were also applied.

The Null hypothesis asserts that there is no statistical distinction between the values obtained from both analytical techniques. The difference of the corresponding variances was checked with an *F*-test in which S_1 is the larger of the reference or test variances [22]:

$$F_{exp} = \frac{s_1^2}{s_2^2} \quad (2)$$

The corresponding ratio of variances (F_{exp}) was compared with the two-sided F tabulated value (5.05) for $\alpha = 0.05$ and the $v_1 = (n_1 - 1)$ and $v_2 = (n_2 - 1)$ degrees of freedom. Since $F_{exp} < F_{tab}$ in all test samples (Table 3), it was concluded that the null hypothesis could not be rejected. After that, CIE method trueness with respect to certified reference standards was investigated with the following t-test [22]:

$$t_{exp} = \frac{\left|\overline{C_{ref}} - \overline{C_{test}}\right|}{\sqrt{\frac{s_{test}^2 (n_{test} - 1) + s_{ref}^2 (n_{ref} - 1)}{n_{test} + n_{ref} - 2} \cdot \left(\frac{1}{n_{test}} + \frac{1}{n_{ref}}\right)}} \quad (3)$$

The t experimental value was compared with the two-sided t tabulated (2.228) for $\alpha = 0.05$ and the ($n_{test} + n_{ref}$ -2 = 10) degrees of freedom. The trueness of the CIE method was not rejected since $t_{exp} < t_{tab}$ (Table 3).

In addition, a graphical treatment was also performed for assessing bromide and nitrite trueness. When the experimental results obtained with both analytical procedures were compared, a straight regression line was obtained. A proportional systematic error leads to a change in $b = 1$(slope) and a constant systematic error shows up in a value of the intercept (a) different from zero [22]. The fit test equation ($y = a + bx$) was used, where y is the result of the CIE method and x the result obtained from the IC method in the same spiked samples. The usual least squares regression was employed to investigate whether ordinate and slope differ significantly from zero and one, respectively. Figure 3 shows the linear regression fitting performed for each sample and collects the least squares parameters obtained. The uncertainty values listed in Figure 3 for slope and ordinate were calculated by means of Equation 4 [22].

$$\chi_i = t \cdot s \quad (4)$$

Here χ_i is the uncertainty associated with the mean concentration value, t is the Student´s t-test parameter for a significance of 95%, and s is the SD of slope and ordinate in the least squares regression, respectively. Both bromide and

nitrite data were close to those assumptions because slope and ordinate were not significantly different from zero and one, respectively. The validity of the test required a normal distribution of the difference of the values obtained using both analytical procedures.

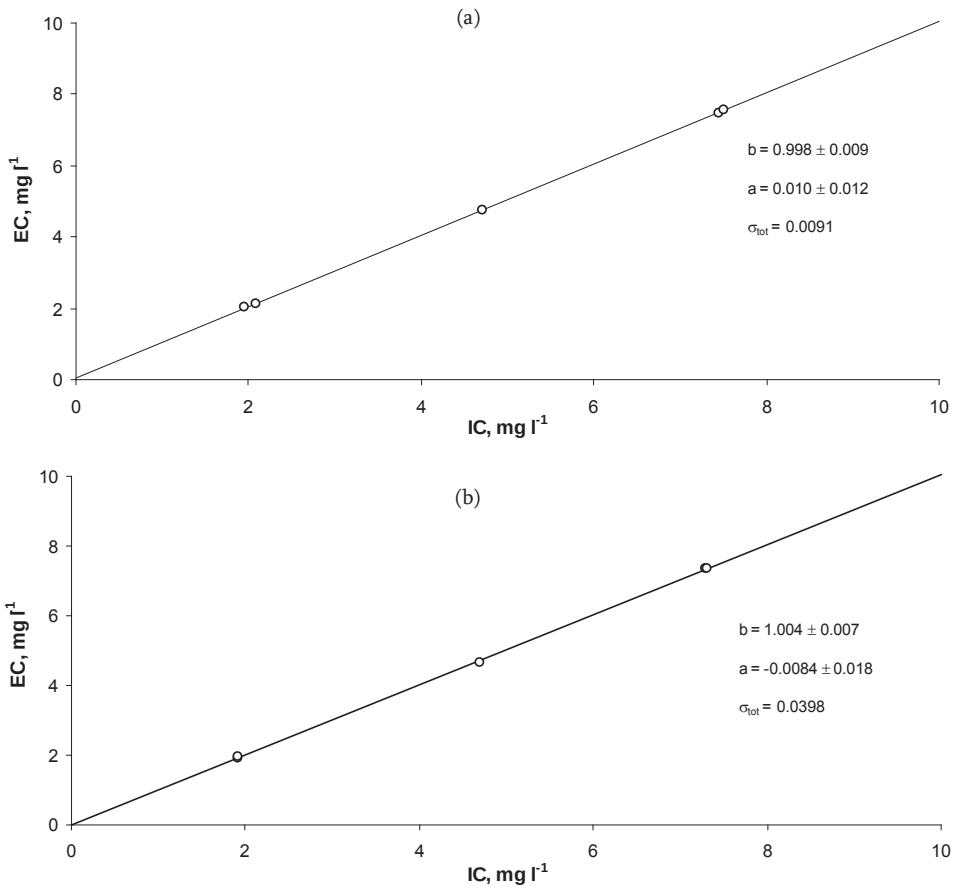

Figure 3. *Least squares fitting for reference (Ion Chromatography) and test (Capillary Ion Electrophoresis) methods for nitrite (a) and bromide (b) anions, b: slope, a: intercept, and σ_{tot}: total standard deviation of the regression. (–) Theoretical curve (b = 1 and a = 0).*

II.3.2.2 Precision

Precision is the closeness of agreement between independent test results obtained under stipulated conditions [13]. Repeatability (n = 10) [23] was

obtained under repeatability conditions as *RSD* < 1.0% for migration times and RSD less than 3.0% for corrected peak areas in all the inorganic anions of interest at intermediate concentration level (20 mg l^{-1}). In addition, the run-different intermediate precision was also obtained by changing the factors affecting the analytical results, such as day or calibrations. The number of replicates per day was equal to two (n = 2) and the effort was focused on the number of runs (n = 10) as Kuttatharmmakul recommended in 1999 [24]. The use of analysis of variance (ANOVA) provided the run-different intermediate precision and the precision between runs [22]. Moreover, ANOVA defined the significant difference between different days (F-test). For all the inorganic anions of interest, no statistically significant difference was found between series (n = 2) since $F_{exp} < F_{crit}$ = 4.414 (Table 4). The critical value was also defined from ANOVA in the output results.

Table 4. *Calculation of the precision parameters obtained from the ANOVA*

Br^-	Cl^-	SO_4^{2-}	NO_3^-	NO_2^-	F^-	HPO_4^{2-}
Repeatability variance, s_r^2						
$8.185\ 10^{-9}$	$1.016\ 10^{-6}$	$5.551\ 10^{-7}$	$8.574\ 10^{-7}$	$3.305\ 10^{-7}$	$4.704\ 10^{-6}$	$1.730\ 10^{-7}$
Repeatability limit, r						
$2.292\ 10^{-8}$	$2.844\ 10^{-6}$	$1.554\ 10^{-6}$	$2.401\ 10^{-6}$	$9.254\ 10^{-7}$	$1.317\ 10^{-5}$	$4.843\ 10^{-7}$
Internal reproducibility variance, s_{run}^2						
$3.169\ 10^{-5}$	$2.784\ 10^{-5}$	$1.402\ 10^{-5}$	$2.768\ 10^{-5}$	$3.232\ 10^{-5}$	$2.543\ 10^{-4}$	$7.328\ 10^{-5}$
Internal reproducibility limit, R						
$8.874\ 10^{-5}$	$7.791\ 10^{-5}$	$3.925\ 10^{-5}$	$7.751\ 10^{-5}$	$9.048\ 10^{-5}$	$7.119\ 10^{-4}$	$2.052\ 10^{-4}$
F_{exp} parameter						
0.005	0.657	0.713	0.557	0.184	0.333	0.043

Finally, the repeatability ($r = 2.8\ s_r$) and internal reproducibility ($R = 2.8\ s_{run}$) limits were also calculated (Table 4) as the absolute difference between two single test results obtained under repeatability or run different intermediate conditions expecting to lie with a probability of 95% [25].

II.3.2.3 Uncertainty

Uncertainty is defined as a parameter, associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measure [24]. It is necessary to take each source of uncertainty in estimating the overall uncertainty and treat it separately to obtain the contribution from that source. Each separate contribution to uncertainty is referred to as an uncertainty component. Uncertainty can be calculated using the information generated in the assessment of trueness ($u_{trueness}$) and intermediate precision (s_{run}) [15]. Therefore, uncertainty can be calculated as the sum of four terms:

$$U = 2 \cdot \sqrt{s_{run}^2 + u_{trueness}^2 + u_{pretreatment}^2 + u_{other}^2} \quad (5)$$

The third component considers the uncertainty of subsampling or pretreatments not carried out in the assessment of trueness such as dilution of samples. This component also includes filtration, weighing or drying, etc. Finally, the fourth component involves all the sources of uncertainty not considered in the former terms.

For uncertainty of the assessment of trueness equation 6 was used.

$$u_{trueness} = \sqrt{\frac{s_{run}^2}{n} + u_{C,ref}^2} \quad (6)$$

Here, n is the number of times that the reference sample is analyzed in the assessment of trueness and $u_{c,ref}$ is the standard uncertainty of the reference sample a) CRM as $U_{CRM}/2$ or b) reference method as $s_{ref}/(n_{ref})^{1/2}$ (Table 5).

Table 5. *Total and individual term of uncertainty for each inorganic anion.*

Br^-	Cl^-	SO_4^{2-}	NO_3^-	NO_2^-	F^-	HPO_4^{2-}
Analyte concentration level (mg l^{-1})						
4.66	1.42	3.42	1.55	4.75	1.00	1.50
			$u_{trueness}$			
0.0255	0.01955	0.05215	0.02867	0.02915	0.00632	0.01265
			s_{run}			
0.07	0.05	0.07	0.09	0.07	0.02	0.04
			u			
0.0038	0.0054	0.0124	0.0170	0.0106	0.00084	0.0034
			U			
0.0076	0.0108	0.0248	0.0340	0.0212	0.00168	0.0068
Relative Uncertainty (%) $100\left(\frac{u(x)}{X}\right)$						
0.08	0.38	0.36	1.09	0.22	0.08	0.22

The sample pretreatment component can be calculated using a sample with the same characteristics as the routine samples. The replicates should be analyzed by changing all the factors that may affect them: operator, day or material. The uncertainty can be calculated as:

$$u_{pretreatment} = \sqrt{s^2_{pretretament} + s^2_{conditions}} \quad (7)$$

Here $s^2_{pretreatment}$ is the variance of the results and $s^2_{conditions}$ depends on the analysis conditions: a) under repeatability s^2_r or b) under intermediate precision s^2_{run}.

Uncertainties of each component were calculated using a spreadsheet method. Table 5 shows results from uncertainty calculations for the determination of each inorganic anion using the CIE method. $s_{pretreatment}$ and u_{other} terms were not included in the uncertainty calculation because the sources of uncertainty defined by these terms were representatively varied in the estimation of the intermediate precision. For example, all the standards and samples are routinely filtered in the analysis and no dilution step was necessary in the samples

included. In any case, if any dilution step must be implemented for some real sample, the uncertainty of the pretreatmet term can be calculated as:

$$C\left(mgl^{-1}\right) = C_{exp} \cdot F_c \quad (8)$$

$$\frac{u_c^2}{C^2} = \frac{u_{C,exp}^2}{C_{exp}^2} + \frac{u_{Fc}^2}{F_C^2} \quad (9)$$

Here, F_c is the dilution factor with its corresponding uncertainty such as pipette, balance or flask.

II.3.2.4 Linear and working ranges

If possible, one should attempt to work with a first order calibration function. Second order calibration functions should only be used in justified exceptions. For estimation of the working and linear ranges, six concentration levels using at least three replicates of each level plus blank were needed. The preliminary concentration range was from 0.5 to 40 mg l^{-1} for each inorganic anion since this range include the regulatory standard specified by *NPDWS* [1-2].

It is important to point out that the calibration function is usually found using the ordinary least squares method and assuming a homogeneous variance of measured values over the range. In order to verify the homogeneity of the variances, n = 10 standard samples of each analyte in both the lowest s_1^2 and highest s_6^2 concentrations of the calibration range were analyzed separately. In all cases, the absence of possible outliers in the two corresponding concentration levels was verified by means of Q-Dixon [26] and Grubbs [27] tests. The variances of both concentration levels were checked for homogeneity using an F-test [22]:

$$F_{exp} = \frac{s_1^2}{s_6^2} \quad (10)$$

For all the inorganic anions considered, F_{exp} showed no a significant difference between the variances since $F_{exp} < F_{tab} = 5.35$ [9] ($f_1 = n_1 - 1 = 9$, $f_6 = n_6 - 1 = 9$, P = 99%). From this comparison, the normal calibration curve was used in order to

obtain the characteristic data of the calibration instead of weighted regression [22].

The linearity of the calibration was assessed using both correlation coefficient ($r^2 > 0.995$) and the plot of the residual values. A high value of the correlation coefficient and a good plot of the residual values were not enough to assess linearity. Additionally, Mandel´s fitting test was used as mathematical verification of the linearity [28]. This test was based on the assumption that relatively large deviations of measured values from a straight line are caused by nonlinearity and may be reduced through the selection of a second order function. The difference of the variances DS^2 was calculated using the residual standard deviation s_{y1} (from the first order calibration function) and s_{y2} (from the second order calibration function) with the degree of freedom $f = 1$ and N calibration points:

$$DS^2 = (N-2)\cdot s_{y1}^2 - (N-3)\cdot s_{y2}^2 \quad (11)$$

The Mandel's test value was calculated for the F test:

$$F_{exp} = \frac{DS^2}{s_{y2}^2} \quad (12)$$

F_{exp} showed no a significant difference between the variances since $F_{exp} < F_{tab} = 16.26$ [9] ($f_1 = 1$, $f_2 = N - 3 = 5$, $P = 99\%$). From this comparison, the first order calibration function was only considered for each analyte. This fact was also confirmed by plotting the residual values in order to look for evidence of trends with respect to analyte concentration or other tendency to exhibit non-randomness. Possible outliers in the experimental points were carefully examined using a specific Cook test for that detection [29]. Figure 4 shows plots of residual values for chloride, sulphate, and nitrate where randomly distribution of the residuals was obtained. Similar tendencies were found for the other inorganic anions considered (Br^-, NO_2^-, F^-. and HPO_4^{2-}).

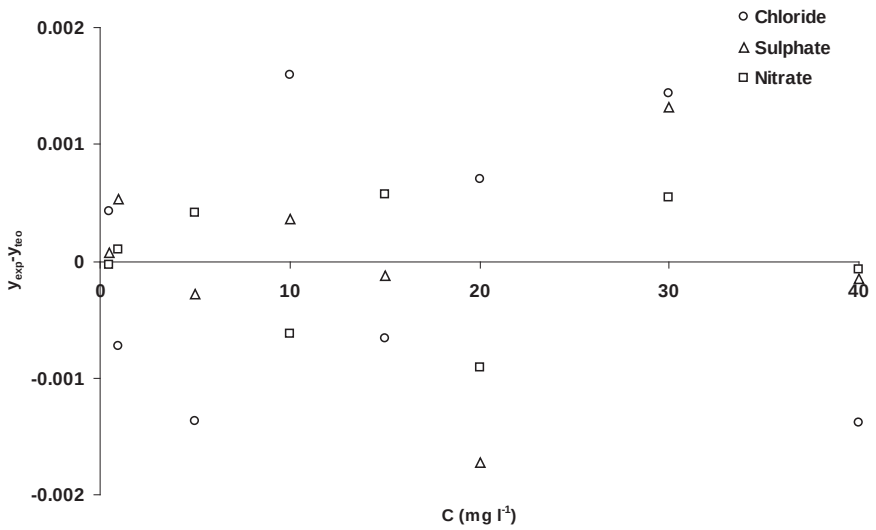

Figure 4. *Plot of residual values for chloride, sulphate and nitrate linear calibrations.*

II.3.2.5 Limit of detection and quantification

The limit of detection (minimum detectable net concentration, X_{MDV}) is commonly defined as the minimum amount or concentration of analyte that can be reliably detected by a given analytical method [30]. Additionally, the limit of quantification (X_{QL}) is the lowest amount or concentration of analyte that can be determined with an acceptable level of precision and trueness [14].

Both detection and quantification limits could be calculated from the decision limit (X_{DL}) values defined as Equation 13, where the signal of 10 measurements of the calibration blank is used [31, 32]:

$$X_{DL} = \frac{s_B \cdot t_{f,\alpha} \sqrt{\frac{1}{N_a} + \frac{1}{N_B}}}{b} \quad (13)$$

Here b was the slope of the regression line and N_a the number of replicates per sample used. Additionally, S_B was the standard deviation of N_B measurements of blank, and t was the Student´s t parameter defined for 95% of confidence level and f degrees of freedom (N_B - 1).

In our case, the decision and quantification limits were calculated from the signal to noise ratio ($S/N = 3$) since it is also suitable procedure for chromatographic methods. In a normal electropherogram, the calibration blank ought to be zero and Equation 13 is not possible to use properly. From the decision and quantification limits, the numerical values of X_{MDV} (minimum detectable value) and X_{QL} (quantification value) were calculated taking into account the chosen levels of significance and the number of degrees of freedom. For equal significance levels, the X_{MDV} and X_{QL} values [9] were calculated as:

$$X_{MDV} = 2 \cdot X_{DL} \quad (14)$$

and

$$X_{QL} = K \cdot X_{DL} \quad (15)$$

Here K is a factor defined depending on the maximum relative result uncertainty ($K = 10$ for a maximum acceptable relative result uncertainty considered ($U = 10\%$) [9]. In this case, the quantification limit was calculated as $X_{QL} = 3 \cdot X_{DL}$ whereby the factor 3 corresponds to a 33.3% relative result uncertainty [9]. These detection and quantification limits obtained for each inorganic anion are listed in Table 6.

Table 6. *Decision limit (X_{DL}), minimum detectable value (X_{MDV}) and quantification (X_{QL}) limits for each inorganic anion considered and expressed in mg l^{-1}.*

	X_{DL}	X_{MDV}	X_{QL}
Br^-	0.076	0.152	0.76
Cl^-	0.026	0.052	0.26
SO_4^{2-}	0.039	0.078	0.39
NO_3^-	0.018	0.036	0.18
NO_2^-	0.047	0.094	0.47
F^-	0.004	0.008	0.04
HPO_4^{2-}	0.012	0.024	0.12

II.3.2.6 Selectivity

Selectivity can be defined as the ability of a method to accurately and specifically determine the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test [14]. In order to check this quality parameter, different water matrix and method blanks were analyzed for the background signals assessing, therefore, the significance of any interference relative to the lowest analyte level specified in the working range was also evaluated. Responses of the different blanks were less than 1.0% of the response in the lowest concentration measured for all the analytes considered.

II.3.2.7 Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small variations in the method parameters and provides an indication of its reliability during a normal usage [14] from the examination of the effect of these changes on the trueness and precision of the final results. Typical parameters can be subdivided in automatically controlled (voltage, temperature, etc.) or not automatically controlled as pH of the samples. This last parameter was tested to assess the robustness of the analytical method by analyzing different certified reference water materials with different pH values from 4.5 (acid rain, *AES 05*), 7.7 (natural water, *ION 915*) to 8.1 (waste water, *SPS NUTR WW1*), and high matrix concentration levels. Figure 5 shows the typical electropherogram obtained for these CRMs considered focusing on chloride, sulphate, and nitrate since they are the only anions present in the 3 CRMs considered.

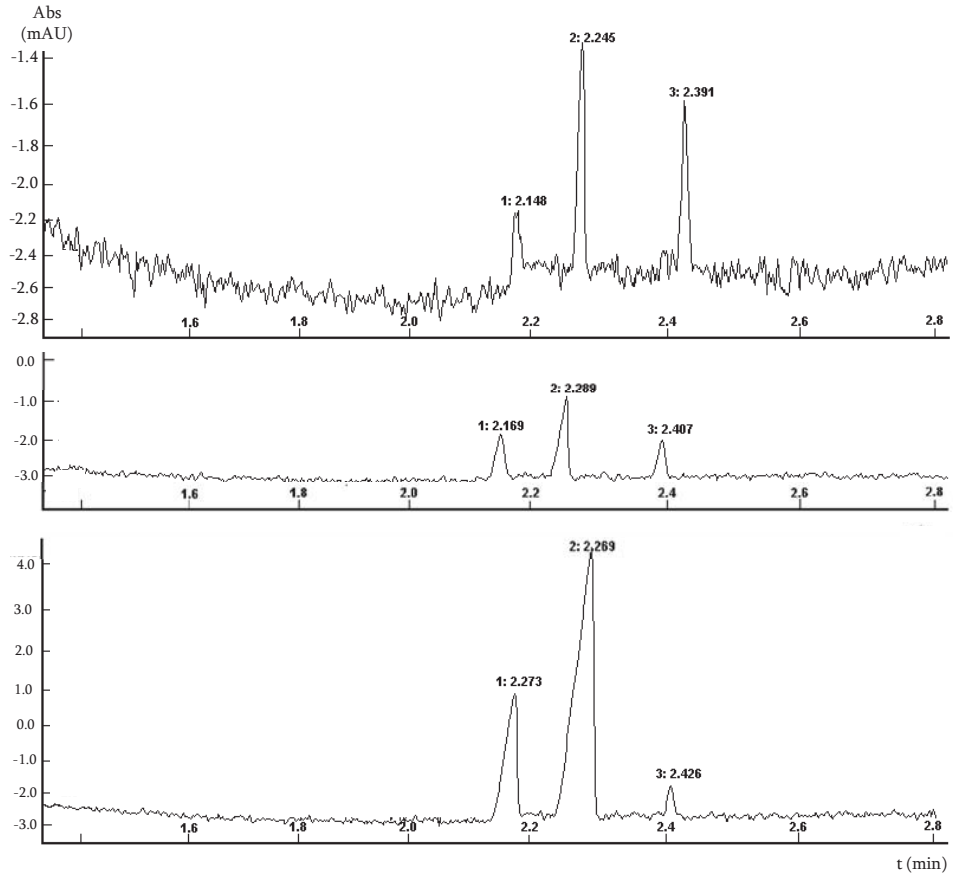

Figure 5. *Electropherograms obtained for the different Certified Reference Materials considered a) AES 05, b) ION 915 and c) SPS NUTR WW1. Peaks are 1: chloride, 2: sulphate and 3: nitrate. Signal 350nm/80 and Reference 245nm/10.*

As can be seen, no significant influence on the retention times and peaks resolution were obtained between the samples. The RSD variation obtained in the retention times between the samples was 0.62%, 0.97%, and 0.73% for chloride, sulphate, and nitrate, respectively. Therefore, composition and pH values of the CRM samples had a negligible effect on the robustness with similar trueness and precision values in all the samples for those expected in the analytical method.

II.4. CONCLUSIONS

The concept of the analytical method validation for inorganic anion determination by capillary ion electrophoresis was deeply discussed in this work. The EURACHEM guide was proved as an efficient tool to assess the fitness for purpose of this analytical method. The basic principles of the method validation were followed and all the requirements were successfully fulfilled.

Once this analytical method was validated, it was used for routine analysis of inorganic anions in different matrixes for the Central Service of Analysis Laboratory of the Basque Country University (UPV/EHU). Proper validation documentation was generated in order to obtain results in good agreement with the quality requirements listed in this work. Inconsistencies were detected and, therefore, avoided by proper reporting and application of the validation processes.

II.5. ACKNOWLEDGEMENTS

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DETERMINATION OF ANIONIC IMPURITIES IN HEXAFLUOROSILICIC ACID BY CAPILLARY ZONE ELECTROPHORESIS

Chapter III

Chapter III- DETERMINATION OF ANIONIC IMPURITIES IN HEXAFLUOROSILICIC ACID BY CAPILLARY ZONE ELECTROPHORESIS

III. 1. INTRODUCTION

Fluorinated compounds production is a great important social and economic sector due to its numerous applications, especially in the development of products which are essential for modern life, such as integrated circuits, solar panels, Gore-tex and Teflon materials and toothpaste [1-4].

Fluoride is found in minerals such as fluorite, fluorapatite and cryolite in nature, being fluorite the most abundance mineral which is used in fluorine industry as raw material [5]. After extracting the fluorite from the mine, it is transported to the company where the reaction with sulphuric acid occurs to obtain hydrogen fluoride or hydrofluoric acid gaseous.

From hydrofluoric acid, a great number of fluorinated compounds are obtained as fluorides, bifluorides and fluorinated salts and acids [6].

Hexafluorosilicic acid (FSA), one of the fluorinated products, can be obtained using three different methods [7]. Samples analyzed in this work were synthesized by the company using the method based on the reaction between hydrofluoric acid and silica, SiO_2 (reaction 1).

$$6HF\ (g) + SiO_2\ (s) \rightarrow H_2SiF_6\ (aq) + 2H_2O\ (aq) \quad (1)$$

There is considerable controversy regarding its acidity. It is considered a very strong, strong or moderate acid, depending on the literature consulted [8-10].

Hexafluorosilicic acid is an acid whose equilibria and chemical species have been studied in depth. A variety of models have been proposed by different authors [11-14]. However, the equilibria that take place are not fully established, there are still discrepancies between the results obtained up to date [15].

The existence of ion-octahedral SiF_6^{2-} has been reported, since has been identified by spectroscopic (Raman and nuclear magnetic resonance) and potentiometric techniques [16]. However, there is no conclusive data regarding the type of intermediate species $Si(OH)_{4-x}F_y^{x-y}$, which are involved in the equilibria.

Hexafluorosilicic acid is used for the tanning of animal skins, removing mildew and rust stains and textiles at unspecific concentrations [10], while at concentrations of 20-25% is used for the fluoridation of drinking water because the fluoride is appropriate for the prevention of dental caries at certain concentrations, according to the guidelines set by the Public Health Service [17]. Moreover, at concentrations of 30-35%, is utilized for sterilization, preservation of wood, hardening for ceramics and cement, in metallurgical industry [18] and to synthesize fluorosilicates and fluorocarbons of magnesium, lead, potassium and sodium [19].

The use of FSA in metallurgical industry requires the determination of the concentration levels of chloride and sulphate impurities due to two main reasons: the interference produced in the selective attack to be carried out to the surface to be treated and the difficulty to reprocess and /or recycling or disposal the acid baths produced.

This control of impurities in commercial products demands the development of analytical methods of easy implementation in industrial laboratories in order to classify samples depending on their impurities level to assure their proper performance.

Nowadays, in the industry, the analysis of chloride and sulphate in FSA is carried out using Volhard method and gravimetry respectively, although interferences are present in both methods.

The analytical methods used to determine trace levels of inorganic anions, including chloride and sulphate, in different matrices, are preferably chromatographic (ion chromatography) [20-24] and electrophoretic (capillary electrophoresis) methods [25-32].

The great separation power and applicability of capillary electrophoresis for routine analysis of inorganic anions led us to choose the capillary electrophoresis as analytical technique in this work.

The goal of the research carried out is the application of this technique for the determination of inorganic impurities in a very complex matrix, FSA, with high conductivity and high acidity. No references have been found dealing with the

determination of inorganic anionic impurities in fluorinated compounds by capillary electrophoresis apart from the work of Boden et al. [33] in which capillary zone electrophoresis (CZE) with an isotacophoresis (ITP) initial state was applied to determine anionic impurities on the silicon wafer surfaces treated with hydrofluoric acid vapour.

Therefore, the aim of this work is the development of a capillary electrophoretic method as an alternative analytical methodology to be implemented in industrial laboratories for the determination of chloride and sulphate in hexafluorosilicic acid.

III.2. MATERIALS AND METHODS

III.2.1. Apparatus

In this work two capillary electrophoretic instruments have been used: Quanta 4000E Capillary Electrophoretic System from Waters Chromatography Division (Barcelona, Spain) with Millennium 32 Chromatography Manager software for data collection and treatment; and HP^{3D} Capillary Electrophoresis System from Agilent Technologies (Barcelona, Spain) with diode array detector and HP ChemStation A.06.03 software for acquisition and data processing.

Fused silica capillaries (75 μm id x 375 μm od), were supplied by Composite Metal Services LTD (Worcester, England).

A Crison model GLP22 pH-meter (Barcelona, Spain), employing a combined Crison glass electrode, with a reference system Ag/AgCl, KCl (3.0 M) was used for pH measurements.

Basic 30 Crison conductimeter (Barcelona, Spain) was used to measure the conductivity of the electrolytes and FSA samples.

Solid phase extraction Supelco Visiprep™ Vacuum Manifold system (Bellefonte, PA, USA) connected to a Millipore vacuum pump (Bedford, Massachusetts) was employed to transform the tetradecyltrimethylammonium bromide used as electroosmotic flow modifier (EFM) to its hydroxilated form.

A Sartorius CP 224S balance (Madrid, Spain) with an accuracy of ± 0.0001 g was used for weighing the reagents.

Polypropylene material was used for the solutions preparation to avoid the glass deterioration by free HF containing FSA samples.

III.2.2. Reagents

Standard solutions of chloride, nitrate, bromide and sulphate at concentration of 1000 mg/L were provided by Merck (Barcelona, Spain). Working solutions of these anions were prepared by dilution from standard solutions.

Sodium chromate tetrahydrated pro-analysis quality was provided by Fluka (Madrid, Spain), and used for the background electrolyte (BGE) preparation.

The pH adjustment of the BGE was carried out with analytical reagent quality phosphoric acid, purchased from Panreac Quimica S.A. (Barcelona, Spain).

The organic additives were HPLC grade methanol and acetonitrile provided by Scharlab S.L. (Barcelona, Spain).

The electroosmotic flow modifier employed was tetradecyltrimethylammonium bromide (TTAB), Cia-Pak FFM Anion-BT, supplied by Waters (Barcelona, Spain) at a concentration of 20 μg/mL. The hydroxide form of this EFM was obtained by anion-exchange system with a solid phase extraction, using Oasis Max 1cc cartridges supplied by Waters (Barcelona, Spain).

The BGE was prepared weighing the amount of sodium chromate necessary for a final concentration of 20 mM, together with the addition of 3 mL of MeOH and 1 mL of tetradecyltrimethylammonium hydroxide (TTAOH), and making up to 50 mL with Milli-Q water.

Pro-analysis quality sodium hydroxide from Merck (Barcelona, Spain) was used throughout the work.

Ultra-pure quality water obtained from a Milli-Q instrument Model 185, from Millipore (Bedford, Massachusetts, USA) was used for the solutions preparation.

III.2.3. Preparation of tetradecyltrimethylammonium hydroxide (TTAOH)

The hydroxilated form of TTAB was prepared following these steps, according to Röden´s [34] work: firstly the cartridge was activated with 3 mL of MeOH. After conditioning with 3 mL of 1 M NaOH, the cartridge was washed with 3 mL of deionized water. Then, 1 mL of TTAB was passed through the anion exchange cartridge, the eluate (TTAOH) was collected and finally the cartridge was cleaned with 3 mL of NH_3:MeOH (95:5 v/v). This procedure was repeated several times and all the eluates were mixed.

III.2.4. Hexafluorosilicic acid samples

The FSA samples were supplied by the company Derivados del Flúor S.A. (DDF) (Cantabria, Spain), with a content ranging between 20 and 42% of H_2SiF_6. The samples were from industrial process, named DTO 208 and REF 674. The electrophoretic system optimization was performed with both industrial samples of FSA.

III.2.5. Capillary conditioning

The capillary was conditioned with an initial wash cycle consisting of 1 M NaOH for 20 min, deionized water for 20 min and running electrolyte for 20 min. Between injections, the capillary was washed with 1 M NaOH for 3.5 min, deionized water for 3.5 min and running electrolyte for 4.5 min. The electrolyte was refreshed after 5-6 runs. Daily, after finishing the experiments, the capillary was washed with deionized water for 10 min and purge with air for 10 min.

III.2.6. Electrophoretic conditions

Samples were injected hydrodynamically (10 cm of height in Quanta electrophoretic system and 50 mbar for 10 s in HP^{3D} Agilent equipment) onto a

fused silica capillary of 63.5 cm x 75 micrometers id, x 375 micrometers od, with an effective length of 55 cm. The capillary temperature was kept constant at 25 ± 1 ºC throughout the experiments. A voltage of -25 kV was applied (in an anionic mode). The electrophoretic zones were detected with a fixed-wavelength UV detector at 254 nm and diode array detector at 370 and 254 nm. Indirect photometric detection was used. The final BGE consisted of 20 mM sodium chromate at pH 8.0 containing 6% MeOH and 1.5 μM TTAOH.

III.2.7. Study of Capillary Stability

The number of runs which could be made without changing the capillary was studied. In the optimum electrophoretic conditions, sequences of six runs of the sample were made, refreshing the electrolyte each sequence. The same procedure was followed introducing a water gap before each sample injection. The gap of water was introduced previous to inject the FSA sample both at a pressure of 50 mbar. The sample injection time was set at 10 s and the water injection time at 25 s.

III.2.8. Quantitative Determination

The linear concentration range was calculated spiking the FSA samples (DTO 208) with solutions containing different chloride and sulphate concentrations from 0.1 to 40 mg/L. Detection limit (LOD) and quantitation limit (LOQ) were calculated as the anion concentration which produced an electrophoretic peak with a signal to noise ratio (S/N) of 3 and 10 respectively.

Industrial FSA samples were quantified using the standard addition method. Additions of solutions containing chloride and sulphate were made. Solutions of chloride and sulphate varying from 2.5 to 12 mg/L for each analyte were prepared. Linear regression method was applied to the electrophoretic peak areas obtained. Average concentration was obtained by extrapolation from four different calibration curves using standard addition method and the same capillary.

Two samples, corresponding to the zero and maximum addition of standard addition method, were assayed in sets of four replicates in order to evaluate the intraday repeatability of the method using the same capillary. Normalized areas (peak area/migration time) were used to calculate the repeatability [35]. Relative standard deviation (RSD%) was used to express the intraday repeatability. Assays have been also carried out to evaluate the method repeatability, injecting the FSA samples using four different capillaries.

III.3.RESULTS

III.3.1. Optimization of electrophoretic system

The influence of different electrophoretic and matrix variables on electrophoretic response was studied in order to optimize the electrophoretic system. Industrial production FSA samples were utilized for this optimization study.

The electrophoretic variables studied were: concentration and pH value of electrolyte, EFM concentration, the addition of additives (acidifying agents, organic additives) and injection time. Moreover, known the high conductivity of samples, the dilution factor was also studied.

To evaluate the influence of dilution factor on the electrophoretic response, conductivity and pH values of REF 674 sample diluted solutions were measured, Table 1, and electropherograms of these solutions were obtained in the electrophoretic conditions reported for the determination of inorganic anions in aqueous solutions [36].

Table 1. *Conductivity and pH values of REF 674 sample diluted solutions and the background electrolyte.*

Solution	Dilution Factor	χ (mS/cm)	pH
1	1/5000	0.470	2.92
2	1/2500	0.974	2.27
3	1/1250	1.876	2.20
4	1/625	1.928	2.02
5	1/500	4.550	1.98
6	1/250	8.150	1.73
BGE employed		**1.082**	**8.0**

Fig. 1 shows the electropherograms obtained for different dilution factors and the peaks identification.

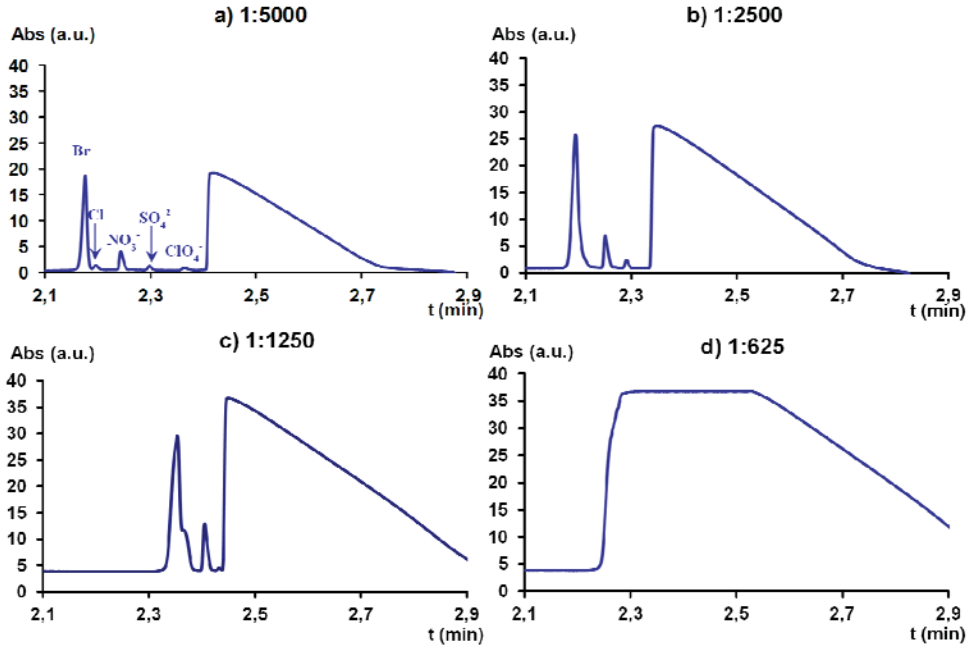

Fig. 1 *Electropherograms obtained for REF 674 sample solutions of FSA with different dilution factor a) 1:5000, b)1:2500 and c) 1:1250, d) 1:625 using the CZE technique and the electrophoretic conditions: 10 mM chromate + 0.59 μM TTAB, 50 s hydrodynamic injection (10 cm height), -20 kV potential applied between the ends of capillary (50 cm of effective length) and detection λ=254 nm.*

A loss of electrophoretic peaks resolution with the increase of the solution injected concentration is obtained. For 1:625 and lower dilution factors the high signal due to the fluorinated species overlaps the response corresponding to anionic impurities.

A sample with 1:5000 dilution factor was spiked with 10 mg/L of several inorganic anions and the different electrophoretic peaks could be identified as bromide, chloride, nitrate and sulphate

To achieve an adequate peak resolution for more concentrated FSA samples, avoiding the great quantitation error associated with high dilution factor; the influence of several instrumental electrophoretic variables as well as the

composition of the background electrolyte, was studied for different dilution factors.

For the background electrolyte composition optimization, the concentration of chromate and TTAB solutions was varied in the range 5-25 mM and 0.7-1.8 μM, respectively. Certain concentration of EFM reverses the electroosmotic flow (EOF) making faster the analysis because the anions go through the capillary to the anode faster than without EFM. The increase of chromate and TTAB solutions concentrations gives rise to well define electrophoretic peaks for more concentrated FSA samples. Concentrations of 20 mM chromate and 1.5 μM TTAB solutions were chosen as the most suitable for the anions separations. The influence of pH value of electrolyte (7-11) was also tested, using phosphoric acid as acidifying agent. A pH value of 8 was chosen as the most adequate for the electrophoretic separation.

The instrumental electrophoretic variables were optimized using the lowest dilution factor which allows obtaining an acceptable peak resolution without affecting considerably the capillary life (1:250). The effect of the injection time on the electrophoretic response was studied in the range 7 to 30 s on the FSA sample. An injection time of 10 s was selected as adequate since the fluorinated species signal decreased and a good electrophoretic separation was obtained, although with a high system current (near 100 μA). To mitigate this effect, the addition of methanol and acetonitrile to the electrolyte (2-8%) was studied, known the improvements achieved with organic additives because of the increase of BGE produced by these organic solvent addition [37-40]. Methanol was chosen as organic additive since, as was expected, decreases the intensity more than the acetonitrile. The addition of 6% MeOH to the electrolyte allowed an improvement of the bromide and chloride peak separation with a reduction of system current (lower than 80 μA).

In the optimum electrophoretic conditions achieved, the qualitative analysis of the different FSA samples with a dilution factor of 1:250 can be carried out in less than 3 min, Fig. 2.

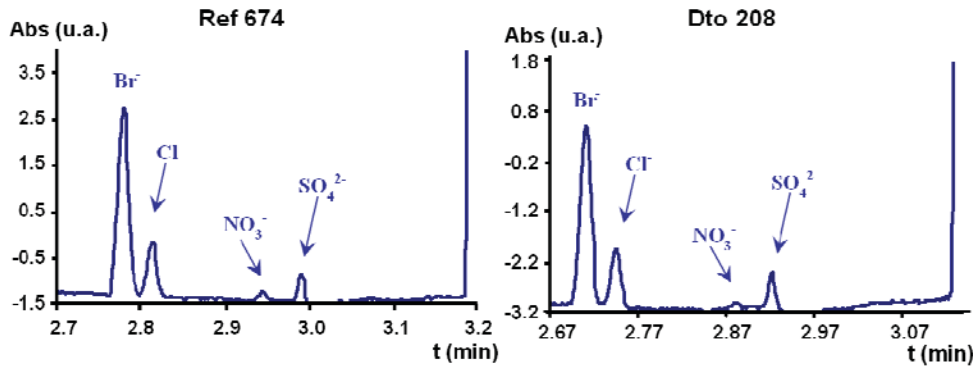

Fig. 2 *Electropherograms for FSA samples diluted (1:250): a) REF 674, b) DTO 208. The composition of electrolyte was 20 mM chromate, pH 8.0 (adjust with H3PO4 50 mM), 1.2 μM TTAB and 6.0% MeOH. The potential applied between the ends of the capillary (effective length 55 cm) was -25 kV, λdetection = 254 nm, and hydrodynamic injection (10 cm height) during 50 s.*

As can be seen for the samples analyzed a bromide electrophoretic peak was obtained. Known the no existence of bromide anion in these industrial samples (information given by Derivados del Fluor, S.A.) led us to think on the possibility of a system peak [34] in the migration time of the bromide, which could be attributed to the electroosmotic flow modifier used (TTAB). This fact makes necessary to transform the tetradecyltrimethylammonium bromide in its hydroxilated form (TTAOH), as is described in section "Material and Method". The elimination of bromide ion makes easy the quantitation of chloride ion especially for samples with high levels of chloride concentration.

Taking into account that this method should be implemented in an industrial laboratory and used as a routine methodology, the robustness of the method was studied. Along the optimization studies, the difficulty to work with FSA samples has been noticeable; only six analyses could be done without changing the capillary, probably due to the interaction between the fluorinated species and the silanol groups of wall capillary [41]. In an attempt to solve this problem, the introduction of a water gap in the electrophoretic system which increases the capillary life, was assayed. For this purpose it was necessary the transference of the electrophoretic method developed up to now to HP3D Capillary

Electrophoresis equipment with diode-array detection, due to the former equipment is not able to carry out this injection mode.

With this new equipment, detection wavelengths of 370 and 254 nm were selected, since correspond to the absorption maxima of electrolyte UV-vis spectrum. The method transference gives rise to higher migration times, so a change in the voltage potential applied to -25 kV was made since the higher potential is applied the greater velocity is obtained. In these electrophoretic conditions, the migration order obtained was chloride, nitrate and sulphate, following the electrophoretic separation rules: first the small anions migrate followed by the biggest ones, then the neutrals and finally the cations.

The water gap assays were made with a sample injection time of 10 s and varying the water injection time from 10 to 300 s. The electrophoretic separation was not affected for the water gap introduction, so the influence of this introduction was evaluated using the electrophoretic peak area of chloride and sulphate impurities. A water injection time of 25 s was chosen since the maximum electrophoretic peak area for both analytes was obtained. With this optimum time, sample injection time was varied from 1 to 40 s. A time of 10 s was chosen taking into account both the sensitivity and the capillary life. In these conditions, the capillary life is increased up to at least 20 runs, enough time to carry out the quantitative determination of chloride and sulphate. In Fig. 3 the electropherograms obtained at the start and end of capillary life, in the optimum electrophoretic conditions without and with water gap use, are shown.

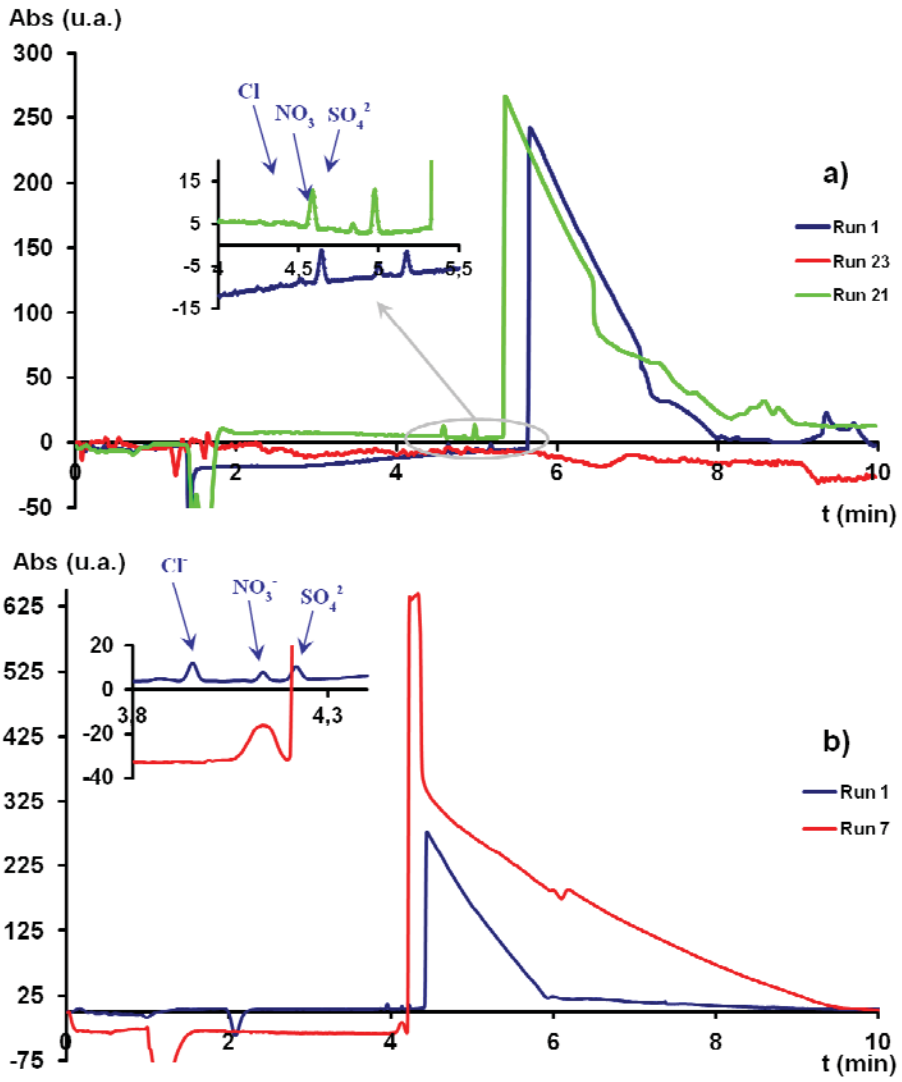

Fig. 3 *Electropherograms obtained in the optimum electrophoretic conditions at the start and end of capillary life a) with water gap use and b) without water gap use. Electrophoretic conditions described in corresponding section.*

A variation of migration times is observed with the number of sample injections, moving the selectivity factor from 1.17 to 1.13. An increase in capillary life is also observed probably due to a partial rehydratation of the silanol groups of the capillary wall. The end of capillary life would be attributed

to loss of its active points due to the reaction of fluorinated species with the internal silanol groups of the capillary wall.

III.3.2. Quantitative determination of FSA samples

Linear concentration range obtained from spiked DTO 208 sample was from 1.9 up to at least 40 mg/L and from 1.3 up to at least 40 mg/L for chloride and sulphate, respectively.

Quantitation limits obtained were 1.9 and 1.3 mg/L for chloride and sulphate respectively while the detection limits were 0.41 and 0.57 for chloride and sulphate respectively.

The electrophoretic method developed was applied to industrial production FSA samples. The regression lines obtained, using the standard addition method, for four replicates of each sample were collected in Table 2.

Table 2. *Regression lines equations of standard addition method for chloride and sulphate determination in industrial production FSA samples.*[a)]

		Regression Equation	r^2	Regression Equation	r^2
	Repl	Cl^-		SO_4^{2-}	
DTO 208	1	Y = (21 ± 7)+(4.8 ± 0.9)*X	0.9966	Y = (19 ± 7)+(4 ± 1)*X	0.9940
	2	Y = (17 ± 3)+(4.3 ± 0.4)*X	0.9968	Y = (18 ± 2)+(3.0 ± 0.4)*X	0.9953
	3	Y = (15 ±6)+(4.1 ± 0.8)*X	0.9862	Y = (18 ± 3)+(2.9 ± 0.7)*X	0.9929
	4	Y = (16 ± 1)+(3.3 ± 0.3)*X	0.9937	Y = (16 ± 11)+(4 ± 1)*X	0.9892
REF 674	1	Y = (21 ± 4)+(3.3 ± 0.5)*X	0.9784	Y = (12 ± 5)+(3.8 ± 0.9)*X	0.9724
	2	Y = (21 ± 3)+(3.9 ± 0.4)*X	0.9966	Y = (15 ± 3)+(3.5 ± 0.5)*X	0.9966
	3	Y = (17 ± 6)+(3.3 ± 0.8)*X	0.9825	Y = (10 ± 5)+(3.2 ± 0.8)*X	0.9910
	4	Y = (13 ± 3)+(3.3 ± 0.5)*X	0.9993	Y=(10.7 ±0.9)+(2.3 ± 0.1)*X	0.9626

[a)]Expressed as: Peak Area = (Intercept ± t*s) + (Slope ± t*s)*Added Concentration, where t is Student t value for a confidence level of 95% and 4 degrees of freedom.

The concentration calculated for both samples, expressed in mg/L as mean value ± t*s/√N, were for chloride: 1228 ± 252 and 1209 ± 201 and for sulphate: 1039 ± 221 and 1222 ± 158 for REF 674 and DTO 208 samples, respectively.

The intraday repeatability obtained with normalized area was 5.7% and 6.7% (RSD%) for chloride and sulphate at low concentration level and 4.6% and 2.3% (RSD%) for chloride and sulphate at high concentration level respectively using the same capillary and 4.3% y 6% for chloride and sulphate at low concentration level using different capillaries.

III.4. CONCLUSIONS

Capillary zone electrophoresis has shown its suitability for the qualitative analysis of inorganic impurities in a tough matrix as hexafluorosilicic acid with high acidity and conductivity.

The introduction of a water gap in the electrophoretic system means an important advantage when a very complex matrix is injected, since an increase of the capillary life is produced without affecting, neither the separation nor the repeatability.

The modified electrophoretic method makes possible the quantitation of the chloride and sulphate in industrial production FSA samples. This method could be also applied to the determination of other inorganic anions as bromide and nitrate.

The electrophoretic method developed supposes a new advance in the analytical methodology to be implemented in industrial laboratory. For the first time, capillary electrophoresis has been applied to the determination of inorganic anions in this kind of matrix, which would allow the future application to other inorganic fluorinated acids (hexafluorozirconic, hexafluorotitanic and tetrafluoroboric acids) with the same chemical characteristics.

The main advantages of the electrophoretic method proposed consist of simplicity, so it can be carry out by unqualified personal, without any sample treatment, minimum consume of sample, minimum generation of residues,

environmentally compatible and allows the simultaneous determination of several inorganic anions.

Apart from these advantages, it is worthwhile to mention the elimination of the common error associated to the visual measurement of final point in volumetry and to the co-precipitation in gravimetry, which are common in the analysis of chloride and sulphate in this kind of complex matrix.

III.5. ACKNOWLEDGEMENTS

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DETERMINATION OF ANIONIC IMPURITIES IN FLUORINATED INORGANIC COMPOUNDS BY ION CHROMATOGRAPHY

Chapter IV

Chapter IVa- INDUSTRIAL APPLICATION OF ION CHROMATOGRAPHY TO THE QUALITY CONTROL OF FLUORINATED INORGANIC ACIDS.

IVa. 1. INTRODUCTION

Fluorinated compounds industry is a very important socio-economical sector due to its numerous applications, especially in the development of products which are essential for modern life, such as integrated circuits, solar panels, Gore-tex and Teflon materials, and toothpaste (Gupta 2003, Chukwuemeka 2006, Togni 2007, Park 2010).

The raw material for all of these fluorinated compounds is fluorite (Highley 2005), a mineral which is used in fluorine industry to obtain hydrogen fluoride or gaseous hydrofluoric acid by reaction with sulphuric acid. From hydrofluoric acid, a great number of fluorinated compounds are obtained such as fluorides, bifluorides and fluorinated acids and salts (Aigueperse 2005).

Some of these fluorinated products are hexafluorosilicic acid (FSA) and tetrafluoroboric acid (FBA), compounds studied in this work. Both acids can be obtained using different methods although industrial samples analysed in this work were synthesized by the chemical company using the method based on the reaction between hydrofluoric acid and silica (SiO_2) or boric acid (H_3BO_3), respectively (Turner 1960).

The applicability of these compounds ranges from organic synthesis to industrial applications. Within the last ones, FSA is used for the tanning of animal skins, removing mildew and rust stains in textiles at unspecific concentrations (Masten 2001), while at concentrations of 20-25% is used for the fluoridation of drinking water (Committee on Fluoride in Drinking Water 2006). Moreover, at concentrations of 30-35%, it is utilized for sterilization, preservation of wood, hardening for ceramics and cement in metallurgical industry (Harben 2004) and to synthesize fluorosilicates and fluorocarbons of magnesium, lead, potassium and sodium.

In the case of FBA, its main application is as reagent to produce ionic liquids (IL), which have a wide range of applications due to their special properties (Wilkes 2004). Among IL, those made from BF_4^- have a relevant importance in aluminium and chromium industry. They are used as electrolyte in metallic plating process, as well as in processes of electrodeposition and electrolytic

stripping to obtain specific metal alloys. In ILs, impurities have been recognized as affecting both physical and chemical properties (Seddon 2000). Generally, they have a poisoning effect on transition metal catalyzed reactions. For example, chloride, in specific ILs, causes deactivation of the cluster used as pre-catalyst in the hydrogenation of arenes (Dyson 2003). FBA is also used as an electrolyte in galvanic cell oxygen sensor systems and, in organic chemistry, as a catalyst for alkylations and polymerizations, for the stabilisation of diazo salts, and for the manufacture of inorganic fluoroborate salts (Papcun 2000).

All the industrial applications in which these acids are involved require a quality control management of anionic impurities content due to the drawbacks produced by these anions. In this sense, analytical techniques are more effective approach than chemical methods used up to now to obtain quantitative information about anionic impurities in fluorinated inorganic acids.These impurities may increase the difficulty of reprocessing, recycling or disposing the acid baths from the industrial processes. In this case, the presence of certain elements is not desirable from the economic and environmental point of view, since it may complicate the treatment to be performed (Liu 2011).

Specifically, the presence of chloride and sulphate impurities at trace levels in fluorinated acids is monitored due to several reasons. Chloride can interfere with the selective attack on the surface to be treated and generate localized corrosion in the structure (Uhlig 1985, Ma 2011). This is also induced by microorganisms in presence of sulphate, which is reduced to H_2S causing the corrosion of the metallic surfaces (Heitz 1996). Since the existence of sulphur species from industrial effluents can affect the bioavailability of heavy metals due to complexation or precipitation reactions, especially the oxidized forms of sulphur as sulphate, which are undesirable in the environment (Pagnanelli 2010, Singh 2011). Moreover, it has also been reported that the electrochemical cells efficiency decreases in the presence of chloride and sulphate ions (Curtis 1978) when FBA is used as electrolyte in these cells.

Currently, the control of anionic impurities in fluorinated products is carried out by tedious and time consuming chemical methods such as volumetry and

gravimetry. These methods suffer from substantial matrix interferences which make difficult to obtain reliable results.

Thus, the development of new analytical methodologies of easy implementation is an important task for the quality control in laboratories of fluorine industry. The impurities level obtained from this control allows the classification of FSA and FBA which assure their proper performance with the consequent economical and environmental benefits.

In literature, the analytical techniques reported for the determination of trace levels of inorganic anions in different matrixes are preferably ion chromatography (IC) (Liu 1999, Curran 2001, Santoyo 2002, Peter 2002, Lu 2002, Haddad 2004, Michalski 2006) and capillary electrophoresis (CE), (Jandik 1993, Sádecká 1999, Kaniansky 1999, Pacáková 2003, Galli 2003).

Different authors as (Vanderford 1992, Dionex1998, Kaiser 1999, Wang 2002) have developed ion chromatographic methods to determine trace anions in hydrofluoric acid, using multidimensional suppressed IC which involves pre-concentration of anions and separation from the matrix. It has also been reported a capillary zone electrophoretic method with an isotacophoresis initial step to determine anionic impurities on the silicon wafer surfaces treated with hydrofluoric acid vapour (Boden 1995). No references have been found dealing with the analysis of inorganic anionic impurities in the fluorinated acids studied, apart from a previous work of our group (Ayarza 2012). In this work, the content of chloride and sulphate was analyzed in FSA by capillary zone electrophoresis (CZE) with indirect photometric detection. Problems with capillary life were reported associated with high corrosive nature of the matrix. Indeed, CZE is not the best analytical approach to solve the industrial problem proposed.

In this work ion chromatography has been chosen as analytical alternative to chemical methods. The research carried out tries to overcome the inherent difficulties associated with the complexity of fluorinated matrixes. These matrixes contain high levels of fluorinated species at low pH values. The main goal of this work consists on the application of ion chromatography technique

for the quantitative determination of the inorganic impurities chloride, bromide, nitrate and sulphate in FSA and FBA. The analytical methodology developed will be used as quality control procedure in industrial laboratories.

IVa.2. EXPERIMENTAL

IVa.2.1. Apparatus, Reagents and Samples

The ion chromatographic separation was performed using an ICS-1000 chromatographic system by Dionex (Sunnyvale, CA, USA) consisting of an ICS-1000 pump, an ICS-1000 column heater, an Anion Self Regenerating Suppressor (ASRS 300 Ultra II) and a heated conductivity detector. The equipment was coupled with an AS-DV autosampler, for 50 plastic vials of 5 ml each one, PolyVial™, which includes a 20 μm filter. Data acquisition and control were performed using Chromeleon version 6.8 software by Dionex (Sunnyvale, CA, USA) which incorporates Virtual Column Separation Simulator 2 for simulating the chromatographic separation of anions in aqueous solutions for the analytical column chosen. An IonPac AS9-HC (4 x 250 mm) analytical column with 9 μm polystirene-divinylbenzene substrate agglomerated with a 0.09 μm diameter anion-exchange aminated latex particles and a guard column, an IonPac AG9-HC (4 x 50 mm), supplied by Dionex (Sunnyvale, CA, USA) were used and thermostated at 35 ºC.

Anhydrous sodium carbonate of proanalysis quality supplied by Merck (Damstadt, Germany), was used for the preparation of different eluent concentration solutions which were prepared dissolving the salt in ultrapure water and filtering the solution immediately before use. The filtration of the eluent was made using a Millipore vacuum pump (Bedford, MA, USA) and employing Durapore® membrane filters of 45 μm from Millipore Ibérica S.A.U. (Madrid, Spain).

Standard solutions of chloride, bromide, nitrate, phosphate and sulphate at a concentration of 1000 mg/L were provided by Merck (Barcelona, Spain). Working solutions of these anions were prepared by dilution from standard solutions.

Commercial FSA and FBA samples were supplied by Alfa Aesar GmbH & Co KG (Karlsruhe, Germany) with a content ranging between 23 and 48% of H_2SiF_6 and HBF_4 and by Sigma-Aldrich Co (St. Louis, MI, USA) with a content of 48% of HBF_4. While industrial FSA and FBA samples were supplied by the company Derivados Del Fúor S.A. (DDF) (Cantabria, Spain), with a content ranging between 4 and 42% of H_2SiF_6 and 4% of HBF_4. These samples were used for the chromatographic system optimization due to the lack of blank matrixes and certified reference materials. The preparation and storage of these solutions was made using polypropylene material to avoid glass deterioration caused by free HF containing FSA and FBA samples.

All solutions were prepared using ultra-pure quality water obtained from a Milli-Q Element A10 water system, from Millipore (Mildford, MA, USA). A Sartorius CP 224S balance (Madrid, Spain) with an accuracy of ± 0.0001 g was employed for the weight of the reagents, standards and samples.

The pH measurements were made using a Crison model GLP22 pH-meter (Barcelona, Spain), employing a combined Crison glass electrode, with a reference system Ag/AgCl, KCl (3.0 M).

IVa.2.2. Chromatographic Conditions

The separation of chloride, bromide, nitrate, phosphate and sulphate was performed using 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 μL. The suppressed conductivity detection was used applying a current of 45 mA to the suppressor column, to reduce the background conductivity of the eluent. The conductivity of the base line in these conditions was approximately 22 μS.

IVa.2.3. Method Validation

The initial step for the quantification of the anions was a qualitative analysis of commercial and industrial samples of FSA and FBA. For this purpose, a

comparison of the chromatographic peak areas obtained for all the samples of each kind of acid was made. Samples with the lowest chromatographic peak areas for each analyte ("zero" samples) were chosen for building the calibration curves for the quantification of anion impurities. The standard addition method was applied in order to take into account the influence of the complex matrixes analyzed. Linear regression data and the "zero" sample concentration obtained once applied the standard addition method were transferred to build external calibrations. These calibration curves were used for the quantitative determination of the rest of samples.

The linear concentration ranges were obtained spiking "zero" samples with solutions containing different chloride, bromide, nitrate and sulphate concentrations. For FSA the linear ranges assayed were from 0.19 to 7 mg/L for chloride, from 0.02 to 7 mg/L for bromide, from 0.05 to 4.5 mg/L for nitrate and from 0.1 to 21 mg/L for sulphate. For FBA the linear ranges assayed were from 0.05 to 0.30 mg/L for chloride, from 0.25 to 5 mg/L for bromide, from 0.15 to 5 mg/L for nitrate and from 0.05 to 3 mg/L for sulphate.

Linear regression method was applied to the chromatographic peak area–concentration data. Quantification limits (LOQs) were calculated as the minimum concentration which give rise to a S/N ratio equal to 10 and can be determined with an accuracy in terms of relative error percentage (RE%) and a repeatability in terms of RSD%, both lower than 15% (Hautman 1997). These LOQs correspond to the first points of the calibration curves. Inter-day and intra-day repeatability of the method were calculated for medium and high concentration levels within the calibration curve range. RSD% was used to express their repeatability. Samples spiked with different concentration values within the calibration curve were used for accuracy calculation in terms of RE%. The repeatability of retention times of standard solutions and samples was calculated in terms of RSD%. Mean concentrations of samples were obtained by interpolation from three replicates and expressed as mean $\pm$ $t^*s/\sqrt{N}$ where t is the student factor, N is the number of replicates and s is the standard deviation, at a confidence level of 95%.

IVa.3. RESULTS AND DISCUSSION

IVa.3.1. Optimization of chromatographic system

The high capacity AS9-HC column was chosen for anionic impurities analysis in fluorinated acids due to the column applicability for trace ions analysis in high ionic strength matrixes. In a first step, the experimental conditions proposed for the separation of chloride, bromide, nitrate, phosphate and sulphate in water by the Virtual Column Separation Simulator 2 (Madden 2002) were used. The initial chromatographic conditions were 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 µL. These conditions are expected to be useful for water matrixes but they should be optimized in complex matrixes like the fluorinated acids studied in this work. In this sense, the variables dilution factor, flow rate and eluent concentration were studied by applying the OVAT (one variable at time) method for the system optimization. The number of chromatographic peaks and the chromatographic resolution were chosen as response of the system to be optimized. A special importance was driven in the chromatographic resolution between fluorinated species and chloride anion, since samples studied contain a huge amount of fluoride and fluorinated species.

The effect of the dilution factor on the chromatographic response of the two acids for a constant injection volume was the first assay done. FSA and FBA samples were diluted with ultrapure water at different dilution factors and analyzed in the initial chromatographic conditions. Corresponding final acid percentages with the dilution factor assayed ranged between 0.1% and no dilution from the original solutions of the different acids. The resulting percentages from the dilution factors assayed for the acids samples which provided the highest number of chromatographic peaks and good resolution was 0.2% in both cases, for FSA and FBA. In Figure1, chromatograms for FSA at different final acid percentage are shown.

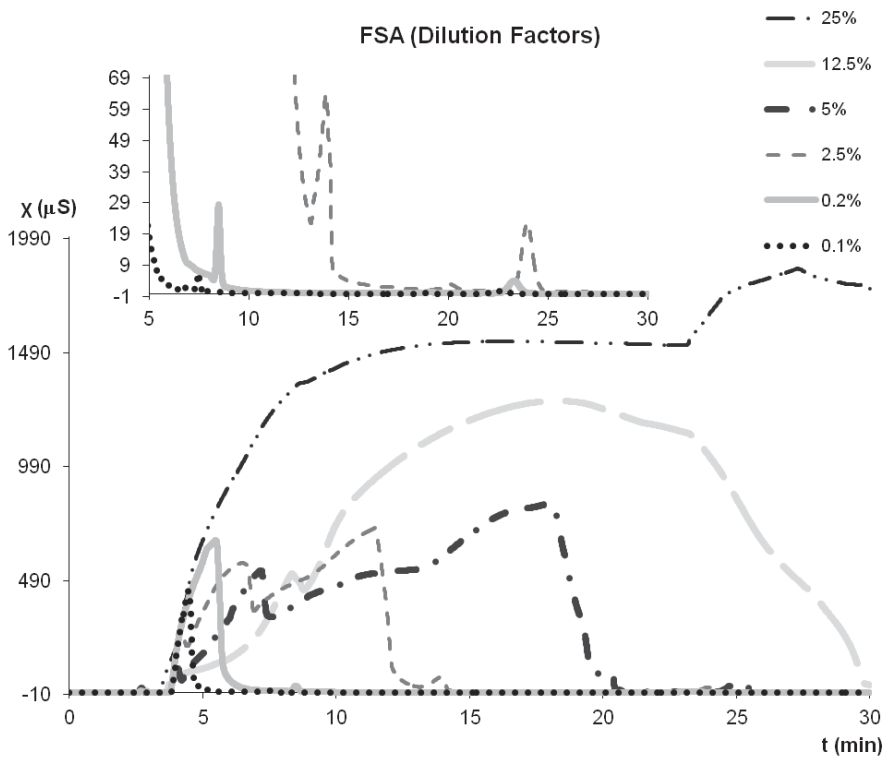

Figure 1. *Chromatograms of FSA at different final acid percentages with the amplification of the time zone in which anion studied elute. Chromatographic conditions: an eluent concentration at 9.0 mM Na_2CO_3 with a flow rate of 1.0 mL/min and an injection volume of 25 µL, applying a current of 45 mA in the suppressor column.*

For FSA, low dilution factors do not give rise to well-defined chromatographic peaks and the column is saturated. Same chromatographic behaviour was observed for FBA.

Once fixed the dilution factor, the chromatographic profiles of the spiked acids samples studied with different concentrations of anions were obtained in the initial chromatographic conditions. As can be seen in Figure 2, well defined chromatographic peaks with good resolution were obtained for bromide, nitrate, phosphate and sulphate. However, the fluorinated species from the

matrix made difficult to obtain an adequate separation of chloride anion, especially for FBA.

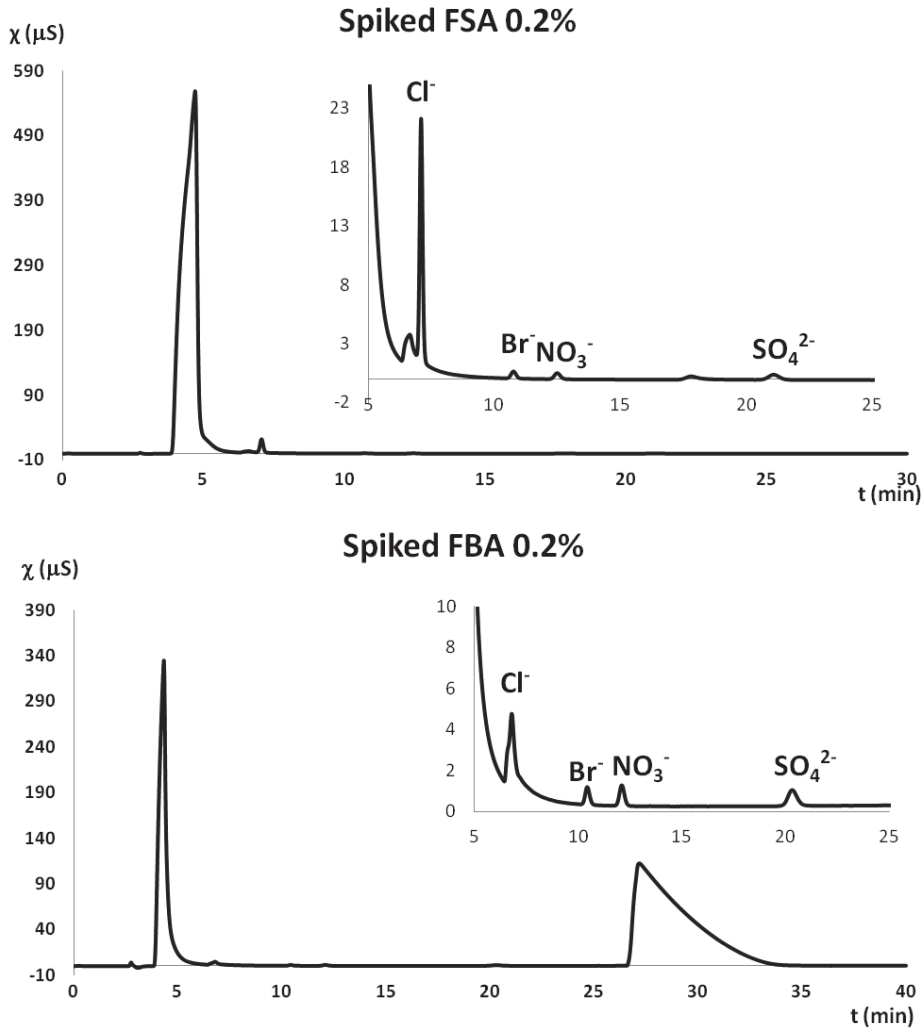

Figure 2. *Chromatograms of FSA and FBA spiked with the studied inorganic anions in the optimum chromatographic conditions: 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 μL, applying a current of 45 mA in the suppressor column. Amplification of the time zone in which anion studied elute is included.*

In order to improve the chromatographic separation between chloride and the fluorinated species, the influence of parameters such as flow rate and eluent concentration on this separation was studied. The injection volume was 25 μL. Variations of flow rate from 0.4 to 1.2 mL/min and eluent concentrations from 5 to 9 mM sodium carbonate were assayed. However, the resolution between chloride and the fluorinated species chromatographic peaks did not improve at any conditions assayed. Since the changes in the response observed did not result in significant improvement, a flow rate of 1 mL/min and a sodium carbonate concentration of 9.0 mM were chosen as final chromatographic conditions.

In these chromatographic conditions the elution order of anionic impurities for both acids were chloride, bromide, nitrate, phosphate and sulphate. On the other hand, the resolution values obtained for these anions were greater than 1.5, except for the separation between fluorinated species and chloride chromatographic peaks in FSA and FBA.

IVa.3.2. Method performance

"Zero" samples were selected for each analyte in FSA and FBA and the external calibration curves were built.

The retention time for each anion in the acids studied, the quality parameters of the calibration curves, the regression line equations, the correlation coefficients, repeatability of the areas and retention times, and accuracy are listed in Table 1.

Table 1 *Quality parameters of the calibration curves, the regression line equations, the correlation coefficients, repeatability of the areas and retention times and the accuracy for the assayed anions in both studied matrixes.*

Hexafluorosilicic Acid (FSA)					
Analyte	Linear Range (mg/l)	Regression Equation Y = a + bx	r^2	t_r (min)	% RSD
Chloride	0.21-4.96	Y = 0.1641x	0.9984	7.187	2.35
Bromide	0.31-4.99	Y = 0.0038 + 0.0051x	0.9993	10.769	1.73
Nitrate	0.38-2.49	Y = 0.0004 + 0.043x	0.9951	12.532	1.25
Sulphate	0.31-21.10	Y = 0.0021 + 0.125x	0.9998	20.986	1.06

	Accuracy (RE %)[a]		Intraday Repeatability (RSD %)		Interday Repeatability (RSD %)	
	Medium	High	Medium	High	Medium	High
Chloride	1.51	0.38	2.12	5.13	1.55	4.14
Bromide	5.79	0.34	10.32	6.49	12.58	4.83
Nitrate	3.14	1.77	4.19	4.51	3.74	3.30
Sulphate	1.87	4.51	4.16	5.12	3.07	3.87

Table 1 *Continuation*

Tetrafluoroboric Acid (FBA)					
Analyte	Linear Range (mg/l)	Regression Equation Y = a + bx	r^2	t_r (min)	RSD %
Chloride	-	-	-	-	-
Bromide	0.25-5.01	Y = -0.0063 + 0.0745x	0.9998	9.257	1.04
Nitrate	0.38-5.38	Y = 0.0857x	0.9920	10.53	0.75
Sulphate	0.39-3.41	Y = 0.1207x	0.9979	20.742	0.20

Analyte	Accuracy (RE %)[a]		Intraday Repeatability (RSD %)		Interday Repeatability (RSD %)	
	Medium	High	Medium	High	Medium	High
Chloride	-	-	-	-	-	-
Bromide	0.95	1.54	2.95	0.61	2.23	0.36
Nitrate	4.46	3.1	4.10	0.42	3.13	0.28
Sulphate	1.34	2.73	2.13	0.85	8.75	5.77

[a] RE% calculated as [(Cc-Cr)/Cr]*100 where Cc is the concentration calculated with the external calibration and Cr is the real concentration.

The ion chromatographic method validated allows determining concentration levels for the four anions with quantification limits ranging between 0.21 and 0.39 mg/L with repeatability for retention times lower than 2.5% and for samples concentration lower than 10 % both in terms of RSD%

IVa.3.3. Application to Real Samples

The developed and validated method was applied to determine the anionic impurities of FSA and FBA samples. Figure 3 shows the chromatograms obtained for real samples of FSA and FBA analyzed.

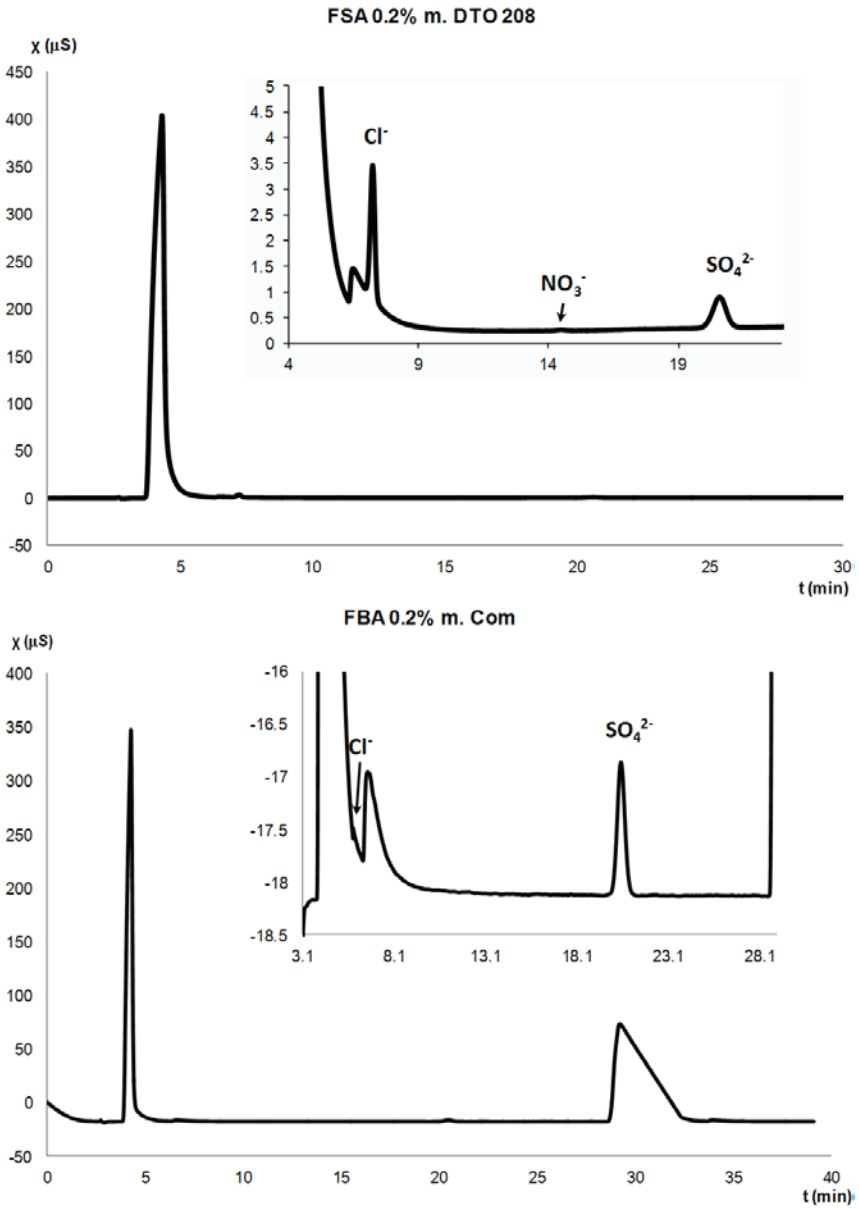

Figure 3. *Chromatograms of real samples of FSA and FBA with the amplification of the time zone in which anion studied elute. Samples analyzed in the optimum chromatographic conditions: 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 µL, applying a current of 45 mA in the suppressor column.*

Relative errors associated with the concentration values obtained, applying the external calibration method proposed, were lower than 15% for all the analytes in FSA and FBA. Chloride, bromide, nitrate and sulphate concentrations obtained for the fluorinated acids studied in the dilution factors applied are collected in Table 2.

Table 2 *Mean concentrations values of FSA and FBA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Hexafluorosilicic Acid (FSA)				
Sample (0.2%)	Chloride		Bromide	
	c (mg/l) ± s*t/√N	RSD (%)	c (mg/l)	RSD (%)
Dto 208	n.a.[a]	-	< 0.31	-
Op 73	0.269 ± 0.008	7.40	< LOD	-
Ens 4	0.313 ± 0.004	2.98	< LOD	-
Ref 674	n.a.	-	< 0.31	-
Com	n.a.	-	0.404 ± 0.002	3.99
Op 251	0.297 ± 0.002	1.54	< LOD	-
Si 1/08	< 0.21	-	< LOD	-
Si 2/08	< 0.21	-	< LOD	-
Si 3/08	< 0.21	-	< LOD	-

Sample (0.2 %)	Nitrate		Sulphate	
	c (mg/l) ± s*t/√N	RSD (%)	c (mg/l) ± s*t/√N	RSD (%)
Dto 208	0.448 ± 0.004	9.13	10.1 ± 0.1	4.2
Op 73	0.411 ± 0.001	2.33	1.384 ± 0.006	1.32
Ens 4	< 0.38	-	1.06 ± 0.02	6.57
Ref 674	< 0.38	-	8.60 ± 0.02	0.81
Com	< 0.38	-	< 0.31	-
Op 251	< 0.38	-	< 0.31	-
Si 1/08	< 0.38	-	0.391 ± 0.005	3.56
Si 2/08	< 0.38	-	< 0.31	-
Si 3/08	< 0.38	-	< 0.31	-

Table 2 *Continuation.*

Tetrafluoroboric Acid (FBA)				
Samples (0.2%)	Chloride		Bromide	
	c (mg/l) ± s*t/√N	RSD (%)	c (mg/l) ± s*t/√N	RSD (%)
Op1627	-	-	< LOD	-
Com	-	-	< LOD	-
Com 2	-	-	< LOD	-
B 7/11	-	-	< LOD	-
B 805	-	-	< LOD	-

Samples (0.2%)	Nitrate		Sulphate	
	c (mg/l) ± s*t/√N	RSD (%)	c (mg/l) ± s*t/√N	RSD (%)
Op1627	< 0.38	-	1.76 ± 0.04	0.85
Com	< 0.38	-	0.95 ± 0.05	2.13
Com 2	< 0.38	-	0.52 ± 0.04	3.05
B 7/11	< LOD	-	0.414 ± 0.03	2.53
B 805	< LOD	-	0.551 ± 0.07	4.91

[a] n.a.: Value out of the linear range obtained

IVa.3.4. Discussion

The present work represents the first approach of applicability of ion chromatography to solve the fluorine chemical industry problem for the analysis of anionic impurities in FSA and FBA.

The method developed allows the analysis of chloride, bromide, nitrate and sulphate in FSA. In spite of the resolution value obtained (Rs = 1.4) for

fluorinated species and chloride chromatographic peaks in FSA the quantitative determination of chloride could be carried out.

The ion chromatographic method was applied to the determination of bromide, nitrate and sulphate in FBA. In this matrix the quantitative analysis of chloride was interfered by the fluorinated species given a resolution value of 1.0, lower than the usual chromatographic resolution threshold. However, the ion chromatographic method developed in isocratic mode can be used for qualitative purposes.

The phosphate anion has a retention time of 17.79 minutes and a resolution value of 3.71 taking nitrate as reference. It is important to point out this anion did not give rise to a Gaussian chromatographic peak in spite of analysing spiked samples with a concentration as great as 50 mg/L, making unable to determine this anion using the analytical method developed. Moreover, it was observed that the sensitivity for this anion decreased with the number of runs made.

This chromatographic behaviour has been observed in other matrixes with high salt content (Singh 1996). The presence of these anions is associated with a consequent high concentration of counterions, which produces a significant accumulation of H^+ on the surface of the suppressor during the ion-exchange process (Mercurio-Cason 1986). This large amount of exchanged hydrogen ions will not be neutralized and will co-elute with matrix anions to obey electroneutrality demands. These hydrogen ions will cause the formation of the strong ion pair with anions of weak acids, such as phosphate ions. The process of proton–phosphate association may result in reducing the charge of the phosphate and then cause the loss of the phosphate chromatographic peak.

IVa.4. CONCLUSIONS

The ion chromatographic method developed has showed its suitability for the quantitative determination of inorganic anionic impurities in FSA and FBA. Chloride, bromide, nitrate and sulphate were analyzed in less than 30 minutes without using any sample treatment apart from dilution. This method solves the

vast problems associated with the determination of anions in presence of huge concentration of fluorinated species and covers a specific necessity of the fluorine industry.

The ion chromatographic method proposed is simple, does require neither multidimensional ion chromatography techniques nor a sample-treatment. The ion chromatographic method in isocratic elution mode has also turned to be a low-cost alternative since the reagents consume is minimum and the equipment necessary is not expensive. Therefore, it is an easy implementation method to industrial laboratories for quality control analysis.

The method developed represents a green alternative to the chemical methods used up to now in the quality control of these products. It is also worth mentioning its advantages like the minimum residues generated and the low sample volume required.

The IC method is free of matrix interferences observed in the chemical methods currently used for anionic impurities analysis in this kind of acids. In addition, difficulties in the final point visualization in volumetry and co-precipitation problems in gravimetry are avoided.

The IC method offers advantages against the capillary electrophoretic method previously applied to one of this kind of matrixes (Ayarza 2012) such as: sensitivity, with LOQs lower than those obtained by CE and sample throughput, problems with capillary life in CE are not present.

The ion chromatographic method developed opens the possibility of being applied to the analysis other inorganic fluorinated products.

IVa.5. ACKNOWLEDGEMENTS

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Chapter IVb- INDUSTRIAL APPLICATION OF ION CHROMATOGRAPHY TO THE QUALITY CONTROL OF FLUORINATED INORGANIC ACIDS. PART II

IVb.1. INTRODUCTION

European hydrofluoric acid (HF) production was 240000 Tons in 2012, which represents a value of 240 millions of Euros at just nine HF production industries in four European countries (CTEF 2012). High amount of HF produced shows a great demand for its use as acid by itself or as a fluorinated derivative. This sector has a great importance not only for the economical aspect but also for the applicability of product produced in it.

HF is the precursor to a lot of essential products for modern life such as well-known pharmaceuticals as fluoxetine (Prozac) or materials such as the omnipresent polytetrafluorethylene (Teflon).

Inorganic fluorinated acids as hexafluorotitanic acid (FTA) and hexafluorozirconic acid (FZA) are also produced using HF as raw material. Both acids can be obtained using different methods, although the reaction between hydrofluoric acid and titania (TiO_2) or zirconia (ZrO_2) as source of the metal is employed by the chemical company to synthesize the samples analysed in this work.

The applicability of these compounds ranges from organic synthesis to industrial applications, being used as electroplating agents, as metal cleaning, to make diazo salts and to produce fluorotitanate (Czika 2008) and fluorozirconate (Sheka 1972) salts. However, hexafluorotitanic and hexafluorozirconic acids are preferably used in the metal surface treatment process.

Hexafluorozirconic acid based surface pretreatments is used as phosphorous free alternative pretreatment technique on metal surfaces to get better adhesion and improved corrosion protection, (Puomi 1999, Puomi 1999, Puomi 2001, Verdier 2005, Verdier 2006, Stromberg 2006, Moore 2008, Adhikari 2010, Eivaz 2012, Khun 2013, Eivaz 2014). This surface treatment can be used for a variety of industrially-important metal surfaces like steel, aluminum and zinc. This process plays a relevant role in extending the life of metals and it is used in automotive, construction, containers and electrical industries, industrial equipment, industries using laboratory equipment, aerospace, and several other industries. The substitution of iron phosphate in the coating pretreatment by hexafluorozirconic acid involves reduction of energy consumed in the chemical

process and the reduction or elimination of phosphate discharge which produce 'green' products.

Hexafluorotitanic acid based surface pretreatments is predominantly used in aluminum surfaces and in electroplating field or galvanization (Lee 2005, Sitthichai 2008, Rahme 2011).

The utilization of baths containing a mixture of both fluorinated acids is used in surfaces treatment as chromium free technique (Oppen 1981, Hallman 1992, Ruiz Garzo 2006, Davies 2008). Some of the results looked for with these treatments are to achieve brightness of the surfaces, in the electrolytic coating of surfaces or in those processes which require to remove oxide layers bonded to metal surfaces. Chromium substitution is compulsory because the hexavalent chromium species are forbidden by the Restriction of Hazardous Substances Directive 2002/95/EC adopted by the European Union in 2003 (European Union 2003).

The fluorine chemical industry requires the quality control of its products, especially regarding anionic impurities contents of the final products due to drawbacks produced by these inorganic anions in the application of them.

These impurities may increase the difficulty to reprocess, recycle or disposal the acid baths produced from the industrial processes and may also complicate the surface treatment to be performed (Liu 2011).

From an economical point of view, the presence of chloride and sulphate produces corrosion which generates additional costs in production process. On the one hand, chloride can interfere the selective attack on the surface to be treated and generate localized corrosion in the structure (Ulig 1985, Ma 2011). This fact makes necessary to maintain chloride concentration levels below 200 mg/l in order to prevent the corrosion in the cooling towers.On the other hand, the presence of sulphate can also cause the corrosion of the metallic surfaces in presence of microorganisms (Heitz 1996).

Regarding environmental point of view, the existence of sulphur species in industrial effluents can affect the bioavailability of heavy metals by complexation or precipitation reactions, especially the oxidized forms of

sulphur as sulphate, which, moreover, are undesirable in the environment (Pagnanelli 2010, Singh 2011).

In the fluorine industry the control of anionic impurities in FTA and FZA is usually carried out by chemical methods as volumetric titration of chloride with silver nitrate and gravimetric determination of sulphate as barium sulphate. These methods suffer from substantial matrix interferences, previously described (Ayarza 2012), which make difficult to obtain reliable results. Thus, the development of new analytical methodologies is a crucial task to assure the reliable classification of samples depending on their impurities level which will allow an effective use of FTA and FZA in the final applications.

Ion chromatography and capillary electrophoresis have been previously applied to similar matrixes with different results. Inorganic anionic impurities in hexafluorosilicic acid have been determined by capillary zone electrophoresis (Ayarza 2012) finding problems with capillary life due to the high corrosive nature of the matrix. Therefore, CZE seems not to be the best analytical approach to solve the industrial problem proposed. Ion chromatographic method allows the determination of sulphate, nitrate and bromide in hexafluorosilicic and tetrafluoroboric acids but the analysis of chloride and phosphate was not able to be implemented due to the high content of fluorinated species in these matrixes. The suitability showed by ion chromatography to determine some anionic impurities in similar matrixes, led us to apply this analytical technique to FTA and FZA, studied in this work.

The great free hydrofluoric acid (HF) content in FTA and FZA and previous results obtained by our research group have been taking into account for the development of an ion chromatographic method for the determination of anionic impurities in these two fluorinated acids. In this sense, the research carried out tries to overcome the problem proposed by fluorine chemical company relative to quality control of FTA and FZA. The final aim of this work will be the application of ion chromatographic as routine analysis method of chloride, nitrate bromide and sulphate in quality control laboratories.

IVb.2. EXPERIMENTAL

IVb.2.1. Apparatus, Reagents and Samples

The ion chromatographic separation was performed using an ICS-1000 chromatographic system by Dionex (Sunnyvale, CA, USA) consisting of an ICS-1000 pump, an ICS-1000 column heater, an Anion Self Regenerating Suppressor (ASRS 300 Ultra II) and a heated conductivity detector. The equipment was coupled with an AS-DV autosampler, for 50 plastic vials of 5 ml each one, PolyVial™, which includes a 20 μm filter. Data acquisition and management of the system were performed using the software Chromeleon version 6.8 by Dionex (Sunnyvale, CA, USA).

An IonPac AS9-HC (4 x 250 mm) analytical column with 9 μm polystirene-divinylbenzene substrate agglomerated with a 0.09 μm diameter anion-exchange aminated latex particles and a guard column, IonPac AG9-HC (4 x 50 mm), supplied by Dionex (Sunnyvale, CA, USA) were used and thermostated at 35 ºC.

The pH measurements were made using a Crison model GLP22 pH-meter (Barcelona, Spain), employing a combined Crison glass electrode, with a reference system Ag/AgCl, KCl (3.0 M).

A Sartorius CP 224S balance (Madrid, Spain) with an accuracy of ± 0.0001 g was employed for the weight of the reagents, standards and samples.

Anhydrous sodium carbonate of proanalysis quality supplied by Merck (Damstadt, Germany), was used for the eluent preparation. The different concentrations of eluent were prepared dissolving sodium carbonate in ultrapure water and filtering the solution immediately before use.

The filters used were Durapore® membrane filters of 45 μm from Millipore Ibérica S.A.U. (Madrid, Spain). The filtration of the eluent was made using a Millipore vacuum pump (Bedford, MA, USA).

Ultra-pure quality water obtained from a Milli-Q Element A10 water system, from Millipore (Mildford, MA, USA) was used for the solutions preparation.

Standard solutions of chloride, bromide, nitrate and sulphate at a 1000 mg/L concentration were provided by Merck (Barcelona, Spain). Working solutions of these anions were prepared by dilution from standard solutions.

Commercial FTA and FZA samples were supplied by Alfa Aesar GmbH & Co KG (Karlsruhe, Germany) with a content of 60 and 45% of H_2TiF_6 and H_2ZrF_6 respectively and by Sigma-Aldrich Co (St. Louis, Missouri, United State of America) with a content ranging between 60 and 50% of H_2TiF_6 and H_2ZrF_6 respectively.

Diluted industrial FTA and FZA samples were supplied by the company Derivados Del Fúor S.A. (DDF) (Cantabria, Spain), with a content of 4% of H_2TiF_6 and H_2ZrF_6. These samples were used for the chromatographic system optimization due to the lack of blank matrixes or certified reference materials.

Polypropylene material was used for the solutions preparation and storage to avoid the glass deterioration by free HF containing FTA and FZA samples.

IVb.2.2. Chromatographic Conditions

The separation of chloride, bromide, nitrate and sulphate was performed using 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 μL. The suppressed conductivity detection was used applying a current of 45 mA to the suppressor column, to suppress the background conductivity of the eluent. The conductivity of the base line in these conditions was approximately 22 μS.

IVb.2.3. Validation Features

The initial step for the quantification of the anions was a qualitative analysis of commercial and industrial samples. This was made by a comparison of the chromatographic peak areas of all the samples of each acid. Samples with the lowest chromatographic peak areas for each analyte (“zero” samples) were chosen to build the calibration curves for the quantification of anion impurities.

The standard addition method was applied in order to take into account the complex matrixes analyzed. An external calibration was calculated from the linear regression data and the "zero" sample concentration obtained once applied the standard addition method. These calibration curves were used for the quantitative determination of the rest of samples.

Linear concentration ranges assayed were determined upon the basis of chromatographic peak areas obtained in the previous qualitative analysis of samples carried out. The linear concentration ranges were obtained spiking "zero" samples with solutions containing different chloride, bromide, nitrate and sulphate concentrations. For FZA the linear ranges assayed were from 0.21 to 6.21 mg/l for chloride, from 0.25 to 5 mg/l for bromide, from 0.14 to 5.07 mg/l for nitrate and from 0.05 to 3.56 mg/l for sulphate. For FTA the linear ranges assayed were from 0.20 to 6.10 mg/l for chloride, from 0.25 to 5.10 mg/l for bromide, from 0.16 to 5.05 mg/l for nitrate and from 0.15 to 5.11 mg/l for sulphate.

Linear regression method was applied to the chromatographic peak area–concentration data. Quantification limits were calculated as the minimum concentration which give rise to a S/N ratio equal to 10 and can be determined with an accuracy in terms of RE% and a repeatability in terms of RSD%, both lower than 15% (Hautman 1997). Inter-day and intra-day repeatability of the method were calculated for medium and high concentrations levels within the calibration curve range. RSD% was used to express the repeatability. Samples spiked with different concentration values within calibration curve were used for accuracy calculation in terms of RE%. The repeatability of retention times of standard solutions and samples was calculated in terms of RSD%. Mean concentrations of samples were obtained by interpolation from three replicates and expressed as mean $\pm$ $t^*s/\sqrt{N}$ where t is the student factor, N is the number of replicates and s is the standard deviation. A confidence level of 95% was used.

IVb.3. RESULTS AND DISCUSSION

IVb.3.1. Chromatographic System

The AS9-HC column was chosen for anionic impurities analysis in fluorinated acids based on its suitability demonstrated for similar matrixes with less amount of free HF in a previous work of our research group (Ayarza 2014). The chromatographic conditions were 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 µL. In these conditions, a baseline drift was observed probably due to the huge amount of fluoride and fluorinated species contained in these acids.

The criteria to choose the chromatographic conditions were based on the number and resolution of chromatographic peaks between fluoride and chloride anions and baseline drift.

The great content fluorinated species present in these types of matrixes force us to study the influence of dilution factor on the chromatographic response. For this purpose, FTA and FZA samples were diluted with ultrapure water at different final acid percentage. The optimum final acid percentage was established at 0.2% for FTA and 0.4% for FZA since chloride, bromide, nitrate and sulphate peaks were identified with adequate resolution and low baseline drift was obtained. Besides, higher baseline drift due to the matrix influence was observed for FTA than for FZA. Indeed, when hexafluorotitanic acid samples were analysed, the base line value after the 20 minutes necessary for the elution of all anions was 1.3 µS higher than that for FZA, as can be seen in Figure 1.

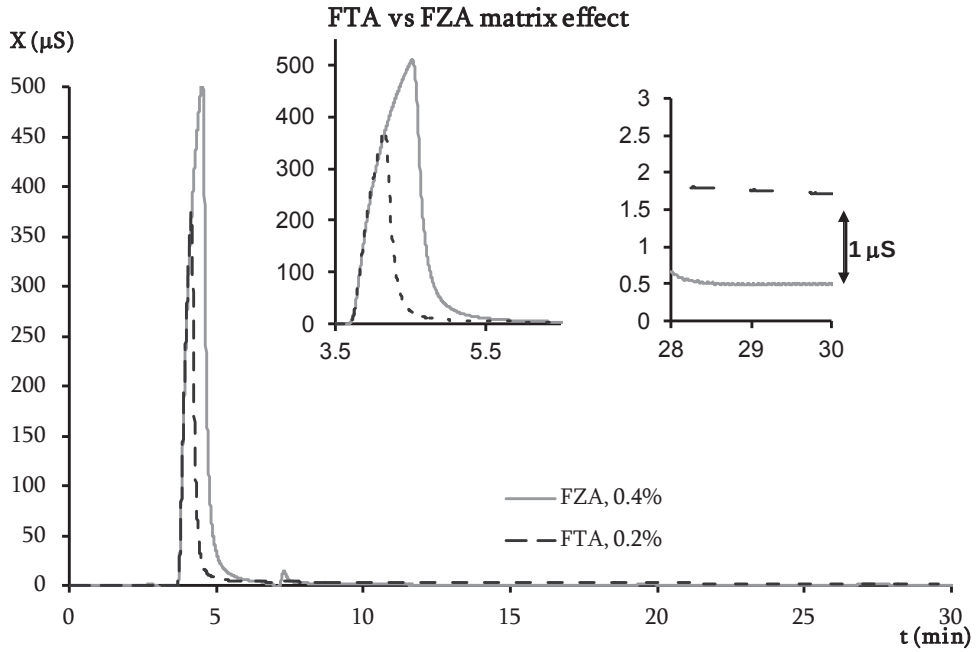

Figure 1. *Chromatograms of FTA and FZA at the optimum final acid percentage. Chromatographic conditions: an eluent concentration at 9.0 mM Na_2CO_3 with a flow rate of 1.0 mL/min and an injection volume of 25 µL, applying a current of 45 mA in the suppressor column.*

Apart from this, a matrix peak was also observed in FTA analysis after 30 minutes of the end of the analysis time. In order to solve these problems, a cleaning step after each analyses of FTA was optimized. Flow rates from 1.0 to 1.5 mL/min were tested in order to decrease cleaning time and considering the pressure of the system as the limiting variable.

Pressure obtained for different flow rates assayed are listed in Table 1.

Table 1 *Pressure and cleaning time necessary for the elution of the matrix peak of the FTA and baseline stabilization at different flow rates tested.*

Flow Rate (mL/min)	Pressure (psi)	Cleaning Time (min)
1.0	1983	48
1.2	2240	37
1.3	2375	33
1.5	2727	30

A time of 48 minutes flowing the eluent at 1 mL/min is necessary to achieve the cleaning of the column and the stabilization of baseline in order to avoid the overpressure in the analytical column and, therefore, increasing the column lifetime. A larger analysis time was set to achieve the complete cleaning and stabilization of the system for FTA matrix.

The chromatographic profiles of the different acids studied can be observed in Figure. 2.

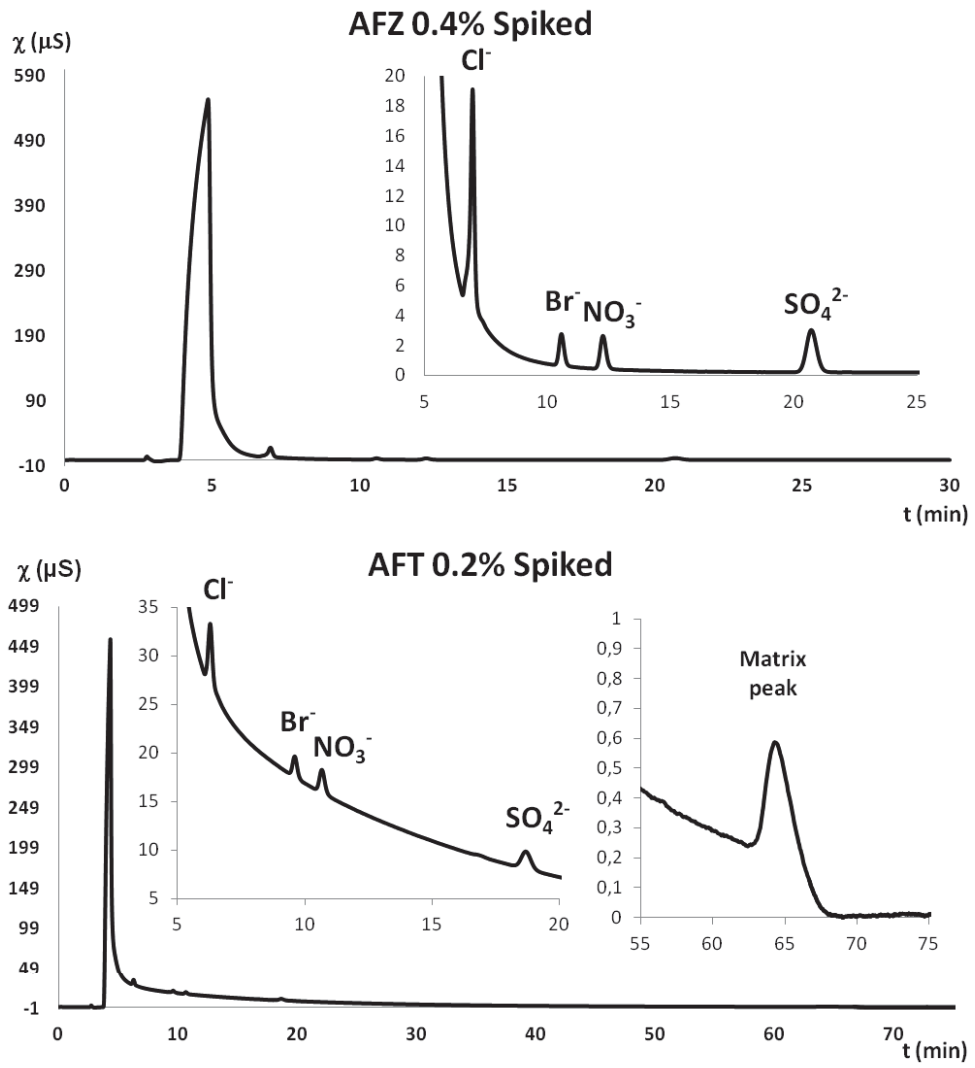

Figure 2. *Chromatograms of FTA and FZA samples spiked with the inorganic anions studied. Chromatographic conditions: 9.0 mM Na_2CO_3 as eluent with a flow rate of 1.0 mL/min and an injection volume of 25 µL, applying a current of 45 mA in the suppressor column.*

Well defined chromatographic peaks and resolution values higher than 1.5 were obtained for bromide, nitrate and sulphate in both acids. A stable baseline after FTA analysis was achieved with an extra time for the cleaning of the system.

However, the fluorinated species from the matrix made difficult to obtain an adequate separation of chloride anion in FZA.

IVb.3.2. Validation Features

The figures of merit of the ion chromatographic method developed for the quantitative determination of anionic impurities in the fluorinated acids are listed in Table 2.

Table 2 *Quality performance of the ion chromatographic method developed.*

Hexafluorotitanic Acid (FTA)					
Analyte	Linear Range (mg/l)	Regression Equation Y = a + bx	r^2	t_m (min)	% RSD
Chloride	1.02 - 7.12	Y = 0.2137x	0.9984	6.186	0.64
Bromide	0.25 - 5.10	Y = 0.0925x - 0.0072	0.9998	9.159	0.79
Nitrate	0.16 - 5.05	Y = 0.1165x - 0.0019	0.9998	10.437	0.91
Sulphate	0.85 - 5.97	Y = 0.1494x	0.9960	18.011	0.72

	Accuracy (RE %)[a]		Intraday Repeatability (RSD %)		Interday Repeatability (RSD %)	
	Medium	High	Medium	High	Medium	High
Chloride	4.06	5.67	1.15	4.14	3.24	4.65
Bromide	3.94	6.17	1.92	3.84	5.64	5.84
Nitrate	4.24	5.23	2.62	3.60	6.32	6.40
Sulphate	5.89	5.67	3.18	3.95	5.47	5.04

Table 2 *Continuation.*

Hexafluorozirconic Acid (FZA)					
Analyte	Linear Range (mg/l)	Regression Equation Y = a + bx	r^2	t_m (min)	% RSD
Chloride	-	-	-	-	-
Bromide	0.25 - 5.01	Y = -0.0060 + 0.0736x	0.9998	10.407	0.485
Nitrate	0.15 - 5.07	Y = 0.0125 + 0.0903x	0.9999	12.099	1.326
Sulphate	0.34 - 3.91	Y = 0.1202x	0.9994	20.530	0.243

	Accuracy (RE %) [a]		Intraday Repeatability (RSD %)		Interday Repeatability (RSD %)	
	Medium	High	Medium	High	Medium	High
Chloride	-	-	-	-	-	-
Bromide	3.08	1.17	3.59	2.9	3.21	3.2
Nitrate	4.46	0.38	4.62	0.42	1.06	2.72
Sulphate	1.34	0.55	3.74	0.85	0.41	3.57

Relative errors associated with the concentration values obtained applying standard addition method and the external calibration method proposed were lower than 10% for all the analytes in FTA and FZA.

A unique quantitative determination method using "zero" sample was demonstrated to be valid for any sample of each fluorinated acids.

IVb.3.3. Application to Real Samples

The developed and validated method was applied to the determination of the inorganic anionic impurities chloride, bromide, nitrate and sulphate in FTA and FZA samples.

The chloride, bromide, nitrate and sulphate concentrations obtained for the different fluorinated acids, taking into account the dilution factors applied are collected in Table 3.

Table 3 *Mean concentrations values of FTA and FZA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Hexafluorotitanic Acid (FTA)						
	Chloride			Bromide		
Sample (0.2%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Com	1.17	0.07	2.33	n.d.	-	-
Com 2	1.20	0.08	2.55	n.d.	-	-
m. 20	n.d.	-	-	n.d.	-	-
	Nitrate			**Sulphate**		
Sample (0.2 %)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Com	0.81	0.07	3.55	0.84	0.10	4.76
Com 2	n.d.	-	-	n.d.	-	-
m. 20	n.d.	-	-	<0.84	-	-

n.d. = Not detectable.

Table 3 *Continuation.*

Hexafluorozirconic Acid (FZA)						
Samples (0.4%)	Chloride			Bromide		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Com	-	-	-	n.d.	-	-
Com 2	-	-	-	n.d.	-	-
m. 1	-	-	-	n.d.	-	-
m.15	-	-	-	n.d.	-	-
m. 16	-	-	-	n.d.	-	-
m. 24	-	-	-	n.d.	-	-
m. 25	-	-	-	n.d.	-	-
m. 127	-	-	-	n.d.	-	-
m. 135	-	-	-	n.d.	-	-

Samples (0.4%)	Nitrate			Sulphate		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Com	n.d.	-	-	-	-	-
Com 2	0.22	0.02	8.37	n.a	-	-
m. 1	n.d.	-	-	4.25	0.03	0.76
m.15	n.d.	-	-	<0.34	-	-
m. 16	n.d.	-	-	n.a	-	-
m. 24	n.d.	-	-	<0.34	-	-
m. 25	n.a	-	-	1.42	0.02	1.73
m. 127	<0.15	-	-	2.84	0.06	0.2
m. 135	<0.15	-	-	<0.34	-	-

n.a. = not applicable because the concentration is out of linear range; n.d. = Not detectable.

IVb.3.4. Discussion

Huge ratios between the analyte chromatographic response and the corresponding one of fluorinated species, 1:250000 and 1:900000 for FTA and FZA respectively, have complicated the development of a successful IC method. An extra effort was necessary to overcome the effects of the matrix observed.

The method developed allows the analysis of chloride, bromide, nitrate and sulphate in FTA, in spite of the high carry over observed in the anaysis of this matrix.

The ion chromatographic method was applied to the quantitative determination of bromide, nitrate and sulphate in FZA and to the qualitative analysis of chloride because in this matrix this quantitative analysis was interfered by the fluorinated species.

The method developed has been successfully applied in the industrial quality control of the company supplier of the samples. This has substituted the chemical methods previously utilized for the analysis of sulphate in all acids the company produces, with the consequent advantages of decreasing analysis time and costs.

Although the current work represents an advance in the applicability of ion chromatography to solve the fluorine chemical industry problems for the analysis of anionic impurities in inorganic fluorinated acids, there are some aspects worthy to mention. On the one hand, difficulty in the analysis of chloride in FZA matrix was observed due to the interferences produced by the fluorinated species which makes difficult its quantitative determination. However, the ion chromatographic method developed in isocratic mode can be used for qualitative purposes. On the other hand, when hexafluorotitanic acid samples were analysed, a high base line value and one late matrix chromatographic peak were obtained. A larger analysis time was necessary to avoid these problems and the overpressure in the analytical column.

IVb.4. CONCLUSIONS

The ion chromatographic method developed has showed its suitability for the quantitative determination of inorganic anionic impurities in FTA and FZA. Chloride, bromide, nitrate and sulphate were analyzed without using any sample treatment apart from dilution. This method solves the vast problems associated with the determination of anions in presence of huge concentration of fluorinated species.

The ion chromatographic method proposed is simple, does not require neither multidimensional ion chromatography techniques nor a sample-treatment. The ion chromatographic method in isocratic elution mode has also turned to be a low-cost alternative since the reagents consume is minimum and the equipment necessary is not expensive. Therefore, it is easily implemented in industrial laboratories.

The method developed represents a green alternative to the chemical methods used up to now in the quality control of these products. It is also worth mentioning its advantages like the minimum residues generated and the low sample volume required.

The IC method can be implemented in industrial laboratories for the analysis of sulphate in this kind of acids, eliminating the co-precipitation problems present in the gravimetry, method previously used.

The IC method offers advantages against the capillary electrophoretic method previously applied to FSA (Ayarza 2012) such as sensitivity, with LOQs lower than those obtained by CE, sample throughput, problems with capillary life in CE are not present, and linear regression fit of calibration curves, with higher correlation coefficients.

The ion chromatographic method developed offers the possibility of being applied to the analysis of salts as potassium hexafluorotitanate and potassium hexafluorozirconate.

IVb.5. ACKNOWLEDGEMENTS

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Chapter IVc- INDUSTRIAL APPLICATION OF GRADIENT ION CHROMATOGRAPHY TO THE QUALITY CONTROL OF FLUORINATED INORGANIC ACIDS.

IVc. 1. INTRODUCTION

Fluorinated compounds production sector has a great social and economic importance due to not only its numerous applications but also the economical amount of money it involves. On the one hand, products as essential for modern life as Gore-tex and Teflon or toothpaste are among fluorinated compounds (Gupta 2003, Chukwuemeka 2006, Togni 2007, Park 2010). On the other hand, this industrial sector represents an amount of 240 millions of Euros in 2012, what is even more relevant if it is taken into account that this production is only generated at nine companies in four European countries (CTEF 2012). In this sense, problem solving in this kind of industry is a great relevance challenge.

In this work, the study is focus on final products of fluorine industry such as hexafluorosilicic acid (FSA), tetrafluoroboric acid (FBA), hexafluorotitanic acid (FTA) and hexafluorozirconic acid (FZA), all of them coming from a reaction of hydrofluoric acid with the corresponding inorganic source of metal, these are silica (SiO_2), boric acid (H_3BO_3), titania (TiO_2) and zirconia (ZrO_2) respectively.

Organic synthesis (Papcun 2000, Czika 2008, Sheka 1972), refrigerants, special polymers for the chemical industry, surface treatment (Lee 2005, Sitthichai 2008, Rahme 2011, Puomi 1999, Puomi 1999, Puomi 2001, Verdier 2005, Verdier 2006, Stromberg 2006, Moore 2008, Adhikari 2010, Eivaz 2012, Khun 2013, Eivaz 2014, Harben 2004), lead-free gasoline, glass, semiconductors, ionic liquids (Wilkes 2004), fluoridation (Committee on Fluoride in Drinking Water 2006), and solar cells are some of the applications of the products objects of interest. All of these acids trend to be used as green alternative in surface treatment industry to substitute chromium or phosphate use (Oppen 1981, Hallman 1992, Ruiz Garzo 2006, Davies 2008). These new technologies promote as two major benefits, the reduction of energy consumed in the chemical process implying economical benefits and the reduction or elimination of phosphate discharge touting environmental benefits. Nowadays, this environmentally friendly tendency is the one followed in all fields of our society and industry overall when it implies costs reduction.

The presence of impurities in final products, as those studied in this work, is not desirable from the economic and environmental point of view since these can complicate the proper performance of the application to be developed (Liu 2011 Seddon 2000 Curtis 1978). From an economical point of view, additional costs are generated when phenomenon as corrosion are provoked by impurities as chloride or sulphate (Ulig 1985, Heitz 1996, Ma 2011). Chloride levels down 200 mg/l used to be enough to prevent the corrosion in the cooling towers. Regarding environmental point of view, the existence of sulphur species in industrial effluents can affect the bioavailability of heavy metals by complexation or precipitation reactions, especially the oxidized forms of sulphur as sulphate, which, moreover, are undesirable in the environment (Pagnanelli 2010, Singh 2011).

All the industrial applications in which these acids are involved require a quality control management of anionic impurities content due to the drawbacks produced by these anions. This control of impurities in final products in industrial laboratories is carried out using Volhard method for chloride and gravimetry for sulphate. These chemical methods are tedious and time consuming and suffer from substantial matrix interferences associated to the visual detection of final point in volumetry and to the co-precipitation in gravimetry. These disadvantages make difficult to obtain reliable results. In this sense, instrumental analytical techniques are more effective approaches than chemical methods to obtain reliable quantitative information about anionic impurities in fluorinated inorganic acids. Therefore, the development of analytical methods of easy implementation in industrial laboratories in order to control the impurities in commercial products is a valuable and an unmet task for the quality control in laboratories of fluorine industry.

In literature, the analytical techniques reported for the determination of trace levels of inorganic anions in the different fluorinated matrixes studied are preferably ion chromatography (IC) and capillary electrophoresis (CE), all of these works have been reported by our research group.

First step in analytical approach to these matrixes was to develop a capillary electrophoresis method (Ayarza 2012) as an alternative to chemical

methodology implemented in industrial laboratories up to that time. This first try tackles the determination of chloride and sulphate in hexafluorosilicic acid, what presented serious difficulties in terms of capillary life due to the high corrosive nature of the matrix. Then, the research carried out tried to overcome the problematic found by applying the ion chromatography technique using isocratic mode addressing FSA and FBA first (Ayarza 2014) and FTA and FZA after. This approach showed better results than capillary electrophoresis one although problems were still found related to chloride determination due to the huge ratio between chloride and fluorinated species in these matrixes. This was reflected in low chromatographic resolution, implying impossibility of quantitative chloride determination. In spite of having developed these new methodologies, fluorine chemical industry problems had not been completely solved.

In this sense, the main goal of this work is the development of an ion chromatographic method using gradient elution mode for the quantitative determination of chloride, bromide, nitrate and sulphate in FSA, FBA, FTA and FZA, in order to overcome the inherent difficulties associated with the complexity of fluorinated matrixes. The analytical methodology developed will be transferred to industrial laboratories as quality control procedure.

IVc.2. EXPERIMENTAL

IVc.2.1. Apparatus, Reagents and Samples

The ion chromatographic separation was performed using an ICS-1000 chromatographic system by Dionex (Sunnyvale, CA, USA) consisting of an ICS-1000 pump, an ICS-1000 column heater, an Anion Self Regenerating Suppressor (ASRS 300 Ultra II) and a heated conductivity detector. The equipment was coupled with an AS-DV autosampler, for 50 plastic vials of 5 ml each one, PolyVial™, which includes a 20 μm filter. The hydroxide eluent was electrolytically generated with an Eluent Generator system, RFIC-EG, equipped with an EluGen KOH cartridge and a RFC -30 Reageant-Free Controller. The eluent was purified in line using continuously electrically regenerated trap columns (CR-ATC). A self-regenerating suppressor (ASRS 300 Ultra II, 4mm)

was employed in recycle water mode for eluent suppression. Data acquisition and management of the system were performed using the software Chromeleon version 6.8 by Dionex (Sunnyvale, CA, USA).

An IonPac AS11-HC (4 x 250 mm) analytical column and a guard column, IonPac AG11-HC (4 x 50 mm), supplied by Dionex (Sunnyvale, CA, USA), were used and thermostated at 35 ºC. These are latex-agglomerated columns composed of highly cross-linked divinylbenzene (DBV).

Ultra-pure quality water obtained from a Milli-Q Element A10 water system, from Millipore (Mildford, MA, USA) was used for the solutions preparation.

Standard solutions of chloride, bromide, nitrate, phosphate and sulphate at concentration of 1000 mg/L were provided by Merck (Barcelona, Spain). Stock solutions of all inorganic anions were prepared by dissolving pure standards in deionized water

Commercial FSA, FBA, FTA and FZA samples were supplied by two commercial brands. Alfa Aesar GmbH & Co KG (Karlsruhe, Germany) with a content 23% of H_2SiF_6, 48% of HBF_4, 45% of H_2ZrF_6 and 60% of H_2TiF_6 and Sigma-Aldrich Co (St. Louis, MI, USA) with a content of 48 % of HBF_4, and 60% of H_2TiF_6, and 50% of H_2ZrF_6. Industrial samples were supplied by the company Derivados Del Fúor S.A. (DDF) (Cantabria, Spain), with a content ranging between 4 and 42% of H_2SiF_6 and 4% of HBF_4, H_2TiF_6 and H_2ZrF_6. Some of these samples were used for the chromatographic system optimization due to the lack of blank matrixes and certified reference materials. The preparation and storage of solutions was made using polypropylene material to avoid glass deterioration caused by free HF containing FSA, FBA, FTA, and FZA samples.

IVc.2.2. Optimization of Chromatographic Conditions

The high-capacity Dionex IonPac AS11-HC column was used for the analytical separation due to it allows the injection of more concentrated samples without column overloading or peak broadening, what provides improved separation. The mobile phase required for this column was KOH. All experiments were carried out using gradient elution. The mobile phase was pumped at a flow rate

of 1.50 mL/min. The column was thermostated at 35 °C. The injection volume was 25 μL. All standards and samples were injected in triplicate. Initial chromatographic conditions used were adapted from those graciously provided by Dionex Corp. The initial KOH gradient used was: 0-7 min isocratic at 2 mM, 7-35 min from 2 to 22.5 mM linearly, 35-42 min isocratic at 22.5 mM, and 42-45 min isocratic at 2 mM of KOH. Optimization of the gradient elution mode of ion chromatographic method was made using one variable at a time (OVAT) method. Parameters to be optimized inside the gradient elution mode were: initial and final concentration of mobile phase, the time interval. KOH initial concentration ranged from 1 - 15 mM and final concentration of KOH was varied from 20 - 55 mM, using time interval from10 to 25 minutes. The number of chromatographic peaks, chromatographic resolution and analysis time were chosen as response of the system to be optimized. Dilution factor of samples was not included in system optimization keeping constant this parameter previously optimized (Ayarza 2014).

IVc.2.3. Validation Features

Samples with the lowest concentration of the majority analytes, “zero” samples, were used to build the calibration curves for the quantification of anionic impurities. These samples were chosen based on the information from previous works (Ayarza 2014).

The linear concentration ranges were obtained spiking “zero” samples with solutions containing different chloride, bromide, nitrate, phosphate and sulphate concentrations. For FSA, FBA, FTA, and FZA the linear ranges assayed were from 0.25 to 10 mg/L for chloride, bromide, nitrate, and sulphate.

Linear regression data and the “zero” sample concentration obtained once applied the standard addition method were transferred to build external calibrations. These calibration curves were used for the quantitative determination of the rest of samples. Quantification limits (LOQs) were calculated as the minimum concentration which gives rise to a S/N ratio equal to 10 and can be determined with an accuracy in terms of relative error percentage (RE%) and a repeatability in terms of RSD%, both lower than 15%

(Hautman 1997). These LOQ values correspond to the first points of the calibration curves.

The precision analysis comprised inter- and intra-day repeatability studies for samples containing low, medium, and high inorganic anions concentration levels. The interday repeatability was determined by analyzing each sample at the three mentioned levels on three different days over about one month.

Samples spiked with different concentration values within the calibration curve were used for accuracy calculation in terms of RE%.

The repeatability of retention times of standard solutions was calculated in terms of RSD%

IVc.2.4. Real Samples Analysis

46 samples of FSA, FBA, FTA and FZA, coming from industrial process production and different commercial suppliers, were analyzed. Mean concentrations of samples were obtained by interpolation from three replicates in the external calibration curve and expressed as mean ± $t^*s/\sqrt{N}$ where t is the student factor, N is the number of replicates and s is the standard deviation, at a confidence level of 95%.

Potential applicability of the developed method to the commercial corresponding salts of studied acids was tested.

Results obtained using the ion chromatographic method developed and the isocratic one (Ayarza 2014) were compared using the paired sample t-test.

The trueness of the current method could only be assessed by comparison of the experimental data with the corresponding results obtained from isocratic ion chromatography previously reported, considered as a reference method due to the lack of CRM and standard method in this kind of matrixes. Linear regression method was also applied to perform this comparison.

IVc.3. RESULTS AND DISCUSSION

IVc.3.1. Optimization of Chromatographic Conditions

The AS11-HC analytical column has demonstrated to be adequate for the separation of chloride, bromide, nitrate, sulphate and phosphate impurities. Among different gradients assayed, the best chromatographic separation and shorter analysis time for all studied inorganic anions present in fluorinated samples were obtained with the following KOH gradient: 0-12 min isocratic at 10 mM, 12-32 min from 10 to 25 mM linearly, 32-38 min isocratic at 25 mM and 38-40 min isocratic at 10 mM. During whole gradient program the suppressor current used was proportional to the highest mobile phase concentration, concretely 96 mA obtaining a base line conductivity of 0.53 μS. The flow rate and injection volume used were 1.5 mL/min and 25 μL respectively.

The acid percentages, 0.2% for FSA, FTA and FBA and 0.4% for FZA, were fixed to lengthen the life of the column. The optimum gradient provided well defined chromatographic peaks with resolution values, Rs, higher than 1.5 for chloride, bromide, nitrate, phosphate and sulphate. In these chromatographic conditions the elution order of anionic impurities for all the acids studied were chloride, bromide, nitrate, sulphate and phosphate. Table 1 summarizes the retention times and the RSD% of all anions studied in the four different matrixes.

Table 1 *Retention times and the RSD% of all analytes in FSA, FBA, FTA and FZA.*

	FSA		FBA		FTA		FZA	
	t_r	RSD%	t_r	RSD%	t_r	RSD%	t_r	RSD%
Chloride	5.204	0.30	5.008	0.18	5.042	0.14	5.331	0.11
Bromide	10.647	0.40	10.335	0.27	10.201	0.56	10.876	0.05
Nitrate	11.186	3.38	11.120	0.29	10.974	0.59	11.715	0.06
Sulphate	20.630	0.10	20.306	0.18	20.125	0.41	20.835	0.04
Phosphate	35.698	0.73	-	-	-	-	35.358	0.12

In Figure 1 a sample of each kind of matrix spiked with the studied anions is shown.

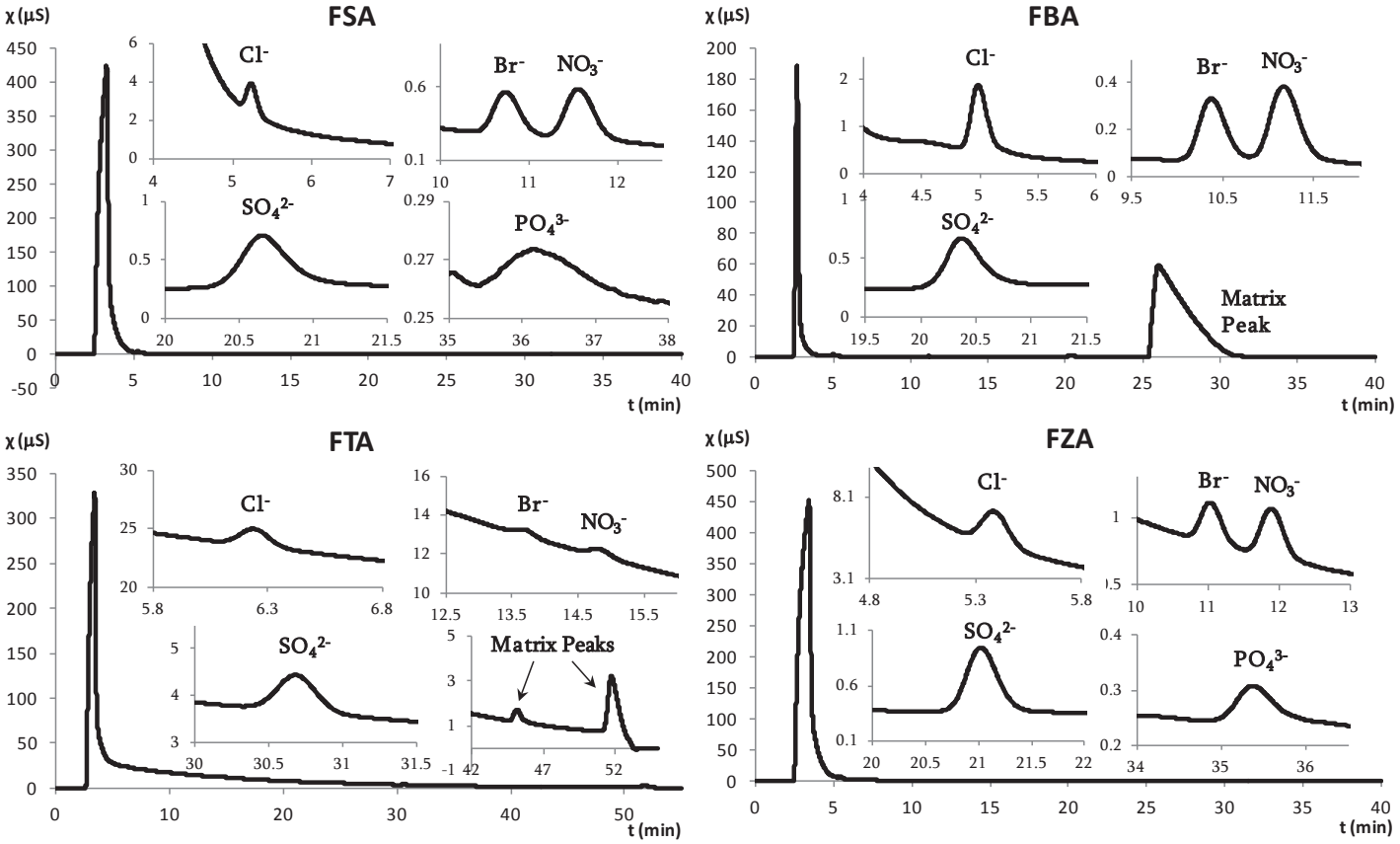

Figure 1. *Chromatograms of FSA, FBA, FTA and FZA samples spiked with the inorganic anions studied at 1.25 mg/L obtained with the optimum chromatographic conditions.*

IVc.3.2. Validation Features

The linear concentration range obtained ranged from the quantification limit to at least 10 mg/L for all inorganic anions in the different matrixes studied.

Linear regression equations for each compound in all matrixes, correlation coefficients and ordinary quantification limits are listed in Table 2.

Table 2 Limits of quantification, *regression equations, and correlation coefficients of the calibration curves for all analytes in FSA, FBA, FTA and FZA.*

Hexafluorosilicic Acid (FSA)

Analyte	LOQ (mg/L)	Regression Equation Y = bx + a	r^2
Chloride	0.25	Y = - 0.0151 + 0.1925x	0.9992
Bromide	0.25	Y = - 0.0074 + 0.0869x	0.9978
Nitrate	0.26	Y = - 0.0114 + 0.1143x	0.9971
Sulphate	0.16	Y = - 0.0234 + 0.1328x	0.9971
Posphate	0.26	Y = - 0.0038 + 0.018x	0.9883

Tetrafluoroboric Acid (FBA)

Analyte	LOQ (mg/L)	Regression Equation Y = a + bx	r^2
Chloride	0.25	Y = -0.0017 + 0.1972x	0.9998
Bromide	0.25	Y = -0.0035 + 0.0803x	0.9991
Nitrate	0.25	Y = -0.0032 + 0.1062x	0.9983
Sulphate	0.15	Y = 0.0000 + 0.1259x	0.9971

Hexafluorotitanic Acid (FTA)

Analyte	LOQ (mg/L)	Regression Equation Y = a + bx	r^2
Chloride	0.25	Y = -0.0120 + 0.1948x	0.9906
Bromide	0.25	Y= -0.0054 + 0.0845x	0.9920
Nitrate	0.25	Y= -0.0055 + 0.1104x	0.9924
Sulphate	0.13	Y= 0.0059 + 0.1606x	0.9902

Table 2 *Continuation.*

Hexafluorozirconic Acid (FZA)			
Analyte	LOQ (mg/L)	Regression Equation Y = a + bx	r^2
Chloride	0.26	Y = 0.0469 + 0.1856x	0.9998
Bromide	0.25	Y = 0.0012 + 0.1087x	0.9967
Nitrate	0.28	Y = 0.0000 + 0.1062x	0.9998
Sulphate	0.22	Y = 0.0326 + 0.1466x	0.9970
Posphate	0.28	Y = 0.0088 + 0.0309x	0.9903

The results of the precision analysis and the accuracy data of inorganic anions in FSA and FBA, and FTA and FZA, are collected in Tables 3 and 4 respectively.

Table 3 *Quality parameters of the ion chromatographic method for all analytes: repeatability of areas and accuracy at three concentration levels for the assayed anions in FSA and FBA matrixes.*

Hexafluorosilicic Acid (FSA)

Analyte	Accuracy (RE %)			Intraday Repeatability (RSD %)			Interday Repeatability (RSD %)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Chloride	-0.97	-1.12	1.61	0.84	0.45	0.06	1.93	0.60	0.22
Bromide	7.81	-0.14	1.84	0.87	0.91	0.19	2.35	2.67	0.25
Nitrate	3.61	-1.34	1.21	1.32	1.64	0.89	9.33	14.17	0.87
Sulphate	-11.16	-4.55	3.17	1.44	0.24	0.04	12.33	1.40	0.67

Tetrafluoroboric Acid (FBA)

Analyte	Accuracy (RE %)			Intraday Repeatability (RSD %)			Interday Repeatability (RSD %)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Chloride	4.43	1.18	1.68	4.06	0.06	0.24	4.27	0.67	0.99
Bromide	2.25	2.16	5.46	2.16	0.34	0.09	5.08	1.52	0.88
Nitrate	-2.07	2.18	5.16	4.48	1.26	0.06	5.60	2.29	1.33
Sulphate	5.10	5.14	10.25	2.45	0.73	0.73	4.01	2.97	2.11

Table 4 *Quality parameters of the calibration curves for all analytes, repeatability of areas and accuracy at three concentration levels for the assayed anions in FTA and FZA matrixes.*

Hexafluorotitanic Acid (FTA)

Analyte	Accuracy (RE %)			Intraday Repeatability (RSD %)			Interday Repeatability (RSD %)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Chloride	17.62	-4.51	0.90	6.48	2.93	2.56	11.40	3.78	2.50
Bromide	7.49	-0.94	3.49	17.37	3.65	2.07	16.30	4.70	1.86
Nitrate	15.65	2.85	5.10	13.22	2.98	1.59	10.08	3.20	2.20
Sulphate	9.63	4.29	5.44	1.96	4.49	2.46	1.63	2.90	2.02

Hexafluorozirconic Acid (FZA)

Analyte	Accuracy (RE %)			Intraday Repeatability (RSD %)			Interday Repeatability (RSD %)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Chloride	3.03	-1.55	0.98	0.65	0.85	0.03	2.04	0.78	0.61
Bromide	8.82	1.74	1.54	3.20	0.54	0.18	2.12	1.70	1.16
Nitrate	-3.59	-0.52	1.44	2.16	0.33	0.17	1.76	0.71	0.71
Sulphate	1.49	-5.04	-0.45	6.26	1.93	1.02	6.61	1.51	0.89

Values of RSD% of the inter- and intra-day repeatability and RE% lower than 15% were obtained at different concentration levels for all the anions studied in the four matrixes tested. Phosphate analysis was discarded because of the loss in sensitivity due to the high amount of H^+ accumulated on the surface of the suppressor concentration due to the huge concentration of counterions (Mercurio-Cason 1986).

IVc.3.3. Real Samples Analysis

The chromatograms obtained for one sample of each kind of acid at optimum ion chromatographic conditions are shown in Figure 2.

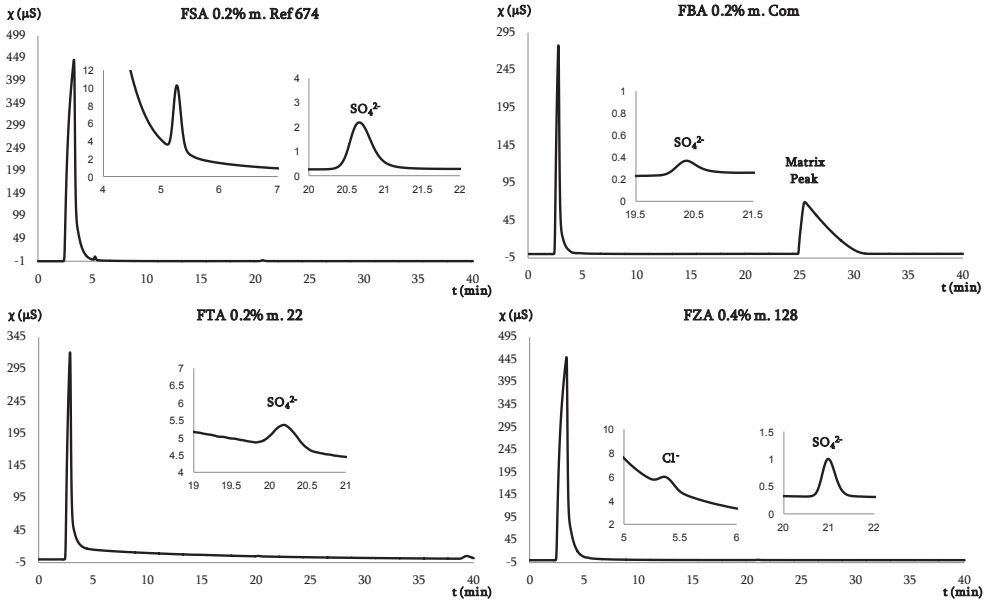

Figure 2. *Chromatograms of FSA, FBA, FTA and FZA samples spiked with the inorganic anions studied at 1.25 mg/L obtained with the optimum chromatographic conditions.*

The calculated concentrations expressed as mean value and their statistical interval of confidence and RSD% are displayed in Tables 5 – 8.

Table 5 *Mean concentrations values of FSA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Samples (0.2%)	Hexafluorosilicic Acid (FSA)•											
	Chloride			Bromide			Nitrate			Sulphate		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Si. Dto 208	9.83	0.09	0.39	n.d.	-	-	n.d.	-	-	9.99	0.08	0.31
Si. Op 73	n.d.	-	-	n.d.	-	-	n.d.	-	-	1.48	0.04	1.09
Si. Ens 4	<0.25	-	-	n.d.	-	-	n.d.	-	-	1.18	0.02	0.70
Si. Ref 674	5.81	0.05	0.38	n.d.	-	-	n.d.	-	-	5.3	0.2	1.67
Si. Com	n.a.	-	-	0.33	0.02	2.16	<0.26	-	-	<0.16	-	-
Si. 1/08	<0.25	-	-	n.d.	-	-	<0.26	-	-	0.32	0.01	1.40
Si. 2/08	n.d.	-	-	n.d.	-	-	n.d.	-	-	<0.16	-	-
Si. 3/08	n.d.	-	-	n.d.	-	-	n.d.	-	-	<0.16	-	-

•n.a. = Not applicable, the result is out of linear range; n.d. = Not detectable.

Table 6 *Mean concentrations values of FBA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Tetrafluoroboric Acid (FBA)

Sample (0.2%)	Chloride			Bromide			Nitrate			Sulphate		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
B. 6/11	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.42	0.04	4.24
B. 7/11	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.23	0.02	3.15
B. 8/11	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.24	0.07	11.22
B. 1/12	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.43	0.05	4.44
B. 2/12	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.42	0.04	3.55
B. 3/12	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.44	0.05	5.06
B. 4/12	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.44	0.06	5.19
B. 5/12	<0.25	-	-	n.d.	-	-	<0.25	-	-	0.5	0.1	11.40
B. 805	<0.25	-	-	n.d.	-	-	n.d.	-	-	0.31	0.05	7.17
B. 328	<0.25	-	-	n.d.	-	-	n.d.	-	-	0.39	0.06	6.55
B. com	<0.25	-	-	n.d.	-	-	n.d.	-	-	0.51	0.09	6.81
B. com2	<0.25	-	-	n.d.	-	-	n.d.	-	-	<0.15	-	-

•n.a. = Not applicable, the result is out of linear range; n.d. = Not detectable.

Table 7 *Mean concentrations values of FTA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Hexafluorotitanic Acid (FTA)

Sample (0.2%)	Chloride			Bromide			Nitrate			Sulphate		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Ti. 4	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.87	0.09	3.99
Ti. 5	<0.25	-	-	n.d.	-	-	n.d.	-	-	0.6	0.1	8.43
Ti. 6	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.3	0.1	14.65
Ti. 17	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.249	0.007	1.19
Ti. 20	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.21	0.08	13.44
Ti. 21	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.20	0.08	13.76
Ti. 22	n.d.	-	-	n.d.	-	-	n.d.	-	-	1.5	0.2	6.00
Ti. 23	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.5	0.1	9.38
Ti. 511	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.53	0.08	6.34
Ti. com	n.d.	-	-	n.d.	-	-	n.d.	-	-	0.3	0.1	13.66
Ti. Com 2	<0.25	-	-	n.d.	-	-	n.d.	-	-	0.9	0.1	4.93

•n.a. = Not applicable, the result is out of linear range; n.d. = Not detectable.

Table 8 *Mean concentrations values of FZA samples obtained from three replicates and expressed as mean ± t*s/√N where t is the student factor, N is the number of replicates and s is the standard deviation at a confidence level of 95%.*

Hexafluorozirconic Acid (FZA)•

Samples (0.4%)	Chloride			Bromide			Nitrate			Sulphate		
	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)	c (mg/L)	±s*t/√N	RSD (%)
Zr. 1	<0.26	-	-	n.d.	-	-	<0.28	-	-	3.9	0.04	0.38
Zr. 2	<0.26	-	-	n.d.	-	-	<0.28	-	-	3.1	0.07	0.87
Zr. 3	n.d.	-	-	<0.25	-	-	<0.28	-	-	2.7	0.3	4.67
Zr. 14	n.d.	-	-	n.d.	-	-	<0.28	-	-	2.5	0.6	10.23
Zr. 15	0.34	0.01	3.50	n.d.	-	-	<0.28	-	-	0.29	0.02	3
Zr. 16	4.72	0.07	0.63	n.d.	-	-	<0.28	-	-	n.a.	-	-
Zr. 24	0.3	0.01	1.73	n.d.	-	-	<0.28	-	-	0.42	0.01	1.16
Zr. 25	0.45	0.02	1.42	n.d.	-	-	n.d.	-	-	1.26	0.05	1.71
Zr. 127	0.45	0.01	1.04	n.d.	-	-	n.d.	-	-	2.53	0.09	1.45
Zr. 128	0.55	0.02	1.61	n.d.	-	-	n.d.	-	-	1.65	0.09	2.13
Zr. 129	0.49	0.01	0.93	n.d.	-	-	n.d.	-	-	0.51	0.01	1.12
Zr. 130	0.48	0.03	2.25	n.d.	-	-	<0.28	-	-	1.12	0.03	1.13
Zr.135	0.3	0.02	3.25	n.d.	-	-	n.d.	-	-	0.27	0.01	1.9
Zr. com	n.a.	-	-	n.d.	-	-	<0.28	-	-	n.a.	-	-
Zr. com 2	5.89	0.08	0.56	n.d.	-	-	<0.28	-	-	4.9	0.2	1.7

•n.a. = Not applicable, the result is out of linear range; n.d. = Not detectable.

The comparison of sample concentration results obtained by the method developed and the isocratic IC method previously reported for inorganic anions in the studied matrixes was difficult due to the low number of samples analyzed by both methods. Chloride, bromide and nitrate concentration values were not able to be compared due to the low concentration values obtained for samples of all the acids analyzed. Sulphate concentration values obtained for all comparable samples did not show significant differences for a significance level of 0.01.

The representation of sample concentration data of gradient IC method versus those of isocratic one for 12 samples provided a regression equation with a slope of 1.0005, an intercept of 0.1010 and a correlation coefficient of 0.9971, being these values not far from 1 and 0 respectively, what demonstrates the trueness for sulphate of the questioned method in FSA, FBA, FTA and FZA.

Chromatographic profiles obtained for commercial corresponding salts at the same purity percentage of the acids are shown in Figure 3.

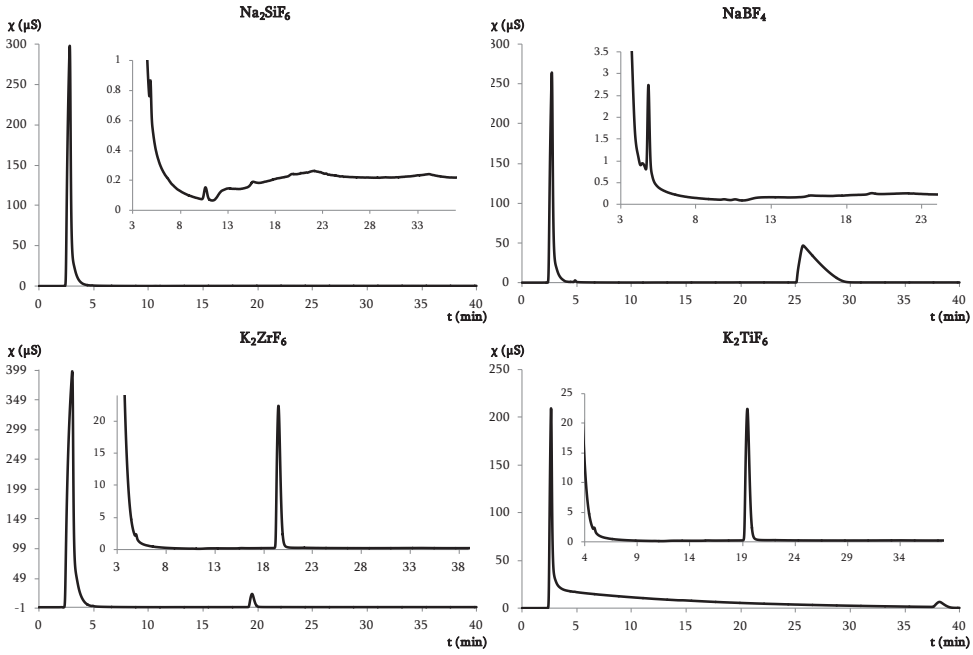

Figure 3. *Chromatograms of the diluted salt solutions corresponding to FSA (0.2%), FBA (0.2%), FTA (0.2%) and FZA (0.4%) obtained with the optimum chromatographic conditions.*

These profiles demonstrate the applicability of the ion chromatography to the quality control of mentioned salts.

IVc.3.4. Discussion

For the first time the gradient ion chromatographic method developed allows the quantitative chloride determination in all of the studied samples free of interferences from fluorinated species. Moreover, it is worthy to mention that sulphate concentration values did not show significant differences compared to those results obtained with isocratic ion chromatographic method.

Phosphate determination is still not possible, due to phenomena earlier described (Mercurio-Cason 1986), in spite of having been able to build calibration curves in FSA and FZA from 0.25 to 5.0 mg/L.

Gradient ion chromatographic method developed spends 40 minutes to perform the complete analysis of the anionic impurities for the industrial quality control procedure, including the stabilization of baseline in FTA matrix, which shortens the analysis time necessary in isocratic elution mode.

The developed method for gradient based on the use of hydroxide eluent presents slightly lower LOQs than the ion chromatographic method previously developed, which is based on the use of carbonate as eluent. The explanation of this fact is that the use of hydroxides offers better sensitivity than carbonate-based eluents, as the former are converted to water by the suppressor, which results in low background conductance, whilst carbonate eluent suppression produces carbonic acid (De Borba 2004).

The developed methods do not provide the concentration value of some anions in all samples at dilution factor employed. However, this method is useful to cover the needs of the fluorine industry since the linear range covers the maximum concentration limits required for the different matrixes. FZA restriction for sulphate is 400 mg/L at 45% (2.67 mg/L at 0.4%) and 100 mg/L for FTA (0.40 mg/L at 0.2%) whilst for chloride at real purity of samples the restriction is 200 mg/L (1.78 mg/L at 0.4% of FZA, 0.8 mg/L at 0.2% of FTA, 1.6 mg/L at 0.2% of FSA and 0.67 mg/L at 0.2% for FBA).

IVc.4. CONCLUSIONS

A single gradient ion chromatographic method allows the simultaneous determination of chloride, bromide, nitrate and sulphate impurities in four different matrixes with huge concentration of fluorinated species, FSA, FBA, FTA and FZA.

The easily transferable and low cost ion chromatographic method developed solves the industrial problem and represents a green alternative in the quality control. On the one hand, it does require neither multidimensional ion

chromatography techniques nor complex sample-treatment. Therefore, the equipment necessary is not expensive. On the other hand, the reagents consumption and residues generation are minimum providing savings. This agrees with cost-benefits criteria necessary to apply the developed method to quality control analysis in industrial laboratories (King 2003).

This fully automated quantitative method developed does not suffer from the matrix interferences associated with difficulties in the final point visualization (volumetry) and co-precipitation problems (gravimetry) observed in the chemical methods currently used for anionic impurities analysis in this kind of acids.

The proposed IC method offers some advantages against separation methods previously applied to these matrixes (Ayarza 2012, Ayarza 2014), CE and IC methods respectively. Advantages versus isocratic ion chromatographic method are: higher number of analytes able to be determined, larger linear range and better repeatability values in the majority data for the three concentration levels assayed. Furthermore, the reduction of total analysis time in case of FTA is other advantage of the proposed method.

The ion chromatographic method developed offers the possibility of being applied to the analysis of other inorganic fluorinated products, such as the corresponding salts as sodium hexafluorosilicate, sodium tetrafluoroborate, potassium hexafluorozirconate, and potassium hexafluorotitanate. However, deeper research needs to be performed.

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DETERMINATION OF SOME HERBICIDES IN SURFACE WATER BY ION CHROMATOGRAPHY-ELECTROSPRAY MASS SPECTROMETRY AND POST COLUMN ADDITION OF ACETONITRILE

Chapter V

Chapter V- DETERMINATION OF SOME HERBICIDES IN SURFACE WATER BY ION CHROMATOGRAPHY-ELECTROSPRAY MASS SPECTROMETRY AND POST COLUMN ADDITION OF ACETONITRILE

V.1. INTRODUCTION

The growth in population in recent decades has increased the need to produce more food in progressively shorter deadlines. Farmers have been forced to find methods to improve the performance of their production and to eliminate potential losses. Currently, the application of pesticides is being used worldwide for satisfying that production demand by successful performance of their harvest.

Herbicides are an important group of agricultural pesticides that every year increases their volume of use, replacing mechanical tillage operations in field. As the less selective the pesticides are the more effectiveness they have, a widespread use of non-selective herbicides has been observed. Glyphosate (GLYP), glufosinate (GLUF) and bialaphos (BIAL) belong to this kind of herbicides.

GLYP is an herbicide with a broad spectrum and a foliar systemic action. Into the plant, it is hardly metabolized, being accumulated until it causes the death of plants. The only metabolite is the amino methyl phosphonic acid (AMPA). GLYP is assigned to a toxicity class III by US Environmental Protection Agency (EPA) [1] and World Health Organization (WHO) [2, 3]. Toxicity and effects of GLYP in health are very controversial topics since there are a huge number of publications both for and against it. In spite of this, GLYP is the active substance of Roundup®, one of the still most sold herbicides in the world.

GLUF or its ammonium salt is an active ingredient in several non-selective systemic herbicides containing the active ingredient phosphinothricin. The action of GLUF results in an increase in concentrations of ammonia leading to cell death. GLUF also inhibits glutamine synthase in animals [4]. Recent toxicological studies have shown that GLUF causes neurological, respiratory, and gastrointestinal damage as well as birth defects in mammals and humans. It was also found to be toxic for reproduction [5] and was included in a biocide ban proposed by the Swedish Chemicals Agency [6] and approved by the European Parliament on January 13, 2009 [7]. In Europe, it will be prohibited on October 1st 2017. In the meantime, products based on this substance have

been marketed in 20 European countries, including Germany, Belgium, Spain and the Netherlands. The main metabolite of GLUF is 3-[hydroxy(methyl)phosphinoyl]propionic acid (MPPA).

Lastly, BIAL is an analog of glutamic acid. It is used as herbicide but it has also antibacterial and antifungal activity [8]. In microorganisms and plants, it is converted in the degradation product phosphinothricin, which acts in the same way as GLUF [9]. The half life time of this metabolite is 5 h so it is not included in this study.

The presence of pesticide residues in the environment, the agricultural workers exposition and the public concern about their possible toxic effects have forced official international institutions to establish maximum allowable concentration levels of these chemicals. The European Union has strict legislation on the occurrence of pesticides in water intended for human consumption. Maximum concentration of one pesticide should not exceed 0.1 μg/L while the sum of all pesticides must be below 0.5 μg/L [10, 11].

These facts show that it is important to determine the concentration of the three herbicides mentioned above in environmental samples and their main metabolites, apart from phosphinothricin. Therefore analytes of interest in the current work are GLYP, GLUF, BIAL, AMPA and MPPA.

Different methods for the determination of the mentioned compounds and its metabolites have been found in bibliography, most of them for GLYP and AMPA. Techniques employed up to now are thin layer chromatography (TLC), colorimetry [12], polarography [13], enzyme-linked inmunosorbent assay (ELISA) [14-16], capillary electrophoresis (CE) [17-21], ion chromatography (IC) [22-27], liquid chromatography (LC) [28-31], gas chromatography (GC) [32-38], inductively coupled plasma (ICP) [39-42] and even nuclear magnetic resonance (NMR) [43]. Within these techniques, liquid chromatography is predominantly used, principally coupled to UV, fluorescent and MS detectors. One of the main drawbacks of liquid chromatography applied to pesticides studies is the need of an extensive sample treatment, which frequently implies derivatization and/or preconcentration. In contrast, ion chromatography has shown to be suitable for the separation and determination of GLYP and AMPA

without intricate sample treatment steps. Marques et al., developed an IC method coupled to a suppressed conductivity detector [44] while Bauer et al. [24] used an electrospray interface mass spectrometry detector. Both works describe the analysis of GLYP and AMPA at concentration levels of 1 μg/L in water samples. However, these methods are not sensitive enough to determine these compounds at the levels required by law. The sensitivity problem can be overcome by the use of organic solvents. The post column addition of organic solvent enhances the analytes S/N in the ESI interface by increasing the generation of gas-phase analyte ions and/or the rate of neutral solvent evaporation what enhances the ESI-MS response [45]. For this reason, organic solvent effect was taken into account in this paper. Only acetonitrile (ACN) was taken into account based on results obtained by Mawhinney et al. [46].

The aim of this work is the development of a method for the simultaneous quantification of three herbicides GLYP, GLUF and BIAL and their main metabolites AMPA and MPPA in surface water samples by ion chromatography-ESI-mass spectrometry. Post column addition of an organic solvent was evaluated as a tactic to overcome problems associated to the use of a non volatile mobile phase with high concentration of ionic species.

V.2. EXPERIMENTAL

V.2.1. Standards and Materials

Ultrapure water (18.2 MΩcm) purified with a Milli-Q Advantage system (Millipore, Milford, Massachusetts) was used for eluent generation and working solutions preparation.

AMPA: ((Aminomethyl)phosphonic acid)), GLYP: (N-phosphonomethyl)glycine, GLUF: ((RS)-2-Amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid) and MPPA: (3-(hydroxy-methylphosphinyl)propionic acid)) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands) and were of pestanal® quality. BIAL: ((2S)-2-[[(2S)-2-[[(2S)-2-amino-4-

[hydroxy(methyl)phosphoryl]butanoyl]amino]propanoyl]amino]propanoic acid) was obtained from Apollo Scientific, (Cheshire, United Kingdom).

Weighing of analytes for standards preparation was made on a Sartorious ME 254 S analytical balance (Goettingen, Germany).

Stock solutions (250 mg/L) of each compound were prepared by dissolving them in ultrapure water. The working solutions of GLYP, GLUF, BIAL and their metabolites were freshly prepared by dilution with ultrapure water from their stock solutions.

Anion stock solutions (F^-, Br^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-} at 1000 mg/L each) were obtained from Merck (Darmstadt, Germany) and used for the preparation of working solutions for the interferences study.

All solutions were prepared in PFA flasks purchased from Brand (Wertheim Germany) and were stored at 4 °C. Solutions were found to be stable for at least 6 months when these were kept in those conditions.

ACN used as post column solvent was ULC/MS quality obtained from Biolsolve Chimie SARL (Dieuze, France).

V.2.2. Instrumentation

A Dionex ICS-5000 Reagent-Free Dual (analytical and capillary) ion chromatograph (Sunnyvale, California) was coupled to a MSQ Plus Mass Detector (Thermo Fisher Scientific Inc., Waltham, Massachusetts). The IC system consisted of a gradient pump with on line degassing, an EG50 electrolytic eluent generator producing extra pure KOH eluent, a CR-ATC (Continuously Regenerated Anion Trap Column) for eluent purification, an ASRS-II micro-membrane suppressor in external water mode and a thermostated conductivity detector. Chromeleon® 6.8 data management system was used for full control of the system and data treatment. Electrospray Ionization was used in negative mode for the detection of the ionized analytes. Xcalibur™ version 1.4 software was used for MS management. Samples were kept in plastic vials and injected using the autosampler (Dionex AS).

A Dionex ICS-2500 ion chromatograph was used to deliver ACN as a post column solvent with a flow-rate of 0.3 mL/min to perform the post-column addition of the organic solvent. The coupling between the IC column outlet and the MS inlet was obtained by a static mixing Tee from Upchurch Scientific Ltd (Silsden, United Kingdom). Several restrictor coils were used in the ACN flow-path to obtain a back pressure of about 1500 psi.

V.2.3. Mass Spectrometric Detection

Mass spectrometric detection was optimized by one variable at the same time (OVAT) method, keeping some parameters constant at default values (probe temperature: 400 °C; nitrogen nebulizer gas pressure: 80 psi; needle voltage: 1.5 kV and cone voltage: 50 V). Single negatively charged deprotonated molecule $[M-H]^-$ were selected for each compound using full scan mode by independently injection of a 500 µL loop with standard solutions at a concentration of 100 µg/L.

The ion transmission process was optimized by studying cone and needle voltages parameters independently for each analyte. Cone voltages ranging between 20 and 120 V were studied with 20 V increments, while the needle voltage was changed from 1 to 5 kV with 0.5 kV increments. The cone was cleaned after every sample series. In the ionization process optimization, probe temperature and organic solvent addition were the parameters studied. Probe temperature was varied from 400 to 650 °C with 50 °C increments. Addition of ACN ranging from 0 to 70% of organic solvent was examined. Selected ion monitoring (SIM) mode with a span and dwell time at 0.3 amu and 0.8 s respectively was used for the quantification.

V.2.4. Chromatographic Conditions Optimization

Chromatographic separation was performed using a Dionex IonPac AS18-4µm (2 mm × 150 mm) column, packed with quaternary ammonium anion exchange resin. Dionex IonPac AS24 (2 mm × 250 mm), IonPac AS24A (2 mm × 250 mm)

and AS19 (2 mm × 250 mm) columns were also used during the chromatographic optimization study at 30 ºC. The analysis was carried out without a guard column. A flow rate of 0.2 mL/min was used. Finally, the loop size effect on the sensitivity was studied. Different injection volumes from 10 to 250 µL were tested.

V.2.5. Organic Solvent Effect

ACN was added post column at different flow-rates to obtain different organic percentages in the eluent stream in order to improve sensitivity. The flow-rate of the IC was kept constant at 0.2 mL/min. In this way H_2O:ACN ratios of 30:70, 40:60, 50:50 and 60:40 were investigated.

The gain in sensitivity for all analytes was determined using standard solutions and calculated by the ratio of $Area_{ACN}/Area_{No\ ACN}$.

V.2.6. Method Validation

The quantitative determination method for pesticides in surface water using ion chromatography-mass spectrometry was evaluated. The linear concentration ranges and the experimental detection and quantification limits were calculated from blank solutions spiked with solutions containing concentrations from 0.02 to 1.5 µg/L of each analyte. The limit of quantification was calculated as 10 times S/N and set as first point of the linear range, while the detection limit was established as a concentration which provided a chromatographic signal equal to 3 times S/N. External calibration mode using ultrapure water as matrix was used. Linear regression method was applied to the chromatographic peak area–concentration data.

Intra-day repeatability and accuracy of the method were calculated for three concentrations levels: low, medium and high within the calibration curve range in ultrapure, tap and surface waters. These low medium and high levels were fixed at 0.5, 1.0 and 1.5 µg/L, respectively. Accuracy was calculated spiking ultrapure, tap and surface water with known concentrations of analytes and

interpolating the area obtained for them in the calibration curve built on ultrapure water.

Repeatability was evaluated using RSD% values and RE% values were used to express the accuracy.

V.2.7. Matrix Effect

In every samples series a standard solution containing the analytes of interest at a concentration of 0.9 μg/L was injected after the analysis of 10 samples to check the response of the MS. The absence of sample carryover was checked by injecting ultrapure water between samples.

V.2.8. Samples Analysis

About 80 surface water samples from points all over the Netherlands were determined. Sampling was carried out in plastic bottles and samples were directly filtered in the laboratory by 0.45 μm Polydisc GW (polyamide) from Whatman™ ('s-Hertogenbosch, The Netherlands) in order to avoid possible adsorption of analytes on the suspended solids and humic matter of the sample. Samples were stored at -20 °C and analyzed directly after defrosting with the developed ion chromatography-mass spectrometric method.

Concentrations of the surface water samples analysed were calculated by interpolation of the chromatographic peak areas obtained in the calibration curve.

V.3. RESULTS

V.3.1. Mass Spectrometric Detection

The most abundant ions for MS detection were found at m/z (a.m.u.) 110.1 for AMPA, 151.1 for MPPA, 168.1 for GLYP, 180.2 for GLUF and 322.3 for BIAL, representing the deprotonated molecules $[M-H]^-$.

Evidences to choose optimum values for parameters related with ion transmission are shown in Figure 1.

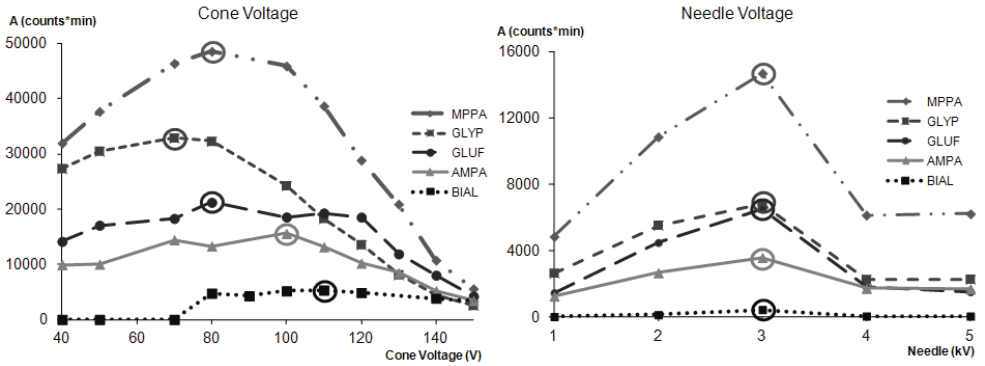

Figure 1. *Influence of cone and needle voltages on chromatographic peak areas obtained for the different analytes studied. The optimum conditions for each analyte are highlighted with a circle.*

Cone voltages were set at 100 V for AMPA, 80 V for MPPA, 70 V for GLYP, 80 V for GLUF and 110 V for BIAL, being these values which provide the highest chromatographic area for each compound while a unique needle voltage of 3 kV was chosen for all compounds. A compromise value for the probe temperature for all analytes, which provided the highest chromatographic area, was 500 °C.

V.3.2. Chromatographic Conditions

First step in the chromatographic system optimization was the selection of column used. Columns tested were AS18-4µm, AS24A, AS24 and AS19. The complete resolution of the chromatographic peaks of pesticides was pursued in the optimization process. The optimization of the elution gradient was made in order to achieve that the most abundant inorganic anions expected in surface waters, such as chloride and sulphate eluted at retention times far from the pesticides. Thereby, the possible matrix effect of these anions is reduced before reaching the detector. The IonPac AS18-4µm anion-exchange column was the only column that meets the separation criteria fixed and was used as analytical separation column. The KOH gradient (0-10 min isocratic 12 mM, 10-20 min to

30 mM concave upward, 20-29 min from 30-50 mM linearly) provided the best chromatographic separation between the pesticides and the common anions present in surface waters. During the gradient program the suppressor current used was proportional to the highest mobile phase concentration.

Increments in injection volume increase the sensitivity as well as decrease column life. Large amount of sample during the injection provokes overloading of column which generates double peaks due to the double separation caused by the length of the injection. This effect was observed at the maximum loop size tested, 250 µL. The use of lower loop sizes makes this effect negligible, so 100 µL was selected as optimum injection volume.

V.3.3. Organic Solvent Effect

Post column addition of ACN to the main flow from IC not only improves the MS response by assisting the desolvation process but it also dilutes the analytes in the total stream to the MS detector. The best results due to the compensation of these phenomena were obtained with a 40:60 H_2O:ACN ratio. This ratio increases the sensitivity obtained with ESI mass spectrometric detection for analytes studied and improves the gaussian shape of the chromatographic peaks.

The gain in sensitivity keeps constant at values of 2.85 for AMPA, 4.2 for MPPA, 6.15 for GLYP, 5.25 for GLUF and 3.0 BIAL, taking into account the dilution factor introduced with the organic solvent addition. Under these conditions the method validation was performed.

V.3.4. Method Validation

Linear concentration ranges and calibration curves expressions together with experimental detection and quantification limits, as first point of the linear range, for analytes are collected in Table 1.

Table 1 *Quality parameters of the developed ion chromatography- mass spectrometric method for the herbicides studied and their degradation products.*

	AMPA	MPPA	GLYP	GLUF	BIAL
Linear Range (µg/L)	0.12 – 1.5	0.06 – 1.5	0.06 – 1.5	0.12 – 1.5	0.4 – 1.5
Linear regression expressions	Y = 1050.5x -40.121	Y = 6847.6x -479.19	Y = 4666.7x -125.09	Y = 4285.5x -187.8	Y = 1575.8x -319.1
r^2	0.9909	0.9949	0.9974	0.9943	0.9899
LOD (S/N=3)	0.06	0.02	0.02	0.06	0.12

In Figure 2 the differences observed for chromatograms obtained for blank samples and solutions at LOQ concentration levels are shown at optimum chromatographic conditions.

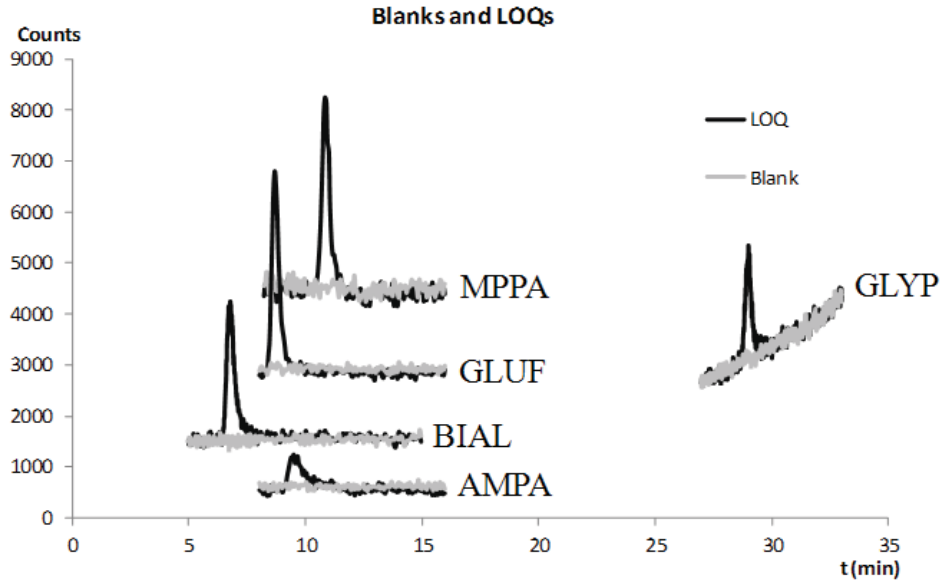

Figure 2. *Zoom of the chromatographic areas of GLYP, GLUF, BIAL, AMPA and MPPA obtained for blank sample and one sample spiked at LOQ level for each analyte.*

Intra-day repeatability and accuracy data of the method for three concentrations levels in ultrapure, tap and surfaces waters are listed in Table 2.

Table 2 *Intra-day repeatability and accuracy results for three concentrations levels in ultrapure, tap and surface water.*

		Surface Water			Tap Water			Ultrapure Water		
		RSD %		RE %	RSD %		RE %	RSD %		RE %
		t	A		t	A		t	A	
Low	AMPA	0.54	4.58	1.05	0.00	2.60	-10.12	0.87	6.79	12.75
	MPPA	0.30	6.10	7.88	0.00	11.28	-12.16	0.00	2.95	9.36
	GLYP	0.03	2.43	-5.25	0.03	0.37	2.38	0.07	2.20	-1.01
	GLUF	0.00	10.74	2.30	0.00	1.51	-3.50	0.32	3.56	-1.72
	BIAL	n.d.	n.d.	n.d.	0.39	7.95	n.d.	0.63	2.54	-9.12
Medium	AMPA	2.64	-8.77	0.31	2.98	-0.96	0.36	0.36	4.52	-11.02
	MPPA	1.47	-1.54	0.29	0.18	-3.84	0.00	0.00	2.40	2.68
	GLYP	1.03	-12.78	0.10	2.84	6.19	0.06	0.06	0.71	-2.27
	GLUF	0.33	7.34	0.25	0.62	-6.13	0.00	0.00	5.13	-11.02
	BIAL	n.d.	n.d.	0.11	0.57	n.d.	0.13	0.13	6.72	-19.34
High	AMPA	0.31	3.52	-1.67	0.31	1.95	-3.63	0.29	6.62	0.79
	MPPA	0.29	1.98	-5.62	0.00	1.37	1.95	0.00	3.34	4.44
	GLYP	0.05	2.10	-13.05	0.07	1.06	13.23	0.07	1.05	-1.20
	GLUF	0.25	4.10	8.21	0.00	0.95	-7.13	0.00	1.94	3.04
	BIAL	0.26	1.33	n.d.	0.11	1.19	n.d.	0.36	3.77	-22.27

n.d.= Not detectable

Inter-day repeatability at three concentrations levels are collected in Table 3.

Table 3 *Inter-day repeatability data of five calibrates at three concentrations levels.*

	Low RSD %		Medium RSD %		High RSD %	
	t	A	t	A	t	A
AMPA	2.04	10.47	0.67	10.45	0.92	8.58
MPPA	0.73	8.18	0.73	4.14	0.73	4.77
GLYP	0.09	5.16	0.07	10.43	0.08	10.48
GLUF	0.54	7.27	0.63	6.85	0.39	6.62
BIAL	0.56	9.46	0.22	5.92	0.6	9.74

Results obtained for BIAL in surface water forced us to discard the analysis of this anion.

V.3.5. Matrix Effect

The high concentration levels of interferent anions present in surface water is a challenge to overcome in the determination of pesticides in this kind of water. The average concentration of chloride and sulphate ions in samples analyzed was 112 ± 24 mg/L. Several precautions are taken to obtain reliable concentration results for the studied compounds in such heavy matrixes.

Standard solution at 0.9 µg/L injected after 10 analyses gave information about the reduction of the response of the MS. Therefore, as first precaution, the cone of the mass spectrometer was cleaned after each sample series. Secondly, after about 500 injections also the extraction cone and the RF lens were cleaned. In this way, repeatability was assured day to day. Besides, the injection of ultrapure water between samples did provide chromatograms without peaks what corroborated the absence of sample carryover.

Another consequence observed due to the matrix was related with the chromatographic peak shape for AMPA. It is a tailed peak and suffers from shifts of retention time up to 2 min when real samples are injected into the column. An explanation for this fact is given in this point. Injections which

consume 10% of the column capacity might generate anions elution 10% earlier in the retention time within the chromatogram. The column used has a capacity of 45 μeq. Assuming an injection of about 100 mg/L anions (Cl^- and SO_4^{2-}) due to the average composition of surface water samples, around 10 μeq are injected. This is about 20% of the total capacity of the column, therefore a 20% decrease in retention time is expected because of this phenomenon. In this research, shifts in the retention time of AMPA were observed and matches the explanation given above. This could be avoided with lower injection volumes what will require more sensitive detection to get the sensitivity required, like an MS/MS detection system.

After being taken the proper precautions cited above, the developed method is suitable to apply to all samples analyzed. Within samples analyzed, it is worthwhile to mention that two samples with about 450 mg/L of salts gave successful results, while only a sample containing more than 5000 mg/L of salts could not be analyzed.

V.3.6. Samples Analysis

River Meuse and the river Meuse basin

Samples were collected from January until September 2013. At Eijsden, where the river Meuse flows into the Netherlands, 22 samples were collected. In winter, the concentration of AMPA was below 0.3 μg/L while in the course of spring and summer it gradually raised to 1.77 μg/L. The mean concentration of AMPA at Eijsden was 0.80 μg/L. The same trend was observed for GLYP but at a five- to tenfold lower level. The mean concentration of GLYP at Eijsden was 0.14 μg/L and was roughly the same as in 2011, when the mean was 0.18 μg/L [47]. Figure 3a displays the values and trends of GLYP, GLUF, AMPA and MPPA found in samples taken in Eijsden. These values are consistent with the kinetic of the GLYP since it fastly degradates in AMPA [48] and present seasonality probably due to its use in agriculture.

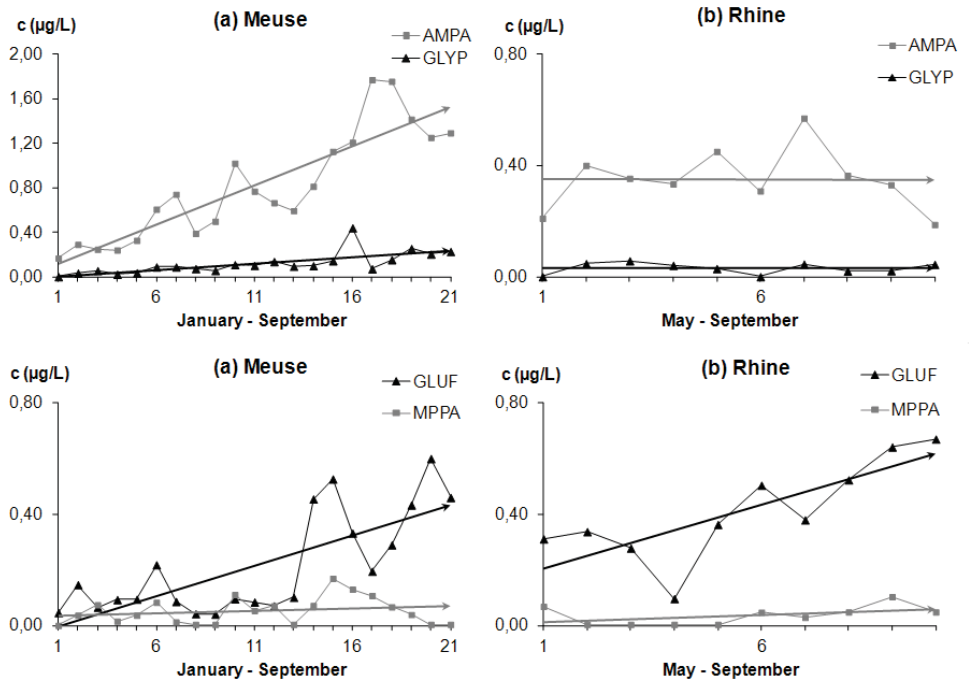

Figure 3. *Concentration values and trends of GLYP, GLUF, AMPA and MPPA found in samples from (a) Eijsden and (b) Lobith, corresponding to the entrance of Meuse and Rhine River, respectively, into the Netherlands.*

For MPPA and GLUF, 14 and 19 samples respectively showed concentrations above the LOD. The mean concentration in Eijsden of MPPA was 0.09 µg/L and of GLUF was 0.39 µg/L. For GLUF the same trend was observed as for AMPA, with concentrations ranging from lower than LOD to 0.60 µg/L.

Another 21 samples were collected in the Dutch river basin of the Meuse. The data showed that the concentrations of pesticides increased along the course of the river in the Netherlands, up to 2.78 µg/L for AMPA and 1.09 µg/L for GLUF. That indicates that these compounds are being introduced in the Netherlands.

It is worthwhile to mention that for AMPA all samples in the river Meuse and the river basin showed concentrations higher than 0.1 µg/L, for GLYP 54%, for GLUF 66% and for MPPA just 10% of the samples. The data for AMPA and GLYP are in agreement with results of 2012 Dutch surveys [49]. Data for all

samples collected in the river Meuse and the river basin are listed in Tables 4 and 6.

Table 4 *Concentration values of pesticides GLYP and GLUF, their metabolites AMPA and MPPA and the most common inorganic anions chloride and sulphate for surface water samples collected in different points in the Meuse River and Meuse River basin.*

	Meuse					
Meuse locations	AMPA	GLYP	MPPA	GLUF	Chloride	Sulphate
	c (µg/L)				c (mg/L)	
BELFBVN 1	0.40	0.06	<LOQ	0.27	39	42
BELFBVN 2	1.73	0.14	0.16	0.35	51	64
BRAKL	0.82	0.04	0.06	0.39	40	40
EIJSDPTN 1	0.17	<LOD	<LOD	<LOQ	17	22
EIJSDPTN 2	0.29	<LOQ	<LOQ	0.15	36	31
EIJSDPTN 3	0.24	<LOQ	0.07	<LOQ	30	25
EIJSDPTN 4	0.24	<LOQ	<LOQ	<LOQ	40	30
EIJSDPTN 5	0.33	<LOQ	<LOQ	<LOQ	30	33
EIJSDPTN 6	0.61	0.09	0.08	0.22	33	37
EIJSDPTN 7	0.74	0.09	<LOD	<LOQ	23	29
EIJSDPTN 8	0.39	0.07	<LOD	<LOD	26	29
EIJSDPTN 9	0.50	0.06	<LOD	<LOD	18	23
EIJSDPTN 10	1.02	0.11	0.11	<LOQ	27	28
EIJSDPTN 11	0.77	0.10	<LOQ	<LOQ	32	35
EIJSDPTN 12	0.67	0.14	0.07	<LOQ	30	34
EIJSDPTN 13	0.59	0.09	<LOD	<LOQ	23	29
EIJSDPTN 14	0.81	0.10	0.07	0.45	39	37
EIJSDPTN 15	1.13	0.14	0.17	0.53	39	50
EIJSDPTN 16	1.21	0.44	0.13	0.33	53	53
EIJSDPTN 17	1.77	0.07	0.11	0.19	43	62
EIJSDPTN 18	1.76	0.15	0.07	0.29	53	56
EIJSDPTN 19	1.42	0.25	0.04	0.43	51	62

Meuse locations	Meuse (continuation)					
	AMPA	GLYP	MPPA	GLUF	Chloride	Sulphate
	c (µg/L)				c (mg/L)	
EIJSDPTN 20	1.25	0.20	<LOD	0.60	76	60
EIJSDPTN 21	1.29	0.23	<LOD	0.46	67	60
EIJSDPTN 22	0.34	0.11	<LOD	0.59	53	50
HEEL 1	0.87	0.13	0.11	0.15	37	36
HEEL 2	1.15	0.06	0.15	0.50	37	42
HEEL 3	1.63	0.21	0.07	0.28	54	59
NEDWT 1	0.64	0.16	0.17	0.51	32	32
NEDWT 2	0.97	0.20	0.31	0.62	46	48
NEDWT 3	1.17	0.27	<LOQ	0.41	54	58
STEVWT 1	0.71	0.07	<LOD	<LOQ	24	28
STEVWT 2	0.61	0.06	<LOQ	0.23	25	32
STEVWT 3	2.00	0.15	0.11	0.56	47	57
STEVWT 4	2.78	0.17	<LOQ	0.25	51	70

River Rhine and the river Rhine basin

From the river Rhine most of the samples were collected after spring. At Lobith, where the Rhine flows into the Netherlands, 10 samples were collected. The mean concentration of AMPA at Lobith is 0.36 µg/L. The concentrations of GLYP and MPPA were at quantification level but for GLUF the mean concentration was 0.41 µg/L (range 0.10-0.67 µg/L). Figure 3b displays the values and trends of GLYP, GLUF, AMPA and MPPA found in samples taken in Lobith.

Another 29 samples were collected in the river Rhine basin. The data showed that only for AMPA the downstream concentration increased compared to the concentration at Lobith. The mean concentration of AMPA in the river Rhine basin was 0.78 µg/L and the highest concentration obtained was 1.86 µg/L.

For AMPA all samples in the river Rhine and the river basin showed concentrations above 0.1 µg/L. For GLYP this relates to 10%, for MPPA to 39% and for GLUF to 97% of the analyzed samples. The data for AMPA and GLYP

are in accordance to the results of Dutch surveys in 2012 [50]. Data for samples collected in the river Rhine and the river basin are listed in Tables 5 and 6.

Table 5 *Concentration values of pesticides GLYP and GLUF, their metabolites AMPA and MPPA and the most common inorganic anions chloride and sulphate for surface water samples collected in different points in the Rhine River and Rhine River basin.*

Rhine locations	AMPA	GLYP	MPPA	GLUF	Chloride	Sulphate
	c (μg/L)				c (mg/L)	
EEMDK23	1.14	0.15	0.14	0.27	77	52
GENMDN 1	1.03	0.09	0.08	0.20	61	47
GENMDN 2	0.96	0.09	0.14	0.16	56	45
GENMDN 3	0.99	0.12	0.15	0.29	55	45
GENMDN 4	1.67	0.08	0.16	0.22	58	40
GENMDN 5	1.86	0.08	0.10	0.25	59	43
GENMDN 6	1.71	0.13	0.14	0.22	58	41
GENMDN 7	1.02	0.07	0.16	0.16	70	44
KAMPN 1	0.36	<LOQ	<LOQ	0.28	58	46
KAMPN 2	0.35	<LOQ	0.07	0.08	36	31
KAMPN 3	0.31	<LOD	0.06	0.37	52	47
KAMPN 4	0.54	<LOQ	0.10	0.30	59	52
KAMPN 5	0.47	0.06	<LOQ	0.30	59	52
KAMPN 6	0.83	0.07	0.15	0.22	61	50
KAMPN 7	0.51	0.09	0.16	0.62	85	66
KETMWT	0.66	<LOD	<LOQ	0.31	75	62
LELSHVN 1	0.19	0.07	<LOQ	0.45	105	78
LELSHVN 2	0.15	<LOQ	0.09	0.70	115	85
LOBPTN 1	0.33	<LOD	0.07	0.31	52	37
LOBPTN 2	0.40	<LOQ	<LOD	0.34	47	43
LOBPTN 3	0.35	0.06	<LOD	0.28	46	39
LOBPTN 4	0.33	<LOQ	<LOD	0.10	32	35

Rhine (continuation)

Rhine locations	AMPA	GLYP	MPPA	GLUF	Chloride	Sulphate
	c (µg/L)				c (mg/L)	
LOBPTN 5	0.45	<LOQ	<LOD	0.36	57	47
LOBPTN 6	0.31	<LOD	<LOQ	0.50	66	51
LOBPTN 7	0.57	<LOQ	<LOQ	0.38	68	48
LOBPTN 8	0.36	<LOQ	<LOQ	0.52	78	58
LOBPTN 9	0.33	<LOQ	0.10	0.64	84	62
LOBPTN 10	0.19	<LOQ	<LOQ	0.67	72	67
RAMSDP	0.85	0.07	0.11	0.24	71	46
VURN 1	0.41	0.06	<LOQ	0.20	41	42
VURN 2	0.42	<LOD	0.09	0.59	95	68

Table 6 *Concentration values of pesticides GLYP and GLUF, their metabolites AMPA and MPPA and the most common inorganic anions chloride and sulphate for surface water samples collected in different points in which water coming from the Meuse River and Rhine River are mixed.*

Meuse and Rhine

Meuse and Rhine locations	AMPA	GLYP	MPPA	GLUF	Chloride	Sulphate
	c (µg/L)				c (mg/L)	
BOVSS	0.44	<LOQ	0.15	0.53	67	62
BRIENOD 1	0.27	<LOQ	0.09	0.48	44	43
BRIENOD 2	0.48	<LOQ	0.11	0.88	116	60
BRIENOD 3	0.44	<LOQ	0.15	1.09	155	73
PUTTHK 1	0.32	<LOQ	<LOD	0.24	50	45
PUTTHK 2	0.31	<LOQ	<LOD	<LOQ	33	33
PUTTHK 3	0.40	<LOQ	<LOQ	0.20	53	47
PUTTHK 4	0.64	0.06	0.09	0.25	59	57

From the data of the river Meuse and river Rhine it can be concluded that reference value marked by law as maximum tolerated one for pesticide in drinkable water, 0.1 µg/L was being exceeded very often.

Comparison of data with other laboratories

In order to prove the reliability of the developed method, 31 of our concentration results were compared with the results obtained by 2 other laboratories using different analytical methods.

One of the laboratories uses HPLC with Fluorescence (FL) detection after a derivatization step with Fluorenylmethyloxycarbonyl (FMOC). Results for AMPA and GLYP of 24 samples were compared with those obtained by this laboratory. The comparison of the results obtained by both methods was made in terms of recovery, defined as the percentage ratio between concentration given by developed method and by HPLC-FL. Mean recoveries of 88% for AMPA and 70% for GLYP were obtained.

Besides, another laboratory using an IC-MS/MS method without any sample clean-up analyzed the other 7 samples, collected in regional water bodies. Only AMPA results could be compared because GLYP was not analyzed by this laboratory. The mean recovery for AMPA was 87%.

These 7 samples were also analyzed using the developed method and results were obtained not only for AMPA, but for GLYP and GLUF.

Within these samples from the regional water bodies, it is worthy to mention two specific cases. In one particular sample AMPA was measured to be only 0.7 μg/L while GLYP was so high as 9.3 μg/L. This sample came from an effluent from a sewage treatment plant where GLYP concentrations might be very high, taking into account data previously reported in the Netherlands, [51, 52]. In another sample the concentration of AMPA was 4.1 μg/L and GLYP 1.4 μg/L. This sample turned out to be an overflow from sewerage.

In these 7 samples also GLUF was detected and the concentrations ranged from lower than LOD to 1.06 μg/L with a mean value of 0.59 μg/L.

V.4. CONCLUSIONS

An ion chromatography-ESI-mass spectrometric method with post column addition of ACN for the simultaneous quantification of three herbicides: GLYP,

GLUF and BIAL and two main metabolites AMPA and MPPA in surface water samples has been developed.

The IC-ESI-MS method does not require any sample treatment apart from filtration. This represents an important advantage with respect to liquid chromatographic methods which need preconcentration and/or derivatization steps.

Post column addition of ACN resulted in an increase in response by a factor of 2.85 for AMPA, 4.2 for MPPA, 6.15 for GLYP, 5.25 for GLUF and 3.0 for BIAL.

The IC-ESI-MS method gives limits of detection ranging from 0.02-0.12 μg/L for all analytes. This method is sensitive enough for the determination of these pesticides at concentrations required by legislation (0.1 μg/L) in different types of water.

The reliability of the method developed was demonstrated using AMPA and GLYP results for 31 water samples. Concentration results were confirmed by other analytical methods used in 2 laboratories.

The method has been successfully applied for the analysis of some herbicides in 80 samples of surface water from different parts of the Netherlands revealing a seasonality profile with higher concentration of pesticides in summer time than in spring.

A constant input of AMPA, GLYP and GLUF from the bordering countries, besides an increase in their concentration in the Netherlands in the river basins of the river Rhine and the river Meuse is observed. In none of the samples BIAL has been found. This fact denotes an environmental concern dealing with water quality which would require an international solution.

The concentration level of 0.1 μg/L was exceeded in all water samples for AMPA analysis, in a high percentage of samples for GLUF and in a low percentage for GLYP. Up to now no monitoring program for GLUF in the Netherlands is in operation.

V.5. ACKNOWLEDGEMENTS

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CONCLUSIONS

Chapter VI

Chapter VI- CONCLUSIONS

VI.1. CONCUSIONS

From the results obtained in this research work the following conclusions can be deduced.

1. The capillary electrophoretic method has been fully validated according to the requirements of Eurachem guide for the determination of fluoride, bromide, nitrite, nitrate, chloride, sulphate and phosphate in different types of water matrixes such as natural water, waste water and acid rain. This method has been implemented in SGIker´s Analytical Research Service (SCAB) for routine analysis of this kind of matrixes.

2. Capillary zone electrophoresis, first approach the determination of anionic impurities in hexafluorosilícico acid, allowed the analysis of chloride, bromide, nitrate and sulphate in this acid. However, several difficulties related to capillary life were obtained due to the tough matrix studied in spite of employing a water gap as a modification of the method.

3. Ion chromatography using isocratic elution mode can be also used to determine the inorganic anionic impurities: bromide, nitrate and sulphate in complex fluorinated matrixes.

4. Ion chromatography using gradient elution mode has shown the best results to solve the problem proposed by industrial laboratories, Derivados del Flúor S.A. This method has been successfully applied to the quality control of inorganic impurities, chloride, bromide, nitrate and sulphate in fluorinated acids, hexafluorosilicic acid, tetrafluoroboric acid hexafluorotitanic acid and hexafluorozirconic acid.

5. Instrumental approach to solve the determination of anionic impurities in fluorinated acids through the use of both capillary electrophoresis and ion chromatography has shown advantages versus the chemical methods used in industrial laboratories up to now.

6. Isocratic and gradient elution methods in ion chromatography open the application field to other inorganic fluorinated products such as the corresponding salts of the fluorinated studied acids.

7. A simple and fast ion chromatography coupled to electrospray mass spectrometric method has been successfully applied for the determination of some herbicides in surface water. This method represents an alternative to the established one which requires liquid chromatography with derivatization step.

Scientific discoveries are present in all areas of our life. For this reason, promoting research is necessary since the knowledge acquired in this way is useful for different fields: from designing bridges to slowing climate change, developing new technologies or solving practical problems. A usual way of researching is to apply the concept of research and development (R&D), which involves the presence of an appreciable element of novelty and the resolution of problems and uncertainties using scientific or technological means. Nowadays, companies need R&D to compete internationally in order to succeed. This competence requires to introduce the concept of quality in their processes and products. Quality involves different perceptions and meanings, although its objective is meeting the expectations and needs of customers. The assurance of this parameter is commonly carried out by quality control procedures.

Concretely, along this work, the research carried out has been focus on three tasks related with quality control procedures, being the main objective of this dissertation to employ analytical chemistry as a tool to provide solutions to different problems exposed by official and industrial laboratories, in order to improve the methods used up to now with the consequent economical and environmental benefits.

The main objective has been fulfilled through next sub-objectives: -analytical validation of a method for inorganic anion analysis in different kind of water by capillary ion electrophoresis; -determination of anionic impurities in fluorinated inorganic acids by both capillary electrophoresis and ion chromatography; and -determination of some herbicides in surface water by ion chromatography-electrospray mass spectrometry and post column addition of acetonitrile.

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