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TRABAJO FIN DE GRADO
GRADO EN QUÍMICA

APPLICATION OF ION CHROMATOGRAPHY IN DETERMINATION OF CARBOXYLIC ACIDS, INORGANIC ANIONS IN SELECTED DRUGS

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1-. INTRODUCTION AND OBJECTIVES

The main objective of this project is the analysis of inorganic anions and various organic acids from selected drugs by the use of suppressed ion chromatography with conductivity detector with different conditions and instruments focusing in the analysis of L-Ascorbic acid because of the potential therapeutic uses in different kind of treatments.

The carboxylic acids, are weak organic acids caracterised by having a caboxylic group which is formed by a C=O with a –OH also bonded to the same carbon. They can be easily found in our daily life, either way in a natural state or in artificial products. [1]

This can be easily found in the nature as in human made products; from short chains like methanoic acid, to really long carbon chains like the fatty acids which have a –COOH group at the end of the chain or humic acids; Naturally they appear from biogeochemical reactions produced by bacterias, algae, plants, animals and even dead biomass in the case of the humic acids. Some of the carboxylic acids even are utterly necessary for the healthy sustainment of the life, as for example the ascorbic acid also known as vitamin C.

The ascorbic acid is a highly soluble water carboxylic acid, which at room temperature can be found as a white fine powder. The term vitamin C is refered specifically when full or partial biological activity is exhibited by the L – ascorbic acid, including its derivates. These can be natural such as the ascorbyl palmiate with a 100% of relative activity or synthetic as the 6-deoxy-l-ascorbic acid with 33% relative activity. Since it can not be produced naturally by the human body, it is obtained primarily by dietary sources where it is found as L-ascorbic acid and L-dehydroascorbic acid.

L-Ascorbic acid is a widely used food additive with many functional roles, many of which are based upon its oxidation–reduction properties. Its functional roles include its uses as a nutritional food additive, antioxidant, browning inhibitor, reducing agent, flavor stabilizer, modifier and enhancer, color stabilizer, dough modifier, and in many other capacities. [2]

Apart from the properties for the alimentary industry, as mentioned before it has a mayor importance in the correct function of the human body; Vitamin C is needed for for the growth and repair of tissues in all parts of your body. It helps the body make collagen, an important protein used to make skin, cartilage, tendons, ligaments, and blood vessels. Vitamin C is needed for healing wounds, and for repairing and maintaining bones and teeth. It also helps the body absorb iron from nonheme sources. Additionally, low levels of vitamin C have been associated with a number of conditions, including high blood pressure, gallbladder disease, scurby, stroke, some cancers, and atherosclerosis, the build up of plaque in blood vessels that can lead to heart attack and stroke. [3]

In the last years the use of Vitamin C as a complement to other active pharmaceutical ingredients (API) in treatments for ailments such as heart diseases, common cold, cancer and osteoarthritis among others has been widely researched; additionally the capacity as antioxidant of the ascorbic acid can be used (sometimes is used []) as an antioxidant for the API of drugs.

On the other hand, the use of carboxylic acids has been increasingly used in the pharmaceutical industry as counter ions in conjunction with other anions like sulfate because of the high suitability of the ionic compounds in salt form; now a days more than 50% of the pharmaceuticals in the market. The actual composition of the counterions affects the safety and efficacy of drugs so that is one of the fundamental reasons for the rapid prediction and the assertion of their efficacy. Even though the prediction of the degradation is still in its early stages, diverse analytical methods help us know what kind of counter ion is binded to the API giving us the possibility of being aware about impurities that could affect either the physical properties of the drug rendering it useless before it expiration date, a lower yield than the expected or other possible products of the reaction of the formation of the drug that could not have any impact on the patient or even a secondary or opposite effect that could harm its well being [4].

That is because the development of methods of analysis for new pharmaceuticals is now an indispensable procedure within the quality control process for these products.

Since the pharmaceuticals are a mix of various compounds (API, excipients, possible impurities…), it must be considered as a complex matrix where de components must be carefully separated and a selective method so doesn't get any kind of interference in the measurement. This can be obtained through a thorough clean up for its posterior analysis or using a chromatographic technique such as liquid chromatography; this way the analytes could be separated and analyzed without being necessary much sample treatment which could end up as a loose of analyte or contaminate the sample.

For the analysis on one hand of there the carboxylic acids, usually reverse phase liquid chromarography is used to analyze them along with spectroscopic and mass spectrometry detectors although it can be also coupled with fluorescence detectors, for example in the case of the ascorbic acid. [2] [5]

On the other hand there are the anions that as the caroylic acids work as a counterion in the drugs; for this analysis the most standardized method is the Ion Chromatography with conductivity or amperometric detectors because of the great sensitivity and selectivity that it provides for this analytes compared to other liquid chromatography techniques. It is also extensively used for the analysis of anions in other fields such as the food industry and environmental analysis. [6]

Since the carboxylic acids are easily ionizable, using a column such as the Dionex AS19 for example, will give us the opportunity to separate and analyze the carboxylic acids and the anions contained in the drugs.

Ion chromatography is a method developed in a similar way to the classical reverse phase HPLC which is focused in the separation of ions so they can be analyzed by a coupled detection method. That separation is performed by a solid ion-exchange material packed in a column which provokes a difference in the migration of the ions.

It allows separating and analyzing in a relatively short time individual ions that come from complex matrix mixed with another cations and anions. However, when the concentration of the sample is too high they require some kind of dilution; in fact the detection limit of the technique generally goes down to the parts per billion ($\mu g\ L^{-1}$). Generally, the ion chromatography is applied in the

analysis of inorganic ions although some organic anions and cations may be determined as well, for example the (small) carboxylic acids.

In the half of the 1970s appeared the term ion chromatography, a new analitytical method which involved a new detection scheme for some inorganic cations and anions. By the end of the decade IC techniques were used to analyze organic ions for the first time; in the 80s the efficiency of the columns developed highly with the reduction of the diameter of the particles of the stationary phase to a size between 5 and 8μm resulting in a significant reduction of the time of analysis and in the 90s the stationary phases started aiming to special selectivities.

Nowadays, IC can be classified mainly in three separation mechanism; Ion exchange chromarography (HPIC), Ion-exclusion Chromatography (HPICE) and Ion-Pair Chromatography (HPIC). In this work the column used for the Dionex Chromatograph is the Dionex Ionpac AS19® and for the Metrohm Chromatograph …; This columns are Anion exchange Chromatography columns.

This kind of columns employs a resin carry functional groups with a fixed charge, the counter ions are located close to these functional group making them be electroneutral. The most used ion-exchange groups are quaternary ammonium bases, although in strong acid cation exchange sulfonate groups are used and for weak acid cation exchangers carboxyl or phosphate (or a mix or both). The capacity that the resin has to exchange ions with the analyte is determined by the sites of ion exchange and is expressed in mmol/g; this is the reason of the importance of the reduction in the size of the particle used in the column and it's research. The smaller the particle, larger is the number of them that you can fit inside of a column which having a bigger number of ion exchange sites and therefore a higher retention. The ion exchange capacity is directly tied to the retention time, being completely correlated and making the material play a minor role when the anion exchangers are equivalents in exchange capacities. With the exception of the latex – based anion exchangers, the surface – functionalized (organic polymers functionalized with tertiary amines) have demonstrated to have a much higher chromatographic efficiency than fully functionalized resins. [7]

Although is not necessary, during the determination of anion with ion chromatography is highly advisable the suppression of the background conductivity in order to get good chromatograms without any interference. [8]

The detector that is being used is of conductivity, which is based on the conductance; a property of a solution that contains a salt that conducts electricity across two electrodes; when a n electric field is applied between the two electrodes, the anions in the solution move towards the anode and the cations towards the cathode. Although conductivity and conductance are not the same, they are closely related by the law of Ohm:

$$V = I \cdot R \qquad (1)$$

The conductance is expressed in terms of electrolytic resistance of the solution as

$$G = \frac{1}{R}\ (S) \qquad (2)$$

The concentration of the salt in a solution is directly tied to the conductancy of the solution and the ion mobility coefficient (which can be calculated from tabulated equivalence conductances)

$$\Lambda = \frac{G \cdot 1000\ K}{C}\ (cm^2 \cdot \Omega \cdot mol) \qquad (3)$$

Where C is the concentration of the solution and K is the cell contant which is inherent and constant to each machine depending on the area and the distance between the electrodes.

A conductivity detector is considered since all ions will conduct electricity although all of them do not work out well in all the cases; In the case of the suppressed conductivity detector, only strong acid anions or weak acids which pKa is smaller than 6. The ones with a pKa higher than 6 are converted to the acid form by the suppressor making them unable to be detected. [9]

This method provides a quick and easy way to quantify the API and possible impurities in the drugs that are in salt form, being the mayor difference with reverse phase HPLC the simplicity and the selectivity of the method;

independently of the matrix or the API, you can use the same chromatographic method for all the analites.

A similar analytical method to IC, is capilar electrophoresis. Although this method is used to analyze the same it can be taken as a complementary technique to IC rather than a competitor; the strengths of one technique can help overcome the weaknesses of the other. For example compared to IC, CE has a better separation efficiency and tolerance to sample matrices especially at higher pH, but it lacks of stability and reproducibility of results (limiting the possible routine applications), has lower sensitivity. [10]

2-. EXPERIMENTAL PROCEDURE

2.1 -.MATERIALS AND EQUIPEMENT

2.1.1-. Materials

- Micropipete
  - 0,1 – 1mL
  - 1 – 5mL
- Volumetric Flask
  - 10 mL (±0,05 mL)
  - 50 mL (±0,1 mL)
  - 100 mL (±0,20 mL)
  - 250 mL (±0,30 mL)
- Analytical weight Rad Wag® (±0,1mg)
- 0,45 µm filters Acrodisc®
- 10 mL syringe
- Mortar
- Beaker
  - 50 mL
  - 100 mL
  - 20 0mL
- Spatula

2.1.2-. Equipement

Chromatographs

- Dionex ICS-3000 Bomb SP, Conductivity detector, AS-AP Autosampler

- 930 Compact IC Flex ion chromatograph (Metrohm, Switzerland) equipped with: IC 818 pump, IC 837 eluent degasser, IC 830 interface, IC 820 separation center, Valco injection valve, Metrodata 2.3 software, IC 919 IC autosampler Plus and IC 819 conductivity detector.

Columns

Table 1: Used columns and their technical specifications

Parameter	Column			
Manufacturer	Metrohm		Dionex	
Column name	Organic acid	Anion Dual 2	AS19	AS20
Column dimension [mm]	250 x 7,8	75 x 4,6	250 x 4,0	250 x 4,0
pH range	ene-13	03-dic	0-14	0-14
Resistance to organic solvents [%]	<20	20	100	100
Column capacity [µval/column]	-	-	240	310
Particle diameters [µm]	9	6	7,5	7,5

2.2-. SOLUTIONS AND REAGENTS

2.2.1-. Reagents

- L-Ascorbic Acid Standard Puriss quality (99,7 – 100,5%) Sigma-Aldrich®
- Fluoride 1000 mg/L Ion Chromatography Standard. Prolabo®
- Chloride 1000 mg/L Ion Chromatography Standard. Accustandard®
- Acetate 1000 mg/L Ion Chromatography Standard. Accustandard®
- Tartarate 1000 mg/L Ion Chromatography Standard. Accustandard®
- Formiate 1000 mg/L Ion Chromatography Standard. Accustandard®
- Nitrate 1000 mg/L Ion Chromatography Standard. Accustandard®
- Sulfate 1000 mg/L Ion Chromatography Standard. Fluka®
- Oxalate 1000 mg/L Ion Chromatography Standard. Accustandard®
- Phosphate 1000 mg/L Ion Chromatography Standard. Fluka®
- Sulfuric Acid Solution 0,17M
- Acetone solution

- Disodium carbonate 0,1M Solution
- Sodium acid carbonate 0,1M Solution

2.2.2-. Solutions

Water used for standards' and eluents' preparation was from the Millipore deionizer (Merck, Germany); its electrical conductivity was < 0.05 µS/cm. Calibration solutions were made by diluting appropriate standard solutions right before their application. All solutions were kept in glass or high-density polyethylene containers at ºC in a fridge temperature.

2.2.3-. Standards

Since the L-Ascorbic acid is a solid, a 1000 mg/L solution was prepared. From that solution the rest of the L-ascorbic acid solutions where prepared with differen concentrations.

First of all, for each column 50 mg/L individual solutions of all the standards were prepared so that the peaks for each analytes could be identify. After that a multistandard solution (WAKO17) with all the anions and carboxylic acids was analyzed to check if there was any incompatibility between the analytes and how they interact between them.

Tabla 2: The concentration of the different anions and carboxylic acids in the solution Wako17

	Concentration (mg/L)								
Solution	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA
WAKO17	10	10	10	100	50	100	10	30	100

In order to prepare the calibration curves, a similar solution was prepared but the L-ascorbic acid was not included since it coeluted with the oxlate.

Table 3: The concentration of the different anions and carboxylic acids in the solutions for the multistandard calibration curve

	Concentration (mg/L)							
Solution	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}
WAKO1	0,1	0,1	0,1	1	0,5	1	0,1	0,3
WAKO2	0,5	0,5	0,5	5	2,5	5	0,5	1,5
WAKO3	1,25	1,25	1,25	12,5	6,25	12,5	1,25	3,75
WAKO4	2,5	2,5	2,5	25	12,5	25	2,5	7,5
WAKO5	5	5	5	50	25	50	5	15
WAKO6	7,5	7,5	7,5	75	37,5	75	7,5	22,5
WAKO7	10	10	10	100	50	100	10	30

The calibration curve for the L-ascorbic acid was prepared apart from the multistandard calibration with the following solutions.

Table 4: Concentrations of the calibration curve solutions for L-Ascorbic acid

	Solution						
	1	2	3	4	5	6	7
L-AA (mg/L)	1	5	12,5	25	50	75	100

2.2.4-. Mobile phases

For the columns Dionex Ionpac AS19 and AS20, in bothe cases a KOH gradient was used alongside deionized water [11] [12]

For the analysis with the column Metrohm Organic Acids a 0,5mmol/L H_2SO_4 with a 15% of acetone was prepared from the 0,17M solution of H_2SO_4 and pure acetone. [13]

A Na_2CO_3 1,3mmol/L and $NaHCO_3$ 2 mmol/L solution was prepare from 0,1M solutions of Na_2CO_3 and $NaHCO_3$ for the analysis with the column Metrohm Anion Dual 2. [14]

2.2.5-. Subject of analysis

The subject of the study were inorganic anions (F^-, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) and ascorbic acid present in selected hypertension and painkillers drugs, a s well as dietary supplements, listed in table.

Table 5: List of the analyzed drugs and their type.

Drugs for hypertension	Painkillers drugs	Dietary supplements	Other
Drug name	Drug name	Name	Propanolol
Zofenil	Apap Extra	Skrzypovita	Solfadeina
Primacor	Polopiryna S	Biotyna z Labovital	Nebicard
Tritace	Pyralgina	Cynk organiczny z Apteo	travisto
Milocardin	Ibuprofen Teva	Vitaminer	Verdin Complex
Carvedilol	Solpadeine	Calcenato	Agen
Valtab	Pyralgina Sprint	Vigantoletten	Asparagnina extra
Propranolol	Polopiryna Max HOT	LadeeVit	Naproxen
Corectin	Zaldiar	Chela-Cynk	Olten
Tolucombi	Parancetamol LGO	Vitaminum B1 z Polfarmex	Collaplex
Tensart	Aspiryna	Plusssz Multiwitamina	Enareiva
Nitrendipina		Żeń-szeń z Labovital	Padolten
Agen		Vitamina C 1000	Enarenal
Nebicard		Chela-Mag B6 Shot	Hepatosan
Avedol		Linea Detox 10	Kilmakt
		Linea 40+	Urosan
		Linea Nox 5	Calcenato

2.2.6-. Sample preparation

All the samples were prepared by weighting one pill of each drug, then is smashed to a fine powder in a mortar and dissolved in 20mL of deionized water (which is weighted because this way is possible to dissolve the smashed drug in a beaker). Finally is filtered with a 0,45µm filter using a syringe. Some of the drugs substances did not fully dissolve because probably where insoluble in water (a problem for the analysis because some of those insoluble substances could be salts); probably an acid digestion would have helped to the dissolution of this substances but they would have interfered in the analysis so the idea was

discarded. Apart from the possible analyte loose, these drugs were difficult to filter demanding the use of more than one filter to be able to complete the filtration.

2.3-. ANALYSIS

2.3.1-. Chromatographic conditions

For each columns a different chromatographic conditions must be applied for an optimal separation of the analysis; these are usually recommended by the fabricants for a specific matrix or analyte. These are the conditions used for each column:

- Dionex Ionpac AS19 [11]

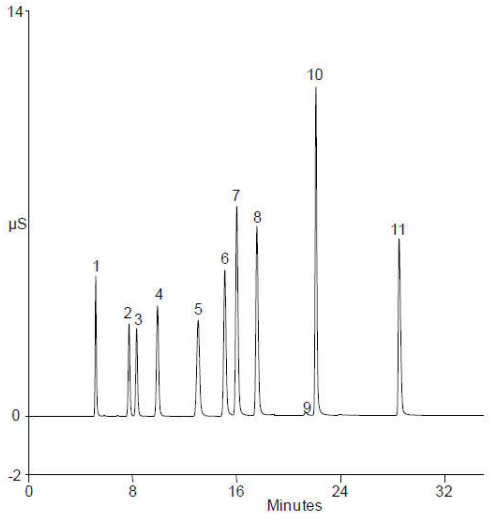
  Mobile phase: 10 mM KOH from 0 to 10 minutes,10-45 mM KOH from 10 to 25 minutes

  Flow: 1mL/min

  Injection Volume: 10µL

  Detection: Conductivity with chemical suppression

  Figure 1: Theoretical peaks for analiytes in the column AS19 (1-Fluoride, 2-Chlorite, 3 Bromate, 4-Chloride, 5-Nitrite, 6-Chlorate, 7-Bromide, 8-Nitrate, 9-Carbonate, 10-Sulphate, 11-Phosphate)



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- Dionex Ionpac AS20 [12]

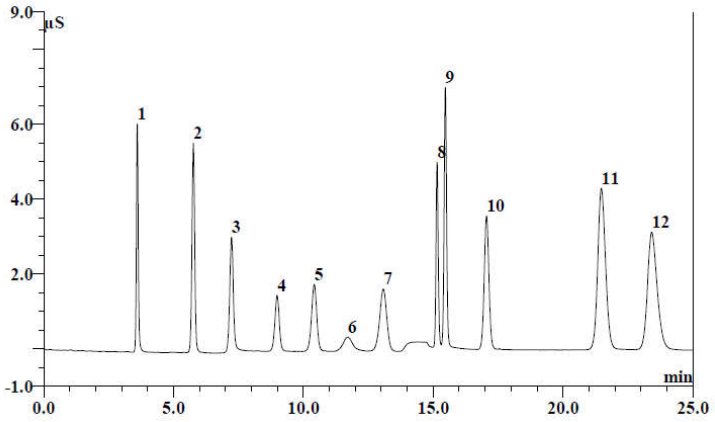
  Mobile phase: 15 mM KOH from 0 to 11 minutes,15-45 mM KOH from 11.1minutes

  Flow: 1mL/min

  Injection Volume: 10µL

  Detection: Conductivity with chemical suppression

  Figure 1: Theoretical peaks for analiytes in the column AS19 (1-Fluoride, 2-Chloride, 3-Nitrite, 4-Bromide, 5-Nitrate, 6-Carbonate, 7-Sulphate, 8-Phosphate, 9-Thiosulfate, 10-Iodide, 11-Thiocyanate, 12-Perchlorate)



- Metrohm Organic Acids [113]

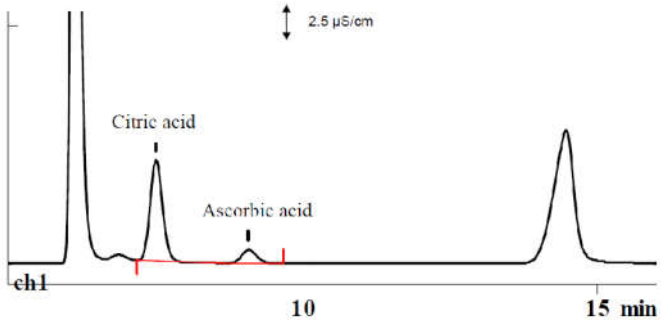
  Mobile phase: 0,5mmol/L H_2SO_4 with a 15% of acetone

  Flow: 0,5 mL/min

  Injection Volume: 100 µL

  Detection: Conductivity with chemical suppression

  Figure 3: Theoretical peaks for Citric acid and ascorbic acid



- Metrohm Anion Dual 2 [14]

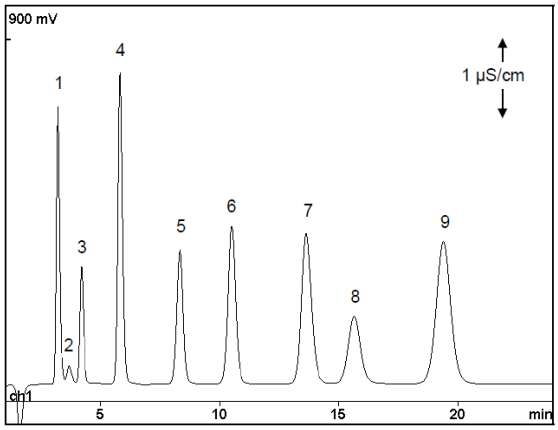
  Mobile phase: Na_2CO_3 1,3mmol/L, $NaHCO_3$ 2 mmol/L

  Flow: 0,8 mL/min

  Injection Volume: 20 µL

  Detection: Conductivity with chemical suppression

  Figure 4: Theoretical peaks for analiytes(1-Fluoride, 2-Acetate, 3-Formate, 4-Chloride, 5-Nitrite, 6-Bromide, 7-Nitrate, 8-Ortophosphate, 9-Sulfate)



2.3.2-. Procedure

First, when a new column is installed in the chromatograph, all the standards were analyzed individually and superposed to check the exact position of the peak from each analyte that the different peaks would not coelute. Then a solution with all the analytes to confirm that there are no coelutions and that all the peaks appear tall and sharp.

Once the adequacy of the chromatogram is verified the calibration solutions are analyzed and the calibration curve is checked.

After that, the analysis of the samples is done and finally of the spiked drugs.

All the solutions were introduced in the chromatogram loop by the autosamplers from the chromatografs. In the case of the Dionex chromatograph the vials are of 8 mL although the minimum sample volume needed is around 0,5mL; the software used for the peak analysis and measurement was Chromaleon 7®. On the

metrohm chromatograph the vials are 12 mL and the minimum volume sample needed is 8 mL; the software used is MagicNet 3.1®

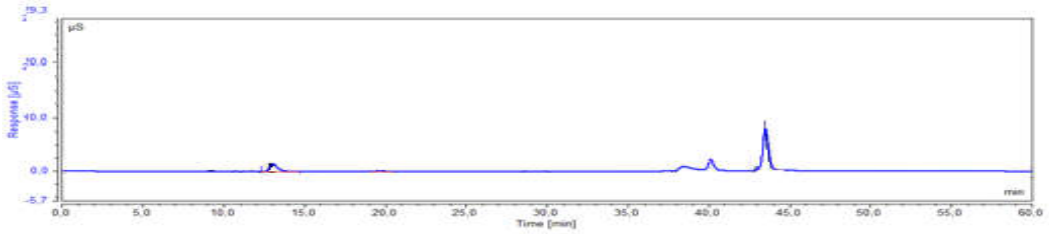
2.4-. RESULTS AND DISCUSION

2.4.1-. Dionex IonPac AS20

Standards and Calibration

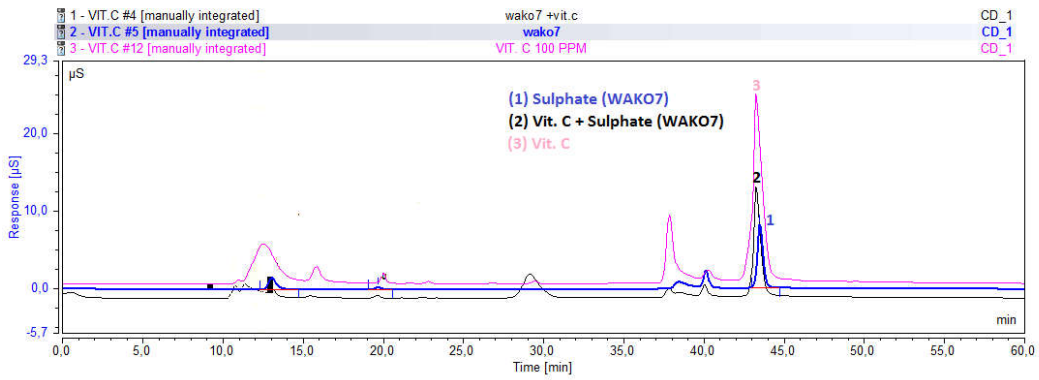
The Dionex IonPac AS20 was the first column used for the analysis, at first the solution WAKO7 was analyzed obtaining a mediocre separation where is not possible to discern all clearly the peaks of each one of the analytes.

Figure 5: The chromatogram of the solution WAKO7 in the column Dionex Ionpac AS20



Apart from the low resolution, the retention time of the sulfate and the L-Ascorbic acid are the same producing a coelution and making impossible the measurement of any of both analytes. Being these one of the most abundant anions present in drugs in the case of the sulfate and the L-ascorbic acid which is one of the main reasons of analysis in this work, the separation of this two analytes was mostly necessary.

Figure 6: The superposed chromatograms of the solution WAKO7, WAKO7 with L-ascorbic acid and a 100 mg/L L-ascorbic acid standard in the column Dionex Ionpac AS20



Different chromatographic conditions were tried such as different eluent % or a bigger flow but none of these changes made a significant difference so the decision of stop using this column was made.

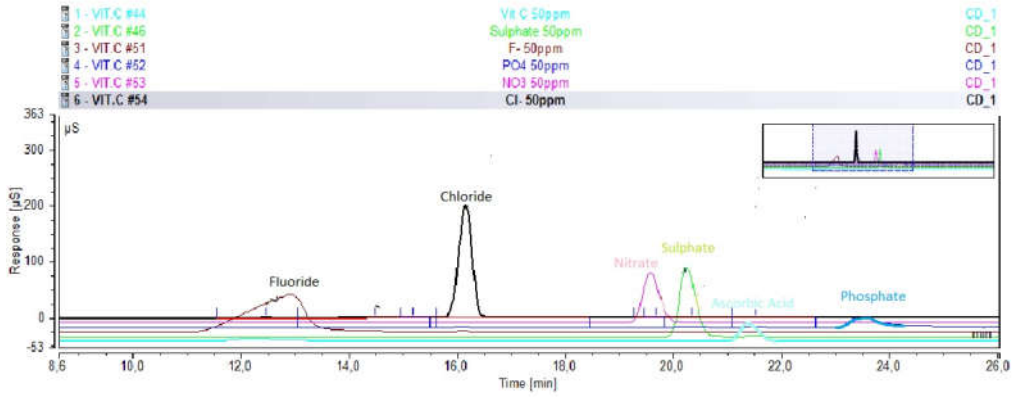
Additionally, as can be seen in the figure X the response is low even at high concentrations as 100 mg/mL of L-ascorbic acid or sulfate; leading this to a bigger chance of intereference in chromatogram because of the noise inherent to the base line, especially in the case of samples with low concentrations. Due to this issue the idea of multiple analysis of each sample in order to do an average and reduce the noise was considered but it was discarded because the chromatogram time is an hour becoming the analysis of one sample very long and not being word the time and economic investment for only one sample.

2.4.2-. Dionex IonPac AS19

Standards and calibration

First of all the standards were analyzed so it could be possible to identify all the peaks easily; superposing all the chromatograms the theoretical full chromatogram for the AS19 was obtained

Figure 7: Superposed chromatograms of 50 mg/L anions and L-ascorbic acid standards in the column Dionex Ionpac AS19

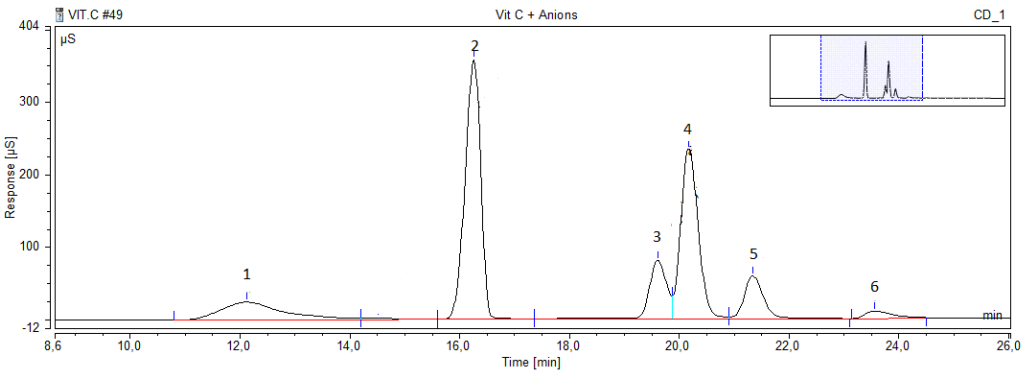


The peaks obtained except for the fluoride (in this case) are quite symmetric and in the case of the chloride, nitrate and sulfate are quite sharp. The response of the peaks has good heights making the noise inherent to the baseline of the chromatogram of little to no relevance for the chromatogram even at low

concentrations. Also can be stated that there is a good separatation between the peaks in general with the exception of the lower parts of the nitrate and sulfate, indicating that the sulfate peak probably will start before the nitrate peak has reached the level of response of the base line leading to a couelution of a small part of the peaks; but this was not considered an actual problem because the main part of both peaks were well separated.

After the analysis of the separate standard solutions, an multistandard solution's, which contained the anions previously analyzed and the L-ascorbic acid (excluding for now the rest of the carboxylic acids) , analysis was carried out in order to determinate how the interactions between the analytes could affect to the real chromatogram.

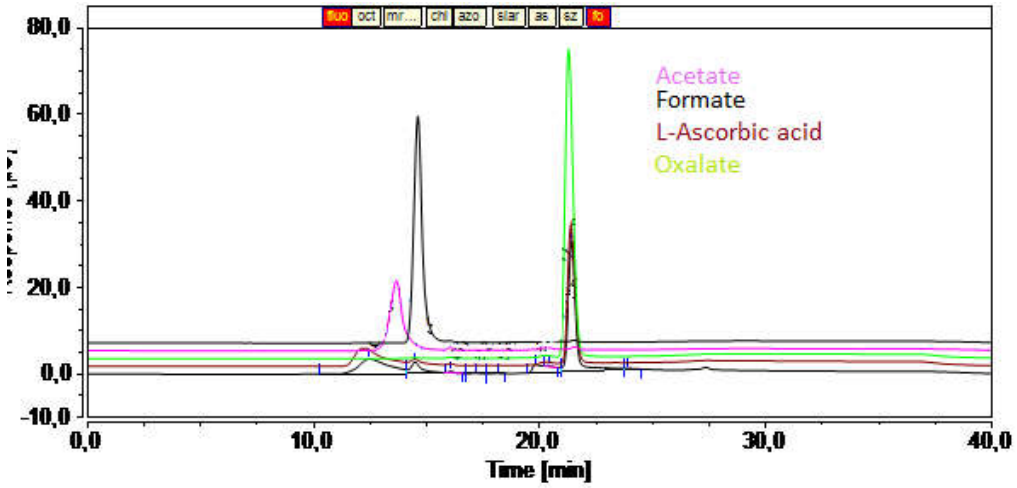
Figure 8: L-Ascorbic Acid + Anions Standards analysis; (1) Fluoride (2) Chloride (3) Nitrate (4) sulfate (5) L-Ascorbic Acid (6) Phosphate



After checking that there were no incompatibilities between the anions, the carboxylic acids were tested. In the figure can be seen that there is not any kind of coelution between them.

On one hand can be seen by the retention times that the peaks of the acetate and formate between appear between the fluoride and the chloride; on the other hand there is the oxalate that appears after the sulfate peak.

Figure 9: Carboxylic acids 50 mg/L standard chromatograms superposed.



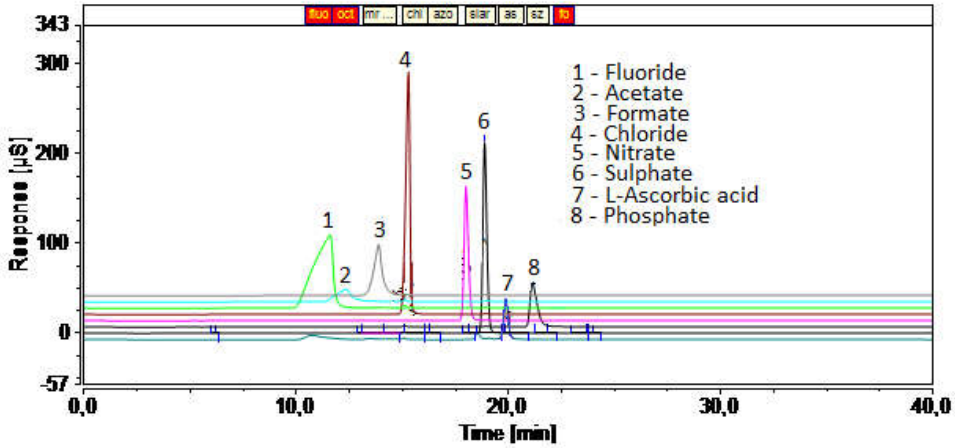
Upon checking the position of the oxalate and the L-ascorbic acid, can easily be seen in the figure that both have practically the exact same retention time.

In the same way that with the column AS20 with the L-ascorbic acid, a change in the chromatographic conditions was tried in order to separate both analytes but this was utterly futile; in any case, a coelution with the oxalate is a smaller inconvenient due to a smaller use of it in the drugs as a counterion so the columns AS 19 was used for the following analysis of drugs.

Finally an analysis was overrun with all the analytes (except oxalate) in one solution.

Once the column needed to be changed, after the change the column was reaconitioned; because of the recondition a standards were analyzed. Upon doing this the retention times became a shorter in general for all the analytes.

Figure 10: Soperposed chromatograms of the analyzed standards after reconditioning



Once compared all the standards the calibration solution were analyzed in order to do the calibration curve. Since the calibration solutions are multistandard solutios, a calibration curve for each analyte was obtained.

The calibration curve was analyzed each time the column was changed; this happened once the column needed to be reconditioned.

Table 6: Results of the calibration curves

Calibration	Analyte	O.O	Slope	R^2	σ	%DER
1	F^-	-	1,92	0,9998	0,01	0,53
2		-	1,92	0,9997	0,01	0,69
1	Acetate	-	0,28	0,9923	0,01	4,39
2						
1	Formate	-	0,72	0,9956	0,02	2,72
2						
1	Cl^-	-	1,19	1,0000	0,00	0,15
2		-	1,20	0,9996	0,01	0,78
1	NO_3^-	-	0,68	1,0000	0,00	0,14
2		-	0,64	0,9997	0,00	0,73
1	SO_4^{2-}	-	0,87	0,9999	0,00	0,36
2		-	0,86	0,9997	0,01	0,71
1	Oxalate	-	0,71	0,9967	0,02	2,36
2						
1	PO_4^{3-}	-	0,33	0,9973	0,01	2,14
2		-	0,37	0,9988	0,01	1,42
1	L-AA	-	0,23	0,9991	0,00	1,25
2		-	0,22	0,9966	0,01	2,38

After doing the lineal estimation of each calibration curve, in all the cases was decided to remove the origin since the %RSD was bigger than the 5%; doing this, the standard deviation of the curves was improved as well as reduced the relative standard deviation of the slopes, improving this way the precision of the curves. In all the cases the linear regression coefficient is bigger than 0,990 indicating that all the curves are of good quality although among them, clearly can be seen that the carboxylic acids (which peaks in the chromatograms are less sharper) have an inferior quality than the chloride, nitrate and sulfate, getting less than three nines in the R^2 while the anions get even closer to 1. This can be noted in the %RSD where the carboxylic acids have it higher than 1% in all the cases even getting closer to the 5%, which is the limit of acceptance, in the case of the acetate.

Here is noticeably that although the column Dionex IonPac AS19 is able to work with anions and carboxylic acids with good results and reliability, is a column more recommended for anion analysis.

Checking the retention times, the %RSD was calculated for all the analytes; in all the cases it was inferior than 4% included here the analysis where the retention time of the peaks changed after the recondition) which increases the standar deviation and proportionally the %RSD. By this results can be said that the method is selective for all the analytes (except as mention before if there is L-ascorbic acid and oxalate in the same sample)

In order to obtain the limit of detection and the limit of quantification water was analyzed 3 times to obtain the baseline for each anion and carboxylic acid, and with the average concentration and the standard deviation, using the following expressions to calculate them.

$$L_D = \bar{Y}_0 + 3\sigma_0 \qquad (4)$$

$$L_Q = \bar{Y}_0 + 10\sigma_0 \qquad (5)$$

Since at the retention time of some of the analytes there is no signal, with the calibration curves the theoretical limit of detection and the limit of quantification of each analyte were calculated; the origin was deleted in the equations so Y_0 was considered 0 and σ_0 was obtained using the equation of miller

$$\sigma_0 = \frac{\sigma_{y/x}}{b_1} \cdot \sqrt{\frac{1}{m} + \frac{1}{N} + \frac{\bar{x}^2}{\sum_{i=1}^{N}(x_i - \bar{x})^2}} \qquad (6)$$

Table 7: Limit of detection and limit of quantification for the different analytes

Analyte	LOD (mg/L)	LOQ (mg/L)
F^-	0,01	0,02
Acetate	0,54	1,80
Formate	0,19	0,29
Cl^-	0,42	0,75
NO_3^-	0,61	1,36
SO_4^{2-}	0,41	1,16
Oxalate	0,12	0,38
PO_4^{3-}	0,04	0,12

The LOD and LOQ as said before are obtained from the analysis of water and measured the area in the retention time, the problem is that in general all the water analysis have different chromatograms so the results obtained can be said that are completely accurate. In some cases like for example the nitrate results under the LOD have been obtained; although doing the the LOD with the equation (6) are lower but those have been obtained in a theoretical way.

Since the L-ascorbic acid was not present in the application note of the Dionex Ionpac AS19, the adequacy of its analysis is not as well established as for the rest and being the main focus of this work the validation for the method was made.

L-Ascorbic acid method validation [15]

First of all for the validation, the linearity of the method is checked with the results from the calibration; The %RSD of the retention times from all the calibration solutions is of 0,35% which is within acceptable limits (<5%) . In both calibration curves, the origin was deleted because its %RSD was higher than 5% improving this way the %RSD and the R2 of the slope. Can be stated that the calibration curves are of good quality and keep linearity since the fulfill that %RSD<5% and R^2>0,99.

To check the accuracy of the method 3 groups of 3 solutions with 25 mg/L, 50 mg/L and 75 mg/L were analyzed; in average the recovery was 102%, a good result within an acceptable range but the %RSD is bigger than the 5%, this is because there is a big difference n the accuracy of the measurement at low (25mg/L) and high (75 mg/L) concentrations. The relative error at low concentration is around 10% while for the rest of the solutions is inferior to 4%, because of this the standard deviation is increases and proportionally the %RSD. For the case of the indivual groups of solutions in all the cases the %RSD is smaller than 5% although still the biggest one is the 25 mg/L group.

The Cochran G test was made to determine the influence of the concentration in the variance of the results with the next expression:

The influence of the concentration in the variance of the results with the next expression:

$$G_{EXP} = \frac{S^2_{max}}{S_1^2+S_2^2+S_3^2} \qquad (7)$$

The G_{EXP} obtained is 0,48 which is smaller than G_{Tab} (α = 0,05; k = 3; n = 3) = 0,87 meaning that the concentration does not have a significant impact in the variance of the measurements.

In order to know if there were significant differences between the real concentration and the obtained results, the test T is applied to each group with the following expression with n-1 liberty grades and α=0,05:

$$T_{EXP} = \frac{|100-\bar{R}|}{\frac{\sqrt{n}}{\%RDS}} \qquad (8)$$

The results obtained are $T_{25mg/L} = 20,20$, $T_{50mg/L} = 0,43$ and $T_{75mg/L} = 3,14$; the only T value that is bigger than the $T_{crit} = 4,30$ is the one for 25 mg/L meaning that is the only one that has significative differences with the real concentration of L-ascorbic acid. From this results can be stated that although the method can be used at lower concentrations, has are better precision and accuracy at higher concentrations.

In order to check the selectivity of the method the retention time of 8 spiked sample and 3 samples that is known that have L-ascorbic acid was tested; The %RSD of the retention time is 0,22% so can be stated that the matrix does not interfere with the analyte and the method is selective always that the drug does not contain oxalate which has the same retention time that the L-acorbic acid. When the recovery of the spiked drugs was checked was obtained that the students T was higher than the critical and the %RDS was over 5%; but this spiking were at low concentrations (20-25mg/L) that as stated before do not generally give good results with this method.

In order to check the precision, 3 50mg/L solutions were analyzed one day and other 3 the next day; First of all, the analytic response repeatability the %RSD of the 6 analysis; it was under the 5% so it is within an acceptable range. By separate, neither of the analysis of each day exceeded the 5% limit of %RDS.

Additionally the test F and test T were made to the results to check if there were significative differences between the results from each day; the used expressions are the following respectively:

$$F_{EXP} = \frac{S_1^2}{S_2^2} \qquad (9)$$

So F_{EXP} is ≥1

$$T_{EXP} = \frac{X_1 - X_2}{S \cdot \left[\frac{1}{n_1} + \frac{1}{n_2}\right]} \qquad (10)$$

Where X_1 and X_2 are the average concentration of each day, n the number of determination each day (in this case 3 both times) and S is

$$S = \sqrt{\frac{(n_1 - 1) \cdot S_1^2 + (n_1 - 1) \cdot S_2^2}{n_1 + n_2 - 2}} \qquad (11)$$

The result for F_{EXP} is 16,55, which is smaller than F_{TAB} =39 so its proved that there are no significant differences between the variances of the results of each day. On the other hand cannot be said that there are not significant differences between the results from each day because the F_{EXP} is 4,67 which is slightly bigger than F_{Tab}=4,604 so the good repeatability of the method cannot be confirmed with this results.

For the sensitivity was taken the area that was at the retention time of the L-ascorbic acid of 3 water analysis and were taken as a white. In order to calculate the limit of detection and the limit of quantification the equations (4) and (5).

The results obtained for the LOD and LOQ are 1,13 mg/L and 2,36 mg/L respectively.

In conclusion can be said that the method can be used for the analysis of L-ascorbic acid in drugs although is recommendable using it with drugs known to have relatively high concentration of L-ascorbic acid since it has been established that the results at smaller concentrations are not as reliable as they should be. About the repeatability is worth mention that the samples were not stored under cold conditions which probably produced a analyte loose; in some samples left for 10 days at room temperature a significant L-ascorbic acid (around 40%) of lose was found most likely due to degradation of the sample.

Sample results

For the analyzed drug samples the next results in the next were obtained

Table 8: Results of the analysis of drugs (1)

	Concentration (mg/L)								
Drug	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA
Chela-Mag B6	-	-	24,67	43,04	-	76,35	-	-	-
Cynk organiczny z Apteo	210,14	234,33	2,04	29,27	22,83	51,01	-	0,64	-
Zofenil	-	-	-	5,57	13,84	7,29	-	-	-
Apap Extra	-	-	3,86	1,53	1,51	3,93	0,52	-	-
Polopiryna S	-		-	7,00	-	10,86	-	-	-
Propanolol	-	-	-	60,06	-	3,38	0,46	-	-
Ibuprofen Teva	-	11,56	6,38	7,17	2,31	4,96	-	-	-
Solpadeina	NGP	8,67	1,89	-	1,49	6,37	0,45	53,40	-
Corectin	-	-	1,12	2,53	-	1,71	-	-	-
Vita-miner	-	-	170,73	34,78	87,67	749,14	-	-	1363,62
Skrzypovita	51,27	-	50,39	530,62	257,49	554,27	-	84,09	44,79
Primacor	-	-	0,39	34,87	-	5,03	-	0,35	-
Viganotoletten	-	-	-	1,08	-	1,35	-	-	-
Carvedilol	1,28	-	0,49	13,10	6,36	13,32	-	2,70	-
Nebicard	3,37	-	0,56	34,19	17,02	34,79	-	8,97	-
Vitaminum B1 z Polfarmex	4,99	-	0,57	51,10	25,54	51,74	-	14,34	-
Zaldix	7,14	-	0,58	73,98	37,01	74,35	-	21,78	-
Pyralgina Sprint	-	12,21	992,49	-	26,68	3095,94	-	-	-
Rutinacea Complete	NGP	NGP	161,63	3,92	866,65	-	-	-	-
Parancetamol LGO	NGP	NGP	2,94	-	1,96	4,45	-	-	-

Table 9: Results of the analysis of drugs (2)

	Concentration (mg/L)								
Drug	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA
Pyralgina	-	-	-	1500,53	812,81	-	-	5,70	-
Travisto	6,42	8,50	113,19	101,94	24,78	168,08	-	5,65	255,71
Polopiryna Max Hot	2,42	498,93	73,08	145,58	20,16	9,68	?	-	312,17
Dexak 50	-	-	9,59	160,76	-	4,20	-	-	-
Chela Cynk	-	-	5,75	5,45	1,51	7,43	-	-	-
Verdin Complexx	26,61	-	14,81	-	92,18	-	-	1,95	
Valtab	-	NGP	-	2,78	-	61,02	-	0,41	-
Agen	0,16	-	-	3,90	36,18	2,96	0,90	-	-
Tolucombi	2,94	-	12,57	3,69	-	-	-	0,47	-
Asparaginian extra	-	-	-		-	-	-		-
Naproxen	-	-	0,27	4,90	-	2,82	0,85	-	-
Proliver	1,66	-	97,40	-	48,15	18,78	Big broad peak	1,21	Big broad peak
Milocardin	-	-	-	-	1,44	1,73	-	-	-
Collaflex	-	-	5,69	82,38	43,93	142,72	?	0,15	385,28
Linea Detox	0,29	-	0,89	-	3,95	58,05	-	0,70	-
Nimesil	0,51	-	-	-	1,65	0,77	-	-	-
Aleve	-	-	7,60	-	2,55	3,13	-	24,43	-
Enarenal	0,61	-	0,53	-	1,44	88,51	-	-	-
Linea 40+	11,15	-	26,12	-	56,41	7,03	-	5,93	2,73

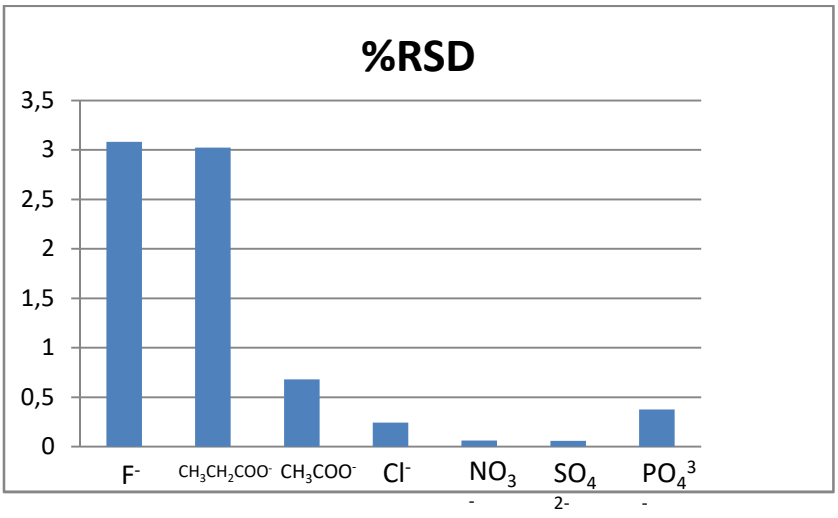
Table 10: Results of the analysis of drugs (3)

	Concentration (mg/L)								
Drug	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA
Tensart	-	-	-	4,73	3,42	3,31	?	-	-
Tritace	0,45	-	-	-	2,11	2,23	-	-	-
Avedol	0,51	-	2,57	-	6,45	-	-	-	-
Zaldiar	-	-	36,57	-	-	-	-	-	-
Olfen	-	-	8,77	-	1,74	-	-	-	-
Collaplex	4,89	NGP	0,55	-	5,65	16,55		0,53	-
Enareiva	-	-	0,51	0,91	-	11,85	?	-	?
Padolten	0,59	-	0,15	106,81	18,62	-	-	-	-
Linea Nox	-	-	3,28	-	12,90	-	?	0,98	?
Nitrendipina/2	-	-	1,07	1,897	-	4,02	-	-	1,81

In some of the analysis of anions, there is a signal for the analyte but the results are below of the LOQ, in those cases the block is colored in yellow.

From the results obtained what can only be said with certainty is that the obtained results (since the exact composition of the drugs is in many cases of doubtful sources or non existant) are within the margin of error in terms of accuracy and precision listed in the following graphics, from data obtained from the different standards analyzed.

Figure 11: Percentage of relative standard deviation of the analysis of standards of the different analysis

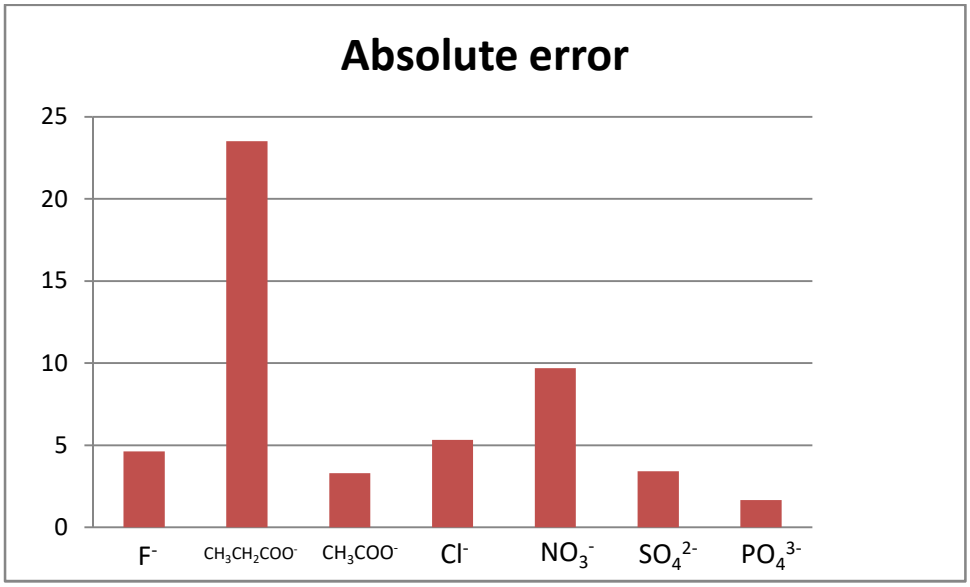


On one hand in the graphics can be seen that in general there is a great precision, all the analytes except the F^- and the acetate have a %RSD inferior to 1% whereas this 2, do not exceed the 5% of limit, are in the 3%.

On the other hand there is the accuracy, which is not as good as it is the precision since in the case of the Cl^-, NO_3^- and acetate the relative error is bigger than the 5%, being even close to the 10% in the case of the nitrate and 25% for the acetate.

The problems with the acetate is more expected since in genera (and specially in the drugs chromatograms the peaks obtained are more irregular and broad than in the other cases. Although the nitrate usually has high and sharp peaks (this is not definitive in terms the accuracy) the difference with the real value.

Figure 11: Percentage of absolute error of the analysis of standards of the different analysis



In the same way, in order to see how the L-ascorbic acid would work on a real matrix of different drugs, some of the previously analyzed drugs were analyzed with different concentrations of L-ascorbic acid between 5 – 25mg/L; low concentrations so the signal obtained is not bigger that the onces obtained for the other analytes present in the different drugs.

Table 11: Results of the analysis of the spiked drugs (1)

	Concentration (mg/L)										
Drug	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA	$AA_{Theoretical}$	% Recovery
Cynk organiczny z Apteo + L-AA	161,30	-	72,67	67,25	93,47	224,85	-	-	1,39	5,00	28
Propanolol + L-AA	-	-	0,88	39,32	-	3,19	?	0,25	6,80	6,66	102
Zofenil + L-AA	-	-	1,02	-	4,40	2,58	-	-	6,06	5,00	121
APAP Extra + L-AA	-	-	2,45	-	1,46	3,22	-	-	5,15	10,00	52
Avedol + L-AA	0,51	-	0,49	4,70	11,85	6,15		-	11,77	10,00	118
Aleve+ L-AA	0,95	7,34	4,13	-	3,89	5,60	-	-	10,71	10,00	107
Zen - szen vita complex + L-AA	1,51	-	0,64	-	1,41	11,88	-	-	22,75	10,00	228
Naproxen+ L-AA	-	-	-	-	4,46	4,91	-	-	9,38	10,00	94
Tensart + L-AA	-	-	0,32	-	4,06	5,93	-	-	11,33	10,00	113
Tritace + L-AA	0,26	-	1,11	-	3,04	5,61	-	-	10,71	10,00	107
Olfen + L- AA	0,41	-	-	-	5,05	3,65	-	-	6,99	10,00	70
Proliver + L-AA	20,64	-	12,99	-	4,12	350,10	23,00	-	33,37	10,00	334
Padolten + L-AA	-	-	23,56	518,75	21,82	357,85	-	-	34,30	10,00	343
Zaldiar + L-AA	-	-	4,01	-	3,21	20,58	-	-	39,44	10,00	394
Nimesil + L-AA	6,67	-	-	-	26,90	53,30	-	-	10,18	10,00	102
Enareiva + L-AA	2,96	-	-	-	33,09	122,94	-	-	23,55	10,00	235
Chela-cynk+ L-AA	-	31,33	6,62	1,83	-	35,37	-	-	12,52	25,00	50
Nebicard+L-AA	1,44	-	0,68	11,32	-	-	-	0,21	22,17	20,00	111
Milocardin+ L-AA	4,39	66,75	-	4,43	-	-	-	-	33,70	20,00	168

Table 12: Results of the analysis of the spiked drugs (2)

	Concentration (mg/L)										
Drug	F^-	Acetate	Formiate	Cl^-	NO_3^-	SO_4^{2-}	Oxalate	PO_4^{3-}	AA	$AA_{Theoretical}$	% Recovery
Nitrendipina+L-AA	2,97	-	3,29	2,56	-	3,94	-	-	22,72	20,00	114
Avedol+ L-AA	1,07	-	-	1,38	-	2,02	-	-	16,94	25,00	68
Carvedilol+ L-AA	2,42	-	-	-	-	-	-	0,33	21,12	20,00	106
Asparaginian extra + L-AA	1,62	-	-	70,60	-	-	-	-	25,40	25,00	102
Polopyrina max hot + L-AA	39,13	247,85	61,21	153,96	-	-	-	19,89	46,39	25,00	186
Olfen + L-AA	16,07	-	14,46	58,62	-	14,30	-	-	24,39	25,00	98
Zaldiar + L-AA	21,69	-	10,88	236,26	-	-	-	1231,51	27,22	25,00	109
Pyralgina +L-AA	175,76	45,30	147,10	144,27	32,17	6980,45	-	-	24,22	20,00	121
Enarenal + L-AA	12,07	-	3,02	10,05	-	1,43	-	5,40	25,20	25,00	101
Dexak 50 + L-AA	9,36	-	11,20	173,15	1,41	1,58	-	5,20	26,75	25,00	107
Pyralgina Sprint + L-AA	108,40	-	858,39	63,53	25,31	4952,24	-	204,63	15,03	20,00	75
Polopyrina +L-AA	-	1380,99	-	4,92	-	20,84	-	4,40	19,44	20,00	97

In general, there are results that obtain a recovery within a margin of 10% error, which is to be expected at this kind of low concentrations as is stated in the method validation. There are outliers where a recovery much bigger than 100% is obtained or with a much lower recovery than expected; this can possibly be because the column retained some amount of L-ascorbic acid; all the analysis with excessive recoveries were made the same day.

Simply comparing the value of the concentration for the anions in the in the tables of the spied and non spiked samples, can be said that in general there is no reproducibility for the method; but must be taken into account that the analysis were made with some time between them, which can lead to a sample degradation even though it was preserved in cold conditions or that are completely new samples so the systematic error must be taken also into account. The sample preparation should be also optimized in order to obtain a better solubilization of the sample, with an ultrasonic bath of the sample for 10 minutes for example.

Anyway, at this point with the results obtained in this project, cannot be said that the method as a whole has a good reproducibility; not over time and not with two different samples of the same drug.

2.4.3-. Metrohm Organic Acid

Standards and calibration

This column only is capable separate carboxylic acids so any of the standards for anions were analyzed perse, separatedly acetate, formate, oxalate, citrate, tartarate and L-ascorbic acid 50 mg/L standards were analyzed in order to know the retention time of each of the carboxylic acids.

In all the chromatograms can be seen a peak with negative response and another broad peak around 15 minutes; this peaks are both system peaks inherents to the column itself independently of the analyte.

The obtained retention times for the peaks were the following

Table 13: Retention times of the different carboxylic acids in the column Metrohm Organic Acid

Carboxylixc acid	RT (min)
L-AA	9,35
Oxalate	11,55
Formiate	12,7
Citrate	7,75
Tartarate	8,5
Acetate	13,54

The selectivity of the column could be said is good, in all the cases the %RSD of the retention time of all the samples analyzed was below 3%.

Then a calibration curve of L-ascorbic acid was analyzed 4 times and the following results.

Table 12: Results of the calibrations of L-ascorbic acid with the column Metrohm Organic acids

	Slope	Origin	R^2	σ	%RSD
Calibration 1	0,0081	-	0,996	0,00020	2,46
Calibration 2	-	-	-	-	-
Calibration 3	0,0069	-	0,997	0,00014	2,07
Calibration 4	0,0058	-	0,997	0,00014	2,36

By themselves all the calibrations seemed to be of good quality, all of them (except the calibration 2) have a correlation coefficient of more than 0,99 and the %RSD is less than 5% but upon checking the areas at the same concentration between the calibrations could be seen that all of them were different, in fact the %RSD of between them is in a range between 10% and 20% indicating that the method doesn't have a good linearity. Also in all the calibrations the solutions below 10 mg/L did not get any signal and in the case of the calibration 2 not even the solution of 10mg/L so there were not enough points to make a calibration curve.

Although it this column does not is not useful for quantification purposes, it can be used complementarily with the Dionex Ionpac AS19 in the cases where there is a doubt

between oxalate and L-ascorbic acid in a drug using it as a qualitative analysis and letting know this way whether the sample has oxalate, L-Ascorbic acid or both.

Because of this the analyses made with this column were merely qualitative, for the drugs where there was some doubt if there was oxalate or L-Ascorbic Acid.

Sample results

Table 13: The qualitative results of the drugs samples for the organic acid sample analysis

	L-AA	Oxalate	Formiate	Citrate	Tartarate	Acetate
Tolucombi						
Zofenil						
Nitrendipina						
Nebicard						
Propanolol						
Milocardin						
Agen						
Primacor						
Carvedilol						
Avedol						
Tensart						
Calcenato						
Skrzypovita						
Ladeevit						
Viganoletten						
Chela cynk						
Hepatosan						
Kilmakt						
Urosan						
Aspiryna						
Corectin						
Parancetamol LGO						
APAP Extra						
Solfadeina						
Naproxen						
Valtap						

In order to confirm the unavailability of the use of this column for quantitative analysis, 3 L-ascorbic acid standards were analyzed 3 times each; as suspected the results were no good, having a %RSD from 10% to 33% and having the obtained results significative differences with the concentration that the solution really had.

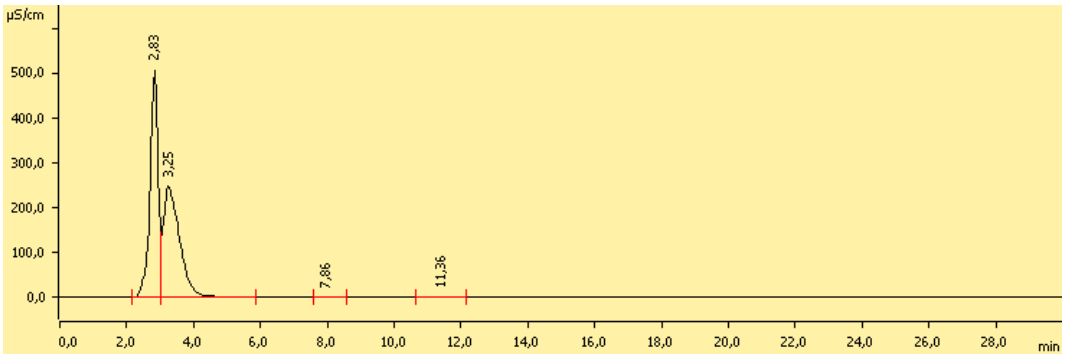
The results obtained only confirm the presence of any of the 5 carboxilyc acids analyzed in the drugs samples which can be used for a confirmation of the results obtained with the column Dionex Ionpac AS19, specially as a confirmation of the presence of L-ascorbic acid and oxalate.

2.4.4-. Metohm Anion Dual 2

Standards and calibration

In this column first of all a 50mg/L L-Ascorbic acid standard was analyzed and the peak appeared at a retention time of 2,72 minutes; after that the WAKO17 solution as well as WAKO7 were analyzed and only two peaks were obtained at around 2,8 and 3,2 minutes without getting any kind of separation.

Figure 12: WAKO7 solution analysis on Anion dual 2



The analysis was repeated 2 times more obtaining the same results. Because of this the use of this column was directly discarded. Is probably that the column did not make any separation at all because since it was old there is a possibility that it use time had expired.

3-. CONCLUSIONS

In conclusion based on the results obtained cannot be said that a viable method as a whole to analyze anions and carboxylic acids in drugs has been found. All of the different columns used, have not been able to pass different test to prove that using them for the analysis of these drugs is accurate, precise and with a good reproducibility. Must be noted that in general the columns were not in the time of their peak performance but the results expected were better than the ones obtained.

The most successful results were with the Dionex Ionpac AS19, which in general specially in the case of the anions with the standards obtained fairly good results in terms of accuracy and precision, but was not able to deliver good results for the reproducibility; ultimately the results for the L-ascrobic acid were partially successful, obtaining only satisfactory results for relatively high concentrations which makes it less useful for small concentration samples (although not completely useless since working that the method used in case of low concentration has an error close to 10%, so could be use as a method with lower confidance limit) and useless in cases where the drug contains oxalate since both coelute exactly at the same time.

The results obtained with the Dionex Ionpac AS20 were disappointing because the low resolution obtained in the chromatograms and specially the coelution of the sulfate and the L-ascorbic acid which rendered it useless for this project because of its focus on the carboxylic acid, although further tests with this columns probably would deem it useful in the analysis of anions in drugs as well as it is for this kind of analysis in environmental samples.

As well as with the AS20, the results with the metrohm Anion Dual 2 are disappointing, but in this case because there was no separation at all; must be noted that this is not probably because of the model of column perse but because the column that was available was old and probably had expired or lost retention capacity and since is a short column is not able to separate anlytes anymore.

Finally the Metrohm organic acids did not obtain good results neither in terms of quantification of the L-ascorbic acid, but even after this it can be used for a

complementary analysis with the Dionex Ionpac AS19 in order to be able to know in case of doubt if the sample has only oxalate, L-ascorbic acid or both.

In general I think that the main issue with this project has been that it was too ambitious for the time that was available, not being able because of this to complete all the wanted analysis with different conditions, specially using the colomns Dionex Ionpac AS14 and Metrohm Metrosep Supp 5 which were good candidates as well to obtain good results in this kind of analysis.

As well as new columns, also would be even more important to improve the sample treatment, which I think that is mainly the reason for the bad reproducibility of the results (apart of the degradation of the samples over time). In most cases the solubilization is only partial obtaining a lot of solids in suspension; a ultrasonic bath of the samples probably would help to at least dissolve better those parts of the drugs in suspension.

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