DIFFERENT TYPES OF ION CHROMATOGRAPHY: A COMPARATIVE STUDY WITH EDUCATIONAL PURPOSES

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Abstract. In this work we propose that students do a comparative study of different types of Ionic Chromatography from a point of view based on active learning and constructivism. In our laboratory principles of interactive learning were applied so that undergraduate students evaluated the characteristics, applications, as well as the advantages and disadvantages of different types of ion chromatography. The use of so-called chemical suppressors for the reduction of eluent's conductivity was a special focus. The proposed study is suitable as a teamwork exercise for enhancing some analytical concepts. It also provides instructors feedback on the degree of assimilation by students.

Keywords: upper-division undergraduate, analytical chemistry, hands-on learning, chromatography, HPLC

1. Introduction

Laboratory instruction is a significant component of science education since it serves for multiple purposes. It has the potential for promoting the understanding of science concepts by fostering inquiry, intellectual development, problem-solving skills and ma-

nipulative skills. We propose a study of chromatography for the related items. Chromatography methods are defined by the chief separation mechanism used. Ion Chromatography is one member of this family of chromatographic methods. Today there are three types of Ion Chromatography: Ion-Exchange Chromatography is simply known as Ion Chromatography (IC), while Ion-Pair Chromatography (IPC) and Ion-Exclusion Chromatography (IEC) are regarded as being more specialized applications.

In the recently years, science educators have focussed on active learning to improve student's learning achievement and attitudes [1-5]. To become functional as scientists, chemistry students must integrate concepts learnt in their classes and apply them to novel, "real life" situations. The laboratory provides an important place for students to practice integrating concepts. An approach for these purposes was to describe study and compare the advantages and disadvantages of different types of Ion Chromatography.

There are a lot of research in laboratory instruction [6-8] and many papers about education on ion chromatography and its history and evolution in 20th century [9-13]. Nevertheless, to the best of our knowledge, no papers dealing with different educational aspects of three types of ion chromatography have been reported.

Chromatic graphic methods are defined by the chief separation mechanism used [14]. Analytes are separated by means of their different retention in an anion or cation-exchange column. Typical operating procedures use isocratic and very small sample injection volumes. This technique has been applied successfully for the determination of numerous inorganic and organic anions and cations, as well as nucleotides, biogenic amines, carbohydrates, sugar alcohols and so on, using Ion-Exchange Chromatography (IC) [15,16], Ion Pair Chromatography (IPC) [17-20] and Ion Exclusion Chromatography (IEC) [21-25]. Conductivity, integrated pulsed amperometry, voltammetry, fluorescence, UV-vis spectrometry (direct, indirect, post-column derivation, multiwavelenght with diode arrays) and coupled mass spectrometry have been used widely as suitable detection systems. In its earliest applications, Ion Chromatography was employed without eluent conductivity suppression. The most widely used eluent was phthalate. Nevertheless, for better detection of charged species the eluent conductivity has to be reduced to as low a level as possible with a chemical or electrochemical suppressor system. The first application of this item was in 1975 [26]. In this case, the most frequently used eluent was the carbonate/bicarbonate system. This way, conductivity of analytes is enhanced and, simultaneously, eluent conductivity is minimized (typical values of eluent conductivity about 2-3 μs/cm). Nowadays, several companies[1-3)] have developed different systems of suppression enabling quantification of some toxic anion in many water samples according to the U.S. EPA[4,5)] and European Commission.[6)] More over, sensitive techniques such as HPLC and ICP-MS need ultra pure water. The levels of as many as 15 different anions are of concern. Nowadays, with the eluent conductivity suppression, typical ionic chromatographic

analysis yield nowadays separations and quantifications at ppbs levels, even ppts in some cases, in cations and anions have been performed. Therefore, the high sensitivity of Ion Chromatography and the minimal sample preparation involved make Ion Chromatography a powerful and very usable tool for the analysis of ions in an undergraduate setting.

The work with the students was composed of four independent phases and now forms part of the laboratory courses in chemical instrumentation and instrumental chemical analysis in our department. In our laboratory this experiment is scheduled in five to six three-hour lab sessions, but, as the experiment is modular, work can be easily tailored to the structure of each course and the time availability. The main teaching steps are: (i) *an introduction to ion chromatography methods*. The goal is for students to become familiar with some aspects of instrumentation used with ion chromatography and to demonstrate the utility of this knowledge in a real-world chemical experiment; (ii) *comparison between the three types of ion chromatography*. The goal is for students to evaluate and discuss the most important characteristics and advantages and disadvantages each one; (iii) *analysis of some anions in real samples using suppression during chemical analysis*. Students analyzed real samples and the results obtained were compared with the corresponding reference methods using t-test; (iv) *evaluation of knowledge gained by students*. After the instruction, a post-test was applied to determinate their knowledge related to Ion Chromatography. The test was running using the e-learning system Moodle.

The proposed study is suitable as a teamwork exercise for enhancing some analytical chemistry concepts and is also useful to instructors by providing information and feedback on the degree of assimilation by the students of the taught material.

2. Reagents and equipment

2.1 Apparatus

A Metrohm Ion Chromatograph 861 IC was used for all determinations. Metrodata PC software (IC Net 2.3) was used for the acquisition and manipulation of data. Metrosep A Supp 4/5 guard column was employed prior to analytical column. An Ion Chromatography column Metrosep A Supp 5-250 (4.0x250 mm) for anions from Metrohm was running.

2.2 Reagents and solutions

All reagents used were HPLC grade and purified water from a Milli Q system (resistivity 18 MΩ cm and TOC <10 ppb) was used throughout the experiments. Standard solutions of fluoride, nitrate, nitrite, sulphate, phosphate and chloride were purchased from Fluka. Working solutions for chromatographic and other comparative methods were prepared by dilution of the corresponding standard solutions with the purified water. All the other reagents used were of analytical reagent grade.

2.3 Chromatographic method

The chromatography method was performance following the instructions provided by the manufacturer.

2.4 Photometric and potentiometric procedures

The recommended procedures for reference methods were carry out according to "*Standard Methods for the Examination of Water and Wastewater*".

3. Results and Discussion

In a previous stage a group of 24 students was selected for the proposed study. Pupils were divided into two groups: a group for traditional learning and other for active learning. Selected students for active learning were included in all stages of the teaching process. For the study of three types of chromatography teachers have to explain some concepts such as retention time, retention factor, peak asymmetry factor, resolution, distribution coefficient, selectivity coefficient, height equivalent to a theoretical plate (HETP), tailing, injection peak and system peak. For simplicity we have chosen a retention model for one eluent with one anion [16] to take into account the eluent concentration and exchange capacity of resin in order to approach the problem of separation in Ion Chromatography. In addition, an excellent review about the origin and characteristics of the system peak appearing in ion chromatography with the most commonly used eluent, bicarbonate and carbonate, was provided to students [27].

If electroneutrality is assumed, then the simplest approach to a retention model for isoionic displacement is one in which a single eluent ion E^{y-} competes with one analyte anion A^{x-} for the functional groups of the stationary phase. The concentration of the eluent anions E^{y-} remains constant with time (isocratic elution). The exchange sites on a separating column with a capacity Q are occupied by eluent anions E^{y-} at the start of the chromatographic process. If a sample containing the analyte ion A^{x-} is added to the column, then the following equilibrium becomes established between the stationary phase (index S) and the mobile phase (index M):

$$yA_M^{x-} + xE_S^{x-} \rightarrow yA_S^{x-} + xE_M^{y-} \quad (1)$$

According to the law of mass action this equilibrium can be described by a thermodynamic equilibrium constant. Then the following thermodynamic equilibrium constant is obtained:

$$K_{A,E} = [A_S^{x-}]^y [E_M^{y-}]^x / [A_M^{x-}]^y [E_S^{y-}]^h \quad (2)$$

The retention model proposed above attempts to make predictions about the retention behaviour of participating analytes under particular chromatographic conditions based on the law of mass action. If the resulting model is suitable for explaining the macroscopic observations then it is possible, for example, to optimize an elution system for a particular separation problem. With this model pupils can see why monovalent ions elute before than divalent or trivalent anions, why increasing the eluent concentration, the elution accelerates and why higher exchange capacities, *Q*, increase the retention times of analytes.

In the second stage of the study pupils focused on the underlying principles of IC, IPC and IEC. For this purpose, they made some posters explaining the three different types of ion chromatography. Figs. 1-3 show the different mechanisms of separation inside the chromatographic column.

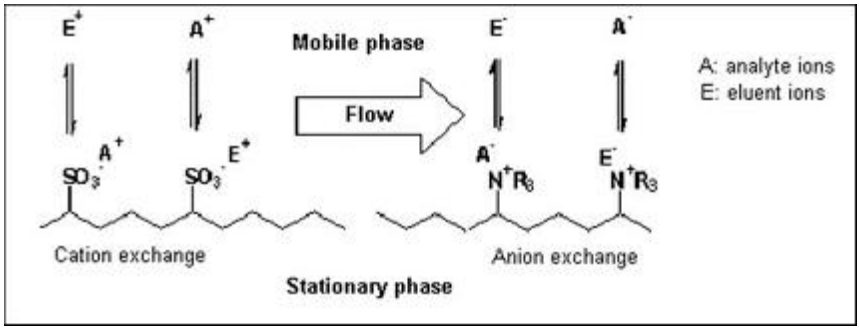

Fig. 1. Mechanism of separation in Ion Exchange chromatography for cations and anions

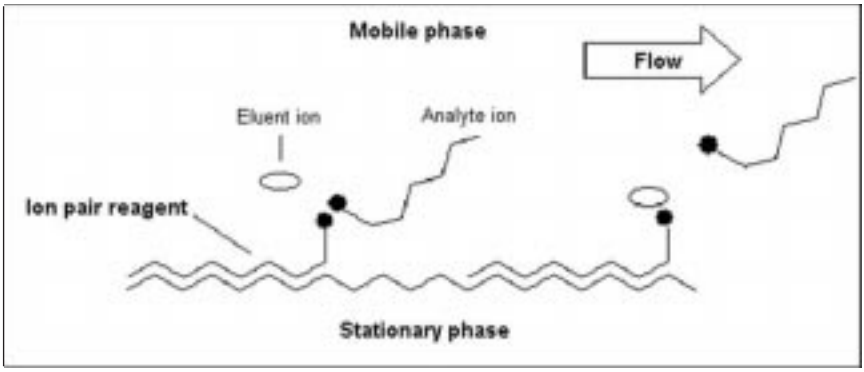

Fig. 2. Mechanism of separation in Ion pair chromatography

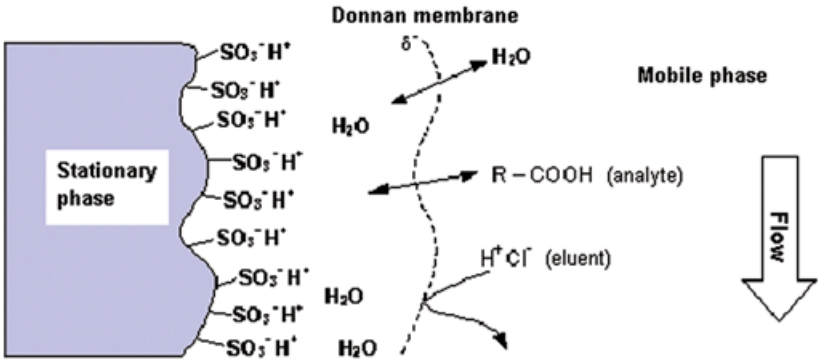

Fig. 3. Mechanism of separation in Ion Exclusion chromatography

After this, pupils were asked to construct some tables with the abbreviations, characteristics, and the more suitable analytical applications of each version of Ion Chromatography (Table 1).

Once the methods were described, students were led in a discussion of the major advantages and disadvantages of each technique. At the end of this section, students were asked to present what they had made.

The third stage consisted of the chemical analysis of real samples with the chromatograph. Two real samples of drinking water were analyzed using Ion-Exchange Chromatography with chemical suppression and elimination of CO_2. Previously both samples were filtered through a 0.45 µm membrane filter to prevent the column from clogging. The experimental conditions and the values of chromatographic variables selected are gathered in Table 2. Molecular absorption spectroscopy and potentiometric methods were carried out by pupils for comparison with the chromatographic results. In both cases, no previous treatment prior to analysis was necessary.

Table 1. Comparison and applications of three types of Ion chromatography

Type of Ion Chromatography	Analytical applications	Matrix	Detector	Measured signal
Ion Exchange chromatography (IC)	Determination of inorganic and organic cations and anions	Wastewaters, drinking waters and mineral waters	Conductimetry	conductivity
Ion Pair chromatography (IPC)	Organic acids and bases very polar, biogenic aminoacids	Wine and fish	Pulsed amperometry	Intensity of current
Ion Exclusion chromatography (IEC)	Weak acids such as carboxylic acids, carbohydrates, phenols or aminoacids	Chocolate, milk, chewing gum	UV-visible spectrometry	Quantity of light

Table. 2. Experimental conditions for separation and determination of some anions

Chromatographic variable	Value
Flow rate	1 ml min^{-1}
Volume injected (loop)	20 µl
Detector	Thermostated conductivity meter
Phase mobile	Sodium hydrogen carbonate (1.0 mmol)/Sodium carbonate (3.2 mmol)
Suppressor	Diluted sulphuric acid
Column	Anion exchanger (Capacity = 94 µ mol Cl^-)
Column working pressure	15 MPa
Guard column	yes
Elimination of CO_2	yes

A typical chromatogram is shown in Fig. 4. Students could see that the peak system corresponding to the elution of carbonate from the mobile phase and that the retention time for the peak system depends on the column used.

The analytical results obtained are summarized in Table 3. If we assume only errors in the dependent variable (conductivity) and precision changes with concentration, *id est*, the standard deviation of the data is proportional to the magnitude of the value being measured, experimental data can be fitted with non-weighted linear least-squares regression. A linear relationship was found in all cases with a correlation coefficient (r) between 0.98-0.99. In addition, student could see that the number of plates is different for each analyte (Table 4).

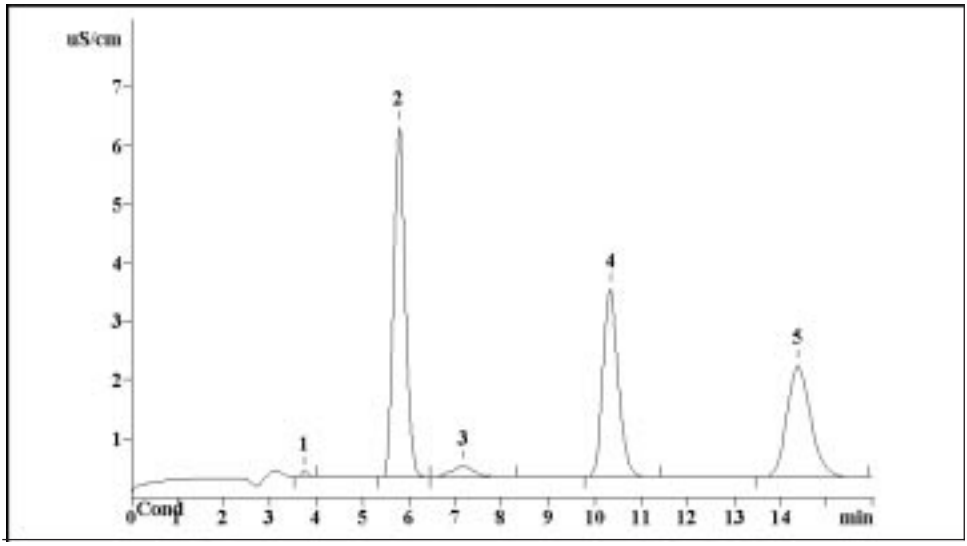

Fig. 4. Chromatogram for the analysis of drinking water 2. Peak 3 is the system peak

Table 3. Analysis of some anions in drinking water with the use of IC

Sample	Found[a]	Reference method[b]
1		
Fluoride	3.4	4.1
Nitrate	16.7	16.0
Nitrite	11.2	11.6
Chloride	17.1	17.7
Phosphate	20.4	19.7
2		
Fluoride	0.05	1.0
Sulphate	14.4	14.9
Nitrate	10.3	11.0
Chloride	5.8	6.4

[a]Mean value (n=5)

[b]Nitrate, Nitrite, Fluoride and Phosphate: Visible Spectroscopy; Chloride: Ion Selective Electrode

Student applied unpaired parametric t-test [28] under the assumptions of normal distribution and equality of variances of data. A 95% confidence level was used. No significant differences between IC and the corresponding reference method were found.

Finally, in order to evaluate the extent of understanding gained by students in experimental aspects of Ion Chromatography, a multiple-choice test was developed by the teacher. The test was based on twenty typical situations relating to classical problems found with columns, detectors, phase polarities and other variables of importance in Ion chromatography. The proposed test was performed using the course management system Moodle. Results obtained from this study show the advantages of the active learning compared with the traditional learning-teaching (Fig. 5).

Table 4. Data of analysis of drinking water

Peak	Component	t_R (min)	Conc. (mg/L)	Numberof plates
1	Fluoride	3.7	0.05	2680
2	Chloride	5.8	6.5	2500
3	System peak	6.8		
4	Nitrate	10.3	8.4	4560
5	Sulphate	14.4	14.5	3670

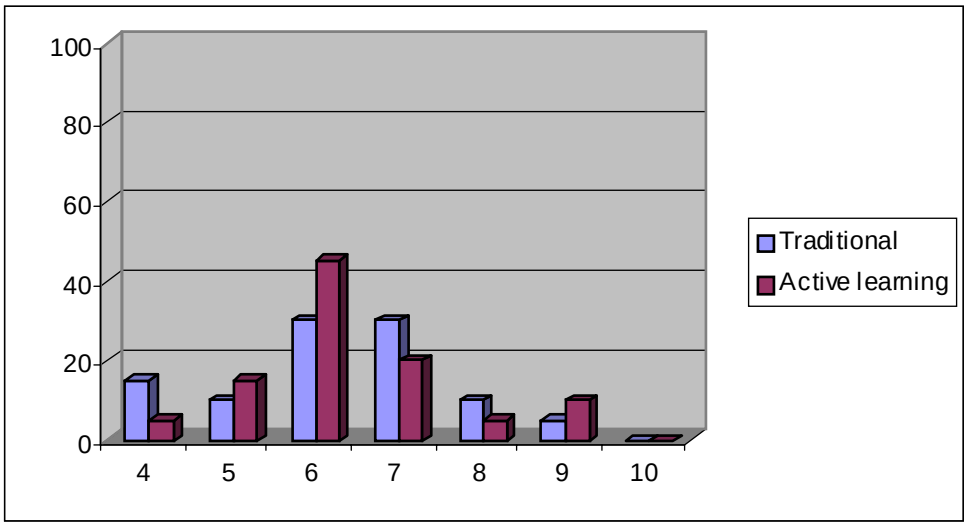

Fig. 5. Qualifications obtained by both groups of pupils. Range from 0 to 10

4. Conclusions

Ion Chromatography measurements have been used by students to improve their analytical concepts in this technique. As students performed this activity they acted like scientists: they designed their own experiments, control variables, took measurements, represented their results in tables and graphs, and made claims based on an analysis of their results.

Through an analysis of the three types of Ion Chromatography, students are able for themselves to point out the most important variables involved in the analysis of different samples.

The results of this study suggested an effective teaching-learning process. Performing this type of study the students begin to appreciate the importance of using the three types of IC for the analysis of some anions of analytical and environmental interest.

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NOTES

1. Dionex Corporation. Application Note 187. http://www.dionex.com

2. Metrohm AG. Technical Poster: Sequential suppression for conductivity detection in ion chromatography http://www.metrohm.com

3. Waters Corporation. http://www.waters.com/waters/library.htm?cid=10011431&lid=1512139

4. US EPA Method 300.1

5. US EPA Method 314.0

6. Directiva 98/83/CE del Consejo, de 3 de noviembre de 1998, relative a la calidad del aqua destinada al consume humano.

REFERENCES

1. Gabel, D. Improving Teaching and Learning Through Chemistry Education Research. *J. Chem. Educ*. **76**, 548-554 (1999).

2. Acar, B., L. Tarhan. Effects of Cooperative Learning Strategies on Students' Understanding of Concepts in Electrochemistry. *Intern. J. Science & Mathematics Education* **5**, 349-373 (2007).

3. Sivan, A,, R.W. Leung, C.-c. Woon, D. Kember. An Implementation of Active Learning and Its Effect on the Quality of Student Learning. *Innovations Education & Teaching Intern*. **37**, 381-389 (2000).

4. De Volder, M.L., H.G. Schmidt, W.S. De Grevem J.H.C. Moust. Motivation and Achievement in Cooperative Learning. In.: H.C. Van Der Berchen, T.C.M. Bergen, E.E.I. De Bryun (Eds.). *Achievement and Task Motivation.* Swets North America, Berwyn, 1989, pp. 123-127.

5. Lazarowitz, L., R. Hetz-Lazarowitz, J.H. Baird. Learning Science in Cooperative Setting: Academic Achievement Affective Outcomes. *J. Research Science Teaching* **31**, 1121-1131 (1994).

6. Cooley, J.H. A Problem-Solving Approach to Teaching Organic Laboratory. *J. Chem. Educ*. **68**, 503-504 (1991).

7. Domin, D.S. A Content Analysis of General Chemistry Laboratory Manuals for Evidence of Higher-Order Cognitive Tasks. *J. Chem. Educ*. **76**, 109-111 (1999).

8. Okebukola, P.A. An Investigation of Some Factors Affecting Students' Attitudes Toward Laboratory Chemistry. *J. Chem. Educ*. **63**, 531-532 (1986).

9. Sinniah, K., K. Piers. Ion Chromatography: Analysis of Ions in Pond Waters. *J. Chem. Educ*. **78**, 358-362 (2001).

10. Small, H. Ion Chromatography: An Account of Its Conception and Early Development. *J. Chem. Educ*. **81**, 1277-1284 (2004).

11. Rakestraw, D.J., R.J. Whelan, T.E. Hannon, R.N. Zare. Application of Ion Chromatography to the Investigation of Real-World Samples. *J. Chem. Educ.* **81**, 1299-1302 (2004).

12. Evans, B. The History of Ion Chromatography: The Engineering Perspective. *J. Chem. Educ*. **81**, 1285-1292 (2004).

13. Bello, M.A., A.G. Gonzalez. Determination of Phosphate in Cola Beverages Using Nonsupressed Ion Chromatography: An Experiment Introducing Ion Chromatography for Quantitative Analysis. *J. Chem. Educ*. **73**, 1174-1175 (1996).

14. Schwedt, G. *Chromatographische Trennmethoden.* Thieme, Stutgart, 1994.

15. Fritz, J.F. Ion Chromatography. *Anal. Chem.* **59**, 335A-344A (1987.

16. Haddad, P.R., P.E. Jackson. *Ion Chromatography: Principles and Applications.* Elsevier, Amsterdam, 1990.

17. Hou, S., M. Ding, J. Zhu. Simultaneous Determination of Silicon and Phosphorus in Soils and Plants be Reversed-Phase Ion-Pair Chromatography. *Talanta* **75**, 178-182 (2008).

18. Zheng, J., Y. Shibata, N. Furata. Determination of Selenoamino Acids Using Two-dimensional Ion-Pair Reversed Phase Chromatography with On-line Detection by Inductively Coupled Plasma Mass Spectrometry. *Talanta* **59**, 27-36 (2003).

19. Wolf, C., T. Storm, F.T. Lange, T. Reemtsma, H.-J. Brauch, S.H. Eberle, M. Jekel. Analysis of Sulfonated Naphtalene-Formaldehyde Condensates by Ion-Pair Chromatography and Their Quantitative Determination from Aqueous Environmental Samples. *Anal Chem*. **72**, 5466-5472 (2000).

20. Werner, A., W. Siems, G. Gerber, H. Schmidt, S. Gruner, H. Becker. Application of Reserved-Phase and Ion-Pair HPLC for Investigation of Nucleotide Matabolism in Erythrocytes, Reticulocytes and Tumour Cells. *Chromatographia* **25**, 237-241 (1988).

21. Bylund, D., S.H. Norstr?m, S.A. Essen, U.S. Lundström. Analysis of Low Molecular Mass Organic Acids in Natural Waters by Ion Exclusion Chromatography Tandem Mass Spectrometry. *J. Chromatography A* **1176**, 89-93 (2007).

22. Ohta, K., M. Ohashi, J.-Y. Jin, T. Takeuchi, C. Fujimoto, S.-H. Choi, J.-J. Ryoo, K.-P. Lee. Separation of Aliphatic Carboxylic Acids and Benzenecarboxylic Acids in a Weakly Acidic Cation-Exchange Resin by Elution with Benzoic Acid-β-Cyclodextrin. *J. Chromatography A* **997**, 117-125 (2003).

23. Johnson, S.K., L.L. Houk, J. Feng, D.C. Johnson, R.S. Houk. Determination of Small Carboxylic Acids by Ion Exclusion Chromatography with Electrospray Mass Spectrometry. *Anal. Chim. Acta* **341**, 205-216 (1997).

24. Mori, M., H. Itabashi, M. Ikeda, K. Tanaka. Ion-Exclision Chromatography with the Direct UV Detection of Non-absoprbing Inorganic Cations Using an Anion-Exchange Conversion Column in the Iodide-form. *Talanta* **70**, 174-177 (2006).

25. Ohta, K., K. Tanaka. Ion-Exclusive Chromatography of Carboxylic Acids on Silica Gel with Aluminium. *J. Chromatography A* **850**, 177-185 (1999).

26. Small, H., T.S. Stevens, W.C. Bauman. Novel Ion Exchange Chromatographic Method Using Conductometric Detection. *Anal. Chem*. **47**, 1801-1809 (1975).

27. Watanabe, H., H. Sato. Retention Behavior of the Second System Peak in Ion-Exchange Chromatography with Dibasic Acid Eluent Systems. *Analytical Sciences* **11**, 923-927 (1995).

28. Miller, J.C., J.N. Miller. *Statistics for Analytical Chemistry*. Prentice Hall, Upper Saddle River, 1993.

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