DIONEX

Application Note 106

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Ion Chromatography in the Pharmaceutical Industry

INTRODUCTION

The U.S. Food and Drug Administration (FDA) protects the consumer by ensuring that product quality and safety are maintained. To achieve this objective, the FDA requires that prescription drugs be produced only according to approved procedures and that the manufacturing equipment meets cleanliness requirements. To conform to these FDA requirements, pharmaceutical companies need to ensure that their product was manufactured according to approved methods and that it meets certain specifications. The authenticity can be determined by testing the final product; however, if a problem is present it then needs to be traced, possibly even back to contaminants in the ingredients.

In addition to ensuring that the drug has been manufactured according to approved procedures, the pharmaceutical companies must also certify that the equipment used during the manufacturing process is not only cleaned of the residues from the previous production batch, but that the cleaning solutions have also been rinsed away.

Ion chromatography is a well established and popular technique for the analysis and quantification of charged species. Although reversed-phase chromatography is the most commonly used chromatographic method for the analysis of drugs in the pharmaceutical industry, ion chromatography serves as an alternative method for ionic species that is often used to provide confirmation for the analysis of drugs, including sulfa drugs. This application note describes the use of ion exchange chromatography and ion exclusion chromatography in the pharmaceutical industry for the determination of counterions and excipients in “off-the-shelf” drugs. The topic of cleaning validation is also discussed as it applies to charged species.

EQUIPMENT

A Dionex chromatographic system consisting of:

- Gradient Pump
- Chromatography Module
- Conductivity Detector
- Absorbance Detector
- Eluent Organizer

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm resistance or better

Anion Analysis

Sodium hydroxide, 50% w/w (Fisher Scientific)

Sodium chloride

Sodium phosphate, dibasic

Acetonitrile

Methanol

Cation Analysis

Methanesulfonic acid (Fluka Chemika-BioChemika)

Organic Acid Analysis

Perfluorobutyric acid (Fluka Chemika-BioChemika)

Tetrabutylammonium hydroxide (Dionex)

PREPARATION OF SOLUTIONS AND REAGENTS

100 mM Sodium Hydroxide

Weigh 992 g (992 mL) of 17.8-MΩ deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the scale and add 8.00 g (5.25 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

5.00 mM Sodium Hydroxide

Weigh 999.6 g (999.6 mL) of 17.8-MΩ deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the scale and add 0.400 g (0.262 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

1.00 M Sodium Chloride

Weigh 58.45 g of sodium chloride and place into a 1-L volumetric flask. Add approximately 500 mL of deionized water and swirl until the chloride is dissolved. Dilute to the mark and mix thoroughly.

1.00 mM Sodium Phosphate

Weigh 0.142 g of sodium phosphate and place into a 1-L volumetric flask. Add approximately 500 mL of deionized water and swirl until the phosphate is dissolved. Dilute to the mark and mix thoroughly.

100 mM Methanesulfonic Acid

Weigh out 9.61 g of methanesulfonic acid (MSA). Carefully add this amount to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly.

0.400 mM Perfluorobutyric Acid

Perfluorobutyric acid (heptafluorobutyric acid) is supplied by FLUKA in 10.0-mL bottles. Dilute the entire contents of one 10.0-mL bottle in 1 L to obtain a 0.0772 M stock solution. Dilute 5.20 g of the stock solution in 1 L to obtain the 0.400 mM working eluent.

5 mM Tetrabutylammonium Hydroxide

Dilute 200 mL of the Dionex 0.1 M TBAOH ion-pairing reagent (P/N 35360) to 4 L with water. Alternatively, dilute 10 mL of 55% tetrabutylammonium hydroxide in 4 L of water.

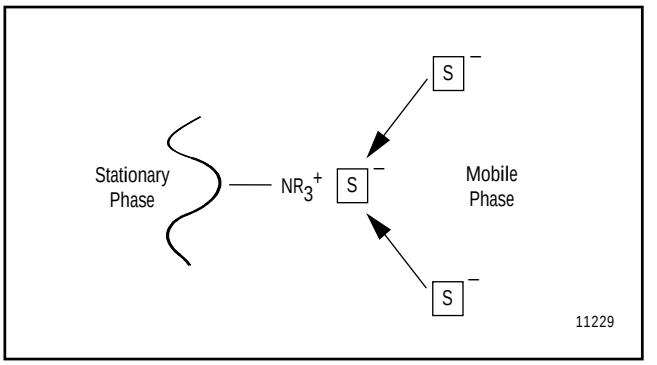

Figure 1 *Typical anion exchange resin.*

RESULTS AND DISCUSSION

The FDA ensures that product quality and safety are maintained by requiring that drugs are manufactured according to approved procedures, and that the equipment is clean. To comply with these FDA requirements, most pharmaceutical companies test samples of the finished product for authenticity, and clean and test equipment for cleanliness following the manufacturing process. If a problem is detected in the final product, the source of the problem must be identified. The problem could range from incorrect concentrations of the various ingredients to the presence of contaminants introduced from impure ingredients.

Ion exchange chromatography is a well established technique for the analysis of charged species such as the strong acid anions, fluoride, chloride, sulfate, and phosphate. The exchange functional group in anion exchange chromatography is generally a quaternary ammonium base, as illustrated in Figure 1. Separation occurs as a result of the different affinities of the analytes and counterions for the fixed charge. In cation exchange chromatography, the functional group is usually a sulfonate or carboxylate ion, but the separation mechanism is analogous. Uncharged species are unretained.

The mechanism of retention in ion exclusion chromatography is illustrated schematically in Figure 2. In ion exclusion chromatography, uncharged species are proposed to be separated as a result of three mechanisms: (1) Donnan exclusion, (2) adsorption, and (3) steric exclusion processes. Charged species pass through the column unretained. Thus, this technique is well suited for the separation of weakly charged species such as weak carboxylic acids. The Donnan

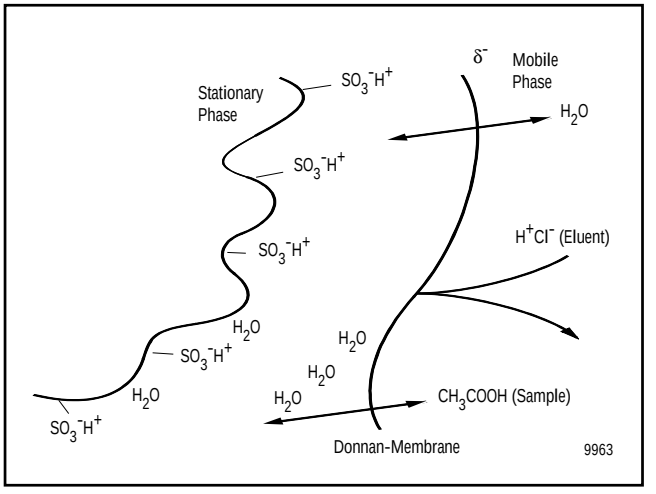

Figure 2 *Mechanism of separation with ion exclusion chromatography.*

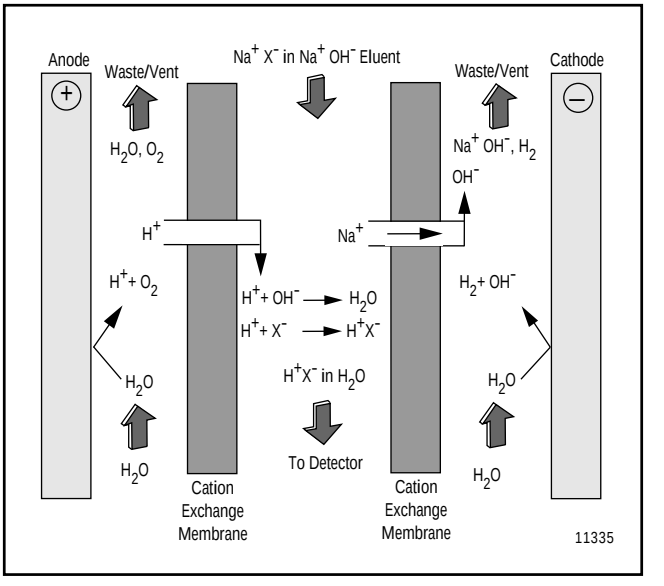

Figure 3 *Schematic diagram illustrating the mechanism of suppression.*

membrane can be envisaged to be an invisible shell around the resin particle that allows the passage of neutral species, but prevents the passage of negatively charged species. Using this separation mechanism, weakly ionized acids are separated based on differences in their pK_as. Strong inorganic acid anions are not retained by the stationary phase but are eluted in the void volume of the column. Uncharged species will cross to the resin and be separated by adsorption and steric exclusion processes. The ion exclusion columns used for the separation of organic acids contain high-capacity, macroporous, sulfonated cation exchange resins as illustrated in Figure 2.

After separation, the nonchromophoric ions are detected using suppressed conductivity detection. A schematic diagram of an anion suppressor with sodium hydroxide as the eluent is shown in Figure 3. The suppressor serves two purposes: (1) to lower the background conductivity caused by the eluent, and (2) to increase the conductivity of the analyte. In this example, the sodium counterion from the eluent is exchanged for a hydronium ion. As a result of this exchange, the sodium hydroxide is converted to water, which has a much lower conductivity. Simultaneously, the sodium counterion associated with the analyte is also exchanged for a hydronium ion. Since the hydronium ion has a higher conductivity than the sodium ion, the analyte will have an overall higher response. The net result of suppression, therefore, is a higher analyte signal against a lower eluent background.

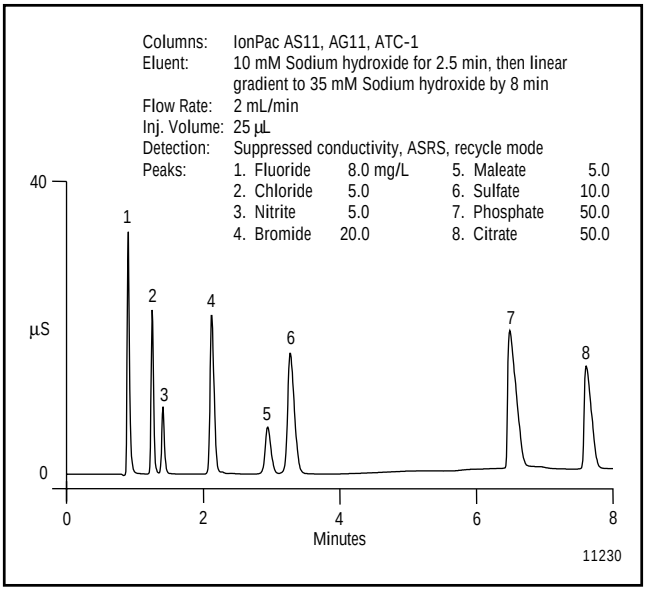

Figure 4 *Separation of eight standard anions by anion exchange chromatography.*

Figure 4 shows the separation of six standard anions and two organic acids that might be found in pharmaceutical formulations by anion exchange chromatography using the IonPac® AS11 column. Using a hydroxide gradient ensures that all eight peaks are eluted within 8 minutes. Reproducibility for this method is on the order of 0.5% or better for retention times and 2% or better for peak areas.

Linearity is good over the range tested (1.5 orders of magnitude), with a coefficient of determination of $r^2 = 0.999$ for most of the analytes.

Figure 5 shows the use of a different anion exchange column, the OmniPac® PAX-100, for the separation of a series of aromatic acids. Sodium hydroxide was added to the eluent system to maintain a high pH for the ionization of the acids. Acetonitrile was added to modify the selectivity. The Cl^-/OH^- ratio was kept constant throughout the gradient to minimize the reequilibration time. Because the analytes are chromophoric, they were monitored using absorbance detection at 254 nm.

Figure 6 shows the separation of six standard cations by cation exchange chromatography using the IonPac CS12A column with suppressed conductivity detection. This column was developed for use with methanesulfonic acid or sulfuric acid, giving similar selectivity with either eluent. A suppressor was used to lower the conductivity of the eluent by removing the methanesulfonate ions from the eluent stream before they reached the detector.

Figure 7 shows the separation of several weak organic acids by ion exclusion chromatography. The IonPac ICE-AS6 column is an ion exclusion column designed for the efficient separation of low molecular weight aliphatic organic acids, such as hydroxy-substituted organic acids, as well as species such as aliphatic alcohols and glycols.

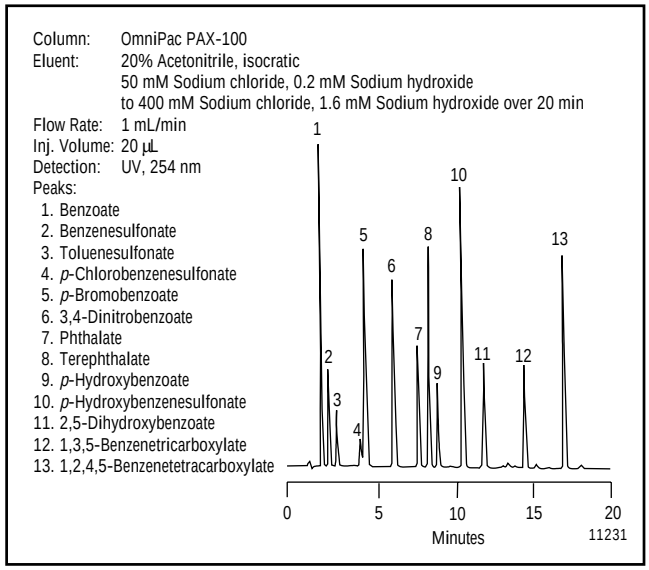

Figure 5 *Separation of standard aromatic acids by anion exchange chromatography.*

The standard eluent for use with the IonPac ICE-AS6 is 0.4 mM heptafluorobutyric acid (perfluorobutyric acid). Other monoprotic acids can be used; however, the background conductivity will be higher.

IC can be used to help the pharmaceutical industry check the quality of the ingredients. Ingredients range from the chemicals used as active ingredients or excipients to pharmaceutical grade water and water for injectables.

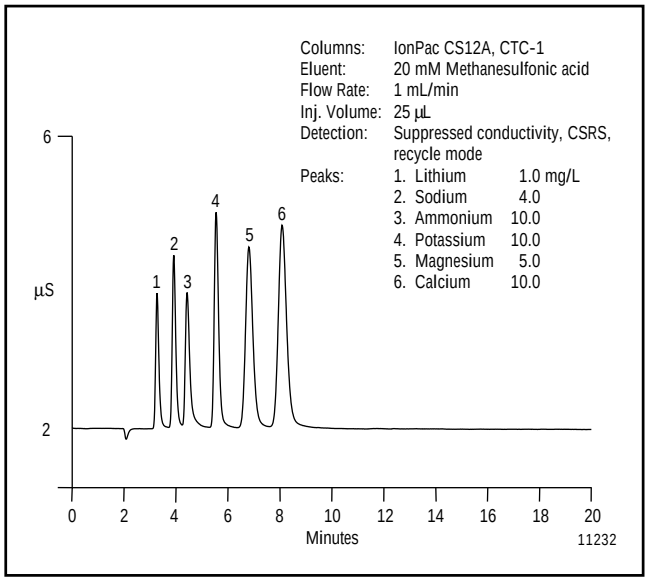

Figure 6 *Separation of standard cations by cation exchange chromatography.*

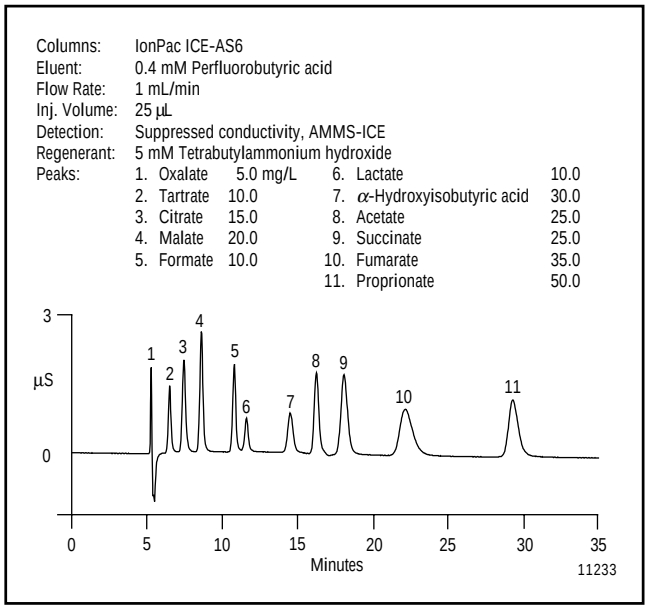

Figure 7 *Separation of standard organic acids by ion exclusion chromatography.*

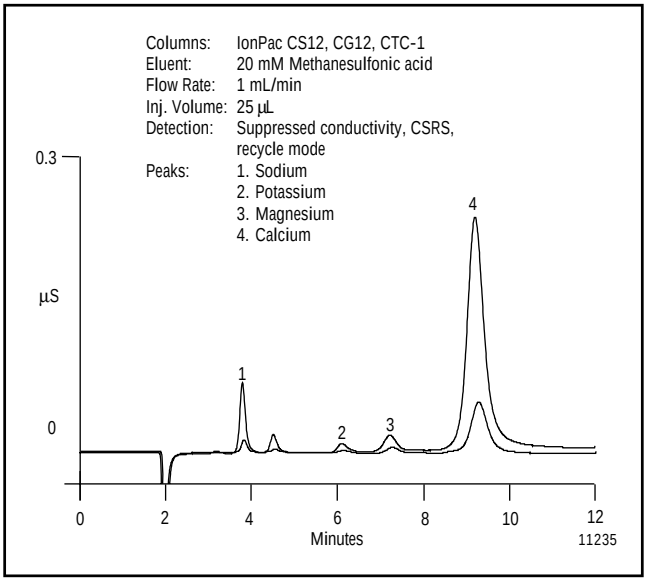

Figure 8 *A chromatogram showing the separation of cations in a solution of water for injectables by cation exchange chromatography is superimposed on a water blank.*

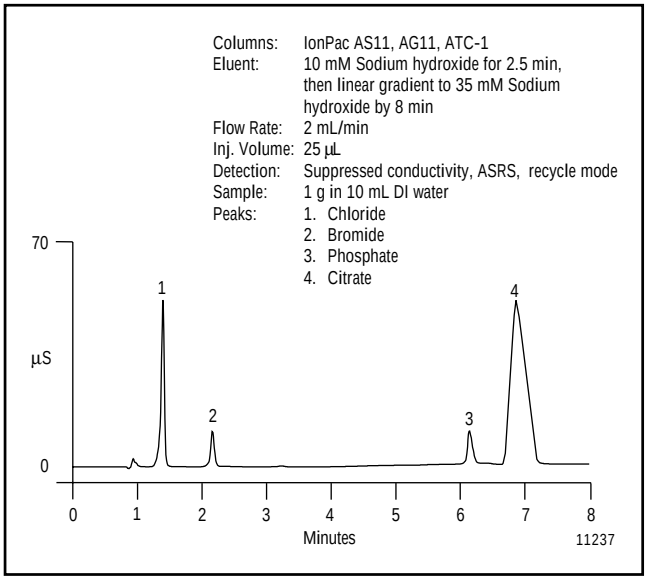

Figure 9 *Separation of anions in an analgesic/decongestant by anion exchange chromatography.*

Figure 8 shows the separation of cations in a solution of water for injectables. The lower trace shows a water blank with very low levels of sodium and calcium. The upper trace shows the water for injectables. Low levels of potassium and magnesium are apparent in the water for injectables in addition to higher levels of sodium and calcium.

IC also can be of use in the analysis of the final formulation. Since the FDA requires drugs to be produced only according to approved procedures, they often confirm authenticity by testing the nature of the excipients or fillers. Since manufacturing processes use distinct excipients such as phosphate or citrate buffers in injectables, or sorbitol, calcium sulfate, or dibasic calcium phosphate as fillers, IC provides a simple approach.

Figure 9 shows the separation of anions in an analgesic/decongestant using anion exchange chromatography. A guard column was used to protect the analytical column and extend its lifetime. An anion trap column was used to prevent contaminants in the hydroxide eluent from reaching the analytical column. This particular formulation had listed as its active ingredients dextromethorphan hydrobromide (HBr) and pseudephedrine hydrochloride (HCl). As can be seen in the chromatogram, both of these counterions give fairly large peaks. Also listed as inactive ingredients were citric acid and tribasic calcium phosphate, both of which appear as peaks in the chromatogram. Note that there are no interferences from the uncharged ingredients.

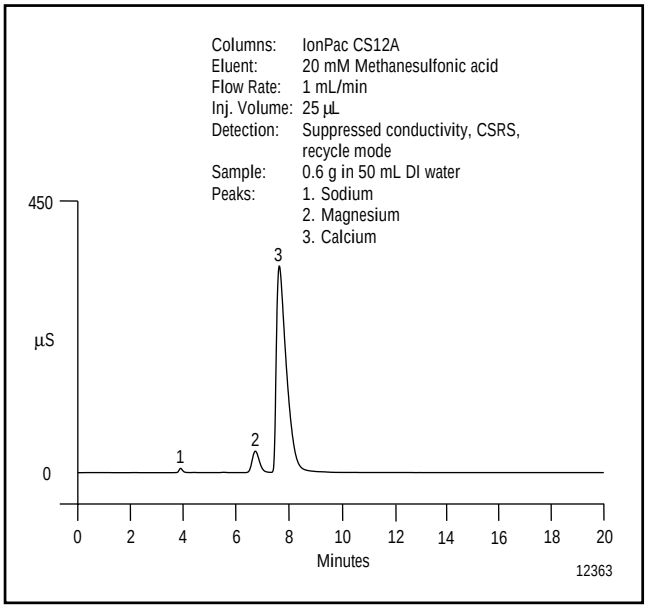

Figure 10 *Separation of cations in an antihistamine/decongestant by anion exchange chromatography.*

Figure 10 shows the separation of cations in an antihistamine/decongestant using cation exchange chromatography. In this formulation, no counterions were detected because they are anionic. However, the inactive ingredients included calcium sulfate and magnesium silicate, as well as several uncharged binders and fillers.

Figure 11 shows the use of ion exclusion chromatography to monitor the presence of organic acids without interference from the strong acid anions. All the strong acid anions are eluted together as a single peak at the beginning of the run and do not interfere with the rest of the separation. The only identified organic acid present is citric acid.

Finally, IC is of use in the area of cleaning validation. There are two common ways to test that the equipment has been rinsed clean. One is to monitor for the last rinsable component, while the other is to test for the most toxic. Samples are typically collected by swabbing, or by using a known volume of water to rinse the surface of the equipment. The problem with the first approach is that generally swabs are taken from hard-to-reach places where chemicals tend to accumulate, thus resulting in falsely high readings. The problem with the second approach is that the chemicals that tend to accumulate in hard-to-reach places are often missed. To overcome these problems, both approaches are often used and both results are considered.

Figure 12 shows the separation of dimethylbenzenesulfonate (DBS) from the other ingredients in MICRO®, a solution used by some pharmaceutical companies for cleaning equipment. DBS is ionic and therefore can be retained on an ion exchange resin. Since it absorbs UV light, it can be monitored with UV detection. The minimum quantifiable limit, that is the signal at 10X the baseline noise, is 0.1 mg/L.

CONCLUSION

Ion Chromatography is a simple and accurate technique for the analysis of ions in ingredients and final products. In addition, it can be used to help with cleaning validation whenever the cleaning solution contains charged species.

LIST OF SUPPLIERS:

Fisher Scientific, 711 Forbes Ave., Pittsburgh,
Pennsylvania, 15219-4785, USA.
Tel: 1-800-766-7000

Fluka, Industriestrasse 25, CH-9471 Buchs, Switzerland.
Tel: +81 755 25 11

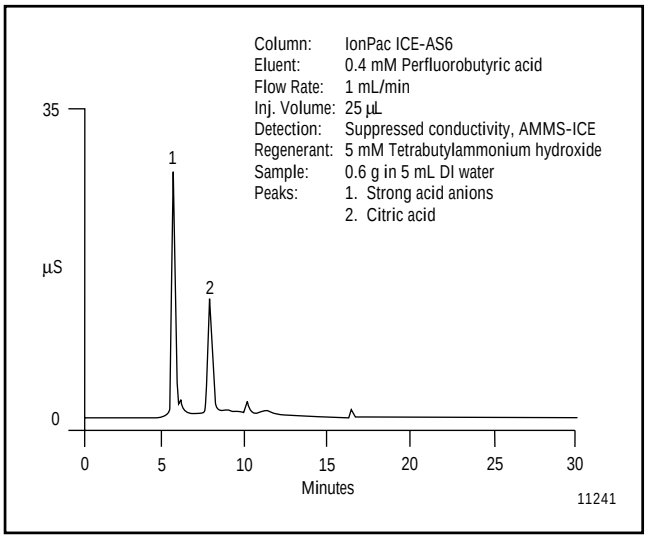

Figure 11 *Analysis of an analgesic by ion exclusion chromatography.*

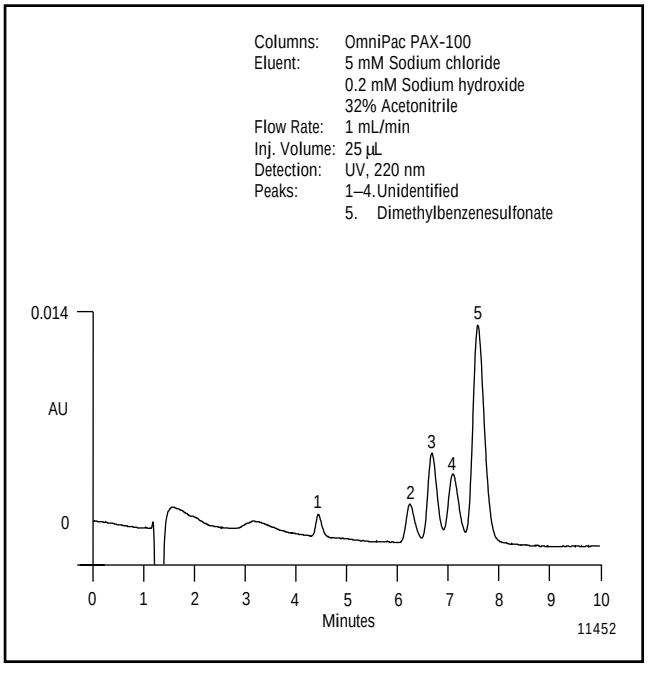

Figure 12 *Chromatogram of a 1 in 360 dilution of a 2% solution of MICRO®.*

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

Dionex Corporation
Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
84119-1484
(801) 972-9292

Dionex U.S. Regional Offices
Sunnyvale, CA (408) 737-8522
Westmont, IL (708) 789-3660
Smyrna, GA (770) 432-8100
Houston, TX (713) 847-5652
Marlton, NJ (609) 596-0600

Dionex International Subsidiaries
Belgium (015) 203800 *Canada* (905) 844-9650 *France* (1) 39 46 08 40 *Germany* (06126) 991-0 *Italy* (06) 30895454
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