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Forensic applications of coupling non-suppressed ion-exchange chromatography with ion-exclusion chromatography

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ABSTRACT

Non-suppressed ion-exchange chromatography coupled with ion-exclusion chromatography separates carboxylic acids and inorganic anions in one isocratic run. The sample is injected into an ion-exclusion column, which provides separation of weak acid anions. The void volume of the ion-exclusion column is collected on-line and injected into an anion-exchange column, which separates the strong acid anions found there. Samples are described in which inorganic anion as well as carboxylic acid interferences were removed by coupled ion chromatography. Additionally, the practicality of the coupled system for forensic sample screening is discussed.

INTRODUCTION

Forensic investigations performed by the National Forensic Chemistry Center (NFCC) frequently require immediate analysis of samples ranging from relatively pure drugs to complex food matrices. Limited time is available for method development. Often, a particular sample matrix has not been worked with previously, nor is it likely to be encountered again. In some cases, analysis of a specific poisonous substance is required. More frequently, inorganic anions and carboxylic acids found in forensic samples are used with the results of other analytical techniques to link or differentiate suspect samples. Which analyte(s) will prove useful is often unknown.

Ion chromatography is a very useful technique for the rapid analysis of multiple ionic analytes in a wide variety of sample matrices. Dilution and filtration is often the only sample preparation necessary; however, in complex matrices the trace analysis of contaminants by ion chromatography can be hindered by the presence of major sample components. While carboxylic acids or other ions may be interferences in the analysis of inorganic anions, their elimination (*e.g.* by solid phase extraction) may also result in analyte losses. For example, the use of a strong cation-exchange resin in the silver form to remove high levels of chloride would simplify the analysis of fluoride and acetate but would also eliminate iodide. In addition, when sample volume is limited, extensive sample pretreatment is not a viable approach. In some forensic cases, only a few hundred microliters of diluted sample have been available for ion chromatographic analysis.

Excellent separations of weak carboxylic acids and inorganic anions in a single analysis have been obtained using gradient ion chromatography [1]. However, gradient analysis requires precisely controlled re-equilibration time between runs, and carbonate contamination of sodium hydroxide eluents can cause baseline drift and poor retention time reproducibility [1]. In many of the forensic samples we have analyzed, there are large differences in the levels of some ions, especially between those naturally occurring and contaminants. When there are disparate levels of ions which have similar retention times, it may be necessary to alter gradient ramps after an initial run to obtain adequate separations in forensic samples, therefore increasing analysis time.

Another approach to the separation of carboxylic acids and inorganic anions is coupling ion exclusion to anion exchange. A system using suppressed ion chromatography was described by Rich *et al.* [2]. However, Cl^- could not be quantified since hydrochloric acid used as ion-exclusion eluent caused a Cl^- system peak. Additionally, packed-bed suppressors in the Ag form were used [2], however packed-bed suppressors suffer from down-time and cause band broadening. Modern membrane suppressors also could not remove the system peak caused by the ion-exclusion eluent (hydrochloric acid or octane sulfonic acid).

A similar system using non-suppressed coupled ion chromatography was developed by Jones *et al.* [3]. The sample is injected onto an ion-exclusion column which provides separation of weak acid anions. The void volume of the ion-exclusion column is collected on-line and injected into an anion-exchange column which can separate strong acids found there. In this way, strong acid anions are analyzed free from carboxylic acid interference. The non-suppressed coupled system does not have a system peak since the eluent for ion exchange is the sodium salt of the acid used for ion exclusion.

In this work, the non-suppressed coupled system was evaluated using actual samples encountered in our lab. Examples will be given to demonstrate the usefulness of this coupled system for samples in which an inorganic anion would interfere in the analysis of a carboxylic acid, as well as cases where an excess of a carboxylic acid would normally hinder the analysis of an inorganic anion by ion exchange. Additionally, the practicality of the coupled system for sample screening will be discussed.

EXPERIMENTAL

Apparatus

The instrumentation used included a Waters (Millipore, Milford, MA, USA) Model 625 pump with system controller, Model 510 pump, two Model 431 conductivity detectors, a Model 700 autosampler, and an automated six-port switching valve. System control and data acquisition were accomplished with a Maxima 825 data station and WD24 interface board (Millipore).

A Waters Ion-Exclusion column (300 × 7.8 mm I.D.) with IC-Pak ion-exclusion Guard-Pak precolumn insert and an IC-Pak A anion-exchange column (50 × 4.6 mm I.D.) were used.

Coupled ion chromatography

Samples were injected onto the ion-exclusion column via the autosampler (100 μl injected) which also triggered the automatic switching program and data acquisition from both detectors. The transfer time of the ion-exclusion void volume peak was determined from injections of 100 μl distilled deionized water. The automated switching valve was triggered to inject a 500-μl fraction of the ion-exclusion void volume onto the anion-exchange column at the time that the void volume peak from the ion-exclusion column was detected (retention time, t_r = 4.65 min). The timed events program used has been described elsewhere [4]. The eluents used were 1 m*M* octanesulfonic acid (pH 3) and 3 m*M* octanesulfonate (pH 6) for the ion-exclusion and anion-exchange separations, respectively. Experiments with anion-exchange only separations used 3 m*M* octanesulfonate eluent. A flow-rate of 1 ml/min was used in all cases.

Reagents, standards and samples

Eluents for ion exclusion and ion exchange were prepared from the same lot of octanesulfonic acid sodium salt (98%, Aldrich, Milwaukee, WI, USA) to minimize sulfate contamination in the ion-exclusion eluent. Concentrated octanesulfonic acid eluent was prepared as described by Jones *et al.* [3] by mixing the sodium salt with cation-exchange resin (Bio-Rad AG 50W-X12, 200–400 mesh, hydrogen form) and filtering through a 0.2-μm filter (Anodisc 47, Alltech, Deerfield, IL, USA).

Individual 1000 mg/l standards were prepared from weighed amounts of salts. Standard mixtures for calibration were prepared by dilution of stock single-ion standards.

Samples were prepared by dilution in 18 MΩ distilled deionized water and filtration through 0.2-μm filter. Food samples were also passed through activated C_{18} cartridges (Maxi-Clean, Alltech) prior to injection.

RESULTS AND DISCUSSION

Fig. 1 illustrates the separation of a mixture of fifteen inorganic anions and carboxylic acids

obtained using coupled ion chromatography. Ion-exclusion separations are based not only on Donnan exclusion (neutral species migrate through the water phases toward the resin core, whereas ionized species are excluded), but are also based upon steric exclusion, and adsorption effects [5,6]. Thus, fluoroacetate, fluoride, glycolate, formate, acetate, propionate, adipate, azide and butyrate elute generally in order of increasing pK_a (Table I and Fig. 1a). However, glycolate (pK_a 3.83) elutes before formate (pK_a 3.75). Strong acids iodate, chloride, nitrate, iodide and sulfate are separated by anion exchange, generally by size and charge (Table I and Fig. 1b). Retention time reproducibility of ten replicate injections on the same day was better than 0.6% relative standard deviation for all compounds except adipate (1.2%). Repeatability of response and linear range are presented in Table I. Detection limits (defined as three times the standard deviation of baseline data points in a blank) range from 0.007 mg/l for fluoride to 1 mg/l for butyrate. Trace enrichment prior to separation can be used to lower detection limits [3]; however, typically sample volume is limited in our work, and thus only detection limits obtainable by direct injection are reported. Repeatability of peak areas ranged from 0.8 to 2.8% R.S.D. at concentrations from 20 to 200 times the detection limit.

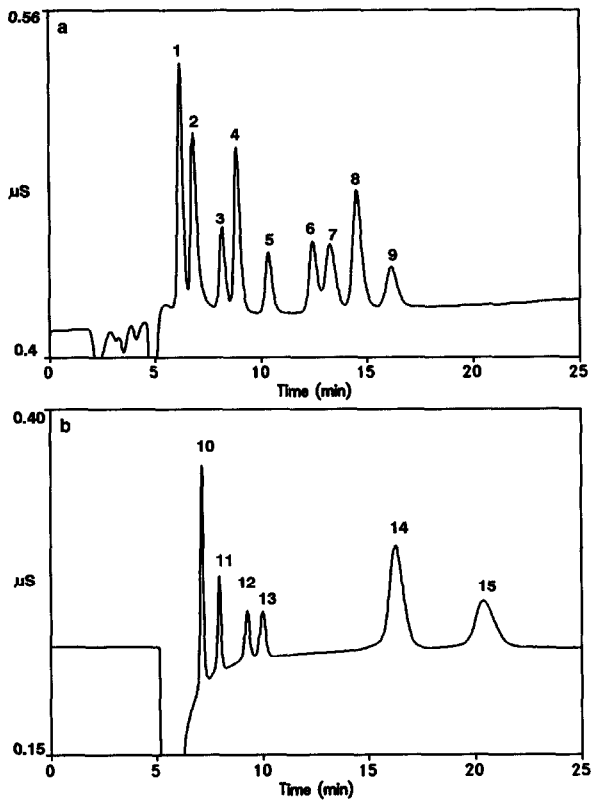

Fig. 1. Separation of carboxylic acids and inorganic anions by the coupled system. (a) Ion-exclusion chromatogram; peaks: 1 = fluoroacetate (1.26 ppm); 2 = fluoride (0.5 ppm); 3 = glycolate (2 ppm); 4 = formate (2 ppm); 5 = acetate (10 ppm); 6 = propionate (20 ppm); 7 = adipate (20 ppm); 8 = azide (11.6 ppm); 9 = butyrate (20 ppm). (b) Anion-exchange chromatogram; peaks: 10 = iodate (50 ppm); 11 = chloride (1 ppm); 12 = bromide (2 ppm); 13 = nitrate (2 ppm); 14 = iodide (20 ppm); 15 = sulfate (5 ppm).

A positive peak was observed in the void volume of the ion-exclusion chromatogram at higher concentrations of inorganic anions (*e.g.* 20 mg/l Cl^- or 100 mg/l Br^-). This is not an indication of non-reproducible transfer onto the anion-exchange column at higher concentration. Rather, it occurred when the conductance of the inorganic anions was sufficient to be observed above the negative signal of water in the ion-exclusion chromatogram. Nitrite and phosphate were not determined using this system. According to Jones *et al.* [3], nitrite and phosphate are not quantitatively transferred to the anion-exchange column in the 500-µl fraction chosen in this system and are not well retained by ion-exclusion mechanisms.

Inorganic anion interferences

The forensic analysis of pharmaceuticals is often complicated by high levels of chloride which can typically mask trace levels of weak carboxylic acids such as acetate or formate. In one poisoning case, traces of acetate and formate were readily detected in a drug which was a hydrochloride salt using the coupled system. Since Cl^- eluted in the void volume of the ion-exclusion column, the coupled system effectively removed Cl^- allowing the detection of acetate and formate which were separated by ion exclusion. Other ions of interest, such as I^- and SO_4^{-2} were determined in the same run on the anion-exchange column.

Salad dressing is another matrix in which chloride can interfere in the analysis of acetate (from vinegar). As stated above, the strong acid anion Cl^- does not interfere with the weak acid acetate in the coupled system. Additionally, F^- (pK_a 3.18) and

TABLE I

LINEARITY, RELATIVE STANDARD DEVIATION (R.S.D.) AND DETECTION LIMITS

Compound	pK_a[a]	Linearity correlation coefficient (concentration range)	R.S.D. (area) (%) (n = 10) (concentration)	Detection limit (mg/l)
Weak acids				
Fluoroacetate	2.59	0.9997 (0.09–3.8 ppm)	0.8 (1.3 ppm)	0.015
Fluoride	3.18	0.995 (0.05–2 ppm)	1.6 (0.5 ppm)	0.007
Glycolate	3.83	0.9989 (0.1–10 ppm)	1.8 (2 ppm)	0.06
Formate	3.75	0.9987 (0.05–2 ppm)	1.1 (2 ppm)	0.011
Acetate	4.76	0.9985 (0.5–50 ppm)	2.2 (10 ppm)	0.22
Propionate	4.78	0.9998 (0.65–65 ppm)	2.2 (20 ppm)	0.40
Adipate	4.42	0.9997 (2–200 ppm)	0.9 (20 ppm)	0.90
Azide	4.72	0.9999 (0.5–50 ppm)	1.1 (12 ppm)	0.30
Butyrate	4.82	0.9996 (2–200 ppm)	2.4 (20 ppm)	1.0
Strong acids				
Iodate	0.80	0.9972 (1–100 ppm)	1.4 (50 ppm)	0.60
Chloride	−6.1	0.9986 (0.1–5 ppm)	1.8 (1 ppm)	0.03
Bromide	−9	0.9997 (0.15–15 ppm)	1.1 (2 ppm)	0.075
Nitrate	−1.38	0.9999 (1–100 ppm)	2.1 (2 ppm)	0.08
Iodide	−9.5	0.9979 (1–100 ppm)	2.3 (20 ppm)	0.40
Sulfate	*ca.* −3	0.9997 (0.5–50 ppm)	2.8 (5 ppm)	0.20

[a] From ref. 7.

azide (pK_a 4.72) contamination in salad dressing were determined in the same analysis (Fig. 2). While acetate, fluoride, and azide can all be determined by anion exchange, interferences can occur due to co-elution with other common anions under typical isocratic conditions. Also, disparate levels of closely eluting species may require weaker eluents and therefore longer run times, or gradient analysis. Since the three species are anions of weak acids they are retained and separated on the ion-exclusion column away from the strong acid anion, Cl^-.

In another case, saline eye solution suspected of containing HF was analyzed by ion chromatography. Elemental analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) was to be used in order to trace the source of HF. High levels of HF can damage an ICP torch, thus quantitation of F^- was necessary to determine the minimum dilution of the sample for ICP-OES analysis. As saline eyedrops contain percentage levels of sodium chloride and boric acid, a weak eluent would have been needed for isocratic anion exchange in order to resolve fluoride from the major sample components. No changes in operating conditions were necessary for the analysis of F^- by coupled chromatography, enabling a rapid solution to the problem. The sample was not found to contain HF as suspected, but instead contained sulfuric acid. Although low levels of F^- would have been detected, the source of the low pH was easily determined in one run. Sources of H_2SO_4 rather than HF were then investigated.

Carboxylic acid interferences

In forensic analyses, it is helpful to determine trace contaminants in a relatively pure substance. A drug of abuse was analyzed by anion exchange; however, the major component obscured the beginning of the chromatogram as shown in Fig. 3a. When analyzed on the coupled system, the major peak was retained on the ion-exclusion column (which was consistent with the drug's identity) while trace levels of Cl^-, NO_3^-, and SO_4^{-2} were now observed in the anion-exchange chromatogram. These anions, in conjunction with trace element patterns, were used to determine lot to lot variability.

Carboxylic acids are common in foods. Pyruvate

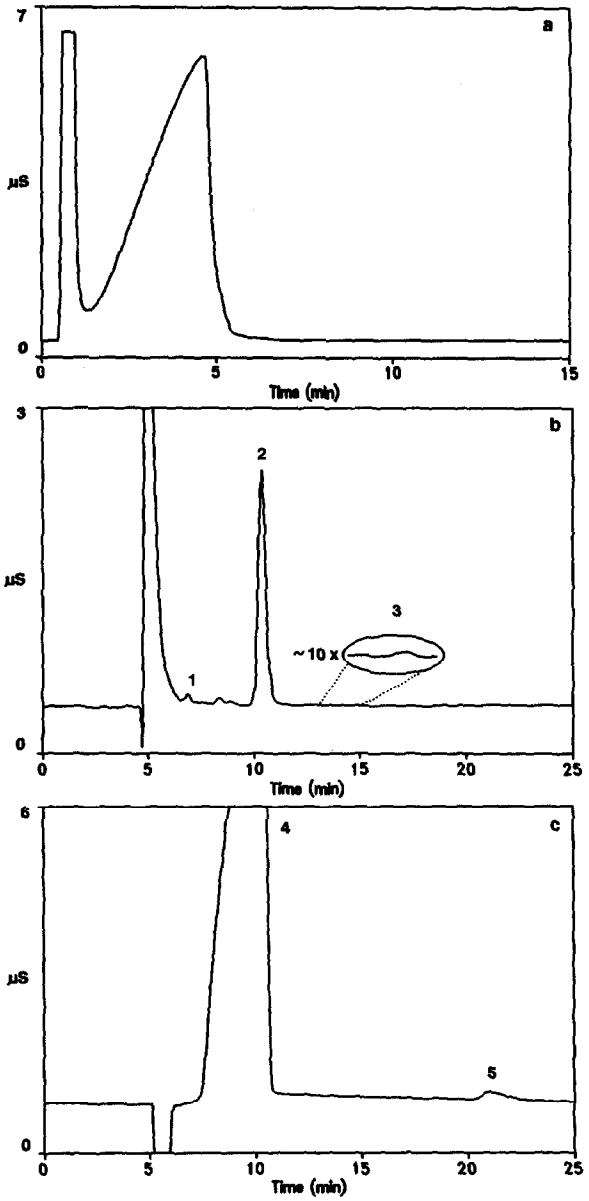

Fig. 2. Sample chromatograms for salad dressing, spiked with 5 ppm fluoride and 6 ppm azide. (a) IC Pak-A column only, 3 m*M* octanesulfonate at 1 ml/min; (b) ion exclusion, and (c) anion exchange in the coupled system. Peaks: 1 = fluoride; 2 = acetate; 3 = azide; 4 = chloride; 5 = sulfate.

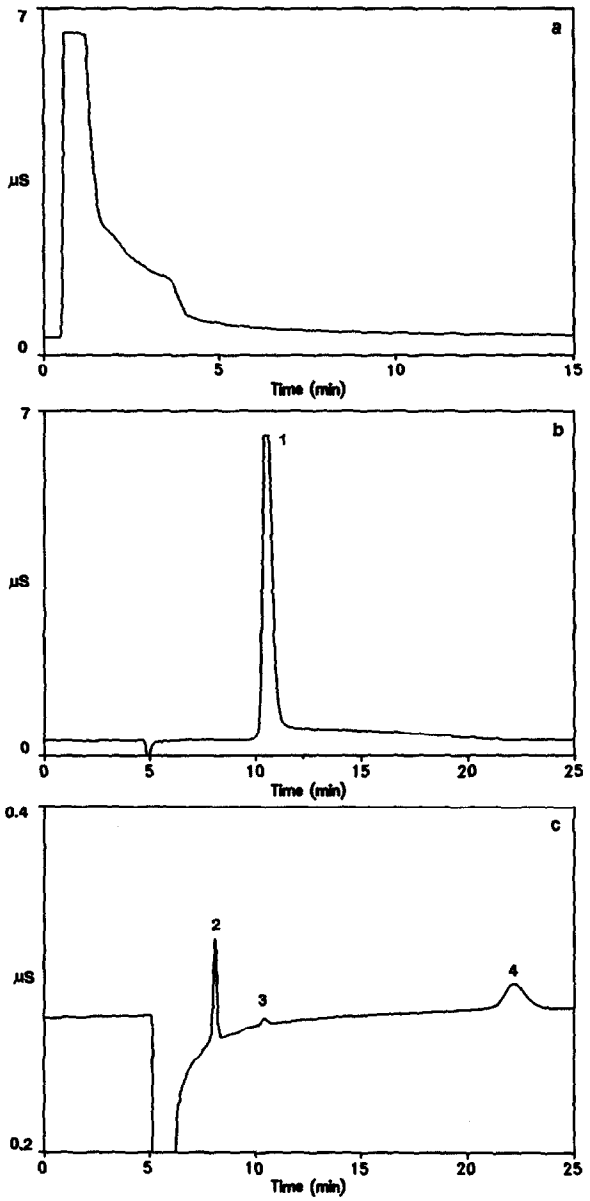

Fig. 3. Comparison between anion-exchange and coupled system in the analysis of a drug of abuse. (a) IC Pak-A column only, 3 m*M* octanesulfonate at 1 ml/min; (b) ion exclusion, and (c) anion exchange in the coupled system. Peaks: 1 = active ingredient; 2 = Cl^-; 3 = NO_3^-; and 4 = SO_4^{-2}.

and lactate elute near the void volume in isocratic anion exchange, complicating the detection of F^- in milk. While F^- can be determined with an ion-selective electrode, analysis by coupled ion chromatography enables the detection of additional anions such as Cl^- which may indicate reconstitution.

Pyruvate, lactate and fluoride were separated by ion exclusion and F^- in milk was determined at a spike level of 2 μg/ml.

Practicality of the coupled system for sample screening

Several anions are toxic and can be screened for in a variety of matrices using coupled ion chromatography. Sodium azide is a commonly used preservative in clinical laboratories and at least two deaths have been reported following accidental ingestion [8]. Azide is difficult to resolve from Br^- and NO_3^- in anion exchange separations [9]. However, using coupled chromatography, the weak acid azide (pK_a 4.72) was separated by ion exclusion, while strong acids Br^- (pK_a −9) and NO_3^- (pK_a −1.38) were separated by anion exchange (Fig. 1; peaks 8, 12 and 13, respectively). Thus, even high levels of Br^- or NO_3^- do not interfere in the analysis of azide. While adipate may co-elute with azide via ion exchange, they are separated by ion exclusion. Nitrates in such complex matrices as meat, cheese or forages would not interfere with azide analysis.

Whereas in some cases emphasis is on obtaining the maximum information available, detection of specific anions is also necessary. In anion-exchange separations fluoroacetate and fluoride elute near the void volume along with other weak acids and small inorganic anions. Although both compounds are toxic, fluoroacetate is more poisonous than fluoride, necessitating separation of these species. Fig. 4 illustrates the separation of weak acids fluoroacetate and fluoride by ion exclusion, as well as other toxic anions lactate, azide, and chlorate in buttered popcorn. High levels of Cl^- observed in the anion-exchange chromatogram would have obscured the weak acids fluoroacetate, fluoride, and lactate in an isocratic anion exchange only separation. Popcorn was freeze dried, ground, and then extracted with distilled deionized water. These analytes have also been determined in seafood.

In some cases a suspect sample may be compared to a matrix control to detect contamination, or sometimes the sample composition is completely unknown. For example, a liquid in an unlabelled bottle collected by an investigator was identified as lactated ringers solution, a veterinary solution, with the help of coupled ion chromatography. The detection of lactate and Cl^- provided much of the

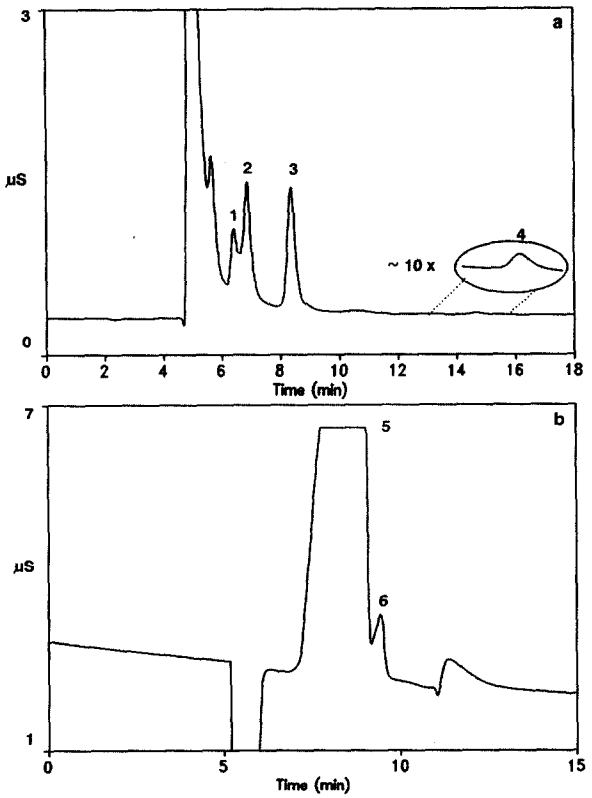

Fig. 4. Analysis of contaminants in buttered popcorn by the coupled system: (a) ion exclusion, (b) anion exchange. Peaks: 1 = fluoroacetate; 2 = fluoride; 3 = lactate; 4 = azide; 5 = chloride; 6 = chlorate.

evidence necessary to identify the solution. More commonly, however, it is not possible to identify by ion chromatography alone a peak which occurred in the suspect sample. Yet, the two modes of separation used in conjunction with one another provides valuable information about pK_a as well as the size/charge of the analyte. An unknown peak detected in the ion-exclusion chromatogram would indicate a weak acid, generally eluting in order of increasing pK_a (such as those in Fig. 1a). Whereas an unknown peak detected on the anion-exchange chromatogram would indicate a strong acid, such as those in Fig. 1b. An early-eluting peak by ion exchange would tend to be a small singly charged anion; a late-eluting peak would tend to be a larger multi-charged anion. Using the information from

the two modes of separation, the focus of investigation by other techniques can then be narrowed.

In other cases, although the sample is not suspected of containing toxic substances, information is required to link or dissociate samples of interest. For example, a juice sample which smelled strongly of liquor was investigated. While it was not possible to identify the liquor using ion chromatography, the coupled system was used to determine the amount of juice in the sample. A peak observed in the ion-exclusion chromatogram of the complaint sample and a control juice was not detected in any of the liquors suspected as the source of the liquor in the juice. That peak, malic acid (retention time 6.6 min), was quantitated and used to calculate the percentage of juice in the sample. This information facilitated the identification of the liquor by gas chromatography and high-performance liquid chromatography.

CONCLUSIONS

While no one method can solve all ion chromatographic separation problems, the coupled system has become a useful part of the overall scheme at the National Forensic Chemistry Center where a variety of analytical techniques are used to solve cases as quickly as possible. Weak acid and strong acid anions are separated on the coupled system, often eliminating matrix interferences. The two modes of separation (ion exclusion and anion exchange) used in conjunction with one another provide valuable information about pK_a as well as the size/charge of an analyte. Additional information obtained using the two modes may narrow the focus of investigations of unknowns by other analytical techniques. Additionally, potentially useful information is not lost in sample preparation.

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