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Anion screening for drugs and intermediates by capillary ion electrophoresis

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ABSTRACT

A method is described to screen bulk pharmaceuticals and their synthetic intermediates for common anions. Capillary electrophoresis (CE) is a rapid separation technique which operates on the basis of the differential migration of charged and uncharged species in an electric field. Capillary ion electrophoresis utilizes the principles of CE in combination with an osmotic flow modifier and indirect UV detection for the rapid determination and quantification of ions. A Waters Quanta 4000 capillary electrophoresis system, equipped with a 60 cm × 75 μm I.D. fused-silica capillary in conjunction with a patented chromate buffer and osmotic flow modifier is used. The detection limit of the assay is 0.5 μg/ml for most anions. Parameters have been optimized so that all analytes migrate within 5.0 min. A number of optimization experiments for parameters such as loading time, voltage, linearity, precision and detection limit of the system are demonstrated. The effects of the presence of organic solvents in the sample diluents on the migration times of anions are examined. Applications of the technique to pravastatin sodium, taxol and iopiperidol are illustrated. Comparison to ion chromatography, advantages and disadvantages, is discussed.

INTRODUCTION

The safety of pharmaceuticals is critically related to their purity, thus the pharmaceutical industry spends a great deal of effort to minimize impurities in bulk drugs and to control degradation of the formulated products [1]. In the past 25 years, impurity analysis has received increasing attention due to the ability of contemporary analytical methodology to yield detailed information on trace impurities.

Impurities typically originate from raw materials, solvents, intermediates, and by-products [2], as well as degradation products and contaminants in the synthetic pathway [2–4]. Thus, there exist many opportunities for impurities to arise in the synthetic process, and despite sample cleanup, many residues remain in the end product in varying amounts.

The present study is useful in screening for anionic impurities in bulk drugs and intermediates. The goal of this screening is the qualitative or the semi-quantitative determination of anionic impurities. Once anions are found using this screening method, individual ion chromatographic (IC) or capillary ion electrophoresis (CIE) (Waters' trade name: Capillary Ion Analysis, CIA) assays are developed for each drug substance specifically for routine monitoring or assay transfer to other labs.

HPLC with refractive index (RI) detection and low-wavelength UV detection have been applied to the analysis of non-chromophoric impurities in pharmaceutical compounds. RI detection has sensitivity problems with polar ionic species, and UV detection is not universally selective. UV detector quality is a critical factor in the success of assays utilizing end absorption, a common feature of many compounds. The separation of ionic species can also be a problem in HPLC since they are usually not well retained. Ion pairing is the most commonly used approach to circumvent this.

IC is the most commonly used method for monitoring ionic species. A gradient suppressed ion

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chromatographic method for screening anions in pharmaceutical compounds is reported [5]. Runtimes are typically more than half an hour for this technique.

Capillary electrophoresis (CE) is an extremely powerful technique with many advantages over gel electrophoresis and fluid chromatographies. An asset of primary importance is the time efficiency of CE separations. Most research using CE in the eighties focused on biomolecules, *i.e.*, proteins, peptides and DNA. Schoots *et al.* [6] analyzed organic anions and quantitated hippuric acid in the blood serum of patients with chronic renal failure. The analysis time for their assay by CE was 8 min, whereas that of the corresponding HPLC assay was 90 min. Furthermore, CE allows smaller sample volumes and greater resolution than other techniques, and no gels, columns, or other complex separation media are required. Upon completion of an analysis, sample residues may be removed from the capillary by purging under vacuum for a few minutes, eliminating the need for more time consuming column washes. Also, minimal sample preparation (filtering through a syringe filter) is required (for an introductory discussion on CE, see ref. 7). The use of CE for the analysis of certain low-molecular-mass ionic species has been documented [8–10].

CIE is a relatively new offshoot of CE. It utilizes the basic principles of CE accompanied by indirect UV or fluorescence detection. An ion with UV absorptive or fluorescent properties in the carrier electrolyte serves as the "visualization agent" which creates a high background signal [11]. Analyte ions are then detected as negative peaks against the constant background. The resolution of complex mixtures is optimized by adding cationic surfactants as osmotic flow modifiers (OFM) [12]. These OFMs control the electroosmotic flow of buffer through the capillary. The Waters Quanta 4000 capillary electrophoresis system combines indirect UV detection, OFM electrolytes, and CE instrumentation to facilitate automated CIE [13] (for a discussion of the theory and range of applications of CIE, see ref. 14).

The effects of various parameters on CE separations have been studied by several investigators [15]. CIE separations can be optimized by varying different parameters such as loading time, run voltage, sample concentration, and sample diluent. In the present study we examined the effects of these parameters. Using optimized parameters, applications of this screening technique are demonstrated in the case of three drug substances.

This anion screening can be helpful in several situations. Such screening method will aid Chemical Process Research departments in determining what assays may be required for characterizing a given drug or intermediate and for setting specifications during the drug development process. Analytical Research and Development can identify potential impurities that are transparent to other chromatographic methods and thereby prevent mass balance concerns before they become issues. In other words, this screening can eliminate the possibility (or establish the presence) of certain anions when mass balance questions arise in certain batches. For synthetic organic chemists and pilot plant chemical engineers, this screening thus give useful information for troubleshooting chemical processes. Finally, it aids in the complete characterization of research reference standards.

The three drug substances illustrated in the present study are pravastatin sodium, taxol and iopiperidol. Pravastatin sodium, a member of the HMG-CoA reductase enzyme inhibitor class, is used to treat primary hypercholesterolemia when diet alone is insufficient. Taxol is a very promising new anticancer agent. Iopiperidol is a new iodinated radiographic contrast imaging agent.

As part of testing the usefulness and efficiency of this screening method, we screend these three compounds, established the absence or the presence of anions in them. If anions were found, individual isocratic IC or sprecific CIE assays were developed to monitor them on a regular basis. Not all the anions detected in this screening had to be quantified at all times. In consultation with the synthetic organic chemists and toxicologists, judgements were made on which anions were important to be monitored routinely.

EXPERIMENTAL

Instrumentation

A Waters Quanta 4000 capillary electrophoresis system (Waters division of Millipore, Milford, MA, USA) equipped with a Waters "AccuSep" 60 cm × 75 μm I.D. fused-silica capillary was used. Data ac-

quisition was performed using a VG Multichrom System on a Microvax computer (Digital Equipment Corporation, Maynard, MA, USA).

Reagents

Sodium chromate AR crystals were obtained from Mallinckrodt (Paris, KY, USA). Sulfuric acid and sodium acetate trihydrate crystals were purchased from J. T. Baker (Phillipsburg, NJ, USA), and sodium nitrite, sodium fluoride, and dibasic sodium phosphate were obtained from Fisher Scientific (Pittsburgh, PA, USA). Sodium bromide, sodium chloride, sodium sulfate, and sodium citrate were obtained from the Aldrich (Milwaukee, WI, USA). Waters Anion-BT electroosmotic flow modifier was obtained from the Waters division of Millipore. Deionized water was from a Waters Milli-Q System. Bulk drug substances were obtained from the Chemical Process Research and Technology Laboratories at the Bristol-Myers Squibb Company (New Brunswick, NJ, USA).

Electrophoretic conditions

Experiments were performed to optimize the CIE system. Unless otherwise specified, the standard conditions used for individual parameters of the system are: 20 kV run voltage, 20 s hydrostatic loading time, and a working electrolyte consisting of a mixture of chromate, dilute sulfuric acid, and Waters Anion-BT OFM [12]. Detection was by UV absorbance at 254 nm. Under these conditions, all the analytes of interest elute wihtin 5.0 min, after which the capillary is purged for 2.0 min under vacuum to remove any remaining drug substance components. Thus, samples can be screened for these anions with a total run time of seven minutes.

Stock standard mixture

The anion screen standard was made by weighing 65.0 ± 2 mg of sodium bromide, 83.0 ± 2 mg of sodium chloride, 75.0 ± 2 mg of sodium sulfate, 75.0 ± 2 mg of sodium nitrite, 78.0 ± 2 mg of sodium citrate, 112.0 ± 2 mg of sodium fluoride, 75.0 ± 2 mg of dibasic sodium phosphate, and 115.0 ± 2 mg of sodium acetate trihydrate crystals into a 100-ml volumetric flask, and diluting to volume with water. This is a concentrated stock solution (approximately 500 μg/ml of each anion) from which appropriate dilutions are made. This stock standard mixture is stable in the refrigerator for up to two months.

Anion screen standard

A fifty-fold dilution of the stock standard mixture gives the 10 μg/ml anion screen standard. Unless otherwise specified, this is the solution used as the standard in all the experiments in this study.

RESULTS AND DISCUSSION

Several parameters can be optimized for CIE. In this study we chose loading time, run voltage, linearity, effect of organic diluents, precision and detection limit. Some of these studies helped us to grasp the nuances of the technique and to test the limits of the system in reality *versus* what was claimed by the manufacturer. Some studies such as the effect of the organic diluent shows the extreme care a user needs to take for preparing standards even for qualitative work with this technique.

Loading time experiment

The purpose of this optimization study is to yield analyte peaks large enough to be quantified reproducibly with baseline separation. The Quanta 4000 is capable of two modes of sample introduction: hydrostatic and electromigration. In this study we used only hydrostatic loading due to the inherent advantages in precision [14]. Loading time is defined as the time in which the inlet end of the capillary is raised to a specific height above the output end. The Quanta 4000 has this height fixed at 10 cm. Loading time is directly proportional to the volume of sample introduced into the capillary in this mode. The effects of different hydrostatic loading times on peak shape and peak area were studied by injecting the anion screen standard at 10, 20, 30, 50, 70 and 90 s loading times. All other parameters were as specified in the experimental section.

The effect of loading time on peak area for some common anions is shown in Fig. 1. The volume of sample loaded was not measured experimentally. Loading time proved to be a good indirect measure of the volume of sample injected for all practical purposes of our study. As expected, peak area increases with increasing loading times. As seen in Fig. 1, for some anions such as bromide, there is a direct linear relationship up to 50 s loading time.

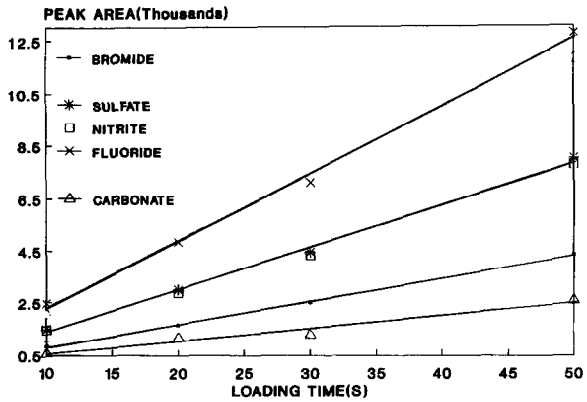


Fig. 1. Effect of loading time on peak areas of some common anions.

Beyond this, the capillary becomes overloaded, resulting in peak overlap. Representative electropherograms are shown in Fig. 2. Fig. 2A shows the electropherogram of the injection at 10 s loading time. Although the peaks are well resolved, the areas are relatively small for these analytes. This is due to the small volume of sample loaded into the capillary relative to longer loading times. This loading time is therefore not acceptable. Fig. 2B–F shows the results of the injections at 20, 30, 50, 70 and 90 s loading time, respectively. From these electropherograms, we arrived at an optimum loading time of 20 s, which, as evidenced in Fig. 2B, results in acceptable peak areas and shapes with little or no peak overlap. We thus confirmed that the default setting on the Waters Quanta 4000 (20 s) is indeed the optimum loading time. In all other optimization experiments in this study, 20 s loading time is used. At 30 s, the overlap between sulfate and nitrite peaks, as well as that between fluoride and phosphate, becomes visibly evident (Fig. 2C). The peaks retain their proper shapes. This loading time can be useful for applications where increased detection limits are required. At 50, 70 and 90 s loading time (Fig. 2D–F), the problems of overlap and skewed peaks become more pronounced. The electropherograms show that 70 and 90 s loading times are clearly unacceptable for this reason.

Run voltage experiment

The purpose of this experiment was to optimize the run voltage. Our objective was to have all the analytes in the standard elute before 5.0 min without peak overlap. The manufacturer-recommended run voltage is 20 kV, therefore we analyzed the range of voltage around this. Samples were loaded at 15, 18, 19, 20, 21, 22, 23, 24 and 25 kV.

A plot of run voltage *vs.* retention rime is shown in Fig. 3. It is evident that each analyte displays a linear, inverse relationship between these parameters. Representative electropherograms of these loadings are shown in Fig. 4. Note that in the 15-kV run, shown in Fig. 4A, the phosphate peak fails to elute within the 5.0-min run time. Running at 25 kV, as evidenced by Fig. 4C, results in shorter elution times, however the resolution suffers. In fact, as the voltage increases, although relative migration times remain the same, the separation deteriorates, as all the analytes migrate sooner. The analytes have less time to be subjected to separatory forces in the capillary, and progressively higher voltages result in poorer resolution.

We determined that 20 kV was indeed the optimum run voltage for our applications, as separation of the analytes of our standard mixture using this voltage are rapid enough to occur within 5.0 min without sacrificing resolution between the peaks (Fig. 4B).

Linearity experiment

The stock standard mixture was diluted to make solutions of the analytes at concentrations of 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml. Each of these solutions were then injected three times on the CIE system using standard conditions. The resulting peak area values were then averaged, and the mean value for each concentration of a single analyte was used in the linear regression calculation for that analyte. Plots of concentration *vs.* peak area for each analyte are shown in Fig. 5. From this figure it can be seen that the dynamic linear range for the analytes in general ranges from 1 μg/ml to 20 or 30 μg/ml. Bromide is an exception, with an upper limit of its linear range at about 80 μg/ml. If the samples have higher anion concentration, they must be diluted to bring down the analyte concentration within the linear dynamic range. This range is rather very narrow as seen from Fig. 5. The coefficient

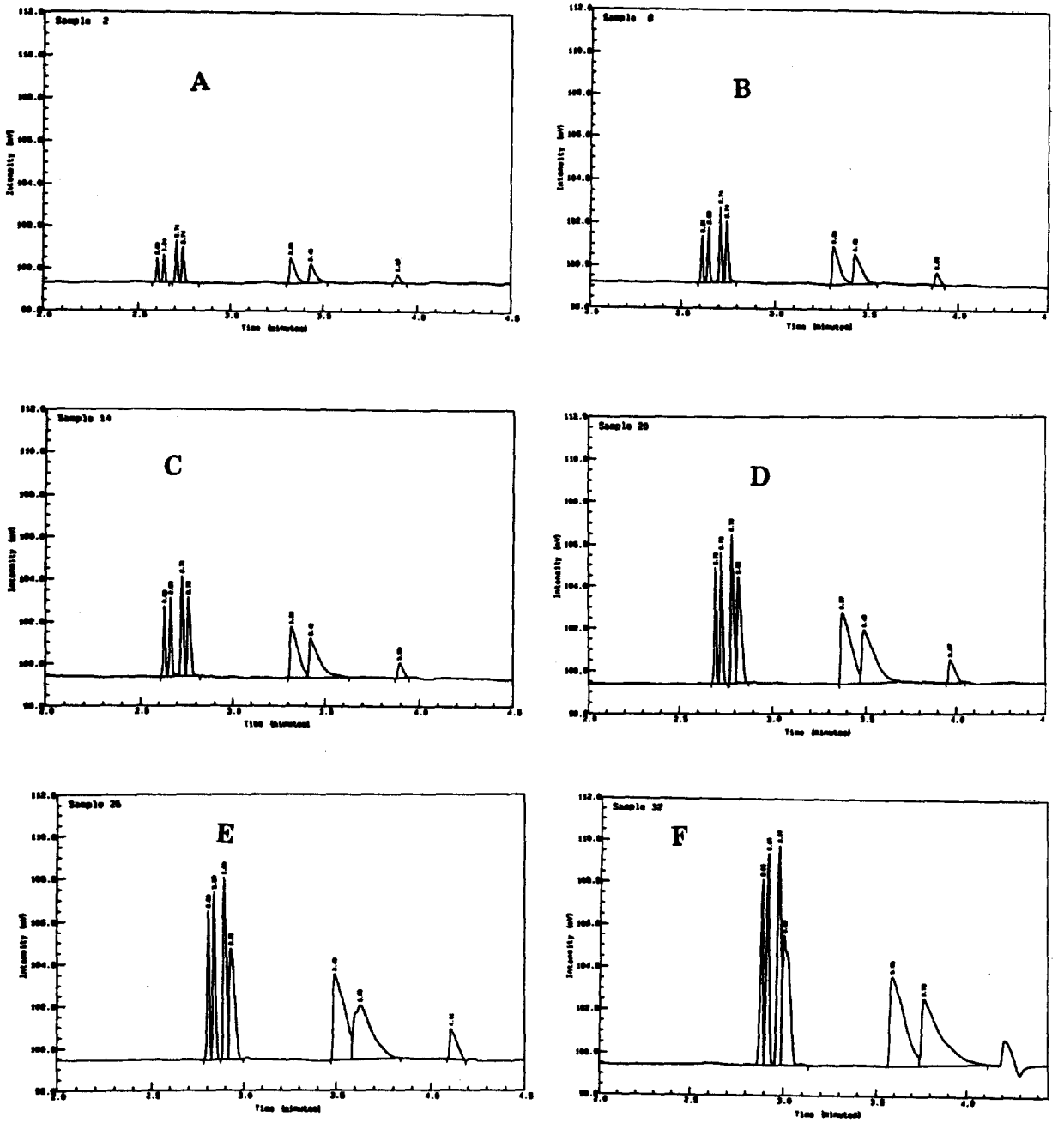


Fig. 2. Anion screen standard (10 μg/ml) at 20 kV run voltage and hydrostatic loading times of (A) 10 s, (B) 20 s, (C) 30 s, (D) 50 s, (E) 70 s and (F) 90 s. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate fluoride and phosphate.

of variation for bromide, chloride and citrate are 0.999 or better. This may be due to their peak symmetry and ease of proper integration at higher concentrations compared to the other anions.

The electropherograms of representative injections at each concentration are shown in Fig. 6. This figure strikingly shows the peak migration time shift with respect to concentration. Thus it is impor-

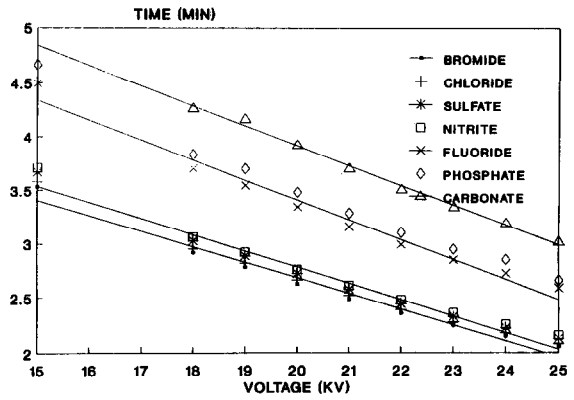


Fig. 3. Effect of run voltage on the migration times of some common annions.

tant to compare unknown samples to standards at similar concentration levels not only for quantitation but also for peak identification. This is particularly important in CIE since peak migration times are very close. Also in Fig. 6, it can be seen that the deviations from linearity at high concentrations for many of the analyte peaks, including fluoride and phosphate, are due to the fact that these concentrations overload the capillary.

The plots of concentrations *vs.* migration time for each analyte are shown graphically in Fig. 7. A "quantitative" picture of the above-mentioned shift in migration times can be seen in Fig. 7. As expected, the anions migrating late are the one most shifted with concentration. The early migrators, bromide, chloride, sulfate and nitrite are less affected by concentration. Calculation of the relative shift in migration times and the correlation with the theoretical prediction of migration times (or their shifts) are beyond the scope of the present study. On the other hand, a good understanding of the extent of this phenomena is essential for useful practical applications of this techniques.

As shown in Fig. 5, the working concentration range (range of interest from 1 to 10 μg/ml) is linear for practical purposes. The results of the linear regression calculations for each analyte in this range showed that all seven anions gave a correlation coefficient of 0.999 or better (Table I). This is a rather narrow linearity range. For qualitative as well as semi-quantitative applications of this technique, up to 30 μg/ml may be acceptable for most anions. If the analyte peaks in the sample fall above this range, then the sample was diluted down to ensure that all anion concentrations fell within this range. It is to be stressed that this is important not only for semi-quantitative work but also for quantitative identification as demonstrated in the above studies.

Effect of organic diluents

Many drugs and intermediates are insoluble in water or other aqueous solutions. Therefore they require organic solvents such as tetrahydrofuran (THF), dimethylacetamide (DMA) etc. to solubilize them. In such cases, anion screening by CIE can be performed by dissolving the samples and anion screen standard in the appropriate solvents. The addition of these solvents to the standard and sample solutions can, however, alter the profile of the analyte peaks and their migration times as shown below.

The effects of differing proportions of organic solvents in the sample diluent on peak shapes, retention times, and baseline stability were studied. The stock anion screen standard was diluted to make several 10 μg/ml solutions, each with 10% (v/v) of the respective organic solvent. The solvents examined were acetonitrile, methanol, isopropanol, THF and DMA. The effects of these organic solvents are shown in Fig. 8A–F. The screens with 10% (v/v) of acetonitrile, methanol, and isopropanol result in peak contamination by co-migration, while those made with THF (Fig. 8E) and DMA (Fig. 8F) result in better separation than the aqueous screen standard (Fig. 8A). The separation between fluoride and phosphate is better in THF and DMA (Fig. 8E and F) than the other solvents. Moreover it was observed that, the screen with 10% (v/v) of DMA increases peak migration times the most without resulting in co-migration or detrimental effects on peak shape (Fig. 8F). Therefore the effect of this solvent on migration times of anions was further investigated. A new set of solutions of anion screen standard (10 μg/ml) at varying proportions (0, 10, 20, 30, 40, 50, 60, and 80%, v/v) of DMA were made from the stock standard mixture. Fig. 9 graphically shows that migration times for all peaks increased with increasing amounts of DMA. The screens consisting of 40% or more DMA resulted in unacceptable baseline disturbances.

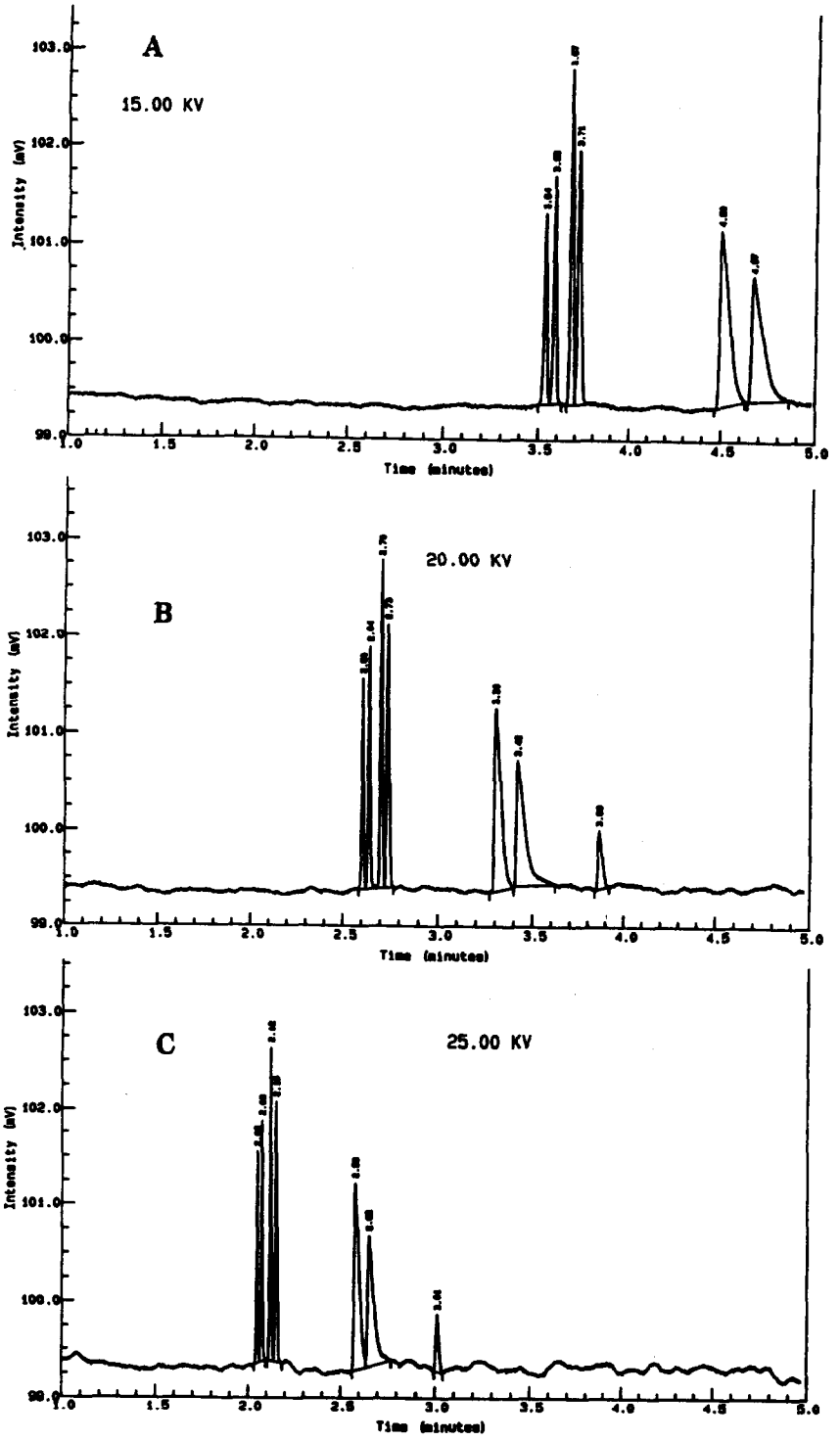


Fig. 4. Anion screen standard (10 μg/ml) at 20 s hydrostatic loading and run voltages of (A) 15 kV, (B) 20 kV and (C) 25 kV. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride and phosphate.

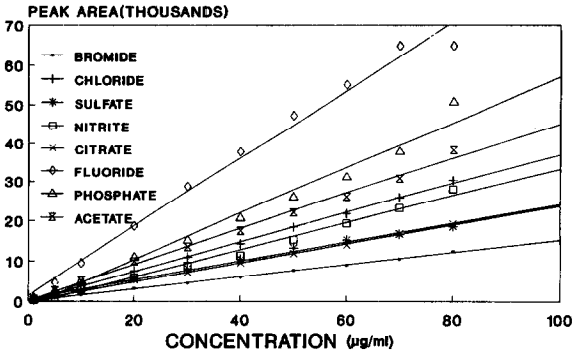


Fig. 5. CIE linearity: the effect of analyte concentration on peak area for eight common anions.

These shifts in migration times may be explained by the established theories of changes in electroosmotic flow when different molecules are introduced into the capillary. They illustrate a very important practical consideration. Even for qualitative identification purposes, it is of extreme importance that the standards are prepared in the exact same diluant as the sample. This may be a slightly unusual consideration for practitioners of HPLC or IC, where usually the retention time changes with sample diluents are minimal. This change in migration time of the anions is exemplified in the case of anion screening of taxol discussed below.

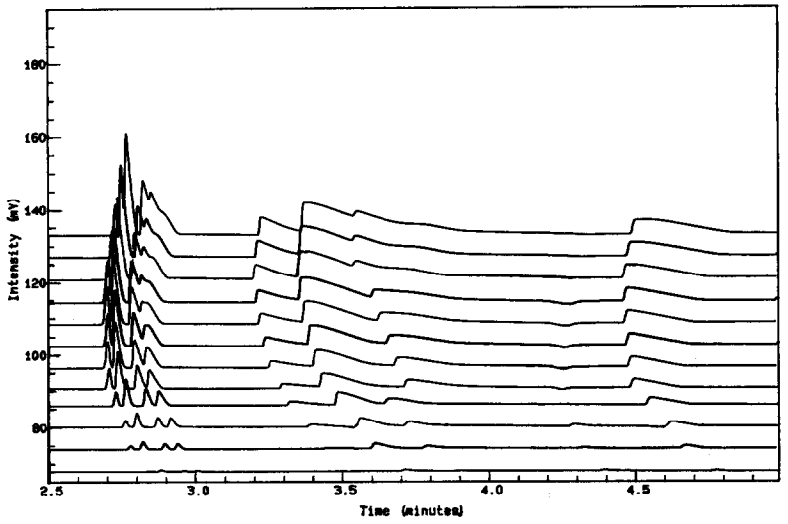


Fig. 6. Electropherogram of the anion screen standard at concentrations of (from bottom to top) 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml. The run was made at 20 kV with a hydrostatic loading time of 20 s. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride, phosphate and carbonate.

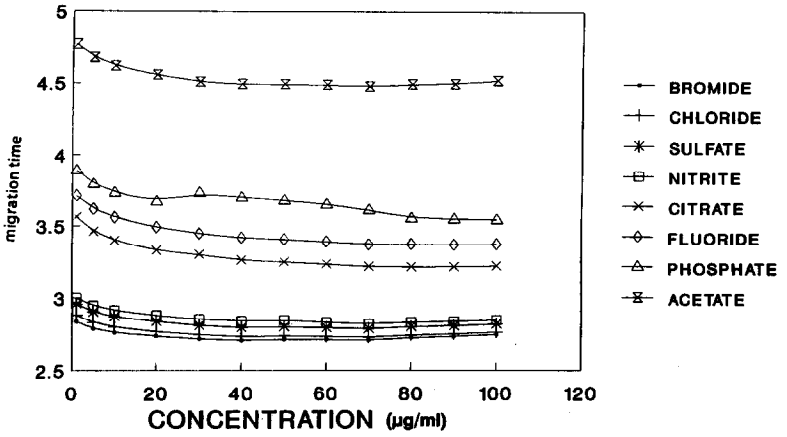


Fig. 7. Effect of analyte concentration on the migration times of common anions.

TABLE I

LINEARITY DATA FOR ANIONS IN THE RANGE 1–100 μg/ml

Concentration (μg/ml)	Mean peak area (n = 2)							
	Bromide	Chloride	Sulfate	Nitrite	Citrate	Fluoride	Phosphate	Acetate
1	140	439	320	344	162	980	456	526
5	868	2037	1496	1583	1165	4561	2377	2655
10	1650	3842	2858	2957	2514	9483	5096	5150
20	3161	7510	5502	5815	5239	18 664	10 981	9895
30	4533	10 938	8119	8793	7054	28 668	15 225	13 474
40	5967	14 320	10 470	11 372	9535	37 880	20 738	17 522
50	7671	18 492	13 244	15 354	11 817	47 343	26 081	22 095
60	9031	21 881	15 242	19 432	13 962	55 187	31 245	26 096
70	10 580	25 865	16 748	23 253	16 781	64 886	37 988	30 830
80	12 454	30 395	18 629	28 113	19 274	64 898	50 849	38 358
90	13 733	33 975	19 573	32 659	21 378	69 989	58 588	43 432
100	15 085	38 114	20 198	38 116	23 551	73 792	67 697	48 141
Intercept	55.5	−180.2	1250.6	−1531.0	84.2	3804.2	−3038.6	−360.4
Slope	151.5	377.6	211.1	370.8	236.4	774.6	654.3	472.1
r	0.999	0.999	0.989	0.994	0.999	0.987	0.989	0.997

Precision

The goal of the precision experiment was to assess the peak area reproducibility of the CIE system. Standard conditions were used. Solutions containing 1 mg/ml of lopiperidol spiked with 1 μg/ml of the anion screen were made. The solution was injected eight times. The peak areas were then statistically analyzed. The peak areas of individual injections and the results of the statistical analysis for each analyte are shown in Table II. Standard deviation ranged between 2–7% for the anions. At 1 μg/ml, these standard deviations are acceptable for screening.

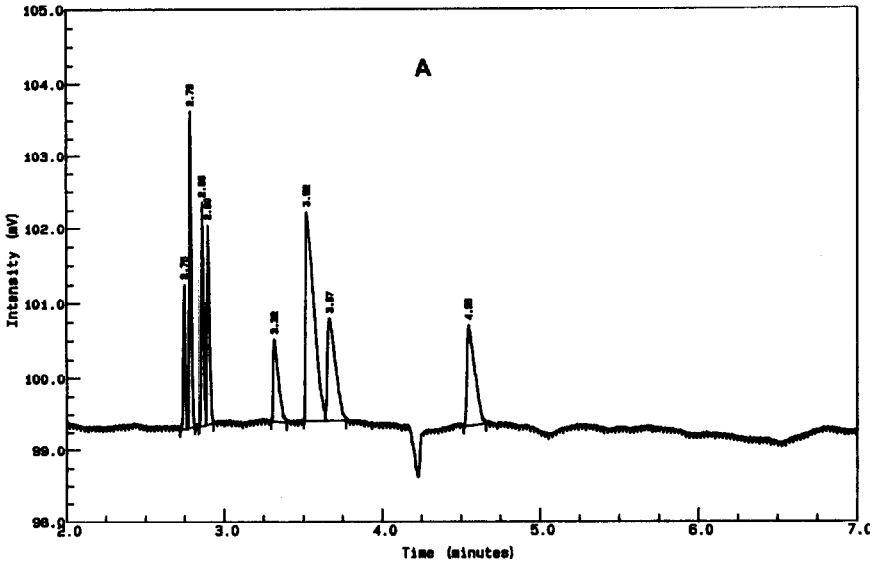
Detection limit and minimum quantifiable limit

The electropherogram of the detection limit standard mixture at 0.5 μg/ml of each anion, is shown in Fig. 10. Below 0.5 μg/ml, the signal to noise ratio is often less than acceptable. For drug concentrations of 1 mg/ml, 0.5 μg/ml corresponds to 0.05% (w/w). The detection limit of 0.05% (w/w) is sufficient for monitoring innocuous impurities such as common anions for mass balance purposes. If necessary, this detection limit can be lowered by increasing the drug concentration from 1 to 10 mg/ml or more. Solubility of the drug in a suitable solvent is the only limiting factor to this approach. The minimum quantifiable limit of the common anions is set at 1.0 μg/ml. An electropherogram of anion screen standards at this level is shown in Fig. 11 under optimized conditions.

COMPARISON OF CIE AND IC

The runtime savings in CIE is probably the most significant advantage over IC. CIE runtimes are typically less than 5 or 6 min. When the 2-min vacuum suction of the capillary is added to this time, the total analysis time is still less than 10 min. Standard IC methods may take almost twice as much analysis time for all these anions.

A more significant advantage of CIE over IC is the ease of sample preparation. The drug can be eliminated from the capillary easily by vacuum suction. Vacuum suction works well with most drug compounds or intermediates we have tried. Exceptions may be strong amine containing drugs. They may not be eliminated from the capillary readily by vacuum suction. In IC, either sample preparation to eliminate the drug beforehand or an elution protocol to elute off the drug from the column is necessary. This can be done by different means such as



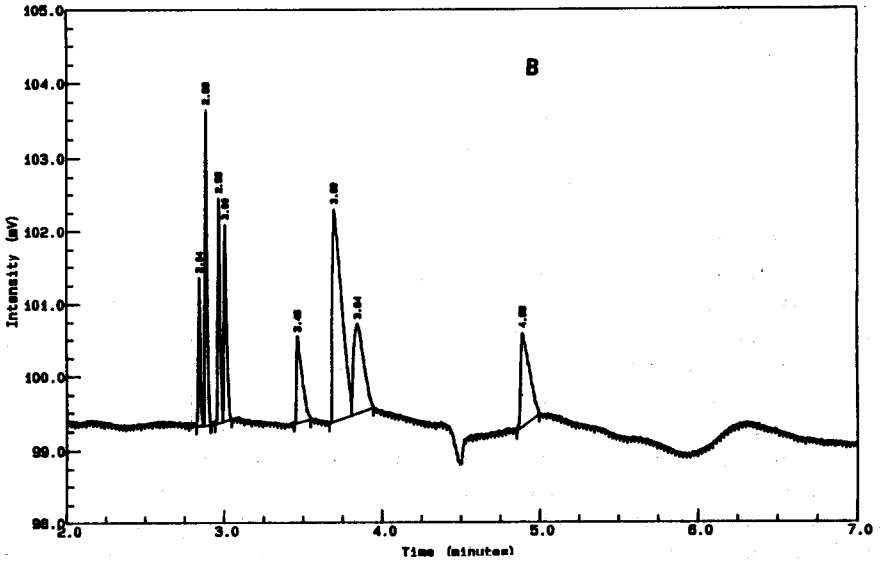
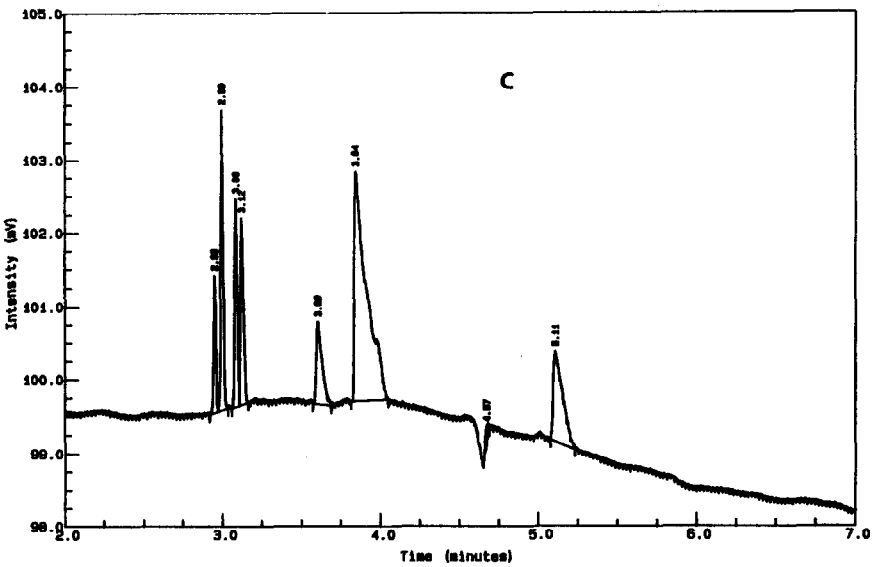


Fig. 8.



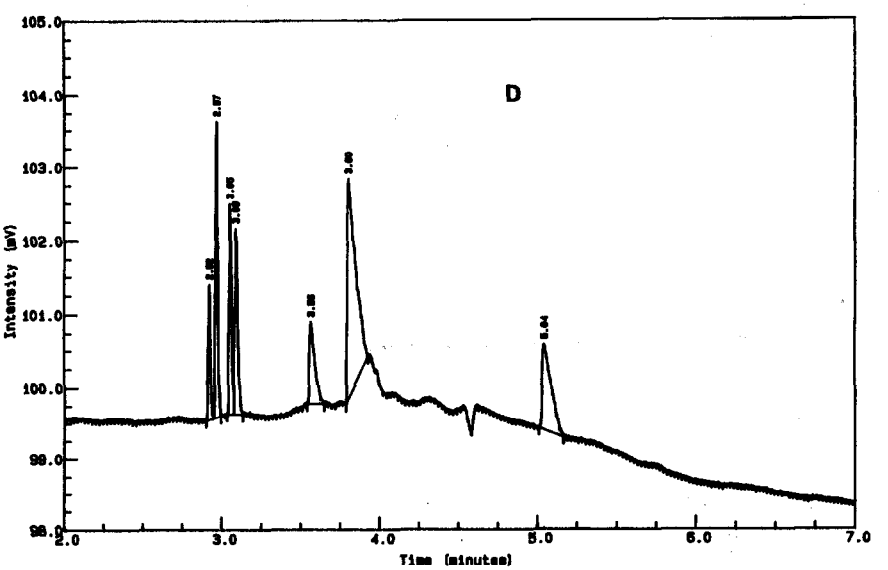


Fig. 8.

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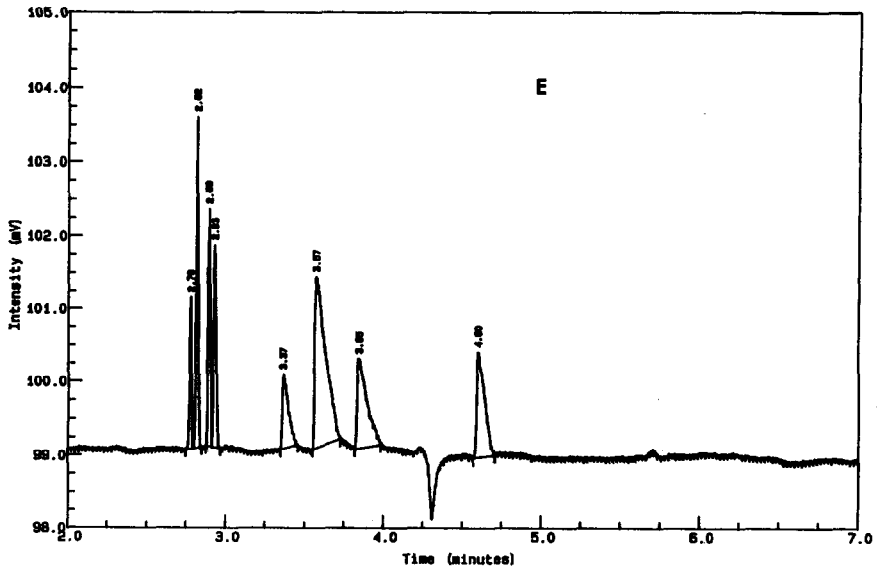

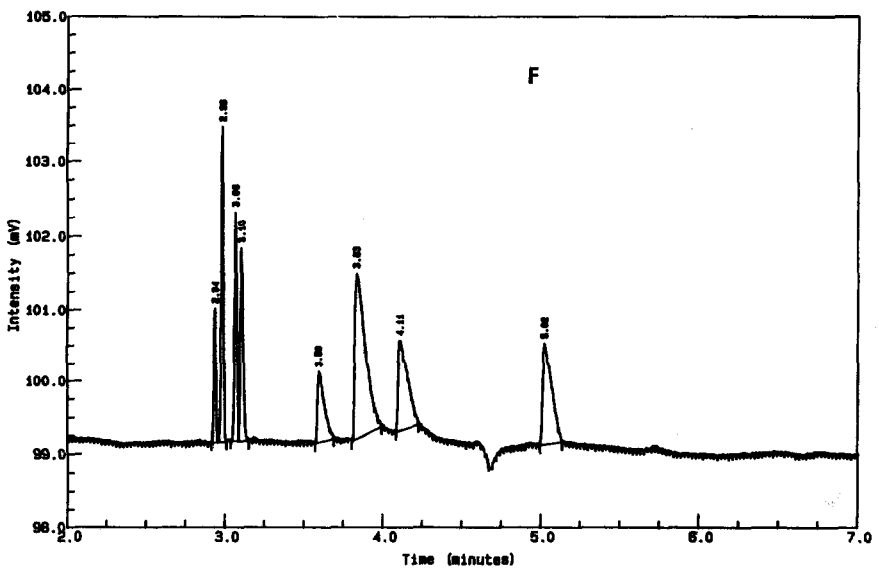

Fig. 8. Electropherograms of 10 μg/ml anion screen standard prepared in: (A) 100% water, (B) 10% acetonitrile, (C) 10% methanol, (D) 10% ispropanol, (E) 10% tetrahydrofuran and (F) 10% dimethylacetamide in water showing the effect of organic solvents in the sample diluent on peak shapes and retention times. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride, phosphate and carbonate.

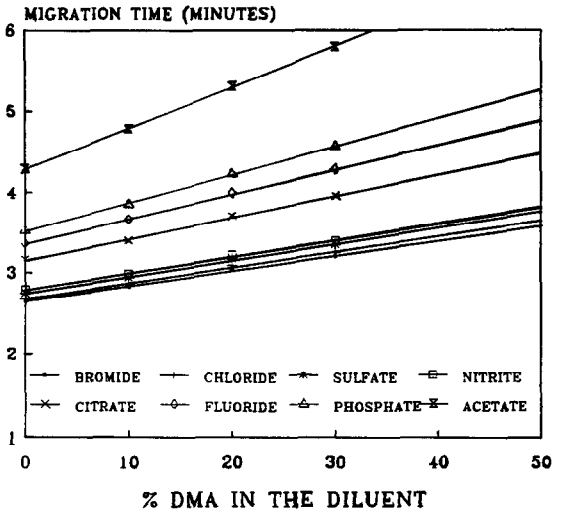

Fig. 9. Effect of amount of dimethylacetamide (DMA) in the sample diluent on peak migration times.

gradient techniques [5]. These procedures increase the time required per analysis. The unavoidable reequilibration time of an ion chromatographic column after any sort of gradient will also contribute to increase in total analysis time.

CIE has the intrinsic baseline noise. As shown in Fig. 12, this can be like a *grass noise*. Changing the buffer and cleaning the capillary can reduce this noise. Fig. 12 shows the worst case scenario for a water loading. This noise can cause lack of absolute sensitivity over suppressed IC. In fact in our experience it was hard to detect below 0.5 μg/ml level for the anions studied. Wherever lower absolute sensitivity is desired, suppressed IC ought to be the method of choice. Integration of the peaks from CIE also took more time and effort from the analyst (because of this inherent baseline noise) than performing the same functions on IC peaks. This extra effort could vary from one data acquisition system to another.

Varying migration times in CIE, necessitates frequent loading of standards. If proper temperature controls are maintained, this is usually not as much of a problem in IC. An example of varying migration times is shown in Fig. 10. In addition, IC methods do not require such stringent equality of sample and standard diluents.

The Quanta 4000 has a carousal with 20 positions for sample vials. Random access to the vials would have increased productivity because three standard concentrations would have needed only three positions. Currently most positions are taken up by the three standard vials bracketing four sample vials.

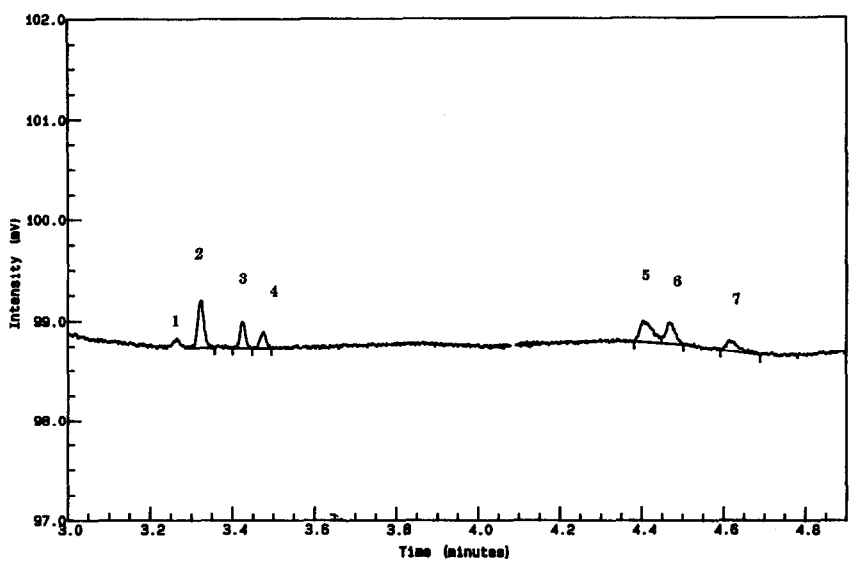

Fig. 10. Detection limit of anions by CIE. All anions at 0.5 μg/ml. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride, and phosphate.

TABLE II

PRECISION OF THE CIE SYSTEM FOR IOPIPERIDOL AT 1.0 mg/ml SPIKED WITH ANIONS AT 0.1% (w/w)

Injection	Mean peak area (n = 2)						
	Bromide	Chloride	Sulfate	Nitrite	Fluoride	Phosphate	Acetate
1	131	1467	234	272	801	205	975
2	128	1456	236	261	809	207	1077
3	131	1478	237	273	814	209	985
4	133	1492	236	260	808	209	1039
5	133	1444	262	263	850	212	957
6	127	1447	217	265	808	205	1031
Mean	131	1464	237	266	815	208	1011
S.D.	2.51	18.67	14.39	5.57	17.64	2.71	45.67
R.S.D. (%)	1.9	1.3	6.1	2.1	2.2	1.3	4.5

Applications of CIE anion screening

The CIE screening procedure was utilized for checking mass balance issues on several Bristol-Myers Squibb drugs. Three typical examples are shown. An electropherogram of a batch of deuterated pravastatin sodium at 1 mg/ml in water is shown in Fig. 13. Comparison of this electropherogram with the anion screen standard diluted tenfold (1 μg/ml) allowed us to detect above 0.1% (w/w) each of bromide, fluoride, and acetate. The other peaks in the sample electropherogram were not further investigated.

A batch of taxol was screened using this technique. Taxol is soluble in 100% methanol. A methanol blank gave the CIE trace shown in Fig. 14A. Two solutions were made, one consisting of 1 mg/

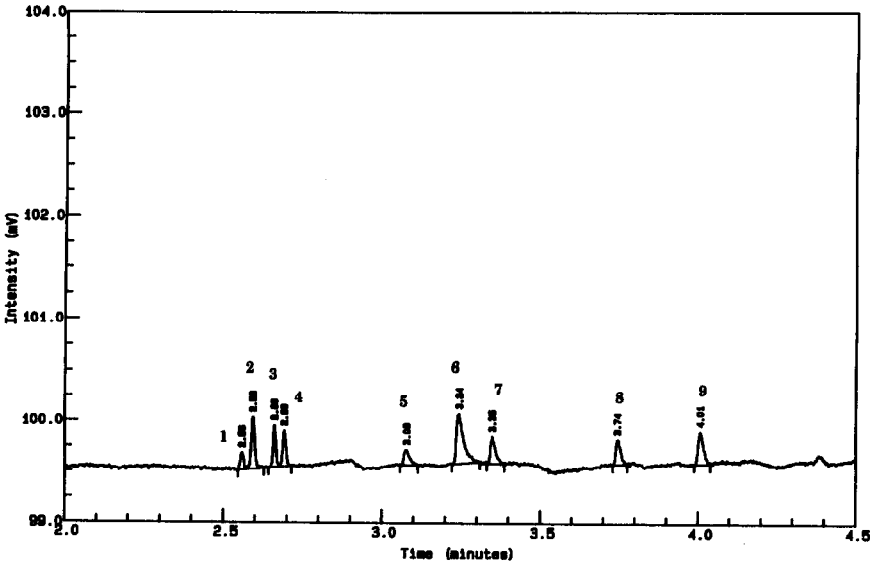

Fig. 11. Minimum quantifiable limit of anions by CIA. All anions at 1.0 μg/ml. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride, phosphate, carbonate and acetate. The experimental conditions are the optimized conditions explained in the experimental section.

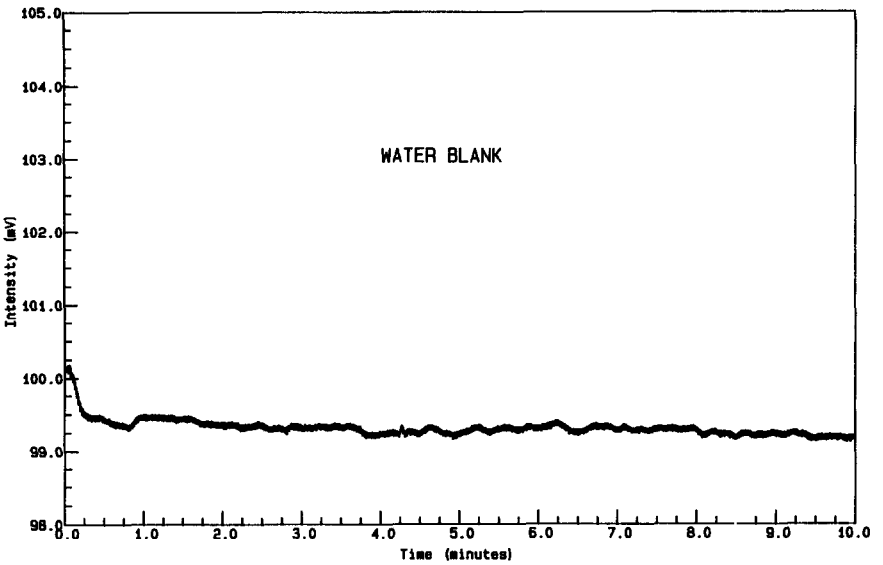

Fig. 12. Electropherogram of water blank under an extreme condition of baseline noise. Standard experimental conditions were used.

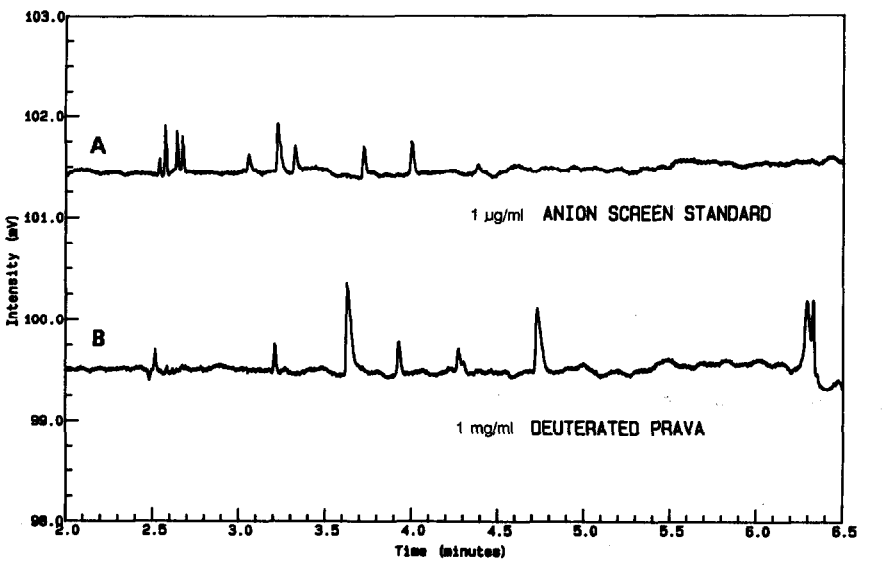

Fig. 13. Electropherograms of (A) 1 µg/ml anion screen standard. The migration order of anions is bromide, chloride, sulfate, nitrite, citrate, fluoride, phosphate, carbonate and acetate. (B) 1 mg/ml deuterated pravachol.

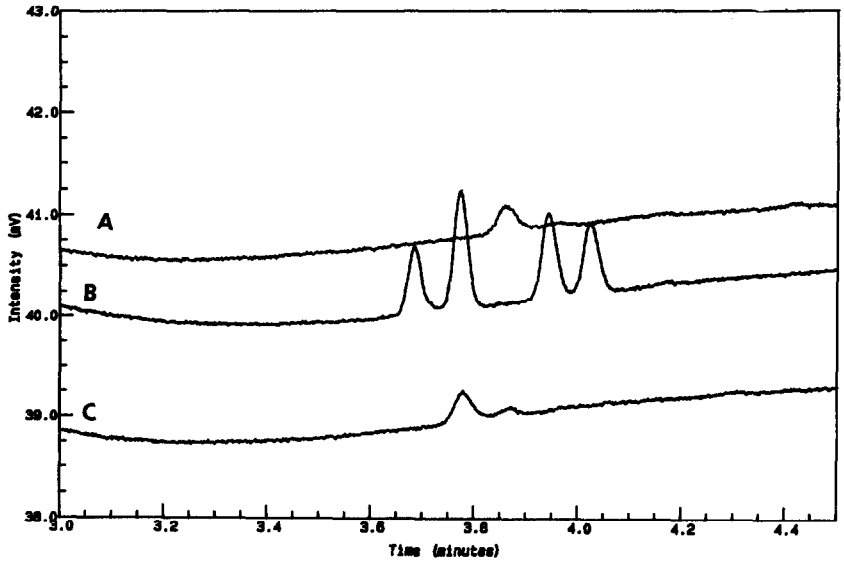

Fig. 14. Electropherograms of (A) a methanol blank (B) 1 mg/ml taxol spiked at 0.5% with bromide, chloride, sulfate and nitrite in methanol, and (C) 1 mg/ml taxol in methanol.

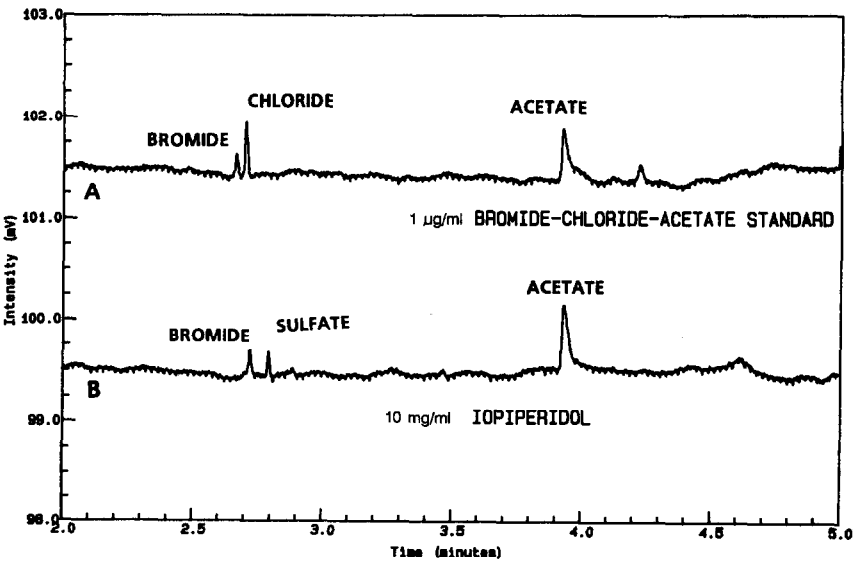

Fig. 15. Electropherograms of (A) 1 µg/ml of bromide, chloride, and acetate standards (B) 10 mg/ml iopiperidol in water.

ml taxol, and another of 1 mg/ml taxol spiked with 5 μg/ml of bromide, chloride, sulfate and nitrite. The electropherograms of these samples are shown in Fig. 14B and C. Note the shift in migration times for all peaks. Bromide, chloride, sulfate, and nitrite migrated within 5.0 min but with later migration times. This experiment demonstrates the profound influence of an organic diluent on analyte migration times. As shown in Fig. 14C, this batch of taxol has no bromide, chloride, sulfate, or nitrite in it. The peak at 3.75 min is seen in the methanol blank too.

Iopiperidol, a novel X-ray contrast agent, is soluble in water. Solutions of the drug were subjected to anion screening by CIE. The electropherogram of one batch is shown in Fig. 15B. Comparison of this sample to anion screen standard at 5 μg/ml (shown in Fig. 15A) enabled us to identify bromide and acetate in the sample solution.

As demonstrated by these examples, this technique facilitates detection of common anions at 0.1% (w/w) level. CIE thus compares favorably with IC for this purpose. In the cases where anions were detected by this screening technique, appropriate specific IC or CIA assays were developed and validated for routine quantitation.

REFERENCES

1 M. W. Dong, P. V. Passalacqua and D. R. Choudhury, *J. Liq. Chromatogr.*, 13 (1990) 2135.
2 S. Ahuja, *Chem. Anal.*, 85 (1986) 195.
3 G. F. Fhillips, *Pharm. Ind.*, 51 (1989) 1282.
4 J. Van Rompay, *J. Pharm. Biomed. Anal.*, 4 (1986) 725.
5 J. Nair, *Pharm. Research*, February (1993) submitted for publication.
6 A. C. Schoots, T. P. E. M. Verheggen, P. M. J. M. De Vries and F. M. Everaerts, *Clin. Chem*, 36 (1990) 435.
7 J. W. Jorgenson and K. D. Lukacs, *Science*, 222 (1983) 266.
8 F. E. P. Mikkers, F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, 169)1979) 11.
9 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
10 X. Huang, J. A. Luckey, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 61 (1989) 766.
11 T. Wang and R. A. Hartwick, *J. Chromatogr.*, 589 (1992) 307.
12 W. R. Jones, P. Jandik and R. Pfeifer, *Am. Lab.*, 23 (1991) 42.
13 A. L. Heckenberg, P. G. Alden, B. J. Wildman, J. Krol, J. P. Romano, P. E. Jackson, P. Jandik and W. R. Jones, *Waters Innovatie Methods For Ion Analysis*, Millipore Corporation, Milford, MA, 1989.
14 P. Jandik, W. R. Jones, A. Weston and P. R. Brown, *LC · GC*, 9 (1992) 634.
15 G. M. McLaughlin, J. A. Nolan, J. L. Lindahl, R. H. Palmieri, K. W. Anderson, S. C. Morris, J. A. Morrison and T. J. Bronzert, *J. Liq. Chromatogr.*, 15 (1992) 961.