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Determination of monovalent and divalent cations and chloride in the carbacephalosporin loracarbef by ion chromatography

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ABSTRACT

Sensitive determination of monovalent and divalent cations and chloride in the carbacephalosporin antibiotic loracarbef can be achieved by separation on a high-capacity cation-exchange column followed by chemically suppressed conductivity detection. The methods developed in our laboratory for these determinations are characterized by the absence of matrix interference, rapid separation, ease of sample preparation and good sensitivity. Of these benefits, it is the minimization of matrix interference supplied by micromembrane chemical suppression that has made this technique so powerful for this application. Advantage is taken of the acid–base chemistry of loracarbef, resulting in a drastic reduction in its concentration in the eluent prior to detection of the analytes of interest. Linearities in the presence of the matrix (coefficient of determination > 0.995) and recoveries from the matrix are excellent ($100 \pm 2\%$). However, when the suppression components exhibit decreased efficiency, systems employing eluents composed of both hydrochloric and diaminopropionic acids are susceptible to problems such as poor peak shape, high background conductivity, and noise thus making accurate determinations difficult. Both the suppression system and the eluent are assessed in relation to the sources of high background conductivity and noise.

INTRODUCTION

The determination of sodium, ammonium, potassium, calcium and chloride is of great importance to the pharmaceutical industry. These ions may be introduced with raw materials or as salts of acids or bases, and at times serve as a counterion to the bulk drug. In our laboratories, determination of these cations is required for both environmental monitoring and the research and development of new drugs. The methods used for these assays must be reproducible on a day-to-day basis with respect to accuracy and sensitivity.

In general, ion chromatography has found widespread application in many areas, especially environmental and water analysis [1–7]. Applications of ion chromatography with photometric [8,9] and direct conductivity [10,11] detection to pharmaceutical analysis are numerous, but those utilizing suppressed conductivity detection have received somewhat limited focus. Herbranson *et al.* [12] determined sulfite and sulfate in injectable formulations by this technique. Brown *et al.* [13] utilized a fiber eluent suppressor to alleviate potential interferences from amino acid type matrices. A column-switching technique has also been used to remove matrix interference in pharmaceutical samples containing high levels of sodium and potassium [14]. Potentiometric, titrimetric, and spectroscopic methods have found wide usage for quantitating sodium, potassium, calcium and chloride, but ammonium can be a particularly troublesome analyte because other modes of detection such as colorimetry and voltammetry [15] require derivatization. Here, chemically suppressed ion chromatography has been shown to be an excellent analytical tech-

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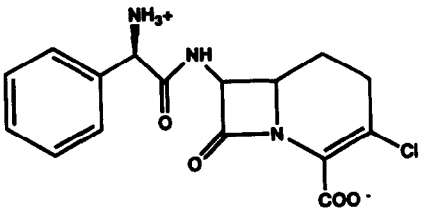

Fig. 1. Loracarbef.

nique for the determination of these common ions in loracarbef, a new carbacephalosporin antibiotic (Fig. 1). The acid–base chemistry of loracarbef, $pK_1 = 2.3$ and $pK_2 = 7$, plays an important role in its analysis.

EXPERIMENTAL

Purified water obtained from a Milli-Q (Millipore, Bedford, MA, USA) water system was used to prepare all solutions. The chromatographic system employed included a Dionex Series 4500i gradient pump and a pulsed electrochemical detector operating in the conductivity mode (Dionex, Sunnyvale, CA, USA). Sample injection was achieved with a Micromeritics 728 autosampler (Norcross, GA, USA) and a 25-μl sample loop. Plastic autosampler vials were used to minimize background signals. All data were collected on a Hewlett-Packard 1000 computer system with custom chromatography software capable of peak integration.

For monovalent cation chromatography, a Dionex IonPac CS2 analytical column (250 mm × 4 mm) was used. For the determination of calcium, the analytical column was an IonPac CS10 (250 mm × 4 mm). Eluents were prepared from ultrapure HCl (99.999%, Aldrich) and 2,3-diaminopropionic acid hydrochloride (DAP) (Dionex). The regenerant was 100 m*M* tetrabutylammonium hydroxide (TBAOH) which was prepared by diluting 65 ml of 40% stock solution (Aldrich, Milwaukee, WI, USA) in 1 l water. Chemical suppression of the eluent was achieved by continuously recycling 100 m*M* TBAOH at a flow of 3–5 ml/min through a CMMS-II micromembrane suppressor and an AutoRegen cartridge (Dionex). This system will be called the "suppression equipment" throughout the paper when referring to cation chromatography. Cation standard solutions were prepared from anhydrous, reagent-grade, chloride salts. All chromatography was performed isocratically at room temperature with an eluent flow-rate of 2.0 ml/min for the monovalent cations and 1.0 ml/min for calcium.

For chloride determinations, a Dionex IonPac AS4 column (250 mm × 4 mm) was used. The eluent was 1.5 m*M* $NaHCO_3$ and 1.2 m*M* Na_2CO_3, prepared from dry, reagent-grade salts (EM Science, Cherry Hill, NJ, USA). The regenerant was 12.5 m*M* sulfuric acid which was prepared by diluting 0.7 ml of the concentrated acid in 1 l water. Chemical suppression of the eluent was achieved with an AMMS-II micromembrane suppressor in the same manner as the cation system. Standard solutions were prepared from reagent-grade KCl. Chromatography was performed isocratically at room temperature with an eluent flow-rate of 1.5 ml/min.

RESULTS AND DISCUSSION

Determination of ions in loracarbef

Determination of Cl^-. Sample solutions were prepared by dissolving 0.6 mg/ml loracarbef in purified water. Under the given experimental conditions, chloride was eluted in 2.9 min (see Fig. 2). Decreasing the concentration of the hydrogencarbonate–carbonate eluent will increase the retention time of Cl^-, giving better selectivity by moving the peak further away from the

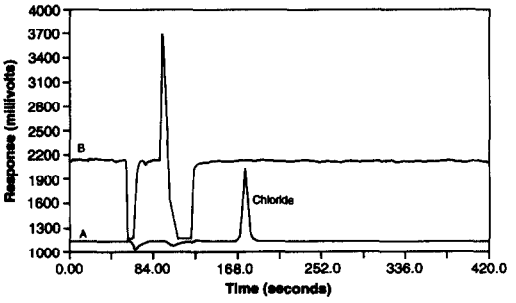

Fig. 2. (A) Chromatogram obtained for Cl^- asay. The absence of the large baseline dip seen when a micromembrane suppressor follows the AS4 analytical column is also shown. Chloride peak represents 4.5 μg chloride/ml. (B) Non-suppressed chromatogram has been baseline offset by −300 μS. Eluent: 1.5 m*M* $NaHCO_3$, 1.2 m*M* Na_2CO_3. 1 mV = 0.01 μS.

void volume and any fluoride ion that may be present. Data relating the retention of Cl^- and other anions on an AS4 column to hydrogencarbonate–carbonate eluent concentrations appear elsewhere [16]. The only other feature in the chromatogram obtained with chemical suppression due to the sample is a second, small baseline dip at 100 s following the water dip. Without chemical suppression, two large dips are seen, the second presumably from loracarbef. Although the size of the second baseline dip is directly proportional to the amount of loracarbef injected (coefficient of determination, COD = 0.987 for injections of 8 to 40 μg), the result of assaying this analytical column effluent fraction by our laboratory's standard HPLC–UV method shows that the dip is not caused by loracarbef directly. Only trace and relatively constant amounts of loracarbef were recovered in the region of the second dip.

Although loracarbef present in the sample solution passes through the analytical column unretained, the suppressor serves to remove a large fraction of it before reaching the detector. Analytical column effluent was collected for three minutes subsequent to sample injection and assayed by HPLC–UV. Quantitative recovery of loracarbef was observed, demonstrating no retention of the matrix on the AS4 column. Assaying suppressor effluent fractions collected over that same time span that first passed through the analytical column yields only a very small concentration of loracarbef and this concentration is also found at later times during the chromatogram. Assaying the regenerant solution shows low levels of loracarbef to be present. Loracarbef enters the suppressor as an anion and subsequently gets protonated to a zwitterion. As a zwitterion, it can be retained in the anion suppressor as the suppressor is essentially a cation exchanger in the H^+ form, similar to a CS2 column on which retention has been demonstrated (see next section). Since loracarbef can both be retained in the micromembrane suppressor and can diffuse across the membrane into the regenerant stream, an interference-free chromatogram is obtained. The displacement of H^+ by loracarbef is likely the cause of the second baseline dip. The association of this displaced H^+ with free HCO_3^- would result in a temporary decrease in the background conductivity and hence a baseline dip.

Linearity in the presence of loracarbef (COD = 0.9999) and recovery (see Table I) of Cl^- from the matrix are excellent from 1 to 20 μg/ml. The within-run R.S.D. (multiple sample preparations, $n = 12$) for Cl^- determined at 4.2 μg/ml in loracarbef is 1.3%. For low-level determinations, it is crucial that sample solutions do not come into contact with low-quality glass such as that found in autosampler vials due to leaching of variable amounts of chloride into solution. The variable background due to this caused the R.S.D. to be 7.9% within the run, or approximately six times worse than when plastic vials were used.

Determination of Na^+, K^+, and NH_4^+. Sample solutions were prepared by dissolving 3 mg/ml loracarbef in purified water for sodium and potassium determination and 8 mg/ml for ammonium determination. These sample loadings do not reflect a significant difference in analyte sensitivity, but are a result of the need to assay appreciably lower levels of ammonium ion. A chromatogram obtained under the given experimental conditions is shown in Fig. 3. An eluent concentration of 7.5 m*M* HCl was found to provide good balance between resolution, analysis time, background conductivity and long-term suppression equipment efficiency. The high resolution of these analytes on the CS2 allows for widely varying amounts of these analytes to be present and still allow for quantitation.

Studies employing ultraviolet detection show that loracarbef, a singly charged cation at the eluent pH, is retained on the analytical column approximately 300 s longer than the most strongly retained analyte, K^+. Analytical column effluent and suppressor effluent (representative of what is measured by the conductivity cell) fractions were collected in 100-s increments beginning 800 s after injection. These fractions were then assayed for loracarbef by HPLC–uv and the results plotted versus time. The results in Fig. 4 indicate that less than 5% of the injected loracarbef eventually makes it to the conductivity detector. Furthermore, the retention time of loracarbef is the same with and without the suppressor

TABLE I

RECOVERY OF IONS IN LORACARBEF

Amount in sample (μg)	Amount added (μg)	Total (μg)	Found (μg)	Recovery (%)
Chloride				
106	25.0	131	125	95
104	125.2	229	231	101
104	250.4	354	353	100
106	375.5	481	483	100
103	500.7	604	614	102
			Average:	100
Sodium				
3.9	128.8	132.7	138	104
3.9	257.5	261.4	258	99
3.9	386.3	390.2	404	104
3.9	515.5	519.4	527	101
			Average:	102
Ammonium				
4.0	10.1	14.1	14.0	99
4.1	50.5	54.6	50.7	93
4.0	100.9	104.9	119	113
4.1	151.4	155.5	156	100
4.1	201.9	206.0	204	99
4.0	252.3	256.3	258	101
			Average:	101
Calcium				
16.9	9.6	26.5	28.2	106
16.9	38.4	55.3	50.9	92
17.0	57.5	74.5	70.7	95
16.7	76.7	93.4	92.4	99
16.9	95.9	112.8	109.3	97
			Average:	98

in-line, showing that it is not retained in the suppressor. Its elution causes a slight, broad baseline depression beginning at approximately

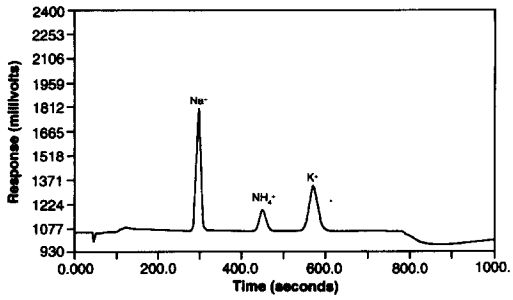

Fig. 3. Separation of monovalent cations. Injected solution: 25 μl of 10 μg Na^+, 7 μg NH_4^+, 9 μg K^+/ml, and 8 mg/ml loracarbef prepared in eluent. Eluent: 7.5 m*M* HCl. 1 mV≡ 0.01 μS.

800 s, returning to baseline at about 1000 s. Any loracarbef that passes through the membrane into the closed-loop TBAOH regenerant stream is quickly decomposed ($t_{1/2}$ = 8 min) due to the high alkalinity and cannot affect subsequent injections. Loracarbef enters the suppressor as a singly charged cation and is subsequently deprotonated to its zwitterionic form which, as such, can diffuse across the membrane. Its eventual elution produces minimal response at the conductivity detector due to its drastically reduced concentration and low conductance of the zwitterionic form. The result is an interference-free chromatogram. The CS2 column has retention only for species that are cations at the pH of the eluent, which is about 2. Neutral and negatively charged species that could possibly

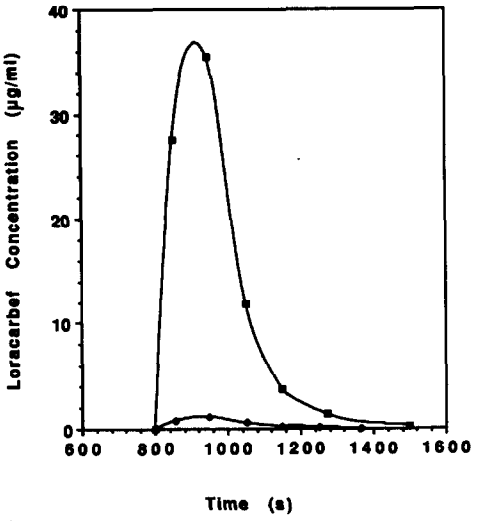

Fig. 4. Concentration of loracarbef in effluent fractions without (□) and with (◆) the micromembrane suppressor following the analytical column. Conditions same as for monovalent cations (see Experimental). Fractions were assayed by HPLC with UV detection.

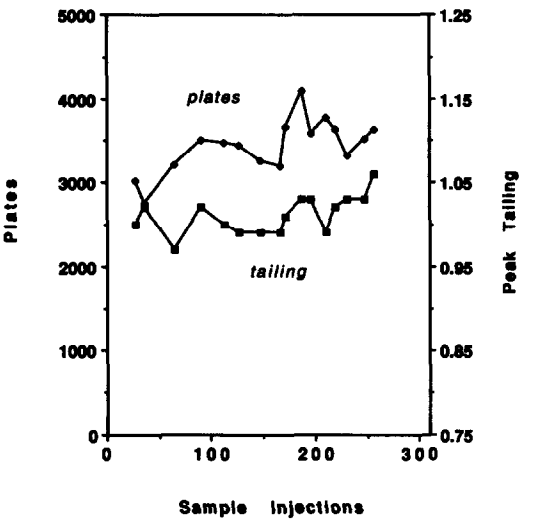

Fig. 5. Column performance over time: plates (◆) and tailing (□) data for ammonium ion on a Dionex CS2 cation-exchange column when assayed for in Loracarbef. Injected: 5 µg NH_4^+/ml.

interfere with these monovalent cations elute in the void volume.

Suitability of the chromatographic system is based upon two criteria: background conductivity and ammonium peak tailing. The system is deemed suitable when both the background conductivity of the system is less than 20 µS and the tailing of the ammonium peak obtained from a 5 µg/ml solution, determined at 10% of the peak height, is between 0.85 and 1.2. Tailing was chosen as a suitability criterion because it is sensitive to the condition of the analytical column and becomes a problem before insufficient resolution of possible interfering analytes does. Additionally, tailing can be indicative of the condition of the micromembrane suppressor (see section *Problems encountered with divalent cation system*). The data shown in Fig. 5 demonstrates the durability of a CS2 cation column used intermittently (for loracarbef exclusively) over the course of a year. Causes and remedies of high background conductivity are addressed in a subsequent section of this article.

Linearity in the matrix (COD = 0.9977) of sample solutions containing from 1.0 to 25 µg/ml NH_4^+ is obtained with excellent recovery (see Table I). The recovery studies for ammonium and sodium ion were performed on loracarbef samples that had low levels of these analytes. The within-run R.S.D. (multiple sample preparations, $n = 12$) for NH_4^+ at 5.6 µg/ml is 5.1%. The detection limit, defined as the ammonium ion level producing a peak amplitude 3× of the noise level, is approximately 0.4 µg/ml (50 ng/mg in the matrix); the limiting factor being the solubility of loracarbef in the mobile phase. (This detection limit is well below the level that our laboratory needs to determine.) Linearity in the matrix (COD = 0.9992) and recovery of Na^+ (at 3 mg/ml loracarbef) and K^+ are also excellent in this working range.

Determination of Ca^{2+}. Sample solutions were prepared by dissolving 4 mg/ml loracarbef in purified water. For the determination of calcium, an added "pusher", DAP, is added to the eluent to elute this species from the CS10 analytical column. Fig. 6B is the chromatogram obtained when determining Ca^{2+} in loracarbef. As with the monovalent cations, linearity in the matrix (COD = 0.9956) and recovery are excellent (see Table I). Rocklin *et al.* [4] have published a general discussion of DAP/HCl eluents and have shown the relation of retention of cations to

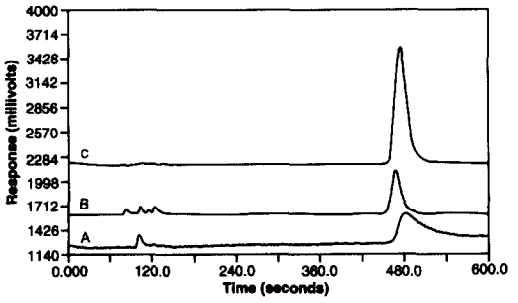

Fig. 6. (A) Chromatographic behavior of Ca^{2+} on a new CS10 separator column and poorly operating suppression equipment and (C) on a new CS10 separator column and new suppression equipment. Injected solution: 25 μl of 23 μg Ca^{2+}/ml, prepared in eluent. (B) Chromatogram with Ca^{2+} present at 9.2 μg/ml and loracarbef present at 4.3 mg/ml. Eluent: 12 m*M* DAP · HCl, 40 m*M* HCl. 1 mV ≡ 0.01 μS.

DAP/HCl eluent concentrations under conditions similar to those described here. Campbell *et al.* [5] give the details of the CS10 column.

Problems encountered with divalent cation system

After relatively short periods of use, the chemically suppressed system for determining divalent cations is susceptible to problems such as poor peak shape and high background conductivity. These problems, as well as system noise, are examined here by assessing the contributions of the analytical column, eluent and suppression system, with focus on the latter.

Poor peak shape. In any chromatographic system, poor peak shape can be attributed to improper chromatographic conditions relating to either the mobile phase or the separator column. When chemical suppression is used, peak shape is also dependent on the condition of the suppressor. Fig. 6A shows the chromatogram obtained by injecting a solution containing 23 μg Ca^{2+}/ml on a system made up of a new IonPac CS10 column and a micromembrane suppressor and AutoRegen cartridge that had been in general service for pharmaceutical assays (loracarbef and other compounds) for approximately 100 h over the course of several months. This amount of use corresponds to slightly less than one-third of the expected lifetime of the AutoRegen cartridge. The peak tails badly, perhaps due to adsorption of Ca^{2+} in the suppressor, and the sensitivity is poor. The poor peak shape can be attributed to poorly functioning suppression equipment rather than the separator column. As shown in Fig. 3, this same "poorly functioning" equipment performed satisfactorily under the monovalent assay conditions. The system employing the DAP/HCl eluent for the determination of a divalent cation such as Ca^{2+} is much more sensitive to the condition of the suppression equipment. This is due to the greater demands put on the suppressor by both the higher [HCl] and the nature of the Ca^{2+} ion. Replacement of all suppression components with new components resulted in satisfactory peak shape for Ca^{2+} (tailing factor = 1.2) and restoration of sensitivity (as shown in Fig. 6C). A sufficient, steady regenerant flow is necessary for efficient chemical suppression. Drug matrices irreversibly bound to the suppressor and iron-containing environmental samples which will precipitate as iron hydroxides in the suppressor may lead to both increased backpressures and analyte adsorption.

High background conductivity. The inability to efficiently suppress the background conductivity

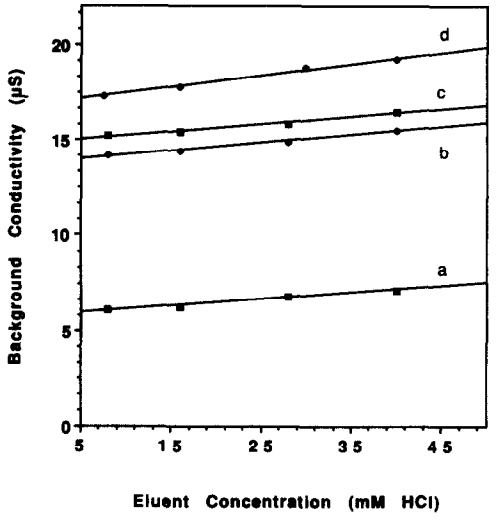

Fig. 7. Dependencies of the background conductivity on the concentration of HCl in the eluent measured on suppression equipment in various stages of use: (a) new, (b) used suppressor after cleaning with 1 *M* HCl, (c) used suppressor before cleaning with 1 *M* HCl, (d) poorly operating suppressor (used in Fig. 6).

leads to decreased sensitivity, non-linearity, and standard calibration curves with negative intercepts [17]. The contributions of HCl, DAP and the suppression equipment as primary sources of high background conductivity were examined.

Plots of [HCl] *versus* background conductivity on suppression equipment that was in various stages of use are shown in Fig. 7. When using poorly functioning suppression equipment, increasing the [HCl] in the eluent caused an increase in the already high background conductivity; the slope of the line corresponding to a 60 nS/m*M* HCl increase (Fig. 7d). On a new micromembrane suppressor and AutoRegen cartridge, the background conductivity decreased significantly, indicating a much higher efficiency (Fig. 7a) and the slope of the line was 35 nS/m*M* HCl, indicating greater capacity. Fig. 7c shows that after receiving fairly light use, the background conductivity displayed a marked increase over when the suppression equipment was new. Despite the increase, Ca^{2+} peak shape was comparable to that obtained when new equipment was part of the system. Cleaning the suppressor per the manufacturer's instructions with 1 *M* HCl did lower the background conductivity slightly, but not to levels seen on new equipment. The capacities to suppress the increase in [HCl] are comparable to new suppressors as the slopes of the lines, Fig. 7b and c, for used and used/cleaned suppressors are 40 and 42 nS/m*M* HCl, respectively.

At constant added [HCl] on poorly functioning suppression equipment, the background conductivity was examined as a function of the [DAP · HCl] in the eluent. At all eluent concentrations studied here, the primary form of DAP is H_3DAP^{2+} ($K_a = 0.047$ [18]). With a [DAP] of 0, 1, 3 and 6 m*M*, the background conductivity was 19.2, 19.5, 18.8 and 18.1 μS, respectively. This constant, even slightly decreasing background conductivity parallels the decrease in the calculated equilibrium [H^+] which is 40, 39, 37 and 31 m*M*, respectively. The same trend was also observed qualitatively on suppression equipment in various conditions, indicating that DAP is converted to an anion and removed from the eluent independent of suppressor condition, and is not contributing to the background conductivity. Since DAP did not contribute to the background conductivity on "poorly operating" suppression equipment, its contribution on a new system was believed to be minimal and therefore not studied.

Noise. Average baseline noise was measured by calculating the average peak-to-peak amplitude of the noise spikes in the region of the chromatogram between 200 and 300 s after injection. For [HCl] between 10 and 40 m*M* (DAP absent) on all suppressor conditions studied here ($n = 16$), the average baseline noise remained under 0.03 μS, still allowing for detection limits stated previously. In general, marked increases in the magnitude of the noise are not related to the condition of the suppression equipment at any eluent concentration of HCl.

CONCLUSIONS

Due to the lack of matrix intereference, ion chromatography with chemical suppression is an excellent technique for determining common ions in a bulk drug such as loracarbef. Advantage is taken of the acid–base chemistry of loracarbef, resulting in a drastic reduction in its concentration in the eluent prior to detection of the analytes of interest. Linearities in the presence of the matrix (COD > 0.995) and recoveries from the matrix are excellent.

Premature exhaustion of suppression efficiency for the divalent cation system can lead to deteriorated peak shapes and high background conductivity. This work provides the analyst with a rationale for system adjustments when the problems studied here are present but not severe.

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