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Analysis of potassium counter ion and inorganic cation impurities in pharmaceutical drug substance by capillary electrophoresis with conductivity detection

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Abstract

Capillary electrophoresis with conductivity detection is a selective and quantitative method for the analysis of counter cations, such as potassium, in pharmaceutical drug substances. It is also a sensitive and specific technique for screening and measuring inorganic impurities in drug substance samples. Both counter ion and inorganic impurities can be measured in the same test. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; Conductivity detection; Potassium; Counter ion; Inorganic cations; Pharmaceutical

1. Introduction

Many polar drug substances are developed in the salt form to promote solubility and stability. The salt counter ion usually needs to be determined as part of the testing necessary for the release of the drug substance for use in clinical supplies. Current methods for determining counter ion content of drug substance include titration, ion chromatography, or spectroscopic methods such as atomic absorption. Since only small amounts of drug substance are often available early in development, separation methods such as ion chromatography may be preferred at this stage. Spectroscopic techniques or titration may be preferred later in development or in the manufacturing quality control lab. It is also desirable to screen for unknown inorganic impurities, which may be present as by-products of the synthesis process in the early development of the drug substance. Although there are reliable, efficient methods for the screening and measuring synthetic impurities by gradient HPLC and for volatile solvents by GC, there are few separation methods for profiling and identifying trace inorganic impurities. Capillary ion electrophoresis (CIE) with indirect UV detection [1,2] has been investigated as a method to determine the content of inorganic counter ions and impurities in pharmaceutical drug substances [3–7] and inorganic impurities in waste water. However, the calibration is linear only over a limited concentration

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range and there is relatively poor limit of detection [4,5].

CIE with conductivity detection [8–10] is a more sensitive and linear method for separation and measurement of inorganic ions and has been applied in the analysis of inorganic and pharmaceutical drug substances [8,10]. We have evaluated CIE with conductivity detection as a method for determining potassium counter ion content and for screening for inorganic impurities in pharmaceutical drug substances. This technology is very useful in the early development of drug substance and the results of our evaluation are included in this paper.

2. Experimental

2.1. Equipment

A Crystal 300 capillary electrophoresis system equipped with a Crystal 1000 conductivity detector [9] from Thermo Bioanalysis (Santa Fe, NM) was used for separation and analysis of inorganic cations. The detector output was interfaced to a Fisons Multichrom software program (version 1.8–3.3) on a Vax 6000 Series computer for data reduction and generation of chromatograms and electropherograms. The ConCap capillaries used with the conductivity detector were obtained from Thermo Bioanalytical. These fused silica capillaries are 60 cm in length and 50 μ ID and have a stainless steel tip which attaches into the detector cell. Each capillary was preconditioned by washing with 1 N NaOH for 5 min at 2000 mbar presure and this was followed by a 5-min wash with water. This treatment was repeated each time a different run buffer was used. All samples were injected hydrodynamically at a pressure of 40 mbar for a period of 0.20 min. A volume of approximately 12 nl was injected into the capillary and this corresponded to about 1% of the internal volume of the capillary. It was necessary to wash the capillary with water before each sample to remove retained drug substance and maintain migration time reproducability.

A Sorvall Model 600 Centrifuge (New Haven, CT) equipped with 10 ml conical polyethylene centrifuge tubes were used for centrifugation of samples. Drug substance samples dissolved in aqueous solutions were centrifuged at 2000 rpm for 5 min in polyethylene centrifuge tubes in order to remove particulates that might plug the capillary. Water-insoluble drug substances were dissolved in methanol at a concentration of 10 mg/ml and then diluted 1:10 with distilled water and the precipitated drug substance removed from solution by centrifuging at 3500 rpm for 5 min. Centrifugation was used for sample preparation since we found that contamination of samples with low levels of cationic impurities was a problem with many types of filter devices. In general we also found it best to use freshly distilled water and to rinse plasticware before use in CIE analysis.

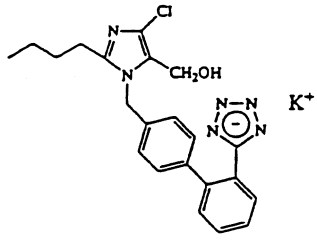
A Perkin Elmer Model 5000 Atomic Absorption Spectrometer (Norwalk, CN) was also used for determination of potassium in aqueous solutions of drug substance samples. All samples were tested at a wavelength of 766.5 nm and slit width of 1.4 nm. An air/acetylene oxidizing flame was used with measurement time of 5 s. Five replicate readings were made of standards, samples and blanks. Potassium standards were made by dilution in distilled water of a 1000-ppm aqueous standard from Spex CertiPrep (Metuchen, NJ).

2.2. Reagents and run buffers

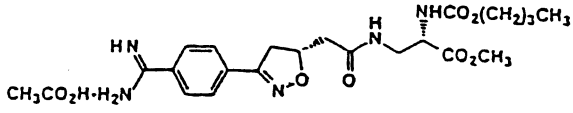
The creatinine, and 18-Crown-6 were obtained from Sigma (St Louis, MO). The LiOH (lithium hydroxide) and KCl (potassium chloride) were obtained from Aldrich (Milwaukee, WI). The glacial acetic acid was from EM Science (Gibbstown, NJ).

The creatinine-acetic acid run buffer was prepared by dissolving 1.70 g creatinine, 310 mg of 18-Crown-6, and 435 μl of glacial acetic acid in a 500-ml volumetric flask and then filling to mark with distilled water.

The losartan potassium, Roxifiban (structures shown in Fig. 1), Etanidazole (*N*-[2-Hydroxyethyl]-2-nitro-1H-imidazole-1-acetamide), and the proprietary water insoluble drug substance were obtained from the DuPont Pharmaceuticals (Wilmington, DE).



Losartan Potassium



Roxifiban

Fig. 1. The structure of losartan potassium, etanidazole, and roxifiban.

3. Results and discussion

3.1. Inorganic cations

The first goal of this work was to establish if CIE with conductivity detection had aufficient sensitivity, precision and accuracy to be used as a method for determining inorganic impurities in drug substances. A variety of inorganic cations can be separated and an electropherogram with conditions is shown in Fig. 2. The cations are attracted to the cathode which is at the detector end of the capillary and separation times are typically less than 8 min.

The precision of the CIE separation can be determined by injecting a solution of cation standards (Fig. 2) six times and measuring the migration times and peak areas as shown in Tables 1

Table 1
Migration time precision

Cation (ppm)	% RSD of migration time for six injections			
	1	10	20	30
NH_4	0.30	0.33	0.20	0.30
K	0.27	0.30	0.21	0.38
Na	0.39	0.36	0.18	0.32
TMAH	0.35	0.34	0.29	0.16
Li	0.39	0.29	0.16	0.32

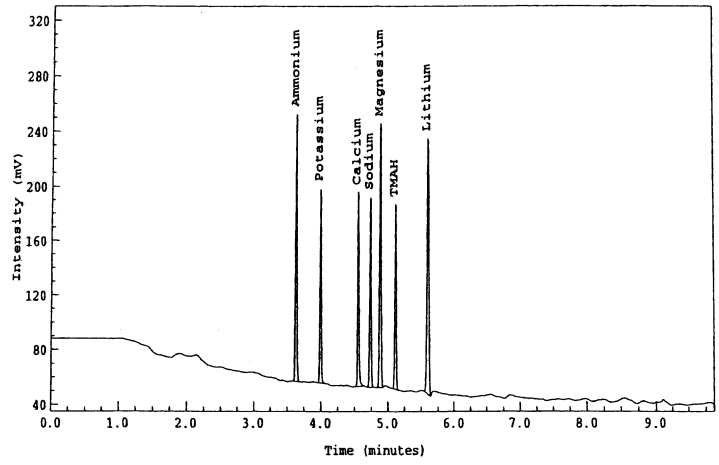


Fig. 2. Separation of inorganic cation standards. Separation conditions: Concap capillary 60 cm by 50 μ; run buffer of 30 mM creatinine, 30 mM acetic acid, 4.5 mM 18-Crown-6; 25 kV run voltage; 30°C oven temp; conductivity detection; pressure injection of 40 mBar for 12 s. Flush at 2000 mBar with water for 1 min and then run buffer for 1.5 min before each injection. Sample contains 10 ppm of ammonium, potassium, sodium, lithium, and 50 ppm TMAH and magnesium in water.

Table 2
Peak area precision

Cation	% RSD of peak area for six injections			
	1 ppm	10 ppm	20 ppm	30 ppm
NH_4	1.67	2.04	0.65	0.45
K	14.77	4.23	1.45	0.81
Na	45.21	7.88	1.04	1.59
TMAH	7.15	2.98	1.27	0.47
Li	2.43	3.21	0.99	0.82

and 2. Precision of the five cations (ammonium, potassium, sodium, tetramethylammonium, and lithium) was measured in standard solutions containing 1, 10, 20, and 30 ppm of each cation. The % RSD of each migration time was less than 0.4% at all concentrations for all cations. The peak area precision of all cations was 2.0% RSD at concentrations of 20 ppm and greater. However, the RSD of sodium and potassium increased dramatically at lower concentrations and approached 50% RSD at concentrations of 1 ppm.

The limit of detection (LOD) is illustrated in Fig. 3 where cation standards were spiked at concentrations of 500 part per billion (ppb). Samples were introduced into the capillary by direct injection of 12 nl of sample solution; this volume is approximately 1% of the internal volume of the capillary. The LOD for this separation with conductivity detection is approximately 10–20 ppb. This is an order of magnitude more sensitive than similar cation separations with indirect UV detection [9] as reported in the literature and this was confirmed in our lab. This sensitivity is useful when doing trace analysis in complex mixtures. Lower LODs have been obtained using isotachophoresis stacking techniques [9,11] but this was not necessary for our work with pharmaceutical drug substances.

The calibration curves of peak area versus concentration are linear from 20 ppb to 50 ppm for these inorganic cations in this run buffer. Although the peak area calibration is linear for three orders of magnitude, peak widths increase significantly at concentration above 2 ppm with these conditions and peak height calibrations are not linear above that value. Calibration curves of lithium, potassium, sodium, ammonium, and TMAH are all linear through zero with correlation coefficients of 0.9997 or higher.

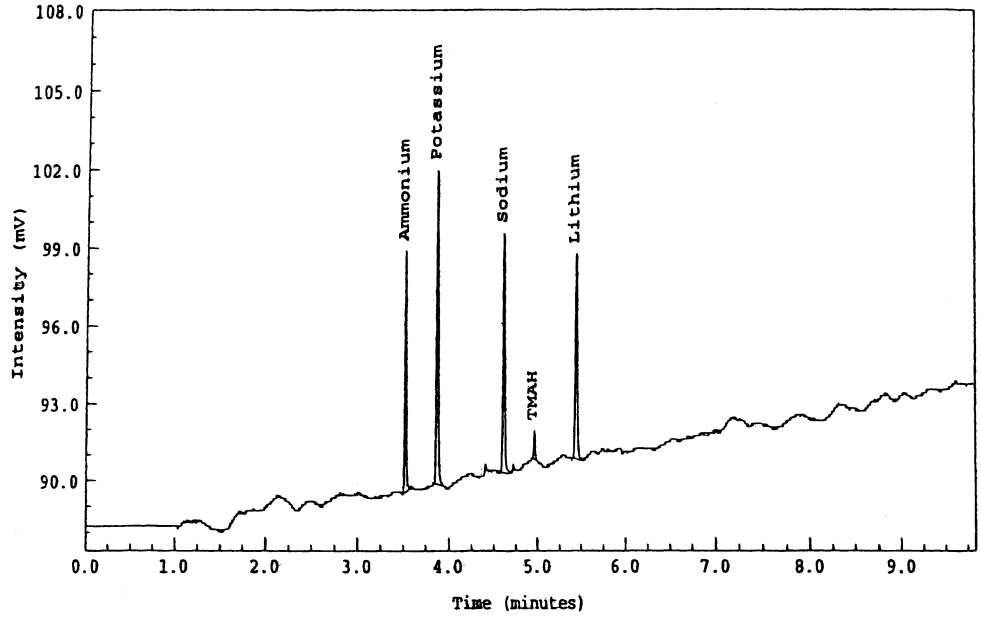


Fig. 3. Limit of detection of inorganic cation standards. Same separation conditions as Fig. 2. Sample contained 500 ppb of labeled cations.

Table 3
Recovery studies in drug substance

Cation	% Recovery from Etanidazole (six samples each concentration)	
	5 ppm	30 ppm
NH_4	93.8 ± 1.3	94.5 ± 2.4
K	108.4 ± 7.3	100.7 ± 2.8
Na	102.8 ± 16.7	100.2 ± 3.3
TMAH	103.2 ± 3.9	97.2 ± 1.5
Li	102.6 ± 2.7	99.9 ± 2.4

Table 4
Recovery studies in water-insoluble drug

Cation	% Recovery from water-insoluble drug substance (six samples each concentration)	
	5 ppm	30 ppm
NH_4	81.2 ± 2.9	97.6 ± 7.1
K	76.0 ± 9.2	103.6 ± 8.6
Na	73.4 ± 12.5	119.7 ± 12.8
TMAH	88.6 ± 4.1	97.6 ± 2.1
Li	87.6 ± 4.0	104.3 ± 6.5

Capillary ion electrophoresis can be used to measure trace amounts of cations in drug substance samples as shown in the recovery study in Table 3. A water-soluble drug, Etanidazole, was dissolved at a concentration of 1 mg/ml in aqueous mixtures (5 or 30 ppm) of cation standards. Recovery was calculated by comparing peak area of the cations in dissolved drug substance samples to peak areas of aqueous standard mixtures. Recoveries are listed in Table 3 and ranged from 93 to 108% with RSDs of less than 4%. The Etanidazole drug substance was retained on the capillary but was flushed away by the water wash before the injection of each sample. Although the drug substance is in much greater concentration in the sample, it does not affect migration time or quantitation of the cations.

A second recovery study was done with a proprietary water-insoluble drug substance. This drug substance was first dissolved in methanol and then precipitated by addition of distilled water. The precipitate was then removed by centrifugation as described in Section 2. The cation concentrations in the drug substance supernatants were then compared to an aqueous standard mix (in 90:10 water/methanol) by CIE. Recoveries were calculated to be from 73 to 120% with RSDs between 2 and 13% and are listed in Table 4. Precipitation of the water-insoluble drug apparently reduces recovery of the cations at a concentration of 5 ppm although recovery and quantitation is satisfactory with the exception of sodium.

A simple method for determination of potassium in losartan potassium drug substance was then developed. A 71-mg sample of drug substance was weighed into a 200-ml flask and dissolved in distilled water containing lithium internal standard at 20 ppm. Standards for the CIE were prepared by weighing dry potassium chloride into volumetric flasks and dissolving in distilled water with a lithium internal standard at 20 ppm to make a 1000-ppm potassium standard solution. A 30-ppm potassium standard was made by dilution in distilled water with lithium internal standard at 20 ppm. Samples and standards were centrifuged at 3500 rpm in polyethylene centrifuge tubes to remove particulates before analysis. Fig. 4 shows the separation of a drug substance sample by CIE. Six samples from one lot of drug substance were analyzed by CIE to determine the method precision for potassium determination and results are shown in Table 5. The RSD of 1.72% is typical for the CIE method and is adequate for this analysis. The potassium content of this lot was determined to be 8.28%; the theoretical amount of potassium in losartan potasium is 8.48%

Both CIE and atomic absorption spectroscopy (AAS) were than used to determine the potassium content of seven different lots of losartan potassium. The same drug substance samples used for CIE measurements as described above were diluted in distilled water to a concentration of 1 ppm for the AAS testing. potassium standards (0.5, 1.0 and 2.0 ppm) were made and used for AAS testing with procedures and conditions as described in Section 2. The potassium content of drug substance samples measured by CIE and AAS is shown in Table 6 and compare well.

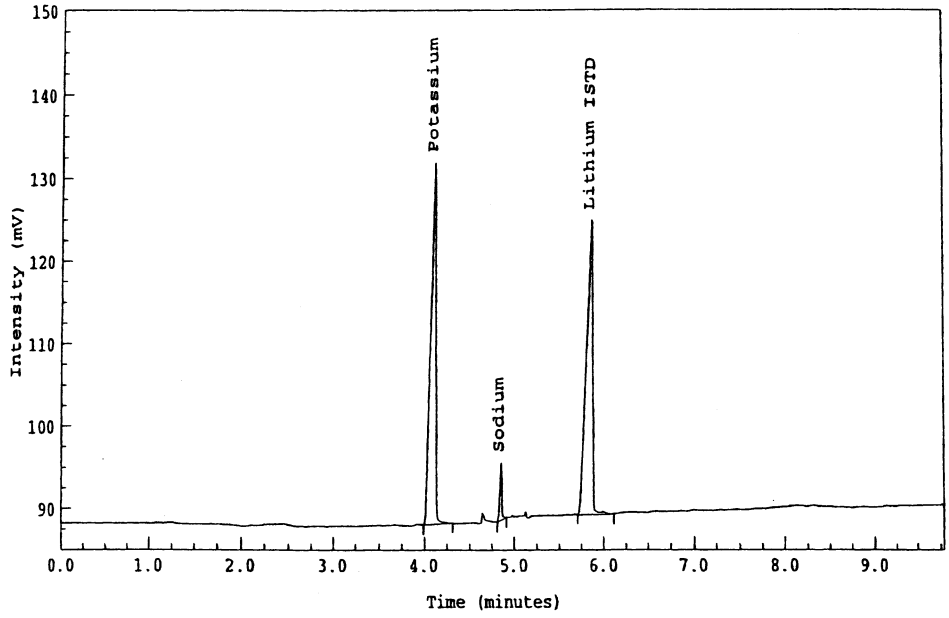


Fig. 4. Separation of potassium in losartan potassium drug substance sample. Same separation conditions as Fig. 2. Lithium internal standard at 20 ppm added to sample solution.

The advantages of the CIE method is that inorganic cation impurities can be separated and measured in the same electropherogram as used for determination of the counter cation. This can be seen in the electropherogram in Fig. 4 which shows a significant amount of the sodium impurity in losartan potassium. The same CIE technique has been used to screen other types of drug substances as shown in Fig. 5 where a significant (0.4%) ammonium impurity was found in a sample of Roxifiban drug substance (structure in Fig. 1) that was dissolved in water with a lithium internal standard. This information can be useful to the process chemist who can then adjust synthesis conditions to eliminate undesirable impurities.

4. Conclusions

Capillary ion electrophoresis with conductivity detection is a precise and accurate method for the determination of potassium counter ion in pharmaceutical drug substances. Creatinine and acetic acid were used in the run buffer. The limit of detection for potassium was approximately 20 ppb in aqueous solution and the calibration was linear to 50 ppm. A peak area precision of less than 2.0% was measured by replicate injections of a potassium standard at sample concentrations of 10 ppm or larger although a greater %RSD was observed at lower concentrations. Method precision for the potassium determination in drug substance was 1.72%. Potassium content of losartan

Table 5
Precision of counter ion analysis

Sample	Losartan — lot 1
	% Potassium
1	8.53
2	8.32
3	8.15
4	8.22
5	8.15
6	8.33
Mean	8.28
S.D.	0.14
%RSD	1.72

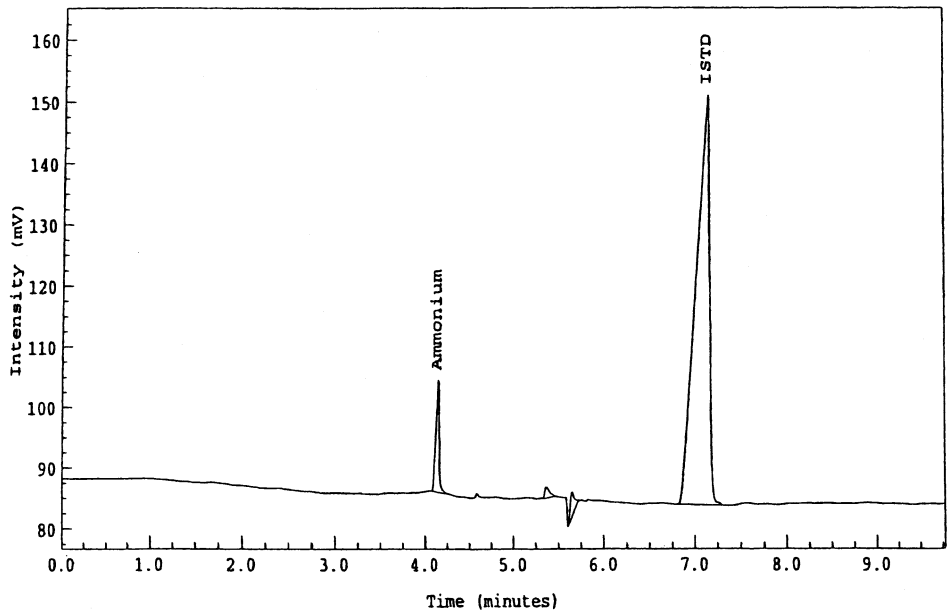


Fig. 5. Separation of ammonium impurity in Roxifiban drug substance. Same conditions as Fig. 2. Lithium internal standard was added to sample solution at 40 ppm.

potassium lots determined by CIE and atomic absorption compare well. Sample preparation is simple and only milligram amounts of drug substance are required.

A major advantage of CIE with conductivity detection is that small amounts ($<0.1\%$) of inorganic impurities can be separated and profiled with the same conditions used for potassium determination. This makes a convenient method for screening for unknown inorganic impurities in the same CIE analysis used for determining the counter ion. Trace inorganic cation impurities can be identified by comparison to migration times of standards.

Table 6
Comparison of counter ion methods

Sample	Potassium in losartan (%)	
	CIE	AAS
Lot 1	8.28	8.27
Lot 2	8.47	8.48
Lot 3	8.85	8.78
Lot 4	8.48	8.80
Lot 5	8.76	9.05
Lot 6	8.51	9.00
Lot 7	8.54	8.22

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