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Analysis of acetate counter ion and inorganic impurities in pharmaceutical drug substances by capillary ion electrophoresis with conductivity detection

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Abstract

Capillary electrophoresis with conductivity detection is a versatile new method for the analysis of counter ions in pharmaceutical drug substances. It is a sensitive and linear technique for determining inorganic ions and short chain carboxylic acids such as acetate. Both acetate counter ion and inorganic impurities can be separated and determined in the same assay. © 1997 Elsevier Science B.V.

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1. Introduction

The testing of pharmaceutical drug substance purity is one of the primary responsibilities of the analytical laboratory in the drug development process. Since many polar drug substances are developed in the salt form, the salt counter ion needs to be determined as part of the release testing. Current methods for determining counter ion content of drug substances include titration or ion chromatography. The ion chromatography (IC) method is often preferred early in the development stage when only small amounts of the drug substance may be available for testing and method development.

Early in the development process it is also desirable to screen for unknown inorganic impurities which may be present as by-products of the synthesis. Although there are reliable, efficient methods for screening and measuring synthetic organic impurities by gradient HPLC and for volatile solvents by GC, there are no comparable convenient separation methods for profiling and identifying trace inorganic impurities. Capillary ion electrophoresis (CIE) with indirect UV detection is a relatively new analytical technique [1,2] which has been used to identify and measure trace

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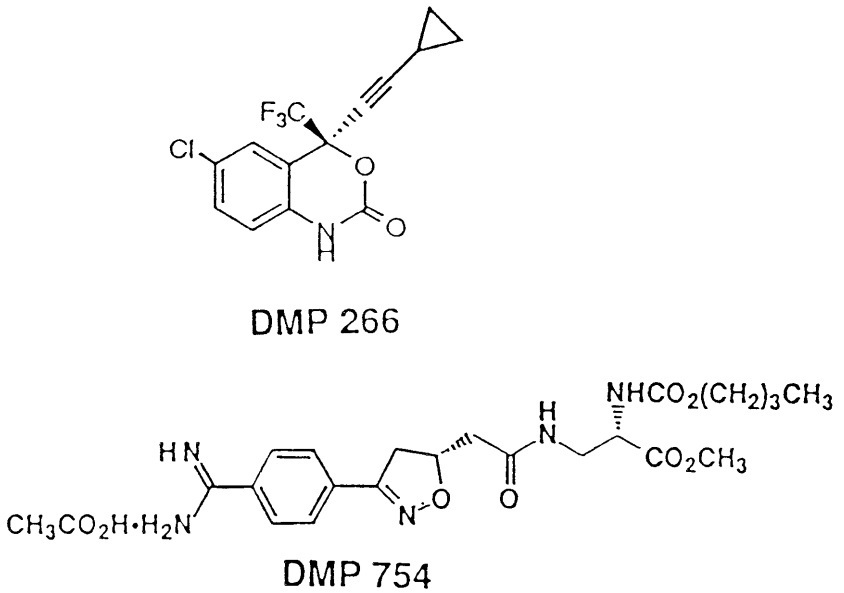

Fig. 1. The structures of DMP 266 and DMP 754.

inorganic impurities in waste water and cosmetics [3,4] and to determine the content of inorganic counter ions and impurities in pharmaceutical drug substances [5–7]. However, CIE with indirect UV detection has a relatively poor limit of detection and calibration is linear only over a limited concentration range [4,5]. Differences in ion mobility of sample and run buffer co-ion can also cause distortion of ion peaks [5].

Recently CIE with conductivity detection [8,9] has been developed as a more sensitive and linear method for separation and measurement of inorganic ions and short chain carboxylic acids and has been applied in the ion analysis of concentrated acids and urine samples [8]. We have evaluated CIE with conductivity detection as a method for determining acetate counter ion content and screening for inorganic impurities in pharmaceutical drug substances. We have found this new technology to be very useful in our analytical lab and the results of our evaluation and examples of applications are included in this paper.

2. Experimental

2.1. Equipment

The separations were done on a Crystal 300 capillary electrophoresis system equipped with a Crystal 1000 conductivity detector from Thermo CE (Boston, MA). A Sorvall Model 600 Centrifuge (New Haven, CT) equipped with 10 ml conical glass centrifuge tubes was used for centrifugation of samples. The detector output was interfaced to a Fisons Multichrom software program (version 1.8-3) 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 CE. These fused silica capillaries are 60 cm in length and 50 micron i.d. and have a stainless steel tip which attaches into the detector cell. Each capillary was preconditioned by washing with 1 N NaOH for 10 min at 2000 mbar pressure and this was followed by a 20 min wash with water. This treatment was repeated at the end of each work-

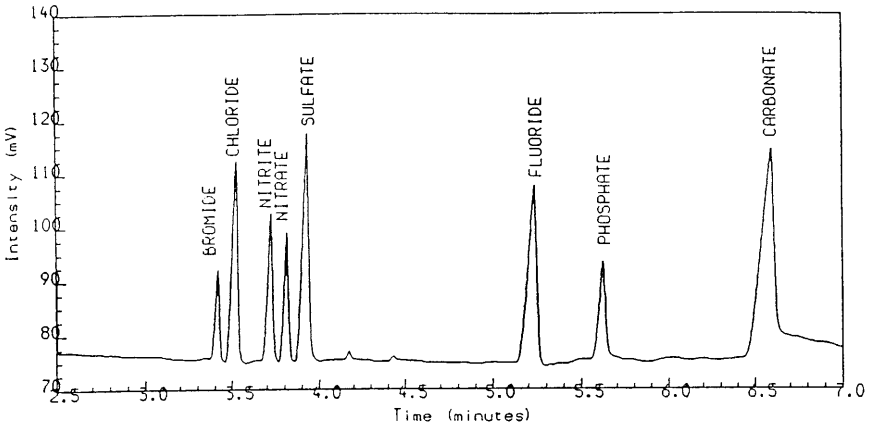

Fig. 2. Separation of inorganic anion standards. Separation conditions: concap capillary 60 cm × 50 μm; run buffer of 50 mM CHES, 20 mM LiOH monohydrate, 0.03% Triton X-100; 25 kV run voltage; 30°C oven temp; conductivity detection; pressure injection of 40 mbar for 12 s. Flush at 2000 mbar with 1 mM CTAB for 1 min and then run buffer for 1.5 min before each injection. Sample contains 2 ppm each anion in water.

ing day or each time a different run buffer was used. Separate capillaries were used for anion and cation separations.

Samples were filtered with 0.45 μm PVDF membrane Autovial® syringeless filters from Whatman (Clifton, NJ). These filters did not introduce any significant amount of inorganic impurities into the samples. In general we found that contamination of samples with low levels of inorganic impurities, particularly chloride and sulfate, was a problem with many types of filter devices.

2.2. Reagents and run buffers

The MES (2-[*N*-morpholino]-ethanesulfonic acid) and CHES (2-[*N*-cyclohexylamino]-ethanesulfonic acid) buffers, 18-Crown-6, L-histidine (His) and Triton X-100 were obtained from Sigma (St. Louis, MO). CTAB (cetyltrimethylammonium bromide) and TTAB (tetradecyltrimethylammonium bromide) were obtained from Fluka Chemical (Ronkonkoma, NY). LiOH (lithium hydroxide monohydrate) was obtained from Aldrich (Milwaukee, WS). Glacial acetic acid, used for preparing standards, was from EM Science (Gibbstown, NJ).

The CHES run buffer was prepared by dissolving 2.59 g of the buffer and 0.21 g LiOH monohydrate in 100 ml of water in a 250 ml volumetric flask and adding 0.75 ml 10% w/v aqueous solution of Triton X-100 and then filling to the mark with deionized water. This run buffer was then vacuum filtered through a Millipore Millicup HV 0.45 μm filter unit (Bedford, MA); it was necessary to make up the run buffer solution daily before use. The 1 mM CTAB solution was prepared by dissolving 182 mg of CTAB in 500 ml deionized water in a 500 ml volumetric flask.

The MES run buffer for anion separation was prepared by adding 3.19 g of buffer and 2.33 g L-histidine to 100 ml water in a 250 ml volumetric flask. A 7 ml aliquot of 1 M TTAOH (hydroxide form of TTAB) and a 0.75 ml aliquot of 10% aqueous solution of Triton X-100 were then

Table 1
Precision of anion standards

Anion	Migration	Peak area	Peak area ratio
Bromide	1.20	1.01	0.67
Nitrite	1.31	1.32	0.74
Sulfate	1.37	1.33	0.58
Fluoride	1.69	1.69	0.62

Values are percent R.S.D. of eight injections with CHES–CTAB run buffer.

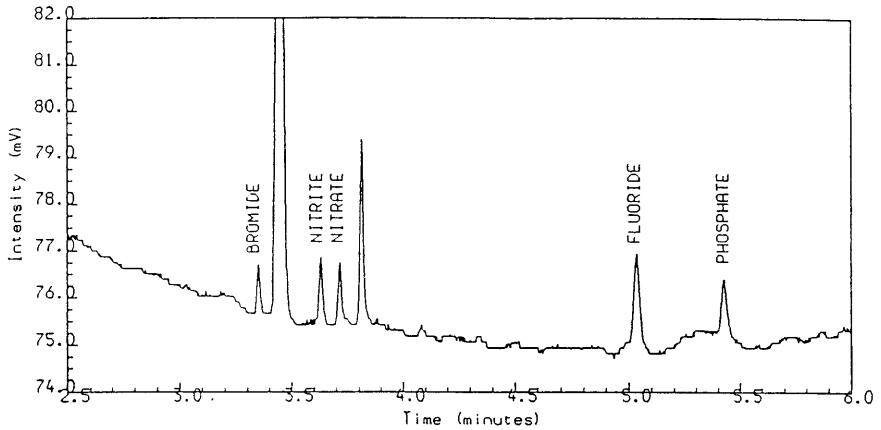

Fig. 3. LOD of inorganic anion standards. Same separation conditions as Fig. 2. Sample contained 2 ppm chloride and 50 ppb of labeled anions.

added and the flask was filled to the mark with deionized water. The 1 M TTAOH solution was prepared by converting TTAB to the hydroxide form by the following procedure. A Dionex On Guard Sample Pretreatment cartridge (Sunnyvale, CA) was washed with 10 ml water from a 10 ml syringe followed by 10 ml 1 N NaOH followed again by 10 ml water.

A 10 ml aliquot of 1 M aqueous TTAB solution was then slowly (one drop every 2 s) forced through the cartridge with a 10 ml syringe; the first ml was discarded and the next 7 ml of the TTAOH solution were dispensed into the 250 ml volumetric flask containing the MES solution as described above. A 0.75 ml aliquot containing 10% Triton X100 solution was added and the volumetric flask was then filled to the mark with distilled water and mixed. This MES run buffer was prepared fresh daily and vacuum filtered with a Millicup 0.45 μm filter before use.

The MES run buffer for the cation separation was prepared by dissolving 1.30 g MES buffer, 2.20 g L-histidine, and 70 mg 18-Crown-6 in a 250 ml volumetric flask and filling to the mark with distilled water. This run buffer was prepared fresh daily and vacuum filtered through a Millicup 0.45 gμm filter before use.

DMP 754 and DMP 266 whose structures are shown in Fig. 1 were obtained from DuPont Merck Pharmaceutical (Wilmington, DE).

3. Results and discussion

3.1. Inorganic anions

The first goal of this work was to establish if CIE with conductivity detection had sufficient sensitivity, precision and accuracy to be used as a method for determining inorganic impurities in drug substances. A variety of inorganic anions can be separated and analyzed under the conditions shown in Fig. 2. To separation anions by CIE it is necessary to reverse the electroosmotic flow (EOF) in the capillary; this can be done by rinsing the capillary with a quaternary ammonium surfactant, such as CTAB, which binds to the ionized silanols to form a monolayer coating. Additional surfactant (CTAB) then adsorbs on the first layer, resulting in a positive charge on the wall [10]. This reversal in wall charge results in a reversal of the EOF towards the anode. The polarity of the power source is reversed and the anions are then attracted to the anode which is at the detector end of the capillary. It is necessary to rerinse the capillary with the CTAB before each sample analysis in order to maintain a reproducible EOF.

The precision of the CIE separation can be determined by injecting the standard solution (Fig. 2) eight times and measuring the migration times and peak areas as shown in Table 1. There

Table 2
Recovery of spiked standards

Sample	Bromide	Nitrite	Sulfate	Fluoride	Phosphate
1	99.0	100.9	100.4	99.3	90.3
2	97.7	102.4	101.8	99.1	93.1
3	94.9	105.2	97.7	99.3	94.3
4	99.0	100.9	103.1	99.5	94.3
5	97.5	102.4	99.1	100.2	97.0
6	98.4	101.6	100.4	100.7	95.6
Mean (%)	97.8	102.2	100.4	99.7	94.1
R.S.D. (%)	1.57	1.57	1.90	0.63	2.29

Values are percent recovery of 2 ppm anions spiked in drug substance samples.

was a slow decrease in migration time which is caused by ion depletion and pH change in the run buffer inlet vial during the electrophoresis. This migration time decrease causes a similar decrease in measured peak areas. Better precision can be obtained either with more frequent replenishment of run buffer or by use of an internal standard.

The limit of detection (LOD) is illustrated in Fig. 3 where anion standards were spiked at concentrations of 50 parts per billion (PPB) in a solution containing 2 parts per million (PPM) chloride. The sample was introduced into the capillary by direct injection of 12 nl sample solution; this volume is approximately 1% of the internal volume of the capillary. The LOD for this separation with conductivity detection is 10–20 ppb and is an order of magnitude lower than similar anion separations done with indirect UV detection [9]. This sensitivity is useful when doing trace analysis in complex mixtures. It is possible to obtain an even lower LOD 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 20 ppm for these inorganic anions in this run buffer. Although the peak area calibration is linear for three orders of magnitude, peak widths increase significantly at concentrations above 2 ppm with these conditions and peak height calibrations are not linear above that value.

Capillary ion electrophoresis can be used to measure trace amounts of anions in drug substance samples as shown in the recovery study in Table 2. A standard mixture of anions was spiked at a concentration of 2 ppm into a 1 mg ml^{-1} solution of a water soluble drug substance sample, DMP 754, and electropherograms of a standard and spiked sample are shown in Fig. 4. Recovery was calculated by comparing the peak area of anions in a drug substance sample to that of the unspiked 2 ppm standard. Recoveries ranged from 97–102% with R.S.D. of less than 2%. The acetate counter ion in DMP 754 elutes at about 7 min. Although the drug substance and counter ion are in much greater concentration, neither affects the migration time or quantitation of trace levels of anions in the same sample.

A second recovery study was done with a water insoluble drug substance DMP 266. The drug substance was dissolved in methanol at a concentration of 10 mg ml^{-1} and then diluted 1:10 with an aqueous anion standard mixture. The precipitated drug substance was then removed from solution by centrifugation at 3500 rpm. The anion concentrations in the drug substance supernatents were then compared to the standard mix by CIE. Recoveries were calculated to be from 97–104% with an R.S.D. of less than 4%. Centrifugation was preferred as the method of sample cleanup for this application because of difficulties in filtering the precipitated drug substance samples. In general we also found it best to use freshly distilled water and to rinse any glass with distilled water before use in CIE analysis.

An example of the use of screening for unknown inorganic impurities is shown in Fig. 5

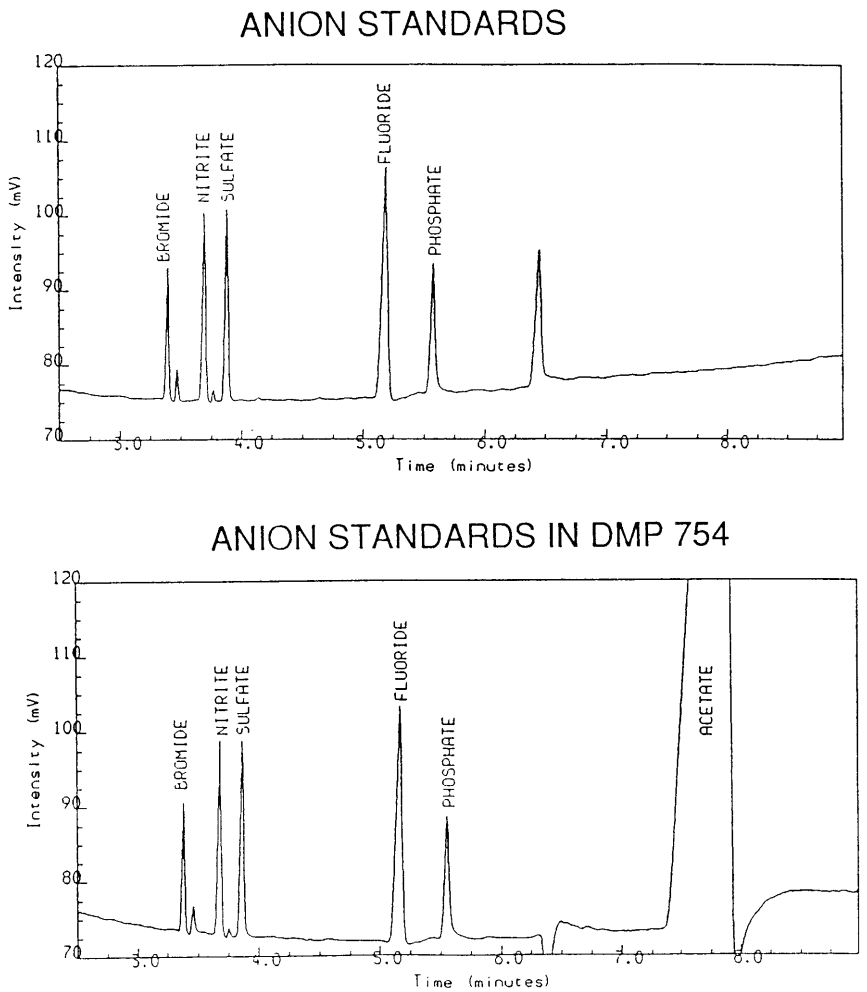

Fig. 4. Separation of anion standards in drug substance sample. Same separation conditions as Fig. 2. Anion standards at 2 ppm in water and in sample of DMP 754 at 1 mg ml^{-1} in water.

where a lot of DMP 266 drug substance was analyzed for the presence of inorganic anions. Significant amounts (greater than 0.5% total) of chloride, nitrate and sulfate impurities were found; the same lot was purified and the new profile showed that the inorganic impurities were significantly reduced. This type of information on inorganic impurities is valuable in the early development stages and can be used by the process chemist to adjust the synthetic process so that higher drug substance purity is achieved and maintained.

3.2. Inorganic anions and short chain carboxylic acids

A second goal of this work was to evaluate whether capillary ion electrophoresis with conductivity detection could be used to accurately and precisely determine the counter ion content of drug substance salts (such as the acetate content of DMP 754). However, the electropherogram in Fig. 4 showed that the acetate anion had a poorly shaped peak with CHES–CTAB run buffer and this caused problems in accuracy and precision of

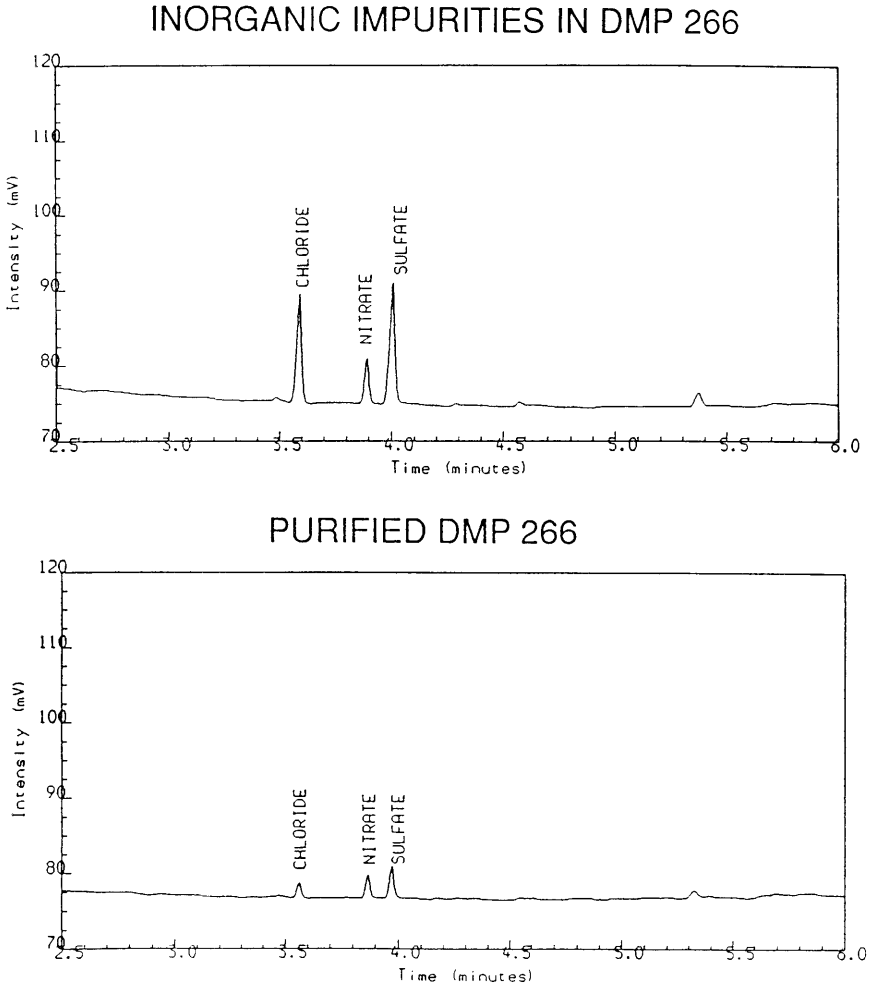

Fig. 5. Separation of inorganic anion impurities in DMP 266 drug substance. Same conditions as Fig. 2.

peak integration. This problem was also true of other more strongly retained anions such as methanesulfonate and formate. We then evaluated other run buffers to find a more suitable candidate for analysis of counter-ions.

The electropherogram in Fig. 6 shows the separation of a mixture of inorganic and carboxylic anions with a run buffer of MES and TTAOH. The separation and peak shape of the anions in the mixture was significantly improved. There was no problem with poor peak shape resulting from mobility mismatch of sample and run buffer as was previously reported when using indirect UV detection [5]; the increased concentration of coanions in MES–TTAOH run buffer overcomes the peak distortion caused by mobility differences. This is an advantage when profiling a sample for a variety of anionic impurities.

There were some problems with the MES–TTAOH run buffer for the analysis of inorganic anions; this included incomplete resolution of bromide and chloride and poor detection of fluoride. Since few of our compounds contain bromide or fluoride, this is not considered a sig-

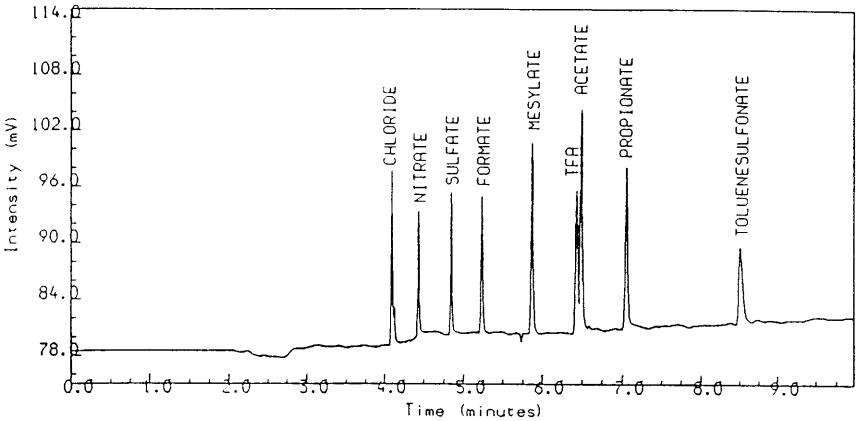

Fig. 6. Separation of inorganic and carboxylic anion standards. Separation conditions: Concap Capillary 60 cm × 50 μm; run buffer 60 mM MES, 60 mM His, 0.7 mM TTAOH, 0.03% Triton X-100; 15 kV run voltage; 30°C oven temp; conductivity detection; pressure injection of 40 mbar for 12.5. Flush for 1.5 m with run buffer at 2000 mbar before each injection. Sample contained 5 ppm chloride, nitrate, sulfate, and 10 ppm formate, methanesulfonate (mesylate), trifluoroacetate, acetate, toluenesulfonate.

nificant disadvantage for the analysis of drug substances. If necessary the CHES–CTAB run buffer could be used for identification and measurement of bromide and fluoride.

Table 3 shows the results of a precision study of the MES–TTAOH run buffer with some inorganic and carboxylic anions. The precision of migration was less than 0.2% for eight injections; there was no significant drift in migration because the pH of the run buffer in the inlet vial does not change during the electrophoresis of the samples. The precision of peak area and area ratio measurement was between 0.5 and 1.0% under these conditions. The LOD was 10–20 ppb for inorganic anions such as chloride and is 40 ppb for carboxylic anions such as acetate.

Table 3
Precision of anion standards

Anion	Migration time	Peak area	Peak area ratio
Chloride	0.19	0.53	0.78
Formate	0.16	0.51	0.36
Methanesulfonate	0.14	0.97	0.86
Acetate	0.13	0.51	0.58

Values are percent R.S.D. of eight injections with MES–HIS–TTAOH run buffer.

The calibration of peak area versus sample concentration was linear to 80 ppm for both inorganic and organic anions in the MES–TTAOH run buffer. This was greater than with the CHES–CTAB buffer and was apparently the result of an improved peak shape with the greater concentration of coanions in the run buffer [11]. Peak height calibration was linear to only 20 ppm with these conditions since peak widths begin to increase at concentrations greater than 20 ppm.

A simple method for determination of acetate in DMP 754 was developed. The drug substance was dissolved in distilled water at a concentration of 0.2 mg ml^{-1} and an internal standard (propanesulfonic acid sodium salt) was added at a concentration of 0.4 mg ml^{-1}. Standards were prepared by weighing glacial acetic acid into distilled water and adding internal standard. Samples and standards had to be analyzed within 24 h in order to avoid microbial degradation of the acetate. Samples and standards were filtered with the PDVF membrane filters described in Section 2. It was also necessary to wash the capillary with both 0.1 N NaOH and water before each sample to maintain migration time reproducibility. Fig. 7 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

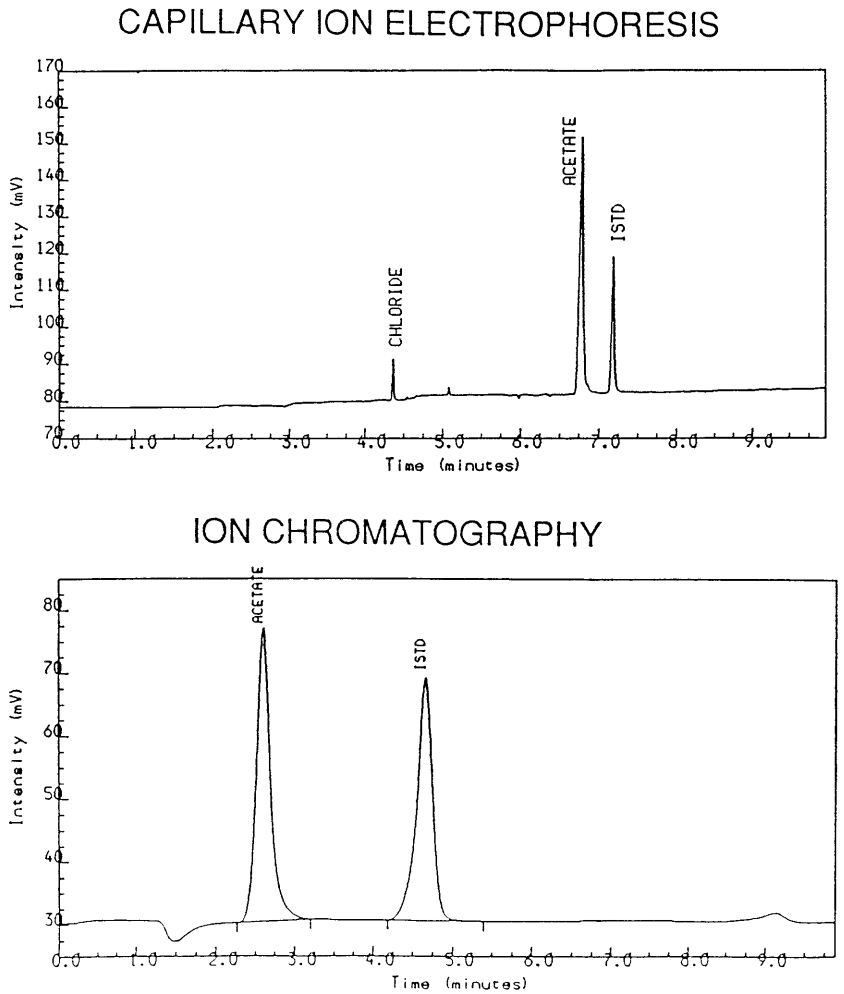

Fig. 7. Separation of acetate in DMP 754 by CIE and IC. CIE separation conditions are the same as Fig. 6 except flush at 2000 mbar for 0.7 min with 0.1 N NaOH, 0.7 min with water and 1.5 min with run buffer before sample introduction. IC separation conditions: Dionex AS4 anion exchange column; 1.7 mM sodium bicarbonate mobile phase; 1 ml min^{-1}; ambient temperature; suppressed conductivity detection; 20 μl sample injection. Sample contained 0.20 mg ml^{-1} of DMP 754 and 0.40 mg ml^{-1} of sodium propanesulfonate (ISTD) in water.

method precision for acetate determination and results are shown in Table 4. The R.S.D. of 1.25% is typical for the CIE method and is adequate for this analysis. The acetate content of this lot was determined to be 11.8%; the theoretical amount of acetate in DMP 754 is 11.8%. Both CIE and IC (see Fig. 7) were then used to determine the acetate content of nine different lots of DMP 754 and the results, shown in Table 5, compare well.

An advantage of the CIE method is that inorganic anionic impurities can be separated and measured in the same electropherogram as used for determination of the counter anion. This can be seen in the electropherogram in Fig. 7 which shows a significant (0.4%) and unexpected impurity which was identified as chloride by comparing migration time to that of the standards. This was confirmed by running the same sample with the

Table 4
Precision of acetate determination by CIE

Sample	Acetate (%)
1	11.6
2	11.7
3	11.9
4	12.0
5	11.9
6	11.8
Mean	11.8
R.S.D. (%)	1.25

CHES–CTAB run buffer discussed above and comparing migration time to a chloride standard.

3.3. Inorganic cations

It was important for the process chemist to know the identity of the cation associated with the chloride impurity shown in Fig. 7. this cation was identified with a CIE cation screen. For the cation separation a MES run buffer without a quaternary amine modifier was used; since cations were being separated, it was not necessary to reverse the EOF in the capillary. The separation was run with the cathode on the detector end of the capillary and a mixture of inorganic cations (Na, K, Li, Mg, NH_4) was separated. This separation was used to determine that ammonium was the cation associated with the chloride impurity in lot 9 of DMP 754. Since ammonium chloride is volatile at elevated temperatures, it was not detected in the residue of the ignition test that is normally used to detect excess amounts of inorganic impurities in drug substances. However, the CIE method did identify this impurity and this information was used by the process chemists to adjust the synthesis conditions so that ammonium chloride could be eliminated in later lots of the drug substance.

Table 5
Determination of acetate in drug substance

Sample lot No.	CIE (%)	IC (%)
1	11.8	11.8
2	11.8	11.8
3	11.5	11.9
4	12.0	11.9
5	12.0	11.6
6	11.5	11.6
7	11.5	11.9
8	11.8	11.7
9	12.1	12.0

4. Conclusions

Capillary ion electrophoresis with conductivity detection is a precise and accurate method for the determination of acetate counter-ion in pharmaceutical drug substances. The run buffer contained an amphoteric buffer (MES) and a TTAPH modifier to reverse the EOF in the capillary. The limit of detection for acetate was 30 ppb in aqueous solution and calibration was linear up to 80 ppm. A system precision of less than 0.7% was measured by replicate injection of an acetate standard; method precision for the acetate determination in drug substances was less than 1.3%. Acetate content of DMP 754 lots determined by CIE and ion chromatography compared well. Sample preparation is simple and only milligram amounts of drug substance are required. CIE with conductivity detection has also be used in our labs for the determination of other counter anions such as methanesulfonate.

An advantage of CIE with conductivity detection is that small amounts ($< 0.1\%$) of inorganic impurities can be separated and profiled in the same electropherogram used for the acetate determination. Amphoteric run buffers can be used in sufficient concentration in the run buffer to suppress peak distortion caused by mobility mismatch of the sample and run buffer. This results in excellent peak shape for a wide variety of inorganic and carboxylic anions so that these anions can be separated and measured in the same electropherogram. This gives a convenient and simple method for screening for unknown inorganic impurities that may be present during development lots of drug substances. Trace inorganic impurities can be identified by comparison to migration times of standards and confirmed if necessary by using a second anion screen that contains CHES and CTAB in the run buffer and

is optimized for separation of the inorganic anions. Unknown inorganic cation impurities can also be identified at trace levels with a cation screen that uses MES and L-histidine in the run buffer. These CIE methods can be used to identify and measure trace ionic impurities in both water soluble and insoluble drug substances.

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