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# Determination of quaternary alkylammonium compounds by capillary zone electrophoresis and indirect UV detection as a real alternative to ion chromatography with suppressed conductivity detection

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**Abstract**

A capillary electrophoretic (CE) method for the determination of residual mid-chain alkyltrimethylammonium compounds in the pharmaceutical product Welchol™ (an alkylated, crosslinked polyallylamine) was developed, validated and compared with the existing ion chromatographic (IC) method with suppressed conductivity detection. Excellent reproducibilities of migration times (RSD<0.5% within a series of 55 sample injections) and relative peak areas (RSD<2%) make the method suitable for quality control as a real alternative to IC. Limits of quantification of 0.01% w/w of each impurity in the active substance were achieved. Buffer systems for indirect UV detection based on creatinine as visualization reagent with different inorganic and organic acids (phosphoric, sulfuric, formic, acetic, oxalic and citric acid) and their effect on selectivity to ten quaternary ammonium compounds were studied. Selectivity changes were observed for the di- and trivalent analytes depending on the buffer applied. Also, the influence of acetonitrile, methanol, 1,4-dioxane and tetrahydrofuran on selectivity was investigated. In addition, CE–MS experiments were carried out in order to identify several impurities in the product.


*Keywords:* Indirect detection; Detection, LC; Quaternary ammonium compounds

## 1. Introduction

Elevated cholesterol (hypercholesterolemia) has been widely recognized as a significant risk factor for coronary heart disease. Bile acid sequestrants are an alternative therapy to absorbed agents such as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) [1]. Bile acids are synthesized by the liver from cholesterol and secreted into the intestine to aid digestion of fats. Bile acid sequestrants bind to bile acids in the intestinal tract and increase their excretion from the body. To replenish the bile acid pool, the liver draws cholesterol from the bloodstream, resulting in a reduction in blood cholesterol levels. Bile acid sequestrants work without entering the bloodstream and are generally regarded as safer than absorbed agents such as statins, which require frequent liver function tests. Since cholesterol-reduction therapy typically involves a life-long drug regimen, the National Cholesterol Education Program guideline recom-

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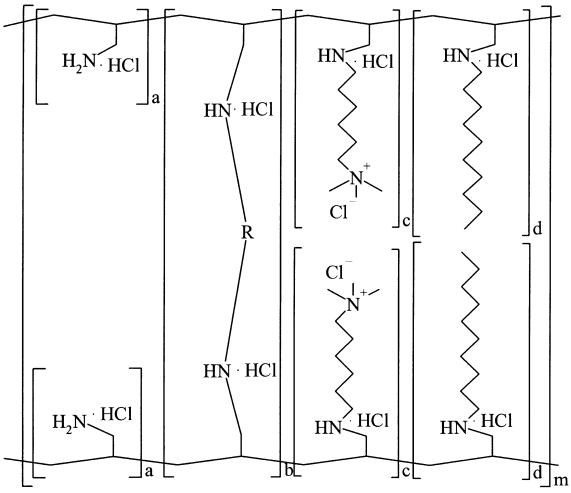



Fig. 1. Structure of the colesevelam hydrochloride gel.

mends that physicians prescribe bile acid sequestrants as first-line drug therapy [2].

Colesevelam hydrochloride (Fig. 1), a crosslinked polyallylamine alkylated with decylbromide and 6-bromohexyltrimethylammonium bromide is the active pharmaceutical ingredient of the new cholesterol reducing drug Welchol™. This nonabsorbed hydrogel binds the bile acids and their conjugates through an anion-exchange mechanism combined with a hydrophobic interaction between the hydrophobic part of the bile acids and the hydrophobic binding site of the polymer [3]. Within the quality control of this bile acid sequestrant, one quality parameter is the determination of the non-volatile impurities by ion chromatography (IC) with suppressed conductivity detection.

These impurities (Table 1), determined by an IC gradient method, result on the one hand from the alkylation process and on the other hand from a possible Hofmann elimination during drying, storage and milling.

In general, quaternary ammonium compounds like those mentioned above can be analyzed either by GC after derivatization [4], by RP-HPLC [5–9], ion-exchange chromatography [10,11] or ion-pair chromatography [12–17]. During the last years mass spectrometry (MS) has gained importance for the determination of quaternary ammonium compounds in various contexts. Besides GC–MS [18], especially the combination of HPLC or IC with MS using an appropriate interface proved to be a powerful technique for the determination of these positively charged compounds [19–22].

Beside the chromatographic separation techniques, isotachophoretic [23,24] and capillary electrophoretic (CE) separations [6,25–40] were successfully applied for the determination of quaternary ammonium compounds. In comparision with chromatography electrophoretic separations of these compounds offer the advantages of high efficiency, short analysis times and low running costs.

The present work shows the development, optimization and validation of a CE method for the analysis of the mentioned compounds.

Within the product specification of the active substance, the known and unknown non-volatile impurities are specified with a maximum of 0.05% w/w each; the sum of all impurities must not exceed 0.30% w/w. Therefore the limit of quantification was set to 0.01% w/w and the upper limit of the working range of the method to 0.10% w/w for minor impurities and 0.30% w/w for major impurities. All other limits (precision, accuracy, selectivity, linearity, reproducibility etc.) were set by regulatives of cUSP and cGMP. This new CE method was compared to the existing IC method and should have the potential to replace the IC method in pharmaceutical quality control analysis.

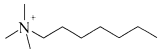
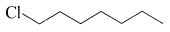
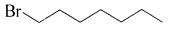
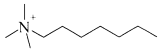
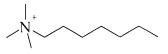
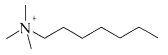
In addition, the combination of CE and MS was used as alternative on-line detection method to the indirect UV detection. The structural information from obtained electrospray ionisation mass spectrometry (ESI–MS) was used to confirm the results of the structure elucidation of unknown impurities by IC–MS in previous work [41].

## 2. Experimental

### 2.1. Instrumentation

The CE measurements were carried out on a HP$^{3D}$ CE (Agilent, Waldbronn, Germany) with a UV–Vis diode array detector. MS measurements were done on a quadrupole system HP 5989B using an pneumatically assisted electrospray interface HP 59987A (Agilent, Palo Alto, CA, USA) equipped

Table 1
Impurities in colesevelam hydrochloride originating from alkylation and Hofmann elimination

| Structure | R1 | R2 | R3 | R4 | (trivial-) name |
|---|---|---|---|---|---|
|  | H– | H– | H– | H– | ammonium |
| | $CH_3$– | $CH_3$– | $CH_3$– | H– | trimethylammonium |
| | $C_{10}H_{21}$– | H– | H– | H– | decylammonium |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | 6-aminohexyltrimethylammonium (aminoquat) |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | hexamethylenbis(trimethylammonium) (diquat) |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | 6-chlorohexyltrimethylammonium (chloroquat) |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | 6-bromohexyltrimethylammonium (bromoquat) |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | 6-hydroxyhexyltrimethylammonium (hydroxyquat) |
|  | | $CH_3$– | $CH_3$– | $CH_3$– | 6-methoxyhexyltrimethylammonium (methoxyquat) |
|  |  | | H– | H– | aminodi(hexyltrimethylammonium) (amino(dihexylquat)) |
| $C_{10}H_{21}$– |  | | H– | H– | decyl-6-aminohexyltrimethylammonium (decylaminoquat) |

with a radiofrequency (RF)-only hexapole (Analytica of Branford, Branford, CT, USA).

For sample preparation a Wrist-Action shaker Model 75 (Burrell Scientific, Pittsburgh, PA, USA) and a GS-15 centrifuge (Beckmann Coulter, Fullerton, CA, USA) were used.

### 2.2. Chemicals

1 *M* sodium hydroxide (analytical-reagent grade), creatinine (>99%), acetic acid (>99.5%), citric acid (>99.5%), tetrahydrofuran (HPLC grade) and triethylamine hydrochloride (>99.5%) were purchased from Fluka (Buchs, Switzerland); 0.1 *M* hydrochloric acid, phosphoric acid, 0.5 *M* sulfuric acid and methanol (all analytical-reagent grade) from J.T. Baker (Deventer, Netherlands); formic acid (98–100%), oxalic acid dihydrate (>99.5%), acetonitrile (gradient grade) and 1,4-dioxane (for HPLC) from Merck (Darmstadt, Germany) and 18-crown-6 (99%) and tetradecyltrimethylammonium bromide (TTAB, 99%, analytical-reagent grade) from Sigma–Aldrich (Steinheim, Germany). High-purity water was prepared by a Milli-Q water purification system (Millipore, Milford, USA).

The reference substances trimethylamine, decylamine, aminoquat, diquat, chloroquat, bromoquat, hydroxyquat, methoxyquat, amino(dihexylquat) and decylaminoquat were either supplied by DSM-Fine Chemicals Austria (Linz, Austria) or GelTex Pharmaceuticals (Waltham, MA, USA) as their bromide salts with a purity better than 99%.

### 2.3. Capillaries and carrier electrolytes

All separations were carried out at 25°C with a separation voltage of +30 kV in fused-silica capillaries with I.D. 50 μm and 64.5 cm total length (56 cm to detector) or 80.5 cm total length (72 cm to detector), or in permanently poly(vinyl alcohol) (PVA)-coated capillaries with I.D. 50 μm and 64.5 cm total length (56 cm to detector). All capillaries were equipped with bubble cell and were purchased from Agilent.

Hydrodynamic injection with a pressure of 50 mbar for 5 s at the capillary inlet was used for every determination.

During method development the fused-silica capillaries were rinsed for 10 min with water, 20 min with 1 *M* NaOH, 15 min with water and 10 min with electrolyte before each use and when the carrier electrolyte system was changed. Using the PVA-coated capillaries no conditioning was necessary. For cleaning purposes the coated capillaries were flushed with 10 m*M* phosphoric acid for a few minutes.

The carrier electrolyte solutions were prepared from stock solutions of the free acids and creatinine by dilution. After filling to the volume, the pH of each carrier electrolyte was measured.

### 2.4. Sample preparation

An amount of sample corresponding to 1 g dry active substance is extracted with 10 ml of 10 m*M* hydrochloric acid containing the internal standard (IC: hexquat, CE: triethylammonium). After extracting for 30 min with a wrist action shaker the gel is centrifuged. A portion of the clear solution is filtered through a syringe-driven filter unit (pore size 0.22 μm) and used for the determination.

## 3. Results and discussion

### 3.1. Method development and optimization

The existing method for the determination of quaternary ammonium compounds within the quality control of colesevelam hydrochloride is based on suppressed IC with conductivity detection using an IonPac CS14 column with a gradient of methanesulfonic acid, 2,3-diaminopropionic acid and acetonitrile as the mobile phase. During the separation the methanesulfonic acid concentration is increased from 3.6 m*M* to 27 m*M* within 40 min and the acetonitrile content is increased from 1% v/v to 45% v/v within the same period of time; the concentration of 2,3-diaminopropionic acid is kept constant at 4 m*M*. With this method it was possible to separate the possible impurities hydroxyquat (6.5 min), methoxyquat and aminoquat (14.0 min), aminodihexylquat (28.5 min) and decylaminoquat (38.2 min) besides other cations such as sodium, ammonium and trimethylammonium. Including the reequillibration of the system one IC run has a runtime of 60 min.

#### 3.1.1. Carrier electrolyte composition

Lacking a chromophore, the analytes are detected using indirect UV detection. Taking into account mobility, p$K_a$ and charge, creatinine [$\mu = 36.8 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ [42], p$K_a$ (protonated form) = 4.8] was selected as visualization reagent. The pH-dependence of the creatinine UV–Vis absorbance shows at pH 5.5 an absolute maximum at 200 nm and a local maximum at 235 nm. These maxima shift with a decreasing pH to shorter wavelengths and are nearly constant at 190 nm for the absolute maximum and 220 nm for the local maximium for pH-values smaller than 3.0.

The influence of the carrier electrolyte counterions starting with acetate, phosphate, sulfate, formate, oxalate and citrate was investigated at different pH values. Since pH values higher than 4.0 result in poor resolutions caused by electrodispersive effects from the conductivity difference between sample solution and electrolyte, the investigated pH range was set between 2.0 and 4.0. Further advantages of this low pH are the lower adsorption of the surface active analytes and a small electroosmotic flow (EOF) with the use of bare fused-silica capillaries.

Fig. 2 shows the influence on separation selectivity depending on the counter-ion present in the carrier electrolyte.

Using acetate, phosphate and formate, diquat and amino(dihexylquat) could not be separated; using oxalate and citrate, sodium ions present in the sample matrix were not separated from aminoquat; and with sulfate as counter-ion, the resolution between decylaminoquat and hexquat as well as the

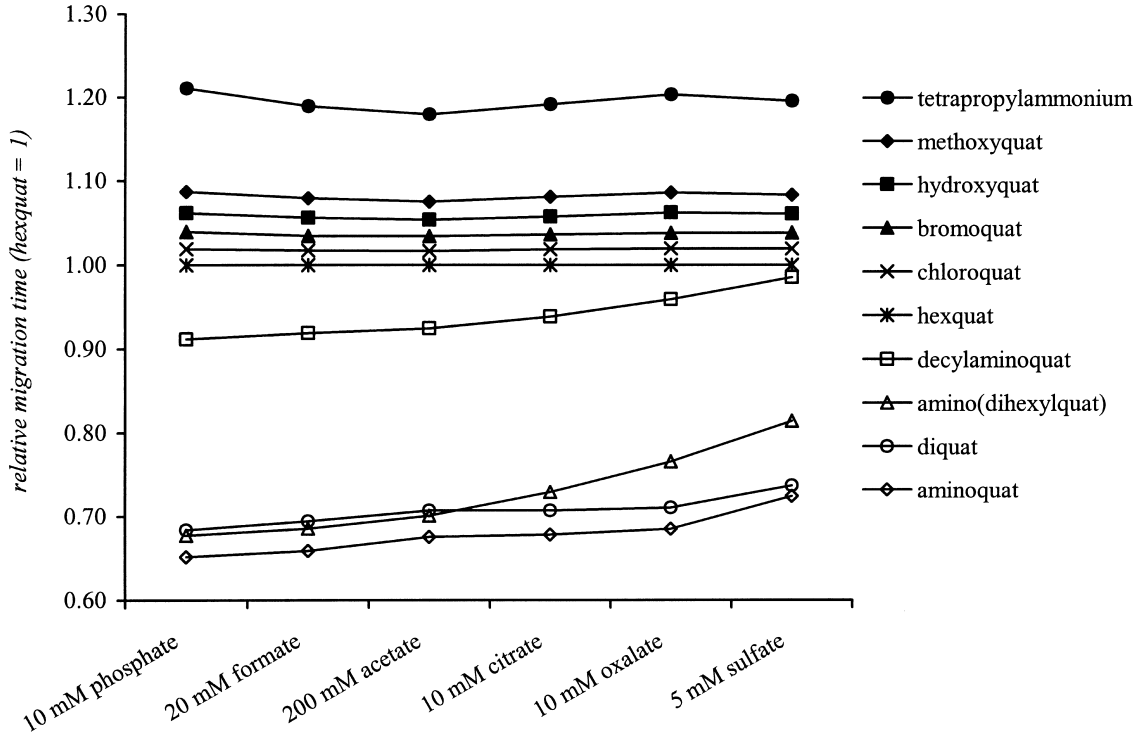



Fig. 2. Influence of the counter-ion present in the carrier electrolyte on separation selectivity; carrier electrolyte: 10 m*M* creatinine (pH approx. 3.5); voltage: +30 kV.

resolution between calcium (also present in the sample matrix), and amino(dihexylquat) was too poor.

A common way to alter the separation selectivity in CE is the use of organic modifiers. Thereby, the main parameters that can be manipulated are viscosity and solvation ability of the carrier electrolyte. The modifiers are selected according to their dielectric constant, dynamic viscosity and dipole moment. Commonly used modifiers are acetonitrile, methanol, tetrahydrofuran (THF), 1,4-dioxane, ethanol and acetone [27,30,34–36,38,39]. In addition, the use of these modifier can improve peak shapes [30,39].

To enhance the resolution of sodium and aminoquat, the influence of acetonitrile, methanol, THF and 1,4-dioxane on the separation was investigated within a range of 0–50% v/v modifier. The effect of the organic modifiers were studied with oxalate and citrate as counter-ions. All separations showed an increase of the migration times due to the higher viscosities of the electrolytes. At contents higher than 25% v/v acetonitrile or methanol, a change in migration order was observed. Decylaminoquat migrated behind of hexquat and the order of hydroxyquat and methoxyquat was reversed. In general, a loss of separation selectivity was observed using acetonitrile and methanol. Stronger effects were observed for 1,4-dioxane and THF, which could be anticipated due to their physicochemical properties. All ions were separated by the use of either 15% v/v 1,4-dioxane or 12.5% v/v THF. The extent of the effect of the modifier on the analytes was increased with the number of quaternary ammonium groups present in the substance. At high contents of THF (20% v/v) or 1,4-dioxane (35% v/v) decylaminoquat migrated between chloroquat and bromoquat. The disadvantage of the use of these two modifiers was the very large baseline drift of about 350 mAU $h^{-1}$ for 15% v/v dioxane and about 900 mAU $h^{-1}$ for 12.5% v/v THF. Fig. 3 shows the effect of the various organic modifiers on the effective mobility.

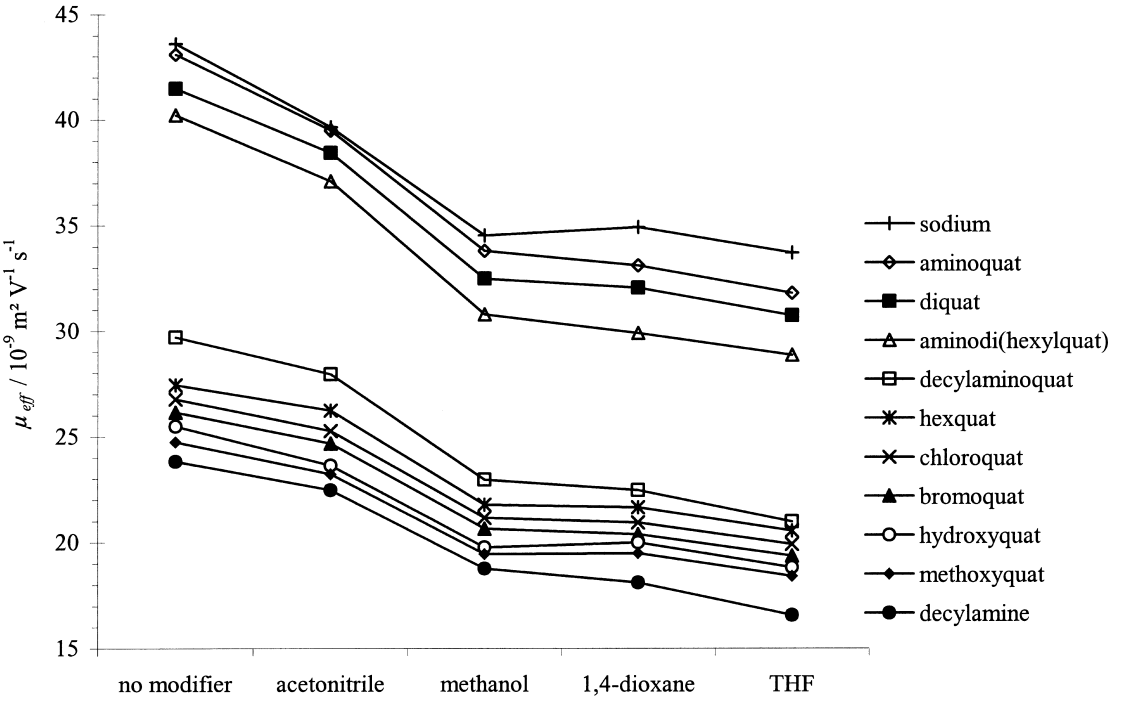



Fig. 3. Influence of 15% v/v organic modifier in the carrier electrolyte on mobility; PVA coated capillary; carrier electrolyte: 10 m*M* creatinine, 10 m*M* oxalic acid, 15% v/v modifier; voltage: +30 kV.

Furthermore, the influence of temperature was studied from 15°C to 50°C when using organic modifiers. All experiments showed a decrease of migration time and resolution at an increase of temperature. Aminoquat migrated at low temperatures in front of trimethylammonium and at high temperatures behind trimethylammonium.

For final method optimization the analyte set of interest was ammonium, sodium, trimethylammonium, amino-, aminodihexyl-, decylamino-, chloro-, hydroxy-, methoxyquat and decylammonium. The critical resolutions were between sodium and aminoquat, between aminoquat and trimethylammonium and between decylaminoquat and hexquat (the internal standard of IC). The change of the internal standard from hexquat to triethylammonium solved the problem with decylaminoquat. For the separation of sodium, aminoquat and trimethylammonium carrier electrolyte systems containing formate and sulfate were investigated.

To ensure a separation of ammonium and potassium (eventually present in some samples) 18-crown-6 was added to the carrier electrolyte, which might also improve the resolution of the analytes. The separation was investigated in a range from 0.5 to 10 m*M* 18-crown-6 with an optimum at a concentration of 2 m*M*. At high crown ether concentrations the resolution of sodium and aminoquat decreases beside the known increase of resolution of ammonium and potassium.

After optimizing the creatinine concentration, the formate concentration, pH and temperature the optimum resolutions were found for the PVA-coated capillaries with a carrier electrolyte containing 12.5 m*M* creatinine, 4.5 m*M* formic acid, 2 m*M* 18-crown-6 at a pH of 3.10 set with sulfuric acid. The optimum resolution for bare fused-silica capillaries could be achieved with a carrier electrolyte containing 12.5 m*M* creatinine, 5 m*M* formic acid, 2 m*M* 18-crown-6 at a pH of 3.0 set with sulfuric acid.

### *3.1.2. Capillary conditioning*

Due to the polyallyl amine oligomers present in the sample solution, bare fused-silica capillaries

Table 2
Optimized method and conditioning procedures for bare fused-silica capillaries

| CE Method | | Capillary conditioning | |
|---|---|---|---|
| *Capillary* | fused-silica, 64.5 cm (effective length 56 cm) ×50 μm I.D. bubble cell (150 μm) | *New capillary* | 2 min water<br>10 min 5 *M* NaOH |
| *Electrolyte* | 12.5 m*M* creatinine; 5 m*M* formic acid, 2 m*M* 18-Crown-6 with sulfuric acid set to pH 3.00 | | 10 min water<br>10 min 0.1 m*M* TTAB<br>5 min 5 *M* NaOH |
| *Voltage* | +30 kV; 8 min run time | | 10 min water |
| *Temperature* | 25°C | *Before each run* | 0.2 min water |
| *Injection* | 250 mbar s (5 s 50 mbar) | | 1 min 0.5 *M* NaOH |
| *Detection* | indirect, 220 nm; reference 450 nm | | 2 min water<br>2 min electrolyte<br>30 s, 30 kV<br>1.5 min electrolyte |

showed an on-going decrease of electroosmotic flow finally resulting in reversal. This effect is caused by the oligomers adsorbed to the capillary surface. To minimize this adsorption, the capillaries were deactivated by flushing once with tetradecyltrimethylammonium bromide. The capillary pretreatment has also been optimized to ensure an optimum reproducibility of migration times. The optimized conditions for the separation (resolution) and capillary conditioning (migration time reproducibility) are shown in Table 2.

Generally, one must also take into consideration possible adsorption phenomena of the analytes at the inner surface of the capillary, which frequently occurs for quaternary alkylammonium compounds. Nevertheless, such negative effects were not observed in the present case, probably due to the relatively short alkyl chain length of the analytes.

Using the PVA-coated capillaries to avoid adsorption, a blockage was observed after about 100 runs. In addition, outliers occured from time to time that could not be completely explained. For these reasons, the validation was performed with the optimized method for the bare fused-silica capillary.

### *3.2. Method validation and comparision to IC*

The developed and optimized method is according to cUSP ⟨1225⟩ a category II–quantification method. To fulfill the regulatory requirements, the validation parameters given in Table 3 were determined. The limits were taken from the IC method validation plan. Analytical methods used in cGMP require system suitability tests. For this reason a sample solution of a product within the specification spiked with sodium, trimethylammonium, aminoquat,

Table 3
Validation parameters, results and comparision of IC and CE

| Validation parameter | IC | CE | Limit |
|---|---|---|---|
| Precision | | | |
| System precision/RSD ($n=10$) | 0.70% | 0.8%–1.5% | ≤3% |
| Migration time/RSD ($n=10$) | – | 0.30% | ≤2% |
| Reproducibility/RSD ($n=10$) | 0.6%–5.1% | 1.1%–3.6% | ≤10% |
| Laboratory precision/RSD ($n=6$) | 2.7%–5.4% | 0.4%–2.7% | ≤10% |
| Accuracy/recovery | 89%–114% | 92%–109% | 100%±20% |
| Limit of quantitation | 0.02% w/w | 0.01% w/w | – |
| Selectivity/$R^2$ | 0.997–0.999 | 0.999–1.000 | ≥0.97 |
| Linearity/$R^2$ | 0.997–1.000 | 0.999–1.000 | ≥0.97 |
| Robustness/RSD ($n=6$) | 2.7%–5.4% | 1.2%–5.5% | ≤10% |
| LOD (% w/w) ($S/N=3$) | 0.001 | 0.003–0.007 | – |
| $C_p$ (method capability index) | 3.3–6.7 | 5.7–13.1 | >2.5 |

amio(dihexylquat) and decylaminoquat is injected six times. From these injections reproducibility of response and migration time, relative response factors and resolutions are calculated and compared to the limits in order to provide an ongoing performance.

Ensuring an optimum reproducibility, ten sample injections are followed by an injection of 10 m*M* hydrochloric acid and an injection of a system suitability test solution.

During validation a representative production batch was used for the determinations within the sample matrix. The spiked sample solutions were prepared by adding the impurities to the extraction solution. The amounts of the added impurities were calculated as % w/w of the active substance concerning the sample mass (1 g dry substance) and the volume of the added extraction solution (10 ml). The analytes are quantified by multiplying the response (quotient of peak area of analyte and peak area of internal standard, both corrected by the migration time) with the corresponding relative response factor. Table 4 shows the relative response factors, relative migration times and effective mobilities of the analyte ions. The numbers given for $rrf_i$ and $rt_{m,i}$ are valid if the internal standard trimethylammonium migrates between 5.3 min and 6.15 min.

The higher RSD of system precision of CE in comparision to IC (see Table 3) results from the determination within the sample matrix in the case of CE, whereas in IC the value was obtained by calculating the RSD from the peak areas of ten injections of the internal standard solution (without extraction). All other validation parameters of CE were determined in the same way as in IC and are equal or slightly better than IC (Table 3).

Fig. 4 shows the long term reproducibility of the relative migration times (RSD 0.04–0.10%, $n=30$) and the response (quotient of the corrected peak area of the analyte and the corrected peak area of the internal standard; RSD 1.07–1.52%, $n=30$) within a series of 57 injections. The relative standard deviations of the migration times within this series was 0.23–0.33%, of the corrected peak areas approx. 5.4–6.1%. Typical electropherograms of a sample solution before and after spiking at the 0.01% w/w level of impurities are shown in Fig. 5.

In addition, this new developed CE-method was tested in routine analysis on great extent, analyzing approx. 80 samples of drug stability testing. Comparing the impurity profiles of random samples analyzed by IC and CE, excellent accordance was observed.

### *3.3. CE–ESI–MS measurements*

The aim of these investigation was a confirmation of structure elucidations done in previous work [41] with IC–MS and GC–MS.

Due to the capillary conditioning procedures the use of fused-silica capillaries was not possible therefore all MS measurements were performed with permanently PVA-coated capillaries. Lacking a sufficient high EOF, the necessary flow for the electrospray interface was generated by applying a pressure at the capillary inlet. The optimum value for a constant spray was 20 mbar.

Table 4
Relative response factors ($rrf_i$), relative migration times ($rt_{m,i}$) with respect to triethylammonium ($t_m=5.3$ to 6.15 min) and effective mobilities ($\mu_{eff}$) of the analytes

| Analyte | $rrf_i$ | $rt_{m,i}$ | $\mu_{eff}/10^{-9}$ m$^2$ V$^{-1}$ s$^{-1}$ |
|---|---|---|---|
| Ammonium | 0.00803 | 0.49–0.52 | 56.9±0.1 |
| Sodium | 0.0104 | 0.69–0.71 | 40.4±0.2 |
| Trimethylamine | 0.0253 | 0.72–0.74 | 38.5±0.2 |
| Aminoquat chloride hydrochloride | 0.0495 | 0.75–0.77 | 37.3±0.2 |
| Amino(dihexylquat) chloride hydrochloride | 0.0599 | 0.85–0.87 | 32.5±0.2 |
| Triethylamine | 0.0364 | 1 | 27.7±0.2 |
| Decylaminoquat chloride hydrochloride | 0.0716 | 1.08–1.07 | 25.5±0.3 |
| Chloroquat chloride | 0.0599 | 1.13–1.11 | 24.6±0.3 |
| Hydroxyquat chloride | 0.0652 | 1.19–1.16 | 23.3±0.3 |
| Methoxyquat chloride | 0.0965 | 1.22–1.18 | 22.7±0.3 |
| Decylamine | 0.101 | 1.31–1.25 | 20.8±0.3 |

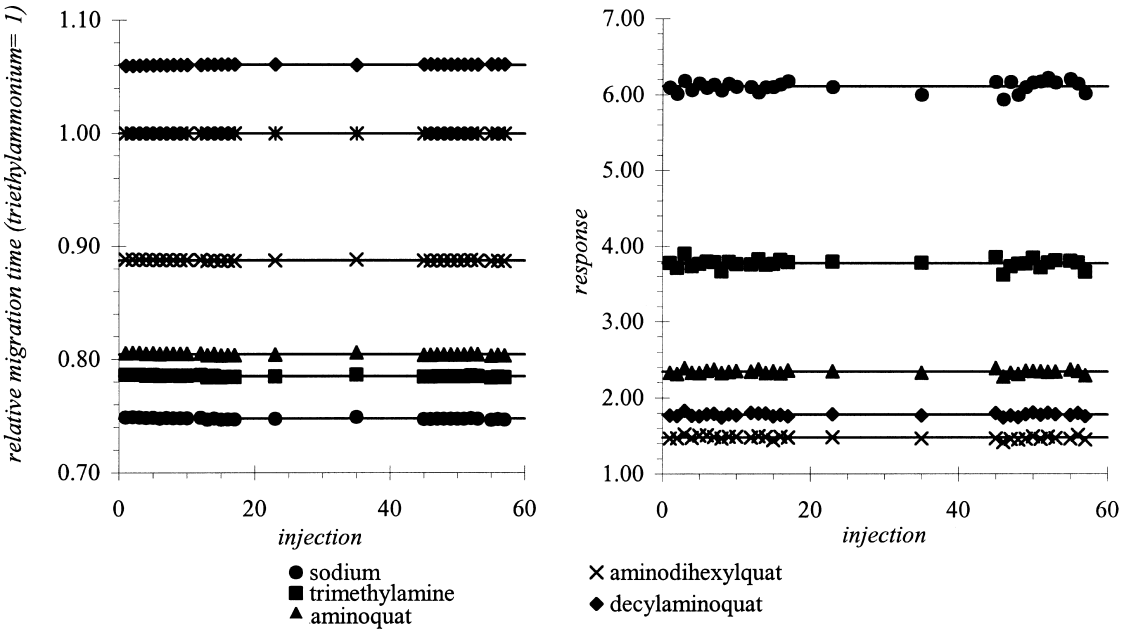



Fig. 4. Long term reproducibility of 30 system suitability test injections within a series of 57 injections.

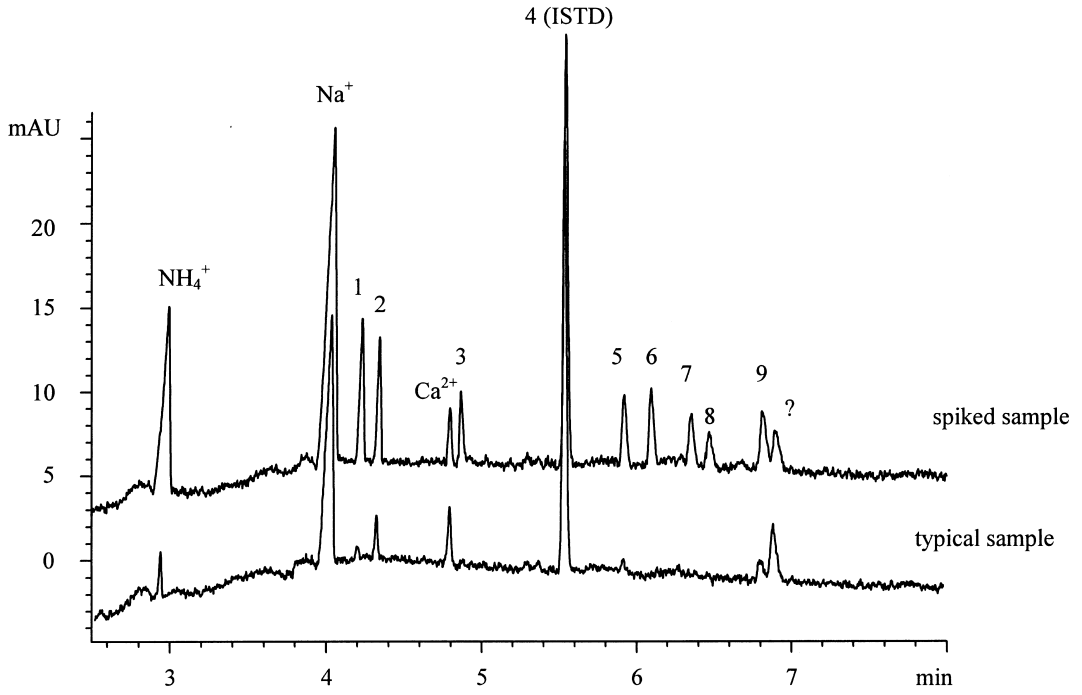



Fig. 5. Typical electropherograms of a sample solution before and after spiking with impurities (0.01% w/w); (1) trimethylammonium, (2) aminoquat, (3) aminodihexylquat, (4) triethylammonium (internal standard), (5) decylaminoquat, (6) chloroquat, (7) hydroxyquat, (8) methoxyquat, (9) decylammonium; conditions see Table 2.

During method development three carrier electrolytes compatible with MS in terms of volatility were investigated. Comparing electrolyte solutions containing 10 m*M* creatinine buffered to the p$K_a$ of the used acid, a decrease of sensitivity of the MS detection was observed when going from formate to acetate and oxalate. Creatinine causes a high background signal at $m/z$ 114 ($MH^+$) during scan measurements. For this reason the detected mass range was divided into a region from $m/z$ 50 to 113 and from 116 to 350.

Using a sheath flow consisting of 20% v/v water and 80% v/v 2-propanol acidified with 0.1% w/w formic acid, the current showed a steady decrease from approximately 8 μA to 2 μA. Similar phenomena had been observed before [43]. The current remains constant when a sheath flow of 20% v/v water and 80% v/v 2-propanol containing 2 m*M* formic acid and 1 m*M* ammonia is applied. The flow-rate of the sheath flow was always 4 μl $min^{-1}$. The benefits of buffered sheath flow compositions has also been reported in the recent literature [44].

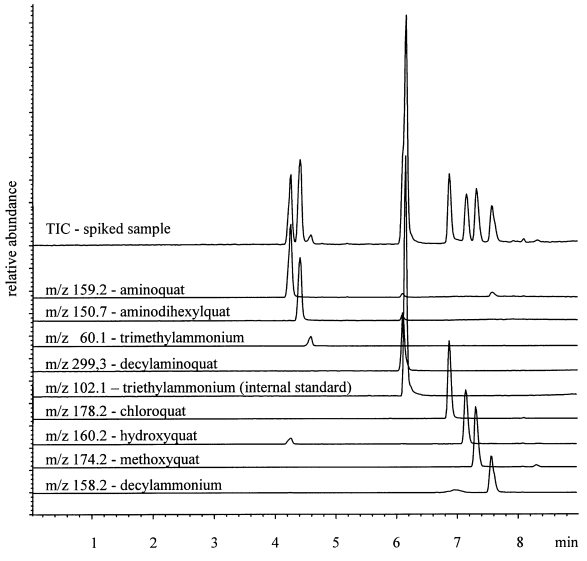



Fig. 6. CE–ESI–MS electropherogram of a sample spiked with 0.02% w/w impurities in the positive ion mode; capillary: 64.5 cm PVA-coated fused-silica, I.D. 50 μm; carrier electrolyte: 20 m*M* formic acid, 10 m*M* creatinine; voltage: + 30 kV at 25°C; injection: 250 mbar s; external inlet pressure: 20 mbar; sheath flow: 2 m*M* ammonia, 4 m*M* formic acid in water: 2-propanol (20:80, v/v).

Fig. 6 shows a typical total ion current electropherogram and the ion traces of a spiked sample (0.02% w/w, each impurity). With ESI–MS detection, correlation coefficients between 0.995 and 1.000 within a range of determination from 0.02% w/w and 0.10% w/w were achieved. Substances containing a tetraalkylammonium group showed limits of detection (LODs, $S/N=3$) of about 0.1 ppm. Higher LODs were observed for trimethylammonium (0.6 ppm) and decylammonium (0.2 ppm), which might be due to their volatility.

Analyzing samples in the scan mode beside the known impurity profile, some unknown impurities could be detected. Comparing $m/z$ values and fragmentation patterns of these compounds to previously measured IC–MS chromatograms, the most prominent impurities were found also in CE–ESI–MS. Fig. 7 shows the TIC-electropherogram of a sample containing unknown impurities and the ion traces of selected impurities. The $m/z$ values of 174.2 and 160.2 are the molecular ions of the co-migrating impurities 5-methoxyhexyltrimethylammonium and 5-hydroxyhexyltrimethylammonium. This fact is underlined by electropherograms of samples where the $m/z$ 174.2 peak occurs without the $m/z$ 160.2 peak; absence of the $m/z$ 160.2 fragment at CID experiments using IC–MS; and a missing fragmentation of the analogous 6-methoxy compound in IC–MS and CE–MS. Finally, the peak at a migration time of 6.6 min was found to be 5,6-dihydroxyhexyltrimethylammonium. These results confirmed the presence of impurities suggested by IC–MS experiments done in previous work.

## 4. Conclusions

The improved general performance demonstrated in this work for CE in comparision with IC, the reduction of the running analysis costs to a third, as well as the cutting in half of the time consumption for the analysis should lead to a preferred employment of the new CE method in routine analysis of colesevelam hydrochloride.

Using MS–detection as alternative to indirect UV, the sensitivity of the CE method could be improved by two orders of magnitude.

Altogether, CE is not only an alternative to IC for

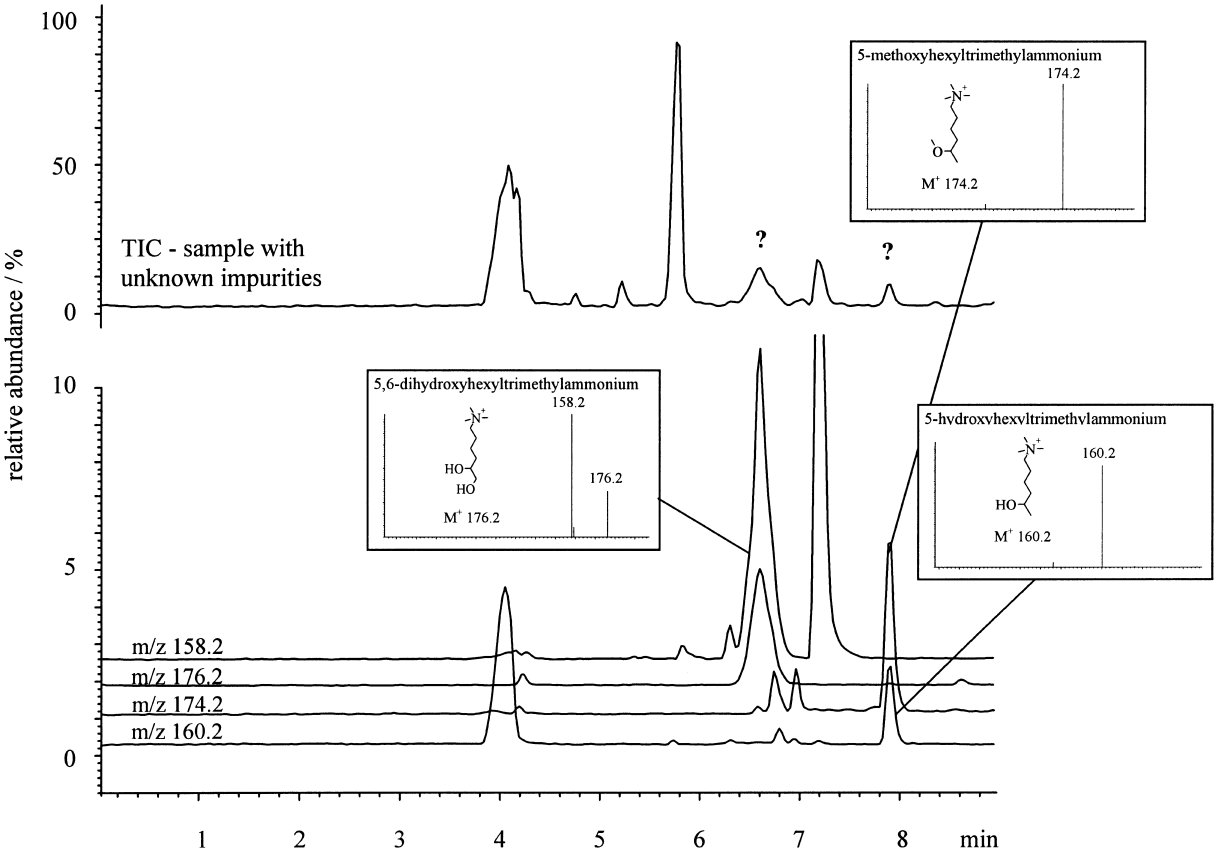



Fig. 7. CE–ESI–MS electropherogram of a sample containing unknown impurities; total ion current and ion traces of selected impurities in the positive ion mode; conditions see Fig. 6.

the problem described. It should be a convenient, inexpensive and fast technique to determine various kinds of alkyl ammonium compounds in agricultural chemicals and detergents in routine analysis.

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