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The determination of bromide in a local anaesthetic hydrochloride by capillary electrophoresis using direct UV detection ☆

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Abstract

The determination of residual amounts of bromide in a local anaesthetic hydrochloride by capillary electrophoresis was developed. Direct UV detection at 200 nm was used for the determination of the bromide content. The separation capacity of the system must be sufficient when bromide is determined in the presence of a large excess of chloride since electromigration dispersion of the highly concentrated chloride peak may impair the resolution. The background electrolyte (BGE) contained both acetonitrile and methanesulphonic acid in order to improve the selectivity and minimise the electromigration dispersion. The system was optimised with respect to resolution of the chloride and the bromide peaks by statistical experimental design using a multivariate optimisation program. The developed method was validated in accordance with the ICH guidelines and proved to be suitable for its intended use.

Keywords: Background electrolyte composition; Anaesthetics; Bromides; Potassium bromide

1. Introduction

Capillary electrophoresis (CE) is an analytical technique that has become an alternative and complementary technique at pharmaceutical laboratories. The high efficiency and the versatility of separation mechanisms that can be used for control of the selectivity are important factors that make the technique attractive. CE of inorganic anions has been reviewed by Kaniansky et al. [1].

The determination of small inorganic anions by CE is usually performed by indirect UV detection [2–4]. The application of indirect detection in the detection of small inorganic anions such as bromide by means of LC has been described [5]. Some of these anions have intrinsic UV responses and they have been separated by LC and detected at 190–214 nm [6,7]. CE has also been used for separation of anions with a moderate UV response, where the ions were detected at 214 nm [8–10]. The determination of trace amounts of an anion simultaneously with an excess of another anion may be difficult, especially when the resolution is insufficient. However, a

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sufficient resolution can be accomplished by control of the electromigration dispersion or by increasing the resolution by adding α-cyclodextrin [11,12]. The selectivity can also be changed by the addition of organic solvents, and this technique has been used in the separation of both positional isomers and inorganic anions [13–17].

The injection of a sample containing complex sample matrices, e.g., an equally charged ion, may also cause problems such as anti-stacking [18]. This effect can be minimised if the sample additives are carefully chosen [19]. The electromigration dispersion can also be minimised if the co-ion/UV-absorbing ions in the BGE have a matching mobility compared with the analyte ions [20]. The response of the peaks is affected by the mobility and concentration of all ions present in the zone, according to the Kohlrausch regulating function [21].

Quantification performed by CE requires certain steps to be taken in order to improve the quantitative capabilities of the technique. Quantitative precision in CE depends largely on the reproducibility of sample introduction [22,23]. Two major factors influencing precision in CE are sample concentration and the temperature in the capillary, although other subtle factors also have an impact on the precision of analysis [24]. Minimisation of sample adsorption is also an important factor in controlling the precision [25].

Important contributions have been made to the validation of different CE methods, and a thorough study of the validation of a CE method for determining basic drugs is one example [26]. An inter-company cross-validation of a method for determining drug counter-ion levels has been published [27]. An experimental design approach has been applied to the validation of a CE method for determining a drug and its related substances [28]. A method for determining the chiral impurity in a local anaesthetic drug has been validated and compared with an established LC method [29]. Validated methods for the determination of cations by indirect detection have also been published [30,31]. Accuracy in these studies was tested by comparison with alternative techniques such as ion chromatography (IC) and titrimetric analysis, and results obtained with the different methods showed close agreement. The robustness of an indirect detection method using an experimental design approach has been described [32]. It was shown in this study that the method was robust with respect to the evaluated parameters.

Ion chromatography is the major technique in the analysis of inorganic ions, although CE is an increasingly used technique for these applications. A comparison of the two techniques was presented by Haddad [33]. It was concluded in this review article that each of the two techniques have benefits and drawbacks, and it was suggested that the two techniques be considered as complementary rather than competitive.

In this paper a new method for the determination of bromide in a local anaesthetic hydrochloride is presented. Bromide is determined by direct UV detection at 200 nm in the presence of a large excess of chloride, and these conditions put great demands on the separation capacity of the separation system. The method presented here was optimised using an experimental design program and was validated in accordance with the ICH guidelines [34,35].

2. Experimental

2.1. Instrument and settings

A Hewlett-Packard capillary electrophoresis $HP^{3D}CE$, Waldbronn, Germany, was used during the separations using the HP-ChemStation software for data processing. The detection wavelength was 200 nm with a bandwidth of 5 nm; reference 245 nm (bandwidth 10 nm). The fused-silica capillary had an internal diameter of 50 μm with an extended light path (bubble factor 3) and an effective length of 41.5 cm. The cassette temperature was set at 20 °C and the voltage applied was −15 kV. Preconditioning of the capillary was performed prior to each run by flushing for 2 min with an acetonitrile–water (60:40) solution, followed by a 3-min flush with the background electrolyte and completion of the conditioning by applying a voltage of −15 kV for 1 min.

The replenish system was used for changing the background electrolyte in inlet/outlet vials prior to each injection. The samples/standards were injected by pressure for 15 s and 30 mbar, followed by injection of the inlet run buffer, for 10 mbar and 2 s. The run time of the analysis was set to 10 min. New

capillaries were flushed with 0.1 *M* sodium hydroxide for 10 min, followed by a 2-min flush with water.

2.2. Reagents

Acetonitrile gradient grade for LC was purchased from Merck (Darmstadt, Germany), methanesulphonic acid 99% from Sigma–Aldrich (Steinheim, Germany), triethanolamine (AG) and sodium hydroxide (AG) from Fluka Chemie (Buchs, Germany) and potassium bromide (working standard) from Merck. Purified water (ultra grade) was obtained from an Elga Maxima ultrapure water system.

The buffer solution that was used in the optimisation study was prepared by adjusting a solution of 250 m*M* methanesulphonic acid to pH 1.3 with sodium hydroxide. For the validation, the buffer solution was adjusted to pH 1.3 with triethanolamine. The BGE was prepared by mixing 200 ml of 250 m*M* methanesulphonic acid, pH 1.3, with 300 ml of acetonitrile in a 500-ml volumetric flask.

2.3. Procedures

Two standard stock solutions were prepared by weighing potassium bromide into the volumetric flasks. The standard solutions were prepared by diluting the two stock solutions with 6 m*M* NaCl to the proper concentrations.

The sample solutions (6 m*M* local anaesthetic salt) were prepared by weighing the local anaesthetic into a volumetric flask and diluting to volume with 6 m*M* NaCl.

2.4. Experimental design software

The software used for the factorially designed separation optimisation study was MODDE® 4.0 from Umetrics (Umeå, Sweden).

3. Results and discussion

3.1. Method development

3.1.1. Detection mode and BGE

The determination of bromide in the presence of a large excess of chloride was initially tested using an indirect UV-detection system. It was possible to separate and detect the bromide and chloride ions in this system. When the chloride concentration was increased to the level that is present in the local anaesthetic samples (6 m*M*), the electromigration dispersion effects caused band broadening and the separation deteriorated.

The limited loadability of indirect UV-detection systems and the knowledge that bromide possesses an intrinsic UV response resulted in the investigation proceeding by the detection of bromide at 200 nm.

A sample matrix containing a high concentration of an ion such as chloride may cause anti-stacking problems [18]. If, however, the mobility of the chloride ion is lower than the mobility of the bromide ion, the high chloride concentration may contribute to a sample self-stacking effect [19]. Furthermore, the buffer co-ion must be carefully chosen with respect to its mobility in order to achieve an efficient separation system. Different buffers were tested in order to find a co-ion with a suitable mobility. It was possible to detect bromide using a citric acid or sulphate buffer, although an unstable baseline or an increased noise level was observed in these systems. A new system was therefore tested where the buffer components originated from a non-aqueous CE system [36]. This system was buffered at pH 1.3–2.3 with methanesulphonic acid solution and diluted with various amounts of acetonitrile. Methanesulphonate proved to be a suitable buffer co-ion having a matching mobility. Acetonitrile was added to this system in order to improve the selectivity.

Initial experiments were made using a BGE containing 30% acetonitrile and 50 m*M* methanesulphonic acid at pH 2.3, obtaining a plate number of the bromide (10 μM) peak of $N > 1.5 \times 10^5$ (Fig. 1).

On this system it was possible to separate the bromide and the excess of chloride in the local anaesthetic drug. A 1 m*M* solution of the drug showed sufficient resolution of the two peaks. However, the separation between the chloride peak and the impurity, bromide, had to be improved in order to increase the loadability. The limit of quantification (LOQ) is improved when the sample concentration is increased, and this puts greater demands on the separation capacity of the method since the over-

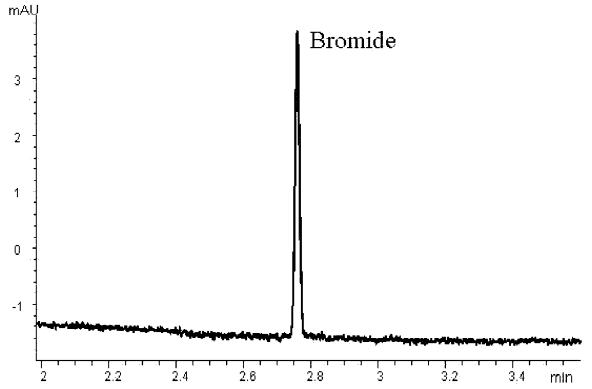


Fig. 1. The background electrolyte contained 50 m*M* methanesulphonic acid adjusted to pH 2.3 with sodium hydroxide and 30% acetonitrile. The sample concentration was 10 μ*M* and UV detection was used at 200 nm (see Section 2 for additional parameters).

loaded chloride peak is band-broadened by electromigration dispersion.

Addition of a higher content of acetonitrile improved the resolution and this made it possible to introduce higher sample concentrations with sufficient resolution.

3.1.2. Selectivity

Addition of organic solvents such as acetonitrile to the BGE may improve the selectivity for inorganic anions. Several authors have shown that difficult separations can be resolved if an organic solvent is used in the BGE [13–17]. However, only a few of them have given a possible explanation of the mechanism for the change in selectivity. The most probable explanation for the altered selectivity is related to the solvatisation of the ions. There will be a change in the hydrodynamic radius of the ions and the effect may be more or less pronounced for different ions. This effect can be used for alteration of the effective mobility of the ions.

The results obtained in this study show that it was possible to improve the selectivity between chloride and bromide by adding acetonitrile to the BGE. The buffering component in the system was methanesulphonic acid and this buffer ion has recently been used in non-aqueous CE systems. It was possible to use acetonitrile contents up to at least 70% (v/v) without obtaining precipitation in the BGE.

3.1.3. Multivariate optimisation

The methanesulphonic acid–acetonitrile system described above was then optimised with a statistical experimental design program (MODDE®, Umetrics Sweden) using a central composite face-centred (CCF) design. The parameters that were chosen for variation were: pH (1.3–2.3), concentration of $MeSO_3^-$ (25–100 m*M*), sample chloride concentration (1–6 m*M*) and amount of acetonitrile (30–70%). The responses that were optimised were the resolution between bromide and chloride and the plate number for bromide.

The parameters that were varied and the responses that were obtained during the optimisation are presented in Table 1.

The multifactorial design and optimisation software fit the data through a second-degree polynomial function. To start with, all linear, interaction and quadratic terms were included. The fraction of variation of the response that can be explained by the model (R^2) and the fraction of variation of the response that can be predicted by the model (Q^2) were then examined. For a good model, R^2 and Q^2 should be as close as 1 as possible. The model estimated the coefficients, which represent half the effect of a factor. A coefficient that did not have a significant effect was then removed from the model and a new model was made. If R^2 and Q^2 decreased when an insignificant coefficient was removed from the model, the coefficient was added to the model again. If R^2 and/or Q^2 increased, the coefficient was left out and the procedure was repeated with the next insignificant coefficient. The effects (the change in response when going from a low level to a high level for each factor) of all factors and interaction terms in the final model are shown in Fig. 2.

The confidence limits shown in Fig. 2 are calculated from $\pm\sqrt{((X'X)^{-1})} \cdot \mathrm{RSD} \cdot t(\alpha/2,\ \mathrm{DF}_{\mathrm{resid}})$. Therefore the sizes of the confidence limits depend on the structure of the *X*-matrix, the size of residual standard deviation and the number of degrees of freedom. Significant effects in the coefficient plot are those with confidence intervals not covering zero.

The effect on the two responses resolution (R_s) and efficiency (N) is low when the pH is varied in the range 1.3–2.3. The effect on the responses is moderate when the methanesulphonic acid concentration is varied in the range 25–100 m*M*. The

Table 1
Statistical experimental design for the method optimisation of the bromide determination in a local anaesthetic hydrochloride salt

Exp. name	Run order	%ACN	pH	$[MeSO_3^-]$ (m*M*)	$[Cl^-]$ (m*M*)	*N*	R_s
N1	5	30	1.3	25	1	112 582	4.00
N2	13	70	1.3	25	1	139 349	13.95
N3	15	30	2.3	25	1	133 972	4.05
N4	19	70	2.3	25	1	134 603	14.06
N5	3	30	1.3	100	1	157 454	5.60
N6	14	70	1.3	100	1	102 119	21.12
N7	18	30	2.3	100	1	160 436	5.38
N8	25	70	2.3	100	1	126 999	21.92
N9	8	30	1.3	25	6	46 516	2.77
N10	4	70	1.3	25	6	87 582	8.80
N11	17	30	2.3	25	6	29 006	2.60
N12	24	70	2.3	25	6	86 599	9.28
N13	16	30	1.3	100	6	124 777	4.22
N14	26	70	1.3	100	6	84 595	15.91
N15	21	30	2.3	100	6	124 203	4.30
N16	23	70	2.3	100	6	112 158	16.95
N17	11	30	1.8	62.5	3.5	116 201	3.78
N18	2	70	1.8	62.5	3.5	70 648	14.04
N19	10	50	1.3	62.5	3.5	144 614	8.04
N20	9	50	2.3	62.5	3.5	132 016	8.66
N21	7	50	1.8	25	3.5	122 246	6.06
N22	27	50	1.8	100	3.5	136 397	9.67
N23	6	50	1.8	62.5	1	159 533	9.53
N24	12	50	1.8	62.5	6	132 740	6.92
N25	22	50	1.8	62.5	3.5	139 592	7.58
N26	1	50	1.8	62.5	3.5	137 890	7.54
N27	20	50	1.8	62.5	3.5	138 714	7.51

Method parameters (factors): amount of acetonitrile (%ACN), pH, buffer concentration ($[MeSO_3^-]$) and sample chloride concentration ($[Cl^-]$). Responses: plate number for the bromide peak (*N*) and resolution between bromide and chloride (R_s). Other conditions are as in Section 2.

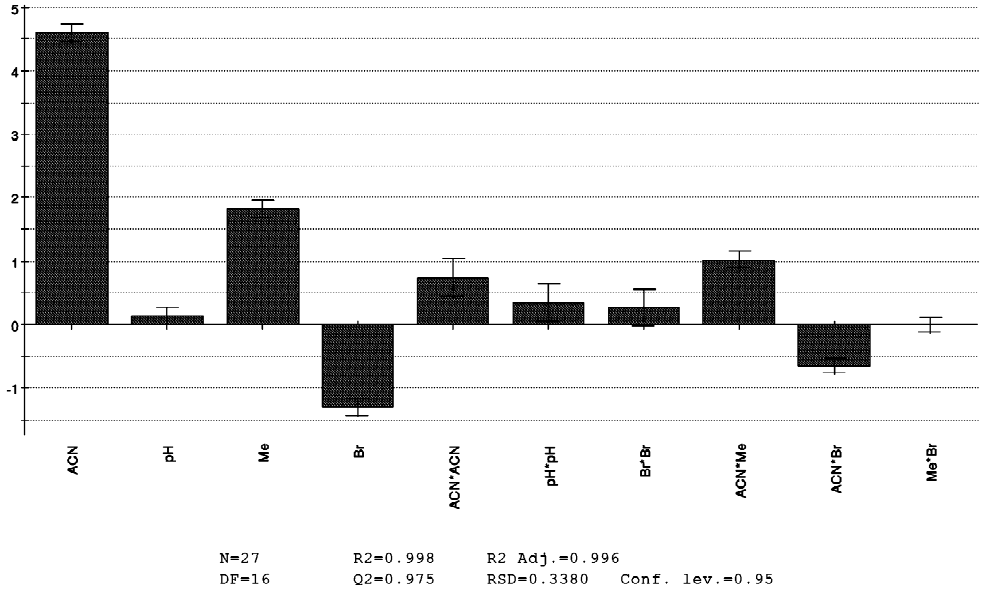
sample concentration affected the responses as expected, with decreasing efficiency and resolution as the chloride concentration is increased.

The amount of acetonitrile used in the BGE proved to have a significant effect on both *N* and R_s. The resolution was improved with increasing acetonitrile concentration from 30 to 70%, but the efficiency decreased with increasing ACN concentration. The ACN×ACN quadratic term proved to have a large negative effect on efficiency, but it had a moderate positive effect on resolution. The interaction term ACN×Me has a moderate positive effect on the resolution, but it has negative effect on the efficiency.

The ACN×Br also showed counteracting effects on resolution and efficiency, where *N* increased and R_s decreased with the interaction term between the acetonitrile and bromide concentration. The quadratic terms pH×pH and Br×Br have minor positive effects on both *N* and R_s. The Me×Br interaction term has a moderate positive effect on the plate number, but no significant effect on the resolution.

The results in Fig. 2 can be displayed in several different plots by experimental design program. The response prediction plot is one example that could be used in different combinations of the factors and responses. In Fig. 3 is a response prediction plot of the significant effect of acetonitrile concentration on efficiency given. The efficiency reached a maximum at 50% acetonitrile. One possible explanation for the optimum may be that if the bromide zone is sharpened due to a sample self-stacking effect, a point may be reached where the two zones migrates apart more rapidly and the stacking effect is reduced.

A



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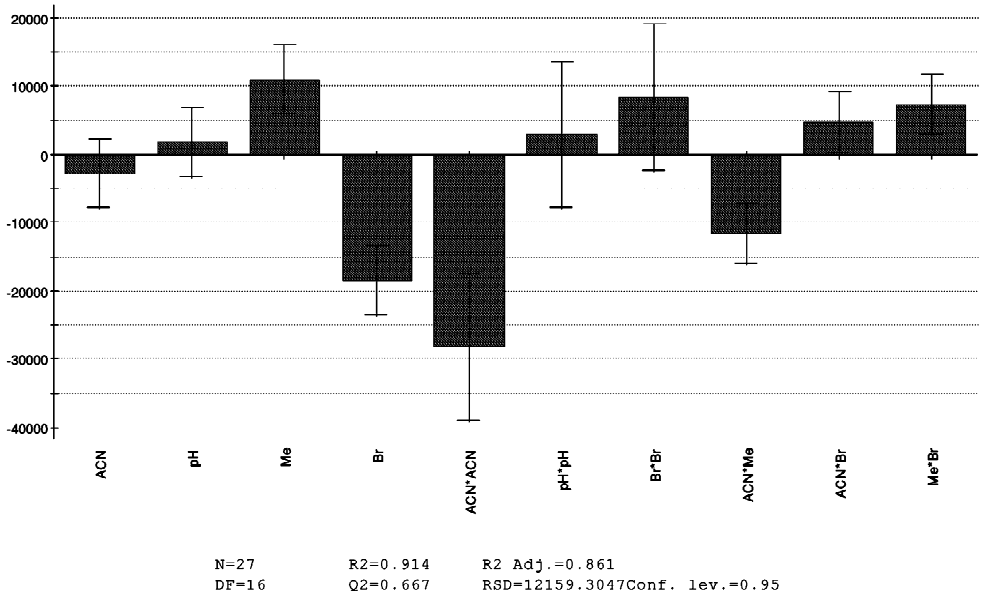

Fig. 2. Terms included in the models for (A) the resolution between bromide and chloride and (B) the plate number of bromide.The main factors are: ACN, acetontrile concentration pH; Me, methane sulphonic acid; and Br, concentration of bromide (sample concentration). The interaction terms are: ACN×ACN, pH×pH, Br×Br, ACN×Me, ACN×Br and Me×Br.

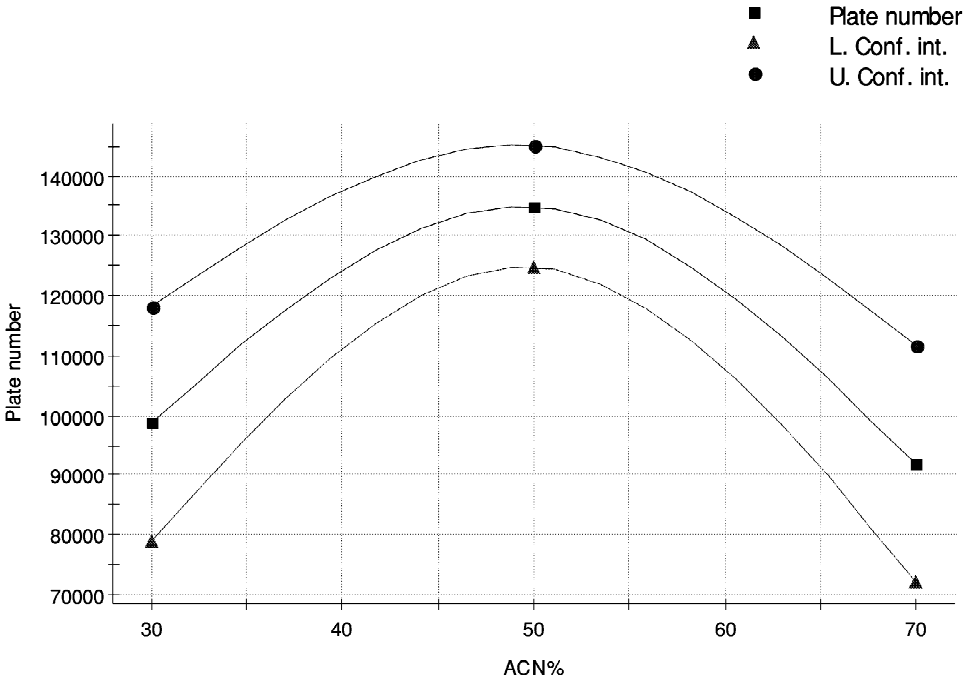

Fig. 3. Resolution between bromide and chloride and plate number of bromide as a function of the amount of acetonitrile. Responses and 95% confidence interval (ConfInt) predicted from the experimental results by the MODDE program. Other conditions are as in Section 2.

Electropherograms from the optimisation are presented in Fig. 4, which clearly show the effect on resolution by increasing the acetonitrile content in the BGE.

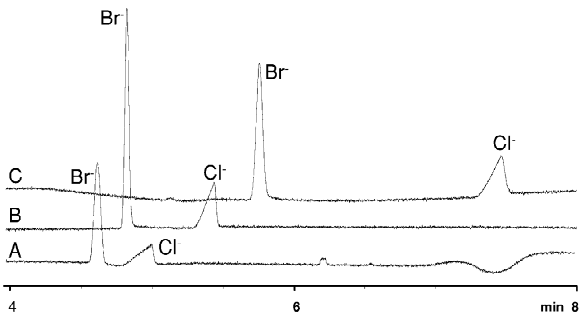

Fig. 4. The effect of variation of the acetonitrile content. Conditions: (A) 30% ACN, 100 m*M* methanesulphonic acid, pH 1.3, and injection of a 6 m*M* local anaesthetic sample containing 0.3% (w/w) bromide; (B) 50% ACN, 62.5 m*M* methanesulphonic acid, pH 1.3, and injection of a 3.5 m*M* local anaesthetic sample containing 0.3% (w/w) bromide; (C) 70% ACN, 62.5 m*M* methanesulphonic acid, pH 1.3, and injection of a 3.5 m*M* local anaesthetic sample containing 0.3% (w/w) bromide (see Section 2 for the remaining conditions).

The experimental design program was then used to find the optimal conditions for the separation of bromide and chloride. The sample concentration was set at 6 m*M*. The suggested composition of the BGE was: an acetonitrile concentration of 60% and a methanesulphonic acid concentration of 100 m*M*. The pH had a minor effect on the efficiency and a pH of 1.3 was used in order to obtain a sufficient buffer capacity.

3.1.4. Peak distortion

During the initial steps of validation of the method, tailing of the bromide peak was found. This problem usually originates from adsorption of the sample to the capillary surface. The adsorption may be minimised if a suitable surface-active agent is added to the BGE.

Triethanolamine is a surface-active additive that has been used in a CE system for the separation of anions [37]. Triethanolamine was added to the BGE for titration of the pH to 1.3 instead of sodium hydroxide. This change in BGE composition reduced the peak tailing and this new BGE was then used

during the validation of the method. This change in BGE composition had minor effects on other parameters such as N, R_s and t_m and it was concluded that it was not necessary to use the experimental design program again for optimisation. In Fig. 5 an electropherogram is shown where an authentic local anaesthetic sample was injected (6 m*M*) in the triethanolamine containing BGE.

This method was then tested on its ability to determine bromide in local anaesthetic samples. The percentage (w/w) of bromide was calculated with external standardisation using a five-level calibration curve. No internal standard was needed, as external standardisation gives sufficient precision in using the developed CE method for the analysis of bromide as an impurity counter-ion in a drug.

3.1.5. Analysis with IC and CE

Bromide was determined with ion chromatography in three up-scaling batches of the local anaesthetic hydrochloride salt before the development of the CE-method described in this paper. The determined bromide was at the same level with both techniques, but it was observed that IC produced more waste solution and was more expensive to operate. The selectivity between highly concentrated chloride peak and the bromide peak was better with the optimised CE-method. It was concluded that the CE could be applied advantageously for monitoring assay of the bromide in local anaesthetic hydrochloride salt.

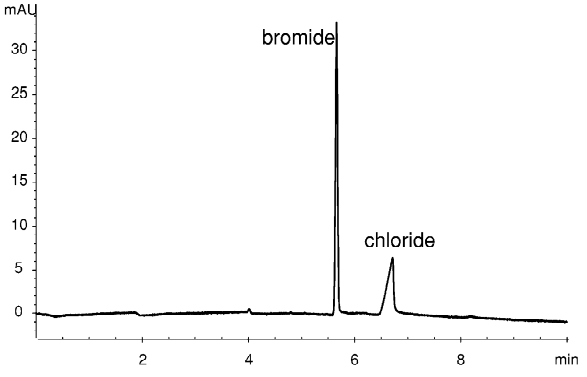

Fig. 5. Optimised separation between bromide and chloride in a 6 m*M* local anaesthetic hydrochloride sample containing 0.3% (w/w) bromide. The BGE consisted of 60% acetonitrile and 100 m*M* methanesulphonic acid adjusted to pH 1.3 with triethanolamine.

3.2. Validation

3.2.1. Specificity

The resolution between the bromide and chloride peak was sufficient as shown in Fig. 5, although other possible interfering anions had to be studied. Iodide is an ion that may interfere with the chloride and bromide peaks when detection is performed at 200 nm. The separation capacity of the developed system was tested by injection of a sample spiked with iodide. Fig. 6 shows that sufficient selectivity is obtained between the iodide peak and the bromide peak.

3.2.2. Detection and quantification limits

Based on a signal-to-noise ratio of 3:1, the detection limit is 0.36 μ*M* in a 6 m*M* solution of the local anaesthetic hydrochloride salt. The LOQ is set at a signal ratio of 10:1, which means that the LOQ for this method is 1.2 μ*M*. However, it is possible to determine even lower amounts of bromide in the local anaesthetic hydrochloride. This can be accomplished by injection of a more concentrated sample of the drug. The high resolution between the bromide and chloride peaks makes it possible to determine bromide in the presence of a larger and more band-broadened chloride peak.

3.2.3. Range

The validated range of the method is 13–167 μ*M*, corresponding to 0.1–0.6% (w/w) bromide impurity in a 6 m*M* solution of the local anaesthetic hydrochloride salt. The precision, linearity and accuracy have been confirmed within and at the extremes of this range.

3.2.4. Precision

3.2.4.1. Repeatability: six replicate injections

The standard solution in the middle of the standard curve was injected six times with relative standard deviations (RSDs) of 0.6% for the corrected area and 0.04% for the migration time, both well under the limit of 1.5% RSD.

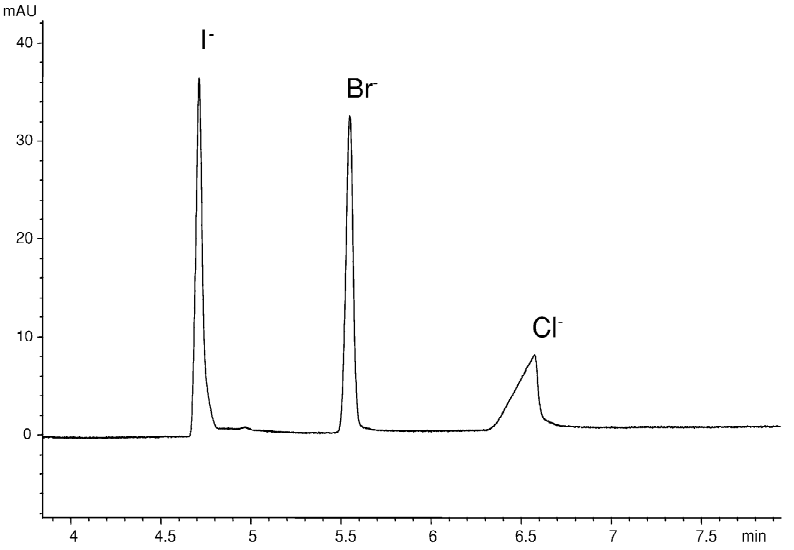

Fig. 6. The background electrolyte contained 100 m*M* methanesulphonic acid adjusted to pH 1.3 with triethanolamine and 60% acetonitrile. The sample concentration was 6 m*M* local anaesthetic hydrochloride spiked with 0.12 m*M* potassium iodide and UV detection was used at 200 nm (see Section 2 for remaining parameters).

3.2.4.2. Reproducibility: six replicate sample preparations

Six replicate sample preparations from a homogeneous sample containing 0.3% (w/w) bromide were injected. Two persons using two different CE systems at two laboratories injected the same sample solution. The RSD of this reproducibility test was 1.4%.

3.2.5. Linearity

Duplicate injections were made at five concentration levels ranging from 13 to 167 μ*M*. The intercept was −0.004 (±0.009), the slope 0.0035 (±0.0001) and the correlation coefficient (*r*) 0.9996. The origin was within the intercept range and the correlation coefficient was ≥0.999.

3.2.6. Accuracy

The accuracy was determined by addition of known amounts of bromide to a local anaesthetic sample containing a low content of bromide. Bromide was added in order to obtain three concentration levels (0.1, 0.35 and 0.6%) of the impurity. The results are presented in the Table 2. The theoretical content and the experimentally determined contents showed close agreement (90–110% recovery). These results confirm that the developed method is accurate.

3.2.7. Comparison of CE and IC

The developed CE system gave bromide contents at the same level as ion chromatography when the results were compared. Three batches of the local

Table 2
Accuracy of the determination of trace levels of bromide in a local anaesthetic hydrochloride drug

Theoretical content (%, w/w)	Found content (%, w/w)	Recovery (%)
0.101	0.105	104
0.103	0.104	101
0.101	0.105	104
0.110	0.113	103
0.347	0.342	98.5
0.353	0.350	98.2
0.347	0.347	99.2
0.350	0.355	102
0.607	0.598	100
0.617	0.615	98.6
0.617	0.601	99.0
0.604	0.625	104
Mean + RSD =	___	101±2.31%

Table 3
Method comparison between capillary electrophoresis and ion chromatography for the determination of bromide in a local anaesthetic hydrochloride drug

LA sample (lot)	Result CE (%, w/w)	Result IC (%, w/w)
b.13	0.33	0.32
b.18	0.12	0.11
b.25	0.13	0.16

anaesthetic hydrochloride were analysed by the two techniques and the results are shown in Table 3.

3.2.8. Robustness

Information about the robustness of the method was obtained during the optimisation of the method. The method is robust with respect to pH and the concentration of methanesulphonic acid versus resolution, but is sensitive to variations in acetonitrile concentration. The BGE is stable for a month if the solution is kept in a closed flask. It is important to use the replenish system or change BGE in run vials between runs. Otherwise, evaporation of acetonitrile may occur and cause a loss in selectivity.

The final method has successfully been in use at the quality control lab for more than 3 years. It was concluded that the method is robust and gives reliable results.

4. Conclusions

A new capillary electrophoresis method has successfully been developed for the determination of bromide in local anaesthetic hydrochloride. Bromide was detected at 200 nm and external standardisation was used in the bromide determinations. Methanesulphonic acid proved to be a suitable buffer co-ion to prevent anti-stacking from the high concentration of chloride in the sample. A sufficient selectivity between bromide and chloride was obtained by the addition of acetonitrile. Triethanolamine proved to be an efficient additive in the BGE for minimisation of peak tailing due to adsorption. The system was optimised with respect to plate number and resolution using a statistical experimental design program. The developed method was validated according to the ICH guidelines and proved to be suitable for the intended use.

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