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Application of capacitively coupled contactless conductivity detection for the analysis of aminoglycoside antibiotics

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ABSTRACT

Capacitively coupled contactless conductivity detection (C^4D) is an interesting tool to detect molecules with no strong UV-absorbing chromophore. It is mainly combined with capillary electrophoresis (CE) and was proven to be a versatile mode of detection. After a short technical explanation, the paper will focus on the possible use of CE-C^4D in quality control of aminoglycoside antibiotics which in general, do not show UV absorption and thus pose a serious challenge for direct detection. It will be shown that CE-C^4D can be applied for the separation of kanamycin, tobramycin and amikacin from their respective impurities. Baseline separation was obtained using similar background electrolytes composed of 2-(*N*-morpholino) ethanesulfonic acid and histidine. As the methods should be useful for the determination of small amounts of impurities, special attention was paid to the sensitivity of the CE-C^4D methods.

KEYWORDS: capillary electrophoresis, C^4D, pharmaceutical analysis, quality control, aminoglycosides

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1. INTRODUCTION

Aminoglycoside antibiotics (AGs), a family of polycationic pseudo-oligosaccharide compounds, consist of two or more aminosugars joined by a glycosidic linkage to an aminocyclitol nucleus usually in a central position. This aminocyclitol is 2-deoxystreptamine, as in most aminoglycosides (Fig. 1), or streptamine. As they are obtained by fermentation, they can contain several closely related substances as impurities which are intermediates or by-products from the biosynthetic pathway. Some AGs are chemically modified so that they can also contain side products from the reaction. Aminoglycoside antibiotics are bactericidal inhibitors of protein synthesis [1]. The AGs are potentially oto- and nephrotoxic. Therefore the content, stability, decomposition and impurity profile need to be carefully monitored.

The presence of the closely related substances, starting materials and by-products makes separation difficult due to their similarity in structure and physicochemical properties. Detection of AGs and their impurities is complicated by the lack of a significant UV absorbing chromophore. Several analytical methods have been used, such as paper and thin-layer chromatography [2, 3], colorimetry after derivatization with ninhydrin [4] and spectrophotometry [5]. Liquid chromatography (LC) coupled with PED has been used successfully for the analysis of several AGs [6-10]. Beside PED, various other direct detection techniques have been coupled with LC: indirect UV detection,

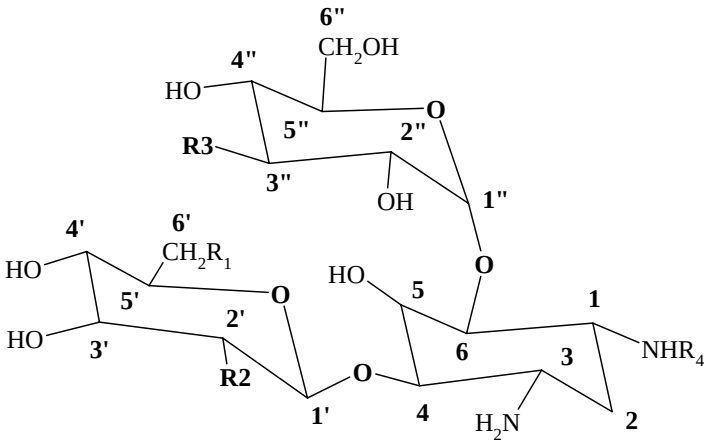

Fig. 1. Representative aminoglycosides containing 2-deoxystreptamine. R1, R2, R3 and R4 differ in structure according to the type of aminoglycoside.

conductivity and refractive index detection [2, 11]. LC combined with mass spectrometry (MS) as detection technique has also been reported [12]. Some investigators have used capillary electrophoresis (CE) in the analysis of AGs combined with borate complexation [13], indirect detection methods [14] and amperometric detection [15]. Micellar electrokinetic capillary chromatography (MEKC) with UV detection for simultaneous determination of amikacin, tobramycin and kanamycin A, was performed in Tris buffer at pH 9.1 with high concentration of sodium pentanesulphonate as an anionic surfactant [16]. However, except PED these methods are not very sensitive and selective for related substances.

LC and CE using pre and post-column derivatization of AGs have been performed using 2,4-dinitrofluorobenzene and 1,2-phthalic dicarboxaldehyde/mercaptoacetic acid or dansylchloride with either UV or fluorescence detection [17-24]. Although these detection methods are quite sensitive, the derivatization step is time consuming and often giving problems with quantitation because of additional sample processing, variability of reaction completeness, possible instability of derivatized products and toxicity of some derivatization agents.

In order to replace the tedious and time-consuming derivatization methods and to avoid the drawbacks of PED, CE with C^4D was investigated. In this paper, examples of analysis methods which were developed in our lab will be given for kanamycin, tobramycin and amikacin.

2. Contactless conductivity detection

Conductivity detection (CD) measures an electrical signal (conductance) between electrodes contacting the solution. In this detection mode, analytes have to be charged in solution for the determination. A conductivity detector cell comprises two inert electrodes across which a high frequency alternating current (AC) signal is applied. The signal arises from the difference in conductance between the analyte and the background electrolyte (BGE). The higher the conductivity differences between the analyte molecules and background co-ion, the larger the detector response. However, main problem of contact CD is fouling of the electrodes. CD can also be carried out in contactless mode, which was first proposed in 1980, with four radial electrodes placed around the separation capillary [25-27]. The modern concept of this mode of detection is called capacitively coupled contactless conductivity detection (C^4D) and was introduced in 1998 [28, 29].

2.1. C^4D detection cell

In C^4D two metallic cylindrical electrodes are placed outside, axially to the capillary (Fig. 2a). This feature makes the assembling of the capillary in C^4D even easier than using UV detectors (where a detection window is needed) and avoids deleterious effects commonly present in conventional conductometry, such as flow interference due to the presence of the electrodes inside the capillary, the polarization of the electrodes by the separation electric field or

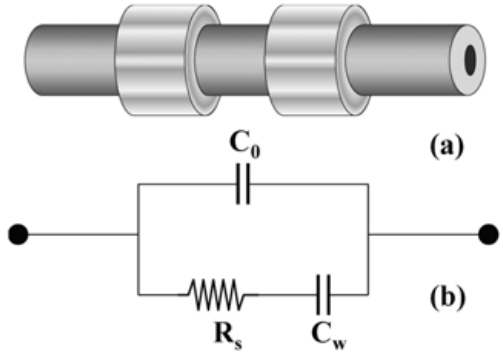

Fig. 2. C^{4}D detection cell (a). The C^{4}D cell can be represented by an electrically equivalent circuit (b) where C_w and C_0 stand for the capacitances of the capillary wall and inter-electrode region, respectively and R_s represents the resistance of the BGE.

chemical modification of the electrodes, causing changes in the electrode activity and/or decrease in the electrode activity (electrode passivation). In conventional CD an alternate potential, in the order of 1-2 kHz is applied to the electrodes simply to avoid electrolysis. In C^{4}D, in order to overcome the high impedance of the capillary wall, an alternate potential in the order of hundreds of kHz is applied. The conductivity cell (capillary + BGE + electrodes surrounding) can be represented as an electric circuit, as schematized in Fig. 2b.

The capacitor C_0 corresponds to the signal transmission between the electrodes (stray capacitance) and C_w represents one serial association of two capacitors formed by the capillary wall between the electrode and the BGE. The resistor R_s (the signal to be measured) corresponds to the section of BGE between the two electrodes.

After the transmission between the electrodes, the signal is applied to a converter current/voltage, transformed to direct current (DC) and amplified. After analog to digital conversion, the data can be computed. Not only the excitation frequency, but also the amplitude of the high frequency signal to be applied to C^{4}D [30, 31] has to be optimized, which can be done by using eDAQ C^{4}D software (Denistone East, Australia).

2.2. Mechanisms of detection

The mechanism of conductometric detection is based on the displacement of the BGE ions by the analyte. In a very simplified way, when the capillary is filled with the BGE, by applying a suitable excitation voltage and frequency to the electrodes, a signal proportional to the solution conductivity is generated. This signal is the baseline signal and it is proportional to the BGE concentration and composition. If a plug of sample is injected, the introduced ions displace some of the ions of the BGE. When the separation potential is applied, the analytes acquire an electrophoretic velocity and transient zones migrate along the capillary heading toward the detector. Variation in solution conductance due to analytes will result in changes in the detected signal. Three situations are possible.

First: If the analyte has higher mobility (μ_a) than the mobility of the BGE co-ion (μ_s), it expels some of the BGE co-ion from its zone. The concentration of the analyte in the migration zone is equimolar to the injected concentration. However, the concentration of the displaced co-ion is lower than the analyte concentration. In order to keep the electroneutrality, some of the BGE counter-ion is incorporated into the formed zone. The result is a zone of higher conductivity, which produces a positive peak when it passes through the C^{4}D cell.

Second: If the μ_a is lower than the μ_s, it also expels some of the BGE co-ion from its zone. Again, the analyte zone has the same concentration as injected and the BGE co-ion is depleted in this zone. However, in this case, the co-ion depletion is larger than the analyte concentration and some of the BGE counter-ion leaves the zone to keep electroneutrality. The result is a zone of lower conductivity, resulting in a negative peak when it passes through the C^{4}D cell.

In a third situation, the mobilities (μ_a and μ_s) are exactly the same and in this condition the signal observed is small, promoted only by differences between the concentrations of the analyte and the BGE co-ion or other phenomena.

The extent of displaced co- and counter-ions depends upon the concentrations and mobilities of all the involved substances and the C^{4}D signal can be obtained by equation 1, as described in the literature [31].

$$\Delta G = \frac{C_a(\mu_a - \mu_s)(\mu_a + \mu_o)}{\mu_a} \frac{F}{10^{-3} K} \quad \text{(Equation 1)}$$

Where ΔG is the admittance variation, *F* is Faraday's constant, K is the cell constant, C_a and μ_a are the analyte ion concentration and absolute mobility respectively and μ_s and μ_o are the absolute mobilities of the BGE co-ion and counter-ion respectively.

In practice, BGEs with high mobility co-ions are chosen to determine low mobility analytes and vice-versa. It is common to take a low mobility counter-ion, despite the expression above. The use of a high mobility counter-ion could also be a valid strategy, but higher conductivity in the BGE leads to higher baseline noise and finally it is the S/N ratio which really matters. Higher sensitivity is obtained for higher differences in mobility between the analyte and its BGE co-ion, which at the same time leads to distortions in the peak shape due to electrodispersion. Therefore, the optimization must compromise sensitivity and peak shape.

To achieve optimum operation, some considerations have to be taken into account. Better results are obtained by using lower conductivity buffers, smaller CE capillary internal diameters, a higher excitation voltage, by reducing vibration and movement and by keeping the temperature stable.

C^4D can be considered a universal detection mode for charged species, with an achieved status of well-established technique [32-35]. Several classes of substances including small inorganic and organic ions (halogens, alkali metals, transition metals, carboxylic acids, amines, etc), alcohols, carbohydrates, amino acids, peptides and proteins, DNA fragments, pharmaceuticals, etc. have been determined by this technique [36-40]. C^4D can be considered as an interesting option for quality control and bioanalysis. The most significant advantage of this detector is the determination of non-UV absorbing molecules without derivatization.

3. CE instrumentation and conditions

The CE experiments were performed on a P/ACE MDQ instrument (Beckman Coulter, Inc. Fullerton, CA, USA), coupled to an eDAQ C^4D system (eDAQ, Denistone East, Australia). Uncoated fused silica capillaries (75 µm internal diameter (i.d.) and 375 µm outer diameter (o.d.)) were purchased from Polymicro Technologies (Phoenix, AZ, USA). New capillaries were conditioned at 45°C by rinsing with 1 M NaOH (10 min), 0.1 M NaOH (30 min), waiting for 30 min and then rinsing with water (10 min). Daily at the start of analysis, the capillary was rinsed at 45°C with 1 M NaOH (5 min), 0.1 M NaOH (5 min), water (5 min) and BGE (5 min). The capillary was rinsed between runs for 1 min with 0.1 M NaOH, 1 min with water and 3 min with buffer. The inlet/outlet vials were replaced every 3 runs. All rinsing steps were performed at 25°C and pressure of 137.9 kPa.

The eDAQ C^4D detector was employed at different peak-to-peak amplitudes and different frequencies were used dependent on the BGE compositions as will be mentioned in each application. The data were acquired using licensed PowerChrom v2 software (eDAQ). Further data processing was done by both PowerChrom v2 and 32 Karat™ 4.0 softwares.

4. Applications

The buffer pH as well as the ionic strength influence the electrophoretic mobility of the analytes through the capillary. Hence the choice of the BGE constituents is crucial. Accordingly, in the present work the buffer constituents morpholinoethanesulfonic acid (Mes) (MES)-monohydrate and L-histidine (L-His) were used and cetyltrimethyl ammonium bromide (CTAB) was added to the mixture as (1) an electroosmotic flow modifier in a concentration less than 1.3 mM, the critical micellar concentration (CMC) and (2) to reduce the adsorption of poly cationic compounds (like AGs) on the capillary wall.

4.1. Kanamycin sulphate

Kanamycin is an aminoglycoside antibiotic which is mainly used as its sulphate or acid sulphate salt. As it is a fermentation product, it can contain several closely related substances such as kanamycins B, C and D, intermediate substances as paromamine, and degradation substances as 2-deoxystreptamine, 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl) deoxystreptamine [6-O-(3-AG)DS] and 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl) deoxystreptamine [4-O-(6-AG)DS]. All these components were well separated by using a BGE of 40 mM MES and 0.6 mM CTAB, at pH 6.35 adjusted by adding L-His [41]. Samples were

hydrodynamically introduced at a pressure of 3.45 kPa for 5 s and a separation voltage of -30 kV (cathode at the injection capillary end) was applied on a fused silica capillary with a total length of 65 cm (41 cm to C^{4}D detector) at 25°C. The eDAQ C^{4}D detector was employed at a peak-to-peak amplitude of 100 V and the frequency was 1200 kHz. The optimized separation was obtained in less than 6 min. It is noteworthy that also sulphate can be determined using this technique. The ammonium acetate was used as internal standard (I.S.). The linearity (R^2 = 0.9999) was found to be good with as regression equation: y = 0.00855x + 0.00134, $S_{y,x}$ = 0.02, where y: relative area, x: concentration (mg.L^{-1}) and $S_{y,x}$: standard error of estimate. The intercept was found to be not statistically different from zero. The repeatability was evaluated by the relative standard deviation (% RSD) of the relative corrected peak areas, migration time and relative migration time. These intraday precisions were 0.3 %, 0.8 % and 0.8 % (n = 6) respectively. The respective interday precisions on these items over 3 days were 1.1 %, 0.1 % and 1.7 % (n = 18). The mean of migration time and relative migration time were 4.6 min and 3.1 (n = 6) for intraday precision and 4.7 min and 3.1 (n = 18) for interday precision respectively. For quantitative analysis of kanamycin sulphate, an amount of 23 ng was used by injecting 23 nL of a 1.0 g.L^{-1} solution dissolved in water prepared by using ultrapure MilliQ-water. The limit of detection (LOD) and the limit of quantification (LOQ) for kanamycin A were 16 pg and 54 pg determined at a signal-to-noise ratio (S/N) of 3 and 10 respectively.

4.2. Tobramycin

Tobramycin (TOB) is produced by fermentation or it can also be synthesized from kanamycin B. Beside the main component, it can contain as related substances: kanamycin B, nebramine and neamine (also known as neomycin A), which are the three impurities of tobramycin reported in the European Pharmacopoeia (Ph. Eur.) [42]. The BGE used is similar to that for kanamycin, but with different concentrations of MES, CTAB and pH. All components were well separated by using a BGE of 25 mM MES and 0.3 mM CTAB, at pH 6.4 adjusted by adding L-His [43]. Samples were hydrodynamically introduced at a pressure of 3.45 kPa for 5 s and a separation voltage of -30 kV (cathode at the injection capillary end) was applied on a fused silica capillary with a total length of 65 cm (43 cm to C^{4}D detector) at 25°C. The eDAQ C^{4}D detector was employed at a peak-to-peak amplitude of 80 V and the frequency was 600 kHz. The optimized separation was obtained in less than 7 min showing good linearity (R^2 = 0.9995) with regression equation: y = 0.0131x - 0.0072, $S_{y,x}$ = 0.13, where y: relative area, x: concentration (mg.L^{-1}) and $S_{y,x}$: standard error of estimate. The intercept was found to be not statistically different from zero. The repeatability was checked by the relative standard deviation (% RSD) of the relative corrected peak areas, migration time and relative migration time. Intraday precisions were 0.2 %, 0.6 % and 0.5 % (n = 6) respectively, and interday precisions over 3 days were 0.7 %, 0.8 % and 0.8 % (n = 18) respectively. The mean of migration time and relative migration time were 5.7 min and 3.5 (n = 6) and 5.7 min and 3.5 (n = 18) for intraday and interday precision respectively. For quantitative analysis of tobramycin, an amount of 4.6 ng was used by injecting 23 nL of a 0.2 g.L^{-1} solution dissolved in water. The limit of detection (S/N = 3) and the limit of quantification (S/N = 10) for tobramycin were 9 pg and 31 pg respectively.

4.3. Amikacin

Amikacin or BB-K8 is a semi-synthetic aminoglycoside antibiotic derived from kanamycin A by the acylation of the C-1 amino group of the 2-deoxystreptamine moiety with L-(-)-γ-amino-α-hydroxybutyric acid (L-HABA) [44]. Since kanamycin A has four acylatable amino groups, it is possible to obtain by-products that differ only in the position of the acyl group. These three positional isomers of amikacin, which are acylated with L-HABA at the C-3, C-6’ or C-3” amino groups of kanamycin A are described as BB-K29, BB-K6 and BB-K11, respectively. Kanamycin A acylated with L-HABA molecules on the C-1 and C-3 position (*di*-HABA kanamycin) can also be formed. Beside those components, amikacin can also contain starting materials of the synthetic reaction as kanamycin A and L-HABA. All these components were well separated by using a BGE of 20 mM MES and 0.3 mM CTAB, at pH 6.6 adjusted by adding L-His [45]. Samples were hydrodynamically introduced at a pressure of

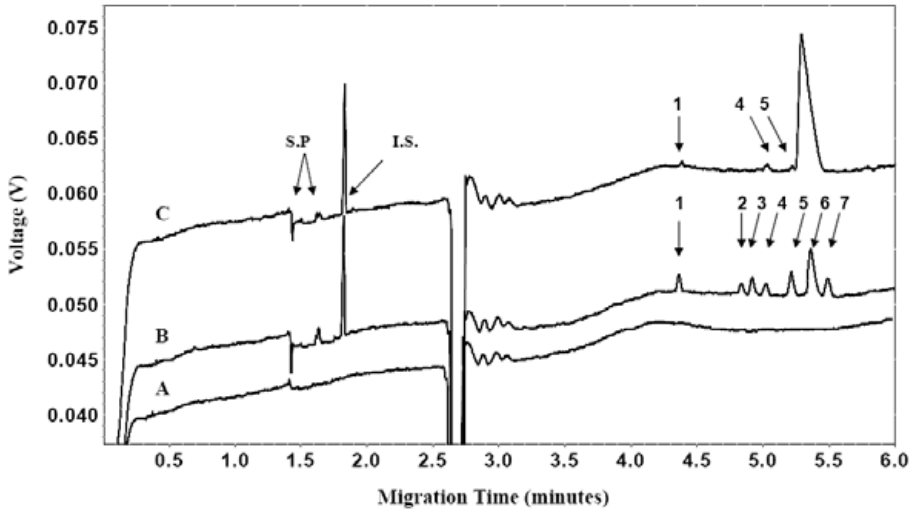

Fig. 3. Electropherograms **A**; Blank (water), **B**; amikacin (50 mg.L^{-1}) spiked with impurities (10 mg.L^{-1} each) and ammonium acetate 20 mg.L^{-1} as internal standard (I.S.) and **C**; amikacin commercial sample (0.5 g.L^{-1}). Capillary, uncoated fused silica 75 µm i.d., 375 µm o.d., (73 cm total length, effective 48 cm to C^{4}D detector); voltage, 30 kV in reverse polarity; temperature, 25°C; injection, inlet pressure 3.45 kPa for 5 sec; BGE, 20 mM Mes and 0.3 mM CTAB, adjusted by L-His to pH 6.6; samples were dissolved in water, 1: L-HABA, 2: BB-K6, 3: BB-K11, 4: BB-K29, 5: kanamycin A, 6: amikacin BB-K8, 7: *di*-HABA kanamycin, I.S. : internal standard and S.P: system peaks.

3.45 kPa for 5 s injection time and a separation voltage of -30 kV (cathode at the injection capillary end) was applied on a fused silica capillary with a total length of 73 cm (48 cm to C^{4}D detector) at 25°C. The eDAQ C^{4}D detector was employed at a peak-to-peak amplitude of 100 V and the frequency was 700 kHz.

Typical electropherograms are shown in Fig. 3. The optimized separation was obtained in less than 6 min with good linearity (R^2 = 0.9996) and as regression equation: y = 0.0224x + 0.0174, $S_{y,x}$ = 0.17, where y: relative area, x: concentration (mg.L^{-1}) and $S_{y,x}$: standard error of estimate. The intercept was found to be not statistically different from zero. The repeatability was examined by the relative standard deviation (% RSD) of the relative corrected peak areas, migration time and relative migration time. Intraday precisions were 0.4 %, 0.2 % and 0.2 % (n = 6) respectively, and interday precisions over 3 days were 1.6 %, 0.8 % and 0.5 % (n = 18) respectively. The mean of migration time and relative migration time were 5.3 min and 2.9 (n = 6) and 5.3 min and 2.9 (n = 18) for intraday precision and interday precision respectively. For quantitative analysis of tobramycin, an amount of 10 ng was used by injecting 20 nL of 0.5 g.L^{-1} solution dissolved in water. The limit of detection (S/N = 3) and the limit of quantification (S/N = 10) for amikacin were 10 pg and 33 pg respectively.

CONCLUSION

The combination of CE with C^{4}D has shown a promising future for the analysis of substances, which are difficult to be detected directly by conventional UV/Vis or fluorescence detectors because of their poor UV absorption. CE-C^{4}D is sensitive (picogram range, which is comparable to a UV detector), universal, environment-friendly and operating costs are low. However, the major drawback is choosing the right BGE which has to be a good compromise between co-ion conductance and the mobility of the BGE and the analyte to be detected. This rather limits the choice of the BGE. Commercially available software allows to choose the optimum frequency and excitation voltage peak-to-peak amplitude.

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REFERENCES

1. Chambers, H. F. The aminoglycosides, In Goodman & Gilman's The Pharmacological Basis of Therapeutics, Hardman, J. G., Limbird, L. E., Gilman, A. G. (Eds.), 11th Edition, Pergamon Press, McGraw Hill, New York, USA, 2001, 1219.
2. Rothrock, J. W., Guegelman, R. T., and Wolf, F. J. 1959, Antibiot. Annu., 776, 1958.
3. Eneva, G., Nikolova-Damyaniva, B., Spassov, S., and Haimova, M. 1990, J. Planar Chromatogr., 3, 232.
4. Inouye, S. and Ogawa, H. 1964, J. Chromatogr., 13, 536.
5. Confino, M. and Bontchev, P. 1990, Mikrochim. Acta, 3, 305.
6. Adams, E., Dalle, J., De Bie, E., De Smedt, I., Roets, E., and Hoogmartens, J. 1997, J. Chromatogr. A, 766, 133.
7. Szùnyog, J., Adams, E., Roets, E., and Hoogmartens, J. 2000, J. Pharm. Biomed. Anal., 23, 891.
8. Adams, E. and Hoogmartens, J. 2004, Curr. Top. Electrochem., 10, 63.
9. Zawilla, N. H., Li, B., Hoogmartens, J., and Adams, E. 2007, J. Pharm. Biomed. Anal., 43, 168.
10. Manyanga, V., Dhulipalla, R. L., Hoogmartens, J., and Adams, E. 2010, J. Chromatogr. A, 1217, 3748.
11. Inchauspé, G. and Samain, D. 1985, J. Chromatogr., 303, 277.
12. Li, B., Van Schepdael, A., Hoogmartens, J., and Adams, E. 2009, J. Chromatogr. A, 1216, 3941.
13. Flurer, C. L. 1995, J. Pharm. Biomed. Anal., 3, 809.
14. Ackermans, M. T., Everaerts, F. M., and Beckers, J. L. 1992, J. Chromatogr., 606, 229.
15. Fang, X. M., Ye, J. N., and Fang, Y. Z. 1996, Anal. Chim. Acta, 329, 49.
16. Yeh, H. H., Lin, S.-J., Ko, J.-Y., Chou, C.-A., and Chen, S.-H. 2005, Electrophoresis, 6, 947.
17. Fabre, H., Sekkat, M., Blanchin, M. D., and Mandrou, B. 1989, J. Pharm. Biomed. Anal., 7, 1711.
18. Dash, A. K. and Suryanarayanan, R. 1991, J. Pharm. Biomed. Anal., 9, 237.
19. Russ, H., McCleary, D., Katimy, R., Montana, J. L., Miller, R. B., Krishnamoorthy, R., and Davis, C. W. 1998, J. Liq. Chromatogr. & Rel. Technol., 21, 2165.
20. Kaale, E., Van Schepdael, A., Roets, E., and Hoogmartens, J. 2002, Electrophoresis, 23, 1695.
21. Kaale, E., Van Schepdael, A., Roets, E., and Hoogmartens, J. 2001, J. Chromatogr. A, 924, 451.
22. Kaale, E., Van Schepdael, A., Roets, E., and Hoogmartens, J. 2003, Electrophoresis, 24, 1119.
23. Wienen, F. and Holzgrabe, U. 2003, Electrophoresis, 24, 2948.
24. Lin, F., Wang, C., and Chang, Y. 2008, J. Chromatogr. A, 1188, 331.
25. Gas, B. and Vacik, J. 1980, Chem. Listy, 74, 652.
26. Gas, B., Demjanenko, M., and Vacik, J. 1980, J. Chromatogr., 192, 253.
27. Vacik, J., Zuska, J., and Muselasova, I. 1985, J. Chromatogr., 320, 233.
28. Fracassi da Silva, J. A. and do Lago, C. L. 1998, Anal. Chem., 70, 4339.
29. Zemann, A. J., Schnell, E., Volgger, D., and Bonn, G. K. 1998, Anal. Chem., 70, 563.
30. Fracassi da Silva, J. A., Guzman, N., and do Lago, C. L. 2002, J. Chromatogr. A, 942, 249.
31. Brito-Neto, J. G. A., Fracassi da Silva, J. A., Blanes, L., and do Lago, C. L. 2005, Electroanalysis, 17, 1198.
32. Brito-Neto, J. G. A., Fracassi da Silva, J. A., Blanes, L., and do Lago, C. L. 2005, Electroanalysis, 17, 1207.
33. Kubáň, P. and Hauser, P. C. 2004, Electrophoresis, 25, 3387.
34. Kubáň, P. and Hauser, P. C. 2004, Electrophoresis, 25, 3398.
35. Kubáň, P. and Hauser, P. C. 2004, Electrophoresis, 16, 2009.
36. Solinova, V. and Kasicka, V. 2006, J. Sep. Sci., 29, 1743.
37. Kubáň, P. and Hauser, P. C. 2008, Anal. Chim. Acta, 607, 15.

38. Kubáň, P. and Hauser, P. C. 2009, Electrophoresis, 30, 176.
39. El-Attug, M. N., Lutumba, B., Hoogmartens, J., Adams, E., and Van Schepdael, A. 2010, Talanta, 83, 400.
40. Kubáň, P. and Hauser, P. C. 2011, Electrophoresis, 32, 30.
41. El-Attug, M. N., Hoogmartens, J., Adams, E., and Van Schepdael, A. 2011, J. Sep. Sci., 34, 2448.
42. European Pharmacopoeia, 7th Ed., EDQM, Strasbourg, 2011, p. 3103.
43. El-Attug, M. N., Hoogmartens, J., Adams, E., and Van Schepdael, A. 2012, J. Pharm. Biomed. Anal., 58, 49.
44. Kawaguchi, H., Naito, T., Nakagawa, S., and Fujisawa, K., 1972, J. Antibiot., 25, 695.
45. El-Attug, M. N., Hoogmartens, J., Adams, E., and Van Schepdael, A. 2012, Electrophoresis, in press.