Short communication

Hsin-Hua Yeh[1]
Shun-Jin Lin[1]
Ju-Yun Ko[2]
Chia-An Chou[2]
Su-Hwei Chen[1, 3]

[1]Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University
[2]School of Medicine, Kaohsiung Medical University
[3]Department of Pharmacy, Kaohsiung Medical University, Chung-Ho Memorial Hospital, Kaohsiung, Taiwan

Rapid and selective micellar electrokinetic chromatography for simultaneous determination of amikacin, kanamycin A, and tobramycin with UV detection and application in drug formulations

A simple and selective micellar electrokinetic chromatography (MEKC) with UV detection is described for simultaneous determination of amikacin, tobramycin, and kanamycin A, performed in Tris buffer (180 mM; pH 9.1) with 300 mM sodium pentanesulfonate (SPS) as an anionic surfactant. Under this condition, good separation with high efficiency and the required short analysis time is achieved. The linear ranges of the method for the determination of amikacin, tobramycin, and kanamycin A were 0.1–0.5 mg/mL, 0.4–2.0 mg/mL, and 0.4–2.0 mg/mL, respectively; the detection limits (signal-to-noise ratio = 3; injection, 0.5 psi 5 s) were 0.08, 0.2, and 0.2 mg/mL, respectively. The small amount of sample required and the expeditiousness of the procedure allow content uniformity to be determined in individual commercial products.

Keywords: Amikacin / Kanamycin A / Micellar electrokinetic chromatography / Tobramycin
DOI 10.1002/elps.200410178

Aminoglycoside antibiotics have a hexose ring, either streptidine or 2-deoxystreptamine, and various glycosidically linked amino sugars. Aminoglycosides are used most widely against Gram-negative enteric bacteria, especially in bacteremia, sepsis, and tuberculosis. At present, amikacin and tobramycin are most widely administered parenterally for serious infections resistant to gentamicin. Kanamycins have three closely related structural forms: kanamycin A, B, and C. Commercially available kanamycin is almost pure kanamycin A, the least toxic of the three forms. Kanamycin A is now largely limited to topical or oral use; it is widely used as a second-line antituberculosis drug. Aminoglycoside antibiotics resemble each other in chemical structure, solubility, pharmacokinetic properties, narrow safety margin, and severe nephrotoxicity and ototoxicity [1]. It is necessary to monitor accurately their concentrations in plasma. Moreover, bulk drugs and pharmaceutical dosage forms described in official pharmacopoeias, such as the US Pharmacopoeia, require potency and purity testing for certification to ensure therapeutic efficacy and to avoid the possible risk of toxicity.

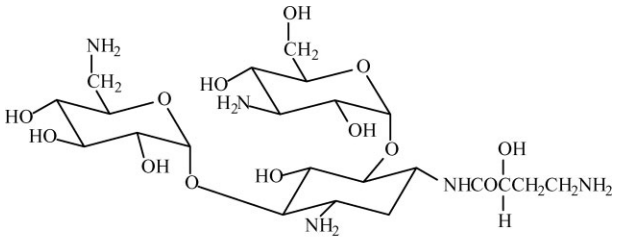
Numerous methods are available for the determination of aminoglycoside antibiotics. High-performance liquid chromatographic (HPLC) techniques are the most widely used and accurate technique for the analysis of the aminoglycoside antibiotics in various matrices [2–7]. Aminoglycoside antibiotics have neither a strong chromophore nor a conjugated system in the structure. Precolumn chemical derivatization with fluorimetric or UV detection is commonly used for increasing sensitivity and improving chromatographic properties. However, chemical derivatization usually requires a complicated treatment step before analysis. The HPLC method with chemical derivatization and detection at 365 nm has been used to assay tobramycin in bulk and pharmaceutical preparations in US Pharmacopoeia 27, while amikacin and kanamycin A are detected by electrochemical detection [7]. The precolumn chemical derivatization coupling with HPLC separations is generally time-consuming and entails a large amount of solvent waste. CE is increasingly being viewed as an alternative technique to HPLC for the determination of aminoglycoside antibiotics. Previous literatures reported on the use of CE in the analysis of aminoglycosides with indirect detection at low pH under

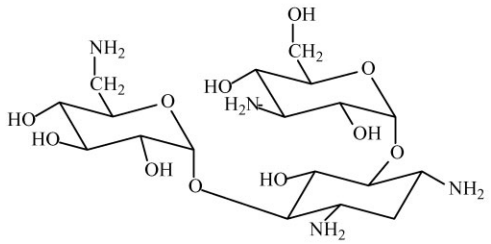
Correspondence: Dr. Su-Hwei Chen, Graduate Institute of Pharmaceutical Science, Kaohsiung Medical University, Kaohsiung 807, Taiwan
E-mail: suhwch@kmu.edu.tw
Fax: +886-7-3210683

Abbreviations: IS, internal standard; **SPS**, sodium pentanesulfonate sulfate

reversed-polarity condition [8], precapillary derivatization with UV detection [9–13], and borate complexation for direct UV detection [14]. The borate complexation method utilized the formation of negatively charged complexes between hexose ring and borate. The stability of the complex depended on the structure of carbohydrate and a longer migration time was needed for separation. In this study, a speedy and selective MEKC with UV detection was developed for simultaneous determination of structure highly related of amikacin, tobramycin, and kanamycin A (structures shown in Fig. 1). The application of the proposed method to analysis of amikacin, kanamycin A, and tobramycin in commercial products is demonstrated.

A Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with UV detector and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary of 40.2 cm (effective length 30 cm) $\times$ 50 μm ID. Samples were injected by pressure (0.5 psi) for 5 s. Preliminary tests of amikacin, tobramycin, and kanamycin A standards (Sigma, St. Louis MO, USA) by capillary zone electrophoresis (CZE) were briefly studied at 10 kV with





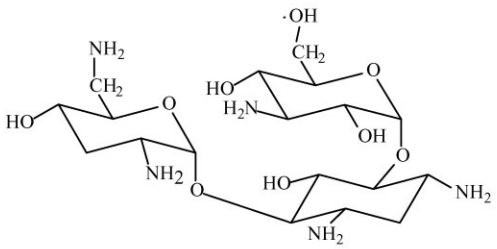


Figure 1. Chemical structures of amikacin, kanamycin A, and tobramycin.

Tris or borate buffer under various pH in the absence of surfactants. Amikacin, tobramycin, and kanamycin A did not separate in these background electrolytes. This indicates that by using the simple separation mode of CZE, based mainly on the differences of charge-to-mass ratios of the analytes in the tested conditions, it is difficult to resolve the highly ionized compounds, amikacin, tobramycin, and kanamycin A. The commonly used anionic surfactant, sodium dodecyl sulfate (SDS), as a micellar source with borate buffer or Tris buffer was utilized to study the resolution of the analytes. SDS at various concentrations in borate or Tris buffer in the pH range 7.0–9.5 was tested to separate the analytes. Although amikacin, tobramycin, and kanamycin A can differentiate in high concentration of borate buffer with SDS as background electrolytes, band-broadening with low theoretical plate numbers and insufficient resolution of the highly ionized compounds (amikacin, kanamycin A, and tobramycin) was observed under the tested conditions (results not shown). Neutral β-cyclodextrin, individually added to SDS with Tris or borate buffer, was investigated but there was no improvement. Therefore, surfactants with carbon numbers less than SDS, such as sodium octane sulfonate, sodium hexanesulfonate and sodium pentanesulfonate (SPS) (Sigma, St. Louis MO, USA) were investigated. These anionic surfactants in Tris buffer yielded improvement of selectivity and resolution between the analytes, especially SPS in Tris buffer. According to the peak efficiency (theoretical plate number *N*) and symmetry factors calculated, SPS as a micelle has a better separation efficiency. As a consequence, simple parameters affecting the MEKC using SPS as a surfactant for amikacin, tobramycin, and kanamycin A separation were studied, including concentrations of the buffer, SPS, and pH of Tris buffer. Different voltages (8, 10, and 12 kV) were tested; 12 kV can provide suitable separation with a shorter migration time and better column efficiency. After MEKC separation of amikacin, tobramycin, and kanamycin A in Tris buffer, the eluted compounds were monitored at 200 nm (cathode at the detection side).

Ionic strength or concentration of buffer have significant effects on solute mobilities and separation efficiency. The retention behavior of amikacin, tobramycin, and kanamycin A in Tris buffer (pH 9.1) in a concentration range of 80–200 mM with 300 mM SPS as anionic surfactant was studied. MEKC of amikacin, tobramycin, and kanamycin A in Tris buffer (pH 9.1) in the concentration range of 80–200 mM can give complete separation (Fig. 2). High-ionic-strength buffers have been used to enhance efficiencies in separation. The highest separation efficiency between analytes was obtained in 180 mM. Tailing peaks of analytes were observed in 200 mM Tris buffer, possibly due to the ineffective heat dissipation at this high concentration buffer.

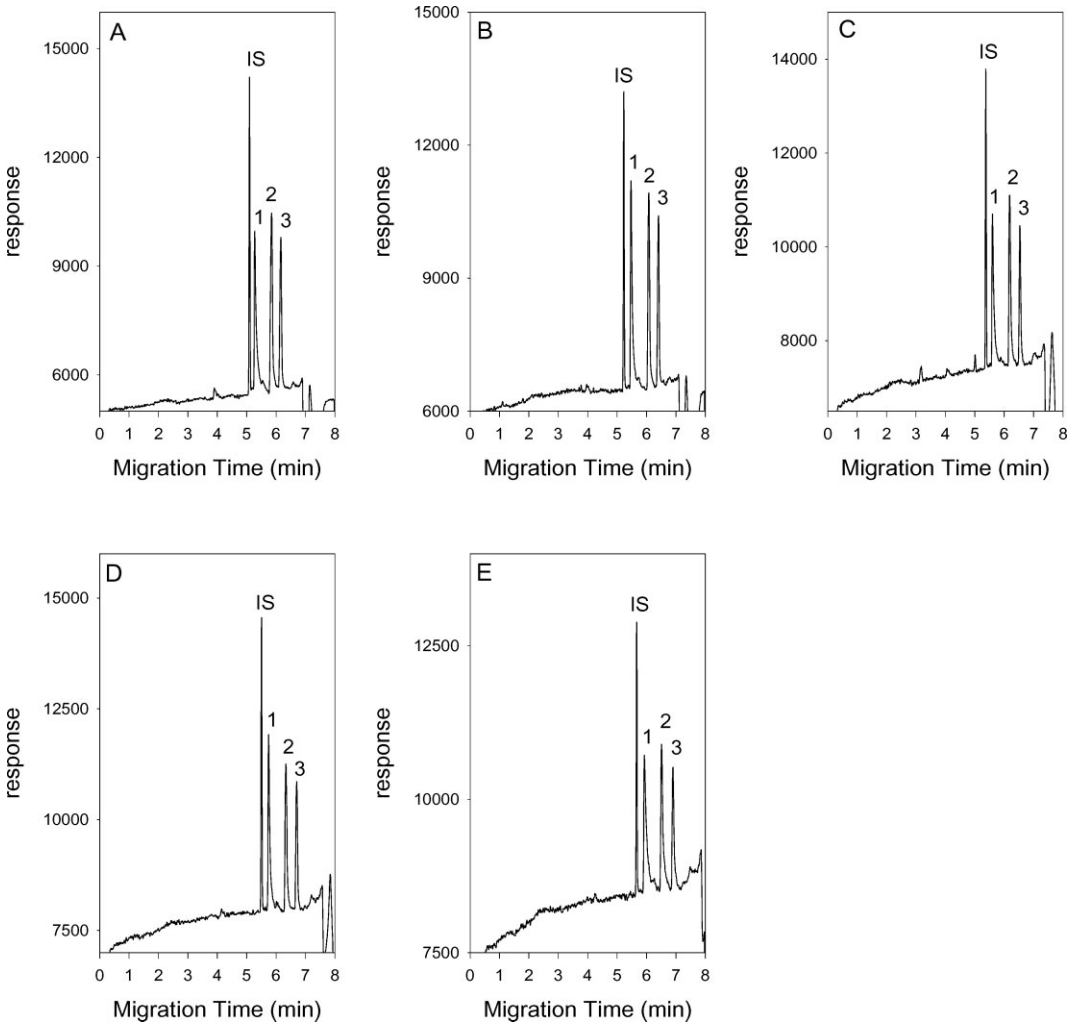


Figure 2. Effect of concentration of Tris buffer (pH 9.1) with 300 mM SPS on the migration of amikacin, tobramycin, kanamycin A, and thiamine (IS). (A) 80 mM, (B) 120 mM, (C) 150 mM, (D) 180 mM, (E) 200 mM. Peaks: 1, 2, and 3 for amikacin, tobramycin, and kanamycin A, respectively. CE conditions: applied voltage, 12 kV (detector at cathode side); uncoated fused-silica capillary, 40.2 cm (effective length 30 cm) $\times$ 50 μm ID; sample injection, 0.5 psi, 5 s; wavelength, 200 nm.

To prevent generation of too much Joule heating resulting in decrease of *N*, 180 mM Tris buffer was chosen. 180 mM Tris buffers with SPS (300 mM) at different pH (8.0, 8.5, 9.0, 9.1, and 9.5) were studied. The resolution of the tested drugs shows significant changes at various pH values (Fig. 3). Amikacin, tobramycin, and kanamycin A completely overlapped at pH 8.0. Partial overlap between amikacin and tobramycin was observed at pH 8.5 and there was complete resolution in pH 9.0, 9.1, and 9.5. In the pH 9.5 Tris buffer system, kanamycin A migrated to near the electroosmotic flow (EOF) marker and could not be analyzed reliably. Higher theoretical plate numbers and narrower peak width was observed at pH 9.1. The migration times of amikacin, tobramycin, kanamycin A, thiamine (internal standard, IS), and EOF were 5.746, 6.329, 6.696, 5.504, and 8.033 min, respectively, and all analytes migrated in front of the EOF.

From the pH effect on separation efficiency of analytes studies, amikacin is obviously mostly affected by pH. This may be due to the more polar functional groups in amikacin's chemical structure. The efficiency of the electrophoretic system is gauged by the number of theoretical plates *N*. Comparing pH 9.1 to pH 9.5 on separation efficiency for amikacin, an approximately threefold theoretical plate number at pH 9.1 was obtained. Therefore, a 180 mM, pH 9.1 Tris buffer was the choice for optimal buffer concentration and pH for simultaneous determination of amikacin, tobramycin, and kanamycin A.

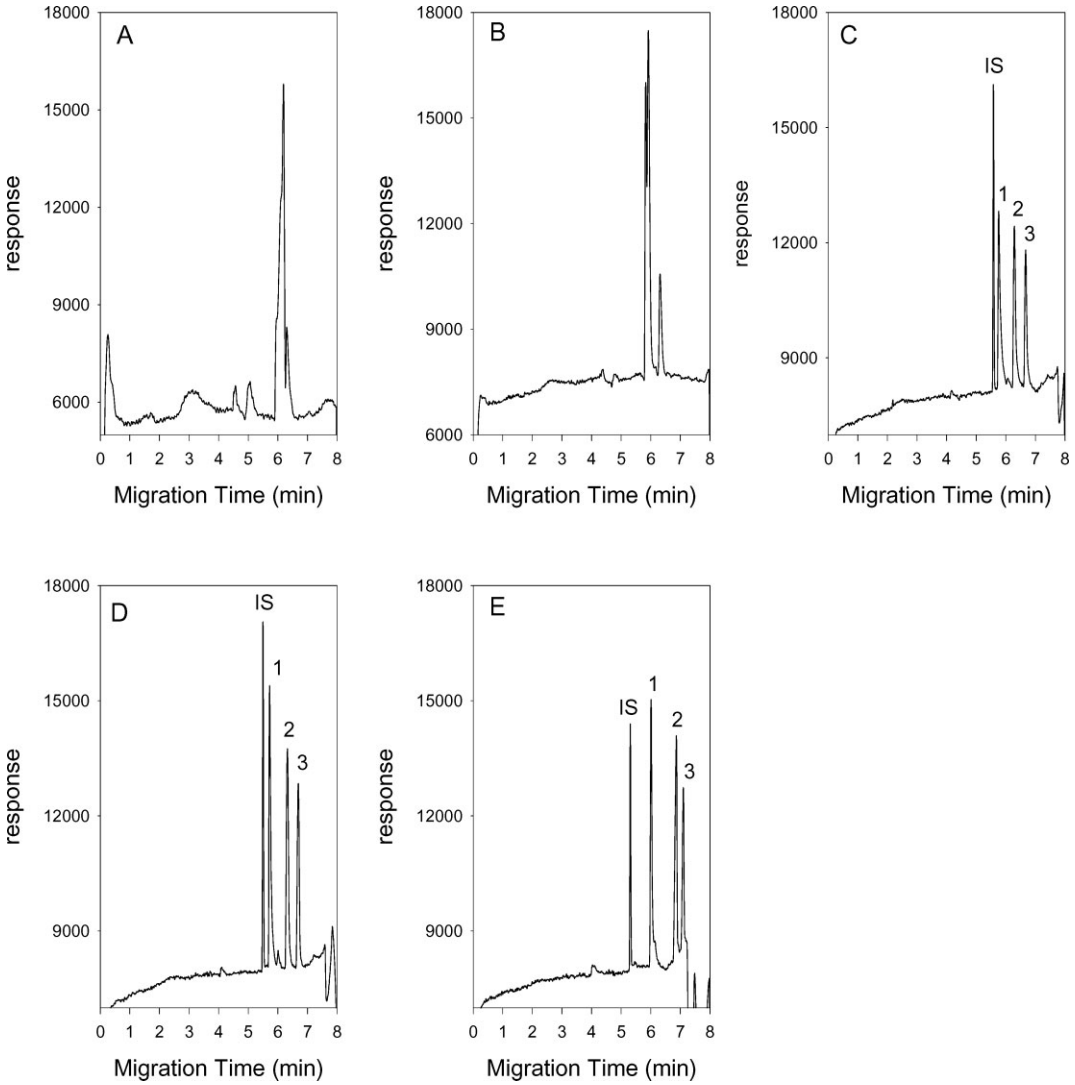


Figure 3. Effect of pH of Tris buffer with 300 mM SPS on the migration of amikacin, tobramycin, kanamycin A, and thiamine (IS). (A) pH 8.0, (B) pH 8.5, (C) pH 9.0, (D) pH 9.1, (E) pH 9.5. Peaks: 1, 2, and 3 for amikacin, tobramycin, and kanamycin A, respectively. For other CE conditions see Fig. 2.

The effect of SPS in the concentration range of 100–300 mM in Tris buffer (180 mM; pH 9.1) on the separation was studied and results indicated that electrophoresis of the drugs at 100 mM of SPS results in partial resolution of amikacin, tobramycin, and kanamycin A (Fig. 4). The values of resolution (R_S) obtained for 100 mM SPS in Tris buffer are 0.505 for amikacin-tobramycin and 0.510 for tobramycin-kanamycin A, compared with 0.818 and 0.763, respectively, for the same pairs in a 150 mM SPS with Tris buffer (180 mM; pH 9.1) system. With the concentration of SPS $\geq$ 200 mM, a baseline resolution electropherogram of the tested drugs was observed. However, a significant peak shape improvement is obtainable by increasing the concentration of SPS, leading to sharper peaks and higher theoretical plate numbers. Owing to the solubility of the surfactant, the concentration of SPS above 300 mM was not tested. An SPS concentration of 300 mM was selected for this study. The optimization of the MEKC mode conditions for analysis of amikacin, tobramycin, and kanamycin A was set at 180 mM Tris buffer (pH 9.1) with 300 mM SPS as running buffer and the analytes were monitored at 200 nm.

Under 12 kV as separation voltage, the current (μA) was about 120 μA in this background electrolyte. The typical electropherograms of the MEKC separation of amikacin, tobramycin, kanamycin A, and thiamine (IS) (reference standard and blank, respectively) are shown as Figs. 5B and A. Peaks 1, 2, and 3 represent amikacin, tobramycin, and kanamycin A, respectively.

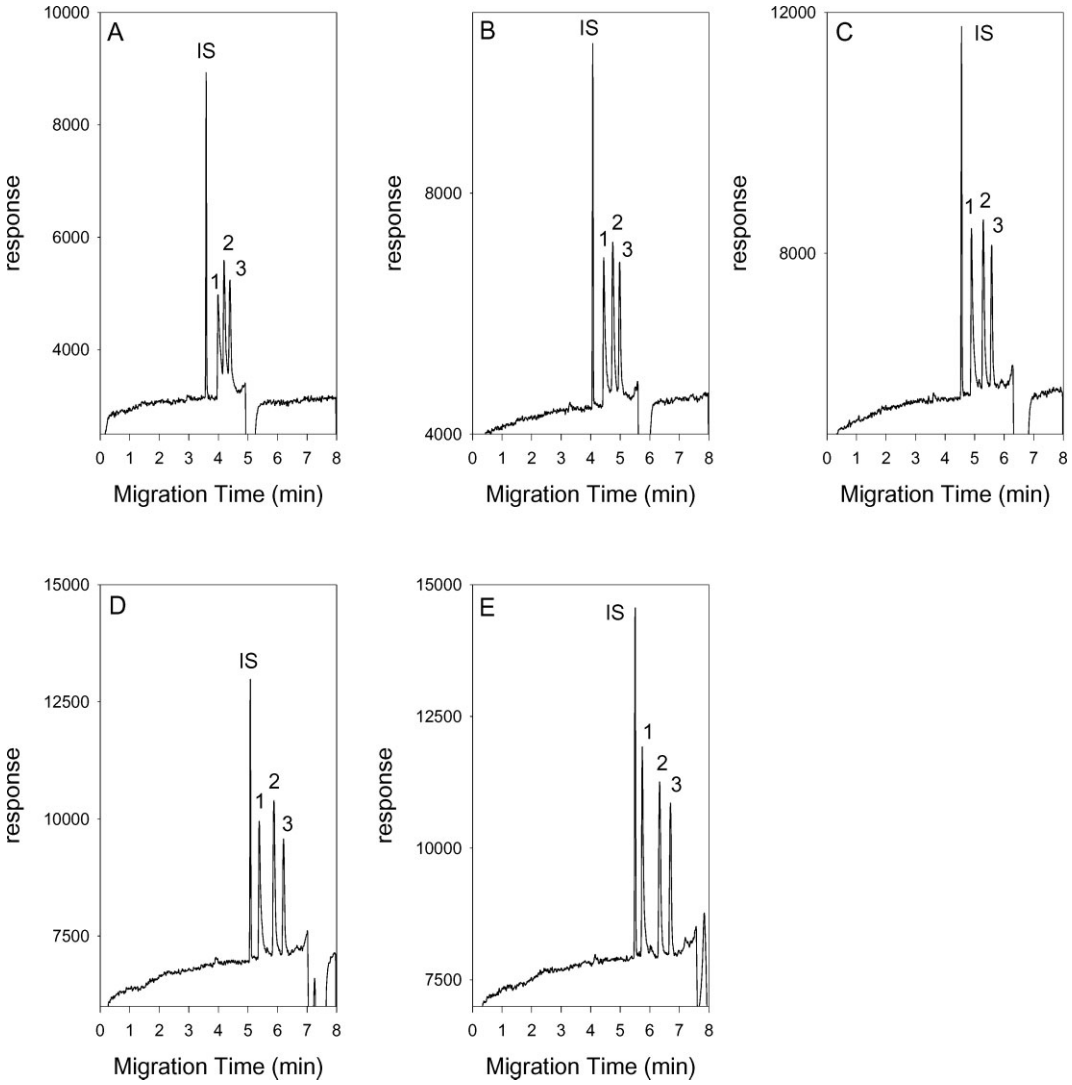


Figure 4. Effect of SPS concentration with 180 mM Tris buffer (pH 9.1) on the migration of amikacin, tobramycin, kanamycin A, and thiamine (IS). (A) 100 mM, (B) 150 mM, (C) 200 mM, (D) 250 mM, (E) 300 mM. Peaks: 1, 2, and 3 for amikacin, tobramycin, and kanamycin A, respectively. For other CE conditions see Fig. 2.

The migration velocity of an analyte depends on the distribution coefficient between the micellar and the nonmicellar phase. The distribution coefficient of the analytes depends on hydrophilic or hydrophobic properties. Among the tested drugs, amikacin has the most highly polar functional groups, containing four primary amino and eight hydroxyl groups compared to tobramycin having five primary amino and five hydroxyl groups and kanamycin A which has four primary amino and seven hydroxyl groups. Therefore, the distribution coefficient of amikacin is less than tobramycin and kanamycin in the SPS micellar phase and therefore amikacin is migrating faster under this running condition. Acetone is used as an electroosmotic flow (EOF) marker in CE. The apparent mobility (μA) was calculated according to the equation: $\mu A = \mu E + \mu\text{EOF} = (lL/tV)$ where l is the length of the capillary (cm) to the detector, V is the voltage, t is the migration time (s) and L is the total length (cm) of the capillary [15]. Under optimized CE conditions, the apparent mobility values of amikacin, tobramycin, kanamycin A, thiamine, and EOF are 2.92×10^{-4} cm^2 V^{-1}s^{-1}, 2.65 10^{-4} cm^2 V^{-1}s^{-1}, 2.51×10^{-4} cm^2 V^{-1}s^{-1}, 3.04×10^{-4} cm^2 V^{-1}s^{-1}, and 2.09×10^{-4} cm^2 V^{-1}s^{-1}, respectively. The electrophoretic mobility values (μE) of amikacin, tobramycin, kanamycin A, and thiamine are 0.83×10^{-4} cm^2 V^{-1}s^{-1}, 0.56×10^{-4} cm^2 V^{-1}s^{-1}, 0.42×10^{-4} cm^2 V^{-1}s^{-1}, and 0.95×10^{-4} cm^2 V^{-1}s^{-1}, respectively.

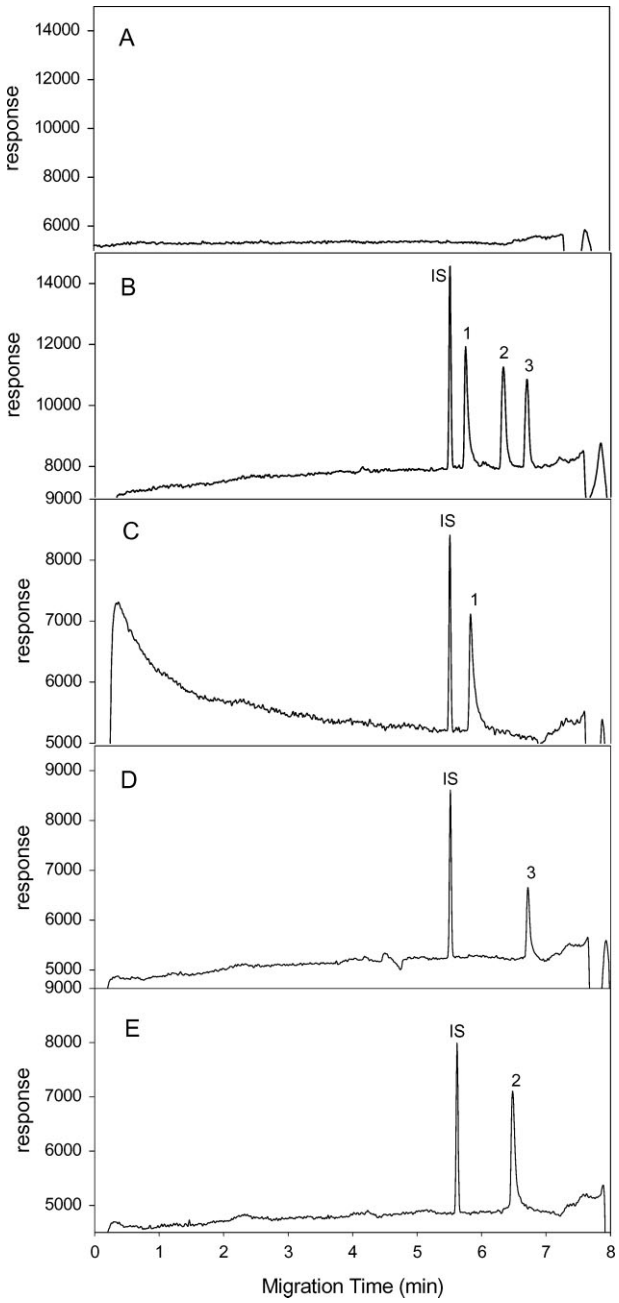


Figure 5. Typical electropherograms of (A) blank, (B) simultaneous determination of analyte standards, (C) amikacin in commercial injection, (D) kanamycin A in commercial capsule, (E) tobramycin in commercial injection. Peaks: 1, 2, and 3 for amikacin, tobramycin, and kanamycin A, respectively. CE conditions: 180 mM Tris buffer (pH 9.1) with 300 mM SPS. For other CE conditions see Fig. 2.

To evaluate the quantitative applicability of the method, five different concentrations of amikacin, tobramycin, and kanamycin A over the ranges 0.1–0.5 mg/mL, 0.4–2.0 mg/mL, and 0.4–2.0 mg/mL were analyzed using thiamine (25 μg/mL) as an IS. The linearity between the peak-area ratios (y) of the related analyte to IS and the concentration (x, mg/mL) of the analyte was investigated. The linear regression equations were obtained as follows: for amikacin, y (4.5717 ± 0.2173) x + (0.0094 ± 0.0002) for intraday (n = 6, r = 0.999) and y = (4.5191 ± 0.3852) x + (0.0243 ± 0.0001) for interday (n = 5, r = 0.999); for tobramycin, y = (1.1165 ± 0.0205) x − (0.0345 ± 0.0006) for intraday (n = 6, r = 0.999) and y = (1.1124 ± 0.0081) x − (0.033 ± 0.0001) for interday (n = 5, r = 0.999); for kanamycin A assay, y = (0.8695 ± 0.0055) x − (0.0049 ± 0.0001) for intraday (n = 6, r = 0.999) and y = (0.8781 ± 0.0173) x − (0.0129 ± 0.0001) for interday (n = 5, r = 0.999). The data indicate good linearity of the proposed method. The detection limits for amikacin, tobramycin, and kanamycin A (signal-to-noise ratio = 3; 0.5 psi, 5 s) were 0.08 mg/mL, 0.2 mg/mL, and 0.2 mg/mL, respectively. The reproducibility and reliability of the proposed method were assessed with three different concentrations of amikacin, tobramycin, and kanamycin A and evaluated as relative standard deviation (RSD) and relative error (RE). The precision of the method for amikacin, tobramycin, and kanamycin A for both intraday and interday analyses at three concentrations are all less than 5.3% for RSD and 6.6% for RE.

The application of the proposed method to the assay of amikacin or kanamycin A or tobramycin in commercial products was studied. For the assay of amikacin in commercial injections, the sample solutions were prepared as follows: an accurate portion of amikacin (Amikacin®, labeled amount 0.5 g/2 mL/vial), equivalent to about 20 mg amikacin, was transferred to a 100 mL volumetric flask containing 50 mL 50 μg/mL thiamine (IS). For the assay of kanamycin in commercial capsules (Kanamycin®, labeled amount 250 mg/capsule), an accurate portion of the powder equivalent to 16 mg kanamycin A was transferred to a 20 mL volumetric flask containing 10 mL 50 μg/mL thiamine (IS). For the assay of kanamycin in injections, an accurate portion of the kanamycin (Kanamycin®, labeled amount 250 mg/mL), equivalent to about 16 mg kanamycin, was transferred to a 20 mL volumetric flask containing 10 mL 50 μg/mL thiamine (IS). For the assay of tobramycin in injections or in ophthalmic solution, an accurate portion of tobramycin (Tobcin® injection, labeled amount 40 mg/mL) or (Tobrex® sterile ophthalmic solution, labeled amount 3 mg/mL), equivalent to 1.6 mg tobramycin, was transferred to a 2 mL volumetric flask containing 1 mL 50 μg/mL thiamine (IS). All of the above solutions in volumetric flasks were diluted to volume with deionized water. The solutions were pipetted into 0.2 mL minivials that could be placed into an autosampler for CE analyses.

Table 1. Analytical results for content uniformity of aminoglycosidic antibiotics in preparations obtained from a commercial source

	Concentration found[b)]	Percentage of claimed content (%)
Amikacin injection[a)] (g/mL)		
1	0.290 ± 0.005	116
2	0.281 ± 0.003	112
3	0.281 ± 0.006	112
4	0.277 ± 0.008	111
5	0.273 ± 0.006	109
Kanamycin capsule[a)] (g/capsule)		
1	0.275 ± 0.003	110
2	0.255 ± 0.003	102
3	0.263 ± 0.003	105
4	0.260 ± 0.005	104
5	0.260 ± 0.001	104
Kanamycin injection[a)] (g/mL)		
1	0.256 ± 0.008	103
2	0.255 ± 0.008	102
3	0.262 ± 0.008	105
4	0.252 ± 0.008	101
5	0.258 ± 0.008	103
Tobramycin ophthalmic solution[a)] (mg/mL)		
1	3.60 ± 0.031	119
2	3.54 ± 0.106	118
3	3.50 ± 0.053	117
4	3.51 ± 0.063	117
5	3.53 ± 0.061	118
Tobramycin injection[a)] (g/mL)		
1	0.045 ± 0.001	112
2	0.046 ± 0.001	114
3	0.044 ± 0.001	111
4	0.045 ± 0.001	113
5	0.045 ± 0.001	112

a) Labeled amount of amikacin in injection, kanamycin A in capsule, kanamycin A in injection, tobramycin in ophthalmic solution, and tobramycin in injection are 0.25 g/mL, 0.25 g/capsule, 0.25 g/mL, 3 mg/mL, and 0.04 g/mL, respectively.

b) Mean ± SD of three replicate analyses

The uniformity test (a test to evaluate the content variation of the drug in formulations) is usually required by an official pharmacopoeia for quality control of the drug in formulation. The results of the percentage of claimed content (%) are 109–116% for amikacin in injections, 102–110% for kanamycin A in capsules, 101–105% for kanamycin A in injections, 117–119% for tobramycin in ophthalmic solution, and 112–114% for tobramycin A in injections (Table 1). All the analytical values fell within the labeled amount required by the USP27 [7]. The typical electropherograms for analysis of amikacin in injection, kanamycin A in capsules, and tobramycin in injection are shown in Figs. 5C, D, and E, respectively. Many formulations contain excipient components, which may strongly retain and unduly affect the chromatographic performance of HPLC columns. Therefore, it is often necessary to pretreat sample solutions prior to HPLC analysis, such as solid-phase extraction or liquid-liquid extraction. However, in the CE analysis of a formulation containing drugs, after separation, the majority of excipients will be removed during a rinsing step.

In conclusion, we demonstrated a simple, speedy, and specific MEKC method for simultaneous determination of amikacin, tobramycin, and kanamycin A. The CE method is based on the anionic surfactant SPS as a micelle to differentiate the tested drugs and detection at UV 200 nm. The MEKC method has been successfully applied to the assay of amikacin, kanamycin A, and tobramycin in commercial products. We offer a completely different selectivity and simpler method for separation of structurally highly similar analytes as a complementary and alternative technique to HPLC in pharmaceutical assays.

The authors are grateful to the National Science Council, ROC, for financial support of the work (NSC 91-2745-p-037-001) and (NSC 93-2113-M-037-019).

Received July 21, 2004

References

[1] Katzung, B. G. (Ed.), *Basic & Clinical Pharmacology*, McGraw-Hill Companies, New York 2004, p. 765.

[2] Šoltés, L., *Biomed. Chromatogr.* 1999, *13*, 3–10.

[3] Adams, E., Vaerenbergh, G. V., Roets, E., Hoogmartens, J., *J. Chromatogr. A* 1998, *819*, 93–97.

[4] Lung, K. R., Kassal, K. R., Green, J. S., Hovsepian, P. K., *J. Pharm. Biomed. Anal.* 1998, *16*, 905–910.

[5] Morovján, G., Csokán, P. P., Németh-Konda, L., *Chromatographia* 1998, *48*, 32–36.

[6] Feng, C. H., Lin, S. J., Wu, H. L., Chen, S. H., *J. Chromatogr. B* 2002, *780*, 349–354.

[7] *U. S. Pharmacopoeia 27*, NF 22 Revision, United States Pharmacopoeial Convention, Inc., Rockville, MD 2004, pp. 112, 1052, 1856.

[8] Ackermans, M. T., Everaerts, F. M., Beckers, J. L., *J. Chromatogr.* 1992, *606*, 229–235.

[9] Kaale, E., Van Schepdael, A., Roets, E., Hoogmartens, J., *Electrophoresis* 2002, *23*, 1695–1701.

[10] Wienen, F., Holzgrabe, U., *Electrophoresis* 2003, *24*, 2948–2957.

[11] Oguri, S., Miki, Y., *J. Chromatogr. B* 1996, *686*, 205–210.

[12] Kaale, E., Van Schepdael, A., Roets, E., Hoogmartens, J., *J. Chromatogr. A* 2001, *924*, 451–458.

[13] Long, Y. H., Hernandez, M., Kaale, E., Van Schepdael, A., Roets, E., Borrull., F., Calull, M., Hoogmartens, J., *J. Chromatogr. B* 2003, *784*, 255–264.

[14] Flurer, C. L., *J. Pharm. Biomed. Anal.* 1995, *13*, 809–816.

[15] Altria, K. D. (Ed), *Capillary Electrophoresis Guidebook: Principle, Operation, and Application*, Humana Press, Totowa, NJ 1996, p. 7.