Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 810 (2004) 313–318
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Bioanalysis of tobramycin for therapeutic drug monitoring by solid-phase extraction and capillary zone electrophoresis

Humphrey Fonge[a], Eliangiringa Kaale[a,1], Cindy Govaerts[a], Koenraad Desmet[b], Ann Van Schepdael[a,*], Jos Hoogmartens[a]

[a] *Laboratory of Pharmaceutical Chemistry and Drug Analysis, K.U. Leuven, E. Van Evenstraat 4, B-3000 Leuven, Belgium*
[b] *Department of Clinical Pathology, UZ-K.U. Leuven Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium*

Received 27 May 2004; accepted 16 August 2004

Abstract

A method based on solid-phase extraction (SPE) and capillary zone electrophoresis (CZE) for the analysis of tobramycin in human serum is presented. An off-line SPE employing a carboxypropyl bonded phase (CBA) cartridge was used for the extraction of tobramycin from human serum. Adsorbed tobramycin was eluted from the CBA cartridge using a mixture of NH_3 (25%, w/v)–methanol (30:70, v/v). After evaporation, the analyte was reconstituted and derivatized with *o*-phthaldialdehyde (OPA)/3-mercaptopropionic acid (MPA). The resulting tobramycin–OPA/MPA derivative was purified, and then identified by mass spectrometry. The tobramycin–OPA/MPA derivative was then analysed by CZE with a background electrolyte (BGE) comprising of 30 mM sodium tetraborate pH 10.0-acetonitrile (ACN) (80:20, v/v) with ultraviolet detection at 230 nm. A linear response was observed in the range of 0.3–30 μg/ml with r^2 = 0.992. The sensitivity of the method was determined by its limit of quantitation (LOQ) and limit of detection (LOD) of 0.3 μg/ml and 0.1 μg/ml, respectively. SPE recovery ranged from 68 to 79% at the trough levels to 98% at the peak levels found in serum. Furosemide has been added as internal standard (IS) to improve precision. For the therapeutic range of tobramycin in serum (2–10 μg/ml) the relative standard deviation (R.S.D.) was less than 11% for the entire SPE/CE process. The method demonstrated excellent selectivity as shown by the lack of interference from a total of 20 drugs investigated. The method was then used in therapeutic drug monitoring of patients receiving the drug.

Keywords: Tobramycin; Bioanalysis; Solid-phase extraction

1. Introduction

Tobramycin is a broad spectrum aminoglycoside antibiotic produced by *Streptomyces tenebrarius* [1]. It is indicated for the treatment of aerobic Gram-positive and some aerobic Gram-negative bacteria for which less toxic antibiotic are ineffective or contra-indicated. Like all other aminoglycosides, its narrow therapeutic index is implicated by its oto- and nephrotoxicity especially when therapy is prolonged [2]. Careful monitoring of this substance is therefore required in order to detect elevated and appropriate levels in serum for therapy so as to improve efficacy.

Quantitative methods currently used for the analysis of tobramycin in biological matrices [3,4] include radioimmunoassay (RIA) [5], high-performance liquid-chromatography (HPLC) [6–10], fluorescence polarization immunoassay (FPIA) [11], microbiological assays [5,12], radiochemical assay [13] and enzyme immunoassay (EIA) [14,15]. These methods each have merits and demerits. The use of microbiological assay (as described by United States Pharmacopeia Drug Information (USPDI) [16]) for therapeutic drug monitoring and pharmacokinetic studies of

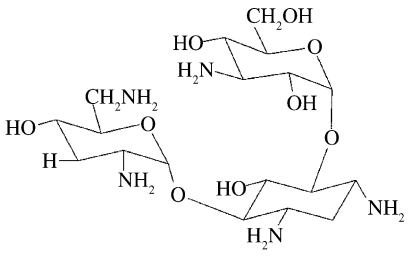
* Corresponding author. Tel.: +32 16 323443; fax: +32 16 323448.
E-mail address: ann.vanschepdael@pharm.kuleuven.ac.be (A. Van Schepdael).
[1] Current address: Department of Medicinal Chemistry, School of Pharmacy, MUCHS, P.O. Box 65013, Dar Es Salaam, Tanzania.

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.08.020

aminoglycosides has massively declined because of its non-specificity, narrow linear range and at times poor sensitivity. The LC method is preferred but relatively expensive and time consuming due to long analysis time and large volumes of organic solvents used. CE was recently reported for the analysis of tobramycin in bulk pharmaceuticals [17] but this method cannot be directly applied in bioanalysis, as there is the need for an effective and quantitative sample cleanup prior to analysis and for a very sensitive assay able to quantitate trough (1–2 μg/ml) and peak (8–10 μg/ml) levels in serum. The major hurdle confronted in analysis of aminoglycosides is their lack of UV chromophore and fluorophore due to their lack of chemical unsaturation (Fig. 1). To analyze these compounds by CE and LC, it is therefore necessary to improve UV and/or fluorimetry detection sensitivity by chemical derivatization. Many reagents have been used in CE and LC for the derivatization of amine compounds [18]. These include OPA/MPA [17,19], dimethylaminoazobenzene sulphonyl chloride [20], 1-fluoro-2,4-dinitrobenzene [21], 9-fluoro-2,4-dinitrophenyl-5-L-alanine amide [22], dansyl chloride [23], OPA/*N*-*tert*-butyloxycarbonyl-L-cysteine [24], OPA/2-mercaptoacetic acid (MAA) [25]. Pre- and post-column derivatizations have been reported. The complexity and cost of installation of a post-column derivatization system discourages its routine use. The most popular remains the pre-column derivatization approach, but the stability of the derivative must be considered when employing this approach.

Biological samples usually consist of high concentrations of organic and inorganic salts, and proteins, which greatly affect CE separation. Thus to obtain sensitive assays for pharmacokinetic and therapeutic drug monitoring studies, it is necessary to eliminate these interfering substances. With the introduction of improved formats and sorbent phases, SPE has become the most popular sample preparation method in bioanalysis. Some aminoglycosides have been quantitatively extracted from biological matrices by the use of a weak cation exchanger CBA [25,26] and C-18 SPE cartridges [27].

In this paper, we report a sensitive and selective assay method for qualitative and quantitative determination of tobramycin in human serum for therapeutic drug monitoring and pharmacokinetic studies by pre-capillary derivatization with OPA/MPA and CZE. An effective SPE employing a CBA cartridge for sample pre-treatment and cleanup of tobramycin from human serum yielded good analyte recovery.



Chemical structure of tobramycin

Fig. 1. Reaction scheme for the derivatization of primary amines with OPA/MPA.

2. Materials and methods

2.1. Reagents

Sodium tetraborate, MPA, acetonitrile (ACN), OPA, ascorbic acid, sodium carbonate and disodium hydrogen phosphate were from Acros Organics (Geel, Belgium). Methanol HPLC-grade was from Fischer chemicals (Leicester, UK). Sodium dihydrogen phosphate was from Merck (Darmstadt, Germany). Potassium hydroxide, sodium hydroxide, ammonium hydroxide and sodium hydrogen carbonate were from Riedel-de Haën (Seelze, Germany). Furosemide and boric acid were from Merck Eurolab (Leuven, Belgium). Tobramycin sample was from Biogal (Debrecen, Hungary) and was >99.8% pure. Benzylpenicillin, cephradine and kanamycin were from the European Pharmacopoeia (Strasbourg, France). Cefadroxil and acetylsalicylic acid were from Certa (Braine-l'Alleud, Belgium). Pen V and cefoxitin were from Merck Sharp and Dohme international (NJ, USA). Paracetamol was from Tramedico (Sint Niklaas, Belgium). Cefazolin was from Bristol (Brussels, Belgium). Cloxacillin, amoxicillin and flucloxacillin were from Beecham (Brussels, Belgium). Ampicillin was from Gist-Brocades (Delft, Holland). Streptomycin was from VMD Chemie (Arendonk, Belgium). Cephalexin and gentamicin were from the United States Pharmacopeia (USP) (Rockville, MD, USA). Cefotaxime sodium was from Hoechst (Frankfurt am Main, Germany). Amikacin was from Bristol Sermoneta (Latina, Italy). Human sera were obtained from Sigma (Bornem, Belgium). Ultrapure Milli-Q water (Millipore, Milford, MA, USA) was used and solutions were filtered with a 0.2 μm filter (Euroscientific, Lint, Belgium) where necessary.

2.2. Instrumentation

The experiments were carried out on a Beckman P/ACE MDQ equipped with photo diode array detection (Beckman, Fullerton, CA, USA). Data were acquired by means of 32 Karat™ version 5.0 Software (Beckman). pH measurements were performed on a Metrohm 691 pH meter (Herisau, Switzerland). Uncoated fused silica capillary was obtained from Polymicro Technologies (Phoenix, AZ, USA). The CBA and aminopropyl bonded phase SPE cartridges were from International Sorbent Technology (IST) (Mid Glamorgan, UK). The SPE was performed in a 10-well VacMaster sample processing station from IST (Mid Glamorgan, UK). The MS data were acquired with an LCQ ion trap mass

spectrometer equipped with an electrospray interface operated in positive ion mode (Thermo Finnigan, San Jose, CA, USA). Ionization source and MS parameters were tuned automatically. A source voltage of 4.5 kV was applied to the electrospray ionization (ESI) needle. The temperature of the heated capillary was set at 270 °C. Nitrogen was supplied by a Nitroprime™ membrane unit type SNIFF (Hoek Loos, Niel, Belgium) and was used as sheath and auxiliary gas. The flow rate of the sheath and the auxiliary gas was set at 80 and 20 (arbitrary units), respectively. Helium was used as the damping and collision gas at a pressure of 0.133 Pa. An X-calibur software package was used for instrument control, data acquisition and processing.

2.3. Preparation of derivatization reagent and standards

The reagent was prepared according to earlier studies by Molnàr-Perl et al. [28,29] and Kaale et al. [17]. OPA (0.44 g) was dissolved in 2.0 ml of methanol and 15 ml of a 30 mM boric acid solution, adjusted to pH 10.4 with 8 M potassium hydroxide was added, followed by 1.0 ml of MPA. The pH of the resulting solution was adjusted to 10.4 with 8 M potassium hydroxide. The solution was then diluted to 20.0 ml with 30 mM boric acid, previously adjusted to pH 10.4 with 8 M potassium hydroxide. The reagent was used after a post-preparation period of at least 90 min. Once prepared the reagent was stored at 4 °C, in the absence of light and used within a period of nine days. The reagent was stable over this period [28,29]. 0.1–30 μg/ml stock solutions of tobramycin were prepared by dissolving tobramycin in methanol–water (50:50, v/v).

2.4. Solid-phase extraction from human serum

A CBA SPE cartridge was used for cleanup of the serum sample. The CBA cartridge was conditioned with 1.0 ml of methanol followed by 2.0 ml of 20 mM phosphate buffer, previously adjusted to pH 7.4, at a flow rate of 1–2 ml/min. 1.0 ml of serum was spiked at different concentrations (0.1–30 μg/ml) with tobramycin stock solutions and diluted to 4.0 ml with water and was applied to the cartridge at a flow rate of 1 ml/min. Interference elution was with 2.0 ml of 20 mM phosphate buffer pH 7.4, then 4.0 ml of 0.2 M borate buffer previously adjusted to pH 9.0 with 8 M KOH followed by 2.0 ml of water. The cartridge was dried by sucking air for 5 min. The analyte was eluted with 2.0 ml of NH_3 (25%, w/v)–methanol (30:70, v/v). The solvent was evaporated in a RotaVapor (Büchi, Flavil, Switzerland) and dried in an oven for 30 min at 105 °C to remove the remaining ammonia.

2.5. Sample derivatization

The reaction scheme for the derivatization is shown in Fig. 1. To 0.1 ml of the different stock solutions of tobramycin was added 1 ml of methanol–water (50:50, v/v) containing 0.01 mg/ml of furosemide (internal standard, IS) and 0.17 ml of the derivatization reagent. To the human sera extracts was added 0.17 ml of the derivatization reagent, 0.1 ml of methanol–water (50:50, v/v) and 1 ml of methanol–water (50:50, v/v) containing 0.01 mg/ml furosemide. The tube was agitated to allow thorough mixing and placed in a water bath at 60 °C for 10 min. The optimum conditions for the derivatization, reagent concentration, time of reaction and temperature had been investigated earlier [17].

2.6. Mass spectrometry

Prior to mass spectrometry it was necessary to clean the derivatized sample from borate and phosphate salts, which would otherwise block the capillaries and damage the mass spectrometer. We used an aminopropyl bonded phase (a weak anion exchanger) SPE cartridge for cleanup of the derivatized sample. The aminopropyl cartridge was conditioned with 1.0 ml of methanol followed by 2.0 ml phosphate buffer (20 mM, pH 7.8). One ml of derivatized sample was diluted four times with Milli-Q water, the pH adjusted to 7.8 with 0.1 M HCl, and applied to the SPE cartridge. The cartridge was then washed with 4.0 ml phosphate buffer (20 mM, pH 7.8) followed by 4.0 ml Milli-Q water. In all these steps the flow rate was maintained at 1 ml/min. The cartridge was sucked to dryness for 5 min. The tobramycin–OPA/MPA derivative was then eluted with 2.0 ml of NH_3 (25%, w/v)–methanol (30:70, v/v) at a flow rate of 1 ml/min, where after the cartridge was sucked dry. The eluate was directly infused (by syringe pump) into the MS.

2.7. CE analysis

Uncoated fused silica capillary with internal diameter (ID) of 50 μm, total length of 40 cm and effective length to detector of 30 cm, was used. A new capillary was conditioned by flushing with 1 M NaOH, followed by 0.1 M NaOH, then water, each for 5 min at 60 °C at a pressure of 20 psi. Each day prior to analysis, the capillary was washed with 0.1 M NaOH for 5 min, followed by water for 2 min and then with background electrolyte (BGE) for 5 min at 30 °C at a pressure of 20 psi. To ensure repeatability of migration times, the capillary was rinsed between injections with 0.1 M NaOH, then water and BGE, each for 1 min. The derivatized sample was injected hydrodynamically at a pressure of 0.8 psi for 15 seconds and a voltage of 23 kV was applied. Capillary temperature was maintained at 30 °C. The BGE was made up of borate buffer–acetonitrile (80:20, v/v). The borate buffer consisted of 30 mM sodium tetraborate prepared by dissolving 1.145 g of sodium tetraborate in Milli-Q water and adjusting the pH to 10.0 with 1 M NaOH, followed by dilution to 100.0 ml with water.

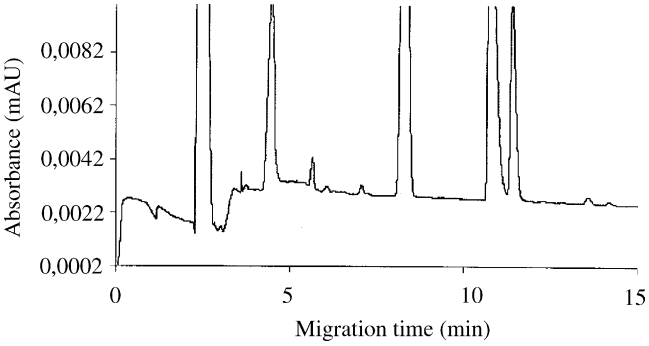


Fig. 2. Electropherogram of blank human serum. Electrophoretic parameters: BGE: 30 mM borate pH 10.0-acetonitrile (80:20, v/v), capillary length 40 cm, ID 50 μm, voltage 23 kV, hydrodynamic injection 15 s, 0.8 psi, UV detection at 230 nm, temperature 30 °C, current 69–73 μA.

3. Results

3.1. Stability of tobramycin/OPA derivative

Pre-capillary derivatization reactions have been complicated by the instability of the resulting thioisoindole derivative(s). It was therefore necessary to investigate the degradation kinetics of the tobramycin–OPA/MPA derivative in order to have a precise assay determination. This was performed by analyzing aliquots of the derivatized mixture, kept at ambient temperature over a period of 6 h. A plot of $\ln A_t/A_0$ against post-derivatization time confirms first order degradation kinetics with $\ln A_t = -0.0025\,t + 0.0941$ with coefficient of determination $r^2 = 0.962$, t = post-derivatization time, A_0 and A_t are the ratios of corrected peak area of tobramycin derivative/corrected peak area of internal standard (at time $t = 0$ and t after derivatization, respectively). A post-derivatization time of no longer than 2 h at room temperature allows a determination with acceptable precision (relative standard deviation, R.S.D. <11%).

3.2. SPE/CE recovery

Electropherograms of blank human serum and human serum spiked with tobramycin, cleaned by SPE, are shown in Figs. 2 and 3. Recoveries of tobramycin for the entire process were obtained by external standardization i.e. comparison of the mean ratio ($n = 3$) of corrected peak areas of tobramycin/IS of standard solutions without SPE, with the mean ratio ($n = 3$) of corrected peak area tobramycin/IS of spiked human serum (subjected to SPE). Recovery ranged from 68% at trough concentration to 98% at the peak levels normally found in serum and is presented in Table 1.

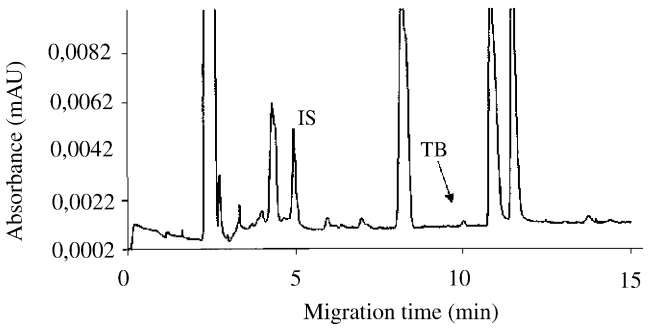


Fig. 3. Electropherogram of human serum spiked with 0.3 μg/ml (LOQ) of tobramycin (see Fig. 2 for electrophoretic parameters). TB: tobramycin, IS: internal standard (0.01 mg/ml furosemide).

4. Method validation

4.1. Sensitivity

The analytical sensitivity was assessed by determination of the limit of quantitation (LOQ) and the limit of detection (LOD). As defined by European Pharmacopoeia [30], the LOQ and LOD are the concentrations at which the signal to noise ratio is 10 or 3, respectively. The LOQ was 0.3 μg/ml ($n = 3$, R.S.D. 12.5%) and the LOD was 0.1 μg/ml.

4.2. Calibration curve and repeatability

One ml of human serum was spiked with tobramycin stock solutions and diluted to 4 ml with water. A 5-point calibration was investigated in the range of 0.3–30 μg/ml, with the lowest level of the analytical calibration curve corresponding to the limit of quantitation. The calibration curve was linear with $y = 0.0461x + 0.0162$ and $r^2 = 0.992$ where y is the ratio of tobramycin corrected peak area/corrected peak area of IS ($n = 3$) and x is the concentration of tobramycin spiked (μg/ml), with $S_{y,x} = 0.00243$, where $S_{y,x}$ is the standard error of the y estimate. This calibration curve was used for the analysis of clinical samples. The differing recoveries with respect to concentration are thus taken into account. The analytical precision was assessed at both intra-day and inter-day at 2 and 10 μg/ml and is presented in Table 1. The highest R.S.D. was 11% ($n = 6$).

4.3. Selectivity

To investigate the selectivity of the method, stock solutions of several drugs that are sometimes co-administered with tobramycin were prepared in methanol–water (50: 50, v/v). Solutions containing 10 μg/ml of interference and 10 μg/ml of tobramycin were derivatized and analysed by CE. None of the following 20 drugs were found to interfere with analysis: acetylsalicylic acid, amikacin, amoxicillin, ampicillin, ascorbic acid, benzylpenicillin, cefadroxil, cefazolin, cefotaxime, cefoxitin, cephalexin, cephradine, cloxacillin, diazepam, flucloxacillin, gentamicin, kanamycin, paracetamol, pen V and streptomycin.

4.4. Confirmation by mass spectrometry

A mass spectrum of the standard tobramycin derivative obtained by ESI in the positive ion mode yielded a [M +

Table 1
Recovery and precision of SPE/CE analysis of tobramycin from human serum

Concentration spiked (μg/ml)	Concentration found (μg/ml)	Recovery (%) (n = 3)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
0.3	0.2	68.5	12.5 (n = 3)	
2	1.6	78.5	11.0 (n = 6)	9.9 (n = 6)
8	7.0	87.0	4.3 (n = 3)	
10	9.8	98.3	10.6 (n = 6)	7.2 (n = 6)

R.S.D.: relative standard deviation.

$H]^+$ ion at m/z = 1488 (Fig. 4). The molecular mass of fully derivatized tobramycin is 1487, where all five primary amine functions reacted with OPA/MPA reagent. Collision-induced dissociation in the ion trap with 60% collision energy of the $[M + H]^+$ ion yielded two product ions in the following pattern: m/z = 1488 $[M + H]^+ \rightarrow m/z$ 1382 and 1276 by loss of 106 and 212 mass units, which corresponds to loss of one and two mercaptopropionic acid (MPA) groups, respectively (spectrum not shown).

4.5. Analysis of clinical samples

The method was used in therapeutic drug monitoring of 10 patients being treated with tobramycin.

5. Discussion

The study presents a linear, selective, sensitive and repeatable SPE/CE method for the determination of tobramycin in human serum. The major advantages of the method over other existing methods include low cost and good selectivity.

In the present study, serum samples of 10 patients were assayed by the novel SPE/CE method. A typical electropherogram is shown in Fig. 5. The electropherogram is similar to that obtained from spiked human serum (Fig. 3). With the exception of one patient, all peak serum samples were within the therapeutic range (8–10 μg/ml). The trough concentrations were below the recommended maximum value (2 μg/ml). According to the USPDI, it can be concluded that these patients have a safe trough concentration of tobramycin. The specificity of the SPE/CE method was investigated using a total of 20 drugs. The method is highly specific as none of the drugs investigated migrated with tobramycin or the IS (furosemide) peaks.

The low LOD (0.1 μg/ml) and LOQ (0.3 μg/ml) mean that the SPE/CE method can be used for TDM and pharmacokinetic studies of tobramycin.

To confirm the identity of what is determined and quantitated in human serum, MS was used to study the

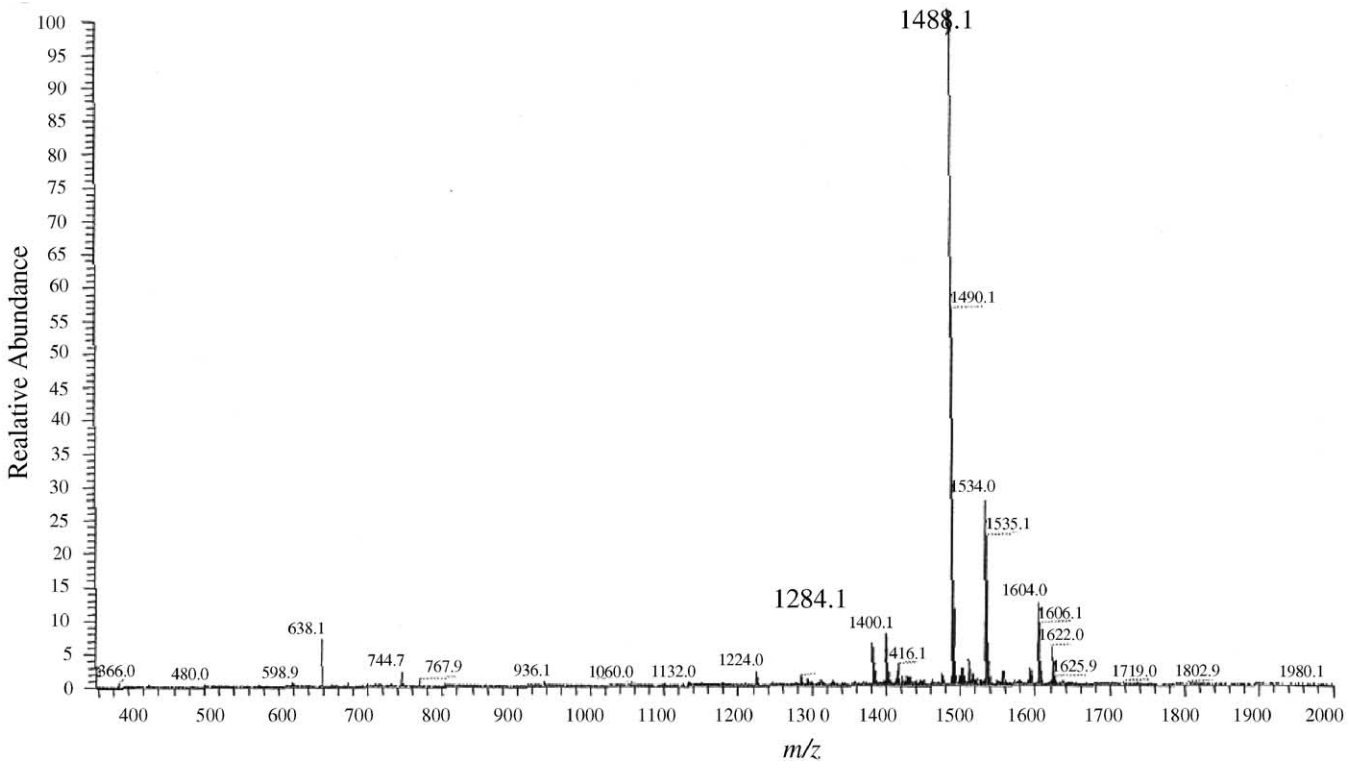


Fig. 4. A mass spectrum of OPA/MPA derivatized tobramycin as acquired by ESI in the positive ion mode. The $[M + H]^+$ ion is identified as m/z 1488.

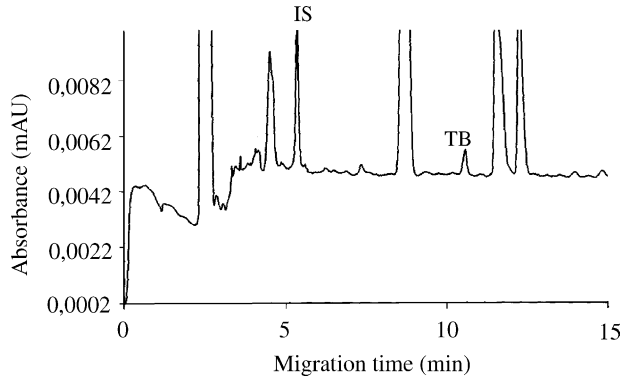


Fig. 5. An electropherogram of a patient serum containing 2.9 μg/ml tobramycin (see Fig. 2 for electrophoretic parameters). IS: Internal standard, TB: tobramycin.

tobramycin–OPA/MPA derivative. Following SPE of the serum (using a CBA cartridge), the sample was derivatized and SPE of the tobramycin–OPA/MPA derivative (using an aminopropyl cartridge) was performed prior to MS. The resulting spectrum (not shown) showed the most abundant peak as *m*/*z* 1488 which corresponds to the $[M + H]^+$ ion of fully derivatized tobramycin. This confirms that for the main peak all five amine functional groups have reacted with the OPA/MPA reagent. $[M + H]^+$ ion peaks of tobramycin, mono-, di-, and tri-substituted thioisoindole derivatives are absent. The approach is a novel approach and is aimed at providing an insight in OPA/MPA derivatization reactions of aminoglycosides.

6. Conclusion

A sensitive, selective and repeatable SPE/CE method for the determination of tobramycin in human serum was developed. An SPE procedure employing a CBA cartridge with elution solvent NH_3 (25%, w/v)–methanol (70:30, v/v), followed by pre-capillary derivatization and then CZE was successfully developed. Recoveries were good, ranging from 68% at trough levels to 98% at peak concentrations. MS scans revealed that for the main peak all five primary amine functions of tobramycin were derivatized at the reaction conditions. The validation parameters cover the range of tobramycin found in serum at both peak and trough levels. The method offers the advantages of high specificity, good linear range, and good repeatability. In addition, sample preparation was simple. The high sensitivity means that the method can be applied for TDM as well as pharmacokinetic studies of tobramycin.

Acknowledgement

E. Kaale acknowledges the financial support from K.U. Leuven.

References

[1] C.E. Higgins, R.E. Kastner, Antimicrob. Agents Chemother. 7 (1967) 324.
[2] H.F. Chambers, in: J.G. Hardman, L.E. Limbird, A.G. Gilman (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed., Pergamon Press, McGraw Hill, New York, 2000, p. 1099.
[3] D.A. Stead, J. Chromatogr. B 747 (2000) 69.
[4] S.K. Maitra, T.T. Yoshikawa, L.B. Guze, M.C. Schotz, Clin. Chem. 25 (1979) 1361.
[5] T. Koeda, M.Y. Umemura, in: H. Umezawa, I.R. Hooper (Eds.), Aminoglycoside Antibiotics, Springer-Verlag, Berlin, 1982, p. 293.
[6] P.M. Kabra, P.K. Bhatnagar, M.A. Nelson, J.H. Wall, L.J. Marton, Clin. Chem. 29 (1983) 672.
[7] C.-H. Feng, S.-J. Lin, H.-L. Wu, S.-W. Chen, J. Chromatogr. B 780 (2002) 349.
[8] L. Essers, J. Chromatogr. 305 (1984) 345.
[9] F. Lai, T. Sheehan, J. Chromatogr. 609 (1992) 173.
[10] H. Russ, D. McCleary, R. Katimy, J.L. Montana, R.B. Miller, R. Krishnamoorthy, C.W. Davis, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2165.
[11] S.K. Banerjee, A. Wells, A. Dasgupta, Ther. Drug Monit. 21 (1999) 540.
[12] M.S. Brady, S.E. Katz, J. Assoc. Off. Anal. Chem. 70 (1987) 641.
[13] S.E. Charm, R. Chi, J. Assoc. Off. Anal. Chem. 71 (1988) 304.
[14] E. Martlbauer, E. Usleber, E. Schneider, R. Dietrich, Analyst 119 (1994) 2543.
[15] A.I. Darwish, J. Pharm. Biomed. Anal. 30 (2003) 1539.
[16] United States Pharmacopeia Drug Information, USPC, 12601 Twinbrook Parkway, Rockville, MD, 2003, 20852.
[17] E. Kaale, A. Van Schepdael, E. Roets, J. Hoogmartens, Electrophoresis 23 (2002) 1695.
[18] I.S. Krull, Z. Deyl, H. Lingeman, J. Chromatogr. B 659 (1994) 1.
[19] K. Li, J. Chromatogr. 579 (1992) 209.
[20] A. Watanabe, J. Semba, A. Kurunaji, S. Kumashiro, M. Toru, J. Chromatogr. 583 (1992) 241.
[21] E.A. Papp, C.A. Knupp, J.H. Barbhaiya, J. Chromatogr. 574 (1992) 93.
[22] Y. Nagata, T. Yamamoto, T. Shimojo, J. Chromatogr. 575 (1992) 147.
[23] R.H. Pullen, A.A. Fatmi, J. Chromatogr. 574 (1992) 101.
[24] A. Hashimoto, T. Nishikawa, T. Oka, K. Takahashi, T. Hayashi, J. Chromatogr. 582 (1992) 41.
[25] Y.H. Long, M. Hernandez, E. Kaale, A. Van Schepdael, E. Roets, F. Borrull, M. Calull, J. Hoogmartens, J. Chromatogr. B 784 (2003) 255.
[26] R.E. Hornish, J.R. Wiest, J. Chromatogr. A 812 (1998) 123.
[27] A.I. Al-Amoud, B.J. Clark, H. Chrystyn, J. Chromatogr. B 769 (2002) 89.
[28] I. Molnàr-Perl, B. Imre, J. Chromatogr. A 798 (1998) 37.
[29] I. Molnàr-Perl, V. Anikó, J. Chromatogr. A 835 (1999) 73.
[30] European Pharmacopoeia, fourth ed., European Department for the Quality of Medicines, Strasbourg, France, 2002.