Determination of tobramycin in eye drops with an open-source hardware ion mobility spectrometer

Nattapong Chantipmanee1 · Peter C. Hauser1

Received: 23 December 2021 / Revised: 25 March 2022 / Accepted: 28 March 2022 / Published online: 5 April 2022
© The Author(s) 2022

Abstract
The analysis of tobramycin was demonstrated successfully as an example for electrospray ionization on an open-source hardware ion mobility spectrometer. This instrument was assembled inexpensively in-house, and required only very few purpose-made components. The quantitative determination of tobramycin required 20 s for a reading. The calibration curve for the range from 50 to 200 μM was found to be linear with a correlation coefficient of $r = 0.9994$. A good reproducibility was obtained (3% relative standard deviation) and the limit of detection was determined as 8 μM. As the concentration of the active ingredient in the eye drops (ophthalmic solutions) is too high for the sensitivity of the instrument, the samples had to be diluted appropriately.

Keywords Ion mobility spectrometry · Electrospray ionization · Open-source hardware (OSH) · Tobramycin · Eye drops

Introduction
Ion mobility spectrometry (IMS) is an alternative to mass spectrometry, or the separation methods of chromatography or electrophoresis, where ionic species are separated in the gas phase by application of an electric field. In contrast to chromatographic or electrophoretic techniques, the gas-phase separation is much faster. The mobilities of the ions in the inert drift gas are dependent on the collision cross sections of the species, which are largely determined by their size.

A drift tube IMS instrument essentially consists of an ionization source, an ion shutter, a drift tube for separation, and a Faraday detector. The separation is based on the different mobilities of the analyte ions in the electric field in a drift gas (usually nitrogen) due to their different collision cross sections. An IMS instrument is substantially simpler in construction than a mass-spectrometer as it can be operated at ambient pressure and does not require the application of modulated electric fields. It also does not need the high-pressure pumps and manifolds of a high-performance liquid chromatography (HPLC) system. In similarity to CE [1], it is therefore possible to build IMS instruments with limited resources and inexpensively, making the analytical technique available to parties who cannot afford state-of-the-art commercial mass-spectrometers or HPLC instruments. Recently, detailed designs of an instrument [2] and the electronic pulser circuitry [3], required for ion injection, have been published by Clowers and coworkers in the spirit of open-source hardware (OSH), further facilitating in-house construction. We have built such an instrument in our laboratory with some modifications concerning mainly the electronic data acquisition. The electrodes and electrode spacers required for the drift tube could be ordered from a supply house for printed circuit boards, and the shutter grids for the ion injector from a company specializing in micromachining. The electronics for sequencing and data acquisition were based on commercially available units, so that only a few mechanical and electronic parts were needed to be made in-house. The total cost was approximately 5000 Swiss Francs.

Commercially available IMS instruments usually employ a radioactive beta-emitter for analyte ionization of gaseous analytes [4]. However, alternative ionization methods are possible in the form of corona discharges [5, 6], UV-ionization [7, 8], or low-temperature plasmas [9–11]. Electrospray ionization, as a further option, has the advantage of being suitable for liquid samples containing non-volatile species [2, 12]. The study reported herein is a contribution to the exploration of the scope of potential applications of
an in-house constructed IMS instrument with electrospray ionization.

Tobramycin is one of the aminoglycoside antibiotics, which is derived from Streptomyces tenebrarius [13]. It is widely used to treat Gram-negative infections, particularly against species of Pseudomonas aeruginosa [13]. Tobramycin was discovered by Eli Lilly in 1967 [14] and is effective by interrupting ribosomal cell functions [15, 16]. For medical use, tobramycin is typically used in eye drops (ophthalmic solutions) at 0.3% (w/v) for treatment of eye infections [17–19]. Like other aminoglycosides, therapy with tobramycin for a long-duration time has potential dose–related side effects of ototoxicity and nephrotoxicity and therapeutic drug monitoring may be applied [13, 15].

Several analytical approaches have been reported for the determination of tobramycin. Immunoassays are one option, but require expensive reagents [20, 21]. For the common separation methods of HPLC, and the alternative capillary electrophoresis (CE), detection by the common UV-absorbance measurement is hampered as tobramycin has no strong chromophore. Chemical derivatization is therefore usually required to enable optical detection by absorbance or fluorescence [15, 22–26]; only when the required sensitivity is low, direct UV-absorbance detection at 210 nm may be employed [27]. Alternatively, evaporative light scattering detection [28, 29], contactless conductivity detection [30, 31], or mass spectrometric detection [32, 33] are employed with HPLC or CE for the determination of tobramycin.

IMS is suitable for analytes which are ionic, or can easily be ionized, and the method has indeed been employed for the determination of pharmaceuticals [34]. Tobramycin can readily be protonated and can therefore be separated and determined as cation. Herein we report on an investigation into the use of the low-cost open-source hardware IMS instrument for the direct analysis of tobramycin in eye drops without requiring derivatization. Electrospray ionization (ESI) was used for ion generation. The detection of other aminoglycosides by ESI-IMS has been reported previously [35], but to the best of our knowledge, this is the first report on the use of the method for the direct quantitative determination of tobramycin in eye drops.

Materials and methods

Chemicals

All chemicals were of analytical grade. Tobramycin sulfate (TBM), tetrabutylammonium bromide (T4), and acetic acid were purchased from Sigma-Aldrich (Buchs, Switzerland). Benzalkonium chloride (BAC) was also obtained from Sigma-Aldrich (product no. 12060) and consists of ~70% benzylidimethyltetradecylammonium chloride and ~30% benzylidimethyltetracylammonium chloride. Methanol (HiPerSolv CHROMANORM) was bought from VWR (VWR Chemicals, Schlieren, Switzerland), and water used throughout the experiment was purified with a Milli-Q system from Millipore (Bedford, MA, USA). All electrosprayed solutions were prepared in 50% (v/v) methanol in water with the addition of 0.01% acetic acid. The diluted samples were filtered through 13-mm syringe filters, Nylon 66, and 0.45 μm (SF1303-2, BGB Analytik, Böckten, Switzerland) before introduction into the ESI-IMS system.

**Ion mobility spectrometer**

The instrument is based on the design reported by Reineke and Clowers and on our earlier implementation [2, 12]. A schematic diagram of the instrument and its periphery is shown in Fig. 1, and the operating parameters are summarized in Table 1. The IMS tube is divided into two regions, viz. the desolvation and drift zones with 10.6 cm in length each. The drift tube was constructed from stacked ring electrodes (1.6 mm thickness) and spacers (2.0 mm thickness) made from printed circuit board material [2], and these were ordered from PCBway (www.pcbway.com), as well as the two alignment boards fitted with 1-MΩ surface mount resistors (±1% resistor, Stackpole Electronics, Raleigh, NC, USA) to create the drift field. The high-voltage generator (CZE1000R) was obtained from Spellman (Hauppauge, NY, USA). The set of electrodes and spacers were stacked together on four PEEK rods (24.0 cm in length each) through holes at the corners of the PCBs. The injection grids according to the Reineke and Clowers design [2] were obtained from Newcut (Newark, NY, USA). The pulse generator for driving the injection was described by Garcia et al. [3] and purchased from GAA Custom Electronics, Benton City, WA, USA (www.mstar2k.com/gaace-home). Ion detection was carried with a Faraday plate connected to a current-to-voltage converter based on a LMC6001 operational amplifier from Texas Instruments (Dallas, TX, USA) with an amplification of 4.7 × 10^9 V/A. The signal was recorded by a 16 bit-resolution PC oscilloscope (Picoscope 4262, Picotech, St. Neots, UK). An Analog Discovery 2 unit (Digilent, Pullman, WA, USA) was employed to sequence the injections and trigger the signal acquisition. Nitrogen (N2) gas (99.999% purity, PanGas, Pratteln, Switzerland) was employed as the drift gas, and its flow was regulated with a mass-flow controller with a range of 0–1000 mL min⁻¹ (Bronkhorst, Aesch, Switzerland).

A fused silica capillary of 5 cm length, 50 μm i.d., and 375 μm o.d. (BGB Analytik, Böckten, Switzerland) was employed to create the electrospray. The solutions were pumped by applying compressed nitrogen at a regulated pressure (using a DR-2–1 valve from Clippard, Cincinnati, OH, USA) to a sealed container (LS-BBRES-1ML, Springer
LabSmith, Livermore, CA, USA). Connection to the high-voltage generator (CZE1000R, Spellman, Hauppauge, NY, USA) was made via a T-piece (T116-203, LabSmith) with a 0.5-mm-diameter platinum wire (Advent, Oxford, UK). Measurements were commenced about 5 s after turning on the electrospray voltage as it was found that this time period was necessary for equilibration of the system before stable signals were obtained.

**Mass spectrometry**

The instrument employed was a LCQ Deca 3D ion trap mass-spectrometer (Finnigan MAT, San Jose, CA, USA). The full-scan positive ion mode with low range (200–600 m/z) was employed to obtain the mass spectrum using the Tune Plus software vs. 2.0 (Thermo Fisher Scientific, Waltham, MA, USA). A fused silica capillary of 100 μm inner and 365 μm outer diameter (TSP-100365-M-10, BGB), inserted inside a stainless steel tube under high voltage (4.5 kV) and a syringe pump (KDS 100 legacy, KD Scientific, Holliston, MA, USA), was used to create the electrospray. The flow rate was set at 0.2 mL/min.

**Calulations**

The reduced mobilities, \( K_0 \) (cm\(^2\) V\(^{-1}\) s\(^{-1}\)), which can be considered to be a normalization of the measured drift times against the instrumental parameters of drift tube length and

---

**Table 1  Operating parameters**

| Operating parameters | Value          |
|----------------------|----------------|
| Pressure (bar)       | 0.125          |
| Flow rate (μL/min)   | 2.0            |
| ID of capillary (μm) | 50             |
| OD of capillary (μm) | 375            |
| Length of capillary (mm) | 5.0         |
| Electro spray voltage (kV) | 11.0       |
| Injection gate       |                |
| Injection time (μs)  | 250            |
| Gate pulse voltage (V) | 50           |
| Distance between grids (μm) | 300         |
| Drift tube           |                |
| Desolvation length (cm) | 10.6         |
| Separation drift length (cm) | 10.6       |
| Field strength (V/cm) | 425.0         |
| Electrode thickness (mm) | 1.6           |
| Spacer gap (mm)      | 2.0            |
| \( N_2 \) counter gas flow rate (mL/min) | 500     |
| Drift tube temperature (°C) | 23±2       |
| Drift tube pressure (Torr) | 765±5      |
| Ion detection        |                |
| Total gain (V/A)     | \( 4.7 \times 10^9 \) |
| Number of averages (times) | 200       |
| Analysis time (s)    | 20             |
field strength as well as the temperature and pressure, were calculated according to the following equation [4]:

\[ K_0 = \left( \frac{L}{t_d} \right) \cdot \left( \frac{E}{p/p_0} \right) \cdot \left( \frac{T/T_0}{T_0} \right) \]  

(1)

where \( L \) is the length (cm) of the drift tube; \( E \) the field strength (V/cm); \( t_d \) the drift time (s); \( p \) and \( p_0 \) are the drift tube pressure and standard pressure (760 torr), respectively; and \( T \) and \( T_0 \) are the drift tube temperature and standard temperature (273.15 K) respectively.

The resolving powers, \( R_p \), were calculated according to the following equation:

\[ R_p = \left( \frac{t_d}{W_{1/2}} \right) \]

(2)

where \( t_d \) is the drift time (s) and \( W_{1/2} \) is the full width at half maximum (FWHM) height.

The linear regression equation with its precision limits was obtained from the raw data by using the function built into Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

**Results and discussion**

**Detection of tobramycin**

The detection of tobramycin with the in-house constructed ESI-IMS instrument is illustrated in Fig. 2A. The general operating conditions are summarized in Table 1. A spectrum acquired with the ESI generation turned off is flat except for an early electronic artifact caused by the injection pulse. The background spectrum for the blank electrolyte shows several peaks at drift times between 10 and 20 ms. The solution consists of a 1:1 mixture of methanol and water with the addition of 0.01% acetic acid for protonation of the analyte. The two main peaks in this background spectrum have reduced mobilities \( (K_0) \) of 1.90 cm² V⁻¹ s⁻¹ and of 1.65 cm² V⁻¹ s⁻¹ and are most likely due to protonated clusters of water [36] with the possible inclusion of methanol. Tetrabutylammonium (T4) was to be used as an internal standard for quantification, and at 5 μM, it gave a single peak at 19.8 ms. This corresponds to a reduced mobility in nitrogen of 1.24 cm² V⁻¹ s⁻¹, which is very close to the value of 1.25 cm² V⁻¹ s⁻¹ reported previously by our group for a different instrument [12] and within the range from 1.19 to 1.42 cm² V⁻¹ s⁻¹ found by Fernandez-Maestre in a literature survey [37]. As can also be seen from Fig. 2A, the injection of tobramycin at 50 μM yielded one main peak at 25.1 ms as well as a series of much weaker peaks at longer drift times, which presumably are adducts. The reduced mobility for the main peak of tobramycin was calculated as 0.97 cm² V⁻¹ s⁻¹. The last trace of Fig. 2A shows the successful injection of tobramycin together with the internal standard tetrabutylammonium.

In order to confirm the peaks in the ion mobility spectrum, the solution containing the mixture of tobramycin and tetrabutylammonium was also analyzed by electrospray ionization mass spectrometry. As can be seen in Fig. 2B, the main peak for tobramycin is the protonated molecular ion (MH⁺) with its mass-to-charge ratio (m/z) at 468.5. Some smaller adduct peaks are found at higher masses, with the most prominent one being the sodium adduct \( (M + Na^+) \). This matches the pattern found with IMS. A single peak is found for tetrabutylammonium, again in agreement with the
IMS spectrum. The two fragments for tobramycin at 324 and 205 m/z units were also reported for mass spectrometry by other groups [32, 33]. Two barely recognizable peaks are present between the T4 peak and the main peak for TBM, which possibly may be due to fragments. However, fragmentation under the IMS conditions is expected to be less pronounced due to the milder working conditions (e.g., working at room temperature).

**Optimization**

The effect of the injection time (50–300 μs) for 50 μM of tobramycin (TBM) with tetrabutylammonium (T4) of 5 μM on the mobility spectra was studied. It can be seen in Fig. 3 that when the injection time is increased, as expected, the peak intensity of tobramycin and of T4 is enhanced. However, on the other hand, the resolving power for TBM was found to decrease from 50 to 36 when increasing the injection time from 150 to 300 μs. As a compromise, an injection time of 250 μs was adopted for the subsequent measurements. For further optimization also, the effects of the field strength on the sensitivity and the resolving power were investigated, and the results are given in Fig. 4. The field strength was found to have an effect on the resolving power. This is expected and the theory predicts a maximum, which is depends on the mobility of the species [38]. The reason why here no maximum is found must be due to the also expected strong loss of sensitivity at low field strength [39]. For the low end of the examined field strength range, the signal was indeed very small (leading to poor precision for the lowest data point) and not measurable for lower applied voltages. The use of field strengths at the higher end is therefore desirable as the signal intensity increases strongly, but as the resolving power might be critical, this will not always be possible. However, for the highest applied voltage, a significant loss of precision is also observed.

**Selectivity**

The eye drops to be analyzed with the method contain other active ingredients and a number of excipients which are either specified on the packaging or simply referred to as inactives. Of the stated species, only the benzalkonium (BACs) leads to cations and therefore potential interferents. The benzalkonium ions are quaternary amines with an alkyl chain of varying lengths. According to Brignole-Baudouin et al. [40], only the benzyldimethylhexadecylammonium and benzyldimethyltetradecylammonium homologues are used in ophthalmic solutions. These are added as chloride salts and act as preservatives. Thus, a mobility spectrum was acquired for a standard mixture of the two BACs in order to ascertain that there is no spectral overlap with tobramycin or the internal standard. As can be seen in Fig. 5, the two benzalkonium cations appear in the mobility spectrum between the peaks for T4 and tobramycin, and therefore, they do not interfere.

**Quantification**

A calibration curve was acquired for the concentration range from 50 to 200 μM (5 points, 3 replicates each) of tobramycin by using the internal standardization method to compensate for variations in the production of the electrospray. T4 was used as internal standard and added to a concentration of 5 μM to calibration standards and samples. A linear calibration was obtained for this range ($y = (0.00304 \pm 0.0$
$y = \frac{0.00524 \pm 0.00764}{0.006} \times x$, where $x$ is the concentration in μM, $y$ is the ratio of the peak areas, and $r = 0.9994$. The relative standard deviation for measurements at the concentration of 100 μM was found to be 3% by taking 6 measurements. The limit of detection was determined as 7.9 μM ($3 \times$ standard deviation). Note that the high end of the calibration curve was limited by a loss of linearity. At the low end, it should be possible to extend the working range by averaging more readings.

**Determination of tobramycin in eye drops**

Five commercial eye drops containing tobramycin were bought from a pharmacy in Bangkok, Thailand, and the proposed ESI-IMS method was applied for its determination in these commercial formulations. The samples were diluted by a factor of 125 with the 50% methanol in water (v/v) mixture, containing 0.01% (v/v) acetic acid, to bring the analyte concentrations according to the labels to within the working range of the calibration curves. The stated concentration was 0.3% (w/v) in all cases. T4 was added as internal standard at a concentration of 5 μM. The resulting ion mobility spectra for the eye drop samples are shown in Fig. 6. These spectra also show the internal standard (T4), the benzalkonium peaks, and some unidentified peaks, which may be from excipients subsumed under inactives on the labels. The tobramycin concentrations determined in the products with the internal standardization method are given in Table 2, which are all close to the concentration of 0.3% (w/v) stated on the labels. However, the tobramycin peak for sample I appears to be slightly broadened, which might be due to an overlap with an unknown compound and this might be the reason why the recovery for this sample is slightly high (107%).

**Conclusions**

It has been demonstrated that tobramycin used as antibiotics in eye drops can be quantified with a simple IMS device employing electrospray ionization. The method is sensitive and requires only minimum sample preparation and no analyte derivatization. The analysis time is short, even if averaging several readings requiring 20 s each. However, the peak resolution in IMS is generally not as good as that of HPLC or MS, as illustrated by the possible interference observed for one of the samples. ESI-IMS is therefore not

| Table 2 | Determination of tobramycin concentration in eye drops |
|---------|-------------------------------------------------------|
| No      | Label content % (w/v) | Determined content % (w/v) ± SD |
|---------|-----------------------|------------------------------|
| Sample I| 0.3                   | 0.32 ± 0.02                   |
| Sample II| 0.3                  | 0.26 ± 0.01                   |
| Sample III| 0.3                 | 0.27 ± 0.01                   |
| Sample IV| 0.3                  | 0.30 ± 0.01                   |
| Sample V| 0.3                   | 0.29 ± 0.01                   |

---

Fig. 5 Mobility spectra for the benzalkonium chloride (BACs) cations (5 μM), and a mixture of the BACs (5 μM) with T4 (5 μM), and TBM (50 μM) obtained with the operating parameters as listed in Table 1

Fig. 6 Mobility spectra for the eye drop products obtained with the operating parameters as listed in Table 1
suit for complex samples. On the other hand, the application demonstrated serves to illustrate the potential of open-source analytical hardware. The instrument can be built by interested laboratories with little effort.

**Author contribution** Nattapong Chantipmanee: conceptualization, investigation, writing—original draft; Peter C. Hauser: supervision, writing—review and editing.

**Funding** Open access funding provided by University of Basel This study received funding from the University of Basel and Development and Promotion of Science and Technology Talents Project of Thailand (DPST 532133).

**Data availability** Not applicable.

**Code availability** No request from the authors.

**Declarations**

**Competing interests** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Furter JS, Boillat MA, Hauser PC. Low-cost automated capillary electrophoresis instrument assembled from commercially available parts. Electrophoresis. 2020;41(24):2073–82. https://doi.org/10.1002/elps.202000211.

2. Reinecke T, Clowers BH. Implementation of a flexible, open-source platform for ion mobility spectrometry. HardwareX. 2018;4:e00030. https://doi.org/10.1016/j.hx.2018.e00030.

3. Garcia L, Saba C, Manocchio G, Anderson GA, Davis E, Clowers BH. An open source ion gate pulsor for ion mobility spectrometry. Int J Ion Mobil Spectrom. 2017;20(3–4):87–93. https://doi.org/10.1007/s12127-017-0223-x.

4. Eiceman GA, Karpas Z, Hill HH, Jr. (2013) Ion mobility spectrometry, third edition. Ion Mobility Spectrometry

5. Hill CA, Thomas CLP. A pulsed corona discharge switchable high resolution ion mobility spectrometer-mass spectrometer. Analyst. 2003;128(1):55–60. https://doi.org/10.1039/b207558j.

6. Tabrizchi M, Khayaman T, Taj N. Design and optimization of a corona discharge ionization source for ion mobility spectrometry. Rev Sci Instrum. 2000;71(6):2321–8. https://doi.org/10.1063/1.1150618.

7. Chen H, Chen C, Li M, Wang W, Jiang D, Li H. Achieving high gating performance for ion mobility spectrometry by manipulating ion swarm spatiotemporal behaviors in the vicinity of ion shutter. Anal Chim Acta. 2019;1052:96–104. https://doi.org/10.1016/j.aca.2018.11.045.

8. Chantipmanee N, Hauser PC. Determination of ethylene by field asymmetric ion mobility spectrometry. Int J Ion Mobil Spectrom. 2020;23(2):161–6. https://doi.org/10.1007/s12127-020-00267-y.

9. Hayen H, Michels A, Franke J. Dielectric barrier discharge ionization for liquid chromatography/mass spectrometry. Anal Chem. 2009;81(24):10239–45. https://doi.org/10.1021/ac902176k.

10. Jafari MT. Low-temperature plasma ionization ion mobility spectrometry. Anal Chem. 2011;83(3):797–803. https://doi.org/10.1021/ac1022937.

11. Chantipmanee N, Furter JS, Hauser PC. Ambient ionization source based on a dielectric barrier discharge for direct testing of pharmaceuticals using ion mobility spectrometry. Anal Chim Acta. 2022;1195:339432. https://doi.org/10.1016/j.aca.2022.339432.

12. Chantipmanee N, Hauser PC. Development of simple drift tube design for ion mobility spectrometry based on flexible printed circuit board material. Anal Chim Acta. 2021;1170:338626. https://doi.org/10.1016/j.aca.2021.338626.

13. Hammert-Stabler CA, Johns T. Laboratory guidelines for monitoring of antimicrobial drugs. Clin Chem. 1998;44(5):1129–40.

14. Isoherranen N, Soback S. Chromatographic methods for analysis of aminoglycoside antibiotics. J AOAC Int. 1999;82(5):1017–45. https://doi.org/10.1093/jaoac/82.5.1017.

15. El-Zaher AA, Mahrouse MA. Utility of experimental design in pre-column derivatization for the analysis of tobramycin by hplc fluorescence detection: application to ophthalmic solution and human plasma. Anal Chem Insights. 2013;8(1):9–20. https://doi.org/10.4137/ACIS11612.

16. Kumar PJ, Clark ML. Kumar & Clark’s clinical medicine. New York: W. B. Saunders, Edinburgh; 2005.

17. Kernt K, Martinez M, Bertin D, Stroman D, Cupp G, Martinez C, Tirado M, Guasch J. A clinical comparison of two formulations of tobramycin 0.3% eyedrops in the treatment of acute bacterial conjunctivitis. Eur J Ophthalmol. 2005;15(5):541–9. https://doi.org/10.1177/112067210501500504.

18. McCormick C, Caballero A, Tang A, Balzli C, Song J, O’Callaghan R. Effectiveness of a new tobramycin (0.3%) and dexamethasone (0.05%) formulation in the treatment of experimental Pseudomonas keratitis. Curr Med Res Opin. 2008;24(6):1569–75. https://doi.org/10.1185/03007990802079877.

19. Protzek O, Bowman L, Abelson M, Shapiro A. Phase 3 safety comparisons for 1.0% azithromycin in polymeric mucoadhesive eye drops versus 0.3% tobramycin eye drops for bacterial conjunctivitis. Invest Ophthalmol Vis Sci. 2007;48(8):3425–9. https://doi.org/10.1167/iovs.06-1413.

20. Darwish IA. Development of generic continuous-flow enzyme immunoassay system for analysis of aminoglycosides in serum. J Pharm Biomed Anal. 2003;30(5):1539–48. https://doi.org/10.1016/S1053-0718(02)00543-5.

21. White LO, Holt HA, Reeves DS, MacGowan AP. Evaluation of Innoluor fluorescence polarization immunoassay kits for the determination of serum concentrations of gentamicin, tobramycin, amikacin and vancomycin. J Antimicrob Chemother. 1997;39(3):355–61. https://doi.org/10.1093/jac/39.3.355.

22. Barends DM, Brouwers JCAM, Hulshoff A. Fast pre-column derivatization of aminoglycosides with 1-fluoro-2,4-dinitrobenzene and its application to pharmaceutical analysis. J Pharm Biomed Anal. 1987;5(6):613–7. https://doi.org/10.1016/0731-7085(87)80073-0.
23. Caturla MC, Cusido E, Westerlund D. High-performance liquid chromatography method for the determination of aminoglycosides based on automated pre-column derivatization with o-phthalaldehyde. J Chromatogr A. 1992;593(1–2):69–72. https://doi.org/10.1016/0021-9673(92)80268-Y.

24. Fonge H, Kaela E, Govaerts C, Desmet K, Schepdael AV, Hoogmartens J. Bioanalysis of tobramycin for therapeutic drug monitoring by solid-phase extraction and capillary zone electrophoresis. J Chromatogr B. 2004;810(2):313–8. https://doi.org/10.1016/S1570-0232(04)00064-6.

25. Kaela E, Van Schepdael A, Roets E, Hoogmartens J. Development and validation of a simple capillary zone electrophoresis method for the analysis of kanamycin sulfate with UV detection after pre-capillary derivatization. J Chromatogr A. 2001;924(1):451–8. https://doi.org/10.1016/S0021-9673(01)00774-9.

26. Kaela E, Van Schepdael A, Roets E, Hoogmartens J. Development and validation of capillary electrophoresis method for tobramycin with precapillary derivatization and UV detection. Electrophoresis. 2002;23(11):1695–701. https://doi.org/10.1002/1522-2683(200206)23:11%3c1695::AID-ELPS1695%3e3.0.CO;2-6.

27. Ruckmani K, Shaikh SZ, Khalil P, Muneeva MS. A simple and rapid high-performance liquid chromatographic method for determining tobramycin in pharmaceutical formulations by direct UV detection. Pharm Methods. 2011;2(2):117–23. https://doi.org/10.4103/2229-4708.94455.

28. Clari M, Storme-Paris I, Chaminade P, Estevenon O, Nicolas A, Rieutord A. Simultaneous quantitation of tobramycin and colistin sulfate by HPLC with evaporative light scattering detection. J Pharm Biomed Anal. 2009;50(1):64–7. https://doi.org/10.1016/j.jpba.2009.03.013.

29. Megouas NC, Koupparis MA. Development and validation of a novel HPLC/ELSD method for the direct determination of tobramycin in pharmaceuticals, plasma, and urine. Anal Bioanal Chem. 2005;382(2):290–6. https://doi.org/10.1007/s00216-004-2948-8.

30. Law WS, Kubn P, Yuan LL, Zhao JH, Li SFY, Hauser PC. Determination of tobramycin in human serum by capillary electrophoresis with contactless conductivity detection. Electrophoresis. 2006;27(10):1932–8. https://doi.org/10.1002/elps.200500819.

31. Le TL, Hauser PC, Pham TNM, Kieu TLP, Le TPQ, Hoang QA, Le DC, Nguyen TAH, Mai TD. Low-cost and versatile analytical tool with purpose-made capillary electrophoresis coupled to contactless conductivity detection: application to antibiotics quality control in Vietnam. Electrophoresis. 2020;41(23):1980–90. https://doi.org/10.1002/elps.202000163.

32. Attema-de Jonge ME, Bekkers JM, Oudemans-van Straaten HM, Spardians RW, Fransen EJF. Simple and sensitive method for quantification of low tobramycin concentrations in human plasma using HPLC-MS/MS. J Chromatogr B. 2008;862(1–2):257–62. https://doi.org/10.1016/j.jchromb.2007.12.008.

33. Jariwala FB, Hibbs JA, Zhuk I, Sukhishvili SA, Attygalle AB. Rapid determination of aminoglycosides in pharmaceutical preparations by electrospray ionization mass spectrometry. J Anal Sci Technol. 2020;11(1):2. https://doi.org/10.1186/s40543-019-0202-4.

34. O’Donnell RM, Sun X, Harrington PD. Pharmaceutical applications of ion mobility spectrometry. TRAC-Trends Anal Chem. 2008;27(1):44–53. https://doi.org/10.1016/j.trac.2007.10.014.

35. Li S, Jia J, Gao X, He X, Li J. Analysis of antibiotics from liquid sample using electrospray ionization-ion mobility spectrometry. Anal Chim Acta. 2012;720:97–103.

36. Kaur-Atwal G, O’Connor G, Aksenov AA, Bocos-Bintintan V, Paul Thomas CL, Creaser CS. Chemical standards for ion mobility spectrometry: a review. Int J Ion Mobil Spectrom. 2009;12(1):1–14. https://doi.org/10.1007/s12127-009-0021-1.

37. Fernandez-Maestre R. Accuracy of reduced mobilities and measurement of instrumental parameters in ion mobility spectrometry. Int J Mass Spectrom. 2017;422:8–13. https://doi.org/10.1016/j.ijms.2017.05.018.

38. Kirk AT, Bakes K, Zimmermann S. A universal relationship between optimum drift voltage and resolving power. Int J Ion Mobil Spectrom. 2017;20:105–9.

39. Kirk AT, Zimmermann S. An analytical model for the optimum drift voltage of drift tube ion mobility spectrometers with respect to resolving power and detection limits. Int J Ion Mobil Spectrom. 2015;18(3–4):129–35. https://doi.org/10.1007/s12127-015-0176-x.

40. Brignole-Baudouin F, Desbenoit N, Hamm G, Liang H, Both JP, Brunelle I, Guerineau V, Legouffe R, Stauber J, Toublout D, Wyszorts M, Salzet M, Laprevote O, Baudouin C. A new safety concern for glaucoma treatment demonstrated by mass spectrometry imaging of benzalkonium chloride distribution in the eye, an experimental study in rabbits. PLoS ONE. 2012;7(11):e50180. https://doi.org/10.1371/journal.pone.0050180.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.