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Development and validation of HPLC method for the determination of tobramycin in urine samples post-inhalation using pre-column derivatisation with fluorescein isothiocyanate

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ABSTRACT

A reversed-phase liquid chromatography method involving pre-column derivatisation with fluorescein isothiocyanate (FITC, isomer I) for determination of tobramycin in urine samples after inhalation has been developed. FITC reacts with the primary amino groups of tobramycin and other aminoglycosides under mild conditions to form a highly fluorescent and stable derivative. The chromatographic separation was carried out on a Phenomenex Luna C_{18} column at ambient temperature using a constant flow rate of 1 ml/min and mobile phase of acetonitrile–methanol–glacial acetic acid–water (420:60:5:515, v/v/v/v). The tobramycin–FITC derivative was monitored by fluorescent detection at an excitation wavelength 490 nm and emission wavelength 518 nm. The linearity of response for tobramycin was demonstrated at 11 different concentrations of tobramycin extracted from spiked urine, ranging from 0.25 to 20 μg/ml. Tobramycin and neomycin were extracted from spiked urine by a solid phase extraction clean-up procedure on a carboxypropyl-bonded phase (CBA) weak cation-exchange cartridge, and the relative recovery was >99% ($n = 5$). The limit of detection (LOD) and limit of quantitation (LOQ) in urine were 70 and 250 ng/ml, respectively. The method had an accuracy of <0.2%, and intra-day and inter-day precision (in term of %coefficient of variation) were <4.89% and 8.25%, respectively. This assay was used for urinary pharmacokinetic studies to identify the relative lung deposition of tobramycin post-inhalation of tobramycin inhaled solution 300 mg/5 ml (TOBI®) by different nebuliser systems.

1. Introduction

Tobramycin is a broad spectrum aminoglycoside antibiotic produced by *Streptomyces tenebrarius* [20]. It exhibits a broad spectrum against aerobic gram-negative bacteria, especially *Pseudomonas* and indole positive *Proteus* [32] that makes it the antibiotic of choice in the treatment of pulmonary infections. Like other aminoglycosides, the use of tobramycin can create potential side-effects of ototoxicity and nephrotoxicity. Therefore, careful monitoring of the drug level in the serum of patients receiving tobramycin is necessary, especially when therapy is of long duration.

Several analytical techniques have been reported for the analysis of tobramycin in biological fluids [26,32] including radioimmunoassay (RIA) [21], fluorescence polarization immunoassay (FPIA) [4], microbiological assays [8,24], radiochemical assay [9], enzyme immunoassay (EIA) [10,29], and chromatographic methods [13,15,22,26,31]. High-performance liquid chromatography (HPLC) and gas chromatography (GC) have been used to detect the aminoglycosides at low concentrations in biological fluids. However, these antibiotics do not have a significant UV absorbance chromophore, or fluorescence, therefore derivatisation with a suitable absorbance-enhancing or fluorescence-producing agent is required for the detection by chromatographic techniques.

A number of HPLC pre-column derivatisation methods have been demonstrated with 2,4,6-trinitrobenzenesulfonic acid (TNBS) [11,17,22], 1-fluro-2,4-dinitrobenzene (FDNB) [2,4–7,27], *o*-phthaladehyde (OPA) [3,12,26–28] and 1-naphthyl isothiocyanate (NITC) [15]. The limitation of use of TNBS and FDNB is due to their high toxicity, and the main disadvantage of NITC is the length of time to achieve reaction. Post-column derivatisation methods with OPA have been reported [1,14,25,32], even though OPA has a poor stability resulting derivative.

Many HPLC methods for determination of aminoglycosides in pharmaceutical preparations and biological fluids directly, without derivatisation, have been developed. Szúnyog et al. [34] have determined tobramycin in a number of commercial samples by liq-

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uid chromatography directly using pulsed electrochemical detector (PED). In a more recent study, Hanko and Rohrer [20] have reported a determination method of tobramycin and its major impurities (kanamycin B and neomycin A), using high-performance anion exchange chromatography with integrated pulsed amperometric detection. Nevertheless, the baseline instability, noise and the long stabilization time affect the reliability of these methods. Keevil et al. [23] have measured tobramycin concentrations in serum samples using liquid chromatography tandem mass spectrometry (LC–MS–MS), but this method not readily available in all laboratories.

Recently, Megoulas and Koupparis [30] have developed and validated a direct HPLC method for determination of tobramycin in pharmaceutical preparations, plasma, and urine, with evaporative light scattering detector (ELSD).

In this study, a simple, stable, sensitive, and validated HPLC method for determination of tobramycin in urine post-inhalation, following pre-column derivatisation with fluorescein isothiocyanate (FITC, isomer I) has been developed.

2. Experimental

2.1. Materials and chemicals

Tobramycin (98% purity), neomycin sulfate, and glycine (99% purity) were purchased from Sigma–Aldrich (Louis, USA). Fluorescein isothiocyanate (isomer I) derivatising reagent and pyridine (HPLC grade) were obtained from Sigma–Aldrich (Gillingham, UK). Acetonitrile (HPLC grade) was supplied by Fisher Scientific UK Limited (Loughborough, UK). All solutions were prepared with ultra-pure milli-Q water and obtained from a Milli-Q water Millipore Purification System (USA). Carboxypropyl-bonded weak cation-exchange (CBA 100 mg/3 ml) solid phase extraction cartridges were purchased from IST (Isolute, Mid Glamorgan, UK).

2.2. Inhalation delivery systems

Different nebuliser delivery systems have been used to assess their performance to deliver TOBI® (300 mg/5 ml) inhaled solution: two different designs of jet nebulisers. The two jet nebulisers were breath-enhanced Pari LC Plus® jet nebuliser attached to a PariBoy*N*® compressor (Pari GmbH, Starnberg, Germany), and the constant output Sidestream® jet nebuliser (Medic-Aid Ltd., West Sussex, UK) attached to a Porta-Neb® compressor (Profiles, UK). The nebuliser fill volume was 5 ml of tobramycin inhaled solution 300 mg/5 ml (TOBI®).

2.3. Chromatographic conditions

The HPLC system consisted of a Hewlett-Packard (HP) 1050 pump and autosampler connected to an on-line membrane degasser (Thermo Separation Products, California, USA). The Shimadzu fluorescence detector model RF-551 (Tokyo, Japan) set at an excitation wavelength 490 nm and emission wavelength 518 nm, and the detector linked to Prime Multi-channel Data Station Software Version 4.2.0. (HPLC Technology Ltd., Herts, UK).

The chromatographic separation was carried out on a 5 μm Luna C_{18} HPLC column (150 mm × 4.60 mm i.d., Phenomenex, Macclesfield, UK) protected by a C_{18} Phenomenex Security Guard cartridge column (4 mm × 3 mm i.d.). The mobile phase consisted of acetonitrile–glacial acetic acid–water (420:60:5:515, v/v/v/v). The mobile phase was filtered and degassed through a nylon membrane filter 0.45 μm pores (Gelman Sciences, Germany) under vacuum. 100 μl of the derivatised samples were injected into the HPLC system and separated at ambient temperature, using a constant flow rate of 1 ml/min.

2.4. Preparation of tobramycin spiked urine (TSU) samples

A master solution of 100 mg/ml of tobramycin free base was prepared in water. Blank urine samples were collected from 12 subjects, which were then used to prepare urine standards for the method validation.

Urine standards were prepared by mixing 50 ml of the 100 mg/ml stock with 5000 ml of blank urine to produce a concentration of 1000 μg/ml, which were then used to prepare calibration standards ranging from 0.250 to 20 μg/ml.

2.5. Solid phase extraction

The CBA cartridge (100 mg/3 ml) was conditioned with 2 ml methanol and 2 ml phosphate buffer (20 mM, pH 7.4), respectively, at a flow rate of 1–2 ml/min, then the sample containing 1 ml spiked urine mixed to 1 ml of internal standard (neomycin sulfate) was applied, and passed slowly through the CBA cartridge at a flow rate of 1 ml/min. Afterward, the CBA cartridge was rinsed with 2 ml of phosphate buffer (20 mM, pH 7.4), borate buffer (200 mM, pH 9), and methanol, respectively. The sorbent bed was then allowed to dry for 5 min, using a full air-vacuum. Finally, the analyte was eluted with 1 ml of eluent containing methanol with concentrated ammonia (90:10, v/v), and collected in a 3-ml glass test tube at a flow rate of 1 ml/min, followed by passing a volume of air to dry the sorbent bed. The collected eluent was evaporated to dryness at 55 °C under a gentle stream of nitrogen and then the resulting residue was reconstituted with 1 ml deionised water.

2.6. Pre-column derivatisation procedure

In a HPLC glass vial, 300 μl of reconstituted residue was derivatised with 300 μl of FITC (20 mM). The mixture was vortex for 40 s, and incubated at 80 °C in the oven for 20 min. After cooling, 100 μl of the derivatised sample was injected into the HPLC system and separated at ambient temperature, using a constant flow rate of 1 ml/min.

2.7. Clinical study

Ethical approval was obtained from the University of Bradford, and all volunteers gave signed informed consent. Twelve (three females) healthy adults, non-smoking subjects completed the study. Their mean (S.D.) age, weight, and height were 32.5 (6.23) years, 76.11 (9.48) kg, and 173.58 (3.84) cm, respectively.

All subjects received inhaled tobramycin 300 mg/5 ml dose from Pari LC Plus® and Sidestream®, on separate randomized study days, each separated by 7 days apart. Urine samples were collected at 0.5, 1, 2, 3, 4, 6, 9, 12 and 24 h post-inhalation of each study dose.

2.8. Biological assay validation

The developed method was validated to determine the tobramycin in urine sample post-inhalation, and the validation procedure was based on FDA (2001) and ICH (1996) guidelines for bioanalytical method for human studies [16,19].

2.8.1. Specificity

Specificity is the ability of the method to quantify the analyte in the presence of other component in the urine matrix [16]. The blank urine samples collected from 12 (three females) volunteers were individually spiked with tobramycin. Both the blank and spiked

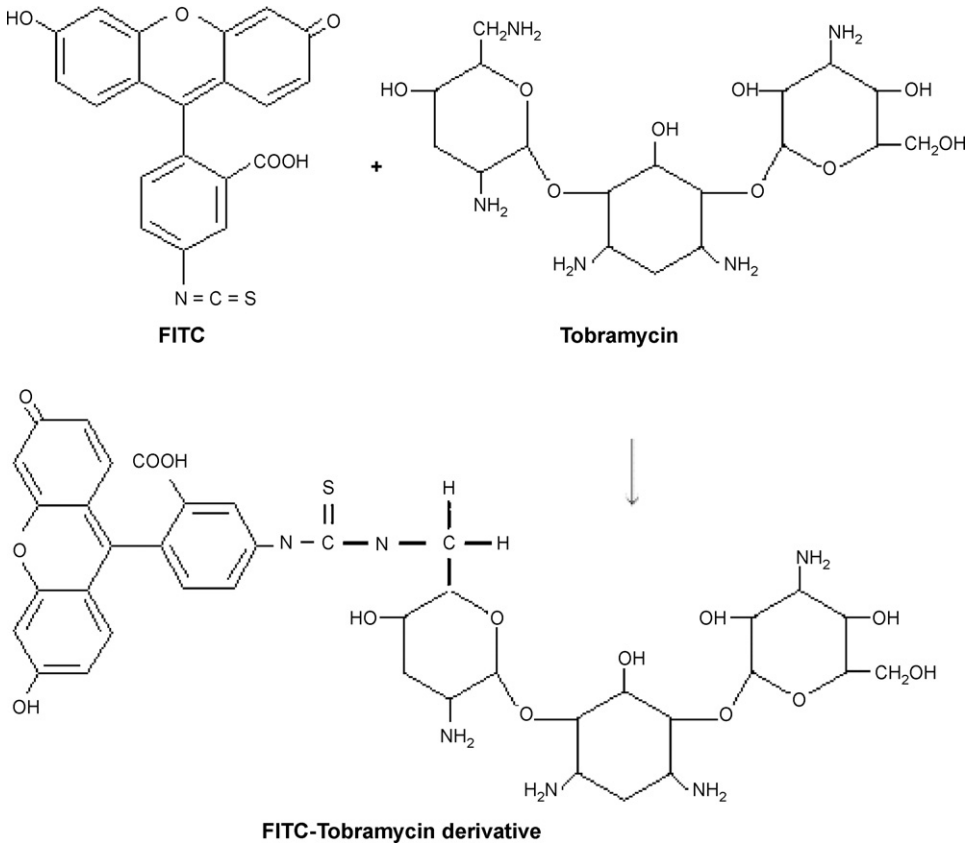

Fig. 1. Reaction of FITC with tobramycin.

urine samples were derivatised with FITC and then assayed by HPLC.

2.8.2. Method linearity

Eleven different concentrations were prepared to range from 0.25 to 20 μg/ml including the lower limit of quantification (LOQ) and covering the expected range. The standards were prepared in urine matrix and then tobramycin extracted and derivatised with FITC. Blank samples were also analysed along with the calibration standards.

2.8.3. Sensitivity

The sensitivity was expressed as the limit of quantitation (LOQ), which is the injected amount that results in a peak with a height at least ten times as high as the baseline noise level, and the limit of detection (LOD) as peak height to base line ratio of 3:1. The LOQ is accepted if the analyte peak response is identifiable and reproducible with a precision of 20% and accuracy of 80–120%. Tobramycin in urine at a concentration of 0.25 μg/ml was extracted and derivatised with FITC and then injected on 5 separate days to determine the precision of the tobramycin peak.

2.8.4. Precision

Precision was examined by five determinations at known concentrations corresponding to low (0.25 and 2 μg/ml), medium (10 μg/ml) and high (20 μg/ml) levels in the calibration range. This study was repeated for 5 days to determine the inter-day variation.

2.8.5. Accuracy

Accuracy of the method was assessed by replicate analysis ($n = 5$) of the extracted tobramycin urine standards at known concentrations that include low (0.25 μg/ml), medium (10 μg/ml) and high (20 μg/ml), and then compared with the true concentration of tobramycin.

2.8.6. Recovery

The recovery was studied by comparing the detector response of extracted tobramycin urine standard at three concentrations levels {(low (0.25 μg/ml), medium (10 μg/ml) and high (20 μg/ml)} with the detector response of the tobramycin standards spiked in extracted blank urine matrix. The measurements were performed by using five determinations per concentration.

2.8.7. Robustness

The robustness of the method was evaluated by introducing small variation from the optimum condition. This variation includes mobile phase, pH, temperature, and flow rate. Moreover, different column from different suppliers were examined.

2.8.8. Stability

The stability of the aqueous and urine standards was evaluated by assaying the analyte during sample collection, handling, after short-term storage and after going through freeze–thaw cycles. On the other hand, three urine samples of tobramycin were frozen at −20 °C for 24 h. The samples were thawed unassisted at room temperature and analysed. The samples were refrozen for 24 h under the same conditions. The freeze–thaw cycle was repeated two more times, and then analysed on the third cycle.

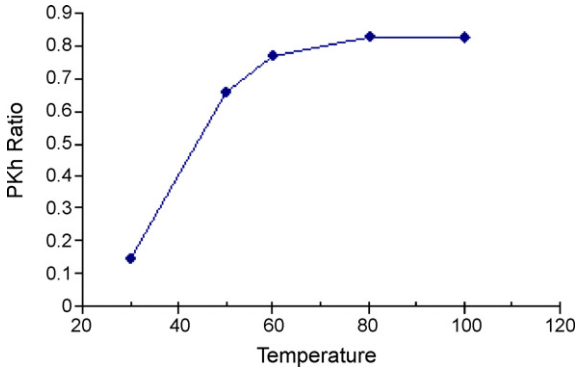

Fig. 2. Effect of reaction temperature on FITC–tobramycin derivatisation yield.

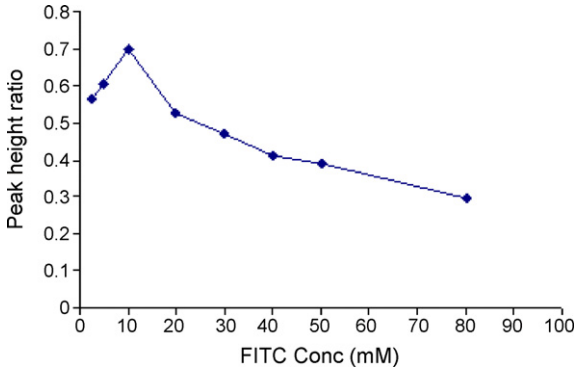

Fig. 4. Effect of FITC concentration on FITC–tobramycin derivative yield.

3. Result and discussion

3.1. Method derivatisation

3.1.1. Principle of reaction

Fluorescein isothiocyanate reacts with amines at alkaline conditions to form highly fluorescent derivatives. The reaction is completed within 15 min in a dark place, and the resulting derivatives detected at excitation and emission wavelengths of 496 and 518 nm, respectively. As shown in Fig. 1, FITC reagent reacts with the amino group in tobramycin, and other aminoglycosides, to form FITC–tobramycin derivative which is highly detectable on a fluorescence detector.

3.1.2. Optimisation of FITC derivatisation method

Derivatisation of tobramycin with FITC derivatising reagent was optimised for various factors such as the reaction temperature, reaction time, FITC concentration, and detection for fluorescence and UV-wavelengths.

Reaction temperature was found to be a critical parameter for the derivatisation reaction. Therefore, the effect of temperature was thoroughly examined by allowing the reaction to proceed at different incubation temperatures ranging from 30 to 100 °C. Fig. 2 shows that the rate of reaction of tobramycin with FITC was increased with temperature and the optimum FITC–tobramycin derivatisation yield was at 80 °C.

The effect of the reaction time was examined by allowing the derivatisation to proceed for various times at 80 °C, ranging from 10 to 120 min. The reaction was completed within 10 min, but maximum yield was obtained at 20 min, as shown in Fig. 3.

The optimum emission fluorescent spectrum was obtained by fixing an excitation wavelength of 496 nm, and detecting the repeated injections of standard tobramycin solution after FITC derivatisation at different emission wavelengths, ranging from 510 to 524 nm. With the same procedure, the optimum excitation wavelength was obtained by fixing an emission wavelength of 518 nm, and detecting the repeated injections at different excitation wavelengths, ranging from 475 to 498 nm. The maximum yield of FITC–tobramycin derivative was obtained at excitation and emission wavelengths of 490 and 518 nm, respectively.

The relationship between the concentration of FITC reagent and reaction yield was studied by using a range of FITC reagent concentrations from 2.5 to 80 mM. The maximum yield was obtained at 10 mM FITC, while a further increment of FITC concentration did not significantly improve the peak height of tobramycin, as shown in Fig. 4.

Effect of buffer concentration on the derivatisation reaction of FITC with tobramycin was examined by dissolving tobramycin in borate buffer at different concentrations, ranging from 10 to 0.2 M. Deterioration of FITC–tobramycin derivative peak occurred at 0.2 M borate buffer, while the optimum yield was observed at 10–25 mM, as shown in Fig. 5.

The influence of pH on the reaction yield was examined in the pH range 7–10.5. As shown in Fig. 6, the optimum reaction occurred at pH 7.5–8. Multiple hydrolysis product peaks were observed with higher pH.

Moreover, the effect of reaction matrix was examined by dissolving tobramycin in water and 25 mM borate at pH 7.5. No significant difference was observed in reaction yield of FITC–tobramycin derivative.

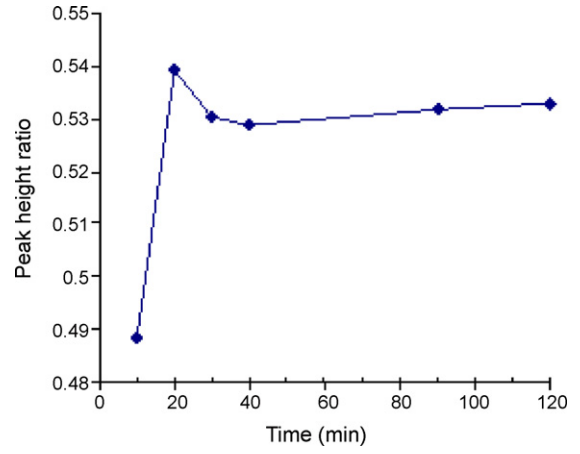

Fig. 3. Effect of reaction time on FITC–tobramycin derivatisation yield at 80 °C.

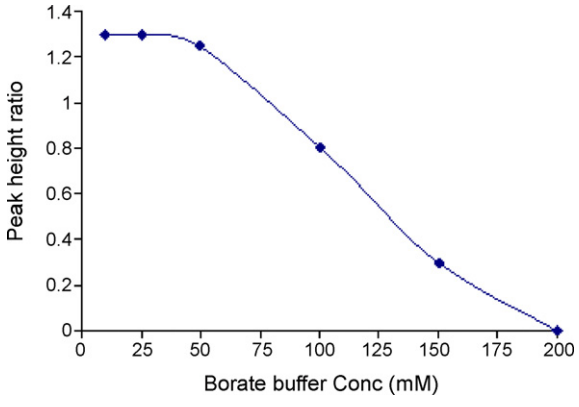

Fig. 5. Effect of buffer concentration on FITC–tobramycin derivative yield.

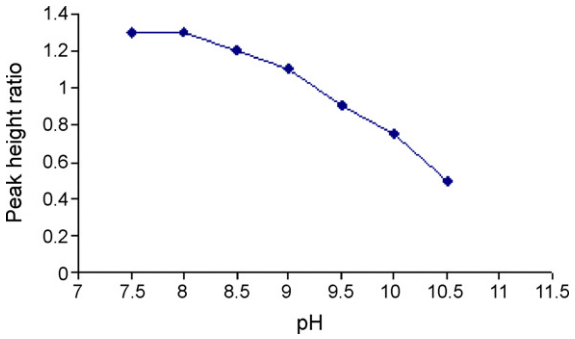

Fig. 6. Effect of buffer pH on FITC–tobramycin derivative yield.

The optimum emission fluorescent spectrum was obtained by fixing an excitation wavelength of 496 nm, and detecting the repeated injections of standard tobramycin solution after FITC derivatisation at different emission wavelengths, ranging from 510 to 524 nm. With the same procedure, the optimum excitation wavelength was obtained by fixing an emission wavelength of 518 nm, and detecting the repeated injections at different excitation wavelengths, ranging from 475 to 498 nm. The maximum yield of FITC–tobramycin derivative was obtained at excitation and emission wavelengths of 490 and 518 nm, respectively.

3.2. Optimisation of HPLC Chromatographic conditions

The method was optimised based on alteration the composition of mobile phase, pH detection wavelengths and other parameters. The presence of residual silanol groups on the silica surface caused peak tailing problems and an extended runtime when operated at higher pH values (pH > 5). 2% glacial acetic was used to lower the pH of mobile phase to pH 3. At lower pH (<3.0) the silanol groups become protonated, thus eliminating the attractions between the ionised silanol groups and the NH_2 group in tobramycin [36].

Acetonitrile percentage was altered from 30% to 90% of the mobile phase composition. The best separation was obtained at 90%. It was found that below this percentage an interference with urine endogenous was observed. The base line resolution for tobramycin from urine matrix was obtained using the mobile phase of acetonitrile–glacial acetic acid–water (420:60:5:515, v/v/v/v).

3.3. Assay validation

3.3.1. Specificity

The specificity of the FITC derivatisation method was examined to ensure that there were no interferences from components of endogenous material. Furthermore, the specificity of FITC was examined with other aminoglycosides and amino compounds, and common medications. Peaks of tobramycin and internal standard were well resolved and were separated from the co-extracted endogenous urine components (Fig. 7).

3.3.2. Linearity

The linearity of the calibration standards was evaluated over the range 0.250–20 μg/ml (see Section 2.8.2). The extracted tobramycin samples were injected in duplicates. The calibration curve (Fig. 8) showed a linear response and gave a regression coefficient (r^2) of 0.9989, the standard deviations for the slope and intercepts were 0.0118 and 0.0418, respectively [$Y = 0.0677(\pm 0.0118)X + 0.0012$ (± 0.0873)].

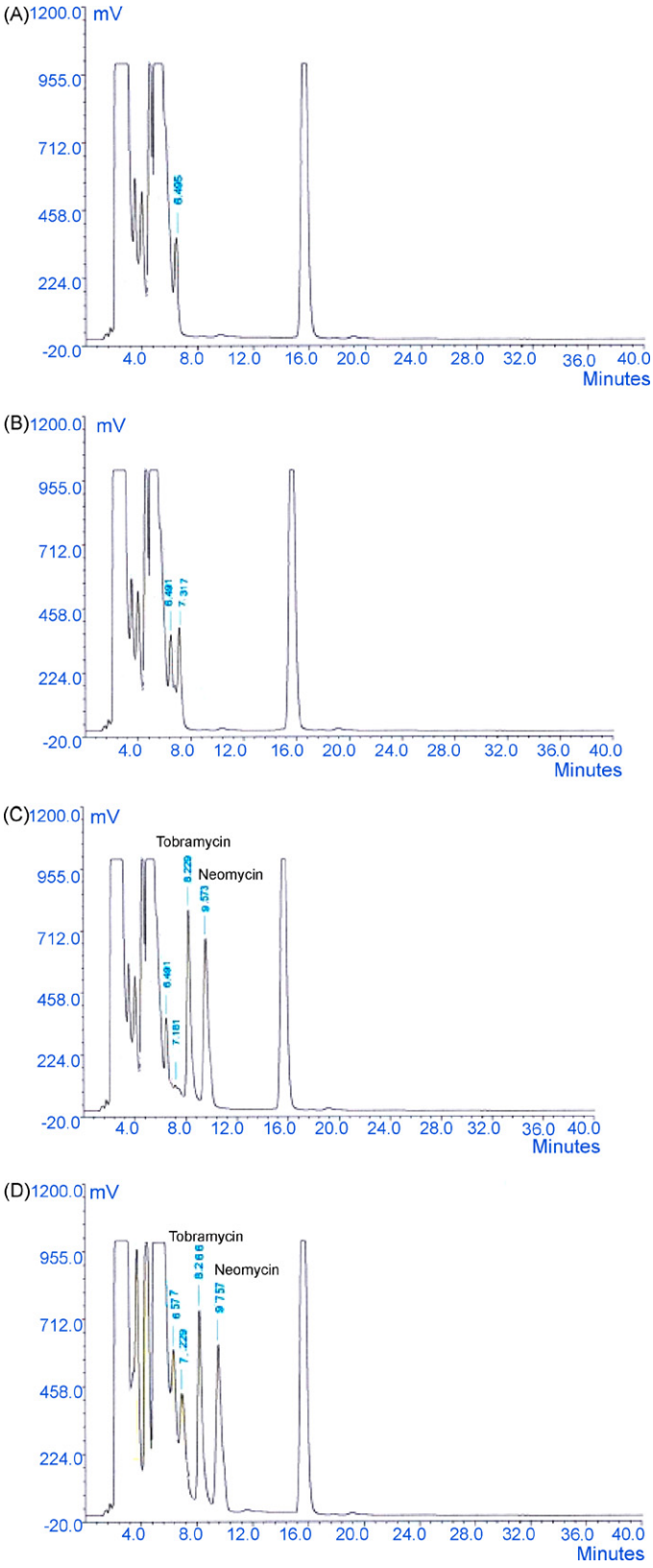

Fig. 7. Chromatograms obtained for (A) a blank (water), (B) extraction of blank human urine after derivatisation with FITC, (C) derivatisation of tobramycin with FITC after extraction from TSU, (D) derivatisation of tobramycin with FITC after extraction from volunteer urine post-inhalation of tobramycin inhaled solution (TOBI®).

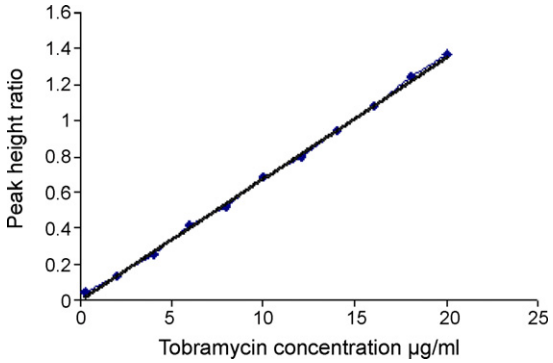

Fig. 8. Calibration curve of TSU after FITC derivatisation.

Table 1a
Intra-day precision for TSU after FITC derivatisation method

Extraction within day	%CV			
	0.25 μg/ml	2 μg/ml	10 μg/ml	20 μg/ml
1	0.91	3.69	1.94	3.14
2	0.85	1.03	1.71	5.33
3	1.28	2.15	1.42	6.77
4	0.08	1.08	3.18	2.07
5	1.77	2.28	0.88	7.18
Mean	0.98	2.05	1.83	4.90

3.3.3. Sensitivity

The LOD and LOQ of the HPLC pre-column FITC derivatisation method for tobramycin spiked urine (TSU) samples were calculated using detection limit (DL = 3.3 s/m) and quantitation limit (QL = 10 s/m) equations, respectively. Where, *s* is the standard deviation of *y*-intercept and *m* is the slope of the calibration curve. The LOD and LOQ were 70 and 250 ng/ml, respectively.

3.3.4. Precision

Inter-assay CVs ranged from 1.26% to 8.21% and the intra-assay CVs ranged from 0.98% to 4.89% at concentrations ranging from 0.25 to 20 μg/ml (Table 1). These determinations were done using pooled urine from healthy individuals to which appropriate doses of tobramycin were added (see Section 2.8).

3.3.5. Recovery and accuracy

Recovery was determined by comparing the peak area of the extracted tobramycin urine standard with the peak area of the tobramycin standard externally spiked with extracted blank urine matrix. The method showed excellent and consistent recoveries,

Table 1b
Inter-day precision for TSU after FITC derivatisation method

Extraction between days	%CV			
	0.25 μg/ml	2 μg/ml	10 μg/ml	20 μg/ml
1	2.69	12.01	1.14	1.06
2	0.87	6.58	1.10	0.33
3	3.18	10.08	5.27	2.03
4	0.35	4.93	1.98	0.09
5	4.58	7.44	1.56	2.82
Mean	2.34	8.21	2.21	1.27

and the mean recoveries for the studied concentrations ranged from 98.13% to 99.52% (Table 2).

The accuracy was assessed by comparing the calculated concentration of the extracted tobramycin urine standard with the true concentration of tobramycin. The accuracy of the bioanalytical method was greater than 98.1% (Table 2). The accuracy of the method was further assessed by the standard addition method. In this assessment, the extracted urine samples were spiked with 0.25, 10 and 20 μg/ml. The accuracy determination of tobramycin in urine samples was greater than 98.35% (Table 2). Each test was repeated three times.

3.3.6. Stability and robustness

Stability studies evaluate the situations that likely to be encountered during the sample processing from being collected from subject to final analysis. Therefore, this study had examined both the stability of FITC–tobramycin derivative and the stability of tobramycin in the biological fluid.

The stability of FITC–tobramycin derivative was examined by repeated injections of the same sample at different times over a period of 5 days, when stored at room temperature in an umber vial, and by comparing with freshly prepared standard. No significant change was found (RSD < 2%), indicating favourable stability of the derivative.

Stability in a biological fluid, urine, is a function of the storage conditions, the chemical properties of the active pharmaceutical ingredient (API) and the urine matrix effect. The stability of tobramycin urine sample was assessed as described in Section 2.8. The stability of tobramycin solutions was evaluated at room temperature for 24 h, and at 2 °C in a refrigerator for 1, 3 and 6 months, then compared to freshly prepared solutions after FITC derivatisation. No significant difference was observed.

Short-term stability test performed at room temperature showed that the samples were stable for 24 h, and the mean recoveries were higher than 98.97; similarly, the long term stability study

Table 2
Recovery and accuracy data for tobramycin urine samples/standards after FITC derivatisation method

Accuracy and recovery for tobramycin urine standard			
Actual concentration (μg/ml)	Observed concentration (μg/ml)	%Accuracy	Recovery%
0.25	0.25	100	98.40
2	1.99	99.50	99.52
10	9.81	98.10	98.13
20	19.83	99.15	99.14

Accuracy for tobramycin urine samples using standard addition method		
Amount added (μg/ml)	Total found (μg/ml)	%Accuracy
0	5.82	
0.25	5.97	98.35
2	7.79	99.62
10	15.62	98.74
20	25.76	99.77

Table 3
Stability data of TSU samples using FITC derivatisation method

Actual tobramycin concentration (μg/ml)	Stored at room temperature for 24 h		Stored at −20 °C for 1 month		Three freeze/thaw cycle	
	RSD%	Recovery%	RSD%	Recovery%	RSD%	Recovery%
2	3.21	99.65	9.66	95.18	7.96	98.86
10	2.28	99.19	6.139	98.33	4.77	98.33
20	5.98	98.97	5.32	96.12	4.62	98.46

showed that tobramycin urine samples were stable for 1 month when stored at −20 °C, with an average recovery >95.18%. No significant decrease of tobramycin concentration in urine was detected after exposing samples to three freeze/thaw cycles and mean recovery ranged from 98.33% to 98.86%. The stability data (extraction $n = 5$) are summarised in Table 3.

3.3.7. Robustness

The robustness of an analytical procedure is a measure of its capacity to resist changes due to small variations in method conditions. The method was robust with respect to small variation in the method parameters. Different lots of the same analytical column, and two different batches of mobile phase solvents were also tested but no significant difference was observed. Moreover, The reproducibility of the retention time of FITC–tobramycin derivative was examined during the validation procedure of the proposed method after more than 50 consecutive injections. The RSD% of the retention time ($n = 50$) was calculated to be 0.456%.

4. Pharmacokinetic study

This method can be used to study the pharmacokinetic profile where tobramycin concentrations in urine are greater than 250 ng/ml. The method was used for determination of the relative lung bioavailability of inhaled tobramycin solution (TOBI®) to the lungs in healthy volunteers, following inhalation from different nebuliser systems, using a urinary pharmacokinetic method that had been developed in-house [35]. Figs. 9 and 10 show the urine concentration versus time profile obtained following inhalation of tobramycin from the nebuliser systems.

The urinary pharmacokinetic data showed that the cumulative amount of tobramycin over 24 h following administration of 300 mg TOBI® delivered from Pari LC Plus® and Sidestream® nebulisers was significantly increased ($p < 0.05$) in breath-enhanced Pari LC Plus® when compared to the constant output Sidestream® jet nebuliser. Besides, the relative lung bioavailability data reflect better relative lung bioavailability of tobramycin following Pari LC Plus® nebulisation.

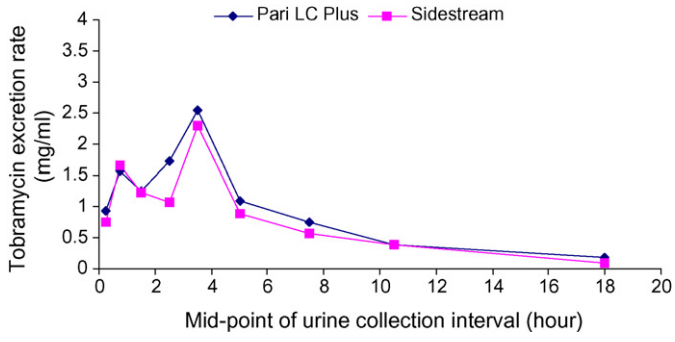

Fig. 9. The mean urinary excretion rate time profile for tobramycin obtained from 12 subjects following nebulised dosing of TOBI®, using the different nebuliser systems.

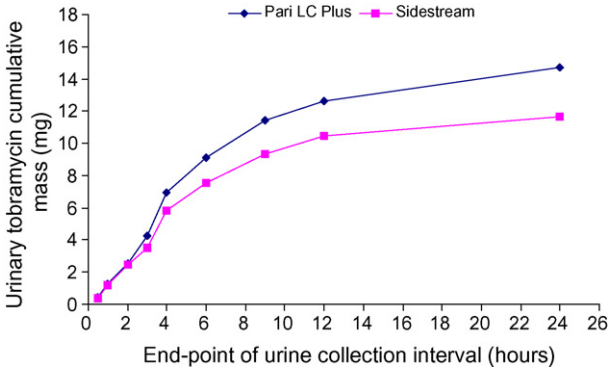

Fig. 10. The mean (S.D.) cumulative amounts (mg) of tobramycin excreted in the urine following administration of nebulised dose of TOBI®, using the different nebuliser systems.

5. Conclusions

A simple, sensitive and validated HPLC method for the determination of tobramycin in spiked human urine following FITC pre-column derivatisation has been developed. The good validation results of the proposed method show its suitability to use in the clinical and research laboratories for the measurement of tobramycin and other aminoglycosides in biological fluids or pharmaceutical formulations. One of clinical applications of this assay was to determine the relative lung deposition of tobramycin inhaled solution 300 mg/5 ml (TOBI®) by different nebuliser systems, using urinary pharmacokinetic method. The higher sensitivity, stability of FITC–tobramycin derivative, and short runtime of analysis are the main advantages of the proposed method.

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