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Determination of kanamycin in serum by solid-phase extraction, pre-capillary derivatization and capillary electrophoresis

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Abstract

An effective method based on solid-phase extraction (SPE) and capillary electrophoresis (CE) for the determination of kanamycin in human serum was developed and validated. Off-line SPE was employed for the isolation of kanamycin from serum on a carboxypropyl-bonded phase (CBA) weak cation-exchange cartridge. A mixture of 0.2 *M* borate (pH 10.5)–methanol (50:50, v/v) was used as analyte eluting solvent. After pre-capillary derivatization with *o*-phthalaldehyde/mercaptoacetic acid reagent, the sample was analyzed by CE with a separation buffer of 30 m*M* borax, pH 10.0, containing 16% (v/v) methanol. A linear response over the concentration range 5–40 μg/ml was obtained with a detection limit of 2 μg/ml. Intra-day and inter-day precision were 6.2 and 10.3% RSD, respectively. Recoveries of approximately 90% were found. For the determination of lower levels of kanamycin (<5 μg/ml), NH_4OH (25%, w/v)–methanol (30:70, v/v) was used for analyte elution. After evaporation, reconstitution and derivatization, the sample was analyzed by on-line field-amplified sample stacking (FASS) CE. Good linearity in the concentration range 0.4–5 μg/ml was obtained with a detection limit of 0.1 μg/ml. Intra-day and inter-day RSD were 3.4 and 11.2%, respectively. Recoveries of approximately 60% were found. The method was successfully applied to the analysis of kanamycin in sera of tuberculosis patients at peak level and trough level concentrations.

Keywords: Field amplified sample stacking; Kanamycin

1. Introduction

Kanamycin is a broad spectrum aminoglycoside antibiotic produced by certain strains of *Streptomyces kanamyceticus* [1]. It contains as major component kanamycin A and as minor components kanamycin B, C and D [2]. Due to its clinically useful activity against *Mycobacterium tuberculosis*, kanamycin is widely used as a second-line anti-tuberculosis drug [3]. Like other aminoglycosides, the use of kanamycin can create potential side-effects of ototoxicity and nephrotoxicity. Therefore, careful

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monitoring of the drug level in the serum of patients receiving kanamycin is required, especially when therapy is of long duration. Knowledge of the serum drug level is used to adjust the patient's dose to ensure therapy efficacy and avoid possible risk of toxicity.

The existing methods for the determination of kanamycin in blood samples include microbiological methods [4], fluorescent immunoassay [5], fluorescent polarization immunoassay [6,7], fluorimetric determination [8] and chemiluminescence immunoassay [9]. However, these methods are non-specific and non-separative, the results being affected by the presence of other aminoglycoside antibiotics.

Methods for the separation of kanamycin from other aminoglycoside antibiotics have been described. Thin-layer chromatography (TLC) was first reported as a semiquantitative method for the determination of kanamycin in serum and urine [10]. It is used for the determination of kanamycin B in kanamycin by the European Pharmacopoeia [11]. However, the sensitivity, resolution and accuracy of TLC are not sufficiently high for the analysis of kanamycin in biological samples.

Combinations of HPLC, post-column derivatization and fluorescence detection have been reported for the determination of kanamycin and other aminoglycosides in biomatrices [12–14]. Electrochemical detection was employed in kanamycin analysis by HPLC [15].

Due to its high resolution and low cost of operation, capillary electrophoresis (CE) has been increasingly viewed as a promising method for the analysis of drugs. Reports on the analysis of kanamycin by CE have been published [16–18]. Since kanamycin has no UV or fluorescence absorption, complex formation [16], indirect UV methods [17] and precapillary derivatization [18] were employed for detection. The sensitivity of the above CE methods is not a problem for the analysis of bulk pharmaceuticals and their formulations where the concentration of kanamycin ranges from 1 to 250 mg/ml. However, these methods cannot be directly applied to clinical analysis, where kanamycin is present in serum in the concentration range 5–35 μg/ml [19]. Because of the complexity of the sample matrix, the low injection volume and the very short optical path length for on-capillary UV detection, the concentration sensitivity of CE is low. Electrochemical detection methods have been reported for the determination of aminoglycoside antibiotics by CE [20,21] with detection limits sufficiently low to determine amikacin and kanamycin in urine. However, electrochemical detection in CE requires a special setup and technique. It is not as convenient as UV detection, which is the most commonly used detection mode in capillary electrophoresis. No report could be found in the literature on the analysis of kanamycin in serum by CE with UV detection and this initiated the present investigation.

In order to quantify low levels of kanamycin in the presence of a complex biological matrix, appropriate sample clean-up, matrix effect elimination and analyte enrichment procedures are required prior to CE separation and quantitation. Solid-phase extraction (SPE) can be used to simultaneously enrich the trace analytes and remove salts and protein. Due to its speed, higher reproducibility, cleaner extract and lower solvent consumption, it has gained prominence for biological sample handling in recent years [22]. Since aminoglycosides are polar and water-soluble compounds, they are chemically ideal for aqueous extraction. Successful solid-phase extractions of aminoglycoside antibiotics from biomatrices using a weak cation exchanger [23–25] and a C_{18} column [26] have been reported.

Field-amplified sample stacking (FASS) is the simplest on-column concentration technique to increase CE sensitivity [27]. It is based on the principle that the mobility of an ion is dependent on the applied electric field strength. If the conductivity of the sample zone is lower than that of the separation buffer, the ions in the sample region will experience a higher electric field and migrate rapidly, and they will stack up as a sharp band at the boundary between the sample plug and the separation buffer. With this technique, the concentration of analyte in the sample zone can increase from 10- to 1000-fold without any modification of the commercial instrument [27].

In the present study, an effective method based on solid-phase extraction on a carboxypropyl-bonded phase (CBA) weak cation-exchange sorbent, precapillary derivatization with *o*-phthalaldehyde (OPA)/mercaptoacetic acid (MAA) reagent and capillary electrophoresis for the determination of

kanamycin in human serum was developed and validated. Optimization of the extraction parameters was studied. FASS was employed for the analysis of low levels of kanamycin. The applicability of the combination of SPE and CE was investigated. The advantages and limitations of the methods developed are given and discussed.

2. Experimental

2.1. Materials and chemicals

Sodium tetraborate decahydrate, 1,2-phthalic dicarboxaldehyde and mercaptoacetic acid were obtained from Acros Organics (Geel, Belgium), sodium dihydrogen phosphate from Merck (Darmstadt, Germany), potassium hydroxide and ammonia solution (ca. 25% NH_3) from Riedel de Haën (Seelze, Germany), sodium hydroxide and methanol (HPLC grade) from BDH (Poole, UK), boric acid from Merck Eurolab (Leuven, Belgium), and picric acid from UCB (Brussels, Belgium). Commercial kanamycin sulfate was obtained from Fluka (Buchs, Switzerland). All solutions were prepared with ultrapure Milli-Q water (Millipore, Milford, MA, USA) and filtered through a 0.2-μm filter (Euroscientific, Lint, Belgium). Solid-phase extraction cartridges (CBA, 100 mg/3 ml) were obtained from IST (Isolute, Sopachem, Brussels, Belgium).

Controlled human sera were obtained from Sigma (Bornem, Belgium). Serum samples from tuberculosis patients in Bangladesh receiving kanamycin were provided through the Damien Foundation. Peak samples were collected 1 h after the injection, trough samples were collected just before the next injection. The samples were stored in plastic tubes and kept frozen until analysis.

2.2. Derivatization reagent and separation buffer

The derivatization reagent consisted of 27 mg/ml OPA and 25 μl/ml MAA [18]. The reagent was freshly prepared by dissolving 540 mg of OPA in 2 ml of methanol and adding 15 ml of 30 m*M* borate buffer (pH 11.5) and 500 μl of MAA. The resulting solution was adjusted to pH 11.5 with 8 *M* potassium hydroxide before making up to 20 ml with the 30 m*M* borate buffer.

The separation buffer was composed of 30 m*M* borax, pH 10.0, containing 16% (v/v) methanol [18]. The buffer was prepared by dissolving 1.144 g of sodium tetraborate decahydrate in 80 ml water and after the pH was adjusted with 1 *M* sodium hydroxide, 16 ml methanol was added before making the solution volume up to 100 ml with water.

2.3. Instrumentation

An Agilent G1602 CE system equipped with a diode-array detector was used for the electrophoretic experiments. An HP 3D Chemstation (Rev. A. 09.01) was used for data acquisition. An uncoated fused-silica capillary of 50 μm I.D.×42.5 cm (34 cm effective length) (Polymicro Technologies, Phoenix, AZ, USA) was used. A system with adjustable vacuum was used for solid-phase extraction.

2.4. Electrophoretic conditions

CE separation conditions were adapted from the method previously developed in this laboratory [18]. For a new capillary, a 10-min flush with 1 *M* sodium hydroxide under a pressure of 100 kPa at 60 °C was carried out. Prior to analysis, the capillary was flushed with 0.1 *M* sodium hydroxide for 5 min, Milli-Q water for 5 min, both at 60 °C, and running buffer for 5 min at 20 °C. During analysis, the capillary temperature was set at 20 °C with a separation voltage of 23.5 kV and UV detection at 335 nm. The derivatized sample was hydrodynamically injected at a pressure of 5 kPa for 8 s.

2.5. Solid-phase extraction procedure

Detailed procedures for the extraction of kanamycin from serum with a CBA-bonded phase sorbent were as follows: the SPE cartridge was conditioned with 1 ml methanol followed by 1 ml phosphate buffer (20 m*M*, pH 7.4) at a flow-rate of 1–2 ml/min. 1 ml serum sample was diluted four times with deionized water and was applied to the SPE cartridge at a flow-rate of 1 ml/min. The cartridge was then washed with 2 ml phosphate buffer (20 m*M*, pH 7.4) followed by 4 ml of borate buffer (20

m*M*, pH 9.0) and 2 ml Milli-Q water at a flow-rate of 1–2 ml/min. The cartridge was dried by passing air for 3 min before the trapped analytes were eluted into a 3-ml test tube with 1 ml of borate buffer (0.2 *M*, pH 10.5)–methanol (50:50, v/v) or NH_4OH (25%, w/v)–methanol (30:70, v/v) at a flow-rate of 1 ml/min, whereafter the cartridge was sucked dry.

2.6. Field-amplified sample stacking

The procedure for on-line sample stacking involved the following steps. (a) The capillary was filled with the sample by hydrodynamic injection at a pressure of 5 kPa for 300 s, so that the whole capillary was filled with sample. (b) The capillary inlet was moved from sample vial to buffer vial, and a negative voltage of 23.5 kV was applied. The sample buffer was pushed out of the capillary by electroosmotic flow (EOF) while the negative sample ions were stacked inside the capillary. (c) When the sample buffer was almost completely pushed out of the capillary, the observed current reached a value of approximately 95% of the current generated when the whole capillary is filled with background electrolyte, and the negative voltage was stopped. (d) The polarity of the high voltage was switched back to normal configuration (positive at capillary inlet) and CE separation started.

3. Results and discussion

3.1. Optimization of extraction conditions

A weak cation exchanger containing carboxypropyl-bonded silica was reported for the successful extraction of gentamicin from plasma and biological samples [23,24]. Due to the similar strongly basic nature of aminoglycosides, these existing extraction procedures, in principle, can be used for the extraction of kanamycin from serum. In order to be compatible with subsequent derivatization and CE analysis, some modifications were necessary.

Since salts are present in serum at a concentration around 0.15 *M*, in order to obtain maximum retention of the analytes on CBA sorbent, the serum sample was diluted four times with deionized water to reduce the viscosity and make the salt concentration less than 0.05 *M*.

SPE cartridge solvation, equilibration, sample loading and interference elution were performed as described in the literature [23]. A solution containing 0.2 *M* borate buffer (pH 10.5)–methanol (50:50, v/v) was employed as elution solvent in the extraction of kanamycin from serum in this study. The use of an eluent containing 50% (v/v) methanol increased the efficiency of elution, but also maintained the optimum methanol concentration in the final extract for the subsequent pre-capillary derivatization.

One hundred microliters of OPA–MAA derivatization reagent and 10 μl picric acid (0.1 mg/ml) were added to the SPE extract and mixed well. After reaction at 40 °C for 5 min, the derivatized sample was injected into the capillary for CE separation and quantitation. The addition of picric acid as internal standard increases the precision of quantitation by accounting for variation of the final volume of the extract and for the variation in sample injection. The purity of the kanamycin peak in the electropherogram was determined by comparison of the UV spectra at different peak positions. The results showed that the SPE clean-up procedure developed in this study yielded a very clean extract, and no substance was found interfering with kanamycin in the CE separation.

The recovery of kanamycin extracted from spiked human serum was calculated by corrected peak area comparison with the same concentration of kanamycin in elution solvent which was derivatized directly. Using the procedure developed in this study, recoveries of kanamycin in the concentration range 5–40 μg/ml were found to be from 92.9 to 95.8% (Table 1).

3.2. Calibration and validation

Intra-day and inter-day precision were 6.2 and 10.3% RSD, respectively (30 μg/ml kanamycin-spiked serum, $n=6$). A five-point calibration was constructed for kanamycin in this study. Calibration standards spanning the 5–40 μg/ml range were made up based on the known weight of kanamycin spiked into 1 ml serum. External standard calibration was used with the analytical signal based on the

Table 1
Recovery and repeatability at different kanamycin levels by SPE–CE

Conc. (μg/ml)	Mean recovery (%)	RSD ($n=3$)
5	92.9	9.5
20	95.8	10.1
40	94.4	14.7

For experimental conditions, refer to Fig. 1.

corrected peak area obtained from the integration. The lower end of the working range was taken as the limit of quantitation (LOQ), which was defined as 10 times the background noise. The upper limit was taken as 5 μg/ml greater than the maximum peak kanamycin concentration in serum (35 μg/ml) described in United States Pharmacopeia Drug Information (USPDI) [19]. The limit of detection (LOD) was calculated based on a S/N ratio of 3. A linear response over the concentration range 5–40 μg/ml was obtained with a LOD of 2 μg/ml and a LOQ of 5 μg/ml ($y = 0.037x + 0.021$, $R = 0.9977$, SD 0.042) for the determination of kanamycin in serum using the developed SPE–CE method. The results showed the method covers the kanamycin blood concentration range (5–35 μg/ml) described in USPDI [19]. Thus, this method can be applied directly to the analysis of kanamycin in real serum samples.

3.3. Stability of kanamycin in serum

Because the serum samples had to be delivered from Bangladesh to Belgium, the stability of kanamycin in serum stored at room temperature was investigated. A 30 μg/ml kanamycin-spiked serum was divided into five batches. Serum aliquots were stored at ambient temperature for 3, 4, 5 and 7 days before analysis. The stability was tested by studying the recoveries of kanamycin from the spiked serum over a given time interval. An average recovery of 91.7% (normal recovery 93.8±8.6%) was obtained with a RSD of 10.5%. The results showed that serum containing kanamycin can be stored at ambient temperature for 1 week without change in the concentration of kanamycin.

3.4. Applicability of the SPE–CE method

To determine the kanamycin concentration in serum samples from TB patients, the serum was pretreated with the SPE procedure described above and after pre-capillary derivatization, the resulting sample was analyzed by CE. A typical electropherogram of kanamycin in serum of a TB patient is shown in Fig. 1, and it is very similar to the electropherogram obtained from the kanamycin-spiked serum sample. The peak kanamycin serum levels of the three selected patients were found to be 26.4, 21.9 and 26.9 μg/ml, respectively (corrected for a recovery of 90%).

However, the results of all three trough samples were below the detection limit of the SPE–CE method described above, which corresponds to 2.0 μg/ml. According to the USPDI guideline [19], it can be concluded that the patients have safe trough concentrations. This is due to the fact that the patients were injected kanamycin once daily. The trough samples were collected just before the next injection. Since the half-life of kanamycin is 2.1–2.4 h [28], the trough concentration should be less than 1 μg/ml in an adult with normal renal function.

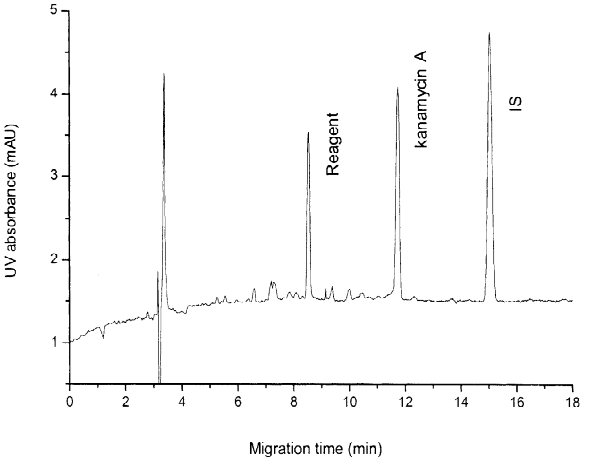

Fig. 1. Electropherogram of kanamycin in peak serum sample of a TB patient by SPE–CE. SPE analyte elution solvent, 1 ml borate (0.2 *M*, pH 10.5)–MeOH 50:50 (v/v); uncoated fused-silica capillary, 50 μm I.D.×42.5 cm (34 cm to detector); separation buffer, 30 m*M* borax buffer, pH 10.0, containing 16% methanol (v/v); applied voltage, 23.5 kV; detection wavelength, 335 nm; capillary temperature, 20 °C; injection, 5 kPa/8 s.

3.5. Optimization of field-amplified sample stacking

In order to determine low levels of kanamycin in the trough serum sample, an appropriate preconcentration method is needed to increase the sensitivity of the CE analysis. On-line FASS is the simplest technique to solve this problem.

3.5.1. The volume of derivatization reagent

Since OPA–MAA derivatization required the reaction medium to contain 50% methanol [18] and since FASS required a sample prepared in low-conductivity buffer (at least 10 times lower than the separation buffer), the experiment was carried out using a 2 μg/ml solution of kanamycin in Milli-Q water–methanol (50:50, v/v). However, salts were inevitably introduced into the sample solution by the addition of derivatization reagent and led to an increase in conductivity of the sample buffer. Excess reagent not only introduced more salts into the sample buffer, but also caused degradation of the kanamycin derivatives [18]. Therefore, as little reagent as possible was added to the kanamycin sample while maintaining the alkaline medium necessary for derivatization: the minimum volume of derivatization reagent to obtain a maximum reaction yield was found to be 15 μl.

3.5.2. pH of the derivatization reagent

The sensitivity of the CE method obviously increased after on-line sample stacking, but the peak of kanamycin A split into two peaks when using OPA–MAA reagent at pH 10.4 [18] for derivatization, as shown in Fig. 2. However, the UV spectra of these two peaks were identical. Upon increase of the pH of the derivatization reagent, the height of the smaller peak decreased. When the reagent pH was increased to 11.5, no peak splitting was observed. A tentative explanation could be that, due to the stacking process, the pH of the stacked sample zone became lower in the case of the pH 10.4 reagent, so that the carboxylic groups of the kanamycin derivatives were not fully ionized.

3.5.3. Buffer removal

After a negative voltage was applied, the positively charged ions and neutral molecules in the sample zone were pumped out of the capillary by the EOF,

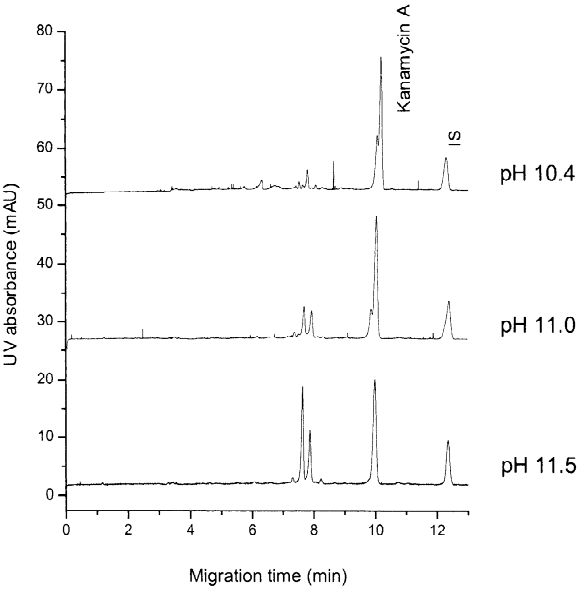

Fig. 2. Influence of the pH of the derivatization reagent on the peak splitting of kanamycin by FASS–CE. Injection time, 5 kPa/300 s. Other electrophoretic conditions are given in Fig. 1.

while the negative sample ions were stacked behind as a narrow zone. It was found that switching the polarity back when the observed current reached 95–98% of the current generated when the whole capillary is filled with background electrolyte, resulted in better peak shape. This can be explained because the more sample buffer removed, the narrower the sample band obtained.

3.5.4. Capillary loading length

The length of the capillary sample load was measured as follows: hydrodynamically injecting the sample into the capillary under a pressure of 5 kPa, the time needed to fill the effective length was obtained by monitoring the UV absorbance. By repeating the above experiment, the filling rate could be calculated. It took 195 s to fill the effective length in the present study. However, since the peak height increased non-linearly with sample loading, and in order to obtain as high as possible sensitivity, the whole capillary was loaded with sample at 5 kPa with an injection time of 300 s in this study.

With field-amplified sample stacking followed by CE separation, the concentrations of kanamycin and

picric acid were found to increase 28- and 36-fold, respectively. This is due to the fact that the smaller picric acid ion moves faster than kanamycin derivatives in the sample zone and results in better stacking.

3.6. Optimization of SPE for FASS–CE

FASS cannot be used for the direct injection of biological samples because of the high concentration of salts and the presence of proteins. Thus, a sample pretreatment procedure for salt and proteins removal is necessary. Although the SPE procedure described above could remove the proteins and the salts existing naturally in serum, it introduced a high concentration of borate at the analyte elution step. The borate salt in the final extract resulted in too high conductivity of the sample buffer to perform FASS.

There are two methods to obtain a low-conductivity sample buffer. One is using a low-conductivity solution as analyte elution solvent, and the other is desalting the SPE extract. Although the ion-exchange mechanism indicates that a buffer adjusted to two pH units above the pK_a of the analyte or a buffer with pH <2.8 can be used for the elution of the analytes from CBA sorbent, only 16.4% recovery was found when using 10 m*M* sodium hydroxide–methanol (10:90, v/v) as elution solvent, and no kanamycin was recovered with 30 m*M* HCl–methanol (10:90, v/v) solution. Thus, desalting had become the only way to obtain a low-conductivity sample buffer in this study. The Millipore drop dialysis method was used before to desalt a sample with a high ionic strength of sodium chloride [29]. However, the large size of borate salt is not as readily dialyzed as small ions. The use of concentrated NH_4OH in methanol for the elution of gentamicin from a CBA cartridge was reported by Cherlet et al. [24]. The ammonia was later removed by evaporation. Thus, in principle, a solution of NH_4OH–methanol would be ideal to elute kanamycin and to avoid the introduction of salt.

Since salts were retained on the CBA sorbent and the wall of the tubing, a diluted borate buffer (20 m*M*, pH 9.0) was used following phosphate buffer as solvent to elute interference compounds. To avoid these non-volatile salts being eluted in the final extract, a small volume of water was applied to the cartridge before drying and analyte elution.

3.6.1. Optimization of NH_4OH content in elution solvent

Solutions containing different volume ratios of NH_4OH (25%, w/v) and methanol were used to elute kanamycin from the CBA cartridge. The dependence of recovery on the NH_4OH content in the elution solvent is shown in Fig. 3. The results show kanamycin recovery increases significantly from 27% to more than 60% with the content of NH_4OH in methanol increasing from 10 to 25%. A further increase in NH_4OH content did not yield higher recovery. The greater the content of NH_4OH solution in the extract, the longer the evaporation time needed. As a compromise between sample preparation time and kanamycin recovery, a mixture of NH_4OH (25%, w/v)–methanol (30:70, v/v) was chosen for analyte elution in the present study. The elution volume was optimized by comparison of the recoveries from different volumes of elution solvent. No significant difference was found between the recoveries from 1 and 2 ml elution solvent. To shorten the sample preparation time, 1 ml of elution solvent was used in this study.

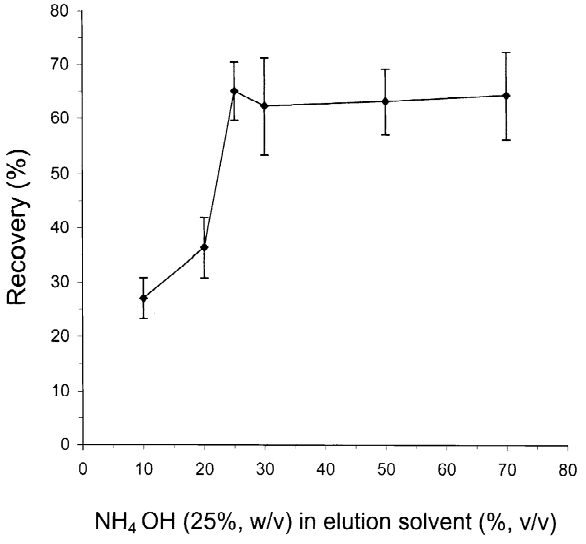

Fig. 3. Effect of NH_4OH on the recovery of kanamycin from serum.

3.6.2. Extract evaporation and residue re-dissolution

In order to eliminate the interfering effect of ammonium on the derivatization procedure and to obtain a low-conductivity sample buffer for sample stacking, ammonium removal is required. Thus, the SPE extract containing NH_4OH and methanol was evaporated to dryness at a temperature of 100 °C in a water bath. The residue was re-dissolved in 1 ml of methanol–water (50:50, v/v) before derivatization.

3.7. Analytical parameters of SPE–FASS–CE

With the SPE–FASS–CE procedure developed, the extract of kanamycin-free serum was very clear and showed no peaks that would interfere with the determination of kanamycin by CE. Electropherograms of kanamycin-free serum and kanamycin-spiked serum are shown in Figs. 4 and 5. Good linearity in the concentration range 0.4–5.0 μg/ml was obtained ($y = 0.820x - 0.035$, $R = 0.9995$, $n = 5$, SD 0.0569), with a LOD of 0.1 μg/ml and a LOQ of 0.4 μg/ml. Although the pre-concentration factor for kanamycin by SPE–FASS–CE with respect to SPE–CE is 25-fold, the higher noise level resulting from sample stacking decreased the sensitivity so that the LOD and LOQ values for SPE–FASS–CE were higher than expected. Intra-day and inter-day precision for the whole analysis procedure, including solid-phase extraction, evaporation, pre-capillary derivatization and on-line sample stacking capillary electrophoresis, were 3.4 and 11.2%, respectively (1 μg/ml kanamycin-spiked serum). The recoveries of different concentrations from solid-phase extraction and evaporation ranged from 61.9 to 68.7% (Table 2).

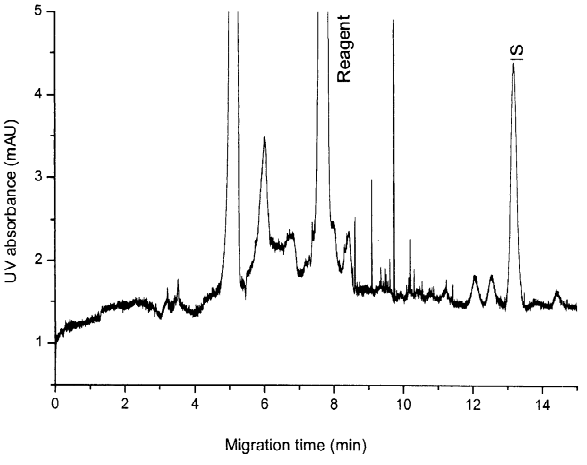

Fig. 4. Electropherogram of kanamycin-free serum by SPE–FASS–CE. SPE analyte elution solvent, 1 ml NH_4OH (25%, w/v)–methanol (30:70, v/v); CE injection time, 5 kPa/300 s. Other CE conditions are given in Fig. 1.

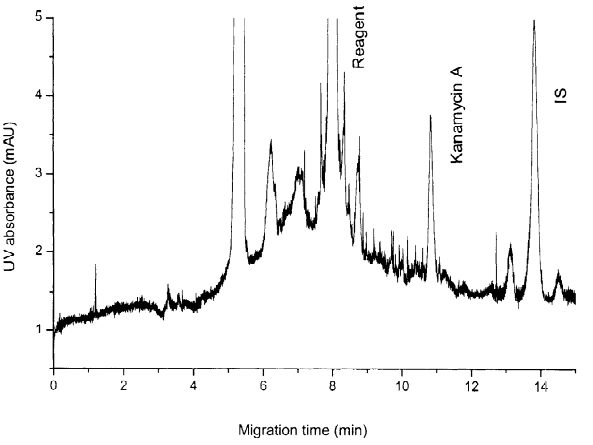

Fig. 5. Electropherogram of 1 μg/ml kanamycin-spiked serum by SPE–FASS–CE. Experimental conditions are given in Fig. 4.

3.8. Application of SPE–FASS–CE

A typical electropherogram of a trough serum sample from a TB patient on kanamycin is shown in Fig. 6. The kanamycin concentrations in the trough serum samples from the three selected patients were found to be 0.9, 1.9 and 0.8 μg/ml, respectively (corrected for a recovery of 60%).

Table 2
Recovery and repeatability at different kanamycin levels by SPE–FASS–CE

Conc. (μg/ml)	Mean recovery (%)	RSD ($n = 3$)
0.5	68.7	8.2
1.0	62.4	11.2
5.0	61.9	10.5

For experimental conditions, refer to Fig. 4.

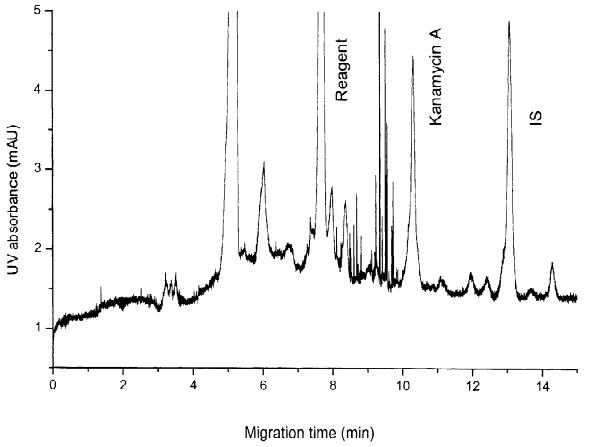

Fig. 6. Electropherogram of kanamycin in a trough serum sample of a TB patient by SPE–FASS–CE. Experimental conditions are given in Fig. 4.

3.9. Interpretation of the kanamycin levels in serum of patients

For the analysis of kanamycin by the methods developed in the present study, serum samples from three patients were selected based on different doses, age and clinical status, according to the patient information provided by the hospitals. The results are shown in Table 3. It was found that all the patients' peak serum kanamycin concentrations were in the recommended therapeutic range (15–30 μg/ml). Results showed that the trough serum kanamycin concentrations of all patients were below the recommended maximum value of 5 μg/ml. The reasons for the occurrence of low kanamycin levels in trough serum samples are the short kanamycin half-life and the once-daily drug injection therapy.

All trough concentrations in the selected patients in this study were higher than the theoretical value of 0.05 μg/ml (calculated from peak concentration, kanamycin half-life and sampling time), which indicated the accumulation of kanamycin in patient serum due to long-term therapy. The higher trough concentration found in patient SSK-1 can be explained by the fact that this patient received 90 doses of kanamycin by the time of sampling, while patients T-66 and MDR-4 received 53 and 35 doses, respectively. Similar trough drug levels were found in patients T-66 and MDR-4, although patient T-66 received more and higher doses of kanamycin. This may be due to the fact that patient T-66 was younger than MDR-4, resulting in better kanamycin excretion.

4. Conclusions

Two hyphenated techniques based on solid-phase extraction and capillary electrophoresis were developed in this study. An SPE procedure with borate buffer (0.2 *M*, pH 10.5)–methanol (50:50, v/v) as elution solvent followed by pre-capillary derivatization and capillary electrophoresis was developed and has been successfully applied to the analysis of kanamycin in peak serum samples from TB patients. The results showed that this SPE–CE method can be used directly in clinical analysis. Due to its simplicity, short analysis time and high recovery, this method is recommended for treatment failure and toxicity monitoring.

In SPE–FASS–CE, NH_4OH (25%, w/v)–methanol (30:70, v/v) was used to elute the trapped kanamycin from the SPE cartridge. After evaporation, residue re-dissolution, and pre-capillary derivatization, the kanamycin sample was analyzed by

Table 3
Kanamycin level in serum of three selected patients

Patient	Age (years)	Weight (kg)	Dose (mg)	No. of doses received	Peak conc.[a] (μg/ml)	Trough conc.[b] (μg/ml)
T-66	37	53	1000	53	26.4	0.9
SSK-1	40	39	750	89	21.9	1.9
MDR-4	45	49	750	35	26.9	0.8

[a] Analysis by SPE–CE. For experimental conditions, refer to Fig. 1.
[b] Analysis by SPE–FASS–CE. For experimental conditions, refer to Fig. 4.

Table 4
Analytical parameters of the two methods developed in this study

	Linear range (μg/ml)	Linear equation	Correlation coefficient	LOQ (μg/ml)	LOD (μg/ml)	Recovery (%)	Intra-day RSD (%) ($n=5$)	Inter-day RSD (%) ($n=5$)
SPE–CE	5–40	$y = 0.037x + 0.021$	0.9977	5	2	90	6.2	10.3
SPE–FASS–CE	0.4–5.0	$y = 0.820x - 0.035$	0.9995	0.4	0.1	60	3.4	11.2

For experimental conditions for SPE–CE, refer to Fig. 1, for SPE–FASS–CE, refer to Fig. 4.

on-line field-amplified sample stacking capillary electrophoresis. This method has been successfully used for the analysis of kanamycin in a trough serum sample. Due to its low LOD and LOQ, this method is recommended for pharmacokinetic study.

The analytical parameters of the two methods developed for determination of kanamycin in serum are shown in Table 4. The results indicate that the two methods developed in the present study cover the concentration range of kanamycin present in serum at both peak level and trough level and demonstrate the applicability for therapeutic kanamycin monitoring and pharmacokinetic study.

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