Evaluation of dynamics of derivatization and development of RP-HPLC method for the determination of amikacin sulphate

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Abstract: The absence of chromophore and/or conjugated system, prerequisite for UV and florescent light detection, or absorbance at very low wavelength necessitates the development of simple and reliable methods for the determination of amikacin sulphate. Therefore, the present study describes for the first time dynamics of the drug derivatization using ninhydrin reagent and development and validation of a simple RP-HPLC method, using diode array detector (DAD). The variables such as heating time, heating type, drug-reagent ratio, reagent composition and storage temperature of the derivative were optimized. The analyte and aqueous ninhydrin solution upon heating for 2.00-5.00 min produced the colored drug-derivative which was stable for one month at refrigeration. The derivatized drug (20.00µL) was eluted through a column – Eclipse DB-C_{18} (5.00 µm, 4.60×150.00 mm), maintained at 25°C- using isocratic mobile phase comprising water and acetonitrile (70:30, v/v) at a flow rate of 1.00 mL/min, and detected at 400 nm. The method was found to be reliable (98.08–100.72% recovery), repeatable (98.02–100.72% intraday accuracy) and reproducible (98.47–101.27% inter day accuracy) with relative standard deviation less than 5%. The results of the present study indicate that the method is easy to perform, specific and sensitive, and suitable to be used for the determination of amikacin sulphate in bulk and pharmaceutical preparations using less expensive/laborious derivatization.

Keywords: Amikacin sulphate, DAD-Detection, Derivatization, Ninhydrin, RP-HPLC.

INTRODUCTION

Amikacin sulphate (AS), D-streptamine, O-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-O-(6-amino-6-deoxy-α-D-glucopyranosyl- (1→4)) -N1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-(S)-sulphate, is an aminated cyclitol containing 4 primary and 1 secondary amino groups, which protonate at neutral pH (Feng *et al*., 2001; Jiang *et al*., 2011). The drug does not have any chromophore and/or conjugated system prerequisite for UV and/or florescent light detection (Chang *et al*., 2010). However, fortunately, the drug has functional groups that can be derivatized to produce UV/florescence detection-oriented derivatives (Ovalles *et al*., 2005). Several types of chemical reactions such as Rimini test (Confino and Panayot, 1990), Hantzsch reaction (Gupta *et al*., 1984, Churagulova, 1987), cyanoacetamide (Zakhari, 1990), Ninhydrin test (Feng-ming *et al*., 2003) and charge transfer complex formation reaction (Al-Sabha, 2010) have been used to prepare UV active derivatives of the drug.

Stead (2000) comprehensively reviewed the literature regarding the use of diverse approaches including microbiological and radiochemical assays for the estimation of the drug. A variety of chromatographic assay had also been developed for the determination of the drug (Wong *et al*., 1982; Caturla and Cusido, 1992; Lung *et al*., 1998; Feng *et al*., 2001; Oertel *et al*., 2004; Ovalles *et al*., 2005; Nicoli and Santi, 2006; Baranowska *et al*., 2006; Chang *et al*., 2010; Baietto *et al*., 2010). Though, every method had its pros and cons, liquid chromatography with pre- or post-column derivatization using various reagents – for both UV and florescent light detection – remained the most favorite method for the determination of the drug (Wong *et al*., 1982; Caturla and Cusido, 1992; Lung *et al*., 1998; Tawa *et al*., 1998; Isoherranen and Soback, 1999; Soltes, 1999; Feng *et al*., 2001; The United States Pharmacopoeia USP28-NF22, 2005; Ovalles *et al*., 2005; Nicoli and Santi, 2006; Baranowska *et al*., 2006; Chang *et al*., 2010).

Most of the derivatization processes introduced till date is not suitable for quality control and analysis due to time factor, laborious work needed for derivatization, expensive reagents and stability of the complex under mild reaction conditions (Caturla and Cusido, 1992; Liu *et al*., 2001). To overcome these issues, other instrumental techniques like pulsed electrochemical detection on gold electrode (Adams *et al*., 1995; Brajanoski *et al*., 2008), mass spectrophotometric detection (Jiang *et al*., 2011), evaporative light scattering detection (Galanakis *et al*., 2006), post-column ligand displacement reaction with fluorescence detection (Yang and Tomellini, 2001) and chemiluminescence detection (Serrano and Silva, 2006) have been used. But all such techniques need special and expensive instrumentation; therefore there is need of developing simple methods that can be employed in less

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developed or equipped laboratories. Keeping all the aforementioned factors in view, the present study aimed to evaluate dynamics of complex formation of AS with ninhydrin as a derivatization agent, and develop a RP-HPLC method for the determination of the drug.

MATERIALS AND METHODS

Chemicals, reagents and other supplies

Analytical grade amikacin sulphate (standard), certified to contain 99.80% of drug, was gifted by M/S Global Pharmaceuticals, Islamabad, Pakistan. The amikacin sulphate injections (Brand A; Anonymized- 125 mg/mL, Batch No. 09 M and Brand B; Anonymized- 250 mg/mL, Batch No. 022, SAMI Pharmaceuticals (Pvt.) Ltd. Karachi, Pakistan) were purchased from local pharmacies. Ninhydrin (Simpsons Ltd. UK) and analytical/HPLC grade solvents such as acetone and acetonitrile (E-Merck) were procured from the local market. Other materials included in-house prepared purified/distilled water, nylon-H filters (0.45 µm, Micropore) and polytetrafluoroethylene syringe filters (0.45 µm, Whatman, Maidstone, England).

Instrumentation

UV/Visible analysis was performed using a double beam UV/Visible spectrophotometer (Shimadzu Scientific Instruments, Japan), Serial No. A10844400603 LP, CAT 206-24401-93, Model UV-2550, connected to Fujitech computer and equipped with UV Probe 2.21 operating software. The absorption spectra of the standard and test solutions were recorded in 1cm quartz cells over the whole range of UV/Visible spectrum (800–200 nm).

High performance liquid chromatography system used in the present study was 1200 series (Agilent Technologies, Waldron, Germany), equipped with isocratic pump (G1310 A), auto sampler (G1329 A), column oven (G1316 A) and diode array detector (DAD) (G 1315 B). ChemStation LC/LCMS for Windows was used for data acquisition.

Preparation of standard solutions of non-derivatized and derivatized drug

Standard stock solutions

Standard stock solution of non-derivatized AS (ndAS) of concentration 125.00 mg/mL was prepared in distilled water. Then, the standard solutions of the drug for derivatization were prepared by two methods which are given as follows:

Without extraction

Two hundred microliters of the standard stock solution (125.00 mg/mL) was added in 2.00 mL ninhydrin solution. The reaction mixture was heated until the development of Ruhemann purple color. Then the contents were transferred to a 10.00 mL volumetric flask and made up the volume with distilled water to get the final derivatized standard stock solution having concentration 2.50mg/mL.

With extraction

Two hundred microliters of the standard stock solution (125.00 mg/mL) was loaded on pre-conditioned cartridge containing 1.0 g silica gel and washed with 4.00 mL of methanol to remove impurities, if any. The sample was eluted by 3.00 mL of ninhydrin solution and derivatization was carried out as stated above.

Working standard solutions

The standard stock solution of derivatized AS (dAS) obtained with and without extraction having concentration 2.50 mg/mL were diluted with distilled water to get a range of working standard solutions having a concentration 0.0625, 0.125, 0.187, 0.250, 0.500 and 0.750 mg/mL.

Determination of λ_{max} and optimization of variables

The standard stock solutions of dAS and ndAS, having concentration 2.50 mg/mL and 125.0 mg/mL, respectively, were scanned at 800–200 nm using distilled water and ninhydrin solution (without analyte) as blank to determine λ_{max}. Various variables such as heating time for effective derivatization, heating type (direct on flame and water bath), drug-reagent ratio, reagent composition and storage temperature of the derivatized drug were optimized.

Chromatography conditions

Each of the working standard derivative solutions (20.00 µL) was eluted through a column – Eclipse DB-C_{18} (5.00 µm, 4.60 × 150.00mm) – using isocratic mobile phase comprising water and acetonitrile (70:30, v/v) at a flow rate of 1.00 mL/min. The temperature of the column was maintained at 25°C and detection was carried out at 400 nm using DAD.

System suitability

To ensure whether the system is working accurately during the analysis, system suitability parameters – capacity factor (k'), selectivity (α), resolution (Rs), tailing factor, peak asymmetry (As) etc. – were evaluated.

Validation of the method

Linearity

The linearity was assessed visually by plotting six concentrations from 0.0625–0.750 mg/mL versus their responses in peak height. Correlation of data points was assessed by correlation coefficient (R^2).

Range

The Beer's range was derived from the linearity studies carried out as discussed earlier.

Sensitivity: limit of detection (LOD) and limit of quantification (LOQ)

Six standards solutions having concentration 0.0625 to 0.750 mg/mL were analyzed in quintuplicate and the data were utilized to construct five standard curves. LOD at a

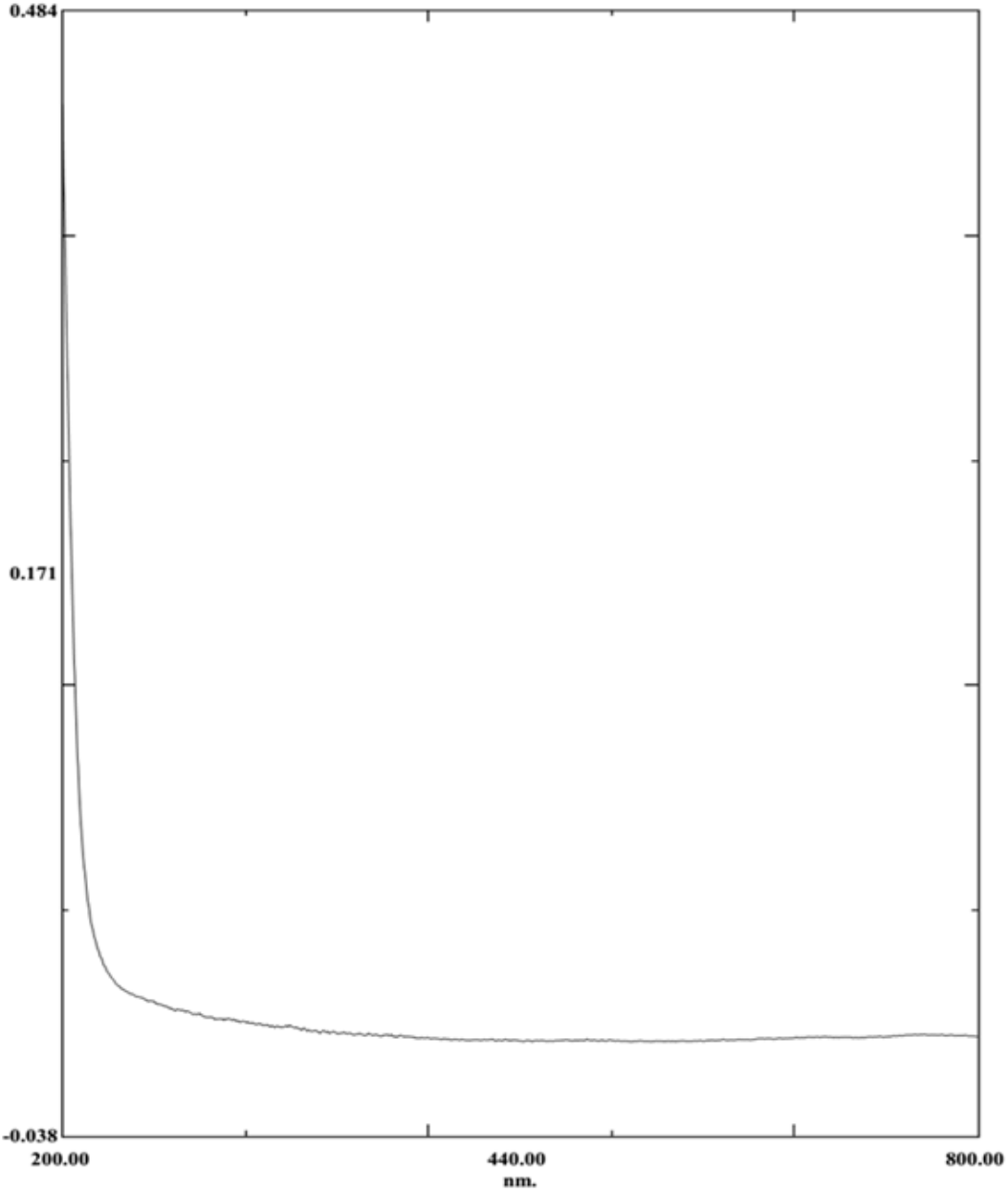

Fig. 1: UV/Visible spectrum of amikacin sulphate in distilled water

signal to noise (S/N) ratio 3:1 and LOQ at S/N ratio 10:1 were determined statistically.

Recovery, intra- and inter- day accuracy and precision

Three working standard solutions (0.125, 0.250 and 0.750 mg/mL) were used to determine recovery, intra- and inter-day accuracy and precision. For recovery, the standard solutions were analyzed in triplicate and their concentrations were determined from the calibration curve. The percentage recovery was determined by comparing obtained values to the true values.

In order to assess the quality of the assay, accuracy and precision estimates i.e. repeatability, reproducibility were carried out at three concentration levels of the standard solution. For intra- and inter- day accuracy and precision, each of the three standard solution concentrations was analyzed six times in a single day and once daily for six consecutive days, respectively. The amounts were determined from calibration curves, constructed each day.

Specificity

Specificity is the ability of the assay to estimate unequivocally the compound of interest in the presence of other components e.g. excipients, impurities, degradants and matrix etc. For evaluating the specificity of the method, commercial injections containing AS were used. Volume (200.00 µL) of commercial injections was treated

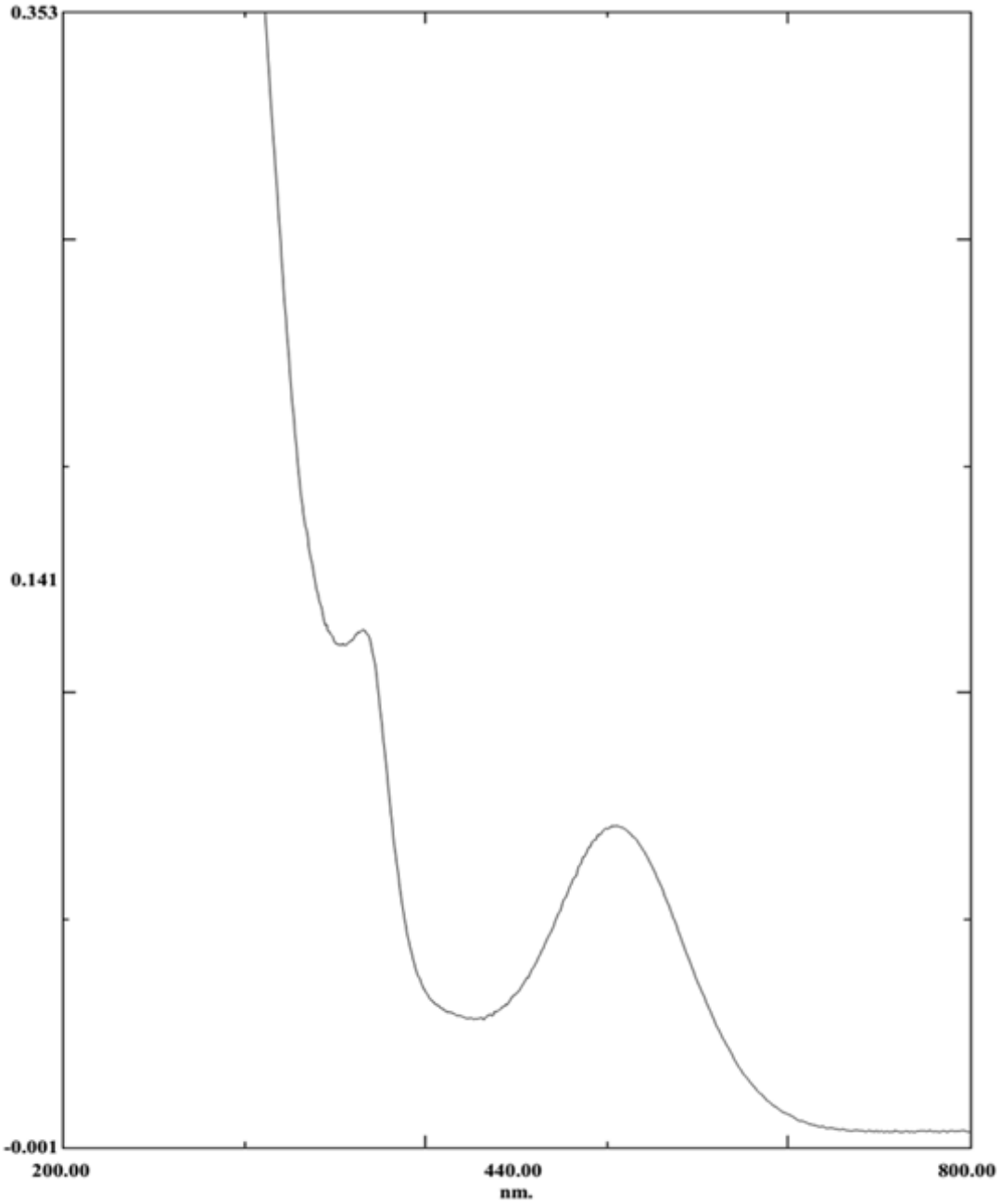

Fig. 2: UV/Visible spectrum of the derivatized amikacin sulphate (dAS)

like the standard stock solution. The working sample solution was prepared by diluting the stock solution (2.50 mg/mL) appropriately so that the concentration fell within the range of the calibration curve. Then six standard solutions and two sample solutions were analyzed in triplicate for the aforementioned chromatographic conditions, and amount of the drug was quantified from the standard curve (linear regression equation).

Robustness

The effect of slight deliberate changes in column temperature, detection wavelength and mobile phase composition on the recovery of the drug was investigated to find the robustness of the method.

Stability of the complex

The stability of the standard and sample solution was evaluated by storing them in screw capped test tubes, protected from light at room temperature and in the refrigerator/freezer. Recovery of these solutions was compared with the freshly prepared solution.

RESULTS

Derivatization of the drug and spectral changes

The UV/Vis absorption spectrum of ndAS solution is shown in fig. 1 which indicated that the drug did not possess any UV absorbing species in the structure. However, the UV/Vis scan of AS after derivatization with

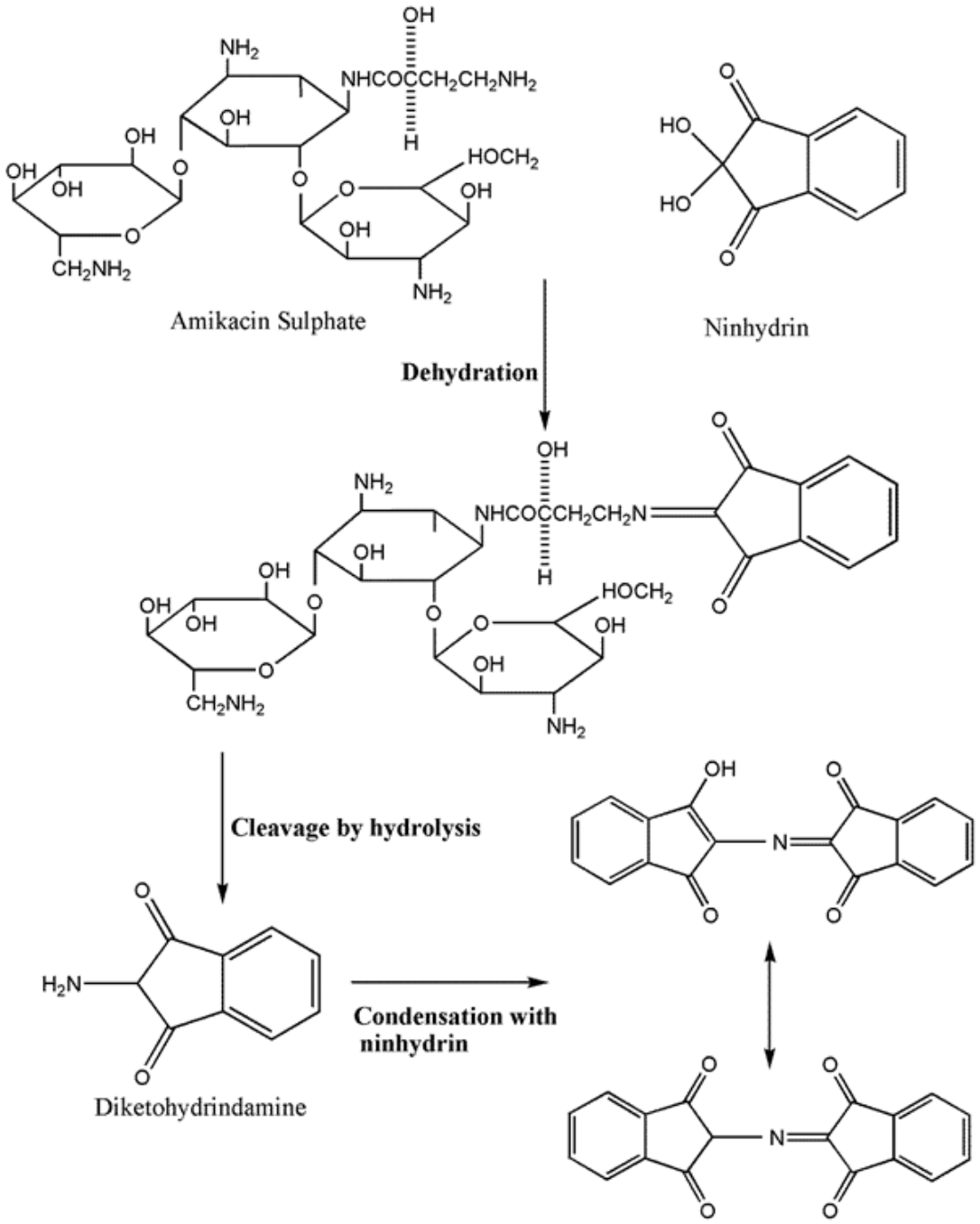

Fig. 3: Reaction sequence indicating mechanism of derivatization of amikacin sulphate

ninhydrin reagent (fig. 2), using distilled water as well as ninhydrin reagent solution as blanks, indicated presence of two chromophores giving maximum absorption at 400 nm and 567 nm.

Derivatization reaction sequence

In the proposed method, the mechanism of derivatization involved amination of ninhydrin by reacting with AS to form diketohydrindamine, which then underwent condensation reaction with another ninhydrin molecule to form an intense purple colored complex (fig. 3).

Dynamics of derivatization

The Ruhemann purple color of the reaction mixture depends on the type of amino group (primary, secondary, tertiary), the concentration of analyte, pH (acidic environment is suitable), temperature and strength of the ninhydrin solution. Therefore, all such variables need to be optimized for good results. Hence, we investigated and optimized the variables in the formation of the drug's complex with ninhydrin reagent. The optimum reagent composition and amount, analyte's concentration, heating conditions and heating time, and storage temperature of the complex are summarized in table 1.

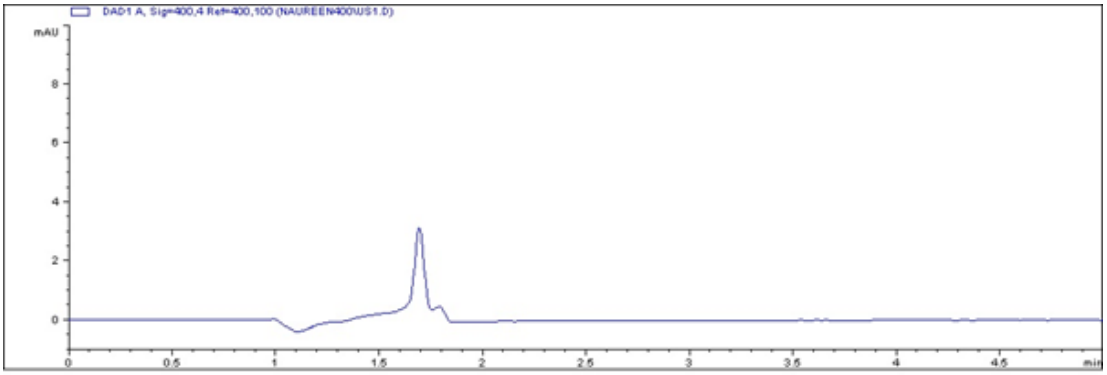

Fig. 4: Chromatogram of amikacin sulphate (AS) standard solutions (0.25 mg/mL) after derivatization with ninhydrin reagent

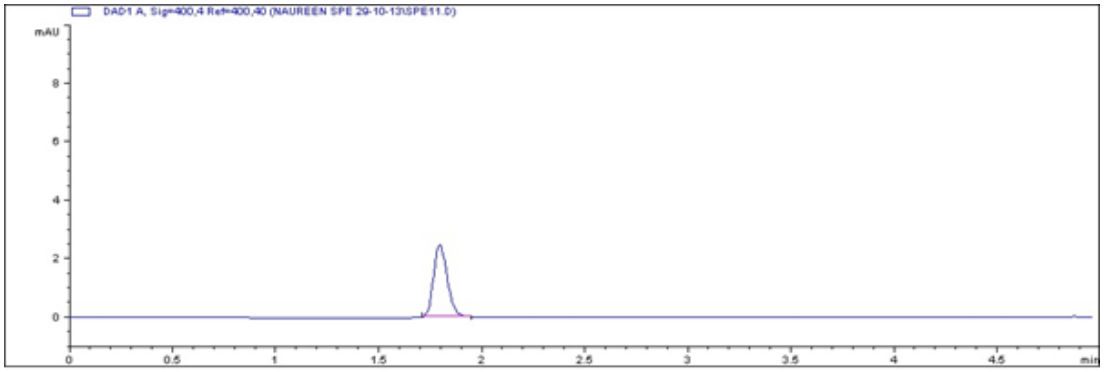

Fig. 5: Chromatogram of amikacin sulphate (AS) standard solutions with extraction (0.25 mg/mL) after derivatization with ninhydrin reagent

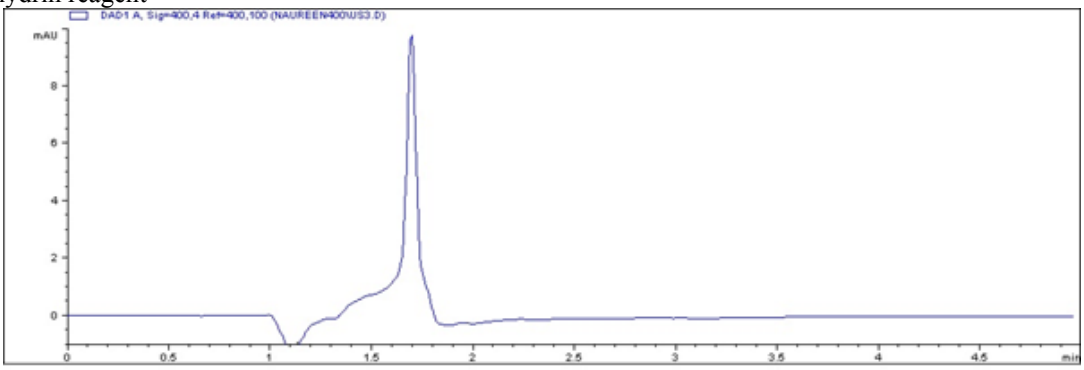

Fig. 6: Chromatogram of amikacin sulphate (AS) commercial sample without extraction (0.75 mg/mL) after derivatization with ninhydrin reagent and detection at 400 nm

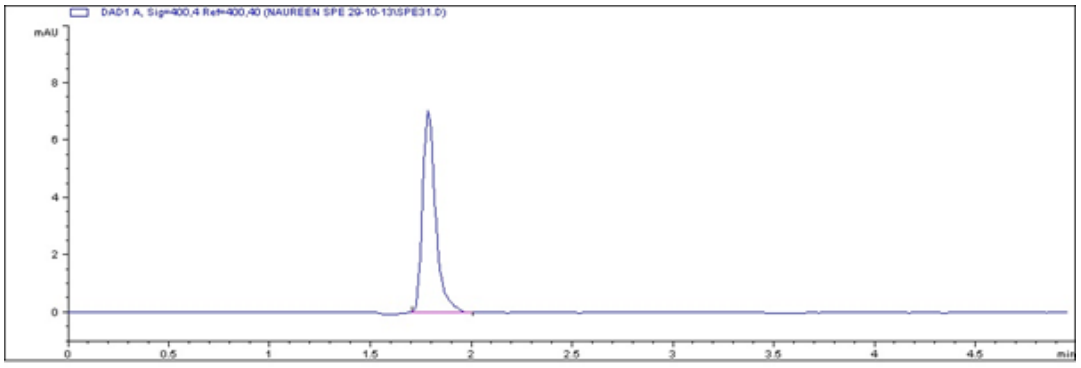

Fig. 7: Chromatogram of amikacin sulphate (AS) commercial sample with extraction (0.75 mg/mL) after derivatization with ninhydrin reagent and detection at 400 nm

Table 1: Optimized derivatization conditions

Derivatization factors	Optimized reaction condition
Reagent composition	0.20 g of ninhydrin in 94.00 mL water and 6.00 mL acetone
Reagent amount	2.00–3.00 mL
Analyte concentration	200.00 µL
Heating type	Direct heating on Bunsen burner flame
Heating time	Approx. 2.00–5.00 min
Storage condition	1h at room temperature, 7 days in refrigeration and 1 month in freezer

Table 2: System suitability parameters for the determination of amikacin sulphate

Parameters	Values
Number of theoretical plate (column efficiency)	5416.96
Height equivalent to theoretical plate	0.0276
Reduced plate height	5.538×10^{-3}
Peak symmetry	1 at 1/10h of the peak
Tailing factor	0.916 at 1/20h of the peak
Capacity factor	0.72
Peak repeatability response	0.40

Table 3: Results of calibration, LOD and LOQ of amikacin sulphate by HPLC method

Standard curve	Calibration range mg/mL	Linear regression equation	Slope	Intercept
1	0.0625–0.750	Y= 9.3269x + 0.1379 R^2=0.9899	9.3269	0.1379
2	0.0625–0.750	Y= 9.3813x - 0.0922 R^2=0.990	9.3813	0.0922
3	0.0625–0.750	Y= 9.3864x - 0.0924 R^2=0.9897	9.3864	0.0924
4	0.0625–0.750	Y= 9.284x - 0.0895 R^2=0.9953	9.284	0.0895
5	0.0625–0.750	Y= 9.1817x - 0.0865 R^2=0.9986	9.1817	0.0865
Mean slope (S)			9.3121	
Standard deviation (SD)				0.0215
LOD = 3.3 SD/S				0.0076
LOQ = 10 SD/S				0.0231

Table 4: Recovery, intraday and inter day accuracy and precision values of amikacin sulphate by HPLC method.

Concentration (mg/mL)	Recovery (n=3)		Intraday (n=6)		Inter-day (n=6)	
	(%)	SD	Accuracy	Precision	Accuracy	Precision
0.125	100.72	0.0081	100.72	2.59	101.27	2.48
0.250	98.08	0.0381	98.02	2.45	98.47	3.14
0.750	99.14	0.0089	99.07	0.43	99.15	2.39

Table 5: Percentage contents of amikacin sulphate in commercial injections determined at 400 nm (n=3)

Name of injection	Amount stated (mg)	Amount calculated (mg)	% age contents ± SD
(Anonymized) Amikacin sulphate	250.00	248.48	99.39 ± 3.42
	500.00	497.87	99.57 ± 3.67

RP-HPLC method development

A working standard solution of dAS without and with extraction is shown in fig. 4 and fig. 5. The dAS prepared without and with extraction eluted through the column at 1.70 min and 1.80 min respectively. It is noteworthy that although no significant difference was observed in the retention time of the peaks, however, the chromatogram of the drug with extraction was better because the baseline was smooth and impurities causing interference in the determination of AS were disappeared.

System suitability parameters

The system suitability parameters calculated from the peak of analyte having concentration 0.25 mg/mL are given in table 2. As per CDER (1994) guidelines, the tailing factor, peak asymmetry, column efficiency values

and peak repeatability response indicated that the method is suitable.

Validation of the RP-HPLC assay

Linearity

The linearity of the assay was assessed by analyzing six concentrations, ranging from 0.0625–0.750 mg/mL, in three replicates and plotting them versus their responses (peak height). The linearity of the calibration curve and adherence of the system to the Beer-Lambert's law was confirmed by the high value of correlation coefficient (R^2) and y-intercept value that was approaching to zero. The method was found to be linear over the whole range investigated with correlation coefficient 0.9899–0.9989 and standard deviation less than 5% (table 3).

Calibration range

The range of calibration was established by considering the practical range necessary, according to the drug concentration in the pharmaceutical products, in order to provide linear, accurate and precise estimations (table 3).

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ)

For the estimation of LOD and LOQ of the drug, various approaches can be used such as visual evaluation, signal-to-noise ratio and statistical method, using SD of the intercept and mean of the slope. In the present study, we have used a statistical approach to determine sensitivity of the method. The results shown in table 3 indicated LOD (0.0076 mg/mL) and LOQ (0.023 mg/mL).

Recovery, intra- and inter-day accuracy and precision

The results of recovery, intra- and inter-day accuracy and precision of the method are given in table 4. Mean recovery was found to be 98.08–100.72% with relative standard deviation less than 5%, which indicated that the method was reliable. Intra- and inter-day accuracy values were 98.02–101.27% with relative standard deviation less than 5%, which proved that the method was repeatable and reproducible.

Specificity

The method was successfully applied to the determination of contents of the drug in commercially available injections. The chromatograms of the derivatized sample solutions prepared without and with extraction are given in fig. 6 and 7, respectively, which also show results similar to those stated above. The results shown in table 5 indicate that the method is suitable as contents of the sample correspond to the label claim.

Robustness

For estimating the robustness of the method, percentage of organic strength of the mobile phase was altered (± 2.00) purposefully and no significant effect on chromatographic resolution was noticed. Furthermore, small deliberate changes in the flow rate (± 0.20mL/min), column oven temperature (± 2.00) did not affect the results.

Solution stability

Recovery of the standard and sample solutions kept in screw capped test tubes, protected from light, stored at room temperature and in refrigerator/freezer was compared with the freshly prepared solution. The solutions stored in the laboratory at room temperature, refrigerator and freezer were found to be stable for one hour, seven days and one month, respectively.

DISCUSSION

Consistent to the reports of the earlier studies (Wong *et al*., 1982; Gupta *et al*., 1984; Churagulova, 1987; Confino and Panayot, 1990; Lung *et al*., 1998; Feng *et al*., 2001; Yang and Tomellini, 2001; Feng-ming *et al*., 2003; Ovalles *et al*., 2005; Galanakis *et al*., 2006; Nicoli and Santi, 2006; Serrano and Silva, 2006; Brajanoski *et al*., 2008; Chang *et al*., 2010; Al-Sabha, 2010), we also observed that the scan of AS solution in UV/Vis region (800–200 nm) using distilled water as blank showed no absorption in this region. However, highly concentrated solution the drug gave a peak at 210 nm, which could not be used for quantification because UV/Vis detectors did not give smooth baseline at such a low wavelength. Therefore, the chromophore was introduced in the drug, by reacting it with ninhydrin reagent, to allow reliable detection and quantification of AS in the bulk and dosage form.

From the scans of the drug, after derivatization with ninhydrin reagent, two peaks (one at 400 nm and second at 567 nm) were observed. The literature contained no report about the behavior of derivatized AS at 400-403 nm. As far as the 2nd peak is concerned, our results were consistent with that reported earlier, wherein ninhydrin-derivatized drug has shown maximum absorbance at 568 nm (Feng-ming *et al*., 2003). It was further noted that the spectra of the complex were similar using both of the blanks (distilled water and ninhydrin solution).

Ninhydrin was used for derivatization of AS since the reagent upon heating in the presence of α-amino group containing compounds give a purple color product, which can be used for qualitative and quantitative purposes (Harding and Warneford, 1916). The change in the color of ninhydrin was due to the major disturbances in the electron confinement that led to the formation of an anion, wherein negative charge delocalized to form an immense number of resonant structures. The disturbances in the electron confinement are also reported to be associated with acid-base indicator color changes (Omer *et al*., 2015).

Our extensive and exhaustive literature review indicated the use of ninhydrin solution as a detecting agent for

various amino containing compounds. Ethanolic solution of ninhydrin was used for detection of aminoglycoside antibiotics by spraying on the thin-layer chromatographic plates and subsequent determination using densitometer at 500 nm (Fred, 1997–2010). Another report was found on the development of spectrofluorimetric method for determination of AS and related compounds through their condensation with ninhydrin and phenyl acetaldehyde and determination of numerous factors affecting the complex formation (Hubicka *et al*., 2009). Regarding the dynamics of the derivatized drug complex with ninhydrin reagent alone, no data reports were available in the literature, hence the present investigation is the first report, describing dynamics of amikacin sulphate derivatization.

Ninhydrin test solution can be prepared by using a variety of solvents e.g. rectified spirit, methanol, ethanol, acetone and heptane etc. Often, it is prepared by dissolving 0.20 g ninhydrin in 100.00 mL ethanol. So, for the present study, initially the reagent solution was prepared in ethanol, but severe bumping of the reaction mixture was observed during the heating. So, the reagent/solvent composition was optimized i.e. 0.20 g ninhydrin was dissolved in a mixture of water and acetone (94.00 mL water and 6.00 mL acetone). Also, it was noted that derivatization of the drug by ninhydrin dissolved in ethanol and in our solvent system yielded the same scans. Rationale behind using the water in higher proportion in our optimized solvent system was to prevent any complication/bumping of the reaction mixture while heating. It was observed that the amount of sample and the quantity of the reagent were other variables affecting the derivatization of the drug. The use of more quantity of the reagent (4.00–5.00 mL) didn't produce better results perhaps due to dilution of the drug in water and more water means increase in time required for the mixture to convert into complex, since water takes time to boil (slowed frequency of collision of the molecules and decreased rate of reaction to produce optimum results). The optimum amount of the reagent was found to be 2.00–3.00 mL to derivatize 200.00 µL of the drug's solution having concentration 125.00 mg/mL. It has been observed that derivatization occurred at higher temperatures e.g. heating at 80-100°C for 5.00 min in water bath or by direct heating on the lowest region of the flame for 2.00 min. The heating of solution at low temperatures for longer duration resulted in the development of orange or yellow colored products (false results). It was further noted that the color of the complex once developed tends to fade with the passage of time. However, the complex was found stable for seven days in the refrigerator and approximately one month in freezer.

During the development of the method, two types of columns (Eclipse DB-C_{18}, 5 µm, 4.6×150 mm and Zorbax Stablebond analytical SB-C_{18}, 5 µm, 4.6×150 mm) were used and changes in the column temperature were made to optimize drug response. It was found that the drug elution through the former column, maintained at 25°C temperature, gave the best results. Several types of mobile phases (tetrahydrofuran: water: methanol, 50:40:20; tetrahydrofuran: water: methanol: acetic acid, 50:30:15:5; acetonitrile: water: acetic acid, 35:60:5; acetonitrile: water: acetic acid, 50:49:01) were investigated. Changes in the pH of the mobile phase and the flow rate were made to improve resolution (Rs), retention time (t_R) and peak symmetry. The optimum separation was achieved by mobile phase consisting of water and acetonitrile (70:30, v/v) at 1.70 min. Based on UV/Vis profile of the complex, detection was carried out at two wavelengths, 400 nm and 567 nm, using DAD. But the response of the detector was found to be good at the former as compared to that of the latter. These results indicated that a 567 nm wavelength was not suitable for quantitative purposes because the detector did not give the required response. The chromatograms of the dAS without and with extraction using optimized chromatographic conditions described above are shown in fig. 4 and 5, respectively, which show that the peaks are Gaussian. However, the chromatogram of the drug with extraction was better because the baseline was smooth and a small peak in fig. 4 (t_R=1.80 min) was disappeared.

System suitability parameters indicated that the method was suitable for the intended purpose. Moreover, all the parameters of the method validation were within the standard range. This indicated that the method is reliable, specific, sensitive and robust to be used in quality control, pre-formulation and stability analysis of AS without any lengthy steps of drug derivatization and sophisticated instruments.

CONCLUSION

It is concluded from the results of the study that the method developed for analysis of AS is simple, reliable, repeatable and reproducible. Hence, may be applied for routine analysis and stability studies of AS without any interference by excipients. Furthermore, the method is advantageous owing low cost, economy, time consumption and environmental protecting. Also, the proposed method is found to be easier than the published techniques for estimation of AS. It is also indicative from the study that the aqueous ninhydrin solution is better as compared to ethanol solution for complex formation. The complex, if stored at room temperature, needs to be analyzed within one hour or stored in refrigerator if intended to be used later. The method is completely validated and its suitability indicates that this method can be used in less equipped quality control laboratories where economy and time factor is essential.

REFERENCES

Adams E, Kibaya R, Roets E and Hoogmartens J (1995). Identification of neomycin and framycetin sulphate and

determination of neamine by thin-layer chromatography. *Pharmeuropa*, **7**: 807-810.

Al-Sabha TN (2010). Spectrophotometric determination of amikacin sulphate via charge transfer complex formation reaction using tetracyanoethylene and 2, 3-dicholoro-5, 6-dicyano-1, 4-benzoquinone reagents. *Arab. J. Sci. Eng*., **35**(2): 27-40.

Baietto L, Avolio AD, Rosa FGD, Garazzino S, Michelazzo M, Ventimiglia G, Siccardi M, Simiele M, Sciandra M and Perri GD (2010). Development and validation of a simultaneous extraction procedure for HPLC-MS quantification of daptomycin, amikacin, gentamicin, and rifampicin in human plasma. *Anal. Bioanal. Chem.*, **396**(2): 791-798.

Baranowska I, Markowski P and Baranowski J (2006). Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reverse-phase high-performance liquid chromatography method. *Anal. Chim. Acta*, **570**(1): 46-58.

Brajanoski G, Hoogmartens J, Allegaert K and Adams E (2008). Determination of amikacin in cerebrospinal fluid by high-performance liquid chromatography with pulsed electrochemical detection. *J. Chromatogr. B*, **867**(1): 149-152.

Caturla MC and Cusido E (1992). High-performance liquid chromatography method for the determination of aminoglycosides based on automated pre-column derivatization with o-phthalaldehyde. *J. Chromatogr. A*, **593**(1-2): 69-72.

CDER (1994). Reviewer Guidance, Validation of Chromatographic Methods. FDA, Rockville MD.

Chang XJ, Peng JD and Liu SP (2010). A simple and rapid high performance liquid chromatographic method with fluorescence detection for estimation of amikacin in plasma- application to preclinical pharmacokinetics. *J. Chin. Chem. Soc.*, **57**(1): 34-39.

Churagulova NK (1987). Spectrophotometric determination of amikacin and sisomicin in ambient air by the Hantzsch reaction. *Antibiot. Med. Biotekhnol.*, **32**(5): 344-345.

Confino M and Panayot B (1990). Spectrophotometric determination of amikacin, kanamycin, neomycin and tobramycin. *Mikrochim. Acta*, **102**(4-6): 305-309.

Feng CH, Lin SJ, Wu HL and Chen SH (2001). Trace analysis of amikacin in human plasma by high-performance liquid chromatography. *Chromatographia*, **53**(1-supplement): S-213-S-217.

Feng-ming D, Yang Y and Ying-Biao T (2003). Quantitative determination of Amikacin Sulfate in Injection by UV- Spectrophotometer. *J. Zunyi Med. Col.*, **26**: 3.

Fred S (1997-2010). What is a simple test for the presence of amino acids? http://antoine.frostburg.edu/chem/senese/101/organic/faq/amino-acid-test.shtml (Accessed May 16, 2015).

Galanakis EG, Megoulas NC, Solich P and Koupparis MA (2006). Development and validation of a novel LC non-derivatization method for the determination of amikacin in pharmaceuticals based on evaporative light scattering detection. *J. Pharm. Biomed. Anal.*, **40**(5): 1114-20.

Gupta VD, Stewart KR and Gunter JM (1984). Quantitation of amikacin, kanamycin, neomycin, and tobramycin in pharmaceutical dosage forms using the Hantzsch reaction. *J. Pharm. Sci.*, **72**(12): 1470-1471.

Harding VJ and Warneford FHS (1916). The ninhydrin reaction with amino-acids and ammonium salts. *J. Biol. Chem.*, **25**: 319-335.

Hubicka U, Krzek J, Woltyska H and Stachacz B (2009). Simultaneous identification and quantitative determination of selected aminoglycoside antibiotics by thin-layer chromatography and densitometry. *J. AOAC Int.,* **92**(4): 1068-1075.

Isoherranen NS and Soback J (1999). Chromatographic methods for analysis of aminoglycoside antibiotics. *J. AOAC Int*., **82**(5): 1017-1045.

Jiang W, Appelblad P, Jonsson T and Hemstrom P (2011). Analysis of amino glycosides with a zwitterionic HILIC stationary phase and mass spectrometry detection. *Chromatography Today*, **36**(5): 26-28.

Liu X, Wang H, Liang SC and Zhang HS (2001). Determination of primary and secondary aliphatic amines by N-hydroxysuccinimidyl 4, 3, 2'-naphthapyrone-4-acetate and reversed-phase high-performance liquid chromatography. *Anal. Chim. Acta*, **441**(1): 45-52.

Lung KR, Kassal KR, Green JS and Hovsepian PK (1998). Catalytic precolumn derivatization of amikacin. *J. Pharm. Biomed. Anal.*, **16**(5): 905-910.

Nicoli S and Santi P (2006). Assay of amikacin in the skin by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.*, **41**(3): 994-997.

Oertel R, Neumeister V and Kirch W (2004). Hydrophilic interaction chromatography combined with tandem-mass spectrometry to determine six aminoglycosides in serum. *J. Chromatogr. A*, **1058**(1-2): 197-201.

Omer MA, Hammad MA, Nagy DM and Aly AA (2015). Development of spectrofluorimetric method for determination of certain amino glycoside drugs in dosage forms and human plasma through condensation with ninhydrin and phenyl acetaldehyde. *Spectrochim. Acta A*, **136**(Part C): 1760-1766.

Ovalles JF, Brunetto MR and Gallignani M (2005). A new method for the analysis of amikacin using 6-aminoquinolyl-N-hydroxysuccinimidyl Carbamate (AQC) derivatization and high-performance liquid chromatography with UV-detection. *J. Pharm. Biomed. Anal.*, **39**(1-2): 294-298.

Serrano JM and Silva M (2006). Determination of amikacin in body fluid by high-performance liquid chromatography with chemiluminescence detection. *J. Chromatogr. B*, **843**(1): 20-24.

Soltes L (1999). Aminoglycoside antibiotics - Two decades of their HPLC bioanalysis. *Biomed. Chromatogr.,* **13**(1): 3-10.

Stead DA (2000). Current methodologies for the analysis of aminoglycosides. *J. Chromatogr. B*, **747**(1-2): 69-93.

Tawa R, Matsunaga H and Fujimoto TJ (1998). High-performance liquid chromatographic analysis of aminoglycoside antibiotics. *J. Chromatogr. A*, **812**(1-2): 141-150.

The United States Pharmacopoeia (USP28-NF22) (2005). United States Pharmacopeial Convention, Inc., Rockville MD.

Wong LT, Beaubien AR and Pakuts AP (1982). Determination of amikacin in microliter quantities of biological fluids by high-performance liquid chromatography using 1-fluoro-2, 4-dinitrobenzene derivatization. *J. Chromatogr.*, **231**(1): 145-154.

Yang M and Tomellini SA (2001). Non-derivatization approach to high-performance liquid chromatography-fluorescence detection for aminoglycoside antibiotics based on a ligand displacement reaction. *J. Chromatogr. A*, **939**(1-2): 59-67.

Zakhari NA (1990). Spectrophotometric assay of certain aminoglycosides using cyanoacetamide. *Anal. Lett.*, **23**(10): 1843-1856.