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Development and validation of a novel LC non-derivatization method for the determination of amikacin in pharmaceuticals based on evaporative light scattering detection

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Abstract

A novel method for the direct determination of the aminoglycoside antibiotic amikacin and its precursor component kanamycin was developed and validated, based on reversed phase LC with evaporative light scattering detector (ELSD). ELSD response to amikacin was found to be enhanced by: (a) use of ion-pairing acidic reagents of increased molecular mass, (b) increase of mobile phase volatility and (c) decrease of peak width and asymmetry (obtained by controlling the mobile phase acidity and/or ratio of organic solvent to water). Utilizing a Thermo Hypersil BetaBasic C_{18} column, the selected optimized mobile phase was water–methanol (60:40, v/v), containing 3.0 ml l^{-1} nonafluoropentanoic acid (18.2 mM) (isocratic elution with flow rate of 1.0 ml min^{-1}). ELSD experimental parameters were: nitrogen pressure 3.5 bar, evaporation temperature 50 °C, and gain 11. Amikacin was eluted at 8.6 min and kanamycin at 10.4 min with a resolution of 1.5. Logarithmic calibration curves were obtained from 7 to 77 μg ml^{-1} ($r > 0.9995$) for amikacin and 8 to 105 μg ml^{-1} ($r > 0.998$) for kanamycin, with a LOD equal to 2.2 and 2.5 μg ml^{-1}, respectively.

In amikacin sulfate pharmaceutical raw materials, the simultaneous determination of sulfate ($t_R = 2.3$ min, LOD = 1.8 μg ml^{-1}, range 5–40 μg ml^{-1}, %R.S.D. = 1.1, $r > 0.9997$), kanamycin and amikacin was feasible. No significant difference was found between the results of the developed LC–ELSD method and those of reference methods, while the mean recovery of kanamycin from spiked samples (0.5%, w/w) was 97.3% (%R.S.D. ≤ 2.0, $n = 6$). Further, the developed method was applied for the determination of amikacin in pharmaceutical formulations (injection solutions) without any interference from the matrix (recovery from spiked samples ranged from 95.6 to 103.8%).
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1. Introduction

Amikacin (1-*N*-[L(−)-4-amino-2-hydroxybutyryl]kanamycin A) is a semisynthetic, broad spectrum antibiotic with an aminoglycosidic structure (Fig. 1). In cases of resistance to other aminoglycosides, amikacin is widely administrated (parenterally) as the second line treatment of gram-negative bacteria, since it appears to be less susceptible to enzymatic degradation. Dose-dependant side-effects of ototoxicity and nephrotoxicity have been observed, establishing relatively narrow safety levels in blood during long-term therapy [1].

Amikacin is a derivative of kanamycin A (solely obtained by bacteria fermentation), which is produced by selective acylation of the C_1 amino group with L-(2)-g-amino-a-hydroxybutyric acid (L-HABA) (Fig. 1) [2,3]. Kanamycin A and side products that differ in the position and number of acyl groups have been reported to be present as impurities in amikacin (sulfate) raw materials [4,5].

Liquid chromatography (LC) appears to be the prevailing technique for the determination of amikacin in various matrices, however, due to the low UV–vis absorptivity of amikacin and the absence of native fluorescence, pre- or post-column derivatization is required. The European Pharmacopoeia (EP) method [5] for the assay of amikacin in pharmaceutical raw materials is a LC–UV method (340 nm) after pre-column derivatization with 2,4,6-trinitrobenzene sulfonic acid. Several broadened

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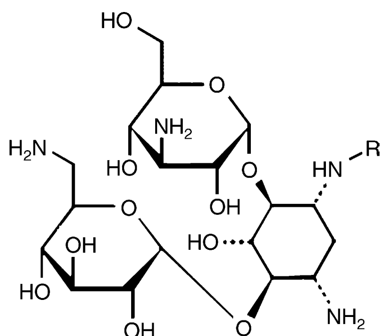

Fig. 1. Chemical structure of amikacin (R = (2*S*)-4-amino-2-hydroxybutanoyl, —CO—CH(OH)—CH_2—CH_2—NH_2) and kanamycin (R = H).

peaks due to the unreacted reagent appear in the first 6 min. The EP method is cable for the simultaneous determination of kanamycin A (limit 0.5%) and side products of acylation reaction. In addition, amikacin sulfate raw materials are tested for sulfates content by an indirect complexometric titration (from 23.3 to 25.8% for EP [5] or amikacin:H_2SO_4 molar ratio 1:2 or 1:1.8 for USP [6]). Other available LC methods are based on precolumn derivatization with 1-fluoro-2,4-dinitrobenzene [7,8], 1-naphthylisothiocyanate [9], 1-naphthoyl chloride [10] and pre- or postcolumn derivatization with *o*-phthalaldehyde [11,12].

In the frame of routine work, the drawbacks of derivatization techniques are widely recognized (e.g. influence by various experimental parameters, incompleteness of derivatization reaction, appearance of additional peaks due to unreacted reagent, use of salt laden mobile phases, prolonged analysis time, additional cost for derivatization system and reagents). Especially for amikacin determination, it has been reported that precolumn derivatization with *o*-phthalaldehyde or 1-fluoro-2,4-dinitrobenzene results in unstable derivatives [11–13]. Additionally, the LC–UV–vis methods are not suitable for the simultaneous determination of sulfates in raw materials and therefore an extra titrimetric procedure has to be performed. A direct LC method based on pulsed electrochemical detection (PED) [4] and an indirect fluorimetric method [14] have also been proposed, while the LC–PED method has been adopted in the recent edition of US Pharmacopoeia [15]. Apart from LC methods, capillary electrophoresis [16], thin layer chromatography [17], kinetic fluorimetry [18] and immunoassays [19] have been successfully applied for the determination of amikacin in various matrices.

Evaporative light scattering detector (ELSD) is increasingly being used in LC as a quasi-universal detector eliminating the need for derivatization of non-absorbing analytes. ELSD operation principle mainly consists of three successive processes: (a) nebulization of chromatographic eluent using an inert gas (e.g. nitrogen), (b) evaporation of mobile phase at relatively low temperature and (c) light scattering by the residual particles, which ideally consist of analytes molecules [20,21]. In the field of pharmaceutical analysis, it has already been proposed as an effective alternative for the determination of the aminoglycosidic antibiotics gentamicin [22,23], neomycin [24] and isepamicin [25], and of, among others, cyclodextrins [26], polyethylene glycols [27], products of combinatorial and parallel synthesis [28] and inorganic ions [29].

In this study, experiments considering the influence of mobile phase composition on amikacin retention and ELSD response factor were carried out. A novel reversed phase LC–ELSD method based on ion pairing retention mechanism for the determination of amikacin was developed and validated. The new method eliminated the requirement for derivatization steps, while it enabled the simultaneous determination of sulfates. The method was applied for the content assay of pharmaceutical raw material and commercial formulations (injection solutions).

2. Experimental

2.1. Instrumentation and software

Chromatographic separations were carried out on a Shimadzu VP Series HPLC (Duisburg, Germany) modular system consisting of: a DGU-14A Online Vacuum-Degasser, a LC-10 AD VP micro-double piston pump, a 7725i Rheodyne manual sample injector equipped with a 20 μl loop, a Thermo Hypersil-Keystone BetaBasic C_{18} analytical column (4.6 mm × 250 mm, spherical particles of 5 μm and 80 Å pore size), a ss-420x A/D converter board and a Class VP 4 data processing software for the recording and integration of the chromatograms.

The detector was a Sedex 75, SEDERE (Cedex-France) low temperature evaporative light scattering detector. The nebulizing gas was nitrogen of industrial purity grade. Separations were carried out using isocratic elution at controlled room temperature (22–25 °C).

A pH meter (Metrohm Herisau) equipped with a glass combination electrode was used for pH measurement of mobile phase.

2.2. Reagents and standards

All chemicals were of analytical reagent grade unless otherwise stated. HPLC-grade water (specific resistance >17.8 MΩ cm) was obtained by a Milli-Q water purification system (Millipore). For mobile phases preparation, nonafluoropentanoic acid (NFPA) (Fluka, ≥94%), heptafluorobutyric acid (HFBA) (Fluka, ≥99.0%), trifluoroacetic acid (TFA) (Sigma, >99%, spectrophotometric grade), formic acid (Merck, ≥98.0%), acetone and methanol (Lab-scan, HPLC grade) were used.

Amikacin pure substance (936 mg g^{-1}), kanamycin A acid sulfate pure substance (Kanamycin A base 689 mg g^{-1}), amikacin and amikacin sulphate raw materials were provided by local pharmaceutical companies, accompanied with certificate of analysis based on European Pharmacopoeia procedures [5]. Commercial formulations (injections) were also provided by local pharmaceutical companies. Standard stock solutions of amikacin (5.00 mg ml^{-1}), kanamycin (5.00 mg ml^{-1}) and ammonium sulfate (SO_4^{2-}, 1.00 mg ml^{-1}) were prepared in water and stored at 4–8 °C.

Table 1
Influence of molecular mass and concentration of ion pairing agent, chromatographic peak broadening and asymmetry on ELSD response to amikacin (detector gain 11)

Mobile phase H_2O–ACN (50:50, v/v) containing acid in ml l^{-1} (mM)	log a (±S.D.)	b (±S.D.)	Peak area[a] ($\times 10^6$) (%R.S.D.)	t_R (min)	Width (min)	Asymmetry factor	r (n = 5)[b]
HCOOH (M_r = 45.0)							
45 (1.17×10^3)	4.754 (±0.014)	1.470 (±0.012)	0.63 (2.2)	5.9	0.34	1.5	0.9999
60 (1.56×10^3)	4.854 (±0.53)	1.403 (±0.044)	0.67 (1.9)	4.4	0.24	1.4	0.996
80 (2.08×10^3)	5.160 (±0.012)	1.233 (±0.011)	2.42 (1.7)	3.5	0.20	1.3	0.9998
TFA (M_r = 113.0)							
0.50 (6.4)	5.008 (±0.096)	1.563 (±0.083)	2.07 (2.1)	4.0	0.24	1.7	0.997
HFBA (M_r = 213.1)							
0.75 (5.7)	5.511 (±0.048)	1.120 (±0.043)	1.74 (2.0)	5.7	0.27	1.8	0.997
1.00 (7.6)	5.695 (±0.042)	1.128 (±0.041)	2.69 (1.8)	4.0	0.23	1.6	0.9990
1.25 (9.6)	5.948 (±0.065)	0.960 (±0.063)	4.48 (1.6)	3.4	0.25	0.9	0.996
NFPA (M_r = 264.1)							
1.5 (9.1)	6.104 (±0.025)	1.154 (±0.051)	8.25 (1.7)	3.5	0.22	1.5	0.9992
2.5 (15.2)	6.002 (±0.069)	1.123 (±0.085)	6.0 (1.9)	6.2	0.25	1.7	0.998

[a] Arbitrary units; determined at the lower concentration level (5.0 μg ml^{-1}), n = 3.
[b] Each point is the average of three replicates, at five concentration levels in the range 5.0–25.0 μg ml^{-1}.

2.3. Procedures

2.3.1. Study of influence of mobile phase composition and peak shape on ELSD response factor

For the evaluation of the influence of ion pairing agents and peak shape on ELSD response factor, flow rate was 1.0 ml min^{-1}, nitrogen pressure 3.5 bar, evaporation temperature 50 °C and detector gain 12. The examined mobile phases are shown in Table 1.

For the evaluation of the influence of mobile phase solvents on ELSD response factor a flow injection set-up was utilized by replacing the analytical column by a stainless steel coil (200 mm length, 1/16 in. i.d.). Flow rate was 0.5 ml min^{-1}, nitrogen pressure 3.5 bar, evaporation temperature 50 °C and detector gain 11. The examined solvents are shown in Table 2.

Using the above experimental configurations and mobile phases, logarithmic calibration curves were established, by the mean values of three replicates at five concentration levels.

2.3.2. Routine determination of amikacin (and sulfates) in raw material and formulations

Using the C_{18} chromatographic column the optimized mobile phase was water–methanol (60:40, v/v) containing 3.00 ml NFPA per liter (18.2 mM). Optimized flow rate was 1.0 ml min (isocratic mode), nitrogen pressure 3.5 bar, evaporation temperature 50 °C and detector gain 11. Mobile phase was filtered through HVLP Millipore filters (diameter 47 mm, pore size 0.45 μm) under vacuum for removing particles and dissolved air. Before measurements, flow path was rinsed with mobile phase for about 30 min, until baseline noise became negligible (less than 3 mV at detector gain 11). Standard and sample working solutions, in mobile phase, were measured in triplicate. Quantitations were carried out using calibration curves of at least two standards corresponding to the 50% and 150% of the labeled content lying in the middle of the dynamic range of the method 7–77 μg ml^{-1} amikacin (i.e. 20 and 60 μg ml^{-1}).

Raw materials of amikacin (sulfate) were simply dissolved in mobile phase, while commercial formulations (only injections) were diluted, in order the expected concentration of the run solution to be in the middle of the calibration curve (40 μg ml^{-1}) and so, to obtain better accuracy. Also, run solutions were filtered through HVLP Millipore 0.45 μm for removing the undissolved particles.

For the determination of kanamycin in raw material of amikacin, a concentrated sample solution (2000 μg ml^{-1}) was required, allowing a detection limit of kanamycin of 0.12%. Calibration was performed with 8 and 25 μg ml^{-1} kanamycin standard solutions.

Sulfates in raw materials were determined after sample dissolution and appropriate dilution in mobile phase in order the expected concentration of the run solution to be about 20 μg ml^{-1}. Calibration was performed with 10 and 30 μg ml^{-1} (ammonium) sulfate standard solutions.

Table 2
Influence of solvent composition of mobile phase on ELSD response to amikacin, in a flow injection system (detector gain 12)

Mobile phase	log a (±S.D.)	b (±S.D.)	Peak area ($\times 10^6$) (4.0 μg ml^{-1})	r (n = 5)[a]
Water	5.979 (±0.035)	1.012 (±0.067)	3.77	0.998
Water–acetonitrile 40:60 (v/v)	6.116 (±0.056)	0.860 (±0.095)	4.45	0.998
Water–methanol 40:60 (v/v)	6.142 (±0.005)	0.878 (±0.010)	4.72	0.9997

[a] Each point in the range 1–5 μg ml^{-1}, is the average of three replicates.

3. Results and discussion

3.1. Study of the influence of mobile phase composition on ELSD response

Due to the polar and basic properties of the aminoglycosidic structure of amikacin (it bares four primary and one secondary amino-groups per molecule, Fig. 1) the ion-pair reversed phase mechanism was found to be the most suitable one for chromatographic separation, consisting of the non polar C_{18} analytical column along with mobile phases of polar solvents and volatile ion pairing acidic agents. The influence on ELSD response factor of both components of the mobile phase (i.e. solvents and ion-pairing agents) was investigated.

Concerning the effect of the ion-pairing agent, four volatile (in order to be compatible with the ELSD) acids (formic, TFA, HFBA, NFPA) were examined, in appropriate concentration ranges (so that the corresponding elution time of amikacin to be in a useful range, such as 2.5–6 min). The peak areas (A) were correlated to the analyte mass (m) by the well-established exponential curve of ELSD response [20,21]:

$$A = am^b \Rightarrow \log A = b \log m + \log a \quad (1)$$

where a and b are coefficients depending on instrumental parameters, nature and concentration of analyte, gas and liquid flow rates, evaporation temperature, etc. The determined values of the coefficients a and b are presented in Table 1, from which the following conclusions can be drawn:

(a) Increase of the mobile phase concentration of the acidic ion-pairing agent resulted in decrease of the amikacin retention time, with the exception of NFPA. This kind of behavior is commonly observed at reversed phase retention mechanism of basic compounds and it may be attributed to the increase of mobile phase acidity and the consequent protonation of the free silanolic groups of stationary phase, resulting in inhibition of cation-exchange interactions with positively charged groups of analyte. In contrast, increase of NFPA concentration resulted in increase of the amikacin retention time due to the higher molecular volume and lipophilicity of NFPA ion pairs. The increased adsorption of lipophilic NFPA anions on the column rendered the non-polar stationary phase a dynamic cation exchanger, which resulted in enhanced ion-exchange interactions with amikacin cations, and therefore stronger retention.

(b) Increase of the mobile phase concentration of the acidic ion-pairing agent resulted in an increase of coefficient a (again with the exception of NFPA), which in turn led to an increase of the area of the chromatographic peak, regardless of the decrease of coefficient b. This behavior can be attributed to the decrease of amikacin retention time, width and asymmetry, which has been reported to influence ELSD response factor, as a result of the exponential correlation between peak area and analyte mass (Eq. (1)) [30]. Actually, in the cases of Gaussian distribution of analyte concentration, Eq. (1) becomes equivalent to

$$A = \alpha^* \sqrt{\frac{2\pi}{b}} \frac{\sigma^{(1-b)}}{(F\sqrt{2\pi})^b} m^b \quad (2)$$

where F is the mobile phase flow rate, σ is the standard deviation of concentration distribution and α^* is a coefficient independent of the peak shape. Since in most ELSD applications $b > 1$, it is derived that ELSD response is enhanced by the decrease of peak broadening (standard deviation).

(c) For similar peak shape (equivalent values of peak width and asymmetry), the area of the chromatographic peak was found to be dependant on the molecular mass of the organic acid. In fact, the peak area increased according to the order of increase of the molecular mass of the organic anion: formic ($M_r = 45.0$) < TFA ($M_r = 113.0$) < HFBA ($M_r = 213.1$) < NFPA ($M_r = 264.1$). The base of the relation between the ELSD response and the molecular mass of the ion pairing agents possibly lay on the inclusion of anions in the amikacin particles resulting in increase of particles mass (and so increase of the analytical signal, Eq. (1)), possibly due to electrostatic interactions with the amikacin cationic amino groups.

The influence of mobile phase solvents was examined, in the absence of acidic ion pairing agent and the chromatographic column (Section 2.3.1). Coefficients a and b of the logarithmic calibration curve (Eq. (1)) were determined for each solvent. From the data presented in Table 2, it is clear that coefficients a and b as well as the peak area are dependant on solvents identity. In fact, coefficient a increased according to the order: water < acetonitrile < methanol, which is identical to the order of increase of the solvents volatility (vapor pressure at 25 °C (kPa): water 3.2, acetonitrile 11.8, methanol 17). The increase of coefficient a resulted in an increase of the area of the chromatographic peak, regardless of the value of coefficient b. The increase of mobile phase volatility has been reported to favor the reduction of droplets condensation on the walls of the nebulization chamber [20], and this is probably the reason for the ELSD response enhancement.

3.2. Development and validation of LC–ELSD method for the assay of amikacin and the control of kanamycin and sulfates in raw materials

The selected and optimized mobile phase was water–methanol (60:40, v/v) containing 3.00 ml nonafluoropentanoic acid (NFPA) per liter (18.2 mM). NFPA appeared to be the most efficient ion-pairing agent for the separation of amikacin from the closely related aminoglycoside kanamycin (the precursor compound). The high molecular mass (and thus lipophilicity) of NFPA resulted in differentiated retention, while it induced the greatest enhancement in ELSD response. Accordingly, methanol was selected because it appeared the highest volatility amongst the examined volatile polar organic solvents. The proportion of methanol to water was selected based on two criteria: Firstly, increase of methanol portion results in increase of mobile phase

Table 3
Characteristics of chromatographic peaks of amikacin, sulfate and kanamycin and logarithmic regression of peak areas towards analyte mass concentration

	Sulfates	Amikacin	Kanamycin
Retention time, t_R (min)	2.3	8.6	10.4
Width at half height (min)	0.08	0.26	0.33
Retention factor[a]	0.77	5.6	7.0
Asymmetry factor (at 5% of peak height)	1.7	1.7	1.9
Theoretical plates, N	4.6×10^3	6.1×10^3	5.5×10^3
Resolution	8.7	1.5	
Intercept of logarithmic calibration, log a (±S.D.)	3.518 (±0.078)	4.624 (±0.064)	3.865 (±0.094)
Slope of logarithmic calibration, b (±S.D.)	2.201 (±0.032)	1.301 (±0.042)	1.395 (±0.069)
Correlation coefficient, r (n = 5)	0.9997	0.9995	0.998
Range (μg ml^{-1})	5–40	7–77	8–105
Detection limit (μg ml^{-1})[b]	1.8	2.2	2.5
%R.S.D. ($n = 3 \times 3$)[c]	1.1 (10 μg ml^{-1})	1.9 (9.6 μg ml^{-1})	1.6 (20.8 μg ml^{-1})
Assay of raw material/comparison between LC–ELSD and reference method			
EP method [5]	24.4%	68.9%	0.50%[d]
HPLC/ELSD method ($n = 3 \times 3$)[e]	24.6 ± 0.4%	68.3 ± 1.2%	0.49 ± 0.01%

[a] Void time = 1.3 min.
[b] Corresponds to analyte concentration producing a peak area equal to 3.3 times the standard deviation of the most dilute standard and is practically equal to the concentration having S/N ratio equal to 3.3.
[c] Three working days (within a week), three injections per day.
[d] Amikacin raw material was fortified with kanamycin standard material.
[e] Three independent sub-samples with three injections per working sample solution.

volatility and so of ELSD response factor. Secondly, the proportion of methanol to water controls the retention time of amikacin and its separation from kanamycin. The proportion was selected to be 60:40 (corresponds to t_R 8.6 min for amikacin and 10.4 min for kanamycin) in order to achieve adequate resolution and to avoid interferences from anions or other compounds with weak retention to C_{18} column. Greater (than 8.6 min) retention time, which may be achieved by increasing the portion of water, is disadvantageous, since it increases the analysis time, while it reduces mobile phase volatility and so the ELSD response factor.

The optimum values of the other experimental parameters (flow rate 1.0 ml min^{-1}, nitrogen pressure 3.5 bar and evaporation temperature 50 °C) were selected based on a univariate optimization procedure.

Table 3 summarizes the chromatographic and analytical characteristics of amikacin, kanamycin and sulfate. In all cases, very good correlation was achieved with the exponential relationship between peak area and analyte mass. A linear calibration curve can be obtained by adopting double logarithmic coordinates (Eq. (1)). A linear calibration curve can also be obtained by raising the area of chromatographic peak to (1/b) [31]:

$$A^{1/b} = a^{1/b}m = km \quad (3)$$

This equation provides a linear calibration curve passing through the origin and can be used for routine quantitations using the single-point calibration approach, provided that the coefficient b has been previously determined from Eq. (1), using a series of standards (at least two).

The weak retention of sulfates, due to polar interactions of HSO_4^- with the free silanolic (–OH) groups of the non polar C_{18} column, enables the good resolution from amikacin and kanamycin, and also from the common anion chloride (R_s = 2.2). Other interfering anions are not expected in raw material.

The results of the assay and the determination of sulfates and kanamycin based on the LC–ELSD method (Fig. 2) were in very good agreement with those obtained by analysis based on European Pharmacopoeia procedures [5] (Table 3). The accuracy of the determination of kanamycin in amikacin sulfate raw material was examined with recovery experiments. Samples of raw material were spiked with kanamycin standard material at for-

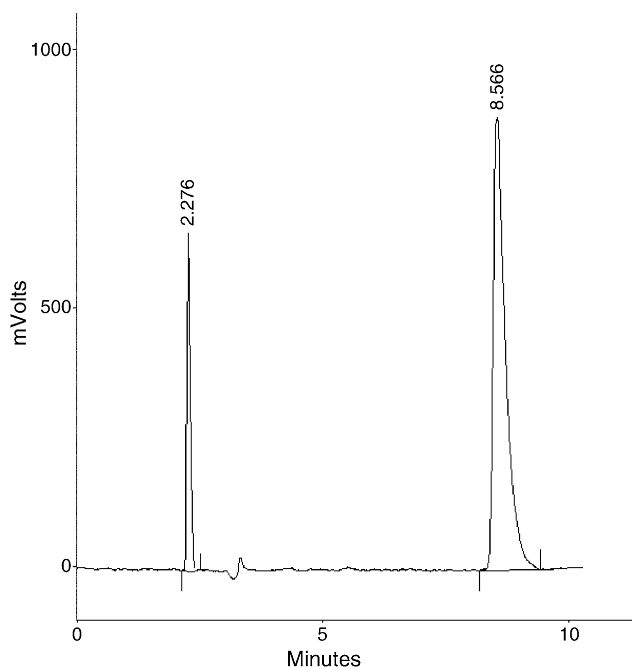

Fig. 2. Typical chromatogram of amikacin sulfate raw material (100 μg ml^{-1}) (sulfate 2.28 min, amikacin 8.57 min).

Table 4
Assay of content and recovery results of amikacin commercial formulations

Formulation, label content (excipients)	Content found (±S.D., $n=6$)	Recovery range (%) (mean, $n=6$)
Briklin 500 mg/2 ml (*p*-dihydroxybenzoic methylester, *p*-dihydroxybenzoic propylester, sodium citrate, sodium sulfite)	506 ± 5	96.0–103.4 (100.3%)
Briklin 250 mg/2 ml (*p*-dihydroxybenzoic methylester, *p*-dihydroxybenzoic propylester, sodium citrate, sodium sulfite)	250 ± 4	95.6–102.6 (99.1%)
Uzix 500 mg/2 ml (methyl paraben, propyl paraben, sodium metabisulfite, disodium edetate, sodium citrate, sulfuric acid)	490 ± 7	96.4–103.8 (99.9%)
Flexelite 500 mg/2 ml (methyl paraben, propyl paraben, sodium metabisulfite, sodium citrate)	504 ± 6	98.0–103.4 (100.2%)

tification level equal to the EP concentration limit (0.5%, w/w) (Fig. 3). Recovery values (mean 97.3%, %R.S.D. ≤ 2.0, $n=6$) confirmed the good accuracy of the LC–ELSD method.

3.3. Application to pharmaceutical formulations

The proposed LC–ELSD method was further applied for the determination of amikacin in commercial pharmaceutical formulations (injections, the only available type). The only required sample treatment was the appropriate dilution with the mobile phase in order to obtain concentration of amikacin in the range 7–77 μg ml^{-1}, preferable about 40 μg ml^{-1}. No other chromatographic peaks were recorded except for the amikacin peak, due to the extended dilution of the formulations (Fig. 4). Retention time of possible excipients was examined and no interference (peak overlapping) with amikacin was observed. Most of them (sodium citrate, sulfuric acid and sodium metabisulfite) are inorganic anions of low molecular mass and so they appear negligible retention on the non-polar C18 column (their elution time is close to the void time).

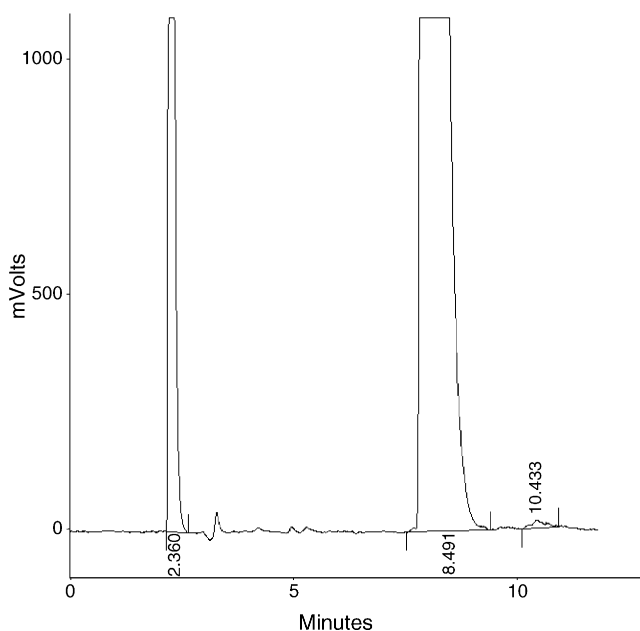

Fig. 3. Typical chromatogram of amikacin sulfate raw material (2000 μg ml^{-1}) fortified with kanamycin 0.5% (w/w) (sulfate 2.36 min, amikacin 8.49 min, kanamycin 10.4 min).

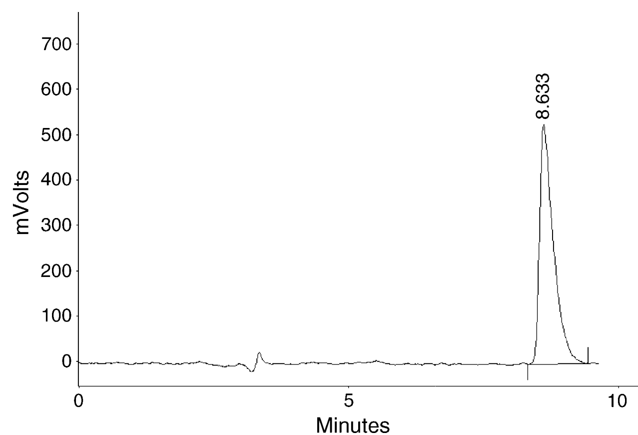

Fig. 4. Typical chromatogram of diluted amikacin formulation (Briklin injection 250 mg ml^{-1}) corresponding to 40 μg ml^{-1} amikacin (t_R 8.63 min).

The experimental results are presented in Table 4. Good precision and conformance to the Pharmacopoeia requirement for content within the range 95–105% of the label content was achieved.

The accuracy of the proposed method was evaluated by recovery experiments of fortified sample solutions. The recovery values (99.1–100.3%) revealed sufficient accuracy (Table 4). Further study of the matrix (excipients) effect on the determination was carried out by dilution experiments (determination of amikacin content in commercial formulations using a varying dilution factor D ($V_{\text{initial}}/V_{\text{final}}$) at three different levels). The correlation curves of the concentration found (in the diluted solution) versus D were very linear ($r > 0.997$) with a slope equal to the content of the formulation and a statistically (proven by t-test) zero intercept. Similarly, the correlation curves of content found versus D were very linear with statistically (proven by t-test) zero slope. These results confirmed the absence of any constant or proportional determinate error due to the matrix effect.

4. Conclusions

LC with evaporative light scattering detection appeared to be efficient for the determination of amikacin and its precursor compound kanamycin. The proposed LC–ELSD method did not require any derivatization step and also enabled the simultaneous determination of the inorganic co-ion (sulfates).

ELSD response factor was strongly dependant on mobile phase composition. The optimized mobile phase for the determination of amikacin was water–methanol (60:40, v/v), containing 3.00 ml NFPA per liter (18.2 mM). Good correlation with the double logarithmic relationship of ELSD signal, sufficient detectability, precision and accuracy were obtained. The proposed LC–ELSD was applied successfully for the determination of amikacin, kanamycin and sulfates in pharmaceutical raw materials and for the assay of pharmaceutical formulations (injections) without tedious pretreatment, and without matrix interferences.

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