Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 73–79
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Development and validation of a novel LC/ELSD method for the quantitation of gentamicin sulfate components in pharmaceuticals

Nikolaos C. Megoulas, Michael A. Koupparis*

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece

Received 13 January 2004; accepted 19 May 2004

Available online 30 July 2004

Abstract

The equivalent response of evaporative light scattering detector (ELSD) for compounds of similar structure is exploited to develop an LC/ELSD method for the simultaneous quantitation of the four main components of gentamicin sulfate, using as external standard the one main component kanamycin. A C_{18} column was used along with a mobile phase consisting of H_2O (containing 35.4 μg/ml of trichloroacetic acid and 0.89 μl/ml of trifluoroacetic acid)–methanol–acetonitrile (990:5:5, v/v/v), in an isocratic mode at 1.1 ml/min. Parameters of ELSD were 50 °C for evaporation temperature and 3.0 bar for pressure of carrier gas (N_2). A logarithmic calibration curve was obtained for sulfate ($t_R = 1.9$ min) from 4.2 to 150 μg/ml ($r > 0.994$) with a precision of 0.18%R.S.D. Kanamycin and the four gentamicin components (C_{1a}, C_2, C_{2a} and C_1) were eluted at 3.2, 4.6, 5.9, 7.1 and 8.7 min, respectively, with good resolution (Rs > 1.5). Logarithmic calibration curve was obtained for each component ($r > 0.99$) with statistically equal slopes varying from 2.457 to 2.558. The mass range of total gentamycin was 35–240 μg/ml. The proposed method was applied for the determination of gentamicin components and sulfate in raw materials and pharmaceutical formulations (injection, drops and cream) without any pretreatment except cream, for which liquid–liquid extraction was required. Recovery from standard addition experiments in commercial formulations was 99–100% regarding total gentamicin and 89–108% regarding individual components, with a precision (%RSD, $n = 4$) 0.7–5.8%.

Keywords: Gentamicin; Kanamycin; Sulfate; LC; Evaporative light scattering; ELSD; Simplex

1. Introduction

Gentamicin (sulfate), an aminoglycoside antibiotic, effective against a wide spectrum of gram-negative and gram-positive bacteria, consists mainly of four closely related components designated as C_1, C_{1a}, C_2 and C_{2a} (Fig. 1). It is administrated in the form of injection, cream, ointment, suspension and it is also used in veterinary medicine [1,2].

The official method for the assay of gentamicin in pharmaceuticals (raw material and formulations), food and tissues is a microbiological one [1,3] and therefore it is time consuming, with low detectability and precision and appears no specificity on the individual chemical components. Raw materials are also tested for methanol (GC, limit 1%), water (coulometric titration, limit 15%), sulfate (indirect complexometric titration, limits 32–35%) and composition (LC with precolumn derivatization with phthalaldehyde reagent, relative limits being set for the components C_1, C_{1a}, and sum of C_2 and C_{2a}) [1,3]. European Pharmacopoeia requires the use of gentamicin sulfate chemical reference standard (CRS) with designated component ratio for the calculation of the relative response factor of each component [3].

Various LC methods have been proposed for the determination of gentamicin [4–7] but due to the low UV absorptivity and the absence of native fluorescence, pre-column or post-column derivatization is required. LC methods with electrospray ionization/ion-trap tandem mass spectrometry [8], pulsed electrochemical detector [9] and laser-based polarimeter [10] have also been reported.

In the frame of routine work, the drawbacks of derivatization techniques are widely recognized (influence by various experimental parameters, incompleteness of derivatization reaction, use of salt laden mobile phases, prolonged analysis time, additional cost for derivatization system and reagents). Especially for gentamicin determination, the lack of real reference standard material of gentamicin components makes

* Corresponding author. Tel.: +30 210 7274559; fax: +30 210 7274750.
E-mail address: koupparis@chem.uoa.gr (M.A. Koupparis).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.05.018

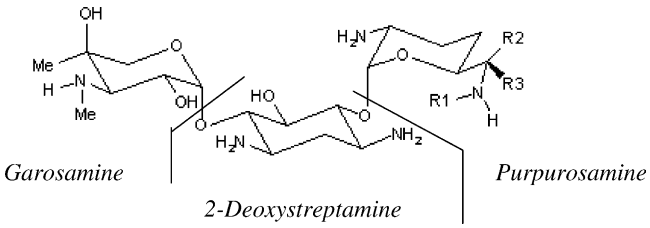

Fig. 1. Structure of gentamicin components (C_{1a}: $R_1 = R_2 = R_3 = H$; C_{2a}: $R_1 = R_2 = H$, $R_3 = CH_3$; C_2: $R_1 = R_3 = H$, $R_2 = CH_3$; C_1: $R_1 = R_2 = CH_3$, $R_3 = H$).

the direct quantitation of each component not feasible, and the chromatographic analysis is limited to the determination of the relative fraction of each component, which is influenced by the relative differences of components absorptivity (response factors). Additionally, the chromatographic conditions and detection techniques, which are applied, are not suitable for the simultaneous determination of sulfate and therefore an extra titrimetric procedure is required.

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 [11]. Its ability to perform quantitation of substances with lack of standard material, since it shows nearly equal response factors for molecules with about equal molecular mass and similar structure formula, is well-established [12]. In the field of pharmaceutical analysis, it has already been proposed as an effective alternative for the determination of, among others, cyclodextrins [13], polyethylene glycols [14], products of combinatorial and parallel synthesis [15] and inorganic ions [16], including sulfates in gentamicin raw material [17].

In this study, the equivalence of ELSD response factors of major gentamicin components and kanamycin (kanamycin A) was examined. Based on the equivalence of responses a novel LC/ELSD method was developed and validated for the resolution and simultaneous direct determination of the four major gentamicin components (and sulfate) without derivatization step to be required. The composition and flow rate of mobile phase and the ELSD parameters were optimized using simplex and univariate techniques. The method was applied for content assay of pharmaceutical raw material and commercial formulations.

2. Experimental

2.1. Instrumentation and software

Chromatographic separations were carried out on a Shimadzu VP Series LC (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, Waters Spherisorb ODS-2 C18 analytical column (4.6 mm × 250 mm, spherical particles of 5 μm and 80 Å pore size), ss-420× A/D converter board and a Class VP 4 data processing software for the recording and integration of the chromatograms.

The detector used was a Sedex 75, S.E.D.E.R.E. low temperature evaporative light scattering detector. The nebulizer 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.

MultiSimplex 2.1 Software (Grabitech solutions AB, Sweden) was utilized to apply multivariate modified simplex algorithm for the optimization of composition and flow rate 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 phase preparation, trichloroacetic acid (TCA) (Merck, >99.5%), trifluoroacetic acid (TFA) (Sigma, >99%, spectrophotometric grade), acetonitrile and methanol (Lab-scan, HPLC grade) were used.

Gentamicin sulfate pure substance and formulations (injection, eye-drops and cream) were provided by local pharmaceutical company. Pure substance was tested for identification, assay and impurities (sulfate, water and methanol) according to European Pharmacopoeia procedures [3]. After standardization using kanamycin CRS, this substance can be used as secondary standard of gentamicin components for routine analysis. A 5.00 mg/ml (total gentamicin) standard stock solution was prepared in water and stored protected from light in the refrigerator. Working standard gentamicin solutions in the range 35–240 μg/ml of total gentamicin were daily prepared in mobile phase.

Kanamycin acid sulfate (CRS) (689 IU/mg, chemical purity in kanamycin A 70.5%) was obtained from European Pharmacopeia. A 1.00 mg/ml standard stock solution was prepared in water and stored protected from light in the refrigerator. Working standard solutions in the range 4.5–75 μg/ml were daily prepared in mobile phase.

For sulfate calibration, a 1.0 mg/ml (SO_4^{2-}) standard stock solution was prepared from potassium sulfate. Working standard solutions in the range 4.2–150 μg/ml were daily prepared by appropriate dilution in mobile phase.

2.3. Procedures

Analytical column, which was utilized, is not compatible with pH below 1.5. Since the applied mobile phases contained strong acids, pH was measured before usage and analytical column was very carefully washed with acetonitrile at the end of each day and stored in the same solvent. Also,

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).

2.3.1. Optimized mobile phase and ELSD parameters

Mobile phase: H_2O (containing 35.4 μg/ml of TCA and 0.89 μl/ml of TFA)–MeOH–ACN (990:5:5, v/v/v), flow rate = 1.1 ml/min. ELSD parameters: nitrogen pressure = 3.0 bar, evaporation temperature = 50 °C, detector gain = 11.

2.3.2. Calibration curve using kanamycin CRS

A series of working standard solutions (4.5–75 μg/ml) in mobile phase were measured in triplicate and the peak areas (A) were used to construct the logarithmic calibration curve $\log A = b \log C_{\mu g/ml} + \log \alpha$ (Eq. (1)).

2.3.3. Standardization of gentamicin pure substance

A series of working solutions of a pure raw material, in the range of total mass (M) 35–240 μg/ml in mobile phase, were measured in triplicate and the peak areas (A) of each eluted component were used to construct the corresponding logarithmic working curve $\log A_i = b \log M + (\log \alpha + b \log x_i)$ (Eq. (2)). The fraction x_i of each component is calculated using the known values of α and b from the calibration curve of kanamycin CRS.

2.3.4. Analysis of commercial raw material and formulations

Raw gentamicin material was dissolved in mobile phase and injections and eye-drops were simply diluted with the mobile phase at a concentration within the (total) mass range of the method (35–240 μg/ml). Cream formulation (quantity equivalent to 2.0 mg of gentamicin) was slightly heated and mixed with 20 ml of dichloromethane to dissolve. Gentamicin was extracted successively with two 5 ml portions of 0.05% v/v TFA aqueous solution. Sample working solutions were injected in the LC system in triplicate and gentamicin individual components were determined from the corresponding calibration curves obtained from kanamycin CRS or the gentamicin secondary standard.

Sulfates in raw materials were determined by the external calibration curve of potassium sulfate after sample dissolution in mobile phase and appropriate dilution to adjust the concentration in the range 4–170 μg/ml.

3. Results and discussion

3.1. Selection and optimization of mobile phase

Mobile phases which are described for the separation of the four major gentamicin components in the official as well as in other published methods are not compatible with the ELSD. Since ELSD demands the evaporation of the mobile phase prior to light scattering step, mobile phases of high volatility are required. Therefore, sodium heptanesulfonate, which is used as ion-pair reagent in the official method, or other non volatile reagents are not applicable.

Various organic solvents (dichloromethane, methanol, acetonitrile, ethanol, propionitrile, ethyl acetate, etc.) and ion pair reagents (formic acid, acetic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, sodium methanesulphonate, sodium butane-1-sulfonate) were tested for their compatibility with ELSD and the successful elution and separation of the four gentamicin components and sulfate. TFA and TCA were found to be compatible with ELSD, due to their high volatility, as well as efficient ion-pair reagents for the separation of gentamicin components. A small portion of polar organic solvents (MeOH and ACN) acting as modifiers was found to improve the symmetry of the chromatographic peaks.

After a preliminary crude optimization, multivariate modified simplex algorithm [18,19] was applied for the final optimization of composition (TFA, TCA, MeOH and ACN) and flow rate of the mobile phase (totally five parameters). Six response variables were considered for the evaluation of the efficiency of the chromatographic determination: (i) sum of peak areas, (ii) precision, (iii) asymmetry factor, (iv) retention time of the first peak (sulfate), (v) retention time of the last peak (component C_1) and (vi) resolution (Table 1). The selected values for the simplex algorithm constants were: reflection $r = 1$, contraction c^+ and $c^- = 0.5$ and expansion $e = 2$.

Since the six response variables (y_i) correspond to different unit scale, a mathematical transformation to a modified response variable $m(y_i)$ was automatically performed by the software, in order the values, which derive from different variables, to be evaluated in an equivalent way. In this transformation, low and high limits and shape constant R ($R = 1$: proportional increase, $R > 1$: the first derivative of $m(y_i)$ increases with y_i) were selected for each variable. An overall one-dimensional value ($M(y)$, weighted geometric average of $m(y_i)$) of each vertex was then calculated, using selected influence values (β_i) of each response variable, in order to compare the successive trials.

The selected low and high limits, R and β_i for each response variable are presented in Table 1. The algorithm stopped after 16 experiments and the optimum results were obtained using a mobile phase consisting of (per liter): 0.88 ml of trifluoroacetic acid, 35 ml of 1.0 mg/ml trichloroacetic acid aqueous solution, 954.1 ml of water, 5 ml of methanol and 5 ml of acetonitrile, in an isocratic mode at a rate of 1.1 ml/min.

3.2. Selection of ELSD parameters

ELSD parameters (evaporation temperature of mobile phase and pressure of carrier gas-N_2) were not included

Table 1
Response variables of multivariate simplex algorithm and the related constants

Target	Sum of peak areas Maximization	Sum of %R.S.D.[a] Minimization[d]	Mean |AF-1|[b] Minimization[d]	$t(SO_4) - 1.5$ min Maximization	20 min $- t(C_1)$ Maximization	Σ(Rs)[c] Maximization
Influence βi	1	0.67	1	0.33	0.67	1
Low limit	200[e]	3	0.2	0.2	4	1
High limit	330[e]	18	1.2	1.3	13	2.5
R	3	3	3	1	1	2

[a] %R.S.D. of peak areas ($n = 3$).
[b] Mean value of the deviations of the asymmetry factors (AF) from unity.
[c] Sum of the two worst resolutions.
[d] Minimization of y_i is equivalent to maximization of $-y_i$.
[e] Arbitrary units.

in the simplex optimization, since they appear a minor influence on the chromatographic separation comparing to mobile phase composition. However, depending on the nature of the analyte, they may appear a greater impact on the detectability and sensitivity of the method. Using the optimized mobile phase, a two step univariate optimization was conducted. In the first step, the influence of evaporation temperature on peak areas was studied (range 40–55 °C) and in the second step, using the optimum evaporation temperature, the N_2 pressure was selected (range 2.5–3.5 bar). No considerable influence on the detector response (peak area) was observed, however best results were obtained for 50 °C and 3.0 bar, respectively.

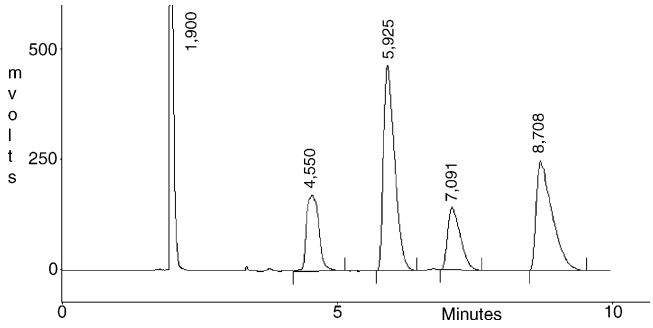

Fig. 2. Typical chromatogram of gentamicin sulfate raw material (175 μg/ml) (sulfate: 1.90 min, C_{1a}: 4.55 min, C_2: 5.92 min, C_{2a}: 7.09 min and C_1: 8.71 min).

3.3. Validation data and quantitation technique

Fig. 2 illustrates a typical chromatogram of gentamicin sulfate, using the optimum chromatographic conditions. Five chromatographic peaks were obtained with good resolution (Rs > 1.5). Chromatographic characteristics of sulfate and the four gentamicin component peaks are summarized in Table 2. The elution order of the four major gentamicin (non-derivatized) components (C_{1a}, C_2, C_{2a}, C_1) being transferred as ion pairs mainly with TCA, as expected, is not in agreement to that observed with USP or EP derivatization method (C_1, C_{1a}, C_{2a}, C_2). The order in the proposed method is confirmed by: (a) the relative polarities of gentamicin components and the corresponding trichloroacetate ion pairs, which determines the relative affinities to C18 column, (b) the agreement with published methods based on reversed phase HPLC without any derivatization step [20,21] or post-column derivatization [22] and (c) the relative height of chromatographic peaks.

Although the ELSD response varies with the scattering domain in most cases it is assumed that the measured peak area (A) can be related to the analyte mass (m) by the logarithmic relation [23]

$$A_i = a_i \times m_i^{bi} \Rightarrow \log A_i = b_i \log m_i + \log a_i \quad (1)$$

where α_i and b_i are coefficients depending on droplet size and nature of solute, gas pressure, evaporation temperature, flow rate, etc.

Standardization of sulfate, using external potassium sulfate standards, appeared good correlation to logarithmic

Table 2
Chromatographic characteristics of kanamycin, gentamicin components and sulfate peaks

	Sulfate	Kanamycin	C_{1a}	C_2	C_{2a}	C_1
Retention factor[a]	0.5	1.5	2.5	3.5	4.5	5.7
Asymmetry factor (at 5% of peak height)	1.5	1.6	1.4	1.5	1.6	2.0
Theoretical plates (N)	4.1×10^3	3.9×10^3	2.0×10^3	4.0×10^3	4.1×10^3	4.4×10^3
Resolution[b]	–	2.1	1.6	1.5	1.6	1.9
%R.S.D. of peak area, $n = 4$ (C μg/ml)	0.18 (5)	1.5 (35)	0.43 (43)	2.2 (41)	0.59 (25)	1.9 (44)
LOD[c] (μg/ml)	1.4	1.5	1.2	1.3	2.4	1.9

[a] Void time = 1.3 min (determined using a non-volatile solvent).
[b] From the previously eluted peak.
[c] 20 μl injection volume.

Table 3
Logarithmic regression of peak areas (arbitrary units) towards analyte mass concentration of gentamicin components, kanamycin and sulfate and comparison between ELSD and Pharmacopoeia method for the assay of a raw material

	Sulfate	C_{1a}	C_2	C_{2a}	C_1	Kanamycin A standard
Calibration/working curves						
Intercept ($\log a + b \log x_i$) logarithmic calibration	3.412 (±0.095)	0.720[a] (±0.046)	1.224[a] (±0.073)	0.49[a] (±0.29)	1.03[a] (±0.21)	2.33 (±0.12)
Calculated x_i equal to component fraction for secondary standard	–	0.230 (±0.026)	0.364 (±0.047)	0.186 (±0.055)	0.305 (±0.070)	–
			Total: 1.08 (±0.10)			
Slope (b) logarithmic Calibration	1.858 (±0.071)	2.558[a] (±0.022)	2.505[a] (±0.035)	2.54[a] (±0.14)	2.459[a] (±0.098)	2.457 (±0.070)
Logarithmic correlation coefficient r^2 ($n = 5$)	0.994	0.9998	0.9996	0.991	0.995	0.998
Range (μg/ml)	4.2–150	3.6–65	3.9–85	7.2–50	5.7–90	4.5–75
Assay (%) of raw material						
ELSD method based on kanamycin A CRS	31.9[b] (±0.1)	23.0[c] (±0.2)	33.4[c] (±0.4%)	17.5[c] (±0.3)	26.1[c] (±0.2)	–
			Total: 50.9 (±0.5)			
ELSD method based on gentamicin secondary standard		21.7[c] (±0.2)	33.4[c] (±0.4)	17.0[c] (±0.3)	27.9[c] (±0.2)	–
			Total: 50.4 (±0.5)			
Pharmacopoeia method	32.4	16.4[c]		49.8[c]	33.8[c]	–

[a] Determined towards mass of total gentamicin, Eq. (2).
[b] Using K_2SO_4 calibration curve, expresses percentage of gentamicin sulfate.
[c] Expresses percentage of total gentamicin.

regression and gave equivalent results for gentamicin raw material with the USP or EP titrimetric method (Table 3).

Due to lack of reference standard material of individual gentamicin components, external standard calibration curves can not be constructed and so their quantitation seems to be impossible. However, taking advantage of the ELSD inherent characteristic to appear approximately equal response factors for molecules with approximately equal molecular mass and similar structural formula (i.e. gentamicin and kanamycin), the quantitation of individual gentamicin components becomes feasible. Should the previous statement be correct, the coefficients a_i and also the coefficients b_i of the logarithmic calibration curves of the various gentamicin components and kanamycin must be statistically equal.

Furthermore, for gentamicin components (and generally for a mixture of compounds of similar structure) Eq. (1) provides

$$\log A_i = b \log(x_i M) + \log a \Rightarrow \log A_i = b \log M + (\log a + b \log x_i) \quad (2)$$

where, x_i is the proportion of individual gentamicin component to total gentamicin and M is the mass concentration of total gentamicin.

A reference standard material of kanamycin (kanamycin A, CRS) was used and a logarithmic calibration curve based on Eq. (1) was constructed using a series of working standard solutions. From the intercept and slope of the calibration curve, the coefficients α and b were determined (Table 3). In order to standardize a gentamicin pure substance, a series of working solutions of pure material of gentamicin sulfate with known total gentamicin concentration (calculated by subtraction of sulfate, methanol and water content) was analyzed and working curves based on Eq. (2) for each gentamicin component were constructed. From their intercepts (equal to $\log a + b \log x_i$) x_i values, and therefore the corresponding percentages of gentamicin components, were determined (Table 3). Therefore, it became feasible, the pure material of gentamicin sulfate to be further utilized as in house secondary standard of individual gentamicin components.

The correctness of the proposed quantitation technique and the validity of the obtained results are supported by: (a) the very good correlation coefficients of the logarithmic regressions, (b) the statistically equal slopes (b_i) of the calibration/working curves among the individual gentamicin components and kanamycin (proven by t-test at 95% confidence level) and (c) the fact that the sum of x_i values determined by independent working curves for each component (independent experiments), appears no statistical difference from unity (it is 1.085 ± 0.105), which indirectly reveals that the coefficients a_i for gentamicin components and kanamycin are statistically equal.

Routine analysis of gentamicin raw material can be performed using one sample working solution and the calibration curves obtained from kanamycin CRS or secondary gentamicin standard. As it is shown in Table 3 equivalent results were obtained. These results are not in good agree-

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

Formulation/claimed content	Content found, mg/ml or mg/g (%R.S.D.)[a]				Mean % recovery[b]			
	C_{1a}	C_2	C_{2a}	C_1	C_{1a}	C_2	C_{2a}	C_1
Dexamytrex Ophtiole® eye drops (Bausch & Lomb) 3 mg/ml (containing also disodium-dexamethasone-21 phosphate)	0.685 (4.1)	0.967 (5.8)	0.561 (3.2)	0.820 (3.9)	105	101	89	99
	Total: 3.033 mg/ml				Total: 99			
Garamat® eye/otic drops (Schering-Plough) 3 mg/ml (containing also Betamethasone sodium phosphate)	0.929 (1.9)	0.743 (2.1)	0.615 (1.9)	0.829 (0.69)	90	107	106	102
	Total: 3.116 mg/ml				Total: 100			
Garamycin® injection (Schering-Plough) 40 mg/ml	12.53 (1.2)	10.03 (2.5)	8.20 (1.2)	11.2 (0.89)	91	108	104	100
	Total: 41.96 mg/ml				Total: 100			
Celestoderm-V® cream (Schering-Plough) 1 mg/g (containing also disodium-dexamethasone-21 phosphate)	0.301 (0.9)	0.210 (1.9)	0.191 (1.2)	0.316 (1.1)	92	107	102	101
	Total: 1.018 mg/g				Total: 100			

[a] %R.S.D. of formulation content found from three different dilution levels.
[b] Four recovery experiments at three different spiking levels.

ment with EP method for C_1 and C_{1a} components, mainly due to the fact that EP method is based on the normalization procedure on absorbance peaks and therefore is influenced by the differences of the relative absorptivities of gentamicin derivatives. This official method is currently under revision to adopt a pulse amperometric detector without any derivatization [24].

3.4. Application to pharmaceutical formulations

The proposed LC/ELSD method was applied for the determination of gentamicin components in commercial formulations (injection, eye/otic drops and cream). Retention time of co-existing active substances and excipients was examined in order to assure that they do not overlap with gentamicin components. Fig. 3 shows a typical chromatogram of a gentamicin formulation (Dexamytrex Ophtiole®) sample solution. Dexamethasone and betamethasone, the main coexisting drugs in many gentamicin formulations, are not eluted with the mobile phase used and a periodical washing of the column with acetonitrile must be performed. The external calibration procedure using kanamycin or secondary gentamicin standard can be used with very similar results. The results shown in Table 4 reveal that all the commercial formulations conform with the common Pharmacopoeia requirement for a content within the range of 95–105% of the label content.

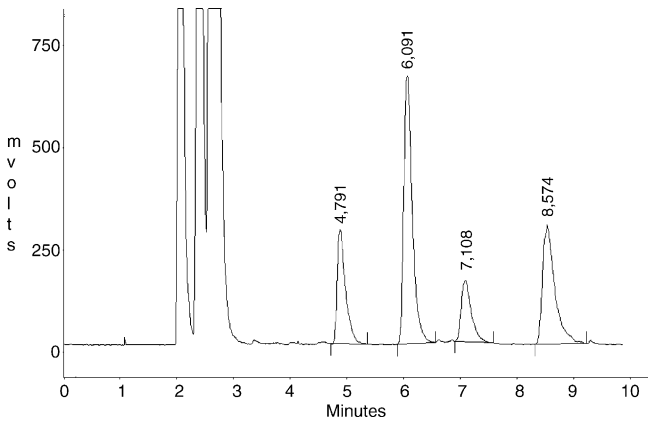

Fig. 3. Typical chromatogram of gentamicin formulation (Dexamytrex Ophtiole®), 200 μg/ml sample solution. Peaks successively to sulfate peak correspond to sodium and excipients.

The accuracy of the method was evaluated by recovery experiments. The recoveries shown in Table 4 (89–108% for individual gentamicin components and 99–100% for total gentamicin) reveal sufficient accuracy. Further study of the matrix effect on the determination was carried out by dilution experiments (determination of gentamicin content in commercial formulations using a varying dilution factor D ($V_{initial}/V_{final}$) at three different levels). The correlation curves of the concentration found (in the diluted solution) versus D were linear ($r > 0.99$) with a slope equal to the content of the formulation and a statistically (proven by t-test) zero intercept. Similarly, the correlation curves of formulation content found versus D were very linear with statistically (proven by t-test) zero slopes. These results reveal the absence of any constant or proportional determinate error due to matrix effect.

4. Conclusions

The quantitation of the four main components of the aminoglycoside antibiotic gentamicin can be performed by LC/ELSD, using a C_{18} column and volatile ion-pairing reagents (trifluoroacetic and trichloroacetic acids). The non-availability of standards for gentamicin components is

overcome by the use of the equivalent ELSD response factors for the similar (one main component) aminoglycoside kanamycin, for which a Chemical Reference Standard is available. The proposed LC/ELSD method does not require any derivatization step and also enables the simultaneous determination of the inorganic co-ion (sulfates). Despites the logarithmic relationship of ELSD signal to the analyte concentration, sufficient detectability, precision and accuracy were obtained.

The proposed method was applied successfully for the determination of gentamicin components and sulfate in raw materials and pharmaceutical formulations (injection, drops and cream) without any pretreatment, and with sufficient recoveries.

Acknowledgements

We are grateful to the Greek General Secretariat of Research and Technology for financial support.

References

[1] The United States Pharmacopoeia and The National Formulary, USP 23, NF 18, 1995, p. 702.
[2] Goodman & Gilman's The Pharmacological Basis of Therapeutics, nineth ed., 2002, p. 1114.
[3] European Pharmacopoeia, fourth ed., 2002, p. 1238.
[4] N. Isoherranan, S. Soback, Clin. Chem. 46 (2000) 837–842.
[5] J. Pick, L.L. Olson, W.Y. Ellis, P. Lim, J. Pharm. Biomed. Anal. 16 (1997) 131–137.
[6] J.H. Albracht, M.S. De-Wit, J. Chromatogr. 389 (1987) 306–311.
[7] P.J. Claes, R. Busson, H. Vanderhaeghe, J. Chromatogr. 298 (1984) 445–457.
[8] D.N. Heller, S.N. Clark, H.F. Righter, J. Mass Spectrom. 35 (2000) 39–49.
[9] L.A. Kaine, K.A. Wolnik, J. Chromatogr. A 674 (1994) 255–261.
[10] K. Ng, P.D. Rice, D.R. Bobbitt, Microchem. J. 44 (1991) 25–33.
[11] M. Dreux, M. Lafosse, L. Morin-Allory, LCGC Int. 9 (1996) 148–153.
[12] L. Fang, M. Wan, M. Pennacchio, J. Pan, J. Comb. Chem. 2 (2000) 254–257.
[13] A. Salvador, B. Herbreteau, M. Lafosse, M. Dreux, Analysis 25 (1997) 263–266.
[14] R.S. Porter, T.K. Chen, J. Chromatogr. A 732 (1996) 399–402.
[15] B.H. Hsu, E. Orton, S.Y. Tang, R.A. Carlton, J. Chromatogr. B 725 (1999) 103–112.
[16] D.S. Risley, J.A. Peterson, J. Liq. Chromatogr. 18 (1995) 3035–3048.
[17] H. Liu, Y. Pan, S.L. Qiu, H.W. Wasg, Yaowu Fenxi Zazhi 21 (2001) 429–434.
[18] E.R. Aberg, A.G.T. Gstavsson, Anal. Chim. Acta 144 (1982) 39–53.
[19] M. Otto, Chemom. Intell. Lab. Syst. 4 (1988) 101–120.
[20] A.E. Graham, E. Speicher, B. Williamson, J. Pharm. Biomed. Anal. 15 (1997) 537–543.
[21] T.A. Getek, M.L. Vestal, T.G. Alexander, J. Chromatogr. 554 (1991) 191–203.
[22] P.J. Kijak, J. Jackson, B. Shaikh, J. Chromatogr. B 691 (1997) 377–382.
[23] M. Lafosse, M. Dreux, L. Morin-Allory, J. Chromatogr. 404 (1987) 95–105.
[24] European Pharmacopoeia, Suppl. 4.5, 2003, p. 3700.