Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1031 (2004) 281–287
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of gentamicin sulfate and related compounds by high-performance liquid chromatography with evaporative light scattering detection

I. Clarot [a,*], P. Chaimbault [b], F. Hasdenteufel [a], P. Netter [c], A. Nicolas [a]

[a] *Laboratoire de Chimie Analytique, UMR 7561 CNRS-UHP, Faculté de Pharmacie, Université Henri-Poincaré–Nancy I, 5 Rue Albert Lebrun, F-54000 Nancy, France*
[b] *Institut de Chimie Organique et Analytique, CNRS UMR 6005, Université d'Orléans, B.P. 6759, F-45067 Orléans Cedex 2, France*
[c] *UMR 7561 CNRS-UHP, Faculté de Médecine, 9 Avenue Forêt de Haye, F-54500 Vandoeuvre les Nancy, France*

Abstract

A rapid and simple method for the separation and quantitation of gentamicin sulfate by HPLC coupled with evaporative light scattering detection (ELSD) has been developed. Detection of the different components of gentamicin is problematic because of the lack of UV absorbing chromophore. The use of the universal ELSD avoids the need for sample derivatization or use of specific detector such as pulsed amperometry. Separation was performed on a highpurity C_{18} 125 mm × 4 mm i.d., 3 μm, reversed phase column with 48.5 mM trifluoroacetic acid–methanol (97:3, v/v), as mobile phase at a flow rate of 0.7 ml/min. The influence of the gas nature, gas pressure and temperature of the drift tube of the detector on the detection response was investigated. Optimization was performed with the help of a specific experimental design software. This method allows the determination of the composition in components C1, C1a, C2, C2a and C2b of gentamicin sulfate samples. Mass spectrometry was employed to confirm the ELSD chromatographic profile. The method was validated using methodology described by the International Conference of Harmonization in the field of Medicinal Substances. Commercial samples of different sources were analyzed and results were in good agreement with specifications of both European and United States Pharmacopoeia.

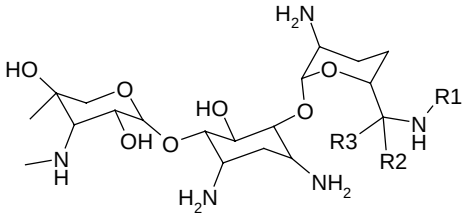
Keywords: Gentamicin sulfate; Aminoglycosides; Antibiotics; Glycosides

1. Introduction

Gentamicin sulfate belongs to a class of compounds known as aminoglycoside antibiotics. Gentamicin is a broad spectrum antibiotic produced by fermentation of *Micromonospora purpurea* [1]. It is a complex mixture of four major components designated as C1, C2, C1a and C2a and minor ones like sisomicin [2], sagamicin (gentamicin C2b [3]) and dihydroxy C2a (antibiotic JI-20 B) which is a precursor of C2a, C2 and C1. Structures and molecular masses of gentamicins are shown in Fig. 1. These structures are closely related to each other and do not possess UV absorbing chromophores, leading to problematic quantitation. Several methods have been developed to determine gentamicin content based on microbiological assay [4], immunoassay [5] or gas–liquid chromatography [6]. For determination of the composition of gentamicin, high-performance liquid chromatographic methods were preferred and 1,2-phthalic dicarboxaldehyde (OPA) was more often used as post or pre-column derivatization agent with either fluorescence [7,8] or UV detection [9]. Other derivatization agents could also be employed such as dansylchloride [10] or 2,4,6-trinitrobenzenesulphonic acid [11]. Such methods, which need sample treatment, make more complex the HPLC system (reaction coil, extra pump, etc.) and were very time-consuming. In fact, several drawbacks could be listed against a sample derivatization process: introduction of non controlled impurities, degradation products and the most important, impurities of the analyte lacking of the specific functional group required for derivatization could not be detected. To avoid sample derivatization, a universal detector could be used as previously shown by Inchaupse and Samain [12] for several aminoglycoside antibiotics with refractive index detection. Mass spectrometry could also

* Corresponding author. Tel.: +33-3-83-68-23-47; fax: +33-3-83-35-23-83.
E-mail address: igor.clarot@pharma.uhp-nancy.fr (I. Clarot).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.12.032



Gentamicin	MW	R1	R2	R3
C_1	477.31	CH_3	CH_3	H
C_{1a}	449.28	H	H	H
C_2	463.30	H	CH_3	H
C_{2a}	463.30	H	H	CH_3
C_{2b}	463.30	CH_3	H	H

Fig. 1. Structure and molecular masses of the gentamicin components.

be employed and was generally used for analysis of complex matrices [13]. Pulsed amperometric detection has been shown to be suitable for the determination of the gentamicin sulfate composition [14,15]. However, this method was described to be difficult in a routine use: because of a problematic signal stability, several experiences were required to obtain a good repeatability [15,16]. Recently, capillary electrophoresis has been used coupled to gentamicin OPA pre-derivatization [17].

Electrochemical detection is prescribed by the European Pharmacopoeia [18] for the determination of gentamicine sulfate composition, the amounts of C1, C1a and the sum of C2, C2a and C2b were limited to 20.0–35.0, 10.0–30.0 and 40.0–60.0%, respectively [19]. The United States Pharmacopoiea [20] prescribe an OPA derivatization with UV detection [21], the content prescribed of gentamicin C1 is between 25 and 50%, of C1a is between 10 and 35% and the sum of C2 and C2a is between 25 and 55%.

Evaporative light scattering detection (ELSD) is described as a universal detection mode suitable for non-absorbing analytes [22]. The chromatographic mobile phase is nebulized with an inert gas and evaporated in a drift tube. The remaining particles are detected by light scattering. The response does not depend on the solute optical properties, any compound less volatile than the mobile phase could be detected. The detector response is now well described [23] and shows a double logarithmic relationship between the signal and the analyte concentration. Such response allows all molecules of the sample to give a proportional signal (same sensitivity). This principle is in good agreement with the search of impurities in pharmaceutical products.

The principle aim of this work was to develop a rapid and simple chromatographic method which allows a direct sample introduction without any derivatization. The method was validated with respect to validation criteria according to ICH guidelines [24]. The detector response was optimized in term of gas nature, gas pressure and drift tube temperature. Mass spectrometry was employed to confirm the analyte chromatographic profile. This method was used to evaluate the composition of gentamicine sulfate of commercial samples from different sources.

2. Experimental

2.1. Chemicals

Methanol (HPLC grade) was obtained from Carlo Erba (Milan, Italy). Trifluoroacetic acid (TFA) was purchased from Acros Organics (Geel, Belgium) and was flushed with nitrogen between each utilization. Barium chloride was obtained from Merck (Darmstadt, Germany). Ultrapure Water was obtained in a Milli-Q system from Millipore (Bedford, MA, USA).

Gentamicin standard (mixture of components C1, C1a, C2 and C2a) and sisomicin were provided by the European Pharmacopoeia laboratory (Strasbourg, France).

Commercial gentamicin sulfate samples were generous gifts from Vetoquinol (Lure, France) and Virbac (Carros, France).

2.2. Instrumentation

2.2.1. LC apparatus

The isocratic HPLC system consisted of an 515 HPLC pump (Waters, Milford, MA, USA), a TSP model AS100 autosampler (Thermoseparation Products, Fremont, CA, USA) set to inject 20 μl and an electronic integrator SpectraPhysics SP4290 coupled with a chromatographic data system Spectra-Physics Winner on Windows (Spectra-Physics, San Jose, CA, USA). The evaporative light scattering detector was a Sedex 75 model from Sedere (Alfortville, France) equipped with a low flow nebulization head (Sedere, Alfortville, France). The analytical column was a Hypurity RP 18, 3 μm, 125 mm × 4 mm i.d. (Thermo Hypersil, Runcorn, UK). The mobile phase consists of 48.5 mM TFA–MeOH (97:3, v/v) at a flow rate of 0.7 ml/min and was filtered with Millipore filter model HVLP 0.45 μm (Millipore, Molsheim, France) before use.

2.2.2. Mass spectrometry

Mass spectrometry (MS) and LC–MS experiments were carried out on a Perkin-Elmer Sciex API 300 mass spectrometer (Perkin-Elmer, Toronto, Canada) equipped with an ionspray source. The instrument was tuned by direct infusion (10 μl/min) of a Gentamicin sulfate solution using a syringe pump Harvard model 22 (Harvard Apparatus, Holliston, MA, USA). The following tune parameters were retained for optimum gentamicin detection in positive ion mode: ionspray voltage, 5.7 kV; orifice, 100 V; focusing ring, 400 V; curtain gas, 7; Q0 voltage, −5 V; ion energy, 1 eV. For negative ion scanning mode, polarity of the electronical optic has been inverted and ionspray voltage decreased to −4.3 kV in order to avoid corona discharges in the ion source.

The LC system consist of a Perkin-Elmer model LC-200 system binary pump (flow rate: 0.7 ml/min) and a Perkin-Elmer series 200 autosampler fitted with a 10-μl loop.

2.2.3. Software

Optimization of the detection was obtained with the help of the experimental design software, Modde 5.0 (Umetrics, NJ, USA).

2.3. Sample preparation

Concentrations of gentamicin sulfate solutions used were those specified by the European Pharmacopoeia corresponding monograph [19]: for the quantitation and the signal-to-noise ratio, 0.500 and 0.025 mg/ml were used, respectively. All dilutions were made in the mobile phase. For LC–MS analysis, 1.0 mg/ml solution diluted in the mobile phase was employed.

2.4. Peak assignment

Peak assignments were made by elution of a gentamicin sulfate standard solution. The relative proportions described for the standard composition have permitted a peak identification. LC–MS experiments (Section 3.3) confirmed the elution order observed.

A sisomicin solution was also injected in the chromatographic system. This known gentamicin impurity was well separated from the major components and was eluted before the first C1a peak.

3. Method development

3.1. Detection optimization

The eluting mobile phase from the HPLC column and the carrier gas are nebulized and volatilized in the drift tube. The temperature of this tube and the gas pressure are of critical importance to insure complete vaporization of the solvents. The nature of the gas used for the nebulization has been shown to play a key role in the heat transfer and in the detection sensitivity [25].

The influence of the gas nature (air, nitrogen and helium), the gas pressure and the drift tube temperature on gentamicine response was studied. A full factorial design at three levels was used. Three replicates of the central point were included in the design to give a final experimental matrix of thirty experiments. The values of this design are given in Table 1.

Table 1
Factors and nominal values used for the detection optimization

Parameter	Low value (−1)	Central value (0)	High value (+1)
Gas pressure (bar)	2.5	3.0	3.5
Gas nature	Helium	Nitrogen	Air
Tube temperature (°C)	40	60	80

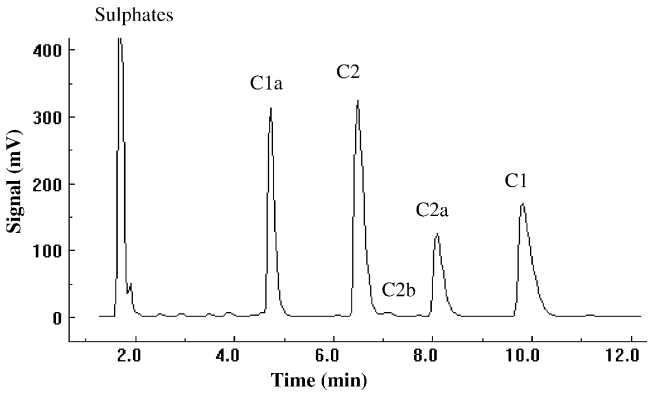


Fig. 2. Chromatogram example of a 0.500 mg/ml gentamicin sulfate solution.

Both sample solutions (0.500 and 0.025 mg/ml) were tested and the software optimization was applied to maximize the peak areas and the signal-to-noise ratio. Maxima were obtained for a drift tube at 60 °C and a pressure of 3.5 bar. No differences were evidenced between the three gases under study; for practical and economical reasons, air was chosen for all the experiments. A type-chromatogram obtained with these conditions is given in Fig. 2.

3.2. Sample treatment

In the related substances assay of the European Pharmacopoeia monograph [18], specified limits were described for impurities eluting before the C1a gentamicin peak. ELSD has the capacity to detect all the non volatile components contained in the sample. So, a peak could be observed in the dead volume (Fig. 2) corresponding to sulfates present in great amount in the analyte (32.0–35.0%) [19]. Further experiments were made when sulfates were precipitated with barium chloride [26]. The non-soluble barium sulfate precipitate was filtered through a 0.45 μm filter (Millipore, Molsheim, France) before injection. The chromatogram obtained shows the absence of the dead volume peak as illustrated in Fig. 3A. The barium quantity used for the precipitation must be very well adjusted because of the apparition of a barium dead volume peak when too much barium chloride was added, Fig. 3B. A 2:1 barium chloride:gentamicin sulfate molar ratio should be used. This procedure was very problematic and was not employed for the validation study. Gentamicin sulfate samples were diluted in the mobile phase and injected without any precaution in the chromatographic system. Void volume peaks were not taken into account in both composition and related substances determinations by the normalization process.

3.3. Mass spectrometry

Liquid chromatography and mass spectrometry were used to make sure that the ELSD method allows the characterization of all products and impurities contained in the gentam-

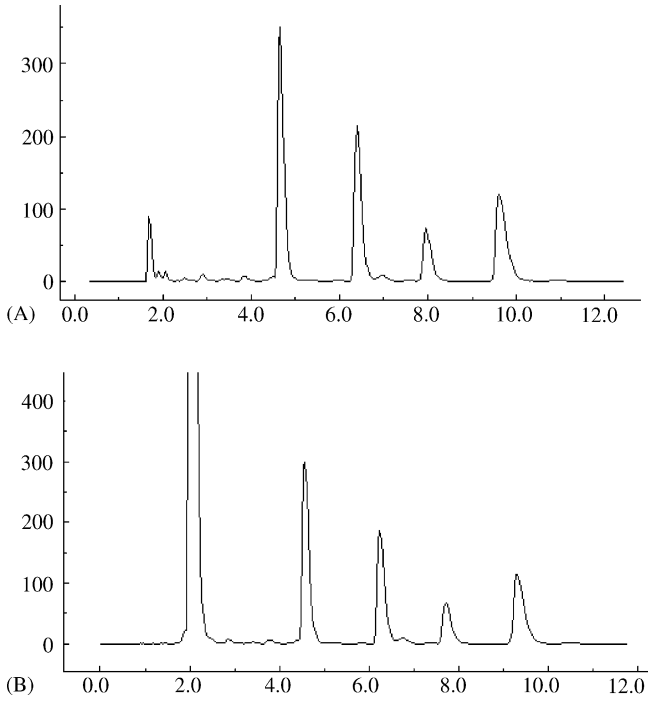


Fig. 3. Chromatograms obtained for a gentamin sulfate sample when sulfates were precipitated with a barium chloride solution. (A) Near the stoichiometric proportions. (B) With an excess of barium.

icin sulfate samples. The mobile phase used for these experiments was a binary mixture of 48.5 mM TFA–methanol (90:10, v/v). This eluent is slightly different from the one described above for the LC–ELSD method (97:3) because of the necessity to increase methanol content to gain a sufficient sensitivity in a full scan MS detection.

Fig. 4 shows the mass spectrum (positive ion mode) obtained after direct infusion of a 1 mg/ml solution of gentamicin sulfate in the ion source without any chromatographic column. Protonated molecular ions of the five gentamicins ($M+H^+$) were produced at *m*/*z* 450.5 (C1a), 464.5 (C2, C2a and C2b) and 478.5 (C1), respectively. The triplet of peak observed at *m*/*z* 548.5, 562.0 and 576.5 exhibits a mass difference of 98 amu with the triplet of gentamicin protonated ions. This difference could be explained as a production of cluster ions constituted of a sulfuric acid molecule and gentamicin molecular ions ($M + H + H_2SO_4$). This interpretation was supported first by the high proportion of sulfate ion in the gentamicin sample (33%, *m*/*m*) and second by the fact these [$M+98$] peaks disappear from the full scan spectra in LC–MS (sulfates are then eluted in the void volume whereas gentamicins are retained by the chromatographic support). Peak observed at *m*/*z* 160.0, 205.0 and 322.0 could be attributed to the mutual fragmentation of gentamicins, a fragmentation scheme is proposed on Fig. 5. However, the *m*/*z* 322 fragment could also correspond to garamine, an impurity listed in the gentamicin sulfate monograph [18].

Gentamicins samples were able to contain impurities that cannot be evidenced only by a single MS detection. For example, *m*/*z* 322 is corresponding to the $[M + H]^+$ ion of garamine but also to a fragment ion of gentamicins as previously evoked. This is a typical indetermination case that require the coupling between a separative method to mass spectrometry to be resolved.

LC–MS full scan in the negative ion mode was rather employed to characterize the sulfate peak (sulfates are naturally anionic) eluting in the void volume. Therefore, no interpretation could be made because of the great proportion of TFA

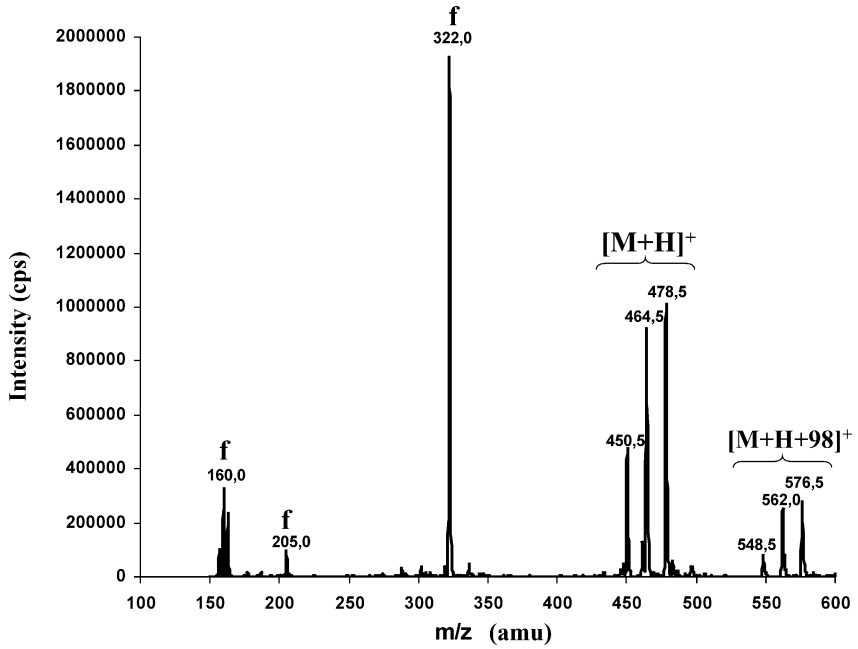


Fig. 4. Spectrum of a gentamicin sulfate solution (1 mg/ml) obtained by infusion in positive ion mode (for mass parameters see Section 2). Peaks indexed with an "f" are corresponding to fragments obtained by collisionally induced dissociation in the curtain gas interface of the mass spectrometer.

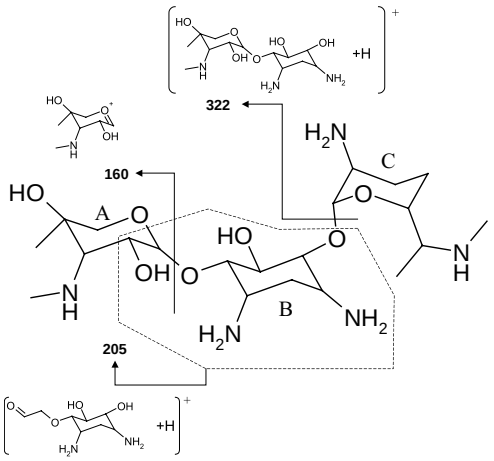


Fig. 5. Fragmentation scheme proposed for the gentamicin products. The fragment ions at *m*/*z* 160 and 205 originate in the fragmentation of the A ring of gentamicins classically observed with sugars [27].

in the mobile phase leading to a possible ionization competition. An another problem was the quick contamination of the spectrometer electronic optic by the trifluoroacetate ions in negative ion mode.

By contrast, LC–MS full scan in the positive mode brings more information than the negative one. No interference was found at the retention time corresponding to the void volume. Obviously, anions (such as sulfates) cannot be detected in positive ion mode mass spectrometry. Thus, the lack of peak for the void volume in positive ion mode is first leading to the confirmation of a sulfate interpretation and second to absence of major impurities hidden by sulfates. In addition, three minor impurities were also easily evidenced. Beside, these peaks have been sooner observed in Fig. 2 with the LC–ELSD method. One of them (3.22 min with LC–MS eluent) is characterized by the presence of the ion at *m*/*z* 322 belonging to its mass spectrum and is a result a good candidate for garamine. Sisomicin was not evidenced by LC–MS (*m*/*z* 447) in the analyzed gentamicin sulfate samples.

In conclusion to the MS study, related substances observed with the LC–ELSD method were confirmed by the MS detection and no additional impurities were observed. The LC–ELSD method was demonstrated suitable to control the main components from gentamicin sulfate samples as well as the related substances. MS detection confirms the elution order proposed in Fig. 2 for the ELSD chromatogram. C1a (*m*/*z* 450.5) is the first eluted gentamicin component whereas C1 (*m*/*z* 478.5) is the last eluted one. Positional isomers C2, C2a and C2b (*m*/*z* 464.5) were identified by their relative proportion in the gentamicin sulfate mixture.

4. LC–ELSD method validation

The method was validated using methodology described by the International Conference of Harmonization (ICH) [24]. Specificity, intermediate precision and linearity were evaluated. Limits of detection were also calculated. The composition of the gentamicin sulfate samples were determined using a normalization process [18].

Table 2
R.S.D. values obtained for six injections of a 0.5 mg/ml gentamicin sulfate solution (repeatability) and 12 injections on 2 days (intermediate precision inter-days)

	Gentamicins				
	C1a	C2	C2b	C2a	C1
Repeatability (R.S.D., %)	0.83	1.31	7.01	1.76	1.18
Intermediate precision (R.S.D., %)	3.86	3.57	10.76	4.38	4.01

4.1. Specificity

A chromatogram of the mobile phase used in the LC–ELSD method shows no interferences in the range of retention times under study. The MS study confirmed that no impurities co-elute with the five gentamicins present in the sample and shows that gentamicin samples were completely described by the LC–ELSD method.

4.2. Repeatability

The method repeatability was determined using six determinations at 100% of the test concentration (0.500 mg/ml), results were shown in Table 2. Relative standard deviation (R.S.D.) values are given for each gentamicin. In every case, R.S.D. values were better than 2%, except for gentamicin C2b which is described as a minor component (1%, *m*/*m*).

Table 3
Regression data for the linearity study of the four major gentamicin sulfate components in a 0.1–1.0 mg/ml concentration range

Gentamicin	Regression equation	r^2
C1a	$y = 1.3523x + 7.1320$	0.9991
C2	$y = 1.3737x + 7.2041$	0.9991
C2a	$y = 1.3954x + 6.8654$	0.9996
C1	$y = 1.3529x + 7.0601$	0.9993

The regression curves were obtained by plotting log (concentration in mg/ml) vs. log (peak area). r^2 is the corresponding coefficient of determination.

Table 4
Regression data for impurities detected with the LC–ELSD method

Impurity	Regression equation	r^2
1	$y = 1.2921x + 5.201$	0.9868
2	$y = 1.2827x + 5.326$	0.9930
3	$y = 1.2889x + 5.401$	0.9964

See Table 3 for the description of the linear model used.

Table 5
Composition of gentamicin sulfate commercial samples determined by normalization

Samples	%C1a	%C2	%C2b	%C2a	%C1	Sum of C2
A	23.6	34.5	0.8	13.8	26.2	49.1
B	27.1	31.9	0.6	12.5	24.2	45.0
C	28.2	32.3	0.5	14.1	23.7	46.9
European Pharmacopoeia	10.0–30.0				20.0–35.0	40.0–60.0
United States Pharmacopoeia	10–35				25–50	25–55

The sum of C2 corresponds to %C2 + %C2a + %C2b.

4.3. Intermediate precision

The intermediate precision was determined by six injections of the test solution on 2 days. The inter-day precision was then calculated, results were given in Table 2 and were satisfactory.

4.4. Linearity

It is now well known that ELSD gives non direct linear response [28]. Light scattering is a complex process involving several mechanisms. It is usually described as a mixture of Rayleigh scattering, Mie scattering, diffraction and reflexion phenomena. The intensity of the scattered light I is a function of the mass of the scattering particles and generally follows an exponential relationship described by the following equation:

$$I = km^b \quad (1)$$

with I the intensity of light, m the mass of the scattering particles, k and b were constants determined principally by the nature of the mobile phase and the detector parameters [29]. Generally b varies between 1 and 2 depending on the apparatus conception [30]. If it is equal to 1, the relation becomes linear. Over two orders of magnitude, Eq. (1) is no longer valid and more complex models have to be used [31]. The theoretical Eq. (1) allows the ELSD to give equivalent responses for related structure substances [32], but also whatever the classes of compounds studied [33].

A plot of $\log I$ versus $\log m$ provides a linear response as a plot of the peak area versus the sample concentration in double logarithmic co-ordinates. Such mathematical transformation is allowed by the ICH validation description [24].

The linearity study was made by preparing five calibration samples covering the concentration range of 0.1–1.0 mg/ml. Each sample was injected in triplicate. The validity of linear models was assessed using classical statistical tests ($n = 3$, $\alpha = 5\%$). Results of regression curves were summarized in Table 3 and indicates good linearity whatever the gentamicin studied.

A similar model was applied for impurities observed in the test solution (Table 4). It could be observed that the double logarithmic model is suitable to follow the related substances. The detector sensitivity, described by the linear regression slopes, was in the same magnitude order than those previously observed for gentamicins (Table 3). The response factors were thus demonstrated equivalent whatever the compound studied in the gentamicin sample.

4.5. Limit of detection (LOD)

The limit of detection is defined as the lowest concentration of analyte that can be clearly detected. Its determination could be made by the calculation of the signal-to-noise ratio [24]. A ratio of 3 was selected and successive dilutions of the test solution gave a LOD relative to the C1a peak of 0.16% (m/m). Such limit is in good agreement with that specified by the European Pharmacopoiea [19].

5. Analysis of commercial samples

Three commercial samples were analyzed. The composition of these samples was determined by normalization on a 0.500 mg/ml solution test. Results are summarized in Table 5. As can be seen, the composition of commercial gentamicins is quite variable. Therefore, each sample composition was in good agreement with specifications required by both Pharmacopoeias (European and USA) [19,21].

6. Conclusion

The method described in this report allows to well separate gentamicins C1, C1a, C2, C2a and C2b and other minor components. The void volume peak is demonstrated to be due to sulfates ions present in great proportion in the gentamicin sulfate samples. The use of ELSD is shown to be suitable to describe a complex pharmaceutical product as well as its related substances without any derivatization in less than 12 min.

Acknowledgements

The authors are very grateful to Thermohypersil for the column gift. They also thanks Sedere for the technical assistance.

References

[1] M. Weinstein, G. Luedemann, E. Oden, G. Wagman, Antimicrob. Agents Chemother (1963) 1–7.

[2] J. Bredy, J. Kadar-Paunez, Z.M. Vajna, G. Horvath, J. Gyimesi, I. Kuczka, J. Antibiot. 30 (1977) 945.
[3] R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato, T. Nara, J. Antibiot. 27 (1974) 793.
[4] L.D. Sabath, J.I. Casey, P.A. Ruch, L.L. Stumpf, M. Finland, J. Lab. Clin. Med. 78 (1971) 457.
[5] J.M. Andrews, R. Wise, J. Antimicrob. Chemother. 14 (1984) 509.
[6] J.W. Mayhew, S.L. Gorbach, J. Chromatogr. 151 (1978) 133.
[7] J. Anhalt, Antimicrob. Agents Chemother. 11 (1977) 651.
[8] S. Maitra, T. Yoshikawa, J. Hansen, I. Nilsson-Ehle, W. Palin, M. Schutz, L. Guze, Clin. Chem. 23 (1977) 2275.
[9] M. Freeman, P. Hawkins, J. Loran, J. Stead, J. Liq. Chromatogr. 2 (1979) 1305.
[10] G.W. Peng, M.A.F. Gadalla, A. Peng, V. Smith, W.L. Chiou, Clin. Chem. 23 (1977) 1838.
[11] P. Gambardella, R. Punziano, M. Gionti, C. Guadalupi, G. Mancini, A. Mangia, J. Chromatogr. 348 (1985) 229.
[12] G. Inchaupse, D. Samain, J. Chromatogr. 303 (1984) 277.
[13] M. Cherlet, S. De Baere, P. De Backer, J. Mass Spectrom. 35 (2000) 1342.
[14] L. Kaine, K. Wolnik, J. Chromatogr. A 674 (1994) 255.
[15] E. Adams, W. Roelants, R. De Paepe, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 18 (1998) 689.
[16] F. Wienen, R. Deubner, U. Holzgrabe, Pharmeuropa 15 (2) (2003) 286.
[17] E. Kaale, S. Leonard, A. Van Schepdael, E. Roets, J. Hoogmartens, J. Chromatogr. A 895 (2000) 67.
[18] European Pharmacopoeia, European Department for the Quality of Medicines, Strasbourg, fourth ed., 2002.
[19] Gentamicin sulphate, monograph 07/2003: 0331, European Pharmacopoeia, European Department for the Quality of Medicines, Strasbourg, 2003.
[20] United States Pharmacopeia 26, United States Pharmacopoeial Convention, Rockville, MD, 2003.
[21] Gentamicin sulphate, Official Monograph, United States Pharmacopeia 26, United States Pharmacopoeial Convention, Rockville, MD, 2003.
[22] M.K. Park, J.H. Park, S.B. Han, Y.G. Shen, I.H. Park, J. Chromatogr. A 736 (1996) 77.
[23] M. Dreux, M. Lafosse, L. Morin-Allory, LC GC Int. 9 (3) (1996) 148.
[24] ICH, Topic Q2B, Technical Coordination, London, UK, 1997.
[25] Y. Mengerink, H.C.J. de Man, Sj. Van der Wal, J. Chromatogr. 552 (1991) 593.
[26] R. Vogel, K. Defillipo, V. Reif, J. Pharm. Biomed. Anal. 24 (2001) 405.
[27] Spectrométrie de Masse, De Hoffmann, Charrette, Stroobant, Dunod, Paris, second ed., 1999, p. 280.
[28] K. Petritis, C. Elfakir, M. Dreux, J. Chromatogr. A 961 (2002) 9.
[29] M. Dreux, M. Lafosse, Spectra 2000 151 (1990) 16.
[30] M. Righezza, G. Guiochon, J. Liq. Chromatogr. 11 (1988) 2427.
[31] P. Van der Moeren, J. Vanderdeelen, L. Baert, Anal. Chem. 64 (1992) 1056.
[32] C.E. Kibbey, Mol. Diversity 1 (1996) 247.
[33] P.A. Asmus, J.B. Landis, J. Chromatogr. 316 (1984) 461.