Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Journal of Chromatography B, 833 (2006) 191–198
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Analysis of neomycin using an improved liquid chromatographic method combined with pulsed electrochemical detection

N.H. Zawilla [a,b], J. Diana [b], J. Hoogmartens [b], E. Adams [b,*]

[a] *National Organization for Drug Control and Research, Cairo, Egypt*
[b] *Laboratory for Pharmaceutical Chemistry and Drug Analysis, Pharmaceutical Sciences, K.U.Leuven, O&N 2, PB 923, Herestraat 49, B-3000 Leuven, Belgium*

Received 23 September 2005; accepted 27 January 2006
Available online 28 February 2006

Abstract

An isocratic liquid chromatographic method with pulsed electrochemical detection is described for the determination of neomycin in the presence of its impurities. The mobile phase is composed of an aqueous solution containing 35 g/l of sodium sulphate, 1 g/l of sodium 1-octanesulfonate, 14 ml/l of tetrahydrofuran (THF) and 50 ml/l of 0.2 M phosphate buffer pH 3.0. Sodium hydroxide was added post column to enhance the detection. An investigation of different reversed-phase columns indicated that the Discovery (C18 5 μm, 250 mm × 4.6 mm I.D.) column was the most suitable. The proposed method shows high efficiency, allowing the separation of the main component neomycin B from neomycin C and 15 other impurities. A central composite design was used to assess the robustness of the method. The method showed good selectivity, repeatability, linearity and sensitivity. This method was applied to analyse commercial samples.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Pulsed electrochemical detection; Neomycin sulphate; Purity testing

1. Introduction

Neomycin, which is usually used as the sulphate, is a broad spectrum aminoglycoside antibiotic. It inhibits the growth of both Gram-positive and Gram-negative bacteria [1,2]. It is mainly used in the treatment of topical infections.

Neomycin sulphate is mainly composed of neomycin B and its stereoisomer neomycin C. Other impurities that can also be present in commercial samples are: paromamine, paromomycin I, paromomycin II, neomycin LP-A (LP = low potency) and neomycin LP-B. The structures of neomycin and its most common impurities are shown in Fig. 1.

Direct UV detection of neomycin and its impurities cannot be performed because of the lack of a strong UV absorbing chromophore. The European Pharmacopoeia (Ph. Eur.) describes a microbiological assay and a liquid chromatographic (LC) method with pulsed electrochemical detection (PED) for the determination of impurities [3].

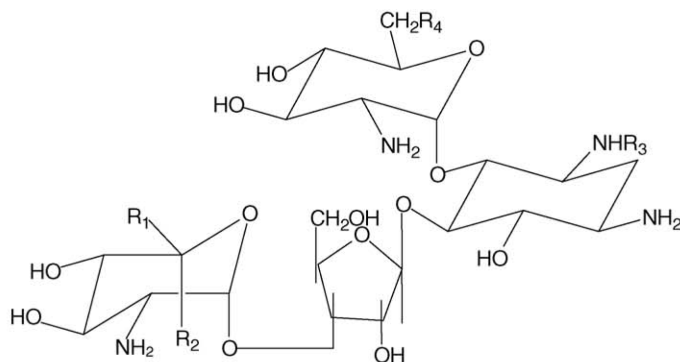
Several chromatographic methods combined with different detection techniques have been reported for the determination of neomycin: thin-layer chromatography with detection after derivatization [4,5], LC combined with PED [6–8] or evaporative light scattering detection (ELSD) [9,10], pre-column derivatization prior to reversed-phase LC [11–13], reversed-phase LC with post-column derivatization [14,15], LC–tandem mass spectrometry using hydrophilic interaction chromatography [16], ion exchange chromatography using refractometric detection [17] and capillary zone electrophoresis with indirect UV detection [18].

PED was found to be the method of choice for the detection of aminoglycoside antibiotics [19]. "Pulsed" electrochemical detection is necessary to avoid fouling of the working electrode surface, what would result in a gradual decrease of the output signal. Beside neomycin, PED was also used successfully to detect other aminoglycosides like kanamycin [20], amikacin [21], tobramycin [22], gentamicin [23] and netilmicin [24].

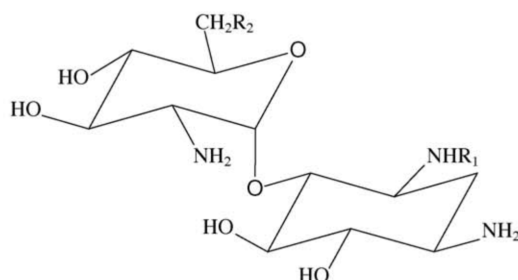
The ion-pair LC method published in 1996, made use of a poly(styrene–divinylbenzene) (PSDVB) column combined with PED [6]. At that time, this reversed phase polymer packing showed a better stability compared to C8 and C18 columns, but its efficiency was lower. In the mean time, new types of columns

* Corresponding author. Tel.: +32 16323443; fax: +32 16323448.
E-mail address: erwin.adams@pharm.kuleuven.be (E. Adams).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.01.034



	R_1	R_2	R_3	R_4
Neomycin B	H	CH_2NH_2	H	NH_2
Neomycin C	CH_2NH_2	H	H	NH_2
Paromomycin I	H	CH_2NH_2	H	OH
Paromomycin II	CH_2NH_2	H	H	OH
LP-B	H	CH_2NH_2	Ac	NH_2



	R_1	R_2
Neamine	H	NH_2
Paromamine	H	OH
LP-A	Ac	NH_2

Fig. 1. Structures of neomycin and its impurities.

like C18 derivatized polymer became available on the market and properties of classical reversed phase columns (C18) improved a lot. In this paper, the performance of different reversed-phase LC columns towards the analysis of neomycin will be examined in order to improve the selectivity so that more impurities can be separated. This will also improve the stability-indicating properties of the method.

2. Experimental

2.1. Reagents and samples

A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to purify glass-distilled water. The buffer was prepared by mixing a 0.2 M solution of phosphoric acid and a 0.2 M solution of potassium hydrogen phosphate till pH 3.0 was reached. These solutions were prepared using 85% phosphoric acid (m/m) from Acros (Geel, Belgium) and potassium dihydrogen phosphate from Merck (Darmstadt, Germany). Sodium 1-octanesulfonate, HPLC grade, was also from Acros. THF (stabilised with 2,6-di-*tert*-butyl-4-methyl phenol) and sodium sulphate anhydrous were obtained from Merck and helium from Air Liquide (Machelen, Belgium). The 0.5 M sodium hydroxide solution was prepared using 50% sodium hydroxide (m/m), aqueous solution (Baker, Deventer, Netherlands). The Neomycin B reference substance was a USP standard (chromatographic purity: 97.1%). Neomycin C, LP-B, LP-A and paromamine reference substances were prepared in

the laboratory from commercial samples according to a previously published paper [25]. A mixture of paromomycin I and paromomycin II was obtained from Carlo Erba (Milan, Italy). Samples were obtained from Alcon (Puurs, Belgium), Alconcusi (Barcelona, Spain), Schering Plough (Hérouville-St-Clair, France) and Upjohn (Kalamazoo, Mi, USA). Sample concentrations of 0.75 mg/ml were used to determine the related substances, respectively. The samples and the related substances were dissolved in the mobile phase.

2.2. Instrumentation

The chromatographic procedure was carried out using a L-6200 Intelligent pump (Merck–Hitachi, Darmstadt, Germany), an autosampler AS100 Spectra Series (San Jose, CA, USA) equipped with a 20 μl loop, a laboratory prepared pneumatic device, allowing pulse-free post-column addition of the sodium hydroxide solution and Chromeleon 6.50 software (Dionex, Sunnyvale, CA, USA) for data acquisition. The pulsed electrochemical detector (PED) was a Decade II from Antec (Leyden, Netherlands). The electrochemical cell consisted of a gold working electrode, a hydrogen reference electrode and a carbon filled polytetrafluoroethylene counter electrode. This electrochemical cell was kept at 35 °C in the detector oven. The following columns were investigated (unless indicated otherwise, all the column dimensions were 250 mm × 4.6 mm I.D., with 5 μm particles): Discovery C18 (Supelco, Bellefonte, PA, USA), PLRP-S 8 μm, 1000 Å (Polymer Laboratories, Shropshire, UK), PLRP-S 5 μm, 1000 Å (Polymer Laboratories), PLRP-S 3 μm, 100 Å, 150 mm × 4.6 mm (Polymer Laboratories), XTerra RP 18 (Waters, Milford, USA), Gemini C18 (Phenomenex, Macclesfield Cheshire, UK), Luna 5 μm, 150 mm × 4.6 mm (Phenomenex), YMC-Pack Pro (YMC, Milford, USA), Hypersil BDS C18 (Thermo, Bellefonte, PA, USA), Astec C18 polymer (Agilent, Wilmington, DE, USA), Zorbax SB (Agilent), Supelcosil LC-C18-DB (Supelco), Supelcosil 3 μm, 150 mm × 4.6 mm (Supelco). The columns were maintained at 35 °C in a water bath heated by means of a Julabo EM thermostat (Julabo, Seelbach, Germany).

2.3. Chromatography

The mobile phase, consisting of an aqueous solution containing 35 g/l of sodium sulphate, 1 g/l of sodium 1-octanesulfonate, 50 ml/l of 0.2 M phosphate buffer (pH 3.0), 14 ml/l of THF, was degassed with helium before use. The flow rate was 1 ml/min. Through a mixing tee, 0.5 M sodium hydroxide was added post-column from a helium-pressurized reservoir (1.6 bar) and mixed in a packed reaction coil (1.2 m, 500 μl) from Dionex. Indeed, the pH of the mobile phase has to be raised to 13 to improve the sensitivity of the detection. The 0.5 M NaOH solution was prepared starting from a 50% (m/m) aqueous solution which was pipetted in helium degassed water. Water was degassed in order to avoid the formation of carbonates that foul the electrode.

The time and voltage parameters for the detector were provided by Dionex [26] and were set as follows: E_1 (+0.05 V), E_2 (+0.75 V) and E_3 (−0.15 V) with the assigned pulse durations t_1 (0–0.40 s), t_2 (0.41–0.60 s) and t_3 (0.61–1.00 s). Integration of the signal occurred between 0.20 and 0.40 s.

2.4. Experimental design

A robustness study was performed by means of an experimental design and multivariate analysis using Modde 5.0 software (Umetrics, Umea, Sweden). A central composite design was applied. A central composite design is composed of a full or fractional factorial design, star points and replicated centre points. The star points enable the model to estimate the curvature response. These star points are located at the centre and both extreme levels of the experimental domain. For a complete central composite design, which includes the points of a two level full factorial design, the number of runs is equal to $2^k + 2k + n$, where k is the number of parameters and n is the number of centre points. In this study, five parameters (amount of sodium sulphate, amount of sodium 1-octanesulfonate, volume of THF, temperature of the column, pH of the phosphate buffer) were investigated. With this number of parameters and three centre points, a complete central composite design would result in a number of runs equal to 45. In order to reduce the number of runs, a central composite design which includes the points of a two level half fractional factorial design was chosen, with a number of runs equal to $2^{k-1} + 2k + n = 29$. The statistical relationship between a response Y and the experimental variables $X_i, X_j, \ldots$ is of the following form:

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_i X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \ldots + E \quad (1)$$

where the β's are the regression coefficients and E is the overall experimental error.

The linear coefficients β_i and β_j describe the quantitative effects of the respective variables. The cross coefficient β_{ij} measures the interaction effect between the variables and the square terms $\beta_{ii} X_i^2$ and $\beta_{jj} X_j^2$ describe the non-linear effects on the response.

3. Results and discussion

3.1. Method development

The Ph. Eur. method for the determination of neomycin impurities is an LC method with PED. This method uses a mobile phase containing 20 ml of trifluoroacetic acid, 6 ml of carbonate-free sodium hydroxide solution diluted to 1 litre with water (the amount of sodium hydroxide in the mobile phase can be varied till reaching the system suitability requirements) and a base-deactivated octadecylsilyl silica gel column (5 μm) 250 mm × 4.6 mm, maintained at 25 °C. Under these conditions LP-B is not separated from neomycin B and the separation of the pair neamine–LP-A is not complete. Previously in our laboratory a method was developed using a polystyrene–divinylbenzene PLRP-S 8 μm, 1000 Å (250 mm × 4.6 mm) column (PSDVB), maintained at 35 °C [4,5]. The mobile phase is composed of an

aqueous solution containing 70 g/l of sodium sulphate, 1.4 g/l of sodium 1-octanesulfonate and 50 ml/l of 0.2 M phosphate buffer pH 3.0. This polymer packing gives very good stability, but the efficiency is rather poor. Nevertheless, it separates neamine from LP-A and LP-B from neomycin B. Hence, this method was used as starting point to develop a more selective method.

3.1.1. Comparison of stationary phases

It was now tried to improve the separation by using other columns than the described PSDVB 8 μm stationary phase. First, polymer columns with a smaller particle size were investigated: PLRP-S 5 μm, 1000 Å and PLRP-S 3 μm, only available with pore sizes of 100 Å. Astec, a C18 derivatised polyvinyl alcohol column, was also included in this study since this stationary phase combines the separation efficiency of traditional reversed phase columns with the stability of polymer columns. In our laboratory, the Astec column was previously used with success for the analysis of erythromycin [27]. Different C18 silica-based stationary phases were also investigated since these columns are known to give higher selectivity compared to polymer stationary phases. The selection of these columns was essentially based on a column classification system previously developed by our laboratory [28]. This system ranks columns according to their properties using only four chromatographic parameters: the retention factor of amylbenzene (k'_{amb}), the relative retention factor benzylamine/phenol at pH 2.7 ($rk'_{ba/ph\ 2.7}$), the relative retention factor triphenylene/*o*-terphenyl ($rk'_{tri/ter}$) and the retention factor of 2,2′-dipyridyl ($k'_{2,2'\text{-d}}$). This allows the selection of columns with different chromatographic properties since columns with different properties show different parameters. The Gemini stationary phase was also included since it is claimed to combine the excellent performance of silica-based columns and the pH stability of polymer columns. Hence the following columns were investigated: PLRP-S 5 μm 1000 Å, PLRP-S 3 μm 100 Å, XTerra RP C18, Gemini C18, Discovery, Luna, YMC-Pack Pro, Hypersil BDS, Astec C18 polymer, Zorbax SB, Supelcosil 5 μm and Supelcosil 3 μm. The different columns were investigated for their selectivity towards the neomycin components, using the chromatographic conditions mentioned above. PLRP-S 5 μm and PLRP-S 3 μm columns gave a selectivity similar to that of the PLRP-S 8 μm column previously used in the original method. The Astec C18 polymer column showed selectivity similar to that of the PLRP-S 8 μm column. The silica-based columns showed better selectivity overall. The selectivity obtained with the Discovery, Gemini, Luna, Hypersil BDS, Zorbax SB and Supelcosil 3 μm was superior to that of the other silica-based columns. A somewhat better separation, due to better peak shape, was obtained with the Discovery column and so this endcapped stationary phase was chosen for further investigation.

3.1.2. Further development using Discovery column

In further method development it was tried to reduce the amount of salt in the mobile phase since this can cause stability problems for silica-based stationary phases. Using a mobile phase containing only 35 g/l of sodium sulphate resulted in a considerable increase in the analysis time. Sodium sulphate was used in the original method to avoid the use of organic modifier since these are not compatible with PED [6]. The only one that was found suitable was THF and so this was incorporated in the mobile phase. To reduce the analysis time, 14 ml/l of THF was found to be a good compromise between the resolution of the different peaks and the analysis time.

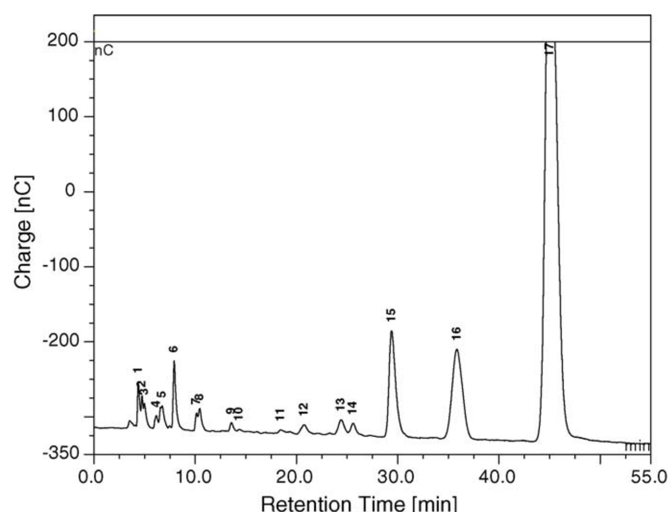


Fig. 2. A typical chromatogram of a commercial neomycin sample. 1: Unknown 1; 2: Unknown 2; 3: Unknown 3; 4: Unknown 4; 5: Paromamine; 6: LP-A; 7: Neamine; 8: Unknown 5; 9: Unknown 6; 10: Unknown 7; 11: Unknown 8; 12: Paromomycin II; 13: Unknown 9; 14: Paromomycin I; 15: LP-B; 16: Neomycin C; 17: Neomycin B. Column: Discovery C18, 5 μm, 250 mm × 4.6 mm I.D. Mobile phase: aqueous solution containing 35 g/l of sodium sulphate, 1 g/l of sodium 1-octanesulfonate, 14 ml/l THF and 50 ml/l of phosphate buffer pH 3. Column temperature: 35 °C.

Lower amounts of sodium 1-octanesulfonate were also investigated. 1 g/l was chosen as a compromise between analysis time and peak shape, since a lower amount showed peak shape distortion and higher amounts showed increased retention time. An acidic pH is required for better interaction between the analyte molecules and the ion-pairing reagent. However, the exact pH is less important since neomycin is always protonated at a pH lower than 5 [29] and this was also found in our previous experiments [6]. Therefore, the pH was kept constant at 3.0. The temperature was investigated between 30 and 40 °C. 35 °C was chosen as a compromise between good separation and column stability. A typical chromatogram obtained by analyzing a commercial neomycin sample using the chosen chromatographic conditions is shown in Fig. 2. It is observed that the main component neomycin B is well separated from its known impurities. Several impurities of unknown identity are also separated. Compared to the previously published LC method using a polymeric stationary phase [6], the method described here shows better selectivity and higher efficiency, but the analysis time is longer (50 min versus 30 min).

Further, the applicability of this method to other silica-based columns, reported in Section 2.2 was investigated. Good results, similar to those obtained with the Discovery column, were observed for the separation of the main components. For the unknown impurities, the separation pattern was variable.

Table 1
Chromatographic parameter settings applied in the central composite design, corresponding to low (−), central (0) and high (+) levels

Chromatographic parameter	Low value (−)	Central value (0)	High value (+)
Amount of sodium sulfate (g/l)	33	35	37
Amount of sodium octane sulfonate (g/l)	0.9	1	1.1
Amount of THF (ml/l)	13	14	15
pH of the buffer	2.5	3	3.5
Temperature (°C)	33	35	37

3.2. Robustness study

The robustness study was performed by means of an experimental design as mentioned under Section 2.4. The different chromatographic parameter settings of the design are given in Table 1. The individual, interaction and quadratic effects on the resolution for the pairs paromamine–LP-A (Rs_1), Unknown 9–paromomycin I (Rs_2), Neomycin C–Neomycin B (Rs_3) are summarized in Fig. 3. The plots consist of bars, which correspond to the regression coefficients and which are proportional to the magnitude of the variable effects. The 95% confidence interval limits are expressed by using error lines. A regression coefficient smaller than the error line interval shows that the variation of the response caused by the variable change is smaller than the experimental error. Therefore, in this case the effect of variable change would be considered insignificant when compared to the response. The magnitude of the effect is proportional to the regression coefficient (see Eq. (1)).

It is observed that sodium sulphate has the most important effect on Rs_1, Rs_2 and Rs_3. This effect is negative for the three separations, which means that an increase of the amount of sodium sulphate will decrease the resolution of the peak pairs studied. Sodium 1-octanesulfonate has a positive effect on the resolution of the three pairs, which means that an increase of the amount of ion-pairing agent improves resolution. However, this will also increase the retention times and so the total analysis time. THF has a negative effect on Rs_1 and Rs_3, but a positive effect on Rs_2. The pH and the temperature have no significant effect on the resolution of the different pairs. No important interactions were found. All the quadratic effects were not found to be significant. In order to better estimate the influence of the most important parameters on Rs_1, Rs_2 and Rs_3, response surface plots were constructed. Fig. 4 shows the variation of Rs_1, Rs_2 and Rs_3 as a function of sodium sulphate, sodium 1-octanesulfonate and THF, while the other parameters are kept constant. It is observed that in the ranges examined Rs_1 and Rs_3 are always well above 1.8. An increase of sodium sulphate in combination with a decrease of THF or sodium 1-octanesulfonate may result in a decrease of Rs_2 down to 0.8. This means, that these parameters should be monitored carefully to ensure sufficient separation of the pair Unknown 9–paromomycin I (Rs_2). Small changes of the chromatographic parameters do not have a detrimental effect on the separation of the other pairs of compounds.

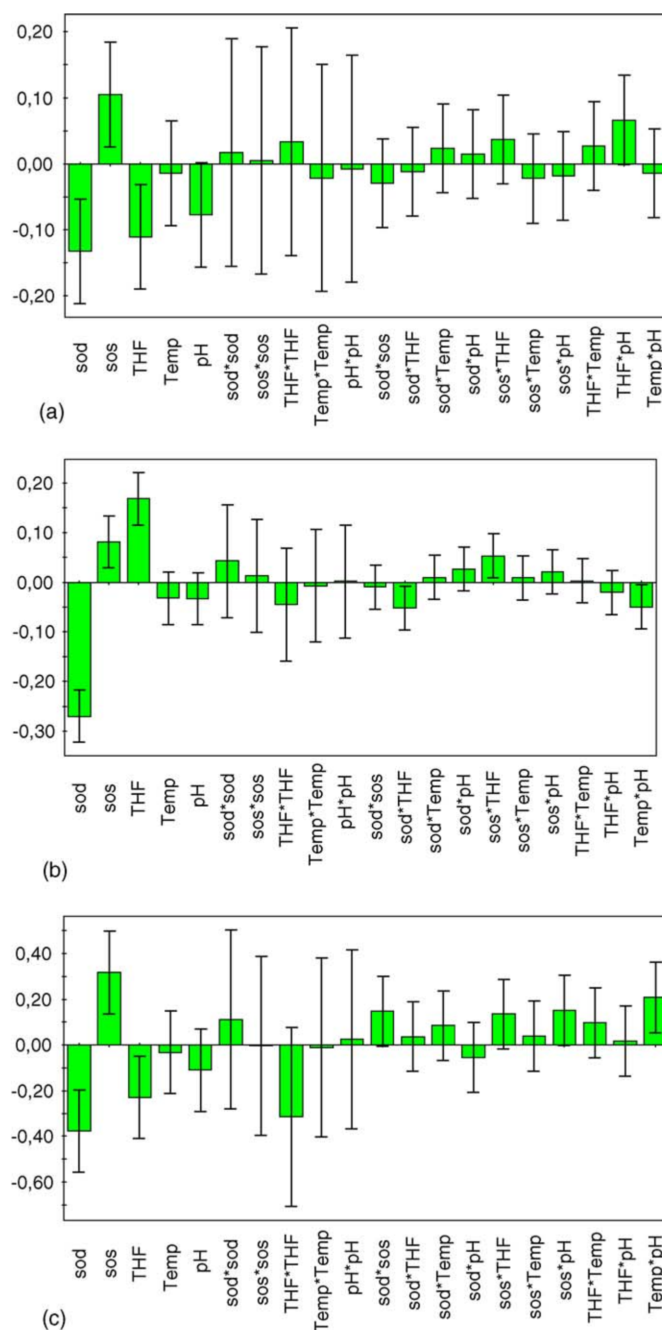


Fig. 3. Regression coefficient plots for the separation of the pairs (a) paromamine–LPA (Rs_1), (b) Unknown 9–paromomycin I (Rs_2) and (c) neomycin C–neomycin B (Rs_3). Sod = sodium sulphate, sos = sodium 1-octanesulfonate, Temp = temperature.

3.3. Quantitative aspects

The repeatability of the method was assessed by analyzing a 0.75 mg/ml solution of a commercial neomycin sample ($n = 6$). The levels of the different components studied and the relative standard deviation (R.S.D.) of the peak areas are given in Table 2. The results indicate good repeatability of the method.

The limit of quantitation (corresponding to a S/N ratio of 10) for the main component neomycin B was found to be 0.2% (R.S.D.: 4.3%, $n = 6$) and the limit of detection (LOD) 0.06%.

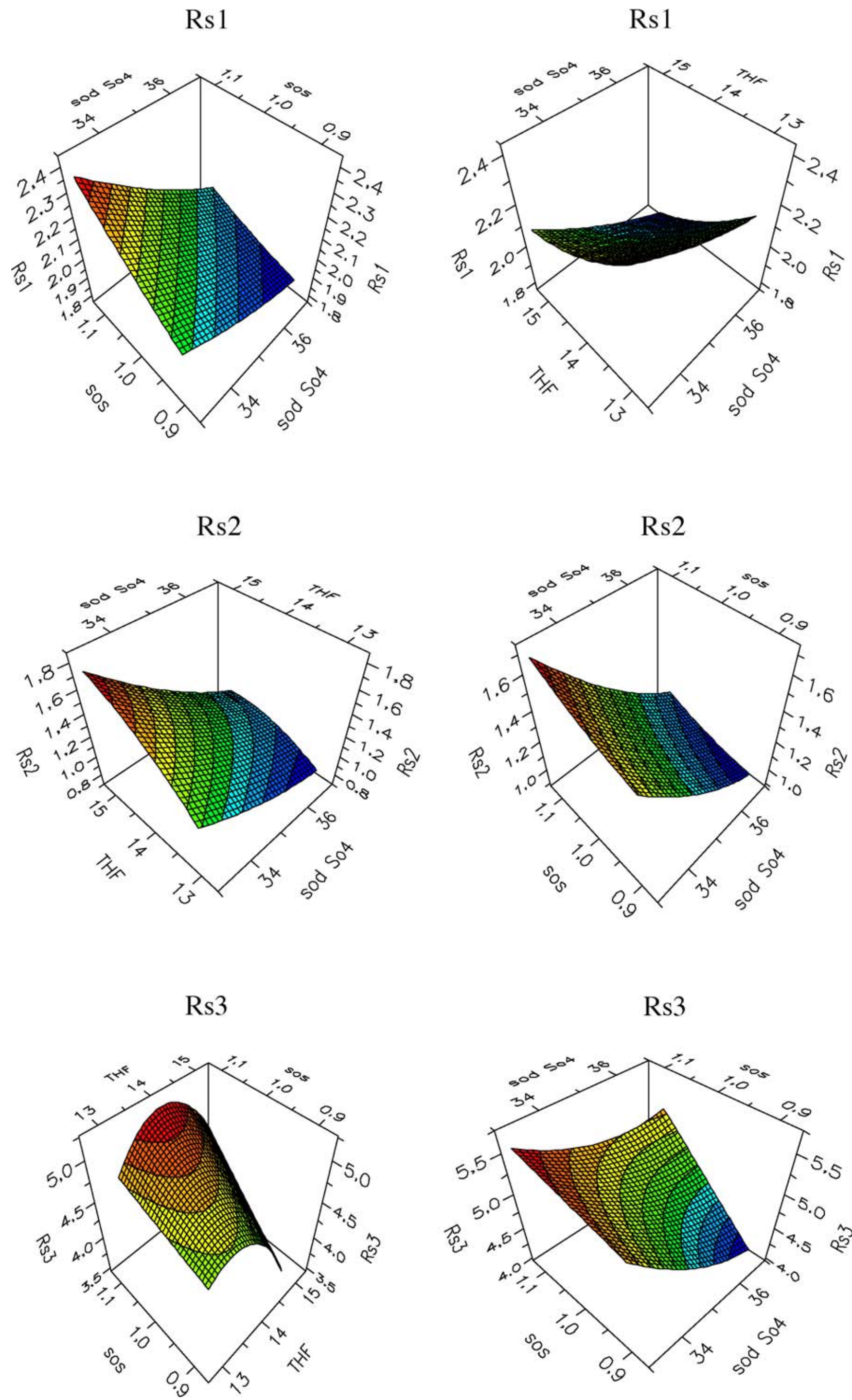


Fig. 4. Response surface plots for the separation of the pairs paromamine–LPA (Rs_1), Unknown 9–paromomycin I (Rs_2) and neomycin C–neomycin B (Rs_3) as a function of sodium sulphate (sod SO_4), sodium 1-octane sulfonate (sos) and tetrahydrofuran (THF).

Table 2
Repeatability data for neomycin impurities

	Paromamine	LP-A	Neamine	Paromomycin II	Paromomycin I	LP-B	Neomycin C
Component level (%)	0.8	1.7	0.5	0.6	0.7	6.5	8.3
R.S.D.	1.5	5.9	7.6	5.4	1.2	2.8	2.3

Table 3
Related substances commercial samples (%m/m)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Unknown 1	0.6 (5.2)	0.5 (15)	0.6 (6.2)	0.5 (4.6)	<LOQ	<LOQ	0.9 (1.3)
Unknown 2	0.4 (4.6)	0.4 (13)	0.4 (11)	1.0 (2.2)	<LOQ	<LOQ	0.5 (3)
Unknown 3	0.4 (10)	0.5 (16)	0.6 (9.8)	0.4 (6.5)	<LOQ	<LOQ	0.5 (1.6)
Unknown 4	0.5 (11)	0.5 (3.0)	0.4 (7.4)	0.5(5.4)	ND	ND	0.4 (5.1)
Paromamine	0.7 (5.3)	0.6 (6.1)	0.6 (5.6)	1.0 (1.0)	ND	ND	0.8 (1.5)
LP-A	0.4 (1.8)	0.3 (9.4)	0.3 (5.6)	2.0 (4.2)	0.5 (8.4)	0.2 (3.5)	1.7 (5.9)
Neamine	<LOQ	0.2 (11)	<LOQ	0.3 (2.4)	ND	ND	0.5 (7.6)
Unknown 5	0.4 (1.6)	0.4 (11)	0.5 (12)	0.5 (3.7)	0.2 (10)	0.2 (5.7)	0.6 (11)
Unknown 6	0.2 (5.8)	0.2 (1.4)	0.3 (13)	0.2 (6.5)	0.2 (14)	<LOQ	0.3 (4.3)
Unknown 7	0.2 (8.5)	<LOQ	<LOQ	0.2 (15)	ND	ND	0.2 (10)
Unknown 8	<LOQ	<LOQ	<LOQ	0.3 (7.2)	ND	ND	0.2 (6.2)
Paromomycin II	0.2 (3.5)	<LOQ	0.3 (6.0)	0.6 (3.9)	ND	ND	0.6 (5.4)
Unknown 9	0.2 (2.4)	0.2 (3)	0.2 (10)	0.6 (7.5)	ND	ND	0.9 (2.5)
Paromomycin I	0.9 (5.9)	0.4 (7)	0.9 (8.1)	0.6 (7.2)	ND	ND	0.7 (1.2)
LP-B	0.8 (2.7)	0.6 (1)	0.7 (2.6)	1.4 (7.9)	ND	ND	6.5 (2.8)
Neomycin C	3.9 (4.5)	5.4 (0.9)	5.0 (8.6)	6.2 (4.4)	2.0 (12.8)	1.0 (4.5)	8.3 (2.3)
Total of impurities	9.8	10.2	10.8	16.3	2.9	1.4	23.6

ND = not detected (<LOD). The R.S.D. values ($n = 3$) are given in parentheses.

The linearity was checked by analyzing neomycin B in the range starting from 0.2% (LOQ) to (100% corresponds to 15 μg injected). Five concentrations were prepared and each concentration was injected three times. The following results were obtained: $Y = 19.957X + 6.388$; $R^2 = 0.9968$; and $S_{y,x} = 8.14$, where Y = peak area; X = concentration, R^2 = coefficient of determination and $S_{y,x}$ = standard error of estimate. The results indicate that the method is linear in the range studied. On the other hand, the linearity data in a range that covers the 100% concentration (15 μg of neomycin B brought onto the column) were not satisfactory, probably due to electrode overload. It is therefore suggested to use a one-tenth dilution of the 100% concentration for assay. This dilution indeed falls in the linear range described above.

The proposed method was applied to the analysis of commercial samples. Data obtained are summarized in Table 3. Results were calculated in terms of neomycin B.

4. Conclusion

An improved LC method was developed for the analysis of neomycin. This method allows complete separation of neomycin from 16 components of which 7 are known neomycin related substances and 9 are unidentified peaks found in commercial samples.

Acknowledgment

E. Adams is a postdoctoral fellow of the Fund for Scientific Research-Flanders (Belgium).

References

[1] S.A. Waksman, H.A. Lechevalier, Science 109 (1949) 305.
[2] M. Barza, R.T. Scheife, Am. J. Hosp. Pharm. 34 (1977) 723.
[3] European Pharmacopoeia, fifth ed., Council of Europe, Strasbourg, France, 2005, monograph 197.
[4] E. Roets, E. Adams, I.G. Murithi, J. Hoogmartens, J. Chromatogr. A 696 (1995) 131.
[5] J. Krzek, M. Starek, A. Kwiecień, W. Rzeszutko, J. Pharm. Biomed. Anal. 24 (2001) 629.
[6] E. Adams, R. Schepers, E. Roets, J. Hoogmartens, J. Chromatogr. A 741 (1996) 233.
[7] E. Adams, R. Schepers, L.W. Gathu, R. Kibaya, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 15 (1997) 505.
[8] Y. Cai, Y. Cai, J. Cheng, S. Mou, L. Yiqiang, J. Chromatogr. A 1085 (2005) 124.
[9] N.C. Megoulas, M.A. Koupparis, J. Chromatogr. A 1057 (2004) 125.
[10] I. Clarot, A. Regazzeti, N. Auzeil, F. Laadani, M. Citton, P. Netter, A. Nicolas, J. Chromatogr. A 1087 (2005) 236.
[11] A. Posyniak, J. Zmudzki, J. Niedzielska, J. Chromatogr. A 941 (2001) 59.
[12] D.A. Stead, R.M.E. Richards, J. Chromatogr. B 693 (1997) 415.
[13] B.H. Kim, S.C. Lee, H.J. Lee, J.H. Ok, Biomed. Chromatogr. 17 (2003) 396.
[14] J.A. Apffel, J. Van Der Louw, K.R. Lammers, W.Th. Kok, U.A.Th. Brinkman, R.W. Frei, C. Burgess, J. Pharm. Biomed. Anal. 3 (1985) 259.
[15] B. Shaikh, J. Jackson, G. Guyer, W.R. Ravis, J. Chromatogr. B Biomed. Sci. Appl. 571 (1991) 189.
[16] R. Oertel, U. Renner, W. Kirch, J. Pharm. Biomed. Anal. 35 (2004) 633.
[17] W. Decoster, P. Claes, H. Vanderhaeghe, J. Chromatogr. A 211 (1981) 223.
[18] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. A 606 (1992) 228.
[19] E. Adams, J. Hoogmartens, Research trends, Curr. Top. Electrochem. 10 (2004) 63.
[20] E. Adams, J. Dalle, E. De Bie, I. De Smedt, E. Roets, J. Hoogmartens, J. Chromatogr. A 766 (1997) 133.
[21] E. Adams, G. Van Vaerenbergh, E. Roets, J. Hoogmartens, J. Chromatogr. A 819 (1998) 93.
[22] J. Szúnyog, E. Adams, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 23 (2000) 891.
[23] E. Adams, W. Roelants, R. De Paepe, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 18 (1998) 689.

[24] E. Adams, D. Puelings, M. Rafiee, E. Roets, J. Hoogmartens, J. Chromatogr. A 812 (1998) 151.
[25] P. Claes, F. Compernolle, H. Vanderhaeghe, J. Antibiot. 27 (1974) 931.
[26] Application Note 66R, Neomycin in Topical Lotions, Dionex, Sunnyvale, CA, 1991.
[27] P. Dehouck, E. Roets, J. Hoogmartens, Chromatographia 57 (2003) 671.
[28] D. Visky, Y. Vander Heyden, T. Ivanyi, P. Baten, J. De Beer, Z. Kovács, B. Noszál, P. Dehouck, E. Roets, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 1012 (2003) 11.
[29] N. Isoherranen, S. Soback, J. AOAC Int. 82 (1999) 1017.