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Comparative study on the analytical performance of different detectors for the liquid chromatographic analysis of tobramycin

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ABSTRACT

Pulsed electrochemical detection (PED) with a triple step potential–time waveform has been used successfully for the direct detection of several non-chromophoric aminoglycosides at alkaline pH following ion-pair liquid chromatography (LC). This direct detection method has helped to overcome the various flaws observed in the analysis of aminoglycosides using time consuming derivatization procedures. However, this technique also suffers from some disadvantages. Hence, improvements of PED as well as alternative detection techniques would be welcome for the detection of drug molecules lacking UV absorbing chromophores. In this study a comparison was made between the analytical performance of different brands of PED detectors and two different evaporative light scattering detectors (ELSD) from the same manufacturer. Different PED waveforms (triple, quadruple and six potential–time waveforms) were also examined for the detection of tobramycin, a representative example of the group of non-chromophoric aminoglycosides. As starting point, the LC–PED method of the European Pharmacopoeia (Ph. Eur.) was taken. As the Ph. Eur. method prescribes the use of a non-volatile mobile phase, an alternative one had to be used in combination with ELSD. The analytical performance was compared with regards to sensitivity, linearity and long-term stability.

1. Introduction

Tobramycin or factor 6 of the nebramycin complex is a broad spectrum water soluble aminoglycoside antibiotic produced by fermentation of the actinomycete *Streptomyces tenebrarius*. It is used for the treatment of infections of aerobic Gram positive and some aerobic Gram negative bacteria. It has a narrow therapeutic index because of its ototoxic and nephrotoxic effects. As it is produced by fermentation, beside the main component it also contains some related substances that result either from the incomplete purification or from degradation of the drug. Kanamycin B, nebramine and neamine (also known as neomycin A) are three known impurities of tobramycin reported in the Ph. Eur. [1].

The major obstacle in the analysis of tobramycin using LC has been its detection due to the absence of a significant UV-chromophore (Fig. 1). Methods involving pre-column and post-column derivatization of tobramycin with various derivatizing agents have been used to introduce a chromophore [2–6]. However, these methods can be tedious, time consuming and give problems with quantitation because of additional sample processing, variability of reaction completeness, possible instability of derivatized products and toxicity of some derivatisation agents. Direct detection of tobramycin was achieved by amperometry combined with LC as reported by Polta et al. and Statler and co-workers [7,8]. This provided a good alternative method for the analysis of tobramycin that did not involve the drawbacks encountered in derivatization procedures. Later, Szunyog et al. developed an ion-pair LC method using a poly(styrene-divinylbenzene) column as stationary phase combined with PED [9]. This method is currently prescribed in the Ph. Eur. for the analysis of tobramycin. Beside reversed phase chromatography, PED has also been used in combination with anion exchange chromatography for the detection of tobramycin and its impurities [10,11]. PED is considered superior to pre- or post-column derivatisation so that it is actually the method of choice for the detection of tobramycin and its impurities, but also for aminoglycosides in general. However, PED also suffers from some disadvantages. Experience is required to obtain repeatable quantitative results and after cleaning the electrodes of the electrochemical cell, often long equilibration times are noticed. Hence, improvements of PED or alternative detection techniques would be interesting for the direct detection of drug molecules that lack chromophores, like tobramycin.

Abbreviations: BHT, butylhydroxytoluene; DTT, drift tube temperature; ELSD, evaporative light scattering detector; Hy-REF, hydrogen reference electrode; MS, mass spectrometry; NGF, nebulizer gas flow; OPA, o-phthaldehyde; PED, pulsed electrochemical detection; Ph. Eur., European Pharmacopoeia; QPW, quadruple potential–time waveform; SPW, six potential–time waveform; SOS, Sodium 1-octanesulphonate; THF, tetrahydrofuran; TPW, triple potential–time waveform.

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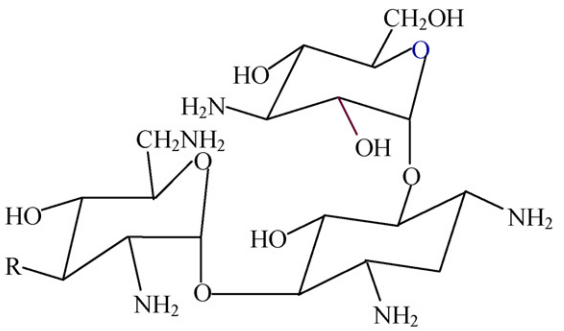
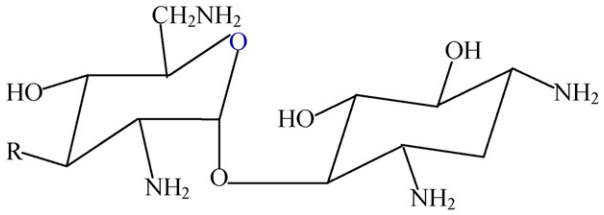


	R
Tobramycin	H
Kanamycin B	OH



	R
Nebramine	H
Neamine	OH

Fig. 1. Structure of tobramycin and some of its related substances.

Beside PED, various other direct detection techniques have been coupled with LC for the analysis of aminoglycosides. Indirect UV detection, conductivity and refractive index detection were not further considered here because of poor sensitivity and reproducibility. Several LC methods combined with mass spectrometry (MS) as detection technique have also been reported [12–15]. However, although LC–MS is highly sensitive and informative, it is a quite complicated and expensive technique to be used for routine analysis. Charged aerosol detection has also been described for analysis of aminoglycosides [16]. Another direct detection technique for non-chromophoric molecules is ELSD in which various steps take place: (1) the effluent from the chromatographic system is nebulized with nitrogen, (2) the droplets enter the drying or the heated drift tube where the solvent evaporates, (3) the dried particles then enter the detection chamber where a laser beam is focused onto the particles and the light scattered by the particles is measured [17]. This implies that mobile phase components have to be volatile. Determination of tobramycin in pharmaceuticals, urine and plasma using LC–ELSD has been reported by Megoulas and Koupparis. In their method, only the main peak tobramycin was studied, eluting within 4.5 min. So, this method would have to be adapted for the determination of impurities, resulting in a longer analysis time and consequently in broader peaks and reduced sensitivity [18]. Clarot et al. reported a LC–ELSD method for the simultaneous determination of tobramycin and colistin. However, they did not report any sensitivity data [19]. Although ELSD has been suggested as a suitable detector for tobramycin in both studies, no comparison with other techniques was made.

The methods using ELSD described in literature mainly focus on the determination of the main compound rather than on the impurities. Therefore, in this study, the impurities in tobramycin in combination with ELSD will be studied, using the volatile mobile phase of the LC–MS method of Li et al. [15]. This method uses a C18 reversed phase column and a mobile phase with heptafluorobutyric acid (HFBA) as volatile ion-pairing reagent. The use of ion-pairing reagents can cause signal suppression in MS leading to a reduced sensitivity [15,20]. It will be checked whether this is also the case with ELSD. Ion-pairing reagents are required to promote the retention of the tobramycin molecules by the stationary phase. Tobramycin is a hydrophilic and a basic molecule with pKa in the range of 7.0–8.8 [18]. As it is a polar molecule it is not retained by the non-polar reversed phase. The approach is to use an acidic mobile phase along with a negatively charged ion-pairing reagent. In the acidic medium the tobramycin molecules get protonated and form apolar ion-pairs that are retained by the reversed phase. This is the approach of most of the methods that have been reported for the analysis of tobramycin using reversed phase LC.

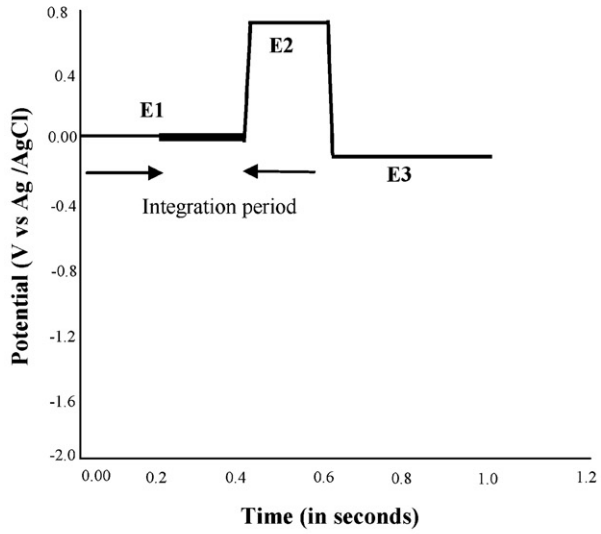
The suitability of PED as detection technique for the non-chromophoric aminoglycosides was demonstrated in the past. On the market, electrochemical detectors from different manufacturers are available. In this study two PED detectors (from different manufacturers) were compared in order to investigate if one performs better than the other under similar LC conditions. One of the two PED detectors could work only with the conventional triple potential–time waveform (TPW) whereas the other one could be used to apply different waveforms. This gave the opportunity to try other waveforms mentioned in the literature beside the TPW that is recommended by the Ph. Eur. for the analysis of tobramycin. Although PED is a good technique for the detection of molecules that lack a chromophore and can be oxidized or reduced, it is not so easy to work with.

ELSD has been suggested as one of the alternatives. However, it is known to be a nonlinear detector and the mobile phase has to be volatile. Recently introduced ELSDs are claimed to be better in sensitivity and linearity as compared to the older types. Hence, it was also decided to compare the PED detectors with an old and a new version of an ELSD detector from the same manufacturer in order to ascertain if it would provide an alternative to PED. Thus, the aim of this study was to compare the analytical performance of two different brands of PED detectors and two different ELSD detectors. To the best of our knowledge, this was not described before. In order to improve PED, different potential–time waveforms (see further) were applied and compared for the detection of tobramycin. For comparison of the PEDs and their waveforms, the LC method of the Ph. Eur. was taken. For ELSD the volatile mobile phase described by Li et al. was used [15]. One of the major problems is to find a suitable ion-pairing reagent. To avoid the use of ion-pairing reagents the performance of columns with extended polar selectivity was also examined. The analytical performance of the different approaches was compared by evaluating the sensitivity, linearity and long-term stability.

2. About the use of PED

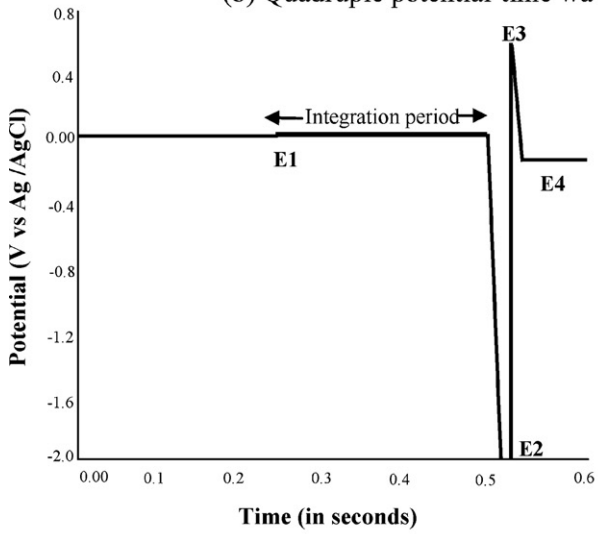
In PED, a three-electrode system is used consisting of a working electrode (usually gold), a reference electrode and an auxiliary electrode. Analyte molecules such as aminoglycosides are electro catalytically oxidized at the surface of the noble metal electrode at high alkaline pH by application of a distinctive potential. The current that is produced as a result of the analyte oxidation is proportional to the analyte concentration, thus allowing quantification of the analyte. To prevent signal loss due to fouling of the electrode, it is cleaned online by application of consecutive potentials. A series of potentials applied for defined time periods is referred to as waveform. The settings of this waveform are important to achieve good sensitivity and reproducibility as they have a considerable influence on the signal response, background noise and

(a) Triple potential-time waveform



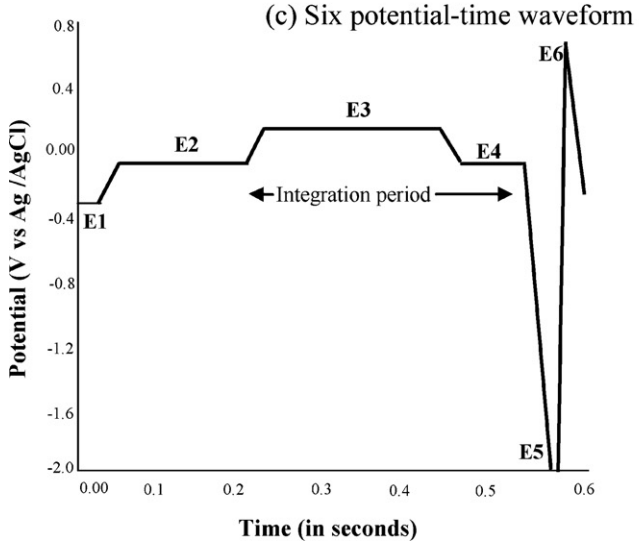
Time	Potential	Integration
0.00	+0.05	
0.20	+0.05	start
0.40	+0.05	end
0.41	+0.75	
0.60	+0.75	
0.61	-0.15	
1.00	-0.15	

(b) Quadruple potential-time waveform



Time	Potential	Integration
0.00	+0.05	
0.24	+0.05	start
0.50	+0.05	end
0.51	-2.00	
0.52	-2.00	
0.53	+0.60	
0.54	-0.15	
0.60	-0.15	

(c) Six potential-time waveform



Time	Potential	Integration
0.00	-0.25	
0.04	-0.25	
0.05	-0.05	
0.21	-0.05	start
0.22	+0.22	
0.46	+0.22	
0.47	-0.05	
0.56	-0.05	end
0.57	-2.00	
0.58	-2.00	
0.59	+0.55	
0.60	-0.25	

Fig. 2. The three waveforms used for the pulsed electrochemical detection of aminoglycosides (a) triple potential–time waveform; (b) quadruple potential–time waveform; (c) six potential–time waveform.

signal stability. Most of the research work done until now for aminoglycosides using PED involved the use of a TPW [21–23]. This is also recommended in the current Ph. Eur. method for the analysis of tobramycin [2]. It consists of a detection, cleaning and reactivation potential (Fig. 2(a)). At the cleaning potential, also inert gold oxide is formed which is reductively removed during the reactivation step. This leads to gradual loss of gold from the working electrode surface resulting in slow recession of the electrode surface. This leads to a slow decrease in detector response over a considerable period of time.

Ding et al. proposed a waveform that is known as quadruple potential–time waveform (QPW). In this waveform, a negative cleaning potential was used to remove the oxidized analytes and contaminants from the surface of the gold electrode (Fig. 2(b)). So, the loss of gold as gold oxide was reduced. This is supposed to help in improving the sensitivity and long-term stability [24]. However, it was observed that the analytical performance of QPW is almost the same as for TPW. So, the six potential–time waveform (SPW) introduced by Clarke et al. [25] for the detection of amino acids and amino sugars was further optimized by Cai et al. [26]. In SPW (Fig. 2(c)), the detection potential is not kept constant, but varies between a higher (E3) and lower value (E4). Simultaneous oxidation of the analytes and the gold takes place at the higher potential. This is followed by the application of a lower potential where the gold oxide formed previously is reduced. As a result, the net signal results only from the oxidation of the analyte. The SPW was reported to improve many analytical performances such as sensitivity [25].

3. Experimental

3.1. Reagents and samples

All reagents were of HPLC grade. Tetrahydrofuran (THF), stabilized with butyl hydroxytoluene (BHT), and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). Sodium 1-octanesulphonate (SOS), phosphoric acid (85% m/m), potassium dihydrogen phosphate and methanol were obtained from Acros (Geel, Belgium). Helium gas was purchased from Air Products (Brussels, Belgium) and nitrogen (nebulizer gas flow) was supplied by Air Liquide (Liège, Belgium). Sodium hydroxide solution 50% (m/m) was purchased from J.T. Baker (Deventer, The Netherlands); ammonium formate from Fluka AG (Buchs SG, Switzerland); formic acid from VEL/Merck Eurolab (Leuven, Belgium) and heptafluorobutyric acid (HFBA) from Interchim (Montluçon, France). Water was produced in-house using a Milli-Q Gradient water purification system (Millipore, Bedford, MA, USA). Tobramycin and its related substances kanamycin B sulphate, neamine and nebramine were available in the laboratory.

3.2. Liquid chromatographic instrumentation and conditions

3.2.1. LC PED instrumentation and chromatographic conditions

The Decade II (Antec, Leyden, The Netherlands) and the ED 50 (Dionex, Sunnyvale, USA) were integrated in a comparable LC set-up with elements from Merck-Hitachi (Darmstadt, Germany) and Thermo Fisher (Waltham, MA, USA). The electrochemical cells of both ED 50 and Decade II have a three-electrode configuration. The working electrode in both cells was made of gold. However, they differ in the counter and reference electrode. The ED 50 cell uses a silver–silver chloride reference electrode and a titanium counter electrode whereas the Decade II has a hydrogen reference electrode (Hy-REF) and carbon filled polytetrafluoroethylene as auxiliary electrode. The mobile phase was delivered at a flow rate of 1.0 ml/min. The samples were injected (20 μl) by an autosampler. As column, PLRP-S, 250 mm × 4.6 mm i.d., 100 nm, 8 μm was used. It was kept in a water bath heated at 55 °C by an immersion circulator (Julabo EC, Germany). Sodium hydroxide (0.5 M) was added post-column using a helium-pressurized reservoir to increase the pH of the column effluent above 12. The post-column solution was added at a flow rate of 0.3 ml/min. After mixing both solutions in a mixing coil (1.2 m, 500 μl) (Dionex) the resulting solution entered into the electrochemical cell. The latter was kept in a hot air oven at 35 °C. The Decade II detector has a built-in oven while the cell of the ED 50 detector was kept in a laboratory-made heating device. For the detector and waveform comparison, the TPW used was the one described by Szunyog et al. [9] and the SPW and QPW used were those described by Cai et al. [26]. Data acquisition software (Chromeleon version 6.8, Dionex) was used to record the signals.

The mobile phase used for the analysis of tobramycin using LC–PED was that prescribed in the Ph. Eur. It consisted of 52 g/l of sodium sulphate, 1.5 g/l of sodium octane sulphonate, 50 ml/l of phosphate buffer (pH 3) and 3 ml/l of THF.

3.2.2. ELSD instrumentation and chromatographic conditions

The LC instrument consisted of a L-6200 Intelligent pump (Merck-Hitachi), a Gilson 234 autosampler equipped with a 20 μl loop (Middleton, WI, USA), a narrow bore C18 column (YMC-Pack PRO C18, 250 mm × 2.1 mm i.d., S-5 μm, 120 Å) kept at 30 °C in a water bath with an immersion circulator (Julabo EC), an ELSD 2000 detector (Alltech Associates, IL, USA) and ChromPerfect software (Justice laboratory software, Fife, UK) for data acquisition. For detection, the drift tube temperature (DTT) of the detector was set to 95 °C and the nebulizer gas flow (NGF) to 2.6 l/min. The mobile phase consisted of an aqueous solution containing 1.2 ml/l of HFBA and 50 mM of ammonium formate, adjusted to pH 3.0 with formic acid before bringing to volume/methanol (68:32, v/v). The flow rate was 0.2 ml/min. For evaluation of the ELSD 3300 the same set-up as with the ELSD 2000 was used. As column with higher selectivity for polar compounds, a Platinum EPS C18 (Grace, Lokeren, Belgium) with extended polar selectivity, 250 mm × 4.6 mm i.d., 100 Å, 5 μm, kept at room temperature was used.

4. Results and discussion

4.1. Comparison of detectors

To evaluate the linearity for the response of the different detectors, a stock solution having a concentration of 1 mg/ml (which is 100% of the test concentration, as described in the method for tobramycin in the Ph. Eur.) was prepared in the respective mobile phase. Serial dilutions were made from this stock solution to find the concentration that gave the limit of quantitation (LOQ) at a signal-to-noise ratio (S/N) of 10. The linearity for the detectors was checked for 5 concentrations from their respective LOQ to 12.5% of the test concentration since a 10% (0.1 mg/ml) dilution is used for assay in the Ph. Eur. monograph. For waveform comparison, 10 concentrations were injected. Triplicate injections of each concentration in the linearity range were injected. The repeatability was checked for six injections at 10% (0.1 mg/ml) of the test concentration and calculating the relative standard deviation (RSD) on the peak areas. For the ELS detectors, sample solutions were prepared in water. The data obtained for the analytical performance of ELSD 2000, ELSD 3300, ED 50 and Decade II are shown in Table 1.

4.1.1. Sensitivity

The two PED detectors were observed to be equally sensitive as both had the same LOQ (Table 1). However, the ELS detectors were found to have higher LOQ values, indicating that they are less sensitive than the PED detectors.

Table 1
Comparison of the analytical performance of ED 50, Decade II, ELSD 2000 and ELSD 3300.

Detectors		ED 50 (TPW)	Decade II (TPW)	ELSD 2000	ELSD 3300
Sensitivity (LOQ)		0.002 mg/ml	0.002 mg/ml	0.015 mg/ml	0.3 mg/ml
Linearity	Range	25–125 μg/ml	25–125 μg/ml	25–125 μg/ml	25–125 μg/ml
	Y=	91.8x – 578	257.5x + 1235	1.535x + 2.5	1.639x + 3.7
	R^2	0.9996	0.9953	0.9996	0.9987
	S_{yx}	80.2	809.78	0.01	0.02
Repeatability	Conc	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml
	RSD	0.95%	0.22%	1.58%	2.25%

x: concentration of analyte in μg/ml for Decade II and ED 50 and log (concentration in μg/ml) for ELSD 2000 and 3300; Y: response obtained by injecting analyte for Decade II and ED 50 and log (response) for ELSD 2000 and 3300; S_{yx}: standard error of estimate; R^2: coefficient of determination, RSD: relative standard deviation.

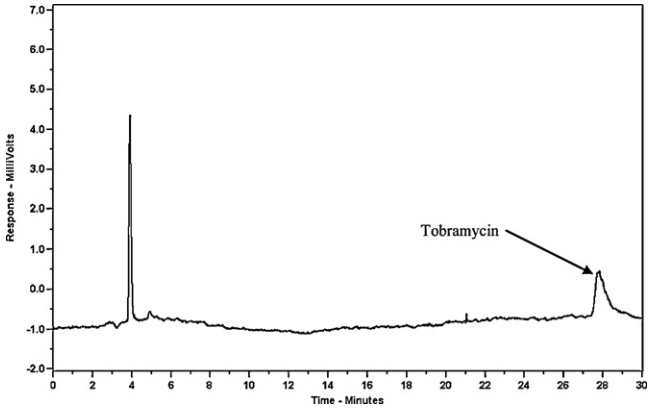


Fig. 3. Typical chromatogram obtained for tobramycin with ELSD 2000 using the chromatographic conditions described in Section 3.2.2.

Among the ELS detectors, the ELSD 3300 had much lower sensitivity as compared to the ELSD 2000. This was due to the fact that when the ELSD 3300 was used with the same LC and detection conditions as for the ELSD 2000, the chromatogram showed a lot of spikes and a high noise level. Figs. 3 and 4 show the respective chromatograms. Spikes can result from the incomplete nebulization of the mobile phase. Hence, it was decided to optimize the NGF and DTT. For this purpose, sample (25% of the test concentration) was injected with different combinations of DTT and NGF and the S/N was calculated. The original DTT of 95 °C and NGF of 2.6 l/min yielded a S/N of 5. The best S/N was obtained with a DTT of 100 °C and NGF of 1.5 l/min (S/N = 9). Even under these detection conditions, the spikes did not disappear completely.

Another reason of the spikes could be the quality or the high quantity of the ion-pairing reagent (HFBA) used in the mobile phase as it was observed that when a lower quantity of ion-pairing reagents was used, the spikes decreased. So, it would be better to exclude the use of ion-pairing reagents in order to increase the sensitivity. Hence, it was tried to develop a method for the analysis of tobramycin using a column with higher selectivity for polar compounds, combined with ELSD.

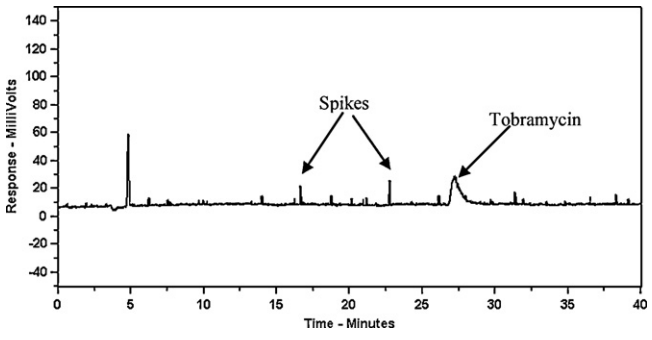


Fig. 4. Typical chromatogram for tobramycin using ELSD 3300 using the chromatographic conditions described in Section 3.2.2.

As starting point, a Platinum EPS stationary phase, which has extended polar selectivity, was used in combination with the ELSD 2000 for which the DTT and NGF were 100 °C and 2.6 l/min respectively. The mobile phase flow rate was 1 ml/min. The initial mobile phase consisted of 5% (v/v) of acetonitrile and 95% (v/v) of 250 mM ammonium acetate adjusted to pH 2 with formic acid. With this mobile phase, tobramycin and its impurities co-eluted within 5 min. Improvements by using different buffer pHs, by changing the amount of acetonitrile, by adapting the column temperature or by replacement of acetonitrile by methanol were not successful. When ion-pairing reagent was added to the aqueous part of the mobile phase, the separation got somewhat better, but it was still not sufficient to be used for determination of impurities. Next, the mobile phase of Li et al. [15] was tried with the Platinum EPS column. Some separation between tobramycin and its impurities was obtained, but decreasing the concentration of HFBA to 700 μl/l led to poorly separated or co-eluted peaks. Thus, it was concluded that it is difficult to separate tobramycin from its impurities without using ion-pairing reagents.

The selectivity obtained with the two detectors, ELSD and PED, using two different methods, volatile and non-volatile, could not be compared as the ELSD was observed to be not very sensitive in comparison to PED. As a result of the poor sensitivity of the ELSD, the smaller impurity peaks were not detected, making it difficult to compare the selectivity.

4.1.2. Linearity

The linearity data for the detectors showed that the two PED detectors exhibit an acceptable linear response in the range examined as they had correlation coefficients close to one and small y-intercepts (Table 1). The ED 50 performed somewhat better than the Decade II. As expected, for ELSD a nonlinear response was observed. This is because the relationship between the analyte mass and area response for ELSD can be described by

$$A = aM^b$$

where A is the area response of the detector, M is the mass of the analyte injected, a and b are numerical coefficients which depend on the analyte and chromatographic conditions.

However, the log–log plot of the peak area response versus analyte quantity is linear for ELSD [27]. It can be described by the equation:

$$\log A = \log a + b \log M$$

The results which are shown in Table 1 for the two ELS detectors were obtained after applying the log–log equation shown above.

The upper limit of the range examined (125 μg/ml) was found to be the maximum concentration for both the PED and ELSD detectors to be within the linear range.

Table 2
Comparison of the analytical performance of the three waveforms.

Waveforms		TPW	QPW	SPW
Sensitivity (LOQ)		0.002 mg/ml	0.001 mg/ml	0.0005 mg/ml
Linearity	Range	0.002–0.125 mg/ml	0.001–0.125 mg/ml	0.0005–0.125 mg/ml
	Y=	$95.48x - 0.24$	$345.7x + 0.04$	$1459x + 1.0$
	R^2	0.9990	0.9994	0.9983
	S_{yx}	0.14	0.40	2.94
Repeatability	Conc	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml
	RSD	0.95%	0.44%	0.94%
Peak area (0.1 mg/ml)		23.23	34.44	151.26
Peak height (0.1 mg/ml)		18.35	27.44	114.94

x: concentration of analyte in μg/ml; Y: response obtained by injecting analyte; S_{yx}: standard error of estimate; R^2: coefficient of determination, RSD: relative standard deviation.

4.1.3. Repeatability

It was observed (Table 1) that the two PED detectors showed RSD values less than 1%, with the lowest RSD for the Decade II. The ELSD 2000 showed a RSD greater than 1% whereas the ELSD 3300 showed a RSD greater than 2%. Thus, it can be concluded that PED detectors are more repeatable than the ELSD detectors.

4.2. Comparison of the waveforms

This comparison was done using the ED 50 detector as with the Decade II it is not possible to apply QPW or SPW. The analytical performance of the three waveforms was compared with regard to sensitivity, repeatability, linearity, peak area and peak height. Sample solutions were prepared in the same manner as described in Section 4.1 for the PED detectors. Data obtained are shown in Table 2.

The sensitivity for the different waveforms was compared by evaluating the LOQ values. It was observed that the LOQ obtained for QPW was two times lower than that for TPW. However, the LOQ for SPW was four times lower than TPW, indicating that it is the most sensitive waveform.

For the linearity, it was observed that all the three waveforms were linear in the range examined. The repeatability for the three waveforms was found to be good as all had RSD values less than 1%.

Another comparison that was performed for the three waveforms was the peak area and peak height for a particular concentration (10% (0.1 mg/ml) of the test concentration). It was observed that the peak areas and the peak heights were a little bit higher for QPW than for TPW, but it was considerably higher for SPW. An overlay of the chromatograms obtained with the three waveforms is shown in Fig. 5. Hence it was concluded that the analytical performance of the SPW is better as compared to the QPW and TPW.

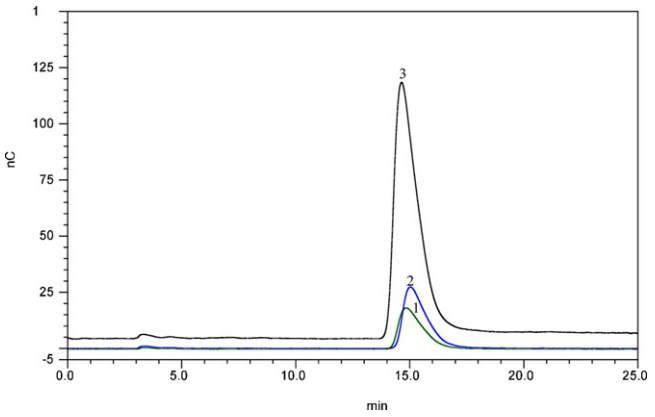


Fig. 5. Overlay of the chromatograms obtained for different waveforms (1) TPW, (2) QPW and (3) SPW after injecting a 10% dilution (0.1 mg/ml) of the test concentration. Tobramycin.

5. Conclusion

Among the detection techniques, PED with SPW was found to show the best analytical performance. Another advantage of PED is that it shows a linear relationship between the quantity of analyte injected and the response obtained whereas for ELSD, a log-log transformation of the data is necessary to obtain a linear relationship. The ELSD 2000 was found to have a better sensitivity than the ELSD 3300, nevertheless ELSD can only be used as an alternative to PED for assay purpose. When quantification of related substances is required, PED should be preferred.

Ion-pairing reagents play a crucial role in the separation of tobramycin from its impurities and it was not possible to develop a method for purity control without using ion-pairing reagents.

For the different waveforms examined, it can be concluded that the SPW was better as LOQ obtained for it was lower compared to the TPW and QPW. In this respect, the ED 50 was found to be the more versatile detector as compared to Decade II since besides TPW also QPW and SPW can be applied.

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