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Short Communication

Quantitation of gentamicin sulfate in injectable solutions by capillary electrophoresis☆

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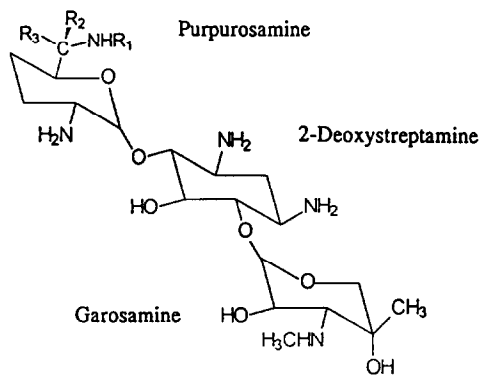
Abstract

The results presented in this communication establish the use of capillary electrophoresis as a tool in the analysis of injectable solutions containing the antibiotic complex gentamicin sulfate. The utilization of a borate buffer leads to the separation of the individual components and their visualization by direct UV detection. This rapid and straightforward procedure yields qualitative and quantitative information simultaneously, and could be utilized as an alternative to the multiple assays required by current US Pharmacopeia protocols.

1. Introduction

Gentamicin is an aminoglycoside antibiotic complex produced by *Micromonospora purpurea*, *M. echinospora*, or variants thereof [1] and is used as an antibacterial agent in humans and animals. Each of the three major components, C_1, C_{1a} and C_2, is comprised of the aminocyclitol 2-deoxystreptamine and two additional amino sugars, as shown in Fig. 1. Other minor components such as gentamicins C_{2a} and C_{2b} may be produced as well [2].

Current US Pharmacopeia (USP) methods for gentamicin sulfate require separate tests for identification and quantitation [3]. The composition of the bulk drug is determined by its derivatization with *o*-phthalaldehyde and the separation of the three major components and



Gentamicin	R_1	R_2	R_3	MW
C_1	CH_3	H	CH_3	477
C_{1a}	H	H	H	449
C_2	H	CH_3	H	463
C_{2a}	H	H	CH_3	463
C_{2b}	CH_3	H	H	463

Fig. 1. Structures of components of gentamicin sulfate.

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C_{2a} by high-performance liquid chromatography (HPLC). Gentamicin contained in creams, ointments and injectables is identified by the appearance of three bands using thin-layer chromatography (TLC). Quantitation in all matrices is accomplished by performing a microbial assay, which requires either 2–4 h (turbidimetric method) or 16–18 h (cylinder-plate method) for analysis, in addition to the 24-h incubation period for the preparation of the inoculum.

Suspect injectable solutions were received at the National Forensic Chemistry Center (NFCC) and required the identification of gentamicin sulfate and its subsequent quantitation. Microbial assays cannot be performed at the NFCC, so an alternative analytical procedure was desired. In addition to the USP HPLC method, on-line HPLC–thermospray mass spectrometry [4] has been utilized to resolve, identify and quantitate the components. Gentamicin sulfate has been studied by capillary electrophoresis (CE) using an indirect detection method at low pH, but without the resolution of the individual components [5]. Previous work concerning carbohydrate complexation of underivatized mono- and oligosaccharides with borate buffers [6,7] suggested the use of direct UV detection for the analysis of gentamicin sulfate in a straightforward and rapid manner. The CE method presented in this communication does not require sample derivatization, yields qualitative and quantitative information simultaneously, and needs only 10 min per analysis.

2. Experimental

2.1. Reagents

Sodium tetraborate decahydrate (borate) was purchased from Aldrich (Milwaukee, WI, USA), and the reference standard gentamicin sulfate was obtained from US Pharmacopeial Convention (Rockville, MD, USA). Distilled deionized water (DDW) was produced in the laboratory using a Milli-Q water purification system (Millipore, Milford, MA, USA). Buffer solutions were filtered through 0.2-μm nylon 66 filters (Alltech, Deerfield, IL, USA).

2.2. Methods

Sample preparation

Approximately 20 mg total mass of USP reference standard gentamicin sulfate were placed in a vial and dried for at least 3 h at 110°C as directed [3]. This standard was dissolved in 2.00 ml DDW to prepare a gentamicin sulfate stock solution, and was diluted as required. Based on the current USP lot (I-1) in which 1 mg standard is equivalent to 0.682 mg gentamicin base, a 2.00 mg/ml gentamicin sulfate working solution contains 1.36 mg gentamicin base/ml. Aliquots of the injectable solutions were diluted with DDW so that the amount of gentamicin base contained in the final working solution was approximately 2.0 mg base/ml. All solutions were stored at 4°C.

Capillary electrophoresis

An ISCO Model 3140 capillary electropherograph (ISCO, Lincoln, NE, USA) was used for all electrophoretic separations. An uncoated, 70 cm length (45 cm to detector) × 50 μm I.D. fused-silica capillary (ISCO) was installed.

Separations occurred in a 0.150 *M* borate buffer, pH 9.4 at +15 kV and 34°C. Direct detection was accomplished at 195 nm. The capillary was rinsed with borate buffer for 180 s at the start of every analysis. Samples were introduced into the capillary by vacuum injection at 25.0 kPa · s. Each analysis required 600 s, and data were collected at a rate of 10 points/s. Peak areas were normalized with respect to their migration times [8].

Gentamicin identification was accomplished by comparing the retention times of the peaks seen in the sample to those of the three peaks visualized with the USP Standard. Sample quantitation utilized total peak area as a single point comparison to the total peak area generated by the USP Standard.

3. Results and discussion

Fig. 2 is a typical electropherogram obtained from the USP Standard working solution. As a confirmation of the presence of gentamicin base,

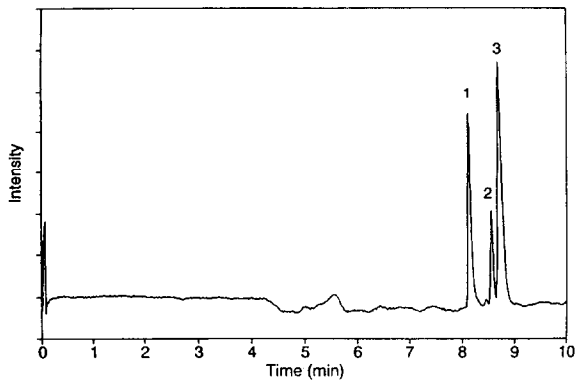

Fig. 2. CE analysis of 1.51 mg gentamicin base/ml from USP reference standard gentamicin sulfate. Separation conditions are given in the Experimental section. Peaks 1, 2 and 3 are discussed in Table 1, and are identified as components C_1, C_{1a}, and $C_2 + C_{2a}$, respectively.

the migration times of the components in the unknown samples are compared to those in the USP Standard. The presumed component migration order is C_1 (peak 1), C_{1a} (peak 2), and C_2 and C_{2a} co-migrating (peak 3). Under the present buffer conditions, it is unlikely that these latter two components would be resolved (see Fig. 1), so co-migration is expected. The unknown solutions exhibit similar electrophoretic behavior, which is verified by the average values for migration times obtained for each peak, found in Table 1. The "control" sample indicated in Table 1 is a manufacturer's declared 100 mg gentamicin base/ml veterinary solution. The three "unknown" samples are injectable solutions that originated with the same source; "unknown 1" and "unknown 2" were collected on the same day, and "unknown 3" was collected three days later. The relative standard deviations (R.S.D.s) of migration times seen with all solutions are less than 0.5%, and demonstrate reproducibility that is comparable to that obtained with HPLC. The migration data in Table 1 indicate that gentamicin is present in all three unknown injectables.

Table 1
Migration data for gentamicin sulfate

	Migration time (min)[a]		
	Peak 1	Peak 2	Peak 3
USP Standard[b]	8.07 ± 0.03 (0.37%)	8.50 ± 0.03 (0.35%)	8.63 ± 0.03 (0.35%)
Control	8.08	8.51	8.64
Unknown 1	8.04	8.46	8.60
Unknown 2	8.06	8.48	8.62
Unknown 3	8.12	8.54	8.68

[a] $n = 4$ unless otherwise noted.
[b] $n = 8$; value in parentheses is relative standard deviation.

Total peak area responses were used for quantitation of gentamicin base in the control and unknown samples by a simple ratio:

$$\frac{\text{mg base/ml USP Standard}}{\text{total peak area USP Standard}} = \frac{\text{mg base/ml sample}}{\text{total peak area sample}}$$

The resulting calculated concentrations for the control and the three unknowns are given in Table 2. For example, when the dilution factor is taken into account, the quantity of gentamicin base in the control injectable solution is 98.8 mg/ml, and is within USP guidelines of 90–125% for acceptable concentration ranges [3]. The values obtained for the three unknown samples using the CE method are in excellent agreement with the results of the microbial assay, with peak area R.S.D.s between 2.1 and 3.6%. The concentration ranges possible, based on standard deviations of the USP Standard and sample solutions, encompass the values obtained with the microbial assay. Based on the amount of gentamicin base present (Table 2), the data indicate that unknowns 1 and 2 were prepared in a similar manner, and unknown 3 was prepared separately.

The data in Table 3 indicate the reproducibility of the method when the buffer and/or capillary are changed. The decrease in the concentration of the control in system 2 is probably due to the age of the USP Standard solution. The same standard was used in systems 1 and 2; a fresh standard solution was prepared and utilized in systems 3 and 4. It is also unclear whether the USP reference standard gentamicin sulfate must be dried beforehand as directed [3]; the USP Standard used in systems 1 and 2 was *not* dried, as opposed to the USP Standard in systems 3 and 4.

Table 2
Determination of gentamicin base in injectable solutions

	Dilution	Gentamicin base concentration (mg/ml)		
		CE[a]	Range[b]	Microbial assay (mg/ml)[c]
USP Standard[d]	–	1.51 (2.56%)	–	–
Control[e]	1:50	98.8 (3.09%)	93.4–104	–
Unknown 1	1:100	114 (2.12%)	108–119	116
Unknown 2	1:100	108 (2.69%)	102–114	111
Unknown 3	1:100	93.6 (3.58%)	88.0–99.5	90.2

[a] $n = 4$ unless otherwise noted; values in parentheses are relative standard deviations.
[b] Values represent concentration range based on standard deviations of USP Standard and sample.
[c] Assays performed by Denver District Office.
[d] $n = 8$; (2.22 mg USP Standard/ml)(0.682 mg gentamicin base/mg USP Standard) = 1.51 mg gentamicin base/ml.
[e] Label declaration 100 mg gentamicin base/ml veterinary solution.

4. Conclusions

The method described in this communication establishes the use of CE in the analysis of gentamicin sulfate in injectable solutions. This method is much simpler and more rapid than the TLC procedure for identification and the microbial assay for quantitation. Because it yields

Table 3
Reproducibility of method

	Date of preparation	Gentamicin base concentration in control (mg/ml)
System 1[a]		98.8
Capillary	October 27th, 1992	
Borate buffer	November 2nd, 1992	
System 2[b]		92.0
Capillary	October 27th, 1992	
Borate buffer	December 15th, 1992	
System 3[c]		99.4
Capillary	December 21st, 1992	
Borate buffer	December 15th, 1992	
System 4[c]		104 (day 1)
Capillary	December 21st, 1992	102 (day 2)
Borate buffer	December 23rd, 1992	103 (day 6)

[a] $n = 8$ for USP Standard; $n = 4$ for control.
[b] $n = 10$ for both solutions.
[c] $n = 6$ for both solutions.

qualitative and quantitative information in one experiment, this CE procedure greatly reduces the amount of time required for routine analyses. The utilization of total peak area for quantitation of gentamicin base in injectable solutions leads to values that are in excellent agreement with those obtained via the accepted USP microbial assay, and demonstrates reproducibility over different capillary/buffer combinations. At this time, means of verifying the presumed migration order are being explored. Preliminary results obtained with bulk gentamicin sulfate samples [9] indicate that component percentage composition can be used for same-source verification. Because pre-column derivatization is not necessary, this method does not discriminate among components based on the presence or absence of primary amine groups, unlike the USP HPLC procedure [10], and therefore is more representative of the true composition of the sample.

5. Acknowledgement

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6. References

[1] S. Budavari (Editor), *The Merck Index*, Merck & Co., Rahway, NJ, 11th ed., 1989, pp. 686–687.
[2] B.E. Rosenkrantz, J.R. Greco, J.G. Hoogerheide and E.M. Oden, in K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 9, Academic Press, New York, 1980, pp. 295–340.
[3] *The United States Pharmacopeia*, United States Pharmacopeial Convention, Rockville, MD, 22nd revision, 1989, pp. 603, 604, 1488–1493; *Supplement 5*, pp. 2620–2621.
[4] T.A. Getek, M.L. Vestal and T.G. Alexander, *J. Chromatogr.*, 554 (1991) 191.
[5] M.T. Ackermans, F.M. Everaerts and J.L. Beckers, *J. Chromatogr.*, 606 (1992) 229.
[6] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, *Anal. Chem.*, 63 (1991) 1541.
[7] A.M. Arentoft, S. Michaelsen and H. Sørensen, *J. Chromatogr. A*, 652 (1993) 517.
[8] K.D. Altria, *J. Chromatogr.*, 634 (1993) 323.
[9] C.L. Flurer, presented at the *5th International Symposium on High Performance Capillary Electrophoresis, Orlando, FL, January 25–28, 1993*, poster.
[10] P.J. Claes, R. Busson and H. Vanderhaeghe, *J. Chromatogr.*, 298 (1984) 445.