Journal of Chromatography, 603 (1992) 298–301
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 227

Short Communication

Quantitation of *l*-epinephrine and determination of the *d*-/*l*-epinephrine enantiomer ratio in a pharmaceutical formulation by capillary electrophoresis

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(First received January 17th, 1992; revised manuscript received April 1st, 1992)

ABSTRACT

A procedure for the quantitation of *l*-epinephrine and the determination of the *d*-/*l*-epinephrine ratio in a pharmaceutical formulation containing *l*-epinephrine is described. The optical isomers of epinephrine were resolved by capillary electrophoresis with a buffer containing heptakis-(2,6-di-*o*-methyl)-*β*-cyclodextrin. Quantitation was achieved with the use of an internal standard, l-pseudoephedrine. Results with and without internal standard correction illustrate the improved reproducibility possible with an internal standard.

INTRODUCTION

There are very few reports in the literature which show that capillary electrophoresis can be used for quantitation in a formulated product [1–3]. This report describes the quantitation of *l*-epinephrine and the determination of the *d*-/*l*-epinephrine ratio in a pharmaceutical formulation. This formulation contains *l*-epinephrine and is a sterile opthalmic solution for the control of simple glaucoma. An analytical method which can distinguish between *d*- and *l*-epinephrine is necessary to insure that minimal racemization occurs over time. Although there are a number of high-performance liquid chromatography procedures in the literature which can distinguish between both isomers of epinephrine, they can involve a time-consuming derivatization step [4–6].

Cycodextrins have been used in capillary isotachophoresis to resolve ephedrine alkaloid enantiomers [7]. Recently, Fanali [8] reported the optical isomer resolution of epinephrine and related compounds by capillary electrophoresis with a 10 m*M* Tris–H_3PO_4/18 m*M* heptakis-(2,6-di-*o*-methyl)-*β*-cyclodextrin (Me-*β*-CD) pH 2.4 buffer. These separations were performed with a coated capillary. In the procedure described here an uncoated fused-silica capillary was used to perform the separation and an internal standard was added to correct for the imprecise injection system of this instrument.

EXPERIMENTAL

Chemicals

Orthophosphoric acid, hydrochloric acid and sodium hydroxide were obtained from J.T. Baker

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0021-9673/92/$05.00

(Phillipsburg, NJ, USA). Tris(hydroxymethyl)amino methane (Tris), *d*-/*l*-epinephrine, *l*-epinephrine, *l*-pseudoephedrine and Me-β-CD were obtained from Sigma (St. Louis, MO, USA).

Apparatus

The experiments were performed with a Dionex CES system (Sunnyvale, CA, USA) equipped with a UV detector set at 206 nm (0.1 a.u.f.s.). Separations were performed in an unmodified fused-silica capillary (50 cm × 0.075 mm I.D., 45 cm to detector) Polymicro Technolgies, Phoenix, AZ, USA). Samples were gravity injected for 10 s at 50 mm. The injection end was the anode (+). The applied voltage was 15 kV and the observed current was 30–40 μA. Separations were achieved with a 10 m*M* Tris/18 m*M* Me-β-CD buffer adjusted to pH 2.4 with concentrated H_3PO_4. Electropherograms were recorded with a Spectra-Physics Chrom-Jet integrator (San Jose, CA, USA).

Procedure

At the beginning of each day, and whenever the buffer solution was changed, the capillary was pressure rinsed two times for 180 s with 0.1 *M* H_3PO_4 and two times with 0.5 *M* NaOH. Then the entire system (capillary, source and destination vials) was rinsed four times with purified water and five times with the buffer solution. These rinse cycles were performed automatically by the instrument. Once this procedure was complete, it was only necessary to rinse the entire system with buffer one time after each injection.

Solutions

All solutions were diluted to volume with 0.01 *M* HCl to prevent epinephrine from oxidizing. Standards were stable for up to one week. The *l*-epinephrine standard solutions contained 25 ppm *l*-epinephrine and 100 ppm *l*-pseudoephedrine as the internal standard. Standard curve solutions contained approximately 12.5, 25 and 37.5 ppm *l*-epinephrine with 100 ppm *l*-pseudoephedrine added as the internal standard. The vehicle standard solutions were prepared from a vehicle solution containing everything in the formulation except *l*-epinephrine. The vehicle standard solutions contained 0.0025% *l*-epinephrine, boric acid, ascorbic acid, acetylcysteine, 0.00005% benzalkonium chloride and sodium carbonate (monohydrate) to adjust pH.

A *d*-epinephrine standard curve at 2, 5, 8 and 10% of the *l*-epinephrine assay concentration was prepared in duplicate by spiking an *l*-epinephrine standard with a *d*-/*l*-epinephrine standard. The solution had to be prepared this way because there was no *d*-epinephrine standard available. The *l*-epinephrine standard used to prepare this curve was contaminated with about 2% (area%) *d*-epinephrine so 2% was the lowest point on the curve.

The formulations containing *l*-epinephrine were prepared so that they contained 100 ppm *l*-pseudoephedrine and 25 ppm, *l*-epinephrine after dilution with 0.01 *M* HCl.

Calculations

The percentage of *l*-epinephrine in the sample solutions can be calculated by

$$l\text{-epinephrine (\%)} = 100\,[(A_f/A_p)/(A_s/A_p)]C_s/C_f$$

where A_f, A_s and A_p are the peak areas for *l*-epinephrine in the formulation, *l*-epinephrine in the standard and *l*-pseudoephedrine, respectively. C_s and C_f are the concentrations of *l*-epinephrine in the standard and formulation solutions, respectively.

The ratio percentage of *d*-epinephrine in the pharmaceutical formulation can be calculated by

$$d\text{-epinephrine (\%)} = \frac{0.945\,A_d}{0.945\,A_d + A_l} \cdot 100$$

where A_d and A_l are the peak areas for *d*- and *l*-epinephrine, respectively, and 0.945 is the peak area ratio (area% *l*/area% *d*) for a racemic mixture of *d*- and *l*-epinephrine. Fanali and Boček [1] showed that a correction factor must be introduced into the equation used to calculate the percentage *d*-epinephrine because the UV absorbance coefficients of *d*- and *l*-epinephrine may not be the same when cyclodextrin is present. The presence of cyclodextrin may shift the absorbance spectra of the complexes.

RESULTS AND DISCUSSION

Fig. 1 shows the electropherogram of a *d*-/*l*-epinephrine standard and a formulation solution containing the internal standard *l*-pseudoephedrine. The elution order of *d*- and *l*-epinephrine in Fig. 1 agrees with Fig. 2 of Fanali's paper [8].

This method was found to be reproducible and precise. Ten separate preparations of standard were prepared on two days. The standard solutions gave relative standard deviations (R.S.D.) of 1.4 and 1.8% with internal standard correction and 3.0 and 3.4% without internal standard correction. Ten separate preparations of vehicle standard gave a mean recovery of 99% with an R.S.D. of 2.6% with internal standard corerction and 101% with an R.S.D. of 6.7% without internal standard correction.

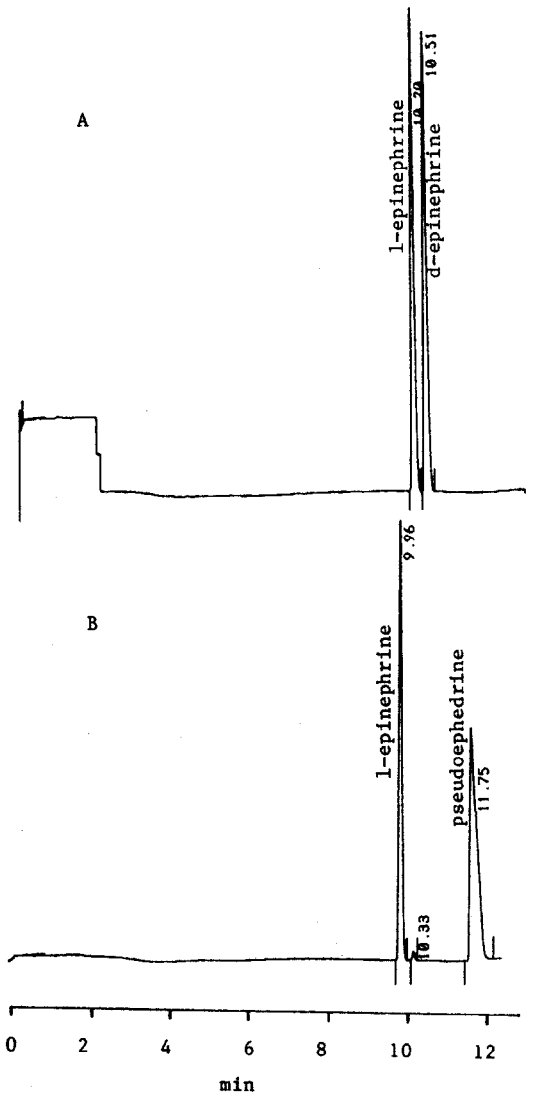

Fig. 1. Electropherograms of (A) 50 ppm *d*-/*l*-epinephrine standard and (B) pharmaceutical formulation diluted to 25 ppm *l*-epinephrine with pseudoephedrine added as an internal standard. See Table I for conditions.

To show that the *l*-epinephrine response is linear in this concentration range, two standard curves and two vehicle standard curves ranging from 12.5 to 37.5 ppm *l*-epinephrine were prepared in duplicate. The results with and without internal standard correction are illustrated in Table I. The two standard and vehicle standard curves for *l*-epinephrine were linear and passed near the origin. The curves had better R.S.D. values with internal standard correction than without. The R.S.D. values ranged from 0.9 to 2.5% with internal standard correction and 1.8 to 8.1% without internal standard correction. The recovery of *l*-epinephrine from vehicle standard solutions, with internal standard correction, was 99 and 101% with R.S.D. values of 1.2 and 2.0%, respectively. Without internal standard correction, the recovery was 86 and 97% with R.S.D. values of 7.9 and 2.2%, respectively.

A *d*-epinephrine standard curve was prepared at 2, 5, 8 and 10% of the *l*-epinephrine assay concen-

TABLE I

LEAST SQUARES REGRESSION ANALYSIS FOR PLOTS OF RELATIVE PEAK AREA *VS.* CONCENTRATION FOR *l*-EPINEPHRINE

Conditons: buffer, 10 m*M* Tris–H_3PO_4/18 m*M* Me-β-CD pH 2.4; fused-silica capillary 50 cm × 0.075 mm (45 cm to detector); injection (at anode) by gravity 50 mm for 10 s; applied voltage, 15 kV; detection wavelength, 206 nm (0.1 a.u.f.s.).

l-Epinephrine	With internal standard		Without internal standard	
Standard curve	1	2	1	2
Correlation coefficient	0.9998	0.9998	0.9988	0.9985
R.S.D. (%)	2.5	0.9	2.7	2.7
Intercept (%)[a]	3.0	1.2	0.2	4.5
Vehicle standard curve	1	2	1	2
Correlation coefficient	0.9988	0.9985	0.9474	0.9972
R.S.D. (%)	1.3	1.9	8.1	1.8
Intercept (%)[a]	0.2	4.5	9.4	2.2
Recovery (%), (R.S.D., %)	99,(1.2)	101,(2.0)	86,(7.9)	97,(2.2)

[a] The intercept calculation is based on the response of a 25 pppm *l*-epinephrine standard.

TABLE II

ANALYSIS OF THE PERCENTAGE OF *l*-EPINEPHRINE AND RATIO PERCENTAGE *d*-EPINEPHRINE IN A PHARMACEUTICAL FORMULATION

Conditions as in Table I. The specification for the percentage of *l*-epinephrine in this formulation is 90–115%. The formulation expires after 12 months.

Lot	Age of sample (months)	n^a	*l*-epinephrine (%)		Ratio percentage *d*-Epinephrine
			Mean value	R.S.D. (%)	
A	5	3	110	1.4	1.4, 1.2, 1.4
B	10	3	102	1.1	1.3, 1.4, 1.4
C	29[b]	3	84	2.5	2.3, 2.2, 2.2

[a] Number of replicate analyses.
[b] This sample past expiration of 12 months.

tration to show that small amounts of *d*-epinephrine could be determined in the presence of 25 ppm *l*-epinephrine and that the *d*-epinephrine response is linear in this region. The curve passed through the origin and had a correlation coefficient of 0.9998 with an R.S.D. of 1.3% after internal standard correction. Without internal standard correction the correlation coefficient was 0.9992 with an R.S.D. of 3.3%.

In all cases, internal standard correction improved the reproducibility of the method. The imprecise injection system of most commercial instruments warrants the use of an internal standard for quantitation. This method was used to assay three samples from three lots of the pharmaceutical formulation containing *l*-epinephrine. The results in Table II illustrate the reproducibility of the method. Lot C, which is 29 months old and 17 months past the expiration date, still shows very little *d*-epinephrine (2.3% or less) indicating very little racemization occurred in this formulation.

ACKNOWLEDGEMENTS

We thank Dr. Karen B. Sentell at the University of Vermont for her many helpful comments on this manuscript. We also thank Alcon for providing us the time and facilities to complete this work.

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