Journal of Chromatography, 319 (1985) 419–426
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

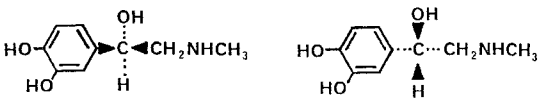
CHROM. 17,443

Note

High-performance liquid chromatographic analysis of epinephrine enantiomers using a UV detector in series with an optical activity detector*

BRITT S. SCOTT and DANNY L. DUNN*
Alcon Laboratories, Inc., Analytical Chemistry, 6201 S. Freeway, Fort Worth, TX 76134 (U.S.A.)
(Received November 27th, 1984)

Aqueous ophthalmic solutions of *l*-epinephrine are administered topically to treat glaucoma. The *d*-isomer of epinephrine is pharmacologically inactive (see Fig. 1). The current United States Pharmacopeia (U.S.P.) assay for epinephrine in epinephrine ophthalmic solution[1] determines only the total epinephrine present and is not specific for *l*-epinephrine. The U.S.P. assay for epinephrine in epinephrine bitartrate ophthalmic solution and in epinephrine borate ophthalmic solution is specific for *l*-epinephrine, but requires a very tedious and time consuming procedure involving acetylation, extraction, gravimetric determination of the residue and finally an optical rotation (OR) measurement. A high-performance liquid chromatographic (HPLC) method suitable for routine use which specifically determines *l*-epinephrine in ophthalmic formulations is desirable to assure the inactive *d*-isomer is not present due to racemization or contamination.



l-EPINEPHRINE d-EPINEPHRINE

Fig. 1. The chemical structures of *d*- and *l*-epinephrine.

Besides its ophthalmic applications, *l*-epinephrine is also used as a cardiac stimulant, a vasoconstrictor and a bronchodilator. Its analysis by HPLC has been reported in a wide variety of pharmaceutical preparations, biological fluids and mammalian organs. Several different detection methods have been used which include electrochemical detection[2–10], fluorometric detection[11] and UV detection[12]. Nimura *et al.*[13] have reported the HPLC resolution of *d*- and *l*-epinephrine enantiomers on

* This work was presented in part at the *Eighth International Symposium on Column Liquid Chromatography, New York, NY, May 20–25, 1984*. The proceedings of this symposium have been published in *J. Chromatogr.*, Vols. 316 and 317 (1984).

0021-9673/85/$03.30

reversed-phase columns using pre-column chiral derivatization with acetylglycosyl isothiocyanates. No separation of *d*- and *l*-epinephrine has been reported using chiral columns, probably due to difficulties in effectively masking the catechol functionality[14].

As an alternative to pre-column derivatization or chiral columns, enantiomer analysis can also be accomplished using optical activity detectors. Yeung and Kuo and co-workers have used a laser-based optical activity detector to detect sugars[15–17], free and esterified cholesterol[15,18] and to characterize shale oil[15,19]. It has recently been suggested that such a detector used with optically active mobile phases could serve as a sensitive universal HPLC detector[20]. A circular dichroism (CD) spectrophotometer equipped with an HPLC flow cell was used to detect pyrethrins[21] and a conventional polarimeter equipped with a flow cell was used to detect sugars[22,23].

Boehme *et al.*[24] have reported an elegant analysis for the enantiomers of permethrinic acid esters using a UV detector in series with a polarimeter detector. The UV detector response quantitated the total amount of enantiomer and the polarimeter response determined the ratio of the enantiomers. We report here a similar HPLC analysis for *l*-epinephrine in an ophthalmic formulation using a UV detector in series before a polarimeter detector. Calculations are developed using the OR/UV detector response ratio of an *l*-epinephrine standard to determine the percent of each enantiomer in unknown mixtures. The method requires minimal sample preparation and is readily automated. Twelve to fifteen samples can be analyzed per hour.

EXPERIMENTAL

Reagents

d,l-Epinephrine hydrochloride was purchased from Pfaltz and Bauer (Stamford, CT, U.S.A.) and *l*-epinephrine free base was obtained from C. H. Boehringer (Ingelheim, F.R.G.). 1-Heptanesulfonic acid sodium salt was purchased from Kodak (Rochester, NY, U.S.A.). The acetonitrile was HPLC grade from Baker (Phillipsburg, NJ, U.S.A.). All other chemicals were reagent grade.

HPLC equipment

Analyses were performed on a 15 × 0.46 cm 5-μm Sprint column containing Spherisorb ODS from Analytical Sciences (Santa Clara, CA, U.S.A.). The HPLC system consisted of a Waters Assoc. (Milford, MA, U.S.A.). M-6000A pump, a Waters 481 variable-wavelength UV detector, a Waters 710B WISP auto-injector and a Spectra-Physics (Houston, TX, U.S.A.) 4200 data system. A Perkin-Elmer (Norwalk, CT, U.S.A.) 241 MC polarimeter was modified using an upgrade kit, No. 141-0041, from the manufacturer to serve as an HPLC detector. The flow cell was 1 dm in length with a volume of 40 μl. The polarimeter signal was passed through a Spectrum (Newark, DE, U.S.A.) 1021A electronic filter and amplifier before being sent to the SP 4200 data system. The polarimeter flow cell was placed in series after the UV detector.

Procedure

The aqueous portion of the mobile phase was 0.11 *M* sodium dihydrogen phosphate, 0.14 m*M* sodium heptanesulfonate and 0.44 m*M* Na_2EDTA. The pH was adjusted to 4 with phosphoric acid, if necessary. The mobile phase was acetonitrile–

water (3:97). The flow-rate was 2.0 ml/min. UV detection was at 250 nm and OR detection was at 303 nm.

In general, 50 μl of a 10 mg/ml epinephrine solution were injected. Commercial 1% epinephrine ophthalmic solutions were injected neat, but 2% epinephrine ophthalmic solutions were diluted 1:2 with mobile phase before injection. The 10 mg/ml *l*-epinephrine standard had a few drops of 20% hydrochloric acid added before final dilution with mobile phase.

Calculations

The calculations to determine % label *l*-epinephrine were derived from Fig. 2. As indicated by Boehme *et al.*[24], the ratio of OR response to UV response (R_{std}) for the pure enantiomers is a constant value and differs only in sign. The value of R_{std} can be found experimentally by injecting a pure *l*-epinephrine standard. The slope for the theoretical line shown in Fig. 2 is:

$$M = \frac{-2R_{std}}{100} \tag{1}$$

Since the *y*-intercept is equal to R_{std}, the percentage *l*-epinephrine (%*l*) in an unknown sample can be found from the experimentally determined sample OR/UV ratio (R_s) by the following:

$$y = Mx + b \tag{2}$$

$$-R_s = \left(\frac{-2R_{std}}{100}\right)(\%l) + R_{std} \tag{3}$$

Solving for %*l*:

$$\%l = \left(\frac{R_s + R_{std}}{R_{std}}\right) 50 \tag{4}$$

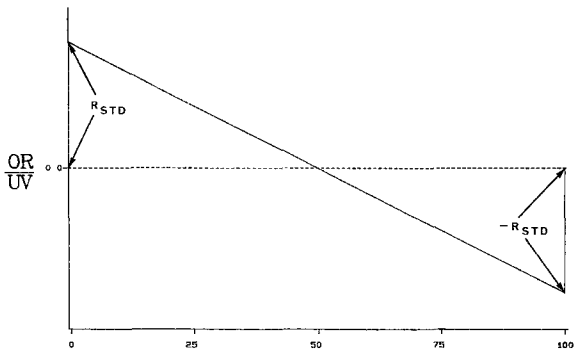


Fig. 2. A theoretical plot of OR/UV response ratios *versus* the percentage *l*-epinephrine in a *d,l*-mixture. R_{std} is the OR/UV response ratio for both the pure *d*-and the pure *l*-isomer.

The total epinephrine concentration (C_s) can then be determined from the UV response:

$$C_s = (C_{std}) \frac{A_s}{A_{std}} \tag{5}$$

where C_{std} is the concentration of the pure *l*-epinephrine standard, A_s is the UV response for the sample and A_{std} is the UV response for the pure *l*-epinephrine standard. The percent label *l*-epinephrine is:

$$\% \text{ label } l\text{-epinephrine} = \frac{C_s}{C_T} (\%l) \tag{6}$$

where C_T = the theoretical labeled concentration of the sample.

RESULTS AND DISCUSSION

A typical chromatogram for an *l*-epinephrine standard solution showing both the UV and polarimeter detector responses is shown in Fig. 3. The epinephrine elutes

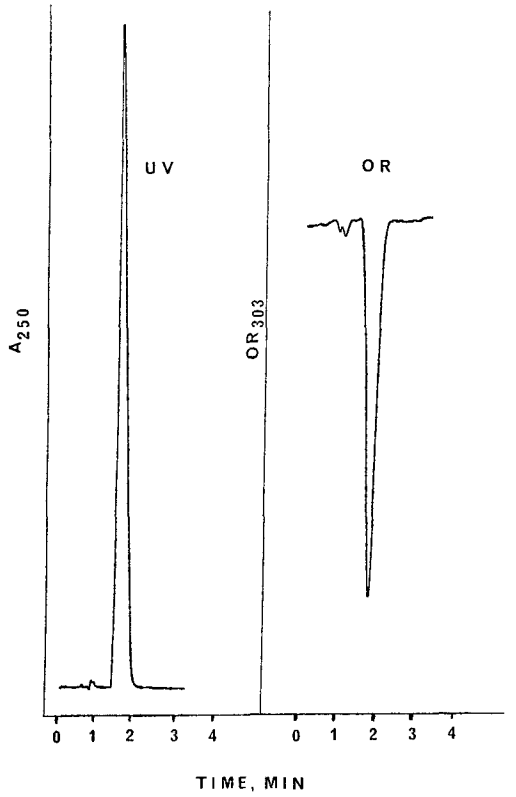


Fig. 3. Chromatograms for an *l*-epinephrine standard injection.

at about 1.5 min on the UV detector and at about 1.8 min on the OR detector.

Pure *l*-epinephrine solutions prepared over the concentration range 6.0–14.0 mg/ml (60–140% label) gave linear response curves which intercepted zero for both the UV and OR detectors (Fig. 4). The correlation coefficient for the UV detector was 0.9985 and for the OR detector it was 0.9999. This shows that the OR/UV ratios for pure *l*-epinephrine will be a constant for all concentrations and that a single point *l*-epinephrine standard will be sufficient to determine the value of this constant (R_{std}).

An epinephrine ophthalmic vehicle (Glaucon®, Alcon Labs., Fort Worth, TX, U.S.A.) containing benzalkonium chloride, sodium metabisulfite, disodium edetate (Na_2EDTA) and sodium chloride was prepared. Mixtures of *d*- and *l*-epinephrine were diluted with vehicle and analyzed. The concentrations ranged from 60% *l*-epinephrine–40% *d*-epinephrine to 100% *l*-epinephrine. In all cases the total epinephrine concentration was held constant at 10 mg/ml. A linear curve was obtained when the OR/UV response ratios (R_s) were plotted versus percent *l*-epinephrine (%*l*) in the mixtures (Fig. 5). The correlation coefficient was 0.9983 and the curve had a y-intercept of zero at a ratio of approximately 50:50 for *d*-epinephrine: *l*-epinephrine. The average recovery of *l*-epinephrine for all points determined *versus* a single point *l*-epinephrine standard was 99.2%. Since the curve presented in Fig. 5 is similar to the theoretical curve presented in Fig. 2, the percentage *l*-epinephrine may be determined by comparing the OR/UV response ratios (R_s) to a standard *l*-epinephrine OR/UV response ratio (R_{std}).

We are currently using a modification of the U.S.P. analytical method to analyze for percent label *l*-epinephrine[25]. The samples are first placed on an acid-washed Celite column. The epinephrine binds to the Celite and some of the excipients are washed from the column with diethyl ether. The epinephrine is then eluted with diethyl ether containing bis(2-ethylhexyl)phosphoric acid. It is then extracted from the diethyl ether eluent with a fixed volume of dilute hydrochloric acid. The concentration of total epinephrine is determined by comparing the UV absorbance at 280 nm to a standard. The apparent *l*-epinephrine concentration is determined by measuring the optical rotation at 405 nm. The actual percent label concentration of *l*-epinephrine may then be calculated. The Celite column method specifically analyzes

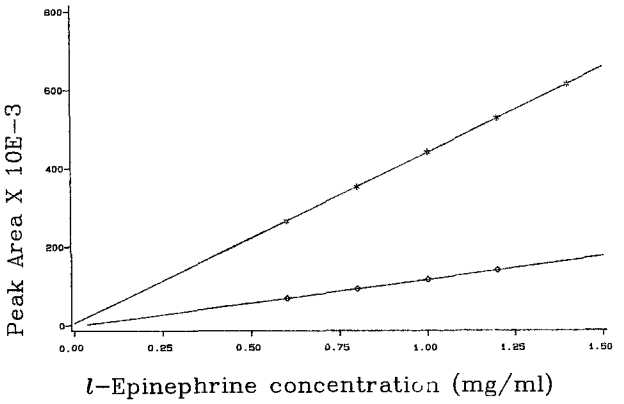


Fig. 4. A plot of peak area *versus* *l*-epinephrine concentration (mg/ml) showing both UV response (▲) and OR response (◇).

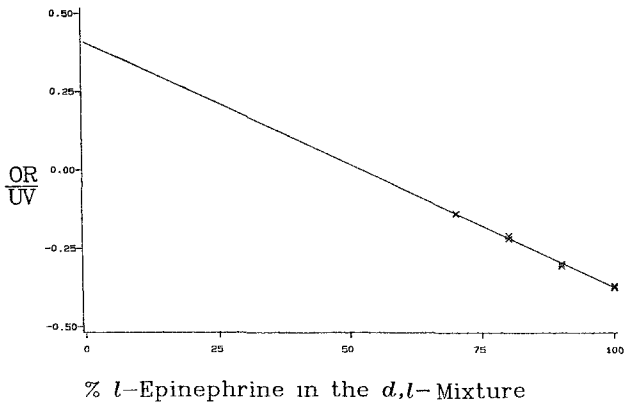


Fig. 5. A plot of OR/UV response ratios *versus* the percentage *l*-epinephrine in *d,l*-vehicle standard mixtures.

for *l*-epinephrine, but it is tedious and very time consuming. Typically 5–6 h are required to analyze 6–8 samples.

The analyses of four lots of *l*-epinephrine ophthalmic solution commercially available for the treatment of glaucoma (Glaucon 1% and 2%) by both the Celite column method and the HPLC method are compared in Table I. Both methods gave similar results.

TABLE I

ANALYSIS OF *l*-EPINEPHRINE OPHTHALMIC SOLUTIONS

Sample	*Lot number*	*Celite column method (% label)*	*HPLC method (% label)*
Glaucon 1%	HBAP	106	107
Glaucon 1%	FKW	110	109
Glaucon 1%	DHAM	100	101
Glaucon 2%	HBAR	110	111

CONCLUSIONS

It has been demonstrated that *l*-epinephrine can be specifically analyzed in ophthalmic formulations by HPLC using a UV detector in series with an optical activity detector. A plot of OR/UV detector response *versus* the percent *l*-epinephrine in a *d,l*-mixture was found to be linear. Calculations were developed which use the OR/UV detector response of a single-point *l*-epinephrine standard to determine the percentage *l*-epinephrine present in an unknown sample. Four lots of commercially available epinephrine ophthalmic solution were analyzed by the HPLC method and gave comparable results when analyzed by the Celite column method. The method is readily automated using an auto-injector and 12–15 samples can be analyzed per

hour. HPLC analysis is very fast compared to the Celite column method which requires virtually a whole working day to analyze half a dozen samples.

One drawback of the method is sensitivity. For pharmaceutical formulations containing 1% epinephrine, the polarimeter signal can be amplified and filtered so that maximum optical rotations of approximately 0.125° can be reproducibly observed. This does not approach the sensitivity needed for the analysis of epinephrine in biological samples. However, Yeung and Kuo and co-workers[15–20] have demonstrated the feasibility of using a sensitive laser based optical activity detector. Optical rotations of $1 \cdot 10^{-5}$ degree and detection of biological compounds at the 100-ng level have been repeatedly reported.

Another experimental optical activity detector worthy of note was described by Drake *et al.*[26]. The detector was originally designed for column liquid chromatography and not HPLC. It was capable of simultaneously monitoring absorbance (A) and circular dichroism (differential absorbance for left- and right-circularly polarized light, $\Delta A = A_L - A_R$). ΔA/A ratios (analogous to our OR/UV ratios) could be determined with a single detector eliminating the additional band broadening and expense of two HPLC detectors in series.

The analysis of biological samples by HPLC using UV and optical activity detectors in series awaits the commercial development of these experimential detectors.

ACKNOWLEDGEMENTS

We thank Robert King and Ken Potter, Alcon Laboratories, for their help in programming the SP 4200 data system. Thanks are also due Alan Marks of the Perkin-Elmer Corporation for helpful advice and assistance.

One of us (B.S.S.) received the 1982 Analyst of the Year Award from the Dallas Society of Analytical Chemists (Dallas, TX, U.S.A.) for participation in this project.

REFERENCES

1 *The United States Pharmacopeia*, United States Pharmacopeial Convention, Rockville, MD, 21st Rev., 1980, p. 377.
2 V. Hammond and D. Johnston, *Clin. Chim. Acta*, 137 (1984) 87.
3 E. Caliguri and I. Mefford, *Brain Res.*, 296 (1984) 156.
4 P. Lin, M. Bulawa, P. Wong, L. Lin, J. Scott and C. Blank, *J. Liquid Chromatogr.*, 7 (1984) 509.
5 M. Patthy and R. Gyenge, *J. Chromatogr.*, 286 (1984) 217.
6 R. J. Martin, B. A. Bailey and R. G. H. Downer, *J. Chromatogr.*, 278 (1983) 265.
7 W. Hunt and T. Dalton, *Anal. Biochem.*, 135 (1983) 269.
8 C. Kim, C. Campanelli and J. M. Khanna, *J. Chromatogr.*, 282 (1983) 151.
9 M. A. Elchisak and J. H. Carlson, *J. Chromatogr.*, 233 (1982) 79.
10 G. Lachatre, G. Nicot, C. Magnet, J. Rocca, L. Merle and J. Valette, *J. Liquid Chromatogr.*, 5 (1982) 1947.
11 N. Abeling, A. Van Gennip, H. Overmars and P. Voute, *Clin. Chim. Acta*, 137 (1984) 211.
12 J. Subert and K. Slais, *Cesk. Farm.*, 32 (1983) 343; *C.A.*, 100 (1984) 109185y.
13 N. Nimura, Y. Kasahara and T. Kinoshita, *J. Chromatogr.*, 213 (1981) 327.
14 T. Doyle, Food and Drug Administration (Washington, DC, U.S.A.), personal communication.
15 J. C. Kuo, *Ph.D. Dissertation*, Iowa State University, Ames, IA, 1982.
16 E. S. Yeung, L. Steenhoek, S. Woodruff and J. C. Kuo, *Anal. Chem.*, 52 (1980) 1399.

17 J. C. Kuo and E. S. Yeung, *J. Chromatogr.*, 223 (1981) 321.
18 J. C. Kuo and E. S. Yeung, *J. Chromatogr.*, 229 (1982) 293.
19 J. C. Kuo and E. S. Yeung, *J. Chromatogr.*, 253 (1982) 199.
20 E. S. Yeung, *Eighth International Symposium on Column Liquid Chromatography, New York, NY, May 20–25, 1984*, Paper 1a-B1; D. R. Bobbitt and E. S. Yeung, *Anal. Chem.*, 56 (1984) 1577.
21 S. A. Westwood, D. E. Games and L. Sheen, *J. Chromatogr.*, 204 (1981) 103.
22 W. Boehme, *Chromatogr. Newslett.*, 8 (1980) 38.
23 J. L. DiCesare and L. S. Ettre, *J. Chromatogr.*, 251 (1982) 1.
24 W. Boehme, G. Wagner and U. Oehme, *Anal. Chem.*, 54 (1982) 709.
25 *Test Procedure 90.1820.2ASP.1261.R01, Epineprhine Assay by Column Ion Pair Extraction*, Alcon Labs., Fort Worth, TX, unpublished.
26 A. F. Drake, J. M. Gould and S. F. Mason, *J. Chromatogr.*, 202 (1980) 239.