Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 4, pp. 357–364, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Determination of enantiomeric purity of ephedrine and pseudoephedrine by high-performance liquid chromatography with dual optical rotation/UV absorbance detection

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Abstract: A reversed-phase high-performance liquid chromatography (HPLC) method with dual optical rotation/UV absorbance detection has been developed for the determination of enantiomeric purity of ephedrine hydrochloride and pseudoephedrine hydrochloride using an achiral column. The method gave a correlation coefficient of 0.9997 for the plot of log(optical rotation response) versus log (concentration) over the range of 0.06–10 mg ml^{-1} of (+)-ephedrine hydrochloride (20 μl injection). The limit of detection was 1.0 μg. Enantiomeric purity is shown to be most readily determined by measuring optical rotation, α, and absorbance, A, responses for standard and unknown samples, and using the equation

$$(\alpha/A)_u/(\alpha/A)_s = (2x_u - 1)/(2x_s - 1),$$

where x is the mole fraction of one of the enantiomers and subscripts s and u refer to standard and unknown, respectively. In blind trials using unknown mixtures of (+)- and (±)-ephedrine hydrochloride and a (+)-ephedrine hydrochloride standard, enantiomeric purities were determined to ±0.4% (95% confidence level) with five or six replicate 50 μg injections. The method has also been applied to the determination of the enantiomer mole fraction of (+)-pseudoephedrine hydrochloride in a cough linctus, giving $x_u = 0.99 \pm 0.01$ with seven replicate injections of 20-fold diluted linctus samples containing 7.5 μg of the chiral compound being assayed. Unlike conventional polarimetry, the method does not require chemically-pure samples and can be orders of magnitude more economical in material.

Keywords: *Ephedrine; pseudoephedrine; reversed-phase high-performance liquid chromatography; enantiomeric purity; optical rotation detection; polarimetry; chiral purity; optical activity.*

Introduction

Ephedrine [1-phenyl-2-(methylamino)-1-propanol], a main alkaloid of ephedra which has been used for thousands of years in Chinese traditional medicine under the name Ma-Huang [1], is widely used as an analeptic and antiasthmatic. Because of the therapeutic [2], forensic and toxicological importance [3], its analysis has received considerable attention. The enantiomers of both ephedrine and its diastereoisomer pseudoephedrine possess different pharmacological activities with (+)-pseudoephedrine having about seven times more vasopressor activity than (−)-pseudoephedrine [4]. The enantiomers also show different rates of clearance from the body [3]. Analytical methods to determine enantiomeric purity are therefore of importance, and may also provide information concerning the origin or route of synthesis [3] of the materials in drug samples.

Several high-performance liquid chromatography (HPLC) methods for chemical and enantiomeric purity determination of ephedrine and pseudoephedrine have been developed. In these methods, chiral resolution has been performed using a chiral stationary phase [5], a chiral complexing agent as liquid stationary phase [6], a chiral mobile phase [7], or pre-column conversion of the enantiomers to diastereomers using chiral derivatizing agents [3, 8, 9] or metal chelate formation [10].

In this paper we describe an alternative approach to chiral purity determination which does not require enantiomer separation. Achiral HPLC is used to separate ephedrine and pseudoephedrine from chemical impurities

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in the sample, with UV absorbance and polarimetric [11] detection. The UV detector quantitates the total amount of ephedrine/pseudoephedrine present. The polarimetric detector gives a measurement of the overall optical rotation (OR) of the sample, due to the total quantity present and to the enantiomeric purity. By taking the ratio of the OR and the UV response a value for the enantiomeric purity alone may be determined. This approach has already been the subject of several brief studies [11–13] and has recently been reviewed [14]. A detailed account of the technique has not yet appeared, and in this article we report a systematic study of the accuracy and the scope of the achiral HPLC/dual detector approach to enantiomeric purity determination.

Experimental

Reagents and chemicals

Samples used were (+)- and (−)-ephedrine hydrochloride (chemical purity >99%) and (+)- and (−)-pseudoephedrine hydrochloride (Sigma, Poole, UK). The cough linctus preparation was Sudafed Elixir (Wellcome). The HPLC mobile phases were prepared using deionized water (from an Elgastat UHQ water purifier), methanol, hexane, propan-2-ol (all HPLC grade), heptane sulphonic acid sodium salt (HSAS), diethylamine, glacial acetic acid and ammonium nitrate (all AR grade) (FSA, Loughborough, UK). All sample solutions were filtered through a 0.45 μm filter before use.

Instrumentation

The HPLC system consisted of a ternary gradient pump (ACS, model 352), an injection valve (Rheodyne 7152) with a 20 μl loop, a variable wavelength UV detector (ACS 750/12) operating at 254 nm, and a polarimetric HPLC detector (ACS ChiraMonitor) with a diode laser light source at 820 nm. The UV data was collected and analysed on an integrator (Trivector Trio). A chart recorder (Chessell Ltd, UK) was used to record the output from the polarimetric detector. A polarimeter (Perkin–Elmer 141) with a 1 dm pathlength silica cell thermostatted at 20°C was used to measure the optical rotation of both (+)- and (−)-ephedrine hydrochloride at the sodium D-line (589 nm) and mercury arc wavelengths (579, 546, 436, 405, 365, 313 and 302 nm).

Chromatographic conditions

Two different achiral HPLC separations were performed. The first used a Spherisorb 5 ODS 2 column (250 × 4.6 mm) with a 50 × 4.6 mm ODS guard column and a mobile phase consisting of methanol–0.0075 M aq. HSAS–glacial acetic acid (79:20:1) [1] at a flow rate of 1.7 ml min^{-1}. The other used a Spherisorb 5 C8 column (250 × 4.6 mm) and MeOH–aq. ammonium nitrate (0.8% by wt) (55:45) as mobile phase at a flow rate of 1.0 ml min^{-1}. All analyses were performed at ambient temperature.

Chiral separations were carried out on a Daicel OD column (250 × 4.6 mm). The mobile phase was hexane–propan-2-ol–diethylamine (90:10:0.15) at a flow rate of 0.8 ml min^{-1}. Since the ephedrine hydrochloride samples are only slightly soluble in the mobile phase, the ephedrine free base had to be prepared. 0.5 g samples of (+)- and (−)-ephedrine hydrochloride standards and racemic ephedrine hydrochloride were dissolved in 10 ml of water, with excess added KOH. Ten millilitres of hexane were then added to extract the ephedrine free base, and aliquots of this were taken for injection onto the HPLC system.

Results and Discussion

Polarimetry

Measured optical rotations, α, were converted to specific rotation, [α], using Biot's law [15]:

$$\alpha = [\alpha]cl, \qquad (1)$$

where c is the mass concentration (g ml^{-1}) and l the cell path length (dm). From our data at the sodium D-line wavelength, $[\alpha]_{589}^{20} = -35.5 \pm 0.7$ and $+34.4 \pm 1.3$ ° ml g^{-1} dm^{-1} for (−)- and (+)-ephedrine hydrochloride, respectively, in agreement with literature values ($|[\alpha]_{589}^{20}| = 34.9$ to 36.6 ° ml g^{-1} dm^{-1} in water) [1, 16].

The dependence of optical rotation on wavelength for ephedrine hydrochloride in water was found to accord with the simple Drude equation (equation 2) [15, 17]:

$$[\alpha]_\lambda = \frac{K}{\lambda^2 - \lambda_0^2}, \qquad (2)$$

which may also be expressed as

$$\lambda^2[\alpha]_\lambda = \lambda_0^2[\alpha]_\lambda + K, \qquad (3)$$

where λ is the wavelength of measurement, $[\alpha]_\lambda$ is the specific rotation at this wavelength, and λ_0 and K are constants for the material. For a species with one dominant chiral chromophore, λ_0 is the absorption wavelength for that chromophore.

A plot of $\lambda^2[\alpha]_\lambda$ against $[\alpha]_\lambda$ (Fig. 1) gives a straight line with slope λ_0^2 and intercept K. λ_0 was found to be 172 ± 4 nm for ephedrine hydrochloride. From equation (3) we can obtain the specific rotation at 820 nm, the wavelength at which the polarimetric HPLC detector operates. This gave $[\alpha]^{20}_{820} = -17.7 \pm 0.2$ ° ml g^{-1} dm^{-1} for (−)-ephedrine hydrochloride in water.

For (+)-pseudoephedrine hydrochloride, similar measurements in MeOH–H_2O (55:45) gave $[\alpha]^{20}_{589} = +63.5 \pm 3.7$, $[\alpha]^{20}_{820} = +31.2 \pm 0.4$ ° ml g^{-1} dm^{-1}, and $\lambda_0 = 189 \pm 8$ nm (literature value $[\alpha]^{20}_{589} = 61.6$ ° ml g^{-1} dm^{-1} in water [16]).

Chiral chromatography

Chiral chromatography was carried out to establish the enantiomeric purity of the (+)- and (−)-ephedrine hydrochloride standards. Baseline resolution of racemic ephedrine was achieved under the conditions described in the Experimental section. Multiple separations of the enantiomer standards were made. Also, standards spiked with 0.5% by volume racemic ephedrine solution were chromatographed, to confirm the identity of the minor enantiomer peak in both sets of standards chromatograms.

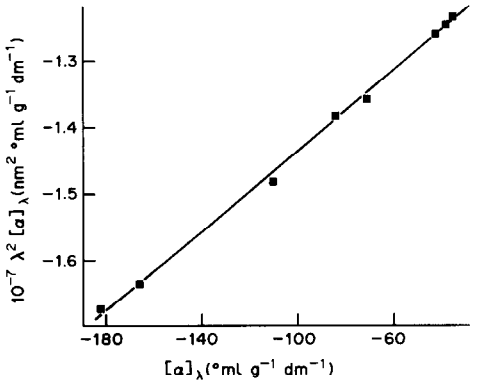

Figure 1
Wavelength dependence of the optical rotation of (−)-ephedrine hydrochloride in water at 20°C.

This was necessary as there were several small chemical impurity peaks which elute close to the enantiomers. Figure 2(a) shows a chromatogram of the (+)-ephedrine standard, and Fig. 2(b) shows an expansion of this chromatogram, which reveals the (−)-ephedrine impurity. From measurements on standards with and without spiking (four repeats for each standard) the enantiomeric purity of the (+)-ephedrine hydrochloride standard was found to be 99.89 ± 0.10%, and the enantiomeric purity of the (−)-ephedrine hydrochloride standard was found to be 99.95 ± 0.05%. Error estimates are given as 95% confidence limits on the mean value [18].

Achiral chromatography

Representative chromatograms of (+)-ephedrine hydrochloride separated on the ODS column are shown in Fig. 3. The reten-

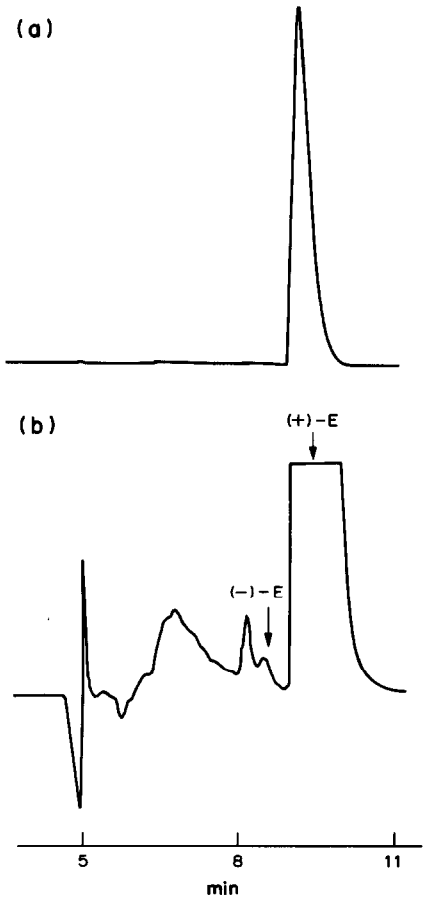

Figure 2
Analysis by chiral HPLC of (+)-ephedrine standard. Column: Daicel OD (250 × 4.6 mm). Mobile phase: hexane–propan-2-ol–diethylamine (90:10:0.15). Flow rate: 0.8 ml min^{-1}. (b) is an expansion of (a); (+)-E, (−)-E are (+)- and (−)-ephedrine peaks, respectively.

Figure 3
Chromatograms of (+)-ephedrine hydrochloride with dual optical rotation and UV absorbance detection. Column: Spherisorb 5 ODS 2 (250 × 4.6 mm). Mobile phase: methanol–0.0075 M aq. HSAS–glacial acetic acid (79:20:1). Flow rate: 1.7 ml min^{-1}.

tion time of ephedrine was about 3 min at a concentration of 1.0 mg ml^{-1}, and it increased a little with decreasing sample loading.

The plot of log(OR peak height) versus log(concentration) shows the linear range of the technique (Fig. 4). The slope is 1.01 ± 0.02, and the correlation coefficient is 0.9997 over the range of 0.06–10.00 mg ml^{-1} of (+)-ephedrine hydrochloride (20 μl injection). The

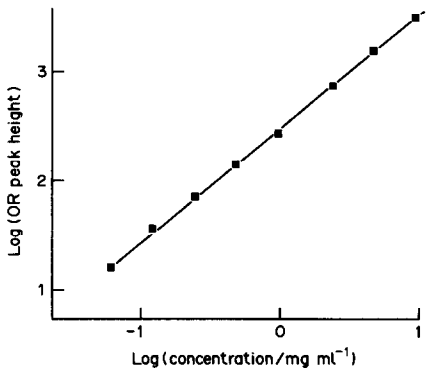

Figure 4
Linear response of OR detector. Plot of log(OR peak height) versus log(concentration/mg ml^{-1}) for (+)-ephedrine hydrochloride.

limit of detection (LOD) was calculated using equation (4) [18]:

$$\mathrm{LOD} = 3s_{y/x}/b, \qquad (4)$$

where $s_{y/x}$ is the RMS error on the data points, and b the slope of the plot of OR peak height versus concentration. Using the lowest concentration data points of Fig. 4, a linear regression analysis gave $s_{y/x} = 4.1$ and $b = 257$ ml mg^{-1}. Hence LOD = 0.048 mg ml^{-1}, which with the volume injected of 20 μl corresponds to a loading of 1.0 μg.

Enantiomeric Purity Determination

From measurements on peaks eluting from an achiral column using polarimetric and spectrophotometric detectors in series the enantiomeric purity can be calculated from the ratio of the optical rotation to the absorbance response, α/A, for unknown and standard samples. The basis for the method will be outlined prior to its application for ephedrine hydrochloride, and an error analysis is given in the Appendix.

For a pair of enantiomers, the specific rotation should be the same in magnitude and opposite in sign. In polarimetric measurements on mixtures of enantiomers, their individual rotational contributions sum as follows to give the overall rotation α for a mixture of (+)- and (−)- forms of mole fractions x and $(1 - x)$, respectively.

$$\alpha = [\alpha]lxc + (-[\alpha])l(1 - x)c, \qquad (5)$$

where c is the total mass concentration, l the cell pathlength and [α] the specific rotation of the pure (+)-enantiomer. Rearranging equation (5),

$$\alpha = [\alpha]l(2x - 1)c. \qquad (6)$$

By measuring the rotation it is therefore possible using equation (6) to find the enantiomer mole fractions.

In determining enantiomeric purity it is necessary to have a standard, s, of known enantiomer mole fraction x_s. This typically would be found by resolution of the enantiomers on a chiral stationary phase. Alternatively, this may be done using the method of Mannschreck *et al.* [19], with coupled OR and UV detection and partial chiral resolution.

Comparison of the optical rotation of unknown and standard samples may then be carried out using a polarimetric detector coupled to an achiral column. From equation (6), with subscripts for unknown, u, and standard, s:

$$\alpha_u = [\alpha]l(2x_u - 1)c_u, \qquad (6.1)$$

$$\alpha_s = [\alpha]l(2x_s - 1)c_s. \qquad (6.2)$$

Dividing equation (6.1) by equation (6.2) gives

$$\frac{(\alpha_u/c_u)}{(\alpha_s/c_s)} = \frac{(2x_u - 1)}{(2x_s - 1)}. \qquad (7)$$

The ratio α_u/α_s is taken from OR peak heights or areas.

To eliminate errors in c, OR and UV detectors may be used in series, and the ratio of their responses calculated, since both α and absorbance, A, are directly proportional to c. From equation (7),

$$\frac{(\alpha/A)_u}{(\alpha/A)_s} = \frac{2x_u - 1}{2x_s - 1}. \qquad (8)$$

It should be noted that this method is free from uncertainties arising from flow rate variation [20], since any change will cause the same proportional effect on both OR and UV peak heights or areas.

For chemically-pure samples, calibration graphs can be prepared by making mixtures of (+)- and (−)-standards at a constant total concentration and plotting α/A versus the composition of the mixture. If both standards are pure enantiomers, α/A should be zero for a 50:50 mixture, i.e. a synthetic racemate. A successful method should show no bias away from zero in this "racemic response" [13].

If the fraction of the (+)-standard is f, then it may be shown that a graph of α/A versus $(2f - 1)$ gives

$$\text{intercept/slope} = \frac{x_2 - x_1}{(x_2 + x_1) - 1}, \qquad (9)$$

where x_2 and x_1 are the (+)-enantiomer and (−)-enantiomer mole fractions in the (+)- and (−)-standards, respectively. Since we are dealing with standards with $x_2, x_1 \approx 1$,

$$\text{intercept/slope} \approx x_2 - x_1. \qquad (10)$$

To test the quality of this method, mixtures were prepared by volume from stock solutions of (+)- and (−)-standards at constant total ephedrine hydrochloride concentration. Achiral chromatographic conditions were as described in Fig. 3.

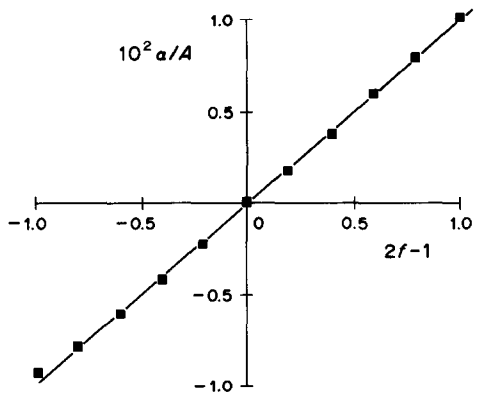

Figure 5
Plot of α/A versus $(2f - 1)$ for ephedrine hydrochloride at a total concentration of 2.5 mg ml^{-1}.

A plot of α/A versus $(2f - 1)$ is shown in Fig. 5 for the 2.5 mg ml^{-1} mixtures. The quality of fit is evident, and correlation coefficients were 0.9985, 0.9992 and 0.9995 for 1.0, 2.5 and 10.0 mg ml^{-1} mixtures. Intercept/slope values with their 95% confidence limits were 0.050 ± 0.026, 0.004 ± 0.019 and 0.007 ± 0.015. The error is seen to decrease somewhat with increasing sample concentration and, apart from the figure at the lowest concentration, values are within their error bounds equal to the value $(x_2 - x_1) = -0.0006 \pm 0.0015$ determined from chiral chromatography. We conclude that at loading of 50 μg or above the α/A method may be successfully used for enantiomeric purity determination of ephedrine hydrochloride.

For quality control of enantiomeric pharmaceuticals, it is of particular importance to be able to make an accurate determination of enantiomeric purity in the mole fraction region approaching a pure single enantiomer. In blind trials, mixtures of (+)-ephedrine hydrochloride and racemic (±)-ephedrine hydrochloride were prepared from appropriate volumes of 2.5 mg ml^{-1} stock solutions of each form to give samples with x_u in the range 0.90–1.00. Assays were then carried out by using OR and UV peak heights to compare $(\alpha/A)_u$ with $(\alpha/A)_s$ for the (+)-ephedrine hydrochloride standard (equation 8). A number, n, of replicate injections for each sample were made (n typically 5–6), and an error analysis carried out

as described in the Appendix. The achiral chromatographic conditions for these experiments entailed the use of the C8 column and MeOH–aq. ammonium nitrate as mobile phase, giving better peak shape and less tailing than in the earlier experiments with the C18 column and MeOH–aq. HSAS–glacial acetic acid. The results of the blind trials are shown in Table 1. The quality of this technique for enantiomeric purity determination is evident, with mean values within 0.2% of the actual values, and 95% confidence limits of 0.4% on the chromatographic measurements.

Table 1
(+)-Ephedrine hydrochloride mole fraction in the mixtures prepared for blind trials

x_u (actual)	$\bar{x}_u \pm t_{95}s_{x_u}/\sqrt{n}$ (determined)	Number of injections
0.974	0.972 ± 0.004	5
0.950	0.950 ± 0.003	6

Enantiomeric purity may be determined in pharmaceutical preparations as well as in pure materials. Figures 6(a) and 6(b) show the UV and OR chromatograms of the cough linctus Sudafed diluted by a factor of 20 and filtered prior to injection. Using OR and UV peak heights to compare $(\alpha/A)_u$ with $(\alpha/A)_s$ for the (+)-pseudoephedrine hydrochloride standard (equation 8), the enantiomeric purity of pseudoephedrine hydrochloride in the Sudafed sample was found to be 0.99 ± 0.01 (+)-form (7 replicate injections of sample, 10 of standard). The method of standard additions [18] was used to determine the concentration of pseudoephedrine hydrochloride in Sudafed. Plots of either OR peak height or UV peak height versus concentration of added (+)-pseudoephedrine hydrochloride showed the concentration in the linctus sample to be 7.5 mg ml^{-1}. Thus with an injection of 7.5 μg of the active compound in the pharmaceutical sample, the enantiomeric purity has been determined with 95% confidence limits of ±1%. The loading in this case was substantially less than with ephedrine hydrochloride (7.5 μg instead of 50 μg), which accounts in part for the greater uncertainty in the purity determination.

There are several other points worthy of comment in Fig. 6. The shape of the OR peak of sucrose may be indicative of an artefact caused by beam defocussing at the solute front,

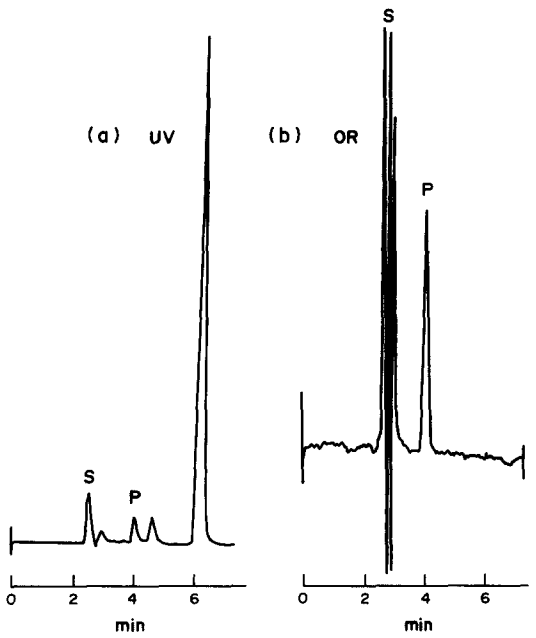

Figure 6
Chromatograms of Sudafed cough linctus with dual OR/UV detection. Column: Spherisorb 5 C8. Mobile phase: methanol–water (0.8% ammonium nitrate by wt) (55:45). Flow rate: 1.0 ml min^{-1}. Pharmaceutical preparation diluted 20 times and filtered before injection. Sucrose (S) and pseudoephedrine hydrochloride (P) peaks labelled.

which causes a lens-shaped refractive index boundary during entry and exit of the slug of concentrated solute [21]. Sucrose is present at concentration 70% (w/v) in Sudafed, giving 700 μg on column for each injection, 100 times the loading of pseudoephedrine hydrochloride. The strongest UV absorbance is due to the anti-microbial preservative methyl-4-hydroxybenzoate, which is not chiral and therefore not present in the OR chromatogram.

Conclusion

Using an optical rotation detector in series with a UV detector after an achiral HPLC column, the enantiomeric purities of ephedrine hydrochloride and pseudoephedrine hydrochloride have been determined both for solutions of the pure compounds and in a pharmaceutical preparation. α/A ratios of unknown and standard samples may be combined to give the enantiomeric purity of the unknown, provided that the purity of the standard has been previously established — for instance using a chiral stationary phase.

Ninety-five per cent confidence limits are somewhat less favourable than those from

chiral chromatography, 0.4% on enantiomeric purity with 50 μg injections of ephedrine hydrochloride in comparison with the value for the (+)-ephedrine standard from measurements using the Daicel OD CSP of 0.1%. However, the achiral chromatographic technique with dual OR/UV detection should be satisfactory for quality control, not least because of the greater robustness and lower replacement costs of the achiral column, together with compatibility with reversed-phase solvents and tolerance to sample overload. The OR/UV method carries less uncertainty than does polarimetry on bulk samples (typically 95% confidence limits of ~2% were found in our polarimetric measurements). Perhaps of greatest importance when considering applications in quality control of pharmaceuticals is that the OR/UV method does not require chemically-pure samples. It can be orders of magnitude more economical in material. Using samples at concentration 2.5 mg ml^{-1}, 50 μg was loaded for each HPLC injection, whereas about 15 mg was required to fill the 6 ml polarimeter cell.

Acknowledgements — We wish to thank the Shandong Teachers' University and the Chinese Government for a visiting scholarship to Zecai Wu, and Gary Bowden for technical assistance.

References

[1] S.L. Ali, *Anal. Profiles Drug Subst.* **15**, 233–281 (1986).
[2] British Pharmacopoeia, London (1988).
[3] F.T. Noggle Jr and C.R. Clark, *J. Forensic Sci.* **31**, 732–742 (1986).
[4] C.O. Wilson, O. Gisvold and R.F. Doerge, *Textbook of Organic Medicinal and Pharmaceutical Chemistry* (15th edn), pp. 438–447. Lippincott Co., (1966).
[5] I.W. Wainer, T.D. Doyle, Z. Hamidzadeh and M. Aldridge, *J. Chromatogr.*, **261**, 123–126 (1983).
[6] C. Pettersson and H.W. Stuurman, *J. Chromatogr. Sci.* **22**, 441–443 (1984).
[7] G. Schill, I.W. Wainer and S.A. Barkan, *J. Chromatogr.* **365**, 73–88 (1986).
[8] T.D. Doyle, W.M. Adams, F.S. Fry Jr and I.W. Wainer, *J. Liq. Chromatogr.*, **9**, 455–471 (1986).
[9] I.W. Wainer, T.D. Doyle and F.S. Fry Jr, *J. Chromatogr.*, **355**, 149–156 (1986).
[10] M. Moriyasu, Y. Hashimoto and M. Endo, *Chem. Lett.* 761–764 (1980).
[11] D.K. Lloyd, D.M. Goodall and H. Scrivener, *Anal. Chem.* **61**, 1238–1243 (1989).
[12] B.D. Scott and D.L. Dunn, *J. Chromatogr.* **319**, 419–426 (1985).
[13] B.H. Reitsma and E.S. Yeung, *J. Chromatogr.* **362**, 353–362 (1986).
[14] D.K. Lloyd and D.M. Goodall, *Chirality* **1**, 251–264 (1989).
[15] C. Tanford, *Physical Chemistry of Macromolecules*. Wiley, New York (1961).
[16] G. Harris (Ed.), *Dictionary of Organic compounds*, Vol. 3. Eyre and Spottiswoode, London (1965).
[17] J.T. Yang and P. Doty, *J. Am. Chem. Soc.* **79**, 761–775 (1957).
[18] J.C. Miller and J.N. Miller, *Statistics for Analytical Chemistry* (2nd edn). Ellis Horwood, Chichester (1988).
[19] A. Mannschreck, A. Eigelsperger and G. Stuhler, *Chem. Ber.*, **115**, 1568–1575 (1982).
[20] E. Grushka and I. Zamir, in *Lasers, Molecules and Methods*, p. 549. Wiley, New York (1989).
[21] B.H. Reitsma and E.S. Yeung, *Anal. Chem.* **59**, 1059–1061 (1987).

[Received for review 4 December 1989; revised manuscript received 6 March 1990]

Appendix

Errors in enantiomer mole fraction determination

Theory

The (+)-enantiomer mole fraction in the unknown, x_u, is determined from that in the standard, x_s, and the observed α/A ratios:

$$\frac{(\alpha/A)_u}{(\alpha/A)_s} = \frac{2x_u - 1}{2x_s - 1}. \tag{8}$$

This may be rearranged to

$$x_u - 0.5 = \frac{(\alpha/A)_u}{(\alpha/A)_s}(x_s - 0.5). \tag{11}$$

Equation (11) may be abbreviated to

$$y = \frac{u}{v} w, \tag{12}$$

where $y = (x_u - 0.5)$, $u = (\alpha/A)_u$, $v = (\alpha/A)_s$, and $w = (x_s - 0.5)$.

For uncorrelated errors in all quantities, the population standard deviation in y, σ_y, is given by:

$$\left(\frac{\sigma_y}{y}\right)^2 = \left(\frac{\sigma_u}{u}\right)^2 + \left(\frac{\sigma_v}{v}\right)^2 + \left(\frac{\sigma_w}{w}\right)^2. \tag{13}$$

Absolute rather than relative errors are of interest, and multiplication by y^2 (equation 12) gives

$$\sigma_y^2 = w^2\left\{\left(\frac{\sigma_u^2}{v^2}\right) + \frac{u^2}{v^2}\left(\frac{\sigma_v^2}{v^2}\right)\right\} + \frac{u^2}{v^2}\sigma_w^2. \tag{14}$$

Whilst equation (14) is of general applicability, it is instructive to consider the two limiting cases:

(i) $u/v \approx 0$ (near racemate).

Here equation (14) reduces to

$$\sigma_y^2 \approx w^2 \frac{\sigma_u^2}{v^2} = \frac{\sigma_u^2}{u^2} y^2, \qquad (15)$$

(ii) $|u/v| \approx 1$ (nearly pure single enantiomer)

$$\sigma_y^2 \approx w^2 \left(\frac{\sigma_u^2 + \sigma_v^2}{v^2}\right) + \sigma_w^2. \qquad (16)$$

In comparing equations (15) and (16) we note that the errors are greater in case (ii), with the uncertainty in enantiomer mole fraction for the standard in case (ii) but not case (i). Additionally, since detector errors are the same for standard and unknown, it would be expected that the standard deviations in the parent populations of u and v would be equal, i.e.

$$\sigma_u = \sigma_v.$$

Thus it follows that the leading term in equation (16), which allows for uncertainty in α/A measurements, is twice that in equation (15).

When dealing with statistics of small samples the analogue of equation (14) is used with $t_{95}s/\sqrt{n}$ values in place of σ, where $s/\sqrt{n}$ is the standard error on the mean and t_{95} the t-value for 95% confidence limits.

Application to ephedrine hydrochloride

Experimentally determined values from one of the blind trials reported in Table 1 were:

$$u \pm t_{95}s_u/\sqrt{n} = (1.365 \pm 0.005_0) \times 10^{-3}, \quad (n = 6)$$

$$v \pm t_{95}s_v/\sqrt{n} = (1.515 \pm 0.008_1) \times 10^{-3}, \quad (n = 7)$$

and

$$w \pm t_{95}s_w/\sqrt{n} = (0.4989 \pm 0.0010), \quad (n = 14).$$

Substituting in equation (14) with $t_{95}s/\sqrt{n}$ in place of σ throughout:

$$(t_{95}s_y/\sqrt{n})^2 = 0.4989^2 \left\{\left(\frac{0.005_0}{1.515}\right)^2 + \left(\frac{1.365}{1.515} \times \frac{0.008_1}{1.515}\right)^2\right\} + \left(\frac{1.365}{1.515} \times 0.0010\right)^2$$

$$= 9.3_0 \times 10^{-6}$$

$$\therefore t_{95}s_{x_u}/\sqrt{n} = t_{95}s_y/\sqrt{n} = 3.0 \times 10^{-3}$$

$$\bar{x}_u \pm t_{95}s_{x_u}/\sqrt{n} = 0.950 \pm 0.003.$$