of the following in a volume of 1 liter: naphthalene (150 g), 2,5-diphenyloxazole (8 g), 1,4-bis(4-methyl-5-phenyl-oxazol-2-yl)benzene (0.6 g), ethylene glycol (20 ml), 2-ethoxyethanol (100 ml), and toluene to make 1 liter. The radioactivity was measured in the liquid scintillation counter with an 18% gain setting. Each vial was counted twice, and the data represent the average of two counts. Results are shown in Table I.

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Optical Purity Determination by NMR: Use of Chiral Lanthanide Shift Reagents and a Base Line Technique

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Abstract □ A method for optical purity determination of a range of chiral drug molecules by NMR spectroscopy is reported. This technique involves the use of optically active lanthanide shift reagents and a newly developed base line analysis. Its applicability was demonstrated for a variety of drugs including nonsteroidal antiinflammatory agents and some adrenergic agents. It is established that successful application of the method depends on a constant shift reagent to sample molar ratio, constant instrumental conditions for all solutions, and the use of a calibration curve derived from solutions containing the same total concentration of the two enantiomers. For the examples cited, the correlation coefficient is not <0.97, and a mathematical treatment is included which supports the basis of the method.

Keyphrases □ Optical purity determination—by NMR spectroscopy, use of chiral lanthanide shift reagents and a base line technique □ Chiral lanthanide shift reagents—determination of optical purity by NMR spectroscopy, base line technique □ NMR spectroscopy—determination of optical purity, use of chiral lanthanide shift reagents and a base line technique

The application of NMR spectroscopy to the quantitative analysis of pharmaceuticals has become widespread (1, 2) since the publication of Hollis (3) on the determination of aspirin, phenacetin, and caffeine mixtures. Provided that careful consideration is given to solvent, internal standard, and instrumental conditions (including spinning sidebands and carbon 13 satellites), mixtures, often of some complexity, can be analyzed with a high degree of accuracy. Optical purity determination is another aspect of the analysis of any drug presented in a resolved form (enantiomer) or the racemate of a particular diastereoisomer. Apart from polarimetry, which has been extensively employed despite its drawbacks, optical purity determination has been achieved by chromatographic methods such as GLC (4, 5) and HPLC (6, 7), isotope dilution (8), kinetic resolution (9), and NMR. Even before the discovery of chiral lanthanide shift reagents, the NMR method was used for optical purity measurement either by diastereoisomer formation (10, 11) or the application of chiral solvents (12, 13). Since the publication of Whitesides and Lewis (14) on the relatively large frequency differences between corresponding resonances of enan-

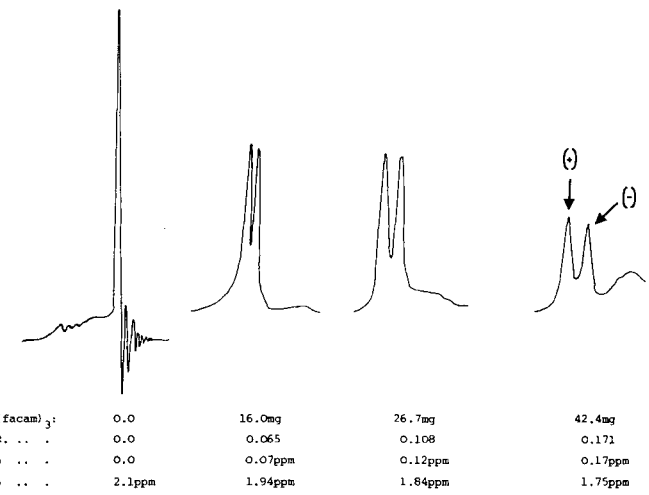

Figure 1—*Resonance of N—CH_3 of (±)-ephedrine in deuterated benzene on incremental addition of III.*

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Table I—Lanthanide Induced Shift for Quantitatively Useful Groups of (±)-Ephedrine[a]

	Molar Ratio	Lanthanide Induced Shift, ppm[b] C—CH_3 δ	C—CH_3 Δv	N—CH_3 δ	N—CH_3 Δv	CH—N[c] δ	CH—N[c] Δv	O—CH δ	O—CH Δv	Aromatic H[d] δ	Aromatic H[d] Δv
(A)[e]	0.000	0.74	0.00	2.10	0.00	2.54	0.00	4.71	0.00	—	—
	0.060	0.04	0.70	0.84	1.26	1.68	0.86	3.85	0.86	—	—
	0.093	−0.27	1.01	0.74	1.36	1.23	1.31	3.35	1.36	—	—
	0.136	−0.64	1.38	0.67	1.43	0.67	1.87	2.78	1.93	—	—
(B)[f]	0.000	0.67	0.00	2.05	0.00	—	—	4.68	0.00	7.15	0.00
	0.045	0.90	0.23	1.97	−0.08	—	—	4.57	−0.11	7.00	−0.15
	0.096	1.02	0.35	1.88	−0.17	—	—	4.52	−0.16	6.85	−0.30
	0.136	1.11	0.44	1.81	−0.24	—	—	4.47	−0.21	6.75	−0.40
	0.182	1.20	0.53	1.72	−0.33	—	—	4.46	−0.22	6.63	−0.52
	0.240	1.39	0.72	1.56	−0.49	—	—	4.47	−0.21	6.47	−0.68
	0.292	1.53	0.86	1.40	−0.60	—	—	4.75	0.07	6.30	−0.85

[a] Concentration of 0.45 *M* in deuterated benzene. [b] Lanthanide induced shifts of I are routinely downfield, II upfield. Negative values of Δv indicate a shift effect uncharacteristic of the lanthanide agent (*e.g.*, upfield for I). [c] The C—*H*—N resonances were broad and featureless. [d] Average results for the aromatic protons. [e] Data for varying shift reagent II–drug molar ratio. [f] Data for varying shift reagent I–drug molar ratio.

Table II—Shift Data for (+)- and (−)-Ephedrine[a] in the Presence of Chiral Lanthanide Shift Reagents at Different Molar Ratios

	Molar Ratio	(−)-isomer δ	(−)-isomer Δv	(+)-isomer δ	(+)-isomer Δv	ΔΔv
(A)[b]	0.00	0.74	0.00	0.74	0.00	0.00
	0.076	0.05	0.69	0.18	0.56	0.13
	0.119	−0.34	1.08	−0.16	0.90	0.18
	0.153	−0.59	1.33	−0.35	1.09	0.24
	0.182	−0.86	1.60	−0.57	1.31	0.29
	0.212	−1.19	1.93	−0.86	1.60	0.33
(B)[c]	0.00	2.10	0.00	2.10	0.00	0.00
	0.065	1.91	0.19	1.98	0.12	0.07
	0.090	1.84	0.26	1.93	0.17	0.09
	0.108	1.77	0.33	1.89	0.21	0.12
	0.120	1.75	0.35	1.88	0.22	0.13
	0.172	1.64	0.46	1.81	0.29	0.17

[a] Concentration of 0.40 *M* in deuterated benzene. [b] Data for C—CH_3 signal at varying IV–drug molar ratios. [c] Data for N—CH_3 signal at varying III–drug molar ratios.

tiomeric amines in the presence of 3-(*tert*-butylhydroxymethylene)-*d*-camphorato europium, much attention has been given to the use of the method in routine optical purity determination (15). The problem with this approach is the difficulty in achieving complete separation of corresponding resonances of the enantiomers before serious line broadening occurs. The purpose of this report is to describe a method of optical purity determination which does not require complete separation of resonance indicative of each enantiomer in a mixture.

EXPERIMENTAL[1]

Materials—Hydrochloride or sulfate salts of (±)-, (+)-, and (−)-ephedrine[2] and (−)-ephedrine base[2] were used, as well as (±)-, (+)-, and (−)-propranolol hydrochloride[3]; (±)-, *S*(+)-, and *R*(−)-albuterol sulfate[4]; (±)-, (+)-, and (−)-ibuprofen[5]; (±)-, (+)-, and (−)-naproxen[6]; (±)- and (+)-ketoprofen[7]; and (±)- and (−)-fenoprofen calcium[8].

The lanthanide shift reagents tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium (I)[9], tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)praseodymium (II)[9], tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato], europium (111) derivative (III)[9], and tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato], praseodymium (111) derivative (IV)[9] were stored over phosphorus pentoxide in a desiccator.

NMR: Determination of Lanthanide Induced Shifts[10]—A solution (~0.2–0.4 *M*) of the racemic compound in the appropriate solvent (0.7 ml) was prepared and the NMR spectrum recorded. Compound I or II in quantities of 10–20 mg was added incrementally and the spectrum recorded after each addition.

Optical Purity Determination[10]—Different quantities of (+)- and (−)-isomers, or alternatively the racemate and either pure isomer or suitable derivatives, were mixed to give differing optical purities, but approximately the same final concentration of total drug when dissolved in the appropriate solvent (0.7 ml). The quantity of chiral lanthanide shift reagent necessary to give the desired shift reagent: drug molar ratio was added (see text and Tables II, III, and V–VIII for explanation and data) and the NMR spectrum of the various solutions recorded under identical instrumental conditions.

Derivatives[11]—*Ketoprofen Methyl Ester*—(±)-Ketoprofen (50 mg) in a mixture of methanol (20 ml) and concentrated hydrochloric acid (1 ml) was refluxed for 1 hr. Evaporation to dryness *in vacuo* gave an oil sufficiently pure for use in subsequent NMR analyses. IR (ν_{max}): 1740, 1690, 1590, and 1500 cm^{-1}; NMR ($CDCl_3$): δ 1.40 (3H, d, C—CH_3), 3.60 (3H, s, $COOCH_3$), 3.62 (1H, q, CH—CH_3), and 7.20–7.90 (9H, m, Ar—*H*) ppm.

Fenoprofen Methyl Ester—(±)-Fenoprofen calcium salt (0.5 g) was dissolved in warm methanol (40 ml), and concentrated hydrochloric acid (1 ml) was added. Concentration of the solution *in vacuo* and extraction with ether (3 × 15 ml) gave the ester as an oil. IR (ν_{max}): 1735, 1590, and 1500 cm^{-1}; NMR ($CDCl_3$): δ 1.48 (3H, d, CH—CH_3), 3.61 (3H, s,

[1] All NMR spectra were recorded on a Perkin-Elmer R12B instrument operating at 60 MHZ with a probe temperature of 37 ± 1°. Unless otherwise stated, tetramethylsilane was employed as internal standard. Abbreviations used in the experimental section to describe resonance appearance are as follows: s, singlet; d, doublet; dd, double doublet; q, quartet; m, multiplet. Solvents were dried over molecular sieve type 4A. IR spectra, obtained as liquid films or as potassium chloride discs (for solids), were recorded on a Pye Unicam SP200 instrument. Low resolution mass spectra were obtained from an automatic electron impact mass spectrometer 12 instrument at 70 eV.

[2] Lake and Cruickshank, Buckhaven, Fife, Scotland.

[3] ICI Limited, Alderley Park, Macclesfield, Cheshire, U.K.

[4] Allen and Hanbury Ltd., Bethnal Green, London, U.K.

[5] Boots Company Ltd., Nottingham, U.K.

[6] Syntex Laboratories, Inc., Calif.

[7] May and Baker Limited, Dagenham, Essex, U.K.

[8] Lilly Research Center, Basingstoke, U.K.

[9] Aldrich Chemical Co. Ltd., Gillingham, Kent, U.K.

[10] Ephedrine and propranolol proton salts were converted to the corresponding free amine by basifying an aqueous solution of the salt with 5 *M* NaOH. Ether or chloroform extraction was performed rapidly in the usual way. Final drying of samples was achieved *in vacuo* at 50°.

[11] The corresponding derivatives of optically pure material were similarly prepared.

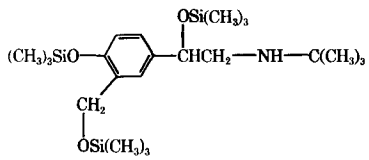
Table III—Application of the Base Line Technique to Ephedrine [a] of Varying Optical Purity

Optical Purity[b], %	Separation[c], mm	$\left(1 - \frac{\text{optical purity}}{100}\right)$
0.0	4.70	1.00
25.6	3.80	0.74
47.3	2.71	0.53
76.0	1.48	0.24

[a] Ephedrine concentration of 0.30 *M* and shift reagent IV–drug molar ratio of 0.216 in deuterated benzene. [b] An optical purity of 0.00 refers to the racemate. The difference between the percentage of the two isomers in a mixture is the percent optical purity. Thus, a sample containing 99% (+)- and 1% (−)-isomer is 98% optically pure. If the percent optical purity is X, then that isomer enriched in the mixture has a percentage given by (X + 100)/2. [c] Using C—CH_3 signal at δ −1.0–−1.5 ppm.

$COOCH_3$), 3.62 (1H, q, CH—CH_3), and 6.72–7.53 (9H, m, Ar—*H*) ppm.

Albuterol Tri(trimethylsilyl)ether (*V*)—(±)-Albuterol (60 mg) was



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heated with *N,N*-bis-trimethylsilyltrifluoroacetamide (VI; 0.4 ml) at 130° for 15 min. The excess of VI and trifluoroacetamide were removed *in vacuo* at 50° to leave V as an oil. IR (ν_{max}): 3000, 1620, and 1508 cm^{-1}; NMR [$CDCl_3$; most shielded $(CH_3)_3Si$ 9H singlet resonance taken as δO]: δ 0.11 [9H, s, $(CH_3)_3Si$], 0.25 [9H, s, $(CH_3)_3Si$], 1.05 [9H, s, $—C(CH_3)_3$], 2.41–2.76 (3H, m, N*H* + CH_2N—, deuterated water reduces to 2H), 4.61–4.76 (3H, m, O—CH_2Ar + $ArCH$—), and 6.60–7.47 (3H, m, Ar—*H*) ppm. Distillation of the oil gave a fraction, bp 140–142°/0.8 mm Hg, of analytical purity; mass spectrum: *m/z* (%), 457 (2), 442 (5.7), 370 (34), 368 (98), 192 (3.4), 148 (18), and 147 (100).

Anal.—Calc. for $C_{22}H_{45}NO_3Si_3$: C, 58.3; H, 9.9; N, 3.1; Si, 18.5. Found[12]: C, 57.9; H, 9.9; N, 3.7; Si, 18.0.

***N*-Acetylpropranolol**—Freshly distilled acetic anhydride (0.3 ml) was added to (±)-propranolol (0.85 g) in tetrahydrofuran (5 ml) and the mixture stirred for 15 hr. The solvent was removed *in vacuo* and the residue dissolved in chloroform (20 ml). The organic layer was washed with 0.1 *M* HCl (10 ml), 0.1 *M* NaOH (10 ml), and then evaporated in the usual way. The residual oil crystallized from petroleum ether (40–60)–ether (1:3) as colorless needles of (±)-*N*-acetylpropranolol. Recrystallization from the same solvent mixture gave a sample (0.45 g; 46%) mp 104°[13], suitable for analysis. IR (ν_{max}): 2950, 1690, 1603, and 1470 cm^{-1}; NMR ($CDCl_3$): δ 1.11–1.42 (6H, overlapping dd, $—CH(CH_3)_2$) 2.07 (3H, s, $COCH_3$), 3.41–4.27 (7H, m, $ArOCH_2$ + $CH(OH)$ + CH_2NCO + $CONCH$), and 6.73–8.34 (7H, m, Ar—*H*) ppm.

RESULTS AND DISCUSSION

It was routine practice in this work to study the influence of the nonchiral shift reagents of I and II[9] on the NMR resonances of racemic molecules, before attempting to use the much more expensive chiral shift reagents, III and IV. Table I shows the lanthanide induced shift for 0.45 *M* (±)-ephedrine in deuterated benzene at varying II–ephedrine and I–ephedrine ratios. By this means it was possible to demonstrate the susceptibility of quantitatively important groups such as C—CH_3 and N—CH_3, which show sharp lines, to the shifting influence of the lanthanide agents. The absence of serious line broadening of the various

[12] Elemental analyses were performed by Butterworth Laboratories Ltd., Teddington, Middlesex, U.K.

[13] Melting points, which are uncorrected, were obtained on a Gallenkamp melting point apparatus Number 889339, supplied by Gallenkamp, Birmingham, U.K.

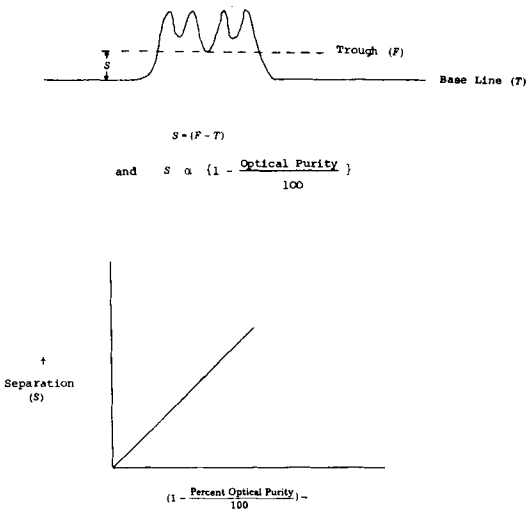

Figure 2—*The base line technique.*

resonances of (±)-ephedrine even at I–drug molar ratio of 0.292, was also observed. A plot of lanthanide induced shift (ppm) against lanthanide agent–ephedrine molar ratio for both nonchiral shift agents yields a linear standard curve for the important C—CH_3 and N—CH_3 groups in the concentration range studied (Table I). The magnitude of observed shifts for N—CH_3 and C—CH_3 in ephedrine, particularly with agent II, indicated their potential use in experiments with chiral lanthanide compounds.

Table II gives data for shifts and differential shifts of the (+)- and (−)-isomers of ephedrine in deuterated benzene in the presence of IV and III. The change in spectral appearance in the δ 1.75–2.10 (N—CH_3) region for 0.4 *M* (±)-ephedrine in deuterated benzene by addition of increasing quantities of III is shown in Fig. 1. Thus, the complexation of the optically active shift reagent with (+)- and (−)-ephedrine gives diastereoisomeric species with slightly different chemical shifts for corresponding groups within the drug molecule. In this case, the relatively large ΔΔv value of 0.33 for the overlapping C—CH_3 doublets at a molar ratio of 0.212 IV–drug, even though line broadening was slightly greater than with III, made IV the shift reagent of choice. It has been observed that for ephedrine, studied under the conditions shown in Table III and in the examples subsequently cited, the distance between the true base line (*T*, Fig. 2) and the trough between the overlapping resonances associated with corresponding groups in the two isomers (*F*, Fig. 2) is proportional to (1 − percent optical purity/100). The distance (*F* − *T*) is *S*, the separation. The principles of this base line technique are outlined, as applied to the analysis of the overlapping C—CH_3 doublets of ephedrine, in Fig. 2. An appropriate calibration curve then allows a sample of unknown optical purity to be determined.

The successful application of the method depends on three important factors:

1. The use of solutions containing a constant shift reagent–drug molar ratio once that ratio has been selected.
2. Determination under constant instrumental conditions for all solutions.
3. The use of a standard total concentration of the two isomers in the

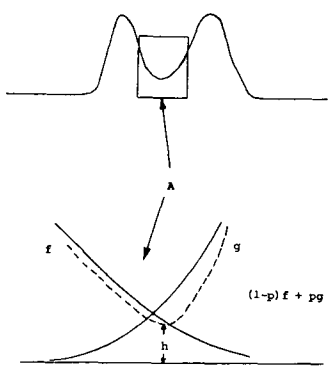

Figure 3—*Mathematical analysis of the overlapping portion of curves similar to the response appearance obtained in the base line technique.*

Table IV—Effect of Dilution with Deuterated Benzene on Lanthanide Induced Shift of (±)-Ephedrine in the Same Solvent

	Volume of deuterated benzene added, ml	C—CH_3		N—CH_3		O—CH	
		δ	Δν	δ	Δν	δ	Δν
(A)[b]	Original[a]	0.90	0.00	1.84	0.00	4.41	0.00
	0.1	1.00	0.10	1.93	0.09	4.53	0.12
	0.2	1.04	1.14	1.96	0.12	4.61	0.20
	0.3	1.05	0.15	1.98	0.14	4.63	0.22
	0.4	1.05	0.15	1.98	0.14	4.67	0.26
(B)[c]	Original[a]	1.13	0.00	1.75	0.00	4.37	0.00
	0.1	1.23	0.10	1.79	0.04	4.52	0.15
	0.2	1.34	0.21	1.89	0.14	4.68	0.31
	0.3	1.42	0.29	1.92	0.17	4.80	0.43
	0.4	1.47	0.34	1.95	0.20	4.89	0.52
	0.5	1.52	0.39	1.97	0.22	4.97	0.60

[a] 0.7-ml volume. [b] Data for compound I–drug molar ratio of 0.106. [c] Data for compound I–drug molar ratio of 0.212.

mixture for the calibration curve. In practice the concentration need not be the same for all solutions studied, provided that appropriate conversion to the standard concentration is effected for purposes of calibration.

It is considered that the relaxation phenomena associated with corresponding groups in such a diastereoisomeric complex need not necessarily be identical, and consequently, a mathematical analysis has been applied to similar overlapping curves. This is presented to support the basis of the method (Fig. 3):

In the region of the minimum (A), suppose that the left hand curve is $f(x)$, the right hand curve is $g(x)$; when it is assumed that $f' < 0, g' > 0$, $f'' > 0, g'' > 0$, and f, g such that for all relevant p, $(1 - p)f + pg$ has a minimum.

Suppose the minimum is at z, with a minimal value, h. The consequence of perturbing p to $(p + \delta p)$ is then considered. Write $\phi(x)$ for $(1 - p)f(x) + pg(x)$. Thus, z is governed by $\phi'(z) = 0$. Therefore:

$$(1 - p)f'(z) + pg'(z) = 0 \qquad \text{(Eq. 1)}$$

Thus, to find δz:

$$(1 - p - \delta p)f'(z + \delta z) + (p + \delta p)g'(z + \delta z) = 0 \qquad \text{(Eq. 2)}$$

where:

$$\phi'(z + \delta z) + \delta p[g'(z + \delta z) - f'(z + \delta z)] = 0 \qquad \text{(Eq. 3)}$$

And on expanding and using $\phi'(z) = 0$

$$\phi''(z)\delta z + 0(\delta z^2) + \delta p[g'(z) - f'(z) + 0(\delta z)] = 0 \qquad \text{(Eq. 4)}$$

Table V—Shift Data for (+)- and (−)-*N*-Acetylpropranolol[a] Using Reagent III

Molar Ratio	(+)-isomer δ	(+)-isomer Δν	(−)-isomer δ	(−)-isomer Δν	ΔΔ[b], ppm
0.000	2.10	0.00	2.10	0.00	0.00
0.183	3.07	0.97	2.92	0.82	0.15
0.276	3.64	1.54	3.42	1.32	0.22
0.367	4.15	2.05	3.87	1.77	0.28
0.521	4.70	2.60	4.39	2.29	0.31

[a] Concentration of 0.25 M acetylated drug in carbon tetrachloride, using $COCH_3$ signal. [b] Using the same shift reagent, the ΔΔν values in deuterated benzene and deuterated chloroform at ~0.5 molar ratios were 0.15 and 0.02 ppm, respectively.

Table VI—Application of the Base Line Technique to *N*-Acetylpropranolol[a] of Varying Optical Purity

Optical Purity, %	S[b], mm	$\left(1 - \frac{\text{optical purity}}{100}\right)$
0.0	4.90	1.00
24.9	3.90	0.75
50.4	2.80	0.50
75.4	1.40	0.25
100.0	0.20	0.00

[a] Concentration of 0.248 M in carbon tetrachloride, using agent III–acetylated drug molar ratio of 0.40. [b] Resonance of $COCH_3$ at δ3.9–4.2 ppm employed in the analysis.

where:

$$\delta z = \frac{-[g'(z) - f'(z)]}{\phi''(z)}\delta p + 0(\delta p^2) \qquad \text{(Eq. 5)}$$

Therefore:

$$h + \delta h = (1 - p - \delta p)f(z + \delta z) + (p + \delta p)g(z + \delta z)$$
$$= \phi(z + \delta z) + \delta p[g(z + \delta z) - f(z + \delta z)] \qquad \text{(Eq. 6)}$$

and:

$$\delta h = \phi'(z)\delta z + \tfrac{1}{2}\phi''(z)\delta z^2 + 0(\delta z^3) + \delta p\{[g(z) - f(z)] + [g'(z) - f'(z)]\,\delta z + 0(\delta z^2)\}$$
$$= \{\phi'(z)\delta z + [g(z) - f(z)]\delta p\} + \tfrac{1}{2}\phi''(z)\delta z^2 + [g'(z) - f'(z)]\delta p\delta z + 0(\delta p\delta z^2\delta z^3)\}$$
$$= [g(z) - f(z)\delta p] + \left\{\tfrac{1}{2}\phi''(z)\frac{[g'(z) - f'(z)]^2}{\phi''(z)^2} + [g'(z) - f'(z)]\frac{-g'(z) - f'(z)]}{\phi''(z)}\right\}\delta p^2 + 0(\delta p^3)$$
$$= [g(z) - f(z)]\delta p - \tfrac{1}{2}\frac{[g'(z) - f'(z)]^2}{\phi''(z)}(\delta p)^2 + 0(\delta p^3)$$

[note that $\phi'(z) = 0$] (Eq. 7)

Thus, δh is approximately linearly related to δp, with coefficient $g(z) - f(z)$, but the quadratic term does not vanish, and indeed the coefficient:

$$-\frac{[g'(z) - f'(z)]^2}{\phi''(z)}$$

need not even be particularly small; it is known that $g' < 0$ and $f' < 0$.

Results for the application of the base line technique to ephedrine of varying optical purity are shown in Table III and the corresponding calibration plot[S *versus* (optical purity/100)] yields a standard linear curve.

In accordance with previous work (16), the lanthanide induced shift produced by nonchiral and chiral shift reagents was found to be altered by dilution of prepared samples with more solvent. For a fixed shift reagent–ephedrine molar ratio, incremental addition of deuterated benzene caused a downfield shift proportional to the amount of solvent added.

Table VII—Application of the Base Line Technique to V[a]

Optical Purity, %	S[b], mm	$\left(1 - \frac{\text{optical purity}}{100}\right)$
0.0	7.00	1.00
25.0	5.20	0.75
50.0	3.70	0.50
75.3	1.80	0.25
100.0	0.15	0.00

[a] Concentration of 0.188 M in carbon tetrachloride employing III–derivatized drug molar ratio of 1.20. [b] ΔΔν (ppm) values of 7.8, 4.8, and 6.5 were obtained at III–drug derivative molar ratios of 1.20 in carbon tetrachloride, deuterated chloroform, and deuterated benzene, respectively. The analytical peak was that for $ArCH_2OSi\leqslant$ in all three cases, but least line broadening occurred in carbon tetrachloride.

Table VIII—Application of the Base Line Technique to Various Nonsteroidal Anti-inflammatory Agents or Their Derivatives

	Optical Purity, %	S, cm	$\left(1 - \frac{\text{optical purity}}{100}\right)$
Ibuprofen[a]	0.0	0.59	1.00
	23.7	0.44	0.76
	47.6	0.29	0.52
	75.0	0.15	0.25
	100.0	0.00	0.00
Naproxen[b]	0.0	1.61	1.00
	24.4	1.39	0.76
	49.0	1.05	0.51
	70.1	0.79	0.30
	100.0	0.40	0.00
Ketoprofen methyl ester[c]	0.0	0.90	1.00
	20.0	0.72	0.80
	45.0	0.48	0.55
	70.0	0.27	0.30
	78.0	0.19	0.22
Fenoprofen methyl ester[d]	0.0	1.80	1.00
	20.0	1.45	0.80
	55.0	0.86	0.45
	80.0	0.46	0.20

[a] Concentration of drug used was 0.23 *M* in carbon tetrachloride, using III–drug molar ratio of 0.366. The analytical peak was $ArCHCH_3$ in the region δ3.14–3.52 ppm. [b] Drug concentration was 0.29 *M* in deuterated chloroform–carbon tetrachloride (3:4), using III–drug molar ratio of 0.355. The analytical peak was at δ1.80–2.60 ppm ($ArCHCH_3$). [c] Drug derivative concentration was 0.30 *M* in carbon tetrachloride, using III–ester molar ratio of 0.406. The analytical peak was $ArCHCH_3$ in the region δ1.90–2.15. [d] Concentration of derivatized drug was 0.215 *M* in carbon tetrachloride using III–ester molar ratio of 0.919. The analytical peak was the $COOCH_3$ resonance in the δ5.20–5.30 region.

Higher molar ratios produced greater downfield shifts. Table IV shows results for two different molar ratios, using I as shift reagent, on successive addition of 0.1 ml volumes of deuterated benzene to a mixture prepared in 0.7 ml of the same solvent.

Data obtained on application of the base line technique to *N*-acetylpropranolol, compound V, ibuprofen, naproxen, ketoprofen methyl ester, and fenoprofen methyl ester are presented in Tables V–VIII and the corresponding calibration curves, when plotted, are linear. Appropriate derivatization was required in those cases of poor solubility of the parent drug in the solvents available, or where the number of points of complexation with the shift reagent was large, with consequent line broadening.

If authentic, optically pure samples of a drug are available, this method offers a useful means of routine optical purity determination up to ~90% optical purity. The susceptibility of a molecule to pseudocontact shifting influences of a lathanide agent can be quickly established using a nonchiral compound, and the appropriate shift reagent–drug molar ratio necessary in a given case found from incremental addition of the shift reagent (Table II).

It has also been observed that, in certain cases, the peak height difference (δh) of overlapping resonances from corresponding groups of optical isomers in the presence of a chiral shift reagent bears a linear relationship to optical purity up to levels of ~50%. However, there was little success in establishing optical purity at levels >50%. The instrumental and other conditions necessary for successful application of the base line technique similarly apply to the peak height difference method.

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Liposome Dialysis for Improved Size Distributions

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Abstract □ A technique is described which allows reproducible preparation of liposomes with improved size–frequency distributions. The recent procedure of extrusion of crude liposome dispersions through controlled-pore polycarbonate membranes is used to control the upper limit of liposome diameter. Subsequent dialysis, using the same type of membrane, can remove the majority of liposomes smaller than a predetermined size. The pattern of dialysis of a liposome preparation is a function of the size–frequency distribution (as well as the membrane pore size) and can be used to approximate the distribution and/or used to monitor the reproducibility of liposome preparations.

Keyphrases □ Liposomes—dialysis for improved size–frequency distribution □ Polycarbonate membranes—dialysis of liposomes, size–frequency distribution □ Dialysis—liposomes, improved size–frequency distribution □ Distribution—size–frequency, liposome dialysis

It is recognized that liposome properties, both as model membranes and drug carrier systems, are dependent on their size (1–4). The differences in the plasma time course and tissue distribution seen between large multilamellar and small unilamellar vesicles are now well established (5), and even different size classes of large multilamellar vesicles can have significantly different pharmacokinetics (6). Unfortunately, the size distribution of the original multilamellar preparation previously described (7) is very heterogeneous and poorly reproducible. The use of this

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