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Validated chiral high-performance liquid chromatographic method for the determination of *trans*-(−)-paroxetine and its enantiomer in bulk and pharmaceutical formulations

R. Ferretti, B. Gallinella, F. La Torre*, L. Turchetto

Istituto Superiore di Sanità, Laboratorio di Chimica del Farmaco, Viale Regina Elena 299, 00161 Rome, Italy

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Abstract

A stereospecific high-performance liquid chromatography method for the determination of *trans*-(−)-paroxetine and its enantiomer in bulk raw material and pharmaceutical formulations was developed and validated. The enantiomeric separation was achieved, without any derivatization, on a carbamate derivative-based column (Chiralpak AD). The effect of the organic modifiers, 2-propanol and ethanol, in the mobile phases was optimised to obtain enantiomeric separation. Limits of detection and quantitation of 2 and 6 ng, respectively, were obtained for both of the enantiomers. The linearity was established in the range of 5–41 μg for *trans*-(−)-paroxetine and in the range of 10–160 ng for *trans*-(+)-paroxetine. The accuracy of the method was 102.3% (mean value) for *trans*-(−)-paroxetine and 99.9% (mean value) for *trans*-(+)-paroxetine. For the precision (repeatability), a relative standard deviation value of 1.5% (mean value) for *trans*-(−)-paroxetine and of 2.1% (mean value) for *trans*-(+)-paroxetine was found. The method is capable of determining a minimum limit of 0.2% of *trans*-(+)-isomer in commercial samples. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Paroxetine

1. Introduction

Over the last few years, optical resolution by high-performance liquid chromatography (HPLC) has become an important and useful method, not only for determining the optical purity of pharmaceutical compounds, but also for obtaining pure optical enantiomers.

A wide variety of chiral stationary phases (CSPs) are now available on the market, which allow enantiomeric separations either at analytical or preparative levels [1–7].

Cellulose and amylose trisphenylcarbamate derivatives, prepared by simple reaction of cellulose and amylose with phenyl isocyanate derivatives, provided useful CSPs for HPLC when coated [8–10] or bonded [11] to silica gel.

trans-(−)-Paroxetine, [*trans*-(−)-3-[(1,3-benzodioxol-5-yloxy) methyl]-4-(4-fluorophenyl)piperidine], a potent, selective 5-hydroxytryptamine uptake inhibitor, is currently in use as an antidepressant (Fig. 1). It was claimed that *trans*-(−)-paroxetine is as effective as tricyclic antidepressants [12], but has much reduced side effects [13]. There is also clinical

*Corresponding author.

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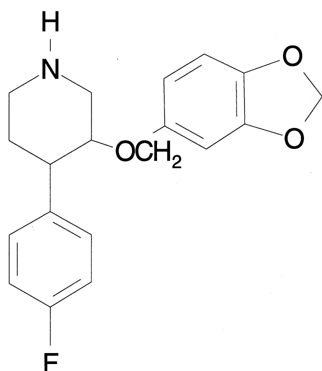

Fig. 1. Structure of *trans*-(±)-paroxetine.

evidence that paroxetine possesses an anxiolytic action in addition to its established antidepressant effect [14–16].

trans-(−)-Paroxetine was determined in blood, serum or plasma by gas chromatography (GC) using maprotiline as the internal standard [17,18].

HPLC assays of *trans*-(−)-paroxetine in human plasma were reported, after derivatization with dansyl chloride, using maprotiline as the internal standard and subsequent fluorescence detection [19] or, after solid-phase extraction, using dibucaine as the internal standard and fluorescence detection [20]. HPLC and ultraviolet (UV) detection were deemed to be more rapid and sensitive than either of the previously reported methods to support repeat-dose studies [21]; an accurate and reliable method was developed for the kinetic and therapeutic monitoring of *trans*-(−)-paroxetine in human plasma using UV detection [22] and a pre-column technique for on-line liquid–solid extraction with direct injection of serum samples was used for the UV determination of *trans*-(−)-paroxetine in human serum [23].

Many drugs or physiologically important compounds are chiral molecules and the optical isomers of these compounds may differ in their pharmacological activity. For this reason, *trans*-paroxetine is dispensed and administered as a single (−)-enantiomer for the treatment of depression; the chiral resolution of the enantiomers, as far as we know, has never been attempted.

In this paper, we describe a simple and convenient procedure for the determination of *trans*-(−)-paroxetine and its enantiomeric impurity in bulk raw material and pharmaceutical formulations.

2. Experimental

2.1. Materials

Stainless-steel Chiralpak AD, AS, Chiralcel OC, OD, OF, OG, OJ (250×4.6 mm I.D.) and Chiralpak AD (250×10 mm I.D.) (Daicel Chemical Industries, Tokyo, Japan) were used.

HPLC-grade solvents were purchased from Carlo Erba (Milan, Italy). Diethylamine (DEA; Uvasol-grade) was obtained from Fluka (Buchs, Switzerland).

A standard of *trans*-(−)-paroxetine–HCl and a racemic mixture of *trans*-(±)-paroxetine were kindly supplied by Chemi S.p.A. [Patrica (Fr.) Italy].

Standards of pure enantiomers of *trans*-(−)-paroxetine and *trans*-(+)-paroxetine were obtained by preparative HPLC.

2.2. Apparatus

Two different sets of instruments were used to determine the reproducibility of the method: The first comprised a Waters 600-MS pump, equipped with a Waters model U6K injector and a Waters model 996 programmable multi-wavelength diode array detector operated at 296 nm (Waters, Milford, MA, USA); data were collected on a NEC Power Mate 433 Workstation (HPLC 1). The second comprised a series 410 liquid chromatograph from Perkin Elmer (Norwalk CT, USA), equipped with a Waters model U6K injector and a Waters model 991 programmable multi-wavelength diode array detector operated at 296 nm (Waters, Milford, MA, USA); data were collected on a Power Mate 386/25 Waters Workstation (HPLC 2).

2.3. Operating conditions

The following operating conditions were used: With the Chiralpak AS column, the mobile phase used was *n*-hexane–2-propanol (90:10, v/v); with the Chiralpak AD column, the mobile phase was

n-hexane–ethanol–DEA (94:6:0.5, v/v); with the OC column, the mobile phase was *n*-hexane–2-propanol (90:10, v/v); with the OD column, the mobile phases used were *n*-hexane–2-propanol (90:10 and 70:30, v/v), *n*-hexane–2-propanol–DEA (90:10:0.8, v/v) and *n*-hexane–ethanol–DEA (90:10:0.4, v/v); with the OF column, the mobile phases used were *n*-hexane–2-propanol–DEA (90:10:0.1 and 80:20:0.1, v/v); with the OG column, the mobile phases used were *n*-hexane–2-propanol–DEA and *n*-hexane–ethanol–DEA (90:10:0.1, v/v) and with the OJ column, the mobile phase used was *n*-hexane–2-propanol (90:10, v/v).

The solvents, used as mobile phases, were degassed with an ultrasonic bath before use. The flow-rate used was 0.5 ml min^{-1}; the columns were operated at ambient temperature; the volume injected was 20 μl and the detector wavelength was set to 296 nm.

Samples of pure enantiomers of *trans*-(±)-paroxetine were obtained, from a racemic mixture, by preparative HPLC (Chiralpak AD; 250×10 mm I.D.), with a mobile phase of *n*-hexane–2-propanol–DEA (90:10:0.1, v/v) and a flow-rate of 2.0 ml min^{-1}; the fractions were collected and analysed on an analytical Chiralpak AD column to assess the chiral purity.

Columns, operated with mobile phases containing DEA, were easily regenerated at the end of every day by washing them with ca. 100 ml of *n*-hexane–2-propanol (90:10, v/v) at a flow rate of 0.2 ml min^{-1}. In this way, no efficiency loss was observed throughout our work. After cleaning, the columns were reconditioned with ca. 100 ml of the mobile phase.

2.4. Sample preparation

2.4.1. Tablets

In a mortar, ten tablets (previously weighed) of a pharmaceutical product available on the Italian market were pulverised and, taking into account the average mass of the tablets, an amount of powder corresponding to 20 mg of *trans*-(−)-paroxetine was weighed exactly. The powder was suspended in methanol (3×5 ml) and stirred for 30 min. The methanol was then filtered through a paper filter and dried with a gentle stream of nitrogen and the residue was dissolved in 20 ml of 2-propanol. Following the conditions described, recoveries of 97.0% were obtained.

3. Results and discussion

CSPs containing cellulose and amylose derivatives were used extensively to solve chiral separation problems [24–28]. The most important forces, responsible for the formation of the diastereomeric solute–CSP complex, are the hydrogen bonding, dipole–dipole and π–π interactions, together with the rigid linear structure (cellulose-based CSP) or helical structure (amylose-based CSP) of the chiral polymer bound to the support.

Poor resolution or no elution of compounds would be due to poor affinity of the compounds in the cellulose- or amylose CSP, or to the difficulty of inclusion of solute in the chiral cavity.

Our initial efforts were directed towards the direct HPLC separation of the enantiomers of *trans*-(±)-paroxetine with CSPs such as Chiralcel OD and Chiralpak AD using mobile phases consisting of *n*-hexane–2-propanol and *n*-hexane–ethanol of different compositions, with or without small amounts of DEA (less than 1%).

When the Chiralcel OD column was used with mobile phases consisting of *n*-hexane–2-propanol (90:10 and 70:30, v/v) elution times were long and there was no enantiomeric resolution, whereas poor values of enantioselectivity and resolution factors were obtained with a mobile phase consisting of *n*-hexane–2-propanol–DEA (90:10:0.8, v/v). Disappointing results were obtained with ethanol as the organic modifier in a mobile phase consisting of *n*-hexane–ethanol–DEA (90:10:0.4, v/v).

Attempts to separate the enantiomers using different columns (Chiralpak AS, Chiralcel OF, OG and OJ) did not succeed.

The Chiralpak AD column was more effective in separating the enantiomers of *trans*-(±)-paroxetine; the mobile phase was optimised to obtain enantiomeric separation. A surprising effect was observed using ethanol, instead of 2-propanol, as the organic modifier. In fact, an inversion of the order of elution of the enantiomers of *trans*-(±)-paroxetine was

observed: The *trans*-(+)-enantiomer, which is the chiral impurity, eluted before the main peak of *trans*-(−)-paroxetine, allowing a more accurate determination of the chiral purity of the *trans*-(−)-paroxetine (Fig. 2); data from this study are listed in Table 1.

The use of a linear photodiode array detector turned out to be useful in confirming that chiral separation of a pure racemic compound had, in fact, taken place. As enantiomers behave identically in symmetrical environments, they will absorb non-polarised light in exactly the same way, giving identical spectra (Fig. 3).

3.1. Test method

The separation of enantiomers of *trans*-(±)-paroxetine was achieved on the Chiralpak AD column using a mixture of *n*-hexane–ethanol–DEA (94:6:0.5, v/v) at room temperature and at a flow-rate of 0.5 ml min^{-1}. *trans*-(−)-Paroxetine eluted after 45 min, while its enantiomer eluted after 39 min, with *a*, R_s and *k* values being 1.19, 2.78 and 5.40, respectively.

Table 1
Chromatographic data for *trans*-(±)-paroxetine on a Chiralpak AD column

Compound	First eluted enantiomer	k'_1 [a]	α [b]	R_s [c]	Eluent[d]
trans-(±)-Paroxetine	(+)	7.20	1.16	2.72	A
	(+)	5.40	1.19	2.78	B
	(+)	1.72	1.16	1.23	C
	(+)	4.73	1.13	1.83	D
	(+)	5.33	1.16	2.02	E
	(+)	4.23	1.08	1.16	F
	(±)	5.72	1.00	0	G
	(−)	4.05	1.11	1.47	H
	(−)	3.66	1.17	2.26	I
	(−)	2.47	1.21	2.60	L

[a] The capacity factor of the first eluted enantiomer.
[b] The enantioselectivity factor.
[c] The resolution factor.
[d] Eluents employed were: (A) *n*-hexane–ethanol–DEA (95:5:0.1, v/v); (B) *n*-hexane–ethanol–DEA (94:6:0.5, v/v); (C) *n*-hexane–ethanol–DEA (90:10:0.1, v/v); (D) *n*-hexane–2-propanol–ethanol–DEA (94:2:4:0.5, v/v); (E) *n*-hexane–2-propanol–ethanol–DEA (94:3:3:0.5, v/v); (F) *n*-hexane–2-propanol–ethanol–DEA (94:3.5:2.5:0.5, v/v); (G) *n*-hexane–2-propanol–ethanol–DEA(94.5:3:2.5:0.5, v/v); (H) *n*-hexane–2-propanol–ethanol–DEA (94:4:2:0.5, v/v); (I) *n*-hexane–2-propanol–ethanol–DEA (94:5:1:0.5, v/v) and (L) *n*-hexane–2-propanol–DEA (90:10:0.1, v/v).

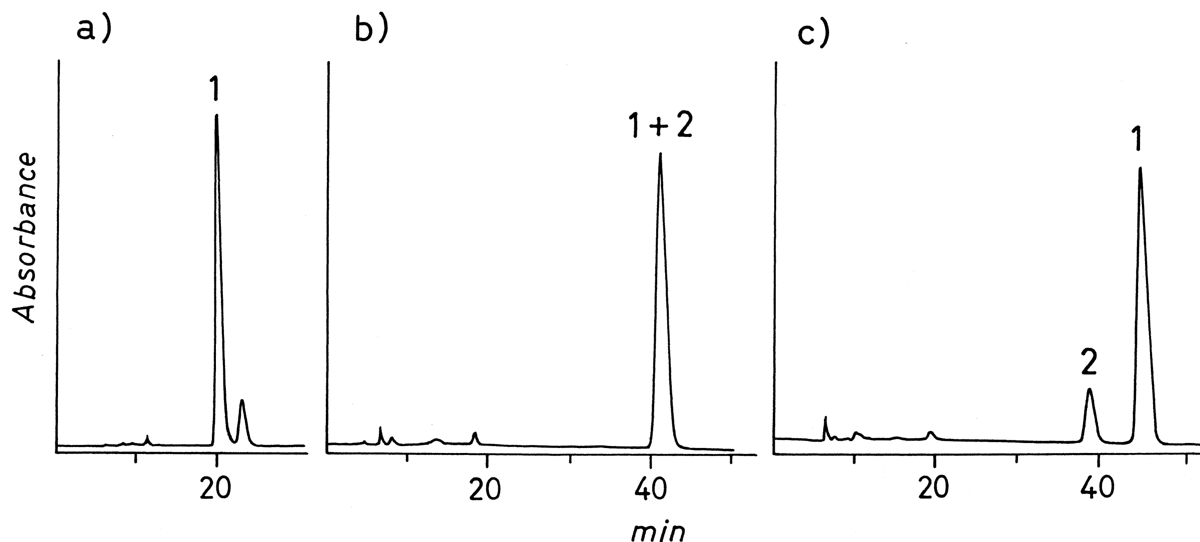

Fig. 2. HPLC of *trans*-(±)-paroxetine. (a) Eluent, [*n*-hexane–2-propanol–DEA (90:10:0.1, v/v)]; (b) eluent, [*n*-hexane–2-propanol–ethanol–DEA (94.5:3:2.5:0.5, v/v)]; (c): eluent [*n*-hexane–ethanol–DEA (94:6:0.5, v/v)]; flow-rate, 0.5 ml min^{-1}; column, Chiralpak AD; detection wavelength, 296 nm.

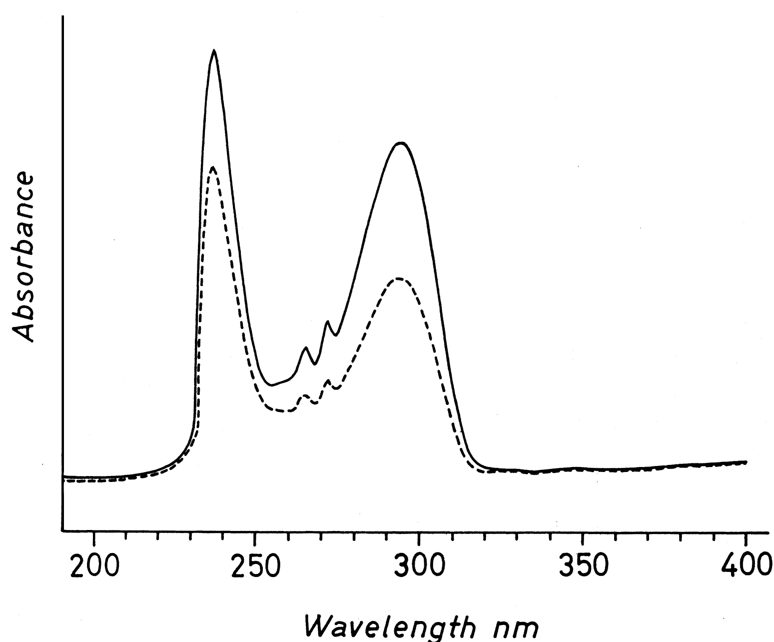

Fig. 3. UV spectra of *trans*-(−)-paroxetine (———) and *trans*-(+)-paroxetine (– – –).

3.2. Limit of detection and limit of quantitation

The limits of detection and quantitation of *trans*-(−)-paroxetine and its enantiomer were calculated according to the methods of Foley and Dorsey [29] and Knoll [30].

By comparing the detector response with the baseline noise, the limits of detection and quantitation were estimated to be 2 and 6 ng, respectively.

3.3. Linearity

The linearity of the method for *trans*-(±)-paroxetine was calculated in the range of 5–41 μg for *trans*-(−)-paroxetine and in the range of 10–160 ng for *trans*-(+)-paroxetine. The data were subjected to linear regression analysis. The calibration graphs showed good linearity, with a correlation coefficient of 0.9992 for *trans*-(−)-paroxetine ($c=7.1293\ 10^{-7}\ A-0.6034$) and 0.9987 for *trans*-(+)-paroxetine ($c=7.8702\ 10^{-4}\ A-1.4451$) ($c$=sample concentration, A=area counts).

3.4. Accuracy

The accuracy of the method for *trans*-(±)-paroxetine in bulk raw material was determined (as % recovery) using two different set of instruments, by the assay of known amounts of samples, precisely weighed, of *trans*-(+)-paroxetine and *trans*-(−)-paroxetine in the range of linearity of the method. Average recoveries were 99.9% (mean value) and 102.3% (mean value), respectively, with relative standard deviations (R.S.D.s) of 2.1 and 1.5% (Table 2).

The accuracy of the method for *trans*-(+)-paroxetine, in pharmaceutical formulations, was determined (as % recovery), using two different sets of instruments, by assaying tablets spiked with known amounts of *trans*-(+)-paroxetine in the range of 0.2–2.4% (Fig. 4). The average recoveries, obtained using the two sets of instruments, were 99.2% (mean value) and 101.9% (mean value), respectively, with R.S.D.s of 1.9% (mean value) and 3.0% (mean value) (Table 3).

3.5. Precision (repeatability and reproducibility)

The repeatability of the method was calculated using the R.S.D. of the areas of the peaks of *trans*-(−)-paroxetine and its enantiomer, which were used for the determination of accuracy; whereas the

Table 2
Inter-day precision and accuracy for *trans*-(±)-paroxetine in bulk raw material

Compound	Parameter[a]	Amount of sample injected (ng/20 μl)					
		160.8	120.6	80.4	40.2	20.1	10.5
trans-(+)-Paroxetine HPLC 1	Mean	157.9	123.0	80.8	43.1	20.8	9.0
	R.S.D. (%)	0	1.1	0	4.1	3.7	1.6
	Recovery (%)	98.2	102.2	100.5	107.2	103.5	85.7
trans-(+)-Paroxetine HPLC 2	Mean	160.8	123.3	76.6	41.0	22.0	9.8
	R.S.D. (%)	0	2.4	0.9	2.0	2.8	4.5
	Recovery (%)	100.0	102.6	95.3	102.0	109.4	93.2
		Amounts of sample injected (μg/20 μl)					
		41.7	31.3	20.8	10.4	5.2	
trans-(−)-Paroxetine HPLC 1	Mean	42.0	32.3	21.7	11.0	5.6	
	R.S.D. (%)	1.2	0.6	1.0	0.6	4.7	
	Recovery (%)	100.7	103.2	104.3	105.8	107.7	
trans-(−)-Paroxetine HPLC 2	Mean	41.7	31.3	20.9	10.3	5.3	
	R.S.D. (%)	1.5	1.0	0.6	2.1	1.9	
	Recovery (%)	100.0	100.0	100.5	99.0	101.9	

[a] R.S.D.=relative standard deviation; $n=3$ in each instance.

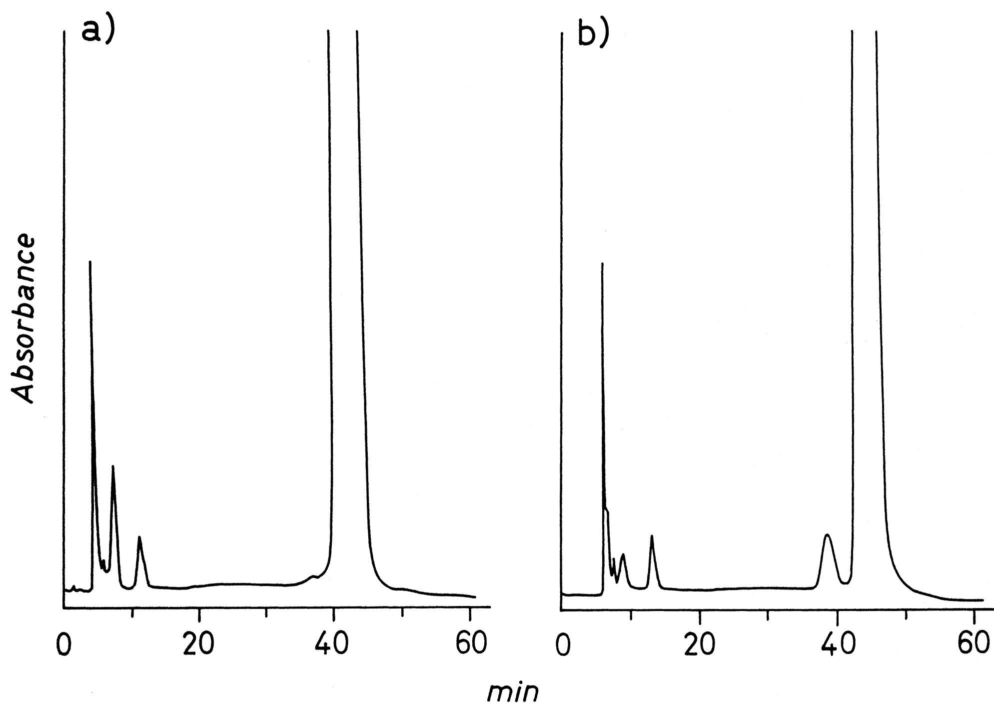

Fig. 4. HPLC of pharmaceutical formulations: Tablets (a), sample injected, 50 μg of *trans*-(−)-paroxetine; (b) sample injected, 50 μg spiked with 1 μg (2%) of *trans*-(+)-paroxetine. Eluent, *n*-hexane–ethanol–DEA (94:6:0.5, v/v/v); flow-rate, 0.5 ml min^{-1}; column, Chiralpak AD; detection wavelength, 296 nm.

Table 3
Inter-day precision and accuracy for *trans*-(+)-paroxetine obtained by spiking pharmaceutical formulations

Compound	Parameter[a]	Amount of compound spiked			
		0.2%	0.4%	0.8%	2.4%
trans-(+)-Paroxetine	Mean	0.19	0.42	0.81	2.3
HPLC 1	R.S.D. (%)	5.00	1.07	1.38	0.30
	Recovery (%)	95.0	105.0	101.24	95.8
trans-(+)-Paroxetine	Mean	0.21	0.41	0.84	2.29
HPLC 2	R.S.D. (%)	3.81	3.31	3.00	2.19
	Recovery (%)	105.0	102.5	105.0	95.4

[a]R.S.D.=relative standard deviation; $n=3$ in each instance.

reproducibility was expressed as the R.S.D. of the same solutions analysed with different HPLC systems in different laboratories.

The data showed good agreement (Tables 2 and 3).

3.6. Range

The method developed was suitable for the determination of *trans*-(−)-paroxetine and its enantiomer in concentration ranges of 5–41 μg and 10–160 ng, respectively, in bulk raw material and pharmaceutical formulations.

4. Conclusion

The HPLC method described for the assessment of the chiral purity of *trans*-(−)-paroxetine was validated to meet the requirements of the pharmaceutical industry [31] and regulatory authorities [32–35] in terms of limits of detection and quantitation, linearity, accuracy, precision and range.

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References

[1] G. Gubitz, Chromatographia 30 (1990) 555.
[2] E. Francotte, A. Junker-Buchheit, J. Chromatogr. 576 (1992) 1.
[3] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[4] D.R. Taylor, K. Maher, J. Chromatogr. Sci. 30 (1992) 67.
[5] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.
[6] Y. Okamoto, R. Aburatani, K. Hatada, J. Chromatogr. 389 (1987) 95.
[7] W.H. Pirkle, C.J. Welch, M.H. Hyun, J. Org. Chem. 48 (1983) 5022.
[8] T. Shibata, I. Okamoto, K. Ishii, J. Liq. Chromatogr. 9 (1986) 313.
[9] E. Yashima, P. Sahavattanapong, Y. Okamoto, Chirality 8 (1996) 446.
[10] K. Oguni, H. Oda, A. Ichida, J. Chromatogr. A 694 (1995) 91.
[11] Y. Okamoto, R. Aburatani, S. Miura, K. Hatada, J. Liq. Chromatogr. 10 (1987) 1613.
[12] J.B. Cohn, C.S. Wilcox, J. Clin. Psychiatry 53(Suppl. 2) (1992) 52.
[13] W.F. Boyer, C.L. Blumhart, J. Clin. Psychiatry 53(Suppl. 2) (1992) 61.
[14] G.C. Dunbar, J.B. Cohn, L.F. Fabre, J.P. Feighner, R.R. Fieve, J. Mendels, R.K. Shrivastava, Br. J. Psychiatry 159 (1991) 394.
[15] J.P. Feighner, W.F. Boyer, J. Clin. Psychiatry 53(Suppl. 2) (1992) 44.
[16] A.A. Kiev, J. Clin. Psychiatry 53(Suppl. 2) (1992) 27.
[17] E.N. Petersen, E. Bechgaard, R.J. Sortwell, L. Wetterberg, Eur. J. Pharmacol. 52 (1978) 115.
[18] S. Dawling, N. Ward, E.G. Essex, B. Widdop, Ann. Clin. Biochem. 27 (1990) 473.
[19] M.A. Brett, H.D. Dierdorf, J. Chromatogr. 419 (1987) 438.
[20] R.N. Gupta, J. Chromatogr. B 661 (1994) 362.
[21] J. Knoeller, R.R. Vogt-Schenkel, M.A. Brett, J. Pharm. Biomed. Anal. 45 (1995) 635.
[22] J.P. Foglia, D. Sorisio, M. Kirshner, B.G. Pollock, J. Chromatogr. B 693 (1997) 147.

[23] M. Bagli, M.L. Rao, T. Sobanski, G. Laux, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 283.
[24] Y. Okamoto, M. Kawashima, K. Hatada, J. Am. Chem. Soc. 106 (1984) 5357.
[25] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, Chem. Lett., (1988) 1125.
[26] Y. Okamoto, R. Aburatani, T. Fukumoto, K. Hatada, Chem. Lett., (1987) 1857.
[27] E. Yashima, M. Yamada, Y. Kaida, Y. Okamoto, J. Chromatogr. A 694 (1995) 347.
[28] Y. Okamoto, R. Aburatani, K. Hatano, K. Hatada, J. Liq. Chromatogr. 11 (1988) 2147.
[29] J.P. Foley, J.G. Dorsey, Chromatographia 18 (1984) 503.
[30] J.E. Knoll, J. Chromatogr. Sci. 23 (1985) 422.
[31] M.N. Cayen, Chirality 3 (1991) 94.
[32] H. Shindo, J. Caldwell, Chirality 3 (1991) 191.
[33] A.J. Hutt, Chirality 3 (1991) 161.
[34] W.H. De Camp, J. Pharm. Biomed. Anal. 11 (1993) 1167.
[35] A.G. Rauws, K. Groen, Chirality 6 (1994) 72.