ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 123–130

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

An enantioselective assay for (±)-modafinil

Quezia B. Cass [a,*], Cristiane K. Kohn [a], Silvana A. Calafatti [b], Hassan Y. Aboul-Enein [c]

[a] *Departamento de Química, Universidade Federal de S. Carlos, Cx. Postal 676, São Carlos, SP, Brazil*
[b] *Unidade de Farmacologia, Universidade São Francisco, Bragança Paulista, 12900-000, SP, Brazil*
[c] *Biological and Medical Research Department, Pharmaceutical Analysis Laboratory, MBC-03, King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh 11211, Saudi Arabia*

Received 21 July 2000; received in revised form 19 January 2001; accepted 29 January 2001

Abstract

Enantiomers of modafinil, (diphenylmethyl)sulphinyl-2-acetamide, were separated by an amylose tris[(*S*)-1-phenylethylcarbamate] chiral stationary phase, using acetonitrile: water (25:75 v/v) as mobile phase, with excellent selectivity ($\alpha = 1.4$) and resolution factors ($R_s = 1.5$). The assay involved a solid-phase extraction of the enantiomers of modafinil from plasma using a C_{18} Cartridge. A good linear relationship was obtained in the concentration of 0.15–3 μg ml^{-1} for each enantiomer. The method developed is sufficiently accurate and precise to be used for clinical samples and has a good selectivity with the two main circulating metabolites: the (diphenylmethyl)sulphinyl-2-acetic acid and (diphenylmethyl)sulphonyl-2-acetamide. The use of a polysaccharide-based column on multimodal elution was explored in developing the method.

Keywords: Modafinil; Enantiomers; Polysaccharides stationary phases; Plasma sample

1. Introduction

Modafinil, (diphenylmethyl)sulphinyl-2-acetamide (Fig. 1), is a novel wake-promoting drug developed for the treatment of narcolepsy, making it an alternative to dexamfetamine for this indication. It is a chiral compound due to the asymmetric sulphoxide function but it is used as a racemate [1–4].

Droin et al. [5] developed a method which was used for assessing enantiomeric purity of modafinil enantiomers but failed for pharmacokinetic purpose. Only recently Gorman [6] developed a method that was able to quantify both enantiomers of modafinil in human plasma using a β-clyclodextrin column.

Derivatised polysaccharide-based stationary phases have shown to be very efficient in the resolution of a series of chiral suphoxides. The use of amylose tris[(*S*)-1-phenylethylcarbamate] showed the best performance in the separation of a wide variety of chiral suphoxides [7] and was

* Corresponding author. Tel.: +55-16-2608207; fax: +55-16-2608350.
E-mail address: quezia@dq.ufscar.br (Q.B. Cass).

PII: S0731-7085(01)00400-9

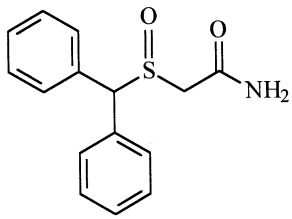

Fig. 1. Structure of (±)-modafinil.

selected for evaluation in the development of an enantioselective assay for (±)-modafinil.

This paper reports on the developed method for analysis of (±)-modafinil in human plasma samples.

2. Material and methods

2.1. General

The column was prepared as described elsewhere [7,8] and consisted of amylose tris[(*S*)-1-phenylethylcarbamate] coated onto APS-Nucleosil (7 μm particle size and 500 Å pore size, 20% w/w, 15 × 0.46 cm ID). HPLC dead times (t_0) were estimated by using 1,3,5-tri-*tert*-butylbenzene on the normal mode and acetonitrile on the reversed phase mode of elution. Solvents were either HPLC grade from Merck (Darmstadt, Germany) or Chromar HPLC grade from Mallinckrodt Baker (St. Louis, Missouri, USA) or were purified as usual [9].

The analysis of the plasma samples were carried out using acetonitrile: water (25:75 v/v) as mobile phase with a flow rate of 0.5 ml min^{-1} at $\lambda = 228$ nm.

The (±)-modafinil used was donated by Laboratoire L. Lafon (Maisons Alfort, France). The cartridges used for extraction were a C18 Varian (100 mg).

The modafinil sulphone was obtained by the oxidation of (±)-modafinil (15.0 mg; 0.055 mmol) at −40° C in dry dichloromethane (2 ml) with *meta*-chloroperbenzoic acid (28.4 mg; 0.165 mmol). After addition of the acid the reaction mixture was stirred at room temperature and

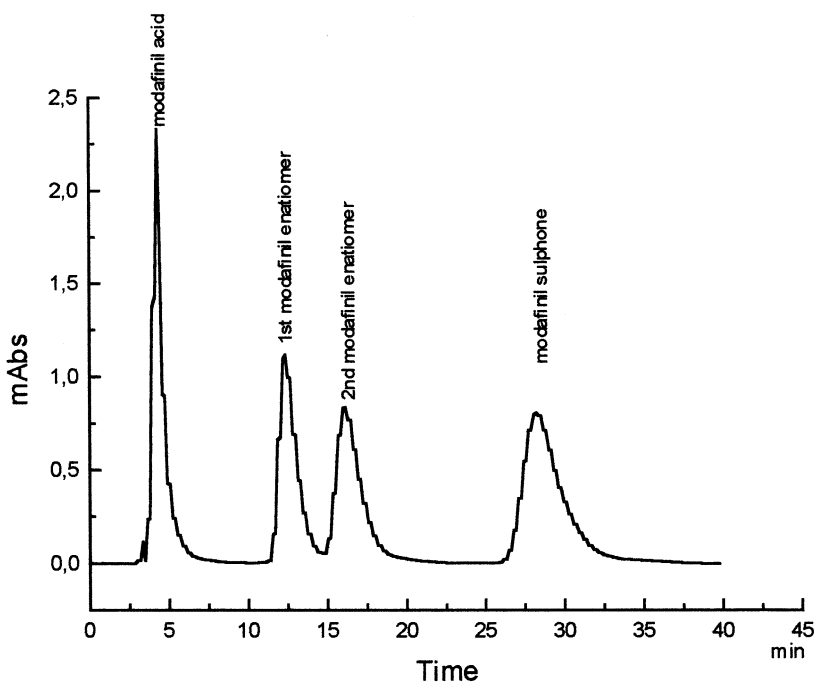

Fig. 2. Chromatogram of the analysis of modafinil enantiomers and its two major metabolites.

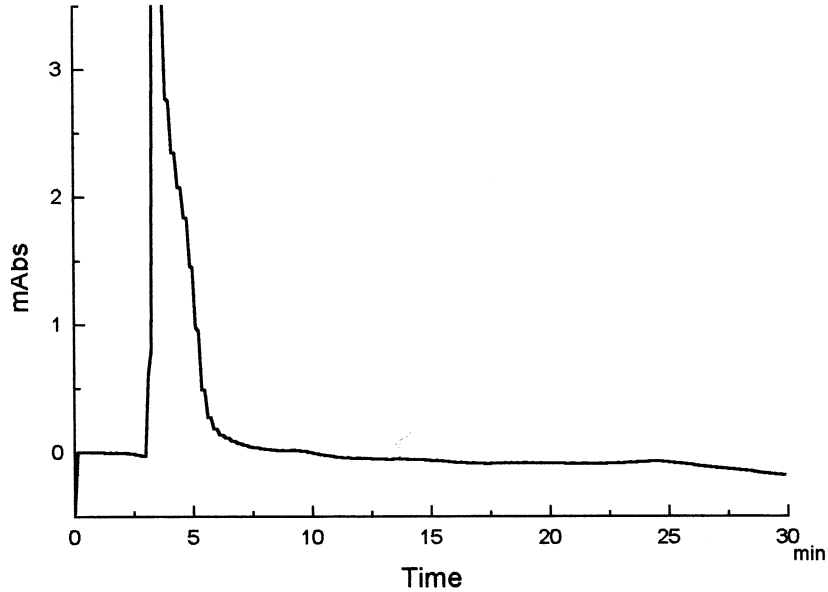

Fig. 3. Chromatogram of a blank pool plasma.

followed by t.l.c. The reaction product was dissolved in dicloromethane and the organic layer was then extracted with a saturated aqueous solution of sodium hydrogen carbonate (3×50 ml), water (50 ml) and dried with anhydrous sodium sulphate and then evaporated in vacuo. The ($\pm$)-modafinil acid was obtained by the basic hydrolysis of the parent amide. The hydrolysis was carried out as follows: ($\pm$)-modafinil (33.6 mg; 0.123 mmol) was dissolved in ethanol and then a 10% solution of sodium hydroxide (0.25 ml) was added. After 30 min of refluxing, the condenser was removed while keeping the heat for about 4 min to loose residual ammonium. The reaction mixture was then cooled in an ice bath and acidified with concentrated hydrochloric acid. A white precipitate was formed and it was extracted with ether. The organic solvent was separated, washed with water, dried with anhydrous sodium sulphate and then evaporated in vacuo.

The modafinil sulphone and ($\pm$)-modafinil acid were characterized by H NMR, IR and also by microanalysis.

Pooled control human plasma was supplied by the S. Francisco University Hospital, Bragança Paulista, SP, Brazil.

2.2. Equipment

The HPLC system was formed by one Shimadzu LC-10AD pump, an auto injector model SIL 10A, a SPD 10A UV detector with a CBM 10A interface. Data acquisition was done on CLASS LC 10 software.

2.3. Stock solutions

A stock solution of ($\pm$)-modafinil (1000 μg ml^{-1}) was prepared in methanol. From this a solution of 80 μg ml^{-1} was prepared and then by serial dilution, solutions of 40, 20, 10, 5.0 and 1.0 μg ml^{-1} were prepared. A solution 60 μg ml^{-1} was also prepared by diluting 7.5 ml of the 80 μg ml^{-1} to 10 ml with methanol.

2.4. Sample preparation

Aliquots (50 μl ml^{-1}) of the appropriate ($\pm$)-modafinil stock solutions were placed in a culture tube and the solvent evaporated under a nitrogen stream at room temperature. To each tube, a 0.5 ml of plasma was added, vortexed for 10 s and then 0.5 ml of water was added. The blank

plasma samples and the spiked plasma were transferred to preconditioned Varian C18 cartridges. The cartridges were conditioned by passing methanol (2 ml) followed by water (2 ml). After the plasma samples were passed through the cartridges they were washed with a saturated solu-

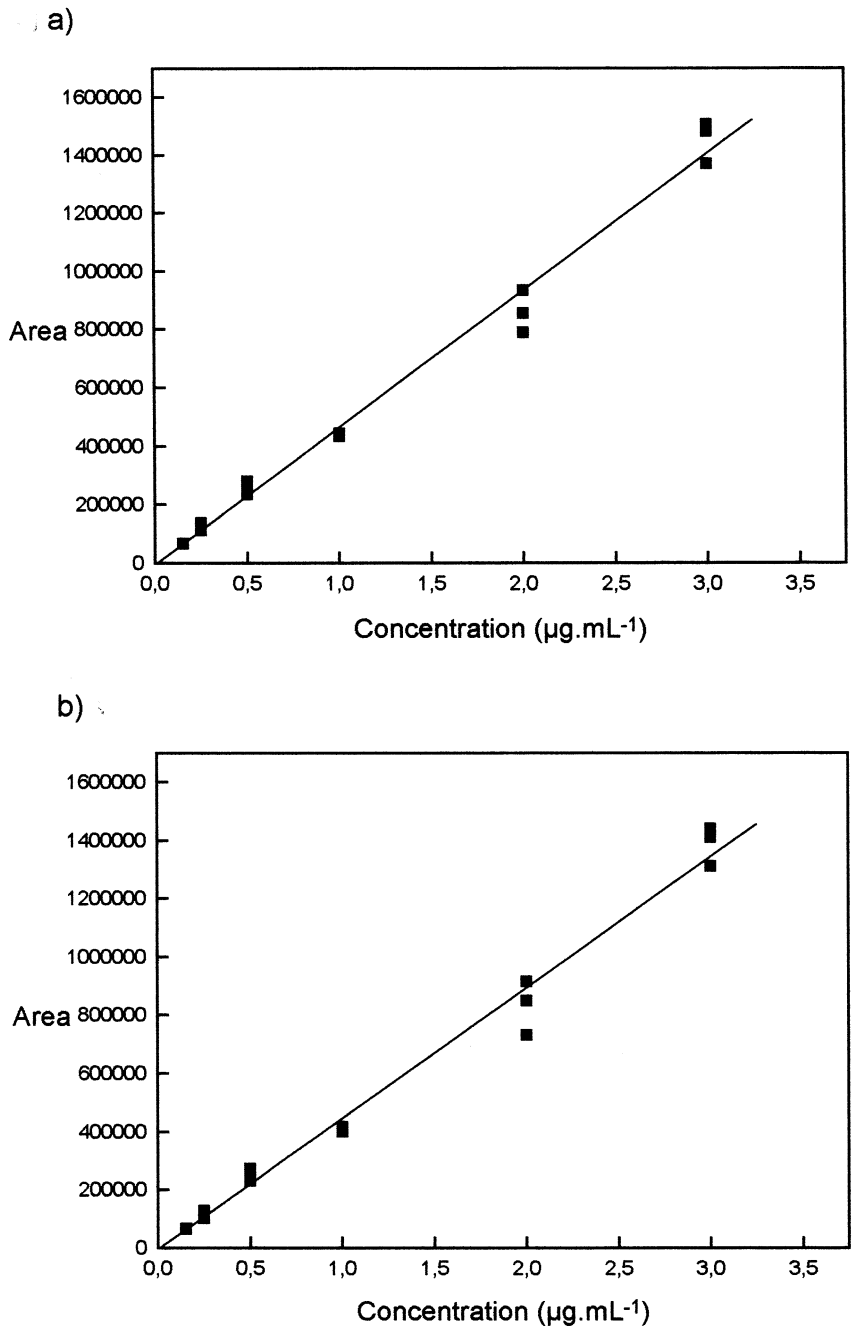

Fig. 4. Calibration curves for the: (a) first; and (b) second eluted enantiomers of modafinil.

Table 1
Recovery of (±)-modafini in human plasma

Concentrations (μg ml^{-1})	First enantiomer (%)	Second enantiomer (%)
0.2	90.3	93.0
0.8	94.4	95.2
2.4	90.6	86.6

tion of sodium chloride (1 ml), water (1 ml), an aqueous acetonitrile 20%v/v solution (1 ml) and then water (1 ml). The cartridges were dried prior to their extraction with methanol (1 ml). The methanol extracts were evaporated under a stream of nitrogen. The dried samples were reconstituted with methanol (250 μl) and then an aliquot (150 μl) was placed in an autosampler vial (200 μl) and an aliquot (50 μl) was injected onto the chromatographic system.

2.5. Standard curve

Using the appropriate stock solutions of (±)-modafinil, spiked plasma samples were prepared at the following concentration: 6.0, 4.0, 2.0, 1.0, 0.5 and 0.3 μg ml^{-1} (or 3.0, 2.0, 1.0, 0.5, 0.25 and 0.15 μg ml^{-1} for each enantiomer). The samples were prepared in triplicate.

Calibration curves were constructed by plotting the peak area against the concentration of each enantiomer.

2.6. Recovery

The recovery was determinated using spiked plasma at 4.8, 1.6 and 0.4 μg ml^{-1} of (±)modafinil (or 2.4, 0.8 and 0.2 μg ml^{-1} for each enantiomer). The peak-area ratios of five-extracted sample were compared to five unextracted samples to derive a percent recovery.

2.7. Precision

The same three concentration samples used for the recovery experiments were used for evaluating the inter-day and intra-day variability. Five samples of each concentration were prepared and analyzed on each of 3 different day.

2.8. Limits of detection and quantification

The limit of detection was calculated taking a signal-to-noise ratio of three as criteria while the limit of quantification was measured by preparing spiked plasma sample with serial diluted solutions. The acceptance criteria for the LOQ was that the CV for three extracted sample was under 20% variability.

3. Results and discussion

The previous results obtained with amylose tris[(*S*)-1-phenylethylcarbamate] coated onto APS-Nucleosil (7 μm particle size and 500 Å pore size) (20% w/w, 15 × 0.46 cm ID) for the enantioseparation of a series of chiral sulphoxides [7] prompted us to investigate its enantioselectivity towards modafinil enantiomers.

An excellent enantioselectivity for modafinil enantiomers was obtained with a selectivity factor (α) of 2.2 and a resolution factor of (R_S) 4.5 using hexane:ethanol (65:35 v/v). Under the chromatographic conditions used the enantiomers of modafinil had retention factors, k, of 4.52 and 9.48 for the first and the second enantiomers, respectively. The selectivity of the method was evaluated by running spiked plasma against blank sample plasma and no endogenous interference was noticed, however, under these conditions, the modafinil sulphone metabolite had the same capacity factor of the first eluted enantiomer of modafinil.

Knowing that different selectivity could be gained by altering the elution mode [10], the selectivity was first investigated under polar elution conditions with either 100% of methanol or ethanol. No marked difference in the capacity factor of the modafinil sulphone was observed. By moving to the reversed phase mode of elution an increase in the capacity factor of the sulphone was, however, observed. The desired selectivity, although, was not achieved using methanol: water as mobile phase. The use of 30% v/v of water in

methanol made the capacity factor of the sulphone the same as that of the second enantiomer. By increasing the amount of water to 50% v/v the k of the sulphone increased but no selectivity was still obtained. The use of acetonitrile: water as mobile phase showed that the desired selectivity could be obtained. The use of 75% v/v of water in acetonitrile gave the following capacity factors: 0.3, 2.8, 3.9 and 7.6 for the modafinil acid, first and second modafinil enantiomers and modafinil sulphone, respectively. A good selectivity factor (α) of 1.4 with a resolution (R_s) of 1.5 was also obtained for the enantiomers under these chromatographic conditions. Fig. 2 is a typical chro-

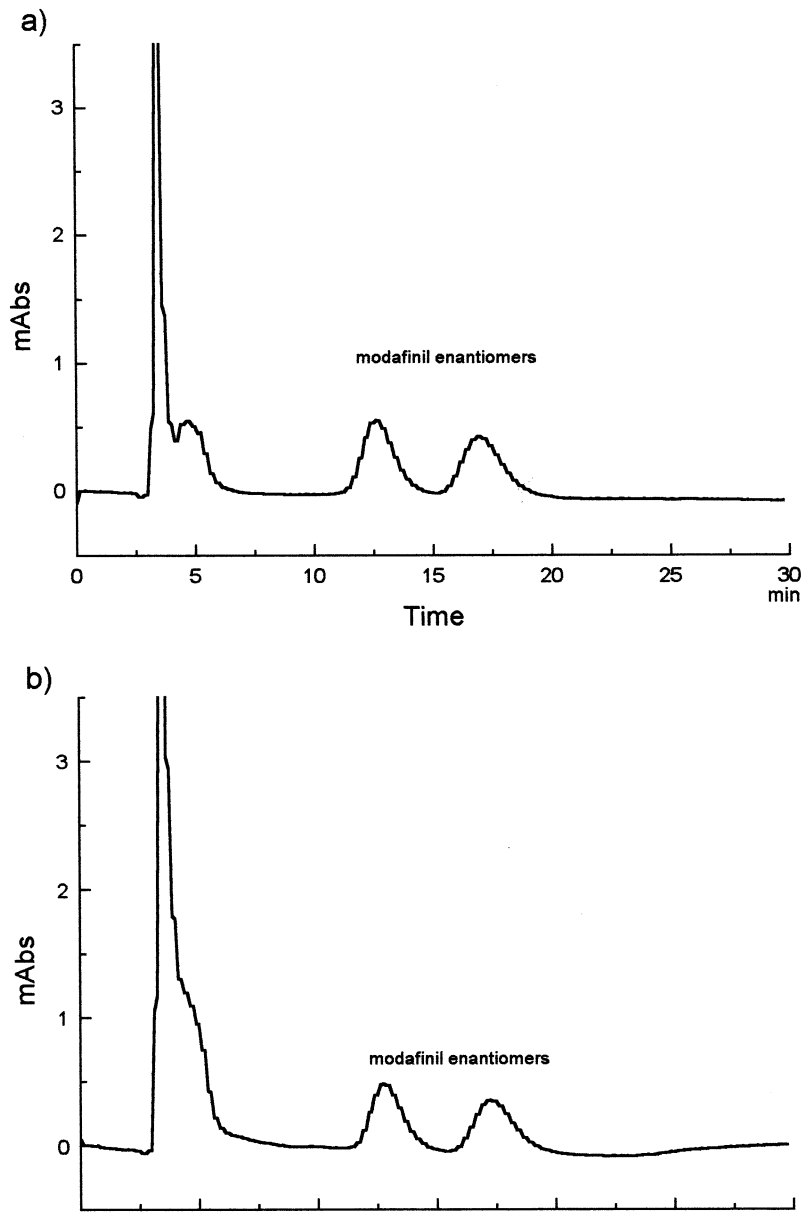

Fig. 5. Chromatograms of the analysis of: (a) plasma-free drug; (b) a plasma sample containing (±)-modafinil.

Table 2
Precision and accuracy data of (±)-Modafinil, inter-day ($n = 5$)

	Concentration	1st day		2nd day		3rd day	
	(μg ml^{-1})	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
1st Enantiomer	0.2	5.3	101	1.5	110	8.6	101
	0.8	8.6	99.9	8.5	93.0	6.1	104
	2.4	5.1	101	1.7	107	6.4	106
2nd Enantiomer	0.2	8.1	88.8	2.0	107	7.0	105
	0.8	9.7	100	7.2	94.5	3.1	109
	2.4	5.7	99.0	2.0	110	6.2	109

matogram of the modafinil enantiomers and its two major metabolites showing the excellent separation obtained between them. The chromatogram of a blank pool plasma (Fig. 3) showed that there were also no endogenous interfering compounds. The method thus could be validated for the quantification of (±)-modafinil in human plasma.

The linearity was studied in a concentration range to cover the one reported for (±)-modafinil in clinical samples [2,3]. The external calibration method was preferred, since the method employed a solid-phase extraction as sample preparation [11].

Regression analysis of the least-square line for the data obtained from the standard calibration curve showed good linearity for both enantiomers on the concentration range examined of 0.15–3.0 μg ml^{-1} with $r = 0.994$ and of 0.993 for the first and the second enantiomer, respectively. Fig. 4 shows the calibration curves obtained for both enantiomers.

The absolute recovery of each enantiomer was examined at three concentrations by comparing the mean peak area obtained after extraction with the ones from direct injection of the working solutions used for preparing the spiked plasma samples. Table 1 shows the results obtained in each concentration and the chromatograms showed in Fig. 5 were obtained during the recovery experiments.

The intra and inter-day precision were evaluated using the data of three quality controls analyzed over a 3 day period. The results are given in Table 2 and are expressed as coefficients of variation. The accuracy was evaluated from back calculation and expressed as the percent deviation between amount found and amount added for each enantiomer at the three concentrations examined.

The limit of quantification for each enantiomer was 0.02 μg ml^{-1} with CV of 17.3% for the first enantiomer and CV of 13.8% for the second enantiomer, while the limit of detection was 0.005 μg ml^{-1}.

Accurate determination of pharmacokinetic parameters from plasma concentration data can thus be obtained by the use of the method described for the analysis of modafinil enantiomers.

Under the normal phase elution conditions described also here, the method can be used efficiently for assessing enantiomeric purity in bulk pharmaceutical. For this application, the mobile phase strength can be increased to hexane:ethanol (60:40 v/v)

4. Conclusion

The methods presented here showed an excellent enantioselectivity for modafinil enantiomers. The good linearity, precision, accuracy, sensitivity and selectivity obtained under reversed phase elution mode allow it to be used for collecting data to pharmacokinetic studies.

Acknowledgements

We thank CNPq and FAPESP for grants and financial support. One of the authors (HYA-E) would like to thank King Faisal Specialist Hospi-

tal and Research Centre Administration for their support to the Pharmaceutical Analysis Laboratory research programs.

References

[1] Th. Becue, M. Broquaire, J. Chromatogr. A557 (1995) 489–494.
[2] G. Moachon, D. Matiner, J. Chromatogr. B654 (1994) 91–96.
[3] P. Burnat, F. Robles, B. Do, J. Chromatogr. B706 (1998) 295–304.
[4] P.B. Mitchell, Psychopharmacol. Bull. 313 (1995) 509–519.
[5] J.E. Drouin, M. Broquaire, J. Chromatogr. A605 (1992) 19–31.
[6] S.H. Gorman, J. Chromatogr. A730 (1999) 1–7.
[7] S.A. Matlin, M. Elizabeth Tiritan, Q.B. Cass, D.R. Boyd, Chirality 8 (1996) 147–152.
[8] Q.B. Cass, A.L.G. Degani, M.E. Tiritan, S.A. Matlin, D.P. Curran, A. Balog, Chirality 9 (1997) 109–112.
[9] D. Perrin, D. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, 2nd, Pergamon Press, Oxford, 1980.
[10] Q.B. Cass, A.L.G. Degani, N.M. Cassiano, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 1029–1038.
[11] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical Method Develop, 2nd, Wiley and Sons, John, New York, 1997.