ELSEVIER

Journal of Chromatography A, 865 (1999) 227–233

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Direct, preparative enantioselective chromatography of propranolol hydrochloride and thioridazine hydrochloride using carbon dioxide-based mobile phases

Fiona Geiser[a,*], Martin Schultz[b], Linda Betz[b], Mohamed Shaimi[a], James Lee[a], William Champion Jr.[a]

[a]*Chiral Technologies, Inc., 730 Springdale Drive, P.O. Box 564, Exton, PA 19341, USA*
[b]*Widener University, Chemistry Department, Chester, PA 19013, USA*

Abstract

In this paper, we describe the direct, preparative enantioselective chromatography of racemic (*rac*)-propranolol hydrochloride (HCl) and *rac*-thioridazine·HCl using Chiralpak AD chiral stationary phase and mobile phase systems containing carbon dioxide and methanol without the use of basic or acidic additives. Isolated fractions of propranolol·HCl were positively identified by mass spectrometry, Beilstein flame test, melting point, and chemical analysis to be HCl enantiomers of propranolol·HCl salts exhibited characteristic mass spectra peaks at 36 and 38 mass-to-charge ratio in the expected 3:1 isotopic ratio for the solute that were absent in the mass spectra for the free-base forms. To our knowledge, the direct, preparative enantioselective isolation of HCl enantiomeric salts of *rac*-propranolol and of *rac*-thioridazine have not been previously demonstrated and published.

Keywords: Enantiomer separation; Pharmaceutical analysis; Preparative chromatography; Propranolol; Thioridazine

1. Introduction

Racemic (*rac*)-propranolol hydrochloride (HCl) (Fig. 1A) is a β-adrenergic blocking agent in which only the (*S*)-(−)-enantiomer is biologically active [1]. The direct chromatographic isolation of (*S*)-(−)-propranolol·HCl would be highly desirable as a model system for other biologically-active HCl salts. To our knowledge, direct preparative enantioselective chromatography of *rac*-propranolol·HCl has not been previously demonstrated and published. In 1991, Lee et al. resolved free bases of *rac*-propranolol, *rac*-pindolol, *rac*-metoprolol, as well as HCl salts of *rac*-betaxolol and *rac*-cicloprolol [2]. The chiral stationary phase (CSP) and mobile phase system in all cases were Chiralcel OD and CO_2–MeOH (80:20, v/v), respectively. They compared these results to resolutions obtained on the same CSP using high-performance liquid chromatography (HPLC) mobile phase systems of hexane, ethanol (EtOH) and 2-propranol. Asymmetry factors were found to improve for all solutes using CO_2 and MeOH mobile phase systems. The implication of Lee et al.'s results is that enantiomeric HCl salts of secondary amines could be directly recovered using mobile phase systems containing CO_2 and MeOH without the use of additives. Reports subsequent to Lee et al. typically included an amine additive in the CO_2 mobile phase systems to improve asymmetry factors for the separation of *rac*-propranolol. For example, Kot et al. reported that they were unable to directly resolve *rac*-propranolol free base using

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)01117-6

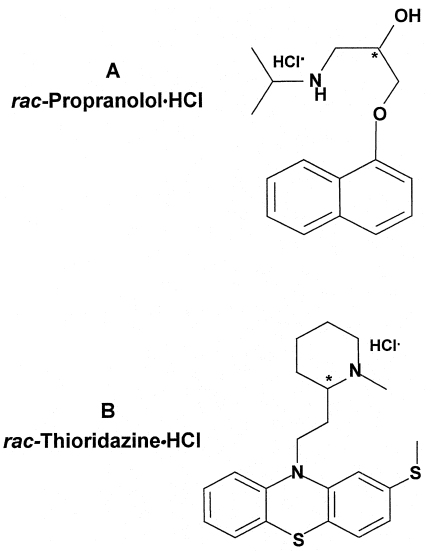

Fig. 1. Chiral center indicated by asterisk for (A) *rac*-propranolol·HCl and for (B) *rac*-thioridazine·HCl.

Chiralpak AD without the addition of diethylamine (DEA) to the mobile phase system of CO_2–MeOH (70:30, v/v) [3]. Medvedovici et al. reported enantioselective resolution of *rac*-propranolol on several CSPs by adding both triethylamine (TEA) and trifluoroacetic acid (TFA) to the CO_2 and MeOH mobile phase system [4]. Bargmann-Leyder et al. also reported the necessity of adding *n*-propylamine to CO_2 mobile phase systems in order to resolve *rac*-propranolol on ChyRoSine-A CSP [5].

We herein describe the direct, preparative enantioselective chromatography of (*S*)-(−)-propranolol·HCl using Chiralpak AD CSP with a mobile phase of CO_2–MeOH (78:22, v/v). This method was also applied to the direct, preparative enantioselective chromatography of *rac*-thioridazine·HCl (Fig. 1B) using Chiralpak AD CSP with a mobile phase of CO_2–MeOH (62:38, v/v). *rac*-Thioridazine·HCl is a piperidyl-substituted phenothiazine neuroleptic agent administered as a racemic mixture in clinical practice in which either (*R*)-(+)- or *S*-(−)-thioridazine may be associated with the intended antipsychotic effects of the administered racemate [6,7]. Thioridazine enantiomers were quantified by Jortani and Poklis [8] using Spherisorb Chiral 1 CSP and a mobile phase system of hexane–methylene chloride–MeOH–1 *M* ammonium acetate in MeOH [8]. To our knowledge, the direct, preparative enantioselective chromatography of the HCl enantiomeric salts of *rac*-thioridazine has not been previously demonstrated.

2. Experimental

2.1. Chromatography apparatus

Supercritical fluid chromatography (SFC) was performed with a modular supercritical fluid chromatograph supplied by Gilson (Middleton, WI, USA). The chromatograph was configured similarly to the supercritical fluid chromatograph described by Verillon and Coleman [9] except that 25 SC pump heads limited maximum pressure to 4000 p.s.i. (1 p.s.i.=6894.76 Pa). Briefly, the chromatographic system consisted of: a 308 pump with 25 SC pump head and thermostatic kit for cooling CO_2 to −15°C; two 306 modifier pumps with 25 SC pump heads; one 307 post-column solvent delivery pump with 10 SC for use during fraction collection. Other components consisted of an 811C dynamic mixer (1.5 ml), 821 pressure regulator, 831 temperature regulator with column switching (six columns), 7037 pressure relief valve (6-μl internal volume), 402 dilutor/syringe pump, 119 ultraviolet–visible (UV–Vis) detector with SFC flow cell, and 233 XL sampler/collector. The system was controlled by UniPoint software with a 506 C system interface. Fractions were collected using 250-ml glass solvent bottles (Gilson, No. 2954663) capped with P/E solvent bottle caps (Gilson, No. 2954668). To prevent damage to the injection needle, the bottom of the P/E solvent bottle cap was removed and replaced with a prepunctured reagent soft cap (Gilson, No. 2950824) that was secured to the collection bottle using taped aluminum foil, and then mounted in a metal rack (Gilson, No. 2954692) with securing clips. The chromatograph was placed inside a fume hood and CO_2 levels were monitored [10] with Model 8560 InspectAIR nondispersive infrared CO_2 meter supplied by TSI (St. Paul, MN, USA). Chromatographic columns were supplied by Chiral Technologies (Exton, PA, USA). Chiralpak AD is an amylose tris(3,5-dimethylphenyl carbamate) polysaccharide CSP manufactured by Daicel (Tokyo, Japan).

2.2. Solutes and eluents

Liquid CO_2 was supplied by MG Industries (98% ''bone-dry'' grade, Malvern, PA, USA) and was cooled to −15°C with a Model 1156 recirculating bath supplied by VWR Scientific (West Chester, PA, USA). HPLC-grade EtOH, MeOH, TFA and DEA were supplied by Fisher Scientific (Fair Lawn, NJ, USA). The CO_2 tank was equipped with an in-line power-to-open solenoid valve supplied by Precesion Dynamics (New Britain, CT, USA, Valve No. A2012-S98) that would close in the event of power outage. HPLC-grade hexane was supplied by Burdick and Jackson (Muskegon, MI, USA). *rac*-Propranolol·HCl was purchased from Aldrich (Milwaukee, WI, USA). *R*-(+)-Propranolol·HCl and USP-grade *rac*-thioridazine·HCl were supplied by Sigma (St. Louis, MO, USA). Free bases of *rac*-propranolol·HCl and *rac*-thioridazine·HCl were prepared by dissolving the salts in distilled water and adding an aqueous solution of saturated sodium bicarbonate followed by filtration or decantation and drying in a vacuum oven at 50°C. All ·HCl salts were dissolved in HPLC-grade MeOH at ambient temperature. Appropriate precautions were taken in handling thioridazine, which was found to be photosensitive.

2.3. Sample characterizations

Mass spectrometry was conducted with a Finnigan 4000 supplied by Finnigan (San Jose, CA, USA) in which approximately 2 μg of solid was placed into a glass capillary sample vial. The vial was then placed into the top of the direct thermal probe, which was inserted into the ion source region of the mass spectrometer. After reducing the pressure to 5×10^{-8} torr, data collection and heating of the probe to sublime the sample was initiated. Copper wire was used for the Beilstein flame test. Optical rotation was confirmed with the laser-based (650 nm) optical rotation HPLC detector supplied by PDR-Chiral (Palm Beach Gardens, FL, USA). Elemental analyses were done by Micro-Analysis (Wilmington, DE, USA). Chloride precipitation titrations [11] using silver nitrate (certified ACS grade) and 0.1% alcoholic dichlorofluorescein were supplied by Fisher Scientific (Philadelphia, PA, USA) and dextrin was supplied by Aldrich. Melting points were determined using a Mel-Temp II from Laboratory Devices (Holliston, MA, USA).

3. Results and discussion

3.1. Preparative chromatography

Results for the preparative chromatography of *rac*-propranolol·HCl and of *rac*-thioridazine·HCl are shown in Figs. 2 and 3, respectively. Chromatographic, physical and chemical characterization, and spectral results are summarized in Table 1. Isolated fractions from the enantioselective separations of both *rac*-propranolol·HCl and *rac*-thioridazine·HCl were positively identified by mass spectrometry, Beilstein flame test, and melting point to be HCl enantiomers of the respective solutes. HCl salts exhibited characteristic peaks at 36 and 38 mass-to-charge ratio in the expected 3:1 isotopic ratio for the solutes. These peaks were absent in the mass spectra for the free-base forms of both propranolol and thioridazine. In the Beilstein copper-wire flame test, the characteristic green color was present for all HCl salts, but was absent for the free bases. The presence of chloride was confirmed for propranolol enantiomers by chemical analysis.

3.2. Additive comparison for rac-thioridazine·HCl

The effects of DEA and of TFA additives on enantiomeric resolution of *rac*-thioridazine·HCl were compared using a single Chiralpak AD analytical column (25 cm×0.46 cm I.D., 20 μm CSP) with hexane and with CO_2 mobile phase systems. Mobile phase conditions and chromatographic results are reported in Table 2. The experiments using EtOH modifier without additives were investigated first, followed by EtOH modifier containing DEA additive, and then by EtOH modifier containing TFA additive. The column was flushed with a mobile phase of hexane–EtOH (60:40, v/v) for at least 30 column volumes in order to remove most of the residual DEA before evaluation of EtOH modifier containing TFA additive. *rac*-Thioridazine·HCl was resolved by use of a mobile phase of CO_2–EtOH (60:40, v/v), but not by the use of a mobile phase of

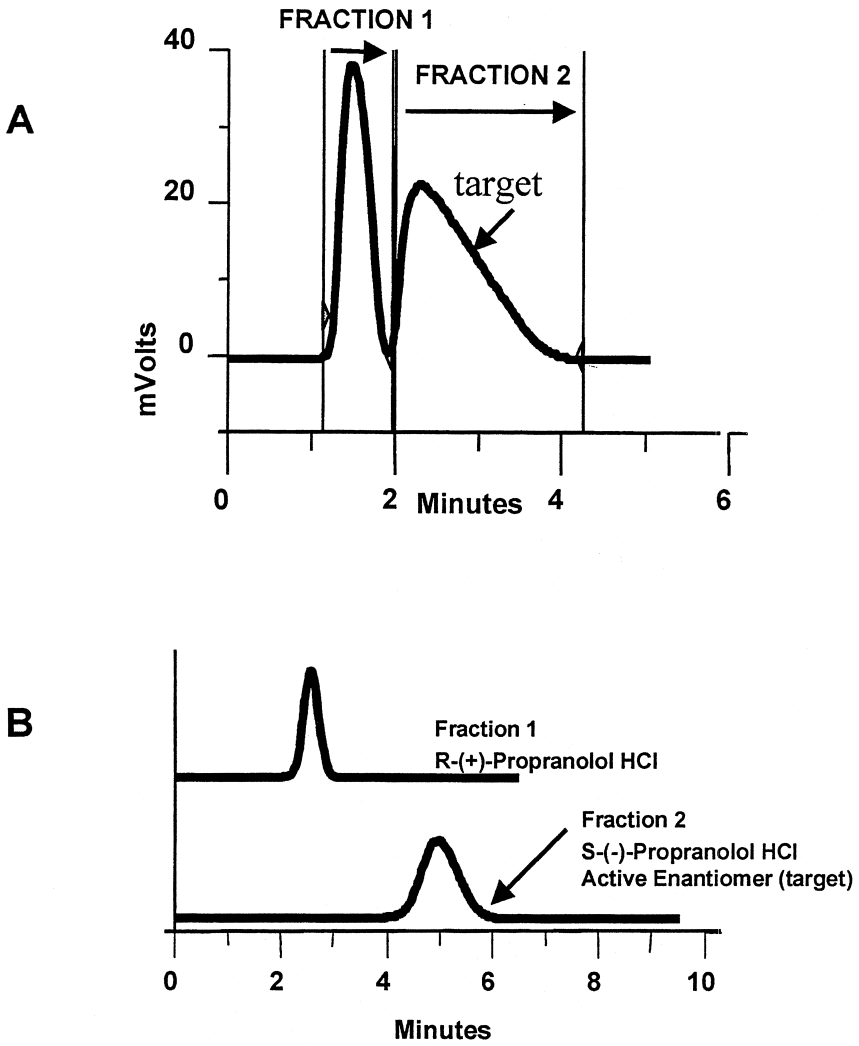

Fig. 2. (A) Preparative enantioselective separation of 40 mg of *rac*-propranolol·HCl (160 mg dissolved per 1 ml MeOH at ambient temperature, 250 μl injection) on a Chiralpak AD column (25 cm×1 cm I.D., 20 μm CSP) using CO_2–MeOH (78:22, v/v) mobile phase, 25 ml/min flow-rate (pre-column pressure of 1813 p.s.i. and post-detector pressure of 1203 p.s.i.), 40°C column temperature, UV detection at 320 nm, and post-detector make-up solvent of 100% MeOH at 0.5 ml/min. (B) Analytical separation of 0.1 mg propranolol·HCl fractions 1 and 2 (20 mg dissolved per 1 ml MeOH at ambient temperature, 5 μl) on a Chiralpak AD column (25 cm×1 cm I.D., 20 μm) using CO_2–MeOH (78:22, v/v) mobile phase, 20 ml/min flow-rate (pre-column pressure of 1522 p.s.i. and post-detector pressure of 1131 p.s.i.), 40°C column temperature, UV detection at 280 nm. Chromatographic results and chemical characterization are summarized in Table 1.

hexane–EtOH (60:40, v/v). Selectivity was suppressed using a mobile phase of CO_2–EtOH–DEA (60:39.9:0.1, v/v); whereas, selectivity was increased for mobile phase systems of CO_2–EtOH–TFA (60:39.9:0.1, v/v) and of hexane–EtOH–TFA (60:39.9:0.1, v/v).

It is interesting to note that for the mobile phase system CO_2–EtOH (60:40, v/v), selectivity in the absence of TFA additive was intermediate between that obtained with the same mobile phase in the presence of TFA. Tang et al. [12] similarly reported increased selectivity and capacity factors for the

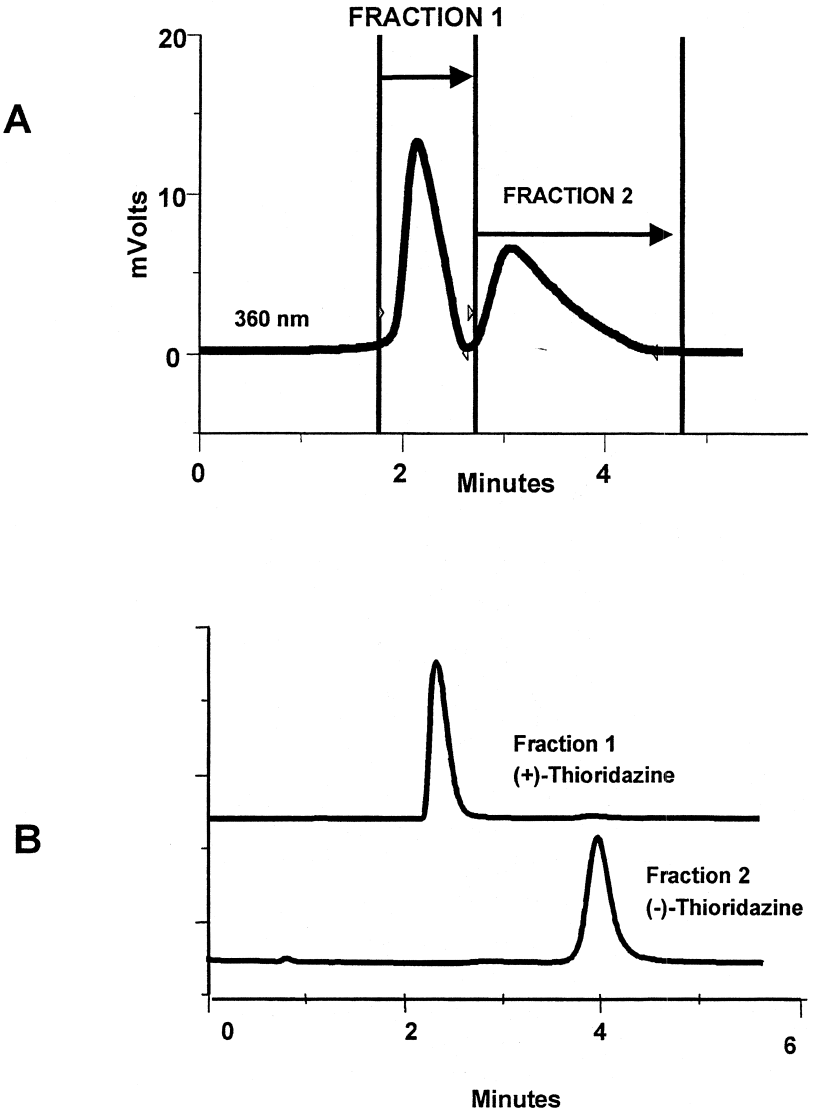

Fig. 3. (A) Preparative enantioselective separation of 32 mg of *rac*-thioridazine·HCl enantiomers (160 mg dissolved per 1 ml MeOH at ambient temperature, 200 μl injection) on a Chiralpak AD column (25 cm×1 cm I.D., 20 μm CSP) using a mobile phase of CO_2–MeOH (62:38, v/v), 20 ml/min flow-rate (pre-column pressure of 3200 p.s.i. and post-detector pressure of 1580 p.s.i.), 40°C column temperature, UV detection at 360 nm with post-detector make-up solvent of 100% MeOH at 0.5 ml/min. (B) Analytical separation of 0.1 mg thioridazine·HCl fractions (20 mg dissolved per 1 ml MeOH at ambient temperature, 5 μl injection) on a Chiralpak AD column (25 cm×0.46 cm I.D., 10 μl CSP) using CO_2–EtOH (60:40, v/v), 5 ml/min (pre-column pressure of 3200 p.s.i. and post-detector pressure of 2000 p.s.i.), 40°C column temperature, UV detection at 280. Chromatographic results and chemical characterization are summarized in Table 1.

enantioselective separation of nicotine using Chiralcel OJ CSP when TFA was added to a mobile phase of hexane–EtOH (95:5, v/v). Tang et al. attributed increased enantioselectivity to enhanced enantioselective hydrogen-bonding interactions of the protonated solutes with the CSP. Although we

Table 1
Characterization results for enantiomeric fractions (chromatographic conditions in Figs. 2 and 3 for propranolol·HCl and thioridazine·HCl, respectively), racemic·HCl salts and racemic free bases

	Propranolol	Thioridazine
Enantiomer 1		
k_1	2.3	2.8
% ee[a]	>99.5	98.5
Melting point (°C)	190–193	163–164
Beilstein flame test	+ (green flame present)	+ (green flame present)
Mass spectrum	36/38 ratio, 3:1 intensity	36/38 ratio, 3:1 intensity
% Chloride	12.5 (Cl titration)	–
Optical rotation	Dextrorotatory	Dextrorotatory
Enantiomer 2		
k_2	5.6	5.6
% ee	99.2	98.2
Melting point (°C)	190–193	163–165
Beilstein flame test	+ (green flame present)	+ (green flame present)
Mass spectrum	36/38 ratio, 3:1 intensity	36/38 ratio, 3:1 intensity
% Chloride	11.7 (elemental analysis)	–
Optical rotation	Levorotatory	Levorotatory
Racemic·HCl mixture		
Melting point (°C)	163–164	162–164
Beilstein flame test	+ (green flame present)	+ (green flame present)
Mass spectrum	–	36/38 ratio, 3:1 intensity
% Chloride	11.8 (Cl titration)	–
Optical rotation	–	–
Racemic free base form		
Melting point (°C)	93–94	Waxy solid
Beilstein flame test	− (green flame absent)	− (green flame absent)
Mass spectrum	36/38 masses absent	36/38 masses absent

[a] ee=Enantiomeric excess.

Table 2
Comparison of the effects of DEA and of TFA additives on the enantiomeric resolution of *rac*-thioridazine·HCl using Chiralpak AD column (25 cm×0.46 cm I.D., 20 μm CSP)[a]

Mobile phase systems	k_1	k_2	α
CO_2–EtOH (60:40, v/v)	4	8.3	2
CO_2–EtOH–DEA (60:39.9:0.1, v/v)	4	5	1.25
CO_2–EtOH–TFA (60:39.9:0.1, v/v)	4	15.6	3.9
Hexane–EtOH (60:40, v/v)	1	1	1
Hexane–EtOH–DEA (60:39.9:0.1, v/v)	1	1	1
Hexane–EtOH–TFA (60:39.9:0.1, v/v)	1	4	4

[a] Chromatographic conditions for all separations were: mobile phase flow-rate of 10 ml/min, ultraviolet detection at 280 nm, column temperature of 40°C, sample concentration of 20 mg/ml in a solution of EtOH–MeOH (50:50, v/v), 20 μl injection.

speculate that protonated-amine species may be forming in the alcoholic CO_2-containing mobile phase system, mechanistic studies are in progress.

4. Conclusions

These results demonstrate the preparative, enantioselective separation and recovery of HCl salts for *rac*-propranolol and for *rac*-thioridazine using Chiralpak AD with mobile phase systems containing CO_2 and MeOH without the use of basic or acidic additives. Solute requirements are that HCl salts should exhibit high solubility and stability in the modifier (i.e., >50 mg/ml). *rac*-Thioridazine·HCl and *rac*-propranolol·HCl were found to exhibit high solubility in MeOH. We anticipate that the use of

mobile phase systems containing CO_2 would be highly useful for both chiral and achiral manufacturing processes for the direct isolation of HCl salts without the use of reactive acidic or basic additives.

Acknowledgements

Appreciation is expressed for the technical assistance of Scott Van Bramer, Mark Timken and Elizabeth Thornton of Widener University Department of Chemistry and of Todd Anderson and Joan Stevens of Gilson Co. We also gratefully acknowledge helpful technical discussions with John Blackwell and Mary Ellen McNally of DuPont Co., with Thomas Chester of Proctor and Gamble Co., and with Geoff Cox of Chiral Technologies – Europe.

References

[1] R. Howe, R.G. Shanks, Nature 210 (1966) 1336.
[2] C.R. Lee, J.-P. Porziemsky, M.-C. Aubert, A.M. Krstulovic, J. Chromatogr. 539 (1991) 55.
[3] A. Kot, P. Sandra, A. Venema, J. Chromatogr. Sci. 32 (1994) 439.
[4] A. Medvedovici, P. Sandra, L. Toribio, F. David, J. Chromatogr. A 785 (1997) 159.
[5] N. Bargmann-Leyder, C. Sella, D. Bauer, A. Tambute, M. Caude, Anal. Chem. 67 (1995) 952.
[6] C.N. Svendsson, M. Froimowitz, C. Hrbek, A. Campbell, N. Kula, R.J. Baldessarini, B.M. Cohen, S. Babb, M.H. Teicher, E.D. Bird, Neuropharmacology 27 (1988) 1117.
[7] C.B. Eap et al., Clin. Pharm. Ther. 59 (1996) 322.
[8] S.A. Jortani, A. Poklis, J. Anal. Toxicol. 17 (1993) 374.
[9] F. Verillon, K. Coleman, in: K. Anton, G. Berger (Eds.), Supercritical Fluid Chromatography with Packed Column, Marcel Dekker, New York, 1998, p. 59.
[10] Carbon Dioxide(CO(2) Asphixation Hazard When Filling Stationary Low Pressure CO(2) Supply System, OSHA Hazardous Information Bulletin, 5 June 1996. http://www.osha-slc.gov/OshDoc/HIB_data/HIB19960605.html
[11] D.A. Skoog, D.M. West, F. Holler, in: Fundamental of Analytical Chemistry, 7th Edition, Harcourt Brace College Publishers, 1996, p. 828.
[12] Y. Tang, W.L. Zielinski, H.M. Bigott, Chirality 10 (1998) 364.