Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Enantioselective Supercritical Fluid Extraction from Racemic Mixtures by Use of Chiral Selectors

Roberto Bauza[ab]; Angel Ríos[ac]; Miguel Valcárcel[a]
[a] Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Córdoba, Spain [b] Department of Chemistry, Faculty of Sciences, Universidad del Zulia (LUZ), Venezuela [c] Department of Analytical Chemistry and Food Technology, University of Castilla, La Mancha, Spain

Online publication date: 08 July 2010

To cite this Article Bauza, Roberto , Ríos, Angel and Valcárcel, Miguel(2005) 'Enantioselective Supercritical Fluid Extraction from Racemic Mixtures by Use of Chiral Selectors', Separation Science and Technology, 39: 2, 459 — 478
To link to this Article: DOI: 10.1081/SS-120027569
URL: http://dx.doi.org/10.1081/SS-120027569

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 39, No. 2, pp. 459–478, 2004

Enantioselective Supercritical Fluid Extraction from Racemic Mixtures by Use of Chiral Selectors

Roberto Bauza,[#] Angel Ríos,[‡] and Miguel Valcárcel[*]

Department of Analytical Chemistry, Faculty of Sciences,
University of Córdoba, Córdoba, Spain

ABSTRACT

A method for the selective extraction of camphorsulfonic, phenylpropionic, and mandelic acid enantiomers from racemic mixtures is proposed. The method uses supercritical fluid extraction (SFE) and in situ chiral precipitation by the addition of specific alkaloids. The SFE extracts obtained were analyzed by high performance liquid chromatography (HPLC) with indirect UV detection. Two different ways of introducing the reagents into the extraction cell were compared. One way was the conventional involving spiking onto diatomaceous earth; in the other,

[#]Current address: Department of Chemistry, Faculty of Sciences, Universidad del Zulia (LUZ), P.O. Box 526, Venezuela.
[‡]Current address: Department of Analytical Chemistry and Food Technology, University of Castilla, La Mancha, Spain.
[*]Correspondence: Miguel Valcárcel, Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Campus de Rabanales, Edificio Anexo 3C-3, E-14071 Córdoba, Spain; Fax: 34-957-218616; E-mail: qa1meobj@uco.es.

DOI: 10.1081/SS-120027569 0149-6395 (Print); 1520-5754 (Online)

reagents were dissolved in the modified-CO_2 fluid for pumping and trapping in the extraction cell, using different programmed cycle times. The latter procedure was found to provide much better precision than the former. In some cases, one enantiomer was made more readily soluble in CO_2 and was extracted with it, while the other, insoluble enantiomer, remained in the extraction cell. Racemic acid mixtures supported on diatomaceous earth as inert support were selectively extracted with high recoveries for the enantiomers of interest (viz. 98% and 2% for *R*- and *S*-10-camphorsulfonic acid, respectively, with strychnine; 1% and 99% for *R*- and *S*-phenylpropionic acid, respectively, with brucine; and 98% and 2% for *R*- and *S*-mandelic acid, respectively, with quinine). The proposed method enables enantioselective extraction by in situ SFE as a preparation and analysis technique for the production of enantiomers in a high optical purity.

Key Words: Supercritical fluid extraction; Racemic acids; Drug design; Chiral selectors.

INTRODUCTION

Optical resolution via diastereomeric salt formation is very useful for the production of optically active chiral compounds on both the laboratory scale and the industrial scale.[1,2] The increasing importance assigned to chirality in drug development is shifting the pharmaceutical industry from the "racemate" vs. enantiomers debate to new prospects arising from fundamental insights into molecular recognition and the exploitation of these new levels of understanding in drug design.[3–5] One important use of chemical chiral precipitation is the obtainment of chiral products with adequate enantiomeric purity for use on the preparative scale.[1]

Molecular chiral recognition is used mainly to produce and isolate enantiomers of the desired purity level[6,7] or to determine the enantiomeric purity of samples. Chromatographic techniques are highly valuable with a view to reaching these goals and have developed rapidly in this context over the last few years, especially in response to the need for new biomedical applications. The resolution of enantiomers as diastereomeric derivatives by HPLC[8–10] or gas chromatography,[11,12] or with chiral stationary phases[13–15] is currently quite common-place.

The advent of supercritical fluid chromatography (SFC) brought about a powerful tool for chiral separations.[16] While the analytical chiral applications of SFC have grown in recent years, its sister technique, supercritical fluid extraction (SFE), has rarely proved capable of providing reliable chiral discrimination during the extraction. Simándi et al. obtained better resolution

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

of racemic acids in 25 different binary mixtures by using various resolving agents with supercritical CO_2 instead of conventional solvents.[17] They also studied various solvents in the supercritical state with a view to assessing their influence on molecular chiral recognition[18] and found that differences between diastereomers in the supercritical fluid state were very large compared to traditional solvents. Recently, Kordikoswki et al. studied the variables with a direct influence on crystal formation in diastereomeric salts by using SFE technology.[19] The results revealed a direct influence of pressure and temperature on the formation of insoluble diastereomeric salts. In previous work, we also showed that enantiomeric acids can be selectively extracted by SFE from a neutral adsorbent support using an *R*-(+)-chiral base.[20] Coupling derivatization reactions with SFE incorporates the advantages of this technique (e.g., a lower viscosity and higher diffusion rate relative to liquid extraction, all of which, and results in the fluid-exhibiting gaslike, transfer properties).[21–25] The fluid most commonly used in SFE is supercritical CO_2, which is fairly inexpensive, nontoxic, and nonflammable; also, its supercritical state (31°C, 73.4 bar) is easy to reach. Moreover, as laboratories increasingly cut down on organic solvents, alternatives such as sc-CO_2 are gaining appeal.[26]

This article reports a selective method for the supercritical fluid extraction of enantiomers from racemic mixtures using alkaloids as chiral selectors. In situ reactions are used to form diastereomeric salts with differential extractability in supercritical CO_2, which allows the subsequent extraction of the pure enantiomers. An interesting approach to the insertion of reagents into the pressurized extraction cell by using the modifier-CO_2 stream is also reported. The proposed method allows toxic solvents to be replaced with green analytical procedures involving CO_2 technology, which is more environmentally benign.[26]

EXPERIMENTAL

Apparatus

The SFE system is a Hewlett-Packard 7680-T (Wilmington, USA) supercritical fluid extractor furnished with a 7-mL extraction cell. A Hewlett-Packard quaternary HPLC pump (model 1050A, Woldbronn, Germany) was used to insert the modifier and reagents into the CO_2 line. Chromatographic analysis of the extracts was performed on a Hewlett-Packard HPLC system consisting of a quaternary HPLC pump (model 1050A), a diode array detector (model 1756, Woldbronn, Germany), and a Rheodyne injection valve (model 7725i, sample loop 20 μL, COTAH, CA).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Reagents and Materials

R-(−)- and *S*-(+)-mandelic acid, *R*-(−)- and *S*-(+)-2-phenylpropionic acid, 1*R*-(−)-10-camphorsulfonic acid, and 1*S*-(+)-10-camphorsulfonic acid were used for the enantiomeric extraction studies. On the other hand, quinine, (−)-cinchonidine, quinidine, (+)-cinchonine, brucine (dried at 100°C for 1 h prior to use), and (−)-strychnine were tested as selective extraction reagents. Diatomaceous earth (Sigma) was used as support. SFE/N38-grade CO_2 supplied by Air Liquide in a cylinder with a dip-tube was used as the extraction fluid. HPLC-grade acetonitrile and methanol (Panreac) were also used as solvent. Preliminary HPLC analyses were conducted with a view to determining the enantiomeric purity of all the chiral compounds used in this work.

SFE Recovery Test of Alkaloids and Racemic Acid Mixtures

Each racemic acid compound (8 mg) was dissolved separately in 2 mL acetone/methanol (1 : 1) at 25°C. An identical amount of each chiral base was also dissolved separately in (1 : 1) acetone/methanol. The extraction cell was prepared as follows: 500 μL of the previous solutions was spiked individually onto 1.2 g of diatomaceous earth as inert support (which filled up the dead volume of the cell). After drying, the reaction cell was kept open for 20 min and then sealed and extracted its contents with supercritical CO_2 modified with 0, 5, 15, 20, or 25% (v/v). Variable extraction temperature (40, 50, or 60°C) pressures (and hence densities) and extraction times were used to determine their effects on the formation and recoveries of diastereomeric salts. Three solid traps consisting of C18, Porapak, and stainless steel beads were tested as trapping material; the trap temperature was kept at 70°C when methanol was used as the modifier and at 45°C when pure CO_2 was used for this purpose. The HPLC mobile phase, acetonitrile or methanol was used as rinse solvent. Quantitative measurements of the analytes were obtained from the peak areas provided by an HP 3396 series integrator. External calibrations were done with higher purity standards of the enantiomers of each racemic acid studied.

Formation of Diastereomeric Salts and Simultaneous Extractions

The feasibility of obtaining the diastereomers within the extractor was studied by examining two different manners of introducing the reactants into the extraction cell, viz. by spiking and in the dynamic mode.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Spiking Procedure

Volumes of 100 to 500 μL of 4 mg mL^{-1} racemic mixture in 1 : 1 methanol/acetone were spiked onto 1.2 g of diatomaceous earth in the extraction cell, followed by 200 to 600 μL of 4 mg mL^{-1} chiral base in 1 : 1 methanol/acetone. The extraction cell was kept open for 45 min to evaporate the solvent. Then, the cell was sealed and the extraction run under different SFE conditions (temperature, pressure, equilibration time, and extraction time). The trapping conditions were the same as those described in the previous section. An aliquot (20 μL) of the SFE extract thus obtained was acidified with 10 μL of 0.1-M HCl and the resulting solution diluted with 1 mL of the HPLC mobile phase.

Dynamic Procedure

This procedure involved inserting the reagents (racemic acids and chiral base) into the extraction cell by the quaternary pump in a series of dosing steps programmed by the software of the pump. Chiral bases and enantiomers were individually dissolved in methanol and premixed in the modifier pump head at 50 : 50 (v/v) before pumping into the CO_2 stream. To ensure that the compound was efficiently trapped on the support under these condition, the flow rate of the modifier (10 to 300 μL min^{-1}) was also carefully adjusted. Breakthrough measurements were made by pumping a dilute solution of the racemic acids or chiral bases (concentration range 10 to 500 μg mL^{-1} for each solution) through the extraction cell containing 1.2 g of diatomaceous earth mixed with two cellulose strips and evaluating the response of the extracts as a function of the concentration delivered by the pump. Once the reagents were placed on the diatomaceous earth, different time steps in the static and dynamic mode were tested. The SFE system was operated in such a way that the modifier pump was started immediately after the extractor to establish the extraction conditions. The products were collected in a solid trap after depressurization. HPLC analyses were performed as just described and the diastereomeric salts formed identified by comparison of their HPLC retention times with those of a appropriate standards.

HPLC Determination

A previous HPLC method, in a slightly modified form, was used to asses the reaction efficiently.[8,9] Modifications affected flow rate and concentration values mainly. This method is based on indirect detection. A Lichrosorb Diol 5 μm column (Merck) was used that was kept at room temperature

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(26 ± 1°C). The mobile phase used was a solution of quinidine acetate salt (3×10^{-4} M) in a 98 : 2 dichloromethane-pentanol mixture as mobile phase. The injected volume was 20 μL and the flow rate 1.0 mL min^{-1}.

RESULTS AND DISCUSSION

Figure 1 shows the chemical structures of the racemic acids and chiral bases studied. These racemic acids and chiral bases were chosen as modes on the grounds of their significance to the pharmaceutical industry, which uses them as effective resolving agents in classic precipitation methods. Thus, 10-camphorsulfonic acid has found large-scale industrial use in the manufacturing of d-phenylglycine, and dextropropoxyphene (an industrial intermediate in the manufacturing of ascorbic acid).[1] Mandelic acid is used as a resolving agent for chiral cyanohydrins (potential sources of commercially interesting optically pure compounds).[27] Finally, the alkaloids are also widely used as

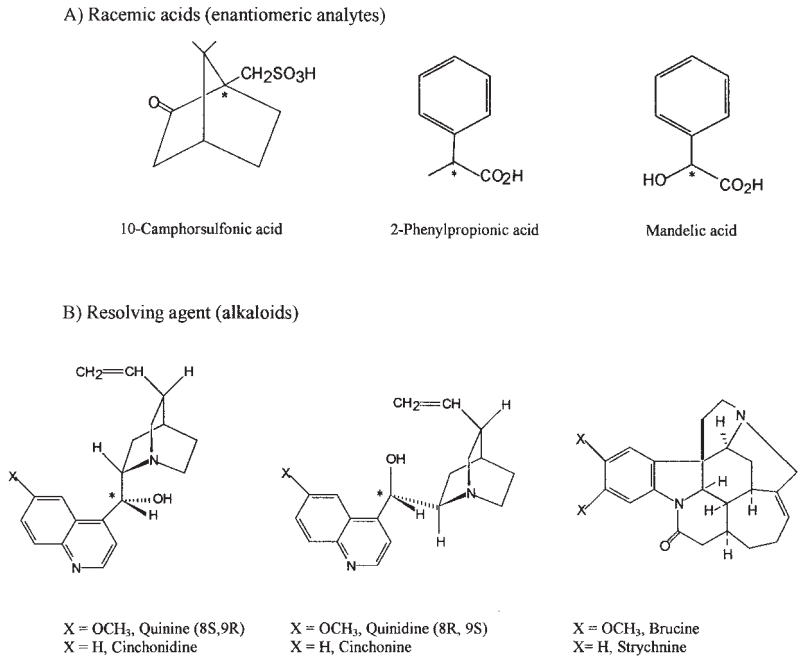

Figure 1. Molecular structures of (A) racemic acid and (B) resolving agent studied in this work.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

resolving agents and have the advantage that they are relatively inexpensive; they are available as natural products in a high degree of chiral purity. Figure 2 depicts the two basic steps involved in the process. In the first step, the diastereomeric salts are formed and extracted entirely in the SF extractor. In the second, they are determined by HPLC. To maximize extractability in supercritical CO_2 during the reaction, recovery test on the individual racemic acids and chiral bases were preliminary conducted.

Recoveries of Alkaloids and Racemic Mixtures

The primary factors influencing solubility (viz. density and temperature) were examined by spiking 500 μL aliquots of the racemic and chiral base solutions, respectively, onto 1.2 g of diatomaceous earth. The results were used to construct a series of isotherms at three working temperatures (40, 50, and 60°C) for both racemic acids and chiral bases. Figure 3 shows the results for the racemic acids, which were nearly always quantitatively recovered with pure CO_2 (or at least after extraction with CO_2/methanol). Figure 3(a) shows the recoveries for 2-phenylpropionic acid over the range densities studied, at three different temperature as can be seen, extraction of this acid was quantitative. 2-Phenylpropionic acid was rendered soluble after 35 min of extraction at a density of 0.65 g mL^{-1} and a temperature of 60°C, which facilitated its extraction. No modifier or static time were required for this acid. A density of 0.65 g mL^{-1} and an extraction temperature of 60°C were thus adopted for further tests with 2-phenylpropionic acid. Figure 3(b) shows the recovery obtained for mandelic acid under the same conditions as for 2-phenylpropionic acid except that methanol was added to the supercritical CO_2 and a static time of 20 min was needed on account of its moderate polarity. The mandelic acid recoveries shown in Fig. 3(b) were obtained with 15% (v/v) methanol in the supercritical CO_2, which was found to be the optimum content of the three tested (viz. 10, 15, and 20%). Using higher modifier contents resulted in no appreciable differences. A high supercritical density (0.8 g mL^{-1}) was required to ensure quantitative extraction mandelic acid. Significant differences were observed at the three temperature levels studied. Finally a temperature of 60°C was selected. Figure 3(c) shows the recovery for 10-camphorsulfonic acid. The extractability of this acid was similar to that of mandelic acid. The addition of 15% (v/v) methanol to the supercritical CO_2 and the inclusion of a static time of 15 min at 60°C were required to maximize recovery. Figure 3(c) was obtained with that methanol content. A density above 0.75 g mL^{-1} and an extraction temperature of 60°C were adopted for further testing with this acid. Similar recovery tests were conducted on the chiral bases. These bases are typically used as resolving agents via chiral

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

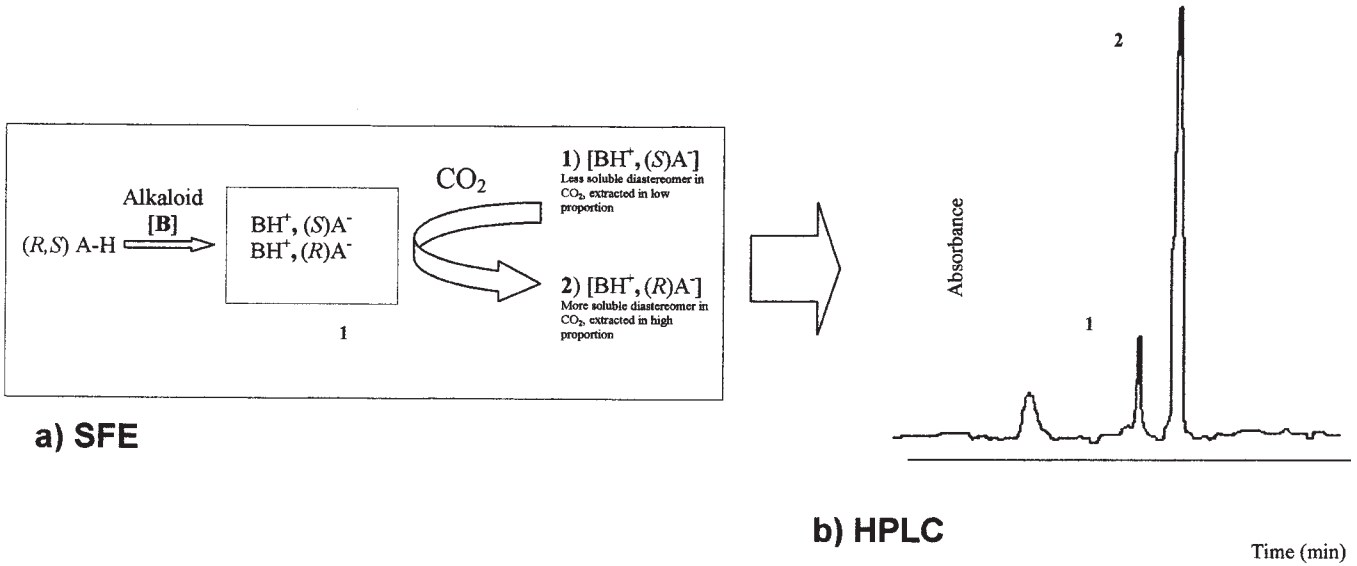

Figure 2. Schematic diagram of basic principles involved in this work, illustrating a hypothetical example, (a) SFE process assuming the higher solubility of *R*-enantiomer, (as diastereomeric salt) in CO_2 and (b) separation and detection of enantiomers extracted in part (a).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

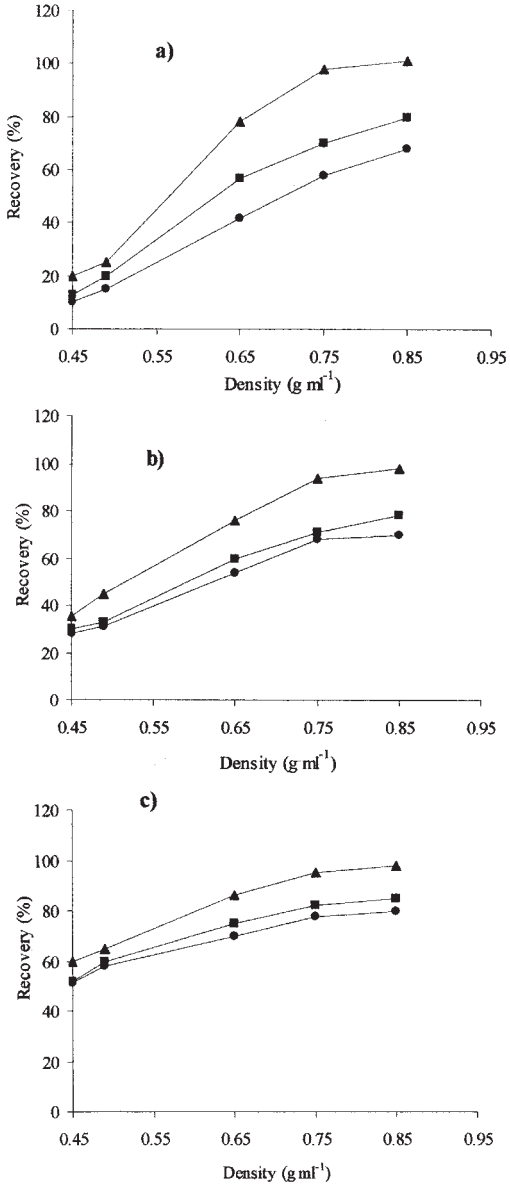

Figure 3. Recoveries for: (a) 2-phenylpropionic acid (500 μL of 4000 mg L^{-1} in acetone, evaporated before SEE; 1 mL min^{-1} pure CO_2; extraction time 35 min; C18 trap at 40°C; rinse with acetonitrile); (b) mandelic acid (the same previous conditions, except: static time 20 min and 15% (v/v) methanol in CO_2, Porapak trap); and (c) 10-camphorsulfonic at the same conditions as (b). (▲) 60°C; (■) 50°C; (•) 40°C; $n = 6$.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

precipitation.[2] Based on their molecular structures (Fig. 1), quinine and quinidine bear a diastereomeric relationship, and so do cinchonidine and cinchonine pair. It is interesting to note that the results for the bases exposed an apparent relationship between structure and extractability. As can be seen from Fig. 4, quinidine and quinine exhibited tended to be more efficiently extracted in pure supercritical CO_2 than did cinchonidine and cinchonine. Cinchonidine and cinchonine can be quantitatively extracted above a density of 0.75 g mL^{-1} at 60°C. A similar recovery-density behavior of cinchonidine and cinchonine was also observed for brucine and strychnine, which differ only in the methoxyl group present in brucine. In general quantitative recovery for this pair can also be accomplished above 0.75 g mL^{-1} at 60°C.

The good recoveries achieved for these substrates reflect their good solubility and ensure a homogeneous medium for the precipitation reaction in the extraction cell. The following section discusses the feasibility of the reactions used to obtain the diastereomeric salts.

Reaction for Obtaining Diastereomeric Salts in Supercritical CO_2

Systematic in situ reaction tests were carried out with a view to exposing the effect of (a) the reaction cell geometry; (b) the reagent and substrate addition mode; and (c) the presence of modifiers. Finally, the in situ reaction with simultaneous extraction of the products with CO_2 was also examined.

Effect of the Spiking Procedure and Reaction Cell Geometry on the In situ SFE Reaction

A stainless steel reaction cell of 10 cm × 10 mm i.d. (7 mL) was first tested. Preliminary results confirmed the selective extraction of some enantiomers, consistent with the HPLC analyses of the extracts. Based on these results, the following conclusions can be drawn: (a) in general, the extraction of *R*- and *S*-enantiomers was scarcely selective for the three racemic acids; (b) no significant differences (paired *t* test, $n = 10$, $P = .05$) between the data obtained with the cell open and sealed were observed; and (c) the repeatability was always poor (23 to 35% RSD, $n = 10$). The spike was mixed in slurry form with the support, which, however, did not improve repeatability. This prompted us to decrease the cell volume to reduce dispersion of the sample and increase contact among the reagents. To confirm this hypothesis, tests similar to that just described were performed, but using two reaction cells of different dimensions (6.7 cm × 0.5 cm i.d. and 2 cm × 0.5 cm i.d.). The cells, made of stainless steel, were accommodated

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

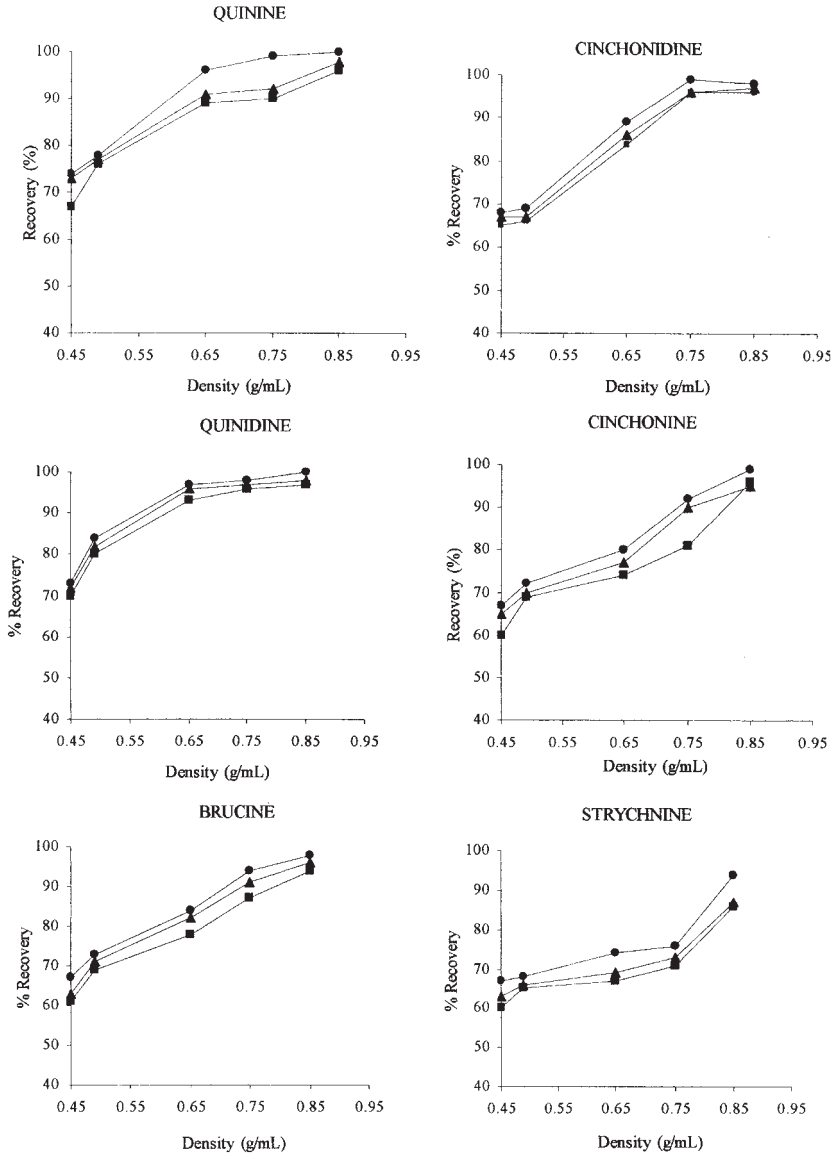

Figure 4. Effect of temperature and density of supercritical CO_2 on the SFE recovery of chiral bases from diatomaceous earth. (●) 60°C; (▲) 50°C; (■) 40°C; $n = 6$.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

within the original 7-mL cell. A substantial improvement in repeatability (5.45 to 8.23% RSD, $n = 10$) was thus obtained. However, the main goal (i.e., selective extraction of one enantiomer in each pair) was still inefficient and recoveries were low (29%, $n = 10$). These findings led us to optimize the insertion of reagents and substrates with a view to improving mixing of the reactants in the SFE system. To overcome the repeatability problem, dynamic incorporation of the reagent into the CO_2 stream was tested.

Effect of the Addition of Reagents and Substrates to the CO_2

Inserting the reagents through a pump and using a modifier solvent in the CO_2 allows one to develop interesting processes in the flow stream for various analytical purposes. In this particular case, reagents were trapped in the support (similar to those used in SPE), which was accommodated in the reactor cell and, simultaneously, polarity modifiers were introduced into the reaction medium. For this purpose, various procedures were tested that provided the results of Table 1. Note that each extraction mode had some associated time steps. The first of two dynamic events allows the sequential

Table 1. Steps of modifier-pump to systematically introduce reagents into the extraction cell through of the CO_2 stream (dynamic procedure).[a]

Step	Extraction mode	Percentage of methanol in CO_2 stream (v/v)	Flow-rate of modifier ($\mu L\,min^{-1}$)[b]	Modifier methanol composition	Step time (min)
1	Dynamic	5	25[c]	50% (v/v) of 300 $\mu g\,mL^{-1}$ racemic acid; 50% of 400 $\mu g\,mL^{-1}$ chiral base	10
2	Dynamic	5	25[c]	100%	0.8
3	Equilibration	5	25[c]	—	5–15
4	Dynamic	15	150[d]	100%	10–40

[a]Extraction cell was packed with 1.2 g diatomaceous earth mixed with two cellulose trips (details in text).
[b]Flow-rate calculated by the software based on percentage of methanol and the flow-rate of CO_2 ($d = 0.5\,g\,mL^{-1}$, $T = 35^{\circ}C$) taken as liquid in the head pump.
[c]Based on 5% of modifier and flow-rate CO_2 of $0.5\,mL\,min^{-1}$.
[d]Based on 15% of modifier and flow-rate CO_2 of $1.0\,mL\,min^{-1}$.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

insertion of the chiral base and racemic acid, both through the quaternary HPLC pump as programmed by a table of events. To increase its trapping efficiency in the extraction cell and avoid early breakthrough as a result, the support was placed on two cellulose strips mixed with diatomaceous earth. The cellulose support allowed a stronger retention of the analytes in comparison to the direct use of diatomaceous earth as support. In this way, higher efficiencies of the reactions taken place within the thimble were achieved. But, on the other hand, 1.2 g of diatomaceous earth was used in each experiment to reduce the dead volume in the thimble and, hence, better precision was reached for the final recoveries. Therefore, two cellulose strips previously spiked with the sample were inserted into the diatomaceous earth located in the extraction thimble. A delay time of 0.5 min with methanol was used between steps to flush the line. Each step was carefully examined by analyzing the extracts until the optimum breakthrough was reached (i.e., until no signal detected). Equilibration and dynamic times were optimized for each case (no more than 15 min of static mode or 40 min of dynamic mode were required in any case).

Methanol solutions of the enantiomers ($300\,\mu g\,mL^{-1}$) and chiral bases ($400\,\mu g\,mL^{-1}$) were mixed as individual pairs (enantiomer-chiral base) in a 50 : 50 (v/v) ratio at the modifier pump head. Using a flow rate of modifier methanol of $25\,\mu L\,min^{-1}$ was found to be indispensable to avoid cell breakthrough. The pump was programmed to deliver 5% of a methanol–CO_2 mixture at $0.5\,mL\,min^{-1}$ to obtain the previous flow rate ($25\,\mu L\,min^{-1}$).

Preliminary results of the dynamic procedure for the reagents revealed substantially improved repeatability and reactivity. This can be ascribed to the improved homogeneity in the mixing of the reagents involved in the reaction. The density of the supercritical fluid was also found to have a strong effect on the extraction selectivity, so further tests were conducted with a view to examining its effect on the racemic resolution.

In Situ Reaction Between Chiral Bases and Racemic Acids

Figure 5 shows the influence of the supercritical CO_2 density on the resolution for each enantiomer recovered. The effect of the SFE conditions was examined and an extraction temperature of 60°C was found to allow all compounds to be efficiently extracted and the optimum static/dynamic time ratio (s/d) was found to be 15/30 for 10-camphorsulfonic acid, 20/40 for mandelic acid, and 20/40 for 2-phenylpropionic acid. The extraction selectivity thus achieved was good with almost all chiral bases. The final results are shown in Table 2. The best resolving agent for the selective extraction of 10-camphorsulfonic acid was strychnine, which provided a recovery of 98% for the *R* enantiomer and 2% for the *S* enantiomer. On the other hand, brucine

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

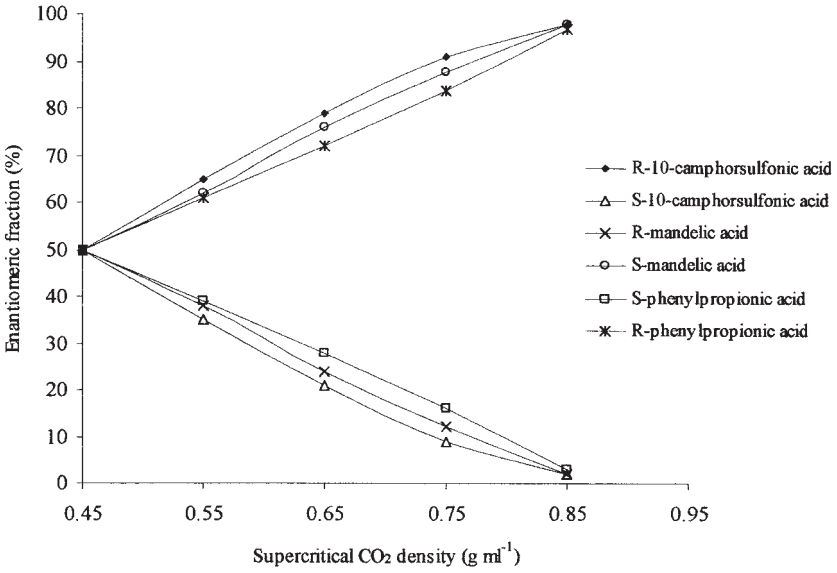

Figure 5. Influence of supercritical CO_2 density on the enantiomeric fraction obtained for each racemic compound (T = 400°C); 500 μL of 2000 mg L^{-1} of each enantiomeric mixture. Chiral bases used according to Table 2.

was the best resolving agent for racemic 2-phenylpropionic acid, with recovery of 1% for the *R* enantiomer and 99% for the *S* enantiomers. Finally, quinine was the best agent for racemic mandelic acid with an extraction selectivity of 98% for the *R* enantiomer and 2% for the *S* enantiomer. The underlying mechanism of the selective extraction remains somewhat unclear, however, density is known to play a crucial role in the formation of crystals of the salt and hence in its solubility. Kordikowski et al. found the resolution of racemic ephedrine to increase with increasing density up to 0.95 g mL^{-1} (ca. 383 bar)[19] and concluded that the large difference in enthalpy of fusion (ΔH_f) was the main source of resolution. These investigators found the melting point (T_m) for diastereomeric salts to differ by more than 50°C and ΔH_f by 20 kJ mol^{-1}. Fogassy et al. drew similar conclusions on the influence of the supercritical CO_2 density on the resolution of racemic acids mixtures in a CO_2 medium.[18] More hydrophobic structures may form when the salt is obtained within the extraction cell. It may, in principle, act as neutral molecules (ion pairs). Further studies are required in this respect with a view to understanding the mechanism through which the diastereomer forms in the supercritical fluid.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 2. Results of optimization study carried out for the selective extraction for racemic acids by in situ reaction-SFE with resolving agents.

Racemic acid compounds	Resolving agent	Total recovery of racemic acid (%)	Enantiomeric fraction (%) ($R:S$)
10-Camphorsulfonic	Quinine	94	43 : 57
	Cinchonidine	90	49 : 51
	Quinidine	93	73 : 27
	Cinchonine	91	70 : 30
	Brucine	89	91 : 9
	Strychnine	99	98 : 2
2-Phenylpropionic	Quinine	98	41 : 59
	Cinchonidine	100	44 : 56
	Quinidine	97	50 : 50
	Cinchonine	99	51 : 49
	Brucine	100	1 : 99
	Strychnine	97	53 : 47
Mandelic	Quinine	100	98 : 2
	Cinchonidine	83	43 : 57
	Quinidine	81	50 : 50
	Cinchonine	82	49 : 51
	Brucine	85	60 : 40
	Strychnine	85	49 : 51

Applications of the Proposed Approach to the Analysis of Synthetic Racemic Mixtures

The previous procedures were validated by using samples containing variable enantiomer proportions (from 80 to 20% of each enantiomer) and analyzing them under the previously established optimum SFE conditions. As can be seen from Table 3, SFE can be used to effectively increase or decrease the proportion of one enantiomer in a racemic mixture. For example, SFE combined with in situ reaction enriched a 80 : 20 R/S mixture of 10-camphorsulphonic acid to 95 : 5 R/S.

Analytically, it is interesting to establish the experimental conditions under which one enantiomer can be determined in the presence of the other with the proposed methodology. For this purpose, the variation of the error in the determination of *R*-10-camphorsulfonic, *S*-2-phenylpropionic, and *R*-mandelic acids was studied as a function of the enantiomer proportion in the sample. Figure 6 shows the corresponding graph for each enantiomeric analyte. The double scale on the x axis including both the *R*- and the *S*-enantiomer

Table 3. Analysis of synthetic samples of 10-camphorsulfonic, 2-phenylpropionic, and mandelic acids with different enantiomeric proportion by the proposed method.

Racemic acid compounds	Initial enantiomeric composition added in the sample (%) (*R* : *S*)	Final enantiomeric composition after the SFE extraction (%) (*R* : *S*)	Enantiomer mainly extracted
10-Camphorsulfonic[a]	80 : 20	95 : 5	*R*-
	75 : 25	94 : 6	
	50 : 50	92 : 8	
	25 : 75	89 : 11	
	20 : 80	81 : 19	
2-Phenylpropionic[b]	80 : 20	11 : 89	*S*-
	75 : 25	7 : 93	
	50 : 50	1 : 99	
	25 : 75	4 : 96	
	20 : 80	4 : 96	
Mandelic[c]	80 : 20	97 : 3	*R*-
	75 : 25	96 : 4	
	50 : 50	98 : 2	
	25 : 75	89 : 11	
	20 : 80	83 : 17	

[a]With strychnine as chiral base.
[b]With brucine as chiral base.
[c]With quinine as chiral base.

composition in the sample. As can be seen, all enantiomeric analytes behave similarly. The errors obtained in the determination of the low concentrations of the *R*-enantiomers (viz. 10-camphorsulfonic and mandelic acid) were very high but decreased dramatically as the proportion of the *R*-enantiomer increased. One can assume (as suggested by the experiments with the pure enantiomers) that the errors will tend to zero as the proportion of *R*-enantiomer tends to 100%. The dashed lines in Fig. 6 show assumed error trends. The results of the determination of *S*-2-phenylpropionic acid was to that of the *R*-enantiomers, but viewing the graph from right to left (i.e., from 0 to 100% of the *S*-enantiomer in this case).

Two representative error levels are shown in Fig. 6 corresponding to 5% and 10%. Even higher errors are admitted in some enantiomeric discriminations. By fixing the error level, one can identify the conditions under which each procedure will be applicable. Such information is provided in Table 4 as the analytical application range based on the *R* : *S* ratio in the sample, as well

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

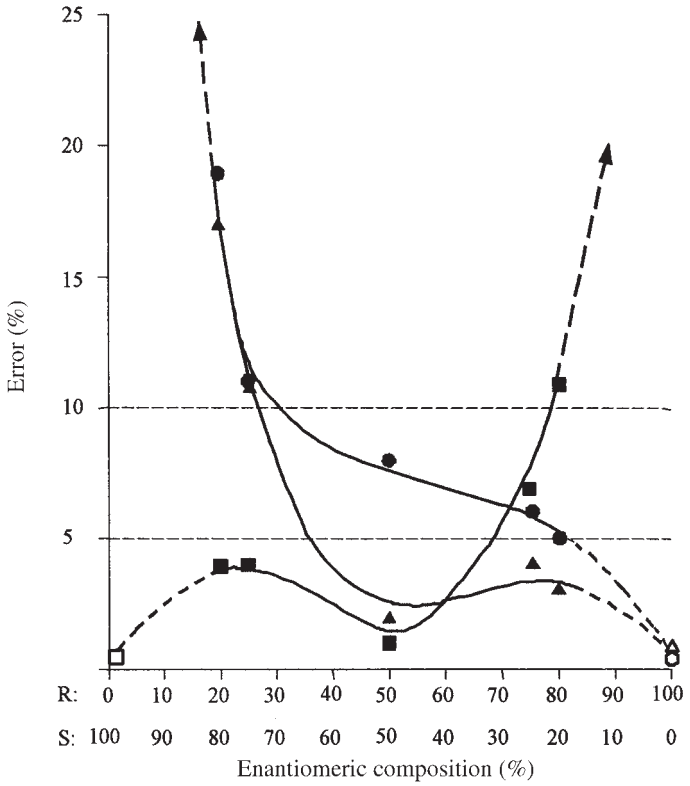

Figure 6. Variations of the errors obtained for the determination of *R*-10-camphorsulfonic acid (●), *S*-2-phenylpropionic acid (■), and *R*-mandelic acid (▲) according to the enantiomeric ratio in synthetic samples. Errors obtained from pure enantiomers standards have been represented by open symbols.

as the maximum tolerated enantiomeric ratio in the determination of one enantiomer in the presence of the other at a preset error level. It is interesting to note the excellent characteristics of the determination of *S*-2-phenylpropionic acid with the proposed method. Samples in $S:R$ ratios up to 31 : 69 can be analyzed with a maximum error of 5%; this means tolerating an $S:R$ ratio of 2.23 at 5% error, which can be increased to 3.55 if a maximum error of 10% is accepted.

Based on the data of Table 4, *R*-10-camphorsulfonic and *R*-mandelic acids can be determined in the presence of the corresponding *S*-enantiomers in proportions up to 69% (w/w) at the 10% error level, but only up to 20% (w/w) at the 5% error level. The maximum tolerated $R:S$ enantiomeric ratio for the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 4. Conditions for the analytical application of the proposed method for the determination of *R*-10-camphorsulfonic acid, *S*-2-phenypropionic acid, and *R*-mandelic acids in synthetic samples at two different error levels.

Compound	Error reference level (%)	(*R* : *S*) application range	Maximum tolerated enantiomeric ratio
10-Camphorsulfonic acid	5	(100 : 0)–(80 : 20)	(*R* : *S*) 0.25
	10	(100 : 0)–(31 : 69)	(*R* : *S*) 0.45
2-Phenylpropionic acid	5	(100 : 0)–(69 : 31)	(*S* : *R*) 2.23
	10	(100 : 0)–(78 : 22)	(*S* : *R*) 3.55
Mandelic acid	5	(100 : 0)–(36 : 64)	(*R* : *S*) 1.78
	10	(100 : 0)–(27 : 73)	(*R* : *S*) 2.70

determination of *R*-mandelic acid is 1.78 (5% error) and 2.70 (10% error); and that for 10-camphorsulfonic acid (0.25 at 5% and 0.45 at 10%).

CONCLUSION

In this work, additional evidence of the potential of analytical supercritical extraction in enantioselective reactions was obtained. In fact, the solubility of one enantiomer can be boosted through of the formation of two diastereomeric salts, due to the fact that precipitation occurs upon addition of the enantiomeric acids and the alkaloids, thus the solubility of the salts in CO_2 determine extractability. In situ SFE with supercritical CO_2 was found to be a flexible method (with regard to the density of the SC fluid) for the resolution of racemic acids with a view to producing material of a high enantiomeric purity. Resolution was found to be a function of the density and temperature of supercritical CO_2.

Additional studies about the me chanism of the enantiospecific reaction in the supercritical medium are needed for further development of selective extraction methods. These results testify to the high selectivity provided by the joint use of chiral selectors and SFE. Applications such as the obtainment of optically active materials and the pretreatment of samples with chiral activity are among the analytical goals that can be explored with the proposed approach.

ACKNOWLEDGMENTS

Funding from Spain's Interministerial Commission for Science and Technology within the framework of Project BQU2001-1815 is gratefully

acknowledged. RB is also indebted to CONICIT-LUZ (Venezuela) for the award of a doctoral fellowship.

REFERENCES

1. Collins, A.N.; Sheldrake, G.N.; Crosby, J. Eds. *Chirality in Industry II*; John Wiley and Sons: Chichester, New York, 1992; 1–63.
2. Lacques, A.; Collet, S.; Willen, H. *Enantiomers, Racemates and Resolutions*; Krieger: USA, 1994.
3. Wainer, I.W.; Ducharme, J.C.; Granvil, P.; Parenteau, H.; Abdullah, S. Using chirality as a unique probe of pharmacological properties. J. Chromatogr. A **1995**, *694* (1), 169–179.
4. Caldwell, J. Importance of stereospecific bioanalytical monitoring in drug development. J. Chromatrogr. A **1996**, *719* (1), 3–13.
5. Aboul-Enein, H.Y.; Stefan, R.I. Enantioselective sensors and biosensors in the analysis of chiral drugs. Crit. Rev. Anal. Chem. **1998**, *28* (1), 259–266.
6. De Camp, W.H. Chiral drugs: the FDA perspective on manufacturing and control. J. Pharm. Biomed. Anal. **1993**, *11* (11–12), 1167–1172.
7. Wai, C.M.; Hunt, F.; Ji, M.; Chen, X. Chemical reactions in supercritical carbon dioxide. J. Chem. Educ. **1998**, *75* (12), 1641–1545.
8. Pettersson, C.; No, K. Chiral resolution of carboxylic and sulphonic acids by ion-pair chromatography. J. Chromatrogr. A **1983**, *282*, 671–684.
9. Ahn, H.; Shiu, G.; Trafton, W.F.; Doyle, T.D. Resolution of the enantiomers of ibuprofen: comparison study of diastereomeric method and chiral stationary phase method. J. Chromatogr. B **1994**, *653* (2), 163–169.
10. Stephani, R.; Cesare, V. Enantiomeric enrichment of non-racemic antihistamines by achiral high-performance liquid chromatograph. J. Chromatogr. A **1998**, *813* (1), 79–84.
11. Schurig, V. Enantiomer separation by gas chromatography on chiral stationary phases. J. Chromatogr. A **1994**, *666* (1–2), 111–129.
12. Sponsler, S.; Biedermann, M. Optimization of chiral separations using capillary gas chromatography. Int. Lab. **1998**, *28* (2), 8A–8B, 8D, 8E–8F.
13. Armstrong, D.W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, L.R. Macrocyclic antibiotics as a new class of chiral selectors for liquid chromatography. Anal. Chem. **1994**, *66* (9), 1473–1484.
14. Van Overbeke, A.; Baeyens, W.; Dewaele, C. Enantiomeric separation of six 2-arylpropionic acids after precolumn derivatization with various amines and alcohols on a cellulose-based chiral stationary phase. Anal. Chim. Acta **1996**, *321* (2–3), 245–261.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

15. Belloli, E.; Foulon, C.; Yous, S.; Bonte, J.P.; Vaccher, C. Diastereomeric and enantiomeric resolution of methoxytetrahydronaphthalene derivatives, melatonin ligand receptors, by HPLC on amylose chiral stationary phases. Chromatographia **2001**, *53* (3–4), 216–219.
16. Williams, K.L.; Sander, L.C.; Wise, S.A. Comparison of liquid and supercritical-fluid chromatography for the separation of enantiomers on chiral stationary phases. J. Pharm. Biomed. Anal. **1997**, *15* (11), 1789–1799.
17. Simándi, B.; Keszei, S.; Fogassy, E.; Sawinsky, J. Supercritical fluid extraction, a novel method for production of enantiomers. J. Org. Chem. **1997**, *62*, 4390–4394.
18. Fogassy, E.; Àcs, M.; Szili, T.; Simándi, B.; Sawinsky, J. Molecular chiral recognition in supercritical solvents. Tetrahedron Lett. **1994**, *35*, 257–260.
19. Kordikoswki, A.; York, P.; Latham, D. Resolution of ephedrine in supercritical CO_2: a novel technique for the separation of chiral drugs. J. Pharm. Sci. **1999**, *88* (8), 786–791.
20. Bauza, R.; Ríos, A.; Valcárcel, M. Supercritical fluid extraction for selective extraction of enantiomers. Anal. Chim. Acta **1999**, *391* (3), 253–256.
21. Field, J.A. Coupling chemical derivatization reactions with supercritical-fluid extraction. J. Chromatogr. A **1997**, *785* (1–2), 239–249.
22. Hartonen, K.; Riekkola, M.L. Detection of beta-blockers in urine by solid-phase extraction supercritical-fluid extraction and gas chromatography-mass spectrometry. J. Chromatogr. B **1996**, *676* (1), 45–52.
23. Meissner, G.; Hartonen, K.; Riekkola, M.L. Supercritical-fluid extraction combined with solid-phase extraction as sample preparation technique for the analysis of beta-blockers in serum and urine. Fresen. J. Anal. Chem. **1998**, *360* (5), 618–621.
24. Jessop, G.Ph.; Laitner, W.; Eds. *Chemical Synthesis Using Supercritical Fluids*; Wiley-VCH: Weinheim, Germany, 1999.
25. Luque de Castro, M.D.; Valcárcel, M.; Tena, M.T. *Analytical Supercritical Fluid Extraction*; Springer-Verlag: Berlin, 1994.
26. Hjeresen, D.L.; Schutt, D.L.; Boese, J.M. Green chemistry and education. J. Chem. Ed. **2000**, *77* (12), 1543–1547.
27. Effenberger, F.; Hörsch, B.; Föster, S.; Ziegler, T. Enzyme-catalyzed synthesis of (*R*)-ketone–cyanohydrins and their hydrolysis to (*R*)-α-hydroxy-methyl-carboxylic acid. Tetrahedron Lett. **1991**, *32* (23), 2605–2608.

Received November 2002
Revised May 2003

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016