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Supercritical fluid extraction: a novel method for the resolution of tetramisole

Sándor Keszei,[a] Béla Simándi,[a,*] Edit Székely,[a] Elemér Fogassy,[b] János Sawinsky [a] and Sándor Kemény [a]

[a] *Technical University of Budapest, Department of Chemical Engineering, H-1521 Budapest, Hungary*
[b] *Technical University of Budapest, Department of Organic Chemical Technology, H-1521 Budapest, Hungary*

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Abstract

A new resolution method, based on the selective distribution of enantiomers between a chiral solid and an achiral supercritical fluid phase, is reported. The chiral solid phase is formed from the optically active dicarboxylic acid derivative, (2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid, and the racemic base (tetramisole). A new method is also described for the enrichment of enantiomeric mixtures which have an enantiomeric ratio other than 1:1. This is based on the partial salt formation of the enantiomeric mixture with an achiral substance, which is then followed by supercritical fluid extraction of the free enantiomer. The extract has an enantiomeric composition which is different from the starting mixture. The method is applied to an enantiomeric mixture of tetramisole with hydrochloric acid.

1. Introduction

The production of chiral compounds in an optically active form is continuously growing, due to demand by both the pharmaceutical and agrochemical industries, as well as researchers.[1] The fact that stereoisomers of certain molecules may have different physiological effects has been known for some time.[2] Many chiral drugs are still administered as racemic mixtures, which usually include the enantiomers with lower therapeutic activities and possible toxic side-effects. Even if an 'unwanted' enantiomer of a racemate is inactive it should be considered as metabolic ballast.[3] This is also true for agrochemicals where the use of enantiomerically pure pesticides could reduce pollution in the environment.[4] Therefore, utilization of enantiomerically pure substances is preferred.

Supercritical fluid chromatography (SFC), is a recently developed method. In this method a chiral complexing agent, bonded to a stationary phase, enables analytical and preparative scales, and separation

* Corresponding author. E-mail: simandi.vmt@chem.bme.hu

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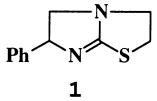
for several important types of racemic compounds[5] (e.g. racemic bases[6]). SFC has several advantages over liquid chromatography, for example, highly selective enantiomeric separation, rapid mass transfer in the supercritical mobile phase, and high-speed separation with high resolution.

The high efficiency of SFC suggests that a supercritical fluid extraction (SFE) may also be developed as a resolution method applicable to a preparative and, perhaps, even an industrial process. In earlier publications, we have shown that SFE can be applied to the resolution of some acids (e.g. chrysanthemic and permetric acids).[7]

In supercritical fluid extraction, the solvent is a fluid at a temperature and pressure above its critical temperature and pressure. It has been widely applied commercially in the food, pharmaceutical and cosmetic industries.[8] SFE is quite simple and offers an easy extraction technique. Carbon dioxide is the most widely used SFE solvent because it is inexpensive, nontoxic, nonflammable, and has a comparatively low critical temperature of 31.3°C and pressure of 7.3 MPa. Changing the extraction conditions, temperature, and pressure of carbon dioxide, results in extracts of different components as though several different solvents were being used in the solvent extraction. Furthermore, separation of the extract from carbon dioxide is achieved by reducing the pressure. As this is done at room temperature, the extract is cold when recovered, which is very desirable when thermally sensitive substances are extracted.

Compound 6-phenyl-2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazole **1** is prescribed as tetramisole (racemate) or as levamisole (*S*)-(−)-enantiomer. The latter is the biologically active enantiomer known to be an effective anthelmintic drug with an immunomodulatory activity. On the other hand, systemic toxicities of (*S*)-(−)-**1** and (*R*)-(+)-**1** are of approximately the same magnitude. It follows that administration of pure (*S*)-(−)-**1** (levamisole) gives pharmaceutical activity with substantially reduced risks of toxic reaction.[9]

In the last few decades, separation of the isomers of synthetic tetramisole (*RS*-**1**) has come into prominence. Conventional resolution methods which are based on diastereomeric salt formation and fractional crystallization using various optically active acids have been described in the literature (see Table 1). To our knowledge, resolution of racemic bases with SFE has not yet been reported. The present study was undertaken with the purpose of demonstrating the applicability of this method, and also to investigate the influence of some parameters on the resolution process of racemic **1**.



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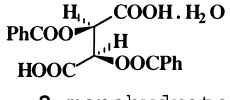

2-monohydrate

2. Results and discussion

2.1. Solubility of the components

In the preparatory stage, the loading capacity of the supercritical fluid was obtained by extraction of pure components under different extraction parameters. The loading concentrations were calculated from mass of extracts and mass of CO_2 passed through the system (see Table 2).

The solubility tests showed that the resolving agent (2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid monohydrate and the supporting material (Perfilt) are insoluble in supercritical carbon dioxide under the conditions of the resolution experiments.

Table 1
Production of levamisole (*S*)-(−)-**1** or its hydrochloric salt by conventional resolution method

Resolving agent	Solvent	Product	Optical purity	Yield
(1*R*)-10-camphorsulfonic acid (a)	Chloroform	(−)-**1** salt	$[\alpha]_D^{25} = -120$ (c=10, H_2O)	80%
L-phenylsulfonyl-glutamic acid (b)	Water	(−)-**1**	$[\alpha]_D^{20} = -85.3$ (c=10, $CHCl_3$)	75%
(2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid (c)	Methanol	(−)-**1** salt		55.2%
N-(*p*-toluenesulfonyl) - L-pyroglutamic acid (d)	Acetone	(−)-**1** salt	$[\alpha]_D^{25} = -119$ (c=5, H_2O)	
(2*R*,3*R*)-*O*,*O*′-di-*p*-toluoyltartaric acid (e)	Methanol-Water	(−)-**1** salt	$[\alpha]_D^{25} = -125$ (c=5, H_2O)	45%
(2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid (f)	Ethanol-Water	(−)-**1** salt		69%
(2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid (g)	Water	(−)-**1** salt	$[\alpha]_D^{20} = -135$ (c=1, H_2O)	67%
(2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid (h)	Acetone	(−)-**1**	88%	85%
L-*N*-[(4-methoxyphenyl)--sulfonyl]glutamic acid (i)	Water	(−)-**1** salt	$[\alpha]_D^{25} = -127$ (c=5, H_2O)	88%
(2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid (j)	Water	(−)-**1**	$[\alpha]_D^{20} = -125.5$ (c=1, H_2O)	54.2%
(*R*)-mandelic acid (k)	Ethanol Ethyl-acetate	(−)-**1**	97.9%	57.5%

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Table 2
Loading concentration of **1** in supercritical CO_2

p [bar]	*T* [°C]	*wt* [%]
120	33	0.12
160	33	0.17
200	33	0.23
120	39	0.11
160	39	0.18
200	39	0.22
120	45	0.07
160	45	0.14
200	45	0.27

Table 3
Effects of the resolving agent (acid)/racemic mixture (base) molar ratio on the yield, the enantiomeric excess (*ee*) and the Fogassy parameter (*S*) at the resolution of **1** (p=160 bar, T=39°C)

Acid / base			**0.125**	**0.25**	**0.35**	**0.50**	**0.75**
Extract :	yield	[%]	58	33	23	7	2
	ee	[%]	3	40	56	60	65
	S	[%]	+0.0348	+0.2640	+0.2576	+0.0840	+0.0260
Raffinate:	yield	[%]	23	48	66	75	80
	ee	[%]	5	26	18	6	2
	S	[%]	–0.0230	–0.2496	–0.2376	–0.0900	–0.0320

*2.2. Partial resolution of tetramisole **1** by SFE*

In the new method, the well-known technique for resolving the racemic mixture (in our case **1**) with less than the equivalent of the resolving agent (in our case **2**-monohydrate) was used.[10] In this procedure, a complex equilibrium is established which involves both the enantiomers of the free base **1** and two diastereomeric complexes **1** and **2**.

The higher stability of one of the diastereomeric complexes results in the preferential formation of that product. Consequently, the free base becomes optically active. Thus, the poor solubility of the complexes in the supercritical CO_2 permits the selective extraction of the free base. Also, if the difference in stability of the complexes is large enough, a remarkable resolution can be achieved in a single extraction step. The extent of the enantiomeric separation depends on the temperature and pressure, since the dissociation constant of the diastereomeric complex, an important factor influencing the enantiomeric purity of the extract, also depends on the state of the solvent.

Mixtures of **1**- and **2**-monohydrate in different molar ratios were prepared and extracted with supercritical carbon dioxide at the selected pressure and temperature. The extract contained the enantiomeric mixture of the free base, whereas the raffinate was the mixture of the diastereomeric complexes. After a single extraction, the (+)- and (−)-enantiomers were enriched in the extract and the raffinate, respectively. For comparison, the extractions at different pressures and temperatures, and the resolving capability of the resolution method, were characterized by the modified Fogassy parameter (*S*). This parameter is the product of the chemical yield (yield=mass of the recovered product/mass of the initial racemic acid) and the enantiomeric purity of the material (S=2×yield×*ee*/10^4). The *S*-value is negative for the (−)-enantiomer.

2.3. Effects of molar ratio, pressure and temperature

Variations of the resolving agent:racemic mixture (**2**:**1**) molar ratio led to establishing the optimum at the ratio of 0.25. Any further increase of the resolving agent fraction resulted in considerably reduced yields, although the *ee* values were higher (see Table 3).

Designed SFE experiments were carried out to map quantitative effects of the pressure and temperature on extraction at an optimum molar ratio of **2**:**1**. The well-known 3^2 full factorial design[11] was realized and three repeated experiments were performed in the centre of design (Table 4). In these tests the *S*-parameter was used as the dependent variable. The three-dimensional response surface plots for experiments at the molar ratio of 0.25 are shown in Fig. 1. The results indicate that the best choice for producing both the (+)-**1**- and (−)-**1**-enantiomers is the combination of the highest temperature (45°C) with the highest pressure (200 bar).

Table 4
Effects of the pressure (p) and temperature (T) on the enantiomeric excess (ee), yield and Fogassy parameter (S) at the resolution of **1** at the molar ratio of 0.25

p [bar]	T [°C]	Extract			Raffinate		
		Yield [%]	ee [%]	S_E	Yield [%]	ee [%]	S_R
120	33	28	23	0.1288	54	12	–0.1296
160	33	29	27	0.1566	53	14	–0.1484
200	33	29	25	0.1450	51	13	–0.1326
120	39	33	40	0.2640	48	28	–0.2688
160	39	33	40	0.2640	48	26	–0.2496
160	39	34	35	0.2380	46	24	–0.2208
160	39	36	39	0.2808	45	29	–0.2610
200	39	34	35	0.2380	46	25	–0.2300
120	45	39	55	0.4290	42	49	–0.4116
160	45	39	59	0.4602	43	51	–0.4386
200	45	40	60	0.4800	42	54	–0.4536

2.4. Improving the enantiomeric purity of partially resolved mixtures

Since the best *ee* values achieved by the single extraction step at the optimal conditions were only 60% for the (+)-enantiomer, and 54% for the (−)-enantiomer, it was necessary to purify the partially resolved base. As the racemic **1**-hydrochloride is much more stable than the correspondingly resolved hydrochloride, and is insoluble in CO_2, addition of a limited quantity of hydrochloric acid may allow for the recovery of the enantiomer in excess by SFE. This procedure was used to achieve a satisfactory resolution of the (+)- and (−)-enantiomers. The results of the purifications obtained from the experiments involving a single extraction at the optimal pressure and temperature are summarized in Table 5.

3. Conclusion

Supercritical fluid extraction was found to be an efficient separation method for resolution of tetramisole. The first extraction of the base-resolving agent mixture resulted in ‘breaking’ the racemic composition. Enantiomerically pure products were obtained by repeated extraction of the partially resolved mixtures, after addition of an achiral salt-forming reagent. The choice for carrying out this separation, with a supercritical fluid replacing toxic industrial solvents, is advantageous for implementing environmentally benign separation processes.

4. Experimental

4.1. General

Both the racemic mixture of the base (**1**-hydrochloride) and the resolving agent (**2**-monohydrate) were purchased from Sigma–Aldrich Chemie, GmbH. Other analytical-grade reagents were obtained from Reanal Ltd, Budapest. The samples were prepared by mixing chiral acid with a racemic base (liberated from its hydrochloric salt) in different molar ratios. Perfilt, a porous supporting material, was impregnated with the mixtures and placed into the extractor vessel. Carbon dioxide of 99.5% (w/w) purity was supplied by Messer Griesheim, Hungaria. Optical rotations of extracts and raffinates were measured

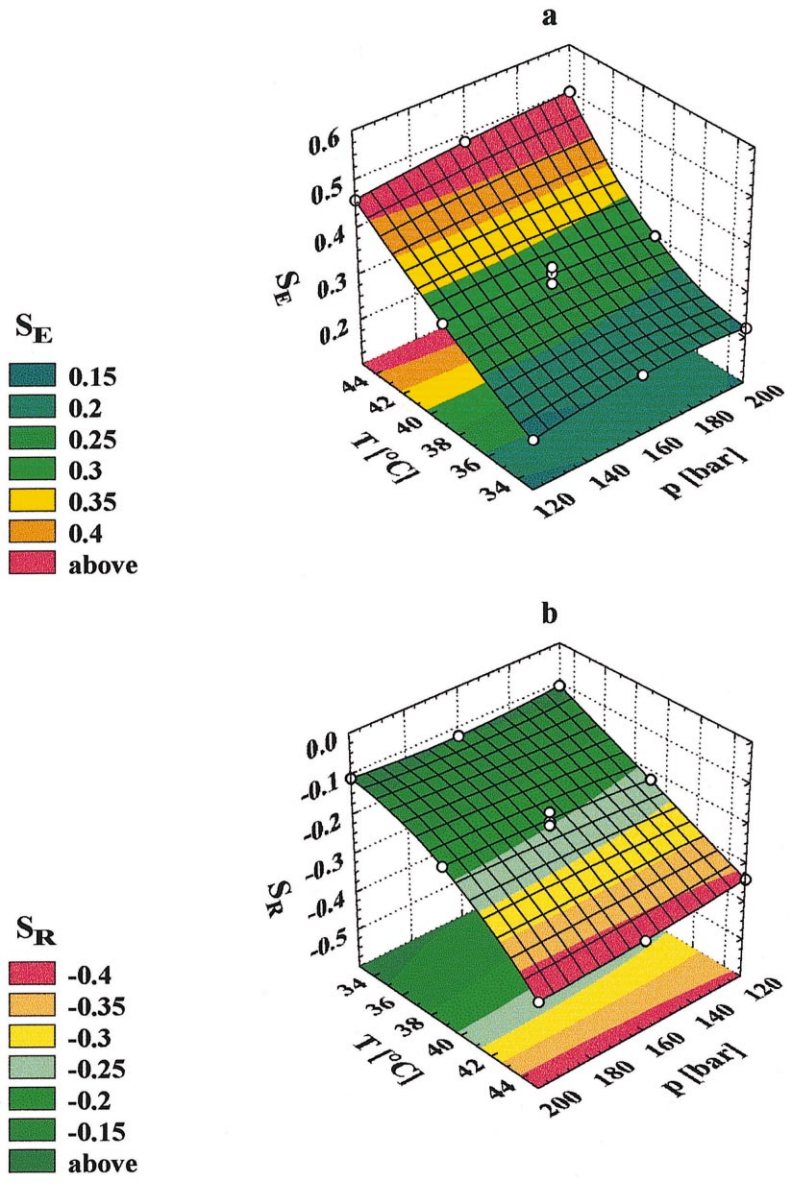

Figure 1. Effects of the pressure and temperature (a) on the Fogassy parameter of the extract (S_E); and (b) on the Fogassy parameter of the residue (S_R) obtained by the resolution of tetramisole (**1**) by SFE (specific CO_2 consumption: 600 g g^{-1})

Table 5
Resolution of the enantiomeric mixture of **1** by SFE using an achiral reagent (hydrochloric acid; p=200 bar, T=45°C)

Starting mixture		Extract			Raffinate		
ee [%]	Confgn.	*ee* [%]	Confgn.	Yield [%]	*ee* [%]	Confgn.	Yield [%]
60	*R*	99	*R*	45	5	*S*	37
54	*S*	99	*S*	50	7	*R*	32

on a Perkin–Elmer 241 polarimeter. Enantiomeric excesses (*ee*s) were determined from optical rotations based on calibration with enantiomeric mixtures of known purities. Experimental setup and extraction methods have been extensively described in earlier publications.[7]

*4.2. Resolution of tetramisole **1***

Racemic **1** (1.50 g, 7.40 mmol) and (2*R*,3*R*)-*O*,*O*′-dibenzoyltartaric acid monohydrate (0.69 g, 1.85 mmol), Perfilt (2.00 g) and ethanol (30 mL) were mixed and then evaporated. The residue was extracted with supercritical carbon dioxide. Extraction was done at 45°C and 200 bar. The quantity of carbon dioxide used for extraction was 900 g. After the removal of carbon dioxide, (+)-**1** (0.60 g, 40%) was obtained. $[\alpha]_D^{20}$=+64 (*c* 5, MeOH). The raffinate was suspended in water (40 mL) and cc. NH_4OH was added to pH 9.5. The reaction mixture was stirred for 30 min, and cooled to 10°C. The precipitate was filtered to give (−)-**1** (0.63 g, 42%). $[\alpha]_D^{20}$=−58 (*c* 5, MeOH).

To a solution of (−)-**1** (1.50 g, 7.40 mmol, 54% *ee*) in ethanol (30 mL) was added 1 M aqueous HCl (3.0 mL) and Perfilt (2.00 g) and then the solvent was evaporated. The residue was extracted with supercritical carbon dioxide (900 g, 45°C, 200 bar). After the removal of carbon dioxide, (−)-**1** (0.75 g, 50%), $[\alpha]_D^{20}$=−107 (*c* 5, MeOH) and (+)-**1**-hydrochloride (0.56 g), $[\alpha]_D^{21}$=+9 (*c* 0.9, H_2O) were obtained in the extract and raffinate, respectively. Similarly, the mixture of (+)-**1** (1.50 g, 7.40 mmol, 60% *ee*), 1 M aqueous HCl (2.6 mL) and Perfilt (2.00 g) was extracted to give (+)-**1** (0.68 g, 45%), $[\alpha]_D^{20}$=+107 (*c* 5, MeOH) in the extract and (−)-**1**-hydrochloride (0.65 g), $[\alpha]_D^{21}$=−6 (*c* 0.9, H_2O) in the raffinate.

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