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Influence of chemical structure of tricyclic tertiary dimethylamines on chiral separation by reversed-phase high-performance liquid chromatography after derivatization with (−)-menthylchloroformate

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ABSTRACT

In the present study (−)-menthylchloroformate was used as a chiral derivatizing agent for promethazine, trimeprazine, trimipramine and N-(2-dimethylaminopropyl)iminodibenzyl. (−)-Menthylchloroformate reacted with the tertiary dimethylamine moiety in these tricyclic antihistamines and antidepressants, resulting in the formation of diastereoisomers. Owing to the reaction conditions, during the derivatization with (−)-menthylchloroformate, the possibility of racemization had to be established. For this purpose different ratios of (+) and (−)-promethazine were prepared. Enantiomeric separation of these mixtures took place on a Chiral α AGP column or, after derivatization with (−)-menthylchloroformate, on a C_{18} column. The results from these two independent separation systems were compared with trace racemization. No racemization was found during the experiments. To study the effects of changes in the molecular structures of the tertiary dimethylamines on the chromatographic behavior of the derivatization products, four tertiary dimethylamines [promethazine, trimipramine, trimeprazine and N-(2-dimethylaminopropyl) iminodibenzyl] were derivatized and analyzed. With these amines the effects on resolution and capacity factor of replacing a phenothiazine ring by an iminodibenzyl ring or insertion of a carbon molecule between the chiral centre in the chain and the place where (−)-menthylchloroformate reacts were studied. Not only the distance between the chiral centres in the diastereoisomers (a longer distance caused less resolution and higher capacity factors) but also the kind of ring influenced resolution and capacity factor. Finally, the influence of eluent composition on resolution and capacity factor was studied. Three different mixtures of methanol and acetic acid were tested. More acetic acid in the eluent caused a better resolution and higher capacity factor. The higher capacity factor, however, resulted in unacceptable retention times.

INTRODUCTION

The recent interest in enantioselectivity in the pharmacological behavior of pharmaceutics is causing a growing need for enantioselective bioanalytical methods. High-performance liquid chromatography (HPLC) is a method with numerous applications for the quantitation of drugs in biological fluids. With direct chiral HPLC methods enantiomeric separation is possible, at least for pure drugs. However, most

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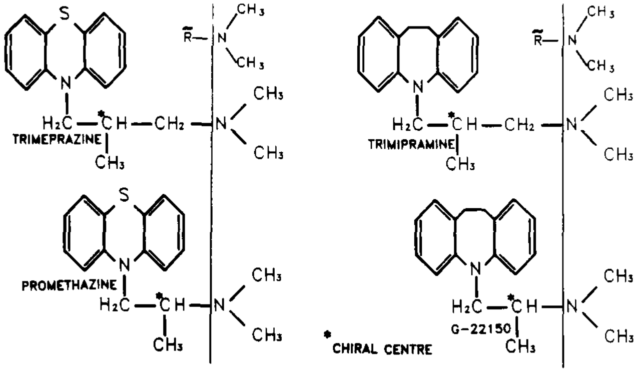

Fig. 1. Structures of the four tertiary dimethylamines.

chiral stationary phases are easily impaired by endogenous compounds from biological matrices such as plasma or urine.

A different approach, the indirect one, is the use of an optically pure derivatizing agent which results in the formation of diastereoisomers. Diastereoisomers have different chemical and physical properties which allow, at least in theory, separation on non-chiral HPLC systems. For the derivatization reaction a suitable functional group in the racemic drug is required. For various easily accessible groups, such as alcohols and primary and secondary amines, suitable derivatization agents exist [1]. Another functional group, present in many pharmaceuticals, is the tertiary amine. For this group only a few derivatization reactions have been described [2].

We recently used (−)-menthylchloroformate to derivatize tertiary dimethylamines, using promethazine as an example [3]. In this paper we have further evaluated the potential of this derivatizing agent. Racemization, due to the applied reaction conditions, was checked for promethazine by comparing the results of the derivatization with the results of an independent direct separation system.

The influence of changes in the chemical structure of the tertiary amine on resolution and capacity factor was also studied. Four amines with comparable structures consisting of a phenothiazine or an iminodibenzyl ring, and a 2-(dimethylamino)-2-methylethyl or a 3-(dimethylamino)-2-methylpropyl side-chain were analyzed. Their structures are given in Fig. 1 and the derivatization reaction with (−)-menthylchloroformate is depicted in Fig. 2.

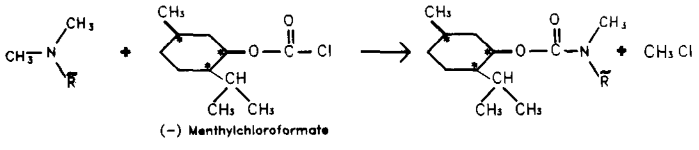

Fig. 2. Derivatization reaction of the tertiary dimethylamines with (−)-menthylchloroformate.

EXPERIMENTAL

Chemicals

Racemic tertiary dimethylamines. Promethazine hydrochloride was obtained from Brocacef (Maarsen, The Netherlands) and was of Ph. Eur. quality. Trimipramine maleate was from Centrachemie (Etten-Leur, The Netherlands). Trimeprazine tartrate came from May & Baker (Manchester, U.K.), and was of B.P. quality.

N-(2-Dimethylaminopropyl)iminodibenzyl, "G-22150", was synthesized according to a modification of the method in ref. 4 with iminodibenzyl from Janssen (Beerse, Belgium) and 1-dimethylamino-2-propylchloride from Aldrich-Chemie (Steinheim, Germany). The reaction product was tested and its identity verified with mass spectrometry, electron impact (EI) and chemical ionization (CI), and ^{1}H nuclear magnetic resonance. The structures of the tertiary amines are given in Fig. 1.

Other reagents. Acetonitrile and methanol were from Westburg (Leusden, The Netherlands) and were of HPLC grade. Triethylamine was purchased from Janssen and was of analytical grade. The (−)-methylchloroformate {$[\alpha]_D^{18}$: 80° (1 g per 100 ml chloroform)} was obtained from Aldrich-Chemie. Acetic acid, diethyl ether, toluene, xylene and sodium dihydrogenphosphate 1-hydrate, all of analytical grade, were from E. Merck (Darmstadt, Germany).

Chromatographic equipment

A reversed-phase HPLC (RP-HPLC) system with a 150 × 4.6 mm I.D. stainless-steel column, filled with Nucleosil C_{18} 5 μm (Macherey Nagel, Düren, Germany) was used to separate the diastereoisomers formed during derivatization. For the direct chiral separations a Chiral α AGP column, 100 × 4 mm I.D. (ChromTech, Stockholm, Sweden) was used.

In the experiments to check racemization during or after the derivatization, the solvent delivery system was a 2150 LKB HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), injections were made by a 710 A WISP (Waters, Milford, MA, U.S.A.) and the UV detector was an HP 1040A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA, U.S.A.) operating at 258 nm. For recording and integrating the chromatograms a C-R3A Chromatopac (Shimadzu, Kyoto, Japan) was used.

In the experiments to study the influence of the chemical structure of the tertiary dimethylamine on resolution and capacity factor, the same RP-HPLC stationary phase was used.

The solvent delivery system here was an M 6000 A (Waters). The injector was a Model 7125 manual loop injector equipped with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.) and the detector was a Spectraflow 757 UV detector (Kratos, Ramsey, NJ, U.S.A.) at 252 nm. For recording the results a BD 40 recorder (Kipp, Delft, The Netherlands) was used.

Derivatization

The derivatization of the tertiary dimethylamines was carried out according to a modification of the method in ref. 3. To a 250-μl sample of a 0.1 mg/ml solution of the tertiary dimethylamine in acetonitrile were added 500 μl of 10% (v/v) (−)-menthylchloroformate in acetonitrile and 25 μl of triethylamine. The solution obtained was heated for 1.5 h at 70°C. After cooling the mixture to room temperature, a 20-μl sample was injected into the RP-HPLC system.

Check for racemization

Solutions of (+)- and (−)-promethazine were prepared in acetonitrile (50 μg/ml) and mixed to give samples with various ratios of the (+) and (−) enantiomers. The enantiomers of promethazine were obtained by crystallization [5] and the enantiomeric purity of the enantiomers thus obtained was verified with the use of a Chiral α AGP stationary phase.

The samples were analyzed by both a direct and an indirect HPLC method under the following conditions.

Direct method: no derivatization, enantiomeric separation on a chiral α AGP with an eluent consisting of 11% acetonitrile and 89% 0.1 *M* sodium dihydrogen phosphase in distilled water, pH 6.0. Eluent flow-rate 0.9 ml/min.

Indirect method: derivatization with (−)-menthylchloroformate and the separation of the formed diastereoisomers on a C_{18} HPLC column with an eluent consisting of 75% acetonitrile and 25% 0.1 *M* acetic acid in distilled water, pH 2.9. Eluent flow-rate 1.0 ml/min.

The percentages of the (+) area and the (−) area relative to the total area [(+) + (−)] were calculated.

Influences of chemical structure of the tertiary dimethylamines on capacity factors and resolutions of the derivatization products in three eluent compositions in RP-HPLC

The capacity factors of the first eluting diastereoisomers were used. The resolution between the two diastereoisomers was measured according to ref. 6. The effects of changes in ring and chain structure on resolution and capacity factor were calculated. Eluent compositions used were mixtures of methanol and 0.1 *M* acetic acid (v/v) with ratios (a) 90:10; (b) 85:15; and (c) 80:20. The flow-rate for all three eluent compositions was 1.0 ml/min.

RESULTS AND DISCUSSION

Check for racemization

To check for racemization during or after the derivatization reaction two methods were compared. The indirect method, derivatization with (−)-menthylchloroformate, was compared with a direct method, separation on a Chiral α AGP column.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF THE INDIRECT METHOD AND THE DIRECT METHOD FOR RACEMIC PROMETHAZINE

Method	Enantiomer	Retention time (min)	Resolution	Plate number
Indirect	−	17.97	2.31	6580
	+	20.28		5167
Direct	+	18.01	1.67	1072
	−	21.68		1554

In Table I the chromatographic characteristics of the indirect and the direct method are compared for racemic promethazine.

The inversion of the elution order for the promethazine enantiomers between the two methods is advantageous for the assessment of optical purity. In practice, this means that a small amount of (+) in a large amount of (−) can be determined more accurately with the direct method, whereas the indirect method is more accurate when a small amount of (−) in a large amount of (+) is to be determined. The results of the experiments with the mixtures containing different ratios of (+)- and (−)-promethazine are summarized in Table II.

The lines for the percentage (+) in the sample could be calculated for the two methods according to the equation $y = ax + b$ where y is the percentage of (+) analyzed and x is the percentage of (+) attempted. Direct method: $y = 0.980\,x - 0.104$ ($r = 0.9990$); indirect method: $y = 0.992\,x + 1.848$ ($r = 0.9993$); indirect/direct: $y = 0.998\,x + 2.877$ ($r = 0.9986$).

The linearity of the lines with a slope near 1 showed that no racemization occurred during these experiments. This was further confirmed by the fact that when only one enantiomer was derivatized (samples 1 and 13) only one peak was observed.

Fig. 3A and B shows that there were difficulties when a small percentage of one enantiomer had to be determined in a high percentage of the other enantiomer. These problems were more significant when the first eluting enantiomer was present in a high percentage relative to the second enantiomer. The changed elution order between the two methods makes the combination of the methods serviceable for enantiomeric purity determinations.

TABLE II

PERCENTAGES OF (+)- AND (−)-PROMETHAZINE IN SAMPLES 1–13

See Fig. 3A and B.

Sample No.	(+)-Promethazine			(−)-Promethazine		
	Attempted	Analyzed		Attempted	Analyzed	
		Direct	Indirect		Direct	Indirect
1	100	100	100	100	100	100
2	99	100	99.6	99	99.0	99.6
3	90	86.2	90.6	90	89.3	88.0
4	80	76.2	80.5	80	80.8	77.0
5	70	66.7	71.7	70	69.8	65.8
6	60	57.6	61.7	60	60.5	57.6
7	50	48.6	52.9	50	51.4	47.1
8	40	39.5	42.4	40	42.4	38.3
9	30	30.2	34.2	30	33.3	28.3
10	20	19.2	23.0	20	23.8	19.5
11	10	10.7	12.0	10	13.8	9.4
12	1	1.0	0.4	1	0.1	0.4
13	0	0	0	0	0	0

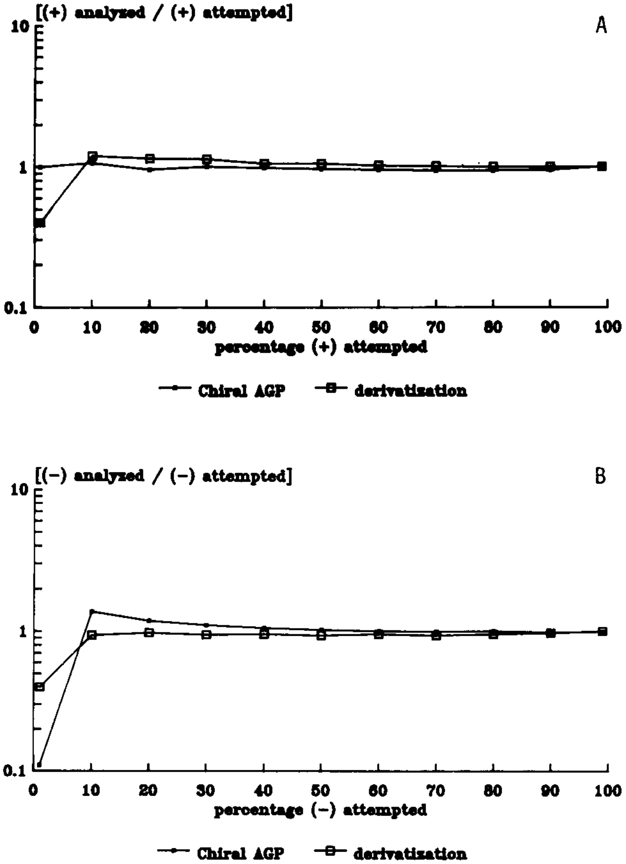

Fig. 3. (A) Percentage (+) attempted divided by the percentage (+) analyzed relative to the percentage (+) attempted in two different separation systems. (B) Percentage (−) attempted divided by the percentage (−) analyzed relative to the percentage (−) attempted in two different separation systems.

TABLE III

RESOLUTION (*R*) BETWEEN THE DIASTEREOISOMERS OF THE FOUR DERIVATIZED AMINES AND THE LOG CAPACITY FACTORS (LOG k') OF THE FIRST ELUTING DIASTEREOISOMER IN THREE ELUENT COMPOSITIONS

Amine	Eluent A		Eluent B		Eluent C	
	R	log k'	*R*	log k'	*R*	log k'
Promethazine	2.0	0.60	2.5	0.97	3.2	1.38
Trimeprazine	1.0	0.81	1.2	1.16	1.4	1.59
Trimipramine	0.0	0.88	>0.1	1.27	0.6	1.73
G-22150	1.1	0.65	1.8	1.02	2.3	1.44

TABLE IV

EFFECTS OF CHANGES IN THE RING STRUCTURE OR THE CHAIN LENGTH OF THE DERIVATIZED AMINE ON RESOLUTION AND LOG k'

Change	Mean R			Mean log k'		
	Eluent A	Eluent B	Eluent C	Eluent A	Eluent B	Eluent C
Phenothiazine	1.5	1.85	2.3	0.705	1.065	1.485
↓	↓	↓	↓	↓	↓	↓
Iminodibenzyl	0.55	0.90	1.45	0.765	1.145	1.585
Effect on R	−0.95	−0.95	−0.85			
Effect on log k'				+0.060	+0.080	+0.100
2-(Dimethylamino)-2-methylethyl	1.55	2.15	2.75	0.625	0.995	1.410
↓	↓	↓	↓	↓	↓	↓
3-(Dimethylamino)-2-methylpropyl	0.50	0.60	1.00	0.845	1.215	1.660
Effect on R	−0.95	−1.55	−1.75			
Effect on log k'				+0.220	+0.220	+0.250

Influences of chemical structure of the tertiary dimethylamines on capacity factors and resolutions of the derivatization products in three eluent compositions in RP-HPLC. The four amines depicted in Fig. 1 were derivatized with (−)-menthylchloroformate. The influences of the differences in chemical structure between these amines on resolutions and capacity factors were calculated. The results are summarized in Tables III and IV.

Tables III and IV show that the resolution between the diastereoisomers was influenced by the distance between the chiral centres: the shorter the distance the better the resolution. The type of ring structure also had a significant effect on resolution and capacity factor.

It is clear that resolution increased with an increasing amount of acetic acid in the eluent and that the capacity factors increased at the same time, resulting in excessively long retention times at acetic acid concentrations > 15%. The reproducibility of the resolution of diastereoisomers on different columns with the same stationary phase is a common problem. For the promethazine derivatives two batches of Machery-Nagel Nucleosil C_{18} 5 μm and one Merck LiChrospher 100 RP-18 5 μm were tested. No differences between the batches or between the brands were observed.

CONCLUSIONS

During the derivatization of promethazine with (−)-menthylchloroformate no racemization occurred. The chromatographic results of the indirect method showed a higher resolution (R) and plate number (N) than the direct method. The difference in elution order for the promethazine enantiomers between the two methods makes the combination of these two methods very useful in the determination of enantiomeric purity of the promethazine enantiomers.

The resolution of the diastereoisomers in the indirect method was influenced not only by the distance between the chiral centres in the diastereoisomers, but also by the ring structure. The iminodibenzyl derivatives showed higher capacity factors than the corresponding phenothiazines. On the other hand, the phenothiazine derivatives were better resolved than the corresponding iminodibenzyls.

More acetic acid caused a better resolution. However, the influence on capacity factor was such that higher amounts of acetic acid in the eluent resulted in unacceptable retention times.

The indirect method is simple and fast and therefore a good and cheap alternative to the direct one. The applicability of the derivatization method in biological samples has shown to be promising in our hands.

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