Before routine use in bioanalysis, two main questions have to be answered:

1) Can metabolites of IF interfere with IF when derivatization is not applied?
2) Is the IF peak related with unchanged IF or a degradation product?

Addition of known IF metabolites did not result in interferences with the IF peak (**Figure 3**).

Mass spectrometry revealed that intact IF entered the MS; this in contrast to CY. The mass spectra are shown for IF (*m/z* 260) in **Figure 4,** for the intra-alkylating product of CY (*m/z* 224), 1st peak, after loss of HCl and intraalkylation, in **Figure 5 a,** and for intact CY (*m/z* 260), 2nd peak, in **Figure 5 b.**

Fragmentation starts with loss of CH_2Cl *m/z* 211: IF–CH_2Cl; *m/z* 211: CY–CH_2Cl.

In conclusion, it is seen that capillary gas chromatography of the new oxazaphosphorine IF can be performed without any derivatization and without use of polar columns.

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Chiral Derivatization of Promethazine with (–)-Menthyl Chloroformate for Enantiomeric Separation by RP-HPLC

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RP-HPLC
Racemate separation
Chiral derivatization
Promethazine

1 Introduction

The enantiomeric separation of drugs and metabolites is of great analytical and biological interest. High Performance Liquid Chromatography (HPLC) has proved to offer a number of suitable alternatives for this purpose, as exemplified by the utilization of chiral stationary phases. A different approach to obtain chiral separation is chemical derivatization of enantiomers to form diastereoisomers which can be separated, at least in theory, on non-chiral HPLC systems. Because many pharmaceuticals contain an amine group, the latter is an important target for derivatization. For primary and secondary amines, various derivatization methods are known. This paper describes a method for derivatizing a tertiary amine, racemic promethazine, with optically pure (–)-menthyl chloroformate

2 Experimental

2.1 Materials

Racemic promethazine · HCl was obtained from Brocacef, Maarssen, The Netherlands and was of Ph. Eur. quality. Acetonitrile and methanol were from Westburg, Leusden, The Netherlands and were of HPLC grade. Triethylamine was purchased from

Janssen, Beerse, Belgium and was of analytical grade. The (−)-menthyl chloroformate {$[\alpha]_D^{18}$: −80° (1 g/100 ml chloroform)} was obtained from Aldrich-Chemie, Steinheim, FRG. Acetic acid and ether both of analytical grade were from E. Merck, Darmstadt, FRG. The sodium carbonate anhydrous and the sodium hydroxide pellets, both of analytical grade, were from E. Merck, Darmstadt, FRG.

2.2 Extraction of the Promethazine Base

Promethazine · HCl was dissolved in distilled water. To this solution were added sodium hydroxide (2 M) and ether. The resulting suspension was shaken vigorously and the ether layer was separated. The water layer was extracted two more times with ether and the combined ether layers were dried (sodium carbonate). Evaporation of the ether layer took place under a gentle stream of nitrogen at room temperature. The resulting promethazine base was used in the derivatization reactions.

2.3 Derivatization

The following solutions were prepared:

Solution **1:** Promethazine base 0.1 mg/ml in acetonitrile/triethylamine 90 : 10 (v/v).
Solution **2:** Acetonitrile/menthyl chloroformate 90 : 10 (v/v).

For derivatization, 50 µl of **1** and 100 µl of **2** were mixed in a conical WISP vial (Waters Associates Inc. Milford, Massachusetts, USA) of 400 µl. The vial was sealed with a teflon seal and heated for 1.5 h at 70 °C. The mixture was cooled to room temperature and a 20 µl sample was injected in the HPLC system. All treatments with promethazine were performed in subdued light to prevent decomposition.

2.4 HPLC System

The RP-HPLC system contained a (150 × 4.6 mm, i.d.) stainless steel column, filled with Nucleosil C18 5 µm (Macherey Nagel, Düren, FRG). The solvent delivery system was a 2150 LKB HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), injections were made by a 710 A WISP (Waters Associates Inc, Milford, Massachusetts, USA) and detection was carried out by a HP 1040 A diode array spectrophotometer (Hewlett Packard, Palo Alto, CA, USA) operating at 258 nm, or from 200 to 600 nm in the scanning mode.

Two mobile phases were used:

A: methanol-0.03 M aqueous acetic acid pH 3.2, 85 : 15 (v/v) for the separation of the diastereoisomers in a short time.
B: acetonitrile-0.1 M aqueous acetic acid pH 2.9, 75 : 25 (v/v) to test for reaction completeness.

3 Results and Discussion

The reaction (**Figure 1**) was complete within 1.5 hours. No promethazine was left after derivatization (**Figure 2**) and no

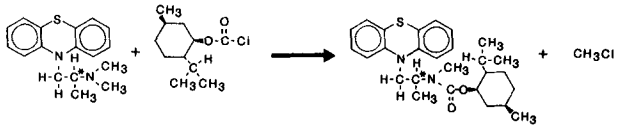

Figure 1

The derivatization reaction.

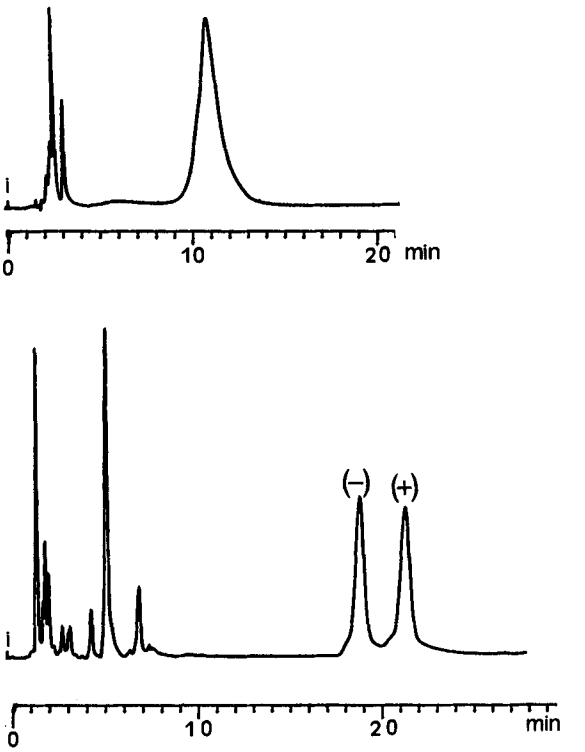

Figure 2

Chromatograms of promethazine (top) and its (−)-menthyl chloroformate diastereoisomers (bottom). Mobile phase B.

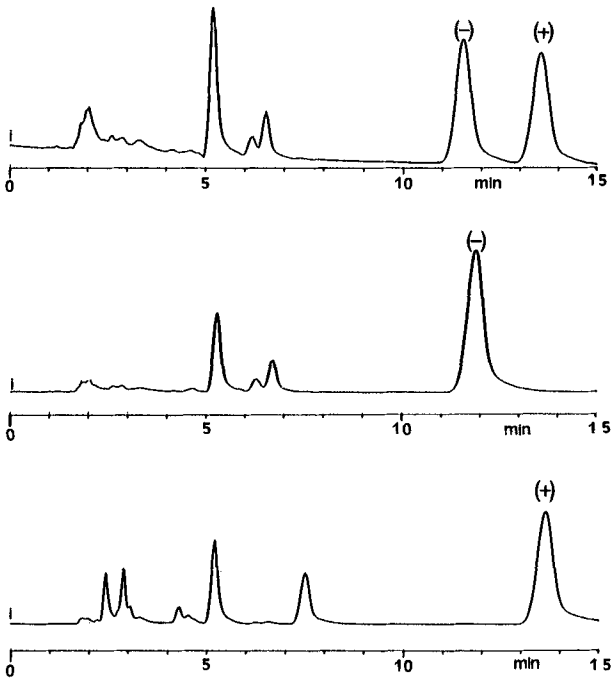

Figure 3

Chromatograms of promethazine (−)-menthyl chloroformate diastereoisomers.
Top: Derivatization of racemic promethazine
Middle: Derivatization of (−)-promethazine
Bottom: Derivatization of (+)-promethazine. Mobile phase A.

decomposition was observed. The reaction completeness was checked with mobile phase **B** because in mobile phase **A** the promethazine peak, *Rt* 3.10 min, did not separate from other peaks. A baseline separation was obtained for the diastereoisomers with mobile phase **A** (**Figure 3**) with a resolution of 2.0. Mobile phase **B** gave a similar separation but with longer retention times (Figure 2). The chromatographic position of the individual enantiomers was checked with the pure promethazine enantiomers. The latter were obtained by crystallization [1]. The enantiomeric purity of the promethazine enantiomers thus obtained was verified with a Chiral α-AGP stationary phase (ChromTech, Stockholm, Sweden). After derivatization with (−)-menthyl chloroformate the (−)-promethazine derivative eluted before the (+) derivative in the RP-HPLC system.

So far, derivatizations with (−)-menthyl chloroformate have been described for secondary amines [2, 3] and for one tertiary amine, namely encainide [4]. The procedure described here is quicker and easier and has the advantage that a RP-HPLC column can be used, which facilitates the handling of biological samples. Since many relevant drugs are secondary or tertiary amines, it may be expected that this approach can be useful for other substances as well. The procedure described is fast, reproducible, and apparently insensitive to side reactions or interfering products.

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Relationship between GC Retention and Physicochemical Parameters of Amino Acids on Chirasil-Val

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Amino acids
Electronic and structural effects

1 Introduction

Many chromatographic methods (TLC, HPLC, GC, capillary zone electrophoresis) [1–6] have been developed for the separation of amino acid enantiomers. Some chiral phases have been developed for the GC separation of derivatized amino acid enantiomers [7, 8], while the retention order of enantiomers has been calculated in GC [9]. To our knowledge there has so far been no detailed study of the relationships between the retention characteristics of amino acid derivatives and their physicochemical parameters.

2 Experimental

A Perkin Elmer gas chromatograph (Model F22) equipped with an FID was interfaced with a Perkin Elmer Sigma 10 integrator. The column was a 25 m × 0.22 mm i.d. fused silica column coated with Chirasil-L-Val. Nitrogen was used as carrier and as make-up gas. The carrier gas flow rate was 1 ml/min. The injector and detector temperatures were 240 °C. A split ratio of 1 : 100 was selected and 1 μl was injected. The volatile amine acid derivatives were prepared as described in ref. [10] without adding ethyl mercaptan to the reaction mixture. The retention time of amino acid derivatives was determined isothermally between 70 and 220 °C column temperatures in steps of 10°. The correlation between the retention time (t_{min}) and column temperature (T °C) was calculated separately for each amino acid and for both configurations:

$$\log t = a - b \cdot \log T \quad (1)$$

To relate the retention parameters of eq. (1) to the physicochemical parameters of amino acids, stepwise regression analysis was used. The parameters of eq. (1) (a and b values) were taken as dependent variables, and the pI, p$K_{\alpha COOH}$, p$K_{\alpha NH_2}$, pK value of the third polar group taken from ref. [11], the z scales characterizing the lipophilicity (z_1), side chain bulk (z_2), and electronic properties (z_3) of amino acids taken from ref. [12], served as independent variables. The acceptance level of the single independent variable was set to 95 %.