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Capillary Gas Chromatography/Mass Spectrometry of Oxazaphosphorines

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1 Introduction

Oxazaphosphorines number among the most widely used anti-cancer agents in clinical oncology. The agents require metabolic activation by hepatic microsomal enzymes, resulting in a wide spectrum of metabolites [1]. The latest oxazaphosphorine to be used is ifosfamide (IF), an isomer of cyclophosphamide (CY) (Figure 1).

Extensive use of these agents has raised the demand for (bio) analytical assays in order to describe the pharmaceutical and pharmacokinetic (including metabolism) phase of the oxazaphosphorines. Several methods were introduced, based on TLC, GC, HPLC, and MS. For routine analysis, HPLC and GC [2, 3] are to be preferred but have some disadvantages: HPLC lacks sensitivity whereas GC generally needs derivatization. Capillary gas chromatography on polar columns proved to be capable of elution of CY and some of its metabolites without derivatization [4]. Capillary GC of underivatized CY resulted in partial degradation thereof, yielding intra-alkylated CY [4]. Nevertheless, the formation of intra-alkylated CY was linear over a sufficient range allowing application of the assay in experimental and clinical sessions [5–7]. This contribution describes CGC and CGC-MS analysis of IF.

Figure 1
Ifosfamide (IF) and cyclophosphamide (CY).

2 Experimental

2.1 Materials

CY, IF, and metabolites [3, 5] used for interference studies were kindly supplied by ASTA (Bielefeld, FRG).

2.2 Apparatus and Methods

2.2.1 GC/FID

The chromatographic system consisted of a Carlo Erba HRGC 5160 Mega Series gas chromatograph (Carlo Erba Strumentazione, Milano, Italy) equipped with an FID detector. The GC was operated with a methylsilicone column (Hewlett-Packard, HP-1, 5 m, 0.53 mm i.d., 2.65 μ m). Helium was used as a carrier gas. Sample introduction was performed by on-column injection at 85 $^{\circ}$ C. The oven temperature was programmed to 100 $^{\circ}$ C at 15 $^{\circ}$ /min $^{-1}$, then to 160 $^{\circ}$ C at 50 $^{\circ}$ /min $^{-1}$, from 160 $^{\circ}$ C to 180 $^{\circ}$ C at 1 $^{\circ}$ /min $^{-1}$, and then to 220 $^{\circ}$ C at 5 $^{\circ}$ /min $^{-1}$. Data acquisition was performed on a Spectra Physics 4290 integrator.

2.2.2 GC/MS

Capillary gas chromatography-mass spectrometry was performed on a Hewlett-Packard 5790 A (MSD) instrument interfaced to a Hewlett-Packard 9825 B data system. Electron ionization mass spectra at 70 eV were obtained at a rate of 2 s $^{-1}$. Samples were injected splitless at 60 $^{\circ}$ C during 30 s, the oven ballistically heated to 150 $^{\circ}$ C (2 min), and then programmed from 150 $^{\circ}$ C to 300 $^{\circ}$ C at 10 $^{\circ}$ /min $^{-1}$.

Purge off-time was 0.50 min; head pressure was 20.0 psi and interface temperature was 290 $^{\circ}$ C. An OV-1 column (Hewlett-Packard, HP-UP, 25 m, 0.20 mm, 0.32 μ m) was installed.

3 Results and Discussion

Capillary gas chromatography may be considered for the determination of oxazaphosphorines as illustrated in Figure 2 a + b.

Figure 2

a) Chromatogram of cyclophosphamide (CY), two peaks are observed. CP_{IA} : intra-alkylated CY. b) Chromatogram of ifosfamide (IF), only one peak is observed.

Figure 3

Chromatograms of two metabolites of IF: 4-keto-ifosfamide and dechloroethyl-ifosfamide.

Figure 4

MS data of ifosfamide (IF).

Figure 5

a) MS data of intra-alkylated cyclophosphamide (CY_{IA}). b) MS data of intact cyclophosphamide (CY).

IF elutes within 5 min using the 5 m methylsilicone column without additional peak formation; this in contrast to CY (Figure 2 a versus 2 b). Both for IF and CY, peaks are linearly related with the amount introduced. The limit of detection was 5 ng for FID. When thermionic N-P detection or electron capture detection is used pg levels are reached. The assay was applied for the determination of CY in saline solutions prepared for administration to cancer patients.

It appeared that the method allows rapid determination of CY in a concentration range of 1 μ g/ml to 100 mg/ml without any sample pretreatment except dilution in the high concentration range [8].

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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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 deri-

vatization 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