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Review

Hyphenated techniques in anticancer drug monitoring I. Capillary gas chromatography–mass spectrometry

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Abstract

Most anticancer agents are relatively unstable substances and are subjected to intensive metabolism in vivo and degradation during sample pretreatment. Hyphenated techniques including a separation technique and, most frequently, mass spectrometry are therefore chosen to obtain insight into the in vivo behavior of anticancer agents. Once established, simpler assays can be derived from those based on hyphenation, which are less expensive. Capillary gas chromatography (cGC)–mass spectrometry (MS) is amongst the most frequently applied hyphenated analytical technologies in anticancer drug monitoring. Here a selection has been made of: (i) cGC–MS applied to the analysis of agents frequently used in clinical oncology (e.g. tamoxifen, oxazaphosphorines); (ii) cGC–MS applied to the development of new agents (Swainsonine and Brefeldin); (iii) cGC–MS applied to the analysis of agents for which comparisons with other frequently applied hyphenation technologies are possible (see Part I of this series). cGC–MS played a key role in the elucidation of the in vivo behavior of the oxazaphosphorine cyclophosphamide, historically the most frequently applied anticancer agent. cGC–MS appeared to be of special interest in the analysis of cyclophosphoramide and congeners in human erythrocytes by coupling of the hyphenated technique with a measurement of sediment technique. This resulted in the quantitative and qualitative analysis of oxazaphosphorine-related mustard gas moieties in human erythrocytes for the first time.

Keywords: Reviews; Drugs

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1. Introduction

The emergence of hyphenated techniques in anticancer drug monitoring in the past 10 years, particularly mass spectrometry coupled to liquid chromatography, has been extensive, and is therefore difficult to review in depth. Many hyphenated techniques used to monitor anticancer drugs involve the coupling of capillary (c) separation techniques, such as gas chromatography (cGC), liquid chromatography (LC) and electrophoresis (CE), with mass spectrometry (cGC–MS, LC–MS and CE–MS). With the exception of cGC–MS [1,2], the use of these techniques was limited until the early 1990s. However, the latest coupling devices used in LC–MS and CE–MS now allow routine monitoring of most anticancer agents, which are often labile, without derivatization. Nevertheless, cGC–MS remains an important tool in pharmaceutical and pharmacological studies of anticancer agents, and has recently produced new and interesting data concerning established agents, such as tamoxifen, thiotepa and the oxazaphosphorines cyclophosphamide and ifosfamide.

The present review describes a selection of application of cGC–MS in anticancer drug monitoring, most reported in the last decade. The use of these techniques in studies of carcinogenesis has been published previously [3].

2. Anticancer drug monitoring by capillary gas chromatography–mass spectrometry

2.1. Tamoxifen

cGC–MS has been elegantly applied to the analysis of various anticancer agents, including tamoxifen, a synthetic non-steroidal anti-estrogen. Tamoxifen is a cornerstone in the treatment of breast cancer, especially in post-menopausal patients. The mechanisms of action of tamoxifen and its metabolism have been studied extensively during the last two decades, resulting in the development of several more powerful anti-estrogens. Both cGC–MS [4–6] and LC–MS were important in these studies. The more limited use of CE–MS has nevertheless led to significant advances. 4-Hydroxytamoxifen, the major metabolite of tamoxifen, has been investigated in the palliative treatment of estrogen receptor positive human breast cancers and other endocrine tumors. Quantitative analysis of 4-hydroxytamoxifen at the femtomole level enables correct evaluation of its relative distribution between plasma and mammary tissue. A highly sensitive and specific assay was developed by Girault et al. [5] for the quantitative measurement of 4-hydroxytamoxifen in biological matrices. The drug and deuterated internal standard (Fig. 1A and B) were monitored by cGC–negative chemical ionization MS with methane as the reactant gas.

The substances were isolated from the complex biological matrices using a solid-phase extraction procedure with Extrelut 1 columns. Fluorinated derivatives were obtained by derivatization with pentafluorobenzyl chloride under soft operating conditions. The abundant and stable molecular ions at m/z 581 and 585, which were generated in the ion source, were analyzed by an electron capture process. cGC was carried out on a fused-silica capillary column (12.5 m × 0.32 mm O.D.), wall coated with a CP Sil 5 non-polar stationary phase (Chrompack, Middelburg, Netherlands). The film thickness and internal diameter of the column were 0.33 and 0.25

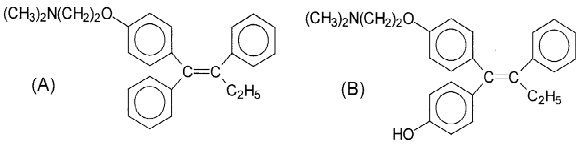

Fig. 1. Chemical structures of (A) tamoxifen and (B) hydroxytamoxifen.

μm, respectively. One end of the capillary column was connected to the injector (320 °C), less than 1 mm from the bottom of the moving glass injection needle. The opposite end was introduced directly near the ion source of the mass spectrometer via a 1/16 in. stainless-steel transfer tube held at 320 °C (1 in. = 2.54 cm). A HP5900 cGC–MS system operating in the NCI mode, with an electron energy of 80 eV, an emission current of 300 μA, and an ion source temperature of 200 °C, was used. cGC–MS has also been utilized to screen for tamoxifen and its metabolites in athletes, as tamoxifen can stimulate the production of androgenic steroids [6].

The oxazaphosphorines cyclophosphamide and ifosfamide, derived from mustard gas, are amongst the most frequently administered anticancer agents. Cyclophosphamide is given as a first line agent in numerous cancers, for example breast cancer. Both cyclophosphamide and ifosfamide are metabolized extensively (Fig. 2).

The use of cyclophosphamide, methotrexate and fluorouracil (the CMF regimen) in the adjuvant treatment of pre-menopausal breast cancer patients results in a significant increase of 5–10% in the 5 and 10 year survival rates. Methods based on hyphenated technologies, capable of following the metabolic pattern of the oxazaphosphorines, became available in the 1990s, and cGC–MS has been applied routinely by various groups [7–9]. Ifosfamide and its metabolites 4-hydroxyifosfamide, *N*-2-dechloroethylifosfamide, *N*-3-dechloroethylifosfamide and ifosphoramide mustard can be measured with ion-trap mass spectrometry in the plasma of cancer patients. The cytotoxic moiety, 4-hydroxyifosfamide, in equilibrium with aldofosfamide, can be trapped by the formation of a cyanohydrin adduct. Deuterium-labeled analogues of these analytes were synthesized and used as internal standards (Fig. 3). A Finnigan IT S40 Ion Trap mass spectrometer, directly coupled to a 3300/3400 Varian GC system with a capillary splitless injector, was used to analyze substances of interest.

The method allows the in-vivo monitoring of ifosfamide behavior using plasma, even in small laboratory animals. The analysis was carried out using chemical ionization, with ammonia as the reagent gas, and the emission current was set at 10 μA. Helium was used as the carrier gas with a head pressure of 15 p.s.i. (10^5 Pa). Separation of the analytes was performed on a DB-5 fused-silica capillary column (30 m × 0.25 mm I.D.) coated with a 0.25 μm thick film of methylsilicone plus 5% phenyl/methylsilicone (J&W Scientific, Folsom, CA, USA). The oven temperature program was 150 °C for 2 min, increasing to 190 °C at a rate of 5 °C/min. Subsequently, the temperature was increased to 250 °C at 15 °C/min, and the final temperature was held for 3 min, resulting in a total elution time of 17 min.

Momerency et al. developed assays for both cyclophosphamide and ifosfamide and their metabolites using cGC–MS [8,10]. Stable trifluoroacetyl derivatives were formed and subjected to electron capture chemical ionization. To achieve maximum detection sensitivity in the single ion monitoring (SIM) mode of cGC–MS of cyclophosphamide and six of its metabolites, it is necessary to monitor a single ion mass within each retention window interval. Several capillary columns with different stationary phases were tested to check for partial overlap of metabolic peaks. A 25 m × 0.25 mm I.D. column coated with a 0.2 μm film of the methylphenylsiloxane Sil 13 (Chrompack) was selected for routine analysis. The column is slightly polar and allows the elution of all trifluoroacetylated metabolites without overlap within the temperature interval used (150–250 °C). Negative ion chemical ionization by electron-capture detection (ECD) was the most sensitive technique for cyclophosphamide, carboxycyclophosphamide, alcocyclophosphamide and dechloroethylcyclophosphamide. Splitless injection of 1 μl of solution from 50 μl of the final derivatized plasma extract allows determination down to 600 pg/ml of plasma, with an absolute limit of detection for most metabolites of 3 pg, at a signal-to-noise ratio of 3:1 [8]. With the techniques described, cyclophosphamide and its metabolites of interest have been profiled in breast cancer patients treated with the oxazaphosphorines [8]. Partitioning of ifosfamide and its metabolites between red blood cells, plasma and plasma water has been studied with a coupling of a measurement of sediment technique (MESED) and cGC–MS [11,12]. With MESED, red blood cells of patients treated with oxazaphosphorines have been isolated quantitatively and plasma, plasma water and red blood cell sediments analyzed by cGC–MS. It

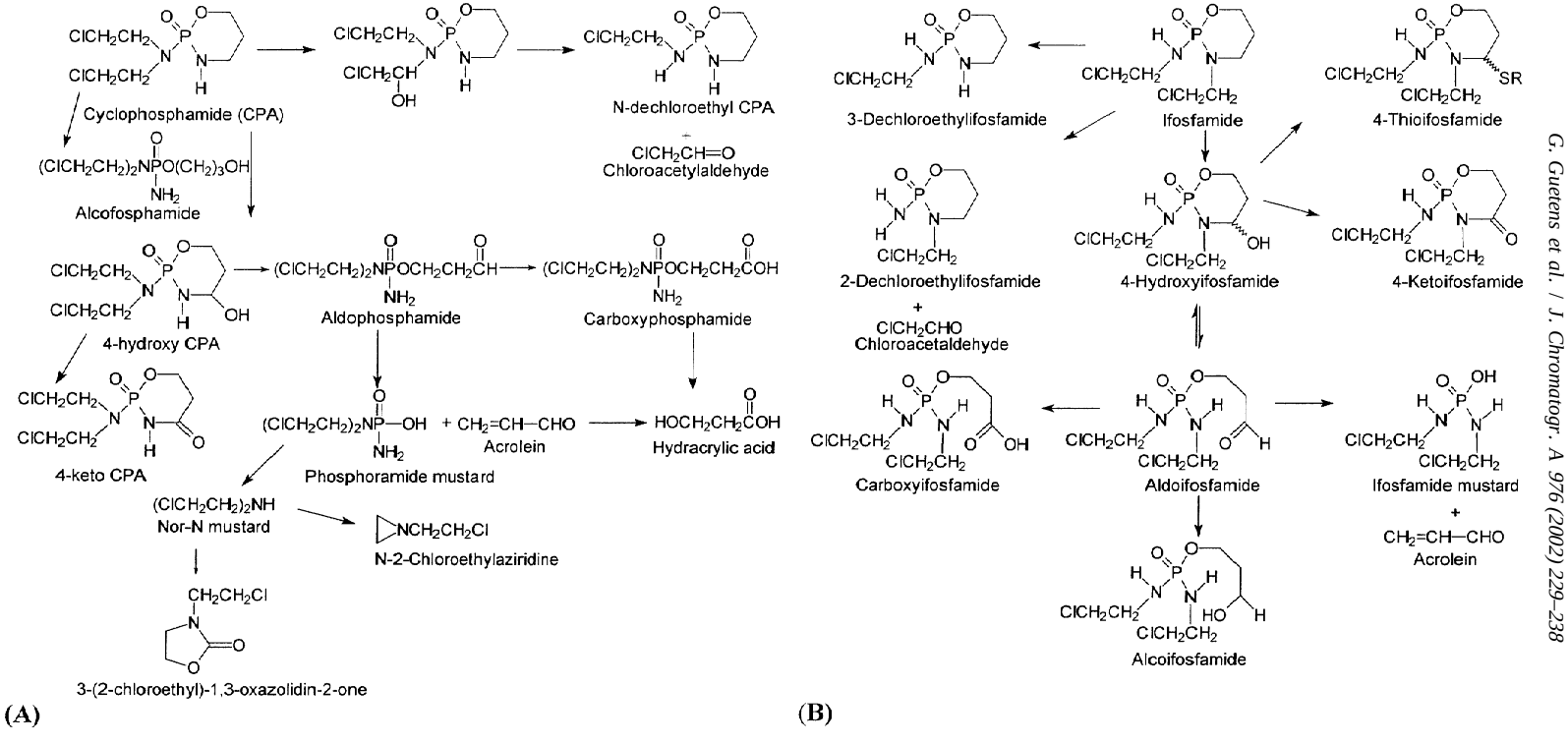

Fig. 2. Metabolic pathways of cyclophosphamide (A) and ifosfamide (B).

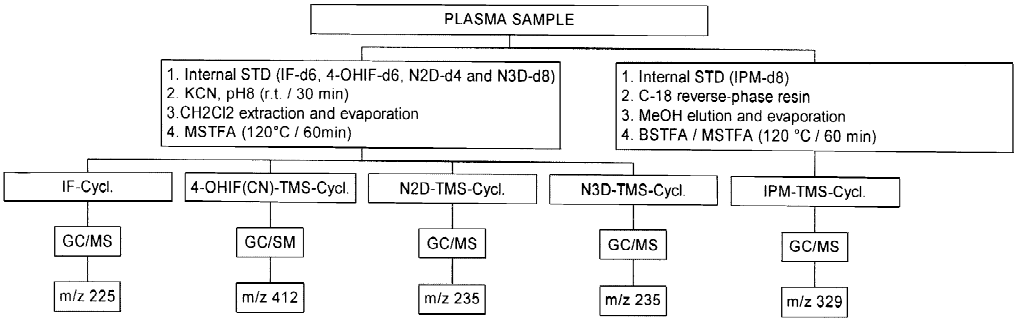

Fig. 3. Comprehensive analytical scheme for the analysis of ifosfamide and major metabolites in plasma. STD = standard; IF-Cycl. = cyclic dehydrochlorinated ifosfamide; 4-OHIF(CN)-TMS-Cycl. = cyclic silylated dehydrochlorinated 4-hydroxyifosfamide cyanohydrin; N2D-TMS-cycl. = cyclic silylated dehydrochlorinated *N*-2-dechloroethylifosfamide; N3D-TMS-cycl. = cyclic silylated dehydrochlorinated *N*-3-dechloroethylifosfamide; IPM-TMS-Cycl. = cyclic silylated dehydrochlorinated ifosforamide mustard.

has been demonstrated that erythrocytes fulfill a crucial role in the transport of ifosfamide moieties and their delivery to plasma water. cGC–MS has also been used to estimate the impact of exposure to oxazaphosphorines on occupational health [13]. Some of the substances of interest were introduced into the cGC–MS system following derivatization with trifluoroacetic anhydride (TFA). The cGC–MS system consisted of a Fisons GC 8065 instrument, equipped with a Fisons A 200S autosampler, and a mass spectrometer MD 800. For separations of both derivatized and underivatized samples, a capillary column (Permabond SE-52-DF, 0.25 μm film thickness, 25 m × 0.25 mm I.D.; Machery–Nagel) connected to a deactivated fused-silica retention gap (350 mm × 0.5 mm I.D., type 160-2537; J&W; Fisons Scientific, Mainz-Kastel, Germany) was used with automated on-column injection. Injection volumes ranged from 0.5 to 2 μl and were corrected for quantification with an external standard (1,2,3,5-tetrachlorobenzene) for underivatized samples and trofosfamide for derivatized samples. Helium was used as the carrier gas at an inlet pressure of 89 kPa. Injection started at 87 °C with secondary cooling for 4 min. The temperature was held for 2 min, then increased to 180 °C at a rate of 5 °C/min, held there for 1 min, and then raised to 185 °C at a rate of 1 °C/min. The column was finally heated at a rate of 20 °C/min to 280 °C, which was maintained for 2 min. The transfer line to the mass spectrometer was maintained at 280 °C. Ionization was achieved by electron impact at 70 eV, and the source temperature was set at 250 °C. Bracketing with standards was used in the analysis of underivatized samples, since the signal response of standards rapidly changed from one analysis to another. Typical mass spectra for ifosfamide, cyclophosphamide and trofosfamide are shown in Fig. 4. The use of cGC–MS–MS results in a somewhat more simplified method with similar results ; the limits of detection are in the low ng/μl for both methods [13,14].

2.2. Busulfan

cGC–MS plays an important role in the routine monitoring of chronopharmacological studies, as in the case of busulfan (Fig. 5) [15], in the detection and identification of metabolites of approved anticancer agents [16,17], and in the analyses of new anticancer agents. In the analysis of busulfan (Fig. 5), Vassal et al. [15] used a deuterated analogue, [$^{2}H_{4}$]busulfan, as an internal standard.

The internal standard demonstrated similar behavior with respect to extraction (partition coefficient), conversion into diiodobutane, and cGC–MS analysis. Split injection was used (split ratio 1:100) and chromatography was performed on CP Sil 5 CB wall-coated open tubular (WCOT) fused-silica capillary columns (25 m × 0.23 mm I.D., film thickness 0.13 μm). Helium was the carrier gas with an inlet pressure of 1.0 bar. The instrument was operated using an injector temperature of 220 °C, and an oven temperature gradient of 30 °C/min from 60 to

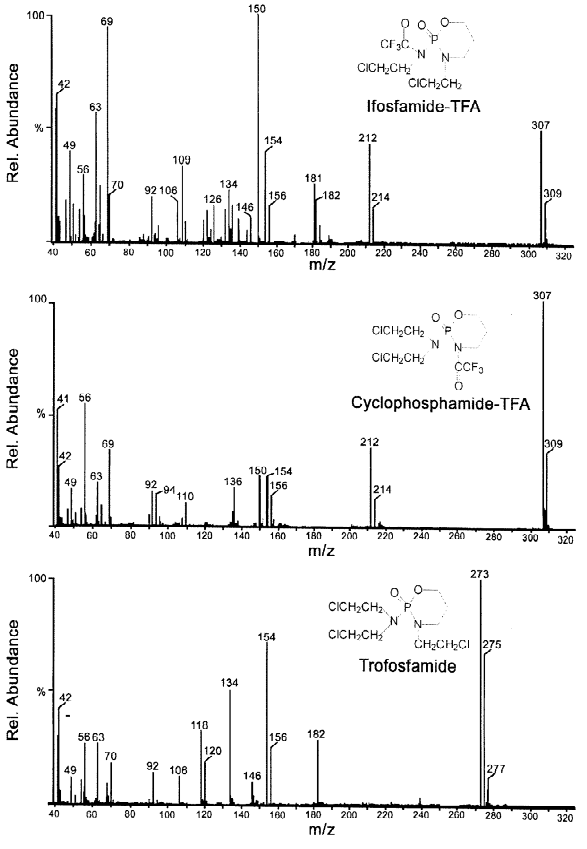

Fig. 4. Mass spectra of derivatized ifosfamide, cyclophosphamide and trofosfamide.

150 °C, subsequently maintained at 150 °C for 6 min. Mass spectra were recorded in the electron impact ionization (EI) mode, at a source temperature of 250 °C. Busulfan was treated with sodium iodide, and the monitored ions were focussed at m/z 182.9671 for transformed busulfan, m/z 186.9922 for transformed [$^2H_4$]busulfan, and m/z 191.0173 for transformed [$^2H_8$]busulfan. These positive ions correspond to the M−127 fragments arising from the loss of one iodine atom [15]. The synthesis of [$^2H_4$]busulfan and [$^2H_8$]busulfan was characterized by fast atom bombardment MS. The method allows the detection of a 10 pg injection, which corresponds to a limit of determination of 0.5 ng/ml [15]. Subsequently, other groups have used cGC–MS to monitor busulfan [16–18], with a slight modification to either the sample preparation [16], or to the chromatographic processes [18]. In an attempt to relate clinical outcome to plasma busulfan concentration, a cGC–MS assay has been developed with non-isotopic pusulfan as an internal standard. After extraction into ethyl acetate, busulfan and pusulfan were iodinated to 1,4-diiodobutane and 1,5-diiodopentane, respectively [17]. cGC–MS analysis was carried out on a Hewlett-Packard (HP) 5890 II gas chromatograph, with a 30-m 100% methylsilicone narrow bore fused-silica capillary column, interfaced to a HP 5970 A mass spectrometer. Helium was used as carrier gas. Total ion monitoring was executed, and substances of interest were quantified by selective ion monitoring of m/z 183 and 197. The limit of quantification was 0.04 mg/l with an analytical recovery of 97%.

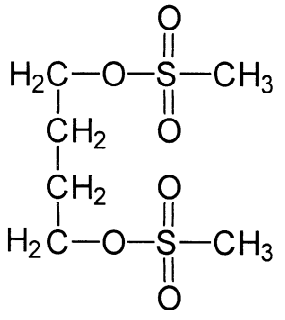

Fig. 5. Structure of busulphan.

2.3. 6-Mercaptopurine

cGC–MS has been used successfully to identify an unknown compound associated with the hypoxanthine antimetabolite 6-mercaptopurine (6MP) in patients with acute lymphoblastic leukemia [19]. The compound was collected from plasma by HPLC. To produce the trimethylsilyl derivative, the isolated and lyophilized material was dissolved in 50 μl of an equivolume mixture of chloroform and *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide containing 10 ml/l trimethylchlorosilane. Calibrators were subjected to the same procedures. CP-sil-8CB columns (25 m× 0.32 mm I.D., Chrompack), installed in a HP 5890 GC coupled to a VG-trio-2 quadrupole mass spectrometer (Fisons Instruments, Cheshire, UK), were used to separate the derivatives. The metabolite was identified by the MS system in the electron impact

ionization mode at 70 eV and a source temperature of 200 °C. Scan measurements were carried out from 40 to 650 u with a scan time of 1 s and an inter-scan delay of 0.1 s. Selected ion recording measurements were executed at the specific ions 254 ($M^+ - 1TMS$) and 326 ($M^+ - 2TMS$) by using a span of 0.4 u, a dwell time of 0.08 s, and an inter-channel delay of 0.02 s [19] (TMS = trimethylsilyl). The formation of 8-hydroxy-6-mercaptopurine was demonstrated, and further studies to characterize this particular aspect of 6MP metabolism need to be performed.

2.4. Dechloroethylazaridine

A sensitive and specific cGC–MS method for the quantitative analysis of *N*-2-dechloroethylaziridine (CEA), a volatile cytotoxic metabolite of cyclophosphamide, has been developed using cGC–MS and stable isotope dilution techniques [20] (Fig. 2). To overcome the problem of high volatility, a deuterium-labeled analogue was used as the internal standard, with a Snyder column-concentrator assembly. cGC–MS analysis of CEA and its deuterium-labeled analog was performed on a Finnigan ITS 40 ion trap mass spectrometer, directly coupled to a 3300/3400 Varian GC system, via a capillary splitless injector. The mixture was separated on a DB-5 fused-silica capillary column [30 m × 0.25 mm I.D., bonded with a 0.25 μm film thickness of 5% methylsilicone (J&W Scientific)]. Helium was the carrier gas with a head pressure of 15 p.s.i. (10^5 Pa). The chemical ionization mode was utilized with ammonia as the reagent gas. The injection port, transfer line and ion source operated at 160, 220 and 230 °C, respectively. With the initial oven temperature maintained at 60 °C for 4 min, and increased to 150 °C at a rate of 10 °C/min, the retention times were 3.48 and 3.58 min for [$^2H_4$]CEA and CEA, respectively. Quantitation was performed by calculating the ion area ratio *m/z* 103 to *m/z* 110, corresponding to CEA and [$^2H_4$]CEA, respectively [19].

2.5. Swainsonine and brefeldin

Swainsonine [21] is an indolizidine alkaloid which competitively inhibits Golgi α-mannosidase II (EC 3.2.1.114). It reduces tumor growth and may stimulate immune function. A method for the therapeutic drug monitoring of Swainsonine based on cGC–MS has been described by Baptista et al. [21]. The method comprises extraction, acetylation and quantification of the indolizidine moiety in human serum samples. cGC–MS experiments on a Varian Saturn GC–MS PC system, utilizing the SPI programmed temperature injector with a packed sleeve for biological samples, revealed a serum concentration of the agent of 3–11.8 mg/l. Samples were run in chemical ionization mode, with isobutane as reagent gas. The limit of detection was 0.1 mg/l with a recovery efficiency of 90%. The mass fragmentation patterns of the analytes were confirmed by comparing the data with the National Institute of Standards and Technology Mass Spectral Library.

Brefeldin A (BFA) is an antitumor macrolide isolated from *Penicillum brefeldianum* [22]. The biological activities of BFA in mammalian cells are based on the inhibition of protein transport from the endoplasmic reticulum to the Golgi complex, and the reversible disassembly of the Golgi complex. A specific method to assay the compound in biological fluids has been developed in which BFA and 1-eicosanol, added as the internal standard, are extracted from plasma specimens with diethyl ether, and analyzed further by cGC–MS and cGC–ECD [22]. The derivatization of both secondary hydroxyl groups of BFA was monitored by cGC–MS, performed using a HP 5860 series II with a 5971 A mass-selective detector controlled by a DOS series MS Chemstation. Nominal resolution electron ionization mass spectra and exact mass measurements were recorded by a Finnigan MAT 90 high-resolution mass spectrometer. Samples were introduced into the ion source using a high-temperature direct exposure probe. Exact mass measurements were obtained on selected ions at a static resolution of 10 000 using perfluorokerosene as internal standard. The ion source temperature was 250 °C, and an accelerating potential of 5 kV, an ionizing potential of 10 eV, and an ionizing current of 1 mA were applied. The exact mass measurement of the ions at *m/z* 280 was consistent with a molecular formula of $C_{16}H_{24}O_4$.

Separations in the routine analysis of plasma samples were performed on a 12 m × 0.2 mm I.D. fused-silica capillary column, wall coated with 0.33

μm HP-1 cross-linked 100% dimethylpolysiloxane gum. The analyte, the heptafluorobutyrylimidazole derivative of BFA, was detected by cGC–ECD at the low ng level, resulting in a lowest concentration of BFA, quantified with acceptable reproducibility in 50 μl of plasma, of 100 ng/ml [22].

2.6. Thiotepa and melphalan

N′,*N′*,*N′*-Triethylenethiophosphoramide (thioTEPA) and melphalan, both of which are related to the nitrogen mustards and are polyalkylating, are currently the subject of renewed interest. Melphalan is now the most popular chemotherapeutic agent in the loco-regional therapy of cancer, as in isolated limb perfusion, when it is combined with tumor necrosis factor (TNF). In an attempt to unravel the metabolic profile of thioTEPA, Van Maanen et al. developed an assay based on solid-phase extraction and liquid–liquid extractions, followed by cGC–MS on a Varian cGC–MS combination [23]. cGC separation was performed on a 10 m×0.25 mm DB5-MS capillary column (J&W Scientific), with a film thickness of 0.25 μm, using helium as carrier gas at a flow-rate of 1 ml/min. The oven temperature program was 2 min at 65 °C, increasing to 230 °C at 12 °C/min, and then to 280 °C at 50 °C/min, and maintained at this temperature for 150 s. A temperature programmed injector was set at 80 °C for 45 s, increased at 100 °C/min to 290 °C, which was maintained for 3 min. MS measurements were performed on a Varian Saturn-2, operating in the positive chemical ionization mode, with methane as reaction gas and the multiplier at 2 kV [23]. The mass spectra of thioTEPA and TEPA are presented in Fig. 6.

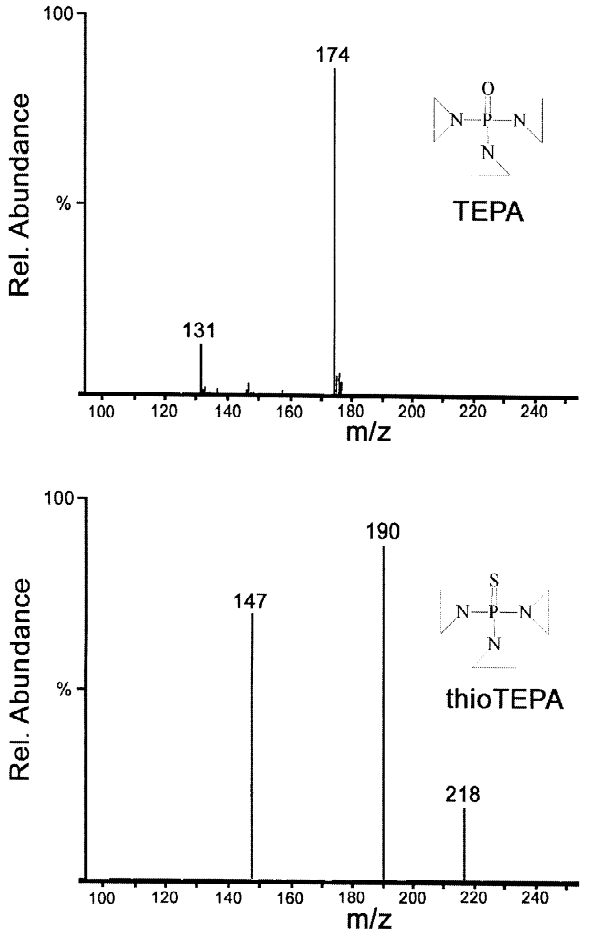

Fig. 6. Mass spectrum of TEPA and thioTepa in a spiked plasma sample. Fragments *m*/*z* 174 and *m*/*z* 131 in the TEPA spectrum originate from $[MH]^+$ and $[M-NC_2H_4]^+$, respectively. Fragments *m*/*z* 190 and *m*/*z* 147 in the thioTEPA spectrum originate from $[MH]^+$ and $[M-NC_2H_4]^+$, respectively. Fragment *m*/*z* 218 corresponds to $[M+C_2H_5]^+$, which is an adduct of the molecular ion and the reaction gas.

A new analytical method for the determination of phenylalanine mustard (melphalan, L-PAM) (Fig. 7), based on cGC–MS, was introduced by De Boeck et al. [24]. The assay includes trifluoroacetylation of the amino and hydroxyl functions of the phenylalanine moiety with trifluoroacetic anhydride. The carboxylic function was methylated with diazomethane in ether. It is important that trifluoroacetylation is performed before methylation, otherwise the trimethyl derivative of melphalan is formed. The cGC column was

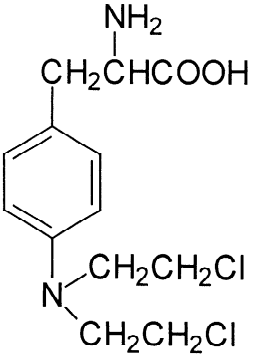

Fig. 7. Structure of melphalan.

heated from 200 to 265 °C at a rate of 8 °C/min. From the complete EI+ and negative chemical ionization (CI−) mass spectra of standard compounds, the specific ions for detection in the SIM mode were determined.

Two diagnostic ions were used, corresponding to the ^{35}Cl and ^{37}Cl isotopes. Integration of the SIM peaks revealed that the peak areas for the reference compounds and L-PAM obtained by EI+ ionization were respectively about three and 16 times higher than those using CI−. The detection limit of L-PAM in plasma was 20 ng/ml, with a signal-to-noise ratio of 10 [24].

2.7. Fluoropyrimidines

Fluorinated pyrimidines and related nucleosides have significant, and in certain cases unique, anticancer activity. Among these compounds, 5-fluorouracil (FUra) is one of the most active anticancer drugs and is frequently used in the treatment of breast and gastrointestinal cancers. Fluorinated pyrimidine analogues, such as 5′-deoxy-5-fluorouridine (doxifluoridine, dFUrd) and capecitabine, have been tested as a means of improving the therapeutic index of FUra. Two studies have shown cGC–MS to be a powerful technique for monitoring the conversion of 5′-dFUrd into FUra, and subsequent catabolism [25,26]. Sample preparation was relatively simple, and both methods used derivatization prior to cGC. Mass spectrometry was usually performed in the EI+ ionization mode (70 eV), with a source temperature of 200 °C [25], whilst for gas chromatography, temperature programming was applied requiring total run times between 10 [25] and 60 min [26]. Using these techniques, it is possible to determine catabolic deficiencies in FUra metabolism in patients swiftly, prior to treatment, thereby preventing serious toxicity.

3. Conclusions

These examples of the use of cGC–MS give only a limited impression of the significance of hyphenated techniques in anticancer drug monitoring. It is likely that the importance of cGC–MS in this field will diminish, as a consequence of the growth of LC–MS(MS) and EC-MS, since most anticancer drugs are thermolabile agents, and even when derivatization is possible, numerous derivatization steps are often required.

References

[1] E.A. de Bruijn, U.R. Tjaden, J. Chromatogr. B 531 (1990) 235.
[2] E.A. de Bruijn, in: E.A. de Bruijn (Ed.), Monitoring Anticancer Agents, Pasmans Press, The Hague, 1992, p. 24.
[3] G. Guetens, G. de Boeck, K. Van Cauwenberghe, E.A. de Bruijn, U.R. Tjaden, LC·GC Int. 12 (1999) 115.
[4] J. Salamoun, M. Mackay, M. Nechatal, M. Matousek, L. Knesel, J. Chromatogr. A 514 (1990) 179.
[5] J. Girault, B. Istin, J.B. Fourtillan, Biol. Mass Spectrom. 22 (1993) 395.
[6] R. Michailescu, H.Y. Aboul-Enein, M.D. Efstatide, Biomed. Chromatogr. 14 (2000).
[7] J.J. Wang, K.K. Chan, J. Chromatogr. B 674 (1995) 205.
[8] G. Momerency, K. Van Cauwenberghe, P.H.Th.J. Slee, A.T. Van Oosterom, E.A. de Bruijn, Biol. Mass Spectrom. 23 (1994) 149.
[9] M. Martino, V. Gilard, R. Martino, Curr. Pharm. Res. 5 (1999) 561.
[10] G. Momerency, K. Van Cauwenberghe, E.A. de Bruijn, A.T. Van Oosterom, M.S. Highley, P.G. Harper, J. High Resolut. Chromatogr. 17 (1994) 655.
[11] G. Momerency, K. Van Cauwenberghe, M.S. Highley, P.G. Harper, A.T. Van Oosterom, E.A. de Bruijn, J. Pharm. Sci. 85 (1996) 262.
[12] H. Dumez, G. Guetens, G. De Boeck, M. Highley, U.R. Tjaden, R. Maes, A. Hanauske, A.T. van Oosterom, E.A. de Bruijn, J. Sep. Sci. 24 (2001) 123.
[13] T. Steger-Hartmann, K. Kümmerer, J. Schecker, J. Chromatogr. A 726 (1996) 179.
[14] P.J. Sessink, R.P. Bos, Drug Saf. 20 (1999) 347.
[15] G. Vassal, M. Ré, A. Gouyette, J. Chromatogr. B 428 (1998) 357.
[16] M.H. Quernin, B. Poonkuizhali, C. Montes, R. Kishnamoorthly, D. Dennison, A. Srivatava, E. Vilmer, M. Chandy, E. Jacqz-Aigrain, J. Chromatogr. B 709 (1998) 47.
[17] W.K. Lai, C.P. Lang, L.K. Law, R. Wong, C.K. Li, P.M. Yuen, Clin. Chem. 44 (1998) 2506.
[18] C.A. Ritter, F. Bohnenstengel, U. Hofmann, H.K. Kroemer, B. Sperker, J. Chromatogr. B 730 (1999) 25.
[19] C.W. Keuzenkamp-Jansen, J.M. van Baal, R.A. De Abreu, J.G.N. de Jong, R. Zuiderent, J.M.F. Trijbels, Clin. Chem. 42 (1996) 380.
[20] H. Lu, K.K. Chan, J. Chromatogr. B 670 (1996) 219.
[21] J.A. Baptista, P. Goss, M. Nghiem, J.J. Krepinsky, M. Baker, J.W. Dennis, Clin. Chem. 10 (1994) 426.
[22] L.R. Philips, J.G. Supko, L. Malspeis, Anal. Biochem. 211 (1993) 16.

[23] R.J. van Maanen, R.D. van Ooijen, J.W. Zwikker, A.D. Huitema, S. Rodenhuis, J.H. Beijnen, J. Chromatogr. B 719 (1998) 103.
[24] G. De Boeck, K. Van Cauwenberghe, A.M. Eggermont, A.T. van Oosterom, E.A. de Bruijn, J. High Resolut. Chromatogr. 20 (1997) 697.
[25] C.G. Zambonin, A. Guerrieri, F. Palmisano, Anal. Chim. Acta 329 (1996) 143.
[26] D. Anderson, D.J. Kerr, C. Blesing, L.N. Seymour, J. Chromatogr. B 688 (1997) 87.