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Analysis of cyclophosphamide and five metabolites from human plasma using liquid chromatography–mass spectrometry and gas chromatography–nitrogen–phosphorus detection

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Abstract

An assay method for the quantification of cyclophosphamide (CY) and five metabolites from human plasma is presented. The procedure is adapted to the chemical properties of the compounds of interest: non-polar compounds are extracted into methylene chloride, concentrated and analyzed by GC–NPD after derivatization, and the remaining aqueous fraction is deproteinated with acetonitrile–methanol prior to separation via reversed-phase HPLC and detection using atmospheric pressure ionization (API)-MS. Standard curves are linear over the required range and reproducible over five months. Plasma concentration–time profiles of CY and metabolites from a patient receiving CY by intravenous infusion (60 mg/kg, once a day for two days) are presented.

Keywords: Cyclophosphamide; Deschloroethylcyclophosphamide; 4-Oxocyclophosphamide; Carboxyethylphosphoramide mustard; Phosphoramide mustard; Hydroxypropylphosphoramide mustard

1. Introduction

Cyclophosphamide (CY) is a phosphoramide form of nitrogen mustard commonly used in cancer treatment. In addition, its toxicity to lymphocytes has made it an important immunosuppresent agent in hematopoietic stem cell transplant therapies and, more recently, in treatment of inflammatory conditions such as systemic lupus erythematosis and rheumatoid arthritis, etc. [1]. Dose escalation of CY is limited by myelosuppression, cardiotoxicity, hemorrhagic cystitis and venoocclusive disease (VOD) of the liver. The parent drug itself is not cytotoxic. It must be activated, primarily by cytochrome P450 2C9 and 3A4 in the liver, to ''transport intermediates'' 4-hydroxycyclophosphamide (HCY) [2] and, possibly, 3-hydroxypropylphosphoramide mustard (HPPM) [3] which enter target cells and

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degrade chemically/enzymatically to produce the ultimate DNA alkylator-phosphoramide mustard (PM). There are a variety of alternative metabolic pathways for CY and HCY which result in deschloroethylcyclophosphamide (DCCY), carboxyethylphosphoramide mustard (CEPM), and 4-oxocyclophosphamide (KetoCY) all of which show very low cytotoxocity. A partial scheme of CY metabolism is shown in Fig. 1.

Attaining therapeutic versus toxic outcomes in patients treated with CY requires an understanding of the individual variation in both activation and detoxifying pathways [4,5] of the drug, which can be obtained through prospective studies analyzing all the compounds shown in Fig. 1. There are many analytical procedures for CY. Most use a gas chromatographic separation of derivatized CY with either mass spectrometry (MS) or nitrogen–phosphorus detection (NPD). The other compounds provide more of a challenge; they are UV transparent, highly polar and demonstrate varying degrees of instability. Over the years, a variety of methods have been developed using thin-layer chromatography (TLC) [6–8], colorimetry [9,10], high-performance liquid chromatography (HPLC) [11], gas chromatography (GC)–NPD or GC–MS [3,12–15], and direct insertion probe MS analysis for some or all of the compounds of interest. GC has emerged as the method of choice for analysis of these compounds. However, the multiplicity of products resulting from derivitization [14], poor extraction efficiency [12], and the instability of the individual metabolites [15] have all served to diminish the utility of this approach. These problems are compounded in high dose therapy, where concentrations of cyclophosphamide may be up to a thousand times those of the metabolites.

We have recently developed a method for HCY analysis using HPLC with UV detection after bedside stabilization as the *p*-nitrophenylhydrazone [5]. Herein we describe methodology which allows the non-polar (and generally more stable) components to

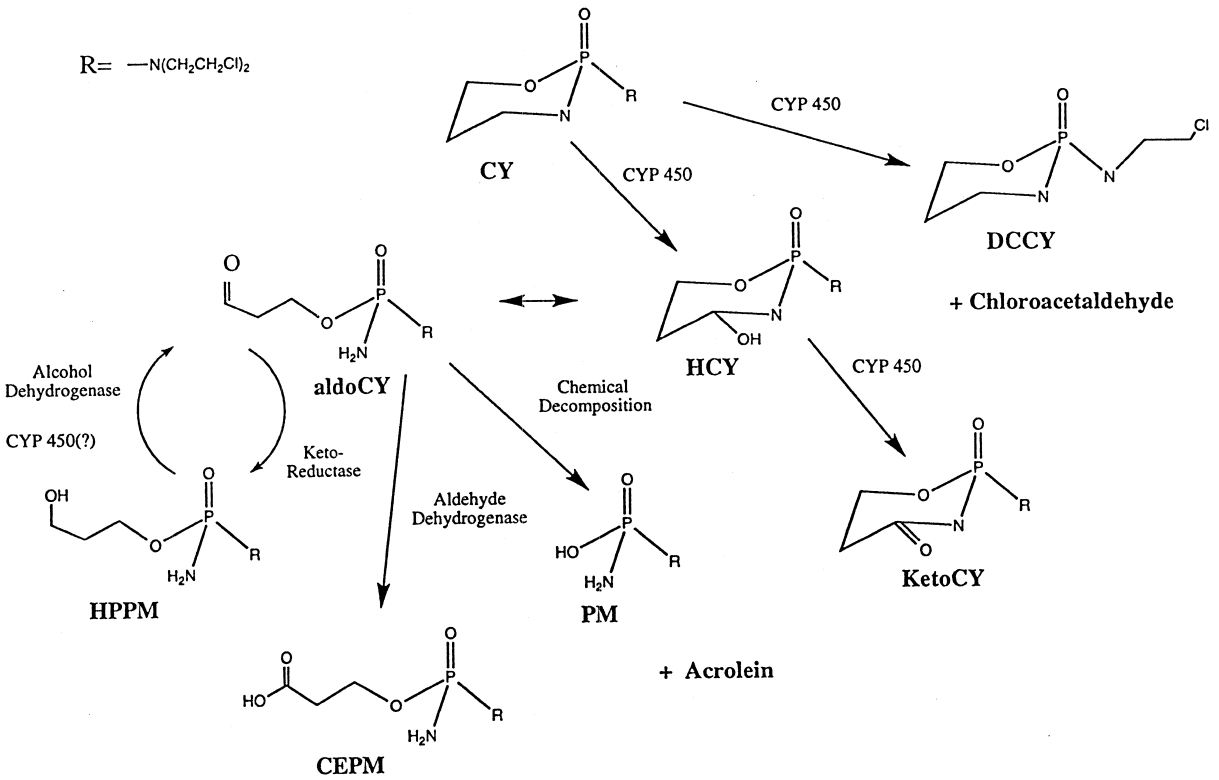

Fig. 1. Partial metabolic scheme of CY.

be extracted from plasma, derivatized, and analyzed by GC using either NPD or MS. The extracted aqueous phase is then deproteinated and analyzed for the more polar compounds by LC–MS using deuterated internal standards. Detection limits obtained for all compounds are in the low to sub-micromolar range and all analyses can be performed using a single 0.5-ml plasma sample.

2. Experimental

2.1. Materials

CY was obtained from Sigma as the monohydrate; DCCY, CEPM and PM were the generous gift of ASTA Medica (Frankfurt, Germany); HPPM was synthesized in the laboratory by the method of Takamizawa et al. [16]. β-Tetradeuterocarboxyethyl phosphoramide mustard (D_4-CEPM) and β-tetradeutero phosphoramide mustard (D_4-PM) were synthesized in the laboratory by the methods of Takamizawa et al. [17] and Grigg and Jarman [18], respectively, using β-D_4-mustard prepared by the method of Jardine et al. [15]. Analysis of the labeled mustard by MS and nuclear magnetic resonance (NMR) showed greater than 99% incorporation of deuterium into the mustard.

KetoCY was synthesized from 4-hydroperoxycyclophosphamide according to Takamizawa et al. [16] using a modified biphasic Fenton reaction. To 290 mg (1 mmol) of 4-hydroperoxycyclophosphamide dissolved in 10 ml methylene chloride at room temperature in a 50 ml roundbottomed flask equipped with a magnetic stirring bar was added 550 mg (2 mmol) ferrous sulfate heptahydrate dissolved in 10 ml water. The reaction flask was sealed and stirred vigorously. The aqueous phase immediately changed from pale green to bright orange. After 30 min the phases were separated and the aqueous phase was extracted exhaustively with methylene chloride (5×10 ml). The combined organic was dried over sodium sulfate, filtered and the solvent removed under vacuum. The resulting pale white solid was recrystallized from acetone resulting in 140 mg of white crystals, m.p. 147–149°C uncorrected, (literature values 148–149°C, [17] 149–150°C [18]). NMR and fast atom bombardment (FAB) MS were consistent with the proposed structure.

The internal standard, 2-dipropylamino-1,3,2-oxazaphosphorine-2-oxide (DPCY) the N',N' dipropyl analogue of CY used in the GC analysis, was synthesized by reacting bis-dipropyl amidophosophoroxydichloride with one equivalent of 3-amino-1-propanol in the presence of triethylamine. After filtration, the product was purified using flash chromatography with ether as the mobile phase.

2.2. Sample preparation

Samples were obtained from patients undergoing high dose CY treatment preceding bone marrow transplantation. Blood was collected over ethylenediaminetetraacetic acid (EDTA), and centrifuged and the plasma frozen within 5 min of blood sampling. Plasma was stored at −70°C until analyzed.

The sample preparation procedure is shown schematically in Fig. 2. Plasma samples and standards were thawed in water at room temperature. A 0.5-ml aliquot was transferred to a screw top tube

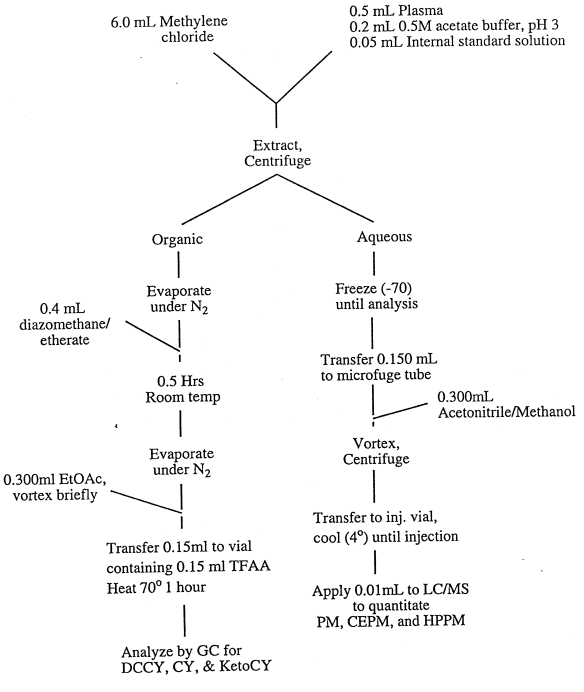

Fig. 2. Schematic of the sample work-up procedure for analysis of CY and five metabolites from plasma.

containing 6.0 ml methylene chloride at 0°C. Internal standard solution (50 μl) containing 300 μg/ml DPCY, and 100 μg/ml D_4-PM and D_4-CEPM was added to each tube and the pH adjusted to 4.0 by the addition of 200 μl 0.5 *M* ammonium acetate buffer, pH 3.0. Samples were sealed, extracted for 5.0 min, centrifuged at 4°C at 1700 *g* for 6 min and placed on ice. A 300-μl portion of the aqueous fraction containing PM, CEPM and HPPM was transferred to a microcentrifuge tube, frozen on dry ice immediately, and stored at −70°C until analysis.

The remaining aqueous portion and protein pellet was aspirated to waste. The methylene chloride phase containing DCCY, DPCY, CY and KetoCY was transferred to a clean tube, evaporated to dryness under nitrogen and the residue dissolved in 0.4 ml ethereal diazomethane. The samples were allowed to react at room temperature for 15 min at which time the solvent and excess diazomethane were evaporated under nitrogen. Samples were taken up in a mixture of ethyl acetate–trifluoroacetic anhydride (1:1) (300 μl), sealed in injection vials, and heated at 70°C for 1 h prior to GC analysis.

The aqueous fractions were thawed in water at room temperature immediately before analysis. A 150-μl fraction was vortexed with an equal volume of cold acetonitrile–methanol (4:1), centrifuged at 1200 *g* for 4 min, and the supernatant transferred to an injection vial.

Standards were prepared in blank plasma, and for GC analysis ranged from 12–400 μ*M* for CY, 0.6–20 μ*M* for Keto, and 0.75–25 μ*M* for DCCY. Those for LC analysis ranged from 3–100 μ*M* for PM and 1.5–50 μ*M* for CEPM and HPPM. These ranges were determined empirically from initial results in patients. Standards sufficient to construct thirty to fifty standard curves were prepared in a batch and stored under the same conditions as the samples such that each was thawed only once – just prior to use.

2.3. Instrumentation and analysis

GC analysis was carried out on a HP 5890 equipped with a Supelco SPB-1 30 m×0.32 mm column with 1 μm film thickness and a nitrogen–phosphorus detector. One microliter of the derivatized samples was injected onto the instrument using a 15:1 split. Initial temperatures were 250°C, 100°C, and 280°C for injector, column and detector, respectively. The column was ramped to 220°C at 40°C/min and then to 260°C at 5°C/min. Compounds of interest eluted between 5 and 9 min. The signal was integrated using HP Chemstation software.

LC–MS involved a Shimadzu LD-10AD solvent delivery system and an Alcott 738R autoinjector equipped with a cooled (4°C) sample tray. Ten microliters of the processed sample were applied to a Zorbax SB-C_8 15 cm×4.6 mm column. The samples were eluted isocratically with a mobile phase of MeOH–10 m*M* ammonium acetate (40:60), pH 4.0 at a flow of 1.0 ml/min. Five percent (50 μl/min) of the column effluent was introduced into a Micromass Quattro II tandem quadrupole mass spectrometer using electrospray (ES) ionization. The ES probe was maintained at 3.8 kV; the cone voltage at 30 eV; the source temperature was 80°C; and nitrogen was used as the nebulizing and bath gases. During assay development, the instrument was operated in the scan mode (50–500 Da); for quantitative purposes we used the single ion recording (SIR) mode monitoring *m*/*z* 221, 223 and 227 for PM and D_4-PM; *m*/*z* 279 and 281 for HPPM; and *m*/*z* 293, 295 and 299 for CEPM and D_4-CEPM. Data processing was carried out using MassLynx (Micromass, Manchester, UK) software.

3. Results and discussion

The synthesized compounds were all >95% chemically pure by NMR analysis. The isotopically labeled internal standards were prepared with four deuteriums on the β (halogenated) carbons of the nitrogen mustard moiety. The deuteriums in this site are very stable (pK_a~20) and exchange is impossible without total fragmentation of the molecule.

The extraction procedure described is based on earlier work by other investigators [12,15] that shows efficient, pH independent, extraction for CY and DCCY. KetoCY (pK_a~6.0) can only be extracted from acidic solutions. The subsequent derivatizations by treatment with diazomethane and trifluoroacetic anhydride are required to impart good chromatographic properties to the compounds and prevent their breakdown during GC analysis. The KetoCY, as a weak acid, forms the N-methyl derivative upon

treatment with diazomethane. The parent, CY and DCCY react with trifluoroacetic anhydride to form the mono and diacylated derivatives, respectively. Initial work with GC–MS confirmed the formulae of these derivatives. In all cases, with electron ionization (EI), the mass of the base peak was the putative derivative – 49 Da (CH_2Cl). When chemical ionization was used, the mass of the base peak was consistent with the suggested derivative – 36 Da HCl. The more polar metabolites, PM, HPPM and CEPM, are poorly soluble in organic solvent. Because of this, the difficulties involved in consistent derivatization procedures and the questionable stability of some of these derivatives, we opted for a mild LC–MS method.

The inter-day accuracy and precision data for four clinically relevant concentrations of each compound are shown in Table 1. The limit of quantification (LOQ) for DCCY and Keto is 0.75 and 0.6 μ*M*, respectively (bias <10%, RSD <15%). We set the limit of quantification for CY at 10 μ*M* using the range of standard concentrations (12–400 μ*M*) relevant to our clinical studies. The absolute limit of quantification is <1.0 μ*M*, but this requires an additional, low value standard curve. Under the current conditions, we monitor elimination of ca. 95% of the CY in patient plasma. Chromatograms of the compounds at the LOQ vs. a relevant blank are shown in Fig. 3.

Gas chromatograms of patient plasma are shown in Fig. 4. The first sample, taken before CY administration, showed that there were no endogenous

Table 1
Accuracy and precision of GC analysis (n=9 for all analyses)

Actual (μ*M*)	Found (μ*M*)	RSD (%)	% Bias[a]
DCCY			
25	25	0.16	0.02
7.5	7.47	2.27	−0.35
2.5	2.52	4.97	0.61
0.75	0.75	11.76	0.34
CY			
400	400.89	0.21	0.22
120	119.70	2.88	−0.25
40	41.51	4.27	3.76
12	12.62	10.43	5.19
Keto			
20	20.03	0.29	0.14
6.0	6.20	3.89	3.32
2.0	1.96	7.93	−1.99
0.6	0.65	13.31	8.96

[a] Bias=(Found−Actual)/Actual·100.

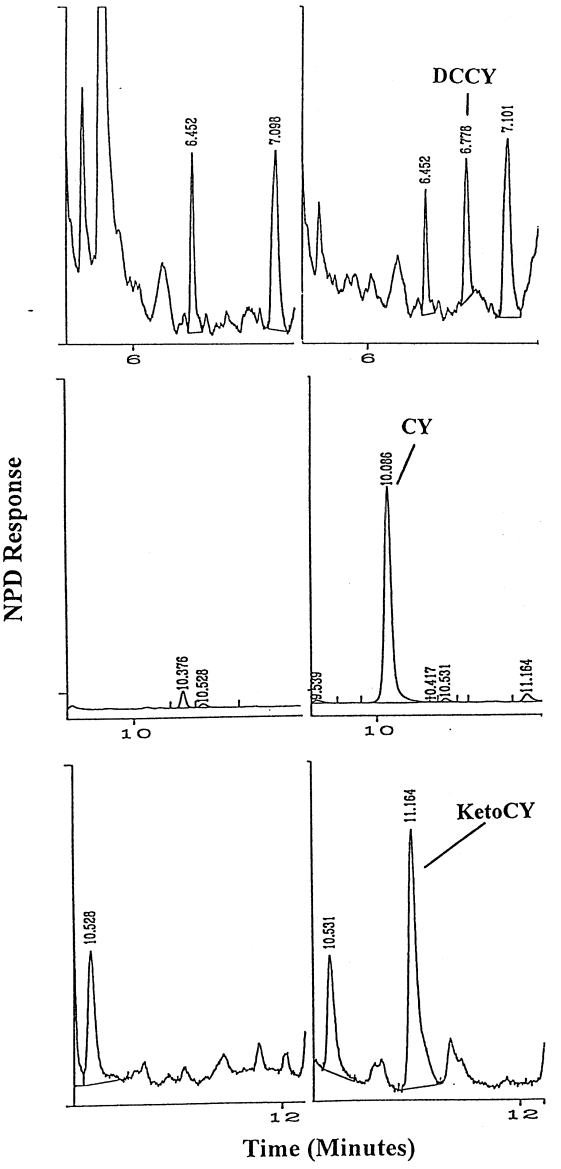

Fig. 3. Right panel: GC–NPD traces of plasma spiked with metabolites at LOQ: 0.6 μ*M* DCCY (top), 12 μ*M* CY (middle), and 0.6 μ*M* KetoCY (bottom). Left panel: corresponding sections of GC traces of blank plasma. The attenuation of the blank chromatograms is adjusted to correspond to the spiked samples.

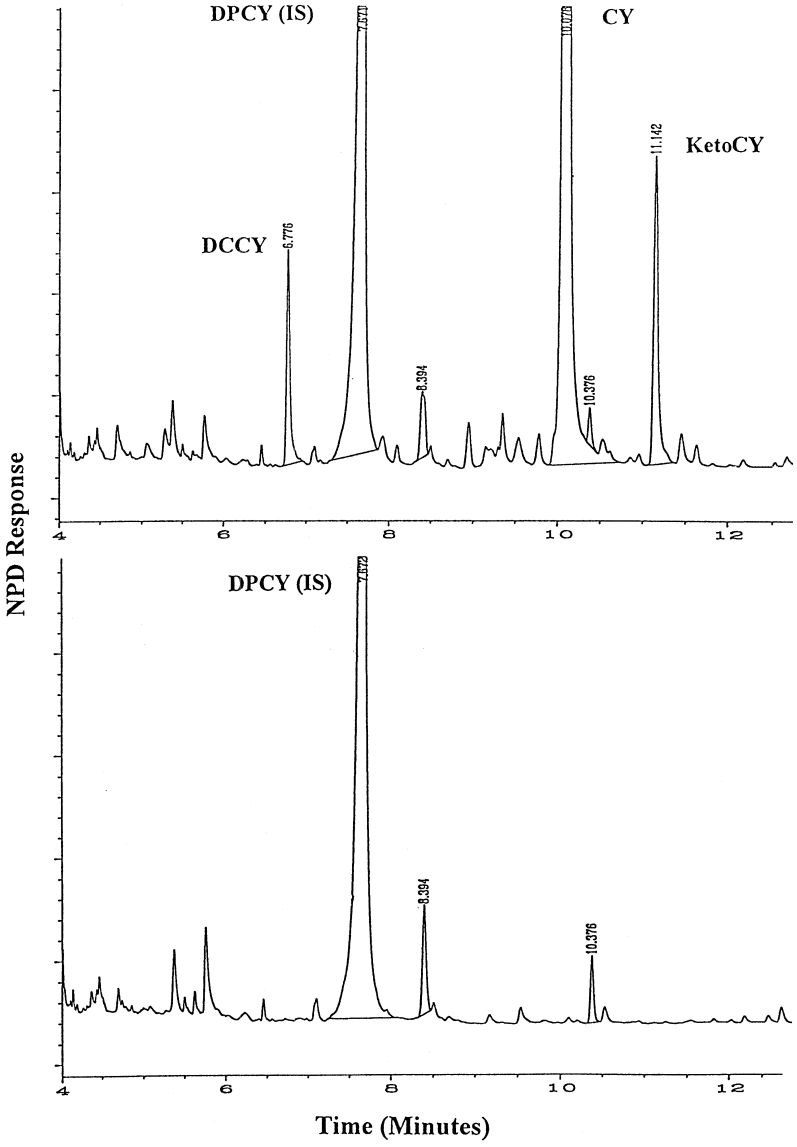

Fig. 4. Top panel: GC–NPD of patient plasma 33 h into CY therapy (9 h after the second 60 mg/kg dose). Retention times are 5.4 min (DCCY, 7.97 μ*M*), 6.0 min (DPCY), 7.7 min (CY, 90.6 μ*M*) and 8.6 min (KetoCY, 5.97 μ*M*). Bottom panel: GC–NPD of patient plasma prior to administration of CY. DPCY (internal standard) elutes at 6 min.

compounds interfering with the analysis. The second sample, taken 33 h into high-dose CY therapy, showed the signals well above background resulting from the compounds of interest. Elution times were: 6.5–6.8 min for DCCY, 7.4–7.7 min for DPCY, 9.9–10.2 min for CY, and 11.0–11.3 min for KetoCY. Ifosfamide, a structural isomer of CY, is frequently used as an internal standard for GC analyses of CY. However, the internal standard we synthesized gave more reproducible results than ifosfamide, which we found less than optimal for two reasons: (1) ifosfamide is less stable the cyclophosphamide and (2) the trifluoroacetic acid (TFA) derivative of ifosfamide chromatographs as a broad peak eluting

very close to CY. The DPCY was very stable and chromatographs as a sharp peak which did not interfere with any of the compounds of interest. The system had a broad dynamic range: the highest CY concentration (400 μ*M*) was over 650-times that of the lowest KetoCY concentration (0.6 μ*M*) yet both compounds could be quantified and the response was linear.

The MS scans of PM, HPPM and CEPM taken in the electrospray mode are shown in Fig. 5. All of the

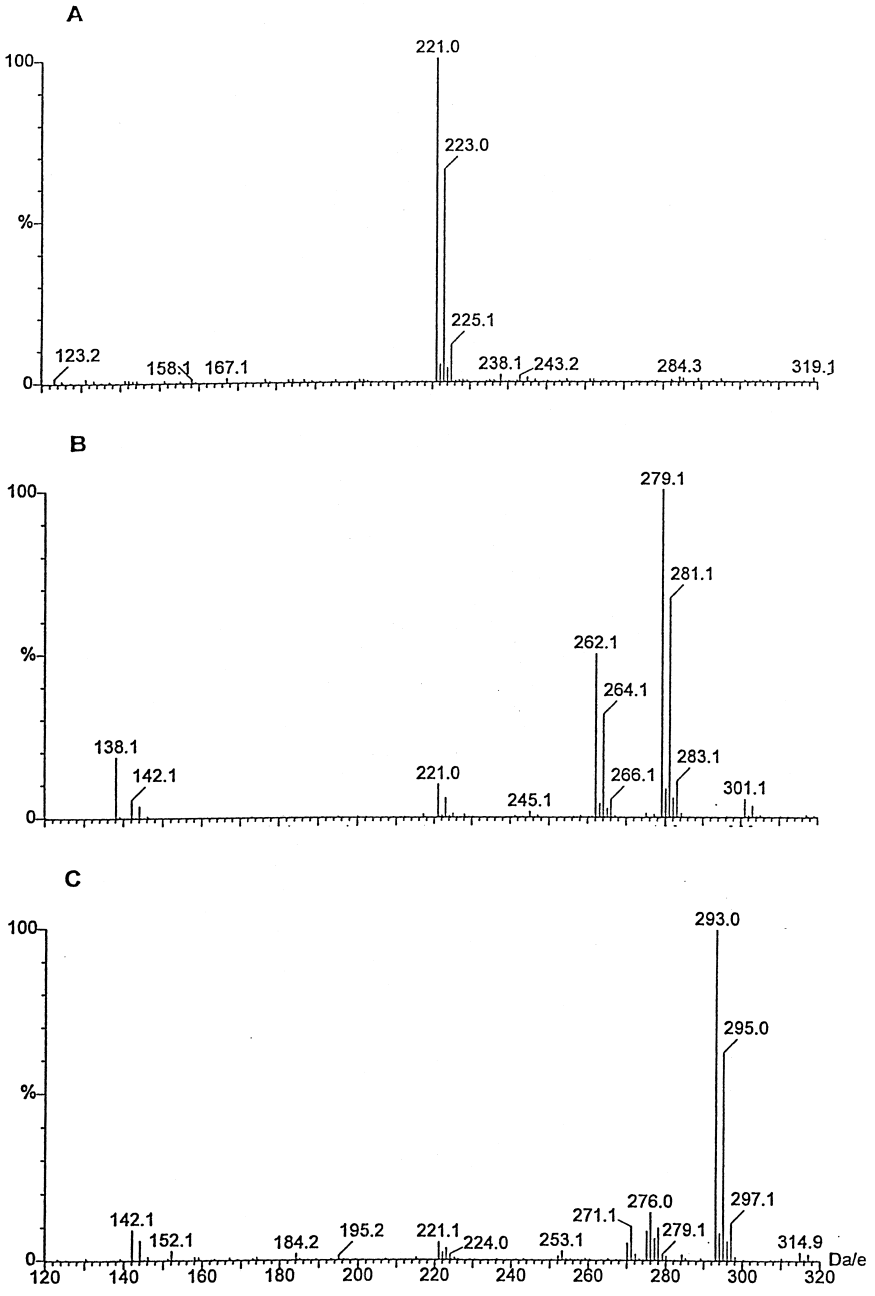

Fig. 5. Electrospray scans of PM, CEPM and HPPM, respectively, taken as described in Experimental.

compounds showed base peaks corresponding to the $^{35}Cl_2+1$ parent ion (i.e., *m/z* 221, 279 and 293, respectively) with the $^{35}Cl^{37}Cl+1$(b+2) signal equivalent to 66% the intensity of base peak. The b+4 peak resulting from $^{37}Cl_2+1$ is 10% of the parent peak. Fragmentation was minimal under the conditions shown: all compounds showed signals at *m/z* 142 and 144 consistent with nitrogen mustard cation. HPPM and CEPM both demonstrated peaks at *m/z* 221 and 223, corresponding to PM formed from phosphate ester hydrolysis and loss of a three carbon fragment (the propionic acid radical in the case of CEPM and the *n*-propanol radical in the case of HPPM). CEPM and HPPM also demonstrated clusters of signals at b-17 and b-18 consistent with deamination and dehydration, respectively. Fragmentation decreased with cone voltage and source temperature. Absolute sensitivity, however, also decreased as these variables were lowered. The preferred settings depended on the quality of the chromatography, the analyte concentration in the samples, and the interference from sample components.

The D_4-CEPM and D_4-PM material produced similar scans, with all fragments including the nitrogen mustard increased by 4 Da (data not shown). The

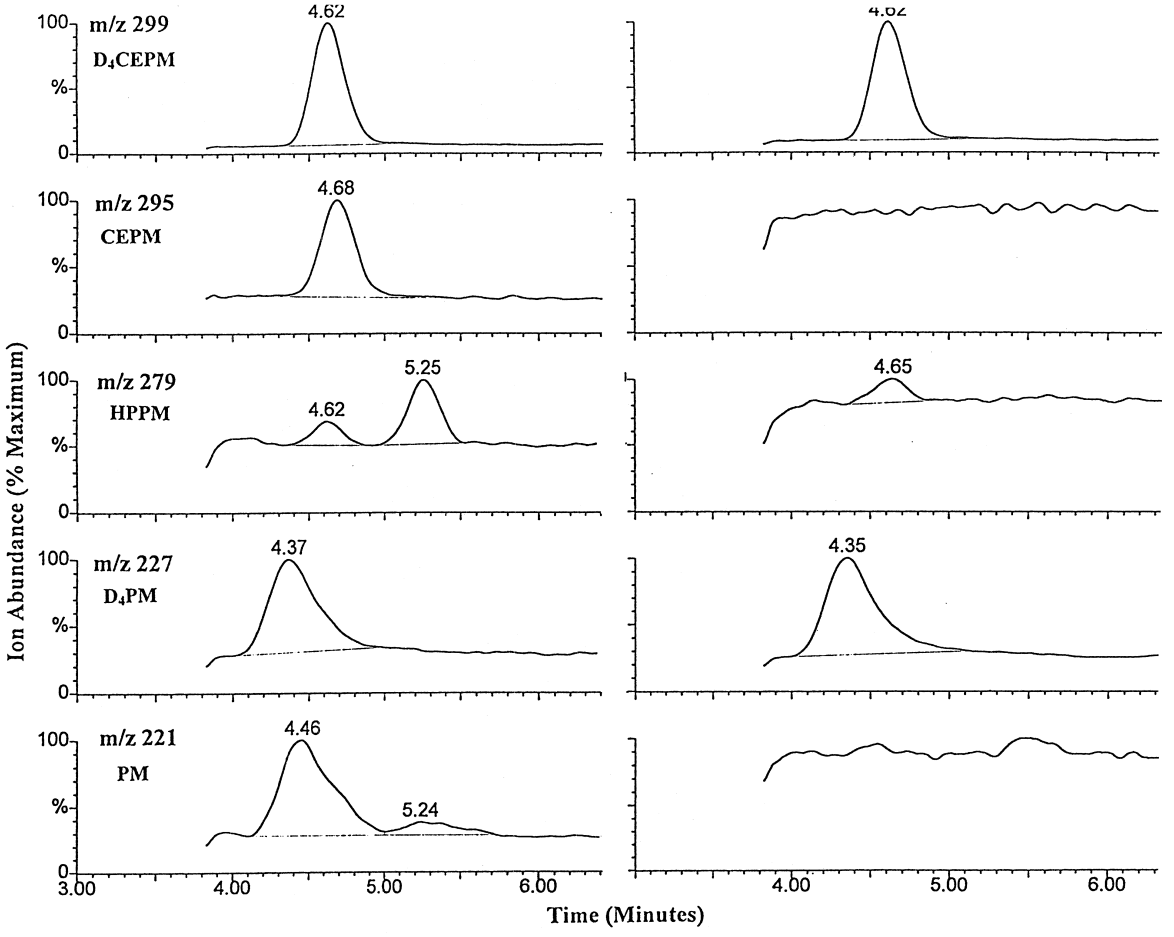

Fig. 6. Left panel: LC–MS of patient 33 h into CY therapy (9 h after the second 60 mg/kg dose). Peaks are: 4.6 *m/z* 299 D_4-CEPM, 4.6 min, *m/z* 295, CEPM (15.5 μ*M*); 5.2 min, *m/z* 279, HPPM (2.89 μ*M*); 4.4 min, *m/z* 227, D_4-PM and 4.4 min *m/z* 221, PM (33.6 μ*M*). Right panel: LC–MS chromatogram of same patient samples obtained prior to CY infusion. The peak at 4.62 min, *m/z* 299 is D_4-CEPM, and that at 4.4 min, *m/z* 227 is D_4-PM.

parent peaks of the internal standards, the D_4-$^{35}Cl_2$ CEPM (m/z 297) and PM (m/z 225) were the same as the $^{37}Cl_2$ signals from the protonated analogues. To avoid the corrections such overlap would require, we monitored signals resulting from the D_4-$^{35}Cl^{37}Cl$ species, m/z 299 for CEPM and m/z 227 for PM as internal standards. These channels showed less than 1% interfering signal from the proteo analogues even at the highest metabolite concentrations monitored in patient plasma.

Because both HPPM and CEPM undergo hydrolysis at the source, chromatographic separation of these compounds is essential to avoid overestimation of PM. There is also potential for cross contamination from the D_4-CEPM into the HPPM channels resulting from dehydration, which reinforces the need for separation between these two compounds. Using a mobile phase of methanol–acetate buffer, pH 4 we achieved virtually baseline separation between the peaks while limiting the total run time to 8.0 min. Phosphoramide mustard elutes first at 4.3–4.6 min followed by CEPM (4.6–5.0 min) and HPPM (5.2–5.5 min). Fig. 6 shows chromatograms of patient plasma prior to and 33 h into high-dose CY therapy (9 h after the second 60 mg/kg dose) both with internal standard added. The blank shows that there was virtually no interfering material and that there was no detectable protonated species contaminating the deuterated internal standards. Analysis of double blank samples proved that there were no indigenous compounds coeluting with the internal standards (data not shown). Both the parent and the parent+2 ions are routinely assayed. Over several months, we occasionally have found the p+2 channels (m/z 223 for PM, m/z 295 for CEPM, and m/z 281 for HPPM) had less interference from plasma constituents. Therefore, we have sometimes used them for quantification despite the fact they have a lower abundance. The accuracy and precision data on the MS aspect of assay is shown in Table 2. The limit of quantification has been established at 3.0 μM for PM and 1.50 μM for CEPM and HPPM. The RSDs of repeated assays have been less than 15% in all cases. Chromatograms of blank plasma spiked with compounds at the LOQ concentrations are shown in Fig. 7. The assay is linear to the low μM range, easily covering the requirements of high dose cyclophosphamide therapy. There has been concern about the stability of these compounds in the injection solution. Any breakdown has been minimized by using a cooled (4°C) injector tray. While there is some loss of peak response over time, this is probably due to chromatographic deterioration and/or loss of MS sensitivity with time. Reanalysis of standard curves after 24 h shows no significant changes in the slopes.

Table 2
Accuracy and precision of LC–MS analysis (n=9 for all analyses)

Actual (μM)	Found (μM)	RSD (%)	% Bias[a]
PM			
100	99.4	0.22	−0.60
30	29.12	2.25	−2.93
10	9.96	4.25	−0.43
3.0	3.27	14.94	9.10
CEPM			
50	50.38	0.17	0.76
15	15.06	2.24	0.38
5	5.06	3.48	1.26
1.5	1.47	8.84	−2.02
Keto			
50	50.02	0.45	0.05
15	14.67	5.46	−2.23
5	5.0	5.42	−0.09
1.5	1.60	12.73	6.93

[a] Bias=(Found−Actual)/Actual·100.

Ideally, we would have had adequate samples to reanalyze samples to confirm the stability of patient samples over prolonged storage periods. Because of the stress these patients were undergoing in cancer therapy, this was not possible. Instead, multiple standard curves were made up in plasma and analyzed over a five-month period. The reproducibility of both the GC and LC–MS aspects of the assay (Tables 3 and 4, respectively) over this period demonstrates not only the stability of the compounds at −70°C, but also the over all ruggedness of the method.

Table 3
Inter-day variability of response for GC–NPD analysis of CY and metabolites over five months

Metabolite	N	Average slope	SD	RSD (%)
DCCY	21	0.0023	0.00023	9.97
CY	21	0.0112	0.00085	7.65
KetoCY	21	0.0120	0.00092	7.68

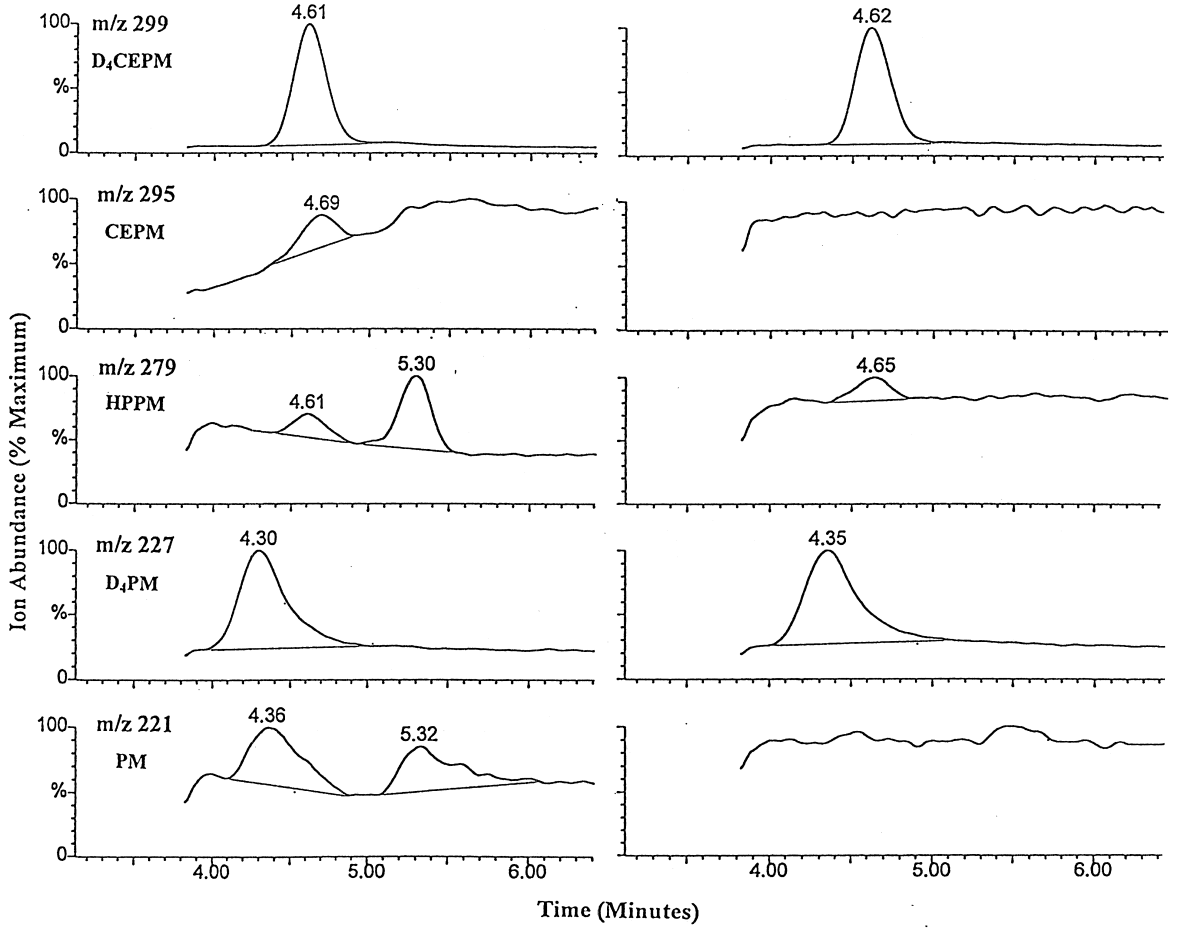

Fig. 7. Left panel: human plasma samples spiked with metabolite standards at the limit of detection; *m/z* 295 1.5 μ*M* CEPM, *m/z* 279 1.5 μ*M* HPPM, and *m/z* 221 3 μ*M* PM, *m/z* 299 and 227 D_4-CEPM and D_4-PM, respectively. Right panel: blank plasma with deuterated internal standards (PM and CEPM).

Table 4
Inter- and intra-day variability of response parameters for LC–MS analysis of CY metabolites over five months.

Day	No. of runs[a]	PM		CEPM		HPPM	
		Mean slope	RSD (%)	Mean slope	RSD (%)	Mean slope	RSD (%)
1	3	0.035	3.30	0.029	3.65		
2	3	0.034	4.02	0.027	0.89		
3	4	0.045	3.14	0.033	5.13	0.046	17.0
4	4	0.032	3.67	0.031	3.50	0.039	8.0
Inter-day		0.036	15.90	0.30	8.61	0.042	11.6

[a] Number of standard curves on a given day.

While it would be possible to use solely LC–MS for the analysis of all the relevant compounds, this would be less than desirable for a number of reasons. First, the amount of time required for each sample would be doubled, if not tripled, requiring a gradient system and a post run reequilibration. Secondly, such a procedure could not have involved an extraction; deproteinated plasma including various amounts of lipids and lipophilic compounds would be applied to the mass spectrometer decreasing its performance with time. Finally, we have found the GC–NPD to be very reliable, the column is exposed to only ~0.04 μl derivatizing solution per injection and has lasted over thousands of analyses.

The MS system we used is a triple quadrupole instrument capable of multiple reaction monitoring (MRM). This was not necessary for the analysis of the metabolites of interest, and its was to the general benefit to make the system adaptable to as many laboratories as possible. Initial investigations into the utility of MS–MS techniques implied a propensity of the parent ion of PM to cleave into several low-abundance-mass fragments limiting the benefit of the more sophisticated procedure.

In conclusion, the method described herein provided a convenient assay for cyclophosphamide and five major metabolites. The procedures were simple, complementary and fast. No solid-phase extraction columns were used (as is the case with many of the alternatives); the procedures were inexpensive, easily batched, and did not require concentration of the analytes. Complete drying of extracts containing PM, HPPM and CEPM prior to the addition of water-sensitive derivatizing reagents was not necessary as is required by GC assays. The small (0.33 μl) volumes of plasma introduced into the MS minimized loss of sensitivity in large runs. The concentration of acetonitrile in the sample relative to the mobile phase limited the maximum that could be applied to the column – larger injections resulted in fronting of the peaks. In test runs, we removed some of the acetonitrile under nitrogen prior to analysis. We were then able to make larger injections of the slightly concentrated solution to increase sensitivity about four-fold (data not shown). We have not done this as a matter of course because currently, analyte concentrations of patient samples fall within the limits of the assay. A pharmacokinetic profile of CY and five metabolites in a patient is shown in Fig. 8. Overall, the method maximizes speed, sensitivity and precision to make possible complete clinical studies on large numbers of patients.

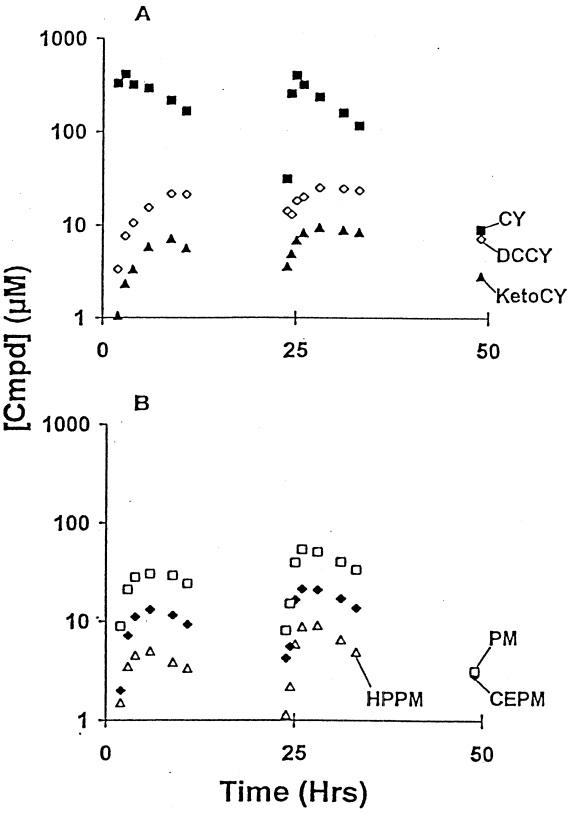

Fig. 8. Top panel (A): time course of CY, DCCY and KetoCY concentrations in plasma analyzed by GC–NPD and bottom panel (B): CEPM, HPPM and PM analyzed by LC–MS.

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References

[1] N.E. Sladek, in: G. Powers (Ed.), Anticancer Drugs: Reactive Metabolism and Drugs Interactions, Pergamon Press, New York, 1994, pp. 79–156.

[2] S. Ren, J.-S. Yang, T.F. Kalhorn, J.T. Slattery, Cancer Res. 57 (1997) 4229–4235.
[3] P.S. Hong, K.C. Chan, Biomed. Environ. Mass Spectrom. 14 (1987) 167–172.
[4] L.J. Ayash, J.E. Wright, O. Tretyakov, R. Gonin, A. Elias, C. Wheeler, J.P. Eder, A. Rosowsky, K. Antman, E. Frei, J. Clin. Oncol. 10 (1992) 995–1000.
[5] J.T. Slattery, T.F. Kalhorn, G.B. McDonald, K. Lambert, C.D. Buckner, W.I. Bensinger, C. Anasetti, F.R. Appelbaum, J Clin. Oncol. 14 (1996) 1484–1494.
[6] A.F.A. Hadidi, J.R. Idle, J. Chromotogr. 427 (1988) 121–130.
[7] A.F.A. Hadidi, C.E.A. Coulter, J.R. Idle, Cancer Res. 48 (1988) 5167–5171.
[8] R.F. Struck, D.S. Alberts, K. Horne, J.G. Phillips, Y. Peng, D.J. Roe, Cancer Res. 47 (1987) 2726.
[9] O.M. Friedman, E. Boger, Anal. Chem. 33 (1961) 906–910.
[10] F.Y.F. Lee, Br. J. Cancer 63 (1991) 45–50.
[11] R.W. Hardy, M.J. Moore, C. Ehrlichman, S.J. Soldin, Ther. Drug Monit. 9 (1987) 221–226.
[12] G. Momercy, K. Van Cauwenberghe, P.H. Slee, A.T. Van Oosterom, E.A. DeBruijn, Biol. Mass Spectrom. 23 (1994) 149–158.
[13] P.S. Hong, K.C. Chan, J. Chromotogr. 495 (1989) 131–138.
[14] I. Jardine, R. Brundrett, M. Colvin, C. Fenselau, Cancer Treat. Reports 60 (1976) 403–408.
[15] I. Jardine, C. Fenselau, M. Appler, M.-N. Kan, R.B. Brundrett, M. Colvin, Cancer Res. 38 (1978) 408–415.
[16] A. Takamizawa, Y. Tochino, Y. Hamashimi, T. Iwata, Chem. Pharm. Bull. 20 (1972) 1612–1616.
[17] A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi, O. Shiratori, J. Med. Chem. 18 (1975) 376–383.
[18] L.J. Grigg, M. Jarman, J. Med. Chem. 18 (1975) 1102–1106.