Simultaneous determination of ifosfamide and its metabolite ifosforamide mustard in human plasma by high-performance liquid chromatography

Summary

Because ifosforamide mustard is the active alkylating metabolite of ifosfamide, it is of particular interest in the pharmacokinetic analysis of patients undergoing ifosfamide treatment. This paper presents an assay for the simultaneous determination of ifosforamide mustard and ifosfamide after derivatization with diethyldithiocarbamate (DDTC), subsequent liquid-liquid extraction of the plasma with acetonitrile and using reversed phase high-performance liquid chromatography with ultra-violet detection at 276 nm. Structural confirmation of the analytes was accomplished using mass spectrometry (MS). Reaction conditions such as incubation duration, temperature and concentration of derivatization agent were investigated; 30 min at 70°C with 100 mg/ml DDTC was optimal. The presented analytical method proved to be accurate, precise and linear for ifosforamide mustard and ifosfamide concentrations, ranging from 0.100-50.0 and 0.100-100 µg/ml, respectively, and with lower limits of quantification of 0.100 µg/ml for both analytes. A typical patient pharmacokinetic profile is presented to demonstrate the applicability of the assay in clinical samples. The analytical method could be employed in high-throughput clinical analysis of ifosforamide mustard and ifosfamide patient samples.

Introduction

Ifosfamide, \textit{N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine 2-oxide}
Chapter 2.3

(Holoxan®, Ifex®) is commonly used for the treatment of various solid tumours, soft tissue sarcomas and haematological malignancies in adults and children. Ifosfamide is a prodrug, which needs activation via the cytochrome P450 enzymatic system in the liver to exert its alkylating activity. First, ifosfamide is hydroxylated to 4-hydroxyifosfamide as represented in figure 1.

![Diagram of ifosfamide metabolism](image)

**Figure 1.** Metabolism of ifosfamide. 4-Hydroxyifosfamide exists in equilibrium with its tautomeric form aldoifosfamide. Aldoifosfamide can be converted into the final alkylating agent ifosforamide mustard with concurrent acrolein formation. Besides activation de-activation occurs, yielding 2-dechloroethylifosfamide and 3-dechloroethylifosfamide with equimolar release of neurotoxic...
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chloroacetaldehyde. In the (tumour)cell ifosfamide mustard reacts with DNA causing interstrand cross-links, leading to cell death.\[^2\] Ifosfamide is known to increase its own metabolism (autoinduction).\[^3\]

![Chemical structures of ifosfamide mustard and ifosfamide with diethyldithiocarbamate (DDTC).](image)

\[^2\] Ifosfamide is known to increase its own metabolism (autoinduction).

\[^3\] Derivatization of ifosfamide mustard and ifosfamide with diethyldithiocarbamate (DDTC).

Recently, an assay was reported for ifosfamide mustard.\[^4\] This method consisted of direct derivatization of ifosfamide mustard in plasma with diethyldithiocarbamate (DDTC) and subsequent solid phase extraction (SPE) of the resulting derivative. The samples were stabilized by adding semicarbazide and sodium chloride, which prevent auto-catalytic breakdown of 4-hydroxyifosfamide to ifosfamide mustard and further ifosfamide mustard breakdown, respectively. During large scale implementation of this method various problems were encountered. These were mainly clogging of the SPE columns, low analytical column half-life, and late co-eluting peaks, preventing rapid use in clinical investigations with high sample throughput. A faster more reproducible method has now been developed for ifosfamide mustard, which also permitted simultaneous determination of ifosfamide. No easy applicable assay for both ifosfamide mustard and ifosfamide by high-performance liquid chromatography has been described thus far. Derivatization of ifosfamide mustard (IFM) and ifosfamide (IFO) with DDTC leads to IFM(DDTC)\(_2\) and IFO(DDTC) as depicted in figure 2. Structural confirmation of the analytes and assay validation were performed for both
Materials and Methods

Equipment

The high-performance liquid chromatographic equipment consisted of an integrated system with a model SCM1000 in-line degassing unit, a model P1000 pump, a model AS3000 automated injector with a 100-µl loop, a model UV150 ultra-violet (UV) detector operated at 276 nm and a DataJet integrator (Thermo Separation Products, Breda, The Netherlands). Data were analysed using PC1000 software (Thermo Separation Products, Breda, The Netherlands) using a Dell optiplex Gs Pentium personal computer. A 250 x 40 mm I.D. RP8 5 µm particle size Prodigy 5 C8 column with a Security Guard C8 pre-column (Phenomenex, Torrance, CA, USA) was operated at ambient temperature. The analytical column was washed monthly with 1-propanol in order to maintain high separation efficiency. The mobile phase consisted of acetonitrile (AcN):water (32:68 v/v) containing 25 mM K₂HPO₄ buffer pH 8.0. For identification of the derivatives a mass spectrometer (MS) was used operating with direct continuous split-injection (Sciex, Thornhill, ON, Canada). Electrospray ionization (1500 V) was achieved using a Turbolon™ sample inlet. Ions were created at atmospheric pressure and were transferred to a model API 365 triple quadrupole mass spectrometer (LC/ESI-MS/MS) with a dwell-time of 0.1-1 msec. The channel electron multiplier was set at 1900 V. For optimising the wavelength of detection, the UV-absorption spectrum was recorded on-line using a Waters model 996 Photodiode-Array (PDA) detector (Waters Chromatography, Milford, MA, USA).

Chemicals

Ifosforamide mustard (D-18847, Lot 034.5333) and all other ifosfamide and cyclophosphamide metabolites were a kind gift of Dr J. Pohl, ASTA Medica AG (Frankfurt, Germany). Sodium diethyldithiocarbamate was obtained from Sigma (St. Louis, Mo, USA). AcN and methanol (HPLC supra-gradient) were purchased from Biosolve (Valkenswaard, The Netherlands). K₂HPO₄, sodium chloride, hydrochloric acid and sodium hydroxide (suprapure grade) were obtained from Merck (Darmstadt, Germany). Semicarbazide hydrochloride (p.a. grade) was purchased from Acros (Geel, Belgium). Distilled water was used throughout.

Preparation of standards

Blank, drug-free plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands). Plasma was centrifuged at 1,000 g for 5 min and the pellet was discarded. To the supernatant a solution containing 2 M semicarbazide, 1 M sodium chloride pH 7.40 (SCZ*) was added, yielding a mixture of plasma:SCZ* 10:1 v/v (plasma*). After storage of at least one hour at -70°C the plasma* was thawed and centrifuged again at 1,000 g for 5 min. The supernatant was used as blank plasma in the assay. Ifosforamide mustard was dissolved as a 1 mg/ml solution in a phosphate buffer (1 M sodium chloride, 0.1 M K₂HPO₄, pH=8.00)(PPB) immediately prior to preparation of the
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standards. Ifosfamide was also dissolved as a 1 mg/ml solution in PPB. 350 µl Ifosforamide mustard and ifosfamide solutions in PPB were added to 500 µl plasma* in a 2.0 ml Eppendorf cup, resulting in combined calibration curves of 0, 0.100, 0.250, 0.500, 1.00, 2.50, 5.00, 10.0 and 50.00 µg/ml ifosforamide mustard and 0, 0.100, 0.500, 1.00, 5.00, 10.0, 25.0, 50.0 and 100 µg/ml ifosfamide. Quality control samples were prepared similarly but from different stock solutions and separate weighting of the standards at 0.100, 0.250, 5.00 and 50.0 µg/ml ifosforamide mustard and 0.100, 0.500, 50.0 and 100 µg/ml ifosfamide.

Sample pretreatment

The derivatising agent was added as a 50-µl volume of 100 mg/ml DDTC in PPB to 500 µl plasma* and 350 µl PPB. After whirl-mixing for 15 sec the samples were placed for 30 min at 70°C in a thermostatically controlled waterbath. After incubation the samples were placed on ice water in order to stop the derivatization reaction. Liquid-liquid extraction was performed by adding 1,000 µl AcN. After whirl-mixing thoroughly three times for 1 min and subsequent centrifugation at 3,000 g for 10 min at 4°C, a 700-µl volume of the AcN fraction was transferred to a clean 1.5 ml Eppendorf cup and evaporated to dryness under a gentle stream of nitrogen at 40°C. The samples were reconstituted by adding 150 µl mobile phase. After whirl-mixing for 1 min and subsequent centrifugation at 3,000 g for 5 min at ambient temperature, the supernatants of the samples were transferred to 200 µl inserts in vials. The injected volume was 50 µl.

Patient sample collection and pretreatment

Whole blood samples were collected and immediately placed on ice water, centrifuged at 1,000 g for 5 min at 4°C and 1 ml plasma was transferred to a 2-ml Eppendorf cup containing 100 µl SCZ*, yielding plasma*. After thorough vortex mixing the samples were stored at -70°C. The entire sample handling was always performed within 10 min. After thawing, a 500-µl volume of patient plasma* was prepared in duplicate, identical to standard sample pretreatment.

Identification

Identification of derivatized ifosforamide mustard was achieved by derivatising an 850 µl volume of 5 mg/ml ifosforamide mustard in PPB with 50 µl 500 mg/ml DDTC in water and incubation and extraction with AcN as described for the plasma samples. After evaporation of the organic extract, the sample was reconstituted in 100 µl mobile phase and injected in the high-performance liquid chromatographic system. The analyte was isolated by collecting the fraction containing the ifosforamide mustard-derivative. The eluate was again extracted with 2:1 (v/v) AcN after addition of 1 g sodium chloride. After whirl-mixing and centrifugation the AcN-layer was transferred and evaporated as described above. Since, the residue could be contaminated by sodium chloride dissolved in co-extracted water in the AcN-layer, the dry residue was again extracted with 1 ml AcN. After transfer of the AcN-layer and subsequent evaporation the clean residue was stored at -70°C pending identification by MS. Derivatization
of ifosfamide yields two products as represented in figure 2, with two corresponding peaks in
the high-performance liquid chromatographic chromatogram. The ifosfamide-derivatives were
isolated similarly by collecting both peaks separately. Partial re-injection of the final AcN-layer
on the high-performance liquid chromatographic system confirmed the purity of the isolated
ifosfamide mustard and ifosfamide-derivatives. The isolated derivatives were reconstituted
in 80% methanol solution and analysed by MS for identification.

**Optimization of derivatization**

Derivatization conditions were changed to find the optimal incubation temperature, duration
and DDTC concentration. Variations in conditions were tested with 0.500 and 5.00 µg/ml
ifosfamide mustard spiked plasma in duplicate. Derivatization temperature was investigated
at ambient temperature (20°C), 40°C and 70°C for 5, 10, 30 and 60 min and the reaction
duration at 70°C for 0, 5, 10, 20, 30, 60 and 120 min. To find the optimal derivatization reagent
concentration tests were performed at 70°C for 30 min with 0, 0.100, 0.500, 1.00, 5.00, 10.0,
50.0, 100 and 500 mg/ml DDTC.

**Specificity and selectivity**

Potential interference from endogenous compounds was investigated by analysing six
different blank plasma samples. The following compounds were investigated for interference
with the analytical method: 2-dechloroethylifosfamide, 3-dechloroethyl-ifosfamide,
didechloroethylifosfamide, 4-ketoifosfamide, carboxyifosfamide, 4-hydroxy-ifosfamide with
and 4-hydroxyifosfamide without semicarbazide stabilization. Possible co-medication was
also tested for interference. Tested substances were topotecan, paclitaxel, ketoconazole,
granisetron, dexamethasone, acetaminophen, oxazepam, temazepam, caffeine, furosemide
and sodium 2-mercaptoethane sulphonate (MESNA). All compounds were tested at a
concentration of 20 µg/ml.

**Limit of quantification**

The lower limits of quantification (LLQ) of ifosfamide mustard and ifosfamide were
investigated in plasma samples from three different donors by a five-fold determination in
three analytical runs. For the concentration to be accepted as the LLQ, the percentage
deviation from the nominal concentration (measure of accuracy) and the relative standard
deviation (measure of precision) were to be less than 20%. The upper limits of quantification
(ULQ) of ifosfamide mustard and ifosfamide were defined as 50.0 and 100 µg/ml,
respectively, based upon the expected clinical concentration range.

**Validation: accuracy, precision and linearity**

Accuracy, between-day and within-day precisions of the method were determined by
assaying five replicate quality control samples in plasma at four different ifosfamide
mustard and ifosfamide concentrations (0.100, 0.250, 5.00 and 50.0 µg/ml and 0.100, 0.500,
50.0 and 100 µg/ml, respectively) in three different analytical runs. Accuracy was measured
as a percentage of the nominal concentration. The within-day and between-day precisions were obtained by analysis of variance (ANOVA) for each test concentration using the analytical run as grouping variable. For the construction of each calibration curve eight spiked plasma samples were analysed in duplicate. After optimization of their weighting factors, linearity of the plasma calibration curves was tested with the F-test for lack of fit.

**Extraction recovery**

Recovery of extraction of the ifosforamide mustard-derivative was determined by dissolving the isolated ifosforamide mustard-derivative in PPB, resulting in theoretical 1.00 and 10.0 μg/ml solutions which were diluted further. Full recovery (without sample extraction) was achieved by addition of AcN to the derivative solution in PPB (68:32 v/v), producing a solution identical to the mobile phase, which was injected directly into the high-performance liquid chromatographic system. Spiked plasma samples were extracted, transferred, evaporated and subsequently reconstituted in mobile phase and injected on the high-performance liquid chromatographic system as described above. Furthermore, 25 mM K₂HPO₄ pH 8.00 was also added to the AcN-layer after extraction (68:32 v/v) of additional samples, producing a solution identical to the mobile phase. Recoveries were calculated by comparing the directly injected ifosforamide mustard-derivative to the extracted samples with and without the evaporation-step.

**Statistics**

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1 (SPSS, Chicago, IL, USA). Correlations were considered statistically significant if calculated p-values were 0.05 or less.

**Results and discussion**

**Chromatography and detection**

The chromatographic method enabled sufficient separation of the analytes. No ion-exchange modifier was used since it did not prove to be essential and better column endurance could be obtained without it. Figure 3 represents typical chromatograms of a blank sample, a patient sample of 3.20 and 14.9 μg/ml ifosforamide mustard and ifosfamide, respectively, and a spiked sample of 5.00 and 25.0 μg/ml ifosforamide mustard and ifosfamide, respectively.
Figure 3. Typical chromatograms of a blank (bottom), a patient (middle) and a spiked (top) plasma sample with 0, 3.20 and 5.00 µg/ml ifosfamide mustard (11.0 min) and 0, 14.9 and 25.0 µg/ml ifosfamide (29.8 and 31.5 min), respectively.

The patient sample was taken at the end of a 1-hour 1.2 g/m² ifosfamide intravenous (i.v.) infusion. Noteworthy are the large injection peak consisting of non-retained DDTC and (derivatized) matrix components, the IFM(DDTC)₂ peak at 11.0 min and the IFO(DDTC) peaks at 29.8 and 31.5 min, respectively. Since the ratio of the exo- and endocyclic ifosfamide-derivative peaks was always equal to 5:1 and it can be expected that the molar absorptivities of the compounds are identical, the sum of both peaks was used in the quantification of ifosfamide.
Sample pretreatment

Besides stabilization of underivatized ifosfoamide mustard,[4] the addition of sodium chloride in PPB to the plasma sample also prevented mixing of the aqueous and organic-phases during the liquid-liquid extraction enabling easy transfer of the organic-layer and preventing undesired co-extraction of water-soluble plasma components. SCZ* addition prevented autocatalytic degradation of 4-hydroxyifosfamide. After a freeze-thaw cycle and subsequent centrifugation, addition of SCZ* to blank or patient plasma resulted in a clear plasma layer and a pellet. If the plasma was not subjected to this procedure no reproducible separation of the aqueous and organic-layer could be obtained. The pellet possibly contained plasma components that otherwise prevent a clear separation of the phases.

Identification

The peak of the ifosfoamide mustard-derivative was identified as double derivatized IFM(DDTC)$_2$, as depicted in figure 4. The molecular weight of IFM(DDTC)$_2$ is 446.1, as depicted in figure 2. Consequently, the parent-peak (MH$^+$) can be observed at a mass-to-charge ratio (m/z) of 447. The sodium and potassium-adducts were observed at m/z 469 and 485, respectively. A dimer (M$^+_2$) was observed at m/z 892 with its typical sodium-adduct at m/z 915. The fragment at m/z 116 and m/z 176 may be explained by CSN(C$_2$H$_5$)$_2$ and C$_2$H$_5$SCSN(C$_2$H$_5$)$_2$ fragments, respectively. The two ifosfamide peaks were identified as isomeric monomers of IFO(DDTC), as depicted in figure 2. The DDTC could react with either the chloroethylgroup attached to the exo- or endocyclic nitrogen atom. The molecular weight of IFO(DDTC) is 373.1. Figures 5 and 6 demonstrate similar MS patterns for both isomers. Although differences in relative intensity between fragments were observed, no unique fragments could be distinguished between the two isomers. The MH$^+$ was observed at m/z 374 with its typical Cl atom isotope peaks. The sodium and potassium-adducts were observed at m/z 396 and 411. A dimer (M$^+_2$) was observed at m/z 746 with its sodium and potassium-adducts at m/z 769 and 785. Since none of the theoretical possible fragments of either the exo- or endocyclic derivatized ifosfamide were observed uniquely with MS, further identification was performed by refragmentation of the parent-peak (MS$^3$). MS$^3$ of peak 1 yielded fragments at m/z 92 and 120. These fragments may be explained by CH$_2$NHC$_2$H$_4$Cl and C$_3$H$_6$NHC$_2$H$_4$Cl, respectively.
**Figure 4.** Mass-spectrum of ifosfamide mustard di-derivative (IFM(DDTC))₂. IFM(DDTC)₂ was isolated at 11.0 min.

**Figure 5.** Mass-spectrum of the exocyclic ifosfamide mono-derivative (IFO(DDTC)) isolated at 29.8 min.
Figure 6. Mass-spectrum of the endocyclic ifosfamide mono-derivative (IFO(DDTC)) isolated at 31.5 min.

The exocyclic derivative can yield these fragments, the endocyclic derivative cannot. Therefore, it was concluded that ifosfamide peak 1 was the exocyclic derivatized ifosfamide and ifosfamide peak 2 was the endocyclic derivatized ifosfamide. The UV-absorption spectrum is shown in figure 7. A maximum is observed at 276 nm.

Figure 7. Ultra-violet absorption spectrum of ifosfamide mustard di-derivative.
Specificity and selectivity

After sample pretreatment, plasma samples spiked with 2-dechloroethylifosfamide, didechloroethylifosfamide, carboxyifosfamide, topotecan, paclitaxel, acetaminophen, caffeine, furosemide and MESNA did not display any interference with the method. Non-interfering peaks were detected for 3-dechloroethylifosfamide (9.0 min), 4-ketoifosfamide (19.8 min), ketoconazole (83.5 min), granisetron (6.0 min), dexamethasone (12.5 min), oxazepam (14.5, 21.3 and 26.4 min), temaze pam (22.8 min), compared to ifosforamide mustard (11.0 min). Stabilized 4-hydroxyifosfamide (with semicarbazide) resulted in a peak at 11.4 min. Unstabilized 4-hydroxylfosfamide was spontaneously converted to ifosforamide mustard (11.0 min). Blank plasma samples of six different individuals showed no interfering endogenous substances in the analysis. Addition of semicarbazide did not interfere with the chromatography.

Optimization of derivatization

The derivatization temperature did not demonstrate an optimum, but the variability with 0.500 and 5.00 µg/ml ifosforamide mustard at 70°C was less than at 20°C and 40°C. Therefore, 70°C was selected as derivatization temperature. Optimization of derivatization duration scaled to 100% is presented in figure 8.

![Figure 8. Effect of incubation duration on derivatization of ifosforamide mustard (IFM) with diethylidithiocarbamate.](image-url)
The optimum derivatization duration was set at 30 min. The effect of DDTC concentration on the derivatization of ifosforamide mustard is presented in figure 9. Between 0 and 5.00 mg/ml no ifosforamide mustard-derivative could be detected. Between 25.0 and 500 mg/ml DDTC a log-linear relationship between derivative response and DDTC concentration is observed. Although no optimum was reached, chromatographic separation between the unretained DDTC peak and the ifosforamide mustard-derivative deteriorated at reagent concentrations of 500 mg/ml. Therefore, 100 mg/ml DDTC was chosen as the most optimal concentration for derivatization.

**Limit of quantification**

In three analytical runs the LLQs of ifosforamide mustard and ifosfamide were determined in five-fold. The mean percentage deviation from the nominal concentration at 0.100 µg/ml ifosforamide mustard and ifosfamide were 10.2 and 1.9% with a relative standard deviation of 17.2 and 11.0%, respectively. Therefore, both LLQs were determined to be 0.100 µg/ml.

**Validation: accuracy, precision and linearity**

The results from the validation of the method in human plasma are listed in table 1. The use of the weighting factor of \(1/(\text{conc.})^2\) resulted in a minimal sum of squares of residuals from the nominal concentrations. The method proved to be accurate for both analytes (average accuracy at four different concentrations between 89.5 and 110.0% of the nominal concentrations) and precise (within-day precision ranged from 2.8 to 15.8% and between-day precision ranged from 2.0 to 10.0%). Correlation coefficients \(r^2\) of calibration curves were always higher than 0.995 as determined by least sum of squares analysis. Calibration curves of ifosforamide mustard and ifosfamide proved to be linear in the range of 0.100-50.0 and
0.100-100 µg/ml, respectively, without lack of fit. Furthermore, no systematic proportional or additive errors were observed.

Table 1. Accuracy, within-day and between-day precisions of ifosfamide mustard (IFM) and ifosfamide (IFO) analysis in human plasma.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentrations (µg/ml)</th>
<th>Accuracy (%)</th>
<th>Precision (%)</th>
<th>Within-day</th>
<th>Between-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFM</td>
<td>0.100</td>
<td>110.0±17.0</td>
<td>100.6-119.7</td>
<td>15.8</td>
<td>N.A.</td>
</tr>
<tr>
<td>IFM</td>
<td>0.250</td>
<td>107.2±15.2</td>
<td>98.7-115.4</td>
<td>11.3</td>
<td>10.0</td>
</tr>
<tr>
<td>IFM</td>
<td>5.00</td>
<td>106.6±8.0</td>
<td>102.1-111.0</td>
<td>4.5</td>
<td>7.2</td>
</tr>
<tr>
<td>IFM</td>
<td>50.0</td>
<td>101.4±8.3</td>
<td>96.8-106.0</td>
<td>2.8</td>
<td>9.2</td>
</tr>
<tr>
<td>IFO</td>
<td>0.100</td>
<td>101.9±11.0</td>
<td>95.8-108.0</td>
<td>11.2</td>
<td>N.A.</td>
</tr>
<tr>
<td>IFO</td>
<td>0.500</td>
<td>94.6±5.1</td>
<td>91.8-97.4</td>
<td>5.1</td>
<td>2.0</td>
</tr>
<tr>
<td>IFO</td>
<td>50.0</td>
<td>90.6±5.3</td>
<td>87.6-93.5</td>
<td>4.2</td>
<td>4.9</td>
</tr>
<tr>
<td>IFO</td>
<td>100</td>
<td>89.5±3.6</td>
<td>87.5-91.5</td>
<td>4.0</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

Abbreviations: S.D.= standard deviation, C.I.= confidence interval, N.A.= not applicable, between-day variation did not exceed within-day variation.

Extraction recovery

Extraction recoveries (mean ± coefficient of variation) of the ifosfamide mustard-derivative from plasma with the evaporation-step were 62.2 ± 6.7 and 62.4 ± 13.4% for 1.00 and 10.0 µg/ml ifosfamide mustard (n=3), respectively. Extraction recoveries of the ifosfamide mustard-derivative from plasma without evaporation-step were 62.9 ± 8.4 and 59.8 ± 3.3% for 1.00 and 10.0 µg/ml ifosfamide mustard spiked sample (n=3), respectively. It is obvious that there is no significant extraction-loss of ifosfamide mustard due to the evaporation-step.

Analysis of patient samples

Figure 10 represents a typical pharmacokinetic profile of a patient receiving 3 g/m² ifosfamide i.v. in three hours on two consecutive days. Ifosfamide and ifosfamide mustard concentrations accumulated over time, but were also eliminated faster on the second day. This resulted in similar maximum ifosfamide concentrations on day 1 and 2. But ifosfamide mustard concentrations were substantially higher on day 2, because more ifosfamide was metabolized to ifosfamide mustard on day 2. This phenomenon can be explained by the autoinduction of ifosfamide and its effect on the metabolism.
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Figure 10. Ifosfamide mustard (IFM, lower curve) and ifosfamide (IFO, upper curve) concentrations in plasma in a patient receiving 3 g/m² ifosfamide i.v. in three hours on two consecutive days.

Conclusions

An analytical method for the simultaneous determination of ifosfamide mustard and ifosfamide in human plasma was described. This technique employed derivatization with DDTC followed by deproteinization with AcN. Quantification was achieved by reversed phase high-performance liquid chromatographic with UV-detection. Identification of the derivatized analytes was accomplished using MS and LC-PDA. Incubation settings were optimized. It is our experience that the presented assay can be readily used in a hospital laboratory environment for simultaneous monitoring of ifosfamide mustard and ifosfamide concentrations in patients.

References
