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# Liquid chromatographic analysis and preliminary pharmacokinetics of methotrexate in cancer patients co-treated with docetaxel

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**Abstract**

A new HPLC method has been developed for the quantitative determination of methotrexate (MTX) and its 7-hydroxyl metabolite in human plasma. Samples were purified by protein precipitation with acetone and methanol, and a sample clean-up with a mixture of *n*-butanol and diethyl ether. The analytes were separated on an RP Inertsil ODS-80A column and eluted in a solvent system containing 5% (v/v) tetrahydrofuran in water (pH 2.0). UV absorption measurement was performed at 313 nm, and the detector response was linear in a concentration range of 10–10 000 ng/ml. The lower limit of quantitation of MTX was 10 ng/ml using 1 ml sample aliquots. Values for accuracy and (within-run and between-run) precision were between 95.5–111% and 3.69–11.0%, respectively, at four concentrations analyzed in quintuplicate on four separate occasions. The assay was applied to study the effects of docetaxel co-administration on the pharmacokinetics and metabolism of MTX in cancer patients. 

*Keywords:* Methotrexate; Docetaxel

## 1. Introduction

Methotrexate (MTX) is a folic acid analogue having an amino group substituted for the hydroxyl function at the C4 position on the pteridine ring (Fig. 1). This substitution converts the molecule to a tight-binding inhibitor of the enzyme dehydrofolate reductase, thereby preventing cancer cells from maintaining levels of reduced folates required to sustain purine and pyrimidine synthesis [1]. Clinical trials conducted during the last few decades demonstrated the efficacy of MTX in the treatment of various human neoplastic disorders, including childhood acute leukemia [2], head and neck cancer [3], and micrometastases of osteosarcoma [4].

The concept of high dose MTX administration with folinic acid (leucovorin) rescue to mitigate toxic side effects, first described by Goldin et al. [5], has been successfully applied in the treatment of various tumors, and high-dose MTX treatment regimens are now commonly included in therapeutic programs. The pioneering work conducted by Evans and coworkers has shown that there is a concentration effect relationship for high-dose MTX in pediatric patients with acute lymphocytic leukemia [6], and that outcome of treatment could be improved if doses were individualized to prevent low systemic expo-

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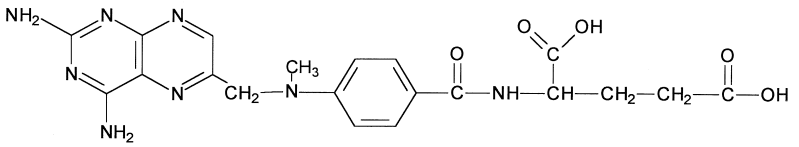
**Methotrexate (MTX)**



**7-Hydroxy-methotrexate (7-OH-MTX)**

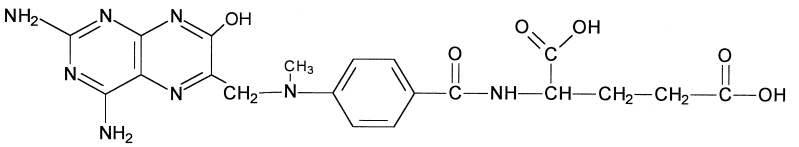


Fig. 1. Chemical structures of MTX and its metabolite 7-OH-MTX.

Table 1
HPLC methods available for the analysis of MTX in human plasma

| Sample Pretreatment[a] | IS[e] (nm) | Column (ng/ml) | Detection | LVC[e] | Ref. |
|---|---|---|---|---|---|
| LLE (PA/EA-IP)[e] | DMBA[e] | Partisil 10-SAX | UV (254) | 90 | [15] |
| LLE (ACN/E-B) | – | μBondapak $C_{18}$ | UV (303) | 10 | [16] |
| LLE (ACN/EA) | – | Partisil 10-PXS | UV (313) | 100 | [17] |
| LLE (TCA) | Sulphafurazole | LiChrosorb RP18 | UV (254+313) | 40 | [18] |
| LLE (EA) | Theophylline | LiChrospher RP18 | UV (313) | 40 | [19] |
| LLE (EA) | Acetophenone | Novapak RP18 | UV (313) | 40 | [20] |
| LLE (ACN/CF) | – | LiChrospher RP8 | UV (307) | 200 | [21] |
| LLE (TCA) | 2-OH-folic acid | μBondapak $C_{18}$ | FL (275/410)[b] | 10 | [22] |
| none | – | Silasorb $C_{18}$ | FL (360/417)[c] | 15 | [23] |
| SPE (Certify-II) | – | PE $C_{18}$ | FL (350/435)[c] | 0.2 | [24–26] |
| LLE (ACN/EA) | – | ODS/TM | FL (367/463)[d] | 50 | [27] |
| SPE (Certify-II) | – | ODS/TM | FL (367/463)[d] | 50 | [28] |
| SPE (Certify-II) | Aminopterin | PRP-1 | FL (350/435)[c] | 1 | [29] |
| Column-switching | – LiChrospher RP18 | alkyl-diol C8 and | UV (307) | 10 | [30] |

[a] LLE procedures: PA/EA-IP=perchloric acid protein precipitation plus ethyl acetate–isopropanol extraction; ACN/E-B=acetonitrile protein precipitation plus ethyl ether–*n*-butanol extraction; ACN/EA=acetonitrile protein precipitation plus ethyl acetate extraction; TCA=trichloroacetic acid protein precipitation; EA=ethyl acetate extraction; ACN/CF=acetonitrile protein precipitation plus chloroform extraction.

[b] Potassium permanganate oxidation.

[c] Photooxidation at 254 nm in the presence of hydrogen peroxide.

[d] Cerium (IV) trihydroxyhydroperoxide oxidation.

[e] Abbreviations: I.S., internal standard; LVC, lowest validated concentration; DMBA, *n*-[4[[2,4-diamino-6-quinazolinyl)methylamino]benzoyl]]aspartic acid; PP, protein precipitation; LLE, liquid–liquid extraction; SPE, solid-phase extraction; FL, fluorescence with excitation and emission wavelength in parenthesis.

sure [7]. In cancer patients, attempts to individualize MTX doses based on pharmacokinetic data in order to decrease toxicity with the dosing of leucovorin have also been used [8].

To enable pharmacokinetic analysis, numerous methods have been developed for the determination of MTX in biological specimens, including enzyme-inhibition assays [9,10], protein-binding assays [11], radio- and enzyme immunoassays [12,13] and HPLC assays [14–30]. Due to the lack of specificity and accuracy of non-chromatographic procedures for MTX analysis, HPLC (Table 1) has emerged as the technique of choice for accurate pharmacokinetic drug monitoring [14]. The most sensitive methods (with quantitation limits below 1 ng/ml) involve solid-phase extraction coupled with pre- or post-column derivatization of MTX by a (photo)chemical oxidative cleavage to highly fluorescent products. These methods, however, have limited clinical applicability because of their time-consuming and expensive sample preparation and required equipment. Here, we describe a new method for the quantitative determination of MTX and its inactive 7-hydroxyl metabolite (7-OH-MTX) in human plasma samples using solvent extraction prior to RP-HPLC with UV detection. The method has been subjected to a rigorous validation procedure [32], and was applied to a clinical pharmacokinetic study in cancer patients receiving MTX either alone or in combination with docetaxel.

## 2. Experimental

### 2.1. Materials

MTX (4-amino-10-methylfolic acid; batch: 17H0704) with a purity of >98.0% was obtained from Sigma Chemicals (St. Louis, MO, USA). An analytical reference standard of the metabolite 7-OH-MTX was prepared using a biosynthetic procedure similar to that reported by Cairnes and Evans [31]. For this purpose, four Wistar rat livers were homogenized at 4°C in 250 ml Tris-hydrochloride (pH 7.6) containing 10 m*M* magnesium chloride (Sigma) using an Ultra-Turrax T25 blender (IKA-Labortechnik, Dottingen, Germany). After preincubation at 37°C for 30 min in a shaking water-bath, 50 mg MTX was added to the homogenate. The metabolic reaction was terminated by protein precipitation with 1 *M* aqueous trichloroacetic acid (Sigma). Dimethyl sulfoxide (DMSO), diethyl ether, *n*-butanol, methanol and acetone were all of the highest grade available from Rathburn (Walkerburn, UK). Perchloric acid [70–72% (v/v) in water], 98% formic acid, ammonium hydroxide [20% (v/v) in water], tetrahydrofuran and sodium hydroxide were supplied by Baker (Deventer, The Netherlands). All water used in the experiments was filtered and deionized with a Milli-Q-UF system (Millipore, Milford, MA, USA). Blank human plasma samples for construction of calibration curves originated from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands).

### 2.2. Analytical standards

Stock solutions of MTX were prepared in triplicate by dissolving *X* mg of MTX in *X* * 454.4/508.5 ml DMSO to correct for the presence of molecular hydrates (3 mole water per mole MTX), resulting in solutions containing 1.00 mg/ml. Spiked plasma samples used as calibration curves were prepared daily by addition of 25 μl of serial dilutions in water, in duplicate, of the stock solution of MTX to 975 μl drug-free human plasma, resulting in calibration standards of 10, 25, 100, 500, 1000, 5000 and 10 000 ng/ml. Three pools of quality control (QC) samples of MTX were prepared in human plasma in the concentrations of 50, 3500 and 7500 ng/ml, by addition of 500 μl of a 5000 ng/ml dilution in water, 175 μl of the 1 mg/ml stock solution or 375 μl of the 1 mg/ml stock solution, respectively, to a 50 ml volumetric flask filled to the mark with human plasma. Samples for determination of the lower limit of quantitation (LLQ) were also prepared on a daily basis identical to the standards of the calibration curve, in blank plasma specimens from five different individuals, at a concentration of 10 ng/ml.

### 2.3. Sample preparation

An aliquot of 1 ml acetone was added to 1000 μl human plasma in a 2.0 ml polypropylene vial (Eppendorf, Hamburg, Germany). After vigorous

mixing for 5 min on a multi-tube vortex-mixer, the sample was centrifuged for 5 min at 23 000 *g* at ambient temperature. The clear supernatant was then further processed with 5 ml of *n*-butanol–diethyl ether (3:4, v/v) in a 12 ml glass tube supplied with a poly(tetrafluoroethylene)-covered screw cap by vortex mixing for 5 min, followed by centrifugation for 5 min at 4000 *g*. Next, approximately 0.5 ml of the lower water phase was transferred to a clean 1.5 ml tube (Eppendorf), which was centrifuged for 5 min at 23 000 *g*. In a final clean-up step, a 100 μl volume of the water phase was accurately transferred to a clean 1.5 ml tube and 1 ml methanol was added. After vortex-mixing for 1 min, the sample was centrifuged again for 5 min at 23 000 *g* and the supernatant dried under a gentle stream of nitrogen at 80°C for a fixed time period of 20 min. The dried residue was reconstituted in 100 μl aqueous ammonium hydroxide (0.02%, v/v), centrifuged for 5 min at 4000 *g*, and an aliquot of 75 μl was injected into the HPLC system using low volume inserts of glass.

### 2.4. HPLC instrumentation and conditions

The HPLC system consisted of a constaMetric 3200 solvent delivery system (LDC Analytical, Riviera Beach, FL, USA), a Waters Model 717 Plus autosampling device (Milford, MA, USA) and a Spectra Physics Model UV-2000 detector (San Jose, CA, USA). Separations were achieved at 60°C on a stainless steel analytical column (150×4.6 mm, I.D.) packed with 5 μm (particle size) Inertsil ODS-80A material (Alltech, Breda, The Netherlands). The mobile phase was composed of 5% (v/v) tetrahydrofuran in water, with the pH adjusted to 2.0 using perchloric acid. The column effluent was monitored by UV absorption measurements with the detector set at 313 nm. Peak recording and integration were performed with the ChromCard data analysis system (Fisons, Milan, Italy). Calibration curves were fitted by weighted ($1/x^2$) linear regression analysis by using the peak area of MTX versus the concentrations of the nominal standards.

### 2.5. Method validation

The validation procedures were performed according to the guidelines recorded in the conference report on 'Analytical Methods Validation: Bioavailability, Bioequivalance and Pharmacokinetic Studies' [32], with minor modifications as described previously [33]. All validation runs were performed on four consecutive days, and included a calibration curve processed in duplicate and a set of QC samples in quintuplicate analyzed with repeated cycles of freezing and thawing. The accuracy or percentage deviation (DEV) was calculated by the formula:

$$\text{DEV} = (\text{observed concentration}/\text{nominal concentration}) \times 100\% \quad (1)$$

The precision was calculated by one-way analysis of variance (ANOVA) for each test concentration, using the run-day as the classification variable. The between-group mean square (bgMS), the within-group mean square (wgMS) and the grand mean (GM) of the observed concentrations across run days were calculated using the software package Number Cruncher Statistical System version 5.X (J.L. Hintze, Kaysville, UT, USA), on an IBM compatible computer. The between-run precision (BRP) was calculated as:

$$\text{BRP} = \{[(\text{bgMS} - \text{wgMS})/n]^{0.5}/\text{GM}\} \times 100\% \quad (2)$$

where $n$ is the number of replicates within each analysis day. The within-run precision (WRP) was calculated as:

$$\text{WRP} = \{(\text{wgMS})^{0.5}/\text{GM}\} \times 100\%$$

### 2.6. Pharmacologic studies

The pharmacokinetics of MTX and 7-OH-MTX were studied in six adult patients with histologically confirmed diagnosis of a malignant solid tumor that was refractory to standard forms of therapy. MTX was provided by Pharmachemie (Haarlem, The Netherlands) and was administered as a 5 min i.v. infusion at a dose level of 30 mg/m$^2$ on days 1 and 15. Each patient received docetaxel (Rhône-Poulenc Rorer, Antony Cedex, France) as a 1 h i.v. infusion at a dose level of 75 mg/m$^2$ either on day 1 (immediately following MTX administration; $n=3$) or on day 2 (24 h after MTX administration; $n=3$). Premedication for hypersensitivity reactions was

uniform for all patients and consisted of dexamethasone (8 mg p.o., twice daily), starting 24 h before docetaxel administration, for a total of 3 days. The clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent forms before entering the pharmacokinetic study.

In each patient, sufficient plasma was obtained before drug administration to evaluate possible interfering peaks in the HPLC analysis. Blood samples for the analysis of MTX and 7-OH-MTX were obtained at the following time points: before MTX infusion; at the end of MTX infusion (5 min); and 0.5, 2, 4, 6, 24, and 48 h after the end of MTX infusion. All blood samples were drawn from a vein in the arm opposite to that used for drug administration. Samples were collected in glass tubes containing lithium heparin and centrifuged immediately for 5 min at 3000 *g* to yield plasma, which was stored frozen at −80°C until the time of analysis. Evaluation of docetaxel pharmacokinetics was evaluated using samples obtained before docetaxel infusion, and 0.5, 1, 2, 4, 6, and 24 h after start of docetaxel infusion, using HPLC analysis as described [33].

The plasma concentration–time curves of MTX, 7-OH-MTX and docetaxel were analyzed using the pharmacokinetic program Siphar version 4.0 (SIMED, Créteil, France). The area under the plasma concentration–time curve (AUC) was calculated by model-independent analysis up to the last sampling time point with a detectable drug level using the linear trapezoidal rule, with extrapolation to infinity using the observed concentration at the last sampling time point. The peak plasma concentration was put on par with the observed drug level in the sample taken at the end of the infusion. The elimination rate constant ($k_{el}$) was obtained from log-linear regression analysis of the final disposition phase, and served to calculate the terminal disposition half-life ($t_{1/2}$, i.e. $0.693/k_{el}$) The total plasma clearance (CL) was calculated by dividing the total dose administered (expressed in mg per square meter body surface area) by the AUC. Parameters for all compounds are reported as mean values±standard deviation. The difference in pharmacokinetic parameters between the MTX administration days and between patient cohorts was evaluated statistically using a non-parametric matched-pairs test and the 90% confidence intervals. Probability values (two-sided) of less than 0.05 were regarded as statistically significant. All calculations were performed using the NCSS statistical package.

## 3. Results and discussion

### *3.1. Chromatography and validation*

Chromatographic analysis was performed using an Inertsil ODS-80A column, in combination with a mobile phase containing tetrahydrofuran. The present HPLC assay was based on a previous extraction and isolation technique consisting of protein precipitation with acetone followed by a clean-up procedure with solvent extraction using a mixture of *n*-butanol–diethyl ether [16]. Using this earlier procedure, however, the extraction mixtures consistently caused a gradual increase in pressure through blocking of the column by particulate matter. To assure sufficient selectivity, acceptable extraction efficiency, and minimize endogenous interferences from the plasma matrix, an additional protein precipitation step with neat methanol was required.

Blank human plasma samples obtained from six different individuals showed no substances after extraction interfering with MTX or 7-OH-MTX. Plasma samples collected from patients immediately prior to drug administration were also free from interfering endogenous compounds (Fig. 2). Interference analysis with a number of drugs, including docetaxel, commonly co-administered with MTX did also not reveal the presence of chromatographic peaks with retention times similar to that of MTX or the metabolite (Table 2). In fact, among the drugs studied, only 5 demonstrated measurable retention on the analytical column and of these only one (metoclopramide) could still be detected (at $t_R = 18.3$ min) following extraction, further pointing to the selectivity of the sample pretreatment procedure. Under the applied conditions, MTX ($t_R = 9.30$ min) and 7-OH-MTX ($t_R = 16.5$ min) were well resolved and adequately separated from minor endogenous plasma components (Fig. 2). The overall chromatographic run time was established at 30 min.

Blank human plasma obtained from five volunteers was initially spiked at a concentration of 10 ng/ml to assess the lower limit of quantitation,

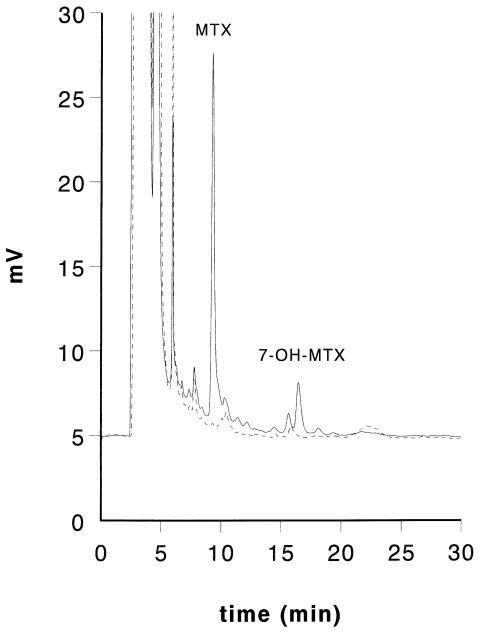



Fig. 2. Representative chromatographic tracings of patient plasma extracts from samples obtained immediately prior to MTX administration (dotted line) and 4 hours after an i.v. bolus of MTX (solid line). Identified peaks in the latter extract represent the parent compound MTX (472 ng/ml) and its metabolite 7-OH-MTX (105 ng/ml).

defined as the lowest standard concentration in the analytical run with a definite level of certainty [32]. At this spiked level, the mean percentage deviation from the nominal concentration and the intra-assay variability (between-run precision) were +3.78% and 5.84%, respectively, which are well within the acceptable ±20% deviation limits [32].

The results of the formal validation of the analytical method in terms of precision and accuracy are listed in Table 3. The use of the MTX peak area in combination with a weight factor of $1/x^2$ for the calibration resulted in the minimal deviation from nominal concentrations, with linear regression coefficients ≥0.995 in all chromatographic runs with MTX spiked in a range of 10 to 10–000 ng/ml. The coefficient of variation of the slope of the four standard curves was 11.9%, indicating minor between-assay variability in peak response. The method was shown to be accurate over the entire range, with an average accuracy at three tested concentrations between −4.51% and +10.6%, and precise with interassay and intra-assay variabilities ≤11.0% (Table 3).

### 3.2. Pharmacokinetics

The described method was applied to a pharmacokinetic study of MTX given in combination with docetaxel in patients with advanced solid tumors. Plasma concentration-time curves of MTX and 7-OH-MTX in patients treated with MTX alone (30 mg/m$^2$) as a 5 min i.v. infusion or in combination with docetaxel (75 mg/m$^2$) given i.v. over 1 h on day 1 or 2 are displayed in Fig. 3. The plasma

Table 2
Drugs (10 μg/ml) evaluated for potential interference with the analysis of MTX

| Compound | Supplier | $t_R$ (min) | Extractable |
|---|---|---|---|
| Acetaminophen | Various | 6.55 | no |
| Alizapride | Lorex (Maarssen, The Netherlands) | 6.03 | no |
| Codeine | Various | none | – |
| Dexamethasone | MSD (Haarlem, The Netherlands) | none | – |
| Docetaxel | Rhône-Poulenc Rorer (Antony, France) | none | – |
| Domperidon | Janssen-Cilag (Tilburg, The Netherlands) | none | – |
| Leucovorin | Pharmachemie (Haarlem, The Netherlands) | none | – |
| Lorazepam | AHP Pharma (Hoofddorp, The Netherlands) | none | – |
| Metoclopramide | Lorex (Maarssen, The Netherlands) | 18.3 | poorly |
| Morphine | ASTA-Medica (Diemen, The Netherlands) | none | – |
| Paroxetine | SB-Farma (Rijswijk, The Netherlands) | 2.75 | no |
| Ranitidine | Glaxo Wellcome (Zeist, The Netherlands) | 3.42/3.70 | no |

Table 3
Accuracy, between-run and within-run precision for the analysis of MTX in human plasma[a]

| Nominal (ng/ml) | Recovered (ng/ml) | DEV[a] (%) | WRP[a] (%) | BRP[a] (%) | *n* |
|---|---|---|---|---|---|
| 10 | 10.4 | +3.78 | 8.60 | 5.84 | 16 |
| 50 | 55.3 | +10.6 | 5.44 | 5.45 | 16 |
| 3500 | 3343 | −4.48 | 3.69 | 11.0 | 20 |
| 7500 | 7162 | −4.51 | 4.24 | 9.36 | 19 |

[a] Abbreviations: DEV, percent deviation (accuracy); WRP, within-run precision; BRP, between-run precision; *n*, number of replicate observations within each validation.

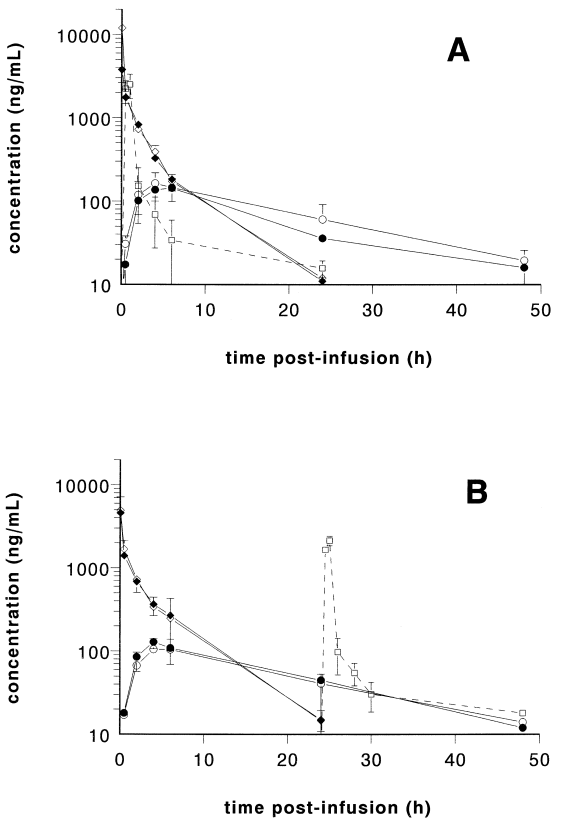



Fig. 3. Plasma concentration-time profiles of MTX (diamonds) and 7-OH-MTX (circles) in patients treated with MTX alone as a 5 min i.v. infusion (open symbols) or in combination with docetaxel (closed symbols) given on day 1 (*panel A*) or on day 2 (*panel B*) at 75 mg/m$^2$ as a 1 h i.v. infusion. Plasma concentration-time profiles of docetaxel are indicated by squares and dotted lines [infusion from 0 to 1 h after MTX (*panel A*) or from 24 to 25 h after MTX (*panel B*)]. Data are presented as mean values (symbol)±standard deviation (error bar).

time course of MTX was in all cases best described with a tri-exponential function after zero order input using weighted least squares regression [weighting factor $1/y$(calc)] and the Powell minimization algorithm. In keeping with recent reports [34], concentrations of 7-OH-MTX increased slowly after i.v. MTX administration and peaked consistently at 4 h after dosing. The metabolite data best fitted a two-compartment model with a lag-phase of approximately 10 min preceding the appearance of 7-OH-MTX in plasma. A summary of the pharmacokinetic parameters for MTX, 7-OH-MTX and docetaxel is presented in Table 4. There were no statistically significant differences in MTX and 7-OH-MTX pharmacokinetics between the two patient cohorts, and the kinetic behavior of docetaxel in both groups was similar, with mean clearance values of 24.9±3.63 vs. 22.1±7.73 l/h/m$^2$, and consistent with previous findings obtained in patients treated with docetaxel alone or in combination with cisplatin [35,36]. The pharmacokinetics of MTX were not significantly altered by docetaxel administration, although with both the day 1 and day 2 schedules of docetaxel dosing there was a trend toward a higher AUC and a slower clearance (Table 4). Similarly, the formation and subsequent disposition of 7-OH-MTX was not substantially altered by docetaxel co-treatment given on either day, although it is possible that minor alterations were obscured by interpatient variation in the generated data.

In conclusion, a thoroughly validated analytical method for the determination of MTX in plasma of cancer patients has been described. The method proved to be specific, accurate and precise and is selective and sensitive enough to be used in clinical trials, and is currently in use to further investigate

Table 4
Paired pharmacokinetic parameters of MTX, 7-OH-MTX and docetaxel[a]

| Parameter | MTX alone | MTX + docetaxel | Difference | 90% CL | *P* |
|---|---|---|---|---|---|
| *docetaxel given on day 1* | | | | | |
| $AUC_{MTX}$ (μg h/ml)[b] | 6.14±0.943 | 7.27±1.82 | −1.12±1.33 | −5.01–2.77 | 0.490 |
| $CL_{MTX}$ (l/h/m$^2$)[b] | 5.13±0.405 | 4.29±0.955 | 0.840±0.629 | −0.995–2.68 | 0.313 |
| $t1/2_{MTX}$ (h)[b] | 2.84±2.41 | 2.68±1.35 | 0.161±0.757 | −3.60–5.11 | 0.662 |
| $AUC_{7\text{-OH-MTX}}$ (μg h/ml) | 3.58±1.59 | 3.10±0.84 | 0.480±0.711 | −0.596–3.56 | 0.173 |
| $t1/2_{MTX}$ (h) | 6.88±0.238 | 7.12±0.349 | −0.243±0.293 | −1.10–0.613 | 0.494 |
| $CL_{docetaxel}$ (l/h/m$^2$) | – | 24.9±3.63 | – | – | – |
| *docetaxel given on day 2* | | | | | |
| $AUC_{MTX}$ (μg h/ml) | 6.40±0.480 | 8.08±1.38 | −1.01±0.812 | −3.38–1.36 | 0.340 |
| $CL_{MTX}$ (l/h/m$^2$) | 4.71±0.364 | 3.80±0.716 | 0.913±0.574 | −0.763–2.59 | 0.253 |
| $t1/2_{MTX}$ (h) | 2.53±0.526 | 2.49±1.01 | 0.061±0.225 | −1.50–1.35 | 0.795 |
| $AUC_{7\text{-OH-MTX}}$ (μg h/ml) | 3.15±0.154 | 3.68±1.03 | −0.533±0.578 | −2.22–1.15 | 0.454 |
| $t1/2_{MTX}$ (h) | 7.39±1.29 | 7.37±1.29 | 0.027±0.054 | −0.130–0.183 | 0.668 |
| $CL_{docetaxel}$ (l/h/m$^2$) | – | 22.1±7.73 | – | – | – |

[a] Data (mean values±standard deviation) were obtained from cancer patients treated on day 1 with MTX at a dose level of 30 mg/m$^2$ as single agent, or in combination with 75 mg/m$^2$ docetaxel administered also on day 1 ($n=3$) or on day 2 ($n=3$).
[b] Abbreviations: AUC, area under the plasma concentration-time curve; CL, total plasma clearance; $t_{1/2}$, terminal disposition half-life.

the influence of docetaxel co-administration on MTX pharmacokinetics in patients.

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