Anal Bioanal Chem (2010) 398:3033–3042
DOI 10.1007/s00216-010-4243-1

ORIGINAL PAPER

Simultaneous quantification of ten cytotoxic drugs by a validated LC–ESI–MS/MS method

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Received: 6 August 2010 / Revised: 20 September 2010 / Accepted: 21 September 2010 / Published online: 7 October 2010

Abstract A liquid chromatography separation with electrospray ionisation and tandem mass spectrometry detection method was developed for the simultaneous quantification of ten commonly handled cytotoxic drugs in a hospital pharmacy. These cytotoxic drugs are cytarabine, gemcitabine, methotrexate, etoposide phosphate, cyclophosphamide, ifosfamide, irinotecan, doxorubicin, epirubicin and vincristine. The chromatographic separation was carried out by RPLC in less than 21 min, applying a gradient elution of water and acetonitrile in the presence of 0.1% formic acid. MS/MS was performed on a triple quadrupole in selected reaction monitoring mode. The analytical method was validated to determine the limit of quantification (LOQ) and quantitative performance: lowest LOQs were between 0.25 and 2 ng mL^{-1} for the ten investigated cytotoxic drugs; trueness values (i.e. recovery) were between 85% and 110%, and relative standard deviations for both repeatability and intermediate precision were always inferior to 15%. The multi-compound method was successfully applied for the quality control of pharmaceutical formulations and for analyses of spiked samples on potentially contaminated surfaces.

Keywords Cytotoxic · Antineoplastic drugs · LC–MS/MS · Pharmaceutical formulation · SRM · Validation

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Introduction

During the last decades, the number of patients receiving anticancer chemotherapy treatments based on cytotoxic drugs has steadily increased. Simple analytical methods are thus required in different pharmaceutical fields, such as quality control or environmental monitoring. Different methods have already been published such as simple flow injection analysis and high-performance liquid chromatography (HPLC)-UV/Vis assays for cytotoxic drugs by Delmas et al. for quality control of cytotoxic preparations in a centralised parenteral preparation unit [1] or simultaneous determination of three anthracycline drugs (doxorubicin, daunorubicin and idarubicin) in serum samples by capillary electrophoresis (CE) with laser-induced fluorescence by Pérez-Ruiz et al. for therapeutic drug monitoring [2]. Due to their toxicity, the analysis of cytotoxic drugs is also useful for environmental monitoring and control of cytotoxic traces in wastewater. As an example, a CE-DAD method for the quantification of 5-fluorouracil in wastewater of hospital effluents was published by Mahnik et al. [3].

Despite safety standards for handling cytotoxic agents, it has been shown that health care professionals are still exposed to these toxic compounds. For instance, several studies reported low-level contamination of these compounds on workbenches, floors, vials, gloves and isolators [4–12].

Moreover, cytotoxic traces have been found in urine of health care professionals [12–15]. Rapid, reliable and validated analytical methods are thus needed for the safety of the operator handling these hazardous drugs and to reduce the exposure at the lowest possible level [16]. A review about analytical methods used for biological and environmental monitoring of hospital personnel exposed to antineoplastic agents was published by Turci et al. [17]. Different instrumental techniques were used depending on the studied analyte. Most of the presented studies used a specific method for the determination of a single cytotoxic drug. For example, a very sensitive voltammetry for platinum drugs, as well as GC–MS methods for the quantification of cyclophosphamide, ifosfamide and fluorouracil, was developed by Schmaus et al. for environmental and biological monitoring [18]. These methods presented very good quantitative performance and detection limits (i.e. 0.1 to 1 pg per sample) and are thus ideal for establishing target guideline values for cytotoxic contamination or for selecting a single compound as a model marker for potential contaminations. On the other hand, such methods are time consuming and not very cost-effective to get an overview of several cytotoxic contaminations. For the latter, multi-compound methods are required. Different approaches have

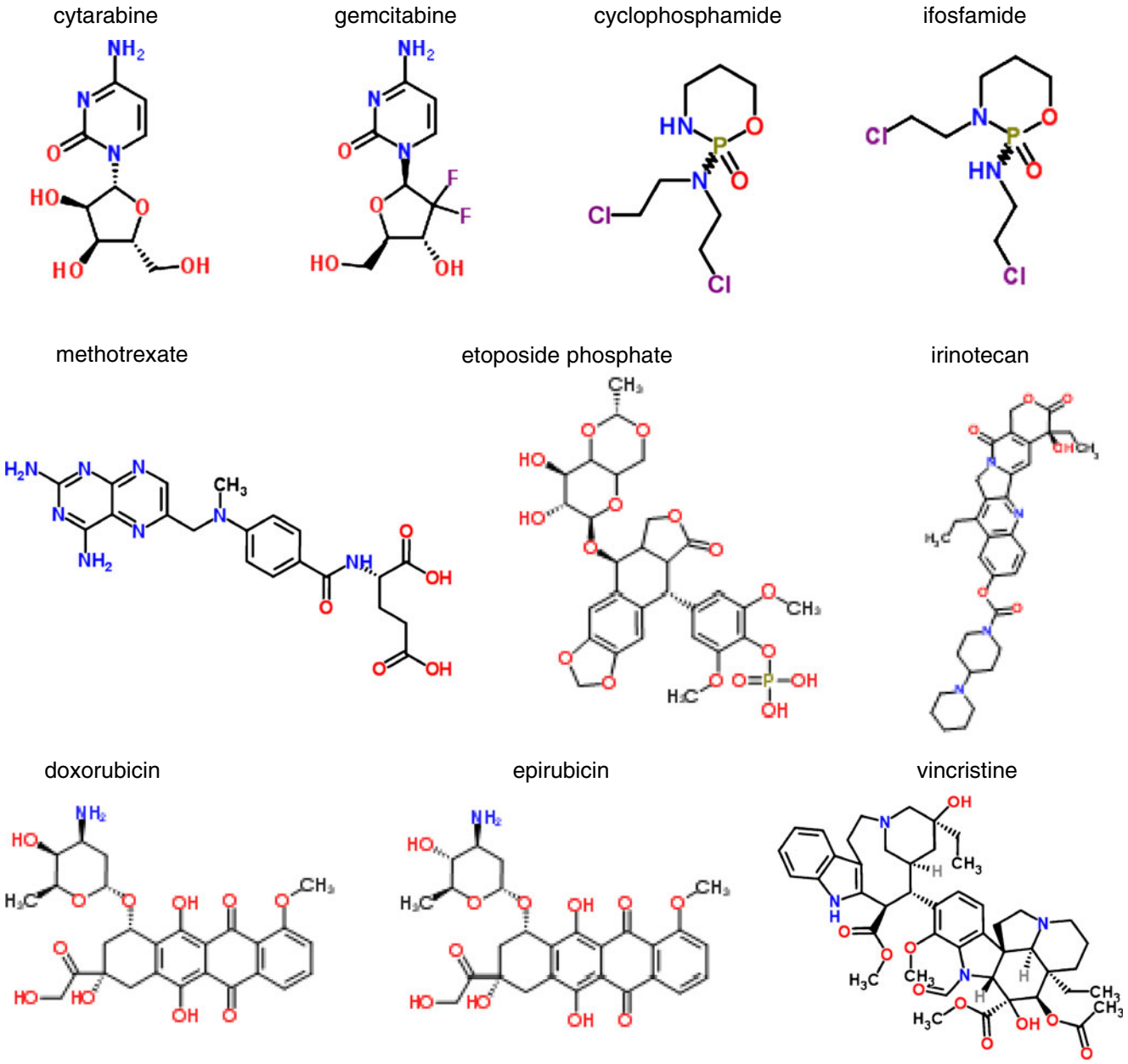

Fig. 1 Chemical structure of the ten studied cytotoxic drugs

Table 1 Preparation of cytotoxic stock solutions

Brand name	Manufacturer	Drug	Drug concentration (mg mL^{-1})	S1 (20 μg mL^{-1}) (in 100 mL H_2O)
Vincristine Teva®	Teva (Aesch, Switzerland)	Vincristine	1	2.000 mL
Doxorubicin Ebewe®	Ebewe Pharma Schweiz (Cham, Switzerland)	Doxorubicin	2	1.000 mL
Epirubicin Actavis Solution®	Actavis (Regensdorf, Switzerland)	Epirubicin	2	1.000 mL
Methotrexate Farmos®	Orion Pharma (Zug, Switzerland)	Methotrexate	2.5	0.800 mL
Endoxan®	Baxter AG (Volketswil, Switzerland)	Cyclophosphamide	20	0.100 mL
Cytosar®	Pfizer AG (Zürich, Switzerland)	Cytarabine	20	0.100 mL
Etopophos®	Bristol-Myers Squibb SA (Baar, Switzerland)	Etoposide phosphate	20	0.100 mL
Gemcitabine Teva®	Teva (Aesch, Switzerland)	Gemcitabine	20	0.100 mL
Campto®	Pfizer AG (Zürich, Switzerland)	Irinotecan	20	0.100 mL
Holoxan®	Baxter AG (Volketswil, Switzerland)	Ifosfamide	40	0.050 mL

been developed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the simultaneous determination of gemcitabine, taxol, cyclophosphamide and ifosfamide by Sottani et al. [19] and for cyclophosphamide, methotrexate and 5-fluorouracil for surface contamination by Sabatini et al. [11].

However, to our knowledge, there is still a lack of simple validated methods for the simultaneous determination of different cytotoxic agents. For drug treatment monitoring or quality control of pharmaceutical formulations, single-compound strategies are often sufficient, but generic multi-compound methods allow simplifying the control. In addition, multi-compound methods are mandatory for environmental analyses. The aim of this work was to develop and validate a simple and sensitive LC–MS/MS method for the simultaneous quantitative determination of ten cytotoxic drugs contained in aqueous samples.

Table 2 Gradient elution programme

Time [min]	Solvent A (%)	Solvent B (%)	Solvent C [%]
0	88	2	10
2.0	88	2	10
2.5	69	21	10
10	69	21	10
13	60	30	10
13.5	40	50	10
15.5	40	50	10
16	88	2	10
21	88	2	10

Mobile phase: *A* water, *B* acetonitrile, *C* formic acid 1%
Flow rate of 200 μL min^{-1}

Experimental

Chemicals and reagents

The study was performed with the following commercially available cytotoxic drugs (see Fig. 1 for their structure and Table 1): Campto® (irinotecan, 20 mg mL^{-1}) and Cytosar® (cytarabine, 20 mg mL^{-1}) were purchased from Pfizer AG (Zürich, Switzerland), gemcitabine Teva® (gemcitabine reconstituted in water at 20 mg mL^{-1}) and vincristine Teva® (vincristine 1 mg mL^{-1}) from Teva Pharm AG (Aesch, Switzerland), Holoxan® (ifosfamide reconstituted in water at 40 mg mL^{-1}) and Endoxan® (cyclophosphamide reconstituted in glucose 5% at 20 mg mL^{-1}) from Baxter AG (Volketswil, Switzerland), methotrexate Farmos® (methotrexate 2.5 mg mL^{-1}) from Orion Pharma (Zug, Switzerland), Etopophos® (etoposide phosphate reconstituted in water at 20 mg mL^{-1}) from Bristol-Myers Squibb SA (Baar, Switzerland), Doxorubine Ebewe® (doxorubicin 2 mg mL^{-1}) from Ebewe Pharma (Cham, Switzerland) and Epirubicin Actavis Solution® (epirubicin 2 mg mL^{-1}) from Actavis (Regensdorf, Switzerland).

The reconstitution of Etopophos, Gemcitabine Teva and Holoxan was done with water for injectables, obtained from Bichsel Laboratories (Interlaken, Switzerland); glucose 5% for the reconstitution of Endoxan was from Sintetica-Bioren SA (Couvet, Switzerland). The internal standard (IS) [^{13}C, $^{2}H_3$]-methotrexate was purchased from Alsachim (Illkirch, France).

Equipment

Analyses were carried out with a high-performance liquid chromatography system Accela from Thermo Fisher Scien-

Table 3 Instrument method for the LC–MS/MS analysis for ten cytotoxic drugs with [^{13}C, $^{2}H_3$]-methotrexate as internal standard

Time segment (min)	Scan event	Drug	Parent (*m*/*z*)	Product (*m*/*z*)	CE (eV)	Mean RT (min)
0–2.2	1	Cytarabine	244.0	112.3	15	1.9
2.2–4	2	Gemcitabine	264.7	112.3	20	2.6
4–7	3	Methotrexate	455.2	308.0	20	6.0
	4	[^{13}C, $^{2}H_3$] methotrexate	459.2	312.2	20	6.0
7–10	5	Etoposide phosphate	691.0	691.0	15	7.5
10–13	6	Ifosfamide	261.1	92.3, 140.2 154.1, 232.9	20	11.4
		Cyclophosphamide	261.1	92.3; 140.2 154.1, 232.9	20	12.2
13–14	7	Irinotecan	587.9	587.3	20	13.7
14–21	8	Doxorubicin	544.6	379.2, 397.1	15	14.8
		Epirubicin	544.6	379.2, 397.1	15	15.3
	9	Vincristine	413.3	353.2	30	15.2

tific Inc. (Waltham, MA) consisting of a quaternary pump equipped with an online degasser, an auto-sampler and a solvent platform. The chromatographic system was coupled to a triple quadrupole Quantum Discovery MS from Thermo Fisher Scientific equipped with an ion max electrospray ionisation (ESI) interface and operated with Xcalibur software (Thermo Fisher Scientific).

Separations were done on a ZORBAX SB-C18 RR 2.1× 100 mm 3.5-μm column from Agilent Technologies (Waldbronn, Germany).

Solutions

Mobile-phase solutions

Chromatography was performed using Lichrosolv® HPLC-grade acetonitrile (ACN) and ultrapure water from Merck (Darmstadt, Germany) and formic acid (FA) 99% from Biosolve (Valkenswaard, the Netherlands). The mobile phase constituted of three solutions: ultrapure water (solution A), ACN (solution B) and FA 1% (solution C).

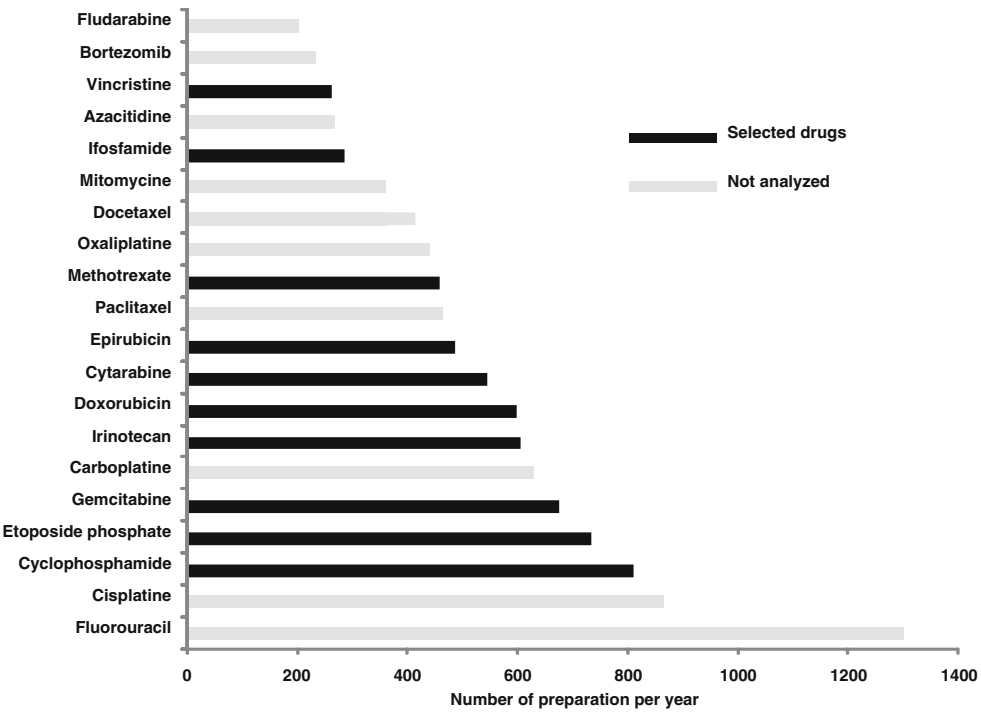

Fig. 2 Overview of the 20 most prepared cytotoxic drugs at the pharmacy of Geneva University Hospitals in 2009. *Black backgrounds* represent the selected compounds

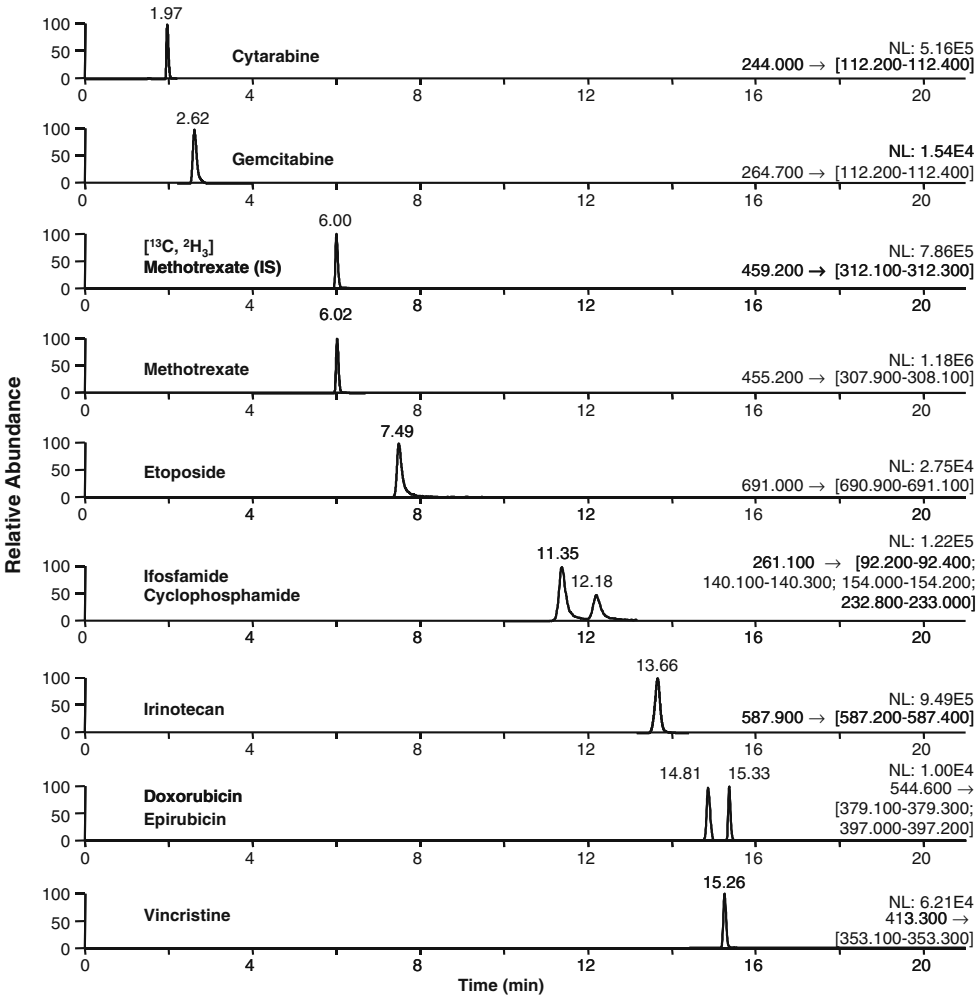

Fig. 3 LC–MS/MS chromatogram of a sample containing ten cytotoxic drugs and the internal standard at 50 ng mL^{-1}. Column: ZORBAX SB-C18 RR 2.1×100 mm 3.5 μm; flow rate 200 μL min^{-1}; gradient conditions and scan events are reported in Tables 2 and 3, respectively

New solvents were prepared for each series of analysis. Washing of the needle and the injection loop was performed with 5% ACN in water after each injection.

Cytotoxic stock solutions, calibration standards, validation standards and internal standard

The operator prepared all solutions (i.e. drug reconstitutions and sample dilution) in appropriate conditions for handling hazardous compounds as cytotoxic agents. Moreover, the development of the method was performed with drug specialities to avoid direct contact of the operator to cytotoxic powder and to minimise contamination risk by preparing working solutions.

A main stock solution (S1) containing the ten cytotoxic drugs was prepared by diluting each compound in water at a concentration of 20 μg mL^{-1} (see Table 1). This solution was further diluted to obtain two independent intermediate stock solutions: S2 at 2 μg mL^{-1} and S3 at 200 ng mL^{-1} in FA 0.1%. The calibration standards and validation standards were prepared by diluting S2 and S3 to 12 concentration levels in FA 0.1% (0.25–200 ng mL^{-1}). All samples were immediately stored at 15 °C in the LC auto-sampler and analysed within the day.

Aliquots of the IS, [^{13}C, $^{2}H_3$]-methotrexate, were prepared with a mixture of ACN and water (75:25 *v*/*v*) at 250 μg mL^{-1} and stored at −22°C for 6 months. No sample degradation could be observed. Stock solutions of IS were regularly diluted at 1 μg mL^{-1} in water, and they were stable for at least 2 weeks at 2–8 °C.

LC–MS/MS conditions

The mobile-phase flow rate was set at 200 μL min^{-1} using the gradient elution programme described in Table 2. The thermostated auto-sampler was maintained at 15 °C, and the injection volume was 25 μL.

Positive ESI conditions were capillary temperature set at 325 °C, spray voltage at 4 kV and sheath and auxiliary gas (nitrogen) flow rate at 45 and 2 psi, respectively. MS/MS was acquired in selected reaction monitoring (SRM) mode in Q1 and Q3. The Q2 collision gas (argon) pressure was set at 1.5 mTorr. Determination of Q2 potential settings and MS/MS transitions (Q1 and Q3) was carried out by direct

Table 4 Validation results for the simultaneous analysis of ten cytotoxic drugs by LC–MS/MS

	Cytarabine	Gemcitabine	Methotrexate	Etoposide phosphate	Ifosfamide	Cyclophosphamide	Irinotecan	Doxorubicin	Epirubicin	Vincristine
Trueness (%)	99–102	96–109	97–104	99–106	98–101	99–104	90–106	86–103	89–103	91–100
Repeatability (%)	1–3	1–5	1–2	1–3	1–8	1–4	1–4	2–10	2–9	1–7
Intermediate precision (%)	1–4	2–9	1–2	1–7	2–8	2–5	3–10	3–10	2–13	1–10
Limit of detection (ng mL^{-1})	0.025	0.25	0.01	0.5	0.25	0.25	0.025	0.5	0.5	0.25
Limit of quantification (ng mL^{-1})	0.25	0.5	0.25	1	1	0.5	1	2	2	1
Linearity range (ng mL^{-1})	1–200	1–200	1–200	1–200	1–200	1–200	1–200	2–200	2–200	1–200
Determination coefficient (r^2)	0.9996	0.9994	0.9997	0.9994	0.9993	0.9990	0.9992	0.9962	0.9976	0.9988

Trueness, repeatability and intermediate precision established at 12 concentration levels ranging from lowest to upper LOQ

infusion of each cytotoxic drug solution at a concentration of 1 μg mL^{-1} diluted in 50:50 of water/methanol with 0.1% FA. Selected *m*/*z* transitions and collision energy for each analyte are reported in Table 3. Seven segments with nine scan events of data acquisition were programmed in the positive mode during the entire analytical run (Table 3).

Chromatographic data acquisition, peak integration and quantification were performed using the Xcalibur software (ThermoQuest, San Jose, CA, USA).

Method validation

Method validation was performed to estimate quantitative performance of the analytical method. The validation was carried out over three series: each series involved (1) freshly prepared calibration, validation samples and solvents, (2) washing of the column and LC system, (3) LC shutoff, (4) cleaning the capillary and cone of the MS with water and methanol and (5) tuning/calibration of the MS system. Calculations were performed using area ratios of the ten cytotoxic drugs on the IS ([^{13}C, ^{2}H$_3$]-methotrexate). There were two independent sample preparations (calibration and validation samples) at 12 concentration levels (0.25–200 ng mL^{-1}) with injections in triplicate.

Application to cytotoxic formulations and environmental samples

For quality control, cytotoxic drugs were determined in formulations prepared at the Geneva University Hospitals pharmacy. Therefore, formulations were diluted in 0.1% FA to obtain a final concentration of 200 ng mL^{-1} with 50 ng mL^{-1} of the IS.

For environmental applications, a standard solution of the ten cytotoxic drugs was spiked over a polypropylene infusion bag (NaCl 0.9% 50 mL) and over a stainless steel surface (100 cm^2) to obtain a concentration of 2.5 ng cm^{-2}. The spiked area was wiped with a blotting paper (Whatman 903®) and an aqueous solution of IS was added. Then, drugs were extracted from the paper in 5 mL of FA 0.1% in glass vials by ultrasonication for 15 min. The procedure was repeated in triplicate (N=3) for each surface.

Results and discussion

Method development

Selection of the cytotoxic agents and internal standard

The developed LC–MS/MS method allows the determination of ten cytotoxic drugs, namely cytarabine, gemcitabine,

methotrexate, etoposide phosphate, cyclophosphamide, ifosfamide, irinotecan, doxorubicin, epirubicin and vincristine. These ten compounds were selected among the 20 most prepared cytotoxic drugs at the pharmacy of the Geneva University Hospitals (Fig. 2). The selection was a compromise between the most prescribed drugs and their toxicity and analytical considerations. For example, 5-fluorouracil, which is the most prepared chemotherapy drug in 2008, was not included because it is not classified as a carcinogen for humans [20], and a contamination of this compound is less hazardous for exposed personal. Furthermore, 5-fluorouracil is a very polar compound poorly retained in our analytical conditions. Three platinum compounds (i.e., cisplatin, carboplatin and oxaliplatin) presented a high percentage of prescribed chemotherapies and belong to the most toxic compounds, but they need other detection techniques such as ICP–MS or voltammetry [17, 18, 21, 22] and were thus not included in this study. Finally, the ten selected drugs belong to different cytotoxic families with different toxicities, giving an excellent overview of possible contamination.

In order to exclude a cross contamination of the IS, a deuterated compound was chosen: [^{13}C, ^{2}H$_3$]-methotrexate was used as IS as it exhibited high ESI–MS/MS response and was eluted in the middle retention time window of the ten investigated cytotoxic drugs.

Optimisation of LC–ESI–MS/MS conditions

The described method enables the quantification of ten cytotoxic compounds with a mobile-phase composition of water, acetonitrile and 0.1% formic acid. With the selected gradient (Table 2), vincristine and epirubicin were the only unresolved cytotoxic drugs but presented different scan events in SRM mode. All other compounds were separated in less than 16 min (21 min including the column reconditioning). A typical chromatogram, obtained from the analysis of a calibration sample with 50 ng mL^{-1} of each analyte and 50 ng mL^{-1} of IS in FA 0.1%, is shown in Fig. 3.

ESI–MS/MS conditions were optimised for each analyte, and the best compromise for all compounds was chosen. All drugs were analysed in positive mode. Different values for capillary temperature, spray voltage, sheath and auxiliary gas were tested (data not shown). Response signals of compounds increased with capillary temperature, but

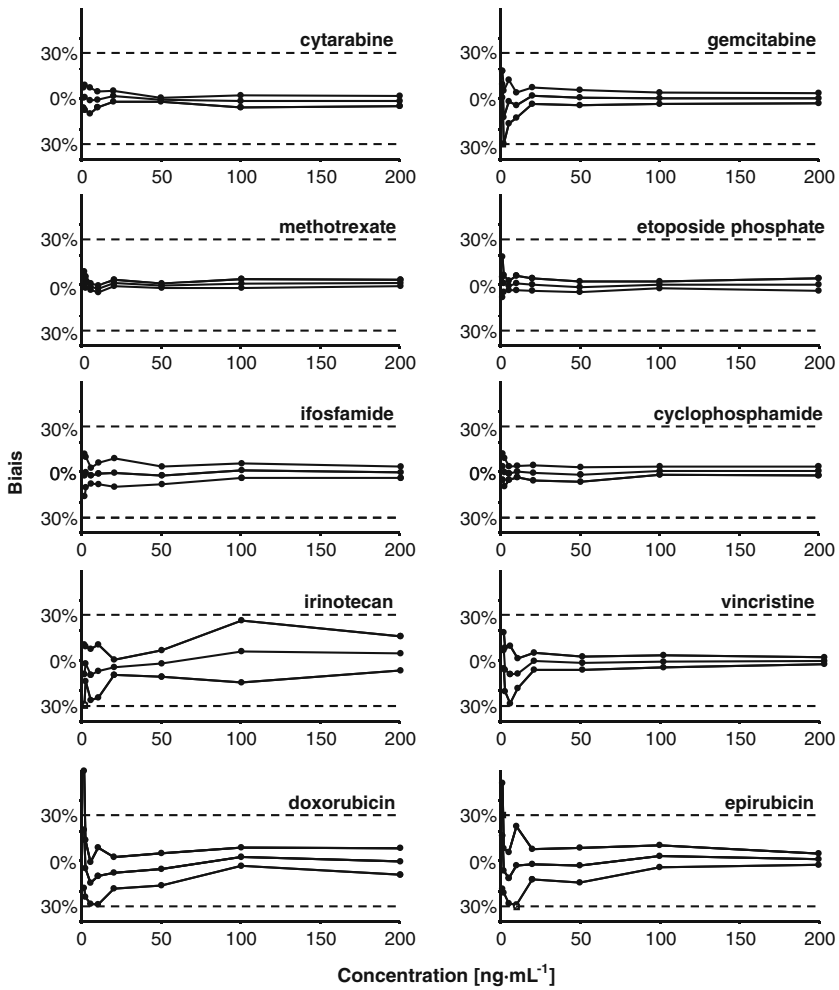

Fig. 4 Accuracy profile for the quantification of ten cytotoxic drugs by LC–MS/MS in the concentration range from 1 to 200 ng mL^{-1}, calculated from the trueness and intermediate precision reported in Table 4; *dashed lines* represent the acceptance limits of ±30%

Table 5 Analysis of the ten cytotoxic drugs by LC–MS/MS in quality control samples and on a spiked surface

	Cytarabine	Gemcitabine	Methotrexate	Etoposide phosphate	Ifosfamide	Cyclophosphamide	Irinotecan	Doxorubicin	Epirubicin	Vincristine
Quality control of drug formulations										
CYT/10-115058 gemcitabine 1,800 mg in 340 mL NaCl 0.9%	–	97±2%	–	–	–	–	–	–	–	–
CYT/10-114026 methotrexate 30 mg in 62 mL glucose 5%	–	–	97±2%	–	–	–	–	–	–	–
CYT/10-113521 ifosfamide 2,316 mg in 1,080 mL glucose 5%	–	–	–	–	97±2%	–	–	–	–	–
CYT/10-116360 cyclophosphamide 860 mg in 293 mL glucose 5%	–	–	–	–	–	100±2%	–	–	–	–
CYT/10-116778 doxorubicin 44 mg in 72 mL NaCl 0.9%	–	–	–	–	–	–	–	106±4%	–	–
CYT/10-115322 epirubicin 190 mg in 145 mL NaCl 0.9%	–	–	–	–	–	–	–	–	98±4%	–
CYT/10-116578 vincristine 1 mg in 51 mL in glucose 5%	–	–	–	–	–	–	–	–	–	93±2%
Environmental applications										
Wiping sample 1:250 ng of the 10 drugs spiked on a polypropylene infusion bag (NaCl 0.9% 50 mL)	94±10%	91±10%	82±2%	85±2%	84±4%	84±8%	49±12%	33±10%	29±10%	18±12%
Wiping sample 2:250 ng of the 10 drugs spiked on a stainless steel surface (100 cm^2)	89±10%	89±10%	67±8%	74±12%	86±12%	85±10%	34±18%	25±8%	23±12%	20±14%

doxorubicin and epirubicin were degraded at temperatures above 325 °C. Final optimised conditions were obtained with a spray voltage of 4 kV, a capillary temperature at 325°C, sheath gas at 45 psi and auxiliary gas at 2 psi. The *m*/*z* transitions and collision energy for each analyte were successfully determined and reported in Table 3.

Triple quadrupole instruments provide excellent sensitivity and selectivity in SRM. Additionally, the MS/MS method can be segmented into various time windows containing different SRM events to increase the signal-to-noise ratio. Thus, seven time segments with nine scan events of data acquisition were used during the entire analytical run. This approach is particularly useful in environmental monitoring because of the relatively low concentration of each individual analyte. With the presented method, cytarabine, gemcitabine, etoposide phosphate and irinotecan were detected in their own time segment with a specific scan event. Doxorubicin and epirubicin, as cyclophosphamide and ifosfamide, were analysed with the same scan event in the same segment, but they were well separated by LC. Vincristine was detected in the same segment as doxorubicin and epirubicin, but with another scan event. Therefore, all cytotoxic drugs could be quantified in satisfied analytical conditions.

Method validation

The method was validated with calibration and validation samples in 0.1% formic acid. Quantitative performance was estimated in three separate series at 12 concentration levels, with three repetitions for calibration standard and three repetitions for validation standard. As a result, 72 injections were carried out per series, for a total of 216 analyses.

Calibration model and concentration range

From all calibration standards, different regression models were tested to determine the best response function for the ten cytotoxic drugs: a weighted linear regression model with a weight equal to $1/x$ gave the best quantitative performance in the studied concentration range with a determination coefficient (r^2) of about 0.999 and superior to 0.996 for all tested compounds (Table 4).

The limit of detection was set at a signal-to-noise ratio of 3:1. LODs were significantly below 1 ng mL^{-1} for all cytotoxic drugs (Table 4). The lowest limit of quantification (LOQ) was determined to ensure relative standard deviation (RSD) inferior to 15%. Lowest LOQs were determined between 0.25 and 2 ng mL^{-1} for all cytotoxic drugs (Table 4). The upper LOQ was set at 200 ng mL^{-1} for all cytotoxic drugs. Higher LOQ values could theoretically be used, but in order to avoid unnecessary exposure of the operator to the cytotoxic agents, concentrations above 200 ng mL^{-1} were not analysed.

Due to practical considerations, the concentration range for all cytotoxic drugs was limited from 1 to 200 ng mL^{-1}.

Accuracy, trueness and precision

Concentrations of validation standards were calculated from the calibration model. Trueness was expressed in percent as the ratio between theoretical and average measured values at each concentration level. Trueness values were between 85% and 110%, as reported in Table 4.

Repeatability and intermediate precision were expressed as RSD of the ratio of the intra-day standard deviation (sr) and between-day standard deviation (sR), respectively. The sr and sR values were obtained using ANOVA analyses. RSD values below 15% were obtained for each compound (Table 4).

To visualise the overall method variability, the accuracy profile of each cytotoxic drug was built from 1 to 200 ng mL^{-1}, combining trueness and intermediate precision as the confidence interval [23]. As presented in Fig. 4, the total error did not exceed ±30% for all compounds in their quantification concentration range. Only doxorubicin and epirubicin presented a superior limit at 1 ng mL^{-1} outside the tolerance of 30%, further justifying the selected LOQ of 2 ng mL^{-1} for these two compounds ("Calibration model and concentration range").

Consequently, the developed LC–ESI–MS/MS method presents quantitative performance fully compatible with environmental monitoring of cytotoxic drugs on surfaces or in combination with a sample preparation for biological sample analyses. Alternatively, the method can be used for quality control or stability studies of pharmaceutical formulations due to the very satisfactory performance at 200 ng mL^{-1} with a total error inferior to 5%.

Applications

In order to demonstrate the applicability of the LC–ESI–MS/MS method to real samples, determination of the ten cytotoxic agents was achieved in pharmaceutical formulations for quality control and in wiping samples for environmental monitoring. Concentrations of the cytotoxic agents were calculated with reference to a calibration curve constructed the same day with five levels of calibration standard (1, 2, 10, 50 and 200 ng mL^{-1}) containing the ten drugs and weighted linear regression with a weight equal to $1/x$ for each compound.

For quality control, pharmaceutical formulations were analysed by diluting the samples to a target value of 200 ng mL^{-1}. As shown in Table 5, concentrations of the tested pharmaceutical formulations were found to be ±10% (including both trueness and precision) of the prescribed concentration which corresponds to the acceptance limit for preparations of the HUG pharmacy.

The method was also successfully applied to environmental samples. The recovery rate was determined for each cytotoxic compound according to the surface type (see Table 5).

Conclusions

A simple LC–ESI–MS/MS method was successfully developed for the simultaneous quantification of ten cytotoxic drugs (cytarabine, gemcitabine, methotrexate, etoposide phosphate, cyclophosphamide, ifosfamide, irinotecan, doxorubicin, epirubicin and vincristine) in 21 min, gradient equilibration time included. This method was validated and exhibited satisfactory quantitative performance in terms of limit of quantification, domain range, trueness and precision: the accuracy profile showed total errors inferior to ±30% for all compounds in their quantification domain range, from 1 or 2 ng mL^{-1} up to 200 ng mL^{-1}, and total errors inferior to ±5% at 200 ng mL^{-1}. Therefore, the method can be used for different applications, as shown by its successful utilisation for quality control of pharmaceutical formulations and environmental monitoring.

Acknowledgements We kindly thank the Proteomics Core Facility at the Faculty of Medicine, University of Geneva (Switzerland) for the loan of the LC–MS/MS instrument.

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