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Development and application of a liquid chromatography coupled to mass spectrometry method for the simultaneous determination of 23 antineoplastic drugs at trace levels

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ABSTRACT

The goal of this study was to develop a method for the simultaneous quantification of 23 commonly used antineoplastic drugs in a hospital pharmacy, using ultra-high pressure liquid chromatography separation coupled to tandem mass spectrometry detection (UHPLC-MS/MS). The following drugs were investigated: 5-fluorouracil, cytarabine, ganciclovir, gemcitabine, dacarbazine, methotrexate, pemetrexed, busulfan, topotecan, rentitrexed, ifosfamide, cyclophosphamide, etoposide, irinotecan, doxorubicin/epirubicin, vincristine, docetaxel, paclitaxel, daunorubicin, idarubicin, vinblastine, oxaliplatin and carboplatin. The chromatographic separation was performed on a phenyl-hexyl column (2.1 ×100 mm, 1.7 μm) with a gradient elution of methanol and water containing 10 mM ammonium formate adjusted to pH 4.9. All compounds were analyzed in less than 13 min and detected with a triple quadrupole mass spectrometer operating in MRM mode. Limits of detection (LODs) and limits of quantification (LOQs) were comprised between 0.01 and 5 ng.mL^{-1}, and between 0.5 and 5 ng.mL^{-1}, respectively. Accuracies ranged between 117% and 83% at the LOQ, intermediate and upper LOQ concentrations, with relative standard deviations (RSD) inferior to 8%, for all the antineoplastic drugs. Finally, the UHPLC-MS/MS method was successfully applied to the analysis of surface samples to evaluate the chemical contamination by these highly toxic compounds in a chemotherapy preparation unit in a hospital pharmacy with the purpose of monitoring the exposure of health care professionals.

1. Introduction

Antineoplastic drugs are the most toxic pharmaceutical molecules. Their high reactivity and lack of specificity toward tumor cells are responsible for a wide range of side effects for patients. In addition, these drugs are harmful to health care professionals and the environment. To evaluate exposure to antineoplastic drugs, two different strategies can be applied. The first is based on an analysis of biological samples from potentially exposed humans. Either indirect or direct biological analysis can be performed. An indirect analysis consists of highlighting a physiological effect caused by the presence of antineoplastic molecules, such as urinary mutagenicity [1,2], DNA damage [3,4] and micronuclei induction [5,6]. The primary drawback of direct analysis is a lack of specificity of indirect tests, and therefore, the detection of antineoplastic drugs directly in biological fluids of health care professionals is preferred. Exposure is clearly detected under these conditions, because the presence of toxic agents in the body is unambiguously demonstrated [6–8]. However, biological monitoring is an invasive test that raises an ethical problem due to the absence of limit values and associated short- or long-term physiological effects. The source of the contamination is also not clearly defined, which is why an alternative strategy can also be considered. This alternative strategy consists of the determination of antineoplastic drugs in the environment using the wipe sample technique [9,10]. The interest of this approach is highlighted by the statement of the first limit values of surface contamination by hazardous drugs, in recent years, by the USP (with the example of cyclophosphamide and a limit of 1.0 ng.cm^{-2}) [11] and the European Biosafety Network (with a limit value of 0.1 ng.cm^{-2} for any hazardous drug)

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Table 1
MS optimized parameters.

Analyte	Adduct	DP	Q1	Q3		CE	CXP
Cytarabine	$[M+H]^+$	51	244.061	95	Q	57	10
				112	q	17	12
Ganciclovir	$[M+H]^+$	11	256.098	152	Q	17	18
				134.9	q	45	14
Gemcitabine	$[M+H]^+$	41	264.056	112	Q	23	12
				95	q	57	10
Dacarbazine	$[M+H]^+$	16	183.122	166	Q	15	18
				68	q	37	8
Methotrexate-[$^{13}C^2H_3$]	$[M+H]^+$	91	459.115	312	Q	27	16
				175	q	47	20
Methotrexate	$[M+H]^+$	81	455.098	308	Q	27	34
				175	q	47	20
Pemetrexed	$[M+H]^+$	46	428.089	281	Q	27	32
				163	q	45	18
Busulfan	$[M+NH_4]^+$	21	264.042	150.9	Q	15	16
				247	q	11	24
Topotecan	$[M+H]^+$	81	422.099	377	Q	27	34
				218.1	q	97	24
Raltitrexed	$[M+H]^+$	36	459.049	312.051	Q	21	20
				173.051	q	45	20
Ifosfamide	$[M+H]^+$	51	261.003	153.9	Q	29	18
				232.9	q	23	28
Cyclophosphamide-[$^2H_8$]	$[M+H]^+$	76	269.074	147	Q	31	16
				150	q	27	16
Cyclophosphamide	$[M+H]^+$	66	261	140	Q	29	14
				106.1	q	29	12
Etoposide	$[M+NH_4]^+$	31	606.141	229	Q	25	26
				185	q	41	22
Irinotecan-[$^{13}C_6$]	$[M+H]^+$	186	593.236	124	Q	45	14
				167.1	q	51	20
Irinotecan	$[M+H]^+$	181	587.209	124.1	Q	43	14
				167	q	51	20
Doxo/Epirubicin	$[M+H]^+$	91	544.11	397	Q	17	14
				361	q	35	18
Vincristine	$[M+2H]^{2+}$	61	413.272	392	Q	25	18
				362	q	27	18
Docetaxel	$[M+Na]^+$	161	830.226	549	Q	35	18
				304	q	33	36
Paclitaxel-[$^2H_5$]	$[M+Na]^+$	196	881.207	313.1	Q	39	16
				591.1	q	35	22
Paclitaxel	$[M+Na]^+$	176	876.218	308	Q	39	36
				591	q	35	18
Daunorubicin	$[M+H]^+$	41	528.143	321.043	Q	27	36
				363.011	q	21	34
Idarubicin	$[M+H]^+$	51	498.127	291	Q	37	14
				130	q	17	16
Vinblastine	$[M+2H]^{2+}$	21	405.908	271.4	Q	33	12
				375.8	q	23	18
Oxaliplatin	$[M+Na]^+$	151	418.986	346.9	Q	29	38
				135	q	29	14
Carboplatin	$[M+H]^+$	1	372.011	294	Q	23	34
				310.8	q	17	14
5 Fluorouracil	$[M+H]^+$	-35	128.942	42	Q	-26	-19
				59.1	q	-32	-7
5 Fluorouracil [$^{13}C^{15}N_2$]	$[M+H]^+$	-30	131.928	44.1	Q	-22	-21
				60	q	-34	-7

DP: declustering potential, Q1: parent ion, Q3: product ion (Q: quantifier ion, q: qualifier ion), CE: collision energy, CXP: collision cell exit potential

[12]. To measure contamination by antineoplastic drugs, the surfaces in contact with antineoplastic drugs and products such as workbenches, floors, computer mice, phones must be sampled with a moistened swab and the solvent used for compound extraction from the swab is directly analyzed by a suitable analytical method. The technique of choice is undoubtedly liquid chromatography coupled to mass spectrometry (LC-MS), due to its high selectivity and sensitivity associated with its versatility.

Despite the high performance of LC-MS, the development of a wipe sampling method for antineoplastic agents monitored in the environment remains a challenging task. Over fifty different antineoplastic drugs at varying doses are commonly used in the hospital and these compounds present great diversity in terms of physico-chemical properties. Thus, several analytical methods usually must be developed to detect all analytes of interest, or a single method should be used by renouncing to analyze certain compounds. A typical example of this problem is the analysis of platinum compounds that are usually detected by inductively coupled plasma-mass spectrometry (ICP-MS) [13–15] or by the absorptive voltammetry technique after ultraviolet photolysis digestion [16]. Although several recent studies have reported the simultaneous analysis of anticancer agents by LC-MS at trace levels [17–20], only a few analytical methods allowing the simultaneous analysis of numerous antineoplastic drugs including platinum compounds, by LC-MS have been published. For example, an LC-MS method was developed and applied for the analyses of nine antineoplastic drugs including carboplatin compounds at the order of $ng.mL^{-1}$ concentration in wiping samples by Dal Bello et al. [21]. Oxaliplatin and five other antineoplastic drugs were also determined by a wipe sampling LC-MS

method with a limit of quantification of 4.55 ng.mL^{-1} [22].

The aim of tis study was to develop a simple and sensitive UHPLC-MS/MS method for the simultaneous quantitative determination of 23 commonly used antineoplastic agents in hospital settings including platinum derivatives. This multitargeted method is dedicated to surface monitoring in chemotherapy preparation units to control the exposure of health care professionals and the environment to antineoplastic drugs.

2. Materials and methods

2.1. Chemicals and reagents

All solvents were of MS grade (except dimethyl sulfoxide), and all chemicals were obtained in the highest analytical quality available. Formic acid and ammonium hydroxide were purchased from Merck (Darmstadt, Germany). Methanol (MeOH) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Buchs, Switzerland). Ultrapure Type 1 water was obtained from a Milli-Q purification system from Millipore (Bedford, MA, USA).

5-Fluorouracil and dacarbazine were purchased from Tokyo Chemical Industry (Zwijndrecht, Belgium). cyclophosphamide monohydrate, etoposide, idarubicin hydrochloride, raltitrexed and topotecan hydrochloride were obtained from Toronto Research Chemicals (North York, ON, Canada). busulfan, carboplatin, cytarabine, daunorubicin hydrochloride, doxorubicin hydrochloride, epirubicin hydrochloride, ganciclovir, gemcitabine hydrochloride, methotrexate, oxaliplatin, paclitaxel, vinblastine sulfate and vincristine sulfate were obtained from the European Pharmacopoeia (Strasbourg, France). pemetrexed was purchased from Pharmaserv (Stansstad, Switzerland). Irinotecan hydrochloride and docetaxel trihydrate were obtained from Merck (Darmstadt, Germany). Ifosfamide was purchased from Baxter AG (Opfikon, Switzerland) as Holoxan® lyophilisate for injection.

[$^{2}H_{8}$]-cyclophosphamide monohydrate, [^{13}C,$^{2}H_{3}$]-Methotrexate, [$^{2}H_{5}$]-paclitaxel, [^{13}C,$^{15}N_{2}$]− 5-fluorouracil and [$^{13}C_{6}$]-irinotecan were purchased from Alsachim (Strasbourg, France).

Stock solutions of standards and internal standards were prepared by dissolution of standard compounds in DMSO at 1 mg·mL^{-1} and 0,2 mg·mL $^{-1}$, respectively and were kept at − 80 °C until use.

2.2. Safety consideration for antineoplastic agent handling

Because antineoplastic agents are highly toxic compounds, their handling requires strict safety precautions to limit analyst and environmental exposure. In this context, all experiments were performed in a laboratory dedicated to the manipulation of toxic compounds, named Cytoxlab. Specific ventilation was used to ensure a lower pressure inside the laboratory to contain any potential contamination. All powders were weighed and solubilized in a horizontal laminar airflow safety cabinet equipped with HEPA H14 filters. Most dilutions were performed with an automated liquid handling workstation (Tecan Freedom EVO®, Männedorf, Switzerland). All instruments and materials in contact with toxic compounds were treated as hazardous waste. Personal protective equipment (i.e., gloves, gown, mask, etc.) were used based on recommendations in the literature and official guidelines.

2.3. UHPLC-MS/MS instrumentation

The UHPLC system consisted of an Agilent 1290 binary pump, autosampler with thermostat and column oven (Agilent, Waldbronn, Germany). The separation was performed with an Acquity Premier CSH Phenyl-Hexyl Column 1.7 μm, 2.1 × 100 mm from Waters (Milford, MA, USA). The mobile phase was composed of two solutions: mobile phase A consisted of aqueous formic acid 10 mM pH 4.9 adjusted with ammonia and mobile phase B consisted of pure methanol (MeOH). The flow rate was set at 0.34 mL/min, and the following gradient elution program was applied: 0–0.5 min, 2% MeOH; 0.51–2 min, 2–50% MeOH; 2.01–7 min, 50–95% MeOH; 7.01–8 min, 95% MeOH; 8.01–10 min, 95–2% MeOH; 10.01–13 min, 2% MeOH. The autosampler and column temperatures were maintained at 8 °C and 25 °C, respectively. The injection volume was 2 μL.

The chromatographic system was coupled to a triple quadrupole mass spectrometer (MS/MS) 6500 + (AB Sciex, CA, USA) equipped with an electrospray ionization interface (ESI). MS was operated in both negative mode (ESI-) for the detection of 5-fluorouracil and positive mode (ESI+) for all other analytes. Solutions of each antineoplastic drug in a mixture of methanol:water (1:1, *v/v*) (at 100 μg.mL^{-1}) were separately injected at 7 μL.min^{-1} into the ESI source by continuous infusion. First, the molecular weight of the precursor was determined in Q1 and the declustering potentials that produced the most intense signal were recorded for each compound. Then, the generated fragment ions were monitored to identify the two most intense product ions for each compound: quantifier and qualifier ions in Q3. The optimized multiple reaction monitoring (MRM) fragmentation transitions and MS parameters of all analytes are reported in Table 1.

The other MS parameters (source parameters) were as follows: ion spray voltage, 5000 V for positive mode and - 2500 V for negative mode; curtain gas, 25 psi; ion source gas (GS1), 60 psi; ion source gas (GS2). 70 psi and temperature, 350 °C. The entrance potential was set at 10 V.

2.4. Sample preparation

Stock solutions of standards were prepared by dissolution of standard compounds in dimethyl sulfoxide (DMSO) at 1 mg.mL^{-1} and were kept at − 80 °C until use. Stock solutions of internal standards were prepared by dilution of individual isotopically labeled compounds in DMSO at 0.2 mg.mL^{-1} and were kept at − 80 °C until use. Stock solutions were thawed at room temperature for 30 min and vortexed a few seconds before use.

Calibration and quantification samples were obtained by pooling and diluting 23 standard stock solutions and 5 internal standard stock solutions. The concentration of each antineoplastic drug *per* level was 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 200 ng.mL^{-1}, while the final concentration of internal standards was 5 ng.mL^{-1}. To determine the limit of detection (LOD) and limit of quantification (LOQ), samples from 0.01 to 5 ng.mL^{-1} containing a final concentration of internal standards at 5 ng.mL^{-1} were also prepared.

Three quantification samples were obtained by dilution of a freshly prepared solution of each drug at the limit of quantification, at the upper limit of quantification (ULOQ) and at the intermediate concentration. All quantification samples contained a final concentration of internal standards of 5 ng.mL^{-1}.

For the application of the developed method, real samples were obtained according to the following procedure: a swab (TX716, Texwipe, Kernersville, North Carolina, USA) made of polyester was moistened with 75% isopropanol and wiped on the defined surface. After complete drying (minimum 2 h at room temperature), the swab was desorbed in 2 mL of diluent containing the 5 internal standards at 5 ng.mL^{-1}. After vortexing for 10 min, the solution was transferred into a glass vial and analyzed by UHPLC-MS/MS.

2.5. LC-MS method performance

In this study, the analytical performance of the developed method was assessed to elaborate an applicable method for the needs of the service. The evaluated parameters included the response function, carry-over effects, selectivity, LOD, LOQ, precision and accuracy by means of two product ions selected for each compound during the MS parameter optimization: the most sensitive one for quantitation and the second most sensitive one for qualitative confirmation.

To estimate the response function, six standard samples (0.5, 1, 5, 10, 50 and 200 ng.mL^{-1}) and three consecutive blank samples (i.e., water

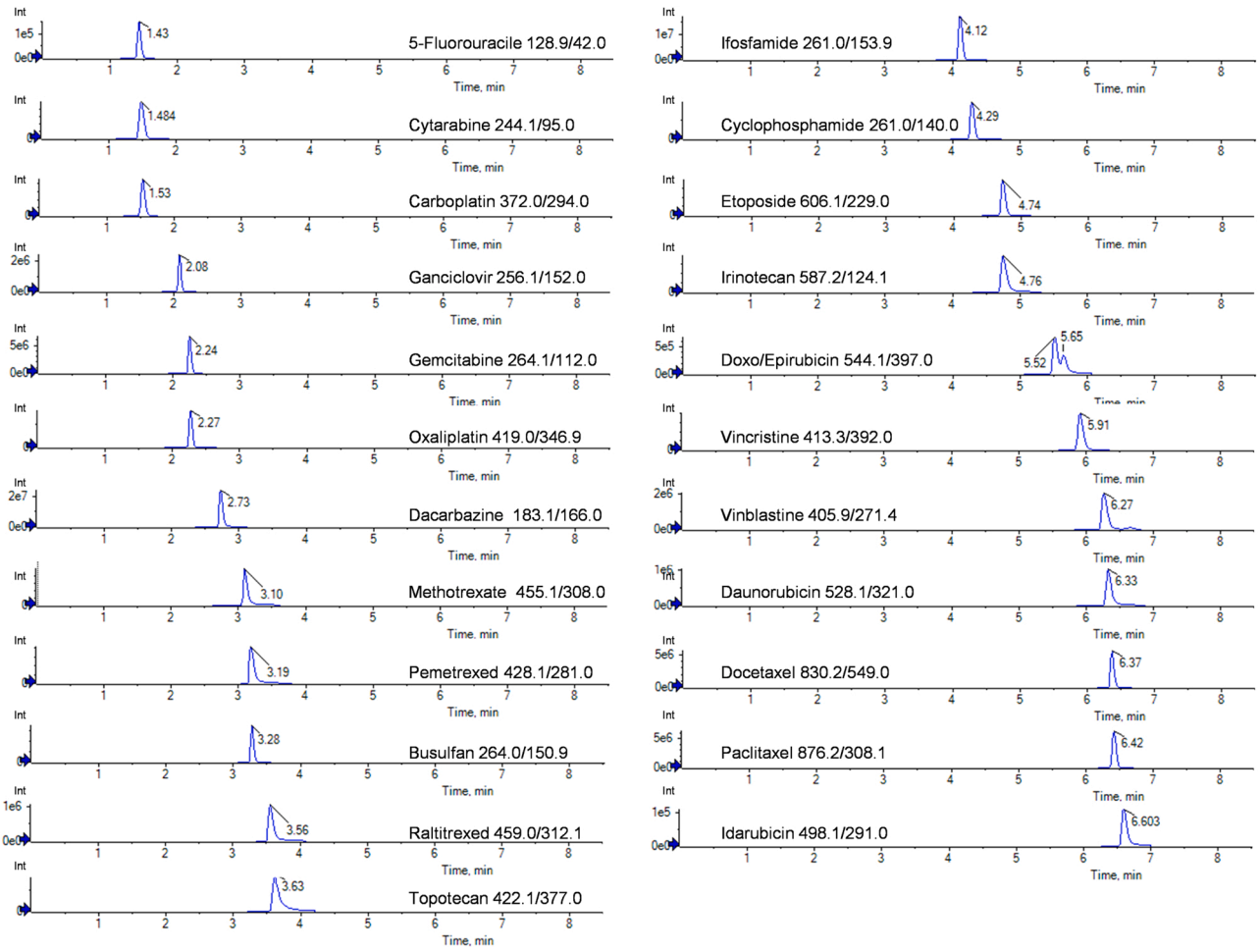

Fig. 1. UHPLC-MS/MS chromatogram of a calibration sample containing the 23 compounds at 100 ng.mL^{-1} in water (Analytical conditions are reported in 2.3 UHPLC-MS instrumentation section).

sample) were analyzed. Each calibration sample contained the five internal standards at a concentration of 5 ng.mL^{-1}. The response function ranges were calculated from the area ratios of the quantifier ion of each drug on the most intense fragment of the respective internal standard. The three blank samples were injected after the ULOQ sample to evaluate analyte carry-over between the analytical runs.

To properly define the method sensitivity, quantification samples containing a decreasing concentration of antineoplastic drugs (from 5 to 0.01 ng.mL^{-1}) and a constant concentration of IS (5 ng.mL^{-1}) were injected six times. LOD was set at a signal-to-noise ratio (S/N) of 3:1 and a constant qualifier-quantifier ion ratio (bias lower than 20% to the average ratio obtained with the calibration). LOQ was determined to ensure relative standard deviation (RSD) less than 30%, an accuracy between 70% and 100% of the expected concentration and an S/N higher than 10:1.

Accuracy and precision were determined with the analysis of quantification samples. The concentration of quantification samples (in triplicate) was calculated from the calibration model. Accuracy was expressed as percentage, as the ratio between theoretical and average measured values at each concentration level (LOQ, intermediate, ULOQ). Precision was defined as the relative standard deviation (RSD).

Given the simplicity of the sample matrix, the matrix effect was systematically considered negligible.

2.6. Method application

To demonstrate the applicability of the developed UHPLC-MS/MS method to real samples, analyses of the 23 antineoplastic drugs were performed with wiping samples for environmental monitoring. Several surfaces were evaluated in a hospital pharmacy chemotherapy compounding unit. The wipe sampling procedure was applied for all locations [23]. For each location, a surface representing approximately 100 cm^2 was wiped with a moistened swab. For nonplanar locations, the entire surface was wiped. After complete drying, the swab was desorbed, and the solution was transferred into a glass vial for further analysis by UHPLC-MS/MS. Several dozen samples were obtained. Concentrations of the antineoplastic drugs were calculated with reference to a calibration curve constructed on the same day.

3. Results and discussion

3.1. Selection of antineoplastic drugs

The objective of this study was to develop a UHPLC-MS/MS method for the simultaneous analysis of several antineoplastic drugs that were implicated in the exposure of health care professionals and the environment. More than 50 different antineoplastic drugs were routinely used in the hospital oncology units and a selection of target analytes was needed. Among these toxic compounds, some (e.g., melphalan, vindesine or dactinomycin) are rarely used; therefore, including them in the proposed method was irrelevant. Some antineoplastic drugs are highly unstable from a chemical perspective and the analysis of the native molecule is unwise. Azacitidine is a typical example, with a water stability of less than 1 h at room temperature. Cisplatin was also not

Table 2
Regressions and coefficients of determination.

Analytes	Concentration range (ng.mL^{-1})	Equations	Determination coefficient (r^2)
5-Fluorouracil	5–200	$y = 1.412e^{-4}\ \ x^2 + 0.268x + 0.047$	0.9991
Cytarabine	5–200	$y = \frac{-1.667e^6 + 5.872e6x^{1.020}}{1414.427 + x^{1.020}}$	0.9997
Ganciclovir	0.5–200	$y = -19.387\ \ x^2 + 7.114e^4x + 762.720$	0.9985
Gemcitabine	0.5–200	$y = -308.712\ \ x^2 + 2.312e^5x + 19291.470$	0.9987
Dacarbazine	0.5–200	$y = -1017.430\ \ x^2 + 9.583e^5x - 3184.691$	0.9995
Methotrexate	0.5–200	$y = -5.858e^{-5}\ \ x^2 + 0.164x + 0.021$	0.9983
Pemetrexed	5–200	$y = 3.355e^{-5}\ \ x^2 + 0.010x - 0.001$	0.9994
Busulfan	0.5–200	$y = -8.784e^{-6}\ \ x^2 + 0.029x - 3.499e^{-4}$	0.9994
Topotecan	0.5–200	$y = -6.434e^{-4}\ \ x^2 + 0.703x - 0.018$	0.9966
Raltitrexed	5–200	$y = 3.577e^{-5}\ \ x^2 + 0.050x - 0.057$	0.9973
Ifosfamide	0.5–200	$y = -1.144e^{-4}\ \ x^2 + 0.191x + 2.834e^{-4}$	0.9992
Cyclophosphamide	0.5–200	$y = -1.424e^{-4}\ \ x^2 + 0.227x - 3.090e^{-4}$	0.9995
Etoposide	1–200	$y = 1.451e^{-4}\ \ x^2 + 0.154x + 0.020$	0.9980
Irinotecan	0.5–200	$y = 7.630e^{-5}\ \ x^2 + 0.200x - 0.014$	0.9999
Doxo/Epirubicin	5–200	$y = 42.566\ \ x^2 + 4.589e^4x - 6.925e^4$	0.9992
Vincristine	1–200	$y = 0.001\ \ x^2 + 0.060x + 0.069$	0.9965
Docetaxel	0.5–200	$y = 1.413e^{-4}\ \ x^2 + 0.141x - 0.007$	0.9990
Paclitaxel	0.5–200	$y = 2.190e^{-4}\ \ x^2 + 0.162x + 0.004$	0.9989
Daunorubicin	5–200	$y = 2.260e^{-5}\ \ x^2 + 0.053x - 0.041$	0.9986
Idarubicin	5–200	$y = 4.472\ \ x^2 + 5107.547x - 2083.159$	0.9989
Vinblastine	5–200	$y = 351.238\ \ x^2 + 5.835e^4x + 1.321e^5$	0.9970
Oxaliplatin	5–200	$y = \frac{-0.068 + 0.250x^{0.932}}{169.908 + x^{0.932}}$	0.9989
Carboplatin	5–200	$y = 1.352e^{-8}\ \ x^2 + 5.057e^{-4}x - 3.714e^{-4}$	0.9991

selected because of its predisposition to hydrolysis in aqueous media and to produce numerous hydrated complexes, which are hardly detected by ESI-MS as clusters [24,25]. Platinum antineoplastic drugs, including organic ligands, such as carboplatin and oxaliplatin, are preferred because they offer greater chemical stability. Their lower polarity also constituted an advantage for reversed-phase liquid chromatographic separation. Under these conditions, selected antineoplastic drugs were reported in Section 2.1. Chemicals and reagents. Even though ganciclovir is not an antineoplastic drug, this agent was also added because it is commonly used in hospitals and is considered by the European Chemicals Agency to be a toxic compound with mutagenic properties [26].

3.2. Liquid chromatography development and internal standard selection

Due to the wide range of physico-chemical properties for the 23 drugs, the development of a chromatographic method for their simultaneous analysis is challenging. The use of a phenyl-type stationary phase appears to be a promising strategy because most analytes of interest contain aromatic, polycyclic or unsaturated structures. In this context, a Waters Acquity Premier CSH Phenyl-Hexyl Column 1.7 μm, 2.1 × 100 mm was selected. Given the diversity of target analytes, a gradient elution had to be optimized. Due to the presence of very polar compounds such as 5-fluorouracil, cytarabine and carboplatin, the gradient began with an initial isocratic step at a very low organic solvent content (0.5 min with 2% MeOH). Then, the percentage of methanol increased to elute the other more lipophilic drugs. A high percentage of organic solvent was required to elute the most hydrophobic analytes (i.e., daunorubicin, docetaxel, paclitaxel and idarubicin). A final isocratic step at 95% MeOH was applied for 2 min to flush the column before a 3 min re-equilibration. A typical LC-MS chromatogram is shown in Fig. 1. Given the high selectivity of the MS detector, a baseline resolution between all the compounds was not mandatory. Only the lack of separation (Rs was lower than 0.5) between two particular compounds (i.e., epirubicin and doxorubicin) could constitute a real problem, because these two diastereoisomers produce the same fragment ions. However, in the context of this study dedicated to the evaluation of surface chemical contamination, the differentiation between these two antineoplastic agents should not be considered essential. Doxorubicin and epirubicin have the same physicochemical properties, and therefore exhibit similar behavior during a chemical decontamination procedure.

For other isobaric compounds, the chemical structure and chromatographic behavior were sufficiently different to obtain a baseline resolution in LC. As an example, a resolution greater than 1.5 was obtained for two structural isomers (e.g., ifosfamide and cyclophosphamide). All other isobaric compounds produced fragment ions with specific masses.

The selection of internal standards was directed by the elution profile obtained under optimized LC conditions and their commercial availability. Because, 5-fluorouracil is the only compound that is detected in negative mode, [^{13}C,^{15}N$_2$]− 5-fluorouracil was selected as an internal standard for this particular drug. For the other target analytes, detected in positive mode, radiolabeled compounds such as [^{2}H$_8$]-cyclophosphamide monohydrate, [^{13}C,^{2}H$_3$]-methotrexate, [^{2}H$_5$]-paclitaxel, and [^{13}C$_6$]-irinotecan were chosen based on the physico-chemical properties and elution times of the analytes.

3.3. Method performance

The aim of this study was to develop an LC-MS/MS method that allows for the qualification and quantification of several antineoplastic drugs at trace levels. Under these conditions, the developed method must be sensitive enough and reliable. The detection of antineoplastic drugs must performed out with a high level of selectivity, allowing quantitative estimation without any ambiguity. Thus, the MRM mode was selected, and the ratio qualifier-quantifier was also considered. Concerning the quantitative performance of the developed method, two aspects must be distinguished. First, no exposure limit was clearly defined for antineoplastic drugs in any work environment, except a recommendation from the United States Pharmacopeia (USP), which indicates only a maximum threshold for cyclophosphamide. These compounds are also notorious for their instability (a property on which their pharmacological action is based). Therefore, the quantitative criteria that the LC-MS/MS method must fulfil may be broader than what is required for the quality control or therapeutic drug monitoring (TDM) analysis.

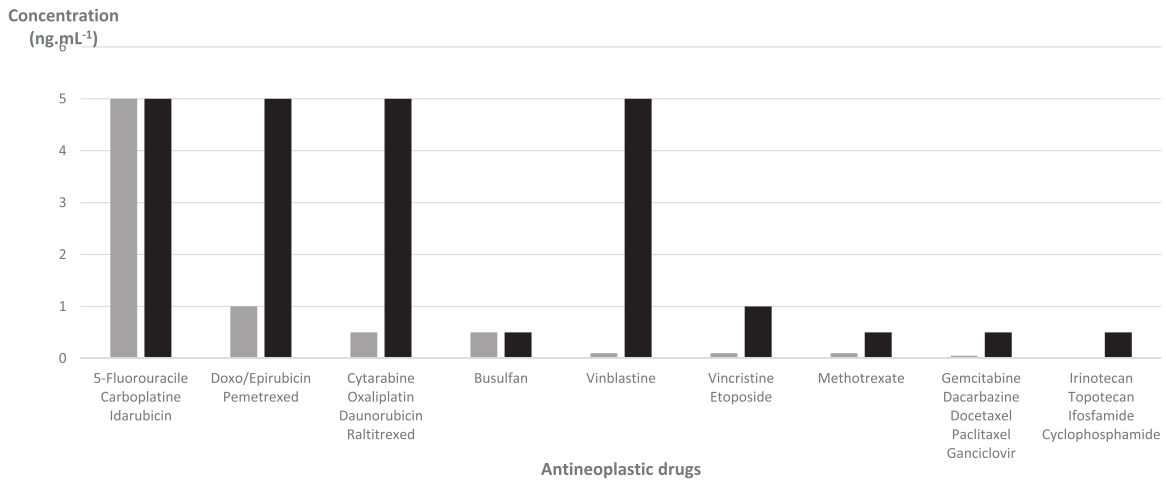

Fig. 2. Limits of detection (in grey) and limits of quantification (in black) for all antineoplastic drugs analysed by the UHPLC-MS/MS method.

Table 3
Accuracy and precision at LOQ, intermediate and ULOQ concentrations.

Analytes	LOQ*		Intermediate (100 ng.mL^{-1})		ULOQ (200 ng.mL^{-1})	
	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
5-Fluorouracil	99	5.2	96	2.5	100.	3.6
Cytarabine	97	2.9	98	3.5	95	1.9
Ganciclovir	113	7.2	103	5.5	106	4.2
Gemcitabine	115	1.1	99.	4.2	104	6.0
Dacarbazine	106	2.3	102	1.6	95	2.1
Methotrexate	111	7.3	102	2.7	98	2.1
Pemetrexed	113	3.4	88	2.3	98	2.6
Busulfan	111	4.4	100	2.0	94	0.6
Topotecan	111	1.9	104	4.0	111	2.9
Raltitrexed	107	0.8	92	1.6	97	2.0
Ifosfamide	106	3.7	100	2.8	96	3.2
Cyclophosphamide	107	1.6	99	2.1	97	2.0
Etoposide	117	3.7	98	1.4	109	1.6
Irinotecan	89	5.7	91	0.1	92	1.1
Doxo/Epirubicin	84	1.9	90	0.8	101	1.5
Vincristine	96	3.0	91	1.9	106	0.9
Docetaxel	110	4.8	100	3.7	100	2.8
Paclitaxel	107	1.2	98	2.8	102	1.3
Daunorubicin	103	4.6	92	1.8	96	4.3
Idarubicin	107	6.6	93	1.8	93	1.2
Vinblastine	83	6.9	86	2.4	97	2.2
Oxaliplatin	91	3.4	99	3.4	94	5.9
Carboplatin	102	7.1	103	3.7	101	1.6

* LOQ was 0.5 ng.mL^{-1} for ganciclovir, gemcitabine, dacarbazine, methotrexate, busulfan, topotecan, ifosfamide, cyclophosphamide, irinotecan, docetaxel and paclitaxel; LOQ was 1 ng.mL^{-1} for vincristine and etoposide; LOQ was 5 ng.mL^{-1} for 5-fluorouracil, cytarabine, pemetrexed, raltitrexed, doxo/epirubicin, daunorubicin, idarubicin, vinblastine, oxaliplatin and carboplatin.

From all calibration standards, different regression models were tested to determine the best response function for the 23 compounds. The selection of the model was based on a regression model with the best determination coefficient (r^2) in the concentration range defined between the LOQ and 200 ng.mL^{-1} for each compound. In most cases, a quadratic regression produced the best performance with r^2 above 0.996, except for cytarabine and oxaliplatin (Table 2). For these compounds, r^2 less than 0.980 were obtained with quadratic regression. The most suitable regression model for these molecules was Hill regression, allowing to obtain r^2 above 0.998.

The LOD was set at a signal-to-noise ratio of 3:1 and a constant qualifier-quantifier ion ratio (bias less than 20% to the average ratio obtained with the calibration). As reported in Fig. 2, LODs were between 0.01 ng.mL^{-1} for the most sensitive compounds (i.e., cyclophosphamide, topotecan, irinotecan, and ifosfamide) and 5 ng.mL^{-1} for 5-fluorouracil, idarubicine and carboplatin. The lowest LOQs were determined to ensure that the relative standard deviation (RSD) remained below 30%, the accuracy was between 70% and 100% of the expected concentration, and the S/N ratio higher than 10:1. With these constraints, LOQs were between 0.5 and 5 ng·mL^{-1} for all compounds, while the upper LOQ was 200 ng.mL^{-1} for all antineoplastic drugs.

The potential carry-over was also evaluated for all analytes by injecting three successive blank samples after the upper LOQ samples. No significant carry-over was observed for any antineoplastic drug.

The concentration of quantification samples was calculated from the most suitable calibration model for each antineoplastic drug. Accuracy was expressed as percentage as the ratio between theoretical and average measured values at the three different concentration levels (i.e., LOQ, intermediate and ULOQ). As reported in Table 3, accuracy values were between 117% and 83%. The precision was estimated using the RSD obtained for the analysis of the quantification samples at the three concentration levels (in triplicate). RSD values less than 8% were

Table 4
Analysis of surfaces in logistic room of the chemotherapy preparation unit in hospital pharmacy by LC-MS/MS (in ng per sample).

Analytes	Wipe samples					
	Computer keyboard and mouse	Refrigerator handle	Phone	Middle of bench	Door Handle	Storage box
5-Fluorouracil	1299	n.d.	n.d.	n.d.	n.d.	< 10
Cytarabine	< 10	n.d.	< 10	< 10	n.d.	n.d.
Ganciclovir	11	< 1	2.2	< 1	< 1	43.4
Gemcitabine	< 1	< 1	< 1	< 1	n.d.	n.d.
Dacarbazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methotrexate	n.d.	n.d.	n.d.	n.d.	n.d.	10.6
Pemetrexed	10.6	n.d.	n.d.	n.d.	n.d.	n.d.
Busulfan	n.d.	< 1	n.d.	n.d.	n.d.	n.d.
Topotecan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Raltitrexed	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ifosfamide	3	n.d.	< 1	< 1	n.d.	2
Cyclophosphamide	1.2	< 1	< 1	n.d.	n.d.	1.8
Etoposide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Irinotecan	n.d.	n.d.	n.d.	n.d.	n.d.	1.6
Doxo/Epirubicin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vincristine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
Docetaxel	n.d.	< 1	< 1	n.d.	n.d.	< 1
Paclitaxel	2.4	n.d.	n.d.	< 1	n.d.	1
Daunorubicin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Idarubicin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vinblastine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oxaliplatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carboplatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

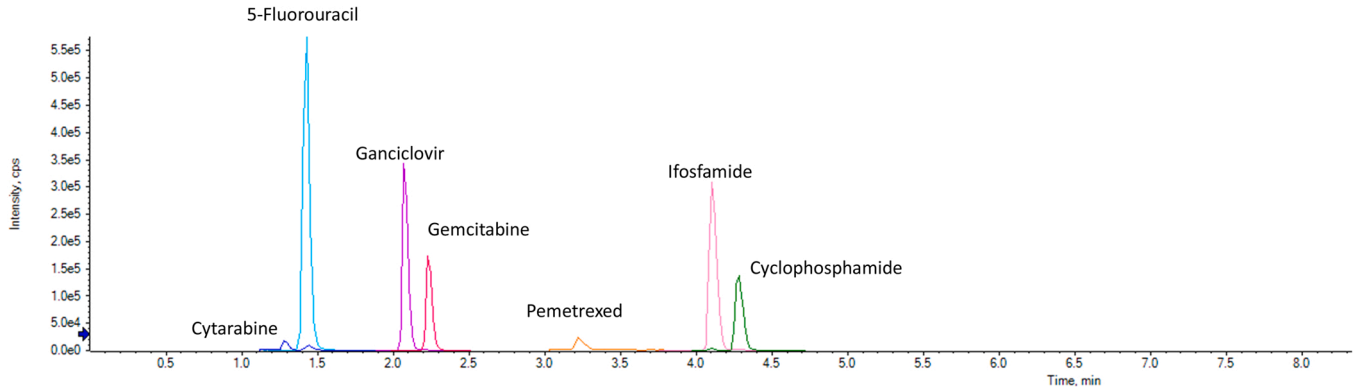

Fig. 3. TIC chromatogram of the UHPLC-MS analysis of the real sample obtained by wiping a computer and a mouse in a chemotherapy preparation unit in hospital pharmacy.

obtained for all antineoplastic drugs.

With these preliminary quantitative results, the developed UHPLC-MS/MS method appeared to be a promising tool for the simultaneous quantification and identification of the 23 antineoplastic drugs as traces in the environment.

3.4. Application of the UHPLC-MS/MS method to wipe samples

To demonstrate the applicability of the UHPLC-MS/MS method to real samples, the contamination rate of 23 antineoplastic drugs was determined at different places in the chemotherapy preparation unit at the pharmacy of Geneva University Hospitals. The sample locations included places in the logistic area (e.g. bench surfaces, storage of antineoplastic products, and equipment regularly handled by operators such as computer keyboards, mice and phones). Several dozen of samples were analyzed by the developed UHPLC-MS/MS method. In most samples, no trace of antineoplastic drugs was detected. Some samples with positive results expressed as ng per sample were reported in Table 4. The total ions chromatogram of the sample "computer keyboard and mouse" is shown in Fig. 3. The most commonly detected drugs were 5-fluorouracil, cytarabine and ganciclovir. These compounds are among the most widely used and administered in large quantities (on the order of mg-g), which may explain their presence. Low quantities of antineoplastic drugs were always detected, except on the computer (keyboard and mouse) with more than 1 μg of 5-fluorouracil. Based on these results, some actions should be applied, such as a more efficient cleaning procedure with different cleaning solvents or an enhanced cleaning frequency to reduce surface contamination. More generally, surface analysis should be applied on a regular basis to monitor potential chemical contamination and, thus, reduce to a minimum, health care professional exposure to these highly toxic agents.

4. Conclusion

A generic LC-MS/MS method was developed for the simultaneous analysis of 23 commonly used antineoplastic drugs, including two platinum derivatives, at trace levels, within 13 min per sample (the time between two sample injections). The proposed method exhibited satisfactory qualitative and quantitative performance in terms of limits of detection, limits of quantification, concentration range, accuracy and

precision. The developed method was applied for the analysis of surface samples in a chemotherapy preparation unit in a hospital pharmacy. As demonstrated, the monitoring of potential surface contamination by the most administered antineoplastic drugs can therefore be easily performed, allowing control of health care professional exposure to these highly toxic compounds.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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