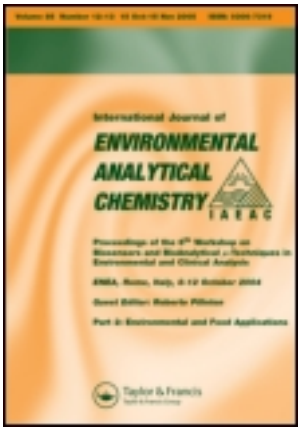

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Development and validation of an LC-MS/MS procedure for environmental monitoring of eight cytostatic drugs in pharmacies

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Development and validation of an LC–MS/MS procedure for environmental monitoring of eight cytostatic drugs in pharmacies

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An LC–MS/MS multi-method for the simultaneous determination of the structurally different and frequently used cytostatic drugs 5-fluorouracil, gemcitabine, methotrexate, cyclophosphamide, ifosfamide, etoposide, docetaxel and paclitaxel was developed and validated. In order to perform repeated ambient monitoring in 130 German pharmacies all steps of the monitoring procedure such as sample collection, transport, storage, sample preparation and HPLC–MS/MS analysis have been adapted and optimised. Thus sensitivity and reliability as well as sample throughput were increased. The final method consists of wipe sampling from 900 cm^2 surfaces and extraction of the tissues with an aqueous pH 3 solution. The limits of quantification range from 3.7 to 37 $pg\,cm^{-2}$. Validation showed that sampling via the individual pharmacy personnel does not affect the overall results. Recovery rates below 70% were observed on rough surfaces for the taxanes docetaxel and paclitaxel. Likewise, neither the storage nor the shipping conditions affected the results significantly.

Keywords: liquid chromatography mass spectrometry; cytostatic drugs; wipe samples; environmental monitoring; occupational exposure; surface contamination

1. Introduction

Apart from patients' well-being, the potential health risk for other persons getting in contact with hazardous drugs such as antineoplastics must be considered. Occupational exposure of health care workers to cytotoxic drugs has been studied intensively [1–3] and has resulted in guidelines for the safe handling of these substances in many countries [4–7]. However, despite high safety standards especially in pharmacies where cytostatic drugs are handled, numerous monitoring studies have revealed that contamination of the workplace and of personnel still frequently occurs [8–11]. European and national regulations like the German Hazardous Substances Ordinance (GefStoffV) stipulate measurements of carcinogenic substances at workplaces.

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Antineoplastic drug applications for the treatment of cancer are prepared in about 800 German pharmacies. Most of these drugs have carcinogenic, mutagenic, and/or adverse developmental or reproductive properties [12,13]. For environmental monitoring wipe sampling and analytical methods for some important substances have been developed and applied during the last two decades.

Regarding the available analytical methods, cyclophosphamide (CP) and ifosfamide (IF) have been investigated frequently using GC-MS after derivatisation with trifluoro acetic acid [2,14]. However, in recent years liquid chromatography tandem mass spectrometry (LC-MS/MS) is used for analysis of occupational exposure [15–20]. 5-fluorouracil (5-FU) can be analysed using HPLC-UV [3,21–23] or after derivatisation with N-tert-butyl-dimethylsilyl-N-methyltrifluoracetamide (TBDMS) with a limit of detection of 1 ng/wipe sample by GC-MS [24–26]. Applications in the pharmaceutical industry [27,28] demonstrate that also LC-MS/MS can be a suitable method for the analysis of 5-FU in the area of environmental monitoring. Regarding MTX a typical trend of the last decade can be observed: Insensitive HPLC-UV methods [29,30] are replaced by more sensitive LC-MS/MS methods [18,31,32]. Single compound methods for the analysis of paclitaxel in urine and wipe samples have been published so far [22,33]. Rubino *et al.* introduced a HPLC-UV method to determine the three nucleosid analogues 5-fluorouracil, cytarabine and gemcitabine [34]. For those polar compounds and their metabolites Kovalova *et al.* showed a remarkable separation using hydrophilic interaction chromatography tandem mass spectrometry for the analysis of waste water samples [35]. First multi-methods for analysis of cyclophosphamide (CP), methotrexate (MTX) and 5-fluorouracil in wipe samples were developed by Sabatini *et al.* [31]. Sottani *et al.* developed an LC-MS/MS multi-method after solid phase extraction for gemcitabine, paclitaxel, cyclophosphamide and ifosfamide [36].

A substantially simpler LC-MS/MS multi-method was developed and applied in previous studies on antibiotics and antineoplastics [37,38], instead of the more common substance specific cytostatic drug samplings and GC-MS single compound analysis. The structurally different compounds 5-fluorouracil, cyclophosphamide, docetaxel, etoposide, gemcitabine, ifosfamide, methotrexate and paclitaxel were chosen for method development, hence the substances were identified as most frequently used cytostatic drugs in a preliminary survey. Due to decreasing application numbers, cytarabine and chlorambucil were excluded from the previously published method [38] and the more frequently used taxanes docetaxel and paclitaxel were included. All investigated drugs have been associated with adverse reproduction effects [6,12,13].

The developed multi-method was applied on a large-scale study in order to determine the contamination level in German cytotoxic drug preparing pharmacies and to investigate the effects of regular wipe sample monitoring.

2. Experimental

2.1 *Chemicals*

Acetonitrile, methanol (both HPLC-grade) and gemcitabine (USP) were purchased from LGC Standards (Wesel, Germany). The other reference standards and chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). High-purity deionised water was produced in house by an Elix 10-Milli-Q Plus water purification system (Millipore, Eschborn, Germany).

2.2 *Preparation of stock solutions, calibration and dilution*

Stock solutions (0.5 g L^{-1}) were prepared in ACN-water (1 : 1, v/v) and stored at 4°C up to 3 months. Only methotrexate was dissolved in water (1 g L^{-1}) at pH 9 (adjusted with sodium hydroxide) and than diluted with acetonitrile (1 : 1, v/v). Calibration standards were prepared by diluting with a blank tissue extract. This external matrix calibration was freshly prepared daily. Real samples with concentrations above the linear range between LOQ and 100 ng mL^{-1} were diluted also with blank tissue extracts and remeasured.

In order to perform the different validations of wipe sampling surfaces were spiked with stock solutions diluted in methanol.

2.3 *Procedure of sampling and transport*

Samples were taken from 30 × 30 cm surfaces (900 cm^2) using three 20 × 21 cm Kimtech Science No. 7102 tissues (Kimberly-Clark, Koblenz, Germany) to wipe each sampling area. Tissues were wetted with 1 mL sterilised phosphate buffer at pH 3 each. Surfaces were thoroughly swept clean in vertical and horizontal strokes, changing the direction with every new tissue. The three tissues were combined to one sample, stored and transported in a 100 mL urine beaker (Uritop S, B.Braun Petzold GmbH, Melsungen, Germany).

Wipe samples were taken by the individual pharmacy personnel after being introduced to the standard operation procedures as described previously [37,38]. Samples were shipped to the laboratory in an A5 EPS box (Contact Impex, Hannover, Germany) containing two thermal packs, which had been frozen for at least 24 hours. Thus, samples are cooled at less than 4°C during transportation. In the laboratory, the samples were stored at –20°C at longest for one week before extraction.

2.4 *Sample preparation and LC-MS/MS analysis*

The samples were extracted with 30 mL of deionised water (adjusted with HCl to pH 3) for 15 minutes by sonification in the urine beaker used for shipment. Prior to injection the extracts were filtered through a 0.45 µm regenerated cellulose acetate syringe filter (Macherey-Nagel, Dueren, Germany). Analysis of all samples were carried out with a 1100 binary pump (Agilent Technologies, Waldbronn, Germany) with an HTS-PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a stack cooler for sample storage at 4°C until injection of 20 µL. Separation of the eight compounds was performed on a 50 × 3 mm Shim-Pack XR-ODS, 2.2 µm column (Shimadzu, Duisburg, Germany) at 30°C and a flow rate of 300 µL min^{-1}. The binary gradient of 0.1% formic acid in water (v/v, phase A) and 0.1% formic acid in acetonitrile (v/v, phase B) consisted of the following steps: 0–0.55 min 5% B, 12 min 80% B, 13 min 80% B, 20 min 5% B. For the detection of the antineoplastic drugs an API 3000 triple quadruple mass spectrometer (Applied Biosystems MDS Sciex, Darmstadt, Germany) equipped with TurboIonSpray™ interface operating at 450°C in positive and negative mode with ion spray probe voltages of 5000 V and −4500 V was used. For measurements in positive and negative mode within one experiment, a settling time of 700 ms was adjusted. The parameter settings for nebuliser, curtain and collision gases (nitrogen each) were 15, 12 and 6 arbitrary units, respectively. Orifice and focusing ring voltage were optimised by continuous flow experiments. The antineoplastic drugs were detected by multiple reaction monitoring

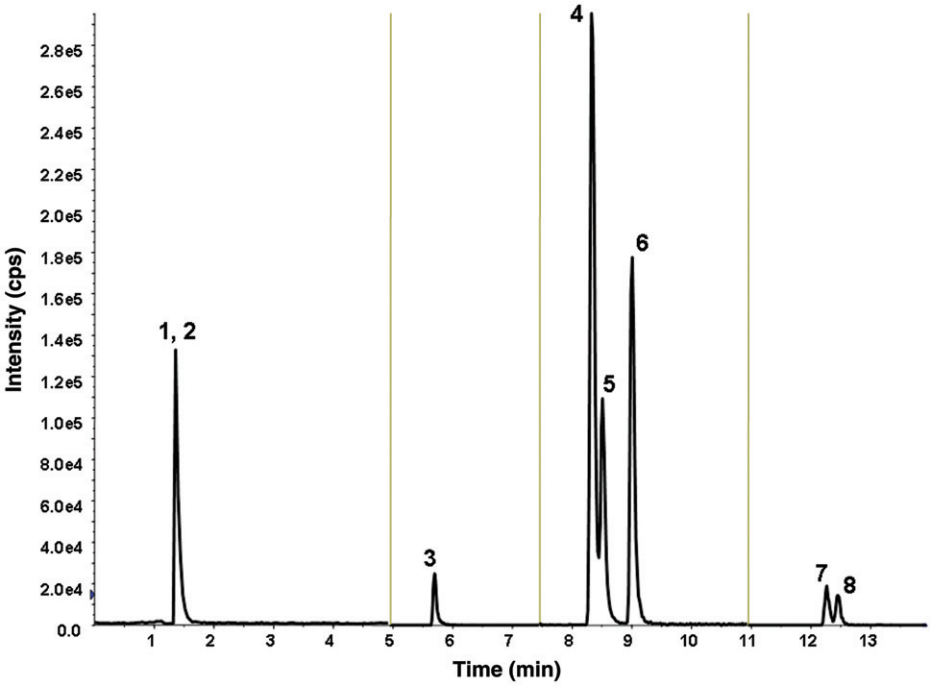

Figure 1. HPLC-ESI-MS/MS chromatogram of a 25 ng mL^{-1} matrix standard on a 50 × 3 mm Shim-Pack XR-ODS, 2.2 µm HPLC column. Temperature: 30°C, flow rate: 300 µL min^{-1}, gradient: 0–0.55 min 5% B, 12 min 80% B, 13 min 80% B, 20 min 5% B, mobile phase A: 0.1% HCOOH in deionised water, mobile phase B: 0.1% HCOOH in acetonitrile. **1**: 5-fluorouracil, **2**: gemcitabine, **3**: methotrexate, **4**: ifosfamide, **5**: cyclophosphamide, **6**: etoposide, **7**: paclitaxel, **8**: docetaxel.

(MRM). The pause time was set at 5 ms and the dwell times at 100 ms. In order to obtain only in the first part of the chromatogram the time consuming and sensitivity decreasing positive-negative switching and also a minimum of MRM transitions the mass spectrometry measurements were split into four periods (Figure 1). Instead of the protonated molecule ions, the more intensive sodium adducts were selected as precursor ion of the MRMs for the two taxanes.

To improve the limits of detection and verification for low concentrated 5-FU samples, a single HPLC-APCI-MS/MS method using a 3200 Q Trap® mass spectrometer (Applied Biosystems/MDS Sciex, Darmstadt, Germany) was applied (Figure 2). The chromatographic separation was done using a LC-20AD Prominence HPLC-pump with a DGU-20A degaser, a CTO-20 AC column oven and a SIL-20 AC autosampler (all Shimadzu, Duisburg, Germany) isocratically with 95 : 5 (v/v) H_2O + 0.1% HCOOH: ACN + 0.1% HCOOH on a 125 × 2 mm Nucleodur 100-5 C18 ec HPLC-column (Machery-Nagel, Dueren, Germany) with a flowrate of 500 µL min^{-1} at 30°C. Ionisation was performed with atmospheric pressure chemical ionisation (APCI) in the negative MRM mode. The ion source temperature was set to 400°C with a needle current of 1 µA. Dwell time was 150 ms. Numeric gas-settings were 30, 40; 6 and 40 for the nebuliser gas, curtain gas, collision gas and turbogas, respectively. A summary of the MS/MS parameter settings for the APCI single and ESI multi-method is shown in Table 1.

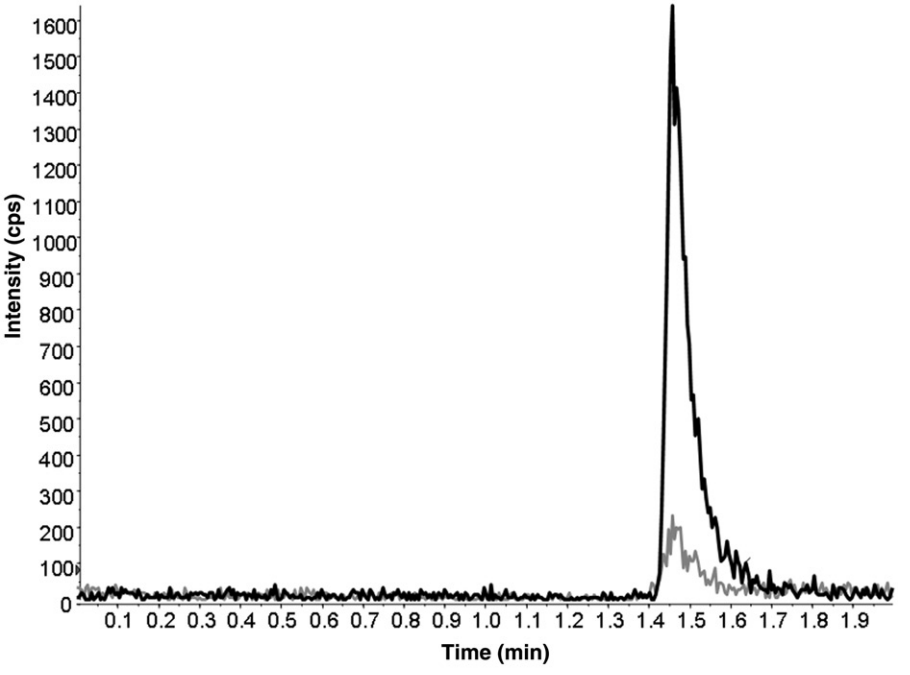

Figure 2. HPLC-APCI-MS/MS chromatogram of 5-fluorouracil ($10\,ng\,mL^{-1}$). $125 \times 2\,mm$ Nucleodur 100-5C18ec HPLC column; temperature: 30°C; flow rate: $500\,\mu L\,min^{-1}$ 95 : 5 (v/v) $H_2O + 0{,}1\%$ HCOOH: CAN + 0,1% HCOOH isocratically; black: MRM 1: $129 \rightarrow 42$ (quantification transition); grey: MRM 2:$129 \rightarrow 59$ (verification transition).

2.5 *Method validation*

2.5.1 *Precision and accuracy*

In the run-up to setup a large scale study extensive investigations were performed to validate different influences on the analytical results. Limits of detection were determinated at a signal to noise ratio of 3 : 1. Limits of quantification were defined as the lowest level of the matrix calibration from a weighted (1/x) regression analysis. In addition to the validation of the instrumental method, inter- and intra-day variations of the laboratory procedure were investigated. For this purpose a triplicate experiment on three different days ($c = 10$, 50 and $100\,ng\,mL^{-1}$) with spiked wipe samples was performed. Accuracy is expressed as recovery rate and the maximum value of the relative error (R.E.).

2.5.2 *Influence of sampler and analysed surface material*

Validation of the wipe sampling procedure was performed in-house. The test surfaces ($10 \times 20\,cm = 200\,cm^2$) were spiked with 2,000 ng in 1 mL methanol resulting in a contamination of $10\,ng\,cm^{-2}$ for each of the eight investigated compounds. Methanol evaporated completely at room temperature within one hour. To distinguish the effect of different persons taking the samples, 28 volunteers performed the wipe sampling from spiked glass surfaces after being introduced to the standard operation procedure. The samples were extracted and analysed as described above. The average recovery efficiencies and standard deviations (error bars) are presented in Figure 3.

Table 1. Optimised MS/MS - detection parameters of the APCI single and ESI multi method.

		Q/V[1]	Q_1 [amu]	Q_3 [amu]	DP [V]	EP [V]	CEP [V]	CE [eV]	CXP [V]
5-Fluorouracil single method: APCI	$[M-H]^-$	Q	129	42	−30	−10	−14	−28	−4
		V	129	59	−30	−10	−14	−36	−4
		Q/V[1]	Q_1 [amu]	Q_3 [amu]	DP [V]	FP [V]	–	CE [eV]	CXP [V]
5-Fluorouracil multi-method: ESI	$[M-H]^-$	Q	129	42	−50	−350		−28	−5
		V	129	59	−50	−350		−28	−5
Cyclophosphamide	$[M+H]^+$	Q	261	140	31	60		31	10
		V	261	233	31	60		23	16
Docetaxel	$[M-Na]^+$	Q	830	549	100	330		35	38
		V	830	248	100	330		45	18
Etoposide	$[M+H]^+$	Q	589	229	16	130		21	16
		V	589	185	16	130		47	12
Gemcitabine	$[M+H]^+$	Q	264	112	56	280		27	8
		V	264	95	56	280		59	6
Ifosfamide	$[M+H]^+$	Q	261	92	36	200		37	8
		V	261	154	36	200		33	10
Methotrexate	$[M-H]^-$	Q	453	324	−56	−300		−30	−23
		V	453	174	−56	−300		−46	−23
Paclitaxel	$[M-Na]^+$	Q	876	308	100	310		43	18
		V	876	591	100	310		35	36

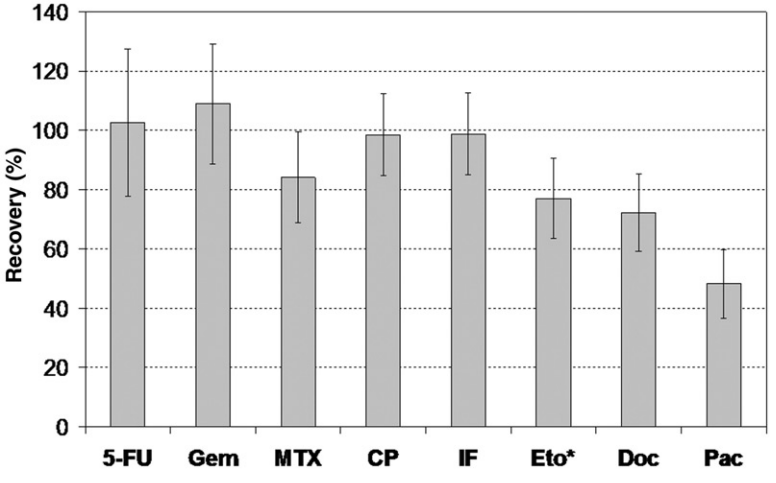

Figure 3. Influence of the sampling person ($n=28$) on the wipe sampling efficiency. (Mean recovery rate, the standard deviation is expressed by the bars; $c=10\,ng\,cm^{-2}$; $n=112$; *because of technical and stability problems with the etoposide standard during this validation trial only the values of 12 persons with 48 single values could be gathered).

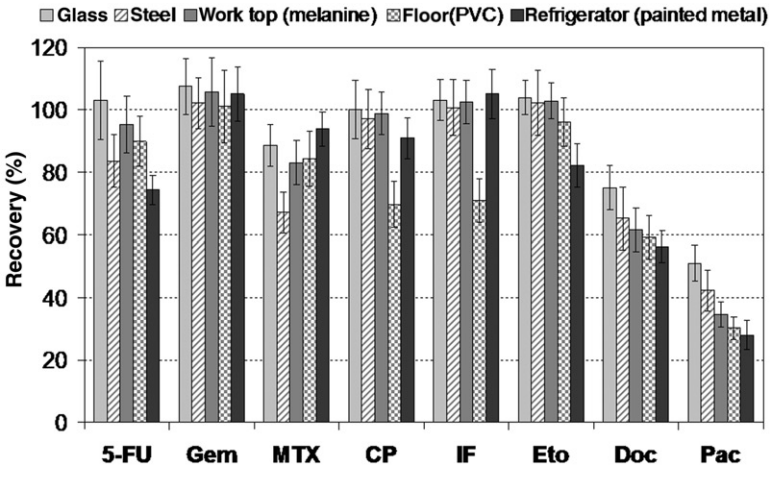

Figure 4. Mean recovery rates and standard deviations (bars) for wiping of different surfaces ($n = 9$, $c = 10\,\mathrm{ng\,cm^{-2}}$).

In addition, the influence of the different sample surfaces was examined on the following common surface materials present in pharmacies: glass, steel, melamine work top, white painted metal (refrigerator door) and PVC floor. Thus nine spiked samples of each material were evaluated in the laboratory. The recovery rates are shown in Figure 4.

2.5.3 *Influence of shipping and storage*

Furthermore, the influences of shipping and storage conditions were validated by simulating three different standard situations relevant for the study:

(A) spiked samples are stored according to the SOP in the transport box together with two frozen freeze packs for 24 hours at room temperature and analysed directly afterwards;
(B) storage conditions as described above, (A), but over 48 hours;
(C) storage conditions similar to (A), with an additional storage at −18°C for 7 days.

The respective recovery rates are shown in Figure 5.

3. Results and discussion

3.1 *Development and validation of the instrumental method*

A robust and reliable LC-MS/MS multi-method for eight structurally different cytostatic drugs was developed and successfully validated. The instrumental limits of detection ($s/n = 3:1$) range from 0.07 to $1.2\,\mathrm{ng\,mL^{-1}}$. Electrospray positive-negative switching with settling times of 700 ms and co-elution of gemcitabine and 5-FU resulted in a poor LOD for 5-FU. Therefore, a second single compound method using APCI was developed and used for analysis of sample extracts with concentrations less than $10\,\mathrm{ng\,mL^{-1}}$ of 5-FU. This was found to be necessary because 5-fluorouracil is clearly the most frequently used (and in the largest amounts handled) antineoplastic drug. LOD and LOQ for this single method are seven-times and eight-times respectively lower than that of the multi-method. Limits of quantification were defined as the lowest level of the weighted (1/x) regression.

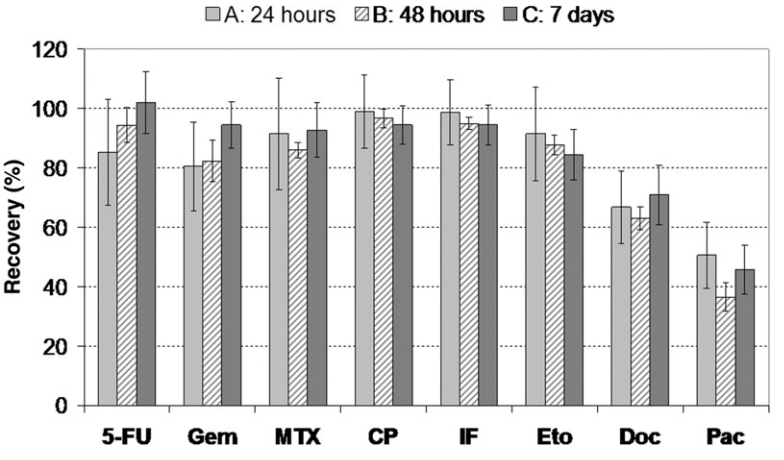

Figure 5. Influence of transport time and storage conditions on the mean recovery rates ($n = 4$; standard deviations are expressed by the bar). **A**: Simulation of sample shipment to the laboratory with spiked samples stored in the transport box together with two frozen freeze packs for 24 hours at room temperature and analysed directly afterwards; **B**: storage conditions similar to A), but 48 hour storage instead of 24 hours; **C**: storage conditions similar to A), but analysed after storage at −18°C for 7 days.

Table 2. Limits of detection (signal-to-noise ratio = 3 : 1) and limits of quantification (lowest calibration level) of the APCI single method for 5-FU and the ESI multi method (8 compounds).

	LOD [ng mL^{-1}]	LOQ [ng mL^{-1}]	LOQ [ng/sample]	LOQ [pg cm^{-2}]
5-Fluorouracil				
– APCI single method	0.17	0.3	9.9	11
– ESI multi method	1.2	2.5	83	92
Gemcitabine	0.10	0.2	6.6	7.3
Methotrexate	0.08	0.1	3.3	3.7
Ifosfamide	0.07	0.1	3.3	3.7
Cyclophosphamide	0.08	0.1	3.3	3.7
Etoposide	0.10	0.1	3.3	3.7
Paclitaxel	0.21	0.5	17	18
Docetaxel	0.35	1.0	33	37

Correlation coefficients (r^2) were better than 0.99 for a calibration from LOQ to 100 ng mL^{-1}. Besides instrumental limits of quantification (0.1 to 1 ng mL^{-1}) also sample specific calculated LOQs (based on an extraction volume of 30 mL and a surface area of 900 cm^2) are summarised in Table 2.

In comparison to the established GC-MS single-methods the LODs for CP, IF, and 5-FU are in the same range. Only analysis of CP and IF using enrichment techniques or high resolution mass spectrometry are more sensitive [39,40]. LODs for MTX single methods using Flx/TDx analysers, HPLC-UV or LC-MS/MS are quite different. Other methods could detect only 580 ng/sample [41,42], which is not useful for analysis of occupational exposure in pharmacies. The most sensitive reported method by Turci *et al.*

has an LOD of 2 ng/wipe sample and an LOQ of 5 ng/wipe sample [32]. Sabatini *et al.* achieved limits of detection of 33 ng mL^{-1} for 5-FU as well as 1.1 ng mL^{-1} for CP and MTX [31]. Sottani *et al.* developed the first LC-MS/MS multi-method after solid phase extraction for gemcitabine, paclitaxel, cyclophosphamide and ifosfamide. Limit of quantification range from 12.5 to 25 ng/wipe sample [36]. Total analysis time for this four compound multi-method was a little bit longer than those of the multi-method for eight cytostatic drugs reported in this paper.

Because it requires no derivatisation or solid phase enrichment, the developed method is a simple and reliable tool for the application of a regular environmental monitoring of structurally different antineoplastics in pharmacies as required in the Monitoring-Effect Study of Wipe Sampling in Pharmacies (MEWIP).

3.2 *Precision and accuracy*

Relative standard derivations for the LC-MS/MS analysis (nine repeated injections of a spiked wipe sample) ranged between 1.1% for cyclophosphamide and 6.4% for docetaxel. Precision and accuracy of the total analytical method are shown in Table 3 (intra-day) and Table 4 (inter-day). Except for the inter-day analysis of etoposide at 10 ng mL^{-1} and paclitaxel at a concentration of 100 ng mL^{-1} all coefficients of variation (C.V.) were below 20%. In combination with the recoveries and relative errors the validation data show the suitability of the developed method. Except for paclitaxel the validation data show good recovery rates above 70%. Therefore the results for paclitaxel were corrected by the specific recovery rate. In accordance with the overall good precision and accuracy the developed multi-method is regarded as a well suited tool for environmental monitoring of ambient contaminations with multiple cytotoxic drugs in pharmacies.

3.3 *Validation of the wiping procedure*

Since sampling had to be performed by the individual pharmacy personnel, the influence of the sampling person was validated as shown in Figure 3. Relative standard deviations were between 14 and 24% for the whole procedure. This indicates that sampling by untrained personnel following the developed SOP does not influence the study results in a critical way overall.

The influence of common surface materials on the recovery rates of the sampling procedure was determined as shown in Figure 4. Best recovery rates can be achieved from glass surfaces while the biggest losses were observed on painted metal (refrigerator door). In addition a slight reduction of the recovery rate was found for 5-FU and MTX on steel surfaces. The recovery rate of gemcitabine was not influenced by the surface material. Due to possible adsorption effects the recovery rates of cyclophosphamide and the isomeric ifosfamide were decreased down to 70% on PVC. This effect has also been described by Pethran *et al.* [43]. For methotrexate, the known light sensitivity of the compound may contribute to the reduced recovery rates [44,45]. In addition the recovery rates for the non-polar taxanes docetaxel and paclitaxel are reduced because a polar sampling and extraction solvent was used. This observation is consistent throughout all following validation experiments.

Figure 5 shows that the different shipping and storage conditions which occurred in MEWIP had no relevant effect on the results. The slightly lower recovery rates for

Table 3. Intra-day accuracy and precision for the analysis of spiked wipe samples ($n = 3$).

	Target conc. [ng mL^{-1}]	Detected conc. mean ± SD [ng mL^{-1}]	Precision C.V. [%]	Accuracy REC [%]	Accuracy R.E. [%]
5-FU	10	8.0 ± 0.4	5.5	80	−5.8
	50	42 ± 2	4.7	83	4.9
	100	76 ± 5	7.1	76	−8.2
CP	10	9.3 ± 0.4	4.7	93	5.0
	50	46 ± 3	7.2	92	−7.7
	100	97 ± 2	2.0	97	−2.2
Doc	10	8.6 ± 0.6	7.5	86	−8.6
	50	43 ± 4	9.2	86	−9.2
	100	90 ± 11	12	90	−13
Eto	10	6.0 ± 0.5	9.0	60	9.2
	50	48 ± 2	3.9	95	4.4
	100	97 ± 3	3.4	97	−3.9
Gem	10	9.4 ± 0.2	2.6	94	2.7
	50	49 ± 0.3	0.6	98	0.7
	100	92 ± 6	6.3	92	7.1
IF	10	9.6 ± 0.5	5.0	96	−5.7
	50	49 ± 0.3	0.6	98	−0.6
	100	100 ± 2	1.9	100	−2.2
MTX	10	7.8 ± 0.9	12	78	−13
	50	39 ± 3	6.7	78	−7.6
	100	81 ± 3	3.8	81	−3.9
Pac	10	5.6 ± 0.9	16	56	−18
	50	27 ± 3	10	53	12
	100	58 ± 9	15	58	−16

5-fluorouracil and gemcitabine during the simulation of transport conditions A and B are within the standard deviation of the total analytical method.

All validation experiments showed consistently reduced recovery rates for docetaxel and paclitaxel. This illustrates the difficulties of one multi-method for polar and non-polar compounds. The efficiency of sampling and extraction of non-polar compounds with a polar solvent like the used aqueous pH 3 solution has strong limitations. Moreover, precision and accuracy resulted in measurement uncertainties above 20% for paclitaxel and docetaxel. Even with a value correction by the recovery, the results for the two taxanes could be not used for an absolute evaluation. However, the taxanes were not excluded form the method since the simultaneous analysis did not require additional efforts. If a specific analysis of non-polar drugs such as the taxanes shall be performed, non-polar solvents like isopropyl alcohol or ethyl acetate must be used for sampling and extraction [22,33].

During MEWIP 1269 samples from 130 pharmacies were analysed with the developed method. Results obtained from the novel LC-MS/MS multi compound analysis in this study were comparable to those of single analysis data of environmental monitoring.

Table 4. Inter-day accuracy and precision for the analysis of spiked wipe samples ($n = 9$, three samples each day on three different days).

	Target conc. [ng mL^{-1}]	Detected conc. Mean ± SD [ng mL^{-1}]	Precision C.V. [%]	Accuracy REC [%]	Accuracy R.E. [%]
5-FU	10	8.7 ± 0.7	8.3	87	−13
	50	42 ± 3	7.3	84	−16
	100	81 ± 9	11	81	19
CP	10	9.6 ± 0.8	8.4	96	19
	50	47 ± 3	7.1	95	−11
	100	94 ± 4	4.2	94	5.5
Doc	10	8.8 ± 0.9	11	88	−20
	50	44 ± 4	8.8	88	14
	100	81 ± 10	13	81	22
Eto	10	7 ± 2	26	70	52
	50	47 ± 7	14	95	20
	100	89 ± 8	8.5	89	12
Gem	10	8.2 ± 1.4	17	82	−29
	50	40 ± 7	17	81	−24
	100	84 ± 8	10	84	17
IF	10	9.8 ± 0.7	7.6	98	16
	50	49 ± 2	4.7	97	−7.6
	100	95 ± 5	4.9	95	6.8
MTX	10	8.1 ± 1.4	17	81	34
	50	40 ± 5	13	80	24
	100	77 ± 4	5.5	77	−10
Pac	10	6.7 ± 1.3	19	67	−31
	50	33 ± 5	15	65	−23
	100	62 ± 15	24	62	39

Contamination levels range over several orders of magnitude. The detailed MEWIP results will be published as well as best practice recommendations elsewhere.

4. Conclusions

A simple multi-method was developed for the simultaneous sampling and LC-MS/MS analysis of structurally different cytostatic drugs. Validation showed that the method is robust and precise for six of the eight investigated antineoplastics. Only for the two taxanes docetaxel and paclitaxel recovery rates are too low and measuring uncertainties too high for an absolute evaluation. Regarding the sample transport and storage conditions, different surface materials, as well as different persons performing the sampling according to the developed SOP the method is well suited for the comparison of different pharmacies. The sampling-sets used for shipping are qualified for the relevant surface materials and a transport up to 48 hours. The application of an ambient monitoring at 1269 samples showed that the developed method can be used as a

reliable tool. As stated by Sottani et al. future work has to be done to increase the number of drugs monitored [36]. Special attention must be considered to the polarity of investigated compounds, solvents and recovery rates from uneven surfaces.

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References

[1] C. Minoia, R. Turci, C. Sottani, A. Schiavi, L. Perbellini, S. Angeleri, F. Draicchio, and P. Apostoli, Rapid Commun Mass Spectrom **12**, 1485 (1998).
[2] G. Schmaus, R. Schierl, and S. Funck, Am. J. Health Syst. Pharm. **59**, 956 (2002).
[3] R. Turci, C. Sottani, G. Spagnoli, and C. Minoia, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **789**, 169 (2003).
[4] Occupational Safety and Health Information Series (Occupational Safety and Health Service, Department of Labour, Wellington, New Zealand, 1997).
[5] HSE Information Sheet MISC 615: *Safe Handling of Cytotoxic Drugs* (Health and Safety Executive, Sudbury, Suffolk, UK, 2003).
[6] NIOSH Alert: *Preventing Occupational Exposures to Antineoplastic and other Hazardous Drugs in Healthcare Settings* (NIOSH – National Institute for Occupational Health and Safety, Cincinnati, OH, USA, 2004).
[7] A. Heinemann, *BGW topic: Cytostatic drugs in the health service. Information for safe handling of cytostatic drugs (M620)* (Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege – BGW, Hamburg, Germany, 2009).
[8] T.A. Connor, P.J. Sessink, B.R. Harrison, J.R. Pretty, B.G. Peters, R.M. Alfaro, A. Bilos, G. Beckmann, M.R. Bing, L.M. Anderson, and R. Dechristoforo, Am. J. Health Syst. Pharm. **62**, 475 (2005).
[9] G. Dranitsaris, M. Johnston, S. Poirier, T. Schueller, D. Milliken, E. Green, and B. Zanke, J. Oncol. Pharm. Pract. **11**, 69 (2005).
[10] E.E.M. Brouwers, A.D.R. Huitema, E.N. Bakker, J.W. Douma, K.J.M. Schimmel, G. van Weringh, P.J. de Wolf, J.H.M. Schelens, and J.H. Beijnen, Int. Arch. Occup. Environ. Health **80**, 689 (2007).
[11] M. Hedmer, H. Tinnerberg, A. Axmon, and B.A.G. Jonsson, Int. Arch. Occup. Environ. Health **81**, 899 (2008).
[12] T.H. Connor and M.A. McDiarmid, CA Cancer J. Clin. **56**, 354 (2006).
[13] IARC, *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 7 (Lyon, France, 1987).
[14] P.J. Sessink, M.M. Scholtes, R.B. Anzion, and R.P. Bos, J. Chromatogr. **616**, 333 (1993).
[15] C. Sottani, R. Turci, L. Perbellini, and C. Minoia, Rapid Communications in Mass Spectrometry **12**, 1063 (1998).
[16] M.E. de Jonge, S.M. van Dam, M.J. Hillebrand, H. Rosing, A.D. Huitema, S. Rodenhuis, and J.H. Beijnen, J. Mass Spectrom. **39**, 262 (2004).

[17] D. Kasel, A. Jetter, S. Harlfinger, W. Gebhardt, and U. Fuhr, Rapid Commun Mass Spectrom. **18**, 1472 (2004).
[18] M. Hedmer, A. Georgiadi, E.R. Bremberg, B.A. Jonsson, and S. Eksborg, Ann. Occup. Hyg. **49**, 629 (2005).
[19] A. Barbieri, L. Sabatini, P. Indiveri, R. Bonfiglioli, V. Lodi, and F.S. Violante, Rapid Commun. Mass Spectrom. **20**, 1889 (2006).
[20] C. Sottani, G. Tranfo, P. Faranda, and C. Minoia, Rapid Commun. Mass Spectrom. **19**, 2794 (2005).
[21] P.J. Sessink, K.A. Boer, A.P. Scheefhals, R.B. Anzion, and R.P. Bos, Int. Arch. Occup. Environ. Health **64**, 105 (1992).
[22] R.R. Larson, M.B. Khazaeli, and H.K. Dillon, Am. J. Health Syst. Pharm. **59**, 270 (2002).
[23] B. Favier, L. Gilles, C. Ardiet, and J. Francois, J. Oncol. Pharm. Pract. **9**, 15 (2003).
[24] M. Kubo, H. Sasabe, and T. Shimizu, J. Chromatogr. **564**, 137 (1991).
[25] L. Thiberville, P. Compagnon, N. Moore, G. Bastian, M.O. Richard, M.F. Hellot, C. Vincent, M.M. Kannass, S. Dominique, and C. Thuillez, Cancer Chemother. Pharmacol. **35**, 64 (1994).
[26] S. Funck, Ph.D. thesis, Ludwig-Maximilians University Munich, 2003.
[27] G. Remaud, M. Boisdron-Celle, A. Morel, and A. Gamelin, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **824**, 153 (2005).
[28] A. Salvador, L. Millerioux, and A. Renou, Chromatographia **63**, 609 (2006).
[29] P.J. Sessink, N.S. Friemel, R.B. Anzion, and R.P. Bos, Int. Arch. Occup. Environ. Health **65**, 401 (1994).
[30] L. Floridia, A.M. Pietropaolo, M. Tavazzani, F.M. Rubino, and A. Colombi, J. Chromatogr. B Biomed. Sci. Appl. **726**, 95 (1999).
[31] L. Sabatini, A. Barbieri, M. Tosi, and F.S. Violante, J. Mass Spectrom. **40**, 669 (2005).
[32] R. Turci, G. Micoli, and C. Minoia, Rapid Commun. Mass Spectrom. **14**, 685 (2000).
[33] C. Sottani, R. Turci, G. Micoli, M.L. Fiorentino, and C. Minoia, Rapid Commun. Mass Spectrom. **14**, 930 (2000).
[34] F.M. Rubino, L. Floridia, A.M. Pietropaolo, M. Tavazzani, and A. Colombi, Med. Lav. **90**, 572 (1999).
[35] L. Kovalova, C.S. McArdell, and J. Hollender, J. Chromatogr. A **1216**, 1100 (2009).
[36] C. Sottani, R. Turci, R. Schierl, R. Gaggeri, A. Barbieri, F.S. Violante, and C. Minoia, Rapid Commun. Mass Spectrom. **21**, 1289 (2007).
[37] J. Tuerk, M. Reinders, D. Dreyer, T.K. Kiffmeyer, K.G. Schmidt, and H.M. Kuss, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **831**, 72 (2006).
[38] W. Fransman, D. Huizer, J. Tuerk, and H. Kromhout, Int. Arch. Occup. Environ. Health **80**, 396 (2007).
[39] M. Hedmer, B.A.G. Jonsson, and O. Nygren, J. Environ. Monit. **6**, 979 (2004).
[40] J. Yoshida, G. Tei, C. Mochizuki, Y. Masu, S. Koda, and S. Kumagai, Ann. Occup. Hyg. **53**, 153 (2009).
[41] M. Zeedijk, B. Greijdanus, F.B. Steenstra, and D.R.A. Uges, Eur. J. Hosp. Pharm. Sci. **11**, 18 (2005).
[42] J.F. Bussieres, Y. Theoret, S. Prot-Labarthe, and D. Larocque, Am. J. Health Syst. Pharm. **64**, 531 (2007).
[43] A. Pethran, R. Schierl, and G. Schmaus, Krankenhauspharmazie **22**, 11 (2001).
[44] A. Jacolot, Ph. Arnaud, D. Lecompte, S. Alamercery, E. Caroff, C. Naveau, Th. Guillot, M. Bousselet, and F. Brion, Int. J. Pharm. **128**, 283 (1996).
[45] I. Kraemer, in *Pharmaceutical Photostability and Stabilization Technology*, edited by Joseph T. Piechocki and Karl Thoma (Informa Healthcare, New York, NY, 2007), pp. 397–427.