Wipe sampling method and evaluation of environmental variables for assessing surface contamination of ten antineoplastic drugs by liquid chromatography/tandem mass spectrometry

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Abstract

This paper describes a novel wipe sampling and high performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) method capable of simultaneously detecting ten antineoplastic drugs (5-fluorouracil, oxaliplatin, methotrexate, vindesine, ifosfamide, cyclophosphamide, vincristine, vinblastine, docetaxel, and paclitaxel). The good overall recoveries and sensitivity values of this method along with the comparatively short run time (8 min) allows for its use in routine monitoring in healthcare facilities. The long-term behavior of the studied drugs on contaminated surfaces and the effect of surface roughness on drug recoveries were studied to gain insights about how these environmental variables influence the detection, cleaning and occupational exposure of these drugs. Surfaces with higher roughness parameter (R_a) values (rougher) had the lowest recoveries while those with lower R_a (smoother) presented the highest recoveries. Long-term assessments evidence distinctive drug behaviors with oxaliplatin, vindesine, vincristine and vinblastine being the less persistent drugs (~ 20 % was recovered after 24 h) and docetaxel and paclitaxel the most persistent drugs with recoveries of 40% and 80% after one month. This information indicates the importance of collecting ancillary information about drug usage (throughput, timing, cleaning procedures, etc.) to interpret the results in the context of potential exposure. Finally, the method was successfully applied to evaluate trace surface contamination down to the single picogram per square centimeter in multiple work areas within three local healthcare centers on Vancouver Island, Canada.

Key words: Occupational exposure, environmental monitoring, antineoplastic drugs, surface wipe, stability, LC-MS/MS.

Introduction

Antineoplastic drugs (also known as chemotherapy or cytotoxic drug) are primarily used for the treatment of cancer. Studies have shown that a range of healthcare workers who are involved with a hospital's medication circuit are at risk of exposure (Hon et al., 2011; Hon et al., 2013). Such exposure may result in genetic damage, toxic reproductive effects, and cancer amongst healthcare workers (Ritchie and Mcadams, 2000; Dranitsaris et al., 2005; Connor and McDiarmid, 2006). Despite these widely accepted health risks, there are no current regulated limits in Canada or elsewhere regarding an acceptable or 'safe' surface concentration. Nevertheless, environmental monitoring using surface wipe testing is now an explicit requirement in safe drug handling documents, notably the United States Pharmacopeia General Chapter <800> (USP 800; USP, 2014), and is intended for risk management purposes (Connor et al., 2016). Specifically, the regular screening of antineoplastic drugs will help to identify contamination sources (i.e. hotspots and pathways) in addition to assessing potential occupational exposure with the ultimate goal to ensure prevention of worker exposure in health care settings.

Since no occupational exposure limit exists it is best practice to follow the principle of maintaining occupational exposure to antineoplastic drugs As Low As Reasonably Achievable (ALARA). To achieve this, accurate sampling and analytical methods must be developed to assess trace levels of drug contamination on surfaces. To this end, we previously reported the development of a novel wipe sampling method to identify and quantify such contamination for six commonly administered antineoplastic drugs (Jeronimo et al., 2015).

However, it has been noted by Connor et al. (2010) that "every published [field] surface contamination study has identified at least one (antineoplastic) drug present by wipe sample analysis". Recognizing that cancer treatments often include more than one drug at a time, and in order to increase the probability of having at least one positive finding, we have decided to add to the library of agents that we can simultaneously analyze. Furthermore, studies have found that wipe recovery can vary greatly depending on the drug, type of wipe used, and environmental variables such as type and condition of

surface to be sampled (Pretty et al., 2012; Hymer et al., 2015). An exploration of these factors is necessary in order to meet the Occupational Safety and Health Administration's guidelines for surface wipe sampling methods (Occupational Safety and Health Administration (OSHA), 2001).

In order to address the need for an accurate and practical method to facilitate the routine screening of these hazardous chemicals in hospitals and health care facilities, we describe herein the validation of an extended method for wipe sampling of ten different antineoplastic drugs: 5-fluorouracil, paclitaxel, cyclophosphamide, vincristine, oxaliplatin, methotrexate, ifosfamide, vindesine, vinblastine, and docetaxel. In addition, we investigate the effect of environmental variables such as surface roughness and the long-term behavior of the drugs on the method efficiency and their impacts on occupational exposure, aspects that have not been previously discussed in the literature.

This sampling procedure and analytical method was successfully applied in the field to evaluate surface contamination of multiple locations within three local healthcare centers on Vancouver Island in British Columbia, Canada.

Materials and Methods

Chemicals and Materials

Methotrexate-methyl-d3 (CAS 432545-63-6), and paclitaxel-d5 (CAS 1129540-33-5) were purchased from Santa Cruz Biotech (Dallas, Texas). Cyclophosphamide (CP; CAS 50-18-0) and 5-fluorouracil (5-FU; CAS 51-21-8) were purchased from Fisher Scientific. Vindesine sulfate (VND; CAS 59917-39-4), vinblastine sulfate (VNB; CAS 865-21-4), vincristine sulfate (VNC; CAS 2068-78-2), vincristine-d3 sulfate (no CAS number, TRC catalog number V314253), oxaliplatin (OXP; CAS 61825-94-3), paclitaxel (PTX; CAS 33069-62-4), docetaxel (DTX; CAS 700367-34-6), ifosfamide (IF, CAS 3778-73-2) and cyclophosphamide-d4 (CAS 173547-45-0) were purchased from Toronto Research Chemicals (Toronto, Ontario). Ammonium formate was purchased from MP Biomedicals (Santa Ana, California). High performance liquid chromatography-grade methanol (HPLC-MeOH) and formic acid (FA) were obtained from Fisher Scientific (Vancouver, Canada). Ultrapure (18MΩ cm) water (H_2O) was

generated in situ using a Barnstead NANOpure Analytical Deionization System. Standards were prepared at nominal concentrations of 1, 5, 15, 50, 100, and 200 ng mL^{-1} for 5-FU and OXP and 0.2, 1, 5, 15, 50, 100, and 200 ng mL^{-1} for all other compounds. All stock solutions were stored at -20°C.

Chromatographic Conditions

An Agilent 1200 series high performance liquid chromatography system (Agilent Technologies, Santa Clara, California) consisting of a G1312B binary pump, a G1379B degasser, and a G1367D refrigerated autosampler was employed in this study. Twenty microliters of sample was injected onto a Phenomenex Kinetex Biphenyl column (50 x 4.6mm, 2.6µm particle size; Phenomenex, Torrance, California). The column oven was maintained at 45 °C and the mobile phase flow was set at 0.6 mL min^{-1}. The mobile phase consisted of (A) 1 mM ammonium formate buffered to pH 2.3 with formic acid and (B) methanol. The timetable was as follows: 50% A from 0-1.3 min, 15% A at 3 min, 5% A from 4-5.5 min, and 50% A from 7-8 min. Total run time for each sample was 8 min.

Mass Spectrometry Conditions

A triple quadrupole Agilent 6410 mass spectrometer (Agilent Technologies, Santa Clara, California) was used for detection in this study. The mass spectrometer (MS) was operated in multiple reaction monitoring mode and utilized positive electrospray ionization. MS parameters of each drug (see Supporting Information Table S1 in the supplementary online material) were individually optimized manually during syringe pump infusion. Agilent Masshunter Workstation Data Acquisition B.02.01 was used for data acquisition and Agilent Masshunter Quantitative Analysis B.07.00 was used for data processing.

Analytical Validation

To assess intra-day variability, ten samples at the same nominal concentration and run on the same day were analyzed. This procedure was done at two concentrations, 5 ng mL^{-1} and 50 ng mL^{-1}. To assess inter-day variability, ten samples at each concentration were run on five separate days. The analytical limit of detection (LOD) was determined by analyzing standards at decreasing concentrations

approaching the LOD. The LOD was set as the lowest concentration of each compound that could be detected at a 3:1 signal to noise ratio (SNR), by peak-to-peak height. The limit of quantification (LOQ) was set as the lowest concentration of each compound that could be detected at a 10:1 SNR). The method detection limit (MDL) was calculated from the LOD, taking into account the recovery in the wiping and desorption steps.

Sample processing

Desorption method development. Desorption step was performed using filter paper (No. 42 ashless, 70mm) from Whatman (Baie d'Urfe, Quebec); each filter was cut in half in order to facilitate its handling during wiping procedures. The extraction was performed in 20 mL scintillation vials from Fisher Scientific (Vancouver, British Columbia) and filtered using 10 mL syringes (Luer-Lok tip) from BD (Mississauga, Ontario) and syringe filters (Millex-HV, 0.45 μm, Millex-GV, 0.22 μm, Millex-GP, 0.22 μm) from EMD Millipore (Etobicoke, Ontario). In order to test the desorption for all ten compounds, inter-stock solution containing the ten antineoplastic drugs at a concentration of 5 μg mL^{-1} was spiked on the filter paper and desorbed using the procedure described in Jeronimo et al. (Jeronimo et al., 2015), with the exception that in this work the syringe filtration step was excluded to improve the desorption efficiency. The final desorption method consisted of sonicating the filter, which was previously spiked with 55 μL of the internal standard (IS) solution, in 5 mL of the desorption solution (H_2O/MeOH 50:50 with 0.1% FA) for 35 min. Then, the solution was centrifuged at 4500 RPM for 15 min. One milliliter of the clear solution was placed in an autosampler vial and analyzed using LC–MS/MS.

Wiping method. Drug recovery efficiencies were evaluated on brand new 10 cm x 10 cm stainless steel (type 304) plates. These plates were purchased for this study; before starting the experiments, these plates were cleaned with Sparkleen detergent, and rinsed with ultrapure deionized water (18.0 MΩ-cm). The wiping tests were conducted using the Whatman filter papers. Before performing the wiping procedures, blank plates (without inter-stock spike) were run multiple times throughout the project and results were always below LOD. Recovery values and relative standard deviations were calculated based on the theoretical (expected) concentration for each drug. 50 μL of inter-stock solution was spread over a

stainless steel plate with a digital micropipette following a line (striped) pattern across the plate to simulate the random dispersion of the drugs. The final concentration on the plate was 2.5 ng cm^{-2} for each drug. After waiting 15 min for solvent evaporation, wiping was performed following the procedure described in Jeronimo et al. (2015). Briefly, the plate was wiped once with half of a Whatman filter paper wetted with 0.5 mL of H_2O/MeOH (20:80) with 0.1% FA. The surface was wiped initially with an up and down motion (vertical) then with a side-to-side motion (horizontal) after folding the paper filter or wipe tissue (ensuring that the wiped area is on the "inside" of the fold). A new pair of gloves was used for each sample.

Wipe method application

Overall quantitative performance of the wipe method on stainless steel surfaces. The performance of the wipe method was evaluated using brand new stainless steel plates (10 x 10 cm) which were spiked with 50 µL of inter-stock solution containing the ten antineoplastic drugs at 5 µg mL^{-1}. After solvent evaporation the plates were wiped and the wipes were desorbed following the protocol previously described. In order to assess the performance of the wipe method in a real scenario, three individuals, including the technician who was involved in the project and two external personnel, wiped the plates on three different days. The technician wiped 14 plates and external personnel wiped 5 plates each (24 replicates in total). A typical chromatogram obtained from the analysis of an extracted wipe sample using the described method is shown in Supporting Information Figure S1 in the supplementary online material.

In addition, wipe kits obtained from two commercial labs in Canada were used to wipe plates spiked in the same manner. The same three individuals each wiped 5 plates using the materials and method described in this paper, 5 using the materials and method from Lab A, and 5 using the materials and method from lab B; a total of 15 samples for each method being compared. After wiping the plates, the samples were immediately sent to the respective labs for analysis. The commercial labs were able to analyze a cluster of 2 to 3 drugs, but CP was the only drug in common among the three wiping methods.

Effect of surface roughness on drugs recoveries: an extended study. Jeronimo et al. (2015) first reported the effect of the surface roughness of stainless steel on wipe recovery of antineoplastic drugs. In this paper an extended study was performed to examine the variation of drug recoveries based on the surface roughness. For this purpose, three different types of plates were used in this experiment: 1- brand new plates, 2- plates used in a previous study, and 3- plates used in multiple studies. The used plates were employed in similar wiping experiments with antineoplastic drugs. All the plates used in this experiment were individually labeled, and then the surface roughness parameter (R_a) of the plates was measured using a Bruker Dektak XT profilometer at the University of British Columbia Advanced Nanofabrication Facility clean room. The scanning stylus radius was 12.5 microns. Three 10-mm scans were made on each plate at 0.3 samples per micron and R_a was calculated by the instrument for each scan. It should be noted that the plates were not scratched intentionally in order to have an evenly distributed gradient of the surface roughness. In addition to the quantitative data of the surface roughness, the plates were classified qualitatively in 3 different categories based on their time of use: 1- brand new plates which were obtained for this study ("category 1"), 2- similar plates used in a previous study ("category 2"), 3- plates used in multiple studies ("category 3"). The qualitative categorization allowed an evaluation of the differences in the recovery of the drugs among plates that have similar R_a, but were more used. For instance, cycles of cleaning using various solvents and/or heating may affect the surface chemistry of the plates, without affecting the surface roughness. After spiking the plates with 50 µL of inter-stock solution, the recoveries of the ten cytotoxic drugs were analyzed following the protocol described above. The wiping procedures in this roughness experiment were performed by the same technician to reduce inter-individual variability isolating the effect of surface roughness on drug recoveries. The relationship between the recovery and the surface roughness was study plotting the recovery values of each plate against its R_a keeping track of their qualitative categories. Moreover, an ANOVA was performed to evaluate the recoveries values among the plates with different categories; when the ANOVA revealed a significant P value ($P<0.05$), a multiple comparison test was used to identify the sample means that were significantly different from each other.

Stability assessment. The stability of the 10 drugs on the wipes was assessed over a week at 5 °C in order to replicate the shipping conditions from the place where the samples are taken to the laboratory. For this purpose, 12 new stainless steel plates were spiked with 50 μL of the inter-stock solution. After solvent evaporation, the plates were wiped by the same technician following the protocol described in the Wiping method section. After wiping, the filter papers were put in scintillation vials and kept at 5 °C. Clusters of three samples were extracted at 0, 24, 48, and 168h and analyzed by LC–MS/MS. An ANOVA test and a multiple comparison test (post hoc test) were used to evaluate the recovery values of the stability test.

Moreover, the long-term behavior of the studied drugs on the stainless steel was evaluated over a period of one month. This experiment was developed to study the behavior of antineoplastic drugs once deposited on a surface and its possible implication to occupational exposure and sampling accuracy. Previous surface wipe assessments have suggested that CP and IF may be stable on surfaces (Connor et al., 2002; Connor et al., 2005; Hedmer et al., 2008; National Institute for Occupational Safety and Health (NIOSH), 2012) but, to our knowledge, this phenomenon has not been explored in depth with these or other drugs. In this study, 18 brand new plates were spiked with 50 μL of the inter-stock solution. After solvent evaporation, cluster of 3 plates were wiped and extracted at 0 and 24 h and 1, 2, 3 and 4 weeks. In addition to the monthly experiment, an additional 24 h surface stability test was carried out following the same procedures described above. After solvent evaporation, cluster of 3 plates were wiped and extracted at 0, 2, 4, 8, 20 and 24 h. During the experiment period the plates were kept in a fume hood under ambient conditions.

Environmental assessment. The developed method was applied to evaluate surface contamination of multiple locations within three local healthcare centers on Vancouver Island. For this purpose a sampling kit containing all required supplies was sent to each facility. The wiping procedures were performed by local staff at each facility following instructions provided in the sampling kit.

Statistical Analysis

The calibration curves and antineoplastic drug recoveries were calculated using Agilent Masshunter Quantitative Analysis, and the data was stored and organized in Excel sheets. The statistical analysis and graphics were developed using MATLAB 7.10.0.

Results

Analytical validation

Table 1 depicts the average accuracy and intra- and inter-day variability of each analyte at two concentrations.

Table 1. Accuracy, Intra- and inter-day variability (% relative standard deviation, RSD, for the developed method)

Drug	Accuracy		Intra-day variability RSD (%)		Inter-day variability RSD (%)	
	5 ng mL $^{-1}$	*50 ng mL^{-1}*	*5 ng mL $^{-1}$*	*50 ng mL^{-1}*	*5 ng mL $^{-1}$*	*50 ng mL^{-1}*
5-Fluorouracil (5-FU)	104%	94%	13%	5%	8%	9%
Oxaliplatin (OXP)	98%	104%	11%	4%	7%	4%
Ifosfamide (IF)	99%	101%	1%	1%	1%	2%
Cyclophosphamide (CP)	92%	102%	3%	3%	5%	2%
Methotrexate (MTX)	100%	100%	2%	1%	1%	2%
Vindesine (VND)	100%	101%	1%	1%	1%	2%
Vincristine (VNC)	105%	100%	11%	5%	8%	2%
Vinblastine (VNB)	100%	100%	2%	5%	4%	6%
Paclitaxel (PTX)	110%	99%	5%	2%	2%	5%
Docetaxel (DTX)	102%	100%	2%	2%	2%	3%

The calculated sensitivity for each compound is summarized in Table 2. The limit of detection (LOD) and limit of quantitation (LOQ) apply to the analytical method sensitivity. The method detection limit (MDL) is presented with respect to surface area wiped and total detectable per wipe and takes into account the calculated recovery for each compound.

Table 2. Limit of detection, limit of quantification and method detection limit (LOD, LOQ, MDL) for the analysis of target antineoplastic drugs

Drug	**LOD (ng mL^{-1})**	**LOQ (ng mL^{-1})**	**MDL$_{sample}$ (ng/sample)**	**MDL$_{surface}$ (pg cm^{-2})**
5-fluorouracil	1.382	4.61	17.64	176.41

Oxaliplatin	0.909	3.03	8.69	86.89
Methotrexate	0.048	0.16	0.39	3.92
Vindesine	0.010	0.03	0.08	0.79
Ifosfamide	0.190	0.63	1.07	10.66
Cyclophosphamide	0.031	0.10	0.18	1.80
Vincristine	0.002	0.01	0.13	0.01
Vinblastine	0.005	0.02	0.36	0.04
Docetaxel	0.071	0.24	3.98	0.40
Paclitaxel	0.009	0.03	0.53	0.05

MDL_{sample} was calculated for each compound using the following equation: ([LOD * extraction volume]/overall method recovery); extraction volume = 5.55 mL, overall method recovery in Table S3

$MDL_{surface}$ was calculated for each compound using the following equation: (MDL_{sample}/surface area); surface area = 100 cm^2

Wipe Desorption

The final desorption values of the studied analytes were close to 100 % for MTX, VND, IF, CP, VNC, VNB, PTX and DTC, and 73% for OXP and 44% for 5-FU, Table 3.

Table 3. Quantitative performance for the desorption of the ten antineoplastic drugs on Whatman filter paper with 5 mL of MeOH 50 % with FA 0.1 % (n=6)

Drug	Recovery (%)	RSD (%)
5-fluorouracil	44%	15%
Oxaliplatin	73%	22%
Methotrexate	100%	1%
Vindesine	97%	3%
Ifosfamide	101%	3%
Cyclophosphamide	101%	3%
Vincristine	95%	3%
Vinblastine	98%	2%
Docetaxel	113%	5%
Paclitaxel	98%	2%

Overall quantitative performance of the wipe method on stainless steel surfaces

The overall quantitative performance of the method is summarized in Figure 1. These data represent the results of a realistic application of the method; five replicates each were done by two external personnel who had no previous experience with the method and limited laboratory experience in

general. The external personnel were provided with instructions and a brief demonstration of the method. Fourteen replicates also were done on two separate occasions by experienced lab personnel.

The performance of the wiping method developed by the School of Population and Public Health (SPPH) was compared with established methods developed by private laboratories in Canada. The results of CP recoveries from the inter-laboratory comparison are summarized in Table 4.

Table 4. Comparison of CP recovered from a stainless steel plate spiked at 2.5 ng cm^{-2} using three methods. (n=15 for each lab)

	Recovery	RSD
SPPH Lab	94%	6%
Lab A	94%	12%
Lab B	86%	3%

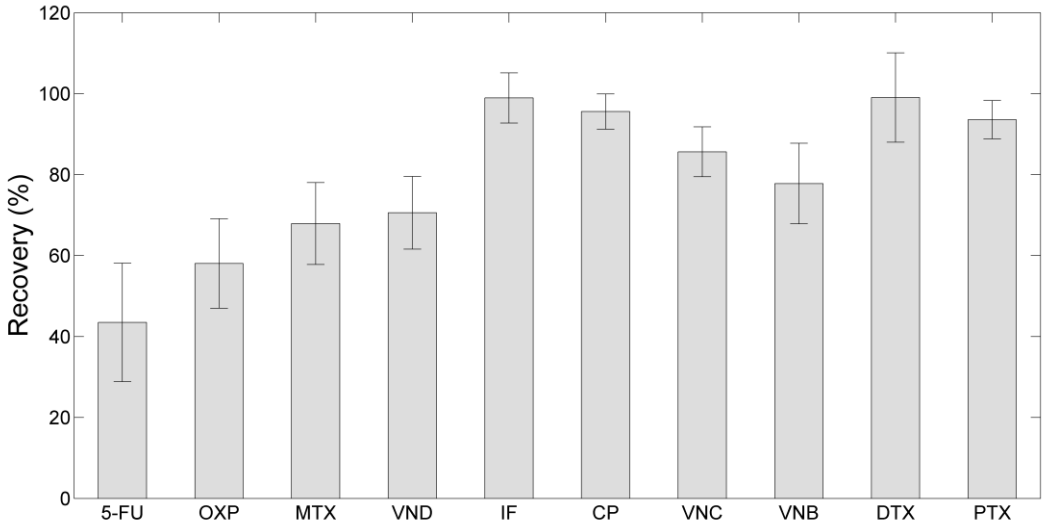

Figure 1. Overall method recoveries of the ten antineoplastic drugs after spiking 50 µL of inter-stock solution on brand new stainless steel plates (n=24) representing average recoveries achieved by personnel with no previous experience and experienced personnel over multiple days

Effect of surface roughness on drug recoveries: an extended study

Results from the surface roughness experiment are presented in Figure 2; the plates with higher R_a values (category 3, red color) had the lowest recoveries while those with lower R_a had the highest recoveries. As mentioned in the experimental section, the R_a values are not uniformly distributed and

there is a gap in the R_a data from approximately 0.8 to 1.9. For this reason, we did not attempt to quantitatively fit the data.

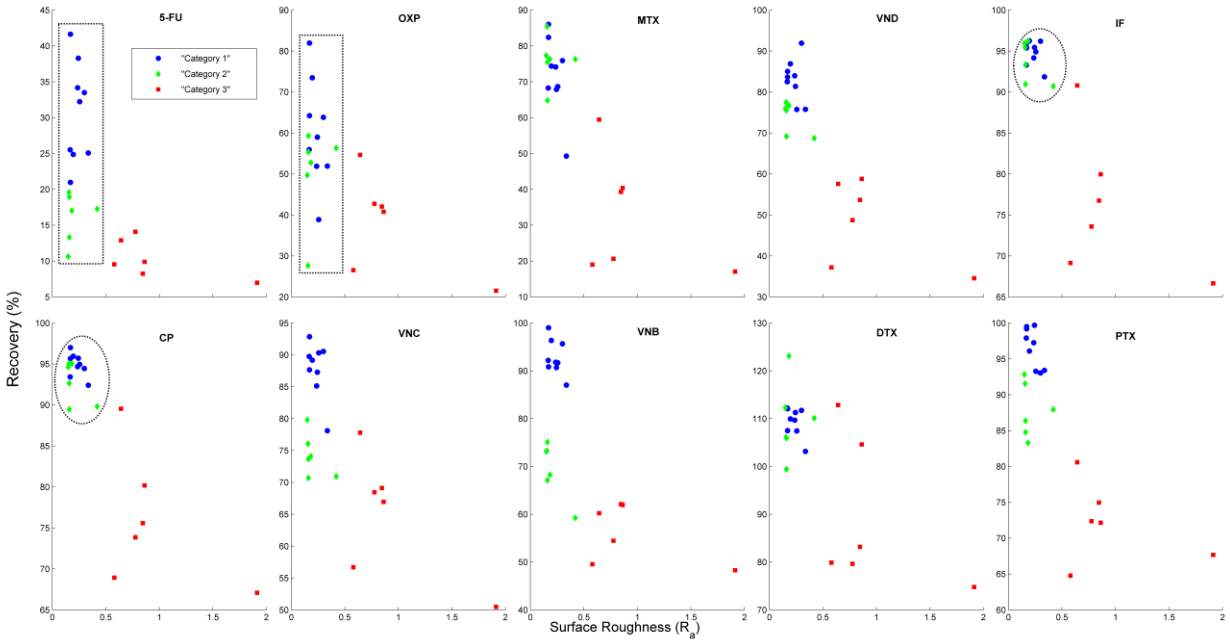

Figure 2. Assessment of the effect of surface roughness on drug recoveries. Plates are classified based on their use time: "Category 1" (new plates, blue circles), "Category 2" (plates used in another study, green diamonds) and "Category 3" (plates used in multiple studies, red squares). Dotted rectangles: drugs which exhibited large variability in drug recoveries from stainless steel plates with similar R_a. Dotted ellipses: drugs which exhibited very small variability in drug recoveries.

Stability assessment

No significant degradation of the evaluated drugs ($P>0.05$) was observed during the first 24– 48 h of the stability assessment. Additionally, after one week no significant change was observed in eight of 10 drugs ($P>0.05$); 5-FU and IF were found to have higher recoveries when stored for a week before desorption.

An assessment of the recovery of the selected antineoplastic drugs on stainless steel versus time after contamination/spiking was carried out over a period of 1 month, Figure 3.

It should be noted that the processes that affect the recovery of the drugs over time (e.g. photo-degradation of the drugs, increased adsorption, surface chemistry, etc.) were not investigated in this experiment.

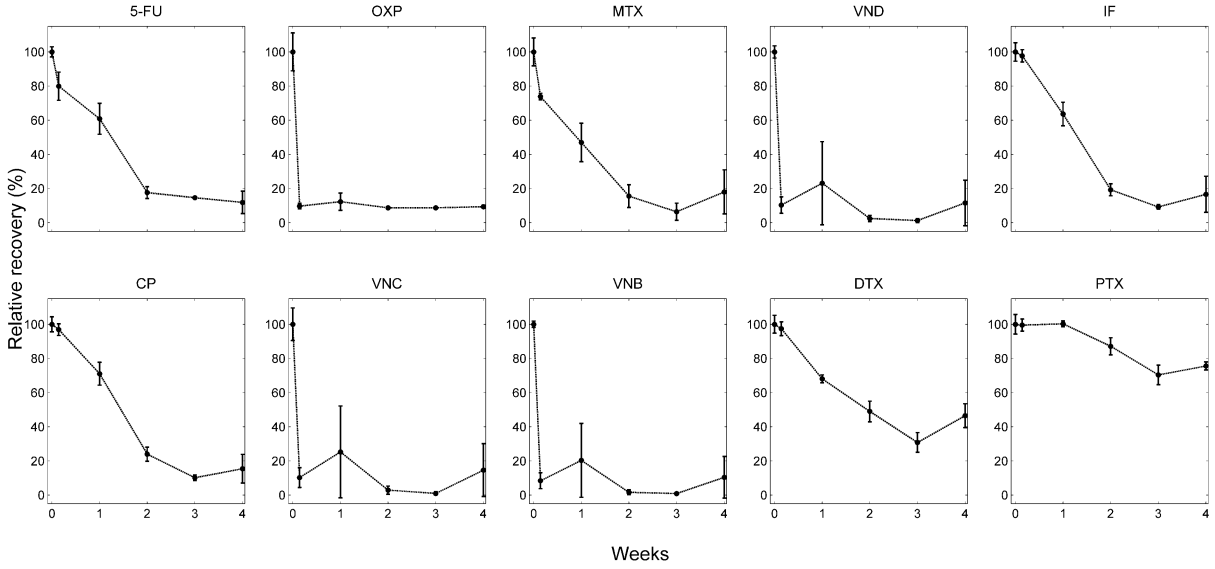

Figure 3. Recovery study of the selected antineoplastic drugs on stainless steel versus time

Environmental assessment

The described method was employed to analyze surface contamination in three local healthcare facilities on Vancouver Island, Canada, where 73 surface samples were collected and analyzed for antineoplastic contamination. The sampled areas were broadly organized into three categories (non-pharmacy staff areas, oncology pharmacy, patient areas) and the results are shown in Table 5.

Table 5. Antineoplastic drugs detected in surface wipes from three local healthcare facilities. Values given as range [min - max] in ng cm^{-2}

	5-FU	OXP	MTX	VND	IF
Non-pharmacy staff areas	<LOD - 26.5	<LOD	<LOD	<LOD	<LOD
Oncology pharmacy	<LOD - 33.0	<LOD	<LOD - 0.463	<LOD	<LOD

Patient areas	<LOD - 4.20	<LOD	<LOD	<LOD	<LOD
	CP	**VNC**	**VNB**	**DTX**	**PTX**
Non-pharmacy staff areas	<LOD - 0.003	<LOD - 0.005	<LOD	<LOD	<LOD
Oncology pharmacy	<LOD - 0.023	<LOD - 0.014	<LOD	<LOD	<LOD - 0.012
Patient areas	<LOD - 0.009	<LOD - 0.006	<LOD	<LOD	<LOD

Discussion

Analytical validation

The measurement sensitivity of all compounds (Table S2) compares favourably with previous work (Nussbaumer et al., 2010; Tuerk et al., 2011; Bussières et al., 2012; Pretty et al., 2012; Bobin-dubigeon et al., 2013; Maeda and Miwa, 2013; Merger et al., 2014; Jeronimo et al., 2015; Hetzel et al., 2016; Müller-Ramírez et al., 2016). For most compounds, the variability of the instrument method, both inter- and intra-day, is low, <5% RSD (Table 1). At the lower concentrations, 5-FU, OXP, and VNC have higher, but still acceptable intra-day variability up to 13%. Overall, 5-FU has the highest variability in every category, possibly due to the fact that it is the least retained analyte on the LC column as seen in Figure S1.

Wipe Desorption

The OSHA evaluation guidelines for surface sampling (Occupational Safety and Health Administration (OSHA), 2001) specify that the desorption of analyte from the wipe should be over 75%, indicating in this case that the desorption for 5-FU requires further improvement. As seen in Table 3, DTX has an average recovery that exceeds 100%. This is likely due to the fact that we used isotopically labelled PTX as an internal standard for DTX, which is analogous but not a perfect internal standard.

In this work the filtration step described in the original paper (Jeronimo et al., 2015) was omitted. This approach was taken after evaluating the desorption efficiency of all 10 analytes using 3 different

syringe filters (Millex-HV, 0.45 μm, Millex-GV, 0.22 μm, Millex-GP, 0.22 μm). Adsorption of the selected antineoplastic drugs was observed for at least one compound on each evaluated syringe filter, compromising the efficiency of the desorption step. In order to obtain as quantitative a desorption as possible while still avoiding injecting particulate into the sensitive LC equipment, the filtration step was replaced with simply centrifuging the desorbed samples (4500 RPM, 15 minutes) and pipetting 1mL of the supernatant to an LC vial.

Overall quantitative performance of the wipe method on stainless steel surfaces

This method proves to yield good recoveries in real-case scenarios (multiple personnel collecting samples over multiple days) which match or exceed those reported in the literature (Larson et al., 2002; Sottani et al., 2007; Tuerk et al., 2011; Nussbaumer et al., 2012) for every compound except 5-FU, Figure 1. The calculated removal efficiency from the surface (overall method recovery [Table S3] divided by desorption recovery [Table 3]) for 5-FU is 98%, which suggests that the relatively low overall recovery of this drug is limited due to poor desorption. Overall, the calculated removal efficiency for all drugs meet the OSHA guideline (>50%). Future work should include finding an improved desorption solution or method that is more efficient for 5-FU and OXP while not affecting the excellent desorption and recovery values for the rest of the drugs.

The accuracy of our group's wiping and analysis method was assessed by comparing it with two commercial laboratories. This comparison was carried out only based upon CP, as that was the only compound all three methods had in common. Our results for CP (Recovery: 94%, RSD: 6%) are in good agreement with those reported by the commercial lab A (Recovery: 94%, RSD: 12%), and also with Lab B, which has a slightly lower recovery (Recovery: 86%, RSD: 3%). These results emphasize the robustness of our approach, with satisfactory sample recovery comparable to the commercially available analysis (which are only able to assess 2-3 compounds per analysis).

Effect of surface roughness on drug recoveries: an extended study

As it is observed in Figure 2, there is a large variability in drug recoveries for the plates grouped in categories 1 and 2, despite the fact they have similar R_a values. This occurred most noticeably with 5-FU and OXP (dotted rectangles); there is a very large range of recoveries (from 27%-82% for OXP) for plates that have little difference in roughness. On the other hand, the recoveries of IF and CP for both plate categories (1 and 2) have very small variability, appearing tightly grouped (dotted ellipses). Between these two distinct distributions, there is a gradient in the recoveries of the other drugs among the plates with lower R_a values.

Statistical analyses emphasize the results displayed in the previous figure; the recoveries for all studied drugs were always significantly different ($P<0.05$) between the rougher plates (category 3) and the new plates (category 1). Similarly, the recoveries of eight out of ten drugs were significantly different ($P<0.05$) between category 3 and category 2 plates. Only four drugs have shown significantly different ($P<0.05$) recoveries between category 1 and category 2 plates, this was expected due to the similarity in the R_a coefficient for these two types of plates.

From these results it is apparent that the roughness of the surface plays an important role in determining recovery of wipe sampling for some drugs; specifically, there is a decrease in recoveries with an increase in surface roughness. However, as can be seen in Figure 2 (specified by dotted boxes), a large variation in recovery can also been observed within plates with similar surface roughness, indicating that other parameters (e.g. surface chemistry) are also important. These results regarding surface roughness are useful when designing procedures for exposure assessment in workplaces at risk for antineoplastic drug contamination or during cleaning activities of contaminated surfaces. Additionally, this new data highlights the importance of gathering ancillary information (surface roughness, throughput, timing, cleaning procedures, etc.) to help interpret the analytical results. For instance, surface roughness information could help to identify false negative results: apparent low values of antineoplastic drugs while the sampling is performed on rough or porous surfaces. Underestimating the amount of cytotoxic drugs present on rough surfaces will hinder a full assessment of drug contamination and exposure in health care

facilities, an issue not previously addressed in guidelines such as USP 800 (United States Pharmacopoeia (USP), 2014).

In order to achieve a higher recovery on surfaces that are known to be rough or otherwise have known low recoveries for some or all drugs, the analyst may decide to perform a second wipe on the same sampled area. However, previous work in our lab has shown wiping again with the same filter will actually yield a lower recovery (likely due to physical degradation of the wipe, especially on a rough surface). A second (new) wipe yields an additional 10% average recovery, which must be balanced against cost of the analysis.

Stability assessment

The shipping of the samples from hospitals and healthcare centers to the laboratories, where the samples are analyzed, is a critical step during antineoplastic drug analysis. In order to evaluate the integrity of the samples a stability test was performed replicating the shipping conditions. In agreement with our previous work (Jeronimo et al., 2015), there was no significant ($P>0.05$) degradation in eight of 10 drugs after a week at 5 °C while 5-FU and IF were found to have higher recoveries in these tests. Similar behavior for 5-FU was observed by Tuerk et al. (2011). Although the samples prove to be stable over a period of a week, the shipping time used during our field analysis was overnight (24–48 h) and the samples were cooled with icepacks during shipping.

When assessing the long term behavior of antineoplastic drugs on the surface, three distinctive patterns can be deduced from the Figure 3: 1) for OXP, VND, VNC and VNB an extremely rapid decrease in their recoveries (below the 20 %) was observed after 24 h, and then remaining constant close to the LOD levels for one month. 2) for 5-FU, MTX, IF and CP a steadier decrease of the recoveries was observed, declining to values of approximately 20% after 2 weeks. 3) DTX and PTX prove to be the more stable drugs; DTX display a smooth decrease of its recovery, reaching values of 40% after 3 weeks

staying nearly stable till the end of the study. PTX's recovery began to decrease in a slow fashion after a week, and around 80% of this drug was recovered from the surface after a month.

The same overall pattern was observed in the 24 h experiment, with OXP, VND, VNC and VNB exhibiting the same sharp decrease in their recoveries compared to the other drugs. However, the recoveries for OXP, VND, VNC and VNB after 24 h declined less in the second experiment than in the original experiment (by approximately 40% versus approximately 85% initially).

These surface stability experiments demonstrate a first approach to investigate the long-term behavior of antineoplastic drugs on stainless steel. Relevant insights can be drawn from these experiments, for instance, the stability of the 10 selected drugs on the surface is not homogeneous, with some drugs exhibiting noticeable lower recoveries than others over the same time interval. This information may be considered when designing sampling approaches and evaluating surface contamination data, as the likelihood of detecting antineoplastic drugs is not only dependent on the analytical approach taken, but also on when each drug was last used in the sampled environment.

Additionally, when assessing occupational contamination of antineoplastic drugs, it is important to consider the behavior of these hazardous drugs in the environment, especially for the more stable drugs such as DTX and PTX which could experience a cumulative effect on the surfaces where successive contamination is added reaching higher concentration. Consequently, the probability of a surface being contaminated by these drugs (e.g. DTX and PTX) may be higher. Moreover, these results may help to elucidate the spreading/contamination pathways in the work environment. Persistent drugs are more likely to be spread to different departments and areas in the healthcare facilities during shipping/receiving, preparation, transportation, and administration of the drugs.

4.6 Environmental assessment

The developed wiping method was successfully used to screen for antineoplastic drugs in three local healthcare facilities. It worth mentioning that this was a single-blinded study where the healthcare

facilities did not release information about drug usage (types of drugs and quantities). In this scenario, wiping methods which are capable of targeting as many compounds as possible are preferred since the chance of having positive results is increased. The fact that this was a single-blinded study also may explain why many drugs were reported <LOD, since the healthcare facilities were not necessarily using all ten of the drugs we analyzed for. Surface contamination above the LOD was detected in six out of ten drugs, being highest for 5-FU. Unsurprisingly, surface contamination was highest inside the pharmacy area where the drugs are prepared. Nevertheless, trace contamination was detected in patient and staff areas adjacent to areas with active drug preparation or administration, emphasizing the potential of this method to conduct routine monitoring throughout a facility with a wide range of surface contamination levels in different areas as well as investigate the dispersion mechanisms of hazardous drugs in the work environment. It should be noted that the actual 5-FU contamination in these facilities is predicted to be higher than reported due to the low (43±14 %) overall method recovery for this drug, which is under the OSHA recommendation (Occupational Safety and Health Administration (OSHA), 2001).

In future routine analysis the authors of this study suggest gathering drug usage information along with additional ancillary data (number and condition of safety cabinets, ventilation, personal protective equipment, cleaning techniques, etc.) as reported by Yoshida et al. (2011), in order to improve the study and interpretation of antineoplastic surface contamination in health care facilities.

Conclusions

The analytical approach discussed in this article, developed to incorporate drugs selected by oncology pharmacy representatives in Canada, is an effective approach for the assessment of surface contamination. The method yields good overall method recoveries for almost all the 10 evaluated drugs except OXP and 5 FU.

The method validation includes a determination of the variability in results within and between days, sensitivity, extraction efficiency, stability of the drugs during storage and shipping and overall

method recovery, which are important parameters for any analytical wipe method (Connor et al., 2016). Additionally, the developed method yields CP recoveries that are in excellent agreement with those reported by external commercial labs. The comparatively short run time (8 min) and expanded number of drugs also allows for more comprehensive routine analysis. The low MDLs achieved in this work allow for analysis of trace contamination and the method was successfully used in three healthcare facilities to evaluate surface contamination to the single picogram per square centimeter level.

We confirmed our previous findings regarding the effect of surface roughness on drug recoveries, with higher R_a values (rougher) having the lowest recoveries while those with lower R_a (smoother) presented the highest recoveries. Additionally, substantial variability in drug recoveries was observed among plates with similar R_a values suggesting that, despite the fact that surface roughness plays an important role in drug recovery, other parameters are also involved. When performing wiping procedures to detect antineoplastic drugs in health care facilities, it is advisable to note the type and condition (roughness) of the surface as these parameters greatly influence the overall recovery efficiencies. Interpretation of surface contamination results should take into account the possibility of variability or underestimation due to surface roughness or porosity.

The results from our study of long term recoveries of the selected antineoplastic drugs on stainless steel show that different drugs have distinctive patterns; OXP, VND, VNC and VNB being the less persistent drugs and DTX and PTX being the most persistent drugs on contaminated surfaces over time with recoveries of 40% and 80% after one month. This information indicates the importance of collecting ancillary information about drug usage to appropriately interpret the contamination results in the context of potential occupational exposure.

Finally, the method was successfully applied to analyze surface contamination in three local healthcare facilities. The fact that surface contamination above the LOD was detected in six out of ten

drugs in this single-blinded study, where information about the drug usage was not provided, highlights the applicability of this method for routine monitoring.

Acknowledgements

Partial support for this work was provided by the Worksafe BC Innovation at Work Grant (RS2014 – IG11). Additional support was provided by the School of Occupational and Public Health at Ryerson University. Thanks to Avital Jarus Hakak for assistance.

Declaration

The authors declare no conflict of interest relating to the material presented in this Article. Its contents, including any opinions and/or conclusions expressed, are solely those of the authors.

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