Development and validation of methods for environmental monitoring of cyclophosphamide in workplaces

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Methods to monitor contamination of workplaces with antineoplastic drugs have been developed and validated. Cyclophosphamide (CP) was used as a model compound as it is one of the most commonly used antineoplastic drugs. A wipe sampling method to detect contamination with CP at surfaces was developed. A personal air sampling method to sample gas and vapour on solid sorbent tubes and particles with filters was also developed. Wipe and filter samples were extracted and sorbent samples were eluted, all with ethyl acetate. The samples were analysed with liquid chromatography tandem mass spectrometry. $^2H_6$-labelled cyclophosphamide was used as an internal standard. The between-day precision was 2–5% for wipe samples, 4–6% for sorbent samples and 3–8% for filter samples. The limit of detection was 0.02 ng CP per sample for the wipe and filter methods and 0.03 ng CP per sample for the solid sorbent method. Wipe sampling on surfaces made of different materials resulted in mean recoveries between 78–106%. The desorption recovery was between 97–102% for the wipe samples, 97% for the sorbent samples and 101% for the filter samples. Samples were stable for up to 2 months at 5 °C and −20 °C and for about 2 d at room temperature. The developed methods were applied to the measurement of contamination with CP in a hospital pharmacy. Trace amounts of CP, 1.3 and 1.4 ng, were detected on surfaces in the pharmacy.

Introduction

Antineoplastic drugs are widely used around the world in medical treatment of different diseases, e.g. cancer. Every day large amounts of antineoplastic drugs are given to patients treated in hospitals, nursing homes and in primary health care. Many antineoplastic drugs are classified as carcinogenic, mutagenic or teratogenic to humans. There are several groups of health care workers including nurses, pharmacy technicians, laboratory staff and cleaners that may be occupationally exposed to antineoplastic drugs. Exposure of workers can occur during preparation or administration of antineoplastic drugs, during nursing and taking care of patients or during cleaning. Absorption of antineoplastic drugs occurs mainly through skin or through inhalation of gases or particles. Occupationally exposed workers have earlier been studied and the levels of antineoplastic drugs or their metabolites in both urine[1–5] and blood[6] have been monitored.

Cyclophosphamide (2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide; CP), a very common antineoplastic drug, was introduced in the 1950s.[7] CP is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC).[8,9] CP is a prodrug and its metabolites are genotoxic due to their ability to interact with DNA, causing cross-linking. CP is used in the treatment of many cancer diseases, as a single drug or in combination with other antineoplastic drugs. CP can therefore be considered as a model compound for identification of exposure during work with antineoplastic drugs.

Contamination by CP has been detected in work environments at hospitals e.g. in pharmacies and in hospital departments handling antineoplastic drugs. Wipe sampling is a common way to monitor surface contamination. Several wipe sampling methods have been used in earlier studies and CP was detected as a surface contaminant in many of the workplaces that were investigated.[10–15] However, validation of these methods has been scarce. Larson et al. evaluated different types of tissues but only two types of wipe tissues were tested.[16] Furthermore, among the previous studies only Schmaus et al. used an internal standard which is not present in the studied work environments.[15] Most of the methods used either no internal standard or ifosfamide, another widely used antineoplastic drug, as internal standard. In the study by Schmaus et al. the stability of wipe samples containing CP was investigated but only over four days.[15] In addition, the sensitivities of the previous methods have not been high enough to detect and evaluate trace contamination with CP.

For measurements of particulate matter of CP in air, stationary and personal methods have previously been developed.[10,11,13,17–19] These methods have been used to study air concentrations of CP at different workplaces. In previous studies different types of filters have been used to collect particles of CP. However, it may not be sufficient to measure particles in the air. The vapour pressure of CP has been determined by Opiolka et al. and was found to be 3.3 mPa at 20 °C.[20] The vapour pressure is low but not negligible. Thus, gaseous CP may also be present in the work environments where CP is handled. In one recent study a stationary method based on a filter connected to a cryogenic-trap was developed to determine both particulate and gaseous CP in air.[21] Measurements with the developed method were carried out in two hospital pharmacies. In some samples concentrations of gas up to 13 μg m^{-3} were detected. However, it is important that the sampling method is portable and easy to use. A method for air sampling of gaseous CP based on solid sorbent media was developed by Larson et al.[22] The method for air monitoring was accurate for measurements of CP concentrations from less than 1.0 μg ml^{-1} to >1.0 mg ml^{-1}, but a more sensitive method is needed to detect trace amounts of CP in the air. Several air methods used either no internal standard or ifosfamide as internal standard.

The aim of this current investigation has been to develop and validate a sensitive method to determine trace contamination with CP at surfaces. In addition, a method for personal sampling of CP in air, both as particles and gas, has also been developed and validated. The developed methods have been used in an application to measure contamination of CP in a hospital pharmacy.

Experimental

Chemicals

Cyclophosphamide monohydrate (purity 99.5%) was purchased from Sigma-Aldrich Chemie (Schnelldorf, Germany). $^2H_6$-labelled cyclophosphamide (CP-D6; purity 97%) was from Phychem (Bergisch Gladbach, Germany) and ifosfamide (Holoxan; purity >98%) was obtained from ASTA Medica (Frankfurt am Main, Germany). Ethyl acetate (HPLC 99.8%) and methanol (HPLC) were from Lab-Scan (Dublin, Ireland). Acetic acid glacial (p.a. >99.8%), sodium chloride (p.a. >99.5%) and sodium hydroxide (p.a. >99.0%) were all from Merck (Darmstadt, Germany). Water was purified in a Milli-RQ Water Purifier (Millipore, Billerica, MA, USA).

Wipe tissues and filters

Wipe tissues used to collect wipe samples were Care Facial tissue, 20.1 × 21.1 cm (Katrin, Metsä Tissue, Mariestad, Sweden), Easi-Tex Master Plus, 31 × 41 cm (Gunnar Engstrand, Stockholm, Sweden), nonwoven swab, 5 × 5 cm (Hartmann-ScandiCare, Anderstorp, Sweden), sterile compress, 10 × 7.5 cm (AKLA, Danderyd, Sweden), Kimcare medical wipe, 10.6 × 20.5 cm (Kimberly-Clark®, Kimberly-Clark, Irving, TX, USA) and Swedish filter paper, 9 cm diameters (Munktell 50, Munktell Filter, Grycksbo, Sweden). Filters were Fluoropore membrane filter (polytetrafluoroethylene (PTFE, Teflon)), Durapore membrane filter with pore size 0.45 µm and 0.65 µm (Millipore, Billerica, MA, USA), glass fiber filter type AE (SKC, Eighty Four, PA, USA) and polycarbonate filter 0.1 µm (Nuclepore, Pleasanton, CA, USA). All filters had a diameter of 13 mm.

Preparation of standards

Two stock solutions of CP were prepared by dissolving weighed amounts of CP in water. Several working solutions were made from these stock solutions. A stock solution of CP-D6 was prepared by dissolving weighed amounts in water, which was further diluted in water. In the initial experiments ifosfamide was used as internal standard.

Determination of CP on surfaces

Sampling procedure. Nonwoven swabs were chosen for the collection of wipe samples. A wipe sample was collected within a defined surface area. Plastic frames with internal size of either 10 × 10 cm (100 cm^2) or 20 × 20 cm (400 cm^2) were used to define the surface area to be sampled. Each wipe tissue was moistening with 1 ml volume of 0.03 M sodium hydroxide solution. All wipe samples were collected with a uniform sampling procedure by careful wiping in two different directions (up and down, right and left) inside the plastic frame. Two wipe tissues were used in the sampling procedure, one in each direction. After sampling the wipe tissues were placed in wide mouth polyethylene bottles (50 ml; Kautex Textron, Bonn, Germany) and stored at −20 °C until the work-up procedure.

Work-up procedure. Aliquots of 20 ml ethyl acetate and 100 µl of internal standard (1.0 µg CP-D6 ml^{-1} water) were added to each bottle containing two wipe tissues. The bottles were shaken upright for 30 min with a shake apparatus 4010 Multi-Tube Vortexer (Corning, Medfield, MA, USA). Aliquots of 10 ml of the extracts were transferred into glass test tubes. The samples were then evaporated to dryness under a stream of nitrogen gas at room temperature. Next, 0.15 ml 0.5% acetic acid was added to each sample, which were then sonicated for 5 min and transferred into microvials. The samples were stored at 5 °C until analysis. Standards were prepared by adding 100 µl aliquots of the working solution to 1 ml 0.03 M sodium hydroxide solution.

Determination of CP in air

Sampling procedure of solid sorbent samples. Solid sorbent tubes containing a styrene divinyl benzene phase, Bond Elut LMS with a mass of 50 mg and volume of 1 ml (Varian, Harbor City, CA, USA), were used to collect gaseous CP. A flow rate of 140 ml min^{-1} was applied. The solid sorbent samples were sealed with Parafilm on both sides after sampling.

Work-up procedure of solid sorbent samples. The Bond Elut columns were connected to a vacuum system VacMaster 20 (Sorbent, Västra Frölunda, Sweden) and eluted with 1.5 ml ethyl acetate into test tubes, to which 100 µl internal standard were then added. The samples were evaporated to dryness under a stream of nitrogen gas at room temperature. Next, the samples were dissolved in 0.15 ml of 0.5% acetic acid, sonicated for 5 min and finally transferred into microvials. The samples were stored at 5 °C until analysis. Standards of CP were prepared from the working solutions by adding 100 µl to 1.5 ml ethyl acetate.

Controlled air system. Air with a defined controlled airflow and relative humidity was obtained by using equipment developed at the National Institute for Working Life in Umeå, Sweden.[23,24] This was used for the evaluation of the collection of CP on the solid sorbent tubes. The air producing system consisted of three different glass bubblers containing water, which were coupled in series. The system was driven by compressed air cleaned by a particle filter (0.01 µm) and with charcoal. The humidified air stream was branched into seven outlets from which sampling experiments could be performed. The airflow was measured with a rotameter (Rota, Wehr, Germany) and the relative humidity was measured by a hygrometer (HUMICAP 14, Vaisala, Helsinki, Finland).

Sampling procedure for filter samples. The filter used for particle sampling was Durapore membrane filter made of polyvinylidene fluoride (PVDF) and with a pore size of 0.65 µm (Millipore, Billerica, MA, USA). The filters had a diameter of 13 mm and the flow rate was 140 ml min^{-1}. The filters were transferred to plastic test tubes after sampling.

Work-up procedure for filter samples. 1 ml ethyl acetate and 100 µl internal standard were added to the test tubes containing the filters which were then shaken upright for 30 min with an IKA-VIBRA-VXR (IKA Labortechnik, Staufen, Germany). The extracted liquid was then transferred to glass test tubes. The samples were evaporated to dryness under a stream of nitrogen gas at room temperature. The samples were dissolved in 150 µl 0.5% acetic acid, sonicated for 5 min and then transferred into microvials. The samples were stored at 5 °C until analysis. Standards were prepared by adding aliquots of 100 µl working solutions to 1.0 ml ethyl acetate.

Analytical procedure

Analysis was performed using a Perkin-Elmer Series 200 liquid chromatography system with a Series 200 autosampler

(Applied Biosystems, Norfolk, CT, USA). The column was a Genesis C_{18} (50 × 2.1 mm) with a particle size of 4 μm (Jones Chromatography, Lakewood, CO, USA). The column outlet was coupled to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems/MDS-SCIEX, Toronto, Canada) equipped with an electrospray ionisation (ESI) source. In the beginning of the method development samples were analysed on an API 2000 triple quadrupole mass spectrometer (PE Biosystem, Foster City, CA, USA) instead of the API 3000. The analytical instruments have been validated against each other and found to give similar results.

Gradient elution was carried out with water (solvent A) and methanol (solvent B), both containing 0.5% acetic acid. A gradient profile including column regeneration with the following proportions (v/v) of solvent B was applied (t(min), %B): (0, 40), (6, 40), (8, 100), (8.1, 40), (11, 40). The flow rate of the mobile phase was 0.2 ml min^{-1} and the injection volume was 20 μl. The analyses were performed by ESI with ion spray voltage at 3000 V and temperature at 400 °C. The instrument operated in a positive ion mode using multiple reaction monitoring (MRM) at m/z 263.1/142.1 (analyte fragment) and m/z 261.0/140.3 (control fragment) for CP and m/z 267.1/140.3 for CP-D6. Declustering potential for CP analyte fragment, control fragment and CP-D6 was 40, 42 and 44 V, respectively. Collision energy was 31 V for CP and 34 V for CP-D6, respectively. Peak-area ratios between CP and internal standard were used for quantification. The ratio between the analyte and control fragment of the samples was compared with the ratio between the analyte and control fragment of the standard samples and was not allowed to exceed 20%.

Application of the developed methods

To test the developed methods in an authentic work environment, a hospital pharmacy in Sweden was contacted. The hospital pharmacy is located at a university hospital and prepares antineoplastic drugs for many different departments at the hospital. The pharmacy prepares all antineoplastic drugs inside microbiological safety cabinets (BSC), class II with a vertical laminar air flow (danLAF®-o-matic VFRS 1206 E, Claus Damm, Humlebæk, Denmark). The pharmacy handles drugs according to the ordinance from the Swedish Work Environment Authority.[25] Personal protective equipment such as specially adapted protective clothing and double protective gloves of two different materials are used. The pharmacy also used CODAN Spike (CODAN Medizinische Geräte, Lensahn, Germany) to prevent the formation of particles in the BSC during drug preparation.

The sampling device consisted of a filter in a filter cassette connected to a solid sorbent. Low flow pumps of model 222 from SKC (Eighty Four, PA, USA) were used in air sampling. Stationary air samples were collected at four different spots in the pharmacy unit. Air was sampled in the BSC, outside the BSC but near the person working in the BSC (about 50 cm from the breathing zone), above the waste bin and above a shaker used to prepare stock solutions of CP. Eight-hour sampling was performed. In addition, two wipe samples were collected in the BSC after preparation of an infusion mixture containing 1000 mg CP. Two spots of 400 cm^2 on the working area were wiped. The samples were worked-up according to the procedures described above.

Results and discussion

Wipe samples

There are a large number of wipe tissues available on the market manufactured in different types of materials and sizes. In previous studies where CP contamination on surfaces has been examined, different types of wipe tissues, solutions and volumes to moisten wipe tissues with have been used. Also,

different surface areas have been sampled and different work-up procedures and analytical methods have been applied. So far a standardised sampling procedure for collection of wipe samples on contaminated surfaces has not been developed.

In this study six different types of wipe tissues; Easi-Tex Master Plus, Care Facial tissues, nonwoven swabs, sterile compresses, Kimcare medical wipes and Swedish filter papers were evaluated. Two wipe tissues of each type were spiked with 5, 10 and 50 ng of CP and treated according to the work-up procedure. The mean recoveries for these six wipe tissues ranged between 88–112%. From the result it was possible to exclude three types of tissues. Sterile compresses and filter paper were excluded due to low mean recoveries (88–93%) and Easi-Tex Master Plus was excluded due to its large size. The three remaining wipe tissues had sufficient desorption capacities. The mean recoveries were between 94–102% for Care Facial tissues, between 96–106% for Kimcare medical wipes and between 97–102% for nonwoven swabs.

The three remaining types of wipe tissues were evaluated to test whether the wipe tissues had to be extracted once or twice. Two wipe tissues of each type were spiked with either 25 or 100 ng of CP and extracted once with 20 ml or twice with 10 ml organic solvent. The recoveries were equal and thus the wipe samples were only extracted once with 20 ml ethyl acetate.

In work environments, *e.g.* hospital pharmacies and departments, surfaces are generally made of laminate, stainless steel or plastic. Working areas in BSC are frequently made of stainless steel and working areas outside BSC are often made of stainless steel or laminate. The floors are mostly made of some type of plastic material. The absorption capacity of the wipe tissues Care Facial, Kimcare medical wipes and nonwoven swabs were investigated by wiping surfaces made of different materials. CP was dissolved in either water or methanol and 100 μl CP solutions containing either 5 or 100 ng of CP were added to the surfaces. The solution was spread out within an area of 100 cm^2 limited by a plastic frame and wipe sampling were performed on both liquid and evaporated spillage of CP. Liquid spillage consisted of CP dissolved in water. Evaporated spillage consisted of CP dissolved in methanol. The methanol was allowed to evaporate before the surface was wipe sampled. The results of the investigation are presented in Table 1. Nonwoven swabs had the highest recoveries, between 87–102%, and the coefficient of variation (CV) ranged between 1–7%, for liquid spillage. For evaporated spillage the recoveries were between 78–106% and the CV ranged between 3–10%. Wipe sampling on plastic surface material resulted in lower recoveries than the other surface materials due to the rough and uneven surface. Nonwoven swab had the best and the highest absorption capacity of CP from these different surface materials. This wipe tissue was also most practically applicable. Thus, nonwoven swab was chosen and was used throughout in the rest of this investigation.

The size of the sampling area was also evaluated. To study if the mean recovery of CP differs between different sampling areas, 5 ng CP were dissolved in 100 μl methanol and spiked to surfaces areas of either 100 or 400 cm^2, both limited by plastic frames. Wipe sampling of evaporated spillage was performed on surfaces made of laminate, stainless steel and plastic flooring material. Wipe sampling of surfaces within an area of 100 cm^2 resulted in mean recoveries between 78–95%. Wipe sampling of surfaces within an area of 400 cm^2 resulted in mean recoveries between 84–92%. Slightly higher recoveries were obtained for the smaller area for sampling on surface materials made of laminate and stainless steel. The recovery was higher for the larger sampling area made of plastic flooring material than for the smaller area. If possible it is preferable to sample a larger area compared to a smaller one. The likelihood of detecting spillage of CP increases with increasing sampling area but it is unpractical to sample too large a surface area.

Table 1 Summary of wipe sampling on surfaces made of different materials with (a) liquid and (b) evaporated spillage of CP

Tissue	Surface material	Spike amount/ng	Mean recovery[a] (%)	CV[a] (%)
(a)				
Kimcare medical wipes	Laminate	5	93	4
Kimcare medical wipes	Plastic flooring	5	86	3
Kimcare medical wipes	Stainless steel	5	94	8
Kimcare medical wipes	Laminate	100	93	2
Kimcare medical wipes	Plastic flooring	100	90	1
Kimcare medical wipes	Stainless steel	100	95	2
Nonwoven swabs	Laminate	5	95	4
Nonwoven swabs	Plastic flooring	5	87	3
Nonwoven swabs	Stainless steel	5	102	4
Nonwoven swabs	Laminate	100	94	1
Nonwoven swabs	Plastic flooring	100	93	1
Nonwoven swabs	Stainless steel	100	102	7
Care Facial	Laminate	5	88	5
Care Facial	Plastic flooring	5	85	6
Care Facial	Stainless steel	5	98	8
Care Facial	Laminate	100	94	4
Care Facial	Plastic flooring	100	92	2
Care Facial	Stainless steel	100	97	2
(b)				
Kimcare medical wipes	Laminate	5	85	7
Kimcare medical wipes	Plastic flooring	5	88	11
Kimcare medical wipes	Stainless steel	5	94	4
Kimcare medical wipes	Laminate	100	85	3
Kimcare medical wipes	Plastic flooring	100	72	11
Kimcare medical wipes	Stainless steel	100	100	2
Nonwoven swabs	Laminate	5	89	3
Nonwoven swabs	Plastic flooring	5	78	9
Nonwoven swabs	Stainless steel	5	95	6
Nonwoven swabs	Laminate	100	99	3
Nonwoven swabs	Plastic flooring	100	85	10
Nonwoven swabs	Stainless steel	100	106	3
Care Facial	Laminate	5	84	6
Care Facial	Plastic flooring	5	70	8
Care Facial	Stainless steel	5	93	4
Care Facial	Laminate	100	88	2
Care Facial	Plastic flooring	100	74	8
Care Facial	Stainless steel	100	102	2

[a] Mean of 10 samples.

When wipe sampling different types of surfaces it is important to use a solution that has the capacity to effectively clean the surfaces. In many other studies and publications a weak sodium hydroxide solution has been used in wipe sampling.[10,12,14] Thus, we chose to moisten the wipe tissues with a 0.03 M sodium hydroxide solution. The recoveries have been high for all three surface materials. Therefore sodium hydroxide seems to be a suitable solution for wipe sampling.

Air samples

Work-up procedure of solid sorbent tubes. Initial results indicated that CP binds irreversibly to glass surfaces and thus sorbent tubes without glass were chosen. Two types of plastic tubes with different polymeric sorbent phases, Bond Elut LMS and Abselut NEXUS with a mass of 30 mg and volume of 1 ml (Varian, Harbor City, CA, USA), were chosen and evaluated. To test the adsorption and desorption capacity of these tubes, 10 μl methanol containing 200 ng CP were injected into two sorbent tubes of each type. The tubes were kept at room temperature for a couple of minutes until the methanol had evaporated. The samples were then eluted according to the

Table 2 Amounts of CP quantified in sorbent tubes spiked with either 5 or 200 ng of CP. Air with a flow of 200 ml min^{-1} and a relative humidity of either 5 or 60% was passed through the sorbent tubes for 8 h

Solid sorbent tube	Spike amount/ng	Relative humidity (%)	Mean recovery of CP[a]/ng	CV[a] (%)
Bond Elut	5	5	86	7
Bond Elut	5	60	87	5
Bond Elut	200	5	98	3
Bond Elut	200	60	99	2

[a] Mean of 6 samples.

work-up procedure. The mean extraction recoveries of Bond Elut and Abselut were 97 and 89%, respectively. Thus, Bond Elut was chosen and used further on in this investigation.

Sampling procedure of sorbent tubes. Different parameters such as relative humidity (5 and 60%) and spike amount of CP (5 and 200 ng) were evaluated using the controlled air system. Air with a constant flow of 200 ml min^{-1} was passed through the sorbent tubes for 8 h. Sampling was performed at a temperature of 20 °C. After sampling each sorbent tube was worked-up according to the described procedure. The results are shown in Table 2. Mean recoveries ranged between 86–99%. Tubes spiked with 5 ng of CP provided lower mean recovery and higher CV than tubes spiked with 200 ng of CP. The relative humidity did not appear to have an effect upon the recovery of CP.

To study how the sampling time affects the recovery, four sorbent tubes were spiked with 200 ng of CP and connected to the outlets of the controlled air system. Air with a relative humidity of 20% was passed through the tubes. Two tubes were sampled for 1 h and two tubes were sampled for 8 h. The mean recovery was 99% and 97% for the tubes sampled for 1 h and 8 h, respectively. This indicates that it is possible to sample CP for up to 8 h.

To study how temperature affected the evaporation of CP, solid sorbent tubes were connected to Teflon tubes with a length of 5 cm. These tubes were connected to each outlet of the controlled air system. A wad made of Teflon wool was placed into each tube. Aliquots of 10 μl methanol containing 200 ng CP were injected into the wads. Two Teflon tubes were at room temperature and two tubes were placed in a heater at 140 °C. An air stream with a relative humidity of 20% and a flow of 200 ml min^{-1} passed through the Teflon tubes and into the solid sorbent tubes for 1 h. The mean extraction recoveries from the solid sorbent tubes are presented in Table 3. The tubes at room temperature had a very low mean recovery as only 11% of the CP was converted into gaseous form and transferred with the air stream into the solid sorbent tubes. At the higher temperature a higher recovery was received as 66% was found in the solid sorbent tubes. This shows that CP may be present in air as a vapour at room temperature.

Filter samples

The usual way to sample particles in air is to use a filter placed in a filter cassette, which is connected to an air-sucking pump.

Table 3 Recovery of CP quantified in sorbent tubes after 1 h sampling

Temperature/°C	Mean recovery[a] (%)	CV[a] (%)
20	11	24
140	66	11

[a] Mean of 2 samples.

Table 4 Recovery of filters spiked with 10 ng of CP

Filter type	Mean recovery[a] (%)	CV[a] (%)
PTFE	97	7
PVDF (0.65 μm)	101	6
PVDF (0.45 μm)	102	4
Polycarbonate	84	19
Glass fiber	105	7

[a] Mean of 10 samples.

There are many different types of filters commercially available and in previous studies different types of filter have been used to sample particles of CP. In this investigation filters made of four different types of materials, PTFE, PVDF, polycarbonate and glass fiber, were evaluated.

The filters were evaluated by spiking ten filters of each type with 10 ng of CP. CP was dissolved in methanol and added in 10 μl aliquots to the filters. After a few minutes when the methanol had evaporated the filter was placed in a plastic test tube and stored at −20 °C until analysis. The mean extraction recoveries of the filters ranged between 84–105% (Table 4). Filters made of polycarbonate had the lowest mean recovery, 84% and the highest CV, 19%. Filters made of glass fiber were not suitable for use because of break down of the filters during the work-up procedure. The remaining filters, one filter made of PTFE and two filters made of PVDF, showed a mean recovery between 97–102% and a CV between 4–7%. These filters therefore seemed to be equally applicable. The PVDF Durapore filter, however, was much cheaper, and, due to that, the Durapore filter with a pore size of 0.65 μm was chosen.

Quantitative analysis

Standard curve. Standards were prepared by adding different amounts of CP to the same matrix as the sample, and the work-up procedure was then performed. To make standard curves, weighted $(1/x)$ linear regression was used. Linear standard curves with $r > 0.99$ were always obtained.

Limit of detection. The limit of detection was defined and calculated as the analyte concentration giving a peak-area ratio equal to the mean of the blank peak-area ratio plus three times the standard deviation in the blanks.[26] Ten blanks of wipe samples, solid sorbent samples and filter samples were analysed for the determination of the mean and the standard deviation of the blank peak-area ratio. The limit of detection was determined to be 0.02 ng CP per wipe sample (0.05 pg cm^{-2} for 400 cm^2 area), 0.02 ng CP per sample (0.02 ng ml^{-1}) for the filter methods and 0.03 ng CP per sample (0.02 ng ml^{-1}) for the solid sorbent method. Limits of detection of the developed methods in this study were much lower compared with earlier studies for wipe and air methods.[10–19]

Precision. The within-day precision of the methods was evaluated by analysing six wipe, solid sorbent and filter samples containing 5 ng CP. The CV was 2% for the wipe samples, 5% for the solid sorbent samples and 4% for the filter samples. Analysing duplicates of samples containing 1, 2, 3, 4, 5 and 20 ng CP evaluated the between-day precision of the methods. The duplicates were analysed on 5 different days within a period of four weeks. The CV was between 2–5% for the wipe samples, between 4–6% for the sorbent samples and between 3–8% for the filter samples.

Stability of wipe samples and air samples

Storage of wipe and air samples containing CP under different conditions was performed to test the stability of CP. Storage

Table 5 Mean recovery and standard deviation of six spiked (a) wipe, (b) sorbent tubes and (c) filter samples stored at different temperatures and time

Storage time/days	Storage temperature/°C		
	20	5	−20
(a)			
0	98 ± 2	98 ± 2	98 ± 2
1	96 ± 2	97 ± 2	97 ± 1
2	94 ± 4	96 ± 3	96 ± 2
7	89 ± 4	99 ± 2	99 ± 2
14	83 ± 5	101 ± 3	100 ± 4
21	75 ± 5	95 ± 3	100 ± 3
28	66 ± 3	100 ± 3	104 ± 2
56	52 ± 15	108 ± 13	107 ± 5
(b)			
0	106 ± 3	106 ± 3	106 ± 3
1	99 ± 2	106 ± 4	104 ± 6
2	90 ± 6	98 ± 5	97 ± 5
7	94 ± 4	101 ± 10	104 ± 3
14	88 ± 3	92 ± 3	93 ± 3
21	88 ± 2	86 ± 10	93 ± 5
28	77 ± 4	90 ± 11	90 ± 4
56	93 ± 4	101 ± 3	106 ± 3
(c)			
0	102 ± 4	102 ± 4	102 ± 4
1	96 ± 10	105 ± 3	101 ± 1
2	101 ± 3	101 ± 2	93 ± 4
7	94 ± 1	100 ± 7	92 ± 2
14	95 ± 2	95 ± 5	92 ± 5
21	86 ± 6	88 ± 4	88 ± 3
28	87 ± 1	90 ± 3	89 ± 3
56	99 ± 3	107 ± 3	106 ± 5

time was up to 2 months. Preparation of wipe samples was performed by moistening two absorbent tissues with 1 ml sodium hydroxide (0.03 M) each. The tissues were placed in bottles and 5 ng CP dissolved in 100 μl water was added to the tissues. The plastic bottle was then capped. The stability of the air samples was tested by adding 5 ng CP dissolved in 10 μl methanol to the solid sorbent tubes and to the filters. The methanol evaporated before the solid sorbent tubes were sealed with Parafilm and the filters were placed in plastic sample tubes with screw caps. Wipe samples and air samples were stored at approximately 20 °C, 5 °C and −20 °C for 0, 1, 2, 7, 14, 21, 28 and 56 d. On each occasion six wipe and air samples from each storing condition were analysed. The samples stored for 0 d were prepared and analysed after sample preparation. The results of the wipe samples showed that CP is a quite stable compound (Table 5a). Wipe samples can be stored at temperatures of 5 °C or −20 °C for at least 2 months. No loss of CP was observed in wipe samples stored at room temperature for 2 d. The results from the storage of sorbent tube and filter samples are shown in Table 5b–c. Sorbent and filter samples can be stored for at least 2 months without any loss of CP. After a week at room temperature 90% of the added CP was still present. The results showed that wipe and air samples can be transported for a couple of days at room temperature without significant losses of CP. Schmaus *et al.* stored wipe samples for one and four days at room temperature and 4 °C and found that wipe samples were stable for four days at 4 °C and one day at room temperature.[15]

Application of the developed methods

During the exposure measurement at the hospital pharmacy 5 infusion mixtures containing CP were prepared. In this

procedure a defined volume of CP from a stock solution was transferred into infusion bags containing *e.g.* 250 ml sodium chloride solution. A total of 13.8 g CP were handled in the BSC during the measurement.

The stationary air measurements were performed over 8 h. The air sampling device consisted of a filter that was in series with a solid sorbent since it is important to measure both particles and gas of CP in air. This has previously been demonstrated by Kiffmeyer *et al.*[21] A mean air volume of 67 L (range 64–73 L) was sucked through the air sampling device. No CP above the limit of detection could be found in any of the filter samples nor in any of the sorbent samples. In this study it has previously been demonstrated that only a small fraction of CP is converted into gaseous form at room temperature. The likelihood of finding CP in air during the measurements at the hospital pharmacy was assessed to be largest inside the BSC. If any gaseous CP was present inside the BSC during the air measurement, it may have been ventilated, since the BSC is connected to the exhaust ventilation, or it may have been attached to surfaces inside the BSC since the initial studies showed that vaporized CP was efficiently attached to surfaces. A reason why no particles of CP were detected in the air in the BSC may be explained by the use of spikes. The spikes contained a filter with a pore size of 0.2 μm, which is expected to prevent the particles from entering the air in the BSC in association with drug preparation.

After one preparation of an infusion mixture containing 1000 mg CP, two wipe samples were collected in the BSC. Amounts of 1.3 and 1.4 ng CP were detected in the two samples and these correspond to 3.3 and 3.5 pg cm^{-2}, respectively. The pharmacy prepared antineoplastic drugs on disposable absorbent sheets, which had a plastic-coated underneath. These sheets almost covered the whole working area in the BSC. The sheet was removed before wipe sampling. This can explain why no high amounts of CP were found on the working area in the BSC. In this application it was demonstrated that the developed method for wipe sampling could detect trace contamination of CP in an authentic work environment.

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