Toxicology Letters 403 (2025) 40–49

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.journals.elsevier.com/toxicology-letters

Development and validation of a multicompound LLE–LC–MS/MS method for biomonitoring of hazardous medicinal products in urine of exposed workers

Maria Francisca Portilha-Cunha [a,b], Arminda Alves [a,b], Ana R.L. Ribeiro [b,c], Adrián M.T. Silva [b,c], Pedro Norton [d,e], Mónica S.F. Santos [d,e,*]

[a] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal
[b] ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal
[c] Laboratory of Separation and Reaction Engineering—Laboratory of Catalysis and Materials (LSRE– LCM), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal
[d] EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas 135, Porto 4050-600, Portugal
[e] Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas 135, Porto 4050-600, Portugal

ARTICLE INFO

Editor : Dr. Angela Mally

Keywords:
Antineoplastic drugs
Cytotoxics
Human biomonitoring
Analytical method
Occupational exposure

ABSTRACT

Antineoplastic drugs are carcinogens, mutagens, or teratogenic substances, which can pose serious risks to professionals. Concerns about chronic exposure to these hazardous medicinal products (HMPs) have led to their prominence in the EU strategic framework on health and safety at work 2021–2027. To estimate and mitigate human exposure to HMPs, regular monitoring programs and, consequently, reliable, sensitive, multicomponent methods are crucial. In this study, an unconventional liquid-liquid extraction coupled with liquid chromatography–tandem mass spectrometry analysis is proposed to simultaneously identify and quantify seven HMPs of high concern in urine: cyclophosphamide, etoposide, ifosfamide, paclitaxel, megestrol, mycophenolate mofetil, and tamoxifen, the last three for the first time. Recoveries of all drugs from urine samples were close to 100 %, and method detection limits (0.6–4.1 ng/L) were noticeably lower than most previously reported. This novel, non-invasive method for biomonitoring is thus suitable to unequivocally identify the target drugs at the expected trace levels in urine and to infer about workers' exposure. The method contributes to the conception of regular monitoring programs for antineoplastic drugs, in line with recommendations under EU Directive 2004/37/EC. This is especially relevant in Portugal, where neither analytical methods nor exposure data exist due to lack of formal surveillance.

1. Introduction

Antineoplastic drugs (ADs) have been used for cancer treatment for decades and their prescription is expected to rise due to the increase of new cancer cases (47 % between 2020 and 2040) (IARC, 2020). They are considered hazardous medicinal products (HMPs), whose genotoxicity, carcinogenicity and mutagenicity has been demonstrated (González-Román et al., 2021; Suspiro and Prista, 2011). Concerns associated to long-term exposure, even at low doses, have been rising, but both acute and chronic effects have been reported, mainly in pharmacy professionals and nurses (ISOPP Standards Committee, 2022; Sessink et al., 2016; Suspiro and Prista, 2011). Still, anyone (other workers, family members or caregivers of patients) may also be at risk when in contact with contaminated objects, air, or biological excreta. Despite the numerous guidelines and good occupational hygiene practices published worldwide (ISOPP Standards Committee, 2022; Mathias et al., 2019; Sessink et al., 2016), no occupational exposure limit values (OELs), biological limit values (BLVs) nor biological guidance values (BGVs) have been set for these drugs. Nevertheless, employing monitoring programs of carcinogenic, mutagenic and/or reprotoxic (CMR) substances is mandatory in the European Union under Directive 2004/37/EC, in which ADs are included since an amendment in 2022. In fact, regular monitoring programs are crucial, as they have been shown to improve the effectiveness of control measures and to increase

* Corresponding author at: EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas 135, Porto 4050-600, Portugal.
E-mail address: monica.santos@ispup.up.pt (M.S.F. Santos).

https://doi.org/10.1016/j.toxlet.2024.11.012
Received 29 December 2023; Received in revised form 7 November 2024; Accepted 27 November 2024
Available online 28 November 2024

workers' awareness. Thus, they contribute to reducing contamination levels (Korczowska et al., 2020), which, nowadays, are still recommended to be "as low as reasonably achievable" (ALARA).

It is known that human absorption of ADs is a reality in occupational contexts due to their reported measurable traces in urine of healthcare workers (Leso et al., 2022). For example, 55 % of 201 urine samples of healthcare workers from six Canadian facilities tested positive for cyclophosphamide (CYC; 75th percentile was 129 ng/L) in 2010/2011, and all eight job categories evaluated were at exposure risk (Hon et al., 2015). On the other hand, Sottani et al. (2010) reported a reduction in positive urine samples, which were around 30 % in the 1990s, 2 % in the 2000s and null in 2006/2007, after implementation of ADs' safe handling procedures. Other studies have reported no positive urine samples for the ADs monitored (Leso et al., 2022), but an absence of risk should not be presumed in such cases since factors such as the fast metabolization or poor sensitivity/selectivity of the analytical methods might have hampered drug detection. Therefore, reliable and validated analytical methods for biological monitoring of ADs and other HMPs are crucial. Indeed, the European Commission (2023) has recently highlighted (in their "Guidance for the safe management of hazardous medicinal products at work") that advances in analytical chemistry would be crucial to expand the implementation of regular biomonitoring of HMPs, thus advising for the development of new techniques and methods. Under this context, multianalyte detection is essential to correctly estimate exposure risk due to the wide variety of drugs in use, their different impacts on health and their different chromatographic behaviors (due to their different physical-chemical properties).

Up to date, CYC and ifosfamide (IFO) are the ADs most frequently investigated in urine samples of professionals, but others such as 5-fluorouracil, platinum compounds, methotrexate, irinotecan, daunorubicin, doxorubicin and epirubicin have also been reported (Leso et al., 2022). All of these drugs are of high concern, according to their inclusion in "The European Trade Union Institute's list of HMPs" for meeting the criteria for classification as category 1A or 1B of the EU Classification, Labelling and Packaging (CLP) system of CMR substances (Lindsley and Musu, 2022). Particularly, CYC is carcinogenic 1B, mutagenic 1B and reprotoxic 1A, and IFO is carcinogenic 1A, mutagenic 1A and reprotoxic 1A (Lindsley and Musu, 2022). Other ADs that also meet these criteria but have been investigated to a lesser extent include etoposide (ETO; carcinogenic 1B) and paclitaxel (PAC; carcinogenic 2, mutagenic 1B, reprotoxic 1B), particularly ETO for which only one method exists (Fabrizi et al., 2016). Still, other drugs of relevant concern have never been monitored due to the lack of analytical methodologies, such as megestrol (MEG; carcinogenic 1B, reprotoxic 1A), tamoxifen (TAM; carcinogenic 1A, reprotoxic 1A), and the immunosuppressant mycophenolate mofetil (MMF; reprotoxic 1B). Hence, CYC, ETO, IFO, MEG, MMF, PAC, and TAM were chosen as target drugs based on: their CMR classifications as category 1A or 1B; their frequent use in current chemotherapy preparations by the partner hospital; and/or their consumption in Portugal (Santos et al., 2017). The rationale for including all seven target drugs lies in the need for a robust method that covers a broad spectrum of drugs commonly used in chemotherapy and with the potential to be hazardous, in various scenarios where healthcare workers might come into contact with them, to ensure that no occupational exposure risk associated with handling HMPs is overlooked.

It is further valuable to note that the renal excretion of the target drugs is an important consideration, but such values should be considered with some caution, given the expected variability due to differences in exposure, and in individual metabolism and health status. For the seven drugs included in the method, it has been reported that the percentage of the parent drug excreted unchanged in the urine is: 10–40 % for CYC (FDA, 2024; IARC, 1981); 7–61 % for IFO (FDA, 2018a; IARC, 1981; Kerbusch et al., 2001); 45–55 % for ETO [Drugs.com, 2024; FDA, 2019; IARC, 2000); 4–14 % for PAC (FDA, 2011; FDA, 2023); 57–78 % for MEG (Canetta et al., 1983; FDA, 2018b); negligible for MMF, despite 93 % of the administered dose being recovered in urine (87 % as mycophenolic glucuronide and < 1 % as mycophenolic acid) (Bullingham et al., 1998; FDA, 2022); and very little for TAM (IARC, 1996; de Vos et al., 1998), although one study reported 27 % (Kisanga et al., 2005). Nevertheless, similar occupational exposure studies have detected parent drugs in workers' urine despite the expectedly low excretion rates (Leso et al., 2022; Turci et al., 2003). Furthermore, although renal excretion of the parent compounds might be minimal, even small amounts of these drugs are of interest, since low levels of a drug with residual excretion as unchanged compound may suggest a high level of exposure.

Given that HMPs are often metabolized, it would also be relevant to biomonitor some metabolites of these drugs in combination with their parent compounds to better understand the exposure of an individual. However, unstable metabolites would not be the best choices as possible biomarkers for exposure assessment of workers, thus limiting the possibilities. For example, many of the metabolites of CYC and IFO are not stable in urine according to B'Hymer and Cheever (2010). Also, to properly develop an analytical method for the unequivocal identification and quantification of a given compound, it is necessary to first obtain an analytical standard of that compound; currently, almost no standards for metabolites of these drugs exist. Therefore, the inclusion of metabolites in the present method was not considered at this point.

In the past decades, several methods have been reported in the literature for analysis of ADs in urine (Mathias et al., 2017; Nussbaumer et al., 2011; Sabourian et al., 2020; Stokvis et al., 2005; Turci et al., 2003), but most were developed/applied for therapeutic drug monitoring in patients, where ADs' concentrations in the urine are significantly higher than those expected in potentially exposed professionals. In this sense, detection and quantification of very low levels of contaminants (trace analysis) with a high degree of specificity and sensitivity is fundamental for a better understanding of the exposure of healthcare workers. To do so, sample preparation is crucial to concentrate the analytes and to remove interfering components (since urine samples include proteins, metabolites, and salts, among others), which may also significantly reduce column lifetime and cause contamination of the ionization source of the instrumental equipment. For the extraction procedure, either solid-phase extraction (SPE) or liquid-liquid extraction (LLE) is usually employed, often depending on the target drugs (Mathias et al., 2017; Palamini et al., 2020; Villa et al., 2020). SPE tends to use lower organic solvents' volumes, but it is a complex procedure comprising several sequential steps and different sorbents are needed to extract drugs with dissimilar physical-chemical properties. On the other hand, LLE needs simpler operations and apparatus, avoiding the use of expensive cartridges, and provides good repeatability and high recoveries. Nevertheless, it typically applies to a reduced number of compounds because of the low polarity solvents used (such as ethyl acetate and diethyl ether) and it is preferred for lipophilic drugs (such as CYC, IFO and PAC), which migrate from the aqueous biologic sample to the organic solvent (Sabourian et al., 2020). Therefore, a step forward is envisaged in this work by proposing an LLE extraction procedure that replaces the typically nonpolar solvents by a polar solvent (acetonitrile (ACN)), allowing the simultaneous extraction of lipophilic and hydrophilic compounds. Regarding the instrumental analysis, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been preferred for simultaneous determination of multiple, dissimilar ADs in urine of exposed workers (Mathias et al., 2017; Sabourian et al., 2020) – since early LC methods using ultraviolet, fluorescent and electrochemical detection lacked specificity, whereas gas chromatography is unsuitable because most ADs are non-volatile and thermolabile compounds (Nussbaumer et al., 2011; Stokvis et al., 2005; Turci et al., 2003). Still, it is important to develop methods that further lower the detection limits reported nowadays (mostly at μg/L sensitivity in urine samples (Mathias et al., 2017)), since no safe value can be defined for CMR compounds. Moreover, most studies report only some validation parameters (Mathias et al., 2017), despite full validation of analytical methods (linearity, specificity, sensitivity, precision, accuracy,

uncertainty) being always recommended. This is a relevant gap in this research field, which limits the possible comparisons among methods and the accurate quantification and exposure risk assessment.

The objective of the present study was to develop and fully validate a multicompound analytical methodology to detect trace amounts of HMPs in urine, with high specificity and sensitivity, aiming for lower detection limits than those previously reported in the literature. The unconventional LLE procedure with ACN coupled to LC–MS/MS analysis allows the simultaneous identification and quantification of seven HMPs in urine: CYC, ETO, IFO, MEG, PAC, and TAM, as well as the immunosuppressant MMF. Method validation, including the estimation of global uncertainty, was performed, and a preliminary evaluation of the presence of the target drugs in the urine of a few exposed healthcare workers from a Portuguese tertiary hospital was conducted. To the authors' best knowledge, this is the first method to biomonitor MEG, MMF and TAM in occupational contexts. Another novelty is the replacement of the typically nonpolar solvents by a polar solvent that allows extraction of lipophilic and hydrophilic compounds, avoiding multiple extraction procedures of the same urine sample.

2. Materials and methods

2.1. Chemicals and reagents

CYC, ETO, IFO, MEG, MMF, PAC, and TAM analytical standards of 98–99 % purity, as well as cyclophosphamide-d4 (CYC-d4) were supplied by either Sigma-Aldrich (St. Louis, MO, USA) or Cayman Chemical Company (Ann Arbor, MI, USA). Stock standard solutions were prepared at a concentration of 100 mg/L in ACN and working solutions were prepared at 10 mg/L in ACN. LC–MS grade ACN, methanol and Milli-Q water were acquired from VWR (Radnor, PA, USA), as were isopropanol, dichloromethane, ethyl acetate and diethyl ether. Formic acid was purchased from Merck (Darmstadt, Germany).

2.2. Safety considerations on AD's handling

Exhaustive controls on handling procedures, storage conditions, and safety rules were followed, as recommended by the manufacturers, for standards' preparation. All AD's handling procedures were accomplished in a safety hood with vertical laminar airflow and work surfaces were protected by absorbent paper. Materials that contacted with ADs were cleaned with isopropanol and all dischargeable materials were treated as hazardous waste.

2.3. Extraction of target drugs from urine

Urine samples were collected in proper containers and kept cool (~ 4 °C) until being processed (in less than 24 h after collection). For the extraction of drugs from urine, the final LLE procedure was: (i) 20 mL of urine was centrifuged at 15,000 *g*, 4 °C, for 15 min (Centrifuge 5804R; Eppendorf, Hamburg, Germany) and the supernatant was transferred to a new Falcon tube; (ii) the supernatant was mixed with 20 mL ACN and vortexed for 3 min; (iii) the mixture was refrigerated for 1 h at − 20 °C and the organic phase collected; (iv) the aqueous phase was again mixed with 20 mL ACN and the mixture vortexed for 3 min; (v) the solution was refrigerated for 30 min at − 20 °C and the organic phase was collected and merged with the previous one; (vi) the organic phase was evaporated to a reduced volume and centrifuged at 30,000 *g*, 4 °C, for 10 min (Centrifuge 5430R; Eppendorf, Hamburg, Germany); (vii) the supernatant was collected and evaporated to dryness; (viii) the dry residue was reconstituted in 100 µL of ACN, vortexed and transferred to a glass insert; (ix) the extract was centrifuged at 30,000 *g*, 4 °C, for 10 min (Centrifuge 5430R; Eppendorf, Hamburg, Germany); (x) the clean extract was transferred to a new glass insert and stored at − 20 °C until analysis.

2.4. Instrumental analysis

A liquid chromatograph (Shimadzu Corporation; Tokyo, Japan) equipped with two Pumps LC-30AD, an Autosampler SIL-30 AC, an Oven CTO-20 AC, a Degasser DGU-20A5, a System Controller CBM-20A, and an LC solution version 5.41SP1, coupled to a triple quadrupole mass spectrometer detector (Shimadzu LCMS-8040) was employed for instrumental analysis (LC–MS/MS). Data were acquired and processed using the LabSolutions software package. A Luna C18 column (150 × 2.1 mm ID, particle size 5 µm; Phenomenex) was employed for separation, the flow rate was 0.2 mL/min, and the injection volume was 5 µL. The mobile phase consisted of a binary mixture of water (A) and methanol (B), both acidified with 0.1 % formic acid, and an elution gradient was used: by starting at 5 % B, which was increased to 20 % B in 15 min and to 45 % B in another 15 min, reaching 100 % in 9 min (39 min); after 2 min, the initial conditions were regained in 4 min, and the system was stabilized for 5 min (50 min). The electrospray ionization source was operated in positive mode and the precursor ions $[M + H]^+$ and the two most abundant fragments were used for identification (transition 2) and quantification (transition 1) of the target analytes – chromatographic and mass spectrometry information are detailed in Table 1. As previously optimized, cone voltage was 4.5 V, collision energy was 10–50 eV, nebulizing gas flow was 3.0 dm^3/min, drying gas flow was 7.5 dm^3/min, block temperature was 400 °C, and desolvation line temperature was 250 °C (Portilha-Cunha et al., 2021).

2.5. Method validation

Calibration was performed over a concentration range from 1 to 1000 µg/L, using nine calibration points. The internal standard quantification methodology was applied using CYC-d4 as a surrogate for all target drugs, with a concentration of 100 µg/L. Instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) for each drug were obtained from a signal-to-noise ratio of 3 and 10, respectively, based on the analytical responses obtained for the 50 and 100 µg/L calibration points.

Validation assays were performed for three concentration levels in the final extracts: 10, 50 and 100 µg/L. All these assays were replicated on different days, using the same pooled urine sample of several non-exposed individuals, thus average values of the assay repetitions were obtained for the three concentration levels and for each drug. Interday precision was given by the determination of the coefficient of variation for the three levels on different days. Recovery tests were performed by

Table 1
Chromatographic and mass spectrometry information obtained for the instrumental analysis of the target drugs by LC–MS/MS.

Drug	rt (min)	Molecular Ion (*m/z*) (Cone Voltage, V)	Transition 1 (CE, eV)	Transition 2 (CE, eV)
CYC	31.260	260.90 $[M + H]^+$ (4.5)	260.90 → 139.95 (− 23)	260.90 → 106.05 (−19)
ETO	34.601	589.20 $[M + H]^+$ (4.5)	589.20 → 228.95 (− 20)	589.20 → 185.10 (−37)
IFO	29.546	260.90 $[M + H]^+$ (4.5)	260.90 → 92.05 (− 26)	260.90 → 153.95 (− 23)
MEG	39.432	385.10 $[M + H]^+$ (4.5)	385.10 → 267.10 (− 20)	385.10 → 325.15 (− 15)
MMF	31.983	434.10 $[M + H]^+$ (4.5)	434.10 → 114.05 (− 27)	434.10 → 194.95 (− 36)
PAC	38.719	876.20 $[M + H]^+$ (4.5)	876.20 → 308.00 (− 30)	876.20 → 591.15 (− 28)
TAM	37.174	876.20 $[M + H]^+$ (4.5)	372.20 → 72.15 (− 26)	372.20 → 44.05 (− 49)
CYC-d4	31.173	265.00 $[M + H]^+$ (4.5)	265.00 → 140.00 (− 24)	265.00 → 63.00 (− 43)

rt—retention time; CE—collision energy; CYC—cyclophosphamide; ETO—etoposide; IFO—ifosfamide; MEG—megestrol; MMF—mycophenolate mofetil; PAC—paclitaxel; TAM—tamoxifen; CYC-d4—cyclophosphamide-d4.

adding the target analytes (1, 5 or 10 ng of each, depending on the concentration level) plus 10 ng of internal standard (CYC-d4) to the urine samples, which were then processed according to the extraction procedure described in Section 2.3. Matrix effects were evaluated by extraction of urine samples as received, with addition of target drugs (1, 5 or 10 ng of each) and internal standard (10 ng) only prior to LC-MS/MS analysis. Control samples correspond to analytical standards prepared in "pure" solvent (ACN), which also contain 1, 5 or 10 ng of each investigated drug, depending on the concentration level, and 10 ng of CYC-d4. Blank samples (extraction of urine samples added with 10 ng internal standard) were also obtained.

Recoveries were calculated according to Eq. (1):

$$\%R = Cs/C_{\mathrm{ME,average}} \times 100, \quad (1)$$

where C_S is the drug concentration measured in an extract obtained from a recovery test and $C_{ME,average}$ corresponds to the concentrations measured in the extracts from matrix effect assays.

Matrix effects were calculated according to Eq. (2):

$$\%\text{Matrix Effect} = C_{\mathrm{ME}}/C_{\mathrm{c,average}} \times 100, \quad (2)$$

where C_{ME} is the drug concentration measured in an extract obtained from a matrix effect assay and $C_{C,average}$ corresponds to the concentrations measured in the control samples.

Accuracy was obtained by comparing the analytical response in the extracts obtained from recovery tests with the analytical response in the respective control samples (*i.e.*, it considers both recoveries and matrix effects phenomena). Method detection limits (MDLs) and method quantification limits (MQLs) were calculated from the instrumental limits (IDLs and IQLs, respectively) taking into consideration the sample concentration factor of 200 times, and the average accuracy obtained for each drug.

2.6. Global uncertainty

The bottom-up approach proposed by the International Organization for Standardization and adopted by the EURACHEM-CITAC Guide was applied to estimate the global uncertainty associated with the quantification of the target analytes in urine by LC–MS/MS (Ellison and Williams, 2012). The four sources of uncertainty considered are related to: the preparation of standards (estimated using the error propagation law for the several dilution steps of the stock standard solution); the calibration curve (calculated for each calibration point); the precision of the method (estimated as the average result of the relative standard deviation of recovery assays at different concentrations); and the accuracy (calculated as the average analytical response for the different concentrations). Detailed equations can be found in the Supplementary material.

2.7. Analysis of healthcare workers' urine

The method developed and validated was employed for the detection of the target drugs in the urine of healthcare workers from the pharmacy and the oncologic day-care hospital of a Portuguese tertiary hospital (over 35 thousand chemotherapy preparations per year). First, urine samples of eleven workers from the pharmacy were collected during their working shifts; later, urine of three workers from the pharmacy and other three from the day-care hospital were collected at the end of their working shift and at the following day (first morning urine). Urine spot samples were collected in 120 mL polypropylene containers with a screw cap by the individuals and samples were kept cool (~ 4 °C) until being processed (on the same day). Each sample was analyzed in duplicate.

3. Results and discussion

3.1. Extraction methodology

In the present study, urine was the selected biological matrix, particularly due to its easy and non-invasive collection, unlike blood collection through venipuncture. Despite some known problems related to hydration variation and specificity, analyzing the pharmaceuticals in urine also provides a wider evaluation time window in comparison to blood sampling, where the concentration of nonpersistent chemicals like ADs usually rapidly declines after exposure (Leso et al., 2022). Regarding sample type, the use of 24-h or spot urine samples has not been formally compared (Chauchat et al., 2019). However, a research group noted that collecting 24-h urine samples for large-scale surveillance programs would be unrealistic and reported no urinary traces of ADs when analyzing both types of samples in three Canadian hospitals (Chauchat et al., 2019; Palamini et al., 2020; Poupeau et al., 2017). Moreover, workers may forget to collect urine for 24-h samples and external contamination seems likely. Hence, urine spot samples were chosen in this study, for being less cumbersome and costly and more "user friendly".

As a first clean-up step, an initial centrifugation of the urine samples was performed for separation of unwanted compounds (mainly proteins), taking into consideration literature information regarding analysis of urine matrices (Mathias et al., 2017; Sabourian et al., 2020). This greatly helps cleaning the samples and, consequently, improves phase separation during the LLE. Regarding the LLE solvents, a few conventional solvents were initially tested. Although dichloromethane, diethyl ether and ethyl acetate showed acceptable recoveries, the extraction process was problematic: the formation of bubbles in the organic phase when using ethyl acetate and diethyl ether (to a lower extent) hindered their proper collection and, consequently, made the evaporation step harder/longer due to the presence of water mixed with the organic solvent; dichloromethane was also difficult to separate from the aqueous phase due to its superior density in comparison to water, thus consisting of the bottom layer, which turned white, likely due to sedimentation of matrix components. Furthermore, these conventional nonpolar solvents do not allow the simultaneous extraction of lipophilic and hydrophilic compounds, as previously explained. Hence, the use of a polar solvent, such as ACN, was considered for the extraction of ADs from urine, taking advantage from the knowledge of the research team on the extraction of these HMPs from surface waters and wastewaters by LLE (Gouveia et al., 2020). Besides the advantage of potentially recovering and detecting a wide range of drugs with different physical-chemical properties, ACN is also preferable over the conventional solvents (dichloromethane, ethyl acetate and diethyl ether) for being more environmentally friendly. Taking into consideration the partial miscibility of ACN and water (urine is mainly constituted of water), the phase separation had to occur at a low temperature (-20 °C), since ACN has a lower melting point than water, and a ratio of 1:1 ACN:urine was needed. Considering that ADs' concentration in the urine of exposed healthcare workers is expected to be in the ng/L level (or even lower), a double extraction of urine samples was considered, similarly to the method used for analysis of wastewaters (Gouveia et al., 2020). This second extraction with ACN was an important step since it enhanced the recovery percentages of the target analytes (data not shown). After the LLE procedure (extraction, phases separation, and evaporation to a reduced volume), the introduction of a centrifugation step was fundamental to further remove unwanted compounds and clean the reconstituted extract, since the extraction of interferents with ACN was noticeable (a significant dry residue was generated during evaporation of the organic phase). After evaporation to dryness and reconstitution, some particles in suspension were still observed in some extracts, thus another centrifugation was deemed necessary to protect the instrumental equipment from clogging and contamination. These two final centrifugations had a positive impact in reducing matrix interferences, while still providing good recoveries.

Therefore, as detailed in Section 2.3, the final extraction methodology briefly consists in: centrifuging 20 mL of urine; mixing the supernatant with 20 mL ACN, cooling it to allow phase separation and recovering the organic phase; repeating the previous step with the remaining aqueous phase; evaporating the organic solvent collected to a reduced volume and centrifuging it; recovering the supernatant and evaporating it to dryness; reconstituting in ACN; and centrifuging and storing the final extract in a glass insert.

Regarding recoveries, which were calculated according to Eq. (1), average values of the six assays performed in two days with the same pooled urine sample were obtained for the seven drugs, for three concentrations (10, 50, 100 μg/L in the final extracts). As displayed in Fig. 1, recoveries of around 100 % were generally obtained for all drugs, which demonstrates that the extraction methodology is very effective (*i.e.*, the target analytes are completely extracted from urine samples). Relatively lower recoveries were attained for TAM, particularly at lower concentrations but still very satisfactory: (62 ± 10) % and (58 ± 8) % for 10 and 50 μg/L, respectively. Across the three concentrations, average recoveries were: (104 ± 9) % for CYC; (100 ± 17)% for ETO; (99 ± 11) % for IFO; (109 ± 13) % for MEG; (108 ± 21) % for MMF; (111 ± 30) % for PAC; and (73 ± 25) % for TAM.

Fig. 2 shows the matrix effects, calculated according to Eq. (2), for all drugs. As observed, there are some compounds present in the extracts that interfere with the quantification of the target analytes by LC-MS/MS but most values are between 80 % and 120 %, which is acceptable. The matrix effects are residual for CYC, IFO, MEG, MMF, and TAM; but for ETO and PAC there is some suppression.

3.2. Method validation

The validation parameters concerning the analysis of the seven drugs by LC–MS/MS are compiled in Table 2. Good linearity (R > 0.999) was verified in the range of 1–1000 μg/L (in the extract) for all compounds using the internal standard calibration approach. The IDLs were very low (all below 1 μg/L). Precision and accuracy for the three concentration levels, obtained from repeated assays performed in different days, were considered acceptable as seen in Table 2. The average interday precision of all target drugs was (10 ± 7) %, while average accuracy of the three concentrations was: (102 ± 18) % for CYC; (73 ± 20)% for ETO; (93 ± 12) % for IFO; (97 ± 15) % for MEG; (113 ± 11) % for MMF; (47 ± 15) % for PAC; and (58 ± 21) % for TAM.

Considering these accuracy values and the sampling concentration factor of 200 times, MDLs are in the range of 0.6–4.1 ng/L (Table 2). Concerning methods for analysis of urine samples of exposed workers (*i.e.*, occupational contexts rather than methods for therapeutic drug monitoring in patients), no values were found in the literature for MEG, MMF, and TAM. The only study analyzing ETO in urine reported a detection limit of 170 ng/L (Fabrizi et al., 2016), which is much higher than that attained here (1.8 ng/L). PAC has not been extensively studied in this biological matrix, with values ranging from 50 to 500 ng/L (Mathias et al., 2017; Leso et al., 2022) and the lowest value reported being 5 ng/L (Lema-Atán et al., 2022), which is still above the MDL in this study (3.9 ng/L). CYC and IFO are some of the most studied drugs in this field and a significant range of detection limit values was found, with most being in the hundreds ng/L level (Mathias et al., 2017; Leso et al., 2022]: 10–8100 ng/L for CYC; and 10–7700 ng/L for IFO. However, recent studies have already reported lower detection limits for both drugs: 9.0 ng/L (Palamini et al., 2020; Poupeau et al., 2017), 2.5 ng/L (Izzo et al., 2018) and 1 ng/L (Villa et al., 2021) for CYC; and 9.7 ng/L (Palamini et al., 2020; Poupeau et al., 2017), 5 ng/L (Izzo et al., 2018), and 1 ng/L (Villa et al., 2021) for IFO. Therefore, the MDLs achieved in the present study are lower than most values previously reported in the literature. Furthermore, when comparing with the recent more sensitive analytical methods, only two values slightly lower than the MDL of CYC (4.1 ng/L) were reported in the literature (1–2.5 ng/L) and only one value lower than 2.3 ng/L for IFO was found in the literature (1 ng/L). Considering that ADs' concentration in the urine of exposed healthcare workers is expected to be in the ng/L level (Leso et al., 2022), it can be stated that this method is adequate to be employed for biological monitoring programs for the analysis of the target drugs in the urine of exposed professionals.

It is also relevant to address the topic of creatinine measurements: although it is a quality marker of the urine (and could be measured in urine samples, even if not used to adjust the concentration values), it does not seem particularly relevant for ADs quantification in this context. Indeed, recent reviews regarding both biological monitoring and urinary methods for ADs exposure of healthcare professionals do not mention this parameter (Leso et al., 2022; Mathias et al., 2017; Sabourian et al., 2020), and other analytical methods for ADs analysis in urine were successfully developed and validated without considering this parameter (Izzo et al., 2018; Lema-Atán et al., 2022; Poupeau et al., 2017). Furthermore, a mixture of real urine samples from unexposed individuals to the target HMPs were used to determine validation parameters, ensuring that precision and accuracy reflect potential uncertainty caused by different creatinine levels.

3.3. Global uncertainty associated with the results

In this study, the global uncertainty associated with the results was estimated and is illustrated in Fig. 3. It is an important validation parameter, although not often reported since it is very relevant when comparing results from different methods or when a maximum legal limit is under consideration. The global uncertainty should be made available and considered when interpreting results. In this study, the EURACHEM methodology was employed, which considers four sources of uncertainty (individual contributions to the global uncertainty are depicted in Fig. S1 from the Supplementary material). Both Fig. 3 and Fig. S1 reveal one of the main difficulties in uncertainty measurement:

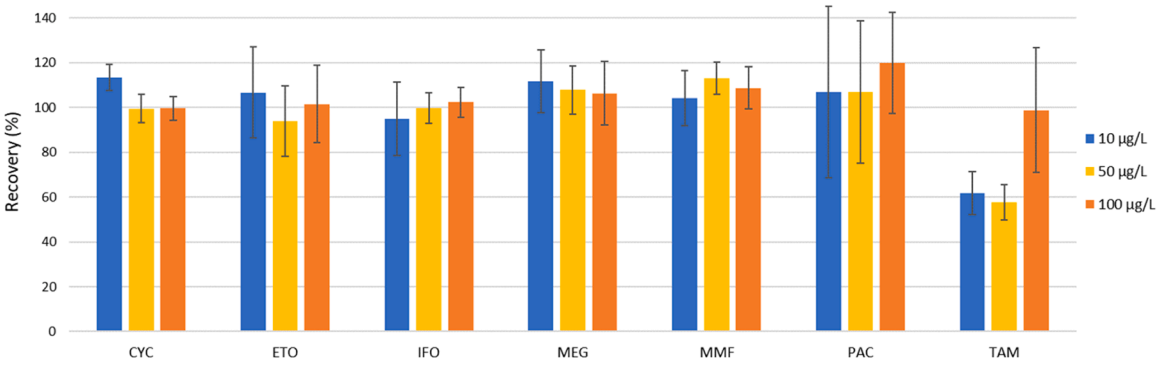

Fig. 1. Mean recoveries for the seven target drugs (cyclophosphamide (CYC), etoposide (ETO), ifosfamide (IFO), megestrol (MEG), mycophenolate mofetil (MMF), paclitaxel (PAC), tamoxifen (TAM)) extracted from spiked urine using the final LLE procedure. Error bars represent standard deviation of the several assays.

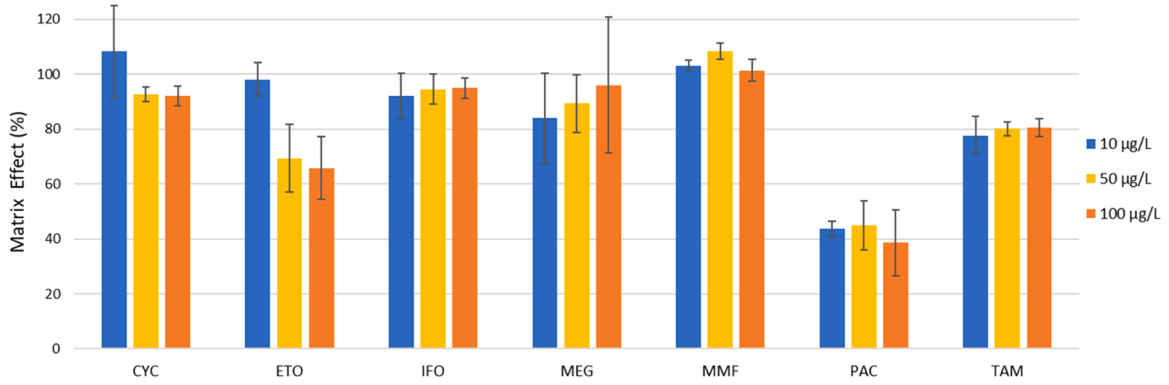

Fig. 2. Mean matrix effects for the seven target drugs (cyclophosphamide (CYC), etoposide (ETO), ifosfamide (IFO), megestrol (MEG), mycophenolate mofetil (MMF), paclitaxel (PAC), tamoxifen (TAM)) extracted from spiked urine using the final LLE procedure. Error bars represent standard deviation of the several assays.

Table 2
Validation parameters obtained for the instrumental analysis of the seven target drugs by LC–MS/MS.

Drug	Linearity (in extracts, µg/L)	IDL (µg/L)	IQL (µg/L)	MDL[a] (ng/L)	MQL[a] (ng/L)	Precision (CV%)			Accuracy (Mean ± SD, %)		
						10 µg/L	50 µg/L	100 µg/L	10 µg/L	50 µg/L	100 µg/L
CYC	2.8–1000	0.8	2.8	4.1	13.5	5	6	4	123 ± 18	92 ± 5	92 ± 4
ETO	1–1000	0.3	0.9	1.8	5.9	16	12	8	104 ± 16	65 ± 16	66 ± 7
IFO	1.4–1000	0.4	1.4	2.3	7.7	12	5	2	88 ± 16	94 ± 9	97 ± 7
MEG	1–1000	0.3	0.8	1.3	4.4	9	9	6	93 ± 16	96 ± 13	99 ± 15
MMF	1–1000	0.1	0.5	0.6	2.1	4	3	4	108 ± 13	123 ± 8	110 ± 6
PAC	1.2–1000	0.4	1.2	3.9	13.1	27	19	17	47 ± 15	47 ± 11	47 ± 18
TAM	1–1000	0.1	0.4	1.1	3.8	7	4	22	48 ± 10	46 ± 6	80 ± 21

CYC—cyclophosphamide; ETO—etoposide; IFO—ifosfamide; MEG—megestrol; MMF—mycophenolate mofetil; PAC—paclitaxel; TAM—tamoxifen; IDL—instrumental detection limit; IQL—instrumental quantification limit; MDL—method detection limit; MQL— method quantification limit; CV—coefficient of variation; SD—standard deviation.

[a] Considering the sample concentration factor (200 ×) and the average accuracy for each drug.

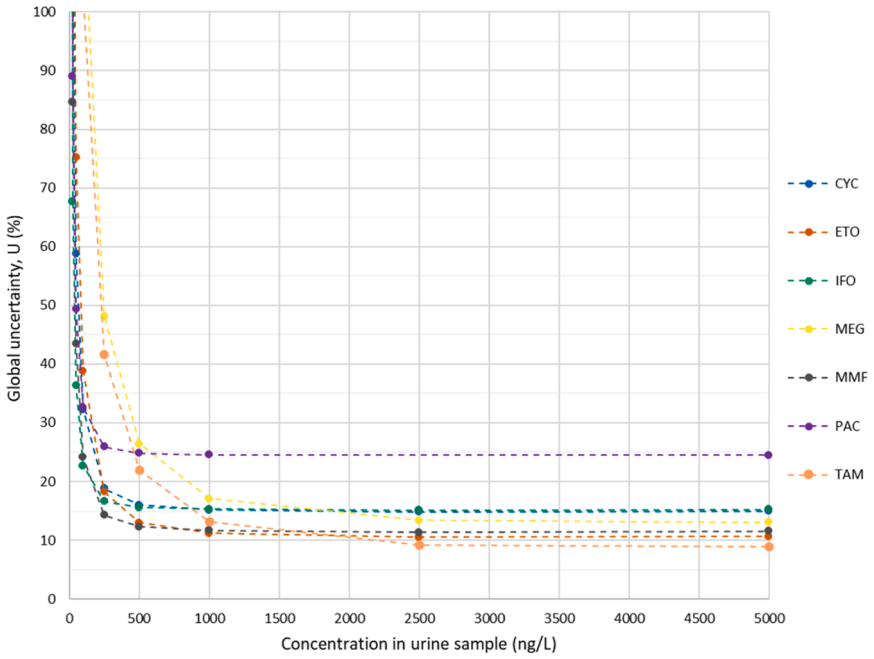

Fig. 3. Global uncertainty of the analytical methodology for the quantification of seven drugs in urine by LC–MS/MS: cyclophosphamide (CYC); etoposide (ETO); ifosfamide (IFO); megestrol (MEG); mycophenolate mofetil (MMF); paclitaxel (PAC); tamoxifen (TAM). Dashed lines are merely illustrative of the data trend. The range of 5–5000 ng/L in urine samples corresponds to the range of 1–1000 µg/L in the extract.

the dependence of the uncertainty with the concentration, which is particularly high in the vicinity of the MDLs, which was observed for all analytes. This is especially relevant since, in the analysis of ADs from urine spot samples, it is expected that most positive samples will fall in the lowest concentration range. In fact, global uncertainty is below 25 % for most of the target analytes for concentrations above 250 ng/L; but it increases significantly for lower concentrations, reaching over 100 % for concentrations close to the MDLs. This means that the target drugs can be unequivocally identified in this lower range, which is extremely important to infer about the exposure of individuals to these HMPs, although their concentrations may not be accurately and precisely determined.

3.4. Analysis of healthcare workers' urine

Regarding the application of the validated analytical methodology, all 23 analyzed samples were negative (either in the end-of-shift or the morning urine samples), meaning that no evidence was found for the presence of the target drugs in the urine of the participating healthcare workers. It should be highlighted that the main objective of this study was to develop and validate an analytical method, rather than performing extensive biomonitoring. As such, this preliminary evaluation of the presence of the target drugs in the urine of a few exposed healthcare workers was performed with the intent to show method application and to act as a starting point for the execution/implementation of a larger biological monitoring campaign/program. The present section demonstrates the method's capability and performance, even if most urine samples were collected from pharmacy personnel. Therefore, although this preliminary sampling may not fully capture all potential exposure scenarios, the methodology itself remains relevant and could be extended to other professional groups in future research, given its broader applicability.

This section holds some limitations, which should be considered when interpreting the results. Particularly, it is known that CYC, ETO, IFO and PAC are frequently used in current chemotherapy preparations by the partner hospital, while the remaining target drugs are not, but detailed records of drug handling on specific days were not available. Hence, MEG, MMF and TAM were not anticipated to be present in the urine of the workers enrolled in this preliminary evaluation. However, it is relevant to consider that occupational exposure to HMPs is not always related to direct drug reconstitution or administration. In fact, workers may come into contact with drug residues or surfaces previously contaminated with HMPs, even if they were not handling those specific drugs on the day of urine collection. Indeed, an environmental assessment in the workplace of the participating professionals (a couple months prior; results unpublished) seems to corroborate the non-detection of ADs in their urine, since no concentrations were found above the "action limit" of 10 ng/cm^2 (Crul and Simons-Sanders, 2018; Sessink, 2011). As observed in Fig. 4, although 91 % of work surfaces sampled from the pharmacy and 40 % from the oncologic day-care hospital were found contaminated with at least one target drug (CYC, ETO, IFO, PAC), no values fell in the red range of the code model used, above which surface contamination is considered "not acceptable" and corrective measures are critical. Still, five concentration values were above 0.1 ng/cm^2, a "safe" (substance-independent) reference/guidance value (Crul and Simons-Sanders, 2018; Kiffmeyer et al., 2013; Korczowska et al., 2020; Sessink, 2011), above which the exposure risk should be estimated and a follow-up monitoring performed.

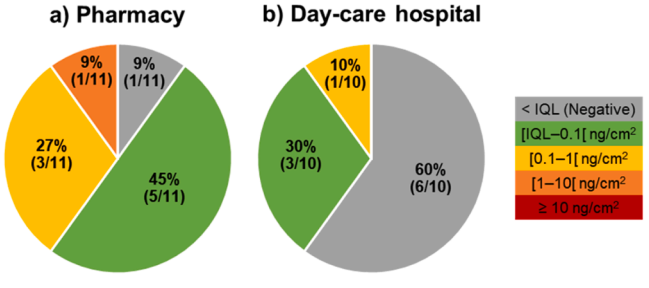

Fig. 4. Surface contamination levels with cyclophosphamide, etoposide, paclitaxel, and ifosfamide in a Portuguese tertiary hospital (April 2023): a) in the pharmacy; b) in the day-care hospital. Sampled locations are considered positive if at least one drug was detected above the IQL; negative samples are color coded grey. Each location was attributed to a color range (green, yellow, orange, or red) based on the highest contamination value of the four target drugs found.

Moreover, exposure to these drugs can still occur through transdermal delivery from contaminated containers/packaging (Hilliquin and Bussières, 2020; e Silva et al., 2023), and surface contamination with HMPs can persist in work environments, even when detailed cleaning protocols/guidelines are in place, contributing to continuous low-level exposure (Delafoy et al., 2023; Korczowska et al., 2020; Lancharro et al., 2016; Portilha-Cunha et al., 2025; Simon et al., 2019). Therefore, urine samples taken after these workers' shifts could still reflect potential exposure, even if they did not handle the drugs directly on the day of collection.

Nonetheless, results from biological monitoring are difficult to interpret, since the likelihood of detecting each specific HMP in the urine of exposed workers strongly depends on the specific tasks and handling procedures, specific drugs handled and their quantity, the frequency and duration of exposure, and adherence to safety protocols (collective control systems, (correct) use of personal protective equipment, and cleaning practices). Additionally, individuals' metabolic rates may cause variation, and the exposure risks obtained from biomonitoring might be underestimated, since only a part of each drug is excreted unchanged in urine. In this preliminary evaluation, the activities of exposed healthcare workers were taken into consideration for their selection as participants (pharmacy workers that prepared pharmaceutical formulations in a clean room and day-care hospital workers that administered the pharmaceutical formulations) but no formal analysis was performed to correlate workers' tasks and drug uptake, since there were no positive samples.

Concerning results from biomonitoring studies of ADs in the literature, there is a great variability among studies and data extrapolated is quite fragmented, which limits the interpretation and generalization of the findings. Older reviews (10–20 years ago) on this topic reported that most of the reviewed studies found measurable levels of ADs in urine samples of exposed workers (Turci et al., 2003; Suspiro and Prista, 2011). However, more recently, Leso et al. (2022) revealed that several studies did not find any positive urine samples, although others still reported up to 55 % (but ADs' concentrations were not always reported). These findings seem to suggest that although the analytical methodologies nowadays available provide higher sensitivity, a reduction in positive urine samples may have occurred over the last decades, likely due to the implementation and adherence to ADs' safe handling procedures, just like conjectured by Sottani et al. (2010) (that found a reduction in positive urine samples from around 30 % in the 1990s to 2 % in the 2000s and to no positive samples in 2006/2007).

Although surface contamination has been the preferred indicator of occupational exposure risk to ADs (having been investigated on surfaces from oncologic healthcare settings worldwide (Delafoy et al., 2023; Kiffmeyer et al., 2013; Korczowska et al., 2020; Petit et al., 2017), it is an indirect indicator. Indeed, even if biomonitoring can be cumbersome (Palamini et al., 2020), it is considered a better approach to human exposure assessment to ADs. As there are no safe levels for CMRs (according to Directive 2004/37/EC), the more appropriate practice would be to complement environmental monitoring (surface contamination) with biological monitoring to carry out an integrated analysis of the exposure risk to these drugs. Still, Kibby (2017) has reported that few studies have concurrently assessed the presence of ADs on surfaces and in urine of exposed professionals.

Therefore, a biomonitoring campaign with a larger sample size will be designed and performed in the partner hospital, simultaneously with an environmental monitoring program. Information concerning exposed subjects, their tasks and working environment will be gathered through individual questionnaires, thus future studies will benefit from improved tracking of workers' tasks to correlate handling with biomonitoring results more accurately. Also, the enrollment of unexposed subjects (preferably from other departments within the same hospital) will be considered, since the inclusion of control groups is considered fundamental to extrapolate definite conclusions, although these have been rarely reported in the literature concerning biological monitoring of ADs (Leso et al., 2022). Concerning sample collection time, it was decided to use urine spot samples collected at the end of the shift. This decision was based on available literature (most studies that employ spot samples collect them at the end of working shifts rather than in the next day(s) (Leso et al., 2022)), easiness of sample collection and delivery from workers, and sample processing timings.

4. Conclusions

An adequate, non-invasive, multicompound method for biological monitoring of HMPs was successfully developed and validated for the identification and quantification of seven HMPs of high concern in urine by LC–MS/MS: CYC, ETO, IFO, MEG, MMF, PAC, and TAM. This is the first method for the analysis of MEG, MMF and TAM in urine in occupational contexts, and for the analysis of ETO by using an unconventional LLE procedure. The LLE method using double extraction with ACN was very effective in recovering the target analytes from urine samples (recoveries of around 100 % for all drugs, except TAM at lower concentrations, which still showed very satisfactory values). The inclusion of centrifugations as clean-up steps helped achieving a final clean extract, which also led to acceptable matrix effects (between 80 % and 120 %).

Full method validation was reported and very low IDLs were achieved for all seven drugs. Average interday precision of all target drugs was (10 ± 7) %, and average accuracy of the three concentrations evaluated was (84 ± 28) %. The MDLs were in the range of 0.6–4.1 ng/L, which are sufficiently low to detect the target drugs at the expected ADs' concentration in the urine of exposed healthcare workers (in the ng/L level). Global uncertainty associated with contamination values reached over 100 % for concentrations around the MDLs but the target drugs can still be unequivocally identified in this lower range (even if concentrations may not be accurately and precisely determined). That is extremely important as it allows to infer about the exposure of individuals to the target HMPs, making this a suitable method for biomonitoring of professionals exposed to them.

The method developed was thus employed for analysis of 23 urine samples from workers of the pharmacy and the oncologic day-care hospital of a Portuguese tertiary hospital. Both end-of-shift and following day (first morning) urines were collected, but no positive urine samples were found. These findings seem consistent with a prior environmental contamination assessment in their workplaces.

The availability of this novel validated biological monitoring method also contributes to the implementation of a better occupational exposure risk assessment in the partner hospital, by complementing the environmental monitoring (surface contamination) already recommended and in use. This is especially important as no formal surveillance or regular monitoring program for ADs exist in our country, despite the recommendation of the EU (CMR Directive 2004/37/EC).

CRediT authorship contribution statement

Maria Francisca Portilha-Cunha: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization. **Arminda Alves**: Resources, Writing – review and editing, Supervision, Funding acquisition. **Ana R. L. Ribeiro**: Resources, Writing – review and editing. **Adrián M. T. Silva**: Resources, Writing – review and editing. **Pedro Norton**: Resources, Writing – review and editing. **Mónica S. F. Santos**: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

Ethical approval

The study was approved by the Ethics Committee and the Data Protection Officer of the partner hospital.

Data accessibility statement

The data underlying this article are available from the corresponding author on reasonable request.

Declaration of Competing Interest

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

Funding/Acknowledgements

This work was financially supported by: national funds through FCT/MCTES (PIDDAC) – LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020), LSRE-LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020), and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020); FCT – Fundação para a Ciência e a Tecnologia, I.P. through the projects with references UIDB/04750/2020 and LA/P/0064/2020 and DOI identifiers https://doi.org/10.54499/UIDB/04750/2020 and https://doi.org/10.54499/LA/P/0064/2020. Maria Francisca Portilha-Cunha is grateful to FCT for her Ph.D. grant (2021.05219.BD). ARLR acknowledges the FCT funding received under the Scientific Employment Stimulus-Individual Call (2022.00184.CEECIND/CP1733/CT0001, DOI: 10.54499/2022.00184.CEECIND/CP1733/CT0001).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2024.11.012.

Data availability

The authors are unable or have chosen not to specify which data has been used.

References

Bullingham, R.E.S., Nicholls, A.J., Kamm, B.R., 1998. Clinical pharmacokinetics of mycophenolate mofetil. Clin. Pharmacokinet. 34 (6), 429–455.

B'Hymer, C., Cheever, K.L., 2010. Evaluation of a procedure for the simultaneous quantification of 4-ketocyclophosphamide, cyclophosphamide, and ifosfamide in human urine. J. Chromatogr. Sci. 48 (5), 328–333. https://doi.org/10.1093/chromsci/48.5.328.

Canetta, R., Florentine, S., Hunter, H., Lenaz, L., 1983. Megestrol acetate. Cancer Treat. Rev. 10 (3), 141–157. https://doi.org/10.1016/0305-7372(83)90029-4.

Chauchat, L., Tanguay, C., Therrien, R., Dufour, A., Gagné, S., Caron, N.J., Bussières, J.F., 2019. Biological monitoring of 4 antineoplastic drugs in health care workers from 2 adult hospitals: a pilot study. Can. J. Hosp. Pharm. 72 (1), 56–59. https://doi.org/10.4212/cjhp.v72i1.2870.

Crul, M., Simons-Sanders, K., 2018. Carry-over of antineoplastic drug contamination in Dutch hospital pharmacies. J. Oncol. Pharm. Pract. 24 (7), 483–489. https://doi.org/10.1177/1078155217704990.

Delafoy, C., Roussy, C., Hudon, A.F., Cirtiu, C.M., Caron, N., Bussières, J.F., Tanguay, C., 2023. Canadian monitoring program of the surface contamination with 11

antineoplastic drugs in 122 centers. J. Oncol. Pharm. Pract. 29 (2), 338–347. https://doi.org/10.1177/10781552211072877.
Drugs.com, 2024. Etoposide Prescribing Information. Available from: ⟨https://www.drugs.com/pro/etoposide.html⟩ (Accessed 10 October 2024).
Ellison, S.L.R., Williams, A., 2012. EURACHEM/CITAC Guide – Quantifying Uncertainty in Analytical Measurement, third ed. London, UK.
European Commission, 2023. Guidance for the safe management of hazardous medicinal products at work. Available from: ⟨https://osha.europa.eu/sites/default/files/KE0322175ENN_0.pdf⟩ (Accessed 17 May 2023).
Fabrizi, G., Fioretti, M., Rocca, L.M., 2016. Dispersive solid-phase extraction procedure coupled to UPLC-ESI–MS/MS analysis for the simultaneous determination of thirteen cytotoxic drugs in human urine. Biomed. Chromatogr. 30 (8), 1297–1308. https://doi.org/10.1002/bmc.3684.
FDA, 2011. Approval Label: Prescribing Information – TAXOL® (paclitaxel). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020262s049lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2018a. Approval Label: Prescribing Information – IFEX (ifosfamide). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/019763s020lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2018b. Approval Label: Prescribing Information – MEGACE®ES (megestrol acetate). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/021778s024lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2019. Approval Label: Prescribing Information – ETOPOPHOS® (etoposide phosphate). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/020457s019lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2022. Approval Label: Prescribing Information – CELLCEPT® (mycophenolate mofetil). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/050722s049s051,050723s049s051,050758s047s049lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2023. Approval Label: Prescribing Information – PACLITAXEL protein-bound particles for injectable suspension (albumin-bound). Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/216338s000lbl.pdf⟩ (Accessed 10 October 2024).
FDA, 2024. Approval Label: Prescribing Information – CYCLOPHOSPHAMIDE for Injection. Available from: ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/012142Orig1s116lbl.pdf⟩ (Accessed 10 October 2024).
González-Román, M.M., García, P.P.H., Otero, D.P., 2021. Cytostatic drugs and risk of genotoxicity in health workers. A literature review. Enferm. Clin. (Engl. Ed.) 31 (4), 247–253. https://doi.org/10.1016/j.enfcle.2019.07.004.
Gouveia, T.I., Silva, A.M., Ribeiro, A.R., Alves, A., Santos, M.S., 2020. Liquid-liquid extraction as a simple tool to quickly quantify fourteen cytostatics in urban wastewaters and access their impact in aquatic biota. Sci. Total Environ. 740, 139995. https://doi.org/10.1016/j.scitotenv.2020.139995.
Hilliquin, D., Bussières, J.F., 2020. External contamination of antineoplastic drug containers from a Canadian wholesaler. J. Oncol. Pharm. Pract. 26 (2), 423–427. https://doi.org/10.1177/1078155219868525.
Hon, C.Y., Teschke, K., Shen, H., Demers, P.A., Venners, S., 2015. Antineoplastic drug contamination in the urine of Canadian healthcare workers. Int. Arch. Occup. Environ. Health 88 (7), 933–941. https://doi.org/10.1007/s00420-015-1026-1.
IARC, 1981. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans: Volume 26 – Some Antineoplastic and Immunosuppressive Agents. ISBN 978-92-832-1226-3. Available from: ⟨https://publications.iarc.fr/44⟩ (Accessed 10 October 2024).
IARC, 1996. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans: Volume 66 – Some Pharmaceutical Drugs. ISBN 978-92-832-1266-9. Available from: ⟨https://publications.iarc.fr/84⟩ (Accessed 10 October 2024).
IARC, 2000. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 76 – Some Antiviral and Antineoplastic Drugs, and Other Pharmaceutical Agents. ISBN 978-92-832-1276-8. Available from: ⟨https://publications.iarc.fr/94⟩ (Accessed 10 October 2024).
IARC, 2020. Press Release Nº 292. Available from: ⟨https://www.iarc.who.int/wp-content/uploads/2020/12/pr292_E.pdf⟩ (Accessed 4 December 2023).
ISOPP Standards Committee, 2022. ISOPP standards for the safe handling of cytotoxics. J. Oncol. Pharm. Pract., vol. 28(no. 3(Suppl.)), pp. 1–126. ⟨https://doi.org/10.1177/10781552211070933⟩.
Izzo, V., Charlier, B., Bloise, E., Pingeon, M., Romano, M., Finelli, A., Vietri, A., Conti, V., Manzo, V., Alfieri, M., Filippelli, A., Dal Piaz, F., 2018. A UHPLC–MS/MS-based method for the simultaneous monitoring of eight antiblastic drugs in plasma and urine of exposed healthcare workers. J. Pharm. Biomed. Anal. 154, 245–251. https://doi.org/10.1016/j.jpba.2018.03.024.
Kerbusch, T., van Putten, J.W., Groen, H.J., Huitema, A.D., Mathôt, R.A., Beijnen, J.H., 2001. Population pharmacokinetics of ifosfamide and its 2- and 3-dechloroethylated and 4-hydroxylated metabolites in resistant small-cell lung cancer patients. Cancer Chemother. Pharmacol. 48, 53–61. https://doi.org/10.1007/s002800100277.
Kibby, T., 2017. A review of surface wipe sampling compared to biologic monitoring for occupational exposure to antineoplastic drugs. J. Occup. Environ. Hyg. 14 (3), 159–174. https://doi.org/10.1080/15459624.2016.1237026.
Kiffmeyer, T.K., Tuerk, J., Hahn, M., Stuetzer, H., Hadtstein, C., Heinemann, A., Eickmann, U., 2013. Application and assessment of a regular environmental monitoring of the antineoplastic drug contamination level in pharmacies—the MEWIP project. Ann. Occup. Hyg. 57 (4), 444–455. https://doi.org/10.1093/annhyg/mes081.
Kisanga, E.R., Mellgren, G., Lien, E.A., 2005. Excretion of hydroxylated metabolites of tamoxifen in human bile and urine. Anticancer Res. 25 (6C), 4487–4492.
Korczowska, E., Crul, M., Tuerk, J., Meier, K., 2020. Environmental contamination with cytotoxic drugs in 15 hospitals from 11 European countries – results of the MASHA project. Eur. J. Oncol. Pharm. 3 (2), e24. https://doi.org/10.1097/OP9.0000000000000024.
Lancharro, P.M., Iglesias, N.C.A., González-Barcala, F.J., González, J.D.M., 2016. Evidence of exposure to cytostatic drugs in healthcare staff: a review of recent literature. Farm. Hosp. 40 (6), 604–621. https://doi.org/10.7399/fh.2016.40.6.9103.
Lema-Atán, J.Á., Lendoiro, E., Paniagua-González, L., Cruz, A., López-Rivadulla, M., de-Castro-Ríos, A., 2022. LC–MS-MS determination of cytostatic drugs on surfaces and in urine to assess occupational exposure. J. Anal. Toxicol. 46 (9), e248–e255. https://doi.org/10.1093/jat/bkac073.
Leso, V., Sottani, C., Santocono, C., Russo, F., Grignani, E., Iavicoli, I., 2022. Exposure to antineoplastic drugs in occupational settings: a systematic review of biological monitoring data. Int. J. Environ. Res. Public Health 19 (6), 3737. https://doi.org/10.3390/ijerph19063737.
Lindsley, I., Musu, T., 2022. The ETUI's list of hazardous medicinal products (HMPs) including cytotoxics and based on the EU CLP classification system of Carcinogenic, Mutagenic and Reprotoxic (CMR) substances. Available from: ⟨https://www.etui.org/sites/default/files/2022-10/The%20ETUI%27s%20list%20of%20hazardous%20medicinal%20products%20%28HMPs%29_2022.pdf⟩ (Accessed 4 December 2023).
Mathias, P.I., Connor, T.H., B'Hymer, C., 2017. A review of high performance liquid chromatographic-mass spectrometric urinary methods for anticancer drug exposure of health care workers. J. Chromatogr. B 1060, 316–324. https://doi.org/10.1016/j.jchromb.2017.06.028.
Mathias, P.I., MacKenzie, B.A., Toennis, C.A., Connor, T.H., 2019. Survey of guidelines and current practices for safe handling of antineoplastic and other hazardous drugs used in 24 countries. J. Oncol. Pharm. Pract. 25 (1), 148–162. https://doi.org/10.1177/1078155217726160.
Nussbaumer, S., Bonnabry, P., Veuthey, J.L., Fleury-Souverain, S., 2011. Analysis of anticancer drugs: a review. Talanta 85 (5), 2265–2289. https://doi.org/10.1016/j.talanta.2011.08.034.
Palamini, M., Dufour, A., Therrien, R., Delisle, J.F., Mercier, G., Gagné, S., Caron, N., Bussières, J.F., 2020. Quantification of healthcare workers' exposure to cyclophosphamide, ifosfamide, methotrexate, and 5-fluorouracil by 24-h urine assay: a descriptive pilot study. J. Oncol. Pharm. Pract. 26 (8), 1864–1870. https://doi.org/10.1177/1078155220907129.
Petit, M., Curti, C., Roche, M., Montana, M., Bornet, C., Vanelle, P., 2017. Environmental monitoring by surface sampling for cytotoxics: a review. Environ. Monit. Assess. 189 (2), 52. https://doi.org/10.1007/s10661-016-5762-9.
Portilha-Cunha, M.F., Ramos, S., Silva, A.M.T., Norton, P., Alves, A., Santos, M.S.F., 2021. An improved LC–MS/MS method for the analysis of thirteen cytostatics in workplace surfaces. Pharmaceuticals 14 (8), 754. https://doi.org/10.3390/ph14080754.
Portilha-Cunha, M.F., Norton, P., Alves, A., Ribeiro, A.R.L., Silva, A.M.T., Santos, M.S.F., 2025. Antineoplastic drugs in healthcare settings: occupational exposure and risk graduation. Emerg. Contam. 11 (1), 100418. https://doi.org/10.1016/j.emcon.2024.100418.
Poupeau, C., Tanguay, C., Plante, C., Gagné, S., Caron, N., Bussières, J.F., 2017. Pilot study of biological monitoring of four antineoplastic drugs among Canadian healthcare workers. J. Oncol. Pharm. Pract. 23 (5), 323–332. https://doi.org/10.1177/1078155216643860.
Sabourian, R., Mirjalili, S.Z., Namini, N., Chavoshy, F., Hajimahmoodi, M., Safavi, M., 2020. HPLC methods for quantifying anticancer drugs in human samples: a systematic review. Anal. Biochem. 610, 113891. https://doi.org/10.1016/j.ab.2020.113891.
Santos, M.S.F., Franquet-Griell, H., Lacorte, S., Madeira, L.M., Alves, A., 2017. Anticancer drugs in Portuguese surface waters – estimation of concentrations and identification of potentially priority drugs. Chemosphere 184, 1250–1260. https://doi.org/10.1016/j.chemosphere.2017.06.102.
Sessink, P.J.M., 2011. Environmental contamination with cytostatic drugs: past, present and future. Available from: ⟨https://www.semanticscholar.org/paper/Environmental-contamination-with-cytostatic-drugs%3A-Sessink/86524d606dbd8e116ca2c26b1ec70d3f81cfe272#paper-header⟩ (Accessed 4 December 2023).
Sessink, P.J.M., Sewell, G., Vandenbroucke, J., 2016. Preventing occupational exposure to cytotoxic and other hazardous drugs – European policy recommendations. Available from: ⟨https://www.europeanbiosafetynetwork.eu/wp-content/uploads/2016/05/Exposure-to-Cytotoxic-Drugs_Recommendation_DINA4_10-03-16.pdf⟩ (Accessed 4 December 2023).
e Silva, L.S., Machado, C.S.B., Linden, R., Antunes, M.V., da Silva, L.C., Wayhs, C.A.Y., Capp, E., Ness, S.L.R., 2023. Residual contamination in antineoplastic drug packaging. J. Oncol. Pharm. Pract. 29 (8), 1862–1867. https://doi.org/10.1177/10781552231151693.
Simon, N., Odou, P., Decaudin, B., Bonnabry, P., Fleury-Souverain, S., 2019. Efficiency of degradation or desorption methods in antineoplastic drug decontamination: a critical review. J. Oncol. Pharm. Pract. 25 (4), 929–946. https://doi.org/10.1177/1078155219831427.
Sottani, C., Porro, B., Comelli, M., Imbriani, M., Minoia, C., 2010. An analysis to study trends in occupational exposure to antineoplastic drugs among health care workers. J. Chromatogr. B 878 (27), 2593–2605. https://doi.org/10.1016/j.jchromb.2010.04.030.
Stokvis, E., Rosing, H., Beijnen, J.H., 2005. Liquid chromatography-mass spectrometry for the quantitative bioanalysis of anticancer drugs. Mass Spectr. Rev. 24 (6), 887–917. https://doi.org/10.1002/mas.20046.

Suspiro, A., Prista, J., 2011. Biomarkers of occupational exposure do anticancer agents: a minireview. Toxicol. Lett. 207 (1), 42–52. https://doi.org/10.1016/j.toxlet.2011.08.022.

Turci, R., Sottani, C., Spagnoli, G., Minoia, C., 2003. Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. J. Chromatogr. B 789 (2), 169–209. https://doi.org/10.1016/S1570-0232(03)00100-4.

Villa, A., Tremolet, K., Martinez, B., Cacao, O.D.S., Atgé, B., Ducint, D., Titier-Debeaupuis, K., Verdun-Esquer, C., Molimard, M., Canal-Raffin, M., 2020. A highly sensitive UHPLC-MS/MS method for urine biological monitoring of occupational exposure to anthracycline antineoplastic drugs and routine application. J. Chromatogr. B 1156, 122305. https://doi.org/10.1016/j.jchromb.2020.122305.

Villa, A., Molimard, M., Sakr, D., Lassalle, R., Bignon, E., Martinez, B., Rouyer, M., Mathoulin-Pelissier, S., Baldi, I., Verdun-Esquer, C., Canal-Raffin, M., 2021. Nurses' internal contamination by antineoplastic drugs in hospital centers: a cross-sectional descriptive study. Int. Arch. Occup. Environ. Health 94 (8), 1839–1850. https://doi.org/10.1007/s00420-021-01706-x.

de Vos, D., Slee, P.H.T.J., Briggs, R.J., Stevenson, D., 1998. Serum and urine levels of tamoxifen and its metabolites in patients with advanced breast cancer after a loading dose and at steady-state levels. Cancer Chemother. Pharmacol. 42, 512–514. https://doi.org/10.1007/s002800050854.