Biological Monitoring of Nurses Exposed to Doxorubicin and Epirubicin by a Validated Liquid Chromatography/Fluorescence Detection Method

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Objectives: Occupational exposure to antineoplastic drugs can represent a potential health risk for hospital staff. Assessing exposure is the first step in providing a safe work environment; the present study aimed to perform a biological monitoring (BM) of nurses exposed to doxorubicin and epirubicin. In order to assure data accuracy and reproducibility, the high-performance liquid chromatography with fluorescence detection method was validated.

Methods: Validation experiments were carried out according to the Food and Drug Administration guidelines. A detailed questionnaire about workplace practices and work organization was administered to 56 nurses of oncology department of two hospitals (A and B) located in southern Italy. End-shift urine samples were collected. Amounts of drugs handled were registered.

Results: The quantification and detection limits were 1.1 and 0.6 pg μl⁻¹ (doxorubicin) and 2.0 and 1.2 pg μl⁻¹ (epirubicin); moreover, the analytical method fulfilled all guidelines requirements. Questionnaire information evidenced that vertical laminar flow hoods were present in both hospitals, surfaces were cleaned with inappropriate detergents, no antispilling devices were adopted, and gloves were not changed during the work shift. A lower percentage of positive samples was found in the hospital where higher amounts of anthracyclines were handled (3.4% in A and 14.8% in B), suggesting individual incorrect working/cleaning practices in hospital A and overall hygienic standards to be improved in hospital B, where ‘critical practices’ were carried out.

Conclusions: Results showed the crucial role of adopting effective safety precautions and handling practices to reduce exposure. Environmental and BM should be performed to discriminate between incorrect personal working modalities and general hygienic standards.

Keywords: doxorubicin; epirubicin; HPLC/FL method validation; nurses biological monitoring

INTRODUCTION

Isolated from strains of Streptomyces, anthracycline glycoside antibiotics represent the largest class of quinoids with antitumoral properties (Arcamone, 1984). Among >200 naturally occurring anthracyclines, daunorubicin, doxorubicin (Doxo), and epirubicin (Epi) are those that are clinically most used for the treatment of a broad spectrum of malignancies (Mugitani et al., 1999; Biganzoli et al., 2003; Friedberg et al., 2003). With respect to daunorubicin, Doxo displayed a wider activity range and is used for the treatment of breast, lung, ovary, head, and bladder tumors (Painter, 1978; Kosugi et al., 2005). Similar to many other cytostatic drugs, side effects are reported for Doxo; the most severe side effect registered for Doxo is a dose-related cardio-toxicity (Hortobagyi, 1997). Epi, a semisynthetic
epimer of Doxo, presents similar response rates but lower cardiotoxicity and is used for the treatment of non-small- and small-cell lung cancer, non-Hodgkin’s lymphoma, and ovarian and gastric cancer (Errante et al., 1999; Zagotto et al., 2001; Friedberg et al., 2003). About 5–10% of the absorbed drugs are excreted unchanged in urine during 24- to 48-h subsequent exposure (Cassinelli et al., 1984; Chin et al., 2002). Due to toxic side effects, the IARC (1987) classifies Doxo in Group 2A, ‘probably carcinogenic to human’. While the appearance of toxic effects is considered ‘acceptable’ in patients considering the therapeutic benefits, the occurrence of primary tumors in healthy subjects, such as the healthcare service team, resulting from handling antiblastic drugs is not acceptable (Fuchs et al., 1995). Exposure risks can be reduced when effective protective measures and correct working/cleaning practices are adopted. Despite this, possible cytostatic absorption cannot be completely eliminated since accidental events (such as penetration by gloves due to their prolonged use and/or their permeability toward cytostatic drugs, contamination of hands due to incorrect gloves wearing, external contamination of flacon surfaces, as well as accidental spilling of drugs during their preparation and/or administration) may be sources of inhalation or skin absorption by personnel. Hence, it is advisable to perform a biological monitoring (BM) of hospital staff in order to measure their exposure levels to cytostatics (Turci et al., 2002; Barbieri et al., 2005; Rekhadevi et al., 2007).

Analytical techniques to quantify the urinary anthracycline levels are reported in literature; various techniques are based on different extraction procedures—in terms of stationary phases of the solid-phase cartridges and/or elution mixture—and detection systems—UV and fluorescence (FL) or mass spectrometry (Mikan et al., 1990; Zagotto et al., 2001; Sottani et al., 2004). Focusing on Doxo and Epi, in the first part of the present study, the overall analytical procedure (from purification to detection and quantification) was optimized and validated according to the Food and Drug Administration (FDA, 2001) guidelines. Validation of an analytical method is indispensable when no official procedure (i.e. methods validated by international agencies) is available as anthracyclines determination is. Several guidelines illustrating validation parameters to be considered, as well as the set of experiments to be performed, are proposed by international agencies. Among these, FDA guidelines are one of the most used in the bioanalytical field and were here used to validate the proposed procedure. Considering the chemical properties of Doxo and Epi, purification from the biological matrix was performed by means of solid-phase extraction (SPE) using Strata-X cartridges. The modified styrene–divinylbenzene polymeric surface of Strata-X cartridges improves selectivity of polar and aromatic compounds with respect to nonpolar stationary phases (such as C18). Since anthracyclines exhibit natural FL, an FL detector was used, enabling sensitivity at sub-ppb levels. The FL detector also offers reduced costs compared to detection by mass spectrometry and greater specificity with respect to UV detection.

In the second part of the study, the validated method was applied to the BM of 56 nurses of two oncology departments of hospitals located in southern Italy. A detailed questionnaire was administered to the personnel, and end-shift urine samples were collected and analyzed.

**MATERIALS AND METHODS**

**Chemicals and apparatus**

Doxo (Adriblastin®, 10 mg 5 ml⁻¹), Epi (Farmorubicin®, 50 mg 25 ml⁻¹), and idarubicin (Ida; Zavedos®, 10 mg, used as internal standard) were purchased from Pharmacia Italia S.p.A. (Milan, Italy). High-performance liquid chromatography (HPLC) grade solvents were obtained from Carlo Erba (Milan, Italy). SPE was carried out with Strata-X cartridges polymeric reversed phase (500 mg 6 ml⁻¹ and 60 mg 3 ml⁻¹, 33 µm) from Phenomenex (St Torrance, CA, USA).

Analyte separation was performed using a BDS HyperClone C8 (150 × 4.60 mm, 5 µm, and 300 Å) column (Phenomenex) and a modular 1100 series HPLC, equipped with a 1100 series autosampler and degasser and with a 1200 series fluorescence detector, all from Agilent (Palo Alto, CA, USA). Data were acquired and processed using the ChemStation program (Rev A 09.03 from Agilent).

**Solutions and validation samples**

Standard solutions of Doxo, Epi, and Ida at a concentration of 2 mg ml⁻¹ were obtained by dissolving the entire drug flacons with bidistilled water. Aqueous working solutions with concentrations 6.5 ng µl⁻¹ (Doxo and Epi) and 1.7 ng µl⁻¹ (Ida) were obtained by progressive dilution of standard solutions. Working solutions of Doxo and Epi were further diluted 1:2 with 0.05 M HCOOH and spiked with the Ida working solution in order to obtain six standard calibrators at concentrations 1635.0, 817.5, 408.7, 204.3, 102.2, and 51.1 pg µl⁻¹ for Doxo and Epi (and 836.0 pg µl⁻¹ for Ida). Aqueous quality control
samples with concentrations of 1635.0, 204.3, and 51.1 pg µl⁻¹ for Doxo and Epi (and 836.0 pg µl⁻¹ for Ida) were prepared following a similar dilution scheme, starting from different drug flacons.

Urinary calibrators were prepared using urine samples from nonexposed volunteers. Urine pH was adjusted to 3.0 with HCOOH. A 6 ml aliquot was centrifuged (at 4000 r.p.m. for 10 min) and spiked with a 2.0 ng ml⁻¹ solution of Doxo and Epi, obtaining a 65.4 pg µl⁻¹ solution, which was progressively diluted 1:2 with the same urine samples in order to obtain the other calibrators (at concentrations 32.7, 16.4, 8.2, 4.1, and 2.0 pg µl⁻¹); all urinary calibrators presented an Ida concentration of 33.0 pg µl⁻¹.

Three urinary quality control samples at concentrations 50.6, 12.6, and 3.2 pg µl⁻¹ for Doxo and Epi (and 33.0 pg µl⁻¹ for Ida) were prepared following the same procedure schematized for the preparation of urinary calibrators but using an independent batch of urine sample from nonexposed volunteers. Each quality control sample was prepared in quadruplicate using an independent urinary batch (three concentration levels each prepared in quadruplicate = four independent set of 3 quality control samples = 12 samples). All samples were maintained at −20°C until the SPE purification step.

**Analytical procedures and HPLC/FL analysis**

Urinary samples were purified by SPE with Strata-X cartridges, preconditioned with 3 ml methanol and 2 ml bidistilled water. The system pressure was constant and equal to 5 mmHg. After loading the urine samples, the cartridges were washed with 1.5 ml of 0.05 M HCOOH and dried for 10 min before eluting analytes twice with 3 ml of a mixture of isopropanol and dichloromethane mixed in equal parts by volume (50/50, v/v). The organic phase was dried under a nitrogen stream and samples were stored at −20°C ± 2°C un- til the HPLC/FL analysis. The chromatographic separation was performed with the following solvents, elution gradient, and detection parameters: 0.05 M HCOOH, solvent A and acetonitrile, solvent B; elution gradient, 30%B → 30%B → 100%B → 100%B → 30%B → 30%B → 0.5 min 3 min 6 min; excitation wavelength, 460 nm; and emission wavelength, 580 nm.

**Method validation**

Long-term stability studies were carried out both for analytes and for the internal standard on aqueous quality control samples (at concentrations of 1635.0, 204.3, and 51.1 pg µl⁻¹ for Doxo and Epi and 836.0 pg µl⁻¹ for Ida). All samples were analyzed in triplicate. For each analyte, the concentration was determined as the mean value of chromatographic peak area. Samples were analyzed immediately after preparation, after 1 or 3 months of storage, at 4 °C or -20°C. In order to exclude possible variations in drug concentration due to freeze-thaw cycles, samples were divided into two aliquots, both analyzed immediately after collection and after storage for one of the two periods and after a single freeze-thaw cycle.

In order to evaluate the stability of the analytes during the extraction procedure, two batches of urinary solutions (at concentrations of 1635.0, 204.3, and 51.1 pg µl⁻¹ for Doxo and Epi and 836.0 pg µl⁻¹ for Ida, each in triplicate) were prepared. The first batch was analyzed immediately after preparation (t₀), while the second batch was analyzed after 6 h of storage at room temperature (t₆h). Short-term stability was calculated as the percentage ratio between the analyte areas registered at t₆h and t₀, as Aₖ₆h/Aₖ₀ × 100.

Linearity was tested by preparing six nonzero urinary calibration standards, which were analyzed in quadruplicate in three separate analytical runs. Linear calibration curves were determined and calibration concentrations were back-calculated. Deviations between calculated and nominal concentrations have to be within ±15% except at the lower limit of quantification (LLOQ), where ±20% deviations can be accepted.

Specificity and selectivity of the method were tested by analyzing six urinary blank samples in order to verify the absence of interfering compounds. Areas of peaks coeluting with the analytes have not to exceed 20% of the area at the LLOQ level.

Recovery from the urinary matrix was calculated as the percentage ratio between the angular coefficient of the urinary (α₀) and standard (αₘ) calibration curves, Rec% = α₀/αₘ × 100. Experiments were repeated in triplicate, and the mean recovery values with the relative standard deviation (Recₐ% ± SD) were calculated both for Doxo and for Epi.

The limit of detection (LOD) and the LLOQ were determined by analyzing six replicates of the blank urinary samples spiked with the internal standard. LOD and LLOQ were, respectively, defined as 3 and 10 times the standard deviation of the HPLC/FL peak areas detected at the retention time (RT) of the analyte of interest.

Inaccuracy (that represents the accuracy’s complement to 100%) was determined by analyzing the previously described four independent set of urinary quality control samples and was reported as the percentage difference between the mean concentration
and the nominal one divided by the nominal concentration; it has to be within $\pm 15\%$ except at the LLOQ level, where they have to be within $\pm 20\%$.

Precision was determined as the ratio between standard deviation and variation coefficient obtained from multiple sampling of the same homogenous sample (intraday) or from independently prepared samples (interday). Each sample was prepared in triplicate and four concentrations were analyzed: three representing the entire range of the calibration curve and one more sample at LLOQ level. Interday precision was evaluated by analyzing samples in three nonconsecutive days. According to the FDA guidelines, both intra- and interday precision have to be $<15\%$ except at the LLOQ, where values $<20\%$ can be accepted.

**BM of occupationally exposed nurses**

The validated analytical procedure was applied to the BM of nurses employed in the oncology wards of two hospitals (referred to as hospitals A and B) located in southern Italy. Each of the 56 nurses involved in antiblastic drug preparation (29 for A and 27 for B) was asked to participate in the study, and there were no refusals. Before starting with the urine sample collection, all nurses were properly informed about the aims of the study, and an informed consent form was obtained from all nurses prior to the beginning of the study. A detailed questionnaire was administered to each subject in order to investigate the number and frequency of drug preparations for each nurse; the amounts of anthracyclines used; training courses attended; working organization and practices, with particular attention to eventual practices with critical consequences on hygienic standards (such as smoking, eating, and preparing coffee in the ward during the shift); adopted preventive measures; and cleaning procedures. Moreover, type and amounts of each drugs (not only antineoplastic) handled by each nurse were registered by copying the relative "preparation register" page. All samples were collected at the end of the shift when nurses prepared/administered antineoplastic and processed respecting privacy. Urine pH was adjusted to 3.0 with 0.05 M HCOOH; 3 ml aliquots were centrifuged (at 4000 r.p.m. for 10 min), spiked with Ida working solution, and stored at $-20^\circ$C until SPE.

**RESULTS**

**Optimization of the analytical procedure**

Both excitation and emission wavelengths were optimized to improve signal intensities. Anthracyclines are natural fluorescent compounds, which show FL at 560 and 580 nm after excitation at 460 or 480 nm (Merk-Index, 1976). For such purpose, a standard solution of the three anthracyclines (450 ng $\mu l^{-1}$ for Doxo and Epi and 600 ng $\mu l^{-1}$ for Ida) was analyzed in HPLC/FL varying excitation or emission wavelengths. Excitation at 460 nm and emission at 580 nm ensured the most intense responses (in terms of peak area) and were chosen for the subsequent HPLC/FL analyses. RTs of 5.0, 5.7, and 8.3 min were registered for Doxo, Epi, and Ida, respectively.

Three different extraction procedures were tested to maximize analyte recovery from the urinary matrix. In all cases, a urinary solution with concentrations 1635 pg $\mu l^{-1}$ for Doxo and Epi and 836 pg $\mu l^{-1}$ for Ida was purified using Strata-X 500 mg 6 ml$^{-1}$ cartridges. Details of the procedures are reported in Table 1. For all three procedures, tested cartridges were conditioned with methanol and water before loading sample. For the first and the third procedures, the urinary sample was directly loaded into the cartridge; on the contrary, for the second procedure, the urinary sample was diluted with 2 ml of phosphate-buffered saline (PBS) buffer (9.6 mM KH$_2$PO$_4$ and 30.3 mM Na$_2$HPO$_4$) and then loaded into the cartridge. The third procedure allowed the higher extraction recovery for both Doxo and EPI (see the following Method validation paragraph). Considering that (i) 3 ml sample was purified by the optimized extraction procedure and (ii) literature data on BM of Italian pharmacy technicians report urinary anthracycline levels in the range 0.8–1.2 pg $\mu l^{-1}$ (Sottani et al., 2008), the Strata-X 500 mg 6 ml$^{-1}$ cartridges were substituted with Strata-X 60 mg 3 ml$^{-1}$, which allowed a higher recovery percentage (see below).

**Method validation**

Long-term stability of aqueous solution was studied using quality control samples at three different concentrations. Samples were prepared in triplicate. Each sample was analyzed immediately and after storage at 4$^\circ$C or $-20^\circ$C for 1 or 3 months, so that a single freeze-thaw cycle was considered. The HPLC/FL chromatograms for both the samples stored at 4$^\circ$C and those stored at $-20^\circ$C did not show any interfering peaks attributable to degradation products. Also, the analyte areas remained constant within experimental error for both samples, thus confirming the high stability of the three molecules under the tested storage conditions.

Short-term stability of the three anthracyclines in the urinary matrix was tested by processing and analyzing two batches of samples at concentrations...
1635.0, 204.3, and 51.1 pg µl⁻¹ for Doxo and Epi and 836.0 pg µl⁻¹ for Ida, each prepared in triplicate. The first batch was processed immediately after preparation (t₀), and the second one was treated after 6 h of storage at room temperature (t₆h). The ratio between peak areas at t₀ and t₆h was used as an index of short-term stability. Results, presented in Table 2, indicated that both Doxo and Epi are stable in the urinary matrix at room temperature for at least 6 h (the time required for the SPE).

Urinary calibration standards in the range 2.0–65.4 pg µl⁻¹ for Doxo and Epi and 33.0 pg µl⁻¹ for Ida were purified by SPE and analyzed by HPLC/FL. The linear fit appeared described the data well, with correlation coefficients in the ranges 0.9942–0.9992 and 0.9972–0.9984 for Doxo and Epi, respectively. The urinary calibrators’ concentrations were back-calculated, obtaining deviations from the nominal concentration (inaccuracy) in the ranges −9.5 to 12.2% for Doxo and −0.9 to 12.2% for Epi and %CV values in the ranges 1.4–12.4% (Doxo) and 1.6–12.5% (Epi).

The extraction procedure concentrated the samples by a factor of 25 (3 ml urine samples were purified and reconstituted in 120 µl of 0.05 M HCOOH). Thus, standard calibration curves at higher concentrations (in the range 51.1–1635.0 pg µl⁻¹ for Doxo and Epi) were prepared, and the ratio between angular coefficients of urinary (a_u) and standard (a_s) calibration curves was used to evaluate the extraction recovery from the biological matrix. The purification of urinary samples was repeated both with Strata-X 500 mg 6 ml⁻¹ and with Strata-X 60 mg 3 ml⁻¹ cartridges; results are summarized in Table 3. A better purification was achieved using the Strata-X 60 mg 3 ml⁻¹ cartridges, which gave a mean percentage recovery of 91.1 ± 2.2% and 93.1 ± 3.1% for Doxo and Epi, respectively. The HPLC/FL chromatograms of urinary blank samples did not show interfering peaks coeluting with the analytes. Nevertheless, the peak areas (noise) at the RTs of Doxo and Epi were measured and LODs and LLOQs were calculated. LODs of 0.6 and 1.2 pg µl⁻¹ and LLOQs of 1.1 and 2.0 pg µl⁻¹ were obtained for Doxo and Epi.

Quality control samples and urine samples spiked with the analytes at the LLOQ levels were used to determine inaccuracy and intra- and interday precision. These values resulted within the FDA requirements and are summarized in Table 4.

**BM of professionally exposed nurses**

Results of the questionnaire administered to 56 nurses of two hospitals located in southern Italy gave the following information: the number and frequency of drug preparations for each nurse was 10–20 day⁻¹ for hospital A and 10–15 day⁻¹ for hospital B; in both hospitals, each nurse prepared/administered antineoplastics for 1 day a week; urine sample collection was made at the end of a shift when nurse only handled cytostatic; the amount of anthracyclines handled in a week was 150 mg Doxo and 700 mg Epi for hospital A and 50 mg Doxo and
150 mg Epi for hospital B; all nurses stated that they had been working in the investigated oncology wards for at least 5 years; all nurses attended a specific training course during the past 3 years; in both hospitals, drugs were transferred from the preparation room to wards by tray or hand; a vertical laminar flow hood was present in the preparation rooms of both hospitals, and neither a luer lock device nor needle protection was used during the handling of drugs; all nurses stated that they cleaned the workbench before and after each preparation using either water or alcohol; nurses wore cotton coats, polyvinyl chloride gloves, and half masks specific for aerosol protection, without changing them during the work shift. Finally, answers collected from hospital B nurses evidenced practices with critical consequences on hygienic standards (i.e. smoking, eating, preparing coffee in the ward during the shift, etc.).

For each investigated subject, end-shift urine samples were collected, spiked with the internal standard, and processed with the previously validated method. All samples gave negative results regarding the presence of Doxo and Epi except for one sample from hospital A, positive to Epi (60.7 pg μl⁻¹; Fig. 1, panel B) and four samples from hospital B: one of which positive to Doxo (33.9 pg μl⁻¹; Fig. 1, panel C), two positive to Epi (60.7 pg μl⁻¹; Fig. 1, panel B) and four samples from hospital B: one of which positive to Doxo (33.9 pg μl⁻¹; Fig. 1, panel C), two positive to Epi (60.7 pg μl⁻¹; Fig. 1, panel B), and one positive to both analytes (17.0 and 84.1 pg μl⁻¹ for Doxo and Epi, respectively; Fig. 1, panel F). The HPLC/FL chromatogram of a negative sample is reported in Fig. 1, panel A. For all five samples, results the High Performance Liquid Chromatography/ElectroSpray Ionization-Mass Spectrometric (HPLC/ESI-MS) full scan analyses confirmed the nature of the eluting compounds (data not shown), excluding, at the same time, the presence of interfering drugs eluting at the same RTs of Doxo and Epi.

**DISCUSSION**

In the first part of the study, the overall analytical technique has been optimized and then validated. In particular, the HPLC/FL separation and detection parameters were varied to obtain well-resolved and intense peaks and a complete elution of both analytes and internal standard within few minutes. Moreover, the chromatographic method allowed a good separation of Doxo and Epi despite their structural analogies. Then, the extraction procedure, based on SPE using Strata-X cartridges, was checked in order to improve analyte recovery from the urinary matrix. In the first extraction procedure tested, the eluting solution (0.05 M HCOOH/MeOH 50/50, v/v) did not allow the discrimination of analytes from the interfering compounds present in the urinary matrix. In the second test, a procedure reported in literature (Sottani et al., 2004) was applied: before loading samples into cartridges, urine samples were diluted with PBS buffer, pH 7.0. The obtained chromatogram presented interfering peaks eluting at RTs proximal to analytes’ ones: while Sottani et al. (2004) could discriminate between analytes and interfering compounds using their relative mass spectra, the method proposed here bases the identification of analytes only on their RTs, so it is crucial to separate analytes from impurities. In the third

<table>
<thead>
<tr>
<th>Nominal concentration (pg μl⁻¹)</th>
<th>Mean calculated concentration ± SD (pg μl⁻¹)</th>
<th>Inaccuracy, %Diff⁵</th>
<th>Intraday precision, %CVᵇ</th>
<th>Interday precision, %CVᵇ</th>
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<tr>
<td><strong>Doxorubicin</strong></td>
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<tr>
<td>1.1 (LLOQ)</td>
<td>1.2 ± 0.2</td>
<td>9.1</td>
<td>16.7</td>
<td>12.0</td>
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<tr>
<td>3.2</td>
<td>3.0 ± 0.4</td>
<td>−6.2</td>
<td>13.3</td>
<td>8.9</td>
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<tr>
<td>12.6</td>
<td>11.7 ± 0.7</td>
<td>−7.1</td>
<td>6.0</td>
<td>6.3</td>
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<tr>
<td>50.6</td>
<td>49.8 ± 1.5</td>
<td>−1.6</td>
<td>3.0</td>
<td>3.0</td>
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<td><strong>Epirubicin</strong></td>
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<tr>
<td>2.0 (LLOQ)</td>
<td>1.9 ± 0.2</td>
<td>−5.0</td>
<td>10.5</td>
<td>11.0</td>
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<tr>
<td>3.2</td>
<td>3.1 ± 0.4</td>
<td>−3.1</td>
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<tr>
<td>12.6</td>
<td>12.9 ± 1.5</td>
<td>2.4</td>
<td>11.6</td>
<td>11.8</td>
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<tr>
<td>50.6</td>
<td>51.8 ± 2.6</td>
<td>2.4</td>
<td>5.0</td>
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⁵%Diff = [(mean calculated concentration − nominal concentration)/nominal concentration] × 100.

⁶%CV = standard deviation/mean calculated concentration × 100.
procedure tested, the PBS buffer was eliminated and the urine sample was acidified to pH 3 with 0.05 M HCOOH; the washing step was performed with the formic acid solution, resulting in a better purification (and specificity) of the extraction procedure. The extraction was further improved by using Strata-X 60 mg 3 ml cartridges: the minor quantity of bounded stationary phase with respect to the 500 mg 6 ml cartridges resulted in a minor number of interaction sites available for impurities present in the urinary matrix. Moreover, the optimized SPE allowed recovery values higher than those reported in the literature for the purification of the same matrix using C18 cartridges (Di Francesco et al., 2007). LODs and LLOQs were calculated by using blank urinary samples as specified in the FDA guidelines and are in line with those reported in the literature for the same (Loadman and Calabrese, 2001) or other (Sottani et al., 2008) detectors.

In the second part of the study, the analytical method was applied to the BM of 56 nurses of two hospitals, and results were commented considering information about work organization and practices obtained from questionnaires administered to each subject. The measured Doxo and Epi levels are three orders of magnitude higher than those reported for a BM study performed in 14 German hospitals (Pethran et al., 2003). That study collected 24-h urine samples from 100 subjects and found 4.5% positive samples (concentrations of Doxo and Epi in the ranges 5–127 and 10–182 pg ml⁻¹, respectively). Moreover, in a BM performed on Italian pharmacy technicians (Sottani et al., 2008), Epi concentrations of 0.8–1.2 pg µl⁻¹ were reported, so results obtained in the present study are ~60-fold higher. Considering that (i) the number of nurses employed in the two hospitals under investigation is quite similar (29 in A and 27 in B), (ii) the amount of both Doxo and Epi handled weekly is higher in hospital A than in hospital B (150 mg Doxo and 700 mg Epi are handled in A, while only 50 mg Doxo and 150 mg Epi are prepared weekly in B), (iii) higher percentage of positive samples was obtained in hospital B (14.8% in B compared to 3.4% found in A), and (iv) practices with critical consequences on hygienic standards were carried out in hospital B, it is reasonable to suppose that the positive result found in hospital A is attributable to the individual (inappropriate care during drug handling/administering or cleaning procedures). Instead, the higher number of positive samples found in hospital B likely indicates that the overall hygienic standards should be improved. Surface cleaning and personal hygiene procedures, including more frequent/thorough hand washing and glove changing, should likely be more strictly enforced in hospital B.

Fig. 1. HPLC/FL chromatograms of urine samples from exposed personnel. Panel A refers to a negative sample; panels B, D, and E show results of nurses positive to Epi; panel C refers to a subject positive to Doxo; panel F refers to a worker positive to both analytes.
Such considerations are in line with comments reported in literature even when different biomarkers are detected. A BM performed in a hospital in southern India (Rekhadevi et al., 2007), for instance, reported the urinary cyclophosphamide levels in 52 nurses, with 42 positive subjects and concentrations in the range 0.08–0.9 μg ml⁻¹. In order to reduce exposure, the authors concluded that the antineoplastic handling practices should be improved. In addition, sufficient safety devices must be adopted and properly used, and adequate training programs for personnel should be designed for employees before they begin working in oncology wards. Likewise, data on the incidence of DNA damage in nurses handling antineoplastics in four German hospitals (Fuchs et al., 1995) highlighted the crucial role of safety precautions such as laminar flow hoods and the use of adequate gloves and facial masks in reducing the risks related to cytostatic manipulation.

CONCLUSIONS

A liquid chromatography with FL detection method has been optimized and validated for the quantification of doxorubicin and epirubicin in urine samples. The optimization of the SPE procedure together with the use of FL detection enabled the required specificity and sensitivity to detect analytes at picogram levels with reduced analysis times (both anthracyclines elute in 10 min).

On the one hand, data on BM of occupationally exposed nurses showed the possibility to adopt the developed method for routine quantitative determination of the two anthracyclines in urine samples as an alternative to the more expensive mass spectrometry-based procedures. The method potentially allows a broader field of applications, ranging from therapeutic drug monitoring to kinetic studies in cancer patients.

On the other hand, results from BM alone could not certainly discern the causes of positive results since all comments made are based on information collected with questionnaires administered to nurses. Pethran et al. (2003) also pointed out the importance of the environmental data ‘to detect the pathway of contamination and to improve hygiene during work’. When planning monitoring campaigns for risk evaluation and risk management purposes, it is advisable to support the biological data with environmental monitoring procedures that are able to depict the general hygienic conditions. Taken together, data from biological and environmental monitoring could enable discrimination between incorrect personal working modalities and general hygienic standards that need improvement.

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