High Performance Liquid Chromatography determination of some antineoplastic agents for environmental monitoring of health care personnel occupationally exposed to cytostatic drugs.

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1. Introduction

Cytotoxic drugs (CDs) are therapeutic agents intended primarily for the treatment of cancer; these drugs are known to be highly toxic to cells principally through their action on cell reproduction. Many of these antineoplastic drugs (ANDs) have proved to be mutagens, teratogens and carcinogens.

In this work the evaluation of occupational exposure of personnel handling or coming in contact with CDs, has been made following the *Guidelines Document for safety and security of hospital personnel exposed to ANDs* published by I.S.P.E.S.L. The aim of these Guidelines is to give practical advice on how to prevent or minimise occupational exposure to CDs, due to the International Agency for Research on Cancer (IARC) has classified some of the ANDs used in oncologycal therapy as carcinogenic to humans (for example cyclophosphamide in Group 1) and some probably carcinogenic to humans (as cisplatin in Group 2A).

In order to assess exposure of hospital personnel involved in the preparation and administration of antineoplastic drugs, environmental monitoring has been carried out in an oncologic department of an hospital. Samples (wipes and pads) have been collected at the beginning and at the end of working day and properly treated before analysis. Wipe samples, consisted on a sterile gauze wetted with water, is passed on surface and laboratory furniture to assess environmental contamination. Pad samples, consisted on a sterile gauze, are positioned under and over protective clothing of nurses. 5-Fluorouracil (5-FU) and methotrexate (MTX) are simultaneously analized by using High Performance Liquid Chromatography (HPLC) with UV detection by means of a diode

array detector (DAD), whereas cyclophosphamide was determined by HPLC coupled with a mass spectrometer.

The aim of this work is to devolope a simple method of sampling and analysis for environmental monitoring of ANDs in hospital drug preparation areas, in order to assess individual and collective occupational exposure of medical personnel, nurses and pharmacy technicians and to focalize how good laboratory practices are significantly important to minimize risk and therefore occupational exposure. Ispesl guidelines became part in this context, because suggest the recommendations on right operating procedures during ANDs manipulation and on routine monitoring analyses as a tool to assess development of necessary policies and procedures and to provide a better understanding of the legislative requirements.

Little is known about the long term effects of occupational exposure to CDs and related wastes, but there is sufficient evidence to indicate adverse health effects in occupational exposure.

The main routes of exposure are inhalation of drug dusts or droplets, absorption through the skin and ingestion by contact with contaminated food or cigarettes. Several studies have reported the following adverse health effects: contact dermatitis, cytogenic abnormalities and mutagenic activity, alterations to normal blood cells count, liver damage, foetal loss in exposed pregnant women and malformations of the offspring of pregnant women.

Therefore it is necessary that even small quantities of CDs should be avoided as much as possible in order to reduce occupational exposure to levels as low as reasonably achievable (ALARA).

2. Experimental

2.1 Chemicals

All solvents and reagents were of analytical highest grade availible.

Methotrexate (MTX), 5-Fluorouracil (5-FU) and Cyclophosphamide (CP) certified were supplied by Novachimica (Italy). Distilled and deionised water was produced from a Milli-Q Plus water purification system.

2.2 .Equipment

An HPLC system consisted on a model HP 1110 with a quaternary pumps and an autosampler "Well Plate" G1367A with a Diode Array Detector was used for the determination of 5-FU and MTX; an HPLC system consisted on a model PE Series 200 with a binary pump and an autosampler Series 200 interfaced with a mass spectrometer API 150 EX with a single quadupole turbo ion spray was used for the detemination of CP.

2.2.1 Liquid Chromatography

Separations for 5 –FU and MTX were accomplished on a reverse-phase column system composed of a Nucleosil 100-5 C18 analytical column (4.6 x 250 mm) Macherey-Nagel equipped with a Nucleosil 100-5 C18 guard column.

Separations for CP were were accomplished on a reverse-phase column system composed of a Hypersil C18 column (4.6 x 150 mm) Supelco.

2.2.2. Mobile phase

A 0.05 M ammonium acetate solution, buffered at pH 4 and a 0.02 M ammonium acetate solution, buffered at pH 4.5 were filtered through 5 µm filter under water jet vacuum.

Separation of MTX and 5 FU was achieved through a gradient elution during about 20 minutes, by using ammonium acetate solution pH 4 (A), water (B), methanol (C) as mobile phases with a flow set to 1 mL/min and with a double detection lenght at 260 nm for 5-FU an 310 nm for MTX.

Determination of CP was performed by using ammonium acetate solution 0.02 M pH 4.5, and methanol (50:50 v/v) as mobile phases, with a flow of 1 mL/min in isocratic conditions.

2.3 Standard solutions

The stock solutions of antineoplastic agents were prepared by appropriately weighing the certified standards and diluting with deionised water to a final concentration of approx. 10 ppm.

The stock solution containing two analytes (5-FU and MTX) was prepared by combining the individual stock solutions to a final concentration of approx. 1 ppm.

Calibration solutions containing seven linearly decreasing concentrations of the drugs (1, 0.75, 0.5, 0.25, 0.1, 0.05, 0.02 ppm respectively) were freshly prepared and analyzed by HPLC system.

2.4 Sampling, sample preparation and analysis

The collection of contaminants on laboratory furniture and surface was performed wetting a sterile gauze (wipe sample) with an appropriate volume of water (10 ml for large surface, 5 ml for small surface) whereas the collection of samples on nurses (pad sample) was accomplished by positioning a sterile gauze over and under protective equipments during the entire working turn.

All the samples were extracted with a solution of ammonium acetate at pH= 4.5, agitated for 30 minutes and analyzed in liquid chromatography. Turbid solutions, collected from dusty surfaces, were preliminary fitered prior analysis.

3. Results

3.1 5-FU and MTX determination

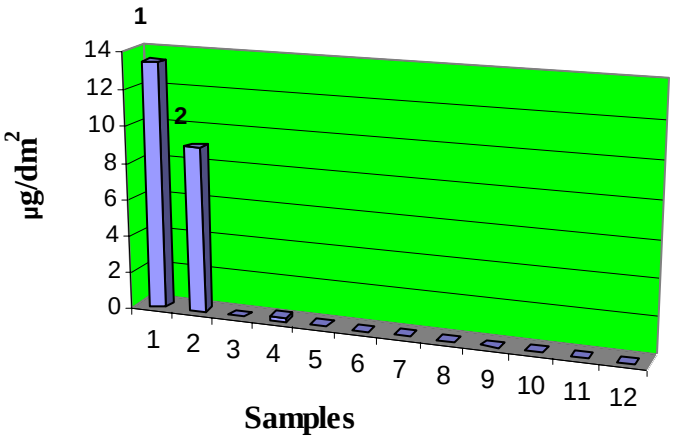
An optimal separation was achieved by using a gradient with 3 mobile phases. Detection at λ 260 nm (5-FU) and 310 nm (MTX) confirms the presence of the analytes at retention times respectively of 5.2 min and 14.4 min for 5-FU and MTX.

The method allows a detection limit of 0.01 ppm (extrapolated signal to noise ratio equals three) a for both the analytes.

Our preliminary monitoring study, on a day hospital oncologic department, shows the trend of a possible environmental contamination, due to ANDs, highligthing which are the critical points of exposure and the right operative procedures to follow during the preparation and the administrationin of these drugs.

Figures 1 – 4 show resuts obtained for the analytes in wipe and pad samples.

Figure 1: 5-FU in wipe samples



1 hood at the beginning

2 hood at the end

Figure 2: 5-FU in pad samples

Pads over clothings
worker a:
1 right forearm.
5 right leg
9 thorax
worker b:
29 tohrax
worker c:
31 right forearm
39 thorax

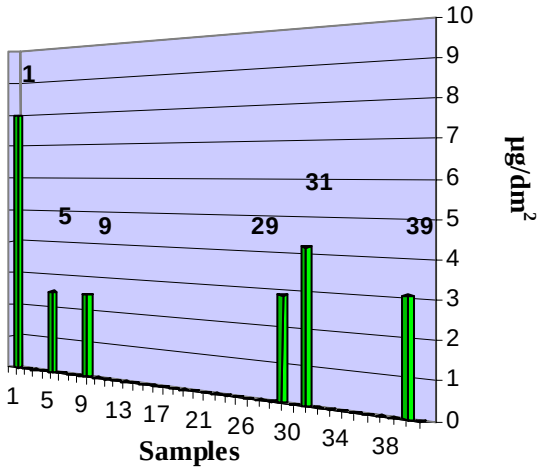


Figure 3: CP in wipe samples

1 piano cappa inizio turno

2 piano cappa fine turno

11 pavimento prep. inizio turno

12 pavimento prep. fine turno

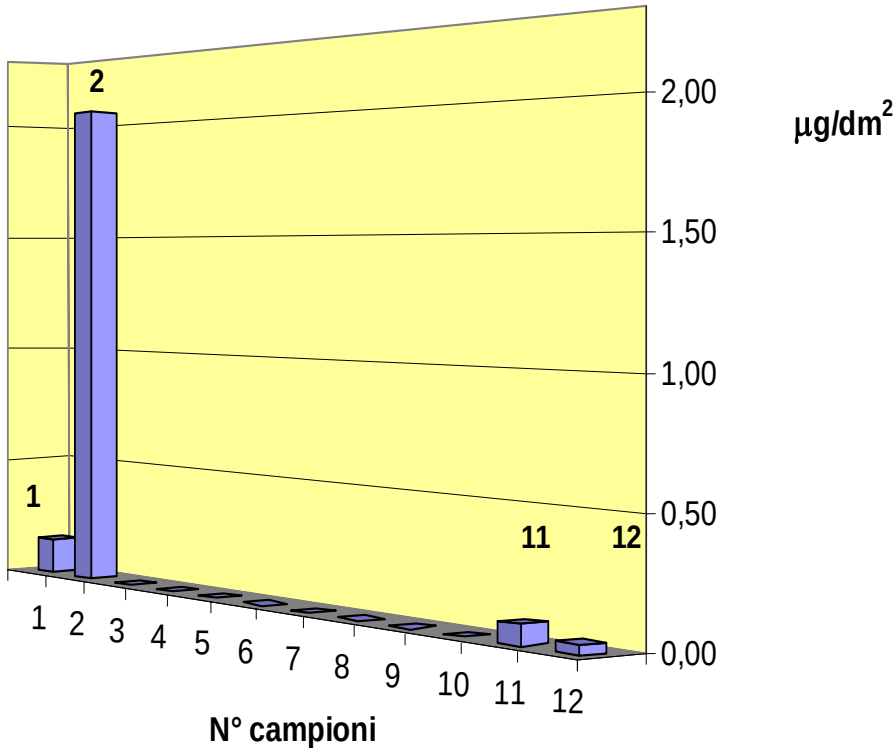


Figure 4: CP in pad samples

Pads over clothings

worker a:

1 right forearm

3 left forearm

5 right leg

7 left leg

9 thorax

worker b:

23 left forearm

29 thorax

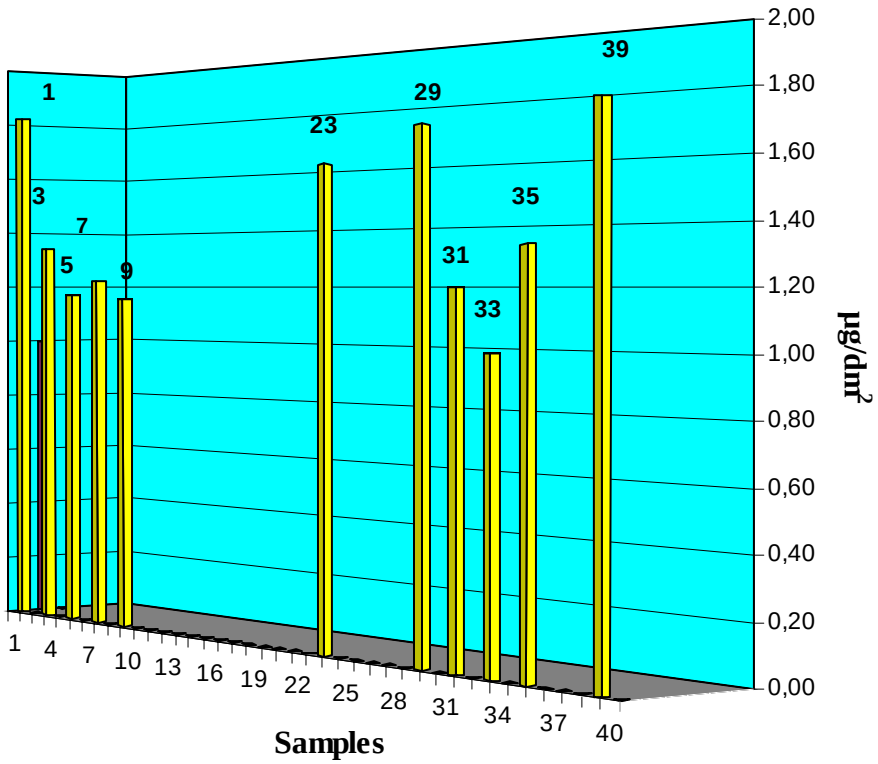
worker c:

31 right forearm

33 left forearm

35 right leg

39 thorax



MTX contamination was not confirmed in any samples, because its concentration was lower than the detection limit of the instrument.
5 -FU was found in hood wipe samples both at the beginning and at the end of the preparation; it is correlated to an unsufficient daily cleaning inside the hood.
In case of pad samples there is a potential personal exposure of the workers dues to an uncorrect operative procedures, as confirmed by results obtained on the forearms and thorax.of nurse.

3.2 CP determination

The selected ion [MH^+] for CP was at m/z 261.2. Under the conditions above mentioned the minimum allowed concentration detectable for CP is 20 ng/mL.
Results obtained for CP show the same trend as 5-FU. Wipe samples evidence a contamination of the hood and of the floor at the beginning and at the end of preparation correlated to an unsufficient daily cleaning procedures. Pad samples show an high personal potential exposure in nurse, probabily due to an uncorrect operative procedures during the preparatin of the antineoplastics.

4. Conclusions

This work, addressed to persons involved in the preparation and administration of ANDs, can be an useful tool for the assement of personal exposure and for the control of operative procedures and the efficiency of personal protective equipments.
Although the preparation of these drugs occured in a prepartion room, under a laminar air flow safety hood, and personnel is equipped with protective clothing and gloves, the environmental monitoring has revealed small quantities of 5-FU and CP. IT This findings suggest that the preparation rooom was not cleaned efficiently such as the hoods, and the drugs are spread in all the room and on protective equipments. Therefore it is necessary the statement of operating cleaning procedures every working day. In order to control the efficacy of these procedures our work propose an HPLC determination that is quite rapid (can be performed in a few minutes) and sensitive (D.L. 20 ng/ml for CP, and 15 ng/ml for MTX and 5-FU).