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Measurement of surface contamination from nucleoside analogue antineoplastic drugs by high-performance liquid chromatography in occupational hygiene studies of oncologic hospital departments

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Abstract

Within the frame of a continuing interest in occupational hygiene of hospitals as workplaces, we describe an automated analytical method by reversed-phase high-performance liquid chromatography for the measurement of contamination from the three most important nucleoside analogue antineoplastic drugs (5-fluorouracil, 5FU; cytarabin, CYA; gemcytabin, GCA) on such surfaces as those of preparation hoods and work-benches in departmental pharmacies of oncologic departments. Our method is characterized by a short analysis time (7 min) under isocratic conditions, by the use of a mobile phase devoid of organic solvent and by high sensitivity (LOD≥40 μg/l for all compounds), adequate to detect surface contamination above a threshold of 4 μg/m^2 for wide surfaces and of 30 μg/m^2 for small irregular objects. We present some results from a preliminary survey study recently performed in seven oncologic departments of two large general hospitals in Milan. To exemplify the contamination levels on various surfaces (such as on handles, floor surfaces and window glass panes, even far from the preparation hood), analyte concentrations in the order of 0.03–0.06 μg/ml, corresponding to 0.8–1.5 μg of 5FU were measured on telephones, of 0.02–0.6 μg/ml (0.85–28 μg/m^2) of CYA were measured on table boards, of 0.05–10.6 μg/ml (1.2–1150 μg/m^2) of GCA on furniture and floors. Spillage fractions up to 1% of the employed ANDs (employed daily 5FU 7–13 g; CYA 0.1–7.1 g; GCA 0.2–5 g) are measured on the polyethylene-backed paper disposable cover sheet of the preparation hood. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: 5-Fluorouracil; Cytarabin; Gemcytabin

1. Introduction

Exposure of hospital staff to the health hazards associated with manipulation of antineoplastic drugs (ANDs) is one among the emerging facets of occupational medicine within hospital premises. Concern about this peculiar source of occupational hazard has been aroused from a long time as a consequence of the epidemiological observation of an increase of infertility, abortion and tumor cases in hospital nursing staff preparing cytostatic drugs, administering antitumoral therapy to patients and disposing of patients' excreta and bed clothing and of alterations

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in some cytogenetic indicators of genotoxicity, although in both cases results of individual studies are often at the threshold of statistical significance [1–4]. However, the absence of NOAEL for genotoxic carcinogenic agents implies that unnecessary (such as occupational) exposure to those compounds should be avoided or limited by use of appropriate procedures. Moreover, a further reason for which exposure of hospital workers (mainly nurses and auxiliary personnel) to antineoplastic drugs should be kept to a minimum may lie also on other than carcinogenic biological properties, such as immunosuppressive activity (nucleoside analogues) or cumulative cardiotoxicity (anthracycline antibiotics), for which hazards no adequate data on adverse health effects following chronic low-dose exposure is available.

Among the number of currently employed ANDs (more than 40 are listed as authorized antineoplastic drugs in the current issue of the ''Prontuario Terapeutico Nazionale'' issued by the Italian National Health Service), seven nucleoside analogues are currently registered. Of these, three (mercaptopurin, thioguanine and tegafur, a lipophilic pro-drug of 5-fluorouracil) are only formulated as capsules for oral administration, one (fludarabine phosphate) has still a very limited use, due to its very recent introduction into use, and three (5-fluorouracil, 5FU; cytarabin, CYA; gemcytabin, GCA) are widely employed in the therapy of many tumor types. Since these drugs are manipulated in relatively large amounts, in the 100–500 kg/yr range, they candidate well as tracers of the level of occupational exposure of nursing personnel to ANDs in oncologic hospital departments, as a consequence of contamination during reconstitution of pharmaceutical preparations for parenteral administration. The exposure level of hospital nursing personnel to ANDs has been recently evaluated for cyclophosphamide, ifosphamide, 5FU and methotrexate [5,6], but exposure to other nucleoside analogue antineoplastic drugs employed as alternatives to 5FU, such as CYA and GCA, has never been measured.

Within the frame of our continuing interest in the occupational hygiene aspects of hospitals as workplaces [7–9], this paper refers on the establishment of a sensitive analytical method by high-performance liquid chromatography (HPLC) for the measurement of surface contamination in hospital premises from these three widely employed injectable nucleoside analogue antineoplastic drugs and on preliminary results of a survey study of seven oncologic departments of two large general hospitals in Milan.

2. Experimental

2.1. General

2.1.1. Chemicals

All solvents and reagents were analytical or the highest grade available. Standard samples of the nucleoside analogue and of some other antineoplastic drugs most commonly employed in the investigated oncologic departments (Table 1) were obtained from involved hospital pharmacies as liophilized (CYA, GCA, MTX, antracyclines) or ready-to-use (5FU) injectable pharmaceutical preparations. Chemical

Table 1
Nucleoside analogue and other antineoplastic drugs analyzed in the study

Compound	Name	CAS No.	Pharmaceutical preparation	Supplier
5-Fluorouracil, 5FU	5-Fluoro-2,4(1H,3H)-pyrimidinedione	51-21-8	Fluorouracile 250 mg	TevaPharma, Milan, Italy
5-Bromouracil, 5BU	5-Bromo-2,4(1H,3H)-pyrimidinedione	51-20-7	Chemical standard	Aldrich, Milan, Italy
5-Iodoouracil, 5IU	5-Iodo-2,4(1H,3H)-pyrimidinedione	696-07-1	Chemical standard	Aldrich, Milan, Italy
Cytarabine, CYA	1-β-D-Arabinofuranosyl-cytosine	147-94-9	Aracitina 100 mg	Pharmacia&Upjohn, Milan, Italy
Gemcitabine, GCA	2′-Deoxy-2′,2′-difluoro-cytidine	95058-81-4	Gemzar 200 mg	Eli-Lilly, Firenze, Italy
Methotrexate, MTX	4-Amino-10-methyl-folic acid	59-05-2	Methotrexate 5 mg	Lederle, Rome, Italy
Aminopterin, AMP	4-Amino-folic acid	54-62-6	Chemical standard	Aldrich, Milan, Italy
Doxorubicin, DOR	14-Hydroxy-daunomycin	25316-40-9	Adriblastina 10 mg	Pharmacia&Upjohn, Milan, Italy
Daunorubicin, DAR	Daunomycin	23541-50-6	Daunoblastina 10 mg	Pharmacia&Upjohn, Milan, Italy
Epirubicin, ERU	4′-Epi-14-hydroxy-daunomycin	56420-45-2	Farmorubicina 10 mg	Pharmacia&Upjohn, Milan, Italy
Idarubicin, IRU	4-Demethoxy-daunomycin	57852-57-0	Zavedos 10 mg	Pharmacia&Upjohn, Milan, Italy

standards (5-bromouracil, 5BU; 5-iodouracil, 5IU and aminopterin, AMP) were purchased from Aldrich (Milan, Italy). All analytical standards and solutions were kept frozen in the dark in the smallest aliquots suitable for daily use.

2.1.2. Equipment

HPLC analyses were performed on a computer-controlled liquid chromatography system composed of an AS1000 autosampler fitted with a fixed-volume 20-μl sample loop and employing 2-ml glass vials, a P1000 isocratic pump, a CrocoCil thermostat oven with temperature controller and a UV1000 UV–Vis detector, all interfaced to the proprietary PC-1000 ver. 3.0 data system (Thermo Separation Products, Rodano, Italy).

2.1.3. Standard solutions

The stock solutions of the individual drugs were prepared by appropriately diluting the pharmaceutical preparations with deionized water for in vivo use to a final concentration of approx. 10 μg/ml. The solutions of the chomatographic internal standards (5BU, 5IU, AMP) were prepared by dissolving a weighted amount of the pure compound (approx. 10 mg) to a final concentration of approx. 10 μg/ml.

The stock solution containing all three analytes (5FU, CYA, GCA) was prepared by combining the individual stock solutions to a final concentration of approx. 1 μg/ml. Appropriate amounts of the stock solutions were further diluted to the required analytical concentrations with deionized water for in vivo use. Calibration solutions containing 0.5 μg/ml of 5BU as the internal standard and six linearly decreasing concentrations of the drugs (1, 0.5, 0.25, 0.125, 0.062 and 0.031 μg/ml, respectively) were freshly prepared and analyzed along with each series of environmental samples.

2.2. Liquid chromatography.

2.2.1. Column

All separations were accomplished on a reverse-phase column system composed of a 0.5 μm frit filter and 10 mm×4.6 mm I.D. Supelguard column placed before a 33 mm long Supelcosil LC-18 analytical column, both packed with spherical 3 μm RP-18 bonded silica particles (Superchrom, Milan, Italy).

2.2.2. Mobile phase

A 5 *M* solution of ammonium acetate, filtered through a 0.20-μm filter under water-jet vacuum and stored at 4°C was employed as stock and freshly diluted to prepare the analytical mobile phase. The final mobile phase was a 10 m*M* ammonium acetate (NH_4OAc) aqueous buffer at pH 6.9. Flow was set to 0.8 ml/min and the column was thermostatted to 30°C. The nucleoside analogues were detected by absorbance monitoring at 272 nm.

k' Values of analytes were calculated as: $k'_{cpd} = (t_{cpd} - t_{void})/t_{void}$. The void elution time was derived, for each autosampler injection of the standard solutions, from the later spike peak due to switch of the automated Rheodyne valve from the ''load'' to the ''inject'' position.

2.2.3. Analytical calculations

The internal standard technique with peak area ratio vs. concentration plot was employed for compound quantification throughout the study. Complete statistical calculations of the regression curve including 95% confidence limits for concentration calculations were performed off-line, on a standard computer spreadsheet. The lower limit for quantification was calculated as the concentration corresponding to a peak ratio (analyte/I.S.) given by the intercept plus three-times its upper confidence limit, calculated on the lower curve of the confidence limit [10]. Intra- and inter-assay accuracy and precision are evaluated from elaboration of the individual calibration curves, each time prepared from the stock solutions.

2.3. Environmental sampling and analysis

2.3.1. Sampling

The collection of contamination on laboratory furniture and surfaces (such as: board and inside surfaces of the preparation hood, nearby floor, window panes, cupboards) and on objects (e.g., drawer handles, laboratory equipment, telephones, etc.) was accomplished by thoroughly rinsing the accurately measured (±1 cm) surfaces with cotton swabs dipped in measured volumes of 0.1 *M* ammonium acetate solution. A typical volume of wash-

ing solution of 20 ml can be employed to sample from as a wide surface as approx. 0.5 m^2. Analyte recovery from wettable surfaces was not explicitly tested, since decontamination of laboratory surfaces with three successive washings of 0.1 *M* ammonium acetate solution and HPLC analysis of each one yielded measurable amounts of drugs only in the first wipe.

Removable objects, such as used pairs of gloves and the disposable cover cloth of the hood board (where employed) were taken to the laboratory, dipped (as a whole or as appropriate representative sub-samples of larger items) into appropriate volumes of washing solution and analyzed.

2.3.2. Sample preparation and analysis

The liquid was collected from the swollen cotton swabs by thoroughly pressing them in a 100-ml plastic syringe and measuring the volume of the recovered solution in graduated glass cylinders. Grossly turbid solutions, such as those collected from the floor or from dusty surfaces were preliminarly filtered through coarse cloth paper and then through disposable syringe filters prior to HPLC analysis. All washings were analyzed as such, after adding an appropriate amount of internal standard (most often 0.5 μg) to a 1 ml sample.

2.3.3. Results calculations

Results referring to contamination of flat surfaces (such as hood surfaces and board, floor, walls) were expressed as mass of drug (i.e., the measured analytical concentration in the chromatographic sample multiplied by the appropriate volume fraction of the washing or extraction sample) per unit surface.

For sampling positions such as the floor or other wide surfaces the extension of the sampling area is not the limiting factor of surface contamination measurement (conveniently wider surfaces can be rinsed with the same or slightly greater volume of solution, if needed): the smallest detectable contamination (limit-of-contamination; LOC; $\mu g/m^2$) can be calculated as:

$$\mathrm{LOC}\ (\mu g/m^2) = \mathrm{LOD}\ (\mu g/ml) \cdot [\text{largest volume of rinsing solution (ml)}/\text{largest rinsable area } (m^2)] \quad (1)$$

i.e., under the current sampling and analysis conditions, around 4 $\mu g/m^2$.

For small objects, such as handles, telephones or gloves, it is the size of the single object, rather than the volume of rinsing solution employed, the factor determining the lowest detectable amount of the analyte, and therefore the minimum value of measurable contamination. A LOC value under the current sampling and analysis conditions can be thus calculated as:

$$\mathrm{LOC}\ (\mu g/m^2) = \mathrm{LOD}\ (\mu g/ml) \cdot [\text{smallest volume of rinsing solution (ml)}/\text{smallest rinsable area } (m^2)] \quad (2)$$

The minimum amount of rinsing solution employed to wipe a small object (or a number of similar objects, such as door handles) is of approx. 10 ml, and the smallest rinsable surface is of 0.015 m^2 (i.e., that of a telephone or of three door handles). Thus the minimum detectable amount of a nucleoside analogue is in the range of approx. 30 $\mu g/m^2$ of sampled surface.

To achieve homogeneity between the two extreme situations shown, samples yielding areic contamination values below a LOC of 10 $\mu g/m^2$ are reported as negative, although the washing samples themselves may yield a chromatographically measurable concentration of the analyte.

3. Results

3.1. Chromatographic separation

A typical separation under the standard chromatographic conditions of a mixture containing 5FU, CYA, GCA and the selected internal standard, 5BU, is reported in Fig. 1. The trace represents the highest point of the calibration curve, a standard solution containing 1 μg/ml of each analyte and 0.5 μg/ml of the internal standard. 5BU was finally preferred to the iodo-analogue as the internal standard, since the latter eluted too close to GCA under a variety of tested chromatographic conditions.

Interference from other cytostatic drugs belonging to different chemical classes was tested with refer-

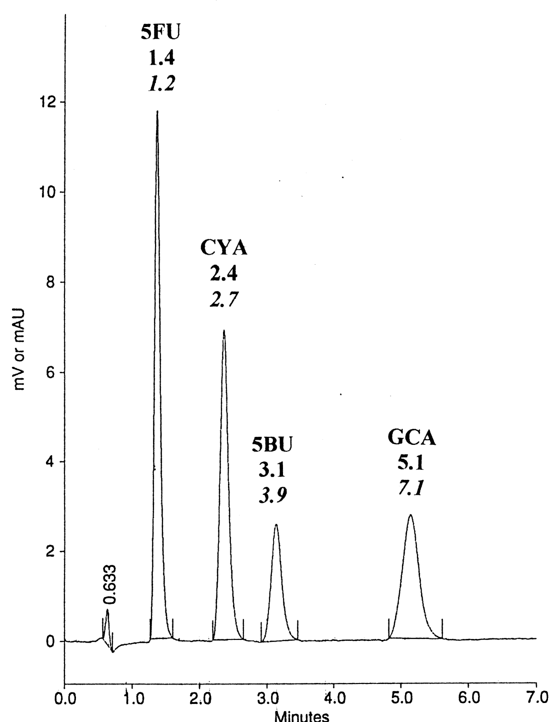

Fig. 1. Typical chromatographic separation of a standard mixture, containing approx. 1 μg/ml of each of the three nucleoside analogue antineoplastic drugs 5-fluorouracil (5FU), cytarabin (CYA), gemcytabin (GCA) and 0.5 μg/ml of the internal standard, 5-bromouracil (5BU). Chromatographic conditions: analytical column: 0.5 μm frit filter + 10 mm × 4.6 mm I.D. Supelguard + 33 mm × 4.6 mm I.D. Supelcosil LC-18 (spherical 3 μg RP-18); mobile phase: 10 m*M* NH_4OAc, pH 6.9; flow: 0.8 ml/min; injection: automatic injector, 20 μl fixed loop; detection: UV 272 nm. Absolute retention times are in minutes; k' values refer to the column void and are italicized.

ence to methotrexate and its analytical internal standard aminopterin 7, and to the four antracycline antibiotics (doxorubicin, daunorubicin, epirubicin and idarubicin) which are among the antitumoral chemotherapeutic agents most commonly employed in the italian oncologic departments. Due to their much stronger lipophilic nature, all six compounds do not elute from the chromatographic column under the conditions of weak eluant strength appropriate for the separation of nucleoside analogues.

3.2. Linearity, sensitivity and reproducibility

Typical calibration curves for quantification of each analyte drug are shown in Table 2. The calibration curves are linear in the range 0.031–1 μg/ml, and the analytical limit of detection (LOD, $S/N>3$) for the three drugs are in the range of 40 μg/l at worse. Therefore, the LOD for all drugs lies between 4 μg/m^2 (large surfaces) and 30 μg/m^2 (small irregular objects) and the contamination value corresponding to the highest point of the calibration curves is of approx. 100 μg/m^2 for surfaces and 1 mg/m^2 for objects. Contamination levels below LOC are considered as not being of interest from the point of view of occupational hygiene, while those above the highest point of the calibration curves point to high-contamination situations imposing immediate remediation.

Intra-assay accuracy and precision are evaluated from a cumulative curve elaborated from three individual sets of calibrators (each composed of six solutions containing 1, 0.5, 0.25, 0.125, 0.062 and

Table 2
Parameters of the cumulative calibration curves for quantification of 5FU, CYA and GCA against 5BU internal standard, each obtained by elaborating together three individual calibration curves in the range 30–1000 μg/l, prepared and analyzed within a two-month period

	5FU	CYA	GCA
Range of calibration (ng/ml)	31.25–1000	31.25–1000	31.25–1000
Intercept	0.03	0.01	0.01
Error-of-intercept	0.01	0.01	0.01
Slope	$2.45\cdot10^{-3}$	$2.09\cdot10^{-3}$	$1.70\cdot10^{-3}$
Error-of-slope	$2.42\cdot10^{-5}$	$1.59\cdot10^{-5}$	$2.01\cdot10^{-5}$
Regression coefficient, r^2	0.9993	0.9996	0.9989
No. of curves	3	3	3
Limit of detection (ng/ml; $S/N>3$)	41.51	32.00	49.82

0.031 μg/ml of analytes and 0.5 μg/ml of internal standard), prepared and analyzed in a single day. Inter-assay accuracy and precision are evaluated from elaboration of the individual calibration curves, each time prepared from the stock solutions, and run as single injections with each lot of samples (Table 3).

3.3. Analysis of environmental samples from field measurements.

A preliminary monitoring study was performed on seven oncologic departments within two large general hospital in Milan. Departments vary widely in lay-out (open vs. restricted-access), operational characteristics (inpatient or day-hospital facility), number of daily treated patients, nature and quantity of ANDs employed (up to 15 different drugs, employed in monthly amounts ranging from a few milligrams to a 100 g). Administered ANDs include a single nucleoside analogue for each department (5FU was employed in three of them, CYA in three, GCA in one).

Fig. 2a–c shows three chromatograms obtained from samples collected in three departments employing 5FU, CYA and GCA, respectively, and selected to illustrate some typical conditions met in the course of the measurement campaign. In chromatograms **a** and **c**, 5IU was employed as the internal standard, since in earlier times it did not interfere with the analysis of 5FU and CYA. No differences in the accuracy and precision of quantification was observed when adding different amounts of internal standard to the samples (chromatograms **a** and **c**) or when switching to the new internal standard, 5BU (chromatogram **b**), which allows one to measure all three drugs in a single run (chromatogram **b**). The first sample (Fig. 2a) contains only 5FU and represents direct injection of a 20-ml eluate from a 10× 10 cm sub-sample taken out of the 42×25 cm

Table 3
Intra- and inter-assay accuracy and precision figures for quantification of 5FU, CYA and GCA

Target concentration (μg/l)	Intra-assay ($n=3$)[a]				Inter-assay[b]			
	Measured	±SD	Accuracy (%)	Precision (%)	Measured	±SD	Accuracy (%)	Precision (%)
5FU ($n=6$)								
31.25	32.43	24.57	3.8	78.6	29.29	30.35	−6.3	97.1
62.50	63.63	24.57	1.8	39.3	60.63	30.35	−3.0	48.6
125.00	126.02	24.57	0.8	19.7	123.31	30.35	−1.4	24.3
250.00	250.80	24.57	0.3	9.8	248.67	30.35	−0.5	12.1
500.00	500.37	24.57	0.1	4.9	499.39	30.35	−0.1	6.1
1000.00	999.50	24.57	−0.0	2.5	1000.82	30.35	0.1	3.0
CYA ($n=3$)								
31.25	32.01	18.95	2.4	60.6	25.96	29.52	−16.9	94.5
62.50	63.22	18.95	1.2	30.3	57.45	29.52	−8.1	47.2
125.00	125.65	18.95	0.5	15.2	120.44	29.52	−3.7	23.6
250.00	250.51	18.95	0.2	7.6	246.41	29.52	−1.4	11.8
500.00	500.24	18.95	0.0	3.8	498.34	29.52	−0.3	5.9
1000.00	999.68	18.95	−0.0	1.9	1002.22	29.52	0.2	3.0
GCA ($n=2$)								
31.25	31.10	29.56	−0.5	94.6	31.04	26.88	−0.2	86.4
62.50	62.36	29.56	−0.2	47.3	62.30	26.88	−0.1	43.1
125.00	124.87	29.56	−0.1	23.6	124.82	26.88	−0.0	21.5
250.00	249.90	29.56	−0.0	11.8	249.86	26.88	−0.0	10.8
500.00	499.95	29.56	−0.0	5.9	499.93	26.88	−0.0	5.4
1000.00	1000.06	29.56	0.0	3.0	1000.09	26.88	0.0	2.7

[a] The intra-day cumulative calibration curves are elaborated from three independent preparations of the calibrators, prepared and analyzed in the same day (same data of Table 2).

[b] Data are elaborated from the individual calibration curves prepared and analyzed with the study samples.

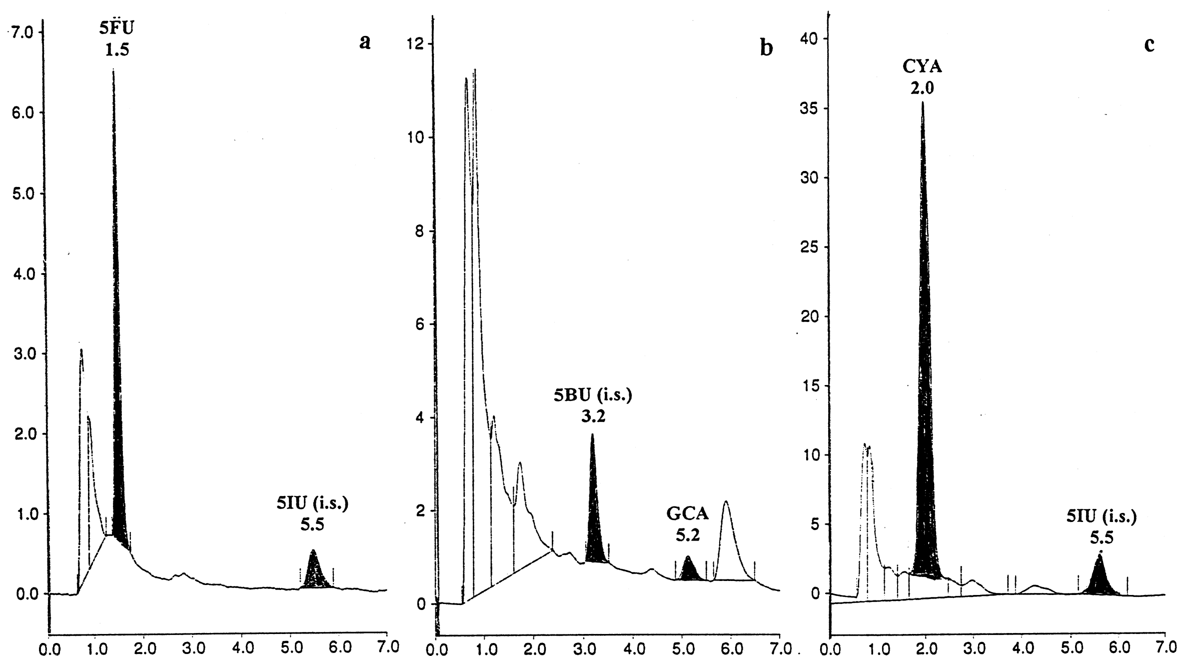

Fig. 2. Typical chromatographic separation of washing samples taken from furniture surfaces in the drug preparation areas of three oncologic hospital departments. Sample **a** represents direct injection of a 20-ml eluate from a 10×10 cm sub-sample taken out of the 42×25 cm disposable paper blanket of the hood board, in a department employing 5FU. Sample concentration is 10.6 μg/ml, corresponding to a contamination of 110 μg/m^2. Sample **b** is a 100-ml washing of the 140×80 cm floor area in front of the preparation hood, in a department employing GCA. Sample concentration is 8.3 μg/ml, corresponding to a contamination of approx. 754 μg/m^2. Sample **c** is a 20-ml washing of the door (0.79 m^2) of the drug refrigerator cupboard in a department employing CYA. Sample concentration is 0.14 μg/ml, corresponding to a contamination of 3.5 μg/m^2, i.e., under the calculated limit-of-contamination. Chromatographic conditions as in Fig. 1; retention times are in minutes; the vertical scale is in milli-absorbance units.

disposable paper blanket of the hood board, in a department employing 5FU. Sample concentration is 10.6 μg/ml, corresponding to a contamination of 110 μg/m^2. The second sample (Fig. 2b) is a 100-ml washing of the 140×80 cm floor area in front of the preparation hood, in a department employing GCA. Sample concentration is 8.3 μg/ml, corresponding to a contamination of approx. 754 μg/m^2. The third sample (Fig. 2c) is a 20-ml washing of the door (0.79 m^2) of the drug refrigerator cupboard in a department employing CYA. Sample concentration is 0.14 μg/ml, corresponding to a contamination of 3.5 μg/m^2, i.e., under the calculated LOC (see above).

Within the preliminary field study, a total of 68 samples were collected from seven preparation rooms, all equipped with a preparation hood (three chemical, with a charcoal filter; four laminar-flow; all hoods recycling ambient air). In all rooms, washing samples were taken from the hood inside walls, the hood board (the disposable blanket cover, when used, was taken as a sample), the floor beneath and around the hood (1 m depth on the front and sides). Representative samples were also taken from and horizontal and vertical furniture surfaces (shelves, cupboard and drawer chests), from window panes and from objects in the room.

A brief synopsis of the results from the monitoring is reported in Table 4. Out of the 68 samples (37 analyzed for 5FU, 25 for CYA and six for GCA), 13 (none for 5FU, 10 for CYA and three for GCA) yielded concentration values below the LOD of the individual analyte. Eleven samples (eight for 5FU, three for CYA and none for GCA) yielded a measurable level of surface contamination, but lower than the cutoff LOC of 10 μg/m^2 (see, e.g., the sample of Fig. 2b).

Table 4
Synopsis of contamination from three nucleoside analogue antineoplastic drugs (5FU, CYA, GCA) measured in seven departmental areas for preparation of cytostatic drug within two large general hospitals

Sampling position within preparation area		Mass of employed drug (min–max; g/wk and No. of vials)[a]								
		5FU 35–65 (50–150 vials)			CYA 0.1–7.5 (1–75 vials)			GCA 5.1 (25 vials)		
		No. samples	No. neg. samples	Min–max ($\mu g/m^2$)[b]	No. samples	No. neg. samples	Min–max ($\mu g/m^2$)[b]	No. samples	No. neg. samples	Min–max ($\mu g/m^2$)[b]
Hoods	Inside	3	0	89–13 000	3	1	30; 14 000	1	0	250
(3 chemical; 4 laminar flow)	Board	0	–	–	1	1	0	0	–	–
	Blanket	4	0	110–111 000	0	–	–	1	0	11200
Surfaces	Chest of drawers	5	1	45–1220	6	4	438–2000	0	–	–
(within 1 m from hood)	Table board	2	1	55	4	2	30; 170	0	–	–
	Shelves	3	0	22–100	0	0	–	0	–	–
	Floor	4	0	127–3800	3	2	750	1	0	972
Other surfaces	Telephone	3	0	–	0	0	–	0	–	–
and objects	Handles	6	3	60–5000	1	0	–	1	1	–
	Window panes	3	2	20	3	1	120; 240	1	1	–
	Others	4	1	320–11 000	4	2	17; 90	1	1	–
Total		37	8	0.5–200[c]	25	11	17–2000[c]	6	3	972[c]

[a] Evaluated from pharmacy inventory data or from daily department worksheets.
[b] Contamination levels <10 $\mu g/m^2$ are considered as negative.
[c] Hood contamination levels are excluded from evaluation.

To exemplify the range of contamination levels measured on various surfaces, very high values were measured – as expected – on the preparation hoods (10 positive samples out of 13; range 30–14 000 $\mu g/m^2$) and especially on the disposable paper blankets under the hoods, where dissolution operations actually take place. The mass of ANDs measured on 10×10 cm samples of five polyethylene-backed paper disposable cover sheet of the preparation hood (approx. 0.45 m^2) rank up to approx. Fifty mg on the whole sheet (in a single case) and correspond to spillages as high as 1% of the employed ANDs (employed daily 5FU 7–13 g; CYA 0.1–7.1 g; GCA 0.2–5 g). Lower drug contamination levels were also measured on floor and furniture surfaces in the immediate proximity of the preparation hoods (18 positive samples out of 28) and on other objects and surfaces, even meters far from the hood (15 positive samples out of 27), where analyte concentrations in the order of 0.03–0.06 $\mu g/ml$, corresponding to 0.8–1.5 μg of 5FU are measured on telephones, of 0.02–0.6 $\mu g/ml$ (0.85–28 $\mu g/m^2$) of CYA are measured on table boards, of 0.05–10.6 $\mu g/ml$ (1.2–1150 $\mu g/m^2$) of GCA are measured on furniture and floors.

The lowest overall contamination levels are found in the Bone Marrow Transplantation (BMT) unit (where only one out of six samples taken outside the preparation hood was found positive, at a contamination level of 15 $\mu g/m^2$). For a merely comparative purpose, in the day-hospital facility of the same department five out of ten samples taken outside the hood yielded measurable concentrations of the tracer, in the range of 0.02–31 $\mu g/ml$, corresponding to contamination levels between 17 and 2000 $\mu g/m^2$.

4. Discussion

To correctly assess the health risks associated to manipulation of ANDs and to rationally advise intervention priorities for exposure abatement, reliable quantitative information on the exposure levels to individual drugs is mandatory, and therefore the

establishment of adequate analytical procedures for the measurement of workplace contamination is needed.

Among the seven nucleoside analogue drugs registered in Italy for use as antineoplastic agents, only those administered by the parenteral route (5FU, CYA, GCA) were considered useful as tracers of exposure, since occupational hazard in hospital drug preparation areas is mainly associated to exposure to aerosolized drug solutions disseminated during reconstitution of liophilized powder formulations for parenteral administration or spilled by accident during preparation. The three target drugs are employed in very large amounts (parenteral therapeutic doses are in the range of 50–200 mg per patient) in several types of solid tumors and leukemias.

5FU is – along with cyclophosphamide, ifosphamide and methotrexate – one of the few antineoplastic drugs for which systematic studies on airborne and surface environmental contamination in production plants and in hospital pharmacies and departments have been published [5,6,11,12], in some cases also associated to biological monitoring of workers exposure through measurement of the urinary metabolite α-fluoro-β-alanine [11,12].

Although a number of analytical methods by liquid chromatography have been published for quantification of 5FU e.g., Refs. [13,14], of CYA [15] and of GCA [16], mainly for pharmacokinetic purposes, few were deemed suitable for our purpose and none proved adequate for the simultaneous separation of all three our target compounds. One major difficulty with 5FU is its very low k' value under reversed-phase conditions, which cannot be modified by eluent pH modification or with the use of different organic solvents [14] and of alternative reversed-phase chromatographic packings (e.g., on phenyl-modified silica; our unreported data). In fact, to overcome this difficulty, most literature methods employ long ($\geq$150 mm) analytical columns, with only marginal improvement of the k' value, but with a substantial increase of sample turnaround time. This is little surprising, if one considers that uracil itself is employed as a void volume marker in RP-HPLC test mixtures. That elution of 5FU under reversed-phase conditions is a critical problem is also clearly stated in a very recent paper, where authors were compelled to derivatize 5FU into a much more lipophilic compound to measure the drug levels in plasma samples by HPLC–tandem mass spectrometry (MS–MS) [17].

Our need for a routine analytical method for the measurement of surface contamination from pharmaceuticals in hospital preparation areas therefore prompted to compromise between several conflicting characteristics: a short analysis time for high sample throughput is necessary to expeditously manage the large number of samples obtained from on-site inspections; specificity and sensitivity should be adequate to trace surface contamination in the μg/m^2 range; finally, switching between different analytical methods without column change should be as fast as possible. These constraints disfavored the set up of gradient separation and the use of ion pairing reagents, but rather led to optimize an isocratic separation on a standard octadecyl silica cartridge column. Our analytical conditions allow to determine surface contamination from the target analytes with a short analysis time of 7 min/sample and a minimum detected concentration, by direct injection of an aliquot of a surface washing, in the 20–40 μg/l range, which compares well with the figures reported by Bos et al. [6].

With the use of our method, we are able to discriminate between departments with very different levels of surface contamination and to trace the diffusion of contamination from the preparation hood into the surrounding space. The contamination levels determined by us are sometimes quite higher than those reported by Bos et al. [6] in similar positions (e.g., the surface contamination of laminar flow hood trays is reported as 620–270 μg/m^2, while we measured values ranging from below the LOC to 13 000 μg/m^2; floor contamination is reported 107–236 μg/m^2, while we measured values up to 3800 μg/m^2) but the important differences in the overall design of the monitoring study makes a direct comparison at best of a qualitative value. We are currently investigating into the relationship between departmental lay-out, organizative procedures and occupational hazard due to exposure of the nursing personnel to the potentially noxious drugs. In particular, the very low level of contamination in the BMT unit is possibly a consequence more of the meticulous care in all operating procedures dictated by the necessity to keep sterile conditions through-

out, rather than of its small size (two rooms) and of the small amount of AND administered.

5. Conclusions

Our analytical method for nucleoside analogue antineoplastic drugs features a fast chromatographic run on a short column, which allows the analysis of a large number of samples within a work-day; its sensitivity is adequate to routine detection of surface contamination from nucleoside analogue antineoplastic drugs in preparation areas in the $\mu g/m^2$ range and, finally, the use of simple isocratic conditions and of a mobile phase without organic solvent allows recycling, unexpensive and unrestricted disposal of exhausted material and minimal exposure of laboratory personnel to potentially noxious chemicals. Its application to evaluation of the surface contamination level of drug preparation areas in oncologic hospital departments contributes to health risk assessment and to rationally advice into safe procedures for manipulation of ANDs and intervention priorities for exposure abatement.

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