

Ricardo Soares Gioda ¹
 Helena Corleta ²
 Edison Capp ³
 Carmen Pilla ¹

MONITORAMENTO DE RESÍDUOS DE FLUOROURACIL EM AMBIENTE DE MANIPULAÇÃO DE FÁRMACOS CITOTÓXICOS

FLUOROURACIL RESIDUES MONITORING OF PHARMACY ENVIRONMENTS IN BRAZILIAN HOSPITAL

MONITOREO DE RESÍDUOS DE FLUOROURACILO EN AMBIENTE DE MANIPULACIÓN DE FÁRMACOS CITOTÓXICOS

1. Hospital de Clínicas de Porto Alegre
 2. Universidade Federal do Rio Grande do Sul

RESUMO

Objetivo: Identificar um método analítico específico para monitorização ambiental de fármacos citotóxicos e avaliar a presença de resíduos de fluorouracila no local de manipulação desses medicamentos no Hospital de Clínicas de Porto Alegre, Brasil.

Métodos: A coleta de amostras foi realizada através da técnica de wipe test e posterior extração. As amostras foram analisadas através da Cromatografia Líquida de alta Eficiência com detector Ultravioleta/Visível.

Resultados: De um total de 96 amostras coletadas para análise do fluorouracil, encontrou-se um percentual de contaminação de 19,8%. Destaque-se os resultados obtidos entre os frascos de medicamento em sua embalagem original e o campo estéril de revestimento da Cabine de Segurança Biológica.

Conclusões: O método mostrou-se adequado para identificar a presença de fluorouracil. Permitiu ainda fornecer informações sobre fontes potenciais de contaminação, possibilitando ajustes nos procedimentos em todas as fases do contato com o medicamento citotóxico.

Descritores: Citotóxicos, Monitoramento de fármacos, Fluorouracila

ABSTRACT

Objectives: Using High Performance Liquid Chromatography, this study suggests an analytical method for monitoring cytotoxic drugs and evaluates the contamination with fluorouracil of the drug handling environment of Hospital de Clínicas de Porto Alegre, Brazil.

Methods: Samples were collected from surfaces, vials and gloves with wipe test technique and subsequent extraction and analysis in High Performance Liquid Chromatography equipment with Ultraviolet/Visible detection.

Results: In 96 samples collected, 19.8 % had the presence of Fluorouracil. Vials taken from the original package and sterile field of Biological Safety Cabine had higher positive results.

Conclusions: Method used was adequate to identify the presence of fluorouracil. This allows a sensitive identification of potential contamination sources by fluorouracil residues in hospital departments, leading to adjustments of proceedings in all phases of the contact with the cytotoxic drugs.

Descriptors: Cytotoxics, Drug Monitoring, Fluorouracil

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RESUMEN

Objetivo: Con auxilio de cromatografía líquida de alto rendimiento, este estudio sugiere un método de análisis para el seguimiento de los fármacos citotóxicos, y evalúa la contaminación con fluorouracilo del entorno de manejo de medicamentos en Hospital de Clínicas de Porto Alegre, Brasil.

Métodos: Se recogieron muestras de superficies, los viales y los guantes con la técnica de ensayo y limpie la extracción y posterior análisis en el Equipo de Cromatografía Líquida de Alta Resolución con detección Ultravioleta/Visible.

Resultados: En 96 muestras recogidas, 19,8% contó con la presencia de fluorouracilo. Viales tomadas desde paquete original y campo estéril de cabina de seguridad biológica mostró resultados más positivos.

Conclusiones: El método utilizado fue suficiente para identificar la presencia de fluorouracilo. Esto permite una identificación sensible de posibles fuentes de contaminación por residuos en los departamentos del hospital, lo que lleva a ajustes de los procedimientos en todas las fases del contacto con los fármacos citotóxicos.

Descritores: Citotóxicos, Monitoreo de fármacos, Fluorouracilo

Autor para Correspondência:
 Ricardo Soares Gioda
 Hospital de Clínicas de Porto Alegre
 rgioda@hotmail.com

INTRODUCTION

Patients submitted to chemotherapy may have secondary malignancies related to the cytotoxic drug used.⁽¹⁾ It has been demonstrated that not just the patients, but also the professionals involved in the handling of these drugs are exposed to health risks.^(2,3)

In 1983, the Occupational Safety and Health Administration (OSHA) started the implementation of measures to protect health of workers exposed to cytotoxic agents. This culminated in a Safe Handling Program, which was published in the American Journal of Hospital Pharmacy.⁽⁴⁾ In 1986, guidelines describing personal protective equipment (PPE), and the handling procedures to be used to avoid the imminent risks to health professionals involved were proposed. The preparation of cytotoxic drugs are performed in a Biological Safety Cabinet (BSC) located in a specific area, usually in the pharmacy, by previously trained professionals.⁽⁵⁾ However, still currently the health risks related to handling antineoplastic drugs have therefore become a major concern for occupational medicine in hospital.⁽⁶⁾

Drug residues can be present in the form of powder, liquid or aerosol, and may be inhaled or percutaneously absorbed.⁽⁷⁾ With the widespread use of BSC, air contamination became less common, usually remaining at levels below detection levels.⁽⁴⁾ During the drug administration period, the exposure is generally due to aerosol or direct skin contact.⁽³⁾ Some of the potential contamination sources are the working surfaces⁽⁸⁾, air⁽⁹⁾, vials and equipment used in the drug preparation and handling.^(10,11)

The main route of exposure to cytotoxics remain the dermal contact.⁽³⁾ It may occur during the preparation of drugs, the opening of ampoules, the contact with needles and syringes containing or that contained the substance, the transference of content from the ampoule to the vial, or the incorrect packaging of partially used vials.

The determination of antineoplastic agents in the environment is commonly done with high performance liquid chromatography with ultraviolet/visible detection (HPLC-UV/VIS). This technique allows adequate detection under routine technical conditions.⁽¹²⁾

Fluorouracil (5-FU) is the most frequently handled antineoplastic at Hospital de Clínicas de Porto Alegre (HCPA) used as the single agent, or combined with other antineoplastic agents, as part of treatment regimens. It is an antimetabolite often used to treat gastrointestinal, lung, and breast tumors. It acts as an inhibitor of thymidylate synthase and needs to be converted into a false nucleotide, in order to inhibit DNA synthesis. It is potentially teratogenic and it is classified as category D in relation to risks in pregnancy and the group 3 of IARC (inadequate evidence of human or animal carcinogenicity).⁽¹³⁾

The aim of this study was to identify a specific analytical method for the environmental monitoring of fluorouracil using HPLC-UV technique and evaluate the presence of drug residues in the handling environment of Hospital de Clínicas de Porto Alegre, Brazil.

METHODS

Samples were collected at the area of pharmacy - Central de Misturas Intravenosas of Hospital de Clínicas de Porto Alegre (CMIV/HCPA). For the collection of environmental samples, the wipe test technique was used: Whatmann paper filter circles (55 mm diameter - Maidstone, Kent, England) soaked in 100 μ L of distilled water were placed in contact with the object or the specified surface⁽¹³⁾.

The surfaces and objects examined were: sterile field for casing (used in the BSC), partially used vials, vials removed from their original storage area, surgical gloves used for handling (samples collected from the inside and outside of the glove), workbench of preparation room and steel bowl used for storage of vials in use. The vials examined were in their original packaging, which should be in good conditions and without evidences of breakage, leakage or humidity. Samples were collected according to the availability of routine work area. Considering an estimate environment contamination of 50 % and a margin of error of 10 % with a confidence level of 95 %, sample size was estimated in 96.

After collection, each sample was placed in a covered PVC test tube, and sent for analysis in the laboratory of Serviço de Patologia Clínica/HCPA.

For the standard solution, Fluorouracil p.a. (Sigma Aldrich Chemical Company - Saint Louis, Missouri, USA) was used. The drug analyzed in this study was Fluoracil, Libbs, São Paulo, Brazil. Prepare of standards and following steps were performed with the use of personal protective equipment for handling chemotherapy drugs.

For the extraction, 2 mL of distilled water were injected inside the tubes with the samples and placed under agitation 30 min at 150 RPM in a Kline horizontal shaker (Fanem, São Paulo-SP). The analysis of the solution after extraction was performed using the High Performance Liquid Chromatography (HPLC) method with UV detection Shimadzu equipment with UV/VIS SPD 10° detector operating in isocratic mode and manual injection - 200 mL loop - was used. The column used for the chromatographic separation of 5-FU was the LiChrospher RP-18 Endocapped (250 mm x 4.6 mm). The analysis was executed with 0.05 M (pH 4.0) sodium acetate as mobile phase, with flow rate of 1 mL/min and wavelength of 195 nm.^(13,14)

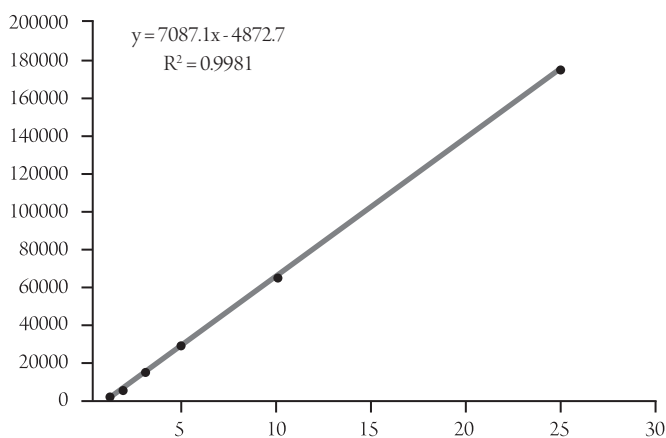
In order to validate the method, recovery tests were conducted aiming to assess the effectiveness of the extraction method of the samples. Tests regarding precision, linearity and quantification limit were conducted.

This study was approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre (GPPG 08-221).

RESULTS

In order to demonstrate linearity of method and define limit of quantification used, a calibration curve was determined. Figure also illustrates the correlation line (R^2) and the equation obtained.

Figure 1. Linearity of method (Fluorouracil calibration curve) AUFS (Absorbance units full scale)



5-FU theoretical concentration (0,5 -25 μ g)

Limit of quantification (LOQ) obtained by successive dilution of the sample containing defined 5-FU concentrations and without significant noise baseline was 0.5 μ g (table 2). Retention time of the sample was 6.4 min.

Table 2. Calibration curve parameters

Parameters	
Linearity (r^2)	0.9981
Range (μ g/mL)	0.5 - 25
Inclination	- 4842.7
Time of retention	6.4 min
Intercept	7087.1
LOQ (μ g/ml)	0.5

The accuracy of technique was tested through repeated measures of samples analyte concentrations. A total of five doses in the same day and during three consecutive days were collected and mean values and variation coefficients were estimated and mean values and variation coefficients were thereafter calculated (table 1).

Table 1. Accuracy of method

	Intra-day (5x)		Inter-day (3 d)	
	mean	CV	mean	CV
5-FU				
2.5 µg	2.4 µg	4.2 %	2.3 µg	5.9 %
5 µg	4.2 µg	2.2 %	4.1 µg	6.5 %
20 µg	18.3 µg	1.3 %	17.3 µg	6.0 %

CV: coefficient of variability.

The 5-FU recovery test with the wipe test technique was performed applying 0.1 mL standard solution with known concentrations on paper filter and the extraction was performed with the same technique used for the samples (table 3).

Table 3. 5-FU recovery test (Wipe test)

5-FU (µg)	TR (%)
20 µg	85 %
10 µg	92 %
5 µg	95 %
2 µg	99 %
0.5 µg	106 %

Method allowed the detection of contamination in handling environments by collecting different sources of sample, as shown in the table below. Contamination rate by 5-FU residues was 19.8 % (table 4).

Table 4. Contamination and environmental samples

Source	Total (n)	Samples >LOQ	Range of concentration
5-FU vials	23	7	0.67 - 90.6 µg
Storage steel bowl	13	2	0.82 - 2.26 µg
5-FU in use vials	16	2	0.5 - 33 µg
Gloves (external side)	12	3	0.62 - 2.9 µg
Gloves (internal side)	08	0	-
Sterile field of BSC (100 cm ²)	11	4	1 - 4.53 µg
Workbench (100 cm ²)	13	1	8.2 - 22.6 µg
Total	96	19 (19.8 %)	

DISCUSSION

High Efficiency Liquid Chromatography (HPLC) is commonly available in analysis laboratories and it is a sensitive and cost-effective technique for monitoring occupational exposure.⁽¹⁵⁾

The collection of samples was conducted by the method known as a wipe test for different sample types, including medicine vials. For the purpose of assessing the external contamination of vials, other methods are also reported, such as the immersion in solution and triple rinsing of the external surface. All these different methods have satisfactorily recovered analyte.⁽¹⁶⁾

The action mechanism of carcinogenic substances is not fully understood, thereby not allowing to determining the safe exposure level in which no toxic effects is observed. It is therefore essential to keep exposure at the lowest possible levels.⁽¹⁷⁾

In a systematic review (14 studies) of women working with cytotoxic agents, there was an increase in the number of spontaneous miscarriages.⁽¹⁸⁾ Nonetheless, it was not possible to determine an association

between exposure and risk of cancer, acute toxic events or congenital malformations. Association between menstrual dysfunctions and the handling of cytostatic drugs in nurses aged between 30 and 45 years has been previously demonstrated.⁽¹⁹⁾ Additionally, occupational exposure to antineoplastic drugs may cause alteration in the immune system, which can lead to health disorders.⁽²⁰⁾

The contamination of the handling room surfaces, such as benches in the preparation room, provides an indirect assessment of appropriate operation of BSC, HEPA filters (high efficiency particulate air) and the pressurization of the room.^(13,21) In our study, only one sample had result above the LOQ (out of thirteen samples examined). The contamination was probably caused by contact of vials or syringes with drug residues. An airborne contamination would have led to a more homogeneous presence of residues in samples of the same surface.

Dermal contact is the main route of exposure to cytotoxic agents and gloves are considered highly important. Protection offered by this PPE must take into account several factors. Permeability of the gloves used in handling cytostatics depends of time on exposure, glove thickness, type of material and chemical characteristics of drugs.⁽²²⁾ In the handling routine of the service, two pairs of sterile latex surgical gloves are utilized. In the samples collected of external glove, no 5-FU residues were detected in the inner surface, even with positive results on the outer face [25 % - 3/12]. In the handling routine of the service, two pairs of sterile latex surgical gloves are utilized.

Recommendations which may reduce the risk of contamination by residues cytotoxic on surfaces such as those found within the BSC (higher frequency in our study) include periodic certification of airflow system and use of syringes with luer-lock connexion and hydrophobic filters for balancing the atmospheric and internal pressure of vial preventing aerosol formation.^(21,23)

External contamination of 5-FU, cyclophosphamide, ifosfamide, etoposide, doxorubicin and docetaxel vials has been previously assessed by water immersion and rotation technique: all vials had contamination levels ranging from 0.5 ng to 2.4 g per vial⁽²⁴⁾. The handling environment might be contaminated with cytotoxic drug residues even in the absence of handling mistakes. Exposure may begin even before the reconstitution or dilution of the medicine vials.^(24,25)

The introduction of decontamination equipment and the use of protective sleeves significantly decreases the contamination degree by antineoplastic agents in hospitals and medical clinics.⁽²⁴⁾ Yet, as evidenced in our study, the way the protective sleeve was utilized in handling the 5-FU vials has not provided a reliable protection. Manufacturers of hazardous drugs such as antineoplastics should make all the efforts to avoid external contamination. Assuming that this is not possible, consumers should be warned of the possibility of contamination and the need to prevent exposure both in documents and reports and the product packaging.^(24,26)

The contamination of drug vials found in our study was reported to the pharmacovigilance system of Hospital de Clínicas de Porto Alegre for registration and appropriate procedures. These results are in accordance with the recommendations of Guidance values for surface monitoring of antineoplastic drugs in German pharmacies where among other are attention on external vial contamination and possible contamination on storage surfaces.⁽²⁷⁾

Results of environmental monitoring should be associated with biological monitoring⁽²⁸⁾ inserted in a surveillance program in order to establish the workers risk exposure and finally, professionals in handling environments should have access to education programs and standards of practice with verified adherence.^(29,30)

CONCLUSION

The HPLC/UV technique associated to the sample collection with wipe test has shown adequate capacity for 5-FU detection. It was possible to identify the contamination level of surfaces and vials in the handling environment of cytotoxic drugs. This study has also indicated possible routes of exposure, thus reinforcing the need to use PPE and special attention to the possibility of contamination with drug residues in all stages of handling process.

REFERENCES

1. Donner AL. Possible risk of working with antineoplastic drugs in horizontal laminar flow hoods. *Am J Hosp Pharm.* 1978, 35 (8) :900.
2. Hirst M, Tse S., White D.F. et al. Occupational exposure to cyclophosphamide. *Lancet.* 1984, 1(8370):186-188.
3. Polovich M. Safe handling of hazardous drugs. *Online J Issues Nurs.* 2004;9(3):6.
4. Stolar MH, Power LA, Viele CS. Recommendations for handling cytotoxic drugs in hospitals. *Am J Hosp Pharm.* 1983;40(7):1163-1171.
5. Yodaiken RE, Bennett D. OSHA work-practice guidelines for personnel dealing with cytotoxic (antineoplastic) drugs. *Occupational Safety and Health Administration. Am J Hosp Pharm.* 1986;43(5):1193-1204.
6. Ndaw S, Denis F, Robert A et al. Biological monitoring of occupational exposure to 5-fluorouracil: urinary alpha-fluoro-beta-alanine assay by high performance liquid chromatography tandem mass spectrometry in health care personnel. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010, 878(27): 2630-2634.
7. Bonassa EMA. *Enfermagem em Terapêutica Oncológica.* 3 ed. São Paulo: Atheneu; 2005: 23-37.
8. Acampora A, Castiglia L, Miraglia N et al. A case study: surface contamination of cyclophosphamide due to working practices and cleaning procedures in two Italian hospitals. *Ann Occup Hyg.* 2005, 49(7):611-618.
9. Larson RR, Khazaeli MB, Dillon HK. A new monitoring method using solid sorbent media for evaluation of airborne cyclophosphamide and other antineoplastic agents. *Appl Occup Environ Hyg.* 2003,18(2):120-131.
10. Favier B, Gilles L, Giammarile F et al. Contamination of syringe plungers during the sampling of cyclophosphamide solutions. *J Oncol Pharm Pract.* 2005,11(1):1-5.
11. Mason HJ, Morton J, Jones K et al. Cytotoxic drug contamination on the outside of vials delivered to a hospital pharmacy. *Ann Occup Hyg.* 2003, 47(8):681-685.
12. Larson RR, Khazaeli MB, Dillon HK. Development of an HPLC method for simultaneous analysis of five antineoplastic agents. *Appl Occup Environ Hyg.* 2003,18(2):109-119.
13. Turci R, Sottani C, Minoia C et al. Biological and environmental monitoring of hospital personnel exposed to antineoplastic agents: a review of analytical methods. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2003, 789(2):169-209.
14. Micoli G, Turci R, Minoia C et al. Determination of 5-fluorouracil in environmental samples by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl.* 2001, 750(1):25-32.
15. McDiarmid MA, Egan T, Watts SR et al. Sampling for airborne fluorouracil in a hospital drug preparation area. *Am J Hosp Pharm.* 1986, 43(8):1942-1945.
16. Connor TH, Sessink PJ, Harrison BR et al. Surface contamination of chemotherapy drug vials and evaluation of new vial-cleaning techniques: results of three studies. *Am J Health Syst Pharm.* 2005, 62(5):475-484.
17. Palazzo S, Bernardo G, Draicchio F et al. [Guidelines for the health protection and safety of workers exposed to antineoplastic drugs]. *Med Lav.* 1996, 87(3):255-264.
18. Dranitsaris G, Johnston M, Poirier S et al. Are health care providers who work with cancer drugs at an increased risk for toxic events? A systematic review and meta-analysis of the literature. *J Oncol Pharm Pract.* 2005,11(2):69-78.
19. Shortridge LA, Lemasters GK, Hertzberg V et al. Menstrual cycles in nurses handling antineoplastic drugs. *Cancer Nurs.* 1995,18(6): 439-444.
20. Biro A, Fodor Z, Tompa A et al. Immunotoxicity Monitoring of Hospital Staff Occupationally Exposed to Cytostatic Drugs. *Pathol Oncol Res.* 2010,17(2):301-308.
21. Connor TH, DeBord DG, Pretty JR et al. Evaluation of antineoplastic drug exposure of health care workers at three university-based US cancer centers. *J Occup Environ Med.* 2010, 52(10):1019-1027.
22. Wallemacq PE, Capron A, Favier B et al. Permeability of 13 different gloves to 13 cytotoxic agents under controlled dynamic conditions. *Am J Health Syst Pharm.* 2006, 63(6):547-556.
23. Martins I, Della Rosa HV. Considerações Toxicológicas da Exposição Ocupacional aos Fármacos Antineoplásicos. *Rev Bras Med Trab.* 2004;2 118-125.
24. Favier B, Gilles L, Latour JF et al. External contamination of vials containing cytotoxic agents supplied by pharmaceutical manufacturers. *J Oncol Pharm Pract.* 2003, 9(1):15-20.
25. Yoshida J, Koda, Kumagai S et al. Association between occupational exposure levels of antineoplastic drugs and work environment in five hospitals in Japan. *J Oncol Pharm Pract.* 2011, 17(1):29-38.
26. Touzin K, Bussieres JF, Gallant C et al. Cyclophosphamide contamination observed on the external surfaces of drug vials and the efficacy of cleaning on vial contamination. *Ann Occup Hyg.* 2008, 52(8):765-771.
27. Schierl R, Bohlandt A, Nowak D. Guidance values for surface monitoring of antineoplastic drugs in German pharmacies. *Ann Occup Hyg.* 2009, 53(7):703-711.
28. Ziegler E, Mason HJ, Baxter PJ. Occupational exposure to cytotoxic drugs in two UK oncology wards. *Occup Environ Med.* 2002, 59(9):608-612.
29. Sottani C, Porro B, Minoia C et al. Occupational exposure to antineoplastic drugs in four Italian health care settings. *Toxicol Let.* 2011, 213(1):107-115.
30. Turci R, Minoia C, Sottani C et al. Occupational exposure to antineoplastic drugs in seven Italian hospitals: the effect of quality assurance and adherence to guidelines. *J Oncol Pharm Pract.* 2011. 17(4):320-332.