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Quantitative and qualitative control of cytotoxic preparations by HPLC-UV in a centralized parenteral preparations unit

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ABSTRACT

The constantly growing incidence of cancer and long-term treatment are leading to an increasing number of cytotoxic preparations in hospital pharmacies. Security and quality standards of cytotoxic preparations are essential to assure treatment efficiency and limit iatrogenic toxicity. In order to secure the process of cytotoxic preparations; we decided to install a quantitative and qualitative High Performance Liquid Chromatography (HPLC) control of cytotoxic preparations carried inside our pharmacotechnic unit. A 100 μl sample of each preparation was assayed by HPLC with ultraviolet/visible–diode array detection, which enabled the identification of all cytotoxic agents thanks to their characteristic UV spectra. We developed rapid and specific HPLC assays that determined qualitatively and quantitatively the presence of 21 different cytotoxic agents in less than 3.5 min. A fifteen per cent tolerance from the theoretical concentration was chosen in agreement with preparation and dosage bias, and a first period control of more than 4400 preparations revealed that around 7.7% preparations did not conform. The main objective of these controls was to avoid the administration of defective chemotherapies to patients and finally to use their results to identify error factors; as a result we will take corrective measures in order to reduce error frequency.

1. Introduction

Cytotoxic treatments present restricted therapeutic index and the prevention of the iatrogeny plays an important role in the improvement of cancer caring [1,2]. The easiest way to reduce this is to fight avoidable iatrogenic events like errors of prescription, preparation or administration. As a result, computerized prescriptions and preparation of chemotherapies in centralized parenteral preparation units, but also double visual or weighing control during manufacturing have become recommended and widespread practices.

Chemotherapies are not manufactured in batch scale but the dose is adapted to each patient according generally to his body surface, that is why they are considered as magistral preparations. Hence, their analytical control is not required by pharmaceutical regulations; nevertheless the quality assurance step seems necessary from an ethical point of view and for accreditation of health institutions. In fact, dose or drug errors expose patients to non-efficacious treatments or major toxicity. While several publications refer to the existence of errors during cytotoxic preparations only few ones deal with controls of those preparations. When this issue is approached, the method of control proposed is mostly double visual control or weighing [3,4]. Those methods appeared largely insufficient due to their lack of specificity. Some units perform *a posteriori* analytical chemotherapy controls for quality assurance objectives and it predominantly concerns a unique drug, 5-fluorouracil or etoposide, which is considered as a quality indicator [4–8]. But in our case we think that cytotoxic controls play a more important part in the certification of preparations conformity before patient administration [9]. Consequently, we would like to control the cytotoxic preparations on-line in order to avoid administration of defective ones.

In our clinical practices, many patients were in day hospitalisation and medical prescriptions were done the morning after blood formulation determination. As a result, chemotherapy could not be prepared in advance and time between medical prescription and chemotherapy administration had to be as short as possible in order to limit patient's waiting. In order to control online cytotoxic preparations, an HPLC device with diode array detection and with precise technical specifications (six column selector system, special reading cell) was recently acquired. Rapid and specific HPLC assays that allowed qualitative and quantitative post-production controls

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were developed to assure on-line conformity certification before preparation delivery.

2. Materials and methods

2.1. Validation of HPLC assays

2.1.1. HPLC system

The HPLC device Dionex Ultimate U3000 (Dionex, Sunnyvale, USA) included a quaternary pump, an auto-sampler equipped with a column oven, a six columns selector system, a semi-preparative reading cell, diode array detector and the Chroméléon software (Dionex, Sunnyvale, USA) which monitored the installation. In practice, one flow path was used for the FIA (Flow Injection Analysis) and the five other paths were connected to reversed phase C18 columns (AQ+ 150 mm × 4.6 mm, 5 μm pore-size, ProntoSIL®, Bischoff Chromatography, Leonberg Germany); in this work only 4 column paths were necessary. Each column was dedicated to a range of mobile phase composition. Supragradient HPLC grade acetonitrile, formic acid, gradient HPLC grade methanol (Sharlau, Sentmenat, Spain) and micro-filtered water were used as mobile phase. The flow was always 1 ml/min. Every morning columns were pre-equilibrated with their specific mobile phase, and every evening all columns were rinsed and conserved during night in acetonitrile or methanol/water 90:10 (v/v).

2.1.2. Standards and quality control preparations

Standards and quality controls were prepared with commercially available drugs and dissolved in the vehicle commonly used for chemotherapy preparation: water for injection (Aguettan, Lyon, France), 5% dextrose or 0.9% NaCl from Viaflo™ infusion bag (Baxter, Maurepas, France). Standard concentrations were chosen to include usual therapeutic concentrations. Vehicle and drug supplier used for standard and quality control preparations are summarized in Table 1 with standard and quality control values. Standards and quality controls were stored at −80 °C except for Melphalan and Etoposide which were unstable and had to be freshly prepared for each assay.

2.1.3. HPLC assay validation

2.1.3.1. Calibration. For each cytotoxic agent, the standard concentrations ($n = 6$) were quantified once for each one, enabling calibration curve to be plotted and regression line to be determined by the method of the least squares regression. Linearity was evaluated by calculating the correlation coefficient, y-intercept, slope of the regression line and the residual sum of squares.

2.1.3.2. Precision: intra- and inter-day repeatability. Each quality control (QC) was quantified six times on 1 day allowing the determination of accuracy, standard deviation, relative standard deviation and the confidence interval which allowed access to intra-day

Table 1
Definition of dilution vehicle, standards and quality control values for each cytotoxic agent.

Cytotoxic agents	Manufacturer	Vehicle	Standard concentrations (mg/ml)						Quality control values (mg/ml)	
5-Fluorouracil	Fluorouracil Dakota 50 mg/ml Dakota Pharm, Paris, France	0.9% NaCl	40	25	15	7.5	5	0	7.5	
Carboplatin	Carboplatin 10 mg/ml Solutas Pharma Gmbh Barleben Germany	5% dextrose	10	5	2.5	1	0.5	0	2	
Cisplatin	Cisplatin 50 mg/ml Oncotec Pharma Produktion Gmbh Rodleben Germany	0.9% NaCl	1	0.5	0.25	0.1	0.05	0	0.3	
Cyclophosphamide	Endoxan 1000 mg Baxter Oncology Gmbh Halle, Germany	0.9% NaCl	10	7.5	5	2.5	1	0	4	
Cytarabine	Aracytine 100 mg Pharmacie Italia S.p.A. Nerviano, Italia	0.9% NaCl	25	20	5	2.5	0.5	0	4	
Dacarbazine	Déticène 100 mg Thissen Braine l'Allaud, Belgique	5% dextrose	10	5	2.5	1	0.5	0	2	
Daunorubicin	Cerubidine 20 mg Thissen Braine l'Allaud, Belgium	5% dextrose	5	2	1	0.5	0.2	0	0.8	
Docetaxel	Taxotere 20 mg Aventis Pharma S.A. Antony, France	Specific solvent and 5% dextrose	5	2.5	1	0.5	0.25	0	0.4	
Doxorubicin	Doxorubicine Teva 2 mg/ml Pharmachemie B.V. Harlem, Neerland	5% dextrose	2	1	0.5	0.25	0.1	0	0.7	
Epirubicin	Farmorubicine 5 mg Pfizer Italia S.r.l., Nerviano, Italia	Water for injection	4	3	2	1	0.5	0	2	
Etoposide	Etoposide 20 mg/ml Oncotec Pharma Produktion Gmbh Rodleben Germany	5% dextrose	20	5	1	0.5	0.1	0		0.3
Fludarabine	Fludara 50 mg Schering A.G., Germany	5% dextrose	5	2	0.5	0.25	0.1	0	0.5	
Ganciclovir	Cymevan 500 mg Roche, Neuilly-sur-Seine, France	5% dextrose	10	5	3	1	0.3	0	3	
Gemcitabine	Gemzar 1000 mg, Lilly, Suresnes, France	0.9% NaCl	20	10	5	2	0.5	0	7	
Idarubicin	Zavedos 10 mg Pfizer Italia S.r.l., Nerviano, Italia	5% dextrose	1	0.5	0.2	0.1	0.05	0	0.15	
Ifosfamide	Holoxan 2000 mg Baxter Oncology Gmbh Halle, Germany	5% dextrose	20	10	5	2.5	0.5	0	7	
Irinotecan	Campto 20 mg/ml Pfizer Italia S.r.l., Nerviano, Italia	5% dextrose	5	2	1	0.5	0.25	0	1	
Melphalan	Alkeran 50 mg GlaxoSmithKline S.p.A. Parme, Italia	0.9% NaCl	5	2.5	1	0.5	0.2	0	1	
Methotrexate	Methotrexate Merck 100 mg/ml Haust Pharm Gmbh, Walfratshausen, Germany	5% dextrose	15	5	1	0.25	0.05	0	10	0.1
Oxaliplatin	Eloxatine 5 mg/ml Aventis Pharma Dogenham, Essex, UK	5% dextrose	1	0.8	0.4	0.2	0.1	0	0.5	
Paclitaxel	Taxol 6 mg/ml Bristol-Myers Squibb S.c.l. Sermoneta, Italia	0.9% NaCl	1.5	1	0.75	0.5	0.25	0	0.6	