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

A. Delmas [a,*], J.B. Gordien [a], J.M. Bernadou [a], M. Roudaut [a], A. Gresser [a], L. Malki [a], M.C. Saux [a,b], D. Breilh [a,b]

[a] *Pharmacie Hôpital Haut-Lévêque CHU de Bordeaux, Pessac, France*
[b] *EA2968 Laboratoire de Pharmacocinétique Clinique et de Pharmacie Clinique (Groupe PK/PD) Université Victor Segalen Bordeaux 2, Bordeaux, France*

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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.

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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

* Corresponding author. Present address: Institut Claudius Régaud, Laboratoire de Biologie Recherche, 20-24, rue du Pont Saint-Pierre, 31052 Toulouse Cedex, France. Tel.: +33 5 61 42 42 42/4541 (Office).
E-mail address: audrey.delmas@ens-lyon.org (A. Delmas).

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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	

repeatability. To evaluate inter-day variability, quality controls were quantified a second time on a different day and the same parameters, as for intra-day repeatability, were calculated.

2.2. Qualitative and quantitative controls

Chemotherapies were prepared most of all for haematology, internal medicine, gastroenterology and dermatology medical units where patients were mostly in day hospitalisation. According to Good Manufacturing Practices [10], chemotherapies were prepared in rigid isolators located in atmosphere controlled rooms to ensure pharmaceutical workers' security and the sterility of preparations. Three kinds of preparations were realised: infusion bags, syringes and cassettes for portable pump. Currently chemotherapy preparations were controlled by double visual control for limpidity and drug volume addition. For the HPLC control, each preparation was sampled in the isolator just after preparation. A 100 μl sample was collected with a fresh needle and a fresh tuberculin syringe (BD, Drogheda, Ireland). As our preparation volumes were greater than 20 ml, sampling 100 μl was insignificant (less than 0.5%). For cytotoxic agents assayed with a column, a rapid equilibration of 2–3 min was realised after each method switch. For cytotoxic agents assayed by FIA, no equilibration was needed. The use of the Chroméléon software permitted to customize an easy to read report. The latter authorized an easy access to the identification of the agent with its corresponding match factor, the measured concentration, and the accuracy relative to the targeted concentration. The identity of the preparation was confirmed when the match factor was greater than 995 and when the measured concentration was between 85 and 115% of the targeted concentration. For each cytotoxic agent the quality control was weekly determined three times. A recalibration was done every time the measured concentration of the quality control was not between 95 and 105%.

3. Results

3.1. Validation of HPLC assays

Cytotoxic agents to be controlled were chosen for their prescription frequency or for the difficulty of their preparation (numerous containers, arduous dissolution,. . .). Taken together the 21 agents that we chose represented 80% of our preparations. The HPLC-UV methods were optimised to be as fast as possible to allow online control of cytotoxic preparation before administration to patient. Flow Injection Analysis (FIA) was used whenever it was possible. Cytarabine, Dacarbazine, Daunorubicin, Docetaxel, Fludarabine, Irinotecan, Doxorubicin, Epirubicin, Methotrexate, Oxaliplatin and Paclitaxel were controlled by using FIA. FIA led to a retention time of 0.13 min and a total analysis time of 1 min, but the latter was not usable for all drugs. For some drugs like Etoposide, Gemcitabin or Melphalan, we suspected the role of excipients already known to interfere with spectroscopic methods as macrogol, PEG or mannitol. But for other drugs like Carboplatin, Cisplatin, Cyclophosphamide or Ifosfamide, no excipients were mentioned by manufacturers even if several peaks were identified in the chromatogram (example of the Cyclophosphamide chromatogram is shown in Fig. 1). For those agents a classical HPLC assay was developed with a C18 150 mm × 4.6 mm column and we searched a mobile phase that reduced as much as possible the retention time. In order to assess the correct separation of agent and excipients

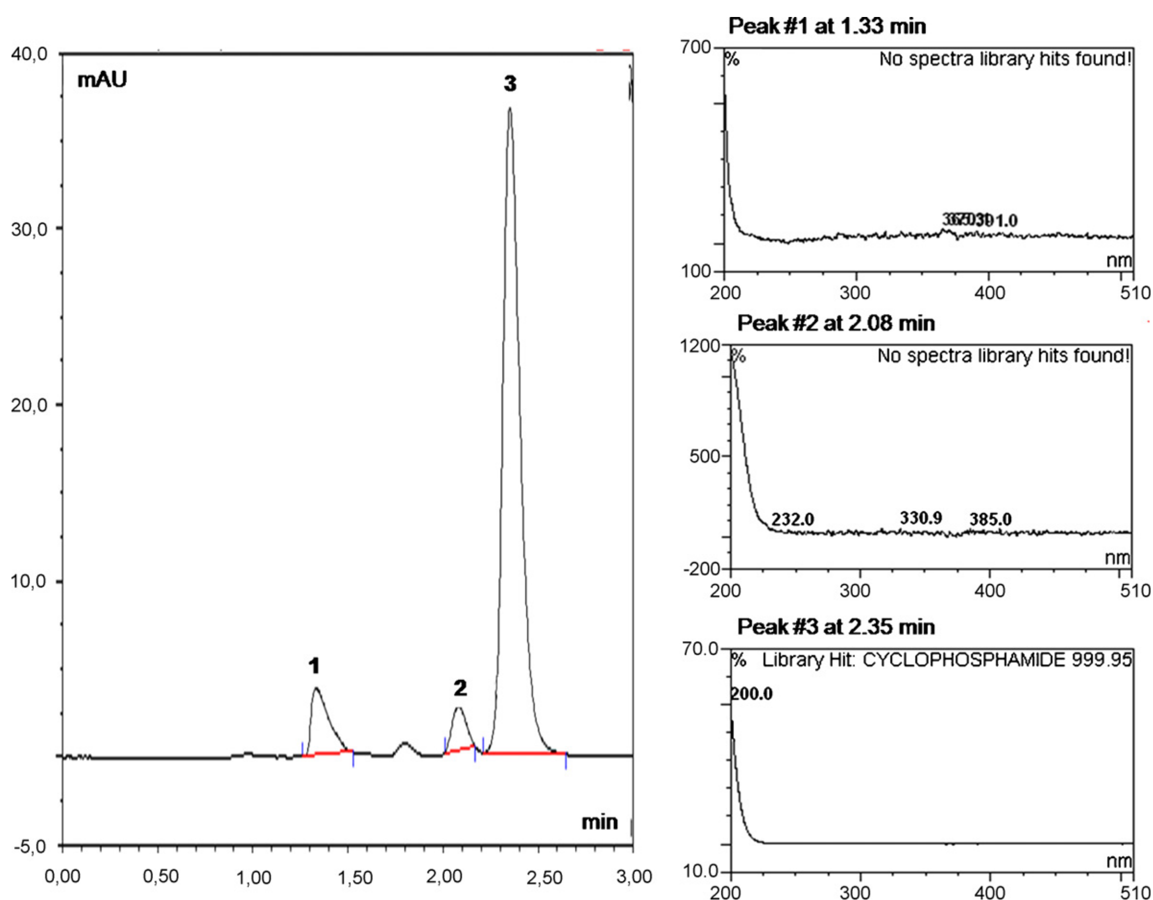

Fig. 1. Chromatogram and spectra of cyclophosphamide. (A) Absorption curve at 200 nm after injection of cyclophosphamide. (B) UV–visible spectra corresponding to the three chromatogram peaks after cyclophophamide injection.

the peak purity was verified. In fact all the assays showed a specific retention time shorter than 3.5 min. To limit equilibration time, an acetonitrile/water mobile phase was preferred. However acetonitrile/water mobile phase was not applicable for only three agents: 5-fluorouracil, idarubicin and melphalan. The mobile phase used for melphalan and idarubicin, was a formic acid 0.1%/acetonitrile whereas a formic acid 0.1%/methanol mixtures were used for 5-fluorouracil because of its polar properties. All these optimised methods required the use of four dedicated pre-equilibrated columns. Column 1 was pre-equilibrated with water/acetonitrile 90:10 (v/v) and used with a mixture containing from 10 to 20% of acetonitrile. Column 2 was pre-equilibrated with water/acetonitrile 40:60 (v/v) and used with a proportion of acetonitrile from 50 to 70%. Column 3 was equilibrated with a formic acid 0.1%/acetonitrile (50:50, v/v) mixture and used with a mixture containing 40 or 60% of acetonitrile. And finally column 4 was equilibrated and used with a formic acid 0.1%/methanol (70:30, v/v) mixture. Mobile phase and column used for each agent are presented in Table 2. In any case, the overload of the column was avoided by injection of small volume that did not produce peak distortion even for the highest standard point. HPLC assays were validated according to ICH guidelines [11].

3.1.1. Specificity

Using the diode array detector, the UV–visible absorption spectrum for each agent was acquired and allowed easy identification. The minimum match score required for identification is 995. In Fig. 2, a comparison of 5 agents' spectra is presented. Specificity data are given in Table 3. The software automatically identified the agent that had the closest spectrum and calculated the match factor. For each agent, a mean of match factors obtained after 10 independent injections is given in Table 3, column mean match factor. Match factors were always over 995 and their average was 999.5. Then to give an indication of the chance of false identifications, a full library spectra comparison was performed. For each agent the mean of the match factors obtained are reported in Table 4. In this case, the mean of those match factors was 547.8. In order to address the problem of specificity, for each agent the maximum non-identification match factor is also indicated in Table 4. In fact, there were two agent couples which could lead to false identifications: ifosfamide/cyclophosphamide and daunorubicin/doxorubicin. Concerning ifosfamide and cyclophosphamide,

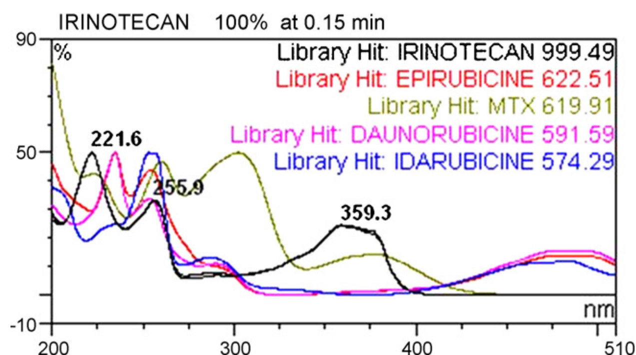

Fig. 2. Comparison of the UV–visible spectra of five agents. In color, spectra of four agents are compared to the irinotecan spectrum with the indication of their respective match factor.

even if they presented very similar UV–visible absorption spectra, they were easily differentiated by HPLC coupled with a column. The spectra of doxorubicin and daunorubicin were also very similar. In this case the chemical structures are so close that it would take at least 5 min to separate them by HPLC-UV. Consequently, identifying these molecules by means of a column could avoid risks of false identification but would also take more time, which is not compatible with our strategy, namely assaying cytotoxic preparations within 4 min.

3.1.2. Linearity

The correlation coefficient, *y*-intercept, slope of the regression line and the residual sum of squares are presented below.

The correlation factors values (R^2) were between 0.997 and 0.999 with an average of 0.999 (14 of the 21 agents presented a correlation factor greater than 0.9990), *y*-intercepts values were between −0.692 and 2.037 with an average of 0.207, slopes of regression line values were between 0.167 and 27.9 with an average of 6.444 and residual sums of squares values were between 0.00085 and 8.06 with an average of 1.16.

3.1.3. Accuracy

According to ICH guidelines, accuracy should be evaluated by a minimum of 9 determinations over a minimum of 3 concentrations levels (e.g. 3 concentrations/3 replicates). In our case, the series

Table 2
Description of HPLC assays.

Cytotoxic agent	Column/FIA	Injection volume (μl)	Mobile phase	Wavelength (nm)	Reference wavelength (nm)	Retention time (min)	Runtime (min)
5-Fluorouracil	Column 4	1	AF/MeOH (70/30)	266	500	2.00	2.8
Carboplatin	Column 1	10	H_2O/ACN (90/10)	230	500	2.07	3.0
Cisplatin	Column 1	10	H_2O/ACN (90/10)	210	500	1.83	2.5
Cyclophosphamide	Column 2	10	H_2O/ACN (40/60)	200	500	2.05	3.0
Cytarabine	FIA	2	H_2O/ACN (50/50)	275	500	0.13	1.0
Dacarbazine	FIA	2	H_2O/ACN (50/50)	325	500	0.13	1.0
Daunorubicin	FIA	5	H_2O/ACN (50/50)	254	Off	0.13	1.0
Docetaxel	FIA	4	H_2O/ACN (50/50)	233	500	0.13	1.0
Doxorubicin	FIA	5	H_2O/ACN (50/50)	253	Off	0.13	1.0
Epirubicin	FIA	2	H_2O/ACN (50/50)	254	Off	0.13	1.0
Etoposide	Column 2	1	H_2O/ACN (50/50)	254	500	2.36	3.0
Fludarabine	FIA	3	H_2O/ACN (50/50)	262	500	0.13	1.0
Ganciclovir	Column 2	5	H_2O/ACN (50/50)	255	500	1.40	2.0
Gemcitabine	Column 1	2	H_2O/ACN (80/20)	270	500	2.29	3.0
Idarubicin	Column 3	10	AF/ACN (60/40)	254	Off	0.13	1.0
Ifosfamide	Column 2	10	H_2O/ACN (30/70)	233	500	2.30	3.0
Irinotecan	FIA	3	H_2O/ACN (50/50)	221	500	0.13	1.0
Melphalan	Column 3	5	AF/ACN (40/60)	261	500	1.60	2.2
Methotrexate	FIA	2	H_2O ACN (50/50)	302	500	0.14	1.0
Oxaliplatin	FIA	20	H_2O/ACN (50/50)	256	500	0.13	1.0
Paclitaxel	FIA	2	H_2O/ACN (50/50)	235	500	0.13	1.0

AF: formic acid 0.1%; H_2O: water; ACN: acetonitrile; MeOH: methanol; FIA: Flow Injection Analysis.

Table 3

Accuracy and precision evaluation of the HPLC method: For each agent, the quality control was assayed six times within 2 days. Then, intra-day and inter-day accuracy but also repeatability were calculated.

Agent	Theoretical quality control concentration (mg/ml)	Accuracy				Repeatability and intermediate variation					
		Measured concentration		Accuracy (%)		Standard deviation		Relative standard deviation (%)		Confidence interval	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
5-Fluorouracil	7.5	7.604	7.51	101.39	100.13	0.195	0.156	2.57	2.08	0.1709	0.0882
Carboplatin	2	2.009	2.02	100.43	101.07	0.0099	0.020	0.49	0.98	0.0078	0.0111
Cisplatin	0.3	0.302	0.308	100.63	102.58	0.0026	0.0064	0.87	2.09	0.0021	0.0036
Cyclophosphamide	4	4.084	4.04	102.09	100.94	0.031	0.054	0.77	1.34	0.0250	0.0305
Cytarabine	4	4.004	4.02	100.09	100.61	0.052	0.093	1.30	2.32	0.0416	0.0527
Dacarbazine	2	1.993	2.05	99.65	102.75	0.057	0.092	2.86	4.46	0.0457	0.0518
Daunorubicin	0.8	0.816	0.775	102.04	96.91	0.030	0.038	3.69	4.88	0.0241	0.0216
Docetaxel	0.4	0.388	0.390	97.06	97.46	0.0034	0.0052	0.87	1.33	0.0027	0.0029
Doxorubicin	0.7	0.716	0.725	102.33	103.59	0.014	0.016	1.94	2.21	0.0111	0.00908
Epirubicin	2	1.981	2.04	99.06	102.07	0.035	0.070	1.78	3.41	0.0282	0.0393
Etoposide	0.3	0.303	0.311	100.89	103.53	0.026	0.021	8.61	6.74	0.0208	0.0118
Fludarabine	0.5	0.527	0.523	105.37	104.52	0.0032	0.0084	0.61	1.61	0.0026	0.0048
Ganciclovir	3	3.114	3.05	103.80	101.75	0.021	0.068	0.69	2.22	0.0171	0.03828
Gemcitabine	7	7.202	6.92	102.89	98.88	0.102	0.303	1.42	4.38	0.0819	0.1714
Idarubicin	0.15	0.146	0.146	97.22	97.44	0.00098	0.0013	0.67	0.87	0.0008	0.00071
Ifosfamide	7	6.915	6.90	98.78	98.62	0.036	0.037	0.53	0.53	0.0291	0.0207
Irinotecan	1	1.006	1.03	100.64	102.93	0.030	0.035	0.75	3.44	0.0064	0.0200
Melphalan	1	0.986	0.999	98.60	99.9	0.012	0.021	1.25	2.10	0.0099	0.0118
Methotrexate	10	10.29	10.3	102.88	102.59	0.012	0.339	0.11	3.36	0.0093	0.1917
	0.1	0.099	0.103	99.00	102.49	0.00044	0.0060	0.44	5.88	0.0003	0.0034
Oxaliplatin	0.5	0.511	0.512	102.23	102.37	0.0024	0.0083	0.47	1.61	0.0019	0.00467
Paclitaxel	0.8	0.598	0.607	99.60	101.15	0.0050	0.014	0.84	2.24	0.0040	0.0077

Table 4
Specificity evaluation of agent identification by the UV–visible spectrum. Identification match factor corresponds to the mean of the match factors obtained after 10 injections of the agent. The non-identification match factor corresponds to the mean of the match factors obtained when the spectrum of each agent was compared to the spectra of all other agent assayed. The maximal non-identification match factor is also indicated. When the latter is greater than 990, the troubleshooting agent is mentioned with, when it is possible, the way to discriminate the two agents.

Agent	Identification match factor	Non-identification match factor mean	Maximal non-identification match factor	Troubleshooting agent	Ways to identify the troubleshooting agent
5-Fluorouracil	999.9	625.0	901.5		
Carboplatin	999.9	605.2	960.4		
Cisplatin	999.9	517.0	923.8		
Cyclophosphamide	999.9	380.5	999.4	Ifosfamide	t_r 3.5 vs 2.05 min
Cytarabine	999.2	711.9	985.8		
Dacarbazine	998.9	207.6	590.3		
Daunorubicin	999.9	527.2	999.5	Doxorubicin	None
Docetaxel	999.3	553.7	962.5		
Doxorubicin	999.8	530.1	999.5	Daunorubicin	None
Epirubicin	999.6	490.6	972.3		
Etoposide	999.7	601.3	959.0		
Fludarabine	999.8	654.0	942.8		
Ganciclovir	999.7	595.3	865.1		
Gemcitabine	999.9	710.9	988.3		
Idarubicin	997.8	538.2	930.8		
Ifosfamide	999.8	393.5	998.7	Cyclophosphamide	t_r 1.5 vs 2.3 min
Irinotecan	999.4	449.9	620.6		
Melphalan	999.6	610.1	960.4		
Methotrexate	999.5	522.9	673.7		
Oxaliplatin	998.9	728.9	949.3		
Paclitaxel	999.8	528.3	963.7		

t_r: retention time.

of therapeutical concentrations that we had to determine were rather narrow. As a result we needed a correct accuracy just in the therapeutical series and not over the whole calibration range. As a consequence and in order to limit cytotoxic manipulations and to simplify the HPLC assay validations, the accuracy was determined by 12 measurements of one concentration: 6 determinations on day 1 and 6 determinations on day 2, except for methotrexate. For methotrexate, two QC concentrations were defined because the latter was usually prescribed at very different doses and concentrations depending on the indication (haematology or autoimmune diseases). For all assayed agents accuracy was between 95 and 105%. All calculated parameters are reported in Table 3.

3.1.4. Precision

Repeatability was assayed by measuring 6 times the QC concentration on two different days for each cytotoxic agent. Then accuracy, standard deviation, relative standard deviation and confidence interval in intra- and inter-day were calculated. All results are reported in Table 3. Inter-day repeatability was very close to intra-day repeatability. According to this result, QC would be quantified only once a week in routine. Finally, the limit of quantification (LOQ) and the limit of detection (LOD) were not determined because they do not pertain to the determination of chemotherapy concentrations which were much greater than LOQ and LOD.

Table 5
Routine controls results classes by cytotoxic agent. During a 130 days of activity, cytotoxic preparations were systematically controlled and results were collected for statistics. Mean accuracy and standard deviation were calculated and non-conform preparations were enumerated in order to identify preparations that need corrective measures.

Cytotoxic agent	Number of controlled preparations	Mean accuracy	Standard deviation	Number of non-conform preparations	Percentage of non-conform preparations
5-Fluorouracil	364	91.3	7.35	52	14.3
Carboplatin	109	95.6	4.49	2	1.83
Cisplatin	129	96.5	4.94	3	2.33
Cyclophosphamide	417	98.2	13.85	23	5.52
Cytarabine	974	101.7	13.50	67	6.88
Dacarbazine	172	97.5	8.45	9	5.23
Daunorubicin	84	93.5	10.15	6	7.14
Docetaxel	23	100.6	14.59	8	34.78
Doxorubicin	222	102.2	38.17	7	3.15
Epirubicin	48	103.9	12.69	11	22.92
Etoposide	255	101.3	14.56	27	10.59
Fludarabine	94	106.3	44.78	14	14.58
Ganciclovir	138	94.4	6.54	3	2.17
Gemcitabine	366	99.6	9.81	22	6.01
Idarubicin	243	95.8	11.34	20	8.23
Ifosfamide	85	103.0	38.64	7	8.24
Irinotecan	310	100.0	9.83	14	4.52
Melphalan	11	92.1	11.5	2	18.2
Methotrexate	112	114.0	43.13	26	23.21
Oxaliplatin	230	103.6	10.08	19	8.26
Paclitaxel	72	101.5	13.67	3	4.17
Total	4458	99.59	14.04	345	7.74

3.2. Results of qualitative and quantitative controls

3.2.1. Results of the preliminary controls

During the first 20 days after controls were set up, controls were voluntary made during low activity periods. These preliminary results revealed that concerning qualitative controls, all controlled preparations conformed. However, concerning quantitative controls, nonconformity affected mostly preparations from powdered drugs (cyclophosphamide, cytarabine, daunorubicin, epirubicin, gemcitabine and idarubicin) with 10% of nonconformity compared to only 3% for drugs from ready-to-use solutions. Generally powdered drugs were directly reconstituted with the liquid of the infusion bag. Consequently the preparation volume was not exactly the one mentioned on the fabrication form and in order to correct this, we chose to reconstitute powdered drugs by volume addition. Concerning preparations of etoposide and docetaxel, they were commercially available in a concentrated viscous ready-to-use solution form. The diluted preparations were difficult to homogenize because the ready-to-use solution and the diluent did not mix instantaneously. This was particularly true for preparations in syringes and it could be responsible of non-representative sampling. As a consequence, a 20% tolerance was accepted for this kind of preparation (syringe of etoposide and infusion bag of paclitaxel).

3.2.2. Routine results of a posteriori controls

After the preliminary controls, all the daily preparations were systematically controlled. The results of a 130 days period are presented in Table 5. The number of daily controls was between 11 and 72 with an average of 34.3. Concerning qualitative controls, as previously, they all conformed. Concerning quantitative controls, global nonconformity rate reached 7.7%. Detailed analysis of those results showed that nonconformity varied according to the agent assayed from 1.83% for carboplatin to 23.21% for methotrexate. However this variation was no more linked to the commercial presentation (powder or concentrated ready-to-use solution) as during the first period of control. The nonconformity rate differed also according to days; the daily nonconformity rate could range from 0 to 30%. We also searched to identify other error factors like manufacturing process, agent prescription frequency or number of preparation done per day, but no correlation could be found.

4. Discussion

The aim of this study was to develop an on-line qualitative and quantitative control of cytotoxic reconstitutions. The majority of patients were in day hospitalisation. In order to reduce patients' waiting, the time spent between computerized medical prescription and patient administration (which includes pharmaceutical validation, manufacturing and quality control) had to be the shortest possible. HPLC was well adapted to fast, one by one, sample control [12]. Moreover, the use of diode array detection allowed qualitative controls with high specificity. For all these reasons, the on-line qualitative and quantitative analysis of a sample with its interpretation had to take less than 3–4 min. As a result the major

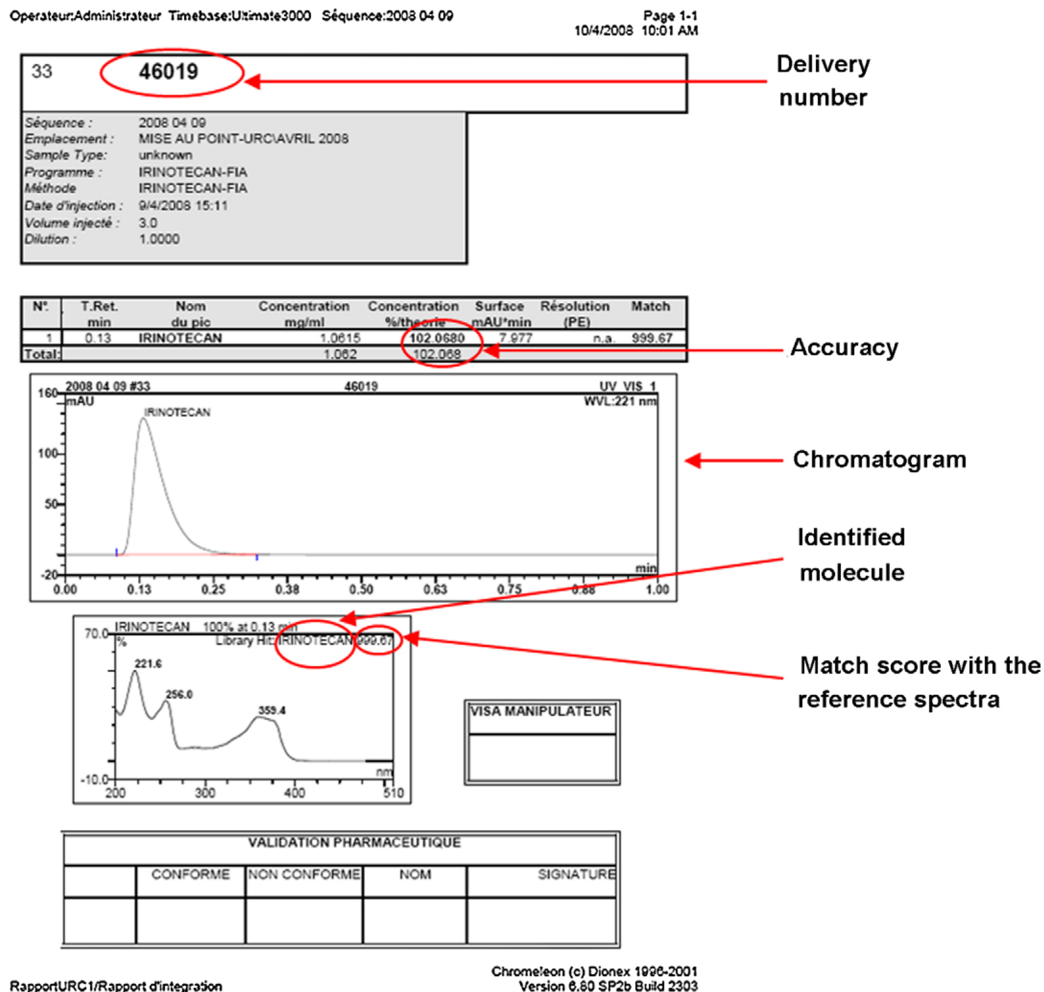

Fig. 3. Editable analysis report.

challenge of this study was, to find for each cytotoxic agent a fast HPLC dosage method, but also to limit the number of different methods to reach this objective. Flow Injection Analysis (FIA) enhanced the speed of the assay, since it took only 1 min. The usual drawback of FIA is that it forbids agent identification by referring to its retention time. In our case, the diode array detection enabled agent identification with more specificity than time retention. Consequently, FIA could be used in order to diminish analysis time without losing the specificity. Over the 21 agents we assayed, we succeeded in controlling 11 agents by FIA which represented more than 50% of our samples. Only one agent needed more than three min to be assayed, but being a very rare prescription (less than 1%), it did not slow the rate of control. Concerning specificity, all agents, except doxorubicin and daunorubicin, were satisfyingly discriminated by choosing a match factor cut off at 995 and in regard to their different retention times. These two agents were not distinguished by FIA but it would be possible if they were assayed with a column. However, this control would take alone, at least 5 min which would slow down our work rate. Finally, because the other 19 agents were readily identified, to avoid any mismatch between doxorubicin and daunorubicin the double visual control was systematically practiced for these two agents.

The majority of cytotoxic agent preparations were very concentrated; as a result, to avoid intermediate dilutions (which would take time, increase risks in manipulation and also introduce new possible errors) our HPLC device was equipped with a semi-preparative reading cell which divided the signal by 30. Consequently, some diluted preparations like vinca-alkaloid chemotherapies could not be controlled with this specific equipment.

For each drug the choice of HPLC-UV or FIA was often linked to the excipients present. So, it appeared dependant of the drug manufacturer. Consequently, the compatibility of agents from other manufacturers with our controls must be considered for the negotiation of the hospital deals. However, the list of excipients is rarely severely modified from one manufacturer to another.

The results presented in Table 5 justified by themselves the importance of an online cytotoxic preparation control. No error of cytotoxic agents had been revealed, but quantitative controls reached 7.7% of nonconformity even with a large tolerance of 15% (and 20% for etoposide and docetaxel preparations). These results were mostly due to insufficient homogenisation or to carelessness in changing syringes and needles. Those poor results underscored the legitimacy of our quality control approach. Even if error rates increased during systematic controls; it seemed that no correlation could be made with cytotoxic agent prescribed frequency, preparation method (with concentrated ready-to-use solution or powdered drug) or daily activity. These results were close to the ones found in other hospitals when controls had been installed [13]. The improvement of defective preparation rates, which values would be between 2 and 3%, could be reasonably expected [5,8,14,15]. For the moment, the cassettes for the portable pump were not controlled because we were not able to sample them without risking an outflow. Next steps will be to control vinca-alcaloids preparations and 5-fluorouracil cassettes. During this first control study an error of 15% was tolerated in order to limit excessive re-manufacturing. After determination of corrective measures, and to improve the precision of preparations, this tolerance will be reduced from 15 to 10%. In order to complete quality assurance of cytotoxic preparations, sterility should be verified, but time factor excluded this kind of control online. Moreover, manufacturing in sterile isolator with sterile drugs was proven to be sufficient to obtain sterile preparations [16]. In practical terms, the short time spent between preparation and administration limits the microbial multiplication.

The Chroméléon software enables the complete customisation of edited report. As a result, we performed an easy to read report presented in Fig. 3: interpretation and validation of the assay were obtained by verifying accuracy and matching score. The investment cost for such a device and its accessories was around 50.000 euros. Routine cost was evaluated at around 10.000 Euros for a total 10.000–12.000 controls per year, which represented less than 1 Euro per analysis and less than 0.2% of the cytotoxic agent budget. In other hospital pharmacies, High Performance Thin Layer Chromatography (HPTLC) is used to make post-production cytotoxic preparation controls, this technique is well-suited to control batches because it assays 50 samples but the time between sampling and results is at least 90–120 min for urgent request and 12–48 h in routine [6]. Moreover, if the nominal investment for the HPLC device we chose was more expensive than an HPTCL one, the routine cost was cheaper. Because over 11 agents could be controlled by FIA, another alternative could be the use of a simple UV measurement with an auto-dilution equipment and a diode array UV instrument. However, this would imply the purchase of two equipments and a higher cost. Moreover, it would also need two kinds of sampling in isolator. Consequently in order to reduce cost and cytotoxic manipulation and to simplify controls we preferred to use for all agents a unique device, an HPLC one.

In conclusion, HPLC was a technique well suited to control chemotherapy preparations, where time between medical prescription, pharmaceutical validation, manufacturing and preparation control had to be as short as possible in order to limit patient's waiting. The cost of these controls appeared to be very reasonable and it should be possible to insert it in the budget of the manufacturing units. This study highlights the feasibility of such a routine control.

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