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Research paper

Contribution of high-performance thin-layer chromatography to a pharmaceutical quality assurance programme in a hospital chemotherapy manufacturing unit

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Abstract

The Department of Clinical Pharmacy (DCP) in the Institut Gustave-Roussy (IGR) is equipped with a high-performance thin-layer chromatography (HPTLC) analytical platform. One of the numerous possible uses of HPTLC is post-production quality control of chemotherapy manufacturing. After 3 years of existence, routine validation of manufactured batches has attained considerable maturity: 24 cytotoxic agents can be controlled in terms of identity, purity and concentration. Approximately 50% of the sampled preparations are assessed. More than 97% were within specifications, 1.6% were not, probably due to incorrect homogenization before sampling; and 1% were not evaluable. Using HPTLC in a hospital manufacturing unit contributes to quality assurance programmes such as accreditation to which the IGR DCP is now committed but also ISO 9001:2000 certification concerning the chemotherapy manufacturing unit.

Keywords: High performance thin layer chromatography; Chemotherapy; Quality assurance; Quality control; Clinical pharmacy

1. Introduction

The Institut Gustave Roussy (IGR) is the leading French research and cancer treatment centre. This institution is deeply involved in a health accreditation process and, as such, is committed to the implementation of a major quality assurance programme. At the end of 2001, this 400-bed comprehensive cancer centre had recorded 91 274 conventional hospitalization days and 12 829 day-hospital stays. The average annual intake of new cases is approximately 10 500 for 55 000 patients on file. Care, research and teaching occupy a major position in such a centre. In addition, the care network policies under development reflect multidisciplinary and holistic patient care for what will become the leading disease, in terms of morbidity and mortality in our regions. During the year 2000, the French government launched a national 5-year plan to fight cancer (Plan National de Lutte Contre le Cancer 2000–2005) focusing on the organization and quality of care so that it is in keeping with the needs and expectations of the population. In parallel, health institutions are involved to a great extent in accreditation procedures [1]. Such a mobilization will bolster the emergence of major quality assurance programmes. The IGR DCP chemotherapy manufacturing unit was submitted to the ISO 9001:2000 Certification Board which approved it in July 2002 [2].

Anti-tumour therapy often combines surgery, radio- and brachytherapy, and even interventional radiology with chemotherapy. With regard to chemotherapy, one of the IGR DCP Functional Units (FU) ensures the production of 75% of the computerized chemotherapy prescriptions which represent approximately 30 000 preparations annually. Ensuring the tracking and quality of the preparations produced by such a system is both demanding and critical given its near-industrial dimensions. Such a process obviously aims at improving the quality of nursing care [3]. In order to give a better response to this major requirement regarding quality, an analytical control interface has been created between both the DCP Production and Quality Assurance and Pharmacotechnical–Pharmacochemical FUs.

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Analytical methods are not presented here. This paper presents the development programme and results after 2 years in service.

2. Material and methods

2.1. Production and Quality Assurance and Pharmacotechnical–Pharmacochemical FU Interface

The IGR Production FU is a centralized technical platform equipped with three laminar air flow cabinets and a huge isolator which provide safe conditions for manufacturing therapeutic solutions. Computerized prescriptions are transmitted from the clinical units via the hospital network to a supervision station for pharmaceutical validation. Respecting manufacturing Standard Operating Procedures (SOPs) allows trained technicians to ensure tracking of dispensed therapeutic objects (bags, syringes, infusors). As the Production FU is itself involved in an ISO 9001 certification process, it has become of paramount importance to enhance prescription safety. This implies the verification and validation (i.e. concentration, identity, purity and physico-chemical properties) of certain preparations (i.e. ready-to-use solutions) so that they are available to the clinical units [4].

During autumn 1998, the Quality Assurance and Pharmacotechnical–Pharmacochemical FU was equipped with an analytical HPTLC (high-performance thin-layer chromatography) platform designed to work in tandem with the Production FU. The qualitative and quantitative analysis involving HPTLC has now attained a remarkably high-performance level and technical maturity [5–8]. Initially designed for industrial purposes, this tool allows the development of post-production quality control for hospital preparations [9]. Running thin-layer chromatography with dosimetric goals (i.e. quantitative analysis) is achieved via the following three successive technical control steps: (1) an automated system intervenes to guarantee the calibrated and reproducible application of the samples to be analysed, (2) the use of diversified, efficient and technologically controlled stationary phases, and (3) the use of a densitometric module allowing multiple wavelength optical reading (i.e. scanner) in order to cover the spectral diversity of the analysed chemical compounds (derivatized or not). Currently, 24 cytotoxic drugs undergo a routine HPTLC analysis.

2.2. Organization of the analytical Quality Assurance System applied to post-production control of chemotherapy

Except for very complex analytical situations, it is now clear that the time required to develop HPTLC dosing methods does not exceed 1 or 2 months. Under the circumstances, 2 years were necessary to validate the current analytical panel (cf. Table 1). Although some new developments are under way, the analytical control interface has been fully operational since January 2001. Briefly, at the end of a codified and controlled manufacturing run, Production FU technicians take a ~500 μl sample of each therapeutic batch. The aqueous or non-aqueous liquid samples (hermetically closed test tubes) are labelled (sampling date, batch number, cytotoxic name abbreviation and dose, solvent nature and volume) and transferred to the control laboratory. Sampling is anonymous (the technician in charge of a manufacturing process is not identified). Samples are treated immediately or stored in adequate conditions (light exposure, temperature i.e. −20 °C, +4 °C or room temperature). According to the physico-chemical characteristics of the solutions (stability, refrigerated/ambient conservation, etc.) and the manufacturing rate (i.e. number of batches manufactured annually), the analyses are submitted to random selection. The time between sampling and the results of analyses varies from 12 to 48 h, according to the laboratory work schedule. A result can be obtained in approximately 90–120 min for a specific urgent request such as incorrect operation during the manufactured process identified by the operator himself. A quantitative assay can be realized on several manufactured batches to identify and stop the delivery of the wrong one. It is important to note that standards and samples can be frozen for some compounds (e.g. paclitaxel, etoposide, cytarabine, gemcitabine, etc.). On the one hand, the use of frozen standards reduces the time required for analysis and, on the other hand, samples that are unstable can be frozen for further assay. The assessment of the temperature storage conditions (i.e. −20 °C, +4 °C or room temperature) has been validated during the analytical validation realized for each developed analytical method.

2.3. Analytical configuration: HPTLC-CAMAG analytical station

The HPTLC-CAMAG analysis station (Merck S.A., Nogent sur Marne, France) is composed of: (1) HPTLC-Vario chamber for mobile phase optimization, (2) TLC Sampler III automated sample applicators, (3) solid Teflon (PFTE) migration chambers, i.e. horizontal tanks enabling sandwich or saturation mode separation, (4) TLC Scanner 3 densitometer driven by CATS 4 software (version 4.05), and (5) Pentium 233 MMX personal computer coupled to an external data backup device.

2.3.1. HPTLC-Vario module

The HPTLC-Vario module is a solid PFTE multi-canal tank. In one operation, six different mobile phases can be assayed simultaneously on line, using a single 10 × 10-cm chromatography plate. In this manner, rapid and semi-empiric optimization of mobile phases becomes possible. The analyst can also use this display for Research and Development purposes with other separation methods, e.g. high-performance liquid chromatography

Table 1
Analytical parameters applied to 24 chemotherapy solutions for post-production quality assessment

Cytotoxic drugs	λ Detection (nm)	LOQ[a] (ng)	*Rf*[b]	Mobile phase composition (v/v)
Fludarabine	270	100	0.15	$CH_3OH/H_2O/CH_3COOC_2H_5$ (60:60:300)
Cytarabine		50	0.28	
Gemcitabine		50	0.49	
Fluorouracil		50	0.69	
Doxorubicin	500	50	0.25	$CH_2Cl_2/CH_3OH/HCOOH/H_2O$ (82:24:2:1)
Daunorubicin			0.35	
Epirubicin	500	50	0.25	$CH_2Cl_2/CH_3OH/HCOOH$ (75:25:1)
Idarubicin			0.35	
Etoposide	290	100	0.20	$CH_3OH/CH_2Cl_2/(C_2H_5)_2O$ (2:45:53)
Carboplatin	510[c]	100	0.30	CH_3COCH_3/H_2O (90:10)
Oxaliplatin		50	0.45	
Cisplatin		50	0.90	
Methotrexate	370	20	0.65	HCl 10^{-3} N/$CHCl_3/CH_3OH$ (16:34:50)
Cyclophosphamide	700[d]	800	0.35	$CH_2Cl_2/CH_3OH/CH_3COOH$ (97:3:2)
Ifosfamide			0.40	
Paclitaxel	227	150	0.25	$CHCl_3/CH_3COCH_3/CH_3OH/NH_4OH$ (80:12.5:7.5:2)
Docetaxel		150		
Irinotecan	366	50	0.45	$CH_2Cl_2/CH_3OH/HCOOH/H_2O$ (82:24:2:1)
Vincristine	274	50	0.25	CH_2Cl_2/CH_3OH (93:7)
Vinorelbine		100	0.40	
Vindesine	274	100	0.10	CH_2Cl_2/CH_3OH (93:7)
Vinblastine		100	0.20	
Melphalan	260	100	0.50	$CH_3COOC_2H_5/CH_3OH/H_2O/NH_4OH$ (60:23:20:2)
Mitoxantrone	608	30	0.45	$CH_2Cl_2/CH_3OH/NH_4OH$ (50:50:7)

[a] Limit of Quantification expressed according to quantity applied and calculated on the first calibration standard adapted to routine use according to batch concentration.

[b] Retention factor.

[c] Reading after derivatization by *para*-nitrosodimethylanilin.

[d] Reading after derivatization by phosphomolybdic acid.

(HPLC). Pre-equilibrating and configuration variants can be studied during the same operating run.

2.3.2. TLC Sampler III automated sample applicators (ATS III)

This module is equipped with a high-precision pneumatic pulverization device involving nitrogen, an inert gas. A mechanized arm, equipped with a syringe and a capillary tube, handles the liquid sample and ensures its application onto adequate-sized chromatography plates (varying from 10 × 10 cm to 10 × 20 cm) [10]. Ten to 3000 nl (between 100 and 500 nl in most cases) of the samples are vaporized in 3–6-mm bands. The analyst programmes the application parameters using ATS III software (version 2.13); it is accurate to within a nanolitre and reproducibility is high (coefficient of variation (CV%) ~0.89) [11]. In order to eliminate cross-contamination, the display is equipped with a capillary rinsing system activated between each application using, in most cases, a methanol/water/isopropanol (1:1:1, v/v/v) mixture in adequate proportions. Several application methods are possible depending on the planned objective. Although we currently use band spraying, a spot contact application is possible, both of which allow up to 64 bands (3 mm) per 10 × 20-cm plate (32 applications on each side of the plate).

2.3.3. Horizontal migration tanks

Using a horizontal migration tank cuts the cost and the time required for the analysis. Convergent migration of a high number of applications can be achieved on the same plate. The PTFE tank is robust and resistant to all kinds of combined solvents and reagents. The frequent choice of sandwich mode vs. saturation mode migration depends on the objective to be achieved. Mobile phase transfer onto the chromatographic support is obtained using a glass junction plate. The mobile phase volume does not exceed 5 ml thus reducing operator exposure risks and of course organic effluent elimination problems. Migration times rarely exceed 5 min for migration distances of about 5 cm.

2.3.4. Stationary phases

Commercially available stationary phases are made of thin uniform silica (or a silica derivative) layers laid on a 0.2-mm glass surface. Phase granulometry is controlled; homogeneous phase granulometry is in fact a key factor in separation quality. A Silicagel 60 F254 support, impregnated with a fluorescence indicator, is used to facilitate the detection of colourless spots of UV absorption. The power and adaptability of HPTLC are also dependent on obtaining phases that are appropriate for separating a large number of chemical compounds [7,12]: (1) normal phases for high molecular weight and apolar substances, (2) reversed phases with ethyl RP2, RP8 or RP18 silica for the separation of diversified species and polar mobile phases, (3) hydroxyproline-impregnated chiral phases for the separation of enantiomers, (4) cyano (CN), diol or amino (NH_2) phases for some specific developments, and (5) various coatings, e.g. aluminum oxide, cellulose or polyamide.

2.3.5. TLC Scanner 3 densitometer

A multi-wavelength densitometer makes quantification possible [13]. Improving the performance of photomultiplicators and the optical quality of scanners has led to the development of reliable systems with wide spectral scanning [9]. The densitometer is driven by a personal computer using the specific CATS 4 software (version 4.05). It is possible to run a large assay palette with this configuration: identifying substances by determining their retention factors ($Rf =$ substance migration distance/solvent migration distance), purity analysis of chromatographic peaks, characterization of absorbance maxima, spectra determination and, of course, quantification. Preferential reading modes involve absorption-reflection or fluorescence-reflection techniques. All wavelengths included between 190 and 800 nm are exploitable, relying on the combined intervention of three sources: (1) a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm, (2) a tungsten lamp emitting a continuous visible spectrum between 350 and 800 nm, and (3) a mercury lamp emitting a discontinuous ray spectrum between 254 and 578 nm (and above all, three high-intensity rays at 313, 366 and 406 nm) adapted for naturally fluorescent substances such as, for example, vinca-alkaloids [14]. CATS 4 software manages data acquisition and optimization, i.e. baseline smoothing and correction, integration window selection, video-integration and reprocessing of chromatography peaks before final integration. Peak surfaces are calculated by the trapeze-log method. Calibration curves are generated with several programmed models which, according to each specific case, depend on a linear or non linear (e.g. Michaelis–Menten) regression linked to an internal or external standard. We then write an exhaustive report of all data collected: statistical information e.g. CV (%), confidence interval, purity rates, which are systematically returned to the operators.

2.4. Summarized procedure for sample treatment for quantification purposes

Samples are placed in snap-ring (PTFE septa) clipped vials. They are arranged in racks housing up to 16 vials. The TLC Sampler III automated device manages the calibrated applications. For a hydro-alcohol–irinotecan (Campto) solution, 16 500-nl bands can be applied in 10 min (30 min for device flushing between applications). After planar migration, plates are dried under a hood (50 °C hot plate) and densitometric reading is performed. The scanner reading rate is approximately 2.5 min/20 samples. Calibration curves are generated by non-linear Michaelis–Menten-like regression and validated by Quality Controls (QC). The limit of quantification (LOQ) calculated for the first calibration standard on the curve is 50 ng for Irinotecan. Due to an analogy between enzymatic and optic saturation phenomena we decided to use this regression model.

3. Results and discussion

The analysis rate is high and can exceed 50 determinations per plate, i.e. up to 250 per day, given the technical capacity of our unit.

The HPTLC-CAMAG device fully adapted for post-production quality control of all types of hospital preparations such as chemotherapy (bags, syringes, infusors), but also morphinomimetic analgesics (infusor pumps) or dry formulations (capsules) [8,15]. It can be used for a broad spectrum of functions: (1) a teaching tool, (2) contributes to intra- or inter-centre validation of cGMP, (3) a mobile phase and spectra optimization system, (4) a development tool for treatment methods for complex matrices, (5) a control platform for generic drugs, (6) a study and optimization tool for container-content interactions.

For an urgent request as mentioned above the manufactured batch whose concentration, identity and purity have been assessed, can only be released for clinical use once the quality control laboratory authorization has been issued. Moreover, for process validation purposes the manufactured batches are assayed after delivery to nurses. Complex solutions can be treated with the device. Fig. 1 shows the simultaneous separation of four DNA nucleotide base analogues during one analytical run: fludarabine (Fludara), cytarabine (Aracytine), gemcitabine (Gemzar) and 5-fluorouracil (Fluoro-uracile Roche). Fig. 2 shows the quantitation of irinotecan (Campto). Table 1 details the elementary chromatographic parameters currently associated with the routine control of 24 cytotoxic drugs in the DCP.

Fig. 3 presents an exhaustive appraisal of HPTLC analyses achieved during the first semester 2002, namely 52.1% of the manufacturing flow (6986 analyses corresponding to 13 412 manufactured batches). Currently, 97.4% of the tested solutions are conform, taking the adopted discrimination criteria into account. The two following points warrant further assessment: (1) although manufacturing rates vary from one drug to another, we have noted that the control rate is not constant and varies from 46 to 97% (average: 61%) and (2) the non-conformity rate of 2.6% can be interpreted as follows: outside specification limits (1.6%) and not evaluable (1.0%). The outside specification limits non-conformity rate (1.6%) include both manufacturing errors and a poor homogenization of the manufactured batches before sampling. Unfortunately, if some of them are not sufficiently homogenized, we cannot attribute the proportion of the manufacturing errors existing

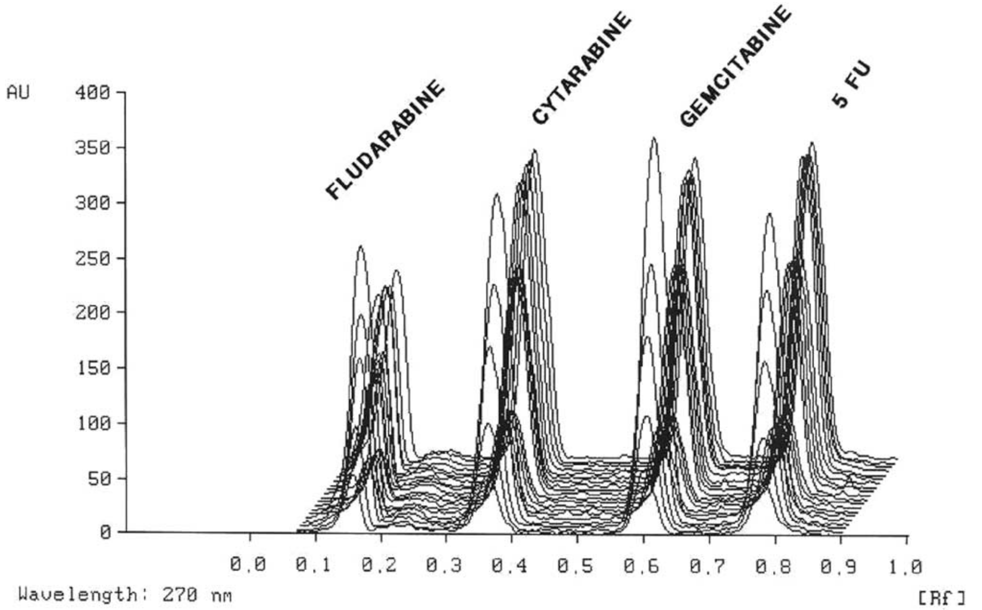
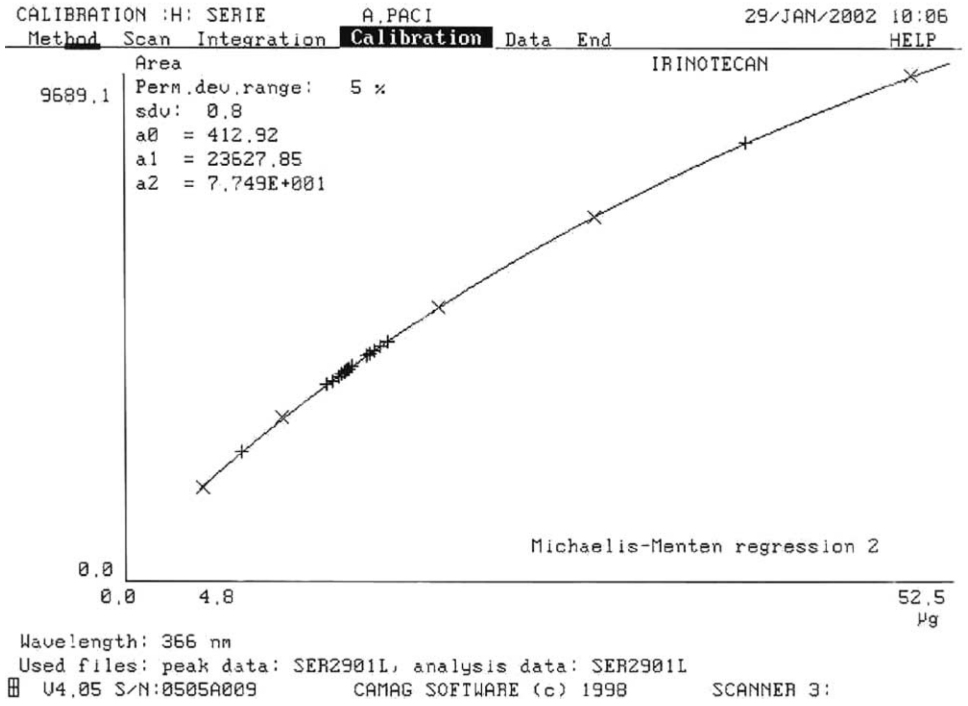


Fig. 1. Simultaneous chromatographic separation of four chemical analogues, i.e. fludarabine, cytarabine, gemcitabine, and fluorouracil. The mobile phase used was: ethyl acetate, methanol, and water (50:10:10 v/v/v).

outside specification limits as non-conformities. Nevertheless, this rate has decreased from 3 to 1.6% between January 2001 and June 2002, which can be considered acceptable in routine practice. This retrospective analysis of non-conformity results has demonstrated that confusion over cytotoxic drug identity never occurred and analyses outside the range (i.e. ± 10% of the theoretical concentration) always seem to be due to incorrect manufactured batch homogenization before sampling. Since July 2001, the HPTLC platform has strongly contributed to operator



CALIBRATION :H: SERIE A.PACI 29/JAN/2002 10:06

IRINOTECAN

Perm.dev.range: 5 %
sdv: 0.8
a0 = 412.92
a1 = 23627.85
a2 = 7.749E+001

Michaelis-Menten regression 2

Wavelength: 366 nm
Used files: peak data: SER2901L, analysis data: SER2901L
V4.05 S/N:0505A009 CAMAG SOFTWARE (c) 1998 SCANNER 3:

Fig. 2. Non-linear calibration with five standards (×) (Michaelis–Menten-like model) (i.e. 100, 200, 400, 600 and 1000 mg/l) used to assay manufactured batches (+) of hydro-alcohol–irinotecan solutions.

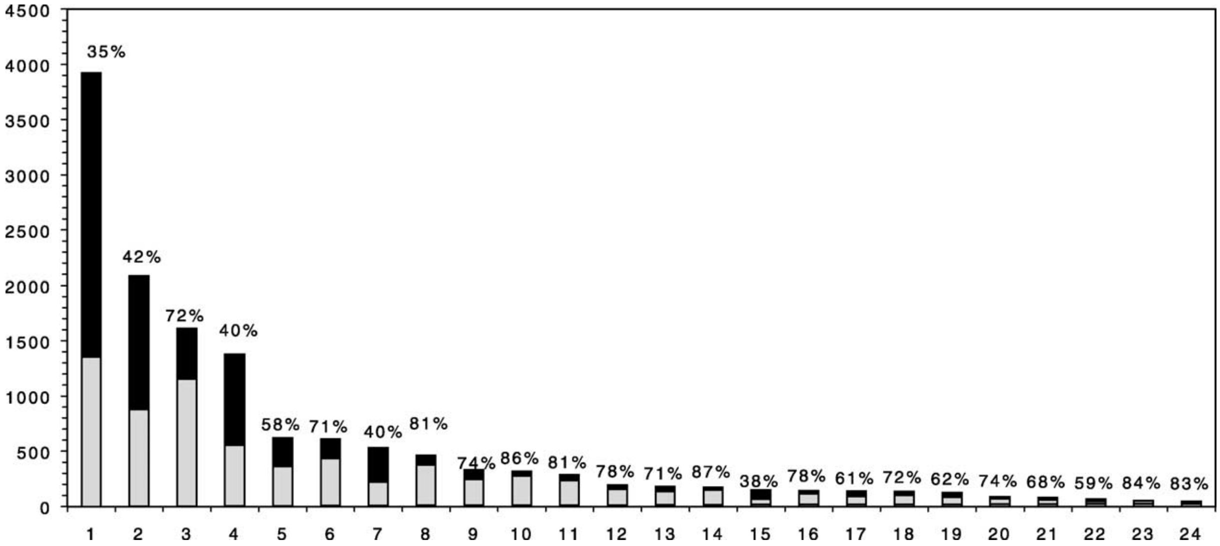


Fig. 3. Exhaustive appraisal of the HPTLC analyses achieved during the first semester 2002 showing the number of manufactured batches (whole bar), the number of completed assays (grey bar) and the corresponding percentage. List of cytotoxic drugs: 1, fluorouracil; 2, cisplatin; 3, etoposide; 4, ifosfamide; 5, cytarabine; 6, cyclophosphamide; 7, carboplatin; 8, doxorubicin; 9, epirubicin; 10, methotrexate; 11, vinorelbine; 12, paclitaxel; 13, oxaliplatin; 14, irinotecan; 15, vincristine; 16, docetaxel; 17, vinblastine; 18, gemcitabine; 19, mitoxantrone; 20, fludarabine; 21, melphalan; 22, vindesine; 23, daunorubicin; 24, idarubicin.

training and involvement in this process and this has resulted in a reduction in the non-conformity rate. This quality management tool is part of the ISO 9001 certification procedure.

We have undertaken an ongoing statistical analysis of non-conformity for each assessed cytotoxic drug in order to determine the optimal control rate of manufactured batches while ensuring the conformity of the whole manufactured flow process.

4. Conclusion

Introducing HPTLC in a hospital pharmacy represents a major step in terms of quality assurance [3,8]. This powerful and adaptable technology now occupies a pivotal position ensuring the identity, purity, concentration conformity and physico-chemical stability studies of injectable drugs prepared in a hospital pharmacy [3]. After 40 months in service and more than 23 500 determinations, of which ~15 000 were conducted in 2002, the HPTLC CAMAG device is now one of the cornerstones of our Quality Assurance System. The nominal investment cost for such a device and its accessories might seem expensive at approximately 14 000 €, with a routine analysis costing less than 1.50 €. Budgetary restrictions that could be an obstacle to acquiring such a system and operating it with qualified analysts can be partially overcome by setting up multi-centric collaborations. Finally, with respect to concerns reducing non-conformity and the joint costs unrelated to quality assurance [16], this analytical tool represents an undeniable contribution to accreditation and certification procedures to which health institutions are now committed in order to improve the quality of nursing care.

Acknowledgements

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