Journal of Pharmaceutical and Biomedical Analysis
30 (2003) 1603–1610

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Identification and quantitation of antineoplastic compounds in chemotherapeutic infusion bags by use of HPTLC: application to the vinca-alkaloids

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Received 24 July 2002; received in revised form 27 August 2002; accepted 4 September 2002

Abstract

An instrumental quantitative high-performance thin-layer chromatographic (HPTLC) method has been developed for the determination of vinca-alkaloids (antineoplastic compounds) in chemotherapeutic infusion bags prepared in a hospital pharmacy. The method uses automated band application onto silica gel plates containing a fluorescent indicator and scanning densitometry of fluorescence-quenched zones of samples and standards. Samples were analyzed to check the content of the active substance against the label declaration of the preparation. The four compounds were separated using the following solvent system CH_2Cl_2–CH_3OH (93:7, v/v). Vincristine (VCR) and vinorelbine (NVB) were assessed in the same run whilst vinblastine (VLB) and vindesine (VDS) were analyzed in a second run. HPTLC allows the identification and the quantitation of more than 20 samples in the same chromatographic run. The analysis of the samples requires 30 min compared with more than 2 h using a typical HPLC method. Moreover, there is no need for a conditioning step, as with HPLC, and each analysis by HPTLC is less expensive.

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

1. Introduction

The Institut Gustave Roussy (IGR) is the leading French center in terms of cancer research and treatment. This institution is deeply involved in a health accreditation process and, as such, is mobilized in favor of the emergence of important quality assurance program.

Antitumoral strategies often combine surgery, radio- and curietherapy, and even intervention radiology to chemotherapy. Concerning chemotherapy, one of IGR's functional units (FU) in its Clinical Pharmacy Department (DCP) presently ensures the production of 70% of computerized prescriptions. This represents approximately 35,000 preparations each year. Ensuring the tracking and production quality of such a quasi-industrial system is both delicate and crucial. The aim of this process is to improve nursing care quality. In order to provide a better response to

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00541-1

this major quality demand, an analytical control interface has been established between both DCP production and quality assurance, and research FUs.

We present here the analytical platform, based on high-performance thin-layer chromatography (HPTLC), which forms an integral part of our pharmaceutical quality assurance program.

High-performance liquid chromatography and gas-phase chromatography are efficient but time-consuming methods for the analysis of numerous samples. HPTLC is a relatively simple and non-expensive assay method, which does not require the use of radio-labeled drug, complex derivatization or expensive equipment. Several methods using thin-layer chromatography have been described in the literature [1–9]. Some of them describe the qualitative analysis of vinblastine (VLB) and some of its metabolites [1,2]. Few works describe the quantitative analysis of VLB in *Catharanthus roseus* [3–5], while Nagy-Turak et al. [6,7] describe the validation of the quantitative planar chromatographic analysis of VLB and deacetoxyvinblastine using one-dimensional over-pressured layer chromatography (OPLC). Nikolova-Damyanova et al. [8] describe a TLC method for quantitative determination of two indole alkaloids which are extract from the plant *Vinca herbacea* as starting material for chemical transformations leading to chemotherapeutic vinca-alkaloids. Wang et al. [9] compared vindesine sulfate quantitation by HPLC and HPTLC. We describe here the use of HPTLC procedures for the simple and rapid quantitative analysis of four vinca-alkaloids for quality program purpose. Although HPTLC is known to have slightly inferior accuracy and repeatability compared with the two most common chromatographic methods (i.e. HPLC and GC), we report here the determination of four vinca-alkaloids (vindesine (VDS), vincristine (VCR), vinblastine (VLB), and vinorelbine (NVB)) used as antineoplastic compounds in a chemotherapeutic regimen in infusion bags. The method has been developed combining thin-layer chromatographic separation and densitometric analysis for the identification and quantitation of each antineoplastic compound.

2. Experimental

2.1. Materials

2.1.1. Chemicals and solvents

The chemical structures of the studied compounds are given in Fig. 1. The substances represented are the commercially available drugs: VDS (Eldisine®, Eli Lilly, France), NVB (Navelbine®, Pierre Fabre Oncology, France), VCR (Oncovin®, Pierre Fabre Oncology, France), and VLB (Velbe®, Eli Lilly, France). Organic solvents were HPLC grade (Carlo Erba, Italy). Furthermore, according to the recommendation of each compound manufacturer, the drugs were diluted in 5% dextrose or 0.9% NaCl solution in polyvinyl chloride (PVC) containers (Maco Pharma, France).

2.1.2. Analytical configuration: HPTLC-CAMAG® analytical station

HPTLC-CAMAG® analysis station (Merck S.A., France) includes: (1) a HPTLC-Vario® chamber for optimization of mobile phases, (2) two TLC Sampler III® automated sample applicators, (3) five solid Teflon (PTFE) migration chambers, i.e. horizontal tanks enabling sandwich or saturation mode separation, (4) a TLC Scanner 3® densitometer controlled by the CATS 4® (4.05 version) software, and (5) a HPTLC-Vario® module for method assessment.

2.2. Methods

2.2.1. Quantitative HPTLC densitometric assay

2.2.1.1. Samples preparations. Standard and working solutions were prepared from commercially available solutions, or crystalline substances, for the construction of calibration curves and quality control (QC) samples. Batch samples were diluted in a hydromethanolic solution (50:50, v/v) allowing the quantitation via the calibration range. Calibration points, QC, and batch samples were contained in snap-ring (PTFE septa) clipped vials. These were arranged in provided racks containing up to 16 vials.

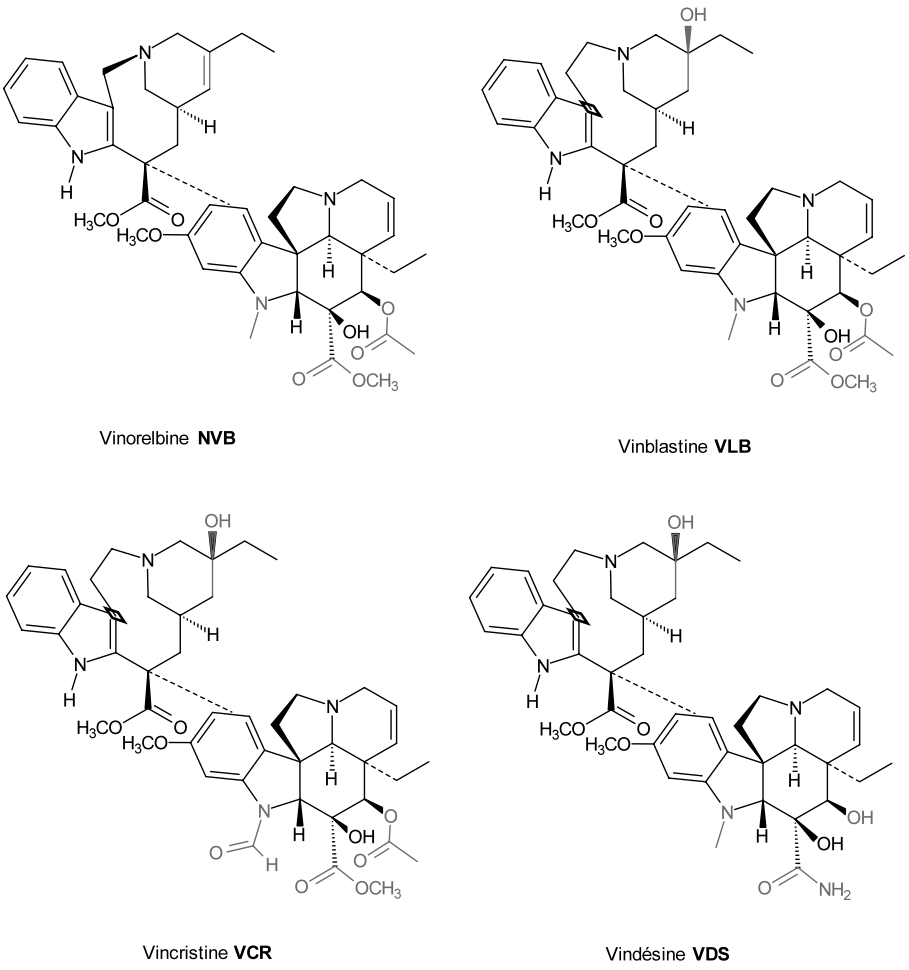

Fig. 1. Vinca-alkaloid structures.

2.2.1.2. Sample application. Two automated TLC Samplers III® devices take into account the calibrated applications. These sample applicators are equipped with a high-precision pneumatic pulverization device, which works in conjunction with an inert gas, nitrogen. A mechanized arm, equipped with a syringe and a capillary tube, takes in charge the liquid sample and its application onto chromatography plates (of adequate size varying from 10×10 to 10×20 cm) [10]. Anything from 10 to 3000 nl (between 50 and 300 nl in most cases) of the samples are vaporized in 3–6 mm bands. Application parameters are programmed by the analyst using the ATS III® software; it is precise up to the nanoliter level and has excellent reproducibility (CV% ~ 0.89) [11]. In order to avoid cross-contamination, the display is equipped with a capillary rinsing system which operates between each application using, in most cases, a methanol–water mixture (50:50, v/v). According to the desired objective, several application methods are possible. If band spraying is used, spot contact applications remain possible. For a hydromethanolic solution, both methods take 10 min for the application of 16 bands measuring 3 mm, equivalent to 300 nl—up to 60 bands measuring 3 mm can be spotted per 10×20 cm plate (30 applications on each side of the plate).

2.2.1.3. Chromatography. The HPTLC-Vario® module is composed of a solid PTFE multicanal tank. This allows simultaneous online testing of six mobile phases, using a single 10 × 10 cm chromatography support. In this way, it is possible to carry out rapid and semi-empiric optimizations of mobile phases.

Stationary phases (manufactured consumable devices) are made of thin uniform silica (or a silica derivative) layers laid on a 0.2 mm glass surface. The phase granulometry is controlled; its homogeneity is one of the key factors contributing to separation quality. A Silicagel® 60 F254 support was used in this study, impregnated with a fluorescence indicator, facilitating the detection of colorless spots absorbing in the ultraviolet (UV).

The choice between the sandwich or saturation migration modes depends on the objective to be reached. Mobile phase transfer on the chromatographic support is obtained using a glass junction plate. Mobile phase volumes do not exceed 5 ml, which minimizes operator exposure risks and significantly reduces disposal problems of the sometimes-toxic organic effluents. Migration times rarely exceed 5 min for migration distances of about 5 cm. Planar migration is followed by plate drying under a fumehood (50 °C hot plate) and by densitometric reading.

2.2.1.4. Densitometric quantitation. Multiwavelength densitometric quantitations are made possible by the use of a TLC scanner 3® densitometer [12]. Obvious improvements in the performances of photomultipliers and the optical qualities of scanners have contributed to the development of reliable systems with large spectral scanning ranges. The device used is driven by a personal computer which operates the specific application CATS 4® (4.05 version). This configuration allows a huge palette of applications to be run: substance identification using retention factor determination (R_f = substance migration distance/solvent migration distance), chromatographic peak purity analysis, absorbance maxima characterization, spectral determination and, of course, quantitation [13]. Preferential reading modes involve absorption-reflection or fluorescence-reflection techniques. All wavelengths included between 190 and 800 nm are exploitable relying on a combination 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 especially three high-intensity rays at 313, 366, and 406 nm) adapted for naturally fluorescent substances (e.g. anthracyclins). CATS 4® software takes in charge data acquisition and optimization, i.e. baseline smoothing and correction, integration windows selection, video integration, and chromatography peak reprocessing before final integration. The peak areas are calculated by the trapeze-log method. Calibration curves are generated with several programmed models which, depending on the cases, rely on a linear or nonlinear (e.g. Michaëlis–Menten) regression, in association with an internal or external standard. The collected data are gathered into a more or less exhaustive report, statistical information, e.g. coefficient of variation (CV), confidence interval, purity levels, etc. being displayed to the operator if required.

2.2.2. Validation of the HPTLC assay

According to "ICH topic Q2A and Q2B" [14,15], the validation of an analytical technique requires selectivity, linearity or regression, accuracy, precision, measurement range limits (LOQ and LOD), robustness, and system suitability. For the quantitation of active chemotherapeutic substances in hospital infusion bags (such combinations being considered as a finished pharmaceutical products), the data elements required according to USP 25 [16] for assay validation and analytical performance are selectivity (specificity), linearity and range, accuracy, and precision.

2.2.2.1. Selectivity. Selective baseline separation was achieved between each chromatographic peak as translated by the densitometer.

2.2.2.2. Calibration function. The calibration function (relationship between peak area and the

amount of substance applied) was determined by Michaëlis–Menten regression over a previously defined range. Each calibration curve was validated using two QCs: the low QC (QC_L) corresponds to the mid-point of the first and the second standard, the medium one (QC_M) corresponds to the mid-point of the third and the fourth while the high one (QC_H) corresponds to the mid-point of the fourth and the last standard.

2.2.2.3. Accuracy. The method accuracy, which gives information about the recovery of the analyte from the sample, was confirmed by analysis using in-system calibration of sample solutions of known substance content. The solutions were spiked with three different known concentrations of each substance, which were assigned as low, medium, and high QC values (QC_L, QC_M, and QC_H). The analysis of each QC sample was repeated six times.

2.2.2.4. Precision. In accordance with ICH guidelines [12,13], precision contains three components: repeatability, intermediate precision, and reproducibility. Here, the last named was not studied.

2.2.2.4.1. Repeatability. Repeatability, expressed as the relative standard deviation (RSD) or coefficient of variation of repeatability (CV_r), consists of multiple measurements of an homogenous sample under the same analytical procedure with the same equipment and in the same laboratory. The analysis of each QC sample was repeated six times.

2.2.2.4.2. Intermediate precision. Intermediate precision evaluates the reliability of the method in a different environment other than that used during development of the method. Determination, expressed as RSD or coefficient of variation of intermediate precision (CV_i), consists of multiple measurements ($n = 6$) of each recommended level studied, i.e. QCs under the same analytical conditions but on multiple days, by different analysts and different equipment except the HPTLC workstation.

3. Results and discussion

3.1. Chromatographic method assessment

3.1.1. Spectroscopic measurement

The UV spectrum of each substance was assessed. Following spectral analysis of each compound, an optimal wavelength was set at 274 nm for the analysis of the four compounds in a single run. The method uses silica gel plates with fluorescent indicator and scanning densitometry of fluorescence-quenched zones of samples and standard.

3.1.2. Mobile phase assessment

Six solvent systems previously developed in our laboratory were tested for the separation of the four vinca-alkaloids using the HPTLC-Vario® module. A dichloromethane–methanol mixture (CH_2Cl_2–CH_3OH, 95:5, v/v) was chosen among these systems. This solvent system was modified in proportions (with or without addition of acetic acid) to improve the chromatographic separation of the four compounds. The finally selected solvent system, CH_2Cl_2–CH_3OH (93:7, v/v) without acetic acid, allows the complete separation of the four compounds as shown in Fig. 2.

3.1.3. Practical optimization for routine use

According to the number of manufactured batches and the number of spotted samples on single silica plates, we decided to analyze NVB and VCR in the same run and VDS and VLB in a second run (a second plate).

3.2. Validation of HPTLC densitometry assay for vinca-alkaloids

3.2.1. Selectivity

Each compound was separated with baseline return as shown in Fig. 2.

3.2.2. Calibration

The calibration function was determined by Michaëlis–Menten regression over 50–500 ng/ml for VCR and 100–500 ng/ml for other compounds such as NVB quantitation as shown in Fig. 3.

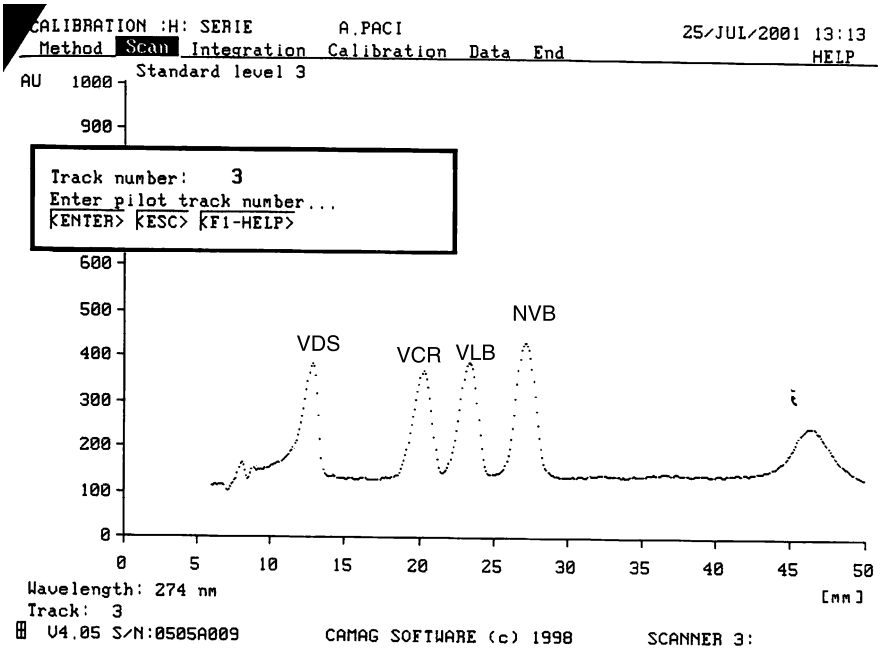

Fig. 2. HPTLC analysis of the four vinca-alkaloids.

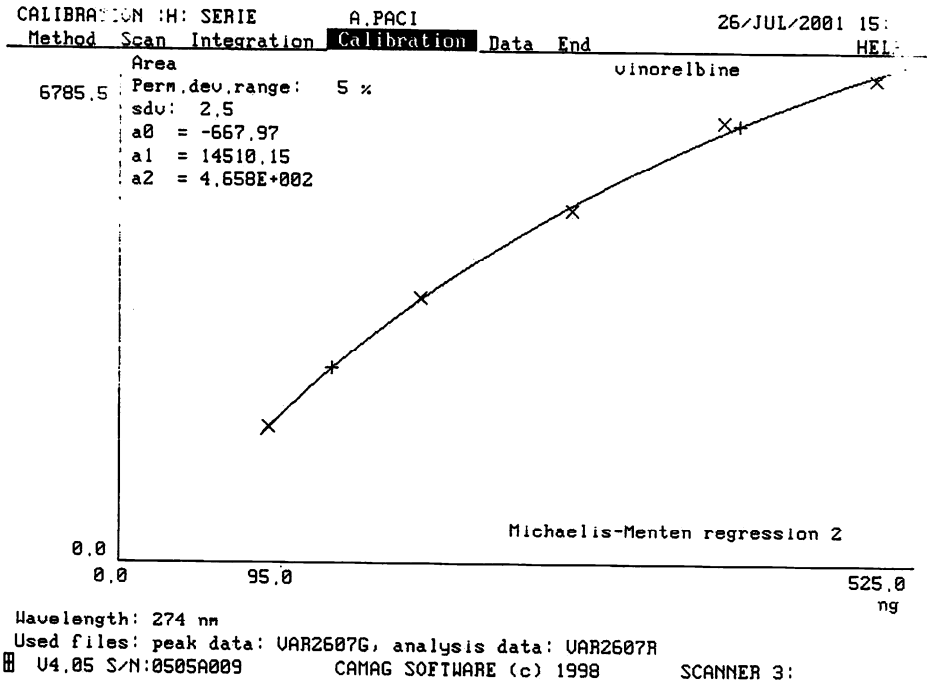

Fig. 3. Nonlinear calibration curve for the five points (i.e. 100, 200, 300, 400, and 500 mg/l) used for the assay of NVB in a methanol–water mixed solution.

3.2.3. Accuracy

The results, summarized in Table 1, show the accuracy of the method according to the mean values and the RSD values calculated from the six analyses for each QC.

3.2.4. Precision

The CV values for repeatability (CV_r) and for intermediate precision (CV_i) for each active substance are summarized in Table 2. All of them were below 5.1% for intermediate precision and repeatability.

This method is accurate and precise, and allows the quantitation of the four vinca-alkaloids. Each compound can be identified through its R_f due to the good selectivity of the method.

3.3. Manufacturing conformity study

Approximately 65.4% of the manufacturing flow of vinca-alkaloids were assayed over 6 months (381 analyses for 583 manufactured batches). Taking into account the adopted discrimination criteria (i.e. $< \pm 10\%$ of the theoretical concentration), 97.8% of the tested solutions conformed. The non-conformity rate of 2.2% (non-conform: 1.15% and invaluable: 1.05%) must be further assessed, although it has been established that such a non-conformity rate can be accepted for routine analyses. From the retrospective analysis of non-conforming results, there was no confusion of cytotoxic drug identity and out of range analyses always appeared to be due to the incorrect homogenization of manufactured batch before sampling. Since July 2001, the HPTLC platform has strongly contributed to operator training and has helped considerably to reduce non-conformity rates for preparations. This analytical method allows the quantitation of the four vinca-alkaloids with good confidence according to the intended purpose. With several other HPTLC methods already developed for a total of 24 cytotoxic agents, the HPTLC platform has become a major quality management tool participating in the ongoing ISO 9001 certification procedure for the production unit.

Table 1
Results of the accuracy study of each active substance, i.e. VCR, NVB, VDS, and VLB

	VCR	NVB	VDS	VLB
QC_L				
Mean	73.4 (75)	152.0 (150)	150.6 (150)	150.9 (150)
RSD	2.58	7.70	3.56	4.36
QC_M				
Mean	181.1 (175)	251.2 (250)	251.5 (250)	245.5 (250)
RSD	6.72	11.05	8.68	5.88
QC_H				
Mean	231.3 (225)	443.7 (450)	452.8 (450)	444.9 (450)
RSD	11.43	8.44	15.38	9.34

Values in brackets correspond to the target values; the mean and RSD values were calculated on six different measurements.

Table 2
Results of the repeatability and the intermediate precision study of each active substance, i.e. VCR, NVB, VDS, and VLB

	VCR	NVB	VDS	VLB
CV_r (%)				
QC_L	2.1	1.4	2.7	2.3
QC_M	1.8	2.0	2.6	1.3
QC_H	0.7	2.2	3.5	2.7
CV_i (%)				
QC_L	3.5	5.1	2.4	2.9
QC_M	3.7	4.4	3.5	2.4
QC_H	4.9	1.9	3.4	2.1

The mean and CV values were calculated on six different measurements.

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