Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Anal. Methods*, 2025, **17**, 3274

Rapid quantification and classification of five vinca alkaloids used in cancer therapy by flow injection analysis with UV detection and chemometrics†

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Vinca alkaloids represent a major class of antineoplastic agents used against cancer. They are prepared in centralized production units by pharmacy technicians. Control of the preparation is indispensable to secure their preparation and avoid any errors, which can have serious consequences for the patient because antineoplastic agents are very toxic. Analytical quality control was proven to be the most efficient control to ensure the right drug at the right dose to the patient. The study focused on vinca alkaloids: vinblastine, vincristine, vindesine, vinflunine, and vinorelbine, in the form of commercially diluted solutions in 0.9% NaCl at therapeutic concentrations. The aim of this study was to develop an analytical methodology for quality control capable of discriminating and quantifying these molecules. The primary objective was to assess the capability of Flow Injection Analysis with UV detection (FIA-UV), combined with chemometrics, for rapid classification and quantification of these alkaloids. A rapid High-Performance Liquid Chromatography with UV-visible detection (HPLC-UV) method was also developed and established as a reference standard. HPLC-UV discrimination was based on retention time, whereas FIA-UV relied on spectral analysis. Therefore, to improve discrimination in FIA-UV, Partial Least Squares Discriminant Analysis (PLS-DA) was incorporated. FIA-UV achieved 100% sensitivity and specificity in discriminating the five alkaloids, demonstrating non-inferiority to HPLC-UV. This method offers a streamlined workflow, reduces iatrogenic risk, and is well suited for antineoplastic agent preparation environments.

Received 26th February 2025
Accepted 25th March 2025

DOI: 10.1039/d5ay00325c

rsc.li/methods

1. Introduction

Cancer chemotherapy is a treatment consisting of the administration of drugs for systemic use which have an action on tumor cells by killing them or preventing them from proliferating.

Antineoplastics are considered high-risk drugs. They may have a narrow therapeutic range associated with high toxicity. Vinca-alkaloid drugs (vincristine, vinblastine, vinorelbine, vindesine, and vinflunine) hold a crucial place in the therapeutic arsenal against cancer.[1] They are the earliest developed microtubule-targeting agents and approved for clinical use, and have been used as agents for many years in the treatment of such cancers as hematological, lymphatic neoplasms and solid tumors of the breast, lung, colorectal or urothelial cancers.[2] Short and long-term side effects constitute a substantial drawback of vinca-alkaloids with high hematologic toxicities or peripheral neuropathy for example. Regular surveillance strategies are necessary and modification of the administered dose, use of alternative treatment or cessation of chemotherapy in case of severe, life-threatening toxicities seem to be the most effective strategies to control the adverse effects. Ensuring the quality of the preparations and limiting the risk of medication errors are of paramount importance in the hospital setting, where the accuracy of treatments is decisive. Safety measures have been taken at each stage of the chemotherapy circuit from prescription to administration. For many years, injectable chemotherapy treatments have been prepared in centralized production units under pharmaceutical responsibility.[3–5] This centralized production in dedicated pharmaceutical units allowed safety in terms of healthcare exposure, management of good preparation practices, cost reduction and patient safety to guarantee the right drug at the right dose and at the right patient. Analytical quality control (AQC) of injectable preparations, performed prior to patient administration, ensures safety by verifying correct drug and dosage, and detecting preparation errors. This involves two primary objectives: precise quantification and accurate molecular discrimination. Because AQC is performed between the end of the preparation and the administration to the patient, rapid analytical techniques are essential

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5ay00325c

to maintain workflow efficiency and avoid delaying chemotherapy. As a result, ultra-fast analytical methods for quality control are developed to cope with the high production flows. Among them, spectroscopic techniques such as UV-visible, infrared, or Raman have been applied to the quality control of cytotoxic drugs[6–13] and were rapid approaches providing spectral information related to the structure of molecules from which discriminative analysis should be based. However, discriminating between molecules with very similar structures using UV-visible spectroscopy can be challenging.[14,15] To address this challenge, chemometrics can be a valuable approach. Chemometrics involves the application of mathematical and statistical tools to chemical analysis. A prominent example is partial least squares (PLS) regression, a widely employed chemometric method for analyzing spectral data. Therefore, separation techniques can be associated to discriminate molecules by their retention time.[16] The choice of a method must be carefully considered, taking into account its quality, reliability, and security.[17,18] These approaches require a rigorous validation of the discrimination before its routine application to avoid the discrimination error corresponding to two situations. The first one corresponds to the non-identification of the wrong drug in the preparation with serious potential consequences for the patient. The second corresponds to a lack of identification of the drug in the preparation, leading to the destruction of the preparation with a significant financial impact regarding the high cost of the anticancer drugs.

In this context, the five commonly prescribed and approved for clinical use vinca-alkaloids were studied: vincristine, vinblastine, vinorelbine, vindesine and vinflunine. Their discrimination by the UV-visible spectrum is challenging due to the close structure of these molecules. Although several HPLC methods have been developed for the analysis for plant or plasma samples,[19–21] they do not simultaneously analyze all five vinca alkaloids used in cancer therapy. More recently, a high-performance thin-layer chromatographic method was proposed for the quantification of vinca alkaloids;[22] however, it requires a minimum analysis time of 30 minutes. These methods predominately focused on quantification, while neglecting the classification of the compounds. Therefore, two distinct analytical approaches using UV-visible spectroscopy were employed. First, a High-Pressure Liquid Chromatography technique with UV detection (HPLC-UV) was established as a reference method, achieving discrimination of the five vinca alkaloids through retention time analysis. Second, a Flow Injection Analysis method with UV detection (FIA-UV) was developed, utilizing spectral discrimination. FIA enables direct UV spectral measurement of molecules injected into a flow stream without prior separation, using the same materials as HPLC but without a column. These two techniques facilitate Analytical Quality Control (AQC) within a closed system, allowing pharmaceutical technicians to perform direct sampling from sealed vials within an isolator at the end of preparation, minimizing the risk of contamination to the user.

The objective of this study was to develop and assess two analytical methodologies, FIA-UV and HPLC-UV, for the simultaneous discrimination and quantification of the five vinca alkaloids. We then aimed to establish the non-inferiority of FIA-UV relative to HPLC-UV for the analytical quality control of chemotherapy preparations within a hospital setting.

2. Experimental

2.1. Chemicals

All the solvents are of analytical grade. Di-sodium hydrogen phosphate dihydrate (Na_2HPO_4, $2H_2O$) and sodium dihydrogen phosphate dihydrate (NaH_2PO_4, $2H_2O$) were obtained from Prolabo VWR (Fontenay-sous-Bois, France). Acetonitrile (ACN) was from Sigma-Aldrich (Saint-Louis, USA). Ultrapure water was obtained from a MiliQ® purification station (Millipore, USA). All solutions were filtered through a 0.45 μm membrane (Durapore® 0.45 μm HV, Merck, Darmstadt, Germany) before use.

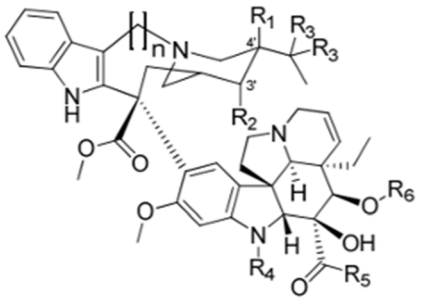
The following pharmaceutical products of the vinca-alkaloids are ready-to-use preparations and their chemical structures are shown in Fig. 1: vincristine sulfate at 2 mg mL^{-1} (VCST from Teva, France), vinflunine ditartrate at 25 mg mL^{-1} (VINF: Javlor®, Pierre Fabre, France) and vinorelbine ditartrate at 10 mg mL^{-1} (VINO: Navelbine®, Pierre Fabre, France). Vinblastine sulfate at 10 mg mL^{-1} (VBST: Velbe®, EG Labo, France) and vindesine sulfate at 1 mg mL^{-1} (VDSN: Eldisine®, EG Labo, France) were reconstituted in 0.9% sodium chloride aqueous solution (NaCl 0.9%) (FreeFlex®, Fresenius Kabi, France).

The excipients of pharmaceutical products were water for injection (VCST, VINF, VINO), sulfuric acid (VBST), sodium hydroxide (VCST) and mannitol (VCST, VBST, VDSN).

2.2. Instrumentation and analytical conditions

The analysis by FIA and by HPLC was carried out on two Ultimate 3000 LC® HPLC systems named "HPLC 1" and "HPLC 2" (Thermo-Fisher Scientific, USA) respectively. The detection was carried out using a UV-visible diode array detector (DAD) equipped with a detection cell with an optical path of 0.4 mm for HPLC 1 and 10.0 mm for HPLC 2. Spectral acquisitions were performed from 200 to 400 nm. HPLC 1 and HPLC 2 were used for HPLC or FIA analysis depending on the concentrations of therapeutic range to avoid sample dilution before analysis. Analysis and data collection were performed using Chromeleon® 7.2 software (Thermo-Fisher Scientific, USA).

2.2.1. Flow injection analysis conditions. The FIA analyses were thus carried out on HPLC 1 for VINF and on HPLC 2 for VBST, VCST, VDSN and VINO. The vector solvent used was ultrapure water at a flow rate of 1.5 mL min^{-1}. The flow rate was optimized to minimize the analysis time while ensuring that FIAgrams were sufficiently resolved regarding the acquisition frequency. Water was selected as the solvent, aligning with the aqueous formulations of medications. This choice mitigates dispersion and peak broadening effects prior to the detection. Furthermore, water is cost-effective, environmentally benign, and does not create clogging within the system, as vinca alkaloids are water-soluble. The FIA injection volumes were set at 5



	n	R_1	R_2	R_3	R_4	R_5	R_6
Vinblastine	2	OH	H	H	CH_3	OCH_3	$COCH_3$
Vincristine	2	OH	H	H	CHO	OCH_3	$COCH_3$
Vindesine	2	OH	H	H	CH_3	NH_2	H
Vinflunine	1	H	H	F	CH_3	OCH_3	$COCH_3$
Vinorelbine	1	$\Delta^{3'-4'}$		H	CH_3	OCH_3	$COCH_3$

Fig. 1 Chemical structure of the five vinca-alkaloid drugs.

μL for VBST, 15 μL for VCST, 6 μL for VDSN, 3 μL for VINF and 1 μL for VINO. The injection volumes were optimized based on 2 parameters: the response factor for each vinca (which are similar) and the therapeutic concentrations of each compound. The goal was to achieve a signal that does not saturate at the highest concentration, while maintaining a sufficient signal-to-noise (S/N) ratio at the lowest concentration to ensure measurements were above the lower limit of quantification. Quantitative analyses were performed at 269 nm for VBST, VDSN, VINF and VINO and at 256 nm for VCST.

2.2.2. High performance liquid chromatography conditions. Chromatographic analyses for the five vinca-alkaloids were carried out on HPLC 2 equipped with a Polaris 3 C18-A column: 50 × 4.6 mm, 3 μm particle size, (Agilent Technologies, Netherlands) at 30 °C. The mobile phase was an ACN/0.025 M phosphate buffer mixture of pH = 7.25 (53/47; V/V) at a flow rate of 1.6 mL min^{-1}. The HPLC injection volumes were set at 10 μL for VBST and VDSN, 20 μL for VCST and 1 μL for VINF and VINO. Quantitative analyses were performed at 269 nm for VBST, VDSN, VINF and VINO and at 256 nm for VCST.

2.3. Sample preparation

Several commonly used vinca-alkaloids drugs have been prepared by diluting the stock solution (ready-to-use or extemporaneously reconstituted solution) with 0.9% chloride sodium at different concentrations corresponding to the therapeutic range: 0.030–0.120 mg mL^{-1} for VBST, 0.010–0.046 mg mL^{-1} for VCST, 0.015–0.070 mg mL^{-1} for VDSN, 2.0–6.0 mg mL^{-1} for VINF and 0.25–0.70 mg mL^{-1} for VINO (see ESI, Table S1†). Dilutions were made with a Microlab 600 series dilutor (Hamilton, Switzerland). All the samples were gathered in glass vials (Interchim, France) and stored at +4 °C before analysis.

Three sets of data were acquired: *i.e.* the calibration set (n = 75, 15 for each molecule) corresponding to the calibration range to develop the quantification and discrimination models with 5 concentration levels, each range being performed every day for 3 consecutive days, (ii) an internal validation set (n = 135, 27 for each molecule) corresponding to the quality controls to validate and optimize the model. It includes 3 levels of concentration repeated 3 times, each sample being analysed each day for 3 consecutive days and (iii) an external validation set (n = 150, 30 for each molecule) to validate the predictive capacity of the quantification and discrimination models. This dataset includes true-life samples within the therapeutic range.

72 samples per vinca-alkaloid giving a total of 360 samples were analyzed by the two methods to assess the performance of FIA and HPLC methods for discriminative and quantitative analyses.

2.4. Data analysis

2.4.1. Discriminant analysis. By HPLC, the discrimination is carried out based on the retention time. A range of retention times are defined for each vinca-alkaloid corresponding to the mean retention time and the 95% confidence interval from the calibration set. The assignment of unknown samples to a class is carried out if its retention time is included in one of the ranges of retention time, otherwise the sample is not assigned.

By FIA, the discriminant analysis was carried out using the open-source R Studio® software: R core team (2023) (R: a language and environment for statistical computing, Vienna, Austria), version 2023.06.0+421. The qualitative analysis was performed using a multivariate analysis approach. Various normalizations of spectra were considered: normalization by the mean spectrum, by the total area and by the absorbance at 269 nm. Partial Least Squares Discriminant Analysis (PLS-DA)[23] was used to reduce the spectral data and thus differentiate the samples of the five vinca-alkaloids. Discriminant analysis, including PLS-DA, was conducted using the Caret[24] and mdatools[25] packages.

PLS-DA, a linear, supervised classification algorithm, enhances the differentiation of sample groups by maximizing

the covariance between independent UV spectra (X) and dependent vinca alkaloid classes (Y). A linear subspace of the explanatory variables is identified, enabling the prediction of Y based on a reduced set of latent variables (LVs).[26] To prevent the risk of overfitting, the optimal number of LVs is determined using the training set to achieve maximum prediction accuracy. A key advantage of PLS-DA lies in its ability to effectively handle highly collinear and noisy data, which are common characteristics of spectral measurements.[27] To determine the optimal number of latent variables, leave-one-out cross-validation was performed on the internal validation set. The number of LVs yielding the highest accuracy, defined as the percentage of correctly classified samples, was selected.

After the selection of the spectral range and the pretreatment by the chemometric approach developed on R Studio®, the method was transposed to the spectral correlation algorithm of the Chromeleon® software. The degree of similarity (match) between two spectra was determined using numerical methods and subtle difference can be quantified allowing correct identification of the five vinca-alkaloids based on spectral matching. Several algorithms are suitable to quantify spectral similarity.[28] Among them, the least square matching method is widely available on HPLC software. This method yields the sum of the squared absorbance deviations at each wavelength to determine an average square deviation between the reference and unknown spectra. This step-by-step wavelength analysis offers precision to measure the similarity between two spectra. For each vinca-alkaloid, 15 spectra of the calibration set were recorded in the library spectra as reference spectra. For each unknown sample, the UV spectrum is compared to the set of 75 spectra recorded in the spectral library on a scale from 0 to 1000. A match score varying from 0 (no match) to 1000 (perfect match) is calculated according to the similarity between the unknown spectrum and the library spectra.

The closer the score obtained is to 1000, the greater the probability of the sample matching the molecule identified by the software. The performance of the model was evaluated using a confusion matrix constructed from the first likelihood score of each sample in the external validation set.

The performance parameters corresponding to the sensitivity, specificity and accuracy were evaluated through a matrix of confusion carried out on the external validation set. Sensitivity is the ability of a method to correctly classify a positive as a positive sample. Specificity measures the ability of a method to correctly classify a negative sample as negative. Then, accuracy assesses the overall performance of the method. They are calculated as follows:

$$\text{Sensitivity}(\%) = \frac{\text{true positive}}{\text{true positive} + \text{false negative}} \times 100$$

$$\text{Specificity}(\%) = \frac{\text{true negative}}{\text{true negative} + \text{false positive}} \times 100$$

2.4.2. Quantitative analysis. Quantitative analysis of the data from the FIA and HPLC was performed using Excel® software. Mean comparisons were made by Student's test using R Studio® software, version 2023.06.0+421. The quantification is carried out by the construction of a linear regression model of the type $Y = \beta_0 + \beta_1 X$ with X corresponding to the concentration and Y the area of the peak at 269 nm for VBST, VDSN, VINF and VINO and 256 nm for VCST obtained by FIA-UV or HPLC-UV. Quantitative analysis methods based on HPLC-UV and FIA-UV have been developed and analytically validated in accordance with ICH guideline Q2 (R1).[29] The chosen validation specifications are for linearity ($R^2 > 0.995$), trueness between 90% and 110%, repeatability and intermediate precision <3%. The tolerance intervals are calculated using the validation set at 90% of the β tolerance and compared to the acceptance limits set at ±10%. The method of Bland–Altman was used to assess the concordance of the assay of vinca-alkaloids (VBST, VCST, VDSN, VINF and VINO) between FIA-UV and HPLC-UV and was performed on the external validation set. The Bland–Altman method is based on the quantification of the agreement between two quantitative measurements by studying the mean difference and constructing limits of agreement. The plot analysis is a way to evaluate the agreement between the FIA and HPLC methods. The resulting graph is a scatter plot XY, in which the Y axis shows the difference between the HPLC and FIA measurements and the X axis represents the average of these measurements. Predicted concentrations were calculated from previously selected linear regression models for HPLC and FIA. The bias (d) and the standard deviation (σ) of the differences 1.96 were calculated. The systematic error (SE) and the total error (TE) of the two methods were predefined at 3% and 15% respectively. The transposability is validated if the 95% confidence interval of d (CId) is equal to or less than the limits of the SE and the lower and upper limits of the 95% confidence interval of $\pm 1.96\sigma$ for differences equal to or less than the limits of the SD.

In addition, on this external validation set and for each sample, the relative error was calculated between the concentration measured by analytical methods and the theoretical concentration. The relative error on the concentrations predicted to release a chemotherapy preparation was routinely set at ±15%. This interval was established based on the variability inherent in the diluent infusion bag filling (5% dextrose or 0.9% sodium chloride), the precision of the syringe, and the analytical error associated with the method.

3. Results and discussion

A total of 72 samples per vinca-alkaloid corresponding to 360 samples were analyzed by HPLC and FIA methods.

$$\text{Accuracy}(\%) = \frac{\text{true positive} + \text{true negative}}{\text{true positive} + \text{true negative} + \text{false negative} + \text{false positive}} \times 100$$

3.1. Discriminant analysis

Two analytical strategies have been studied to allow the discrimination of vinca-alkaloids by a separative technique based on retention times and by a non-separative technique based on a multivariate analysis of spectral data by chemometrics.

3.1.1. Optimization of the HPLC method. A multivariate optimization of ultra-rapid chromatographic separation of vinca alkaloids was done in a previous study. Acetonitrile%, the mobile phase flow rate and the pH of the phosphate buffer were the selected optimization parameters to reduce the sample time analysis with satisfactory resolution. The pH of phosphate buffer was a critical parameter on the retention of vinorelbine and optimal resolution was obtained for a pH of 7.25. The flow rate at 1.6 mL min^{-1} and the ACN/phosphate buffer mixture (53/47; V/V) were the optimal conditions to reduce the sample time analysis to 2.0 min.

Based on the calibration set data, specific ranges of retention times were defined for each drug to guarantee their discrimination. As shown in Fig. 2, the order of the five vinca-alkaloids elution was VDSN (0.913 ± 0.001 min), VCST (1.076 ± 0.002 min), VINO (1.251 ± 0.034 min), VBST (1.570 ± 0.004 min) and VINF (1.602 ± 0.007 min). Despite close retention times between VBST and VINF, their retention times were significatively different at 5% ($p < 0.001$).

3.1.2. Discriminative HPLC validation. The discriminative parameters of the method were assessed through specificity and sensitivity with an external validation set of vinca-alkaloids ($n = 150$) provided to real life samples from compounded bags and covering the therapeutic concentrations. The set was analyzed in parallel by the FIA method (spectral discrimination). No discordant result was obtained between the two methods and 100% of the unknow samples were well predicted by the retention time. The specificity, sensitivity and accuracy were 100% by the HPLC method, which makes it possible to discriminate all five vinca-alkaloids.

3.1.3. Optimization of the FIA method. Except for VCST, spectra have an absorption maximum close to 269 nm and show strong similarities for VBST/VDSN and VINO/VINF (Fig. 3). Two discriminating bands are observed for VCST at 256 and 296 nm. Structurally, VCST is differentiated from other alkaloids by the substitution in R_4 of the methyl group by an aldehyde function (Fig. 1). Carbonyl functions are at the origin of forbidden electronic transitions $n \rightarrow \pi^*$ which are characterized by the appearance of UV bands around 280 nm. The similarities for VBST/VDSN can be explained by the excipients of the pharmaceutical products and especially mannitol; excipients were not used for VINO/VINF products.

To develop discriminative analysis, the spectral data were pretreated to overcome variations linked to concentration. The spectral region between 200 and 220 nm exhibiting a non-specific absorption range with random spectral variations was excluded. The spectra were then normalized by the spectral band at 269 nm to limit the intra-group variations linked to the concentration (Fig. 3).

The discriminant analysis was performed by a method supervised using PLS-DA to separate the five vinca-alkaloids. The best predictive model was obtained after normalization by the wavelength at 269 nm from 220 to 400 nm. Five latent variables (LVs) were selected to develop the model and represent 99.98% of the total variability of the original variables (98.0% for the first LV, 1.6% for the second) (Fig. 4). Despite achieving 99% explained variance with the initial two latent variables (LVs), five LVs were necessary for accurate classification. This finding is consistent with the hypothesis that five LVs are needed to differentiate five distinct molecules. All unknown

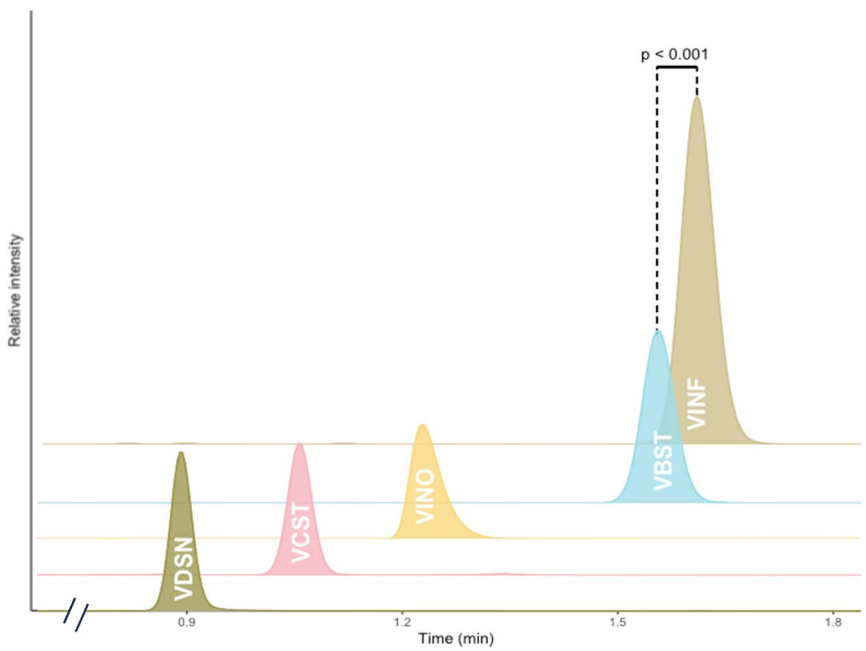


Fig. 2 The overlapped chromatograms (recorded at 269 nm) of the five vinca-alkaloids.

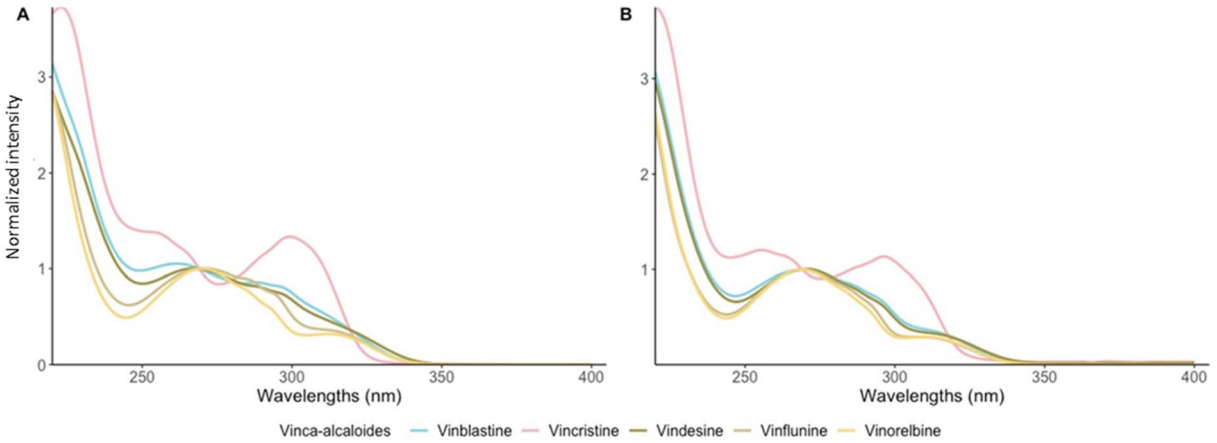


Fig. 3 Mean normalized UV spectra at 269 nm from 220 to 400 nm after analysis by FIA (A) and HPLC (B) for the five vinca-alkaloids.

samples from the external validation set were correctly assigned with 100% accuracy, sensitivity and specificity.

The discriminant analysis by the least square matching method was then based on the results of PLS-DA, and qualitative models of discrimination were developed using Chromeleon® software for each vinca alkaloid.

In Table 1, very high match scores were obtained for each of the five vinca-alkaloid samples analyzed under their own analytical conditions with values >999.0. From this experiment, we concluded the excellent relevance of the highest match score. As a first step, a threshold limit is determined for the processing of the least square matching method. Indeed, as a matching is operated from the spectral library, a limit to specify whether the sample is accepted or rejected is obvious. The value of the threshold was set at 998.0 for the five vinca-alkaloids. As a second step, the qualitative parameters of the method were assessed through specificity and sensitivity with an external validation set of vinca-alkaloids ($n = 150$). The set was analyzed in parallel by the chromatographic method (discrimination by the retention time) and no discordant result was obtained between the two methods and excellent (100%) specificity and sensitivity were achieved under these conditions leading to excellent accuracy (100%).

3.2. Quantitative analysis

A convenient calibration process was carried out for the five vinca-alkaloids with the FIA method and HPLC method (ESI, Fig. S1†). These distinct analytical conditions were validated in

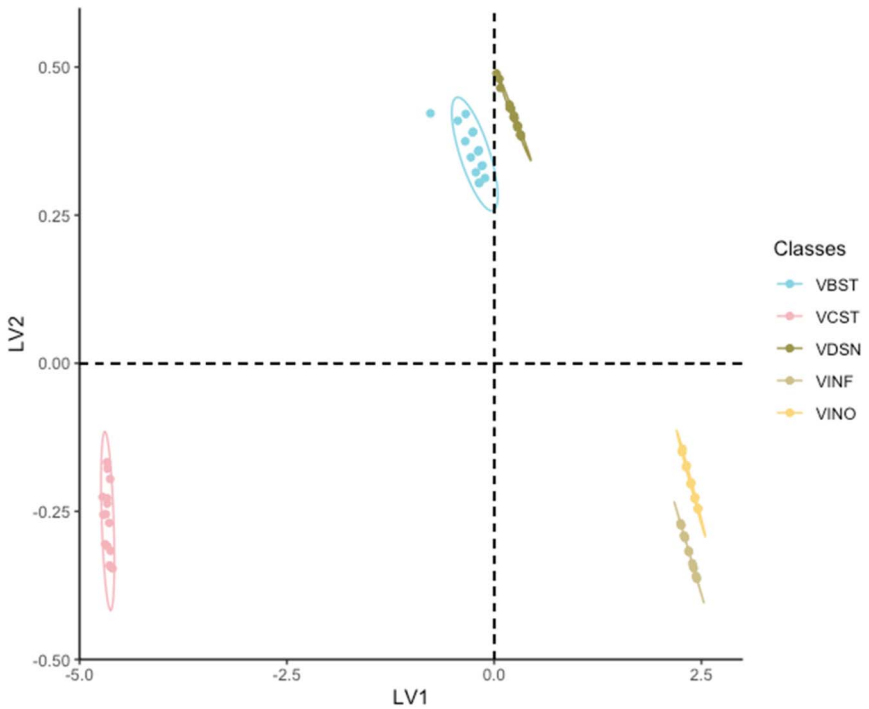


Fig. 4 Score plot of the PLS-DA model for samples of the five vinca alkaloids after FIA analysis (normalization at 269 nm, spectra from 220 nm to 400 nm).

Table 1 Confusion matrix of the external validation set for the discriminant model for the least square matching method (FIA/UV). The grey cells correspond to correct predictions% (match values)

		Predicted classes				
		VBST (n = 30)	VCST (n = 30)	VDSN (n = 30)	VINF (n = 30)	VINO (n = 30)
Real classes	VBST	100% (>999.99)	0%	0%	0%	0%
	VCST	0%	100% (>999.70)	0%	0%	0%
	VDSN	0%	0%	100% (>999.01)	0%	0%
	VINF	0%	0%	0%	100% (>999.98)	0%
	VINO	0%	0%	0%	0%	100% (>999.68)

terms of linearity, accuracy, and precision according to ICH requirements (Q2R1). Based on the FIA results, the linearity of each of the five analytical conditions was very satisfactory (R^2 > 0.997) under the FIA conditions. Satisfactory RSDs of both the repeatability and the precision were reached with values <0.8% and 2.3% respectively (ESI, Table S2†).

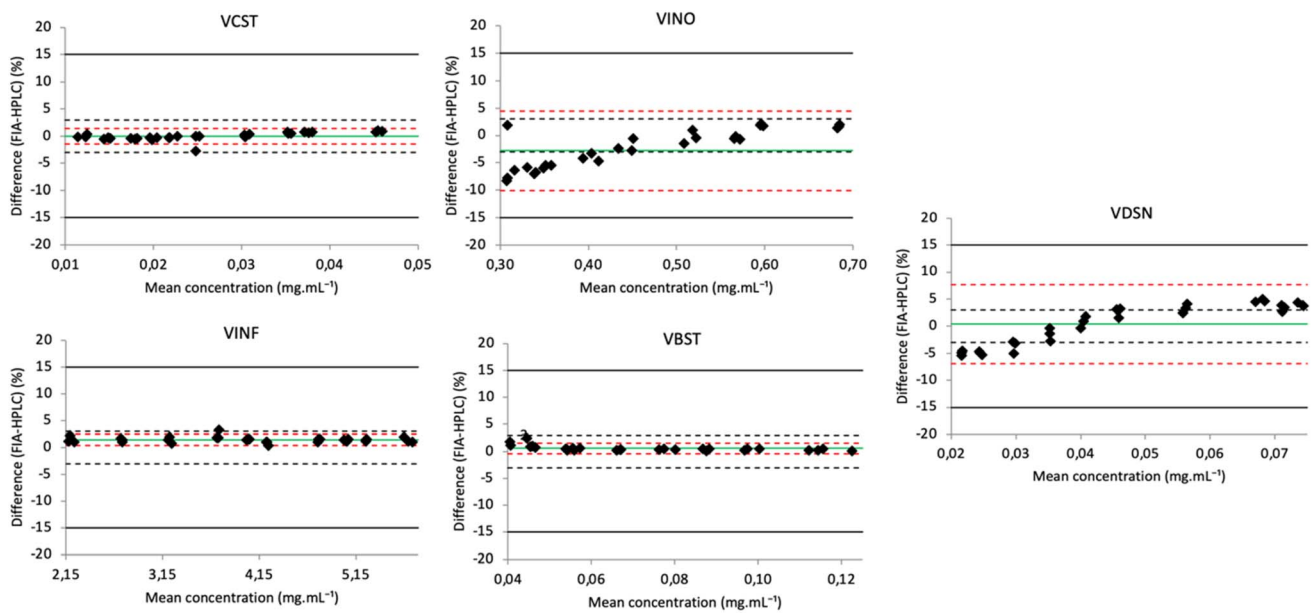


Fig. 5 Bland–Altman plot showing the relative difference between FIA/UV and HPLC/UV (UV) against the mean predicted concentration for the external validation set. The green dotted line is the bias (d), red dashed lines are the limits of agreement (±1.96SD) and the dark dashed and dotted lines are respectively the predefined limits of systematic error (±SE) and total error (±TE). The advantages and drawbacks of the methods and the comments are summarized in Table 2.

Table 2 Advantages and drawbacks of FIA and HPLC methods for quality control of vinca-alkaloids

		FIA	HPLC
Optimization		Chemometric optimization by PLS-DA or by the least square matching method	Chromatographic optimization (resolution and retention time)
		Short time and easy optimization	Long time and tedious optimization
		Easy to apply in current practice	Relatively easy to apply in current practice
In current practice		Time of analysis: 30 s	Time of analysis: 2 min
		HPLC system	HPLC system
		No consumable	Chromatographic column
		Solvent: ultrapure water	Solvent: buffer and organic
		Low cost	Medium cost
Environmental footprint		Low	Medium (organic solvent)
Robustness		Risk of low spectral shift	Alteration of chromatographic support (peak broadening, retention time variations…)
		Risk of variation of the UV lamp intensity	Risk of variation of the UV lamp intensity
Global cost	Equipment	Expensive	Expensive
	Consumable	Cheap	Relatively expensive

From these results, we showed that FIA and HPLC conditions provided accurate quantification of the five vinca-alkaloids.

3.3. Comparison of FIA and HPLC

To demonstrate the transposability of the FIA and HPLC methods and the applicability of the FIA spectral method coupled with chemometric analysis, samples of the external validation set were analyzed with FIA and HPLC procedures. Using the Bland–Altman plot (Fig. 5), the five vinca alkaloid drugs satisfied the predefined limits for SE as well as TE and the methods were interchangeable.

4. Conclusions

This research achieved the development of two analytical techniques for the classification and quantification of five vinca alkaloids, critical in cancer treatment. HPLC-UV was established as the reference method for comparison with FIA-UV. FIA-UV exhibited excellent accuracy in both classification and quantification, demonstrating performance comparable to HPLC-UV. The FIA method provides several benefits over HPLC, such as faster processing, lower costs, reduced environmental burden, and ease of routine use, making it highly suitable for integration into anticancer drug preparation workflows.

Data availability

Data, *i.e.* raw spectra and R code are available at: **https://doi.org/10.5281/zenodo.14924579** (version 2).

Author contributions

Conceptualization E. Caudron and L. M. M. Lê; data curation: E. Caudron, A. Dowek, M. Berge, C. Boughanem and L. M. M. Lê; formal analysis: C. Boughanem; investigation: E. Caudron, C. Boughanem and L. M. M. Lê; methodology: E. Caudron, C. Boughanem and L. M. M. Lê; project administration: E. Caudron and L. M. M. Lê; resources: E. Caudron, J. Saidi and L. M. M. Lê; software: C. Boughanem, A. Dowek and M. Berge; supervision: E. Caudron and L. M. M. Lê; validation: E. Caudron, C. Boughanem and A. Dowek; visualization: E. Caudron, C. Boughanem, L. M. M. Lê and A. Dowek; roles/writing – original draft: C. Boughanem, E. Caudron; and writing – review & editing: C. Boughanem, E. Caudron, A. Dowek, M. Berge and L. M. M. Lê.

Conflicts of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Notes and references

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