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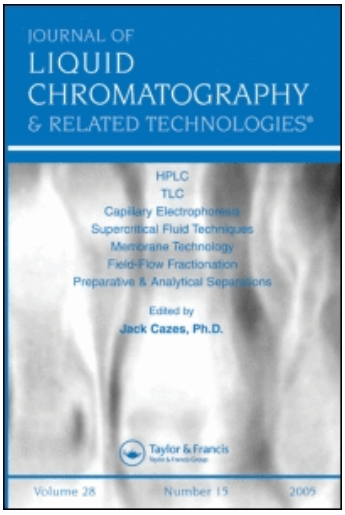

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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To cite this Article Tam, Mai Ngoc , Nikolova-Damyanova, B. and Pyuskyulev, B.(1995) 'Quantitative Thin Layer Chromatography of Indole Alkaloids. II. Catharanthine and Vindoline', Journal of Liquid Chromatography & Related Technologies, 18: 5, 849 — 858
To link to this Article: DOI: 10.1080/10826079508010396
URL: http://dx.doi.org/10.1080/10826079508010396

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JOURNAL OF LIQUID CHROMATOGRAPHY, 18(5), 849-858 (1995)

QUANTITATIVE THIN LAYER CHROMATOGRAPHY OF INDOLE ALKALOIDS. II. CATHARANTHINE AND VINDOLINE

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ABSTRACT

A method is described for the densitometric determination of the indole alkaloids catharanthine and vindoline in plant extract. The alkaloids were separated from each other and from the rest of the components by three-fold development with a mobile phase of petroleum ether / ethyl ether / acetone / ethanol (70+10+20+1, v/v/v/v). Catharanthine was scanned at 280 nm and vindoline - at 310 nm. The calibration curves were linear in the interval 1 μg/ per spot - 5 μg/per spot. A standard deviation of less than 0.1 μg per spot and a relative error not exceeding 3% were found.

INTRODUCTION

Until recently, the plant *Catharanthus roseus* (L.) G. Don has been the only natural source for the production of the medicinally important binary indole alkaloids vinblastine and vincristine. Unfortunately, the content of these two alkaloids in the plant is very

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low, causing serious complications in their isolation. Obviously these factors influence unfavourably the cost of the final product [1]. The high clinical effect of the vinblastine and vincristine as antitumour agents on one hand and their limited availability on the other provoked an increasing scientific interest in their synthesis. A great number of vinblastine analogues has been synthesized. Some of them have been applied in the chemotherapy of cancer [2, 3]. The alkaloids catharanthine (I) and vindoline (II), Figure 1, have been preferably used as starting materials in these syntheses. They are also present in *Catharanthus roseus* , in amounts exceeding 10 - 40 times that of vinblastine and vincristine [4]. Reliable analytical methods are required for the evaluation of the analytical purity of I and II as well as for the determination of their content in the crude plant material and in the appropriate reaction mixture. Different

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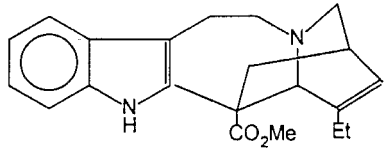

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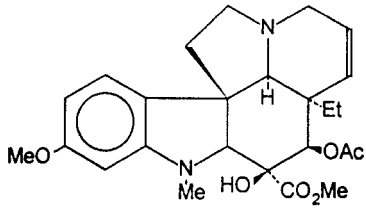

II

FIGURE 1. The chemical structure of the indole alkaloids catharanthine (I) and vindoline (II)

procedures and techniques have been proposed for the quantitation of indole alkaloids and particularly those of *Catharanthus roseus* [4], among them HPLC being rather advantageous [5].

The experience gained in this laboratory in the analysis of *Vinca* alkaloids has shown that TLC densitometric analysis combined with suitable pre treatment of the sample could be successfully applied to a routine simultaneous determination of the indole alkaloids tabersonine and 11-methoxytabersonine in plant extracts [6].

Presently, we reported about a simplified analytical TLC method for routine densitometric quantitation of catharanthine and vindoline.

EXPERIMENTAL

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Materials

All reagents and solvents were of analytical grade and were used without further purification. Petroleum ether had b. p. 40-60 °C.

20 x 20 cm precoated glass TLC plates Silica gel 60 F_{254} (Merck Art. 5715) were used.

Catharanthine and vindoline were isolated in this laboratory from leaves of *Catharanthus roseus* and purified by column chromatography on aluminium oxide 90, Brockmann II-III, 0.063 0.200 mm (Merck, Art. 1097). The crude catharanthine was recrystallized in acetone, while vindoline in ethyl ether. The purity of the isolated material was checked by TLC, melting point, UV, NMR, IR spectroscopy and mass spectrometry.

Stock solutions of the pure alkaloids were prepared by dissolving 12.88 mg of catharanthine and 12.80 mg of vindoline, respectively in abs. ethanol in 25 ml volumetric flasks. Five working solutions of each alkaloid with concentrations in the range 0.512-0.103 mg/ml catharanthine and 0.512-0.1024 mg/ml vindoline, were prepared by dilution and used to plot the calibration graphs.

Isolation of Total Alkaloids from *Catharanthus roseus*

The finely ground dry plant material (4 g) was extracted repeatedly with five portions of 10 ml each methanol 2N citric acid (1:1, v/v). Duration of each extraction was 30 min. The combined extracts (50 ml) were concentrated under vacuum to a volume of 10 ml and the solution adjusted with ammonia to pH 8-9. The alkaloids were then extracted with 5 successive 15 ml portions of ethyl acetate. The combined extracts were washed with 3 ml of distilled water, dried over anhydrous sodium sulphate and evaporated to dryness (vacuum evaporator, 35° C). The residue was dissolved in abs. ethanol (5 ml) and solvent evaporated as described above. The resulting residue was dried at 40° C under vacuum for 1h, the residue was weighed, transferred quantitatively into a 25 ml volumetric flask and brought to volume with abs. ethanol. 10 µl aliquots of this solution were subjected to chromatography.

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Thin Layer Chromatography.

The absorbent layer was cut into two equal parts measuring 20 cm x 10 cm, a double number of samples can be applied on the two opposite wide sides of the plate.

The samples (10 µl aliquots) were applied along a straight line 15 mm above the rim of the plate as spots with diameter not exceeding 6 mm. Spots were spaced at a distance exactly 15 mm from one another. The marginal spots were spaced at a distance of 11 mm from the side edges of the plate.

The plate were then allowed to stay in dark for 15 min to ensure the evaporation of the solvent from the spots. During that time the chromatographic chamber (standard 215 mm x 205 mm x 125 mm) was saturated with the vapours of the mobile phase petroleum ether ethyl ether acetone ethanol (70+10+20+1, v/v). The plates were developed to a solvent front of 85 mm. They were then removed from

the chamber, flushed with cold air for 1 min and allowed to stay in dark at room temperature for 30 min. The developing procedure was repeated three times.

Apparatus

Densitometric measurements were performed on Shimadzu CS 930 dual wavelength scanner equipped with Shimadzu DR 2 data recorder , in zigzag refection mode with a slit size of 1.2 mm x 1.2 mm.

RESULTS AND DISCUSSION.

Thin Layer Chromatography

It is well known that the extracts containing alkaloids isolated from a plant material comprise a great number of individual compounds. More than 90 alkaloids have been isolated, for example, from extracts of *Catharanthus roseus* [7]. A lot of them have similar physico chemical properties and chromatographic behaviour. Generally, the alkaloids of *Catharanthus roseus* can be classified into two main groups: monomeric and bis (or binary) indole alkaloids [7, 8]. Catharanthine and vindoline belong to the monomeric group. They are distinguished from the corresponding bis indole constituents for their relatively low polarity.

Precoated glass Merck TLC Silica gel 60 F_{254} 20 cm x 20 cm were preferred in this study due to our experience and conviction in their high chromatographic quality and reproducible results (see also [9]). It is worth noticing that small differences were observed between separate batches but they had negligible effect on the final results. As a matter of fact excellent results have been obtained on aluminium oxide 60 F_{254} type E (Merck, Art. 5715) plates as well.

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Silica gel plates were, however, preferred because of their convenience and wider use in practice.

The composition of the mobile phase was established empirically. The chromatographic behaviour of catharanthine and vindoline as well as of bis indole alkaloids vinblastine and leurosine against each constituent of the proposed solvent mixture has been studied. With pure ethyl ether a tailing of some alkaloid spots was observed, the spots being spread. Acetone and ethanol did not cause tailing but was not selective as vindorosine could not be resolved from vindoline, the former migrating just ahead. Petroleum ether alone could not move the components from the origine and was, therefore suitable modifier. Thus, a mobile phase of petroleum ether/ethyl ether/ acetone/ ethanol in proportions 70:10:20:1 (v/v/v/v) was used and it provided reliable chromatographic result: well shaped round spots, clear separation of catharanthine and vindoline from the accompanying components, reasonable distances between the spots satisfying the densitometric requirements. Moreover, under these the bis indole alkaloids remained at the start (or close to it), while catharanthine and vindoline were the main spots with R_f values 0.39 and 0.20 respectively. A lot of non polar minor components, which formed series of well defined spots, were spaced between catharanthine and vindoline but were clearly separated and did not interfere with the quantitative densitometric measurements.

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Densitometric quantitation

As already shown [6] the indole alkaloids have strong UV chromofors and can be quantified by measuring the absorbance of the spots directly on the plate via scanning densitometry. In order to determine the optimal wavelength, the respective in situ UV spectra were recorded. Catharanthine and vindoline had maxima at different wavelengths: 280 nm (catharantine) and 310 nm (vindoline) and these were chosen for the quantitative measurements. Thus, the

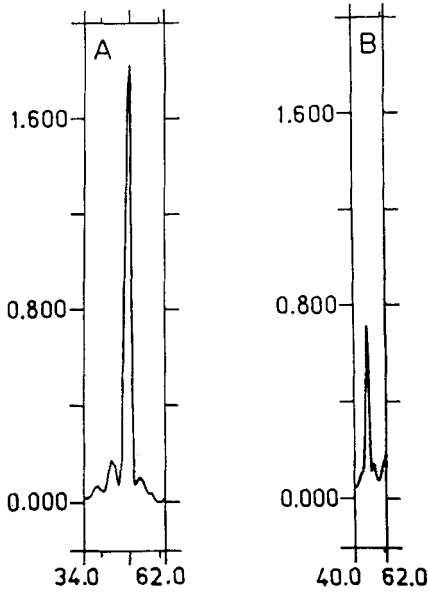

FIGURE 2. Densitograms of Catharntine (A) and vindoline (B) in extracts of *Catharanthus roseus* applaied on silica gel G plates and developed with mobile phase petroleum ether / ethyl ether / acetone / ethanol (70+10+20+1, v/v/v/v).

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respective spots were measured separately, each at its characteristic wavelength the scanned distance being about 20-25 mm, Figure 2.

Plates were scanned not longer than 45 min after development. This time was enough for the mobile phase solvents to evaporate while the densitometric signals did not show any significant decrease or increase of the recorded values.

Calibration graphs were constructed by using series of standard solutions of each alkaloid (see EXPERIMENTAL). Standards were spotted in triplicate and peak areas (as derived from the integrator) were plotted against the respective amounts per spot. Both graphs were linear in the interval 1 μg -5 μg per spot, Figure 3. The correlation coefficients were 0.998 (catharantine) and 0.995 (vindoline).

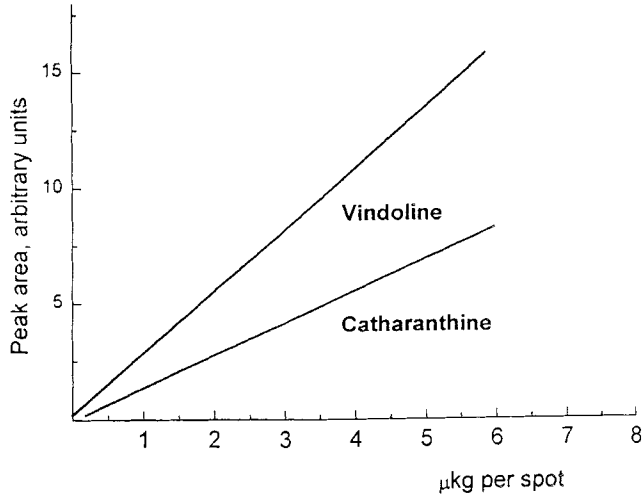

FIGURE 3. The peak area *vs* per spot amounts of catharantine (λ=280 nm and vindoline (λ = 310 nm) as measured by scanning densitometry

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Table 1

Acuracy and Precision of the Densitometric Determination of Catharnthine and Windoline by Silica gel Thin Layer Chromatography

Alkaloid	Known, μg/per spot	Found, μg /per spot[a]	Relative error, %[b]
Catharanthine	1.03	1.00±0.03	2.9
	3.09	3.06±0.03	0.9
	5.15	5.05±0.10	1.9
Vindoline	1.03	1.02±0.01	0.9
	3.07	3.08±0.01	0.3
	5.12	5.13±0.01	0.2

[a] mean ± standard deviation, N=2.

[b] $|x| - |a| / a * 100$, where **x** is the mean value and **a** is the known value.

In order to determine the accuracy and the precision of the densitometric measurements catharantine and vindoline were spotted in three different per spots amounts on two different plates. The respective standards, in concentrations of 2.0 μg/per spot and 4.0 μg/per spot were also applied alongside each plate. Plates were developed as described above and scanned in the automatic external standard mode. The results (in μg per spot as derived from the integrator) are shown in Table 1. It is evident that the standard deviation did not exceed 0.1 μg/per spot and the relative error was not higher than 3%.

This approach has been in use in our laboratory for more than two years and is suitable, in our opinion, for phytochemical screening of plant extracts since it answers all requirements for a reliable analytical method.

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ACKNOWLEDGEMENT

This research was partially supported by the Bulgarian National Research Foundation under contract No X-95. The authors are indebted to Ms K.Chervenkova for the skilful technical assistance

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Received: August 16, 1994
Accepted: August 29, 1994

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