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Short communication

Application of high-performance liquid chromatography to the determination of vinblastine in *Catharanthus roseus*

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Abstract

A method for the determination of vinblastine in *Catharanthus roseus* by HPLC is described. A crude alkaloid extract, obtained by extraction of leaves with toluene, 2% citric or tartaric acid and benzene, was separated by TLC. The vinblastine fraction was cut out and vinblastine was eluted from the sorbent. The amount of vinblastine was determined by HPLC with peak-height measurement. The standard deviation is 0.2 μg/ml. The detection limit is 0.05 ng of vinblastine in a sample applied to the HPLC column.

1. Introduction

Vinblastine (Fig. 1) is a dimeric indole alkaloid from *Catharanthus roseus* (L.) G. Don and is used in the chemotherapy of various neoplastic diseases [1,2]. Several high-performance liquid chromatographic (HPLC) methods have been developed for the determination of vinblastine in biological fluids, in tumour cells and tissues, in cell cultures of *C. roseus* and for the study of the stability of vinblastine under various conditions. One HPLC method was included in the US Pharmacopeia for control of vinblastine preparations [3]. Only three HPLC methods for the determination of vinblastine in plants have been described [4–6], and these have complicated and long sample preparation stages.

This paper describes an HPLC method for the determination of vinblastine in leaves of *C. roseus*.

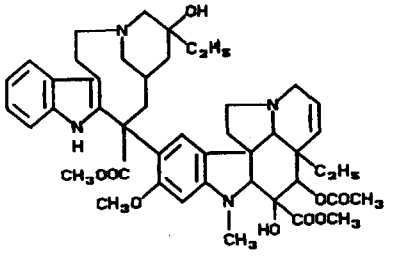

Fig. 1. Structure of vinblastine.

2. Experimental

2.1. Equipment

The chromatographic system consisted of a Waters Model 501 HPLC pump (Millipore, Mil-

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ford, MA, USA), equipped with Rheodyne sample injector with a 20-μl loop (Diagnosticum, Moscow, Russian Federation). Detection was performed with an LCD 2563 UV detector (Laboratórní Přístroje, Prague, Czech Republic) operating at 254 nm. A Separon SIX C_{18} (5 μm) reversed-phase cartridge column (15 cm × 3.3 mm I.D.) (Laboratórní Přístroje) or an RP-18 CGC (5 μm) cartridge column (15 cm × 3 mm I.D.) (Merck, Darmstadt, Germany) was used for separation. The mobile phase was methanol–5 m*M* diammonium hydrogenphosphate (pH 7.3) (65:35). The analysis was carried out under isocratic conditions at a flow-rate 0.5 ml/min at room temperature (about 20–22°C).

2.2. Materials

Vinblastine sulphate was obtained from Eli Lilly (Indianapolis, IN, USA), diammonium hydrogenphosphate from Merck and sodium acetate, citric acid, ethyl acetate, diethylamine, benzene, toluene, methanol, ethanol and ammonia solution from Sojuzreaktiv (Moscow, Russian Federation). Water was distilled from glass before use.

TLC was performed on Sorbfil silica gel plates (Plastmash, Krasnodar, Russian Federation).

2.3. Analytical procedure

The powdered leaves (3.00 g) of air-dried plant material were wetted with 3 ml of 5% sodium acetate for 1 h and extracted with toluene (75 ml) by standing overnight. The filtered toluene solution (50 ml) was extracted with 2% citric acid, the acid extract was adjusted to pH 5.8–6.0 with 5% ammonia solution and alkaloids were extracted with benzene. The crude alkaloid extract was evaporated to dryness at 45°C. The dry residue was dissolved in 1 ml of methanol–benzene (1:1) and 30 μl of the solution obtained were applied to a Sorbfil TLC plate on the 1-cm line. The plate was developed with ethyl acetate–benzene–ethanol–10% ammonia solution (100:5:23:2.3) and the vinblastine zone (R_F 0.25–0.33) was identified by comparison with a sample (10 μl) of a stock solution of vinblastine sulphate (standard sample) and was cut out. The vinblastine fraction was eluted with 10 ml of 1.5% diethylamine in methanol for 5 min at 55°C with continuous shaking. The resulting solution was filtered through a 10 PO 4 porous glass filter (Universil, Nyíregyháza, Hungary) (pore size 9 μm) and evaporated to dryness at 45°C. The dry residue was dissolved in 300 μl of mobile phase and injected into the HPLC column. The amount of vinblastine in the sample was determined from the peak-height by means of a calibration graph.

The purity of the standard sample of vinblastine sulphate was established as 99.9% by HPLC [3]. The water content of vinblastine was determined by drying a weighed standard sample in vacuo at reduced pressure (16 kPa) at ca. 60–61°C over powdered phosphorus pentoxide for 24 h.

A stock solution of standard sample of vinblastine sulphate (1 mg/ml) was prepared in methanol and stored in a glass tube at −18°C. Working standard solutions for constructing the calibration graph were prepared by diluting the stock standard solution with mobile phase as required.

A calibration graph was obtained by measuring the height of the vinblastine peak for various dilutions of the stock standard solution. Samples of vinblastine (from 0.05 to 2 μg) were injected and chromatography was performed in triplicate.

3. Results and discussion

We tried to develop an easier and faster method than those described previously [4–6] for the determination of vinblastine in *C. roseus*. The method presented is a modification of previously published methods described in the Russian Pharmacopoeia [6]. We replaced the spectrophotometric determination of vinblastine by HPLC and replaced column liquid chromatography on Sephadex LH-20 with TLC.

The large number of related alkaloids (more than 200) and the low concentrations of vinblastine (hundredths of a per cent) made it impossible to avoid preliminary purification of

the sample. For this reason, the crude alkaloid extract was purified by TLC. As shown in Fig. 2, purification of the sample by TLC makes it possible to obtain good resolution in subsequent HPLC. The HPLC conditions were chosen from previous publications [7–9]. The retention time of the vinblastine peak was about 20–25 min on both the Separon SIX C_{18} and the RP-18 columns. Increasing the flow-rate to more than 0.5 ml/min occasionally led to compression of the sorbents (especially Separon SIX C_{18}).

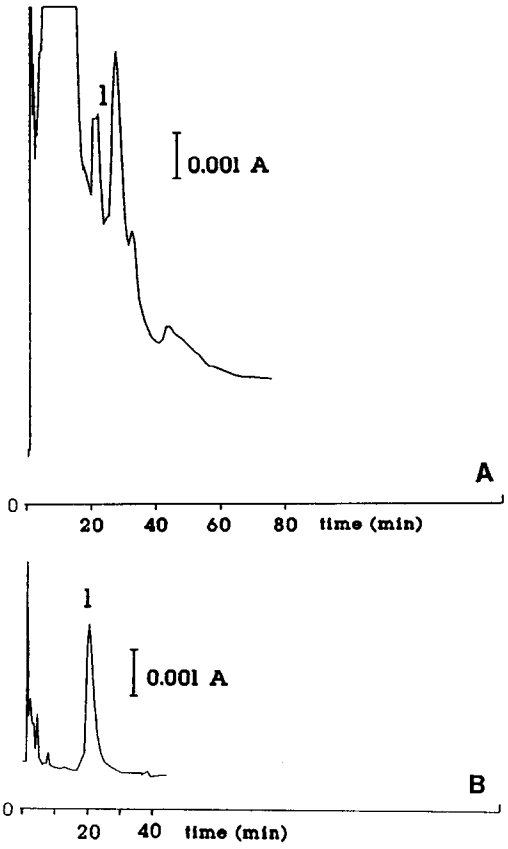

Fig. 2. HPLC of *Catharanthus roseus* extract, (A) before TLC purification and (B) after TLC purification. Peak 1 = vinblastine.

Although calibration via an internal standard is usually more reliable, we used calibration via an external standard as this made the analysis faster. Moreover, as it was determined by repeated analysis of one *C. roseus* sample, the relative standard deviation (R.S.D.) for the present method (via an external standard) was 7.1% and essentially equal to that for the method via an internal standard (about 6.8%).

The stability of the stock standard solution of vinblastine was studied by repeated injections and comparison of peak heights. It was established that vinblastine is stable for about 2 weeks at −18°C. At room temperature vinblastine is degraded after about 2 days.

The devised method provides good accuracy for the determination of vinblastine in the range 0.1–2 μg in an injected sample, i.e. in the concentration range 5–100 μg/ml. This range fits the practical levels of vinblastine contents in *C. roseus* (0.005–0.04%).

The reproducibility (intra-assay variability) is satisfactory; the R.S.D. ranged between 1.2 and 4.8% ($n = 5$). The inter-assay variability was measured by taking a control sample (25 μg/ml) every day. The R.S.D. measured over several days was 7.3% ($n = 6$).

Known amounts of vinblastine sulphate were

Table 1
Recovery of vinblastine from *C. roseus* leaves

Amount added (mg/g)	Final amount[a] (mg/g)	Mean recovery		R.S.D. (%)
		mg/g	%	
0	15	14.5 ± 0.15	96.7	1.03
5	20	19.6 ± 0.21	98.0	1.07
10	25	25.2 ± 0.73	100.8	2.90
20	35	35.4 ± 0.92	101.1	2.59
50	65	64.3 ± 2.03	98.9	3.15

[a] Means ± 95% confidence intervals; $n = 6$.

added to a sample of powdered leaves and the recoveries (after subtracting the result without addition of the vinblastine sulphate from the total results with the addition of the vinblastine sulphate, expressed as mg per g) and the R.S.D. were calculated. The results are given in Table 1.

The detection limit is ca. 2.5 μg/ml of vinblastine at a signal-to-noise ratio of about 7.

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