Journal of Chromatography, 457 (1988) 442–445
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 914

Note

Determination of isoquinoline alkaloids from *Peumus boldus by* high-performance liquid chromatography

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(Received August 12th, 1988)

Peumus boldus has long been used as a folk medicine[1] and it is still reported in many Pharmacopoeias[2]. It has been shown that its pharmacological activity is due to the isoquinoline alkaloid fraction[3] (Fig. 1).

Most published analyses of *Peumus boldus* alkaloids have involved separations by thin-layer chromatography (TLC)[4,5], while the total alkaloid content has been determined by titrimetric[6] and spectrophotometric[7] methods. The main alkaloid boldine has been determined by high-performance liquid chromatography (HPLC)[8] and by voltammetry[9].

All these procedures require time-consuming steps such as liquid–liquid partition, where the complete extraction of the alkaloids from buffered aqueous phases is difficult.

This work was undertaken to develop a rapid and reliable HPLC method for the separation of the main alkaloids bodine, isocorydine and N-methyllaurotetanine in *Peumus boldus* extracts and drugs. The method involves a combined clean-up and concentration procedure by means of a C_{18} Sep-Pak cartridge followed by isocratic reversed-phase HPLC.

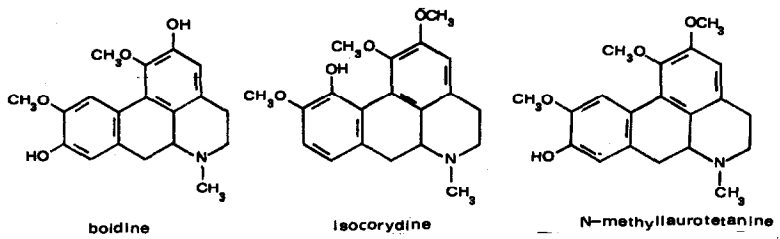

Fig. 1. Structures of boldine, isocorydine and N-methyllaurotetanine.

0021-9673/88/$03.50

EXPERIMENTAL

Materials

Boldine was obtained from Sigma (St. Louis, MO, U.S.A.). Isocorydine and N-methyllaurotetanine were isolated from a commercial extract of *Peumus boldus* leaves by preparative TLC. *Peumus boldus* leaf extracts were purchased from different sources. Methanol, acetonitrile and water were of HPLC grade (Chromasolv; Riedel de Haën, Hannover, F.R.G.). Triethylamine and chloroform were of analytical-reagent grade (Carlo Erba, Milan, Italy). Sep-Pak C_{18} cartridges (Waters Assoc., Milford, MA, U.S.A.) were used for sample preparation.

Preparation of Peumus boldus samples

Peumus boldus leaf extracts (1 ml) were diluted to 5 ml with water and 2 ml were applied to a C_{18} Sep-Pak cartridge. After washing with 4 ml of 0.05 *M* ammonium monohydrogenphosphate buffer and 2 ml of water, the alkaloid fraction was eluted with 2 ml of methanol and the eluate diluted to 5 ml with the same solvent.

Drugs containing *Peumus boldus* extracts were treated similarly.

TLC

TLC was carried out on Merck silica gel 60 F_{254} plates. The samples were developed with chloroform–diethylamine (75:25) and the alkaloids were detected by UV irradiation at 254 nm (R_F values: boldine = 0.25; N-methyllaurotetanine = 0.35; isocorydine = 0.55).

HPLC

Alkaloids were separated using a system consisting of a Waters M-6000 pump fitted with a μBondapak C_{18} column (30 cm × 3.9 mm I.D.) and a U6K injector. A pre-column (Waters Assoc., Part No. 84550) was used to extend the column lifetime.

The mobile phase was acetonitrile–water–triethylamine (87:15:0.2, v/v) adjusted to pH 2.6 with 10% phosphoric acid. Samples were eluted isocratically at a flow-rate of 1.8 ml/min and detected at 270 nm (0.01 a.u.f.s.).

Reference solutions of boldine, isocorydine and N-methyllaurotetanine in methanol (0.07 mg/ml) were prepared and analysed by HPLC. The resulting chromatograms yielded data for the calibration graphs.

RESULTS AND DISCUSSION

In the analysis of basic substances such as alkaloids using reversed-phase HPLC, tailing peaks are obtained owing to retention by free silanol groups when aqueous methanol (acetonitrile) is used as the eluent. To improve the peak shapes and separations, acidic mobile phases or acidic amine–phosphate buffers can be useful. Various systems were therefore evaluated for their ability to resolve *Peumus boldus* alkaloids. Methanol and acetonitrile, when used as organic components of an acidic mobile phase (pH 2–3), were found to give low resolution. With acetonitrile–water–triethylamine (pH 2.6) as the eluent, the peak shapes were much improved and baseline separation of the alkaloids was achieved. Fig. 2 illustrates a chromatogram of standard boldine, isocorydine and N-methyllaurotetanine eluted with this system.

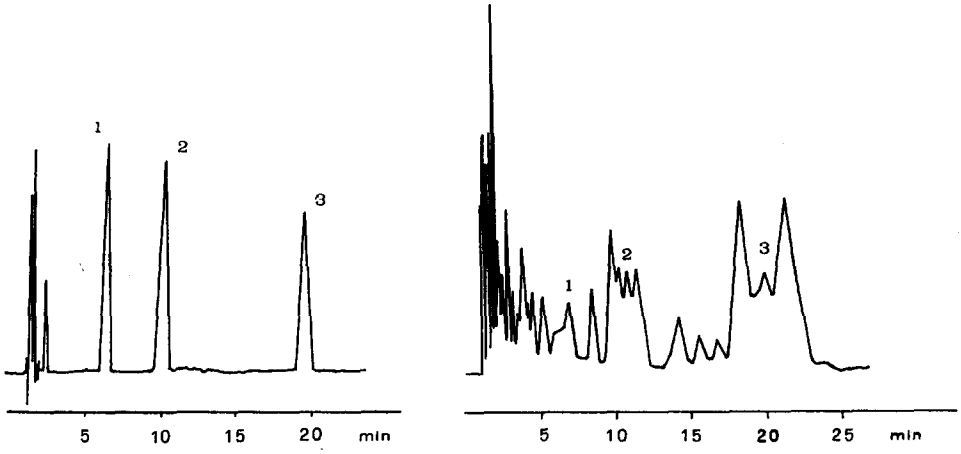

Fig. 2. Chromatogram of (1) boldine, (2) isocorydine and (3) N-methyllaurotetanine standards. Eluent, acetonitrile–water–triethylamine (87:15:0.2), pH 2.6; flow-rate, 1.8 ml/min; UV detection at 270 nm.

Fig. 3. Chromatogram of an unpurified *Peumus boldus* leaf extract. Chromatographic conditions and peaks as in Fig. 2.

However, in the analysis of *Peumus boldus* samples other constituents are coeluted with the alkaloids (Fig. 3). Hence, the identification of these compounds must be preceded by a clean-up procedure. Purification with Sep-Pak C_{18} cartridges strongly reduces the front and the impurities (Fig. 4), and provides a quantitative recovery of the alkaloids compared with extraction with chloroform. It should also be noted that neither long extraction times nor large volumes of toxic solvents are required.

Boldine, isocorydine and N-methyllaurotetanine exhibit different UV absorption maxima[10], and the determination was carried out at 270 nm, which represents the

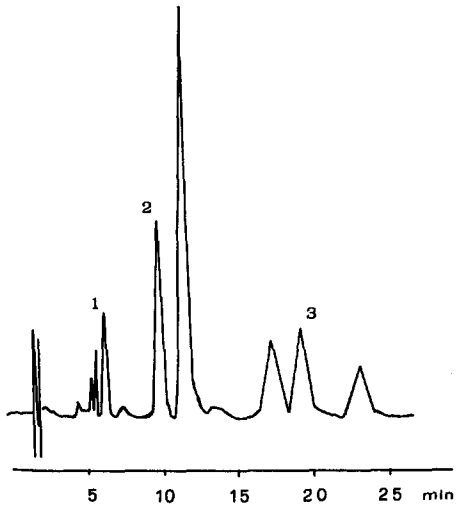

Fig. 4. Chromatogram of a purified (C_{18} Sep-Pak cartridge) *Peumus boldus* leaf extract. Chromatographic conditions and peaks as in Fig. 2.

TABLE I

CONTENTS OF BOLDINE, ISOCORYDINE AND N-METHYLLAUROTETANINE IN THREE COMMERCIAL *PEUMUS BOLDUS* LEAF EXTRACTS

Extract	*Compound (%)*		
	Boldine	*Isocorydine*	*N-Methyllaurotetanine*
I	0.014	0.0033	0.0029
II	0.016	0.004	0.0035
III	0.008	0.0019	0.0015

best compromise. Sets of standard alkaloids covering the range 0.5–2.5 nmol were run, and the following relationships between peak areas (y) and amount injected (nmol) (x) were obtained:

boldine:	$y = 215x + 31$	$r = 0.997$
isocorydine:	$y = 181x$	$r = 0.994$
N-methyllaurotetanine:	$y = 154x + 11$	$r = 0.995$

The determination of *Peumus boldus* alkaloids in commercial extracts was achieved by external standardization with a good relative standard deviation (3.3%; $n = 5$) (Table I). The procedure was successfully applied to the evaluation of commercially available preparations of *Peumus boldus* containing also other extracts, such as cascara and rhubarb.

In conclusion, the proposed method represents a useful approach to the pharmaceutical quality control of *Peumus boldus* alkaloids.

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