Journal of Chromatography, 351 (1986) 140–143
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 195

Note

Comparison of high-performance thin-layer chromatography–densitometry and gas–liquid chromatography for the determination of conessine in *Holarrhena floribunda* stem bark

P. DUEZ* and S. CHAMART

Laboratoire de Chimie Analytique, Chimie Pharmaceutique Inorganique et Toxicologie, CP 205/1, Université Libre de Bruxelles, Campus Plaine, 1050 Bruxelles (Belgium)

M. VANHAELEN and R. VANHAELEN-FASTRÉ

Laboratoire de Pharmacognosie, CP 205/4, Université Libre de Bruxelles, Campus Plaine, 1050 Bruxelles (Belgium)

and

M. HANOCQ and L. MOLLE

Laboratoire de Chimie Analytique, Chimie Pharmaceutique Inorganique et Toxicologie, CP 205/1, Université Libre de Bruxelles, Campus Plaine, 1050 Bruxelles (Belgium)

(Received September 18th, 1985)

Conessine, the main alkaloid from the stem and root bark of Seoulou (*Holarrhena floribunda* Wall.) and Kurchi (*H. antidyssenterica* (G. Don.), Dur. & Schinz.), which are Apocynaceae from West-Africa and India, respectively, is known for its amoebicide, antidysenteric and febrifuge properties[1]. Unquestionable toxic effects, such as restlessness, tremors, insomnia, vertigo or even severe neuropsychopathic effects, have led to the withdrawal of this substance from modern pharmacopoeia[2].

However, *H. floribunda* bark is still in use in Africa either for its antiblennoragic or its supposed diuretic activity[3]. In order to study the possible local use of the amoebicide properties of this plant, it was decided to standardize the total alkaloidal[4] content of the drug, and particularly conessine.

Two analytical procedures were developed for the assay of conessine: gas–liquid chromatography (GLC) on a 3% SE-30 column, and high-performance thin-layer chromatography (HPTLC)–densitometry with Dragendorff and iodo-platinate as chromogenic reagents. The use of high-performance liquid chromatography was also investigated.

EXPERIMENTAL

A Packard-Becker Model 421 gas chromatograph with a flame ionization detector (Delft, The Netherlands) was equipped with a packed column (1 m × 2 mm I.D.) of 3% SE-30 Chromosorb W-AW (100–120 mesh) DMCS. The flow-rate of the helium carrier gas was 30 ml/min, the oven temperature was 230°C, and the detector and injector temperatures were 245°C. The chromatograph was interfaced with a Shimadzu integrator model CR-3A. The internal standard was codeine (Merck, Darmstadt, F.R.G.).

0021-9673/86/$03.50 © 1986 Elsevier Science Publishers B.V.

HPTLC precoated plates of silica gel 60F 254 (10 × 20 cm) were obtained from Merck. The solutions (1 μl, standards or crude extracts) were applied 15 mm from the lower edge of the plates and then developed with ethyl acetate–hexane–diethylamine (75:24:6); this mobile phase was allowed to travel, in a saturated tank, a distance of 100 mm. After development and drying at 105°C for 2 h, the plates were sprayed with 30 ml of a Dragendorff reagent [1.7% bismuth subnitrate in acetic acid–water (1:4)–40% potassium iodide aqueous solution–acetic acid–ethyl acetate (5:5:20:ad 100)] and then with an iodoplatinate reagent (0.03% potassium hexa-iodoplatinate aqueous solution) until dis-colouration of plate background. Plates were covered with a glass plate during measurement of the orange spots with a Shimadzu high speed TLC scanner CS-920, at the following settings: λ_{abs}, 500 nm; zig-zag stroke width, 9 mm; beam size, 1.2 × 1.2 mm; linearizer 1, AZS off. The mean values were calculated from integration of nine spots corresponding to three different standard concentrations, each being analysed twice, and three spots of the solution of unknown concentration.

H. floribunda stem bark powder (100 mg, 315 μm) was weighed into 10-ml glass-stoppered centrifuging tubes, mixed with 10 mg of calcium hydroxide, 0.2 ml of water and 5 ml of methanol; this suspension was shaken for 15 min and centrifuged at 2000 *g*, these last steps being repeated thrice with 5 ml of methanol. Supernatants were combined and evaporated to dryness under reduced pressure; the residue was dissolved in 1 ml of a methanolic codeine solution (0.06%) and filtered through a Millipore HV-4 filter.

Standard solutions were 2:10 to 8:10 dilutions in the same methanolic codeine solution of a stock solution prepared by dissolving 25 mg of conessine (Pfaltz & Bauer, U.S.A.) in 25 ml of methanol containing the same concentration of internal standard.

RESULTS AND DISCUSSION

HPLC

HPLC with UV detection was found to be unsuitable for the determination of conessine. On chemically modified phases, no conditions allowed sufficient separation and resolution of the peaks in plant extracts, even after a specific alkaloid extraction, with either ionic or non-ionic mobile phases. Furthermore, the shape of the conessine peak was irregular and extremely dependent on small variations in the organic content of the mobile phase. With straight phases, the cut-offs of the usable organic solvents were found to be inconsistent with the conessine detection wavelength (205 nm).

GLC

Conessine was easily separated (Fig. 1) without derivatization or temperature programming. No interference appeared in the chromatograms even after the injection of a large number of extracts. This shows that the high temperature required for conessine elution does not limit the separation of this compound from the other constituents of crude extracts; hence preliminary purification of these methanolic plant extracts was not required. The detection limit was 20 ng; codeine was used as an internal standard.

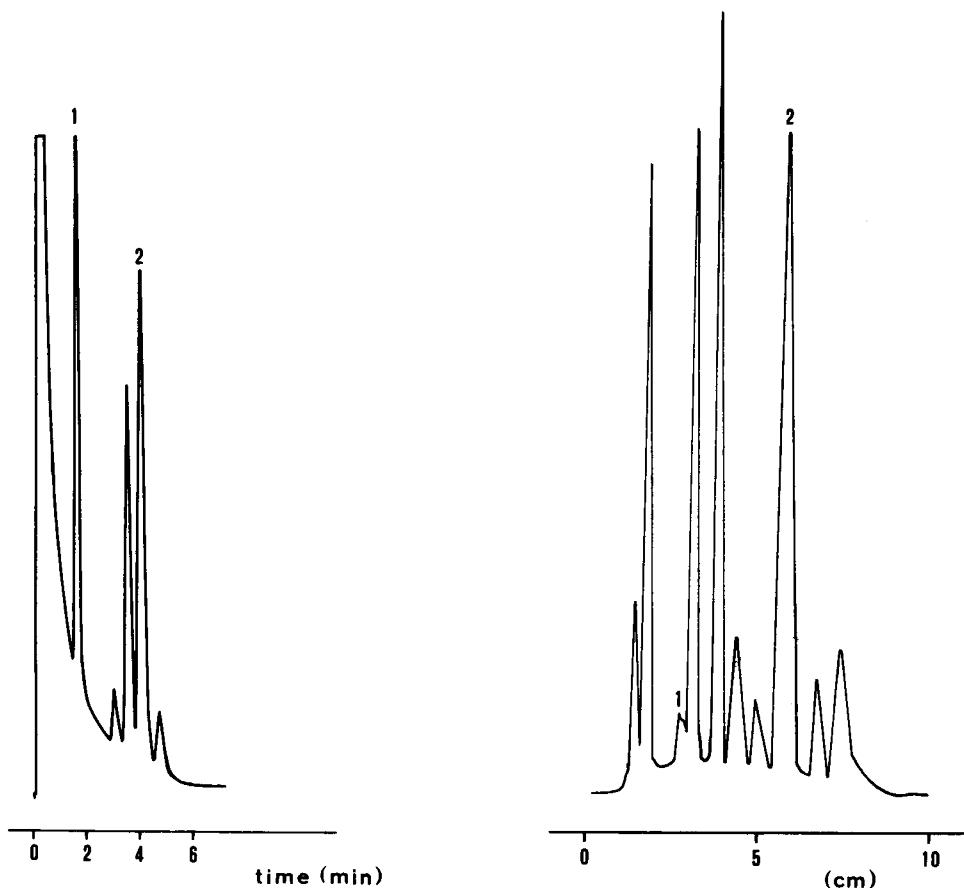

Fig. 1. GLC chromatogram of a *Holarrhena* stem bark extract. Column, 3% SE-30 Chromosorb W AW DMCS (100–120 mesh); carrier gas, helium; flow-rate, 30 ml/min; oven temperature, 230°C; detector and injector temperatures, 245°C. Peaks: 1 = codeine; 2 = conessine.

Fig. 2. TLC scanning profile of a *Holarrhena* stem bark extract. Adsorbent, HPTLC silica gel 60 F254; mobile phase, ethyl acetate–hexane–diethylamine (75:24:6); absorption wavelength, 500 nm. Peaks as in Fig. 1.

HPTLC–densitometry

The TLC system was derived as described previously[5], and led to efficient separation of conessine from other compounds in crude extracts (Fig. 2). None of the previously described colorimetric reactions of conessine[6] showed an acceptable sensitivity, either for detection or for HPTLC–densitometric assays. The combination of the alkaloid reagents Dragendorff and iodoplatinate significantly enhanced the detection limits and reproducibility, with orange derivatives well contrasted against a nearly colourless background. It was found necessary to cover the chromatoplate with a glass plate immediately after spraying of the second reagent and during measurement, in order to avoid recolouration of background as well as the evaporation of acetic acid, which could damage the densitometer. Codeine (internal standard for

TABLE I

DETERMINATION OF CONESSINE IN STEM BARK EXTRACTS; COMPARISON OF THE DENSITOMETRIC AND GLC RESULTS

Sample	*HPTLC results*			*GLC results*		
	Mean conessine (%) (dried powder)	*Standard deviation*		*Mean conessine (%) (dried powder)*	*Standard deviation*	
		Absolute	*Relative*		*Absolute*	*Relative*
1	0.11	0.01	10	0.15	0.01	5
2	0.36	0.02	6	0.37	0.02	4
3	0.07	0.01	15	0.09	0.01	10
4	0.42	0.02	4	0.41	0.01	3
5	0.79	0.01	2	0.76	0.02	3

GLC) reacted poorly with the chromogenic reagents, so it could not be used as an internal standard in TLC; it was completely separated from conessine and did not interfere in measurements. Concentrations of 0.1–0.9 μg of alkaloid base per microlitre spotted afforded a linear calibration graph with r values (correlation coefficient) typically greater than 0.990; the detection limit was *ca.* 40 ng.

Extraction procedure and comparison between HPTLC–densitometry and GLC results

A four-step extraction procedure guaranteed that at least 99% of the total conessine was extracted. The proposed densitometric HPTLC method was applied to conessine determination on stem bark of *H. floribunda* from Burkina-Faso (Africa); data were compared with those obtained by GLC (Table I) and show little difference in mean values or in coefficients of variation. Therefore these two assay methods should both prove to be of use in the rapid determination of conessine in plant material.

ACKNOWLEDGEMENTS

We thank Mr. A. Livaditis for his very skilful technical assistance, and the Administration Générale de Coopération au Développement of Ministère des Affaires Etrangères de Belgique and UNIDO (Vienna) for financial support.

REFERENCES

1 A. Bouquet and M. Debay, *Plantes médicinales de la Côte d'Ivoire*, Travaux et documents de l'Orstom, 32, Orstom, Paris, 1974, pp. 25–27.
2 W. Martindale, *The Extra Pharmacopoeia*, 25th ed., The Pharmaceutical Press, London, 1967, pp. 579–583.
3 R. R. Paris and H. Moyse, *Précis de Matière Medicale*, Vol. III, Masson and Cie, Paris, 1971, pp. 57–64.
4 *British Pharmaceutical Codex*, 5th ed., The Pharmaceutical Press, London, 1949.
5 A. B. Svendsen and R. Verpoorte, *Chromatography of Alkaloids, Part A*, Elsevier, Amsterdam, 1983, p. 27.
6 A. Schmit, *Travaux Lab. Mat. Med. Paris*, 35 (1950) 37.