ELSEVIER

Journal of Chromatography A, 863 (1999) 123–126

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Separation, identification and determination of sanguinarine in argemone and other adulterated edible oils by reversed-phase high-performance liquid chromatography

Sajid Husain*, R. Narsimha, R. Nageswara Rao

Analytical Chemistry and Environmental Science Division Indian Institute of Chemical Technology, Hyderabad 500 007, India

Received 28 May 1999; received in revised form 15 July 1999; accepted 6 September 1999

Abstract

A simple, rapid and reliable reversed-phase high-performance liquid chromatographic method for the separation and determination of sanguinarine in argemone and other edible oils has been developed. The separation has been achieved on a C_{18} column with CH_3OH–CH_3CN–tetrahydrofuran–water as mobile phase using diode array detection at 280 nm. The minimum detection limit of sanguinarine in the adulterated edible oils is 5 μg/g. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Oils; Argemone oil; Sanguinarine; Alkaloids; Benzophenanthridine

1. Introduction

Sanguinarine is one of the benzophenanthridine alkaloids found not only in the plant extracts of *Macleaya microcarpa* but also in the oil extracted from *Argemone mexicana* [1,2]. It has shown antibacterial as well as anti-inflammatory properties and is under clinical trials for the treatment of periodontal disease [3,4]. However, it has recently led to a dropsy epidemic resulting not only in neuroparalysis but also in the death of several people who consumed adulterated mustard oil in India [5]. It is believed that the sanguinarine present in argemone oil is toxic and interferes with the oxidation of pyruvic acid, accumulating in blood and tissues culminating in widespread vascular dilation and oedema of internal organs. Therefore development of rapid and reliable methods for its determination are of great importance for quality assurance of edible oil.

A few methods for the assay of sanguinarine have been reported in the literature [6,7]. High-performance liquid chromatography (HPLC) has been used, but the conditioning of cyano column with triethylamine and acidified methanol was found to be quite tedious [8]. An alternative method using hexyltriethyl ammonium phosphate as an ion-pairing reagent was developed but significant tailing in the peak corresponding to sanguinarine was noticed [9]. Moreover, it has been reported in the literature that the use of ion-pairing reagents is restricted because of baseline artifacts and irregular peak shapes [10–12].

In the present paper, a simple and rapid reversed-

*Corresponding author.

0021-9673/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(99)00964-4

phase high-performance liquid chromatographic method for quantitative estimation of sanguinarine in edible oils using a C_{18} column with methanol–acetonitrile–tetrahydrofuran (THF)–water (21:55:4:20, v/v) as mobile phase and a photodiode array detection at 280 nm at ambient temperature is described.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass distilled water (Nanopure, Barnsted, USA) was used through out the study. HPLC-grade methanol, acetonitrile and tetrahydrofuran (Spectrochem, Bombay, India) were used. Sanguinarine chloride (Sigma, St. Louis, MO, USA) was used as certified reference material. Mustard, palm, til and ricebran oils purchased from a local market and argemone oil extracted from its seeds were used. Edible oils suspected as adulterated with argemone were collected from different sources and used.

2.2. Apparatus

A HPLC system composed of two LC-10AT Vp pumps, an SPM-10Vp diode array detector, an SIL-10 ADVp autosampler, a DGU-12 A degasser, and SIL-6B system controller (all from Shimadzu, Kyoto, Japan) was used. A reversed-phase C_{18} (Shimadzu) column (250 mm×4.6 mm I.D., particle size 5 μm) was used for separation. The chromatographic and integrated data were recorded using the HP-Vectra (Hewlett-Packard, Waldbron, Germany) computer system.

2.3. Chromatographic conditions

The mobile phase was methanol–acetonitrile–tetrahydrofuran–water (21:55:4:20, v/v). Samples were dissolved in the mobile phase and the analysis was carried out under isocratic conditions at a flow-rate of 1 ml/min. Chromatograms were recorded at an absorption wavelength of 280 nm using diode-array detection.

2.4. Analytical procedure

Oil samples (1 ml) were dissolved in the mobile phase (10 ml) and a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures and the adulterated oil samples were analyzed under identical conditions. The amount of sanguinarine was calculated from the area of its corresponding peak in the chromatograms.

3. Results and discussion

Reversed-phase HPLC studies of sanguinarine in large excess of edible oils have been carried out and it has been found that the retention behavior of sanguinarine relied greatly upon the composition of the mobile phase. A complex and highly unusual eluent, i.e., CH_3OH–CH_3CN–THF–water (21:55:4:20, v/v) has been selected for analysis. The small quantity of tetrahydrofuran has been used to increase solubility while methanol and acetonitrile to improve not only the resolution but also the selectivity of sanguinarine on a C_{18} column. The mobile phase constituents have been optimized in such a way that the signal corresponding to sanguinarine is free from interferences of fatty acid components of the edible oils. The retention time of sanguinarine is approximately 5.8 min. Mustard, palm, til and ricebran oils have also been subjected to HPLC under the same conditions and no peak is seen after 4.3 min. The fatty acid components of these oils have not shown any signals either overlapping or interfering with that of sanguinarine. Two different wavelengths of maximum absorption, i.e., 280 and 320 nm were observed in the UV spectrum of sanguinarine chloride in methanol. However the limit of detection using 280 nm has been found to be higher when compared to 320 nm. Therefore the UV detector was set at 280 nm and used for both detection and quantification.

Known amounts of sanguinarine were added to mustard oil to yield concentrations in the range of 0.01–1.0 mg/g and used for calibration. A typical chromatogram of mustard oil spiked with 100 μg/g sanguinarine is shown in Fig. 1. The standard curve for sanguinarine was found to be linear with a regression coefficient (r^2) of 0.9930. The minimum

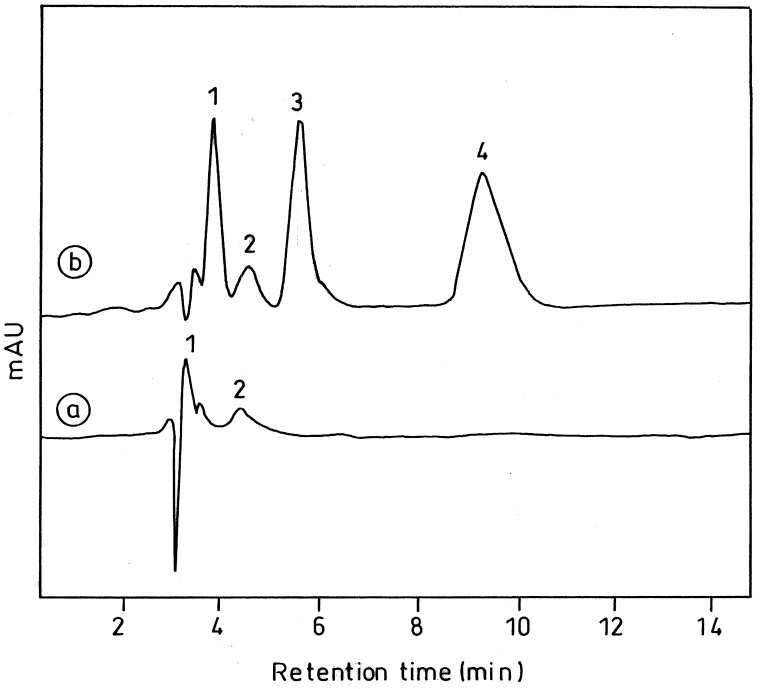

Fig. 1. Typical chromatograms of a mustard oil; (a) unspiked and (b) spiked with argemone. Peaks 1 and 2, mustard; 3, sanguinarine (100 μg) and 4, argemone.

detectable quantity of sanguinarine was found to be approximately 5 μg/g with a signal-to-noise ratio of 4 at 0.001 AUFS. The precision of analysis was assessed by five replicate analyses of edible oils containing sanguinarine in the concentration range of 0.01–1.0 mg/g and then the variations were calculated. The response for a series of five injections of 10 μg of sanguinarine in mustard oil has resulted in a relative standard deviation (RSD) of 3.5% for retention time (t_R) and 2.8 for peak area (i_p) respectively. Similarly, the RSDs in measuring t_R and i_p of sanguinarine in palm, til and ricebran oils have been determined and were found to be in the ranges of 3.9–4.8% and 2.4–3.6%, respectively.

The quality of mustard and other edible oils suspected to be adulterated with argemone were thoroughly checked by HPLC. A typical chromatogram of one of the oils is shown in Fig. 2. The presence of sanguinarine in the referred oils has been confirmed by two different techniques. In the first instance a peak is observed around 5.8 min in the HPLC chromatogram having a λ_{max} of 280 nm in the UV spectrum. Later it has been confirmed by extracting it from the oil and subjecting it to mass spectrometric analysis for identification. The mass spectrum of sanguinarine chloride has been shown as an inset in Fig. 2. The molecular ion (M^+332) and fragmentation patterns were compared with that of reference standard unambiguously. Thus, sanguinarine has been found to be present in some of the oils analyzed by HPLC, however, the degree of adulteration varied significantly. The content of argemone has been found to be as high as 22.7% in the oil extracted from mustard seeds. These results indicate that the argemone seeds might have been camouflaged as mustard and got mixed up with them either at the time of harvesting the crops or at a later stage.

In conclusion, the proposed HPLC method for estimating sanguinarine in adulterated edible oils is simple, rapid and easily adoptable for quantitative determinations. It has eliminated not only the use of ion-pair reagents responsible for baseline artifacts but also time-consuming sample preparation pro-

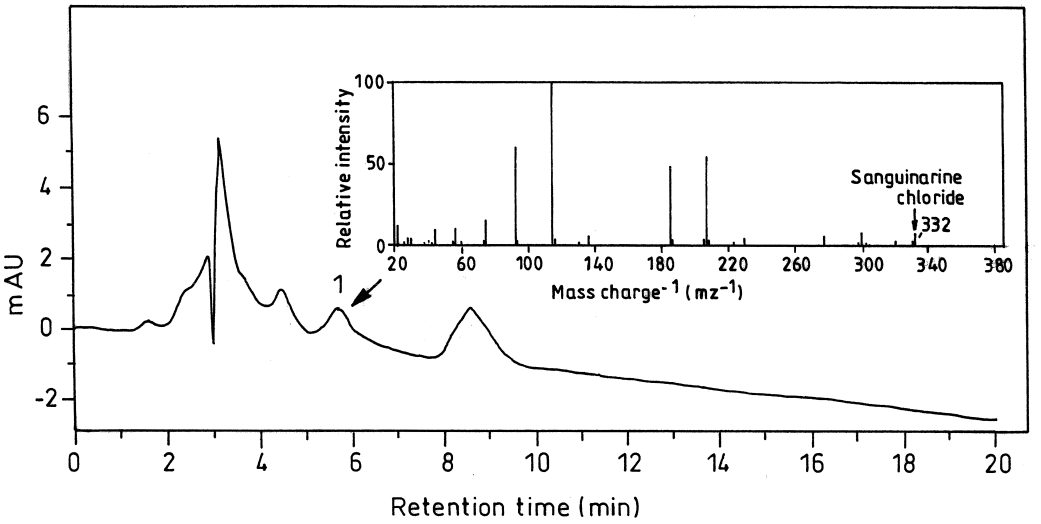

Fig. 2. Typical chromatogram of an adulterated mustard oil collected from a vendor. Peak 1=sanguinarine found 8.2 μg/g. The inset shows the mass spectrum of sanguinarine chloride.

cedures thus making the method simple and cost effective. It is reliable and convenient not only for clinical but also forensic investigations.

Acknowledgements

The authors wish to thank Dr. K.V. Raghavan, Director, IICT, Hyderabad for his keen interest in our studies and kind permission to communicate the results for publication (IICT communication No. 4329).

References

[1] P.B. Gove, in: Webster's Third New International Dictionary, G&C Merriam, USA, 1961, p. 2011.
[2] C. Walker, Can. Dental Assoc. 56 (1990) 13.
[3] J. Lenfield, M. Karoutil, E. Marsalek, J. Slavik, V. Preininger, V. Simanek, J. Med. Plant. Res. 43 (1981) 161.
[4] B.D. Krane, M.O. Fagbull, M. Shama, J. Nat. Prod. 47 (1984) 1.
[5] A. Madhavan, J. Oil. Tech. Assoc. Ind. 30 (1998) 181.
[6] S.A.E. Hakim, V. Mijovie, J. Walker, Nature 189 (1961) 198.
[7] I.S. Shenalikar, S. Babu, G.S. Thaper, B.V. Ramasastri, Ind. J. Med. Res. 73 (1981) 204.
[8] E.M. Thorne, R.T. Boulware, R.J. Harkrader, G.L. Southard, J. Soc. Cosmet. Chem. 37 (1986) 279.
[9] P. Reinhart, R. Harkrader, R. Wylie, G. Yewey, J. Chromatogr. 570 (1991) 425.
[10] H.U. Ehmeke, H. Kelker, K.H. Konigs, H. Uliner, Fresenius Z. Anal. Chem. 294 (1979) 251.
[11] P. Jandera, H. Engelhardt, Chromatographia 13 (1980) 18.
[12] P. Jandera, H. Churacek, J. Chromatogr. 197 (1980) 181.