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Short communication

Reversed-phase high-performance liquid chromatographic separation and quantification of lantadenes using isocratic systems

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Abstract

Isocratic systems of varying suitability are reported for reversed-phase HPLC of lantadenes, the pentacyclic triterpenoids from the hepatotoxic plant *Lantana camara* var. *aculeata*. The column used was a Nova-Pak C_{18} (250×4.6 mm). The detection was at 210 nm and 240 nm. The mobile phase acetonitrile–water–acetic acid (80:20:0.01) provided baseline separation of all the lantadenes except lantadenes A and C. The mobile phase methanol–water–acetic acid (85:15:0.01) also provided resolution of all the lantadenes but the retention time windows were very broad. The mobile phase methanol–acetonitrile–water–acetic acid (68:20:12:0.01) has been found to be the most suitable for the separation and quantitation of lantadenes. © 1997 Elsevier Science B.V.

Keywords: *Lantana camara*; Lantadenes; Triterpenoids

1. Introduction

Lantadenes are pentacyclic triterpenoids having a side chain at C-22 (Fig. 1) and are present in the leaves of the plant *Lantana camara* [1,2]. Lantadenes which have been found to be present in the hepatotoxic taxa of lantana plant are lantadene A (LA), lantadene B (LB), lantadene C (LC), lantadene D (LD), reduced lantadene A (RLA) and reduced lantadene B (RLB) [1–5]. They are the esters of 22-hydroxyoleanonic acid (Fig. 1) which forms the core structure of lantadenes. Previously, we have reported the TLC procedures for the separation of lantadenes using a number of solvent systems [6]. These systems had the limitations of resolution, sensitivity and adoption for quantification in biological samples. HPLC has been the technique of choice for the separation and quantification of natural products including oleanane type triterpenoids [7–9]. Isocratic separations are favoured, whenever possible, as they do not require complex gradient systems, are easily reproduced and eliminate the necessity of re-equilibrating the column between the runs. We report here the protocols for separation and quantification of lantadenes using isocratic reversed-phase HPLC systems.

2. Experimental

Lantadenes were prepared from *Lantana camara* var. *aculeata* (Red flower variety) as described previously [1,3–5]. The identity of the compounds was confirmed by comparison with authentic standards and by spectroscopic analysis. 22-Hydroxyoleanonic acid was prepared by alkaline hydrolysis

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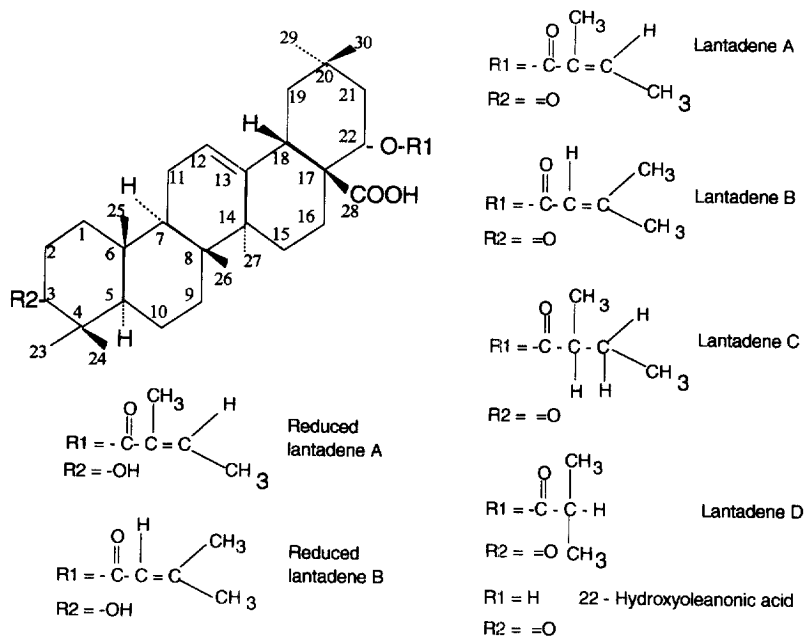

Fig. 1. Chemical structures of lantadenes.

of lantadene A [10]. The solvents for HPLC were of HPLC grade and were purchased from E. Merck (India), and S.D. Fine Chemicals (India). Acetic acid (HPLC grade) was purchased from Sisco Research Labs. (Mumbai, India). Other solvents were of analytical grade and were freshly distilled before use. Silica gel GF_{254} was from Sisco Research Labs., TLC procedures were as described earlier [6,11]. The mobile phase constituents for HPLC analysis were mixed (v/v) and filtered using Millipore filtration assembly with 0.22 μm Durapore membrane filter. For HPLC analysis a Nova-Pak C_{18} (4 μm, 250×4.6 mm) column, a 510 pump, a 490E multichannel detector, a Millennium data processor (Waters, USA) and a Rheodyne injector with 20 μl loop were used. The flow-rate was 1 ml/min. Detection was at 210 nm and 240 nm. Different combinations of the solvents methanol, acetonitrile, water and acetic acid were used to achieve baseline separation of lantadenes.

2.1. Lantadenes samples

A standard solution of lantadenes containing LA (1 mg), LB (1 mg), LC (2 mg), LD (2 mg), RLA (1 mg), RLB (1 mg) and OA (2 mg) was prepared in 10 ml methanol and filtered through a 13 mm Whatman stainless-steel filter assembly using a 0.22 μm Durapore membrane filter. Twenty μl aliquots were used for HPLC injections using different mobile phases. Calibration curves (mass of the lantadenes injected vs. peak area) were linear over the range of 0.05–4 μg for LA, LB; 0.1–4 μg for LC, LD; 0.05–20 μg for RLA, RLB; and 0.1–20 μg for OA. Peak areas showed good reproducibility (average R.S.D. of 0.4%).

2.2. Quantification of lantadenes in lantana leaf samples

Lantana leaf samples were collected from lantana plants (*Lantana camara* var. *aculeata*) in the month of September, in Kangra Valley from the vicinity of IVRI laboratories. The samples were dried at 70°C. Fine power of 1 mm particle size was prepared using Cyclotec grinder (Tecator, Sweden). 2 g leaf powder was extracted with acetonitrile (3×40 ml) with intermittent shaking. Standard LA (5 mg) was added

as the standard to another set of 2 g leaf powder and was processed similarly for recovery studies. Acetonitrile extract was dried in vacuo. The residue was dissolved in 10 ml dichloromethane and 1 ml aliquots were taken for preparative TLC using silicagel GF_{254} plates. Aliquots (10 μl) of lantadenes mixture containing 20 μg of each dissolved in dichloromethane were applied in a parallel lane on the same plate for comparison. The plates were developed in the solvent system light petroleum (b.p., 60–80°C)–ethyl acetate–acetic acid (88:10:2). After development, the plates were air dried and observed in a UV cabinet at 254 nm. The zone corresponding to lantadenes was scraped off and extracted with methanol (3×10 ml). The methanolic extracted was filtered through a 0.22 μm Durapore membrane filter using a 13 mm Whatman stainless syringe filtration assembly. Twenty μl aliquots were used for HPLC analysis.

3. Results and discussion

Mobile phases containing methanol, acetonitrile and water, with or without acetic acid were used. Peaks were very broad and LA, LC and LB, LD were not resolved in the mobile phase methanol–water (90:10). However, addition of acetic acid to this mobile phase slightly improved the resolution and the peaks became sharp. Further manipulation of the proportion of methanol and water provided baseline separation of all the lantadenes but the t_R windows were broad and run times needed were longer (Fig. 2). LD and RLB did not resolve well and LA and LC eluted as single peak in the mobile phase acetonitrile–water (90:10). Addition of acetic acid did not improve the resolution. Baseline resolution of all lantadenes except LA and LC was obtained using the mobile phase acetonitrile–water (80:20). The resolution further improved and the peaks became sharper when acetic acid was added to this mobile phase, but LA and LC again eluted as single peak. The mobile phase methanol–acetonitrile–water–acetic acid (68:20:12:0.01) provided baseline resolution of all lantadenes except LA and LC where resolution approached the baseline and the peaks were well resolved. The peaks were sharp, the t_R windows were narrow and the run time required was also less (Fig. 3). Lantadenes, which did not have an olefinic bond (–C=C–) in the side chain and OA which is not esterified at C-22 did not absorb at 240 nm. The amounts of different lantadenes in lantana leaf powder are given in Table 1. LA was the most abundant followed by LC, LB, LD, RLA and RLB. LA and LC have been found to induce hepatotoxicity in guinea pigs [3,4]. The recovery of LA added exogenously to lantana leaf powder, as standard, was 98±2 (n=3). The methodology pre-

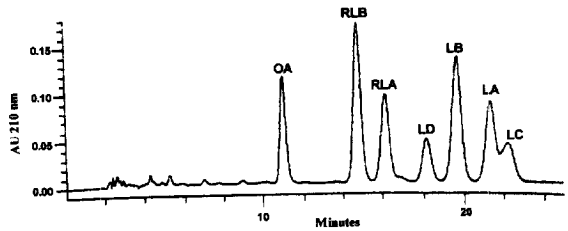

Fig. 3. Elution profile of lantadenes. Mobile phase: methanol–acetonitrile–water–acetic acid (68:20:12:0.01).

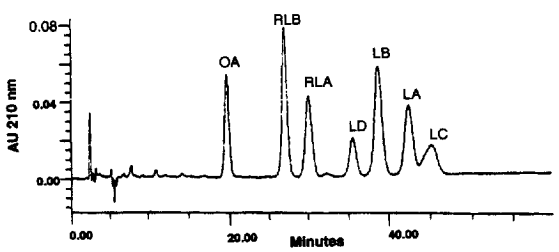

Fig. 2. Elution profile of lantadenes. Mobile phase: methanol–water–acetic acid (85:15:0.01).

Table 1
Quantification of lantadenes in lantana leaves

Lantadene	Amount (mg/100 g dry mass)
Lantadene A	721±18
Lantadene B	499±15
Lantadene C	642±38
Lantadene D	170±4
Reduced lantadene A	27±2
Reduced lantadene B	14±1

Values are ±S.D. (n=3).

sented here can be adopted for quantification of lantadenes in biological samples for metabolism and disposition studies of the animal system.

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References

[1] O.P. Sharma, P.D. Sharma, J. Sci. Ind. Res. 48 (1989) 471.
[2] O.P. Sharma, J. Vaid, P.D. Sharma, J. Chem. Ecol. 17 (1991) 2283.
[3] O.P. Sharma, V. Pattabhi, R.K. Dawra, J. Biochem. Toxicol. 6 (1991) 57.
[4] O.P. Shamna, J. Vaid, V. Pattabhi, K.K. Bhutani, J. Biochem. Toxicol. 7 (1992) 73.
[5] O.P. Sharma, R.K. Dawra, Phytochemistry 29 (1990) 3961.
[6] O.P. Sharma, R.K. Dawra, J. Chromatogr. 587 (1991) 351.
[7] M.C. McMaster, HPLC, a Practical Users' Guide, VCH, New York, 1994.
[8] M.B. Radosevich, N.E. Delfel, J. Chromatogr. 292 (1984) 403.
[9] H.B. Mostad, J. Doehl, J. Chromatrogr. 396 (1987) 157.
[10] S.R. Johnes, J.A. Lamberton, T.C. Morton, H. Saures, R.I. Willing, Aust. J. Chem. 36 (1983) 1895.
[11] E. Stahl, Thin Layer Chromatography, a Laboratory Handbook, Springer, New York, 1969.