Asian Journal of Chemistry; Vol. 23, No. 5 (2011), 2046-2048

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Separation and Simultaneous Quantification of α- and β-Asarone in *Acorus calamus* Linn. From Indian Sub-Continent on Caffeine Modified Silica

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(*Received*: 26 June 2010; *Accepted*: 14 January 2011) AJC-9485

A simple and validated HPTLC method was applied on caffeine modified silica using toluene:ethyl acetate (93:7 v/v) as mobile phase for simultaneous determination of α- and β-asarone, two major isomeric phytoconstituents of *Acorus calamus* Linn (Family: Araceae). The mobile phase gave well seperated and compact spots for both α- and β-asarones at retardation factor (R_f) of 0.67 ± 0.02 and 0.77 ± 0.02, respectively over modified silica plates. Densitometric analysis of asarones (α and β) was carried out in the absorbance mode at 313 nm in the concentration range 50-1000 ng/spot and 10-1000 µg/spot for α and β asarone, respectively.

Key Words: TLC, Asarone, Caffeine modified silica, Quantification.

INTRODUCTION

Dried rhizomes of *Acorus calamus* Linn. contains α- and β-asarone (Fig. 1)[1]. α-Asarone showed hypocholesterolemic[2], antioxidant[3], antidiabetic[4] activity and also used in status epilepticus[5] whereas, β-asarone showed anticonvulsant effect and used in neurological disorders[6,7]. It has been reported as a more potent hypotensive than α-asarone[8].

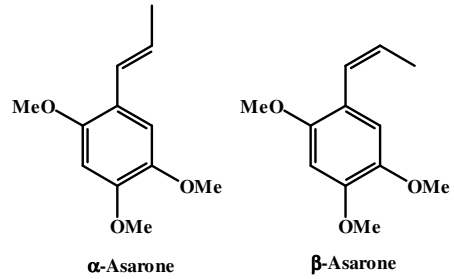

Fig. 1. Structure of α- and β-asarone

Several analytical methods have been reported for isolation of different components by GC-MS[9,10] and quantification of α-asarone by micellar electrokinetic capillary chromatography-UV-Vis specrophotometery[11] and β-asarone by HPTLC and HPLC[12,13]. Till date there is no fully validated method reported for simultaneous separation and determination of asarone isomers by TLC/HPTLC. In the present investigation slightly modified method reported by Widmer *et al.*[12] on separation and quantification on caffeine modified silica was used to separate the both the isomers on the same plate having different R_f. The content of asarones were quantified simultaneously in alcoholic extracts of different samples of calamus rhizome collected from different geographical conditions of India with good linearity. The proposed HPTLC method was validated as per the ICH guidelines similar to the several chromatographic HPLC and HPTLC methods developed and validated in the laboratory for the quality control of herbal drugs[14-21].

EXPERIMENTAL

Standard α- and β-asarones were purchased from Sigma Aldrich and other chemicals and reagents were of analytical grade (AR) and procured from Merck Ltd. India and S.D. Fine Chem., India.

Collection of *Acorus calamus* rhizomes: Samples of *Acorus calamus* were collected from different regions of India: Hamdard University Herbal Garden (Delhi) cultivated, Sambhag (Madhya Pradesh) wild, Lucknow-1 Integral University Herbal Garden (Uttar Pradesh) cultivated, Lucknow-2 (Uttar Pradesh) wild, Chadigarh (Punjab) wild, Banaras (Uttar Pradesh) wild, Kottayam (Kerala) wild, Tamil Nadu (Chennai) wild and Gurgaon (Haryana) wild. The samples collected were identified by Dr H.B. Singh, NISCAIR New Delhi (Ref. NISCAIR/RHMD/consult-2008-09/1149/181/02/01-09).

Sample preparation: Dried powdered rhizome samples were refluxed with ethanol (100 mL) for 2 h and evaporated to dryness in rota vapour. The residue obtained were re-dissolved (20 mg/mL) in chromatographic grade ethanol.

Optimization of mobile and stationary phase: Previously, toluene:ethyl acetate (9:1 v/v) mobile phase was used followed by modification of stationary phase with silver nitrate for isomeric separations. Later, caffeine 5 % in dichloromethane was used to modify stationary phase and finally 10 % caffeine in dichloromethane was selected for separation. Similarly, mobile phase was changed for better separation and toluene: ethyl acetate (93:7 v/v) was finalized.

HPTLC instrumentation: The samples were spotted (2 µL/spot) in the form of band (width 5 mm) with a Camag microlitre syringe on TLC aluminium plate precoated with silica gel 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 120 nL/s was employed and space between two bands was 6.6 mm. The plate was pre-derivatized using 10 % caffeine solution in chloroform and dried in hot air oven at 100 °C for 15 min. Linear ascending development was carried out in twin trough glass chamber (20 cm × 10 cm) saturated with the mobile phase *i.e.*, toluene:ethyl acetate (93:7 v/v). The length of chromatogram run was 80 mm subsequent to the development; TLC plate was dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 313 nm. The source of radiation utilized was tungsten lamp. The slit dimension was kept 4 mm × 0.45 mm Micro and 20 mm/s scanning speed was employed.

Preparation of calibration curves of α- and β-asarone: Stock solution containing α- and β-asarone 100 µg and 1000 µg/mL, respectively were prepared in ethanol and from this 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 µL/spot were spotted in triplicate on TLC plate to obtain concentrations of 50 to 1000 ng/spot for α-asarone and 10 to 1000 µg/spot for β-asarone. The data of peak area *vs.* drug concentration was treated by linear least-square regression by WINCATS software.

Quantification of α- and β-asarone in all samples: Two µL (4 mm band length) of all samples (20 mg/mL) were applied in duplicate on TLC plate (20 cm × 10 cm) for quantification of α- and β-asarone in the samples, collected from different regions of India. The α- and β-asarone were quantified by WINCATS software using regression equation obtained from calibration curve and the mean of duplicate samples were calculated with respect to height and area separately.

RESULTS AND DISCUSSION

The modified caffeine impregnated TLC plates and optimized mobile phase (toluene:ethyl acetate, 93:7 v/v) used in the investigation were found to show good resolution and gave well-separated, sharp and compact spots for α- and β-isomers of asarones at different R_f values 0.67 ± 0.02 for α and 0.77 ± 0.02 for β, respectively (Fig. 2). 20 mL of the mobile phase was used to run the chromatogram up to 80 mm length. The optimized chamber saturation time for mobile phase was 15 min at room temperature. Densitometric analysis of asarones (α and β) was carried out in the absorbance mode at 313 nm using W lamp.

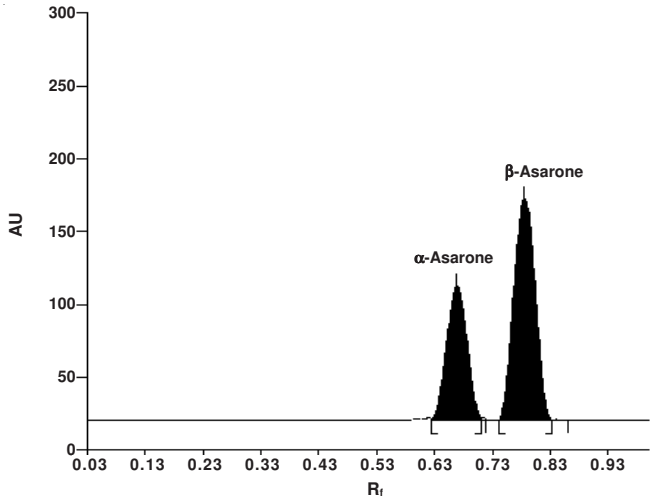

Fig. 2. HPTLC chromatogram of standard α-asarone and β-asarone

The linear regression analysis data of α-asarone for the calibration curve in the concentration range of 50-1000 ng/spot showed good linear relationship with $r^2 = 0.985 \pm 0.0054$ and regression equation Y = 1.789x + 1591 with respect to peak area. The data for calibration curve of β-asarone showed good linear relationship having $r^2 = 0.995 \pm 0.0071$ with regression equation Y = 31.51x + 1331 with respect to peak area in the concentration range of 10-1000 ng/spot. The values for slope and intercept for both the isomers are presented in Table-1.

TABLE-1
LINEAR REGRESSION DATA FOR CALIBRATION CURVE (N = 3)

Calibration parameters	α-Asarone	β-Asarone
Linearity range (ng/spot)	50-1000	10-1000
Regression equation	Y=1.789x+1591	Y=31.51x+1331
Correlation coefficient (r ± SD)	0.985±0.0054	0.995±0.0071
Slope ± SD	1.789±0.088	31.51±0.062
Intercept ± SD	1591.53±2.68	1331.14±1.95

The accuracy was determined in terms of % recovery and was found to be 97.5-103.03 % for α-asarone and 97.5-103.4 % for β-asarone after addition of extra 50, 100 and 150 % of the standard asarones. The intra-day (repeatability) and inter-day (reproducibility) and inter-analyst precisions were found at the concentration levels of 20, 40 ng for α-asarone and 200, 500 ng for β-asarone. The % RSD values for intra-day, inter-day and inter-analyst precisions were found to be 0.49-0.91, 1.22-1.3 and 2.07-2.72 for α-asarone, 1.07-1.32, 1.22-1.61 and 2.12-2.61 for β-asarone, respectively. The low values indicated good precision of the proposed method. The low values of % RSD (< 2) obtained after introducing small deliberate changes in mobile phase composition, mobile phase volume and saturation time at concentrations of 20, 50 ng/spot for α and 200, 500 ng/spot for β asarone in triplicate indicated the robustness of the method. LOD and LOQ, obtained by blank determination method, were found to be 15 ng, 50 ng/spot for α-asarone and 3.4 ng, 10 ng/spot for β-asarone, respectively. The low values of LOD and LOQ indicated the sensitivity of the method proposed. The summary of validation parameters were given in Table-2.

TABLE-2
METHOD VALIDATION OF ASARONE ISOMERS

Validation parameters	Values	
	α	β
Accuracy (n = 6)	97.5-103.03	97.5-103.4
Precision (n = 6)		
Intra-day (% RSD range at two concentration level)	0.49-0.91	1.07-1.32
Inter-day (% RSD range at two concentration level)	1.2-1.3	1.22 – 1.61
Inter -analyst (% RSD range at two concentration level)	2.07-2.72	2.12-2.61
Robustness (n = 6)		
Mobile phase composition (% RSD range at two compositions)	0.86-1.74	1.31-2.00
Mobile phase volume (% RSD range at two different volumes)	1.37-2.07	1.03-1.80
Saturation time (% RSD range at two different time)	1.53-1.91	0.90-1.74
Limit of detection (ng spot $^{-1}$)	15	3.4
Limit of quantification (ng spot^{-1})	50	10

The proposed method was found specific by comparing R_f of samples and standard resolution factor (1.03) as well as the super imposed spectra. The chromatogram showed different R_f for α-asarone and β-asarone in sample as well as in standards.

The content of α-asarone was found highest in the alcoholic extracts of samples collected from Madhya Pradesh (0.79 % w/w) followed by Delhi (0.69 % w/w), Gurgaon (0.57 % w/w) and Lucknow (0.56 % w/w). The samples collected from Banaras showed least amount of α-asarone (0.17 % w/w) followed by Chennai (0.36 % w/w) and Chandigarh (0.39 % w/w).

The content of β-asarone in alcoholic extracts was found to be highest in the samples collected from Kottayam (Kerala) (11.18 % w/w) followed by Delhi samples (10.8 % w/w) and Banaras samples (10.4 % w/w, whereas the samples collected from other regions of India were found to contain 8.69-9.77 % w/w of β-asarone. The least amount of β-asarone was found present in the samples collected from Chandigarh region (8.69 % w/w). The results calculated from the regression equations using WINCATS software and mean value obtained from area is represented in Table-3.

TABLE-3
CONTENT OF α- AND β-ASARONES IN ALCOHOLIC EXTRACTS OF CALAMUS COLLECTED FROM DIFFERENT PLACES OF INDIA

Sample	Content % w/w	
	α-Asarone	β-Asarone
Delhi	0.686	10.812
Madhya Pradesh	0.797	9.777
Lucknow-Cultivated	0.559	9.537
Punjab	0.388	8.695
Varanasi	0.170	10.428
Kerala	0.457	11.176
Tamil Nadu	0.356	9.284
Haryana	0.566	8.773
Lucknow-Wild	0.531	9.576

The present caffeine modified simultaneous HPTLC method for the determination of α-asarone and β-asarone found simple, economic, accurate, precise and reproducible and can be used for the regular quality control and safety evaluation of the *Acorus calamus* Linn. as well as formulations, food stuffs and beverages containing it or its oil/ extract as an ingredient for medicinal purpose (Ayurveda and Unani system of medicine) or as a flavouring agent.

REFERENCES

1. K.R. Kritikar and B.D. Basu, Indian Medicinal Plant (Indian Medical Science Series No. 93). Periodical Experts Book Agency, New Delhi, India, Vol. 11, p. 3617 (2000).
2. L. Garduno, M. Salazar, S. Salazar, M.E. Morelos, F. Labarrios, J. Tamariz and G.A. Chamorro, *J. Ethnopharmacol.*, **55**, 161 (1997).
3. S. Manikandan and R.S. Devi, *Pharmacol. Res.*, **52**, 467 (2005).
4. O. Rau, M. Wurglics, T. Dingermann, M. Abdel-Tawab and M. Schubert-Zsilavecz, *Pharmazie*, **61**, 952 (2006).
5. J.J. Chen, *J. Tradit. Chin. Med.*, **4**, 217 (1984).
6. P.C. Dandiya and M.K. Menon, *Br. J. Pharmacol. Chemother.*, **20**, 436 (1963).
7. H.B. Wu and Y.Q. Fang, *Yao Xue Xue Bao.*, **39**, 836 (2004).
8. J.D. Sharma and P.C. Dandiya, *Indian J. Med. Res.*, **50**, 61 (1962).
9. G. Mazza, *J. Chromatogr. A*, **328**, 179 (1985).
10. R. Oprean, L. Oprean, M. Tamas, R. Sandulescu and L. Roman, *J. Pharm. Biomed. Anal.*, **24**, 1163 (2001).
11. K.M. Hanson, G.M. Ely, L.A. Holland, P.S. Zehr and B.C. Saderberg, *Electrophoresis*, **26**, 943 (2005).
12. V. Widmer, A. Schibli and E. Reich, *J. AOAC Int.*, **88**, 1562 (2005).
13. C. Chen, D. Spriano and B. Meier, *Planta Med.*, **75**, 1448 (2009).
14. S. Ahmad, R. Ahmad, R. Parveen, M. Mujeeb and M. Aquil, *Chromatographia*, **67**, 441 (2008).
15. P. Alam, M. Ali and S. Ahmad, *J. Chromatogr. Sci.*, **47**, 910 (2009).
16. M.J. Ansari, S. Ahmad, K. Kohli, J. Ali and R.K. Khar, *J. Pharm. Biomed. Anal.*, **39**, 132 (2005).
17. R. Parveen, S. Baboota, S. Ahmad, J. Ali and A. Ahuja, *Biomed. Chromatogr.*, **24**, 639 (2010).
18. P. Jha, R. Parveen, S.A. Khan, O. Alam and S. Ahmad, *J. AOAC Int.*, **93**, 787 (2010).
19. S.M. Musthaba, M.T. Athar, S. Baboota and S. Ahmad, *Asian J. Chem.*, **23**, 385 (2011).
20. S. Ahmad, Y.T. Kamal, M. Singh, R. Parveen and S.M. Musthaba, *Asian J. Chem.*, **23**, 469 (2011).
21. Y.T. Kamal, M. Singh, E.T. Tamboli, R. Parveen and S. Ahmad, *Acta Chromatogr.*, **23**, 157 (2011).