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Estimation of boswellic acids from market formulations of *Boswellia serrata* extract and 11-keto β-boswellic acid in human plasma by high-performance thin-layer chromatography

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Abstract

A rapid and sensitive high-performance thin-layer chromatographic (HPTLC) method was developed and validated for the quantitative estimation of boswellic acids in formulation containing *Boswellia serrata* extract (BSE) and 11-keto β-boswellic acid in human plasma. Simple extraction method was used for isolation of boswellic acid from formulation sample and acidified plasma sample. The isolated samples were chromatographed on silica gel $60F_{254}$-TLC plates, developed using ternary-solvent system (hexane–chloroform–methanol, 5:5:0.5, v/v) and scanned at 260 nm. The linearity range for 11-KBA spiked in 1 ml of plasma was 29.15–145.75 ng with average recovery of 91.66%. The limit of detection and limit of quantification for 11-KBA in human plasma were found to be 8.75 ng/ml and 29.15 ng/ml. The developed method was successfully applied for the assay of market formulations containing BSE and to determine plasma level of 11-keto β-boswellic acid in a clinical pilot study.
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Keywords: *Boswellia serrata* extract; 11-Keto boswellic acid; Market formulation; Human plasma; HPTLC method

1. Introduction

The *Boswellia serrata* extract (BSE), used for the treatment of inflammatory diseases in Ayurvedic system of medicine [1,2], is reported to contain monoterpenes, diterpenes and triterpenes [3–5]. The anti-inflammatory activity is attributed to the presence of four-pentacyclic triterpene acids, viz. β-boswellic acid (BA), 3-acetyl β-boswellic acid (ABA), 11-keto β-boswellic acid (11-KBA) and 3-acetyl 11-keto β-boswellic acid (A-11-KBA) [2] (Fig. 1). 11-KBA and A-11-KBA have more pronounced anti-inflammatory activity [6]. Boswellic acid derivative, A-11-KBA, is selective, non-competitive, non-redox and potent inhibitors of 5-lipoxygenase, which is the key enzyme of leukotriene biosynthesis from arachidonic acid [6–8]. It decreases the activity of human leukocyte elastase (HLE) [9]. It was reported that acetyl boswellic acids are effective cytotoxic agents, inhibit human topoisomerases I and IIα activity [10].

For the analysis of boswellic acids from *Boswellia serrata* extract, nonaqueous titration method [11], RP-HPLC method [12] and HPTLC method [13,14] are reported while HPLC [15–17], GC–MS [18] and LC–MS method [19] are reported for their estimation in human plasma. There is no common method reported which can analyze boswellic acids in formulation and in plasma. Previously reported HPTLC methods [13,14] are less sensitive and specific for the analysis of boswellic acids. They were applied only for the analysis of boswellic acids from natural resin material. Therefore, it was thought of interest to develop an HPTLC method for the estimation of boswellic acids both in market formulations and in human plasma. Since however A-11-KBA could not be detected in considerable amounts in plasma [16], a specific method restricted to the determination of the stable analyte, 11-KBA, in human plasma was developed. The developed method was successfully applied to estimate the plasma concentration of 11-KBA in human volunteers after administering single oral dose tablet containing

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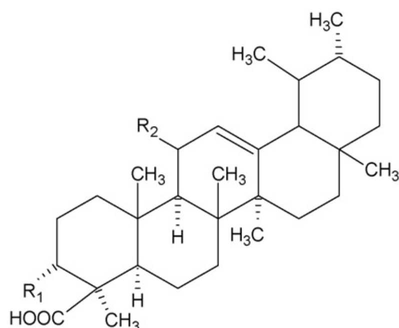

Chemical name	R_1	R_2
β-boswellic acid (BA)	-OH	$-H_2$
3-acetyl β-boswellic acid (ABA)	-OAc	$-H_2$
11-keto β-boswellic acid (KBA)	-OH	=O
3-acetyl 11-keto β-boswellic acid (AKBA)	-OAc	=O

Fig. 1. Pentacyclic triterpenic acids.

500 mg of BSE. The developed method is rapid, sensitive and economic.

2. Experimental

2.1. Instrumentation

The HPTLC system (Camag Sonnenmattstr, Mutenz, Switzerland) consisting of a Linomat IV semi-automatic spotting device, a glass twin-trough TLC chamber (20 cm × 10 cm), a TLC scanner-3 and a data station with CATS4 software and HPTLC syringe (100 μl capacity; Hamilton Company, Reno, Nevada, USA) was used for chromatographic studies.

2.2. Chemicals and reagents

Chloroform, hexane, hydrochloric acid, anhydrous sodium sulphate (s.d.fiNE cHEM Ltd., Mumbai, India), methanol (Ranbaxy Fine Chemicals Ltd., New Delhi, India), methyl tert-butyl ether (Riedel de Haen, Germany) used were of analytical reagent grade. Drug/metabolite free plasma was obtained from B. J. Medical College (Ahmedabad, India). Working standard of BSE (composition: 18.74% β-boswellic acid, 12.59% acetyl-β-boswellic acid, 5.83% 11-KBA, 3.25% A-11-KBA) and human volunteer plasma samples for analysis were supplied by M. P. Shah Medical College (Jamnagar, India).

2.3. Chromatographic conditions

Separation was performed on 10 cm × 10 cm aluminium-backed plates precoated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). The TLC plate was pre-washed with methanol and dried in an oven at 50 °C for 10 min. Samples were spotted on the TLC plate 10 mm from the bottom edge using Linomat IV semi-automatic spotter and analyzed using following parameters: band width, 4 mm (for formulation samples) or 2 mm (for plasma samples); track distance, 4 mm; spraying rate, 10 s/μl; volume of mobile phase, 20 ml; temperature, 27 ± 1 °C; relative humidity, 35–40%; chamber saturation time, 45 min; migration distance, 80 mm; slit dimension, 3 mm × 0.45 mm (for formulation samples) or 1 mm × 0.2 mm (for plasma samples); scanning speed, 5 mm/s; detection wavelength, 260 nm. Mobile phase consisted of hexane–chloroform–methanol (5:5:0.5, v/v).

2.4. Preparation of stock solution of standard BSE

Accurately weighed 12.5 mg standard BSE was dissolved in 25 ml of methanol to get BSE stock solution (500 μg/ml; corresponding to 29.15 μg/ml of 11-KBA and 16.25 μg/ml of A-11-KBA).

2.5. Preparation of working standard solution of BSE

One milliliter of stock solution of standard BSE was diluted to 10 ml with methanol to get final concentration 50 μg/ml of BSE (corresponding to 2.92 μg/ml of 11-KBA and 1.63 μg/ml of A-11-KBA).

2.6. Preparation of sample solution for analysis of tablet formulation

Ten tablets were weighed accurately and finely powdered. Tablet powder equivalent to 100 mg of BSE was accurately weighed and transferred to 50 ml volumetric flask and 15 ml of methanol was added. The mixture was sonicated for 30 min, diluted to the mark with methanol and filtered through Whatman filter paper No. 41. Filtrate (0.25 ml) was diluted to 10 ml with methanol.

2.7. Procedure for extraction of 11-KBA from plasma

One milliliter of plasma (drug/metabolite free or spiked with fixed aliquots of working standard BSE solution or volunteer plasma sample) was transferred to a tapered-bottom glass centrifuge tube (15 ml capacity). Hydrochloric acid (0.05 M, 100 μl) was added and mixed by vortexing for 1 min at high speed. The mixture was allowed to stand for 5 min and was extracted with mixture of hexane–methyl tert-butyl ether (5 ml, 2:3, v/v) by vortexing at high speed for 1 min, followed by centrifugation at 615 × *g* for 5 min. The clear supernatant layer obtained was transferred to another test-tube and dried with anhydrous sodium sulphate. The dried organic layer was evaporated to dryness on water bath (60–70 °C) and the residue obtained was reconstituted in 70 μl of chloroform.

2.8. Chromatographic separation

Working standard solution (30 μl) and sample solution for analysis of tablet formulation (30 μl) or chloroform extracts of

drug/metabolite free plasma or plasma spiked with BSE or volunteer plasma samples (50 μl) were spotted on the pre-washed TLC plate under nitrogen stream using semi-automatic spotter. The plate was dried under IR lamp and developed in a twin-trough chamber previously saturated for 45 min with mobile phase, hexane–chloroform–methanol (5:5:0.5, v/v). After development, the plate was dried in air. Photometric measurements were performed at 260 nm in absorbance/reflectance mode with Camag TLC Scanner-3 using CATS4 software incorporating the track optimization option.

2.9. Identification of boswellic acid

Standard BSE powder (50 mg) was hydrolyzed with sodium hydroxide solution (20%, w/v, 25 ml) by heating on steam bath for 2 h. The content was cooled to room temperature, neutralized with hydrochloric acid and extracted with chloroform (10 ml × 3). The chloroform extract was dried with anhydrous sodium sulphate and evaporated to dryness. The obtained solid (5 mg) was dissolved in methanol (100 ml) and analyzed as described in Section 2.8.

2.10. Preparation of calibration curve

2.10.1. Calibration curve of standard 11-KBA and A-11-KBA

Aliquots of 10, 20, 30, 40 and 50 μl of BSE solution (50 μg/ml, corresponding to 29.15, 58.30, 87.45, 116.60, 145.75 ng of 11-KBA/spot and 16.25, 32.50, 48.75, 65.00, 81.25 ng of A-11-KBA/spot) were spotted on a TLC plate and analyzed as described under Section 2.8. Calibration curve was prepared by plotting peak area of 11-KBA and A-11-KBA against their respective concentration.

2.10.2. Calibration curve for 11-KBA spiked in plasma

Aliquots (10, 20, 30, 40, and 50 μl) of working standard solution of BSE (corresponding to 29.15, 58.30, 87.45, 116.60, 145.75 ng of 11-KBA) were spiked in 1 ml of drug/metabolite free plasma. Spiked plasma samples were extracted using extraction method described under Section 2.7. Fifty microliter of the reconstituted sample was spotted on a TLC plate and analyzed as described under Section 2.8. Calibration curve was constructed by plotting peak area versus corresponding 11-KBA concentration.

2.11. Validation of the proposed method

2.11.1. For tablet formulation

2.11.1.1. Linearity. The linear responses for 11-KBA and A-11-KBA in the range 10–50 μl of BSE solution corresponding to 29.15–145.75 ng of 11-KBA/spot and 16.25–81.25 ng of A-11-KBA/spot were assessed in terms of slope, intercept, and correlation coefficient values.

2.11.1.2. Accuracy. The accuracy was determined by standard addition method. To a fixed amount of pre-analyzed sample of BSE, increasing amount of standard BSE solution was added in all the levels of calibration curve. The recovery of 11-KBA and A-11-KBA was calculated at each level ($n = 3$).

2.11.1.3. Precision.

2.11.1.3.1. Repeatability. Repeatability of measurement of peak area and peak height: Standard BSE solution (30 μl, 50 μg/ml) was spotted on a TLC plate, developed and dried. The separated spot was scanned for seven times without changing plate position and RSD for measurement of peak area was computed.

2.11.1.3.2. Repeatability of sample application. Standard BSE solution (30 μl, 50 μg/ml) was spotted on a TLC plate seven times, developed, dried and photometrically analyzed as described under Section 2.8. The area of seven spots was measured and the RSD of peak area was calculated.

2.11.1.3.3. Inter-day and intra-day precision. The inter-day precision (RSD) was determined by analyzing standard solution of BSE over the entire calibration range for 5 days.

The intra-day precision (RSD) was determined by analyzing standard solution of BSE over the entire calibration range for five times on the same day.

2.11.1.4. Limit of detection. Aliquots of 2, 5, 7, 10, 20 μl of standard BSE solution (50 μg/ml, corresponding to 5.83, 14.58, 20.41, 29.15, 58.30 ng of 11-KBA/spot and 3.25, 8.13, 11.38, 16.25, 32.50 ng of A-11-KBA/spot) was spotted on a TLC plate. The plate was developed and analyzed as described under Section 2.8.

2.11.1.5. Specificity. The spots of boswellic acids (11-KBA and A-11-KBA) from tablet formulation were confirmed by comparing its R_F and absorbance/reflectance spectrum with that of standard 11-KBA and A-11-KBA. The peak purity of boswellic acids was determined by correlating the spectra of 11-KBA and A-11-KBA scanned at peak start, peak apex, and peak end positions of the spot.

2.11.2. For plasma analysis

2.11.2.1. Linearity. The linear response for 11-KBA spiked in plasma in the range 10–50 μl of BSE solution corresponding to 29.15–145.75 ng/ml was assessed in terms of slope, intercept, and correlation coefficient values.

2.11.2.2. Accuracy. The accuracy of the method in terms of the extraction efficiency of the method was determined for 11-KBA from plasma. Drug/metabolite free plasma spiked with BSE solution in the range of calibration curve was analyzed as described in Section 2.10.2. Finally, accuracy [%] was calculated using the formula: Accuracy [%] = [Peak area of 11-KBA spiked in plasma × 70 × 100]/[Peak area of standard 11-KBA × 50].

2.11.2.3. Precision. The precision of the proposed method in terms of intra-day variation (RSD) was determined by analyzing plasma samples spiked with BSE solution over the entire calibration range for five times on the same day. Inter-day precision (RSD) was assessed by analysis of plasma samples spiked

with BSE solution over the entire calibration range daily for 5 days.

2.11.2.4. Limit of detection. For determining limit of detection of 11-KBA, plasma spiked with 3, 7, 10, 20 μl of standard BSE solution (50 μg/ml, corresponding to 8.75, 20.41, 29.15, 58.30 ng of 11-KBA) and analyzed as described in Section 2.10.2.

2.11.2.5. Specificity. The specificity of the method was ascertained by analyzing standard BSE, blank (drug free) plasma and plasma spiked with standard BSE. The spot of 11-KBA from plasma components was confirmed by comparing its R_F and absorbance/reflectance spectrum of the spot with standard 11-KBA. The peak purity for 11-KBA was tested by correlating the spectra of 11-KBA scanned at peak start, peak apex and peak end positions of the spot.

2.11.2.6. Stability of 11-KBA in plasma. Stability of 11-KBA in plasma under storage conditions (−20 °C, 17 days, in glass vial) was studied by analyzing spiked plasma sample containing 145.75 ng of 11-KBA/ml of plasma. The sample was analyzed on the same day as well as on third, eighth and seventeenth day of storage, for the amount of 11-KBA. Ratios of concentration of 11-KBA on different days of storage to that on first day and 90% confidence interval for these ratios were determined.

2.12. Analysis of samples

2.12.1. Analysis of tablet formulation

Working standard BSE solution (30 μl, 50 μg/ml) and sample solution were spotted on a TLC plate in replicate and analyzed as described in Section 2.8. The amount of 11-KBA and A-11-KBA present in sample solution was determined by fitting area values of corresponding peak into the equation representing calibration curve of 11-KBA and A-11-KBA.

2.12.2. Analysis of volunteer plasma samples

A preliminary study was undertaken in six healthy volunteers by administering single oral dose tablet containing 500 mg BSE (Boswell® Tablets, Reneissance Herb, Ayurceutics Ltd., U.S.). The volunteer blood sample was collected after 2 h of administration of tablet dosage form, centrifuged and kept in deep fridge at −20 °C until analysis. The collected plasma samples were analyzed as described under Section 2.10.2.

3. Results and discussion

Owing to its versatility, sensitivity, and speed of analysis, the HPTLC method was considered to be suitable, for analysis of boswellic acids in tablet formulation and human blood plasma.

3.1. Chromatographic conditions

The TLC plates were prewashed with methanol and dried in oven to remove any adsorbed or volatile impurities. Methanol and chloroform were used in mobile phase for migration of drug whereas hexane retards the migration of drug. It was observed that drying of TLC plate under I.R. lamp after spotting and presaturation of TLC chamber with mobile phase for 45 min ensured good reproducibility of R_F value.

3.2. Extraction and separation of 11-KBA from plasma

Plasma samples were acidified with 0.05 M hydrochloric acid to precipitate out 11-KBA. Hexane–methyl tert-butyl ether (2:3, v/v) mixture was selected as the solvent for extraction of 11-KBA from plasma, which yielded 88.13–95.73% recovery. Emulsion formation was observed on vortexing acidified plasma with organic solvent, therefore it was centrifuged at 615 × *g* for 5 min to break emulsion. 11-KBA was well separated from co-extracted material and resolved very well under the described chromatographic conditions at R_F 0.16 without any interference from constituents of the plasma**.**

3.3. Identification of boswellic acids

The spotted working standard solution showed two clear spot at 0.16 and 0.25 R_F. To confirm the spot of A-11-KBA, the standard BSE solution was hydrolyzed with sodium hydroxide and analyzed. Following this treatment the hydrolyzed BSE solution showed only one peak at R_F 0.16 while the peak at R_F 0.25 was abolished confirming that the spot at R_F 0.25 corresponds to A-11-KBA, which was hydrolyzed on treatment with sodium hydroxide to 11-KBA (Fig. 2). Thus it was confirmed that the spots at R_F 0.16 and 0.25 may be attributed to 11-KBA and A-11-KBA, respectively.

3.4. Method validation

3.4.1. Formulation analysis

3.4.1.1. Linearity. Representative calibration curve of 11-KBA and A-11-KBA was obtained by plotting the mean peak

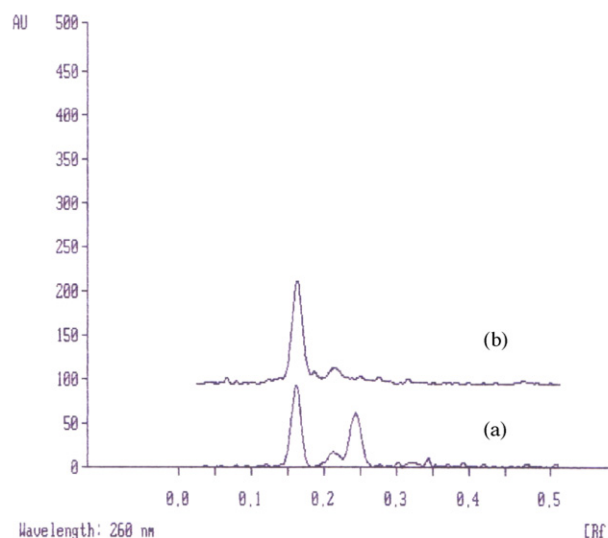

Fig. 2. Comparison of chromatograms obtained from (a) standard BSE solution with (b) standard BSE solution obtained after hydrolysis with sodium hydroxide.

area of 11-KBA and A-11-KBA against concentration over the range of 29.15–145.75 ng/spot and 16.25–81.25 ng/spot, respectively ($n = 5$). They were found to be linear in the above-mentioned range with correlation coefficients of 0.9982 for 11-KBA and 0.9939 for A-11-KBA. The RSD for 11-KBA and A-11-KBA were found to be in the range of 3.56–4.90% and 4.30–5.35%, respectively. The average linear regressed equations for the corresponding curves were $y = 19.94x + 197.98$ (11-KBA) and $y = 27.32x + 11.72$ (A-11-KBA).

3.4.1.2. Accuracy. The recovery of added sample was 98.24 to 104.17% and 94.12 to 105.92% for 11-KBA and A-11-KBA, respectively.

3.4.1.3. Precision.

3.4.1.3.1. Repeatability of measurement of peak height and area. Precision of the instrument was checked by repeated scan of the same spot (Standard BSE solution, 30 μl, 50 μg/ml) seven times without changing the plate position. The RSD for measurement of peak height and peak area of 11-KBA were found to be 0.10 and 0.16, respectively, while for A-11-KBA corresponding values were found to be 0.16 and 0.12, respectively.

3.4.1.3.2. Repeatability of sample application. The repeatability of sample application (RSD) for height and area of 11-KBA were found to be 2.50 and 2.30 and for A-11-KBA corresponding values were found to be 2.70 and 3.40, respectively, which ensures precision of the spotter device.

3.4.1.3.3. Inter-day precision. Inter-day precision (RSD, $n = 5$) was found to be 3.56–4.90% and 4.70–5.35% for 11-KBA and A-11-KBA, respectively. Intra-day precision (RSD, $n = 5$) was found to be 1.29–4.99% and 3.07–4.08% for 11-KBA and A-11-KBA, respectively.

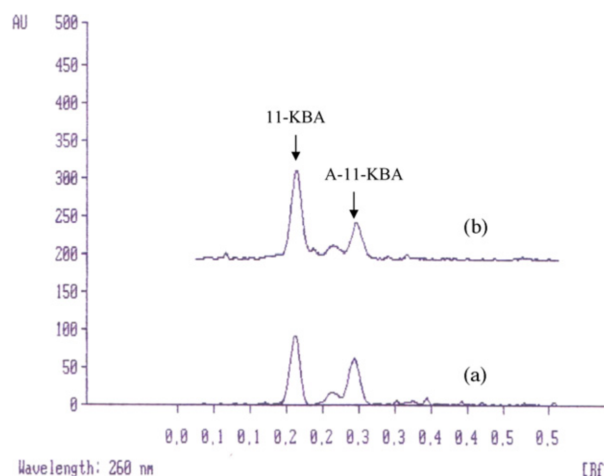

Fig. 3. Comparison of chromatogram of (a) 11-KBA and A-11-KBA obtained from tablet formulation and (b) standard 11-KBA and A-11-KBA of BSE.

3.4.1.4. Limit of detection. The limit of detection was found to be 14.58 ng/spot and 11.38 ng/spot for 11-KBA and A-11-KBA, respectively.

3.4.1.5. Specificity. Comparison of chromatograms of standard boswellic acids (11-KBA and A-11-KBA) and boswellic acids from tablet formulation showed identical R_F values for both acids, i.e. 0.16 for 11-KBA and 0.25 for A-11-KBA. The excipient and other component present in tablet formulation did not interfere in the separation and resolution of 11-KBA and A-11-KBA (Fig. 3). Comparison of the spectra scanned at peak start (s), peak apex (m) and peak end (e) positions of individual spots of 11-KBA and A-11-KBA showed a high degree of correlation (above 0.99), confirmed the purity of the corresponding spots (Fig. 4). Apart from R_F values the spectrum of individual acids were also correlated with spectrum of standard boswellic acids. The correlation obtained was 0.9976 and 0.9842 for 11-KBA and

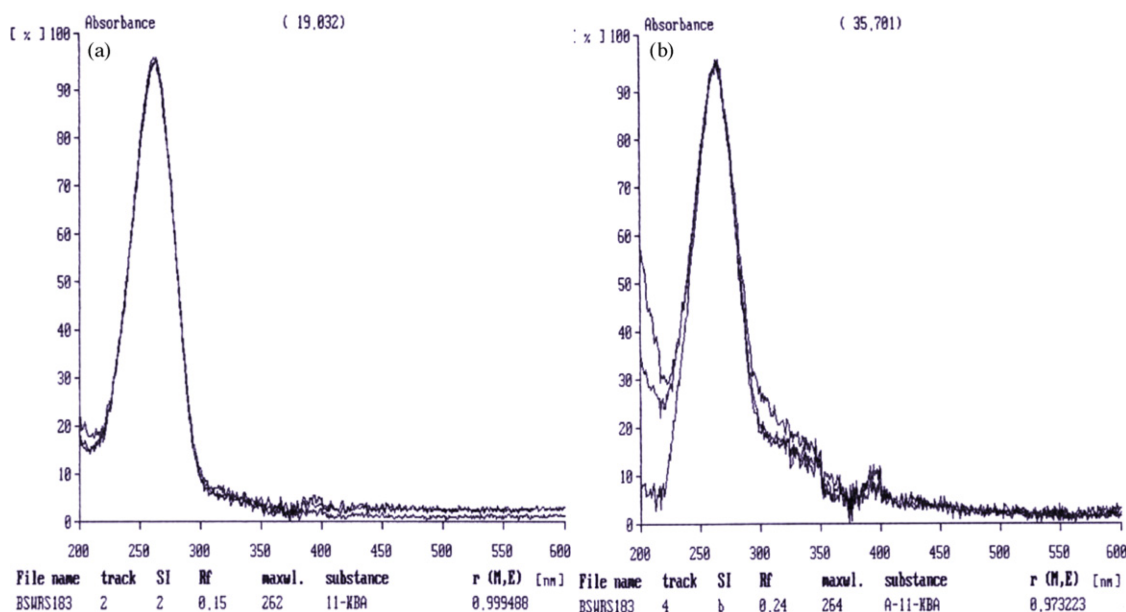

Fig. 4. Absorbance/reflectance spectra showing peak purity of (a) 11-KBA and (b) A-11-KBA of tablet formulation.

Table 1
Calibration data for standard 11-KBA spiked in plasma ($n = 5$)

Concentration of 11-KBA spiked in 1 ml of plasma (ng)	Peak area mean ± S.D.	RSD
29.15	891.28 ± 83.89	9.40
58.30	1705.74 ± 124.39	7.30
87.45	2377.54 ± 151.40	6.40
116.60	2839.56 ± 128.50	4.70
145.75	3540.40 ± 106.62	3.01

A-11-KBA, respectively, confirmed the identity of the spots.

3.4.2. Plasma analysis

3.4.2.1. Linearity. Linear least-square regression analysis of calibration curve demonstrated linearity between the peak areas and concentration of 11-KBA spiked in 1 ml of plasma in the range of 29.15–145.75 ng of 11-KBA with the correlation coefficient 0.9923. The average linear regression equation for curve was $y = 22.06x + 341.27$, where x is the concentration of 11-KBA and y is the peak area. Statistical data for all calibration standards are given in Table 1.

3.4.2.2. Accuracy. The percentage recovery of 11-KBA spiked in plasma was in the range of 88.13–95.73%.

3.4.2.3. Precision. The Inter-day and intra-day precision (RSD, $n = 5$) were varied in the range of 3.01–9.40% and 1.64–11.01%, respectively for 11-KBA spiked in the plasma.

3.4.2.4. Limit of detection. The limit of detection of 11-KBA spiked in plasma was found to be 8.75 ng/ml.

3.4.2.5. Specificity. Comparison of chromatograms of standard BSE, blank (drug-free) plasma, plasma spiked with standard BSE and volunteer plasma confirmed that endogenous plasma component and related compounds did not interfere in separation and resolution of 11-KBA (Fig. 5). Peak purity check of 11-KBA peak showed good correlation between spectra scanned at peak start, peak apex and peak end positions (r(s, m) = 0.9912 and r(m, e) = 0.9786), confirmed the purity of the 11-KBA spot. This was further supported by good correlation ($r = 0.8959$) between the spectrum of standard 11-KBA and the spectrum of 11-KBA spiked in plasma.

3.4.2.6. Stability of 11-KBA in plasma. The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior the analysis. Stability results are shown in Table 2. The concentration ratio of 11-KBA on day 3, day 8 and day 17 with respect to reference sample (day 1) were well within limits (0.91–1.03). The 90% confidence interval was found to be 0.80–1.19 for 11-KBA that is also within prescribed limits.

3.5. Analysis of formulation containing BSE

The formulation powder was sonicated with methanol for 30 min to ensure complete dissolution of boswellic acids. The

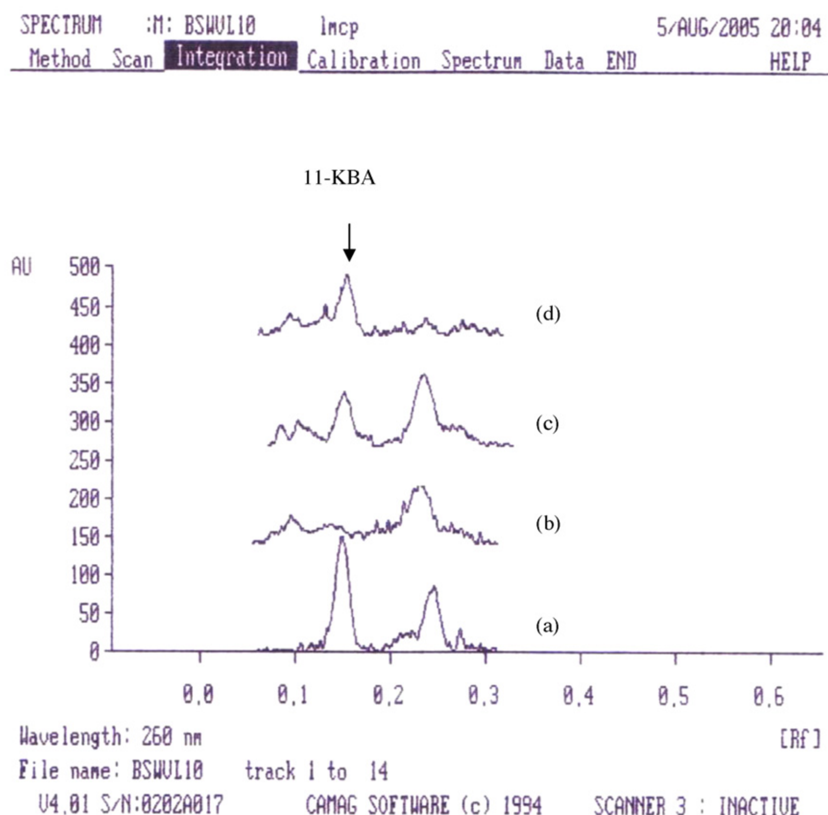

Fig. 5. Comparison of chromatogram of (a) standard 11-KBA; (b) blank plasma; (c) plasma spiked with standard BSE; and (d) volunteer plasma.

Table 2
Data for stability and confidence interval for 11-KBA spiked in plasma (145.75 ng/ml of plasma)

Day	Concentration found (ng/ml of plasma)	Mean ± S.D.	RSD	Concentration ratio (test day/reference day) (0.90–1.10)	90% Confidence interval	
					Lower limit (0.8)	Upper limit (1.2)
1	140.81	136.54 ± 6.62	4.84%	1.00	0.80	1.19
3	131.84			0.94		
8	128.54			0.91		
17	144.95			1.03		

Table 3
Estimation of 11-KBA and A-11-KBA in tablet formulation

Formulation	% of 11-KBA found	% of A-11-KBA found
A	4.84	1.21
B	1.65	1.13
C	4.39	1.28

Table 4
Plasma levels of 11-KBA in human volunteer after 2 h administration of Boswell® tablets

Volunteer number	Plasma concentration (ng/ml)
1	65.96
2	38.94
3	67.04
4	29.87
5	24.02
6	33.44

developed HPTLC method was used to estimate 11-KBA and A-11-KBA in three different market formulations. Amounts of 11-KBA and A-11-KBA were calculated using equations $y = 19.94x + 197.98$ and $y = 27.32x + 11.72$, respectively (where, y = peak area and x = concentration in ng/spot). Results of market formulation assay are given in Table 3.

3.6. Analysis of plasma samples

Plasma levels of 11-KBA after 2 h administration of boswellic acid tablet formulation containing 500 mg BSE (Boswell® Tablets, Reneissance Herb, Ayurceutics Ltd., U.S.) in six human volunteers was estimated using proposed HPTLC method. Table 4 shows the amount of 11-KBA found in volunteer plasma sample.

4. Conclusion

A rapid, sensitive, specific, accurate and precise HPTLC method was developed for estimation of boswellic acids (11-KBA and A-11-KBA) from tablet formulations of BSE and 11-KBA in human blood plasma. Validation assays for intra- and inter-day precision and accuracy as well as for limit of quantification met the international acceptance criteria for bioanalytical method validation. The proposed HPTLC method is more sensitive and specific for the analysis of boswellic acids than the reported methods. It can be used to generate plasma level curves for 11-KBA in pharmacokinetic study.

References

[1] K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants, I, 2nd edition, M/s Periodical Experts, Delhi, India, 1935, p. 521.
[2] G.K. Chatterjee, S.D. Pal, Indian Drugs 21 (1984) 431.
[3] R.S. Pardhy, S.C. Bhattacharya, Indian J. Chem. 16B (1978) 171.
[4] R.S. Pardhy, S.C. Bhattacharya, Indain J. Chem. 16B (1978) 174.
[5] R.S. Pardhy, S.C. Bhattacharya, Indian J. Chem. 16B (1978) 176.
[6] H. Safayhi, T. Mack, J. Sabieraj, M.I. Anazodo, L.R. Subramanian, H.P.T. Ammon, J. Pharmcol. Exp. Biol. 261 (1992) 1143.
[7] H. Safayhi, E.R. Sailer, H.P.T. Ammon, Mol. Pharmacol. 47 (1995) 1212.
[8] E.R. Sailer, L.R. Subramanian, B. Rall, R.F. Hoernlein, H.P.T. Ammon, H. Safayhi, Brit. J. Pharmacol. 117 (1996) 615.
[9] H. Safayhi, B. Rall, E.R. Sailer, H.P.T. Ammon, J. Pharmacol. Exp. Ther. 281 (1997) 460.
[10] T. Syrovets, B. Buchele, E. Gedig, J.R. Slupsky, T. Simmet, Mol. Pharmcol. 58 (2000) 71.
[11] R.K. Gupta, V.N. Gupta, V.K. Gupta, C.K. Atal, Indian Drugs 21 (1984) 532.
[12] M. Ganzera, I.A. Khan, Planta Med. 67 (2001) 778.
[13] K. Krohn, M.S. Rao, N.V. Raman, M. Khalilullah, Phytochem. Anal. 12 (2001) 374.
[14] R.T. Sane, M.M. Phadke, P.S. Hijli, M.C. Shah, P.H. Patel, Indian Drugs 35 (1998) 286.
[15] S. Sharma, V. Thawani, L. Hingorani, M. Shrivastava, V.R. Bhate, S.R. Khiyane, Phytomedicine 11 (2004) 255.
[16] M.A. Tawab, A. Kaunzinger, U. Bahr, M. Karas, M. Wurglics, M. Schubert-Zsilavecz, J. Chromatogr. B: Biomed. Sci. Appl. 761 (2001) 221.
[17] B. Büchele, T. Simmet, J. Chromatogr. B 795 (2003) 355.
[18] A. Kaunzinger, A. Baumeister, K. Cuda, N. Haring, B. Schug, J. Pharm. Biomed. Anal. 28 (2002) 729.
[19] K. Reising, J. Meins, B. Bastian, G. Eckert, W.E. Mueller, M. Schubert-Zsilavecz, M.A. Tawab, Anal. Chem. 77 (2005) 6640.