RESEARCH ARTICLE

Development and Validation of HPLC And HPTLC for Simultaneous Analysis of E and Z Guggulsterone, A-11–KBA And 11–KBA from Herbal Formulation

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ABSTRACT

RP-HPLC and HPTLC are two easy, sensitive, efficient, and accurate procedures that allow for simultaneous estimates of Z and E guggulsterone, A-11-KBA, and 11-KBA. For the HPLC method, we recommend the use of a symmetry C18 column. Using a solvent gradient based on solvent A (orthophosphoric acid) and solvent B (methanol), the effluent was monitored at 250 nm with a 1.0 mL/min flow rate. The peaks of 11-KBA and A-11-KBA were eluted at 5.8 and 6.3 minutes, while those of Z and E-guggulsterone were at 4.8 and 5.3 minutes. For the HPTLC method of separation, a silica gel layer was applied on an aluminum plate prewashed in methanol using a Camag Linomat V applicator fitted with a 100 µL syringe. The linear expansion was carried out using a solvent mixture of n-hexane, chloroform, ethyl acetate, and methanol (v/v/v: 10:3:3:1, respectively). Camag T.L.C. scanner III (V 1.4.3.6336), operating in reflectance-absorbance mode at 254 nm and controlled by win CATS software, was used to carry out the densitometric scanning. The R.F. resolutions for 11-KBA, A-11-KBA, E-guggulsterone, and Z-guggulsterone in the selected mobile phase were 0.68, 0.61, 0.39, and 0.28, respectively. The linearity, accuracy, and precision of the techniques were all confirmed. The proposed methods were successful in estimating E- and Z-guggulsterone as well as 11-KBA and A-11-KBA.

Keywords: RP-HPLC, HPTLC, Z and E –guggulsterone, A-11–K.B.A. & 11–K.B.A., ICH R_1 (Q_2) Guidelines.

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INTRODUCTION

The guggul plant (*Commiphora mukul*) contains the phytosteroid guggulsterone in its resin. Z-guggulsterone and E-guggulsterone are the two possible forms of guggulsterone. It was previously thought that because of its role as an antagonist of the farnesoid X receptor, cholesterol production in the liver would be reduced in humans. Numerous studies have shown that diverse doses of guggulsterone have no consequence on total cholesterol and that low-density lipoprotein ("bad cholesterol") levels actually increased in many cases.[1,2] Yet, guggulsterone can be found in a wide variety of dietary aids. The research set out to find a way to determine Z- and E-guggulsterone, 11-KBA & A-11-KBA in herbal products

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using a simultaneous RP-HPLC and HPTLC approach with ultraviolet detection. Several system appropriateness factors were investigated to ascertain the efficacy of the RP-HPLC and HPTLC technique.[3,4]

MATERIAL AND METHODS

Reagents and Chemicals

Plantex Ltd, Vijaywada, India was the source for the allusion standards of E-guggulsterone (98% purity), Z-guggulsterone (95% purity), 11-KBA & A-11-KBA. Himalaya. Pvt. Ltd. produces a polyherbal anti-arthritic preparation that contains extracts from seven different herbs: *Zingiber officinale, Boswellia serrata, Ricinus communis, Withania somnifera (L.) Dunal, Vitex negundo, and Nyctanthes arbortristis* are only a few medicinal plants used. The following chemicals were purchased from Merck in Mumbai, India: ethyl acetate, n-hexane, methanol and chloroform (analytical grade); chemicals, solvents and reagents were also used.

Chromatographic Condition

RP-HPLC method

For the HPLC analysis, we utilized a Waters 2695 LC system (Milford, Massachusetts, U.S.A.) outfitted with an Alliance 2695 separation module, a Performance PLUS inline degasser, a Waters pump-control module, an automatic injector, a heater/cooler for the column and sample, Waters Empower 2 software and a photodiode array detector. Disposable syringe filters with 0.45 µm; mdi Nylon-66 membranes were used to filter everything. A Symmetry C18 column (4.6 mm 250 mm 5 µm particle sizes; G.L. Sciences Inc., Japan) was used for the separation. The flow rate of the mobile phase was set to be 1.0 mL/min, and the solvent gradient consisted of 0.1% v/v solvent A and solvent B. Filtration with 0.45 µm; MDI Nylon-66 membrane disc filters degassed the mobile phase. At a column temperature of 30.5°C, the run lasted for 35 minutes. At 250 nm, the analytes were detected with an injection volume of 20 µL. Software was used to combine the chromatographic reaction. Z- and E-guggulsterone, A-11-KBA, and 11-KBA all reached their maximum concentrations at times of 9.29, 7.56, 24.46 and 20.57, minutes.

HPTLC method

The samples were labeled in 6 mm bands on methanol-prewashed, silica gel-coated aluminum plate 60 F254 using a Camag Linomat V applicator with a 100 µL syringe. The continuous flow rate was 150 µL/s.[5] At room temperature, the linear growth was nurtured in a 20x10 cm glass twin trough container (25°C ± 2) and relative humidity of 60 ± 5% using a solvent system (17 mL) comprised of n-nexane, chloroform, ethyl acetate, and methanol (10:3:3:1, v/v/v). About 80 mm of the chromatogram was extrapolated from the position of the sample. The plates were put into an air dryer following the developing procedure. Camag T.L.C. scanner III with win CATS software (Version 1.4.3.6336) used for density scanning in reflectance-absorbance mode at 254 nm. The scanning speed was 20 mm/s, and the slit size was 5 by 0.45 mm. An enclosed CAMAG Reprostar 3 and a digital camera were used to take the chromatoplate pictures.

Preparation of standard stock solutions

Solution of E–guggulsterone (100 µg/mL), Z–guggulsterone (115 µg/mL), 11–K.B.A. (220 µg/mL) and A–11–K.B.A. (200 µg/mL) were ready by alone diluting respective quantities with methanol.

Further dilutions of above stock solutions were ready in methanol in concentration 17.5 and 5 µg/mL for E & Z–guggulsterone (P); & 71.5 and 35 µg/mL for 11–K.B.A. & A–11–K.B.A. (Q) correspondingly used for the chromatographic analysis. HPTLC E and Z–guggulsterone were weighed; dissolved in methanol to obtain a solution of 100 µg/mL each. Samples added to methanol to get a solution of 250 µg/mL each. The stock solution of Z & E–guggulsterone, A-11–K.B.A. & 11-KBA was further diluted to get working standard mixture solution 10 µg/mL each of E and Z–guggulsterone, 50 µg/mL each of 11–K.B.A. & A–11–K.B.A. correspondingly. Chromatographic analysis proceeded using this solution as the working standard.[6]

Sample preparation

Average fill weight of 20 capsules was obtained by using a precision balance. Accurately 70 mL methanol taken in 100 mL volumetric flask containing a formulation equivalent to one capsule. After sonicating the flask in an ultrasonic water bath for 30 minutes, methanol was added until the desired concentration was reached.[7]

Method validation[8]

The developed procedure was tested and shown to be reliable in terms of its linearity, intraday, interday precision, accuracy, sensitivity and specificity. Tables 1 and 2 (A and B) display the results acquired in order to determine the efficacy of the RP-HPLC and HPTLC methods.

Recovery Studies

Experiments in recovery were performed to verify the method's efficacy and appropriateness and to examine for interference using the conventional adding technique. Standards were formulated into formula at 3 distinct concentrations (80, 100 and 120%); then analysed. Six separate assessments were made at each recovery tier, with the findings tabulated in Table 1.

RESULTS & DISCUSSION

Figure 1 shows an RP-HPLC chromatogram and Figure 2 shows an HPTLC chromatogram, both of which are typical results from sample preparation.

RP-HPLC

Using a Waters 2695 LC system, we developed an optimal procedure for the simultaneous determination of samples. At 1.0 mL/min flow, a solvent gradient of 0.1% v/v solvent A and B was used to separate the samples on a symmetry C_{18} column. There was excellent peak definition, symmetry, and resolution. Retention time of E and Z–guggulsterone, 11–KBA and A–11–KBA in standard and sample chromatograms were

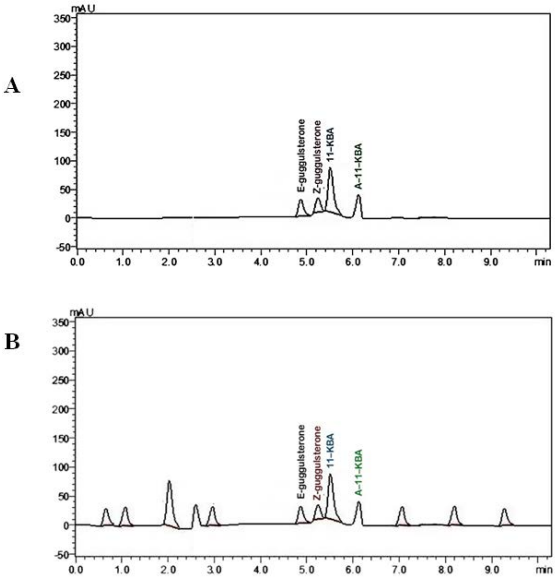

Figure 1 : A. HPLC Chromatogram of standard Z & E–guggulsterone, A-11–KBA & 11–KBA B. HPLC Chromatogram of Formulation

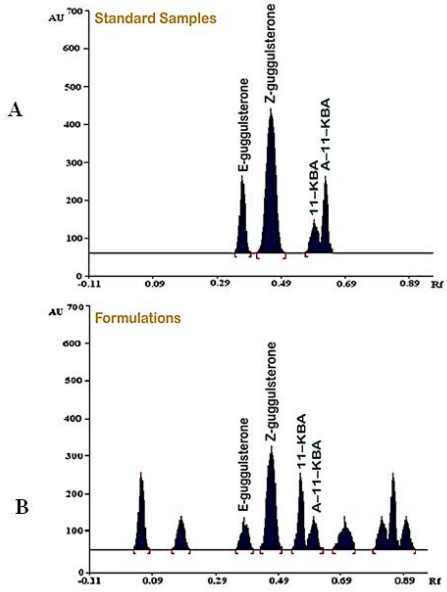

Figure 2 : A. HPTLC Chromatogram of standard E & Z–guggulsterone, 11–K.B.A. & A– 11–K.B.A. B. HPLC Chromatogram of Formulation

found to be 4.8, 5.3, 5.8 and 6.3 minutes, respectively and the optimized wavelength were found to be 250 nm. Technique was linear in the range of 2.6 to 3.6 µg/mL for E and Z–guggulsterone and 12-37.6 µg/mL for 11–KBA and A–11–KBA with regression coefficients of 0.9991, 0.9992, 0.9921 and 0.9993 for E and Z–guggulsterone, 11–KBA and A–11–KBA The recovery values for E and Z–guggulsterone were in the range of 101.32−109.33 % and 96.89−97.78 % with average recovery of 104.02 % and 97.24 % respectively. The recovery was in the range of 96.09−97.97 % and 98.11−98.72 % with average recovery of 97.20 % and 98.33 % for 11–KBA and A– 11–KBA respectively.

The adding, recovery, and percentage recovery values demonstrate the method's ability to recover 100% of the original dose despite the presence of excipients in the formulation.

The low values demonstrate the excellent precision of the procedure.

HPTLC

Many low, medium, and high-polarity solvent solutions were tried out to determine the optimal composition of the HPTLC mobile phase. A mobile phase consisting of 10% each of chloroform, n-hexane, methanol and ethyl acetate yielded the best results. The best possible mobile phase was n-hexane, chloroform, ethyl acetate, and methanol (10:3:3:1, v/v/v/v). Spots with clear borders were formed after 15 minutes of mobile phase saturation at room temperature. Good resolution was seen with the chosen mobile phase (R.F. = 0.61 for E-guggulsterone and 0.68 for Z-guggulsterone, and R.F. = 0.28 for 11-KBA and 0.39 for A-11-KBA). For Z and E-guggulsterone, method was linear between 10 and 90 ng spot^{-1}, while for A-11-KBA & 11-KBA, range was between 50 and 450 ng spot^{-1}, with regression coefficients of 0.9979 and 0.9989 for E and Z–guggulsterone and 0.9987 and 0.9981 for 11–KBA and A–11–KBA respectively. The LoD was found to be 2 ng/spot for E & Z guggulsterone and 10 ng/spot for 11–K.B.A., A–11–K.B.A., respectively. The LoQ was found to be 6.6 ng/spot for E & Z–guggulsterone & 33 ng/spot for 11–K.B.A., A–11 K.B.A., respectively. The recovery values for E and Z–guggulsterone ranged from 96.73–97.22% and 97.45–97.53% with an average recovery of 96.93 and 97.39%, respectively. The recovery was in the range of 97.06–98.03% and 97.01–97.64% with an average recovery of 97.60 and 97.22% for 11BA & A 11 K.B.A. This demonstrates that the procedure is not affected by any excipients in the formulation. Recovery standard deviation and coefficient of variation both have very small values (less than 2%) across all levels, demonstrating the high precision of the technique.

Table 1: Recovery study

Level	*%Recovery**							
	RP-HPLC				*HPTLC*			
	E–guggulsterone	*Z–guggulsterone*	*11–KBA*	*A–11–K.B.A.*	*E–guggulsterone*	*Z–guggulsterone*	*11–KBA*	*A–11–K.B.A.*
80	108.9	97.39	97.42	100.5	97.53	96.46	98.92	97.58
100	101.4	97.00	97.27	98.21	97.82	97.45	97.89	97.52
120	102.2	96.26	96.38	98.74	96.27	97.87	97.19	97.93

*n=6

Table 2A: Method validation with system suitability parameter

Parameter	*RP-HPLC*			
	E guggulsterone	*Z guggulsterone*	*11–KBA*	*A–11–K.B.A.*
Linearity Range	2.6 to 3.6 µg/mL	9 to 26 µg/mL	12.4 to 37.2 µg/mL	10 to 28 µg/mL
Regression Coefficient (r^2)	0.9991	0.9921	0.9992	0.9993
LoD	1.7 µg/mL	0.14 µg/mL	1.1 ng/mL	0.35ng/mL
LoQ	2.1 µg/mL	0.41 µg/mL	3.13 ng/mL	0.82ng/mL
Precision				
Intra-Day (%RSD)	2.93	3.01	2.04	1.17
Inter-Day (%RSD)	3.29	3.40	2.33	1.49
Retention time	4.8	5.3	5.8	6.3
Tailing Factor	01.1	01.1	01.0	01.1
No. of Theoretical Plates	7065	8223	72807	50327

Table 2B: Method validation and system suitability parameter

Parameter	*HPTLC*			
	E–guggulsterone	*Z–guggulsterone*	*11–KBA*	*A–11–K.B.A.*
Linearity Range (ng/mL)	10–90	10–90	50–450	50–450
Regression Coefficient (r^2)	0.9979	0.9989	0.9987	0.9981
Limit of Detection (ng/mL)	2	2	10	10
Limit of Quantification (ng/mL)	6.6	6.6	33	33
Precision				
Intra Day (% R.S.D.)	01.02	01.07	00.66	01.06
Inter Day (%RSD)	01.09	00.96	00.80	01.30

CONCLUSION

The precision, specificity, accuracy, stability suggesting, and robustness of the established HPLC and HPTLC procedures are remarkable. Validation experiments indicate that the proposed approaches for the simultaneous determination of Z & E guggulsterone; A-11 KBA, 11-KBA in bulk and in the pharmaceutical formulation should be accurate. A combination of RP-HPLC and HPTLC was validated according to the ICH requirements. The ICH criteria have been used to verify an RP-HPLC and HPTLC technique. This means they can be used for basic analyses of tablet quality.

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