INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF OLEANOLIC ACID IN ARISTAKA

Komal Khanpara[1]*, Renuka[1], V. J. Shukla[2]
[1]Ph. D. Scholar, Pharmaceutical Chemistry Laboratory, I. P. G. T. and R.A., Dhanvantari Bhawan, GAU, Jamnagar, Gujarat, India
[2]Head, Dept. Pharmaceutical Chemistry, I. P. G. T. and R.A. Gujarat Ayurved University, Jamnagar, Gujarat, India
*Corresponding Author Email: komal.khanpara@gmail.com

Article Received on: 27/02/14 Revised on: 19/03/14 Approved for publication: 03/04/14

DOI: 10.7897/2230-8407.050468

ABSTRACT
A Simple, monetary, reproducible, precise High performance thin layer chromatography (HPTLC) method was used to determine the content of Oleanolic acid in methanolic extract of *Sapindus trifoliatus* Linn. using Toluene: Ethyl acetate: Formic acid (7:2:1) as mobile phase. Quantification was performed by λ= 529 nm. The validated method showed linear response over conc. range of 400-1200 ng. The limit of detection was noticed to be of 10 ng and was stastically tested for repeatability by interday and intraday precision test as per ICH guidelines. Method was found Precise, accurate, specific and recoverable.
Keywords: HPTLC, Oleanolic acid, Validation, *Sapindus trifoliatus* Linn.

INTRODUCTION

Standardization and analysis of the chemical marker of the Ayurvedic and other poly herbal formulation is always very big problem. Quantitative estimation of chemical markers of each ingredient in the poly herbal preparation required ideal separation technique by which these markers are separated with highest purity and with least inferences from each other. For botanicals and herbal preparations, there is a requirement for scientific proof and clinical validation with chemical standardization, biological assays, animal models and clinical trials[1]. In the present study, we report the development of a simple, optimized and validated HPTLC method for the simultaneous estimation of Oleanolic acid in *Sapindus trifoliatus* Linn. Oleanolic acid a chemical marker was selected, one from each medicinal herbs used as raw materials, for the quantification purpose because these markers are responsible for the physiological action of the plants. The method was validated on the basis of its selectivity, linearity, precision and accuracy, limit of detection (LOD) and limit of quantification (LOQ) according to ICH requirements.

MATERIALS AND METHODS

A CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC III scanner, stationary phase used was silica gel G60F254, 20 x 10 cm TLC plate. The Reference standard of Oleanolic acid was obtained from India. Methanol, Toluene, Ethyl acetate, Formic acid was used of AR Grade. The plates were developed in a CAMAG twin trough glass chamber (20 x 10 cm) by ascending method. Distance of solvent front 80 mm, band length 6 mm and detection wavelength 529 nm were used for the present study.

Sample Preparation

Standard stock solution

The std. Oleanolic acid was taken 1 mg in conical flask and extracted twice with 1 ml of methanol, and sonicated for 10 minutes in ultrasonic bath. The solution was filtered through Whatman filter paper No. 44 and the filtrate was used as further analysis.[4,5]

Hydrolyzed extract of sample

The powdered material was first extracted with alcohol. The alcohol extract was acid hydrolyzed to liberate the sapogenins from glycoside. The hydrolyzed extract was dried up to residue. The residue was dissolved in solvent of desired solubility and subjected for further analysis[2].

Preparation of different concentrations stock solutions

The standard solution was prepared by dissolving 10 mg Std. Oleanolic acid in 10 ml methanol solution which gives 1000 µg/ml. The working standard of 100 µg/ml was prepared from standard solution by diluting with methanol. Different concentrations of 2, 4, 6, 8, 10,12 µg/ml were prepared from standard solutions

Chromatographic Conditions

Analysis was performed on 20 cm × 10 cm HPTLC silica gel G60F254 plates with fluorescent indicator. The plate cleaned by predevelopment to the top with methanol, and dried in an oven 105^{0}C for 5 minutes. Sample and standard zones were applied to the layer as bands by means of a CAMAG. Linomat5 automated spray-on applicator equipped with a 100 µl syringe and operated with the settings band length 6 mm, application rate 4 µl/sec, distance between bands 4 mm, distance from the plate and distance from the bottom of the plate 2 cm.

Method Validation

Calibration Curve

2, 4, 6, 8, 10 and 12 µl standard solution of Oleanolic acid was applied onto TLC plate to generate Calibration curve. The chromatograms were developed using said chromatographic conditions. The plate was dried in air and kept in hot air oven at 105°C for 5 minutes. The standard zones were quantified by linear scanning at 529 nm by use of a TLC Scanner III CAMAG with a mercury source[3].

Precision

Repeatability

Repeatability of sample application and measurement of peak area was carried out using the three replicates of same spot 1000 ng/spot. Repeatability is also termed intra-assay precision.

Intermediate precision

The intra-day and inter-day variations for determination of Oleanolic acid were carried out at three different concentration levels 2, 5, 10 µl/spot.

Specificity

The specificity of method was ascertained by standard Oleanolic acid and samples (extracted from powder). The spots of diluent methanol, standard Oleanolic acid, extracted samples (extracted from powder) were spotted on TLC plate in duplicate and run. The spots for Oleanolic acid that eluted in sample extract were confirmed with R_f value[8,9].

Recovery Studies

Recovery Study was performed by spiking 80, 100 and 120 % of standard drug externally to the pre analysed samples. The experiment was conducted in triplicate and applied onto the plate in duplicate. This was conducted to check the recovery of drugs at different levels of concentrations[10,6,7].

RESULT AND DISCUSSION

The solvent system of Toluene: ethyl acetate: Formic acid (07: 02:01 v/v/v) was found to be ideal mobile phase for separation of Oleanolic acid. Standard Oleanolic acid showed single peak in HPTLC chromatogram. Calibration curve of Oleanolic acid was prepared by plotting concentration of Oleanolic acid versus average area of the peak. The methanol extract of formulations shows more number of peaks. The difference of % it may be due to varied factors like drug variety, geographical variation, and age of the plant at the time of harvest, genetic and environmental factors.

Calibration Curves

Calibration graph was found to be linear over the concentration range 0.4-1.2 ug/spots. The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation Y = 699.9X and regression coefficient (r^2) was 0.999 and R.S.D. was found 0.173. Response obtained for Oleanolic acid in preparation of calibration curve as shown in Figure 1 and 2 and spectral comparison of standard as shown in Figure 3.

Linearity

A representative calibration curve of Oleanolic acid was obtained by plotting the peak area of Oleanolic acid against the concentration of Oleanolic acid (0.4-1.2 ug/ml) respectively. The correlation coefficient for Oleanolic acid and sample was found to be 0.999 respectively and thus exhibits good linearity between concentration and area and shown in Figure 4 and 5 and results summarised in Table 1.

Accuracy (Recovery Studies)

To study accuracy of the developed method, recovery studies were carried out using standard addition method at three different level at 80 %, 100 %, 120 % and the % recoveries were calculated.

Recovery = Mean Conc.* 100/Nominal Conc.

Which indicates the sensitivity of method is adequate. The low values of RSD obtained after small deliberate changes of the conditions (mobile phase, composition of mobile phase, saturation time and time from chromatography to scanning). The average % recovery Oleanolic acid was found to be 99.98 % and shown in Table 2.

Precision

Interday Precision

Determination of precision, the same experiment is repeated with 1 sample on 3 days and results summarised for Oleanolic acid and Aristaka in Table 3 and 5 respectively.

Intraday Precision

Determination of precision, the same experiment is repeated with 1 sample for 3 hours in a day and results summarised for Oleanolic acid and Aristaka in Table 4 and 6 respectively.

Table 1: Linearity (Oleanolic acid) by HPTLC

S. No.	Amount in ug/ml	R_f value	Peak Area	R^2	% RSD
1	0.2	0.66	1414.2	0.9989	1.01 %
2	0.4	0.66	1509.2		
3	0.6	0.66	1689.8		
4	0.8	0.66	1705.2		
5	1	0.66	1812.1		
6	1.2	0.66	1916.2		

Table 2: Recovery study

S. No.	Sample	Initial amount (ng/spot)	Amount added (ng/spot)	Conc. Recovered (ng/spot)	Recovery (%)
1	Oleanolic acid	400	80 %	99.55	99.98 %
			100 %	100.54	
			120 %	99.87	

Table 3: Interday Precision of Oleanolic acid by HPTLC method

Concentration	400 ng/spot	600 ng /spot	800 ng /spot
Precision -1	1241.4	1813.6	2149.3
Precision -2	1236.9	1862.2	2147.0
Precision -3	1243.9	1870.1	2131.4
Mean	1240.73	1848.63	2142.56
Standard Deviation	3.56	30.59	9.72
% RSD	0.29%	1.65%	0.45%

Table 4: Intraday Precision of Oleanolic acid by HPTLC method

Concentration	400 ng /spot	600 ng /spot	800 ng /spot
Precision -1	1114.2	1709.4	2124.2
Precision -2	1132.8	1748.6	2143.6
Precision -3	1148.6	1752.8	2112.4
Mean	1131.86	1736.93	2126.7
Standard Deviation	17.22	23.94	15.75
% RSD	1.52%	1.38 %	0.74 %

Table 5: Interday Precision of Aristaka by HPTLC method

Concentration	400 ng /spot	600 ng /spot	800 ng /spot
Precision -1	488.3	1093.0	1341.6
Precision -2	484.6	1045.2	1341.4
Precision -3	484.2	1014.6	1312.8
Mean	485.7	1050.93	1331.9
Standard Deviation	2.26	39.51	18.48
% RSD	0.46 %	3.75 %	1.38 %

Table 6: Intraday Precision of Aristaka by HPTLC method

Concentration	400 ng /spot	600 ng /spot	800 ng /spot
Precision -1	360.8	948.2	1268.4
Precision -2	364.7	947.4	1264.7
Precision -3	367.6	940.7	1268.2
Mean	364.36	945.43	1267.1
Standard Deviation	3.41	4.12	2.08
% RSD	0.93 %	0.43 %	0.16 %

Stability

Stability of the sample during chromatography is investigated by 2-dimensional (2D) development. If the sample is stable during chromatography, all components can be detected on the diagonal line connecting the application position and the intersection of the 2 solvent fronts. Spots located off this line indicate the formation of artifacts. Methods that produce artifacts must be treated with caution. If visualization of the fingerprint requires a derivatization step, the stability of result must be evaluated. In Aristaka and extract and std. Develop in chromatography and diagonal line was observed.

Parameters	Oleanolic acid
Calibration range (ng/spot)	200-1200 ng/spot
Detection weave length	529 nm
Mobile phase (Toluene: E. A.: F.A.)	(7:2 :1v/v /v)
R_f value	0.68
Regression equation	Y = 6.5879 X -3.4749
Slope	6.5879
Intercept	-3.4749
Correlation coefficient	0.9989
Limit of Detection {LOD)	0.21
Limit of Quantification (LOQ)	0.45

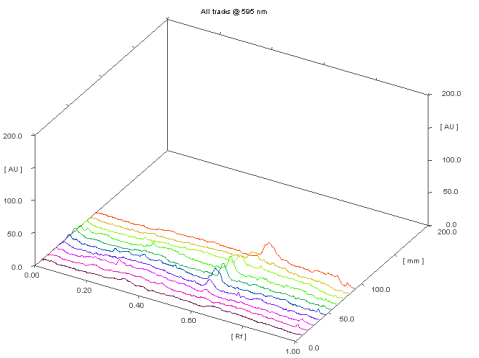

Figure 1: Calibration curve

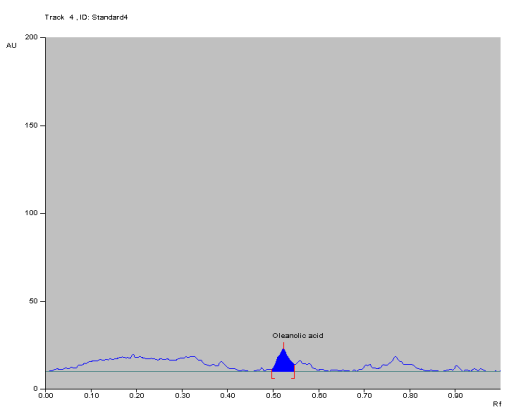

Figure 2: Single peak of Oleanolic acid

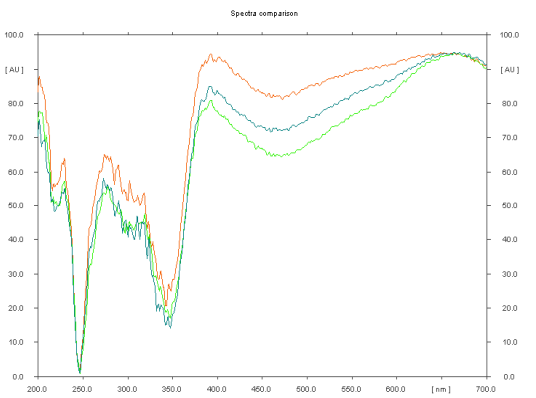

Figure 3: Spectral comparision of Stand Oleanolic acid at R_f 0.64

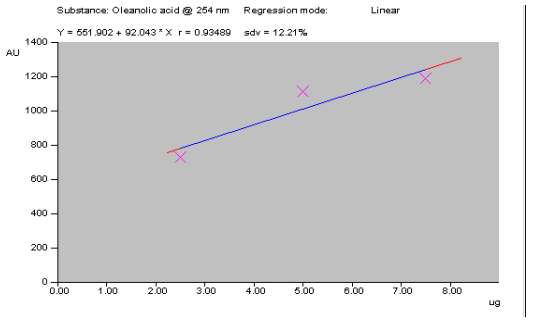

Figure 4: Oleanolic acid linearity With graph height

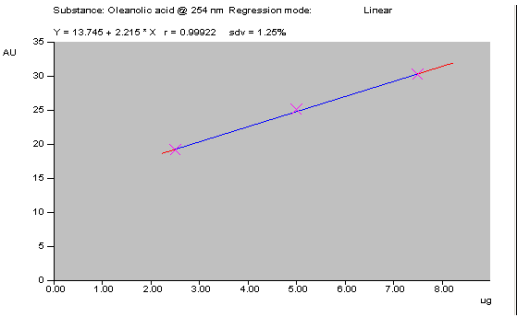

Figure 5: Oleanolic acid linearity with graph area

CONCLUSION

The developed HPTLC method is fast, simple, precise, specific and accurate. Statistical analysis proved that method is repeatable and selective for determination of Oleanolic acid.

REFERENCES

1. Validation of Standardized High-Performance Thin-Layer Chromatographic Methods for Quality Control and Stability Testing of Herbals, Kathrin koll, Eike Reich and Anne blatter, Germany, Switzerland; 2003.
2. Gurdeep R Chatwal, Sham K Anand. Industrial Method of Chemical Analysis, 5th revised and Enlarged Edition, Himalaya Publishing house, 2.272-2.503, 2.599-2.616 , 2.673-2.700
3. Validation of analytical Procedures, Methodology, ICH harmonized triapartite guidelines; 1996.
4. Isao Kitagawa, Pure Applied chemistry, 4th revised edition 2002; 74(7): 1189–1198.
5. M Saeedi, K Morteza Semnani, MR Ghoreishi, Journal of Dermatological Treatment 2003; (14): 153-157. http://dx.doi.org/10.1080/09546630310014369
6. Chauhan SK, Singh BP, Kimothi GP, Agarwal S, Indian Journal of Pharmaceutical sciences 1998; 60(4): 251-252.
7. Hayashi M, Kadowaki E, Takamatsu T, Matsuoka M, Yakugaku Zasshi 1992; 112(7): 496-502.
8. Valéria M Di Mambro, Journal of Pharmaceutical and Biomedical Analysis 2005; 37(2).
9. Cristina Fiorea, Journal of Ethno pharmacology 2005; 99(3), 14: 317-324.
10. Ali Nokhodchi. Validation of chromatographic techniques, IL Farmaco 59; 2004. p. 155-161.

Cite this article as:
Komal Khanpara, Renuka, V. J. Shukla. Development and validation of HPTLC method for estimation of Oleanolic acid in Aristaka. Int. Res. J. Pharm. 2014; 5(4):321-324 http://dx.doi.org/10.7897/2230-8407.050468

Source of support: Nil, Conflict of interest: None Declared