International Journal of Researches In Biosciences, Agriculture & Technology | Dec. 2014, Special Issue | ISSN No. (Online) 2347-517X

HPTLC Method Development for the Simultaneous Estimation of Oleic acid and Linoleic Acid in Rasnadi Guggul Formulations

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Abstract:

In the present study, an attempt was made to the study included standardization of *Rasnadi Guggul* formulations by developing a simple, precise and accurate High Performance Thin Layer Chromatography (HPTLC) method using oleic acid and linoleic acid as chemical markers. Chromatographic analysis was performed on silica gel 60 F254 precoated TLC plates using Toulene: Ethyl acetate: Methanol: Acetic acid (9:1:0.2:0.5 v/v/v/v) for oleic acid and Toulene: Ethyl acetate: Formic acid (9:1:0.3 v/v/v) for linoleic acid as mobile phase. The R_f values of oleic acid and linoleic acid were the basis of confirmation of these markers. The total peak areas of standard markers and corresponding formulations were compared and their contents were estimated in all the formulations. The results demonstrated that, analytical method i.e. HPTLC, for marketed formulations concludes that BM, JM demonstrated lowest concentration of Linoleic acid and oleic acid, while laboratory prepared formulations ET, EOT contained more concentration. In conclusion, the analytical method developed herein for standardization of *'Rasnadi guggul'* preparation including its tablets, will be helpful in obtaining a quality control profile of this formulation.

Keywords:
Oleic acid, Linoleic acid, HPTLC standardization, *Rasnadi guggul.*

Introduction:

The standardization of herbal medicines is essential, as herbal medicines are prepared from material of plant origin that is prone to contamination, deterioration and variation in composition[(1)]. *Rasnadi Guggulu* is used to treat sandhigatavata. Guggulu is proved to have both anti-inflammatory and anti-arthritic properties[(2)]. Guggulu means 'Gunjo Vyadheragati Rakshati' which implies protection against diseases. This word stands for this herb as well as for all the preparation that contain it as the chief ingredients [(3)]. Apart from other active crude drugs present in the formulation castor oil also one of the ingredient. Fixed oil of a number of a plants contains large amounts of oleic and linoleic acids. The fixed oil possesses significant anti-inflammatory, analgesic, antiarithritic, antipyretic, antiulcer properties, it is concluded that castor oil, which is more economical and convenient than oxytocin, can be used safely and effectively to stimulate labor without any noticeable toxicity [(4)].

Extensive literature survey reveals that, till now no any analytical method is developed on Rasnadi Guggul formulation using oleic and linoleic acid as markers so by considering the pharmaceutical application of the

markers, an attempt was made to standardize the 'Rasnadi guggul' marketed and In-house formulation by estimation of oleic acid and linoleic acid using High Performance Thin Layer Chromatography. Thus, the present study will help in developing a quality profile about the different formulations of these crude drugs being currently used in the market.

Material and methods:

Drugs

Crude drugs present in this formulation are *Vanda roxburghii* (Roots of Rasna), *Cedrus devdar* (Woods of Devdar), *Zingiber officinalis* (Rhizomes of Ginger), *Ricinus communis* (Roots of Erandmool), *Tinospora cardifolia* (Stems of Giloy) extracted in laboratory and Purified *Commiphora mukul* (Guggul) were procured from Natural Remedies, Bangalore, India. The three different batches of marketed formulations (tablets) of 'Rasnadi guggul' of three different manufacturers in India were procured for this study from the market of Nagpur, Maharashtra, India. i.e. Formulation 1 (Batch 100285 [BM1], 090271 [BM2], 110291 [BM3]) of company A (BM), Formulation 2 (Batch 01/09 [JM1], 0007 [JM2], 0009[JM3]) of Company B (JM). Formulation 3 (07/09 [CM1], 0004 [CM2], 0006 [CM3]) of company C(CM) Standards linoleic acid and oleic acid were procured from Sigma-Aldrich, St. Louis, MO, USA .All the solvents used in the present study were of analytical grade.

Extraction Process[5]:

About 1000 g of crude drug was taken and subjected to size reduction. Extraction was done by maceration with Hydroalcohol (1:1 ratio) and Alcohol as solvents. Each extract was then concentrated using Rotary Vacuum Evaporator and residue was collected in glass bottles.

Formula of Rasnadi Guggul Tablets [6]:

From the extractive value extract tablets, extract with oil tablets were prepared by direct compression method. The powder tablet were also prepared by powder passing through sieve no. 120. 300 mg of each tablet contains the crude powder drug. 150 mg of each tablet contains the extracts and extracts with oil.

Thin Layer Chromatography (Tlc)[7]:

The presence of markers oleic acid and linoleic acid was confirmed using TLC, pre -coated silica gel 60 F254 TLC plates (E. Merck, 0.2 mm thickness), 20 x 10cm were used as stationary phase where volume of 2, 4, 6, 8, 12, 16µl of standard solution was applied and 5µl & 10µl of sample of inhouse prepared tablet and marketed formulation was applied by using 6 mm band, 4 mm distance and start position 10.The mobile phase used for oleic acid was Toulene :Ethyl acetate :Methanol :Acetic acid (9:1:0.2:0.5 v/v/v/v) and for linoleic acid mobile phase was Toulene : Ethyl acetate : Formic acid (9 :1:0.3 v/v/v)

High Performance Thin Layer Chromatography[8]:

1) Selection of Solvent system

The solvent system which was developed for TLC was used for HPTLC and similar results were obtained.

2) Application of sample

A sample was dissolved in methanol and was applied on precoated plate with the help of Linomat IV applicator.

3) Development of Chromatogram

A rectangular twin trough glass chamber was used in the experiment. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth filter paper was placed in the glass chamber and was allowed to be soaked in the developing solvent. The moistened paper was pressed against the walls of the chamber so that it adheres to the walls. The chamber was allowed to saturate for 30 minutes before use. The experiment was carried out at room temperature in diffused day light.

Procedure:

The plate was dipped in a saturated chromatographic chamber containing the solvent system and was allowed to elute up to 8 cm and was air dried. The bands were scanned in CAMAG TLC scanner-3.

Chromatographic conditions:

Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase.

Stationary phase	:	HPTLC precoated, silica gel G 60 F_{254} (Merck, Germany)
Size	:	20 x 10 cm
Developing chamber	:	Twin trough glass chamber
Mode of application	:	Band
Band size	:	6 mm
Separation technique	:	Ascending
Temperature	:	20 ± 5^{O}C
Saturation time	:	30 min
Scanning mode	:	Absorbance/Reflectance

HPTLC of oleic Acid:

Analytical method- Standard preparation- Stock solution of 0.5mg/ml was prepared in methanol.

Sample preparation- Sample solution of 5mg/ml was prepared in methanol.

Application of solution (TLC plate):

The solvent system which was developed as per TLC of oleic acid and similar results were obtained. The plate was dried in air and scanned at 366nm. The calibration curve concentration verses area under curve for

standard oleic acid was made and percentage of oleic acid in prepared tablet and marketed formulation was calculated from the calibration curve. Rf value - 0.85

HPTLC of Linoleic Acid:

Analytical method- Standard preparation- Stock solution of 0.5mg/ml was prepared in methanol.

Sample preparation- Sample solution of 5mg/ml was prepared in methanol.

Application of solution (TLC plate):

The solvent system which was developed as per TLC of linoleic acid and similar results were obtained. The plate was dried in air and scanned at 366nm. The calibration curve concentration verses area under curve for standard linoleic acid was made and percentage of Linoleic acid in prepared tablet and marketed formulation was calculated from the calibration curve. ***Rf value-0.73***

Result and discussion:

By calculating the extractive values the amount of active constituents in a given amount of medicinal plants materials were determined. From the extractive value, Extractive value of individual crude drug (Table.1) were determined and formula for the Rasnadi Guggul Tablet was prepared (Table.2). The presence of chemical markers were confirmed through TLC (Table.3) the confirmed markers were quantified in all the formulations by HPTLC. (Table.4) The correlation coefficient of respective markers oleic acid and linoleic acid (Fig 1 and 2) (r = 0.99959, r=0.99975). The limit of detection and limit of quantization of oleic acid and linoleic acid was found to be 6.739, 3.300 mg and 20.42 mg ,10mg respectively. (Table 5.)

The HPTLC analysis depicted well resolved peaks of all the formulations showing the presence of respective markers. The spots of the entire chromatogram were visualized under UV and their percentage w/w was reported on the basis of regression equation. Our observations from the above analysis showed that, Marketed formulation BM, JM demonstrated lowest concentration of Linoleic acid and oleic acid , while laboratory prepared formulations ET, EOT contained more concentration. (Table 6)

An attempt was made to develop a selective, validated HPTLC method for the estimation of oleic acid linoleic acid. Physicochemical standards are generally used for determining the identity, purity and strength of the drug source. These characters are also used to check the genuine nature of the prepared tablets and Marketed formulation.. The use of TLC/HPTLC has expanded considerably due to the development of gradient TLC methods, improved stationary and mobile phase selection and as new methods of quantitation methods. From thin layer chromatography analysis, it was

observed that, the marketed formulations showed lesser Rf value as compared to in-house formulations which is mostly likely due to the impact of manufacturing practices. Further, all the formulations were standardized using the chemical markers confirmed in TLC analysis i.e. oleic acid and linoleic acid. Thus, through this study we have optimised the analytical method for standardization of marketed as well as in-house preparations of 'Rasnadi guggul' tablets. The percentage of oleic acid and linoleic acid from prepared and marketed formulation was determined by HPTLC method and was compared by using standard oleic acid and linoleic acid. The method will help in obtaining a quality control profile of the various formulations prepared using these crude drugs and it will act as a source of referential information for researchers having interest in the relevant field. However, further studies are underway to obtain a biological standardization profiling of optimized formulation with respect to their pharmacological potential such as anti-inflammatory, anti-arthritis activities. A simple, accurate, precise, specific HPTLC method was developed for the estimation of oleic acid and linoleic acid. The polynomial regression analysis minimized sampling error and analysis of sample was possible for a wider range of concentration.

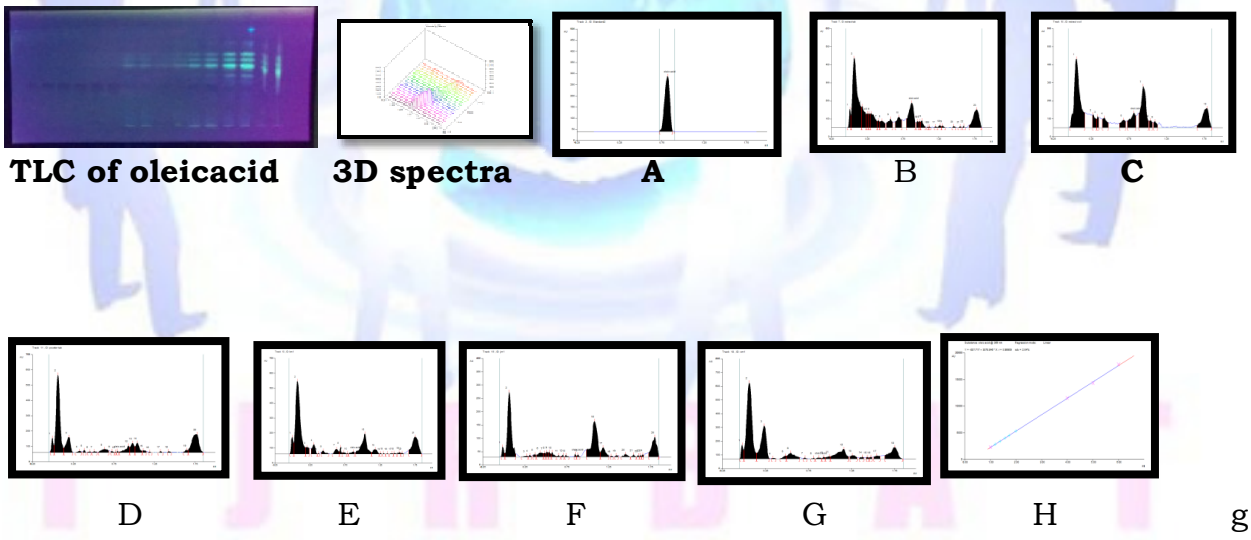

Figure 1. HPTLC chromatogram of oleic acid (b) in different formulations of *Rasnadi guggul* tablets. In figure A: standard peak of oleic acid, B: peak of oleic acid present in extract tablet, C: peak of oleic acid present in extract with oil tablet, D: peak of oleic acid present in powder tablet, E: peak of oleic acid present in BM, F: peak of oleic acid present in JM, G: peak of oleic acid present in CM, H: linearity curve of oleic acid by area.

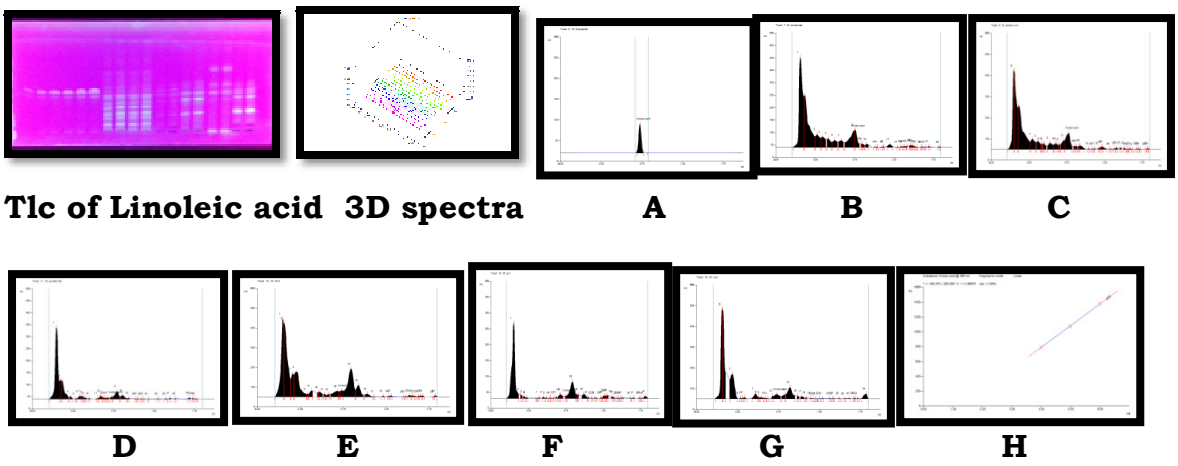

Figure 2. HPTLC chromatogram of linoleic acid in different formulations of *Rasnadi guggul* tablets. In figure A: standard peak of linoleic acid, B: peak of linoleic acid present in extract tablet, C: peak of linoleic acid present in extract with oil tablet, D: peak of linoleic acid present in powder tablet, E: peak of linoleic acid present in BM, F: peak of linoleic acid present in JM, G: peak of linoleic acid present in CM, H: linearity curve of linoleic acid by area

Table no. 1: Extractive value for Calculating Formula

Crude Drugs	Hydroalcoholic extractive value		Methanolic extractive value		
	Giloy	Ginger	Rasna	Devdar	Erandmool
Extractive value (% w/w)	37.9	10.9	4.1	16.8	0.5

Table no.2: Formula of *Rasnadi guggul* tablet

Sr. no.	Ingredient	mg/tab		
		PT	ET	EOT
1	*Tinospora cardifolia*	25	9.4	9.4
2	*Ricinus communis*	25	0.125	-
3	*Castor oil*	-	-	q.s
4	*Vanda roxburghii*	25	1.0	1.0
5	*Cedrus devdar*	25	4.2	4.2
6	*Zingiber officinalis*	25	5.6	5.6
7	Commiphora mukul	125	81.4	81.4
8	MCC	50	50	50

PT: Powder tablet, ET: Extract tablet, OT: Extract with oil tablet

Table no. 3: Development of solvent system

	Sample	**R_f value**	**Solvent system**
1	Oleic acid	0.85	Toulene :Ethyl acetate :Methanol: Acetic acid (9:1:0.2:0.5)
2	Linoleic acid	0.73	Toulene : Ethyl acetate : Formic acid (9 :1:0.3)

Table.no.4: % of oleic acid in *Rasnadi guggul* formulation

Sr.no.	Sample	Rf value	% w/w of oleic acid
1	PT	0.96	3.57
2	ET	0.85	2.73
3	EOT	0.86	3.13
4	BM	0.93	2.38
5	JM	0.95	Not Detected
	CM	0.96	Not Detected

Table no.5: Linearity performance parameter

Sr. No.	Parameters	Height	
		Oleic acid	Linoleic acid
1.	LOD	6.7393 µg	3.300 µg
2.	LOQ	20.42 µg	10.00 µg
3.	Correlation Coefficient	0.99959	0.99975

Table 6. Quantification (in % w/w) of phytoconstituents in *Rasnadi guggul* tablet

Batch	Oleic acid	Linoleic acid
PT	3.57	1.940
ET	2.73	12.52
EOT	3.13	8.430
BM	2.38	2.86
JM	-	19.50
CM	-	-

Acknowledgements :

The authors greatly acknowledge the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, (Maharashtra), for their support to carry out this work.

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