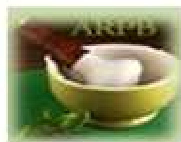
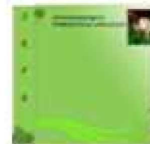


Advance Research in Pharmaceuticals and Biologicals
(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)
ISSN 2250-0774 USA CODEN: ARPBGZ



EVALUATION OF *RASAYANA CHURNA*, AN AYURVEDIC FORMULATION, BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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Received : 12/02/2014 **Revised : 25/03/2014** **Accepted : 29/03/2014**

ABSTRACT:
Rasayana churna is an Ayurvedic formulation, well-known for its adaptogenic and immunomodulatory activities. It consists of three ingredients, viz. Guduchi (stem of *Tinospara cordifolia)*, chhota Gokhru (fruit of *Tribulus terrestris*) and Amla (fruit of *Embelica officinalis*) in equal proportions. Standardization of Ayurvedic formulations by modern analytical methods is the need of the hour. This article reports the development and validation of HPTLC methods for evaluation of *Rasayana churna* by quantification of unique markers for each of its ingredients. Berberine, diosgenin and gallic acid were selected as unique markers for *Tinospora cordifolia*, *Tribulus terrestris* and *Embelica officinalis*, respectively. The analysis was done on precoated silica gel 60 F_{254} TLC plates using a mobile phase composed of Chloroform: Methanol : Ammonia (8 : 2 : 0.1) for berberine, Hexane : Acetone (7 : 3) for diosgenin and Toluene : Ethyl acetate : Formic acid (3 : 3 : 0.8) for gallic acid. The developed methods were validated as per the ICH guidelines for their linearity, precision, accuracy and sensitivity. The methods follow Beer-Lambert law in the specified concentration range. The proposed methods are accurate, reproducible and sensitive and were found to be suitable for quantification of these marker compounds in *Rasayana churna*.

Keywords: Rasayana Churna, HPTLC, Diosgenin, Berberine, Gallic acid, Ascorbic acid.

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INTRODUCTION

India is one of the nations blessed with a rich heritage of Traditional system of medicine. The recent resurgence in the interest in Ayurvedic system of medicine has resulted in large scale manufacturing of Ayurvedic formulations[1]. Most serious draw back with Indian herbal drugs is the absence of standard method for their quality control. Indian Herbal Pharmacopoeia gives quality control parameters for many herbal drugs but not for its polyhedral formulations. So, it is essential to standardize the herbs and herbal formulation as per the WHO guidelinesFor India to play a lead role in the global herbal market, it is essential to standardize the Ayurvedic formulations using sophisticated analytical techniques such as HPLC, HPTLC, GC-MS, Spectrofluorimetry, etc.

Rasayana churna is an Ayurvedic formulation, well-known for its adaptogenic and immunomodulatory activities. It is also having gastrointestinal cytoprotective activity[2]. It consists of three ingredients, viz. Guduchi (stem of *Tinospara cordifolia)*, Gokhru (fruit of *Tribulus terrestris*) and Amla (fruit of *Embelica officinalis*) in equal proportions[3]. An attempt was made to develop a quality control method for Rasayana churna using unique markers for each of its ingredients. Berberine, diosgenin and gallic acid were selected as unique markers for *Tinospora cordifolia*, *Tribulus terrestris* and *Embelica officinalis*, respectively. In the present study, HPTLC methods were developed for analysis of berberine, diosgenin and gallic acid in Rasayana churna and its ingredients.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

All the crude drugs were purchased from a local supplier M/S Lalu Vrajlal Gandhi (LVG), Ahmedabad. Identification and authentication of all crude drugs were done by comparing their morphological and microscopical characteristics with those reported in the literatur[4,5,6]. The voucher specimens of the drugs (voucher specimen no.PH/11/004, PH/11/005 and PH/11/6) were deposited in the pharmacognosy department of K. B. Institute of Pharmaceutical Education and Research, Gandhinagar.

Preparation of Rasayana Churna

All the three ingredients were powdered separately, passed through 60# sieve, and mixed thoroughly in equal proportions to prepare Rasayana churna. The preparation was stored in airtight containers.

Preparation of Standard Solution of Berberine

A stock solution of Berberine was prepared by dissolving 10 mg of Berberine in 100 ml of methanol in a volumetric flask (0.1 mg/ml).

Preparation of Standard Solution of Diosgenin

A stock solution of Diosgenin was prepared by dissolving 5 mg of Diosgenin in 10 ml of methanol in a volumetric flask (0.5 mg/ml).

Preparation of Standard Solution of Gallic Acid

A stock solution of Gallic acid was prepared by dissolving 10 mg of Gallic acid in 10 ml of methanol in a volumetric flask (1 mg/ml).

Preparation of Test Solutions

For analysis of Berberine

The formulation (3 g) was moistened with sufficient quantity of ammonia and dried at temperature below 60ºC. The dried powder was extracted with chloroform (25 ml) under reflux for 1 hr on waterbath at 60ºC. The solution was filtered and filtrate was shaken with 3 successive portions of 10 ml each, of 5% sulphuric acid. The acid layer was separated, basified with ammonia and extracted with 3 successive portions of 10 ml each, of chloroform. The chloroform layer was separated, evaporated to dryness and re-dissolved in 10 ml of methanol in 10 ml volumetric flask and volume was made up with methanol. The same procedure was followed for preparing the test solution of powdered stem of *Tinospora cordifolia*.

For analysis of Diosgenin

The formulation (9 g) was extracted with methanol (50 ml) under reflux for 1 hr on waterbath at 60˚C. The extract was filtered and evaporated to dryness. The residue was heated with 10 ml of 10% aqueous sulphuric acid at 80˚C for 1 hr. The mixture was cooled, neutralized with 10% aqueous sodium hydroxide solution and extracted with 10 ml of chloroform. The chloroform extract was separated and volume made up with chloroform in a 10 ml volumetric flask. The same procedure was followed for preparing the test solution of the powdered fruits of *Tribulus terrestris*.

For analysis of Gallic Acid

The formulation (3 g) was macerated with 10 ml of methanol for 24 hr. From the filtrate 1ml was taken diluted with 10 ml methanol in volumetric flask. The same procedure was performed for separately powdered fruits of *Embelica officinalis*.

Calibration Curve for Berberine, Diosgenin and Gallic Acid

For preparing the calibration curve of berberine, 1 to 6 µl of stock solution of berberine was applied on precoated TLC plate to get a concentration of 100 to 600 ng of berberine per spot. For calibration curve of diosgenin 1, 2, 4-10 (500 – 5000 ng/spot) and for gallic acid 1, 3, 5 to 9 µl
(1000 – 9000 ng/spot) of respective stock solutions were applied.

Chromatographic Conditions

The experiments were performed on precoated TLC plates of silica gel 60 F_{254} (0.2 mm thickness, 20×10 cm, Merck, Switzerland). Samples were applied to the plates as 8 mm bands, 8 mm apart and 10 mm from the edges of the plate, with a Camag Linomat V automatic sample applicator. The plates were developed by the ascending technique, to a distance of 80 mm, at 25 ± 5°C, relative humidity 50–60%, in a Camag twin trough glass chamber with a stainless steel lid, using a mobile phase composed of Chloroform: Methanol : Ammonia (8 : 2 : 0.1) for berberine, Hexane : Acetone (7 : 3) for diosgenin and Toluene : Ethyl acetate : Formic acid (3 : 3 : 0.8) for gallic acid. The TLC chamber was allowed to saturate with mobile phase for 20 min. before development of TLC. After development, plates were air-dried and scanned using the Camag TLC Scanner. The scanning wavelength selected for berberine and gallic acid were 345 nm and 270 nm were, respectively. For analysis of diosgenin, plate was derivatized with 1% Anisaldehyde-sulfuric acid, heated in an oven at 100°C for 5 min. and scanned at 540 nm wavelength.

Method Validation

The developed methods were validated in terms of linearity, accuracy, precision, limit of detection, and limit of quantification as per the ICH guidelines. Precision was measured by repeating the experiment six times a day for intraday precision and on six different days for interday precision by using same chromatographic conditions was expressed in terms of percent relative standard deviation (% RSD). Accuracy

of proposed method was determined by recovery experiments carried out by the standard addition method. This study was performed by addition of known amounts of berberine, diosgenin and gallic acid into a known amount of pre-analyzed formulation and the percentages of added compounds recovered in analysis were calculated.

RESULTS AND DISCUSSION

Rasayana Churna is well known Ayurvedic formulation used as an adaptogenic agent. Standardization of Ayurvedic formulations by modern analytical methods is the need of the hour. Therefore, an attempt was made to develop methods for analysis of *Rasayana churna* by HPTLC using unique markers for each of its ingredient. Berberine, diosgenin and gallic acid were selected as unique markers for *Guduchi*, *Gokhru* and *Amla*, respectively.

The sample of Gokhru and Rasayana churna were heated with 10% aqueous sulphuric acid to liberate diosgenin from its glycosides by hydrolysis. The mobile phases and linearity curve for each of the markers were developed by trial and error method, to get good resolution of markers in the test solutions and appropriate peak area for calibration curve of each marker. The scanning wavelengths for berberine and gallic acid were selected based on the wavelength maxima observed in their respective UV spectra.

The contents of berberine, diosgenin and gallic acid, determined by the proposed method,by HPTLC fingerprinting in *Rasayana churna* and its ingredients, are presented in table 1.(Fig: 1to 3). The developed methods were validated as per the ICH guidelines. The validation data for the developed methods is shown in table 2. The methods follow Beer-Lambert law in the specified concentration range as indicated by their correlation coefficient values, which are above 0.99. The methods were found to be precise as evidenced by the interday and intraday precision values (% RSD) which were less than 2%.

The proposed methods are accurate, reproducible and sensitive and were found to be suitable for quantification of this marker compounds in *Rasayana churna*.

Table. 1: Estimation of Berberine, Diosgenin and Gallic Acid in Rasayana churna and its ingredients.

Drug	Content (% w/w)*		
	Berberine	Diosgenin	Gallic acid
Tinospora cordifolia stem	0.362± 0.05	-	-
Tribulus terrestris fruit	-	0.071 ± 0.001	-
Emblica officinalis fruit	-	-	2.233 ± 0.05
Rasayana Churna	0.115 ± 0.02	0.023 ± 0.001	0.719 ± 0.02

*Mean ± SD

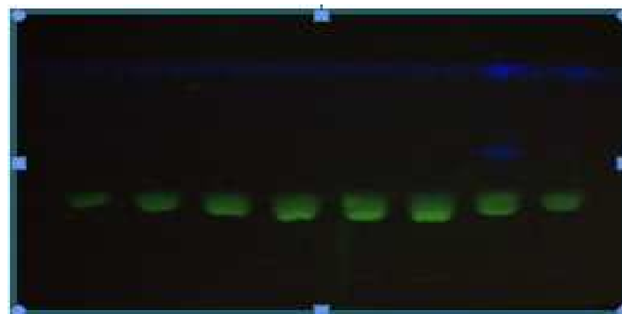

Fig.1: HPTLC fingerprinting of Berberine in Galo and Rasayana churna

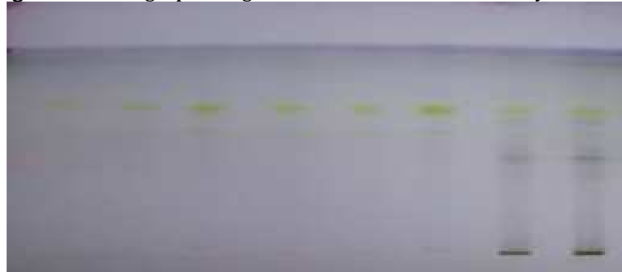

Fig. 2: HPTLC fingerprinting of Diosgenin in Gokharu and Rasayana churna

Fig. 3: HPTLC fingerprinting of Gallic acid in Amla and Rasayana churna

Table. 2: Validation Parameters of HPTLC method for analysis of Berberine, Diosgenin and Gallic acid in Rasayana churna

S. No.	Parameters	Berberine	Diosgenin	Gallic Acid
1	**Linearity**			
	Range	100-600	500-5000	1000-9000
	Linear equation	y=70.847x+ 477.07	y=1.7424x + 82.49	y=2.9649x+ 232.10
	Correlation coefficient	0.991	0.999	0.994
2	**Precision (% RSD)**			
	Repeatability of Measurement	0.807	0.88	0.695
	Repeatability of Application	0.767	1.01	0.701
	Interday precision	1.081	1.41	0.408
	Intraday precision	0.368	0.97	0.325
3	Limit of Detection (ng/spot)	22	156	258
4	Limit of Quantification (ng/spot)	67	473	783
5	Accuracy (% Recovery)	99.32%	99.09%	99.81%

Acknowledgement

I am very grateful to Dr. K. Pundarikakshudu, Director, L. J. Institute of Pharmacy, Ahmedabad, for his kind support during this research work.

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