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Quantitative Analysis of Berberine in an Ayurvedic Formulation-Rasayana Churna by UV Spectrophotometry

H. Joshi¹, N. Kanaki²

¹Assistant professor, L. J. Institute of Pharmacy, Sanand, Ahmedabad, Gujarat.

²Assistant professor, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat.

ABSTRACT:

Rasayana churna is a well known rejuvenating formulation of Ayurveda, consisting of *guduchi* (*Tinospora cordifolia*), *gokhru* (*Tribulus terrestris*) and *amla* (*Embelica officinalis*). A UV- spectrophotometric method for the quantitative determination of berberine in Rasayana churna was developed and validated. The method involved measurement of absorbance of sample solutions at the absorption maxima of berberine (348 nm). The interference from other compounds was eliminated by processing the crude drug and the formulations to isolate alkaloidal fraction from them. The method was validated by determining linearity, precision and accuracy as per the ICH guidelines. Berberine obeyed Beer's law in the concentration range of 2-20 µg/ml. The method proved to be quite accurate with a mean percentage recovery of 99.21%. Thus, the proposed method is simple, rapid, precise, accurate, and suitable for quantification of berberine content in samples of *Rasayana churna*.

KEY WORDS: Berberine, Rasayana churna, UV-Spectrophotometry

INTRODUCTION:

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Rasayana churna is a well known Ayurvedic formulation official in *Bheshaj Samhita* (Ayurvedic Pharmacopoeia). Rasayana churna (RSTD) consist of 3 ingredients viz., *guduchi* (*Tinospora cordifolia*), *gokharu* (*Tribulus terrestris*) and *amla* (*Embelica officinalis*). Traditionally it is used as antioxidant, immunomodulatory and for rejuvenating purpose¹. Most serious draw back with Indian herbal drugs is the absence of standardized parameters and methods for their quality control. So, it is essential to standardize the herbs and herbal formulation as per the WHO guidelines. It is the need of the hour to develop methods for standardization of herbal formulations using modern analytical techniques. A HPLC method was developed for the estimation of berberine in the stem of *Tinospora cordifolia* and *Tinospora sinensis*². The authors have attempted to develop a colorimetric method for estimation of berberine in Rasayana churna.

MATERIAL AND METHODS:

Preparation of Rasayana Churna

The ingredients of Rasayana churna were procured from local supplier and were authenticated by comparing their morphological and microscopical characteristics with those reported in the literature.

Rasayana churna was prepared in the laboratory by mixing the powdered ingredients drugs in equal proportions¹. This in-house formulation was denoted as reference standard (RSTD). The marketed formulations of Rasayana churna were

For Correspondence:

Ms. Hetal R. Joshi

L. J. Institute of Pharmacy

Sanand, Ahmedabad

Gujarat, India.

Email: hetaljoshi7@gmail.com

procured from two different manufacturers, The Zandu Pharmaceutical World LTD, Vapi, Gujarat (RZAN) and Shri Narnarayan Ayurvedic Pharmacy, Ahmedabad, Gujarat (RNAR).

Chemicals

All the chemicals and solvents used were of A.R. grade; standard Berberine (98%) was procured from YUCCA laboratories, Mumbai.

Preparation of sample solutions

Accurately weighed quantity (3 gm) of in-house (RSTD) and each of the marketed formulations (RZAN and RNAR) was triturated separately with ammonia solution and dried at room temperature below 60°C. The dried material was extracted with chloroform for 1 hr. The chloroform extract was separated by filtration and extracted successively with 5% H₂SO₄ (10ml x 3). The combined acidic extract was basified with sodium carbonate solution upto pH 9. The basified solution was extracted with three successive portions of chloroform. The chloroform extract was then concentrated to dryness at temperature not exceeding 50°C. The residue was dissolved in 10 ml of methanol and 1 ml of this stock solution was diluted upto 10 ml in a volumetric flask with methanol. The same procedure was performed for market formulations of Rasayana churna and powdered stem of *Tinospora cordifolia*.

Preparation of standard solution of berberine

An accurately weighed quantity (10mg) of berberine was dissolved in methanol and volume was made up to 10ml with methanol in a volumetric flask. Stock solution of berberine was prepared by diluting 1 ml of this solution with methanol up to 100ml in volumetric flask to give 10 µg/ml concentration of berberine.

Calibration curve of berberine

Appropriate aliquots of the stock solution were taken and diluted with methanol in separate 10 ml volumetric flasks to prepare standard solutions of berberine having concentrations ranging from 2 to 20µg/ml of berberine. The absorbance of each standard solution was measured at 348 nm, using methanol as blank. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted. The linear correlation between these concentrations(x- axis) and absorbance (y-axis) were graphically presented and slope (b), intercept (a), and correlation coefficient (r²) were calculated for the linear equation (y=bx+a).

Estimation of berberine in Rasayana churna

Absorbances were measured at 348 nm for sample solutions of Rasayana churna and stem of *Tinospora cordifolia*. The concentrations of berberine in the samples were calculated using the regression equation for berberine and the content of berberine was expressed as percentage by weight.

Precision and accuracy

The method was validated for precision and accuracy. The method was validated for interday and intraday precision by taking repeated measurements of the absorbance at 348 nm of the sample solution of same concentration on three consecutive days and at three different time-points on a single day, respectively. The accuracy of the method was determined by performing the recovery studies at two different concentration-levels, after adding known amounts of berberine into the Rasayana churna, of which the berberine content was estimated previously.

RESULTS AND DISCUSSION

A UV-spectrophotometric method for quantification of berberine in Rasayana churna was developed. The method involves measurement of UV absorbance at absorption maxima 348 nm for quantification of berberine in herbal formulations. Berberine was found to obey Beer Lambert's law within the concentration range of 2-20 µg/ml at 348 nm with correlation coefficient of 0.991 (Table 1, Figure1). The regression equation for the linearity curve was $y = 0.0612x - 0.0108$. The interference from other compounds was eliminated by processing the crude drug and the formulations to isolate alkaloidal fraction from them. The concentrations of berberine in *Tinospora cordifolia* stem and the formulations of *Rasayana churna* were determined and expressed as percentage by weight of the crude drug or formulation (Table 2). The method was validated for precision by estimating the repeatability and reproducibility (Table 2) and for accuracy by recovery study (Table 3). The method was found to be precise and accurate.

CONCLUSION

The UV-Spectrophotometric method developed for quantification of berberine in *Rasayana churna* is simple, rapid, precise, accurate, and suitable for quantitative monitoring of berberine content in samples of *Rasayana churna*.

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TABLES AND FIGURES

Table 1 Validation parameters of the method for quantification of berberine in *Rasayana churna*

Sr. no.	Parameters	Value
01	Absorption maxima	348 nm
02	Beer's law limit	2-20 µg/ml
03	Regression equation($y=bx+a$)	$0.0612x-0.0108$
04	Intercept(a)	- 0.018
05	Slope(b)	0.0612
06	Correlation coefficients(r^2)	0.9962
07	Precision (% C.V.)	
	Repeatability of Measurement of absorbance (n=6)	0.629
	Interday (n=3)	0.42
	Intraday (n=3)	0.30
08	Accuracy (% recovery)	99.21%

Table 2 Quantification of berberine content in *Rasayana churna*

Sample	Content of Berberine (%w/w) *	% C.V.
<i>T. cordifolia</i>	0.387 ± 0.004	1.16
RSTD	0.102 ± 0.004	1.42
RZAN	0.091 ± 0.003	1.35
RNAR	0.079 ± 0.002	1.26

* Mean \pm SD (n=3)

Table 3 Data of recovery study

Sr. no.	Amount of berberine (µg/ml)			RSD (%)	Recovery (%)
	In sample	added	Estimated*		
01	10	5	14.8 ± 0.86	0.57	98.93
02	10	10	19.8 ± 0.42	0.21	99.26
03	10	15	24.8 ± 0.59	0.04	99.46
			mean	0.27	99.21

*Mean \pm SD (n=3), RSD - Relative standard deviation

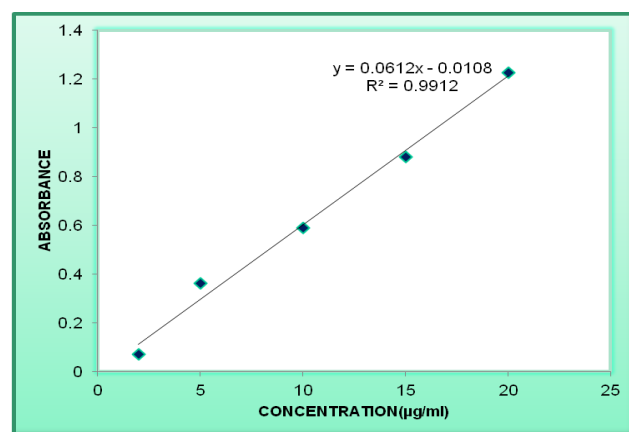


Figure-1: Calibration curve of berberine

