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Chromatographic Analysis and Validation of Berberine in Amrutharistam-A Polyherbal Formulation

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Original Research Article

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Abstract: Amrutharistam is a vital Ayurvedic formulation used for all kind of fever and rheumatic fever, which is prepared by fermenting the decoction of *Tinospora cordifolia* containing berberine as one of the active ingredient. Preliminary analysis indicated the presence of berberine being the largest concentration. The HPLC carried out in Lichrospher 100, RP-8e (250*4.6*5) column, mobile phase consisting of phosphate buffer and acetonitrile (70: 30). The determining wavelength was confirmed as 343nm. Five marketed formulations of Amrutharistam A-I, A-II, A-III, A-IV and A-V showed berberine concentration to be 9.63µg/ml, 10.10µg/ml, 10.20µg/ml, 9.64µg/ml and 10µg/ml respectively. The result showed the peak area response is linear within the concentration range of 10-50µg/ml with a correlation coefficient of 0.9998. Recovery studies 50%, 100% and 150% were conducted by standard addition method and found to be 99.804%. The developed and validated method can be effectively applied to the quantitative determination of berberine in Amrutharistam.

Keywords: Amrutharistam, Berberine, Standard addition method, Validation.

INTRODUCTION

There is a paradigm shift in consumer's inclination from western medicine to herbal in past decade; still there is a segment that is sceptical about using classical ayurvedic preparations. Plant derived products are available in pharmacies over the counter as self-medication [1] as dietary supplements and health tonic.

The herbal industry needs to follow strict guidelines and regulatory statutes of herbal medicine which contributes to its safety and efficacy [2, 3]. Standardization of herbal formulation requires implementation of GMP [4, 5].

Analytical RP-HPLC is widely used in pharmaceutical industry for isolating and purification of herbal compounds. Polyherbal formulations are standardized by fingerprint analysis which is done by HPLC/HPTLC and quantitation of individual chemical markers.

Amrutharistam used for all kind of fever and rheumatic fever, which is prepared by fermenting the decoction of containing berberine as one of the active ingredient. Berberine is an isoquinoline alkaloid isolated from stem of *Tinospora cordifolia*. Berberine are used as an antifungal and Antiprotozoal, anti-arrhythmic [6] antibiotic and antidiabetic [7].

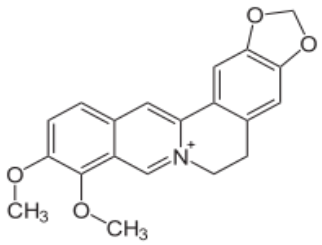

Fig-1: Structure of Berberine

MATERIALS AND METHODS

Chemicals and solvent

Berberine was obtained from Naturals Remedies, Ltd Bangalore. Five marketed formulation of Amrutharistam were purchased from local market of Mangalore. HPLC grade acetonitrile and methanol were obtained from Merck. Analytical grade potassium

dihydrogen phosphate and o-phosphoric acid were obtained from Qualigens, Mumbai.

Instrumentation chromatographic condition

Standard and sample of berberine was analyzed by HPLC technique using the following conditions (Table-1).

MOBILE PHASE

Preparation of buffer (Solvent A)

In 900ml of water, 0.136g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) has been dissolved, and then 0.5ml of ortho phosphoric acid was added. The volume was made up to 1000ml with water and filtered through 0.45 µ membrane and sonicated for 3mins.

Table-1: HPLC Parameters

PARAMETER	DESCRIPTION
Column	Hibar, prepacked column, Lichrospher 100, RP-8e
Column size	4.6mm*250mm*5µ
Detector	Photodiode array detector
Mobile phase	Buffer: Acetonitrile (70: 30 v/v)
Flow rate	1.5ml/min
Wavelength	343nm
Retention time	2.5min
Injection volume	20µl
pH of mobile phase	3 with ortho phosphoric acid
Elution	Isocratic elution
Temperature	Ambient

Acetonitrile (Solvent B)

The column was washed with 100ml of mobile phase at flow rate of 1.5ml/min.

Preparation of standard solution

Standard stock solution of berberine was prepared from 10mg of berberine dissolved in 30ml of methanol by sonicating for 5min, cool and make up to 100ml with methanol.

Preparation of sample preparation

5ml of Amrutharistam was taken in volumetric flask. 20ml of HPLC grade methanol was added, kept on water bath for 5mins and sonicated for 10mins and volume was made by methanol and filtered through PES filter paper and subject to HPLC analysis. All five marketed formulation A-I, A-II, A-III A-IV and A-V were prepared in same manner.

HPLC method devlopment

Method development involves evaluation and optimization of various stages of sample preparation, chromatographic separation, detection and quantification. Optimization of various parameters was performed in order to develop a selective and sensitive method for analysis of berberine on HPLC.

HPLC method validation

The developed RP-HPLC method was validated as per ICH guidelines.

- System suitability studies: System suitability tests are integral part for verification of the reproducibility of the chromatographic conditions. Test was performed on freshly prepared stock solution to ascertain its effectiveness. 20µl of berberine was injected into HPLC system to evaluate the developed method.
- Linearity: The method developed was linear over the whole range of concentration from 10-50µg/ml for three phases of the validation done. Using linear regression equation, all the calibration lines passed all the acceptance criteria (Fig-2, Table-2).
- Accuracy: The accuracy of the standard in the spiked sample was evaluated at three levels for berberine, with recoveries of about 50, 100, and 150% and percentage recovery was calculated (Table-3).
- Precision: Precision studies were verified by intra and interday. Successive six replicates of three different concentrations 10, 30 and 50µg/ml were analyzed. Inter precision studies was analyzed in similar manner on three successive days (Table-4, 5).
- LOD and LOQ: The LOD and LOQ values were calculated from the calibration curves as k*SD/b where k=3.3 for LOD and 10 for LOQ. SD is the standard deviation of the response of the minimum detectable drug concentration and b is the slope of calibration curve.
- Robustness: Robustness was evaluated by factorial design, where rate, wavelength and column temperature. The tested flow rate flow was 1.7ml/min and 1.3ml/min, while wavelength was 341nm, 345nm and temperature was increased and decreased by ±5 C. The experimental runs were repeated three times (Table-6).

Analysis of amrutharistham formulation

Five marketed formulation of A-I, A-II, A-III, A-IV and A-V showed berberine concentration to be 9.63µg/ml, 10.10µg/ml, 10.20µg/ml, 9.64 µg/ml and 10 µg/ml respectively (Table-7).

Table-2: Linearity report

Parameters	Drug	Acceptance criteria
Linearity Range	10-50µg/ml	-
Regression Equation	Y= 4246x-2677	-
Co relation Coefficient (R^2)	0.999	0.99
Slope	4246	-
Intercept	2677	-
LOD	0.24µg/ml	
LOQ	0.737µg/ml	

Table-3: Accuracy

Sl. No	Level	Amount of pure drug	Total Amount of drug	Peak Area	%Recovery	Mean
1	50	10	20	121681	99.528	100.6162
		10	20	123478	101.742	
		10	20	122564	100.616	
2	100	20	40	162110	99.7708	101.098
		20	40	164530	102.753	
		20	40	163187	101.098	
3	150	30	50	205470	103.255	100.3894
		30	50	205470	99.4418	
		30	50	203145	100.389	

Table-4: Inter day Precision

Sl.No	**Peak area**						Mean	%RSD
	1 day	2 day	3 day	4 day	5 day	6 day		
1	40926	40735	40932	40895	41321	40831	**40940**	**0.49**
2	121681	121627	120649	121321	121452	121943	**121398.4**	**0.39**
3	211865	212920	212342	211992	211872	212912	**212317.2**	**0.23**

Table-5: Intraday Precision

Sl. No	**Peak area**						Mean	%RSD
	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour		
1	41256	40562	40935	40678	41249	40923	**40933.83**	**0.69**
2	121958	121802	121654	121321	120975	121943	**121608.8**	**0.32**
3	211865	212920	212342	211992	211872	212912	**212317.2**	**0.23**

Table-6: Robustness of method (tailing factor)

SL. No	**Flow rate ml/ min**		**Column temp**		**Wavelength**	
	1.3ml/min	1.7ml.min	-5 C	+5 C	341nm	345nm
	0.941	0.93	0.943	0.953	0.968	0.932
	0.939	0.90	0.941	0.960	0.941	0.935
	0.942	0.89	0.939	0.949	0.931	0.97
Mean	0.9386	1.873	2.812	2.862	2.84	2.837
% RSD	0.5791	0.3390	0.0229	0.1070	0.0901	0.0808

Table-7: Analysis samples

Sample	A-I	A-II	A-III	A-IV	A-V
Berberine µg/ml	9.63	10.1	10.2	9.64	10

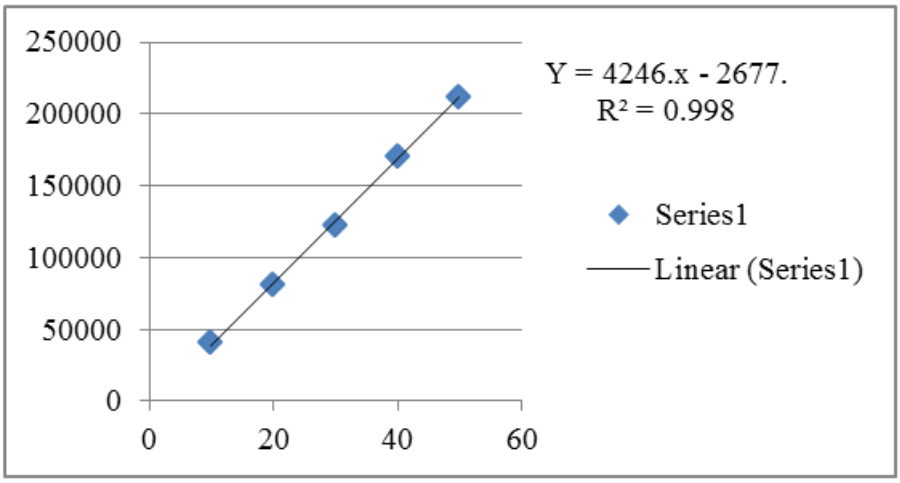

Fig -2: Linearity graph of berberine

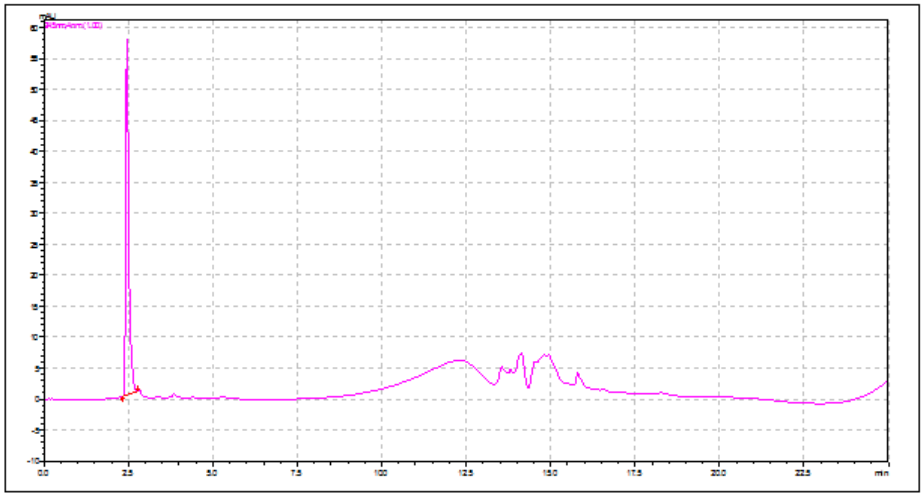

Fig-3: Chromatogram for Retention time of Standard Berberine

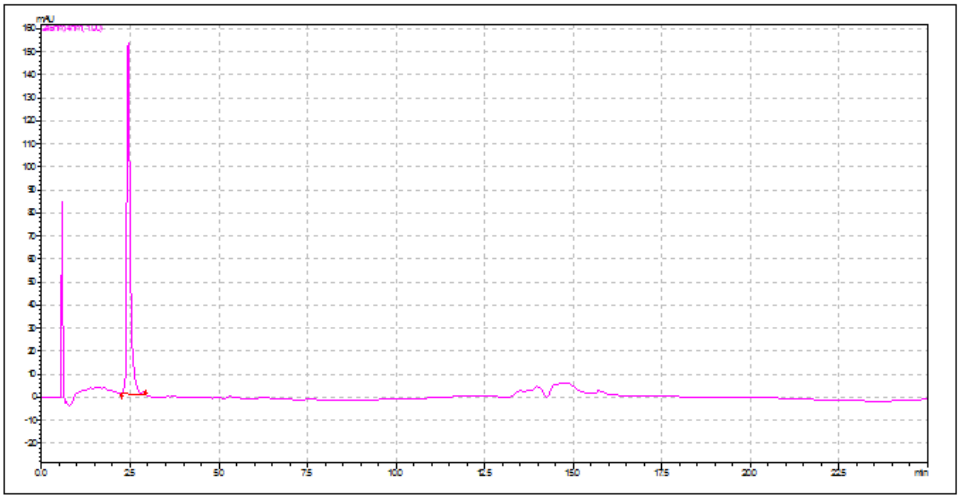

Fig 04: Chromatogram for Retention time of sample Berberine

RESULTS AND DISCUSSION

A good separation and sharp peak were obtained by using buffer and acetonitrile by gradient elution. Quantification was done at 343nm and retention time was found to be 2.5min.The system suitability was performed and reports were satisfied. The linearity was determined by concentration verses peak area, over a concentration range of 10-50µg/ml. The regression equation was Y=4246.9x + 2677.5. The linearity was satisfactory as co relation co efficient was 0.999; results

ensure good relation exists between concentration and peak area. The accuracy was obtained through recovery studies from the different between the area of spike and unspiked drug. The %recovery was 98-102%, within acceptance criteria. The precision study of method was determined by replicate injection of standard solution. For intraday precision % RSD was 0.69, 0.32, and 0.23 and for inter day 0.49, 0.39, 0.23 respectively. All the obtained values are below 2%, so method is précised. The lower limit of detection was found to be 0.23µg/ml and limit of quantification was 0.734µg/ml. The developed method was unaffected by small deliberate changes in flow rate, column temperature and wavelength. % RSD values of data are less than 2.0%, so the method is robust. The assay result obtained for analysis of berberine in five marketed formulation of Amrutharistam A-I, A-II, A-III, A-IV and A-V showed concentration to be 9.63µg/ml, 10.10µg/ml, 10.20µg/ml, 9.64µg/ml and 10 µg/ml respectively.

CONCLUSION

In HPLC method, retention time of berberine was found to be same in formulation and standard. The developed was simple, rapid, accurate and economical and can be used for routine quality control analysis of berberine.

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