RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF BERBERINE CHLORIDE AND GALANGIN IN *TINOSPORA CORDIFOLIA* M. AND *ALPINIA GALANGA* L. AND THEIR FORMULATIONS

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ABSTRACT

Berberine chloride is a quaternary ammonium compound obtained from *Tinospora cordifolia* M. and galangin is a member of the flavonol class of flavonoids from *Alpinia galanga* L. They are reported to have various pharmacological activities. The separation was carried out by using Hypersil BDS 100 RP C18 (5 µm, 250×4.6 mm i.d.) column using an isocratic mobile phase comprising acetonitrile: 10 mM phosphate buffer (45:55 V/V) pH 4.5 adjusted with orthophosphoric acid. The flow rate was maintained at 1.50 mL min^{-1}. The detection was carried out at 365 nm. The method was validated according to ICH guidelines. The retention times of berberine chloride and galangin were found to be 3.41 and 14.13 min, respectively. The developed RP-HPLC method was found to be simple, accurate, precise, sensitive and specific for the simultaneous quantification of berberine chloride and galangin in *T. cordifolia* M. and *A. galanga* L., respectively, and their herbal formulations.

Keywords: Berberine chloride, Galangin, *Tinospora cordifolia, Alpinia galanga*

INTRODUCTION

T. cordifolia M. is called a Gaduchi in Sanskrit and Amrita or Giloya in Hindi[1]. The stem of *T. cordifolia* is an important herb in folk medicine and ayurvedic system of medicine[2]. It has been used for many years as antidiabetic, anti-inflammatory, antiartharitic, antioxidant, antistress, hepatoprotactive and antineoplastic. It contains various chemical constituents of different classes, such as alkaloids, diterpenoids lactone, glycoside and steroids. Berberine chloride (BER-H) is an isoquinoline alkaloid, which is a common constituent of gaduchi and many medicinal herbs and plants[3]. Berberine chloride is a quaternary ammonium salt and is categorized as an isoquinoline alkaloid. Berberine chloride is widely used as an antibacterial, antifungal and anti-inflammatory drug and has been used as a gastrointestinal remedy for thousands of years in China[4].

The rhizome of *A. galanga* L., commonly called greater galanga, is distributed in various parts of India and throughout Southeast Asia. It is commonly known as Rasna in Sanskrit, Kullanjan in Hindi and Galanga in English[5]. It exhibits various activities likes anti-inflammatory, hypoglycaemic, antiallergic, antimicrobial and gastro protective. It contains various chemical constituents which belongs to different classes, such as flavanoids and essential oils[6-8]. Galangin (GAL), a member of the flavonol class of flavanoids, is the active pharmacological ingredient from *A. galanga and* is reported to have a variety of anti-inflammatory properties *in vitro* via negative regulation of NF-κB[9].

The quantification of various constituents is a very important part of standardization. It is needed to produce more and more standardized products for good health and overall well being of people globally as well as to promote herbal heritage for their benefit. Most of these herbal drugs are not included in the Pharmacopoeias. Hence, there is ample scope for the development of proper analytical methods. To date, some analytical methods have been reported for analysis of BER-H and GAL in various plants and formulations by HPTLC[10-12], HPLC[13-17], UV visible spectroscopy[18-19], spectrofluorimetry[20-21] and LC-MS[22-25]. However, no method is available for the simultaneous quantification of BER-H and GAL in *T. cordifolia* and *A. galanga,* respectively, and their polyherbal formulations. Therefore, need for analytical method for the quantification of these active constituents

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Table I: Results of linearity data

Conc. (µg mL^{-1})	Conc. (µg mL^{-1})	Peak area (mean ± S.D, n=3)	Peak area (mean±S.D, n=3)	%RSD	
BER-H	**GAL**	**BER-H**	**GAL**	**BER-H**	**GAL**
5	10	126151 ± 1623.11	129527 ± 1103.94	1.289	0.852
10	20	258657 ± 2423.89	210852 ± 2224.13	0.937	1.055
15	30	433733 ± 3620.43	353635 ± 3436.86	0.835	0.972
20	40	511803 ± 3792.02	487757 ± 5000.02	0.741	1.025
25	50	627554 ± 5395.96	678437 ± 7139.7	0.860	1.052
30	60	735819 ± 3990.81	875571 ± 5497.51	0.542	0.628
			Mean %RSD	**0.867**	**0.931**

in the plants samples and its formulations exists. This study was intended to develop a simple, accurate, precise, sensitive and specific RP-HPLC method for the simultaneous quantification of BER-H and GAL in plant samples and their formulations and to validate the method in accordance with the International Council of Harmonization (ICH) guidelines[26-27].

MATERIALS AND METHODS

Materials

HPLC grade methanol and acetonitrile were used for HPLC analysis. Double distilled water was purified by Milli-Q-system. All other solvents used in this study were of analytical grade. Reference standards of BER-H and GAL (HPLC purity >98.0 %) were purchased from Sigma Aldrich, Bangalore, Karnataka, India. Plant samples of *T. cordifolia* and *A. galanga* were purchased from the local market in Gandhinagar, Gujarat, India. Two marketed formulations were purchased from the local market in Ahmedabad, Gujarat, India.

Instruments

HPLC system, 2230 series (Analytical Technologies Ltd., India) equipped with P 2230 HPLC pump, LU 2230 Plus 4 low pressure gradient unit, Autosampler and UV 2230 UV visible detector was used.

Methods

Preparation of standard stock solution of berberine chloride (100 µg mL^{-1}) and galangin (100 µg mL^{-1})

An accurately weighed amount of 1 mg of BER-H and GAL each was transferred and dissolved using methanol into 10 mL of volumetric flask to get concentration of 100 µg mL^{-1} of each.

Calibration curves of berberine chloride (5-30 µg mL^{-1}) and galangin (10-60 µg mL^{-1})

Suitable aliquots of standard solution of BER-H (50, 100, 150, 200, 250, 300 µL) and standard solution of GAL (100, 200, 300, 400, 500, 600 µL) were transferred to a series of vial and the volume in each vial was adjusted to 1 mL with methanol. The resulting solutions were injected into the chromatography system and the peak areas were measured. Calibration graphs were plotted of the peak area versus concentration of standard solutions injected.

Sample preparation

A required amount of dried powder of *T. cordifolia*, *A. galanga* and their formulations was extracted thrice with methanol (20 mL*3) in steam water bath for 30 min. The extracts of all samples were filtered and combined. The combined extracts were evaporated to dryness on steam water bath to get residue. The residues of all the test samples were dissolved in 10 mL of methanol. The afforded solutions were filtered through 0.45-µm Whatman filter prior to HPLC. All sample solutions were diluted further using methanol. Each of sample solutions was injected and chromatogram and peak area were recorded. Each analysis was performed in triplicate, % assay was reported.

Chromatographic conditions

RP-HPLC was performed on Hypersil BDS 100 RP C18 (5 µm, 250×4.6 mm i.d.) column using an isocratic mobile phase comprising of acetonitrile: 10

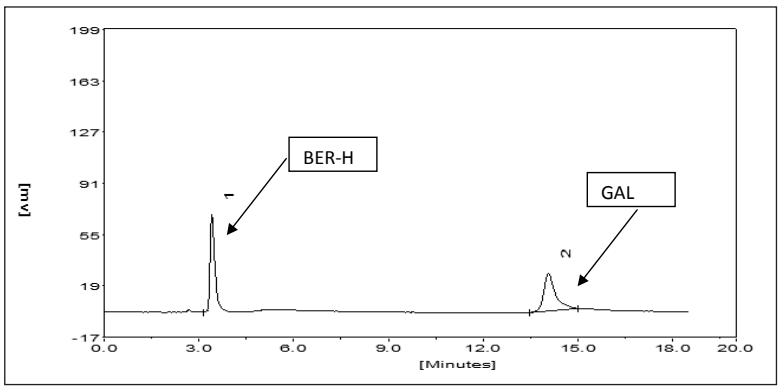

Fig. 1: Chromatogram of BER-H and GAL standard solution (1- BER-H; 2- GAL)

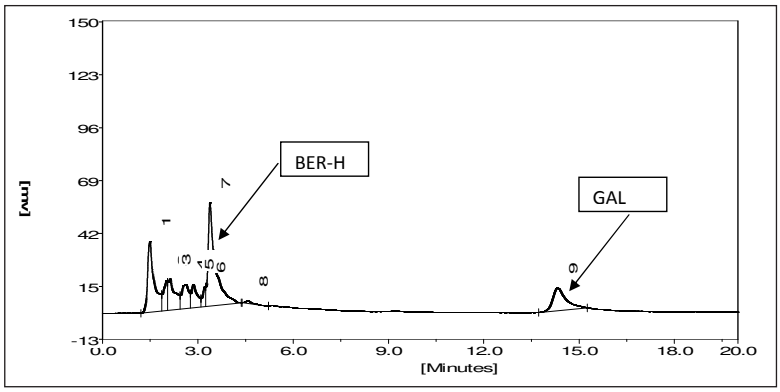

Fig. 2: Chromatogram of test solution (Plant extract)

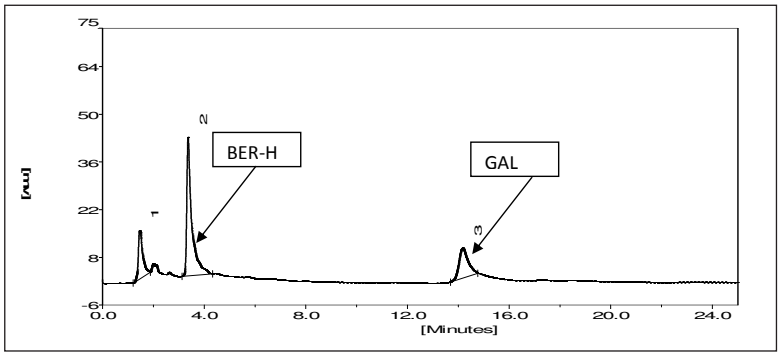

Fig. 3: Chromatogram of test solution (Formulation 1)

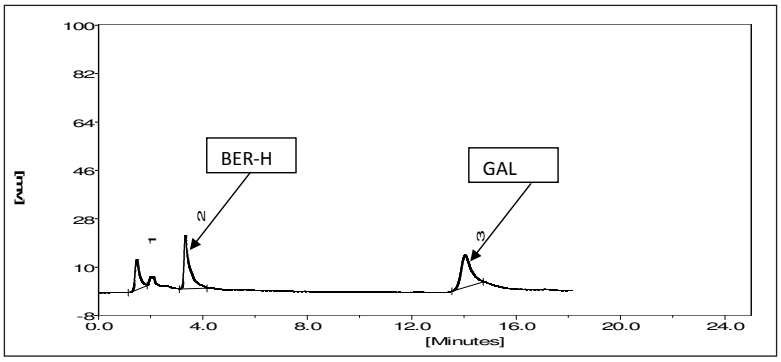

Fig. 4: Chromatogram of test solution (Formulation 2)

mM phosphate buffer (45:55 V/V) pH 4.5, adjusted with orthophosphoric acid. The flow rate was adjusted at 1.50 mL min^{-1} and temperature of the column was kept at room temperature. The samples were injected in a volume of 20 µL and detection was carried out at 365 nm.

METHOD VALIDATION

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity

The linear response was determined by analyzing six independent levels of the calibration curve in the range of 5-30 µg mL^{-1} for BER-H and 10-60 µg mL^{-1} for GAL. Results are expressed in terms of correlation co-efficient.

Precision

Intra-day precision and Inter-day precision were determined for standard solution of BER-H (10, 15 and 20 µg mL^{-1}) and GAL (20, 30 and 40 µg mL^{-1}) for three times on the same day for intraday and on three different days for inter-day precision.

LOD and LOQ

Calibration curve was repeated 5 times and the standard deviation (SD) of the intercepts (response) and slope were calculated. Then LOD and LOQ were measured by using mathematical expressions: LOD = 3.3 δ/S, LOQ = 10 δ/S, where S is the slope of the calibration curve and δ is the standard deviation of y-intercept of regression line.

Accuracy (% Recovery)

Accuracy was expressed as % recovery by the assay of known, added amount of analyte. It is a measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate on previously analyzed test samples with three different concentrations of standards at 80 %, 100 % and 120 %, respectively.

Table II: Results of intraday precision

Conc. (µg mL^{-1})		Peak area (mean ± S.D., n=3)		% RSD	
BER-H	GAL	BER-H	GAL	BER-H	GAL
10	20	258657 ± 2423.89	210852 ± 2224.13	0.937	1.055
15	30	433733 ± 3620.43	353635 ± 3436.86	0.835	0.972
20	40	511803 ± 3792.02	487757 ± 5000.02	0.741	1.025
		Avg %RSD		0.838	1.017

Table III: Results of interday precision

Conc. (µg mL^{-1})		Peak area (mean ± S.D., n=3)		% RSD	
BER-H	GAL	BER-H	GAL	BER-H	GAL
10	20	257722.67 ±3968.42	210339 ± 2316.62	1.540	1.101
15	30	433742.67 ± 4532.36	353743.33 ± 4559.54	1.045	1.289
20	40	511916.67 ± 5850.9	487236 ± 6413.99	1.143	1.316
		Avg %RSD		1.243	1.236

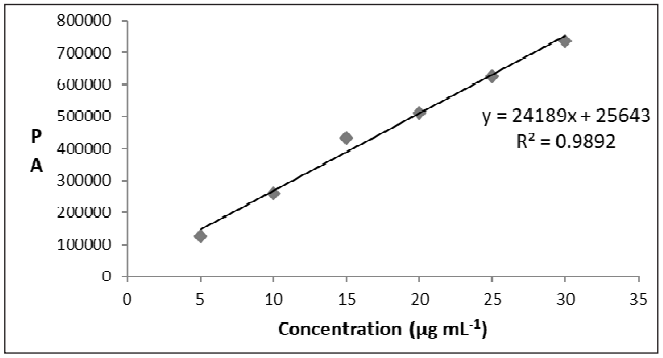

Fig. 5: Calibration curve of berberine chloride (5-30 µg mL^{-1})

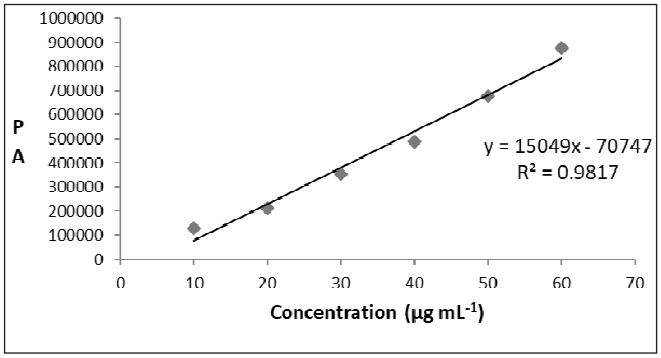

Fig. 6: Calibration curve of galangin (10-60 µg mL^{-1})

Table IV: Results of recovery study of BER-H

% Amt estimated	Amt of BER-H present in pre-analysed sample (µg mL^{-1})	Amt of BER-H standard added (µg mL^{-1})	Amt recovered (µg mL^{-1})	% Recovery ± SD
80	10	8	18.02	100.11 ± 0.160
100	10	10	19.91	99.53 ± 0.077
120	10	12	22.18	101.27 ± 0.209

Table V: Results of recovery study of GAL

% Amt estimated	Amt of GAL present in pre analysed sample (µg mL^{-1})	Amt of GAL standard added (µg mL^{-1})	Amt recovered (µg mL^{-1})	% Recovery ± SD
80	10	8	18.31	101.72 ± 0.160
100	10	10	20.40	101.99 ± 0.077
120	10	12	21.92	99.71 ± 0.209

Robustness study

Robustness of the method was determined by performing small changes in mobile phase ratio, wavelength detection, flow rate and pH.

System suitability

The parameters used in these were asymmetry of chromatographic peak, theoretical plates, retention time and resolution.

RESULTS AND DISCUSSION

HPLC separation optimization

To obtain high resolution, reproducible and symmetric peaks, mobile phase of different compositions were tried for optimization. Different ratios of methanol-water, acetonitrile-water, methanol-acetonitrile were tried, but no satisfied separation was achieved. Hence phosphate buffer pH 4.5 was used with acetonitrile to

Table VI: Results of robustness study

Parameter	%RSD
Mobile phase composition	0.675
Wave length	1.3
Flow rate	0.715

Table VII: Results of system suitability study

Parameter	BER-H	GAL
Asymmetry	1.83 ± 0.026	1.57 ± 0.03
Theoretical plates	15331.67 ± 250.081	27501.67 ± 454.142
Retention time (min)	3.41 ± 0.021	14.13 ± 0.070
Resolution	-	24.04 ± 0.220

obtained good separation. After optimizing separation parameters, acetonitrile: 10 mM phosphate buffer (45:55 V/V) pH 4.5, at flow rate of 1.5 mL min^{-1} was utilised to ensure that each run of analysis was completed within 15 min. Detection wavelength was selected as 365 nm, as it was the isosbestic point with maximum absorbance of both the constituents. The chromatograms of standard solution of BER-H and GAL, plant extract and its formulations are shown in Figs. 1-4. The retention times of BER-H and GAL were found to be 3.41 and 14.13, min respectively.

Validation results of RP-HPLC method

The linearity data of standard BER-H and GAL are given in Table I. The calibration curves for BER-H and GAL were obtained by plotting the peak area of BER-H and GAL versus concentration, and they were found to be linear over the range of 5-30 µg mL^{-1} and 10-60 µg mL^{-1}, with r^2 = 0.989 and r^2 = 0.981, respectively (Figs. 5 & 6). The LOD and LOQ for BER-H were found to be 0.480 µg mL^{-1} and 1.456 µg mL^{-1}, respectively. The LOD and LOQ for GAL were found to be 0.826 µg mL^{-1} and 2.503 µg mL^{-1}, respectively. The % RSD for intraday precision for BER-H and GAL are depicted in Table II. The % RSD for inter day precision for BER-H and GAL are depicted in Table III. The % RSD values were found to be low, and hence, the method is precise. The results of accuracy for BER-H and GAL were found to be 99.53-101.27 % and 99.71-101.99 %, respectively (Table IV & V). The % recovery data show that the proposed method is accurate. The % RSD of different parameters for robustness study was found to be less than 2 % (Table VI). The results of system suitability study are shown in Table VII. The results indicated insignificant difference and thus indicative of a robust method.

Assay results of plant samples and their formulations

The proposed RP-HPLC method was applied for the quantification of BER-H and GAL in *T. cordifolia* and *A. galanga,* respectively, and their herbal formulations. The resolution was good; the contents of BER-H and GAL in plant sample, formulation 1 and 2 are shown in Table VIII.

Table VIII: Assay of BER-H and GAL in plant sample and its formulations

Test sample	% Assay ± SD (n=3) (% w/w)	
	BER-H	GAL
Plant extract	0.246 ± 0.0028	0.276 ± 0.0024
Formulation 1	0.019 ± 0.0008	0.023 ± 0.0007
Formulation 2	0.008 ± 0.0001	0.036 ± 0.0005

CONCLUSION

The developed RP-HPLC method was found to be accurate, precise, sensitive and specific for the simultaneous quantification of berberine chloride and galangin in *T. cordifolia M.* and *A. galanga L.,* respectively, and their herbal formulations.

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