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Research Article

HPTLC METHOD FOR ESTIMATION OF BERBERINE IN AYURVEDIC FORMULATIONS CONTAINING BERBERIS ARISTATA BY AN ACID DYE METHOD

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ABSTRACT

A simple, specific, sensitive and validated high-performance thin layer chromatographic method was developed for the analysis of Berberine from Ayurvedic formulations (Pilex and Diabecon tablets from Himalaya herbals, Punarnawadi gugglu from Baidyanath) containing *Berberis aristata*. An ion pair method using acidic dye like methyl orange was used for the selective extraction of berberine from the ayurvedic formulations. A TLC aluminium sheet precoated with silica gel 60 F_{254} was used as the stationary phase. The mobile phase system consisting of n-butanol: glacial acetic acid: water (12: 3: 4 v/v) gave a good resolution of Berberine at R_f value of 0.55. Spectro-densitometric scanning was performed at an absorbance wavelength of 350 nm. The calibration plot of Berberine exhibited good polynomial regression relationship (r = 0.9988) over a concentration range of 50-250 ng/spot. The Relative Standard Deviation for intra-day and inter-day precision analysis of Berberine was found to be 0.86-3.25% and 2.73-3.99%, respectively. Statistical analysis proved that the proposed method is accurate and reproducible. The proposed method was applied for the estimation of berberine from marketed formulations containing *Berberis aristata* and the contents were determined without interference of excipients.

Keywords: HPTLC, Berberine, Berberis aristata, Acid dye method

INTRODUCTION

Berberine is an isoquinoline alkaloid mostly found in roots of *Berberis aristata* (Family Berberidaceae). Chemically it is 5, 6-dihydro-9, 10-dimethoxybenzo [g] -1, 3 benzodioxolo [5,6a] quinolizium The most important property of berberine is its antibiotic properties [1,2]. It is also used in the treatment of type 2 diabetes [3]. The Ayurvedic formulations like Pilex tablets (S1), Diabecon tablets (S2) and Punarnawadi gugglu tablets (S3) contain *Berberis aristata* as one of the ingredient and these are used for the treatment of piles, diabetes and inflammation, respectively. Various methods are reported in the literature for the estimation of berberine in its marketed formulations like U.V. spectroscopy [4], HPLC [5], HPTLC [6], non aqueous capillary electrophoresis [7] etc. In all the methods mentioned there are possibilities of interference of other ingredients present in the formulation. So the present work aims to develop a selective method for estimation of berberine forming an ion associate. Spectrophotometric method is reported for the estimation of berberine by formation of ion pairs [8]. So far no HPTLC method is reported for the estimation of berberine by formation of ion associate. So an attempt is made to develop a selective, validated HPTLC method for the estimation of berberine by formation of coloured complex of berberine with methyl orange under acidic conditions. The proposed method was further used for the analysis of berberine in its market formulation.

MATERIALS AND METHOD

Berberine was procured from Sigma-Aldrich, Mumbai, India. All reagents and chemicals were of analytical grade. A Camag- HPTLC system comprising of Camag Linomat IV automatic spotter with 100 µL Hamilton syringe, Camag TLC Scanner III with CATS4 software, Camag UV-cabinet and Camag twin trough development chamber with stainless steel lid was employed. The source of radiation used was Tungsten Lamp.

Preparation of standard solution

A stock solution of berberine containing 1mg/ml was prepared in methanol. An acid dye treated standard solution of berberine was prepared under optimized condition. Stock solution of berberine (0.1 ml) was treated with 1.9 ml of 0.1N Hydrochloric acid and 4 ml of methyl orange solution in methanol (2mg/ml) and chloroform (3x3, 9 ml) in a test-tube. The test-tube was placed on a cyclo-mixer for 1 minute. The solution was set aside for 15 minutes. The chloroform layer was separated and transferred to 10 ml volumetric flask. The volume was made up to the mark with chloroform. This solution was coloured and was treated as standard solution of berberine (10µg/ml).

Conditions

Methanol pre-washed TLC aluminium sheets of pre-coated silica gel 60 GF254 with thickness of 200 µm (E- Merck, Germany) were used as stationary phase. A mixture n-butanol: glacial acetic acid: water (12:3:4 v/v/v) was used as mobile phase. Chamber saturation time was 30 minutes. Sample application was maintained at a rate of 10sec/µL, scanning speed was 10 mm/s with 6 mm band. Ascending separation technique was used. Temperature was maintained at 33 ± 5 ºC, with relative humidity 40-60% and migration distance of 55 mm. The scanning mode chosen was absorbance/reflectance. Slit dimension was 3 x 0.45 mm with a detection wavelength of 350 mm. The detection wavelength was selected by insitu overlain spectra of berberine after acid dye treatment.

Preparation of sample solution

For preparation of sample solution 3 tablets of Pilex (S1) and 8 tablets of Diabecon (S2) from Himalaya herbals, India and 5 tablets of Punarnawadi gugglu (S3) of Baidyanath Pharmaceuticals, India were triturated separately using motor and pestle. The powdered samples of S1 to S3 were extracted with 80% ethanol separately under reflux on a constant temperature water bath for 1 .5 hour with occasional shaking. The extract of each sample was allowed to evaporate to obtain a syrupy mass under vacuum. The syrupy mass was dissolved in hot water. The solution of each sample was filtered through Whatmann No 1filter paper and the filtrate was transferred to separate 10 ml volumetric flasks. The solution of each sample was diluted up to the mark with 80% ethanol. The ethanolic solution (0.1 ml) of each sample was treated in the same way as mentioned in the preparation of standard berberine solution. The final chloroform solution of each sample was used for the assay analysis.

Method of analysis

Aliquots of 5, 10, 15, 20 and 25 µl of berberine standard solution (10 µg/ml) along with 70µL (S1 & S2), 90 µL (S3) in triplicate were applied on pre-washed and activated TLC plate with the help of semi automatic spotter. The plate was dried and developed in previously saturated twin trough chamber at constant temperature 20 ± 5˚C using n-butanol :acetic acid: water (12:3:4) as mobile phase. After development plate was dried and photometrically analysed at 350

nm wavelength. Calibration curve was prepared by plotting respective peak areas of berberine against concentration.

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines. The formation of a coloured complex of berberine with methyl orange under acidic condition minimized the interference of the other amines and alkaloids present in the samples. The mobile phase system containing n-butanol: glacial acetic acid: water (12: 3: 4 v/v) gave a good resolution sharp peak of Berberine with R_f value of 0.55 (Fig 1). The method was found to be specific. No interference of other ingredients was found in the analysis of the different formulation of berberine containing Berberis aristata (Fig 1).

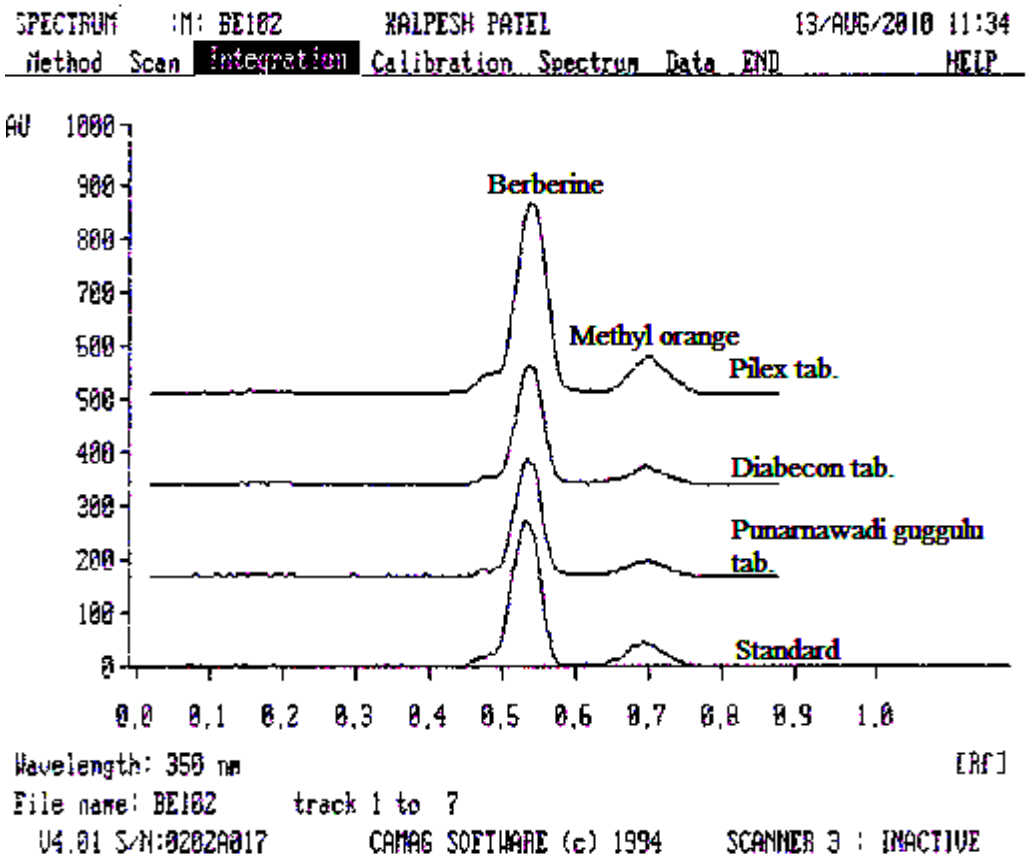

Fig. 1: It shows Densitogram of standard berberine, pilex tablet (S1), Diabecon tablet (S2) and Punarnawadi gugglu (S3) at 350 nm after treatment with methyl orange. Peak 1 in all the tracks indicate that of berberine and peak 2 in all the tracks is of methyl orange.

The calibration plot of Berberine exhibited polynomial regression relationship (r = 0.9988) over a concentration range of 50-250 ng/spot. The polynomial regression analysis minimized sampling error and analysis of sample was possible for a wider range of concentration. The accuracy of the proposed method was ascertained by carrying out the recovery study at three different levels (50, 100, 150 %). The accuracy was found to be in the range of 99.3-101.24%. The inter day and intra day precision was carried out and the results were reported in terms of RSD (Table 1). The limit of detection and limit of quantitation was found to be 5ng/spot and 10 ng/spot respectively. The summary of validation parameter is shown in Table 1. The proposed method was used for the analysis of berberine in different ayurvedic formulations. The amount of berberine was 0.046, 0.021, and 0.083 %w/w in Pilex tablets, Diabecon tablets and Punarnawadi guggulu tablets respectively. (Table 2)

Table 1: Validation Parameters

Parameter	Value
Linearity range	50-250 ng/spot.
Coefficient of correlation	0.9988
Specificity	Specific
Intra day Precision (%C.V)	0.86-3.25%
Inter day Precision(%C.V)	2.73-3.99%

Table 2: Content of Berberine in Market Formulation

Formulation	% of Berberine* ± S. D
Himalaya Pilex tablets (S1)	0.048 ± 0.002
Himalaya Diabecon tablets (S2)	0.023 ± 0.002
Baidyanath Punarnawadi guggulu tablets (S3)	0.089 ± 0.006

* Each reading is mean of three observations. S.D means standard deviation

CONCLUSION

The developed HPTLC method is simple, accurate, precise and specific for the estimation of berberine. The proposed method is successfully used for the estimation of berberine in different ayurvedic formulations. It was found that the acid dye method used minimizes the interference of other closely related constituents in the formulations. The content of berberine in the baidyanath punarnawadi gugglu tablets was found to be higher than Himalaya pilex tablets and diabecon tablets. The method can be used as a valuable analytical tool for the routine standardization of these formulations.

REFERENCES

1. Sun D, Abraham SN, and Beachey EH. Influence of berberine sulfate on synthesis and expression of Pap fimbrial adhesin in

uropathogenic Escherichia coli. Antimicrob Agents Chemother. 1988; 32(8): 1274–1277.

2. Amin AH, Subbaiah TV and Abbasi KM. Berberine Sulphate: antimicrobial action, bioassay and mode of action. Can. J. Microbiol. 1969; 15: 1067-1076.
3. Tang LQ, Wei W, Chen LM and Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J. Ethnopharmacol. 2006; 108: 109-115
4. Chen L, Yantao L, Shen X and Zhang L . Determination of Berberine Hydrochloride in Berberine Prickly- heat Powder by UV Spectrophotometry. China Pharmaceuticals 2007; 16(21):1903.
5. Ya SU, Min LU. Determination of hydrochloric berberine in Quban powder by HPLC. Strait Pharmaceutical J. 2009; 3: 20
6. Rout KK, Pradhan S, Mishra SK. Estimation of berberine in ayurvedic formulations containing Berberis aristata. J. AOAC Int. 2008; 91: 1149-1153.
7. Dong YM, Chen XF, Chen, XG. Rapid determination of berberine in Tianshen capsule by nonaqueous capillary electrophoresis. J. Lanzhou University Natural Sciences. 2006; 42: 73-77.
8. Sakai T, Ohno N, Sasaki H and Hyuga T. Extraction-Spectrophotometric Determination of Berberine in Crude Drugs by the Formation of a New Ion Associate. Analytical Sciences. 1991; 7(1): 39.
9. Upwar N K, Patel R, Waseem, Mahobi N K. Hypoglycemic effect of methanolic extract of *Berberis aristata* dc stem on normal and stretozotocin induced diabetic rats. J. of Pharmacy and Pharm. Sciences 2011; 3(1): 222-224.
10. Adikay S, Koganti B, Prasad, KVSRG. Effect of decoction of root bark of *berberis aristata* against cisplatin-induced nephrotoxicity in rats. J. of Pharmacy and Pharm. Sciences 2010; 2(3):51-56.