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

MIMANSHA C PATEL,*, I. S. RATHOD, SINDHU B. EZHAVA

Department of Quality Assurance, Medisol Lifescience Pvt. Ltd., Aklara, Vapi, India, Department of Quality Assurance, L M College of Pharmacy, Navrangpura, Ahmedabad-380009, India. Email: patelmimansha3@gmail.com

Received: 04 Oct 2012, Revised and Accepted: 19 Nov 2012

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] -I, 3 benzodioxolo [5,6a] quinolinol. 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 aluminum sheets of pre-coated silica gel 60 GF254 with thickness of 200 µm (E- Merck, Germany) were 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 Rf 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

REFERENCES


Acknowledgement

The authors are grateful to the authorities of L M College of Pharmacy for providing the necessary facilities.
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 Rf 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).

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.

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 guggulu 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 Beachy EH. Influence of berberine sulfate on synthesis and expression of Pap fimbral adhesin in


5. Ya SU, Min LU. Determination of hydrochloric berberine in Quban powder by HPLC. Strait Pharmaceutical. 2009; 3: 20


