IJPSR (2023), Volume 14, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 January 2023; received in revised form, 20 March 2023; accepted 25 April 2023; published 01 September 2023

IDENTIFICATION AND QUANTIFICATION OF BERBERINE IN A POLYHERBAL FORMULATION BY HPTLC METHOD

C. R. Kamath [1] and S. A. Salvi [*2]

Department of Chemistry [1], K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai - 400077, Maharashtra, India.
Department of Chemistry [2], Sathaye College (Autonomous), Dixit Road, Vile-Parle East, Mumbai - 400057, Maharashtra, India.

Keywords:

Berberine, Diabetes, HPTLC, Chemical marker, Standardization

Correspondence to Author:
S. A. Salvi

Assistant Professor,
Department of Chemistry,
Sathaye College (Autonomous),
Dixit Road, Vile-Parle East, Mumbai - 400057, Maharashtra, India.

E-mail: swarupa.salvi@sathayecollege.edu.in

ABSTRACT: Diabetes mellitus is a challenging human disorder for medical researchers, and the patients suffering from it are from all age groups and countries. BSL-150 tablets is a polyherbal formulation used for the treatment of diabetes mellitus. It contains seeds of *Syzygium cumini*, bark of *Tinospora cordifolia*, leaves of *Gymnema sylvestre* and seeds of *Phyllanthus emblica*. These plants' dried, powdered parts in the same quantities as marketed tablets were mixed to obtain the in-house formulation. The phytochemical analysis of the methanolic extracts of in-house and marketed samples showed presence of flavonoids, steroids, alkaloids, terpenoids, phenols, saponins and tannins. Berberine, an isoquinoline alkaloid, was identified in samples using HPTLC andn-Butanol: Ethyl acetate: Glacial acetic acid: Water (3:5:1:1) as mobile phaseon silica gel 60 F254 TLC plates. This led to the development of a validated standardization method using Berberine as a chemical marker, following the guidelines of USP and ICH. The R_f of Berberine was found to be 0.322 ± 0.010. In the calibration curve the linearity was found to be in the range of 10 to 90 ng/spot. The detection limit was 0.067 ng/spot, and the Limit of quantification was 8.62 ng/spot. The CV during intra-day and inter-day analysis were 3.3% and 1.96% respectively; this shows the precision. The recovery was calculated and was 85.94%; this shows accuracy. The method also shows specificity for Berberine. Thus a validated method for standardization of BSL-150 using Berberineas chemical marker was developed.

INTRODUCTION: Diabetes mellitus is a disorder caused by increased blood sugar levels, and hyperglycemia. This increased blood sugar level can be due to lack of insulin secretion or lack of insulin action or both [1]. If not treated properly, it causes many ill effects on kidneys, eyes, heart *etc* [2]. According to International Diabetes Federation, approximately 537 million adults (20-79 years) are living with diabetes in 2021.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(9).4544-47

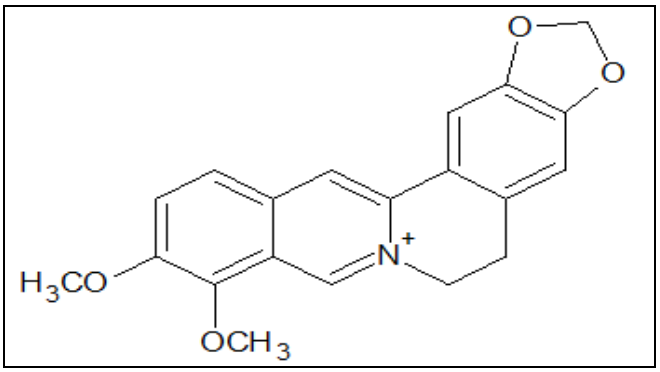
This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(9).4544-47

The total number of people living with diabetes is projected to rise to 643 million by 2030 and 783 million by 2045 [3]. Due to the lesser side effects and more effectiveness, herbal medicines are preferred over synthetic drugs in treating diabetes [4].

The polyherbal formulation selected for present study BSL-150 by Inducare Pharmacontains leaves of *Gymnema sylvestre* 100mg, seeds of *Syzygium cumini* 150 mg, seeds of *Phyllanthus emblica* 100mg and bark of *Tinospora cordifolia* 50mg. These plants are known for their anti-diabetic activity of lowering blood sugar levels [5]. Although Herbal preparations are in demand, they need standardization for better acceptance [6, 7]. Identification of a chemical marker and its quantification in the herbal product is an important

aspect in developing a method of standardization of herbal medicines [8, 9]. For this purpose widely used, modern technique is HPTLC [10, 11]. The bark of *Tinospora cordifolia* is one ingredient in tablet BSL-150. It has been reported that Berberine is one of the phytochemical alkaloids present in the bark of *Tinospora cordifolia* [12, 13]. So it also must be present in these tablets. So, Berberine is selected as the marker for the present study.



First the identification of Berberine in BSL-150 was carried out using HPTLC by developing suitable mobile phase for the same. After this the quantification of berberine followed by its use as the potential chemical marker for standardisation of BSL-150 was explored.

MATERIALS AND METHODS:

Chemicals and Reagents: All analytical grade chemicals were used for the analysis and obtained from Merck Chemicals. The Berberine standard of HPLC grade was procured from Natural Remedies Pvt. Ltd. The BSL-150 Tablets were bought from Gala Ayurved Bhandar, Dombivli, Thane.

Equipment: A Camag HPTLC system with Camag Linomat 5 semiautomatic sample applicator, Camag twin trough development chamber, Hamilton syringe(100µL), Camag TLC scanner – IV, vision CATS version 2.4 and Camag visualizer was used for this study.

Plant Materials: BSL-150 tablets contain *Gymnema sylvestre*, *Syzygium cumini*, *Phyllanthus emblica* and *Tinospora cordifolia*. The plant parts were obtained from the local areas in Mumbai. They were authenticated from Blatter Herbarium, Mumbai. In-house formulation matching the contents of BSL-150 was prepared by mixing the dried powders of the plant Parts in the proportion by weight as mentioned in the contents of the tablets. The powders were prepared by drying the plant parts, grinding and sieving through sieve 45.

Preparation of Sample and Standard Solutions: Sample solutions of six samples- 1. *Gymnema sylvestre* (GS), 2. *Syzygium cumini* (SC), 3. *Phyllanthus emblica* (PE), 4. *Tinospora cordifolia* (TC), 5. BSL-150 Marketed (BSL-M), 6. BSL-150 In-house (BSL-IH) was prepared from 100mg of the powder, 4-5 drops of conc. HCl added 5ml of methanol and sonicated for 30 minutes. The solutions were filtered through the Whatman filter paper no. 1 and the volume was made to 10ml with methanol. The final concentration was 10mg/ml. These six solutions were used for HPTLC method development and validation.

Preparation of Standard or reference berberine solution- 5mg of standard Berberine was dissolved in 1-2 ml of methanol, and this solution was diluted to 5ml with methanol. 5ml of this solution was further diluted to 100ml with methanol to get 50ng/µl solution.

Preparation, Spotting and Development of HPTLC Plate: Aluminium plate precoated with silica gel 60 F 254 of 250 µm thickness and dimensions 200.0 x 100.0 mm was used as the stationary phase. After the prewashing with methanol and activation at 120°C, the samples and reference were spotted on the plates. The spots were applied in a band of 8.0mm in width using Camag microliter syringe in Camag Linomat 5 semiautomatic sample applicator. With the slit dimension of 6.0 x 0.45mm, the scanning speed employed was 20mm/s. 20ml of mobile phase was used in a twin trough chamber. The chamber was saturated with mobile phase using Whatman filter paper no. 1 for 20 minutes at room temperature of 25±2 °C with a relative humidity of 60±5%. The chromatograms were run up to a length of 70mm and air-dried. Vision CATS software operated Camag TLC Scanner IV was used for scanning. The scanner used a deuterium lamp for Wavelength(s) 254 nm, whereas a mercury lamp for 366 nm. Peak areas for different signals were compared for quantification. The validation methods followed, including determining linearity range, LOD, LOQ, Precision, Reproducibility, Specificity, and accuracy, were as per the USP chapter 203 HPTLC SOP.

RESULTS AND DISCUSSIONS: n-Butanol: Ethyl acetate: Glacial acetic acid: Water (3: 5: 1: 1) was the mobile phase developed for identifying and quantifying Berberine on HPTLC. When all 6 samples and references were spotted on the same plate, only TC, BSL 150 M, and BSL 150 IH showed the spot corresponding to the R_f of reference berberine.

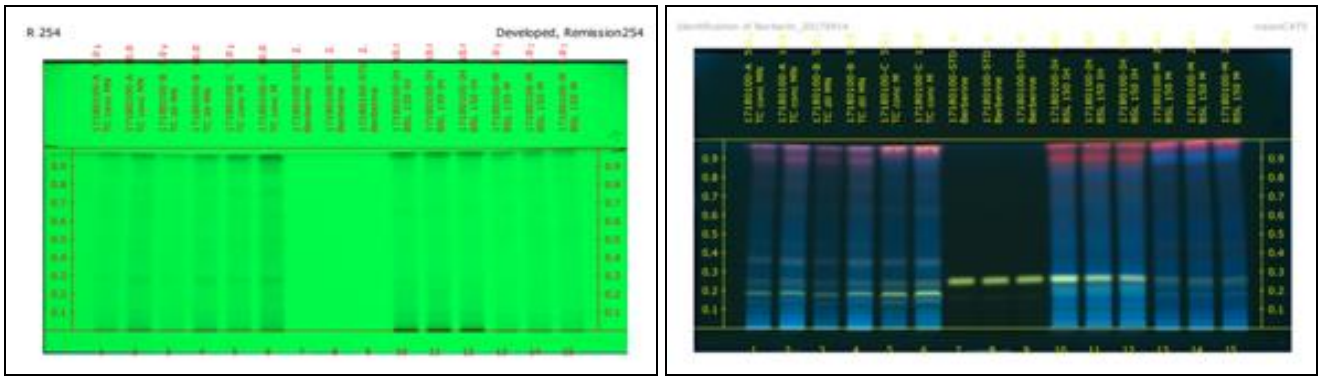


FIG. 1(A): SCANNED FOR REMISSION AT 254nm **FIG. 1(B): SCANNED FOR REMISSION AT 366nm**

FIG. 1: IDENTIFICATION OF BERBERINE

The R_f of Berberine was found to be 0.322 ± 0.010. Hence, berberine was identified to be present in BSL-150 tablets. In **Fig. 1B**, the separated bands of berberine can be easily seen in TC, BSL 150 M and BSL 150 IH. The 15 tracks in the plate for the identification shown are-track 1 to 6 are TC, 7 to 9 are berberine standard, 10 to12 are BSL 150 IH and 13 to 15 are BSL 150 M, as shown in **Fig. 1.** For the quantification, the TC, BSL 150 M and BSL 150 IH were spotted in triplicate and repeated for three days. After regression analysis using least square method for the concentration and area covered by the peaks. $Y= 3.084\ X10^{-6}\ x + 1.169\ X10^{-2}$ was the regression equation where, x is the concentration and y is the corresponding area of peak. This equation is used for finding the concentration of berberine. The TC and BSL 150 IH samples contain 0.08% w/w of Berberine, whereas the BSL 150 M contains 0.008% w/w of Berberine. The correlation coefficient, R was found to be 99.8%. A linearity was obtained for the range of 10 to 90 ng, **Fig. 2.**

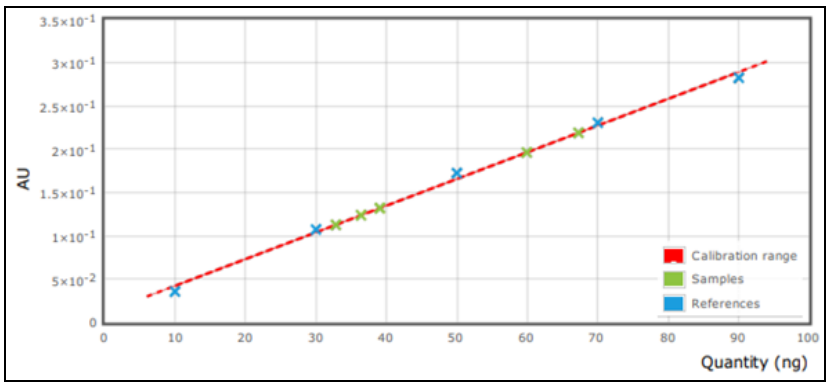


FIG. 2: LINEARITY STUDY OF BERBERINE

Accuracy is assessed by recovery. For the recovery, the concentration of TC spiked samples were compared with theoretical concentration. The recovery was 85.94%, whereas the %RSD was 3.32%. Upon development of the plate where the TC, BSL 150 IH, BSL 150 M, Mobile phase, diluent and reference were spotted, showed berberine band only in samples and reference. Such spots were absent in the mobile phase and diluent. This shows the specificity of the method.

CONCLUSION: The results show that berberine can be used as a marker compound to standardize BSL 150 tablets. A quick, accurate and validated method of standardization by using berberine for these tablets is developed. The method is validated for linearity, precision, accuracy, and specificity.

ACKNOWLEDGMENT: We thank K. J. Somaiya College of Science and Commerce, Sathaye College, for their support during the

research work. We are also thankful to Anchrom Enterprises Pvt. Ltd. For the support in HPTLC analysis.

CONFLICTS OF INTEREST: I am reporting that I have not received funding from any company that may be affected by the research reported in the enclosed paper.

REFERENCES:

1. Sapra A and Bhandari P: Diabetes Mellitus. [Updated 2022 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551501/
2. https://www.who.int/news-room/fact-sheets/detail/diabetes
3. IDF Diabetis Atlas 10th edition 2021.
4. Walia S, Dua JS and Prasad DN: Herbal Drugs with Anti-Diabetic Potential, Journal of Drug Delivery and Therapeutics 2021; 11(6): 248-256.
5. Modak M, Dixit P, Londhe J, Ghaskadbi S and Devasagayam TPA: Indian Herbs and herbal drugs Used for the Treatment of Diabetis. J Clin Biochem Nutr 2007; 40(3): 163–173.
6. Bhanu S, Zafar PS and Panwar R: Herbal drug standardisation. The Indian Pharmacist 2005; 4(35): 19-22.
7. Sachan AK and Vishnoi G: Roopakkumar: Need of standardisation of herbal medicines in modern era. International Journal of Phytomedicine 8 2016; 300-307.
8. Gunawan Indrayanto: The importance of method validation in herbal drug research. J of Pharmaceutical and Biomedical Analysis 2022; 214; 114735
9. Bijauliya RK, Alok S, Chanchal DK and Kumar M: A comprehensive review on standardisation. International J of Pharma Science and Research 2017; 8(9): 3663-3677.
10. Shinde DB and Chavan MJ: HPTLC in Herbal drug Quantification, HPTLC, -Springer 2011; 117-140.
11. Lalhriatpuii T: HPTLC Fingerprint in Herbal Drug Formulations. In: Sen, S., Chakraborty, R. (eds) Herbal Medicine in India - Springer, Singapore 2020; 337-362.
12. Srinivasan GV and Unnikrishnan KP: HPLC estimation of berberine in *Tinospora cordifolia* and tinosporasinesis. Indian J Pharm Sci 2008; 70(1): 96–99.
13. Sharma P and Dwivedee B: The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon 2019; 5(9): 02437.

How to cite this article:
Kamath CR and Salvi SA: Identification and quantification of berberine in a polyherbal formulation by HPTLC method. Int J Pharm Sci & Res 2023; 14(9): 4544-47. doi: 10.13040/IJPSR.0975-8232.14(9).4544-47.