A simple spectrofluorometric estimation of psoralen from <em>Psoralea corylifolia</em> (L.) (Bavchi) seeds.

Khushboo P. Salaskar *, Varsha M. Jadhav, Vilasrao J. Kadam.
Department of Quality Assurance, Bharati Vidyapeeth’s College of Pharmacy, Sector 8, CBD Belapur, Navi-Mumbai – 400614, India

Received on: 20-05-2009; Accepted on:15-07-2009

ABSTRACT

A simple, accurate, precise, sensitive spectrofluorometric method was developed for estimation of psoralen in methanolic extract of <em>Psoralea corylifolia</em> seeds. It showed strong intense blue fluorescence having excitation and emission wavelength 290nm and 427.66 nm, respectively. Linear relationship for the fluorescence intensity was obtained in the range 50-100 µg/mL. Limit of detection and limit of quantification was found to be 6.51 µg/mL and 19.75 µg/mL, respectively. The method was statistically validated and found suitable for estimation of psoralen in a methanol extract of plant <em>Psoralea corylifolia</em>.

Keywords: <em>Psoralea corylifolia</em>, psoralen, spectrofluorometry.

INTRODUCTION

Dry fruits of Leguminous plant <em>Psoralea corylifolia</em> Linn. (syn: <em>Cullen corylifolium</em> Linn.) is one of the most popular Traditional Chinese Medicine and officially listed in Chinese Pharmacopoeia (1). It’s an annual herb growing throughout the plains of India. The plant is of immense biological importance and it has been widely exploited since ages for its magical effect against several skin diseases like psoriasis, leucoderma and leprosy (2). It is reported to contain essential oil, coumarins, alkaloids, flavonoids and terpenoids (3, 4). The literature also records therapeutic action of <em>Psoralea corylifolia</em> against various diseases such as asthama, diarrhea, alopecia areata (5), impotence, menstruation disorder and uterine hemorrhage (6). It shows antitumor (7), anti-allergic (8), antioxidant (9), insecticidal (10) and antimicrobial activity (11). The drug has also been reported for the treatment of enuresis, various kidney problems (12), depression (13, 14), osteoporosis and bone fractures (3, 15), lumbago and tuberculosis (16).

Psoralen (7H-Furo (3, 2-g) (1) benzopyran-7-one) is the major and most active furanocoumarin present in <em>Psoralea corylifolia</em> which promotes pigmentation (17, 18). Psoralen has been found to intercalate into DNA, where they form mono and di adducts in the presence of long wavelength UV light and thus are used for the treatment of hypo-pigmented lesions of the skin like leucoderma (19).

Fluorescence occurs because of the transition from first excited singlet state to ground state by emission of light (20).

MATERIALS AND METHOD

Instrumentation

A Perkin Elmer LS 55 Luminescence spectrometer equipped with a 1 cm fluorescence free quartz cell was employed for all spectral and fluorescence measurements. Instrumental parameters were as follows: Scan speed: Medium. Sensitivity: High.

Reagents and standard used

All chemicals were AR grade were purchased from S.D. Fine chemicals, Mumbai.

Plant material

The seeds of <em>Psoralea corylifolia</em> were procured from local market and authenticated for their correct botanical identity by scientist of Agharkar Research Institute, Pune. The seeds were dried, powdered and kept in an air tight container.

Preparation of standard solution for calibration curve

Standard solution was prepared by dissolving 10 mg psoralen in 10 mL methanol and further dilution was done to obtain concentration (100 µg/mL). Appropriate dilutions were made in methanol to produce standard solutions of 50, 60, 70, 80, 90 µg/mL. The standard curve of psoralen was plotted by measuring fluorescence intensity of solu-
Analysis of Psoralen in Herbal Extract

Preparation of the methanolic extract of *P. corylifolia*: Accurately weighed 2.5-g dried seed powder of *Psoralea corylifolia* was defatted with petroleum ether (60-80 °C) and extracted with methanol (4 x 25 mL) under reflux and dark brown extract was concentrated in vacuum via rotavacuum drier until pourable. Further this extract was taken in the tarred evaporating dish and evaporated to the constant weight. A dark brown sticky extract was then used for further analysis.

Preparation of the sample solution: The methanolic extract (100 mg) was accurately weighed and transferred to a 20 mL volumetric flask containing 10 mL methanol. The mixture was sonicated for 15 mins and diluted to 20 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 10 mins and the clear supernatant was analyzed for drug content.

Estimation of Excitation and Emission Wavelength

Standard solution of psoralen was scanned in the range of 200-600 nm for determination of excitation wavelength and it was found to be 290 nm. Same solution was scanned for emission wavelength in the range of 370-600 nm taking 290 nm as an excitation wavelength and it was found to be 427.66 nm. The fluorescence intensity of standard solution and sample solution was measured at excitation wavelength 366 nm and emission wavelength 427.66 nm.

Method Validation

This method was validated as per the ICH guidelines (21), the method validation parameters checked were linearity, accuracy and recovery, precision, limit of detection, limit of quantification, and specificity.

RESULTS AND DISCUSSION

Psoralen showed intense blue fluorescence under UV light, therefore this necessitated to develop a new and fast spectrophotometric method for the compound. The fluorescence intensity of psoralen has linear relationship in the concentration range of 50-100 µg/mL (Fig. 2. and Table 2). Stability study proved that the fluorescent characteristic of the solution was stable up to 3 hrs at room temperature (28 ± 2. 0°C). After that, fluorescence intensity diminished gradually. Other parameters like excitation wavelength, emission wavelength, regression equation, coefficient of determination ($r^2$), limit of detection (LOD), limit of quantification (LOQ) are listed in Table 1. Recovery study was carried out by adding known amount of psoralen to preanalyzed crude drug and again analyzed to estimate its content (Table 3). The method was found to be highly selective without any interference. The LOD and LOQ were found to be within the detectable and quantifiable limits. The validation parameters reported, assures the competitive performance of the newly developed method for estimating psoralen from the complex matrix. The present studies were performed to evolve a new analytical method, which can help in standardization of various herbal and traditional formulations in practice.

**Determination of marker in dry extract:**

The amount of psoralen in dry extract was found to be 1.092%.

**CONCLUSION**

Psoralen which is present in *Psoralea corylifolia* seeds is one of the important compound used for the treatment of psoriasis, leucoderma and leprosy. In this work a spectrophotometric method has been developed for quantification of psoralen in dry seed powder extract of *Psoralea corylifolia*. This method can be used as rapid quality control method for *Psoralea corylifolia*. The validated method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of psoralen in *Psoralea corylifolia* seed extract and its formulation.

**ACKNOWLEDGEMENT**

The authors are thankful to Dr. K.S. Laddha Sir from UICT, Matunga, Mumbai for providing gift sample of pure Psoralen.
REFERENCES


Source of support: Nil, Conflict of interest: None Declared