Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Studies on extraction, isolation and estimation of psoralen from the fruits of *Psoralea corylifolia*

Rajput S.J.*, Vijaya Zade, Pallavi Rai

Pharmaceutical Quality Assurance Laboratory, Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India 390 001.

Author for Correspondence: srajput@gmail.com

ABSTRACT

Psoralen was isolated by column chromatography from the methanol extract from Psoralea corylifolia seeds. The chemical structure of the compound was established by spectroscopic methods. To analyze bulk drug and formulations, a simple, selective, and precise high performance thin layer chromatographic method was developed and validated. The proposed method employed HPTLC aluminum plates pre-coated with silica gel 60F-254 as the stationary phase. The plates were developed with benzene: chloroform: ethyl acetate (80:10:10 v/v) as mobile phase, this system was found to give compact spots for psoralen (R_f value of (0.61 $\pm$ 0.02). Regression analysis from densitometric analysis at 254 nm showed linear correlation between peak height and peak area (r = 0.9994 and 0.9998, respectively) in the concentration range from 50-250 ng/µL. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 25 ng/µL and 50 ng/µL respectively. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of psoralen.

KEY WORDS: Psoralen, HPTLC, Column chromatography, Psoralea corylifolia.

INTRODUCTION

Psoralen 7H-Furo (3, 2-g) (1) benzopyran-7-one **(Fig.1)** is a naturally occurring furanocoumarin obtained from dried ripe fruits of Psoralea corylifolia Linn. (Family: Leguminosae) used for the stimulation of skin pigment-melanin, in the treatment of vitiligo, psoriasis, cutaneous T cell lymphoma (CTCL), alopecia, eczema and other skin diseases (1-4). The seeds have also been reported to have antifungal and antiprotozoal property (5). Psoralen has been found to intercalate into DNA, where they form mono- and di- adducts in the presence of long wave UV light and thus are used for the treatment of hypopigmented lesions of the skin like leucoderma (6).

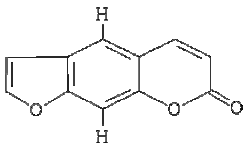

Fig.1. *Chemical Structure of Psoralen.*

The preparative separation and purification of psoralen and related compounds from various plant materials by the reported methods are tedious and usually require highly sophisticated instruments which are not cost effective (7-9). Due to the important biological properties and broad applications of psoralen we have attempted to develop an efficient method to isolate, purify and characterize the pure compounds from the seeds of the plant Psoralea corylifolia.

Literature reveals that a number of methods like UV-Spectrophotometry and HPLC are available for the analysis of psoralen which either lack sensitivity or are very tedious (1-2, 10-11). Estimation of related compounds of psoralen in serum have also been reported using HPTLC and GC-MS which are either lack specificity or are not cost effective(12-13). In the present study, an attempt has been made to develop and validate a HPTLC method for the estimation of psoralen in bulk and in formulation.

The advantage of quantitative thin-layer chromatography (QTLC) methods over other techniques is that large number of samples can be simultaneously analyzed using small volume of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Mobile phases having pH 8 and above can be employed. Suspensions or turbid

samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. The popularity of high-performance thin layer chromatography (HPTLC) is continuously increasing. The HPTLC plates are smaller in size particle size (2-7µm), have narrow particle size distribution, and possess smooth surfaces and thinner layers (0.2mm). Thus, separation and quantification can provide results that are either superior or comparable with other analytical methods such as HPLC (14-15).

MATERIALS AND METHODS

Extraction and Isolation

Materials

Psoralen (reference standard) was obtained from "Franco Pharmaceutical Ltd, Bombay", India. All chemicals and reagents were purchased from M/s Qualigens, Mumbai, India. 1.2 Instrumentation

A Shimadzu 1601 UV, Japan recording spectrophotometer with 10 mm matched quartz cells was employed for UV spectroscopy. IR spectrum of was taken on Shimadzu FTIR 800, using potassium bromide disc method.

Isolation of psoralen

Psoralea corylifolia was purchased from the local area of Baroda and authentified at the department of botany and the voucher specimen is maintained in the herbarium at Pharmacy Department, The M.S. University of Baroda. Oven dried (60°C) powdered seeds (1kg) of Psoralea corylifolia were defatted with petroleum ether (60-80°C) at room temperature. The dried powder was further extracted with methanol by soxhletion. The concentrated crude methanol extract (29.6 g) was chromatographed over silica gel (300 g) and eluted with benzene and chloroform mixtures. Different fractions of elute (50mL) each were collected and subjected to analysis by TLC. The first few eluent of benzene and chloroform consisted of sticky dark brown mass were rejected, while the fraction eluted at, benzene: chloroform (20:80 v/v) consisted of yellow needle shaped crystals which was characterized as psoralen by comparing with authentic sample. The product was further purified by re-crystallization with methanol (yield: 11.2 mg). Identification of the compound was based on the detailed spectral analysis (UV and FT-IR) and comparison with a reference standard.

HPTLC STUDIES OF PSORALEN

HPTLC instrumentation - The samples were spotted in the form of bands of width 6 mm with a CAMAG microlitre syringe on precoated silica gel aluminium plate 60F-254 (10cm x 10 cm with 0.2mm thickness, E. Merck, Germany) using a CAMAG Linomat-V. A constant application rate of 150nL/s was employed with space of 5.5 mm between two bands. The slit dimension was kept at 4 mm x 0.1 mm, with scanning speed of 20 mm/s. The linear ascending development was carried out in trough chamber saturated with the mobile phase mobile phase consisted of benzene: chloroform: ethyl acetate (80:10:10 v/v,20 mL). The optimized chamber saturation time for mobile phase was 15 minutes at room temperature. The length of chromatogram run was 65 mm. Subsequent to the development; TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on CAMAG TLC scanner III in the absorbance mode at 254 nm.

Calibration plots of psoralen.

A stock solution of psoralen (100 µg/mL) was prepared in methanol. Suitable aliquots in the range of 0.1 to 2.5 mL of stock solution in 10 mL volumetric flask, and volumes were made up with methanol to prepare a series of dilutions in the range of 1 to 25 ng/µL. The drug was spotted in duplicate on TLC plate to obtain concentrations of 50-250 ng/spot of psoralen. The data of peak height and area versus drug concentration were treated by linear least-square regression.

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (100 ng/spot of psoralen). The intra- and inter-day variation for the determination of psoralen was carried out at two different concentration levels 100 and 200 ng/spot (16).

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of benzene: chloroform: ethyl acetate (85:10:5v/v) was tried at two different concentration levels of 100 and 200 ng/spot.

Limit of detection and limit of quantitation

In order to determine detection and quantification limits, psoralen concentrations in lower part of the linear range of the calibration plots were used.

Psoralen solutions of 50, 100 and 150ng/µL were prepared and applied in triplicate (1 µL).The amount of psoralen by spot versus average response (peak height and area) was graphed and the equation for this curve was determined, thereby obtaining an estimate of the target response: ybl. The ybl value corresponds to the intersection of the curve. Subsequently, a second curve was graphed showing the amount of psoralen by spot versus standard deviation of the responses. From the equation of this curve, we obtained an estimate of the standard deviation for target: sbl, which corresponds to the intersection of this curve. Detection and quantification limits were calculated by means of the equations:

Detection limit= (ybl+3sbl)/b:

Quantification limit= (ybl+10sbl)/b,

where "b" corresponds to the slope obtained in the linearity study of the method (17-18).

Analysis of psoralen in prepared formulation

To determine the content of psoralen in tablets, the tablets were powdered and powder equivalent to 10 mg of psoralen was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 mL. The resulting solution was centrifuged at 3000rpm for 5 minutes and supernatant was analyzed for drug content. The 2 µL of the filtered solution was applied on TLC plate followed by development and scanning. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied through recovery studies.

Recovery studies

The analyzed samples were spiked with extra 50, 100 and 150% of the standard psoralen and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

RESULTS AND DISCUSSION

Characterization Of Psoralen

Melting point

The crystals obtained by column chromatography had the melting point of 163-165 °C which is comparable with the reported melting point (162-164 °C).

Infrared Spectroscopy

The IR spectral data of the isolated compound showed the characteristic peaks at 1616, 1581, 1557, 1422, 831, 748, which are comparable to that of reference standard.

Molar Absorptivity - The UV spectral data of the isolated compound and marker compound was comparable and the molar absorptivity was calculated at λ_{max} 247.0 nm. The results have been depicted in Table 1.

METHOD DEVELOPMENT

Development of the optimum mobile phase

TLC procedure was optimized with a view to develop quantification method. Initially, benzene: chloroform in varying ratios was tried. The mobile phase benzene: chloroform (98:02 v\v) gave R_f value of 0.16, therefore, a second mobile phase was tried. Benzene: chloroform: ethyl acetate (80:10:10 v\v) gave good resolution as well as a well defined peak at R_f value of 0.62 .Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 minutes at room temperature (Fig. 2).

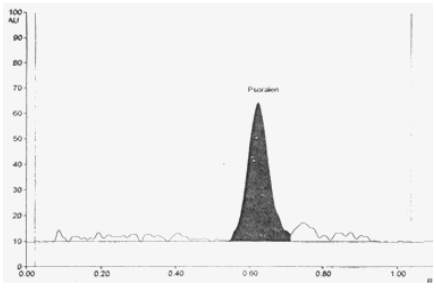

Fig. 2 *HPTLC Chromatogram (Psoralen Standard).*

Calibration plots

The linear regression data for the calibration plots (n=3) as shown in Table 2 showed a good linear relationship over the concentration range 50 ng-250 ng/spot with respect to peak height and area. No significant difference was observed in the slopes of standard plots.

Validation Of The Method

Precision

The repeatability of sample application and measurement of peak height and area were expressed in terms of % RSD and results are depicted in Table 3 which revealed intra-day and inter- day variation of psoralen at two different concentration levels of 100 and 200 ng/spot.

Robustness of the method

The low values of % RSD obtained after introducing small changes in mobile phase composition indicated the robustness of the method Table 4.

Table 1. *Calculations of molar absorptivity(ε) for psoralen.*

S.No	Concentration (µg/mL)	Abs.	Conccentration (g/L)	Molar conc (moles/Lx10^5)	Molar Absorptivity(ε)
1	2	0.315	0.002	10.687	2.9475
2	4	0.564	0.004	21.374	2.6387
3	6	0.799	0.006	32.061	2.4921
4	8	1.155	0.008	42.748	2.7018
5	10	1.338	0.010	53.435	2.5039
6	12	1.606	0.012	64.123	2.5045
7	15	2.007	0.015	80.153	2.5039

Table 2. *Regression analysis for calibration plots. (n=3)*

Linearity Range	50 ng/µL to 250 ng/µL
Coefficient of Determination (Height)	0.99941
Coefficient of Determination (Area)	0.99984
Regression equation (Height)	Y= 1.463 + 0.334 x
Regression equation (Area)	Y= 6.818 + 9.625 x
Slope (Height)	1.463
Slope (Area)	6.818
Intercept (Height)	0.334
Intercept (Area)	9.625

Table 3. *Intra- and inter-day precision of HPTLC method. (n=6)*

Amount (ng/spot)	Intra-day precision		
	Mean area	S.D.	% RSD
100	941.76	39.03	1.14
200	1825.06	78.34	1.06
Amount (ng/spot)	Inter-day precision		
	Mean area	S.D.	% RSD
100	932.13	41.23	1.32
200	1892.35	85.57	1.11

Table 4. *Robustness of the method.*

Amount (ng/spot)	Mobile phase composition	
	Benzene: Chloroform: Ethyl acetate (80:10:10v/v)	Benzene: Chloroform: Ethyl acetate (85:10:5v/v)
100	1.15	1.67
200	0.97	1.54

Table 5. *Recovery studies. (n=3)*

Excess drug added to the analyte(%)	Theoretical content (ng)	Recovery (%)	% RSD
0	100	101.97	1.13
50	150	99.10	1.67
100	200	97.31	1.43
150	250	98.22	1.18

LOD and LOQ

The calibration plot in this study was plotted between amount of analyte versus average response (peak area) and the regression equation was obtained (Y=6.818+9.625X) with a regression coefficient of 0.999. Detection limit and quantification limit was calculated by the method as described earlier and found 25 ng and 50 ng respectively, which indicates the adequate sensitivity of the method.

Recovery studies

The proposed method when used for extraction and subsequent estimation of psoralen in pharmaceutical dosage forms after spiking with 50,100,150 % of additional drug afforded recovery of 97-102% as listed in Table 5.

Analysis of the formulation

A spot at R_f 0.62 was observed in the chromatogram of psoralen samples extracted from tablets (Fig. 3). There was no interference from the excipients commonly present in the tablets. The psoralen content was found to be 98.12% with a RSD of 1.14%.

It may therefore be inferred that degradation of psoralen had not occurred in the formulations. The low % RSD value indicated the suitability of this method for routine analysis of psoralen in pharmaceutical dosage forms.

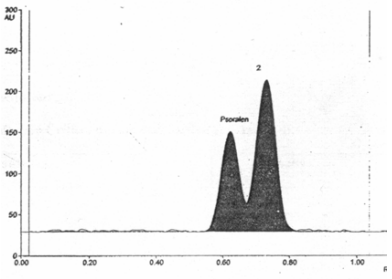

Fig. 3 HPTLC Chromatogram (sample).

CONCLUSION

Although the complete organic synthesis of psoralen has been established, it is not yet economically feasible because of its complexity and low yield. Thus it is essential to develop new methods for the isolation which are simple as well as reliable. Thus the above method for isolation of psoralen can be utilized in future. Further, the proposed high-performance thin layer chromatographic method (HPTLC) can be widely used as a standard technique for rapid and accurate quantitative determination of psoralen in the bulk drug and in pharmaceutical dosage forms. Running time and cost per analysis are relatively low in comparison with other methods. The analytical performance of the proposed HPTLC method was established and the method was validated in terms of precision, accuracy, robustness, detection and quantification limits.

ACKNOWLEGDEMENT

Authors are grateful to Dr. S.H. Mishra, Professor of Pharmacognosy, Pharmacy Department, The M.S. University of Baroda for providing the HPTLC facility. The authors also wish to thank Franco Pharmaceutical Ltd, Bombay for supplying the authentic sample of psoralen as gift sample.

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