Fingerprint Analysis of *Psoralea corylifolia* Linn. Seeds (Babchi) by Ultra Performance Liquid Chromato-graphy with Photodiode Array Detector

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Abstract

chemical fingerprint method for methanolic extract of seeds of *Psoralea corylifolia* Linn. was developed for Quality Control analysis by Ultra Performance Liquid Chromatography with Photodiode Array Detector (UPLC-PAD). *Psoralea corylifolia* Linn., seeds collected from Pharmacy, Central Research Institute of Unani Medicine, Hyderabad and extract was analyzed by using a reverse-phase Waters Acquity BEH C_{18} column (50 mm × 2.1 mm, 1.7 µm). Among mobile phases investigated such as methanol-water, acetonitrile-water, and acetonitrile-buffer (containing Orthophosphoric acid), the water (A) - acetonitrile (B) system was the ultimate choice. Gradient elution was essential with gradient mobile phase consisted of 0.1%OPA in (water: Acetonitrile : 10:1) (A) and acetonitrile (B) using a gradient program at a flow rate of 0.5 mL/min with detection at a wavelength of 254 nm. The chromatographic fingerprints showed different chemical constituents qualitatively in *Psoralea corylifolia* Linn. seeds, out of which psoralen peak identified corresponding to the retention time of standard Psoralen and further confirmed by UV spectrum. The Psoralen content in seeds extract of *Psoralea corylifolia* Linn. which is an active principle is accurately determine corresponding to that of standard Psoralen with shorter analysis time.

Key Words: Fingerprint, *Psoralea corylifolia*, Seeds, Ultra Performance Liquid Chromatography (UPLC)

Introduction

Today the world is impending on herbal medicine and attracting considerable attention because of its excellent qualities such as low toxicity and less side effects, proven medical effects and rare drug tolerance (Wang *et al*., 2010; Kong *et al*., 2009; Feng *et al*., 2006). It is well known that medicinal plants collected at different harvesting times and from different regions may considerably differ in types and quantities of chemical components, which results in affect of quality of pharmaceutical products and in standardization of herbal medicine (Wang *et al*., 2009; Caballero-Ortega *et al*., 2007; Zhang *et al*., 2009). Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy (Straus, 2002; De Smet, 2002).

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The quality assessment and control of herbal medicine is an important concern for both the health authorities and the public (Eisenberg *et al*., 1998; Xu *et al*., 2005; Cardeal *et al*., 2008). Most investigations focused on several markers or pharmacologically active constituents to assess the quality and potency of the herbal medicine or preparations, which cannot stand for the overall quality of herbal drugs. Therefore, developments of reliable, comprehensive quality assessment methods are necessary for herbal drugs (Gu *et al*., 2004). The fingerprint technique, which emphasized on the whole characteristics of samples' compositions and focuses on identifying and assessing the stability of the samples, was accepted by WHO, (Anonymous, 2000). High Performance Liquid Chromatography (HPLC) played the most important role among all the fingerprint techniques (Zhou *et al*., 2009). However, the traditional HPLC fingerprints cannot meet the requirements of high throughput analysis due to the low column efficiency and long analysis time with generally more than an hour (Ye *et al*., 2009; Xu *et al*., 2009a, Xu *et al*., 2009b). In recent years, UPLC has been reported as a viable technique for quantitative and chemical fingerprint analysis of herbal medicines, prior to HPLC analysis (Wang *et al*., 2010; Kong *et al*., 2009; Liu *et al*., 2007).

Psoralea corylifolia L. is a widely used medicinal plant in India and Asia (Yadav and verma, 2005). The seeds of *P. corylifolia* L. exert antioxidative, antimicrobial and anti-inflammatory activities (Haraguchi *et al*., 2002; Karsura *et al*., 2001; Ferrandiz *et al*., 1996). *Psoralea corylifolia* Linn. seeds have been used for the treatment of various kinds of skin disorders such as vitiligo, Psoriasis, eczema, asthma, cough, nephritis, and calvities (Anonymous, 1985). The effective components of the herb are coumarins. Psoralen and isopsoralen are the major components. Pharmacological test revealed that they have antitumor (Wu *et al*., 1998), antibacterial and antivirus activities and can affect metabolism of some remedy (Mi, *et al*., 1998). Psoralen is used as reference standard in the quality control of *Psoralea corylifolia* Linn., seeds. So the isolation and purification of psoralen and isopsoralen are of great interest. Psoralen (7H-Furo (3, 2-g) (1) benzopyran-7-one) is the major and most active furanocoumarin present in *Psoralea corylifolia* which promotes pigmentation (Sebastian, 2006; Khastgir *et al*., 1959). 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 (Vaidya, 2006). In this paper, a UPLC chemical fingerprinting method was developed for quality control of the *Psoralea corylifolia* Linn. seeds.

Materials and Methods

Standards and reagents

Standard Psoralen (purity ≥ 99%) was purchased from Sigma Aldrich (Bangalore). LC grade water, acetonitrile and methanol were purchased from Fischer Scientific (India). AR grade Ortho Phosphoric acid (purity 88%, Himedia) as buffer used in preparation of mobile phase.

Plant materials

Psoralea corylifolia Linn. seeds sample collected from Pharmacy, Central Research Institute of Unani Medicine, Hyderabad as shown in figure 1. Authentification and identification of sample based on morphological characteristics is done from the same institute. For the chemical fingerprinting analysis, sample is pulverized to obtain a uniform powder for extraction used for the study.

Sample preparation

Sample powder (5.0 g) was accurately weighed and extracted with 30mL methanol under ultrasonic water bath for 60 min at 25°C with 17 W and 60 Hz of power (Oscar ultrasonic, India). After cooling, methanol was added for the lost weight. Then the solution was filtered through a 0.2µm membrane filter and 1µL was injected for UPLC analysis.

Preparation of Standard Solution

Standard solution of Psoralen (1000 µg/mL) was prepared by dissolving in 10ml methanol and the aliquots is used for UPLC analysis. The concentrations of standard Psoralen used for calibration were 50, 100, 150 and 200 µg/mL. The standard curve was calibrated using the linear regression equation derived from the peak areas.

UPLC equipment and conditions

Experiments were performed on a Waters Acquity UPLC-H Class system (Waters, USA) equipped with a Quaternary solvent pump, an auto sampler and a PDA detector, and connected to Waters Empower software. The mobile phase consisted of 0.1%OPA in (water: Acetonitrile :: 10:1) (A) and acetonitrile (B) using a gradient program of 80% A in 0-1 min, 80-20% A in 1-7 min, 20% A in 7-8 min. The flow rate was 0.5 mL/min. The detection wavelength and column temperature were 254 nm and 40°C respectively. UV spectrums were acquired in the range 190-400 nm (2 nm resolution).

In this paper the chromatographic profile of psoralen stdandard and *Psoralea corylifolia* Linn. methanolic extract of seeds were analyzed using a binary gradient. Psoralen Characteristic peaks in the chromatogram was obtained at retention time 1.743 min was used as a reference for sample. Correspondingly retention time for Psoralen in the sample was obtained at Rt 1.745

Table 1 : Peak list of Psoralen standard with Retention time.

S. No.	Name	Retention Time	Area	% Area	Height
1	Psoralen	1.743	573051	100.00	200984

Table 2 : Peak list of seeds extract of *Psoralea corylifolia* Linn. with Retention time.

S.No.	Name	Retention Time	Area	% Area	Height
1	Psoralen	1.745	36660	0.24	12940
2	-	3.886	1848366	12.12	1126124
3	-	3.975	216896	1.42	135609
4	-	4.101	258318	1.69	152435
5	-	4.327	347683	2.28	210248
6	-	4.413	250444	1.64	133268
7	-	4.446	206645	1.36	125826
8	-	4.565	265653	1.74	163843
9	-	4.925	194197	1.27	67260
10	-	5.110	422359	2.77	243099
11	-	5.301	361982	2.37	194443
12	-	5.510	1182168	7.75	589662
13	-	6.074	397830	2.61	205156
14	-	6.773	9257610	60.72	3148375

Figure 1: *Psoralea corylifolia* Linn. Seeds sample

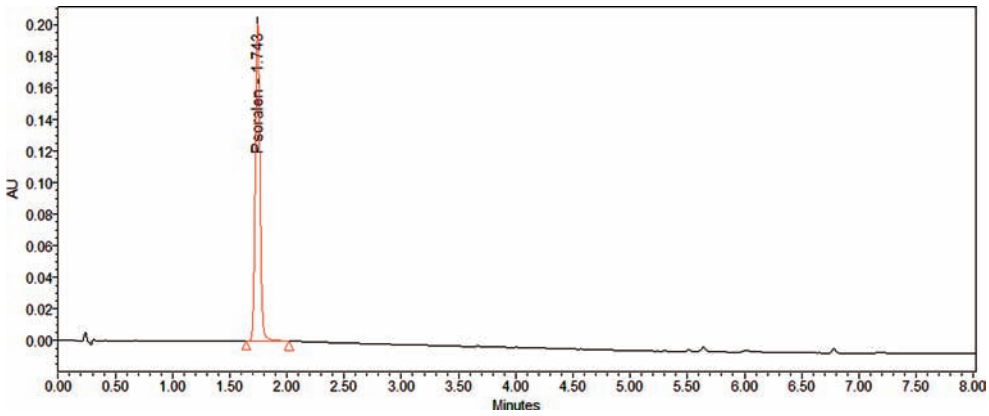

Figure 2: UPLC Chromatogram of standard Psoralen.

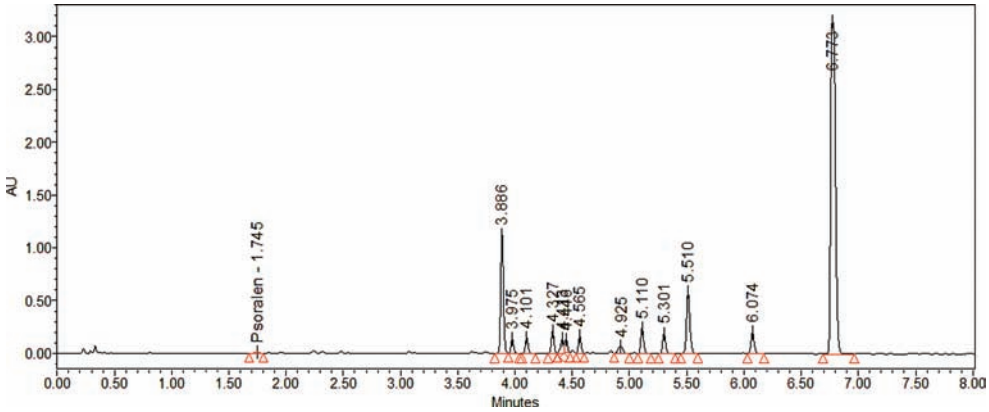

Figure 3: UPLC Chromatogram of seeds extract of *Psoralea corylifolia* Linn.

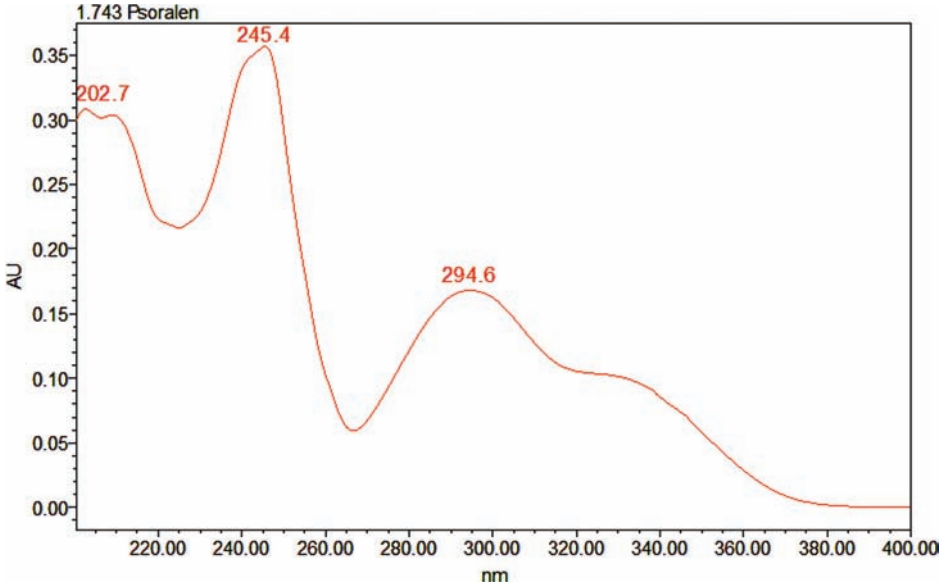

Figure 4: UV spectrum of standard *Psoralea*.

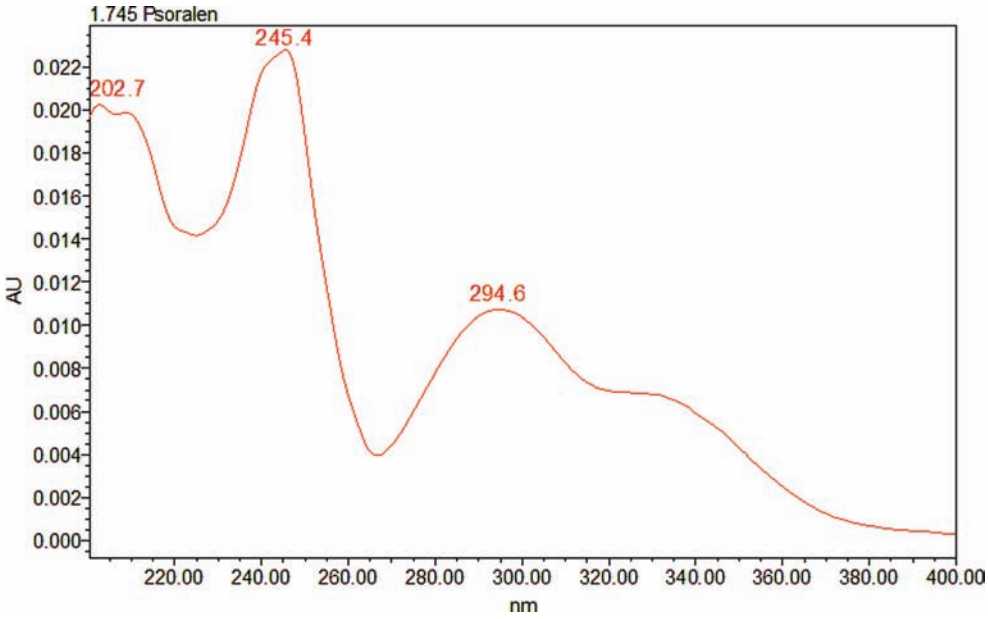

Figure 5: UV spectrum of Psoralen peak in seeds extract of *Psoralea corylifolia* Linn.

Results and Discussion

Optimization of UPLC conditions

Conditions such as the mobile phase, flow rate, column temperature and detection wave length were investigated to get the best resolution for psoralen in *Psoralea corylifolia* Linn. seeds.

Mobile phase was thought to be the main effect on the resolution when the column was selected with Waters Acquity BEH C_{18} columns (50 mm × 2.1 mm, 1.7 µm). Among mobile phases investigated such as methanol-water, acetonitrile-water, and acetonitrile-buffer (containing Orthophosphoric acid), the water (A) - acetonitrile (B) system was the ultimate choice. Gradient elution was essential for the separation. The gradient time, gradient polarity and initial composition of the mobile phase were taken into consideration. The gradient program was finally designed as 80% A in 0-1 min, 80-20% A in 1-7 min, 20 % A in 7-8 min. Column temperature of 40°C and flow rate of 0.5mL/min was resulted good separation. Besides, wave length of maximum absorption has been determined by a photodiode array detector and four wave lengths at 254, 275, 290, 310 nm were selected and compared. Wave length of 254 nm was selected to obtain the lowest baseline noise, a sufficiently large number of detectable peaks and better resolution in the chromatograms. Under the optimal conditions, almost all the components in the extracts of *Psoralea*

corylifolia Linn. seeds were well separated with high peak capacity within 8 min.

UPLC fingerprints development of Psoralea corylifolia Linn. seeds and identification of peaks:

Chromatographic fingerprints obtained for standard Psoralen and for seeds of *Psoralea corylifolia* Linn. are shown in figure 2. In the present study, Psoralen Standard consisting of Peak at Rt 1.743 in the chromatogram having total peak area of 573051 corresponding to 100% area as shown in table 1 whereas methanolic extract of seeds of *Psoralea corylifolia* Linn. consisting of 14 peaks in chromatogram shown in figure 3 whose peak area, area%, height and Rt are shown in table 2. The psoralen peak has been identified in the sample chromatogram by comparison of UV spectrum obtained from the PDA spectrum and extracted at 254nm and the UV spectrum of psoralen standard and psoralen in sample extract are shown in figure 4 and 5 which are identical confirming the peak to be psoralen. The amount of Psoralen content estimated in the sample corresponding to peak area of standard psoralen and was found to be 0.1279 µg%. A UPLC method was developed for the quantification of psoralen in *Psoralea corylifolia* seeds. The analysis of sample can be completed within 8 min. This method possesses the advantages of simplicity, sensitivity and good reproducibility and will be applicable to the quality control of seeds of *Psoralea corylifolia* Linn.

Conclusion

A method of UPLC was developed for chemical fingerprint analysis obtained for seeds extract of *Psoralea corylifolia* Linn. The chromatograms of standard psoralen and the sample containing Psoralen was compared for the identification of psoralen in the sample and its content determined by the UPLC fingerprints method which proved that the established method was suitable for fingerprint analysis of quality control of *Psoralea corylifolia* Linn. Based on the result obtained the content of Psoralen in the sample corresponding to standard psoralen was found to be 0.1279 µg% in the seeds within the shorter run time of 8min. The results also showed that UPLC possessed the advantages of shorter analysis times, higher column efficiency and less solvent consumption for the quality control of seeds of *Psoralea corylifolia* Linn. These superiorities make UPLC an attractive alternative to conventional HPLC technique in herbal medicine fingerprinting analysis. Therefore, the method developed in this study would provide an important reference to establish the quality control method for other related herbal single drugs or preparations.

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References

Anonymous, 1985. Jiangsu New Medicinal College Dictionary of Traditional Chinese Herbs. Shanghai Science and Technology Publishing House, Shanghai, pp. 1178.

Anonymous, 2000. World Health Organization.General guidelines for methodologies on research and evaluation of traditional medicine. Geneva.

Caballero-Ortega, H., Pereda-Miranda, R., Abdullaev Fikrat, I., 2007. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chem*. 100: 1126-31.

Cardeal, Z.L., Souza, P.P., Silva, M.D., Marriott, P.J., 2008. Comprehensive two-dimensional gas chromatography for fingerprint pattern recognition in cachaca production. *Talanta* 74: 793-9.

De Smet PAGM. 2002. Herbal Remedies. *N. Engl. J. Med*. 347: 2046-56.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., Appel, S., Wilkey, S., Van Rompay, M., *et al*., 1998. Trends in alternative medicine use in the United States, 1990-1997. *JAMA* 280: 1569-75.

Feng, Y., Wu, Z.H., Zhou, X.Z., Zhou, Z.M., Fan, W.Y., 2006. Knowledge discovery in traditional Chinese medicine: State of the art and perspectives. *Artif. Intell. Med.* 38: 219-36.

Ferrandiz, M.L., Gil, B., Sanz, M.J., Ubeda, A., Erazo, S., Gonzalez, E., *et al*., 1996. Effect of bakuchiol on leukocyte functions and some inflammatory responses in mice. *J. Pharm. Pharmacol.* 48: 975–980.

Gu, M., Ouyang, F., Su, Z.G., 2004. Comparison of high-speed counter-current chromatography and high-performance liquid chromatography on fingerprinting of Chinese traditional medicine. *J. Chromatogr. A.* 1022: 139-44.

Haraguchi, H., Inoue, J., Tamura, Y. and Mizutani, K., 2002. Antioxidative components of *Psoralea corylifolia* (Leguminosae). *Phytother. Res.* 16: 539–544.

Karsura, H., Tsukiyama, R.I., Suzuki, A. and Kobayashi, M., 2001. In vitro antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrob. Agents. Chemother.* 45: 3009–3013.

Khastgir, H.N., Duttagupta, P.C., Sengupta, P., 1959. Psoralen and Isopsoralen from *Psoralea corylifolia*. *Indian Journal of Applied Chemistry* 22(2): 82- 85.

Kong, W.J., Zhao, Y.L., Xiao, X.H., Jin, C., Li, Z.L., 2009. Quantitative and chemical finger print analysis for quality control of Rhizoma *Coptidischinensis* based on UPLC-PAD combined with chemometrics methods. *Phytomedicine* 16: 950-9.

Liu, M., Li, Y.G., Chou, G.X., Cheng, X.M., Zhang, M., Wang, Z.T., 2007. Extraction and ultra-performance liquid chromatography of hydrophilic and lipophilic bioactive components in a Chinese herb Radix Salviae Miltiorrhizae. *J Chromatogr A* 1157: 51-5.

Mi, S.Q., Hong, X., Huang, T.L., Ye, S.M., Ou, W.P., Feng, M.R., *et al*., 1998. *Tradit. Chin. Drug Res. Clin. Pharmacol.* 9: 147.

Miura, H., Nishida, H. and Iinuma, M., 1996. Effect of crude fractions of *Psoralea corylifolia* seed extract on bone calcification. *Planta. Med.* 62: 150–153.

Sebastian, P., 2006. Ayurvedic Medicine: The Principles of Traditional Practice, Part 2. Elsevier Health Sciences, New York, pp. 135- 136.

Straus, S.E. 2002. Herbal Medicines -What's in the Bottle? *N. Engl. J. Med*. 347: 1997-1998.

Vaidya, A.D.B., 2006. Reverse Pharmacological correlates of Ayurvedic drug actions. *Indian Journal of Pharmacology* 38(5): 311- 315.

Wang, L., Yuan, K., Yu, W.W., 2010. Studies of UPLC fingerprint for the identification of *Magnolia officinalis* cortex processed. *Pharmacogn. Mag*. 6: 83-8.

Wang, Y., Han, T., Zhang, X.G., Zheng, C.J., Rahman, K., Qin, L.P., 2009. LC fingerprint and hierarchical cluster analysis of *Crocus sativus* L. from different locations in China. *Chromatographia* 70: 143-9.

Wu, S.N., Zhang, Z.H., Zhao, J.B., 1998. *Chin. J. Chin. Mater. Med*. 23: 303.

Xu, C.J., Liang, Y.Z., Chau, F.T., 2005. Identification of essential components of *Houttuyniacordata* by gas chromatography/ mass spectrometry and the integrated chemometric approach. *Talanta.* 68: 108-15.

Yadava, R.N. and Verma, Y.A., 2005. A new biologically active flavonol glycoside from *Psoralea corylifolia Linn.*. *J. Asian. Nat. Prod. Res.* 7: 671–675.

Zhang, X.G., Han, T., Zhang, Q.Y., Zhang, H., Huang, B.K., Xu, L.L., *et al*., 2009. Chemical fingerprinting and hierarchical clustering analysis of *Centella asiatica* from different locations in China. *Chromatographia* 69: 51-7.