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QUANTIFICATION OF SANTONIN FROM *ARTEMISIA PALLENS* WALL BY HPTLC

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ABSTRACT

A simple High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the analysis of santonin in different extracts of *Artemisia pallens* Wall. The quantification of analytes has been carried out using a mobile phase hexane: ethyl acetate (3:2) on pre-coated aluminium plates (Silica gel Merck 60 F_{254}) and densitometric determination was carried out. The spots were located at 258 nm. The amount of santonin in the extracts has been estimated by comparing the peak area using the standard. Linearity of the standard was found in the concentration range of 1 to 5 µg/spot. The correlation coefficient value was 0.9835. The proposed HPTLC method was found to be simple, faster and reliable for analysis of santonin.

KEYWORDS

Artemisia pallens Wall, santonin, analytes and HPTLC.

INTRODUCTION

In the past few decades compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. Santonin, a sesquiterpene lactone, is widely distributed in the genus *Artemisia* within the family Asteraceae[1]. It possesses anti-inflammatory, antipyretic and analgesic properties[2]. *Artemisia pallens* Wall. a potent medicinal plant used in Ayurvedic system of medicine, belongs to this family. It is used in Indian folk medicine for the treatment of diabetes mellitus. Oral administration of the methanol

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extract of the aerial parts of *Artemisia pallens* Wall. led to significant blood glucose lowering effect in glucose-fed hyperglycemic and alloxan-induced diabetic rats[3]. Plant is also screened for anthelmintic activity[4]. Earlier, santonin has been estimated using spectrophotometric methods in pharmaceutical preparations and crude drugs[5]. Therefore, an attempt has been made to develop HPTLC method, which is simple and reliable for analysis of santonin in crude extracts.

MATERIALS AND METHODS

Reagents and Chemicals

All solvents used were of AR-grade and were obtained from Merck, Mumbai (India). Standard santonin was obtained from Sigma chemicals.

Plant Material

The plant material was collected from Jejuri, Maharashtra state of India. It was authenticated at Botanical Survey of India, Pune, India. Its voucher number is BSI/WC/Tech/2008/1059.

Chromatographic Conditions

Stationary phase:	Pre-coated silica gel plates Merck 60 F_{254} (10 × 10 cm, 0.2 mm thickness).
Mobile phase:	Hexane: Ethyl Acetate (3:2)
Lamp:	Deuterium
Wavelength:	258 nm
Application mode:	CAMAG Automatic TLC Sampler III
Development mode:	CAMAG Twin Trough Chamber
Scanner:	CAMAG TLC Scanner 3 and CATS software

Experimental conditions: Temperature 25± 2 ^{0}C, relative humidity 40%

Preparation of Standard

A stock solution of santonin (1 mg / ml) was prepared by dissolving 10 mg of accurately weighed sample in chloroform and making up the volume up to 10 ml. The stock solution was further diluted with chloroform for working standard solution of 0.2 mg/ml.

Calibration curve for Standard

The standard solution of santonin (1 µg to 5 µg per respective spot) was applied in triplicate on TLC plate. Quantitative evaluation of the plate was performed in absorption / reflection mode at 258 nm using a slit width of 6.0 × 0.30 mm, scanning speed 20 mm/s with a computerized CAMAG, TLC scanner-3 integrated with CATS – III software. The plate was developed and scanned as per the chromatographic conditions and the peak areas were recorded.

Preparation of Extracts

Shade dried aerial parts of the plant were coarsely powdered (100 g) and extracted with acetone (**A,** 7.08 %) and methanol (**B,** 11.6 %) separately by stirring for 18 hours at room temperature. Solvent was removed under reduced pressure to obtain crude extracts. The crude extract (**A, 7 g)** was broad fractioned on silica gel (60-120, 10 g) using n-hexane (**C**) and Hexane: Acetone (**D**, 90:10). The solvent was removed under reduced pressure to get their extracts. The yield of extracts C and D was found to be 29.8 and 15.6 % respectively

HPTLC Quantification in Test Samples

10 mg of various extracts as prepared above were weighed accurately and dissolved in chloroform to make up the volume of 1 ml. This was further diluted with chloroform to get solutions of 1 mg/ml. 20 µL per spot of these solutions were applied on to a pre-

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coated silica gel plates (in triplicates). The plates were developed by ascending mode to a distance of 10 cm and scanned as per the conditions mentioned above. The santonin content of various extracts was determined by comparing the area of the chromatogram with the calibration curve of working standard. The R_f value of the standard santonin (0.3) was compared with the R_f value of the extracts. The average content of santonin in different extracts was expressed as mg per g of extract.

RESULTS AND DISCUSSION

Different compositions of the mobile phase were tested and the desired resolution was achieved by hexane: ethyl acetate (3:2). Spectral characteristics of the peak of standard and that of the extracts were compared for identification of santonin. Calibration curve of santonin was obtained by plotting peak areas verses concentration applied. It was found to be linear in the range of 1 µg to 5 µg per spot. Equation of the calibration curve is y = 5083x + 4343.8. The correlation coefficient was found to be 0.9835 and thus exhibits good linearity between concentration and area. The amount of santonin in the extract **A** was found to be 31.29 mg/g while in **B** it was 40.71 mg/g of extract. Santonin content of extracts **C** and **D** were found to be 1.940 and 20.87 mg/g of acetone extract respectively. Santonin was isolated by repeated column chromatography of acetone extract (**A**). The results obtained by HPTLC are in accordance with the amount obtained by the column chromatography.

CONCLUSION

In conclusion, the proposed HPTLC method was found to be simple, rapid, accurate and reproducible for the estimation of santonin in *Artemisia pallens* Wall.

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