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Quantitative Determination of Umbelliferone in the Root Extract of *Cichorium intybus* by HPTLC

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A simple sensitive HPTLC method developed for determination of umbelliferone in the methanolic root extract of *Cichorium intybus* by HPTLC. The stationary phase was precoated silica gel 60 F_{254} and the mobile phase used was toluene:ethyl acetate:glacial acetic acid (8:13:1 v/v/v). The plate was scanned and quantified at 356 nm for umbelliferone. The method was validated in terms of linearity, accuracy and specificity. The proposed HPTLC method provides a faster and cost effective control for routine analysis of umbelliferone in the extracts containing *Cichorium intybus* coumarins.

Key Words: *Cichorium intybus*, HPTLC, Umbelliferone, Methanolic root extract, Linearity, Accuracy, Specificity.

INTRODUCTION

Cichorium intybus Linn. (Asteraceae) is commonly known as chicory, is a perennial herb distributed in the temperate parts of the world and found wild in Punjab and Andhra Pradesh regions. The major producing countries of chicory are the United Kingdom, Belgium, Europe, France, Netherlands, Germany, Switzerland and South Africa[1]. It used in Indian system of medicine as a cardiotonic, antiinflammatory, digestive, stomachic, liver tonic and diuretic[2]. The main reported phytoconstituents of chicory roots are phenylacetic acid esters, cichoriosides[3], sonchuside A[4], ixerisoside, magnolialide[5] and endesmanolides[6,7]. The herb is a major component of hepato-herbal formulations such as: Liv-52, Acilvan, Hepex, Livokin and Vimliv. Presently, it is a crop of choice for future genetic manipulation and the valuable phytochemicals may lead to metabolic engineering of secondary pathways. It is also used extensively in coffee blends as a vegetable and in value-added healthcare products[3]. In the present study, a simple HPTLC method was developed and validated for separation and quantification of umbelliferone in methanolic root extract of *Cichorium intybus* by HPTLC.

EXPERIMENTAL

The roots of *Cichorium intybus* were collected from the plants growing in the herbal garden of Jamia Hamdard, New Delhi and identified by Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University), where a voucher specimen (No. PRL/JH/05/28) was deposited and reference standard of umbelliferone were procured from Sigma Aldrich, Delhi, India.

Preparation of plant extract solution: The dried coarse powder of roots of *C. intybus* was extracted to exhaustion with methanol (250 mL) using a soxhlet apparatus. The methanol extract thus obtained was dried under reduce pressure at room temperature (40 ºC). About 100 mg of extract was dissolved in 10 mL of methanol, sonicated and filtered through membrane filter.

Preparation of standard solution: Standard stock solution (1000 µg/10 mL) of umbelliferone was prepared by weighing 10 mg of 99.00 % pure umbelliferone, transferring to 10 mL volumetric flask, dissolving in minimum quantity of methanol and sonicated in ultrasonic water bath to dissolve and volume was made up to 10 mL with the same solvent. Then 1 mL of standard stock solution was taken from the volumetric flask and diluted to 10 mL with methanol. The resulting solutions were used as reference solution for umbelliferone.

Chromatographic conditions: The following chromatographic conditions were used to quantify the umbelliferone: stationary phase: Silica gel 60 F_{254} (E. Merck) precoated TLC plates. Mobile phase: toluene:ethyl acetate:glacial acetic acid (8:13:1 v/v/v). Sample volume: 2 µL. Temperature: ambient room temperatue. Migration distance: 8 cm. Detection wavelength: 356 nm.

Procedure: The precoated TLC plates were pre-washed with methanol. Standard and sample solutions were applied to the plates as sharp bands by means of Camag Linomat V sample applicator. The spots were dried in a current of air. The mobile phase (20 mL) was poured into twin trough glass chamber whole assembly was left to equilibrate for 0.5 h and the plate was placed in the chamber. The plate was then

developed until the solvent front had travelled at a distance of 80 mm above the base of the plate. The plate was then removed from the chamber and dried in a current of air. Detection and quantification were performed with Camag TLC Scanner 3 at a wavelength of 356 nm.

Assay: Standard and sample solutions were spotted on an HPTLC plate (E. Merck). The percentage of umbelliferone presents in roots of *C. intybus* extract was calculated by comparison of the areas measured for the sample and standard solutions; (Figs. 1-4) represent the chromatograms of standard umbelliferone and sample.

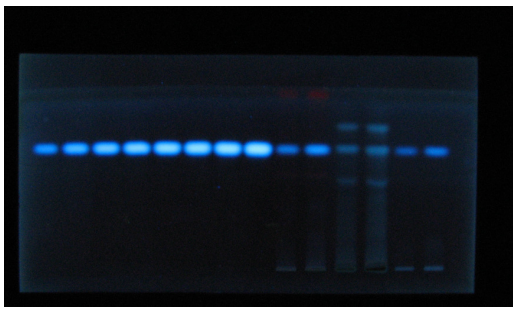

Fig. 1. HPTLC plate of umbelliferone (standard) of different concentration and methanolic root extract of *C. intybus*

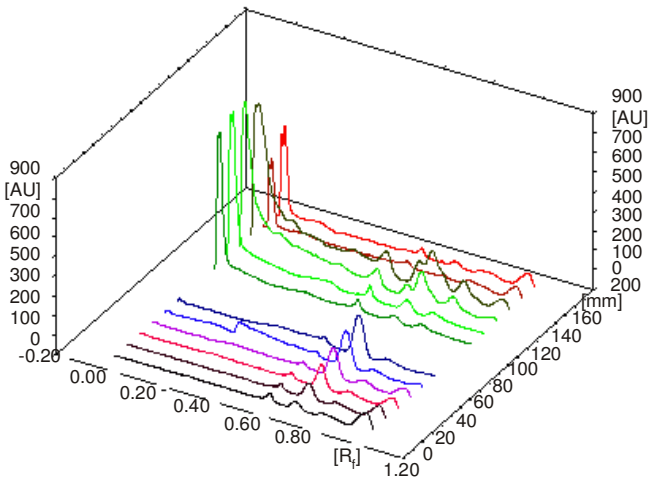

Fig. 2. HPTLC tracing of umbelliferone under UV at 356 nm

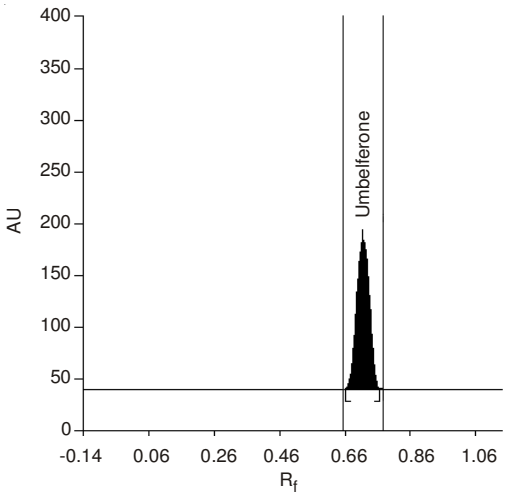

Fig. 3. Chromatogram of standard umbelliferone

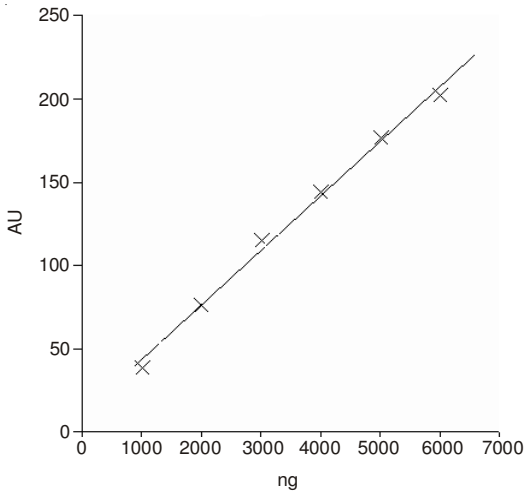

Fig. 4. Calibration curve of umbelliferone

Linearity: Linearity was performed by applying standard solution at different concentrations ranging from 8-40 µg/spot on 20 cm × 10 cm HPTLC plates, precoated with silica gel 60 F_{254} (E. Merck) in the form of sharp 6 mm bands; the distance between two adjacent bands was 10 mm. The plates were developed in a solvent system of toluene:ethyl acetate:glacial acetic acid (8:13:1 v/v/v), up to a distance of 75 mm, at room temperature. The plates were dried in air. The detector response for umbelliferone was measured for each band at wavelength of 356 nm, using Camag TLC Scanner 3 and winCAT software. The peak areas of umbelliferone were recorded for each concentration. The linearity curve of umbelliferone was obtained by plotting a graph of peak area of umbelliferone *vs.* applied concentration of umbelliferone (µg).

Method validation: The method was validated[8] for precision, repeatability and accuracy. The precision was checked by repeated scanning of the same spot of umbelliferone (20.2 µg) three times each and was expressed as relative standard deviation (RSD %). The repeatability of the method was confirmed by analyzing 16 µg and 20.2 µg/spot of standard umbelliferone solution (n = 3) and was expressed as RSD %. The precision of the method was studied by analyzing aliquots of standard solution of umbelliferone (16 µg and 20.2 µg/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as RSD %.

To study the accuracy, the recovery experiment was performed by the method of standard addition. The recovery of the added amount of standard was analyzed at three different levels, each being analyzed in a manner similar to that of described for assay. Each level of additions was repeated three times on three different days and recovery of the added amount of standard was calculated. Limit of detection and limit of quantification was also calculated by the proposed method.

RESULTS AND DISCUSSION

The method described utilizes silica gel 60 F_{254} HPTLC plates as stationary phase and toluene:ethyl acetate:glacial acetic acid (8:13:1 v/v/v) as mobile phase which gives good separation of umbelliferone (R_f = 0.71). The results of method validation parameters are shown in (Table-1). The identity of

TABLE-1
VALIDATION METHOD PARAMETERS FOR QUANTITATIVE DETERMINATION OF UMBELLIFERONE IN THE ROOT EXTRACT OF *C. intybus* BY HPTLC

Parameters	Results
Precision (RSD)	< 2 %
Linearity	8 – 40 µg/spot
Limit of detection	2 µg/spot
Limit of quantification	9.7 µg/spot
Accuracy	97.50-100.05 %
Assay	40.2 % w/w

the band of umbelliferone in the sample extracts were confirmed by overlaying the UV absorption spectra of sample with that of reference standard which showed λ_{max} at 356 nm (Fig. 2). The calibration curve was linear in the range of 8 µg to 40 µg/spot and the correlation coefficient was determined. The correlation coefficient was found to be 0.99730. The limit of quantification was found to be 9.9 µg and the limit of detection was 2.5 µg/spot. The method was validating in terms of precision and reproducible expressed as RSD % which were found to be less than 2 %. The recovery values obtained were 97.50-100.05 %, showing accuracy of method. The average percentage recovery was found to be 99.35 %.

Conclusion

The developed HPTLC method was simple accurate, precise, economic and can be utalised for routine analysis and quantitative determination of umbelliferone from *Cichorium intybus*.

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