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Extraction and Quantification of Some Plants Extracts through HPTLC

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Abstract

A study of quantitative analysis of some extracts is presented. The HPTLC analyses were performed in a normal chromatographic chamber on silica gel plates, with an appropriate mobile phase. Photodensitometric evaluation of the chromatographic plates was performed by UV-visible absorbance. Quantitative analyses of extracts were performed by use of calibration curves. The quantity of compounds extracted depends on the composition of the extraction solvent and on the extraction technique. The best results were obtained by heating under reflux with systems that contained hydroalcoholic solvent.

Introduction

Quality control of medicinal plants is highly essential to ensure authenticity, stability, and consistency. Safety and efficacy studies along with the standardization of bioactive extract on the basis of active principle or major compound(s) as a quality assurance parameter shall open up unlimited possibilities for herbal medicine in pharmacotherapeutics [1–3]. Standardization can be carried out by obtaining a chemical fingerprint/profile in terms of one or more marker compounds (chemical or biomarker). Use of chromatography for standardization of plant products was introduced by the WHO and is accepted as a strategy for identification and evaluation of the quality of plant medicines[4–6]. HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity and speed, the need for minimum sample clean-up, reproducibility, accuracy, and reliability. Here, we have developed HPTLC method for quantification of plant extract.

Materials and method

Extraction of crude drugs [7,8]

Extracts of all the individual drugs were obtained by maceration technique. Hydroalcohol was used as the solvent, since it possess the optimum solubility characteristics required for extraction. A known weight of the powdered drug was taken for extraction with water and ethyl alcohol (50:50) ratio in a flat bottomed maceration flask and subjected to maceration. The maceration flask was kept at room temperature protected from sunlight and shaken several times daily. At the end of the 7th day, the extract was filtered, concentrated and stored in a desiccator for further studies. The percentage yield of the extracts obtained was calculated and reported.

Quantification of the extract by HPTLC

The extracts were quantified using standard drugs in the following stages.

a. Preparation of Samples

100mg of the selected extracts were weighed accurately and dissolved in 10ml of methanol in a volumetric flask. The flask was shaken for 30 min, the solution filtered through Whatman filter paper and the final volume was made up to 10 ml with methanol.

b. Preparation of Standard solutions

Accurately weighed 100 mg of extracts were dissolved in 10 ml of methanol. From this 1.0 ml was diluted to 20ml methanol to produce 0.5 mg/ml. The standard drugs solution was also prepared at a concentration of 0.5 mg/ml with methanol in the similar manner and used for the analysis.

c. Application of standard and sample solutions

For the application of the solutions, pre-coated plates of 4 x 10 cm size (Silica gel 60 F 254, E.MERC) were used. The standards and the sample solutions were applied on different tracks of the plate. A thin band of 6mm width was applied using Linomat IV (Automatic TLC applicator, CAMAG, Switzerland).

d. Chromatogram development and Densitometric scanning

With the help of the suitable solvent system selected for the quantification of the selected extracts the plates were developed in the twin trough chamber. After the development of the chromatogram, plates were taken out, dried using hair drier and observed under UV light. The developed plates were then scanned using densitometer followed by quantification of the extracts with reference to standard solution. The suitable solvent system selected for the quantification of the selected active compounds in their extracts and the wavelengths.

Table 1: Solvent system for the herbal extracts for HPTLC

Sl.No.	Extract	Active Compound	Solvent System	Wavelength
1.	*Andrographis echinoides*	Andrographolide	Acetone: Chloroform: Benzene 7:2:1(V/V/V)	273 nm
2.	*Acorus Calamus*	α-β-Asarone	Toluene: Ethyl acetate 93:7(V/V)	254 nm
3.	*Phyllanthus acidus*	Hypophyllanthin	Hexane: Acetone: Ethyl acetate 74:12:8 (V/V/V)	235 nm
4.	*Rauvolfia tetraphylla*	Reserpine	Toluene: Ethyl acetate: De ethylamine 7:2:1(V/V/V)	254 nm
5.	*Strychnos cinnamomifolia*	Strychnine	Toluene: Ethyl acetate: De ethylamine 7:2:1(V/V/V)	254 nm

Results:

Quantification of Andrographolide, α-Asarone, β-Asarone, Hypophyllanthin, Reserpine, Strychnine

Quantification Analysis of compound separated by HPTLC can be carried by estimating the area of Band detection and results showed that 100mg of Andrographis echinoides, Acorus Calamus, Phyllanthus acidus, Rauvolfia tetraphylla and Strychnos cinnamomifolia, extract contains 4.563mg, 5.1mg, 6.17 mg, 1.16mg, 0.345mg, & 10.0 mg of pure Andrographolide, α- Asarone, β-Asarone, Hypophyllanthin, Reserpine and Strychnine respectively.

Table 2:

Sample	Andrographolide(%w/w)	α-Asarone (%w/w)	β-Asarone (%w/w)	Hypophyllanthin (%w/w)	Reserpine (%w/w)	Strychnine(%w/w)
Extract (100mg)	4.563 mg	5.1 mg	6.17 mg	1.16 mg	0.345 mg	10.0 mg

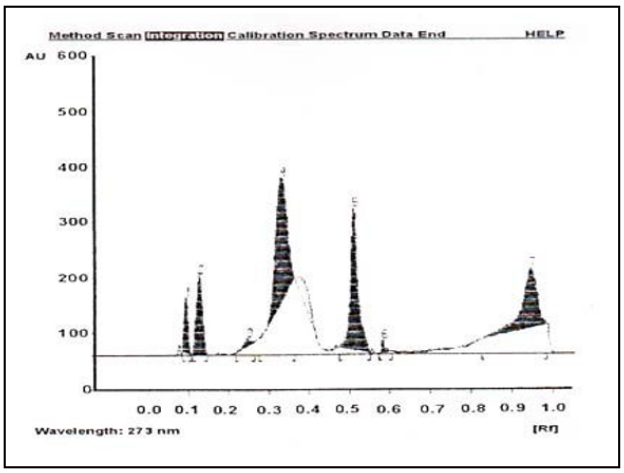

Figure 1: Typical densitogram of *Andrographis echinoides*

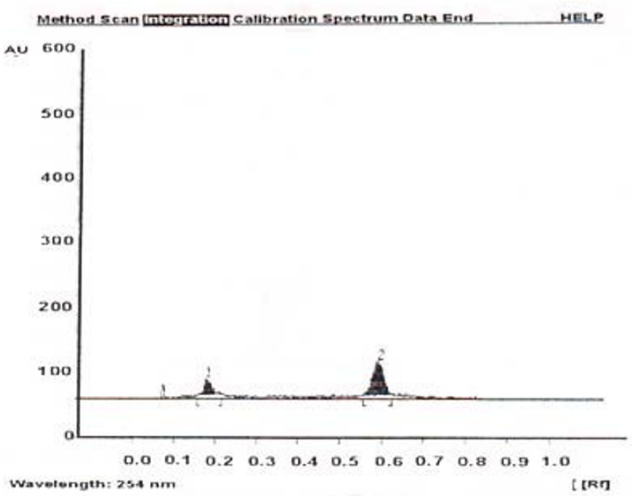

Figure 2: Typical densitogram of *Acorus Calamus*

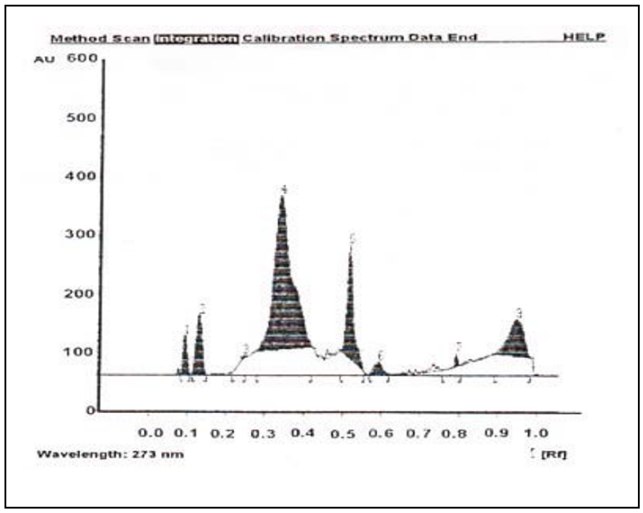

Figure 3: Typical densitogram of *Phyllanthus acidus*

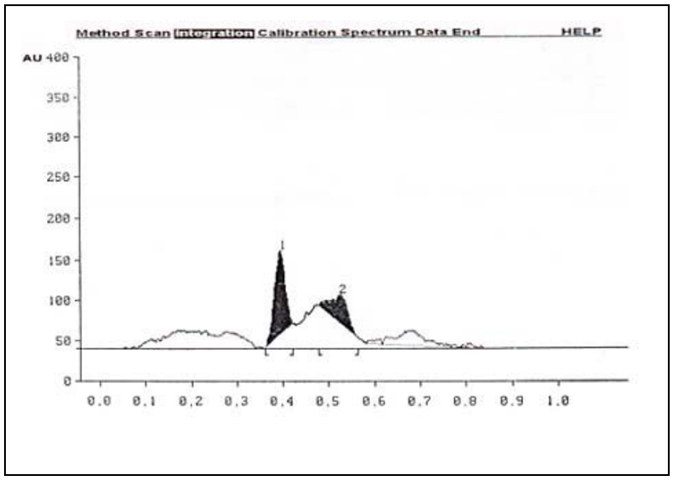

Figure 4: Typical densitogram of *Rauvolfia tetraphylla*

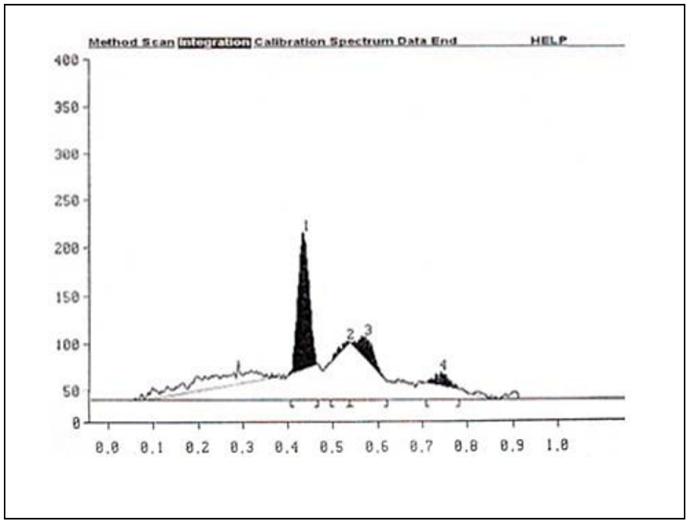

Figure 5: Typical densitogram of *Strychnos cinnamomifolia*

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