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Analysis of powdered calendula officinalis linn

Yashoda Yadav[1]*, Dr. Surendar Karwasara[2]

[1] Research Scholar, Faculty of Basic & Applied Sciences, Maharishi Arvind University, Jaipur

(Rajasthan)-302041

[2] Supervisor, Faculty of Basic & Applied Sciences, Maharishi Arvind University, Jaipur

(Rajasthan)-302041

Abstract - India is well recognized as an ancient culture with a rich tradition of herbal medicine. The vast majority of the raw materials used in the pharmaceutical and perfume industries come from India's forests, where a plethora of fragrant and therapeutic plants are gathered. Calendula officinalis is a member of the daisy family (Asteraceae) that is native to southern Europe or the Eastern Mediterranean. It may be grown as an annual or short-lived perennial. It's been a garden staple for decades, and now it's popping up all over the place in milder temperatures by itself. In this research, a high-performance thin-layer chromatographic technique was modified to be sensitive, straightforward, and accurate in its analysis of powdered Calendula officinalis Linn.

Keywords - Calendula officinalis Linn., Herbal plants, high-performance thin-layer chromatographic technique.

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INTRODUCTION

Traditional medicines that are being used today sometimes consist fully or mostly of plant-based substances. For this reason, they are intrinsic to the product's biological efficacy. Chromatography is a relatively new scientific technique that has shown to be helpful in the standardization of plants and herbal blends. Standardizing a wide range of plant species by testing for marker compounds and fingerprint analysis assists in determining their correct botanical identification.[1]

Analytical chromatography is used to precisely measure the concentrations of specific analytes, whereas traditional chromatography is used on larger quantities of material. For this reason, we cannot say that one precludes the other. Separating constituent parts of a chemical is a typical use of High Performance Thin Layer Chromatography (HPTLC). High-performance thin-layer chromatography is a development of the original thin-layer technique (TLC). Automating its many procedures, increasing its resolution, and allowing for more exact quantitative measurements are all possible enhancements to the core operation of thin layer chromatography. [2-3]

Thin-layer chromatography is superior to other chromatographic techniques because it generates chromatographic fingerprints, which may be used as a reliable identifier. HPTLC analysis is often used to evaluate the components of herbal and animal extracts, fermentation mixtures, medicinal and excipient substances, and formulated herbal products. [4]

establishing standards for the quality of plant materials used in medical applications. These standard compounds are subsequently used as indicators of the sample's genuine botanical origin. Thus, modern HPTLC-based studies are developing ways for standardizing medicinal plants by making use of a number of different marker molecules. [5-6]

Using its full potential, thin-layer chromatography might form the foundation for a very sophisticated instrumental method called as high-performance thin-layer chromatography. It is a helpful analytical tool for acquiring chromatographic data on complicated mixtures of medicines, natural products, clinical samples, foods, etc. because of its automation, scanning, complete optimization, selective detection principle, minimum sample preparation, hyphenation, etc. [7]

The value and importance of HPTLC have been widely acknowledged. Current efforts are mostly focused on standardizing testing protocols. Although there have been advancements in chromatography techniques, HPTLC is still the gold standard. HPTLC is a chromatographic technique used for determining the concentration of an active molecule, identifying and quantifying impurities, and identifying components. Since it is one of the most flexible, reliable, and cost-effective separation methods, HPTLC is ideal for the research of botanicals and

herbal medicines. It's essential for the reliable identification of complex fingerprints on plant extracts and medications, and its usage in tandem with established techniques guarantees repeatable results. [8]

The pharmaceutical sector, clinical chemistry, forensic chemistry, chemistry, cosmetics, drug and food analysis, or environmental analysis all make extensive use of high-performance liquid chromatography. One of its numerous benefits is that, unlike any other chromatographic technique, it provides a pictorial depiction of the data. There is also the potential for a large number of detections, rapid results generation, a large sample capacity, and low costs proven HPTLC to be a reliable method for analyzing salbutamol concentrations in serum samples during clinical trials. [9-10]

In addition to its use in evaluating and studying a wide range of lipids, HPTLC has also been used to successfully separate 20 different lipid subclasses. The field of clinical medicine is well-represented in the many periodicals available, many of which feature reports of research. HPLC is widely used for the analysis of pharmaceuticals in serum and other tissues.[11-12]

ADVANTAGES OF HPTLC

1. One may utilize high performance liquid chromatography (HPTLC) for several types of analysis, including fingerprinting, biological research, and the measurement of herbal drugs.

2. When compared to traditional methods, HPTLC is both quick and cheap.

3. HPTLC is often used for the examination of vitamins, water-soluble food dyes, and pesticides in various foods.

4. The great sample throughput & cheap cost per analysis offered by high-performance liquid chromatography (HPTLC) are only two of its many benefits.

5. Improved resolution across a given range,

6. Improved efficiency in development time and decreased solvent use.

MATERIAL AND METHODS

Water suitable for HPLC analysis, ethyl acetate of analytical grade (99.6% purity), and methanol (99.9% purity) were all purchased from E.Merck in Mumbai, India. Formic acid and glacial acetic acid of analytical quality were purchased from Qualigens Fine Chemicals in Mumbai, India.

A. Preparation of Stock Solutions

- **Preparation of stock (A) solution of Rutin (1000 µg/mL)**

One thousand micrograms of Rutin per milliliter of methanol was made into a stock solution. A 10.0 mg sample of standardRutin was weighed precisely and then transferred to a 10.0 mL standard volumetric flask. The flask's contents were dissolved in 5.0 mL of methanol, sonicated, and then diluted to the appropriate volume with more methanol.

- **Preparation of stock (B) solution of Chlorogenic acid (1000 µg/ mL)**

Methanolic Chlorogenic acid stock solution (1000 g/mL) was made. Accurately weighing 10.0 mg of standardChlorogenic acid, 10.0 mL of standard volumetric flask was subsequently filled with the substance. The flask's contents were dissolved in 5.0 mL of methanol, sonicated, and then diluted to the appropriate volume with more methanol.

- **Preparation of stock (C) solution of Hyperoside (1000 µg/ mL)**

There was made a methanolic stock solution of Hyperoside (1000 g/mL). An precisely weighed sample of standard Hyperoside (10.0 mg) was poured into a 10.0 mLstandard volumetric flask. The flask's contents were dissolved in 5.0 mL of methanol, then sonicated, and finally diluted to the appropriate level with more methanol.

The aforementioned stocks of rutin, chlorogenic acid, and hyperoside were used to create a calibration curve with solutions ranging from 0.1- to 0.05-.

- **Sample Preparation**

Dried flowers from Calendula officinalis Linn., about one thousand milligrams in total, were weighed and placed in a round-bottom flask. Following the addition of 10 mL of methanol, the flask was placed in a boiling water bath and allowed to reflux for 30 minutes. Once the extract was filtered, we used Whatman filter paper no. 1. Finally, the volume was brought to 5.0cm3 using methanol in a volumetric flask after the contents were evaporated to dryness.

B. Method Validation

After extensive testing, it was determined that the newly created technique was suitable for its intended use via a thorough validation procedure. Prior to demonstrating the validity of a technique, validation is a methodical process of identifying, measuring, assessing, recording, and re-evaluating all the important processes involved. One area where focus is directed is on the pharmaceutical industry: the validation of chromatographic procedures. When a technique is validated, it is shown to be accurate,

precise, selective, sensitive, reproducible, and stable, as required by the FDA's recommendations.

These are the factors that were taken into account during the HPTLC method validation for the determination of Rutin, Chlorogenic acid, and Hyperoside concentrations in C.Officinalis Linn. flower extracts. Were,

1. Linearity and range

a) LDR

b) LWR

2. Limit of Detection(LOD) and Limit of Quantification (LOQ)

3. Precision

a) Repeatability

b) Intermediate Precision

RESULTS

Linearity and range

(a) Linear Dynamic Range (LDR) For Rutin, chlorogenic acid and hyperoside.

How closely a calibration plot of detector response vs concentration follows a straight line is how linear a technique is defined. To find out what concentrations of Rutin, chlorogenic acid, and hyperoside are optimal for use, we conducted the following experiment.

Rutin, chlorogenic acid, and hyperoside's linear dynamic ranges were calculated using series of standard solutions containing 0.1 to 1.00 g per band, 0.2-1.20 g per band, and 0.05 to 0.50 g per band, respectively. The plate was developed and scanned after the bands were applied from the bottom at a distance of 8.0mm.

Densitograms were generated after each Rutin, chlorogenic acid, and hyperoside application, and the peak regions of these compounds were measured and recorded.

Table 1. Rutin, chlorogenic acid, and hyperoside linear dynamic range.

Obs.No	Rutin		Chlorogenic Acid		Hyperoside	
	Concentration µg per band	Peak area	Concentration µg per band	Peakarea	Concentration µg per band	Peakzarea
1	0.10	365	0.05	285	0.2	473
2	0.20	648	0.1	700	0.3	978
3	0.30	932	0.15	1119	0.4	1487
4	0.40	1215	0.20	1531	0.5	1999
5	0.50	1500	0.25	1936	0.6	2522
6	0.60	1789	0.30	2358	0.7	3034
7	0.70	2084	0.35	2768	0.8	3564
8	0.80	2370	0.40	3183	0.9	4066
9	0.90	2656	0.45	3608	1.0	4586
10	1.0	2946	0.50	4031	1.2	5518

The concentrations of rutin, chlorogenic acid, and hyperoside used in the experiment were plotted against the peak areas of the three compounds (Y-axis) in a scatter plot (X-axis).

When plotted, concentrations of 0.10 g to 1.0 g per band demonstrate a linear response from Rutin**.** In the concentration range of 0.05 g to 0.50 g per band, the hyperoside response is linear, as shown in the graph**.** Rutin's response is linear between 0.2 and 1.2 micrograms per band, as shown in the graph**.**

(b) Linear Working Range (LWR):

Triplicate applications of standard solutions of rutin (0.10 g to 1.0 g per band), chlorogenic acid (0.05 g to 0.5 g per band), and hyperoside (0.20 g to 1.20 g per band), developed and scanned under the optimum conditions described above, yielded identical results. Peak regions of rutin, chlorogenic acid, and hyperoside were detected in the densitograms.

Linear working range for Rutin,Chlorogenic acid and Hyperoside

The calibration curve was constructed by relating the concentration of rutin, chlorogenic acid, and hyperoside to the average peak area of the resulting graph. For each applied concentration, we determined its mean peak area, standard deviation (S.D.), and percentage relative standard deviation (%R.S.D.).

Table 2: Findings from Rutin's Linear Working Range

Obs.No.	Concentration of Rutin (µg/mL)	Area 1	Area 2	Area 3	Mean	S.D.	% RSD
1	0.10	360	368	364	364	4.00	1.09
2	0.20	647	648	648	647.6	0.57	0.08
3	0.30	933	934	932	933	1.00	0.10
4	0.40	1216	1213	1214	1214.3	1.52	0.12
5	0.50	1511	1516	1513	1513.3	2.51	0.16
6	0.60	1787	1786	1790	1787.6	2.08	0.11

Table 3: Chlorogenic acid's linear working range results

Obs.No.	Concentration of chlorogenic acid (µg/mL)	Area 1	Area 2	Area 3	Mean	S.D.	% RSD
1	**0.05**	282	283	282	282.33	0.57	0.20
2	**0.1**	710	702	714	708.66	6.11	0.86
3	**0.15**	1125	1115	1116	1118.67	5.50	0.49
4	**0.20**	1502	1534	1524	1520	16.37	1.07
5	**0.25**	1956	1966	1934	1952	16.37	0.83
6	**0.30**	2360	2342	2380	2360.67	19.00	0.80

Table 4: Data on Hyperoside's Theoretical Linear Work Range

Obs.No.	Concentration of Hyperoside (µg/mL)	Area 1	Area 2	Area 3	Mean	S.D.	% RSD
1	**0.2**	470	479	482	477	6.24	1.30
2	**0.3**	980	968	979	975.66	6.65	0.68
3	**0.4**	1464	1477	1492	1477.67	14.01	0.94
4	**0.5**	2015	1997	2002	2004.67	9.29	0.46
5	**0.6**	2556	2512	2547	2538.33	23.24	0.91
6	**0.7**	3014	3024	3067	3035	28.16	0.92

Limit of Detection (LOD) and Limit of Quantification (LOQ):

As the name implies, the LOD is the lowest concentration of an analyte that can be identified by a given technique under normal working circumstances. The LOQ is the lowest concentration of an analyte in a sample that can be determined with sufficient precision and accuracy, given the operating parameters of the technique.

The signal-to-noise ratios of 3:1 and 10:1 were used to get the LOD and LOQ values, respectively. The significance of Rutin's limits of detection (LOD) and quantification (LOQ). The concentrations of chlorogenic acid, hyperoside, and rutin per band were 0.03 g, 0.03 g, and 0.01 g, respectively.

Table 5: LOD and LOQ for Rutin ,Chlorogenic acid and Hyperoside

Standard	LOD µg per band	LOQ µg per band
Rutin	0.03	0.1
Chlorogenic acid	0.05	0.2
Hyperoside	0.01	0.05

Precision:

a. Intermediate Precision:

Methanolic floral extract was performed in six duplicates with intermediate accuracy. Officinalis, and it was tested over the course of three consecutive days (interday).

Six separate daily weights were taken of a powdered form of dried flowers averaging about 1000mg. After the methanolic extract was made, it was analyzed. A test solution was made from the extracted sample solution. On the same day, we made a note of the peak area values for Rutin, Chlorogenic Acid, and Hyperoside. We determined the average peak areas, standard deviations, and percentage relative standard deviations (RSDs) for Rutin, Chlorogenic acid, and Hyperoside. You may see the intermediate accuracy findings from three consecutive days.

Table 6: Intermediate precision of Rutin ,Chlorogenic acid and Hyperosidefrom flowers of C.Officinalis Linn.

(DAY 1)

Obs.No	Weight of sample powder (mg)	Peak area ofRutin from sample powder	Peak area of Chlorogenicacid from sample powder	Peak area of Hyperoside from samplepowder
1	1006	1095	3652	750
2	1005	1098	3669	745
3	1001	1102	3674	765
4	1004	1091	3677	752
5	1009	1085	3659	741
6	1002	1078	3667	745
Mean	**1004.5**	**1091.5**	**3666.33**	**749.66**
S.D.	**2.88**	**8.82**	**9.37**	**8.47**
(% R.S.D.)	**0.28**	**0.80**	**0.25**	**1.13**

Table 7: C.Officinalis Linn. flower extracts with intermediate levels of Rutin, chlorogenic acid, and hyperoside.

(DAY 2)

Obs.No	Weight of sample powder (mg)	Peak area ofRutin from sample powder	Peak area of Chlorogenicacid from sample powder	Peak area of Hyperoside from samplepowder
1	1001	1099	3674	741
2	1002	1085	3689	736
3	1004	1098	3669	745
4	1008	1106	3664	730
5	1009	1109	3678	726
6	1006	1087	3681	731
Mean	1005	1097.33	3675.83	734.83
S.D.	3.22	9.72	8.88	7.19
(% R.S.D.)	0.32	0.88	0.24	0.97

The relative standard deviation (RSD) values for the peaks of Rutin, Chlorogenic acid, and Hyperoside in C.Officinalis Linn. flowers are shown below as percentages. Achieving statistical significance for six replicates across three days of testing.

The recovery values obtained for rutin ,chlorogenic acid and hyperoside were in theacceptable range of 85% - 115%. This indicates excellent reliability, accuracy and reproducibility of the method.

Table 8: Summary of method validation parameters

Parameter	Rutin	Chlorogenicacid	Hyperoside
Linearity(µg/ml)	0.1 1.00	0.2 1.20	0.05-0.5
Correlation coefficient	0.9999	0.9999	0.9998
LOD (µg/mL)	0.03	0.05	0.01
LOQ (µg/mL)	0.1	0.2	0.05
Intermediate precision (% R.S.D n=6) flowers powder of C.Officinalis Linn.	0.7966	0.2166	0.9733
Repeatability (% R.S.D n=6) flowers powder of C.Officinalis Linn.	0.80	0.25	1.13

In the current study, a normal phase HPTLC technique was established for the determination of rutin, chlorogenic acid, and hyperoside in the powdered flowers of C.Officinalis Linn.

To date, there is no record of a study that measured rutin, chlorogenic acid, and hyperoside all at once. In the current study, we employ normal mode separation to determine the exact amounts of rutin, chlorogenic acid, and hyperoside in a powder made from the flowers of the C.Officinalis Linn plant.

Solvents with varying degrees of polarity, including polar, mid-polar, and non-polar, were tested. Glacial acetic acid, formic acid, ethyl acetate, and water The dilution conditions of 10:1.1:1.1:2.3 (v/v/v/v) were suitable for the separation of Rutin (Rf = 0.61), Chlorogenic Acid (Rf =0.71), and Hyperoside (Rf = 0.71), which can be separated from the other components of the C.Officinalis Linn. flower powder ethanolic extract by using a volumetric ratio of toluene, ethyl acetate, and formic acid.

CONCLUSION

High performance thin layer chromatography was validated for the simultaneous qualitative and quantitative analysis of rutin, chlorogenic acid, and hyperosidemarkers in C.Officinalis Linn flower powder. Flower powder from the C.Officinalis Linn. Plant can be easily analyzed using the proposed method because it is quick, easy, and accurate.

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Corresponding Author

Yashoda Yadav*

Research Scholar, Faculty of Basic & Applied Sciences, Maharishi Arvind University, Jaipur (Rajasthan)-302041