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Original Research Article

HPTLC and U.V. Spectrophotometer as Tools for the Determination of Curcumin and Piperine in Zandu Haldi drops: A standardization approach

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Abstract:

Zandu Haldi Drops are a one-of-a-kind recipe intended to promote immunity and overall health. It possesses the goodness of saffron, cinnamon, cardamom, black pepper, and Haldi. The present work was designed to estimate Curcumin (Turmeric) by High-Performance Thin Layer Chromatography (HPTLC) as well as Piperine (Pepper) through UV Spectrophotometry, with reference to the marker compounds. A standard curve was prepared separately for Curcumin and Piperine using methanol as solvent. HPTLC plate was scanned in scanner at 421 nm. Sample peak area values were compared with that of standard's area to determine the extent of drug present. The standard plot for Piperine was prepared by scanning the known concentrations at 342 nm and the unknown sample concentration was determined from this curve. The concentration of Curcumin was found to be 5.6 µg/ml and the Piperine concentration in the formulation was 2.5%. From the obtained results, it can be concluded that the formulation of Zandu haldi drops confirmed the presence of labelled ingredients viz, Turmeric and Pepper.

Keywords: Curcumin, HPTLC, Piperine, U.V. spectrophotometry, Zandu haldi drops.

Introduction

From hundreds of years, herbal products have found use as medicinal drugs for a number of diseases. The global health has had a significant impact due to using of plants with medicinal value. In the present era of rapid industrialization and technological advancements, plants continue to play a pivotal role in modern drug development making a significant impact towards health care [1]. Our single and most widespread source of medicines has been herbal products. Nature has been kind to gift earth with plants that is highly capable of producing an infinite number of complex chemical substances whose structures would otherwise be impossible to realize and understand [2]. At present, about 120 different chemical substances have their origin from plant source, while many others comprise of synthetic variations of these herbal products that have immense medicinal potential [3]. Literature survey did not divulge on information pertaining to the standardization of Haldi drops and hence the current work describes the determination of Curcumin and Piperine from the marketed product.

Materials and Methods

Procurement of Zandu Haldi drops: The Zandu Haldi drops was acquired from Zandu care online. Chemicals and solvents used in the experiment: The standard compound Curcumin and solvent Dichloromethane were obtained from Himedia and Loba chem respectivey. Silica plates were from the Merck Company. Piperine was procured from Aldrich Company. Analytical grade chemicals were used for the experimental part. Make and model of Instruments: The instrument CAMAG HPTLC with CAMAG Linomat IV as Sample applicator. CAMAG TLC Scanner III was made use of and Twin trough chamber was used for development. The Win Cats software was used as scanner in the experiment. The U.V. spectrophotometer (SHIMADZU-1650) was used in the experiment and the software was UV PROB 2.

HPTLC of Curcumin:

Standard Stock solution: Small volume of methanol was taken in a 10 mL volumetric flask into which 1 mg of Curcumin was dissolved. Standard stock of 100 µg/mL was achieved by making up the volume to 10 mL using a suitable diluent. From this, 10 µg/mL was prepared as working standard.

Sample solution: An accurately measured amount (1ml) of the formulation was dissolved in methanol in a 10ml volumetric flask. The volume was then increased to 10ml with solvent to get standard stock of 100 µg/ml. In another 10ml volumetric flask, an accurately measured quantity (1ml) of this stock solution was dissolved in methanol. The volume was then increased to 10ml with diluent to get 10 µg/ml concentration of standard stock.

Instrumentation and Chromatographic conditions: The instrument used was CAMAG Linomat IV Sample applicator, development was made using CAMAG Twin trough chamber and scanned by CAMAG TLC Scanner III. The chromatographic conditions were Marker compound: Curcumin (10µg/ml), Sample: Zandu haldi drops (10µg/ml), Solvent: Methanol, Stationary phase: Pre-coated Silica gel plate 60 F254 pre-washed with methanol and the Dichloromethane: methanol (99:1) was used as the mobile phase. Application volume was 10µl, gas used was Nitrogen, oven temp: 120 º C and scanned at the wavelength 421nm.

Procedure: The formulation extract and the marker compound were dissolved in methanol to make the concentration 10 µg/ml. The silica plates were pre-heated to remove the moisture and then the formulation extract and the standard samples were applied to it using the sample applicator. Then the samples were developed by the mobile phase [dichloromethane: methanol (99:1)]. After the development, the TLC plate was dried at room temperature. Then it was scanned in scanner 3 at a wavelength of 421nm. The peak area values of the sample against the standard were analysed to calculate the quantity of drug present.

U.V. Spectrophotometry of Piperine: Standard Stock solution: In a 10ml volumetric flask, 1 mg Piperine was dissolved in methanol. The volume was then increased to 10ml with solvent to get a stock solution containing 100 µg/ml Piperine. From this, working standard of 1 to 5 µg/Ml with 5 different concentrations were prepared.

Preparation of Sample solution: An accurately measured quantity (1ml) of the formulation was dissolved in methanol in a 10ml volumetric flask. To obtain standard concentration of 100 µg/ml, a suitable diluent was used and the volume was adjusted to 10 mL. From this stock solution, an accurately measured quantity (0.5ml) was dissolved in methanol in another 10ml volumetric flask. With the help of diluent, the volume was made up to 10ml to achieve a standard concentration of 5 µg/ml.

Procedure: The standard Piperine was dissolved in methanol to prepare five different concentrations from 1 to 5µg/mL, then the prepared formulation of 5µg/mL was scanned from a wavelength of 200 - 600 nm range and the lambda max was found at 342 nm. Different concentrations were then scanned and calibration curve was constructed with the obtained results.

Standard error of response and standard error of y intercept of regression lines was made use of to determine Limit of Quantification (LOQ) as well as Limit of Detection (LOD).

$LOD = 3.3 \times (\sigma)/S$ and $LOQ = 10 \times (\sigma)/S$

Here, σ = Standard deviation of the response and S = Standard deviation of y intercept of regression lines.

Results and Discussion

The formulation was subjected to various chemical tests as a part of preliminary phytochemical screening.

Table 1 summarizes on the results of preliminary phytochemical tests.

Table 1: Preliminary phytochemical tests for the identification of constituents.

Phytoconstituents	Test/Reagent used	Result
Alkaloids	Mayer's	+
	Dragendorff's	+
	Hager's	+
	Wagner's	+
Carbohydrates	Molisch's	+
	Benedict's	+
	Fehling's	+
Steroids	Liebermann-Burchard	+
	Salkowski's	+
Tannins	Lead acetate	+
	Ferric chloride	+
	Alkaline reagent	+
Saponins	Foam	-
	Sodium bicarbonate	-
Phenols	Alc. Ferric chloride	+
	Lead acetate	+

There are different methods for estimating Curcumin in crude drug powder and several marketed preparations. An effective, subtle and accurate HPTLC technique for determining Curcumin in numerous marketed spices sample of turmeric powder has been developed and equated with an in-house turmeric powder [4].

Simple as well as precise HPTLC methods were developed in one of the studies for simultaneous estimation of Curcumin and Galangin, the two anti-inflammatory drugs. Experiment was so designed to analyse these drugs in their commercial dosage form (capsules) without interference of other ingredients [5]. Curcumin, Piperine, and Thymol in an ayurvedic preparation was simultaneously estimated in another study with the help of a simple, accurate and cost-effective HPTLC method [6].

For Piperine also, various HPTLC methods are available for determination of Piperine in crude drug and its formulations available in the market.

Determination of Curcumin by HPTLC:

Solvent system of the mobile phase possessing dichloromethane: methanol (99:1) gave dense, compact as well as well separated spots of the marker compound from the formulation at 421nm.

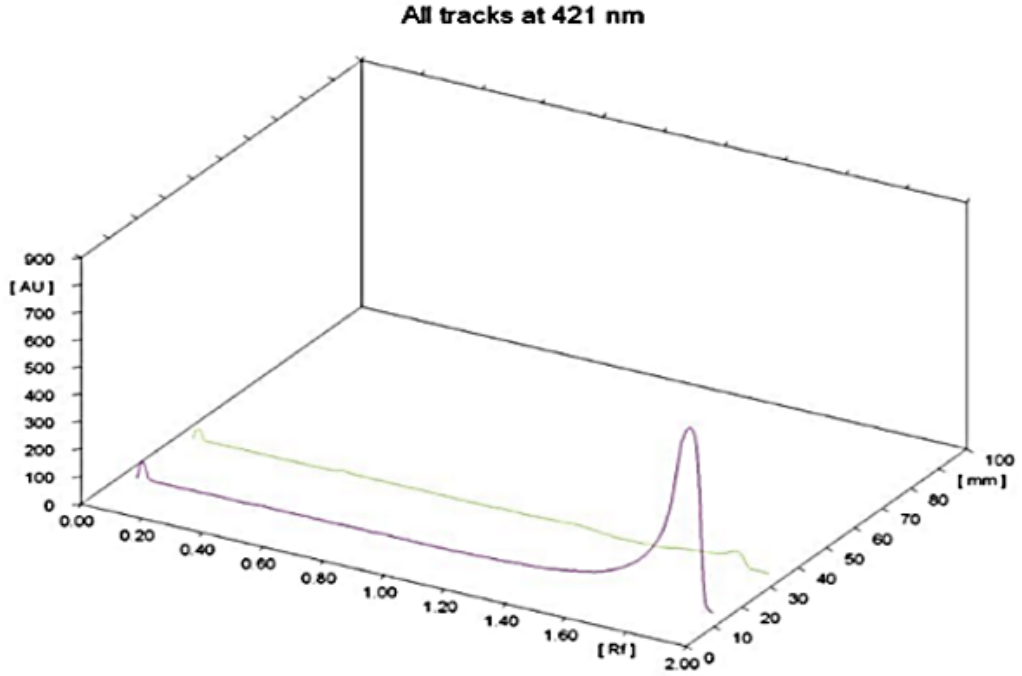

Figure 1: 3D Chromatogram of Curcumin standard

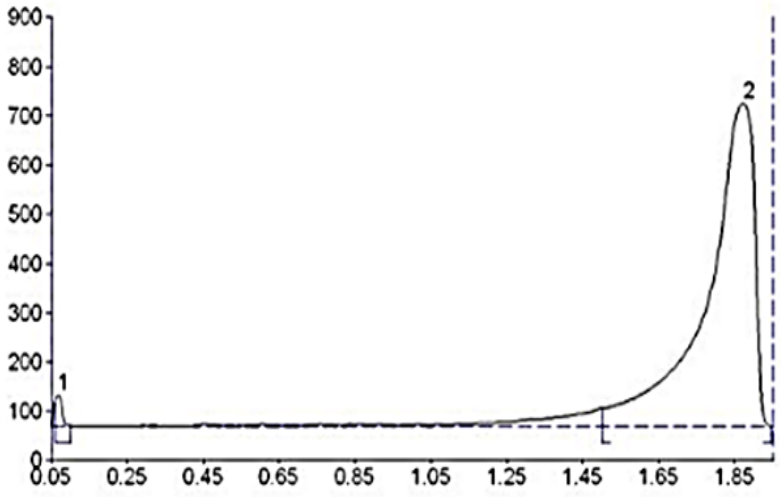

Figure 2: Chromatogram of the standard compound

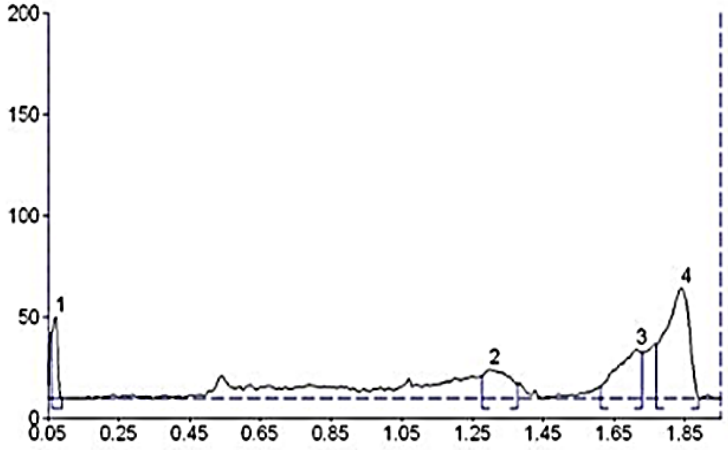

Figure 3. Chromatogram of Zandu Haldi drops formulation

Qualitative analysis was done and the Rf value of Curcumin was found to be 0.84 from standard and the sample.

Quantitative determination was done by plotting the curve of concentration vs peak area of the standard was made and the concentration of curcumin present in the formulation solution used in this method was graphically extrapolated using the peak area of the formulation solution and was found to be 5.6 µg/ml. This indicates that Curcumin is the major ingredient of the formulation and is present in high concentration.

In one of the studies, UV-Visible spectrophotometric method was established through an appropriate solvent system for determining Curcumin and Tetrahydrocurcumin [7]. A UV-Spectrophotometric technique was developed for estimating Curcumin and Quercetin throughout formulation progress, as the other methods such as chromatographic method is time consuming [8]. In another experiment, a study was carried out to develop and evaluate Resveratrol and Curcumin in combination as well as individually by simple, precise, rapid and accurate reverse phase HPLC method [9].

Estimation of Piperine by U.V. Spectrophotometry: Absorbance of 5 µg/ml concentration solution of the formulation was found to be 0.734.

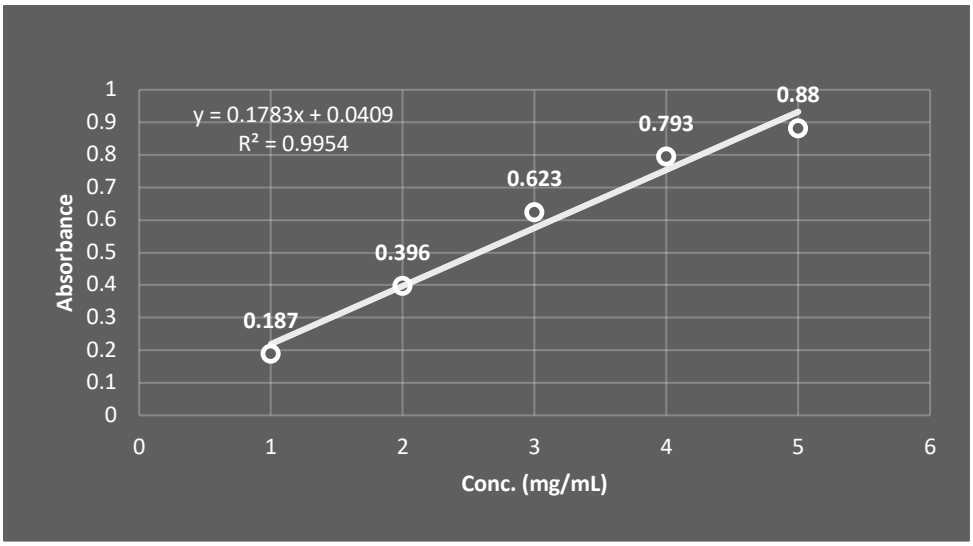

Figure 4. Standard plot of Piperine in methanol.

The plots of concentration vs absorbance demonstrated a linear relationship. The equation of the straight line for Piperine was y = 0.1783x + 0.0409. The linearity range for Piperine was found to be between 1 and 5 µg/ml and the regression coefficient was calculated to be 0.9954, signifying good linearity between the absorbance and concentration. LOD and LOQ were found to be 0.956 µg and 2.897 µg respectively. These results obtained indicate that Piperine is present in the formulation (Zandu haldi drops) and is present in low concentration. For instance, the determination of

Ascorbic acid and Piperine individually in raw materials and in an Ayurvedic formulation Dhathryaristam was carried out using reverse phase C18 eluted with gradient mobile phase. And the developed method can be effectively used to determine these compounds in various formulations [10]. A HPLC method was developed using acetonitrile: water (90:10) as the mobile phase and used to show that multicomponent crystal of piperine-saccharin increased the dissolution of piperine. The experiment was done to show the poor dissolution of Piperine in water [11]. Piperine is well known bio-enhancer as it inhibits drug metabolizing enzymes in rodents and enhances the bioavailability of various drugs. But, Piperine-vitamin A conjugate inhibit human CP450 3A4 was studied that confirmed an increase in bioavailability of Vitamin A [12].

Conclusion

Curcumin was determined by HPTLC and Piperine was determined through UV spectrophotometric method. The developed technique is simple that can find application in routine analysis in formulations.

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