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ESTIMATION OF CURCUMIN IN DIFFERENT TURMERIC SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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INTRODUCTION:

Curcumin is a naturally occurring phytochemical; a bright yellow chemical produced by plants of the ***Curcumalonga L***species. Curcumin is a nutritional compound located within the rhizome, or rootstalk, of the turmeric plant [9]. Curcumin, contained in the rhizome of the plant Curcuma longa Linn, has been used widely in India and Indonesia for the treatment of inflammation. An average turmeric rhizome is about 2% to 5% curcumin.Rhizomes are horizontal underground stems that send out shoots, as well as roots. The bright yellow-orange colour of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids. Curcumin is the active ingredient of the dietary spice turmeric and primary curcuminoids in rhizome of turmeric plant, Zingiberacea family. Other curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin(3)..

In India, phytochemicals, as well as medicinal plants, have remained the most abundant source of health care and life improvement since very long. India is the richest source of traditional herbal plants with their prescriptions. In India, Ayurveda, Unani and Siddha medico-therapeutics are playing a very important role in the society since ancient time[8,12,14]. Ayurveda is approximately 5000 years old and predominantly uses phytochemicals in their preparations and formulations. Now in modern era, about 24%–27% drugs are derived from the plant sources. Several synthetic drugs also have been developed as the analogs/prototype of the natural phytochemicals, which serve as lead compounds for these synthetic drugs. India, being one of the richest plant biodiversity countries in the world, has

Western Ghats and Himalayas as the regions rich in plant biodiversity in the country. About 7500 plant species out of 43,000 (that exist in the country) are recorded in various medicines, and ~1700 species are acknowledged in the Ayurvedic literature. In India, phytochemicals are not limited to medicinal use only, but also they have been used in cosmetics, health and hygiene, fragrance, and food supplements(14).

Curcumin can now regarded as a new drug with great potential and it is being used as a supplement in several countries. In India, for example turmeric containing curcumin has been used in curries; in Japan, it is popularly served in tea; in Thailand, it is used in cosmetics; in China, it is used as a colorant; in Korea, it is served in drinks; in Malaysia, it is used as an antiseptic; in Pakistan, people use it as an anti-inflammatory agent to get relief from gastrointestinal discomfort; and in the United States, it is used in mustard sauce, cheese, butter, and chips, as a preservative and a colouring agent. Curcumin is marketed in several forms including capsules, tablets, ointments, energy drinks, soaps, and cosmetics.

Thebiosynthetic route of curcumin is uncertain. In 1973, Peter J. Roughley and Donald A. Whiting proposed two mechanisms for curcumin biosynthesis. The first mechanism involves a chain extension reaction by cinnamic acid and 5 malonyl-CoA molecules that eventually arylize into a curcuminoid. The second mechanism involves two cinnamate units coupled together by malonyl-CoA. Both use cinnamic acid as their starting point, which is derived from the amino acid phenylalanine(10).

Curcumin is a yellowish crystalline, odourless powder, poorly soluble in water, petroleum ether and benzene; soluble in ethyl alcohol, glacial acetic acid and in propylene glycol; very soluble in acetone and ethyl ether.It consists of three chemical entities in its structure: two aromatic ring systems containing o-methoxy phenolic groups, linked by a seven-carbon linker consisting of an α, β-unsaturated β-diketone moiety[7].

A large number of in vitro and in vivo studies in both animals and man have indicated that curcumin has strong antioxidant[5,8], anti-carcinogenic[4], anti-inflammatory, anti-angiogenic, antispasmodic, antimicrobial, anti-parasitic and other activities. Curcuminhas potential application in the treatment of psoriasis, diabetes, asthma, cataract formation. It is also used in the treatment of neurodegenerative disorder such as Alzheimer[6], arthritis, cardiovascular diseases[14,12] like atherosclerosis and inflammatory bowel disease [2].

Lakadong Turmeric Powder

MATERIALS AND METHOD:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Curcumin, isocratic peak HPLC instrument with Zodiac C18 column (100 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

CHEMICALS AND SOLVENTS:

Methanol : HPLC grade, Merck Specialties Private Limited, Mumbai, India.

Water : HPLC grade, Merck Specialties Private Limited, Mumbai, India.

Reversed phase chromatography*:*

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

HPLCPumps:

The development of HPLC led to the development of the pump system.The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system. An HPLC pump is also called a “solvent delivery system.” The purpose of the pump is to maintain a constant flow of mobile phase through the HPLC system. High-pressure generation is a “standard” requirement of pumps besides which, it should also

to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate. Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces pulses(1).

Injection Port:

An injector is placed next to the pump.The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.The most widely used injection method is based on sampling loops.The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing. The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide an injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000)[6].

HPLC Columns:

Columns are the main important component in HPLC because the column is responsible for the separation of sample components. The sample passes through the column with the mobile phase and separates in its components when it comes out from the column. Generally, silica gel is filled in the high-performance liquid chromatography columns because of its particle size and porosity that helps in the separation of components and silica gel is also an inert material that does not react with any mobile phase. Therefore silica columns can be used to analyze the compounds of different chemical natures. The material filled in the HPLC columns is known as a stationary phase. Columns are usually made of polished stainless steel, are between 50 and 300 mm long, and have an internal diameter between 2 and 5 mm.They are commonly filled with a stationary phase with a particle size of 3–10 µm[6].

Preparation of sample solution:

Six samples of turmeric, three from natural sources obtained from an Ayurveda store and three samples from general store were used for the estimation of curcumin content. Samples obtained from natural source which are Duggirala long fingers , Black turmeric bulb , KasturiHaldi fingers are collected and grinded into a fine powder using motor and pestle. And remaining three samples are already in powdered form which are ready for use. From the powder, am amount of the powder equivalent to 0.5g of Curcuminwas weighed and was dissolved in 50ml of 95% Ethanol which helps in the extraction of curcumin from turmeric because curcumin being a liposoluble compound it can be extracted with organic solvents like acetone and methanol. This extracted solution is filtered using whatman no1 filter paper. The filtered extraction was dissolved again in 50ml of 95% HPLC grade Methanol and allowed to get evaporated. To the evaporated sample 10mlof 95% HPLC grade methanol is added and mixed gently until the crude get dissolved in Methanol.Now these samples are used for analysis.

PreparationofMobilephase:

A mixture of Methanol and Water in the ratio of 50:50 (v/v)was measured accurately.
The solution was sonicated till the solvents mixed completely. Then it was filtered through 0.45µm nylon membrane filter paper using vacuum filtration. The final filtrate solution was used as a mobile phase for the estimation of Curcumin. It was found from the experiments that 1.2ml/min flow rate was ideal forthe successful elution of the analyte.

Table 1: **HPLC Parametres for the Estimation of Curcumin**

S No	Parameter	Condition
1	Elution	Isocratic
2	Mobile phase	Methanol and Water in the ratio of 75:25 (v/v)
3	Stationary Phase	Zodiac C18 column (100 X 4.6 mm, 5µm) column
4	Wavelength	225nm
5	Mobile phase flow rate	1.2ml/min
6	Mobile phase pH	5.5
7	Sample volume	20µL
8	Run time	10.0min

Construction of calibration curve:

From the prepared stock solution, using mobile phase as solvent each of these sample solutions (20µl) was injected into the column, the peak area and retention times were recorded. The calibration curve for **curcumin** was constructed by plotting the mean peak area against the concentration.

Assay sample preparation:

From the prepared formulation solution, 20µl of the sample was injected in to HPLC system, peak area response of the prepared formulation solution was used for the assay of Curcumin in the prepared solution. % assay of the method was calculated by considering peak area response of the sample solution and substituting peak area value in the regression equation.

RESULTS:

HPLC method was used for the estimation of Curcumin in natural turmeric samples.

Standard chromatogram of shows peak at retention time of 10.0min and blank chromatogram doesn't have any peaks. Hence, the method was found to be specific.Calibration curve was obtained with in the injected sample volume of 20µg/ml ;regression equation was found to be Y = 86.839x – 64.613 with correlation of 0.999.

Table 2: LINEARITY RESULTS

S.No	Sample Name	Peak area	Retention Time	% of samples
1	Duggirala long fingers	1683	0.7667	20.124
2	Black turmeric	598	0.9500	7.63
3	Kasturihaldi	489	1.0667	6.37
4	Lakadong	829	1.3333	10.29
5	Dhanalakshmi Turmeric	584	1.3333	7.46
6	GCC Turmeric powder	827	0.8667	10.26
Correlation coefficient: 0.999				

Chromatogram for DUGGIRALA LONG FINGERS

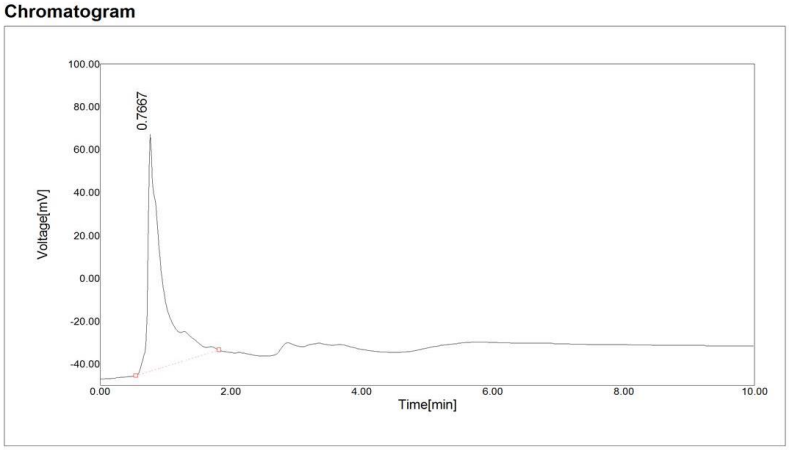
Analysis Report

Post:	Name:

Analysis

Sample Name: long fingers Sample ID: File: 0000.RAW	Date: 2022-05-26 PM 12:08:06 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		0.7667	1683.6373	110.5054	0.0000
Sum			1683.6373	110.5054	0.0000

ChromatogramforBLACKTURMERIC

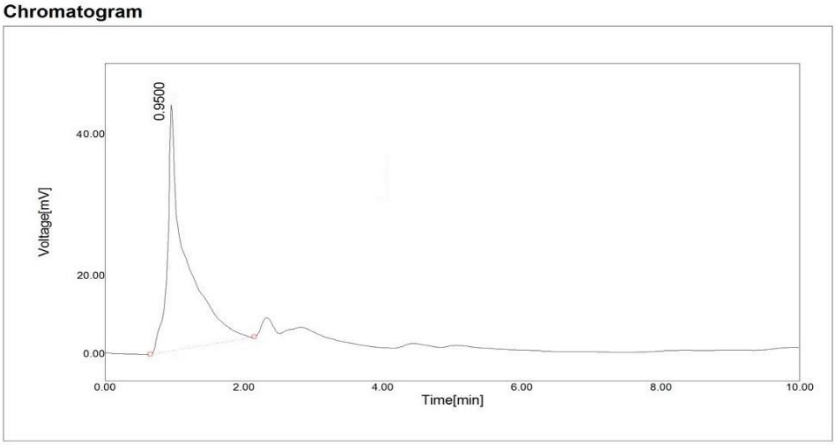
Analysis Report

Post:	Name:

Analysis

Sample Name: black turmeric Sample ID: File: 0002.RAW	Date: 2022-05-26 PM 12:24:16 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		0.9500	598.5189	25.6734	0.0000
Sum			598.5189	25.6734	0.0000

Chromatogram for KASTURI HALDI

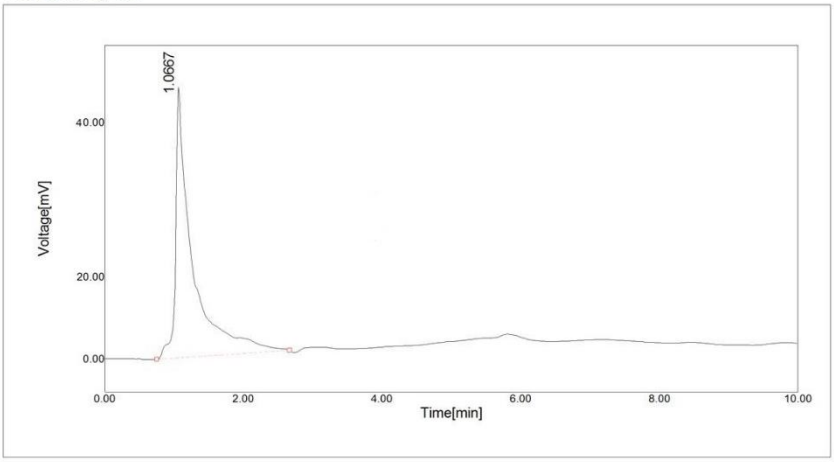
Analysis Report

Post:	Name:

Analysis

Sample Name: kasturi haldi Sample ID: File: 0003.RAW	Date: 2022-05-26 PM 12:36:25 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		1.0667	489.2241	21.1054	0.0000
Sum			489.2241	21.1054	0.0000

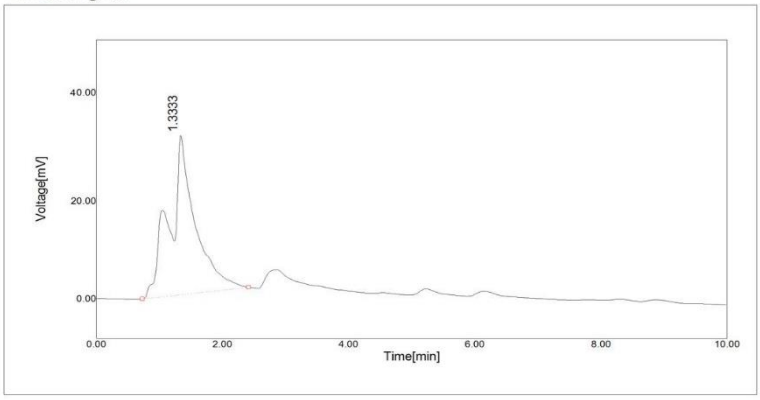
Analysis Report

Post:	Name:

Analysis

Sample Name: lakadong Sample ID: File: 0006.RAW	Date: 2022-05-26 PM 01:11:35 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		1.3333	829.5552	29.3673	0.0000
Sum			829.5552	29.3673	0.0000

Chromatogram for DHANALAKSHMI TURMERIC POWDER

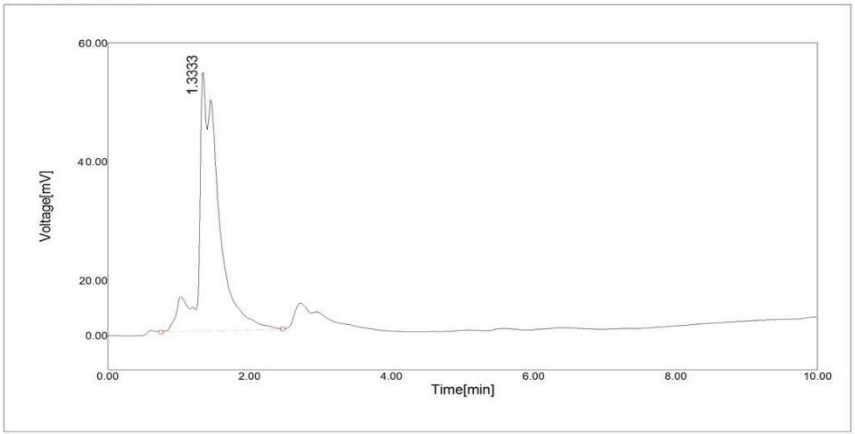
Analysis Report

Post:	Name:

Analysis

Sample Name: Dhanalakshmi Sample ID: File: 0005.RAW	Date: 2022-05-26 PM 01:00:02 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		1.3333	584.8134	23.4283	0.0000
Sum			584.8134	23.4283	0.0000

Chromatogram for GCC TURMERIC POWDER

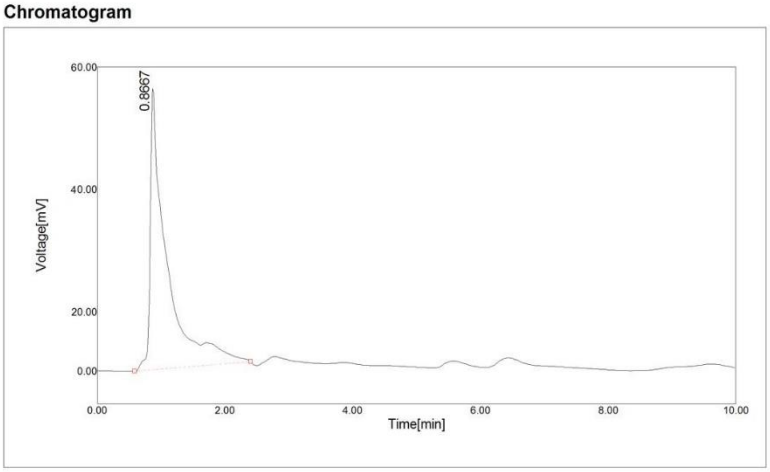
Analysis Report

Post:	Name:

Analysis

Sample Name: GCC Sample ID: File: 0004.RAW	Date: 2022-05-26 PM 12:48:18 Channel: 1. ADM A

Chromatogram



Result

No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]
1		0.8667	827.2760	45.8418	0.0000
Sum			827.2760	45.8418	0.0000

Chromatogram Results:

The % assay was found to be more than 98% was found in the samples under study by the given method. 20.124 % of curcumin in Duggirala long fingers was estimated in the method.

CONCLUSION:

Curcumin, is a natural product isolated as a yellow pigment from the rhizome of Curcuma longa L is a primary bioactive substance; a principle curcuminoid in turmeric with many biological applications. A simple, rapid and sensitive, analytical HPLC method was followed for the estimation of Curcumin from its natural source turmeric. The mobile phase consists of Methanol and Water in the ratio of 50:50 (v/v) at a flow rate of 1.2ml/min, UV detector wavelength of 225nm was found to be suitable for the analysis of Curcumin In these conditions the retention time of Curcumin was found to be 10 minutes. By using the slope formula (Y = 86.839x – 64.613 with correlation of 0.999) obtained in the calibration curve the amount of curcumin present in the samples is calculated. From the calculations the amount of curcumin present in the Duggirala long fingers is 20.124% with high curcumin content. Less amount of curcumin was observed in marketed powder compared to Duggirala long fingers.

CONFLICT OF INTEREST:ll

The authors have no conflict of interest regarding this investigation.

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