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Extraction, isolation and quantification of curcumin for possible diabetis treatment

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***Abstract*---**Anti-HIV, anti-tumor, anti-viral, anti-cancer, anti-fungal, and ant-parasitic properties of curcumin make it an important active ingredient in many medicines. High-purity curcumin can be obtained using a new extraction method. Increases in production were noticeable. This method of isolation and quantification had a low barrier to entry and was quick to execute. Curcumin was extracted using a soxhlet extraction experiment in this study. Diabetes Mellitus research and development can benefit from high-purity curcumin isolates. Analytical tools currently being developed can also play a significant role in the future quantification of novel dosage forms.

***Keywords*---**curcumin, diabetes mellitus, isolation, quantification.

Introduction

Diabetes mellitus is a major and growing health problem on a global scale, as well as a significant contributor to extended periods of poor health and premature death. It is a metabolic disorder that lasts for a long time and is characterized by a high concentration of glucose in the blood (hyperglycemia) that is caused by a lack of insulin, and it is frequently combined with insulin resistance (Badwaik *et al.* 2019; Pivari *et al.* 2019; Rivera-Mancía *et al.* 2018). Due to the multifactorial nature of pathology, patient management, which may include lifelong drug therapy and changes in lifestyle, can be extremely difficult. At this time, there is an increasing body of evidence about the efficacy of using herbal supplements in the prevention and management of diabetes (Baghel *et al.* 2022; Benzie *et al.* 2011; Cefalu *et al.* 2011; Kamdi *et al.* 2021; Kumari *et al.* 2021). Curcumin is a bioactive component that can be found in Curcuma longa. It demonstrates several physiological and pharmacological properties, including antioxidant, anti-inflammatory, anticancer, neuroprotective, and anti-diabetic activities. Curcumin is extracted from the root of Curcuma longa (Anderson *et al.* 2000; Priyadarsini,

K. I. 2014; Zielińska *et al.* 2020). Curcumin was extracted and isolated for the current study. The amount of isolated curcumin was measured using newly developed UV and HPLC analytical techniques.

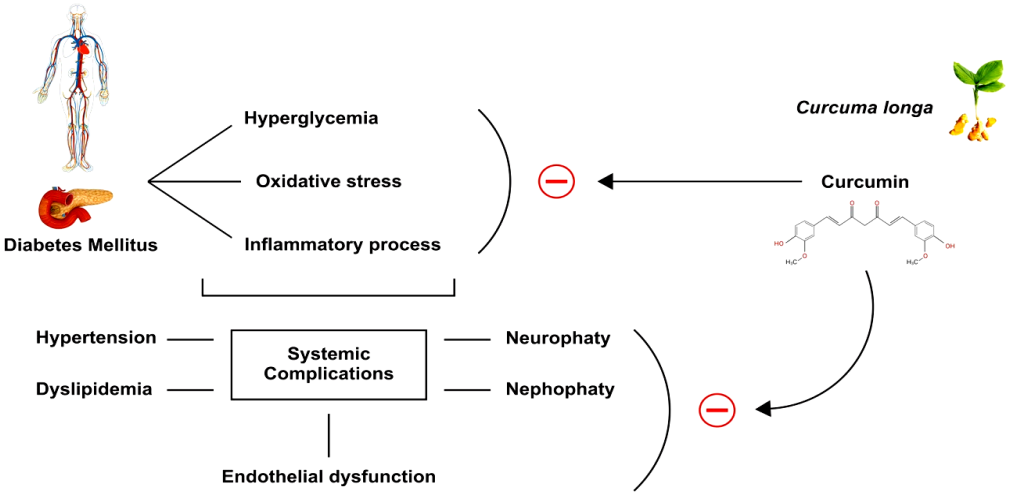

Fig. 1: Effect of curcummin on Dibbetes Mellitus (Marton *et al.* 2021)

Material and Method

Curcummin was received as a gift sample from Veedish Chemicals, pune, India. Tetrahydrofuron, Water, Citric acid Methanol, Dichloromethane, Ethanol, Acetonitrile were purchased from SD fine-Chem. Ltd, Mumbai. All other materials and chemicals used were of either pharmaceutical or analytical grade. Turmeric used was purchased from a traditional market and standard curcumin

Isolation of Curcumin

Turmeric was cleaned and washed under flowing tap water and left to dry at room temperature. The turmeric was crushed and grinded until become powder. A 500 mg of turmeric powder were prepared for extraction with soxhlation method where ethanol and dichloromethane were used as solvents. Extraction was stopped when the solvent no longer turned orange, the results were compared. The isolation was carried out by column chromatography on silica gel with dichloromethane-methanol (97:3) % as mobile phase.

Analysis UV Visible Spectroscopy may be used to estimate the amount of Curcumin in an extract

Analyzing the absorbance at 424 nm, the amount of Curcumin contained in the extract was determined using the calibration curve technique (Verma *et al.* 2017).

Analyzing Curcumin levels in extracts using HPLC

The peak area of the test solution was measured after it was injected. Total curcumin percentage was determined.

Preparation of standard and test solutions

Preparation of stock solution Stock solutions were prepared by dissolving 10 mg of curcumin in 10 ml of methanol and subjected to sonication to give concentration of 1000 µg/ml. Method development Aliquots of the standard stock solutions were further diluted with mobile phase to get 24 µg/ml of curcumin. The samples were injected in the HPLC system and chromatograms were recorded. Various combinations of mobile phase components, column temperature, and columns were tried to get desirable resolved peaks. Method validation System suitability The system suitability test is used to verify that the chromatographic system is suitable for the intended analysis or not. The system suitability of the method was checked by injecting six different injections of 24 µg/ml of curcumin. Various parameters like tailing factor, theoretical plates, peak area and resolution were checked according to USP criteria.

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix (Giri *et al.* 2012). Specificity was carried by dissolving 500 mg of placebo sample (pellets made up of excipients viz. avicel PH 101, carbopol 940, hydroxypropyl cellulose-H and sodium chloride excluding both the drugs) in 100 ml of mobile phase.

Precision

Precision was studied at three levels: repeatability, intermediate precision, and reproducibility. Repeatability: Repeatability study was performed by preparing a minimum of six determinations (n=6) of test concentration (curcumin 24 µg/ml) and was analyzed. It is expressed as the percent relative standard deviation (% RSD). Intermediate Precision: The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. This study was done at two levels (intra-day and inter-day study). Intra-day precision was done by preparing the test concentration (curcumin 24 µg/ml) and assaying for six times (n=6) at 3 different time intervals. Inter-day precision was done by analyzing samples (n=6) for three consecutive days. The percent relative standard deviation (% RSD) values were calculated.

Result and Discussion

The total curcumin content of crude curcuminoid powder was found to be 76.82% W/W whereas in recrystallized powder the purity was increased to 99.45% W/W. The pure curcumin powder obtained Dier recrystallization was orange-yellow coloured crystalline powder with melting point of 183°C.

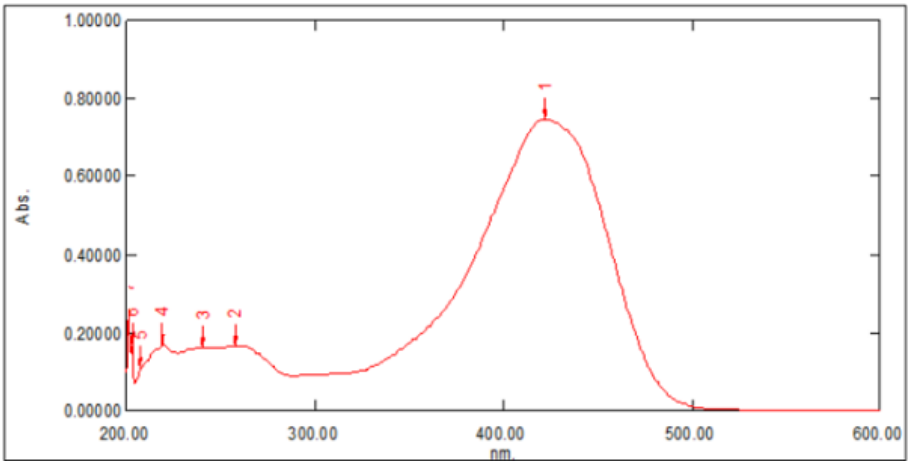

Fig. 2: Determination of maximum wavelength of curcumin by UV

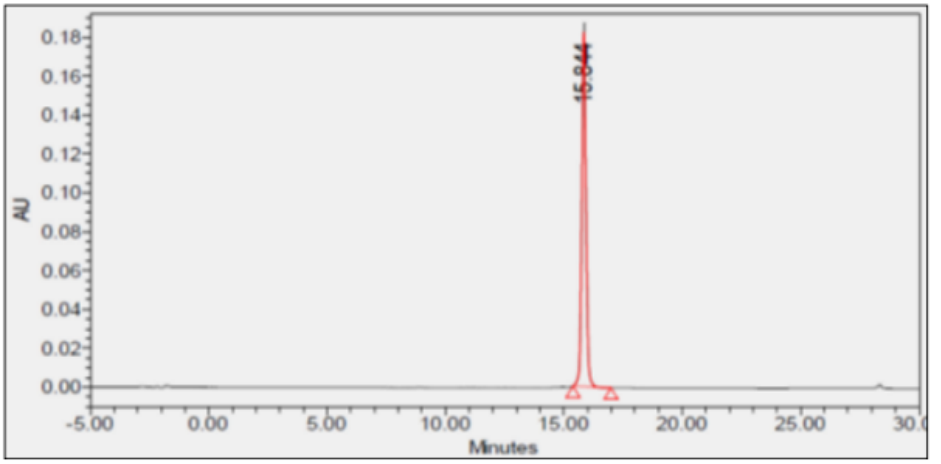

Fig. 3: HPLC chromatogram of Standard Curcumin solution

Analyzing the absorbance at 424 nm, the amount of Curcumin contained in the extract was determined using the calibration curve technique. Table 1 contains the findings. The peak area of the test solution was measured after it was injected. Total curcumin percentage was determined. Table 1 summarizes the findings.

Table 1: Drug Content by UV Visible Spectroscopy and HPLC

UV Visible spectroscopy		HPLC	
Sample No.	% Assay	Sample No.	% Assay
1	98.54	1	95.96
2	96.49	2	94.23
3	97.86	3	94.25
4	97.17	4	94.10
5	95.81	5	93.98
6	95.12	6	93.01

Mean	96.88	Mean	94.25
% RSD	1.32	% RSD	1.01

Analytical Method Validation

Establishing proof that offers a high degree of certainty that an action will consistently generate a desired outcome or product that meets its set criteria and quality features may be characterised as validation (Panda, S., & Das, S. 2022). Following ICH recommendations (Q2) R1, the technique was validated for numerous criteria, such as linearity, accuracies and precision, toughness and robustness, limit of detection (LOD) and limit of quantitation (ICH, 2018)

Linearity

Different stock solution concentrations were analysed to assess the linearity. It demonstrates linearity at 424 nm in the range of 1-7 g/ml for UV curcumin. HPLC has a concentration range of 20-60 g/ml and is visible in spectroscopy. Using UV and HPLC calibration curves, concentrations were plotted against absorbance and peak area, respectively. Using conventional curcumin concentrations, a regression equation and a correlation coefficient were calculated.

Table 3: Linearity

UV Visible spectroscopy		HPLC	
Curcumin (µg/ml)	Absorbance at 424 nm (Mean±SD)	Curcumin (µg/ml)	Peak Area of Curcumin (Mean±SD)
1	0.150	20	925656
2	0.299	24	1120212
3	0.448	32	1482569
4	0.596	40	1845812
5	0.745	48	2221254
6	0.886	56	2578985
7	1.026	60	2755658

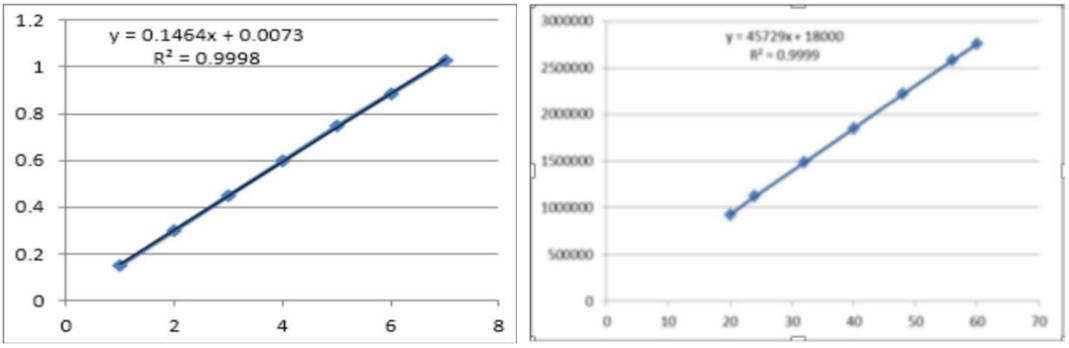

Fig. 3: Linearity Graph for Curcumin by a) UV, b)HPLC

Accuracy

Recovery experiments were used to gauge the precision. At 80, 100, and 120 percent recovery levels of working concentration, the working standard Curcumin was injected into the pre-analyzed extract test solution for each batch. Analysis of the spiked test solution was carried out according to plan. Table 20 lists the percentages of recoveries based on their various levels.

Table 4: Recovery for Curcumin

Analyte	UV Visible spectroscopy			HPLC		
	Recovery level	% Recovery	Average % Recovery	Recovery level	% Recovery	Average % Recovery
Total Curcumin	80%-1	98.76	99.52	80%-1	101.22	101.32
	80%-2	100.28		80%-2	101.61	
	80%-3	99.52		80%-3	101.12	
	100%-1	97.42	97.63	100%-1	100.81	101.17
	100%-2	96.7		100%-2	101.56	
	100%-3	98.79		100%-3	101.43	
	120%-1	98.19	99.01	120%-1	101.08	100.87
	120%-2	100.05		120%-2	100.62	
	120%-3	98.81		120%-3	100.91	

Precision

We used repeatability and intermediate precision to assess accuracy. UV-visible spectroscopy and HPLC were used to assess the repeatability of curcumin. UV Visible spectroscopy and HPLC were used to measure curcumin concentrations at three different levels. Repeatability and intermediate precision were utilised to evaluate accuracy. HPLC was performed six times on the same day (intra-day) using 40 g/mL of curcumin and UV Visible spectroscopy utilised 5 g/mL of curcumin to investigate repeatability. As a result, curcumin doses of 1, 3, or 5 grammes per millilitre (g/mL) were employed to quantify the intermediate precision three times over the course of a week using UV and HPLC, respectively (inter-day).

Table 5: Repeatability (Intra-Day Precision) of the UV Visible spectroscopy and HPLC Method

UV Visible spectroscopy (n=6)			HPLC(n=6)		
Curcumin (µg/ml)	Absorbance at 424 nm (Mean±SD)	%RSD	Curcumin (µg/ml)	Peak Area of Curcumin (Mean±SD)	%RSD
5	0.745±0.001211	0.1625	40	1852371±30980.53	1.6724

Table 6: Repeatability (Intra-day precision) of the UV Visible spectroscopy method for extract

Absorbance at 424 nm (Mean±SD)	%RSD
0.739±0.005033	0.681

Table 7: Intermediate precision (Inter-day precision) of UV Visible spectroscopy and HPLC method

UV Visible spectroscopy (n=3)			HPLC(n=3)		
Curcumin (µg/ml)	Absorbance at 424 nm (Mean±SD)	%RSD	Curcumin (µg/ml)	Peak Area of Curcumin (Mean±SD)	%RSD
1	0.145±0.00251	1.7355	20	9435733±16688.60	1.7686
3	0.445±0.00251	0.5655	40	1856577±11076.14	0.5965
5	0.742±0.00351	0.4026	60	2758486±60919.84	0.220

Table 8: Intermediate precision (Inter-day precision) of UV Visible spectroscopy method

Curcumin (µg/ml)	Absorbance at 424 nm (Mean±SD)	%RSD	Percentage
1	0.145±0.00251	1.7355	94.28
3	0.445±0.00251	0.5655	99.73
5	0.742±0.00351	0.4026	99.95

System precision

Five duplicate injections of a standard were used to test the accuracy of the system using the approach that was presented. Table 25 lists the peak area, average, and percent RSD.

Table 9: Peak Area of Curcumin by HPLC

Injection No	Peak Area of Curcumin
1	1861464
2	1852753
3	1845812
4	1899230
5	1802598
Mean	1852371
%RSD	1.92

Robustness for HPLC

According to ICH recommendations, the method's robustness was verified by varying the chromatographic conditions' parameters by a little amount. Standard solution is injected, and the chromatographic settings are varied to perform the tests.

Table 10: Robustness for Curcumin by HPLC

Parameters		% RSD	Peak tailing	Theoretical Plates	Remark
Wavelength (nm)	420	1.41	1.32	32760	Pass
	425	0.60	1.28	34338	Pass
	430	1.73	1.31	32811	Pass
Column Temperature (°C)	27	0.42	1.26	35139	Pass
	32	0.83	1.38	26276	Pass
	37	0.73	1.30	33692	Pass
Flow (mL/min)	1.0	0.38	1.25	37031	Pass
	1.1	0.52	1.21	44453	Pass
	1.2	0.45	1.27	30454	Pass

LOQ and LOD

The LOD is the smallest amount of analyte that can be detected in a sample. Analyte quantification limits are the lowest quantities of analytes that can be reliably and precisely measured in a sample (LOQ).

The following equation was used to calculate the LOQ and LOD:

LOQ = 10 * S.D. / Slope
LOD = 3.3 * S.D. / Slope

Table 11: LOQ and LOD by UV Visible spectroscopy and HPLC

SN.	UV Visible spectroscopy	HPLC
LOD	0.0617	2.23
LOQ	0.127	6.774

In both standard and test cases, stability

The standard and test solutions were made and kept at room temperature in line with the recommended procedure. At different stages in the testing process, both the standard and experimental solutions were scrutinised. Curcumin and Extract's Percentage Relative Changes are within permissible limits after 24 hours at room temperature.

Conclusion

Curcuminoids are a class of nutraceutical compounds that are used not only in medical treatments but also in the culinary arts. The recently developed extraction method yields curcumin of exceptionally high purity. There was a noticeable rise in the overall yield. The currently employed strategy for isolation and quantification was uncomplicated and cost effective. Curcumin that has been isolated and purified to a high degree can be used effectively in research and development pertaining to diabetes. The currently developed analytical tool has

the potential to play an important part in the quantification of novel dosage forms in the future.

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