SHORT COMMUNICATIONS

Estimation of Curcuminoids in *Curcuma Longa* by HPLC and Spectrophotometric Methods

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A spectrophotometric and reverse phase HPLC method to determine curcumin in different samples of *C. longa* were developed and described. The linearity in the range of 0.1 to 0.8 mcg for HPLC and 2 to 5 mcg for spectrophotometric method was observed. The methods are sensitive, precise and reproducible and can be used for quantitative monitoring of curcumin in *C. longa*.

Curcuma Longa L. rhizome, (Zingiberaceae), commonly used as a spice, is well known for its medicinal value in Indian traditional system of medicine and has been a recipe for several common ailments[1-3]. Curcuminoids, A mixture of curcumin, monodemethoxy curcumin and bisdemethoxy curcumin is perhaps the most important constituent responsible for its Anti-inflammatory. Antiulcer, hepato protective and wound healing activity[4].

Determination of total curcumin is very important for standardisation of *C.longa*. Literature survey reveals that a flurometric[5] and a TLC cum Spectrophotometric[6] method are available for identification of *C. longa* in finished formulations. We report here the development of a precise, sensitive and reproducible methods for quantification of total curcumin i.e. curcuminoids in *C.longa* by spectrophotometer and HPLC.

About 100 g samples were ground to pass through 40 mesh S.S. Sieve. One gram of ground sample was weighed accurately and extracted with methanol (25 ml x 4) over steaming water bath separately. The extracts were filtered and the volume was made upto 100 ml with methanol in both the cases.

A 0.10 mg/ml solution of curcumin reference standard (M/s Sigma Chemical Company, USA) was prepared in methanol.

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A uv- visible recording Spectrophotometer (Beckman DU 64) was employed in the spectrophotometric assay procedure. Methanol extract of test samples was diluted and absorbance at 430 nm was read against methanol. The dilution was made suitably so that the absorbance lies between 0.5 to 1.0. In a similar way, the absorbance of five diluted samples of standard curcumin solution was also read. Curcumin content in different samples was calculated using a linear regression equation of calibration graph plotted between concentration and absorbance. The equation for Curcumin is $y = 170.73 x + 0.0430$ with a correlation coefficient of 0.998 where x is the compound analysed and y is response in absorbance. Curcumin contents in test samples was expressed as percentage of total curcumin.

A Water's HPLC system consisting of a Rheodyne 7125 injector equipped with 996 Photodiode array detector, 510 chromatographic pump and millennium software version 2.1 was used for the analysis of curcumin samples. Methanol extract was diluted (1:2) and 20 ul each of different test samples alongwith five different concentration of standard curcumin (0.005, 0.01, 0.02, 0.03 and 0.04 mg/ml) injected into HPLC using Nova pak RP C18 column (3.9 x 150 mm) with reverse phase guard column. The mobile phase used was methanol : Water : 70 : 30 with a flow rate of 1.0 ml/min. The chromatogram was scanned upto 10 min which was detected at 425 nm. The amount of curcumin was calculated from the linear regression equation of calibration graph plotted between concentration

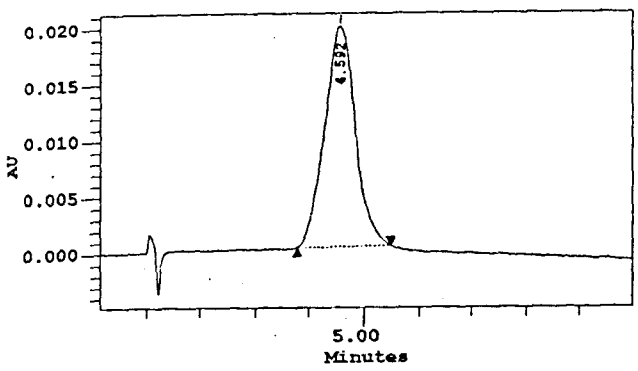

Fig.1: HPLC Chromatogram of Standard Curcumin

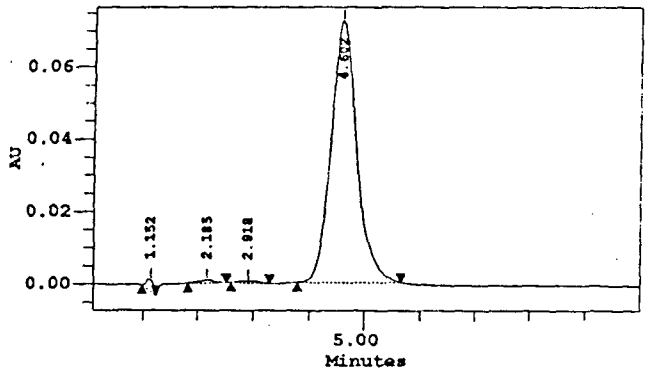

Fig.2 : HPLC Chromatogram of C.longa finger type

Table-1: Spectrophotometric method validation and recovery of Curcumin

S.No.	Description of samples	Amount of samples taken (mg) (A)	Amount of Curcumin present in A (mg) (B)	Amount of Curcumin added to A (mg) (C)	Amount of total curcumin taken (mg) D=(B+C)	Amount of Curcumin found (mg) (E)	% recovery E/D x 100
1.	Finger type	1020	14.38	5.00	19.38	19.28	99.48
2.	Round type	1020	24.51	5.00	29.51	29.39	99.59

Each value is the average of three replicales

Average percentage recovery -99.53%

Table-2: HPLC method validation and recovery of Curcumin

S.No.	Description of samples	Amount of samples taken (mg) (A)	Amount of Curcumin present in A (mg) (B)	Amount of Curcumin added to A (mg) (C)	Amount of total Curcumin taken (mg) D=(B+C)	Amount of Curcumin found (mg) (E)	% recovery E/D X 100
1.	Finger type	1020	14.28	5.00	19.28	19.20	99.58
2.	Round type	1030	24.41	5.00	29.41	28.96	98.47

Each value is the average of three replicales

Average percentage recovery-99.03%

and area. The equation for curcumin was y = 8212698 x + 164708 with a correlation coefficient of 0.999 where 'x' is the compound analysed and 'y' is the response in peak area. Curcumin content in test samples was expressed as a percent of total curcumin.

To validate the methods and to known percentage recovery a know amount of standard curcumin was added to the test samples in which content of total curcumin had been estimated previously by the proposed methods. The samples were extracted and analysed separately as per the proposed methods. The results are mentioned in Table -1 and 2. Total curcumin content estimated by spectrophotometric and HPLC method have been shown in Table-3. A significant difference in the curcumin content

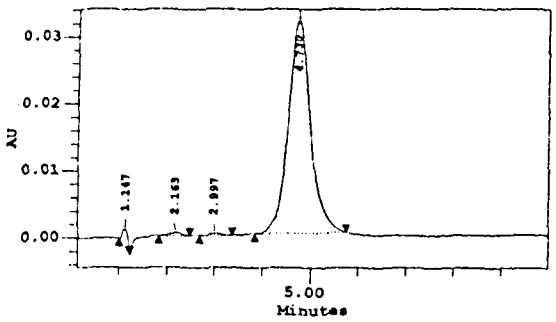

Fig.3: HPLC Chromatogram of *C.Longa* round type.

Table -3 Estimation of total curcuminoids in *Curcuma longa*

Description of Sample	Method of Analysis	
	Spectrophotometric	HPLC
Finger type	1.41	1.40
Round type	2.38	2.37

Each value is the average of three determinations.

was observed in finger and round type *C.longa* by both the methods. Using the HPLC method, curcumin was observed at around 4.5 min. The chromatogram of standard curcumin and that of test samples have been provided in Fig. 1, 2 & 3 respectively. The proposed methods are rapid, simple and reproducible for quantitative monitoring of curcumin in different *C.longa* samples.

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