Development of HPTLC Method and Its Validation for the Estimation of Curcuminoids from Polyherbal Mouth Ulcer Gel Formulation

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Abstract:- A rapid, simple, selective and precise high performance thin layer chromatographic method has been developed for the determination of curcumin in mouth ulcer poly herbal formulation. The separation was performed on TLC aluminium plates Precoated with silica gel G60 F254. Good separation was achieved in the mobile phase of Chloroform: methanol: Glacial acetic acid (7.5: 2.0: 0.5 v/v/v) at Rf = 0.18, 0.31, 0.56 for bisdemethoxy curcumin, demethoxy curcumin and curcumin respectively. The method was validated successfully by checking precision, repeatability and accuracy. The present method can simplify the analysis of Curcuminoids in poly herbal formulations for their routine quality analysis.

Keywords:- Curcuminoids, curcumin, demethoxy curcumin, bisdemethoxy Curcumin, method development, validation, stability, densitometry, Quantification.

I.   INTRODUCTION

Turmeric *Curcuma longa*[1] L. (Zingiberaceae) family is commonly known for its medicinal values in the Indian traditional system of medicine curcuma longa[2] accelerates the process of ulcer healing and has anti inflammatory and anti bacterial properties. Curcumin (C) is main coloring substance in *curcuma longa*[3-10] along with the other two related compounds, demethoxy Curcumin (DMC) and bis demethoxy Curcumin (BDMC) are altogether known as Curcuminoids. The chemical structures of three Curcuminoids are shown in Figure.1.
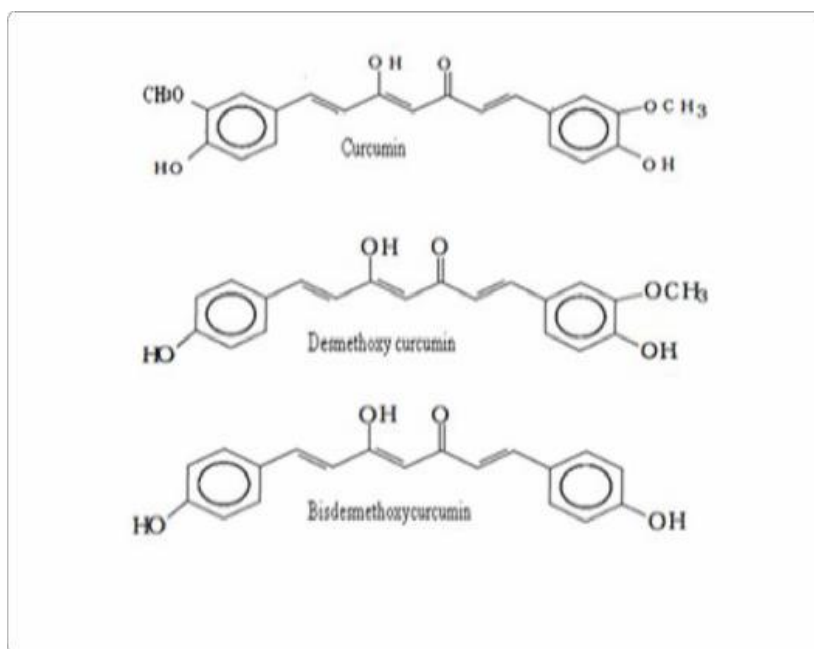

Figure.1: Chemical structure of Curcuminoids.

The pharmaceutical gel which is under taken for study contains *Aloe vera* and *curcuma longa*. A number of studies are undertaken to separate curcuminoids pigments by thin layer chromatography (TLC), High Performance thin layer chromatography[11] (HPTLC), and column chromatography (CC). The stationary phase most commonly used is silica gel with different solvent systems including benzene, ethyl acetate, ethanol, chloroform, acetic acid, hexane, and methanol for chromatographic separations. HPLC[12] method was sensitive, precise and accurate for detection and quantification of curcuminoids in the extract of rhizome *Curcuma longa*.

Separation by high performance liquid chromatography[15-21] (HPLC) was done mostly on reverse phase HPLC method employing mixtures of water, acetonitrile, ethanol, potassium dihydrogen phosphate buffer and methanol since the curcuminoids pigments vary in chemical structures, it is possible that the physico-chemical characteristics as well as the functional properties would vary among them. There are number of method are available for the estimation of curcuminoids from raw herb, their powder mixtures, and other ayurvedic formulations by HPTLC[22-23] method of analysis but no method is available which describes the development and validations of curcuminoids from pharmaceutical poly herbal gel formulations.

II.   MATERIAL AND METHODS

Chemical, Equipments and Instrument:
All chemicals and reagents of analytical grade were purchased from Merck chemical, Mumbai. India. Pure drug sample of Curcuminoids was kindly supplied as a sample from natural remedies labs. HPTLC system used for analysis was of CAMAG HPTLC system. The sample were spotted in the form of bands with a Camag 100microlite sample syringe (Hamilton, Bondouz, Switzerland) syringe on Precoated silica gel aluminium plate $60F_{254}$ (20X10) with 250μm thickness;(Merck") using a Camag Linomat 5 sample applicator (Switzerland).The linear ascending development was carried out in 20cmX 10cm twin trough chamber using mobile phase (Chloroform: methanol: Glacial acetic acid 7.5: 2.0: 0.5 v/v/v). TLC plates were dried on Camag TLC plate heater III.Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode at 430nm after development for all measurement and operated by Camag *WINCATS* software. The source of radiation utilized was tungsten lamp emitting a continuous UV spectrum 400 to 700nm.

Preparation of standard solution:
Stock solutions of curcumin (1μg/μl), demethoxy curcumin and Bisdemethoxy curcumin (0.5μg/μl) were prepared in methanol and different concentrations were applied on TLC plate, Linomat V for preparing calibration curve.

Sample preparation:
Weigh accurately 10.0gm sample in 100ml beaker, add 30ml methanol and stir it for 10minutes. Transfer the dissolved solution in to 50ml volumetric flask and again stir the remaining residue with 15ml methanol transfer the solution to same volumetric and make up the volume to 50ml with methanol.

Optimization of the HPTLC method:
It is well known that the solvent do exert a profound influence on the quality and shape of the peak; the chromatographic separation studies were carried out on the stock solution of curcuminoids. Initially on the plate 4.0μl of stock solution was applied as band 8mm of width. Plates were developed by ascending development using neat solvents like hexane, acetone, ammonia, methanol, chloroform, dichloromethane, ethyl acetate, acetonitrile etc. without chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution between Curcuminoids. After several trials mixture of Chloroform: methanol: Glacial acetic acid (7.5: 2.0: 0.5 v/v/v) was chosen as the mobile phase for analysis then the plate was at 60°C for 5minute. The other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible Rf value, better resolution, and symmetrical peak shape for the drugs. The spot appeared more compact and peak shape more symmetrical when the TLC plate were pretreated first with methanol and then with the mixture of chloroform and methanol (1:1v/v) after that the plate was activated at 110°C for 5.0minutes.

Calibration curve for curcuminoids:
Different amounts of standard solutions were applied (band width: 6mm, distance between bands: 11.5mm, in triplicate on a Precoated silica gel 60 F 254 TLC plate using a CAMAG Linomat V semiautomatic applicator.

Quantification of Curcuminoids in formulation:
6.0μl each of the sample solutions was applied on the same plate using a CAMAG Linomat V semiautomatic auto sampler along with standard. The plate was developed and scanned as mentioned above. The peak areas were recorded and amount of curcuminoids were calculated from the calibration curve.

Method Validation:
The method was validated[14] for precision, repeatability and accuracy. Precision was checked by repeated scanning of the same spots of curcuminoids 6 times (n=6) and was expressed as coefficient of variance

(%CV). The repeatability of the method was affirmed by analyzing different concentration of curcuminoids in standard solution after application and TLC plate (n=6) and was expressed as % CV. Accuracy of the method was tested by performing recovery studies at three different levels. To preanalyzed samples of pharmaceutical poly herbal gel formulation, standard curcuminoids were added in the range of linearity in different amount and mean % recovery were calculated.

III.   RESULT AND DISCUSSION

We report a method for the quantification of curcuminoids (i.e. curcumin, demethoxy curcumin and Bis demethoxy curcumin) in pharmaceutical herbal formulation. Of the various solvent systems tried, the one containing Chloroform: methanol: Glacial acetic acid (7.5: 2.0: 0.5 v/v/v) gave the best resolution of curcuminoids in the presence of other compounds present in the formulation. The identity of the bands in sample formulation was confirmed by overlaying their UV absorption spectrum with that of standards. Purity of the curcuminoids in the sample was confirmed by comparing the absorption spectra at start, middle and end position of the respective bands.

The method was validated in terms of precision, repeatability and accuracy. The relationship between the concentration and peak response was linear within the range of 500 to 5000ng/spot; 200 to 1200ng and 100-700ng for curcumin, demethoxy curcumin and bis demethoxy Curcumin respectively.

The mean % recovery at three different levels for curcumin, demethoxy curcumin and bis demethoxy curcumin was found to be 100.80%, 99.23% and 100.12% for sample formulation respectively. The amount of curcuminoids in the formulation was estimated by the proposed method simultaneously.
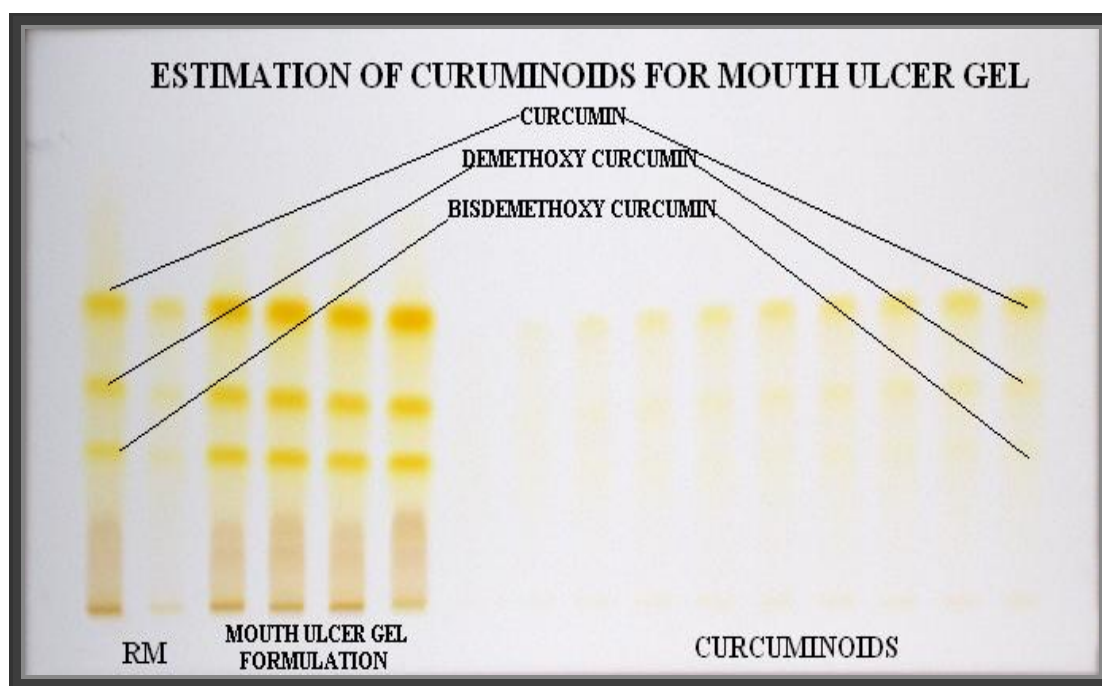

Figure2 (A) chromatogram showing the separated spots of curcuminoids mixture in raw material and in test sample corresponding to standard curcuminoids.
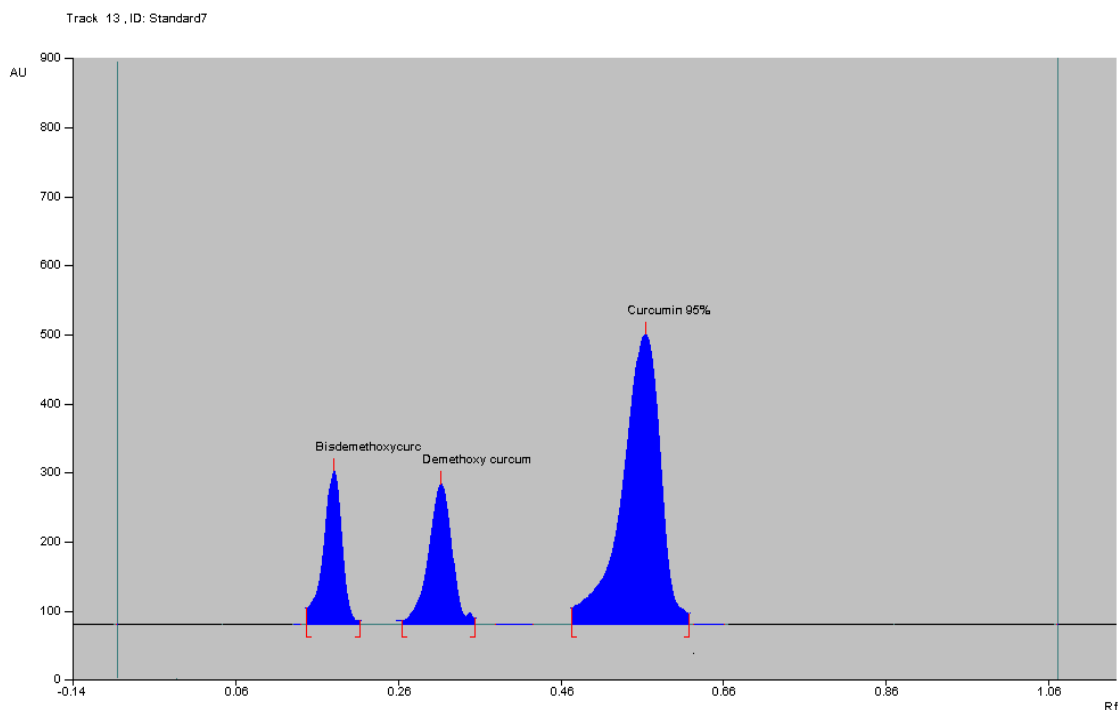
Figure 2(B): Shows densitogram of standard showing curcumin Rf=0.18, demethoxy curcumin Rf= 0.31 and bisdemethoxy curcumin Rf= 0.56 at 430nm in reflection/absorption mode simultaneously.
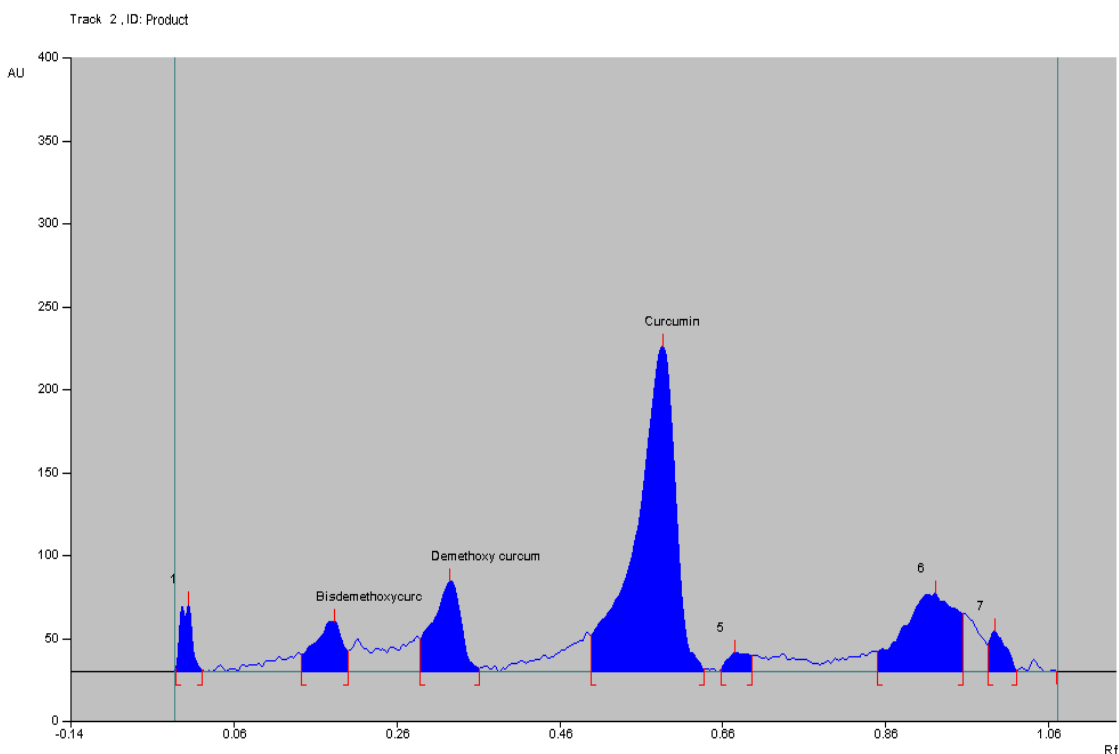

Figure 3: Shows densitogram of product showing curcumin, demethoxy curcumin and bisdemethoxy curcumin simultaneously.

Table 1: Method Validation Parameters.

Parameter	Result for curcumin	Demethoxy curcumin	Bisdemethoxy curcumin
Precision (%CV, n=6)	1.22	1.67	1.29
Repeatability (%CV, n=6)	1.26	1.58	1.63
LOD	100 ng/spot	45.0 ng/spot	52.0 ng/spot
LOQ	250ng/spot	170 ng/spot	80.0 ng/spot
Linearity (Correlation coefficient)	Y = -3505.107+50.275* X	Y = 303.578+8.2584* X	Y = 603.108+12.0516* X
Linearity range (ng/spot)	500 to 5000ng/spot	200 to 1200ng	100-700ng
Retention factor	0.18	0.31	0.56

Table 2: Precision data of Curcuminoids.

Sr. No.	Application of sample (µl)	Area of Standard		
		Curcumin	Demethoxy Curcumin	Bisdemethoxy Curcumin
1	4	15870.95	4560.21	3544.25
2	4	16262.46	4578.25	3606.59
3	4	15860.46	4743.10	3548.54
4	4	15980.78	4516.60	3550.42
5	4	16300.67	4607.05	3566.45
6	4	15931.08	4606.08	3661.45
Average		16034.40	4601.88	3579.61
% RSD		1.22	1.67	1.29

IV. CONCLUSION

The HPTLC method developed was found to be suitable for the quantitation of curcumin, demethoxy curcumin and Bisdemethoxy curcumin in herbal mouth ulcer formulation in presence of other plant constituents and formulation excipients.The proposed method was found to be precise, simple, specific and sensitive and can be used for quality control purposes.

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