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NOVEL SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF CURCUMIN AND DICLOFENAC DIETHYLAMINE IN DEVELOPED DOSAGE FORM

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ABSTRACT: Rheumatoid arthritis is an autoimmune disease that involves inflammation of joints. Diclofenac diethylamine, as well as Curcumin, are widely used for symptomatic relief of pain and inflammation. The present study was aimed at providing a simple, accurate and economical spectrophotometric method for simultaneous determination of curcumin and diclofenac diethylamine from topical gel formulation. Simultaneous determination of both drugs namely, curcumin and diclofenac diethylamine was done by Absorbance correction method using phosphate buffer 5.5: ethanol (1:1) as the solvent. A topical gel containing curcumin microspheres and diethylamine were prepared using carbopo l971 as a gelling agent along with propylene glycol, preservatives, and triethanolamine. Diclofenac diethylamine showed an absorbance maximum at 278 nm whereas curcumin showed at 428.4 nm in phosphate buffer 5. 5: ethanol (1:1). The linearity of both drugs was found in the concentration range 2-26 μg/ml for Diclofenac diethylamine and 1-7 μg/ml for Curcumin respectively. This method was then applied successfully to pharmaceutical dosage form gel and no interference of excipients was found. Recovery for curcumin and diclofenac diethylamine was found to be 99.50% and 98.76% respectively. Validation of the method was done as per ICH guidelines to prove the suitability of the method for quantitative determination of both drugs diclofenac diethylamine and curcumin. These results indicated that the proposed method could be used for successful determination of curcumin and diclofenac diethylamine from pharmaceutical dosage form.

INTRODUCTION: Rheumatoid arthritis is a disease that includes pain, swelling, inflammation *etc.* Various drugs like NSAIDs, biologics, DMARs etc are used for treatment of inflammation associated with arthritis [1]. They can be easily given orally however; they have shortcomings like toxicity when given in high dose.

Hence, topical administration of such drugs is a better alternative as lesser dose with targeted action can be achieved. Also, as topical drugs do not go through first pass metabolism it is a better route for administration of drugs [2]. Curcumin 1, 7-bis [4-hydroxy-3-methoxyphenyl]-1, 6-heptadiene-3, 5-dione **Fig. 1**, is a natural polyphenolic compound.

Curcumin which is the main active constituent is isolated from the rhizome of turmeric, *Curcuma longa* Linn (Zingiberaceae) and is orange-yellow crystalline powder [3 - 6]. Curcumin is found to have many activities including anti-inflammatory activity which is mainly due to inhibition of cyclo-oxygenase, lipoxygenase, metabolism of arachi-

donic acid, cytokines (Interleukins and tumour necrosis factor), nuclear factor-KB and also due to the release of steroidal hormones. It is also found to stabilize lysosomal membrane and cause uncoupling of oxidative phosphorylation along with strong oxygen radical scavenging activity [7-9]. It has an empirical formula $C_{21}H_{20}O_6$ and molecular weight of 368.38 g/mol.
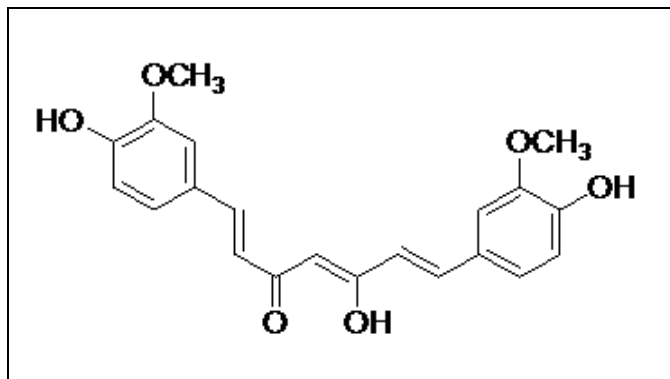

FIG. 1: CHEMICAL STRUCTURE OF CURCUMIN

Dicofenac diethylamine (DDEA), diethyl-ammonium 2-[(2, 6-dichloroanilino) phenyl] ace-tate **Fig. 2**, is white to light beige, crystalline powder. It has empirical formula $C_{18}H_{22}CL_2N_2O_2$ and molecular weight of 369.29 g/mol. It is sparingly soluble in water and acetone and freely soluble in ethanol and methanol. It is commonly used as an analgesic and anti-inflammatory agent and it works as a prostaglandin syntheses enzyme inhibitor thus inhibiting the activity of cyclo-oxygenase (COX) [10-13]. DDEA is official in British Pharmacopoeia wherein it recommends HPTLC and HPLC method for its analysis [14].
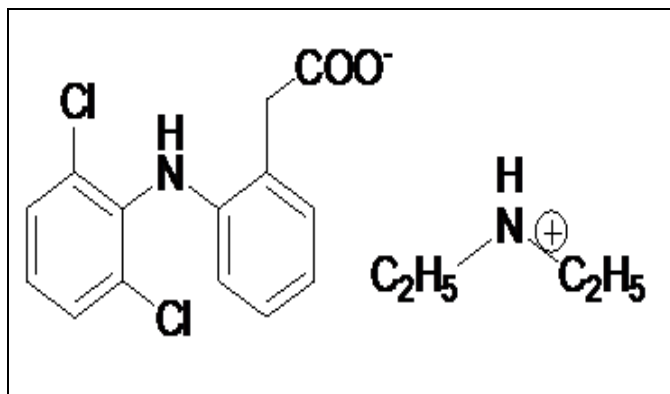

FIG. 2: CHEMICAL STRUCTURE OF DICLOFENAC DIETHYLAMINE

There are no marketed formulations available for this combination of both the drugs. As per the literature there are various analytical methods

available for determination of curcumin [15-18] and DDEA [19-22]. Therefore, for simultaneous deter-mination of these drugs, absorbance correction method was developed for analyzing the developed formulation. The objective of formulation was to develop gel containing DDEA and curcumin that would not only reduce dose and thus dose related toxicity but also provide targeted action as gel could be applied directly at the site of action without undergoing first pass metabolism. The method proposed was simple, accurate and validated as per ICH guidelines [23, 24, 25].

For quantitative determination of curcumin and diclofenac diethylamine from developed pharma-ceutical dosage form. In the present study, phosphate buffer 5.5: ethanol was used as a solvent for simultaneous determination of both the drugs. This method was simple, cheaper and easy to handle as compared to complex methods like HPTLC and HPLC and which also require solvents that are expensive.

MATERIALS AND METHODS: Curcumin was obtained from konarch herbals and healthcare, mumbai and diclofenac diethylamine from Unique Chemicals, Panoli. UV-Visible Spectrophotometer (UV Probe Version 2.33) of 1800 Shimadzu, Digital pH meter of Eutech Instruments and analytical weighing balance of citizon were used in the study. All the chemicals used were of analytical grade.

Preparation of Standard Stock Solution: An accurately weighed quantity of curcumin (10 mg) and diclofenac diethylamine (10 mg) were transferred separately to 10 ml volumetric flask and volume was made up with phosphate buffer 5.5: ethanol to obtain standard solution having concentration of curcumin (1000 µg/ml) and diclofenac diethylamine (1000 µg/ml). The solutions were then further diluted to get concentrations of 100 µg/ml of each drug.

Absorbance Correction Method: For deter-mination of λ_{max}, working standard solutions were scanned from 200 to 500 nm. For DDEA λ_{max} was found to be 278 nm and for curcumin 428.4 nm respectively. It was found that curcumin showed interference at 278 nm whereas DDEA did not show any interference at 428.4 nm **Fig. 3**.
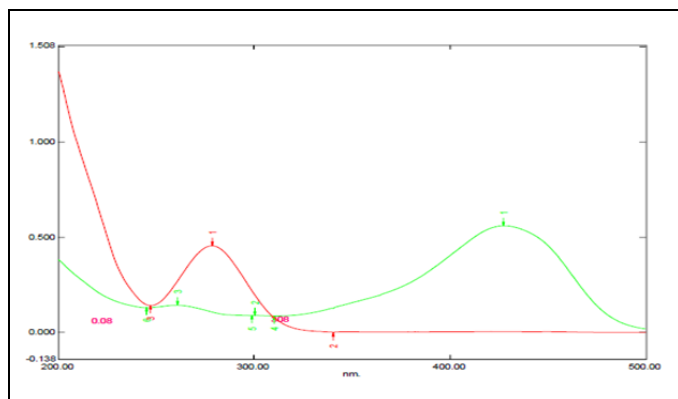
FIG. 3: SPECTRAL OVERLAY OF DICLOFENAC DIETHYLAMINE AND CURCUMIN

To construct Beer-Lambert's plot for curcumin and DDEA, stock solutions of both drugs were prepared in phosphate buffer 5.5: ethanol (1:1). Similarly, Beer-Lambert's plot was obtained for mixture containing both drugs curcumin and diclofenac in the ratio (1:1) at different concentrations.

Quantitative estimation of curcumin & DDEA in the mixture was carried out using the following equations:

$$c = A/ab$$

Where, A = Absorbance, a = A (1%, 1 cm), b = path length (b = 1), c = concentration (gms/100 ml = μg/ml

Corrected absorbance of DDEA =   Absorbance of mixture at 278.0 - Absorbance of curcumin at 428.4nm.

C_x = Corrected absorbance of DDEA at 278 / Absorptivity of it at 278 nm, C_y = Absorbance of mixture at 428.4 / Absorptivity of it at 428.4 nm.

Where, C_x is concentration of diclofenac diethylamine at 278 nm, C_y is concentration of curcumin at 428.4 nm.

Preparation and Analysis of Gel Containing Curcumin Microspheres and Diclofenac Diethylamine: For preparation of gel, curcumin microspheres (1%) and diclofenac diethylamine (1%) were added to Carbopol 971 which was used as gelling agent. Other excipients used were propylene glycol, preservatives, triethanolamine and purified water (q.s) [26 - 29]. For analysis, 0.5 g of gel formulation containing curcumin microspheres and diclofenac diethylamine was weighed and

transmitted to a 50 ml volumetric flask. The sample was dissolved in phosphate buffer 5.5: ethanol (1:1) by sonication for 30 min. It was then filtered and used for determination of curcumin and diclofenac diethylamine. A solution of placebo gel treated by the same procedure was used as blank to avoid interference of other components present in gel by spectrophotometric method.

Method Validation: The method of analysis was validated as per the ICH Q2B guidelines for validation parameters like linearity, accuracy, precision, robustness, LOD and LOQ. The accuracy studies were carried out using standard addition method, where in, percent recovery of curcumin and DDEA was determined. In this, curcumin and DDEA corresponding to 80%, 100% and 120% of label claim were added to drug samples. Then at each level of the concentration, six determinations were obtained and compared.

Precision study was carried out by determination of parameters like repeatability, intra-day precision, and inter-day precision. Accordingly, test solutions were analyzed in three replicates, on the same day andon three different days. For repeatability, same concentration was made six times and checked for repetition of data. The limit of detection (LOD) was calculated using equation LOD = 3.3σ/s whereas, Limit of quantification (LOQ) was calculated using equation LOQ = 10σ/s where σ is standard deviation of y intercept of the calibration curve (n=3) and s is the slope of regression equation. Robustness of the developed method containing curcumin and diclofenac diethylamine was evaluated at different pH values. Accordingly, pH 5.5, pH 5.4 and pH 5.6 were selected for analysis of robustness of developed method.

RESULTS AND DISCUSSION: In UV spectra of drugs curcumin and DDEA using solvent phosphate buffer 5.5: ethanol (1:1), it was found that both drugs absorbed at 278 nm, whereas only curcumin absorbed at 428.4 nm, hence absorbance correction method was applied. Calibration curves for curcumin and DDEA were found linear over the range of 1-7 μg/ml **Fig. 4** and 2-26 μg/ml **Fig. 5** and in mixture with the concentration range Curcumin: DDEA (1:1, 4:4, 7:7, 10:10, 13:13, 16:16, 19:19 μg/ml) at 278 nm and 428.4 nm.

This developed method was then applied for analysis of curcumin and DDEA in combined dosage form and was found to be very accurate at separating other excipients from drugs.
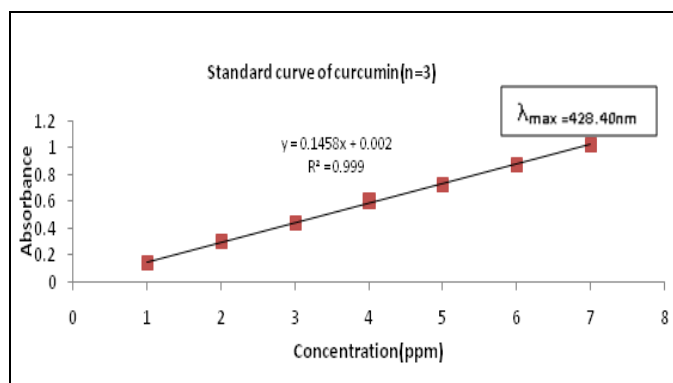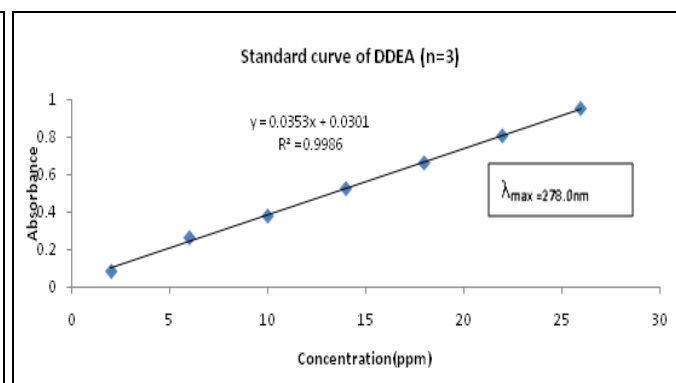

FIG. 4: LINEARITY OF CURCUMIN AT 428 nm

FIG. 5: LINEARITY OF DICLOFENAC DIETHYLAMINE AT 278 nm

LOD (Limit of quantification) was determined to be 0.2652 (µg/ml) for DDEA and 0.2138 (µg/ml) for curcumin, whereas LOQ (limit of quantification) was found to be 0.8038 (µg/ml) for DDEA and 0.6479 (µg/ml) for curcumin. Accuracy was within the range of 99.40-101.92% which ensured that the method is accurate. Repeatability, intra-day and inter-day precision study of the developed method confirmed adequate sample stability and method reliability where all the RSDs were less than 2%. Robustness analysis of the method showed that the results were in acceptable range with a deliberate variation of pH values. The results for validation are summarized in **Table 1**.

TABLE 1: REGRESSION ANALYSIS DATA WITH SUMMARY OF THE VALIDATION PARAMETERS

S. no.	Parameters (Units)	Curcumin	DDEA
1	Concentration range (µg/ml)	1-7 µg/ml	2-26 µg/ml
2	Regression equation	$y = 0.1458x + 0.002$	$y = 0.0353x + 0.0301$
3	Slope	0.1458	0.0353
3	Intercept	0.002	0.0301
4	Correlation coefficient (r^2)	0.999	0.9986
5	LOD (µg/ml)	0.2138	0.2652
6	LOQ (µg/ml)	0.6479	0.8038
7	Accuracy (% Recovery) (n = 6)	99.40-101.73	100.64-101.92
8	Robustness	Robust	Robust
9	Repeatability (% RSD)	1.48	1.66
10	Precision (Method precision, % RSD)		
11	Interday (n = 3)	0.08-0.26	0.63-1.68
12	Intraday (n = 3)	0.09-0.23	0.24-0.45

From the results obtained it was found that the method was developed for simultaneous determination of curcumin and DDEA and also validated according to the guidelines given by ICH. Further, this method was found to be accurate and precise. The validity of the proposed method was evaluated by the simultaneous determination of drug content of curcumin and diclofenac diethylamine present in a gel formulation. Recovery obtained was 99.50% (0.034% RSD) for curcumin and 98.76% (0.035% RSD) for diclofenac diethylamine respectively as shown in **Table 2**. Results obtained from this assay method proved that there was no interference of excipients and that this method could be used effectively for the analysis of curcumin and DDEA. Recovery studies were performed to check the accuracy and validity of the proposed method.

TABLE 2: SUMMARY OF ESTIMATION OF CURCUMIN AND DDEA IN FINAL GEL FORMULATION

S. no.	Drug	Total amount of drug added (µg/ml)	Total amount of drug recovered (mean ± SD)	% RSD	% Recovery
1	DDEA	10	9.876 ± 0.0034	0.035	98.76%
2	Curcumin	10	9.956 ± 0.0034	0.034	99.50%

The results obtained demonstrate that the proposed UV-spectrophotometric method (Absorbance correction method) for simultaneous determination of curcumin and DDEA is simple, reliable and economical. Therefore, this method can be used for the determination of both drugs either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

CONCLUSION: The developed absorbance correction method is foundto be simple, sensitive, accurate and precise and canbe appliedfor routine analysis of Curcumin and Diclofenac diethylamine. The developed method was validated as per ICH guidelines. From statistical analysis it was proved that the method is repeatable and selective for the analysis of Curcumin and Diclofenac diethylamine in combination as a single drug in bulk as well as in pharmaceutical formulations.

CONFLICTS OF INTEREST: The Authors of the article declare there is no conflict of interest

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