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DEVELOPMENT AND VALIDATION OF A SENSITIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR THE ESTIMATION OF *CURCUMIN* IN RAT PLASMA

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ABSTRACT: *Curcumin* is used as a potent anti-inflammatory agent in the treatment of rheumatoid arthritis and its clinical pharmacokinetics requires a sensitive method for estimation of its plasma concentration. Hence the aim of the study was to develop a rapid, selective, and sensitive HPLC method coupled with UV detection for the determination of *Curcumin* in rat plasma. The process of elution was carried out on Phenomenex Luna C18 (250*4.6 mm id, 5 µm particle size, 130 Å) column using acetonitrile and 1 % formic acid mixture in the ratio of 70:30 % v/v as mobile phase delivered with a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 420 nm. The method was found to be linear in the range of 100 to 3000 ng/ml with R2 close to one (0.9969). The limit of detection (LOD) and limit of quantification (LOQ) of CUR was found to be 2.82 ng/mL and 8.5 ng/mL, respectively. The method was validated for accuracy, precision, linearity, LOD, LOQ, and robustness. Hence the method developed can be used in routine analysis of *curcumin* in rat plasma which can be useful to determine the pharmacokinetics of *curcumin*.

INTRODUCTION: *Curcumin* (CUR) (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3, 5-dione), a low-molecular-weight polyphenol derived from the rhizomes of turmeric (*Curcuma longa* Linn). CUR is a golden pigment from turmeric, widely used in Asian cuisine as a spice, and has been used in Ayurvedic and Chinese medicine for centuries [1, 2]. CUR exhibits diverse pharmacological actions in the treatment of cardiovascular diseases, diabetes, Alzheimer's disease, multiple sclerosis, HIV20, cataract, and inflammatory bowel disease [3, 7].

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Potent antioxidant and anti-inflammatory activity of CUR lead to an array of metabolic, cellular and molecular activities that individually or in combination exhibit wide therapeutic activities, including anti-cancer and anti-arthritic activities [8, 9]. A survey of the literature, both *in-vitro* and *in-vivo*, suggests that CUR may have potential against

arthritis. CUR downregulates the activation of transcription factor NF-kB, thus leading to downregulation of the expression of TNF-alpha, adhesion molecules, MMPs, COX-2 , 5 –LOX and other inflammatory intermediates associated with arthritis [10, 11]. The literature survey revealed that numerous methods for estimating pure curcumin have been reported, including HPLC analysis with ultraviolet detection, fluorescence detection, radioimmunoassay and capillary zone electrophoresis with laser-induced fluorescence detection and matrix-assisted laser desorption [12, 15]. Further, various HPLC methods of estimation of *curcumin* have also been established in an animal model such as rat plasma [16, 17]. However, the methods of analysis developed in rat plasma involve complex procedures and lower sensitivity [17]. Hence, this paper describes a simple, economical, and convenient HPLC procedure with UV detection and simple sample preparation to determine CUR in rat plasma samples that have not been effectively tried out. The other objective was to validate the method as per the International Conference on Harmonization (ICH) guidelines [Q2 (R1)] [18].

MATERIALS & METHODS: *Curcumin* was received as a gift sample from Konark herbals and health care (Mumbai). Quercetin was purchased from Hi-media. Formic acid (FA) and acetonitrile of HPLC grade (purity, min 99.8%) were obtained from SD Fine Chem Pvt. Ltd (India). Ultrapure water, obtained from Millipore Direct-Q® 3 water purification system (Millipore Corporation, MA, USA), was employed to prepare samples and mobile phase. All other chemicals and solvents used were of analytical/HPLC grade.

Instrumentation: Chromatographic experiments were performed on Shimadzu LC-2010C HT HPLC system (Shimadzu, Japan) equipped with a quaternary pump, degasser, autosampler, column oven, and UV detector. Data acquisition, surveillance, and peak integration of output chromatograms was executed using the system control software Lab Solutions.

Separation of Plasma From Rat Blood: Rat plasma was collected from healthy female wistar rats (150-200 g). The animals were acclimatized to laboratory conditions and were kept on a standard diet with free access to water. The animals were subjected to fasting overnight prior to blood collection. The blood withdrawal was approved by Institutional Animal Ethics Committee (Approval No: AACP/IAEC/Mar 2019/05). Blood was collected from the retro-orbital plexus by micro capillary technique under light ether anesthesia. Followed collection, it was subjected to centrifugation to extract the plasma. The collected plasma was stored in a deep freezer at -4 °C until further analysis [19, 20].

LC Conditions: After the extensive screening of pH and composition of mobile phase solutions, the best peak separation of *curcumin* was achieved by isocratic elution with acetonitrile and 1 % formic acid mixture in the ratio of 70:30 % v/v as mobile phase delivered with a flow rate of 1.0 mL/min, and Phenomenex Luna C18 (250* 4.6 mm id, 5 µm particle size, 130 Å) column was used as stationary phase. Detection of *curcumin* was obtained using UV detector at 420 nm with 20 µL as injection volume. The temperature of the column was fixed at 25 °C and the autosampler at 4 °C.

Stock Solutions and Standards: CUR standard stock solution (1000 µg/mL) was prepared using methanol as solvent. The prepared stock solution was stored at 4 °C until further use. Intermediate standard solution (100 µg/mL) of CUR was prepared by diluting the standard stock solution with methanol. Quercetin was used as an internal standard (IS). A stock solution (1 mg/ml) of IS was prepared by accurately weighing an appropriate amount of quercetin and dissolving it in the methanol. Working standard solutions of 10 µg/ml were prepared by making appropriate serial dilutions of the IS stock solution in the mobile phase. The standard solutions were stored at -15 °C to -25 °C, protected from light, and were used within seven days.

Preparation of Calibration Standards: The calibration standards were in the range of 100 (standard 1) to 3000 ng/mL (standard 7) and were prepared by the precipitation method. 10 µL of the corresponding aqueous standard of *curcumin* (100-3000 ng/mL) and IS (1 µg/mL) was added to 80 µL plasma and vortexed for 2 min. 400 µL of chilled acetonitrile was added to this mixture and vortexed for 5 min. It was then subjected to centrifugation

(10,000 rpm, 4 ˚C for 5 min), and the supernatant was collected and analyzed using HPLC. All these solutions were stable for seven days when stored in the refrigerator at 4 ºC ± 2.0 ºC and were used within one week. The bulk spiked CC samples were stored at -20 ºC and brought to room temperature before use.

Method Validation: All the validation studies were carried out as per ICH guidelines [Q2 (R1)]. The parameters validated were selectivity and specificity, linearity, accuracy, precision, limit of detection, and quantification [18].

Specificity and Selectivity: The specificity and selectivity of the analytical method were assessed by preparing and examining a blank sample and a standard solution of CUR (3000 ng/mL) in six replicates. It was used to assess the extent of any interference caused by the blank sample at the retention of the analyte. The absence of detectable interfering peaks at the retention time of the analyte was considered as lack of interference.

Linearity: Calibration curves were constructed with seven standard solutions containing the two compounds simultaneously, ranging from 100 to 3000 ng/mL. Linearity was determined by calculating a regression line by the method of least squares, representing the peak area as a function of the standard concentration.

Accuracy and Precision: Precision was determined by repeatability (intraday) and intermediate precision (inter-day) for three consecutive days. Three standard solutions (quality controls), 100, 1000, and 3000 ng/mL, were analyzed according to the proposed method (intraday precision) for three consecutive days (interday precision). The relative standard deviation (RSD) determined at each concentration level should not exceed 15%, except for the lower limit of quantitation, where it should not exceed 20%. The accuracy was determined by measuring six replicates of the three quality controls and by calculating the percentage of bias for each compound according to the equation % accuracy = (observed concentration/nominal concentration) × 100. The mean value should be within 15% of the actual value, except at the LOQ, where it should not deviate by more than 20%.

Ruggedness: From the stock solution, sample solutions of different concentrations of 100, 1000, and 3000 ng/mL were equipped and analyzed by two different analysts employing analogous operational and environmental surroundings. The peak area was calculated for identical concentration solutions six times [21].

Limits of Detection and Quantification (LOD & LOQ): The following formulas were used to measure the LOD and LOQ of the analytical method developed for the estimation of *curcumin*.

$$LOD = (3.3 \times \sigma) / S, LOQ = (10 \times \sigma) / S$$

Where, σ = standard deviation of the response; and S = slope of the calibration curve.

RESULTS AND DISCUSSION:

Specificity and Selectivity: The specificity and selectivity of the analytical method were assessed by preparing and examining a blank sample and a standard solution of *curcumin* (3000 ng/mL). It was used to assess the extent of any interference caused by the blank sample **Fig. 1.** at the retention of the analyte. The absence of detectable interfering peaks at the retention time of the analyte was considered as lack of interference. The mean retention time for *curcumin* was 4.3 ± 0.5 min. IS showed a good sharp peak at 7.5 ± 0.5 min by UV detection at 420 nm **Fig. 2** & **3.** The proposed method is suitable for the quantification of CUR and IS in rat plasma samples. It showed specificity since no interfering peaks were observed from endogenous components of rat plasma at CUR and IS retention time.

Linearity: Standard solutions for calibration were prepared in the 100-3000 ng/mL concentration range and analyzed. The correlation coefficient (R2) was determined from the calibration curve (y=mx+c) represented by the plot of peak area (y) versus concentration (x) of the standard solutions. The calibration curve for quantifying *curcumin* appeared linear over a 100-3000 ng/mL concentration range with a correlation coefficient (R2) of 0.9969. The regression analysis of the calibration curve with the mean of peak area, slope, intercept, and the regression constructed by six different trials is depicted in **Fig. 4.** The results showed the existence of an excellent correlation between response factors and concentration of *curcumin*.

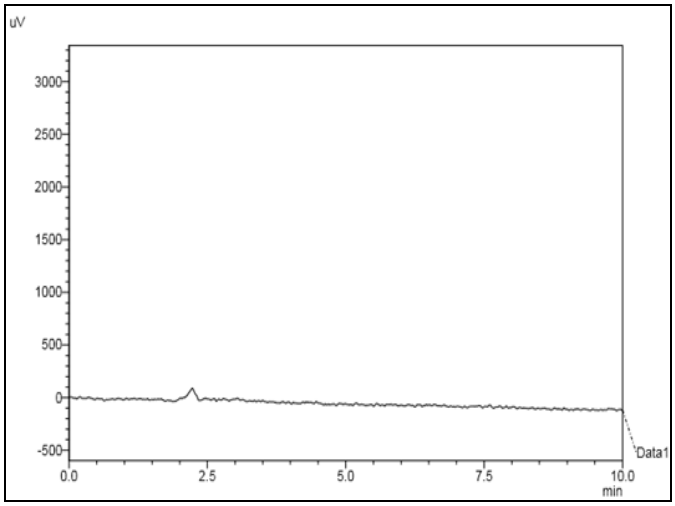

FIG. 1: HPLC CHROMATOGRAM OF BLANK RAT PLASMA

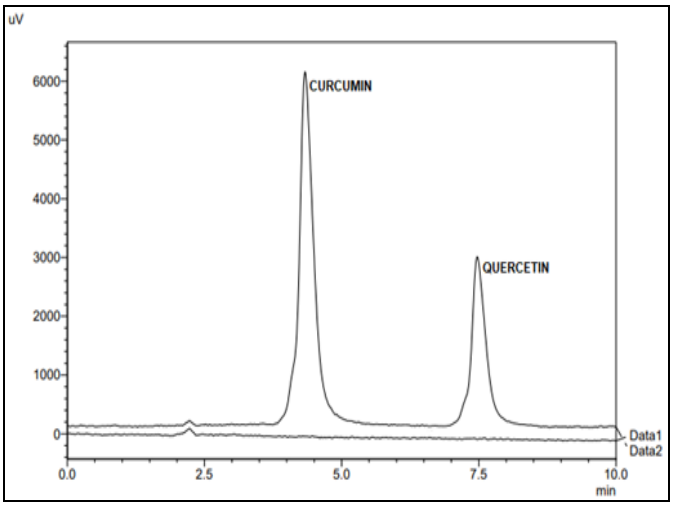

FIG. 2: OVERLAID CHROMATOGRAMS OF *CURCUMIN* & INTERNAL STANDARD (DATA 2) AND BLANK (DATA 1).

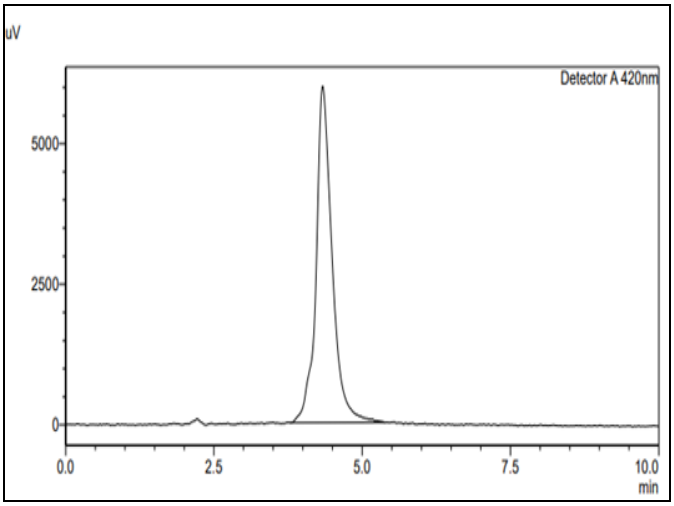

FIG. 3: HPLC CHROMATOGRAM OF RAT PLASMA SPIKED WITH CUR PURE DRUG

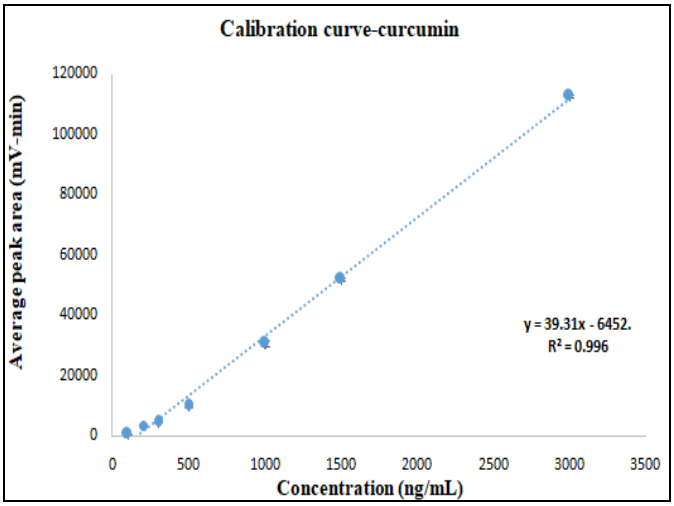

FIG. 4: CALIBRATION CURVE OF *CURCUMIN*

Accuracy and Precision: Samples containing the drug in concentrations of 100, 1000 and 3000 ng/ml were estimated for intra-day and inter-day studies to confirm the precision and accuracy of the developed method; the results are given in Table 1. All the data obtained fulfill the acceptance criteria. Intra-day and inter-day precision (% R.S.D.) of the methods were lower than 5% and were within the acceptable limits to be in concurrence with the guidelines for the united states pharmacopeial norms of method validation. Accuracy was with the deviation between the nominal concentration and calculated concentration for CUR well below the limit of ± 15%. The results obtained for the determination of precision and accuracy were reproducible and robust [20].

TABLE 1: INTRA AND INTER-DAY PRECISION AND ACCURACY DETERMINATION OF CUR CONCENTRATION IN SPIKED RAT PLASMA SAMPLES

Spiked Concentration (ng/ml)	Measured concentration (mean ± SD)		Precision (% RSD)		Accuracy (Relative error, %)	
	Intraday	Interday	Intraday	Interday	Intraday	Interday
100	101.45 ± 2.28	101.34 ± 1.087	2.25	1.07	1.45	1.64
1000	1014.96 ± 11.78	1013.40 ± 11.81	1.16	1.165	1.49	1.34
3000	3103 ± 10.65	3111.68 ± 5.76	0.34	1.85	3.43	3.72

(n = 6 at each concentration for intra-day and n= 6 for interday precision).

Ruggedness: The ruggedness was assessed by performing the analysis under the same experimental conditions by two different analyst. AS depicted in **Table 2,** contents of the drug were not affected by these changes as obvious from the low values of % RSD within the accepted limits, indicating ruggedness of the method developed.

TABLE 2: VALUES OF RUGGEDNESS STUDIES FOR THE DEVELOPED METHOD

Spiked Concentration (ng/ml)	Measured concentration by analyst 1 (mean ±SD)	%RSD	Measured concentration by analyst 2 (mean ±SD)	%RSD
100	100.18±2.42	2.41	101.99±2.69	2.64
1000	1024.39±11.98	1.169	1015.50±12.85	1.26
3000	3000.98±6.95	0.231	3000.43±6.56	0.218

Limits of Detection and Quantification: The LOD and LOQ for *curcumin* were found to be 2.82 ng/mL and 8.5 ng/mL, respectively. This indicates that the amount of drug which HPLC can detect can be as low as 3 ng/ml, and the amount of drug which can be quantified by the same can be as low as 9 ng/ml.

CONCLUSION: A rapid, simple, and sensitive HPLC method for the determination of CUR in spiked rat plasma was developed and validated. Sample preparation was done by protein precipitation technique.

Quercetin was employed as an internal standard, and no interfering peaks were observed at the elution time of CUR and IS. The method developed was accurate, reproducible, specific and provided excellent separation and enable the quantification of CUR in rat plasma.

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CONFLICTS OF INTEREST: None

REFERENCES:

1. Sikha A, Harini A and Hegde PL: Pharmacological activities of wild turmeric (*Curcuma aromatic* Salisb): a review. Journal of Pharmacognosy and Phytochemistry. 2015; 3: 1-4.
2. Sharma A, Raja MKMM and Manne R: Evaluation of physicochemical properties and chemical constituents of rasam, a traditional south indian soup. Journal of Pharmaceutical Research International 2021; 33(17): 18-25.
3. Hewlings SJ and Kalman DS: Curcumin: A review of its effects on human health. Foods 2017; 6: 92.
4. Lim GP, Chu T, Yang F, Beech W, Frautschy SA and Cole GM: The curry spice *curcumin* reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. Journal of Neuroscience 2001; 21(21): 8370-77.
5. Natarajan C and Bright JJ: *Curcumin* inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase-STAT pathway in T lymphocytes. Journal of Immunology 2002; 168(12): 6506-13.
6. Ahmad MZ, Alkahtani SA, Akhter S, Ahmad FJ, Ahmad J and Akhtar MS: Progress in nanotechnology-based drug carrier in designing of curcumin nano medicines for cancer therapy: current state-of-the-art. J Drug Target 2015; 1-21.
7. Kocaadam B and Sanlier N: Curcumin an active component of turmeric and its effect on health. Critical Reviews in Food Science and Nutrition 2017; 57: 2889-95.
8. Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR and Das PK: *Curcumin*, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzenesulphonic acid-induced colitis. British Journal of Pharmacology 2005; 139(2): 209-18.
9. Yadav VR, Prasad S, Kannappan R, Ravindran J, Chaturvedi MM, Vaahtera L, Parkkinen J and Aggarwal BB: Cyclodexrin-complexedcurcumin exhibits anti-inflammatory and anti-proliferative activities superior to those of curcumin through higher cellular uptake. Biochemical Pharmacology 2010; 80(7): 1021-32.
10. Khanna D, Sethi G, Ahn SK, Pandey KM, Kunnumakkara BA and Sung B: Natural products as a gold mine for arthritis treatment. Current Opinion in Pharmacology 2007; 7: 344-51.
11. Maroon CJ, Bost WJ and Maroon A: Natural anti-inflammatoryagents for pain relief. International Journal of Neurosurgery and Neurosciences 2013; 1-16.
12. Yang KY, Lin LC, Tseng TY, Wang SC and Tsai TH: Oral bioavailability of *curcumin* in rat and the herbal analysis from Curcuma longa by LCMS/MS. Journal of Chromatography B 2007; 853: 183-18.
13. Lechtenberg M, Quandt B and Nahrstedt A: Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of Curcuma domestica Val. and Curcuma xanthorrhizaRoxbby capillary electrophoresis. Phytochemical Analysis 2004; 5: 152-58.
14. Sharma K, Agrawal SS and Gupta M: Development and validation of UV Spectrophotometric method for the estimation of curcumin in bulk drug and pharmaceutical dosage forms. International Journal of Drug Development and Research 2012; 4: 375-80.
15. Singh SP and Wahajuddin Jain GK: Determination of curcumin in rat plasma by liquid–liquid extraction using lc–ms/ms with electrospray ionization: assay development, validation and application to a pharmacokinetic study. Journal of Bioanalysis and Biomedicine 2020; 2: 79-84.

16. Ma Z, Shayeganpour A, Brocks D, Lavasanifar A and Samuel J: High-performance liquid chromatography analysis of *curcumin* in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. Biomedical Chromatography 2007; 21(5): 546-52.
17. Liu A, Lou H, Zhao L and Fan P: Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. Journal of Pharmaceutical and Biomedical Analysis 2006; 40: 720-27.
18. ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Methodology, Q2 (R1), International Conference on Harmonization of Technical Requirements for Registrations of Pharmaceuticals for Human Use, ICH, Geneva, Switzerland 2005.
19. Raichur V and Kusumdevi V: Development and validation of a highly sensitive high-performance liquid chromatography (hplc) method for the estimation of methotrexate (mtx) pure drug and marketed formulation in spiked rat. Plasma 2016; 8(3): 313-17.
20. Mustafa S and Kusumdevi V: Liquid chromatographic assay for the analysis of kanamycin sulfate nano-particles in rat after intramuscular administration: application to a pharmacokinetic study. Journal of Applied Pharmaceutical Science 2016; 6(08): 57-66.
21. Mathur M and Kusumdevi V: Design of experiment utilization to develop and validate high-performance liquid chromatography technique for estimation of pure drug and marketed formulations of Atorvastatin in spiked rat plasma samples. International Journal of Pharmaceutical Sciences and Research 2017; 8(4): 1708-16.

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