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SIMPLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF METHOTREXATE AND CURCUMIN IN BULK DRUGS

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Abstract:
A method of estimation for the simultaneous estimation of methotrexate and curcumin in their bulk powder is developed and validated as per ICH guidelines. The method involved measurement of absorbance of the methotrexate and curcumin at the λmax of the drugs i.e., 307nm and 425nm in methanol. Calibration curves of methotrexate and curcumin were found to be linear in the range 2-18µg/mL and 1-7 µg/mL respectively. The correlation coefficient values R^2 was found to be 0.999. LOD and LOQ were 122.23ng/mL and 370.4ng/mL for methotrexate, 12.69ng/mL and 38.48ng/mL for curcumin. The percentage recovery at various concentration levels varied in between 99 to 101% for both the drugs estimated, thereby confirming accuracy of the method developed. In the precision study, the %RSD was found to be less than 2%, indicating the method is precise. Hence it can be concluded that the method developed is simple, accurate, precise, and economical.

Keywords:- *Methotrexate, Curcumin, Simultaneous estimation and Validation.*

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INTRODUCTION:

Methotrexate (MTX) chemically is 4-amino-N_{10}-methylpteroyl glutamic acid. It is an analogue of folic acid. Methotrexate is extensively used in the treatment of cancer and rheumatoid arthritis [1-2]. 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. Being a potent antioxidant and possessing anti-inflammatory activity, CUR led to an array of metabolic, cellular and molecular activities that individually or in combination exhibit diverse therapeutic activities such as anti-rheumatic and anti-cancer [3-4]. Both the drugs show effectiveness in the treatment of rheumatoid arthritis and cancer. Combination of both the drugs can lead to greater therapeutic effectiveness than the single drug alone [5-6]. The effectiveness of combining MTX and CUR has been proved by many researchers in the treatment of rheumatoid arthritis and cancer [7-8]. In addition, literature survey reveals that there was no method of estimation available for the selected drug combination by UV. Hence this works aims at developing a simple, rapid and economic method for simultaneous estimation of methotrexate and curcumin in bulk drug by UV by applying simultaneous equation method.

MATERIALS & METHODS:

Instrument details: - A double beam UV-visible spectrometer (UV-1700, Shimadzu) was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Methotrexate was obtained from Mac-Chem Product India Pvt. Curcumin was obtained from Konark Herbals & Health Care, Mumbai. Methanol was purchased from SD Fine-Chem Ltd. The other chemicals were of analytical grade.

Preparation of Standard Stock Solution: -

A standard stock solution of methotrexate and curcumin was prepared in methanol separately. Accurately weighed, 10mg of methotrexate and curcumin was dissolved in 100 ml of methanol to result in a concentration of 100µg/ml. Working standards were prepared using this stock solution.

Simultaneous Equation Method Development: - Working solutions of both drugs were scanned in the UV range 200– 800 nm. The overlay spectra of both drugs were recorded. From overlain spectra, wavelengths 307 nm (λmax of MTX) and 425 nm (λmax of CUR) were selected for analysis of both drugs using simultaneous equation method (λ1- 307 nm for MTX and λ2-425nm for CUR).

Different concentrations of the drug ranging from 2mcg/ml to 18 mcg/ml for methotrexate and from 1mcg/ml to 7mcg/ml for curcumin were prepared by diluting varying volumes of working standard solution of respected drug with methanol. These solutions were scanned and absorbance was measured at 307 and 425 nanometers respectively using a UV-Visible Spectrophotometer, UV-1700.

The concentration of drugs x (MTX) and y (CUR) in sample solutions were determined by the SE

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1} - ax1ay2$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1} - ax1ay2$$

where Cx and Cy are the concentration of MTX and CUR, $A1$ and $A2$ are the absorbance of sample solution at 307 nm and 425 nm, respectively, $ax1$ and $ax2$ are absorptivity of MTX at 307 nm and 425 nm, and $ay1$ and $ay2$ are absorptivity of CUR at 307 nm and 425 nm, respectively.

Determination of Absorptivity Value. The absorptivity value of MTX and CUR from each solution was calculated using following formula and the results are presented in table 1 and 2:

$$Absorptivity = \frac{Absorbance}{Concentration}(gm/1ooml)$$

Method Validation: The method was validated according to ICH Q2 (R1) guidelines for the following parameters.

Linearity and range: Linearity of the developed method was established from the calibration curves prepared using linear regression analysis. Based on the measurement of the absorbance for the calibration standards, graph was plotted against the respective concentration to obtain the standard calibration graph. The procedure was repeated six times and the average values of absorbance were calculated. The data obtained was statistically evaluated to obtain the standard deviation of the said values and regression coefficient. The calibration curve range was decided depending upon the Beer-lamberts law adherence.

Accuracy: - The accuracy of the developed method was determined by performing recovery studies. The studies were carried out by taking standard mixture solution of MTX and CUR and absorbance was

determined at respective concentration (table 3). Three different concentration were prepared in triplicate at level of 80%, 100% and 120% of its predefined concentration (i.e. 2, 4 & 16 and 1, 2 & 4 for MTX and CUR respectively)

Precision:

Repeatability: - Repeatability was determined by analyzing different levels of drug concentrations in mixed standards from independent stock solutions with minimum three replication within in the same day i.e, 0, 8th and 24th hour (table 4).

Intermediate precision:- Inter-day variations in estimation were determined to assess intermediate precision of the proposed method. The absorbance of six determinations of working solution was taken on different days. Concentration of methotrexate and curcumin in each replicate was calculated. The standard deviation was calculated from the concentrations of methotrexate and Curcumin in six determinations of working solution (table 4).

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by the proposed method were determined using calibration standards. The LOD and LOQ were calculated as per equations 1 and 2 respectively,

LOD = 3.3 (SD Intercept / Slope)..... (1)
LOQ = 10 (SD Intercept / Slope)..... (2)
Where "SD intercept" is the standard deviation of the intercept of regression line and "Slope" is the slope of the calibration curve.

RESULTS AND DISCUSSIONS:

Methotrexate and Curcumin act as anti-rheumatic drugs which have been used in the treatment of rheumatoid arthritis and cancer, hence development of analytical method which is simple and economic is the need of the hour. There are various techniques for estimation of the drugs, of all the simplest and most economic method is UV-spectrophotometric method, because the method is cost effective and less laborious in comparison to the HPLC method of analysis.

Linearity: - The overlain spectra (Fig 1) of MTX and CUR exhibit lambda max of 307nm and 425nm for MTX and CUR respectively**.** The calibration curve was plotted by taking absorbance versus concentration in the range of 2-18µg/mL and 1-7 µg/mL for MTX and CUR respectively (Fig 2 & 3). The curves were linear and the correlation coefficient value (R^2**)** was 0.9992 for MTX and CUR.

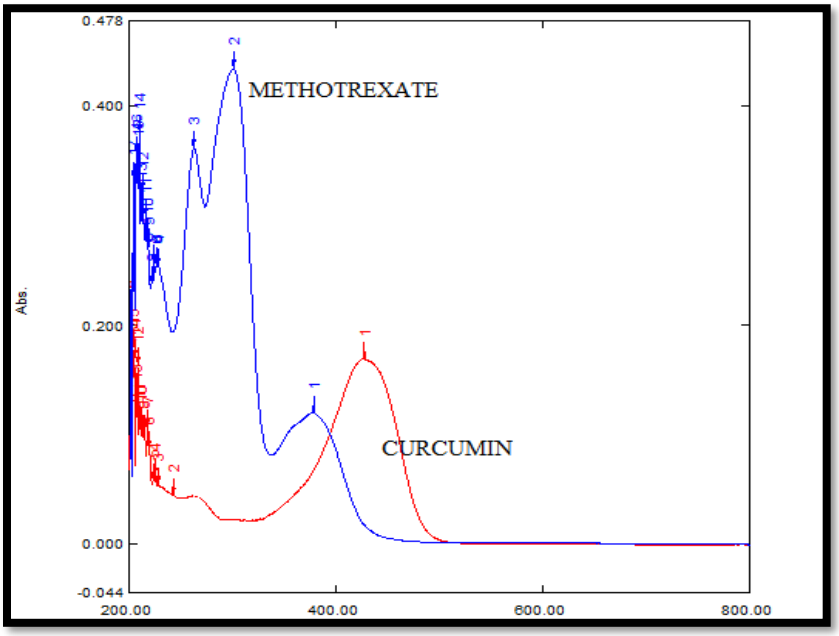

Figure 1: Overlain spectra of methotrexate and curcumin.

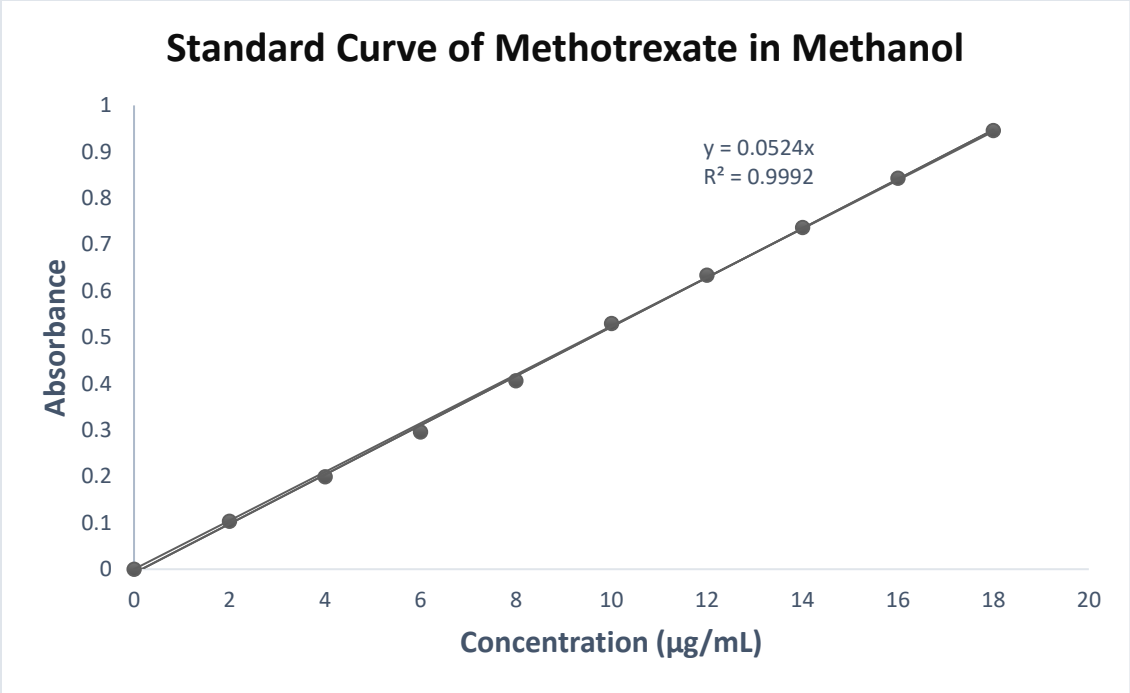

Figure 2: Calibration Curve of Methotrexate

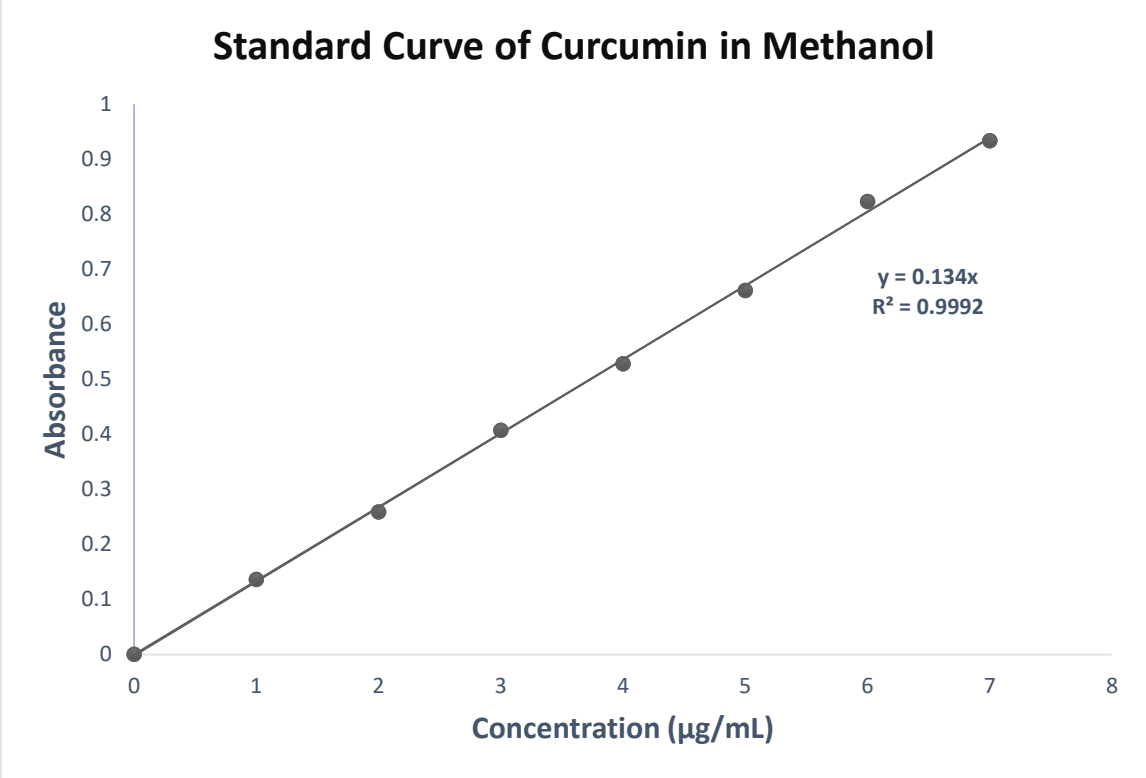

Figure 3: Calibration Curve of Curcumin

Table1:-Absorptivity value for MTX

Concentration (µg/ml)	Absorbance λ_1 -307 nm	Absorptivity λ_1 -307 nm	Absorbance λ_2 -425 nm	Absorptivity λ_2 -425 nm
2	0.103	515	0.004	20
4	0.199	497.5	0.008	20
6	0.296	493.3333	0.014	23.33333
8	0.406	507.5	0.017	21.25
10	0.53	530	0.022	22
12	0.634	528.3333	0.03	25
14	0.737	526.4286	0.036	25.71429
16	0.843	526.875	0.036	22.5
18	0.946	525.5556	0.039	21.66667
	Absorptivity at $\lambda_{1=}$ 516.7251		Absorptivity at $\lambda_{2=}$ 22.38492	

Table 2:- Absorptivity value for CUR

Concentration (µg/ml)	Absorbance λ_1 -307 nm	Absorptivity λ_1 -307 nm	Absorbance λ_2 -425 nm	Absorptivity λ_2 -425 nm
1	0.036	360	0.136	1360
2	0.033	165	0.259	1295
3	0.05	166.6667	0.407	1356.667
4	0.075	187.5	0.528	1320
5	0.076	152	0.661	1322
6	0.103	171.6667	0.823	1371.667
7	0.106	151.4286	0.933	1332.857
	Absorptivity at $\lambda_{1=}$ 193.466		Absorptivity at $\lambda_{2=}$ 1336.884	

Accuracy:- The accuracy of the method is required to be obtained within the linearity range of the method developed. Accuracy of the developed method was dogged from recovery studies. The recovery of MTX and CUR from the standard mixture solution was found to be in the range of 99-101% (table 3) and the %RSD was found to be less than 2%, indicating the method is accurate and reproducible.

Table 3: Recovery results of MTX and CUR

Drug in standard mixture (µg/ml)		Concentration %	Amount of drug added (µg/ml)		Mean % Recovery	%RSD	Mean % Recovery	%RSD
MTX	CUR		MTX	CUR	MTX		CUR	
2	1	80	1.6	0.8	100.01± 0.306	0.306	100.05± 0.862	0.860
2	1	80	1.6	0.8				
2	1	80	1.6	0.8				
4	2	100	2	2	99.95 ± 0.238	0.238	99.87±0.284	0.285
4	2	100	2	2				
4	2	100	2	2				
16	4	120	19.2	4.8	100.01± 0.165	0.165	99.21±0.848	0.854
16	4	120	19.2	4.8				
16	4	120	19.2	4.8				

Precision:- :Precision of the method was verified by repeatability and intermediate precision studies. Intermediate precision of the method was checked by assaying the sample solution on three different days (table 4). This study indicates that the solutions can be analyzed up to 120 hours without affecting the stability of the drug in the solvent. The % RSD value was found to be less than 2%, indicating the method is precise.

Table 4: Precisions results of MTX and CUR

Parameters	Sampling time	Methotrexate		Curcumin	
		% Assay	% RSD	% Assay	% RSD
Repeatability (n=3)	0 hour	100.50±0.899	0.899	100.74±1.055	1.047
	8th hour	100.19±0.77	0.777	100.49±0.703	0.700
	24th hour	101.46±1.19	1.172	101.24±0.930	0.919
Intermediate Precision (n=6)	Day 1	100.62±0.389	0.386	101.24±0.569	0.562
	Day 3	101.30±1.25	1.23	100.87±0.569	0.565
	Day 5	99.71±1.55	1.56	100.37±0.373	0.371

Limit of Detection and Limit of Quantification: - LOD and LOQ was found to be 122.23ng/mL and 12.69ng/mL for methotrexate, 370.4ng/mL and 38.48ng/mL for curcumin respectively. Hence it can be concluded that the method can be utilized to detect the drug at nanogram level.

CONCLUSION:

The method developed was found to be simple, precise and accurate. Further the method was selective for the simultaneous estimation of methotrexate and curcumin in pure form. Hence can be used for the routine analysis in the quality control laboratories.

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