Hygeia.J.D.Med. July 2017 – December 2017 ISSN 2229 3590
Hygeia :: journal for drugs and medicines
July 2017
Open Access www.hygeiajournal.com
Research article section: Pharmaceutical Analysis
A Half Yearly Scientific, International, Open Access Journal for Drugs and Medicines
DOI:10.15254/H.J.D.Med.9.2017.160 Research Article

SIMULTANEOUS DETERMINATION OF CURCUMIN AND GEFITINIB IN PURE FORM BY USING UV SPECTROPHOTOMETRIC METHOD

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Keywords: Curcumin, Gefitinib, simultaneous estimation, UV spectrophotometric method

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Received: 12 January 2017,
Revised: 9 February 2017
Accepted: 25 March 2017,
Available online: 15 July 2017

ABSTRACT

Plan: *Development and Validation of a method for the UV* simultaneous *determination of curcumin and Gefitinib.*

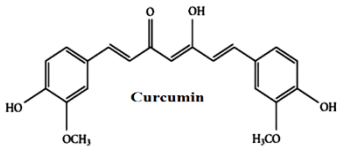
Preface: *U.V Spectrophotometric method have been widely employed in determination of individual components in a mixture or fixed dose combination. For the ternary mixture containing Curcumin and Gefitinib, no spectrophotometric method for simultaneous evaluation has been reported so far. Thus our aim is to develop simultaneous equation method for estimation of the ternary mixture using U.V spectrophotometry.*

Methodology: The method was validated as per ICH guidelines. The recovery studies confirmed the accuracy and precision of the method.

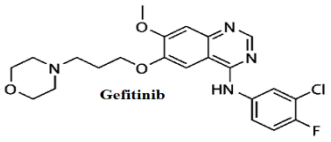
Outcome: *It was successfully applied for the analysis of the drug in bulk and could be effectively used for the routine analysis.*

1. INTRODUCTION

Curcumin, chemically, ((1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione) and Gefitinib, chemically, (N (3 chlorofluorophenyl) 7 methoxy 6 (3 morpholinopropoxy) quinazolin -4-amine)[1,2,3,4]. Whereas curcumin is a polyphenolic compound with molecular weight 368.39 g/mol and gefitinib is a quinazolamine with molecular weight 446.90 g/mol. Both are the anticancer drug is applicable for various brain and lung cancers.UV spectrophotometric studies revealed that maximum light absorption for Curcumin and Gefitinib are at 423 nm and 254 nm respectively [5,6,7,8].



Curcumin



Gefitinib

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Hygeia journal for drugs and medicines, 2229 3590
Rid: G-1426-2017

2. MATERIALS AND METHOD

2.1. Material

Curcumin (CRM) supplied as a gift sample by Sunpure Extracts Pvt. Ltd (Delhi, India) and Gefitinib (GFT) supplied as a gift sample by Khandelwal industries Pvt Ltd (Mumbai, India) both drug was used as working standard.

2.2. Instrumentation

A double beam UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) connected to a computer loaded with spectra manager software UV Probe was used. The spectra were obtained with the instrumental parameters as follows: Wavelength range: 200–800 nm. All weights were taken on an electronic balance (Model Shimadzu AUX 120).

2.3. Selection of common solvent: After the solubility study of both drugs in different solvents, methanol was confirmed as a common solvent for developing spectral characteristic (Figure 1).

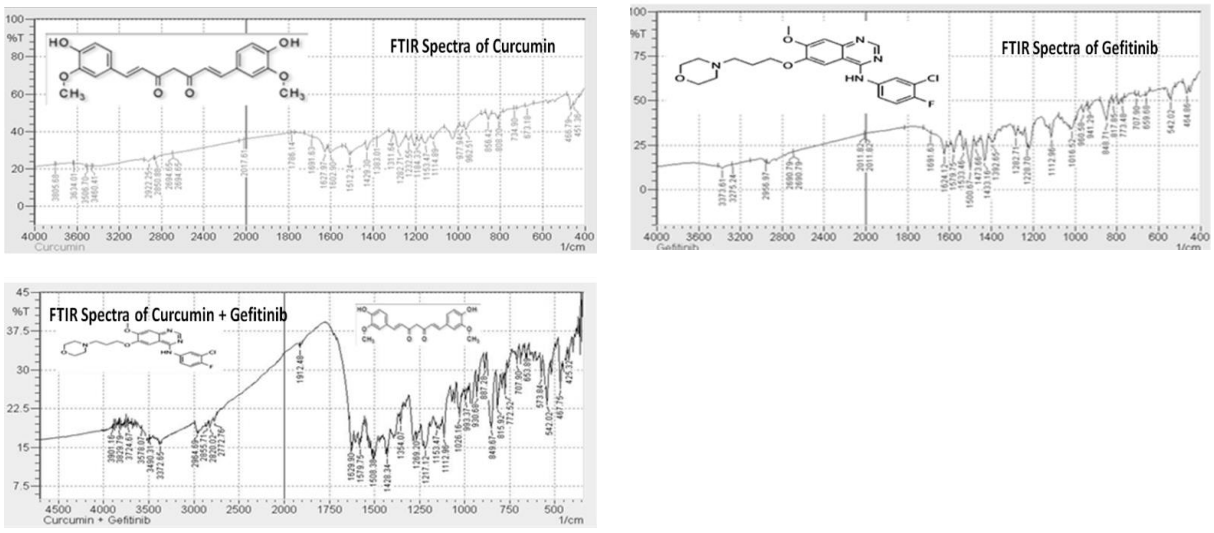

Figure 1. FTIR Spectra of drugs

2.4. Preparation of standard stock solution

According to European pharmacopoeia, 10 mg of curcumin was dissolve in 100 ml of methanol (100 µg/mL). Out of this stock 0.2-1.2 ml was pipetted and diluted up to 10 ml by methanol (2-12 µg/mL) and examined between 200-800 nm and 10 mg of Gefitinib was dissolve in 100 ml of methanol (100 µg/mL). Out of this stock 0.2-1.2 ml was pipetted and diluted up to 10 ml by methanol (2-12 µg/mL) and examined between 200-400 nm. The maximum absorbance was determined using UV-Vis Spectrophotometer (UV-1700, Shimadzu, Japan) to confirm the λmax of the drugs.

2.5. Drug: drug interference study

Standard stock solution (100 µg/ml) of curcumin and Gefitinib was prepared separately in methanol by serial dilution technique. The absorbance values for curcumin and Gefitinib were recorded at 423 nm and 254 nm respectively, using methanol as a blank. Absorptivity values a (1%. 1 cm) were calculated for both wavelengths from absorbance values.

2.6. Simultaneous Equation Method

From the standard stock solutions both drug (100 µg/mL), 0.2-1.2 ml of both the solutions were taken and made it to final concentration of 2-12 µg/ml. Absorbance was measured at both the wavelengths (423 nm and 254 nm) by using methanol as blank.

The reading were taken in triplicate. Absorbance maxima of both the drugs were recorded at both the wavelengths. The concentration was determined by using simultaneous equation method.

$A_1 = ax_1Cp + ay_1Cs$ …… (At 423 nm)
$A_2 = ax_2Cp + ay_2Cs$ …… (At 254 nm)
A_1 = absorbance value of the sample solution at 423 nm
A_2 = absorbance value of the sample solution at 254 nm
ax_1 = absorptivity of curcumin at 423 nm
ax_2 = absorptivity of curcumin at 254 nm
ay_1 = absorptivity of Gefitinib at 254 nm
ay_2 = absorptivity of Gefitinib at 423 nm
Cp = concentration of the Gefitinib in µg/ml
CS = concentration of the curcumin in µg/ml

2.7. Q - Analysis (Absorbance Ratio Method)

Q Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length. In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths: One being the λmax of one of the component (λ2) and the other being a wavelength of equal absorptivities of the two components i.e. an Iso-absorptive point.

2.8. Study of Beer's Lambert Law

The solutions having concentrations in range 2-12 µg/ml for both Curcumin and Gefitinib were prepared in methanol using working standard solution. The absorbances of resulting solutions were measured at 423 nm, 254 nm and 242 nm. Calibration curves were plotted at these wavelengths. Both the drugs obeyed linearity individually and combination within the concentration range of 2-12 µg/ml for both Curcumin and Gefitinib.

2.9. Validation of analytical method

The analytical performance characteristics which may be tested during methods validation: % Recovery, Precision, Ruggedness and sensitivity.

3. RESULTS AND DISCUSSION

3.1. Method Development

The solution of Curcumin in methanol was found to exhibit maximum absorption at 423 nm and The solution of Gefitinib in methanol was found to exhibit maximum absorption at 254 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and Thus the procured drug sample of curcumin complies with the reference spectra (Figure 2) and produce drug sample Gefitinib complies with the reference spectra (Figure 3).

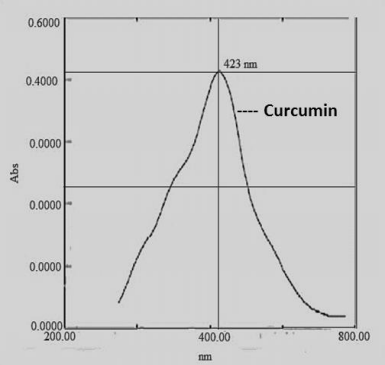

Figure 2. UV spectra of Curcumin

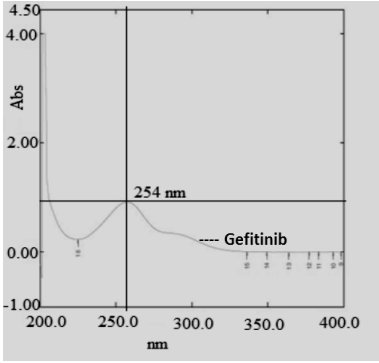

Figure 3. UV spectra of Gefitinib

3.2. Linearity study

Accurately weighted Curcumin (10 mg) and Gefitinib (10 mg) was dissolved in 100 ml of methanol to obtain working standard of 100 µg/ml. Aliquots were pipetted from the stock solution of drug and were transferred to 10 ml volumetric flask, the final volume was adjusted with methanol so that concentration of 2-12 µg/ml could be made. Absorbance of the above solution were taken at 423 nm for curcumin and 254 nm for Gefitinib using UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan) against the blank solution prepared in the same manner without adding the drug. A graph of absorbance vs concentration was plotted (Figure 4, Figure 5 and Table 1).

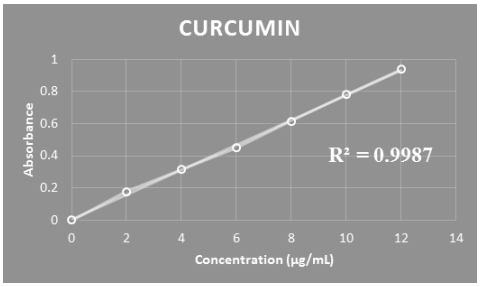

Figure 4. Calibration curve of Curcumin

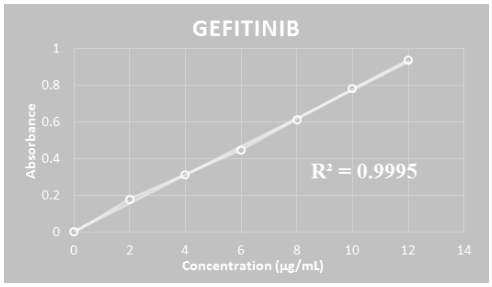

Figure 5. Calibration curve of Gefitinib

Table 1. Linearity study

Parameters	*Curcumin*	*Gefitinib*
λmax(nm)	423 nm	254 nm
Linearity range (µg/ml)	2-12 µg/ml	2-12 µg/ml
Absorptivity	.029	.031
Regression coefficient(r2)	0.9987	0.9995

Estimation of Absorptivity (E 1%, 1cm) values at Selected Wavelengths: The Absorptivity (E 1%, 1cm value) of curcumin and Gefitinib drugs was calculated at 423 nm and 254 nm.

3.3. Determination of Iso-absorptive point and selection of suitable Wavelength

An Iso-absorptive point (a wavelength of equal absorptivity of the two components) was determined by taking overlain spectrum of the solutions curcumin and Gefitinib (10 µg/ml each) in methanol in UV range against the solvent blank. From the overlain spectra of the two drugs, it was found that Curcumin showed λ max at 423 nm and Gefitinib showed λmax at 254 nm. Iso-absorptive point was found out at 242 nm, as Iso-absorptive point was selected for estimation of Drug simultaneously (Figure 6).

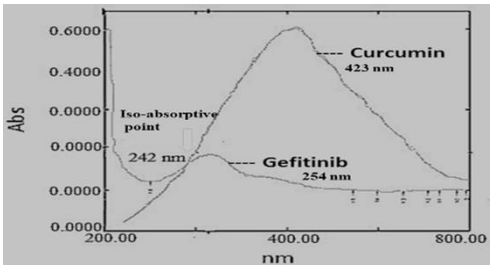

Figure 6. Overlay spectra of Curcumin and Gefitinib with Iso absorptive point

The individual concentration range for beer-lambert was found 2-12 µg/ml for both Curcumin and Gefitinib at 423 nm and 254 nm with correlation coefficient 0.9987 and 0.9995 respectively shown in Table 1. UV scan of 2-12 µg/ml solution of curcumin and Gefitinib combination showed the absorption maxima at 423 nm, 254 nm and 242 nm. The simultaneous estimation was done to check the interference between both the drugs at the λmax of one another.

By substituting absorbance and absorptivity values of table in simultaneous equation, C_1 and C_2 were calculated, C_1: 9.79 µg/ml, C_2: 10 µg/ml. The percentage of curcumin and gefitinib recovered after the combination was found to be 99.9 % and 100 % respectively indicating no interference between both the drugs. The Linearity was observed by the linear regression equation method for Curcumin and Gefitinib in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. It is validated as per ICH guidelines.

3.4. Validation of analytical method

3.4.1. Recovery

Recovery study is performed by standard addition method by adding the known amount of Curcumin and Gefitinib (Working standard) at two different concentration levels i.e 80%, 100% of assay concentration and % recovery for all these drug were calculated. Result was reported in Table 2.

Table 2. Recovery study

Drug	*Initial amount (µg/ml)*	*Added Amount (µg/ml)*	*% Recovery*	*% RSD (n = 3)*
Curcumin	2	1.8	99.25	0.05
	2	2	100.88	0.07
Gefitinib	2	1.8	99.44	0.01
	2	2	101.15	0.05

3.4.2. Precision

Intra-day precision was determined by analysing, the two different concentrations 2 mg/ml, 3 mg/ml containing curcumin and Gefitinib, for three times in the same day (n = 3) Table 3. Inter-day variability was assessed using above mentioned three concentrations analysed on three different days, over a period of one week (n = 3) Table 3.

Table 3. Intra-day and Inter-day Presion

		Intra - Day		*Inter - Day*	
Drug	*Con. (µg/ml)*	*Mean ± SD*	*% RSD*	*Mean ± SD*	*% RSD*
Curcumin	2	1.9 ± 0.0011	0.04	1.9 ± 0.0015	0.01
	3	2.9 ± 0.0020	0.09	3.0 ± 0.0051	0.04
Gefitinib	2	1.8 ± 0.0051	0.06	2.0 ± 0.005	0.07
	3	2.7 ± 0.0030	0.02	3.0 ± 0.0011	0.06

3.4.3. Ruggedness

From stock solution, sample solution containing curcumin and Gefitinib (2 µg/ml) was prepared and analysed by two different analysts using similar operational and environmental conditions (Table 4) (n = 3).

Table 4. Ruggedness study

	% Amount Found		*% RSD*	
Drug	*Analyst I*	*Analyst II*	*Analyst I*	*Analyst II*
Curcumin	99.47	98.75	0.07	0.01
Gefitinib	100.24	100.00	0.09	0.05

3.4.4. Sensitivity

Sensitivity of the proposed method were estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ) (Table 5).

Table 5. Sensitivity study

Drug	*LOD*	*LOQ*
Curcumin	0.18 ± 0.004	0.65 ± 0.010
Gefitinib	0.16 ± 0.002	0.63 ± 0.012

4. CONCLUSION

The proposed UV spectrophotometric method was found very simple, rapid and economical. However, the most important outcome of the simultaneous estimation is that we can formulate and analyse both the drugs in combination for any suitable dosage form in a very safe and effective way. The method is validated in compliance with ICH guidelines is suitable for simultaneous estimation of curcumin and gefitinib with excellent recovery, precision and linearity.

ACKNOWLEDGEMENTS

The authors are grateful to Hon. Principal, SES's, R. C. Patel Institute of Pharmaceutical Education and Research, Dr. S. J. Surana and wish to acknowledge all those who are involved directly or indirectly for compilation of this article.

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Sagar Savale. Simultaneous determination of curcumin and gefitinib in pure form by using UV spectrophotometric method. *Hygeia.J.D.Med* **2017**; 9(1):1-8. Available from http://www.hygeiajournal.com , DOI: 10.15254/H.J.D.Med.9.2017.160