Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Simultaneous Estimation of Curcumin and Gefitinib in Bulk, Plasma and Brain Homogenate by RP-HPLC Technique

SAGAR KISHOR SAVALE[1]*

[1]Department of Pharmaceutics, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur 425-405, MS, India

ARTICLE DETAILS

Article history:
Received on 14 August 2018
Modified on 20 September 2018
Accepted on 24 September 2018

Keywords:
RP-HPLC,
Simultaneous Estimation,
Plasma,
Brain Homogenate,
Curcumin,
Gefitinib.

ABSTRACT

The present study was aimed at developing a reversed phase high performance liquid chromatography (RP-HPLC) method for simultaneously determination of curcumin (CRM) and gefitinib (GFT) in bulk, plasma and brain homogenate and hydrochlorothiazide was used as an internal standard. A new simple, rapid, selective, precise and accurate reverse phase high performance liquid chromatography method has been developed. The separation was achieved by using C-18 column (Qualisil BDS C18, 250 mm x 4.6 mm I.D.) coupled with a guard column of silica, mobile phase was consisting of acetonitrile: water with 1% formic acid (30:70 v/v). The flow rate was 0.2 ml/min and the drug was detected using PDA detector at the wavelength of 242 nm. The experimental conditions, including the diluting solvent, mobile phase composition, column saturation and flow rate, were optimised to provide high-resolution and reproducible peaks. The method was developed and tested for linearity range of 10-60 µg/mL for bulk analysis and 200-800 ng/mL for plasma and brain homogenate. The method was validated as per ICH guidelines, the developed method was validated in terms of linearity, application of the proposed method to bulk sample, recovery, precision, repeatability, ruggedness, sensitivity (LOD and LOQ) and robustness, stability study (short and long-term stabilities, Freeze/thaw stability, post-preparative).The low value of % RSD showed that the method was precise within the acceptance limit of 2%. The developed method was successfully applied for the analysis of the drug in bulk as well as various marketed formulation and drug in plasma and brain distribution studies.

INTRODUCTION

Curcumin ((1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione), a polyphenol known as diferuloylmethane and Gefitinib (N-(3-chlorofluorophenyl) 7methoxy 6 (3 morpholinopropoxy) quinazolin-4- amine) is a type of drug called a tyrosine kinase inhibitor (TKI), also known as a cancer growth inhibitor. Molecular formula of CRM is $C_{21}H_{20}O_6$ and GFT is $C_{22}H_{24}ClFN_4O_3$. Molecular weight of curcumin (CRM) is 368.39 g/mol and gefitinib (GFT) is 446.90 g/mol. CRM and GFT is highly lipophilic drug having a log P value is 1.82 and 3.2 respectively. Dissociation constant of CRM and GFT was 8.3±0.04 and 5.4-7.2. CRM is a bright yellow-orange powder material and GFT is white crystalline powder materials. CRM and GFT have maximum solubility in methanol and acetonitrile. Melting point of CRM and GFT was 185ºc and 194ºc respectively. Reported λmax of CRM is 423 nm and GFT was 254 nm [1-4]. CRM and GFT were identified by Melting point, infrared vibrational spectrophotometry (IR), Differential Scanning Calorimetry (DSC). CRM and GFT interact with various proteins, inhibit the activity of various kinases, and control the activation of transcription factors that are involved in cell proliferation and survival. They inhibit the activity of EGFR by competing with adenosine triphosphate for its binding site on the intracellular tyrosine kinase domain of the receptor [1-4]. This inhibits autophosphorylation of EGFR and blocks downstream signalling. CRM and GFT are effective chemotherapeutic agents against a wide variety of cancer types. The development of MDR (multi dose resistance) is a major factor that results in failure of conventional chemotherapies. CRM enhances GFT induced cytotoxicity via down regulation of nuclear factor (NF)-κB and the Akt pathways,

Author for Correspondence:
Email: savalesagar484@gmail.com

thereby reversing MDR [5-9]. Various bio-analytical methods were developed for individual analysis of CRM [10-14] and GFT [15-18] in plasma and brain homogenate. But there is no bio-analytical method for simultaneous estimation of CRM and GFT in Plasma and brain homogenate. This study was aimed at developing a simple, rapid and sensitive method for simultaneous estimation of analyte (CRM and GFT) in tissue samples (plasma and brain homogenate) by using RP-HPLC.

MATERIALS AND METHODS

Chemicals and Reagents

All solvents used were of HPLC grade. Formic acid, Methanol and acetonitrile were obtained from MERCK. Chem. Ltd (Mumbai, India) and ultrapure water was used for mobile phase preparation. Reference standard of curcumin (CRM) supplied as a gift sample from Sunpure Extracts Pvt. Ltd (Delhi, India) and that of gefitinib (GFT) was supplied as a gift sample by Khandelwal Industries Pvt. Ltd (Mumbai, India).

Tissue Samples

Tissue samples were obtained from Central Animal House Facility, R.C. Patel Institute of Pharmaceutical education and Research Shirpur. Registration No. 651/PO/ReBi/S/02/CPCSEA. The rats were euthanasiazed by using CO_2 chamber (carcass disposal: Deep Burying under Soil). The rats (same animals) were sacrificed and the animals were decapitated immediately after blood collection (Fig. 1) and skull was cut open and the brain was carefully excised (same animals, Fig. 1). Each brain tissue was quickly rinsed with normal saline solution. The brain tissue samples were homogenized with one volume of normal saline solution in a tissue homogenizer. Blood samples were anticoagulated with heparin and centrifuged at 5000 rpm for 10 min to obtain plasma. All plasma samples and brain homogenates were stored in a deep freezer at −70˚C until HPLC analysis.

Instrumentation

Analysis was carried out using an Agilent HPLC system (Agilent technologies, USA). The system was equipped with quaternary pump and photo diode-array detector (PDA). Chromatographic separations were performed using the C-18 column (Qualisil BDS C18, 250 mm x 4.6 mm I.D.). All data were acquired and processed using EZ chrome elite software version 3.3.2.

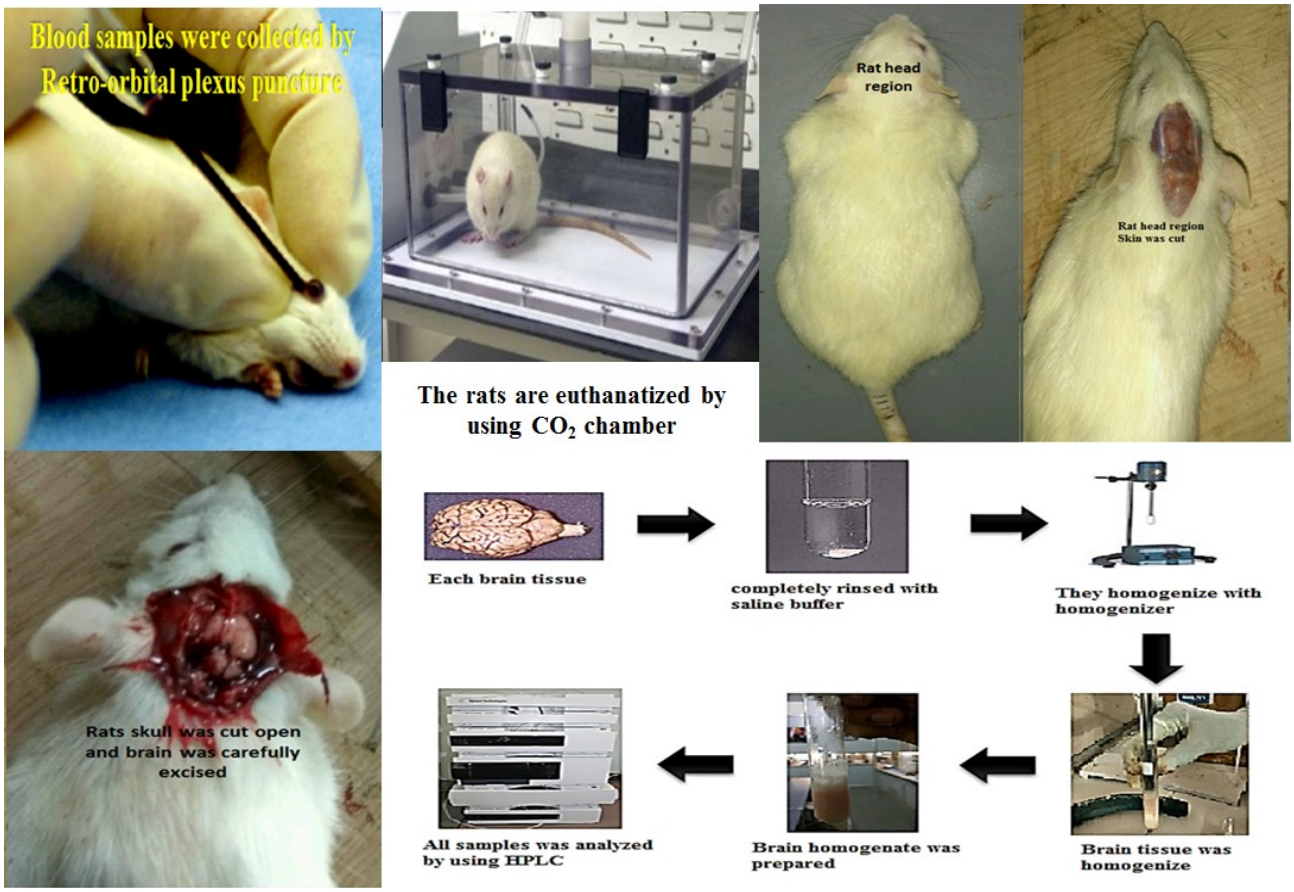

Figure 1: Tissue Homogenate Sample (Plasma and Brain) Collection and Analysis Steps

Chromatographic Conditions

Chromatographic separation was performed by using C-18 column (Qualisil BDS C18, 250 mm x 4.6 mm I.D.) coupled with a guard column. Isocratic elution was performed with acetonitrile: water with 1% formic acid (30:70 v/v) at a flow rate of 0.2 mL /min. The mobile phase was selected to give proper resolution of peaks.

Preparation of Standard Solutions And Quality Control (Qc) Samples:

Preparation of standard solutions for Bulk analysis

A) Preparation of standard solutions of Curcumin

Certified reference standard of CRM was weighed 100 mg accurately and transferred into a 100 ml of volumetric flask and dissolved in 100 ml of methanol to obtain a solution having concentration1000 µg/mL solution. The working standard solution was 10-60 µg/mL solution.

B) Preparation of standard solutions of Gefitinib

Certified reference standards of GFT was weighed accurately and transferred 100 mg accurately and transferred into a 100 ml of volumetric flask and dissolved in 100 ml of methanol to obtain a solution having concentration1000 µg/mL solution. The working standard solution was 10-60 µg/mL solution.

Tissue Homogenate Sample Processing and Quality Control (QC) Samples

P) Preparation of standard solutions of Curcumin

Certified reference standards of CRM was weighed accurately and transferred 10 mg of CRM as working standard into 100 ml of volumetric flask, add about 100 ml of methanol and sonicated (100 µg/mL solution). The working standard solution was 200-800 ng/mL solution.

Q) Preparation of standard solutions of Gefitinib

Certified reference standards of GFT was weighed accurately and transferred 10 mg of GFT as working standard into 100 ml of volumetric flask, add about 100 ml of methanol and sonicated (100 µg/mL solutions). The working standard solution was 200-800 ng/mL solution.

R) Preparation of standard solutions of internal standard (IS)

Internal standard such as hydrochlorothiazide, add 100 mg of IS in 100 ml of methanolic working solution (1000 µg/ml). The working standard solution was 20 µg/mL solution.

S) Preparation of Tissue samples

The whole procedure was carried out at room temperature. To 100 µl of CRM standard solution and 100 µl of GFT standard solution, 100 µl of blank brain homogenate or plasma sample, 100 µl of IS hydrochlorothiazide (20 µg/ml) were spiked and added extraction solvent 2 mL of acetonitrile was added and vortexed mixture for 20 min. This sample was ultra centrifuged at 10,000 rpm for 10 min. The supernatant layer was collected and 20 µl was analyzed by HPLC system.

Method Development

Method development was important to judge the quality, reliability and consistency of analytical results [19-21]. It is the process for proving that analytical method is acceptable for determination of the concentration of drugs [22]. The final chromatographic condition for method development was reported in Table 1.

Table 1: Final Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Standard solution	For Bulk: 100 µg/mL solution in methanol
	For Tissue Samples: 100 µg/mL solution in methanol
HPLC System	Agilent Technologies HPLC system
Pump	Reciprocating Quaternary pump
Detector	Photo Diode Array Detector
Data processor	EZ Chrome Elite Chromatographic data system
Stationary phase	Qualisil BDS C18, 250 mm x 4.6 mm I.D.
Mobile phase	Acetonitrile: water with 1% formic acid (30:70 v/v)
Detection wavelength	242 nm
Flow rate	0.2 mL/min
Sample size	20 µl

Method Validation

Application of the proposed method to bulk sample, linearity, recovery, precision, repeatability, ruggedness, sensitivity, robustness

and stability were determined in method validation [23-29].

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range.

Percent recovery of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The percent recovery as well as average percent recovery was calculated. Recovery should be assessed using minimum 9 determinations over minimum 3 concentrations level covering specified range. Recovery study was performed three different level 80%, 100% and 120%.

The precision is the measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Intra-day precision was determined by analysing, the three different concentrations 20 mg/ml, 30 mg/ml and 40 mg/ml for bulk analysis and 200 ng/ml, 400 ng/ml and 600 ng/ml for tissue samples analysis, for three times in the same day and Inter-day variability was assessed using above mentioned three concentrations of bulk and tissue samples were analysed by three different days, over a period of one week.

Repeatability is measured by multiple time analysis of a homogenous sample of 10 µg/ml solution containing CRM and GFT that indicates the performance of the HPLC instrument under chromatographic conditions.

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. From stock solution, sample solution containing CRM and GFT (10 µg/ml) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration of solutions, three times.

Robustness of the method was determined by making slight changes in the chromatographic conditions like change in pH and change in mobile phase ratio. To evaluate robustness few parameters were deliberately varied.

Sensitivity refers to the smallest quantity that can be accurately measured. It also indicates the capacity of the method to measure small variations in concentration. Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). For bulk analysis, six different concentration ranges 10-60 µg/ml and for tissue sample analysis 200-700 ng/ml. The linear regression equation of the calibration curve was used to determine the LOD and LOQ.

The stability of curcumin and gefitinib in tissue samples (plasma and brain homogenate) was assessed under different storage conditions. Stability was expressed as the concentration ratio of analytes in sample under each storage condition against those in the freshly prepared sample. All stability assessments were assayed at three concentrations. Three samples were determined for short-term stability by putting them on the bench top at room temperature for 12 h and 24 h, respectively, prior to extraction. To evaluate freeze/thaw stability, three samples were subject to three freeze-thaw cycles with each cycle stepping from defrosting at room temperature to freezing at -20°C for 12 h. To determine the post-preparative stability, the extracted samples were stored in the sampler for 24 h. The long-term stability was performed by processing and analysing samples of plasma and brain kept at -20°C for 40 days [30].

RESULTS AND DISCUSSION

A) Method development

Operating conditions of HPLC, such as component of mobile phase and elution, type of column, were carefully optimized. Different mobile phase compositions were tried, first which included acetonitrile and water (0.1% ammonia) (40: 60 % v/v), did not get adequate resolution. Acetonitrile: water (0.1% ammonia) (50: 50 % v/v) tried which did not gave proper resolution of drugs, then acetonitrile: water (0.1% ammonia) (20: 80 % v/v) tried which do not give proper resolution of drugs, the mobile phase for proper resolution of two drugs. Mobile phase consisting of Acetonitrile: water with 1.0 % formic acid in the ratio of (30:70 % v/v) is tried and drug was resolved properly. This method showed the best peak shape and ideal detection response. Furthermore, strong organic solvent in the reversed-phase chromatography

can reduce static retention and shorten analysis time. In addition, with the column, all the reference standard (CRM and GFT) and internal standard (IS) can be completely separated with narrow peaks, high sensitivity and no obvious tailing. Sample preparation is extremely important to the whole method in order to reduce possible interference from the sample matrix and increase sensitivity. To achieve the best analyte extraction, using single solvent, acetonitrile proved to be efficient for analysis of reference standard and internal standard (IS). Overall, the optimized extraction procedures allowed good recovery and selectivity for all reference standards and internal standard (IS) most importantly, were simple and reproducible. Typical chromatograms for simultaneous estimation of CRM and GFT in bulk and tissue samples (plasma and brain homogenate) were shown in Figure 2.

B) Method validation

1. Linearity

For bulk analysis, the linearity concentration was in the range of 10-60 µg/mL for CRM and GFT. The correlation coefficient (R^2) for CRM was 0.999 and GFT was 0.9993. Calibration curve of CRM and GFT in bulk was shown in Figure 3. For tissue sample analysis (plasma and brain homogenate) linearity concentration was in the range of 200-800 ng/mL for both drugs. The correlation coefficient (R^2) of CRM in plasma was 0.9993 and correlation coefficient (R^2) of GFT in plasma was 0.999. Calibration curve of CRM and GFT in plasma was shown in Figure 3. The correlation coefficient (R^2) of CRM in brain homogenate was 0.9995 and correlation coefficient (R^2) of GFT in brain homogenate was 0.9997. Calibration curve of CRM and GFT in brain homogenate was shown in Figure 3.

2. Application of the proposed method to bulk sample

Bulk sample was determined chromatographic standards in laboratory mixture and the concentration of drug was determined from their respective linearity curves and Results are shown in Table 2 and Figure 2.

Table 2: Analysis of Laboratory mixture

Component	Amount taken (µg)	Amount Found µg ± SD (n = 6)	% RSD
CRM	10	9.67 ± 0.057	0.59
GFT	10	9.56 ± 0.068	0.71

3. Recovery Study

The recovery of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies of bulk and tissue samples (plasma and brain homogenate) for the proposed method were carried out respective data is obtained and mentioned in Table 3. Recovery study was determined at three levels 80%, 100%, 120% at each level three determinations were performed.

4. Precision

Intra-day and Inter-day precision of bulk sample analysis and tissue sample analysis was reported in Table 4. The % RSD for CRM and GFT was less than 2.0%. The results are showing that the proposed method was precise.

5. Repeatability, Ruggedness and Robustness of bulk sample analysis

Repeatability expresses the precision under the same operating conditions over a short interval of time. Ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such different instruments, different analysts. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method developed was rugged. Robustness is the measure of the capacity of the analytical method to remain unaffected by Small but deliberate variations in procedure, it was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method developed was robust. Results of repeatability, ruggedness, robustness study are shown in Table 5a, 5b and 5c respectively.

Table 5a: Repeatability

Component	Amount taken (µg)	Amount Found µg ± SD (n = 6)	% RSD
CRM	10	9.78 ± 0.047	0.49
GFT	10	9.65 ± 0.058	0.51

Table 5b: Ruggedness

Drug	% Amount Found		% RSD (n = 3)	
	Analyst I	Analyst II	Analyst I	Analyst II
CRM	97.22	96.58	0.78	0.61
GFT	95.62	95.20	0.77	0.83

Table 5b: Robustness

Chromatographic conditions (change in Mobile Phase)	Conc. (µg/ml)	Retention time (CRM)	Retention time (GFT)
Acetonitrile : Water with 1.0 % formic acid (20:80)	10	9.46	7.34
	10	9.5	7.39
	10	9.49	7.31
Acetonitrile : Water with 1.0 % formic acid (40:60)	10	9.38	7.31
	10	9.32	7.25
	10	9.5	7.39
Chromatographic conditions (change in pH) 1.19	10	9.48	7.34
	10	9.46	7.37
	10	9.5	7.39
Chromatographic conditions (change in pH) 5.42	10	9.38	7.31
	10	9.32	7.25
	10	9.5	7.39

6. Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The linear regression equation of the calibration curve was used to determine the LOD and LOQ. Limit of detection, limit of quantitation of bulk sample analysis and tissue sample analysis (plasma and brain) were reported in Table 6 respectively.

Table 6: Sensitivity Study

Analysis	Drug	LOD	LOQ
Bulk	CRM	0.26 ± 0.01	0.69 ± 0.12
	GFT	0.18 ± 0.08	0.65 ± 0.19
Plasma	CRM	50.65 ± 0.58	158.10 ± 0.25
	GFT	58.40 ± 0.12	147.52 ± 0.82
Brain	CRM	64.41 ± 0.52	168.21 ± 0.14
	GFT	61.88 ± 0.26	165.87 ± 0.56

7. Stability

The results demonstrated that CRM and GFT were stable in tissue sample (plasma and brain) at room temperature for 12 h, in the sampling for 24 h and after three freeze-thaw cycles. All analytes were stable after stored at room temperature for 24 h. Even when stored in a long-term freezer set at -20°C for 40 days, all analytes remained stable. Stability data for CRM and GFT are shown in Table 7.

And the results suggested that the tissue sample containing CRM and GFT can be stored under common laboratory conditions without any significant degradation of all analytes. Stability of CRM and GFT was investigated using different concentrations of QC plasma and brain homogenate samples.

Table 3: Recovery Study

Analysis	Drug	Initial amount (µg/ml)	Added Amount (µg/ml)	% Recovery	% RSD (n = 3)
Bulk	CRM	10	8	100.53	0.93
		10	10	99.41	0.30
		10	12	103.20	0.52
	GFT	10	8	99.04	0.40
		10	10	98.92	0.23
		10	12	100.28	0.73
		Initial amount (ng/ml)	**Added Amount (ng/ml)**	**% Recovery**	**% RSD (n = 3)**
Plasma	CRM	200	188	97.58	0.61
		200	200	94.17	0.29
		200	202	101.33	0.12
	GFT	200	188	95.25	0.54
		200	200	98.74	0.95
		200	202	100.75	0.16
Brain	CRM	200	188	99.44	0.32
		200	200	101.45	0.87
		200	202	97.42	0.52
	GFT	200	188	105.89	0.75
		200	200	95.42	0.96
		200	202	97.89	0.25

Table 4: Presion Study

Analysis	Drug	Con. (µg/ml)	Intra - Day		Inter - Day	
			Mean ± SD	% RSD (n = 3)	Mean ± SD	% RSD (n = 3)
Bulk	CRM	20	19.65 ± 0.10	0.53	19.64 ± 0.10	0.51
		30	28.46 ± 0.20	0.73	28.64 ± 0.24	0.82
		40	37.47 ± 0.37	0.98	37.43 ± 0.40	1.08
	GFT	20	19.84 ± 0.065	0.32	19.88 ± 0.05	0.26
		30	29.71 ± 0.21	0.72	30.02 ± 0.24	0.82
		40	41.07 ± 0.34	0.83	40.1 ± 0.38	0.95
		Con. (ng/ml)	**Mean ± SD**	**% RSD (n = 3)**	**Mean ± SD**	**% RSD (n = 3)**
Plasma	CRM	200	199.25 ± 0.21	0.52	201.22 ± 0.26	0.32
		400	398.29 ± 0.74	0.11	399.74 ± 0.51	0.89
		600	599.11 ± .051	0.62	600.11 ± 0.44	0.46
	GFT	200	197.32 ± 0.14	0.16	199.53 ± 0.32	0.90
		400	399.21 ± 0.28	0.43	400.22 ± 0.69	0.53
		600	598.31 ± 0.41	0.94	601.54 ± 0.28	0.10
Brain	CRM	200	200.41 ± 0.12	0.63	198.56 ± 0.74	0.81
		400	401.52 ± 0.36	0.51	399.41 ± 0.52	0.22
		600	599.42 ± 0.69	0.27	600.89 ± 0.87	0.59
	GFT	200	199.58 ± 0.25	0.19	198.52 ± 0.26	0.11
		400	396.59 ± 0.68	0.54	400.41 ± 0.89	0.34
		600	600.74 ± 0.14	0.35	599.47 ± 0.58	0.62

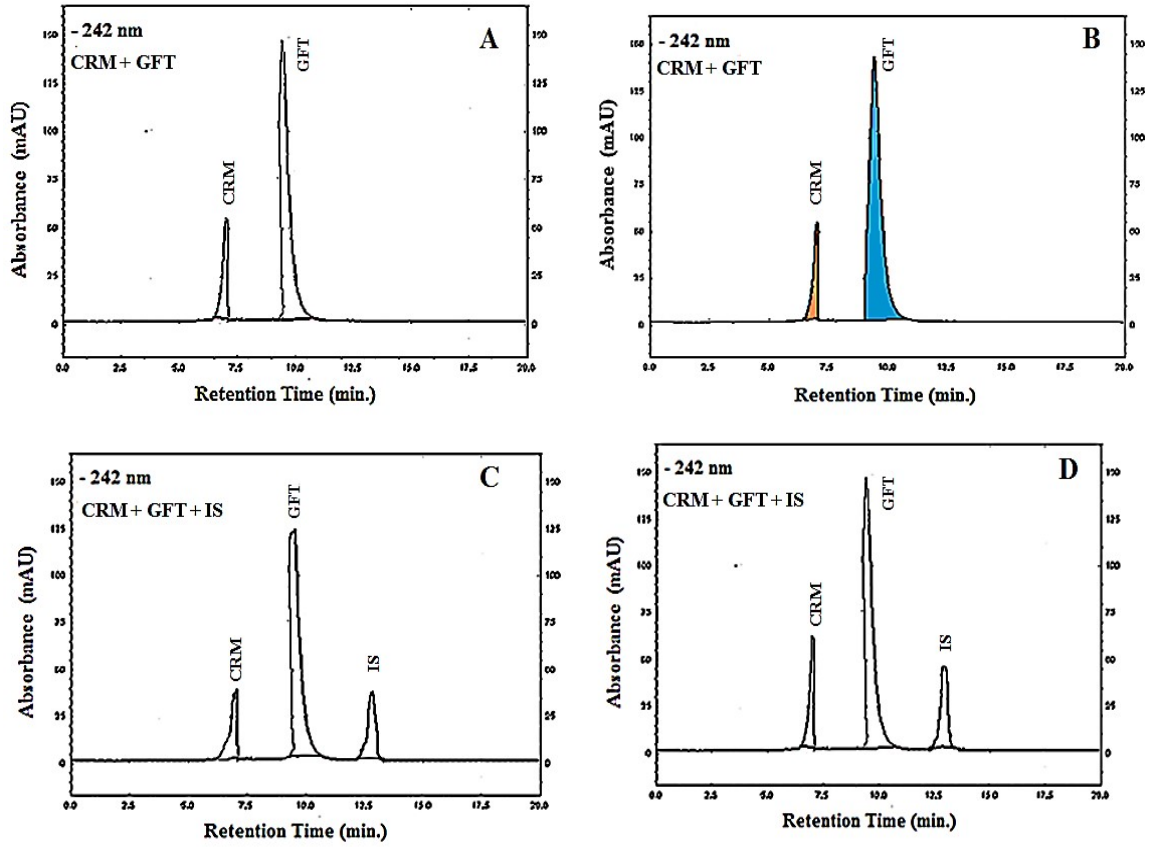

Figure 2: Typical chromatograms for simultaneous estimation Curcumin and Gefitinib (A), Chromatogram standard (Application of the proposed method to bulk sample of laboratory mixture containing two drugs, curcumin and gefitinib) (B), simultaneous estimation Curcumin and Gefitinib in Plasma (C), simultaneous estimation Curcumin and Gefitinib in brain homogenate (D).

Table 7: Stability of Curcumin and Gefitinib in Rat Plasma and Brain Homogenates

Analysis	Drug	Nominal (ng/ml)	3 freeze-thaw cycles	short-term room temperature		post-preparative stability (24 h)	long-term room temperature (40 d)
				(12 h)	(24 h)		
Plasma	CRM	400	99.36 ± 1.43	97.16 ± 3.35	98.13 ± 5.09	103.13 ± 2.19	95.33 ± 6.60
		600	100.18 ± 0.23	100.03 ± 1.31	99.52 ± 3.15	101.32 ± 0.39	102.86 ± 14.20
		800	99.65 ± 1.62	97.56 ± 2.89	98.24 ± 6.12	101.54 ± 0.45	96.89 ± 8.50
	GFT	400	99.81 ± 1.11	96.49 ± 1.56	98.58 ± 7.01	102.73 ± 0.78	95.69 ± 9.59
		600	99.88 ± 1.77	97.83 ± 4.11	98.42 ± 4.74	103.01 ± 1.99	96.87 ± 7.11
		800	100.47 ± 0.74	100.62 ± 1.88	99.74 ± 3.25	101.79 ± 0.52	102.99 ± 13.54
Brain	CRM	400	92.47 ± 12.11	93.57 ± 8.88	94.71 ± 13.01	110.46 ± 4.11	97.82 ± 32.45
		600	107.13 ± 3.52	105.45 ± 4.52	104.68 ± 3.89	97.77 ± 7.89	101.15 ± 2.88
		800	95.74 ± 4.85	96.56 ± 2.87	97.74 ± 1.25	98.59 ± 6.35	103.87 ± 21.77
	GFT	400	106.25 ± 3.87	104.48 ± 3.87	103.87 ± 2.58	98.12 ± 7.52	102.48 ± 2.15
		600	93.85 ± 10.45	94.25 ± 7.15	95.47 ± 13.54	110.50 ± 3.99	97.88 ± 27.41
		800	97.45 ± 3.18	98.41 ± 2.45	99.15 ± 1.95	108.47 ± 7.51	101.51 ± 19.78

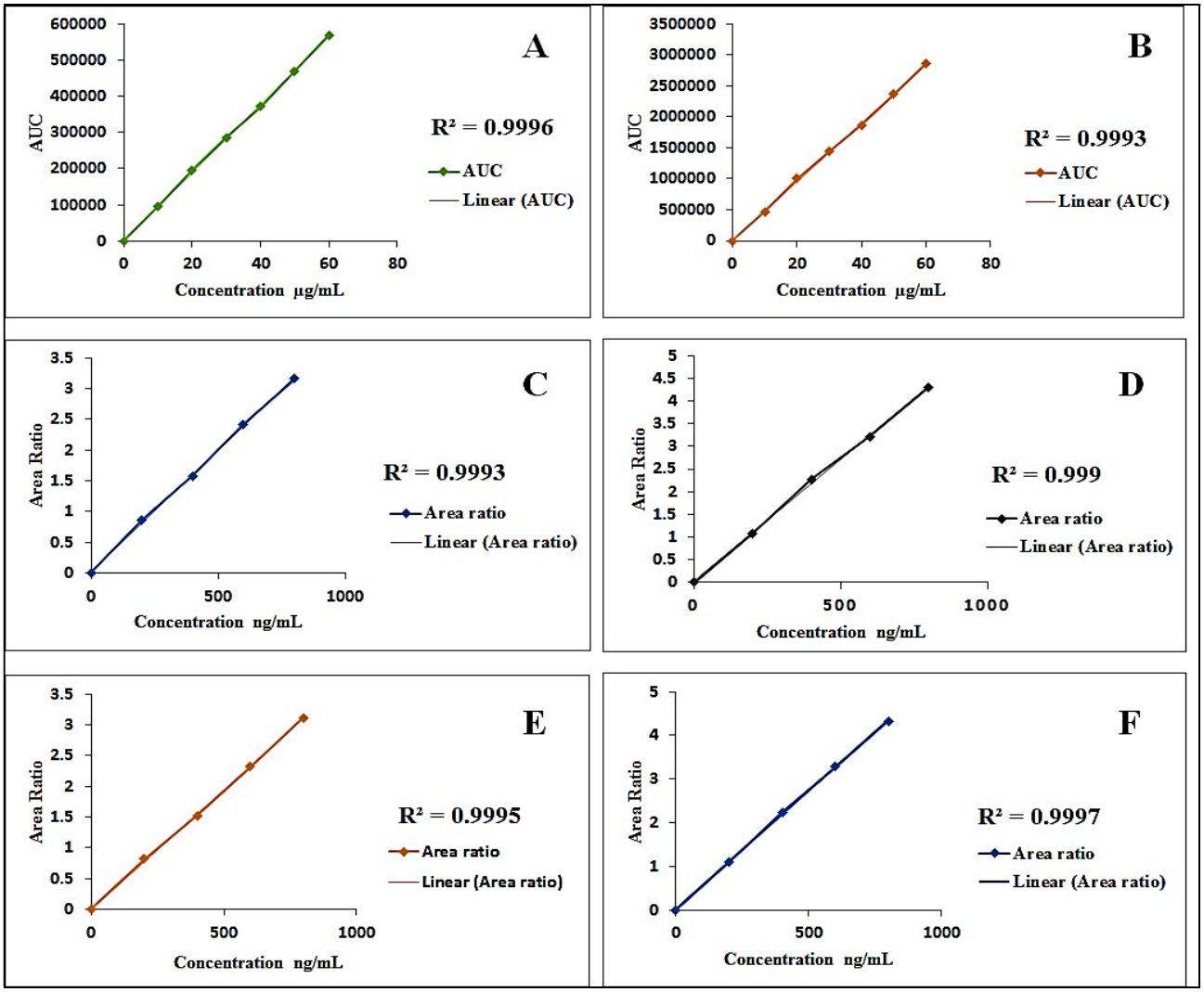

Figure 3: Calibration curve: standard calibration curve of curcumin (A), standard calibration curve of gefitinib (B), standard calibration curve of curcumin in Plasma (C), standard calibration curve of gefitinib in Plasma (D), standard calibration curve of curcumin in brain homogenate (E), standard calibration curve of gefitinib in brain homogenate (F).

Excellent recoveries of CRM and GFT were observed at different storage conditions and no significant loss of CRM and GFT in either plasma or brain homogenate was observed.

CONCLUSIONS

In this study, we developed and validated a highly sensitive and specific RP-HPLC method for the quantitative analysis of CRM and GFT in bulk and tissue samples (plasma and brain homogenate). Validation of analytical method for simultaneous estimation for CRM and GFT was determined by evaluating linearity, precision, recovery, repeatability, ruggedness, robustness, sensitivity (LOD-LOQ) and stability (short and long-term stabilities, Freeze/thaw stability, post-preparative) in order to establish the suitability of analytical method. The method was validated in compliance with ICH guidelines is suitable for simultaneous estimation of analytes with excellent recovery, precision, linearity and stability. Therefore, we suggest that this method can be used for routine analysis of CRM and GFT in bulk, tissue samples (plasma and brain homogenate) and in analysis of pharmaceutical formulations or dosage forms.

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. It has been a great honour to work with such a professional.

REFERENCES

[1] J. Teske, K. Putzbach, W. Engewald, R.K. Müller, Determination of cannabinoids by gas chromatography-mass spectrometry and large-volume programmed-temperature vaporiser injection using 25 microl of biological fluid, J. Chromatogr. B Analyt. Technol, Biomed. Life Sci. 2002; 772(2):299-306.

[2] H. Ahsan, N. Parveen, NU. Khan, SM. Hadi, Pro-oxidant, antioxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin, Chem-Biol Interact. 1999; 121:161-175.

[3] R. Sharma, A. Gescher, W. Steward, Curcumin: the story so far, European Journal of Cancer. 2005; 4 (13):1955-1968.

[4] K. Hao, X. Zhao, X. Liu, G. Wang, LC Determination of Curcumin in Dog Plasma for a Pharma cokinetic Study, Chromatographia. 2006; 64:531-535.

[5] P. Rojsanga, W. Gritsanapan, L. nsuk, Determination of Berberine Content in the Stem Extracts of Coscinium fenestratum by TLC Densitometry, Medical Principles Practice. 2006; 15:373-378.

[6] G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran, P. Srinivas, Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers, Planta Medica. 1998; 64:353-356.

[7] V. Ravindranath, N. Chandrasekhara, in vitro studies on the intestinal absorption of curcumin in rats, Toxicology. 1981; 20:251-257.

[8] A. Liu, H. Lou, L. Zhao, P. Fan, Validated LC=MS=MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin, J. Pharm. Biomed. Anal. 2006; 40:720-727.

[9] C. Ireson, D. Jones, S. Orr, M. Coughtrie, D. Boocock, M. Williams, P. Farmer, W. Steward, A. Gescher, Metabolism of the cancer chemo preventive agent curcumin in human and rat intestine, Cancer Epidemiol. Biomarkers Prev. 2002; 11 (1):105-111.

[10] XM Wang, QZ Zhang, J Yang, RH Zhu, J Zhang, LJ Cai, WX Peng, Validated HPLC–MS/MS Method for Simultaneous Determination of Curcumin and Piperine in Human Plasma,Trop J Pharm Res. 2012; 11:621-629.

[11] J. Li, Y. Jiang, J. Wen, G. Fan, Y. Wu, C. Zhang, A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, validation and application to a pharmacokinetic study of curcumin liposome, Biomed. Chromatogr. 2009; 23:1201–1207.

[12] A. Liu, H. Lou, L. Zhao, P. Fan, 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; 40:720-727.

[13] R.G. Madane, H.S. Mahajan, Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and in vivo study, Drug Deliv, Early Online. 2014; 1-9.

[14] C. Schiborr, P. Eckert, G. Rimbach, J. Frank, A validated method for the quantification

of curcumin in plasma and brain tissue by fast narrow-bore high-performance liquid chromatography with fluorescence detection, Anal Bioanal Chem. 2010; 397:1917-1925.

[15] I. Andriamanana, I. Gana, B. Duretz, A. Hulin, Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS, Journal of Chromatography B. 2013; 926:83-91.

[16] L. Couchman, M. Birch, R. Ireland, A. Corrigan, S. Wickramasinghe, D. Josephs, J. Spicer, R. J. Flanagan, An automated method for the measurement of a range of tyrosine kinase inhibitors in human plasma or serum using turbulent flow liquid chromatography–tandem mass spectrometry, Anal Bioanal Chem. 2012; 403:1685-1695.

[17] N. A. G. Lankheet, M. J. X. Hillebrand, H. Rosing, J. H. M. Schellens, J. H. Beijnen, A. D. R. Huitema, Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry, Biomed. Chromatogr. 2012.

[18] M. Zhao, C. Hartke, A. Jimeno, J. Li, P. He, Y. Zabelina, M. Hidalgo, D. Baker, Specific method for determination of gefitinib in human plasma, mouse plasma and tissues using high performance liquid chromatography coupled to tandem mass spectrometry, Journal of Chromatography B. 2005; 819:73-80.

[19] M. Hutter, S. Kneisel, V. Auwärter, M.A, Neukamm. Determination of 22 synthetic cannabinoids in human hair by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2012; 903:95-101.

[20] S.S. Simões, I. Silva, A.C. Ajenjo, M.J. Dias, Validation and application of an UPLC-MS/MS method for the quantification of synthetic cannabinoids in urine samples and analysis of seized materials from the Portuguese market, Forensic Sci. Int. 2014; 243:117-125.

[21] W. Gritsanapan, Popular natural cosmetics in Thailand and their natural components, Thai J Health Res. 2004; 18:23-9.

[22] RK. Maheshwari, AK. Singh, J. Gaddipati, RC. Srimal, Multiple biological effects of curcumin: A short review, Life Sciences.2006; 78:2081-2087.

[23] SJ Taylor, IJ. McDowell, Determination of the curcuminoid pigments in turmeric (*Curcuma domestica* Val) by reversed-phase high performance liquid chromatography, Chromatographia. 1992; 34:73-77.

[24] CR. Culy, D. Faulds, Gefitinib, Drugs. 2002; 62:2237-2248.

[25] K. Asami, S. Atagi, Comparing the Efficacy of Gefitinib, Erlotinib, and Afatinib in Non-Small Cell Lung Cancer with Activating Epidermal Growth Factor Receptor (EGFR) Mutations, Austin J Lung Cancer Res. 2016; 1(1):01-06.

[26] C. Gridelli, F. De Marinis, M. Di Maio, D. Cortinovis, F. Cappuzzo, T. Mok, Gefitinib as first-line treatment for patients with advanced non-small-cell lung cancer with activating epidermal growth factor receptor mutation: Review of the evidence, Lung Cancer. 2011; 71:249-257.

[27] N. Annette, Y. Poon, S. Simon, M. Ho, W. Yeo, S. Tony, K. Mok, Brain Metastasis Responding to Gefitinib Alone, Oncology. 2003; 67:174-178.

[28] MB. Varanasi, MA. Khan, VR Jangala, BB. Teja, Visible spectrophotometric determination of Gefitinib in bulk drug and pharmaceutical formulations, International Journal of Chemical Sciences. 2009; 7(4):2549-2554.

[29] S. Gowri, A. Rajeswari, B. Nagesh, V. Devi, V. Krishna, High performance liquid chromatographic estimation of Gefitinib in pharmaceutical dosage forms, Asian Journal of Chemistry. 2009; 21(8):5863-5867.

[30] E. Wong, J. Wu, A. Mahadevan, Gefitinib and high-dose fractionated radiotherapy for carcinomatous encephalitis from non-small cell lung carcinoma, Biologics: Targets & Therapy. 2007; 1(3):321-324.