Pharmacophore 2016, Vol. 7 (3), 141-151 USA CODEN: PHARM7 ISSN 2229-5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

SIMULTANEOUS ESTIMATION OF ARTEMETHER & CURCUMIN BY RP-HPLC METHOD

Rupali Kalra*, Anupama Diwan, Jatinder Kumar and Shekhar Sharma

School of Pharmaceutical Sciences, Plot No.23, Sector 32, Apeejay Stya University, Gurgaon-122001, India

ABSTRACT

Malaria still remains one of the killer disease in the world with an estimated 200-400 million cases and one million deaths annually. In the present study, a simple, accurate, rapid and precise reversed-phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Artemether and Curcumin. The chromatographic separation was performed in isocratic mode on Chrombudget C18 (4.6 x 100 mm, 5µm) column with flow rate of 0.7 mL/min using mobile phase consisting mixture of acetonitrile (HPLC grade) and 0.1 % formic acid (in Milli-Q water v/v) in a ratio 60: 40 (v/v) at wavelength of 216 nm. Calibration plots were linear in range over the concentration range of 2500-7500µg/mL for Artemether and 25-75µg/mL for Curcumin. The method was validated for accuracy, precision, specificity, detection limit, quantization limit, linearity, range and robustness. The method showed excellent linearity with Coefficient of determination r2>0.99, % Relative Standard Deviation (RSDs) of intra- and inter-day precisions were satisfactory found 0.32 & 0.32 for Artemether and 0.31 & 2.04 for Curcumin. The recovery averages of Artemether and Curcumin were obtained 100% and 101% respectively. Limit of detection (LODs) and Limit of quantification (LOQs) were calculated as for 352.7 µg/mL & 1068.8 µg/mL for Artemether in linear range 2500-7500 µg/mL and 3.21 µg/mL & 9.75 µg/mL for Curcumin in linear range.25-75 µg/mL. The high recovery and low relative standard deviation confirms the suitability of the method developed for Artemether (ART) and Curcumin (CUR).

Keywords: Curcumin, Artemether, RP-HPLC, Method validation, Linearity, Precisio, Accuracy, Robustness.

INTRODUCTION

Artemisinin also known as *Qinghaosu*, and its derivatives are a group of drugs that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria. Artemether (ART) (Figure I a) is a methyl ether derivative of Artemisinin, which is a peroxide lactone isolated from the antimalarial plant *Artemisia annua*. It is also known as dihydroartemisinin methyl ether, but its correct chemical nomenclature is (+)-(3-alpha,5a-beta,6-beta,8a-beta,9-alpha,12-beta,12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin.[1] Curcumin (Figure I b) (CUR) is a diarylheptanoid, an active constituent mainly derived from *Curcuma longa* (turmeric), which has been used for its medicinal benefits for centuries. Curcumin composition was elucidated as 1, 6-heptadiene-3, 5-dione-1, 7-*bis*-(4-hydroxy-3-methoxyphenyl)-(1E, 6E).[4,5] Drug resistant malaria has become one of the most important problems in antimalarial therapy in recent years. According to World Health organization (WHO), Artemisinin based Combination Therapies (ACTs) is always preferred as first line treatment in malaria.[6] Resistance in vivo has been reported in all anti

malarial drugs, except Artemisinin and its derivatives. However, According to WHO, due to development of resistance against most of the antimalarial drugs, Artemisinin derivative based combination therapies was found to be more effective and safe in preventing parasite recrudescence. As compared to Artemisinin, Artemether (ART), the methyl ether synthetic derivative is preferred because of same action, low cost of molecule and ease of availability. Therefore, Artemether has been tried in combination with various compounds like Curcumin (CUR) to minimize the drug resistance, side effects of monotherapy and also to minimize the long therapy. Curcumin not only synergizes with ART as an antimalarial to kill the parasite, but is also uniquely able to prime the immune system to protect against parasite recrudescence in the animal model.[1,7] Literature survey reveals one of the High performance liquid chromatographic (HPLC) methods for quantification of Artemether alone in pharmaceutical raw material.[8] Literature survey also reveals the HPLC method development for simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage form.[2] Literature survey reveals the simultaneous estimation of Curcumin and Silibinin by Reversed-phase high performance liquid chromatographic method (RP HPLC).[9] However, one of the HPLC/MS method was also developed for simultaneous estimation of Curcumin and Piperine in human plasma.[10] Only liquid chromatographic method was developed for quantification of Curcumin, Arteether, Tetrhydrocurcumin and Dihydroartemisinin.[6] But no simultaneous method was developed for both the drugs in combination. The combination of these two drugs is also not official in any pharmacopeia; hence, no official method is available for simultaneous estimation of both the drugs. So we need to develop a suitable analytical procedure for simultaneous estimation of Artemether and Curcumin. The purpose of our study was to develop a simple and validated proposed HPLC method for simultaneous estimation of Artemether and Curcumin in bulk drugs and in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals

Curcumin (>99% Purity; Figure 1b) was obtained as a gift sample from Chroma Dex, USA and Artemether (>99% Purity; Figure 1a) was kindly obtained as gift sample. All the chemicals used were of analytical grade. Formic acid (about 98% - 100%) (AR grade) and Acetonitrile (99.8%, HPLC grade) were purchased from RANKEM. Milli-Q water was used for the preparation of buffer.

Instrumentation

All standards and samples were analyzed on Shimadzu integrated quaternary gradient HPLC system LC2010 CHT (Kyoto, Japan) equipped with a LC-10ADvp pump, SIL-HTC auto sampler, CTO-10ACvp column compartment and SPD-10Avp photodiode array detector. LC Solution software was utilized for instrument control, data collection and data processing.

Chromatographic Conditions

Chromatographic method development depends upon the nature of the sample, molecular weight and solubility. CUR and ART were polar in nature. Polar compounds can be separated by reverse phase chromatography. Reverse phase high performance liquid chromatography (RP-HPLC) technique was selected for initial separations from the knowledge of properties of the compounds. Chromatographic analysis was carried out on Chrombudget C18 (4.6 x 100 mm, 5µm) column at 30°C temperature. Separation of curcumin and artemether were isocratically done with a mobile phase consisting acetonitrile (HPLC garde) and 0.1 % formic acid (in Milli-Q water v/v) in a ratio 60: 40 (v/v) at a flow rate 0.7 mL/min with injection volume 10 µL. The mobile phase was filtered through a 0.45 µmnyl on membrane filter (Millipore, Bradford, MA), under vacuum and degassed ultrasonically for 30 min. The chromatograms were recorded at wavelength 216 nm. Primary stock standard solutions of Curcumin and Artemether were prepared by dissolving accurately weighed 5 mg of curcumin

and 500 mg of artemether in 50 mL of Milli-Q water : Acetonitrile (HPLC grade) in a ratio 50:50 v/v to achieve final concentration of curcumin : artemether (100 µg/mL: 10mg/mL). Standard stock solution was used for further preparation of sample dilutions. Appropriate working standard solutions of Curcumin and Artemether were prepared by dilution of the stock standard solution with diluent (Milli-Q water and Acetonitrile in 50:50 v/v) to achieve concentration range of curcumin: artemether (50 µg/mL: 5mg/mL). Further sample dilutions were prepared by dilution of the stock standard solution with diluent. Triplicate of each sample solutions for validation study were prepared by dilution of the standard stock solution with linear concentration range of 25-75 µg/mL for curcumin and 2500-7500 µg/mL for artemether.

Linearity

Calibration curves were constructed in the ranges of 25-75 µg/mL and 2500-7500 µg/mL for CUR and ART, respectively, to encompass the expected concentrations in the measured samples. Triplicate of 10 µL injections for each working standard solution were made. To obtain the calibration graph, the peak area for each concentration was recorded and then plotted against the corresponding concentration.

Accuracy

The accuracy of an analytical procedure expresses the degree of closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the observed value. The accuracy was done by taking three samples at about 50 %, 70 %, 100 %, 130 % and 150 % concentration and injecting three times. Then analyze according to chromatographic method and % RSD value was calculated.

$$\%\ \text{Recovery} = \frac{\text{SS Area Mean}}{\text{Std Mean}} \times \frac{\text{Weight of Std (mg)}}{\text{Weight of analyte in SS preparation (mg)}} \times 100$$

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. For repeatability, six replicate samples solutions with standard solution of analyte sample were prepared according to the analytical method. The samples were analyzed according to the analytical method and make two injections of each sample. The assay results (% recovery), the mean and relative standard deviations (% RSD) of the samples were calculated. For intermediate precision, the work was performed on different days, using different operating conditions (e.g. column, apparatus and reagents). Six replicate samples were prepared according to the analytical method. The samples were analyzed according to the analytical method and make two injections of each sample. The assay results (% recovery), the mean and relative standard deviations (% RSD) of the samples were calculated.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present; typically these might include impurities & degradants etc. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The linearity of the method was evaluated by determining the coefficient of determination, the graph of residuals and of linearity. The LOD and LOQ value was calculated. System suitability and specificity of working chromatographic method were achieved by injecting 10µL of blank solution (three injections) and standard solution (five injections) of CUR-ART(Figures II) have shown the chromatograms for blank and standard solution of CUR-ART. Peak purity analysis for CUR and ART were performed on Agilent 6120 single Quadrupole LCMS (shown in Figure III).

RESULTS

In order to affect the simultaneous estimation of CUR and ART under isocratic conditions, different chromatographic conditions (organic modifier, flow rate, and pH) have been investigated.

Our main objective of the chromatographic method development was to achieve a better separation of CUR and ART with peak tailing factor <2, retention time in between 2 and 12 min, along with good resolution. This objective was obtained using mobile phase consisting of acetonitrile-formic acid (0.1% in Milli-Q water) in the proportion of (60:40) v/ with Chrombudget C18 (4.6 x 100 mm, 5µm) column. All the analyte peaks were well defined, resolved and free from tailing, the tailing factors were <2 for all peaks. The separation orders were CUR (retention time, RT 3.3 min) and ART (retention time, RT 10.7 min) at a flow rate of 0.7 ml/min. The optimum wavelength for detection was 216 nm at which much better detector responses for the two drugs were obtained (Figure 2b). System suitability tests are used to verify that the column efficiency (N), selectivity factor (resolution) and reproducibility of the chromatographic system are adequate for the analysis. System suitability tests were carried out on freshly prepared standard stock solutions of CRT and ART. The system was found to be suitable as shown in Table I as per SST limits in CDER guidelines and Hsu and Chien recommendation.[11] Several studies have suggested the use of statistical analysis (e.g., Plackett and Burman or other fractional factorial designs) on data gathered during method optimization or validation. This is in line with guidance from ICH, which regards SST as one of the method validation steps.[12,13]

Calibration plot (Figure IV) was constructed between concentrations of drugs versus the peak area. Results show linear relationship in the range of 25-75 µg/mL and 2500-7500 µg/mL for CUR and ART, respectively. Linear regression equation was calculated for triplicate run of each concentration. According to ICH recommendation,[12-13] the approach based on the standard deviation (SD) of the response and the slope was used for determining the detection and quantitation limits. Determination coefficient (R^2), standard deviation of residuals (SD), slope, intercept, LOD and LOQ were listed in Table II. Accuracy of measurements was determined over five concentration range 25-75µg/mL and 2500-7500µg/mL for CUR and ART respectively, results were assessed by percentage recovery and determination coefficient (R^2)[14] listed in Table III. Precision (repeatability): Relative standard deviation (RSD) of drugs peak area in six triplicate injections of standard drug solution determined each day of 3 consecutive days. Intra-day and Inter-day precision were assessed using three concentration and three replicates of each concentration. Resultant RSD values were found to <2% of % recovery, summarized in Table IV showed good repeatability and reliability of proposed method. Triplicate of blank/diluent and standard sample solution were injected and found no interference of retention time. For the method robustness evaluation, small deliberate variations in different experimental parameters such as mobile phase composition, flow rate and column temperature did not significantly affect the retention time & peak area of CUR and ART indicating, the proposed method is robust accordingly as results mentioned in Table V.

DISCUSSION

Results of several parameters indicate high sensitivity and reliability of proposed RP-HPLC method.

CONCLUSIONS

The method developed is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method is simple, specific, rapid, accurate, reliable and easy to perform for simultaneous estimation of Curcumin and Artemether in bulk as well as in pharmaceutical dosage form.

ACKNOWLEDGEMENT

We are highly thankful to ASRF, Gurgaon and Apeejay Stya University for carrying out my analytical work.

Table I: Analytical parameters for system suitability test (SST) of RP-HPLC method

Parameters	CDER guidelines	Hsu and Chien recommendation	CUR	ART
Retention time (min)	-------	-------	3.3	10.7
%RSD Repeatability of peak response	≤1.0% for five replicates	≤1.5 % general	0.38	0.48
Resolution (Rs)	>2.0 general	>2.0 general	>2	>2
Tailing factor	≤ 2.0	<1.5-2.0	1.19	1.42
Column efficiency (N)	>2000 (N plate count)	-------	4001	6307
Capacity factor (K’)	>2	2-8	2.0	8.5

% RSD = Relative standard deviation, CUR= Curcumin, ART = Artemether

Table II: Selective parameter used for simultaneous estimation of CUR and ART

Parameters (units)	CUR	ART
Linear Range (μg/mL)	25-75	2500-7500
Intercept (a)	-9160.6	9567.4
Slope(b)	45601	458.96
Determination Coefficient (R^2)	0.9992	0.9991
SD (STEy,x)*)	44746.8	48753.5
LOD(μg/mL)	3.21	352.7
LOQ (μg/mL)	9.75	1068.8

*STEy,x (Returns the standard error of the predicted y-value for each x in the regression),
CUR= Curcumin, ART = Artemether,, LOD= limit of detection, LOQ = limit of quantification.

Table III: Accuracy evaluation of proposed HPLC method

Sample No.	Curcumin (CUR)			Artemether (ART)		
	Conc.(μg/mL) Taken*	Conc.(μg/mL) Found*	% Recovery*	Conc.(μg/mL) Taken*	Conc.(μg/mL) Found*	% Recovery*
1	24.98	24.62	98.53	2470.02	2433.30	98.51
2	35.40	36.32	102.6	3500.02	3570.14	102.0
3	50.57	50.44	99.74	5000.03	4944.70	98.89
4	65.74	67.65	102.91	6500.04	6598.84	101.52
5	75.48	76.62	101.52	7499.93	7474.93	99.67
Mean			101.06			100.12
SD			1.88			1.57
%RSD			1.86			1.56
R^2**	0.9992			0.9991		

*Average of three determinations and ** From linearity plot, SD= Standard deviation, RSD = Relative standard deviation, R^2 = Coefficient of determination.

Table IV: Precision evaluation of proposed HPLC method

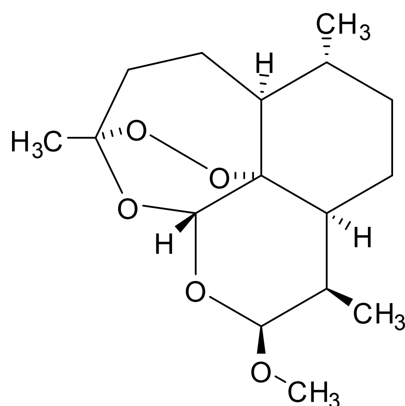
Parameter	Sample No.	Curcumin (CUR)	Artemether (ART)	Parameter	Sample No.	Curcumin (CUR)	Artemether (ART)
		% Recovery*	% Recovery*			% Recovery*	% Recovery*
Intra-day Precision-1	1	96.39	96.78	Inter-day Precision-day1	1	102.8	99.88
	2	97.03	97.44		2	103.48	100.55
	3	96.61	96.76		3	103.03	99.85
	4	97.2	97.11		4	103.66	100.21
	5	96.99	96.57		5	103.44	99.65
	6	96.86	96.99		6	103.29	100.09
	Mean	96.85	96.94		**Mean**	103.28	100.04
	SD	0.29	0.309		**SD**	0.32	0.318
	%RSD	**0.31**	**0.32**		**%RSD**	**0.31**	**0.32**
Intra-day Precision-2	1	96.78	96.67	Inter-day Precision-day2	1	100.11	99.73
	2	96.89	96.45		2	99.69	100.26
	3	96.6	96.39		3	99.50	99.52
	4	97.28	96.78		4	99.73	99.72
	5	96.71	96.57		5	99.06	99.54
	6	97.59	97.54		6	98.52	99.80
	Mean	96.98	96.73		**Mean**	99.44	99.76
	SD	0.38	0.42		**SD**	0.56	0.27
	%RSD	**0.39**	**0.43**		**%RSD**	**0.57**	**0.27**
Intra-day Combined Precision				Inter-day Combined Precision			
	Mean	97.0	97.00		**Mean**	101.00	100.00
	SD	0.30	0.400		**SD**	2.05	0.32
	%RSD	**0.31**	**0.41**		**%RSD**	**2.04**	**0.32**

*Average of three determinations, SD= Standard deviation, RSD = Relative standard deviation

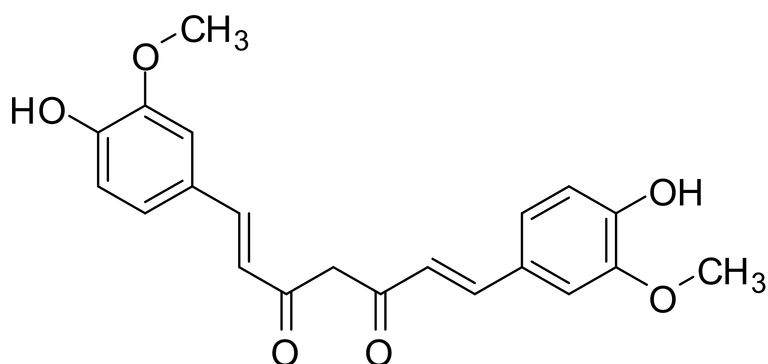
Table V: Robustness evaluation proposed HPLC method

Parameter	Variation	Curcumin (CUR)				Artemether (ART)			
		%RSD		N	T	%RSD		N	T
		RT	Area			RT	Area		
Column temp. Variation (30 ± 5°C)	+ 5°C	0.0	0.5	4083	1.18	0.0	0.18	6484	1.39
	- 5°C	0.0	0.1	4224	1.17	0.18	0.23	6378	1.43
Mobile phase Variation (± 5,10%)	+ 5%	0.0	0.06	3767	1.20	0.0	0.12	6489	1.40
	- 5%	0.0	0.01	4743	1.14	0.08	0.01	6536	1.41
	+ 10%	0.0	0.03	3374	1.20	0.0	0.4	6448	1.38
	- 10%	0.0	0.1	5363	1.11	0.0	0.22	6478	1.43
Flow rate variation (± 10, 25%)	+ 10%	0.0	0.12	3968	1.16	0.0	0.31	6452	1.40
	- 10%	0.0	0.28	4484	1.17	0.08	0.1	6568	1.42
	+ 25%	0.0	0.1	3660	1.17	0.0	0.14	6328	1.38
	- 25%	0.0	0.46	4710	1.16	0.14	0.09	6634	1.42

N; theoretical plates and T; tailing factor, RSD = Relative standard deviation, RT = Retention time

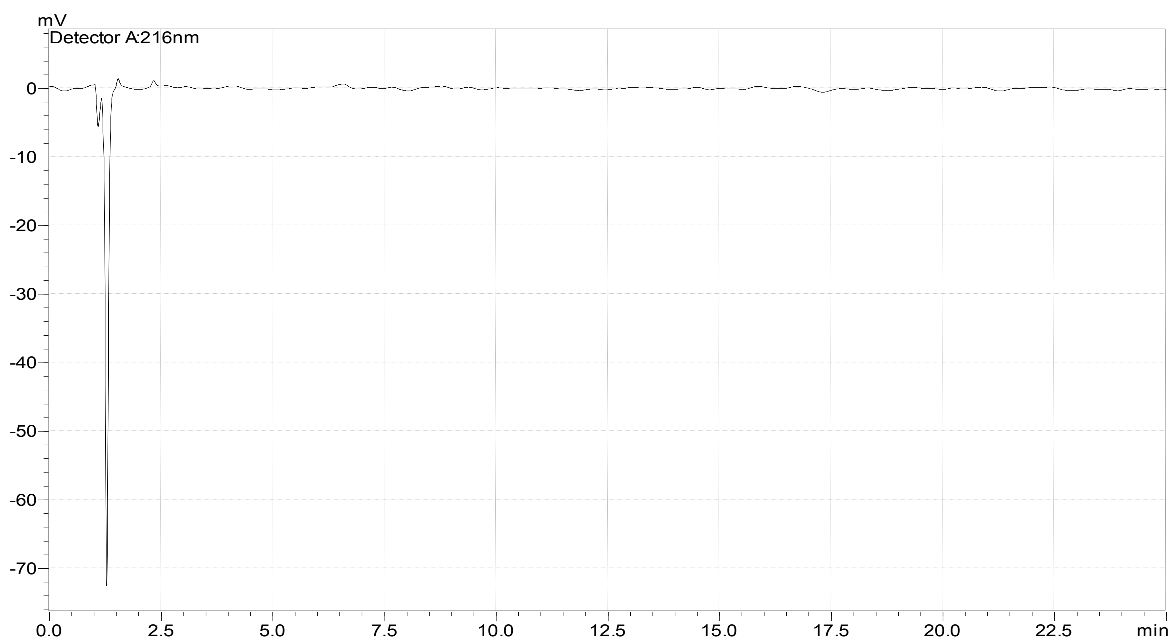


(a) Artemether:(+)-(3-alpha,5a-beta,6-beta,8a-beta,9-alpha,12-beta,12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin

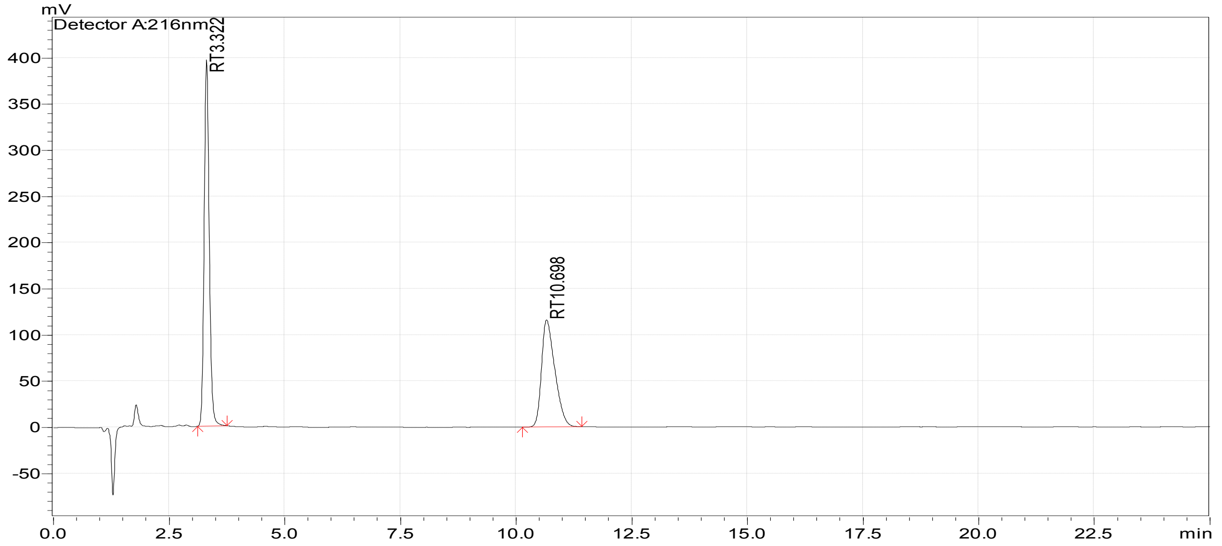


(b) Curcumin: (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

Figure I: Structure of (a) Artemether and (b) Curcumin



(II a) Blank



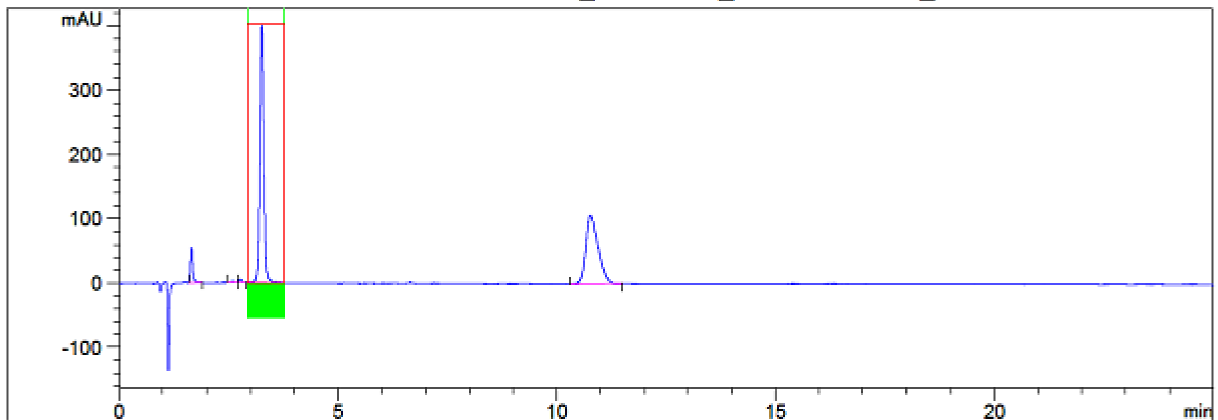
(II b) Standard Solution CUR-ART

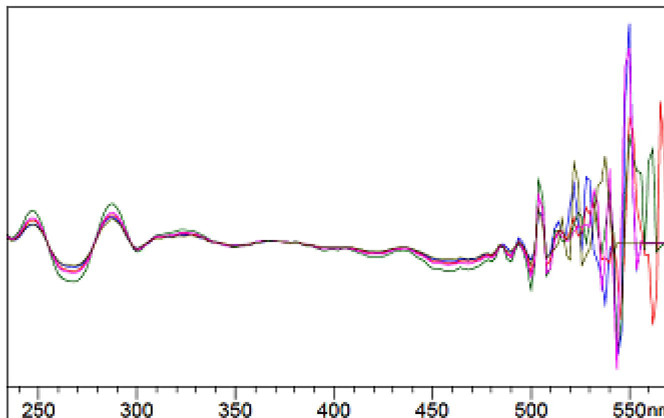
Figure II: HPLC chromatogram: (a) Blank and (b) Standard solution CUR (RT 3.32) and ART (RT **10.69)**

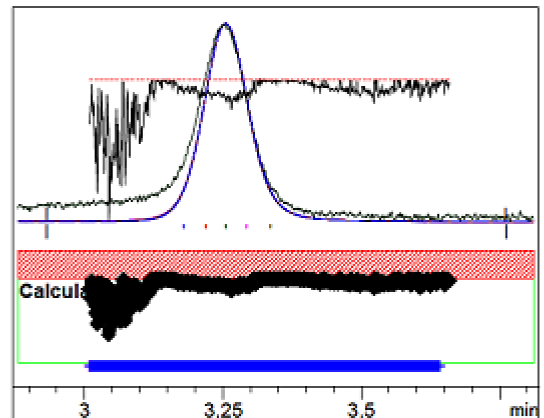
```
Data File C:\CHEM32\1\DATA\MD_ART-CURC_2014\ART50MG_CURC0.D
Sample Name: ART50MG_CURC0.5MG
```

```
Purity results peak 4 at 3.255 min.
```

```
Signal DAD1 C, Sig=216,4 Ref=off (MD_ART-CURC_2014\ART50MG_CURC0.D)
```







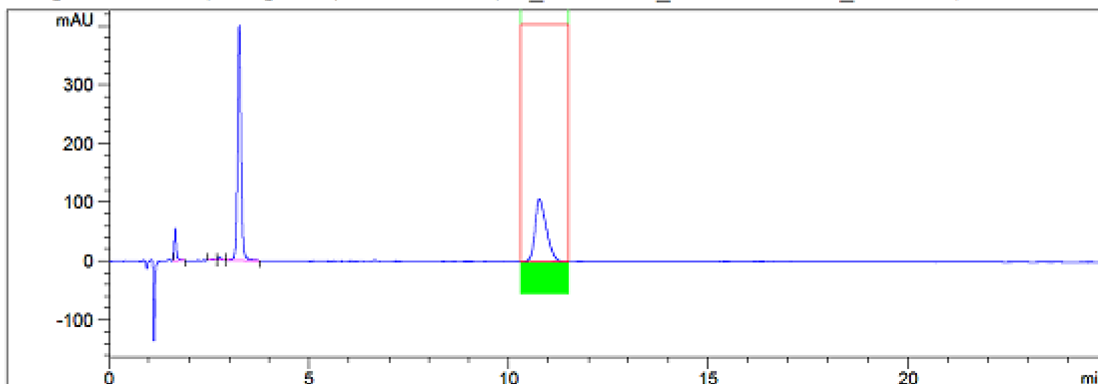

```
-> The purity factor is within the calculated threshold limit. <-
```

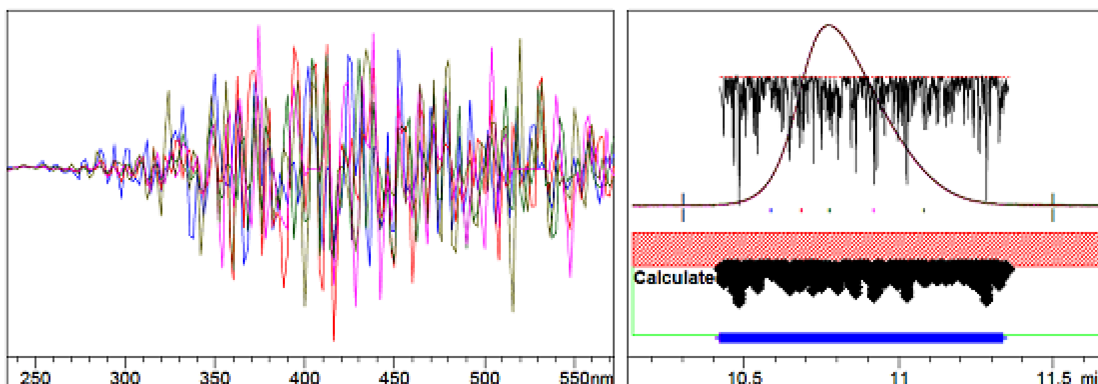
(III a) Peak Purity of Curcumin (CUR)

```
Data File C:\CHEM32\1\DATA\MD_ART-CURC_2014\ART50MG_CURC0.D
Sample Name: ART50MG_CURC0.5MG

        Purity results peak 5 at 10.773 min.

         Signal DAD1 C, Sig=216,4 Ref=off (MD_ART-CURC_2014\ART50MG_CURC0.D)
```

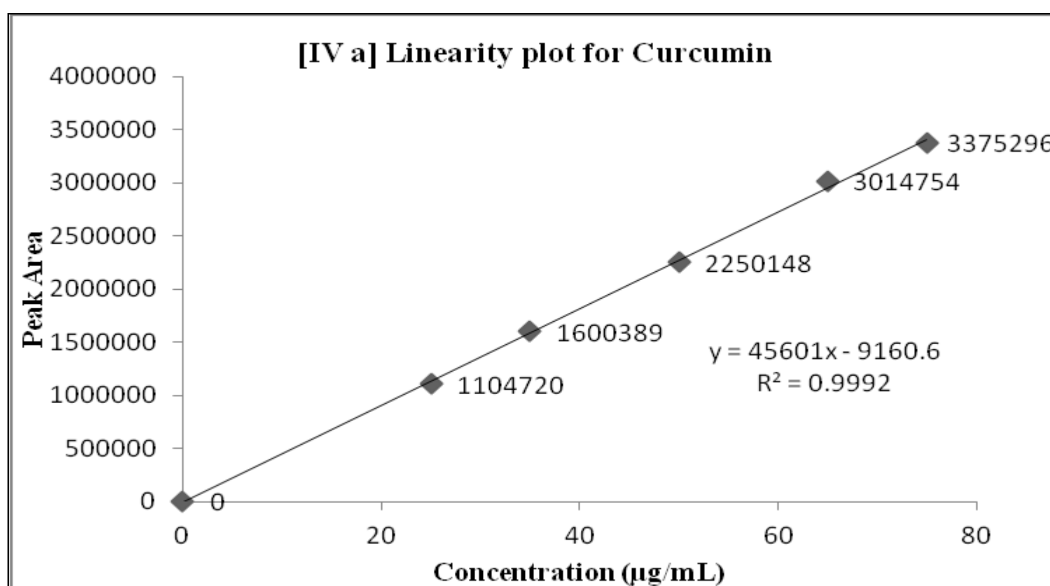





```
-> The purity factor is within the calculated threshold limit. <-
```

(III b) Peak Purity of Artemether (ART)

Figure III: Peak purity analysis by LCMS: (a) CUR and (b) ART



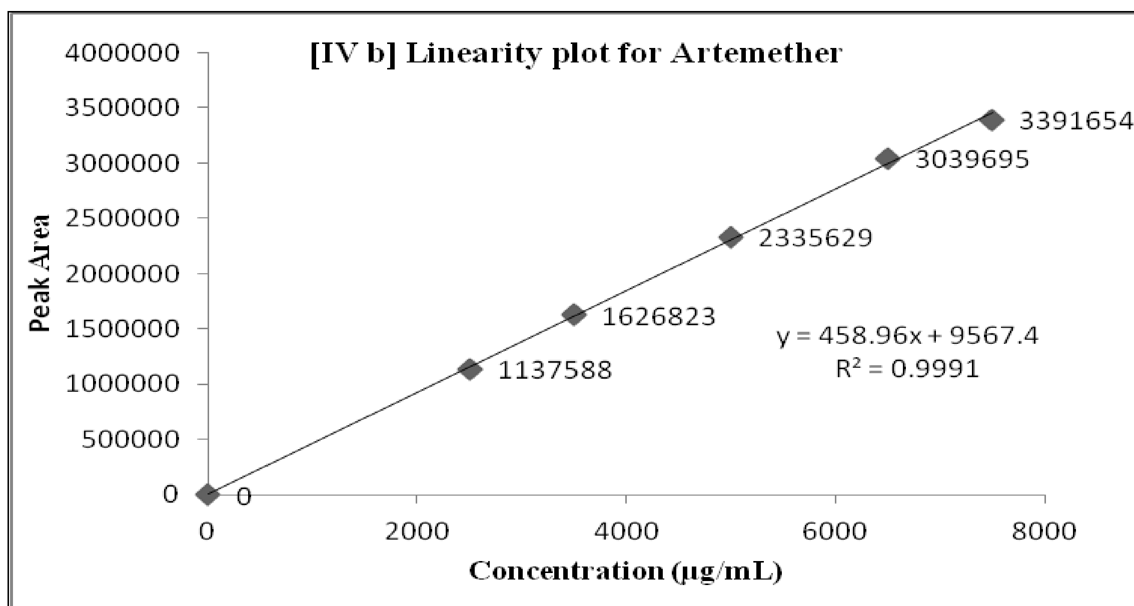


Figure IV: Calibration plot for (a) Curcumin and (b) Artemether: Conc. (µg/mL) vs. Peak Area

REFERENCES

1. ICH Harmonized Tripartie Guidline Q2A (2005), "***Text on Validation of Analytical Procedures***," Incorporated in November 2005.
2. J, Sunil; M, Sanjit Nath and U, Samba Moorthy (2010), "HPLC method development and validation for simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage forms", ***International journal of Pharmacy and Pharmaceutical Sciences***, Vol 2, Issue 4, 93-96.
3. Mandawgade, SD; Sharma, S; Pathak, S and Patravale, VB (2008), "Development of SMEDDS using natural lipophile: application to beta-Artemether delivery", ***International Journal of Pharmaceutics***, 362(1-2), 179-183.
4. Raju, C Reddy; Palakkodu, G Vatsala; Venkateshwar, G Keshamouni; Govindarajan, Padmanaban and Pundi, N Rangarajan (2005), "Curcumin for malaria therapy", ***Biochemical and Biophysical Research Communications***, 326(2), 472-474.
5. Shengpeng, Wang; Miao, Tan; Zhangfeng, Zhong; Meiwan, Chen and Yitao, Wang (2011), "Nanotechnologies for Curcumin: An Ancient Puzzler Meets" , ***Journal of Nanomaterials.***
6. Patrick, B Memvanga; Jeremie, K Mbinze; Eric, Rozet and Phillippe, Hubert *et al.* (2014), "Development of a liquid chromatographic method for the simultaneous quantification of curcumin, B artemether, tetrahydrocurcumin and dihydroartemisinin: Application to lipid based formulations", ***Journal of Pharmaceutical and Biomedical Analysis***, 88, 447-456.
7. Govindarajan, P; Nagaraj, VA and Rangarajan, PN (2012), "Artemisinin-based combination with curcumin adds a new dimension to malaria therapy", ***Current Science***, Vol. 102, No. 5.
8. Isabela, da Costa César and Gerson, Antônio Pianett (2009), "Quantitation of artemether in pharmaceutical raw material and injections by high performance liquid chromatography", ***Brazilian Journal of Pharmaceutical Sciences***, Vol. 45, (4), 737-742.
9. Moorthi, K Kathiresan (2013), "Simultaneous estimation of curcumin and silibinin using validated RP HPLC PDA method and its application in Pharmaceutical nanoformulation", **International *Journal of Pharmacy and Pharmaceutical Sciences*** , Vol 5, Suppl. 3,475-478.

10. Xiu, Mei Wang; Qi, Zhi Zhang Jian Yan; Rong, Hua Zhu, Jun Zhang; Li, Jing Cai and Wen, Xing Peng (2012), "Validated HPLC MS/MS Method for Simultaneous Determination of Curcumin and Piperine in Human Plasma", ***Tropical Journal of Pharmaceutical Research***,11(4), 621-629.
11. (2007),"***The United States Pharcopoeia XXVIIII and NF XXV***," Electronic Version, American Pharmaceutical Association, Washington DC.
12. ICH Harmonized Tripartie Guideline Q2B (R1) (2005), "***Validation of Analytical Procedures: Text and Methodology***," Incorporated in November 2005.
13. Nandakumar, DN; Arun, VN; Vathsala, PG; Rangarajan, PN and Padmanaban, G (2006), "Curcumin-artemisinin combination therapy for malaria", ***Antimicrobial. Agents Chemother***, 1859 1860.
14. The International Conference on Harmonization Q2 (R1) (2005), "***Validation of Analytical Procedure: Text and Methodology***".

Correspondence Author:
Rupali Kalra
School of Pharmaceutical Sciences, Plot No.23, Sector 32, Apeejay Stya University, Gurgaon-122001, India

Cite This Article: Rupali, Kalra; Anupama, Diwan; Jatinder, Kumar and Shekhar, Sharma (2016), "Simultaneous estimation of artemether & curcumin by RP-HPLC method", ***Pharmacophore***, Vol. 7 (3), 141-151.