Academic Sciences International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 7, Issue 1, 2015

Original Article

DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF ARTESUNATE AND CURCUMIN IN LIPOSOMAL FORMULATION

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Received: 24 Feb 2015, Revised and Accepted: 02 April 2015

ABSTRACT

The present study is very expectant towards the development of co-encapsulated liposomal formulation. The assay method for artesunate has a challenge because it doesnot have a readily recognisable absorption chromophore needed for UV spectroscopy. Curcumin, a lipid soluble antioxidant, exhibits solvent and medium sensitive absorption and fluorescence properties. A simple and rapid method involving two reaction steps has been developed for assay of artesunate and curcumin combination in liposomal formulation. Stock solution of artesunate and curcumin were diluted to final concentration of 10 µg/ml. The UV scan of 10 µg/ml solution of artesunate and curcumin combinations showed the absorbtion maxima at 240 nm and 425 nm respectively. The coloured complex obeyed the Beer's law in the concentration range of 2-20 µg/ml. The above methods is rapid tool for routine analysis of artesunate and curcumin in novel carrier system. The recovery studies confirmed the accuracy and precision of the methods.

Keywords: Artesunate, Curcumin, Liposomal .

INTRODUCTION

The artemisinin-based combination therapies (ACTs) are currently recommended by the World Health Organization as the first-line treatment for Plasmodium falciparum malaria. Since 2010, five ACTs are marketed namely: artemether plus luCURantrine, artesunate plus amodiaquine, artesunate plus CURloquine, artesunate plus sulfadoxine-pyrimethamine and dihy-droartemisinin plus piperaquine.[1] Several reasons including cost considerations, pharmacokinetic mismatch, resistance, cross-resistance and side effects due to the partner drug of artemisinin-type compounds, many efforts are invested to discover new companion drug. In the same way, Curcumin loaded lipid-based formulations were also developed to improve the bioavailability of Curcumin at the target site. In order to guarantee the quality, safety and efficacy of curcumin (CUR) and artesunate (ART) in these promising antimalarial lipid-based formulations, it is mandatory to have a suitable analytical procedure.

Curcumin (CUR, 1), 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3, 5-dione, is a polyphenol derived from the herbal remedy and dietary spice turmeric (*Curcuma longa*). It possesses diverse anti-inflammatory and anti-cancer properties following oral or topical administration.[1] Turmeric and curcumin are reported to be potent anti bacterial and anti hepatotoxic agents.[2] Chemically artesunate was (3*R*,5a*S*,6*R*,8a*S*,9*R*, 10*S*,12*R*,12a*R*)-Decahydro-3,6,9 trimethyl-3,12-epoxy-12 *H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol, hydrogen succinate.[3] Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticide active against the ring stage of the parasite.

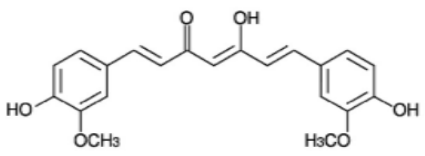

Fig. 1: Structure of Curcumin

There was no any method has been reported for simultaneous estimation of both drug by UV Spectrophotometric method. This paper describes a simple, accurate, sensitive and validated UV Spectrophotometric method for simultaneous quantification of these compounds as the bulk drug and in combined dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [4-5].

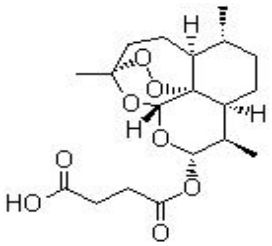

Fig. 2: Structure of artesunate

MATERIALS AND METHODS

Drugs and chemicals

A standard drug sample of curcumin was purchased from Chemdye Lab and artesunate were provided as gift from IPCA Pharma, Mumbai. All chemicals and solvents used of AR grade.

UV-spectrophotometer UV-1700(Shimadzu, Japan) with spectral band-with of 2 nm and 10 nm matched quartz cell was used for the development analytical method over the range of 800-200 nm. Optimized liposomal formulation containing artesunate and curcumin (1:1) was used as sample. Calibrated borosilicate glassware was used during experimental study.

Standard stock solution of ART

1. 0.1 N sodium hydroxide: 0.42 g of sodium hydroxide was weighed and dissolved in distilld water and made up to 100 ml.

2. 0.1 Macetic acid in 20% Methanol: 1.114 ml of glacial acetic acid was measured and diluted to 200 ml with methanol.

Decomposition reaction: Basic decomposition followed by acid reaction

The method applied in this study is modified methods used by Zhao in HPLC assay of artemisinin from plasma and saliva [6]. 10 mg ART

was accurately weighed, dissolved in methanol and made up to 10 ml to give a concentration of 1000 mcg/ml. One mililiter of the solution was transferred to a 10 ml volumetric flask containing 4 ml of 0.1 N Sodium hydroxide. The flask was placed in the water bath at 50±2 °C for 1 hr. The solution was allowed to cool and made up to volume with 0.1 M acetic acid in 20% methanol. The stock solution was diluted to the 10, 20. 60 µg/ml concentrations with 0.1 M acetic acid in 20% methanol.

Standard stock solution of CUR

An accurately weighed quantity of 10 mg of CUR was taken in 10 ml volumetric flask dissolved in sufficient quantity of methanol and diluted to 10 ml with the same solvent so as to get the concentration of 1000 µg/ml.

Determination of λ max of ART and CUR

The standard solution of CUR was scanned in the range of 800-200 nm and the λ max was found to be 425 nm against methanol. Similarly, the standard solution of ART was scanned in the range of 800-200 nm and the λ max was found to be 240 nm against0.1 M acetic acid in 20% methanol.

Analysis of mix standard (ART and CUR) and formulation

The method was checked by analysing a solution congaing the known concentration of both drugs. The mixed standard in Beer-Lambert's range for each drug in the ratio of 1:5 containing 10, 30, 60 µg/ml of ART and 2, 6, 12 µg/ml of CUR respectively was prepared by diluting appropriate volumes of standard stock solution. Liposomes were prepared by film hydration methods by using rotary vacuum evaporator and optimized to the ratio of drug: Cholesterol: PEG: Soya lecithin in 1:10:15:30.

The % entrapment of artesunate and curcumin was found to be 96.42 and 95.78 respectively. Drug associated with liposome was separated from unentraped drug using centrifugation methods. Liposomes were centrifuged at 20000 RPM for 1 hr at controlled temperature 4 °C.

Supernatant containing unentrapped drug suspension was withdrawn and diluted first with 10 ml methanol and second according to decomposition reaction. Various dilutions of the mix standard and formulation stock solutions were scanned and the absorbances of these solutions were measured at 240 nm and 425 nm respectively. The concentration of ART and CUR present in mixed standard was calculated using simultaneous equation.

Method validation

The method was developed and validated according to analytical procedure as per the ICH guidelines [9-10] for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ and accuracy for the analyte.

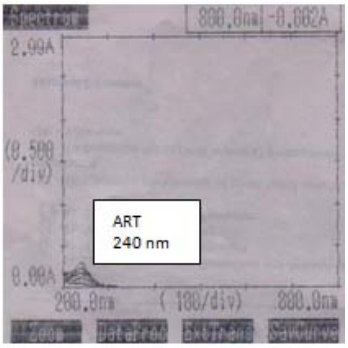

Fig. 3: λ max of ART

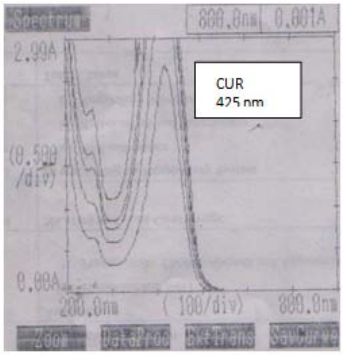

Fig. 4: λ max of CUR

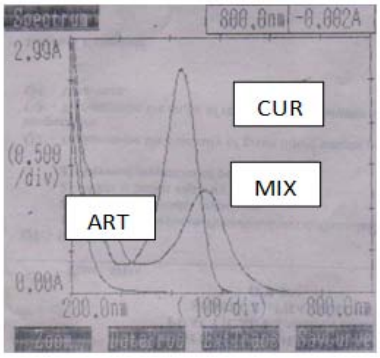

Fig. 5.: Overlay spectrum of ART, CUR and Formulation

Table 1: Standard calibration table of ART and CUR

S. No.	For artesunate conc. (µg/ml)	Abs*at 240 nm	For curcumin conc. (µg/ml)	Abs at 425 nm
1	10	0.007	02	0.162
2	20	0.015	04	0.321
3	30	0.024	06	0.483
4	40	0.032	08	0.658
5	50	0.040	10	0.804
6	60	0.049	12	0.958

* each value is a mean of three observation

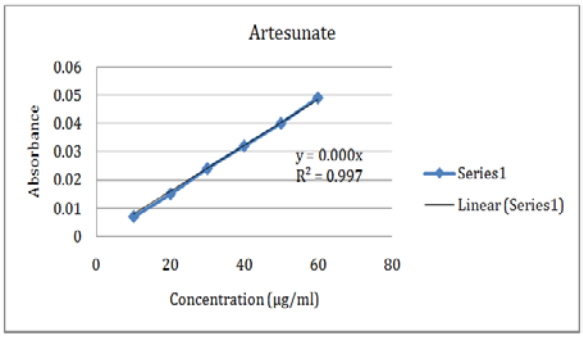

Fig. 6: Calibration curve of Artesunate at 240 nm

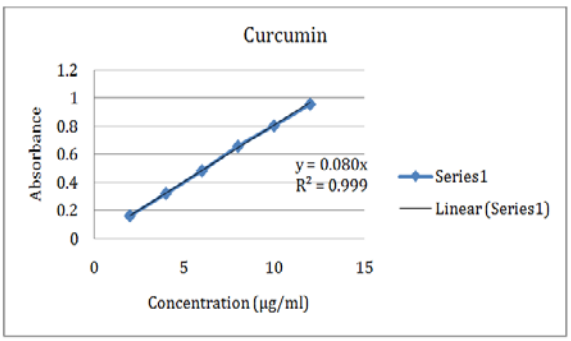

Fig. 7: Calibration curve of curcumin at 425 nm

Table 2: Optical and line equation parameter

Parameter	Artesunate	Curcumin
Working Wavelength	240	425
Linearity Range (µg/ml)	10-60	02-12
MolarAbsorptivity (mol^{-1} cm^2)	(9.153±0.59) x 10^2	48000
Limit of Detection (µg/ml)	0.54	0.45
Limit of Quantitation(µg/ml)	1.79	0.48
Y=mx+c		
Slop	0.000	0.080
Regression Coefficient	0.997	0.999

Table 3: Absorbance of mixed standard (ART+CUR)

S. No.	Mix Standard		Abs at 240 nm	Abs at 425 nm
	Conc. of ART(µg/ml)	Conc. of CUR (µg/ml)		
1	10	02	0.008	0.164
2	30	06	0.025	0.485
3	60	12	0.048	0.956

Table 4: Result of mixture containing ART and CUR

S. No.	Amount present (µg/ml)		Amount found (µg/ml)		% Amount found	
	ART	CUR	ART	CUR	ART	CUR
1	10	02	9.96	1.94	99.6	97.00
2	30	06	29.85	5.78	99.5	96.33
3	60	12	59.90	11.84	99.83	98.66

Table 5: Result of optimized liposomal formulation

S. No	Label claim (mg/ml)		Amount found (mg/ml)		% Label Claim	
	ART	CUR	ART	CUR	ART	CUR
1	1	2	0.98	1.97	98	98.5
2	2	4	1.96	3.98	98	99.5
3	3	6	2.97	5.99	99	99.83
Mean					98.33	99.27
SD					0.578	0.478
%RSD					0.587	0.482

Table 6: Result of recovery studies

Level of % recovery	Amount present(mg/ml)		Amount of standard added (mg)		Total amount recovered (mg)		% Recovery	
	ART	CUR	ART	CUR	ART	CUR	ART	CUR
80	1	2	0.8	1.6	1.78	3.59	98.8	99.7
100	2	4	1.0	2.0	2.98	5.89	99.3	98.2
120	3	6	1.2	2.4	4.19	8.38	99.7	99.7
Mean							99.26	99.2
SD							0.531	0.86
%RSD							0.535	0.87

RESULTS AND DISCUSSION

A) Linearity

From the standard stock solutions containing 1000 µg/ml of ART and 1000 µg/ml of CUR dilutions were made to prepare the range of standard solutions having different concentrations of ART (10-60 µg/ml) and CUR (02-12 µg/ml). The absorbance was measured at 240 nm (λ_{max} of ART) and at 425 nm (λ_{max} of CUR) respectively. The results obtained are shown in table No.1. The linearity of the relationship between absorbance and concentration was determined by plotting the calibration curve for ART and CUR respectively. The calibration curves are shown in fig. 6 and 7.

B) Precision

Repeatability of the method was established by analyzing various replicates samples of ART and CUR. Precision was carried out by performing Interday variation and intraday variation. In Interday variation the sample was analyzed on three consecutive days. In intraday and variation the absorbance were measured three times in a day. The results for intraday and interday precision are shown in table No.7.

C) Limit of detection

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD was calculated using the following formula:

$$LOD = \frac{3.3 \times \text{Std deviation of Y Intercept}}{\text{Slope of calibration curve}}$$

The limit of detection of ART and CUR are as follows:

ART: 0.54 µg/ml, CUR: 0.45 µg/ml

D) Limit of quantitation

The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable

precision and accuracy. LOD was calculated using the following formula:

$$LOD = \frac{10 \times \text{Std deviation of Y Intercept}}{\text{Slope of calibration curve}}$$

The limit of detection of ART and CUR are as follows:

ART: 1.79 µg/mlCUR: 0.48 µg/ml

E) Recovery studies

Recovery studies were carried out by standard addition method at three levels, 80%, 100%, and 120%. In this method, a known amount of standard drug solution were added to Liposomal suspension and absorbance was measured at 240 nm and 425 nm (λ. max of ART and CUR respectively) and the concentration of both drugs can be determined.

At each level three determinations were performed and results were obtained. The results for recovery studies were given in table No.6.

Table 7: Result of intermediate precision

Formulation	Parameter	Intraday precision	Interday precesion
ART	Mean	99.58	99.68
	SD	0.3176	0.3722
	%RSD	0.3189	0.3732
CUR	Mean	100.24	99.59
	SD	0.5656	0.2504
	%RSD	0.5644	0.2516

The linearity of ART and CUR was observed in the range of 20-140µg/ml and 50-350µg/ml respectively with correlation coefficient 0.9954 & 0.994 for ART and CUR respectively. Detection wavelength used was 242 nm for ART and 256 nm for CUR. The LOD and LOQ values for ART were 0.54µg/ml & 1.79µg/ml. For CUR the LOD and LOQ values were 0.45µg/ml & 0.48µg/ml.

CONCLUSION

A simple, precise, accurate and economic UV method was developed and validated for estimation of Artesunate and curcumin from bulk and formulation. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, LOD, LOQ. The LOD and LOQ values for ART were 0.54µg/ml & 1.79µg/ml. For CUR the LOD and LOQ values were 0.45µg/ml & 0.48µg/ml. the method was validated as per ICH guideline.

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