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Estimation of Artemether and Arteether by High Performance Thin Layer Chromatography

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A new, simple, accurate and precise high performance thin layer chromatographic method has been developed for the estimation of artemether and arteether as pure drug powder and in pharmaceutical formulations. The method employs silica gel F_{254} as stationary phase on aluminium foil and mobile phase comprising toluene: butanol (10:1) in case of artemether and toluene: dichloromethane (0.5:10) for arteether. Since artemisinin derivatives are non-UV absorbing compounds, they were converted into UV absorbing derivative using a derivatizing agent. A solution of 10 % v/v sulphuric acid in ethanol gave prominent well-resolved blue coloured spot for artemether and vanillin in sulphuric acid in ethanolic solution gave prominent well-resolved pink coloured spot for arteether. In both cases, the spots were stable for more than a day. The densitometric analysis of both the drugs was carried out in the absorbance mode at 546 nm. The R_f values were 0.45 for artemether and 0.30 for arteether. The linear detector response was observed between 100-600 ng per spot for artemether and arteether and the calibration plots showed good linear relationship with coefficient of correlation, r = 0.9981 and 0.9989, respectively, with respect to peak area. The methods were validated for precision, recovery and robustness. The limits of detection and quantitation were 25 and 28 ng/spot for artemether and 75 and 84 ng/spot for arteether, respectively. The recovery study was carried out by standard addition method. The recovery was found to be 99.49 ± 0.687 for artemether and 99.50 ± 0.590 for arteether.

Key Words: Artemether, Arteether, HPTLC.

INTRODUCTION

Artemisinin and its derivatives are promising and potent antimalarial drugs, which meet the dual challenge, posted by drug resistant parasites and rapid progression of malarial illness[1]. Artemisinin (Quinghaosu) is a novel antimalarial drug, which has been isolated from Chinese plant *Artemisia annua*, belonging to family Asteraceae[2]. It is a sesquiterpene

with an unusual endoperoxide linkage structurally unrelated to other known antimalarials. The use of artemisinin as antimalarial agent is hampered by its poor solubility in oil and water and its poor efficacy on oral administration. Thus, new structural modifications in artemisinin were essential[3]. The formation of short chain ether derivatives such as artemether (methyl ether) and arteether (ethyl ether) have modified the sesquiterpene. Both of these derivatives possess superior lipid solubility and antimalarial activities comparable to parent artemisinin[4].

Literature survey revealed that many methods like TLC[5], HPLC with UV detection[6,7] and with reductive electrochemical detection[8-10], capillary gas chromatography chemical ionization mass spectrometry[11], GC-MS with selective ion monitoring[12] and colorimetric method[13,14] for the estimation of artemether in biological fluids and pharmaceutical formulations are reported. Methods reported for arteether include HPLC with UV detection[15], with reductive electrochemical detection[16] and with mass spectrometry[17], UV spectrophotometric method[18] and liquid chromatography with mass spectrometry[19].

In the present study, a new analytical method by HPTLC was developed and validated, which showed results comparable to that obtained with earlier reported HPLC method. Moreover, this method is more accurate, precise and reproducible over the other available methods.

EXPERIMENTAL

Artemether bulk drug was obtained as a gift sample from IPCA Lab. Ltd., M.P. and arteether from Skymax Laboratories Pvt. Ltd., Gujrat. All other chemicals and reagents were of AR/HPLC grade.

The instrument used in the present study was CAMAG-HPTLC system comprising CAMAG LINOMAT V automatic sample applicator, CAMAG TLC SCANNER III with WINCATS software; CAMAG twin trough glass chambers were used.

Chromatographic conditions: Stationary phase: Silica gel 60 F_{254} TLC precoated aluminium plates, 10 × 10 cm size with 200 µm layer thickness. Mobile phase: toluene: butanol (10:1) for artemether and toluene: dichloromethane (0.5:10) for arteether. Chamber saturation time: 0.5 h. Sample application: 6 mm band. Separation technique: ascending. Temperature: 20 ± 5ºC. Migration distance: 75 mm. Scanning mode: absorbance. Detection wavelength: 546 nm. Source of radiation: deuterium or tungsten lamp.

Calibration curve: A stock solution of artemether (100 µg/mL) in methanol was prepared by dissolving 10 mg of artemether in 100 mL of methanol. Different volumes of stock solution 1, 2, 3, 4, 5 and 6 µL were spotted in duplicate on TLC plate with the help of automatic sample

applicator, to obtain concentrations of 100, 200, 300, 400, 500 and 600 ng/spot of artemether, respectively. The plates were developed in the twin trough chamber, dried and densitometrically scanned at 546 nm. The data of peak height/area *vs.* drug concentration were treated by linear least-square regression. Similarly, stock solution of arteether (100 µg/mL) was prepared and procedure repeated as above.

Validation of the proposed method

Precision: Precision of an analytical method is expressed as SD or RSD of series of measurement. Repeatability of sample application and measurement of peak area were performed by the proposed method using six replicates of the same spot (500 ng/spot of the drug). The intra- and inter-day variation for the determination of artemether and arteether was carried out at three different concentration levels of 200, 600 and 3000 ng/spot. This test was performed to justify the suitability of the proposed method.

Robustness: By altering various experimental conditions like mobile phase composition, amount of mobile phase, plate treatment, time from spotting to chromatography and time from chromatography to scanning, the effects on the results were examined. It was carried out at three different concentration levels of 200, 600 and 3000 ng/spot, in triplicate for artemether and arteether, respectively.

Limit of detection and limit of quantitation: In order to estimate the LOD and LOQ, blank methanol was spotted six times on HPTLC plate and then developed, sprayed and scanned in a similar way as that for calibration curve and then signal-noise ratio was determined.

The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\sigma}{S}$$

where, σ = the standard deviation of the response and S = the slope of the calibration curve.

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

Recovery studies: Stock solution of marketed preparation (having concentration of 800 µg/mL in case of artemether and 750 µg/mL for arteether) was prepared. This solution was first analyzed by the proposed method. In the analyzed sample, an extra 80, 100 and 120 % of standard drug was added and then mixture was analyzed. The experiment was conducted in triplicate. It was done to find out the recovery of drug at different levels in the formulation.

Analysis of artemether and arteether in marketed formulations: To determine the content of artemether in injection (labeled claim: 80 mg/mL), the contents of the vials were pooled and mixed with methanol. The solution was sonicated for 0.5 h and volume was made up to 100 mL with methanol. The resulting solution was filtered twice. Final concentration of 800 ng/μL was obtained and 1 μL of this solution was spotted on plate, developed and scanned.

To determine the content of arteether in injection (labeled claim: 150 mg/2 mL), the contents of the vial was pooled and mixed with methanol. Same procedure was followed as above, to obtain final concentration of 750 ng/μL. 1 μL of this solution was spotted on plate, developed and scanned. Both the experiments were done six times.

RESULTS AND DISCUSSION

The mobile phase optimized for the TLC procedure was selected by a process of trial and error. Toluene was selected as one of the components of mobile phase with acceptable resolution. Since R_f value was too low, butanol was added in varying ratios. Good resolution and reproducible R_f was achieved with ratio of toluene:butanol (10:1). Similarly, mobile phase chosen for arteether was toluene:dichloromethane (0.5:10). Since artemisinin derivatives are non-UV absorbing compounds, they were converted into UV absorbing derivative using derivatizing agent. A solution of 10 % v/v sulphuric acid in ethanol gave prominent well-resolved blue coloured spot for artemether and vanillin in sulphuric acid in ethanolic solution gave prominent well-resolved pink coloured spot for arteether. In both cases, the spots were stable for more than a day. Densitometric quantitation was carried out in the absorbance mode at 546 nm and symmetrical, well-resolved, well-defined peaks were obtained for artemether and arteether. The R_f values were 0.45 and 0.30 for artemether and arteether, respectively. The linear regression data for the calibration curves (n = 3) in Table-1 showed good linear relationship over the concentration range 100-600 ng per spot with respect to peak area for both the drugs with coefficient of correlation values 0.9981 and 0.9961, respectively (Figs. 1 and 2). The developed HPTLC method was validated as per ICH guidelines. The precision of the method was evaluated by repeatability of sample application and measurement of peak area using 6 replicates of the same spot (500 ng/spot of the drug) with % RSD of 0.665 and 0.56 for artemether and arteether, respectively. The intra- and inter-day variation for the determination of artemether and arteether was carried out at 3 different concentration levels of 200, 600 and 3000 ng/spot with %RSD of 1.01 and 0.34, respectively (Table-2). The robustness of the method was evaluated by altering the various experimental conditions. This was

TABLE-1
DATA FOR CALIBRATION CURVE OF ARTEMETHER AND ARTEETHER (n = 3)

Concentration (ng/spot)	Mean peak area ± SD	
	Artemether	Arteether
100	1456.47 ± 15.90	365.61 ± 18.84
200	2096.11 ± 16.82	558.53 ± 35.23
300	2792.91 ± 10.25	941.82 ± 14.00
400	3544.01 ± 13.64	1244.04 ± 20.64
500	4162.57 ± 33.29	1559.48 ± 21.93
600	4724.77 ± 28.01	1853.29 ± 13.45

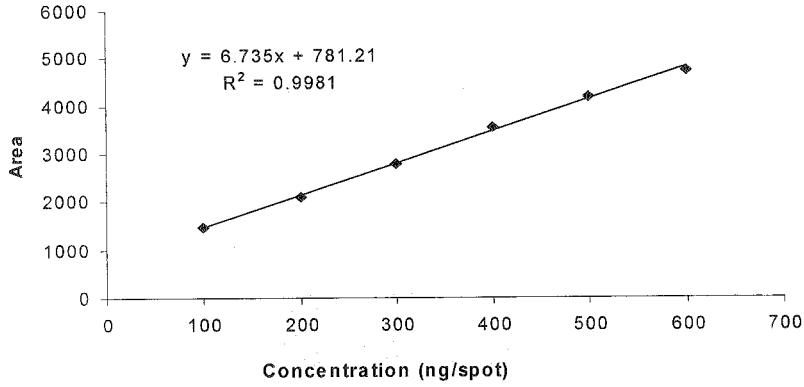

Fig. 1. Calibration plot for artemether

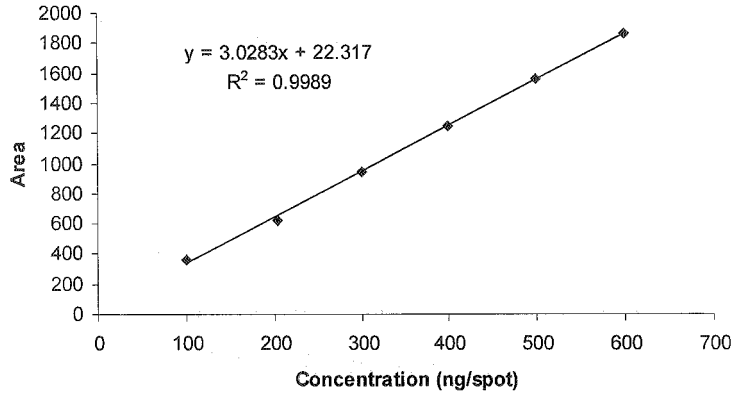

Fig. 2. Calibration plot for arteether

TABLE-2
INTRA- AND INTER-DAY PRECISION OF THE METHOD FOR ARTEMETHER AND ARTEETHER (n = 6)

Amount (ng/spot)	Drug	Intra-day precision			Inter-day precision		
		Mean area	SD	RSD (%)	Mean area	SD	RSD (%)
200	Artemether	2076.01	27.43	1.32	2207.39	23.56	1.06
	Arteether	2165.55	13.34	0.61	2066.26	26.23	1.26
600	Artemether	4862.54	39.83	0.81	4683.43	28.18	0.60
	Arteether	6662.59	25.62	0.38	6742.59	25.85	0.38
3000	Artemether	15100.13	38.52	0.25	15238.02	64.11	0.42
	Arteether	19251.94	31.32	0.162	19376.77	37.24	0.19

carried out at three different concentration levels of 200, 600 and 3000 ng/spot, in triplicate for artemether and arteether, respectively with % RSD values of 0.48 for both drugs (Tables 3 and 4). The limit of detection and limit of quantitation for artemether and arteether are 25 ng/spot, 75 ng/spot and 28 ng/spot, 84 ng/spot, respectively. The accuracy of the method was evaluated by percentage recovery (by standard addition) of the drug. The average recovery was found to be 99.49 ± 0.687 and 99.50 ± 0.590, respectively (Table-5). The developed HPTLC method was also applied to the analysis of artemether and arteether in their pharmaceutical dosage forms. The drug content was found to be 98.81 % with % RSD of 0.64 in case of artemether and 99.03 % with % RSD of 0.32 for arteether (Table-6). Low values of % RSD indicate the suitability of method for routine analysis of artemisinin derivatives in their pharmaceutical dosage forms.

TABLE-3
ROBUSTNESS OF THE METHOD FOR ARTEMETHER (n = 3)

Parameters	RSD (%)			Mean RSD (%)
	200 ng/μL	600 ng/μL	3000 ng/μL	
When plate was activated for 40 min	0.23	0.69	0.52	0.45
When 15 mL of mobile phase was used	0.33	0.20	0.65	0.48
After 10 min of development of plate, scanning was carried out	0.35	0.67	0.59	0.39
After 5 min of spotting, development of plate was carried out	0.50	0.38	0.82	0.56
Mobile phase composition	0.49	0.65	0.23	0.53

TABLE-4
ROBUSTNESS OF THE METHOD FOR ARTEETHER (n = 3)

Parameters	RSD (%)			Mean RSD (%)
	200 ng/μL	600 ng/μL	3000 ng/μL	
When plate was activated for 40 min	0.66	0.25	0.13	0.34
When 15 mL of mobile phase was used	0.35	0.44	0.53	0.44
After 10 min of development of plate, scanning was carried out	0.49	0.62	0.24	0.45
After 5 min of spotting, development of plate was carried out	0.85	0.44	0.60	0.63
Mobile phase composition	0.74	0.52	0.38	0.54

TABLE-5
RECOVERY STUDIES FOR ARTEMETHER AND ARTEETHER (n = 3)

Excess drug added to analyte (%)	Drug	Theoretical content (ng)	Recovery (%)	RSD (%)
80	Artemether	144	98.89	0.211
	Arteether	135	98.93	0.250
100	Arteether	160	99.34	0.203
	Arteether	150	99.48	0.230
120	Artemether	176	100.24	0.314
	Arteether	165	100.11	0.150

TABLE-6
ANALYSIS OF MARKETED FORMULATION OF ARTEMETHER AND ARTEETHER BY HPTLC METHOD (n = 6)

Drug	Theoretical content (mg/mL vial)	Amount of drug recovered (mg/mL vial)	Recovery (%)	RSD (%)
Artemether	80	74.27	98.81	0.647
Arteether	75	79.05	99.03	0.320

The results obtained on analysis by the proposed method for artemether and arteether revealed that the HPTLC method is simple, accurate, precise and reproducible for the estimation of the drugs as pure drug powder and in pharmaceutical dosage forms. Moreover, this method can be used for the routine analysis of artemether and arteether in their respective, commercially available marketed formulations.

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