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The forced degradation and solid state stability indicating study and Validation of method for the determination of Assay of Artesunate by HPLC.

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ABSTRACT

An analytical method based on high-performance liquid chromatography (HPLC) validation of the method for the determination of assay of Artemether, used to malaria control drug. The mobile phase consisted of a mixture of water, acetonitrile and methanol in the ratio of 30:35:35 v/v/v. Chromatography was performed on Symmetry shield RP -18, 4.6 x 150mm, 3.5µm and detector of UV at 210 nm, 1.0 mL/min as a Flow rate, 30 µL as an Injection volume. The chromatogram of Artemether its impurities namely α-artemether, dihydroartemisinin and artemisinin were found. Accuracy satisfactory by % recovery obtained in the range of 100.4 – 100.6 at 80 to 120% level, the linearity results for Artemether linear from 80 – 120 %.(R= 0.9997). An accelerated forced degradation study on Artemether significant degradation was observed when Artemethe sample solution exposed to acid at room temperature, base and peroxide. Rapid degradation observed when Artemether samples exposed to acid at 60^0C and UV light. The degraded samples shows decrease in assay value, indicating that the assay method is stability indicating. The assay values obtained for the solid state in the range of 92.1- 99.9%.The proposed method was found to be specificity, linearity, and precision, intermediate precision, and accuracy, stability in analytical solution and robustness. The validation was performed according to the current requirements as laid down in the ICH guidelines.

Keywords: Artemether; forced degradation; RP-HPLC

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INTRODUCTION

Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the poor countries of the world [1]. One of the greatest challenges facing malaria control worldwide is the spread and intensification of parasite resistance to antimalarial drugs. The limited number of such drugs has led to increasing difficulties in the development of antimalarial drug policies and adequate disease management [2]. Artemisinin-based combination therapy (ACT) is increasingly being advocated as promising treatment. ACT is based on the use of two drugs with different modes of action: an artemisinin-derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action [3]. Malaria is a major health problem in many areas of the world. It is estimated that 300-500 million people are at risk of contracting malaria each year, and 1-2 million deaths are reported annually due to severe, cerebral malaria [4]. The commonly used antimalarials including chloroquine, sulfadoxine- pyrimethamine, and mefloquine, showed drug resistance to the parasites [5]. Semisynthetic derivatives of qinghaosu (QHS), a natural product of the Chinese herb *Artemisia annua*, are highly effective against multiresistant strains of *Plasmodium falciparum*. QHS (artemisinin) derivatives are nitrogen free sesquiterpenes that contain a peroxide linkage, which confers activity against the malaria parasite. These compounds are effective safe and well tolerated. They are rapidly metabolized to the active metabolite dihydroartemisinin. Artemether (Fig. 1) is one of these promising antimalarial compounds [6]. Nevertheless, several analytical techniques have been reported for the qualitative and quantitative determination of these compounds in biological matrices. High-performance liquid chromatography (HPLC) with post-column alkali [7–9] and pre-column acid [10–12] derivatization and UV detection were the most popular techniques. The presence of a peroxide bridge in the structure of these compounds offers the advantage of the use of HPLC with reductive electrochemical detection [13–16].

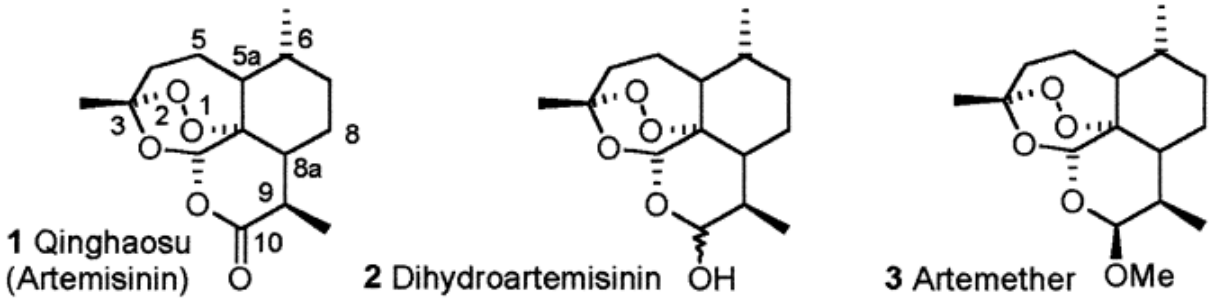

Fig. 1. Structure of artemisinin, artemether, and dihydroartemisinin.

EXPERIMENTAL

Reagents and chemicals

HPLC grade Acetonitrile and Methanol were obtained from Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard volumetric glassware.

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Instrumentation and Chromatographic Conditions

The instrument used was a Waters Model Alliance 2695 separation module equipped with auto sampler, Waters 2998 PDA Detector and the data recorded using empower software. The mobile Phase consisted of acetonitrile and tetrahydrofuran by using the column Symmetry shield RP -18, 4.6 x 150mm, 3.5µm and detector of UV at 210 nm, 1.0 mL/min as a Flow rate.

RESULTS AND DISCUSSION

Method development

Precision

System precision

Six replicate injections of standard solution of Artemether were injected into the HPLC system and analyzed as per the proposed method. The areas of response of the analyte along with % RSD are 0.06.The % RSD observed on the replicates indicates the reproducibility and hence the precision of the system.

Method precision

Six samples of Artemether analyzed as per the method. Each named impurity and total impurities were calculated on these replicates. The % RSD observed 0.18. These results comply with the acceptance criteria and indicating acceptable precision of the system. The % RSD observed in the calculation of known impurities and total impurities indicate the precision of the method.

Specificity

Each known impurity and Artemether solutions were prepared individually at a concentration of 0.10 mg/ml and a solution of all known impurities spiked. All these solutions were analyzed using the PDA detector as per the HPLC method. Artemether and its known impurities (α-artemether, dihydroartemisinin and artemisinin) elute at different retention times and the impurities are adequately resolved as shown in fig 2.

Linearity

The linearity of the HPLC method was demonstrated for Artemether related substances solutions ranging from LOQ 20%, 40%, 80%, 100%, 120% and 150% .Results obtained are shown in Table1. The linearity results for Artemether and impurities in the specified concentration range were found satisfactory, with acorrelation coefficient greater than 0.99.

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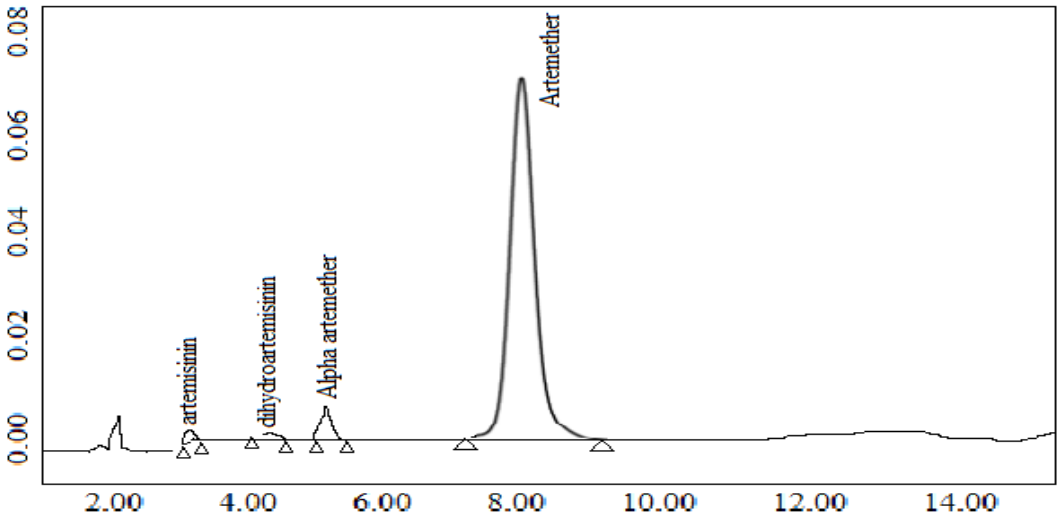

Figure 2: Artemether and spiked with impurities.

Table 1. Linearity method.

Component	Slope	Intercept	Multiple R	R.square
α-artemether	25900285.24	-203.0438	0.9991	0.9982
dihydroartemisinin	15080195.34	-169.0652	0.9992	0.9983
artemisinin	40337599.28	-115.9015	0.9995	0.9990
Artemether	42142633.97	-411.2542	0.9996	0.9993

Accuracy

A known amount of Artemether was taken into volumetric flask and spiked with known quantities of each named impurity at LOQ, 20%, 40%, 80%, 100%, 120% and 150% in triplicates. % recovery obtained in the range of 100.4 – 100.6 at 80.0% to 120.0%.

Robustness

System suitability followed by a sample analysis was run to assess if these changes had a significant effect on the chromatography. A sample of Artemether spiked with known impurities was analyzed for verifying the level of impurities at each variation. The retention time of all the impurities including Artemether were effected by slight variation in the flow, pH and column temperature, however the system suitability criteria for the method were fulfilled. The number of theoretical plates for Artemether peak not less than 3000. The resolution between the peaks due to intermediate and Artemether not less than 2.0. The tailing factor for Artemether peak not more than 2.0.

System suitability

Theoretical plates for Artemether e peak from first chromatogram of standard should be not less than 3000, Tailing factor for Artemether peak from first chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0.

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Solution Stability

Solution stability was checked and Sample solution spiked with impurities is found to be stable up to 1440 minutes at 10°C.% difference of response from initial for each known impurity >0.1% not more than 15 and total impurities not more than10.

Degradation studies

Artemether and its impurities were analyzed individually to verify the retention times. In order to assess the stability indicating nature of the HPLC method, Artemether samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples were then analyzed a photodiode-array detector.

Forced Degradation

Analyze the impurities and Artemether individually as per above method to verify the retention time. In order to assess the stability indicating nature of the HPLC method, Artemether samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples are analyzed using a photodiode-array detector.

Standard preparation

About 100 mg of Artemether standard was transferred into a 50 mL volumetric flask. Dissolved in and diluted to volume with diluent of methanol. Diluted 5.0 mL of this solution to 10 mL with diluent of methanol. Prepared in duplicate.

Acid hydrolysis, Base hydrolysis and Oxidation

At room temperature one solution was prepared by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N hydrochloric acid solution. The solutions were kept at room temperature for 3 hours, then neutralized with 0.2 mL of 1N sodium hydroxide solution and diluted to 10 mL with diluents methanol. At 60^0C one solution was prepared by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N hydrochloric acid solution. The solutions were kept at 60^0C for 3 hours, then neutralized with 0.2 mL of 1N sodium hydroxide solution and diluted to 10 mL with diluent of methanol.

At room temperature four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N sodium hydroxide solution. The solutions were kept at room temperature for 3 hours, 6 hours, 12 hours and 24 hours, then neutralized with 0.2 mL of 1N hydrochloric acid solution and diluted to 10 mL with diluent of methanol. At 60^0C four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL

volumetric flask, dissolved in and diluted to volume with diluents methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 1N sodium hydroxide solution. The solutions were kept at 60^0C for 3 hours, 6 hours, 12 hours and 24 hours, then neutralized with 0.2 mL of 1N hydrochloric acid solution and diluted to 10 mL with diluent of methanol.

At room temperature four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 5% of hydrogen peroxide solution. The solutions were kept at room temperature for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.At 60^0C four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask and added 0.2 mL of 5% of hydrogen peroxide solution. The solutions were kept at 60^0C for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.

Heat degradation, UV degradation and Solid state stability

Four solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask. The solutions were kept at 60^0C for 3 hours, 6 hours, 12 hours and 24 hours and diluted to 10 mL with diluents of methanol.
Three solutions was prepared individually by transferring about 100 mg of substance into a 50 mL volumetric flask, dissolved in and diluted to 100 mL diluent of methanol. 5.0 mL of this solution was transferred into a 10 mL volumetric flask. The solutions were exposed to an integrated near Ultra violet energy (UV light) of not less than 25 watt hours/square meter, 50 watt hours/square meter and 200 watt hours/square meter and diluted to 10 mL with diluents of methanol.

Solid state stability was performed by exposing the fluorescent light, UV light and heat.

Standard preparation

About 100 mg of Artemether standard was transferred into a 50 mL volumetric flask. Dissolved in and diluted to volume with diluent of methanol. Diluted 5.0 mL of this solution to 10 mL with diluents methanol. Prepared in duplicate.

Exposure to white fluorescent light, Exposure to UV light and Exposure to heat at 60^0C

About 300 mg of the sample was taken in a petridish and exposed to white fluorescent light with an overall illumination of not less than 1.2 million lux hours. After exposure, transferring 100 mg of sample into a 50 mL with diluents of methanol and analyzed.
About 500 mg of the sample was taken in a petridish and exposed to an integrated near Ultra violet energy (UV light) of not less than 200 watt hours/square meter. After exposure, transferring about 100 mg of the sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents of methanol. Diluted 5 mL of this solution to 10 mL with

diluents and analyzed.

About 300 mg of the sample was taken in a petridish and exposed to heat at 60^0C for 24 hours. After exposure, transferring about 100 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluents methanol. Diluted 5 mL of this solution to 10 mL with diluents and analyzed.

CONCLUSION

Hence, it can be concluded that the newly developed RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Artemether without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of Artemether for routine quality control and stability studies.

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