Scientific Research and Essays Vol. 7(10), pp. 1165-1168, 16 March, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.2053
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

New HPLC method for the determination of artemether in injections

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Accepted 16 February, 2012

This new economical HPLC method has been developed for the estimation of artemether in injections to reduce the cost of estimation. Previously, one HPLC method is available in European Pharmacopoeia for the estimation of artemether but it is costly due to high price of acetonitrile. Present study replaced acetonitrile with methanol and showed that new method remained as specific, linear, accurate and precise as previous.

Key words: Artemether, injections, HPLC, acetonitrile, methanol.

INTRODUCTION

Artemether, an anti-malarial methyl-ether derivative of artemisinin and isolated from *Artemisia annua* (Acremont et al., 2010; Rottmann et al., 2010), is prescribed for treating multi-drug resistant strains of *falciparum* malaria (Ansari et al., 2010; Murtaza et al., 2009). For better performance, it is formulated with lumefantrine (Kumar and Clark, 2008). Its chemical name is (+)-(3-alpha,5a-beta,6-beta,8a-beta, 9-alpha,12-beta,12aR)-decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12-H-pyrano (4,3-j)-1,2-benzodioxepin. Its molecular formula and weight are $C_{16}H_{26}O_5$ and 298.4 (Krause, 1997; Moody, 2002; Endeshaw et al., 2008). The activity of artemether against all plasmodium is excellent due to its fast schizontocidal action which involves the annihilation of the asexual erythrocytic varieties of *Plasmodium falciparum* and *Plasmodium vivax* (Ansari et al., 2010; Msellem et al., 2009). Artemether accumulates in the food vacuole followed by the splitting of its endoperoxide bridge due to its interaction with haem and thus conversion to haemozoin blocks along with destruction of present haemozoin. It releases haem and a cluster of free radicals into the parasite which causes the retardation of protein formation during growth of trophozoites (Msellem et al., 2009**;** Bisoffi et al., 2009; Skarbinski, 2009; Murray and Jason, 2009).

Artemether consists of white crystals. It is practically insoluble in the water, very soluble in the dichloromethane and acetone, freely soluble in ethyl acetate and dehydrated ethanol (Murtaza et al., 2009).

This study was designed to develop a new economical HPLC method for the estimation of artemether in injections to reduce the cost of estimation. Previously, one HPLC method is available in European Pharmacopoeia for the estimation of artemether but it is costly due to high price of acetonitrile. Present study replaced acetonitrile with methanol and showed that new method remained as specific, linear, accurate and precise as previous.

MATERIALS AND METHODS

Artemether was a gift from Novartis Pharmaceuticals, Lahore,

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Pakistan. Artemether contains not less than 97.0% and not more than 102.0%. Arachis oil was obtained from BDH, UK. All other chemicals were of analytical grade.

Identification of artemether

One ml of dehydrated ethanol and 0.1 g of potassium iodide was added to 30 mg of artemether, heated the mixture using water bath till a yellow color was produced. 30 mg of artemether was dissolved in 6 ml of dehydrate ethanol, placed a few drops of the mixture on a white porcelain and added 1 drop of vaniline/sulfuric acid resulting in the appearance of pink color. The melting point of artemether was 86.0 to 90.0°C. Its specific optical rotation (using 10 mg/ml solution in dehydrate ethanol) was +166° to 173°. Its sulfated ash was not more than 1.0 mg/g, while its loss on drying was not more than 5.0 mg/g.

Determination of solubility

To develop new HPLC method in artemether injection, initially the raw artemether as well as the formulation of artemether injection was assessed. Initially, the solubility of artemether was determined which showed that artemether is very soluble in the methanol, thus in the subsequent study, methanol was used instead of acetonitrile. Thus the mobile phase of the developed method is the ratio of water and methanol (68:32). Then the identification as well as the potency tests for artemether was performed.

Preparation of artemether injection

To prepare 1 L solution of artemether injection, 700 ml of arachis oil was heated upto 130°C in 1 L beaker, cooled it to 70°C and then 80 g artemether was added with continuous shaking until a clear solution was obtained.

Reference HPLC method for artemether in injection

An HPLC method for the determination of artemether in injections is also mention in the European pharmacopoeia. The assay is determined using HPLC, reverse phase C18 column Supelco L1 C18 (4.6 mm × 250 mm, 5 µ) and UV detector set at a wavelength of about 216 nm. Mobile phase consisted of a mixture of acetonitrile and water (62:38, v/v) operated at a flow rate of 1.5 ml/min in ambient temperature.

Validation of HPLC methods

Objective of this protocol is to validate analytical testing method for artemether injection by high performance liquid chromatography technique. This validation study is intended to show that the method is suitable for test assay of artemeher injection. This protocol is applicable to analytical testing method validation of artemether injection which is intended to be used as release procedure.

Method validation will be a three-step process: (i) A validation test procedure is performed for each of the applicable attributes of validation, (ii) experimental results of a given test procedure are compared to established acceptance criteria and (ii) documentation is assembled to support the conclusion that the analytical method is scientifically sound and statistically valid.

Validation of proposed analytical methods means examination of following attributes of HPLC method; Specificity, linearity, precision, accuracy, ruggedness and robustness and limits of quantification and detection (Murtaza et al., 2009).

Preparation of standard solutions

Reference standard of artemether equivalent to 80 mg was added to 20 ml of mobile phase in the flask and shaked well to dissolve artemether. The final volume was made up to 25 ml, mixed and filtered.

Sample solution

An accurate volume of 1 ml of artemether injection equivalent to about 80 mg of artemether was transferred to 20 ml of mobile phase and shaked it mechanically for 15 min to dissolve. The final volume was made up to 25 ml, mixed and filtered.

Percentage determination of artemether in the sample

The area of chromatograms obtained for both (Figure 1), standard and sample solution was used to calculate the percentage of artemether in the sample using the following formula (Murtaza et al., 2009):

Percentage of artemether in the sample = $(A_{Sample} \times C_{Standard} \times 100)/(A_{Standard} \times C_{Sample})$

Where, “A” and “C” are the areas and concentration of solutions.

RESULTS

After preparation, solution of artemether was injected into the HPLC rheodyne (20 µl). Artemether peak was observed at 73 min, which is a very long retention time. To reduce the retention time, the proportion of mobile phase was changed as it is known that artemether is soluble in methanol and insoluble in water so the ratio of methanol in the mobile phase was increased. The new mobile phase ratio became as methanol and water (70:30). The retention time reduced to 32 min keeping the above mentioned conditions constant, which is a very long and time consuming retention time. Then the ratio of methanol in mobile phase was further increased and the new ratio of mobile phase became (80:20) for methanol and water, respectively. This time the retention time was 12 min which is considered as good retention time for an analysis. So, the mobile phase in the ratio of 80: 20 for methanol and water was finalized.

DISCUSSION

Artemether injection formulation was formulated in the arachis oil which is immiscible in the water and slightly miscible in the methanol. Therefore, mobile phase consisting of water and methanol in the ratio of 20 and 80, respectively cannot fully extract the artemether from the arachis oil. On the other side, artemether is insoluble in the water; it means that increase in water contents decrease the solubility of artemether in the mobile phase, due to this reason artemether solubility is much more in the arachis oil than the mobile phase (Murray and Jason,

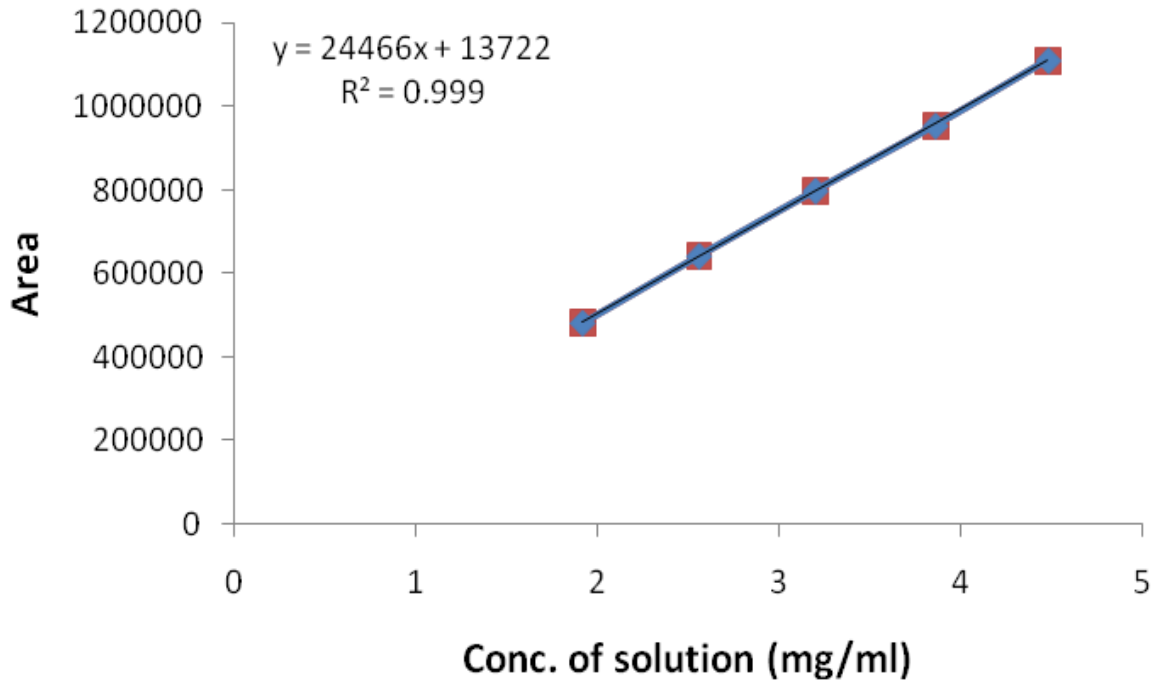

Figure 1. Calibration curve of artemether.

2009). Keeping in mind the above facts, pure methanol was used as diluent.

Method validation

The linearity of an analytical procedure is its ability to produce results that are directly and indirectly proportional to the concentration of the analyte in the sample within a given range, response to be linear on at least 5 to 6 points. The results show that new HPLC method follow linearity in the range of 60 to 140%, as evident from the value of $R^2 = 0.999$.

It relates to the closeness of the test results to true values i.e. measure of exactness of analytical method. It is expressed as % recovery by the assay of known amount of analyte in the linearity range. For the determination of accuracy, the concentrations used are 80, 100 and 120% of injection with percentage recovery 100.94, 101.94 and 101.64%, respectively.

The precision of the analytical method is the degree of agreement among individual results and how the individual test results are scattered from the mean value usually expressed as standard deviation or as the relative standard deviation (Murray and Jason, 2009). The precision divided into the three steps that is system precision, repeatability and reproducibility. The results of the system precision of standard preparations show that relative standard deviation was 0.45% which is well within the limits. The result of repeatability shows that standard deviation is 0.85. The repeatability results also meet specification and are well within the limit. The standard deviation of the three days results is 0.92. The relative standard deviation is 0.92.

The robustness of an analytical method is a measure of its capacity to remain unaffected by a small but deliberate variation in the procedural parameters listed in the procedure documentation and provide an indication of its suitability during normal usage. In this method, a mobile phase composition was changed with reduced percentage of methanol and increases the percentage of water (Murtaza et al., 2009). Apart from this, change in the temperature of system to about 45°C was done. The result of the robustness is 666 which shows that slight change of mobile phase do not change the overall result of the sample. It slight change the retention time (longer than exact mobile phase) but the result are within the limit. Increase in the temperature decrease the retention time of the sample but still the results are not affected and are well within the limit.

Limit of quantitation is the amount of analyte in the sample solution which can be determined quantitatively (Murtaza et al., 2009). The limit of quantitation was 1.92 mg/ml. Limit of detection is the amount of analyte in the sample solution which can be determined quantitatively. The limit of detection was 0.63 mg/ml.

Application of new HPLC method

New analytical method was applied to evaluation of two other brands of artemether injection that is Malart injection

(Medicraft Pharmaceuticals, Peshawar, Pakistan) and artem injection (Novartis Pharmaceuticals, Lahore, Pakistan). The results are 103.12% and 99.05% for the Malart and Artem injections, respectively. The results show that it is applicable to other formulations as well. The results show that the method is stable and reliable for most of the formulations.

Conclusion

New method for the estimation of artemether is linear, specific and sensitive and can be applied to analyze various injection formulations of artemether.

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