WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 1, 609-625. Research Article ISSN 2277– 7105

STABILITY-INDICATING HPLC DETERMINATION OF ARTEMETHER IN BULK DRUG AND PHARMACEUTICAL FORM

[*1]Milind Ubale and [2]Vilas Choudhari

[1]Research Centre of Chemistry, Vasantrao Naik Mahavidyalaya, Aurangabad.

[2]P G Departments of Chemistry, Yogeshwari Mahavidyalaya, Ambajogai, India.

Article Received on
25 Oct 2015,

Revised on 15 Nov 2015,
Accepted on 05 Dec 2015

***Correspondence for Author**
Dr. Milind Ubale
Research Centre of Chemistry, Vasantrao Naik Mahavidyalaya, Aurangabad.

ABSTRACT

An accurate and precise liquid chromatographic method was developed for the simultaneous estimation of Artemether in bulk drug and pharmaceutical form. The chromatographic analysis was performed on Purospher STAR C-18 250 x 4.0 mm 5µm or equivalent with mobile phase consisting of Acetonitrile and water (pH- 3.0) in the ratio 62:38 v/v, at a flow rate of 1.5 ml/min and eluent monitored at 216nm. The retention time for Artemether was found to be 14.641 minutes. The proposed method is simple, accurate and precise and could be successfully employed in routine quality control for the simultaneous estimation of Artemether in bulk drug and pharmaceutical form.

KEYWORDS: Artemether**,** RP-HPLC, Mobile phase, Column etc.

INTRODUCTION

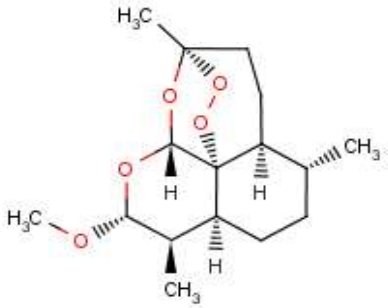
Artemether, an anti-malarial methyl-ether derivative of artemisinin and isolated from Artemisia annua[1-2] is prescribed for treating multi-drug resistant strains of falciparum malaria.[3-4]

For better performance, it is formulated with lumefantrine.[5] 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.[5-6]

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.[7-8] 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.[7-11] 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.[4]

Chemistry of Artemether



Molecular formula	**$C_{16}H_{26}O_5$**
Molecular weight	298.4
IUPAC Nomenclature	3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR) -Decahydro-10-Methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4, 3-j] -1, 2-benzodioxepin.
Colour	white colour powder
Therapeutic Category	Antimalarial agent.

Objective of the work

In the present study the main target for the development of chromatographic method was to get the reliable method for the quantification of Artemether from bulk drug and can be applied for the degradable products Literature survey reveals that a very few researcher as carried and reported systematic study of HPLC and UV methods for estimation of Artemether which were sophisticated, but time consuming.[12-20] Thus, the present study was aimed for development of speedy and cost effective HPLC technique for determination of Artemether as bulk and in dosage forms. The chromatographic method was optimized by changing various parameters, such as the mobile phase composition, pH of the buffer used in the mobile phase. Retention time and separation of peak of Artemether were dependent on pH of the buffer and the percentage of methanol. Various blends of solvent systems in varying proportions were tried as mobile phase as carried by different researchers.[21-22]

MATERIALS AND METHODS

The Objective to validate analytical testing method for selected drugs for the present study by high performance liquid chromatography technique. The methodology and required chemicals for the validation study are described after review of the literature and prescribed by various agency.[23-26] Chemicals used during present study were procured from MERCK, Qualigens, S d fine chemicals and were of HPLC grade, AR grade. Materials and solution, Chromatographic condition, Buffer preparation, Standard preparation. Validation parameter solution preparation such as Method and System Precision, Linearity, Accuracy, Robustness parameter. Preparation of mobile phase, Standard, Sample and mainly Impurity preparation made as prescribed in literature.[27-33]

Instrumentation

HPLC Method has been validated for Assay of Artemether (Specification No: ADL/FP/050) and method involves analysis of Artemether using HPLC column, Purospher STAR C18 250 x 4.6mm, 5µm or equivalent. Validation parameters such as specificity, precision, linearity, accuracy, intermediate precision, robustness and stability of analytical sample solution were studied.

Chromatographic conditions

The high performance liquid chromatographic (HPLC) system Systronics HPLC with Iris32 Lite Software was operated isocratically using column Purospher STAR C-18 250 x 4.0 mm 5µm with temperature maintained 25°C ± 2°C., using a mobile phase composition of Acetonitrile and potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with Orthophosphoric acid) in the ratio of 62:38%v/v at a flow rate of 1.5ml/min within a run time of 35 min. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a millipore vacuum filter system equipped with 0.45µm high vacuum filter and was carried at 216 nm.

Preparation of mobile phase

The mobile phase for the present study was the mixture of Acetonitrile and water in the ratio of 62: 38, before use it is mixed, filtered and degassed.

Preparation of working standard solution (10000 ppm)

250 mg of working standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 25 ml volumetric flask and diluted upto the mark with mobile phase.

Preparation of sample solution (10000 ppm)

250 mg of sample is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 25 ml volumetric flask and diluted upto the mark with mobile phase.

Preparation of working standard solution (50000 ppm)

1.250 g of working standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 25 ml volumetric flask and diluted upto the mark with mobile phase.

Preparation of resolution solution

500 mg of Artemether working standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 50 ml volumetric flask and dissolved in mobile phase. 1.5ml of Artemisinin impurity stock solution (500 ppm), 1.5ml of Dihydroartemisinin impurity stock solution (500 ppm) and 1.5 ml of alpha Artemether impurity stock solution (500 ppm) added to it and diluted upto the mark with diluents.

Mobile phase was injected to record blank into the chromatogram, also the standard solution in six replicate were injected to calculate the average area of standard solution. Similarly test solution was injected individually into the chromatograph. Run the chromatograms for 35 min. mathematical relation used to calculate of the assay are given below.

$$\% \text{ Assay ('as is' basis)} = \frac{At}{As} \times \frac{Ws}{Wt} \times \frac{P}{100} \times 100$$

Where,

At = Area of principal peak in test solution.

As = Average area of principal peak in standard solution.

Ws = Weight of working standard, in mg.

Wt = Weight of test sample in mg.

P = % Potency of reference standard, on 'as is' basis.

Calculate the % assay on dried basis as follows.

$$\text{Assay ("dried" basis)} == \frac{A}{100 - LOD} \times 100$$

Where**,** A = Assay on 'as is' basis.

System suitability

Preparation of standard solution

250 mg of standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 25 ml volumetric flask and diluted upto the mark with mobile phase. To calculate % RSD of the peak area of Artemether working standard. Six replicate injections of Artemether working standard solution were injected.

Method precision

Preparation of sample solution

250 mg of test sample is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 25 ml volumetric flask and diluted upto the mark with mobile phase, six samples solutions were separately prepared in similar manner. Calculation of the Assay of each sample of Artemether is made by comparing against the standard.

Calculated % RSD of assay values

Calculation

$$\% \text{ Assay ('as is' basis)} = \frac{At}{As} \times \frac{Ws}{Wt} \times \frac{P}{100} \times 100$$

Where, At = Area of principal peak in test solution.

As = Average area of principal peak in standard solution.

Ws = Weight of reference standard, in mg.

Wt = Weight of test sample in mg.

P = % Potency of reference standard, on 'as is' basis.

Calculate the % assay on dried basis as follows.

$$\text{Assay ("dried" basis)} = \frac{A}{100 - LOD} \times 100$$

Where, A = Assay on 'as is' basis.

Linearity

Linearity of Artemether was studied by injecting solutions prepared at five different levels of concentration from standard stock solution of working standard and injected these five levels in triplicates.

Preparation of Artemether working standard stock solution (50000 ppm)

5000mg of Artemether working standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 100 ml volumetric flask and diluted upto the mark with mobile phase and mixed well.

Linearity Level-1

Transferred 5.0 ml of Artemether working standard stock solution (50000 ppm) to 50 ml volumetric flask and diluted up to the mark with diluents (5000 ppm).

Linearity Level-2

Transferred 8.0 ml of Artemether working standard stock solution (50000 ppm) to 50 ml volumetric flask and diluted up to the mark with diluents (8000 ppm).

Linearity Level-3

Transferred 10.0 ml of Artemether working standard stock solution (50000 ppm) to 50 ml volumetric flask and diluted up to the mark with diluents (10000 ppm).

Linearity Level-4

Transferred 12.0 ml of Artemether working standard stock solution (50000 ppm) to 50 ml volumetric flask and diluted up to the mark with diluents (12000 ppm).

Linearity Level-5

Transferred 15.0 ml of Artemether working standard stock solution (50000 ppm) to 50 ml volumetric flask and diluted up to the mark with diluents (15000 ppm). To calculate % RSD, average peak area of these levels and Coefficient of correlation (r). These five levels in three replicates are injected.

Accuracy

Accuracy of Artemether was studied in presence of Artemisinin, Dihydroartemisinin and Alpha-Artemether impurities.

Three accuracy levels were prepared for nine determinations of sample.

Preparation of Artemether standard

500.86 mg of standard is accurately weighed and dissolved in minimum amount of mobile phase and transferred in 50 ml volumetric flask, dissolved and diluted up to the mark with diluents.

Preparation of Artemether sample

Table no 1. Preparations of sample solutions are as given below in the table.

Accuracy Levels	Wt. of sample (mg)	Artemisinin Imp. (500 ppm)	Dihydroartemisinin Imp. (500 ppm)	Alpha-Artemether imp (500 ppm)	Dilution with diluents
Level-1	W_1 350.41	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_2 350.52	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_3 350.35	1.5 ml	1.5 ml	1.5 ml	50 ml
Level-2	W_1 497.03	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_2 497.55	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_3 498.01	1.5 ml	1.5 ml	1.5 ml	50 ml
Level-3	W_1 649.74	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_2 649.78	1.5 ml	1.5 ml	1.5 ml	50 ml
	W_3 649.75	1.5 ml	1.5 ml	1.5 ml	50 ml

To calculate % RSD of standard injections and assay of each sample. The Artemether standard in six replicates and above samples were injected separately

Intermediate precision

Intermediate precision of the method was verified by analyzing the sample separately six times by different analyst, different instrument and different days.

Intermediate precision was performed by different analyst on different day. The Artemether standard (10000 ppm) and Artemether sample (10000) ppm were prepared.

To calculate % RSD of standard peak area and also to calculate assay of samples and % RSD of assay values. Six replicate of Artemether standard and that of six Artemether sample separately prepared were injected.

Robustness

Robustness of the method was verified by deliberately changing the parameters like flow rate, Mobile phase composition and column temperature. The Artemether standard (10000 ppm) and Artemether sample (10000) ppm were prepared. To calculate %RSD of standard peak area and also to calculate assay of samples and % RSD of assay values. Six replicate of Artemether standard and that of six Artemether sample separately prepared were injected.

Variation in parameters for Robustness

Table No 2.

Sr. No.	Parameters	Standard parameter	Changes studied	
1	Flow rate	1.5 ml/min.	1.4 ml/min.	1.6 ml/min.
2	Mobile phase composition	CAN: Water 620: 380	ACN: Water 600: 400	ACN: Water 640: 360
3	Column temperature	25°C	20°C	30°C

Solution stability

Stability of analytical sample solution was studied against freshly prepared standard at 0 hrs, 5.0 hrs, 10.0 hrs, 15.0 hrs, 20.0 hrs and 25.0 hrs. The Artemether standard (10000 ppm) and Artemether sample (10000 ppm) were prepared To calculated % RSD of standard injections and also calculated assay at each interval against freshly prepared standard. Inject the standard in six replicates at each interval and sample into the chromatograph.

Limit of Detection

Limit of detection were estimated by injecting serial dilutions of Artemether less than 25 ppm in six replicates and calculated % RSD of Artemether.

Limit of Quantification

The limit of quantification was estimated by injecting serial dilutions of Artemether less than 25 ppm in six replicates and calculated % RSD Artemether.

RESULTS AND DISCUSSION

HPLC Method has been validated for Assay of Artemether (Specification No: ADL/FP/050) and method involves analysis of Artemether using HPLC column, Purospher STAR C18 250 x 4.6mm**,** 5µm or equivalent. Validation parameters such as specificity, precision, linearity, accuracy, intermediate precision, robustness and stability of analytical sample solution were studied.

Specificity and system suitability

System suitability has been studied using six replicate injections of Artemether working standard and relative standard deviation of peak areas for six replicate injections calculated and found to be AVG 4153.10 with SD 1.99 and % RSD of Artemether standard found to be 0.04%. (NMT 2, 0%).

System precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples.[34] The two most common precision measures are 'repeatability' and 'reproducibility'. These are expression of two extreme measure of precision which can be obtained.[35]

In the present finding Standard solution of Artemether were prepared as per testing procedure and injected into the HPLC system in six replicates. The values obtained found to be 0.04 % relative standard deviation (NMT 2.0%) for peak area obtained in six replicate injections and the AVG 4168.28, with SD 2.01 thus showing that the equipment used for the study worked correctly for the developed analytical method, and being highly repetitive.[35]

Method precision

Six replicate solution of Artemether drug (250.91, 250.85, 250.61, 250.53, 250.10 and 250.03 mg) were prepared and the calculated assay of each sample was compared with the standard for calculation of percentage relative standard deviation % RSD of assay values.[36]

Method precision study was carried by using six replicate injections of different weight Artemether sample (250.91, 250.85, 250.61, 250.53, 250.10 and 250.03mg) and relative standard deviation of peak area of six replicate injections found to be % RSD 0.33(NMT 2.0%), whereas Assay for as such basis found to be AVG 99.58 with SD 0.21 and % RSD 0.21 and Assay on dried basis of the sample AVG 99.70 with SD 0.21 and % RSD 0.21. Thus the calculated % RSD of assay values found to be 0.21%. (NMT 1%).

Intermediate Precision

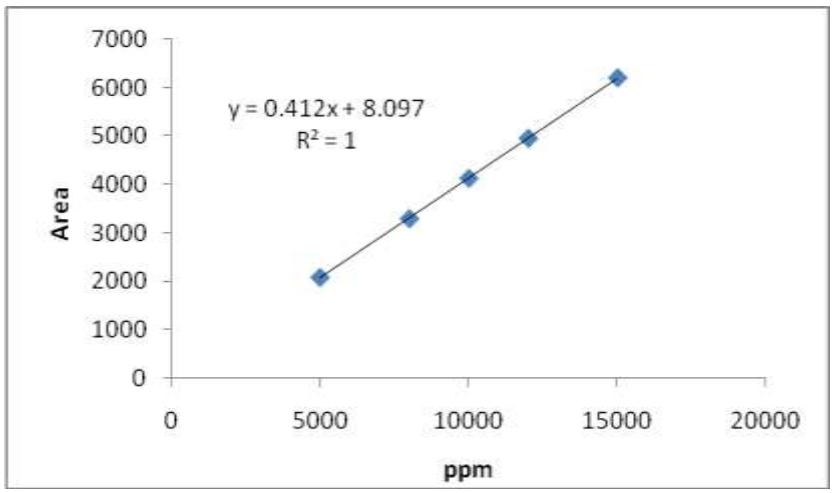
Intermediate precision of the method was verified by analyzing the sample separately 6 times by different analyst, different instrument and different days.[37] The calculated assay of

samples and % RSD of assay values found to be 0.13% (NMT 2%) for Artemether standard and 0.25% (NMT 1%) of assay values.

Linearity

Linearity is the ability of a method to elicit test results that are directly proportional to analyte concentration within a given range. Range is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The accepted criteria for linearity is that the correlation coefficient (R^2) is not less than 0.999 for the least squares method of analysis of the line.[38-39] In present investigation the linear study identifies a specified concentration range where analytes response is linearly proportional to the concentration. The standard curve found to be linear over five levels of concentration range from 5000ppm, 8000ppm, 10,000ppm,12,000ppm and 15,000ppm. The equation of the standard curve relating the peak area to the Artemether concentration in this range was y = 0.412x – 8.097.

Linearity



The drug showed good linearity in the range of five levels of concentration range 50000 to 15,000 ppm with coefficient of correlation value (R^2) 1.0000 for peak area.

Accuracy

Accuracy is popularly used to describe the measure of exactness of an analytical method, or the close of an agreement between the value, which is accepted as a conventional, true value or as an accepted reference value and the value found. It is properly a qualitative concept and the correct term is 'bias.'[40] The bias of a method is an expression of how close the mean of asset of results (produced by the method) is to the true value. Bias is usually determined by

study of relevant reference materials or by spiking studies.[40] Accuracy, sometimes also referred to as recovery is an indicator of the trueness of the test measurements. To determine the accuracy of the method three quality control samples were used.[41] The samples chosen were (Artemisinin, Dihydroartemisinin and Alpha-Artemether impurities) were such to represent the entire range of the standard curve i e lower, middle and higher concentration of the range. (Table no 1).

In the present finding recovery studies were conducted after addition of standard drug solution at three different levels shown in tabular form to pre-analyzed sample solution and to check the accuracy of the method, The calculated % RSD of Artemether standard was found to be 0.05% (NMT 2%) and the accuracy of assay of three levels was between 98.0 – 102%. The results obtained are in good agreement with the standards set by ICH and WHO.[41-42]

Robustness

The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters.[43-44] The percentage recovery of Artemether was good under most conditions and didn't show any significant change when the critical parameters were modified. (Table no 2.). The tailing factor for Artemether was always less than 2.0 and it was well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method it can be concluded that the method was robust. Similar result for other drugs were reported by Souppart et.al.[45]

Solution Stability

Stability of analytical sample solution was studied against freshly prepared standard at 0 hrs, 5.0 hrs, 10.0 hrs, 15.0 hrs, 20.0 hrs and 25.0 hrs.[41] The Prepared Artemether standard (10000 ppm) and Artemether sample (10000 ppm) were Injected the standard in 6 replicates at each interval and sample into the chromatograph. The Calculated % RSD of standard injections found to be (NMT 2.0%) and also calculated assay at each interval against freshly prepared standard. The Sample found to be stable up to 24 hrs and variation in assay value was 0.66%.

LOD and LOQ

Limit of detection (LOD**)** is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.[41,46]

Estimated the limit of detection by injecting serial dilutions of Artemether less than 25 ppm in 6 replicates and calculated % RSD of Artemether. Limit of detection would be the lowest concentration of analyte which on 6 replicate injections gives % RSD more than 10.0%.

Limit of Quantification

Estimated the limit of quantification by injecting serial dilutions of Artemether less than 25 ppm in 6 replicates and calculated % RSD Artemether.

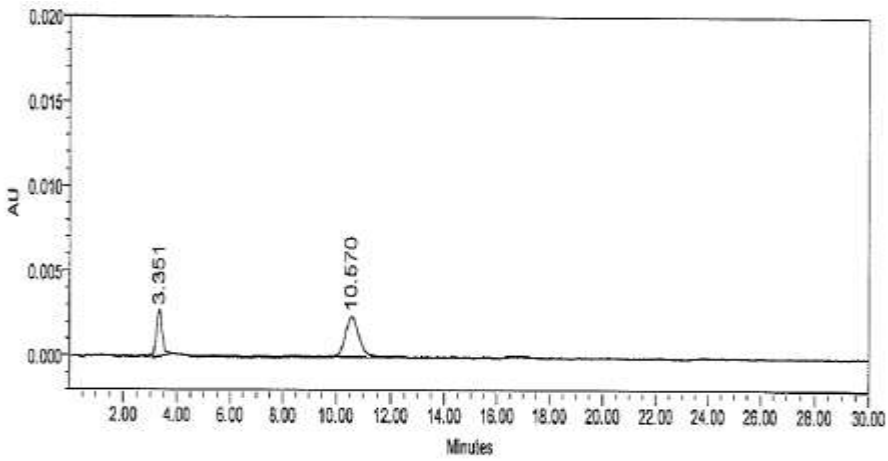
Limit of quantification found to be the lowest concentration of analyte which on 6 replicate injections gives % RSD less than 10.0%.[41,46]

In present study Limit of detection of Artemether is 0.75 ppm whereas Limit of quantification of Artemether was 2.5 ppm.

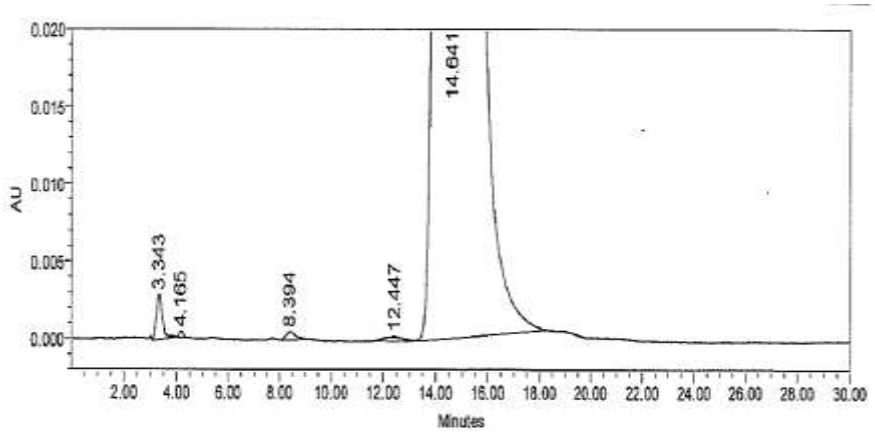
LOD would be the lowest concentration of analyte on which on 6 replicate injections gives % RSD more than 10.0%. LOQ found to be the lowest concentration of analyte on which on 6 replicate injections give % RSD less than 10.0%.

In the present findings these data show that the method is sensitive for the determination of Artemether. The LOD and LOQ were measured by using an equation and were found to be 0.25ppm and 0.5ppm. Our findings are in good agreement with the earlier reported work on other drugs by Mansor et al.[46-47]

A chromatogram of Artemether Standard



A chromatogram of Artemether Sample



CONCLUSION

The proposed multivariate spectrophotometric method for quantification of Artemether, pharmaceutical dosage forms has been developed and validated. The method was selective, precise, accurate, reproducible and linear over the concentration range. The method is simple and suitable for the determination of Artemether, in formulations without interference of excipients.

REFERENCES

1. Acremont V, Lengeler C, Genton B (2010). Reduction in the proportion of fevers associated with Plasmodium falciparum parasitaemia in Africa: A Systematic Review. Malaria J., 9: 240-244.
2. Rottmann M, McNamara C, Yeung B (2010). Spiroindolones, a Potent Compound Class for the Treatment of Malaria. Science, 329: no 5996 1175-1180.
3. Ansari MT, Haneef M, Murtaza G (2010). Solid Dispersions of Artemisinin in Polyvinyl Pyrrolidone and Polyethylene Glycol. Adv. Clin. Exp. Med., 19(6): 745-754.
4. Murtaza G, Ahmad M, Madni MA, Asghar MW (2009). A new reverse phase HPLC method with fluorescent detection for the determination of salbutamol sulphate in human plasma. Bull. Chem. Soc. Ethiop., 23(1): 1-8.
5. B Nasir SNH Shah G Murtaza N Shahid M Akhtar BA Khan S Azhar A Mumtaz A Waseem SA Khan M Ubaid S Noreen B kamal MHHB Asad. New HPLC method for the determination of artemether in injections. Scientific Research and Essays, 2012; 7(10): 1165-1168.
6. Krause PJ (1997). Malaria (Plasmodium). Nelson text book of pediatrics. Ed., 18(1): 1477-1482.

7. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev., 2002; 15: 66–78.
8. Endeshaw T, Gebre T, Ngondi J (2008) Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. Malar J., 7: 118.
9. Msellem MI, Martensson A, Rotllant G, Bhattarai A, Strömberg J (2009). Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, zanzibar—a crossover validation study. PLoS Med., 6(4): 759-768.
10. Mohamed SS, Khalid SA, Ward SA, Wan T, Tang H, et al. (1999) Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography–mass spectrometry-selected ion monitoring. J Chromatogr B Biomed Sci Appl, 731: 251–260.
11. Skarbinski J (2009). Effect of Malaria Rapid Diagnostic Tests on the Management of Uncomplicated Malaria with Artemether-Lumefantrine in Kenya: A Cluster Randomized Trial. Am. J. Trop. Med. Hyg., 80: 919-926.
12. B.M.J. De Spiegeleer, M. D'Hondt, E. Vangheluwe, K. Vandercruyssen, B.G.I. De Spiegeleer, H. Jansen, I. Koijen, J. Van Gompel. (2012). Relative response factor determination of artemether degradants with a dry heat stress approach. Journal of Pharmaceutical and Biomedical Analysis, 70: 111–116.
13. James D Terish, Suresh Kumar, Ramesh N* and Sasijith SL. (2011) Development and Validation of High-Performance Liquid Chromatography-Tandem Mass Spectrometric Method for Simultaneous Determination of Artemether and its metabolite, Dihydroartemisinin in Human Plasma: A Pharmacokinetic Study Application. European Journal of Experimental Biology, 2011; 1(3): 169-179.
14. Hou XY, Mc Manus DP, Gray DJ, Balen J, Luo XS, He YK, Ellis M, Williams GM, Li YS (2008). A randomized, double-blind, placebo-controlled trial of safety and efficacy of combined praziquantel and artemether treatment for acute schistosomiasis japonica in China. Bull World Health Organ., 86(10): 788–795.
15. Jansen, F.H. and S.A. Soomro, (2007). Chemical Instability Determines the Biological Action of the Artemisinins. Current Medicinal Chemistry, 14: 3243-3259.
16. WHO (2006.) monograph on good agricultural and collection practices (GACP) for Artemisia annua L.
17. Artemisinin Monograph. The International Pharmocopeia. (2003) WHO: Geneva. 185-233.

18. Sahai, P and Ram A Vishwakarma. (1998.) HPLC−Electrospray Ionization Mass Spectrometric Analysis of Antimalarial Drug Artemisinin Anal. Chem., 70(14): 3084-3087.
19. Zhao, S. (1987) High-performance liquid chromatographic determination of artemisinine (Qinghaosu) in human plasma and saliva. Analyst., 112: 661-664.
20. Liu, C.-Z., H.-Y. Zhou and Y. Zhao. (2007). An effective method for fast determination of artemisinin in Artemisia annua L. by high performance liquid chromatography with evaporative light scattering detection. Analytical Chimica Acta, 581: 298-302.
21. Rashmi T. R., Francis M. S. and Soumya Murali. (2014) Determination of Artemisinin In Selected Artemisia L. Species By HPLC. Indo American Journal of Pharmaceutical Research (IAJPR), 2014: 4(5): 2637-2644.
22. Debnath C, Haslinger E, Likussar W, Michelitsch A. (2006,). Determination of the antimalaria drug in pharmaceutical preparations by differential pulse polarography. J Pharm Biomed Anal., 41(2): 638-43.
23. Q.A. Xu, L.A. Trissel (Eds.) (1999). Stability-Indicating HPLC Methods for Drug Analysis, American Pharmaceutical Association, Washington.
24. ICH, Good Manufacturing Practices for Active Pharmaceutical Ingredients. International Conference on Harmonization, IFPMA, Geneva, 2000.
25. Vandenberghe, D.R., et al., Simultaneous determination of artemisining and its bioprecursors Artemisia annua. J. Nat. Products, 1995; 58(5): 798-803.
26. Christen P. and J.-L. Veuthey. (2001). New trends in extraction, identification and quantification of artemisinin and its derivatives. Current Medicinal Chemistry, 8: 1827-1839.
27. Tayade NG, Nagarsenker MS. (2007). Validated HPTLC method of analysis for artemether and its formulations. J Pharm Biomed Anal., 43: 839-44.
28. D.D. Hong, M. Shah, Development and validation of HPLC stability indicating assays, Drug stability: principles and practices, J.T. Carstensen, C.T. Rhodes (Eds.) Marcel Dekker, New York, 2000; 329–384.
29. L.R. Snyder, J.J. Kirkland, J.L. Glach (Eds.), Practical HPLC Method Development, John Wiley and sons, New York, 1997; 295.
30. L.R. Snyder, J.J. Kirkland, J.L. Glach (Eds.), Practical HPLC Method Development, John Wiley and sons , New York, 1997; 439–478.

31. ICH Harmonized Tripartite Guidelines (Q2R1). Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, European commission, Japan and USA, 2005.
32. Shrivastava A, Nagori BP, Saini P, Issarani R and Gaur SS. (2008). New Simple and Economical Spectrophotometric Method for Estimation of Artemether in Pharmaceutical Dosage Forms. Asian J. Research Chem., 1(1): 19–21.
33. Gamelin E, Boisdron-Celle M, Turacant A, Larra F, Allain P, Robert J. (1997). Rapid and sensitive high-performance liquid chromatographic analysis of halogenopyrimidines in plasma. J Chromatogr B Biomed Sci Appl., 695: 409-416.
34. T.M. Kalyankar and R.B. Kakde (2011). Reversed-Phase Liquid Chromatographic Method for Simultaneous Determination of Artemether and Lumefantrine in Pharmaceutical Preparation International Journal of Chem Tech Research (IJCRGG) 3(3): 1722-1727.
35. Sandrenan N., Sioufi A., Godbillon J., Netter C., Donker M., Van Valkenburg. (1997). Determination of artemether and its metabolite, dihydroartemisinin, in plasma by high-performance liquid chromatography and electrochemical detection in the reductive mode. Journal of Chromatography B., 691: 145-153.
36. Isabela da Costa Cesar, Fernando Henrique Andrade, Nogueira, Gerson Antonio. (2008). Comparsion of HPLC, UV spectrophotometry and potentiometric titration methods for the determination of lumefantrine in pharmaceutical products. Journal of Pharmaceutical and Biomedical Analysis, 48: 223-226.
37. Sunil J., Sanjith Nath M., Samba Moorthy U (2010). HPLC Method Development and Validation for Simultaneous Estimation of Artemether and Lumefantrine in Pharmaceutical Dosage Forms. International Journal of Pharmacy and Pharmaceutical sciences, 2(4): 93-96.
38. Arun R. and Anton Smith A. (2011.), Simultaneous HPLC-UV method for the estimation of artemether and lumefantrine in tablet dosage form. Int J Pharm Biomed Res, 2(3): 201-205.
39. Nitin G. Tayade, Mangal S. Nagarsenker, (2007) Validated HPTLC method of analysis for Artemether and its formulation, Journal of Pharmaceutical and Biomedical Analysis, 43: 839-844.
40. Pawan K. Saini, Raman M. Singh, Satish C. Mathur, Gyanendra N. Singh, Chotten L. Jain, Roop Khar and Abdul Hafeez. (2010). A Simple and Sensitive HPTLC Method for

Quantitative Analysis of Artemether and Lumefantrine in Tablets. Journal of planar Chromatography, 23: 119-122.

41. ICH Harmonized Tripartite Guidelines (Q2R1). (2005). Validation of analytical. Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, European commission, Japan and USA.
42. World Health Organizations, Antimalarial Drug Combination Therapy, Report of a WHO Technical Consultation, WHO, Geneva, 2001.
43. V. Navaratnam, S.M. Mansor, L.K. Chin, M.N. Mordi, M. Asokan, N.K. Nair. (1995). Determination of Artemether and dihydroartemisinin in blood plasma by high performance liquid chromatography for application in clinical pharmacological studies. J. Chromatogr. B., 669: 289–294.
44. B. Sridhar, K. Hanumantha Rao. (2010). A Validated Reverse Phase HPLC Method For The Simultaneous Estimation of Artemether and Lumefantrine in Pharmaceutical Dosage Forms. An International Journal of Advances In Pharmaceutical Sciences, 1(1).
45. C. Souppart, N. Gauducheau, N. Sandrenan, F. Richard. 2002. Development and validation of a high-performance liquid chromatography-mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. J. Chromatogr. B, 774: 195–203.
46. Mansor SM, Navaratnam V, Yahaya, N, Nair NK, Wernsdorfer WH, Degen PH. (1996). Determination of a new antimalarial drug, benflumetol, in blood plasma by high-performance liquid chromatography. J Chromatogr B Biomed Appl., Jul 12; 682(2): 321-5.
47. ICH, Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva, 1993.