International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Issue 4, 2010

Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN PHARMACEUTICAL DOSAGE FORMS

J.SUNIL[1*], M.SANJITH NATH[1], U.SAMBA MOORTHY[2]

[1]Department of Pharmacy, Krishna University, Machilipatnam, Andrapradesh, India, [2]Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda, Andhra Pradesh, India. E-mail: sunilpharma49@gmail.com

Received: 06 April 2010, Revised and Accepted: 26 May 2010

ABSTRACT

A Simple and precise HPLC method was developed for the estimation of Artemether and Lumefantrine in pure and pharmaceutical dosage forms. The quantification was carried out using symmetry C18, 250 x 4.6 mm, i.d, 5µm particle size in isocratic mode, with mobile phase compressing of buffer and acetonitrile in the ratio of 40:60 (v/v), pH 3 ± 0.5. The flow rate was 1.5 ml/min and the detection was carried out by UV detector dual i.e, 210 and 303 nm. The retention times were 13.887 and 7.218 mins for Artemether and Lumefantrine, respectively. The percentage recovery was found to be 98.87 and 99.78 % for Artemether and Lumefantrine; respectively.The method was validated by evaluation of different parameters.

Key words: HPLC, Artemether, Lumefantrine, UV detector

INTRODUCTION[1-5]

Artemether (figure-1) chemically know as 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. Artemether is a compound with a molecular formula $C_{16}H_{26}O_5$ and molecular weight of 298.4 g mol^{-1} and is a white crystalline powder. Artemether is practically insoluble in water, very soluble in dichloromethane & acetone and freely soluble in ethyl acetate and dehydrated ethanol.

Lumefantrine (figure-2) chemically (1R, S)-2-Dibutylamino-1-{2, 7-dichloro-9-[(Z) (4-chlorobenzylidene)-9H-fluoren-4-yl}-ethanol, Lumefantrine is a compound with molecular formula $C_{30}H_{32}Cl_3NO$ and molecular weight of 528.9 g mol^{-1}, and is a yellow crystalline powder. Lumefantrine practically insoluble in water and aqueous acids.

Pharmacology of Artemether and Lumefantrine:

Both artemether and lumefantrine act as blood schizontocides. Artemether is concentrated in the food vacuole. It then splits its endoperoxide bridge as it interacts with haem, blocking conversion to haemozoin, destroying existing haemozoin and releasing haem and a cluster of free radicals into the parasite. Lumefantrine is thought to interfere with the haem polymerisation process,a critical detoxicifying pathway for the malaria parasite. Both Artemether and Lumefantrine have a secondary action involving inhibition of nucleic acid and protein synthesis within the malarial parasite. An 8 - amino-quinoline derivative such as primaquine should be given sequentially after the combination in cases of mixed infections of P. falciparum and P. vivax to achieve hypnozoites eradication. The combination is also associated with rapid gametocyte clearance.

Branded Drugs: Larither injection - 3 x 1ml, Larither capsules - Strip of 6 capsules. Lumerax tablets, Riamet, Coartem.

The literature survey[6-17] indicates that Artemether and Lumefantrine were estimation by UV, TLC, HPTLC and HPLC in different pharmaceutical dosage forms. There is no method has been reported for estimation of Artemether and Lumefantrine, thus an attempt was made to estimate Artemether and Lumefantrine by using HPLC.

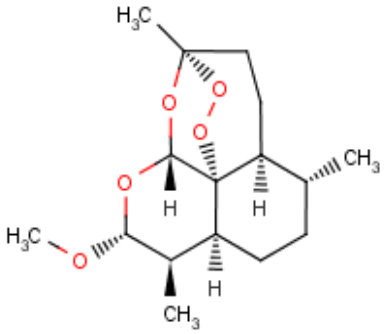

Fig. 1: Artemether

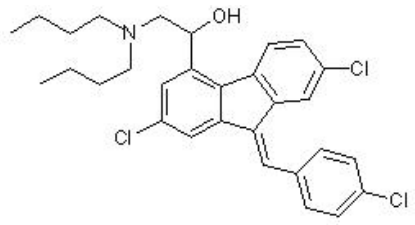

Fig. 2: Lumefantrine

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade. Pure standards of Artemether and Lumefantrine were obtained as gift sample from Hetero Drugs, Hyderabad. The purities of these

standards were 99.85 and 99.76 %, respectively. Hydrochloric acid, tween80, sodium lauryl sulphate, sodium acetate trihydrate, glacial acetic acid, monobasic potassium phosphate, sodium hydroxide, acetonitrile, potassium hydrogen phosphate, potassium hydroxide, ortho phosphoric acid and water used were of HPLC grade.

Preparation of buffer

A 1.36 gm of potassium dihydrogen orthophosphate was dissolved in 900 ml of Milli-Q water. Then the pH was adjusted to 3.0 with ortho phosphoric acid. Then the volume was make up to 1000 ml and was filtered through 0.45µm nylon membrane filter and degassed.

Preparation of mobile phase

A degassed mixture of Buffer and Acetonitrile in the ratio of 40:60 (v/v) was prepared and the mixture was filtered through 0.45 µ membrane filters and it was degassed.

Standard Preparation

A 24.0 mg of Lumefantrine working Standard was weighed and transferred accurately into a 100 ml clean and dry volumetric flask; it was dissolved in 60 ml diluent. Then 5 ml of Artemether standard stock solution was added and diluted to volume with diluent and mixed well.

Sample preparation

Not less than ten tablets of Lumefantrine was weighed and powdered. A tablet powder equivalent to 24 mg of Lumefantrine was accurately weighed and was transferred into 100 ml volumetric flask, Then 60ml of acetonitrile was added and sonicated for 30 minutes with intermediate shaking. Make up then volume was make up with buffer (40 ml). A portion of the solution was filtered through 0.45µm membrane filter and first few ml of the filtrate was discarded.

Chromatographic conditions

Freshly prepared Buffer and acetonitrile 40:60 (v/v) mobile phase and adjust pH to 3 were filtered through 0.45 µ membrane filter and sonicated before use. Flow rate of Mobile phase was maintained at 1.5 ml/min. The column temperature maintained was ambient temperature. The detection was carried out dual i.e, 210 and 303 nm, Injection volume 20 µl and total run time was 20 min. Column was Symmetry C18, 250 x 4.6 mm, 5µ particle size.

System suitability parameters

System suitability tests are an integral part of chromatographic method. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of Artemether and Lumefantrine. In addition to this standard deviation of Artemether and Lumefantrine standards were evaluated by injecting a mixed standard of both Artemether and Lumefantrine (800 and 240 ppm) as internal standard for five times at 20 min interval and the values were recorded. All the above parameters are shown in table -1.

Artemether standard stock preparation

A 40.0 mg of Artemether working standard was weighed and transferred into a 50 ml clean and dry volumetric flask. Then it was dissolved and diluted to volume with diluent.

Assay procedure

A 20 µl of diluent, placebo, standard preparation (5 times) and sample preparation were separately injected into the chromatographic system. Then the chromatograms and the peak responses were measured.

The placebo chromatogram was examined for any extraneous peaks that were observed in the chromatogram of sample preparation. Chromatogram of the standard preparation was recorded and the peak responses were measured. The tailing factor for the principal peak should not more than 4.0 and the number of the theoretical plates should not less than 5000. The % RSD (Relative Standard Deviation) should not be more then 2.0. A 20 µL of standard preparation and assay preparation was separately injected in the chromatogram, the chromatograms were recorded and the responses for the major peaks were measured.

RESULTS AND DISCUSSION

In order to develop simultaneous estimation of two components under isocratic conditions, the mixture of acetonitrile with buffer in different ratios were assayed as the mobile phase. A mixture of buffer and acetonitrile in different ratios were also tried for the assay of combined dosage forms. Finally a mixture of acetonitrile - potssium dihydrogen orthophosphate (buffer) in ratio of 40:60 v/v, proved to be the effective mixture than the other mixture used for the separation. Then the flow rates were tested includes 0.5, 0.8, 1.0, 1.5 and 2.0 ml, among these flow rates 1.5mL was selected for the assay because better resolution of the peaks were observed. System suitability test was applied to freshly prepared stock solution of Artemether and Lumefantrine to check the parameters like Tailing factors, Resolution, Theoretical plates, Relative standard deviation as shown in table-1.

Table 1: System suitability parameters

Parameters	Lumefantrine	Artemether
Tailing factors	3.6	1.1
Resolution	7.218	13.887
Theoretical plates	1992	17863
Relative standard deviation	0.10	1.08

Solubility

The solubility of Artemether in mg/ml in all medias can be calculated by the following formula:

Solubility = Sample absorbance/standard absorbance × std dilution/sample dilution × Purity/100 × wt of sample

From the above observation, it was observed that Lumefantrine showed acceptable solubility in all media exhibiting higher solubility in 0.1N HCl and Tween 80.

From the above observation it was observed that Artemether showed acceptable solubility in all media exhibiting higher solubility in pH 4.5 Acetate buffer and 2% SLS -Table-2,3. The developed method was studied for precision. The precision of the method was demonstrated by at least six determinations in method precision. Standard deviation and the results are given in table-4. The accuracy of the proposed HPLC method was expressed in terms of recovery. The recovery studies was carried out and given in terms of percentage recovery and given in table -5

Table 2: Solubility results of lumefantrine

S.No.	Media	Sample wt. in mg	Sample absorbance	Standard absorbance	Solubility (mg/ml)
1	Purified water	25.39	0.671	0.453	3.90
2	0.1N HCl	70.81	0.516	0.461	6.95
3	0.1N HCl and 1%Tween80	400	0.482	0.487	38.83
4	pH 4.5 Acetate buffer	150.40	0.513	0.437	18.42
5	pH 4.5 Acetate buffer and 2% of Sodium lauryl sulphate	170	0.401	0.479	13.14
6	pH 6.8 Phosphate buffer	25.0	0.627	0.452	3.58
7	pH 7.4 Phosphate buffer	25.0	0.678	0.462	3.791

Table 3: Solubility results of artemether

S.No.	Media	Sample wt.	Sample absorbance	Standard absorbance	Solubility mg/ml
1	Purified water	20.16	0.536	0.325	3.26
2	0.1N HCl	70.90	0.473	0.391	9.69
3	0.1N HCl and 1%Tween80	70.00	0.459	0.345	10.49
4	pH 4.5 Acetate buffer	251.1	0.391	0.309	29.93
5	pH 4.5 Acetate buffer and 2% Sodium lauryl sulphate	520	0.394	0.325	57.35
6	pH 6.8 Phosphate buffer	20.00	0.531	0.384	2.73
7	pH 7.4 Phosphate buffer	20.16	0.592	0.371	3.14

Table 4: Precision of method

Determinations	1	2	3	4	5	6	Mean	Std.dev	% RSD
Lumefantrine	98.9	99.6	97.8	100.1	98.8	99.5	99.11	0.73	0.74
Artemether	99.6	97.8	100.3	99.8	98.9	99.2	99.26	0.79	0.79

Table 5: Recovery studies

Drug	Amount added (µg)	Amount recovered (µg)	Recovery (%)	mean
	12.06	12.02	99.66	
Lumefantrine	24.34	24.28	99.75	99.78
	36.51	36.49	99.94	
Artemether	2.10	2.08	99.1	
	4.23	4.17	98.6	98.87
	6.25	6.18	98.9	

CONCLUSION

The proposed method was found to be simple, species-specific and highly accurate, required less time consumption for analysis and this can be employed for the routine analysis.

REFERENCES

1. Surendra Nath Pandeya., Med. Chem. Vol.2, 3rd ed. 2003; 601-633
2. White NJ, Van Vugt ., Clinical Pharmacokinetics and Pharmacodynamics of Artemether-Lumefantrine, Vol.37, 1997;105-125.
3. M Bindschedler, G Lefevre, F Ezzet, N Schaeffer., E. J. of Clinical Pharmacology. 2000; 56:375-381.
4. Agarwal, Suraj P; Ali, Asgar, Ahuja, Shipra., A. J. of Chemistry. 2007; 19: 4407-4414.
5. Wang, Ziyou, Chen, Zufen, Yaowu Fenxi zazhi., Simultaneous determination of ß- artemether and its metabolite and urine by a HPLC-MS using Electrospray ionization, Pharmaceutical analysis, J. C.A . 2000; 20(3): 178-179.
6. Zeng. Mei-Yi; Lu, Zhi-Liang, Yang, Song-Cheng; Zhang, Min., Determination of lumefantrine in human plasma by RP-HPLC with UV detection, J.C. B & Biomed. Appl.1996; 681(2); 299-306.
7. Mansor, Sharif M; Navaratnam.V; Yahaya Norizah; Nair, N.K. Determinationof new anti malarial drug Lumefantrine in blood plasma by HPLC, J.C. B Biomed Appl. 1996; 682(2):321-325.
8. Annerberg.A , Singtoroj.T ., High throughput assay for the determination of lumefantrine in plasma , J.C.B, Analytical tech. Biomed lifesci. 2005; 1822: 330-332.
9. Gabriels, Plaizier-Vercammen.J., Design of a dissolution system for the evaluation of the release rate characteristics of artemether and dihydroartemisinin from tablets.J. Phar. and Physical pharmacy, 2004; 18(2): 17-19.
10. Chimanuka.C, Gabriels.M, Plaizier-Vercammen J.R., Preparation of beta-artemether liposomes, their HPLC-UV evaluation and relevance for clearing recrudescent parasitaemia in plasmodium chabaudi malaria-infected mice. J.phar. and Biomed. Analysis.2002; 28:13-22.
11. Lindegardha.N, Annerberga.B.A, Blessbornc.D., Development and validation of a bio analytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of Lumefantrine and its metabolite in plasma. J. Pharm. and Biomed. analysis. 2005; 37(5):1081-1088.
12. Sandrenan.N, Sioufi.A, Godbillon.J., Determination of Artemether and its metabolite, dihydro artemisin in plasma by HPLC and Electrochemical detection in the reductive

mode. J. C. B. Biomed. Scie. 1997;691:145-153

13. Souppart.C, Gauducheau.N, Sandrenan.N ., Development and Validation of a HPLC-MS assay for the determination of artemether and its metabolite dihydro artemisinin in human plasma, J.C.B.Analytical Technologies in the Biomedical and Life Scie. 2002;774(2):195- 203
14. E. Vandenkerckhone, J; Van Hemel J; Sas B., Simultaneous determination of Beta-artemether and its metabolite DHA in human plasma and urine by a HPLC-MS using Electrospray ionization. J. Chro. 2005;61 :637-641.
15. Sabchareon A., Comparative clinical trial of artesunate suppositories and oral artesunate in combination with mefloquine in the treatment of children with acute falciparum malaria. A. J. Tropical Medicine.1998;58:11-16
16. Yuexian Huang, Guanghua Xie, Zhongming Zhou., Determination of artemether in plasma and whole blood using HPLC with flow-through polarographic detection, B. J of clinical pharmacology, 1998;145:123-129
17. Gabriels.M, Plaizier, Vercammen.J.A., Densitometric thin-layer chromatographic determination of artemisinin and its lipophilic derivatives, artemether and arteether, Journal of Chromatographic Science. 2003; 41(7): 359-366.