Hacettepe University Journal of the Faculty of Pharmacy
Volume 33 / Number 1 / January 2013 / pp. 41-58

Forced Degradation Profiling of Artemether by Validated Stability-Indicating RP-HPLC-DAD Method

Received : 20.07.2013
Revised : 21.10.2013
Accepted : 25.10.2013

Deepti Jain*, Pawan Kumar Basniwalº**

Introduction

Malaria is a life threatening disease caused by *Plasmodium* strains and transmitted by female anopheles mosquitoes. Artemether is a potent and rapidly acting antimalarial agent which is enlisted in World Health Organization (WHO) list of essential medicines for the treatment of severe multi-resistant malaria[1]. Artemether (ART) (Fig.1), chemically, decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4.3-j]-1,2-benzodioxepin, more active than parent compound artemisinin and it is used in combination therapy for malaria[2].

ART was analyzed in pharmaceutical dosage forms alone or along with combination drug component of dosage form, in plasma samples alone or along with its metabolite. In this context, numbers of analytical methods were reported to determine ART and its metabolite dihydroartemisinin in biological matrix to study pharmacokinetic vis. chromatography–mass spectrometry-selected ion monitoring[3], liquid chromatography–tandem mass spectrometry[4-7], LC method[8-11]. HPLC–ESI-MS/MS method was applied to a pharmacokinetic study in healthy volunteers who received fixed-dose combination tablets of ART, lumefantrine and

* School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Bhopal-462 033, Madhya Pradesh, India
** Lal Bahadur Shastri College of Pharmacy, Jaipur – 302 004, Rajasthan, India
º Corresponding author: E-mail: pawanbasniwal@gmail.com

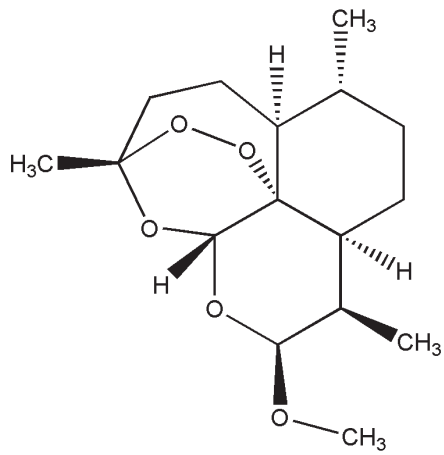

Figure 1
Structure of ART

artesunate[12]. Fourteen antimalarial drugs and their metabolites were simultaneously determined by another LC–MS/MS method[13]. It was determined in dosage forms by LC[14-16], HPTLC[17]; in fixed dosage form with lumefantrine[18-21] and with preservatives (methylparaben and propylparaben) in suspension[22] by LC. Differential pulse polarography was applied to determine drug in pharmaceutical preparations which require neither extensive separation nor extraction of ART[23].

As per knowledge of authors there is no report on inherent stability of the ART, which is still gap in research. Inherent stability of the active pharmaceutical ingredient (API) should be performed according to ICH guidelines which include hydrolytic stability, susceptibility to oxidation, and thermal stability[24]. Forced degradation studies provide information about degradation pathways and degradation products and establish the inherent stability of the API[25,26].

Thus, objective of present work was to develop a stability-indicating LC method for analysis of ART in bulk drug and in its pharmaceutical dosage forms and establish inherent stability by conducting different forced degradation studies as per ICH guidelines. Validated stability indicating assay method would be used for analysis of ART in presence of its degradation products and for evaluation of purity of bulk drug and stability of dosage forms of ART.

Materials and Methods

Instrumentation and chromatograph

HPLC chromatograph was Agilent Infinity 1260 series equipped with 1260 binary pump VL 400 bar, 1260 manual injector 600 bar, Rheodyne 7725i 7-port sample injection valve with 20 µL fixed loop, ZORBAX Eclipse Plus C18 (250 × 4.6 mm, 5 mm), 1260 DAD VL, 20 Hz detector, standard flow cell 10 mm, 13 mL, 120 bar, OpenLab CDS EZChrom Ed. Workstation and syringe 50.0 µL, FN, LC tip. All weighing for analysis was performed on Shimadzu electronic analytical balance AX-200. All dilutions, mobile phase and other solutions were used for the analysis were filter through 0.2 m nylon filter (Ultipor®N66 Nylon 6,6 membrane, Pall Sciences, Pall India Pvt. Ltd. Mumbai, India). Hot air oven with digital controller (Tempo; TI-126B), UV chamber fitted with two long wavelength (365 nm) UV tubes were used of degradation of powder form of ART sample.

Chemicals and reagent

Working standard was used as ART which was supplied by IPCA laboratories, Ratlam as a gift sample. Triple distillation water was prepared from distillation assembly. Methanol, acetonitrile, sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Merck Specialties Private Limited Mumbai, India.

Chromatography

Accurately weighed about 100 mg of ART was dissolved in 50 µL of acetonitrile and volume to 100 ml with triple distilled water to prepare stock P (1000 µg/mL). Aliquot of the stock P was diluted by ten times to prepare stock Q, which was used to prepare standard dilutions of ART containing 5, 10, 15, 20 and 25 µg/mL. All standard dilutions were filtered through 0.2 µ nylon filter (Ultipor®N66 Nylon 6,6 membrane, Pall Sciences) and chromatographed on Agilent Infinity 1260 series with above described conditions of chromatograph. The mixture of acetonitrile and water (70:30) was used as mobile for elution of drug on Zorbax Eclipse Plus C18 column (250 × 4.6 mm, 5 µm) at 1.0 µL/min flow rate. ART was successfully eluted at 13.48 min with run time of 20 min and detection was performed by photodiode-array detector (PDA) at 216 nm.

Method Validation

System suitability

Six replicates of standard ART sample (15 µg/mL) were repeatedly injected subsequently to determine system suitability parameters of the chromatograph for ART analytical method vis. number of theoretical plates, tailing factor, capacity factor, retention time and peak purity of the analytes.

Linearity, range and calibration

A new stock solution (1,000 µg/mL) of ART was prepared in 50% acetonitrile and six replicates of the each standard solution (5, 10, 15, 20 and 25 µg/mL) were prepared from aliquots of the stock solution by diluting with the same solvent. The standard samples were chromatographed and average peak areas of respective chromatograms were plotted against concentration.

Accuracy

Accuracy of the developed method was confirmed by apparent recovery method. The standard dilutions were spiked to pre-analysed ART samples containing concentration (10, 15 and 20 µg/mL). This experiment was repeated three times and statistical parameters were calculated.

Precision

The repeatability was established by peak area of six replicate injections of standard ART samples containing concentration of 10, 15 and 20 mg/mL on same day, while and intermediate precision was confirmed by variation in days of analysis and analyst-to-analyst.

Robustness

The ability to reproduce the developed analytical method in different under different circumstances was confirmed by two different parameters vis. amount of the organic modifier and temperature of real time analysis. The effect of three variations in amount of organic modifier (concentration of acetonitrile 69, 70 and 71%) and temperature variation (20, 25 and 30 °C) were observed on the retention time, peak symmetry as well peak area at three different concentration levels (10, 15 and 20 µg/mL).

LOQ and LOD

The limits of detection (LOD) and quantitation (LOQ) were determined by signal-to-noise ratio (S/N) method; where diluent was injected six times repeatedly and serial standard dilutions were injected, respectively. The LOD and LOQ were determined as the amounts for which S/N was 3:1 and 10:1, respectively.

Specificity

The specificity of developed method was evaluated by comparing the analysis of standard ART dilutions and degraded ART samples in different forced degradation conditions. The retention time, peak symmetry, peak purity and UV spectrum of the recorded chromatogram different samples were confirmed to assure the specificity.

Stability of samples

As above, three concentrations were used for chromatographic analysis and these samples were analysed during all six days; response ratios and statistical parameters were evaluated.

Forced degradation

Six different types of stress conditions were used to study forced degradation profile of the drug. Drug was refluxed at 60 °C temperature under neutral (water), acidic (0.1 N HCl) and alkali (0.1 N NaOH) conditions, respectively. Solid drug powder was degraded in temperature (60 °C) and UV light (265 nm), while oxidative degradation was performed in 3 and 10% H_2O_2 solution. The degradation was performed on the 1 mg/mL of ART in 50% acetonitrile in respective solution condition.

Results

ART was successfully eluted at on C18 column (250 × 4.6 mm, 5 μm) at 13.48 min by using mixture of acetonitrile and water (70:30) at 1.0 μL/min flow rate and detection was performed by photodiode-array detector. The average number of theoretical plates for ART was 19873 with capacity factor of 133.65; while peak purity was unit with tailing factor

of 1.142. The regression equation for drug was AUC = 59998 x – 8385 for 5-25 mg/µL as linearity with correlation coefficient of 0.9998. Accuracy, precision (repeatability and intermediate precision) and robustness (mobile phase composition and temperature) were determined within 100.15 - 99.82%. LOD and LOQ for the ART were assured as 0.5 µg/mL and 1.0 µg/mL with response ratio of 1153.03, respectively. Drug was extensive degraded in hydrolytic conditions viz. 90% in 0.1 N HCl within 8 days; 100% in 0.1 N NaOH within five days and 83% in neutral conditions within four days. No degradation was found at 60 °C, in UV light and in hydrogen peroxide solution (3 and 10%).

Discussion

Optimization of the chromatography

Although number of the LC methods were reported for determination of ART along with its metabolites as well as other antimalarial drugs, but none of them able to resolve the ART from its different degradation products when they were tried for the present study. The separation of degradation products formed during different conditions of forced degradation study was the major challenge for development of RP-HPLC method. The ZORBAX Eclipse Plus C18 (250 × 4.6 mm, 5 µm) column was used for the elution of analytes. Mixture of ammonium salts and acetonitrile was used for pharmacokinetic study for ART [8,17,23]; so, mixture of ammonium acetate (40 mM) and acetonitrile (35:65) was used for separation of ART from its degradation products. The little bit separation was achieved but drug was eluted with diluents peak. When ammonium acetate content was increased from 35% to 40%; the degradants were separated but the shape of the ART peak was not acceptable form and retention time for degradation was far away from 20 min run time. Then, gradient programming was planned for above mobile phase but we have not able to separate the analytes.

Keeping the solubility and nature of the drug; the water and acetonitrile (40:60) was introduced to elute the analytes; the degradants were well separated but ART peak was merged with diluent peak. When water content was increased then more complexity in chromatogram was appeared. So, water content was decreased to 35%; interestingly all

analytes were well separated; but still there was gap for better resolution and finally, the mixture of water and acetonitrile (30:70) was confirmed the separation of all analytes with acceptable resolution and peak symmetry. As the structure of ART (Fig. 1) indicates that there is no prominent chromophore for UV absorption, so the detection wavelength was decided to be 216 nm.

Method Validation

System suitability

System performance consistency was confirmed by analyzing system suitability parameters; where six replicates of ART sample (15 µg/mL) were chromatographed subsequently and retention time (RT), number of theoretical plates, tailing factor, plates per meter, capacity factor and peak purity were evaluated and % RSD values for these parameters were found within acceptance criteria of system performance. The ART drug was eluted at 13.48 min with higher theoretical plates (19873) with lower RSD value (0.023) indicate superior separation of the drug on C18 column in defined chromatographic conditions; while the plates per meter was determined as 79492.25 (Table I). Tailing factors of peak should be in between 0.9-1.4; ART has 1.142 with 0.033 % RSD value which indicates that peak is in symmetrical shape. The capacity factor for ART was found to be 133.65 (Table I); means C18 column has better retaining capacity of the drug. As peak purity of the peak of ART at retention time (13.4 min) was unit (Fig. 2); there was no interference in elution at the time of retention time of drug. It was best explained by the use of PDA detector.

Linearity, range and calibration

The linearity was confirmed over the concentration of 5 – 25 µg/mL and the regression equation was estimated as: AUC (area under curve) = 59998 x – 8385 with higher correlation coefficient near to unit (0.9998). The working range was also confirmed as 10 -20 µg/mL (Table I).

Accuracy

The pre-analysed samples of ART was spiked with 50, 100 and 150% of the drug and recovery was 99.62-100.56 with standard deviation of 0.484 (% RSD ± 0.263) (Table I).

TABLE I
Validation parameters of RP-HPLC method for ART

Validation parameters	**Value[a] ± SD[b] ± RSD[c]**
System Suitability	
RT[d]	13.48 ± 0.114 ± 0.008
No. of Theoretical Plates	19873 ± 456.85 ± 0.023
Tailing Factor	1.142 ± 0.038 ± 0.033
Plates/meter	79492.25 ± 18822.45 ± 0.023
Peak purity	1.00 ± 0.000 ± 0.000
Capacity factor	133.65 ± 1.135 ± 0.008
Linearity	5 – 25 µg/mL
Regression equation	AUC[e] = 59998 XF – 8385
Correlation coefficient	r2 = 0.9998 ± 0.004, 0.100
Range	10 -20 µg/mL
Accuracy	100.02 ± 0.484, ± 0.263
Precision	
Repeatability	100.01 ± 0.532, ± 0.193
Intermediate precision	
Day-to-day	99.98 ± 0.875, ± 0.655
Analyst-to-analyst	99.82 ± 0.759, ± 0.302
Robustness	
Mobile phase composition	100.15 ± 0.963, ± 0.832
Temperature	99.92 ± 0.732, ± 0.504
LOD[g]	0.5 µg/mL ± 1.153, ± 0.879
LOQ[h]	1.0 µg/mL ± 1.096, ± 0.504
Specificity	Ascertained by analyzing standard drug samples and degraded samples of different conditions
Stability in sample solution Response ratio	1153.03 ± 40.73, ± 0.035

a mean of six replicates; b Standard deviation; c % Relative standard deviation; d Retention time; e Area under curve; f concentration in µg/mL; g Limit of detection; h Limit of quantitation

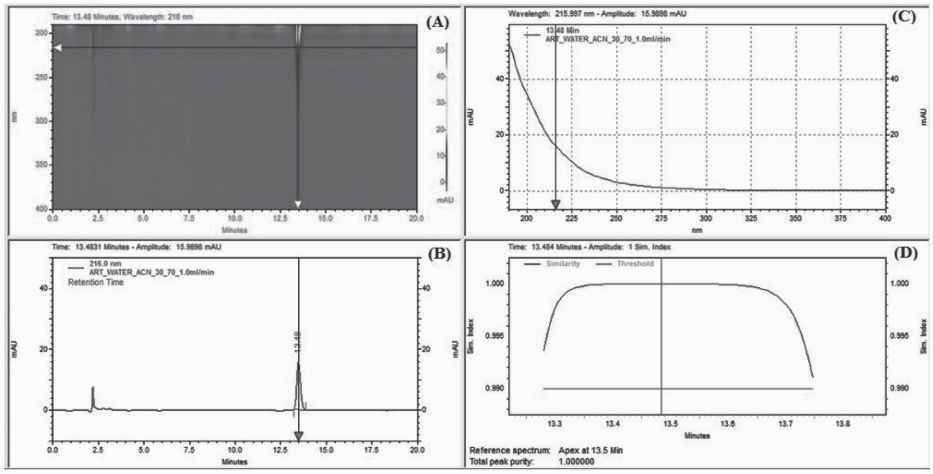

Figure 2
Counter plot [A], chromatogram [B] of ART; spectrum and peak purity curve at 13.48 min of ART in mixture of water and acetonitrile (30:70)

Precision

The repeatability of sample injections was measured as peak area of chromatogram which was expressed 100.01% with RSD of 0.193, while intermediate precision was evaluated on the heads of inter-day analysis as well as analyst-to-analyst. Both parameters were determined more than 99% with less than unit standard deviation (0.759 and 0.875) at all three levels of concentration (Table I).

Robustness

To confirm the reliability of the developed method in slight variation of analysis, the robustness study was conducted where two parameters were taken into consideration such as organic modifier concentration and temperature variation. There was no significant effect of above change in parameters on results; which were 100.15 and 99.92 respectively with far less than two % RSD value (Table I).

LOQ and LOD

The sensitivity of the developed method was ascertained by determining LOD and LOQ. The signal to noise (S/N) ratio of 3:1 was 0.5 µg/mL

(% RSD ± 0.879) for LOD and S/N ratio of 10:1 was 1.0 µg/mL (% RSD ± 0.504). The RSD values for the both parameters were indicated the sensitivity of method was satisfactory (Table I).

Specificity

The standard ART samples were subjected to forced degradation followed by chromatographic analysis. The AUC of ART peak in chromatogram has been decreased with increase in the AUC of degradant's peak. This experimental evidence confirmed that the developed method was specific for the analysis of ART.

Forced degradation

About 90% ART was degraded in 0.1 N HCl at 60 °C within 8 days and it gradually decreases with time and seventeen degradation products were formed during this degradation (Fig 3). Peak area of peaks at 4.1, 5.0, 5.3, 7.1, 8.3, 9.1 and 9.8 min were increased with degradation of ART, while peaks at 4.7, 6.2, 6.6, 8.3, 11.3, 11.8 and 14.3 min were first increased and followed by decreased. It may be due to further degradation of formed degradation products of ART. Peaks at 7.3, 14.3 and 15.3 min have variable fluctuation on degradation pattern (Fig. 4). Degradants at 4.1, 5.3, 8.3 and 9.1 min were prominent acidic hydrolytic products; as their area under curve were more than other degradation products.

ART was completely degraded in basic conditions (0.1 N NaOH) at 60 °C within five days (Figure 5 & Figure 6). Although sixteen degradation products were formed in this degradation but peaks at 4.5, 6.6, 7.6 and 10.1 were major degradation products of ART. These degradation products increase with time except degradant at 6.6 min, which first increased and then decreased. Other degradants at 4.1, 4.7, 6.3 and 9.2 min were also degraded as same fashion as 6.6 min's degradant.

Neutral (water) degradation of ART at 60 °C was faster than acid and basic conditions. It was degraded about 83% in neutral conditions within four days. Although fourteen degradation products were formed during this degradation study, but only few of them were increased with

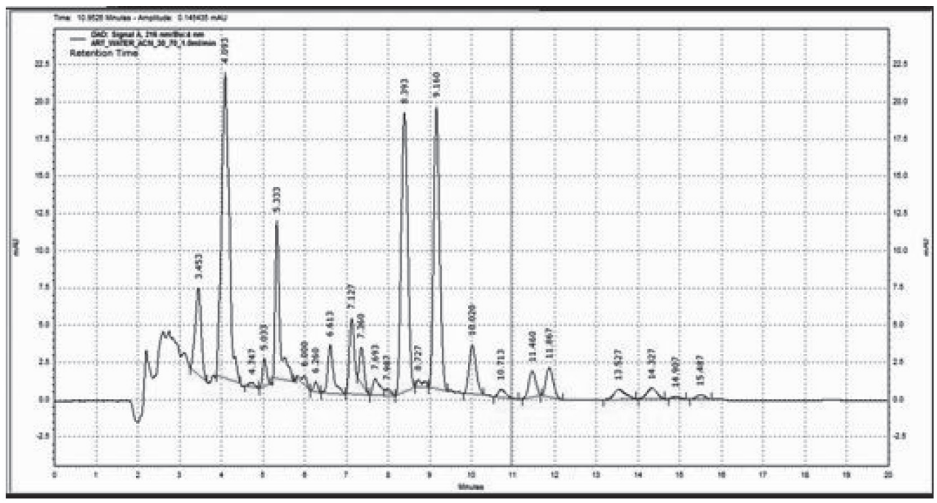

Figure 3
Representative chromatogram of ART in acidic degradation (0.1 N HCl) at 60 °C

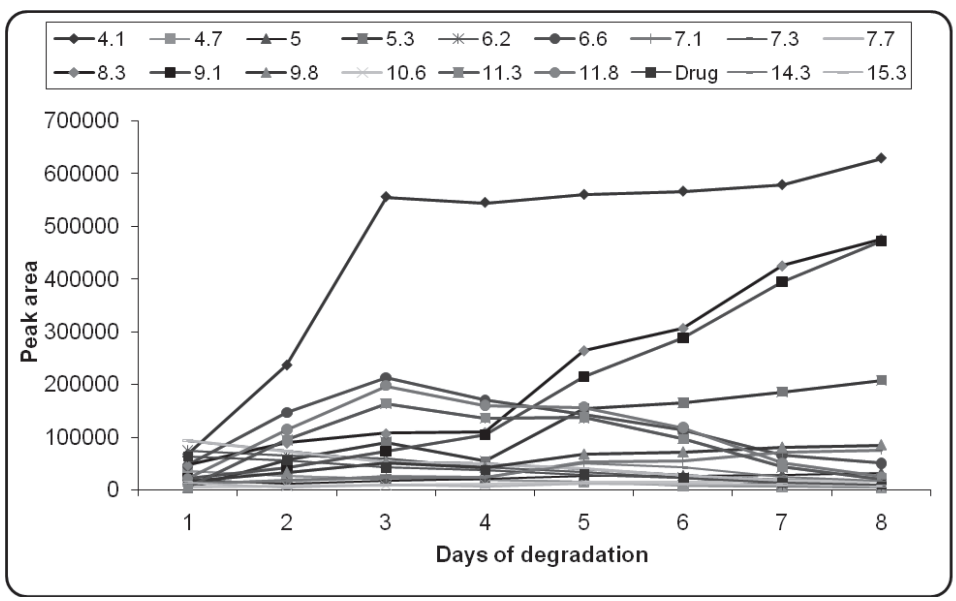

Figure 4
Degradation profile of ART in acidic conditions (0.1 N HCl) at 60 °C

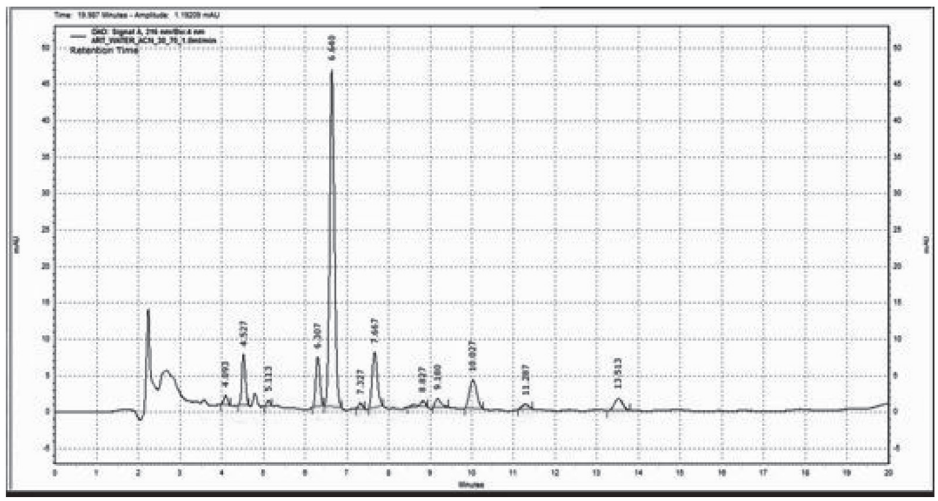

Figure 5
Representative chromatogram of ART in basic degradation (0.1 N NaOH) at 60 °C

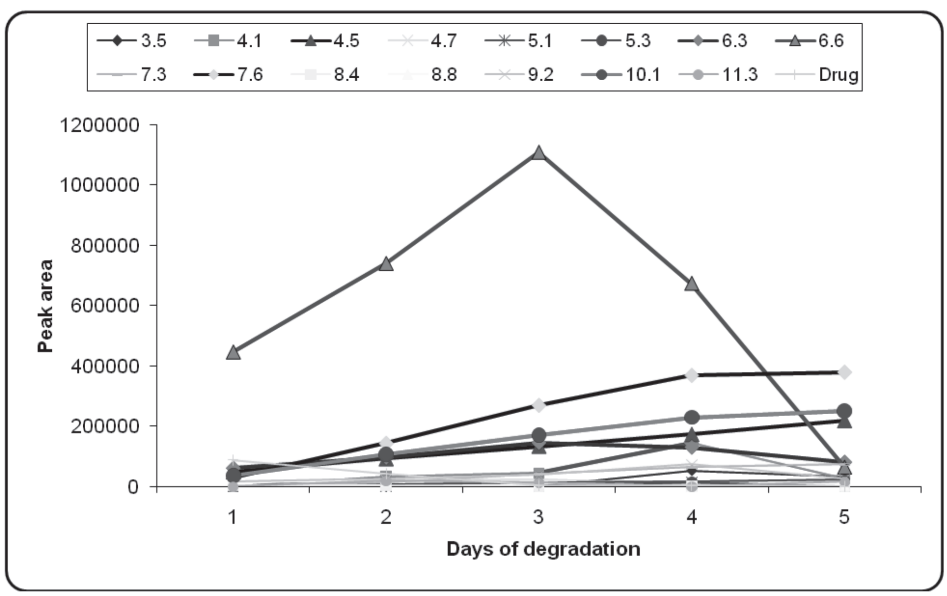

Figure 6
Degradation profile of ART in basic conditions (0.1 N NaOH) at 60 °C

time of stressed conditions. The gradual sharp increase in area of peak at 10.45 min with gradual decrease in ART concentration, which appeared in first day sample (Figure 7 & Figure 8). Peak at 4.45 min was also gradually slow increased. Other peaks were not observed in first day sample and/or subsequent days.

There was no degradation was observed in hydrogen peroxide (3% and 10%) at room temperature for 10 days. The drug was also stable at 60 °C and UV light irradiation for 10 days i.e. there was no degradation product was observed in both conditions. As concern with comparative hydrolytic degradation profile of ART, the fastest degradation was in neutral condition followed by basic and then acidic (Fig. 9).

Conclusion

The RP-HPLC-DAD method has been developed and validated to assure the reliability of assay method and successfully applied to study the forced degradation profile of ART in different conditions. The ART was degraded in all three hydrolytic conditions (neutral, acidic and basic),

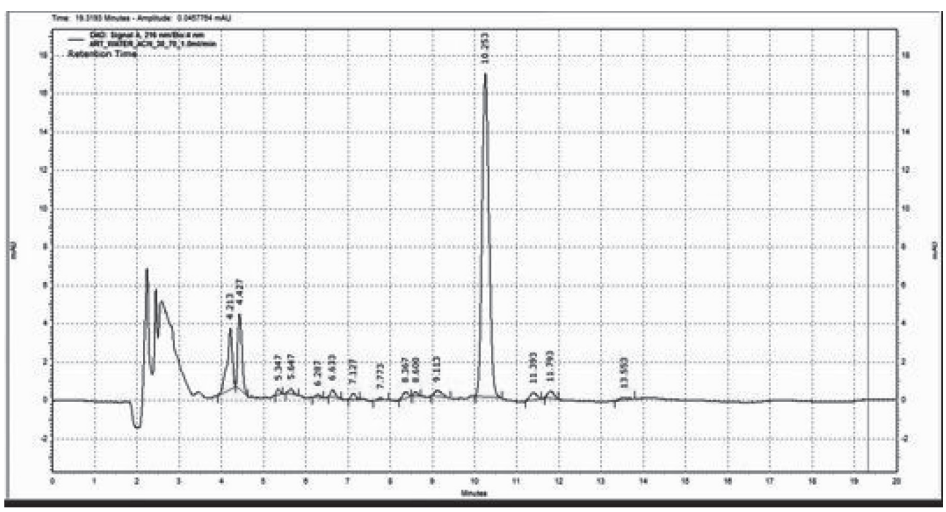

Figure 7
Representative chromatogram of ART in neutral degradation (water) at 60 °C

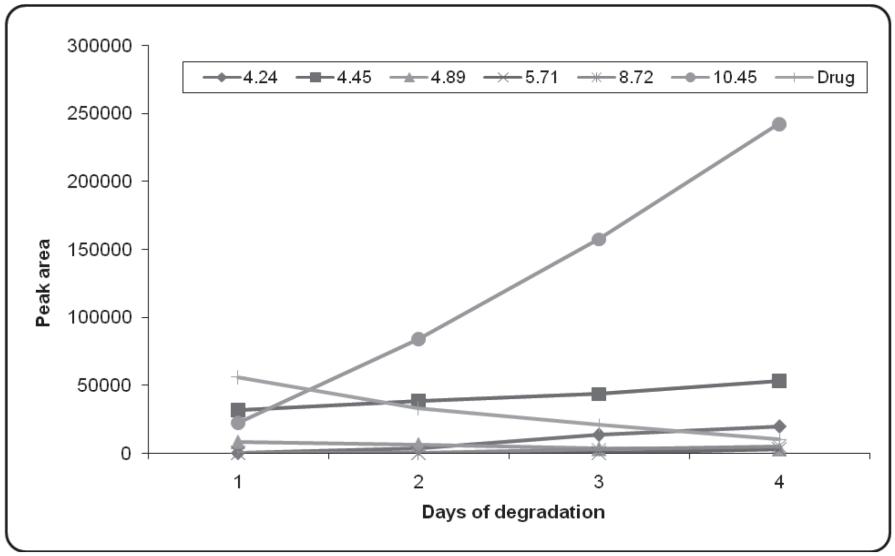

Figure 8
Degradation profile of ART in neutral conditions (water) at 60 °C

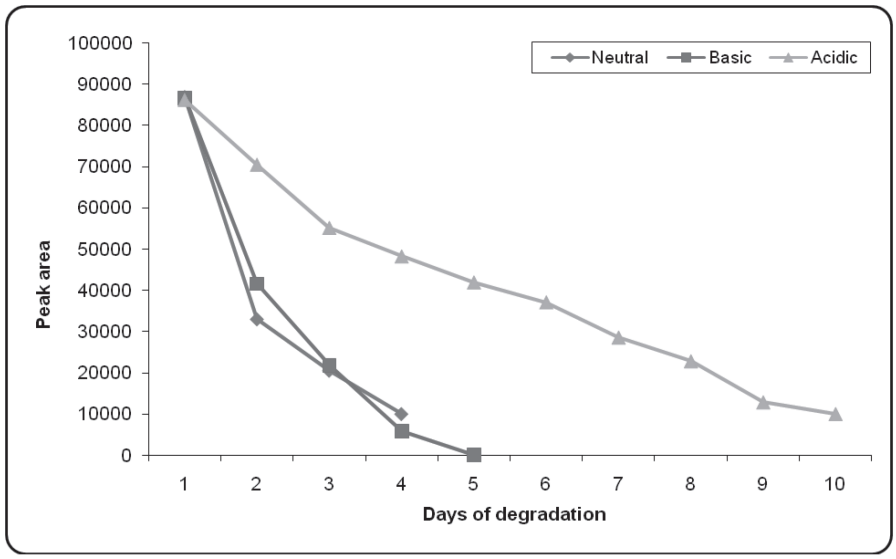

Figure 9
Degradation profile of ART in all hydrolytic conditions

but it was not degraded under temperature (60 °C), UV irradiation and in hydrogen peroxide solution (3 and 10%). As the ART was well separated from its degradation products; the validated chromatographic method may be applied for impurity profiling, routine analysis in pharmaceutical industry, bioequivalence and dissolution studies.

Acknowledgement

One of the authors, Pawan Kumar Basniwal, earnestly indebted to Science and Engineering Research Board (SERB), DST, New Delhi, for the financial support for this research work under Fast Track Scheme for Young Scientists. Authors are highly thankful to Head, School of Pharmaceutical Sciences, RGPV, Bhopal and Principal, LBS College of Pharmacy, Jaipur for providing the experimental facilities for this research work.

Summary

A novel stability-indicating RP-HPLC method for artemether has been developed and validated as per ICH guidelines and applied successfully to establish inherent stability of the drug by performing forced degradation in different stress conditions. The chromatographic separation of artemether and its degradants was achieved on Zorbax Eclipse Plus C18 column (250 × 4.6 mm, 5 μm) by employing the mixture of acetonitrile and water (70:30) at 13.48 min with 1.0 μL/min flow rate. The detection was performed by photodiode-array detector at 216 nm and room temperature was monitored for column. The degradation behavior drug was determined by performing forced degradation profile viz. hydrolysis, oxidation, photolysis and thermal decomposition. Extensive degradation was found in hydrolytic conditions (neutral, acid and alkaline), while it was stable under temperature (60 °C), UV irradiation and in hydrogen peroxide solution (3 and 10%). The validated RP-HPLC-DAD method may be applied for impurity profiling, quality control for purity of drug, routine analysis in pharmaceutical industry, bioequivalence and dissolution studies.

Key words: Artemether, Forced degradation profiling, RP-HPLC-DAD

Özet

Artemether' in Stres Bozundurma Profilinin Stabilite Göstergeli Valide Edilmiş RP-HPLC-DAD Metoduyla Belirlenmesi

Artemether için yeni bir stabilite göstergeli RP-HPLC method geliştirilmiştir ve ICH kılavuzlarına göre valide edilmiştir. Geliştirilen yöntem farklı stres koşullarında Artemether' in kararlılığını göstermek için kullanılmıştır. Artemether ve bozunma ürünlerinin ayrımı Zorbax Eclipse Plus C18 kolon (250 × 4.6 mm, 5 μm) kullanılarak asetonirtil ve su (70:30) karışımı ile 1.0 mL/dak akış hızında 13.48 dakikada gerçekleştirilmiştir. Dedeksiyon, dizi diyot dedektör aracılığıyla 216 nm dalga boyunda gerçekleştirilmiştir ve kolon oda sıcaklığında tutulmuştur. Artemether' in bozunma davranışı stres koşullarında hidroliz, oksidasyon, fotoliz ve termal bozunma uygulanarak incelenmiştir. Düşük sıcaklıklarda (60 °C) kararlı olan Artemether için sulu ortamda (nötr, asidik, bazik), UV ışık altında ve hidrojen peroksit (%3 - %10) ortamında bozunma gözlenmiştir. Geliştirilen valide edilmiş RP-HPLC yöntemi, impürüte profillenmesinde, ilacın kalite kontrolünde, ilaç sanayisinde rutin analizlerde, biyoeşdeğerlik çalışmalarında ve dissolüsyon çalışmalarında kullanılabilir.

Anahtar kelimeler: Artemether, Stres Bozundurma Profili, RP-HPLC-DAD

REFERENCES

1. World Health Organization (WHO) website: http://www.who.int/malaria/ publications/ world_ malaria_report_2012/en/index.html (08.04.2013)
2. Stringham, R.W., Lynam, K.G., Mrozinski, P. et al.: High performance liquid chromatographic evaluation of artemisinin, raw material in the synthesis of artesunate and artemether, J Chromatogr A 1216, 8918–8925 (2009).
3. Mohamed, S.A., Khalid, S.A. Ward, et al.: Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography–mass spectrometry-selected ion monitoring, J Chromatogr B 731, 251–260 (1999).
4. Wiesner, L., Govender, K., Meredith, S.A. et al.: A liquid–liquid LC/MS/MS assay for the determination of artemether and DHA in malaria patient samples, J Pharm Biomed Ana 55, 373–378 (2011).
5. Souppart, C., Gauducheau, N., Sandrenan, N. et al.: 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 (2002).
6. Magalhães, I.R.S., Jabor, V.A.P., Faria, A.M., et al.: Determination of -artemether and its main metabolite dihydroartemisinin in plasma employing liquid-phase microextrac-

tion prior to liquid chromatographic–tandem mass spectrometric analysis, Talanta 81, 941–947 (2010).

7. Huang, L., Jayewardene, A.L., Li, X., et al.: Development and validation of a high-performance liquid chromatography/tandem mass spectrometry method for the determination of artemether and its active metabolite dihydroartemisinin in human plasma, J Pharm Biomed Ana 50, 959–965 (2009).
8. Navaratnam, V., Mansor, S.M., Chin, L.K., et al.: 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 (1995).
9. Sandrenan, N., Sioufi, A., Godbillon, J., et al.: Determination of artemether and its metabolite, dihydroartemisinin, in plasma by high-performance liquid chromatography and electrochemical detection in the reductive mode, J Chromatogr B 691, 145-153 (1997).
10. Karbwang, J., Na-Bangchang, K., Molunto, P., et al.: Determination of artemether and its major metabolite, dihydroartemisinin, in plasma using high-performance liquid chromatography with electrochemical detection, J Chromatogr B 690, 259-265 (1997).
11. Thomas, C.G., Ward, S.A., Edwards, G.: Selective determination, in plasma, of artemether and major metabolite, dihydroartemisinin, by high performance liquid chromatography with ultraviolet detection, J Chromatogr 583, 131-136 (1992) Biomed. Applicat.
12. César, I.C., Ribeiro, J.A.A., Teixeira, L.S., et al.: Liquid chromatography–tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study, J Pharm Biomed Ana 54, 114–120 (2011).
13. Hodel, E.M., Zanolari, B., Mercier, T., et al.: A single LC–tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma, J Chromatogr B 877, 867–886 (2009).
14. César, I.C., Pianetti, G.A.: Quantitation of artemether in pharmaceutical raw material and injections by high performance liquid chromatography, Brazilian J Pharm Sci 45, 737-742 (2009).
15. Nasir, B., Shah, S.N.H., Murtaza, G., et al.: New HPLC method for the determination of artemether in injections, Sci Res Essays 7(10), 1165-1168 (2012).
16. Chimanuka, B., Gabriels, M., Detaevernier, M. R., et al.: Preparation of β-artemether liposomes, their HPLC–UV evaluation and relevance for clearing recrudescent parasitaemia in Plasmodium chabaudi malaria-infected mice, J Pharm Biomed Ana 28, 13–22 (2002).
17. Tayade, N.G., Nagarsenker, M.S.: Validated HPTLC method of analysis for artemether and its formulations, J Pharm Biomed Ana 43, 839–844 (2007).
18. Cesar, I.C., Nogueira, F.H.A., Pianetti, G.A.: Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection, J Pharm Biomed Ana 48, 951–954 (2008).
19. Kalyankar, T.M., Kakde, R.B.: Reversed-phase liquid chromatographic method for simultaneous determination of artemether and lumefantrine in pharmaceutical preparation, Int J ChemTech Res 3(3), 1722-1727 (2011).
20. Arun, R., Smithl, A.A.: Simultaneous HPLC-UV method for the estimation of artemether and lumefantrine in tablet dosage form, Int J Pharm Biomed Res 2(3), 201-205 (2011).
21. Sridhar, B., Rao, K.H., Srinivas, T.V.S., et al.: A validated reverse phase HPLC method for the simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms, Int J Adv Pharm Sci 1(1), 95-99 (2010).

22. Atemnkeng, M.A., Marchand, E., Vercammen, J.P.: Assay of artemether, methylparaben and propylparaben in a formulated paediatric antimalarial dry suspension, J Pharm Biomed Ana 43, 727–732 (2007).
23. Debnath, C., Haslinger, E., Likussar, W., et al.: Determination of the antimalaria drug artemether in pharmaceutical preparations by differential pulse polarography, J Pharm Biomed Ana 41, 638–643 (2006).
24. International Conference on Harmonization, Note for guidance on stability testing of new drug substance and products. In: International conference on harmonization of technical requirement for registration of pharmaceutical for human use, Geneva (2000).
25. Basniwal, P.K., Srivastava, P.K., Jain, S.K., Jain, D.: RP-LC Analysis and hydrolytic degradation profile of racecadotril, Chromatographia 68, 641–647 (2008).
26. Jain, D., Basniwal, P.K.: Forced degradation and impurity profiling: recent trends in analytical perspectives, J Pharm Biomed Ana 96, 11-35 (2013).