December 2008 Volume 7 Issue 12

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(12) 2008 [857-861]

# A report on forced degradation studies of artesunate and amodiquine tablets

**Manisha Phadke*, Vivek K.Jadhav, Datta Patil, R.K.Jadhav**

**Ipca Laboratories Limited, International House, 48, Kandivli Industrial Estate, Kandivli (west), Mumbai -400067, (INDIA)**

**Tel : 912266474460 ; Fax : 00912228686613**

**E-mail : manishap@ipca.co.in**

*Received: 18th October, 2008 ; Accepted: 23rd October, 2008*

## ABSTRACT

The forced degradation of drug product will evaluate the acceptability of analytical method by establishing the specificity of the method by studying acid hydrolysis, base hydrolysis and oxidation. The objective of the current study was to develop a validated stability-indicating assay method (SIAM) for artesunate and amodiaquine tablets in the fixed dosage form after subjecting it to forced decomposition under hydrolysis, oxidation, photolysis and thermal stress conditions. Resolution of drug and the degradation products formed under different stress studies were successfully achieved on a base deactivated C-18, 10 cm × 4.6-mm internal diameter, 3-micron particle size HPLC column with mobile phase composed of buffer, (1.36 gm of monobasic potassium phosphate in 1000ml of water. Adjust pH to 3.0 with Orthophosphoric Acid.) and Acetonitrile in the ratio 500:400 , a flow rate of 0.80 mL/min (run time of 20 minutes), a column temperature Ambient and an injection volume of 20 micro L. Detection of artesunate was by UV detector at a wavelength of 210 nm and 300nm for Amodiaquine.



## KEYWORDS

Artesunate;
Amodiaquine;
Forced degradation;
Stability indicating.

## INTRODUCTION

A stability-indicating assay is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating assay accurately measure the active ingredients, without interference from degradation products, process impurities excipients, or other potential impurities Developing SIAMs is necessary to carry out any stability study. Stress testing of artesunate and amodiaquine tabets was performed according to the International Conference on Harmonization (ICH) guidelines in order to validate the stability-indicating power of the analytical procedures[9-10]. Stress testing showed that artesuante and amodiquine underwent acid, alkaline and oxidative degradation; on the other hand, it showed stability towards photo- and thermal degradation.

Literature survey reveals few analytical methods for determination of single drug component like artesunate or amodiaquine , dihydromisinin but not a single method for simultanaeous determination of artesunate and amodiaquine in combination dosage form[1,2-8].

## EXPERIMENTAL

### Materials and reagents

Artesunate (B.No.5057ASJI and Amodiaquine Hydrochloride B.NO.5009Q2RJ pure drug samples provided by IPCA Laboratories LTD. Ratlam (API Division). A drug product containing 100 mg of

Full Paper

artesunate and 300 mg of amodiaquine per tablet were obtained from R and D (F) ,Ipca Laboratories Ltd.
All preparations were carried out using the following reagents:

Milli-Q grade water, Acetonitrile(ACN) HPLC grade (Merck), Monobasic potassium phosphate ,reagent grade (Merck), Orthophosphoric Acid (Merck) and Glacial Acetic Acid HPLC grade (Spectrochem LTD) .

**Prerparation of standard solution**

Standard solution containing 0.25mg per ml of Artesunate and 1mg per ml Amodiaquine was prepared.

**Sample preparation for forced degradation study**

**Forced degradation study of active ingredients and drug product**

**(a) Acid hydrolysis**

Weigh 20 tablets and calculate average weight. Weigh 5 tablets in 500ml volumetric flask, add 10ml acetonitrile, shake for 2minutes, add about 300ml diluent and sonicate for 15minutes with intermediate shaking, add 50ml 0.1N HCl and heat for 60minutes, cool at room temperature and neutralized with 0.1N NaOH and then dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent.

(Acid Blank : Add 10ml Acetonitrile and 300ml diluent in 500ml volumetric flask, add 50ml 0.1N HCl and heat for 60minutes, cool at room temperature and neutralized with 0.1N NaOH, dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent)

**(b) Alkali hydrolysis**

Weigh 20 tablets and calculate average weight. Weigh 5 tablets in 500ml volumetric flask, add 10ml acetonitrile, shake for 2minutes, add about 300ml diluent and sonicate for 15minutes with intermediate shaking, add 50ml 0.1N NaOH and heat for 60minutes, cool at room temperature and neutralized with 0.1N HCl, dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent.

(Alkali Blank : Add 10ml Acetonitrile and 300ml diluent in 500ml volumetric flask, add 50ml 0.1N NaOH and heat for 60minutes, cool at room temperature and neutralized with 0.1N HCl, dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent)

**(c) Oxidation**

Weigh 20 tablets and calculate average weight. Weigh 5 tablets in 500ml volumetric flask, add 10ml acetonitrile, shake for 2minutes, add about 300ml diluent and sonicate for 15minutes with intermediate shaking, add 50ml 10% $H_2O_2$ and heat for 60minutes, cool at room temperature, dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent.

(Oxidation Blank : Add 10ml Acetonitrile and 300ml diluent in 500ml volumetric flask, add 50ml 10% $H_2O_2$ and heat for 60minutes, cool at room temperature, dilute upto the mark with diluent, filter through GF/C paper. Further pipette out 5ml of filtrate to 20ml diluent)

**(d) Heat Treatment**

Active Artesunate and Amodiaquine was exposed to heat at 60C for 1week. Further sample preparation is same as per parent sample.

**(e) Photo-stability**

Active Artesunate and Amodiaquine was exposed in Photo-stability chamber for stipulated period. Further sample preparation is same as per parent sample.

## RESULTS AND DISCUSSION

The results are presented in TABLE 1.

As no peak from placebo was eluted at the Retention Time (R.T.) of artesunate and its impurity and amodiaquine hydrochloride, indicates that placebo interference is Nil and the method is very specific to simultanaeous estimation of artesunate and amodiaquine combined dosage forms.

From the forced degradation study it was established that no degradant was found to interfere with the retention time of artesunate and amodiaquine hydrochloride and its impurities.

Acid Hydrolysis showed complete degradation of artesunate into a new unknown degradant and increase in the peak response of dihydromisinin. and amodiaquine hydrochloride showed slight decrease in peak area re-

**TABLE 1 : Forced degradation data**

| Sr. no. | Degradation Condition | % Assay | | Purity angle < purity threshold | |
|---|---|---|---|---|---|
| | | Artesunate | Amodiaquin HCl | Artesunate | Amodiaquin HCl |
| 1. | Parent acitve | 99.20 | 100.85 | Pure | Pure |
| 2. | Active exposed to acid hydrolysis | 82.81 | 101.39 | Pure | Pure |
| 3. | Active exposed to alkali hydrolysis | 4.61 | 98.98 | Pure | Pure |
| 4. | Active exposed to oxidation with 10% $H_2O_2$, | 90.13 | 95.78 | Pure | Pure |
| 5. | Active drug exposed to heat | 101.15 | 99.74 | Pure | Pure |
| 6. | Parent drug product Sample | 97.48 | 102.29 | Pure | Pure |
| 7. | Drug product exposed to acid hydrolysis | 77.16 | 96.73 | Pure | Pure |
| 8. | Drug product exposed to alkali hydrolysis | 94.05 | 96.80 | Pure | Pure |
| 9. | Drug product exposed to oxidation with 10% $H_2O_2$ | 84.30 | 89.87 | Pure | Pure |
| 10. | Drug product exposed sample Photostability | 97.39 | 97.34 | Pure | Pure |
| 11. | Sample exposed to heat | 97.94 | 98.74 | Pure | Pure |

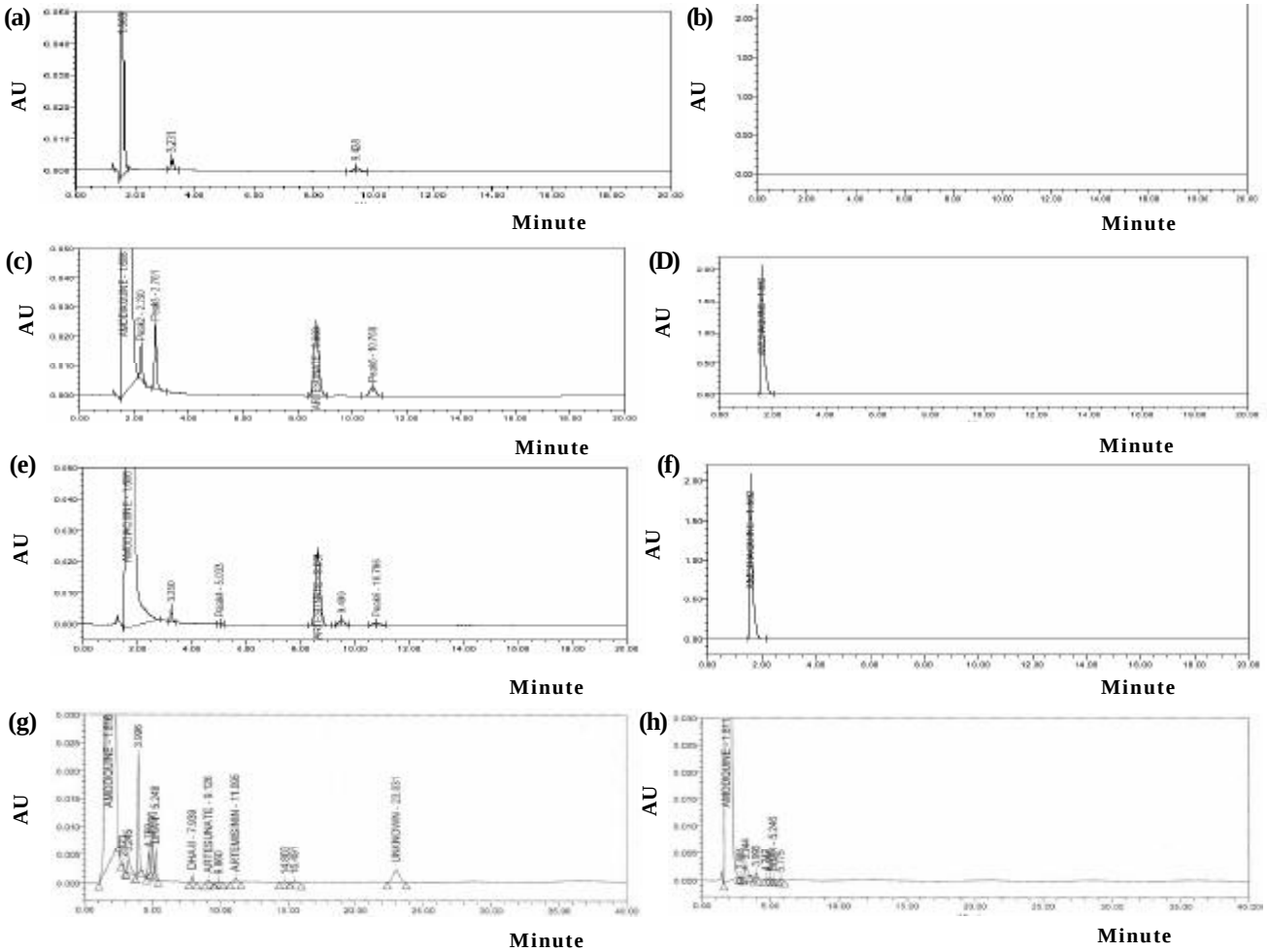


**Figure 1 : Chromatograms of (a) placebo at 210nm (b) placebo at 300nm (c) std mix at 210nm (d) std mix at 300nm (e) precision at 210nm (f) precision at 300nm (g) acid spl 60 min at 210nm (h) acid spl 60 min at 300nm**

sponse resulting in the decrease in the assay value. Alkali hydrolysis also showed drastic decrease in the assay value of both artesunate and amodiaquine hydrochloride and formation of known impurity dihydro artemisinin and artemisinin and unknown impurity at rrt about 2.9 with respect to artesunate. Oxidation method

Full Paper

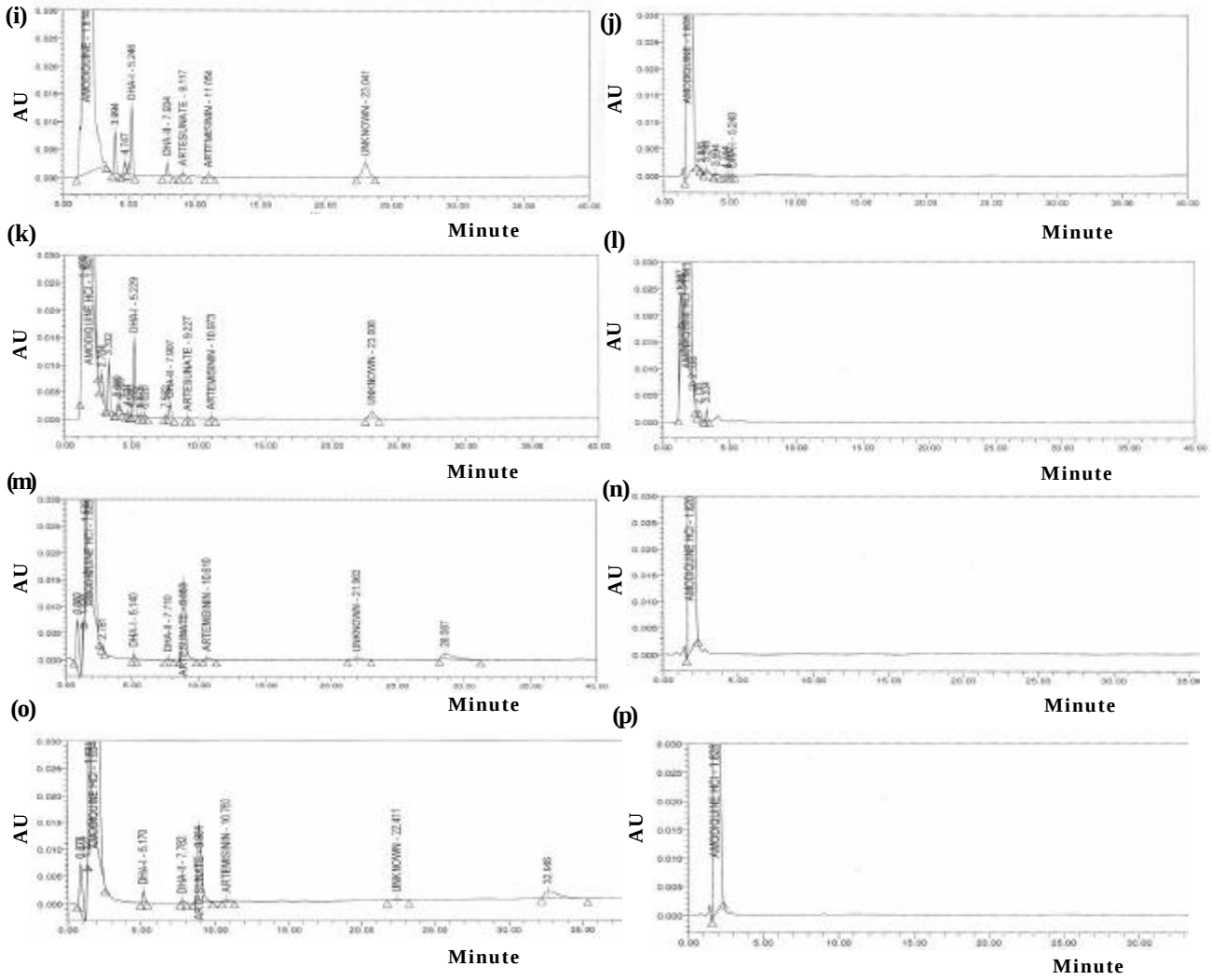


**Figure 1 : Chromatograms of (i) alkali spl 60 min at 210nm (j) alkali spl 60 min at 300nm (k) oxidation spl 60 min at 210nm (l) oxidation spl 60 min at 300nm (m) heat spl 60 min at 210nm (n) heat spl 60 min at 300nm (o) photo stability spl 60 min at 210nm (p) photo stability spl 60 min at 300nm**

of degradation showed decrease in peak area response of artesunate and amodiaquine hydrochloride and at the same time increase in the dihydro artemisinin, artesmisinin and unknown impurity at rrt about 2.9 is observed.

No major degradation was observed in the active drugs and combination dosage form of artesunate and amodiaquine when exposed to Photostability and elevated temperature.

From the studies it was also observed and confirmed that no other formulation components and potential degradation products of known and unknown identity do produce any chromatographic responses that would interfere with the main peak area response of artesunate and ,amodiaquine hydrochloride and its impurities dihydroartemisinin ,artemisinin. Hence this method is said to be selective and specific for the stability indicating of artesunate and amodiaquine combination dosage form. on storage.

For all the degraded samples, the artesunate and amodiaquine hydrochloride peak passed the peak purity testing, leading to a conclusion that the peak is spectrally homogeneous. In the other words none of the degradants formed during the stress study co elute with the artesunate and amodiquine hydrochloride peak. The specificity of the method is confirmed and the method is stability indicating. (Figure 1).

## CONCLUSIONS

A new HPLC method developed for simultanaeous determination of artesunate and amodiaquine in combination dosage form. The method was proved to be stability indicating.

## ACKNWLEDGMENTS

To Ipca Laboratories Limited for providing necessary facilities and my collegues for their help in conducting the study.

## REFERENCES

**[1]** The United States Pharmacopoeia, Published by Authority of The United States Pharmacopeial Convention USP 30, **(2007)**.
**[2]** International Pharmacopoeia, Published by the World Health Organization, Geneva, **1**, **(2005)**.
**[3]** P.Christen, J.L.Veuthey; Current Medical Chemistry, **8**, 1827 -1839 **(2001)**.
**[4]** O.M.Minzi, M.Rais, J.O.Sveensson, L.L.Gustafsson, O.Ericsson; Journal of Chromatography B: Biomedical Sciences and Applications, **783(2)**, 473-480 **(2003)**.
**[5]** ICH Harmonized Tripartite Guideline; ICH Q3A, Impurities in New Drug Substance, **(2003)**.
**[6]** ICH Harmonized Tripartite Guideline, ICH Q3B(R), Impurities in New Drug Products, **(2003)**.
**[7]** Ermer Joachim, H.John., M.C.B.Miller; Method Validation in Pharmaceutical Analysis, **(2004)**.
**[8]** Snyder Lloyed, J.Kirkland, J.Glajch; 'Practical HPLC Method Development', 2nd ed. Wiley, New York, 685-706 **(1997)**.
**[9]** ICH Harmonized Tripartite Guideline, ICH Q2R, Text on Validation of Analytical procedures, **(1995)**.
**[10]** ICH Harmonized Tripartite Guideline, ICH Q2R1, Validation of Analytical Procedures: Methodology, **(1997)**.