Arabian Journal of Chemistry (2022) **15**, 103657

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Innovative stability-indicating LC-Corona CAD method for simultaneous determination of assay in Artesunate and Mefloquine hydrochloride fixed-dose combination product

Wilson Camargo [1], Diogo Dibo [1], Monique Silva dos Santos, Ivone de Jesus do Nascimento Lopes, Flávia Furtado de Mendonça de Sousa, Livia Deris Prado, Camila Areias de Oliveira [*,1]

Laboratório de Desenvolvimento e Validação Analítica, Farmanguinhos, Fundação Oswaldo Cruz, Rua Sizenando Nabuco, 100, Rio de Janeiro 21041-250, RJ, Brazil

Received 14 October 2021; accepted 16 December 2021
Available online 24 December 2021

KEYWORDS

Artesunate;
Mefloquine hydrochloride;
Corona CAD and forced degradation

Abstract This work describes for the first time a stability-indicating HPLC-Corona CAD method for content determination of Artesunate (AS) and Mefloquine hydrochloride (MQ) in coated tablets 100 + 220 mg (ASMQ) developed by Farmanguinhos-Fiocruz. The chromatographic separation was carried out on two Promosil C18 columns in sequence. Chromatography was done using 0.05% formic acid/acetonitrile (80:20) in gradient at flow rate of 1 mL min^{-1}, flow 0.6 mL/min for the right pump, and 0.3 mL/min for the left pump (acetonitrile 100%). The temperature was set at 25 °C for the oven and the detection. The elution time of AS and MQ was found to be 40. 5 ± 0.5 min and 10.5 ± 0.5, respectively. The method was validated for system suitability, selectivity, linearity, precision, accuracy, and robustness. The forced degradation studies indicated that AS instability is the major trigger for product degradation, especially under heat and oxidative conditions. In conclusion, the method validation was in agreement with ICH guideline Q2(R1) and AOAC acceptance criteria. Our findings prospected the Corona-CAD detector as a quality control solution regarding the challenges of stability-indicating methods for fixed-dose products.

* Corresponding author.
E-mail address: camila.a.oliveira@far.fiocruz.br (C. Areias de Oliveira).
[1] Shared co-first authorship.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2021.103657
1878-5352

1. Introduction

According to World Health Organization (WHO), in 2018, an estimated 228 million malaria cases occurred worldwide. Nevertheless, the global incidence of Malaria declined in the last decade; recent years pointed out a slowed reduction rate. Although the WHO African Region had 94% of all malaria death cases in 2018, the Americas region reported an increase due to raises in malaria transmission in Venezuela (GMP/WHO, 2019).

Brazil, especially, had considerable success in reducing Malaria incidence and mortality, pointing out the possibility of moving towards burden elimination (Folegatti et al., 2017). As the main one in Latin America, the Brazilian pharmaceutical market has a leading role in treating this disease (SINDUSFARMA, 2020). Farmanguinhos, a Brazilian public official pharmaceutic laboratory, possess in the portfolio the fixed-dose combination artesunate (AS) + mefloquine hydrochloride (MQ), coated tablets 100 + 220 mg (ASMQ), an antimalarial drug product active against the infection caused by *Plasmodium Falciparum* and *Plasmodium Vivax,* the most incident in America (1).

Artesunate (Fig. 1) is a semi-synthetic derivative of artemisinin. Chemically is 4-oxo-4-[[(1R,4S,5R,8S,9R,10S,12R,13R)-1,5,9-trimethyl-11,14,15,16 tetraoxatetracyclo[10.3.1.04,13.08,13]hexadecan-10-yl]oxy]butanoic acid, its antimalarial mechanism of action involves damage to the parasite membrane. Also, Mefloquine hydrochloride ([2,8-bis(trifluoromethyl)quinolin-4-yl]-piperidin-2-ylmethanol) activity relies on the formation of toxic complexes with heme that damage membranes and interact with plasmodial components (Fig. 1) (Jyothi et al., 2014).

The first-line therapy with products as ASQM (Artesunate and Mefloquine hydrochloride fixed-dose combination) has kept higher treatment efficacy rates in the WHO Region of the Americas (1). However, the analytical effort to develop a stability-indicating method with suitable selectivity for this fixed-dose combination is challenging, especially regarding impurities analysis. Several authors described chromatographic conditions for AS and MQ in HPLC with UV or PDA detectors and lack studies defining MQ degradation profile. A few works discussed the degradation of Artesunate alone or associated with fixed-dose tablets. The most related degradation products are Dihydroartemisinin (DHA) derived from hydrolysis, followed by oxidation of the hemiacetal to Artemisinin. (Hall et al., 2016; Jyothi et al., 2014; Mathew et al., 2018; Nogueira et al., 2004; Rao et al., 2011; Siddiqui et al., 2017).

The simultaneous analyses of both APIs is not described in any pharmacopeias, and this method development is challenging due to the excessive broadening of the MQ peak at a suitable UV wavelength, as stated by Nogueira and co-works. The authors suceed in developing a simultaneous quantification for ASMQ product using a PDA detector, however the method was not used in forced degradation studies (Nogueira et al., 2013). ICH guideline Q2(R1) stress testing identifies the likely degradation products, which further helps determine the intrinsic molecule stability, establishes degradation pathways, and validates the stability-indicating procedures used. This data also supports pharmaceutical development since drug substances, and drug product degradation pathways can be prevented in the early manufacturing and packaging stages (Blessy et al., 2014; ICH, 2003).

Nevertheless, the higher absorbance profile in UV of MQ compared with AS combined with the higher concentration of MQ in the product ASMQ turns unfeasible using a concentration-based detector in the chromatographic assay analyses can fulfill the requirements of a forced degradation study (Blessy et al., 2014; ICH, 2003; Iram et al., 2016; Kogawa and Salgado, 2016).

To face this analytical challenge, our group studied the development and validation of the stability-indicating method for the assay analyses of ASMQ using the Corona® Charged Aerosol Detector (CAD), a universal detector introduced in the earlies 2000s. CAD provides a uniform response to nonvolatile analytes, and its use has been increasing in the last years (Ligor et al., 2013). In this mass-based

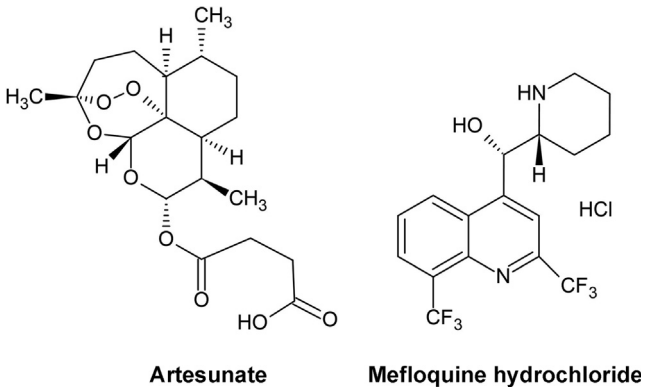

Fig. 1 Chemical structure of Artesunate and Mefloquine hydrochloride.

detector, first, the sample is nebulized with subsequent evaporation of the mobile phase for the production of the dried particles that are charged with an ionized, neutral gas like nitrogen and finally detected are by an electrometer. Moreover, the CAD can detect a broader range of analytes than the UV detector due to its ability to analyze substances lacking chromophoric properties. Recently, CAD use in drug substances analyses as aminoglycosides and excipients states the growing interest concerning non-chromophoric compounds (Ilko, 2015; Jariwala et al., 2020). Also, in the current comparison to mass spectroscopy (MS), Hazotte and co-workers showed that CAD could universally detect all substances of interest while two sources of ionization (pressure chemical ionization -APCI and electrospray ionization -ESI) were required for MS detection (Hazotte et al., 2007). However, the advantage of CAD, the only aerosol-based detector currently used in pharmacopeias is the Evaporative Light Scattering Detector (ELSD) (Ilko, 2015).

Regarding this scenario, our work aims to describe a pioneer analytical development of a stability-indicating method for ASMQ assay through the CAD detector use and support this aerosol-based detector his way into compendial testing.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents are at least of analytical grade. Purified Water was used. Formic acid was purchased from

Table 1 Separation gradient developed for the study of forced degradation by Corona CAD HPLC.

Time (min.)	Left bomb – Isocratic	Right bomb	
	Flow (mL/min) 100% B	Flow (mL/min)	% (A/B)
00,0	0,3	0,6	70:30
08,0	0,3	0,6	70:30
09,0	0,3	0,6	55:45
36,0	0,3	0,6	55:45
37,0	0,3	0,6	15:85
47,0	0,3	0,6	15:85
48,0	0,3	0,6	70:30
60,0	0,3	0,6	70:30

Legend: Phase A: 0.05% formic acid/Acetonitrile (80:20) and Phase B: 100% Acetonitrile.

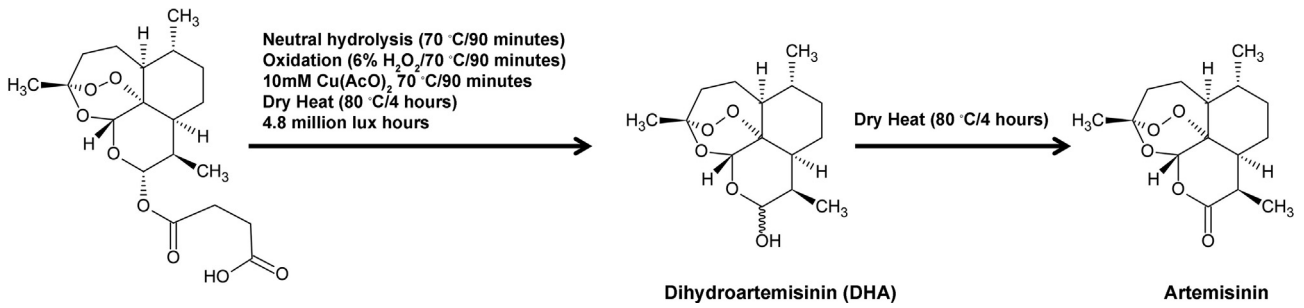

Fig. 2 Degradation pathway of Artesunate in hydrolysis, oxidation and heat.

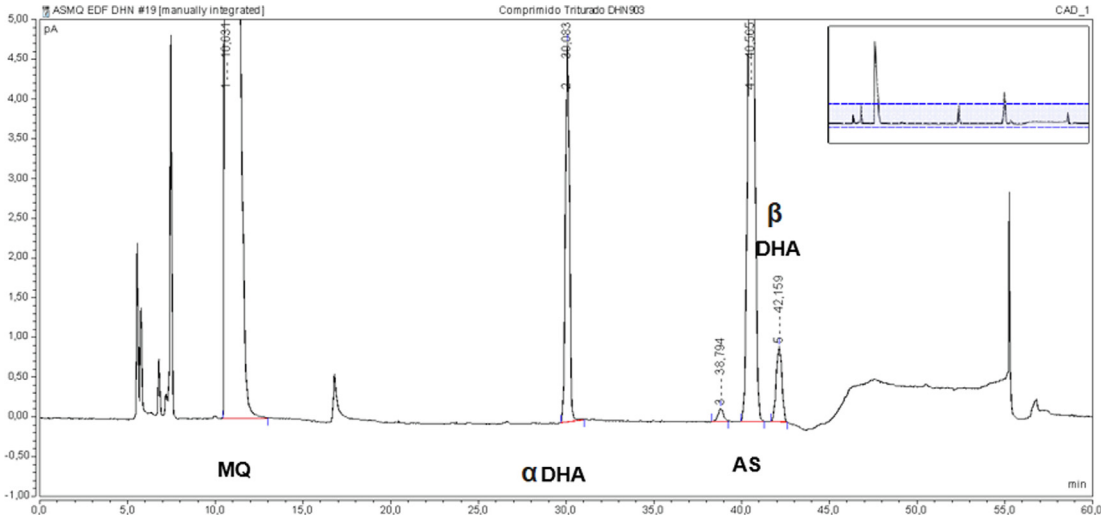

Fig. 3 Chromatogram of neutral hydrolysis (70 °C/90 min) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

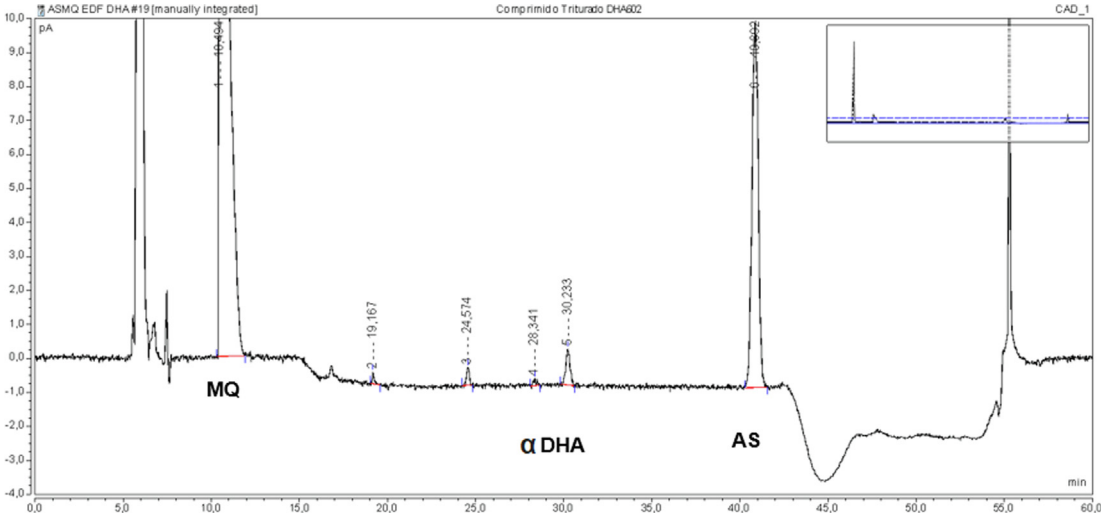

Fig. 4 Chromatogram of acidic hydrolysis (0.1 M HCl/1 h) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

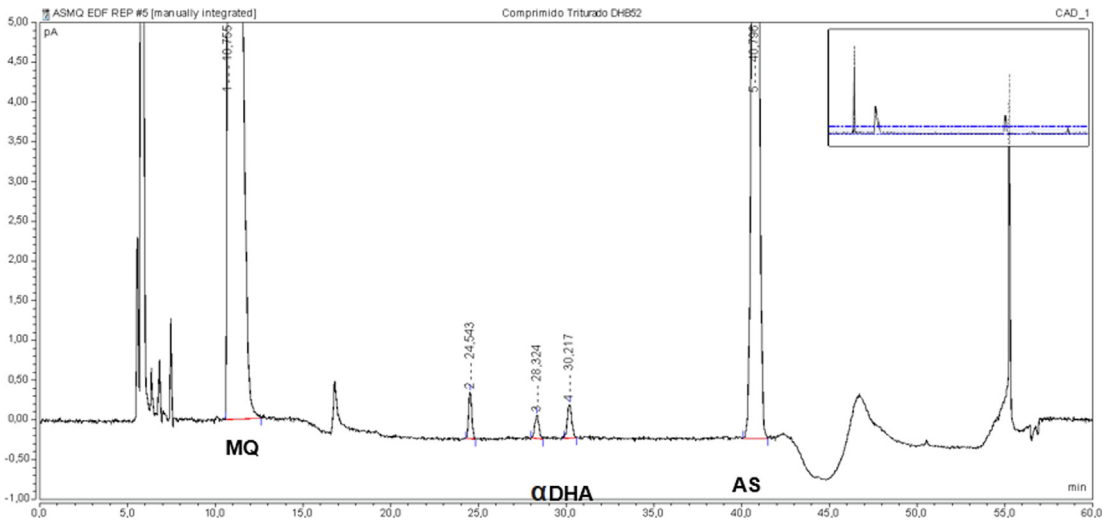

Fig. 5 Chromatogram of basic hydrolysis (0.01 M NaOH/5 min) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

Sharlau (Spain, Barcelona). Acetonitrile HPLC-grade was purchased from Tedia (United States of America, Fairfield). The actives pharmaceutical ingredients Artesunate and Mefloquine Hydrochloride APIs were respectively purchased from Huvepharma (Italy, Garessio) and Olon SPA (Italy, Milan). Artesunate and Mefloquine Hydrochloride standards were respectively purchased from Brazilian Pharmacopeia and European Pharmacopeia. Dihydroartemisinin (mixture of α and β isomers) and Artesimisin standards were purchased from Sigma-Aldrich (Brazil, São Paulo). ASMQ (Artesunate 100 mg + Mefloquine Hydrochloride 220 mg) tablets were developed at Farmanguinhos (Brazil, Rio de Janeiro).

2.2. Instrumentation

Thermo Scientific HPLC series Dionex Ultimate 3000, PDA, and Corona-CAD detectors were used for the chromatographic analyses. UV irradiation chamber, Ethik (424/CF), was used for the photodegradation assay.

2.3. Column

Two Promosil C-18 (250 × 4,6) mm and 5 µm columns were used, in sequence, for the stability indicated method.

2.4. Chromatography

The chromatographic conditions for the for the right pump included the use of 0.05% formic acid/acetonitrile (80:20) in gradient (Table 1), flow 0.6 mL/min. For the left pump a isocratic mode in a flow 0.3 mL/min was used. The chromatographic run was performed in 60 min with a 10 µL injection of the sample and an oven temperature set at 25 °C. The detection was conducted with the PDA (scanning from 200 to

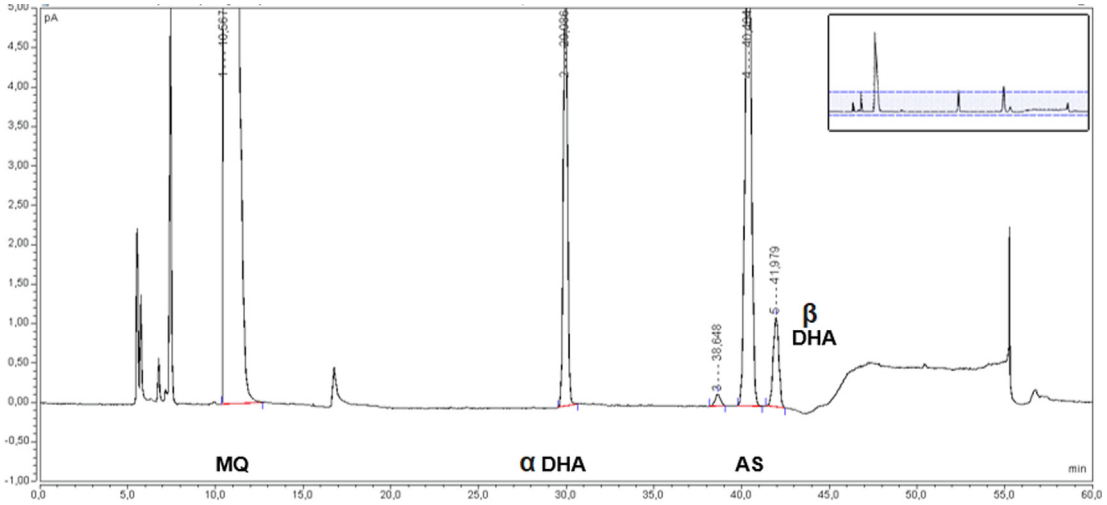

Fig. 6 Chromatogram of oxidative degradation (6% H_2O_2/70 °C/90 min) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

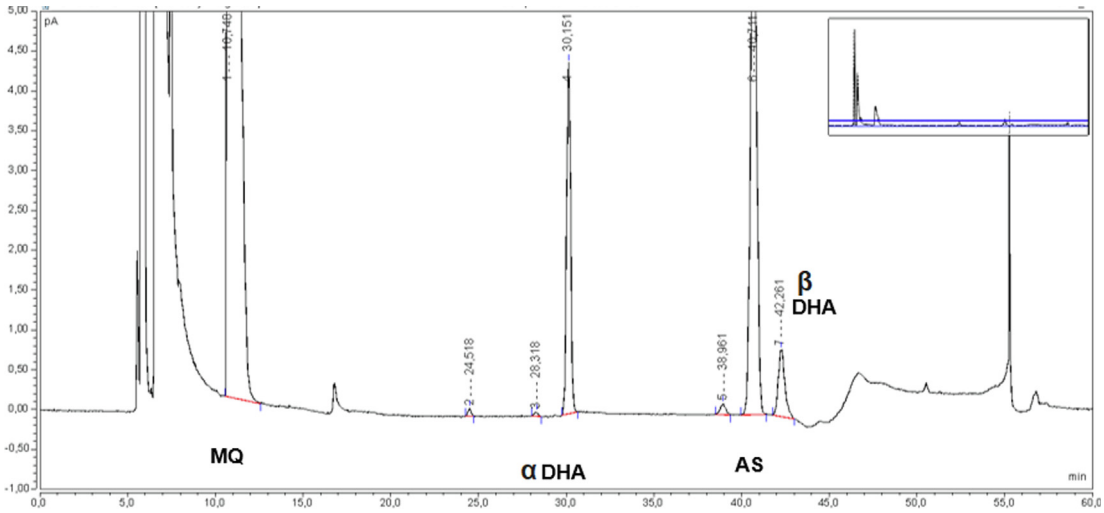

Fig. 7 Chromatogram of metal ions degradation (10 mM of $Cu(AcO)_2$ at 70 °C for 90 min) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

300 nm, determining the peak purity) and in the Corona CAD (data collection rate at 25 Hz, filter at 3.6 s, and vaporization at 25 °C).

2.5. Forced degradation studies

The forced degradation samples (oxidation; metal ions; neutral, acid, and basic hydrolyses) were prepared with 12.5 mg of Artesunate API, 27.5 mg Mefloquine Hydrochloride API alone, or with 17.9 mg placebo and 57.8 mg of the powdered drug product. The samples were solubilized or extracted with acetonitrile, followed by dilution to 25.0 mL in volumetric flasks. 5.0 mL aliquots of these stock solutions were subjected to degradation (ANVISA. Ministério da Saúde. Resolução RDC no 53, December 2015; ICH, 2003; Iram et al., 2016).

The solid form of degradation had a specific sample preparation procedure. Regarding the dry and wet heat degradation, 3.0 mg of Artesunate API, 6 mg Mefloquine Hydrochloride API alone or in combination, 4 mg Placebo, and 12 mg of powdered drug product after the stress condition were solubilized or extracted with acetonitrile, followed by dilution to 25.0 mL in volumetric flasks. Concerning the photochemical degradation, 12.5 mg of Artesunate API, 27.5 mg Mefloquine Hydrochloride API alone or in combination, 17.9 mg Placebo and 57.8 mg of powdered drug product after the stress condition were solubilized or extracted with acetonitrile, followed by dilution to 25.0 mL in volumetric flasks (12,13).

According to the acceptance criteria, the peak purity of the APIs must be not less than 0.99. Also, the tailing factor of the main (API) peaks must be between 1.0 and 2.0, and the resolution between the APIs and their adjacent peaks must be at least 2.0 (USP Organization, 2017).

2.5.1. Neutral, acid and basic conditions

Acid decomposition studies were carried under agitation (ultrasonic bath) of the samples in 0.1 M hydrochloric acid for

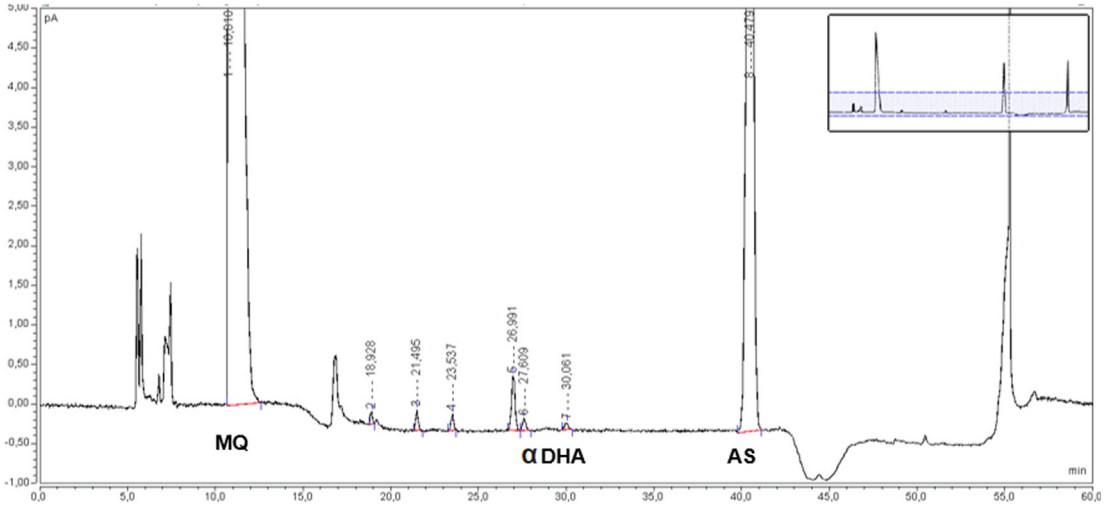

Fig. 8 Chromatogram of photochemical degradation (4.8 million lux hours) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin.

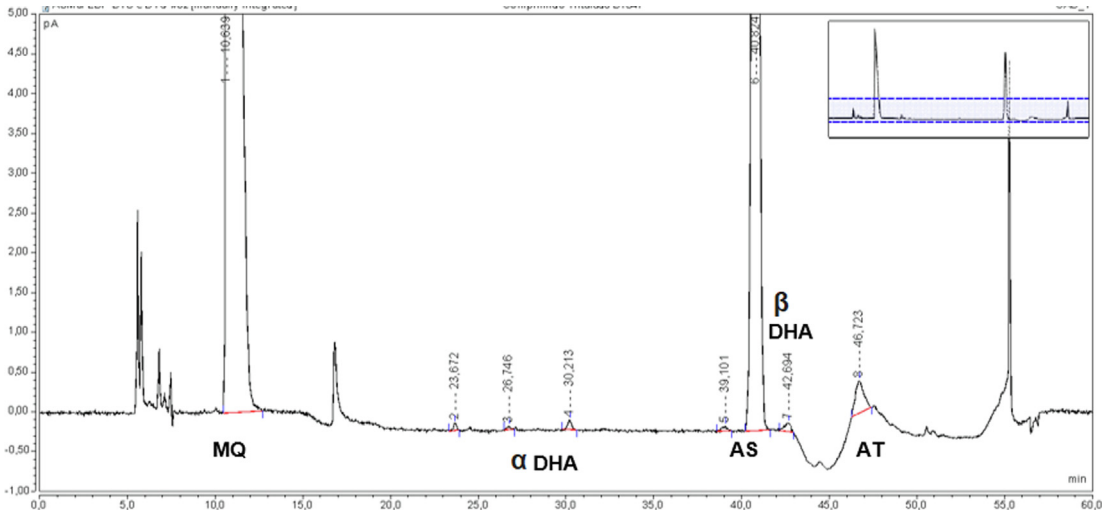

Fig. 9 Chromatogram of dry heat degradation (80 °C for 4 h) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin, AT Artemisinin.

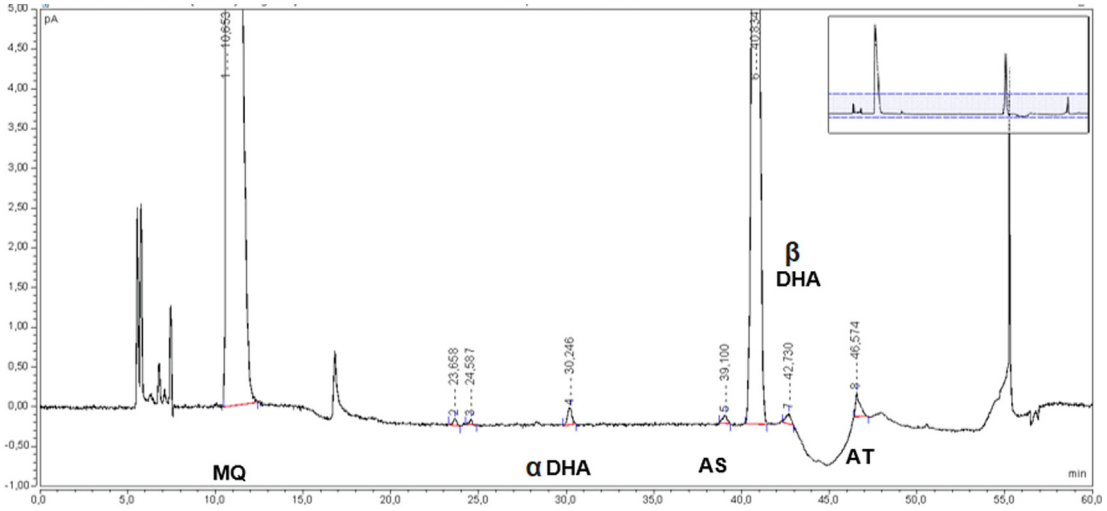

Fig. 10 Chromatogram of humid heat degradation (80 °C for 4 h in a saturated vapor atmosphere) ASMQ sample. Legend: AS Artesunate, MQ: Mefloquine hydrochloride, DHA Dihydroartemisinin, AT Artemisinin.

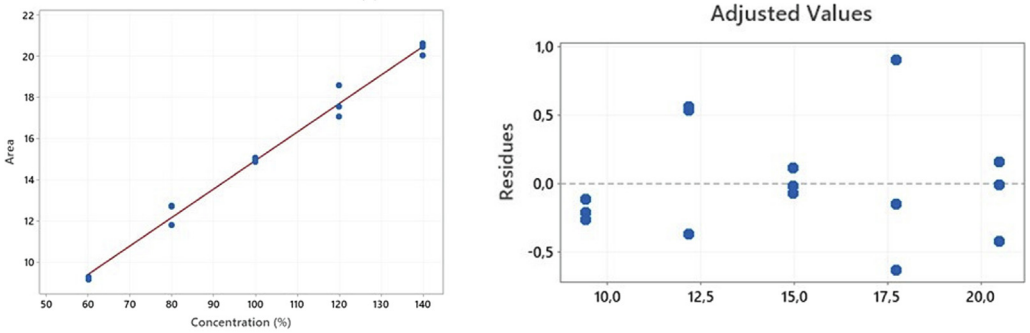

Fig. 11 Responses (area) in relation to analyte concentration (%) and residues versus adjusted values of Artesunate.

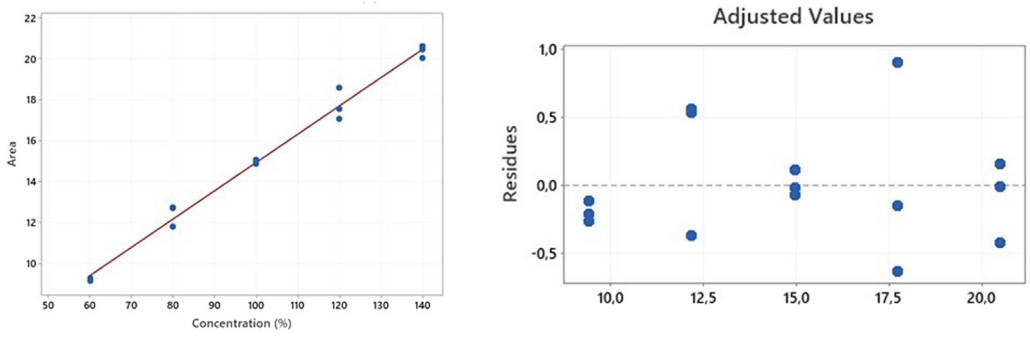

Fig. 12 Responses (area) in relation to analyte concentration (%) and residues versus adjusted values of Mefloquine hydrochloride.

Table 2 Linearity and regression data.

PARAMETERS	RESULTS	
Linearity range (mg/mL)	AS 0.06–0.13	MQ 0.132 – 0.308
Retention Time (minutes)	40.5 ± 0.5	10.5 ± 0.5
Slope	0.057	0.138
Intercept	−0.0328	1.109
Coefficient of determination (R^2)	0.997	0.998
Coefficient of correlation (R)	0.994	0.995
Limit of detection (mg/mL) - LOD	0.007	0.004
Limit of quantification (mg/mL) - LOQ	0.02	0.01

Legend: AS Artesunate, MQ: Mefloquine hydrochloride.

1 h. The studies under alkaline conditions were carried out under agitation (ultrasonic bath) of the samples in 0.01 M sodium hydroxide for 5 min. The neutral hydrolysis was performed at 70 °C for 90 min. The resultant solutions were neutralized if **degration** recommended, followed by a final dilution with acetonitrile/water (1:1) to 25.0 mL in a volumetric flask and filtered through a 0.45 µm PTFE membrane before HPLC injection.

2.5.2. Oxidation with hydrogen peroxide

The samples were exposed to 6% hydrogen peroxide at 70 °C for 90 min to study hydrogen peroxide-induced degradation. The resultant solutions were diluted with acetonitrile/water (1: 1) to 25.0 mL in a volumetric flask and filtered through a 0.45 µm PTFE membrane before HPLC injection.

2.5.3. Metal ions

The samples were exposed to 10 mM of $Cu(AcO)_2$ at 70 °C for 90 min to study oxidation induced by metal ions. After the degradation process, the samples were chelated with EDTA 10 mM. The resultant solutions were diluted with acetonitrile/water (1: 1) to 25.0 mL in a volumetric flask and filtered through a 0.45 µm PTFE membrane before HPLC injection.

2.5.4. Photochemical degradation

The photochemical stability of the samples was studied by exposing samples in solid form to direct sunlight (4.8 million lux hours) for a total period of 10 days. After the stress conditions, the resultant solutions were prepared as described in **item 2.5** and filtered through a 0.45 µm PTFE membrane before HPLC injection.

2.5.5. Dry and wet heat degradation

Different combinations of stress conditions were performed to study dry heat degradation of the samples in solid form. Artesunate and its combinations with placebo and Mefloquine Hydrochloride were placed in an oven at 80 °C for 30 h. Mefloquine Hydrochloride and its combinations with placebo and Artesunate were placed in an oven at 90 °C for 30 h. The drug product was placed in an oven at 80 °C for 4 h. Also, the sam-

Table 3 Precision study for ASMQ proposed assay method.

PRECISION			
REPEATABILITY		INTERMEDIATE PRECISION	
Acceptance Criteria (AOAC INTERNATIONAL., 2016)	Results	Acceptance Criteria (17)	Results
RSD ST1and ST2 ≤ 2.0%	0.9% (ST1) and 0.6% (ST2) - AS 0.7% (ST1) and 0.1% (ST2) - MQ	**RSD ST1 and ST2 ≤ 2.0%**	1.8% (ST1) and 0.6% (ST2) - AS 1.7% (ST1) and 0.5% (ST2)- MQ
- RDS (Analyst A) ≤ 2.7%	1.6% -AS 1.2% - MQ	**RDS (Analyst B) ≤ 2.7%**	1.9% - AS 2.3% - MQ
		RSD between Analyst A and B ≤ 2.7%	1.7% - AS 2.1% - MQ

Legend: AS Artesunate, MQ: Mefloquine hydrochloride.

Table 4 Accuracy study for ASMQ proposed assay method.

Accuracy					
STANDARDS		SAMPLE			
Acceptance Criteria(AOAC INTERNATIONAL., 2016)	Results	Acceptance Criteria (17)	Concentrations (%)	Results AS	Results MQ
RSD ST1and ST2 $\leq$ 2.0%	1.6% (ST1) and 0.2% (ST2) - AS 1.4% (ST1) and 1.1% (ST2) - MQ	Recovery in each level 97–103% RSD of the recovery in each level $\leq$ 2.7%.	60	98.3% RSD 0.3%	102.6% RSD 0.2%
			100	100% RSD 2.3%	99.6% RSD 1.4%
			140	101.3% RSD 0.2%	98% RSD 0.8%

Legend: AS Artesunate, MQ: Mefloquine hydrochloride.

ples were analyzed in solid form in a saturated vapor atmosphere regarding wet heat. Artesunate and its combinations with placebo and Mefloquine Hydrochloride were placed in an oven at 80 °C for 15 h. Mefloquine Hydrochloride and its combinations with placebo and Artesunate were placed in an oven at 90 °C for 15 h. The drug product was placed in an oven at 80 °C for 4 h. After the stress conditions, the resultant solutions were prepared as described in **item 2.5** and filtered through a 0.45 µm PTFE membrane before HPLC injection.

2.6. Validation of the method

Validation of the optimized LC method was done concerning the following parameters (International Council for Harmonisation, 2011).

2.6.1. Standard solutions

To prepare the three standard solutions, the APIs were weighed (0,50 AS + 1,10 MQ mg/mL) and transferred to a volumetric flask. Half of the volume was completed with diluent (acetonitrile), and the flasks were submitted to an ultrasonic bath for 20 min. After complete solubilization, the volume was completed. From the three different solutions, the three differents linearity curves were performed.

2.6.1.1. Linearity. The Linearity of the method was studied by injecting five concentrations of both APIs, Artesunate (60–141 µg/mL) and Mefloquine Hydrochloride (132–312 µg/mL) in acetonitrile in triplicate into the HPLC system, keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.6.2. Precision

Precision was computed here by repeatability and intermediate precision. Repeatability studies were carried out by analyzing the primary standard and also the product sample solutions. In both samples, the Artesunate concentration was 100 µg/mL and the Mefloquine concentration was 220 µg/mL. The standard solution was prepared in duplicate, and one replicate was injected 5 five times (ST1) and the other three times (ST2). Six replicates were prepared for the product sample solution, and one injection of each sample was analyzed. The intermediate precision of the method was checked by repeating studies on another day with a different analyst. The acceptance criteria was determined according to the AOAC INTERNATIONAL (AOAC INTERNATIONAL., 2016).

2.6.3. Accuracy

The linearity samples determined the accuracy of the developed method regarding the recovery of each API in the samples 60% (60 µg/mL of Artesunate and 132 µg/mL of Mefloquine), 100% (100 µg/mL of Artesunate and 220 µg/mL of Mefloquine), and 140 % (130 µg/mL of Artesunate and 308 µg/mL of Mefloquine). The acceptance criteria was determined according to the AOAC INTERNATIONAL (AOAC INTERNATIONAL., 2016). The following equation determined the recovery:

$$\text{Recovery}(\%) = \frac{C_{\text{practical}}}{C_{\text{theoretical}}} \times 100$$

2.6.4. Robustness of the method

Few parameters like variation of flow rate in the left and right pump, oven temperature, and detection temperature (vaporization) were altered to evaluate the robustness of the HPLC method. This parameter was performed by analyzing the primary standard solution (ST1) and a solution with a mixture of placebo (140 µg/mL) and the primary standards. In both samples, the Artesunate concentration was 100 µg/mL, and the Mefloquine concentration was 220 µg/mL. The standard solution was prepared and injected three times. Three replicates were prepared for the solution with a mixture of placebo and the primary standards solution, and one injection of each sample was analyzed in the altered method conditions. For the statistical analyses, a 5% significance level was used. The acceptance criteria was determined according to the AOAC INTERNATIONAL (AOAC INTERNATIONAL., 2016).

2.6.5. Stability of the analytical solution.

To prepare the sample solutions, the APIs were weighed (25 mg for AS + 55 mg for MQ) and transferred to a volumetric flask (50 mL). Half of the volume was completed with diluent (acetonitrile), and the flasks were submitted to an

Table 5 Robustness of ASMQ proposed assay method.

	Oven Temp. (°C)					Flow rate (mL min^{-1}) Left bomb/Flow rate (mL min^{-1}) Right bomb			Detection Temp. (°C)				
Standard Conditions	25		23		27	0.3/0.6			25				
Acceptance Criteria (AOAC INTERNATIONAL., 2016)	23	27				0.27/0.54	0.33/0.66	0.27/0.54	0.33/0.66	23	27	23	27
RSD ST1 ≤ 2.0%	AS		MQ			AS		MQ		AS		MQ	
	0.4%	0.7%	0.6%	0.6%		0.6%	0.4%	0.2%	0.6%	3.1%	1.7%	1.0%	3.1%
RSD of the recovery in solution 100% ≤ 2.7%	6.5%	1.7%	6.8%	1.4%		11%	0.6%	6%	0.2%	9.2%	6.1%	15.7%	6.7%
Recovery in each solution 97–103%	Max.	Max.	Max.	Max.		Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.
	Recovery	Recovery	Recovery	Recovery		Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery
	101%	100%	100%	100%		104%	100%	99%	99%	97%	97%	98%	101%
	Min.	Min.	Min.	Min.		Min.	Min.	Min.	Min.	Min.	Min.	Min.	Min.
	Recovery	Recovery	Recovery	Recovery		Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery
	89%	97%	89%	98%		85%	98%	89%	99%	82%	86%	74%	89%

Legend: AS:Artesunate, MQ: Mefloquine hydrochloride.

ultrasonic bath for 20 min. After complete solubilization, the volume was completed. A 4 mL aliquot was transferd to a 20 mL volumetric flask and the volume was completed with acetonitrile (0.1 AS + 0.22 MQ mg/mL). The stability of the sample and the standard solution was performed by analyzing the primary standard solution (ST1) and a sample solution for 72 h at room temperature. In both samples, the Artesunate concentration was 100 µg/mL, and the Mefloquine concentration was 220 µg/mL. According to the acceptance criteria, the RSD for API areas in the standard and sample solution must be less than 1.0% (17).

3. Results and discussion

3.1. Method development and otimization

The preliminary method development was based on the chromatographic conditions described by Phadke et al. (2010). The column first chosen was C-18 BEH de (50 × 2,1 mm −1,7 µm), endcapped, hybrid technology, low silanol activity, and good selectivity for the UPLC technique. The eluation mode chosen was a gradient, and the mobile phase was 10 mM potassium monophosphate/acetonitrile (63.2:36.8) as phase A (pH value 3,0) and acetonitrile 100% in phase B. The PDA detector was used at 210 and 222 nm. MQ was the first substance to eluate as a weak base with a pKa of approximately 8.6 (Psimadas et al., 2012). However, simultaneous quantification of AS and MQ in the UV proved impossible due to the excessive broadening of the MQ peak combined with a higher work concentration. Since AS was the most unstable drug substance, the method had no selectivity regarding APIs and impurities. Regardless, Nogueira and co-works reported this analytical challenge, and the authors did not perform forced degradation studies to prove the method selectivity, which is a regulatory requirement (Nogueira et al., 2013).

Finally, the detection was performed in a CAD to reduce the UV absorption disparity for the APIs and achieve proportionality in MQ and AS areas. Two columns in series (Promosil C-18, 250 × 4.6 mm and 5 µm) were connected to increase peak resolution and decrease the effects of the sample loading in the column.

3.2. Forced degradation

3.2.1. Hydrolysis

ASMQ was unstable regarding hydrolysis under heating as 49.12% of AS in the product was degraded under neutral conditions. DHA (Dihydroartemisinin) was the mainly related compound formed as described in Figs. 2 and 3**.** The acidic and basic medium did not induce a significant degradation as only 14.68% and 3.27 of AS, respectively, was decreased in the drug product (Figs. 4 and 5). MQ was also stable (degradation less than 10%) in all the conditions alone or associated.

3.2.2. Oxidation

The oxidative condition under heat was a point of attention concerning the ASMQ. ASMQ was unstable at H_2O_2 reaction as 59.51% degradation was identified after exposing the pro-

duct (Fig. 6), with the formation of one unknown degradation product and an expressive formation of DHA (Fig. 3). AS alone and associated with the placed also had a strong degradation of 54.4%. MQ was stable in all the conditions alone or associated. Regarding the oxidation via metal ions under heat, ASMQ was also unstable and degraded 57.52% with three unknown degradation products and a strong formation of DHA (Fig. 7). AS alone and associated with the placed had an increased degradation of 63.7% and 76.2%, respectively. MQ was also stable (degradation less than 10%) in all the conditions alone or associated.

3.2.3. Photochemical degradation

ASMQ was unstable to photochemical degradation as more than 10% of AS (12.9%) degradation was seen after exposing the product (Fig. 8), with the formation of 5 unknown degradation products and DHA. Both APIs alone or associated with each other or placebo showed a degradation profile under 10%.

3.2.4. Dry and wet heat degradation

ASMQ in solid state was unstable to humid heat degradation as more than 10% of AS (10.96%) degradation was identified after exposing the product (Fig. 9), with the formation of 4 unknown degradation products, DHA and Artemisinin. Fig. 3 describes the formation of Artemisin from the hydrolysis of AS to DHA, followed by oxidation of the hemiacetal to Artemisinin. AS alone and associated with the placed had an increased degradation of 23.7% and 28.7%, respectively. MQ was stable in all the conditions alone or associated.

No degradation above 10% was noticed in ASMQ, for both APIs in the dry heat condition (Fig. 10). However, AS when associated with placebo had a degradation of 24.74%. MQ was stable in all the conditions alone or associated.

3.3. Results of method validation

The validation studies on the stability-indicating method developed for ASMQ involving the chromatographic conditions described in items 2.2–2.4 are summarised below.

3.3.1. Linearity

For fulfilling regulatory requirements, the linearity assessment must provid statistical data that proves the suitability of the linear regression. Data homoscedasticity shall be investigated for the use of a proper model. Besides the graphic representation of the responses concerning analyte concentration, the residue dispersion graphic must be accompanied by its statistical evaluation and an evaluation of the significance of the angular coefficient (Brasil, 2017).

To assess the homoscedasticity, the Cochran Test was used. The calculated C value was 0.558 and 0.615 for AS and MQ, respectively. These results were not higher than the table critical values for the Cochrantest for variance outliers (0.684), indicating a homoscedasticity behavior. Also, the residues graph for both APIs (Figs. 11 and 12) confirmed this result since the residues are randomly distributed around zero. The ANOVA F-test examined the significance of the slope. The calculated F value was 1114.521 and for AS and MQ, respectively. These results were higher than the table critical values of F(4.67), which indicates that the slope is different from zero. The limit of quantification and detection was estimated from the residual standard deviation of the regression line. All the regression details are summarized in Table 2 and are in accordance with the acceptance criteria (Brasil, 2017).

3.3.2. Precision

The results of the repeatability and intermediate precision experiments are described in Table 3. It can be seen that RSD values for both repeatability and intermediate precision suitable according to the acceptance criteria of both APIs as recommended by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q2 (R1), illustrating that the developed method was found to be precise.

3.3.3. Accuracy

The accuracy of the method was determined by recovery of three levels of linearity solutions (n = 3) and showed high accuracy with the minimal and maximal recovery of 98.3% and 100%, respectively, for AS. The minimal and maximal recovery of 98% and 102.6% was achieved regarding MQ, as described in Table 4.

3.3.4. Robustness of the method and stability of the analytical solution

According to the results described in Table 5**,** the method was robust for both APIs assay determination in the oven temperature range of 25.0 + 2.0 °C. Regarding the flow of the right and left the pump, the suitable range of work was 0.6 + 0.0 6 mL/min and 0.3 + 0.03 mL/min, respectively. The variation in the detection temperature (vaporization) compromised the method suitability since this parameter affects the droplet size, significantly influences the detector response (Ilko, 2015).

The sample and standard solutions were considered stable for 72 h when stored and in a well-sealed bottle at 15 °C since the RSD between the areas was less than 1%.

4. Conclusion

In conclusion, our forced degradation study results indicated that Artesunate mainly induced the ASMQ drug product degradation. Frequently high temperatures and oxidation conditions generated the hydrolysis of the API and principally formed DHA. Our results highlighted the importance of analytical development as an essential support to selecting process parameters and product packaging. Also, a suitable innovative LC-Corona CAD method for determining AS and MQ content was developed and validated according to ICH recommendations, which provides analytical support for further initiatives corning the application of this mass-based detection device.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

ANVISA. Ministério da Saúde. Resolução RDC nº 53, December, 2015, 2015. This resolution establishes parameters for notification,

identification, and qualification of degradation products in medicinal products with synthetic and semi-synthetic active substances.
AOAC INTERNATIONAL, 2016. Official methods of analysis. Guidelines for standard method performance requirements, Appendix F. Rockville.
Blessy, M., Patel, R.D., Prajapati, P.N., Agrawal, Y.K., 2014. Development of forced degradation and stability indicating studies of drugs - a review. J. Pharm. Anal. 4, 159–165. https://doi.org/10.1016/j.jpha.2013.09.003.
Brasil, 2017. ANVISA. Ministério da Saúde. RDC 166, July/2017, This resolution establishes parameters for analytical validation 2017, pp. 1–21.
Folegatti, P.M., Siqueira, A.M., Monteiro, W.M., Lacerda, M.V.G., Drakeley, C.J., Braga, É.M., 2017. A systematic review on malaria sero-epidemiology studies in the Brazilian Amazon: insights into immunological markers for exposure and protection. Malar. J. 16, 1–15. https://doi.org/10.1186/s12936-017-1762-7.
GMP/WHO, 2019. World malaria report 2019, WHO Regional Office for Africa.
Hall, Z., Allan, E.L., Van Schalkwyk, D.A., Van Wyk, A., Kaur, H., 2016. Degradation of artemisinin-based combination therapies under tropical conditions. Am. J. Trop. Med. Hyg. 94, 993–1001. https://doi.org/10.4269/ajtmh.15-0665.
Hazotte, A., Libong, D., Matoga, M., Chaminade, P., 2007. Comparison of universal detectors for high-temperature micro liquid chromatography. J. Chromatogr. A 1170, 52–61. https://doi.org/10.1016/j.chroma.2007.09.008.
ICH, 2003. Q1A (R2) Stability Testing of new Drug Substances and Products.
Ilko, D., 2015. The use of charged aerosol detection for the analysis of excipients and active pharmaceutical ingredients.
International Council for Harmonisation, 2011. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, Prescrire International.
Iram, F., Iram, H., Iqbal, A., Husain, A., 2016. Forced degradation studies. J. Anal. Pharm. Res. 3, 6 https://doi.org/10.15406/japlr.2016.03.00073.
Jariwala, F.B., Hibbs, J.A., Zhuk, I., Sukhishvili, S.A., Attygalle, A.B., 2020. Rapid determination of aminoglycosides in pharmaceutical preparations by electrospray ionization mass spectrometry. J. Anal. Sci. Technol. 11. https://doi.org/10.1186/s40543-019-0202-4.
Jyothi, P., Geetha, K., Ajitha, A., Uma, V., Rao, M., Ramarao, N., 2014. Stability indicating method development and validation for simultaneous estimation of mefloquine and artesunate in tablet dosage form. Sch. Acad. J. PharmacyOnline) Sch. Acad. J. Pharm. 3, 2320–4206.
Kogawa, A.C., Salgado, H.R.N., 2016. Impurities and forced degradation studies: a review. Curr. Pharm. Anal. 12, 18–24.
Ligor, M., Studzińska, S., Horna, A., Buszewski, B., 2013. Corona-charged aerosol detection: an analytical approach. Crit. Rev. Anal. Chem. 43, 64–78. https://doi.org/10.1080/10408347.2012.746134.
Mathew, N., Jacob, J.T., Nadig, S., 2018. Stability indicating degradation behaviour of artesunate under stress conditions. Res. J. Pharm. Technol. https://doi.org/10.5958/0974-360X.2018.01016.8.
Nogueira, A.C., Nakano, A.K., Joekes, I., 2004. Impairment of hair mechanical properties by sun exposure and bleaching treatments. J. Cosmet. Sci. 55, 533–537.
Nogueira, F.H.A., Reis, Araújo, N.F., Paula Rocha Chellini, Isabela da Costa César, G.A.P., 2013. Development and validation of an HPLC method for the simultaneous determination of artesunate and mefloquine hydrochloride in fixed-dose combination tablets. Brazilian J. Pharm. Sci. 49, 1089–1093. https://doi.org/10.1093/jaoac/94.4.1089
Phadke, M.U., Jadhav, V.K., Patil, D., Narayankar, S., Jadhav, R.K., Bansal, Y.K., 2010. Development and validation of stability indicating UPLC method for determination of related impurities in Artesunate and Amodiaquine fixed dose tablets. Der. Pharm. Lett. 6, 333–341.
Psimadas, D., Georgoulias, P., Valotassiou, V., Loudos, G., 2012. Molecular nanomedicine towards cancer. J. Pharm. Sci. 101, 2271–2280. https://doi.org/10.1002/jps.
Rao, B.T., Krishna, P.M., Kumar, R.K., Venkateswarlu, P., 2011. The forced degradation and solid state stability indicating study and validation of method for the determination of assay of Artesunate by HPLC. Res. J. Pharm. Biol. Chem. Sci. 2, 111–117.
Siddiqui, M.R., AlOthman, Z.A., Rahman, N., 2017. Analytical techniques in pharmaceutical analysis: a review. Arab. J. Chem. 10, S1409–S1421. https://doi.org/10.1016/j.arabjc.2013.04.016.
SINDUSFARMA, 2020. Perfil da indústria farmacêutica Mercado farmacêutico no Brasil.
USP Organization, 2017. Chromatography. United States Pharmacopoeia 40-NF 35 1–12.