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Analytical Lifecycle Management (ALM) and Analytical Quality by Design (AQbD) based Analytical Method Development for Separation of Related Substances in Amodiaquine Hydrochloride along with its Degradation Products and Structural Elucidation by LC-Quadrupole-Time of Flight-Tandem Mass Spectrometry

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The current study aimed to develop a robust, regulatory-flexible, stability-indicating ultra high performance liquid chromatography (UHPLC) analytical procedure compatible with mass spectrometry for the determination of impurities in amodiaquine hydrochloride using analytical lifecycle management (ALM) and analytical quality by design (AQbD) principles. The analytical target profile (ATP) and critical method attributes (CMAs) for the analytical method were identified. The pH of the mobile phase, flow rate and column oven temperature were identified as critical method parameters (CMPs) through risk assessment studies and they were screened and optimized using the design of experiments (DoE) to generate the design space (DS). Finally, all the impurities were well separated in a 15 min run time by using an Acquity UPLC BEH-C18 column with 20 mM ammonium acetate pH 8.0 as mobile phase A and acetonitrile as mobile phase B with a gradient program of time (min), % of B: 0/15, 1.0/15, 9/55, 12/55, 12.1/15 and 15/15. The flow rate was 0.3 mL min^{-1} and the column oven temperature were 30 ºC. Evaluated the robustness of the developed analytical method. Degradation products obtained from the forced degradation studies are well separated from process impurities and main compound. Based on the stress studies, impurity-3 was identified as a key degradation product in base degradation, while DP-1, impurity-3, DP-2, DP-3 and DP-4 were in the oxidative degradation. Total 9 impurities out of 4 process related and 5 degradation impurities were well separated in the developed method. Used the same method to LC coupled tandem mass spectrometer for separation and structure elucidation of degradation products. The method validation was carried out in accordance with ICH Q2 (R1) and USP <1225> guidelines.

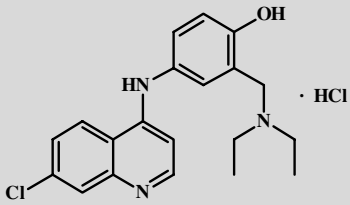
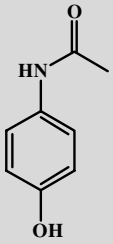
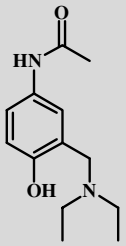
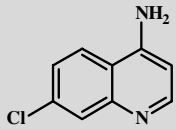
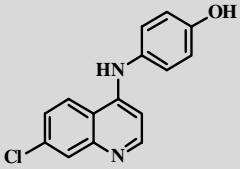
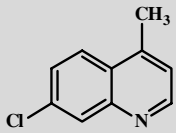
Keywords: Amodiaquine hydrochloride, Design of experiments, Method operable design region, Design space. UHPLC, LC-Q-ToF MS.

INTRODUCTION

Amodiaquine, a 4-aminoquinoline derivative that is orally active and has anti-inflammatory and antimalarial effects [1]. Amodiaquine works against some chloroquine-resistant strains similarly to chloroquine, mainly *Plasmodium falciparum*, the most deadly malaria parasite. Chemically, 4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)phenol hydrochloride is known as amodiaquine hydrochloride (AMD) and used to treat uncomplicated malaria, including malaria causes due to *Plasmodium falciparum*. Table-1 depicts the chemical structures of AMD and its impurities, molecular formula, molecular weight and its type (process related impurities or degradation impurities).

A thorough review of literature survey reveals availability of many reports for simultaneous determination of amodiaquine hydrochloride [2-10] and few for investigations on plasma extraction studies [11-13] and limited reports are available for the related substances separation [14-16] isolation and characterization of hydrolytic degradation product of AMD (impurity-3) [17] and few articles having degradation products characterization [18-24]. Thin layer chromatography is used in the International Pharmacopeia [25] to analyze amodiaquine impurities and the currently United States Pharmacopeia [26] have incor-

TABLE-1
AMODIAQUINE HYDROCHLORIDE AND ITS PROCESS AND DEGRADATION IMPURITIES
CHEMICAL STRUCTURES, MOLECULAR FORMULA, MOLECULAR WEIGHT

Compound name	Chemical structure	m.f. and w.w.	Component type
Amodiaquine hydrochloride		m.f.: $C_{20}H_{22}N_3OCl{\cdot}HCl$ m.w.: 355.8612	Active
Impurity-1		m.f.: $C_8H_9NO_2$ m.w.: 151.1626	Process
Impurity-2		m.f.: $C_{13}H_{20}N_2O_2$ m.w.: 236.3101	Process
Impurity-3		m.f.: $C_9H_7N_2Cl$ m.w.: 178.6183	Degradant
Impurity-4		m.f.: $C_{15}H_{11}N_2OCl$ m.w.: 270.7136	Process
Impurity-5		m.f.: $C_{10}H_8NCl$ m.w.: 177.6302	Process

porated high-performance liquid chromatography (HPLC) method. However, no specific impurities were mentioned in both the pharmacopeias.

Since no impurities method for the drug substance had been published so far to the best of our knowledge, hence, it is aimed to develop an appropriate reversed phase ultra-high performance liquid chromatography (RP-UHPLC) method for determining related compounds as well as its degradation products in the drug substance by applying the analytical quality by design (AQbD) concepts and an analytical lifecycle management strategy.

UPLC is considered as one of the best techniques in liquid chromatographic separations for a variety of analytes. In contrast to HPLC, UPLC increases the efficiency along with sensitivity of analytes separating, proving to be time saving and cost-effective approach [27]. The traditional liquid chromatography method development for an analytical procedure follows a trial-and-error approach employing the common one-factor at a time (OFAT) evaluation. This traditional approach results numerous experiments and also does not provide any information on the interactions among the different factors. The analytical procedures developed by the above-mentioned approach often results in poor performance in terms of robustness [28-30]. Furthermore, drug regulatory agencies have long been concerned about the lack of assurance in the robustness and quality of the analytical methods. This issue was effectively addressed in ICH Q14 "Analytical Procedure Development" [31]. USP <1220> explains a systematic way of approach for analytical procedure life cycle [32]. ICH Q14 and USP <1220> provides a framework for lifecycle management focused on analytical procedures and encouraging the use of advanced scientific approaches like analytical quality by design (AQbD) rather than traditional

methodologies [33-35]. According to <1220> analytical lifecycle management (ALM) has comprised of the analytical target profile (ATP) and three stages to provide comprehensive approach to controlling an analytical procedure throughout its lifecycle.

The AQbD approach, which is a systematic approach for the method development integrating scientific knowledge with an ability to develop a robust, affordable, flexible and appropriate procedures for particular applications and this approach is crucial in ALM. Furthermore, the AQbD approach based generated data and design space will support in the continual development of analytical methods under ALM [36,37]. The present work aims to utilize ALM and AQbD approaches to design and validate an analytical method for the determination of impurities present in amodiaquine hydrochloride in better systematic and scientific manners.

EXPERIMENTAL

The sample of amodiaquine hydrochloride (AMD) and its impurities were acquired from the US Pharmacopeial Convention-India (P) Ltd., Hyderabad, India. Merck supplied analytical reagents (AR) such as ammonium acetate buffer, sodium hydroxide, 30% v/v hydrogen peroxide, hydrochloric acid and HPLC grade solvents acetonitrile (Merck, Mumbai, India). All solutions were prepared using HPLC grade water collected from Sartorius Milli-Q water system (Arium pro-VF).

Instruments: The AMD and its impurities were separated using an UPLC system (Waters H-Class from Waters Corp., Milford, MA, USA) equipped with a quaternary pump having degasser, photodiode array (PDA) detector with an autosampler and a temperature-controlled column compartment controlled by Empower 3 software. The LC-MS and LC-MS/MS analysis was carried out using a SYNAPT G2 Q-TOF Mass spectrometer from Waters Corporation. Used ACQUITY-UHPLC system coupled with mass detector equipped with a binary pump, autosampler, column compartment and UV-PDA detector for LC detection and for mass detection used a Q-TOF and an electrospray ionization (ESI) source. Masslynx 4.1 software was used to collect and process the data.

Chromatography: The separation of the impurities was carried out on an Acquity UPLC BEH C-18 (100 × 2.1) mm, 1.7 µm column. A mobile phase containing 20 mM ammonium acetate buffer pH 8.0 and acetonitrile with a gradient composition. The flow rate of mobile phase was 0.3 mL/min, at a column temperature set at 30 ºC and injection volume is 2 µL. 235 nm wavelength was chosen for UV detection. The gradient elution program used for the separation: (T min/%B): 0/15, 1.0/15, 9/55, 12/55, 12.1/15 and 15/15. Compounds were identified using waters LCMS. Capillary voltage 2.5 kV, source temperature 100 ºC, desolvation temperature 300 ºC, sampling cone 40 V, extraction cone 2.0 V and desolvation gas flow 550 L/h are the typical LC-MS method conditions in positive ionization mode with ESI source considered for detection of AMD and its impurities.

Software: Design expert (v,13.0.5.0; Stat-Ease, Inc. USA) was used to generate the design of experiments and overlay plots.

Sample solutions preparations: For sample solution preparation, a 50:50% v/v combination of water and acetonitrile was adopted as a diluent.

Preparation of organic impurities (OI) standard solution: Prepared a stock solution of 50 µg/mL by dissolving an appropriate quantity of AMD and impurities in diluent. An organic impurity standard solution containing 0.25 µg/mL was prepared from this stock solution.

Preparation of AMD sample solution: Weighed about 10 mg of AMD and transferred in to a 20 mL volumetric flask, dissolved and diluted to volume with diluent (500 µg/mL).

Impurities spiked sample solution preparation: Transferred about 25 mg of AMD into a 50 mL volumetric flask, then added 0.25 mL of above impurities stock solution (50 µg/mL), dissolved and diluted to volume with diluent (concentration of AMD at 500 µg/mL and impurities at 0.25 µg/mL).

Robustness solution preparation: Weighed about 10 mg of AMD to 20 mL volumetric flask and added 2 mL of impurities stock solution (50 µg/mL) dissolved and diluted to volume with diluent (AMD at 500 µg/mL and impurities 5 µg/mL concentration).

Chromatographic method validation: The analytical procedure was validated in accordance with ICH Q2 (R1) [38] for the parameters such as system suitability, specificity, linearity, accuracy and robustness.

System suitability: System suitability criteria for system performance were confirmed. Six replicate injections of OI standard was used to establish system precision in terms of area % relative standard deviation (%RSD) and the resolution was measured.

Specificity: Stress tests were carried out to demonstrate the stability-indicating method and the specificity for AMD drug substance under ICH conditions. Degradation was performed under stress conditions such as, acid (1 N HCl for 24 h reflux at 60 ºC), base (0.1 N NaOH for 24 h at ambient temperature), oxidation (6% peroxide for 4 h reflux at 60 ºC), thermal (105 ºC for 3 days), humidity (85 ºC and 85% RH for 3 days) and photolytic stress (1.2 million lux hours followed by 200 watt-hours per square meter).

Precision: The precision of the procedure was evaluated by injecting six different preparations of AMD samples (500 µg/mL) spiked with 0.05% of each impurity-1, impurity-2, impurity-3, impurity-4 and impurity-5.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ were determined using a signal-to-noise (S/N) ratio of 3 and 10, respectively.

Linearity: Five different concentrations ranging from 0.05 to 0.15% of the analyte concentration were prepared from stock solutions. The data on peak area *versus* concentration was analyzed using linear regression and calibration curve was generated by correlating impurity area *vs*. concentration in µg/mL.

Accuracy: The method accuracy was determined using three different concentration levels by spiking all of the impurities to the drug substance with a concentrations of 0.05%, 0.10% and 0.15% of AMD (500 µg/mL) and calculated the percentage mean recoveries for each impurity. The accuracy

of an analytical procedure shows the degree of agreement between the true and observed values.

Robustness studies: Full factorial design was created to evaluate robustness of the developed analytical procedure. The interaction effect of the variable factors was investigated using deliberate changes in the proposed method conditions.

RESULTS AND DISCUSSION

Procedure design (development and understanding): This step follows the principles of AQbD and can be subdivided into following stages: (1) defining an analytical target profile (ATP) and critical method attributes (CMAs), (2) initial risk assessment and identification of critical method parameters (CMPs), (3) screening and optimization of the critical method parameters (CMPs), (4) design of experiments (DoE) for robustness, (5) identification of method operable design region (MODR).

Defining analytical target profile (ATP) and critical method attributes (CMAs): The ATP specifies an analytical procedure's performance requirements, which matches the quality target product profile (QTPP) established in ICH Q8. Also, the goal of developed analytical procedure is to quantify the organic impurities in AMD drug substances rapidly, accurately and precisely. It should quantify the related substances within the range from reporting threshold (0.05%) to 150% qualification threshold (0.15%) of impurities with an acceptable recovery of 80-120% and the %RSD for repeatability is ≤ 5% with the target sample concentration of amodiaquine hydrochloride at 500 µg/mL.

The analytical method must be capable of separating amodiaquine HCl and its related substances along with its degradation products with a minimum peak resolution of greater than or equal to 2.0. Aimed to develop a more robust stability-indicating UHPLC method by separating all the impurities of amodiaquine hydrochloride along with its degradation products. A UHPLC-UV method with mass compatible mobile phases were identified as the suitable analytical technique based on the defined ATP. Therefore, the critical method attributes (CMAs) were selected as retention time, tailing factor and the resolution between impurities and main compound.

Initial risk assessment and identification of critical method parameters (CMPs): In the development of QbD-based methods, the risks that can influence CMAs must be extensively investigated. As a result, the risk assessment was carried out in order to report the CMPs that have a major impact on CMAs by collecting prior knowledge about the sample materials, chemical structures, physico-chemical characteristics and other related information. Based on the chemical structures, the presence of amine groups in the molecule are very sensitive to pH of the mobile phase and stationary phases. Therefore, summarized risk assessment data in Table-2 and the risk variables are classified as low, medium and high depending on their influence on selected critical method attributes.

The screening and choice of the analytical method conditions involving high-risk variables was done based on the risk assessment. Among the parameters investigated, it was observed that buffer pH of the aqueous component, flow rate of mobile phase, column oven temperature, stationary phase chemistry, organic solvent and the gradient program were the high-risk factors. Other components were categorized as low or medium risk.

Screening and optimization of critical method parameters (CMPs): The mobile phase pH, organic solvents and stationary phases need to be evaluated since they are high-risk factors. Initially, three distinct pH values, 2.5, 5.0 and 8.0, (0.1% formic acid in water pH about 2.5, 20 mM ammonium acetate pH 5.0 and pH 8.0 buffers) were considered. As an organic solvent widely used in reversed-phase liquid chromatography, methanol and acetonitrile were chosen and diverse reverse phase columns such as Acquity UPLC BEH C-18, C-8 and phenyl were investigated. The experiments using methanol as an organic modifier were unsuccessful because of the high column back pressure. As a result, methanol was eliminated as an organic solvent. Among the screening studies, achieved good separation on acquity UPLC BEH C-18 column with a mobile phase pH 8.0 as an aqueous and acetonitrile as an organic solvent. The chosen column has a pH range that can be sustained between 1 and 12 with temperature maximum range of 60 ºC. Based on the initial risk assessment information, the mobile phase flow rate, column oven temperature and gradient program were further optimized. The optimal flow rate was 0.3 mL min^{-1}, the oven temperature was finalized to 30 ºC and the gradient elution time up to 15 min. The gradient program as follows: Time (min)/ % solution B: 0/15, 1.0/15, 9.0/55, 12.0/55, 12.1/15, 15.0/15. Fig. 1 depicts the chromatogram that was obtained utilizing the optimum analytical conditions for determining amodiaquine and its impurities.

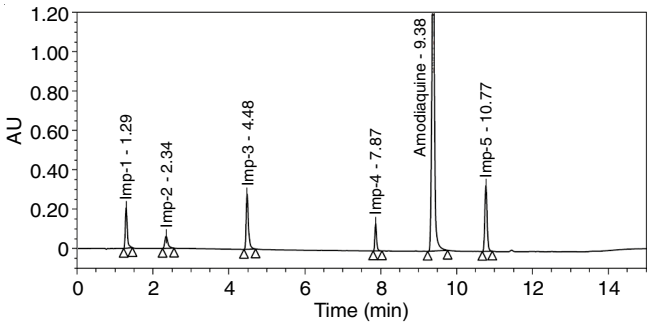


Fig. 1. Obtained chromatogram using the optimized analytical conditions for determination of AMD and its impurities

TABLE-2
RISK ANALYSIS MATRIX FOR RISK ASSESSMENT

CAAs	Buffer concentration	Buffer pH	Flow rate	Column temperature	Stationary phase	Organic solvent	Gradient time
Retention time	Low	High	Low	Medium	Medium	High	High
Resolution	Low	High	High	High	High	High	High
Tailing factor	Medium	Medium	Low	Low	Low	Medium	Low

Design of experiments for robustness: A full factorial design approach is considered to be one of the best available statistical tools for optimization of CMPs, which helps in assessing the effect of individual and multiple factors at a time (MFAT) and it was effective for defining the method operable design space region (MODR). The parameters obtained from the screening studies were thoroughly investigated using a full factorial design (2 levels and 3 factors) by considering factor A is mobile phase pH, factor B is flow rate (mL/min) and factor C is column oven temperature (ºC) as critical method parameters and summarized the experiments with level of variables in Table-3.

TABLE-3
FULL FACTORIAL DESIGN OF EXPERIMENTS AND THEIR VARIABLES FOR ROBUSTNESS STUDIES

Factors	Code levels	Actual levels
A: Mobile phase pH	-1.0	7.8
	0.0	8.0
	+1.0	8.2
B: Flow rate (mL/min)	-1.0	0.28
	0.0	3.0
	+1.0	3.2.
C: Column oven temperature (ºC)	-1.0	25
	0.0	30
	+1.0	35

Selected the critical pair resolutions between Imp-1 & 2 as Response R1, for AMD & Imp-5 as Response R2. The remaining peaks were not taken into consideration for statistical analysis since they were separated by a minimum resolution of > 7. For all responses R1 and R2, the model is significant and the lack of fit is not significant.

Analysis of Variance (ANOVA) statistical tools were used to analyze the model's significance. The obtained p value < 0.05 indicates that the model was significant and summarized these values in Table-4. Fig. 2 depict the 3D surface plots and the effect of chromatographic factors (mobile phase pH, flow rate and column temperature) on responses R1, R2 and Fig. 3 represents for the counter plots of the considered resolutions for the afore said responses.

Identification of method operable design region (MODR): The determination of the method operable design region is a crucial step in the AQbD method development. The proposed design method operable region identified using overlay plots, desirability and graphical optimization. The overlay plots in Fig. 4 demonstrates that the conditions for the developed method are more robust and that there is a more design space for the optimum working point for the responses R1 and R2. The grey area represents an undesirable area, whereas the yellow area is desirable. Therefore, chosen this as working point region.

Procedure performance qualification: Post procedure design phase, the analytical method was validated in accordance with ICH Q2 (R1) for system suitability, specificity, linearity, LOD, LOQ, accuracy, precision and intermediate precision to show the procedure performance met the ATP within the analytical lifecycle management (ALM).

Specificity: Both general and stability-indicating specificity are the part of specificity. Confirmed general specificity by injecting blank, standard, as such sample and impurity-spiked sample. The forced degradation studies were required to confirm the stability-indicating specificity. Summary of the forced degradation conditions and degradation products observed in AMD described in Table-5. The drug has shown significant degradation under peroxide, base stress conditions. The overlaid chromatograms of AMD with all impurity spiked sample, control sample, base degradation and peroxide degradation samples shown in Fig. 5 and the separation of five degradation impurities and four process impurities along with the main compound shown in Fig. 6. The drug showed to be stable under acid, thermal, photolytic and humidity stress conditions. To confirm the mass spectral homogeneity and peak purity of the AMD peak and its related impurities, all of these stress conditions applied samples were evaluated by utilizing the aforesaid UHPLC methodologies with a photo diode array detector coupled with LC Q-ToF mass spectrometry. The mass spectra of the obtained degradation products shown in Fig. 7 and found that they are spectrally homogeneous. Therefore, the developed analytical method is considered to be stability-indicating.

Structural characterization of AMD degradation products (DPs): In present work, a total of 9 impurities (including 4 process related impurities and 5 DPs) were separated from the main compound using UPLC and the degradation impurities structures were characterized based on processed HRMS data. A total of five DPs were obtained in oxidative degradation including Imp-3 and it is a major degradation impurity in alkaline stress conditions as well. Chemical structures of DP1 to DP4 were predicted by LC coupled HRMS.

The degradation studies showed that AMD under peroxide stress conditions led to the formation of degradation products DP-1, DP-2, DP-3 and DP-4 have been illustrated in **Scheme-I**.

The degradation product DP-1 with *m/z* 180 formed under oxidative stress conditions. The lower *m/z* of the degradation

TABLE-4
ANNOVA RESULTS FOR RESPONSES R1, R2 FROM THE FULL FACTORIAL DESIGN STUDY

	Source	Sum of squares	Df	Mean square	Model F-value	Model p-value	Prob > F
Response 1 (R1) Resolution b/w Imp-1&2	Model	32.26	7	4.61	4278.89	< 0.0001	Significant
	Mobile phase pH	27.71	1	27.71	2573.14	< 0.0001	
	Flow rate	0.0435	1	0.0435	40.41	0.0079	
	Temperature	4.22	1	4.22	3918.22	< 0.0001	
Response 2 (R2) Resolution b/w AMD & Imp-5	Model	16.19	7	2.31	476.33	0.0001	Significant
	Mobile phase pH	15.21	1	15.21	3131.92	< 0.0001	
	Flow rate	0.6216	1	0.6216	128.02	0.0015	
	Temperature	0.0153	1	0.0153	3.15	0.0017	

AMD = Amodiaquine hydrochloride

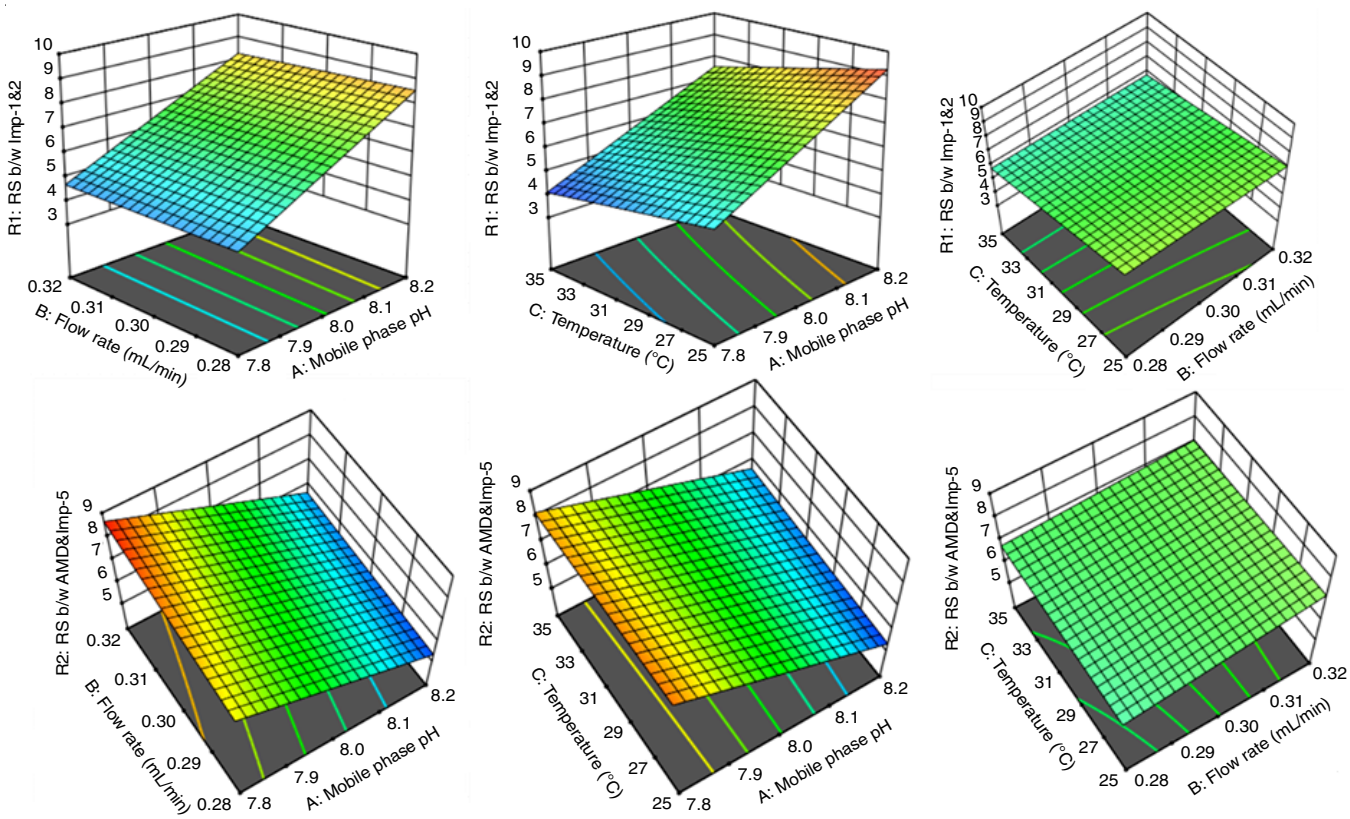


Fig. 2. 3D surface plots obtained from full factorial design for the resolution as responses R1 and R2. (R1: Rs b/w Imp-1 & 2, R2: Rs b/w AMD & Imp-5)

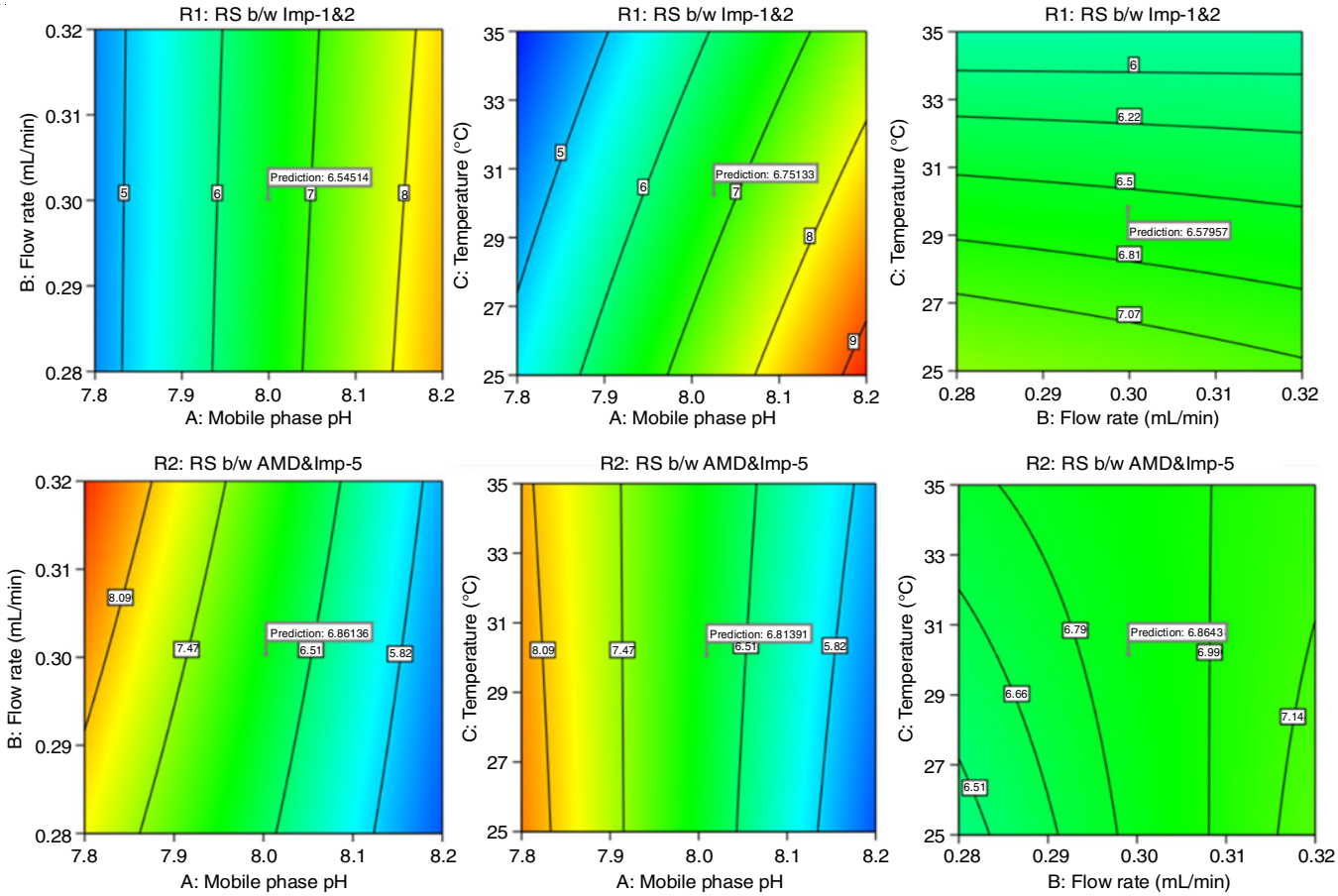


Fig. 3. Counter plots for resolutions as responses R1 and R2. (R1: Rs b/w Imp-1&2, R2: Rs b/w AMD & Imp-5)

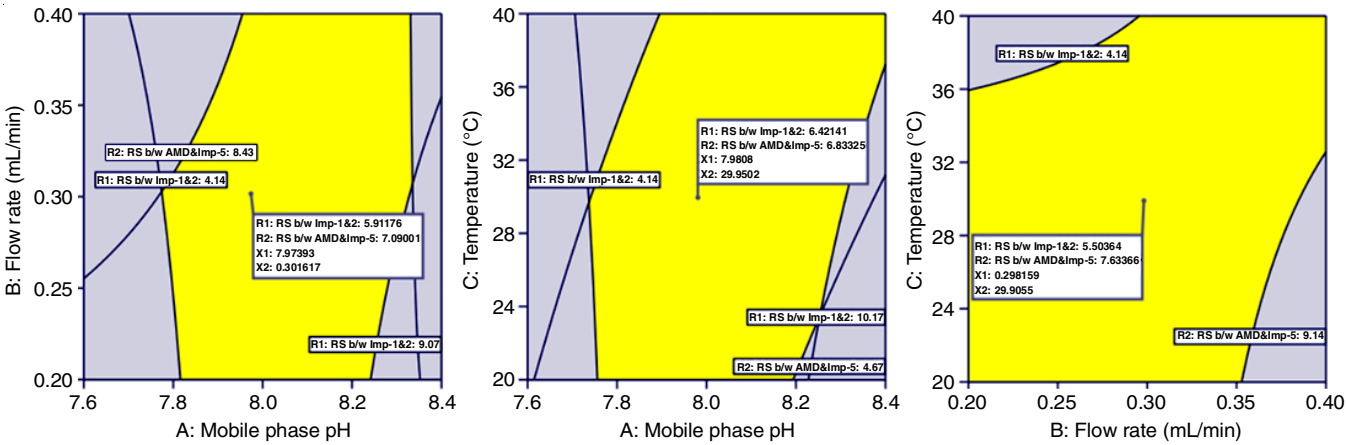


Fig. 4. Overlay plots for the responses R1, R2 and the identified method operable design region (MODR) (yellow region: design space, gray region: undesirable region)

TABLE-5
SUMMARY OF FORCED DEGRADATION CONDITIONS AND DEGRADATION PRODUCTS OBSERVED IN AMODIAQUINE HYDROCHLORIDE

Degradation study	Exposure conditions	Degradation products formed
Acid hydrolysis	1 N HCl reflux at 60 °C for 24 h	No degradation
Base hydrolysis	0.1 N NaOH at RT for 24 h	Imp-3
Oxidative	6% H_2O_2, reflux at 60 °C for 4 h	Imp-3, DP-1, DP-2, DP-3, DP-4
Thermal	Thermal at 105 °C for 3 days	No degradation
UV light	200 $Whrm^{-2}$	No degradation
Fluorescent light	1.2 million lux hours	No degradation
Humidity	85% RH & 85 °C for 3 days	No degradation

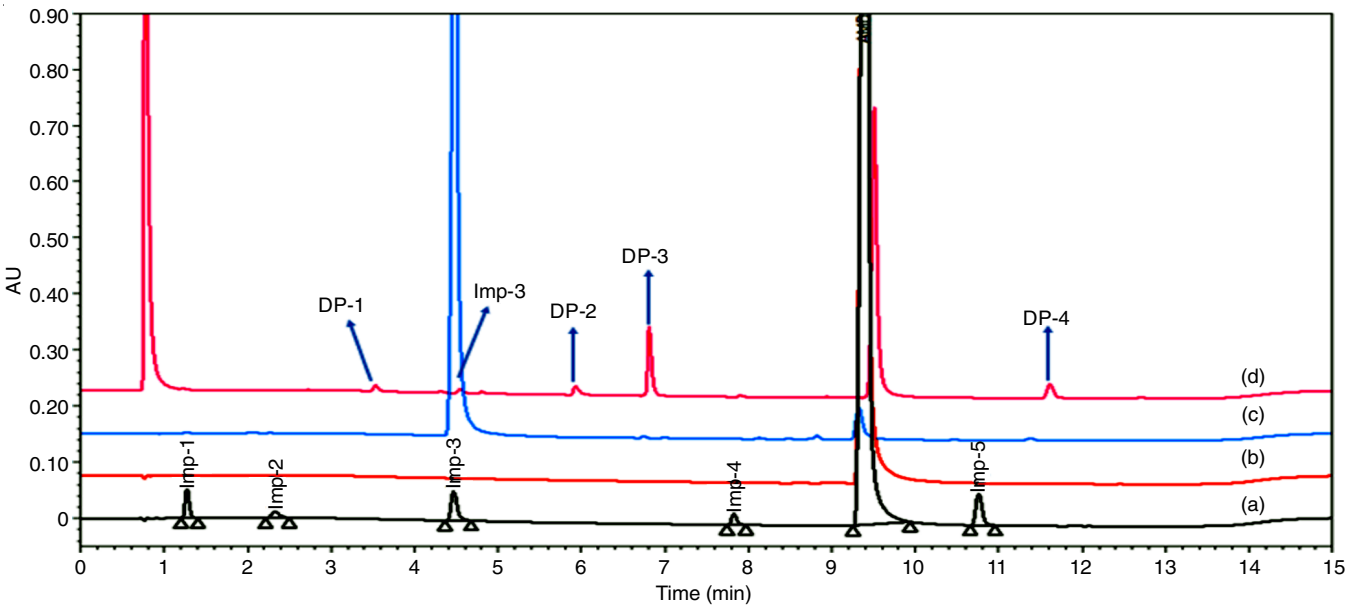


Fig. 5. Overlaid chromatogram of (a) spiked sample, (b) control sample, (c) base degradation sample and (d) peroxide degradation sample

product produced fewer number of fragments, but were sufficient for deducing the structure of the degradation products. The characteristic fragments were formed at *m/z* 162 and 145 which indicated the characteristic losses of OH and Cl groups from the structure of DP-1. The MS/MS and the accurate mass experiments supported the proposed structure of DP-1 (Fig. 8a). Imp-3 with an *m/z* 179 was also observed as one of the major degradation products in the base degradation conditions (Fig. 8b). The structure of DP-2 was characterized with a *m/z* of 372 and fragmentation ions at *m/z* 299 (loss of diethylamine moiety from the parent *m/z* 372), subsequent loss of an -OH radical formed the ion at *m/z* 282. Loss of CHO group from the ion at *m/z* 282 formed the ion at *m/z* 253. The spectrum of the ion at *m/z* 218 clearly indicated the loss of chloride ion radical. These fragment ions and the fragmentation pattern along with the accurate mass experiments clearly indicate the

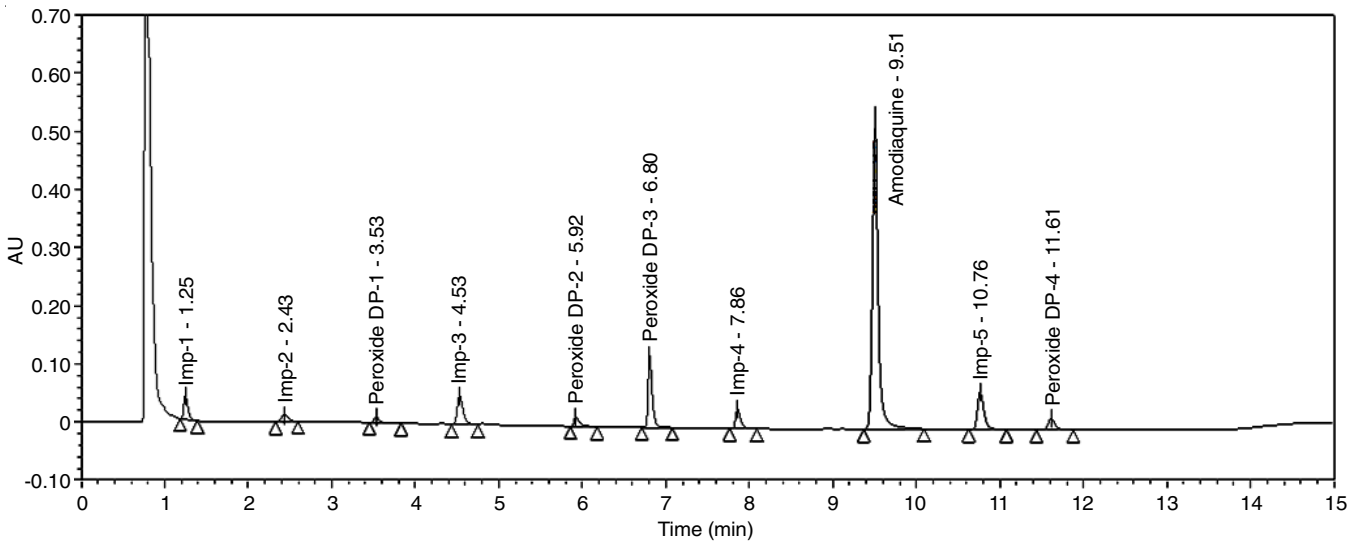


Fig. 6. Typical chromatogram of separation of known impurities and degradation products of the main compound

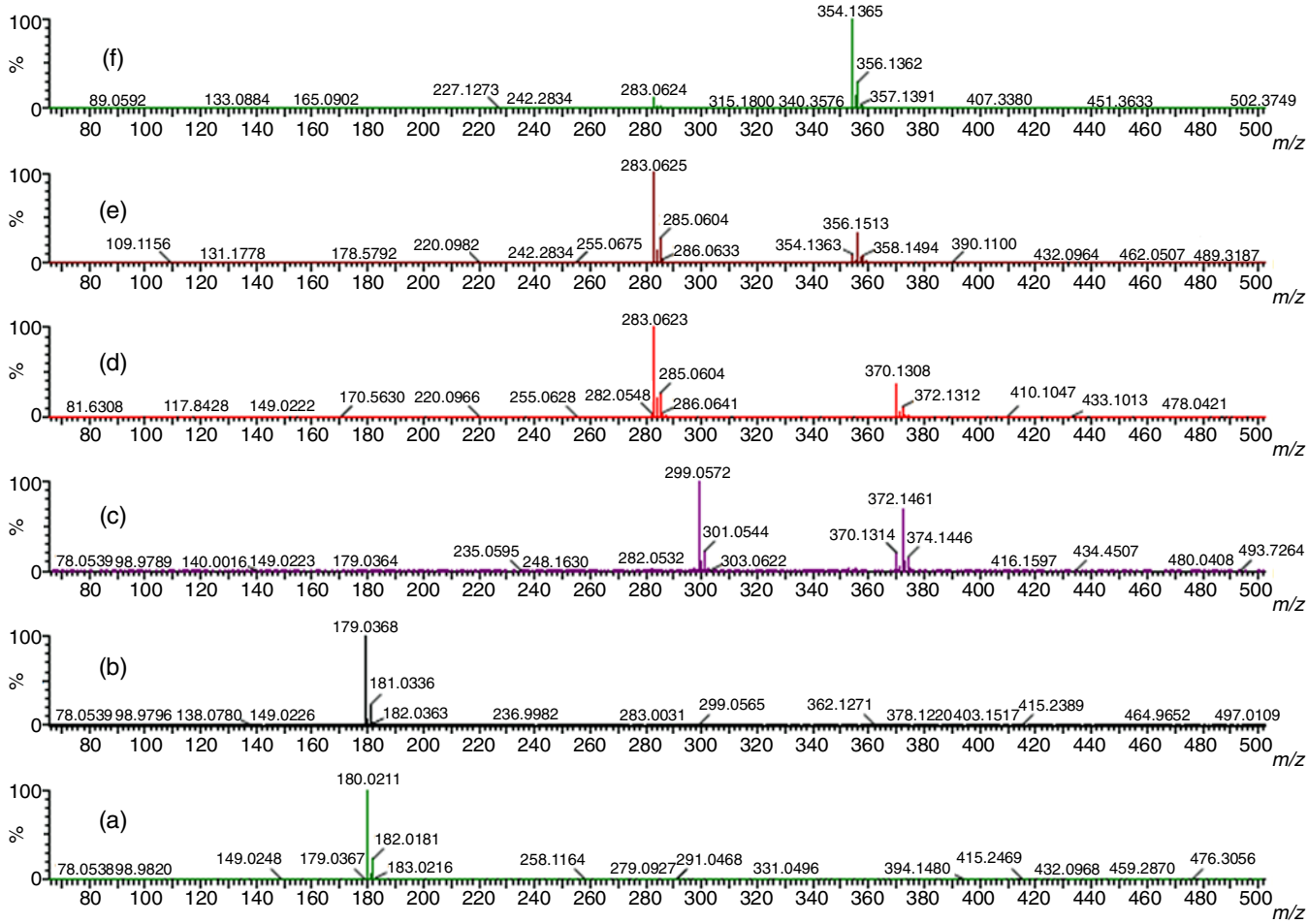
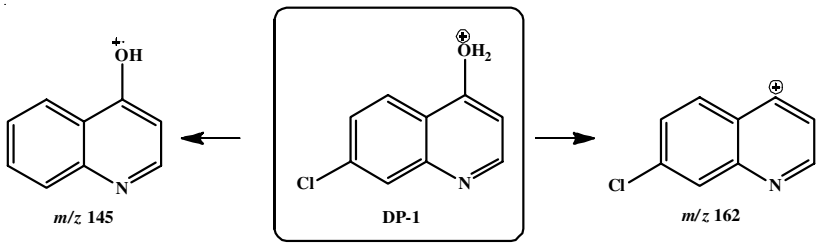


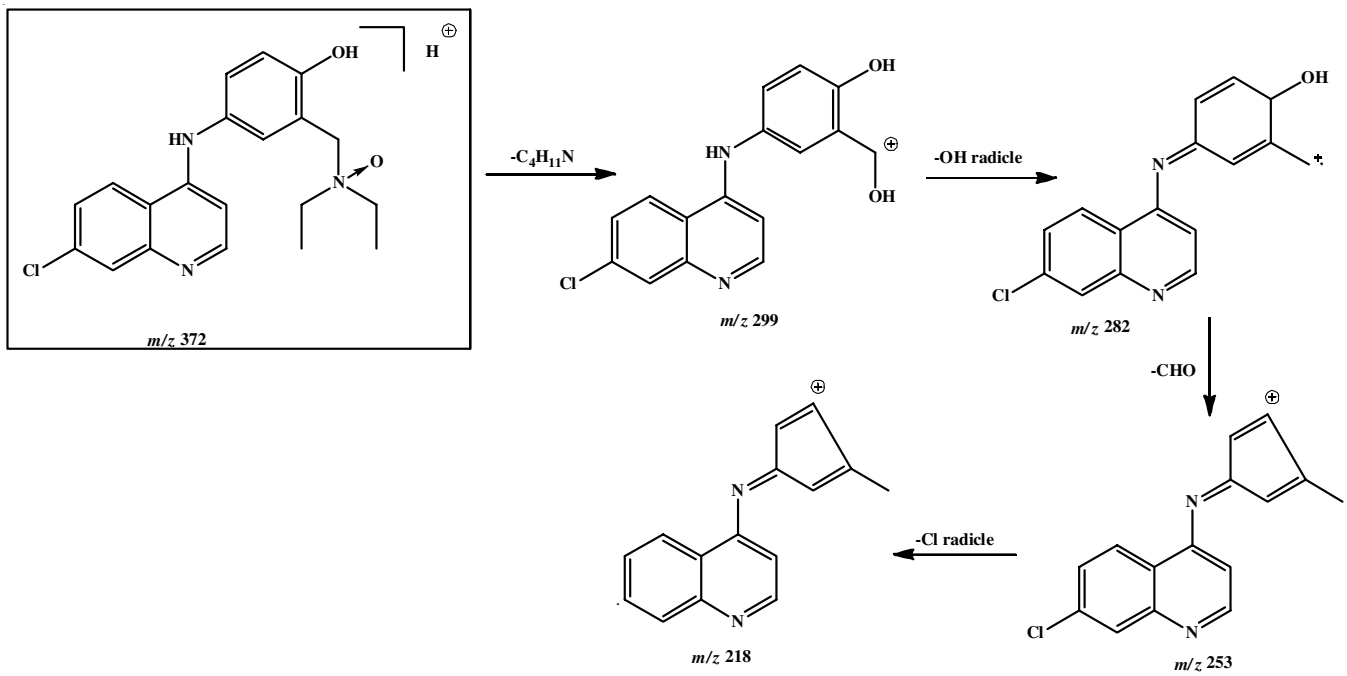
Fig. 7. LC Q-ToF MS spectra of peroxide degradation impurities (a) DP-1, (b) impurity-3, (c) DP-2, (d) DP-3, (e) AMD and (f) DP-4 in AMD

proposed structure of DP-2 (Fig. 8c). The DP-3 was obtained in oxidative stress conditions with *m/z* 372.14 $[M+H]^+$. The MS spectrum of DP-3 showed a *m/z* of 370, two mass units less than DP-2. The loss of two protons were also indicated by the elemental composition of DP-3. The fragmentation of DP-3 was similar to that of the DP-2 with characteristic ions at *m/z* 282, *m/z* 253 and *m/z* 218 (Fig. 8d).

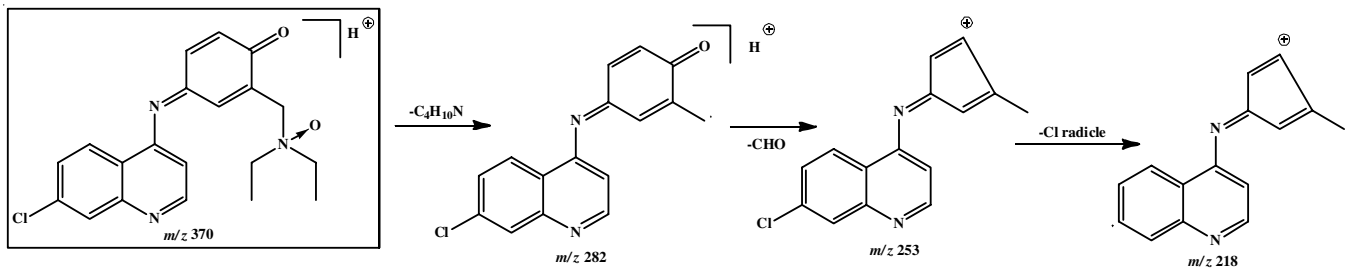
The degradation product DP-4 showed an *m/z* of 354, which corresponds to 2 mass units lower than that of the main drug AMD. Although the fragmentation of the degradation product DP-4 clearly showed similarity to that of DP-2 and DP-3, it was the accurate mass experiments which suggested the unsaturation of DP-4 in the phenolic ring system giving rise to a benzophenone moiety in the structure of DP-4 (Fig. 8f).



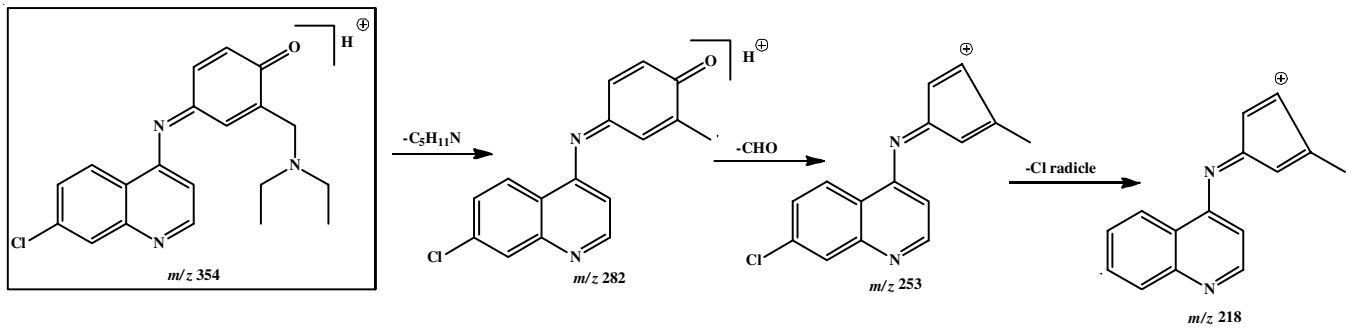
Proposed fragmentation pathway of DP-1



Proposed fragmentation pathway of DP-2



Proposed fragmentation pathway of DP-3



Proposed fragmentation pathway of DP-4

Scheme-I: Proposed LC-QTOF-MS/MS fragmentation pathway of AMD, DP-1, DP-2, DP-3 and DP-4

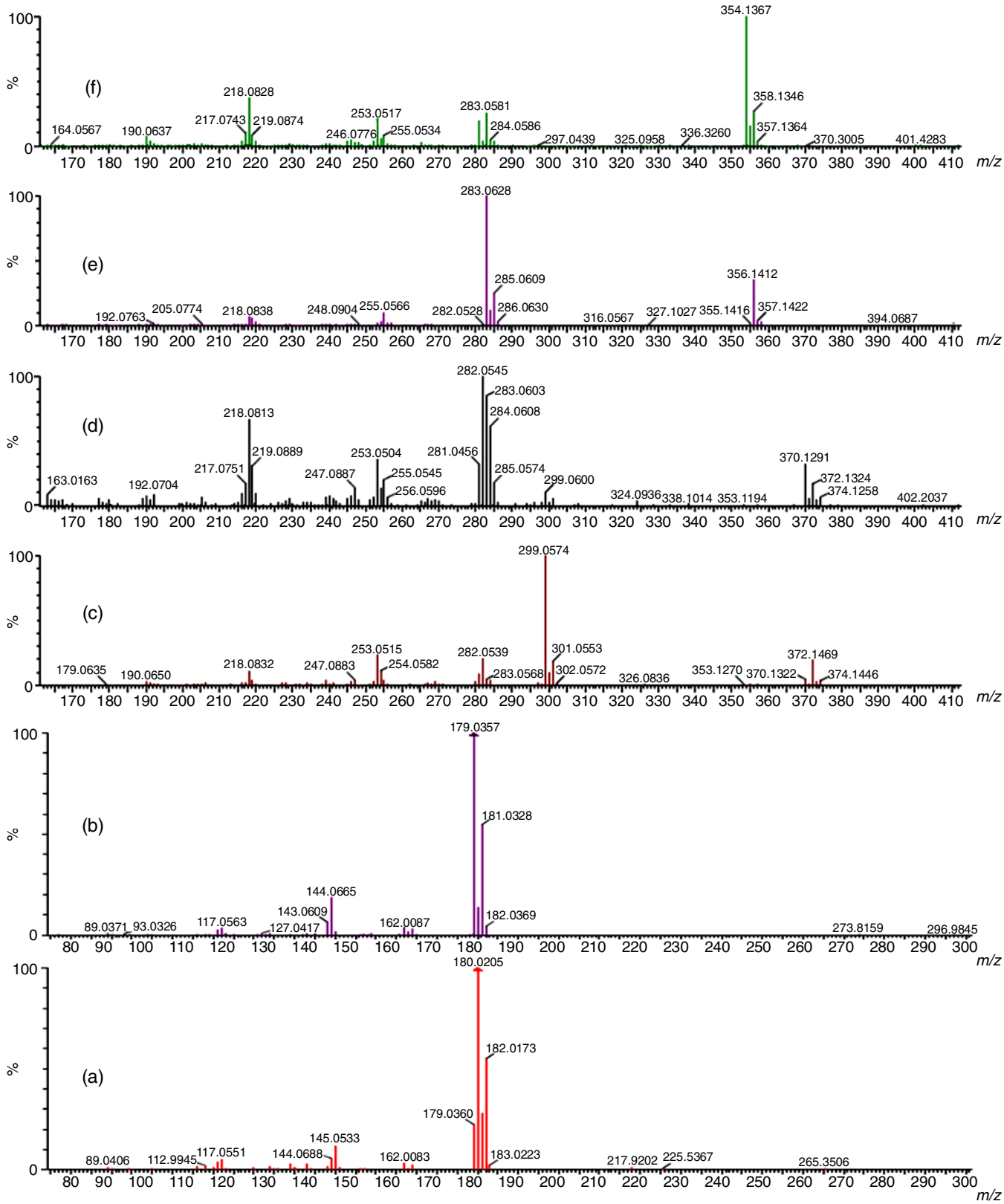


Fig. 8. LC Q-ToF MS/MS spectra of peroxide degradation products (a) DP-1, (b) impurity-3, (c) DP-2, (d) DP-3, (e) AMD and (f) DP-4

Procedure validation: Validation results of linearity, accuracy, precision, intermediate precision, LOD and LOQ studies are shown in Table-6. All the method validation results were found to be acceptable. This demonstrating that the analytical procedure can produce consistent results and always meet the ATP.

Continued procedure performance qualification: During the routine application, shifts in the procedure performance

TABLE-6
PROCEDURE PERFORMANCE QUALIFICATION: VALIDATION DATA OF THE ANALYTICAL PROCEDURE

Parameters	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Amodiaquine hydrochloride
Linearity						
Correlation coefficient	0.99972	0.99946	0.99984	0.99963	0.99951	0.99992
LOD and LOQ						
Detection limit (%)	0.003	0.010	0.002	0.006	0.005	0.002
Quantitation limit (%)	0.009	0.024	0.008	0.018	0.016	0.006
Accuracy (% recovery)[#]						
50% level	96.82	98.88	97.25	99.64	97.51	–
100% level	99.13	97.57	96.94	98.34	99.17	–
150% level	102.41	100.28	101.52	100.73	101.58	–
Intermediate precision						
100% level	100.25	98.57	98.04	99.01	100.54	–

[#]Mean % recovery for six determinations at 100% and three determinations for other levels.

are unavoidable. Therefore, the control strategies must be established to ensure that the procedure's performance always fulfills the ATP. The chromatographic column, which is essential for efficient performance of any analytical method and also decreasing in method's performance in sometimes.

Control charts were used to monitor data for the CMAs (primarily resolution) and the equipment parameter (column pressure) during the analysis time. Monitoring column pressure is important because increasing column pressure indicates decreased column performance that may show impact on separation. Control charts are used to track process performance, enabling analysts to see any unusual or out-of-trend performance in the analytical procedure and to take appropriate actions.

Conclusion

An analytical lifecycle management (ALM) framework and AQbD concept driven RP-UHPLC analytical procedure was developed for the determination of amodiaquine hydrochloride (AMD) and its related substances along with its degradation products within a 15 min run time. Analytical target profile (ATP) was clearly established and critical method attributes (CMAs) were selected in accordance with the procedure performance requirements. The critical method parameters (CMPs) were carefully evaluated and the method operable design region (MODR) was identified using the design of experiments (DoE) and the overlay plots. The success of this study demonstrates the applicability and advantages of ALM and AQbD for the development of analytical procedures. The validation as well as the verification processes were included into the analytical procedure lifecycle, instead of being considered as distinct entities, therefore making the entire method development process a much systematic and meticulous one. The method development process became more scientific and efficient as a result adopting AQbD. The proposed UHPLC method is capable of separating 5 major degradation products (DP-1, Imp-3, DP-2, DP-3 and DP-4) and 4 related substances (Imp-1, Imp-2, Imp-4 and Imp-5) of AMD. Forced degradation products had been identified and characterized using LC-ESI-QTOF tandem mass spectrometry. The mass compatibility of the method enables an easy transition between different detection methods, which facilitates the identification of potential degradation products in amodiaquine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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