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Simultaneous and Trace Level Determination of Six Potential Impurities by UPLC-ESI-MS/MS in Antiarrhythmic Drug: Dronedarone Hydrochloride

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Development and validation of six potential impurities by ultra performance liquid chromatography electro spray ionization tandem mass (UPLC-ESI-MS/MS) method for dronedarone hydrochloride drug was accomplished coherent with ICH guidelines. Successful chromatographic separation of dronedarone with its six impurities was attained by using gradient elution mode on RP-UPLC column using three pump mode system of 0.1 % formic acid in water as mobile phase A, methanol as the mobile phase B and solvent mixture of methanol, acetonitrile and water in the ratio of 65:30:5 v/v/v as the mobile phase C. Chromatographic conditions were set as 0.3 mL min^{-1} flow rate at the column temperature of 45 °C with the injection volume 2 µL. Briefly, the method enabled quantitation of six impurities with high accuracy (recovery > 90 %) and precision (% RSD < 5.0),within the ranges of 0.18-2.82 µg g^{-1}. The regression (r) for each impurity over a range was > 0.99. The detection limit and quantitation limit of impurities were set at 0.09 and 0.18 µg g^{-1}, respectively. The performed validation tests proved the suitability of the method for its intended purposes.

Keywords: Dronedarone hydrochloride, Six potential impurities, Trace level analysis, UPLC-MS/MS, Method development, Validation.

INTRODUCTION

The U.S. Food and Drug Administration approved dronedarone hydrochloride in 2009, which was marketed under the trade name Multaq® by Sanofi-Aventis [1,2]. This antiarrhythmic drug substance chemically recognized as N-{2-butyl-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl}methane sulfonamide hydrochloride [3] and its novel antiarrhythmic agent property with multi channel blocking and anti adrenergic properties is also reported [4]. The oral administration of this drug is categorized under BCS (biopharmaceutics classification system) class-II, which has low solubility and high permeability exhibiting food effect and widespread first-pass metabolism lead to a low unlimited bioavailability (4-15 %) [5,6].

The extent of impurities in the drug substances and drug products are based on the different aspects such as route of synthesis, conditions maintained in the reaction process, starting materials, reagents and solvents superiority, the steps employed in the purification of drug substance, excipients, manufacturing processes of formulated drugs, packaging and storage of the drug product. Owing maximum daily dose of these impurities, the acceptable levels typically administrated below 1.0 % and each individual impurity levels between 0.05-0.1 %. Conversely, they must be quantifiable individually. It is important for the regulatory bodies and pharmaceutical manufacturing organizations to expand the methods at trace quantitation levels, once they identified. All the six potential impurities of dronedarone hydrochloride are reviewed for the possibility of producing genotoxicity. Based on this perception, the expert software tools like QSARs (quantitative structure-activity relationships) and SARs (structure-activity relationships) are very much useful to evaluate genotoxicity for the impurities, in particular when inadequate knowledge of impurities existed. Moreover expert decision support system like Derek Nexus and statistical software tool like Sarah Nexus application systems are employed for prediction of toxicity,

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prediction of mutagenicity, respectively. These software applications are used for the confirmation of genotoxicity [7], for selected impurities according to regulatory submissions under the ICH M7 guidelines [8]. The alert has demonstrated from the predicted ICH M7 classification and these impurities are shown some structural alert based on nitro group presence and leads to resulting control actions against dronedarone drug moiety. According to ICH M7, impurities have to be controlled at low or below acceptable limit (TTC limit: not more than 1.875 μg g^{-1} based on maximum daily dose 800 mg of dronedarone hydrochloride). Due to process capability to remove these structural alert impurities well below the TTC level, a routine control for these impurities required.

A number of methods evaluated for dronedarone hydrochloride, related substances and impurities of dronedarone in the human plasma, dosage forms, characterization, degradation studies, stability studies, pharmacokinetics and some estimations have been noticed in the literature [9-17]. In that spectrometric technique like LCMS/MS and HPLC chromatography with UV detection were mostly used.

Ever since, no study was developed for these six potential impurities at low ppm level in a single method. Hence, this study was to develop and validate (according to ICH) [18] a simple, sensitive, robust and trace level UPLC ESI-MS/MS method for the concurrent analysis of six impurities in dronedarone hydrochloride drug substance.

EXPERIMENTAL

Dronedarone hydrochloride: 99.5 %, required analyte impurities (Imp-1: 98.0 %, Imp-2: 98.9 %, Imp-3:100.0 %, Imp-4: 99.3 %, Imp-5: 95.9 %, Imp-6: 98.7 %) and related substances (hydroxy impurity: 96.8 %, dronedarone hydroxyl methyl impurity: 99.2 %, dronedarone BIS methyl sulfonamide: 99.3 %, N-desbutyldronedarone: 99.1 %) were received as a gift from Aurobindo Pharma Research Centre-II (a division of AurobindoPharma Limited, Hyderabad, India). HPLC grade methanol: 99.1 % and acetonitrile: 99.3 % were obtained from Merck (Mumbai, India). Formic acid: 99.0 % was purchased from Sigma-Aldrich (St. Louis, MO, USA). High purity Milli-Q water was used with the help of Millipore Milli-Q plus purification system (Bedford, MA, USA).

An Acquity® ultra performance liquid chromatography system with a quaternary solvent manager and column holder (Waters, Milford, USA) was coupled with an Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, USA). Chromatographic separation was achieved using an Acquity® UPLC HSS T3, 1.8 μm (100 mm × 2.1 mm) (Waters, Milford, USA). All data manipulation was carried out using the Masslynx V4.1 software (Waters, Milford, USA).

In the synthetic process development of dronedarone hydrochloride, one of the key starting raw material was 2-butyl-3-(4-hydroxy benzoyl)-5-nitro benzofuran. It was processed by the starting material 5-nitro-2-butyl benzofuran (Imp-3) through the intermediate (2-butyl-5-nitro benzo furan-3-yl)-(4-methoxy phenyl)methanone (Imp-6). In this process, lower [2-propyl-3-(4-hydroxy benzoyl)-5-nitro benzo furan (Imp-2)], higher [2-pentyl-3-(4-hydroxy benzoyl)-5-nitro benzo furan (Imp-4)] analogs of 2-butyl-3-(4-hydroxy benzoyl)-5-nitro benzofuran and 2-butyl-3-(3-chloro-4-hydroxybenzoyl)-5-nitrobenzofuran (Imp-5) also can be formed. 5-Nitro-2-butyl benzofuran (Imp-3) was synthesized from the starting material 2-hydroxy-5-nitro benzyl bromide through the intermediate its derivative of phosphonium bromide (Imp-1). The schematic representation of these impurities formation is summarized in Fig. 1.

Preparation of impurity stock, standard and sample solutions: Primary impurity stock solution (0.49 mg mL^{-1}) was prepared individually by sonicate to dissolving each impurity reference sample in methanol. Intermediate impurity stock solution (0.0098 mg mL^{-1}) was prepared by dissolving primary impurity stock each solution 1.0 mL in to 50 mL water-methanol (20:80, v/v). Intermediate impurity stock solution further diluted to (47.04 μg g^{-1}) dissolving 1.2 mL in to 50 mL water-methanol (20:80, v/v). Further standard final solution (1.88 μg g^{-1}) was prepared by dissolving intermediate impurity stock solution (47.04 μg g^{-1}) 2.0 mL in to 50 mL water-methanol (20:80, v/v). Test sample of dronedarone hydrochloride drug substance was prepared by diluting the 5 mg mL^{-1} in water-methanol (20:80, v/v). The standard solutions and test samples were optimized to achieve a preferred signal-to-noise ratio (S/N) and desired peak shapes.

Chromatographic and mass spectrometric conditions: The mobile phases were composed of ultra pure water plus 0.1 % formic acid (mobile phase A), methanol (mobile phase B) and solvent mixture of methanol, acetonitrile and water in ratio 65:30:5 v/v/v as (mobile phase C).

The flow rate was 0.3 mL min^{-1} and the mobile phase (MOP) gradients [time (min)/%MOP-B/%MOP-C: 0.01/30/20, 3.0/30/20, 3.5/0/70, 10.0/0/70, 11.0/70/0, 20.0/70/0, 21.0/30/20, 25.0/30/20] were progressively modified over a run time of 25 min. Sample injection volume of 2 μL and the column temperature was set at 45 °C. The tandem mass spectrometer was operated in positive mode electron spray ionization (ESI) with multiple reaction monitoring acquisition parameters are shown in Table-1. The source and desolvation temperature were set at 150 and 500 °C, respectively. Moreover capillary and cone voltages were fixed at 3.8 kv and 25 v, respectively. Nitrogen was used as a desolvation gas and flow was set at 1000 L/h. Dwell time for each transition was 0.064 s. The competent of dronedarone and six impurities analysis time was set as the

TABLE-1
MULTIPLE REACTION MONITORING (MRM) ACQUISITION PARAMETERS

Compd.	Parent (*m/z*)	Daughter (*m/z*)	Cone (v)	Collision (v)	Comments
Imp-1	414.2 $[M]^+$	183.1	32	54	Quantifier
Imp-1	414.2 $[M]^+$	262.8	32	32	Qualifier
Imp-2	326.2	121.1	8	20	Quantifier
Imp-2	326.2	232.1	8	18	Qualifier
Imp-3	220.1	131.1	18	28	Quantifier
Imp-3	220.1	174.2	18	10	Qualifier
Imp-4	354.2	121.1	32	22	Quantifier
Imp-4	354.2	260.1	32	18	Qualifier
Imp-5	374.2	155.1	10	24	Quantifier
Imp-5	374.2	246.1	10	18	Qualifier
Imp-6	354.2	135.1	56	22	Quantifier
Imp-6	354.2	246.2	56	18	Qualifier

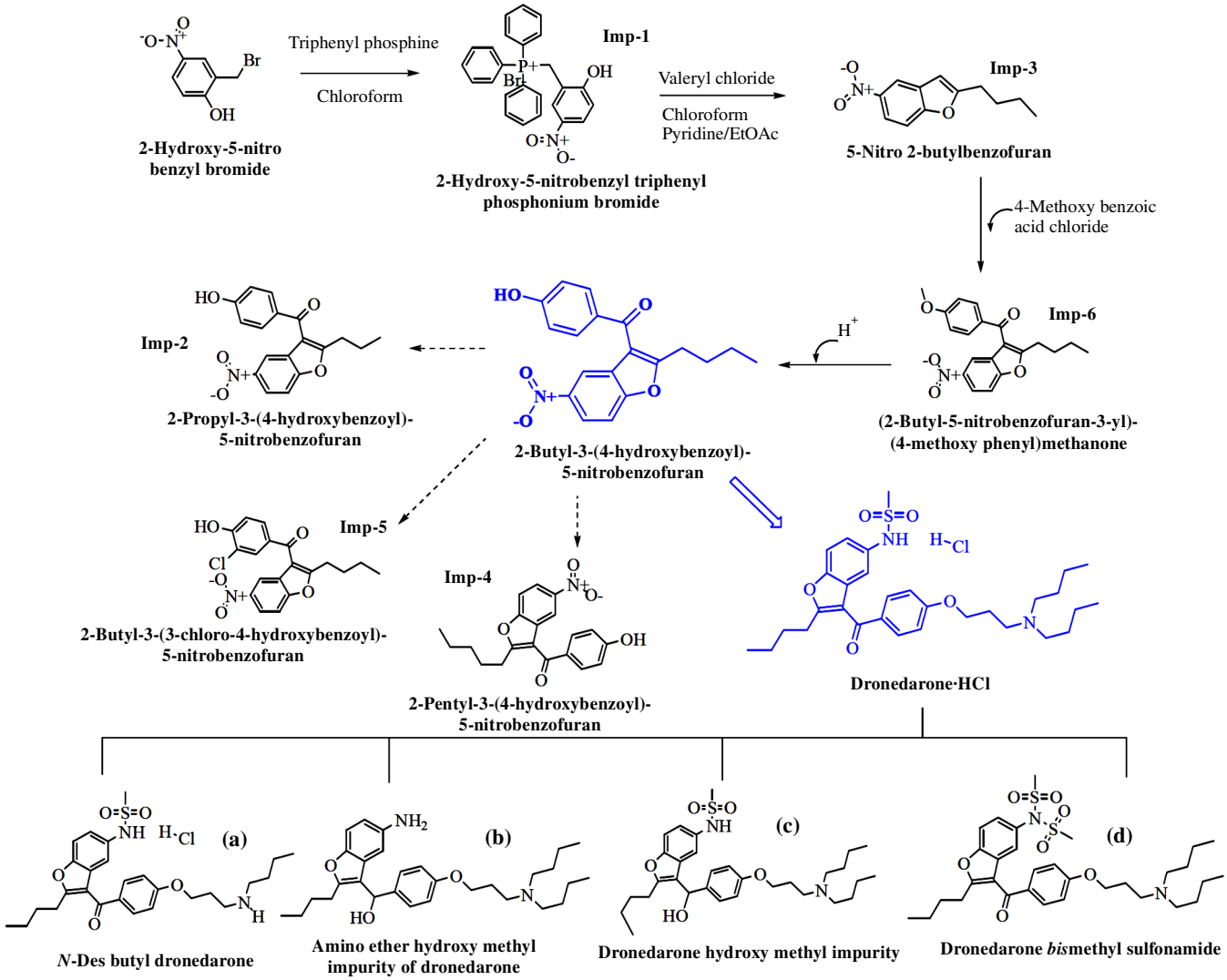


Fig. 1. Schematic representation of possibility impurities formation at different stages in Dronedarone HCl synthesis and (a), (b), (c), (d) related substances of dronedarone HCl

defined analytical target profile, sample matrix and for this perspective operation of a switching valve allowed the flow to be sent to waste (0.01 min-1.3 min and again 4.0 min-8.0 min of time for standard and test sample solution), when test sample peak and its sample matrix was eluted.

Optimization of mass spectrometer conditions: All six impurities stock solutions was prepared individually in methanol and water solvents in ratio (90:10 v/v) and diluted to get final concentration of about 2.0 µg g^{-1}. In the proposed method, we performed positive ESI mode, which has been revealed to be more susceptible to analytical problems such as, elevated baseline, matrix effects than APCI. However, we did not find any of these difficulties during present method validation process and also not found any significant matrix effect for this method.

Selection of UPLC column: The big challenge of the method at trace level determination of all these impurities is separation of impurities with drug substance, related substances and their responses in chromatographic system. Optimization for separation and response, several attempts were made with different columns *viz.*, X-terra ms C18 column (150 mm × 4.6 mm, 5.0 µm) and acquity UPLC BEH C18 column (100 mm, × 2.1 mm and 1.7 µm) using isocratic and gradient elution, but in all the above conditions the separation of related substances of dronedarone with analyte impurities was not satisfactory. Because X-terra ms, BEH C18 columns were found, not to suitable as the response of analyte and related substance peaks were not resolved among themselves and from drug substance peak. But, on acquity UPLC column HSS T3 column (100 mm long, 2.1 mm internal diameter, 1.8 µm diameter), the related substances were well resolved from the analytes and responses of analyte peaks were also found satisfactory.

Optimization of chromatographic conditions: The main difficulty was to obtain sufficient selectivity and sensitivity of impurities due to the similar chemical structures of dronedarone hydrochloride and its related substances. Several attempts were made with different reverse phase columns (X-terra ms C18 column-150 mm × 4.6 mm, 5.0 µm and Acquity UPLC BEH C18 column -100 mm, × 2.1 mm and 1.7 µm) using isocratic and gradient elution. But in all above conditions the separation of the impurities was not satisfactory. Because, X -terra ms C18 column and acquity UPLC BEH C18 column were not found suitable as the response of analytes were found less and impurity peaks were not well resolved among themselves and from the dronedarone peak.

But, on acquity UPLC HSS T3 column (100 mm long, 2.1 mm internal diameter, 1.8 µm diameter) the separation and response of six analyte peaks were found good. Development started with isocratic flow rate 0.3 mL min^{-1} [0.1 % formic acid in water (MOP-A), methanol (MOP-B), acetonitrile (MOP-C) (35:20:45 v/v/v)] and injection volume 5.0 µL. Column oven temperature was 45 °C. Impurity standard solution was 1.88 µg g^{-1} (test sample concentration 5.0 mg mL^{-1}) injected to optimized tuning UPLC-ESI-MS/MS system. The response of Imp-3 peak was very low and all peaks were not much resolved with the drug substance at this chromatographic system. To overcome this, ratio of mobile phase was changed to gradient elution using three pump system [time (min)/% MOP-B/MOP-C) 0.01/0/65, 10.0/10/55, 12.0/10/70, 20/10/70, 21/0/65, 25/0/65] with flow rate 0.3 mL min^{-1} and column oven temperature was 35 °C. Separation of impurities with drug substance still was not observed. With the same above conditions gradient program further optimized to [time (min)/% MOP-B/MOP-C] 0.01/10/55, 2.0/10/55, 2.5/0/65, 10.0/0/65, 11.0/15/65, 20.0/15/65, 21.0/10/55, 25.0/10/55. In this optimized conditions peak shape split of Imp-1 analyte was observed. Solvent mixture of methanol, acetonitrile and water in ratio-65:30:5 v/v/v as (mobile phase C) was used for better separation and peak shapes of analytes. After few attempts of gradient optimization, finalized gradient program was set [time (min)/ % MOP-B/%MOP-C: 0.01/30/20, 3.0/30/20, 3.5/0/70, 10.0/0/70, 11.0/70/0, 20.0/70/0, 21.0/30/20, 25.0/30/20]. Temperature was fixed at 45 °C with the injection volume 2.0 µL. In these conditions all analyte peaks were well separated with symmetrical peak shapes, no interference found at the retention time of analytes in drug substance.

RESULTS AND DISCUSSION

Mass analysis: Each solution was used to generate multiple reaction monitoring (MRM) transition, through the automated MRM method development software (intellistart) of MassLynx. Six impurities were run in Q1 scan, positive and negative modes and ionized intensely in positive mode. In MRM transitions scan mode the parent and daughter fragments were as follows: Imp-1, 414.2 $[M+H]^+$ and 108.0, 183.1, 262.7. Imp-2, 326.1 $[M+H]^+$ and 121.1, 171.0, 232.1. Imp-3, 220.1 $[M+H]^+$ and 131.0, 132.0, 174.1. Imp-4, 354.2 $[M+H]^+$ and 121.1, 176.1, 260.1. Imp-5, 374.1 $[M+H]^+$ and 155.0, 204.1, 246.1. Imp-6, 354.2 $[M+H]^+$ and 135.0, 204.1, 246.1. Stable, intense fragments of these impurities were used for quantitation and a product ion mass spectrum of six impurities is shown in Fig. 2. The ion source parameters were optimized to get proper impurities optimum response.

Most methods of literature use only one mass transition to detect the compound. In this method, we used a second mass transition for the impurity. This allows ion ratio monitoring which adds to method selectivity enabling detection of potential interferences [19]. With this method we found that the ion ratios were consistent across a range of concentrations, with the relative standard deviations (RSDs) generally < 10 %. Isotopic analogs of six impurities are preferred as internal standards for quantitative analysis. Due to unavailability isotopic analogs of each impurity and higher economical values of these isotopes, the use of ion ratio for the appropriate transitions of the impurities is essential for a reliable and precise LC-MS/MS method. The ratio of qualifier ions relative to the quantifier ions (calculated by dividing the lower by the higher response)

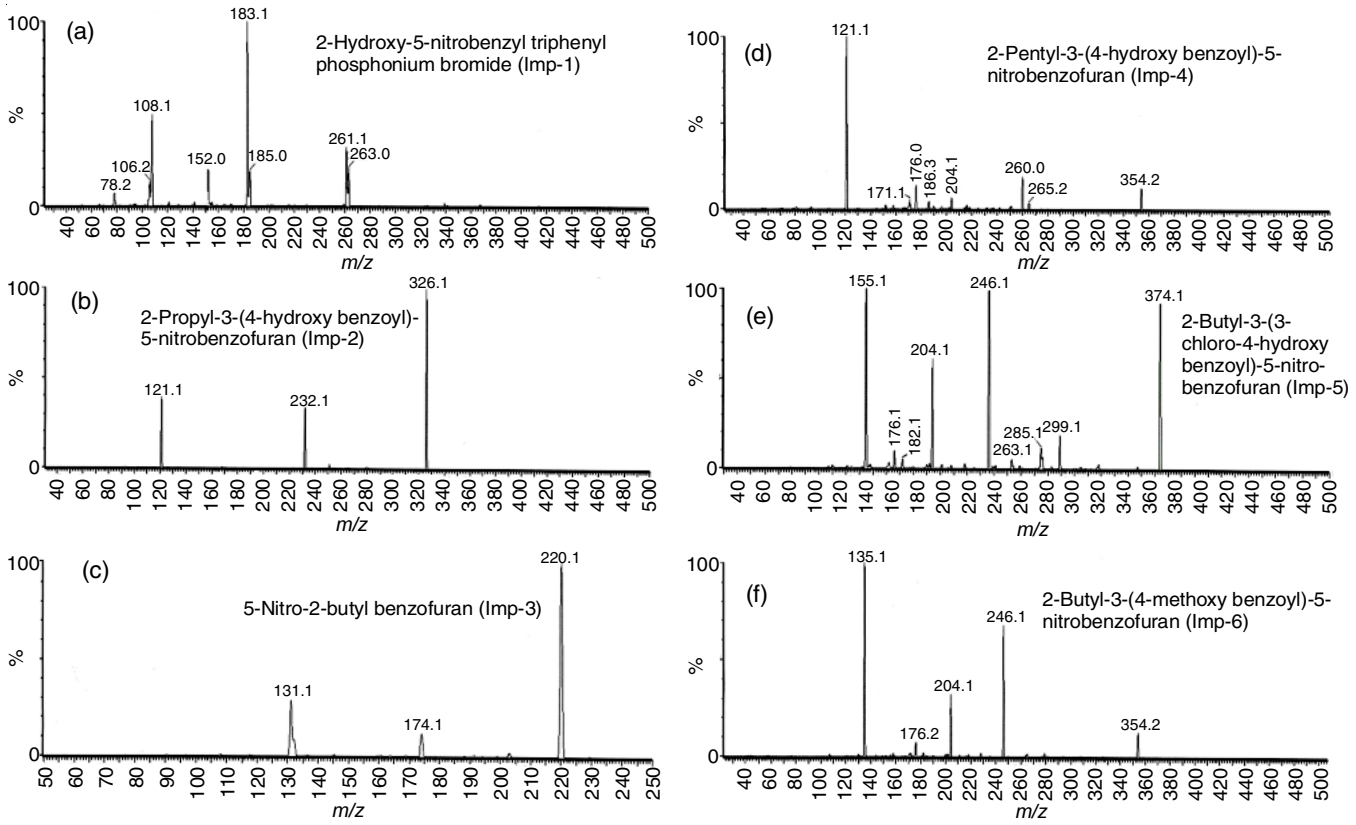


Fig. 2. Product ion mass spectra of six impurities

was used for analyte confirmation. The ratio tolerance was set at ± 20 %.

Method validation

Linearity: Peak area response for the six potential impurities was strictly linear in the concentration range between 0.188 and 2.82 μg g^{-1}. Measured concentrations of six impurities were compared to expected concentrations. Linearity of impurities was confirmed by linear regression analysis. The regression (r) for impurities was > 0.999. The results revealed an excellent correlation between the peak area and analyte concentration. The values of slope, intercept and regression (r) are shown in Fig. 3.

Selectivity*:* An optimized method was developed to separate six potential impurities from dronedarone hydrochloride and its related substances. The method was determined by analyzing the drug substance, six impurities and related substances of dronedarone solutions. The solutions of dronedarone hydrochloride test sample, dronedarone hydrochloride spiked with related substances without six impurities standard solution at specification level and test sample spiked related substances with six impurities standard solution at specification level prepared and injected for analysis. The corresponding chromatograms are shown in Fig. 4. The chromatograms clearly depicts that the gradient method was highly specific in separating the six impurities from drug substance and process related substances.

Limit of detection (LOD) and limit of quantification (LOQ): Determination of limit of detection and limit of quantification based on the response of impurities and showing precision at that level by injecting the solution of impurities in six replicates (n = 6) in to the UPLC-MS/MS system. The s/n ratio of the detection and quantification levels for each impurity was > 3.0 and > 10.0, respectively. The evaluated values of LOD and LOQ were found for each impurity 0.09 and 0.18 μg g^{-1}, respectively. The precision at the LOQ concentration for all impurities was below 4.0 %. It is noted that the LOD values for each impurity was below the required concentration limit (1.88 μg g^{-1}) for dronedarone hydrochloride (Table-2).

Precision: The precision of potential impurities was checked by injecting six individual preparations of dronedarone HCl (5 mg mL^{-1}) spiked with desired concentration (*i.e.* 1.88 μg g^{-1}) of six analytes with respect to dronedarone hydrochloride drug substance concentration. The % relative standard deviation (% RSD) of area for each impurity was calculated. The intermediate precision (reproducibility) of the method was also evaluated by using different analyst, different column and different instrument in the same laboratory conditions. To study the method precision, six replicate mixed sample solutions of six analytes and dronedarone HCl were injected. The % RSD of six impurities was calculated. Results of method precision and intermediate precision studies are summarized in Table-3.

Accuracy: The accuracy study of the impurities was carried out by standard spiking method. A known amount of standard

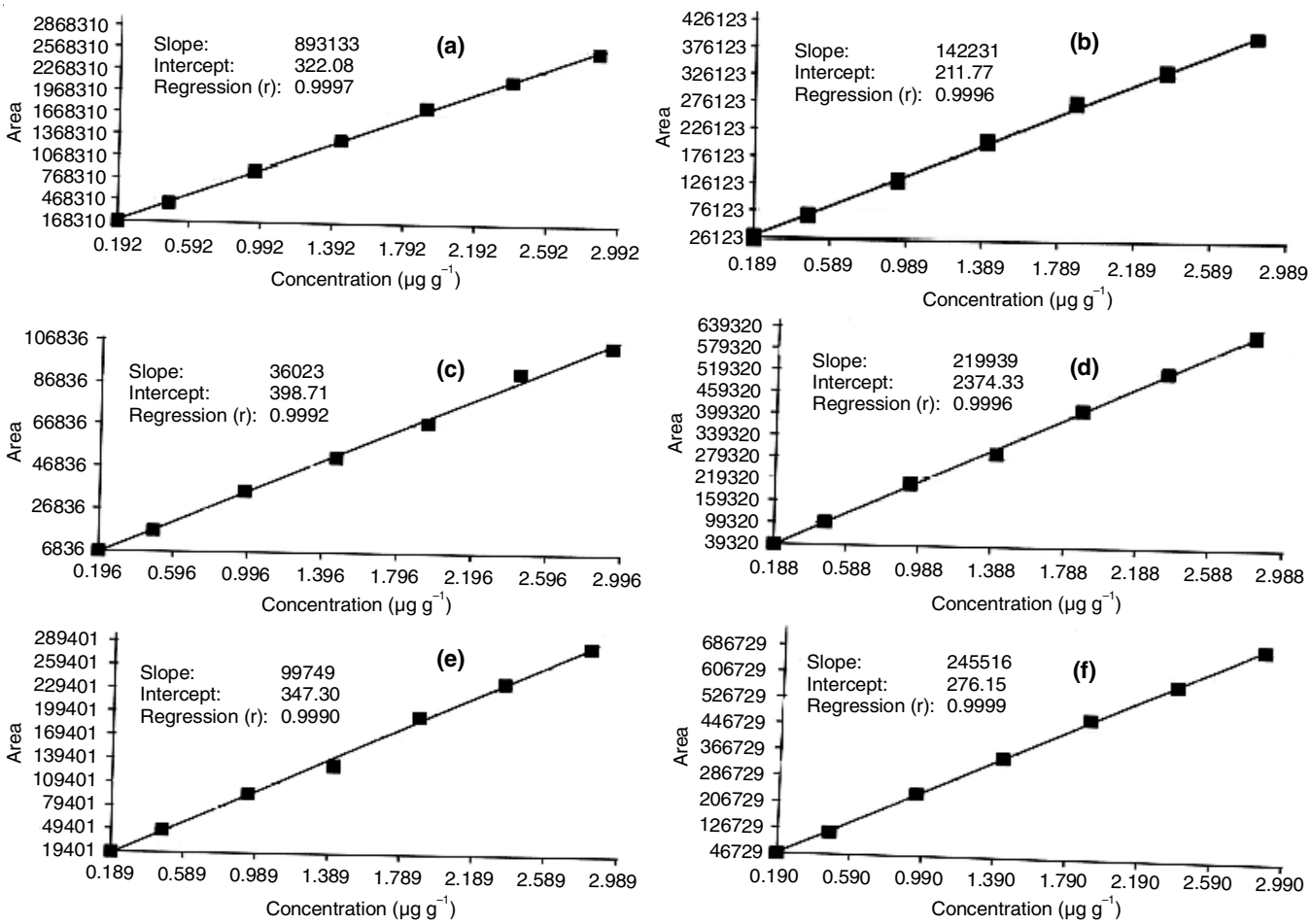
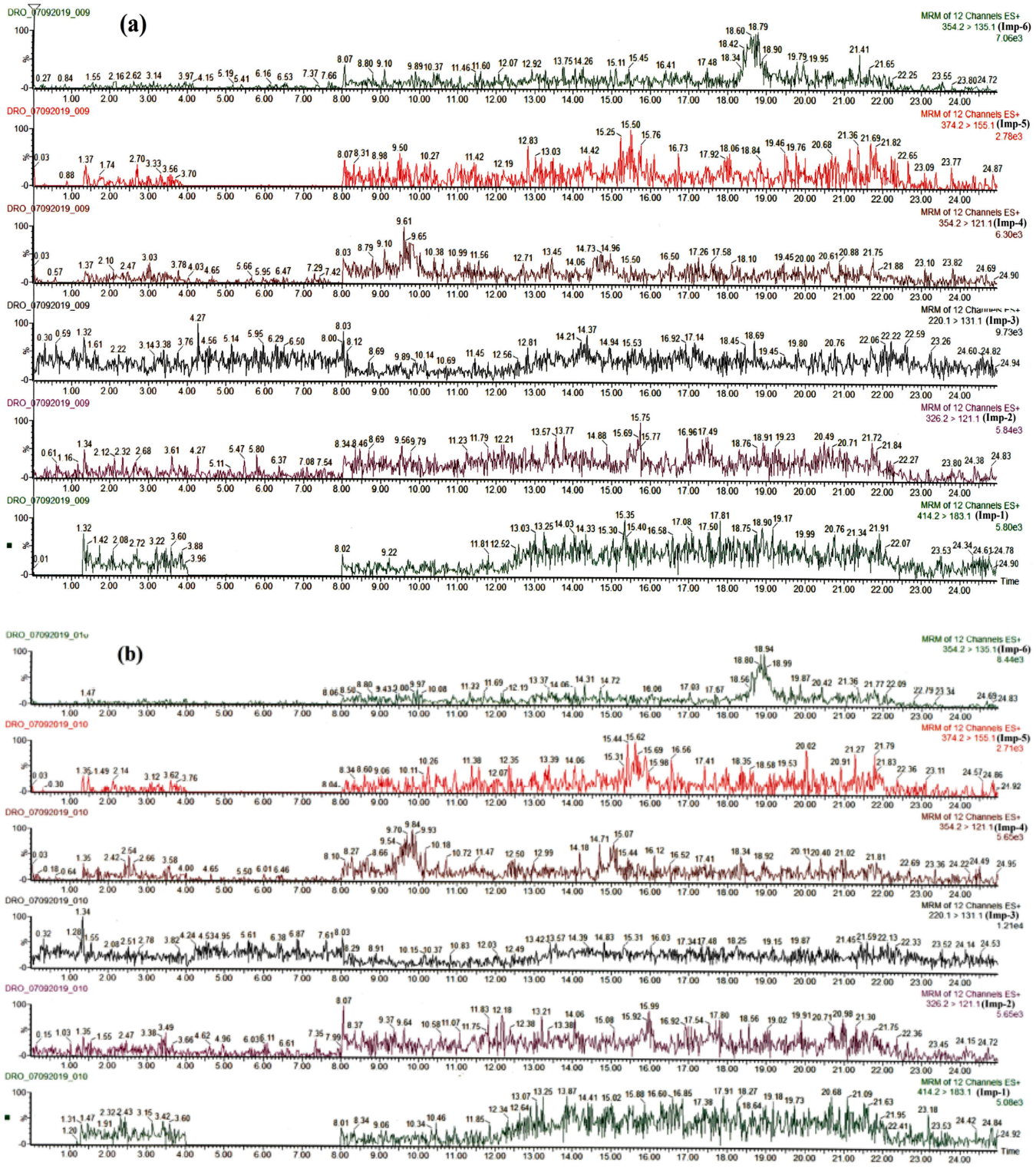


Fig. 3. Linearity plot of (a) Imp-1, (b) Imp-2, (c) Imp-3, (d) Imp-4, (e) Imp-5 and (f) Imp-6

TABLE-2
EVALUATION OF SYSTEM PRECISION, LOD PRECISION AND LOQ PRECISION FOR SIX IMPURITIES

Impurity	Precision experiment*			
	Intra-day (SD ± % RSD)	Inter-day (SD ± % RSD)	LOD (SD ± % RSD)	LOQ (SD ± % RSD)
Imp-1	27621.98 ± 1.6	46882.03 ± 2.6	1078.27 ± 1.3	1963.53 ± 1.2
Imp-2	4580.31 ± 1.8	3765.41 ± 1.4	227.89 ± 1.7	677.08 ± 2.7
Imp-3	3606.45 ± 4.2	3385.51 ± 3.8	130.16 ± 3.7	263.35 ± 3.9
Imp-4	2683.76 ± 0.7	5454.76 ± 1.3	357.62 ± 1.8	879.19 ± 2.3
Imp-5	3416.74 ± 1.8	3073.90 ± 1.6	192.48 ± 2.0	489.44 ± 2.7
Imp-6	3182.99 ± 0.7	7362.60 ± 1.5	587.42 ± 2.5	499.74 ± 1.1

*Precision (n = 6) at concentration 1.88 μg g^{-1} for intra, inter-day and LOD (0.09 μg g^{-1}); LOQ (0.18 μg g^{-1}).



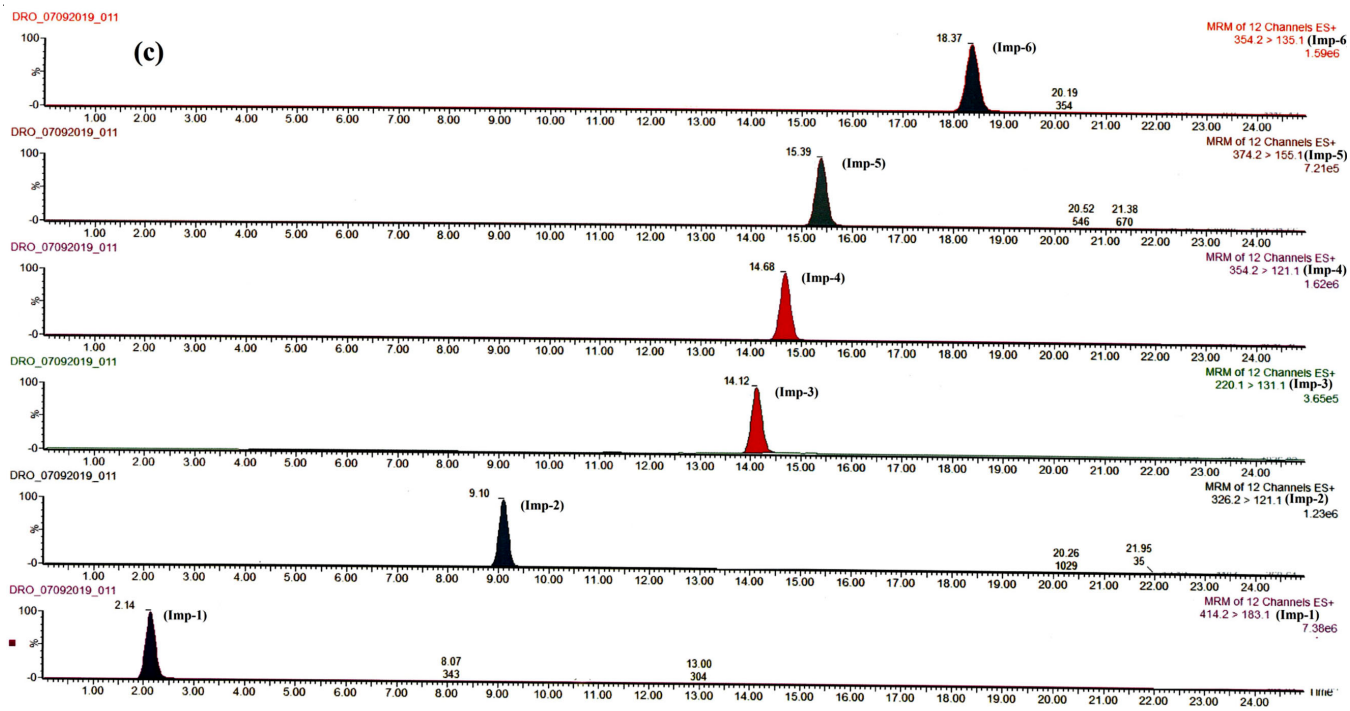


Fig. 4. (a) Test sample chromatogram of dronedarone hydrochloride drug substance, (b) test sample spiked with related substances without six impurities and (c) Test sample spiked related substances with six impurities

TABLE-3
EVALUATION OF ACCURACY, METHOD PRECISION AND INTERMEDIATE PRECISION FOR SIX IMPURITIES USING THE PROPOSED METHOD

Impurity	Concentration (μg g^{-1})	% Recovery* ± SD	Method precision % Recovery** ± SD	Intermediate precision % Recovery** ± SD
Imp-1	0.188	97.9 ± 2.06	N A	N A
	1.884	98.7 ± 0.85	103.2 ± 0.42	100.5 ± 0.90
	2.831	100.1 ± 0.46	NA	NA
Imp-2	0.188	103.0 ± 2.62	NA	NA
	1.884	99.6 ± 0.50	102.8 ± 1.09	101.3 ± 0.89
	2.831	97.8 ± 1.07	NA	NA
Imp-3	0.188	95.3 ± 4.46	NA	NA
	1.884	101.7 ± 4.15	101.5 ± 4.01	96.7 ± 6.52
	2.831	98.1 ± 3.41	NA	NA
Imp-4	0.188	100.8 ± 3.59	NA	NA
	1.884	98.7 ± 0.75	102.4 ± 1.20	99.9 ± 0.99
	2.831	95.9 ± 1.14	NA	NA
Imp-5	0.188	99.1 ± 1.46	NA	NA
	1.884	98.9 ± 0.10	103.4 ± 1.14	99.5 ± 1.74
	2.831	93.1 ± 0.61	NA	NA
Imp-6	0.188	99.6 ± 0.31	NA	NA
	1.884	99.9 ± 1.18	103.5 ± 0.72	100.4 ± 0.63
	2.831	98.9 ± 0.40	NA	NA

*Mean value of three determinations; **Mean value of six determinations; NA: not applicable

solution was added to the permanent amount of pre-analyzed sample solution. Standard spiking method was executed at two plus one concentration levels of LOQ, 100 and 150 %. Recovery experiments for impurities results depicted in Table-3. The percentage recoveries of six potential analytes in the drug substance for LOQ, 100 and 150 % levels ranged from 92.0 to 105.8, 97.1 to 105.1 and from 92.4 to 101.9, respectively. The recoveries at all the three concentrations were satisfactory for all analytes (Imp-1 to Imp-6). Therefore method has been used successfully for the determination of five different batches of dronedarone HCl API (Active Pharma Ingredient) samples, but the concentration of each analyte impurity was found below the detection level shown in Table-4, also there was no interference of sample matrix and related substances of drug substance.

Robustness: Robustness of the method was studied with deliberate modifications in the flow rate of the mobile phase, system suitability at 1.88 μg g^{-1} concentration level before, after source cone cleaning and column temperature. The flow rate of the mobile phase was 0.3 mL min^{-1} and the same was altered by ±10 % of its flow, *i.e.*, from 0.27 to 0.33 mL min^{-1}. The source cleaning before and after the effect on system suitability, no significant impact on areas of each analyte and the % RSD

TABLE-4
ANALYSIS OF FIVE DRUG SUBSTANCE BATCHES IN DRONEDARONE·HCL FOR THE IDENTIFICATION OF SIX POTENTIAL IMPURITIES

ID	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6
Sample-1	ND	ND	ND	ND	ND	ND
Sample-2	ND	ND	ND	ND	ND	ND
Sample-3	ND	ND	ND	ND	ND	ND
Sample-4	ND	ND	ND	ND	ND	ND
Sample-5	ND	ND	ND	ND	ND	ND

ND = Not detected

value of each analyte obtained in standard precision experiment was below 5.0. The effect of column temperature was studied at 44.1 and 45.9 °C instead of 45 °C, while other mobile phase components were kept constant. By all above studies: no significant changes in the chromatographic system, which indicated that method was robust in the specified range.

Solution stability: Stability in solution was evaluated by the standard solution and test sample preparation. The standard solution and test sample solution, spiked with all six impurities at specification level (1.88 μg g^{-1}) were prepared as per test method and analyzed at least 4 h by keeping the solutions at 25 ± 2 °C. The responses for the aged solutions (after 48 h) were evaluated by comparison with freshly prepared solutions. The results were statistically identical with the initial value without measurable loss and moreover % difference between the standard and spiked sample areas of each impurity was below the 10.

Conclusion

The desired goal of this study is to develop highly sensitive, trace level, more accurate and simultaneous determination of analytical method using LC-MS/MS for the quantitation of six analytes in dronederone hydrochloride drug substance. The method was fully validated as per ICH guide lines. Limit of quantitation, low detection levels at ppm and linear range make this LC-MS/MS method suitable for determination of six potential impurities in dronedarone hydrochloride levels in a high throughput laboratory, providing a good laboratory practices (GLP) service with pharmaceutically timely results.

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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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