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A High Sensitive LC-MS/MS Method for the Simultaneous Determination of Potential Genotoxic Impurities Carboxy Phenyl Boronic Acid and Methyl Phenyl Boronic Acid in Lumacaftor

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Abstract

A simple, rapid, and highly sensitive LC-MS/MS method has been developed for the simultaneous and trace level quantification of underivatized boronic acids in lumacaftor active pharmaceutical ingredient. Chromatographic separation of boronic acids and lumacaftor achieved using Agilent Poroshell HPH C18 150 × 4.6 mm 2.7 µ column with 0.1% ammonia in water as mobile phase A and 100% acetonitrile as mobile phase B at a flow rate of 0.25 ml/min. Gradient elution was used with a total method run time of 14 minutes. Boronic acids were successfully ionized and quantified without derivatization using electrospray ionization in negative mode using tandem quadrupole mass spectrometry in multiple reactions monitoring mode. Method validation was performed as per ICH guidelines with good linearity over the concentration range of 0.05 ppm to 5 ppm of Lumacaftor test concentration for both the boronic acids with a correlation coefficient of >0.99. Recoveries were found good at different concentration levels and within the range of 80% - 120%. The developed method can be successfully used for the routine quantification of boronic acids at a concentration level of 20 ng/ml (1 ppm with respect to 20 mg/ml lumacaftor).

Keywords

Boronic Acid, Genotoxic, LC-MS/MS, Lumacaftor

1. Introduction

Boronic acids are used as chemical building blocks during the synthesis of organic compounds and are the most common intermediates used in the preparation of biaryl derivatives using Suzuki-Miyaura coupling [1] [2] [3]. Studies have been conducted to evaluate the mutagenic toxicity of commercially available boronic acids resulted in positive Ames test [3]. Boron containing compounds are potential mutagenic impurities and often controlled in drug substances as per International council of Harmonization (ICH) M7 [4] guidelines using highly sensitive analytical techniques like LC-MS/MS for quantification.

Lumacaftor is a pharmaceutical drug used in combination with Ivacaftor sold under the name of ORKAMBI approved by FDA for the treatment of genetic disease cystic fibrosis (CF) [5]. Cystic fibrosis results due to the defects in the cystic fibrosis transmembrane conductance regulator (CFTR) causes dysregulation of epithelial fluid transport in the lungs, gastrointestinal tract, and sweat glands and can cause progressive multi organ failure [6] [7] [8].

The aim of the current research work is to quantify mutagenic boronic acid impurities in Lumacaftor drug substance. During literature search we could find methods published for determination of boronic acids using SIM ionization mode in single quadrupole LCMS [9] [10], however in the present article we have analyzed boronic acids which using highly sensitive and selective triple quadrupole LC-MS/MS in Multiple reaction monitoring (MRM) mode. We could also find article published for derivatized boronic acids using LC-MS/MS [11] however we could analyze underivatized boronic acids in the present work using highly sensitive LC-MS/MS which can reduce the sample preparation time and efforts of derivatization and increase the throughput during analysis of multiple samples of lumacaftor drug substance. Several LCMSMS methods have published for the determination of Lumacaftor in combination with Ivacaftor in biological fluids [12] [13]. Chromatographic methods published for the determination of Lumacaftor in its bulk dosage form [14] [15]. As per the extensive literature survey performed, there is no data published for the quantification of boronic acids in lumacaftor using LC-MS/MS till date.

Both the possible boronic acids were procured based on the results obtained from computational structure analysis for mutagenicity alerts. The concentrations of boronic acid impurities in lumacaftor must be controlled at concentrations lower than 1 ppm considering the maximum allowable dosage. In this paper, we present the LC-MS/MS method development for the simultaneous determination of carboxy phenyl boronic acid and methyl phenyl boronic acid in lumacaftor. The chemical structures of Carboxy phenyl boronic acid, Methyl phenyl boronic acid and Lumacaftor along with molecular formula and mono isotopic mass considering the most abundant isotope of boron are captured in **Figure 1**. The validation of the method in terms of limit of detection, limit of quantification, repeatability, accuracy, robustness, linearity and specificity is in accordance with ICH guidelines [16].

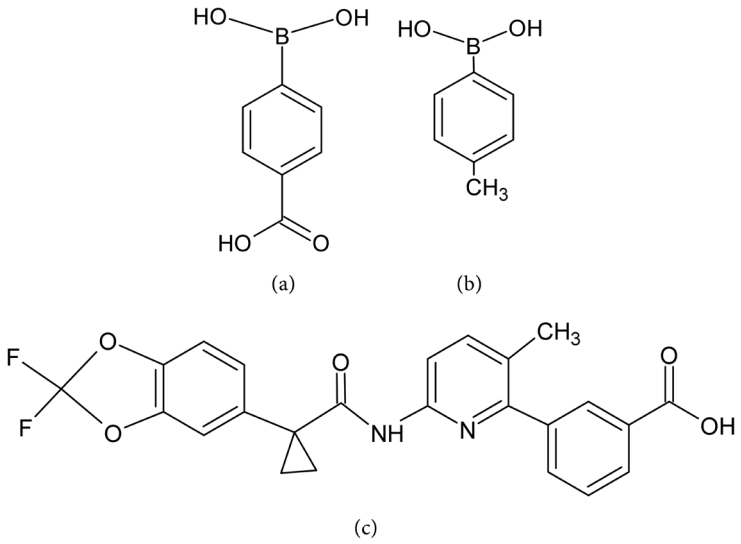

Figure 1. Chemical structures of Carboxy phenyl boronic acid (a); Methyl phenyl boronic acid (b), Lumacaftor (c) compounds. (a) Molecular Formula: $C_7H_7BO_4$, Monoisotopic Mass: 166.04374 Da; (b) Molecular Formula: $C_{24}H_{18}F_2N_2O_5$, Monoisotopic Mass: 452.118378 Da; (c) Molecular Formula: $C_7H_9BO_2$, Monoisotopic Mass: 136.069561 Da.

2. Experimental

2.1. Reagents and Chemicals

LCMS grade with highest purity of >99.8% solvents and reagents were used. Water, Acetonitrile and Methanol were procured from Honeywell (Charlotte, NC, USA). Ammonium hydroxide solution was purchased from Fluka. Lumacaftor drug substance and boronic acids were procured from PS3 labs LLP, Hyderabad, India.

2.2. Mobile Phase Preparation

Mobile phase A was prepared by adding 1 ml of ammonium hydroxide solution in 1000 ml of water and mobile phase B was 100% acetonitrile. Both the mobile phases were sonicated to degas and stored at ambient temperature for further usage. Mobile phases prepared freshly before each set of analysis.

2.3. Preparation of Sample and Standard Solutions

Weighed accurately 10 mg each of carboxy phenyl boronic acid and Methyl phenyl boronic acid and transferred in 10 ml volumetric flasks and made up to mark with 100% acetonitrile to obtain final concentration of 1000 µg/ml. A 10 µg/ml mixture of boronic acids were further prepared by addition of appropriate stock volumes and diluted with 80% Acetonitrile solution. Performed further dilution to 1 µg/ml mixture. Calibration standards were prepared from 1 µg/ml to achieve the final concentrations of 100, 80, 60, 40, 10, 5 and 1 ng/ml.

Recovery experiment required spiking solutions which were prepared by weighing accurately 100 mg of lumacaftor drug substance in 5 ml volumetric flask and then add appropriate volume of boronic acids impurity mix stock to

obtain a concentration of 20 ng/ml (1.0 ppm) and 5 ng/ml (0.25 ppm) with respect to test concentration of 20 mg/ml of lumacaftor. To ensure repeatability experiment performed in triplicates.

2.4. LC-MS/MS Operating Conditions

Chromatographic separation and detection performed using 1290 Infinity II UHPLC (Agilent technologies., Santa Clara, CA) equipped with stack of modules including binary pump, multisampler and diode array detector connected with an Agilent 6470 (Agilent technologies., Santa Clara, CA) LCMSMS triple quadrupole with Agilent Jet Stream (AJS) Electrospray Ionization interface. Poroshell HPH C18 150 × 4.6 mm 2.7 µ column (Agilent technologies., Santa Clara, CA) was used to separate boronic acid impurities and lumacaftor using 0.1% ammonium hydroxide in water as Mobile phase A and 100% acetonitrile as mobile phase B using gradient mode of elution at a flow rate of 0.25 ml/min with a run time of 14 minutes. The column oven temperature maintained at 40˚C and the autosampler temperature maintained at 10˚C with an injection volume of 20 µl. The gradient program used as follows (time in min/%B): 0.00/15, 2.00/15, 6.00/90, 11.00/90, 11.1.00/15, 14/15.

Mass spectrometric conditions were optimized in ESI negative mode using MRM mode of acquisition for both the boronic acid impurities in the form of deprotonated molecular ions $(M\text{-}H)^-$ at m/z 164.9 and 135.1 respectively for carboxy phenyl boronic acid and methyl phenyl boronic acid impurities considering the most abundant isotope of Boron. Ionization source was operated with a capillary voltage 4500 V, Nozzle voltage 2000 V, Drying gas temperature 300˚C, Drying gas flow 12 l/min, Nebulizer pressure 35 psi, Sheath gas temperature 350˚C, Sheath gas flow 10 l/min respectively. All parameters of LC and MS were controlled using Agilent Mass Hunter software 10.1 version.

2.5. Method Validation

Successful validation of the developed method in terms of Specificity, reproducibility, linearity, LOD, LOQ, robustness and solution stability executed, and the validation parameters conducted using ICH guidelines [16]. Initially to verify the sensitivity of the method, individual solutions of the impurities injected at absolute concentrations of 5.0 ng/ml (0.25 ppm wrt lumacaftor 20 mg/ml test concentration) and obtained the S/N ratio values. Further method reproducibility was established at 20 ng/ml (1.0 ppm wrt lumacaftor 20 mg/ml test concentration) by injecting six replicates from the same vial. Next, the method linearity was evaluated from 1 ng/ml to 100 ng/ml (0.05 - 5.0 ppm) using seven different concentration levels. Calculation of slope, intercept and regression coefficient values employed using least square linear regression. Recovery experiment was executed in triplicate sets at two different concentration levels of 20 ng/ml (1.0 ppm wrt test) and LOQ (0.25 ppm wrt test) to establish the efficiency. Assessment of specificity of the developed method in presence of lumacaftor drug sub-

stance performed. Method robustness was tested by altering the mobile phase composition, flow rate and column temperature conditions. Solution stability also established at different time intervals to evaluate the stability criteria of the impurities.

3. Results and Discussion

3.1. Chromatographic Method Development

This study was conducted to develop highly sensitive and selective analytical method that could separate and quantify both the mutagenic boronic acid impurities in Lumacaftor drug substance.

For optimal peak shapes and good separation between lumacaftor and boronic acid impurities, initially started with 5 mM ammonium formate buffer and adjusting several mobile phase pH and gradient conditions were evaluated but methyl phenyl boronic acid and lumacaftor getting coeluted and when altering pH conditions using 0.1% ammonium hydroxide solution provided the better peak shapes and sensitivities finally with Poroshell HPH 150 × 4.6 mm 2.7 µ column after parallelly checking different column options. Both the methanol and acetonitrile were evaluated for mobile phase B and concluded with acetonitrile due to better separation efficiency. Various flow rates were checked and finally concluded with 0.25 ml/min. 40˚C column temperature also helped in achieving the separation. The retention times of boronic acid impurities carboxy phenyl boronic acid and methyl phenyl boronic acid were observed to be 4.026 and 10.726 min respectively and lumacaftor eluted at 9.846 min. Representative chromatograms for standard and spike samples with boronic acid impurities provided in Figure 2 & Figure 3. Lumacaftor diverted to waste using time programmed events in method during the sample analysis using inbuilt divertor valve of MS to avoid the contamination during routine analysis.

3.2. Optimization of MSMS Parameters

Mass spectrometric conditions optimization aimed at developing simple, selective, highly sensitive and robust method for the determination of determination of underivatized boronic acids in lumacaftor drug substance. 1.0 µg/ml impurity mix solution was used to carry out MSMS method development. During initial stages of development negative mode ionization was found to be more sensitive due to the nature of the impurities, limiting the method development to negative ionization only. Critical compound dependent parameters like capillary, Nozzle and fragmentor voltages (V) were optimized for boronic acids to obtain the desired response for parent molecular ions considering the most abundant isotope of Boron which are presented in Table 1. Further collision energies were optimized by checking with different Collison cell voltages to establish sensitive and reproducible MRM transitions for both the boronic acids. The MS/MS spectra for both the boronic acids at different collision energies were captured in Figure 4, Figure 5.

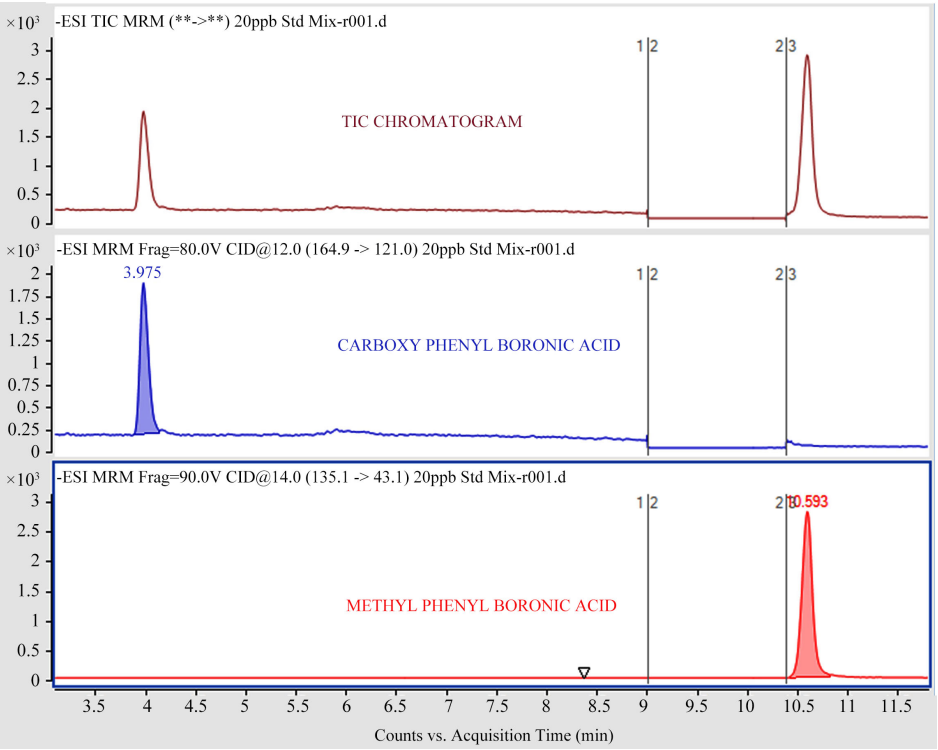

Figure 2. 20 ng/ml (1.0 ppm wrt test) standard chromatogram for carboxy phenyl and methyl phenyl boronic acid impurities.

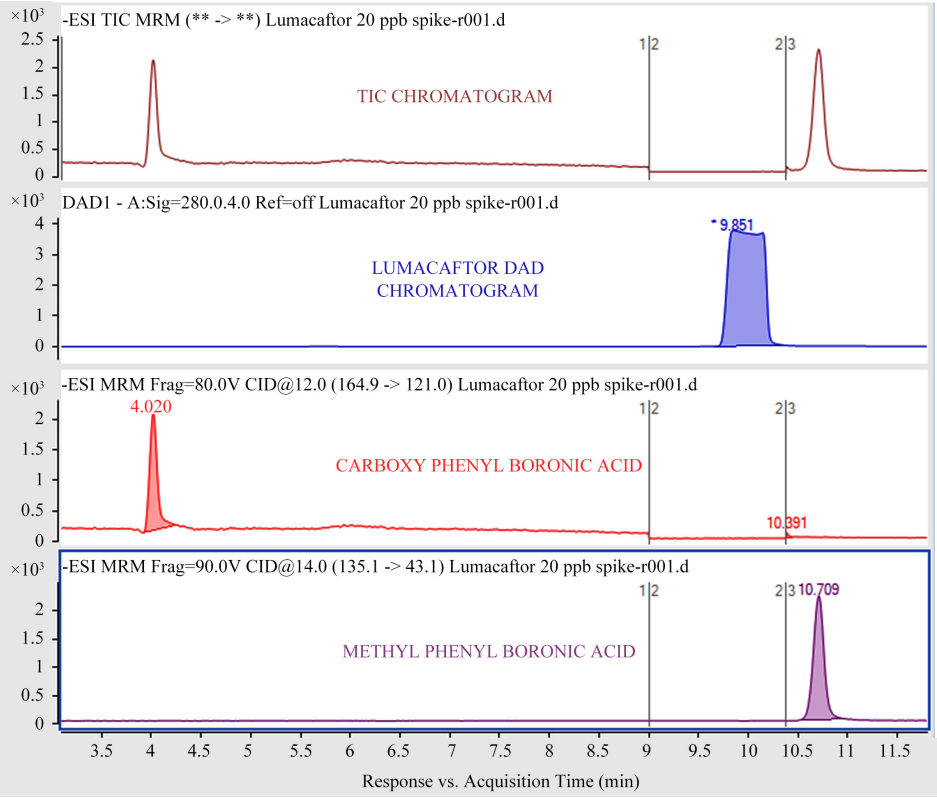

Figure 3. 20 ng/ml (1.0 ppm wrt test) spike chromatogram for carboxy phenyl and methyl phenyl boronic acid impurities.

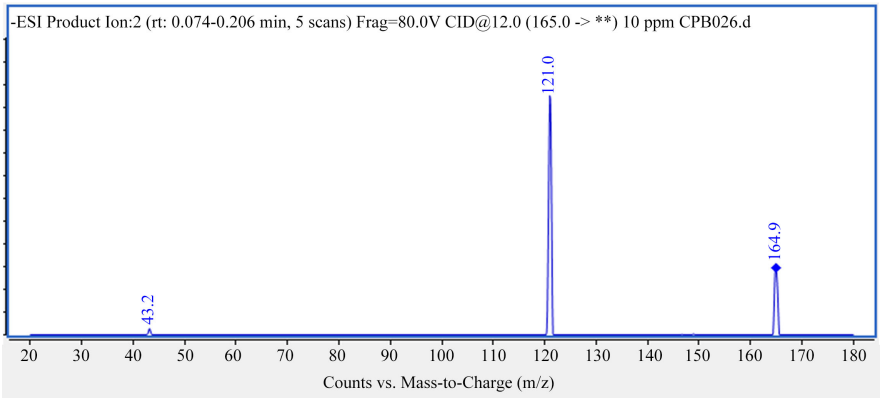

Figure 4. MSMS spectra of carboxy phenyl boronic acid.

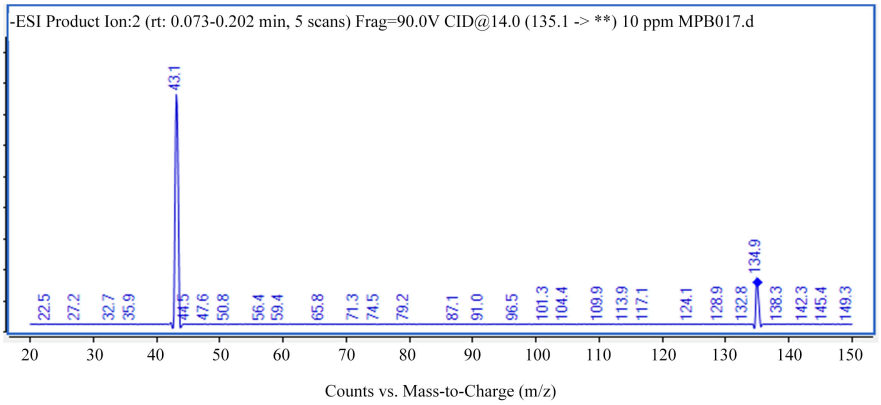

Figure 5. MSMS spectra of methyl phenyl boronic acid.

Table 1. Optimized MSMS parameters for Carboy phenyl and Methyl phenyl boronic acid impurities in ESI negative mode.

S. No	Name of the Impurity	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collison energy (V)
1	**Carboxy phenyl boronic acid**	164.9	121.0	80	12
2	**Methyl phenyl boronic acid**	134.9	43.1	90	14

4. Method Validation

As per ICH recommended guidelines, the developed method was successfully validated and established all the critical parameters required to demonstrate the method efficiency.

4.1. Specificity

Lumacaftor with boronic acid impurities mix solution was prepared at required specification level in the diluent and then subjected to LCMSMS analysis. The results obtained shown that there was no interference of Lumacaftor API with carboxy phenyl and methyl phenyl boronic acid impurities. The chromatogram acquired was presented in **Figure 2**.

4.2. Linearity

Method linearity was established from 1 ng/ml to 100 ng/ml (0.05 - 5 ppm) for both the boronic acid impurities. The regression coefficient, slope, and intercept values were derived using least squares linear regression analysis of average peak areas versus concentration of impurities. Good correlation between peak areas and concentrations of impurities observed as can be seen in Table 2 and linearity figures captured in Figure 6 & Figure 7.

4.3. LOD and LOQ

The LOQ and LOD values for both the boronic acid impurities were determined based on S/N ratios of 10.0 and 3.0 respectively, by injecting known standard concentrations and the results are captured in Table 2. S/N ratio values are derived using peak to peak algorithm for both the boronic acid impurities. Recovery and reproducibility were also evaluated at LOQ level using triplicate injections. Reproducibility data at LOQ concentration for Carboxy phenyl and Methyl phenyl boronic acids also captured in Table 3.

Table 2. Linearity ranges, Correlation Coefficients, Signal to Noise ratios of LOQs and LODs for Carboxy phenyl and Methyl phenyl boronic acid impurities.

				S/N ratio (Peak to Peak basis)	
S. No	Name of the Impurity	Linearity Range (ppm)	Correlation coefficient (R^2)	LOQ (0.25 ppm)	LOD (0.05 ppm)
1	Carboxy phenyl boronic acid	0.05 - 5	0.9974	26.8	7.4
2	Methyl phenyl boronic acid	0.05 - 5	0.9915	150.6	43.4

Table 3. Repeatability data for carboxy phenyl and methyl phenyl boronic acid impurities at 5 ng/ml (0.25 ppm) (LOQ).

	S.NO	Carboxy phenyl boronic acid	Methyl phenyl boronic acid
Initial Replicates	1	3249	4435
	2	2617	4118
	3	2669	4299
	4	2845	4605
	5	2751	4577
	6	2695	4380
	Average	2804.3	4402.3
	STD DEV	231.3	181.5
	%RSD	8.2	4.1

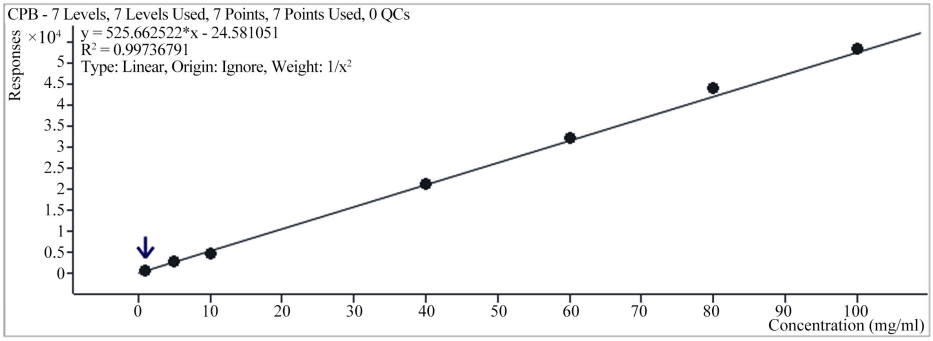

Figure 6. Linearity generated from 1 ng/ml to 100 ng/ml (0.05 - 5 ppm) for carboxy phenyl boronic acid.

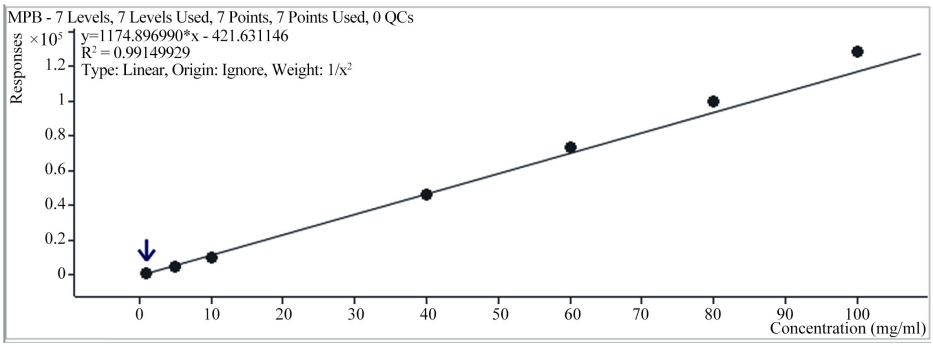

Figure 7. Linearity generated from 1 ng/ml to 100 ng/ml (0.05 - 5 ppm) for methyl phenyl boronic acid.

4.4. Accuracy and Recovery

Deviation from linearity standard concentrations was referred to as accuracy and was evaluated by injecting impurity mixture from LOQ which is 25% of specification limit and on 5 times the specification limit. The acceptance criteria for accuracy is between 80% - 120% for all the linearity standards except LOQ which could be 70% - 130% for such a low concentration range. Accuracy values observed at all levels for both the boronic acid impurities were within 15% which are well within the required acceptance criteria. Recovery was evaluated by standard addition method in triplicate at two concentrations at 0.25 ppm and 1.0 ppm levels in Lumacaftor API. The acceptance criteria for recovery is 80% - 120%. The percentage recoveries for both the boronic acid impurities presented in Table 4.

4.5. Robustness

Method robustness was evaluated changing different conditions of the method including the flow rate and composition of the mobile phase and column oven temperatures. The optimized flow rate of the mobile phase was 0.25 mL/min and the same was altered from 0.225 to 0.275 ml/min. The effect of column oven temperature on resolution was studied at 35˚C and 45˚C (altered by 5.0˚C). There was no impact on chromatographic performance of both the boronic acid impurities due to the mentioned changes proving the method robustness based

on the obtained results.

4.6. Repeatability and Solution Stability

Repeatability of the developed method was evaluated by injecting six replicate injections at 20 ng/ml (1.0 ppm) mixture of boronic acid impurities and observed the %RSD after including multiple bracketing standards. The acceptance criteria for cumulative %RSD is less than 15%. The RSD values achieved for both the boronic acid impurities are less than 6.1% which are well within the acceptance criteria which are presented in Table 5. Repeatability overlaid chromatogram for both the boronic acid impurities captured in Figure 8. The solution stability study of lumacaftor and boronic acid mixture was evaluated by placing spiked and un spiked sample solutions at 25˚C for 24 h and measured against freshly prepared standard solutions and there were no significant changes observed for any of the impurities. Therefore, we confirmed the stability of impurities in sample solution for at least 24 hours.

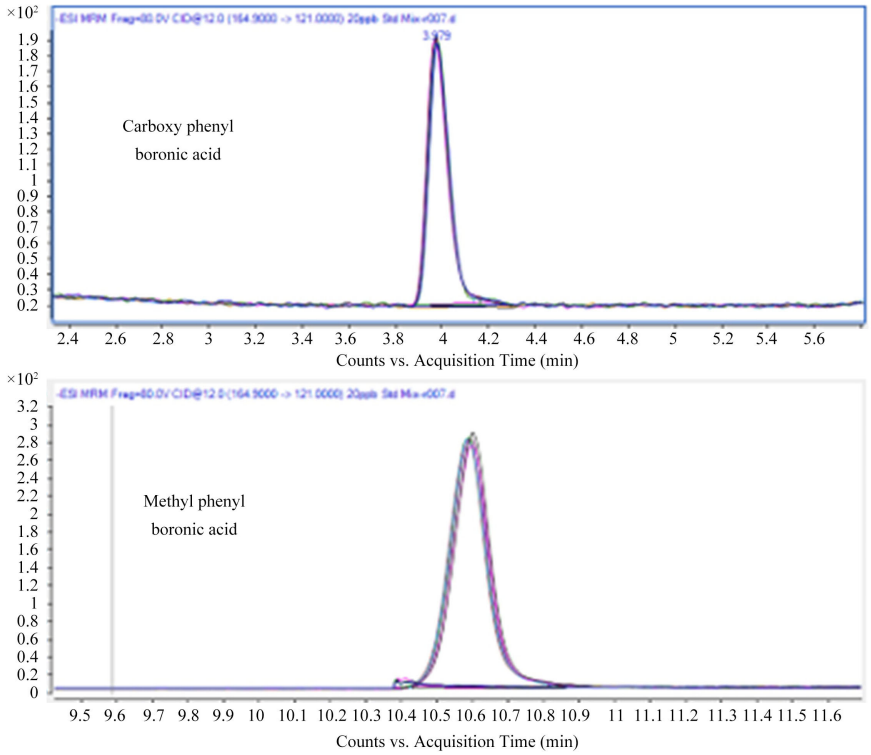

Figure 8. Repeatability overlay of seven injections including bracketing standard for carboxy phenyl and methyl phenyl boronic acid impurities in lumacaftor.

Table 4. Recoveries of carboxy phenyl and methyl phenyl boronic acid impurities at 1.0 ppm and 0.25 ppm (LOQ).

S. No	Name of the Impurity	Recovery at 1.0 ppm	Recovery at 0.25 ppm
1	**Carboxy phenyl boronic acid**	92.8	90.6
2	**Methyl phenyl boronic acid**	88.2	92.0

Table 5. Repeatability data for carboxy phenyl and methyl phenyl boronic acid impurities at 20 ng/ml (1.0 ppm) including bracketing standard.

	S.NO	Carboxy phenyl boronic acid	Methyl phenyl boronic acid
Initial Replicates	1	10,720	21,137
	2	11,323	20,763
	3	11,046	20,749
	4	11,100	20,932
	5	9912	21,248
	6	10,471	20,494
	7	9601	21,683
Bracketing standard	Average	10,596.1	21,000.9
	STD DEV	642.1	393.0
	%RSD	6.1	1.9

5. Conclusion

In summary, the work presented here is novel in terms of simultaneous determination of mutagenic underivatized boronic acid impurities in lumacaftor active pharmaceutical ingredient by single method using LC-MS/MS and there is no literature available for the quantification of underivatized boronic acids in drug substances. We could also perform all the critical parameters to prove the method performance and complete method validation as per ICH recommendations. The LOD and LOQ values determined for both the boronic acid impurities are very low showing the high sensitivity performance of the method. The method is completely validated and presents good reproducibility, linearity, recovery, and robustness. The method developed and presented here could be very useful for the determination of boronic acid impurities in lumacaftor drug substance during routine manufacturing process increasing the throughput and also could help in establishing the safety of the active pharmaceutical ingredient.

Conflicts of Interest

To the best of our knowledge, this is the first method published for simultaneous determination of underivatized Carboxy phenyl and Methyl phenylboronic acids in Lumacaftor drug substance and hold no conflicts to declare.

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