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DEVELOPMENT AND VALIDATION OF A SENSITIVE LC–MS METHOD FOR THE DETERMINATION OF FOUR POTENTIAL IMPURITIES (LAS-I, LAS-II AND THEIR REGIOISOMERS) IN LASMIDITAN DRUG SUBSTANCE AT PPM LEVELS

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Abstract

Lasmiditan is a selective 5-HT1F receptor agonist indicated for treatment of acute migraine attack. The impurities such as trace levels of process related impurities in the drug substance need to be controlled at low ppm levels and this is not detectable by conventional HPLC–UV method. A selective liquid chromatography–mass spectrometry (LC–MS) method was developed and validated for the simultaneous determination of four impurities, LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer, in lasmiditan. It was performed on a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm) with a gradient of ammonium acetate buffer and acetonitrile at 1.0 mL/min, and a positive mode electrospray ionisation with multiple-ion monitoring at m/z 335 and 301. Impurities eluted at 11.0 - 16.6 min which was very well resolved from the lasmidatan peak (about 3.1 min). The method was validated according to ICH Q2(R1) for specificity, system suitability, limit of detection (0.02–0.03 ppm), limit of quantitation (0.06–0.1 ppm), precision at the LOQ (%RSD ≤ 4.89), linearity ($R^2 \geq 0.9970$ from the LOQ to 3.0 ppm) and accuracy (recovery 98.0–103.0% at the LOQ and 96.8–103.2% at 1.8 ppm (120% of the specification limit); %RSD ≤ 2.62). The LOQs range from 15 to 25 times lower than the 1.5 ppm limit, making the method suitable to quantitate the impurities at the Specification limit in the lasmiditan drug substance.

Keywords: lasmiditan; LC–MS; impurities; regioisomers; method validation; ICH Q2(R1)

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1. Introduction

Lasmiditan is selective 5-HT1F receptor agonist being developed for acute treatment of migraines with or without aura (C19H18F3N3O2, M = 377.4 g/mol). It is not vasoconstrictive like the triptans. The effectiveness and safety of OXT have been demonstrated in two phase 3 studies called the SAMURAI and SPARTAN [1,2].

The control of impurities in API is a regulatory requirement (ICH Q3A(R2)) and the control of impurities with a possibility of becoming mutagenic under ICH M7(R1) [3,4] is considerably more stringent. The limits are

usually in the low ppm range when related to the amount of drug substance and for such limits, UV detection is not sufficiently sensitive or selective. The selectivity can be achieved with tandem or single quadrupole mass spectrometry when coupled to liquid chromatography.

There are only some analytical methods for lasmiditan reported. A gradient of phosphate buffer silica Inertsil ODS-3 column HPLC method was developed and validated for separating lasmiditan from its process impurities and degradants, followed by characterization of these impurities by LC–MS/MS [5]. An LC–MS/MS method for bulk drug and formulation (C18 column; acetonitrile–0.1% formic acid, 70:30 v/v; 1 mL/min; [M+H]+ at m/z 378.24; retention time about 2.3 min) was linear over 12.50–75 ng/mL (r = 0.999), with an LOD of 0.66 ng/mL and an LOQ of 2.22 ng/mL [6]. These approaches involve either assay and degradation profiling of lasmiditan. There are no validated ppm levels LC–MS method reported for the four impurities LAS-I, LAS-II and regioisomers of drug substance lasmiditan reported to our knowledge.

The aim of this study was to develop and validate a sensitive, selective and reproducible LC–MS analysis method that allows for quantitation of these impurities at the specification limit NMT 1.5 ppm per impurity, and to show its suitability for the quantitation.

2. Experimental

2.1 Chemicals and reagents

Lasmiditan drug substance and reference standard of LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer were [source, purity and batch numbers to be inserted]. The four impurities to be inserted were [Chemical name/supplier], ammonium acetate [grade/supplier] and water [grade/purification system]. Membrane filters were used to filter solutions before analysis (0.45 µm).

2.2 Chromatographic conditions

Table 1. Liquid chromatographic conditions

Parameter	Setting
Column	Hypersil BDS C18, 250 × 4.6 mm, 5 µm
Mobile phase A	Ammonium acetate buffer [concentration/pH to be confirmed]
Mobile phase B	Acetonitrile
Flow rate	1.0 mL/min
Column temperature	35 °C
Sample cooler temperature	5 °C
Injection volume	30 µL
Diluent	Methanol
Run time	25 min
UV detection (in series)	220 nm

Gradient programme (time, min / %A / %B): 0 / 75 / 25; 5 / 75 / 25; 6 / 45 / 55; 19 / 45 / 55; 20 / 75 / 25; 23 / 75 / 25.

2.3 Mass spectrometric conditions

A [instrument manufacturer and model] triple-quadrupole mass spectrometer with a Turbo Spray electrospray source was operated in positive ion mode, acquiring in Q1 multiple-ion (SIM) scan mode. Source and analyser settings

are given in Table 2. The ions monitored were m/z 335 for LAS-I and LAS-I regioisomer, and m/z 301 for LAS-II and LAS-II regioisomer. Data were acquired and processed with [software and version].

Table 2. Mass spectrometric parameters

Parameter	Setting
Ionisation	ESI (Turbo Spray), positive mode
Scan type	Q1 multiple ions
Nebuliser gas	13 psi
Curtain gas	12 psi
Ion spray voltage	4500 V
Source temperature	325 °C
Focusing potential	260 V
Declustering potential	55 V
Entrance potential	10 V

2.4 Preparation of solutions

Standard solution. About 10 mg each of LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer were accurately weighed into a 100 mL volumetric flask, dissolved and made up to volume with diluent. One millilitre of this solution was diluted to 100 mL with diluent, and 5.5 mL of the resulting solution was further diluted to 100 mL, giving about 55 ng/mL of each impurity. With respect to a sample concentration of 30 mg/mL, this corresponds to about 1.8 ppm. This is 120% of the 1.5 ppm specification limit and is used as the working (100%) level throughout this study.

Sample solution. About 300 mg of lasmiditan was accurately weighed into a 10 mL volumetric flask, dissolved and diluted to volume with diluent (30 mg/mL).

2.5 System suitability and calculation

A blank (diluent) was injected, followed by six replicate injections of the standard solution. The system was considered suitable if the %RSD of the peak area of each impurity was not more than 10.0. The impurity content (ppm) in the sample was calculated as:

$$\text{Impurity (ppm)} = (A_T / A_S) \times (C_S / C_T) \times P \times 10^4$$

where A_T is the impurity peak area in the sample solution, A_S is the mean impurity peak area in the standard solution, C_S is the impurity concentration in the standard solution (mg/mL), C_T is the sample concentration (mg/mL), and P is the percentage purity of the impurity standard (the factor 10^4 converts the fractional ratio to ppm when P is expressed in %). [Formula to be checked by the authors; see verification items.]

2.6 Method validation

Specificity, system suitability, LOD, LOQ, precision at the LOQ, linearity and accuracy were validated using the method according to ICH Q2(R1) [7]. Acceptance criteria were: %RSD of peak area ≤ 10.0 (system suitability and precision at the LOQ and LOQ); S/N value around 3:1 for the LOD and about 10:1 for the LOQ; linearity coefficient (r) ≥ 0.980 and intercept of not more than 10.0% of the response at 100% level, with reporting of slope, intercept, confidence intervals and residual sum of squares; LOQ recovery (70.0–130.0%) and specification level recovery (90.0–110.0%) with %RSD ≤ 10.0.

3. Results and Discussion

3.1 Method development

The stationary phase used to aid in the ability to obtain volatile conditions in MS was a C-18 stationary phase employing an ammonium acetate / acetonitrile gradient. The gradient begins at 25% acetonitrile, to retain and resolve early eluting portion, then moves to 55% acetonitrile, to remove later eluting impurities. Since LAS-I and its regioisomer have m/z of 335, as well as LAS-II and its regioisomer of m/z 301, a chromatographic resolution of each isomeric pair is required. Within each extracted-ion channel, the pairs were separated (m/z 301: 11.0 and 11.9 min; m/z 335: 12.8 and 16.6 min). The multiple-ion monitoring was performed on protonated molecular ions to afford the requisite selectivity at ppm levels with no interference from the lasmidatan matrix, which elutes quickly at ~3.1 min. [Optimisation experiments – if done – may be summarised here.

3.2 Specificity

Individual solutions of lasmiditan and each impurity (approx. 0.1 mg/mL) and a solution containing all of the impurities spiked into lasmiditan were analysed. The retention times and protonated molecular ions are listed in Table 3 and the extracted-ion chromatograms of the spiked solution in Figure 2. No peaks co-eluted with lasmiditan and all other impurity peaks were sufficiently separated from lasmiditan. No peaks appeared on the blank chromatograms at the retention time of impurities (Figure S3).

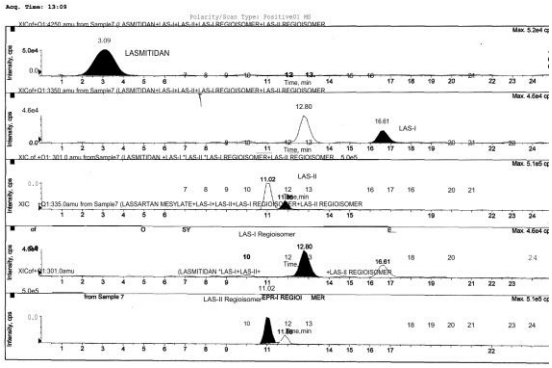


Figure 2. Specificity: extracted-ion chromatograms of lasmiditan spiked with LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer (traces for m/z 425, 335 and 301; time in min). Individual-component chromatograms are given in Figure S1.

Table 3. Retention times and m/z values (specificity)

Component	Retention time (min)	m/z [M+H]+
LAS-II regioisomer	11.03	301
LAS-II	11.88	301
LAS-I regioisomer	12.81	335
LAS-I	16.56	335
Lasmiditan	3.12	425 †

† Value as recorded in the raw data; see verification item 1.

3.3 System suitability

Six replicate injections of the standard solution gave %RSD values of 2.18% (LAS-I), 1.33% (LAS-II), 2.28% (LAS-I regioisomer) and 3.21% (LAS-II regioisomer), all well within the limit of 10.0% (Table 4; Figure 3).

Table 4. System suitability (n = 6, peak area)

Injection	LAS-I	LAS-II	LAS-I regioisomer	LAS-II regioisomer

1	311800	784800	511300	3086000
2	303700	777100	497000	3136000
3	299900	775900	509700	2928000
4	302100	784100	495600	2945000
5	306700	760300	484500	3033000
6	317700	763500	513600	3164000
Mean	306983	774283	501950	3048667
%RSD	2.18	1.33	2.28	3.21

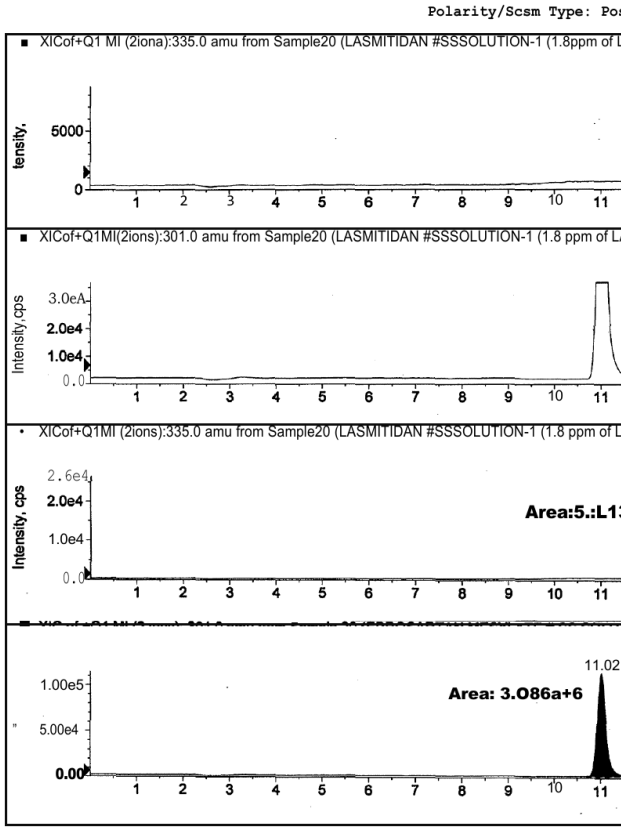


Figure 3. System suitability: representative chromatograms of the standard solution (about 1.8 ppm of each impurity; injection 1 of 6). Chromatograms of all six injections are given in Figure S2.

3.4 Limits of detection and quantitation

LOD and LOQ were established from signal-to-noise ratios (Table 5; Figure 4). The LOD was 0.02–0.03 ppm (S/N 3.1–3.5) and the LOQ 0.06–0.1 ppm (S/N 9.8–10.4) with respect to the sample concentration. At 30 mg/mL, 0.03 ppm and 0.1 ppm correspond to about 0.9 ng/mL and 3 ng/mL in solution. The LOQs are 15-fold (LAS-I, LAS-II, LAS-II regioisomer) and 25-fold (LAS-I regioisomer) lower than the 1.5 ppm limit.

Table 5. LOD and LOQ (ppm with respect to sample concentration)

Component	LOD (ppm)	S/N at LOD	LOQ (ppm)	S/N at LOQ
LAS-I	0.03	3.5	0.1	10.4
LAS-II	0.03	3.1	0.1	9.8
LAS-I regioisomer	0.02	3.3	0.06	10.1
LAS-II regioisomer	0.03	3.5	0.1	9.9

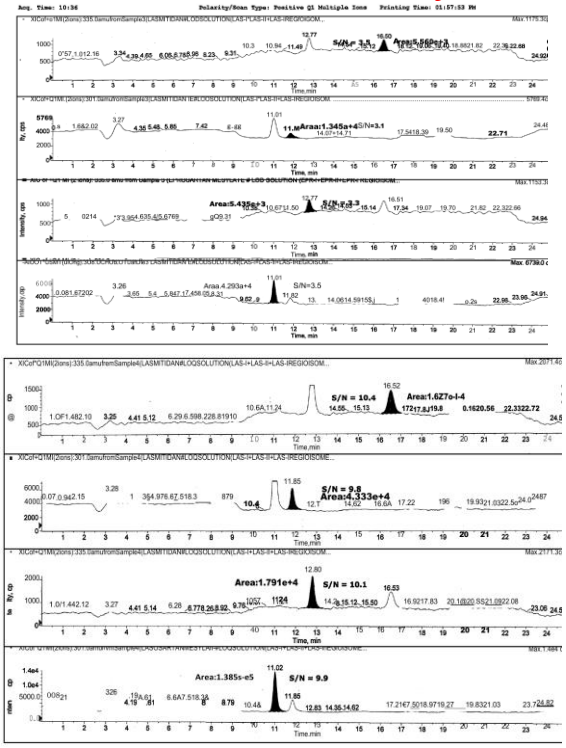


Figure 4. Sensitivity: (a) chromatograms at the LOD level and (b) chromatograms at the LOQ level for the four impurities. Blank chromatograms are given in Figure S3 and further LOQ chromatograms in Figure S4.

3.5 Precision at the LOQ

Six replicate injections of each impurity at the LOQ level gave %RSD values of 4.89, 1.95, 3.89 and 2.25% for LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer, respectively, within the 10.0% criterion (Table 6; Figure 5).

Table 6. Precision at the LOQ level (n = 6)

Component	Mean peak area	%RSD
LAS-I	15387	4.89
LAS-II	43092	1.95
LAS-I regioisomer	18068	3.89
LAS-II regioisomer	129317	2.25

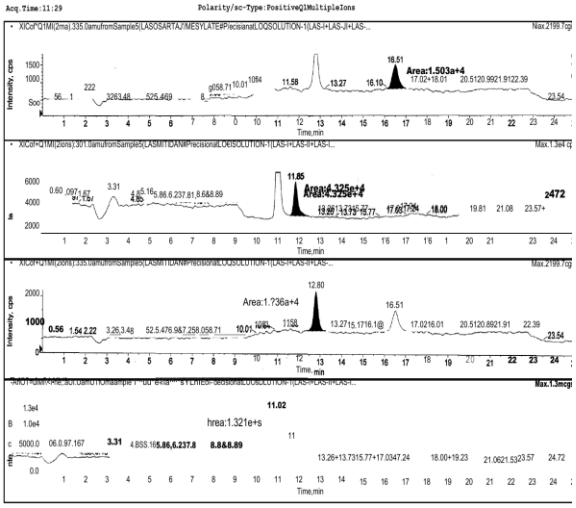


Figure 5. Precision at the LOQ: representative chromatograms of the first of six replicate injections at the LOQ level. The remaining injections are given in Figure S4.

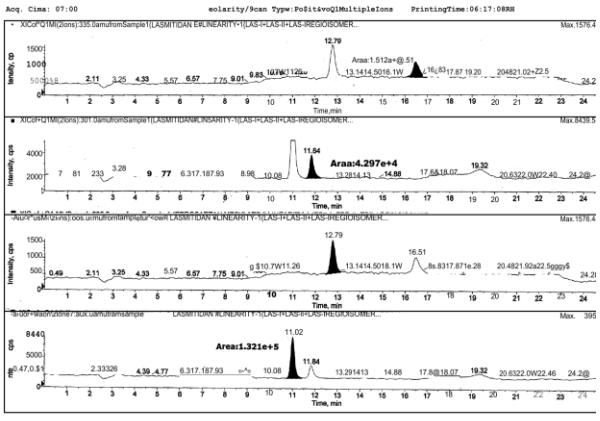
3.6 Linearity

Six concentration levels from the LOQ to 3.0 ppm were analysed for each impurity (LAS-I and LAS-II: 0.1, 0.7, 1.2, 1.8, 2.4 and 3.0 ppm; LAS-I regioisomer: 0.06, 0.6, 1.2, 1.8, 2.4 and 3.0 ppm; LAS-II regioisomer: 0.1, 0.7, 1.2, 1.8, 2.4 and 3.0 ppm). Peak area versus concentration was linear with R^2 of 0.9970–0.9979 ($r \geq 0.9985$), and the intercepts were within ±10% of the 100% response (Table 7; Figure 6). The residual sums of squares (4 degrees of freedom) were 3.49×10^8 (LAS-I), 3.20×10^9 (LAS-II), 1.10×10^9 (LAS-I regioisomer) and 5.41×10^{10} (LAS-II regioisomer).

Table 7. Linearity parameters

Component	Slope	Intercept	r	R^2	95% CI of slope	95% CI of intercept	Intercept (% of 100% response)
LAS-I	160697	−1940.5	0.9988	0.9977	149961–171433	−21512 to 17631	−0.68
LAS-II	426618	17280	0.9985	0.9970	394108–459128	−41985 to 76546	2.20
LAS-I regioisomer	290463	−4943	0.9989	0.9979	271823–309104	−38808 to 28922	−0.95
LAS-II regioisomer	1823707	−144001	0.9986	0.9972	1690055–1957358	−387648 to 99647	−4.59

The confidence intervals of all intercepts include zero, indicating no significant constant bias. The different slopes reflect compound-dependent ionisation efficiency, so each impurity must be quantified against its own reference standard.



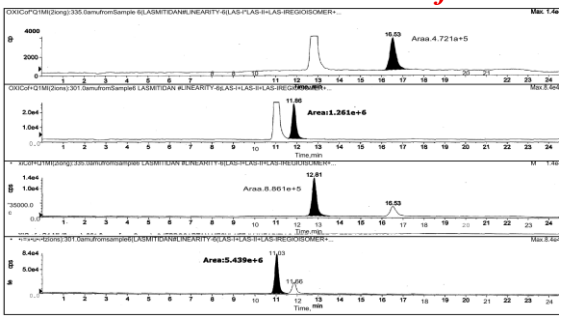


Figure 6. Linearity: chromatograms at (a) the lowest level (LOQ; linearity level 1) and (b) the highest level (3.0 ppm; level 6). Chromatograms of all levels and the calibration plots are given in Figure S5.

3.7 Accuracy

Accuracy was evaluated by spiking lasmiditan with the four impurities in triplicate at the LOQ level and at 1.8 ppm (120% of the 1.5 ppm specification limit; hereafter "the specification level"). Recoveries at the LOQ were 98.0–103.0% with %RSD 0.98–2.00 (Table 8), and at the specification level 96.8–103.2% with %RSD 1.55–2.62 (Table 9), all within the acceptance criteria. Representative chromatograms are shown in Figure 7.

Table 8. Recovery at the LOQ level (n = 3)

Component	Spiked (ppm)	Measured (ppm)	Recovery (%)	%RSD
LAS-I	0.1	0.098 / 0.102 / 0.100	98.0 / 102.0 / 100.0	2.00
LAS-II	0.1	0.102 / 0.099 / 0.100	102.0 / 99.0 / 100.0	1.52
LAS-I regioisomer	0.06	0.059 / 0.061 / 0.060	98.3 / 101.7 / 100.0	1.67
LAS-II regioisomer	0.1	0.102 / 0.101 / 0.103	102.0 / 101.0 / 103.0	0.98

Table 9. Recovery at the specification level (n = 3)

Component	Spiked (ppm)	Measured (ppm)	Recovery (%)	%RSD
LAS-I	1.8	1.743 / 1.834 / 1.771	96.8 / 101.9 / 98.4	2.62
LAS-II	1.8	1.826 / 1.772 / 1.787	101.4 / 98.4 / 99.3	1.57
LAS-I regioisomer	1.8	1.783 / 1.837 / 1.798	99.1 / 102.1 / 99.9	1.55 ‡
LAS-II regioisomer	1.8	1.858 / 1.769 / 1.815	103.2 / 98.3 / 100.8	2.44

‡ Calculated from the three recoveries; not stated in the original report.

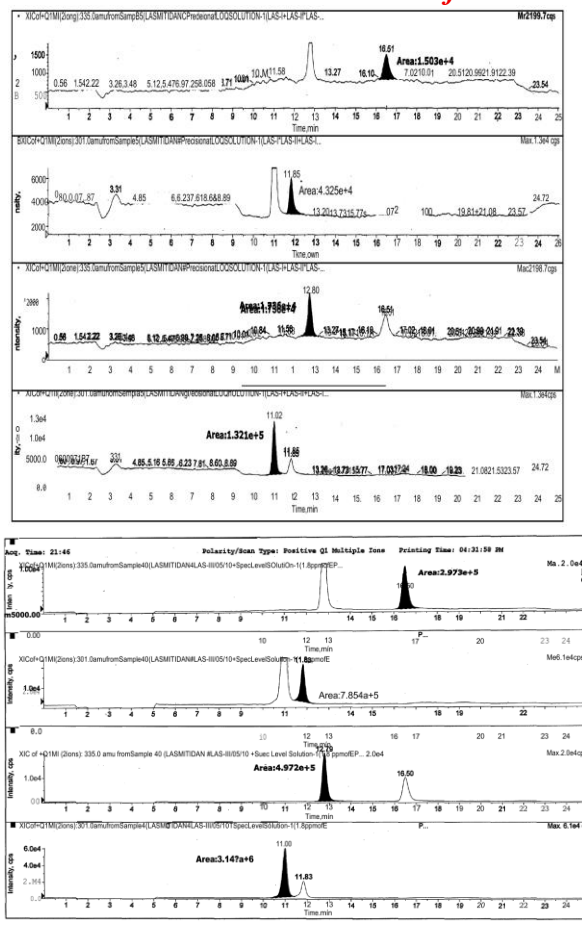


Figure 7. Accuracy: representative chromatograms of lasmiditan spiked with the four impurities at (a) the LOQ level (preparation 1) and (b) the specification level (preparation 1). All chromatograms are given in Figure S6.

3.8 Scope and limitations

The method uses Q1 single-stage monitoring, so selectivity relies on retention time plus the protonated molecular ion; the isomeric pairs are distinguished chromatographically. [Robustness, intermediate precision, solution stability and results for representative lasmiditan batches to be added if available.]

4. Conclusion

A selective and sensitive LC–MS method was developed and validated for the simultaneous determination of LAS-I, LAS-II, LAS-I regioisomer and LAS-II regioisomer in lasmiditan drug substance. The method meets the predefined acceptance criteria for specificity, system suitability, sensitivity (LOQ 0.06–0.1 ppm), repeatability at the LOQ, linearity and accuracy, with quantitation limits well below the 1.5 ppm specification, and is suitable for the quantitation of these impurities in lasmiditan drug substance at the specification limit; intermediate precision, robustness and analysis of production batches should be established before routine release testing.

Declarations

Conflict of interest: None

Funding: None

Author contributions

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