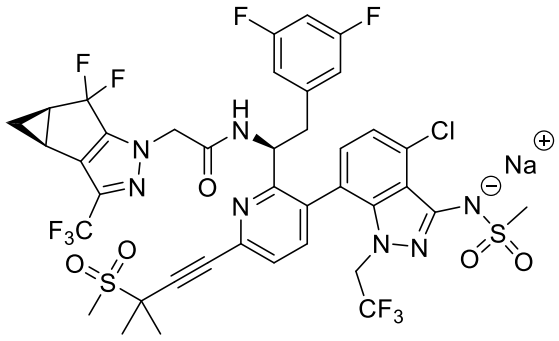
Medicines for All Institute

Summary of Analytical Development Work on Synthesis of Lenacapavir



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Executive Summary

This analytical development report (ADR) describes the results of the analytical method development efforts for lenacapavir synthesis (GFN-002-LEN-PDR) at Medicines for All Institute (M4ALL). Methods for in-process and final API testing are disclosed. Lenacapavir is a first-in-class drug developed by Gilead Sciences Inc. that targets the HIV capsid protein and also demonstrates potent effectiveness for preventing HIV. It has been approved twice by the FDA: once in 2022 for the treatment of multi-drug resistant HIV (marketed under the trade name Sunlenca®) and again in 2025 for HIV pre-exposure prophylaxis (PrEP) (marketed under the trade name Yeztugo®).

At the onset of M4ALL's process research and development work, a single, time-intensive LC-UV analytical method[18] was available in the public domain. To expedite process innovation, M4ALL undertook the development of a full suite of in-house methods. This resulted in reduced analysis times for the characterization of lenacapavir active pharmaceutical ingredient (API), and facilitated project timeline adherence while maintaining robust and reliable data for quality control.

Medicines for All – Contributors to the Research and Report

Contributor	Title/Role
Dr. Ryan Littich	Chief Technology Officer
Dr. Justina M. Burns	Assoc Dir of Analytical Chemistry (Project Lead)
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Contents

1 Introduction

The Human Immunodeficiency Virus (HIV) continues to pose one of the most urgent and enduring challenges to global public health. It is estimated that more than 600,000 people die from HIV-related illnesses each year, with over 40 million deaths recorded since the beginning of the epidemic.[1] Currently, approximately 40 million individuals are living with HIV worldwide, including 1.5 million children; more than 1 million new infections occur, annually.[2,3] Among the most promising therapies for HIV treatment is lenacapavir, a first-in-class antiviral that targets the HIV capsid protein.[4–7] This novel approach disrupts multiple stages of the viral life cycle. Lenacapavir's long-acting nature and availability in both oral and injectable forms have positioned it as a first-line treatment for HIV infection. In 2022, the U.S. Food and Drug Administration (FDA) approved lenacapavir for the treatment of multi-drug-resistant HIV.[8] Notably, the drug also shows strong efficacy in preventing HIV. Marketed under the tradename *Yeztugo*®, lenacapavir was approved by the FDA in 2025 for use as a pre-exposure prophylaxis (PrEP) against HIV.[9]

Despite its clinical success, lenacapavir has faced criticism over its cost.[10–12] According to 2024 data, Gilead Sciences priced the drug between $30,625 and $44,819 per person per year (pppy), an unaffordable range for many individuals, especially those in middle- and low-income countries. The current cost of goods (COG) for the active pharmaceutical ingredient (API) stands at $64,480 per kilogram, with imports sourced from India. [11] To make lenacapavir more accessible, the target pppy price is set below $100. Achieving this goal requires reducing the generic manufacturing cost of the API to $10,000 per kilogram or less. The recent voluntary licensing agreement between Gilead and six pharmaceutical companies represents a major step toward expanding access to lenacapavir for both prevention and treatment in low- and middle-income countries.[13]

With ongoing support from the Gates Foundation,[a] M4ALL has been entrusted with reducing the overall cost of this complex API molecule. A techno-economic analysis of the

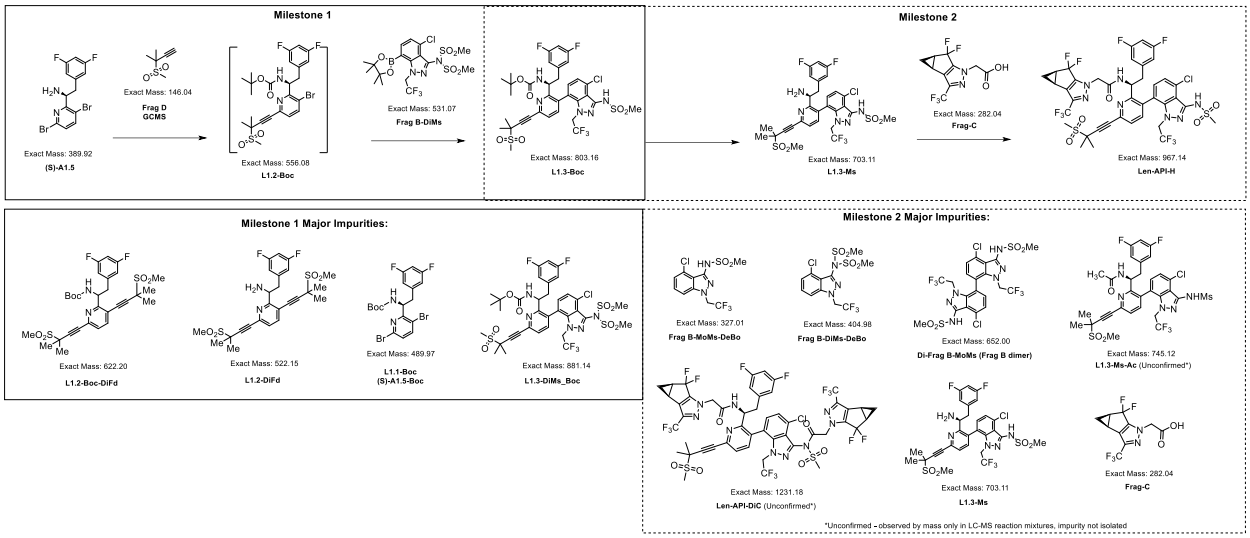
[a] With support from the Gates Foundation during Year 1 (2023-2024), M4ALL successfully developed new chemistry and process that significantly reduced the overall cost of Fragments A, B, and C.

baseline synthesis route identified Fragment C and palladium (Pd) catalysts as the primary cost drivers of lenacapavir's raw material cost (RMC). Consequently, the strategic introduction of Fragment C at a late stage, along with minimizing the use of Pd catalysts, has the potential to significantly lower the production cost of this breakthrough drug.

M4ALL implemented a three-step synthetic approach for the preparation of lenacapavir API, represented in two key project milestones:

- **Milestone 1**: One-pot, sequential Pd-catalyzed Heck and Suzuki coupling to link Fragment A with Fragments D and B
- **Milestone 2**: H_2SO_4-mediated Boc deprotection, followed by a late-stage amidation with Fragment C to furnish the lenacapavir sodium API

As previously outlined, the central objective of this work is to reduce the RMC of the lenacapavir API synthesis through innovative chemistry and process optimization. Minimizing alterations to the incumbent advanced intermediates - to facilitate uptake by generic pharmaceutical manufactures - was another core tenet of the institute's work. Toward this goal, M4ALL developed a cost-efficient process, starting from Frag A. The synthesis begins with the coupling of Frag A and Frag D in the presence of Boc_2O and a catalytic amount of $PdCl_2(PPh_3)_2$. The resulting Heck product undergoes a telescoped Suzuki coupling with Frag B-DiMs (derivatized from Frag B with one step), then treatment with KOH to yield the key intermediate L1.3-K-Boc. Subsequent Boc deprotection and T3P-mediated amidation with Fragment C produce chemically pure lenacapavir API, finalized through recrystallization (Scheme 1.1). The discussion below outlines the analysis efforts related to this work.

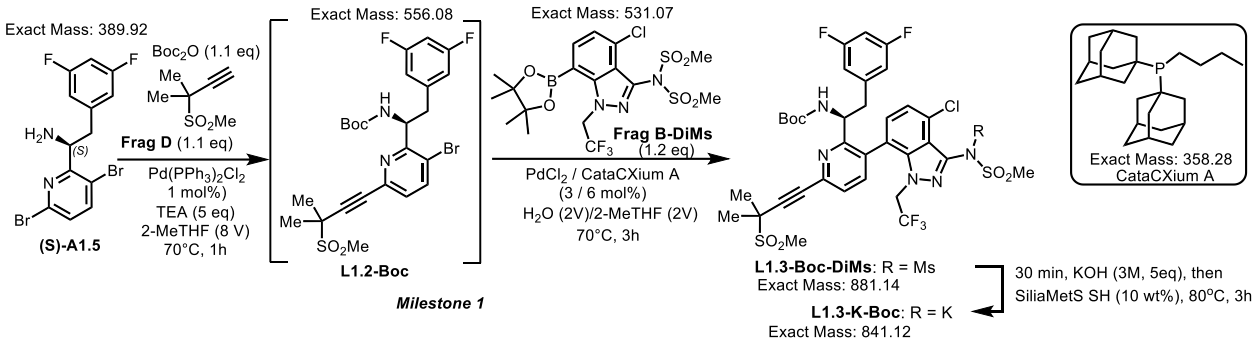


Scheme 1.1 Len-API synthesis and major impurities.

2 Results and Discussion

2.1 One-pot sequential Pd-catalyzed coupling reactions (Milestone 1)

The one-pot sequential synthesis of L1.3-K-Boc involves four key transformations, starting from (*S*)-A1.5 (Frag A). The sequence begins with in-situ Boc protection of the amine, followed by a Heck coupling to install the alkyne moiety. A subsequent Suzuki reaction with Frag B-DiMs introduces the indazole fragment. Demesylation using KOH selectively removed one mesyl group and simultaneously generates the potassium salt of sulfonamide, yielding L1.3-K-Boc. The crude product is then treated with SiliaMetS SH, to reduce residual Pd content to below 10 ppm (Scheme 2.1.1).



Scheme 2.1.1 M4All one-pot double-dose sequential Pd couplings for L1.3-K-Boc synthesis.

2.1.1 Pharmacopoeia Methods

Neither compendial methods nor monographs from the United States Pharmacopoeia and/or the European Pharmacopeia are available for L1.3-K-Boc.

2.1.2 Method Development

2.1.2.1 L1.3-K-Boc In Process and Assay Analysis

Chromatographic analysis was performed on an Agilent 1100/1200 liquid chromatograph equipped with a diode array detector (DAD). Separation was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (2.1×150 mm, 3.5 µm) maintained at a temperature of 30°C. The mobile phase consisted of a binary gradient elution using mobile phases of 0.1% phosphoric acid in water (A) and acetonitrile (B) at a constant flow rate of 0.7 mL/min. Initial conditions were set to 45% B, ramping to 65% B over 12.00 min with a 2 min hold followed by a final ramp to 95% B over 2 min with a 4 min hold. A post-run equilibration of 4 minutes was applied. Samples were prepared at 1 mg/mL in acetonitrile. An injection volume of 1.0 µL was used. The detection was monitored at 275 nm. The full method can be found in Appendix 3.1 (LCUV_Len API_Gradient). Figure 2.1.2.1.1 is a representative chromatogram for the various intermediates and impurities analyzed in the current method. It should be noted that L1.3-Boc exists as atropisomers in solution and is observed as a pair of peaks on the chromatogram.

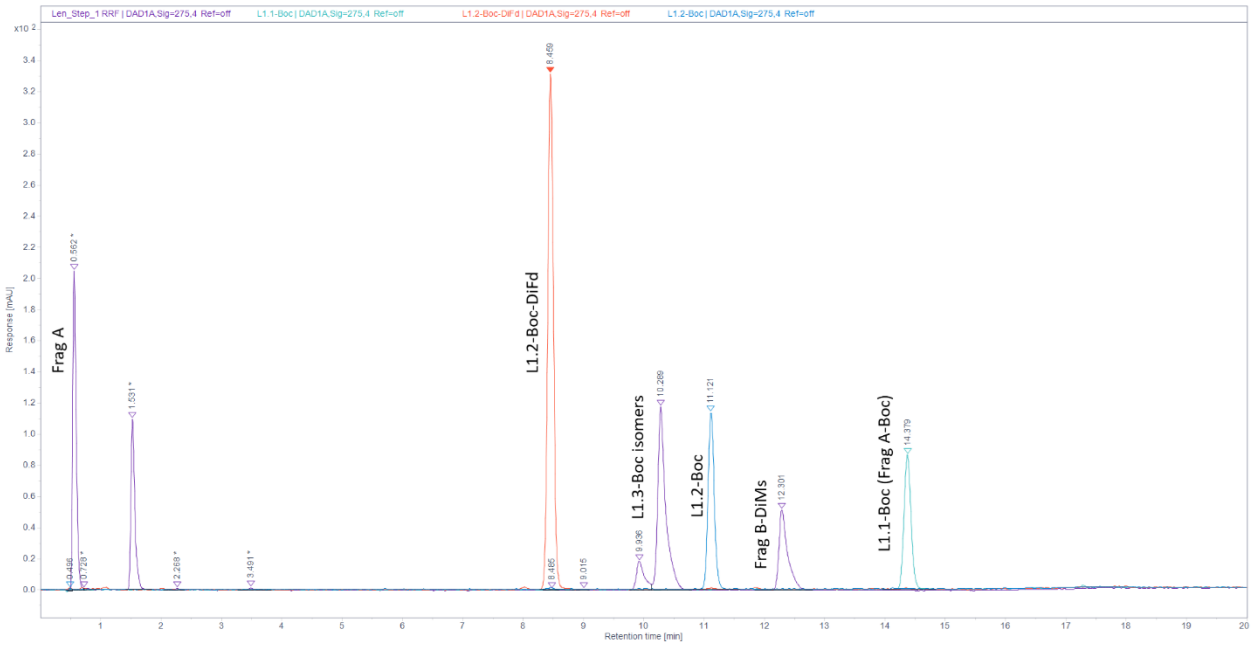


Figure 2.1.2.1.1. Representative chromatogram for L1.3-Boc synthesis.

2.1.2.1.1 Relative Response Factors for L1.3-K-Boc Synthesis

Samples of L1.3-Boc, impurities and synthetic intermediates were prepared at known concentrations and injected using the above method with a range of detection wavelengths to select an optimal wavelength for purity evaluation and reaction monitoring Figure 2.1.2.1.1.1. No true isosbestic point exists for this suite of analytes. As such, 275 nm was chosen for quantitation and monitoring purposes.

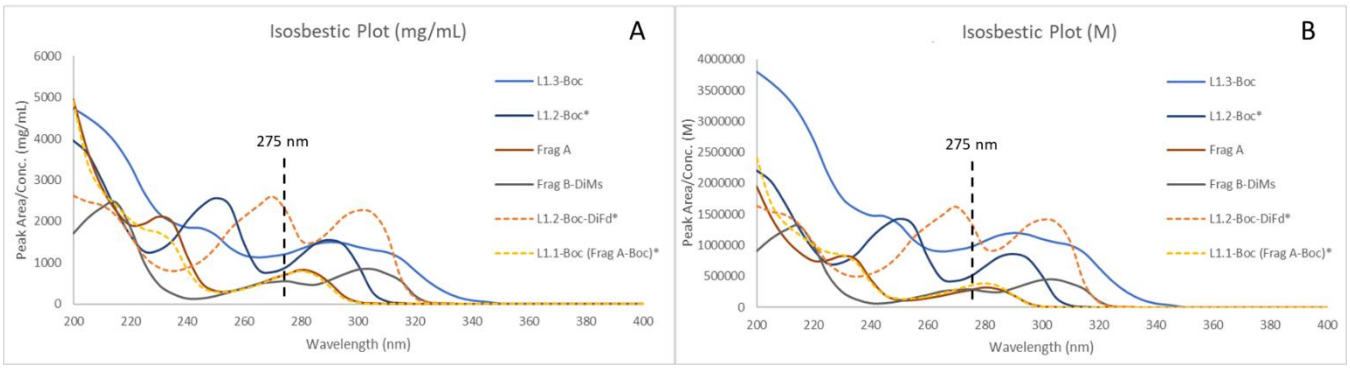


Figure 2.1.2.1.1. A) Isosbestic plot of L1.3-Boc and associated analytes based on mg/mL. B) Isosbestic plot of L1.3-Boc and associated analytes based on molarity (M).

Relative response factors (RRFs) were subsequently determined for starting materials and each available known impurity using the method put forth in Section 2.1.2.1 (Table 2.1.2.1.1). Impurities were synthesized and purified in-house (refer to GFN-002-PDR). Samples were prepared at 1 mg/mL in acetonitrile and relative response factors were calculated using Equation 2.1.2.1.1. Table 2.1.2.1.1 lists the approximate retention times and RRFs (based on mg/mL and M basis) for each analyte.

$$\mathrm{RRF} = \frac{\left({}^{\mathrm{Area}}/_{\mathrm{Concentration}}\right)_{\mathrm{Analyte}}}{\left({}^{\mathrm{Area}}/_{\mathrm{Concentration}}\right)_{\mathrm{L1.3-Boc}}} \qquad \text{Eqn. 2.1.2.1.1}$$

Table 2.1.2.1.1. Relative response factors for Milestone 1 are calculated based on concentration (mg/mL)

Compound	Retention Time (min)	RRF at 275 nm (mg/mL)	RRF at 275 nm (M)
Frag A	0.56	0.54	0.26
L1.2-DiFd	0.67	-[b]	-[b]
L1.2-Boc-DiFd	8.46	1.83	1.42
L1.3-Boc (Product)	9.94 / 10.28[a]	1.00	1.00
L1.2-Boc	11.12	0.75	0.52
Frag B-DiMs	12.3	0.41	0.27
L1.3-DiMs-Boc	12.09 / 12.67[a]	-[b]	-[b]
L1.1-Boc (Frag A-Boc)	14.38	0.60	0.37

[a]Isomers, all RRFs were calculated using the sum of the isomer peak areas. [b]Sufficient quantity not available for determination

2.1.2.1.2 L1.3-K-Boc Linearity

L 1.3-K-Boc response at 275 nm was linear between 0.05 – 1.35 mg/mL. A minimum of 5 standard levels were used to calculate the curve with a linear fit of $R^2 > 0.99$ (Figure 2.1.2.1.2.1).

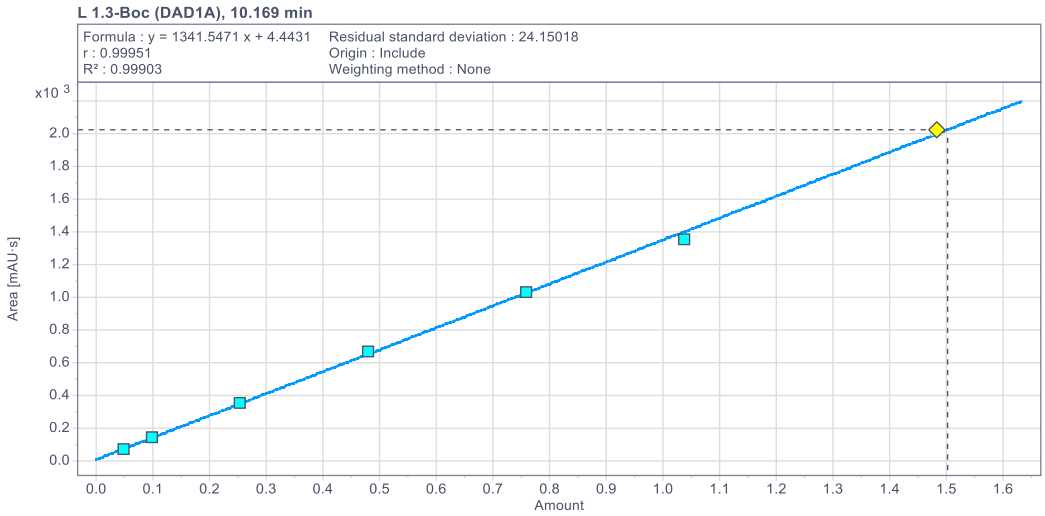


Figure 2.1.2.1.2.1. Linear range of L1.3-Boc.

2.1.2.1.3 L1.3-K-Boc Impurity Limits of Detection (LOD)

The sample preparation workflow for L 1.3-K-Boc was established at an initial concentration of 1 mg/mL in acetonitrile to ensure the analyte was within the linear range for weight percentage analysis. Impurity standards were also prepared at an initial concentration of 1 mg/mL and then serially diluted to determine their limit of detection (LOD). The impurities were diluted to a concentration of 0.0016 mg/mL, at which the signal-to-noise (S/N) ratio was well above 10 for all impurities. The S/N was calculated automatically by the Agilent OpenLab CDS software, with the noise region set for a duration of no more than one minute, immediately following each peak.

Based on an impurity concentration of 0.0016 mg/mL, the L 1.3-K-Boc sample would need to be prepared at 3 mg/mL to observe impurities at a 0.05% level 0r 1.5 mg/mL to observe impurities at the 0.1% level. The final LOD values for the impurities have not yet been determined at the time of this report.

2.1.2.2 L1.3-K-Boc Potassium Analysis

Potassium salt analysis was performed using a Hydrophilic Interaction Liquid Chromatography (HILIC) method with an Agilent InfinityLab Poroshell 120 HILIC-Z column (3.0×150 mm, 2.7

µm). The mobile phases were: 10 mM ammonium acetate, pH 4 (Mobile Phase A) and 90:10 acetonitrile: 10 mM ammonium acetate, pH 4 (Mobile Phase B). A gradient elution was employed, starting at 90% B, transitioning to 80% B over 5 minutes, and then to 20% B over 5 minutes, which was held for an additional 4 minutes, followed by a 3-minute post-run equilibration period. The flow rate was maintained at 0.8 mL/min, and the column temperature was set to 30° C. A sample injection volume of 1.5 µL was used. Detection was achieved with an evaporative light scattering detector (ELSD). Samples were prepared at a concentration of 8 mg/mL in acetonitrile.

For ELSD detection, the following parameters were applied: evaporator temperature of 30° C, nebulizer temperature of 30° C, and gas flow of 1.6 L/min. The detector operated with a data rate of 80 Hz, a LED intensity of 100%, smoothing set to 30, and a PMT gain of 1.00. The full method can be found in Appendix 3.5 (Salts-HILIC_ELSD Method). Figure 2.1.2.2.1 is a representative chromatogram for L1.3-K-Boc.

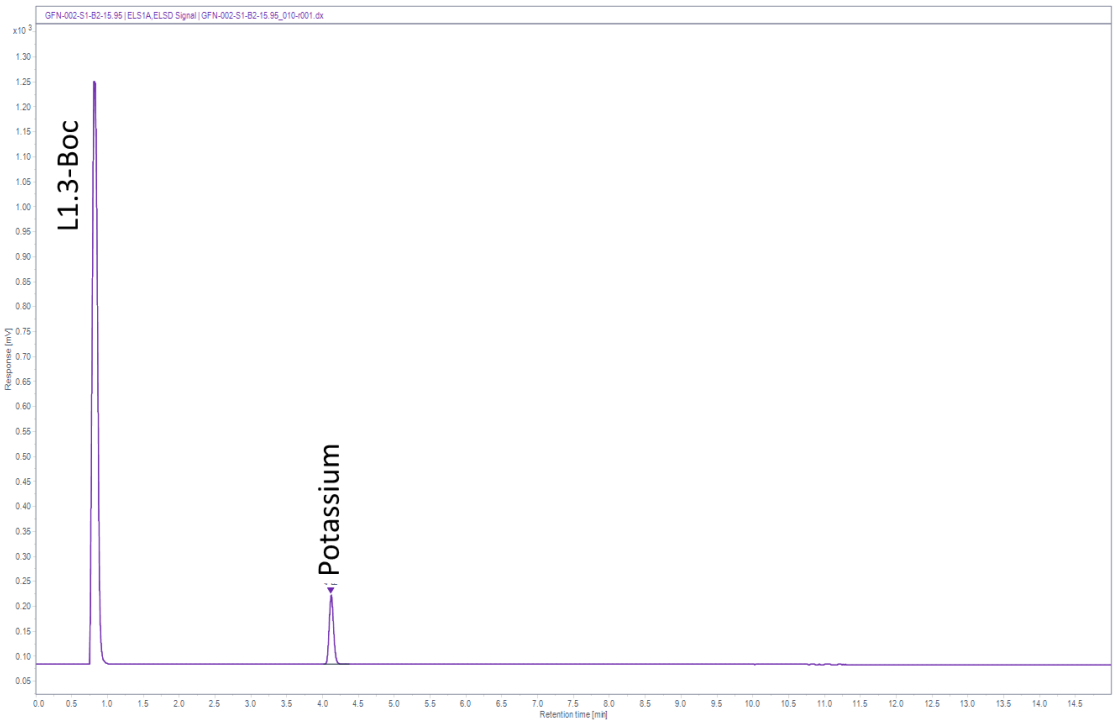


Figure 2.1.2.2.1. Representative chromatogram for L1.3-K-Boc using the HILIC method for salt analysis.

2.1.2.2.1 L1.3-K-Boc Potassium Linearity

The potassium linearity was evaluated using the Salts-HILIC method. A minimum of five standard concentrations were used to establish the calibration curve. The data was best described by a quadratic fit with a coefficient of determination (R^2) of 0.9965 over the concentration range of 0.05 to 0.35 mg/mL, satisfying the acceptance criterion of $R^2 > 0.99$ (Figure 2.1.2.2.1).

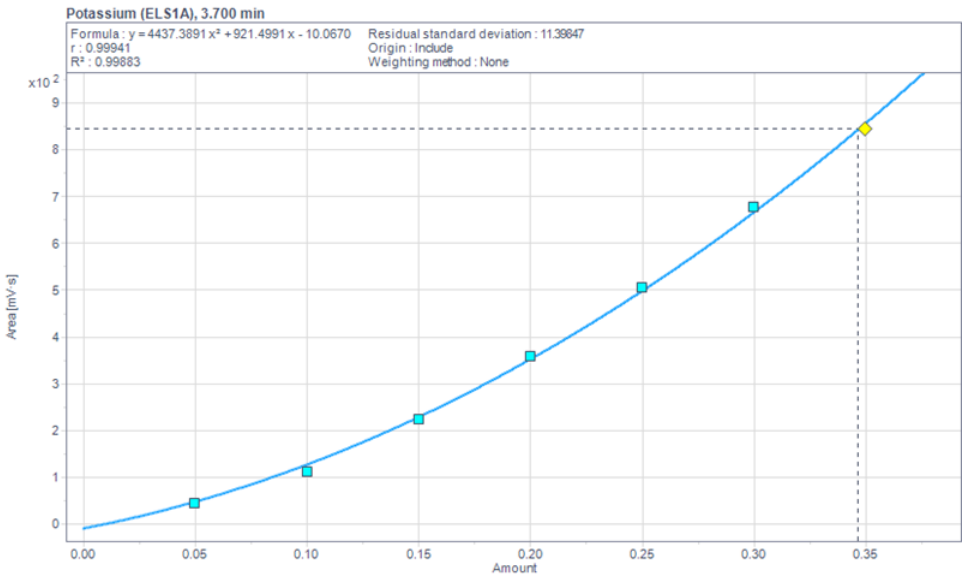


Figure 2.1.2.2.1. Linear range of potassium.

2.1.2.2.2 L1.3-K-Boc Potassium Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection (LOD) and limit of quantitation (LOQ) were not determined for potassium.

2.1.2.3 L1.3-K-Boc Solvent Analysis

Solvents were analyzed by gas chromatography (GC) - flame ionization detector (FID) with a HP-1 column (30 m × 320 µm, 5 µm film thickness). Inlet pressure was 4.8 psi, and the injection temperature was set to 260° C. A split injection was used with a split ratio of 50:1. The column flow was 0.787 mL/min. The temperature program started at an initial temperature of 50° C, held for 5 minutes, and then ramped at a rate of 20° C/min to a final temperature of 235° C, which was held for 5.75 minutes. The detector was set to 200° C. The detector gas flows were as follows: air flow at 450 mL/min, H_2 fuel flow at 35 mL/min, and N_2 makeup flow at 30 mL/min. Samples were

prepared at a concentration of 5-10 mg/mL in acetonitrile or methanol. The full method can be found in Appendix 3.3 (Solvent Method).

2.1.2.4 L1.3-K-Boc Palladium Elemental Analysis

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) was utilized for the analysis of palladium (Pd) using an Agilent 5800 instrument. The operating conditions were as follows: a Radio Frequency (RF) Power of 1.4 kW was applied to generate a robust plasma with a plasma gas flow rate of 12.0 L/min. The auxiliary gas flow was set to 1.0 L/min, and the nebulizer gas flow was 0.70 L/min, with a pressure of 220 kPa. The sample introduction system featured a glass cyclonic spray chamber and a SeaSpray nebulizer, with an uptake rate of 0.8 mL/min. The analysis was conducted in SVDV (Synchronous Vertical Dual View) mode, which provided both axial and radial views for simultaneous detection of low and high concentrations. Palladium was monitored at the 340.458 nm emission line with a replicate read time of 5 seconds, a stabilization time of 15 seconds, and a sample uptake delay time of 10 seconds.

Samples were prepared with a minimum of 50 mg of sample which was dissolved in 5 mL of 10% HCl in methanol. The mixture was vortexed and/or sonicated to ensure complete dissolution. A 1 mL aliquot of this solution was then diluted to a final volume of 10 mL with a 10% HCl in deionized water matrix. For samples with residual solids, the final solution was filtered using a 0.45µm PTFE syringe filter. The full method can be found in Appendix 3.6 (ICP-OES Method).

2.1.2.5 L1.3-K-Boc LC-MS Analysis

Separation and detection were performed using an Agilent 1260 liquid chromatograph with a diode array detector (DAD) coupled to a mass selective detector (MSD). The chromatographic separation was achieved using a reversed-phase Agilent ZORBAX Eclipse XDB-C18 column (2.1×150 mm, 3.5 µm particle size). The mobile phases were 0.1% formic acid in water (Mobile Phase A) and 0.1% formic acid in acetonitrile (Mobile Phase B). Initial conditions started at 45% B, ramping to 65% B over 12 minutes, followed by a final ramp to 95% B over 2 minutes, where it was held for 11 minutes before re-equilibration. Flow rate was maintained at 0.7 mL/min throughout, and the column temperature was set to 30° C. A 1.0 µL sample injection volume was used.

For MS detection, the following parameters were applied: gas temperature of 350° C, drying gas flow of 11 L/min, and a nebulizer pressure of 35 psig. The quadrupole temperature was set to 100° C. The mass spectrometer operated with a mass range of 40-2000 in the positive mode, a fragmentor voltage of 15 V, and a gain EMV of 1.00. The Vcap was set to +4000 V and -4000 V, with a step size of 0.10 and a % Cycle Time of 50. The full method can be found in Appendix 3.4 (LCMSLen API_Gradient).

2.1.3 L1.3-K-Boc Impurities

2.1.3.1 Starting Material Impurities

Impurities were not specified nor determined for L1.3-K-Boc starting materials.

2.1.3.2 L1.3-K-Boc Synthesis Impurities

The production of L 1.3-K-Boc has the potential to generate impurities during synthesis. These impurities range from unreacted starting materials or intermediates to dimerization of (S)-A1.5 with Frag D (Figure 2.1.3.2.1). The impurities retention times were marked and relative response factors determined. The results are described above in Table 2.1.2.1.1.

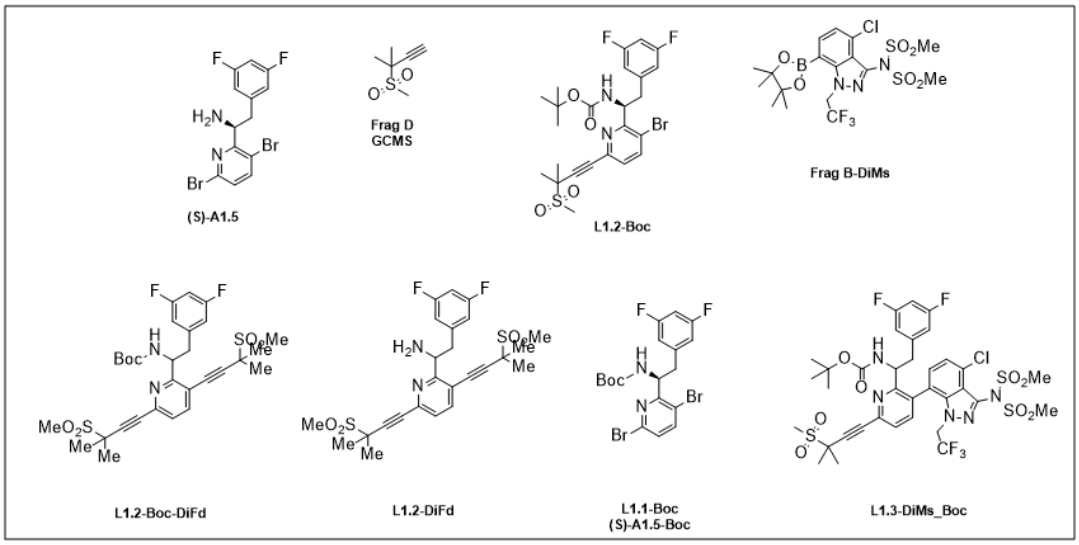


Figure 2.1.3.2.1. Impurities of Milestone 1.

2.1.4 L1.3-K-Boc Forced Degradation Studies

Forced degradation studies were not performed for L1.3-K-Boc nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.1.5 L1.3-K-Boc Stability Testing

Stability studies were not performed for L1.3-K-Boc nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.1.6 L1.3-K-Boc Methods

Analytical methods used to support the synthesis L1.3-K-Boc are appended to this report.

2.1.6.1 L1.3-K-Boc Key Starting Materials

(S)-A1.5 (Frag A) is analyzed via LC-DAD using the method "LCUV_Len API_Gradient" (Appendix 3.1).

Frag D is analyzed via GC-MS using the method "General_ThinFilm_100to1split_MS" (Appendix 3.7).

Frag B-DiMs is analyzed via LC-DAD using the method "LCUV_Len API_Gradient" (Appendix 3.1).

2.1.6.2 L1.3-K-Boc Reagents and Solvents

All reagents and solvents are analyzed via GC-MS using the method "Solvent_v1" (Appendix 3.3).

2.1.6.3 L1.3-K-Boc Intermediates

All intermediates are analyzed via LC-DAD using the method "LCUV_Len API_Gradient" (Appendix 3.1).

2.1.6.4 L1.3-K-Boc In-Process Controls (IPC)

All IPC are analyzed via LC-DAD using the method "LCUV_Len API_Gradient" (Appendix 3.1).

The requirements for the IPC were as follows (LC-DAD peak area % at 275 nm): L1.1-Boc NMT 0.2 %; Frag B-DiMs NMT 0.2 %; L1.3-Boc-DiMs NMT 0.2 A %; L1.2-Boc NMT 0.2 %; L1.2-Boc-DiFd NMT 0.2 %

2.1.6.5 L1.3-K-Boc Final Product Analysis

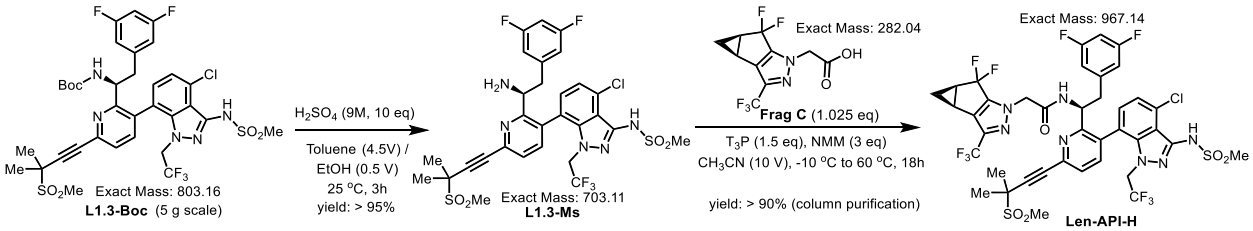
Isolated L1.3-K-Boc was assayed using a multitude of different techniques to determine the quality of the intermediate. This material was evaluated by the above-mentioned methods, LC-DAD for area % of product and impurities and weight % (LCUV_Len API_Gradient), ICP-OES (ICP-OES) for palladium content, Karl Fisher titration for water content, GC-FID for solvent content (Solvent), LC-MS for product and impurity conformation by m/z, and LC-ELSD (Salts-HILIC_ELSD) for potassium content.

2.1.6.6 L1.3-K-Boc Method Appropriateness

During development of the LCUV_Len API_Gradient method, certain performance characteristics were evaluated to select analytical conditions. These results are described above and include linearity. This method was not tested for specificity. Method validation was not performed.

2.2 H_2SO_4-based deBoc reaction and process development (Milestone 2)

Using L1.3-K-Boc as the starting material, Boc deprotection was carried out using 9M of H_2SO_4,[16] yielding the corresponding amine L1.3-Ms. Subsequent coupling with Frag C afforded Len-API-H (Scheme 2.2.1).



Scheme 2.2.1 TFA-promoted Boc-deprotection and the subsequent amidation with **Frag C** for **Len-API-H** synthesis.

2.2.1 Pharmacopoeia Methods

Neither compendial methods nor monographs from the United States Pharmacopoeia and/or the European Pharmacopeia are available for L1.3-Ms.

2.2.2 Method Development

2.2.2.1 L1.3-Ms In Process and Assay Analysis

In process chromatographic analysis was performed using an Agilent 1100/1200 liquid chromatograph equipped with a diode array detector (DAD). Separation was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (2.1×150 mm, 3.5 µm) maintained at a temperature of 30°C. The mobile phase consisted of a binary gradient elution program with 0.1% phosphoric acid in water (A) and acetonitrile (B) at a constant flow rate of 0.7 mL/min. Initial conditions were set to 45% B, ramping to 65% B over 12.00 min with a 2 min hold, followed by a final ramp to 95% B over 2 min with a 4 min hold. A post-run equilibration of 4 minutes was applied. Samples were prepared at 1 mg/mL in acetonitrile. An injection volume of 1.0 µL was used. The detection was monitored at 275 nm. The full method can be found in Appendix 3.1 (LCUV_Len API_Gradient). Figure 2.2.2.1.1 is a representative chromatogram for the various intermediates and impurities analyzed in the current method. L1.3-Boc, L1.3-Ms and L1.3-Ms-Ac exist as atropisomers in solution and are observed as a pair of peaks on the chromatogram.

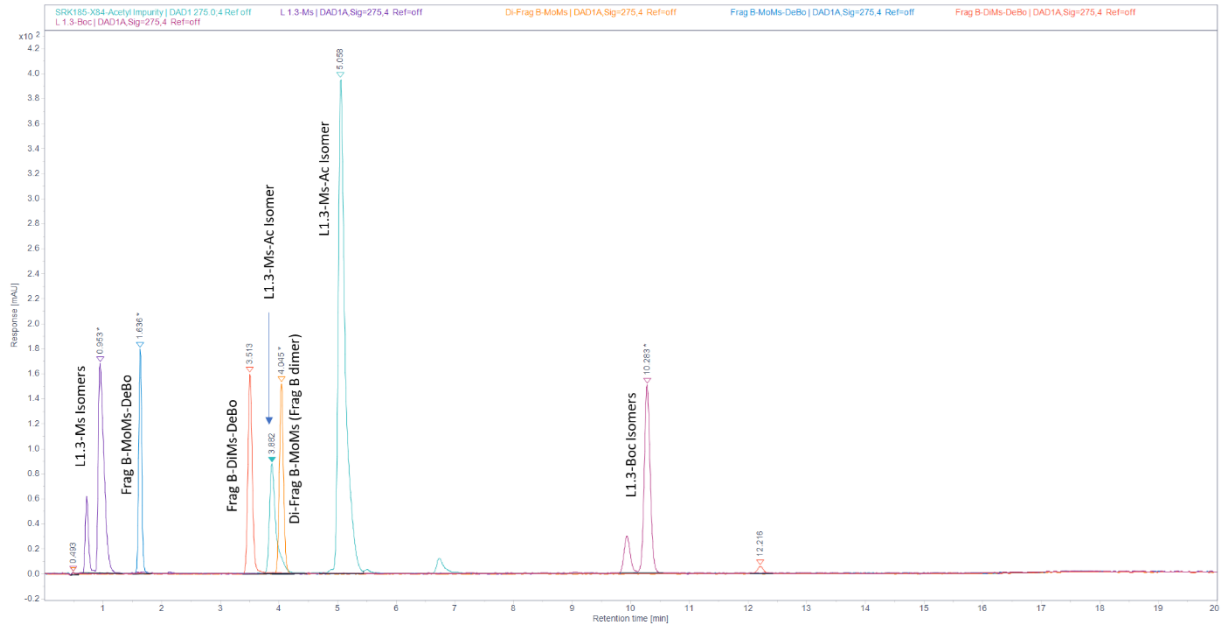


Figure 2.2.2.1.1. Representative chromatogram for L1.3-Ms, starting materials and impurities.

Final L1.3-MS quantitation was performed via chromatographic analysis on an Agilent 1100/1200 liquid chromatograph equipped with a diode array detector (DAD). The separation was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (2.1×150 mm, 3.5 µm) maintained at a temperature of 30°C. The mobile phases were 0.1% phosphoric acid in water (Mobile Phase A) and acetonitrile (Mobile Phase B). Initial conditions were set to 25% B, ramping to 50% B over 10 min and then to 95% B at 1 min. This composition was held until 9 min before a post-run equilibration of 4 minutes was initiated. Samples were prepared at a concentration of 1 mg/mL in acetonitrile, and an injection volume of 1.0 µL was used for all injections. The detection was monitored at 275 nm. The full method can be found in Appendix 3.2 (LCUV_L1-3Ms). Figure 2.2.2.1.2 is a representative chromatogram for the various intermediates and impurities analyzed in the current method. In this quantitative method for L1.3-Ms, only the L1.3-Ms atropisomers are separated while those of L1.3-Boc co-elute.

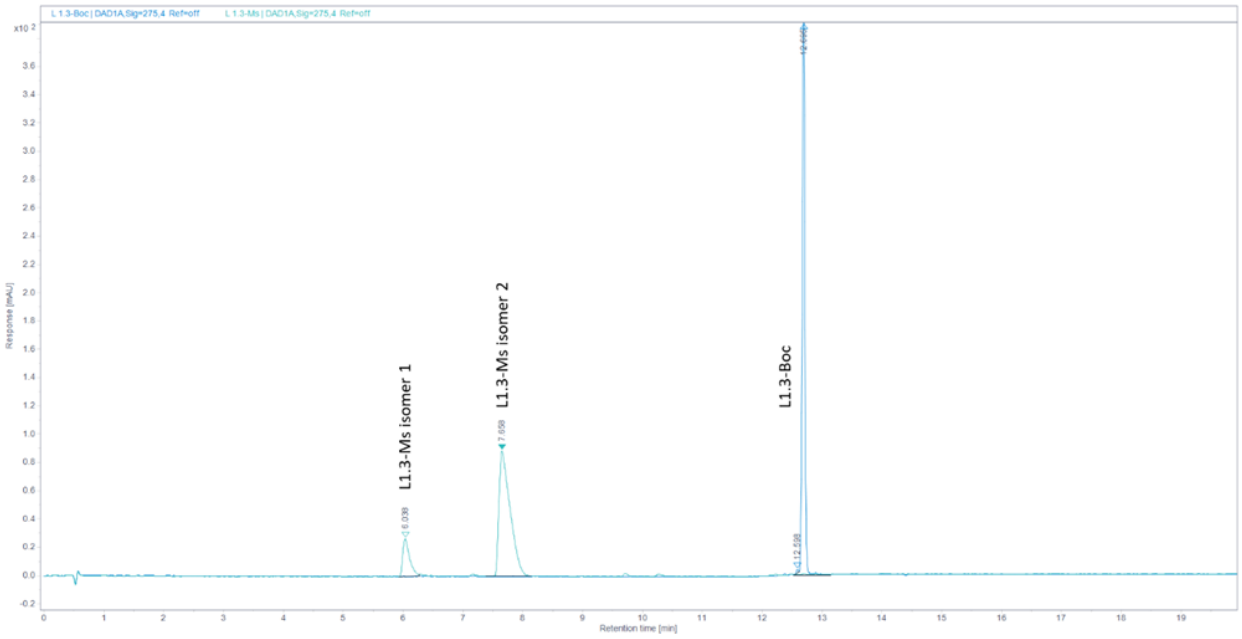


Figure 2.2.2.1.2. Representative chromatogram for L1.3-Ms quantitation.

2.2.2.1.1 L1.3-Ms Relative Response Factors

Samples of L1.3-Ms, impurities and synthetic intermediates were prepared at known concentrations and injected using the above method with a range of detection wavelengths to select an optimal wavelength for purity evaluation and reaction monitoring Figure 2.2.2.1.1.1. No true isosbestic point exists for this suite of analytes. As such, 275 nm was chosen for quantitation and monitoring purposes.

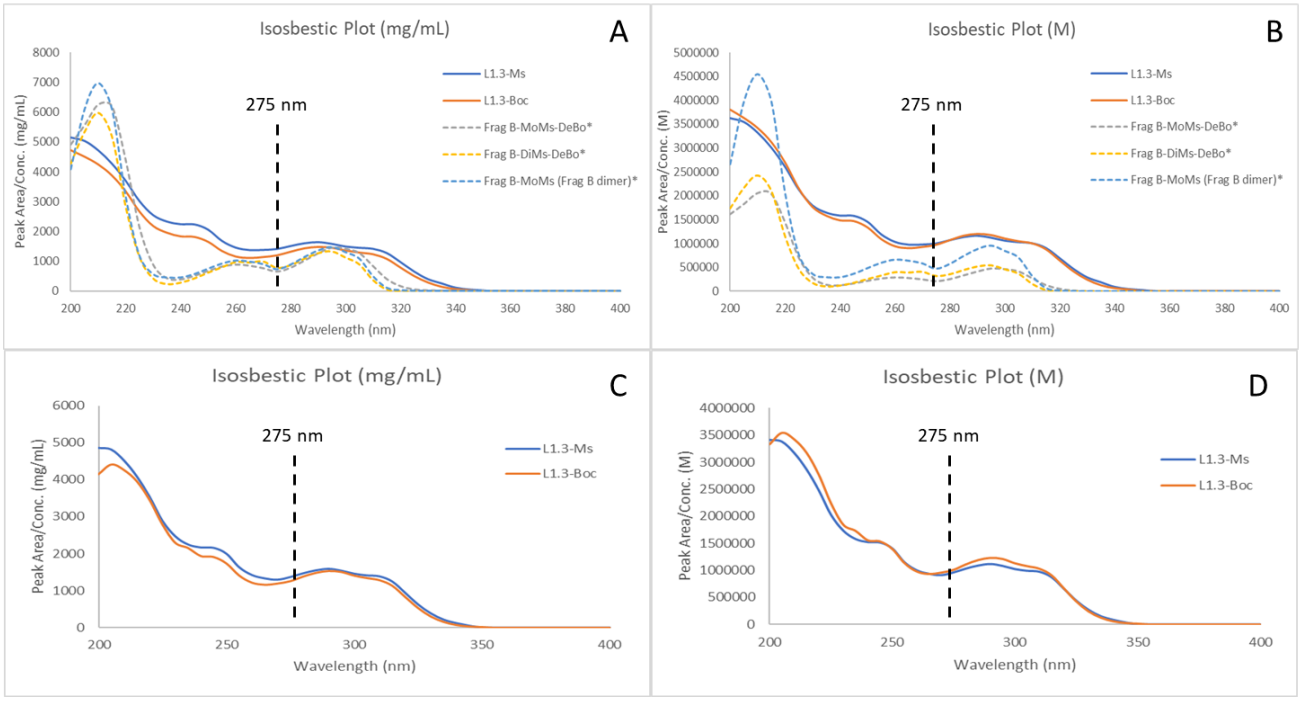


Figure 2.2.2.1.1.1. A) Isosbestic plot of L1.3-Ms and associated analytes based on mg/mL utilizing the in-process method (LCUV_Len API_Gradient). B) Isosbestic plot of L1.3-Ms and associated analytes based on molarity (M) utilizing the in-process method (LCUV_Len API_Gradient). C) Isosbestic plot of L1.3-Ms and L1.3-Boc (starting material) based on mg/mL utilizing the in-process method (LCUV_L1-3Ms). D) Isosbestic plot of L1.3-Ms and L1.3-Boc (starting material) based on molarity (M) utilizing the in-process method (LCUV_L1-3Ms).

Relative response factors (RRFs) were subsequently determined for starting materials and each available known impurity using the method put forth in Section 2.1.2.1 (Table 2.1.2.1.1).

Impurities were synthesized and purified in-house (refer to GFN-002-PDR). Samples were prepared at 1 mg/mL in acetonitrile. Relative response factors were calculated using Equation 2.2.2.1.1. Table 2.2.2.1.1 lists the approximate retention times and RRFs (based on mg/mL and M basis) for each analyte.

$$\mathrm{RRF} = \frac{\left(\mathrm{Area}/\mathrm{Concentration}\right)_{\mathrm{Analyte}}}{\left(\mathrm{Area}/\mathrm{Concentration}\right)_{\mathrm{L1.3-Boc}}} \qquad \text{Eqn. 2.2.2.1.1}$$

Table 2.2.2.1.1. Relative response factors for Milestone 2 are calculated based on concentration (mg/mL)

Compound	Retention Time (min)	In process method (LCUV_Len API_Gradient)		Quantitation method (LCUV_L1-3Ms)	
		RRF at 275 nm (mg/mL)	RRF at 275 nm (M)	RRF at 275 nm (mg/mL)	RRF at 275 nm (M)
L1.3-Ms (Product)	0.80 / 0.98 / 1.19 [a]	1.00	1.00	1.00	1.00
Frag B-MoMs-Debo	1.64	0.46	0.21	-[b]	-[b]
Frag B-DiMs-Debo	3.50	0.56	0.32	-[b]	-[b]
L1.3-Ms-Ac	3.88 / 5.06	-[b]	-[b]	-[b]	-[b]
Di-Frag B-MoMs (Frag B dimer)	4.05	0.51	0.47	-[b]	-[b]
L 1.3-Boc	9.94 / 10.28[a]	0.85	0.97	0.92	1.05

[a]Isomers, all RRFs were calculated using the sum of the isomer peak areas. [b]Not calculated

2.2.2.1.2 L1.3-Ms Linearity

Using the method LCUV_L1-3Ms (Appendix 3.2), quantitation for L 1.3-Ms was done at a response of 275 nm that was linear between 0.09 – 1.24 mg/mL. A minimum of 5 standard levels were used to calculate the curve with a linear fit of $R^2 > 0.99$ (Figure 2.2.2.1.2.1).

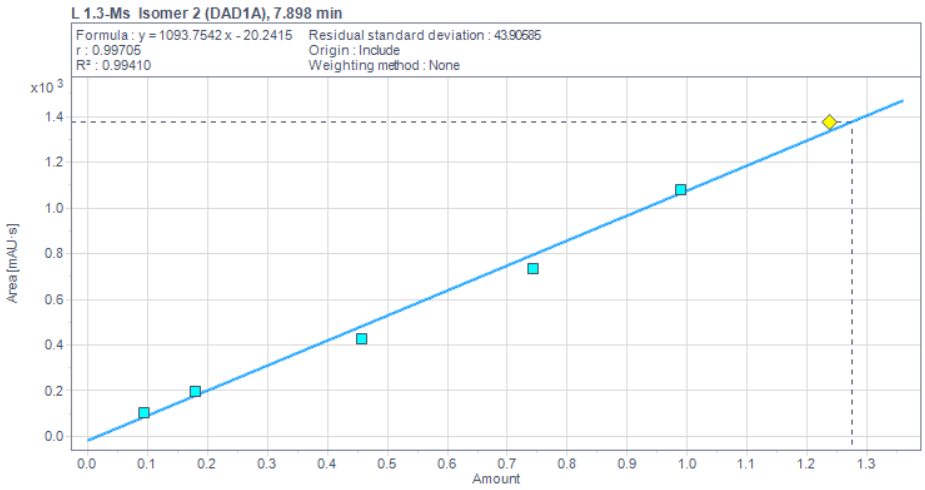


Figure 2.2.2.1.2.1. Linear range of L1.3-Ms.

2.2.2.1.3 L1.3-Ms Impurities Limit of Detection (LOD)

The sample preparation workflow for L 1.3-Ms was established at an initial concentration of 1 mg/mL in acetonitrile to ensure the analyte was within the linear range for weight percentage analysis. Impurity standards were also prepared at an initial concentration of 1 mg/mL and then serially diluted to determine their limit of detection (LOD). The impurities were diluted to a concentration of 0.0016 mg/mL, at which the signal-to-noise (S/N) ratio was well above 10 for all impurities. The S/N was calculated automatically by the Agilent OpenLab CDS software, with the noise region set for a duration of no more than one minute, immediately following each peak.

Based on an impurity concentration of 0.0016 mg/mL, the L 1.3-Ms sample would need to be prepared at 3 mg/mL to observe impurities at a 0.05% level or at 1.5 mg/mL to observe impurities

at the 0.1% level. The final LOD values for the impurities have not yet been determined at the time of this report.

2.2.2.2 L1.3-Ms Potassium Salt Analysis

The same method utilized in Milestone 1 for salt analysis was utilized in Milestone 2. Refer to Section 2.1.2.2 and Appendix 3.5 for further details.

2.2.2.3 L1.3-Ms Solvent Analysis

The same method utilized in Milestone 1 for solvent analysis was utilized in Milestone 2. Refer to Section 2.1.2.3 and Appendix 3.3 for further details.

2.2.2.4 L1.3-Ms Palladium Elemental Analysis

The same method utilized in Milestone 1 for Pd analysis was utilized in Milestone 2. Refer to Section 2.1.2.4 and Appendix 3.6 for further details.

2.2.2.5 L1.3-Ms LC-MS Analysis

The same method utilized in Milestone 1 for LC-MS analysis was utilized in Milestone 2. Refer to Section 2.1.2.5 and Appendix 3.4 for further details.

2.2.3 L1.3-Ms Impurities

2.2.3.1 L1.3-Ms Starting Material Impurities

Impurities were not specified nor determined for L1.3-Ms starting materials.

2.2.3.2 L1.3-Ms Synthesis Impurities

The production of L1.3-Ms has the potential to generate impurities during synthesis. These impurities range from unreacted starting materials or impurities carried over from the Milestone 1 step (Figure 2.2.3.2.1). The impurities retention times were marked and relative response factors determined. The results are described above.

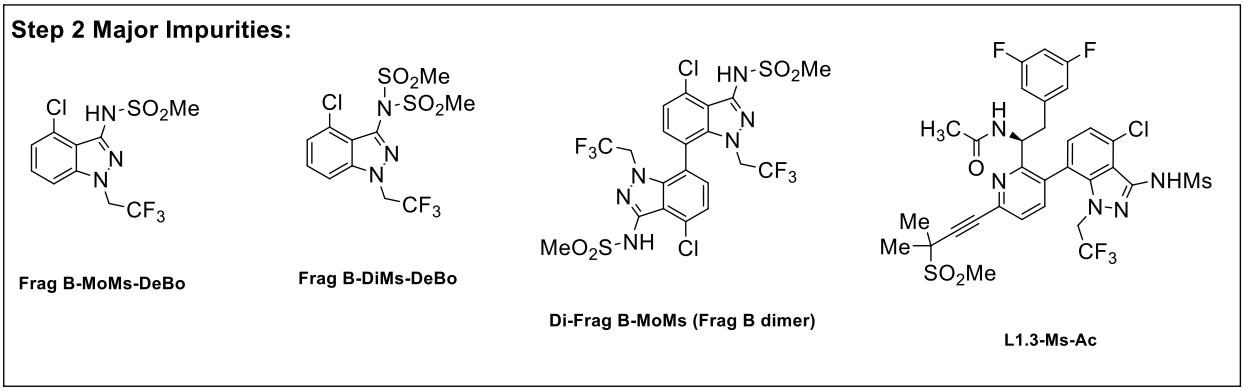


Figure 2.2.3.2.1 Impurities of Milestone 2.

2.2.4 L1.3-Ms Forced Degradation Studies

Forced degradation studies were not performed for L1.3-Ms nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.2.5 L1.3-Ms Stability Testing

Stability studies were not performed for L1.3-Ms nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.2.6 L1.3-Ms Methods

Analytical methods used to support the synthesis L1.3-Ms are appended to this report.

2.2.6.1 Key Starting Materials

L1.3-K-Boc is analyzed via LC-DAD using the method LCUV_Len API_Gradient (Appendix 3.1).

2.2.6.2 L1.3-Ms Reagents and Solvents

All reagents and solvents were analyzed via GC-MS using the method "Solvent" (Appendix 3.3).

2.2.6.3 L1.3-Ms Intermediates

All intermediates were analyzed via LC-DAD using the method "LCUV_Len API_Gradient" (Appendix 3.1).

2.2.6.4 L1.3-Ms In-Process Controls (IPC)

The requirements for the IPC were as follows: L1.3-K-Boc NMT 0.2 % (LC-DAD peak area % at 275 nm, LCUV_Len API_Gradient); Na, K, SO4 NMT 0 % (LC-ELSD, weight %, Salts-HILIC_ELSD)

2.2.6.5 L1.3-Ms Final Product Analysis

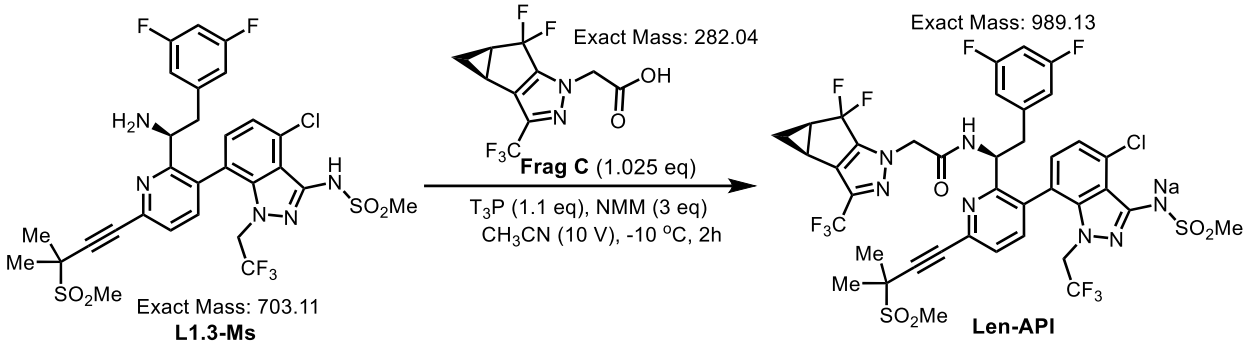
Isolated L1.3-Ms was assayed using a multitude of different techniques to determine the quality of the intermediate. This material was evaluated by the above-mentioned methods, LC-DAD for Area % of product and impurities, and weight % (LCUV_Len API_Gradient), ICP-OES (ICP-OES) for palladium content, Karl Fisher titration for water content, GC-FID for solvent content (Solvent), LC-MS (LCMSLen API_Gradient) for product and impurity conformation by m/z, and LC-ELSD (Salts-HILIC_ELSD) for potassium content.

2.2.6.6 L1.3-Ms Method Appropriateness

During development of the L1.3-Ms certain performance characteristics were evaluated to select analytical conditions. These results are described above and include linearity. This method was not tested for specificity. Method validation was not performed.

2.3 T3P-promoted amidation and process development (Milestone 2)

The synthesis of sodium Len-API was accomplished via a T3P-promoted amide coupling between L1.3-Ms and Frag C, using NMM as the base (Scheme 2.3.1). Following recrystallization from EtOH/heptane, Len-API was obtained with a single major impurity (see Section 2.3.3.2).



Scheme 2.3.1 T3P-promoted amide coupling for synthesis of Len-API.

2.3.1 Pharmacopoeia Methods

Neither compendial methods nor monographs from the United States Pharmacopoeia and the European Pharmacopeia are available for Len-API. A paper published by Wagner *et al.*[18] achieved Len-API at 99.9% by area percent. However, the paper does not state the wavelength to which this area percent corresponds.

2.3.2 Method Development

2.3.2.1 Len-API In Process and Assay Analysis

Chromatographic analysis was performed on an Agilent 1100/1200 liquid chromatograph equipped with a diode array detector (DAD). Separation was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (2.1×150 mm, 3.5 µm) maintained at a temperature of 30°C. The mobile phase consisted of a binary gradient elution program with 0.1% phosphoric acid in water (A) and acetonitrile (B) at a constant flow rate of 0.7 mL/min. Initial conditions were set to 45% B, ramping to 65% B over 12.00 min with a 2 min hold. A final ramp to 95% B over 2 min with a 4 min hold. A post-run equilibration of 4 minutes was applied. Samples were prepared at 1 mg/mL in acetonitrile. An injection volume of 1.0 µL was used. The detection was monitored at 235 nm. The full method can be found in Appendix 3.1 (LCUV_Len API_Gradient). Figure 2.3.2.1.1 is a representative chromatogram for the various intermediates and impurities analyzed in the current method. It should be noted that Len-API, L1.3-Ms, L1.3-Ms-Ac and Len-API-DiC are observed as a pair of peaks due to atropisomerism in solution which was also observed by Wagner *et al.*[18].

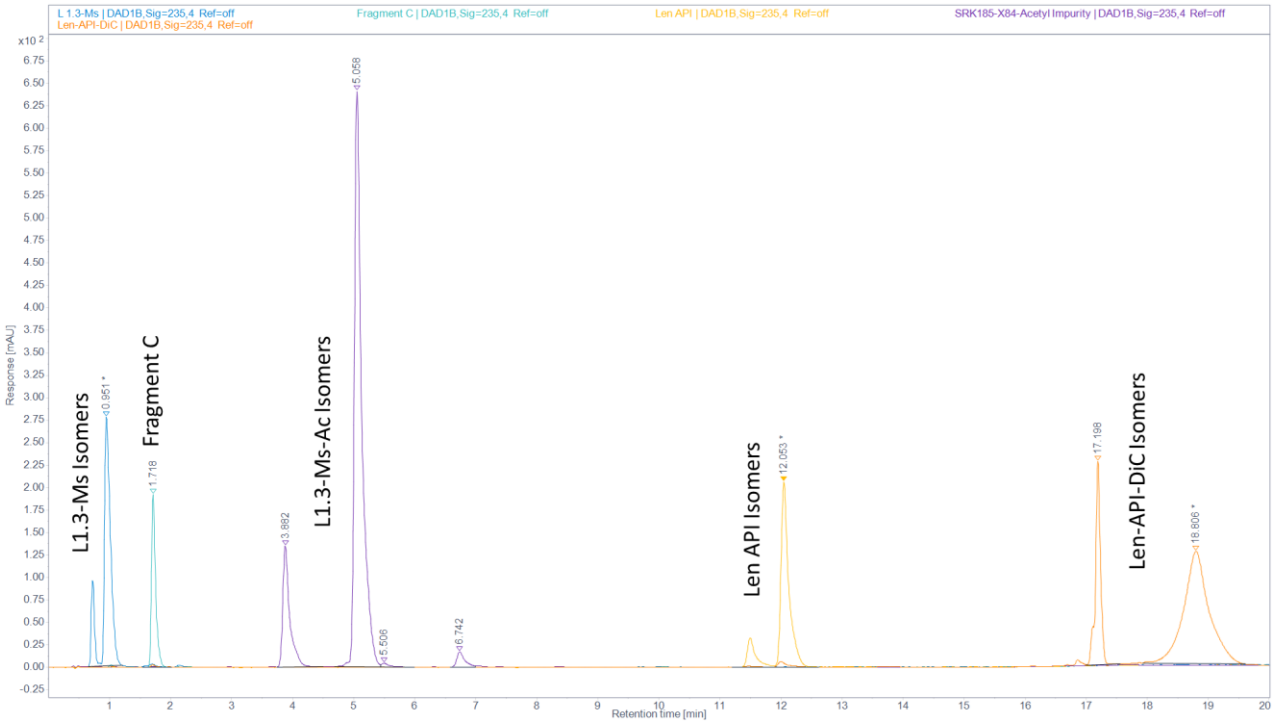


Figure 2.3.2.1.1. Representative chromatogram for Len-API synthesis.

2.3.2.1.1 Len-API Relative Response Factors

Samples of Len-API, impurities and synthetic intermediates were prepared at known concentrations and injected using the above method with a range of detection wavelengths to select an optimal wavelength for purity evaluation and reaction monitoring Figure 2.3.2.1.1.1. No true isosbestic point exists for this suite of analytes. As such, 235 nm was chosen for quantitation and monitoring purposes.

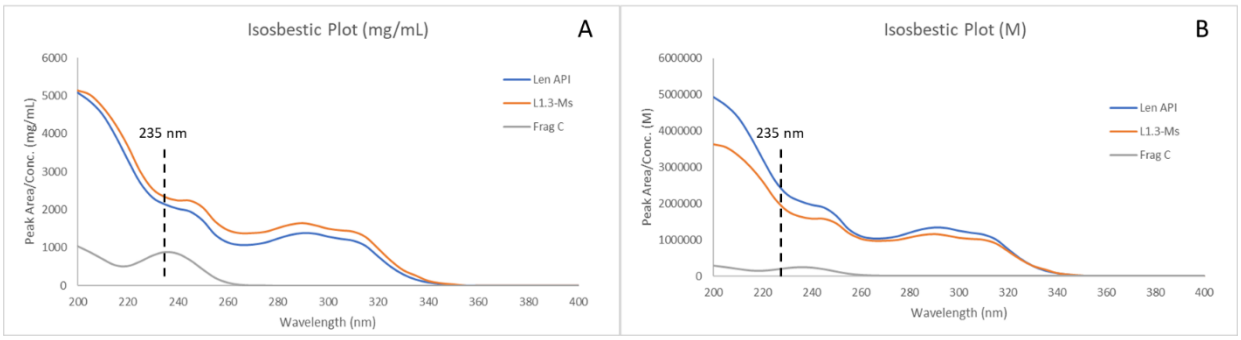


Figure 2.3.2.1.1.1. A) Isosbestic plot of Len-API and associated analytes based on mg/mL. B) Isosbestic plot of Len-API and associated analytes based on molarity (M).

Relative response factors (RRFs) were subsequently determined for starting materials and each available known impurity using the method put forth in Section 2.3.2.1 (Table 2.3.2.1.1). Impurities were synthesized and purified in-house (refer to GFN-002-PDR). Samples were prepared at 1 mg/mL in acetonitrile. Relative response factors were calculated using Equation 2.3.2.1.1. Table 2.3.2.1.1 lists the approximate retention times and RRFs (based on mg/mL and M basis) for each analyte.

$$\mathrm{RRF} = \frac{\left(\mathrm{Area}/\mathrm{Concentration}\right)_{\mathrm{Analyte}}}{\left(\mathrm{Area}/\mathrm{Concentration}\right)_{\mathrm{L1.3-Boc}}} \qquad \text{Eqn. 2.3.2.1.1}$$

Table 2.3.2.1.1. Relative response factors for Milestone 1 are calculated based on concentration (mg/mL)

Compound	Retention Time (min)	RRF at 235 nm (mg/mL)	RRF at 235 nm (M)
L1.3-Ms	0.80 / 0.98 / 1.19[a]	1.09	0.80
Frag C	1.72	0.41	0.12
L1.3-Ms-Ac	3.88 / 5.05	-[b]	-[b]
Len-API (Product)	11.50 / 12.05 [a]	1.00	1.00
Len-API-DiC	17.2 / 18.8	-[b]	-[b]

[a]Isomers, all RRFs were calculated using the sum of the isomer peak areas. [b]Not calculated.

2.3.2.1.2 Len-API Linearity

Len-API response at 235 nm was linear between 0.5 – 1.6 mg/mL. A minimum of 5 standard levels were used to calculate the curve with a linear fit of $R^2 > 0.99$ (Figure 2.1.2.1.2.1).

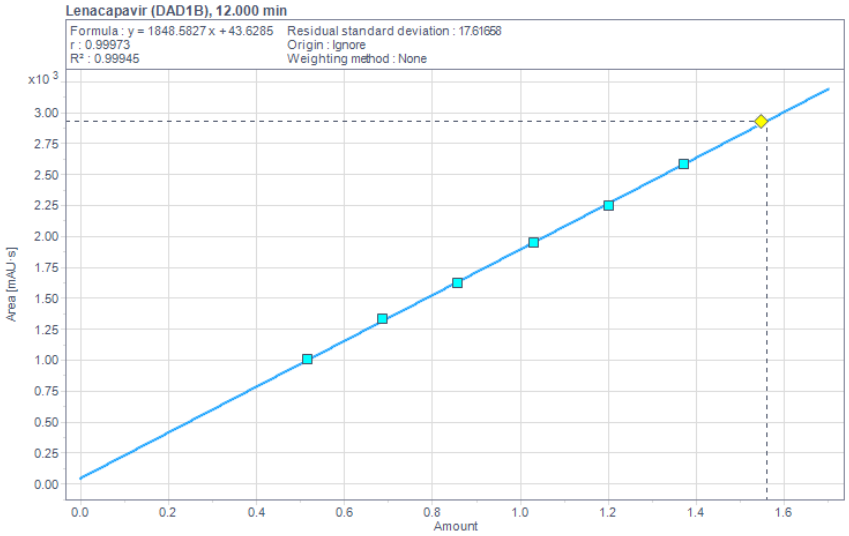


Figure 2.1.2.1.2.1. Linear range of L1.3-Boc.

2.3.2.1.3 Len-API Impurities Limit of Detection (LOD)

Impurity reference standards for the API-forming step of the synthesis were not generated as of the writing of this report.

2.3.2.2 Len-API Sodium Salt Analysis

The same method utilized in Milestone 1 for salt analysis was utilized in Milestone 2. Refer to Section 2.1.2.2 and Appendix 3.5 for further details.

2.3.2.2.1 Linearity

The linearity of the sodium assay was evaluated using the Salts-HILIC method. A minimum of five standard concentrations were used to establish the calibration curve. The data were best described by a quadratic fit with a coefficient of determination (R^2) of 0.9939 over the concentration range of 0.05 to 0.35 mg/mL, satisfying the acceptance criterion of $R^2 > 0.99$ (Figure 2.3.2.2.1.1).

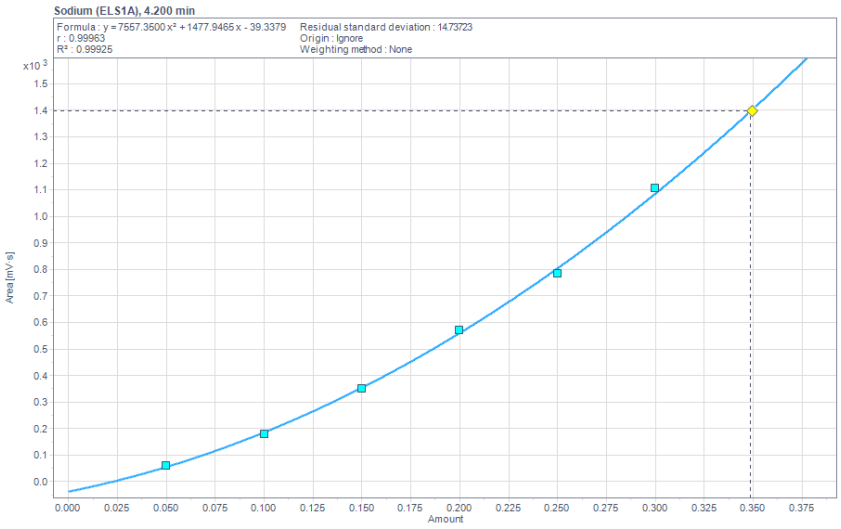


Figure 2.3.2.2.1.1. Linear range of sodium.

2.3.2.2.2 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were not determined for sodium.

2.3.2.3 Len-API Solvent Analysis

The same method utilized in Milestone 1 for solvent analysis was utilized in Milestone 2. Refer to Section 2.1.2.3 and Appendix 3.3 for further details.

2.3.2.4 Len-API Palladium Elemental Analysis

The same method utilized in Milestone 1 for Pd analysis was utilized in Milestone 2. Refer to Section 2.1.2.4 and Appendix 3.6 for further details.

2.3.2.5 Len-API LC-MS Analysis

The same method utilized in Milestone 1 for LC-MS analysis was utilized in Milestone 2. Refer to Section 2.1.2.5 and Appendix 3.4 for further details.

2.3.3 Len-API Impurities

2.3.3.1 Starting Material Impurities

Impurities were not specified nor determined for Len-API starting materials.

2.3.3.2 *Synthesis Impurities*

The production of Len-API has the potential to generate impurities during synthesis. These impurities range from unreacted starting materials, impurities carried over from the Milestone 2 step, or the amide coupling of fragment C on the incorrect amine (Figure 2.3.3.2).

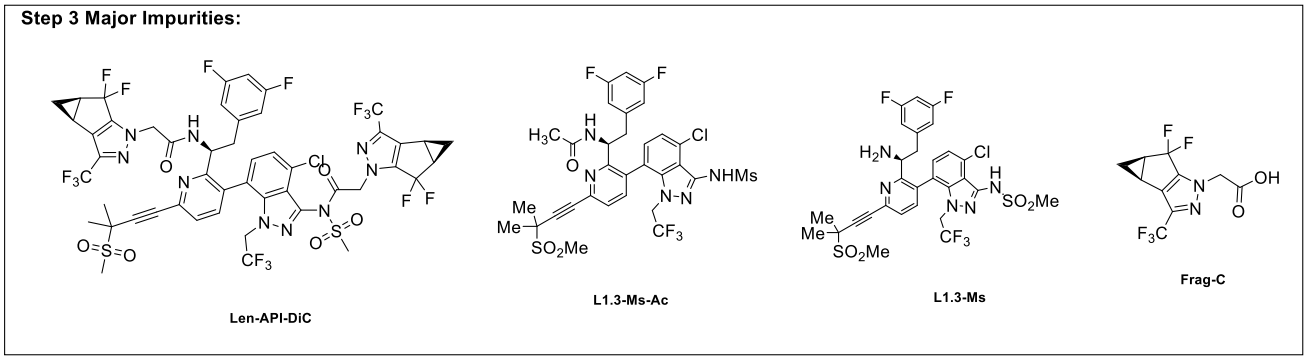


Figure 2.3.3.2 Impurities of Milestone 2.

2.3.4 Len-API Forced Degradation Studies

Forced degradation studies were not performed for Len-API nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.3.5 Len-API Stability Testing

Stability studies were not performed for Len-API nor its starting materials, intermediates and impurities. (Not in project scope of work.)

2.3.6 Len-API Methods

Analytical methods used to support the synthesis Len-API are appended to this report.

2.3.6.1 *Len-API Key Starting Materials*

L 1.3-Ms was analyzed via LC-DAD using the method "LCUV_L13Ms" (Appendix 3.2).

Frag C was analyzed via LC-DAD using the method "LCUV_Len API_Gradient (235 nm)" (Appendix 3.1).

2.3.6.2 Len-API Reagents and Solvents

All reagents and solvents were analyzed via GC-MS using the method "Solvent" (Appendix 3.3).

2.3.6.3 Len-API Intermediates

All intermediates were analyzed via LC-DAD using the method "LCUV_L13Ms" (Appendix 3.2).

2.3.6.4 Len-API In-Process Controls (IPC)

The requirements for the IPC were as follows (LCUV_Len API_Gradient, 235 nm): L1.3-Ms NMT 0.1 %; Len-API greater than 99.5 % Final Product Analysis

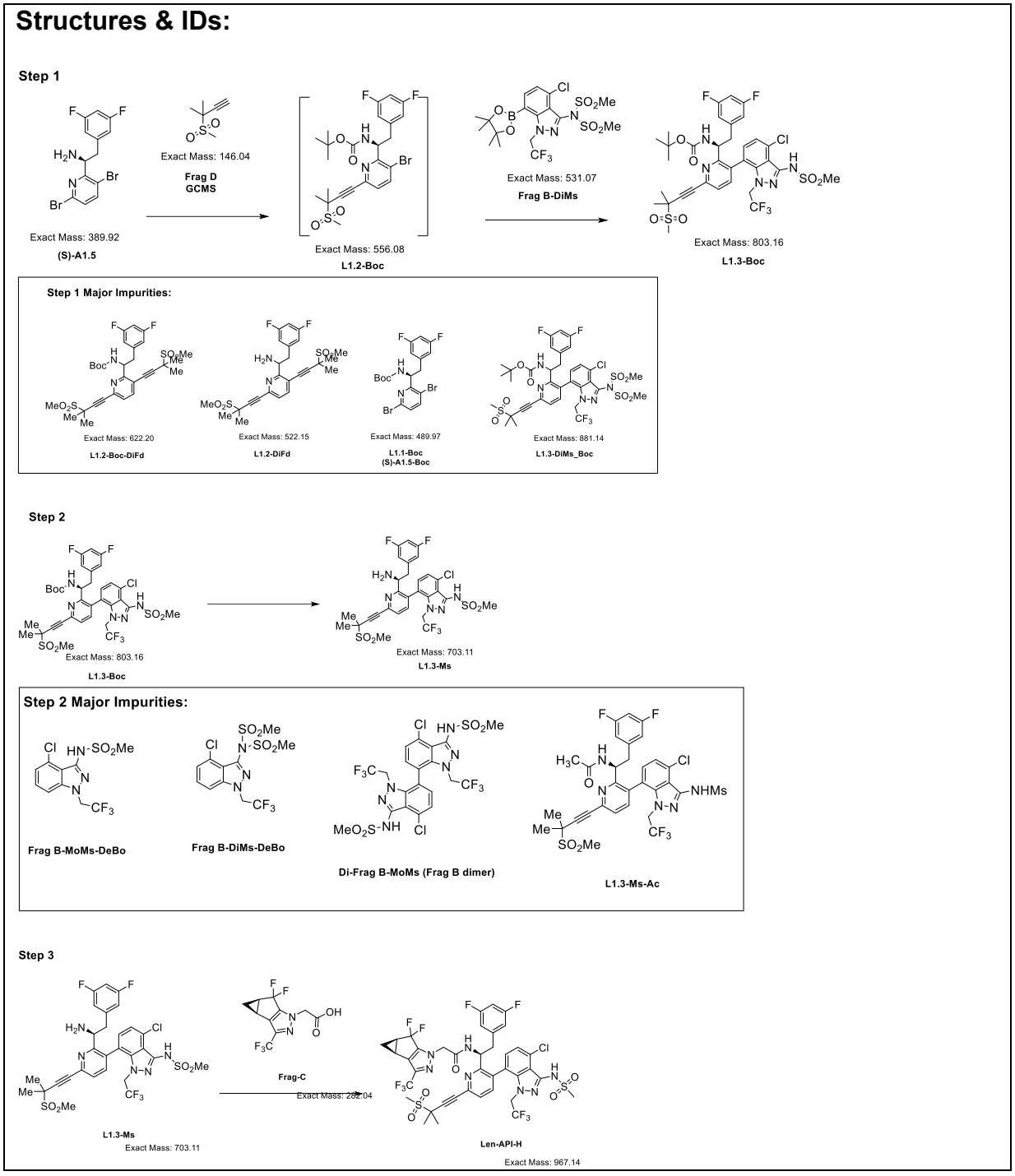
Isolated Len-API was assayed using a multitude of different techniques to assess product quality. This material was evaluated by the above-mentioned methods, LC-DAD for Area % of product and impurities, and weight % (LCUV_Len API_Gradient), ICP-OES (ICP-OES) for palladium content, Karl Fisher titration for water content, GC-FID for solvent content (Solvent), LC-MS (LCMSLen API_Gradient) for product and impurity conformation by *m/z*, and LC-ELSD (Salts-HILIC_ELSD) for sodium content.

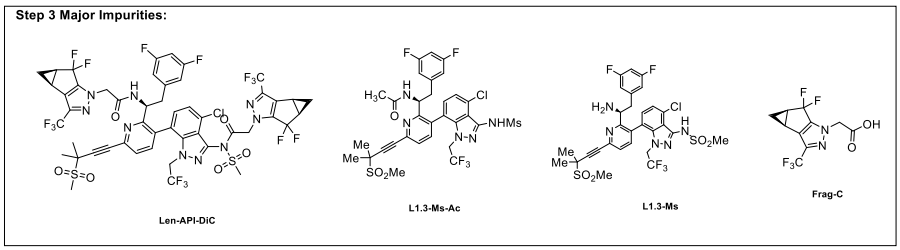
2.3.6.5 Len-API Method Appropriateness

During development of the Len-API certain performance characteristics were evaluated to select analytical conditions. These results are described above and include linearity. This method was not tested for specificity. Method validation was not performed. The latter two aspects were not in the project scope.

3 Appendix

3.1 LC-UV_Len API_Gradient Method





Conditions:

Column: Agilent ZORBAX Eclipse XDB-C18, 2.1 x 150 mm, 3.5 µm

Mobile Phase A: 0.1% phosphoric acid in water

Mobile Phase B: Acetonitrile

Injection volume: 1.0 µL Column temp: 30ºC Flow rate: 0.7 mL/min

Detector wavelength(s): Main: 275 nm (Step 1 and Step 2), 235nm (Step 3)

Sample preparation: Prepare samples at 1 mg/mL in acetonitrile

LC Gradient Table:

Time (min)	%A	%B
0.00	55	45
12.00	35	65
14.00	35	65
16.00	5	95
20.00	5	95

Post-run equilibration: 4 minutes

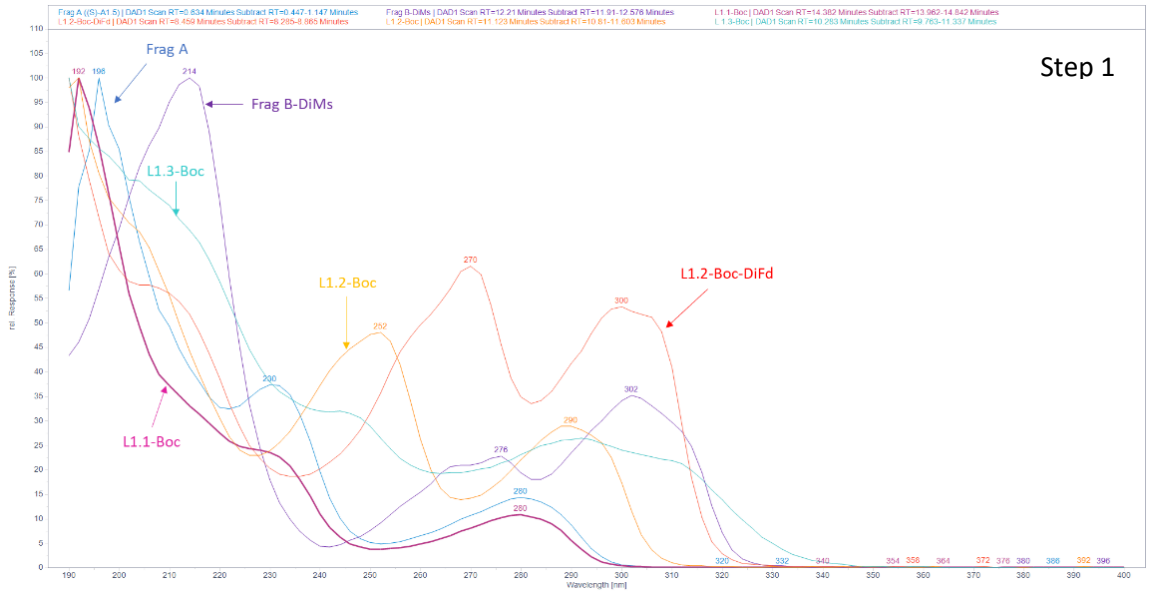
Retention Times			
Compound	**Time (min)**	**Relative RF (mg/mL) ***	**Relative RF (M)***
Step 1 - LenAPI_Step 1_Processing, 275 nm			
Frag A	0.56	0.54	0.26
L 1.2- DiFd	0.67	-	-
L 1.2-Boc-DiFd	8.46	1.83	1.42
L 1.3-Boc	9.94 / 10.28	1.00	1.00
L 1.2-Boc	11.12	0.75	0.52
Frag B-DiMs	12.30	0.41	0.27
L 1.3-DiMs-Boc	12.09 / 12.67	-	-
L 1.1-Boc (Frag A-Boc)	14.38	0.60	0.37
Step 2 - LenAPI_Step 2_Processing, 275 nm			
L 1.3-Ms	0.80 / 0.98 / 1.19	1.00	1.00
Frag B-MoMs-Debo	1.64	0.46	0.21
Frag B-DiMs-Debo	3.50	0.56	0.32

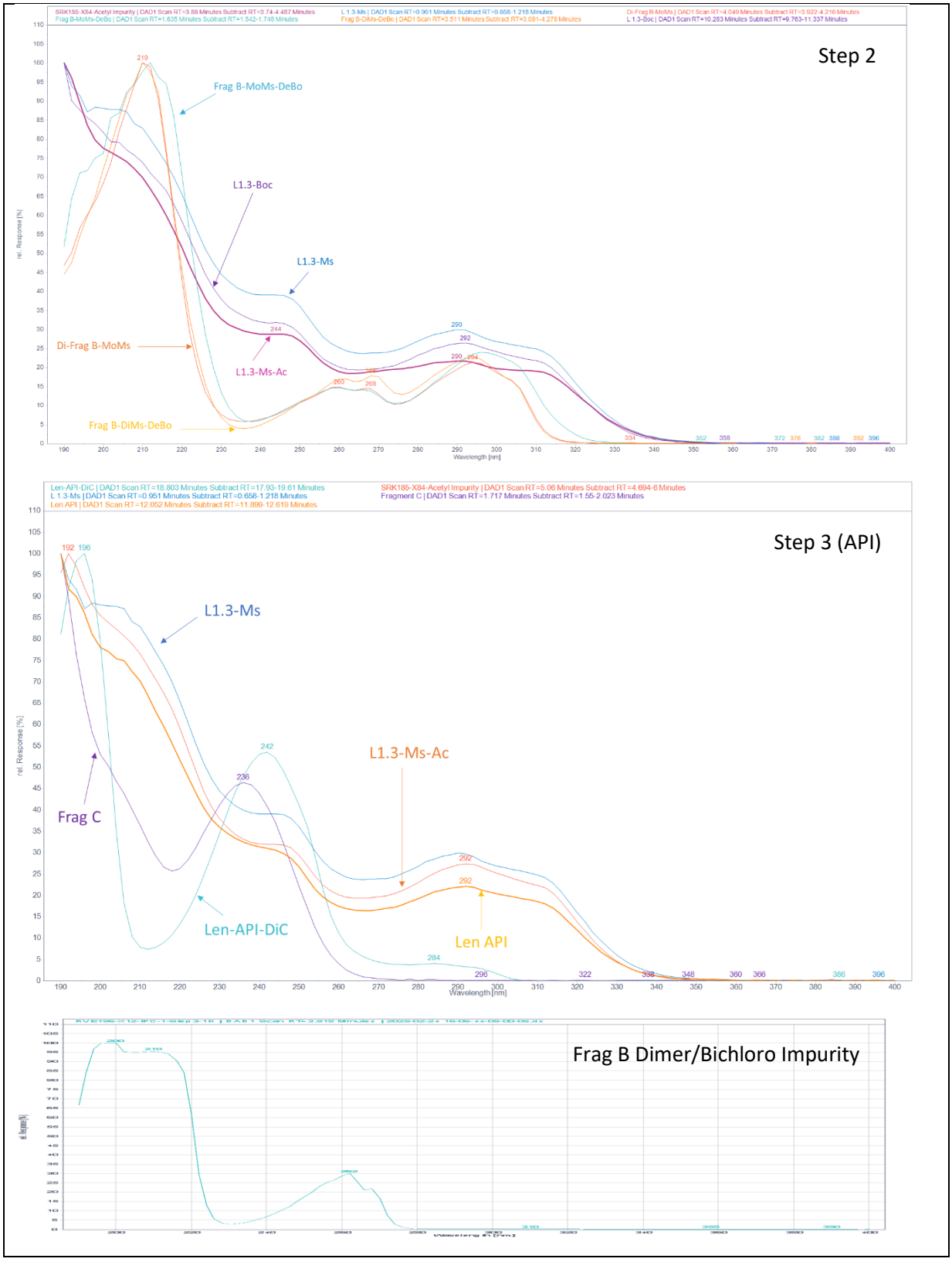
L1.3-Ms-Ac	3.88 / 5.06	-	-
Di-Frag B-MoMs (Frag B dimer)	4.05	0.51	0.47
L 1.3-Boc	9.94 / 10.28	0.85	0.97
Step 3 - LenAPI_Final Step_Processing, 235 nm			
L 1.3-Ms	0.80 / 0.98 / 1.19	1.09	0.80
L1.3-Ms-Ac	3.88 / 5.06	-	-
Frag C	1.72	0.41	0.12
Len API	11.50 / 12.05	1.00	1.00
Len-API-DiC	17.2 / 18.8	-	-

Notes: Frag D has very low absorbance. For processing samples use the processing method associated with the step.

Where an analyte exists as multiple isomers, RRF was calculated using the sum of all isomer peaks.

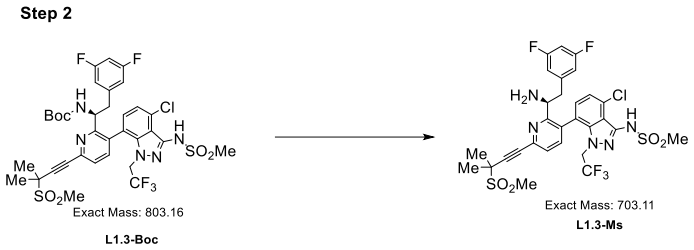
Representative UV Spectra




Step 2
Frag B-MoMs-DeBo
L1.3-Boc
L1.3-Ms
Di-Frag B-MoMs
L1.3-Ms-Ac
Frag B-DiMs-DeBo
rel. Response [%]
Wavelength [nm]
Step 3 (API)
L1.3-Ms
L1.3-Ms-Ac
Frag C
Len-API-DiC
Len API
rel. Response [%]
Wavelength [nm]
Frag B Dimer/Bichloro Impurity

3.2 LCUVA_L 1.3-Ms Method

Structures & IDs:



Conditions:

Column: Agilent ZORBAX Eclipse XDB-C18, 2.1 x 150 mm, 3.5 µm

Mobile Phase A: 0.1% phosphoric acid in water

Mobile Phase B: Acetonitrile

Injection volume: 1.0 µL

Column temp: 30°C

Flow rate: 0.7 mL/min

Detector wavelength(s): Main: 275 nm

LC Gradient Table:

Time (min)	%A	%B
0.00	75	25
10.00	50	50
11.00	5	95
20.00	5	95

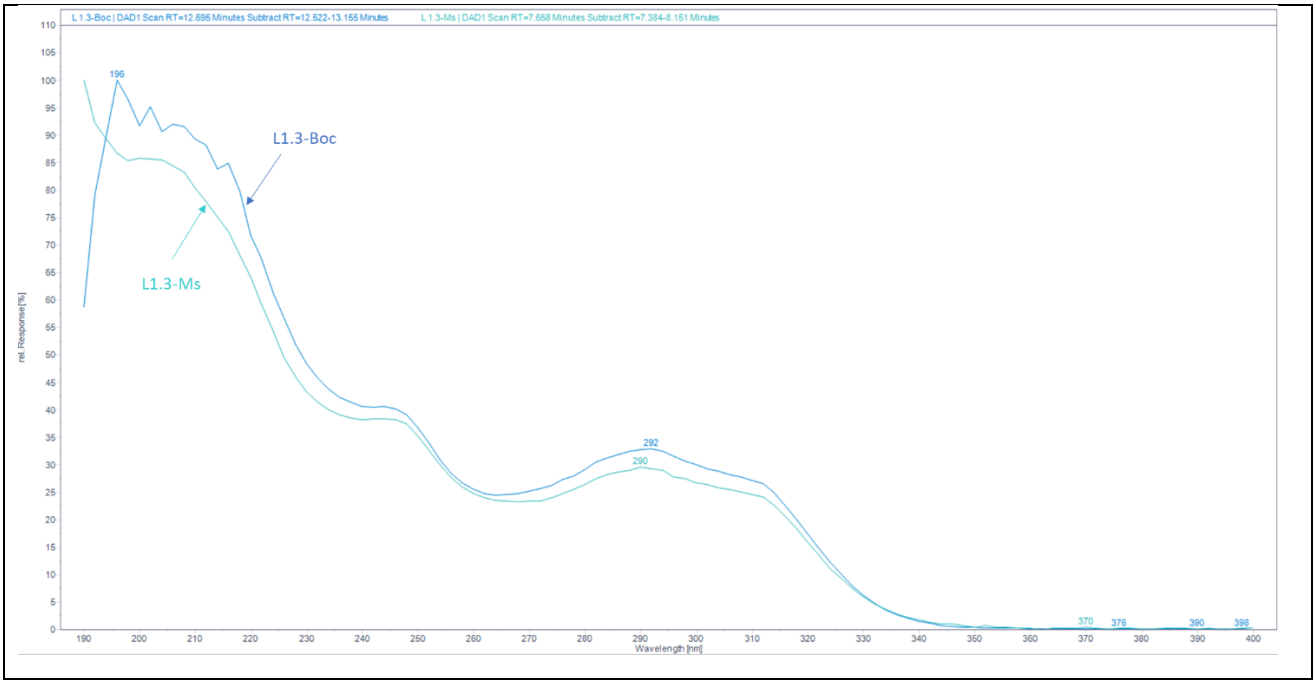
Post-run equilibration: 4 minutes

Sample preparation: Prepare samples at 1 mg/mL in acetonitrile

Retention Times			
Compound	**Time (min)**	**Relative RF (mg/mL) ***	**Relative RF (M)***
Step 2 - LenAPI_Step 2_Processing, 275 nm			
L 1.3-Ms	6.04 / 7.66	1.00	1.00
L 1.3-Boc	12.70	0.92	1.05

Notes: Where an analyte exists as multiple isomers, RRF was calculated using the sum of all isomer peaks.

Representative UV Spectra



3.3 Solvent Method (GC-FID)

Conditions:

Column: HP-1; 30M X 320 µm; 5 µm film

Inlet Pressure: 4.8 psi | Split Ratio: 50:1 | Split Flow: 39.439 mL/min

Column flow: 0.787 mL/min | Injection Temp: 260 °C | Injection volume: 1 µL

Solvent Delay: N/A | Runtime: 20 min

Temperature Program:

Time (min)	Temp (°C)	Ramp (°C/min)	Hold (min)
0	50	0	5
20	235	20	5.75

FID Parameters:

Heater (°C)	200
Air Flow (mL/min)	450
H_2 Fuel Flow (mL/min)	35
N_2 Makeup Flow (mL/min)	30

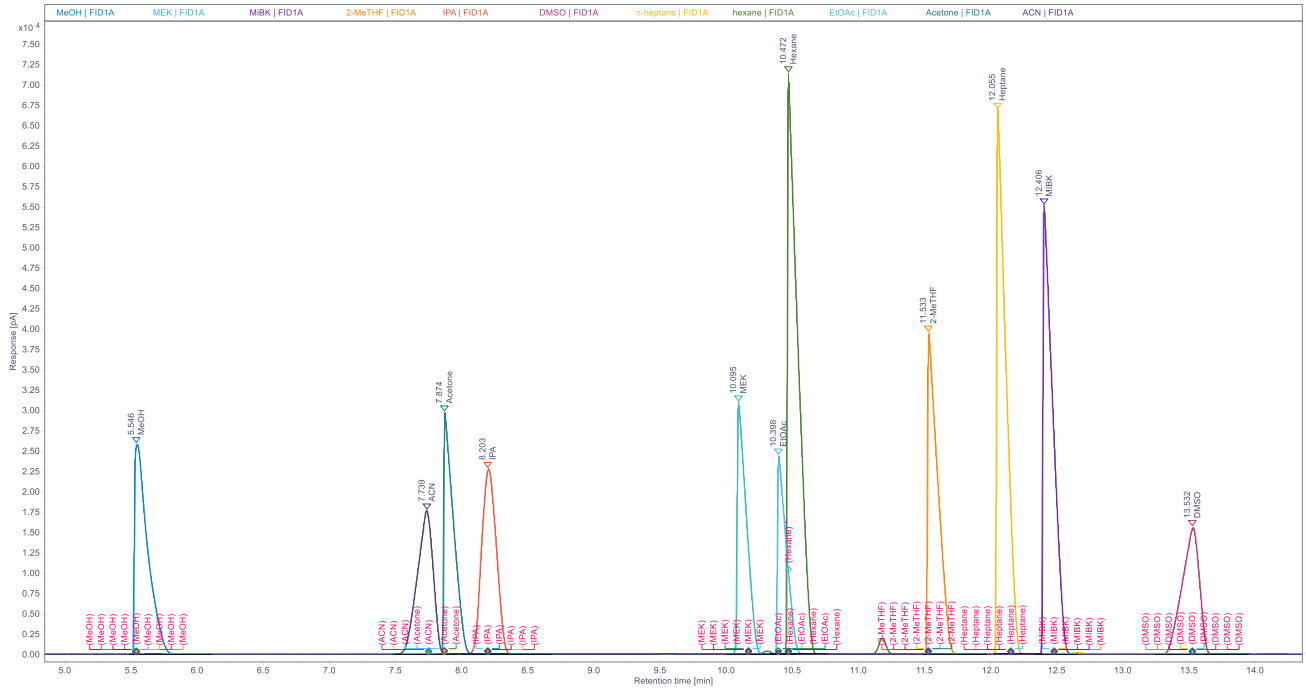
Sample preparation: Prepare samples at 5-10 mg/mL in acetonitrile or other suitable solvent.

Retention Times

Compound	Time (min)	Compound	Time (min)
Methanol (MeOH)	5.46	Heptane (n-heptane)	12.12
Acetonitrile (ACN)	7.65	Methyl isobutyl ketone (MIBK)	12.46
Acetone	7.87	Toluene	13.05
2-Propanol (IPA)	8.2	Dimethyl Sulfoxide (DMSO)	13.37
Methyl *t*-Butyl Ether (MTBE)	9.85	Hexanes	10.26, 10.52, 10.63
2-Butanone (MEK)	10.10		
Ethyl Acetate (EtOAc)	10.41		

n-Hexane (hexane)	10.52		
Chloroform	10.63		
2-Methyl Tetrahydrofuran (2-MeTHF)	11.62		

Representative Chromatograms



3.4 LCMS Len API_Gradient Method (LC-MS)

Instrument Type: Agilent 1260 liquid chromatograph (LC) with diode array detector (DAD) and 6125 mass spectrometer detector (MSD)

Conditions:

Column: Agilent ZORBAX Eclipse XDB-C18, 2.1 x 150 mm, 3.5 µm

Mobile Phase A: 0.1% formic acid in water

Mobile Phase B: 0.1% formic acid in Acetonitrile

Injection volume: 1.0 µL Column temp: 30°C Flow rate: 0.7 mL/min

Detector wavelength(s): Main: 235nm, 275nm

LC Gradient Table:

Time (min)	%A	%B
0.00	55	45
12.00	35	65
14.00	35	65
16.00	5	95
25.00	5	95

Post-run equilibration: 5 minutes

MSD Parameters:

Source Parameters			Signal 1 (+)
Gas Temp.	350 ˚C	Time (min)	0.00
Drying Gas	11 L/min	Mass Range	40-2000
Neb. Pressure	35 psig	Fragmentor	15
Quad Temp.	100 ˚C	Gain EMV	1.00
VCap (+)	4000 V	Threshold	0

VCap (-)	4000 V	Step Size	0.10
		% Cycle Time	50

Sample preparation: Prepare samples at 1 mg/mL in acetonitrile

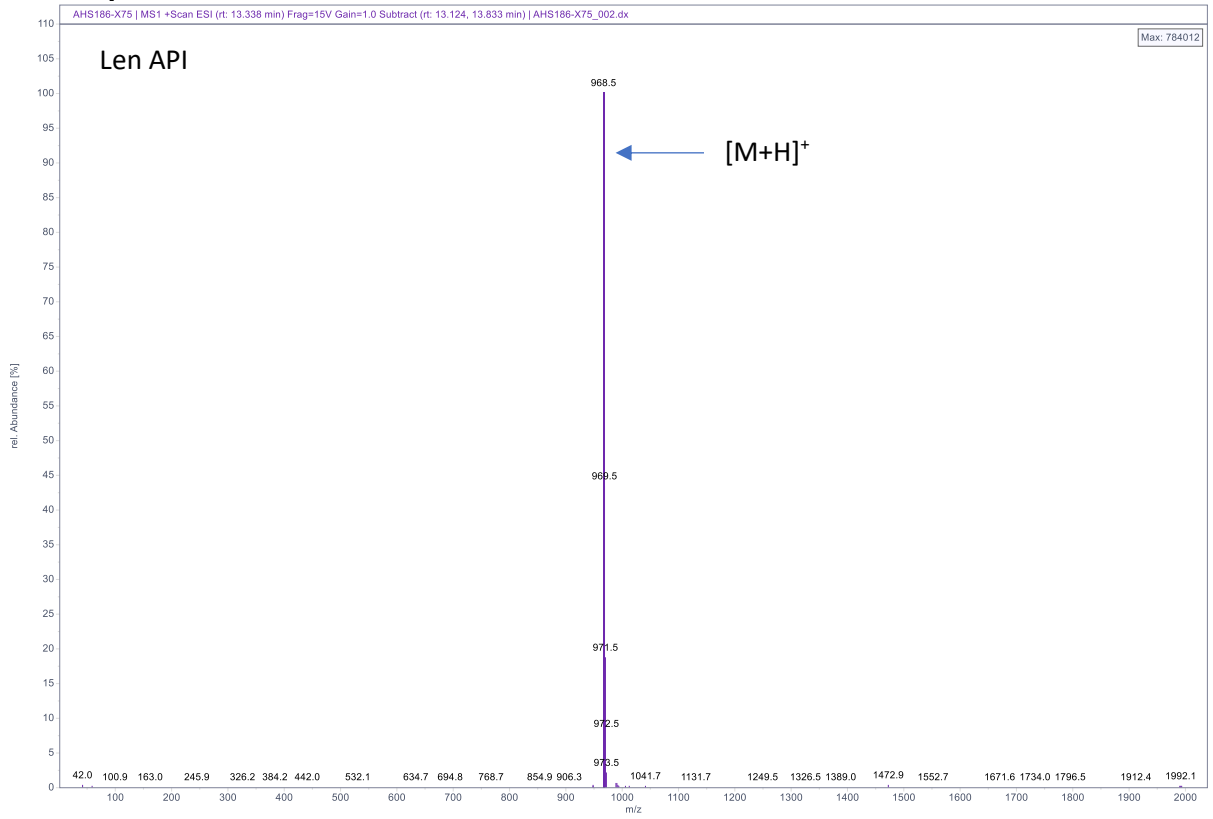
Retention Times

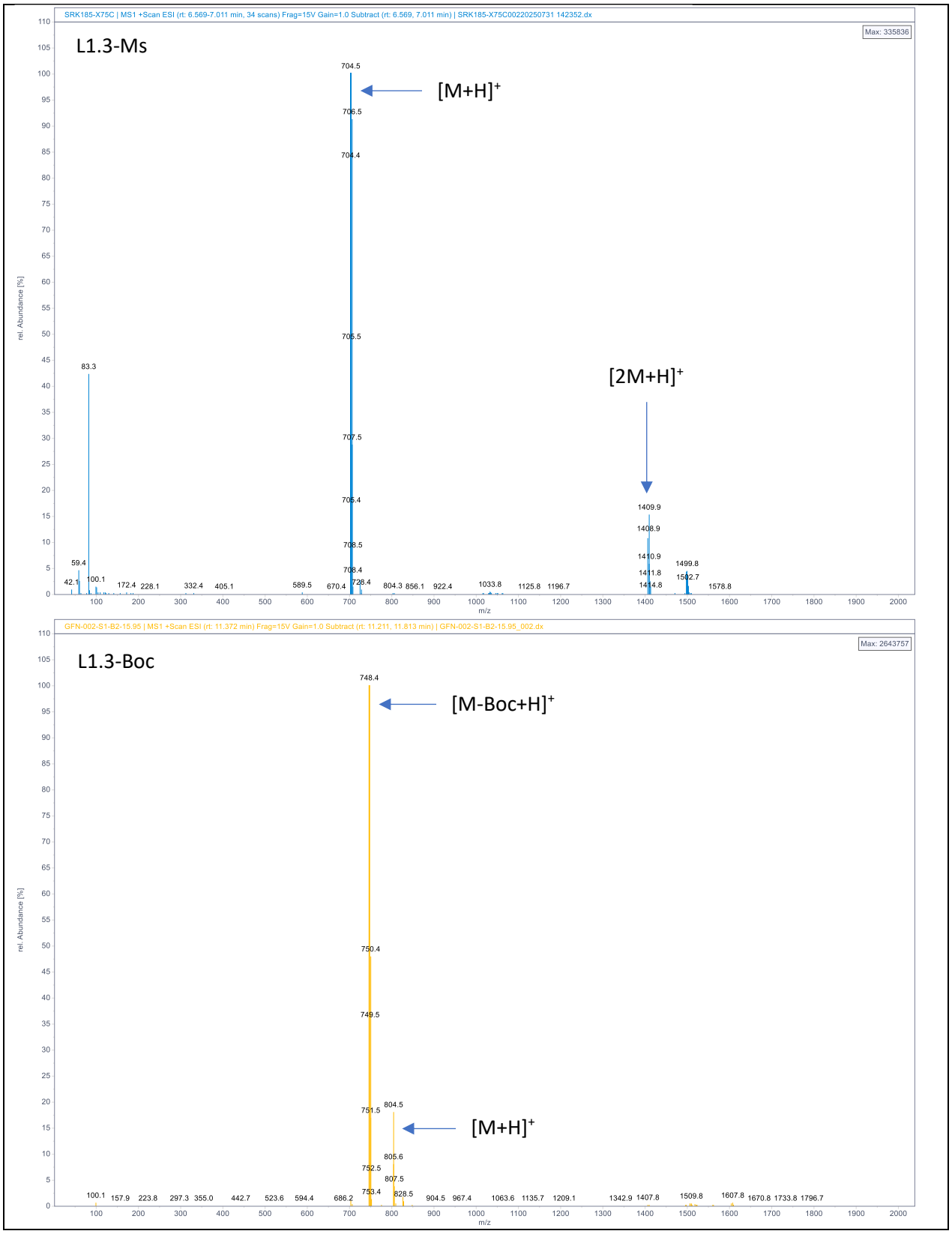
Compound	Time (min)	*m/z*
L 1.3-Ms	0.9 / 1.0 / 1.2	704 $[M+H]^+$; 1407 $[2M+H]^+$
Frag C	2.0	283 $[M+H]^+$
Frag B-DiMs-Debo	3.7	406 $[M+H]^+$
L1.3-Ms	4.8 / 6.1	746 $[M+H]^+$
Di-Frag B-MoMs	5.1	653 $[M+H]^+$; 1327 $[2M+Na]^+$
(S)-A 1.5 (Len A)	5.7	393 $[M+H]^+$; 434 $[M+ACN+H]^+$
L 1.2-Boc	10.6	503 $[M-Boc+H]^+$; 537 $[M+H]^+$
Frag B-DiMs	10.7	532 $[M+H]^+$; 549 $[M+NH_4]^+$
L 1.3-Boc	10.9/11.3	748 $[M-Boc+H]^+$; 804 $[M+H]^+$
Len API (Len API-H)	12.9 / 13.5	968 $[M+H]^+$
L 1.1-Boc	15.5	437 $[M+H]^+$
Len API – DiC*	17.5	1233 $[M+H]^+$

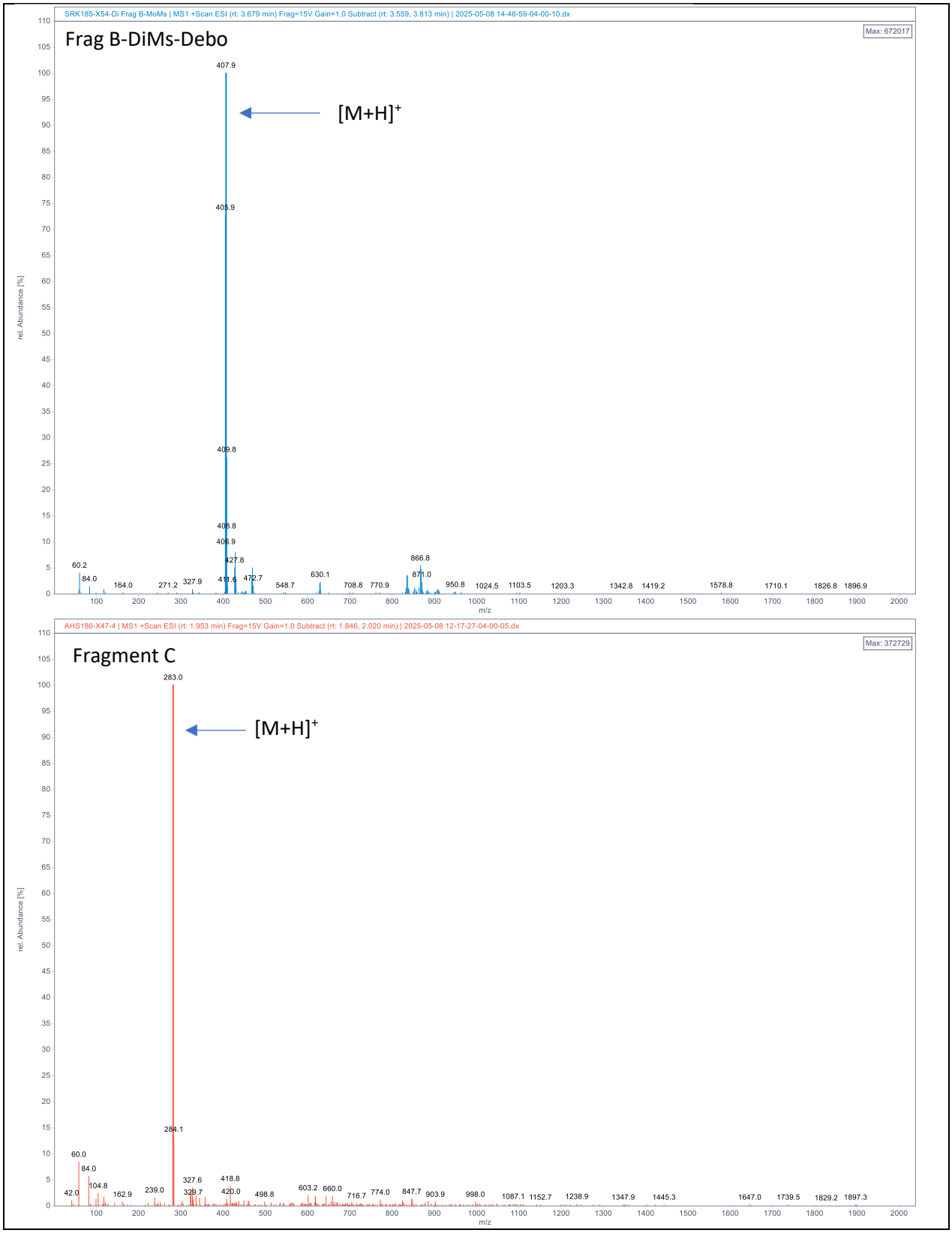
*Speculated based on mass, impurity not isolated for full characterization or other method development.

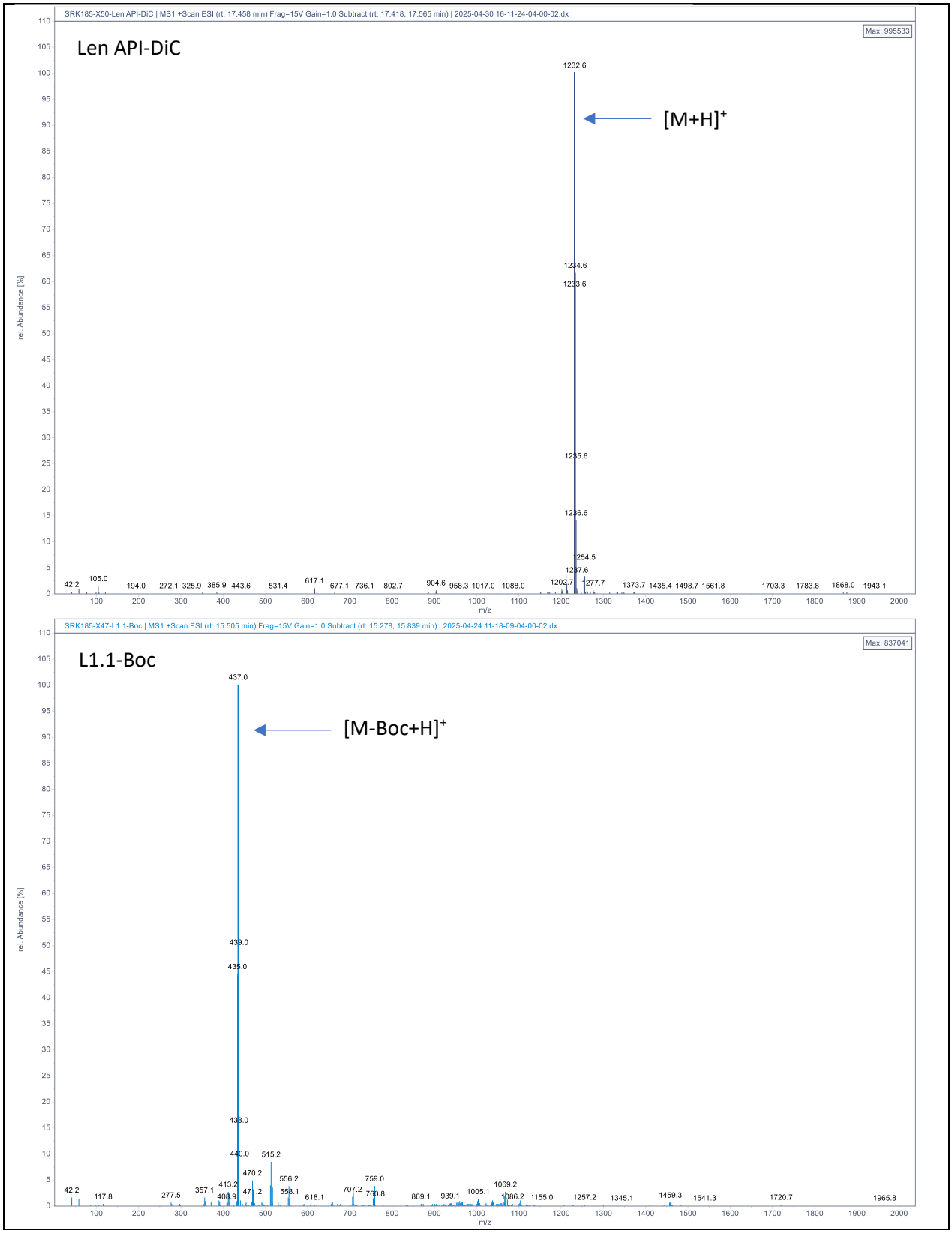
Notes: Frag D exhibits low UV absorbance making it difficult to detect via this method. L 1.3-Ms has three peaks

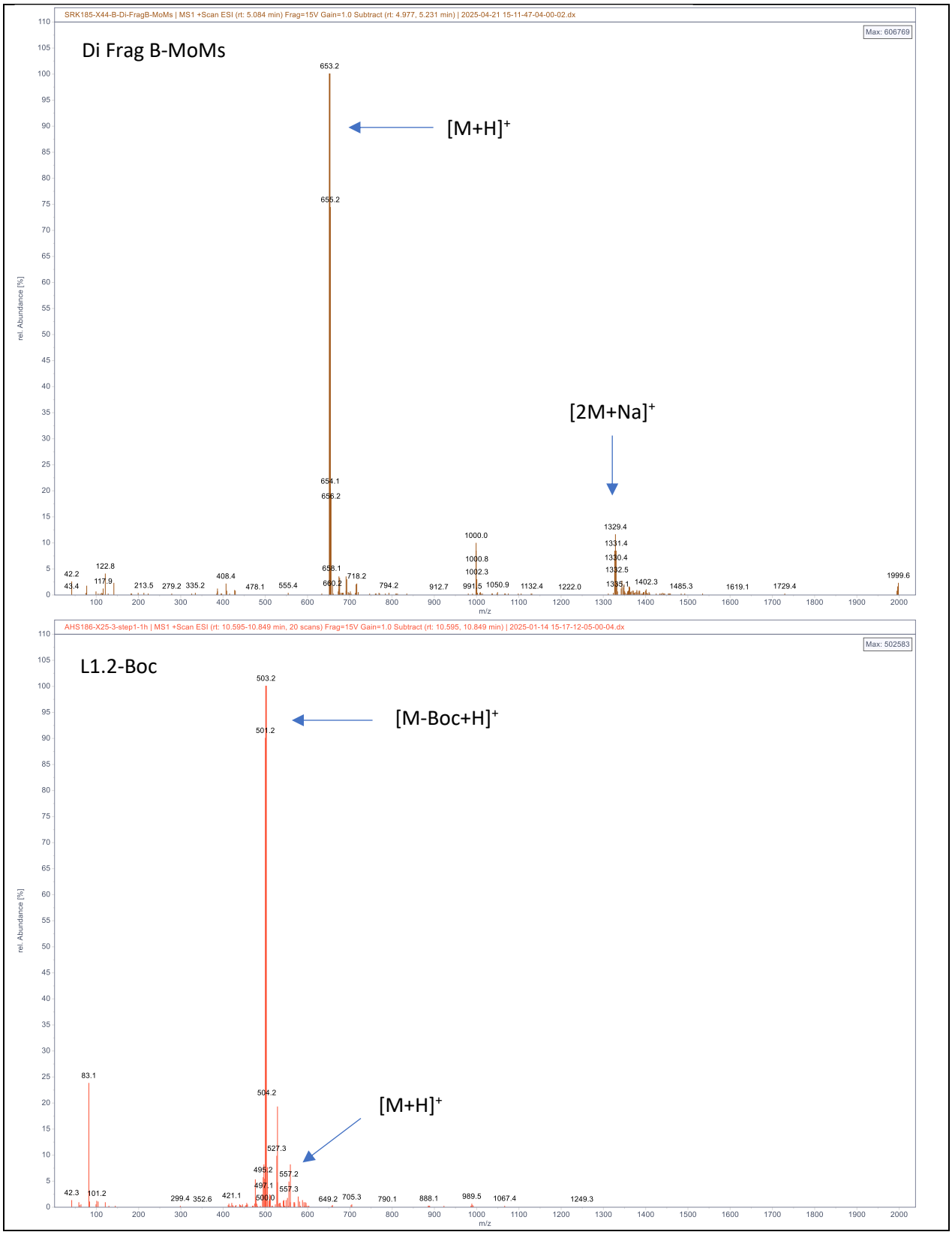
Mass Spectra

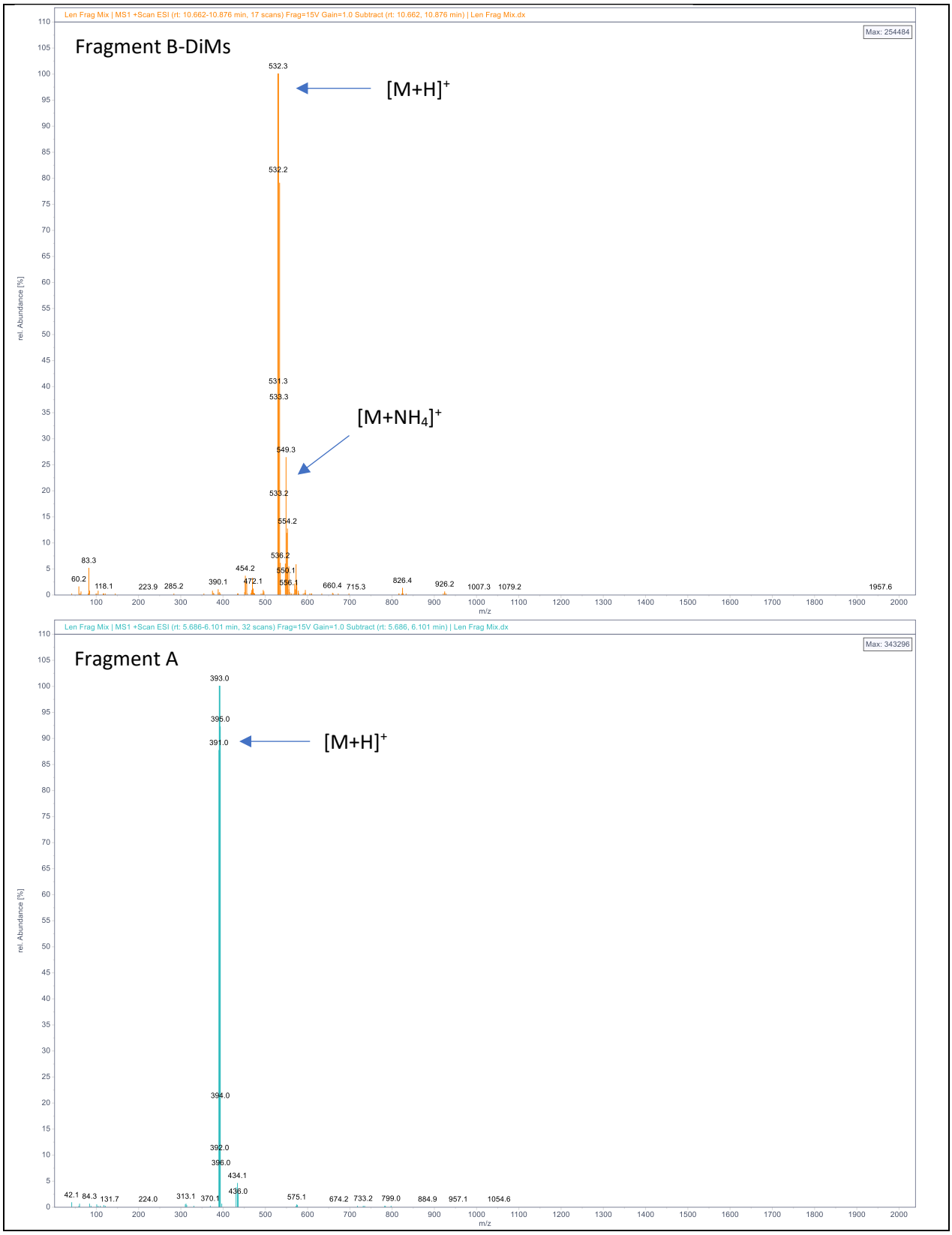



SRK185-X75C | MS1 +Scan ESI (rt: 6.569-7.011 min, 34 scans) Frag=15V Gain=1.0 Subtract (rt: 6.569, 7.011 min) | SRK185-X75C00220250731 142352.dx
Max: 335836
L1.3-Ms
[M+H]+
[2M+H]+
rel. Abundance [%]
m/z
GFN-002-S1-B2-15.95 | MS1 +Scan ESI (rt: 11.372 min) Frag=15V Gain=1.0 Subtract (rt: 11.211, 11.813 min) | GFN-002-S1-B2-15.95_002.dx
Max: 2643757
L1.3-Boc
[M-Boc+H]+
[M+H]+
rel. Abundance [%]
m/z


SRK185-X54-Di Frag B-MoMs | MS1 +Scan ESI (rt: 3.679 min) Frag=15V Gain=1.0 Subtract (rt: 3.559, 3.813 min) | 2025-05-08 14-46-59-04-00-10.dx
Frag B-DiMs-Debo
Max: 672017
[M+H]+
rel. Abundance [%]
m/z
AHS186-X47-4 | MS1 +Scan ESI (rt: 1.953 min) Frag=15V Gain=1.0 Subtract (rt: 1.846, 2.020 min) | 2025-05-08 12-17-27-04-00-05.dx
Fragment C
Max: 372729
[M+H]+
rel. Abundance [%]
m/z


SRK185-X50-Len API-DiC | MS1 +Scan ESI (rt: 17.458 min) Frag=15V Gain=1.0 Subtract (rt: 17.418, 17.565 min) | 2025-04-30 16-11-24-04-00-02.dx
Max: 995533
Len API-DiC
[M+H]+
1232.6
1234.6
1233.6
1235.6
1236.6
1254.5
rel. Abundance [%]
m/z
SRK185-X47-L1.1-Boc | MS1 +Scan ESI (rt: 15.505 min) Frag=15V Gain=1.0 Subtract (rt: 15.278, 15.839 min) | 2025-04-24 11-18-09-04-00-02.dx
Max: 837041
L1.1-Boc
[M-Boc+H]+
437.0
439.0
435.0
438.0
440.0
515.2
rel. Abundance [%]
m/z


SRK185-X44-B-Di-FragB-MoMs | MS1 +Scan ESI (rt: 5.084 min) Frag=15V Gain=1.0 Subtract (rt: 4.977, 5.231 min) | 2025-04-21 15-11-47-04-00-02.dx
Max: 606769
Di Frag B-MoMs
653.2
655.2
654.1
656.2
[M+H]+
[2M+Na]+
1329.4
1331.4
1330.4
1332.5
1000.0
1000.8
1002.3
rel. Abundance [%]
m/z
AHS186-X25-3-step1-1h | MS1 +Scan ESI (rt: 10.595-10.849 min, 20 scans) Frag=15V Gain=1.0 Subtract (rt: 10.595, 10.849 min) | 2025-01-14 15-17-12-05-00-04.dx
Max: 502583
L1.2-Boc
503.2
501.2
[M-Boc+H]+
83.1
504.2
527.3
[M+H]+
rel. Abundance [%]
m/z


Len Frag Mix | MS1 +Scan ESI (rt: 10.662-10.876 min, 17 scans) Frag=15V Gain=1.0 Subtract (rt: 10.662, 10.876 min) | Len Frag Mix.dx
Max: 254484
Fragment B-DiMs
[M+H]+
[M+NH4]+
rel. Abundance [%]
m/z
Len Frag Mix | MS1 +Scan ESI (rt: 5.686-6.101 min, 32 scans) Frag=15V Gain=1.0 Subtract (rt: 5.686, 6.101 min) | Len Frag Mix.dx
Max: 343296
Fragment A
[M+H]+
rel. Abundance [%]
m/z

3.5 LC-ELSD Salts-HILIC_ELSD Method

Instrument Type: Agilent 1100 liquid chromatograph (LC) with 1260 electron light scattering detector (ELSD)

Conditions:

Column: Agilent InfinityLab Poroshell 120 HILIC-Z, 3.0 x 150 mm, 2.7 µm
Mobile Phase A: 10 mM ammonium acetate, pH 4
Mobile Phase B: 90:10, acetonitrile: 10mM ammonium acetate, pH 4:
Injection volume: 1.5 µL Column temp: 30 °C Flow rate: 0.8 mL/min
Detector: ELSD

LC Gradient Table:

Time (min)	%A	%B
0	10%	90%
1	10%	90%
6	20%	80%
11	80%	20%
15	80%	20%

Post-run equilibration: 3 min

ELSD Parameters:

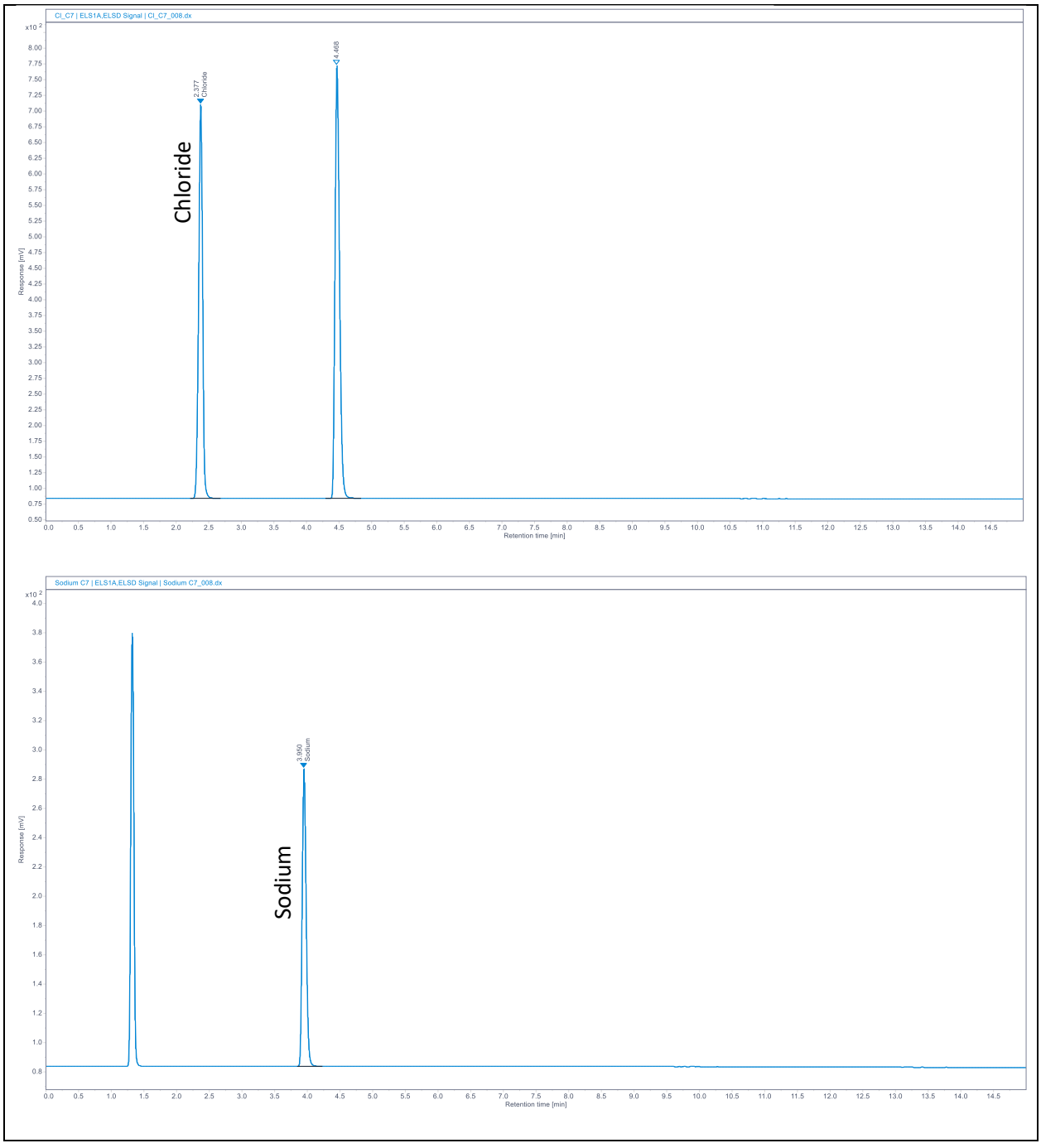
Evaporator Temp.	30 °C
Nebulizer Temp.	30 °C
Gas Flow Rate (SLM)	1.60
Data Rate (Hz)	80
LED Intensity	100%
Smoothing	30
PMT Gain	1.0

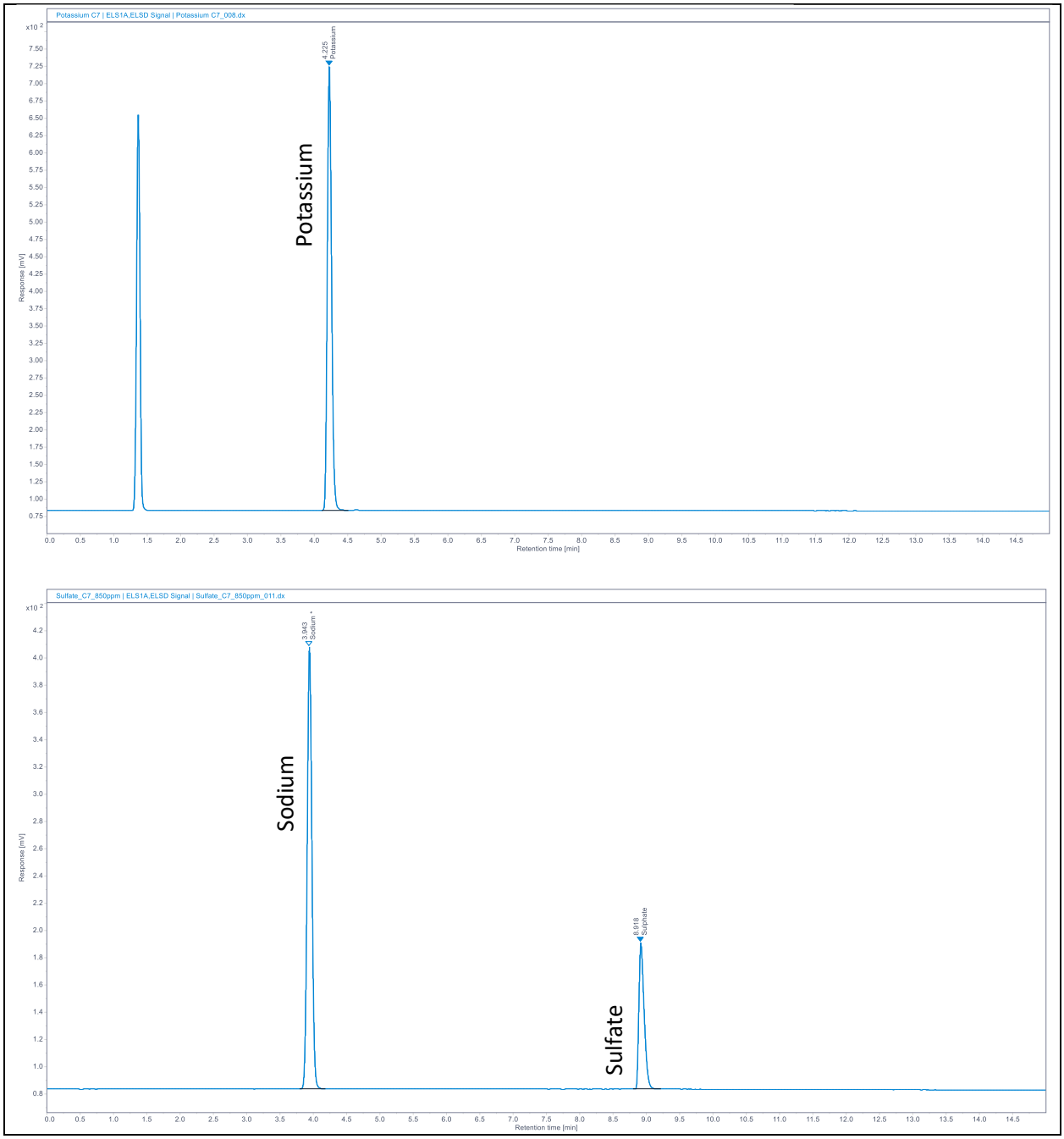
Sample preparation: 2mg/mL in appropriate solvent.

Retention Times

Compound	Time (min)
Chloride	2.38
Sodium	3.95
Potassium	4.23
Sulfate	8.92

Representative Chromatograms


Cl_C7 | ELS1A,ELSD Signal | Cl_C7_008.dx
x10 2
Response [mV]
Retention time [min]
2.377
Chloride
4.468
Chloride
Sodium C7 | ELS1A,ELSD Signal | Sodium C7_008.dx
x10 2
Response [mV]
Retention time [min]
3.950
Sodium
Sodium



3.6 ICP-OES Method

ICP-OES Conditions:			
RF Power (kW)	1.4	Spray Chamber	Glass Cyclonic
Plasm Gas Flow (L/min)	12.0	Nebulizer	SeaSpray
Auxiliary Gas Flow (L/min)	1.0	Uptake Rate (mL/min)	0.8
Nebulizer Gas Flow	0.7	View Mode	SVDV

Pressure (kPa)	220

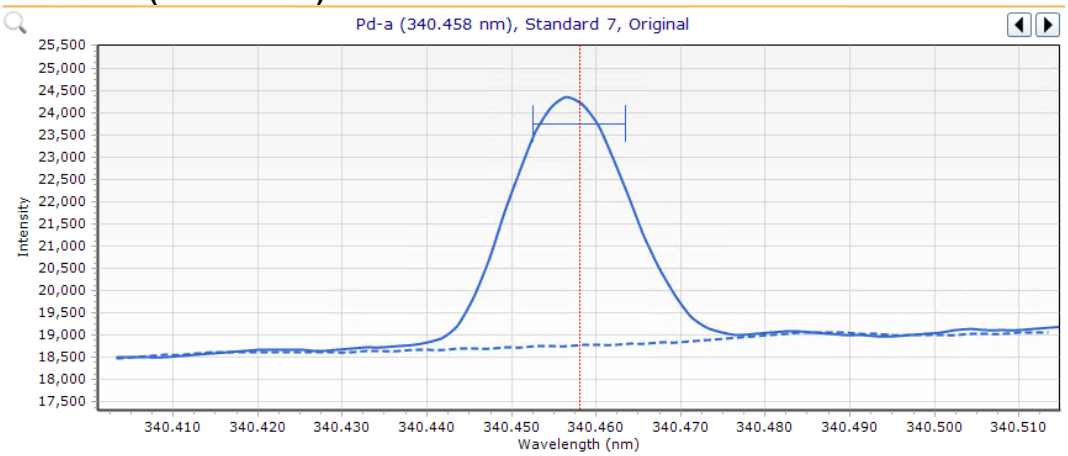
replicate read time (sec)	5
stabilization time (sec)	15
sample uptake delay time (sec)	10

Notes: The most intense emission line is generally selected for optimal sensitivity and a strong signal-to-noise ratio. Check for potential spectral interferences from other elements in the sample matrix, choose a secondary, less intense line that is free from any overlap.

Sample preparation: Weigh at least 50 mg of the sample and add 5 mL of 10% HCl in methanol. To dissolve the contents, vortex and/or sonicate the mixture. Transfer a 1 mL aliquot of this solution to a new tube, then bring the final volume to 10 mL with 10% HCl in deionized water. If any solids remain, filter the final solution using a 32mm PTFE syringe filter (0.45µm).

Representative Spectra

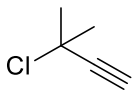
Palladium (340.458 nm)



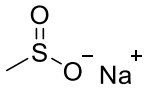
3.7 General_ThinFilm_100to1split_MS (GC-MS)

Instrument Type: Agilent 8890 gas chromatograph (GC) with a 5977 mass spectrometer detector (MSD)

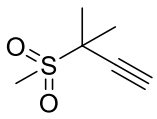
Structures & IDs:



Exact Mass: 102.02
3-CMB



Exact Mass: 101.98
Sodium methanesulfinate



Exact Mass: 146.04
Fragment D

Conditions:

Column: J&W HP-5ms GC Column, 30 m, 0.25 mm, 0.25 µm, 7 inch cage

Inlet Pressure: 6.71 psi | Split Ratio: 100:1 | Split Flow: 91.8 mL/min

Column flow: 0.92 mL/min | Injection Temp: 250°C | Injection volume: 1.0 µL

Total Flow: 95.7 mL/min Solvent Delay: 3.0 min Runtime: 19.0 min

Temperature Program:

Temp (˚C)	Ramp (˚C/min)	Hold (min)
50	-	3.0
250	25	3.0
300	25	3.0

MS Parameters:

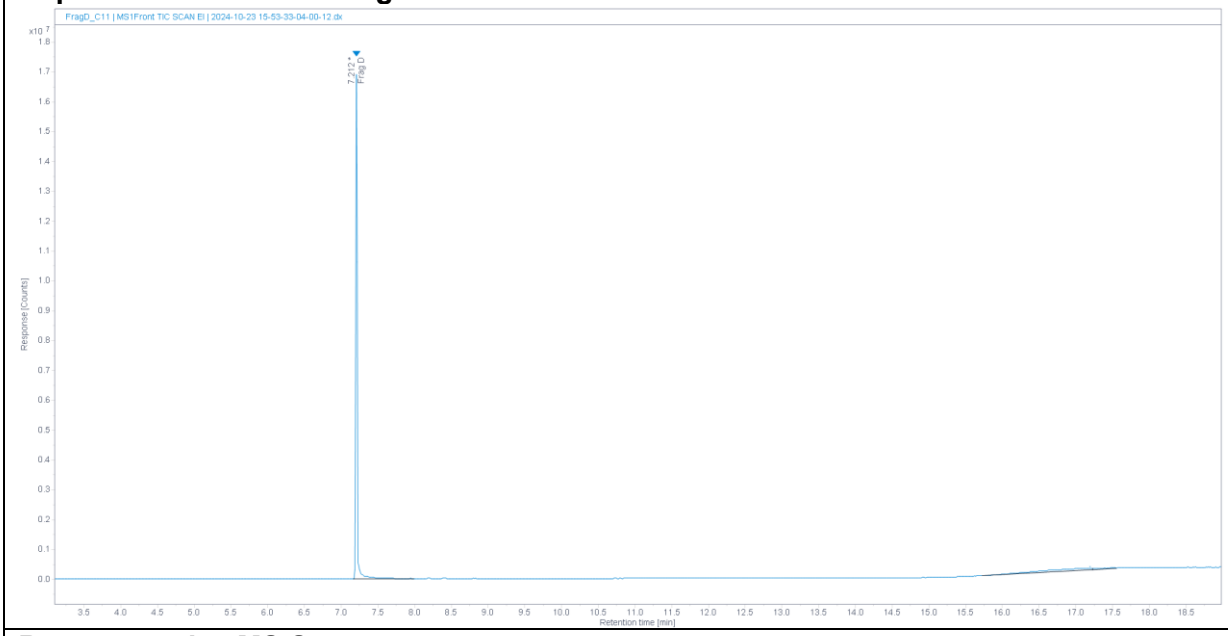
Transfer Line Temp (˚C)	250
Source Temp (˚C)	230
Quad Temp (˚C)	15
Electron Energy (eV)	70
Mass Range	40 - 700

Sample preparation: ~1.0 mg/mL in ACN

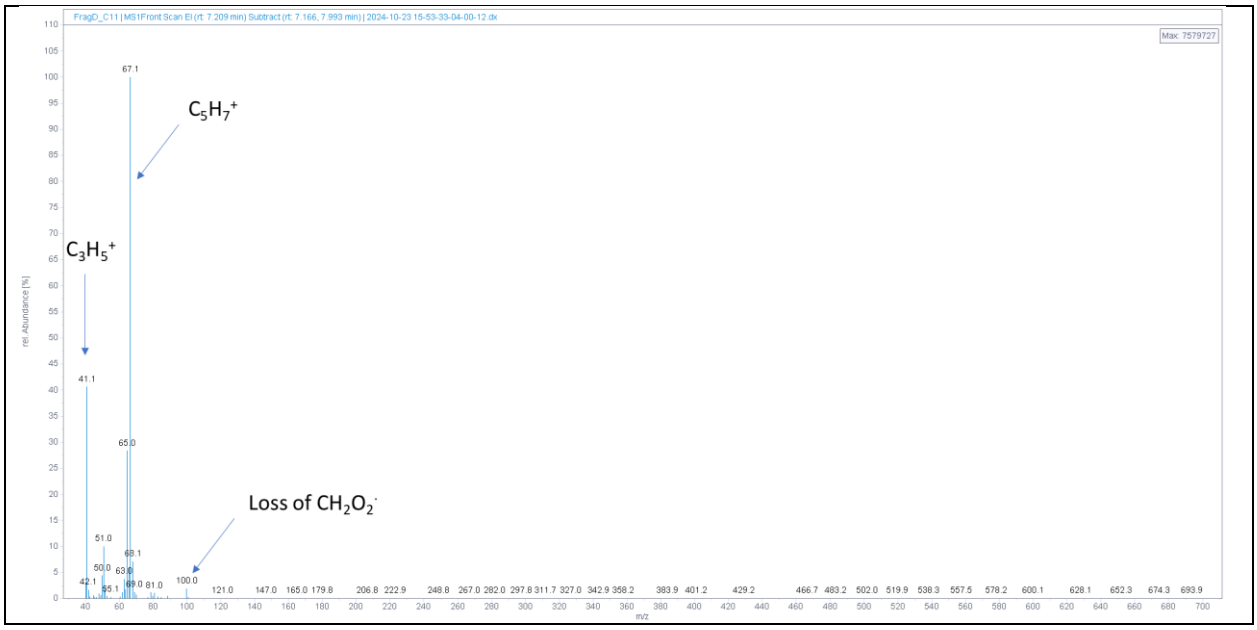
Retention Times

Compound	Time (min)	*m/z*
Fragment D	7.2	100, 67

Representative Chromatogram



Representative MS Spectra



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