Supercritical Fluid Chromatography MS/MS Method Development for the Analysis of GLP-1 Analogues

MP557

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1. Introduction

Supercritical Fluid Chromatography (SFC), originally developed in the 1960s, had faced significant barriers to adoption due to technological limitations. Modern SFC instrumentation has largely overcome these early challenges; however, a major limitation remains the lack of clear guidance regarding optimal stationary phase selection for a given separation. SFC commonly relies on empirical column screening approaches to identify the most effective stationary phase. Once an appropriate column is identified, additional optimization of method parameters can further improve separation performance. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) present a particularly challenging analytical target because they contain both peptide and lipid-like structural features. Given the widespread clinical and commercial interest in GLP-1 therapeutics, it is valuable to facilitate broader adoption of SFC for these analytes through:

- Screening of commonly used SFC stationary phases
- Optimization of LC-MS-compatible SFC method parameters
- Demonstration of GLP-1 RA quantitation and linearity

Establishing this groundwork may help lower barriers to SFC adoption while highlighting key advantages of the technique, including reduced solvent consumption and faster analysis times.

2. Methods

GLP-1 standards, liraglutide (Victoza/Saxenda) and semaglutide (Ozempic/Wegovy) were purchased from Cayman Chemicals. Samples were in suspended in 100% methanol. A Shimadzu Analytical SFC system was coupled to a Shimadzu LCMS-8050 Triple Quadrupole MS (**Figure 1)**. Ultimately, a DCPak® P4VP column was used for quantitative measurements. GLP-1 samples were separated at 1.1 mL/min. Mobile phase consisted of supercritical CO_2 and methanol-based mixes as a modifier. The makeup pump consisted of methanol + 0.1% formic acid to improve ionization efficiency.

Figure 1. Shimadzu Nexera Analytical SFC & LCMS-8050 Triple Quadrupole MS.

Table 1. Chromatographic and MS acquisition parameters.

Nexera Analytical SFC			
Mobile phase	A: CO_2 B: Methanol		
Analytical gradient (%B)	5% (0 min) ⇒ 85% (3-4.5 min) ⇒ 5% (4.6-6min)		
Column Oven temp.	68 ℃		
Flow rate	1.1 mL/min		
Makeup flow	0.1 mL/min		
BPR	150 bar at 50 ℃		
LCMS-8050 Triple-quadrupole MS			
Nebulizer gas flow	3 L/min		
Heating gas flow	10 L/min		
Drying gas flow	10 L/min		
Interface temp.	400 ℃		
DL temp.	300 ℃		
Heatblock temp.	400 ℃		
MRM Transitions			
Liraglutide	**938.8**	**>**	**1128.6**
	938.8	>	1064.3
	938.8	>	1353.9
	1251.3	>	1245.0
	1251.3	>	1772.4
Semaglutide	**1029.3**	**>**	**1238.3**
	1029.3	>	1302.9
	1029.3	>	1110.0

3. Results

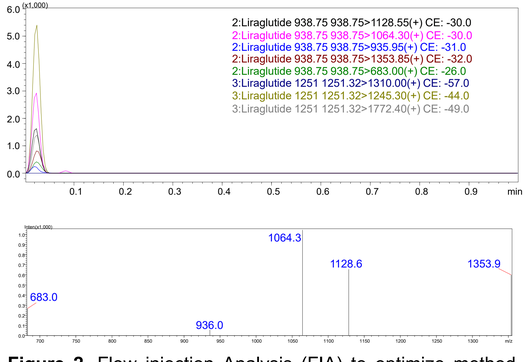

Figure 2. Flow injection Analysis (FIA) to optimize method detection parameters on a Shimadzu LCMS-8050.

Table 2. Columns screened for suitable chromatographic separation.

Shim-pack UC Series			
Phase	Bonded Phase	Phase	Bonded Phase
Phenyl	Phenyl Group	PBr	Pentabromobenzyl Group
CN	Cyanopropyl Group	Sil	NA (bare silica)
Diol II	Diol Group	Amide	Carbamoyl Group
Choles	Cholesteryl Group	NH2	Aminopropyl Group
Py	Pyridinyl Group		
Additional Column			
Scepter C18-120	Octadecyl group	DCPak® P4VP	poly (4-vinyl pyridine)

Table 3. Different modifiers tested for optimal separation and sensitivity. An average signal intensity of three injections per modifier using 50 µg/mL standard.

Modifier	Flowrate (mL/min)	Liraglutide m/z 938	Liraglutide m/z 1251	Semaglutide m/z 1029
Methanol, 2-Propanol, (85/15%, v/v)	1.1	74792	14761	98848
Methanol	1.1	68723	11737	83108
Methanol/2-propanol/water (82.5/14.5/3, v/v/v)	1.1	102769	9378	159160

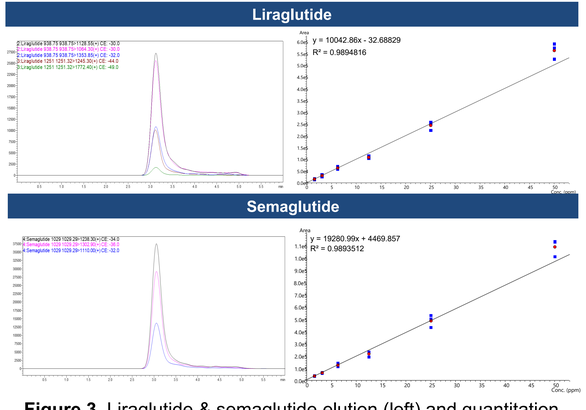

Figure 3. Liraglutide & semaglutide elution (left) and quantitation using most abundant MRM transition.

4. Discussion

When optimizing SFC-MS methods, it is advantageous to determine optimal mass spectrometry parameters prior to adjusting chromatographic conditions, particularly when a UV detector is not utilized. If prior knowledge of GLP-1 transitions are known from HPLC analysis, it was observed that the transitions and charge distributions were consistent between HPLC and SFC. Flow injection analysis (FIA) provided a rapid and straightforward means of maximizing signal intensity for a given flow rate. Once desolvation conditions (e.g., gas settings, probe position, etc.) had been optimized, column screening commenced with confidence that improvements in signal could be reliably attributed to chromatographic conditions.

Although numerous HPLC stationary phases could have been evaluated, limiting the screening to a standard octadecyl (C18) column initially seemed prudent. Ultimately, the poly(4-vinyl pyridine) column produced superior chromatographic performance. While the initial peak shape was promising, several rounds of incremental modifier optimization further improved performance. As is typical in SFC, methanol proved to be a reliable modifier. Although differences were observed following the addition of 2-propanol and water, signal intensity and linearity remained largely unchanged. Furthermore, 2-propanol should be used cautiously, as its addition increased operating pressure by approximately 100 psi.

5. Conclusion

GLP-1 peptide analysis can be performed under SFC-MS conditions using a poly(4-vinyl pyridine) (P4VP) column. Among the evaluated modifiers, methanol-based systems produced the highest signal intensity. These results suggest that SFC is a viable alternative to HPLC for GLP-1 analysis and may be suitable for routine implementation.

6. References

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- https://www.shimadzu.com/an/industries/pharma-biopharma/peptide-therapeutics/index.html. Accessed 13 May 2026.
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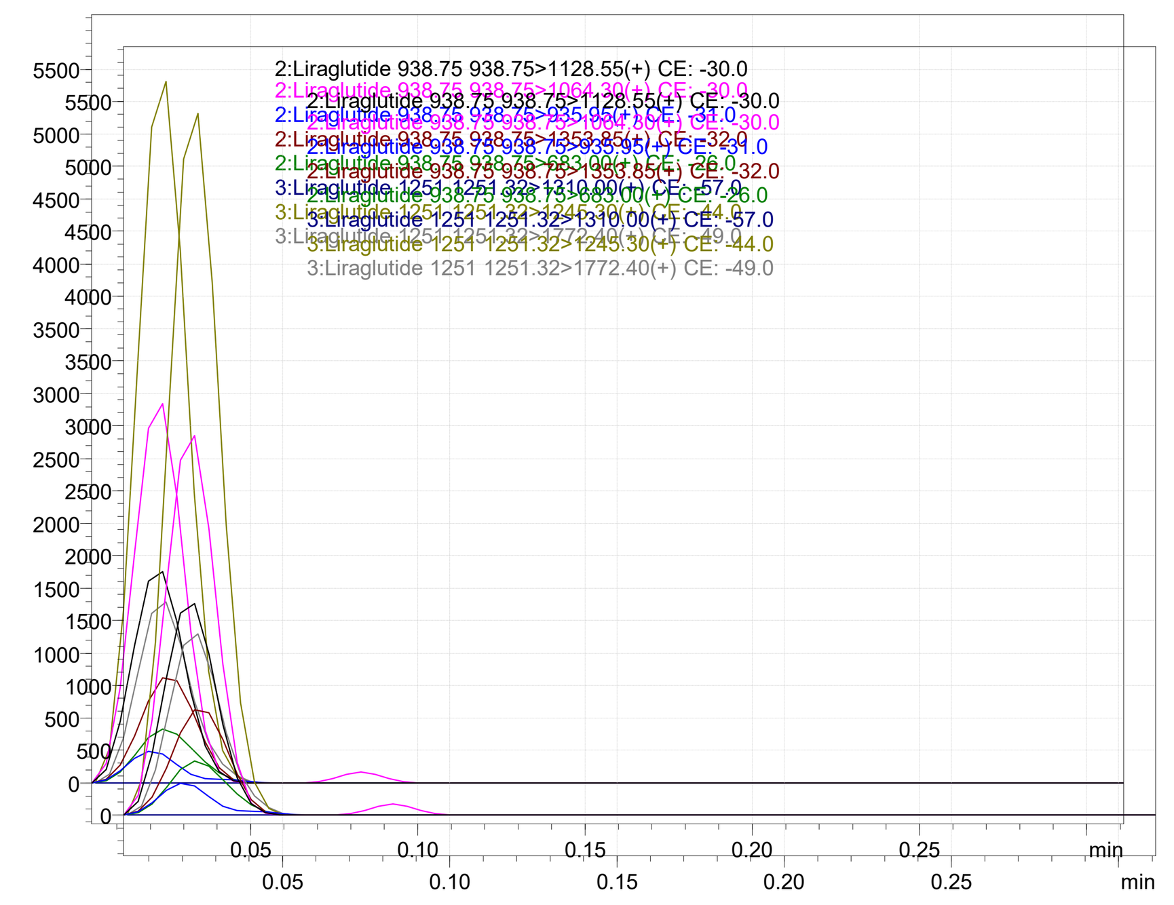
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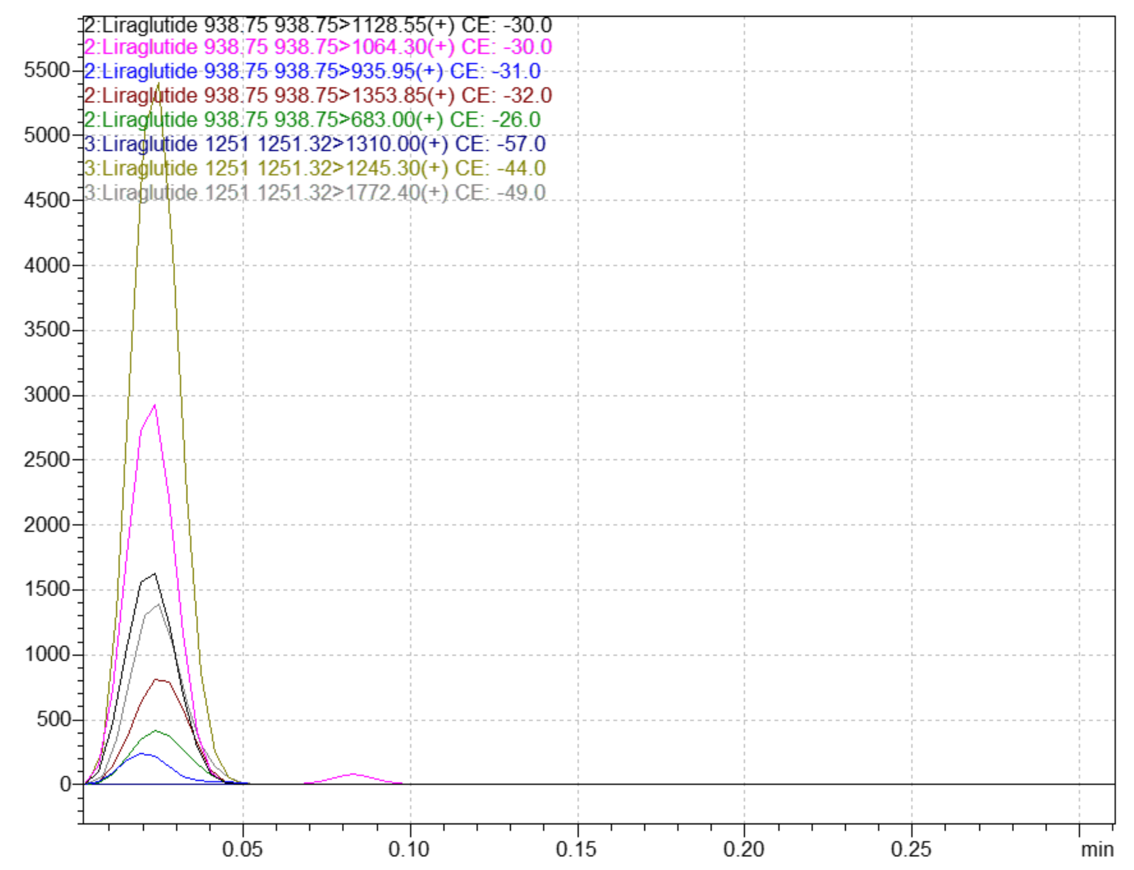
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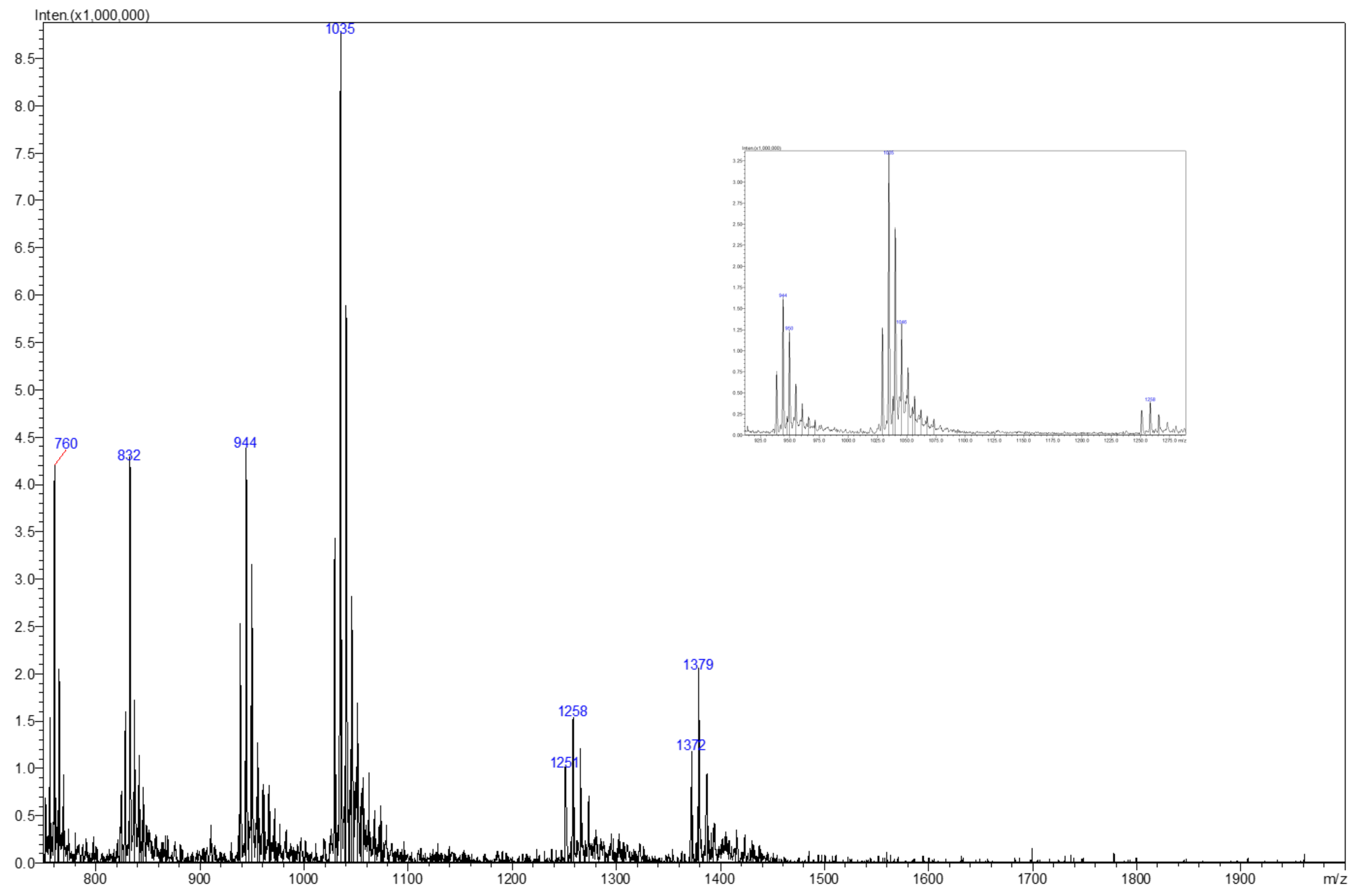
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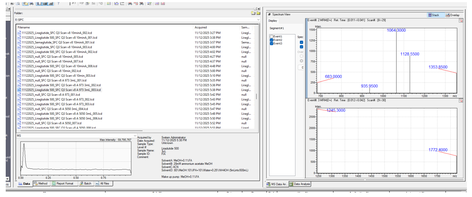
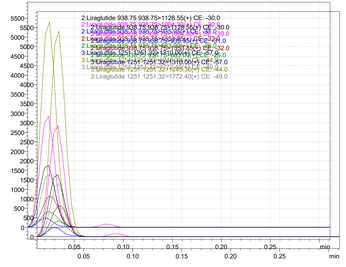
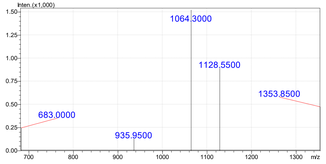
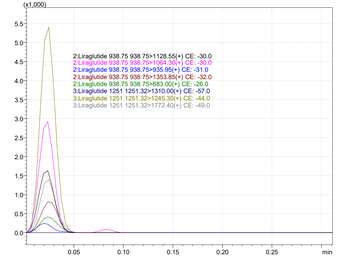
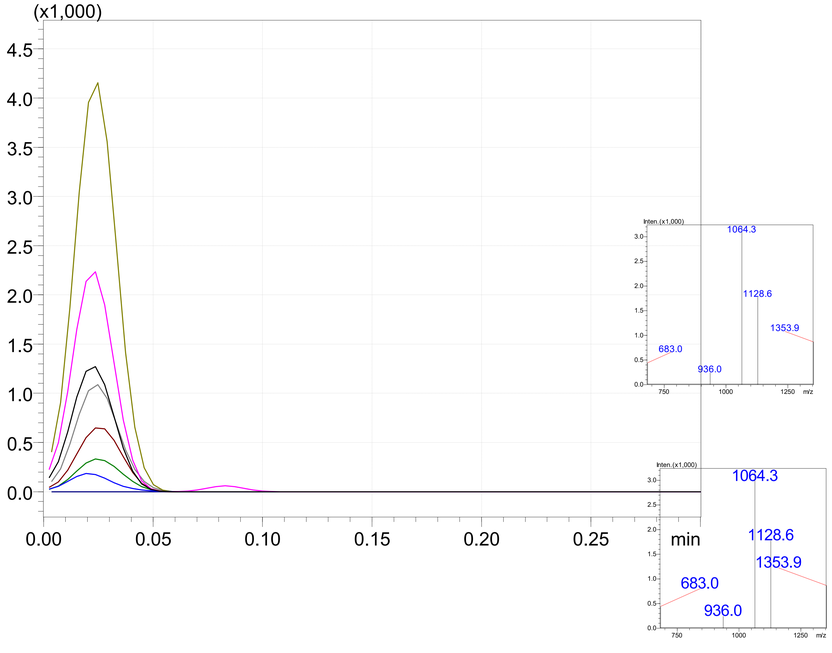
Q3 Scan of the precursor.
This is to show that the ionization is still the same for semaglutide and liraglutide under SFC condition.











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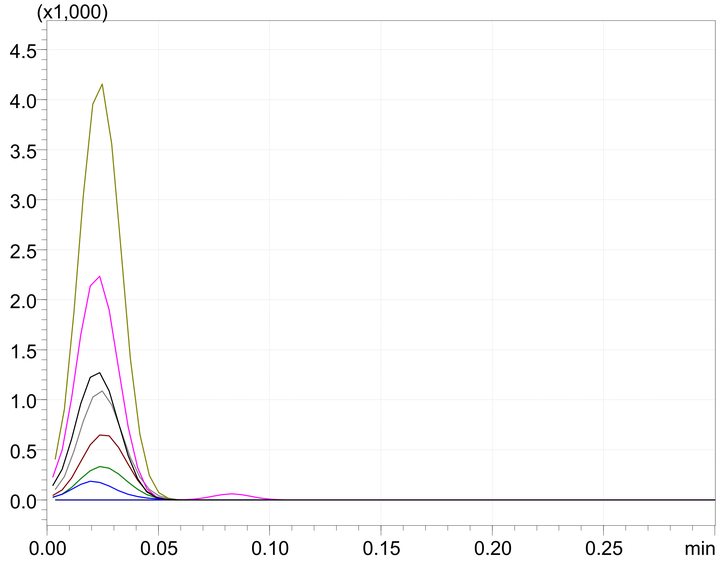

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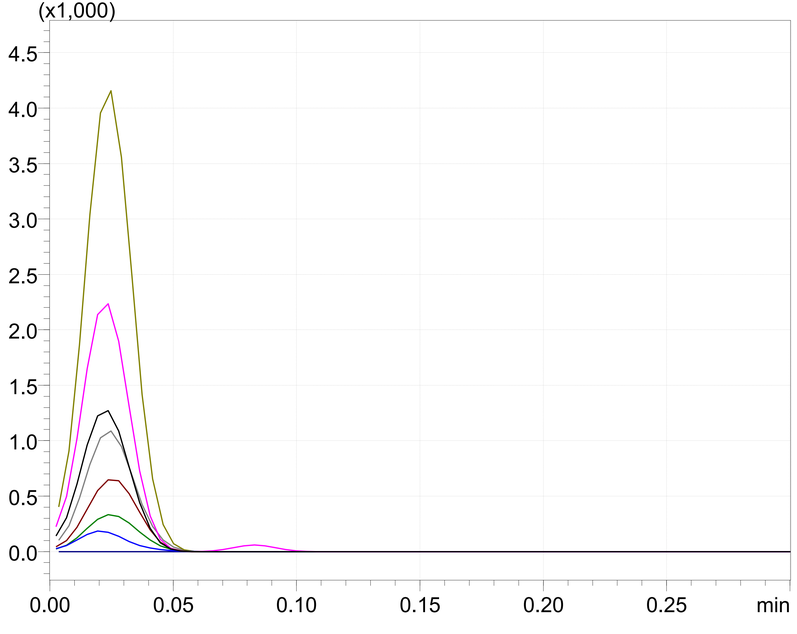

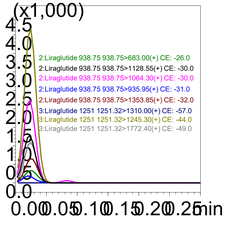

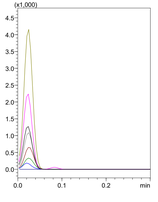

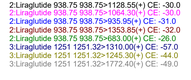

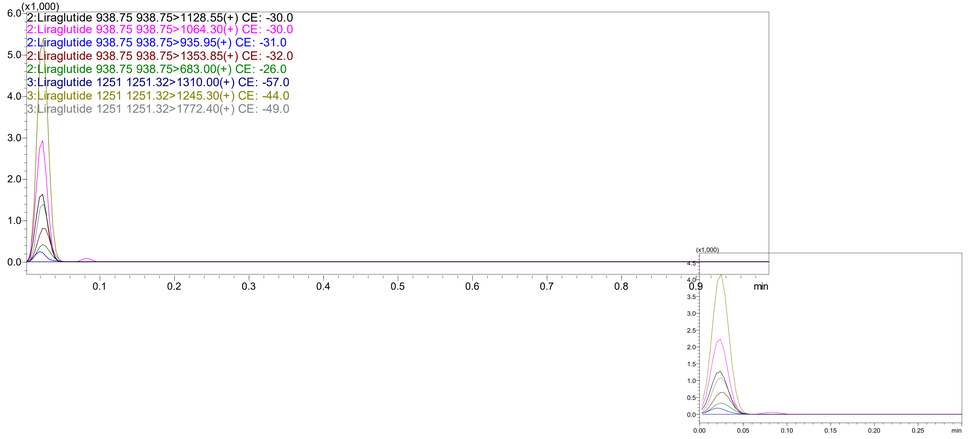
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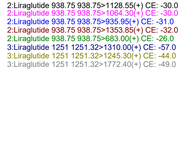
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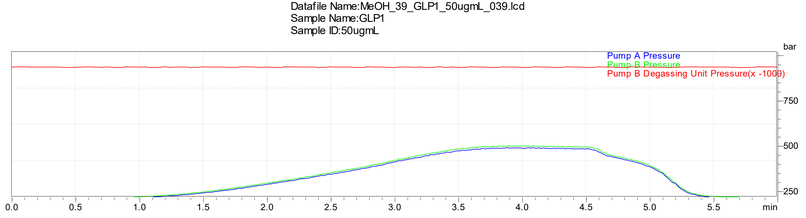
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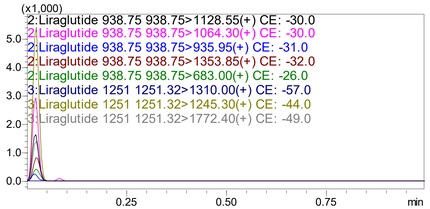
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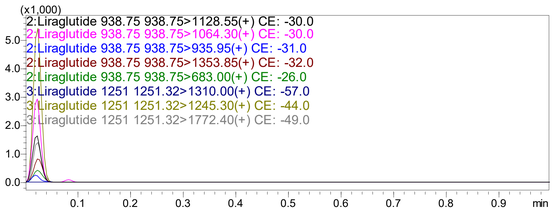
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