

A Sensitive and Rapid Liquid Chromatography-Tandem Mass Spectrometry Method for Quantification of Lacosamide and Desmethyl Lacosamide using Lacosamide-13C,d3 as Internal Standard



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Abstract

Background: Lacosamide (LCM) is an antiepileptic drug (AED) approved by the FDA in October 2008 for the adjunctive treatment of partial onset seizures. The major metabolite in human is O-desmethyl lacosamide (ODL). Monitoring LCM and ODL may help physicians to optimize the therapeutic dosing. Though there are published methods for the quantification of LCM by either liquid chromatography-ultraviolet (HPLC-UV) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) there are few that measure both LCM and ODL simultaneously and none uses an isotope labeled internal standard (IS) for LC-MS/MS methods. Our objective was to develop and validate a simple, sensitive, and rapid LC-MS/MS assay for the quantification of LCM and ODL using lacosamide-13C,d3 as IS. **Methods:** Serum (25μL) and 150μL IS solution (5μg/mL of lacosamide-13C,d3 in methanol) were vortex mixed and centrifuged. Supernatant (10μL) was mixed with 1000μL of 0.1% formic acid in water and 3μL was analyzed on an Accucore C18 column in an LC-MS/MS system. Total chromatographic time was 6.5 minutes. A quantifier and a qualifier transition were monitored for LCM (quantifier, 251.1→108.0 and qualifier, 251.1→116.1) and ODL (quantifier, 237.1→108.0 and qualifier, 237.1→91.1). **Results:** LCM was linear from 0.65 to 44.56 μg/mL with analytical recoveries ranging from 89.7 to 104.8%, while ODL was linear from 0.76 to 48.84 μg/mL with analytical recoveries of 97.8-122.0%. The total coefficient of variation was <4.7% for LCM and <4.0% for ODL at three levels tested. Comparison with an independent LC-MS/MS method showed a mean difference of 0.01μg/mL (-2.4%) for LCM and 0.11μg/mL (-4.9%) for ODL. Deming regressions are presented in **Figure 1**. **Conclusion:** This validated LC-MS/MS method offers a rapid and sensitive quantification of LCM and ODL with simple sample preparation. It has been validated for clinical use.

Background

- Lacosamide (LCM) is a FDA approved antiepileptic drug (AED) for the adjunctive treatment of parital onset seizures.
- O-desmethyl lacosamide (ODL) is the major human metabolite of lacosamide in humans.
- No published LC-MS/MS methods use an isotope labeled internal standard.
- Our objective was to develop and validate a simple, sensitive, and rapid LC-MS/MS assay for the quantification of LCM and ODL using lacosamide-13C,d3 as IS.

Materials and Methods

- Burdick and Jackson high purity Methanol, Acetone, Acetonitrile, 2-Propanol (VWR, West Chester, PA, USA)
- Type I Water (Millipore Synergy, Billerica, MA, USA)
- Fluka ACS grade Formic Acid (Sigma-Aldrich, St. Louis, MO, USA)
- Lacosamide (99.7%; **Figure 1**), lacosamide-13C,d3 (99.3%) (Cerilliant, Round Rock, TX, USA)
- Desmethyl Lacosamide (98%; Figure 1, Toronto Research Chemicals Inc., Toronto, Ontario, Canada)
- Accucore C18 2.6 μ (50x2.1mm, Thermo Scientific, Waltham, MA, USA)
- UltraShield UHPLC Precolumn filter (Restek, Bellefonte, PA, USA)

Sample Preparation:

- Add internal standard (150 μL) and standard, control, or patient sample (25 μL) to 1.5 mL polypropylene microcentrifuge tube.
- Vortex for 15 s
- Centrifuge for 10 min at 15,500 x g
- Add supernatant (10 μL) to injection vial containing 0.1% Formic Acid in Type I water (1 mL)
- Vortex for 10 s

Liquid Chromatography Conditions (**Figure 2**):

- Mobile phase A = 0.1% Formic Acid in Type I water
- Mobile phase B = 0.1% Formic Acid in Methanol
- Injection volume: 3 μL
- Column Temperature: 26°C
- HPLC gradient

Optimized Mass Spectrometry Conditions

- Turbo Spray ESI+
- Spray voltage = 1000 V
- Curtain Gas = 20 U
- Collision Gas = Medium
- Temperature = 400°C
- Ion Source Gas 1 = 45 U
- Ion Source Gas 2 = 40 U

Method Validation

A quantifier and a qualifier transition were monitored for LCM (quantifier, 251.1→108.0 and qualifier, 251.1→116.1) and ODL (quantifier, 237.1→108.0 and qualifier, 237.1→91.1). An ion suppression study was performed, by post-column infusion, to determine if there was matrix effect. A mixing study was performed to evaluate relative ion suppression. Mixing study was performed by mixing, 1:1 (v:v),candidate matrix, BioRad Drug Free Serum, and patient sample (n = 6). Candidate matrix, patient sample, and 1:1 mixture were extracted and run in triplicate. The response ratio of the mixture must be within 20% of the theoretical response. Interference was tested based on the CLSI EP7-A2 guidelines by extracting and analyzing commercial controls as well as lipemic, uremic, hemolyzed, and icteric patient samples. The analytical measurement range (AMR) was determined by serial dilution of spiked patient serum. The AMR samples were then extracted and analyzed in triplicate. The acceptance criteria for AMR is recovery of 100 ± 20%, CV <20%, and S/N >10. Precision was determined based on CLSI- EP10-A3 guidelines by running the sequence mid-high-low-mid-high-mid-low twice a day for five days. Patient samples (n = 24) were compared with a reference laboratory LC-MS/MS assay. Statistics were performed using EP evaluator (Data Innovations, South Burlington, VT, USA) and Excel (Microsoft, Redmond, WA, USA).

Results

No ion suppression or matrix effect was observed (**Figure 4**). There was no observed interference from tested compounds. No carryover was observed at 44.52 μg/mL for LCM and 47.11 μg/mL for ODL. The AMR for LCM and ODL is shown in **Table 1**. Total and intra-assay precision are shown in **Table 2**. Patient comparisons with a reference laboratory LC-MS/MS method are shown in **Figure 5**.

Table 1: AMR Data							
Lacosamide Spiked Concentration (μg/mL)	Mean	Analytical Recovery (%)	% CV	Desmethyl Lacosamide Spiked Concentration (μg/mL)	Mean	Analytical Recovery (%)	% CV
0	0	NA	NA	0	0.00	NA	NA
0.62	0.65	104.8	1.5	0.62	0.76	122.0	3.3
1.24	1.24	100.3	7.6	1.24	1.36	108.5	3.3
2.48	2.44	98.3	2.3	2.48	2.74	109.7	2.1
4.97	4.83	97.1	2.4	4.97	5.32	106.6	1.6
9.93	9.68	97.4	1.8	9.93	10.16	101.7	1.3
19.86	19.08	96.1	2.7	19.86	19.99	100.1	2.1
39.72	36.61	92.2	4	39.72	39.14	98.0	3.1
49.65	44.56	89.7	1.1	49.65	48.84	97.8	1.4

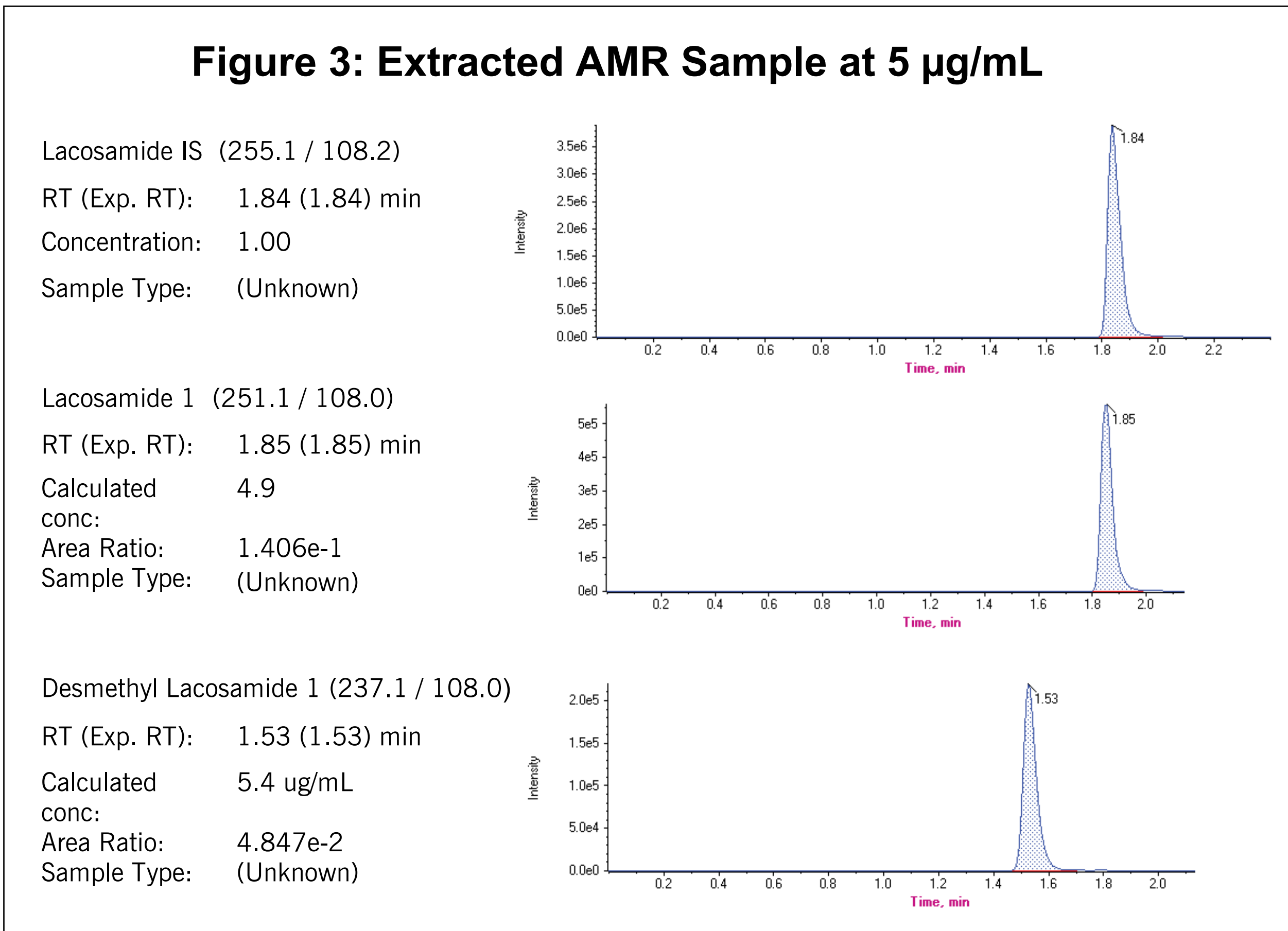
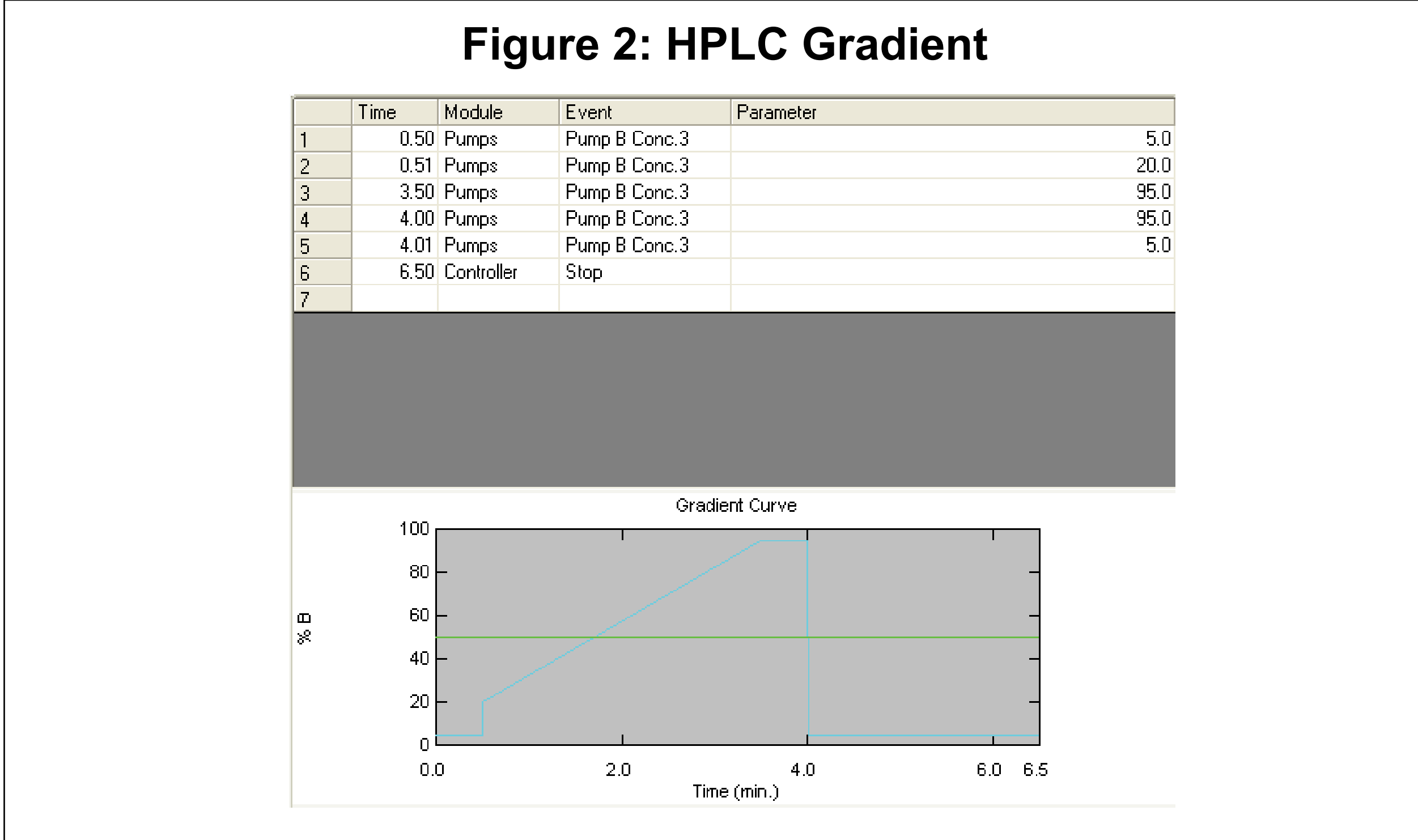
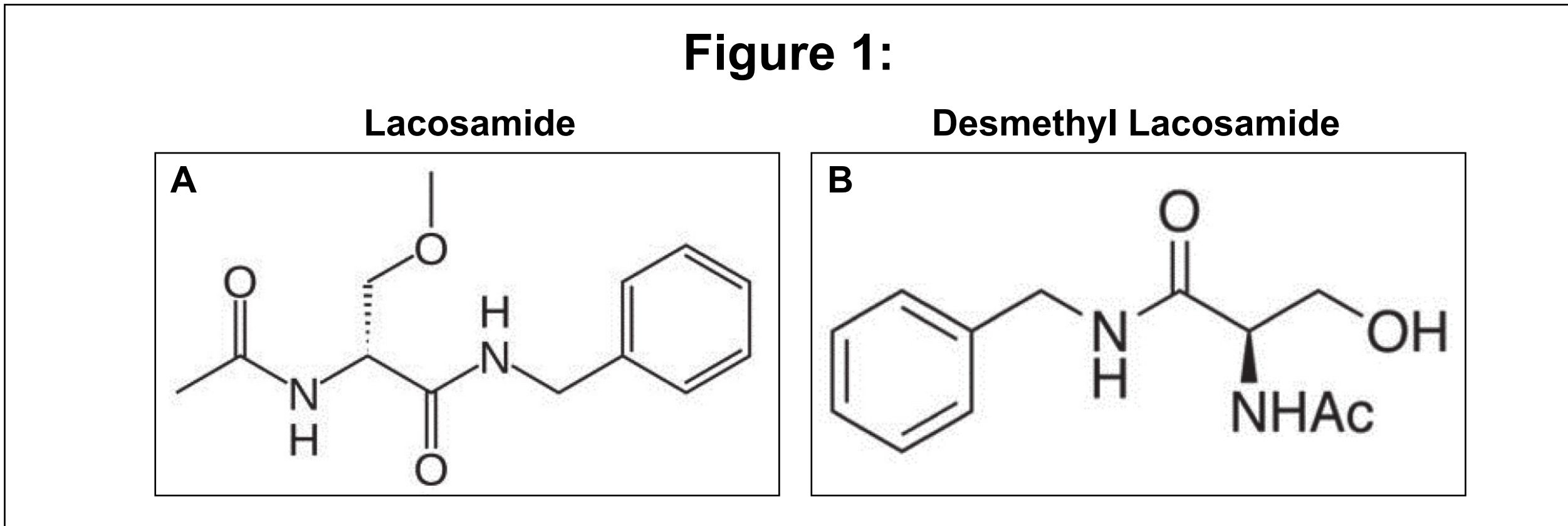
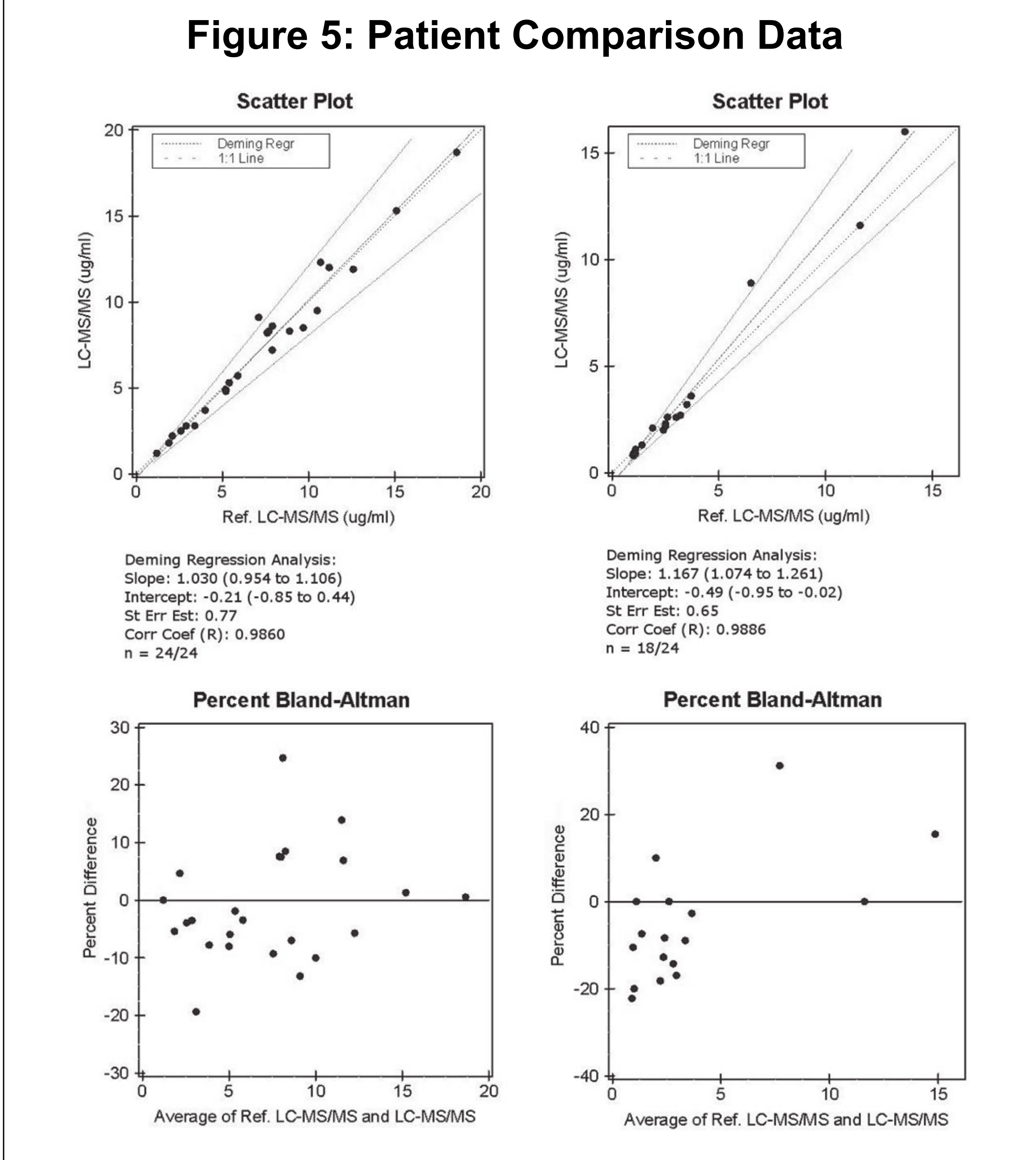
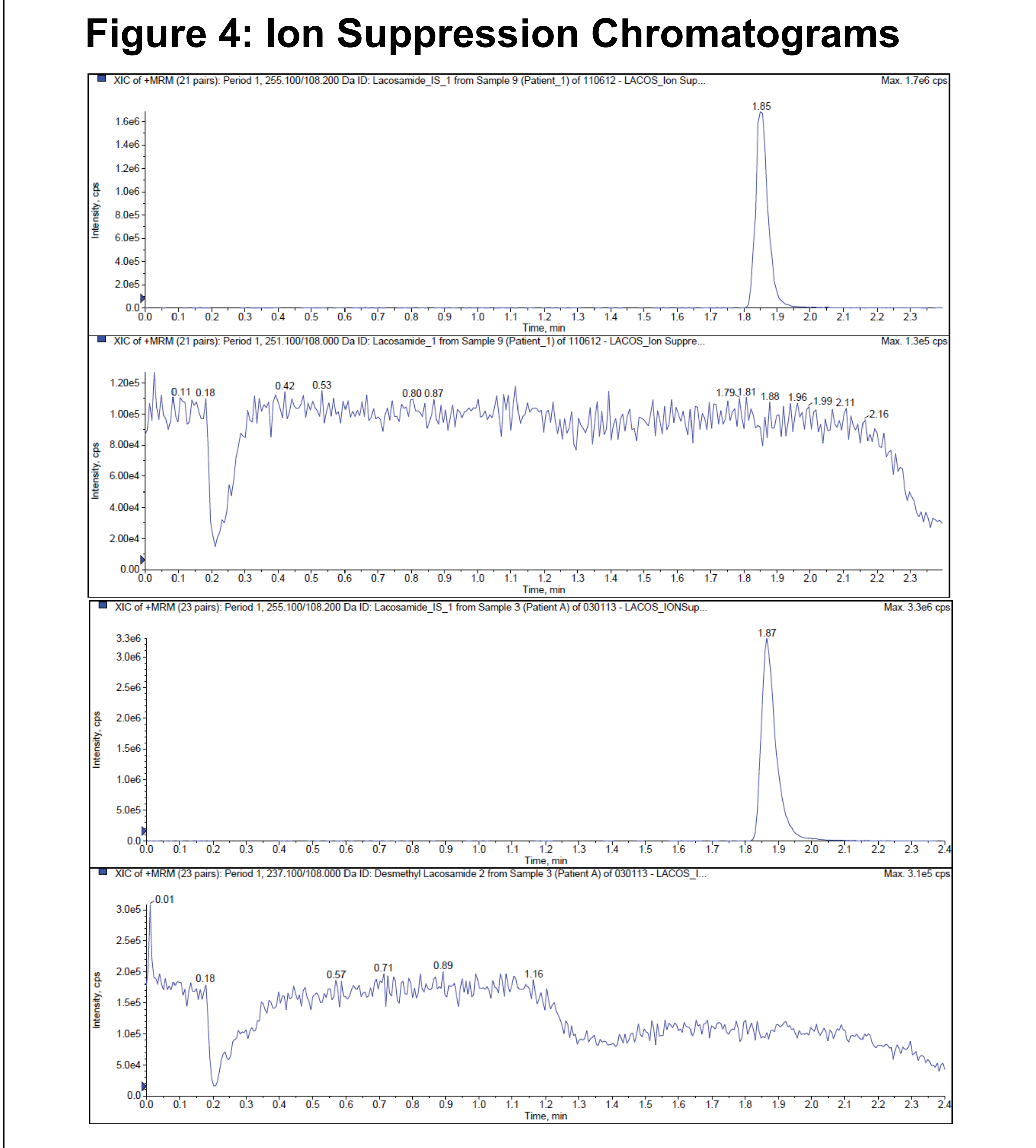


Table 2: Precision Data						
	Lacosamide			Desmethyl Lacosamide		
	Low	Mild	High	Low	Mild	High
N	30	30	30	30	30	30
Grand Mean (μg/mL)	2.15	8.97	15.73	2.10	8.71	15.43
Total %CV	4.7	2.4	2.2	3.6	4	2.7
Total SD	0.101	0.214	0.347	0.076	0.349	0.412
Within Run SD	0.101	0.175	0.347	0.076	0.254	0.372
Within Run %CV	4.7	2	2.2	3.6	2.9	2.4



Conclusion

This validated LC-MS/MS method offers a rapid and sensitive quantification of LCM and ODL with simple sample preparation. It has been validated for clinical use.