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Liquid Chromatography Mass Spectrometry

Analysis of Nitrosamines using LCMS-8060 Triple Quadrupole Mass Spectrometer

LCMS-8060

Summary

Hexanoate ester derivatized 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its D3 labeled internal standard are quantitatively measured out of a urine matrix using the LCMS-8060.

Method

Derivatized standards for both the target and the internal standard were used to optimize the MS and LC method parameters. Mobile phases utilized in this method include 7 mM Ammonium bicarbonate (A) and methanol (B) pumped through a Discovery HSF5 50 x 2 mm column with 3um particles. Analytes were acquired using ESI in positive mode to measure transitions matching the literature with two additional transitions added for each analyte (**Figure 1 A** and **C**). The time program for each injection spans six minutes. Though all calibration curve levels were acquired using a 0.1 μL injection volume, samples C1-C7 as well as C15 presented NNAL levels far higher than the intended bracketed concentration range of the calibration curve (**Table 1**).

Results

Figure 1 panel C provides MS chromatograms for three of the high cal curve levels including C1, C2, and C15 (left to right). In each example the NNAL response is positioned above its corresponding response from the internal standard at that level. The linear calibration curve with 1/C weight utilized the remaining calibration levels C8-C14 and C16-C20, each acquired in triplicate. **Figure 1 A** provides MS chromatograms of three levels through the linear range including C8, C14, and C20 (left to right). The impact of the high concentrations in samples C1-C7 and C15 is observed by comparing panels B and D in **Figure 1**. When the high concentration outliers are removed from the curve good linearity is acheived providing high % Accuracy across the curve with good reproducibility between measurements (**Table 1** and **Figure 1 B**). The amounts on column (using 0.1 μL injections) spanned 10-300 fg (levels C8-C20). Lower concentrations could have been measured as **Figure 1 A (C8)** demonstrates a S/N or 24 leaving more sensitivity to further expand the dyanamic range on the low end.

Samples Q1-Q5 provided similar response to calibration curve in that two of these samples produced saturating signal whereas the other three responded with a mid to high response within the calibrated range of concentrations (**Table 1**).
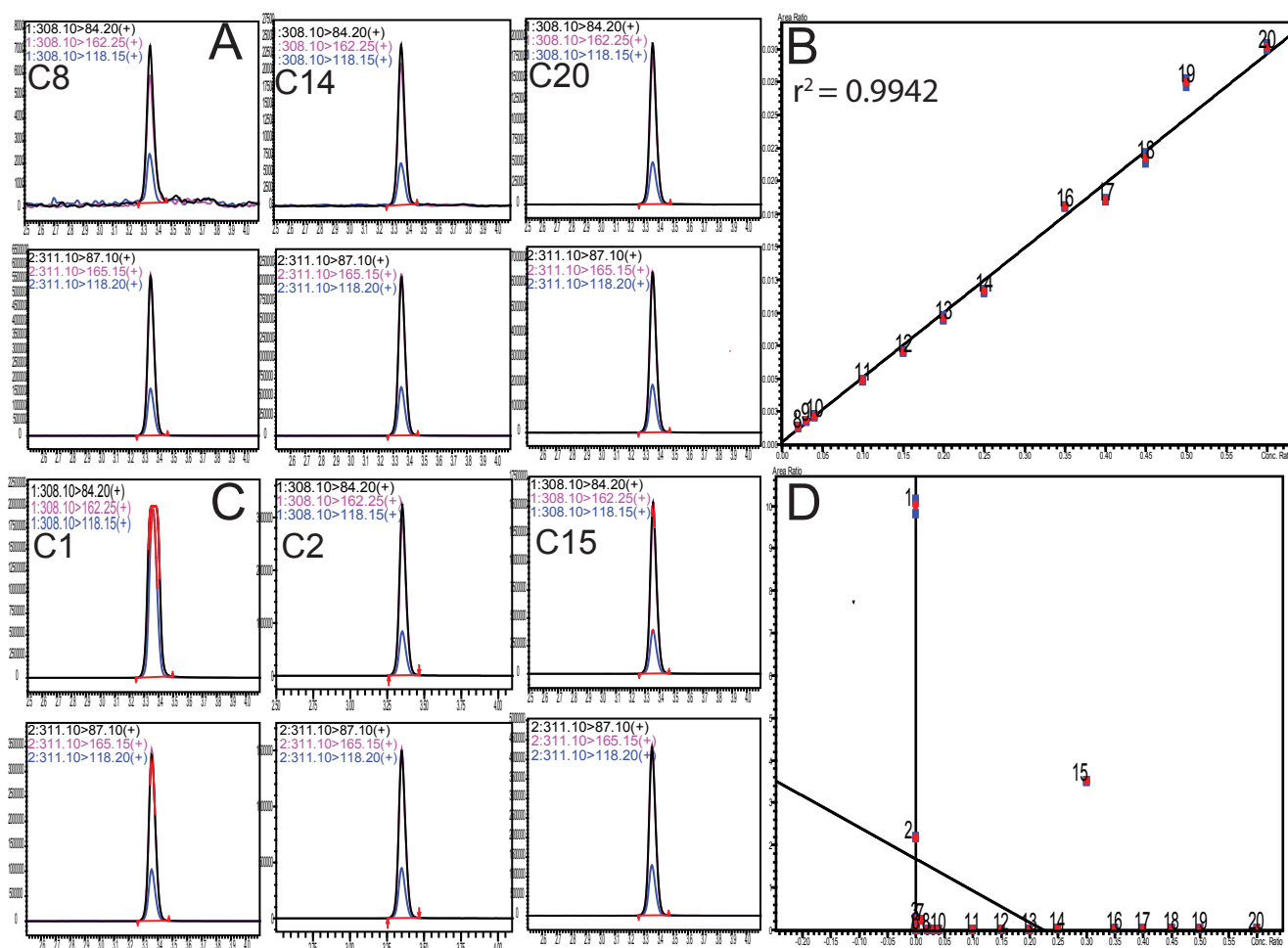

Figure 1: MS Chromatograms and Calibration Curves.

Sample Name	[NNAL] (pg/mL)	[NNAL-D3] (pg/mL)	Calibration Point	NNAL Avg. Peak Area	ITSD. Avg. Peak Area	Accuracy %	Cal. Point Area %RSD
C1	0	500	1	113,427,852	11,323,579		
C2	0.1	500	2	10,331,445	4,744,232	222,753,785	
C3	0.5	500	3	1,906,906	7,274,827	5,317,626	
C4	1	500	4	56,786	19,280,390	27,748	
C5	5	500	5	166,650	16,311,943	20,150	
C6	10	500	6	95,436	4,606,053	21,165	
C7	50	500	7	1,816,351	7,868,779	46,846	
C8	100	500	8	23,459	18,031,048	113	4.77
C9	150	500	9	18,698	10,625,082	106	2.06
C10	200	500	10	33,673	15,826,073	98	5.26
C11	500	500	11	88,870	18,395,072	94	1.83
C12	750	500	12	108,528	15,456,179	92	1.26
C13	1000	500	13	94,821	9,947,298	95	2.40
C14	1250	500	14	79,003	6,802,389	93	1.32
C15	1500	500	15	49,418,471	14,088,705	23814	
C16	1750	500	16	301,560	16,737,820	104	0.32
C17	2000	500	17	330,980	17,887,063	93	0.55
C18	2250	500	18	416,269	19,163,122	97	1.87
C19	2500	500	19	664,057	24,208,988	111	1.09
C20	3000	500	20	626,304	20,778,547	102	0.80

Sample Name	[NNAL] (pg/mL)	[NNAL-D3] (pg/mL)		NNAL Avg. Peak Area	ITSD. Avg. Peak Area	Concentration (pg/mL)	
Q1	TBD	500		65,682,347	21,220,085	315,236	
Q2	TBD	500		131,703,655	25,800,550	Saturated	
Q3	TBD	500		213,489	23,510,318	1668	
Q4	TBD	500		409,440	24,003,933	1717	
Q5	TBD	500		536,121	24,438,267	2142	

Addendum:
To confirm the LOD and LOQ of the LCMS-8060 for NNAL using this method, level C8 was serially diluted to the concentrations orignally described for levels C7-C1, as shown in **Table 1**. The ITSD was used in the diluent for these dilutions in attempt to keep the level of internal standard consistent, though the starting concentration for each sample was truly unknown due to the sample processing conducted prior to recieving these samples. Therefore, while the sensitivity for the analyte can be confirmed, the linearity of the calibration curve based upon the ratio between the analyte and the ITSD is less certain.
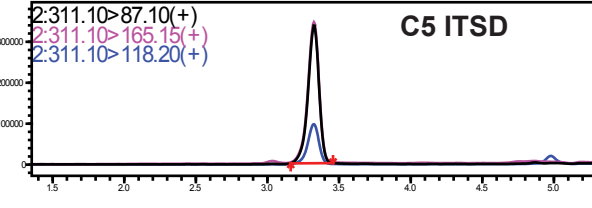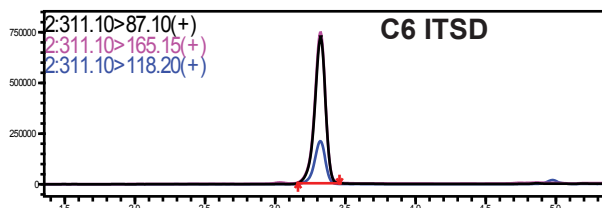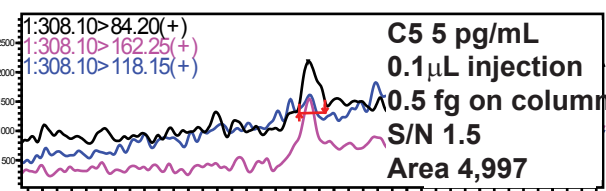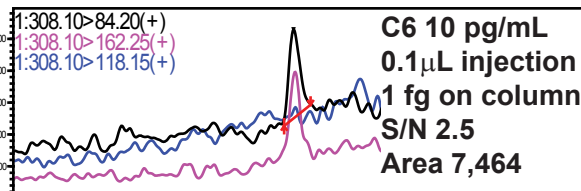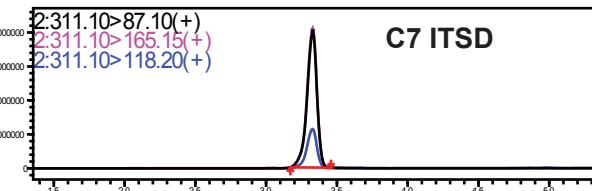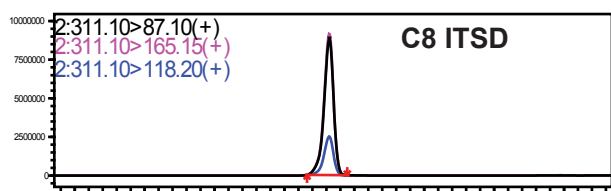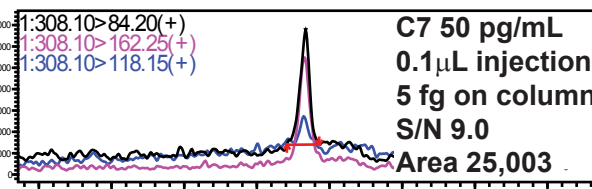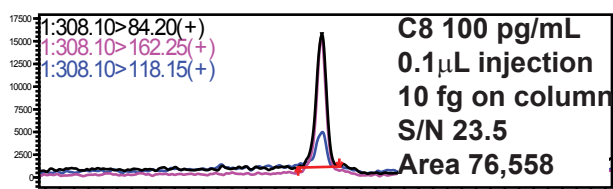
With the adjustment of the probe's lateral position and the capillary's vertical protrusion made since the previous acquistion, the signal intensity for these analytes was improved even further as seen by comparing the peak areas for level C8 between the previous and current measurements using the same method and injection volume.

Conclusions
This supplemental figure provides visual and calculated confirmation of the sensitivity of the LCMS-8060 using this method to measure NNAL. The provided S/N and peak area values demonstrate an LOQ between 1 and 2 fg on column while levels below 1 fg can still be detected. These results now extend the sensitivity by an additional order of magnitude over the previously reported results.

ULTRA FAST MASS SPECTROMETRY

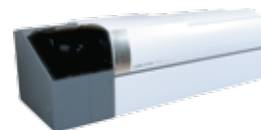LCMS-8030   LCMS-8040   LCMS-8050   LCMS-8060   LCMS-2020   LCMS-IT-TOF

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