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Application News

No. LCMS-109

Liquid Chromatography Mass Spectrometry

Identification and Confirmation of Ten Common Seized Drugs Utilizing an LCMS-2020 Single Quadrupole Mass Spectrometer

■ Introduction

The use of illicit drugs continues to be an epidemic across the United States. Currently most laboratories require the use of a GC or GCMS system and a cumbersome derivatization step to analyze seized drugs. By utilizing a LCMS method, faster detection rates and a larger drug panel could be screened simultaneously. The need for a derivatization step could also be avoided.

■ LCMS Instrumentation

A Shimadzu LCMS-2020 single quadrupole mass spectrometer coupled with an integrated LC-2040C 3D UHPLC system was employed for this evaluation. The LC-2040C 3D was equipped with a PDA detector for simultaneous analysis of analyte absorbance and compound ionization.

■ LCMS Method Development

Ten common seized and illicit drugs were analyzed using a single quadrupole mass spectrometer, LCMS-2020, with electrospray ionization (ESI) in positive mode. A mixture of all 10 analytes was prepared using certified reference materials purchased from Cayman Chemical. Each standard was dissolved in LCMS grade water and diluted to 10ug/mL for initial testing.

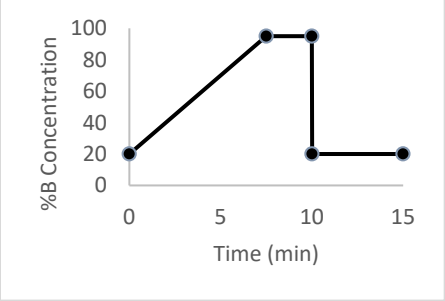
A 1uL injection volume was used with Shimadzu's NexLeaf CBX Potency II column (100mm x 3.0mm, 1.8um) for complete baseline separation of all 10 analytes. Gradient elution was required with mobile phase A being water and Mobile phase B being Methanol both with 5mmol ammonium formate and 0.1% formic acid (Figure 1).

The overall runtime was 15 minutes and utilized selected ion monitoring (SIM) for quantitation and scanning with in-source collision-induced dissociation (in-source CID) for identification and secondary confirmation. The scan event used a scan range of 50 to 500 *m/z* with a scan speed of 5000 u/sec. LCMS method parameters and the final SIM channels used can be found in Table 1.

Calibration curves were prepared with neat standards ranging from 0.1ug/mL to 1ug/mL. The corresponding SIM channel for each analyte was used for external calibration.

Table 1: LCMS-2020 Method parameters

Drying Gas	10.0 L/min
Interface Temperature	350°C
DL Temperature	250°C
Heat Block Temperature	400°C
Flow rate	0.3 mL/min
Column Oven Temperature	40°C
Sample Tray Temperature	10°C
SIM Channels (*m/z*)	337.2; 323.2; 286.1; 328.1; 205.1; 370.1; 304.1; 150.2; 256.2



Time	%B
0	20
7.5	95
10	95
10.01	20
15	20

Figure 1: LC Gradient Parameters

■ Results and Discussion

A Shimadzu LCMS-2020 was used to create a SIM method for 10 common drugs generally run by GCMS. In addition to a SIM method demonstrating chromatographic separation (Figure 2) a scan event was added to monitor in-source fragmentation. Two different Q-Array voltages were applied to fragment all of the analytes of interest, 60V and 75V. Naloxone and 6-MAM have the same mass and therefore could only be differentiated using retention time. With simultaneous in-source CID additional identification could be completed by comparing the fragmentation patterns produced (Figure 3). Fragments *m/z* 310.05 and 268.10 are only seen with Naloxone and are not produced for 6-MAM. in-source CID was also used for secondary confirmation. Figure 4 shows a comparison of the fragmentation pattern produced using in-source CID versus a known library spectrum from Metlin's database[1].

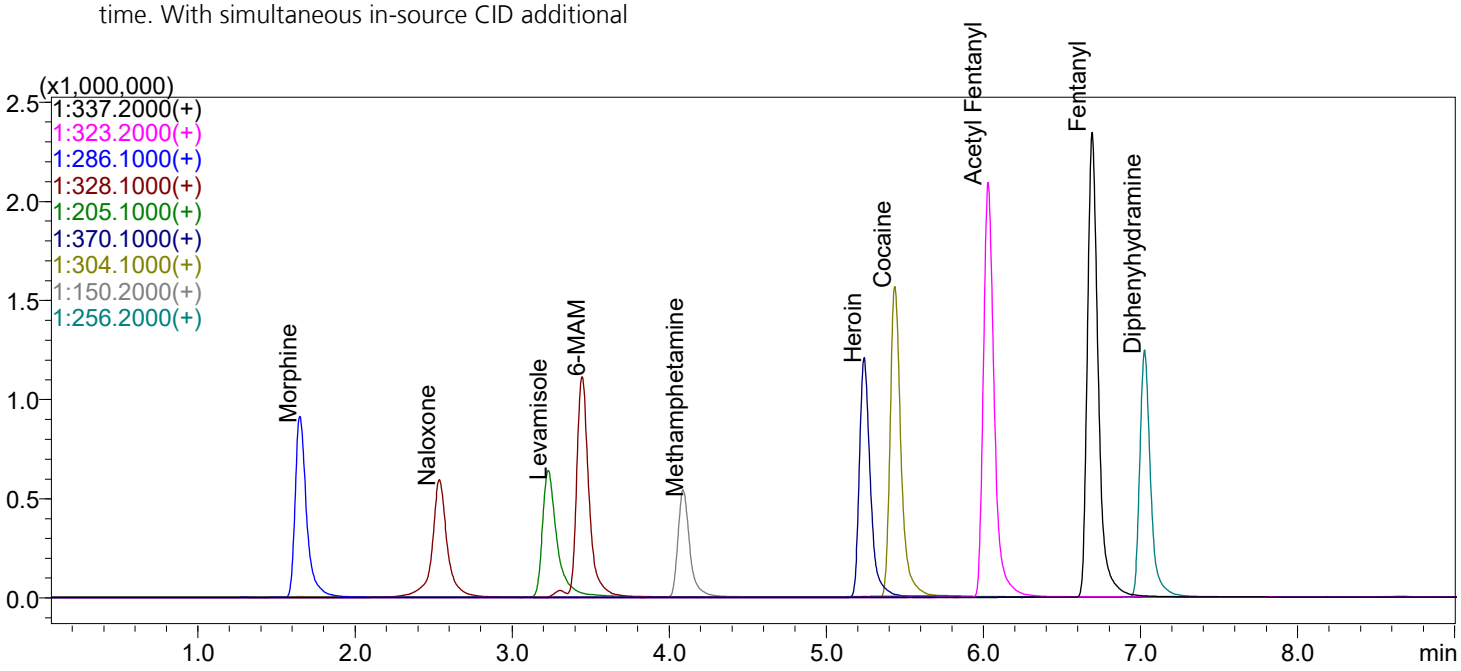


Figure 2: Representative SIM Chromatogram for 10 common seized drugs at 1ug/mL

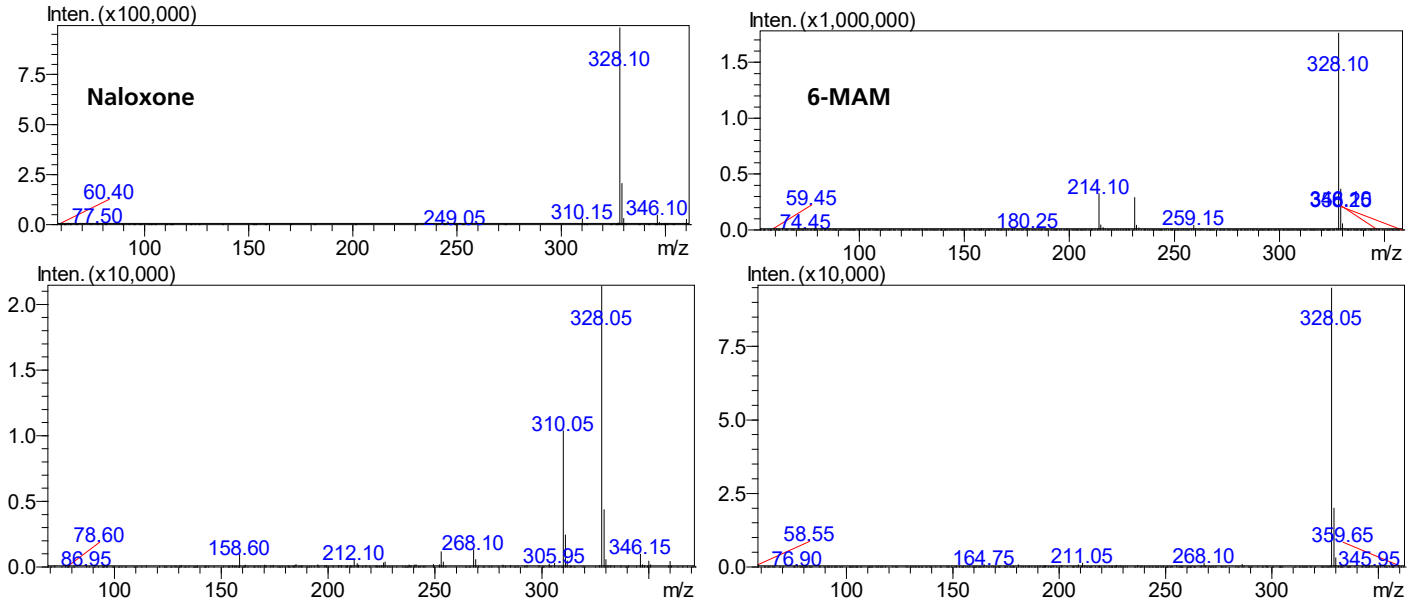


Figure 3: Scan and in-source CID spectra for Naloxone and 6-MAM at 1ug/mL. Top spectrum is a positive scan event with 0V, bottom spectrum is a positive scan event with 60V in-source CID

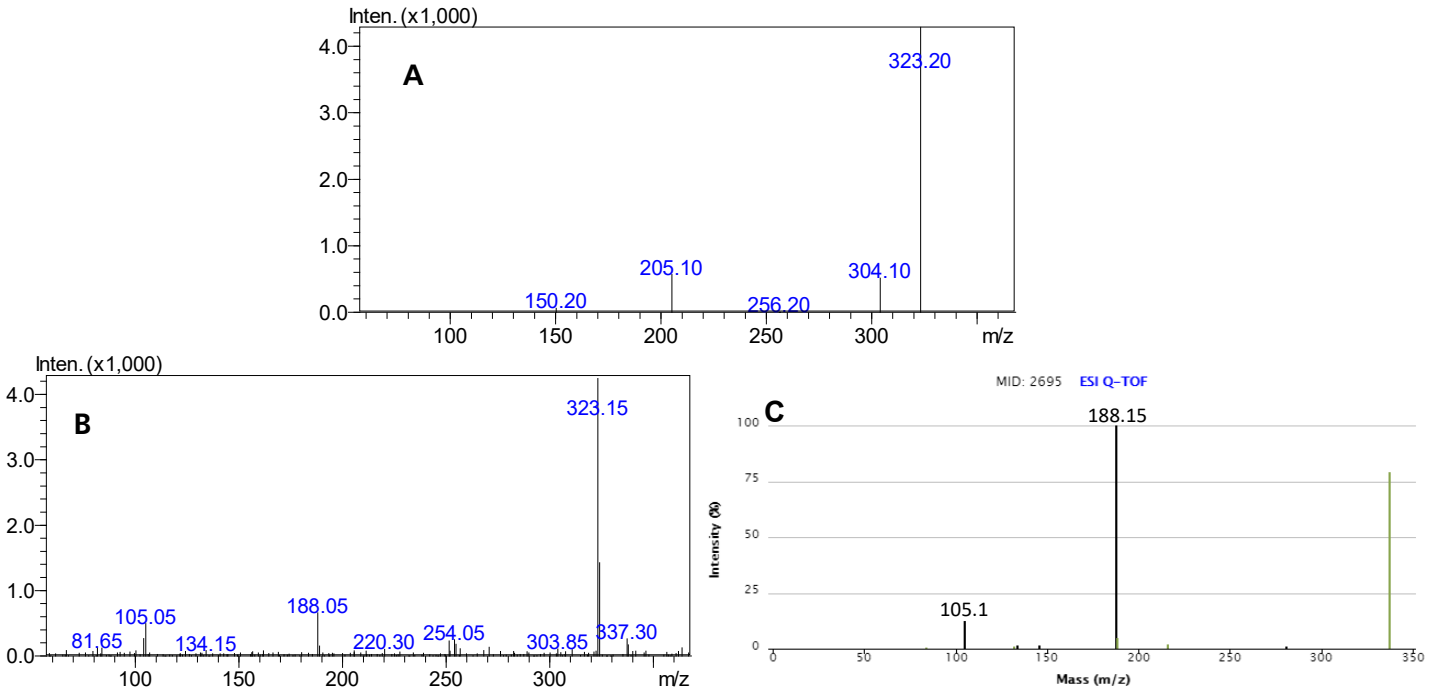
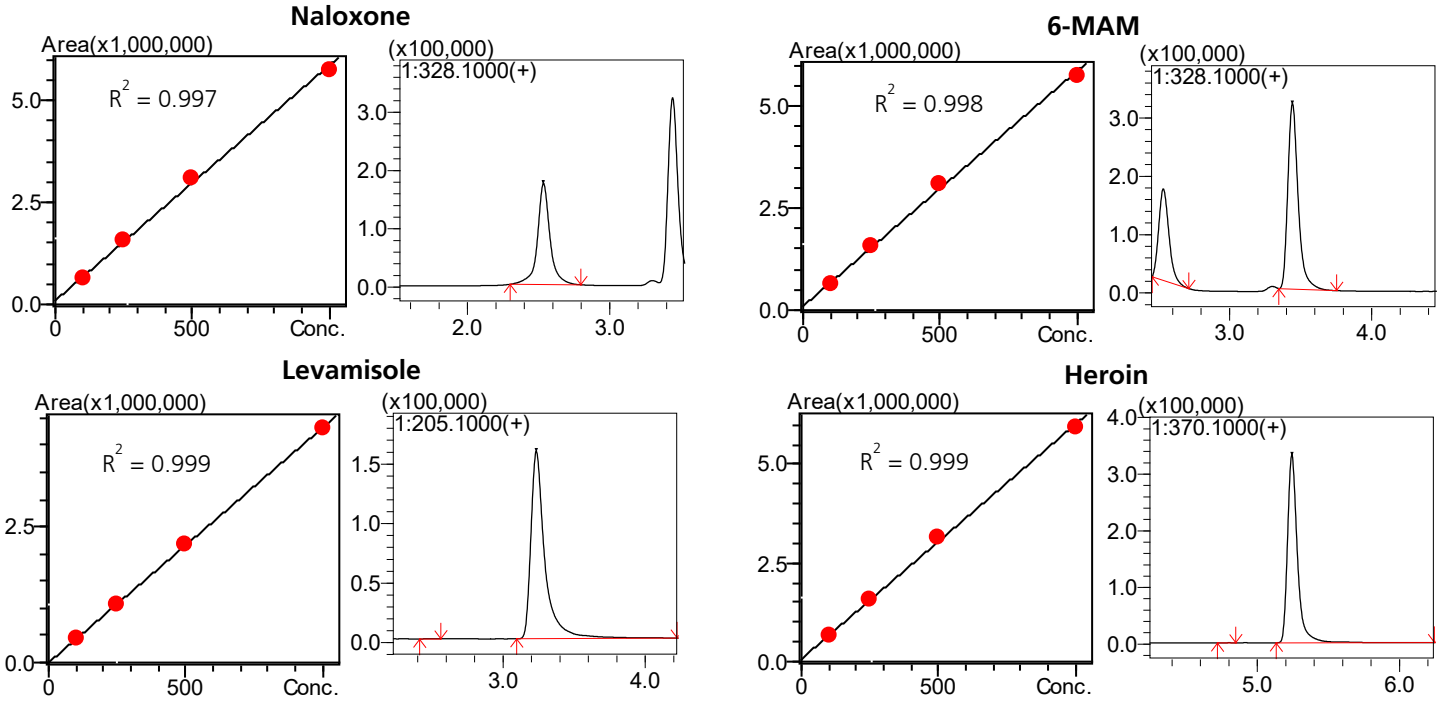


Figure 4: SIM and in-source CID spectra for Acetyl Fentanyl at 1ug/mL. A.) Spectrum from SIM event, B.) Spectrum from scan event with 60V in-source CID on LCMS-2020, C.) Spectrum from Metlin Database[1]

All calibration curves demonstrated linearity with a range from 0.1ug/mL to 1ug/mL. Final calibration curves were determined by plotting peak area versus concentration with a 1/C weighting factor. A correlation coefficient, R^2=0.996 or better was obtained for all analytes. Representative chromatograms and calibration curves for each analyte can be seen in Figure 5 and 6.

All calibration curves were run in triplicate to evaluate reproducibility and accuracy. The final method demonstrated peak area RSDs ranging from 0.15 to 1.40% with the average accuracies between 93 and 106% for every compound.



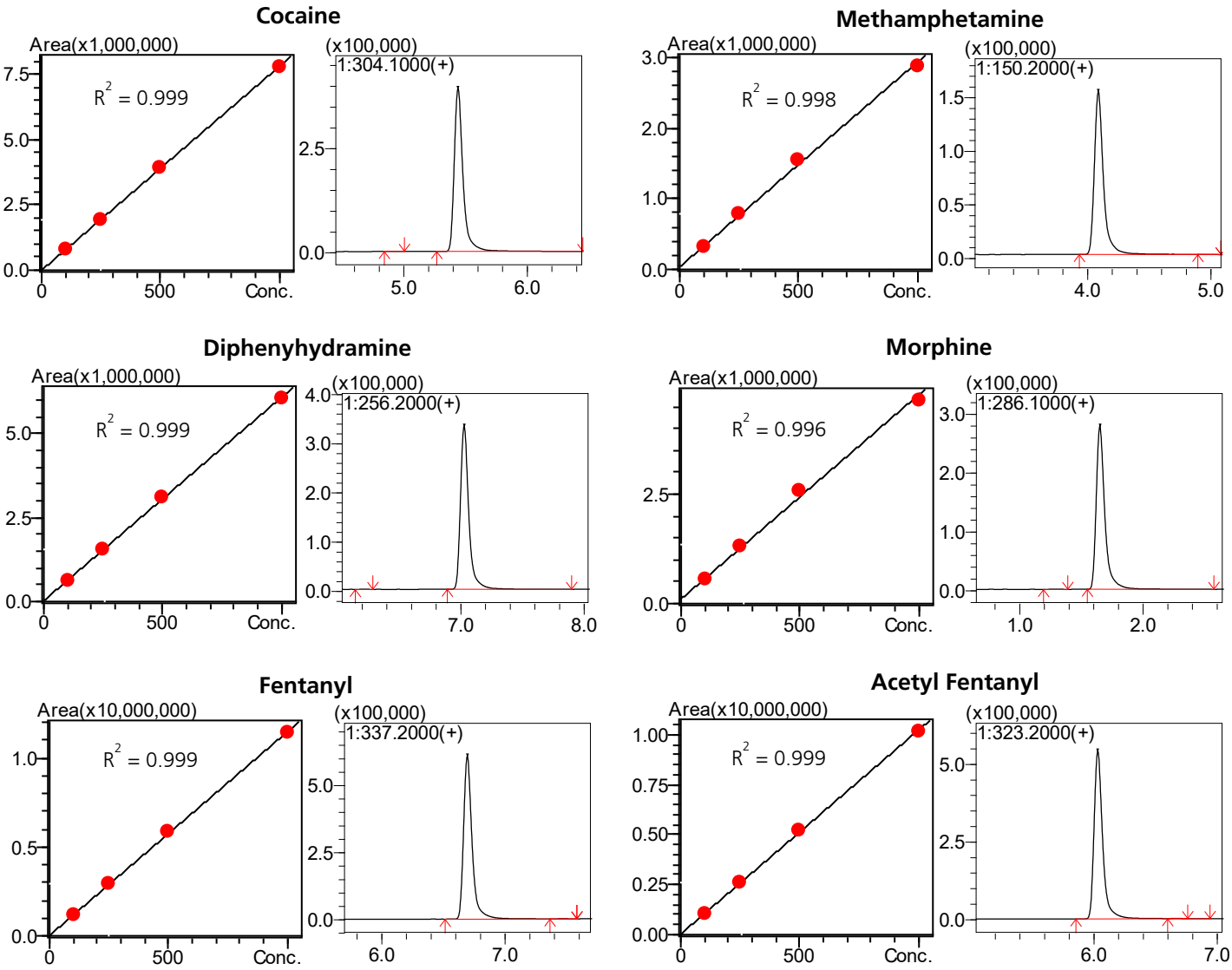


Figure 5: Calibration Curves for each compound and representative chromatograms at 250 ng/mL

■ Conclusion

A single chromatographic method was developed for the separation and quantitation of ten common seized drugs. The single quadrupole mass spectrometer, LCMS 2020, demonstrated its capability for simultaneous detection and confirmation using in source fragmentation of all analytes. Linear calibration curves were acquired for each analyte. Further method development could be explored to increase the panel of drugs being screened as well as determining LOQs for each analyte of interest.

■ Reference

1. Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, xonBrandon TR, Custodio DE, Abagyan R, Siuzdak G. METLIN: a metabolite mass spectral database. Ther Drug Monit [Internet]. 2005;27 :747-51.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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