Sensitive LC-MSMS Method for the Simultaneous Quantification of 32 Drugs in Oral Fluid

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Abstract

- Driving under the influence of drugs may impair one's ability to drive by affecting judgement, coordination, and decision making/reaction times.
- An LC-MSMS method was developed for the simultaneous detection of a panel of 32 drugs in oral fluid collected by Quantisal™ device.
- The method tests for the following target drugs and metabolites: delta-9-tetrahydrocannabinol (THC), morphine, 6-acetyl morphine (6-AM), hydrocodone, oxymorphone, hydromorphone, codeine, oxycodone, fentanyl, buprenorphine, methadone, tramadol, amphetamine, methamphetamine, 3,4,-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), ketamine, phencyclidine (PCP), cocaine, benzoylecgonine (BE), cocaethylene, zolpidem, meprobamate, carisoprodol, oxazepam, lorazepam, 7-aminoclonazepam, nordiazepam, alprazolam, clonazepam, temazepam, and diazepam.
- The method was applied to 14 authentic oral fluid samples

Introduction

- Studies have shown increasing rates of driving under the influence of alcohol and/or drugs, and an increase in cases involving impairment by more than one drug.
- Blood or urine are the traditional matrices collected in driving under the influence of drug cases.
- Oral fluid offers the advantages of quick, non-invasive sample collection that can be performed on site and without the need of medical staff.
- Oral fluid may reflect recent drug exposure.
- The Quantisal™ collection device is a common tool used for collection, preservation, and transportation of oral fluid. Collection involves a pad that absorbs 1 mL of oral fluid, which is preserved and extracted in buffer at a 1:4 ratio.
- The target compounds in the method were selected based on National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) recommendations and prevalence in the NYC area.

Materials

- Strata X polymeric reversed phase SPE cartridges 33 µm, 3 mL, 60 mg (Phenomenex).
- Quantisal buffer (Immunalysis).
- UPLC-Triple Quadrupole LCMS-8030 (Shimadzu).
- Chromatographic column Kinetex C18 100 x 2.1 mm with particle size 1.7 µm (Phenomenex).

Results

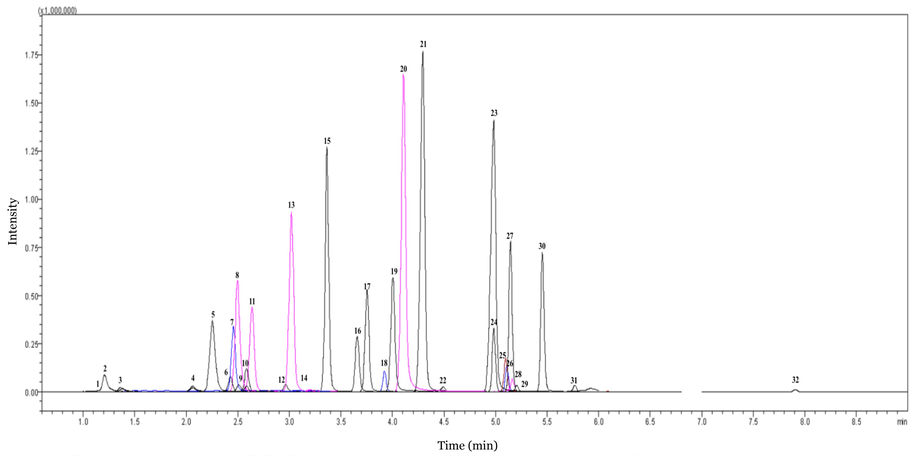

Fig. 1. MRM chromatogram of 32 drugs and metabolites in oral fluid at 15 ng/mL. 1.Morphine 2.Oxymorphone; 3.Hydromorphone; 4.Codeine; 5.Amphetamine; 6.Oxycodone; 7.MDA; 8.Methamphetamine; 9.6-AM; 10.Hydrocodone; 11.MDMA; 12.7-aminoclonazepam; 13.Ketamine; 14.BE; 15.Tramadol; 16.Cocaine; 17.Zolpidem; 18.Meprobamate; 19.Cocaethylene; 20.PCP; 21.Fentanyl; 22.Buprenorphine; 23.Methadone; 24.Oxazepam; 25. Nordiazepam; 26.Lorazepam; 27.Carisoprodol; 28.Alprazolam; 29.Clonazepam; 30.Temazepam; 31.Diazepam; 32.THC.

- **Limits of quantification (LOQ) ranged from 0.5 to 5 ng/mL**, depending on the compound:
  - 0.5 ng/mL: fentanyl, amphetamine, methamphetamine, MDMA, cocaine, cocaethylene, carisoprodol, meprobamate, zolpidem, tramadol, ketamine, and PCP.
  - 1 ng/mL: THC, alprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, methadone, 6-acetylmorphine, and buprenorphine.
  - 5 ng/mL: codeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone, BE, and MDA.
- **Linearity from LOQ to 200 ng/mL** (n=5); linear model, $1/x^2$ weighing.

Table 1. Intra and inter-day precision (CV %) and bias (%) at the limit of quantification (LOQ), the low QC (LQC, 1.5, 3, or 15 ng/mL), and high QC (HQC, 150 ng/mL), (n=15).

LOQ		LQC			HQC		
Inter-day Precision	Bias	Intraday Precision	Inter-day Precision	Bias	Intraday Precision	Inter-day Precision	Bias
0.6 to 12.4	-8.2 to 2.7	3.3 to 18.2	3.3 to 19.9	-11.8 to 19.4	2.1 to 16.3	3.1 to 16.0	-18.3 to 0.6

Table 2. Matrix effect (%) of oral fluid from different sources with Quantisal Buffer at 40 ng/mL (n=3, CV<20%).

Ion Suppression (9 out of 32 analytes)	No Matrix Effect (19 out of 32 analytes)	Ion Enhancement (4 out of 32 analytes)
-93.8 to -27.1	-21 to 18.7	57.8 to 3117.6

✓ Ion Suppression (%): Methadone -93.8; buprenorphine -61.8; clonazepam -57.5; alprazolam -43.1; fentanyl -35.4; PCP -34.5; nordiazepam -32.1; lorazepam -30.5; diazepam -27.1.
✓ Ion Enhancement (%): Oxycodone 57.8; 7-aminoclonazepam 58.3; temazepam 58.3; THC 3117.6.

Table 3. Number of positive cases and range of concentrations each target analyte detected (n=14)

Analyte	# of Positives	Concentration Range (ng/mL)	Percentage of Samples Positive (%)
Methadone	10	47.4 - >200	66.7
Cocaine	8	0.5 - 1.7	57.1
Benzoylecgonine	5	12.8 - 42.1	35.7
THC	5	1.4 - 30.5	35.7
Morphine	2	7.3 - 7.5	14.3
6-Acetylmorphine	2	3.2 - 4.4	14.3
Lorazepam	2	1.2 - 2	14.3
Methamphetamine	1	1.1	7.1
Tramadol	1	5.5	7.1
Buprenorphine	1	27.8	7.1
Alprazolam	1	8.7	7.1
Clonazepam	1	1.3	7.1

Methods

Sample Preparation (Strata-X SPE cartridges):
- Conditioning: 2 mL methanol & 2 mL water
- Load sample: **0.25 mL oral fluid**, **0.75 mL Quantisal Buffer**, 1 mL of pH 10 phosphate buffer, & 50 µL of 0.1 µg/mL internal standard mixture
- Wash: 2 mL water: MeOH (95:5 v/v) & 2 mL of water: methanol: NH_4OH (60:39.5:0.5)
- Elution: 2 mL dichloromethane:2-propanol (75:25)
- Add 50 µL of 1% HCl in methanol (1:99) before evaporation
- Reconstitute in 200 µL 0.1% formic acid in water: acetonitrile (95:5)

LCMS Method:
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: 0.1% formic acid in acetonitrile
- Gradient: 0 min-10% B, 0.5 min-10% B, 5 min -60% B, 6 min-90% B, 9 min-90% B, 9.5 min- 10% B, for a total chromatographic run time of 15 min.
- Flow 0.3 mL/min; 30°C column temperature
- ESI +; 2 MRM transitions per compound

Conclusions

- The method is sensitive, achieving LOQ's between 0.5- 5 ng/mL in 0.25 mL of neat oral fluid, in Quantisal buffer
- The validation has shown the method to be reliable and precise.
- THC showed significant ion enhancement due to Quantisal buffer during preliminary validation
- Methadone was the most commonly detected drug, followed by cocaine, and benzoylecgonine and THC

References

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