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Development and Evaluation of Drug Testing in Urine Using LC-MS/MS in a Clinical Laboratory

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Introduction

- **Increasing Drug Use**: The rise in drug use in Korea has led to an increased need for accurate and efficient drug testing in clinical and forensic laboratories.
- **LC-MS/MS as Gold Standard**: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers high sensitivity and specificity, capable of detecting a wide range of substances.
- **Aims**: Develop and evaluate an LC-MS/MS-based method for detecting 20 commonly abused substances using Waters Acquity UPLC, Xevo TQ-XS, and Chromsystems' MassChrom® Drugs of Abuse Testing in Urine reagent kit.

Methods

- Instruments and Reagents:
  - UPLC System: Waters Acquity UPLC
  - Mass Spectrometer: Xevo TQ-XS tandem MS
  - Reagent Kit: Chromsystems' MassChrom® Drugs of Abuse Testing in Urine reagent kit
- Targeted Substances (20 in total):
  - Amphetamines: Methamphetamine, Amphetamine
  - Ecstasy Derivatives: MDMA, Phencyclidine, MDA
  - Ketamines: Ketamine, Norketamine
  - Cocaine Derivatives: Cocaine, Benzoylecgonine
  - Opioids: Morphine, Fentanyl, Codeine, Hydrocodone, Oxycodone, Dihydrocodeine, Hydromorphone, Oxymorphone, LSD, 6-MAM
  - Cannabis Derivatives: 11-Nor-9-Carboxy-THC
- Testing Protocol:

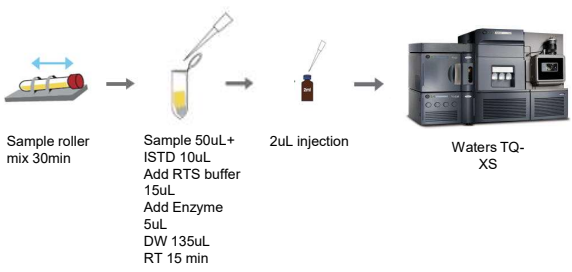
The sample preparation process is shown in figure 1.

Urine samples were analyzed using the Waters Acquity UPLC system coupled with the Xevo TQ-XS mass spectrometer. Separation was carried out using an ACQUITY UPLC HSS PFP column (2.1 × 50 mm, 1.8 µm) with a binary gradient of 0.1% formic acid in water and acetonitrile. The Xevo TQ-XS was operated in MRM (Multiple Reaction Monitoring) mode with electrospray ionization (ESI).

- Performance Parameters Assessed:

Accuracy, precision, linearity, LOD, LOQ, and carryover

Figure 1. Overview of the sample preparation.



Results

1. Precision & Accuracy

Precision, evaluated through intra- and inter-day variability, was within acceptable limits (RSD ≤ 15%), with relative standard deviations below 4% for all analytes.

Accuracy, assessed using Chromsystems' MassCheck® Drugs of Abuse Testing Urine Controls, was consistently within ±10% of nominal concentrations.

2. Linearity

Calibration curves for all analytes were linear over the tested concentration ranges, with correlation coefficients (R^2) exceeding 0.99.

3. LOD & LOQ

LOD and LOQ of each analytes are shown in Table 1.

4. Carryover

Carryover was below 0.1% for all substances tested.

The method demonstrated robust performance in detecting amphetamines, ecstasy derivatives, ketamines, cocaine derivatives, opioids, and cannabis derivatives in random urine samples. The method's comprehensive evaluation ensured reliable performance for forensic and clinical toxicology applications.

Table1. Recovery, RSD, linearity range, LOD, and LOQ of each analytes.

Analytes	MRM Transition	Recovery (%)	RSD (%)	Linearity range (ug/L)	LOD (ug/L)	LOQ (ug/L)
Amphetamine	136→91	100.7	2.3	25.99 – 690.0	2.60	25.99
Methamphetamine	155→92	100.0	2.3	24.11 – 764.0	2.41	24.11
MDA	180→133	99.4	2.3	17.39 – 878.0	1.74	17.39
MDMA	195→164	100.2	1.9	13.93 – 719.0	1.39	13.93
PCP	244→86	98.9	2.4	5.92 – 168.0	0.59	5.92
Ketamine	239→126	100.1	2.1	20.81 – 859.0	2.80	20.81
Norketamine	225→207.9	99.6	2.3	34.28 – 853.0	3.43	34.28
Cocaine	305→183	99.6	2.2	10.7 – 475.0	1.07	10.70
Benzoylecgonine	291→169	100.8	1.8	6.91 – 465.0	0.69	6.91
Morphine	286→165	98.6	2.2	9.93 – 378.0	0.99	9.93
Hydromorphone	286→185	99.9	2.3	0.72 – 169.0	0.07	0.72
Codeine	300→215	100.7	2.4	3.91 – 379.0	0.39	3.91
Hydrocodone	300→199	100.1	2.4	8.11 – 431.0	0.81	8.11
Dihydrocodeine	302→199	100.8	2.4	3.31 – 333.0	0.33	3.31
Oxymorphone	302→227	100.4	1.8	8.83 – 391.0	0.88	8.83
Oxycodone	316→298	100.2	2.1	7.05 – 852.0	0.71	7.05
LSD	324→223	99.6	2.8	0.17 - 14.1.0	0.02	0.17
6-Monoacetylmorphine	328→165	98.7	2.1	2.89 – 86.0	0.29	2.89
Fentanyl	337→188	98.6	2.9	2.89 – 137.0	0.29	2.89
11-Nor-9-carboxy-Δ9-THC	345→299	99.2	2.1	1.56 – 187.0	0.16	1.56

Conclusions

The LC-MS/MS-based method developed for drug testing in urine shows excellent sensitivity and specificity. It enables the rapid detection of multiple drugs with high accuracy and precision, making it a valuable tool for clinical and forensic toxicology applications in Korea.

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