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# Simultaneous Quantification of 15 Drugs of Abuse in Oral Fluid and Plasma by Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry

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# 1. Introduction

Driving under the influence of drugs make troubles to traffic safety. To access the prevalence of psychoactive substance use by drivers is essential for drug monitoring. Plasma or oral fluid such as saliva have been investigated as samples for testing individuals suspected of driving under the influence of drugs. Because only trace concentration of drugs in plasma and limited amount of saliva is available for drug analysis, it is crucial to have a multicomponent method with low detection limit. We describe the validated ultra high performance LC/MS/MS for determination of 15 drugs such as morphine, codeine, dolantin, tramadol, alprazolam, MDMA, ketamine, methadone et. al. in oral fluid and plasma.

This research was conducted following the competent Chinese law.

# 2. Methods and Materials

## Sample Preparation

(1) Fifty microliter of oral fluid or plasma were subjected to protein precipitation using two hundred microlliter acetonitrile.
(2) The sample was mixed with a vortex mixer and the centrifuged at 4500 rpm for 5 minutes.
(3) The supernatant was extracted and injected into the LC-MS/MS instrument.

## LC/MS/MS Analysis

The analysis was performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A5 degasser, and an SIL-30AC autosampler. The separation was carried out on Inertsil ODS-4 (100 µmL. × 3.0 mmi.d., 2 µm, GL Sciences) with the column temperature at 40 °C. A triple quadrupole mass spectrometer (Shimadzu LCMS-8080, Kyoto, Japan) was connected to the UHPLC instrument via an ESI interface.

### Analytical Conditions

**UHPLC (Nexera system)**

Column : Inertsil ODS-4 (100 µmL. × 3.0 mmi.d., 2 µm, GL Sciences)
Mobile phase A : water with 0.1% formic acid and 10 µmol/L ammonium acetate
Mobile phase B : acetonitrile
Gradient program : as in Table 1

Table 1 Time Program

| Time(min) | Module | Command | Value |
|---|---|---|---|
| 2.00 | Pumps | Pump B Conc. | 6 |
| 2.50 | Pumps | Pump B Conc. | 40 |
| 6.00 | Pumps | Pump B Conc. | 90 |
| 7.00 | Pumps | Pump B Conc. | 90 |
| 7.01 | Pumps | Pump B Conc. | 6 |
| 9.00 | Controller | Stop | |

Flow rate : 0.6 µL/min
Column temperature : 40 °C
Injection volume : 10 µL

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**MS/MS (LCMS-8080 triple quadrupole mass spectrometer)**

| | |
|---|---|
| Ionization | : ESI |
| Polarity | : Positive |
| Ionization voltage | : +4.5 kV |
| Nebulizing gas flow | : 5.0 L/min |
| Heating gas pressure | : 12.0 L/min |
| Probe temperature | : 400 °C |
| HSID temperature | : 300 °C |
| Scan mode | : MRM |

Table 2 MRM parameters of 15 drugs

| Compound | Precursor | Product | Dwell Time (ms) | EV (V) | CE(V) | CCL4(V) |
|---|---|---|---|---|---|---|
| Morphine | 286.05 | 153.15* | 100 | 10.0 | -58.0 | -10.0 |
| | | 165.15 | 100 | 10.0 | -56.0 | -8.0 |
| Codeine | 300.10 | 153.10* | 10 | 40.0 | -40.0 | -20.0 |
| | | 199.05 | 10 | 20.0 | -59.0 | -12.5 |
| Dolantin | 248.10 | 220.10* | 10 | 10.0 | -29.0 | -10.0 |
| | | 174.15 | 10 | 10.0 | -27.0 | -15.0 |
| Tramadol | 264.10 | 58.40* | 10 | 20.0 | -52.0 | -3.0 |
| | | 246.20 | 10 | 10.0 | -17.0 | -15.0 |
| Deoxyephedrine | 150.30 | 119.20* | 10 | 10.0 | -15.0 | -10.0 |
| | | 91.20 | 10 | 20.0 | -27.0 | -5.0 |
| MDMA | 194.05 | 163.10* | 10 | 20.0 | -18.0 | -15.0 |
| | | 105.15 | 10 | 20.0 | -36.0 | -6.0 |
| Ketamine | 238.10 | 125.15* | 10 | 10.0 | -24.0 | -10.0 |
| | | 179.10 | 10 | 10.0 | -39.0 | -8.0 |
| Methadone | 310.10 | 265.05* | 10 | 10.0 | -19.0 | -15.0 |
| | | 105.15 | 10 | 10.0 | -38.0 | -8.0 |
| Diazepatn | 284.90 | 222.00* | 10 | 40.0 | -38.0 | -20.0 |
| | | 154.00 | 10 | 30.0 | -42.0 | -12.5 |
| Nitrazepam | 281.95 | 236.05* | 10 | 30.0 | -34.0 | -20.0 |
| | | 207.05 | 10 | 20.0 | -49.0 | -15.0 |
| Clonazepam | 315.90 | 270.00* | 10 | 40.0 | -33.0 | -15.0 |
| | | 241.00 | 10 | 30.0 | -46.0 | -15.0 |
| Alprazolam | 309.00 | 280.95* | 10 | 20.0 | -34.0 | -20.0 |
| | | 274.00 | 10 | 40.0 | -34.0 | -25.0 |
| Estazolam | 295.00 | 266.95* | 10 | 20.0 | -34.0 | -25.0 |
| | | 240.95 | 10 | 20.0 | -32.0 | -12.5 |
| Triazolam | 343.00 | 314.95* | 10 | 40.0 | -36.0 | -8.0 |
| | | 307.95 | 10 | 20.0 | -38.0 | -10.0 |
| Midazolam | 326.00 | 291.00* | 10 | 20.0 | -38.0 | -12.5 |
| | | 249.00 | 10 | 10.0 | -48.0 | -20.0 |

# 3. Results and Discussion

Oral fluids or plasma containing various concentrations of 15 drugs (morphine, codeine, dolantin, tramadol, alprazolam, MDMA, ketamine, methadone, diazepam, nitrazepam, clonzepam, alprazolam, estazolam, triazolam, midazolam). Five standards ranging from 0.5 to 500 ng/mL final oral fluid concentration were prepared and extracted by protein precipitation and the final extracts were analyzed by LC/MS/MS. The MRM chromatograms of 0.5 ng/mL 15 drugs mixture are presented in Fig. 1. The correlation coefficients for 15 drugs were found to be > 0.998.

Excellent precision and accuracy were maintained over four orders of magnitude, demonstrating a linear dynamic range suitable for real-world applications and the calibration curves with human plasma as the matrix were shown in Fig. 2. By analyzing oral fluid or plasma samples containing mixture of 15 drugs at three levels including 0.5 ng/mL, 2.5 ng/mL, 50 ng/mL, excellent repeatability was demonstrated with the %RSD being better than 4.4% for all the compound within six injections as shown in Table 3 (oral fluid samples as matrix) and Table 4 (human plasma as matrix).

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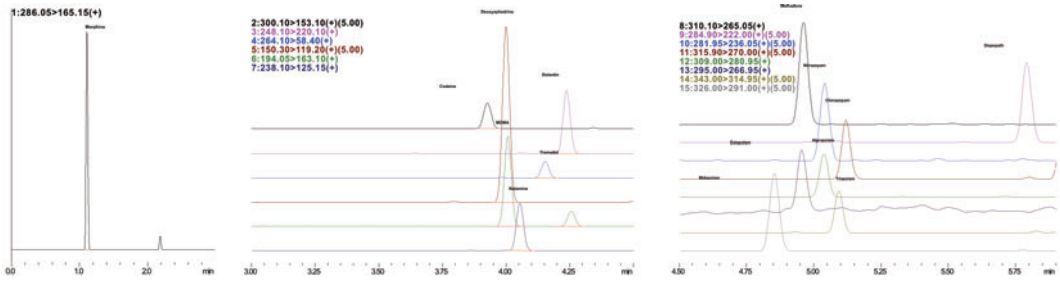


Fig. 1 Representative chromatograms of 15 drugs (0.5 ng/mL each) in human plasma

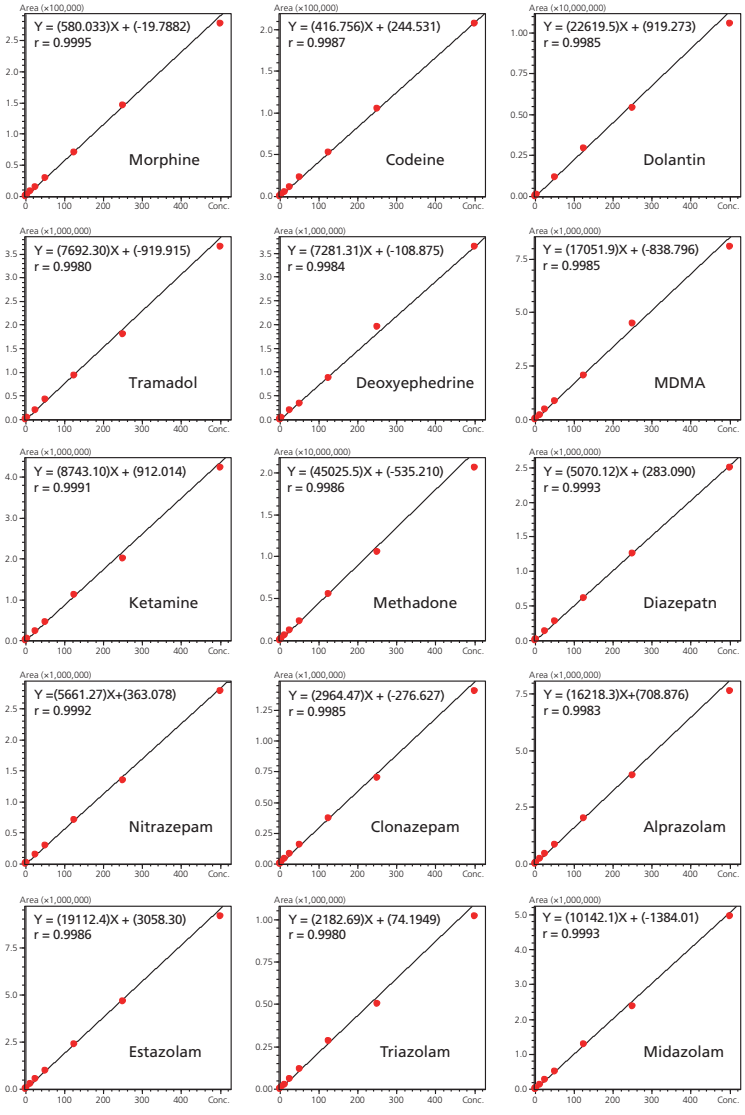


Fig. 2 Calibration curve of 15 durgs in human plasma

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Table 3 Repeatability of 15 drugs of different concentration in oral fluid (n=6)

| Compound | %RSD (0.5 ng/mL) | | %RSD (5.0 ng/mL) | | %RSD (50 ng/mL) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| Morphine | 0.182 | 3.19 | 0.034 | 2.66 | 0.044 | 1.10 |
| Codeine | 0.041 | 3.76 | 0.020 | 2.68 | 0.015 | 2.63 |
| Dolatin | 0.015 | 2.98 | 0.006 | 1.73 | 0.010 | 1.19 |
| Tramadol | 0.020 | 4.10 | 0.010 | 3.77 | 0.009 | 2.61 |
| Deoxyephedrine | 0.022 | 3.10 | 0.031 | 2.11 | 0.027 | 1.81 |
| MDMA | 0.015 | 3.85 | 0.052 | 2.39 | 0.014 | 1.79 |
| Ketamine | 0.020 | 3.35 | 0.013 | 2.26 | 0.015 | 1.40 |
| Methadone | 0.020 | 2.67 | 0.007 | 1.87 | 0.008 | 1.34 |
| Diazepatn | 0.013 | 3.99 | 0.010 | 2.77 | 0.009 | 2.57 |
| Nitrazepam | 0.024 | 2.37 | 0.010 | 1.99 | 0.010 | 1.69 |
| Clonazepam | 0.017 | 4.08 | 0.012 | 2.71 | 0.010 | 1.96 |
| Alprazolam | 0.024 | 2.71 | 0.010 | 2.41 | 0.007 | 1.66 |
| Estazolam | 0.020 | 4.19 | 0.004 | 3.00 | 0.009 | 2.14 |
| Triazolam | 0.041 | 3.51 | 0.029 | 2.01 | 0.027 | 2.34 |
| Midazolam | 0.016 | 4.36 | 0.009 | 2.85 | 0.009 | 1.74 |

Table 4 Repeatability of 15 drugs of different concentration in human plasma (n=6)

| Compound | %RSD (0.5 ng/mL) | | %RSD (5.0 ng/mL) | | %RSD (50 ng/mL) | |
|---|---|---|---|---|---|---|
| | R.T. | Area | R.T. | Area | R.T. | Area |
| Morphine | 0.080 | 2.30 | 0.079 | 1.53 | 0.096 | 0.54 |
| Codeine | 0.089 | 3.81 | 0.031 | 1.95 | 0.015 | 0.59 |
| Dolatin | 0.009 | 1.79 | 0.026 | 1.37 | 0.007 | 0.50 |
| Tramadol | 0.097 | 3.06 | 0.081 | 2.15 | 0.038 | 0.90 |
| Deoxyephedrine | 0.037 | 1.44 | 0.035 | 1.08 | 0.016 | 0.94 |
| MDMA | 0.040 | 1.76 | 0.037 | 1.36 | 0.013 | 0.99 |
| Ketamine | 0.022 | 1.65 | 0.030 | 1.00 | 0.006 | 0.59 |
| Methadone | 0.012 | 1.03 | 0.023 | 1.19 | 0.009 | 0.66 |
| Diazepatn | 0.029 | 1.30 | 0.017 | 1.28 | 0.008 | 0.45 |
| Nitrazepam | 0.039 | 1.93 | 0.018 | 1.34 | 0.008 | 0.39 |
| Clonazepam | 0.023 | 3.06 | 0.019 | 1.89 | 0.008 | 0.92 |
| Alprazolam | 0.010 | 2.01 | 0.020 | 1.09 | 0.008 | 0.89 |
| Estazolam | 0.016 | 3.45 | 0.024 | 1.53 | 0.007 | 0.97 |
| Triazolam | 0.050 | 3.91 | 0.025 | 2.43 | 0.009 | 1.06 |
| Midazolam | 0.035 | 1.91 | 0.024 | 1.65 | 0.006 | 1.25 |

# 4. Conclusions

The sensitive and repeatable LC/MS/MS technique was successfully applied for determination of drugs in oral fluid and plasma. Five ranging from 0.5 to 500 ng/mL final oral fluid concentration were prepared and extracted by protein precipitation and the final extracts were analyzed by LC/MS/MS and the linear regression for 15 drugs was found to be > 0.998. LOQs for these compounds in oral fluids were between 0.160 and 0.323 ng/mL, and LODs were between 0.040 and 0.081 ng/mL. Moreover, LOQs for these compounds in plasma were between 0.075 and 0.239 ng/mL, and LODs were between 0.019 and 0.060 ng/mL. By analyzing oral fluid samples or plasma containing mixture of 15 drugs at three levels including 0.5 ng/mL, 2.5 ng/mL, 50 ng/mL, excellent repeatability was demonstrated with the %RSD being better than 4.4% for all the compound within six injections.

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