INTRODUCTION

- Cannabinoids are among the most commonly detected compounds in toxicology screening programs and workplace drug testing.
- Urine is tested for the 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) metabolite along with its glucuronides.
- Traditionally, this assay involved hydrolysis, solid phase extraction, evaporation of solvents, derivatisation and finally detection by GC-MS, a time- and labour-intensive process.

AIM

- To develop a rapid and simple 2D-LC-MS/MS method for THC-COOH in human urine to be used in routine medical screening and workplace confirmations adhering to AS/NZS 4308:2008 standards.

METHODS

Sample preparation directly in vial:

1.5 µl urine + 25 µl 1 µM D8-THC-COOH
2. Add 125 µl 2% formic acid
3. Mix and inject 20 µl on 2D LC-MS/MS

Online sample clean-up (back flush):

- Column A: Zorbax Eclipse Plus-C18, 2.1 x 12.5 mm, 5 µm, room temp.
- Column B: Zorbax Eclipse Plus-C18, 2.1 x 50 mm, 5 µm
- Mobile phase A: 0.1% formic acid + 10mM ammonium formate in H2O
- Mobile phase B: Acetonitrile

LC conditions

- Pump 1 (loading pump): Flow rate 0.35 ml/min
- Pump 2: Flow rate 0.9 ml/min
- Column A: Load
- Column B: Elute

MS conditions

- Instrument: Agilent 6460 QQQ LC/MS with Agilent JetStream
- MRM
- THC-COOH: 343.2/299.2
- D8-THC-COOH: 353.2/254.1
- qualifier 1: 343.2/245.1
- qualifier 2: 353.2/194.1

RESULTS

Example standard curve:

- External cut-off control (15 ng/ml)
- Patient sample (745 ng/ml)

- Example chromatograms:
- Validation:
  - Range in human urine: 5-1000 ng/ml THC-COOH
  - Calibration curve: Linear weighted 1/x, r2≥0.996
  - Limit of detection: 0.5 ng/ml
  - Matrix effect: 95-105%
  - Recovery: 100%
  - Hydrolysis of glucuronides: 100%
  - Carry-over: <0.3%
  - Intra- and inter-day precision and bias <5% (<10% at LLOQ)
  - External QC program: Results within ±10%
  - Corr. with previous assay: r=0.997, deviation <12% (n=115)
  - Autosampler stability: Stable at 4°C for >3 days

CONCLUSION

- An assay for confirmation and quantification of THC-COOH in human urine was developed:
  - Simple sample preparation due to alkaline hydrolysis directly in the injection vial.
  - 2 min total runtime using robust online sample clean-up (2D-LC-MS/MS).
  - Excellent correlation with previous method and external QC samples.

The assay is to be implemented in routine use analysing >80 samples per day.