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Forensic toxicology

Quantitative analysis of cannabinoids in urine and oral fluid using reactive paper spray tandem mass spectrometry for clinical research and forensic toxicology

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Keywords

Urine, oral fluid, drug testing, THC, THC-COOH, THC metabolite, cannabinoids, reactive PS-MS, TSQ Fortis Plus MS, VeriSpray PaperSpray ion source, TraceFinder software, selected reaction monitoring (SRM), forensic toxicology, clinical research

Application benefits

- Simple on-paper derivatization allows for the sensitive and quantitative analysis of cannabinoids in urine and oral fluid.
- Reduced cost per sample and rapid analysis with minimal sample preparation enables increased sample throughput.

Goal

To develop a reliable and high-throughput quantitative MS-based method/workflow sensitive enough to meet toxicology cut-off values for drug testing of THC in oral fluid and THC-COOH in urine using the Thermo Scientific™ TSQ Fortis Plus™ triple quadrupole mass spectrometer with the Thermo Scientific™ VeriSpray™ PaperSpray ion source.

Introduction

Cannabis is the most widely used recreational drug worldwide and is often the target of workplace drug testing. The global drug testing market was estimated at a value of 8.1 billion USD in 2020 with approximately 25% of this revenue generated by cannabis/marijuana testing.[1] Drug testing for cannabis impairment is primarily accomplished by assaying for (–)-*trans*-Δ9-tetrahydrocannabinol (THC) in blood or oral fluid, or by analyzing its primary metabolite 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) in urine samples. Urine is the biofluid of choice for drug testing, with 40.8% of global revenue from the drug testing market generated from urine samples. Oral fluid samples are desired over blood samples since oral fluid can be easily and non-invasively collected in almost any location. Collection can be directly observed and is considered less

prone to adulteration or specimen substitution. Current drug testing guidelines stipulate that an initial screening test may be an immunoassay or another technology such as spectrometry, and that the confirmatory drug test must use mass spectrometric identification. Cutoff values differ by jurisdiction but are generally recommended as 2 ng/mL confirmatory / 4 ng/mL screening for THC in oral fluid and 15 ng/mL confirmatory / 50 ng/mL screening for THC-COOH in urine. Most often, gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS) is used for confirmatory drug testing. Chromatographic MS methods are considered the gold standard in forensic drug testing due in part to their high sensitivity and selectivity. Unfortunately for many confirmatory tests, extensive sample preparation steps and lengthy run times (typically 10–20 min) are required for adequate analysis. Because of this, many laboratories have extensive sample backlogs (months or longer). Paper spray mass spectrometry (PS-MS) is a potential alternative MS method featuring negligible sample preparation, rapid analysis (e.g., 1–2 min), and adequate sensitivity and selectivity. The use of a simple on-paper chemical derivatization scheme for cannabinoids with the diazonium salt Fast Red RC for reactive paper spray mass spectrometry demonstrates cannabinoid analysis sensitive enough for applications in toxicology.

All experiments were carried out using the VeriSpray PaperSpray ion source on a TSQ Fortis Plus triple quadrupole mass spectrometer (Figure 1A). The VeriSpray system enables robust, rapid, automated paper spray analysis. The VeriSpray plate loader magazine holds up to 10 VeriSpray sample plates, allowing for 240 samples to be run without user intervention (Figure 1B). Samples are deposited and dried on VeriSpray sampling plates, each containing 24 individual PaperSpray strips (Figure 1C).

Experimental

PaperSpray and MS conditions

A TSQ Fortis Plus triple quadrupole mass spectrometer coupled to a VeriSpray PaperSpray ion source was used for all analyses. Both rewet and spray solvents used for PaperSpray analysis were acetonitrile with 0.1% formic acid and were applied according to the settings in Table 1. The paper tip to MS inlet distance was 5.0 mm, and optimum tube lens settings and collision energies were determined by direct infusion of analyte mixtures into the mass spectrometer using a heated electrospray ionization source. Mass spectrometry parameters were optimized and set to a spray voltage of +3.8 kV, Q1 resolution at 0.7 Da FWHM, and Q3 resolution at 1.2 Da FWHM. Additional MS parameters are outlined in Table 2. Optimized SRM transitions for derivatized THC and THC-COOH and the corresponding internal standards are listed in Table 3.

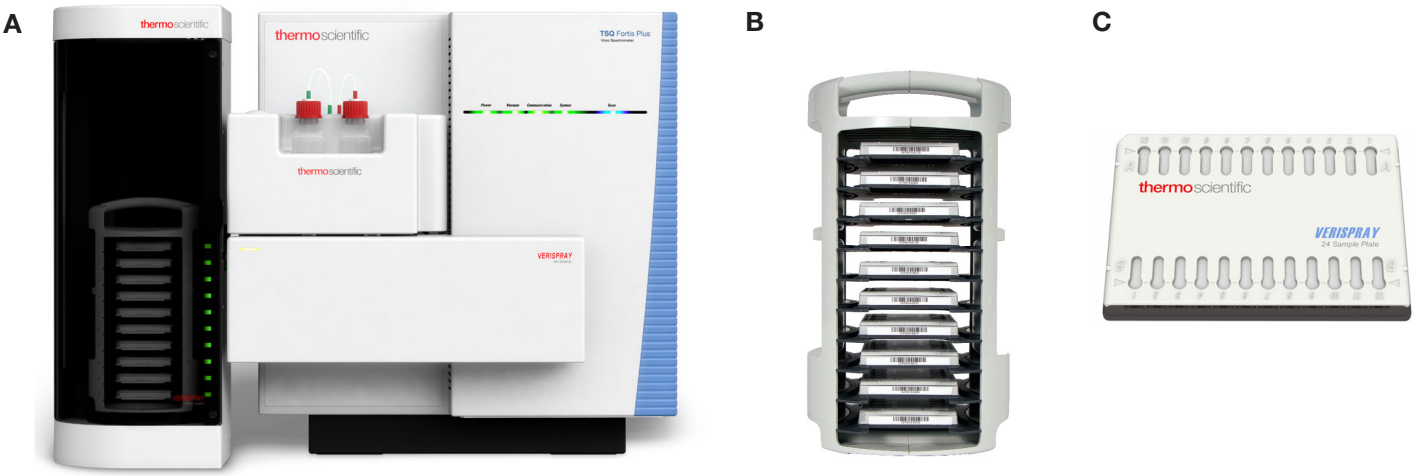

Figure 1. (A) VeriSpray ion source and plate loader mounted on to the TSQ Fortis Plus MS, (B) plate loader magazine, and (C) VeriSpray sample plate

Table 1. VeriSpray solvent application parameters. Each rewetting and solvent dispense is 10 µL.

Rewetting dispense delay		Solvent dispense delay					
Dispense	Delay (s)	Dispense	Delay (s)	Dispense	Delay (s)	Dispense	Delay (s)
1	1	1	1	6	3	11	5
2	1	2	1	7	5	12	7
		3	1	8	5	13	7
		4	1	9	5	14	7
		5	3	10	5		

Table 2A. TSQ Fortis Plus parameters

Ion source parameter	Value
Spray voltage	Time dependent
Positive ion	3,800 V
Sweep gas	0 Arb
Ion transfer tube temperature	300 °C
Q1 resolution	0.7
Q3 resolution	1.2
CID Gas	2 mTorr

Table 2B. Time-dependent spray voltage settings

Time (min)	Voltage (V)
0	0
0.1	3,800
1.1	0

Table 3. Optimized SRM transitions for THC and THC-COOH derivatized with Fast Red RC

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	Tube lens (V)
THC	483.3	141.9	52.7	115
		156.9	37.4	
		361.1	24.5	
THC-d_3	486.3	141.9	52.5	115
		156.9	37.7	
		364.1	23.8	
THC-COOH	513.3	141.9	54.6	130
		156.9	35.5	
		338.1	23.2	
THC-COOH-d_3	516.3	141.9	53.0	130
		156.9	37.8	
		341.1	23.6	

Reactive PaperSpray MS

All measurements used reactive PaperSpray MS (PS-MS). An on-paper chemical derivatization of THC and THC-COOH was accomplished using the diazonium salt Fast Red RC (5-chloro-2-methoxybenzenediazonium chloride hemi (zinc chloride) salt). Ten microliter aliquots of 1.5 mM Fast Red RC in 5.0 mM ammonium acetate were deposited on VeriSpray sample plates and air-dried. Oral fluid and urine samples were spiked with internal standard and diluted with methanol to 70/30 sample/methanol. Ten microliter of sample solution was applied to the VeriSpray cartridges on top of the dried Fast Red RC solution. Two 10 µL aliquots of dichloromethane were deposited onto the dried sample spots. Calibration curves (0.5, 1.5, 4, 10, 25, 100, 250 ng/mL) were generated from six replicates using reactive PS-MS.

Method validation

Accuracy, within-run and between-run precision were assessed according to the Scientific Working Group for Forensic Toxicology's (SWGTOX) guidelines.[2] The low QC sample was prepared near the cutoff value, the medium QC value at the midpoint between high and low QC samples, and the high QC at 80% of the highest calibrator. QC samples were analyzed (n = 5) over a period of five consecutive days to determine accuracy (%bias), within-run precision (%CV), and between-run precision (%CV).

Data acquisition and analysis

Data acquisition and processing was accomplished using Thermo Scientific™ TraceFinder™ software version 5.1 SP3 Clinical Research. All linear regressions used a 1/X weighting. Lower limits of quantitation (LLOQs) were determined from the lowest calibrator that met the criteria: $R^2 \geq 0.98$, %CV ≤ 15, and %bias ≤ ±20, and ion ratios 1 and 2 within 20% of the target ion ratio (average ion ratio of all calibrators).

Results and discussion

The derivatization reaction used has been previously reported for solution-phase chemistry[3] and was easily applied to on-paper chemistry. Dichloromethane addition to dried biofluid spots was found to increase sensitivity and method robustness, although the mechanism is unclear. Calibrations for both THC in human oral fluid and THC-COOH in human urine were achieved with good linearity (R^2 = 0.9977, 0.9919, respectively) and used for the quantitation of quality control samples prepared in pooled human oral fluid and urine samples. The calibration models of THC in oral fluid and THC-COOH in urine are shown in Figure 2. The %RSD of internal standard signal areas over the calibrations was approximately 20%, which is typical for PS-MS measurements and indicates negligible variability arising from the derivatization process. QC samples at 9, 80, and 200 ng/mL analyzed over five days demonstrated acceptable performance for THC in oral fluid (Table 4) and THC-COOH in urine (Table 5). Intra- and inter-day precision was less than 15% and bias values less than ±15% at all QC levels. LLOQ values of 4 ng/mL and 10 ng/mL were determined for THC in oral fluid and THC-COOH in urine, respectively. The total analysis time for a single dried urine or oral fluid spot, including extraction (~1 min) followed by mass spectrometric analysis (1.2 min) was approximately 2.2 minutes.

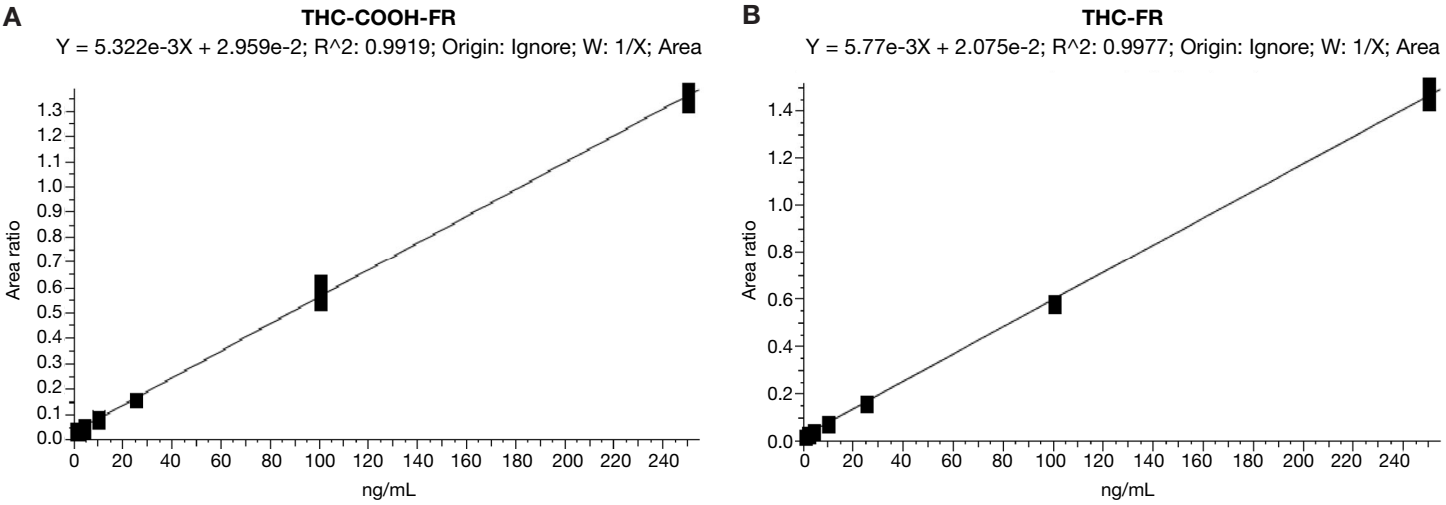

Figure 2. Fast Red RC reactive paper spray mass spectrometry calibration (7 levels, 2–250 ng/mL, n ≥ 6) of (A) THC-COOH in human urine and (B) THC in human oral fluid

Table 4. Quality control data for THC derivatized with Fast Red RC in human oral fluid samples

QC level	Concentration (ng/mL)	%Bias	Within-run precision (%CV)	Between-run precision (%CV)
Low	9	-13.0	8.2	5.5
Medium	80	7.2	2.1	1.4
High	200	11.7	2.4	3.9

Table 5. Quality control data for THC-COOH derivatized with Fast Red RC in human urine samples

QC level	Concentration (ng/mL)	%Bias	Within-run precision (%CV)	Between-run precision (%CV)
Low	9	2.9	13.7	8.8
Medium	80	10.3	3.3	2.0
High	200	6.1	5.9	4.0

Conclusion

PaperSpray MS with the VeriSpray ion source demonstrated sensitive and accurate quantitation of THC in oral fluid and THC-COOH in urine. Sensitivity was dramatically increased using Fast Red RC derivatization with reactive paper spray mass spectrometry. The determined LLOQ of 4 ng/mL for THC in oral fluid is adequate for screening applications, and the determined LLOQ of 10 ng/mL for THC-COOH in urine is suitable for both screening and confirmatory testing. A total analysis time of ~2 minutes coupled with the simple on-paper derivatization allows for a high-throughput and robust analysis of biofluid samples while avoiding lengthy and cumbersome offline derivatization or sample preparation. A detailed evaluation of this approach for cannabinoid measurement by PS-MS that includes a comparison with validated LC-MS measurements has been published.[4]

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