Ultra-sensitive analytical methodology for the quantification of 11-nor-9-carboxy-THC (THC-COOH) in oral fluid

Using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

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With over 192 million consumers worldwide in 2018, cannabis is the most commonly abused recreational drug.[1] Detection of its use can be performed in several biological matrices such as blood, urine, hair and oral fluid. In recent years, oral fluid has gained considerable attention as a quicker and less invasive means of monitoring cannabis use. More specifically, the use of this matrix for drug testing benefits from ease of sampling, observed collection and difficulty of sample adulteration.

Δ9-tetrahydrocannabinol (THC) has previously been used as the marker of choice to monitor cannabis consumption. However, the detection and quantification of THC in drug screening can result in a high-number of false positives since this analyte can be found in subjects passively exposed to cannabis. 11-nor-9-carboxy-THC (THC-COOH) was proposed as a marker of cannabis intake since it is not detected in oral fluid collected from subjects passively exposed to cannabis.[2] The low (pg/mL) concentrations of THC-COOH in oral fluid of active users together with the abundance of matrix interferences present in the stabilization buffer and oral fluid itself pose a considerable analytical challenge. In this technical note, the SCIEX 7500 System is shown to provide the required levels of sensitivity, robustness and performance for the accurate quantification of pg/mL levels of THC-COOH in oral fluid without the need for derivatization.

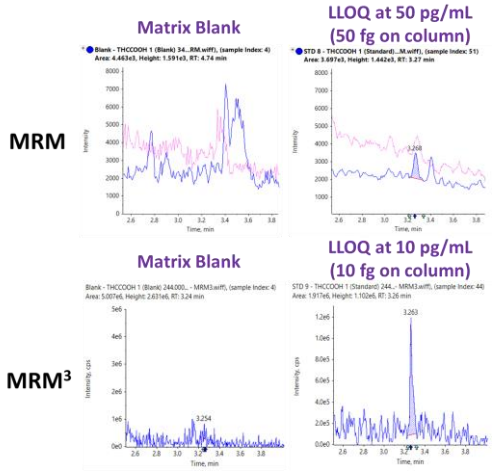

Figure 1. High sensitivity on the SCIEX 7500 System enabled trace level detection of THC-COOH in oral fluid. XICs showing the quantifier ion traces for the matrix blank and THC-COOH at the LLOQ for both the MRM (top) and MRM³ (bottom) workflows.

Key advantages of the SCIEX Triple Quad 7500 System – QTRAP Ready for sensitive detection of THC-COOH in oral fluid

- Hardware improvement on the SCIEX 7500 System provides significant gains in sensitivity by sampling more ions, resulting in greater quantification performance[3]
- OptiFlow® Pro Ion Source with E Lens™ Technology provides improvements in ion generation while D Jet™ Ion Guide improves ion sampling, resulting in greater sensitivity
- Simple dilute and shoot approach significantly reduces sample preparation time and negates the needs for time-consuming procedures such as solid-phase extraction (SPE), evaporation and reconstitution
- Improved ion generation and sampling enables accurate and sensitive quantification of THC-COOH with an LLOQ of 50 pg/mL (50 fg on column) in oral fluid using the MRM workflow
- The ion trap functionality of the SCIEX Triple Quad 7500 System – QTRAP® Ready was leveraged to improve sensitivity, selectivity and specificity of THC-COOH detection in oral fluid
- MRM³ workflow was successfully used to eliminate background and matrix interferences at the low end of the calibration curve, improving signal-to-noise ratio and resulting in an LLOQ of THC-COOH of 10 pg/mL (10 fg on column) in oral fluid
- Overall performance of the system resulted in excellent linearity with optimal precision, accuracy and reproducibility across the calibration range

Experimental details

Stock solution preparation: Stock solutions of THC-COOH and THC-COOH-d3 were prepared separately in methanol at 1000 ng/mL. Blank oral fluid was spiked with the stock solution of THC-COOH to create calibrator solutions at 100000, 50000, 10000, 5000, 1000, 500, 100, 50 and 10 pg/mL. All sample concentrations are expressed as pg of THC-COOH per mL of blank oral fluid or fg of THC-COOH on column throughout this technical note, regardless of the subsequent dilutions performed.

Sample preparation: A dilute and shoot sample preparation approach was used in this experiment. In brief, 250 µL of each of the spiked oral fluid calibrator solutions was mixed with 750 µL of Quantisal stabilization buffer. The resulting solutions were then further diluted 10 times to minimize matrix effects by taking 100 µL of each solution and adding 25 µL of the 1000 ng/mL internal standard stock solution and 875 µL of a 20% methanol solution. The samples were thoroughly vortexed, centrifuged and 40 µL of the supernatant were injected for analysis. No further sample clean-up nor extraction was performed.

Liquid chromatography: HPLC separation was performed on an ExionLC™ system using a Phenomenex C18 column (50 × 2.1 mm, 2.6 µm, 00D-4475-AN) held at 40ºC equipped with a Phenomenex KrudKatcher Ultra fit (AF0-5727). Mobile phases used consisted of water, methanol and modifiers. The LC flow rate was 0.5 mL/min and the total run time was 6 min. The injection volume was 40 µL.

Mass spectrometry: A SCIEX 7500 System equipped with an OptiFlow Pro Ion Source and E Lens Technology was used. The ionization source was operated in negative electrospray ionization (ESI) mode. A looped MS experiment was performed. The first experiment consisted of five MRM transitions of 100 msec dwell time each: three for THC-COOH and two for THC-COOH-d3. The second experiment was an MRM^3 scan using a scan speed of 10000 Da/s with 200 msec fill time, 25 msec excitation time and an 8V Q3 entry barrier. MRM^3 data was collected as full scan data from 180 to 250 Da to simultaneously monitor the 343.1→299.2→244.9 and the 343.1→299.2→191.1 MRM^3 transitions. The Guided MRM^3 automated compound optimization feature in SCIEX OS Software was used for optimizing the parameters for the MRM^3 experiment. Six replicates of each sample were injected to build a data analysis processing method.

Data analysis: Data processing was performed using SCIEX OS Software. Quantitative analysis was performed using AutoPeak Algorithm in the Analytics module of the software where calibration curves, concentration calculations, assay precision and accuracy statistics were generated.

Combination of OptiFlow Pro Ion Source and E Lens Technology results in enhanced workflow analytical sensitivity

Accurate quantification of meaningful (pg/mL) THC-COOH levels in human oral fluid is paramount to confirm THC consumption. However, the low concentration of THC-COOH found in oral fluid of active THC users and the complexity of this matrix make detection of this metabolite challenging. As a result, developing a sensitive workflow that can accurately quantify trace levels of THC-COOH in oral fluid is critical to confirm active cannabis consumption.

The ability to accurately detect low concentration levels of THC-COOH extracted from human oral fluid was assessed using the SCIEX 7500 System. Six replicates of each of the processed calibrator samples were injected to determine the lower limit of quantification (LLOQ) value for THC-COOH and to assess the overall robustness and reproducibility of the instrument. The LLOQ value for THC-COOH was determined to be the lowest concentration calibration level meeting the following analytical performance requirements: signal-to-noise ratio (S/N) ≥10, calculated concentration accuracy within 15% of 100%, precision (%bias) below 10%, and calibrators falling on a linear calibration curve with an R^2 value of at least 0.99. Detection and integration of the peaks was automatically performed using the AutoPeak Algorithm in the Analytics module of SCIEX OS Software. Analyte concentration and ion ratio were calculated automatically in the software.

Figure 2 shows the extracted ion chromatogram (XIC) traces and resulting calibration curves for two of the MRM transitions monitored for THC-COOH. The two series of XIC traces for both the quantifier and qualifier ions of THC-COOH showed a high level of sensitivity and precision across concentrations ranging from 50 to 100000 pg/mL. Eight levels of calibrators were used to determine the ion ratio criteria for the quantifier and qualifier ions of THC-COOH. The results demonstrated excellent correlation of the generated regression curves covering concentration ranges meeting the requirements of pg of THC-COOH per mL of blank oral fluid: lower limits of quantification (LLOQ) was determined to be 50 pg/mL (50 fg on column) for the quantifier ion. In addition, excellent linearity was observed across the concentration range from 50 to 100000 pg/mL for the quantifier ion and from 100 to 100000 pg/mL for the qualifier ion, with R^2 values of 0.99870 and 0.99868, respectively.

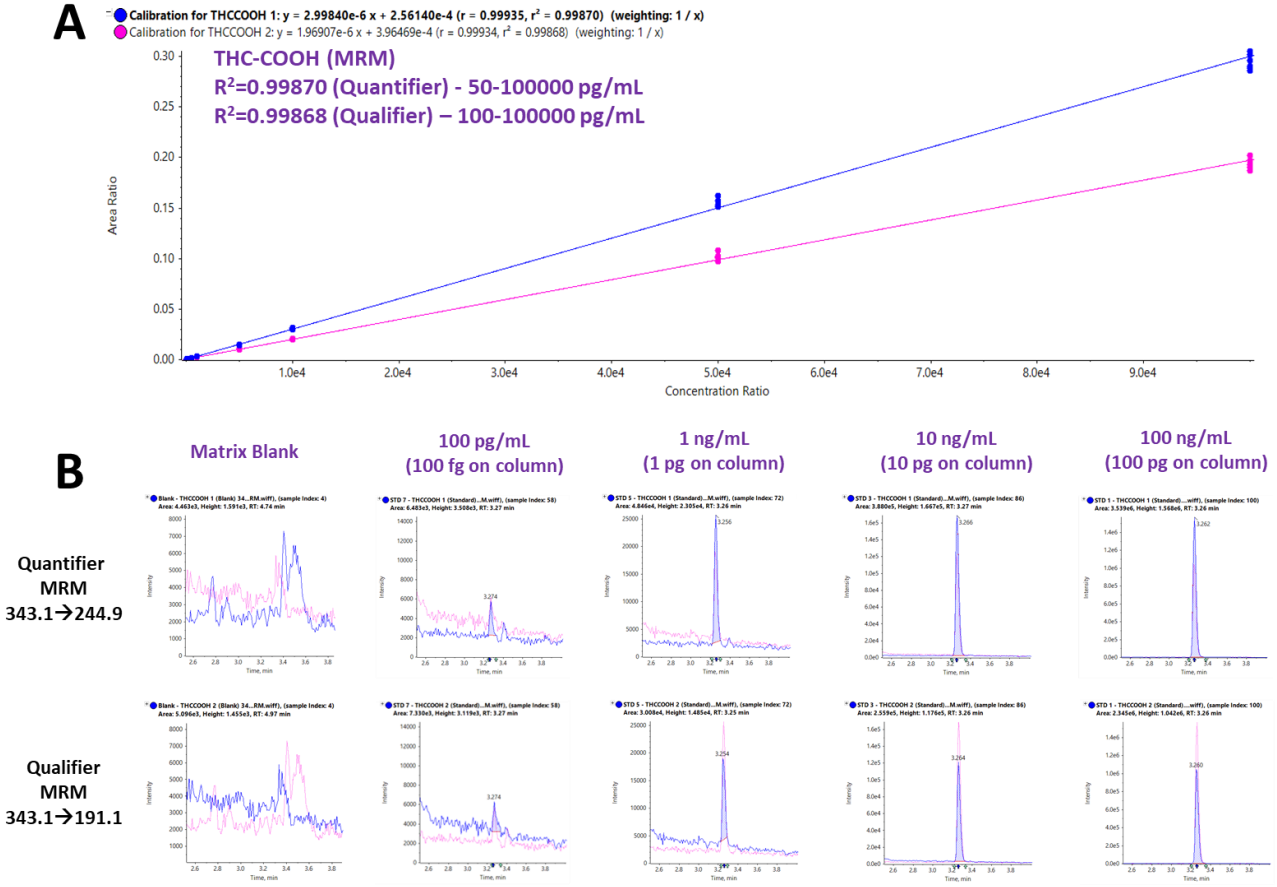

Figure 2. High sensitivity and linearity using MRM for the detection of THC-COOH in oral fluid. (A) Calibration curves resulting from the calibration series for THC-COOH from 50 to 100000 pg/mL and 100 to 100000 pg/mL for the quantifier and qualifier ions, respectively. (B) Selected XIC traces of the quantifier and qualifier ion for the matrix blank and THC-COOH at 100, 1000, 10000 and 100000 pg of THC-COOH per mL of blank oral fluid. A total of six injections were performed for each calibrator sample.

A series of six replicate injections were performed to evaluate the robustness and overall performance of the SCIEX 7500 System. Table 1A summarizes the MRM results from the average (n=6) injections of each of the calibrator solutions spiked with THC-COOH and extracted from human oral fluid. The assay showed excellent precision and accuracy for concentrations ranging from 50 to 100000 pg/mL for the two THC-COOH MRM transitions monitored. All quantified samples had a %CV value below 10% and accuracy values ranging between 91.38 and 111.38%. These results demonstrate that the SCIEX 7500 System can provide robust and reproducible results even for challenging forensic workflows requiring detection of extremely low concentration levels of analytes. The performance showcased by the SCIEX 7500 System prove to be adequate to achieve the required levels of sensitivity, robustness and reproducibility required by the current workflow to accurately quantify THC-COOH down to pg/mL levels.

Improved selectivity and LLOQ using MRM^3

Detecting low amounts of a metabolite present in complex biological specimen without extensive or time-consuming sample cleanup is often prone to high background and matrix interferences. In such cases, accurate quantification of specific analytes using MRM can be impaired due to the high matrix background. What typically ensues is a compromised LLOQ as the detection of these analytes is limited by signal-to-noise rather than raw instrument response. The addition of a third MS stage combining normal LIT functions with normal quadrupole functions using a novel QQQ/LIT workflow coined MRM^3 can significantly reduce these background interferences while providing increased selectivity and sensitivity at the low end of the calibration curve.[3] The principle of MRM^3 analysis as performed on the SCIEX Triple Quad 7500 System – QTRAP Ready is shown in Figure 3.

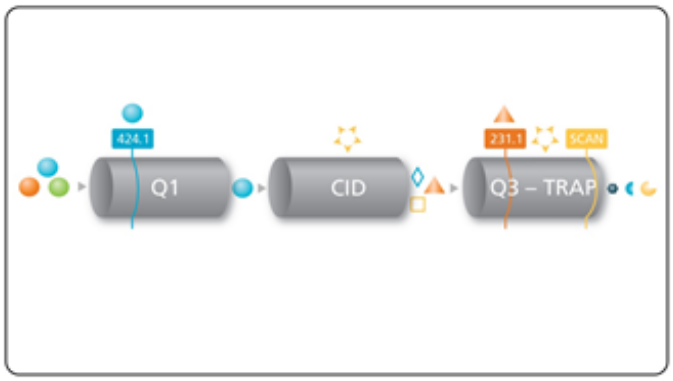

Figure 3. MRM³ scan mode for quantitative analysis by LC-MS. Analyte's precursor ions are selected in the Q1 quadrupole, fragmented in the Q2 collision cell before being collected in the linear ion trap (LIT) in Q3 where a suitable fragment ion is successively being isolated then fragmented using resonance excitation. These second generation product ions are then collected out of the LIT and scanned by the detector, enabling higher specificity analyte detection with better sensitivity from highly complex biological samples.[4]

The MRM³ quantification assay was performed on the same set of calibrator samples. Two secondary product ions of the 343.1→299.2 transition were isolated, fragmented in the linear ion trap, extracted using a width of 1 Da and summed together to produce the MRM³ signal. Figure 4 shows detection of THC-COOH at 50 pg/mL using MRM (top left) and MRM³ (top right). However, due to higher background when running the MRM assay, no signal was observable at 10 pg/mL THC-COOH (bottom left) in either of the two MRM transitions monitored. MRM³ showed much better specificity and sensitivity than the MRM data at this concentration (bottom right). As seen in Figure 4, the use of the MRM³ workflow provided much higher sensitivity compared to MRM alone due to the additional level of selectivity obtained by monitoring secondary product ions for THC-COOH in oral fluid.

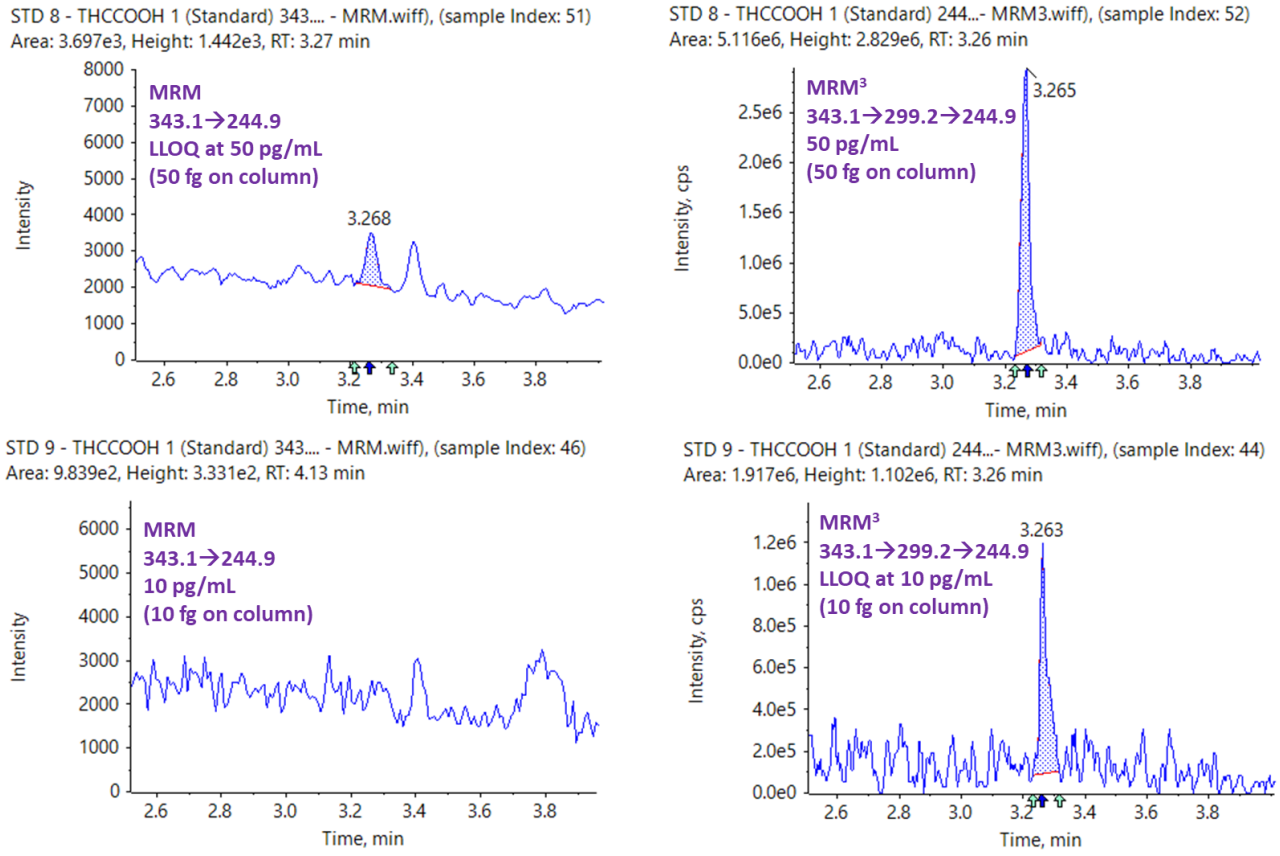

Figure 4. Comparison of MRM vs. MRM³ for the detection of THC-COOH in oral fluid. (Top) Both MRM (left) and MRM³ (right) workflows enabled detection and quantification of THC-COOH at 50 pg/mL in oral fluid. However, the MRM³ workflow enabled significant reduction of background caused by matrix interference, significantly improving selectivity and resulting in a better LLOQ. (Bottom) Using MRM, THC-COOH was not detectable at 10 pg/mL due to higher background (left) however the MRM³ approach provided good detection of THC-COOH at this level (right).

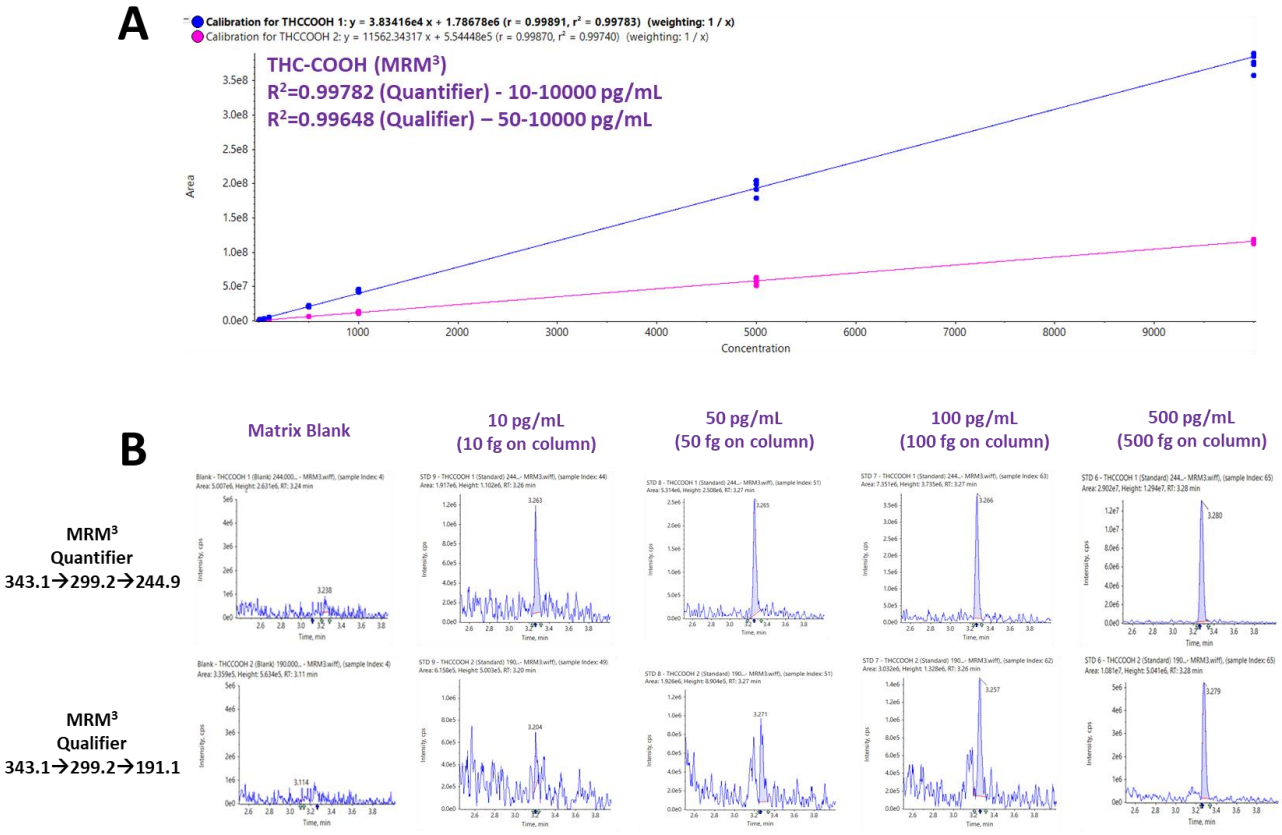

Figure 5. Significant improvement in LLOQ using MRM³ for the detection of THC-COOH in oral fluid. A) Calibration curves resulting from the calibration series for THC-COOH from 10 to 10000 pg/mL and 50 to 10000 pg/mL for the quantifier and qualifier ions, respectively. B) Selected XIC traces of the quantifier and qualifier ion for the matrix blank and THC-COOH at concentrations at the low end of the curve (10, 50, 100 and 500 pg of THC-COOH per mL of blank oral fluid). A total of six injections were performed for each calibrator sample.

The LLOQ for the MRM³ was also determined by processing the MRM³ data in SCIEX OS Software. Figure 5 shows the extracted ion chromatogram (XIC) traces and resulting calibration curves for the two secondary product ions monitored for THC-COOH. The LLOQ was determined to be 10 pg/mL (10 fg on column) for the quantifier ion. Calibration curves were also generated for the MRM³ workflow and showed excellent linearity across the concentration range from 10 to 10000 pg/mL for the quantifier ion and from 50 to 10000 pg/mL for the qualifier ion, with R^2 values of 0.99782 and 0.99648, respectively. Overall, a 5-fold improvement in LLOQ was observed for the MRM³ experiment (10 pg/mL) compared to the MRM experiment (50 pg/mL). While MRM detection suffered from high background caused by matrix interference at low concentrations, MRM³ detection resulted in more selectivity and improved the detection sensitivity. Table 1B summarizes the MRM³ results from the average (n=6) injections of each of the calibrator solutions. The precision values (%CV) were <10% with accuracies between 87.43 and 109.49 for concentrations ranging from 10 to 10000 pg/mL for the quantifier ion and from 50 to 10000 pg/mL for the qualifier ion, respectively.

Conclusions

A highly sensitive workflow for the detection of THC-COOH in oral fluid is described using the SCIEX Triple Quad 7500 System – QTRAP Ready.[4] The use of a rapid and simple dilute and shoot approach without the need for sample pre-treatment or derivatization provided a comprehensive extraction method that is suitable for high-throughput oral fluid drug testing in the forensic toxicology laboratory. The high sensitivity of the SCIEX 7500 System enabled accurate and sensitive quantification of THC-COOH in oral fluid with an LLOQ of 50 pg/mL (50 pg on column). This unparalleled sensitivity was achieved with excellent linearity and without any sacrifice or compromise in data quality, as demonstrated by the excellent precision (<10%) and accuracy (between 91.38 and 111.38%) across the calibration range (50 to 10000 pg/mL). The MRM³ workflow was shown to increase selectivity by significantly reducing background and matrix interference, resulting in a 5-fold improvement in LLOQ compared to the MRM approach. The results of the MRM³ experiment also demonstrated excellent linearity, precision and accuracy across the calibration range.

Overall, these results demonstrate that the levels of sensitivity and robustness achieved by the SCIEX 7500 System enable accurately quantification of low levels of THC-COOH in oral fluid without the need for time-consuming sample preparation or derivatization. Therefore, the presented workflow is readily adaptable for high-throughput toxicology testing and provides a reliable and sensitive means of differentiating passive environmental exposure from active cannabis consumption.

References

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2. K.B. Scheidweiler, S.K. Himes, X. Chen, H.F. Liu, M.A. Huestis. (2013) 11-Nor9-carboxy-Δ9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **405**, 6019.
3. MRM³ quantitation for highest selectivity in complex matrices. SCIEX technical note RUO-MKT-02-2739.
4. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.

A

	Row	Component Na...	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
Quantifier MRM 343.1→244.9	7	THCCOOH 1	50.00000	6 of 6	4.605e1	3.423e0	7.43	92.10
	8	THCCOOH 1	100.00000	6 of 6	8.529e1	3.193e0	3.74	85.29
	9	THCCOOH 1	500.00000	6 of 6	5.505e2	3.337e1	6.06	110.10
	10	THCCOOH 1	1000.00000	6 of 6	1.114e3	2.945e1	2.64	111.38
	11	THCCOOH 1	5000.00000	6 of 6	4.869e3	9.742e1	2.00	97.37
	12	THCCOOH 1	10000.00000	6 of 6	1.024e4	1.787e2	1.75	102.36
	13	THCCOOH 1	50000.00000	6 of 6	5.165e4	1.386e3	2.68	103.29
	14	THCCOOH 1	100000.00000	6 of 6	9.810e4	2.587e3	2.64	98.10
Qualifier MRM 343.1→191.0	22	THCCOOH 2	100.00000	6 of 6	9.138e1	6.346e0	6.94	91.38
	23	THCCOOH 2	500.00000	6 of 6	5.283e2	2.716e1	5.14	105.67
	24	THCCOOH 2	1000.00000	6 of 6	9.988e2	3.219e1	3.22	99.88
	25	THCCOOH 2	5000.00000	6 of 6	4.957e3	1.102e2	2.22	99.15
	26	THCCOOH 2	10000.00000	6 of 6	1.025e4	2.137e2	2.08	102.53
	27	THCCOOH 2	50000.00000	6 of 6	5.162e4	1.760e3	3.41	103.24
	28	THCCOOH 2	100000.00000	6 of 6	9.815e4	2.686e3	2.74	98.15

B

	Row	Component Na...	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
Quantifier MRM³ 343.1→299.2→244.9	6	THCCOOH 1	10.00000	6 of 6	1.003e1	4.745e-1	4.73	100.29
	7	THCCOOH 1	50.00000	6 of 6	4.371e1	1.915e0	4.38	87.43
	8	THCCOOH 1	100.00000	6 of 6	9.935e1	4.827e0	4.86	99.35
	9	THCCOOH 1	500.00000	6 of 6	5.188e2	1.707e1	3.29	103.77
	10	THCCOOH 1	1000.00000	6 of 6	1.095e3	3.944e1	3.60	109.49
	11	THCCOOH 1	5000.00000	6 of 6	5.074e3	2.529e2	4.98	101.49
	12	THCCOOH 1	10000.00000	6 of 6	9.819e3	3.059e2	3.12	98.19
Qualifier MRM³ 343.1→299.2→191.1	21	THCCOOH 2	50.00000	6 of 6	4.572e1	2.874e0	6.29	91.44
	22	THCCOOH 2	100.00000	6 of 6	1.003e2	4.519e0	4.51	100.31
	23	THCCOOH 2	500.00000	6 of 6	5.149e2	1.858e1	3.61	102.98
	24	THCCOOH 2	1000.00000	6 of 6	1.066e3	9.996e1	9.38	106.56
	25	THCCOOH 2	5000.00000	6 of 6	4.947e3	3.700e2	7.48	98.95
	26	THCCOOH 2	10000.00000	6 of 6	9.976e3	1.836e2	1.84	99.76

Table 1. Statistical results table generated in SCIEX OS Software showing accuracy and precision of THCOOH for the series of calibrator samples using (A) MRM and (B) MRM³. Average (n=6) results generated in Analytics show high levels of precision (<10%) and accuracy (within 15% of 100%) for concentrations across the respective calibration curves for the quantifier and qualifier ions for both experiments. Reported concentrations are pg of THC-COOH per mL of blank oral fluid without accounting for the subsequent dilutions performed during the sample preparation procedures.

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