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Forensic

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New Strategy for Easy, Sensitive and Selective Detection of THC Carboxylic Acid Direct from Hair

Use of LC-MRM^3 on SCIEX QTRAP$^®$ 5500 LC-MS/MS System

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Overview

A new, highly selective and sensitive scan function for quantification of 11-nor-9-carboxy-D^9-tetrahydrocanabinol (THC carboxylic acid) in hair is presented. Hair is a very complex matrix and high background and/or interferences around the expected retention time for THC carboxylic acid are observed in the main MRM traces. The ability to use fragments of second order, MS^3 (MS/MS/MS), will show a significant increase in selectivity in comparison to classical MRM mode. The hardware selectivity of LC-MS^3 in combination with the fast scan speeds and extraordinary sensitivity of the latest generation Linear Accelerator^{TM} Trap enables a highly selective and sensitive LC-MS/MS/MS experiment for quantification, LC-MRM^3, which is a new state-of-the-art strategy for quantitation.

Introduction

Cannabis (marijuana) is one of the most widely used drugs in the world and is illegal in most countries. The active ingredient in cannabis is THC and its major metabolite is THC carboxylic acid, which is the target analyte for detecting cannabis use. Urine is the typical matrix analyzed for THC use and LC/MS/MS operating in MRM mode is the usual technique of choice. Analysis of urine or plasma for THC carboxylic acid generally detects use only during a time window of up to thirty days. However, THC carboxylic acid is also stored in hair and, hence, enables monitoring of a person's use of cannabis over an extended period of time. Unfortunately, the hair matrix is quite complex and contains various substances similar in structure and mass to THC carboxylic acid, e.g. some fatty acids. Furthermore, the concentrations of THC carboxylic acid in hair are relatively low, increasing the challenges of this analysis.

In principle, there are different approaches to diminish the negative influence of interfering substances of hair matrix, e.g. sample clean-up (Solid Phase Extraction) or derivatization. As a result of the structural and physical affinity of fatty acids, classical sample clean-up/enrichment will not lead to an appropriate increase of selectivity of the overall method. Investigations into a suitable derivatization process with various common reagents for THC carboxylic acid [1] were unsuccessful in increasing selectivity. As mentioned before, the physical and chemical affinity of compounds from the hair matrix lead to various derivatization products that also have MRM mass transition interferences close to expected retention time of the THC carboxylic acid derivatives. A very selective MS experiment is required to quantify low level analytes in complex matrices that yield MRM interferences. A new MS^3 approach for quantification – MRM^3 – is a new approach for low level quantification when the traditional MRM approach does not have the required selectivity.

Hardware and Methods

The basic principle of exceptional performance of SCIEX QTRAP$^®$ 5500 MS^3 mode is traced back to the redesigned, second generation Linear Accelerator^{TM} Trap, Figure 1. To ensure higher efficiency of trapping, monoisotopic isolation and increased yield of fragmentation in linear ion trap mode, the linear ion trap in the SCIEX QTRAP$^®$ 5500 system has several technical improvements. These improvements allow unique functionality, such as MRM^3 quantitation.
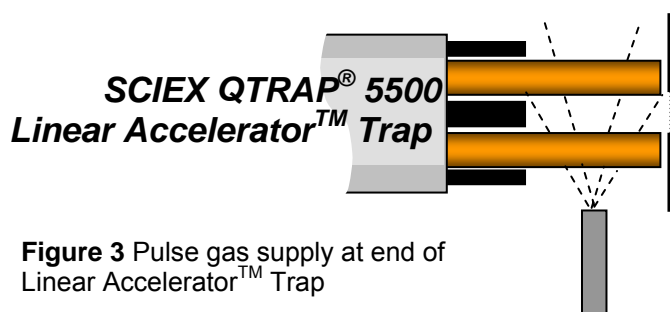

Figure 1
Linear Accelerator^{TM} Trap

A higher RF amplitude of 1,228 MHz is now applied to rods and increases the fragmentation yield by a factor of over 2x in comparison to the 4000 QTRAP$^®$ system [2]. Additionally higher RFs are beneficial for trapping small molecules.
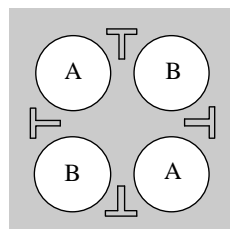
Figure 2 T-shaped auxillary rods

The confinement field is further optimized by 4 additional T-shaped auxiliary electrodes, Figure 2. The decreasing T-shape from the trap entrance to the trap exit causes an electrical field gradient to be induced; which results in higher trapping efficiency and faster scan capabilities.

SCIEX QTRAP® 5500 Linear Accelerator™ Trap

Figure 3 Pulse gas supply at end of Linear Accelerator™ Trap

Last but not least, a pulse gas valve is placed close to the region of Q3 Linear Ion Trap (LIT) and leads to increased pressure during LIT operating modes, Figure 3. Higher pressure reduces excitation time [3] and together with RF significantly increases MS^3 fragmentation efficiency. The technical innovation in combination with unique hardware set-up on hybrid SCIEX QTRAP® 5500 instruments enables the possibility of extraordinary and inimitable sensitive MS^3 experiments. Hardware selectivity can be obtained whenever dedicated second order fragment ion *m/z* is equal to or exceed 200. In those cases MS^3 window width can be decreased down to 1.1 amu without loosing significant sensitivity. Hence, a highly selective and sensitive MS/MS/MS experiment is generated, which is dedicated to quantification, LC-MRM^3.

MS-Conditions

'Compound Optimization' in Analyst® software offers an easy way for the first step in developing an LC-MRM^3 method for detection of THC carboxylic acid directly from hair. This software tool optimizes customary MRM transitions, which can be used as a starting point for seeking appropriate secondary fragments using MS^3 scan capabilities. THC carboxylic acid ionizes best in negative electrospray mode and an [M-H]$^-$ ion is observed at *m/z* 343. A Product Ion Scan of THC carboxylic acid, shown in Figure 4, serves as the basis of applicative secondary precursor ions. Higher mass fragment ions of sufficient intensity, e.g. at *m/z* 299 and/or 245, are typical eligible second precursor ions. In case of THC carboxylic acid the 'Compound optimization' determines the MRM transitions 343/299 and *m/z* 343/245 to be most abundant in negative ionization mode. Using optimized

MRM transition 343/299 and related parameters, e.g. Collision Energy (CE), an MS^3 scan for second order fragment ion at *m/z* 299, Figure 5, enables potential fragments for setting up an MRM^3 experiment to be determined. The ion at *m/z* 245 is a fragment ion of second order with the ion at *m/z* 299 being a secondary precursor ion. After determining that this fragment was detected as one of the most abundant MRM transitions it seems beneficial to use MRM^3 transition of ions at *m/z* 343 to 299 to 245 (343/299/245) to produce the most sensitive and selective trace for quantification of THC carboxylic acid. Comparable to MS/MS fragmentation the corresponding Excitation Energy, AF2, needs to be optimized for LIT fragmentation of the ion at *m/z* 299 selective to the fragment ion at *m/z* 245, Figure 6. However, besides optimizing AF2, which came out to be best at 0.09 V (standard value = 0.1 V), additionally the Excitation Time needs to be adjusted as well. This value defines the time period, within which AF2 will be applied to the second precursors for highest fragmentation efficiency. In terms of LIT fragmentation 299/245 the Excitation time was found to be best at 20 msec (standard value = 25 msec).
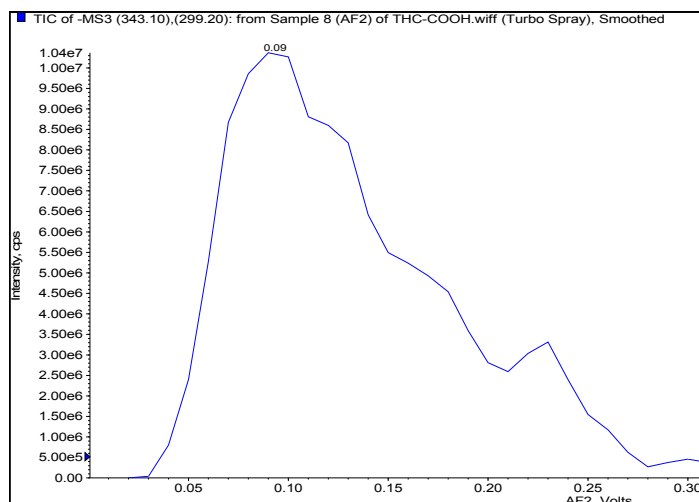
Figure 4. MS/MS of THC carboxylic acid at *m/z* 343 [M-H]$^-$

THC carboxylic acid
Product Ion Scan of m/z = 343 amu
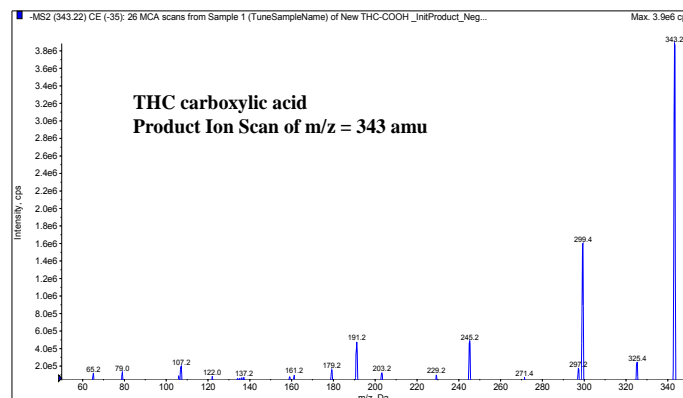
Figure 5. MS^3 scan: fragmentation pattern of 2nd precursor ion at *m/z* 299 of THC carboxylic acid at *m/z* 343 [M-H]$^-$

THC carboxylic acid
MS^3 Scan of m/z = 343 / 299 amu
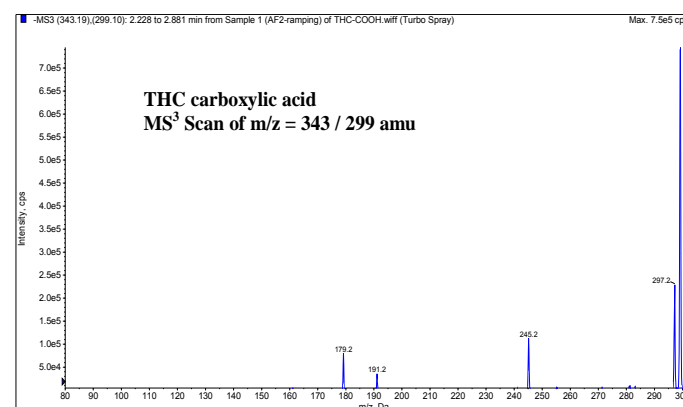
Figure 6. Optimization of AF2, Excitation Energy, for MS^3 fragmentation

TIC of -MS3 (343.10),(299.20): from Sample 8 (AF2) of THC-COOH.wiff (Turbo Spray), Smoothed

Table 1. MS^3- Parameters for THC carboxylic acid

Parameter	
Scan Type	MS/MS/MS (MS^3)
Transition	343.1/299.2/245.1(±1)
Polarity	Negative
1st Precursor	343.10 m/z
DP	-145 V
CE	-30 V
2nd Precursor	299.20 Da
Resolution Q1	Unit
Resolution Q3	LIT
Scan Rate	10,000 Da/s
Q0 trapping	Yes
Center/Width	Yes (245.12 / 2 Da)
LIT fix fill time	250.00 msec
Fragmentation	Yes
AF2	0.09 V
Excitation Time	20.00 msec

Table 1 summarizes the MS-Parameters used for THC carboxylic acid. Additional parameters like AF3 and EXB are instrument/mass dependant and are automatically set by the software. A comparison in terms of sensitivity by varying scan width for the second order fragment ion at *m/z* 245 is shown in Figure 7.

The final MS method includes additional MRM^3 transitions for d$_3$ and d$_6$ internal standards (IS), Figure 8. In deviation from THC carboxylic acid MRM^3 parameters a fixed fill time of 20 msec is applied to the ISs. Mass transitions were optimized to 346.1/302.2/248.1(±1) and 352.15/308.2/254.15(±1) for d$_3$ and d$_9$ respectively.

Despite the use of 3 MRM^3 experiments the total cycle time is less than 0.7 seconds and, hence, makes the option available for using fast LC conditions.

LC-Conditions

An Agilent 1200 SL LC system is used to obtain fast LC conditions. Table 3 shows the flow rates and gradient, which result in chromatographic peak width of about 0.12 min (7.2 sec); injection volume of 20 µL is used. Hence, the total MS cycle time of less than 0.7 sec ensures at least 10 data points across the chromatographic peak. The total LC method run time is 8 min.

Table 2. Final LC conditions

Agilent XDB-C18, 100 x 4.6, 1.8 µm
Flow: 700 µL/min
Solvent: A = H2O
           B = Methanol
           5 mM NH$_4$Ac each

Step Table:		
Step	Total Time(min)	A (%)
0	0.00	70.0
1	4.0	10.0
2	6.00	10.0
3	6.30	70.0
4	8.00	70.0

Sample Preparation

Based on standard procedure published by **Cairns T., Kippenberger D.J., Scholtz H.** and **Baumgartner WA** the following extraction procedure is suggested:

15-25 mg hair is added with 0.1 mL d$_3$ IS (50 ng/mL) and a mixture of 1.9 mL water and 200 µL 10 N NaOH and treated for 1 hour at 60-70°C in an ultrasonic bath. The extract is washed with a mixture of hexane/ethylacetate 9:1, the organic phase than discarded and pH value adjusted to < 1 with HCl. Extract again with hexane/ethyl acetate 9:1, evaporate to dryness and reconstitute in 0.4 mL LC starting solvent. Sample preparation is finalized by adding 0.1 mL d$_9$ IS (50 ng/mL).

Figure 7. Secondary order fragment scan width versus sensitivity
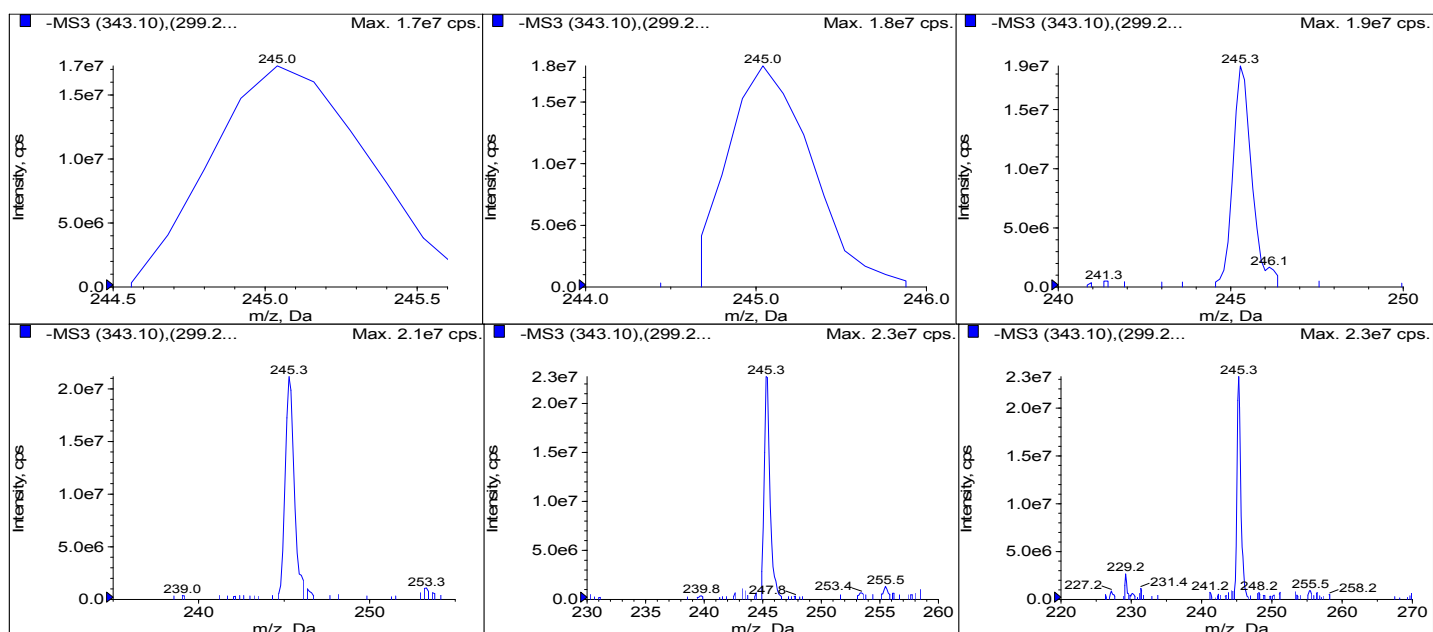
Figure 8. Final LC-MRM^3 method
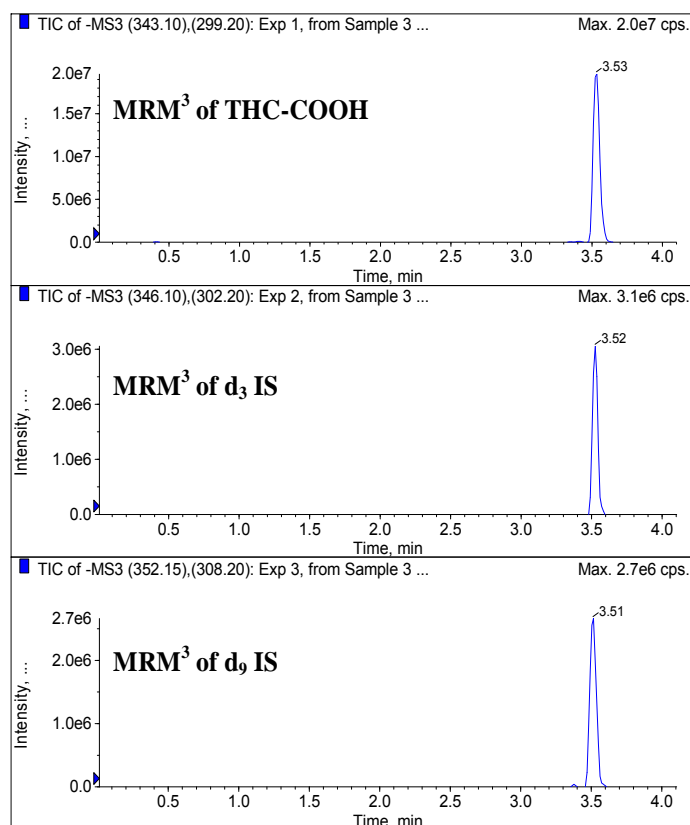
Results and Discussion

Figure 9 (left part) shows the MRM transition of THC carboxylic acid (blue=quantifier, red=qualifier) resulting from standard injection as well as from spiked hair matrix.

As already mentioned the MRM trace of THC carboxylic acid suffers from various intense matrix induced peaks as well as high background on principle. The lack of selectivity makes the detection and quantification of medium concentrations difficult and impossible in the case of lower concentrations.

In comparison to the classical MRM experiment, Figure 9 (right part) presents the chromatogram of THC carboxylic acid as MRM^3 experiment. The advantage of the selectivity of this type of experiment is obvious: only one single peak, THC carboxylic acid, can be identified and the background decreased significantly. The relative intensity, measured as signal-to-noise ratio, clearly increases.

A detection limit lower than 0.1 pg/(mg hair) is illustrated in Figure 10. Linearity of calibration is found to be within 2.5 magnitudes when using fixed fill time. Using quadratic functionality or dynamic fill time (increases cycle time) the range of quantification can be increased up to 3.5 magnitudes.

Thus, a highly selective LC-MS approach for the detection of THC carboxylic acid is introduced, which easily replaces background interfered MRM experiments without loosing any signal-to-noise sensitivity.

Figure 9. Comparison classical MRM versus MRM³; upper row = Std. 100 pg/mL absolute lower row = 100 pg/mL spiked hair matrix
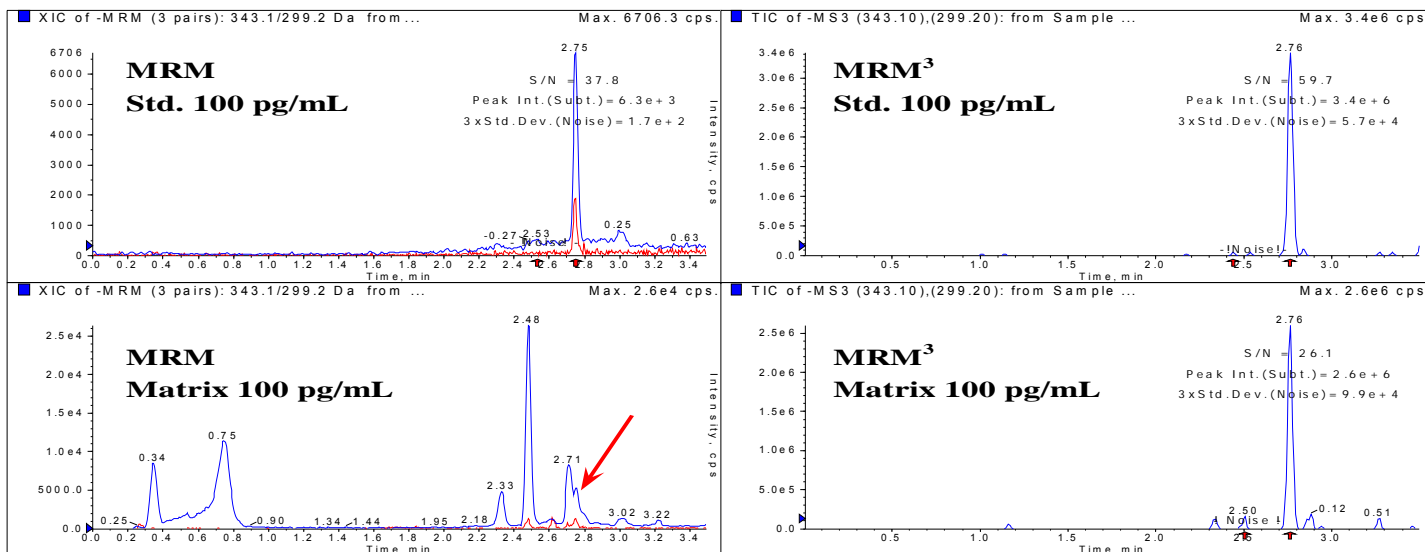

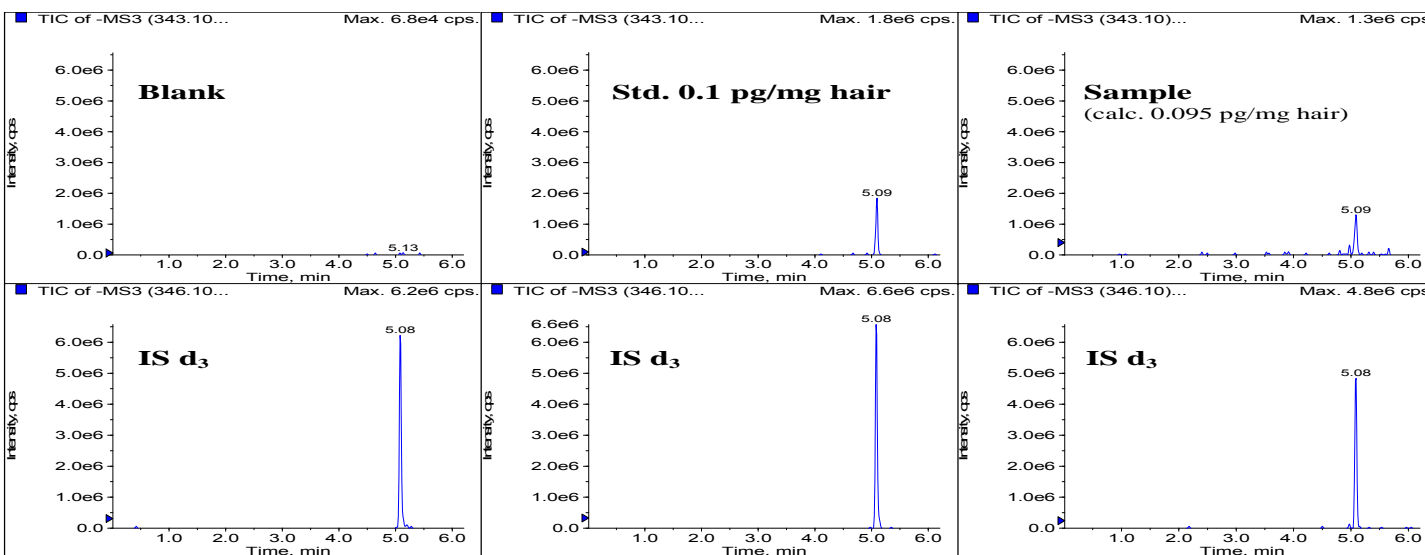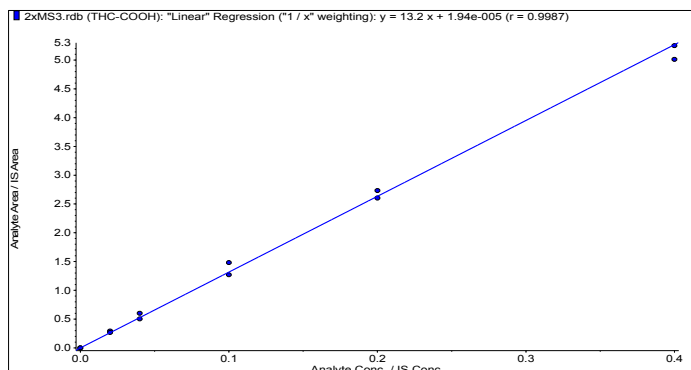**Figure 10. Example of blank, standard and sample injection**

Figure 11. Representative Calibration Curve (r= 0.9987)

Conclusion

- The State-of-the-Art QTRAP® technology in SCIEX QTRAP® 5500 LC-MS/MS system provides improved potentials to enhance selectivity of classical MRM scan mode.

- Highly-selective LC-MRM³ mode with unique sensitivity.

- In comparison to classical MRM no loss of Signal-to-Noise sensitivity.

- The introduced method provides an easy, highly selective and extraordinary sensitive analysis of THC in hair. The method allows the monitoring of THC over an extended period of time when compared to the analysis of urine or plasma.

Contact

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References

[1]   A rapid and simple procedure for the determination of cannabinoids in hemp food products by gas chromatography mass spectrometry (Pellegrini M., Marchei E., Pacifici R. and Pichini S., J. Pharm. Biomed. Anal., 36, 2005, 939-946)

[2]   Increase in drive frequency from 816 kHz to 1.228 kHz increases fragmentation efficiency (Collings B.A., J. Am. Soc. Mass Spectrom., 18, 1459-1466, 2007.)

[3]   MS/MS of ions in a low pressure linear ion trap using a pulsed gas (Collings B.A. and Romaschin A.R., J. Am. Soc. Mass Spectrom., 20, 2009, 1714-1717.)