

Drugs of abuse in hair: high speed and sensitivity demands for LC-MS/MS

Marcos A. Pudenzi¹; Luis O. Junqueira¹; Ichiro Hirano¹
 (1) Shimadzu do Brasil Ltda, Barueri, Brazil

Overview

- ◆ The analysis of drugs of abuse (DoA) in hair has some advantages over other matrices. Due to the larger window of detection these substances can be measured even after several months after usage. Because of that, it has been used as marker of chronic or long-term abuse of drugs, despite the matrix complexities and variabilities, being implemented in Brazil as mandatory for some professional driving license renewal categories (CONTRAN 843/21).
- ◆ Due to the large number of samples/day and low limits of quantitation necessary, a fast LC-MS/MS method is necessary for the screening of these DoA in real samples, with low cost and easy sample preparation. This work presents methodologies for DoA analysis in hair, with high sensitivity and throughput analysis, using LC-MS/MS.



Fig. 1 Shimadzu LCMS-8060NX was used for a DoA determination

1. Introduction

- ◆ Back in 2015, the Federal Law 13.103/2015 established the toxicology testing with a detection window of at least 90 days for certain professional drivers in Brazil. Testing panel was defined by the Brazilian National Traffic Council (CONTRAN) to include several stimulant drugs but also opiate painkillers and marijuana.
- ◆ This law was modified in 2021 by CONTRAN in order to expand the testing by reducing the intervals in which they must be taken, drastically increasing the number of tests. This expansion demanded for the development of incredibly fast methodologies with very high sensitivity to achieve the low levels defined by CONTRAN.

2. Methods

2.1. Screening/Confirmation

- ◆ 10mg of hair are washed with methanol and cut to ~1mm pieces. The hair extraction is done with acidified organic solvent under heating for 1h45min, plus 15 minutes of ultrasonic bath. This procedure can be done in large batches with hundreds of samples simultaneously. Extracts are then transferred to 1.5mL vials and injected in a LCMS-8060NX with C18 column.
- ◆ Fourteen analytes of different drug classes are included in a 1.7min method (2.1min injection to injection) for screening and confirmation, separating benzoylecgonine and norcocaine, two cocaine metabolites that are isomers.

2.2. THC-COOH Confirmation

- ◆ For the THC-COOH confirmation, extraction was done using 30mg and hexane:ethyl acetate was used after hair digestion. Longer run time is necessary to hinder suppression. Samples were analyzed in a LCMS-8060NX with Nexera X3 UHPLC.

3. Result

Drug	Cutoff (pg/mg)	LOQ (pg/mg)
6-Acetyl-morphine	200	100
Codeine	200	100
Morphine	200	100
Amphetamine	200	100
Metamphetamine	200	100
MDA	200	100
MDMA	200	100
Diethylpropione (Anfepramone)	200	100
Mazindol	500	250
Cocaine	500	250
Benzoylecgonine*	50	25
Norcocaine*	50	25
Cocaethylene*	50	25
THC	50	25
THC-COOH*	0.2	0.1

Tab. 1 Cutoff Values and LOQ reached for each analyte.
 * Confirmation only analytes

- ◆ As seen in Table 1, LCMS-8060NX presented a good sensitivity for all the analytes being able to quantitate below cutoff value.

3.1. Screening/Confirmation

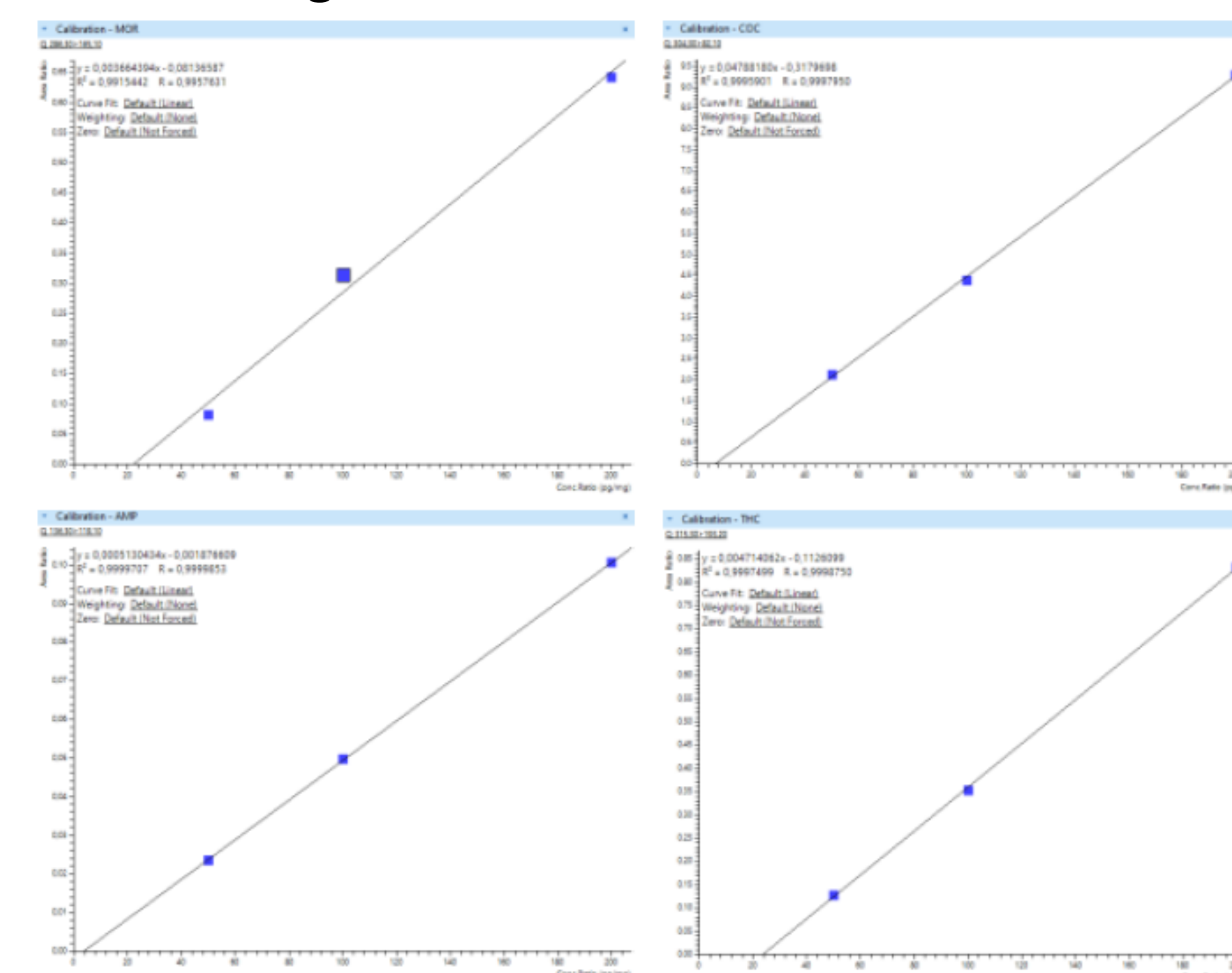


Fig. 2 Calibration curves for morphine, amphetamine, cocaine and THC on 50, 100 and 200% of the cutoff values for screening

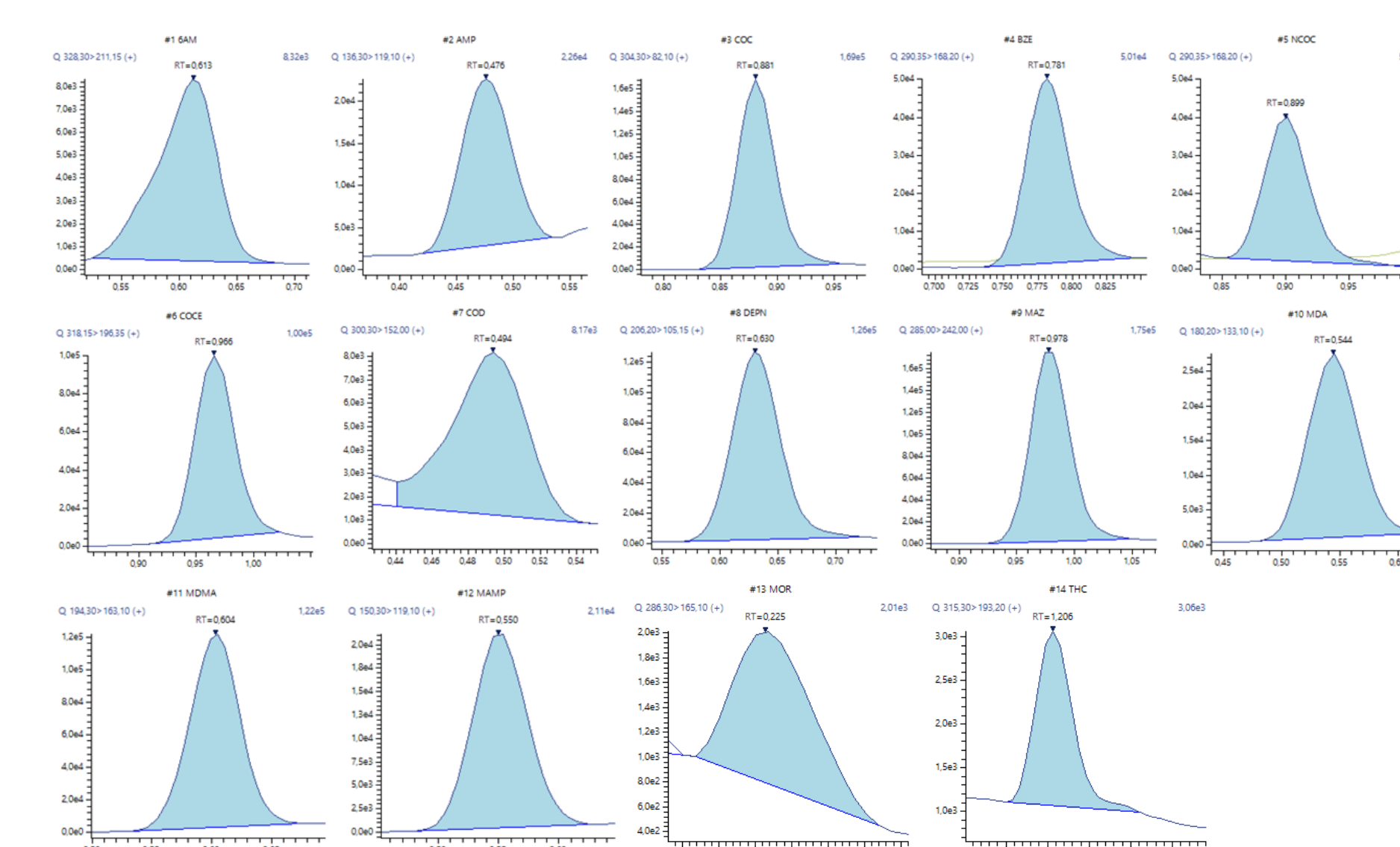


Fig. 3 Chromatogram of all 14 analytes at cutoff value.

- ◆ Results shown a good linearity for all analytes ($R^2 > 0.99$). Figure 2 shows the calibration curves for 4 key analytes and Figure 3 shows all analytes chromatograms for a confirmation run.

3.2. THC-COOH Confirmation

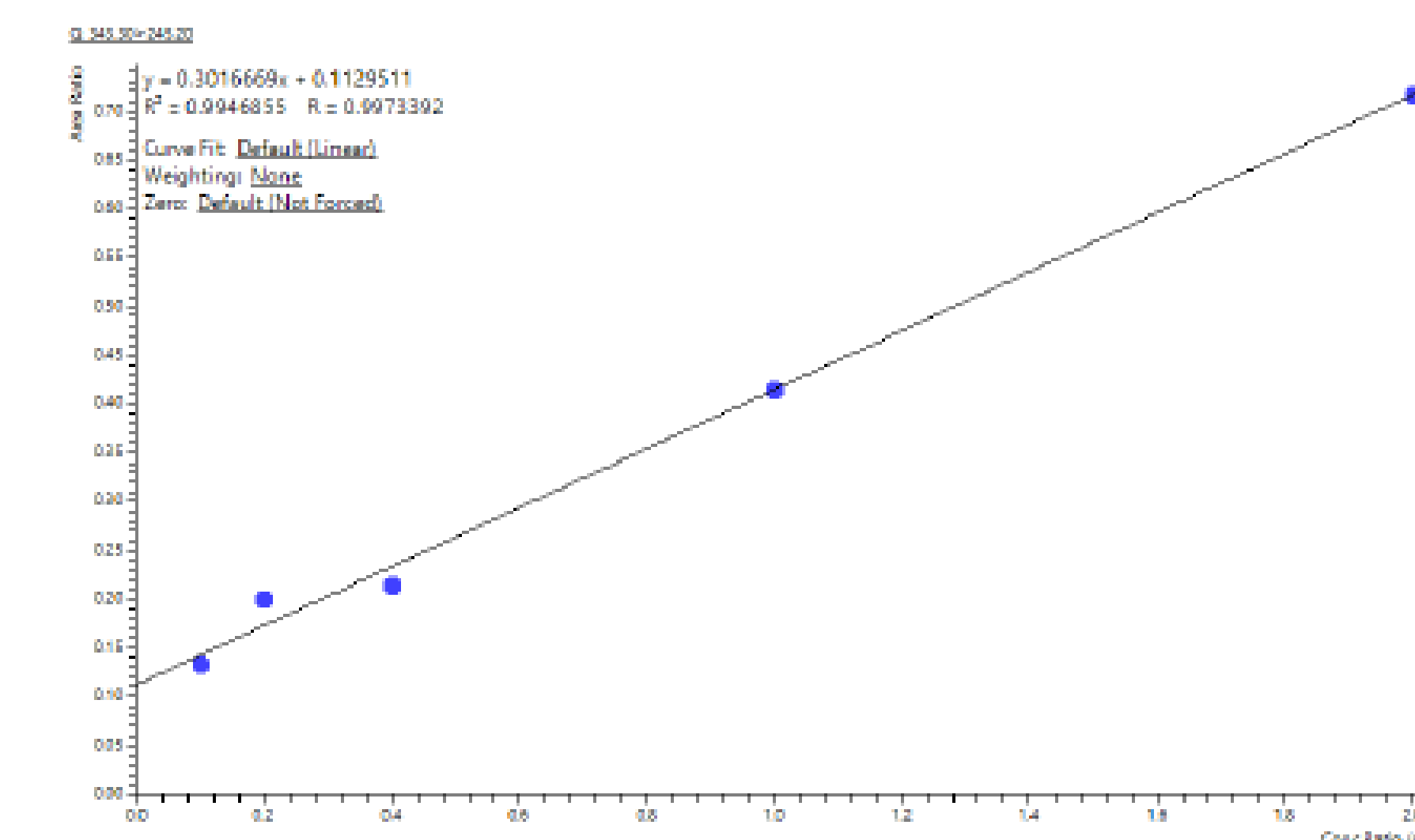


Fig. 4 Calibration curve for THC-COOH starting at 50% cutoff.

- ◆ Data shows that limits of quantitation could be reached for all target compounds using LCMS-8060NX, including THC-COOH for the confirmation of THC (0.2 pg/mg cutoff).

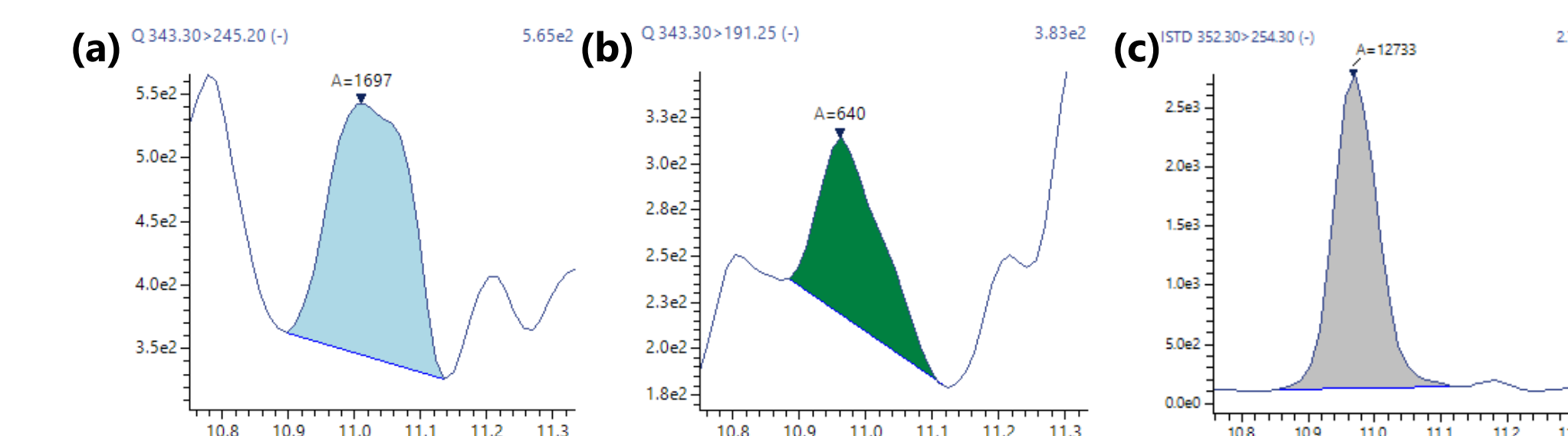


Fig. 5 (a) Chromatogram of THC-COOH quantifier ion at 0,1 pg/mg; (b) Chromatogram of THC-COOH qualifier ion at 0,1 pg/mg; (c) Internal Standard (THC-COOH-D9) chromatogram at 2,0 pg/mg.

4. Conclusion

- ✓ Shimadzu LCMS-8060NX system was able to reliably detect all relevant DoA in hair with appropriate sensitivity providing a complete solution to the Hair analysis.
- ✓ Shimadzu LCMS-8060NX system allows for an extremely fast and reliable method allowing for below 2 minutes methods.