

## Determination of Drugs of Abuse in Hair by UPLC-MS/MS: View from Brazil

Danilo Pereira  
 Waters Corporation, Sao Paulo, Brazil



### GOAL

To develop a robust UPLC-MS/MS method for the analysis of various drugs of abuse in hair that can be used in routine testing to allow the granting of commercial driving licenses in Brazil.

### BACKGROUND

The use of hair as a biological matrix for forensic toxicology testing has increased in popularity over the last decade.

In contrast to traditional matrices, such as blood and urine, hair offers another means of detection and can be used to provide a chronological history of drug exposure over several months to years, if segmental analysis is performed. Human hair is known to grow at approximately one centimeter per month. Drugs can be incorporated into the hair by several mechanisms including passive diffusion from the blood supply at the follicle into the growing hair matrix, diffusion into the hair shaft from sweat or sebum, and through external contamination such as smoke or contaminated hands. Hair collection is a non-invasive technique and can be achieved without the privacy and adulteration issues associated with urine collection and, in contrast to blood samples, hair does not require medically trained staff

A robust and sensitive UPLC-MS/MS method for the determination of a panel of drugs of abuse in hair.

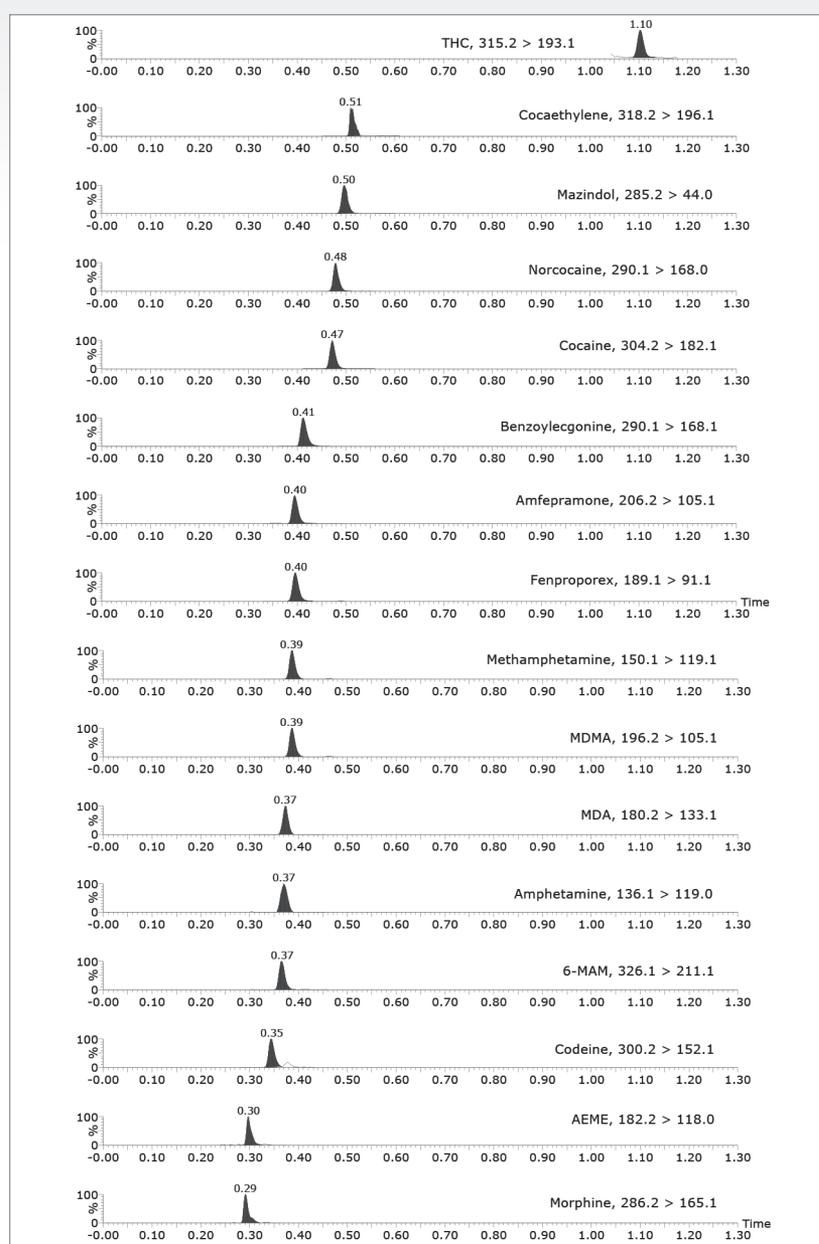


Figure 1. Smoothed and integrated chromatogram showing the analytes spiked into control hair at concentrations equivalent to the confirmation cut-off recommended by the Society of Hair Testing. Displayed is the quantifier-only transition.

to collect the sample. Furthermore, hair samples can be easily stored.

In certain geographies, such as Brazil, the granting of commercial driving licenses is linked to the applicant being able to prove that they have not taken any of the drugs of abuse that are on the government sanctioned list. This list includes opiates, amphetamines, cocaine, and tetrahydrocannabinol (THC) and their metabolites. In Brazil, it is estimated that this leads to more than one million tests per year. To ensure that this extremely large volume of tests can be carried out, a very fast, robust analytical method is required which can also conform to the guidelines recommended by the Society of Hair Testing (SoHT).<sup>1</sup>

## THE SOLUTION

Control hair was collected from volunteers, and following decontamination with methanol, it was finely cut into 1–2 mm segments using scissors. The minced hair was stored at 4 °C until required.

Ten milligrams of chopped hair (spiked or real samples) was placed into polypropylene tubes containing methanol. The sample was pulverized and incubated at 50 °C for 15 hours. Following incubation, the sample was centrifuged, and the supernatant simply transferred to a Waters™ Total Recovery Vial.

To achieve the very fast separation required, an ACQUITY™ UPLC™ I-Class (FTN) System was used and the analytes of interest were separated using a gradient of formic acid/acetonitrile on an ACQUITY UPLC BEH C<sub>18</sub> Column (p/n: [186002349](#)). At least two MRM transitions were monitored for each analyte using the Xevo™ TQ-S micro Mass Spectrometer. Where available, equivalent deuterated internal standards were added to the samples at a concentration of 0.4 ng/mg to ensure robust

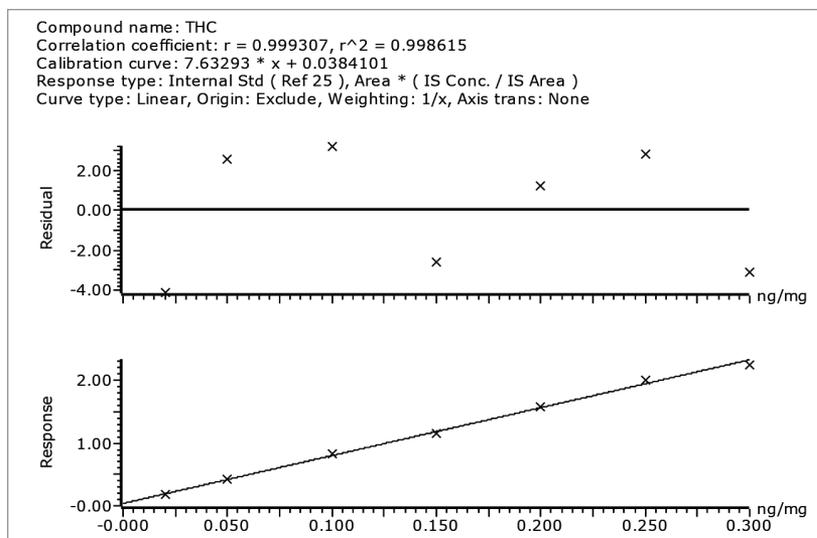


Figure 2. Linearity of THC spiked into control hair over the range 0.02 to 0.3 ng/mg.

quantitation. The run time for the chromatographic method was 1.2 minutes and provided separation of all analytes including the isobaric norcocaine and benzoylecgonine. A chromatogram of the separated analytes is shown in Figure 1. The figure shows the smoothed and integrated quantifier MRM trace for the analytes of interest spiked at concentrations equivalent to the confirmation cut-off concentration recommended by SoHT.

The linearity of the assay was investigated and the calibration curve for THC over the range 0.02 to 0.3 ng/mg (along with the residuals plot) is shown in Figure 2. To confirm the presence of THC in a sample, a separate analytical method, using the same mobile phases and column, was employed to detect carboxy-THC (cTHC); this chromatographic method was 5.5 minutes.

## SUMMARY

The increased use of hair testing to grant commercial driving licenses in certain geographies has highlighted the need for very quick, accurate, reliable, and robust methods to quantify drugs of abuse at very low concentrations. The combination of the ACQUITY UPLC I-Class (FTN) System and the Xevo TQ-S micro Mass Spectrometer allows for the determination of a large number of compounds in under 1.3 minutes, at very low concentrations, ensuring extremely high sample throughput.

## References

- Cooper, G. A. A.; Kronstrand, R.; Kintz, P. Society of Hair Testing Guidelines for Drug Testing in Hair. *Forensic Science International* **2012**, 218, 20–24.

## For Forensic Toxicology Use Only.

# Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters, The Science of What's Possible, ACQUITY, UPLC, and Xevo are trademark of Waters Corporation. All other trademarks are the property of their respective owners.