Using Ion Mobility to separate different designer drug metabolites

The large number of new emerging designer drugs and the need to detect not only the parent analyte but their metabolites poses a big challenge in forensic screening. Ion mobility combined with HRES mass spectrometry allows for the identification and characterization of known as well as unknown analytes extending the scope to address these challenges.

Introduction

Screening for synthetic cannabinoids is often performed by analysing urine as it is a non-invasive matrix. Due to the metabolism in the human body, none or only a small concentration of the parent cannabinoid will be left for detection. Therefore, it is necessary to screen not only for the parent compound but also for the drug metabolites.

One common metabolism for AKB-48 is hydroxylation. The hydroxylation can take place on different carbon positions resulting in a variety of structural isomers. The large number of different metabolites and the introduction of new drug analogs make urine screening a complicated task that requires high confidence for known substances as well as the flexibility of identifying new drug metabolites.

A mass spectrometer with the ability to deliver accurate mass measurements, a high confidence in isotopic pattern distribution and clear MS/MS spectra is the first step to gain insights into a complex sample in order to solve this question. In addition, the timsTOF offers the possibility to use ion-mobility-separation to further increase the capability to identify and differentiate structural isomers.

Keywords: AKB-48 metabolite, Designer Drugs, IMS, timsTOF, Forensic Screening

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Methods

AKB-48 metabolites not commercially available were prepared in-house[1] and used for measurements. Two mixtures of different AKB-48 hydroxylation metabolites with a concentration of 125 ng/mL each were prepared. A chromatographic separation was used prior to the ion mobility separation. Detection was performed on an ion mobility-separation-time-of-flight instrument (timsTOF, Bruker Daltonics).

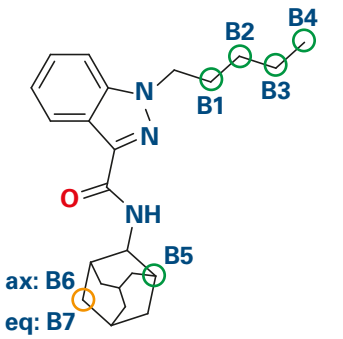


Figure 1: AKB-48 and its hydroxylation sites.

Instrumentation

UPLC	Ultimate 3000 Rapid Separations LC, Thermo Scientific
Column	HSS T3, 2.1 x 150 mm, 1.8 µm, Waters
Temperature	60 °C
Mobile phase	A = H_2O, 10 mM Ammonium formate and 0.05% formic acid B = ACN, 0.05% formic acid
Gradient	Multistep gradient from 57%-93% B in 8 min
Flow rate	0.5 mL/min
Injection	1 µL
MS	timsTOF mass spectrometer, Bruker Daltonics
Scan mode	Full scan TOF MS with IMS in Ultra mode
Ionization	ESI +2500 V
MS-Calibration	sodium formate cluster
IMS-Calibration	tuning mix, low concentration

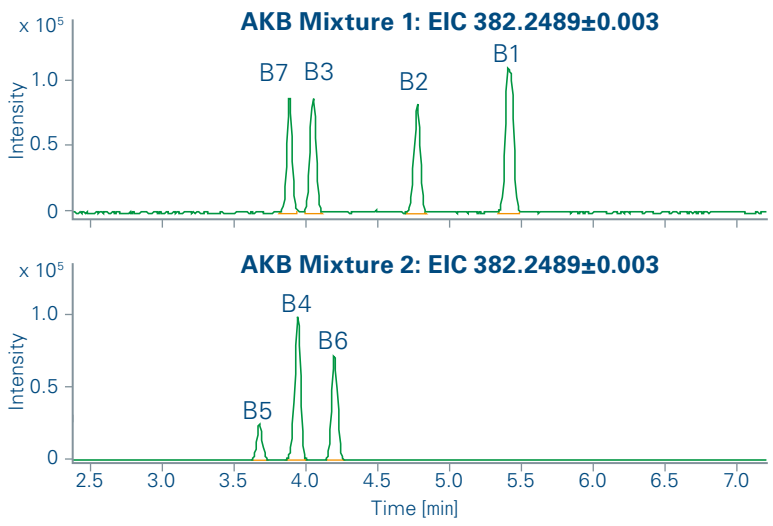


Figure 2: Extracted Ion Chromatograms for $[C_{23}H_{31}N_3O_2+H]^+$ with m/z = 382.2489 ± 3 mDa. Two different mixtures 1 and 2 of hydroxylated AKB-48 metabolites were prepared and measured.

Results and Discussion

In a first step, the focus was on the LC separation of the metabolites to enable a clear characterization of every single metabolite (Figure 2). The metabolites were ionising as a mixture of $[M+H]^+$ and $[M+Na]^+$ adducts both showing excellent mass accuracies and isotope pattern distributions (Figure 3 as one representative example), demonstrating the high data quality for a first identification process.

In a next step Extracted Ion Mobilagram (EIM) traces of different adduct species ($[M+H]^+$ and $[M+Na]^+$) were examined. Both adduct types showed a clear separation on the ion mobility axis. Protonated species showed signals of the inverse mobility $1/K_0$ [V·s/cm²] with values around 1.00-1.04 (Figure 4, blue) while sodium adducts showed a higher value of about 1.04-1.08 [V·s/cm²] (Figure 4, orange).

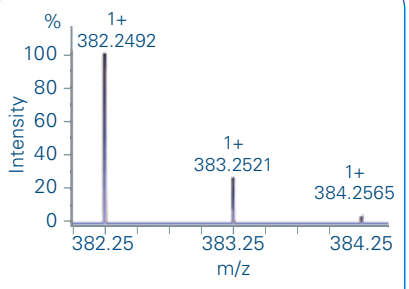
For the metabolites that were derived from hydroxylation at the end of the alkyl chain (B3 and B4), the inverse mobility is shifted to lower $1/K_0$ values compared to the other metabolites.

Using the sodium adducts a clear differentiation of metabolites derived from hydroxylations at the adamantyl moiety could be achieved as shown in Figure 5. These metabolites (B5-B7) show higher $1/K_0$ values than those that show a hydroxylation at the alkyl chain (B1-B4). Interestingly, B1 is different from the other metabolites and does not fit into the grouping of the other isomers. It shows a $1/K_0$ value in the middle of alkyl-chain and adamantyl-moiety.

Two of the main metabolites after human liver microsome incubations (HLM incubation) are B4 and B7[2]. Based on the used LC separation method B4 and B7 are co-eluting. Therefore, a differentiation of both

compounds is not clearly given via LC-MS only methods. Adding IMS separation enables a clear differentiation of both compounds. Both, the $[M+H]^+$ as well as the $[M+Na]^+$ EIM trace show a baseline separation of both isomeric structures (Figure 6).

In combination with the exact mass, the isotope pattern distribution and verification using fragmentation, CCS values are an additional parameter for identification and confirmation of possible metabolites of designer drugs.



■ +MS, 3.8-4.0 min, $1/K_0$=1.017-1.040

m/z	I %, experimental	I %, theoretical
382.2492	100	100
383.2521	27.2	26.4
384.2565	4.1	3.8

Figure 3: Comparison of the experimental data of B4 and the theoretical isotope pattern distribution shows a good overlay. The monoisotopic signal as well as the following isotopes show only a small deviation to the theoretical values (mass accuracy: - 0.8 ppm, mSigma: 4.9).

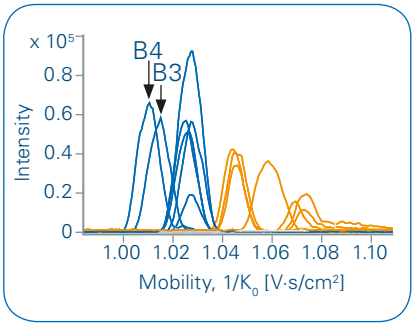


Figure 4: Overlay of all Extracted Ion Mobilograms (EIM) of the protonated species (in blue, m/z = 382.2492) and the sodium adducts (in orange, m/z = 404.2312). A grouping of the different species is clearly given.

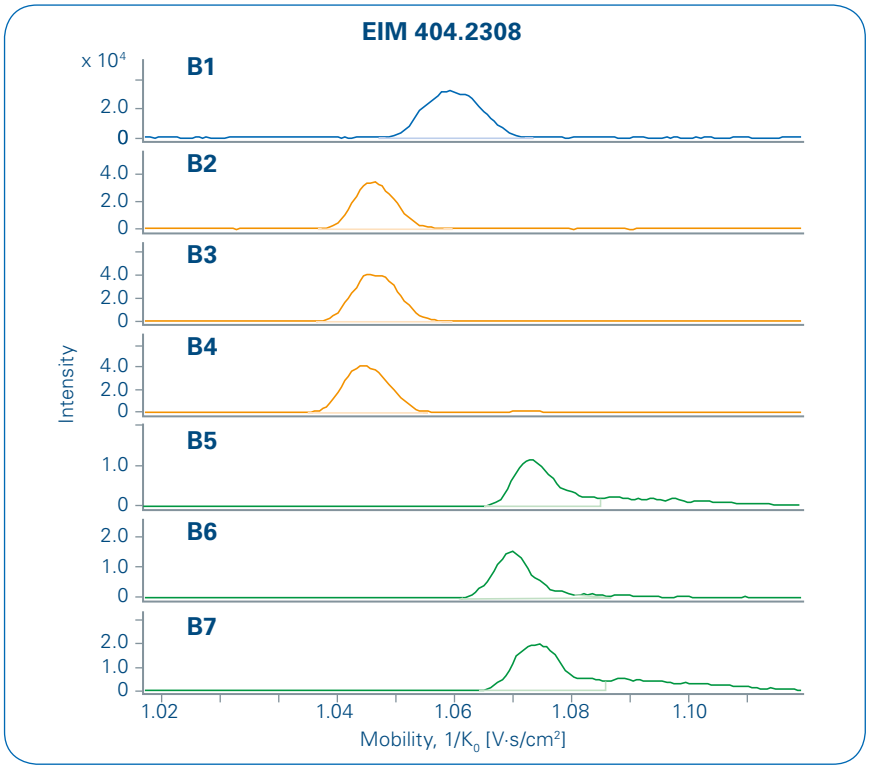


Figure 5: Display of EIM traces of the different metabolites as $[M+Na]^+$. In orange B2-B4 are displayed referring to metabolites showing hydroxylation of the alkyl chain. In green B5-B7 are displayed referring to hydroxylation on the adamantyl moiety. B1 in blue is an exception as it shows $1/K_0$ values between those of the two groups.

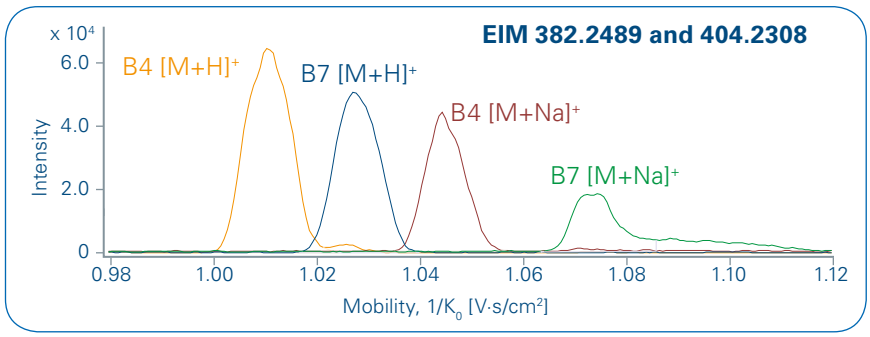


Figure 6: Overlay of EIM traces for B4 and B7. A baseline separation for both adduct types ($[M+H]^+$ and $[M+Na]^+$) is given.

Conclusions

- Ion Mobility with the timsTOF offers an additional separation method to differentiate between different AKB-48 metabolites in combination with excellent values in mass accuracy and isotopic pattern distribution.
- A clear grouping of the CCS values is given based on the region of hydroxylation.
- On the basis of the mobility of the sodium adducts metabolites showing a hydroxylation at the adamantyl group or a hydroxylation on the alkyl chain can clearly be differentiated.

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www.bruker.com/timstof

References

[1] Jakob Wallgren, Svante Vikingsson, Anders Johansson, Martin Josefsson, Henrik Green, Johan Dahlén, Xiongyu Wua, Peter Konradsson, *Synthesis and identification of an important metabolite of AKB-48 with a secondary hydroxyl group on the adamantyl ring*, **2017**, Tetrahedron Letters, (58), 1456-1458.
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