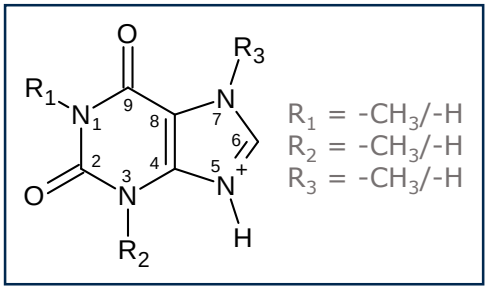
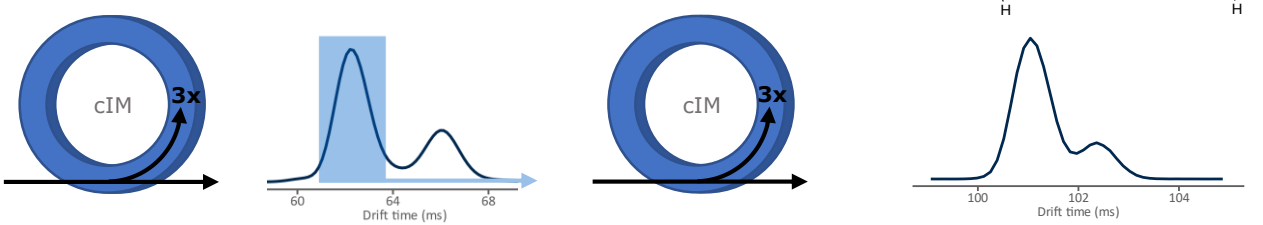


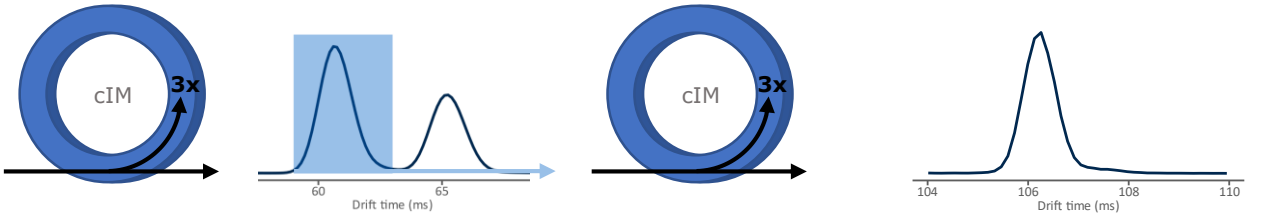
Structure dependant protomer formation can aid identification of caffeine metabolites

IMS² experiments:

Paraxanthine



Theobromine



Indicative **patterns** in structure

- Methyl group present in R_2 and absent in R_1 position resulted **protomers**
- Methyl group absent in R_2 position resulted **tautomers**

Conclusions

- Structural parts present can be associated to protomer formation and help to identify unknown molecules with a higher confidence.
- Thermodynamically unstable protomers in both solution and gas-phase can still be present in ES produced gas-phase ions.
- Further investigation of structural indicators for protomer formation could be used in non-targeted analysis for additional confirmation of proposed structure.

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Protomer formation can aid structural identification of caffeine metabolites

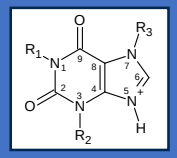
Introduction

In positive electrospray ionization (ESI) mode protonated gas-phase molecules [M+H]+ are produced enabling mass spectrometric and ion mobility characterization. For polyfunctional molecules with multiple protonation sites, proton binding to different thermo-dynamically less stable sites has been observed. The impact of protomer formation to collision induced dissociation and ionization efficiency in ESI is yet unknown.

Methods

Substances:

- Caffeine (1,3,7-trimethylxanthine)
- Theophylline (1,3-dimethylxanthine)
- Paraxanthine (1,7-dimethylxanthine)
- Theobromine (3,7-dimethylxanthine)
- 1-methylxanthine
- 3-methylxanthine
- 7-methylxanthine
- Xanthine
- Hypoxanthine



Cyclic IM-MS

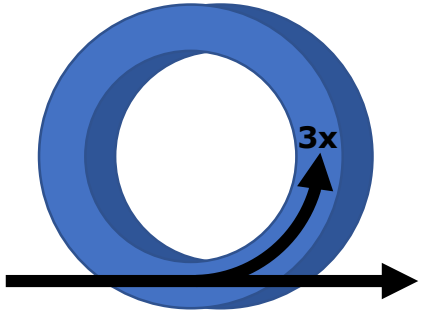
- Solvent effect on arrival time distributions (ATDs) and fragmentational behavior
- Two-dimentional IM experiments

Computational

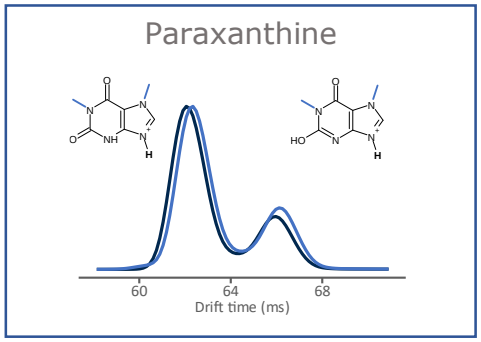
- Gibbs free energies for proposed protonated isomeric structures

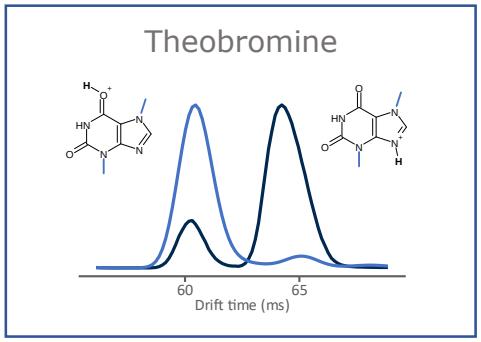
SIRIUS software

- identification and characterization based on MS/MS spectra



Cyclic Ion Mobility Spectrometer





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Results

ATDs

- High- and low-mobility species observed for five compounds
- Paraxanthine, 1-methylxanthine and 7-methylxanthine – no solvent effect
- Theobromine and 3-methylxanthine – strong solvent effect

CID experiments and **computations**:

- Theobromine and 3-methylxanthine – different fragmentation patterns of separated species
- [M+H-H2O]+ was dominating fragment for high-mobility species indicating O-protonation
- No differences in MS/MS spectra of other compounds
- paraxanthine, 1-methylxanthine and 7-methylxanthine – separation of tautomers
- Confirmed with computations and IMS2
- Based on computations, N-protonation to imidazole nitrogen (N-5) is suggested for all investigated compounds in both solution and gas-phase.

Indicative **patterns** in structure:

- Methyl group absent in N-3 position resulted tautomers
- methyl group present in N-3 and absent in N-1 position resulted protomers

SIRIUS **identification**:

- Molecular formula identified with high confidence
- Correct isomer was identified but with very low confidence score

Discussion

- Structural parts present can be associated to protomer formation and help to identify unknown molecules with a higher confidence.
- Thermodynamically unstable protomers in both solution and gas-phase can still be present in ES produced ions.
- Further investigation of structural indicators for protomer formation could be used in non-targeted analysis for additional confirmation of proposed structure.