Isolation of Isomeric Drug Metabolites by Differential Mobility Spectrometry: A Proof of Concept Study using Caffeine

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Abstract

Caffeine is one of the most widely consumed psychoactive substances with well established pharmacokinetic properties. Differential Mobility Spectrometry– Mass Spectrometry (DMS-MS) has been used in the past to separate complicated mixtures of analytes. We envision a proof of concept study whereby DMS can be used to isolate metabolites of caffeine and their tautomer's to quantify their relative abundance. This could prove useful in determining physicochemical properties of metabolites in clinical trials on new drug species.

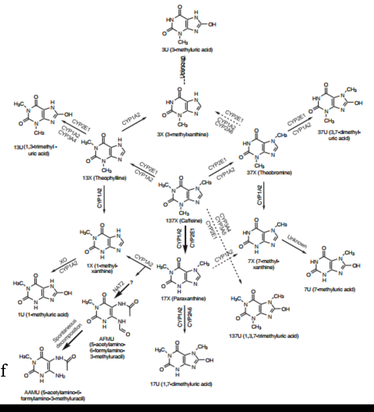


Figure 1: Metabolic pathway of caffeine[1]

Experimental Methods

DMS was performed on a SELEXion equipped with a 5500 QTRAP.

- Compensation voltages (CV) were ramped at particular separation voltage (SV) to generate an ionogram
- Collision induced dissociation (CID) used to generate mass spectra of breakdown products
- Dispersion plots were created by taking the maximum CV of each peak and plotting it against SV
- H_2O modifier was infused into the N_2 carrier gas at 1.5% (v/v)

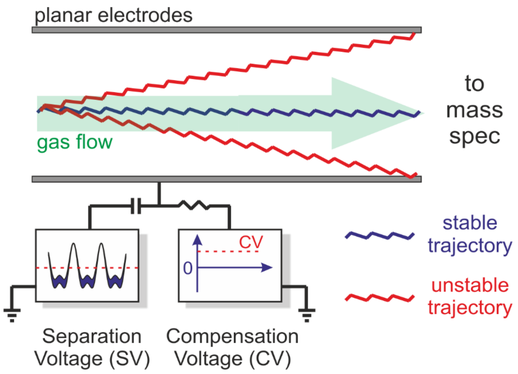


Figure 2: DMS schematic diagram[2]

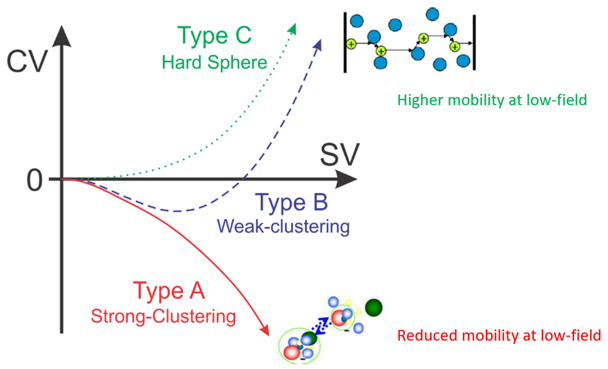
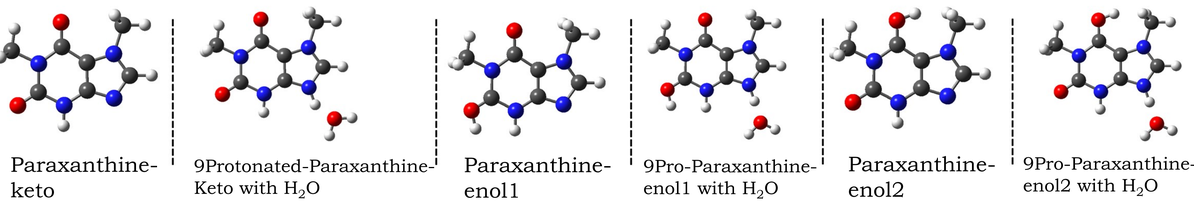


Figure 3: Three behaviours observed in the DMS cell[2]

Computational Methods

Computational portion was completed using Gaussian 09
Caffeine and its metabolites were optimized at the B3LYP/6-311++g(d,p) level of theory
Each possible protonation site belonging to each tautomeric form were explored
Solvent binding energies were determined employing counterpoise and empirical dispersion corrected values



Paraxanthine-keto | 9Protonated-Paraxanthine-Keto with H_2O | Paraxanthine-enol1 | 9Pro-Paraxanthine-enol1 with H_2O | Paraxanthine-enol2 | 9Pro-Paraxanthine-enol2 with H_2O

Figure 4: Global minima of tautomeric $[Paraxanthine]^+$ and $[Paraxanthine{\cdot}H_2O]^+$

Experimental Results

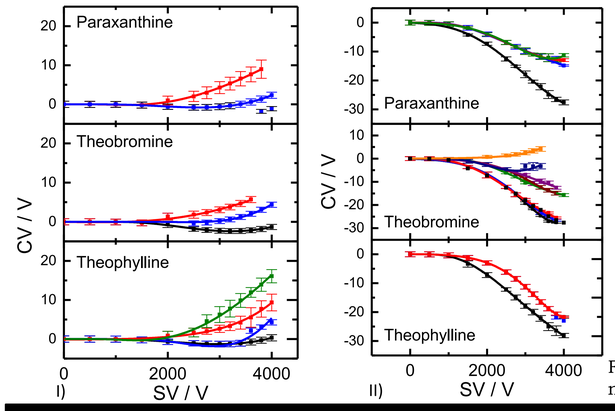


Figure 5: Dispersion plots of primary three metabolites in i) N_2 and ii) H_2O

- Breakdown curves were constructed which indicate unique tautomeric forms or protonation sites (data not shown)
- Primary three Metabolites in Dry N_2 exhibited Type C and Type B clustering behavior
- Primary three Metabolites in H_2O exhibited Type B and A clustering behavior
- In H_2O modifier it appears that many tautomeric forms exist and this will further be investigated

Computational Results

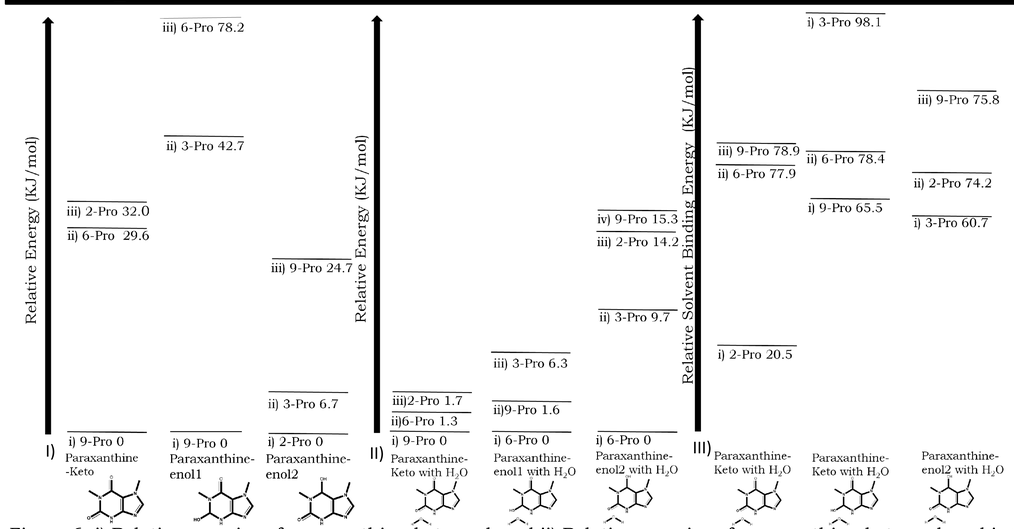


Figure 6: i) Relative energies of paraxanthine-keto and enol ii) Relative energies of paraxanthine-keto and enol in H_2O iii) H_2O solvent binding energies

- The three bare protonated paraxanthine tautomer's that were DFT-optimized are shown ranked in figure 6 based on their relative energies
- Protonated paraxanthine tautomer's with H_2O are shown ranked based on their relative energies
- Results of the relative solvent binding energies found from the protonated tautomer's that were complexed with H_2O are shown ranked

Experimental Directions

- DMS traces and calculations of the remaining metabolites will be obtained
- To explore the validity of DMS to isolate caffeine metabolites, a new technique utilizing coated blade spray (CBS) will be employed

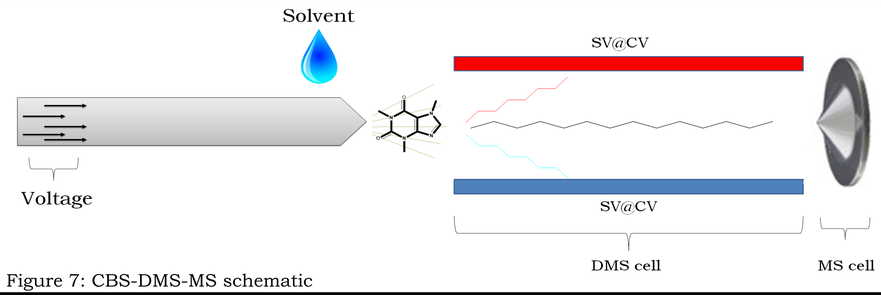


Figure 7: CBS-DMS-MS schematic

References and Acknowledgments

(1) Rybak, M.E, Pao, C., *Anal. Bioanl. Chem.*, **2014**, 406, 771-784.
(2) Hopkins, W.S, *Mol. Phys.*, **2015**, 113 (21), 3151-3158.

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