Investigation of simulated oxidative and conjugative metabolism reactions with liquid chromatography / accurate mass high resolution mass spectrometry

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Motivation

- In metabolic studies, the identification of metabolites in blood or urine samples as well as liver microsome incubations is relatively easy, but the detection of reactive species turns out to be tricky.

- Trapping experiments deliver only indirect proof of identity.

- Electrochemical simulations of metabolic processes, coupled to high performance liquid chromatography (HPLC), have been shown to be a simple and fast alternative to classical approaches.

- Combination of electrochemistry with high resolution accurate mass (HRAM) mass spectrometry may deliver enhanced sensitivity and confidence in metabolite detection, especially for reactive metabolites.
Methods
Experimental Setup

- For the shown experiments a ROXY™ potentiostat equipped with a thin layer ReactorCell™ from Antec (Zoeterwoude, The Netherlands) was used, coupled to a Exactive Benchtop Orbitrap™ mass spectrometer (Thermo Scientific, Bremen, Germany) with ESI source.

- Analyte solutions were provided in water / acetonitrile 1:1 with 0.1% formic acid.

- Protein solutions for conjugation experiments were provided in 10 mM ammonium formiate solution, adjusted to pH 7.8.
The ROXY potentiostat and Exactive
Infusion experiments for Phase I and Phase II studies

only analyte passing EC cell is analysed

Phase I studies

conjugation of reaction products with protein after EC cell

„Phase II“ studies
Setup with chromatographic separation

reaction products are trapped in injection loop

content of injection loop is passed to column
Exactive™

- **Resolution**
  100,000 at 1 scan per second
  10,000 at 10 scans per second

- **Mass accuracy**
  ppm mass accuracy

- **Sensitivity**
  500 fg Buspirone > 10:1

- **Dynamic Range**
  >4000 within a spectrum

- **Scan speed**
  Up to 10 scans per second

- **Mass Range**
  m/z 50 - 4000

- **Polarity switching**
  Yes, 1 full cycle <1 sec
Metabolites of small molecules
Thiomethoxam (insecticide)

Extracted ion voltammograms of Thiamethoxam from 0.2 to 2.5V
"EC spectrum" of Thiamethoxam

summation of upcoming signals at rising voltage in the electrochemical cell
Voltammogram of Thiomethoxam

comparison of spectra at different EC voltages

NL: 3.32E5
thiamethoxam_inf_003_1.8v#1  RT: 10.00  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 3.00E5
thiamethoxam_inf_003_1.9v#1  RT: 10.49  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 2.84E5
thiamethoxam_inf_003_2.0v#1  RT: 10.99  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 1.72E5
thiamethoxam_inf_003_2.1v#1  RT: 11.50  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 2.14E5
thiamethoxam_inf_003_2.2v#1  RT: 11.99  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 2.92E5
thiamethoxam_inf_003_2.3v#1  RT: 12.50  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 2.73E5
thiamethoxam_inf_003_2.4v#1  RT: 12.99  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]

NL: 3.46E5
thiamethoxam_inf_003_2.5v#1  RT: 13.50  AV: 1 T: FTMS {1,1}  + p ESI
Full ms [150.00-500.00]
Voltammogram of Thiomethoxam

So far so good – but where is the benefit of HRAM in this case?
Confirmation of the oxidation product of Thiomethoxam

m/z 308: oxy-Thiamethoxam or Glutathion?
Confirmation of the oxidation product of Thiomethoxam

m/z 308: oxy-Thiamethoxam or Glutathion?
Confirmation of the mono-oxygenation of Thiomethoxam

Resolution of 100.000 is needed!
... and it is not Glutathion
Isotopic resolution on overlapping isotopic signals

easy example is Amodiaquine (anti malaria drug)
Isotopic resolution on overlaying isotopic signals

Di-dehydro amodiaquine
M + 2 ($^{37}$Cl)

Di-dehydro amodiaquine
M + 2 ($^{13}$C)
Irinotecan (anti cancer drug)

Irinotecan also has a di-dehydro metabolite
Irinotecan A0 and Meatbolite A2

voltammogram of Irinotecan from 0.2 to 2 V, the signals don’t interfere at resolution 100.000
Conjugate Protein-drug
MS spectrum of β-Lactoglobulin

isotopic resolution for the multiply charged ions is easily achieved at resolution 100.000
Deconvoluted spectrum of β-Lactoglobulin

Deconvolution gives clear evidence of the conjugation with Amodiaquine
Summary

- Electrochemistry allows easy and fast simulation of oxidative and conjugative metabolism reactions.
- Metabolites can be detected with high sensitivity and specificity.
- High confidence in identification is provided.
- Interferences can be resolved through high resolution of 100,000.
- Protein conjugates can be identified with high confidence through isotopic resolution for proteins.
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