Ionic Liquid GCxGC-TOF-MS Approach for Generic Screening of Aerosol Constituents in Aqueous Tobacco Aerosol Fractions and Analysis of Microsomal Incubates

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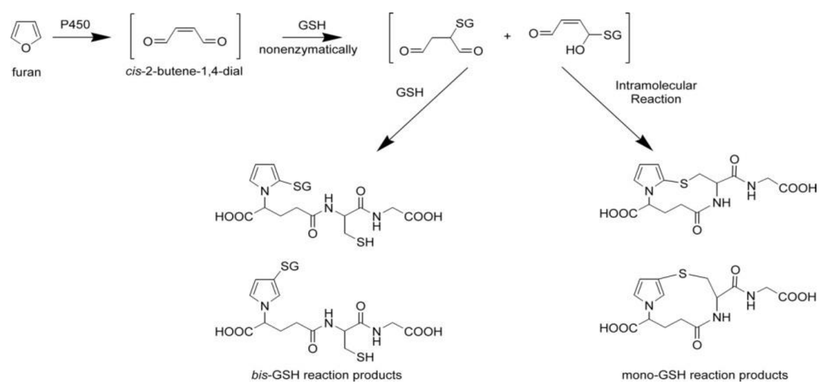
Introduction

Background

- Furans are naturally present in tobacco leaves and are also formed by thermal degradation of carbohydrate precursors (between 300-400°C)[1] during the burning or heating of tobacco within cigarettes
- Biotransformation of simple furans in the body can be described by two major pathways, either by the initial generation of reactive intermediates (cis-2-butene-1,4-dial and derivatives) by CYP2E1 metabolism[2] or, if substituted with a side chain (e.g., furfuryl alcohol), by further oxidation to the corresponding aldehydes and acids prior to Phase II conjugation and excretion from the body
- Furan-derived reactive intermediate metabolites undergo non-enzymatic conjugation with glutathione (GSH)[3]



- Simple furans represent an experimental challenge for *in vitro* metabolism models due to their high volatility (e.g., B.P. furan: 31.3°C) and low water solubility (e.g., $logP_{OW}$ furan: 1.3)
- An analytical approach using water resistant ionic liquid stationary phases for 2-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF-MS) represents a simple and robust alternative to analyze aqueous media directly (e.g., smoke aerosols trapped in phosphate buffered saline (PBS), microsomal incubations) using cool on column injection

Objectives

- To develop an *in vitro* enzymatic model, combined with an innovative analytical approach, to assess the capability of the human liver to metabolize aerosol constituents
- To evaluate this model by following the kinetics of furan parent compounds and the formation of metabolites and their GSH adducts
- To screen the chemical composition of cigarette smoke trapped in aqueous media

Strategy

- Establish an easy to use and robust *in vitro* model to characterize the metabolic fate of furans in human liver
- Use a combination of two water resistant ionic-liquid based analytical columns for GCxGC-TOF-MS[4,5], which avoids any solvent delay and enables coverage of highly volatile furans, amongst other constituents
- Identify reactive intermediate metabolites by determination of their glutathione adducts using liquid chromatography coupled with high resolution mass spectrometry (LC-HR-MS)

Materials and Methods

Sample preparation

- Metabolites were generated *in vitro* using an enzymatic system containing human liver microsomes and S9 fractions combined with a NADPH Regenerating System (NRS) containing NADP, G6P and G6PDH
- Reactive metabolic intermediates were trapped using the soft nucleophile glutathione
- Individual incubation vials were used for each time point to avoid aliquoting and losing volatile compounds
- To avoid adsorption effects at material surfaces silanized glass was used
- Corresponding negative controls followed the same procedure in the absence of the NRS
- Enzymatic activity was stopped by the addition of methanol, containing a set of internal standards, followed by crash freezing using dry ice, thawing and centrifugation
- The supernatant was directly injected onto GCxGC-TOF-MS (cool on-column) and LC-HR-MS systems

Instrumentation

- Kinetic analysis of parent compounds was performed using a Leco Pegasus® 4D GCxGC-TOF-MS instrument
- LC-MS analysis for glutathione adducts of reactive furan intermediates was performed using a Thermo Q Exactive™ instrument

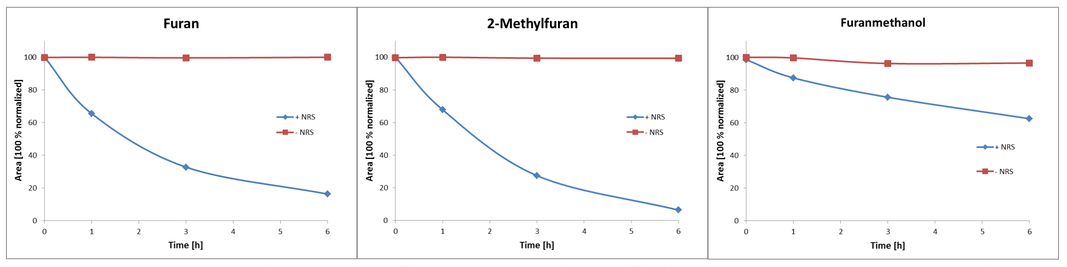
Q Exactive™

Leco Pegasus® 4D

Results

Phase I metabolism kinetics for a furan based substrate mix (furan, 2-methylfuran and 2-furanmethanol)

Experiments were performed using a GCxGC-TOF-MS system with two labeled internal standards, with observed peak areas normalized using the area ratio between compound and corresponding internal standard (furan-d4 for furan and 2-methylfuran; furfural-d4 for 2-furanmethanol). While the furans remained unchanged in the absence of the NRS (negative control), when present (+NRS), extensive metabolism was observed for all tested furans. Furan and 2-methylfuran were rapidly metabolized, with almost 90% of the parent compounds transformed after 6 hours. The kinetics for 2-furanmethanol showed a significantly slower rate of transformation (about 40% after 6 hours).



Elimination rate: **Ke=0,24h^{-1}**
Half life: **$t_{1/2}$=2,86h**

Elimination rate: **Ke=0,37h^{-1}**
Half life: **$t_{1/2}$=1,86h**

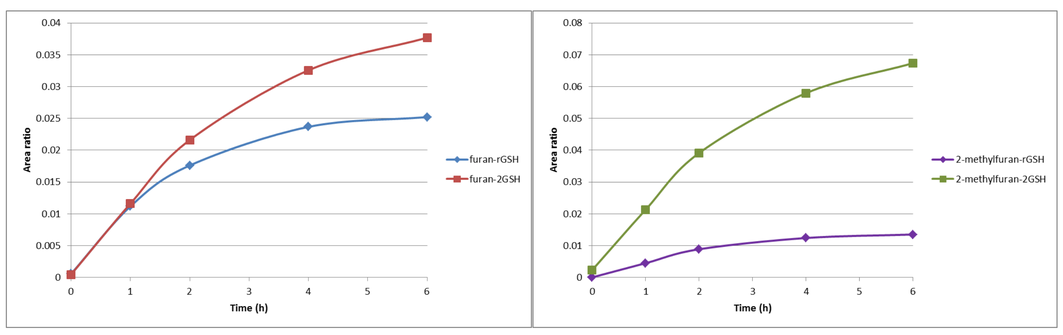
Elimination rate: **Ke=0,06h^{-1}**
Half life: **$t_{1/2}$=10,83h**

Trapping of furan and 2-methylfuran reactive metabolites using GSH

Glutathione was used to trap reactive metabolites derived from furan and 2-methylfuran, where two different types of adduct were generated:

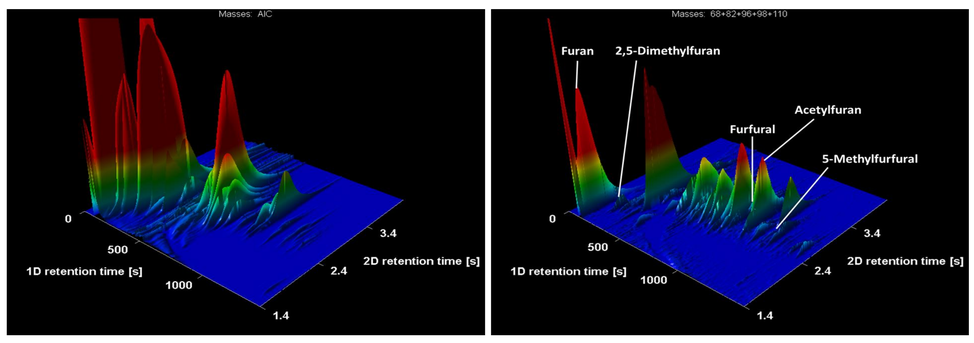
- Adduct formation with a single glutathione moiety followed by intramolecular rearrangement (mono-GSH adduct)
- Adduct formation with two glutathione moieties without any intramolecular rearrangement (bis-GSH adduct)

Monitoring of these adducts was performed using LC-HR-MS, which was the preferred method for thermolabile compounds. Both types of glutathione adduct were generated and therefore both had to be considered.



Screening of cigarette smoke trapped in aqueous media

GCxGC-TOF-MS full-scan chromatograms of standard mixture of furans used for metabolism investigation (right) and whole cigarette smoke trapped in phosphate buffer saline solution (left)



Conclusions

- The application of ionic liquid columns for the analysis of aqueous media using GCxGC-TOF-MS was established
- Metabolic kinetics for highly volatile compounds were assessed using this approach
  - ➢ Kinetics for parent compound transformation were measured
  - ➢ Adduct formation was assessed using a complementary LC-HR-MS approach
- It should be feasible to use these analytical techniques for non-targeted screening of the metabolic fate of complex chemical mixtures such as cigarette smoke in aqueous media

References

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