Screening of aqueous media using GC×GC-TOF-MS

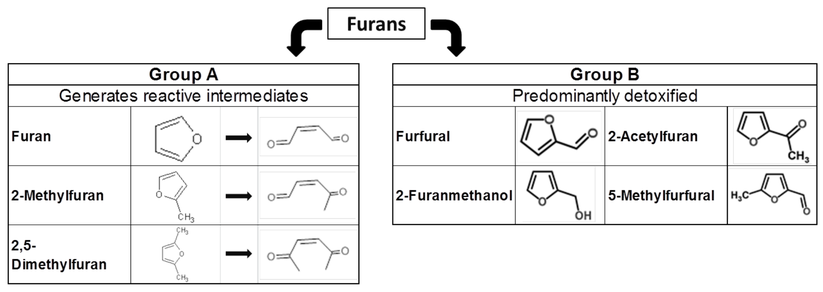
Martin Almstetter, Arno Knorr, Audrey Debrick, Quentin Dutertre, Mark Bentley

Philip Morris International, R&D, Biological Systems Research Department, Metabolomics & Analytical Chemistry, CH-2000 Neuchâtel, Switzerland.

Introduction

Background

- The inherently complex chemical space associated with tobacco and tobacco-related research requires the use of a variety of different experimental strategies
- In gas chromatography (GC), highly volatile compounds are commonly analyzed using headspace techniques or with the use of tailored extraction/trapping strategies
- Aqueous solutions are often used to trap cigarette aerosol fractions or as part of *in vitro* metabolic investigations to study any potential toxification of tobacco aerosol related xenobiotics
- Furans represent a volatile group of constituents that are naturally present in tobacco leaves and are additionally generated during the smoking of tobacco products by thermal degradation
- Biotransformation of furans can be described by two major pathways[1], either by the initial generation of reactive intermediates (cis-1,4-butendial and derivatives) by oxidative CYP (2E1) activation (Group A), or by direct detoxification prior to excretion from the body (Group B)

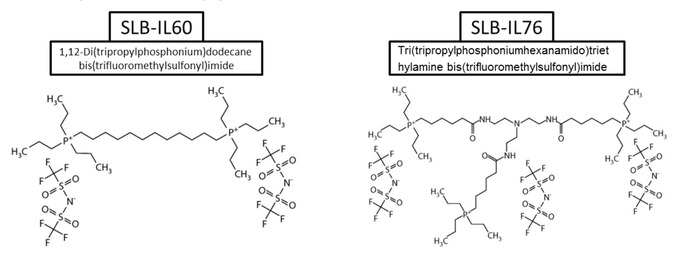


Aim

- To develop and implement a non-targeted GC×GC-TOF-MS approach for aqueous samples, e.g. cigarette aerosol trapped in phosphate-buffered saline (PBS) or microsomal incubation samples containing phosphate-buffered solution

Strategy

- To use a combination of water resistant ionic-liquid based analytical columns[2,3]
  → avoids any solvent delay
  → enables coverage of highly volatile furans, amongst other constituents
- Structures for the ionic liquid stationary phases used for GC:



Materials and Methods

Sample preparation

➢ The durability of the GC columns was prolonged extensively by dephosphating the samples prior to injection

1. Phosphate precipitation

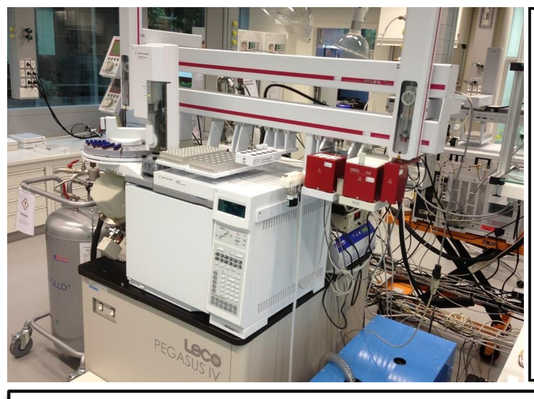
$FeCl_3 + KH_2PO_4 \rightarrow FePO_4 + 2\ HCl + KCl$

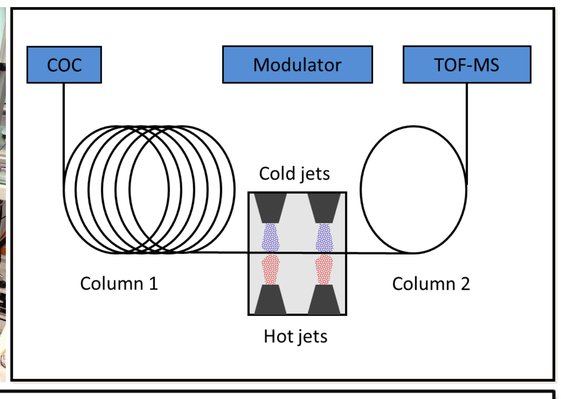
$FeCl_3 + Na_2HPO_4 \rightarrow FePO_4 + HCl + 2\ NaCl$

2. Centrifugation

3. Cool-on-column injection

GC×GC-TOF-MS Setup



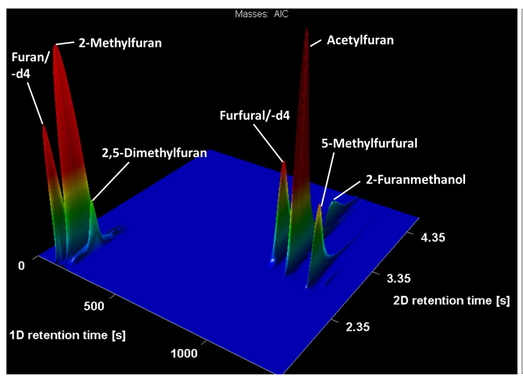


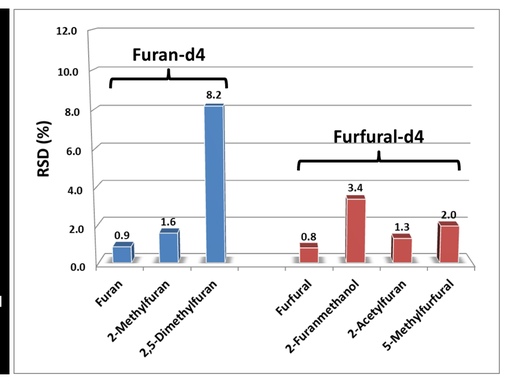
Injection	Cool-on-column, 0.5 µL at 38°C
Column 1	SLB-IL60 (30m × 0.32mm ID × 0.26µm film thickness)
Column 2	SLB-IL76 (2.2m × 0.25mm ID × 0.20µm film thickness)
Temperature program	35°C (2min) – 5°C/min – 255°C (15min) 40°C (2min) – 4°C/min – 48°C – 5.5°C/min – 265°C (15min)
Modulator offset	5°C
Modulation period	6s (1s hot pulse)
MS parameters	m/z 35-500, 200 spectra/s, acquisition delay 0s

Results

Repeatability assessment for furans

➢ Representative Apex Ion Chromatogram (AIC) for a mixture of furans (left)
➢ Repeatability (N=20) was assessed after 100 injections of aqueous sample (right)
➢ Early eluting furans were normalized using furan-d4, and later eluting furans using furfural-d4

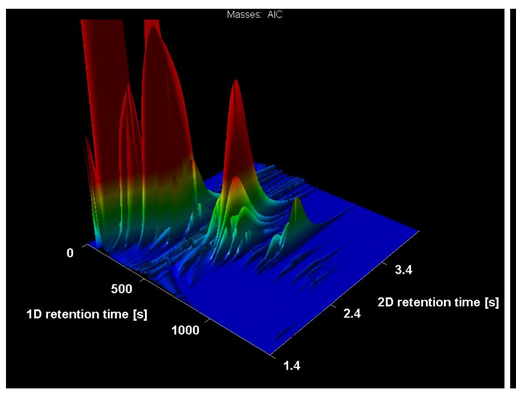


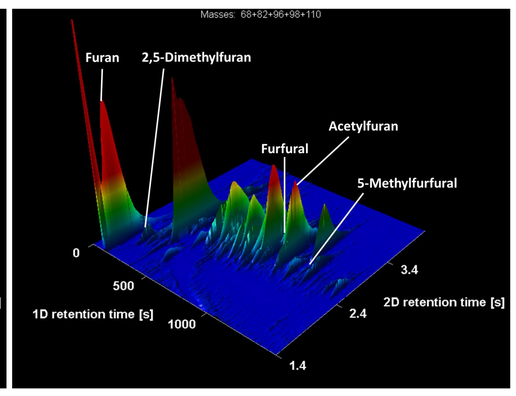


➢ Excellent repeatability (RSD <4%) was demonstrated for six of the seven furans investigated. The inferior repeatability observed for 2,5-dimethylfuran (RSD 8.2%) was considered to be due to differences in hydrophobicity/volatility compared with the internal standard, furan-d4

Non-targeted screening of aqueous cigarette aerosol fractions

➢ AIC of cigarette whole aerosol trapped in PBS (left)
➢ Extracted ion chromatogram (EIC) for investigated furans (right)

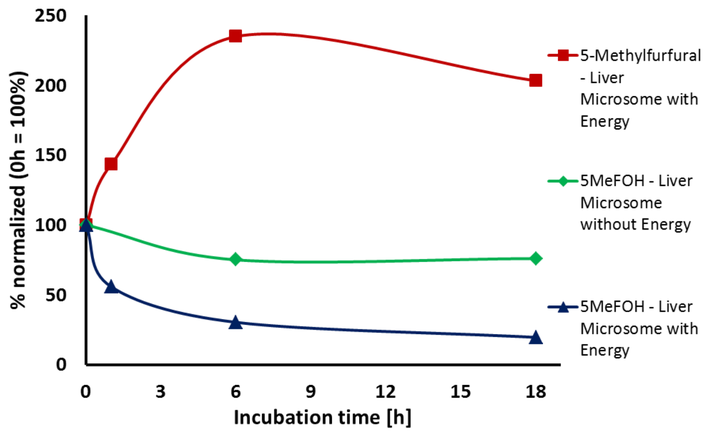




➢ Furthermore the approach enables the evaluation of a broad range of chemical constituents in a comprehensive non-targeted way

Metabolism during microsomal incubation

➢ Example: Monitoring phase I oxidation of (5-methyl-2-furyl)alcohol (5MeFOH) to 5-methylfurfural



Conclusions

➢ Ionic liquid columns make aqueous media applicable for GC
➢ The presented approach
  → allows the detection of highly volatile compounds by avoiding any solvent delay
  → comprises minimal sample preparation for lowest risk of changing chemical constituent profiles
  → enables a broad coverage of volatile compounds without the need for additional strategies
➢ Robustness has been proven for N >100 aqueous samples
➢ Group of furans showed excellent repeatability
➢ Applicability was presented, i.e. cigarette aerosol trapped in phosphate-buffered saline and microsomal incubation samples

References

1) *Peterson L. A. Reactive metabolites in the biotransformation of molecules containing a furan ring. Chem. Res. Toxicol. 26:6 (2013).*
2) *Armstrong D. W. et al. Examination of ionic liquids and their interaction with molecules, when used as stationary phases in gas chromatography. Anal. Chem. 71(17):3873 (1999).*
3) *Anderson J. L. et al. Structure and Properties of High Stability Geminal Dicationic Ionic Liquids. J. Am. Chem. Soc. 127:593 (2005).*

Philip Morris International Research & Development, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland
T: +41 58 242 21 13, **F**: +41 58 242 28 11, **W**: www.pmi.com