Multivariate optimization of method for analysis of emissions from heated tobacco by HS-SPME GC×GC-TOFMS

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INTRODUCTION

Cigarette smoke is a highly complex dynamic aerosol system generated by distillation, pyrolysis and combustion reactions when the tobacco is burnt. As the burning tip of a cigarette reaches temperatures of up to 1000ºC, more than 6800 compounds have been identified in mainstream smoke[1]. Heating tobacco to temperatures lower than 300°C simplifies the composition of emissions by decreasing the production of chemicals[2].

The study focused on developing and optimising an analytical strategy for the chemical characterisation of emissions from heated tobacco. Aerosol was generated using an A14 smoking engine (Borgwaldt, Germany) according to the Health Canada Intense regime (method T-115, Health Canada) applying 12 bell shaped puffs of 55ml volume, 2s puff duration and 30s interval between the puffs. Emissions were collected on Cambridge filter pads for Head Space Solid-Phase Micro Extraction (HS-SPME) analysis. Response-surface experimental design was applied to the optimization of the HS-SPME extraction parameters. Using the optimized SPME parameters, the emissions of heated tobacco were analyzed by means of comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS).

MATERIALS and METHODS

GENERAL

- LECO Pegasus 4D GC×GC-LR TOFMS with Gerstel MPS2 (with SPME and CIS4)
- Rtx-5MS (30m x 0.25mm ID x 0.25µm df) x Rxi17Sil MS (1.0m x 0.15mm ID x 0.15µm df)
- Rxi-624Sil MS (30m x 0.25mm ID x 1.4µm df) x Stabilwax (1.0m x 0.25mm ID x 0.25µm df)
- Emissions captured on 44 mm glass fiber (Cambridge) filter pad
- LECO ChromaTOF® software (4.50) used for processing
- GC Image™ software package (2.5b1) used for multivariate comparison
- MATLAB R2015b used for convex hull calculations
- R version 3.2.3 used for design of experiments

OPTIMIZATION

- 5 types of commercially available SPME fibers were tested:
  - 85 µm polyacrylate (PA),
  - 100 µm polydimethylsiloxane (PDMS),
  - 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB),
  - 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS),
  - 85 µm carboxen/polydimethylsiloxane (CAR/PDMS).
- Fractional factorial 2-level screening design was applied for selection of the most important parameters of HS-SPME. Sample size, incubation time and temperature, extraction time and temperature, desorption time and temperature were selected as factors.
- Central composite design was applied for optimisation of the most relevant HS-SPME parameters: - incubation and extraction temperature s and extraction time.

ANALYSIS

- Evaluation of two column sets was performed *via* comparison of number of detected analytes and determination of the orthogonality with convex hull relative area approach.
- The retention space used was evaluated with the convex hull approach, using the Delanuay triangulation method.
- GC Image was used for comprehensive evaluation of precision expressed as repeatability for SPME fibers.

RESULTS

SELECTION OF SPME FIBER

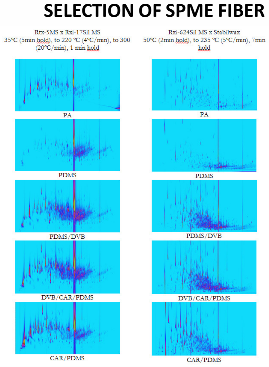

Figure 1. GC×GC contour plots of PP from heated tobacco emission – comparison of column setups studied extracted by means of 5 types of SPME fibers

SELECTION OF COLUMN SET

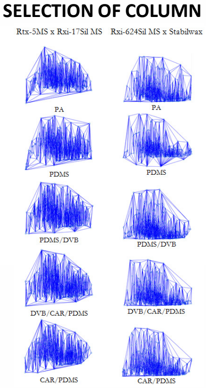

Figure 2. Representation of convex hull computed for GC×GC contour plots from heated tobacco emission extracted by HS-SPME – comparison of two column setups studied

PRECISION OF PRE-SELECTED FIBERS

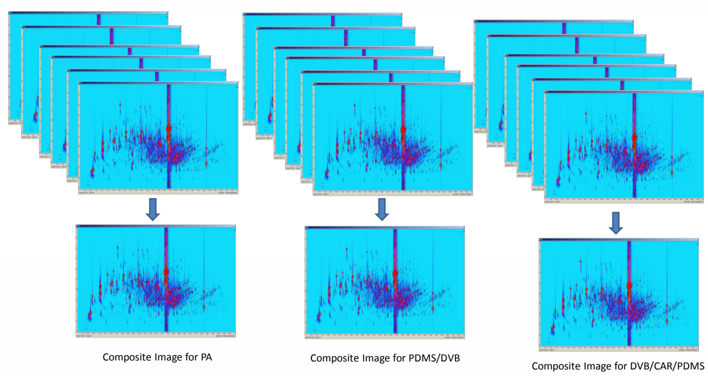

Figure 3. Building Composite Images containing up to 1132 compounds found for 3 selected SPME fibers

EXPERIMENTAL DESIGN - SCREENING

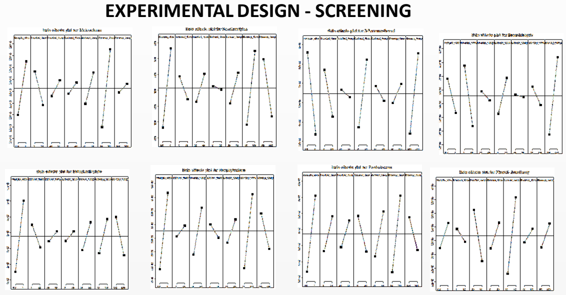

Figure 4. Results of fractional factorial design used for screening

EXPERIMENTAL DESIGN - OPTIMISATION

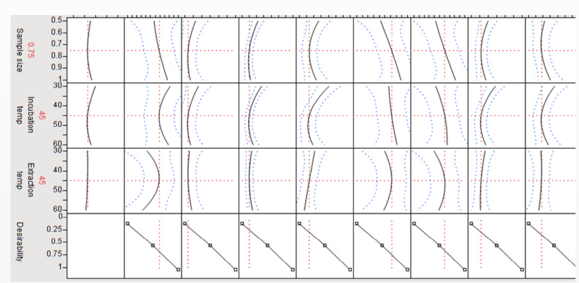

Figure 5. Results of central composite design used for optimisation of HS-SPME

CONCLUSIONS

- The average number of compounds detected when using non-polar x midpolar column set was higher in comparison to midpolar x polar. However, for each type of studied SPME fiber coatings midpolar x polar combination of columns offered better utilization of separation space on GC×GC contour plot according to convex hull relative area approach.
- Based on number of detected analytes PA, PDMS/DVB and DVB/CAR/PDMS fibers were pre-selected for further investigation.
- For PA, PDMS/DVB and DVB/CAR/PDMS fibers 747, 1132 and 1056 records representing agreed sample constituents were considered, respectively (n=6). PDMS/DVB fiber extracted 251 analytes characterised with with blob volume RSD% ≤ 10% and 471 analytes with RSD% of less than15% being the best fiber in this exercise (n=6).
- The strategy of multivariate experimental design was applied in two steps. First selecting the most relevant factors influencing HS-SPME extraction and subsequently optimising them with relatively small number of experiments.
- Maximised performance was achieved at incubation temperature of 60 °C, extraction temperature of 60 °C and extraction time of 10 minutes.
- GC Image software package allowed for comprehensive evaluation of precision (repeatability) for 1132 analytes.

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