Using chemical ionisation in the identification of volatile organic compounds (VOCs) on breath

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Aims

- To identify 15 unknown VOCs on breath which were shown to be markedly altered following exposure to candle emissions.
- To evaluate the effectiveness of CI-MS for structural identification and elucidation of VOCs.

1. Background and Objectives

The World Health Organisation (WHO) estimated that 3.2 million deaths were attributable to indoor air pollution in 2020 with inflammation being central to the pathophysiology. It is now well established that earlier diagnosis of disease improves patient outcome and we are therefore on a mission to create non-invasive breath tests for inflammatory disease using our Breath Biopsy® technology.

The CS1 study seeks to discover VOC biomarkers of inflammation. Breath was analysed from asthmatic patients (n=17) exposed to different inflammation-inducing candles, using thermal desorption-gas chromatography-high resolution mass spectrometry (TD-GC-MS) in electron ionisation (EI) mode. Fifteen molecular features (MFs) were found to be markedly altered by candle emission exposure and library searching suggested several tentative assignments.

A limitation of EI is that MFs are highly fragmented such that molecular ion (MI), which reveals molecular formula, often cannot be detected. Also, an open-access library of GC-Orbitrap mass spectra does not currently exist. Chemical ionisation (CI) mode (Figure 1) is a softer ionisation technique than EI with a greater propensity for generation of MI and associated adducts. This work evaluated CI mode as a complimentary technique to EI for the identification of fifteen MFs in CS1. Reagent gas flow rate, electron energy and ion source temperature were explored.

The objectives of this study were to determine the effect of reagent gas flow rate, ion source energy (electron volts, eV) and ion source temperature on the overall sensitivity and MI yield of VOCs in CI mode, and to generate CI spectra for the fifteen unknown MFs on CS1 exhaled breath to aid structural identification and elucidation.

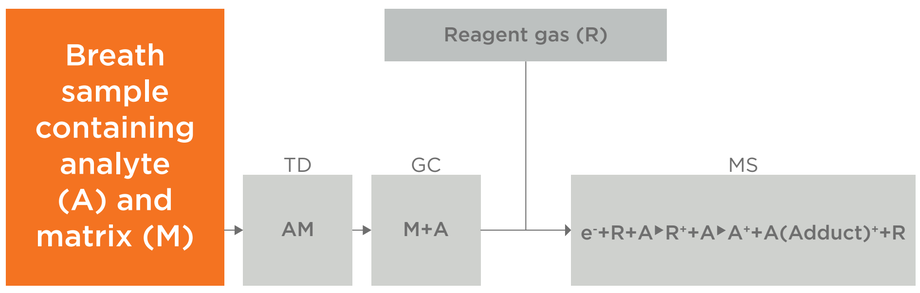

Figure 1. TD-GC-MS in CI mode. Reagent gas is ionised via an EI mechanism at the filament in the MS ion source. Upon collision with ionised reagent gas, unfragmented analyte becomes ionised making it detectable by MS. Theoretically, greater ratios of ionised reagent gas to analyte increase MI production.

2. Methods

Biomonitoring tubes and a TD100-XR (Markes International, UK) were used with a Trace-1310 GC and Orbitrap Exploris 240 (ThermoFisher, USA). QCs were prepared by nitrogen gas-assisted injection with a mixed standard (including C6-C16 alkane ladder for retention indexing) in methanol giving 10ng of each VOC. Empty tubes (containing no sorbent) were analysed between samples and QCs to reduce carryover and solvent blanks (containing methanol only) were used to assess background levels of VOCs. Tube desorption was performed at 250°C and a nitrogen gas flow of 50 mL/min for five minutes. The cold trap was heated from 10°C to 250°C at 40°C per minute with a split flow of 8 mL/min. GC carrier gas was helium with a flow rate of 2.0 mL/min and a Stabilwax-DA column (30m x 0.25mm, 0.25µm). GC oven temperature was held for two-minutes at 40°C followed by a 10°C/min ramp to 250°C. Methane or isobutane were used as MS reagent gas in positive mode, mass resolution was 60,000, scan range was 45-450m/z or 60-450m/z and AGC target was 1e6. Reagent gas flow rate (0.5-3.0mL/min), ion source energy (12-70eV) and ion source temperature (190-210°C) were explored. Data was acquired using Chromeleon software (v7). Compound Discoverer (v3.2) was used for deconvolution and determination of molecular formula and retention index. FreeStyle (v1.8 SP1) was used for manual interpretation. Definitive assignments were based on analysis of authentic standards.

3. Results

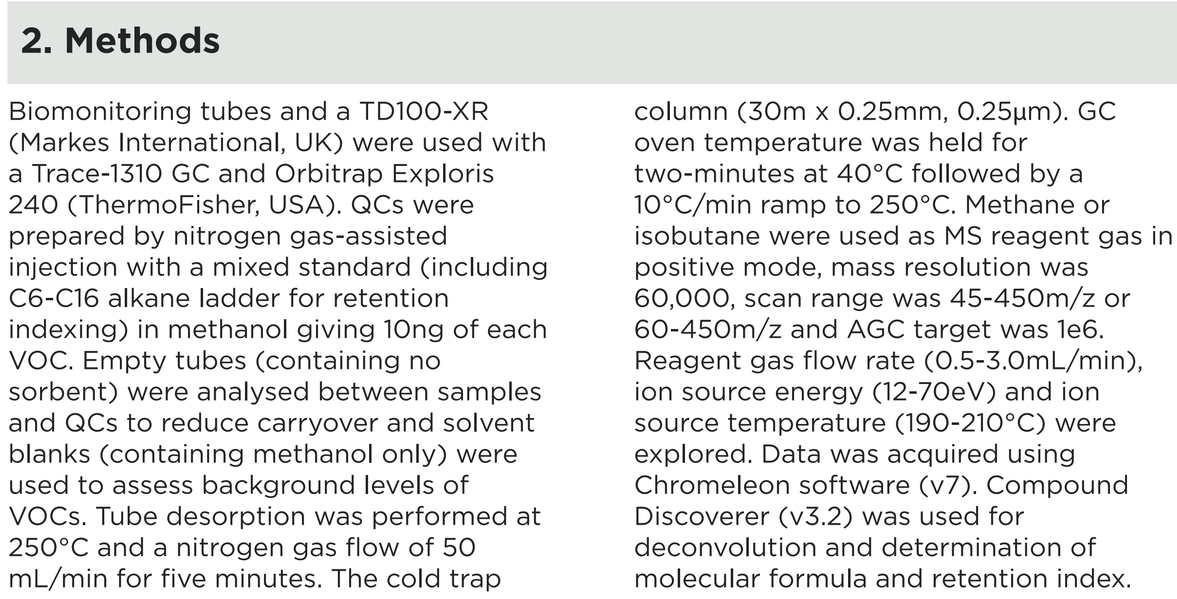

Figure 2. Total ion chromatograms (TICs) of a QC zoomed-in on an aromatic standard target at 5.27min (left panels) and corresponding mass spectrum (right panels) at various reagent gas flows. The ion at 99.026 m/z represents the molecular ion ($M+H^+$) of interest. Ion source temperature of 190°C and 70eV were used.

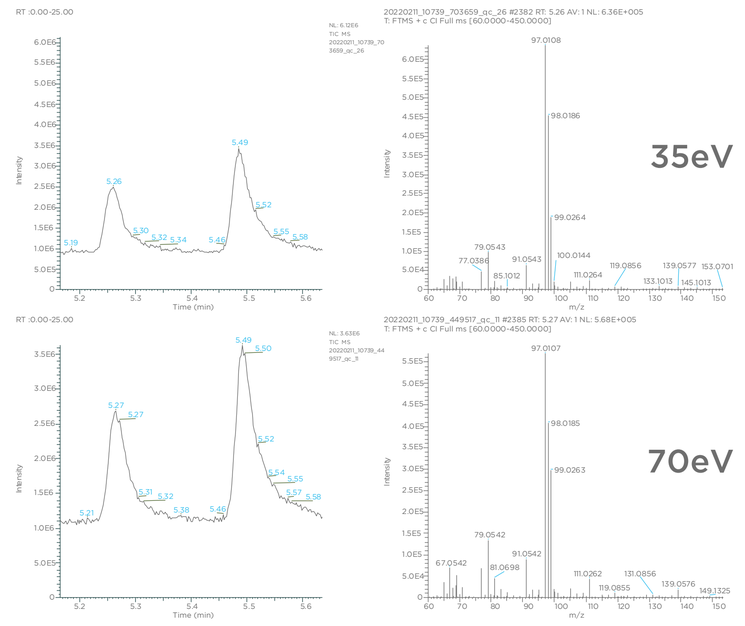

Figure 3. Total ion chromatograms (TICs) of a QC zoomed-in on an aromatic standard target at 5.27min (left panels) and corresponding mass spectrum (right panels) at various electron energies. The ion at 99.026 m/z represents the molecular ion ($M+H^+$) of interest. Reagent gas flow of 1.0ml/min and mass spectrometer source temperature of 190°C were used.

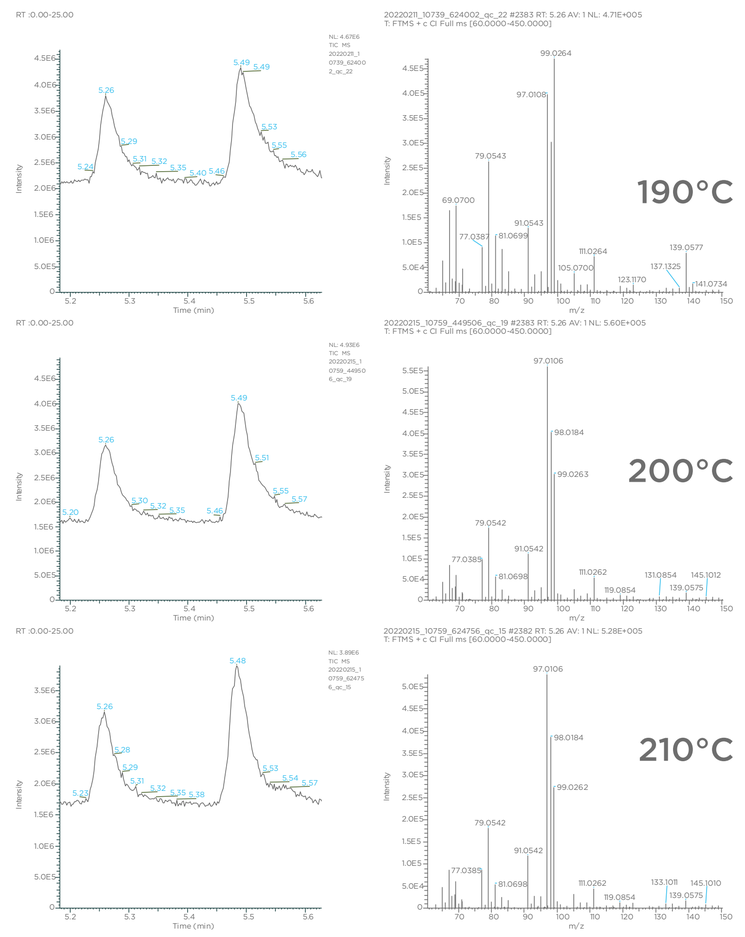

Figure 4. Total ion chromatograms (TICs) of a QC zoomed-in on an aromatic standard target at 5.26min (left panels) and corresponding mass spectrum (right panels) at various ion source temperatures. The ion at 99.026 m/z represents the molecular ion ($M+H^+$) of interest. Reagent gas flow of 1.5ml/min and 70eV were used.

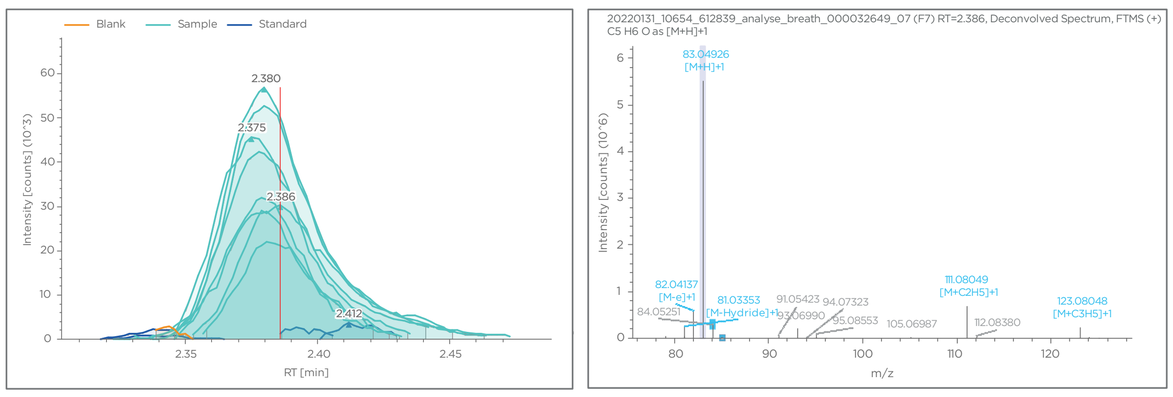

Figure 5. Exemplar CI data of an unknown MF on breath at 2.4min. Left panel: deconvolved and overlaid extracted ion chromatogram (EIC) showing higher signals on breath vs empty tube ('blank') and QCs ('standard'); right panel: deconvolved mass spectrum of a breath sample showing molecular ion ($M+H^+$) and cation radical adducts.

4. Conclusions

- An aromatic target was found to have high sensitivity in CI mode and was used to assess generation of molecular ion and adducts. M+H+ signal was more pronounced at higher reagent gas flow rate, higher ion source energy and lower ion source temperature (Fig 2-4).
- Signal-to-noise of TIC was reduced by increasing reagent gas flow rate (data not shown).
- Robust CI spectra were produced for CS1 breath samples (Fig 5). Definitive structural assignment was expedited for eight unknown MFs including three furans, one benzenic compound, two alkenes, a terpene and a siloxane. The other seven unknown MFs were not detected by CI including an aldehyde, a furan, two ketones, two siloxanes and a low-level unidentified compound. Negative CI mode may improve sensitivity of oxygenated compounds.
- Retention time shifts between original and reanalysed datasets confounded peak alignment. Retention indexing proved to be an effective solution to this and permitted identification of stereoisomers.
- The newly identified MFs are currently being evaluated for their potential utility as inflammatory VOC markers in the clinic using a new targeted breathomics approach.
- Reagent gas flow rate, electron energy and ion source temperature should be optimised for optimal MI generation and TIC sensitivity in CI mode.
- Eight of the fifteen MFs of interest in CS1 generated detectable molecular ions and adducts using CI mode which expedited definitive assignment of their structural identities.
- Retention indexing improves peak alignment between chromatograms with RT differences.