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# Evaluation and application of static headspace–multicapillary column-gas chromatography–ion mobility spectrometry for complex sample analysis

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ABSTRACT

An evaluation of static headspace–multicapillary column-gas chromatography–ion mobility spectrometry (SHS-MCC-GC-IMS) has been undertaken to assess its applicability for the determination of 32 volatile compounds (VCs). The key experimental variables of sample incubation time and temperature have been evaluated alongside the MCC-GC variables of column polarity, syringe temperature, injection temperature, injection volume, column temperature and carrier gas flow rate coupled with the IMS variables of temperature and drift gas flow rate. This evaluation resulted in six sets of experimental variables being required to separate the 32 VCs. The optimum experimental variables for SHS-MCC-GC-IMS, the retention time and drift time operating parameters were determined; to normalise the operating parameters, the relative drift time and normalised reduced ion mobility for each VC were determined. In addition, a full theoretical explanation is provided on the formation of the monomer, dimer and trimer of a VC. The optimum operating condition for each VC calibration data was obtained alongside limit of detection (LOD) and limit of quantitation (LOQ) values. Typical detection limits ranged from 0.1 ng bis(methylthio)methane, ethylbutanoate and (E)-2-nonenal to 472 ng isovaleric acid with correlation coefficient ($R^2$) data ranging from 0.9793 (for the dimer of octanal) through to 0.9990 (for isobutyric acid). Finally, the developed protocols were applied to the analysis of malodour in sock samples. Initial work involved spiking an inert matrix and sock samples with appropriate concentrations of eight VCs. The average recovery from the inert matrix was $101 \pm 18\%$ ($n = 8$), while recoveries from the sock samples were lower, that is, $54 \pm 30\%$ ($n = 8$) for sock type 1 and $78 \pm 24\%$ ($n = 6$) for sock type 2. Finally, SHS-MCC-GC-IMS was applied to sock malodour in a field trial based on 11 volunteers (mixed gender) over a 3-week period. By applying the SHS-MCC-GC-IMS database, four VCs were identified and quantified: ammonia, dimethyl disulphide, dimethyl trisulphide and butyric acid. A link was identified between the presence of high ammonia and dimethyl disulphide concentrations and a high malodour odour grading, that is, $\geq 6$. Statistical analysis did not find any correlation between the occurrence of dimethyl disulphide and participant gender.



## 1. Introduction

Ion mobility spectrometry (IMS) was developed in the latter half of the last century in response to the need by the military agencies for a fast and sensitive technique for the detection of chemical warfare agents, explosives, hazardous chemicals and drugs [1–4]. Ion mobility spectrometry and time-of-flight mass spectrometry (TOF-MS) are similar in the sense that ionized compounds are separated on the basis of their charge and size by passage along a tube, the drift tube, under the influence of an electric field. Larger molecules move more slowly than small ones and a spectrum is generated in time, based upon their arrival at the detector. In the case of TOF-MS, it separates fragment ions whereas IMS being a soft ionization technique only generates and separates molecular ions. In contrast to TOF-MS, IMS is an atmospheric technique and as soft ionization is used, only molecular ions of volatile organic compounds need to be resolved. In addition, IMS is not an identification technique, which is a distinct disadvantage over TOF-MS. However, an IMS instrument is compact, ideal for use in the field, relatively simple to operate, sensitive and produces the results very rapidly. Many instruments are now used by the security operations at airports in addition to the military [5].

In recent years, IMS has been combined with chromatographic systems including gas chromatography (GC) [6,7] and liquid chro-

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matography mass spectrometry (LC-MS) [8,9] as well as TOF-MS [10,11]. Gas chromatography combined with IMS extends the usefulness of IMS by providing the additional dimension of retention time separation of the gas chromatograph to the drift time separation of the spectrometer. Further, the IMS signal intensity provides quantitative data in addition to the qualitative information.

Currently, very rapid chromatographic separations are carried out using short (up to 25 cm) multicapillary columns (MCC) containing approximately a 1000 parallel capillary tubes coated with a stationary phase. Studies have been carried out to investigate the application of SHS-MCC-GC-IMS [6,7,12,13]. The important operating parameters include gas flows (i.e. drift and carrier gas flows) and temperatures (i.e. drift tube, column and injector) each of which has been studied in order to optimize separation [6,7].

Thus, there are two types of separations: the first is chromatographic separation and the second one is drift separation. Headspace volatile or semi-volatile compounds are selectively separated by the GC column and then introduced into the ionization region of the IMS where they are converted into gas phase ions. Ionization occurs under ambient pressure and in the presence of an applied electric field these generated ions then travel through the drift tube region of the IMS and are detected at the Faraday plate according to their mobility, ion cross section and charge [14,15]. Nitrogen is normally used as both the carrier gas and drift gas. Other gases may also be used but it is a prime requirement that the gas used must contains water [16]. The actual amount does not appear to be critical, and certainly in the case of nitrogen the level is very low (<20 ppm). Detection limits in the IMS are typically in the low ppb range for VCs [13,17].

SHS-MCC-GC-IMS has been used in trace analysis for the characterization of biological and clinical samples [12,18,19], medical diagnosis [13,20], food quality control [6,21], safety monitoring [22], biomolecule analysis [23] and fermentation application [24]. For example, using the static headspace approach, MCC-GC-IMS has been used to differentiate virgin and extra virgin olive oil [6]. These two types of virgin olive oils have very similar characteristics and other analytical techniques are unable to distinguish these selectively. According to the results, static headspace coupled to MCC-GC-IMS provided better results than traditional methods (97% of classification and an 87% prediction were achieved). Perl et al. [7] detected and characterized the metabolic volatile profiles of *Aspergillus fumigatus* and four Candida species by SHS-MCC-GC-IMS. In the study, GC–MS was involved as an additional technique to identify unknowns in the MCC-GC-IMS data. However, isoamyl alcohol and cyclohexanone, which both eluted at similar drift time and retention time, were not resolved by SHS-MCC-GC-IMS. Moreover, the authors [7] have concluded that fast analysis of complex volatile organic compound mixtures without sample preparation or pre-concentration is the major advantage of MCC-GC-IMS [25]. Recently, Jünger et al. [18] conducted similar investigations to identify human pathogenic bacteria by the determination of their characteristic metabolic volatile organic profiles. In the study, SHS-MCC-GC-IMS has been used to differentiate 15 bacterial strains by their metabolic VC profiles. Additionally, time-consuming high-resolution gas chromatography (HRGC)–MS was employed for further confirmation of compounds in several selected strains.

Of interest in this research article is the analysis and identification of VCs associated with malodour from pre- and post-laundry garments, that is, garments before and after the laundry process; specifically small sulphur and nitrogen containing molecules which can be difficult to analyse. Numerous factors have been identified for the formation of malodour in laundries such as humidity, indoor drying, chemical oxidation and metabolism of micro-organisms as well as human odour [26–29]. Human odour is generated from different parts of the body, for example, hair, mouth, foot and axillae, due to bacterial degradation (e.g. large-chain fatty acids that can be broken down by microbes to short-chain, volatile fatty acids). Several investigations have been conducted to investigate the occurrence of axillary odour [30,31]. During wearing, laundry can be contaminated by sebaceous lipids, sweat and dead skin cells. These substrates provide the nourishment and facilitate micro-organism survival on laundries [28,32]. The characteristic malodour is generated from the laundries, which can be identified just after washing due to poor hygiene in laundering. The microbial communities and biofilm which build up inside the washing machine have been identified as sources of malodour with potential for cross-contamination of garments [27,33]. Traditionally in Europe, laundry has been washed at a high temperature (>60 °C). However nowadays, for environmental reasons, lower temperatures are recommended and employed. At lower temperature range (30–40 °C), generation of malodour is more prevalent [27,29]. The use of higher temperature washing conditions decreases the risk of micro-organism survival in laundry compared to lower washing temperatures. Nagoh et al. [28] investigated the odorants generated from the indoor drying of garments and identified numerous odorant compounds such as medium-chain alcohols, medium-chain aldehydes, ketones, fatty acids, N-compounds and S-compounds. Munk et al. [32] investigated the compounds attached to laundry soiled with sweat and sebum just after a washing process. Under mild washing condition (low temperature), they identified 14 different odorants, specifically, ethyl-2-methylpropanoate, ethylbutanoate,1-hexen-3-one, 1-octen-3-one, (Z)-4-heptenal, octanal, (E)-2-octenal, methional, (Z)-2- nonenal, (E,Z)-2,6-nonadienal, (E,Z)-2,4-nonadienal, (E,E)-2,4-decadienal, 4-methoxybenzaldehyde.

Adhesion of odorants to different textiles has also been investigated. The determination of how well-selected odorants adhere to cotton and polyester textiles during laundry and the drying process was carried out by Munk et al. [27]. According to their study, odorants were more effectively removed from cotton textiles rather than polyesters during the wash cycle. The removal ability of odorants from textiles is dependent on the hydrophobicity and hydrophilicity of the textile; the hydrophobicity of polyester fabric preventing odorant removal. Conversely, the odour generated in cotton during wet storage was significantly higher than in polyester [27]. This may be caused by the greater water absorbency of (or hydrophilicity) cotton fibres over polyester fibres.

The overall aim of this article was to evaluate the performance of SHS-MCC-GC-IMS for the analysis of volatile compounds in complex matrices. This was achieved by: (a) development of a hypothesis on the formation of analyte monomers, dimers and trimers, (b) investigation of the main operating parameters and their influence on signal, (c) development of a strategy for calibration of VCs on different columns, that is, polar and non-polar and (d) application of the developed methodology to the analysis of foot malodour, specifically socks.

## 2. Experimental

### 2.1. Chemicals/Reagents

Acetone (CAS 67-64-1, ≥ 99.9%), ammonia (CAS 1336-21-6, 28% $NH_3$ in $H_2O$, ≥ 99.99%), bis(methylthio)methane (CAS 1618-26-4, 99%), 1- butanethiol (CAS 109-79-5, 99%), butyric acid (CAS 107-92-6, ≥ 99%), (E,E)-2,4-decadienal (CAS 25152-84-5, ≥ 85%), 1-decanol (CAS 112-30-1, 99%), dimethyl disulphide (CAS 624-92-0, ≥ 98%), dimethyl sulphide (CAS 75-18-3, ≥ 99%), dimethyl trisulphide (CAS 3658-80-8, ≥ 98%), 1-dodecanol (CAS 112-53-8, 98%), ethanethiol (CAS 75-08-1, 97%), ethylbutanoate (CAS 105-54-4, ≥ 98%), 4-fluoroacetophenone (CAS 403-42-9, 99%), guaiacol