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Multidimensional gas chromatography in combination with accurate mass, tandem mass spectrometry, and element-specific detection for identification of sulfur compounds in tobacco smoke☆

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ABSTRACT

A method is developed for identification of sulfur compounds in tobacco smoke extract. The method is based on large volume injection (LVI) of 10 μL of tobacco smoke extract followed by selectable one-dimensional (^{1}D) or two-dimensional (^{2}D) gas chromatography (GC) coupled to a hybrid quadrupole time-of-flight mass spectrometer (Q-TOF-MS) using electron ionization (EI) and positive chemical ionization (PCI), with parallel sulfur chemiluminescence detection (SCD). In order to identify each individual sulfur compound, sequential heart-cuts of 28 sulfur fractions from ^{1}D GC to ^{2}D GC were performed with the three MS detection modes (SCD/EI-TOF-MS, SCD/PCI–TOF-MS, and SCD/PCI–Q-TOF-MS). Thirty sulfur compounds were positively identified by MS library search, linear retention indices (LRI), molecular mass determination using PCI accurate mass spectra, formula calculation using EI and PCI accurate mass spectra, and structure elucidation using collision activated dissociation (CAD) of the protonated molecule. Additionally, 11 molecular formulas were obtained for unknown sulfur compounds. The determined values of the identified and unknown sulfur compounds were in the range of 10–740 ng mg total particulate matter (TPM) (RSD: 1.2–12%, $n = 3$).

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) has been an indispensable technique for identification of volatile compounds. However, one dimensional GC in combination with low resolution mass spectrometry is often insufficient for unequivocal identification of important trace components in complex samples like natural products due to co-elution of various compounds and non-specific electron ionization (EI) mass spectra. GC–MS with simultaneous selective detection (e.g. element-specific detection and/or olfactometry) can help to locate the region of interest within the complex chromatogram, but lack of sufficient resolution may still preclude reliable identification base on a pure mass spectrum, even after mass spectral deconvolution. An effective way to improve the chromatographic resolution and identification capability is through multidimensional (MD) GC with simultaneous mass spectrometric and element-specific detection. There are two established MD GC approaches: heart-cutting two-dimensional (^{2}D) GC (GC–GC) and comprehensive ^{2}D GC (GC × GC) [1–4]. GC × GC is mainly used in exhaustive analysis of a sample for total profiling and is for instance applied to the analysis of sulfur compounds in petrochemical products [5–7], in wine [8,9] and in coffee [8,10]. Heart-cutting two-dimensional GC, on the other hand, is typically used in a "target mode" whereby only selected fractions from the first dimensional separation are transferred to a second dimension for more detailed analysis. Although several injections are often required for the identification of multiple target compounds, heart-cutting ^{2}D GC–MS with parallel selective detection and/or olfactometry has higher ability to obtain a pure mass spectrum for each target solute that respond to specific element detection and/or olfactometry because of a much longer (and thus more efficient) second dimension column and its proper (independent) temperature programming.

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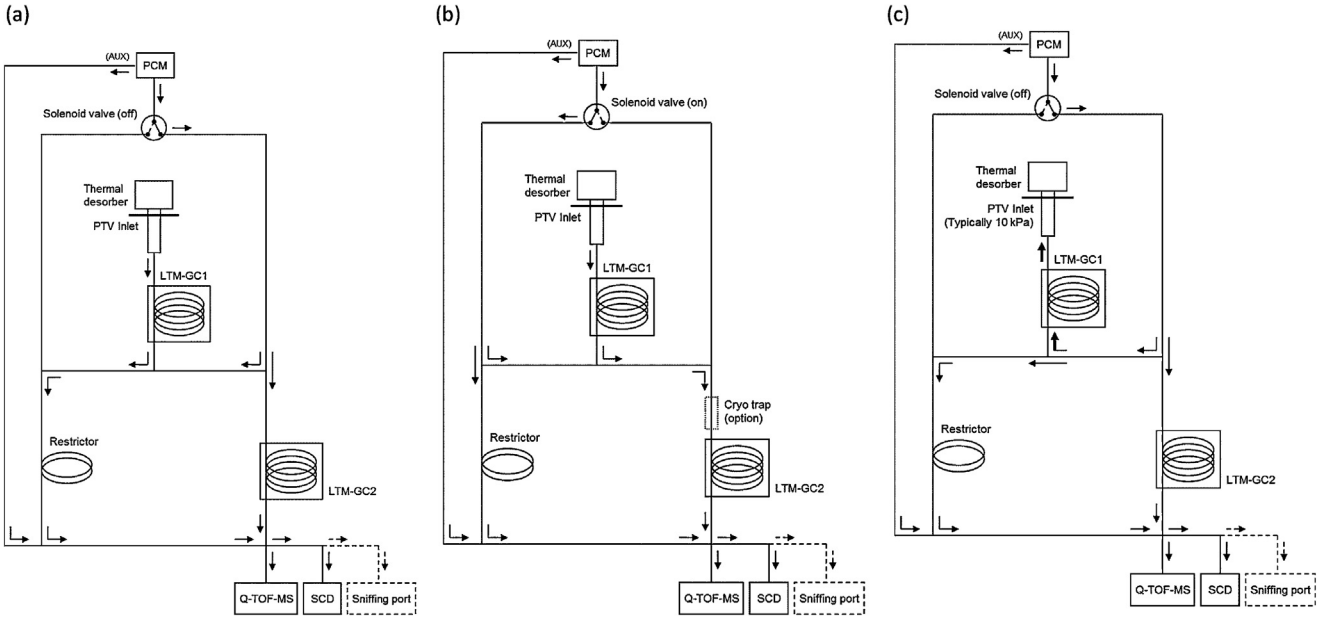

Fig. 1. Schematic flow diagrams for a selectable $^{1}D/^{2}D$ GC–SCD/Q-TOF-MS system. (a) ^{1}D GC–SCD/Q-TOF-MS analysis; (b) heart-cutting; (c) ^{2}D GC–SCD/Q-TOF-MS analysis and ^{1}D GC back flush. Dashed line shows the additional capability for parallel olfactometry using a sniffing port for both ^{1}D and ^{2}D separation (see text).

While the overall peak capacity in GC × GC can be higher than in heart-cut GC–GC [11], the conventional peak width (3–5 s), the higher sample capacity and the possibility for olfactometric detection in the second dimension makes the multiple heart-cut GC–GC approach most suitable for targeted analysis of sulfur compounds in complex samples. In 2010, Sasamoto and Ochiai [12] demonstrated a selectable ^{1}D or ^{2}D GC–MS ($^{1}D/^{2}D$ GC–MS) with parallel olfactometry or element-specific detection for analysis of trace odor compounds in beverages. With this system, simultaneous mass spectrometric detection and olfactometry/element-specific detection can be performed for both ^{1}D GC separation and ^{2}D GC separation, without any instrumental set-up change. Electron ionization (EI) mass spectra obtained by $^{1}D/^{2}D$ GC–MS with parallel element-specific detection provides additional filtering of MS library search results based on elemental information and linear retention indices (LRI) [12,13]. However, in certain cases, no or low MS library match can occur for unknowns. Although the availability of accurate mass spectra provides additional identification power in natural product identification, EI mass spectra often lack an abundant molecular ion that is required for identification of unknowns. In this respect, soft ionization such as chemical ionization (CI) offers interesting possibilities, especially in combination with tandem mass spectrometry (MS/MS) with accurate mass detection as available on a recently introduced GC-hybrid quadrupole time-of-flight mass spectrometry (GC–Q-TOF-MS) system [14,15]. Accurate masses from MS/MS product ion spectra with collision-induced dissociation (CID) {also known as collision deactivated dissociation (CAD)} [16] can help to verify that all the generated fragment ions can be correlated to the proposed structure [17,18]. However, in order to obtain a high quality CAD mass spectrum, it is essential to use a pure precursor ion from well resolved peak in a total ion chromatogram (TIC). In this respect, $^{1}D/^{2}D$ GC–Q-TOF-MS with parallel selective detection can be a very powerful tool for structure elucidation of the selected peak (that is also detected with an element-selective detector and/or olfactometry) in complex matrices. Volatile sulfur compounds in food and beverage have received special attentions due to their extremely low odor threshold levels and high sensory impact [19]. While sulfur compounds contribute to both enzymatically derived flavors and thermally derived flavors, these compounds are most often present at very low levels in complex matrices. Sulfur compounds which are derived from Maillard reaction of amino acids and sugar degradation products also play an important role in the flavor of tobacco smoke. However, the number of identified sulfur compounds in tobacco smoke is still limited due to one of the most complex sample matrices [20]. To analyze volatile sulfur compounds in tobacco smoke, it is therefore essential to have an advanced GC separation technique and instrumentation. Dallüge et al. [21] demonstrated GC × GC-high-speed unit resolution TOF-MS for unraveling the composition of tobacco smoke. Out of a list of several thousands of detected peaks (after mass spectral deconvolution), 14 sulfur compounds could be identified (using NIST library matching). However, it is difficult to confirm the odor character of those sulfur compounds with olfactometry in GC × GC due to very fast elution time in every modulation period (e.g. 6 s). Also, lack of sufficient resolution in ^{2}D separation of GC × GC might still cause co-elution with non-sulfur compounds which have a high or different sensory impact.

In this study, $^{1}D/^{2}D$ GC–Q-TOF-MS with parallel sulfur chemiluminescence detection (SCD) was applied for identification of trace sulfur compounds in tobacco smoke. The $^{1}D/^{2}D$ GC–SCD/Q-TOF-MS system has also the capability to integrate parallel olfactometry in both ^{1}D and ^{2}D separations [12]. To unravel the complexity and identify important sulfur compounds, 28 sulfur fractions selected from ^{1}D GC–SCD on a non-polar pre-column are sequentially transferred onto a polar main-column and then further separated, detected and identified using ^{2}D GC–SCD/Q-TOF-MS. Identification is based on a MS library search, $^{1}D/^{2}D$ LRI, elemental information (sulfur), molecular mass determination with positive CI (PCI) accurate mass spectra, formula calculation with EI and PCI accurate mass spectra, and structure elucidation with CAD of the protonated molecule. Also, sulfur compounds are quantified with the use of the linear and equimolar response of the ^{2}D GC–SCD to sulfur compounds.

2. Experimental

2.1. Reagents and materials

Methanol was high-purity pesticides grade (Kanto Kagaku, Tokyo, Japan). 2-Acetyl-4-methylthiazole and dihydro-2