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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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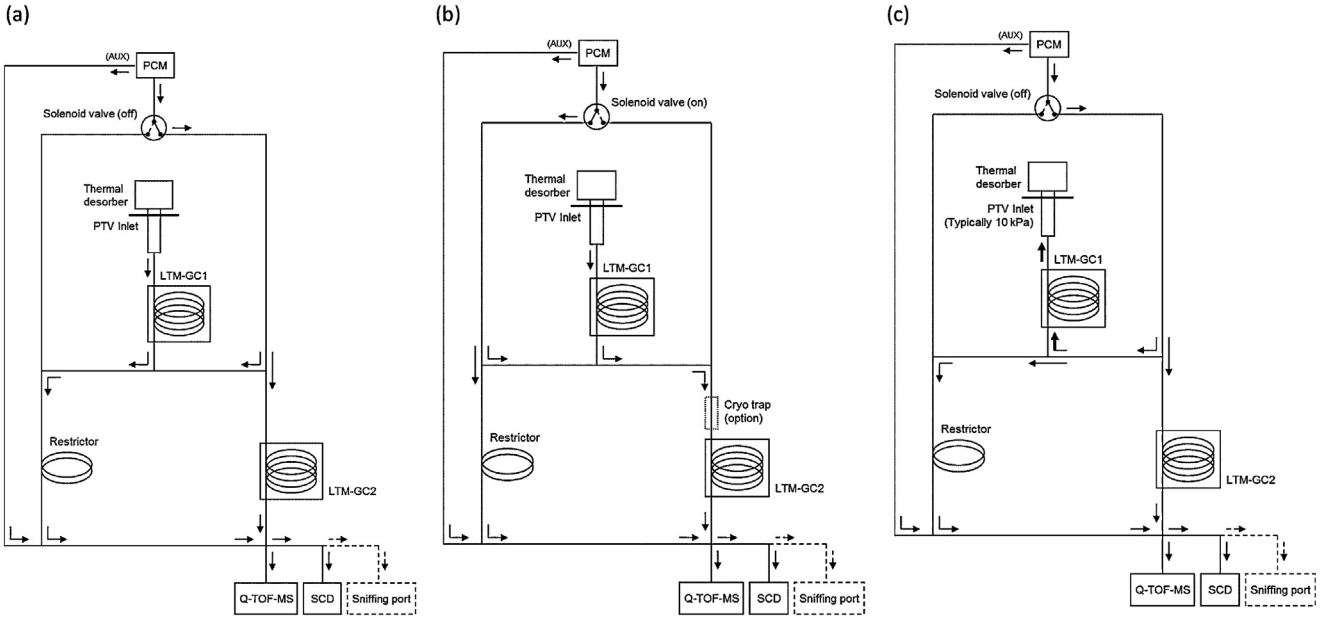

Fig. 1. Schematic flow diagrams for a selectable $^{1}D/^{2}D$ GC–SCD/Q-TOF-MS system. (a) ^{1}D GC–SCD/Q-TOF-MS analysis; (b) heart-cutting; (c) ^{2}D GC–SCD/Q-TOF-MS analysis and ^{1}D GC back flush. Dashed line shows the additional capability for parallel olfactometry using a sniffing port for both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

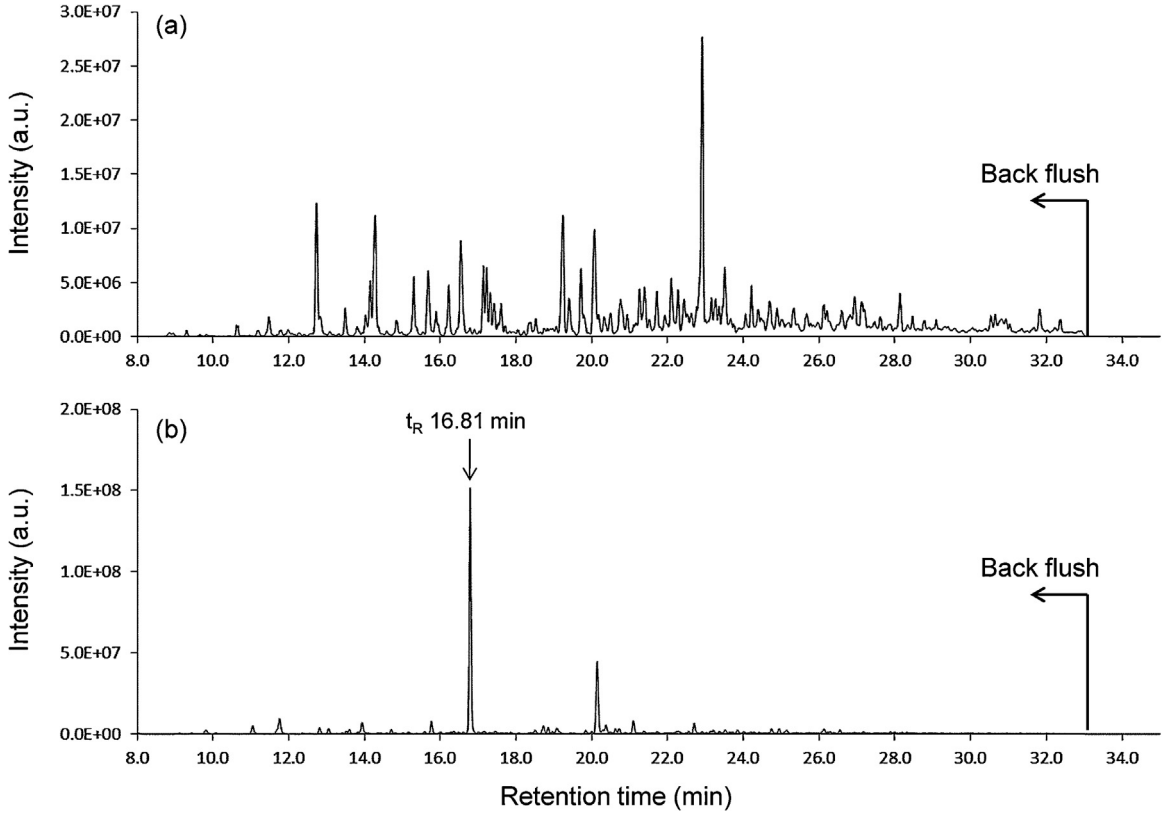

Fig. 2. [1]D total ion chromatogram (TIC) and SCD chromatogram of the tobacco smoke extract. (a) [1]D TIC; (b) [1]D SCD chromatogram.

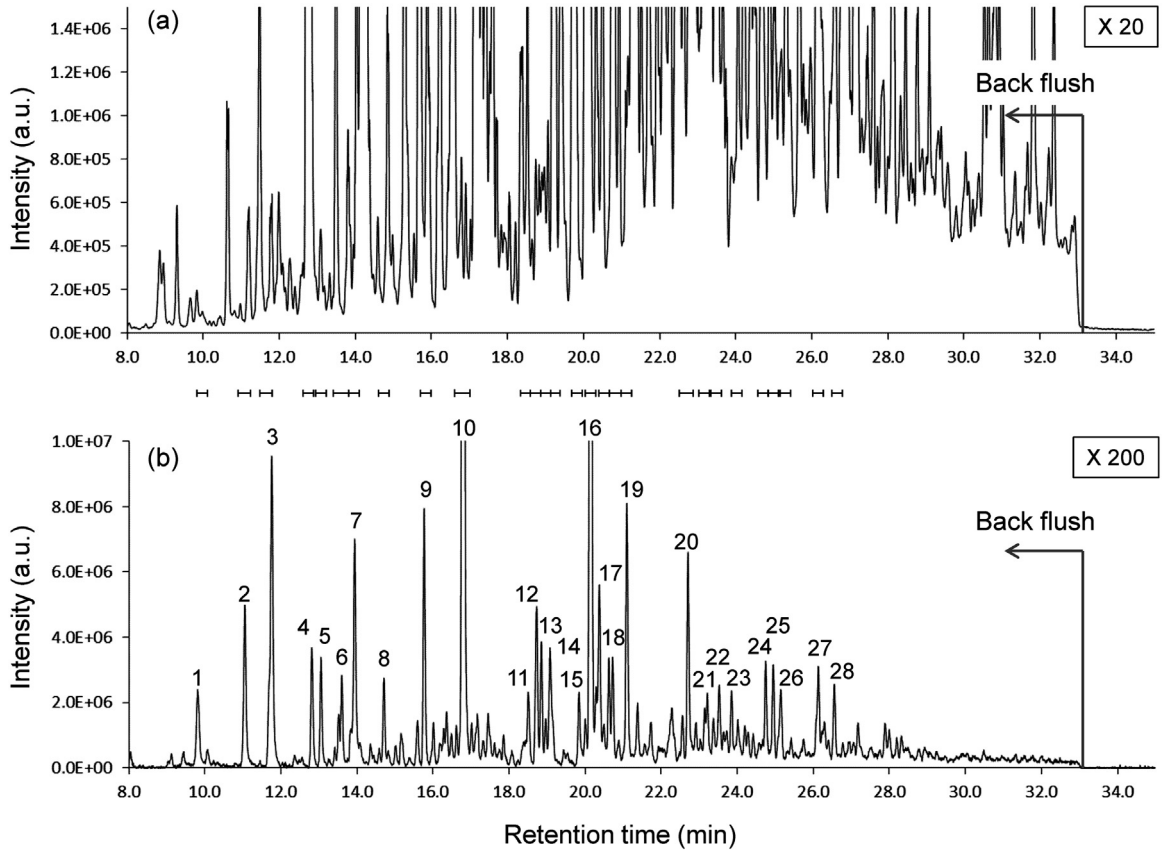

Fig. 3. [1]D total ion chromatogram (TIC) and SCD chromatogram of the tobacco smoke extract with a zoomed *y*-axis (×20 for the [1]D TIC and ×200 for the [1]D SCD chromatogram). (a) [1]D TIC (×20); (b) [1]D SCD chromatogram (×200). The 28 most abundant sulfur compounds (from SCD trace) are marked. Between the TIC and SCD trace, the heart-cut windows are indicated. The start t_R and the end t_R of these sulfur containing fractions 1–28 are listed in Table 1.

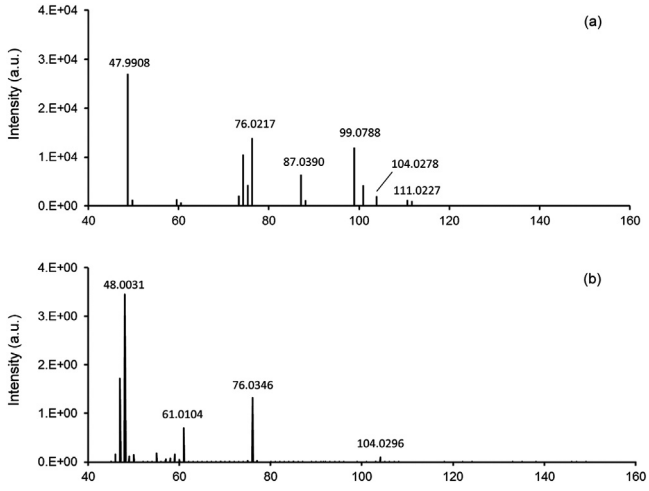

Fig. 4. A comparison of the mass spectrum of the most abundant sulfur compound in the tobacco smoke extract. (a) Deconvoluted mass spectrum (obtained with Mass Hunter deconvolution in high-resolution mode) at ^{1}D t_R of 16.48 min; (b) mass spectrum obtained at ^{2}D t_R of 40.36 min.

(3H)-thiophene were purchased from Sigma–Aldrich Japan K.K. (Tokyo, Japan). Dimethyl disulfide, 4,5-dimethyl thiazole, dihydro-3(2H)-thiophene, dimethyl trisulfide, and 2,4,5-trimethyl thiazole were purchased from Wako Pure Chemicals Ltd. (Osaka, Japan). Methional was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Dimethyl sulfoxide, 3-ethyl thiophene, 4-methyl thiazole, 3-methyl-2-thiophenecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 1-(2-thienyl)-1-butanone, 2-thiophenecarboxaldehyde, and 3-thiophenecarboxaldehyde were obtained from Dr. Katsumi Umano of Takata Koryo Co. Ltd. (Hyogo, Japan). The flue-cured tobacco was obtained from Japan Tobacco Inc. (Tokyo, Japan) as single grade tobacco.

2.2. Instrumentation

Analysis was performed on a ^{1}D/^{2}D GC–SCD/Q-TOF-MS. The Agilent 7890 gas chromatograph (host GC) (Agilent Technologies, Santa Clara, CA, USA) was equipped with a TDU thermal desorption unit (GERSTEL, Mülheim an der Ruhr, Germany), a CIS4 programmable temperature vaporizing (PTV) inlet (GERSTEL), a MPS2 robotic arm (GERSTEL), a CTS2 cryo-trap system (GERSTEL), a dual low thermal mass (LTM)-GC system (Agilent), and a SCD (Agilent). A 7200 Q-TOF-MS with CI option from Agilent was used. The dual LTM-GC–SCD/Q-TOF-MS system was configured as ^{1}D/^{2}D GC–MS with simultaneous selective detection previously described [12], which enables simple and fast operation of both ^{1}D GC–MS and ^{2}D GC–MS with parallel selective detection without any instrumental setup change. The ^{1}D/^{2}D GC–SCD/Q-TOF-MS system was equipped with dual wide format LTM-GC column modules (5 in.; 1 in. = 2.54 cm), an Agilent capillary flow technology (CFT) Deans switch, a 3-way splitter (with make-up gas line), which were controlled with a pressure control module (PCM). PCM has two pressure control capabilities with PCM line controlling flow at the Deans switch and AUX line controlling flow at the 3-way splitter. One is called PCM (main) and the other is called Auxiliary (AUX).

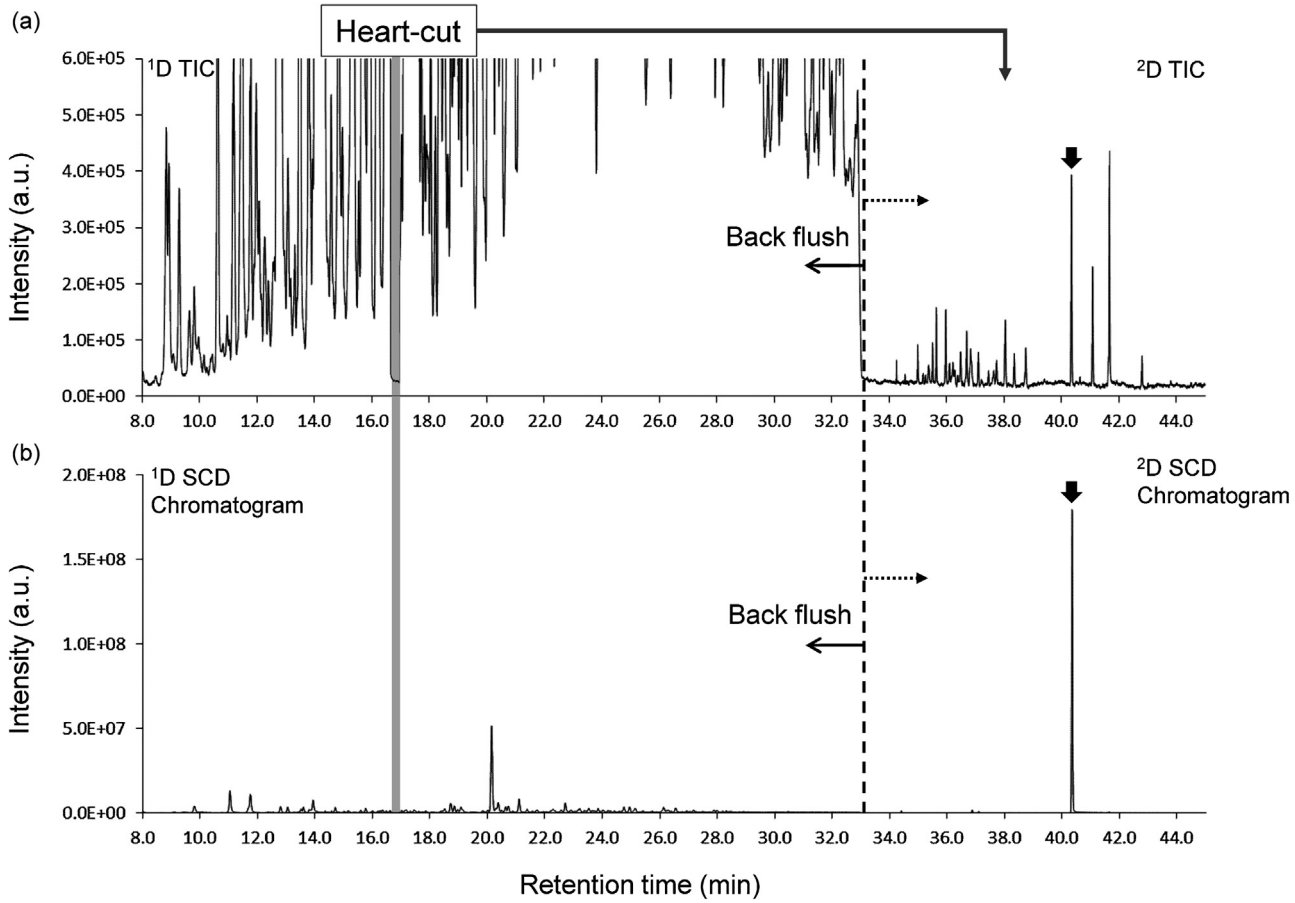

Fig. 5. An example of a heart-cut from ^{1}D t_R 16.65 min to 16.95 min (the sulfur fraction 10 in Fig. 3) and both ^{1}D and ^{2}D TIC and SCD chromatograms. (a) ^{1}D/^{2}D TIC; (b) ^{1}D/^{2}D SCD chromatograms.

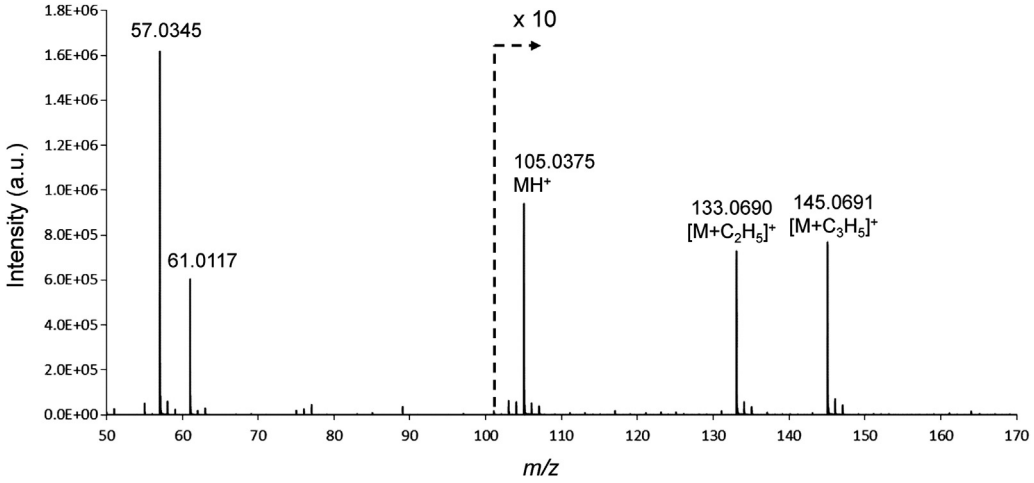

Fig. 6. PCI mass spectrum of the target sulfur peak 10-3 obtained from the ^{2}D t_R of 40.36 min.

2.3. Sample preparation

The smoke from the flue-cured tobacco was collected with a Cerulean SM 410 (Molins PLC, England, UK) smoking machine using 35 mL puff volume, 2 s puff duration, and 60 s puff interval. The machine airflows were tuned for ISO condition [22]. Smoke from three cigarettes was collected on a 44-mm diameter Cambridge filter pad (Borgwaldt GmbH, Hamburg, Germany). Total particulate matter (TPM) was determined at 80 mg by weighing the pad before and after collection. Then, the pad was extracted on a mechanical shaker with 30 mL methanol. The extract was analyzed using the thermal desorption (TD)-^{1}D/^{2}D GC–SCD/Q-TOF-MS system.

2.4. Large volume injection (LVI) using a thermal desorption system and micro-vial insert

Ten micro-liter large volume injection was performed with the TDU system that acts as two-stage inlet. This system allows optimization of inlet conditions for solvent venting, analyte refocusing and transfer to the column independent of the presence of matrix components [23]. After automated injection into a glass micro-vial that can be heated in the TDU, non-volatile sample matrix is left in the micro-vial and never contaminates the inlet. Volatiles are splitless transferred to the inlet where they can be refocused before introduction into the GC column. Finally, the TDU liner containing the micro-vial insert is returned to the auto-sampler tray. The TDU was programmed from 30 °C (held for 0.5 min) to 60 °C at 70 °C min^{-1} (held for 3 min), from 60 °C to 80 °C at 70 °C min^{-1} (held for 3 min) with 100 mL min^{-1} purge flow. Volatiles were focused at 10 °C on a Tenax TA packed liner in the PTV inlet. The PTV inlet was programmed from 10 °C (held for 0.5 min) to 240 °C (held for GC run time) at 720 °C min^{-1} to inject trapped compounds onto the analytical column. The injection was performed in the splitless mode.

2.5. Selectable ^{1}D/^{2}D GC–SCD/Q-TOF-MS

Fig. 1 shows the flow diagrams of the proposed system. Separations were performed on a 30 m, 0.25 mm i.d., 1.0 μm film thickness DB-1 column (Agilent) as the first dimensional (^{1}D) column and a 10 m, 0.18 mm i.d., 0.30 μm film thickness DB-Wax column (Agilent) as the second dimensional (^{2}D) column. The column temperature for the ^{1}D DB-1 was programmed from 40 °C (held for 3 min) to 220 °C (held for 10 min) at 5 °C min^{-1}. After the retention time of 33 min, the capillary column was back flushed. The column temperature for the ^{2}D DB-Wax was kept at 40 °C during ^{1}D GC analysis, and programmed from 40 °C at 10 °C min^{-1} to 240 ◦C (held for 10 min) for ^{2}D GC analysis. The host GC oven was kept at a constant temperature of 250 °C. The inlet pressure was 394 kPa and the pressure of AUX of PCM for the 3-way splitter was 25 kPa, respectively. The Deans switch pressure (for the ^{2}D column) of the PCM was set at 307 kPa. A deactivated fused silica capillary with 1.1 m × 0.25 mm i.d., was used for connecting from the splitter to the SCD, and 1.1 m × 0.20 mm i.d., for connecting from the splitter to the Q-TOF-MS. These pressures and transfer-line capillaries allow simultaneous mass spectrometric and sulfur chemiluminescence detection with minimum delay time (typically less than 0.1 s) at a constant split ratio of 1:2. For parallel olfactometry, SCD and Q-TOF-MS detection in both ^{1}D and ^{2}D separation, an additional 2-way CFT splitter and transfer capillaries are used. In this case, the deactivated fused silica capillary with 1.1 m × 0.20 mm i.d., is used for connecting from the 3-way splitter to the Q-TOF-MS, 0.20 m × 0.25 mm i.d., for connecting from the 3-way splitter to the 2-way splitter, 0.65 m × 0.20 mm i.d. for connecting from the 2-way splitter to SCD, and 1.25 m × 0.32 mm i.d. for connecting from the 2-way splitter to a sniffing port (GERSTEL ODP3). With the same pressures described above, these capillaries allow simultaneous olfactometry, mass spectrometric and sulfur chemiluminescence detection with minimum delay time (typically less than 0.8 s for olfactometry, and 0.1 s for Q-TOF-MS and SCD) at a constant split ratio of 1:1:1

The Q-TOF-MS was operated at a mass range of *m*/*z* 29–500 with dual gain mode (resolution was approximately 7000 (fwhm). No solvent delay time was used for the Q-TOF-MS measurement since back flush mode was used during solvent venting of LVI, resulting in complete elimination of solvent before final splitless transfer to ^{1}D GC column. The data acquisition speed was 5 Hz. The electron accelerating voltage of the EI was 70 V. Methane was used as a reagent gas at 1.0 mL min^{-1} in the PCI. Nitrogen was used as collision gas at 1.5 mL mL^{-1} and the collision energy of 10–30 V was used for MS/MS experiments. Alternate MS mode was used in the MS/MS measurement for simultaneous TOF-MS and Q-TOF-MS (MS/MS) detection. TOF-MS calibration was performed with perfluorotributylamine (PFTBA) for EI and perfluoro-5,8-dimethyl-3,6,9-decane trioxide (PFDTD) for PCI, respectively, in every sequence. The SCD burner temperature was set to 800 °C and its flow rate was 63 mL min^{-1} and 45 mL min^{-1} for air and hydrogen, respectively.

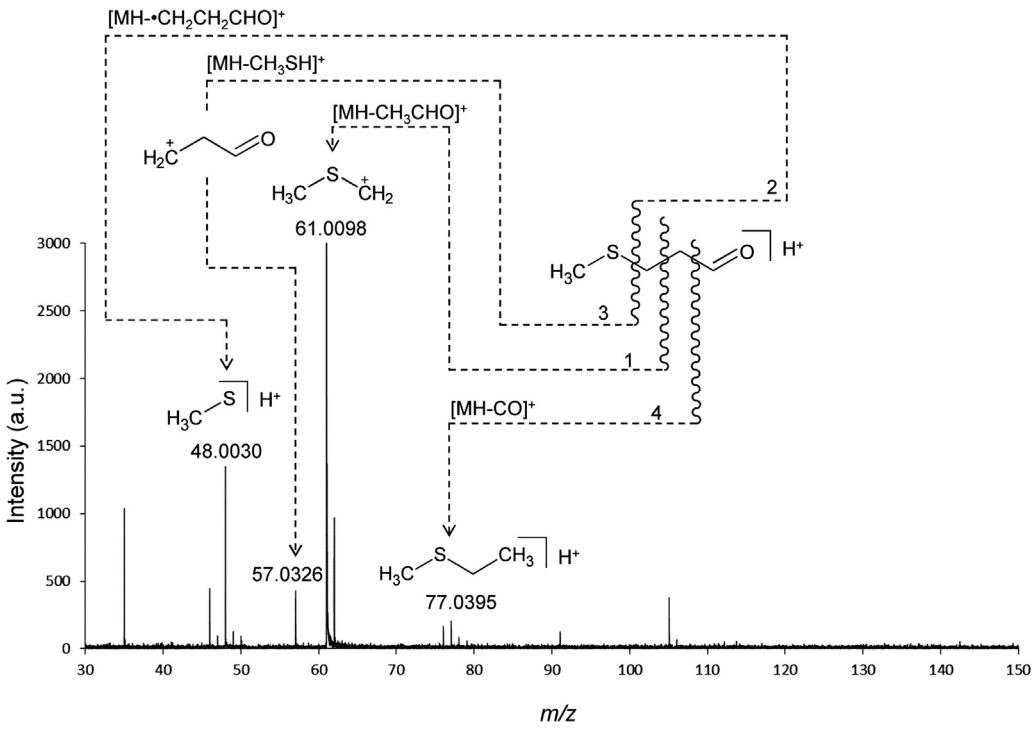

Fig. 7. CAD mass spectrum of the protonated molecule of C_4H_8OS.

2.6. Data analysis

Mass Hunter Acquisition ver. B.06.01.1321 (Agilent), Mass Hunter Qualitative Analysis ver. B.06.00633 (Agilent), Mass Hunter Molecular Structure Correlator (MSC) ver. B.05.00, Automated Mass Spectral Deconvolution and Identification System (AMDIS) ver. 2.70 Build 130.53 (National Institute of Standard and Technology, Gaithersburg, MD, USA), and Aroma Office ^{2}D database ver. 3.01.00 (Gerstel KK, Tokyo, Japan) were used for data analysis. Aroma Office ^{2}D contains the most comprehensive database of odor compounds available (>101,000 entries). This software is a searchable database which contains LRI information for a wide range of odor compounds from many literature references.

3. Results and discussion

3.1. Identification of sulfur compounds

Fig. 2 shows the ^{1}D total ion chromatogram (TIC) (Fig. 2a) and SCD chromatogram (Fig. 2b) of the tobacco smoke extract in full scale *y*-axis. Fig. 3 shows the same chromatograms but with a zoomed *y*-axis (×20 for the ^{1}D TIC and ×200 for the ^{1}D SCD chromatogram). Sample back flush was performed at a retention time (t_R) of 33 min, before the elution of the huge nicotine peak. Although numerous sulfur compounds were detected in the ^{1}D SCD chromatogram (Fig. 3b), these sulfur compounds were completely buried in the ^{1}D TIC (Fig. 3a). It is difficult to extract a clean mass spectrum for each sulfur compound because of significant interference of co-eluting sample matrix. In order to screen for sulfur compounds, mass spectral deconvolution with a NIST AMDIS, and the Mass Hunter using unit resolution and high resolution (±50 ppm mass window) were first performed. Thirty-five sulfur containing candidates were obtained in a list of 865 compounds with the NIST AMDIS search. Eighteen sulfur containing candidates were obtained in a list of 433 compounds with the Mass Hunter search using unit resolution. Twenty-five sulfur containing candidates were obtained in 837 compounds with the Mass Hunter search using high resolution. Fig. 4a shows the deconvoluted mass spectrum at the t_R of 16.81 min in the ^{1}D TIC obtained from the Mass Hunter search using high resolution. This spectrum corresponds to the sulfur peak at the same t_R of 16.81 min in the ^{1}D SCD chromatogram (see Figs. 2b and 3b/sulfur fraction 10). Although this sulfur peak is the most abundant in the ^{1}D SCD chromatogram, the number one candidate from the NIST library search with all deconvolution conditions was always a non-sulfur compound and obviously an incorrect identification, e.g. hexanoic acid methyl ester, 4-methylpentanoic acid methyl ester, or methyl [4-(benzyloxy)-3-chlorophenyl]acetate. Also, there were no sulfur compounds among the other candidate compounds from the library search lists for this sulfur peak. For the second most abundant peak eluting at t_R of 20.16 min in the ^{1}D SCD chromatogram (see Figs. 2b and 3b/sulfur fraction 16), no useful mass spectra were obtained in the ^{1}D TIC from all deconvolution results. Consequently, it can be concluded that ^{1}D GC–TOF-MS in combination with automated deconvolution capabilities, including a high resolution mode is often not enough for identification of trace sulfur compounds in tobacco smoke extract which contains thousands of compounds. Therefore, at least a ^{2}D GC separation needs to be added. A heart-cut from t_R 16.65 min to 16.95 min in the ^{1}D TIC (Fig. 3, sulfur fraction 10) was first performed to transfer the sulfur peak to the second dimension. After heart-cutting, the heart-cut fraction was cryo-focused in the cold trap at −100 °C during the rest of ^{1}D GC run. At the t_R 33 min, ^{1}D GC was back-flushed and cold trap was rapidly heated to start ^{2}D GC. Fig. 5 illustrates both ^{1}D and ^{2}D TIC (Fig. 5a) and SCD chromatogram (Fig. 5b). The sulfur peak eluted at a t_R of 40.36 min in the ^{2}D SCD chromatogram. For this peak, a pure mass spectrum could now be obtained (Fig. 4b), which is remarkably different from the deconvoluted mass spectrum obtained from the ^{1}D separation (Fig. 4a). Also, a ^{1}D LRI of 868 on the DB-1 column and a ^{2}D LRI of 1447 on the DB-Wax column were measured. It is also interesting to observe that in the ^{2}D TIC several other peaks are detected that apparently co-eluted with S-containing compound in the ^{1}D separation, illustrating the complexity of the matrix. Although 8 sulfur candidates (with reverse match factors

Table 1
Identification of sulfur compounds in tobacco smoke by LVI-^{1}D/^{2}D GC–SCD/EI–TOF-MS.

Fraction			No.	Compound	CAS	^{1}D GC–SCD						^{2}D GC–EI–TOF-MS							
No.	Start t_R (min)	End t_R (min)				^{1}D SCD t_R (min)	^{1}D LRI[a] (SCD)	LRI (DB-1)[b] (database)	^{2}D SCD t_R (min)	^{2}D LRI[c] (SCD)	LRI (DB-Wax)[d] (Database)	NIST search				Formula	m/z	Mass error (ppm)	Mass error (mDa)
												Match	Match rank	R. Match	Prob. (%)				
1	9.65	9.95	1-1	Thiocyanic acid, methyl ester	556-64-9	9.83	682	665(2)	37.90	1263	1271(2)	905	1	911	73.4	C_2H_3NS	72.9975	9.9	0.72
2	10.90	11.20	2-1	Thiazole	288-47-1	11.07	715	710(9)	37.67	1246	1248(5)	895	1	895	95.0	C_3H_3NS	84.9982	−1.1	−0.090
			2-2	Dimethyl sulfon	67-71-0	11.07	715	–	38.06	1275	–	746	1	746	29.0	$C_2H_6O_2S$	94.0082	0.86	0.080
3	11.60	11.90	3-1	Dimethyl disulfide	624-92-0	11.78	734	729(11)	35.63	1063	1073(21)	660	1	912	67.9	$C_2H_6S_2$	93.9899	7.4	0.69
4	12.70	12.95	4-1	2-Methyl thiophene	554-14-3	12.83	762	756(5)	35.84	1090	1094(5)	878	1	878	53.5	C_5H_6S	98.0183	1.9	0.18
5	12.95	13.15	5-1	3-Methyl thiophene	616-44-4	13.08	768	–	36.08	1115	1108(5)	888	1	888	59.6	C_5H_6S	98.0183	8.5	0.83
6	13.45	13.70	6-1	2-Methyl thiazole	3581-87-1	13.62	783	781(5)	37.53	1235	1245(5)	857	1	874	95.6	C_4H_5NS	99.0136	1.2	0.12
7	13.85	14.05	7-1	4-Methyl thiazole	693-95-8	13.96	792	789(3)	38.11	1282	1278(2)	887	1	887	82.7	C_4H_5NS	99.0138	0	0
			7-2	Dimethyl sulfoxide	67-68-5	13.96	792	–	41.89	1571	1584(3)	738	1	848	75.3	C_2H_6OS	78.0124	12.8	1.0
10	16.65	16.95	10-1	3,4-Dimethyl thiophene	632-15-5	16.81	868	–	36.87	1184	–	872	1	884	63.2	C_6H_8S	112.0342	3.0	0.33
			10-2	3-Ethyl thiophene	1795-01-3	16.81	868	854(1)	37.11	1204	1210(2)	–	–	–	–	C_6H_8S	112.0341	0.92	0.10
			10-3	Methional	3268-49-3	16.81	868	862(6)	40.35	1447	1456(45)	643	2	643	3.91	C_4H_8OS	104.0296	5.4	−0.56
11	18.45	18.60	11-1	4,5-Dimethyl thiazole	3581-91-7	18.52	913	908(2)	39.31	1368	1372(2)	683	1	858	59.5	C_5H_7NS	113.0291	0.88	0.10
			11-2	Dihydro-3(2H)-thiophenone	1003-04-9	18.52	913	909(5)	41.63	1549	1562(3)	868	1	870	92.2	C_4H_6OS	102.0134	0.22	0.020
13	18.80	18.90	13-1	5-Ethyl thiazole	17626-73-2	18.87	922	–	39.66	1394	–	617	1	788	30.1	C_5H_7NS	113.0284	8.5	0.96
14	19.00	19.20	14-1	2,4-Dimethyl-2-thiazoline	614-40-5	19.10	928	–	39.52	1383	–	579	2	680	11.8	C_5H_9NS	115.0446	4.1	0.47
			14-2	Propane, 1-(methylthio)-	3877-15-4	19.10	928	–	40.25	1439	–	635	1	688	26.0	$C_4H_{10}S$	90.0496	2.3	0.20
16	20.05	20.25	16-1	Dimethyl trisulfide	3658-80-8	20.16	956	949(12)	39.26	1364	1375(27)	828	1	834	94.9	$C_2H_6S_3$	125.9626	0.11	0.010
			16-2	Butanethioic acid, S-methyl ester	2432-51-1	20.16	956	–	41.29	1521	–	761	1	796	91.1	$C_5H_{10}OS$	118.0448	−0.6	−0.070
			16-3	2-Thiophenecarboxaldehyde	98-03-3	20.16	956	964(8)	43.03	1668	–	832	3	833	29.5	C_5H_4OS	111.9968	8.7	0.97
17	20.25	20.45	17-1	Thiophene, 2-(1-methylethyl)-	4095-22-1	20.39	962	–	37.93	1265	–	822	1	833	61.0	$C_7H_{10}S$	126.0494	4.0	0.51
			17-2	Dihydro-2(3H)-thiophenone	1003-10-7	20.39	962	952(1)	42.49	1621	1615(1)	851	1	855	45.3	C_4H_6OS	102.0136	−1.6	−0.17
18	20.55	20.80	18-1	3-Thiophenecarboxaldehyde	498-62-4	20.64	968	973(5)	43.19	1682	1693(2)	829	1	829	24.8	C_5H_4OS	111.9968	8.7	0.97
19	21.00	21.20	19-1	Thiazole, 2,4,5-trimethyl-	13623-11-5	21.12	981	981(1)	39.39	1374	1384(2)	701	1	841	66.0	C_6H_9NS	127.0451	0	0
21	23.08	23.28	21-1	Butanenitrile, 4-(methylthio)-	59121-24-3	23.23	1039	–	44.21	1742	–	640	1	792	60.5	C_5H_9NS	115.0449	0.91	0.10
23	23.80	23.90	23-1	1-(Methylthio)-3-pentanone	66735-69-1	23.86	1057	1052(1)	42.13	1591	–	654	1	694	54.0	$C_6H_{12}OS$	132.0605	−1.1	−0.15
			23-2	3-Methyl-2-thiophenecarboxaldehyde	5834-16-2	23.86	1057	1068(4)	43.54	1708	–	842	2	907	71.7	C_6H_6OS	126.0133	1.9	0.23
24	24.70	24.82	24-1	2-Acetyl-4-methylthiazole	7533-07-5	24.76	1082	1085(2)	43.23	1686	-	–	–	–	–	C_6H_7NOS	141.0245	−1.5	−0.22
26	25.03	25.24	26-1	5-Methyl-2-thiophenecarboxaldehyde	13679-70-4	25.16	1093	1090(5)	44.46	1756	1754(4)	845	1	912	80.5	C_6H_6OS	126.0138	0.80	0.10
28	26.47	26.63	28-1	1,4-Dithiacyclohept-2-ene	70063-50-2	26.56	1134	–	42.81	1650	–	556	1	718	12.1	$C_5H_8S_2$	132.0068	−4.7	−0.62
3	11.60	11.90	3-2	S1	–	11.78	734	–	37.78	1254	–	–	–	–	–	C_3H_6OS	90.0125	9.6	0.86
6	13.45	13.70	6-2	S2	–	13.62	783	–	39.45	1378	–	–	–	–	–	C_3H_4OS	87.9969	9.6	0.85
8	14.65	14.80	8-1	S3	–	14.73	812	–	38.72	1324	–	–	–	–	–	C_4H_8OS	104.0287	2.9	0.31
11	18.45	18.60	11-3	S4	–	18.52	913	–	39.69	1397	–	–	–	–	–	$C_5H_{10}OS$	118.0450	−3.1	−0.36
14	19.00	19.20	14-3	S5	–	19.10	928	–	43.43	1702	–	–	–	–	–	C_4H_7NS	101.0288	5.3	0.54
17	20.25	20.45	17-3	S6	–	20.39	962	–	38.02	1272	–	–	–	–	–	$C_7H_{10}S$	126.0494	3.1	0.39
			17-4	S7	–	20.39	962	–	44.95	1781	–	–	–	–	–	C_3H_7NOS	105.0242	0.75	0.080
22	23.45	23.60	22-1	S8	–	23.54	1048	–	47.96	1905	–	–	–	–	–	C_4H_6OS	102.0127	7.0	0.71
24	24.70	24.82	24-2	S9	–	24.76	1082	–	45.82	1820	–	–	–	–	–	C_5H_4OS	111.9977	0.75	0.080
26	25.03	25.24	26-2	S10	–	25.16	1093	–	47.71	1895	–	–	–	–	–	C_5H_7NS	113.0293	0.64	0.070
27	26.00	26.20	27-1	S11	–	26.15	1122	–	48.02	1908	–	–	–	–	–	C_5H_9NS	115.0439	9.7	1.12

[a] Calculated ^{1}D LRI (DB-1) obtained from ^{1}D GC–SCD.
[b] Average ^{1}D LRI (DB-1) obtained from Aroma Office ^{2}D database.
[c] Calculated ^{2}D LRI (DB-Wax) obtained from ^{2}D GC–SCD.
[d] Average ^{2}D LRI (DB-Wax) obtained from Aroma Office ^{2}D database.

Table 2
Identification of sulfur compounds in tobacco smoke by LVI-^{1}D/^{2}D GC–SCD/PCI–TOF-MS.

No.	Compound	^{2}D GC–PCI–TOF-MS											
		MH^+				$[M+C_2H_5]^+$				$[M+C_3H_5]^+$			
		Formula	*m/z*	Mass error (ppm)	Mass error (mDa)	Formula	*m/z*	Mass error (ppm)	Mass error (mDa)	Formula	*m/z*	Mass error (ppm)	Mass error (mDa)
1-1	Thiocyanic acid, methyl ester	C_2H_4NS	74.0061	−2.7	−0.20	C_4H_8NS	102.0360	12	1.2	C_5H_8NS	114.0360	3.2	0.36
2-1	Thiazole	C_3H_4NS	86.0059	−1.0	−0.080	C_5H_8NS	114.0380	−5.0	−0.57	C_6H_8NS	126.0380	−6.6	−0.83
2-2	Dimethyl sulfon	$C_2H_7O_2S$	95.0168	−6.7	−0.63	$C_4H_{11}O_2S$	123.0480	−4.4	−0.55	$C_5H_{11}O_2S$	135.0479	−3.1	−0.42
3-1	Dimethyl disulfide	$C_2H_7S_2$	94.9988	−7.4	−0.70	–	–	–	–	–	–	–	–
4-1	2-Methyl thiophene	C_5H_7S	99.0266	−3.0	−0.29	$C_7H_{11}S$	127.0575	1.7	0.22	$C_8H_{11}S$	139.0577	−2.8	−0.38
5-1	3-Methyl thiophene	C_5H_7S	99.0270	−7.4	−0.74	$C_7H_{11}S$	127.0584	−5.8	−0.74	$C_8H_{11}S$	139.0587	−7.6	−1.1
6-1	2-Methyl thiazole	C_4H_6NS	100.0219	−3.7	−0.37	$C_6H_{10}NS$	128.0530	−0.47	−0.06	$C_7H_{10}NS$	140.0532	−3.2	−0.44
7-1	4-Methyl thiazole	C_4H_6NS	100.0220	−4.8	−0.48	$C_6H_{10}NS$	128.0531	−2.6	−0.33	$C_7H_{10}NS$	140.0533	−4.9	−0.68
7-2	Dimethyl sulfoxide	–	–	–	–	–	–	–	–	–	–	–	–
10-1	3,4-Dimethyl thiophene	C_6H_9S	113.0426	−4.9	−0.55	$C_8H_{13}S$	141.0736	−3.9	−0.54	–	–	–	–
10-2	3-Ethyl thiophene	C_6H_9S	113.0427	−5.1	−0.57	$C_8H_{13}S$	141.0742	−7.4	−1.0	$C_9H_{13}S$	153.0729	1.9	0.30
10-3	Methional	C_4H_9OS	105.0375	−6.4	−0.67	$C_6H_{13}OS$	133.0690	−6.8	−0.90	$C_7H_{13}OS$	145.0691	−6.0	−0.87
11-1	4,5-Dimethyl thiazole	C_5H_8NS	114.0378	−6.5	−0.74	$C_7H_{12}NS$	142.0699	−13	−1.9	$C_8H_{12}NS$	154.0677	1.7	0.27
11-2	Dihydro-3(2H)-thiophenone	C_4H_7OS	103.0217	−4.3	−0.45	$C_6H_{11}OS$	131.0534	−7.1	−0.93	$C_7H_{11}OS$	143.0530	−5.0	−0.71
13-1	5-Ethyl thiazole	C_5H_8NS	114.0370	−0.17	−0.020	$C_7H_{12}NS$	142.0688	−1.8	−0.26	$C_8H_{12}NS$	154.0665	12.4	1.9
14-1	2,4-Dimethyl-2-thiazoline	$C_5H_{10}NS$	116.0531	−1.7	−0.20	$C_7H_{14}NS$	144.0846	−2.6	−0.38	C_8H_7NS	156.0851	−6.1	−0.94
14-2	Propane, 1-(methylthio)-	$C_5H_{11}OS$	119.0530	−0.86	−0.10	$C_7H_{15}OS$	147.0836	1.5	0.22	$C_8H_{15}OS$	159.0861	−14.0	−2.2
16-1	Dimethyl trisulfide	$C_2H_7S_3$	126.9713	−2.9	−0.36	–	–	–	–	–	–	–	–
16-2	Butanethioic acid, S-methyl ester	$C_5H_{11}OS$	119.0529	−3.2	−0.37	$C_7H_{15}OS$	147.0842	−4.6	−0.67	$C_8H_{15}OS$	159.0842	−3.8	−0.61
16-3	2-Thiophenecarboxaldehyde	C_5H_5OS	113.0059	−2.9	−0.32	C_7H_9OS	141.0364	3.0	0.43	C_8H_9OS	153.0369	−0.46	−0.07
17-1	Thiophene, 2-(1-methylethyl)-	$C_7H_{11}S$	127.0580	−2.5	−0.32	$C_9H_{15}S$	155.0878	−0.29	0	$C_{10}H_{15}S$	167.0902	−7.9	−1.3
17-2	Dihydro-2(3H)-thiophenone	C_4H_7OS	103.0221	−7.9	−0.81	$C_6H_{11}OS$	131.0526	−2.1	−0.28	$C_7H_{11}OS$	143.0530	−3.2	−0.46
18-1	3-Thiophenecarboxaldehyde	C_5H_5OS	113.0063	−5.8	−0.66	C_7H_9OS	141.0384	−11	−1.6	C_8H_9OS	153.0393	−8.5	−1.2
19-1	Thiazole, 2,4,5-trimethyl-	$C_6H_{10}NS$	128.0532	−1.6	−0.21	$C_8H_{14}NS$	156.0849	−4.2	−0.66	$C_9H_{14}NS$	168.0838	−2.4	−0.40
21-1	Butanenitrile, 4-(methylthio)-	$C_5H_{10}NS$	116.0533	−4.9	−0.57	$C_7H_{14}NS$	144.0852	−5.1	−0.73	$C_8H_{14}NS$	156.0849	−1.9	−0.30
23-1	1-(Methylthio)-3-pentanone	$C_6H_{13}OS$	133.0688	−4.4	−0.59	$C_8H_{17}OS$	161.1013	−4.3	−0.69	$C_9H_{17}OS$	173.0984	7.8	1.4
23-2	3-Methyl-2-thiophenecarboxaldehyde	C_6H_7OS	127.0219	−5.3	−0.67	$C_8H_{11}OS$	155.0511	7.3	1.1	$C_9H_{11}OS$	167.0543	−7.1	−1.2
24-1	2-Acetyl-4-methylthiazole	C_6H_8NOS	142.0332	−8.3	−1.2	$C_8H_{12}NOS$	170.0644	−3.1	−0.53	$C_9H_{12}NOS$	182.0645	−9.5	−1.7
26-1	5-Methyl-2-thiophenecarboxaldehyde	C_6H_7OS	127.0220	−5.0	−0.64	$C_8H_{11}OS$	155.0525	−2.2	−0.34	–	–	–	–
28-1	1,4-Dithiacyclohept-2-ene	$C_5H_9S_2$	133.0149	−6.8	−0.90	–	–	–	–	–	–	–	–
3-2	S1	C_3H_7OS	91.0219	−7.1	−0.65	$C_5H_{11}OS$	119.0530	−11	−1.4	$C_6H_{11}OS$	131.0530	−7.7	−1.0
6-2	S2	C_3H_5OS	89.0062	−8.1	−0.72	–	–	–	–	–	–	–	–
8-1	S3	C_4H_9OS	105.0377	−8.7	−0.92	$C_6H_{13}OS$	133.0688	−2.4	−0.32	$C_7H_{13}OS$	145.0693	−10	−1.5
11-3	S4	$C_5H_{11}OS$	119.0529	−3.1	−0.37	$C_7H_{15}OS$	147.0840	−1.9	−0.28	$C_8H_{15}OS$	159.0851	−14	−2.3
14-3	S5	C_4H_8NS	102.0380	−7.9	−0.81	$C_6H_{12}NS$	130.0687	−1.7	−0.22	$C_7H_{12}NS$	142.0689	−2.8	−0.40
17-3	S6	$C_7H_{11}S$	127.0584	−9.0	−1.2	$C_9H_{15}S$	155.0881	4.9	0.76	$C_{10}H_{15}S$	167.0916	−15	−2.6
17-4	S7	C_3H_8NOS	106.0311	9.0	0.96	–	–	–	–	–	–	–	–
22-1	S8	C_4H_7OS	103.0220	−8.0	−0.83	–	–	–	–	$C_7H_{11}OS$	143.0528	−1.8	−0.26
24-2	S9	C_5H_5OS	113.0064	−8.0	−0.91	–	–	–	–	C_8H_9OS	153.0386	−6.1	−0.93
26-2	S10	C_5H_8NS	114.0379	−4.1	−0.47	$C_7H_{12}NS$	142.0700	−5.7	−0.81	–	–	–	–
27-1	S11	$C_5H_{10}NS$	116.0540	−6.9	−0.80	$C_7H_{14}NS$	144.0843	−1.0	−0.15	–	–	–	–

Table 3
Identification of sulfur compounds in tobacco smoke by LVI-^{1}D/^{2}D GC–SCD/PCI–Q-TOF-MS.

No.	Compound	MH^+				MS/MS fragment				
		Formula	m/z	Mass error (ppm)	Mass error (mDa)	Collision energy (V)	Fragment ion	m/z	Mass error (ppm)	Mass error (mDa)
1-1	Thiocyanic acid, methyl ester	C_2H_4NS	74.0058	−2.8	−0.21	20	$[MH-CHN]^+$	46.9957	−15	−0.70
							$[MH-{}^{\bullet}CH_3]^+$	58.9833	−15	−0.88
2-1	Thiazole	C_3H_4NS	86.0067	0	0	30	$[MH-CS]^+$	42.0347	−21	−0.87
							$[MH-CHN]^+$	58.9957	−12	−0.70
2-2	Dimethyl sulfon	$C_2H_7O_2S$	95.0173	−13	−1.3	10	$[MH-CH_4]^+$	78.9862	−17	−1.4
							$[MH-CH_3OS]^+$	62.9906	−11	−0.69
4-1	2-Methyl thiophene	C_5H_7S	99.0266	3.0	0.30	20	$[MH-SH_2]^+$	65.0392	9.2	0.60
							$[MH-{}^{\bullet}CH_3]^+$	84.0039	3.6	0.30
5-1	3-Methyl thiophene	C_5H_7S	99.0246	−7.2	−0.71	20	$[MH-SH_2]^+$	65.0377	14	0.88
							$[MH-{}^{\bullet}CH_3]^+$	84.0000	34	2.8
6-1	2-Methyl thiazole	C_4H_6NS	100.0209	−3.5	−0.35	20	$[MH-{}^{\bullet}CCSH]^+$	42.0331	17	0.73
							$[MH-{}^{\bullet}CN(CH_3)]^+$	58.9944	10	0.60
10-1	3,4-Dimethyl thiophene	C_6H_9S	113.0407	−6.3	−0.71	20	$[MH-SH_2]^+$	79.0534	11	0.83
							$[MH-CH_4]^+$	97.0087	20	1.9
10-2	3-Ethyl thiophene	C_6H_9S	113.0961	−6.3	−0.71	20	$[MH-CHCSH]^+$	55.0532	19	1.0
							$[MH-CH_2CH_2]^+$	85.0088	22	1.8
10-3	Methional	C_4H_9OS	105.0696	−6.1	−0.64	10	$[MH-{}^{\bullet}CH_2CH_2CHO]^+$	48.0030	−3.7	−0.18
							$[MH-CH_3SH]^+$	57.0326	16	0.89
							$[MH-CH_3CHO]^+$	61.0098	14	0.85
							$[MH-CO]^+$	77.0395	32	2.4
11-1	4,5-Dimethyl thiazole	C_5H_8NS	114.0383	−5.4	−0.62	20	$[MH-CNSH]^+$	55.0543	−1.3	−0.07
							$[MH-CHN]^+$	87.0260	3.4	0.30
11-2	Dihydro-3(2H)-thiophenone	C_4H_7OS	103.0214	−4.8	−0.49	10	$[MH-H_2O]^+$	75.0279	−21	−1.6
							$[MH-CO_2]^+$	85.0099	8.8	0.75
14-1	2,4-Dimethyl-2-thiazoline	$C_5H_{10}NS$	116.0525	−2.2	−0.26	10	$[MH-CH_3CHNH]^+$	73.0098	12	0.85
							$[MH-NH_3]^+$	99.0263	0	0
							$[MH-CH_4]^+$	100.0213	−1.5	−0.15
16-2	Butanethioic acid, S-methyl ester	$C_5H_{11}OS$	119.0844	−3.3	−0.39	10	$[MH-CH_2CH_2-H_2O]^+$	61.0104	4.1	0.25
							$[MH-CH_3SH]^+$	71.0486	7.6	0.54
17-1	Thiophene, 2-(1-methylethyl)-	$C_7H_{11}S$	127.0593	−4.8	−0.61	10	$[MH-CH_2CHSH]^+$	67.0527	23	1.5
							$[MH-C_2H_4]^+$	99.0265	−2.0	−0.20
17-2	Dihydro-2(3H)-thiophenone	C_4H_7OS	103.0198	−8.7	−0.90	20	$[MH-CH_2CHCH_3]^+$	60.9734	14	0.86
							$[MH-H_2O]^+$	85.0094	15	1.2
18-1	3-Thiophenecarboxaldehyde	C_5H_5OS	113.0032	−6.6	−0.75	10	$[MH-CO]^+$	85.0094	15	1.3
19-1	Thiazole, 2,4,5-trimethyl-	$C_6H_{10}NS$	128.0517	−2.9	−0.37	20	$[MH-CH_3C(SH)NH]^+$	53.0381	9.0	0.48
							$[MH-CH_3CN]^+$	87.0252	13	1.1
21-1	Butanenitrile, 4-(methylthio)-	$C_5H_{10}NS$	116.0525	−3.9	−0.45	10	$[MH-CH_3SH]^+$	68.0487	11	0.78
							$[MH-CH_3CN]^+$	75.0268	−6.7	−0.50
24-1	2-Acetyl-4-methylthiazole	C_6H_8NOS	142.0302	−7.7	−1.1	20	$[MH-CH_2CO]^+$	100.0205	11	1.1
							$[MH-NCC(O)CH_3]^+$	73.0092	21	1.5
3-2	S1	C_3H_7OS	91.0209	−7.6	−0.69	10	$C_2H_5S^+$	61.0111	−5.8	−0.35
							$C_2H_4OS^+$	75.9969	11	0.84
8-1	S3	C_4H_9OS	105.0370	−8.0	−0.84	10	CHS^+	44.9789	−9.9	−0.45
							$C_3H_5O^+$	57.0332	5.1	0.29
							$C_4H_7S^+$	87.0253	12	1.0

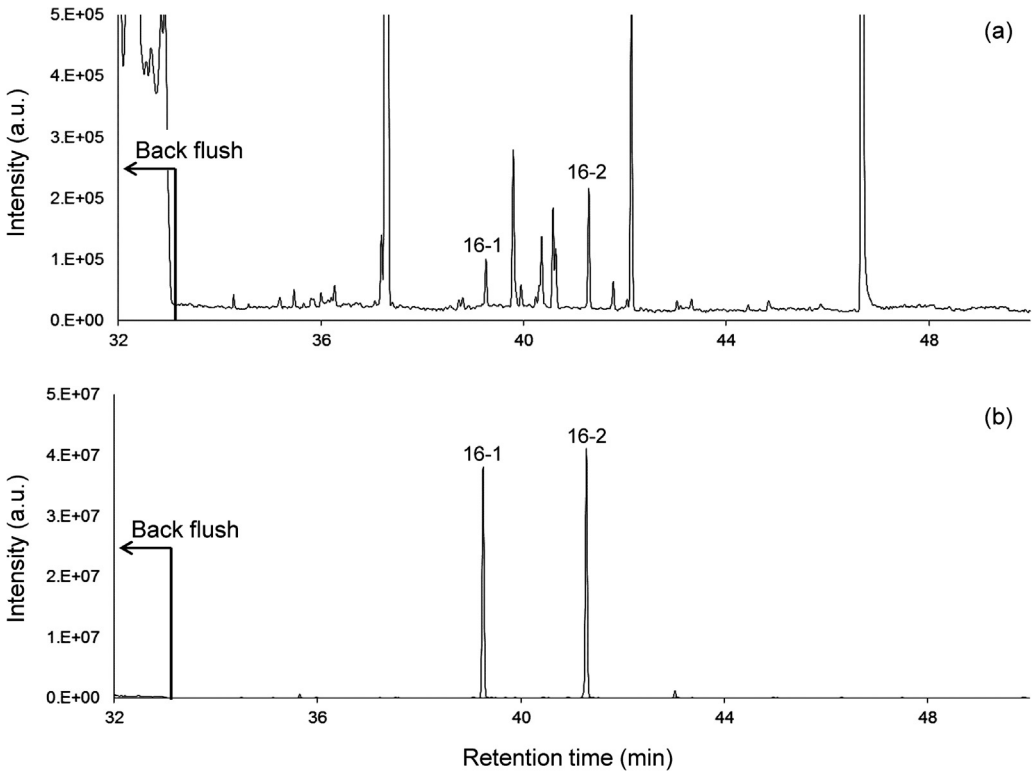

Fig. 8. ^{2}D total ion chromatogram (TIC) and SCD chromatogram obtained from sulfur fraction 16. (a) ^{2}D TIC; (b) ^{2}D SCD chromatogram. 16-1: dimethyl trisulfide, 16-2: butanethioic acid, S-methyl ester.

of more than 600) were obtained from the NIST library search of the spectrum in Fig. 4b, the cross search with two different LRIs (^{1}D LRI: 868, ^{2}D LRI: 1447) could narrow the candidates down to 3 compounds without taking elemental information into account. Using an additional filtering with the elemental information obtained from the SCD (sulfur must be present), only methional remained as possible candidate. Although methional was number 2 candidate in the NIST library search results and the ion of *m*/*z* 104.0296 in the EI mass spectrum corresponds to methional formula of C_4H_8OS with the mass error of −0.56 mDa (5.4 ppm), the match factors and the probability in the library search results showed low values, e.g. 643 for both forward and reverse match, and only 39.1% for the probability. Therefore, PCI was also performed for confirmation of molecular mass of this sulfur compound. Fig. 6 shows the PCI mass spectrum of the target peak. Although the relative abundance of *m*/*z* 105.0375 was 5.86% of the base fragment peak at *m*/*z* 57.0345, this ion corresponds to the protonated molecule (MH^+) of methional with the mass error of −0.67 mDa (−6.4 ppm). Also, *m*/*z* 133.0690 (4.56%) and *m*/*z* 145.0691 (4.93%) corresponding to the $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ ions (which are known as adduct ions from PCI with methane) with respective mass errors of −0.90 mDa (−6.8 ppm) and −0.87 mDa (−6.0 ppm), confirm the molecular ion and molecular formula of this sulfur compound as C_4H_8OS. Finally, structure elucidation was performed with collision activated dissociation of the protonated molecule ($MH^+ \pm 0.5$ mDa). It is known that fragmentation of the protonated molecule generally occurs in the protonated part (function) of a compound [24,25], resulting in simple bond cleavage processes compared to EI. Therefore CAD of the protonated molecule in accurate mass measurement can dramatically help structure elucidation. Fig. 7 demonstrates the CAD mass spectrum of the protonated molecule using the collision energy of 10 V. Also, methional structure and bond cleavages are shown in Fig. 7. Considerable information concerning the methional structure ($CH_3SCH_2CH_2CHO$) can be derived from the fragment ions in the CAD mass spectrum. The most abundant ion at *m*/*z* 61.0098 corresponds to bond cleavage 1 and $[MH-CH_3CHO]^+$ with the mass error of 0.79 mDa (14 ppm) (neutral loss of CH_3CHO). In addition, the ions at *m*/*z* 48.0030, *m*/*z* 57.0326 and *m*/*z* 77.0395 correspond to bond cleavage 2 and $[MH-{}^{\bullet}CH_2CH_2CHO]^+$ (mass error: −0.18 mDa; −3.7 ppm), bond cleavage 3 and $[MH-CH_3SH]^+$ (mass error: 0.95 mDa; 16 ppm), and bond cleavage 4 (which might be accompanied with rearrangement reaction of hydrogen) and $[MH-CO]^+$ (mass error: 2.4 mDa; 32 ppm), respectively. These ions clearly demonstrate that the structures such as CH_3SCH_2—, CH_3S—, —CH_2CH_2CHO, and $CH_3SCH_2CH_2$—, are involved. Thus, this sulfur compound could be identified with high probability as methional based on the combination of LRI cross search, molecular mass determination, formula calculation, and structure elucidation even with the low NIST library match and probability.

In order to identify additional sulfur compounds in the tobacco smoke extract, the other twenty-seven sulfur fractions selected from the ^{1}D GC–SCD chromatogram (the sulfur fractions 1–9, 11–28 in Fig. 3) were sequentially transferred to the second dimensional separation and then measured with ^{2}D GC–SCD/EI–TOF-MS, ^{2}D GC–SCD/PCI–TOF-MS and ^{2}D GC–SCD/PCI–Q-TOF-MS (MS/MS). In some of the fractions, more than one sulfur compound could be detected in the SCD trace (see below, peaks are labeled *X*–*Y* with *X* = heart-cut fraction and *Y* = elution sequence in the ^{2}D within this fraction).

Identification was performed with the NIST library search, ^{1}D/^{2}D LRI, molecular mass determination, formula calculation, and structure elucidation. Although the CAD mass spectra were not obtained for several sulfur compounds because of a lack of sensitivity in the MS/MS measurements, the combined approach with ^{1}D/^{2}D LRI, formula calculation, and the CAD mass spectrum of the protonated molecule, provided highly probable candidates for some sulfur compounds (e.g. 3-ethyl thiophene, 2,4-dimethyl-2-thiazoline, and 2-acetyl-4-methyl-thiazole), which showed no or low NIST library

Table 4
Concentration of the sulfur compounds in tobacco smoke extract.

No.	Compound	SCD		Amount on Q-TOF-MS (pg)	Concentration (ng mg TPM^{-1})
		Amount (pg)	RSD (%), $n = 3$		
1-1	Thiocyanic acid, methyl ester	580	6.6	290	33
2-1	Thiazole	260	12	130	15
2-2	Dimethyl sulfon	540	12	270	30
3-1	**Dimethyl disulfide**	140	10	70	7.8
4-1	2-Methyl thiophene	360	4.7	180	20
5-1	3-Methyl thiophene	400	6.8	200	22
6-1	2-Methyl thiazole	330	8.7	170	19
7-1	**4-Methyl thiazole**	810	7.7	410	46
7-2	**Dimethyl sulfoxide**	290	9.5	145	16
10-1	3,4-Dimethyl thiophene	100	8.4	52	5.8
10-2	**3-Ethyl thiophene**	52	5.9	26	2.9
10-3	**Methional**	13,000	3.5	6600	740
11-1	**4,5-Dimethyl thiazole**	24	3.6	12	1.3
11-2	**Dihydro-3(2H)-thiophenone**	190	7.5	94	11
13-1	5-Ethyl thiazole	33	6.6	17	1.9
14-1	2,4-Dimethyl-2-thiazoline	95	11	48	5.3
14-2	Propane, 1-(methylthio)-	390	7.6	190	21
16-1	**Dimethyl trisulfide**	890	4.1	444	50
16-2	Butanethioic acid, S-methyl ester	3000	3.4	1500	170
16-3	**2-Thiophenecarboxaldehyde**	73	7.6	37	4.1
17-1	Thiophene, 2-(1-methylethyl)-	19	6.6	93	1.0
17-2	**Dihydro-2(3H)-thiophenone**	630	11	310	35
18-1	**3-Thiophenecarboxaldehyde**	240	4.4	120	13
19-1	**Thiazole, 2,4,5-trimethyl-**	46	7.7	23	2.6
21-1	Butanenitrile, 4-(methylthio)-	120	2.7	61	6.9
23-1	1-(Methylthio)-3-pentanone	110	9.3	56	6.2
23-2	**3-Methyl-2-thiophenecarboxaldehyde**	63	6.6	31	3.5
24-1	**2-Acetyl-4-methylthiazole**	230	6.1	110	12
26-1	**5-Methyl-2-thiophenecarboxaldehyde**	19	1.2	9.3	1.1
28-1	1,4-Dithiacyclohept-2-ene	100	12	52	5.8
3-2	S1	290	7.1	150	17
6-2	S2	230	3.2	120	13
8-1	S3	330	2.4	160	18
11-3	S4	90	5.9	45	5.1
14-3	S5	49	9.8	25	2.8
17-3	S6	34	11	17	1.9
17-4	S7	170	7.4	85	10
22-1	S8	260	6.3	130	15
24-2	S9	18	6.9	9.2	1.0
26-2	S10	57	2.4	28	3.2
27-1	S11	110	8.7	53	6.0

Compound in bold was identified with authentic standard.

match quality. Finally the identity of fifteen sulfur compounds (3-1, 7-1, 7-2, 10-2, 10-3, 11-1, 11-2, 16-1, 16-3, 17-2, 18-1, 19-1, 23-2, 24-1, and 26-1) could be confirmed with authentic compounds, and another 15 sulfur compounds were tentatively identified with high probability. Of these 30 sulfur compounds, thirteen (2-2, 7-2, 10-1, 10-2, 11-2, 14-1, 16-2, 16-3, 17-1, 18-1, 21-1, 24-1, and 28-1) have not previously been reported in tobacco smoke [19]. Tables 1–3 summarize the 30 identified sulfur compounds with the parameters used for their identification. Also, Tables 1 and 2 list 11 additional unknown sulfur compounds (S1–S11) with their best candidate molecular formulas. The CAD mass spectral information of the unknown sulfur compounds (S1 and S3) is also included in Table 3. The identified sulfur compounds were also compared to the screening results obtained from the three deconvolution conditions (NIST AMDIS, Mass Hunter using unit resolution, and Mass Hunter using high resolution). Although there are no matches in the NIST AMDIS search and the Mass Hunter search using unit resolution, two sulfur compounds such as methyl thiazole isomers (6-1, 7-1) were found (as the number 1 candidate) in the Mass Hunter search using high resolution.

Although it is necessary to perform three heart-cuts on each sulfur fraction, to allow three different MS detection modes, and this obviously takes much longer than conventional ^{1}D GC–EI/TOF-MS, the proposed approach can greatly improve the identification capability both qualitatively and quantitatively.

3.2. Quantification of sulfur compounds

Finally, quantification of the identified sulfur compounds and the unknown sulfur compounds (S1–S11), was performed using a linear and equimolar response of the ^{2}D GC–SCD to sulfur compounds [26]. 1-(2-Thienyl)-1-butanone, which was not present in the sample, was chosen as a standard and spiked into the sample between 1 and 200 ng mL^{-1} (6.5 and 1300 pmol mL^{-1}). The recovery of 1-(2-thienyl)-1-butanone in the tobacco smoke extract spiked at 100 ng mL^{-1} was calculated at 95% (RSD: 4.5%, $n = 6$) by comparing peak areas with those of a calibration curve prepared by automated direct liquid injection of a standard solution injected into a micro-vial in a thermal desorption liner through a septum head of the TDU. Fig. 8 shows a separation of heart-cut fraction 16, showing the presence of dimethyl trisulfide (16-1) and butanethioic acid, S-methyl ester (16-2) in the ^{2}D TIC (Fig. 8a) and the ^{2}D SCD chromatogram (Fig. 8b). These sulfur compounds co-eluted in the ^{1}D GC–SCD chromatogram as sulfur fraction 16, which was the second abundant sulfur peak (see Figs. 2b and 3b). Although the responses of these sulfur compounds in the ^{2}D TIC are different, those in the ^{2}D SCD chromatogram shows similar values

since the SCD response is proportional to the molar concentration of sulfur. Therefore, the determined value of dimethyl trisulfide (50 ng mg TPM^{-1}) is less than 30% of that of butanethioic acid, S-methyl ester (170 ng mg TPM^{-1}) because of three times higher number of sulfur atoms. Table 4 summarizes the determined values. Concentrations of the target sulfur compounds were in the range of 10–740 ng mg TPM^{-1} (RSD: 1.2–12%, n = 3).

4. Conclusion

The combination of LVI, ^{1}D/^{2}D GC, SCD, and Q-TOF-MS with EI and PCI, offers a very effective synergy for identifying trace sulfur compounds in a highly complex sample such as tobacco smoke. The method allows the combined approach using ^{1}D/^{2}D LRI, molecular mass determination, formula calculation, and structure elucidation as well as the NIST library search. Thirty sulfur compounds were tentatively identified with high probability in the flue-cured tobacco smoke extract by sequential heart-cuts of the 28 sulfur fractions using three MS detection modes (SCD/EI–TOF-MS, SCD/PCI–TOF-MS, and SCD/PCI–Q-TOF-MS), while maintaining greater system cleanliness with LVI using the TDU inlet and column back-flushing. Also, the best candidate molecular formulas could be obtained for 11 unknown sulfur compounds. Forty-one sulfur compounds could thus be determined at ng per mg TPM^{-1} levels.

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