Chapter 4

Emerging Analytical Techniques for the Assessment of Aroma Relevant Sulfur Compounds in Coffee

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Aroma extraction by solid phase micro extraction (SPME) was combined with comprehensive two-dimensional gas chromatography (2D-GC) using two column configurations and detection by time-of-flight mass spectrometry (TOF-MS) for the quantitative assessment of trace sulfur compounds in coffee. Based on the optimization of 2D separation and TOF-MS detection parameters, quantification assays were established for 3-methyl-2-butene-1-thiol (MBT) and 3-(methylthio)propionaldehyde (methional). The quantification of these compounds in roast and ground (R&G) coffee as well as in filter coffee brew resulted in satisfactory detection limits and repeatability of the method. Furthermore, quantitative results highlighted the importance of MBT in the aroma above a freshly ground coffee. Data indicated that it is almost quantitatively lost during preparation of filter coffee beverage due to evaporation and degradation. In contrast, methional was found quite abundant in R&G coffee and is highly recovered in the final beverage. In addition, identification was compared to standard methods comprising aroma isolation by high vacuum transfer and solvent aroma extraction. The evaluation of the solvent extract by 2D-GC-TOFMS as well as GC-olfactometry resulted in fewer sulfur compounds identified, thus indicating high degradation rates of reactive sulfur compounds during

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sample work-up. The study revealed that 2D-GC-TOF-MS is a powerful tool to identify and quantify trace sulfur compounds in coffee. Combined with SPME aroma isolation, it represents a rapid, sensitive, and ecological alternative to conventional methods.

Introduction

The characterization of coffee aroma is a challenging task as many of the important odorants are just present in trace amounts and/or are reactive and unstable. This is valid in particular for sulfur compounds, such as thiols, that are known to be susceptible to oxidative degradation reactions (*1*, *2*). The trapping of thiols with *p*-hydroxymercuri benzoate, adsorption of the resulting product on an anion exchange column, and subsequent release with cysteine has been proven as an efficient method for the enrichment of trace amounts of thiols in food (*3*). Several sulfur compounds could be detected for the first time in coffee by combining this methodology with GC-olfactometry (GC-O) and GC-MS analysis (*4*). As examples, quantitative analysis in various coffee brews revealed 3-mercapto-2-pentanone, 2-mercapto-3-pentanone and 4-methoxy-2-methylbutan-2-thiol contents that are significantly above their odor thresholds in water (*4*).

The approaches for the quantification of trace aroma compounds described above generally involve an excess amount of sample and require labor-intense and time-consuming extraction and pre-separation steps, often in conjunction with the use of large quantities of solvents. Furthermore, co-elution phenomena in GC separation and insufficient sensitivity in MS detection limit their assessment in "routine" analysis and, thus, represent further constraints for a closer evaluation of the role of sulfur compounds in coffee aroma.

Besides the aspect of exploring rapid and sensitive methodologies, the development of environment-friendly procedures has become increasingly important. In recent studies, our group demonstrated the potential of SPME aroma isolation combined with the emerging GC×GC-TOF-MS technique as a relatively rapid and less labor-intense tool to analyze key aroma compounds in coffee beverages. As an example, trace sulfur compounds such as 2-acethylthiazole, 3-(methylthio)propionaldehyde, and 2-methyl-3-furanthiol were detected and positively identified, which could not be achieved using linear GC-MS or heart-cut GC/GC-MS (*5*).

The aim of the present work was to develop a quantification method based on comprehensive GC×GC-TOF-MS using the isotope dilution assay (IDA) method with emphasis on trace sulfur compounds like 3-(methylthio)-propionaldehyde (methional) and 3-methyl-2-butene-1-thiol (MBT).

Experimental

Sample Preparation

Coffee Brews

60 g of roast and ground (R&G) coffee (Arabica, Colombia; 85%; Robusta, Indonesia, 15%) were brewed over a filter paper with 1000 mL tap water. After cooling down to room temperature, 7 mL of the brew was pipetted into a 20 mL headspace vial.

Quantification by Isotope Dilution Assay (IDA)

R&G Coffee

R&G coffee was suspended in hot water to get a slurry and after cooling spiked with defined quantities of isotope labeled analogues of the analytes.

Filter Coffee Brews

The filter coffee was prepared as described above. After cooling down of the brew to room temperature an aliquot of the brew was spiked with defined quantities of labeled isotopes of the analytes.

Isolation of Aroma Compounds by High Vacuum Transfer (HVT) and Solvent Extraction

200 mL of filter coffee were distilled under vacuum at 40 °C using the so-called "Solvent Assisted Flavour Evaporation" apparature (SAFE), in order to separate the volatiles from the non-volatile material. After phase separation, and extraction with solvent, the organic phase was washed with a saturated NaCl-solution and dried over Na_2SO_4. The extract was concentrated on a Vigreux-column (60 cm) at 40 °C to about 5 – 10 mL and then further to 1 mL by means of microdistillation.

Isolation of Aroma Compounds by Solid Phase Micro Extraction (SPME)

The prepared coffee solutions were equilibrated for 60 min at 20 °C in the sealed vials and the aroma compounds were then extracted from the headspace during 10 min at 40 °C using SPME (2 cm fiber coated with PDMS/DVB/Carboxen; Supelco, Bellefonte, PA, USA). Aroma compounds were thermally desorbed into the split-splitless injector (in splitless-mode) heated at 240°C.

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2D-Gas Chromatography Time-of-Flight Mass Spectrometry (GC×GC-TOF-MS)

The system consisted of a Agilent 7890A GC (Agilent, Palo Alto, CA, USA) equipped with a split-splitless injector and a CTC-PAL autosampler (CTC, Brechbühler, Switzerland). Column setup A: 1st column DB-FFAP (30 m × 0.25 mm; film thickness, 0.25 µm; J&W Scientific; Folsom; CA, USA) and 2nd column DB-1701 (2 m × 0.1 mm; film thickness, 0.1 µm; J&W Scientific; Folsom; CA, USA). Column setup B: 1st column Equity-1701 (30 m × 0.25 mm; film thickness, 0.25 µm; Supelco; Bellefonte, PA, USA), and 2nd column DB-WAX (2 m × 0.1 mm; film thickness, 0.1 µm; J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas with a constant flow of 1.2 mL/ min. Same oven program was applied for both column setups: initial temperature of 40 °C was held for 2 min; raised to 140 °C at 4 °C/min, and then raised to 240 °C (for column setup A) or 235 °C (for column setup B) at 10 °C/min and held for 10 min.

Modulation was performed with a four-jet thermal modulator (LECO, St. Joseph, MI, USA) using liquid nitrogen for cooling. The modulation period was set at 5 s in column setup A and at 10 s in column setup B. The modulation temperature was kept 15 °C above the oven temperature for column setup A and 20 °C for column setup B, respectively.

Mass spectrometry was performed on a Pegasus 4D TOFMS (LECO, St. Joseph, MI, USA). The mass spectrometer was operated at a spectrum storage rate of 200 Hz and a detector (multi channel plate) voltage of 1500 V to 1800 V. Chromatograms were processed using the LECO ChromaTOF™ software.

GC-O

This was performed on a Fisons gas chromatograph (Type HRGC MEGA SERIES) using two different fused silica thin-film capillaries; DB-FFAP (J&W Scientific; Folsom, CA, USA) and ZB-1701 (Phenomenex, Aschaffenburg, Germany), each 30 m × 0.25 mm; film thickness, 0.25 µm. Samples were applied by the "cold on-column" injection technique at 40 °C. After 2 min, the temperature of the oven was raised by 6°C/min to 240 °C and held for 10 min. The Kovats retention indices were calculated by co-chromatography of *n*-alkanes. The identification of the compounds was based on retention indices on two columns of different polarity (DB-FFAP and OV-1701), co-chromatography of references and odor quality on the sniffing port (Sniffer 9000 System, Brechbuehler, Switzerland).

Results and Discussion

Identification of Trace Sulfur Compounds

In 2D GC the overall separation is influenced by the type and combination of the two columns, their dimensions (length and diameter), the thickness of the stationary phases, the carrier gas velocity, the temperature regime for both columns, and the modulation time. Hence, the analysis of a complex food

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matrix such as coffee requires optimization in method development to achieve the identification of targeted trace aroma compounds. Therefore, the analysis methodology has to be designed in such a way that the combined first- and second-dimension separation allows both the identification and quantification of the analytes of interest. The major part of coffee aroma compounds ranges in a broad spectrum from semi-polar to polar. As a consequence, a combination of semi-polar and polar columns for an efficient separation in 2D GC seems to be reasonable. Indeed, the results of a former study (*5*) revealed that a better separation was obtained with a polar/ medium polar column set (SolGel-Wax × DB-1701) as compared to the apolar/medium polar configuration (ZB-5MS × DB-1701). Analyte peaks were more efficiently distributed along the primary dimension, resulting also in a better separation in the second dimension. Combined with SPME aroma isolation, sulfur compounds like 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), and 3-(methylthio)propionaldehyde (methional) could be identified. The identification of 3-methyl-2-butene-1-thiol (MBT) was also targeted, but not achieved by any of column setups mentioned. Therefore, in the present study special emphasis was put on the identification and quantitative evaluation of MBT in R&G coffee powder and the filter coffee beverage. Optimization work particularly focused on the column setup, the two-dimensional separation as well as the detection parameters. Two combinations of polar and semi-polar phases were applied for the assessment of coffee aroma, i.e. hyphenation of a polar with a medium polar column (column setup A: DB-FFAP × OV-1701) in comparison to the medium polar-polar configuration (column setup B: Equity-1701 × DB-Wax).

As shown in the 2D contour plot (Figure 1), separation by the column setup A led to a good distribution of the analytes in the first dimension; whereas, the second dimension was less well performing. Nevertheless, the resolution was satisfactory in both dimensions and resulted in the detection of methional (Table 1). Despite of the co-elution with abundant compounds furfural and acetic acid (Figure 2**,** retention at 1360 s), methional was well separated on the second column (1.905 s), and a clean mass spectrum was obtained. The deconvoluted mass spectrum was compared with the NIST library and sufficient similarity was found.

The optimization of the 2D-GC-TOFMS system for the detection of methional started first with the assessment of the optimal carrier gas flow. Different constant carrier gas flow rates (from 0.8 to 2.0 mL/min) were tested, and best flow was found at 1.2 ml/min when a 30 m DB-FFAP with a diameter of 0.25 mm was applied. An optimal flow rate is not possible in 2D-GC since two columns with different inner diameters are connected in series. This means that the volumetric flow through both columns is the same but linear velocities in each of them differ. But due to the increased pressure level in the first column provoked by the high flow resistance of the narrow-bore second-dimension column, diffusion in the first column is slower and, thus, optimum velocity is far lower than in 1D-GC. Therefore, Beens et al. (*6*) stated that in the combination of two columns with different internal diameters, one column should be operated close to its optimum flow conditions, while the second column is operated at sub-optimal conditions. Hence, the optimal flow in 2D evaluation is always a compromise between the flow in 1st and 2nd dimension for given column types and dimensions.

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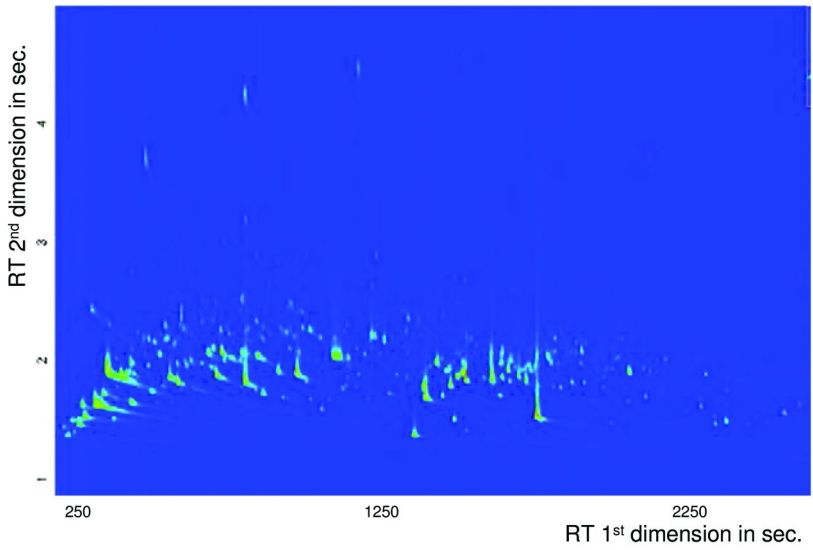

Figure 1. 2D diagram for the column setup A (DB-FFAP/OV-1701), coffee brew extracted by SPME (40 °C for 10 min.); heating rate up to 180 °C: 4 K/min, 15 K offset, modulation time 5 sec; carrier gas flow of 1.2 ml/min. (see color insert)

Table 1. Identification of trace sulfur compounds by different analytical methods and column configurations

	SPME-GC×GC-TOFMS		*HVT GC×GC-TOFMS*	*HVT GC-O*
S-Compound	*A: DB-FFAP × DB-1701*	*B: Equity-1701 × DB-Wax*	*A: DB-FFAP × DB-1701*	*DB-FFAP*
3-Methyl-2-butene-1-thiol	+	-	-	+
3-(Methylthio)-propionaldehyde	+	+	+	+

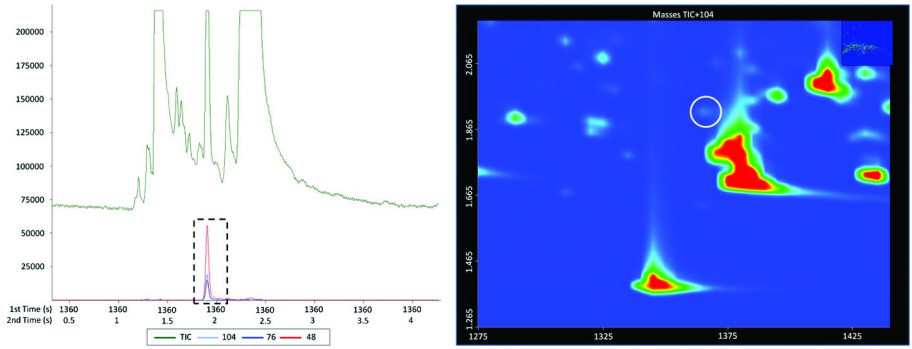

Figure 2. Identification of 3-(methylthio)propionaldehyde in coffee brew using SPME-GC×GC-TOFMS in combination with column setup A; position of the analyte in 1D chromatogram (black frame, left) and in 2D plot (circle, right). (see color insert)

In GC×GC, next to the separation in the first-dimension column, the temperature-programming rate also influences the retention times in the second column, and consequently have to be optimized together with the carrier gas flow. For the polar/medium-polar column setup (setup A) an oven heating rate of 4 °C/min was found adequate when above designated carrier flow rate was applied. The optimization of modulation frequency of the first dimension peak is important for preserving the separation achieved in first dimension and improving sensitivity through peak refocusing. In general, the modulation time should be set slightly higher than the retention time of the analyte with the highest retention in the second dimension. Together with the modulation time the offset-temperature, the temperature difference between first oven and the second oven, has to be optimized in parallel. The combination of modulation time (2-10 s) and off-set temperature (5-15 °C) was tested with given column program of 4 °C/min and column flow of 1.2 ml/min. The described approach resulted in an effective combination setting for column setup A with a modulation time of 5 s and an offset-temperature of 15 °C.

Despite of the above mentioned extensive optimization efforts, the targeted sulfur odorant MBT could not be detected. Thiols such as MFT, FFT, and MBT are known to highly interact with the coffee matrix (i.e. melanoidins) leading to rather low extractable amounts present in the gaseous phase (*7*) above the coffee beverage. Therefore Milo et al. (*8*) suggested the release of reversible bound thiols by the addition of cysteine. In the present study, release of thiols by cysteine was also applied in combination with an increase of the detector voltage from 1500 V to 1700 V to enhance sensitivity of detection.

Figure 3 illustrates the chromatogram with the total ion current (TIC) and the specific masses for MBT m/z 69 and m/z 102. The m/z of MBT are highly overlaid by the TIC of co-eluting 2,3-pentanedione. Actually, the LECO Pegasus software can deconvolute peaks whose apexes are only three data points apart. One illustrative example that demonstrates the need for deconvolution was observed with MBT. However, by deconvolution the specific masses m/z 69 and m/z 102

at 715 s in 1st D and 2.4 s in 2nd D were found to be unique masses, and the MS spectra was identified as that of MBT (Figure 4).

For the medium polar/polar column setup (setup B: Equity-1701×DB-FFAP) oven heating rate and first column flow were set equal to setup A, namely 4 °C/ min and a capillary gas flow of 1.2 ml/min in primary dimension, respectively. In contrast to setup A, the best conditions for the modulation were found at an increased modulation time of 10 s combined with an enlarged offset temperature of 20 °C. Figure 5 shows that the 2nd dimension is fully charged in the rather large range of 10 s. However, the chromatogram showed a better spread of the components in 2nd dimension. But, a significant tailing of the major peaks resulted in the 2nd dimension accompanied by an inferior resolution. At first dimension methional is co-eluting with acetic acid at a retention time of 1070 s, but it is well separated from acetic acid at a retention time of 3.345 s in the 2nd dimension, resulting in a clear MS spectrum due to a considerable improved signal to noise ratio.

In contrast to the column setup A, MBT could not be detected in the application of column setup B. The addition of cysteine into the coffee sample to release bound thiols did not result in its detection either. The reasons therefore may be found in the rather high modulation period that causes a decreased resolution in the 2nd dimension, and thus lower detection sensitivity.

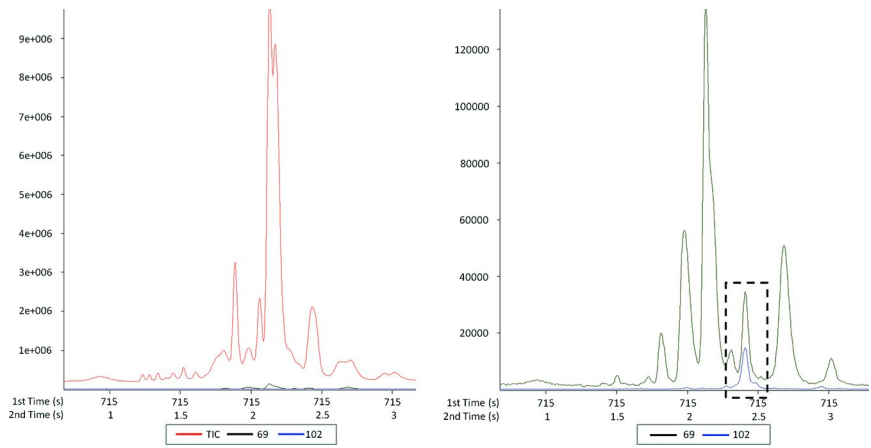

Figure 3. Identification of 3-methyl-2-butene-1-thiol (MBT) in coffee brew using SPME-GC×GC-TOFMS (TIC, left) and (m/z 69 and 102, right); Column setup A. (see color insert)

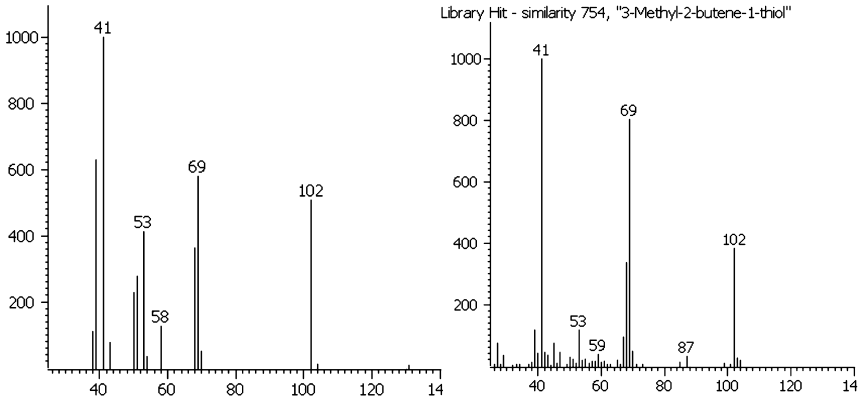

Figure 4. Identification of 3-methyl-2-butene-1-thiol (MBT) in coffee brew using SPME-GC×GC-TOFMS and column setup A; obtained mass spectrum (left) and library reference mass spectrum (right).

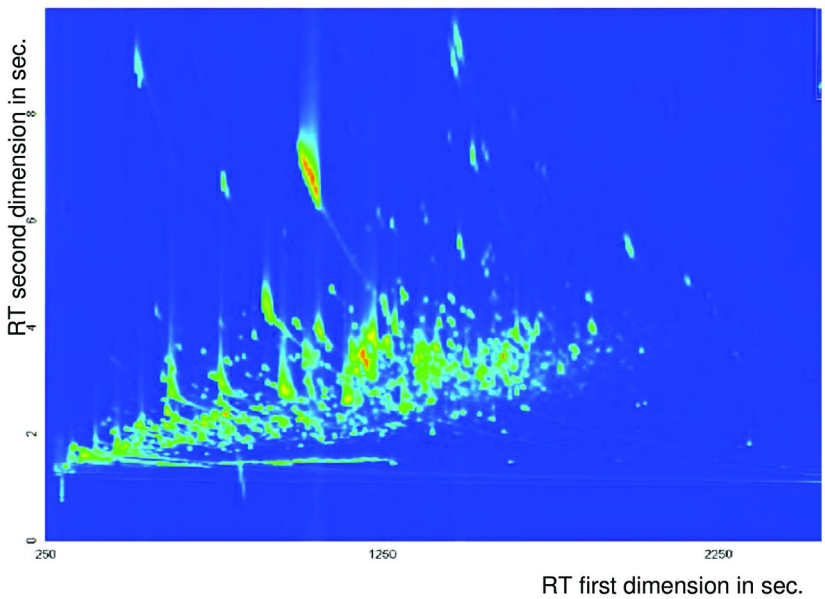

Figure 5. 2D diagram for the column setup B (Equity-1701/DB-FFAP), coffee brew extracted by SPME (40 °C for 10 min.); 4 K/min, 20 K off-set, modulation time 10 s.; carrier gas flow of 1.2 ml/min. (see color insert)

The advantage of the extraction of volatiles by SPME is the rapid aroma isolation (generally from 5 min to 60 min) from a little amount of sample. At the same time, the latter implicates the main drawback as an increase of sample amount is limited. To prove the capacity of the SPME method for the assessment of targeted compounds, it was compared to the more "classical" aroma isolation approach comprising of high vacuum transfer (HVT) distillation with subsequent

solvent extraction. For the aroma isolation by HVT, 200 mL of coffee brew were prepared and distilled. The complete distillate was than submitted to solvent extraction with dichloromethane. After concentration of the extract by distillation, the obtained solvent extract was evaluated by 2D-GC-TOF-MS (column setup A and B) as well as GC-O. Surprisingly, only methional could be positively detected in the solvent extract by 2-dimensional GC-TOF-MS evaluation (Table 1). Despite of the high amount of prepared sample detection of MBT was not achieved; the concentration in the solvent extract is even below the detection level of the TOF-MS. In parallel the solvent aroma extract was assessed by linear GC-O as well. This led to the additional detection of MBT at the sniffing port of the GC-O device. These results clearly demonstrate that sample work-up by solvent extraction and subsequent distillation lead to important degradation of sulfur compounds such as MBT. Despite of the larger sample volume prepared for the solvent extraction, less targeted trace compounds could be detected as compared to SPME isolation. In conclusion, it can be stated that the assets of comprehensive GC×GC-TOF-MS are best used when it is combined with mild isolation techniques.

Quantification of Trace Sulfur Compounds

Methional was shown to be an important shelf life marker in roasted coffee (*9*). Its quantification in a former storage test on R&G coffee (*9*) could only be achieved by a labor-intensive solid phase extraction (SPE) in conjunction with liquid injection and heart-cut GC/GC-MS analysis. Same is valid for MBT that is highly susceptible to oxidative degradation. MBT provides a characteristic fresh odor character and was positively detected in different coffee samples, such as in R&G and brew (*10*). Due to its very low odor threshold and its high volatility it may play an important role particularly in freshly ground coffee samples, but also in coffee brew as an extraction yield of 85% is given in the literature (*10*). Therefore, the development of a more rapid method for the quantification of this sulfur compound in routine analysis is of high interest.

The method developed for the identification of MBT and methional by SPME in combination with 2D-GC×GC-TOF-MS was adapted to quantitative analysis using an isotope dilution assay (IDA) for these sulfur compounds in R&G coffee and coffee brew, respectively. In theory, MBT and methional should be quantified with the column setup A where both compounds were sufficiently separated to obtain clean mass spectra. For the quantitative assessment of a compound in 2D-GC by IDA, it is essential that the peaks of the analyte and its isotopic labeled standard are sufficiently separated in the 2nd dimension. Only then a positive identification of the components is possible by the deconvolution software. Figure 6 illustrates how this condition is fulfilled for MBT, where a slight shift in the retention of only about 0.04 s was enough to be clearly detected by the deconvolution tool as two distinct peaks, i.e. compounds. Both the analyte and labeled standard are almost base-line separated for their quantification masses m/z 102 and m/z 108, respectively. Unfortunately, this requirement was not obtained for methional; m/z 107 of the isotopic labeled standard was not sufficiently separated from m/z 104 to ensure a clear identification. Therefore,

an assay was also tested on the column setup B. As discussed before, MBT can not be detected by this column combination. In the quantification of methional the apexes of m/z 107 and m/z 104 were separated by less than 0.03 s (Figure 7). Nevertheless, automatic detection and thus quantification was feasible. As a conclusion of the quantitative assessment, the targeted sulfur compounds MBT and methional cannot be quantified using the same combination of columns; the polar/ medium-polar configuration was evidenced as suitable for the quantification of MBT, whereas the reversed configuration (setup B) represents the only possibility to quantify methional.

The accuracy and applicability of a new method is generally characterized by detection limits, the level of detection (LoD) and quantification (LoQ), the repeatability as well as the reproducibility of the analysis. For MBT a very low LoD of 0.022 ppb was determined in R&G coffee, and a LoQ of 0.07 ppb resulted thereof (Table 2). The LoD and LoQ of 3-(methylthio)-propionaldehyde were assessed at 1.7 ppb and 5.6 ppb, respectively. These results are fully satisfactory, especially when taking into account that each analyte was split into 2-3 2nd dimension peaks which had to be added up. The low LoD and LoQ levels, particularly for MBT, are only possible by the high resolution capacity of the comprehensive 2-dimensional separation.

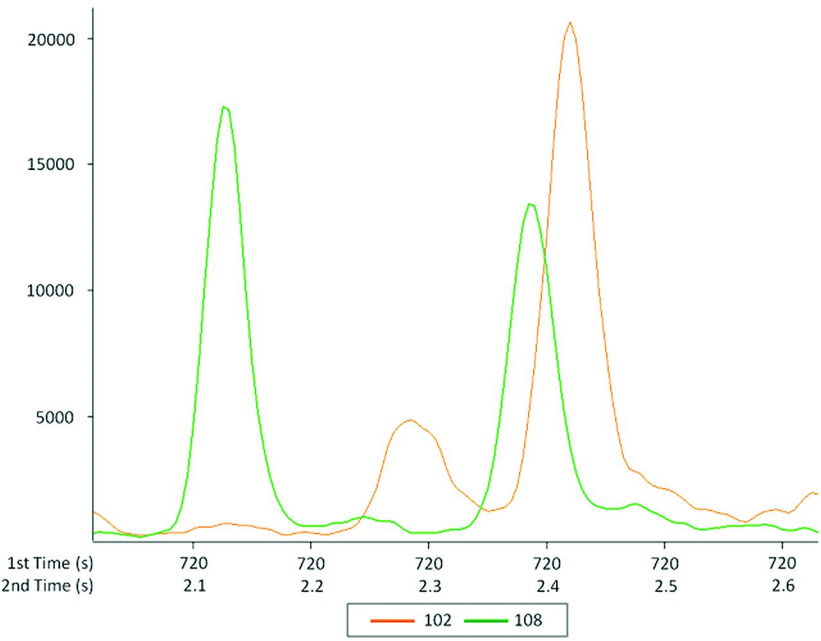

Figure 6. Quantification of 3-methyl-2-butene-1-thiol by IDA; m/z 102 of analyte (orange) and m/z 108 of D_6-labeled standard (green) in R&G coffee; column setup A. (see color insert)

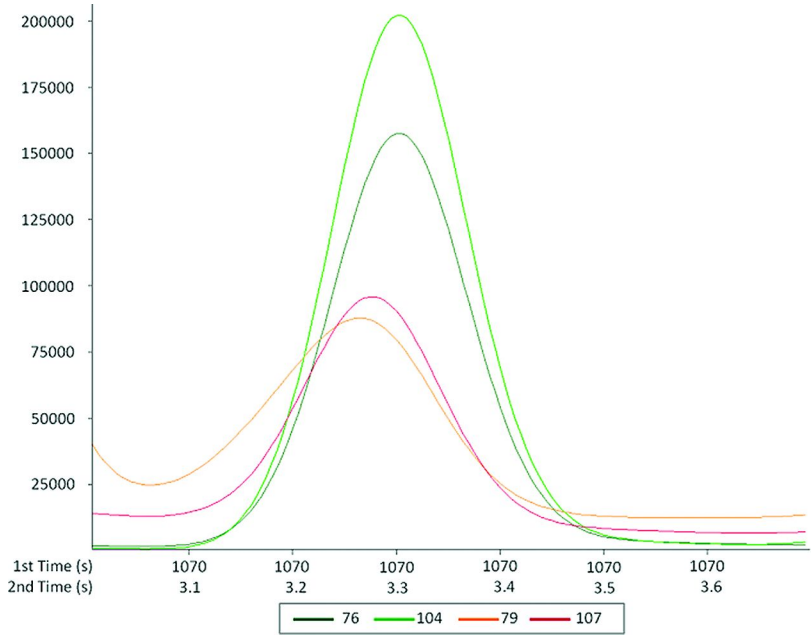

Figure 7. Quantification of 3-(methylthio)propionaldehyde by IDA; molecular mass m/z 104 of analyte (green) and m/z 107 of D_3-labeled standard (red); column setup B. (see color insert)

Table 2. Detection limit (LoD) and quantification limit (LoQ) determined for MBT and methional by SPME GC×GC-TOF-MS in R&G coffee

Compound	*GC×GC-TOFMS*	
	LoD [ppb]	*LoQ [ppb]*
3-methyl-2-butene-1-thiol	0.022	0.07
3-(methylthio)-propionaldehyde	1.7	5.6

For the evaluation of the simple repeatability of the SPME-GC×GC-TOF-MS measurement one R&G coffee as well as one brew sample were quantified by IDA and measured in at least six repetitions. The simple repeatability of the GC×GC-TOF-MS measurements for MBT as well as for methional is given in Table 3. Quantification of methional in both matrices resulted in rather low relative standard deviation of repeatability CV(r). This is easily explained by the conspicuous separation of the analyte in the 2nd dimension, the very low background signal, and thus, the high signal to noise ratio resulting thereof. Due to the considerable lower quantities of MBT in the assessed samples, the CV(r) were determined at higher level of around 14% in the R&G coffee and 12% in the brew, respectively.

Table 3. Simple repeatability for the quantification of MBT and methional in R&G coffee and coffee brew by SPME-2D-GC-TOF-MS (number of measurement repetitions n≥ 6)

	R&G coffee			*Coffee brew*		
Compound	*Range [µg/kg]*	*Mean [µg/kg]*	*CV(r) [%]*	*Range [µg/kg]*	*Mean [µg/kg]*	*CV(r) [%]*
3-methyl-2-butene-1-thiol	31.1 – 39.9	34.8	14.1	0.137 – 0.160	0.145	11.9
3-(methylthio)-propionaldehyde	659.4 – 714.5	678.7	2.93	37.5 – 42.6	39.4	3.74

The concentration of MBT was determined at 31.8 µg/kg in the R&G coffee when measured by SPME in combination with 2D-GC TOF-MS (Table 4) with a relative standard deviation (RSD) of 3.3%. Whereas, the coffee brew revealed a very low amount of only 0.12 µg/kg, with a RSD of 10%. Calculating the concentration in brew normalized to µg per kg R&G coffee, namely 1.60 µg/kg R&G coffee, only 5% of MBT was found to be recovered in the final coffee beverage. In comparison, the reference methodology comprising of HVT for the aroma isolation from the brew and solvent extraction combined with heart-cut GC/GC-MS measurement resulted in a content of 0.18 µg/kg on the same coffee blend (Table 5). The values are in a similar magnitude of concentrations, hence, the method for the quantification of MBT by SPME and 2D HC TOF-MS can be assumed as an accurate and reliable alternative to the conventional approach. However, Mayer et al. (*15*) found a significantly higher value of 0.6 µg/l in a brew by applying solvent liquid-liquid extraction, vacuum distillation, entrapment of the thiols by *p*-hydroxymercury salt with subsequent release, and analysis by dynamic headspace in conjunction with HRGC-MS. Based on the assessed concentration of MBT in R&G coffee of 13 µg/kg, they calculated an extraction yield of 85% when preparing 1 L of brew from 54 g R&G coffee. This value seems to be quite high considering the volatility of MBT as well its rapid degradation behavior. The important analytical differences can simply result from unequal brewing process, drip filter machines or sample treatment afterwards.

Considering the orthonasal detection threshold of 0.3 ppb in water, the concentration of MBT found in coffee brew (0.12 µg/kg) most likely does not contribute to the overall aroma of a filter coffee beverage. However, synergistic effects with other thiols cannot be excluded. In contrast, MBT may play a key role for the aroma of freshly ground coffee considering the very low detection threshold in air. Thiols are supposed to have an important contribution for the fresh coffee aroma; beside other high volatile compounds, the extremely high volatility of MBT may explain the fact that fresh ground coffee powder rapidely loses its desired aroma.

Table 4. Quantification of MBT and methional in R&G coffee and coffee brew; data are means of 3 assays. Extraction yield based on 60 g coffee powder for 1 L brew

Compound	*R&G coffee*			*Coffee brew*			*Extr. yield [%]*
	Range [µg/kg]	*Average [µg/kg]*	*RSD [%]*	*Range [µg/l]*	*Average [µg/l]*	*RSD [%]*	
3-Methyl-2-butene-1-thiol	31.3 - 33	31.8	3.3	0.103 - 0.134	0.12	10.0	5.1
3-(Methylthio)-propionaldehyde	663 - 703	679	3.2	38.5 - 40.8	39.4	3.7	77

Table 5. Comparison of quantitative results to literature data obtained by reference methods (solvent extraction (SE) and GC-MS)

Compound	*R&G coffee [µg/kg]*		*Coffee brew [µg/l]*		
	GC×GC TOF-MS	*SE-GC-MS* [1,2,3,4,5]	*GC×GC TOF-MS*	*HVT-GC-GC-MS* [6]	*SE-GC-MS* [25]
3-Methyl-2-butene-1-thiol	31.8	7 - 17.9	0.12	0.18	0.6
3-(Methylthio)-propionaldehyde	679	213 - 303	39.4	34.1	5.7 - 10

[1] Semmelroch et al. 1995 (*11*), 100% Arabica, Colombia. [2] Semmelroch et al. 1996 (*10*), 100% Arabica, Colombia. [3] Czerny et al. 2000 (*12*), 100% Arabica, Columbia. [4] Mayer et al. 1999 (*13*), 100% Arabica, Columbia. [5] Mayer et al. 2000 (*14*), in 100% Arabica, Colombia. [6] Internal data; 85% Arabica, Colombia, 15%, Robusta, Indonesia.

The quantification of methional in R&G coffee by SPME in combination with 2D-GC TOF-MS resulted in a concentration of 679 µg/kg, with RSD of 3.2 % (Table 4). The brewed beverage from the same coffee revealed a quantity of 39.4 µg/kg of methional with a RSD of 3.7%. The value in the filter coffee corresponds to 525 µg/kg calculated on the base of R&G coffee. When the extracted quantity of the brew is set in relation to the R&G coffee, it becomes obvious that methional is quite efficiently extracted at 77% level during the brewing process. With a detection threshold of 0.43 µg/L in water (*16*) methional must be considered as an aroma relevant odorant in the coffee beverage, but most likely also in R&G coffee. The analysis of methional in the same blend by the reference method resulted in a concentration of about 34 µg/L (Table 5). These results indicate that the SPME-2D-GC-TOF-MS approach represents a reliable alternative for the quantification of methional.

Conclusions

The present study demonstrated the potential of the 2-D GC-TOF-MS technique combined with SPME aroma isolation as a rapid, sensitive, accurate, and ecological method for the quantification of trace sulfur compounds in coffee. Based on its superiority in terms of sensitivity and resolution, this method can be used to quantify sensitive trace odorants like MBT and methional without time consuming clean-up procedures.

As it was shown for MBT and methional, a well defined selection of the column setup for GC×GC as well as optimization of separation parameters, and the modulation conditions, (i.e. modulation time and offset temperature) are essential. However, slowing down of the temperature-programming rate is needed in 2-D GC. Therefore, a practicable solution using the GC×GC approach goes at the expense of the separation time that is slightly longer as compared to 1D-GC analysis. Another and more important draw-back is given by the data treatment of the software that is not designed for routine quantification by IDA. Due to the cutting of peaks, some time-consuming hand operation in data treatment is needed. Therefore, in the future main emphasis has to be put on a more reliable automatic data treatment in the routine quantification of multiple compounds to facilitate the use of this methodology in industrial research.

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