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Fast Screening of Apple Flavor Compounds by SPME in Combination with Fast Capillary GC–MS using a Modular Accelerated Column Heater (MACH) and Quadrupole Mass Spectrometric Detector (qMSD)

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Capillary gas chromatography (CGC), Quadrupole mass spectrometry (qMS), Fast GC analysis, Modular Accelerated Column Heater (MACH), Headspace solid-phase micro extraction (HS-SPME)

Abstract
A fast HS-SPME-CGC-MSD method for the analysis of flavor compounds of apples was developed. A complete profile was obtained after solid phase micro-extraction within 3 minutes GC run-time using a modular accelerated column heater (MACH). Detection with a state-of-the-art quadrupole MSD allowed a data acquisition rate of 20 Hz, while the mass spectral data quality was maintained. The gain in analysis speed was approximately a factor 10 in comparison to the standard method used in the customers laboratory.

Introduction
Speed of analysis in capillary GC can be increased by using fast and ultra-fast temperature programming. In general, the resolution of a separation will be decreased when the temperature gradient is very fast, but for several applications, some loss of resolution can be accepted, especially when the separation is combined with mass spectrometric detection.

Recently, direct resistively heating of the capillary column resulting in very fast heating rates (>200°C/min) has been introduced [1]. The system, available via GERSTEL under the name Modular Accelarated Column Heater (MACH™, figure 1) is mounted onto the door of a standard GC and up to four modules containing separate capillary columns can be controlled independently.

Figure 1. Single column MACH connected to 6890 GC system.

The columns are coupled by short transfer capillaries to the injector and detector of choice. The MACH system was previously used to improve analysis speed by up to a factor 10 and, mainly in combination with split or splitless liquid or headspace injection for the analysis of mineral oil and residual solvents [2,3]. This work describes the use of solid phase micro-extraction (SPME) in combination with fast GC analysis for the determination flavor compounds in apples, the method is used to characterize apples from different producers. Since many samples have to be analysed, a high throughput method is needed. SPME is a relatively fast, miniaturized sample preparation method that enables solute enrichment, resulting in higher sensitivity compared with static headspace analysis. SPME can be fully automated and is compatible with fast GC. Detection was performed with a state-of-the-art single quadrupole mass spectrometric detector (qMSD) that allows acquisition of mass spectral data at scan rates that are compatible with fast capillary GC analysis. Critical aspects of injection, chromatographic and MSD conditions are discussed.

EXPERIMENTAL

Instrumental conditions. A Modular Accelerated Column Heater (MACH™, GERSTEL GmbH, Mülheim an der Ruhr, Germany) was mounted on the oven door of an Agilent 6890 gas chromatograph (GC, Agilent Technologies, Little Falls, DE, USA), which was coupled to an Agilent 5975 Inert mass spectrometric detector (MSD). The system was equipped with a MPS 2 multipurpose sampler for automated solid-phase micro extraction (SPME) sampling and desorption. The MACH column oven module contained a 10 m x 100 µm I.D., 1 µm DB 1-MS capillary column. The inlet of the column was connected to a split-splitless injector using 20 cm of a deactivated fused silica capillary of 100 µm I.D. The injector was equipped with a deactivated liner of 1.5 mm I.D.. The outlet of the column was coupled with a deactivated fused silica capillary (50 cm x 100 µm I.D.) to the MSD. Both transfer capillaries were connected to the analytical column using low dead volume connectors. The injector was used in the split mode (split ratio 1:3), the injector temperature was set to 250°C. Helium was used as carrier gas at a constant pressure of 390 kPa. The MACH oven module was programmed from 25°C (0 sec) to 105°C (0 sec) at a rate of 50°C/min, and to 250°C (30 sec) at a rate of 250°C/min. The Agilent GC oven served only to keep the transfer capillaries heated and was set to a constant temperature of 250°C. The MSD transfer line was equally set to 250°C. The MSD source and quadrupole temperatures were set to 230°C and 150°C, respectively. The mass spectrometer was used in the fast scan mode between m/z 33 and 300. The data acquisition frequency was 21 Hz.

Sample preparation conditions. An apple was peeled and the fruit was homogenized using an Ultraturrax™ blender. A ten gram sample of compote was then weighed into a 20 mL headspace vial and capped. SPME was performed in the headspace of the sample (HS-SPME) for a period of 5 min at 25°C using a 100 µm df polydimethylsiloxane (PDMS) fiber. The SPME fiber was desorbed in the injector for 0.5 min. In order to optimize productivity, the MPS 2 was set up in the 'sample prep-ahead' mode, performing SPME sampling during the GC run of the preceding sample.

Results and Discussion

Figure 2A shows the HS-SPME-GCMSD analysis of a 10 g sample of an apple compote. A complete profile of the aroma compounds was obtained within less than 3 minutes. Figure 2B shows a detailed view of the elution window between 1 and 3 min. The identified compounds are labeled on the chromatogram and are listed in Table 1. The total analysis cycle time, including cooling of the oven module, was less than 4.5 min.

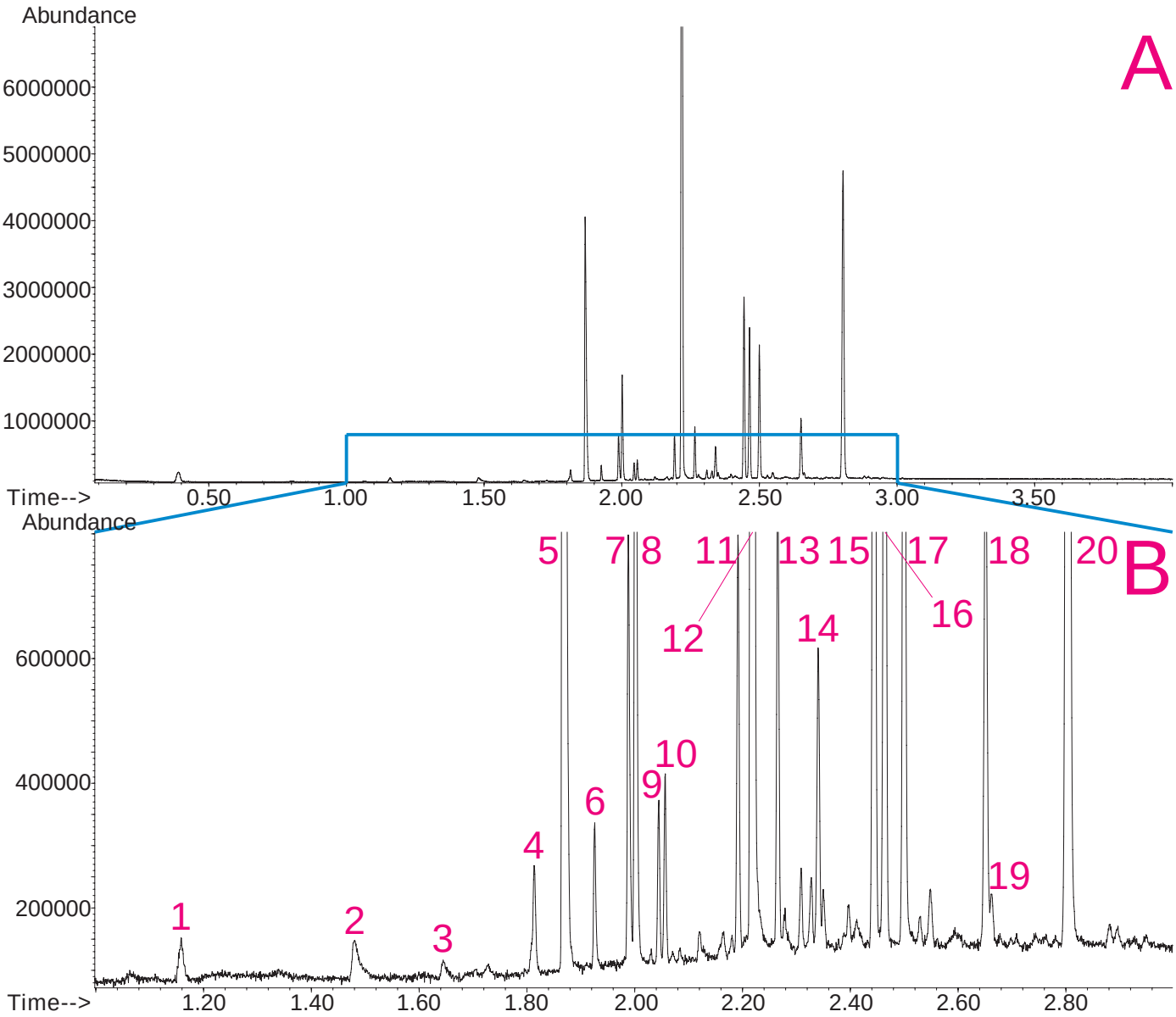

Figure 2. Total ion chromatogram (A) and detail between 1.0 and 3.0 min (B) of the HS-SPME-GC-MSD analysis of 10 g apple compote. Compounds are listed in table 1. For sampling and chromatographic conditions please see text.

Table 1. Peak identification of selected compounds from the HS-SPME-GC-MSD analysis of 10 g apple compote (Figure 2): Identities, retention times, repeatability of the retention times (SD, n=3) and peak widths. Sampling and chromatographic conditions are listed in the text.

Peak No.	Compound	RT [min]	SD [min]	Peak width [min]
1	Hexane	1.159	0.000	0.010
2	Butanol	1.481	0.001	0.013
3	Propyl acetate	1.646	0.001	0.014
4	Hexanal	1.814	0.001	0.007
5	Butyl acetate	1.869	0.000	0.007
6	2-Hexenal	1.926	0.000	0.005
7	1-Hexanol	1.989	0.000	0.005
8	2-Methyl-1-butyl acetate	2.002	0.000	0.005
9	Butyl propanoate	2.045	0.000	0.005
10	Pentyl acetate	2.057	0.000	0.005
11	Butyl butanoate	2.192	0.000	0.005
12	Hexyl acetate	2.218	0.000	0.006
13	2-Methyl-butyl butanoate	2.266	0.000	0.005
14	Hexyl propanoate	2.341	0.000	0.006
15	Hexyl butanoate	2.445	0.001	0.006
16	Estragol	2.464	0.001	0.006
17	Hexyl-2-methyl butanoate	2.500	0.000	0.006
18	Hexyl hexanoate	2.652	0.000	0.006
19	Butyl benzoate	2.663	0.000	0.006
20	α-Farnesene	2.804	0.000	0.008

SPME was performed on a 100 µm df PDMS fiber in order to enable fast enrichment of compounds over a wide range of boiling points. Moreover, the fiber enables a relative fast release of compounds in the hot injector at 250°C. The injector was programmed in the split mode, using a split ratio of 3:1. The column flow, at initial GC conditions, was approximately 0.8 mL/min. Using this small split flow, the flow in the liner is increased to 3 mL/min, allowing fast transfer of the solutes into the column, while barely affecting sensitivity. In splitless mode, some band-broadening for the early eluting solutes was observed. The refocusing of the desorbed solutes in the capillary column was enhanced by using a low initial column temperature of 25°C. In contrast to classical GC ovens, this is feasible using the MACH column oven module without having excessively long cool-down times (in this case only 30 sec). The peak widths at half peak height are around 0.010-0.014 min (600-780 msec) for the most volatile compounds (hexane, butanol and propyl acetate) and between 0.005 and 0.007 min (300-420 msec) for all others. For the fastest peaks (width at baseline around 0.01 min), more than 10 datapoints are taken, as illustrated in Figure 3 for 2-methyl-1-butylacetate (peak 8). This ensures reliable quantification.

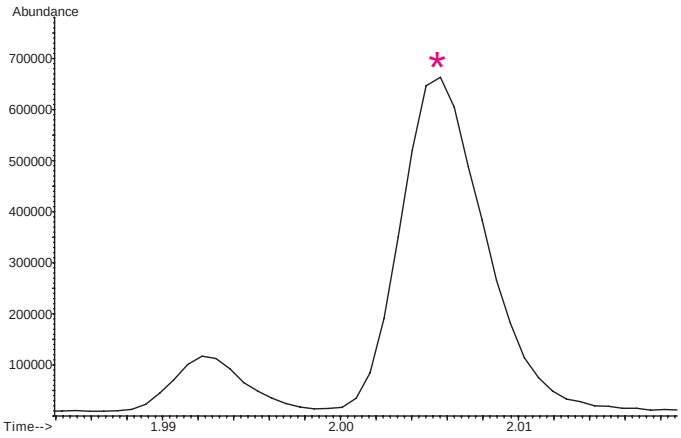

Figure 3. Detail between 1.98 and 2.02 min of the total ion chromatogram of the HS-SPME-GC-MSD analysis of 10 g apple compote. (*) 2-Methyl-1-butyl acetate.

In fast and ultrafast GC, shifts in retention behavior caused by inaccurate column temperature control can results in drastic changes of separation performance. Additionally, relatively slow injection techniques such as thermal desorption can contribute to these changes. The SPME-fast GC-MSD method was repeated 3 times for 3 sub-samples of the apple compote and the standard deviations (SD) on the retention times listed in Table 1 do not exceed 0.001 min.

The data acquisition rates of quadrupole mass spectrometric detectors are relatively slow in comparison to e.g. time-of-flight (TOF) mass spectrometers. However, the Agilent 5975 Inert quadrupole MSD can acquire data at a rate of up to 10000 amu/sec. This improves both the quantitative and qualitative data obtained in fast GC experiments. For this application, the mass spectrometer was used in the fast scan mode, scanning between m/z 33 and 300. The spectra are, in contrast to default qMSD acquisition, not averaged from the raw scan data. Figure 4 shows the spectrum of 2-Methyl-1-butanol acetate (tR=2.002 min), it is very similar to the spectrum in NIST MS spectral library (quality match=90%).

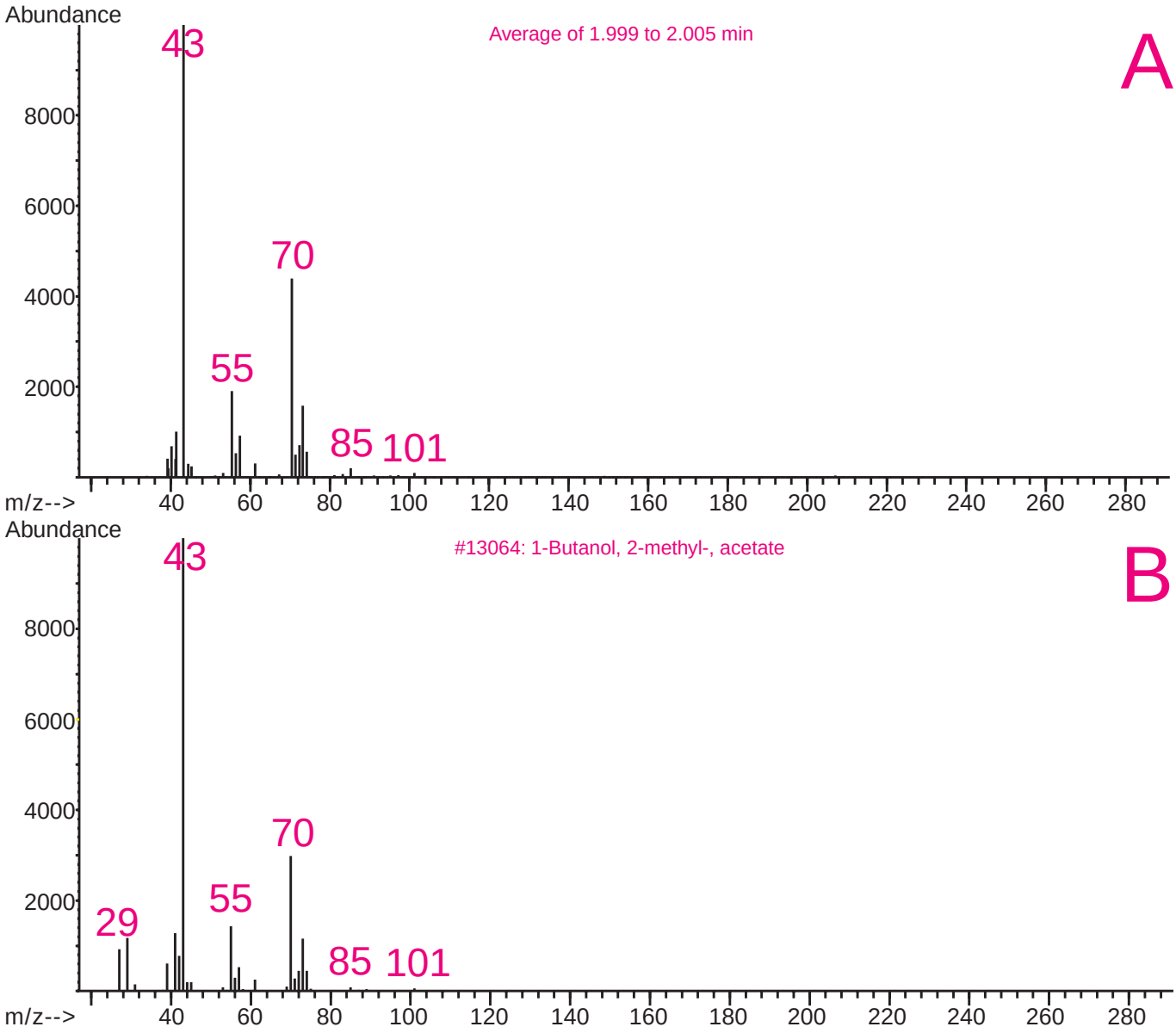

Figure 4. Mass spectrum at tR=2.002 min (A) and NIST spectrum of 2-Methyl-1-butyl acetate (B).

Conclusion

Solid-phase micro extraction was combined with fast GC-qMSD analysis for the determination of flavor compounds. Complete and reliable profiles are obtained within 3 min. SPME does not contribute to peak broadening. The quadrupole mass spectrometric detector was used in the 'fast scan mode' and resulted in 10 data points for peaks of 600 msec wide, while qualitative spectra were obtained.

References

[1] R. Mustacich et al, US patents 6 217 829 (2001), 6 209 386 (2001) and 6 530 260 (2003)

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