



Accelerating apple flavor enjoyment

Fast Apple Flavor screening using SPME coupled with fast GC/MS based on standard columns

Food producers invest large sums in complex flavor analysis to ensure consistent product quality and product flavor. Professor Pat Sandra's Research Institute for Chromatography (RIC) has developed a HS-SPME-GC/MS method that speeds up screening of flavor compounds.

Few fruits are seen as symbol of so many different things and few carry as many different meanings across human cultures as apples. Among other things, apples symbolize fertility, love, and ties to home, family and country. Lest we forget, apples equally symbolize temptation and original sin. In the U.S., the saying “as American as apple pie” refers to apples as almost a symbol of the good American life. Symbolism aside, one can still say apples are healthy and tasty, provided that they have been allowed to fully ripen in the proper way. Most frequent consumers of apples have sufficient experience to recognize a good, ripe and well developed apple when confronted with one. The speed at which an experienced person can make a correct visual and olfactory assessment of an apple cannot be beaten by analytical instrumentation to date.

When, however, a brand name product relies on consistently delivering the same accepted great taste batch after batch - year in year out - more than subjective selection by humans will be required: Enter chemical analysis in the form of gas chromatography coupled with mass selective detection (GC/MSD). Professor Pat Sandra and his team at the RIC accepted the challenge to speed up the analysis of flavor compounds in apples used for a brand name product. Using a fast GC column module, the Modular Accelerated Column Heater (MACH) from GERSTEL, the RIC team was able to speed up apple flavor analysis by a factor of 10 compared with the customer analysis method, reducing the cycle time to 4.5 minutes.

Easy GC modification improves performance

The speed of capillary GC analysis can be improved significantly by increasing the speed of temperature programming. Additional factors are at play, of course. Shorter columns with smaller i.d. provide a big boost. Smaller columns in return require improved pneumatic control at higher carrier gas pressures. Further, a GC inlet is needed that offers adequate dimensions (small internal volume) and a split vent. These properties enable the inlet to deliver analytes to the GC column in a narrow band, the best possible start for high-quality fast separations. Accelerating the GC separation process of course has consequences “downstream”: Faster data ac-

quisition is needed for sufficient peak definition and accuracy of results, for this, an MSD is needed that offers faster scan rates. The RIC used a 6890 GC combined with a state-of-the-art 5975 Quadropole MSD from Agilent Technologies for this work.

The 5975 enables data acquisition rates of up to 21 Hz, 21 full scans per second, while maintaining mass spectral data quality.

To enable fast heating rates, the GC was equipped with a Modular Accelerated Column Heater (MACH) from GERSTEL. MACH is based on column heating modules that enable direct resistive heating at rates of up to 1800 °C / min. Not only is the column heating step accelerated by MACH: The GC column cools down from 240 °C to 40 °C in as little as 30 seconds, depending on the column length. Since there is little material that needs heating or cooling, the GC equilibration time can also be cut to near zero. The overall result: Ultra-short GC cycle times and a significant increase in productivity and throughput.

MACH based on Low Thermal Mass (LTM) technology

Unlike a traditional GC oven, the MACH module has no insulation material and no metal based oven chamber that needs to be heated or cooled along with the column over the GC cycle. MACH is controlled from the Agilent Technologies ChemStation Software or directly through the MAESTRO software. Andreas Hoffmann, GERSTEL Applications Manager: “We don’t drill a hole in the GC oven door to mount the MACH modules; the door is replaced with a dedicated oven door that holds up to 4 MACH modules.” It takes only around 30 minutes to upgrade a 6890 or 7890 GC to MACH, getting it ready to perform faster. “The column module is simply mounted from the

outside through an opening in the door, not inside the GC oven. “This has the added benefit that when operating the GC oven isothermally at the maximum temperature required for the analysis, no special accessories are required to heat the end of the column or the transfer capillaries to the standard GC injector or standard GC detector.”

First peel your apple, and then apply automated sample preparation and high performance column technology

This is how the RIC scientists approached the task: An apple was peeled and homogenized using an Ultraturrax™ blender. A ten gram sample of the resulting apple sauce, or compote, was weighed into a 20 mL vial, which was capped and placed in the GERSTEL MultiPurpose Sampler (MPS) tray. Sample preparation and sample introduction to the GC/MSD system was performed by the MPS using the Headspace Solid Phase Micro Extraction (HS-SPME) technique.

The SPME fibre, coated with 100 µm polydimethylsiloxane (PDMS), was placed in the sample headspace for five minutes to extract the volatile analytes. The sample temperature was held at 25 °C. The fibre was subsequently desorbed at 250 °C for 30 seconds. For maximum productivity, the MPS was set to PrepAhead mode providing overlapping sample preparation and chromatography. In PrepAhead mode, samples are prepared during the GC-run of the preceding sample. When the GC becomes ready, the next sample has been prepared and is ready to be injected. This approach helps to ensure that the GC /MSD system is never idle providing maximum productivity and system utilization.

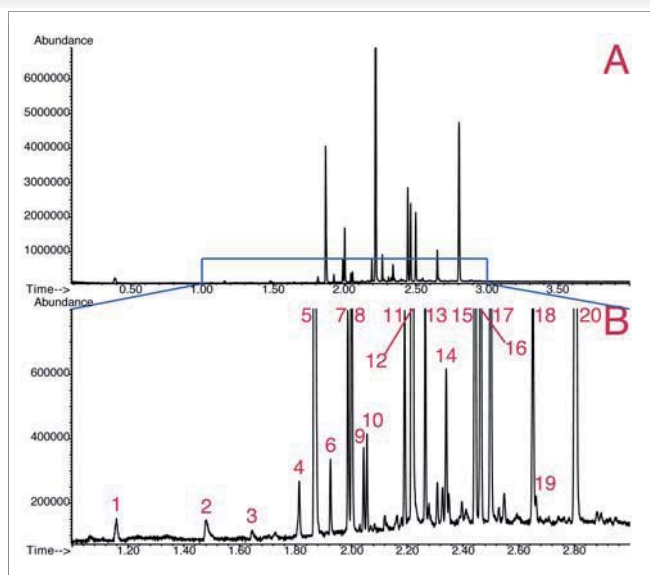
The inlet, a Cooled Injection System (CIS) from GERSTEL was fitted with a 1.5 mm i.d. deactivated liner and set to split mode with a split ratio of 3:1. The Helium carrier gas was initially set to a constant pressure of 390 kPa and the column flow was set to 0.8 mL/min, which translated to a flow of 3 mL/min through the liner, sufficient for fast analyte transfer onto the GC column. The resulting sensitivity met all QC requirements.

The inlet was connected to the column in the MACH module via a 20 cm long, 100 µm i.d. deactivated fused silica capillary. The transfer capillary was kept inside the GC oven at 250 °C isothermal. The

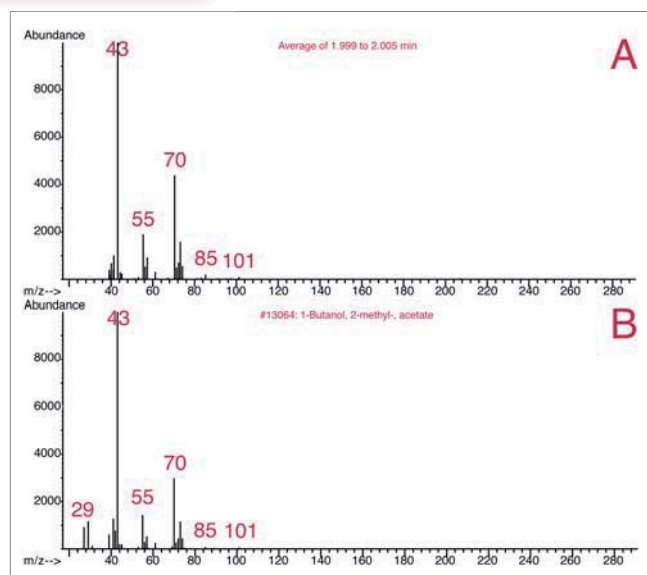


Andreas Hoffmann,
Manager Analytical
Services (Application),
GERSTEL





Total Ion Chromatogram (A) and detail between 1.0 and 3.0 minutes (B) of the HS-SPME-GC/MS analysis of ten grams of apple sauce / compote. For a list of compounds, see table.



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



The RIC used a 6890 GC coupled with a 5975 MSD, both from Agilent Technologies, for fast GC analysis of apple flavor compounds. The GC was equipped with a GERSTEL MACH module providing fast separations and fast cool-down. Sample preparation was performed automatically using the GERSTEL MultiPurpose Sampler. The MPS was also used to introduce the analytes to the GC inlet GERSTEL Cooled Injection System (CIS). The picture shows the newer model 7890 GC with a dual MACH module.

Peak number	Compound	RT [min]	SD [min]	Peak [$w_{1/2}$ min]
1	Hexane	1.159	0.000	0.010
2	Butanol	1.481	0.001	0.013
3	Propylacetate	1.646	0.001	0.014
4	Hexanal	1.814	0.001	0.007
5	Butylacetate	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-butylacetate	2.002	0.000	0.005
9	Butylpropanoate	2.045	0.000	0.005
10	Pentylbutanoate	2.057	0.000	0.005
11	Butylbutanoate	2.192	0.000	0.005
12	Hexylacetate	2.218	0.000	0.006
13	2-Methyl-butylbutanoate	2.266	0.000	0.005
14	Hexylpropanoate	2.341	0.000	0.006
15	Hexylbutanoate	2.445	0.001	0.006
16	Estragole	2.464	0.001	0.006
17	Hexyl-2-methylbutanoate	2.500	0.000	0.006
18	Hexylhexanoate	2.652	0.000	0.006
19	Butylbenzoate	2.663	0.000	0.006
20	alpha-Farnesene	2.804	0.000	0.008

Selected identified flavor compounds from a ten gram apple sauce sample, determined by HS-SPME-GC/MS. The analysis was repeated in triplicate, the standard deviation (SD) of the retention times was always below 0.001 min.

column inside the MACH module was a 10 metre, 100 μm i.d., DB 1 MS column with a film thickness of 1 μm . The column outlet was connected to the MSD via a 50 cm long, 100 μm i.d. deactivated fused silica capillary that was also kept inside the GC oven at 250 $^{\circ}\text{C}$. Both Transfer capillaries were connected to the column using zero-dead-volume connectors.

Flavor profiling in three minutes with a GC cycle time of only 4.5 minutes

The MACH module was operated with the following temperature program:

Initial temperature: 25 $^{\circ}\text{C}$, rate 50 $^{\circ}\text{C}/\text{min}$ to 105 $^{\circ}\text{C}$, held for 0 minutes. Rate 2: 250 $^{\circ}\text{C}/\text{min}$ to the final temperature of 250 $^{\circ}\text{C}$, held for 30 seconds.

The transfer capillaries were kept at a constant temperature of 250 $^{\circ}\text{C}$. The MSD Ion Source and Quadrupole temperatures were set to 230 and 150 $^{\circ}\text{C}$ respectively. The MSD was operated in fast scan mode between m/z 33 and 300. The data acquisition rate was set to 21 Hz. Unlike the classical GC oven, the MACH module cools within seconds, ensuring that the cycle time can be kept to only a few short minutes. "The complete apple flavor profile was available after three minutes and the GC/MS system was generally ready for the next analysis after 4.5 minutes", the RIC scientists reported.

The peak width at half height ($w_{1/2}$) was within the range from

0.010 to 0.014 minutes, equal to around 600 to 780 milliseconds for most of the volatile organic compounds (VOCs) such as hexane, butanol and propylacetate. Peak widths ($w_{1/2}$) for all other compounds were between 0.005 and 0.007 minutes, equal to from 300 to 420 milliseconds. The narrowest peaks had a peak width of around 0.01 minutes at base line. At the set scan rate, more than ten points were taken across the peak, enabling reliable quantitation.

For more information:

AppNote 8/2006, „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)“, <http://www.gerstel.com/p-gc-an-2006-08.pdf>

