Rapid Screening of Volatile and Semi-Volatile Organic Components in Cocoa Beans and Chocolate Products Using a Portable GC/MS System

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The quality of raw materials used to produce chocolate greatly impacts the finished product quality where taste and odour can be greatly influenced by both the source, transport, and storage conditions of the raw materials. Volatile and semi-volatile organic compounds (VOCs, SVOCs) found in products originating from cocoa beans have been previously characterised [1] and compounds indicative of product quality, including acetic acid, nonanaldehyde, tetramethyl pyrazine and trimethyl pyrazine were identified in cocoa beans from the Ivory Coast, Costa Rica, Ghana and Ecuador [2]. Analysis of raw materials prior to use in the chocolate-making process can be advantageous for the manufacturer since the presence of chemical defects causing moulding problems or elevated free fatty acid content resulting from improper storage conditions, can impact a cocoa bean's taste and odour characteristics. By detecting off-flavour compounds early in the supply chain and during the manufacturing process, chocolate makers can eliminate poor quality raw materials and decrease rejection rates of finished products not meeting quality standards.

In this study, we report on a preliminary investigation into the use of headspace SPME (solid phase micro-extraction) coupled with a novel, field-portable GC-MS system (Torion T-9, PerkinElmer Inc, Shelton, CT) for the on-site screening of VOCs and SVOCs prior to shipping and upon receipt of raw materials by the cocoa manufacturer. The ability to quickly fingerprint raw materials for the presence of mould indicator compounds at various points in the supply chain can reduce costs by allowing the procurer (shipper, buyer, broker, etc.) to rapidly evaluate the quality of a shipment before it is purchased. In addition to cocoa beans, prescreening of raw materials and end-products using this technology can be broadly applied to numerous other foodstuffs to determine the quality and safety of food products and materials.

This novel analytical approach integrates a high speed low thermal mass (LTM) capillary gas chromatograph with a miniaturised toroidal ion trap mass spectrometer allowing for high resolution separation and mass spectral detection of a wide range of compounds with a typical analysis time of less than 3 minutes. The instrument uses retention time indices and an on-board spectral library to identify compounds of interest. Its capability is further enhanced by deconvolution algorithms to ensure reliable identification of even co-eluting compounds in complex mixtures. When used in conjunction with an extensive NIST database and custom built target compound libraries, unknown peaks can be positively identified using known standards by non-experienced personnel in the field.

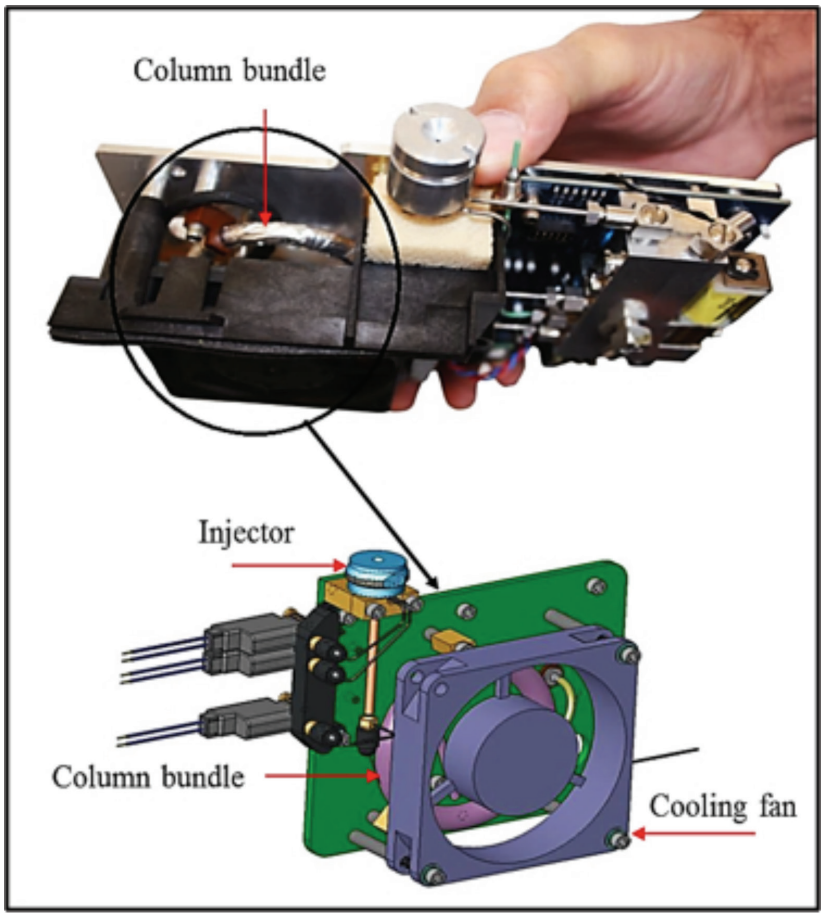


Figure 1: The Low Thermal Mass (LTM) GC used in this study

Methodology

The system and its applicability for field-based analysis has been previously described [3,4,5]. However, to better understand its practical capabilities, it's important to take a closer look at the instrumental components [6]. Although this technology was built for portability and speed, the gas chromatograph was designed to provide comparable chromatographic resolution and performance to a benchtop system. The instrument weighs just 14.5 Kg including the battery, with dimensions of 38 cm x 39 cm x 23 cm. Its compactness and miniature size is achieved by replacing a conventional capillary column oven with a low thermal mass (LTM) column bundle using direct-contact electrical resistive heating. LTM/GC uses a small diameter, metal capillary column, which is bundled with resistive heating and temperature-sensing wires that are braided together with insulator strands. This design provides for more controlled heating, greater heating and cooling speeds and very low power consumption. And since column heating requires considerably less operating power than a conventional GC, longer battery-lifetime is experienced. With its combination of direct resistive heating and rapid temperature ramp rates, the system can separate multi-component analytes in typically less than three minutes. The LTM column is shown in Figure 1.

Improved Spectral Quality

It is well recognised that elevated temperature program methods are required for the determination of many volatile organic compounds. However, when using conventional LTM column technology at this temperature, it is typical to get poor peak shapes and resolution. This problem is mainly caused by the real temperature in the column not matching the values that were set in the method, due to cooler spots existing on the bunched column especially in areas that are located near to the conductive foil covering layer. In addition, there also may be cooler sites on the column, which are not close to where the thermal sensors are located.

This phenomenon is exemplified in a conventional LTM column in Figure 2, which shows a round cross section bundle structure of metal or fused silica column rings, resistive temperature detector (RTD) sensors and insulating material, covered by a conductive aluminium foil in such a way that the heat transfer and distribution can achieve the highest efficiency in two working stages of heating and cooling. However, in reality, heat distribution and transfer of the conventional LTM column are not identical as expected for the entire cross sectional area. Depending on the arrangement of the column rings, heater and sensor there may be heat transfer discrimination or on-site temperature differences especially when the column is used at high temperature. This issue limits the ability of low volatility compound elution

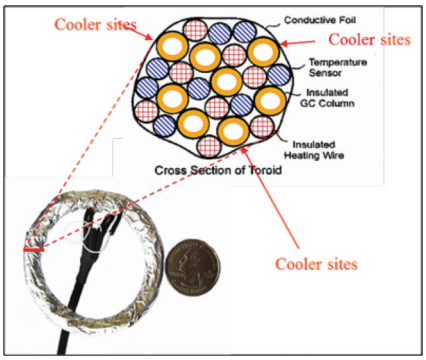


Figure 2: Cooler sites experienced with conventional LTM column technology

Because of this limitation in conventional LTM technology, a new planar LTM column was designed to minimise the cool spots by using thin aluminium covers to wrap around the one-layer regular-arrangement column and insulated heating wire. In this novel planar design, column rings, which can be either metal or fused silica are coiled side by side within single layer on the surface of a swirled aluminium band, compared to the conventional design where the column was bundled in a round cross section. This planar column provides identical heat distribution, but virtually eliminates cooler spots, thus improving the chromatographic separation at high temperature GC runs required for higher boiling point compounds. The principles of this new technology are shown in Figure 3.

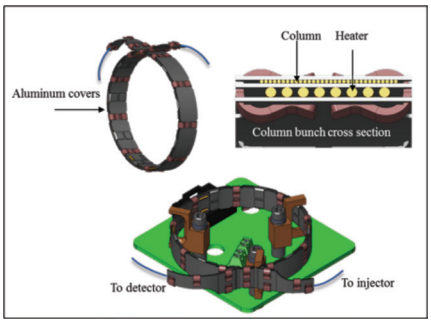
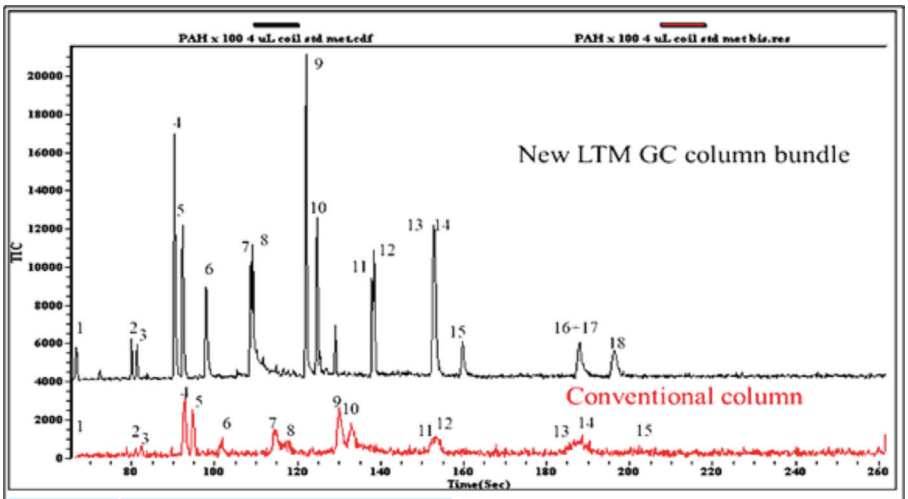


Figure 3: Principles of planar LTM column technology, with the cool spots eliminated



No.	Name	No.	Name
1	Naphthalene	10	Pyrene
2	2-Methylnaphthalene	11	Benzo[*a*]anthracene
3	1-Methylnaphthalene	12	Chrysene
4	Acenaphthylene	13	Benzo[*b*]fluoranthene
5	Acenaphthene	14	Benzo[*k*]fluoranthene
6	Fluorene	15	Benzo[*a*]pyrene
7	Phenanthrene	16	Benzo[*ghi*]perylene
8	Anthracene	17	Dibenz[*a,h*]anthracene
9	Fluoranthene	18	Indeno[*1,2,3-cd*]pyrene

Figure 4: Chromatographic comparison between a conventional LTM column (red) and the planar LTM column (black) described in this study for the separation and analysis of a mix of 18 PAH compounds (labelled and identified 1-18).

To get a better understanding of the practical capabilities of this new technology for the separation and analysis of semi-volatile organic compounds, a comparison was made between conventional LTM column technology and the planar LTM column described here for a standard of 18 polycyclic aromatic hydrocarbons (PAHs) with a boiling point range of 200-570°C. This comparison is exemplified in Figure 4.

The following observations were made.

- Peak shapes are poorer due to lower efficiency and resolution reduced in the conventional column – peaks were wider with a noisier background
- The retention times of the peaks in the conventional LTM column were substantially longer
- Peak intensities in the flat low thermal mass column were significantly higher than those in conventional one
- Conventional column technology could not analyse components 16, 17 and 18 in the PAH mix, they did not elute because of their very low volatility (boiling points 524-550°C)

It is likely that the poor peak shapes were caused by the cooler sites existing on the conventional column, which slowed down the movement of the analytes, decreased their partition coefficient and mass transfer, but increased the longitudinal diffusion band in the mobile phase. As a result the peaks become broadened and took a longer time to elute from the column. In fact, it can be seen that some high boiling point compounds got so broad that they cannot be seen as a normal Gaussian peak shape and as a result, were not detected.

The Mass Spectrometer

The instrument's mass spectrometer uses a novel toroidal ion trap, which is well-suited for miniaturisation compared to other types of mass spectrometers, such as conventional cylindrical ion traps or linear quadrupoles. The benefits of using smaller ion traps is that they can operate at higher pressure so vacuum requirements are less stringent, allowing for smaller pumps which reduces both size and weight. The toroidal ion trap geometry translates into larger trapping volumes despite its miniaturised size, resulting in high ion counts and increased sensitivity, low noise levels and good spectral quality This means the instrument can operate off battery power for longer periods than any other field portable GC/MS. A schematic of the instrument's toroidal ion trap is shown in Figure 5.

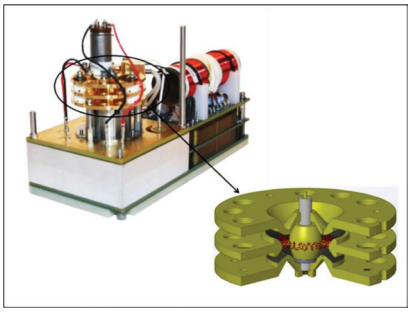


Figure 5: Schematic of the toroidal ion trap

The ion trap mass analyser is heated to 150-200°C depending on the application and operates under vacuum, which results in the electrodes staying clean for long periods of time. This reduces the need for frequent maintenance, while increasing mass spectral quality and reproducibility. Performing at an elevated temperature also leads to long-term MS resolution stability, providing <0.5 m/z mass resolution (FWHM) over the 41-500 Daltons (da) mass range. Most chromatographic peaks are ≥ 1 sec wide, ensuring that a multiple suite of compounds can be fully resolved and analysed in 1 min or less. The scan rate of the mass spectrometer is 10 -15 scans per sec, which provides multiple data points across the narrow chromatographic peaks resulting in excellent mass spectral quality. Typical repeatability at this scan rate is ~ 10% RSD for ppm-ppb levels using the SPME sampling method and lower at higher concentrations. The principle of quantifying with multiple data points across the chromatographic peak is shown in Figure 6.

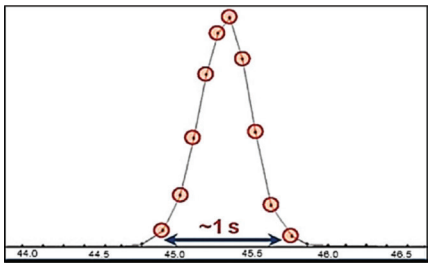


Figure 6: The toroidal ion trap mass spectrometer provides multiple scans across a narrow chromatographic peak

Sample Preparation Module

The capability and flexibility of this portable GC-MS technology can be further improved using a compact, battery-operated, rugged sampling accessory (SPS-3, PerkinElmer Inc, Shelton, CT) for use in the field [7]. The choice of sampling modules includes:

- Heated headspace (HS) for volatiles in solid and liquid samples
- Purge and trap (P&T) for volatiles in liquid (aqueous) samples
- Thermal Desorption (TD) for volatiles using a conventional TD tube
- Internal standard addition module

These modules require the use of the needle trap (NT) to transfer the analytes to the GC/MS. In addition to the SPS-3 sampling module, the needle trap can be used independently to sample gases without the sampling module, while solid phase micro-extraction (can be utilised for gas and liquid samples and a coiled wire filament (CWF) can be used for semi volatiles dissolved in solvent samples. However, for this study, a SPME system was the sampling device of choice.

Experimental

The experimental conditions for the rapid screening of VOCs/SVOCs in cocoa beans and chocolate products are described below.

Sample Preparation

Cocoa beans, cocoa butter, and finished chocolate product were provided by Theo Chocolate (Seattle, WA). Each sample was placed in a 20mL headspace vial and capped. A CUSTODION™ SPME syringe with a 65 µm Polydimethylsiloxane/ Divinylbenzene (PDMS/DVB) fibre was used for extraction by exposing the fibre directly into the headspace above each sample for 10 min at 60°C, as shown in Figure 7. This sampling technique improved sample off-gassing and analyte collection of VOCs and SVOCs in the sample.

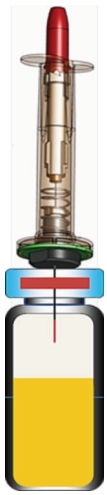


Figure 7: Schematic of the SPME syringe sampling the headspace vial

Analytical Conditions

Following each sample preparation, the SPME syringe was inserted into the injection port of the portable GC-MS where the target analytes were desorbed into a low thermal mass injector (270°C) coupled to a capillary GC column (MXT-5, 5 m x 0.1 mm, 0.4 µm - Restek, State College, PA). After an initial 10 second hold at 50°C, the GC temperature was increased at 2°C/sec to 280°C. The capillary GC was coupled to a toroidal trap MS detector having a mass range of 41-500 m/z and a scan rate of 10-15 scans/sec. The full chromatographic separation conditions are shown in Table 1, while the mass spectrometer parameters are shown in Table 2.

Screening Results and Discussion

Figure 8 shows the GC-MS total ion chromatograms (TICs) for three organically cultivated, fermented, dried cocoa beans from different geographical sources. Characteristic compounds in the headspace eluted in less than 90 seconds under the GC-MS conditions listed in Table 1. Peaks marked with an asterix (*) represent candidate VOC and SVOC markers previously mentioned, which potentially can be used to evaluate the acceptability and confirmation of the authenticity of a raw material shipment [2]. By comparing the chromatographic fingerprints of VOC/SVOC compounds generated by the sample to a representative or reference VOC/SVOC profile from an acceptable lot, the analyst can then make a very rapid assessment of the quality of that cocoa bean/chocolate product or commodity. Additionally, the mass spectral data of these eluted peaks could be further examined for clarity and confirmation and compared with reference data bases if required.

Table 1: The chromatographic separation conditions for cocoa powder samples

Gas Chromatographic Separation Conditions	
Sample delivery	SPME (solid phase micro-extraction)
SPME phase	Divinylbenzene/Polydimethylsiloxane (DVB/PDMS, 65 µm)
Injector temperature	270°C
Transfer line temperature	250°C
Injector split ratio	10:1
Column Technology (Restek®, State College, PA)	MTX®-5: low-polarity phase diphenyl dimethyl polysiloxane; 5 m x 0.1 mm x 0.4 µm
Initial Temperature/ Hold Time	50°C for 10 s
Final Temperature/Hold Time	280°C for 50 s
Temperature Ramp Rate	2°C/s

Table 2: Mass Spectrometer Parameters

Mass Spectrometer Operating Conditions	
Mass spectrometer	Toroidal Ion Trap
Ionisation source	Electron ionisation
MS Operating temperature	200°C
Mass range	41-500 amu
Resolution	< 0.5 m/z at 300 amu
MS scan rate	10-15 Scans/s
Detector	Electron Multiplier

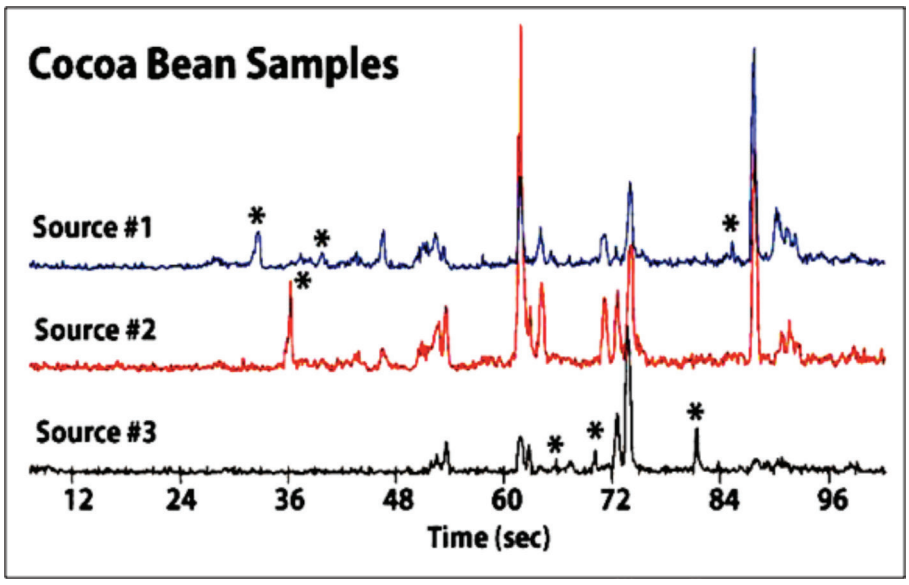


Figure 8: Chromatogram of cocoa bean headspace from three sources. Note peaks shown with an asterix (*) represent compounds marked for quality control identification purposes

Conclusion

This study has shown that VOCs and SVOCs from cocoa beans can be rapidly screened at the source using headspace SPME sampling coupled with a novel portable GC system fitted with a toroidal trap mass spectrometer. Using this combination, both field and laboratory screening of marker compounds can indicate initial raw material quality and support rapid decision making. The ability to analyse a wide variety of organic compounds in cocoa and other natural food products provides timely and important information at the commodity source. In addition, the short analysis time allows the user to quickly analyse multiple samples on-site if required and allow non-experienced personnel to make critical decisions without have to send the samples back to the lab for further clarification. However, it should be emphasised that this has been a preliminary investigation and although very promising, additional works needs to be carried out to characterise and identify a more comprehensive suite of contaminants.

References

1. Identification of the Key Aroma Compounds in Cocoa Powder Based on Molecular Sensory Correlations; F. Frauendorfer, P. Schieberle, Journal of Agriculture and Food Chemistry, 54, 15, 5521–5529, 2006.

2. Development of a GCxGC-TOFMS Method using SPME to Determine Volatile Compounds in Cacao Beans; E.M. Humston et. al., Journal of Separation Science, 32, 2289 – 2295, 2009.

3. Hand-Portable Gas Chromatograph-Toroidal Ion Trap Mass Spectrometer (GC-TMS) for Detection of Hazardous Compounds; J. Contreras, J. Murray, S. Tolley, J. Oliphant, H. Tolley, S. Lammert, E. Lee, D. Later, M. Lee, Journal of American Society of Mass Spectrometry, 19, 10, 1425–1434, (2008).

4. Trace Analysis in the Field Using Gas Chromatography-Mass Spectrometry; T. V. Truong et.al. Scientia Chromatographica, 6(1):13-26, (2014).

5. The Applicability of Field-Portable GC/MS for the Rapid Sampling and Measurement of High Boiling Semivolatile Organic Compounds in Environmental Samples; T.V. Truong et.al., Current Trends in Mass Spec, LC/GC Supplement, 20-26, July, (2016).

6. Torion T-9 Portable GC-MS Product Note, https://www.perkinelmer.com/lab-solutions/resources/docs/PRD_Torion-T-9-GCMS_012311B_01.pdf

7. SPS-3 Sample Preparation Module for Torion T-9, http://www.perkinelmer.com/lab-solutions/resources/docs/PRD_Sample_Prep_Station(013095_01).pdf