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# Extension of a dynamic headspace multi-volatile method to milliliter injection volumes with full sample evaporation: Application to green tea

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ABSTRACT

An extension of multi-volatile method (MVM) technology using the combination of a standard dynamic headspace (DHS) configuration, and a modified DHS configuration incorporating an additional vacuum module, was developed for milliliter injection volume of aqueous sample with full sample evaporation. A prior step involved investigation of water management by weighing of the water residue in the adsorbent trap. The extended MVM for 1 mL aqueous sample consists of five different DHS method parameter sets including choice of the replaceable adsorbent trap. An initial two DHS sampling sets at 25 °C with the standard DHS configuration using a carbon-based adsorbent trap target very volatile solutes with high vapor pressure (>10 kPa) and volatile solutes with moderate vapor pressure (1–10 kPa). Subsequent three DHS sampling sets at 80 °C with the modified DHS configuration using a Tenax TA trap target solutes with low vapor pressure (<1 kPa) and/or hydrophilic characteristics. After the five sequential DHS samplings using the same HS vial, the five traps are sequentially desorbed with thermal desorption in reverse order of the DHS sampling and the desorbed compounds are trapped and concentrated in a programmed temperature vaporizing (PTV) inlet and subsequently analyzed in a single GC–MS run. Recoveries of 21 test aroma compounds in 1 mL water for each separate DHS sampling and the combined MVM procedure were evaluated as a function of vapor pressure in the range of 0.000088–120 kPa. The MVM procedure provided high recoveries (>88%) for 17 test aroma compounds and moderate recoveries (44–71%) for 4 test compounds. The method showed good linearity ($r^2 > 0.9913$) and high sensitivity (limit of detection: 0.1–0.5 ng mL$^{-1}$) even with MS scan mode. The improved sensitivity of the method was demonstrated with analysis of a wide variety of aroma compounds in brewed green tea. Compared to the original 100 μL MVM procedure, this extension to 1 mL MVM allowed detection of nearly twice the number of aroma compounds, including 18 potent aroma compounds from top-note to base-note (e.g. 2,3-butanedione, coumarin, furaneol, guaiacol, *cis*-3-hexenol, linalool, maltol, methional, 3-methyl butanal, 2,3,5-trimethyl pyrazine, and vanillin). Sensitivity for 23 compounds improved by a factor of 3.4–15 under 1 mL MVM conditions.



## 1. Introduction

A number of different and varied factors must be taken into account when considering the analysis of aroma compounds in foods and beverages. In no particular order these can be summarized as low concentration levels (from pg mL$^{-1}$ to ng mL$^{-1}$), an extended volatility range {vapor pressure (*VP*) from single figure Pa to >100 kPa}, a wide range of water solubility (from single figure mg L$^{-1}$ to >10 g L$^{-1}$), and complex matrices, including lipids, proteins, amino acids, sugars, and flavonoids. Many different sample preparation techniques have been documented for isolation and extraction of aroma compounds in food prior to gas chromatography (GC) [1]. These include liquid phase extraction, gas phase extraction/distillation, and solid phase extraction, etc., but it is clear that success will depend on suitable matching of technique to analyte and matrix characteristics.

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Headspace (HS) GC has been frequently used for aroma analysis because of its ability to exploit the volatility of aroma compounds and its many practical advantages of simplicity, amenability to full automation, free from non-volatile contamination and lack of any need for solvent use [2–6]. However, conventional HS techniques such as dynamic HS (DHS) and HS solid phase microextraction (HS-SPME) exhibit greater selectivity for more volatile and/or hydrophobic compounds, often resulting in a partial chromatogram which does not include hydrophilic and/or low vapor pressure aroma compounds. Static HS (SHS) provides a flavor picture of the sample released from the matrix under given experimental conditions. In 2014, a multi-volatile method (MVM) with sequential DHS sampling using different individual trapping conditions on the same sample was developed for uniform extraction and enrichment of a wide range of aroma compounds in aqueous samples [7]. The feasibility and benefits of using the MVM approach have been demonstrated by the determination of key aroma compounds (spanning the range from highly volatile acetaldehyde to much less volatile vanillin) in brewed coffee. There are several important aspects for consideration in the development of MVM procedure: (1) proper water management for successful GC analysis, (2) choice of adsorbent trap for targeting of specific compound ranges, (3) an appreciation of the risk of breakthrough of (very) volatile compounds, (4) thermal desorption efficiency for polar and/or low vapor pressure compounds, (5) recovery of hydrophilic and/or low vapor pressure compounds from aqueous sample. Therefore, the MVM procedure consists of three different DHS sampling steps performed at increasing temperatures, including a final full evaporation DHS (FEDHS) method [8], based on a classical full evaporation technique (FET) developed by Markelov and Guzowski [9]. Firstly, DHS sampling at 25 °C using a carbon-based multi-bed adsorbent is performed for very volatile compounds (*VP* > 20 kPa) from 100 μL aqueous sample, followed by an additional DHS sampling at 25 °C using a second identical trap but with quite different purge/trap conditions for the set of slightly less volatile compounds (*VP* from 1 kPa to 20 kPa). Finally an FEDHS sampling at 80 °C using the third Tenax TA trap with much increased purge flow is performed for the remainder of volatile compounds including hydrophilic and/or low vapor pressure species (*VP* < 1 kPa). After sampling from this same HS vial, the three traps are sequentially thermally desorbed in reverse order of the DHS sampling, recombining and concentrating the desorbed compounds in the programmed temperature vaporizing (PTV) inlet for subsequent single run GC–MS analysis. This MVM approach provides a very representative image of the overall volatile fraction of an aqueous sample, but the downside in comparison to conventional HS techniques is the limited sample volume of 100 μL with corresponding limited sensitivity (usually for more volatile and/or hydrophobic compounds). There are two main reasons for the limited sample volume of 100 μL MVM. Firstly, the required water management program to properly dry the third trap in the final full evaporation step effectively dictates a sample size of 100 μL on all steps in the combined procedure, even though a larger sample size could be treated by the first two steps without the final FEDHS step. The 100 μL full evaporation of an aqueous sample requires 2.6 L of nitrogen purge gas at a flow rate of 100 mL $min^{-1}$ (thereby taking 26 min) in order to remove condensed water from the third trap and the vent line of the DHS system [8]. Secondly, hydrophilic solutes exhibit a high purge efficiency under the large phase ratio conditions of a 100 μL sample in a 10 mL HS vial, but the decrease in phase ratio for these very volatile hydrophilic compounds (e.g. acetaldehyde) when going to mL samples size in a 10 mL HS vial will have a substantial negative effect on the related purge efficiency. These considerations highlight the challenges in developing larger MVM injection of aqueous samples.

In this study, we investigated an extension of MVM technology using the combination of a standard DHS configuration, and a modified DHS configuration incorporating an additional vacuum module in order to overcome the disadvantage of limited sample volume. The use of this additional vacuum unit allows faster elimination of water from both the trap and the DHS vent line by a factor of 3 for 100–1000 μL FEDHS mode. The modified DHS configuration only requires about 5 L of purge gas for a full evaporation of 1 mL aqueous sample but now incorporates 3 sequential DHS sampling sets for recovering hydrophilic and/or low vapor pressure species. The additional vacuum unit can promote not only water elimination but also breakthrough of some compounds. Therefore, the standard DHS configuration (without the additional vacuum unit) was used for the initial DHS sampling set at 25 °C for (very) volatile compounds (*VP* > 1 kPa) prior to the second DHS sampling set at 80 °C and using the enhanced vacuum. Each DHS configuration can be automatically switched under full software control in the extended MVM procedures. Compared to the 100 μL MVM procedure the sampling steps have increased from 3 to 5 and under two different vacuum conditions, but full automation of all steps has been retained. The parameters required for all DHS sampling steps were investigated with the same test compounds, which include a wide variety of aroma compounds from top-note to base-note, spiked into 1 mL water. The improved sensitivity of the proposed method is demonstrated with analysis of aroma compounds in 1 mL of brewed green tea.

## 2. Experimental

### 2.1. Reagents and materials

Acetaldehyde, 2-acetyl pyrrole, benzyl alcohol, butanal, 2,3-butanedione, γ-butyrolactone, coumarin, dimethyl disulfide, 2,5-dimethyl-3-ethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, dimethyl sulfide, 2-ethyl-6-methylpyrazine, furan, furaneol, furfural, indole, maltol, methional, 2-methyl furan, nonanal, pentanal, 2,3-pentanedione, phenethyl alcohol, propanal, pyrrole, and vanillin were purchased from Wako Pure Chemical (Osaka, Japan). β-Damascenone, ethyl decanoate, guaiacol, *cis*-3-hexenol, 1-hexanol, and γ-nonalactone, were obtained from Dr. Katsumi Umano of Takata Koryo Co., Ltd (Hyogo, Japan). Green tea samples were purchased in local stores in Tokyo, Japan.

### 2.2. Instrumentation

MVM was performed using a GERSTEL DHS module (GERSTEL, Mülheim an der Ruhr, Germany) equipped with an additional vacuum unit consisting of ULVAC DA-15S vacuum pump (ULVAC Inc., Kanagawa, Japan), glass manifold with a Teflon lid, vacuum gauge, Nylon tubing (i.d. 1/8 in.), rubber vacuum hose, and a 3-way solenoid valve (Kendrin Kuhnke, Malente, Germany). A common line (COM) of the 3-way valve was connected to the DHS vent line, a normal close line (NC) was connected to the additional vacuum unit. The 3-way valve was switched by an in-house controller unit consisting of a 2 channel relay controller (Numato systems Pvt Ltd, Karnataka, India), OMRON MK2KP DC24 latching relay (OMRON Corp., Kyoto, Japan), and 24 VDC power supply. The controller unit was controlled by TTL (Transistor-Transistor-Logic) signal from an MPS2 autosampler and Maestro software (GERSTEL). Fig. 1 shows the procedure for the extended MVM for 1 mL aqueous sample using a standard DHS configuration (Fig. 1a) and a modified DHS configuration (Fig. 1b). For standard DHS, the DHS vent line was connected to a normal open line (NO) of the 3-way solenoid valve and purge gas was vented from NO. For modified DHS, the DHS vent line was connected to the vacuum unit thorough the NC of the 3-way valve which were controlled by Maestro software. Both