APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Quantification of Rice Aroma, 2-Acetyl-1-Pyrroline (2-AP), Using TurboMatrix Headspace Trap Coupled with GC/NPD and GC/MS

Introduction

Rice is the most important staple food for a large part of the world's human population. Rice varieties with aroma quality, known as aromatic or fragrant rice, have earned a reputation and wide popularity. 2AP was firstly identified by R. Buttery and his co-workers[1] and it was suggested as one of the key characteristic compounds of aromatic rice. It is a five-membered N-heterocyclic ring compound and its structure is shown in the Figure 1.

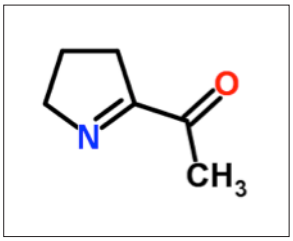

Figure 1. The structural of 2-acetyl-1-pyrroline (2AP).

In the past two decades, many techniques were reported for the extraction of 2AP in rice grains, such as purge and trap, steam distillation-solvent extraction, Likens-Nickerson simultaneous distillation-extraction, solvent extraction and solid phase microextraction. However, some of these methods are time-consuming, which require many steps for sample preparation and thus, are not appropriate to analyze large numbers of rice samples. The method employing headspace (HS) coupled with gas chromatography (GC) requires no sample preparation making it a rapid and efficient analysis technique[2-3].

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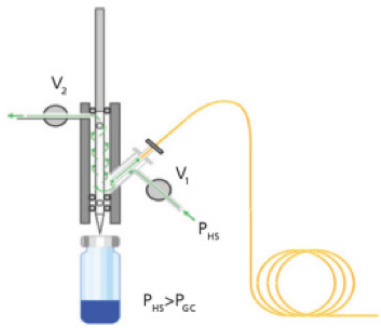
HS/GC is for applications involving the solvent-free extraction of volatile compounds. It is an unsurpassed technique, eliminating the time-consuming steps and risk of human error associated with other GC sample-preparation methods. This technique is engineered to deliver unparalleled precision, sensitivity and productivity in a broad range of specialized applications including forensics, food and beverage, pharmaceuticals, agricultural and environmental.

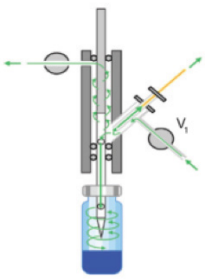
HS/GC coupled to a mass spectrometer (MS) detector provides a sensitive tool for identification of trace level volatile compounds in plant materials without involving complex extraction techniques. HS has the advantage of being a flexible, simple and a relatively economic extraction technique. TurboMatrix™ Headspace (HS) and Headspace Trap samplers are the clear choice for laboratories seeking outstanding throughput and precision.

Pressure-Balanced Technology

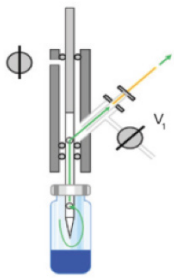
A PerkinElmer exclusive, pressure-balanced technology allows samples to be introduced into the column without using a gas syringe or multiport valves. Instead, carrier gas pressures are precisely regulated to manage transfer, eliminating many of the sources of variability and contamination found in other systems.



Step 1: Standby



Step 2: Pressurization



Step 3: Sampling

Figure 2. Pressure-balanced process.

Experimental

Chemicals and Reagents

All solvents used were analytical-reagent grade and purchased from the following sources: benzene from Merck (Darmstadt, Germany), benzyl alcohol from Fisher (Loughborough, UK), 2,4,6-trimethylpyridine (TMP), used as internal standards from Merck (Schuchardt, Germany) and 2-acetyl-1-pyrroline (2AP), from Toronto Research Chemicals (Ontario, Canada).

Sample Preparation

Rice grain samples were finely ground then the samples (0.50 g) were separately placed in headspace vial with 1.00 µl of 1000 ppm TMP as the internal standard. Headspace vials were sealed before analysis by HS-GC/NPD and GC/MS.

Calibration Procedure

Calibration standards (0.2- 10.00 µg/g) were generated by spiking varying amounts of 1000 µg/g of a standard AP in headspace vials containing 0.50 g of the non-aromatic rice (Supanburi rice). The internal standard solution, 1.00 µl of 1000 µg/g TMP in toluene, was added to each vial using the open vial sample introduction technique.

HS Trap GC/NPD and GC/MS Conditions

Static HS-GC analysis was carried out using an PerkinElmer Ltd, model SQ8 gas chromatograph-mass spectrometer equipped with PerkinElmer Ltd, model TurboMatrix 40 Trap Headspace Sampler (Figure 3). The optimum of HS-GCMS conditions are show in Table 1.

Results and Discussion

Separation and Identification of 2AP in Rice

The Turbomatrix 40 Trap headspace sampler and gas chromatography-mass spectrometric technique were developed for determination of 2AP, in rice grain samples. The chromatograms and mass spectrum of 2AP and TMP as internal standard obtained from HS trap and GC/MS were demonstrated in Figure 4 and Figure 5. The results showed that the good separation of the volatile compounds in rice samples added with TMP in Toluene were obtained from HS-GC/NPD. TMP and 2AP were eluted with the retention time at 5.05 and 5.47 minutes, respectively. Peak purity of these compounds were identified and confirmed by mass spectra data acquired from by HS-GC/MS analysis.

Figure 3. The Clarus 680 (GC/NPD) and SQ8T (GC/MS).

Table 1. Instrumental methodology.

TurboMatrix 40 Trap Headspace Sampler	
Thermostatting Temperature	120 °C
Needle Temperature	125 °C
Transfer Line Temperature	130 °C
Thermos Tatting Time	15 minutes
Pressurization Time	1.5 minutes
Trap and Dry Purge Time	2 minutes
Carrier Gas and Flow Rate	Helium, 15 psi
PerkinElmer Clarus 680 Gas Chromatograph	
Carrier gas and Flow Rate	Helium, 3 mL/min
Column	PerkinEler Elite-5MS (30 m × 320 µm ID×0.25 µm film thickness
Injection Temperature	200 °C
Temperature Programming	45-125 °C
Detector	NPD
Detector Temperature	250 °C
H_2 Flow Rate	4 mL/min
Air Flow Rate	80 mL/min
PerkinElmer Clarus SQ 8 T Single Quadrupole	
Mode	Electron impact (EI)
Inlet Line Temperature	200 °C
Source Temperature	200 °C
Mass Range	29-500 *m/z*
Software	TurboMass 6.1

Method Validation of 2AP

The method of TurboMatrix 40 trap headspace sampler and gas chromatography with mass spectrometer were developed for quantification of 2AP in fragrant rice using HS/GC. Calibration curve for 2AP analysis by headspace was generated by spiking known concentrations of the analyte into a non-fragrant rice variety (Supan Buri). The correlation between detector response was measured in terms of peak area ratios between 2AP and TMP. The response of 2AP standard was linear over a concentration range of 0.10 - 10.00 µg/g of rice samples using NPD detector with a correlation coefficient (r^2) 0.9924, Figure 6. The effective linear concentration ranges of the method were in the range of ranged 0.20-10.00 µg of 2AP/g of rice sample for HS-GC/NPD[4].

The percentage recovery of 2AP in the first headspace extraction step was 45.66%. Method validation performed for this developed SHS-GC/NPD method demonstrated the limits of detection (LOD) and limits of quantitation (LOQ) at 0.10 µg of 2AP and 0.0500 g of rice samples, respectively. The intraday and interday coefficients were 2.25% RSD (n=10) and 4.60% RSD (n=35), respectively.

Analysis of Rice Samples

These developed methods were applied to quantify the amount of 2AP in the rice samples. The concentration of 2AP found in all rice samples are shown in Table 2. It was observed that the amount of 2AP in rice sample were in the range 1.22-2.58 µg of 2AP/g of rice samples[5-6].

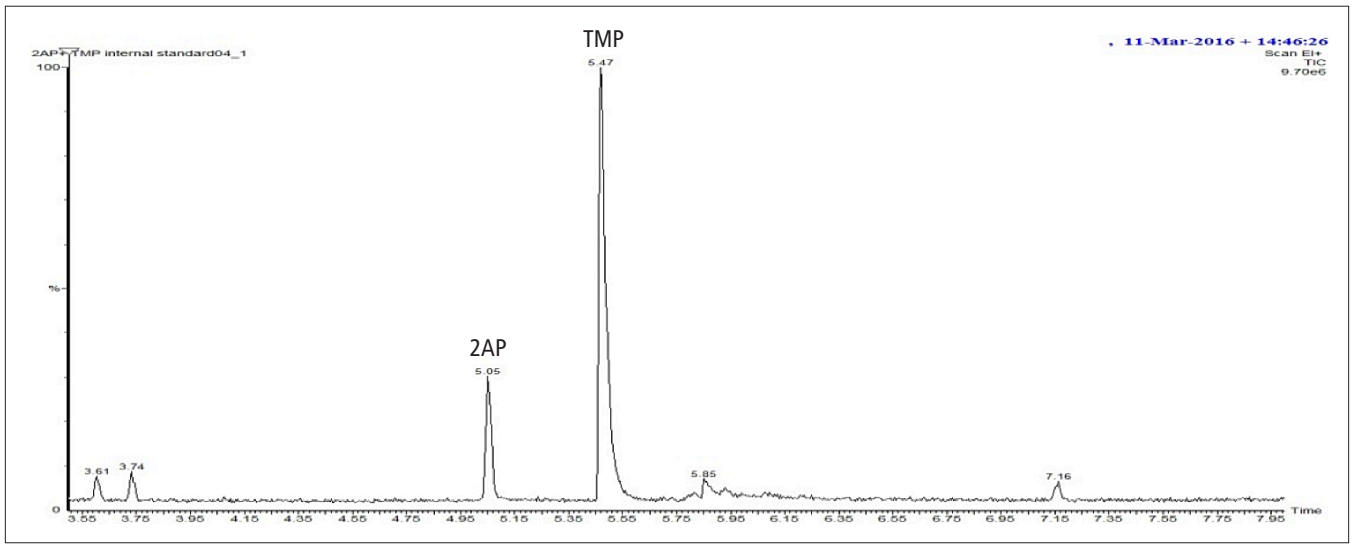


Figure 4. The chromatogram of 2AP and TMP standard obtained from HS trap and GC/MS.

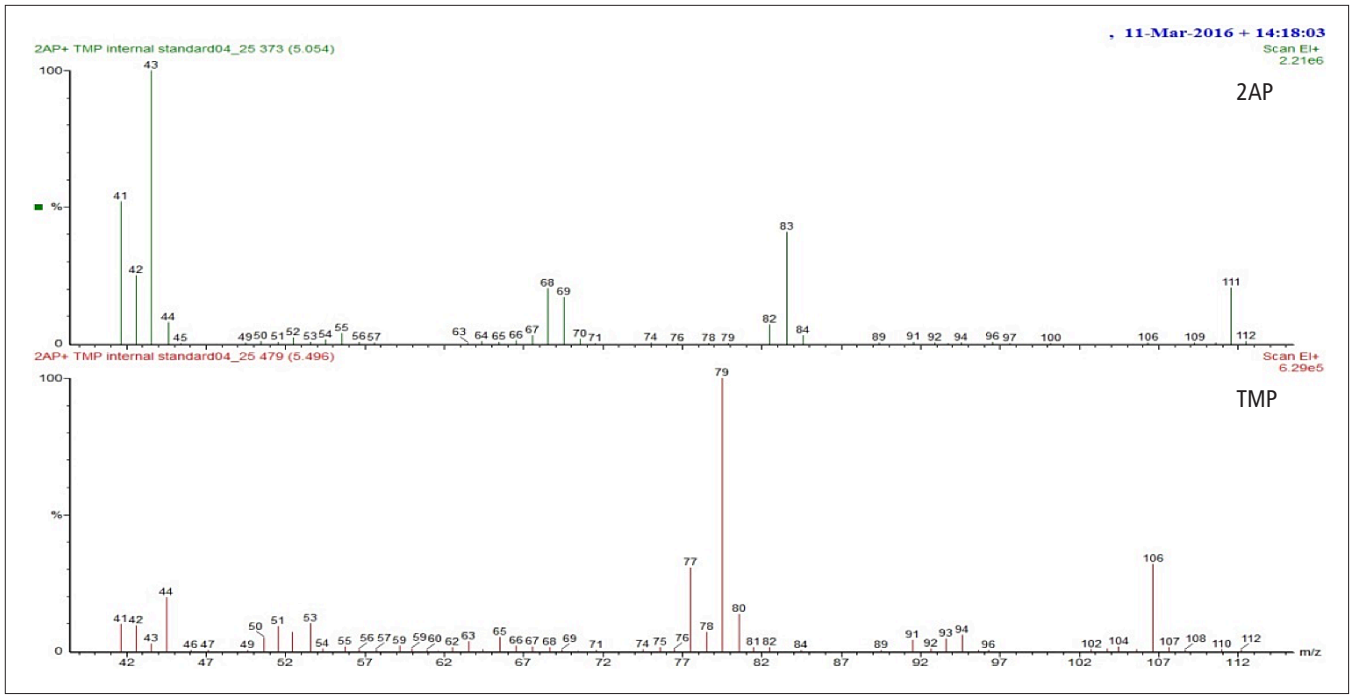


Figure 5. The mass spectrum of 2AP and TMP obtained from HS trap and GC/MS.

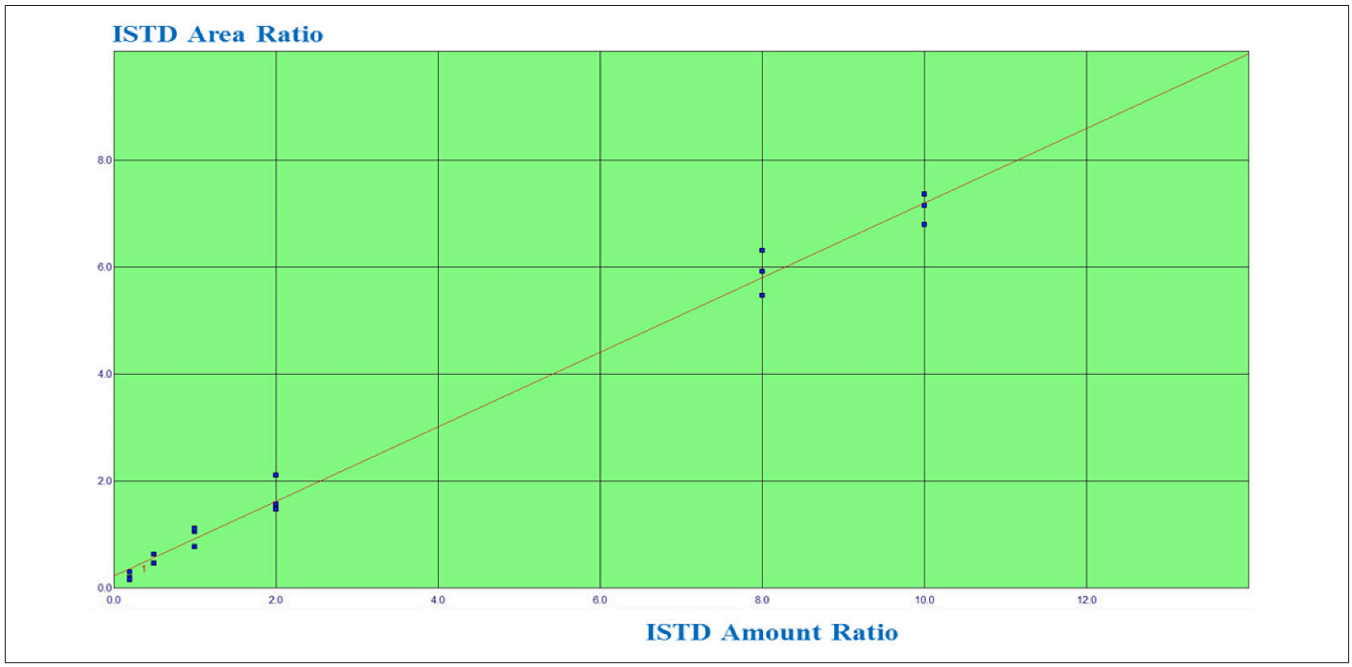


Figure 6. The calibration plot of a standard curve 2AP /TMP obtained from HS trap and GC/NPD concentration at 0.10-10.00 µg 2AP/g of rice sample.

Table 2. Quantification analysis of 2AP in Thai fragrance rice samples using HS-GC/NPD.

Rice Samples	Concentration of 2AP, µg/g, (mean ± SD)
KDML 105 (CR) Rice	2.53 ± 0.08
KDML 105 (Dech Udom) Rice	1.70 ± 0.10
Moo Rice	2.58 ± 0.06
Phakmai Rice	1.22 ± 0.05
Srisaket Rice	1.55 ± 0.01
Yasothron Rice	1.99 ± 0.01

Conclusions

The automated HS-GC/NPD technique was developed and applied for the analysis of various type of rice samples. This method was successful for the determination of a key aroma compound, 2AP, in rice sample with different varieties. The method described in this study is rapid, convenient and requires little sample preparation making it an ideal analysis tool for aroma analysis of rices.

Acknowledgement

The automated HS-GC/NPD technique was developed and The authors thank Assoc. Prof. Dr. Sugunya Mahatheeranont for her thoughtful advice and suggestions. We also would like to take this opportunity to thank Assoc. Prof. Dr. Aphichat Vanavichit for providing laboratory and facilities to make this study possible.

Acknowledgement

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