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Application News

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Food / Nexis GC-2030

Determination of Volatiles in Liquors by GC-FID with Dual Acquisition System

❑ Introduction

Alcoholic beverages contain various volatile compounds that are desirable for their flavors [1]. Unfortunately, some unwanted volatile compounds (due to their toxicities) could be present too and need to be reduced or removed in the products. For alcoholic beverages with low amount of ethanol (below 10%), such as beer, headspace coupled with gas chromatograph (HS-GC) technique could be utilized to monitor the volatile compounds [1]. However, liquor or spirit drinks are not suitable for headspace due to their high ethanol content. Thus, liquid injection was utilized for this experiment. Due to the various volatile components present in liquors, it was not feasible to separate all of them in one column. To avoid frequent downtime, this test was carried out using GC with dual acquisition system available in Shimadzu Nexis GC-2030.

Figure 1: Dual AOC-20i/s Autosampler on Nexis GC-2030

❑ Experimental

Analytical conditions

The experiment was performed using Nexis GC-2030 with Dual AOC-20i/s Autosampler. Since the target volatile compounds require 2 columns for separation, a dual acquisition system was prepared. This comprised of 2 injection ports (SPL), 2 columns, 2 detectors (FID) and a dual injection autosampler (Figure 1). With this configuration, the analyses could be carried out without the inconvenience and downtime of changing column and moving the autosampler.

The target volatile compounds were divided into 2 mixtures (Table 1). The separations of Mixture 1 and Mixture 2 were done using Rtx-502.2 column and SH-Stabilwax column, respectively. The analysis conditions are displayed in Table 2.

Table 1. List of Target Compounds

Mixture 1				Mixture 2	
No.	**Compound**	**No.**	**Compound**	**No.**	**Compound**
1	IPA	6	n-butanol	1	Acetaldehyde
2	Acetone	7	Isoamyl alcohol	2	Methanol
3	n-propanol	8	2-methyl-1-butanol	3	2-butanol
4	Ethyl acetate	9	Isoamyl acetate		
5	Isobutanol				
Internal Standard: 3-pentanol					

Table 2. Analysis Conditions

Instrument	**Nexis GC-2030**		
Autosampler	**Dual AOC-20i/s**		
Analytical Condition 1 (Mixture 1)		**Analytical Condition 2 (Mixture 2)**	
Injection volume	0.5 µl	Injection volume	0.5 µl
Injection temp	200°C	Injection temp	200°C
Injection mode	Split (split ratio = 10)	Injection mode	Split (split ratio = 10)
Flow control mode	Linear velocity	Flow control mode	Linear velocity
Linear velocity	36 cm/s	Linear velocity	57.8 cm/s
High pressure injection	200 kPa for 1.5 min	High pressure injection	200 kPa for 1.5 min
Carrier gas	Helium	Carrier gas	Helium
Column	Rtx 502.2 (30 m length, 0.25 mm ID, 1.4 µm df)	Column	SH-Stabilwax (30 m length, 0.32 mm ID, 1 µm df)
Column temp program	35°C (hold for 5 min) → rate: 30°C/min → 70°C (hold for 5 min) → rate: 30°C/min → 200°C (hold for 3 min)	Column temp program	40°C (hold for 1 min) → rate: 30°C/min →130°C (hold for 5 min)
Detector (FID) temp	220°C	Detector (FID) temp	220°C

Sample Preparation

All calibrations standards were prepared in 40% ethanol as the diluent. The concentrations of the calibration standards were 30, 150, 300 and 600 ppm (v/v) for each mixture. All the calibration standards and liquor samples were spiked with 300 ppm (v/v) IS compound (3-pentanol).

❑ Results and Discussion

The samples consisted of high content of water. Water is known to produce a very high expansion volume when heated in the injection port. This would create a very large pressure, leading to a risk of sample flowing back to the injection port lines.

This phenomenon is called backflash. To prevent backflash, injection was done at a low injection volume (0.5 µl). For this purpose, a 5-µl syringe (P/N: 221-75173) was used to give a better accuracy instead of the usual 10-µl syringe. To reduce the water expansion volume, a high pressure during injection was implemented using the **High Pressure Injection** feature in Shimadzu Nexis GC-2030.

Separation and Calibration

Mixture 1 Standard

Mixture 1 Standard was successfully separated (Figure 2). Internal standard calibration curve of each compound attained good linearity (R^2 > 0.9997), as shown in Figure 3.

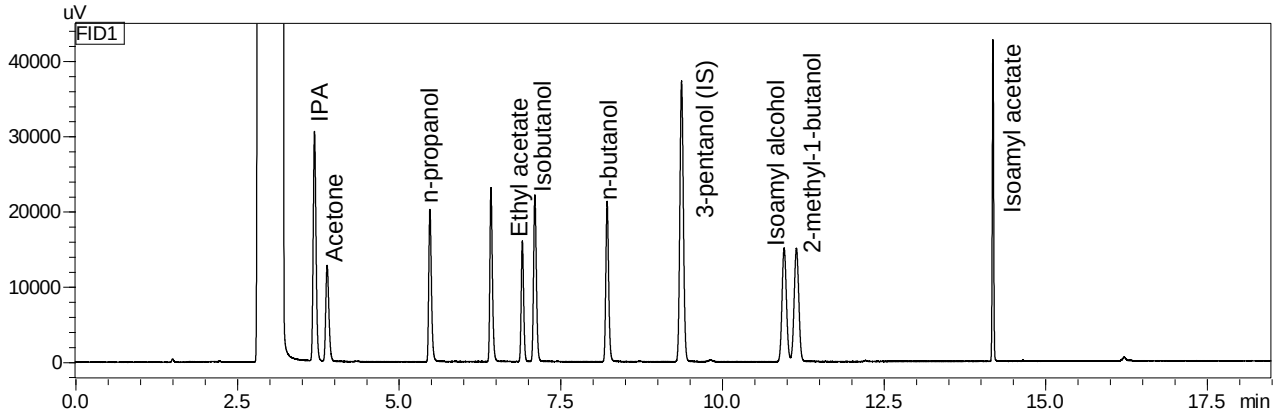

Figure 2: Separation of 150 ppm (v/v) Mixture 1 Standard with 300 ppm (v/v) 3-pentanol (IS) in Rtx-502.2 column

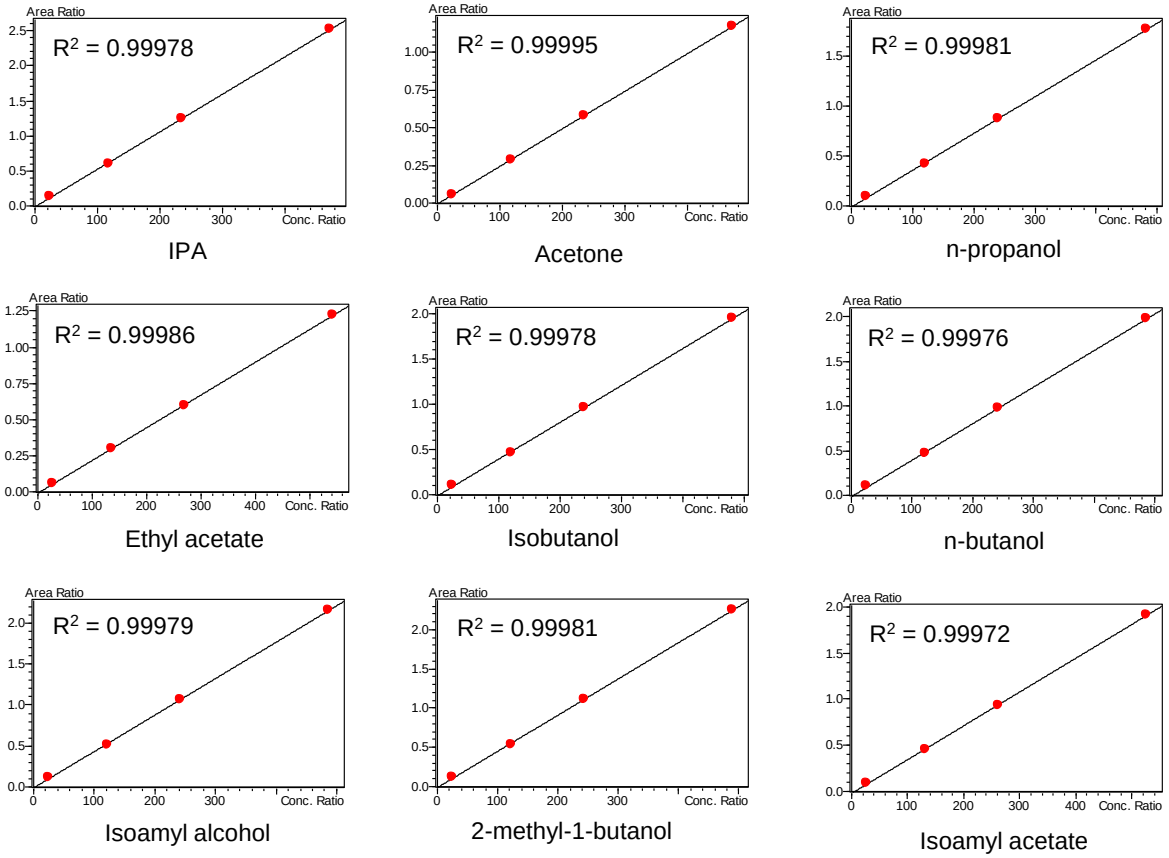

Figure 3: Internal Standard Calibration Curves of Mixture 1 Standard, 30-600 ppm (v/v)

Mixture 2 Standard

Figure 4 shows the well separated compounds in Mixture 2 Standard. Excellent linearity of internal standard calibration curves was achieved (R^2 > 0.9998), as seen in Figure 5.

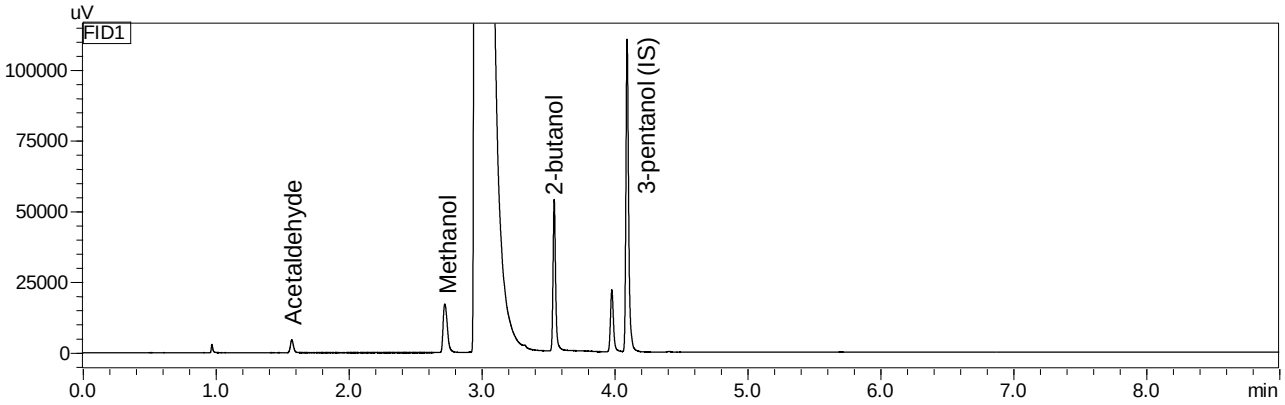

Figure 4: Separation of 150 ppm (v/v) Mixture 2 Standard with 300 ppm (v/v) 3-pentanol (IS) in SH-Stabilwax column

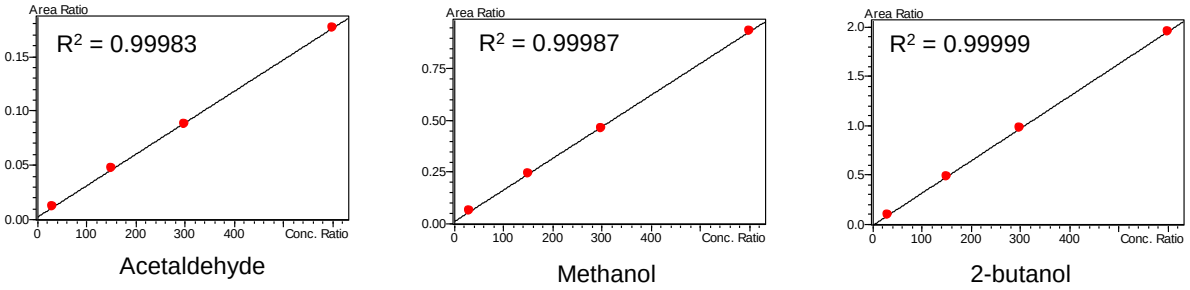

Figure 5: Internal Standard Calibration Curves of Mixture 2 Standard, 30-600 ppm (v/v)

Quantitation

Two samples, Liquor 1 and Liquor 2, were analyzed after spiked with the same amount of internal standard compound. Figure 6 shows the comparison of Liquor 2 and the standard samples.

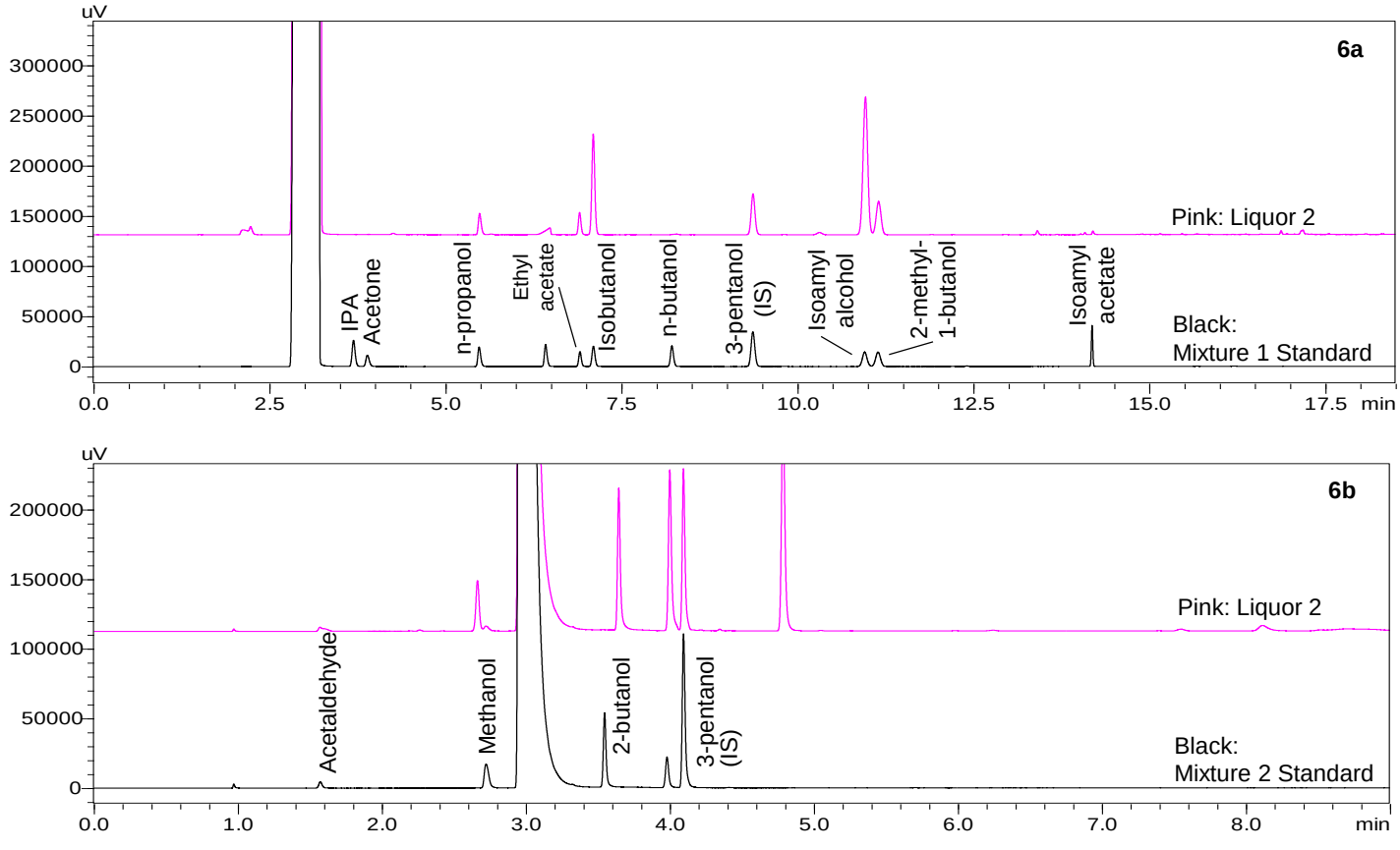

Figure 6: Chromatograms of Liquor 2 compared to Mixture 1 Standard (6a) and Mixture 2 Standard (6b)

Table 3 displays the quantitation results of volatile compounds in the liquor samples (Liquor 1 and Liquor 2). Mainly, the detected compounds are the commonly found fusel oils, esters and aldehydes which give aroma and flavor to liquors. Methanol, a toxic compound, could be present in liquors due to fermentation process. The methanol contents in the 2 samples were 177.47 ppm and 37.72 ppm, respectively.

As a comparison, the EU limit of methanol in traditional fruit spirits is 10 g methanol/l ethanol, which is equivalent to 0.4% (v/v) or 4000 ppm (v/v) at an ethanol concentration of 40% (v/v) [2]. Meanwhile, in ‘‘agricultural spirit’’ which is the starting material for many alcoholic drinks, the EU limit of methanol is 0.02% (v/v) or 200 ppm (v/v). IPA and acetone are unusual to be found in liquors, and if they are present, it might be due to contamination.

Table 3. Quantitation Results

Compound Name	Concentration in ppm (v/v)	
	Liquor 1	Liquor 2
Isopropanol (IPA)	3.71*	Not Detected
Acetone	Not Detected	Not Detected
n-propanol	113.77	121.05
Ethyl acetate	182.49	186.96
Isobutanol	490.13	524.53
n-butanol	8.97*	9.20*
Isoamyl alcohol	974.93*	1048.01*
2-methyl-1-butanol	237.30	254.26
Isoamyl acetate	15.17*	17.47*
Acetaldehyde	130.84	109.88
Methanol	177.47	37.72
2-butanol	Not Detected	Not Detected

*Outside of calibration curve range. Adjustment of calibration curve levels is needed for accurate quantitation.

❑ Conclusion

Separation and quantitation of volatiles in liquor samples were carried out by Nexis GC-2030. Shimadzu GC is capable of using dual acquisition system which reduce downtime and avoid the inconvenience of changing the column and moving the autosampler frequently. Additionally, the occurrence of backlash (due to high water containing sample) was prevented using a 0.5 µl injection volume and High Pressure Injection feature. The resulting method is useful for quality control of flavour and aroma as well as detecting the undesirable volatile compounds in liquor samples.

❑ References

[1] Dragone, C. et al, Characterisation of volatile compounds in alcoholic beverage produced by whey fermentation, Food Chemistry, 112 (2009) 929-935.

[2] Paine, A., Dayan, A., Defining a tolerable concentration of methanol in alcoholic drinks, Human & Experimental Toxicology (2001) 20, 563 –568.

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