APPLICATION NOTE

Gas Chromatography

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A Method for the Quantification of Ethanol Content in Consumable Fruit Juices by Headspace Injection

Introduction

The definition of an alcoholic beverage in the United States of America is a beverage that contains in excess of 0.5% ethanol by volume that is intended for consumption alone or when diluted. Production of alcohol has been long established in society with many styles that take advantage of the metabolism of sugars into ethanol. While the production of ethanol is desirable for alcoholic beverages, it is undesirable for other beverages which contain sugars that do not wish to be sold as an alcoholic beverage. Such sugar metabolism is naturally occurring and is well understood to happen in raw fruit as well as processed juice and can vary by type, variety and maturation in the growing season.

A new application has been developed in the accurate determination of ethanol content in samples of these products utilizing the PerkinElmer® TurboMatrix™ headspace (HS) autosampler for better reproducible results. Additionally, since ethanol is the only desired peak, this method allows for a quick run time giving users the opportunity to analyze high volume throughput samples multiple times. The main focus of this method is intended toward fruit juices and it is confirmed to give accurate results amongst a wide range of store bought juices. This application note outlines the principles and technology of this method in the analysis and quantification of ethanol in consumable juices.

Experimental

System

Gas Chromatograph	PerkinElmer Clarus® 580
Injector	Programmable Split Splitless (PSS)
Detector	FID
Electronic Pneumatics	PPC Carrier for PSS (Hydrogen), PPC FID Gases (Air & Hydrogen)
Column	30 m x 0.32 mmID x 1.8 µm Elite BAC-1 Advantage #N9315071
Headspace Apparatus	TurboMatrix
Data Analysis	Data processed on Waters® Empower® 3 software

Headspace Conditions

Temperature	Oven 60 °C, Needle 110 °C, Transfer Line 120 °C
Timing	Thermostat - 12 min, Pressurize – 1 min, Injection – 0.02 min, Withdraw – 0.3 min
Pressure	16 psig of Hydrogen Gas
Transfer Line Column	Split connection 2 m of 0.32 mmID fused silica, terminated in injector
Options	Operative Mode: Constant Inject Mode: Time

GC Conditions

GC Oven	45 °C Isothermal, Run Time: 2.50 min Equilibration Time: 0 min
Carrier Pressure	12 psig for 2.50 minutes, Split Flow 5.0 mL/min
FID	Range: x1 Attn: x-1 Temp: 250 °C Air: 450 mL/min H2: 45 mL/min

Reagents

Off the shelf juices and deionized water are used for sample preparation. The internal standard solution used is t-butanol in deionized water.

Calibration

Ethanol standards with known amounts over the quantification range of 50 to 500 mg/dl ethanol v/v with an ISTD at a constant concentration. Vail and capped securely with headspace vial crimper.

Sample Preparation A 50 µL volume of t-butanol is diluted in 250 mL of deionized water attached to the automatic dilutor. Precisely 75 µL of a juice sample and 750 µL deionized water/internal standard mixture are combined with an automatic dilutor into a standard autosampler vial. The vial is then securely sealed with a headspace vial crimper.

Table 1. Retention times of BAC compounds.

Compound	Retention Time (min)
Methanol	0.663
Acetaldehyde	0.697
Ethanol	0.806
Isopropanol	0.957
Acetone	1.033
t-Butanol	1.112
n-Propanol	1.268
Ethyl Acetate	1.924

Note: These compounds will not all be present in all fruit juices but are used to show the proper separation from ethanol in the case that an addition peak is present. It was also used to determine a reliable internal standard that would not co-elute with the ethanol. In this method t-butanol was used as the internal standard.

Results

It is necessary to have a good calibration curve and an internal standard for reference. Since ethanol has a very distinct and repeatable retention time, it allows for reliable integration of the area of the peak. The internal standard used is t-butanol, which elutes well after ethanol. The isothermal GC method allows for a minimum time between injections of 3.0 minutes, also referred to as PII (period from injection to injection). As expected the calibration produces an excellent quantitative linearity (0.997) and a high precision (1 % RSD) was seen at 500 ppm of ethanol.

Several commercial juices were analyzed for ethanol content with the results in Table 2.

Table 2. Ethanol content of selected store purchased juices.

Fruit Juice	Concentration of ethanol (mg/dl)
Orange juice A	56.5
Orange juice B	3.7
Mixed berry juice	57.0
Grape juice	236.2
Lemonade	13.2
Apple juice	86.4
Pomegranate juice	39.7

The results would indicate that the ethanol content in all cases is safely below the required limit at which a beverage is considered to be alcoholic. Also, the data suggests that the ethanol content is independent of the variety of fruit in the juice but further analysis at the point of manufacturer would be required to definitively make such a claim. It has been shown that the ethanol content that occurs naturally in different varieties of orange, for example, could be the cause of the different results between the two orange juices examined.

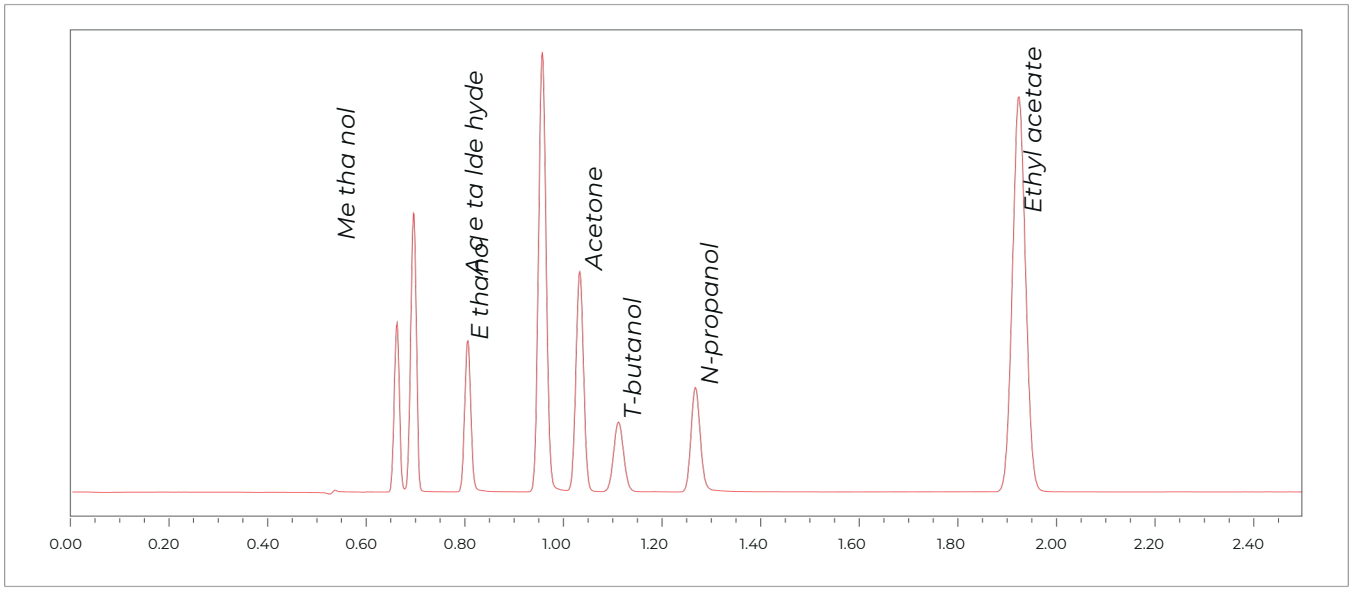

Figure 1. Chromatogram showing the elution order of the BAC mix that identified t-butanol as a suitable internal standard for the ethanol analysis.

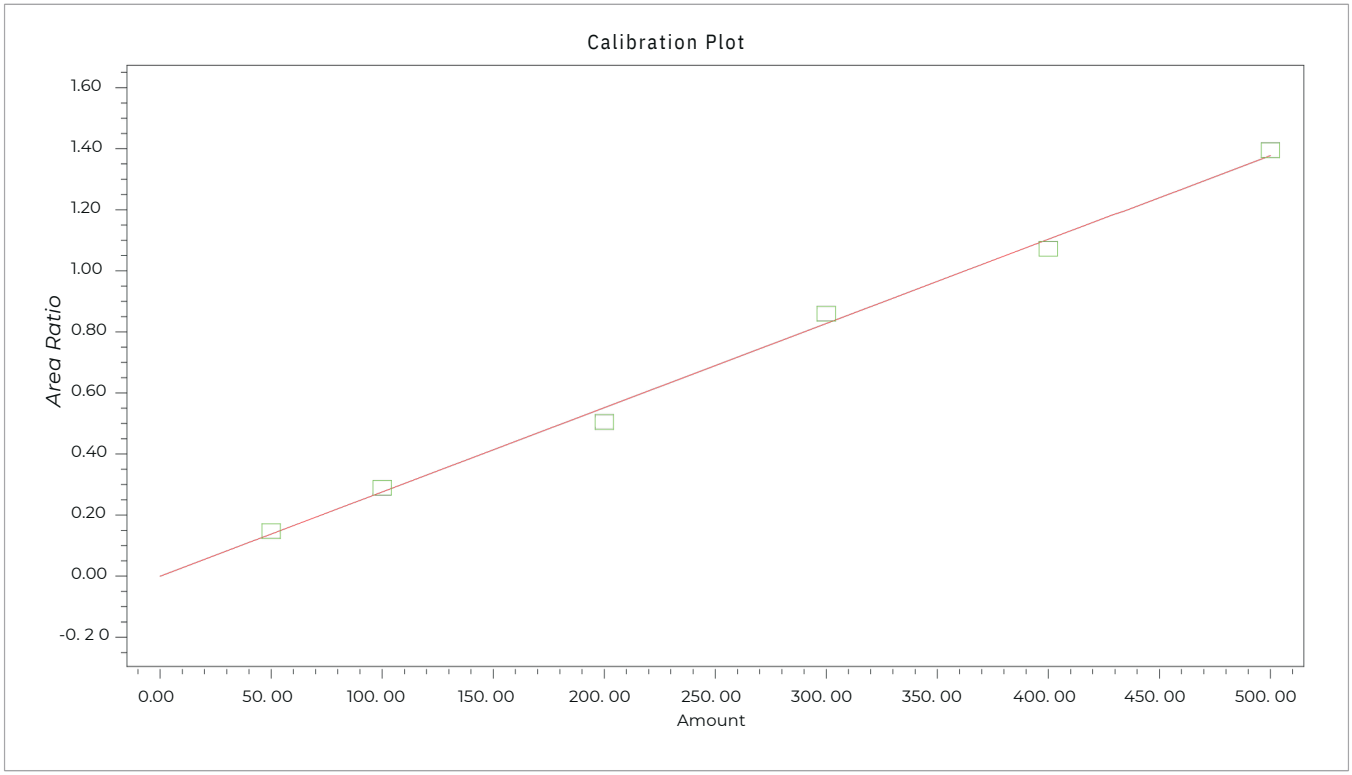

Figure 2. Calibration curve of ethanol used for the analysis of fruit juice.

Conclusion

This simplified method allows for favorable results in the quantification of ethanol content in consumable fruit juices. Additionally, the headspace introduction of the sample to the GC ensures that the amount of sample that gets on to the column is consistent. By decreasing the chance of errors in the preparation leads to concrete results that can be used as valid proof. These results are obtained from a TurboMatrix HS autosampler and the necessary time taken in sample preparation of each individual run due to the sensitivity of the headspace.

For Further Reading:

1. Timothy D. Ruppel; PerkinElmer Field Application Report, "Blood Alcohol Analysis Utilizing Headspace Autosampling and Dual-column GC Confirmation"
2. Timothy D. Ruppel; PerkinElmer Field Application Report, "Simulaneous and Rapid Separation of Blood-Alcohol Compounds and Commonly Abused Inhalants by Headspace-Gas Chromatography"
3. Paul L. Davis; Florida State horticultural Society, 1971, Pg 217- 222

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