APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Method Development for Identification of Adulterated Spirits using Field Portable GC/MS

Introduction

The spirits drink industry is the most valuable European agri-food export sector (€10bn in exports, representing a trade surplus close to €9bn). Distilled spirits are as diverse as the EU's Member States, spanning 46 product categories, and include a host of geographically-specific products that contribute to the culture of their regions and the European Union. Scotch Whisky, for example, is the largest category of whisky in the world, and is sold in almost 200 countries worldwide. The value of Scotch Whisky exports for 2014 were reported at £3.95 billion, equating to around £135 per second to the UK balance of trade. Scotch Whisky and other European spirits are premium global products, and are therefore a major target for illegal counterfeiters.

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Counterfeiting has an impact on direct sales: it damages the reputation of brands and spirit categories. Safety issues are also a concern, and these impacts are therefore relevant to all spirit producers. Counterfeiters use denatured industrial alcohol to produce fake and adulterated spirits, which degrades the product and can pose a significant health risk to those consuming it.

There are chemicals specifically added to denatured alcohol to make it undrinkable; added at trace levels, as required by law, they can pose a significant health risk if consumed. Methyl ethyl ketone and methanol are examples of such compounds added to alcohol to prevent consumption.

A number of analytical techniques have been utilized for detecting adulterated whisky and spirits. These methods are often laboratory-based, and rely on experienced operators to carry out the analysis. A very simple method has been developed using a field-portable GC/MS to detect adulterated Scotch Whiskey and other spirit products. In this application note, we will be focusing on the analysis of various compounds that can aid in the detection of adulterated Scotch Whiskey. Other markers in denatured alcohol are unique in their chemical structure, and can easily be detected and positively identified by GC/MS. The mass range of the Torion® T-9 (41-500 m/z) falls just short of being able to detect methanol which is frequently used in combination with other compounds as a marker for denatured alcohol. However, the method described here provides the ability to reliably detect adulterated spirits without the need for detecting methanol, and doing so directly in the field, either at a port of entry, a distribution warehouse or point of sale, which can help keep these dangerous products from reaching the consumer.

To successfully take a sophisticated technique such as GC/MS to the field requires the development of simple robust hardware, easy-to-use software, and complementary sample preparation and introduction techniques. These systems are often put in the hands of non-chemists who do not have in-depth training on GC/MS sample preparation and data analysis. The PerkinElmer Torion T-9 is a fully self-contained, ruggedized, field-portable GC/MS. Battery-operated, with an internal carrier gas supply, and easy to use software, the system can be easily operated in the field by a non-chemist. Successful deployment of the T-9 to the field is also dependant on development of a sampling method that is compatible for the task, and presenting the data analysis of the results to the analyst in a clear actionable form. In this paper we focus on the complete method development to rapidly detect and identify denatured alcohol in Scotch Whisky and other spirits. The method uses the trace compounds added to denatured alcohol in the 1 -5% concentration ranges for detection and identification. These chemical markers in denatured alcohol are unique in their chemical structure, and can easily be detected, and positively identified, by GC/MS. The use of GC/MS, with its gas chromatography portion as a separation front end, ensures that the flavour and aroma compounds present in spirits do not interfere with the detection, by masking the marker compounds, or giving rise to false identifications. Because counterfeiters may attempt to chemically remove some of the markers, further lowering the concentrations to prevent detection, the high sensitivity of the GC/MS technique ensures positive marker detection, even at very low concentrations.

Method Development

A general purpose GC/MS method was selected for the analysis, based on previous experience. The method parameters are listed in Table 1. Next, standards of the denatured marker compounds at concentrations in the mid-ppm and low-percent range were spiked into a mixture of water with 40 % ethanol. Samples were initially prepared by pipetting 1 ml of sample into a 10 ml headspace vial, which was then sealed with Teflon coated septum. The samples were allowed to equilibrate for 10 minutes. The volatile compounds in the mixture will partition between the liquid and the headspace above the liquid in the sealed container. The headspace above the liquid was sampled for periods of time ranging from 30 seconds to five minutes, to determine an optimum sampling time. Experimentation with the various concentration ranges and sampling times are steps in optimization of the method. The Custodion™ SPME sampling syringe was used to sample the headspace of the sample container The SPME fiber is 1 cm in length, and is coated with an adsorbent material. A general-purpose, wide-boiling-point range, fiber coating was selected for this application (PDMS): polydimethylsiloxane/ (DVB) divinylbenzene. By exposing the fiber to the headspace above the sample, volatile components are collected on the fiber for subsequent injection into the GC/MS.

Table 1. Method Parameters.

SPME	PDMS/DVB
GC Injector	270 °C
GC Column	MXT-5 5 m x 0.1 mm id, 0.4 um df
GC Carrier Gas	50 °C initial hold for 10s, programmed at 2 °C/s to 270 °C
GC Column Temp.	43.92
GC Run Time	2 minutes
Injection	Split 10:1
Mass Analyzer	Toroidal ion trap
Scan Range	41-500
Transfer Line	250 °C
Ionization	-70 eV in trap electron impact

A reference library (target compound method) of denatured marker compounds was built using the prepared standards. The target compound library consists of the GC retention time and MS mass spectra; these two parameters are used for orthogonal identification, meaning that two different sources of information are employed cooperatively to ensure identification accuracy. Table 2 is a list of the reference library for marker compounds that can be found in German, European and United States denatured alcohol products.

Table 2. Target List

Compound	Retention Time	CAS Number	Mass Spectrum
Isopropyl Alcohol	11.30	67-63-0	44, 45*, 47,59,61,69
Methyl ethyl ketone	16.88	78-93-3	43, 57, 72, 73*
Methyl isopropyl ketone	21.99	563-80-4	43*, 71, 86, 89
Methyl isobutyl ketone	29.42	591-78-6	43, 57, 58, 85,100,101*
Ethyl sec-amyl ketone	48.78	541-85-5	43, 57, 71, 72, 73, 99,128,129*

**Represents base peak*

The method was tested for detection of the denaturants by spiking European and German method denatured alcohol into a blended Scotch Whisky. When analyzed, both produced positive detection for the denatured compounds.

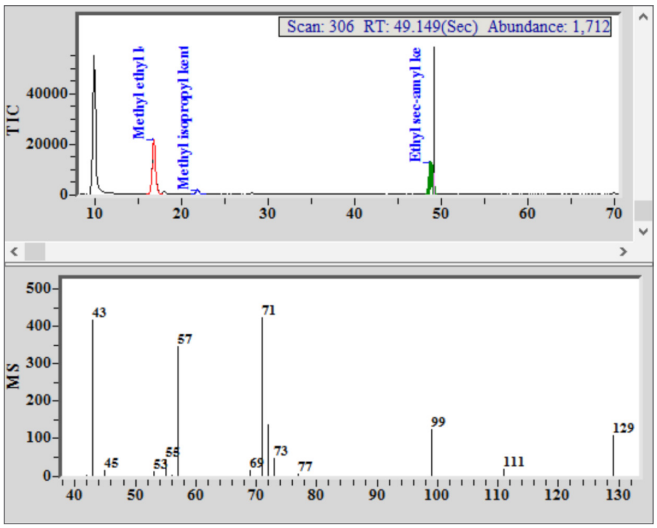

Figure 1. Chromatogram and mass spectrum, with identification of marker components in a Euro spike of a blended Scotch Whisky

GC/MS results are typically presented as a list of chemical compounds that have been detected. In the laboratory an analyst knows how to interpret the data. However, in the field, the non-specialist will not necessarily know that the detection of methyl isopropyl ketone represents the detection of denatured alcohol. The T-9 software includes the ability to flag specific compounds as hazardous, and the use of hazard levels alerts the operator that one or more of the denatured alcohol markers has been detected. Figure 2 is an example of the alert screen that is displayed on the T-9 when one or more compounds in the target library are detected.

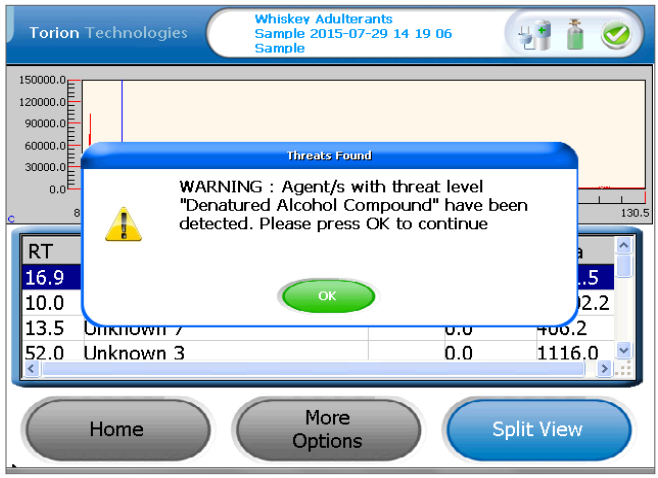

Figure 2. An example of an 'Alert' when a target compound in denatured alcohol is detected.

Method Validation

Tests were then performed to determine the minimum level of identification, and dynamic range of identification, using one of the blended Scotch Whisky products. Denatured alcohol from the USA was spiked into the whisky at levels ranging from .1% (1000 ppm/v) to 10% (100,000 ppm). The Material Safety Data Sheet (MSDS) for the denatured alcohol product lists the component concentration ranges as 1 – 4% for (MIBK) Methyl Isobutyl Ketone in the product. The resultant concentration ranges for the MIBK, when using a spike of 0.1 to 10 % denatured alcohol, is therefore approximately 10 – 4000 ppm/v. The lower level of identification was determined to be 0.25% denatured alcohol or from 25 to 100 ppm/v of MIBK (1- 4% concentration in the denatured alcohol).

Table 3. The spirit drinks employed in this study.

Qty	Spirit Drinks
2	Blended Scotch
2	Single Malt
2	Tennessee whiskey
1	Canadian whiskey
1	Irish whiskey
3	Rums (2 flavoured)
2	Vodka (1 flavoured)
1	Tequila
1	Liquor

During this next stage of method validation, a simplified sample preparation step was developed. The concept was to make sampling as easy, and as cost effective as possible in the field. The use of the 10 ml headspace vials used during initial method development would be costly and cumbersome in the field. Polypropylene autosampler tubes (15 ml), commonly used in laboratories, provided a cost-effective, easy-to-use field sampling device (PerkinElmer part # B0193233). The tubes are graduated so that the spirit drink can be easily poured into the tube, ensuring an accurate volume. Use of the sampling tubes eliminated the need for pipetting, and use of a crimper to seal the vial, thus reducing the cost of consumables required for the analysis.

The sample preparation steps are adding 2 ml of the spirit to be tested to the sampling tube, capping with the screw cap top provided, and equilibrating for five minutes. After five minutes, the cap is then removed, the CUSTODION SPME syringe inserted into the head space and held firmly against the top of the tube. The SPME fiber is then exposed to the headspace for 30 seconds. The sample, adsorbed onto the SPME fiber, is then injected into the GC/MS for analysis.

Figure 3. Sampling with the CUSTODION. The SPME fibre is housed in a durable syringe that is operated like a ball point pen.

The 15 spirit drinks were prepared using this method, sampled and run unadulterated with zero false positive detection events. Denatured alcohol was then spiked into the drinks at a concentration of 0.25%. All 15 samples were then run with 100% positive detection. The spirits were then spiked with at a denatured alcohol concentration of 10%. The denatured marker MIBK was again positively detected. The final step in method development was to further simplify the reporting so that only compounds listed in the target library are reported.

Summary

The use of field-portable GC/MS provides rapid reliable determination of the presence of denatured alcohol when added to spirit drinks to raise the alcohol content. The low detection limits, based on the addition of 0.25% by volume of denatured alcohol, affords confidence that even if steps are taken to partially remove the denatured alcohol markers they will be detected. It should also be noted that the test presents a more challenging scenario than would be expected in practice: that is the use of 100% denatured alcohol as a counterfeit white spirit. The analysis time is approximately 10 minutes per sample. The data analysis software has been setup to provide results in a "non-chemist" format so that there is no ambiguity for the operator.

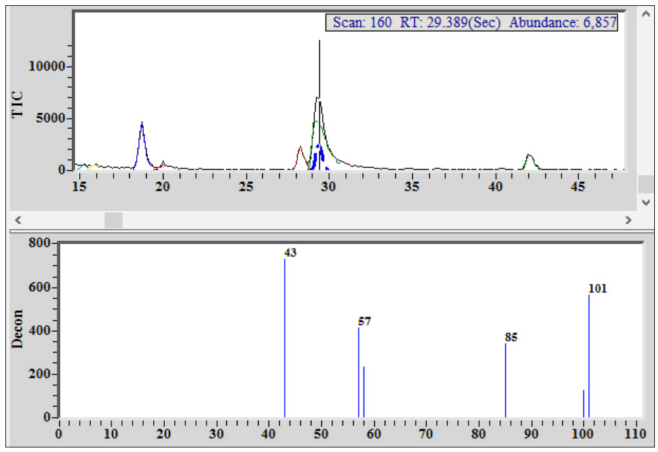

Figure 4. Chromatogram from single malt Scotch whiskey showing detection and identification of methyl isobutyl ketone (MIBK) co-eluting with a flavor/aroma compound.

Acknowledgements

The initial method development was conducted at The Scotch Whisky Research Institute (SWRI), Edinburgh Scotland. Peter Cockburn of SWRI and Dr Andrew Hobson of Quantitech Ltd contributed to the development.

The Scotch Whisky Research Institute is the Research & Technology Organization for the Scotch whisky industry. Its mission is to ensure sustainability and protect Scotch whisky as a premium global product. Its work for this method development was part of the EU FoodIntegrity Project. The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

As well as doing fundamental research for the benefit of the industry, the SWRI undertakes generic Scotch whisky authenticity analysis on behalf of the Scotch Whisky Association's legal department. Their analytical reports are used to support prosecutions against counterfeiters worldwide. Every year they analyze around 150 suspect whisky samples sourced from around the world.

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