BEYOND MEASURE

SCION INSTRUMENTS

# The Analysis of Flavours in Beer with SCION ChromSync Software

Application Note AN0024

## INTRODUCTION

One of the most widely purchased beverages in the world is beer. With the consumer market so large, breweries are developing their products to have its own distinct flavour. It is vital that breweries test and monitor the flavour compounds during the production process to ensure that the same flavours are consistently achieved. The volatile compounds that make up the flavour composition must therefore be profiled batch to batch.

Gas chromatography (GC) is often the instrumentation of choice for the analysis of flavour active volatiles in beer. Compass Chromatography Data System (CDS) is a state of the art chromatography software platform that controls GC instruments whilst offering automated processing and reporting of results. ChromSync is an application add on specifically for the flavour and fragrance industry. Chromsync has the ability to determine the 'fingerprint' of flavour compounds in beer. The individual 'fingerprints' are then compared with a reference standard. ChromSync rapidly compares peak retention time as well as area% profiles of complex chromatograms, making processing volatile flavour profiles effortless. Additionally, ChromSync instantly confirms product batch to batch reproducibility whilst reporting any missing compounds and calculating the degrees of similarity.

This application note demonstrated the ease of using ChromSync with CompassCDS for the comparison of five beer samples analysed via headspace (HS) gas chromatography (GC) with flame ionisation detection (FID).

## EXPERIMENTAL

A SCION 456 GC-FID was coupled with the Teledyne Tekmar HT3 headspace autosampler. Five commercially available beers were prepared in 20mL HS vials; 5mL of each sample was added with 3g of NaCl. The samples were thoroughly mixed, ready for injection onto the analytical system. The analytical parameters used can be found in Tables 1 and 2.

The five beers analysed were labelled as Reference Sample, Sample A, Sample B, Sample C and Sample D.

**Table 1.** Analytical conditions of the Teledyne Tekmar HT3 HS autosampler

| Conditions | |
|---|---|
| **Oven** | 80°C |
| **Sample Loop** | 80°C |
| **Transfer Line** | 100°C |
| **Vial Equib Time** | 20 minutes |
| **Fill Pressure** | 15psi at 50mL/min |
| **Inject Time** | 0.5 minutes |

**Table 2.** Analytical conditions of the SCION GC-FID

| Conditions | |
|---|---|
| **Injector** | S/SL 25:1, 140°C |
| **Column** | SCION-Wax 30m x 0.53mm x 1.0µm |
| **Oven** | 40°C (3 mins), 10°C/min to 120°C , 20°C/min to 200°C |
| **Carrier** | Helium, 2mL/min |
| **FID** | 250°C |

## RESULTS

For the purposes of demonstrating the capabilities of ChromSync it was not required to identify the individual flavour compounds. Figure 1 shows the chromatogram of the reference sample.

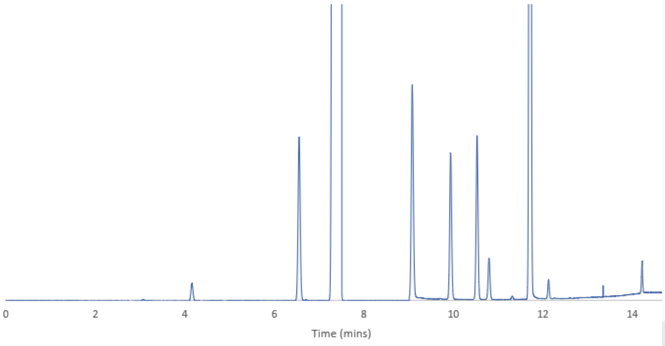


**Fig 1**. Flavour profile of the Reference Sample

Manual comparisons of different flavour profiles is a time extensive process with an increased chance of error in reporting of results, especially in highly complex chromatograms. ChromSync is an automated comparison application that eliminates the need for manual comparisons thus increasing both lab accuracy and productivity.

Chromsync automatically determines tolerance levels for peaks set in the reference sample, however, users can set their own acceptable levels based upon their own methods.

These tolerance levels are then used to determine if peaks present in the sample are identical to those in the reference sample and highlighted if they fall out of the accepted tolerance levels. Smaller peaks within both samples have larger tolerance limits as standard. This is an excellent identification tool for monitoring any changes during the fermentation of beer and comparing batch to batch discrepancies; vital for quality control of beer production. On the comparison charts, the lower markers represent the Reference Sample with the comparative sample being the top markers. The peak area is represented by the size of the markers.

Figure 2 shows the comparison between Reference Sample and Sample A when the tolerance limits were left as predefined.

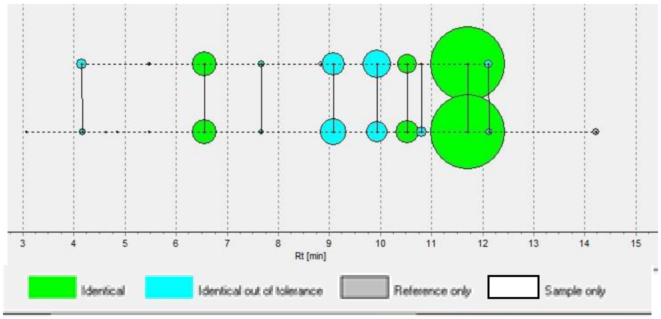


**Fig 2**. Reference beer sample and Sample A comparison

The green markers show the identical peaks within both samples whereas the blue markers show identical peaks that are out of the predefined tolerance levels. Adjusting the tolerance levels to those acceptable to specific brewery quality control limits alters the blue markers to green, showing identical peaks are present, as shown in Figure 3.

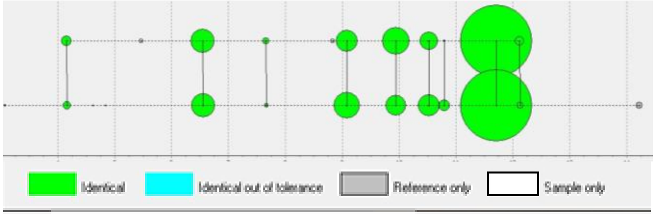


**Fig 3**. Adjusted tolerance levels of Figure 2 samples.

The identical peak match for Figure 2 was 18.75% with 37.50% out of the tolerance level. With adjustment to the processing parameters, the identical peak match increased to 60% with zero out of tolerance peaks. 26.67% of the remaining peaks were present in the Reference Sample only, with the other 13.33% present only in Sample A.

The adjusted tolerance parameters were used for the remainder of the comparisons. Figure 4 shows the comparison between the Reference Sample and Sample B.

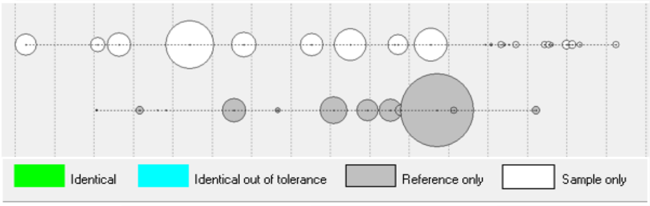


**Fig 4**. Reference beer sample and Sample B comparison

Sample B, a Turkish beer, has a completely different flavour profile when compared to the reference sample. There were no identical flavours between the two samples, as represented by the white and grey markers in Figure 4. Noticeably, Sample B contains more flavour components than the reference sample.

The comparison of the Reference Sample and Sample C is shown in Figure 5. 30% of both samples contained the same flavour compounds, with an additional 10% in the out of tolerance range for Sample C. The larger blue markers show that the peak area of the aromas in Sample C are significantly greater than those in the Reference Sample hence the out of tolerance marker.

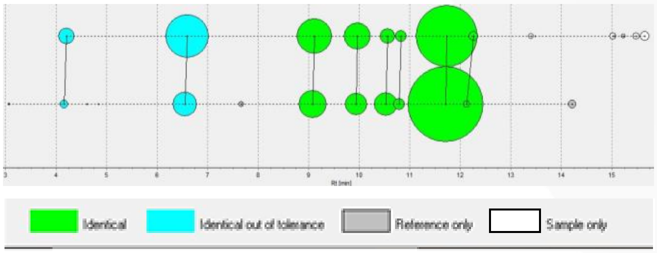


**Fig 5**. Reference beer sample and Sample C comparison

Figure 6 details the comparison between the Reference Sample and Sample D.

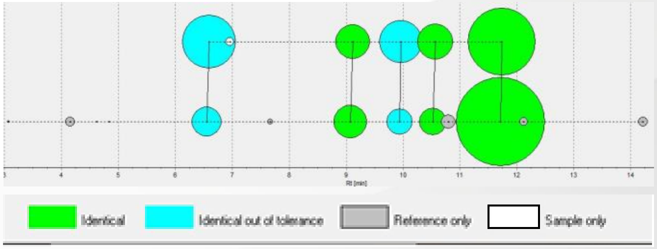


**Fig 6**. Reference beer sample and Sample D comparison

21.43% of the flavour aromas, of both the Reference Sample and Sample D were identical, with 14.29% out of the tolerance range.

As with Sample C, the peak areas of these aromas in Sample D are larger than the defined tolerance limit. Sample D is missing 57.14% of the flavours found in the Reference Sample and contains an additional 7.14% flavours not found in the reference sample.

Figure 7 shows the overlay flavour profile of all beer samples including the reference standard. It is easy to visualise the similarities and differences between not only the different flavours but the different peak areas that constitute the samples.

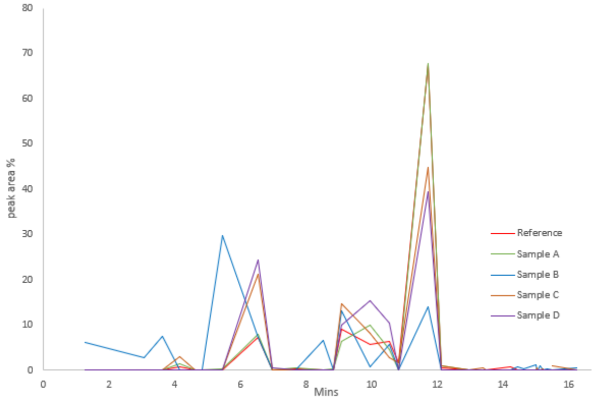


**Fig 7**. Overlay flavor profiles of all beer samples.

Table 3 details the individual flavour compounds found in all beer samples analysed and processed using the ChromSync add on for CompassCDS. Chromsync accurately performs comparisons regardless if there are issues surrounding peak distortion, scaling, column aging, retention time shifts and even changes in experimental conditions.

## CONCLUSION

ChromSync is an application add on for CompassCDS processing software. Chromsync not only defines the 'fingerprints' of flavour profiles but also compares a reference standard chromatogram with a sample chromatogram to identify matching peaks, matching peaks outside of the set tolerance levels and also different peaks present in the two samples. Chromsync is the perfect data processing software for industries involved in flavours and fragrances, eliminating extensive manual processing times and the high risk of errors that are associated with manual comparison.

**Table 3.** Peak area % of each flavour compound, in each beer sample

| RT Ref | Reference | A | B | C | D |
|---|---|---|---|---|---|
| 1.27 | - | - | 6.0552 | - | - |
| 3.07 | 0.0565 | - | 2.7341 | - | - |
| 3.64 | - | - | 7.3901 | - | - |
| 4.16 | 0.7419 | 1.3869 | - | 3.0093 | - |
| 4.62 | 0.0057 | - | - | - | - |
| 4.84 | 0.0198 | - | - | - | - |
| 5.43 | - | - | 29.7991 | - | - |
| 5.47 | - | 0.2133 | - | - | - |
| 6.55 | 7.2185 | 7.8772 | - | 21.2165 | 24.4384 |
| 6.81 | - | - | 7.2185 | - | - |
| 6.96 | - | - | - | - | 0.514 |
| 7.67 | 0.3362 | 0.5217 | - | - | - |
| 8.53 | - | - | 6.5918 | - | - |
| 8.83 | - | 0.301 | - | - | - |
| 9.08 | 9.1647 | 6.369 | - | 14.6471 | 9.9399 |
| 9.50 | - | - | 13.1803 | - | - |
| 9.94 | 5.7562 | 9.968 | - | 8.0822 | 15.2803 |
| 10.53 | 6.334 | 4.5821 | - | 2.7861 | 10.4596 |
| 10.63 | - | - | 0.6511 | - | - |
| 10.72 | - | - | 5.5788 | - | - |
| 10.8 | 1.6139 | 0.0399 | - | 1.6156 | - |
| 11.54 | - | - | 13.9813 | - | - |
| 11.71 | 67.4467 | 67.737 | - | 44.8106 | 39.3737 |
| 12.13 | 0.5905 | 1.0037 | - | 1.0191 | - |
| 12.94 | - | - | 0.0182 | - | - |
| 13.09 | - | - | 0.1813 | - | - |
| 13.40 | - | - | 0.1083 | 0.4114 | - |
| 13.48 | - | - | - | 0.0002 | - |
| 13.53 | - | - | 0.0206 | - | - |
| 14.22 | 0.7153 | - | - | - | - |
| 14.43 | - | - | 0.649 | - | - |
| 14.54 | - | - | 0.5817 | - | - |
| 14.61 | - | - | 0.326 | - | - |
| 14.99 | - | - | 1.1627 | - | - |
| 15.02 | - | - | - | 0.598 | - |
| 15.06 | - | - | 0.0605 | 0.0214 | - |
| 15.13 | - | - | 0.9254 | - | - |
| 15.22 | - | - | 0.0255 | 0.2131 | - |
| 15.33 | - | - | 0.3536 | - | - |
| 15.48 | - | - | - | 0.9753 | - |
| 16.25 | - | - | 0.5112 | - | - |

SCION Instruments
1 Bain Square, Livingston
EH54 7DQ, UK
Tel: +44 1506 300 200
sales-eu@scioninstruments.com
www.scioninstruments.com

SCION Instruments NL BV
Stanleyweg 4
4462GN, Goes, Netherlands
Tel: +31 113 348926
sales-eu@scioninstruments.com
www.scioninstruments.com

SCION INSTRUMENTS


Figure 1:
Two channel "Off-Flavor" Beer analyzer.

Application Note # CA-1818791

# Analysis of volatiles in Beer using the Scion two channel "Off-Flavor" Beer analyzer

## Introduction

The brewing process of making good beer depends on many factors, since it uses organic base materials and natural processes on one hand, while the quality is determined by human smell and taste on the other hand.

To ensure a constant quality of beer, independent from personal preferences, the production time in the year and the production location, analytical equipment like Gas Chromatography (GC) is used to identify and quantify key-flavors. A number of components need to be present with a maximum and minimum concentration to guarantee the typical flavor of the beer. Other components may not exceed a concentration level to avoid an "off–flavor".

Among numerous compounds, monitoring for esters, alcohols, sulfur compounds, and vicinal diketones (VDK, which include 2,3-butanedione (diacetyl) and 2,3-pentanedione ) in the beer, are very important. They are considered extremely important since they are known to affect the taste of the beer.

Validation of the beer quality can be applied using a batch-to -batch product conformance test with a reference beer. This application note describes the headspace analysis of flavors and Off-Flavors in beer using a two channel 456-GC based beer analyzer. Note that the two channel analyzer can be extended with a third channel analyzer to include more low level sulfur compounds in Beer, malt and wort[1].

## Instrumentation

The Scion Off-Flavor Beer Analyzer is configured with two independent GC channels.

| | |
|---|---|
| **Channel 1:** | |
| **Injector:** | S/SL, Split/Splitless Injector |
| **Column:** | SCION-Wax, 60m x 0.32mm x 1.0µm, P/N SC32438 |
| **Detector:** | Flame Ionization Detector (FID) |
| **Channel 2:** | |
| **Injector:** | S/SL, Split/Splitless Injector |
| **Column:** | SCION-5, 60 m x 0.53 mm, df = 0.5 µm (Part no. SC30257) |
| **Detector:** | Electron Capture detector (ECD) |

Sampler: CombiPal-*xt* – headspace injection system. Including a "cooled tray" option

GC control and datahandling: CompassCDS Chromatography Software and ChromSync plug-in software

## Method Settings

| | Channel 1 | Channel 2 |
|---|---|---|
| Carrier Gas | Hydrogen | Hydrogen |
| Flow Rate | 3.5 mL/min | 4.5 mL/min |
| Injector | Split/Splitless | Split/Splitless |
| Inj. Temp | 155° C | 155° C |
| Split Ratio | 1:7 | 1:10 |
| Detector | FID | ECD |
| Det. Temp | 160° C | 160° C |

## Results and Discussion

A test sample was used to calibrate the system. Figures 2 and 3 show the chromatograms obtained with both GC channels.

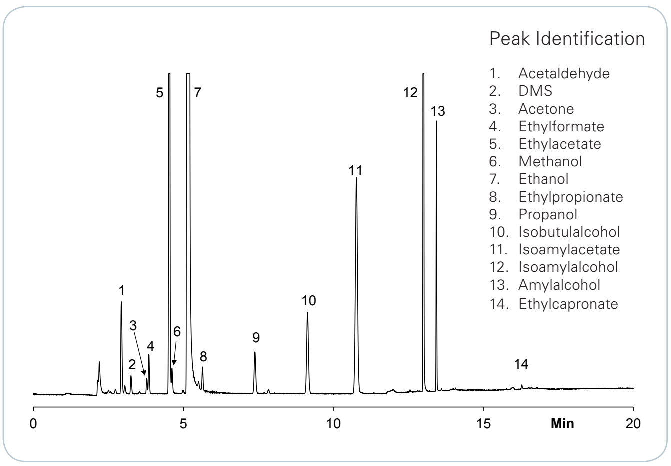


Figure 2: Test sample analysis using FID detection

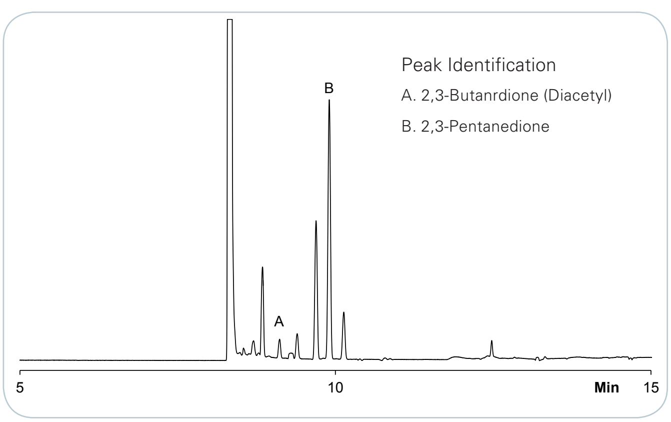


Figure 3: Test sample analysis using ECD detection (VDK)

| | | |
|---|---|---|
| **Vicinal diketones (VDK)** | A | 2,3-Butanedione (Diacetyl) |
| | B | 2,3-Pentanedione |

A standard beer was analyzed. Figures 4 and 5 show the FID and the ECD chromatograms of channel 1 and 2.

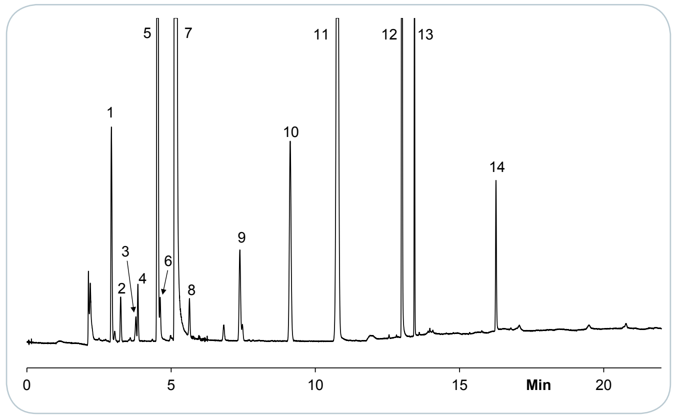


Figure 4. Beer analysis using FID detection

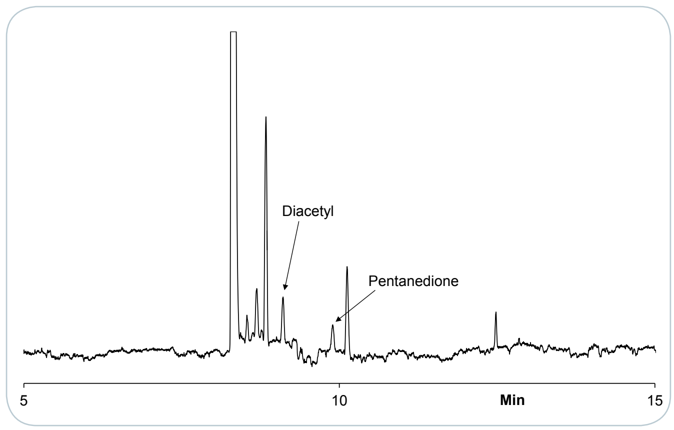


Figure 5. Beer analysis using ECD detection

The concentrations of some of the components of Figures 4 and 5 can be found in Table 2. The Limit of Detection and Limit of Quantification are also presented. The LOD uses a peak definition of 3x noise where the LOD uses 10x noise level.

| Peak No. | Component | Concentration (µg/mL) | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|
| 1 | Acetaldehyde | 5614.4 | 150 | 300 |
| 2 | DMS | 57.68 | 7.8 | 15.6 |
| 4 | Ethylformate | 401.4 | 42 | 84 |
| 5 | Ethylacetate | 25743.9 | 50 | 100 |
| 10 | Isobutylalcohol | 17618.4 | 500 | 1000 |
| 11 | Isoamylacetate | 3501.06 | 28 | 28 |
| 12 | Isoamylalcohol | 60488.9 | 110 | 220 |
| A | Diacetyl | 11.6 | 18 | 36 |
| B | 2,3-Pentanedione | 2.8 | 1.37 | 2.74 |

ChromSync is used for batch-to-batch conformance testing by matching the results of a beer sample against a reference beer. The test includes pre-determined peak area windows resulting in degree-of-match performance values of the analyzed beer. See figure 6.

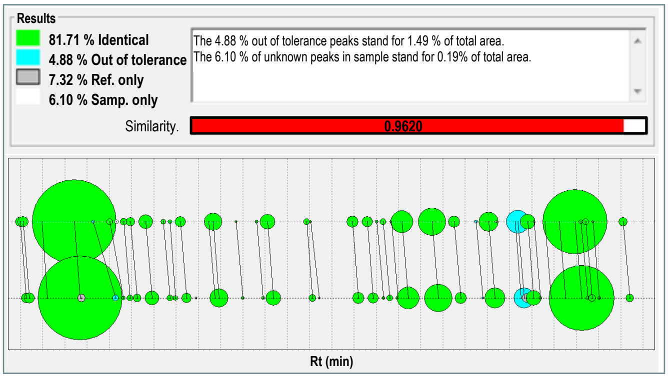


Figure 6: ChromSync compares a sample chromatogram (top) with the reference (bottom) generating a degree of similarity. Peak areas within tolerance limits (green), outside of tolerance limits (blue), peaks missing from the sample (white) and the reference peaks (grey) are shown as color coded disks with the size illustrating the peak area.

## Conclusion

The Scion two channel "Off-Flavor" Beer analyzer is optimized for the analysis of volatiles in beer and is perfectly suited for the analysis of "off flavors" in beer.

Aldehydes, esters, alcohols, and even a sulfur compound such as DMS, can be analyzed on channel 1, equipped with a split injector, SCION-Wax column, and an FID. Detection limits were in the ppm (w/v) range.

The vicinal diketones 2,3-butanedione (diacetyl) and 2,3-pentanedione are analyzed on channel 2, equipped with a split injector, SCION-5 column, and an ECD. Detection limits were in the low ppm (w/v) range.

ChromSync herewith is an excellent tool for conformance testing of the beer quality.


## Authors

Paul Van den Engel


## References


[1]Scion application note CA-1818792, Analysis of volatiles in beer, malt and wort using the Scion three-channel "Off-Flavor" Beer analyzer.


| Keywords |
|---|
| Off flavors |
| Beer |
| Gas chromatography |

| Instrumentation & Software |
|---|
| Scion two channel "Off-Flavor" Beer Analyzer |
| Scion CompassCDS Chromatography Software |
| Scion ChromSync Plug-in Software |
| PAL COMBI-*xt* |




**Scion instruments (NL) B.V.**

Stanleyweg 4
4462GN Goes
The Netherlands
Tel: +31 (0)113348926
sales@scioninstruments.com

**Scion instruments U.K. Ltd.**

1 Bain Square
Kirkton Campus
Livingston, EH54 7DQ
United Kingdom
Tel: +44 (0) 1506 300 200
+44 (0) 1506 425 300

**www.scioninstruments.com**

SCION INSTRUMENTS

Figure 1:
Three channel "Off-Flavor" Beer analyzer.

Application Note # CA-1818792

# Analysis of volatiles in Beer, Malt and Wort using the Scion three channel "Off-Flavor" Beer analyzer

## Introduction

For breweries, quality control of their product is very complex. The quality of expected taste and smell of beer is at the end determined by people's tongue and nose. Disadvantage of these highly selective and sensitive sensors is the capability for quantitation. This is required to ensure a constant quality of the product independent on the production time in the year and production location. In order to maintain a constant quality, analytical equipment like Gas Chromatography (GC) is used to identify and quantify key-flavors. A number of components need to be present with a maximum and minimum concentration to guarantee the typical flavor of the beer. Other components may not exceed a concentration level to avoid an "off–flavor". Therefore a batch-to batch product conformance test is applied using a reference beer to validate the beer quality. Besides their contribution to the flavor, the information on component level of "off-flavors" is also highly valuable in order to identify the cause of an exceeding concentration, like a too high dimethyl sulfide (DMS) content, indicating among others the possible presence of a bacterial infection. Flavors and Off-flavors include a wide range of different chemical groups including alcohols, esters, acids, vicinal diketones and sulfides. This application note describes the headspace analysis of flavors and Off-Flavors in beer using a three channel 456-GC based analyzer.

## Method set up

Due to human sensitivity to flavors, the components' threshold concentration is at the ppm down to the sub ppm range[1]. For GC analysis this means that sensitive and selective detectors are used to meet the required detection limits.

The detectors include:

- The Flame Ionization Detector (FID) highly selective to hydrocarbons including oxygenated hydrocarbons (oxygenates) like alcohols and esters
- The Electron Capture Detector (ECD) highly selective to halogens and ketones especially for VDK
- The Pulsed Flame Photometric Detector (PFPD) highly selective to sulfur containing components like dimethylsulfide (DMS)

Since the matrix of beer contains besides the flavors, a high amount of water and non-volatiles (polypeptides, carbohydrates and salts), the use of a headspace injection is the preferred sampling method.

## Instrumentation

| | |
|---|---|
| **Technique** | The Scion Off-Flavor Beer analyzer is configured using three independent GC channels |
| **Channel 1** | |
| **Injection:** | S/SL, Split/Splitless Injector |
| **Column:** | SCION-Wax, 60m x 0.32mm x 1.0µm, P/N SC32438 |
| **Detection:** | Flame Ionization Detector (FID) |
| **Channel 2** | |
| **Injection:** | S/SL, Split/Splitless Injector |
| **Column:** | SCION-5, 60 m x 0.53 mm x 0.5 µm, P/N SC30257 |
| **Detection:** | Electron Capture detector (ECD) |
| **Channel 3** | |
| **Injection:** | S/SL, Split/Splitless Injector |
| **Column:** | SCION-Wax 30m x 0.32mm x 1µm, P/N SC32435 |
| **Detection:** | Pulsed Flame Photometric Detector (PFPD) |
| **Sampler:** | PAL COMBI-xt headspace injection system with sample tray cooling |
| **Software:** | CompassCDS Chromatography Software ChromSync plug-in software |

## Conditions

| Settings | Chan. 1 | Chan. 2 | Chan. 3 |
|---|---|---|---|
| Carrier gas | Hydrogen | Hydrogen | Hydrogen |
| Flow rate (mL/min) | 3.5 | 4.5 | 5.0 |
| injector | S/SL | S/SL | S/SL |
| Inj. Temp. | 155°C | 155°C | 155°C |
| Split ratio | 1:7 | 1:10 | 1:10 |
| Detector | FID | ECD | PFPD |
| Gate delay | - | - | 6 ms |
| Gate width | - | - | 10 ms |

Table 1: Method parameters

## Results and Discussion

The analysis of beer shows a perfect detection of oxygenates using the FID shown in figure 2 and low level ketones using the ECD in figure 3[2].

ChromSync is used for batch-to-batch conformance testing by matching the results of a beer sample against a reference beer. The test includes pre-determined peak area windows resulting in degree-of-match performance values of the analyzed beer. See figure 6.

**Peak Identification**

1. Acetaldehyde
2. DMS
3. Acetone
4. Ethylformate
5. Ethylacetate
6. Methanol
7. Ethanol
8. Ethylpropionate
9. Propanol
10. Isobutylalcohol
11. Isoamylacetate
12. Isoamylalcohol
13. Amylalcohol
14. Ethylcapronate

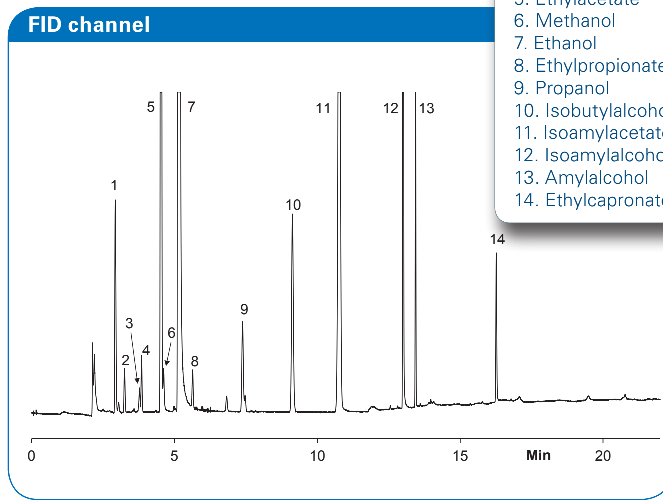


Figure 2: Beer analysis using FID detection

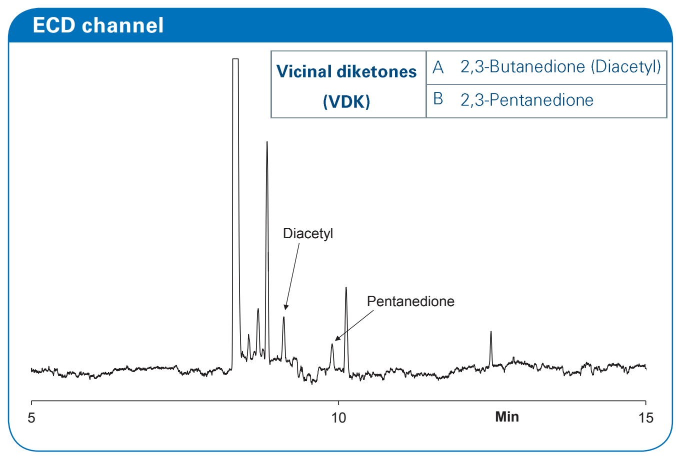


Figure 3: Beer analysis using ECD detection

The analysis of DMS analysis in wort is done using the PFPD channel as shown in figure 4. Although DMS is detected by the FID (peak 2 in figure 2), extra sensitivity is required to meet Limit of Quantitation (LOQ). Using a Sulfur containing internal standard, the analysis with the PFPD is free from any component interference as shown in figure 4. Even when the DMS concentration is low, quantitation is not interfered with other volatiles. Figure 5 shows the analysis of DMS in wort.

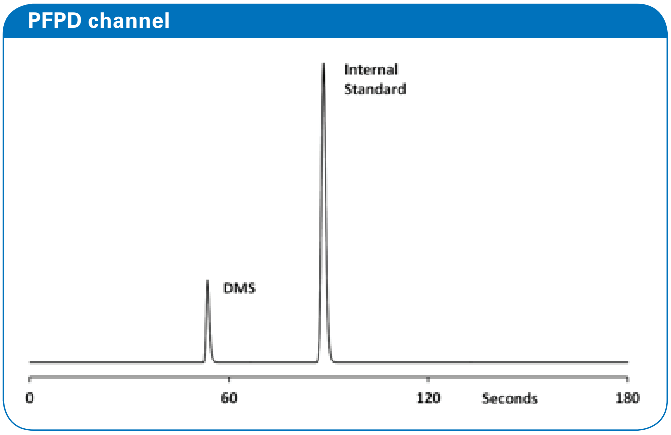


Figure 4: Test analysis using PFPD detection

ChromSync is used for batch-to-batch conformance testing by matching the results of a beer sample against a reference beer. The test includes pre-determined peak area windows resulting in degree-of-match performance values of the analyzed beer. See figure 5.

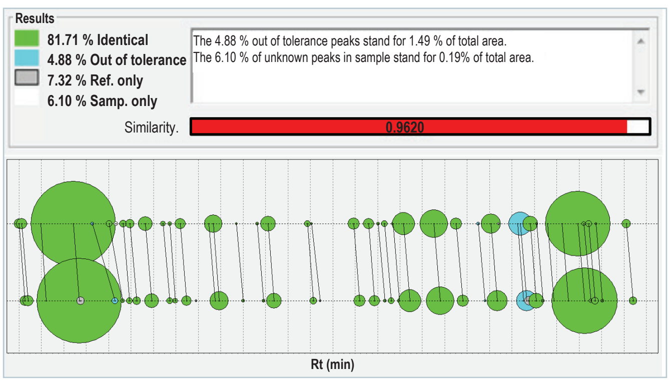


Figure 6: ChromSync compares a sample chromatogram (top) with the reference (bottom) generating a degree of similarity. Peak areas within tolerance limits (green), outside of tolerance limits (blue), peaks missing from the sample (white) and the reference peaks (grey) are shown as color coded disks with the size illustrating the peak area.

| Keywords |
|---|
| Beer |
| Wort |
| Malt |
| Gas chromatography |
| Off flavor |

| Instrumentation & Software |
|---|
| Scion three channel "Off-Flavor" Beer Analyzer |
| Scion CompassCDS Chromatography Software |
| Scion ChromSync plug-in Software |

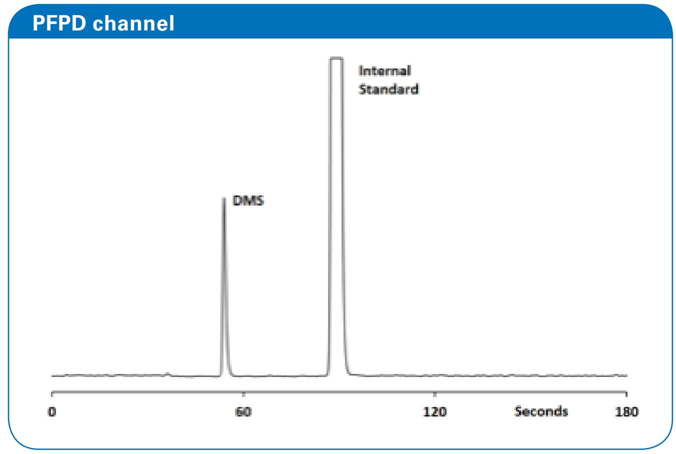


Figure 5: DMS analysis in wort with PFPD. The signal is shown 50 times amplified compared to figure 4.

## Conclusion

The Scion three channel “Off-Flavor” Beer analyzer is optimized for the analysis of volatiles in beer, malt and wort. Aldehydes, esters, alcohols, are analyzed on channel 1 using the FID detector. The vicinal diketones 2,3-butanedione (diacetyl) and 2,3-pentanedione are analyzed on channel 2 using the ECD detector. Dimethyl sulfide is either analyzed on channel 1 using the FID detector in case of beer analysis and on channel 3 using the PFPD detector for low DMS detection in beer and for DMS analysis in wort and malt. ChromSync herewith is an excellent tool for conformance testing of the beer quality.

## Authors

Paul van den Engel and Jos Curvers

## References

[1] Odor measurement review. Page 118-127: "Measurement of Odor Threshold by Triangle Odor Bag Method" Office of Odor, Noise and Vibration Environmental Management, Bureau Ministry of the Environment, Government of Japan

[2] Scion application note CA-1818791 Analysis of “Off-Flavors” in Beer using the Scion two channel Off-Flavor Beer analyzer

For research use only. Not for use in diagnostic procedures.



**Scion instruments (NL) B.V.**

Stanleyweg 4
4462GN Goes
The Netherlands
Tel: +31 (0)113348926
sales@scioninstruments.com

**Scion instruments U.K. Ltd.**

1 Bain Square
Kirkton Campus
Livingston, EH54 7DQ
United Kingdom
Tel: +44 (0) 1506 300 200
+44 (0) 1506 425 300

**www.scioninstruments.com**