A Comparison of ITEX Dynamic Headspace–GC/MS to other Enrichment Techniques for Analysis of Flavoring Compounds

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Overview:

Purpose:

- To demonstrate the new automated PAL ITEX Dynamic Headspace unit is capable of analyzing flavoring compounds.
- Compare the ITEX to other enrichment techniques.
- Automate the analysis of these flavoring compounds using the ITEX.
- Reduce analysts time involved in the analysis.

Methods:

- Analysis of flavoring compounds using a manual purge and trap GC/MS system.
- Analysis of flavoring compounds using automated dynamic headspace.
- Analysis of flavoring compounds using automated headspace and liquid immersion SPME
- Analysis of flavoring compounds using automated ITEX Dynamic Headspace Unit
- PAL RTC robotic sample handler for automated analysis.
- GC/MS analysis using an Agilent 5975C GC-MS

Results:

- The automated ITEX Dynamic Headspace unit demonstrated equivalent accuracy and precision when compared to purge and trap and overall was more sensitive than the other enrichment techniques. The ITEX unit allows for automated analysis and optimization of conditions which drastically reduces analyst's time when compared to performing the experiments manually with the purge and trap without any reduction in sensitivity, accuracy and reproducibility.

Introduction:

Enrichment techniques are commonly used for the analysis of flavoring compounds in different matrices in conjunction with gas chromatography/mass spectrometry. Analysis of flavoring (aroma) compounds is typically done by purge and trap, SPME or headspace, depending on requirements for sensitivity. The In-Tube Extraction (ITEX) Dynamic Headspace uses a micro trap filled with an adsorbent material to efficiently extract and concentrate the compounds. The object of this work was to evaluate if the ITEX Dynamic Headspace can be used to effectively analyze for these compounds and reduce the analyst's time involved.

Methods:

- The purge and trap analysis was conducted using an OI-Analytical PT unit and an Agilent GC/MS
- A novel PAL RTC robotic sample handler was used to prepare samples for inject into the GC/MS for all the other techniques.
- PAL Sample Control Software v. 2.0.1.1 controlled the RTC and data acquisition with the Agilent GC/MS instrument. GC/MS methods were written with Chemstation V.E.02.01.1177.
- All analyses used the same 1000 ppm working standard prepared in methanol of the compounds listed in Table 1.

Compound	CAS
2-Methylpropanal	78-84-2
Diacetyl	431-03-8
3-Methylbutanal	590-86-3
2-Methylbutanal	96-17-3
2,3-Pentanedione	600-14-6
2,3-Hexanedione	3848-24-6
Hexanal	66-25-1
2-Heptenal	18829-55-5
Octanal	124-13-0
Nonenal	18829-56-6

Table 1: Flavor compounds used for the evaluation

Purge and Trap

- 1 µl of the 1000 ppm standard was added to 10 ml of water
- The sample was absorbed at 70°C for 5 minutes, dry purge for 2 minutes, Trap held at 31°C
- Trap desorbed at 180°C for 4 minutes

GC-MS analysis was performed on an Agilent 5973 GC-MS

GC Method
- Initial temperature of 10°C held for 3 minutes,
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- Carrier Gas helium flow rate 1.5 ml/min
- Split ratio of 1:1
- Injection Port 250°C
- Column used was a Restek DB-5MS (30 m x 0.250 mm X 1µm).

MS parameters
- Scan Mode 33-350 m/z
- Source Temperature 250°C
- Transfer Line 290°C

Headspace

- 1 µl of the 1000 ppm standard was added to 2 ml of water
- The sample was heated at 90°C for 30 minutes with agitation at 250 rpm
- Tool Temperature 90°C

GC-MS analysis was performed on an Agilent 5975C GC-MS

GC Method
- Initial temperature of 10°C held for 3 minutes,
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- Carrier Gas helium flow rate 1.5 ml/min
- Split ratio of 50:1
- Injection Volume 1 ml
- Injection Port 250°C
- Column used was an Agilent DB-5MS (30 m x 0.250 mm X 1µm).

MS parameters
- Scan Mode 33-350 m/z
- Source Temperature 220°C
- Transfer Line 280°C

Headspace SPME

- 1 µl of the 1000 ppm standard was added to 8 ml of water
- The sample was heated at 60°C for 30 minutes with agitation at 250 rpm
- Absorption for 30 minutes using a CAR/PDMS (blue) fiber

GC-MS analysis was performed on an Agilent 5975C GC-MS

GC Method
- Initial temperature of 10°C held for 3 minutes,
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- Carrier Gas helium flow rate 1.5 ml/min
- Split ratio of 50:1
- Injection Port 250°C
- Desorption time 5 minutes
- Column used was an Agilent DB-5MS (30 m x 0.250 mm X 1µm).

MS parameters
- Scan Mode 33-350 m/z
- Source Temperature 220°C
- Transfer Line 280°C

SPME – liquid Immersion

- 1 µl of the 1000 ppm standard was added to 10 ml of water
- The sample was heated at 30°C for 30 minutes with agitation at 250 rpm
- Immersion for 30 minutes using a CAR/PDMS (blue) fiber

GC-MS analysis was performed on an Agilent 5975C GC-MS

GC Method
- Initial temperature of 10°C held for 3 minutes,
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- Carrier Gas helium flow rate 1.5 ml/min
- Split ratio of 50:1
- Injection Port 250°C
- Desorption time 5 minutes
- Column used was an Agilent DB-5MS (30 m x 0.250 mm X 1µm).

MS parameters
- Scan Mode 33-350 m/z
- Source Temperature 220°C
- Transfer Line 280°C

ITEX

- 1 µl of the 1000 ppm standard was added to 2 ml of water
- 30 Minute Incubate
- Incubate sample for 30 min at 60°C, then 30 ITEX filling strokes @ 100 µl/sec to 1000 µl hold 5 seconds, inject 500 µl at 100 µl/sec, desorption temp 200°C
- 15 Minute Incubate
- Incubate sample for 15 min at 60°C, then 30 ITEX filling strokes @ 100 µl/sec to 1000 µl hold 5 seconds, inject 500 µl at 100 µl/sec, desorption temp 200°C

GC-MS analysis was performed on an Agilent 5975C GC-MS

GC Method
- Initial temperature of 10°C held for 3 minutes,
- Ramped to 200°C at a rate of 10°C / minute
- Ramped to 280° at a rate of 40°C / minute,
- Held at 280°C for 5 minutes
- Carrier Gas helium flow rate 1.5 ml/min
- Split ratio of 50:1
- Injection Port 250°C
- Column used was an Agilent DB-5MS (30 m x 0.250 mm X 1µm).

MS parameters
- Scan Mode 29-350 m/z
- Source Temperature 220°C
- Transfer Line 280°C

Results:

Figure 1 shows a comparison of the average responses for the different enrichment techniques for each compound. The data shows that for the majority of the compounds the ITEX was more sensitive than the other enrichment techniques. It should be noted that the ITEX has several parameters such as the number of manipulations, speed of adsorption step or the speed of desorption that can be changed to optimize the response of individual compounds.

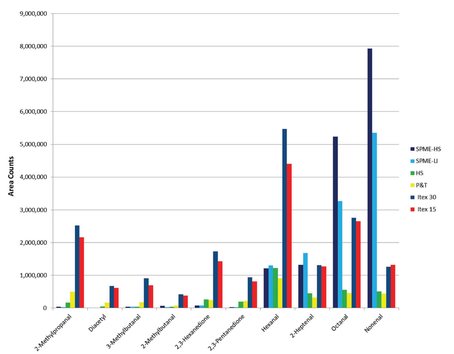

Figure 1: Comparison of the Enrichment Techniques

Figure 2 show a comparison of the responses for purge and trap and ITEX. The data shows that the ITEX is considerably more sensitive than purge and trap and automating analyzed the samples which resulted in a significant savings in analyst's time.

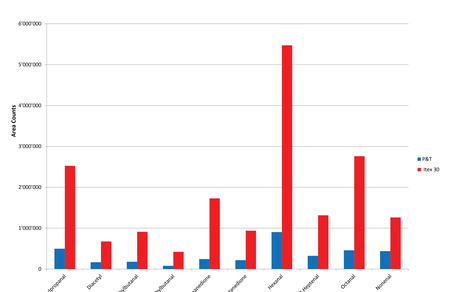

Figure 2: Comparison of Purge & Trap vs ITEX

Tables 2 and 3 shows a comparison the %RSD and percent recovery for purge and trap and ITEX. The data shows that the ITEX Dynamic Headspace unit for the majority of the compounds is equivalent to purge and trap.

Compound	%RSD (P&T)n=6	%RSD (ITEX)n=3
2-Methylpropanal	7.1	15.6
Diacetyl	19.8	35.8
3-Methylbutanal	7.8	8.9
2-Methylbutanal	7.8	9.0
2,3-Pentanedione	2.2	2.8
Hexanal	5.1	5.5
Octanal	5.1	9.9
Nonenal	15.3	14.8
2,3-Hexanedione	4.3	6.4

Table 2: Comparison of %RSD for Purge & Trap vs ITEX

Compound	% Recovery (P&T)n=6	%Recovery (ITEX)n=3
2-Methylpropanal	121.5	113.7
Diacetyl	88.8	67.1
3-Methylbutanal	120.7	113.3
2-Methylbutanal	127.0	126.0
2,3-Pentanedione	101.1	106.4
Hexanal	121.0	177.0
Octanal	93.3	135.7
Nonenal	180.2	85.7
2,3-Hexanedione	116.2	136.3

Table 3: Comparison of %Recovery for Purge & Trap vs ITEX

Figure 3 show a comparison the two ITEX techniques where the incubation time was varied. The data shows that the two incubation times compared closely and the 15 minute incubation was only gave a slightly less response.

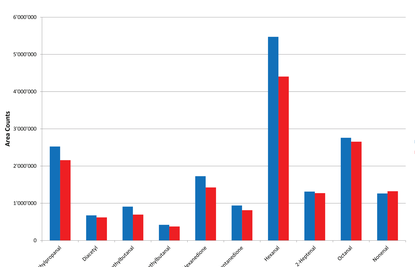

Figure 3 – Comparison of 30 min vs 15 min ITEX incubation

Conclusions:

- The ITEX Dynamic Headspace Unit was more sensitive than the other enrichment techniques for the majority of the compounds.
- The ITEX Dynamic Headspace Unit demonstrated equivalent accuracy and precision when compared to purge and trap and was considerably more sensitive.
- The automation of the ITEX Dynamic Headspace Unit increases sample throughput while reducing the analyst's time involved when compared to the manual Purge and Trap Unit.
- Using the PAL System with Robotic Tool Change, it is possible to switch between HS, SPME and ITEX Dynamic Headspace within one sequence.