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Comparison of headspace techniques for the screening of fragrance compounds in flowers

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Introduction

Flowers scents are commonly used in the fragrance industry as an inspiration for the production of new perfumes. However flower smell profiles can also be useful from a botanical perspective to characterise different species and get a better understanding of the plant volatiles and the chemical ecology behind it.

The most challenging task for these applications is the extraction of a representative fragrance profile that mimics the flower smell being studied. In order to capture a flower smell profile it is crucial to extract and identify the most relevant fragrance contributing compounds.

Due to the nature of the analytes, headspace sampling techniques coupled to gas chromatography mass spectrometry (GC-MS) are the method of choice.

Static Headspace (HS) is well established but often limited by lack of sensitivity for low concentration compounds. On the other hand, Dynamic Headspace (DHS) uses flow of inert gas to exhaustively extract volatile compounds from the sample and preconcentrate them onto an adsorbent trap. Release of the trapped compounds is carried out by thermal desorption. DHS allows fast extraction and short analysis cycle with very high recoveries, increasing significantly the sensitivity of the technique. It's a very performing technique especially for screening applications where it's essential capturing the highest number of compounds regardless their concentration.

Another approach to headspace sampling is the use of Twister® stir bar. Using Twisters in headspace mode the stirbar is contained within an insert fitted inside a headspace vial as shown in Figure 1. The insert has got a slit on the bottom which allows the sample gas phase to enter in contact with the Twister bar. The sample can either be incubated or kept at room temperature for a fixed amount of time to allow reaching of the equilibrium. Once the target compounds have been absorbed on the twister, they can be released by thermal desorption.

Figure 1: Twister Headspace setup

This application note showcases a comparison between DHS and Twister Headspace for the extraction of flowers smell profile.

Instrumentation

Autosampler: GERSTEL MPS Robotic Dual Head, USM tool with gripper
Modules: Vial Tray VT15 20 mL, DHS, TDU tubes Tray VT40
GC-MS: Agilent GC 7890- QTOF 7200, RIS Source

Figure 2: MPS Robotic Dual Head equipped with DHS and ODP and coupled to Agilent GC-QTOF

Methods

Samples

Three different flower species were purchased: Hyacinth (white), Tulip (Red) and Kalanchoe (Red). Where size allowed it, the whole flowers was transferred into a 20mL headspace vial and extracted either via DHS or via Twister Headspace. In case of the Tulip the whole flower was cut into quarters and each quarter was inserted into the vial. Four replicates per flower type were analysed by each investigated technique. Headspace Twister samples were incubated for 24 hrs.

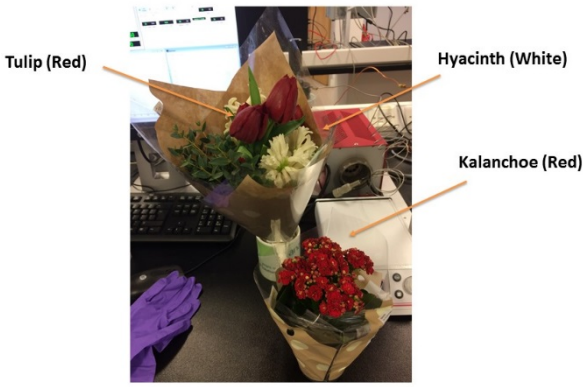

Figure 3: Flower species used for smell profile extraction by DHS and Twister

DHS-TDU-CIS

DHS:
No purge
Incubation: 5 min at 35°C
Trapping: 150 mL at 50 mL/min, Trap 35°C
No drying

TDU: Splitless mode, 30°C for 1 min, 720°C/s to 250 °C held for 5 min. Transfer line 260 °C

CIS: Solvent Vent Mode, Vent Flow 100 mL/min, Split 1:10. Initial Temperature: 10°C for 2 min, 12°C/s to 250 °C held for 10min.
Tenax packed liner

Twister-TDU-CIS

TDU: Splitless mode, 30°C for 1 min, 720°C/s to 300 °C held for 10 min. Transfer line 280 °C

CIS: Solvent Vent Mode, Vent Flow 100 mL/min, Split 1:10. Initial Temperature: 10°C for 2 min, 12°C/s to 250 °C held for 10 min.
Tenax packed liner

GC-MS analysis

GC:
Column: HP-5MS Ultra inert 30 m x 0.25 mm x 0.25 µm
Flow: 1 mL/min
GC ramp: 40 °C held for 2 min, 7 °C/min to 300 °C held for 10 min
Runtime: 49 min

MS:
Auxiliary temperature: 300 °C
EI mode at 230°C, Quadrupole 150°C, Transients Mass range 30-800 *m/z*

Results and Discussion

Samples were run fully randomised to minimise bias. Procedural blanks were acquired to evaluate background contribution. Figure 4 and Figure 5 shows an example of total ion chromatograms (TICs) obtained for the blank and the three flower species for both DHS and Twister, respectively.

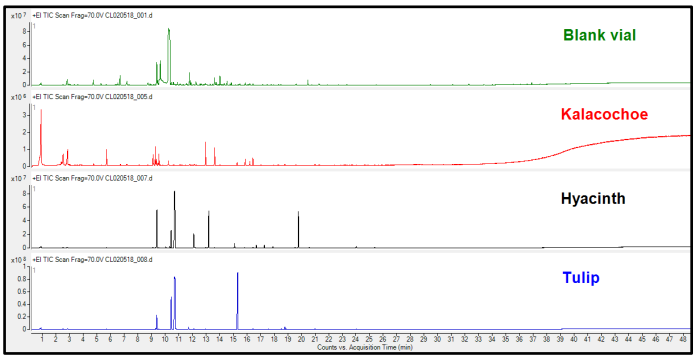

Figure 4: Total Ion Chromatograms (TIC) by DHS-TDU-GC-MS for (from the top): blank vial, Kalanchoe, Hyacinth and Tulip

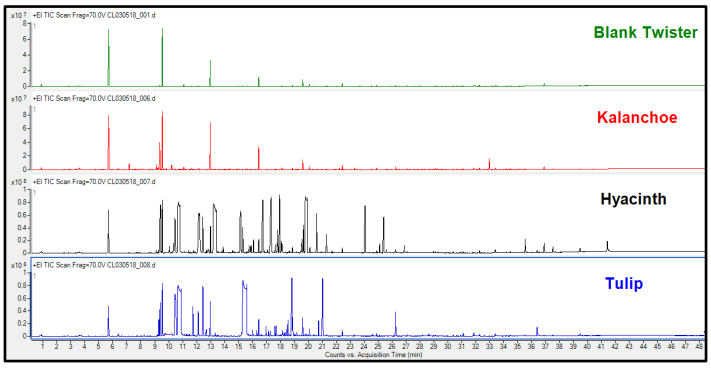

Figure 5: Total Ion Chromatograms (TIC) by Twister-TDU-GC-MS for (from the top): blank vial, Kalanchoe, Hyacinth and Tulip

Acquired data were processed using Agilent Mass Hunter Unknowns Analysis to deconvolute the complex chromatographic information, extract and library search relevant components. Table 1 summarises the average number of components and library hits found for all three flower species with the investigated techniques.

	Kalanchoe	Hyacinth	Tulip
DHS Components	1025	1182	1266
DHS Hits	218	281	306
Twister Components	3963	4554	4808
Twister Hits	907	1148	1226

Table 1: Deconvoluted components and library hits for the three flower species analysed by the two headspace techniques, DHS and Twister Headspace

As shown already by the TIC chromatograms, the Twister samples revealed a significant higher number of peaks in the chromatography than the DHS samples. However the Twisters sampled the headspace for 24 hours while DHS was set to sample only 150 mL of the headspace (trapping time of 3 min at trapping flow of 50 mL/min) therefore performances are not directly comparable as such. Deconvoluted data were then exported to Agilent Mass Profiler Professional (MPP) for statistical evaluation. Principal components analysis (PCA) was chosen to further investigate data since it's a very effective visual way to explore the variance in the data set and it helps in the identification of patterns. Figure 6 and Figure 7 show the Principal Component Analysis graphs for both DHS and Twister data.

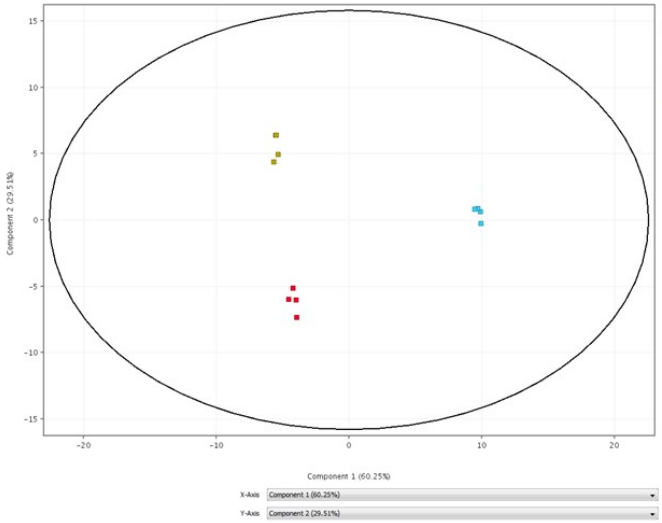

Figure 6: PCA obtained for the analysis of the investigated dataset by DHS-TDU-GC-MS (Hyacinth: red, Kalanchoe: yellow, Tulip: blue)

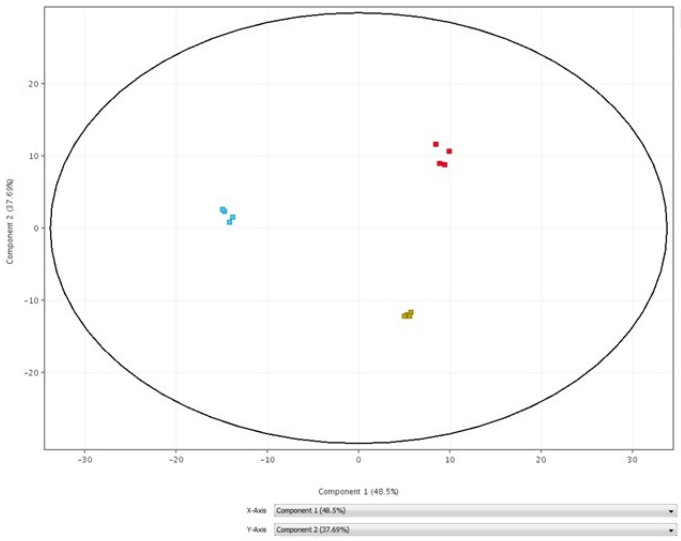

Figure 7: PCA obtained for the analysis of the investigated dataset by Twister TDU-GC-MS (Hyacinth: red, Kalanchoe: yellow, Tulip: blue)

Both techniques could efficiently separate the three flower species in nicely tight clusters, suggesting significant differences in the chromatographic profiles. A very useful analysis tool in MPP is "Find Unique Entities", which allows you to query a specific entity list to find unique entities that are specific to conditions highlighted by the classification model. This function generates a Venn Diagram which lists the number and identity of the unique entities per group. Figure 8 shows an example of the Venn Diagram generated by the Find Unique Entities option for the DHS dataset.

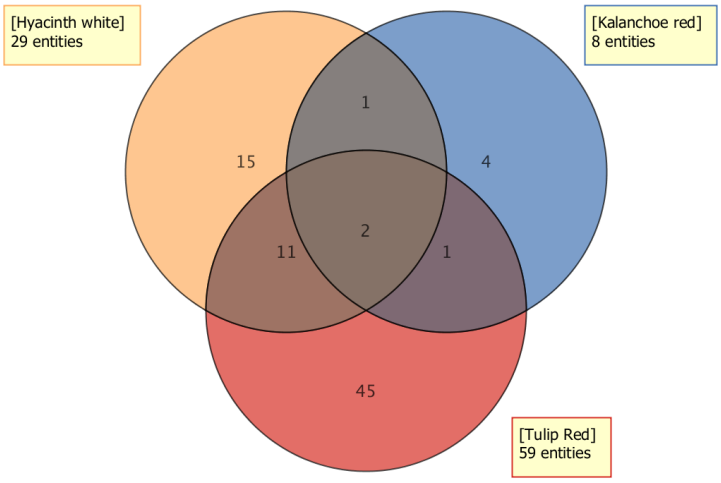

Figure 8: Venn Diagram generated by the Find Unique Entities option for the DHS dataset

The Find Unique entities suggested 15 entities unique to Hyacinth. Out of those 15, 8 compounds were confirmed in

identity and found contributing to the floral smell profile. Table 2 lists the compounds and their smell found on the fragrance database http://www.thegoodscentscompany.com

Compound Name	Compound Smell
p-methyl anisole	Minty, powdery and nutty
Benzyl alcohol	Sweet, floral, fruity
Benzyl acetate	Sweet floral fruity jasmin fresh
Phenylethyl acetate	Floral rose sweet honey fruity tropical
Benzyl isobutyrate	Jasmin oily fruity sweet rose tropical
Methyleugenol	Sweet fresh warm spicy clove cinnamon
Benzyl tiglate	Balsamic earthy mushroom rose undertone
Alpha farnesene	Citrus herbal lavender bergamot myrrh

Table 2: Compounds unique to Hyacinth having floral smell

Figure 9 shows the box plot graphs for the peak areas of the 8 compounds having a floral smell by DHS and by Twisters, respectively. As shown by the graph, those compounds were found significantly higher in the Hyacinth and not in the other two flowers.

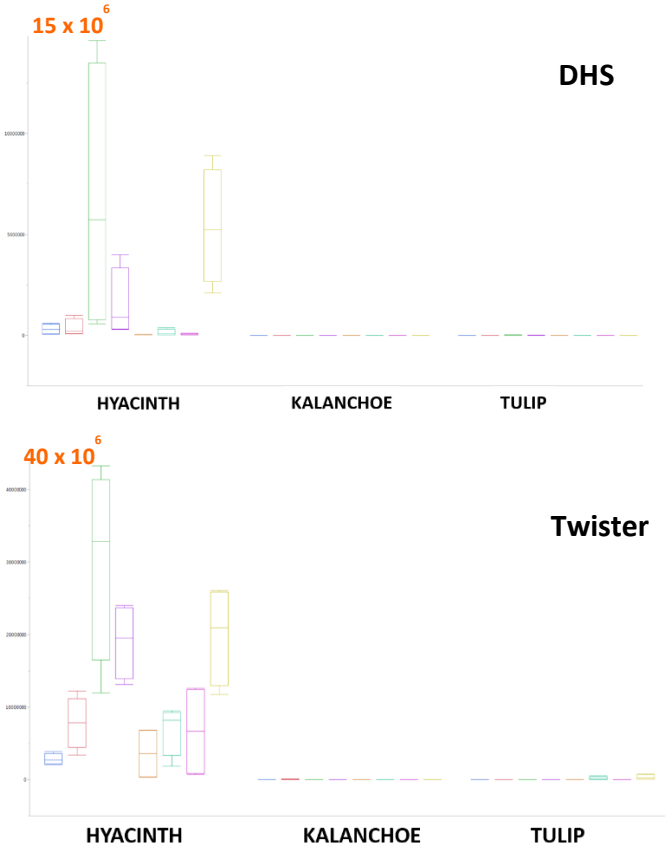

Figure 9: Box plot graph for the peak areas of the eight compounds having floral smell by DHS (top) and by Twisters bottom

From the right p-methyl anisole: blue; benzyl alcohol: red ; benzyl acetate: green; phenethyl acetate: purple; benzyl isobutyrate: orange; methyleugenol: light blue ; benzyl tiglate: pink; alpha farnesene: yellow.

Conclusions

Two very powerful headspace techniques, DHS and Twister Headspace, were used to investigate the smell profile of three flower species. The data allowed to successfully differentiate flower species. Eight compounds having floral smell were found unique in the Hyacinth flower using both DHS and Twisters. This proof of concept study shows the potential of these two techniques for the successful investigation of flower fragrances.