Anatune Ltd
Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA, UK
Tel: +44 (0) 1223279210 Fax: +44 (0) 1223279253
Email: info@anatune.co.uk Internet: www.anatune.co.uk

Dynamic Headspace (DHS) for the Screening of Fragrance Compounds in Flowers by GC/Q-TOF using Chemometrics

Camilla Liscio, Anatune Ltd., Girton, Cambridgeshire (UK).

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. Figure 1 shows the three main steps in a DHS method: firstly, incubation to promote passage of the analytes to the headspace, secondly trapping to concentrate the analytes on the adsorbent material and lastly thermal desorption to transfer the analytes to the GC-MS system. There is also an optional dry purging step to eliminate water in case of very wet samples.

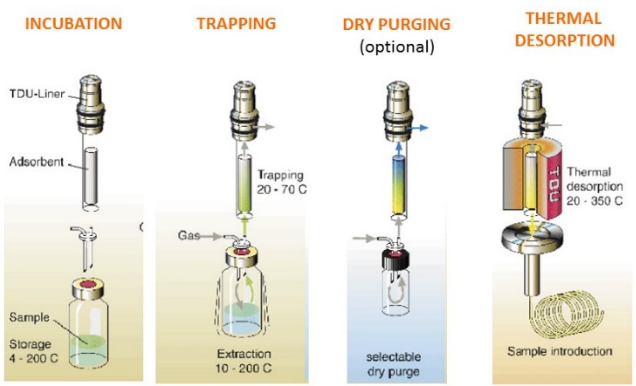

Figure 1: DHS analysis stages

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.

This application note showcases DHS 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 mounted onto Agilent 7200 GC-QTOF

Methods

Samples

Three different flower species were purchased: Hyacinth (white), Tulip (Red) and Kalanchoe (Red) (Figure 3). Where size allowed it, the whole flowers was transferred into a 20mL headspace vial and extracted via DHS. 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.

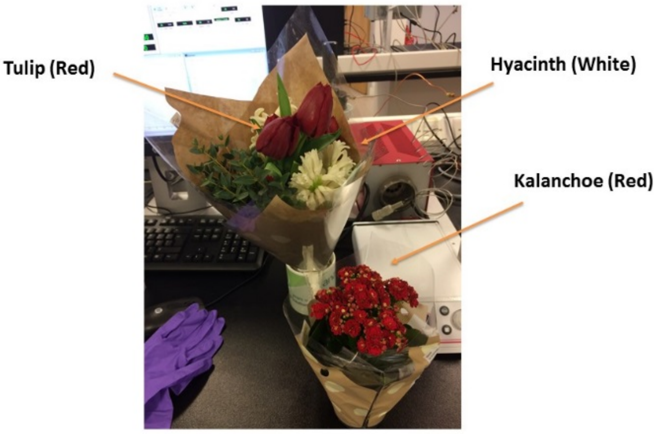

Figure 3: Flower species used for smell profile extraction

DHS-TDU-CIS

DHS:
Incubation: 35°C
Trapping: 35°C
TDU: Splitless mode
CIS: Solvent Vent Mode, Split 1:10. 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, 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 shows an example of total ion chromatograms (TICs) obtained for the blank and the three flower species by DHS. Acquired data were processed using Agilent Mass Hunter Unknowns Analysis to deconvolute the complex chromatographic information, extract and library search relevant components.

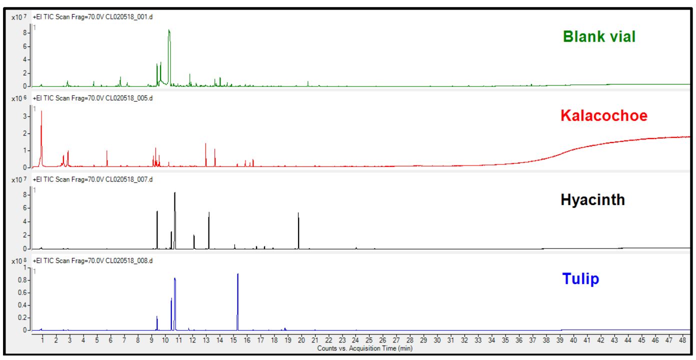

Figure 4: Total Ion Chromatograms (TIC) by DHS-TDU-GC-MS for (from the top): blank vial, Kalanchoe, Hyacinth and Tulip

Table 1 summarises the average number of components and library hits found for all three flower species with the investigated technique.

	Kalanchoe	Hyacinth	Tulip
DHS Components	1025	1182	1266
DHS Hits	218	281	306

Table 1: Deconvoluted components and library hits for the three flower species analysed by DHS

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 5 shows the Principal Component Analysis graphs for the DHS data.

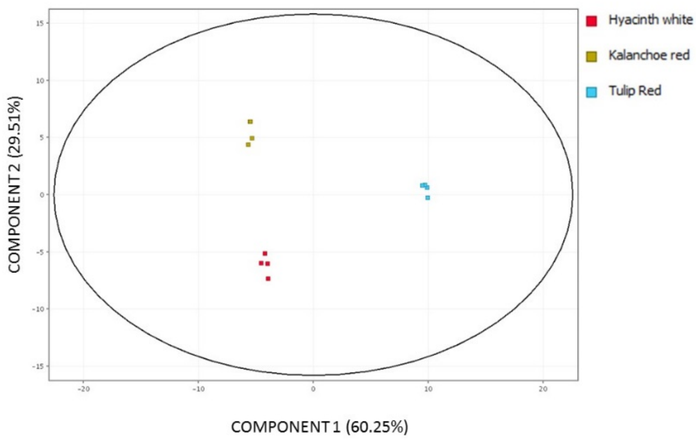

Figure 5: PCA obtained for the analysis of the investigated dataset by DHS-TDU-GC-MS (Hyacinth: red, Kalanchoe: yellow, Tulip: blue)

DHS 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 6 shows an example of the Venn Diagram generated by the Find Unique Entities option for the DHS dataset.

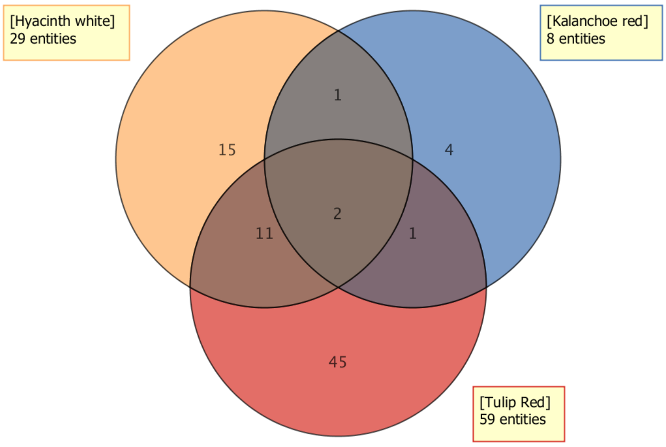

Figure 6: Venn Diagram generated by the Find Unique Entities option for the DHS dataset

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 7 shows the box plot graphs for the peak areas of the 8 compounds having a floral smell by DHS. Box plots are a very useful way to display variation of the dataset population. The spacing between the different parts of the box indicate the degree of dispersion and helps with the identification of outliers. Box and whiskers plot quartiles, and the band inside the box is always the median. As shown by the graph, the compounds identified by the Find Unique Entities option were found significantly higher in the Hyacinth and not in the other two flowers.

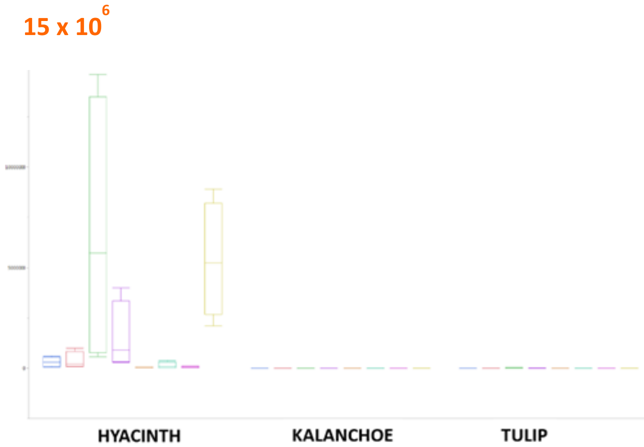

Figure 7: Box plot graph for the peak areas of the eight compounds having floral smell by DHS. From the right p-methyl anisole: blue; benzyl alcohol: red; benzyl acetate: green; phenethyl acetate: purple; benzyl isobutyrate: orange; methyleugenol: light blue; benzyl tiglate: pink; alpha farnesene: yellow.

Conclusions

A very powerful headspace technique, Dynamic Headspace (DHS), was used to investigate the smell profile of three flower species. Mass Profiler Professional offered powerful tools which allowed to successfully differentiate flower species. Eight compounds having floral smell were found unique in the Hyacinth flower. This proof of concept study shows the potential of DHS for the successful investigation of flower fragrances.