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SNIFFING OUT PARKINSON DISEASE USING GERSTEL DYNAMIC HEADSPACE (DHS) AND OLFACTORY DETECTION PORT (ODP)

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INTRODUCTION

Parkinson's disease (PD) is a long-term degenerative disorder of the central nervous system which largely affects the motor system. Generally, symptoms manifest gradually over time. The cause of Parkinson's disease is unidentified, but assumed to implicate both genetic and environmental factors. As yet there is no cure, with treatment focused on improving symptoms. Hence, the importance of an early diagnosis to limit as much as possible neurons loss.

Mrs Joy Milne has recently captured the media attention as a "super smeller". Mrs Milne was able to diagnose PD using her sense of smell even earlier than scientists could diagnose it with conventional medical technology. Mrs Milne's gift manifested when she started smelling an unfamiliar musky odour coming from her husband, who was then diagnosed with PD. As a result of Mrs Milne's super-smelling observations, new research projects aim at identifying volatile molecules secreted through the skin of people in the early stages of PD. In fact, scientists believe that changes in the skin of patients with early PD produces a unique odour linked to the condition. Finding the molecular signature responsible for the characteristic PD odour would be extremely beneficial for the development of early diagnosis tools for the scientific community.

To aid with the characterisation of the distinctive PD smell profile, here at Anatune we developed a Dynamic Headspace (DHS) GC-MS method for the analysis of gauzes used to swipe skin of PD affected individuals. DHS is a sample preparation capability for GC application using the GERSTEL MultiPurpose Sampler MPS. DHS efficiently extracts and concentrates VOCs from liquid or solid samples. The sample is incubated while the headspace is purged with a controlled flow of inert gas through an adsorbent tube. Once extraction and pre-concentration is completed, the adsorbent tube is automatically desorbed using the GERSTEL Thermal Desorption Unit (TDU). Analytes are then cryo-focused on the GERSTEL Cool Injection System (CIS) PTV injector before being transferred to the GC for analysis.

In order to correlate the PD molecular signature to the PD smell, the same setup was used in combination with the GERSTEL Olfactory Detection Port (ODP). The ODP allows recognising of odorous compounds as they elute from the GC by smelling. In fact, the gas flow is split as it leaves the column between the detector of choice (in our case MS) and the ODP to allow simultaneous detection on the two analytical tools. The additional smell profile information can then be acquired. Voice recognition software and intensity registration are also available to allow direct annotation of the chromatogram.

This application note describes the preliminary work done at Anatune in support of the work published by Manchester University. See here. The data was acquired using DHS in combination with ODP for the investigation of the smell of the molecular signature of Parkinson's disease.

INSTRUMENTATION

System 1:

Autosampler: GERSTEL MPS xt Dual Rail, Gripper

Modules: Vial tray VT32-20 mL, DHS

GC-MS: Agilent GC 7890- MSD 5977BC, HES Source

System 2:

Autosampler: GERSTEL MPS Robotic Dual Head, USM tool with gripper

Modules: Vial Tray VT-15 20 mL, DHS, ODP

GC-MS: Agilent GC 7890- Q-TOF 7200, RIS Source

Figure 1: MPS Robotic Dual Head equipped with DHS and ODP and coupled to Agilent GC/Q-TOF.

METHODS

Control patients (n=11), PD patients drug naïve (n=10), PD patients under medication (n=10) were swiped on the skin in the back of their neck using sterile medical gauze dressing. Gauzes were transferred into 20 mL headspace vials and then analysed by DHS-TDU-GC-MS. 11 blank gauzes were also analysed to evaluate background contribution.

DHS-TDU-GC-MS analysis

CIS: Solvent Vent Mode, Vent Flow 80 mL/min, Split 1:10. Initial Temperature: 10°C for 2 min, 12°C/s to 250°C held for 10min. Tenax packed liner
TDU: Splitless mode, 30°C for 1 min, 720°C/s to 250°C held for 5 min. Transfer line 260°C

DHS:

No purge
Incubation: 5 min at 60°C
Trapping: 500 mL at 50 ml/min, Trap 40°C
No drying
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 5 min, 10°C/min to 170°C, 8°C/min to 250°C, 10°C/min to 260°C held for 2 min
Runtime: 31 min
MSD (5977B)

Auxiliary temperature: 300°C
EI mode at 230°C, Quadrupole 150°C, Mass range 30-800 *m/z*
Q-TOF (7200)

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. System blanks and matrix blank were acquired regularly every 10 samples to monitor possible carry over effect. Figure 1 shows an example of total ion chromatograms (TICs) obtained for the analysed samples.

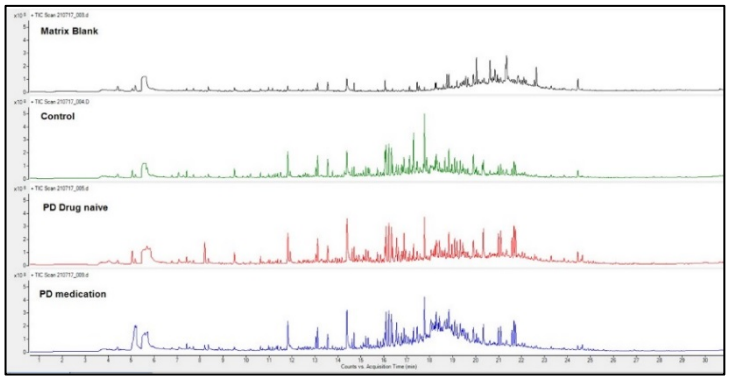

Figure 2: Total Ion Chromatograms (TIC) by DHS-TDU-GC-MS for (from the top): matrix blank gauze, control patient, PD drug naïve and PD under medication.

Acquired data were processed using Agilent Mass Hunter Unknown analysis to deconvolute the complex chromatographic information, extract and library search relevant components. Table 1 summarises the results obtained for all analysed samples.

Deconvoluted data were then exported to Agilent Mass Profiler Professional (MPP) for statistical evaluation. Principal components analysis (PCA) and clustering were chosen to investigate data. PCA is a visual way to explore the variance in the data set and it helps in the identification of patters. Clustering allows to organise entities and experimental conditions based on the similarity of their abundance profiles. Figure 2 and Figure 3 show the Principal Component Analysis graph and the Clustering (Hierarchical) obtained for the study results.

Table1: List of total deconvoluted components, blank subtracted and library hits components for the analysed

Sample Name	Components	Blank Subtracted	Hits
Matrix Blank1	642	NA	390
Matrix Blank 2	624	NA	374
Control	740	200	256
PD drug naive	699	189	274
PD drug naive	698	190	275
Control	705	178	300
Control	700	185	262
PD medication	721	186	273
Control	694	173	273
Control	709	168	311
PD medication	735	185	305
Control	679	176	261
Matrix Blank 3	724	NA	437
PD drug naïve	706	209	232
Control	726	178	299
PD medication	742	189	301
PD drug naïve	705	171	305
Control	638	179	274
Control	733	175	319
PD medication	725	178	271
PD drug naive	708	179	274
Control	695	173	297
PD drug naive	697	174	286
Matrix Blank 3	636	NA	369
PD drug naive	697	178	271
PD medication	703	182	281
PD drug naïve	732	175	289
PD medication	681	170	265
PD medication	732	155	323
PD drug naive	746	183	300
PD medication	720	175	297
PD medication	665	175	256
Control	663	179	253
PD medication	748	158	340
PD drug naïve	709	205	275
Matrix Blank 4	594	NA	302
Matrix Blank 5	641	NA	365

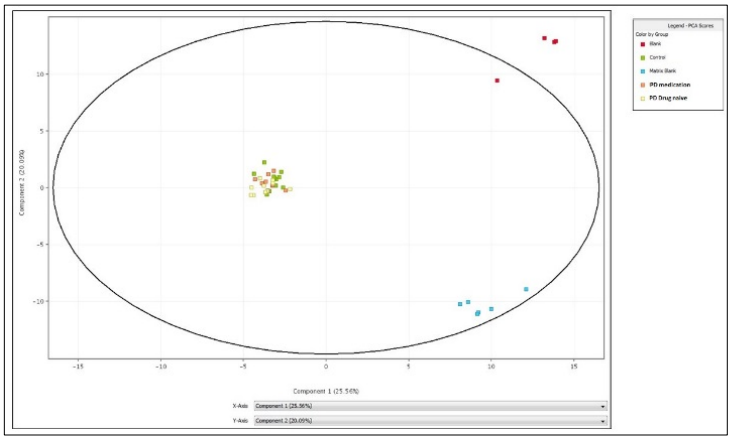

Figure 3: PCA obtained for the analysis of the investigated dataset by DHS-TDU-GC-MS.

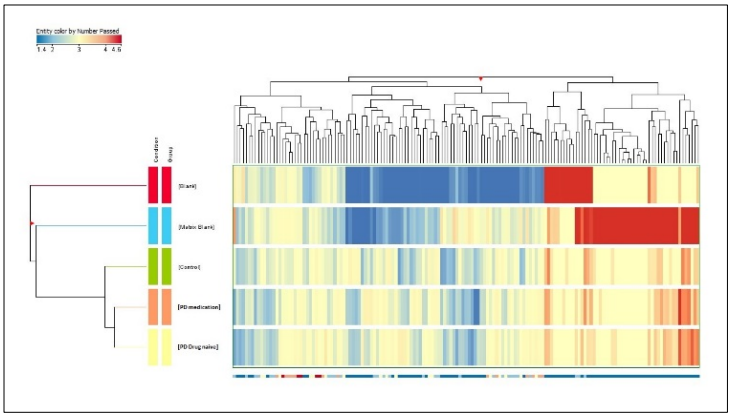

Figure 4: Hierarchical dendrogram obtained for the analysis of investigated dataset by DHS- TDU-GC-MS.

As shown in Figure 4 dendrogram, the unsupervised clustering algorithm could separate matrix blank, control, PD drug naïve and PD medication and differences in the entities patterns can be visualized in the heat map.

MPP generated lists of candidate entities responsible for the clustering. Amongst these entities, some revealed a smell profile as shown in Table 2.

Table 2: List of entities suggested by MPP which showed a smell profile

Compound Name	Retention time	Formula	CAS Number	Smell Profile
Pyrazine	6.174	C4H4N2	290-37-9	Nutty
2-Heptanone	9.546	C7H14O	110-43-0	Woody
alpha.-pinene	10.101	C10H16	80-56-8	Herbal
Acetic acid, phenylmethyl ester	14.039	C9H10O2	140-11-4	floral
Dodecanal	17.575	C12H24O	112-54-9	soapy
Longifolene	17.737	C15H24	475-20-7	woody
7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	24.49	C18H26O	88-29-9	musk

To help with the isolation of the entities potentially relevant as PD biomarker, a selection of matrix blanks and patients samples were run on an analogue DHS-TDU-GC-MS system equipped with the Olfactory Detection Port to allow Joy to smell the eluted peaks and identify relevant areas of the chromatographic space.

Figure 5: Mrs Joy Milne sniffing out PD relevant chromatographic peaks using the GERSTEL Olfactory Detector Port (ODP).

Figure 6 shows the overlaid TIC chromatograms (blank trace) and olfactograms (red trace) for a matrix blank gauze and three PD gauze samples.

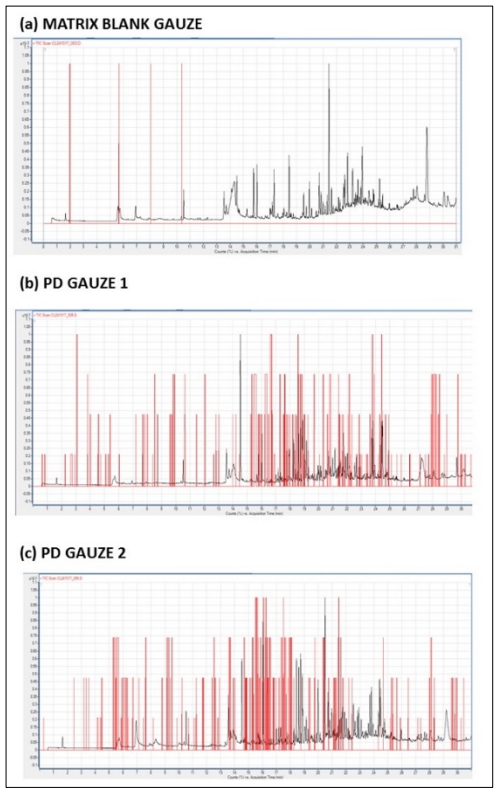

Figure 6: TICs (black) and relative olfactograms (red) for (a) matrix blank (b) PD sample 1 and (c) PD sample 2.

As highlighted by the olfactogram, Joy could isolate several chromatographic areas relevant to the PD smell in the patient samples in comparison to the matrix blank samples.

CONCLUSIONS

Very promising results were obtained by analysing Parkinson's disease patients' sweat sampled on medical gauzes using DHS-TDU-GC-MS. The supersmeller Joy Milne could highlight chromatographic areas relevant to the characteristic Parkinson's smell thanks to the use of the GERSTEL Olfactory Detection Port. Further investigation is planned to deepen understanding of these encouraging data.

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