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ANALYSIS OF HUMAN CELL LINES USING AUTOMATIC TUBE EXCHANGE (ATEX) AND GC/MS

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INTRODUCTION

GlaxoSmithKline were interested in measuring volatile components from cell lines. The non-volatile cell lines were pelleted from a suspension using a centrifuge for analysis. Due to the confidentiality of this project no further information can be given on these samples. However, this application note details the approach taken which could be applied generically to volatile analytes in a non-volatile matrix.

Automatic Tube Exchange (ATEX) is an automated thermal desorption technique where the sample is inserted into a microvial which in turn is inserted into a Thermal Desorption Tube (TDU Tube) for analysis, Figure 1.

Each sample is placed in the thermal desorption tray which is present on the GERSTEL MultiPurpose Sampler (MPS) above the Agilent Gas Chromatograph quadrupole time of flight (GC/Q-TOF), Figure 2.

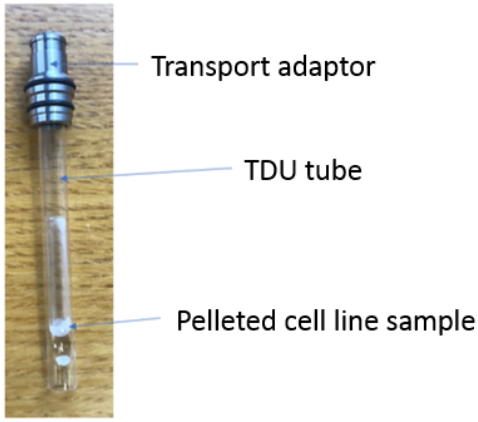

Figure 1 Pelleted sample ready for analysis

Figure 2. GERSTEL MultiPurpose Sampler with GC/Q-TOF (7200)

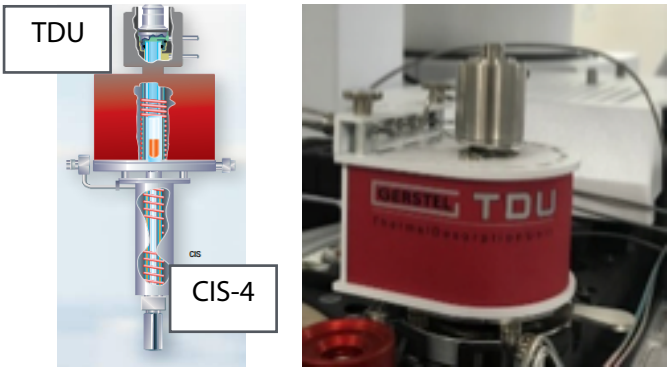

Figure 3. Thermal Desorption Unit on the GERSTEL MultiPurpose sampler

A schematic diagram and a photo of the Thermal Desorption Unit (TDU), Figure 3, which is mounted onto the Cooled Inlet System (CIS-4) shows how the sample flow path is uninterrupted from sample to the GC column. The TDU and CIS are independently heated and the zones between are heated to ensure analytes do not condensate in the flow path.

The desorption process from the sample to the GC starts with the CIS-4 being cooled (either cryogenically or by a Peltier cooler). This remains cool until the TDU program has finished. After inserting the sample, the TDU is thermally ramped typically from 50℃ to 250℃ desorbing the analytes from the TDU tube onto a clean cooled inlet liner within the CIS-4. Once this thermal cycle is complete, the CIS-4 inlet is then rapidly heated above 200℃ to desorb the analytes of interest from the CIS-4 onto the GC column. The non-volatile material left in the TDU tube (Figure 4) is then returned to the tray and the CIS-4 inlet remains clean.

Figure 4. Sample residues after desorption

INSTRUMENTATION

GERSTEL Dual Head MultiPurpose Sampler (MPS) XT

GERSTEL TDU using ATEX

GERSTEL CIS-4 using Tenax

Agilent GC/MS 7890/5975

Agilent GC/Q-TOF 7890B/7200

Both Agilent Technologies GC/MS and GC/Q-TOF have been used for this analysis with Masshunter Software for acquisition and data processing Mass Profile Professional software.

METHOD

Two different pelleted cell lines (Sample A and B) were supplied by GSK. Sample pellets were provided in the microvials at two different concentrations and were stored on dry ice until the analysis was performed. Multiple replicates were received and analysed.

TD-GC-MS Method:

TDU: 70℃ (1 minute) at 200 ℃/min to 300℃

CIS: 10℃ (0.1 minute) at 12 ℃/second to 250℃

GC column: DB5MS 30 x 0.25 mm x 0.25 µm

Oven Program: 40℃ linear thermal gradient to 325℃

Mass Spectrometer range – 50 to 450 m/z (both Single quadrupole and QTOF used for this study).

RESULTS

Due to the complex nature of the samples, it was important to check for any carry over through to the next injection. A comparison of biological sample B and the subsequent blank desorption (Figure 5) shows that no significant carry-over was observed.

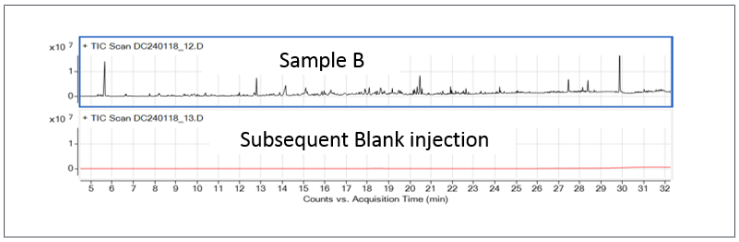

Figure 5. Comparison of Sample B with Blank injection showing no carry over

Indole, 7,9 Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8 dione, Dodecanamide and Cholesterol were tentatively identified in sample A as potential compounds of interest, Figure 6. The identification of Cholesterol was not only good when compared to a library spectra (Figure 7) but was also confirmed when analysed on the GC/Q-TOF with an accurate mass error of less than 5 ppm (for cholesterol M^+).

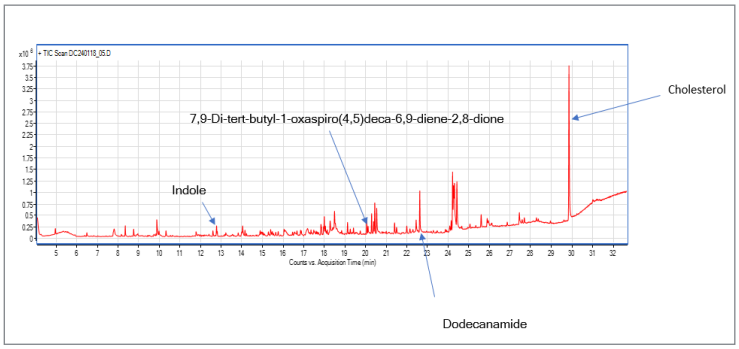

Figure 6. Analysis of Sample A at the lower concentration

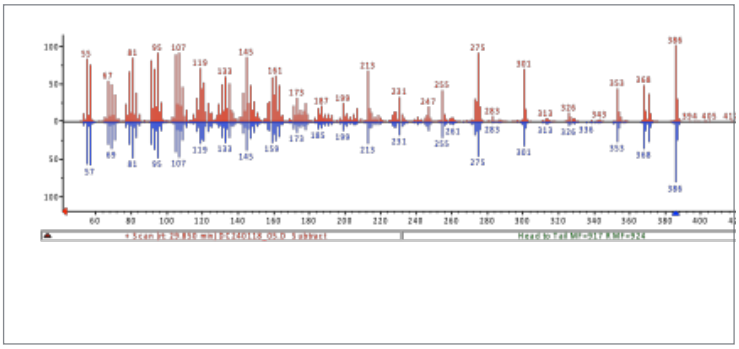

Figure 7 NIST match for Cholesterol (red unknown, blue library match)

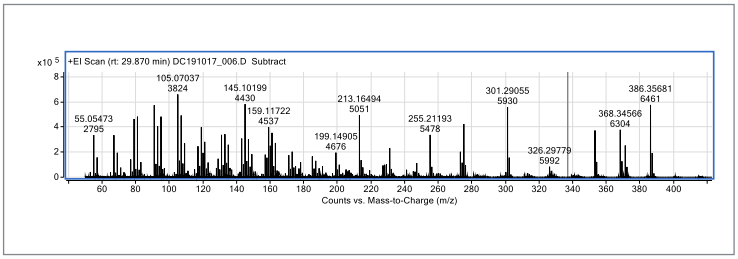

Figure 8. GC/Q-TOF data for analyte at 30 minute

Reproducibility was checked for Samples A and B. Four replicates of each biological sample were analysed, as shown in Figure 9 (Sample A, higher concentration) and Figure 10 (Sample B, higher concentration).

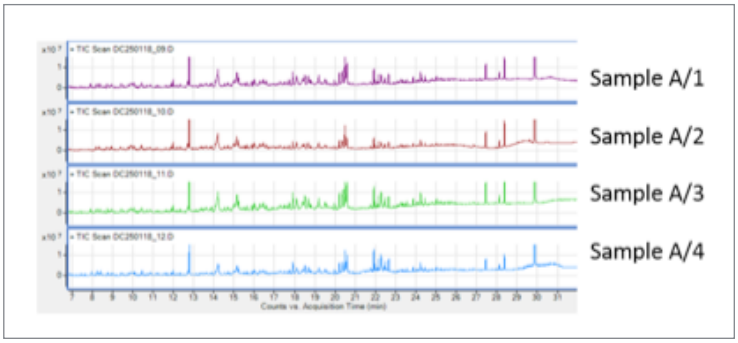

Figure 9. Replicate analysis for Sample A at the higher concentration

The chromatographic peaks at retention times 13 (Indole) and 30 minutes (Cholesterol) in Sample A were integrated in all four chromatograms and the relative standard deviation (%RSD) were calculated to be 20% and 18% respectively.

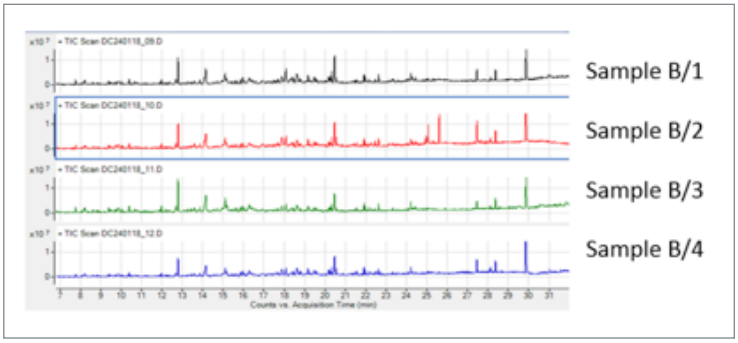

Figure 10. Replicate analysis for Sample B at the higher concentration

The lower concentration samples were also analysed. The same analytes were detected with a lower response (not shown).

Principal Component Analysis (PCA) was performed on the data to find key analytes between the two different cell lines. A scores plot of all the samples analysed shows a clear separation of the cell lines, Figure 11, of Sample A, (green) and Sample B (Blue) and a clear separation of both cell lines from the blank (red).

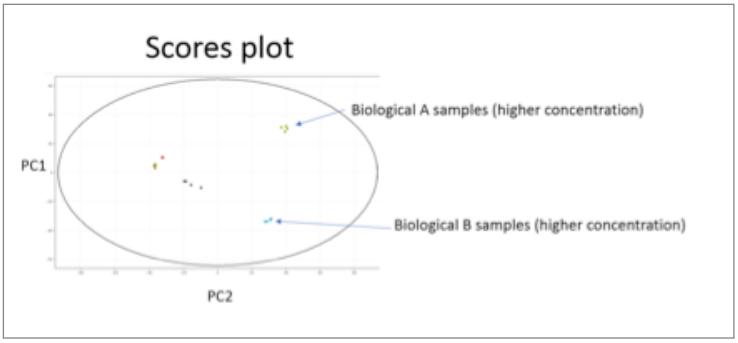

Figure 11. Scores plot displaying visual differences between the two cell lines analysed

From the PCA, it was possible to identify key markers for each cell line. One of the principal differences was identified to have a prominent ion at m/z 98. An extracted ion chromatogram (EIC) Figure 12, showed a number of analytes that were detected for Sample A which were present at a significantly higher concentration than in Sample B.

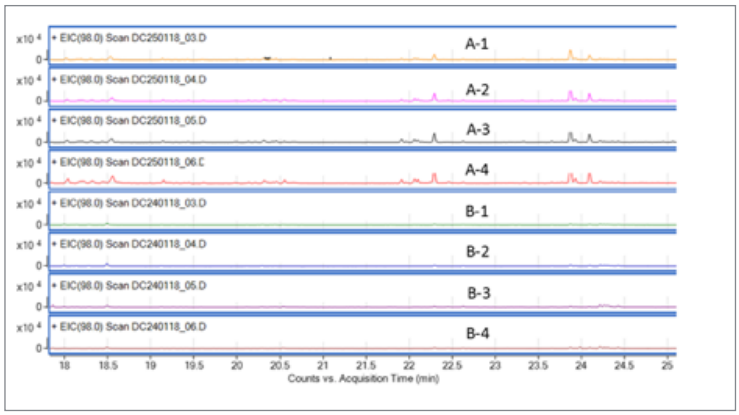

Figure 12. Extracted ion chromatograms at 98 m/z showing differences between the two sets of samples

The analyte detected in the EIC at 22.3 minutes in Sample A gave a good NIST match for Palmidrol. Figure 14 shows the comparison between the NIST library and the unknown at 22.3 minutes.

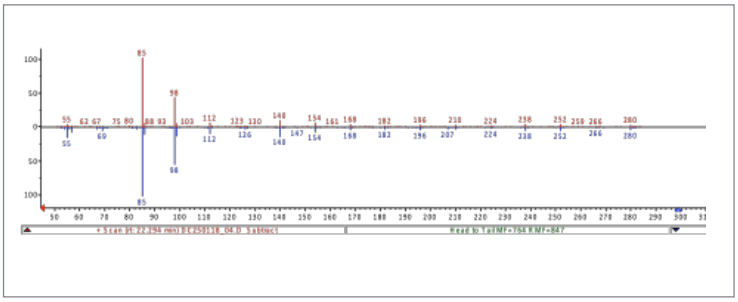

Figure 13 NIST Match for Palmidrol (red unknown, blue library match) in Sample A

Similar fragmentation pattern was shared by another analyte suggested as significantly different in the two sample populations: Oxazole, 2-(8Z)-8-heptadecen-1-yl-4,5-dihydro-. Figure 14 shows the extracted ion chromatograms for the component and the comparison between the deconvoluted spectrum and the library spectrum.

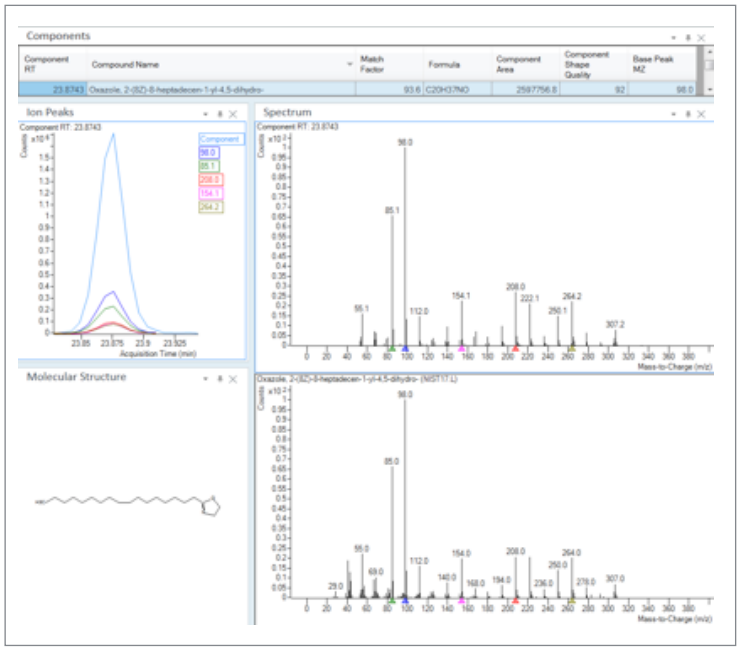

Figure 14 Ion peaks and spectrum comparison for the component identified as Oxazole, 2-(8Z)-8-heptadecen-1-yl-4,5-dihydro-

CONCLUSION

- By using Automated Tube Exchange, it is possible to analyse pelleted cell line samples without introducing the non-volatile components into the inlet liner which will help improve the robustness of the analytical method.
- Using Principal Component Analysis, it is possible to distinguish between the two different human cell lines and it is also possible to find key analytes which are indicative of each group. Further interrogation of the data should yield further differences between the groups.
- Having identified the differences between Samples A and B, this method could be adapted with the addition of a suitable internal standard to quantify the principle components.