



APPLICATION OF AN AUTOMATED LIQUID-LIQUID EXTRACTION (LLE) GCXGC-TIME OF FLIGHT-MS (GCXGC-TOFMS) WORKFLOW TO A SIMULATED EXTRACTABLES STUDY

APPLICATION NOTE AS-302

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Abstract

Data analysis is often the time-consuming step in analytical chemistry, this being made more difficult when complex chromatograms are involved or when comparisons between sample and extraction types are required. In this work principal component analysis (PCA) provided by LECO's ChromaTOF® Tile was used to look for differences in extraction types produced from a simulant solution study of a rubber sample. Dichloromethane (DCM), deionised water and 10% ethanol (aq) were compared with DCM showing clear differences from aqueous matrices. PCA also highlighted that aqueous matrices produced no significant differences when compared to a procedural blank.

INTRODUCTION

Extractables and leachables studies involve the analysis of complex matrices such as rubbers and plastics to assess the presence of potentially harmful compounds and to evaluate the impact of these materials in clinical use. For example, rubber o-rings from nasal spray devices are evaluated for compounds which may leach into the formulation over the lifetime of the device and thus present a potential toxicological hazard. Simulant solutions are used to mimic clinical usage and give the best assessment of leachate potential under carefully designed conditions. These studies can be targeted, looking for known analytes, for example those which may be used in the manufacture of the testing material or untargeted for a full characterisation of the material.

Previous automated Liquid/Liquid extraction methods have been developed and applied to these simulant solution scenarios^{1,2}, showing good recovery and precision of a range of analytes spanning a wide physico-chemical spectrum proving to be suitable alternative to manual sample preparation techniques.

As the data produced from these studies can often be complex due to the nature of the sample types, untargeted data analysis can be challenging and time-consuming as analysts will need to deconvolute and manually process each peak. Multi-dimensional chromatography, such as 2-dimensional Gas Chromatography (2D-GC) can play an important role in such complex analyses by providing greater chromatographic selectivity through the use of serial orthogonal stationary phases. Improvements in selectivity and efficiency achieved with 2D-GC techniques can aid in improved deconvolution and more effective data analysis, leading to an increase in data quality and processing speed.

Recent advances in 2D GC data processing uses tile-based Fisher-Ratio (F-ratio) analysis³ of data, combined with principal component analysis (PCA), to compare data sets and identify differences between samples classes (different sample types). F-ratios are described as the ratio

of variances within classes and between classes. This enables users to identify unique components between classes to enhance the capabilities of targeted analytical approaches.

In this work, a previously developed dispersive liquid-liquid microextraction (DiLLME) approach was used to analyse and compare, water, 10% (aq) ethanol and dichloromethane (DCM) extract solutions for an extractables and leachables study in the investigation of a rubber material. By using Fisher-Ratio and principal component analysis, it was found that both water and 10% ethanol performed similarly in terms of the components extracted, with several unique components identified when using dichloromethane as the extraction solvent for these types of materials.

EXPERIMENTAL

Instrumentation

Gerstel MultiPurposeSampler MPS DualHead Robotic/Robotic^{Pro}

LECO Pegasus BT 4D GCxGC-TOFMS

ChromaTOF[®]

First dimension: RXi-5SiIMS 30 m x 0.25 mm x 0.25 μ m

Second dimension: DB-17MS 0.7 m x 0.18 mm x 0.18 μ m

Modulation period 2.5 seconds



Figure 1: GERSTEL MPS and LECO Pegasus BT 4D

METHOD

One test article of rubber o-ring was extracted in 5 mL of three different solvents: Deionised water, 10% ethanol (aq.) and DCM at 10 °C below the boiling point of each solvent for 3 hours, to replicate an extractables study. The water and 10% ethanol extracts were back extracted with dispersive liquid-liquid extraction using 1 mL DCM as extraction solvent with 0.5 mL propan-1-ol added to the water matrix to act as disperser agent. Five reps of each extraction were carried out. Five replicates of blank lab water were also extracted. Extracts were analysed by GCxGC followed by data processing using ChromaTOF® and ChromaTOF® Tile software to compare the extraction types and investigate potential differences in the extractants from the three solutions. To sure comparability, the same chromatography and mass spectrometry parameters were used for all extraction types.

RESULTS

A GCxGC method was developed, optimising the modulation periods to ensure best separation between sample components. Figure 2 shows a

comparison of chromatograms between DCM extracts and water-based extracts. It was observed that dichloromethane extractions give rise to more intense peaks and a greater number of components between 700-1000 seconds (11-16 minutes).

Chromatograms were visually inspected in ChromaTOF® for retention time drift and peak shape prior to analysis in ChromaTOF® Tile for F-ratio analysis with accompanying principal component analysis (PCA). Figure 3 displays an example of the initial F-ratio assessment where the data must be checked for suitable tile size. A tile being defined as peak widths in the first and second dimension which equates to certain number of modulations and number of spectra collected. Here, each feature can be assessed to ensure the tile size is appropriate as duplicate features may be identified. This may be the case for compounds that produce tailing peaks, or where the tile size chosen is too small and the compound peak has a retention time wider than the tile and so data should be reviewed at this point with the suggested tile size being adjusted to suit.

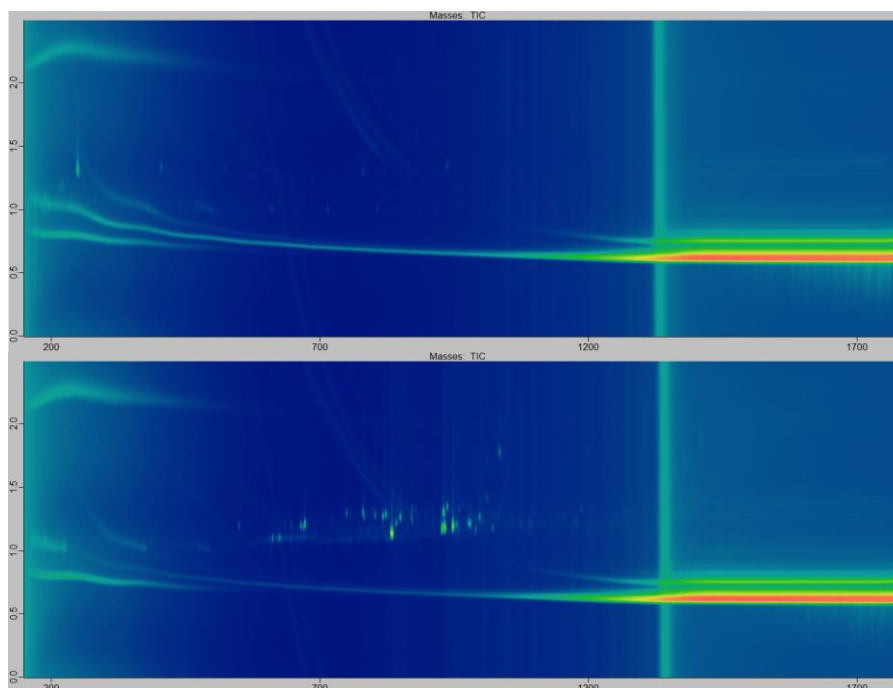


Figure 2: Example Total Ion Chromatogram (TIC) contour plots for 10% ethanol (aq), top and DCM, bottom.



Figure 3: Initial F-ratio analysis showing list of features with associated F-ratio and masses found for each sample class (left). Feature intensities are also depicted with heat maps, red being most intense. The contour plots to the right show a selected feature within a tile (red box) for each sample class.

Figure 4 shows the scores and loading plots from the PCA calculated using the features and library hits from the initial F-ratio analysis. Component 1 shows the largest variation between the extraction types with DCM extractions being clearly distinguishable from both water and 10% ethanol matrices. In addition, results from water and 10% ethanol extracts exhibit little difference

between them and no significant differences to the procedural blank solutions. This may be due to the inability of water-based solutions to extract the material or the presence of compounds below the limit of detection.

Using the scores and loadings plots to further analyse the data, several components were

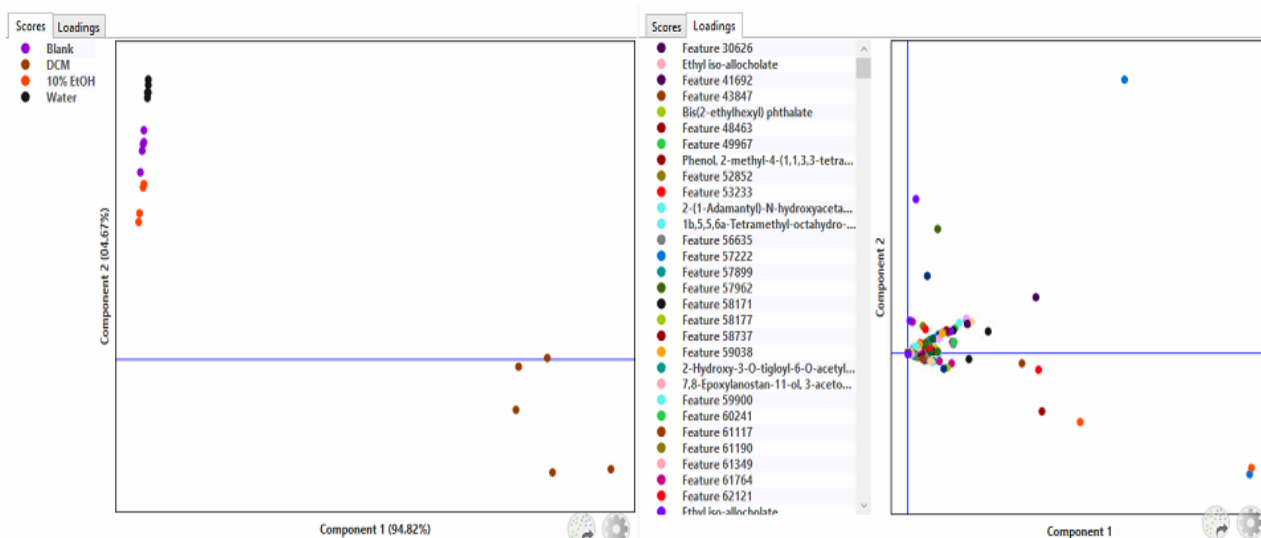


Figure 4: Left: Scores plot of PCA showing DCM extracts as significantly different o all others. Right: loadings plot showing individual features and their importance to each principal component.

revealed as being unique to the DCM extracts. Whilst in many cases TIC comparison may show differences between sample classes by visual checking and chromatographic overlay, where sample classes and chromatograms are similar, the differences may not be so easily seen and important peaks of interest that are smaller in area may be lost. PCA provided by ChromaTOF tile gives a quick comparison that does not rely on judgment by visual differences. The area bar chart for pyrene across all extraction types is shown in figure 5, clearly indicating the uniqueness of this component within the DCM extract. Pyrene has a Log $K_{o/w}$ of 4.88⁴ which explains the preference of this analyte to partition (extract) into the less polar DCM extractant solution, versus the more polar aqueous extraction solvents.

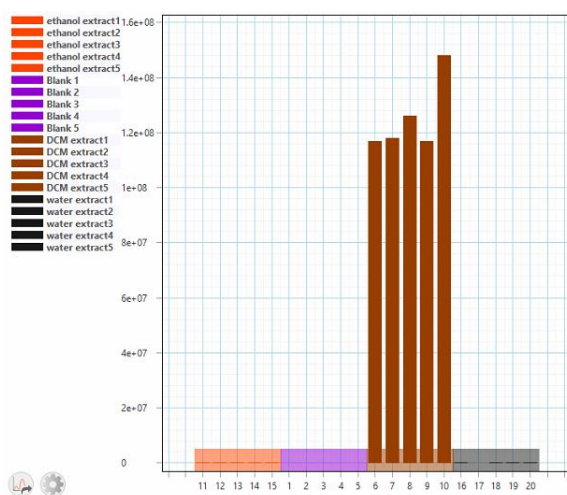


Figure 5: Area bar chart for Pyrene across all samples

A branched alkene, 2,4-dimethyl-1-heptene was also found to be unique to DCM extracts. Figure 6 shows the library spectrum compared to the sample with a similarity score of 946 with the associated chromatogram in figure 7.

To further aid in tentative compound identification, retention time indexing was used. Retention time and retention indices (RI) obtained from an injected standardise alkane mix can be used within ChromaTOF® Tile for confidence in identification. 2,4-dimethyl-1-heptene has a library RI of 836, compared to a

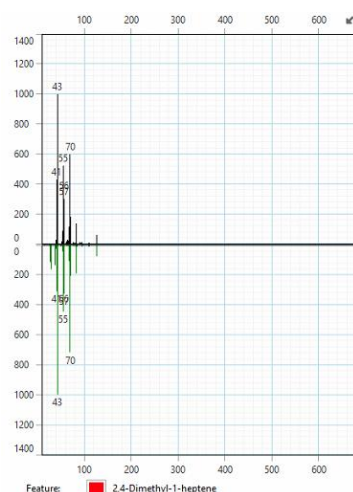


Figure 6: Mass spectrum for 2,4-dimethyl-1-heptene. Library spectrum top, sample spectrum bottom.

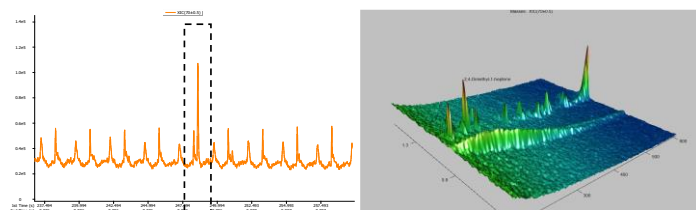


Figure 7: Left: Extraction Ion Chromatogram (XIC) for 2,4-dimethyl-1-heptene, m/z 70. Peak signal-to-noise 123. Right: 3D contour plot for the same mass.

sample generated RI of 841, providing further confidence to the identification. Table 1 provides an example list of ten tentatively identified compounds in the DCM extracts. Aqueous matrices did not provide any unique compounds not identified in the blank. For greater certainty of identification, a user library can be created from analytical standards or from samples known to contain certain compounds.

Table 1: *Tentatively identified compounds*

Tentatively Identified Compound	Library Match Score	Experimental RI value	Δ RI
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	814	1480	8
2-Cyclohexen-1-one	882	934	14
2-tert-Butyl-4-ethylphenol, O-(n-propyloxycarbonyl)-	786	1694	-14
Acenaphthylene	831	1472	17
Benzene, 1-(1,1-dimethylethyl)-4-ethyl-	815	1191	30
Benzene, 1,1'-(1,3-butadiyne-1,4-diyl)bis-	819	2174	32
Decane, 2,3,5,8-tetramethyl-	846	1290	-28
Heptane, 2,4-dimethyl-	820	808	-13
Phenol, 2-(1,1,3,3-tetramethylbutyl)-	835	1628	25
Tetrachloroethylene	873	795	-20

CONCLUSION

This work shows how automated sample preparation techniques combined with 2D GC can provide a comprehensive workflow for extractables and leachables studies, in this case providing an evaluation of a variety of simulant solutions used for comparison of extracting power. These evaluations can then be used to guide more targeted studies.

By using automation for sample preparation, time can be more effectively used on data analysis, which in cases such as these can be time consuming. By using ChromaTOF® Tile, PCA approaches can be used to drive simulation solution selection which does not rely on visual comparison which can lead to missed components which could be of importance. ChromaTOF Tile software approaches can also decrease the data analysis time, which is often the rate limiting step in extractables and leachables analysis.

Automated sample preparation also provides the flexibility to extract and analyse various sample types by several different techniques, providing greater efficiency in leachable study design and reduced method development and validation time.

Two-dimensional gas chromatography provides greater chromatographic separation between components for improved compound identification with the option of swift and seamless switch to 1D GC for studies where extra chromatographic resolution is not needed.

REFERENCES

1. Analysis of Semi-Volatile Leachables by Dispersive Liquid-Liquid Micro Extraction (DLLME); Mark Stanford, GlaxoSmithKline, 2013

2. SIMULANT SOLUTION EXTRACTION USING AUTOMATED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION (DiLLME) IN EXTRACTABLES AND LEACHABLES ANALYSIS; Jonathan Dunscombe, Anatune, Sarah Brophy, VR Analytical
3. Tile-Based Fisher Ratio Analysis of Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GC x GC- TOFMS) Data Using a Null Distribution Approach; Brendon A. Parsons et al, Anal. Chem. 2015, 87, 7, 3812-3819.
4. <https://pubchem.ncbi.nlm.nih.gov/compound/Pyrene#section=Vapor-Pressure>

To discuss implementing this application solution for Extractables and Leachables, contact us and we will be delighted to work with you from conception to method transfer into your laboratory.

We also offer fully validated methods, according to your validation protocol, where required.