

COMPARISON OF THE AGILENT 5977B MSD WITH HIGH EFFICIENCY SOURCE AND 7250 Q-TOF FOR ANALYSIS USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION OF SEMI VOLATILE ORGANIC COMPOUNDS

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INTRODUCTION

Dispersive liquid-liquid microextraction (DLLME) is a technique gaining a lot of attention. Due to increases in sensitivity of both the Agilent 5977B with HES and the 7250 Q-TOF, sample sizes are easily scaled down while achieving the required detection limits. This application note will discuss and compare the chromatographic and mass spectral data from the Agilent 5977B MSD and 7250 Q-TOF.

Anatune Application Note AS186 discusses how the 5977B HES can be applied to a fully automated DLLME workflow for the analysis of Semi-Volatile Organic Compounds (SVOCs) achieving limits of detection that were only previously achievable through manual methods utilising a large concentration factor.

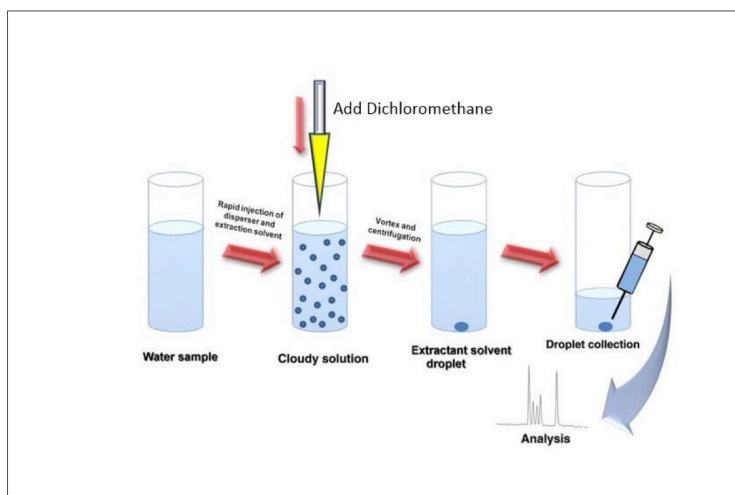


Figure 1: Illustration of Dispersive Liquid-Liquid Micro-Extraction

With any analytical technique the data-analysis is as important as the sample preparation. Software must be used to its full potential to ensure that false positives, which can have serious implications, are not reported. Agilent MassHunter data analysis software covers a wide range of applications to enable both qualitative and quantitative workflows.

The aim of this application note is to explore the benefit of using 7250 Q-TOF compared to 5977B HES for a DLLME SVOC targeted analysis workflow

INTRUMENTATION

Autosampler: Dual Head Multi-Purpose Sampler Robotic, Universal Syringe Module tool equipped with 10 μ L syringe (MPS).
GERSTEL QuickMix
Anatune CF200 Robotic centrifuge

GC-MS: Agilent 7890B GC / Agilent 7250 Q-TOF, Low energy EI source
Agilent 7890B GC / Agilent 5977B HES MSD

Software: Agilent Masshunter B.09 qualitative,
MassHunter B.08 Qualitative,
Masshunter PCDL Manager B.08
GERSTEL Maestro

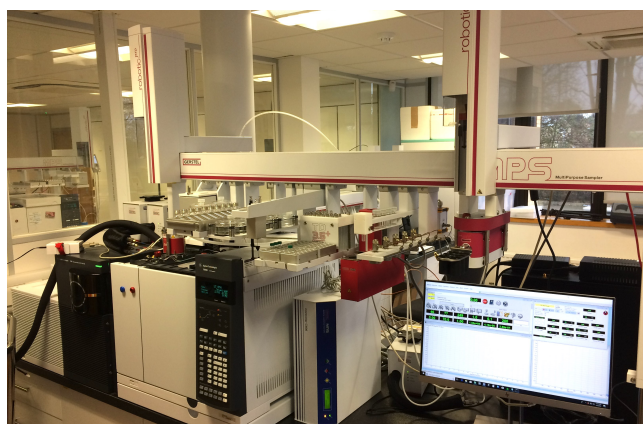


Figure 2: GERSTEL Dual Head Robotic MPS on top of Agilent GC 7890B coupled to 7250 Q-TOF MS

METHOD

Sample Preparation

Water was manually spiked using EPA 8270 stock solution to make up concentrations of 0, 0.10, 0.50, 0.125 and 1.0 μ g/L. 8 ml of each sample was then taken and extracted by DLLME using the MPS. Figure 3 shows the GERSTEL Maestro PrepSequence for a set of six samples, prepared in a batch. Samples were prepared using an offline setup which does not include injection into the GC. For DLLME, a batch of six samples can be prepared in 30 minutes. The extracts produced were injected onto the 5977B MSD HES and 7250 Q-TOF using a 2 μ l splitless injection.

Data Analysis

All data was reviewed using Agilent MassHunter Qualitative Navigator to collect information such as signal to noise. Unknowns Analysis was used for both single quad and Q-TOF data to create a quantitation method for use within MassHunter Quant.

Prior to quantitation, a Personal Compound Database and Library (PCDL) was created for the target compounds acquired on the Agilent GC-Q-TOF using a concentrated standard. This was performed by using the Molecular Feature Extraction option in MassHunter Qualitative Workflows. The created PCDL was then used as reference library for a targeted workflow within MassHunter Quant.

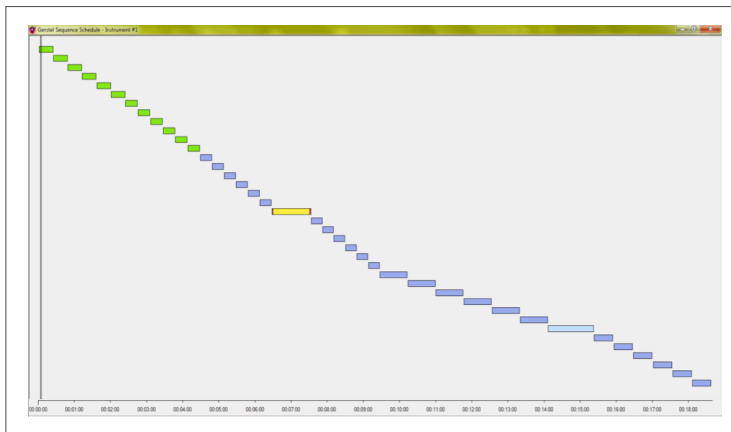


Figure 3: DLLME PrepSequence for six samples

RESULTS

To assess the performance of each instrument a selection of compounds were picked from the suite: 1,2-dichlorobenzene (m/z 146 (145.9690)), nitrobenzene (m/z 123 (123.0320)), 2,4,5-trichlorophenol (m/z 196 (195.9249)) and fluorene (m/z 166 (166.0783)), respectively. The selected ions were extracted from the total ion chromatogram in MassHunter Qualitative Analysis and the signal to noise ratio calculated for each. The ratios comparing 5977B HES and 7250 Q-TOF at 0.05 µg/L and 1.0 µg/L are shown in Table 1 and Table 2, respectively.

Compound	S/N (5977B HES)	S/N (7250 QTOF)
1,2-dichlorobenzene	64.7	37.4
nitrobenzene	13.9	15.6
2,4,5-trichlorophenol	2.2	45
Fluorene	66.4	180

Table 1: Signal to noise ratios at 0.05 µg/L

Compound	S/N (5977B HES)	S/N (7250 QTOF)
1,2-dichlorobenzene	686	762
nitrobenzene	216	291
2,4,5-trichlorophenol	82.4	327
Fluorene	1764	1620

Table 2: Signal to noise ratios at 1.0 µg/L

All single quadrupole data was extracted using a symmetric m/z error of 0.5 mass units. On the other hand, Q-TOF accurate mass data gives the ability to extract the exact mass along with a ppm error window. This results in reduced background noise. Figure 4 compares the extracted ion chromatogram (EIC) of 2,4,5-trichlorophenol at 0.05 µg/L using 0.5 mass unit error window for single quad data and a ppm error of 50 ppm using theoretical mass of 195.9249 m/z. Acquisition of accurate mass data provides a distinct advantage producing a much cleaner extracted ion chromatogram with fewer interfering peaks. As a result, a higher signal to noise ratio is observed together and a reduced chance of seeing interfering ions.

Table 3 shows the mass accuracies for each of the chosen compounds at 1.0 µg/L

Following creation of a library, a quantitation method and batch was created for both single quad and Q-TOF data. Figure 5 displays the calibration curve for 1,2-dichlorobenzene from the 5977B HES on top and 7250 Q-TOF below. It is here that the Q-TOF provides a greater a benefit.

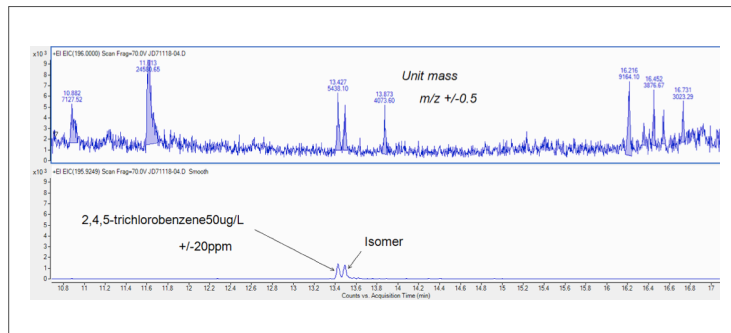


Figure 4: EIC comparison for 2,4,5-trichlorobenzene with 20 ppm window (top) and unit mass extraction (bottom).

Compound	Accurate mass (m/z)	Measured mass (m/z)	Mass accuracy (ppm)
1,2-dichlorobenzene	145.9685	145.9683	1.08
nitrobenzene	123.0315	123.0312	2.27
2,4,5-trichlorophenol	195.9244	195.9245	-0.51
Fluorene	166.0777	166.0777	0.01

Table 3. Mass accuracies for measured masses at 1.0 µg/L

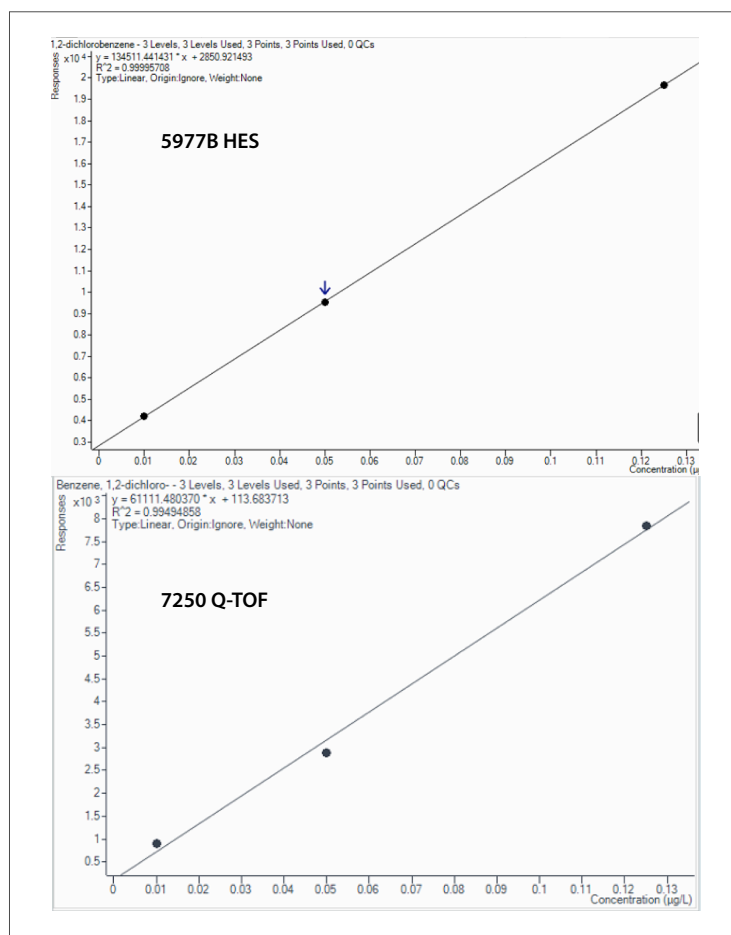


Figure 5. Calibration curve for 1,2-dichlorobenzene for both the Agilent 5977B MSD with HES and 7250 Q-TOF

R² values of greater than 0.990 for both instruments can be achieved with a linear calibration. Repeatability and recovery for this method were not investigated as part of this work. When performing targeted workflows, accurate mass data gives an advantage with the capability to setup outliers for mass accuracy values, with the software flagging an issue when values fall outside user defined parameters.

Name	Data File	Type	Level	Exp. Conc.	RT	Resp.	Final Conc.	Mass Accuracy
blank	JD71118-01.D	Blank			19.004	31186	0.2120	-2.8469
10ng/l	JD71118-02.D	Sample			19.021	2678	0.0143	1.8359
10ng/l	JD71118-03.D	Cal	1	0.0100	19.020	2121	0.0104	-3.1634
50ng/l	JD71118-04.D	Cal	2	0.0500	19.022	7734	0.0494	-2.8216
125ng/l	JD71118-05.D	Cal	3	0.1250	19.020	18672	0.1252	-5.5207
svoc std	svoc1.D	Sample			19.019	373115	2.5838	0.1826
blank	JD71118-06.D	Sample			19.015	264	0.0000	-20.5905

Figure 6: Mass accuracy within a quantitation batch for pyrene

The mass accuracy can be used to determine if the correct compound has been identified along with using qualifier ratios and retention time flagging, which are the only tools available for single quad data. In the example shown in figure 6 for pyrene, mass accuracy values of less than ten are observed across the concentration range, giving good confirmation that the correct compound is present.

For further confirmation of the presence of a particular compound, either in a targeted suite or unknowns workflow, the Low Energy Electron Ionisation (LE-EI) capability of the 7250 Q-TOF can be used. This ionisation mode uses a lower electron energy to reduce fragmentation and potentially increases the molecular ion response used for identification. This is a useful tool and can be used as an alternative to chemical ionisation. Figure 7 shows the confirmation of dibutyl phthalate using LE-EI to produce the molecular ion.

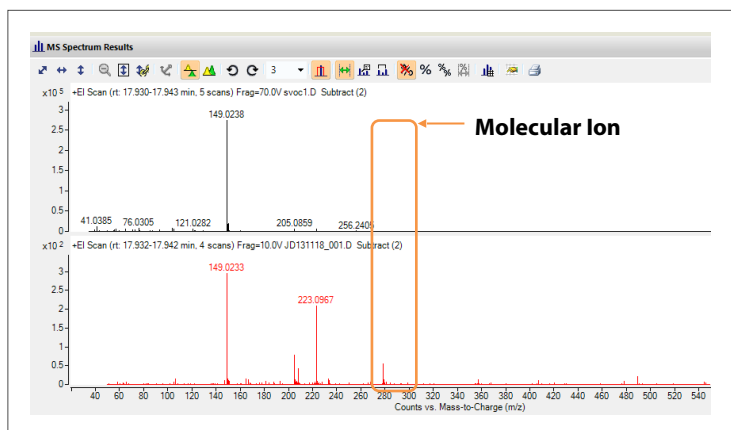


Figure 7: Comparison of mass spectra using standard EI (top) and LE-EI (bottom)

CONCLUSIONS

Both single quadrupole and Q-TOF instrumentation can be used as part of an automated DLLME targeted workflow. Using a Q-TOF for targeted and untargeted work can give higher confidence in results thanks to the accurate mass data to confirm compound identities, achieving mass accuracy of less than 5ppm in most cases. This can be used in combination with Low Energy Electron Ionisation for structural interrogation and further confirmation of the presence of compounds.

For true unknowns investigations where high resolution instrumentation is required for accurate mass data, the option of MS/MS (with Q-TOF) also provides extra benefit for further structural interrogation.

Automation brings an advantage to these workflows in reducing sample volumes and sample extraction times to increase laboratory efficiency reducing extraction times to 30 minutes for six samples.

Limits of detection down to 10 ng/L or lower are possible with the Agilent Q-TOF with automated DLLME extraction. In comparison, method reporting limits of 0.5 ug/L or less could be achieved when using 5977B with HES (in scan mode) on 6ml of sample. This is a marked improvement on current customer work using 200 mL of sample with evaporation step on a 5973 GC-MSD set up for SVOC workflow. Previous work performed at Anatune has shown that LODs of down to 0.01 ug/L are possible for a targeted analysis using the HES.

The extra sensitivity of the 5977B MSD with HES allows the automation of the sample preparation using DLLME; this is beneficial when used for full mass scan analysis for example.

REFERENCES

'Exploring the advantages of automated sample preparation combined with GC-TOF MS for SVOC and pesticides analysis in environmental waters', John Quick, ALS Environmental, Coventry UK

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