TECHNICAL NOTE 10728

Robust analysis of PAHs and PCBs in soil with over 500 repeat injections using Orbitrap Exploris GC

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Goal

The scope of this study was to test a simplified GC-Orbitrap™ analytical method over a large number of consecutive injections of soil samples to assess if it can meet the demands of routine trace analysis in soil samples.

Introduction

Polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) are toxic organic pollutants that can contaminate soils, air, sediments, and water as a result of natural and human processes. PCBs and PAHs are resistant to environmental degradation and can be transported over long distances. PAHs and PCBs from the environment can enter food chains where they are very persistent and very bioaccumulative (vPvB).[1]

PAHs and PCBs have numerous congeners, many of which have identical masses. For this reason, gas chromatography-mass spectrometry is the analytical technique of choice for their separation and quantification. Higher mass PAHs are prone to poor peak shape in gas chromatography, making peak integration challenging by impacting chromatographic resolution and sensitivity, leading to higher limits of detection and quantification. Also, the routine analysis of PAHs and PCBs in complex soil matrices requires consumables and instrumentation that deliver exceptional degrees of stability in terms of peak areas, response factors, ion ratios, and mass accuracy so that multiple batches of samples can be analyzed day in and day out with minimal instrument maintenance, such as liner change, column trimming, MS maintenance, or tuning.

In this work the performance of the Thermo Scientific™ Orbitrap Exploris™ GC was demonstrated for the analysis of PAH and PCBs in complex soil matrices by 500 repeat injections. Orbitrap Exploris GC is ideal for routine environmental screening methods because of its ability to meet the required sensitivity and in full scan, which enables consolidation of methods through combining compound classes into a single acquisition. Robustness and suitability for routine analysis was assessed by looking at the relative response factor, ion ratio, and mass accuracy stability for a low-level calibration QC standard (40 pg/μL in *n*-hexane) interspaced throughout the injection sequence of 500 samples, standards, and blanks over a total period of three weeks of continual high-throughput analysis. The system robustness was assessed by examining the peak area stability of 500 injections of a spiked QuEChERS soil extract (10 pg/μL) over this time period.

Experimental

Calibration standards containing 45 native PCBs and PAHs at twelve concentration levels (full details can be found in Application Note 10731[2]), and 14 (^{13}C-labeled) internal standards were acquired from Fisher Scientific, AccuStandards, and Wellington Laboratories Inc. (Ontario, Canada). For the assessment of relative response factor (RRF) stability, a low-level QC standard (40 pg/μL) was injected directly after every 20 sample injections.

An Orbitrap Exploris GC with mass resolving powers of up to 60k (*m/z* 219, FWHM) equipped with an electron ionization (EI) source and vacuum probe interlock (VPI) was fitted with an Instant-Connect SSL injector. The Thermo Scientific™ ExtractaBrite™ EI source is fully removable without needing to break the vacuum during source cleaning and column changing, and the patented RF lens leads to an excellent level of sensitivity and robustness.

Liquid injections (1 μL) of the QuEChERS soil extracts were performed using a Thermo Scientific™ TriPlus™ RSH™ Autosampler and Thermo Scientific™ LinerGOLD™ single taper with glass wool SSL liner (P/N 453A1925-UI). Chromatographic separation was achieved by a Thermo Scientific™ TraceGOLD™ TG-5 SilMS™ 30 m × 0.25 mm I.D. × 0.25 μm (P/N 26096-1420) film capillary column. Full experimental details, instrument parameters, and consumables used can be found in the Application Note 10731.[2]

Data were acquired using full scan (FS) acquisition mode, processed and reported using Thermo Scientific™ Chromeleon™ 7.3 software, which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting all within one platform.[3] With Automated System Suitability Testing (SST) and Intelligent Run Control (IRC), real time batches can be analyzed and the sequence can be stopped should a QC criterion fall outside of the set limits, saving precious samples from being injected.[4]

Results and discussion

PAH and PCB robustness were assessed based on absolute peak area response for 500 injections of a soil QuEChERS extract spiked at 10 pg/μL (ppb) level. The Orbitrap Exploris GC suitability for routine PAH and PCB analysis was also assessed continuously over three weeks with measurements of analytical parameters, such as stability of relative response factors, ion ratios, and compound mass accuracy. The batch included blanks, calibration standards, and soil sample extracts as well as quality control (QC) low level (40 pg/μL) solvent standards injected every 20 soil sample injections. Additional details of chromatography, sensitivity, linearity, and sample analysis can be found in the supporting application note.

Routine GC-MS analysis

Matrix complexity

Due to the diversity of matrices with various degrees of complexity, achieving enough selectivity can be challenging in routine GC-MS analysis of environmental samples. An example of soil sample complexity is shown in Figure 1 as a TIC of a sonicated unspiked QuEChERS soil extract containing many environmental contaminants including linear and branched alkanes.

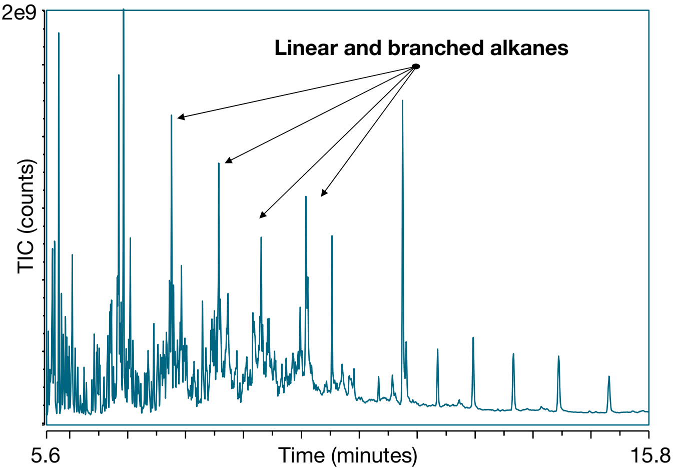
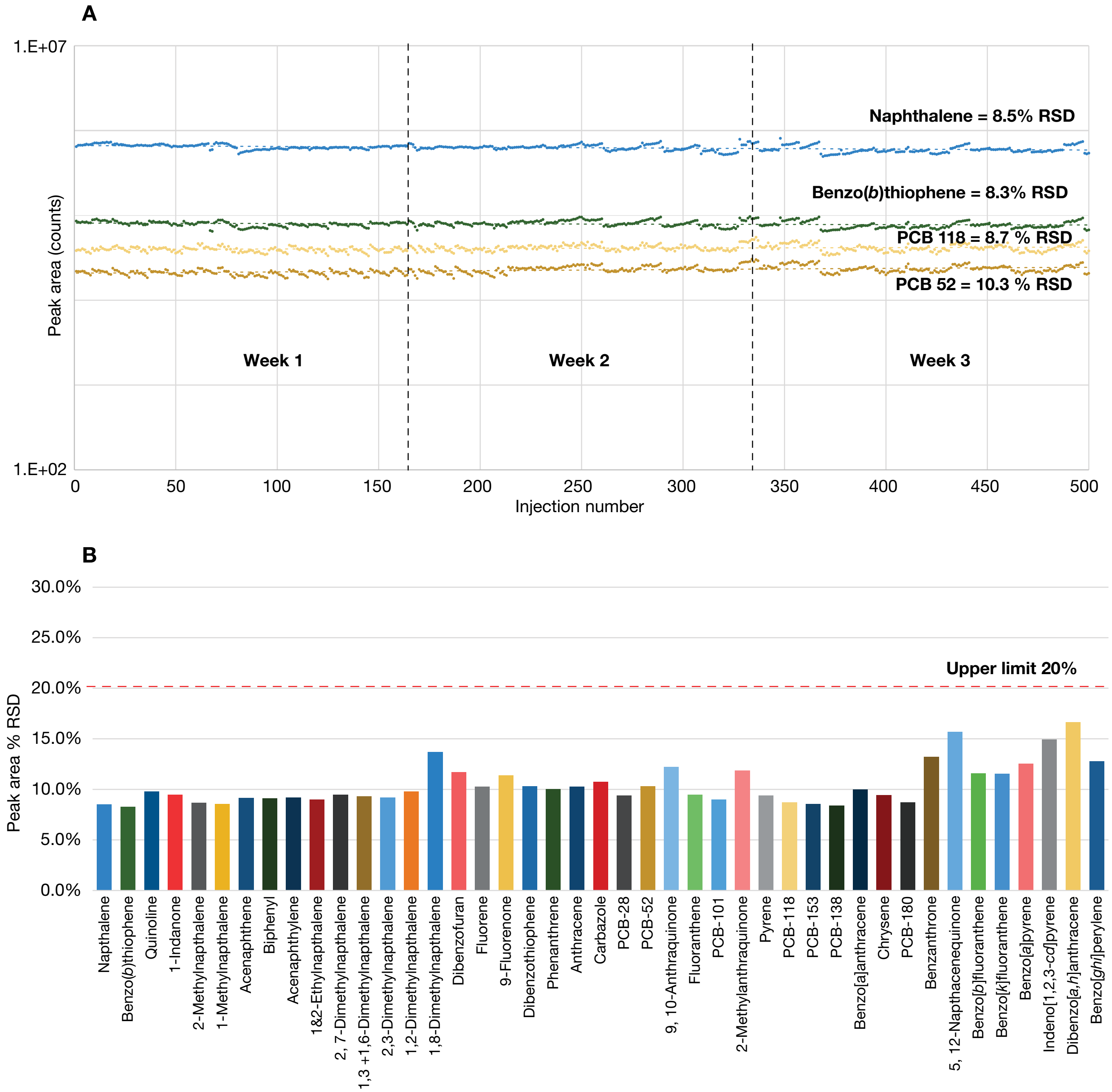


Figure 1. Total ion chromatogram of a QuEChERS soil extract (unspiked) acquired using an Orbitrap Exploris GC in electron ionization (EI), full scan (FS) over a mass range of *m/z* 50–550

Assessment of robustness

To evaluate system robustness, a QuEChERS soil extract post-spiked at 10 pg/µL (ppb) was injected 500 times. The absolute peak areas of target compounds were plotted and used to calculate the stability of response over time (as %RSD) as demonstrated for certain PAHs and PCBs (Figure 2A). The results demonstrate the system robustness with peak area repeatabilities for the incurred residues were <20% RSD over the 500 injections and three weeks of continuous analysis with an average of 10.5% across all compounds. (Figure 2B). Importantly minimal inlet maintenance (septa change every 100 injections) was performed with no liner change, column trimming, MS maintenance, or tuning were performed over the analysis period (the inlet septa were replaced every 100 injections and the Orbitrap system was calibrated weekly).



*Inlet septa were replaced every 100 injections. Apart from this no other inlet maintenance was undertaken.

Figure 2. (A) Repeatability %RSD of absolute peak area response (no internal standard correction), for example PAHs and PCBs from n=500 injections of a QuEChERS soil extract post-spiked at 10 pg/µL (ppb); (B) Absolute peak area %RSDs (no internal standard adjustment) for all PAHs and PCBs from n=500 injections of a QuEChERS soil extract as described in part (A)

Response factor stability in matrix samples

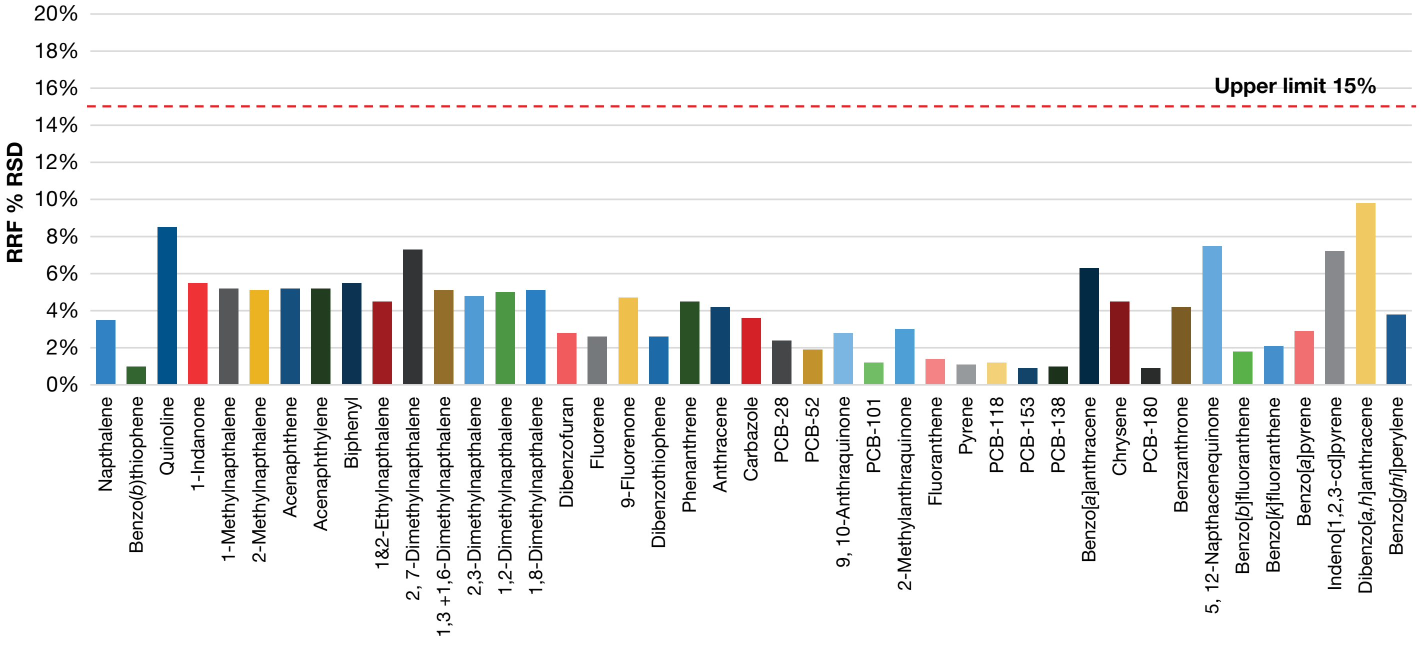
Relative response factor (RRF) is defined as the ratio between the response factors (RF) of both the analyte of interest and the associated internal standard. It can be used to calculate an unknown amount of an analyte in a sample of interest with high accuracy and precision, providing the system of interest is stable in nature, i.e., generating consistent RRFs across an analytical batch. Many labs choose to do this continuously throughout an analytical sequence without having to run an expensive and time-consuming calibration by instead running a quality control (QC) standard to check the RRF %RSD i.e. the precision and accuracy of measurements and the RRF % deviation from the calibration average. For these reasons, a low-level QC standard (40 pg/µL) was injected every 20 injections and the average RRF %RSDs were monitored for each PAH and PCB congener (Figure 3).

The RRF %RSDs were ≤15% for all the targeted PAHs and PCBs calculated over a sequence with an average of 4% across all compounds (which included over 500 sample injections). This demonstrates the excellent system stability with minimal inlet maintenance (septa change every 100 injections), no column trimming, MS maintenance, or system tuning.

RRF % deviation from the calibration average was also calculated for all PAHs and PCBs in the QC standards across the batch including 500 samples. All results were within a tolerance window of ±15%, in the calculated benzo(a)pyrene and PCB 153 examples (Figure 4) the RRF % deviation for n=35 QC injections were 1.2% and 0.5%, respectively.

Ion ratio stability in matrix samples

Another analytical parameter used for confident confirmation of compounds detected in samples is the ion ratio between quantification and qualifier ions. Stability of ion ratios is essential for any mass spectrometer in a routine laboratory setting in order to safeguard against false positive results. One way to indicate how stable the measurements are is the ion ratio of each analyte and its potential deviation from the initially determined value (usually determined as an average value over an external calibration curve injected at the beginning of a sequence). The ion ratio values obtained for all PAHs and PCBs for a low-level QC standard (40 pg/µL) were within ±15% of the expected values calculated as an average across a calibration curve ranging from 0.1-500 pg/µL (corresponding to 0.1–500 µg/kg in extracted soil). (Figure 5). This demonstrates excellent system stability and applicability for routine analysis.



*Inlet septa were replaced every 100 injections. Apart from this no other inlet maintenance was undertaken.

Figure 3. Response factor %RSD for a low-level standard QC standard (40 pg/µL) (n=35) run every 20 sample injections, injection sequence containing blanks, standards, and over 500 matrix (QuEChERS soil extract) injections

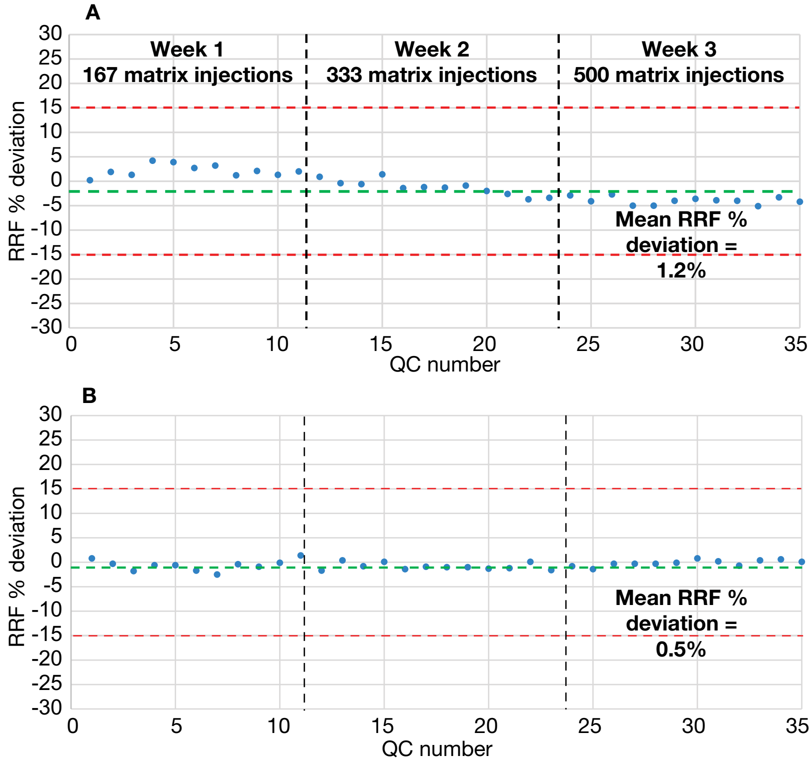


Figure 4. Chart showing the individual RRF % deviation values for benzo(a) pyrene (A) and PCB 153 (B) calculated for a low-level standard QC standard (40 pg/µL) (n=35) run every 20 sample injections. The ±15% tolerance RRF % deviation from the calibration average upper and lower limits are annotated using the red dotted lines and the mean RRF % deviation for the QCs is displayed using the green dotted line.

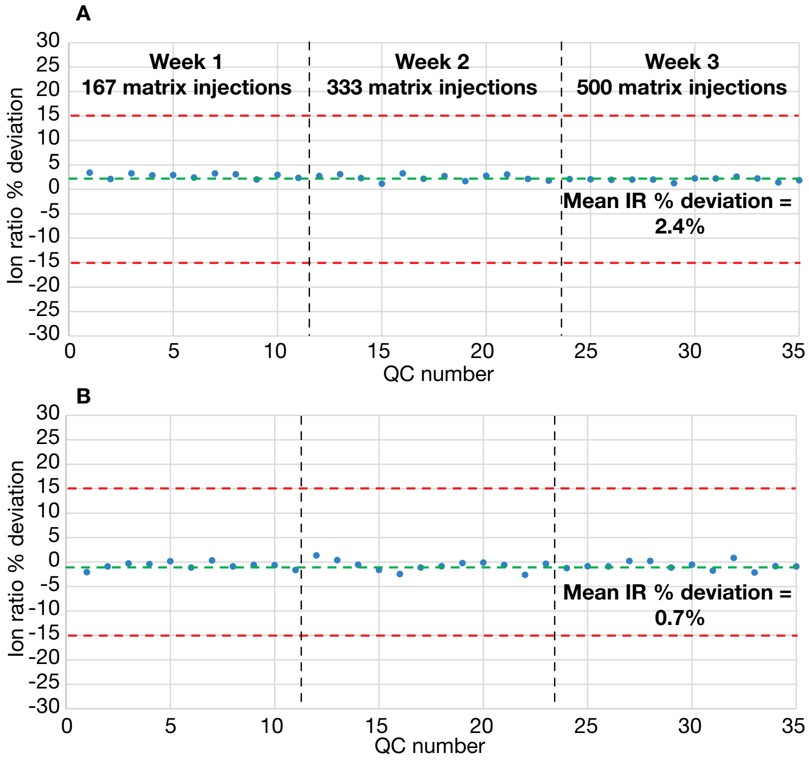


Figure 5. Example of ion ratio stability for benzo(a)pyrene (A) and PCB 153 (B) calculated for a low-level standard QC standard (40 pg/µL) (n=35) run every 20 injections. The ±15% tolerance ion ratio % deviation upper and lower limits are annotated using the red dotted lines and the mean ion ratio % deviation for the QCs is displayed using the green dotted line.

Mass accuracy stability in matrix samples

For high resolution accurate mass spectrometers operating in routine environments, stable mass accuracy is a cornerstone of system stability. Mass accuracy stability was assessed by monitoring the ppm mass error of a QC standard. The results obtained for all PAHs and PCBs for a low-level QC standard (40 pg/µL) were within ±1 ppm of the expected values. A few examples of the mass accuracy stability are shown in Figure 6.

The mass accuracy stability was also demonstrated in matrix (n=500 injections, 10 pg/µL soil QuEChERS extract) where accurate mass data was plotted for low, high, and medium mass compounds 1-indanone, PCB 180, and bezno(*g,h,i*)perylene (*m/z*=132.05697, 393.80195, and 276.09335, respectively), which eluted in the early, mid, and late range of the chromatogram (RT=6.0, 10.8, and 15.5 min) (Figure 7 – A, B, C). The average mass accuracies were 0.3, 0.1, and 0.0 ppm, respectively, which is well within 1 ppm mass accuracy criteria for the Orbitrap system. A QC standard was also used to monitor system performance over the three-week robustness study (40 pg/µL) injected (n=35) every 20 soil samples with mass calibration performed on a weekly basis. Average measured mass accuracy in ppm is denoted by a colored dot for each compound and the associated standard deviation is displayed as error bars. Annotated also is the combined mean mass accuracy and standard error for measurements made for all compounds (Figure 7 – D).

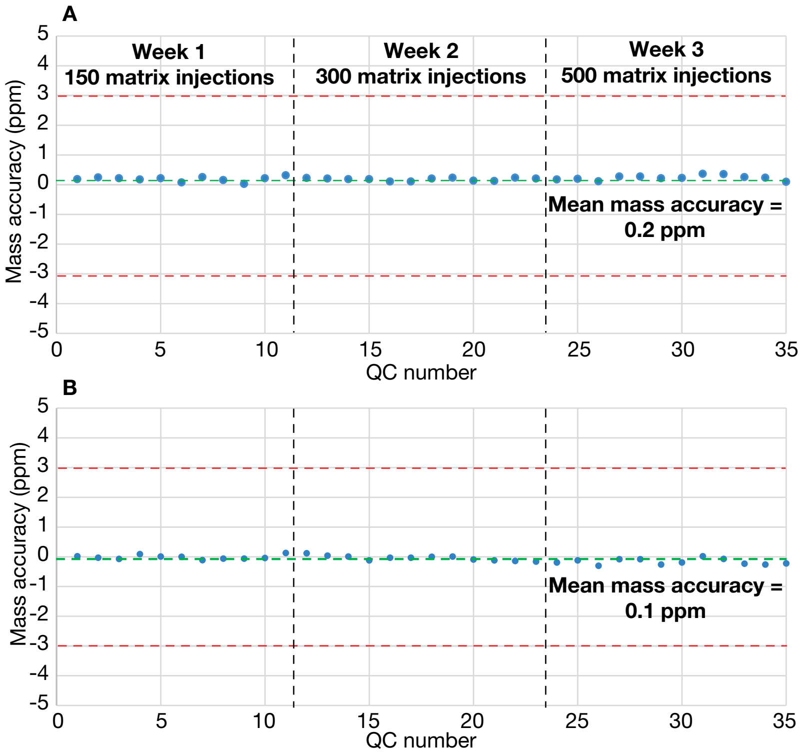


Figure 6. Example of mass accuracy stability for benzo(a)pyrene (A) and PCB 153 (B) calculated for a low-level standard QC standard (40 pg/µL) (n=35) run every 20 injections.

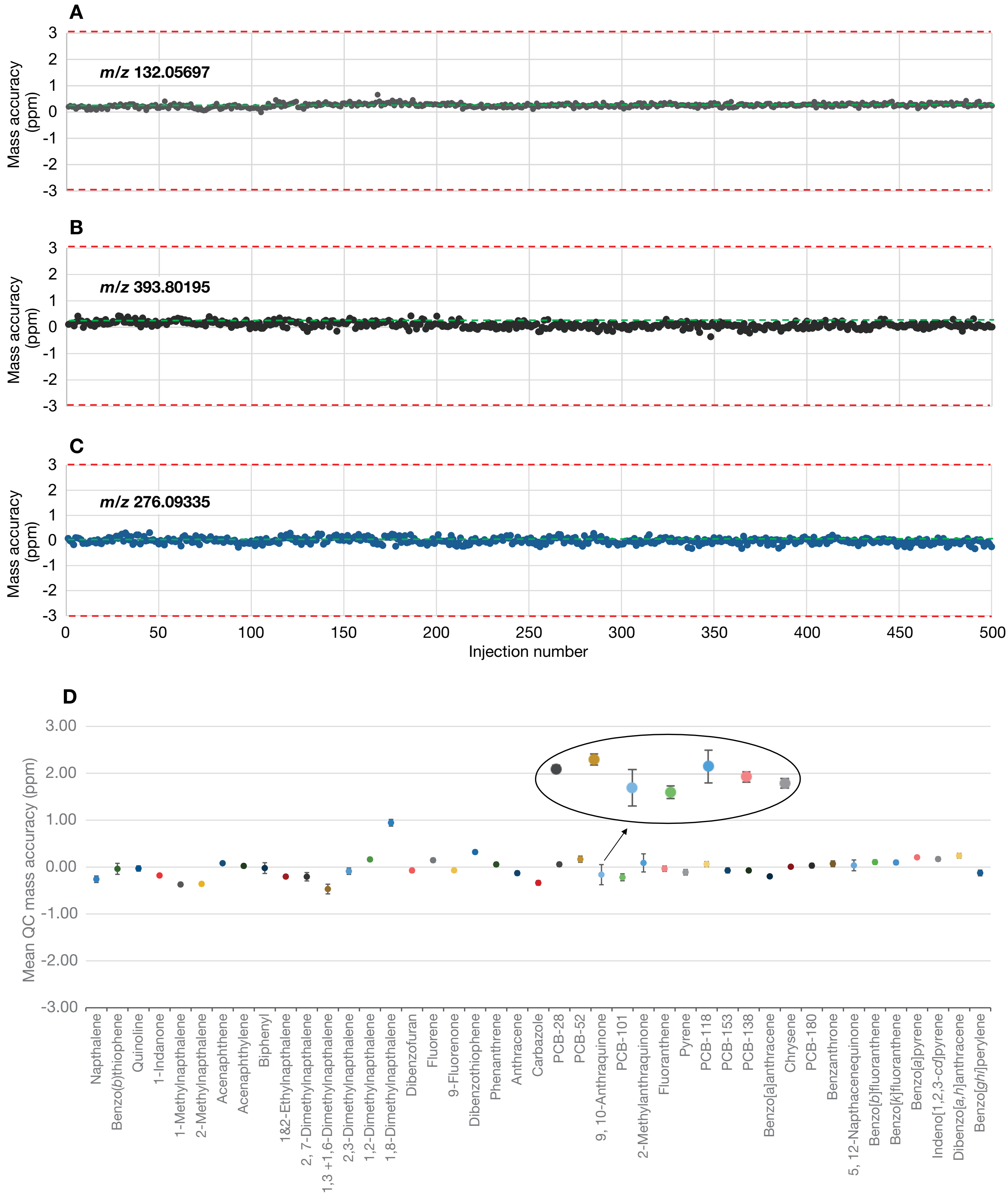


Figure 7. (A) Mass accuracy stability of low mass, early eluting (oxyPAH) 1-indanone, n=500 injections of a 10 pg/µL spiked soil QuEChERS extract (RT=6.0 min, *m/z* 132.05697, average mass accuracy=0.3 ppm); (B) Mass accuracy stability of high mass, mid-eluting PCB 180, n=500 injections of a 10 pg/µL spiked soil QuEChERS extract (RT=10.8 min, *m/z* 393.80195, average mass accuracy = 0.1 ppm); (C) Mass accuracy stability of mid mass, late eluting (PAH) benzo(*g,h,i*)perylene, n=500 injections of a 10 pg/µL spiked soil QuEChERS extract (RT=15.5 min, *m/z* 276.09335, average mass accuracy = 0.0 ppm); (D) Example of mass accuracy stability for all target compounds analyzed in a low-level standard QC standard used to monitor system performance over the three-week robustness study (40 pg/µL) (n=35) injected every 20 soil samples throughout the injection sequence including 500 matrix injections with mass calibration performed on a weekly basis. Average measured mass accuracy in ppm is donated by a colored dot for each compound and the associated standard deviation is displayed as error bars. Annotated also is the combined mean mass accuracy and standard error for measurements made for all compounds.

Conclusions

These results demonstrate that the Orbitrap Exploris GC-MS system provides the consistent, uninterrupted performance needed in fast-paced routine environments looking at increasing productivity while reducing instrument downtime and the cost per sample. In summary, the following performance was demonstrated:

- Excellent system repeatability in routine use. When analyzing modified QuEChERS soil extracts, the peak area repeatabilities for the incurred residues were <20% RSD over the 500 complex soil sample injections and three weeks of continuous analysis with an average of 10.5% across all compounds.

- High RRF stability of low-level QC standards throughout an injection sequence containing 500 sample injections was demonstrated with the RRF %RSDs for all compounds being ≤15% with an average of 4%. This shows long term system stability and applicability for routine GC-MS analysis of PAHs and PCBs in soil.

- Outstanding RRF agreement of QC standards with the measured RRFs % deviation of the n=35 injected QC standards were all within ±15% of the measured value across the calibration curve with minimal inlet maintenance, column trimming, MS maintenance, or tuning.

- Stable QC ion ratios where all were within ±15% of the expected values calculated as an average across a calibration curve ranging from 0.1 to 500 pg/μL (corresponding to 0.1–500 μg/kg in extracted soil).

- Excellent mass accuracy stability was demonstrated for all compounds measured in the 35 QC standards, which had mass accuracies that were within ±1 ppm of the expected values with only weekly calibration of the MS disrupting the instrument up-time.

- Further mass accuracy was demonstrated with the average of all compounds in a QC standard being <1ppm over three weeks of analysis.

- Examples of mass accuracy in matrix were demonstrated for low, high, and medium (m/z)/ early, mid, and late eluting compounds 1-indanone, PCB 180, and benzo(g,h,i)perylene with average (n=500) mass accuracies of 0.3, 0.1, and 0.0 ppm, respectively.

References

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