IMPROVED MHE-SIFT-MS WORFLOW:

EVEN FASTER QUANTITATION OF FORMALDEHYDE IN GELUCIRE EXCIPIENT

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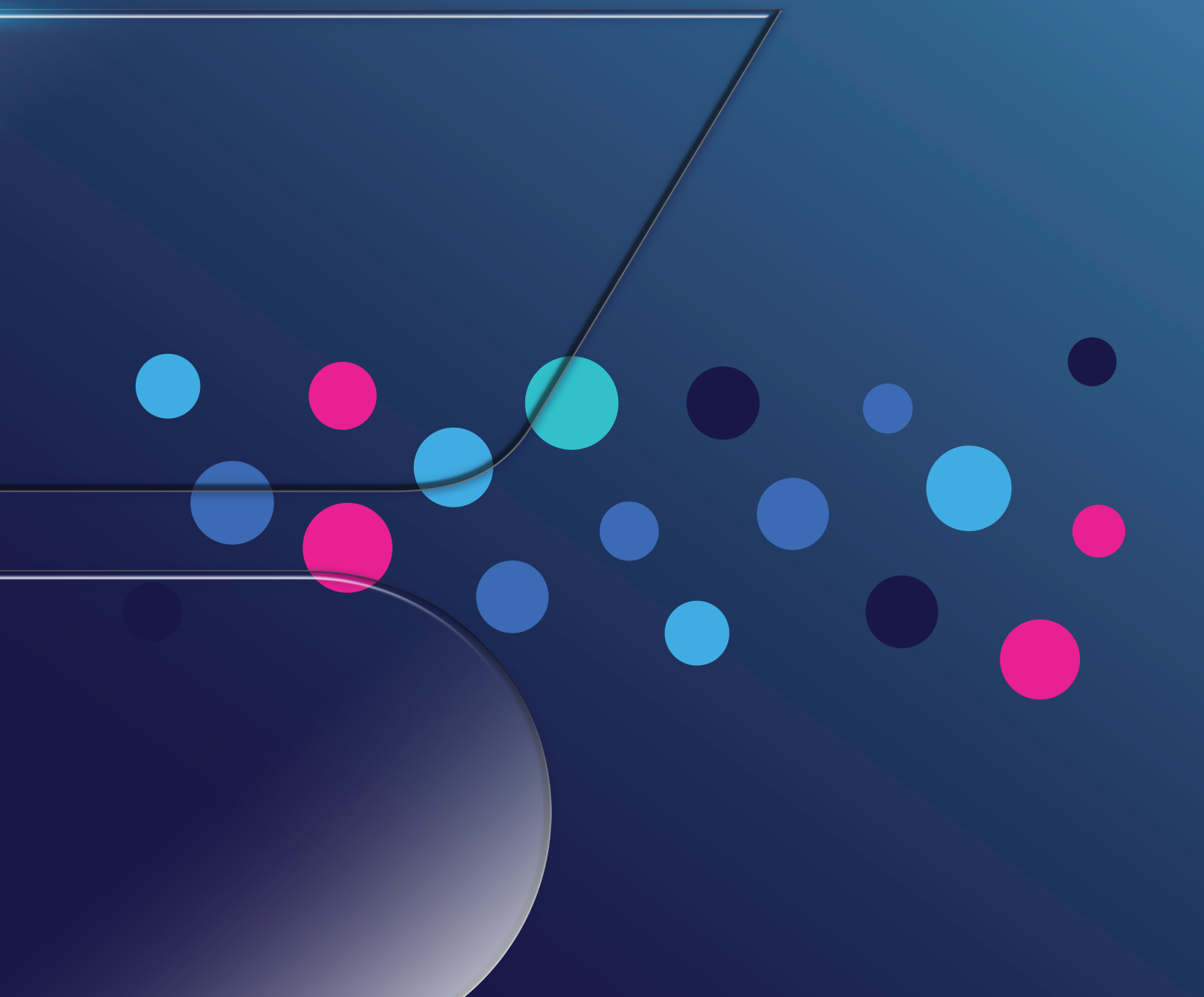


Abstract

Multiple headspace extraction (MHE) is a powerful sample preparation technique because it enables volatile compounds to be quantified in condensed-phase matrices for which reliable calibration standards cannot be prepared. MHE, however, is ordinarily slow (both in time-to-result and in throughput) and expensive due to repeated analysis of the same sample. This application note describes how the efficiency of a MHE workflow can be significantly improved for MHE-SIFT-MS due to the stability of the technique. Formaldehyde impurity is analyzed easily and quantitatively in Gelucire excipient with this improved approach. The 'MHE calibration' holds for at least four weeks when using SIFT-MS. This means that the time-to-result is reduced to 85 minutes for this system (including prep, 60-minute incubation, and analysis of a system suitability test bracketed with two blanks) – six-fold faster than the conventional MHE-SIFT-MS approach. Furthermore, quantitative analysis is achieved at the throughput of static headspace-SIFT-MS analysis: 12 samples per hour (or 220 samples/day including blanks and check standards). The enhanced MHE-SIFT-MS workflow provides unprecedented benefits for quantitative analysis of condensed-phase samples – especially since changing between analytical methods is seamless with SIFT-MS instruments.

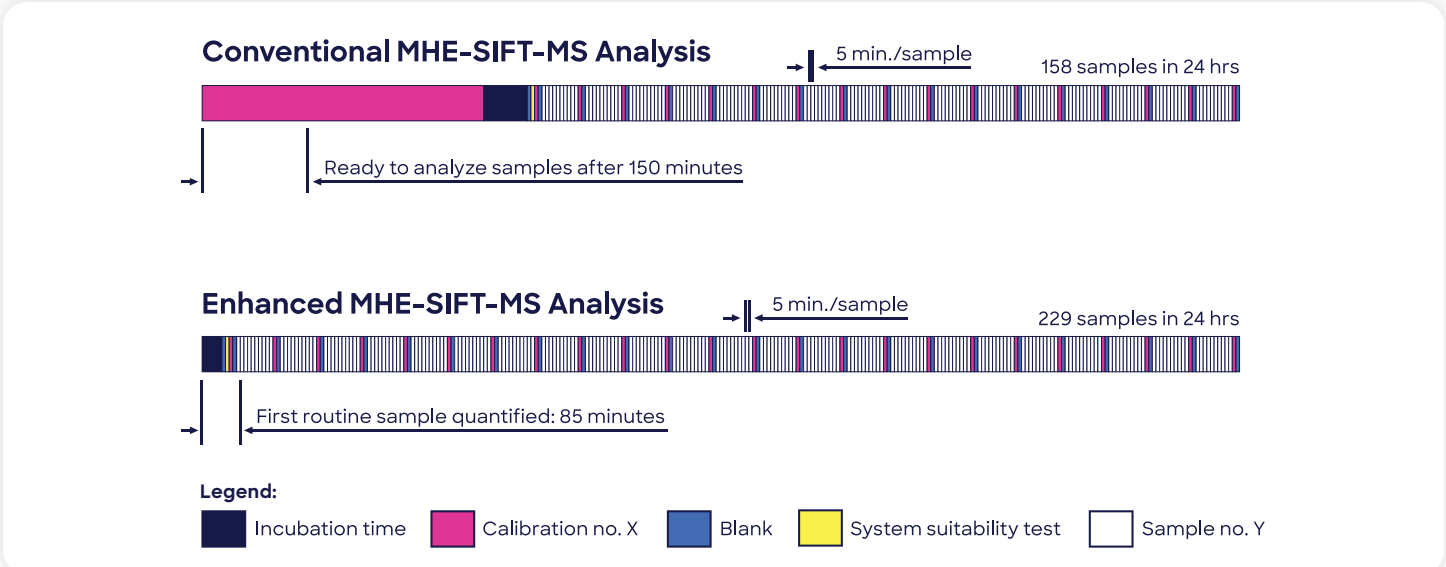
INTRODUCTION

Multiple headspace extraction (MHE) is a powerful sample preparation technique because it enables volatile compounds to be quantified in condensed-phase matrices for which reliable calibration standards cannot be prepared (Perkins and Langford (2022a, 2022b)). The disadvantage of conventional MHE is that it is time consuming due to repeated headspace measurements on the same sample. For well-behaved systems the six headspace generation cycles can be reduced to three or four injections (Kolb and Ettre (2006)).

Previous studies using MHE-SIFT-MS to analyze styrene residue in polystyrene (PS; Perkins and Langford (2022b)) and *N*-nitrosodimethylamine (NDMA) in ranitidine tablets (Perkins and Langford (2022c)) have demonstrated that the ratio of the first MHE injection to the concentration calculated from the full MHE sequence (or 'MHE calibration') is very repeatable. Hence, assuming repeatable samples (e.g., for solids, the same grind size and morphology), it was suggested in the work on PS that analysts could do one MHE calibration daily (Perkins and Langford (2022b)). There are, however, still significant implications for throughput and the time to first quantitative result with the daily MHE calibration approach due to (1) the repeated headspace generation (the rate-limiting step), and (2) the way that the sample scheduling is currently handled by the autosampler software.

Reducing the time taken to achieve a quantitative result is the focus of this application note. Since SIFT-MS instruments have been used for quantitative analysis against workplace exposure limits for many years with only annual recalibration required, the study conducted here describes initial investigation of temporal stability for MHE-SIFT-MS 'calibrations' (i.e., the correlation of the first injection to the concentration determined with full MHE analysis). The system utilized is formaldehyde content in Gelucire excipient (a polyethylene glycol ester), for which method development has previously

Figure 1. The enhanced MHE-SIFT-MS workflow revolutionizes quantitative VOC analysis in condensed-phase samples. It reduces the time to first result six-fold compared to daily calibration, while enabling over 70 additional routine samples to be analyzed in a 24-hour period.



been described (Perkins and Langford (2022d)). The results reported in this application note demonstrate that – for consistent samples – 'MHE calibration' with SIFT-MS holds for at least four weeks within generally accepted criteria. This means that on any other day within the calibration period, a quantitative result can be achieved in less than 90 min for this system (including the 60-min incubation and analysis of a system suitability test (SST) bracketed with two blanks; Figure 1). Equally remarkable, no preconcentration or derivatization is required for SIFT-MS analysis of formaldehyde and a sample throughput of 12 per hour is readily achieved. Hence MHE-SIFT-MS provides unprecedented workflow benefits for MHE and formaldehyde analysis.

METHOD

1. The SIFT-MS technique

This work utilized a Syft Tracer™ SIFT-MS instrument operating on helium carrier gas. SIFT-MS (Figure 2) uses soft chemical ionization (CI) to generate mass-selected reagent ions (Smith *et al.* (2023)) that can rapidly react with and quantify VOCs down to part-per-trillion concentrations (by volume, pptV). Up to eight reagent ions (H_3O^+, NO^+, O_2^+, O^-, OH^-, O_2^-, NO_2^- and NO_3^-) obtained from a microwave discharge in air are available on Syft Tracer™ instruments. These reagent ions react with VOCs and other trace analytes in well-controlled ion-molecule reactions, but they do not react with the major components of air (N_2, O_2 and Ar). This enables direct, real-time analysis of air samples to be achieved at trace and ultra-trace levels without pre-concentration. Rapid switching between reagent ions provides high selectivity because the multiple reaction mechanisms give independent measurements of each analyte (Langford (2023)). The multiple reagent ions frequently remove uncertainty from isobaric overlaps in mixtures containing multiple analytes. Hence Syft Tracer™ sets the standard for sensitive and selective real-time analysis of volatile compounds.

Automated MHE analysis was carried out using a Syft Tracer™ coupled with a multipurpose autosampler (MPS Robotic Pro, GERSTEL; Mülheim, Germany). The autosampler was controlled using GERSTEL's Maestro software. Here, each sample was incubated in a GERSTEL agitator throughout its six-cycle MHE sequence (see Perkins and Langford (2022a)). Headspace was sampled using a 2.5-mL headspace syringe (heated to 150 °C) and subsequently injected at a flow rate of 50 µL s^{-1} into the SIFT-MS instrument's autosampler inlet (heated to 150 °C) via a self-sealing GERSTEL septumless sampling head. Since the nominal sample flow into the SIFT-MS instrument is 420 µL s^{-1}, a make-up gas flow (ultra-high purity nitrogen) is also introduced through the sampling head. This dilution is accounted for in the final concentration calculations below. The analysis time for each sample was 120 s. Figure 3 shows the six injections for a sample incubated at 50 °C for 60 min.

Figure 3. Real-time SIFT-MS analysis of formaldehyde: the headspace injections from six cycles of headspace generation in an MHE study of Gelucire 44/14 incubated at 50 °C (Day 0, replicate 1).

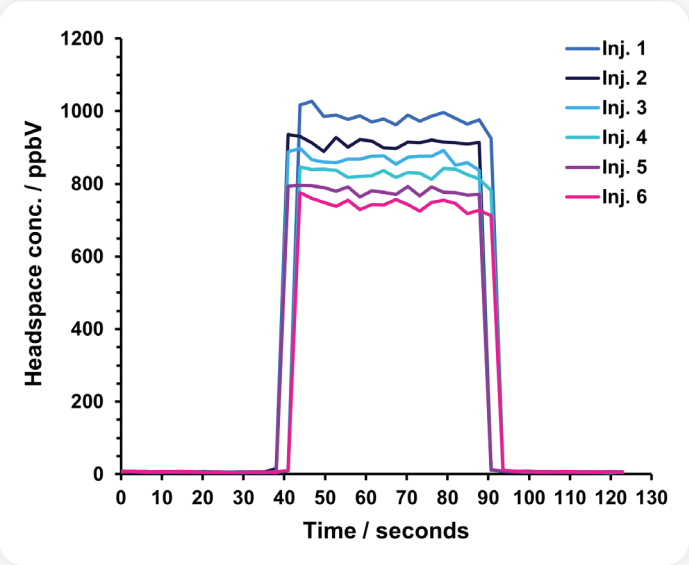
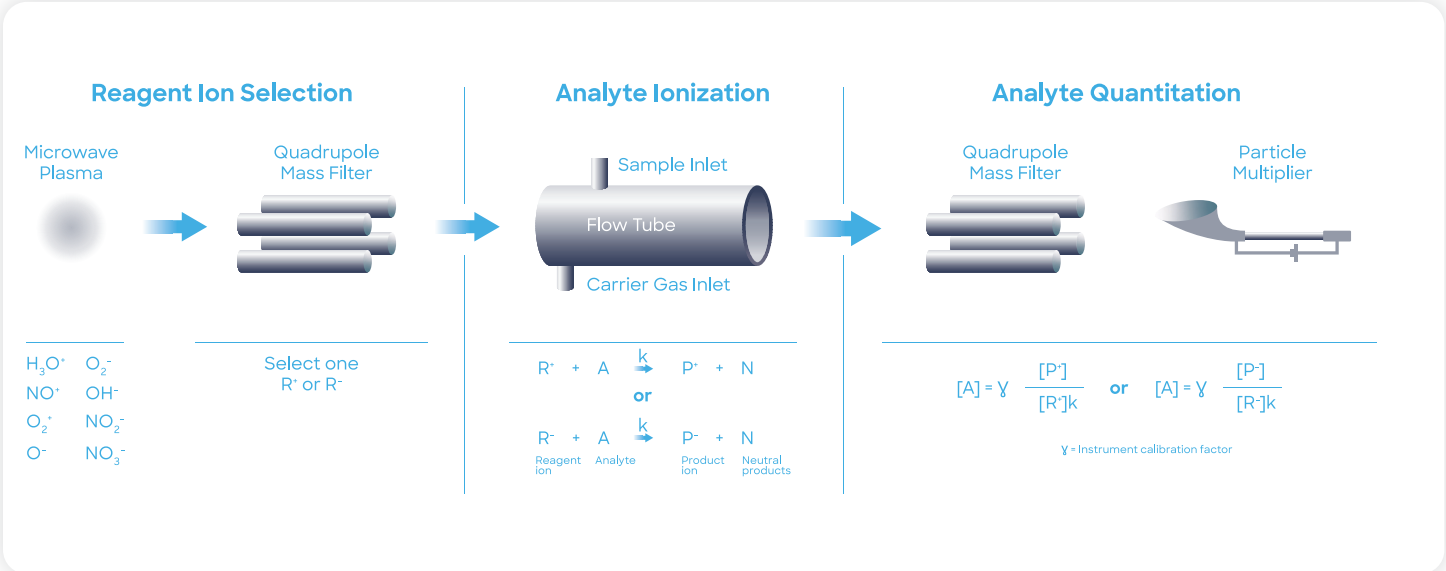


Figure 2. Schematic diagram of SIFT-MS – a direct, chemical-ionization analytical technique.



2. SIFT-MS detection of formaldehyde

SIFT-MS selectively detects formaldehyde *via* the proton-transfer reaction shown in Eqn. 1 (Španěl and Smith (2008)).

$$H_3O^+ + H_2CO \rightarrow H_3CO^+ + H_2O \qquad k = 3x10^{-9}\ cm^3\ s^{-1} \qquad (1)$$

The H_3CO^+ product ion is detected at a mass-to-charge ratio (m/z) of 31. This product ion m/z is specific to detection of formaldehyde due to the soft ionization in SIFT-MS and its infrequent occurrence for other volatiles. Formaldehyde quantitation was conducted using the literature reaction rate coefficient (*k*) above.

In this study, reported concentrations are the mean of the values obtained during injection (i.e., between about 50 and 80 s in Figure 3). Note that no internal standard was utilized.

3. Samples

Commercially available Gelucire 44/14 excipient was supplied for analysis by a third-party. For SIFT-MS headspace analysis, 200 mg was placed in 20 mL headspace vials.

Full six-injection MHE analyses were run in triplicate on six days over a 27-day period. These data are presented and processed in various way in the following section to evaluate the feasibility of infrequent 'MHE calibration'.

RESULTS AND DISCUSSION

1. MHE calculations day-by-day

Figure 4 summarizes the headspace concentrations obtained using SIFT-MS over the 27-day period (all replicates and all MHE injections). The relative standard deviations (RSDs) shown in the figure legend demonstrate the stability of the SIFT-MS technique (≤4%) – formaldehyde quantitation was conducted using the library parameters; no calibration was conducted and no internal standards were used. The MHE plots generated from these data are shown in Figure 5 for four measurement days spanning the 27-day test period. Linearity is excellent, with the regression coefficient, R^2, greater than 0.993 across all samples (including days not shown).

The concentration of formaldehyde in the Gelucire excipient is calculated from the first data point and slope (the area under the curve), as described in Perkins and Langford (2022b) (and references therein). The average daily results are shown in Figure 6 and show the stability of the SIFT-MS technique with overall RSD for the 27-day study of 4.9% (calculated across individual replicates, not the average values shown in the figure). Note that the MHE extrapolation/integration procedure leads to a reduction in repeatability compared to individual headspace injections but it remains well within the range accepted for intermediate precision.

Figure 4. Headspace-SIFT-MS analysis of formaldehyde content in Gelucire 44/14 (incubated at 50 °C) over a 27-day period. Triplicate, six-injection MHE was conducted on each day that measurements were made. The %RSD for each MHE injection was calculated across all 18 measurements.

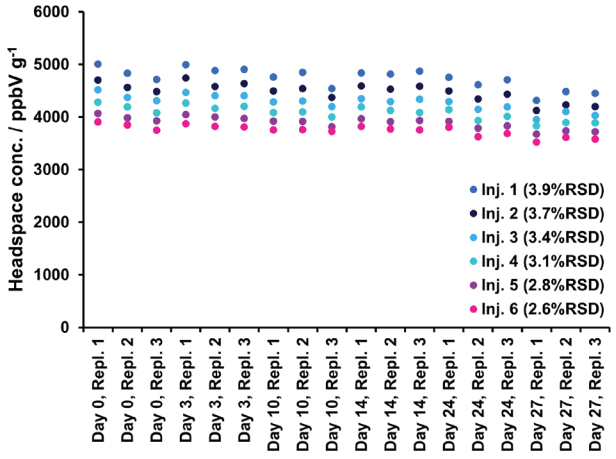
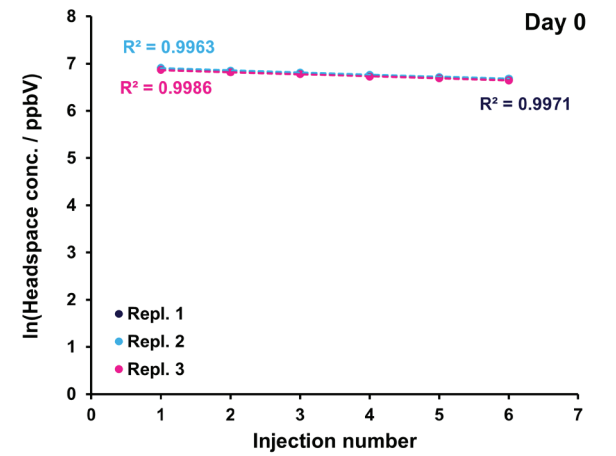
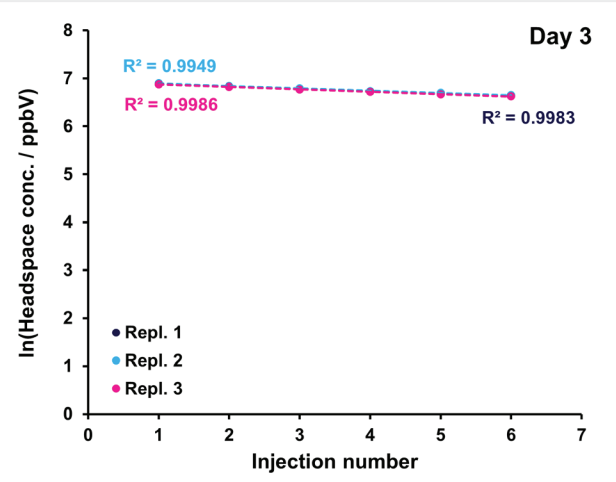
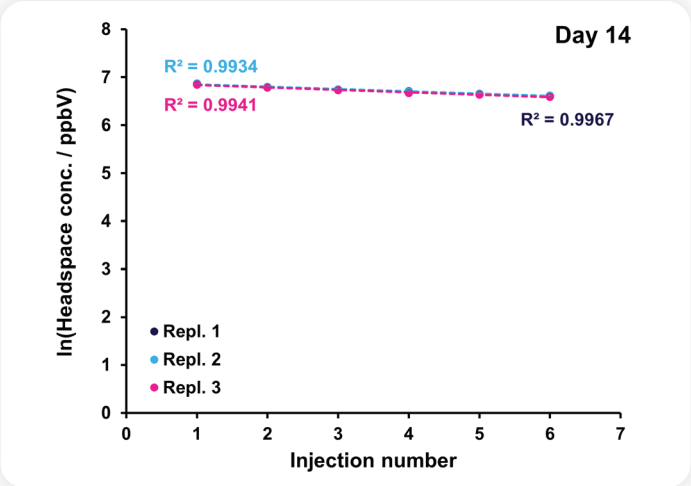


Figure 5. MHE-SIFT-MS data obtained for Gelucire 44/14 at 50 °C on Days 0, 3, 14, and 27 of the study. The three replicate measurements made on each day are shown. Note the logarithmic concentration axis.







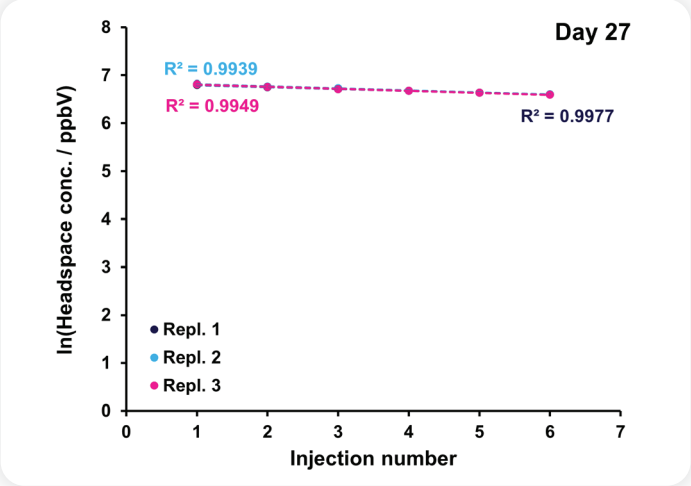
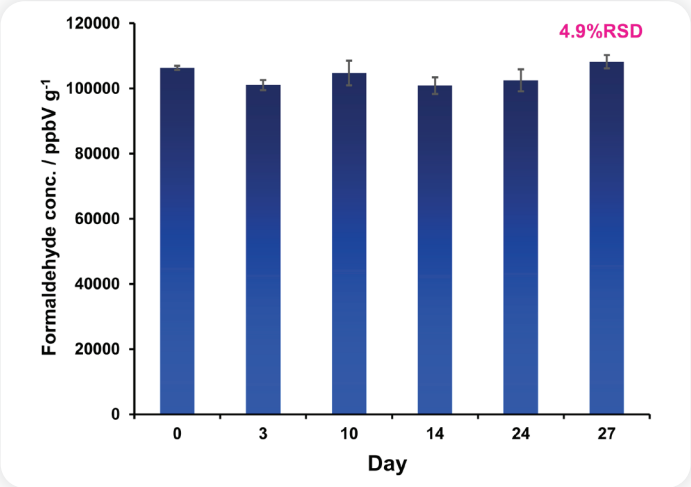


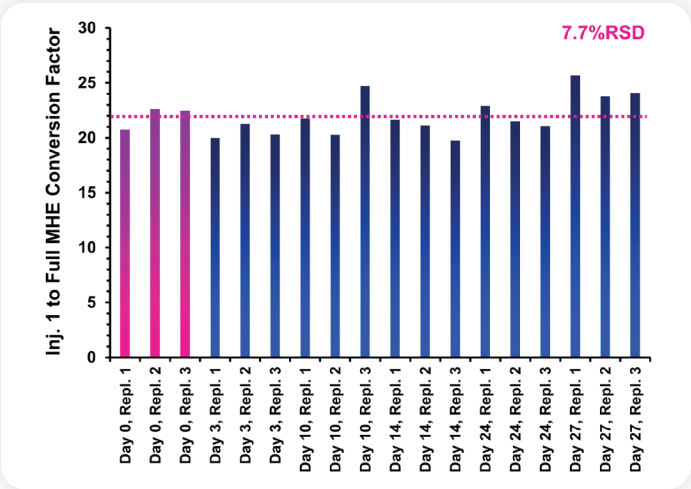
Figure 6. Concentrations of formaldehyde in Gelucire 44/14 over the 27-day period, with the mean of three replicates shown (error bars are one standard deviation).



2. MHE calculations over 27 days

The data presented in Figure 6 were calculated from the individual MHE measurements (Perkins and Langford (2022b)). The use-case being evaluated here involves assessing the reliability of applying the Day 0 calibration (i.e., the ratio of the first headspace injection to the full six-injection MHE) to all data collected in the 27-day period. Figure 7 shows the conversion (calibration) factor obtained for the individual measurements across the study. It is evident that the factor varies somewhat but this is within the acceptable range for intermediate precision (7.7%RSD). These data suggest that this 'MHE calibration' should be conducted in triplicate and the mean value applied to the samples analyzed using a single injection to give the quantitative result.

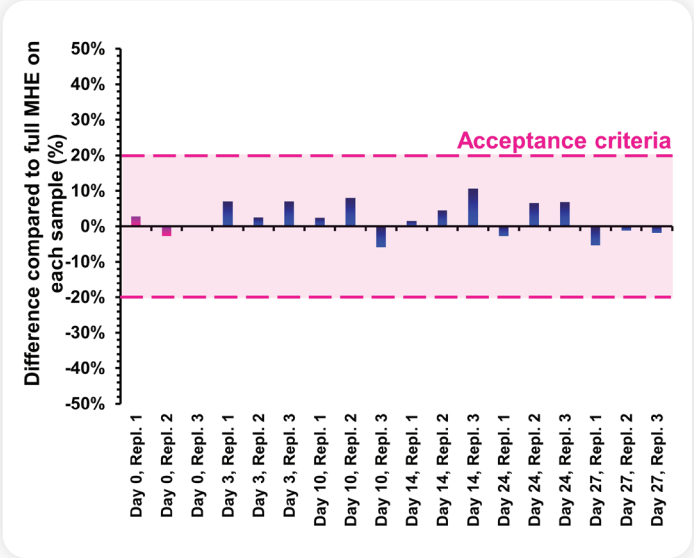
Figure 7. Conversion/calibration factor for 1st MHE injection to the full six-injection MHE calibration, with Day 0 emphasized in purple/pink. The dotted pink line indicates the mean of the three replicate measurements made on Day 0 – the calibration factor applied to all MHE data in the study (Figure 8).



The average Day 0 calibration factor (shown as the pink dotted line on Figure 7) was applied to all first-injection data (Figure 4), including the individual Day 0 measurements. Figure 8 shows the percentage difference in the value calculated from the average Day 0 calibration factor compared to the individual MHE measurements. All data calculated from the Day 0 calibration factor are within 20% of the full MHE measurement. This demonstrates that for samples of consistent particle size and morphology *quantitative analysis – even of a chromatographically challenging species, such as formaldehyde – can be achieved in the condensed phase from a single headspace injection using a calibration made up to four weeks in advance*. Astonishing as this statement sounds, it is realized in practice with Syft Tracer™ because of the flexibility of the analytical platform (see Langford and Perkins (2023)). The instrument that generated these data was utilized in the same configuration for numerous other analyses between MHE runs. This hardware configuration accommodates almost all headspace analyses (and more; e.g., sample bags) for a wide variety of analytes, without any inlet liner, column, or detector changes that are frequently required for chromatographic methods. Combined, the calibration stability and flexibility of Syft Tracer™ can revolutionize workflows, as discussed below.

Future work will consider the robustness of this approach to changes in particle size and morphology. Comparability of single-injection data acquisition with full MHE data may be improved through inclusion of a regular SST, because in the presented data, no instrument interventions, or data corrections were made whatsoever.

Figure 8. Difference in final calculated concentration of formaldehyde in Gelucire when using the Day 0 calibration factor for the 1st MHE injection compared with the full six-injection MHE calculation for each sample (including the individual Day 0 measurements in purple/pink).

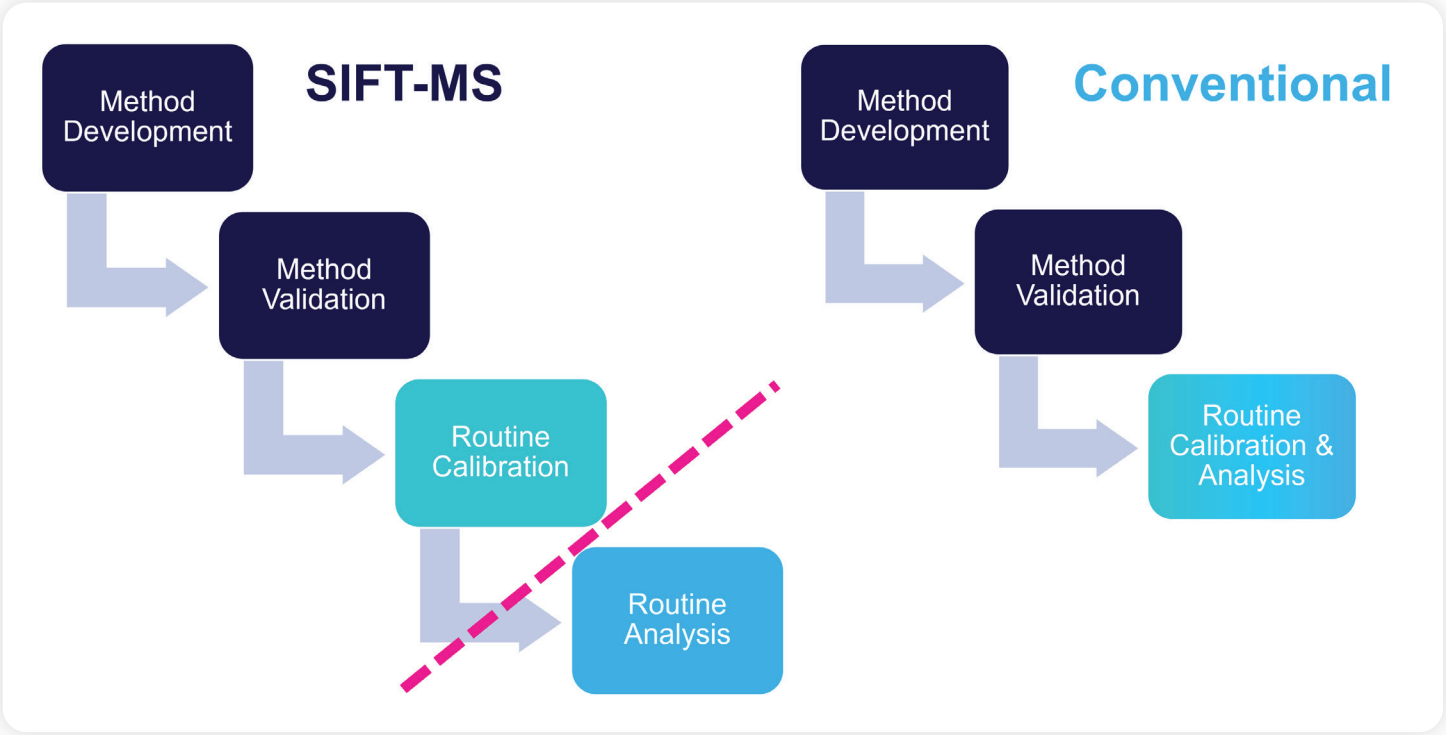


3. Workflow implications

This study demonstrates that when utilizing MHE with SIFT-MS, the calibration factor for full six-injection MHE analysis can be dissociated from the analysis by at least 27 days. This means that quantitative analysis can be conducted using a single headspace injection throughout this period – i.e., the approach has been simplified to static headspace analysis, as shown in Figure 1. This is only possible due to the stability of the SIFT-MS instrumentation and the use of ultra-soft chemical ionization that creates tremendous breadth of analysis with one configuration.

More generally, routine calibration can be uncoupled from routine sample analysis for headspace-SIFT-MS, in contrast to conventional chromatographic methods (Figure 9). Combined with seamless transitioning between analytical methods due to the highly flexible and stable Syft Tracer™ platform, major workflow improvements are achieved compared with chromatographic methods for quantitative analysis of volatile impurities. This will be explored in more detail in a future application note.

Figure 9. High-level comparison of SIFT-MS and conventional chromatography workflows from method development through to routine analysis. With SIFT-MS, routine analysis can be uncoupled from routine calibration, significantly improving workflow.



CONCLUSIONS

• SIFT-MS ionization is very stable – full calibrations do not need to be conducted every day.
• 'MHE calibration' (the ratio of first injection to full MHE) is stable for at least 27 days so can be conducted infrequently.
• Reducing the calibration frequency in routine analysis enables significant workflow benefits to be realized, including six-fold faster time to first result for quantitative analysis of condensed-phase samples.
• Over 220 samples per day can be analyzed quantitatively for formaldehyde impurities using the enhanced MHE-SIFT-MS approach.
• Formaldehyde analysis using SIFT-MS is simple because sample derivatization is eliminated.
• Seamless transitions between test methods make Syft Tracer™ the most efficient and flexible instrument for analysis of volatile impurities.

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