HEAD-TO-HEAD COMPARISON

OF CLASS 2A AND 2B RESIDUAL SOLVENTS ANALYSIS USING SIFT-MS AND GC-FID

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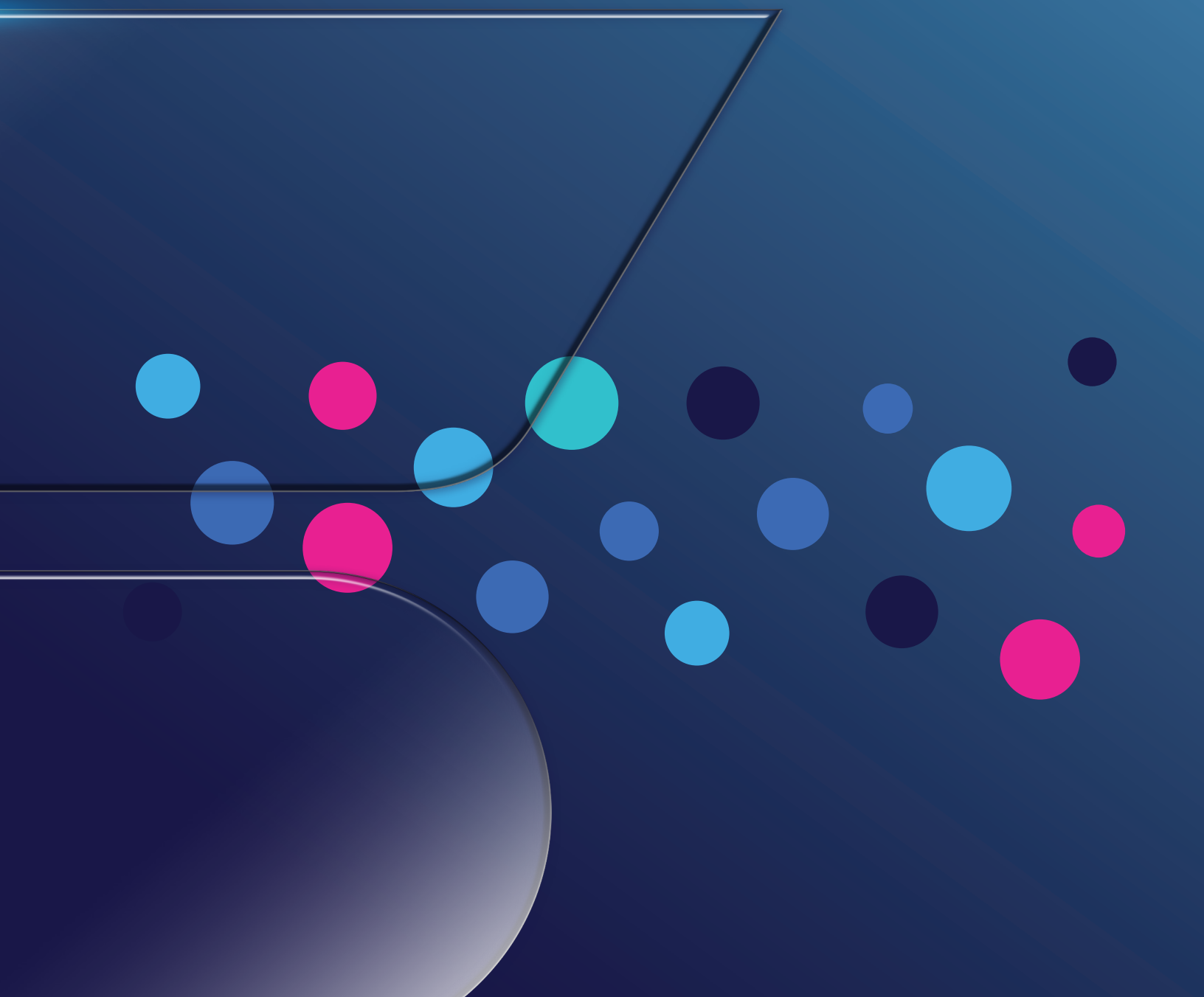


Abstract

Gas chromatography-flame ionization detection (GC-FID), and its mass spectrometry-based counterpart (GC/MS), are the incumbent techniques for analysis of residual solvents in pharmaceutical products. Previous work validated selected ion flow tube mass spectrometry (SIFT-MS) for United States Pharmacopeia (USP) General Chapter <467> *Residual Solvents (USP<467>) according to USP guideline Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures <1467>* (USP<1467>). This application note describes head-to-head comparison of GC-FID and SIFT-MS analyses of the same formulations (in tableted and oral suspension dose forms). The techniques perform similarly for linearity and repeatability, but SIFT-MS provides superior performance for accuracy and recovery. Furthermore, SIFT-MS provides greater than 11-fold increase in sample throughput and significantly reduces the time taken to report quantitative results (over six times faster for a full calibration set).

INTRODUCTION

Pharmacopeial methods for residual solvents analysis utilize gas chromatography-based techniques. However, alternative procedures are permitted if acceptance criteria are met (USP<1467>)). Recently, selected ion flow tube mass spectrometry (SIFT-MS) has been demonstrated as an acceptable alternative procedure for analysis of water-soluble Class 2A and Class 2B residual solvents based on performance criteria being met (Biba *et al.* (2021)). A summary of the full article is provided by Perkins and Langford (2022). This study did not, however, directly compare the SIFT-MS-based alternative procedure to the standard method.

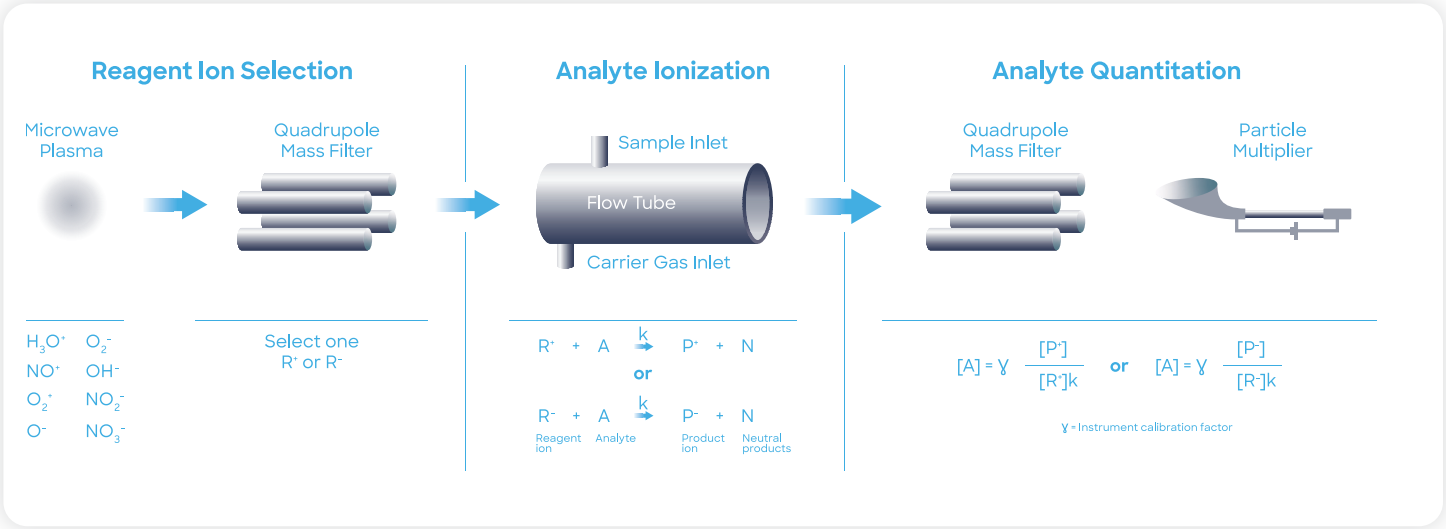
The present study addresses this shortcoming by comparing – for identically prepared samples – the performance of the SIFT-MS alternative procedure (Biba *et al.* (2021)) and the standard <467> procedure (USP<467>) using gas chromatography-flame ionization detection (GC-FID). Specifically, comparison is made of repeatability and linearity of the standard solutions, and the accuracy and recovery for spiked solutions of two products: a tablet and an oral suspension. Overall, analytical performance versus the Chapter <467> performance criteria is slightly better for SIFT-MS than for GC-FID. However, SIFT-MS provides a significant throughput advantage with the batch running over 11-fold faster than GC-FID. SIFT-MS also delivers the first quantitative results over six times faster than GC-FID.

Methods

1. Automated SIFT-MS analysis

This work utilized a Syft Tracer™ SIFT-MS instrument operating on helium carrier gas. SIFT-MS (Figure 1) 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 now applied in commercial SIFT-MS instruments (Smith *et al.* (2023)). 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

Figure 1. Schematic diagram of the SIFT-MS technique, which utilizes soft chemical ionization for direct analysis of samples.



the multiple reaction mechanisms give independent measurements of each analyte. The multiple reagent ions frequently improve selectivity of isobaric compounds in mixtures containing multiple analytes.

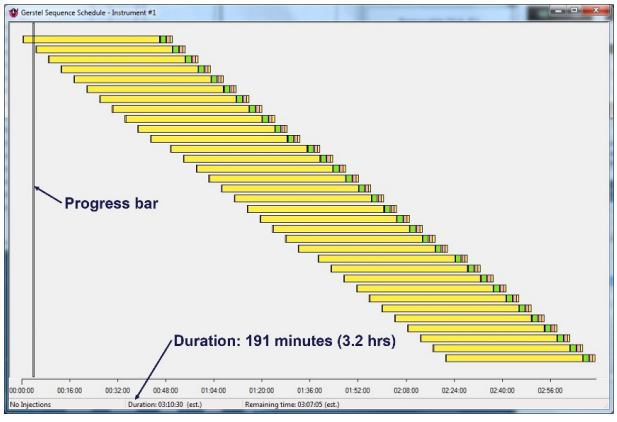
Residual solvents reported in this application note were analyzed using the quantitation ions summarized in Table A1 (Appendix A). Because reagent ions are rapidly switchable in SIFT-MS, all positively charged ions were used in the method to provide the best combination of specificity and sensitivity.

The SIFT-MS instrument was equipped with a GERSTEL MPS autosampler (Robotic Pro; Mülheim, Germany). Samples were incubated in a virtual twelve-place GERSTEL agitator (comprised of two physical six-place agitators) prior to sampling of the headspace and subsequent injection into the SIFT-MS instrument through a GERSTEL septumless sampling head. The GERSTEL Maestro software's "PrepAhead" sequence schedule is shown in Figure 2(a).

The headspace conditions for all analyses used 6 mL of solution in a 20-mL headspace vial incubated at 60 °C for 45 min. A 2.5-mL aliquot of headspace was removed via a heated syringe (150° C) and injected into the SIFT-MS at 25 µL s^{-1}, with a zero-air make-up gas flow through the heated inlet (150° C) to ensure that the total flow into the instrument was 25 mL min^{-1}. After the injection, the syringe was flushed with zero air for 1 min at 200 mL min^{-1}. Figure 3 shows an example headspace injection for Class 2A solvents.

Figure 2. Sequence schedules for automated (a) SIFT-MS and (b) GC-FID instruments running their <1467> and <467> methods, respectively. Rapid sample analysis with SIFT-MS enables 36 samples to be analyzed in just over 3 h rather than 36 h for GC-FID. These schedules were captured while sequences were running, as indicated by the vertical progress bar.

(a)



(b)

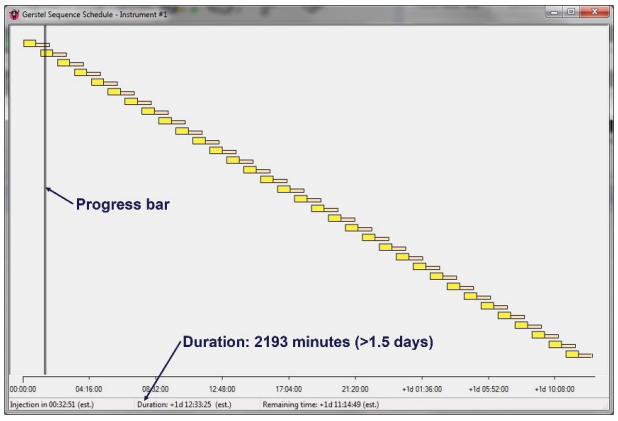
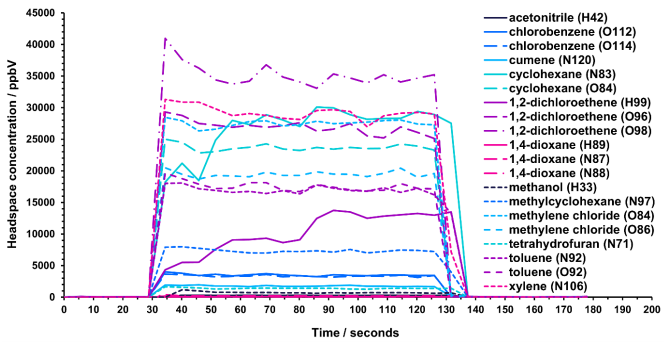


Figure 3. Example headspace-SIFT-MS analysis of a standard showing product ions for Class 2A compounds.

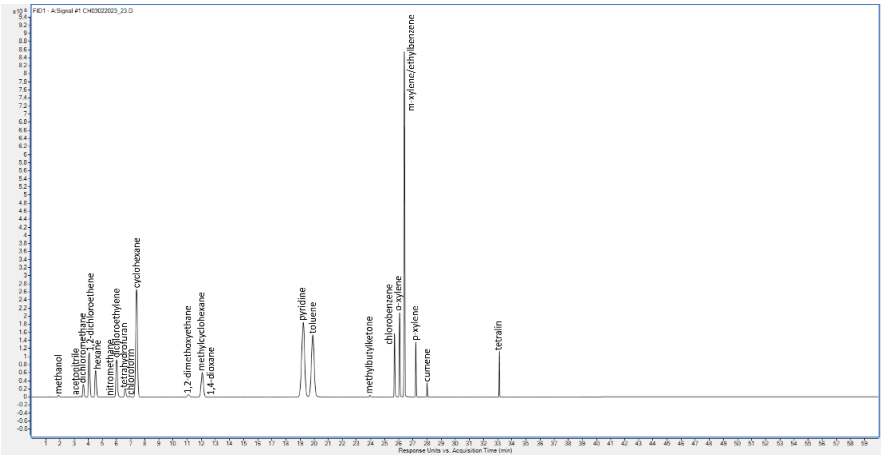


2. Automated GC-FID analysis

GC-FID analysis was conducted using an Agilent 7890 GC system (Agilent Technologies, Santa Clara, CA) coupled with a GERSTEL MPS autosampler (Robotic Pro (dual head); Mülheim, Germany). Samples were incubated for 45 min at 80 °C. The optimized schedule using the GERSTEL Maestro PrepAhead software is shown in Figure 2(b).

A 1-mL aliquot of the headspace from each vial was injected at 200 µL s^{-1} into the split/splitless inlet (5:1 split ratio) of the GC. Separation was achieved using a VF-624ms column (30 m × 0.32 mm × 1.8 µm; Agilent Technologies, Santa Clara, CA) with a helium flow rate of 2.15 mL min^{-1}. The oven was initially held at 40 °C for 20 min before a ramp of 10 °C min^{-1} to 240 °C where it was held for 20 min. Eluting components were detected using flame ionization detection (FID) running at 250 °C with nitrogen makeup gas, hydrogen, and air flows of 25, 30, and 400 mL min^{-1}, respectively. A representative chromatogram is shown in Figure 4.

Figure 4. A representative, annotated GC-FID chromatogram for the combined Class 2A and 2B mixture.



3. Sample preparation and analysis

This evaluation was conducted in two parts and utilized different standards for each. For linearity and repeatability USP Class 2A and 2B standards were used (part numbers 1601281 and 1601292, respectively; USP, Rockville MD, USA), while for accuracy and recovery Sigma-Aldrich Class 2A and 2B standards were used (part numbers PHR1064 and PHR1065, respectively; Sigma-Aldrich, Gillingham, UK).

Two acetaminophen (paracetamol) formulations were selected for the comparative accuracy and recovery exercise: tablets (500 mg; Co-op private label, UK) and an oral suspension (250 mg per 5mL dose; Calpol Six-plus sugar-free, Johnson & Johnson).

For the accuracy and recovery study, test samples were prepared batch-wise. This was to mitigate the progressive loss of poorly water-soluble analytes from aqueous stock solutions over time, which introduces variability into sample preparation. In addition, during sample preparation the standard stock solution was re-capped after addition to vials in each step 2 to 5 below.

1. Addition of all test solutions (sample solutions) and water to appropriate vials
2. Calibrations (triplicate pre-calibration)
3. Tablet and oral suspension – 100% spikes
4. Tablet and oral suspension – 50% spike
5. Calibrations (triplicate post-calibration).

For both techniques, the samples were run in the following sequence:

1. Blank
2. Calibrations (triplicate pre-calibration)
3. Tablet (triplicate)
4. Tablet plus 50% spike (triplicate)
5. Tablet plus 100% spike (triplicate)
6. Oral suspension (triplicate)
7. Oral suspension plus 50% spike (triplicate)
8. Oral suspension plus 100% spike (triplicate)
9. Calibrations (triplicate post-calibration)
10. Blank.

Results and Discussion

This section summarizes linearity, repeatability, accuracy, and recovery results obtained from side-by-side analysis of water-soluble Class 2A and 2B solvents using GC-FID and SIFT-MS. This differs from the previous study (Biba *et al.* (2021), Perkins and Langford (2022)), in which specificity, range, and limit of quantitation (LOQ) were also evaluated for SIFT-MS analysis of Class 2 residual solvents. It is noteworthy that the utility of multiple, rapidly switchable reagent ions – frequently with orthogonal reaction chemistry – underpins SIFT-MS specificity. Here, a matrix interferent for 1,4-dioxane (product ion at m/z 89 formed through proton-transfer reaction with H_3O^+; Table A1) was evident in the oral suspension, but not in the tablet sample. In contrast, the NO^+ product ions (m/z 87 and 88) were unaffected and performed well.

Full data for this study will be included in a forthcoming publication and its supplementary materials (Perkins *et al.* (2023)).

1. Linearity

Selected linearity data for SIFT-MS and GC-FID over the range 0.2 to 1.0 of the solvent limit (USP<467>) are shown in Figure 5. SIFT-MS responses are plotted in part-per-million by volume (ppmV) because this is the "natural" unit of gas-phase concentration measurement for the technique. This unit also has the benefit of internally normalizing reagent ion drift (should it occur) since it is proportional to the ratio of product ion to reagent ion during sample measurement.

Table 1 summarizes the linearity data obtained for SIFT-MS and GC-FID analyses in terms of the linear regression coefficients (R^2). Both techniques easily meet the acceptance criterion, although SIFT-MS usually performs slightly better.

Figure 5. Linearity of SIFT-MS and GC-FID measurements for (a) cyclohexanone, (b) methylbutylketone (MBK), (c) tetralin, and (d) toluene. Linear regression coefficients (R^2) are shown.

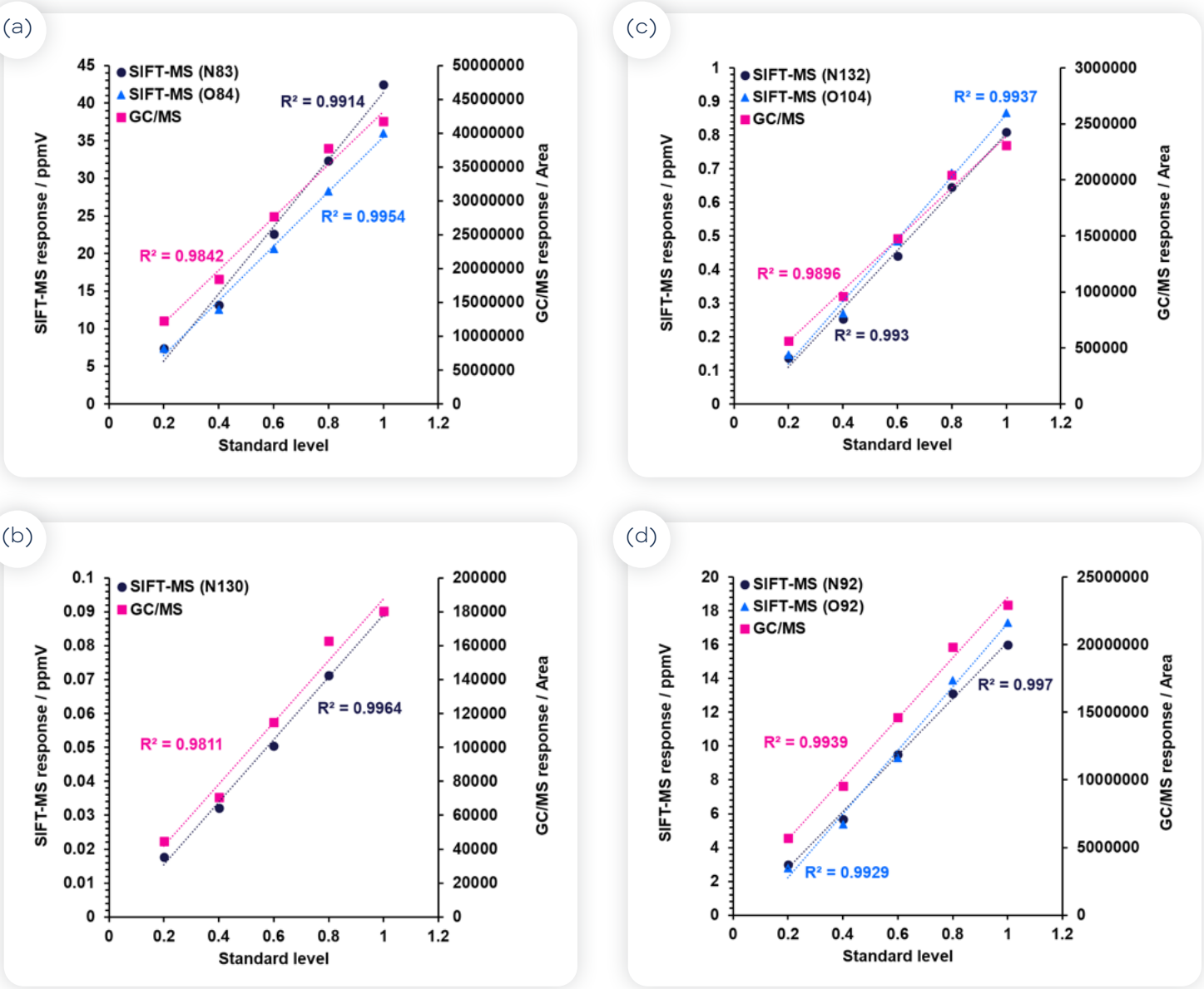


Table 1. Comparative performance of SIFT-MS and GC-FID (versus USP⟨467⟩ acceptance criteria) for linearity, repeatability, accuracy, and recovery on identically prepared and scheduled samples.

Parameters	Acceptance Criteria	Results: SIFT-MS	Results: GC-FID
Linearity	$R^2 \geq 0.970$	$R^2 \geq 0.97$	$R^2 \geq 0.94$
Precision: Repeatability	RSD is ≤20%*	1.4 - 9.5%RSD	2.0 - 16.9%RSD
Accuracy	<20%		
1. Tablet			
· 50% level		-19.2 - 2.3%	15.1 - 3.8% Exceptions: Chlorobenzene -29.7%, methylcyclohexane -22.5%, pyridine +24.8%, 1,2-dimethoxyethane +44.6%
· 100% level		-15.6 - 12.6%	-18.1 - 7.0% Exceptions: Chlorobenzene -29.7%, 1,4-dioxane -28.1%, methanol -25.6%, acetonitrile -20.1%
1. Oral Suspension			
· 50% level		-14.2 - 12.8% Exceptions: 1,4-dioxane 100.3% (1 ion), hexane 25.4%	-16.0 - 14.1% Exceptions: Chlorobenzene -31.5%, 1,2-dimethoxyethane -22.8%, xylene - 20.4%
· 100% level		-11.7 - 13.4% Exceptions: 1,4-dioxane 36.1% (1 ion)	-10.7 - 7.9% Exceptions: Chlorobenzene -30.3%, 1,2-dimethoxyethane -23.1%, methanol -22.9%, 1,4-dioxane -22.8%, MBK -20.8%, acetonitrile -20.7%, chloroform -20.4%
Recovery	"The mean recovery for each spiked sample solution should be 80%-120%"		
1. Tablet			
· 50% level		81.3 - 102.3%	84.9 - 103.8% Exceptions: Chlorobenzene 70.3%, methylcyclohexane 77.5%, pyridine 124.8%, 1,2-dimethoxyethane 144.6%
· 100% level		84.4 - 112.6%	89.4 - 107.9% Exceptions: Chlorobenzene 70.1%, 1,4-dioxane 71.9%, methanol 74.4%, acetonitrile 79.9%
1. Oral Suspension			
· 50% level		85.8 - 112.8% Exceptions: hexane 125.4%, 1,4-dioxane 200.3% (1 ion)	84.0 - 114.1% Exceptions: Chlorobenzene 68.5%, 1,2-dimethoxyethane 77.2%
· 100% level		88.3 - 113.4% Exceptions: 1,4-dioxane 136.1% (1 ion)	88.2 - 107.9% Exceptions: Chlorobenzene 69.7%, 1,2-dimethoxyethane 76.9%, methanol 77.1%, 1,4-dioxane 77.2%, MBK 79.2%, chioroform 79.6%, acetonitrile 79.3%

*For "at least six independent spiked sample solution preparations from the same lot" for each solvent present

2. Precision

Table 1 summarizes the repeatability results obtained for the two techniques. For both SIFT-MS and GC-FID, acceptance criteria are met for all compounds (though the highest value for GC-FID arises due to the summing of individual xylene isomers for comparison with SIFT-MS; the individual isomers perform better).

Note that results obtained for intermediate precision using SIFT-MS (a different analyst using different standards and samples) were described previously (Biba *et al.* (2021)). Reproducibility of SIFT-MS data across multiple geographically separated instruments and analysts will be described in a future application note and publication.

3. Accuracy

Accuracy was calculated as the percentage difference from the expected spiked level (0.5 or 1.0) using the mean of the bracketed 1.0-level standards (pre- and post-run). For 0.5-level spikes, the 1.0-level calibration was halved accordingly.

Accuracy data are summarized in Table 1. Drift was observed between pre- and post-run calibrations for some compounds, so the accuracy and recovery data

are conservative. This will be discussed in more detail in the upcoming publication (Perkins et al. (2023)).

Overall, the SIFT-MS results displayed higher accuracy than did the GC-FID results, primarily in terms of more compounds meeting acceptance criteria. For the tablet samples, SIFT-MS meets the acceptance criteria for all analytes (less than 20%), whereas GC-FID analysis suffered several failures per spike level with chlorobenzene the only compound that failed for both.

The oral suspension was more challenging for both techniques. For SIFT-MS, as mentioned above, a 1,4-dioxane product ion (m/z 89 with H_3O^+) suffers interference giving poor accuracy (though note that the other two product ions meet acceptance criteria with a range from -10.8 to 3.3% across both spike levels). Hexane accuracy fails at the lower (0.5) spike level but meets criteria at level 1.0. Only one product ion was used to target hexane, so this result may be indicative of matrix interference. GC-FID fails to achieve acceptance criteria more frequently than SIFT-MS. Chlorobenzene and 1,2-dimethoxyethane are problematic at both spike levels for GC-FID, while various compounds fall just short of the accuracy criterion at one level.

4. Recovery

Recoveries were calculated against the 1.0 level repeatability data (mean across the pre- and post-run calibration samples). The results for both techniques are given in Table 1. Failures in meeting acceptance criteria largely parallel those described above for accuracy.

Conclusions

- SIFT-MS has been successfully benchmarked alongside the standard GC-FID technique for Class 2A and 2B residual solvent analysis.
- Both techniques comfortably meet acceptance criteria for linearity and precision.
- SIFT-MS out-performed GC-FID for accuracy and recovery measurements (one compound failing for SIFT-MS versus ten for GC-FID).
- Multiple, rapidly switchable reagent ions are very beneficial for overcoming matrix interference in SIFT-MS measurements.
- SIFT-MS has a throughput advantage for residual solvent analysis over 11-fold greater than GC-FID.
- SIFT-MS reports quantitative results over six times faster than GC-FID.

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Table A1. Target solvents and the reagent ions and primary product ions mass-to charge ratios (in Daltons) used to quantify them (product ion branching ratios are shown in parentheses). To avoid unnecessary complications, only the product ions used in the method are shown.

Compound	Reagent and product ions used		
	H3O+	NO+	O2+
Benzene		78 (76%) 108 (24%)	
Acetonitrite	42 (100%)		
Chlorobenzene			112 (75%) 114 (25%)
Chloroform			83 (56%) 85 (38%)
Cumene		120 (60%)	
Cyclohexane		83 (64%)	84 (74%)
1,2-Dichloroethene	99 (20%)		96 (60%) 98 (35%)
1,2-Dimethoxyethane	91 (40%)	89 (80%)	
1,4-Dioxane	89 (100%)	87 (55%) 88 (45%)	
Hexane		85 (100%)	
Methanol	33 (100%)		
Methylbutyketone (MBK)		130 (100%)	
Methylcyclohexane		97 (100%)	
Methylene Chloride			84 (56%) 86 (38%)
Nitromethane	62 (100%)		
Pyridine	80 (100%)		
Tetrahydrofuran		71 (100%)	
Tetralin		132 (100%)	104 (35%)
Toluene		92 (100%)	92 (100%)
Trichloroethylene			130 (42%) 132 (42%)
Xylene (all isomeers; ethylbenzene)		106 (100%)	

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