

Direct Analysis of Class 2 Residual Solvents Using Molecular Rotational Resonance Spectroscopy

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ABSTRACT

Molecular rotational resonance (MRR) spectroscopy utilizes the microwave region of electromagnetic radiation, which, in terms of energy, corresponds to transitions between pure rotational energy levels of molecules. Combined with adiabatic cooling in a gas phase, MRR measurement provides a pure rotational spectrum of a molecule's ground vibrational state, which has absolute frequencies and extremely narrow linewidths. Such a spectrum is unique for each chemical structure because it precisely reflects the absolute positions in space for all atoms in a molecule and the corresponding three-dimensional mass distributions. Any small change in a position in space or the mass of any atom(s) between two different substances will result in a well-resolved change in their MRR spectra. Hence, MRR offers unparalleled chemical selectivity. This unique selectivity gives MRR a key advantage: mixtures, including mixtures of all types of isomers, can be analyzed directly without the need for chromatographic separation. As such, MRR has the potential to reduce the time of analyses of mixtures containing diverse volatile chemicals, including residual solvents listed in [Residual Solvents \(467\)](#).

This *Stimuli* article presents the development and validation of a continuous headspace-MRR method for analysis of selected Class 2 (moderate toxicity) residual solvents (per [\(467\)](#)), including all of the low-volatility solvents from [USP Residual Solvents Class 2—Mixture C RS](#). Solvents for this study were selected based on diversity of their boiling points to demonstrate MRR feasibility for analysis of a representative range of solvents. The data presented suggest that MRR can meet ICH and USP requirements for analysis of most of Class 2 and Class 3 residual solvents, and half of Class 1 residual solvents.

An attractive feature of using MRR for residual solvent analysis as an orthogonal technique is that it can bridge analytical gaps of conventional methods for analysis of low-volatility solvents such as those from [USP Residual Solvents Class 2—Mixture C RS](#), dimethyl sulfoxide (DMSO), water-soluble acids, volatile amines, and others. Further, MRR is an online-capable technique that can be used for analysis of diverse volatile molecules not only in laboratory settings but also on the process floor. Therefore, MRR can be used at any stage of pharmaceutical development and manufacturing including support for process analytical technology (PAT) and quality by design (QbD) initiatives and continuous manufacturing.

INTRODUCTION

[Residual Solvents \(467\)](#) is the USP documentary standard for the control of residual solvents in pharmaceutical products. It has information on the categorization of residual solvents by risk assessment, limits of residual solvents in drug products and dietary supplement products, control strategy, and analytical procedures for evaluating the levels of all Class 1 and most Class 2 residual solvents. These analytical procedures are based on static headspace gas chromatography (SH-GC) with a flame ionization detector (FID) and were validated for all Class 1 solvents (with severe toxicity) and most Class 2 solvents (with less severe toxicity).

The SH-GC methods in [\(467\)](#) cannot detect several residual Class 2 solvents, which are the components of [USP Residual Solvents Class 2—Mixture C RS](#) (2-methoxyethanol; 2-ethoxyethanol; *N,N*-dimethylacetamide; *N,N*-dimethylformamide; ethylene glycol; formamide; *N*-methylpyrrolidone; and sulfolane), as these solvents have lower vapor pressures than most of the other Class 1 and 2 solvents. As stated in [\(467\)](#), “those solvents should be determined using an alternative method that has been appropriately validated to demonstrate that the method is suitable for its intended purpose”. Generally accepted guidances for validation of compendial and alternative procedures include [Validation of Compendial Procedures \(1225\)](#) and ICH Q2(R2) ([1](#)). [Residual Solvents—Verification of Compendial Procedures and Validation of Alternative Procedures \(1467\)](#) provides additional guidance on the specific validation criteria for alternative methods used for identification and quantification of residual solvents.

The only type of gas chromatography (GC) method reported in the literature that is capable of analyzing all the solvents in [USP Residual Solvents Class 2—Mixture C RS](#) utilizes direct injection GC ([2–3](#)). However, this method, despite its simplicity, is not a reliable option for analysis of residual solvents. The biggest disadvantage of direct injection GC is that all formulation matrix components, including active pharmaceutical ingredients and excipients, are also injected into the GC system. Nonvolatile or corrosive sample matrix components, if present, can cause injector contamination, as well as column contamination and deterioration ([4–6](#)). Even if there are no nonvolatile or corrosive components in the injected sample matrix, the matrix must be fully eluted prior to the next injection, resulting in an increased analysis time.

A few alternative methods, including reverse-phase HPLC ([7](#)), SPME-GC ([8](#)), SPME-GC-MS ([9](#)), and SIFT-MS ([10](#)), that are capable of analyzing some of the solvents in [USP Residual Solvents Class 2—Mixture C RS](#) have also been reported. However, to our knowledge, none of these alternative methods can provide a convenient and universal solution for analysis of all the solvents represented in [USP Residual Solvents Class 2—Mixture C RS](#).

Therefore, we studied the feasibility of using molecular rotational resonance (MRR) spectroscopy and validated its suitability as a convenient universal solution for components of [USP Residual Solvents Class 2—Mixture C RS](#), as well as several selected residual solvents from [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#).

MRR is a spectroscopic technique that utilizes microwave radiation to probe the pure molecular rotation of compounds in gas phase, for both pure compounds and mixtures of them. The compounds, both pure and in mixtures, can be precisely and unambiguously identified through their

rotational angular momentum transitions in the gas phase. Simplistically, the rotational angular momentum is a product of the principal moments of inertia of a molecule and the angular velocities of rotation around its principal axes. Hence, the molecular rotational transitions are quantized as determined by the principal moments of inertia that precisely reflect three-dimensional mass distributions of molecules. Even in the most complex general case of asymmetric top molecules, where all three principal moments of inertia are not equal to zero, the process of solving the molecular Hamiltonian using modern quantum chemistry methods is well-developed and highly accurate ([11](#)). Furthermore, as the principal moments of inertia can be directly and accurately calculated for any given equilibrium molecular geometry, it is straightforward to theoretically predict and assign the corresponding molecular MRR spectral patterns.

These MRR spectral patterns are information-rich, well-defined, reproducible, highly characteristic, and extremely sharp with lines routinely determined to a relative frequency precision of $>10^6$ (ratio of frequency to error in frequency, e.g., <10 kHz frequency error on a line frequency of 10 GHz or greater). Even the molecules with highly similar structures, such as regioisomers ([12](#)), diastereomers ([13](#)), and isotopomers ([14–15](#)), have distinct principal moments of inertia to produce unique MRR fingerprints, with essentially no spectral overlaps. Enantiomers, which have identical moments of inertia, can still be easily discriminated using gaseous chiral tag molecules that convert the original enantiomers into the MRR-distinguishable weakly-bonded diastereomeric complexes directly in a gas phase ([16–17](#)). This excellent selectivity means that virtually all compounds, including all types of isomers, can be resolved by MRR without chromatographic separation.

In recent years, advances in measurement technology have enabled the characterization of increasingly complex systems including crude reaction mixtures ([13,20](#)) pharmaceutical raw material impurities ([12](#)), isotopically labeled mixtures ([15,18–19](#)), and mixtures of enantiomers ([19](#)). MRR has also been extended to online measurement applications ([13,21](#)). Its primary limitations are that analytes must have a permanent dipole moment and that the analyte must be analyzed in the gas phase ([11](#)).

This article demonstrates that MRR with continuous headspace sampling is capable of not only accelerating the analysis of residual solvents via direct analysis but also bridging the analytical gap of SH-GC for analysis of low-volatile residual solvents from [USP Residual Solvents Class 2—Mixture C RS](#). MRR meets the analytical detection requirements for a wide array of volatile and semi-volatile impurities for complying with the acceptance criteria using a single instrumental setup. The continuous headspace sampling protocol involves flowing neon carrier gas over a heated sample, from which vapor phase components are introduced into the gas stream. The resulting mixture enters a vacuum chamber through a pulsed supersonic valve, after which the MRR spectra are recorded. Each of the MRR-detectable USP residual solvents, including each of those in [USP Residual Solvents Class 2—Mixture C RS](#), has a distinct MRR spectrum that can be used for extraordinarily selective identification as well as quantitation in the drug product. Each solvent in the sample can be analyzed with a variable measurement time depending on the measurement sensitivity required, and multiple solvents can be analyzed in combination without the risk of misidentification of any of them. The method can also be readily extended to include more volatile residual solvents in any of the other classes as well as other residual impurities.

The use of alternative procedures for residual solvent analysis, even though generally permissible, requires proper validation to demonstrate the adequacy of these procedures and the suitability of their analytical metrics for the purpose. More details about validation procedures, parameters, and definitions that are widely accepted in the pharmaceutical industry can be found in ([1467](#)) and ICH Q2(R2) guidance ([1](#)). Briefly, for quantitative methods it is generally required to demonstrate the method selectivity, linearity and range, repeatability, limit of quantitation (LOQ), and accuracy and recovery.

The MRR prototype used in this study was capable of meeting USP and ICH selectivity, sensitivity, linearity, and range requirements for all solvents studied, including solvents poorly suited for GC analysis such as those in the [USP Residual Solvents Class 2—Mixture C RS](#). Analysis repeatability and accuracy/recovery requirements were met for most of the studied residual solvents as well. With upcoming technology improvements, MRR is expected to fully comply with USP and ICH analytical and method validation requirements for most of residual solvents from Classes 1, 2, and 3.

In addition to bridging a gap of SH-GC for analysis of the solvents represented in [USP Residual Solvents Class 2—Mixture C RS](#), MRR is also capable of direct analysis of other residual solvents that are poorly suited for GC analysis, such as acetic acid, formic acids, and dimethyl sulfoxide (DMSO). Other challenging volatile impurities that are not included in ([467](#)), can also be analyzed using the presented headspace-MRR method without any derivatization required. Examples include but are not limited to ethylene oxide, formaldehyde, acetaldehyde, carboxylic acids, volatile amines, and sulfur-containing species.

INSTRUMENTAL METHODS

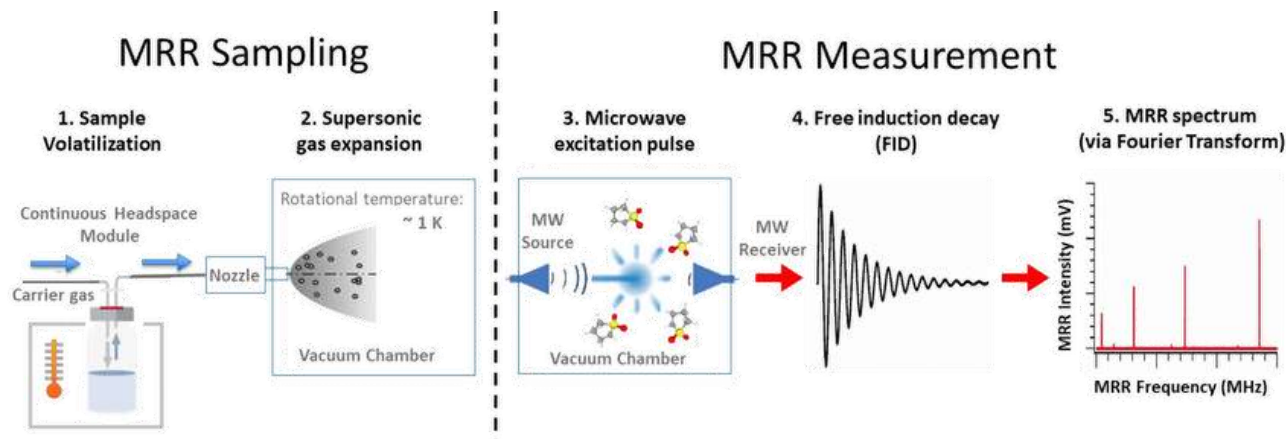
Continuous Headspace Method

MRR uses continuous headspace sample introduction that allows for effective sampling of large headspace volumes, which improves the sensitivity for analytes that are present at very low concentrations. Furthermore, the measurement time can be tailored for each analyte in a sample to meet the sensitivity requirements for that analyte and to optimize the measurement cycle time in case of multi-analyte analyses.

[Figure 1](#) shows a diagram of a continuous headspace-sampling MRR spectrometer, including both the sampling and measurement parts. Research-grade neon ($>99.999\%$ purity) is used as the carrier gas, which flows over a sealed headspace vial where the sample (diluted, if necessary, in a suitable diluent) has been allowed to equilibrate at a specific temperature. This temperature is chosen to optimize sensitivity for the analyte(s) to be characterized. The carrier gas inlet pressure is held to a typical constant value of +5 psig. The resulting mixture of neon and volatilized analytes flows through a heated transfer line and is introduced into a high vacuum chamber ($<10^{-5}$ Torr) through a pulsed solenoid valve. The vacuum pump (a turbomolecular pump with 950 L/s pumping speed, backed by a dry-compression backing pump) maintains a chamber pressure in the $\sim 10^{-4}$ Torr pressure range during measurements, which is suitable for sensitive MRR measurements.

The role of the pulsed solenoid valve is to create a supersonic expansion due to the rapid speed with which it opens and the pressure difference between the vacuum and the analyte mixture being injected. These pulsed supersonic valves have been in regular use in rotational spectroscopy instruments for over 40 years ([22](#)). This allows adiabatic cooling of the rotational and vibrational states of the molecule, which greatly increases the MRR signal strength in the microwave region of the spectrum for the vast majority of the analytes in this study. These valves also prepare

isolated molecules capable of free rotation, so that the transition frequencies and line shapes are independent of any other components in the sample, allowing for excellent selectivity and quantitation.



Click image to enlarge

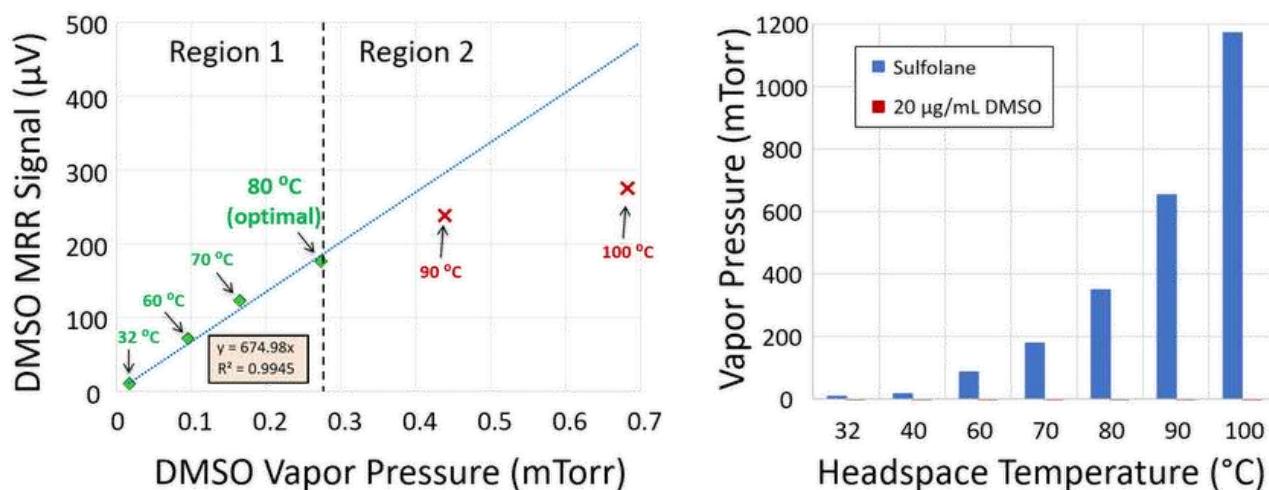
Figure 1. Schematic diagram of the continuous headspace-sampling MRR instrument. See the text for further description.

Threshold Total Vapor Pressure for MRR Measurements

For each component of the sample mixture in the sealed headspace vial, including the diluent, the equilibrium headspace concentration of that component in the analyzed gas mixture is directly proportional to its partial vapor pressure above the solution. We have found that for quantitative MRR measurements, it is important to keep the total gas-phase vapor pressure below about 1 Torr (matrix-dependent), which corresponds to total concentrations of all volatile components of approximately 0.1% in the gas stream. At higher analyte concentrations, MRR signals exhibit a nonlinear relationship with concentration due to the formation of noncovalent complexes in the supersonic expansion; noncovalent complexes exhibit rotation as a unit (rather than as free molecules) and so do not contribute to the total signal of that particular analyte.

It should be noted that about 1 Torr value is given only as a general value. In highly polar sample matrixes, where probability of forming the noncovalent complexes in a gasphase is high, the actual total vapor pressure threshold value could be less than 1 Torr. In contrast, in relatively nonpolar sample matrices, this vapor pressure threshold value may be greater than 1 Torr.

20 $\mu\text{g/mL}$ DMSO in Sulfolane



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Figure 2. Example of experimental determination of the maximum allowable headspace temperature for MRR analysis. On the left, a plot of the observed DMSO signals from a 20-mg/mL solution in sulfolane against the estimated DMSO partial pressure is given. This partial pressure is calculated assuming Raoult's Law (see [Appendix](#)). On the right, the vapor pressure contributions to the solution as a function of temperature are given.

To illustrate the importance of the total vapor pressure of all components for headspace-MRR analysis, [Figure 2](#) shows MRR signals of 20 $\mu\text{g/mL}$ DMSO in sulfolane (>99% purity), as well as the total vapor pressure of the system (predominately, sulfolane), at different headspace temperatures. As evident, at headspace temperatures below about 80°, MRR signals are directly proportional to the partial vapor pressure of DMSO. At headspace temperatures greater than about 80°, there is a deviation from the linear response attributed to the formation of

noncovalent complexes of DMSO (presumably, with sulfolane). As such, 80° is the maximum headspace temperature for this particular MRR analysis. This temperature is chosen because, first, the MRR signal is verified to have a linear response to trace-level DMSO concentrations of up to 20 µg/mL in sulfolane solution, and, second, MRR sensitivity to solution-phase DMSO is greater at this temperature than at lower headspace temperatures. As evident from the right panel of [Figure 2](#), the total vapor pressure threshold value for DMSO in the sulfolane sample is about 0.4 Torr. A practical approach for how to estimate the optimal headspace temperature for quantitation of volatiles in a given sample matrix will be discussed in *Targeted MRR Method Development*.

One consequence of the solution vapor pressure limitation is that it is typically preferable to choose a diluent that has low vapor pressure to maximize the sensitivity of the headspace-MRR measurement. For this reason, in this work, we used an ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate, or BMIMBF₄, as a diluent, which has negligible vapor pressure.

MRR Measurement

The targeted MRR spectrometer used for the measurements in this study has been described in greater detail elsewhere ([11](#)). Briefly, a Fabry-Perot resonator consists of two spherical mirrors that are separated by approximately 30 cm. One of the mirrors is fixed and has the solenoid valve mounted into it, as well as two feed-throughs for coupling microwave radiation in and out of the cavity. The other mirror is mounted on a translation stage capable of precisely tuning the length of the cavity to a value specified by the instrument software. To perform a measurement at a specified frequency, the movable mirror first tunes to a length where a stable cavity mode can be found. A Schottky diode detector is used to confirm that the mirror meets the resonance condition, and small corrections can be made if necessary. Once this is confirmed, the pulsed solenoid valve begins pulsing gas into the chamber at a typical repetition rate of 10 Hz, with each gas pulse having a typical duration of 1 ms.

Following each gas pulse, a series of microwave pulses are transmitted into the chamber that are coincident with the molecular line frequency to be measured. Each pulse, with a typical duration of 1–4 µs, induces a macroscopic coherence between a portion of the molecules of that analyte in the sample chamber. Once the pulse is switched off, the molecules emit a free induction decay signal at their characteristic MRR transition frequency. This emission is downconverted, recorded on a digitizer, and Fourier transformed to yield the resulting frequency spectrum. This measurement scheme is analogous to Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy. The length of a single measurement can be configured by the user, with typical measurement lengths ranging from a few seconds to 10 min per analyte. Once the measurement at a single frequency is complete, the cavity can be automatically retuned to a new frequency within a few seconds, and a new analyte can be characterized. The power of the microwave pulses is automatically adjusted by software for each compound, as the optimal pulse power depends on the dipole moment of the transition being measured.

For the validation study described in this *Stimuli* article, a database of residual solvents and MRR transition frequencies has been compiled. Many of the residual solvents have had their rotational spectra characterized in the past by different laboratories, resulting in a set of transition frequencies that could be used in this study without further modification (the literature references to these data are cited in [Table 2](#)). For solvents that had not been previously characterized by MRR, their spectra were measured using a broadband chirped pulse Fourier transform microwave (CP-FTMW) spectrometer ([23](#)). This spectrometer uses broadband horn antennas to transmit excitation pulses through the chamber to interact with the molecular sample, which reduces the sensitivity of the measurement but enables much larger bandwidth measurements for new compounds.

MRR Spectral Assignment

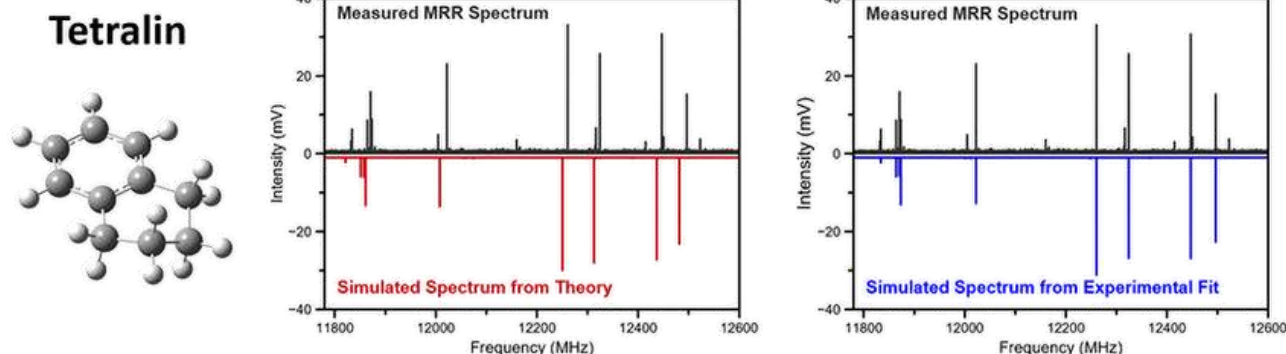
One of the most attractive features of MRR is that, as the spectrum is precisely determined by the three-dimensional structure of the molecule in the gas phase, the molecular identity can be confirmed unambiguously by comparing it to theoretical calculations. Furthermore, the unambiguous spectral assignments can be done directly in crude mixtures without requiring a reference sample, as soon as a list of candidate structures matches the reality ([13](#)). In recent years, developments in quantum chemistry methods, in particular dispersion-corrected density functional theory ([24](#)), have enabled accurate prediction of molecular equilibrium geometry, from which MRR spectroscopy parameters can be accurately derived.

[Figure 3](#) provides an example of an MRR spectrum assignment for tetralin, a Class 2 residual solvent that had not been previously characterized with MRR. In this figure, the black positive-going trace in each panel represents the experimentally measured MRR spectrum. In the left sub-plot, the red negative-going spectrum is simulated using the rotational constants determined from quantum chemical calculations. While there is not an exact match between the line positions, the strong lines show a clear comparable pattern. The theoretical rotational constants can be refined to yield a new simulation (the blue spectrum in the right panel) where the frequencies of the experimental and simulated spectra agree within experimental precision (<10 kHz). The structure is verified by comparing the experimental and theoretically calculated rotational constants ([Table 1](#)). The constants agree to within less than 0.3% so that the identity of the measured compound as tetralin can be unambiguously confirmed.

Table 1. Comparison of Theoretical and Experimental Rotational Constants of Tetralin^a

Rotational Constants	Experimental Fit	Theory	Error
A (MHz)	2776.032	2783.93	−0.28%
B (MHz)	1127.1022	1125.935	+0.10%
C (MHz)	829.8582	829.005	+0.10%

^a Rotational constants were calculated using B3LYP GD3BJ/6-311++G(d,p) basis set.



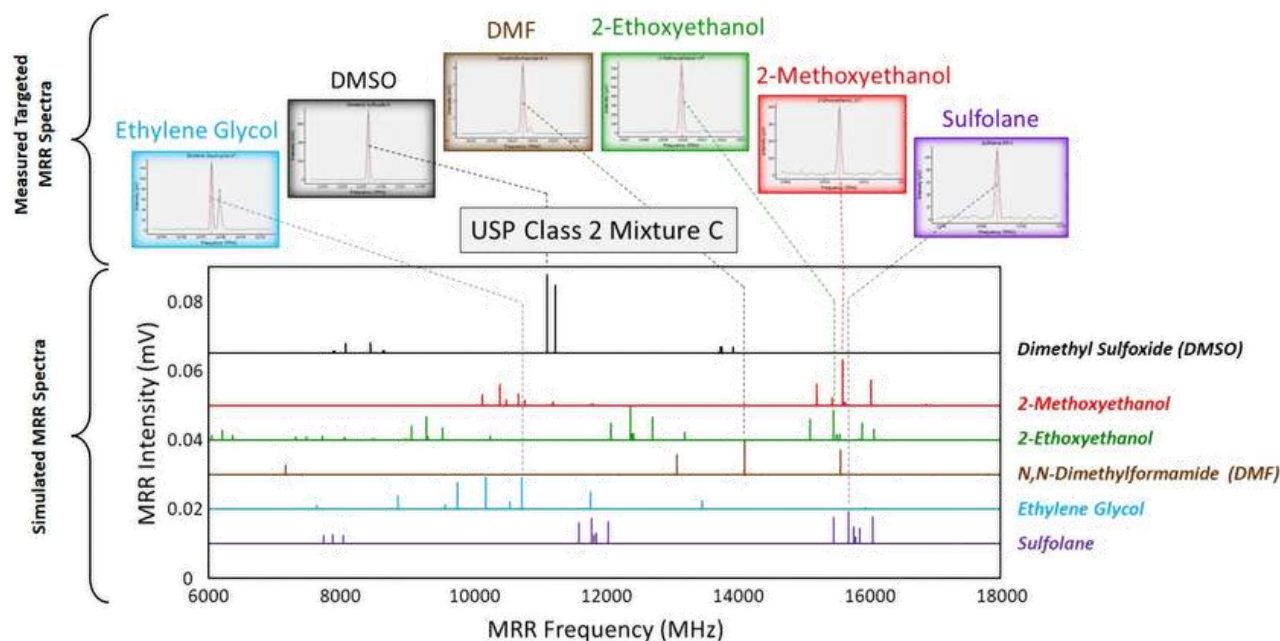
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Figure 3. Assignment of tetralin's MRR spectrum (black: measured MRR spectrum of tetralin; red: simulated MRR spectrum of tetralin using theoretically calculated rotational constants; blue: simulated spectrum of tetralin using experimentally refined rotational constants).

Selectivity of MRR Measurements

In a targeted MRR spectrometer, the typical full-width at half-maximum (FWHM) of an individual transition is ~ 0.1 – 0.2 MHz, while the frequency range of the instrument is 5000–18500 MHz, leading to approximately 10^5 measurement channels. A single molecule, depending on its size and geometry, may have anywhere from a few to several hundred possible detection frequencies that are extremely narrow. As a result, it is possible to identify overlap-free detection frequencies for individual species, even in complex multi-component mixtures.

The main plot of [Figure 4](#) shows MRR spectra for five solvents included in [USP Residual Solvents Class 2—Mixture C RS](#), simulated with experimental linewidth, along with the spectrum of DMSO, the diluent in this standard. The linewidth of the transition is not visible on this scale. The insets show experimental measurements using the targeted spectrometer for each of the six components from this standard, showing that each line is clear and unblended, and the chance of overlap is extremely low; in addition, if there is an unlikely event of a coincidental overlap, each compound has multiple lines that could be used for the analysis. With this selectivity of MRR, the direct quantitative analysis of residual solvent mixtures is straightforward.



Click image to enlarge

Figure 4. MRR selectivity for residual solvents from [USP Residual Solvents Class 2—Mixture C RS](#). Main plot (bottom): simulated MRR spectra for five solvents from [USP Residual Solvents Class 2—Mixture C RS](#) and DMSO diluent. The spectra are simulated using the experimental fits (see [MRR Spectral Assignment](#) for more details). Top subplots: targeted MRR spectra of the same analytes. Note that ethylene glycol has a second line of different species in the measurement window, but this does not interfere with ethylene glycol identification or quantification.

MATERIALS

The list of residual solvents used in this study is presented in [Table 2](#). Out of the 33 Class 2 residual solvents listed in [\(467\)](#), five were not included in the MRR library because they have no dipole moment: cyclohexane; *trans*-1,2-dichloroethene; 1,4-dioxane; *p*-xylene; and *n*-hexane. It should also be noted that 24 out of 26 Class 3 residual solvents are also included in the targeted MRR library.

Table 2. List of Class 2 Residual Solvents (per (467).) Included in the MRR Library

Solvent Name	Purity ^a (%)	Boiling Point (°C)	Respective USP Reference Standard	MRR Source	Frequency (MHz)
2-Methoxyethanol	99.5	124	USP Residual Solvents Class 2 —Mixture C RS	(25)	15609.95
2-Ethoxyethanol	99.5	135.6	USP Residual Solvents Class 2 —Mixture C RS	(26)	15470.76
Dimethylacetamide (DMAC)	99.8	165	USP Residual Solvents Class 2 —Mixture C RS	(27)	14729.29
Dimethylformamide (DMF)	99.9	153	USP Residual Solvents Class 2 —Mixture C RS	(28)	14123.51
Ethylene glycol	99.5	197.3	USP Residual Solvents Class 2 —Mixture C RS	(29)	10747.53
N-Methylpyrrolidone (NMP)	99	202	USP Residual Solvents Class 2 —Mixture C RS	This <i>Stimuli</i> article	15696.07
Formamide	99.5	210	USP Residual Solvents Class 2 —Mixture C RS	(30)	9237.03
Sulfolane	99	285	USP Residual Solvents Class 2 —Mixture C RS	(31)	15698.79
Acetonitrile	99.8	82	USP Residual Solvents Class 2 —Mixture A RS	(32)	18398.00
Chlorobenzene	99.5	132	USP Residual Solvents Class 2 —Mixture A RS	(33)	8350.01
cis-1,2-Dichloroethene	99.2	60	USP Residual Solvents Class 2 —Mixture A RS	(34)	13604.4
Ethylbenzene	99.5	136	USP Residual Solvents Class 2 —Mixture A RS	(35)	13144.75
Isopropylbenzene	99.5	152.4	USP Residual Solvents Class 2 —Mixture A RS	(36)	8276.39
Methanol	99.8	64.7	USP Residual Solvents Class 2 —Mixture A RS	(37)	12178.59
Methylcyclohexane	99.5	100.9	USP Residual Solvents Class 2 —Mixture A RS	This <i>Stimuli</i> article	10366.27
Methylisobutyl ketone	99.3	116	USP Residual Solvents Class 2 —Mixture A RS	(38)	14475.59
Dichloromethane	99.8	39.6	USP Residual Solvents Class 2 —Mixture A RS	(39)	15906.1
Tetrahydrofuran	99.9	66	USP Residual Solvents Class 2 —Mixture A RS	(40)	13385.79
Toluene	99.8	110.6	USP Residual Solvents Class 2 —Mixture A RS	(41)	15834.19
m-Xylene	99.5	139	USP Residual Solvents Class 2 —Mixture A RS	(42)	14708.15
o-Xylene	99.2	144	USP Residual Solvents Class 2 —Mixture A RS	(43)	9214.11
Chloroform	99.5	61.2	USP Residual Solvents Class 2 —Mixture B RS	(44)	13204.28
1,2-Dimethoxyethane	99.5	85	USP Residual Solvents Class 2 —Mixture B RS	(45)	14691.54
2-Hexanone	99.5	128	USP Residual Solvents Class 2 —Mixture B RS	(46)	15989.57
Nitromethane	99	100	USP Residual Solvents Class 2 —Mixture B RS	(47–48)	16420.10

Solvent Name	Purity ^a (%)	Boiling Point (°C)	Respective USP Reference Standard	MRR Source	Frequency (MHz)
Pyridine	99.5	115	USP Residual Solvents Class 2—Mixture B RS	(49–50)	14903.01
Tetralin	98.4	208	USP Residual Solvents Class 2—Mixture B RS	This <i>Stimuli</i> article	10817.28
Trichloroethene	99.5	87.2	USP Residual Solvents Class 2—Mixture B RS	(51)	13424.9

^a From the certificates of analysis provided by suppliers.

Samples and Standards Preparation

Standard stock solution: 200 µL of [USP Residual Solvents Class 2—Mixture C RS](#) were added in a 5-mL volumetric flask and diluted with BMIMBF₄ to volume.

Sample solution: 500.0 mg of respective drug substance were accurately weighed and transferred into a 25-mL volumetric flask and diluted with the ionic liquid to volume.

Spiked sample stock solutions: 200 µL of [USP Residual Solvents Class 2—Mixture C RS](#) were added in a 5-mL volumetric flask and diluted with *Sample solution* (corresponding to 1000% of the USP limit for a 20-mg/mL solution of drug substance) to volume. Three sets of *Spiked sample stock solutions* were prepared: acetaminophen, dextromethorphan, and diphenhydramine, respectively.

Standard solutions: Add the respective volume of *Standard stock solution* (see [Table 3](#)) into a 2-mL volumetric flask and dilute with the ionic liquid to volume.

Spiked sample standard solutions: Add the respective volume of *Spiked sample stock solutions* (see [Table 3](#)) into a 2-mL volumetric flask and dilute with *Sample solution* to volume.

The *Standard solutions* and *Spiked sample standard solutions* were prepared at five different concentration levels (see [Table 3](#)) corresponding to 50%, 75%, 100%, 150%, and 250% of the USP limit for a 20-mg/mL solution of drug substance.

Table 3. Preparation of Standard Solutions at Five Concentration Levels

Standard Solution ID	Level 1	Level 2	Level 3	Level 4	Level 5
Percentage of USP Limit in Solution (%) ^a	50	75	100	150	250
Volume of Standard Stock Solution (µL)	100	150	200	300	500

^a USP limit in solution is calculated assuming 20-mg/mL concentration of a drug substance.

As for the remaining solvent members of USP Residual Solvents Class 2—Mixture C RS (formamide and sulfolane), as well as the selected solvent members of USP Residual Solvents Class 2—Mixture A RS and USP Residual Solvents Class 2—Mixture B RS, we prepared custom standards as described in [Tables 4](#) and [Table 5](#). The [Table 4](#) residual solvents were added into a 5-mL volumetric flask prefilled with 3 mL of diluent and then diluted with a corresponding diluent to volume to prepare the stock solutions 1-A through 1-D. The [Table 5](#) *Standard stock solution* and *Standard solutions* were prepared sequentially by adding a specified volume of a specified stock solution into a 2-mL volumetric flask prefilled with 0.5 mL of diluent and then diluting it with a relevant diluent to volume. As diluents, we used either the ionic liquid (BMIMBF₄) for linearity studies or a solution of a pharmaceutical substance in the ionic liquid at a specified concentration for accuracy and recovery studies. Solvents in both the *Standard stock solution* and *Standard solutions* were grouped by their boiling points to optimize MRR sensitivity.

Table 4. Preparation of Custom Stock Solutions 1-A through 1-D^a

Stock ID ^b	Residual Solvent	Boiling Point (°C)	USP Limit (µg/g)	Substance Concentration (mg/mL)	Reference Standard Concentration in Solution Corresponding to the USP Limit		Volume in Stock Solution 1 (µL)
					µg/mL	ppmv	
Stock solution 1-A	<i>cis</i> -1,2-Dichloroethene	60	1870	10	18.7	14.6	290
	Methanol	64.7	3000	10	30	37.9	760
	Tetrahydrofuran	66	720	10	7.2	8.1	160
	Acetonitrile	82	410	10	4.1	5.2	105
Stock solution 1-B	Nitromethane	100	50	10	0.5	0.44	9
	Toluene	110.6	890	10	8.9	10.3	205
	Pyridine	115	200	10	2	2.04	40
Stock solution	Chlorobenzene	132	360	10	3.6	3.2	65
	Ethylbenzene	136	368	10	3.68	4.3	85

Stock ID ^b	Residual Solvent	Boiling Point (°C)	USP Limit (µg/g)	Substance Concentration (mg/mL)	Reference Standard Concentration in Solution Corresponding to the USP Limit		Volume in Stock Solution 1 (µL)
					µg/mL	ppmv	
1-C	<i>m</i> -Xylene	139	1302	10	13.02	15.1	305
Stock solution 1-D	Formamide	210	220	40	8.8	7.8	155
	Sulfolane	285	160	40	6.4	5.1	100

^a These solutions contain 2–4 residual solvents at concentrations corresponding to 4000× the USP limits. (See [467](#)). All standards are diluted to a 5-mL volume. Solvents are grouped by boiling points.)

^b Ionic liquid (BMIMBF₄) or 10-to 40-mg/mL substance solution in the ionic liquid.

Table 5. Preparation of Custom Standard Solutions at Five Concentration Levels for Selected Solvents Listed in USP Residual Solvents Class 2—Mixture A RS, USP Residual Solvents Class 2—Mixture B RS, and USP Residual Solvents Class 2—Mixture C RS as Specified in [Table 4](#) and the Text

Solution ID	Stock Solution 2	Stock Solution 3	Level 1-A		Level 2	Level 3	Level 4	Level 5 ^a	
Concentration of Reference Standard in Solution (% of USP Limit)	20,000	1000	20	25	50	100	200	400	500
Volume of Stock 1 (µL)	100	—	—	—	—	—	—	—	—
Volume of Stock 2 (µL)	—	100	—	—	—	—	—	—	—
Volume of Stock 3 (µL)	—	—	40	50	100	200	400	800	1000

^a Ionic liquid (BMIMBF₄) or 10- to 40-mg/mL substance solution in the ionic liquid.

HEADSPACE-MRR METHOD DEVELOPMENT

Diluent Considerations

As discussed previously, the diluent for headspace-MRR analysis must meet two criteria. First, this diluent should be capable of dissolving a drug substance of interest to the required concentration level (typically, within the 10-to50-mg/mL range). Second, the diluent should have less than approximately 1 Torr of vapor pressure in the intended range of headspace temperatures. For this reason, an ionic liquid (1-butyl-3-imidazolium tetrafluoroborate or BMIMBF₄) was used in this work.

Solubility Studies

Prior to any MRR measurements, it was verified that all the pharmaceutical drug substances involved in this study, such as acetaminophen, aspirin, ibuprofen, dextromethorphan, and diphenhydramine, can be fully dissolved in the BMIMBF₄ ionic liquid diluent to the required levels that vary during our study between 10 and 40 mg/mL. In some cases, sonication was required for dissolution.

In case of encountering solubility issues while using BMIMBF₄ to dissolve a substance of interest, there are other commercial ionic liquid options, including EMIM DEP (1-ethyl-3-methylimidazolium diethyl phosphate) that are reported in the literature to be capable of dissolving even cellulose ([52](#)). Alternatively, small quantities of a low-volatile cosolvent, such as sulfolane, can be added to BMIMBF₄ or other suitable ionic liquid to resolve possible solubility issues.

Determining Optimal Headspace Temperature for MRR Analysis

For each *Standard solution*, the maximum temperature range was estimated by calculating the total vapor pressure of the solution following a procedure described in the [Appendix](#). All components of the solution were considered, including, for example, the DMSO used as the diluent in the manufacture of [USP Residual Solvents Class 2—Mixture C RS](#). We found that for this sample, DMSO had the largest contribution to the vapor pressure of the solution. For the other stock solutions, which were prepared from individual solvent standards, the vapor pressure was limited by the analytes themselves (since the ionic liquid diluent contributes negligible vapor pressure).

MRR Library for Residual Solvent Analysis

For each Class 2 solvent, a strong spectral line was chosen based on its signal intensity within the range of the MRR spectrometer used in this study (6–18.5 GHz). These frequency values are given in [Table 2](#). Almost all of these solvents have additional transitions in the MRR library that are also suitable for targeted analysis. The library contains the full characterization of the MRR spectrum of each solvent in this frequency range, compiled from the sources given in [Table 2](#). The Class 2 solvents that were not previously in the MRR library were added through the procedure described previously.

Even though spectral overlaps between different molecules are extremely rare for MRR, the measured or simulated spectra of all components of interest can be overlaid on one plot to verify that the selected (strongest) lines are overlap-free in the mixture of interest, similar to that of five solvents from [USP Residual Solvents Class 2—Mixture C RS](#) in [Figure 4](#).

After targeted lines for each analyte of interest were selected, the MRR instrument ran an automated routine to optimize MRR parameters for the selected line to maximize analysis sensitivity. To save time, this routine can be run using a sample mixture with the highest quantifiable analyte concentrations and the optimal headspace temperature for the intended MRR method as discussed.

VALIDATION STUDY DESIGN

There are two main goals of this validation study. The first goal is to verify that headspace-MRR can bridge the analytical gap of conventional SH-GC methods for analysis of low-volatile residual solvents from [USP Residual Solvents Class 2—Mixture C RS](#). Furthermore, we evaluated the possibility of MRR integration into existing residual solvent analysis workflows by preparing residual solvent standards using [USP Residual Solvents Class 2—Mixture C RS](#).

The second goal is to demonstrate MRR feasibility for analysis of most of Class 2 residual solvents. For this purpose, we selected several diverse residual solvents from [USP Residual Solvents Class 2—Mixture A RS](#), [USP Residual Solvents Class 2—Mixture B RS](#), and [USP Residual Solvents Class 2—Mixture C RS](#) that cover most of the boiling point range for Class 2 residual solvents. The selected solvents were grouped by their boiling points and can be classified as high-volatility (1,2-*cis*-dichloroethene, methanol, tetrahydrofuran, and acetonitrile), medium-volatility (nitromethane, toluene, pyridine), medium-to-low volatility (chlorobenzene, ethylbenzene, *m*-xylene), and very low volatility (formamide and sulfolane).

It should be noted that for the second half of the feasibility study we utilized custom standards that were prepared using chemically pure solvents, grouped by their boiling points, and ionic liquid as a diluent for pharmaceutical substances under study. The reason for using the custom standards is that it gives an option to increase the headspace temperature (while still meeting the maximum allowable vapor pressure requirement) to further reduce the time required to reach the desired measurement sensitivity.

The validation study described in this article was designed in accordance with the requirements specified in the ICH Q2(R2) guideline and recommendations specified in [\(1467\)](#) for validation of nonchromatographic alternative procedures for residual solvents.

VALIDATION RESULTS

System Suitability

MRR is an extraordinarily selective analytical technique; the MRR spectral pattern of a molecule cannot be matched by the spectral pattern of any other molecule. There are essentially no spectral overlaps between different residual solvent species or other constituents of the drug substance matrix. For these reasons, the main factor that determines MRR suitability for the task is the analysis sensitivity that, in turn, can be accurately predicted theoretically [\(11\)](#). As a result, once MRR sensitivity for analysis of a particular residual solvent is verified, no specific system suitability test is required besides the routine MRR instrument operational qualification tests.

Specificity and Selectivity

ICH Q2(R2) guidance states that "the specificity or selectivity of an analytical procedure can be demonstrated through the absence of interference, comparison of results to an orthogonal procedure or may be inherently given by the underlying scientific principles of the analytical procedure". MRR is an inherently highly selective analytical technique, and spectral interferences between different species are very rare. However, we recommend a few practical steps to further augment the analysis specificity.

First, if all volatile components of a sample matrix of interest are in the MRR library (including the target analytes, drug substance, impurities, degradation products, excipients, and diluents), then overlaying their library spectra (including all detectable conformers) on the same plot can be used to check for potential spectral overlaps.

Second, if some of the volatile matrix components or their MRR spectra are unknown or if spectral overlap is suspected, we recommend testing those components by the MRR method using blank and spiked samples. If spectral overlap is confirmed for a transition, then another transition from the MRR library can be used for the targeted analysis of this analyte(s).

Alternatively, it is possible to develop MRR methods that use multiple targeted lines for each analyte of interest. However, this approach will result in increased analysis cycle time.

LINEARITY AND RANGE

According to ICH Q2(R2), a minimum of five concentrations appropriately distributed across the range is recommended for assessing the linearity. For residual solvents testing, the reportable range to be demonstrated is 50%–120% of the target concentration (i.e., 50%–120% of the USP concentration limit for each residual solvent). Chapter [\(1467\)](#) recommends for nonchromatographic procedures that the coefficient of determination of the linearity curve, R^2 , is not less than 0.90.

In the first part of our validation study, where [USP Residual Solvents Class 2—Mixture C RS](#) was used for sample preparation [Table 3](#), the demonstrated linear range is 50%–250% of the USP limit ([Figure 5](#) and [Table 6](#)). In the second part of our study, where custom-made residual solvent standards were used, the demonstrated linear range is at least 25%–400% of the USP limit ([Figure 6](#), [Figure 7](#), and [Table 8](#)). Pearson's linear regression coefficient, R^2 , was equal to or greater than about 0.99 for most of the residual solvents and greater than about 0.97 for all solvents. Therefore, the requirements for linearity and range were met for all listed residual solvents.

LIMIT OF QUANTITATION

LOQs were estimated for each solvent from a corresponding linear slope and a standard deviation (SD) of six measurements of blank MRR signals (see [Table 6](#) and [Table 8](#)). The 600 s duration of blank measurements was chosen because 600 s represents the maximum practical measurement time per analyte for headspace-MRR analysis. The mathematical formula for LOQ is provided below.

$$LOQ = 10 \times SD_{\text{Blank}} / \text{Slope}$$

The ICH Q3C(R2) and [\(467\)](#) provide the concentration limits for residual solvents based on the permissible daily exposure (PDE) when the maximum daily dose of the drug product does not exceed 10 g/day. The LOQs for all analytes (residual solvents) of this study were no more than 50% of USP limits per [\(467\)](#) (see [Table 6](#) and [Table 8](#)). Since the spectrometer used to perform the analysis measures selected rotational transitions (corresponding to each analyte) separately, these quantitation limits can be easily and independently adjusted as needed in any direction by shortening or increasing the measurement time for a specific analyte, as needed.

Repeatability

As stated in ICH Q2(R2), repeatability can be determined from either six measurements at 100% of target concentration or at least nine measurements within a reportable range for the procedure (e.g., three concentrations and triplicate analysis). The recommended acceptance

criterion for validation of nonchromatographic alternative procedures in [\(1467\)](#) is a relative standard deviation of not more than 20% for each solvent present.

For the first part of our study, we used three replicates at each of the 75%, 100%, and 150% (of the USP limit) concentration levels to calculate the repeatability (see [Table 6](#)). For the second part of our study, we used three replicates at each of the 50%, 100%, and 200% (of the USP limit) concentration levels (see [Table 8](#)). As evident from [Table 6](#) and [Table 8](#), the repeatability for most of residual solvents is less than 20% and meets the ICH requirements. The exceptions are dimethylacetamide (DMAC), tetrahydrofuran (THF), ethylbenzene, and *m*-xylene.

Accuracy

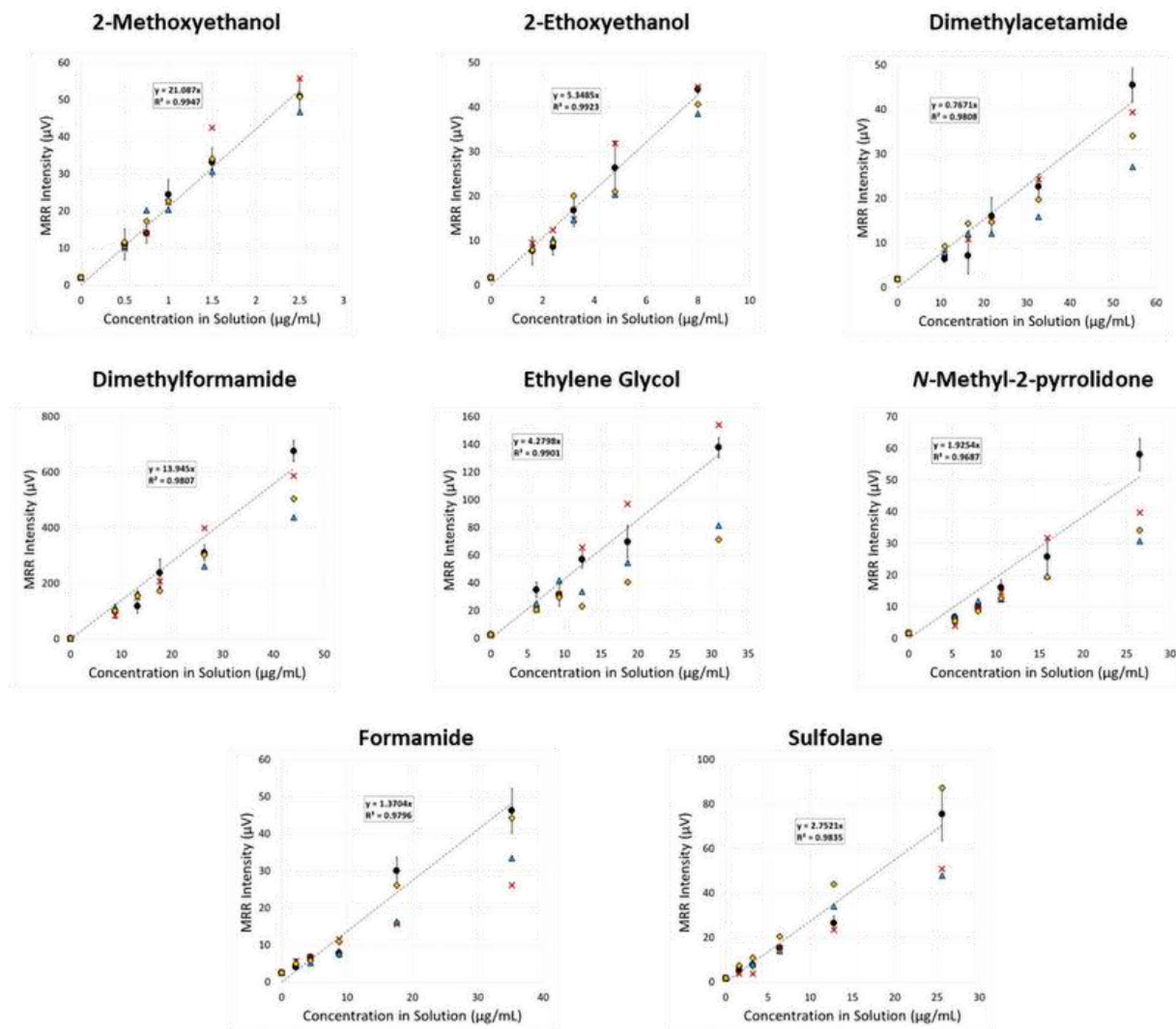
To evaluate accuracy and recovery, three spiked sample solutions were prepared for each concentration level for the three drug substances. For the first part of this study, we used 20-mg/mL solutions of acetaminophen, dextromethorphan, and diphenhydramine spiked at 75%, 100%, and 150% concentration levels using [USP Residual Solvents Class 2—Mixture C RS](#). For the accuracy and recovery of selected components of [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#), we used 10-mg/mL solutions of acetaminophen, aspirin, and ibuprofen spiked at 50%, 100%, and 200% concentration levels, respectively. For the accuracy and recovery for the remaining two components of [USP Residual Solvents Class 2—Mixture C RS](#) (formamide and sulfolane), we used 40-mg/mL solutions of acetaminophen, dextromethorphan, and diphenhydramine spiked at 50%, 100%, and 200% concentration levels, respectively.

Recovery data are summarized in [Table 7](#) and [Table 9](#). Specifically, we calculated the average recovery for three replicates at three concentration levels. The acceptance criterion recommended in [\(1467\)](#) for nonchromatographic procedures is a solvent recovery within the 80%–120% range. For most of these solvents in the specified substances, the recovery acceptance criteria were met. The exceptions are dimethylacetamide and ethylene glycol in diphenhydramine; sulfolane in acetaminophen and diphenhydramine; tetrahydrofuran in aspirin and ibuprofen; nitromethane in ibuprofen; and pyridine in acetaminophen, aspirin, and ibuprofen. Except for pyridine, the variability of recovery data for these analytes is believed to be attributed to the valve performance. The variation in pyridine recovery data strongly suggests that matrix effects may be responsible. However, because of both high MRR sensitivity to pyridine and a good linear dependence for the pyridine signal in different substances, we believe that the standard addition method, similar to that described in [\(467\)](#), [8. Analytical Procedures for Class 1 and Class 2 Residual Solvents, 8.1 Chromatographic Systems, Procedure C](#) will produce accurate results for pyridine quantitation in pharmaceutical substances despite the observed matrix effects.

Solution Stability

Fresh *Standard stock solution* and *Standard solutions* were prepared daily; therefore, the solution stability was not evaluated for this *Stimuli* article.

● No Substance ✕ Acetaminophen ▲ Dextromethorphan ◆ Diphenhydramine --- Linear (No Substance)



Click image to enlarge

Figure 5. MRR validation data for solvents from [USP Residual Solvents Class 2—Mixture C RS](#). For the top six solvents (2-methoxyethanol, 2-ethoxyethanol, dimethylacetamide, dimethylformamide, ethylene glycol, and *N*-methylpyrrolidone), the corresponding standards were prepared using [USP Residual Solvents Class 2—Mixture C RS](#) (see previous sections and [Table 3](#)). For formamide and sulfolane, the custom standards were made as described previously and in [Table 4](#) and [Table 5](#). The regression line is provided for “no substance” data.

Table 6. Validation Data for Eight Solvents from [USP Residual Solvents Class 2—Mixture C RS](#) in Ionic Liquids (BMIMBF₄)

Solvent	Concentration Range in Solution (µg/mL)	s								USP Concentration Limit (µg/g)
		Linearity, <i>R</i> ²	Repeatability (3 × 3, %)	MRR Response Factor (µV per 1 µg/mL)	600s Background Noise (SD of 6 Measurements, µV)	600 s LOQ in Solution (µg/mL)	Estimated 600 s LOQ with Respect to Substance (µg/g) for Concentrations of:			
							10 mg/mL	20 mg/mL	50 mg/mL	
2-Methoxyethanol ^a	0.5–2.5	0.995	14.0	21.1	0.66	0.31	31	16	6	50
2-Ethoxyethanol ^a	1.6–8	0.992	19.0	5.4	0.65	1.21	121	61	24	160
Dimethylformamide (DMF) ^a	8.8–44	0.981	15.4	14.0	1.11	0.79	79	40	16	880

Solvent	Concentration Range in Solution (µg/mL)	s								USP Concentration Limit (µg/g)
		Linearity, R^2	Repeatability (3 × 3, %)	MRR Response Factor (µV per 1 µg/mL)	600s Background Noise (SD of 6 Measurements, µV)	600 s LOQ in Solution (µg/mL)	Estimated 600 s LOQ with Respect to Substance (µg/g) for Concentrations of:			
							10 mg/mL	20 mg/mL	50 mg/mL	
Dimethylacetamide (DMAC) ^a	10.9–54.5	0.981	30.6	0.77	0.41	5.37	537	268	107	1090
Ethylene glycol ^a	6.2–31	0.990	16.6	4.3	1.16	2.70	270	135	54	620
N-Methylpyrrolidone (NMP) ^a	5.3–26.5	0.969	15.7	1.9	0.59	3.06	306	153	61	530
Formamide ^b	4.4–35.2	0.980	10.0	1.4	0.70	5.11	511	255	102	220
Sulfolane ^b	3.2–25.6	0.984	15.3	2.8	0.62	2.25	225	113	45	160

^a [USP Residual Solvents Class 2—Mixture C RS](#) was used.

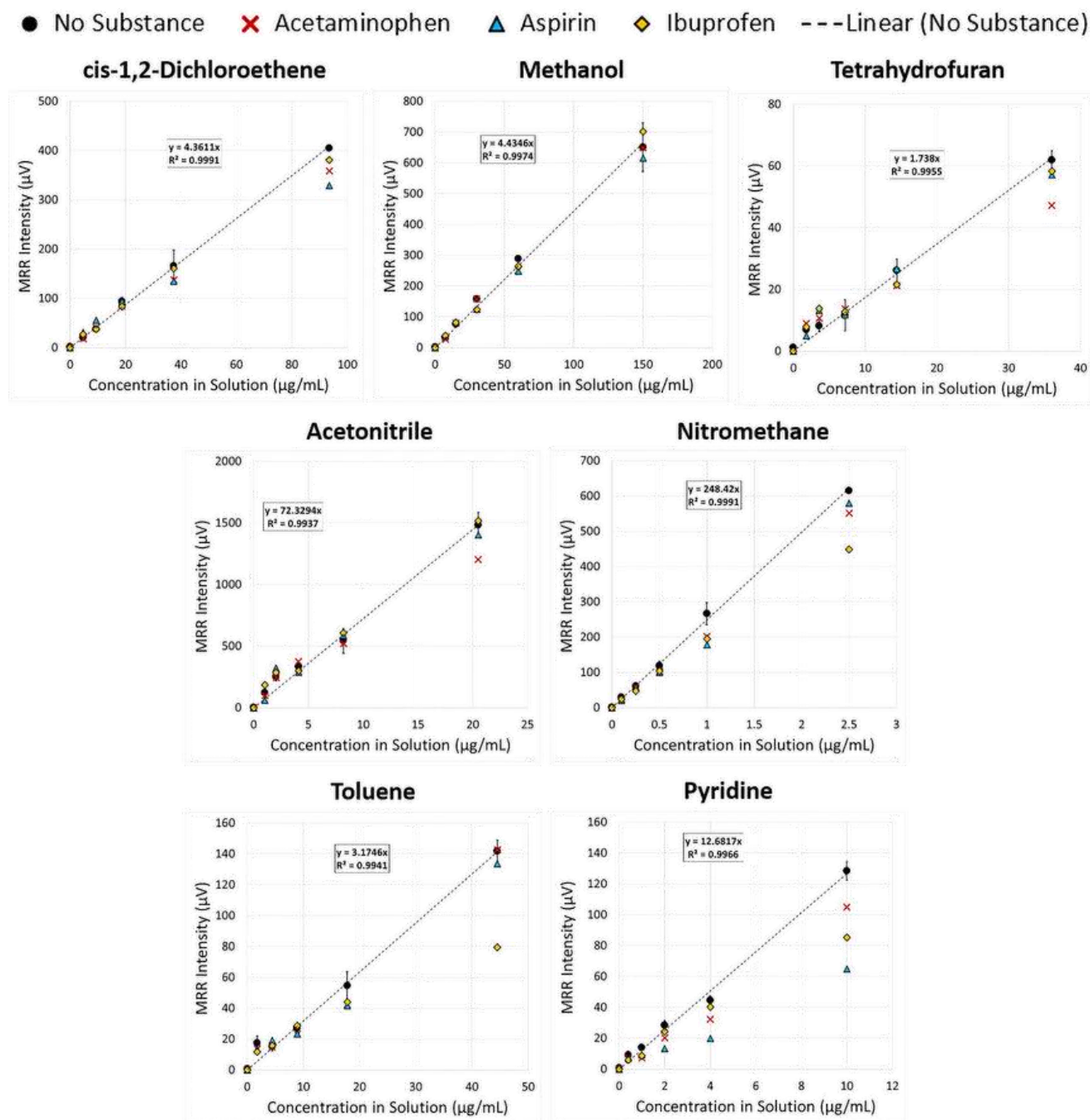
^b Commercial individual neat solvents were used.

Table 7. Mean Recovery Data for Eight Solvents from [USP Residual Solvents Class 2—Mixture C RS](#) in 20-mg/mL Drug Substance Solutions in Ionic Liquids (BMIMBF₄)

Solvent	Mean % Recovery (3 × 3) for 75%, 100%, and 150% Levels for:		
	Acetaminophen	Dextromethorphan	Diphenhydramine
2-Methoxyethanol ^a	106.7	106.9	106.4
2-Ethoxyethanol ^a	117.6	95.0	103.5
Dimethylformamide (DMF) ^a	114.3	99.9	100.8
Dimethylacetamide (DMAC) ^a	117.7	105.3	126.8
Ethylene glycol ^a	118.6	90.3	64.3
N-Methylpyrrolidone (NMP) ^a	105.3	91.8	80.8
Formamide ^b	100.4	76.3	104.3
Sulfolane ^b	78.8	107.5	146.5

^a [USP Residual Solvents Class 2—Mixture C RS](#) used.

^b Commercial individual neat solvents were used.



Click image to enlarge

Figure 6. Validation data for selected solvents of high and medium volatility from [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#). Custom standards were prepared using pure residual solvents, as described previously and in [Table 4](#) and [Table 5](#).

Table 8. Validation Data for Selected Solvents from [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#) in Ionic Liquids (BMIMBF₄)

Solvent ^a	Concentration Range in Solution (µg/mL)		Evaluated Performance Parameter								USP Limit in Substance (µg/g)
			Linearity, <i>R</i> ²	Repeatability (3 × 3, %)	MRR Response Factor (µV per 1 µg/mL)	600 s Background Noise (µV, SD of 6 Measurements)	600 s LOQ in Solution (µg/mL)	Estimated 600 s LOQ in Substance (µg/g) for Solution Concentration			
	Min.	Max.						10 mg/mL	20 mg/mL	50 mg/mL	
<i>cis</i> -1,2-Dichloroethene	1.65	93.5	0.999	10.9	4.36	0.72	1.65	165	83	33	1870

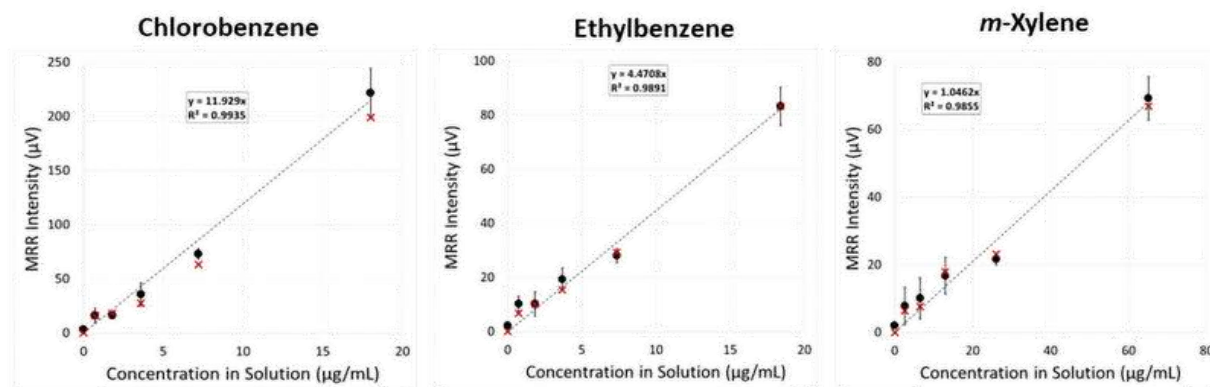
Solvent ^a	Concentration		Evaluated Performance Parameter								USP Limit in Substance (µg/g)
	Range in Solution (µg/mL)		Linearity, <i>R</i> ²	Repeatability (3 × 3, %)	MRR Response Factor (µV per 1 µg/mL)	600 s Background Noise (µV, SD of 6 Measurements)	600 s LOQ in Solution (µg/mL)	Estimated 600 s LOQ in Substance (µg/g) for Solution Concentration			
	Min.	Max.						10 mg/mL	20 mg/mL	50 mg/mL	
Methanol	1.99	150	0.997	6.1	4.43	0.88	1.99	199	99	40	3000
Tetrahydrofuran	5.75	36	0.996	25.6	1.74	1.00	5.75	575	287	115	720
Acetonitrile	0.09	20.5	0.994	11.9	72.3	0.63	0.09	8.7	4.4	1.7	410
Nitromethane	0.03	2.5	0.999	9.2	248	0.66	0.03	2.7	1.3	0.53	50
Toluene	3.15	44.5	0.994	13.8	3.17	1.00	3.15	315	158	63	890
Pyridine	0.38	10	0.997	8.9	12.7	0.48	0.38	38	19	8	200
Chlorobenzene	1.52	18	0.994	16.6	11.9	1.81	1.52	152	76	30	360
Ethylbenzene	1.77	18.4	0.989	25.3	4.47	0.79	1.77	177	88	35	368
<i>m</i> -Xylene	6.10	65.1	0.986	34.6	1.05	0.64	6.10	610	305	122	1302

^a Neat commercial solvents were used (see [Table 2](#)).

Table 9. Recovery Data for Selected Solvents from [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#) in Drug Substance Solutions with a Concentration of 10 mg/mL

Residual Solvent	Average Recovery (%) for 50%, 100%, and 200% Concentration Levels ^a		
	Acetaminophen	Aspirin	Ibuprofen
<i>cis</i> -1,2-Dichloroethene	93.5	110.1	95.2
Methanol	97.3	91.5	92.4
Tetrahydrofuran	110.0	121.7	121.0
Acetonitrile	101.9	107.5	104.8
Nitromethane (2-B)	86.1	82.4	79.4
Toluene (2-A)	86.8	95.8	96.2
Pyridine (2-B)	65.0	52.5	79.1

^a Three preparations per level.



Click image to enlarge

Figure 7. Validation data for selected solvents of medium volatility from [USP Residual Solvents Class 2—Mixture A RS](#): no substance (black dot) vs. 10-mg/mL acetaminophen (red x). Additional studies are being conducted. Custom residual solvent standards were prepared as described previously and in [Table 4](#) and [Table 5](#).

Discussion

In contrast to chromatography, which determines different component compounds of a mixture based on chromatographic separation (i.e., temporal separation because of the distribution of components between two phases), the headspace-MRR identifies and quantifies those components directly in the gas phase based on their pure molecular rotational spectrum resulting from molecular geometry and three-

dimensional mass distributions. All volatile components of a sample are present in the sample gas phase during MRR analysis. To ensure linear response of MRR signals, the total vapor pressure of all volatile components (but not counting the carrier gas) generally should not exceed 1 Torr. This can be achieved by adjusting the headspace temperature and/or changing the composition of a sample matrix (for example, by changing the sample preparation procedure). Therefore, it is beneficial for MRR analysis to use diluents with as low as possible vapor pressure. For this reason, we used ionic liquids or ionic liquids with small amounts of low-volatile cosolvents (such as sulfolane).

One more important consideration that can help to significantly improve the sensitivity and speed of MRR analysis is to reconsider not only the diluent but also the grouping of the residual solvents in standard mixtures. Specifically, the commercially available standard mixtures for residual solvent analysis, such as [USP Residual Solvents Class 2—Mixture A RS](#), [USP Residual Solvents Class 2—Mixture B RS](#), and [USP Residual Solvents Class 2—Mixture C RS](#), are designed for GC. In contrast, to optimize MRR analytical metrics, it would be beneficial to make standard mixtures of residual solvents that are grouped by their boiling points and avoid placing highly volatile and low-volatile solvents into one standard mixture. In this work, we proved the feasibility of this approach using custom-made standards for selected residual solvents from [USP Residual Solvents Class 2—Mixture A RS](#), [USP Residual Solvents Class 2—Mixture B RS](#), and [USP Residual Solvents Class 2—Mixture C RS](#) ([Table 4](#) and [Table 5](#)).

Despite the above, [USP Residual Solvents Class 2—Mixture C RS](#) as described in ([467](#)) can certainly be used with MRR to directly analyze six (of eight) solvents represented in the Reference Standard: 2-methoxyethanol, 2-ethoxyethanol, dimethylformamide, dimethyl acetamide, ethylene glycol, and *N*-methylpyrrolidone; thus filling a gap in the analysis for these solvents as there is no compendial or any other standard procedure available. The same conclusion can likely be derived for many solvents represented in [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#), but the exact list of solvents suitable for the conventional workflow using commercial standard mixtures is still to be determined.

In contrast, if custom standards that utilize low-volatile diluent and residual solvents grouped by their boiling points are made, MRR analysis is certainly capable of meeting analytical technique performance characteristics needed for complying with ICH and USP acceptance criteria (per ([467](#))) for most Class 2 solvents. The exceptions include nonpolar solvents such as *trans*-1,2-dichloroethene; 1,4-dioxane; *p*-xylene; and *n*-hexane.

CONCLUSIONS

This *Stimuli* article demonstrates that MRR spectroscopy can emerge as an alternative method for residual solvent analysis in the near future. This is due to several factors. First, MRR bridges the analytical gaps in conventional SH-GC methods for analyzing low-volatile residual solvents, such as those from [USP Residual Solvents Class 2—Mixture C RS](#). Second, the high chemical selectivity of MRR enables direct analysis of residual solvents in multicomponent mixtures without chemical separation to significantly decrease analysis time comparing to GC and eliminate any potential analyte coelution and identification problems.

The developed MRR method was validated for several selected residual solvents from [USP Residual Solvents Class 2—Mixture A RS](#) and [USP Residual Solvents Class 2—Mixture B RS](#) (see ([467](#))), as well as for all eight low-volatile solvents from [USP Residual Solvents Class 2—Mixture C RS](#). The first-generation headspace-MRR prototype that was used in this study is capable of meeting analytical procedure performance characteristics for complying with USP acceptance criteria (per ([467](#))) for most of the studied residual solvents. In addition, the first tests of a new-generation fully-automated headspace-MRR setup show significantly improved analysis performance characteristics in terms of analysis sensitivity, repeatability, and sample throughput. With simultaneous multi-sample headspace equilibration utilized in this new setup, it is possible to achieve effective residual solvent analysis cycle times between 2 and 20 min per sample, depending on the number and type of analytes and required analysis sensitivity.

In summary, as the sensitivity of MRR analysis can be accurately predicted theoretically, it is possible to state with high confidence that the new-generation, fully-automated, high-temperature headspace-MRR instrument can meet analytical procedure performance characteristics needed for complying with USP acceptance criteria (per ([467](#))) for most of Class 2 and 3 solvents including those from [USP Residual Solvents Class 2—Mixture C RS](#), water-soluble acids, and DMSO, as well as about half of Class 1 solvents. In addition, MRR can directly analyze several other volatile impurities poorly suited to GC analysis that are not listed in ([467](#)). This list includes but is not limited to formaldehyde, ethylene oxide, carboxylic acids, and volatile amines.

Furthermore, MRR is an online-capable technique ([13,21](#)) that can bring the high-resolution and extraordinary chemical selectivity of high-end instrumentation directly to the process floor. For this reason, MRR can support the implementation of continuous manufacturing, as well as PAT and QbD initiatives to enable detection of diverse impurities at any stage of pharmaceutical development and manufacturing.

APPENDIX: THEORETICAL ESTIMATE OF TOTAL VAPOR PRESSURE OF ALL SAMPLE COMPONENTS IN HEADSPACE

For each component of the sample mixture in the sealed headspace vial, including the diluent, the equilibrium headspace concentration of that component in the neon gas that exits the headspace vial during MRR measurement, C_i^{gas} , is directly proportional to its partial vapor pressure, p_i^{gas} :

$$C_i^{\text{gas}} \propto p_i^{\text{gas}} \quad \text{Equation 1}$$

According to Raoult's law ([53](#)), the equilibrium vapor pressure of i^{th} mixture component, p_i^{gas} , is proportional to its molar fraction in solution, μ_i^{solution} . In ideal mixtures, the proportionality constant is equal to the vapor pressure of the pure analyte, $p_i^{100\%}(T)$, at a given headspace temperature T .

$$p_i^{\text{gas}} = p_i^{100\%}(T) \bullet \mu_i^{\text{solution}} \quad \text{Equation 2}$$

In real mixtures, there are deviations from Raoult's law. The matrix effects mainly occur because of the intermolecular interactions in a liquid phase between the i^{th} analyte and the other matrix components, which may change the proportionality coefficient between the partial vapor pressure of a component and its molar fraction in solution. To compensate for these effects, the activity coefficient, γ_i , can be introduced to Raoult's equation (53):

$$p_i^{\text{gas}} = p_i^{100\%}(T) \bullet \gamma_i \bullet \mu_i^{\text{solution}} \quad \text{Equation 3}$$

According to Dalton's law (53), total vapor pressure of components of a gas-phase mixture, $p_{\text{total}}^{\text{gas}}$, can be determined as a sum of partial vapor pressures, p_i^{gas} , of individual components of a mixture:

$$p_{\text{total}}^{\text{gas}} = \sum (p_i^{\text{gas}}) = \sum \left[p_i^{100\%}(T) \bullet \gamma_i \bullet \mu_i^{\text{solution}} \right] \quad \text{Equation 4}$$

In their turn, vapor pressures of pure analytes, $p_i^{100\%}(T)$, can be modeled using Antoine's equation (54):

$$\log_{10} [p_i^{100\%}(T)] = A_i - B_i / [T + C_i] \quad \text{Equation 5}$$

where T is the temperature of the solution and A_i , B_i , and C_i are the Antoine's equation coefficients for the i^{th} component of a mixture. It should be noted that Antoine's coefficients are tabulated for many substances, including residual solvents, and can be found in the literature (55–56).

By substituting Equation 5 into Equation 4, it is possible to derive the equation that is used for estimating the total vapor pressure of volatile components of any given sample mixture and at any given headspace temperature:

$$p_{\text{total}}^{\text{gas}}(T) = \sum \left[10^{(A_i - \frac{B_i}{T+C_i})} \bullet \gamma_i \bullet \mu_i^{\text{solution}} \right] \quad \text{Equation 6}$$

It should be noted that for dilute solutions, it is possible to introduce the direct proportionality coefficient that reflects the equilibrium distribution between the gas-phase and solution-phase molar fractions of a particular analyte, μ_i^{gas} and μ_i^{solution} , respectively. This coefficient, $K_i(T)$, is often called the partition coefficient (or, sometimes, the distribution coefficient) (53). The partition coefficient is temperature-dependent but constant at any given temperature. From comparison to Equation 4, it is obvious that this coefficient is reversely proportional to the vapor pressure of the corresponding analyte:

$$K_i(T) = \mu_i^{\text{solution}} / \mu_i^{\text{gas}} \propto 1 / [p_i^{100\%}(T) \bullet \gamma_i] \quad \text{Equation 7}$$

With the aid of partition coefficients, Equation 4 can be redefined to enable determination of the total molar concentration of all components in the gas phase from their corresponding molar concentrations in the solution phase:

$$\mu_{\text{total}}^{\text{gas}} = \sum_i \mu_i^{\text{gas}} = \sum_i \mu_i^{\text{solution}} / K_i(T) \quad \text{Equation 8}$$

It is evident from Equation 8 that volatile analytes, for example, most solvents from USP Residual Solvents Class 2—Mixture A RS and USP Residual Solvents Class 2—Mixture B RS, will have small values of K_i and, thus, much higher molar fractions in a gas phase compared to those in a liquid phase. In contrast, nonvolatile sample components, such as the drug substance and excipients, will have very large values of K_i (i.e., very low vapor pressures) and, therefore, will not contribute to the gas phase composition.

In this article, we consider all xylene isomers (*o*-xylene, *m*-xylene, *p*-xylene, and ethylbenzene) and the two 1,2-dichloroethene isomers (*cis* and *trans*) to be separate species, as they have distinct MRR spectra.

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