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Research Article

Optimization of the Water-Insoluble Procedures for *USP* General Chapter *Residual Solvents* <467>

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Abstract. The water-insoluble procedures in *US Pharmacopeia* (*USP*) General Chapter *Residual Solvents* <467>, which are based on *European Pharmacopoeia* procedures, were optimized and modified before their inclusion in the chapter to improve their scope, performance, and ruggedness. The optimized procedures use a static headspace introduction system with a gas chromatograph equipped with a flame ionization detector. This article describes some of the key changes made to the USP published procedures, including use of dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) as the solvent, addition of 5 mL of water and 1 mL of sample (dissolved in DMSO or DMF) to the headspace vial, use of a 3:1 GC split ratio, and use of new matrix-matched system suitability solutions. These procedures were verified with two different active pharmaceutical ingredients—hydroxyzine pamoate and prednisone. In the investigation, the more polar material (hydroxyzine pamoate) showed greater recoveries for the optimized procedures when prepared in DMSO. The less polar material (prednisone) typically had greater recoveries in DMF for the optimized procedures. During experimentation, insights into sample preparation, additional types of headspace instrumentation, solvent purity, and other parameters were also gained.

KEY WORDS: GC-FID; limits; method optimization; residual solvents; US Pharmacopeia.

INTRODUCTION

Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients or in the preparation of drug products (1). These solvents are commonly used in the production of drug products and their constituents. Because residual solvents have no therapeutic use, manufacturers must limit their presence in products.

In 1990, *US Pharmacopeia* (*USP*) added a chapter titled *Organic Volatile Impurities* <467> in *USP XXII, 3rd Supplement* (2). The chapter has been revised many times. The most recent revision included the adoption of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) classifications and evaluations (3) and some of the *European Pharmacopoeia* (*EP*) procedures (4,5), as well as a renaming of the chapter to *Residual Solvents* <467>. In General Chapter *Residual Solvents* <467>, the *US Pharmacopeia* provides the ICH-based acceptance limits for residual solvents that may be found in pharmaceuticals. These limits were adopted from ICH, which set them using historical toxicological data (3). Based on their toxicity level, residual solvents are divided into three classes—Class 1, Class 2, and Class 3 (Table I). The use of Class 1 materials should be avoided whenever possible, and the use of Class 2 materials should be limited to the extent possible. Because of their low toxic potential, Class 3 materials may be used more freely and will not be discussed here.

Residual Solvents <467> includes the provision to test only for solvents likely to be present, the inclusion of analytical procedures for identification and quantitation, and guidelines for acceptance limits. In addition, *USP* also states in its *General Notices* Section 5.60.20 that "all *USP* and *NF* articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph" (6). The Food and Drug Administration (FDA) also presented a draft guidance affirming that compendial drug products meet the residual solvents requirements in the new <467> (7). In the same document FDA recommends that new drug application and abbreviated new drug application applicants for noncompendial drug products control and limit residual solvents (7).

The *USP* Chapter includes the EP-based (4,5) testing procedures to assess whether the solvents being measured are within the prescribed limits. The procedures involve the use of static headspace and gas chromatography with flame ionization detection (GC-FID). GC-FID was the technology of choice because residual solvents are volatile and stable, and the technique has the appropriate sensitivity

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Table I. List of Class 1, 2, and 3 Residual Solvents

Classification	Components
Class 1	Benzene; carbon tetrachloride; 1,2-dichloroethane; 1,1-dichloroethene; 1,1,1-trichloroethane
Class 2	Acetonitrile; chlorobenzene; chloroform; cyclohexane; 1,2-dichloroethene; 1,2-dimethoxyethane; *N*,*N*-dimethylacetamide; *N*,*N*-dimethylformamide; 1,4-dioxane; 2-ethoxyethanol; ethylene glycol; formamide; hexane; methanol; 2-methoxyethanol; methylbutylketone; methylcyclohexane; methylene chloride; *N*-methylpyrrolidone; nitromethane; pyridine; sulfolane; tetrahydrofuran; tetralin; toluene; trichloroethylene; xylene (usually 60% *m*-xylene, 14% *p*-xylene, and 9% *o*-xylene with 17% ethyl benzene)
Class 3	Acetic acid, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, *tert*-butylmethyl ether, cumene, dimethyl sulfoxide, ethanol, ethyl acetate, ethyl ether, ethyl formate, formic acid, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethylketone, methylisobutylketone, 2-methyl-l-propanol, pentane, 1-pentanol, 1-propanol, 2-propanol, propyl acetate

(10^{-13} g/mL), a linear response range (up to seven orders of magnitude), and low noise (8). Because of their volatility and their generally high air–water partitioning (9), many residual solvents work well with static headspace injection. Less volatile sample components and matrices are not injected on the column, which results in less complex sample analyses, decreased instrument contamination, and increased GC column life.

In the *USP* approach based on *EP* procedures (5), the solubility of the sample under test is used to determine whether the section for water-soluble articles is used or if the section for water-insoluble articles is followed. For both sections, the sample preparation solvent(s) are described [water for the water-soluble articles section and dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) for the water-insoluble articles section]. Three procedures (A, B, and C) are provided for both water-soluble and water-insoluble substances (Table II). For Classes 1 and 2 residual solvents, Procedure A is both an identification and limit test, Procedure B is a confirmatory test, and Procedure C is a quantitative test. Procedures A and B complement each other because peaks that co-elute using Procedure A are resolved using Procedure B and vice versa. Depending on the compound, the GC and headspace parameters described in Procedure A or B can be used to perform quantitation as described in Procedure C.

The revised version of <467> includes slight modifications to the water-soluble articles (solvent = water) section, for example, the addition of a correction factor when examining 1,1,1-trichloroethane. The currently official water-soluble articles section of <467> can be found in the *First Supplement* to *USP 32–NF 27* (1) and is not presented here.

This article describes the results of the experiments designed to optimize parts of the <467> water-insoluble articles procedures before their official adoption in the *First Supplement* to *USP 32–NF 27* (1). Some of the key changes include solvent choice (DMSO and DMF), different headspace solvent ratios, GC split ratios, and new matrix-matched system suitability solutions. The procedures were tested using two different active pharmaceutical ingredients (APIs) on a GC-FID instrument using loop injection static headspace sample introduction. This article provides insights gained during experimentation, such as sample preparation suggestions, considerations for other types of headspace instrumentation, solvent purity, and other tips.

MATERIALS

Chemicals

USP Residual Solvents Mixture—Class 1 Reference Standard, USP Residual Solvents Class 2—Mixture A Reference Standard, USP Residual Solvent Class 1—Benzene Reference Standard, USP Residual Solvent Class 2—Acetonitrile Reference Standard (United States Pharmacopeia, Rockville, Maryland, USA); organic-free water, HPLC grade (Fisher Scientific, Waltham, Massachusetts, USA); dimethyl sulfoxide, 99.9% for spectroscopy (Acros, Waltham, Massachusetts, USA); 1,3-dimethyl-2-imidazolidinone >98% (To yo Kasei Kogyo Co., Ltd, Osaka, Japan); dimethylformamide, biotech grade (Burdick and Jackson, Morristown, New Jersey, USA); helium, Ultrahigh Purity Carrier Grade, (Roberts Oxygen, Rockville, Maryland, USA); nitrogen, Nitrogen Generator Model UHPN2-1100, Ultrahigh purity grade (Parker Balston, Haverhill, Massachusetts, USA); air, Zero Air Generator Model 76-803, Ultra high purity (Whatman, Kent, UK); hydrogen, Hydrogen Generator Model 75-34 (Whatman, Kent, UK); and prednisone >98% grade and hydroxyzine pamoate (Sigma, St. Louis, Missouri, USA).

Equipment and Materials

Gas Chromatograph 6890N equipped with Flame Ionization Detector and ChemStation data processing software Revision A.09.03 [1417], 7694 Headspace sampler (loop size 1 mL), and 4-mm split, taper, low pressure drop, glass wool, GC liner (all from Agilent, Santa Clara, California, USA); CombiPAL Autosampler (CTC Analytics, Zwingen, Switzerland); CP-3800 Gas Chromatograph equipped with Flame Ionization Detector and Saturn GC/MS Workstation System with MS workstation upgrade, Varian MS, V.6.6 (service pack 1) software, 4-mm split/splitless, no glass wool, GC inlet liner (all from Varian, Palo Alto, California, USA); Zero Air Generator Model 76-803 and Hydrogen Generator Model 75-34 (both from Whatman, Kent, UK); Nitrogen Generator Model UHPN2-1100 (Parker Balston, Haverhill, Massachusetts); Rtx-1301/Rtx-G43, 30 m×0.53 mm, 3-µm film thickness fused silica capillary GC column (Procedure A; Restek, Bellefonte, Pennsylvania, USA); DB-Wax capillary GC column, 30 m×0.32 mm, 0.25-µm film thickness (Procedure B; Agilent, Santa Clara, California, USA). See Tables II, III, and IV. (Note: USP is

Table II. Gas Chromatography with Flame Ionization Detection and Headspace Solvent Parameters for *USP* General Chapter *Residual Solvents* <467>: Water-Insoluble and Water-Soluble Articles

Attributes	Procedure A	Procedure B
Column packing	G43 Crossbond 6% cyanopropylphenyl-94% dimethylpolysiloxane	G16 Crossbond Carbowax-PEG
Column size	30 m×0.53 mm, 3-µm film thickness	30 m×0.32 mm, 0.25-µm film thickness
Run time	60 min	60 min
Carrier gas (helium) linear velocity	35 cm/s	35 cm/s
Split ratio	3:1 (loop injection system) 4:1 (syringe system)	3:1 (loop injection system)
Initial GC oven temperature	40°	50°
Initial hold time	20 min	20 min
Ramp rate	10°/min	6°/min
Final GC oven temperature	240°	165°
Final hold time	20 min	20 min
Detector	Flame ionization detector	Flame ionization detector
Detector temperature	250°	250°
Injection mode	Split	Split
Injector temperature	140°	140°
Headspace solvent ratio for water-soluble articles	1 mL of solution in water and 5 mL of water	1 mL of solution in water and 5 mL of water
Headspace solvent ratio for water-insoluble articles	1 mL of solution in DMF or DMSO and 5 mL of water	1 mL of solution in DMF or DMSO and 5 mL of water

For Procedure C, either the parameters listed in Procedure A or the ones listed in Procedure B may be used. *GC* indicates gas chromatography, *DMF* diethylformamide, *DMSO* diethyl sulfoxide

certified to ISO 9001 and ISO 17025, and all equipment used in this study was qualified by an approved service engineer and met system suitability requirements.)

METHODS

Optimization and Modifications of Solvent Choice (DMSO or DMF) and Headspace Solvent Ratios

To test the procedures presented in *Pharmacopeial Forum* (*PF*) 32(2) <467> (10), we prepared a solution (Class 1 mixture solution) containing all of the Class 1 components in Table I by adding 1 mL of the USP Residual Solvents Mixture Class 1 Reference Standard (Class 1 RS) and diluting it with either water, DMF, or 1,3-dimethyl-2-imidazolidinone (DMI) to the limits provided in Table V. After preparation, 1 mL of the limit concentration solution and 5 mL of water, DMF, or DMI was added to a 10-mL headspace vial. The headspace parameters and Procedure A GC parameters presented in Tables II and III (with the exception of GC split ratio of 5:1) were used to analyze the solutions.

Table III. Static Headspace Parameters for Both Procedures A and B in <467> for Water-Insoluble Articles Using a Loop Injection System Headspace Instrument

Attributes	Values
Equilibration temperature	80°
Equilibration time	45, 60 min
Loop temperature	90°
Transfer line temperature	105°
Pressurization time	0.5 min
Injection volume	1.0 mL
Loop fill time	0.1 min
Loop equilibration time	0.05 min
Injection time	1.0 min
Vial pressure	10 psi
Headspace vial size	10 mL

In addition to the headspace equilibration temperature described in Table III, we tested eight different headspace equilibration temperatures ranging from 80° to 140°—in accordance with USP *General Notices*, all temperatures are given in degrees centigrade—with a Class 1 mixture solution prepared with DMF. One milliliter of the DMF Class 1 mixture solution was added to a headspace vial containing 5 mL of DMF and was injected. All other headspace and GC procedures were as described in the preceding paragraph. During the course of this experiment, a second more highly concentrated Class 1 mixture solution (1,000 times the limit concentrations of Class 1 components given in Table V) was prepared using DMF as the diluting solvent, and then 1 mL of this highly concentrated solution was added to 5 mL of DMF in a 10-mL headspace vial.

In order to optimize peak responses, we prepared a Class 1 mixture solution (five times the limit concentration of the Class 1 component peaks given in Table V) in DMF. One milliliter of this solution was added to a 10-mL headspace vial that contained 5 mL of water and DMF in varying proportions. The GC parameters and headspace parameters were as described in the preceding paragraph with the exception of the headspace equilibration temperatures, which varied (80°, 90°, or 105°; Fig. 1).

After optimizing the headspace solvent ratio, we also tested an alternative solvent, DMSO. A Class 1 mixture solution at the limit concentrations (Table V) was prepared in

Table IV. Static Headspace Parameters for Both Procedures A and B in <467> for Water-Insoluble Articles Using a Syringe Injection System Headspace Instrument

Attributes	Values
Injection volume	1 mL
Sample incubation time	45 min
Headspace vial size	10 mL
Syringe size	2.5 mL heated
Syringe temperature	85°
Agitator temperature (headspace oven)	80°
Agitator speed	500 rpm
Agitator cycle	4 s on, 0 s off
Plunger fill speed	100 µL/s
Fill stroke	0
Viscosity delay	1.0 s
Pre-injection delay	0.5 s
Plunger injection speed	150 µL/s
Post-injection delay	0.5 s
Syringe flush time	30 s
Gas chromatography cycle time (for prep ahead)	59 min 0 s

DMSO, and then 1 mL of this solution was added to 5 mL of water in a 10-mL headspace vial. The headspace and Procedure A GC parameters are shown in Tables II and III. The only exception is the GC split ratio of 5:1.

Optimization and Modification of GC Split Ratios

A Class 1 mixture solution (limit concentrations of components) in DMF was prepared. One milliliter of this solution was added to 5 mL of water in a 10-mL headspace vial. Several headspace vials were prepared and injected using different split ratios (Table VI). All other headspace and Procedure A GC parameters are as found in Tables II and III.

Using the same sample preparation as above, we investigated the GC parameters from Procedure B (Table II) and the headspace parameters presented in Table III.

Modifications of Matrix-Matched System Suitability Solutions

To correct the inconsistency in test material and system suitability solvents, we diluted 1 mL of the Class 1 RS in DMF or DMSO to the limit concentrations of the class 1 components shown in Table V. Then 1 mL of the limit concentration solution and 5 mL of water were added to the headspace vial (Figs. 2 and 3). Besides the Class 1 RS, a Class 2A mixture solution was also prepared. One milliliter of a

Table V. Limit Concentrations of Selected Residual Solvents

Solvent	Class	Concentration limit (ppm)
Benzene	1	2
Carbon tetrachloride	1	4
1,2-dichloroethane	1	5
1,1-dichloroethene	1	8
1,1,1-trichloroethane	1	1,500
Acetonitrile	2	410

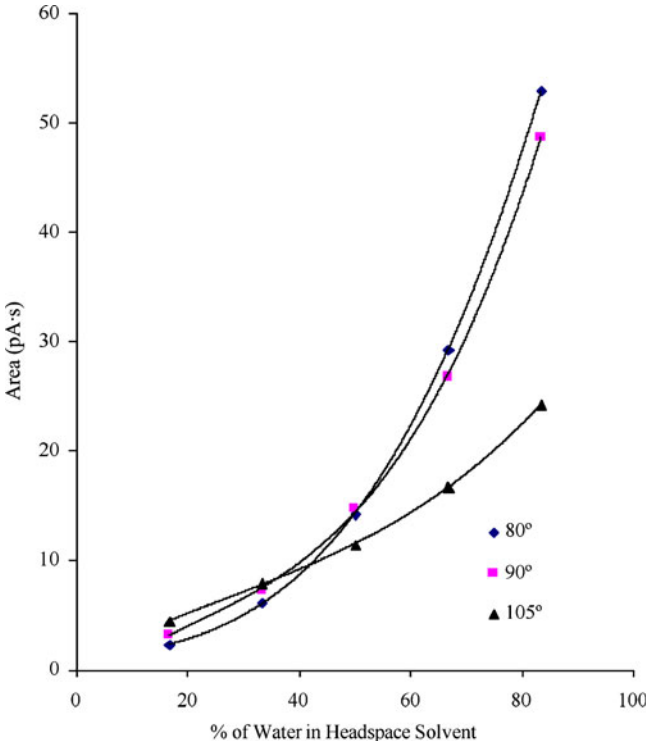

Fig. 1. Effect of headspace equilibration temperature and percent water in the headspace solvent on the peak response of 1,1,1-trichloroethane

USP Residual Solvents Class 2 Mixture A Reference Standard (Class 2A RS) was diluted with DMF or DMSO to the limit concentrations provided in <467> (1). One milliliter of the limit concentration solution and 5 mL of water were added to the headspace vial. The headspace parameters and Procedure A and B GC parameters shown in Tables II and III were used.

To ensure the performance of the optimized procedure, we prepared a solution composed of hydroxyzine pamoate (sample under test) and an intermediate dilution of the Class 1 mixture solution [Class 1 suitability solution (1)] in DMF. A 1-mL aliquot of this solution was added to a headspace vial containing 5 mL of water (Fig. 4). Procedure A GC parameters were utilized (Table III).

Verification of Procedures

Hydroxyzine pamoate and prednisone were selected as representative polar and nonpolar APIs, respectively. These APIs were studied in both DMF and DMSO. A spiked solution (API present) was prepared at the limit concentrations of the Class 1 residual solvents (Table V) in each solvent. This was referred to as the 100% spiked solution. A Class 1 mixture solution (API absent) was prepared with the corresponding solvent, and the area values were compared to the areas of the 100% spiked solution.

Insights Gained during Experimentation

Using DMF as a solvent, we prepared four separate Class 1 mixture solutions in six 10-mL headspace vials (each contained 1 mL of Class 1 mixture solution and 5 mL of

Table VI. Effect of Split Flow Ratios on Class 1 Mixture Residual Solvent Peaks Using Procedure A Gas Chromatography Parameters

	Peak areas				
Split flow	1,1 dichloroethene (pA s)	1,1,1 trichloroethane (pA s)	Carbon tetrachloride (pA s)	Benzene (pA s)	1,2 dichloroethane (pA s)
5:1	49.9	58.8	7.0	36.7	11.2
3:1	70.5	81.6	9.7	50.1	15.5
2:1	80.7	98.8	11.5	62.7	19.6

A 3:1 split ratio was chosen because of difficulty in meeting resolution requirements with a 2:1 split ratio

water). All six vials of each preparation were injected, for 24 injections total. We performed two separate trials of four sample preparations with six injections each for Procedure A (Table II) and performed only one trial for Procedure B (Table II). Headspace parameters were as shown in Table III.

Syringe Headspace. A Class 1 mixture solution and a Class 2A mixture solution were prepared in DMF and DMSO. One milliliter of the limit concentration solution and 5 mL of water were added to the headspace vial and injected using Procedure A GC parameters (Table II) and a syringe headspace instrument (Table IV).

RESULTS AND DISCUSSION

Optimization and Modifications of Solvent Choice (DMSO or DMF) and Headspace Solvent Ratios

In *PF* 32(2) <467> Procedures A and B under water-insoluble articles included test solutions prepared using DMF or DMI and standards prepared using water (10). The solvent in the headspace vial for the test solutions consisted exclusively of DMF or DMI for the water-insoluble materials, and water was the solvent in the headspace vial for the

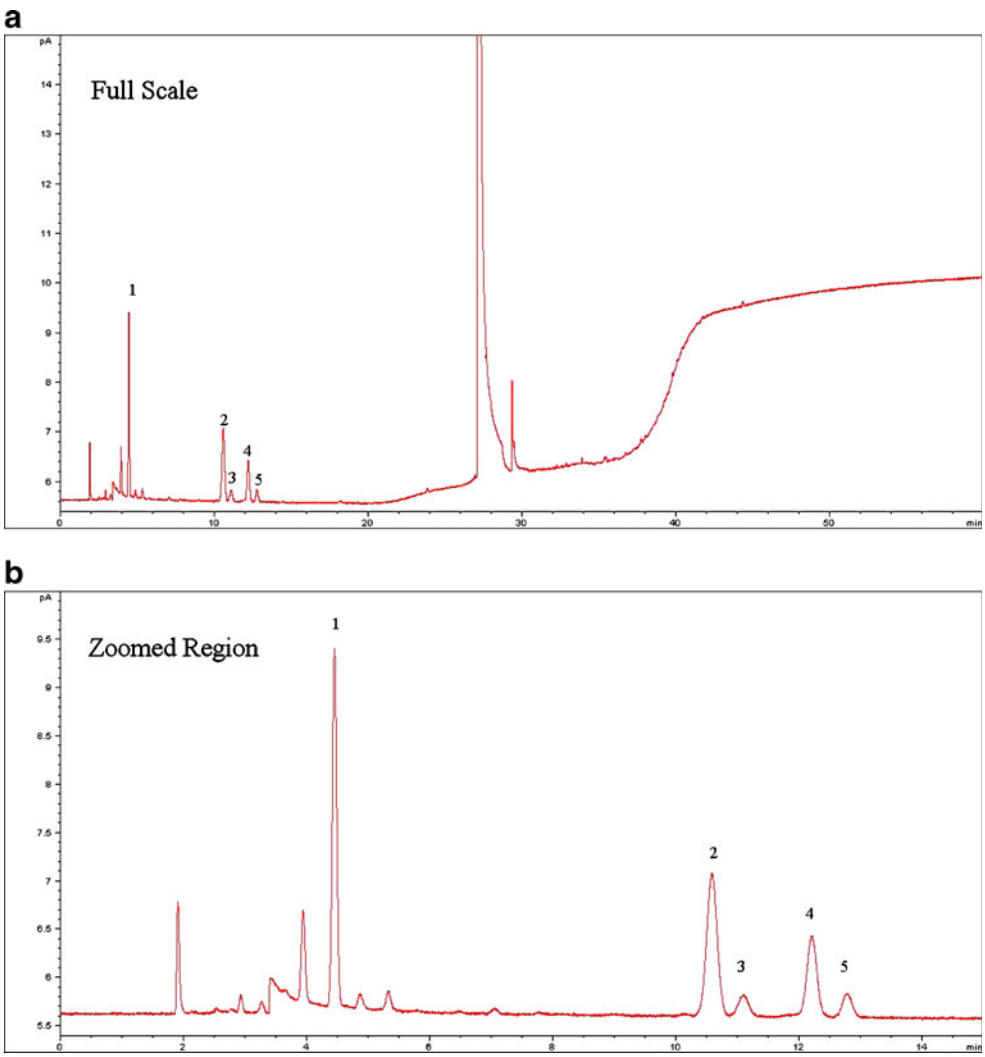

Fig. 2. Class 1 mixture solution in DMF using Procedure A: *1* 1,1-dichloroethene; *2* 1,1,1-trichloroethane; *3* carbon tetrachloride; *4* benzene; *5* 1,2-dichloroethane

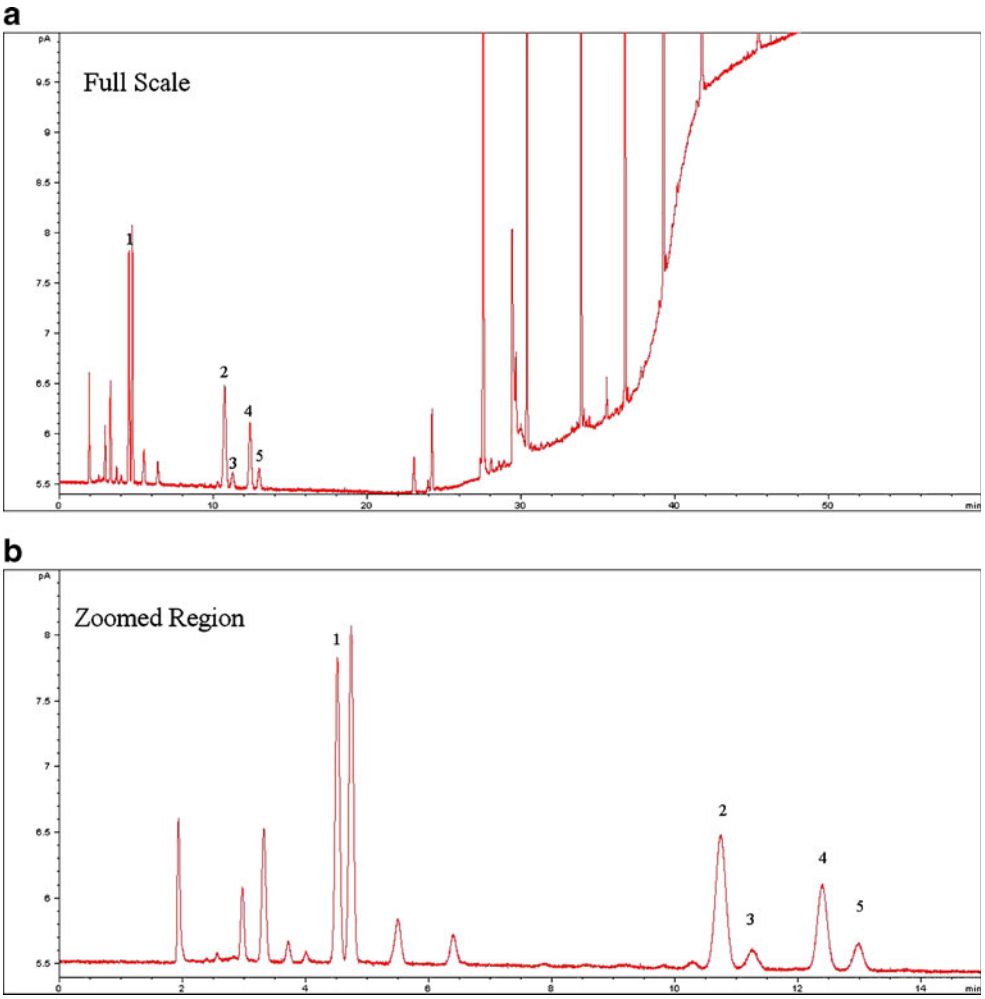

Fig. 3. Class 1 mixture solution in DMSO using Procedure A: *1* 1,1-dichloroethene; *2* 1,1,1-trichloroethane; *3* carbon tetrachloride; *4* benzene; *5* 1,2-dichloroethane

standards. The peak responses of samples containing the Class 1 RS prepared in three solvents—water, DMF, and DMI—were compared. All five component peaks (1,1,1-trichloroethane, benzene, 1,1-dichloroethene, 1,2-dichloroethane, and carbon tetrachloride) of the Class 1 mixture solution prepared in water were observed with signal-to-noise ratios greater than 3. However, none of the Class 1 component peaks were observed in the samples using either DMF or DMI as the solvent. Possibly when DMF or DMI was used as a solvent, the Class 1 components did not

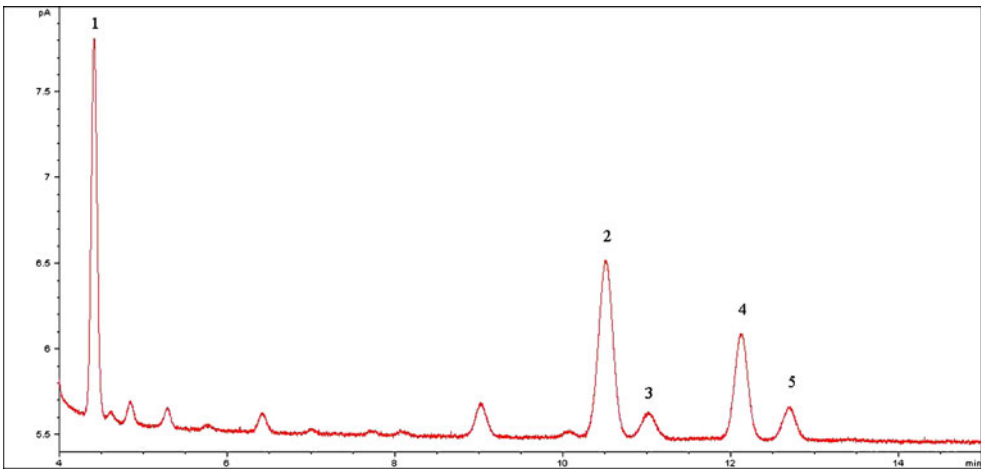

Fig. 4. Class 1 system suitability solution using DMF as a solvent, hydroxyzine pamoate as the test material, and Procedure A GC parameters: *1* 1,1-dichloroethene; *2* 1,1,1-trichloroethane; *3* carbon tetrachloride; *4* benzene; *5* 1,2-dichloroethane

volatilize under the headspace conditions presented in *PF* 32 (2) (10).

In order to volatilize the samples, we tested eight different headspace equilibration temperatures ranging from 80° to 140° with a Class 1 mixture standard solution prepared with DMF. Regardless of headspace temperature, all the injections did not show component peaks from the Class 1 mixture solution. A highly concentrated Class 1 mixture standard solution (1,000 times the limit concentration) was prepared using DMF. This sample was tested to see if the lack of response was a matter of sensitivity. Each residual solvent component of the Class 1 mixture solution yielded a measurable peak area that was at least double when the headspace equilibration temperature was 140° compared to results obtained at 105°. The Class 1 residual solvents are soluble in DMF, so they tend to reside in the solution instead of the headspace.

To increase the amount of Class 1 residual solvents in the headspace, we increased the amount of water in the headspace solution because these solvents are highly insoluble in water. One-milliliter portions of a Class 1 mixture solution prepared in DMF were added to 10-mL headspace vials containing 5-mL solutions consisting of DMF and 17% to 83% water at headspace equilibration temperatures of 80°, 90°, or 105°. All the Class 1 residual solvent peaks (Table I) showed the same trend. The response decreased as the equilibration temperature increased, and the peak response increased as the percent water increased. Figure 1 shows these findings for 1,1,1-trichloroethane. Thus, the ratio of 5 mL of water to 1 mL of DMF in the headspace solvent provided the greatest peak responses for the Class 1 residual solvents.

Because DMF does not dissolve all compounds and is also a residual solvent, an alternative solvent is required for water-insoluble materials. DMSO was chosen as the alternative solvent because of its dissolving capabilities and lower boiling point compared to DMI. The responses were similar for the Class 1 mixture standard solution prepared in DMSO compared to one prepared in DMF, for which 1 mL of solution was added to 5 mL of water in the 10-mL headspace vial. However, additional peaks were observed in the DMSO-prepared solution chromatograms and were attributable to the neat DMSO.

Optimization and Modification of GC Split Ratios

In *PF* 32(2) the recommended GC split ratio was 5:1 (10). However, under these conditions a few of the Class 1 component peaks such as carbon tetrachloride and 1,2-dichloroethane are relatively small, so 2:1 and 3:1 split ratios were investigated in an effort to maximize the peak heights and areas. A Class 1 mixture solution was injected using different split ratios (Table VI) and Procedure A GC parameters (Table II). A 2:1 split ratio provided the largest peak responses, but we encountered difficulty in meeting resolution requirements (resolution between acetonitrile and methylene chloride is not less than 1 for Procedure A)

Table VII. Recovery Determination Using Hydroxyzine Pamoate Dissolved in Dimethyl Sulfoxide and Dimethylformamide with Procedure A Parameters

	1,1 dichloroethene area (pA s)	1,1,1 trichloroethane area (pA s)	Carbon tetrachloride area (pA s)	Benzene area (pA s)	1,2 dichloroethane area (pA s)
Hydroxyzine pamoate dissolved in DMSO					
100% spiked solution					
Global average	13.8	14.3	2.2	8.7	2.6
SD	0.9	0.6	0.1	0.3	0.1
RSD	6.3	4.4	2.9	3.7	3.8
Class 1 mixture standard solution					
Global average	13.9	14.8	2.0	8.7	2.6
SD	1.1	0.5	0.0	0.3	0.0
RSD	8.1	3.1	1.8	3.2	1.3
Recovery (%)	99.4	96.4	109.7	99.9	100.4
Propagated RSD[a]	10.2	5.4	3.4	4.9	4.0
Propagated SD[b]	10.2	5.2	3.8	4.9	4.0
95% CI[c]	14.1	7.2	5.2	6.8	5.5
Hydroxyzine pamoate dissolved in DMF					
100% spiked solution					
Global average	12.9	15.8	2.7	9.0	2.6
SD	0.1	1.1	0.1	0.5	0.2
RSD	0.9	6.8	3.8	5.9	7.8
Class 1 mixture standard solution					
Global average	16.7	19.0	2.7	10.7	3.2
SD	1.0	2.6	0.4	1.5	0.5
RSD	6.1	13.7	16.1	13.8	14.3
Recovery (%)	77.2	83.1	97.9	83.8	81.0
Propagated RSD[a]	6.1	15.3	16.6	15.0	16.3
Propagated SD[b]	4.7	12.7	16.2	12.6	13.2
95% CI[c]	6.6	17.6	22.5	17.4	18.3

[a] Propagated relative standard deviation (RSD)=square root [(RSD of preparations)^2 +(RSD of standards)^2]
[b] Propagated standard deviation (SD)=(recovery%×propagated RSD)/100
[c] 95% confidence interval (CI)
DMSO dimethyl sulfoxide, *DMF* diethylformamide

(results and solutions not discussed here). A 3:1 split ratio provided the best compromise between peak sensitivity and resolution. For example, carbon tetrachloride in the Class 1 mixture solution was not always detected at a 5:1 split ratio, but a split ratio of 3:1 provided increased sensitivity (signal-to-noise ratio greater than 3).

The Procedure B GC parameters and the headspace parameters presented in Tables II and III were tested using a Class 1 mixture solution prepared in DMF. Class 1 mixture solutions were simultaneously run using Procedures A and B (Table II) under the same conditions. Each of the components in the Class 1 mixture solution that did not co-elute had approximately the same average peak area with both procedures. For example, 1,1-dichloroethene had an average area response of 14.2 pA s with Procedure A and 14.5 pA s with Procedure B. DMSO was also tested as a possible solvent with Procedures A and B. The peak responses for the Class 1 mixture solution were similar to those obtained when DMF was used as the solvent.

Modifications of Matrix-Matched System Suitability Solutions

In *PF* 32(2), the standard solutions for the water-insoluble articles for Procedures A and B were prepared in DMSO and water, but the test solutions were prepared in either DMF or DMI (10). In Procedure A (Table II), the user is directed to compare the peak responses from the standards and test solutions. If a peak found in the test solution elutes at the locus of a residual solvent peak, the peak areas of the standard are compared to the peak areas of the test solution. If the peak in the test solution is smaller than the peak in the standard solution, no further testing is required. Based on the solvent inconsistency between the standards and test solutions, a comparison between the component peaks in the different solutions may lead to an erroneous answer. To correct the inconsistency in solvents, we prepared the standard solutions in DMF or DMSO, instead of water and DMSO, but followed the same dilution scheme presented in the water-soluble articles section of <467> (1). One milliliter of the limit concentration solution (in DMF or DMSO) and 5 mL of water were added to the headspace vial. The samples were then injected, and chromatograms were obtained (Figs. 2 and 3).

With these changes, the system suitability criteria for water-soluble articles could be used for water-insoluble articles (10). These criteria for Procedure A are: the resolution between acetonitrile and methylene chloride is not less than 1; the signal-to-noise ratio for 1,1,1-trichloroethane is not less than 5; and the signal-to-noise ratio of each Class 1 peak in a system suitability solution is not less than 3.

In addition to testing Procedure A methodology, we also tested the Procedure B methodology (Table II) using the same standard solutions prepared in DMF or DMSO. The Procedure B system suitability requirements as stated in *PF* 32(2)(10)—resolution between acetonitrile and trichloroethylene is not less than 1, the signal-to-noise ratio for benzene is not less than 5, and the signal-to-noise ratio of each Class 1 component in a system

Table VIII. Recovery Determination Using Hydroxyzine Pamoate Dissolved in Dimethyl Sulfoxide and Dimethylformamide with Procedure B Parameters

	1,1 dichloroethene area (pA s)	1,1,1 trichloroethane/carbon tetrachloride area (pA s)	Benzene area (pA s)	1,2 dichloroethane area (pA s)
Hydroxyzine pamoate dissolved in dimethyl sulfoxide				
100% spiked solution				
Global average	9.7	13.3	9.2	2.5
SD	1.4	1.0	0.6	0.2
RSD	14.0	7.4	6.7	7.1
Class 1 mixture standard solution				
Global average	9.5	15.1	8.5	2.6
SD	0.7	0.6	0.3	0.1
RSD	7.2	3.6	3.2	2.4
Recovery (%)	102.1	88.3	108.1	94.4
Propagated RSD[a]	15.8	8.3	7.5	7.5
Propagated SD[b]	16.1	7.3	8.1	7.0
95% CI[c]	22.3	10.1	11.2	9.7
Hydroxyzine pamoate dissolved in dimethylformamide				
100% spiked solution				
Global average	7.7	7.5	7.5	2.5
SD	0.4	0.8	0.6	0.2
RSD	4.7	10.1	7.4	8.4
Class 1 mixture standard solution				
Global average	9.2	9.3	8.6	2.8
SD	0.8	2.2	1.9	0.6
RSD	8.4	23.2	22.2	22.3
Recovery (%)	83.3	80.8	87.2	89.4
Propagated RSD[a]	9.6	25.4	23.4	23.8
Propagated SD[b]	8.0	20.5	20.4	21.3
95% CI[c]	11.1	28.4	28.3	29.6

[a] Propagated relative standard deviation (RSD)=square root [(RSD of preps)^2 +(RSD of standards)^2]
[b] Propagated standard deviation (SD)=(recovery% ×propagated RSD)/100
[c] 95% confidence interval (CI)

suitability solution is not less than 3—were met using the standard solutions (Class 1 suitability solution was not tested here) prepared in either DMF or DMSO. (In the *First Supplement* to *USP 32–NF 27* the resolution requirement was changed to a resolution between acetonitrile and *cis*-dichloroethene of not less than 1.)

To ensure the performance of the procedure, we prepared a solution composed of a hydroxyzine pamoate test solution and an intermediate dilution of the Class 1 mixture solution [Class 1 suitability solution (1)] in DMF. A 1-mL aliquot of this solution was added to a headspace vial containing 5 mL of water. A representative chromatogram of this Class 1 suitability solution is shown in Fig. 4. All five components of the Class 1 suitability solution (Table I) had signal-to-noise ratios greater than 3, which met the requirement in the Chapter. This Class 1 suitability solution takes into account matrix effects caused by the sample under test and ensures the intended system is sufficiently sensitive to detect the residual solvents. The requirement for the Class 1 suitability solution is that all the signal-to-noise ratios of the component peaks must be greater than 3.

Verification of Procedures

In this set of experiments two preparations of polar (hydroxyzine pamoate) and nonpolar (prednisone) APIs dissolved in DMF or DMSO were used to verify Procedures A and B for water-insoluble articles. These APIs were studied in both DMF and DMSO. A 100% spiked solution (API present) and a Class 1 mixture solution (API absent) were prepared with the corresponding solvent, and the area values were compared. From the area data, a percent recovery was calculated as follows:

$$\text{Recovery}\,(\%) = \frac{\text{global average area of 100\% spiked solution}}{\text{global average area of Class 1 mixture solution}}$$

Using Procedure A parameters with DMSO as the solvent and hydroxyzine pamoate as the API, we found that the recoveries of each Class 1 component ranged from 96% to 110% for the 100% spiked solution (API present) *vs* the Class 1 mixture solution (API absent; Table VII). The same combination of DMSO and hydroxyzine pamoate with Procedure B parameters yielded Class 1 component recoveries ranging from 88% to 108% (Table VIII). For hydroxyzine pamoate dissolved in DMF and spiked at the 100% level, the Class 1 component recoveries were low, ranging from 77% to 98% for Procedure A and 81% to 89% for Procedure B (Tables VII and VIII).

When prednisone was dissolved in DMSO and examined using Procedure A, Class 1 component recoveries for the 100% spiked solution *vs* the Class 1 mixture solution ranged from 92% to 96% except for 1,1-dichloroethene (Table IX), which yielded a recovery of 76%. With Procedure B parameters, prednisone dissolved in DMSO showed Class 1 component recoveries ranging from 77% to 94% (Table X). Conversely, for similar experiments with prednisone dissolved in DMF, the Class 1

Table IX. Recovery Determination Using Prednisone Dissolved in Dimethyl Sulfoxide and Dimethylformamide with Procedure A Parameters

	1,1 dichloroethene area (pA s)	1,1,1 trichloroethane area (pA s)	Carbon tetrachloride area (pA s)	Benzene area (pA s)	1,2 dichloroethane area (pA s)
Prednisone dissolved in dimethyl sulfoxide					
100% spiked solution					
Global average	12.1	16.2	2.4	9.7	2.9
SD	0.7	0.3	0.0	0.1	0.0
RSD	5.4	1.8	0.6	0.6	0.2
Class 1 mixture standard solution					
Global average	15.9	17.6	2.5	10.4	3.1
SD	2.5	2.4	0.5	1.3	0.4
RSD	15.4	13.9	19.2	13.0	12.6
Recovery (%)	75.8	92.2	96.3	93.5	93.2
Propagated RSD[a]	16.3	14.0	19.2	13.0	12.6
Propagated SD[b]	12.4	12.9	18.5	12.1	11.7
95% CI[c]	17.1	17.9	25.7	16.8	16.2
Prednisone dissolved in dimethylformamide					
100% spiked solution					
Global average	13.7	14.6	2.4	8.1	2.5
SD	0.9	0.8	0.1	0.4	0.0
RSD	6.6	5.4	2.1	5.2	1.2
Class 1 mixture standard solution					
Global average	14.8	14.6	2.3	8.2	2.6
SD	0.6	0.3	0.0	0.1	0.1
RSD	4.2	2.1	0.1	1.3	5.6
Recovery (%)	93.2	99.8	103.8	99.1	98.3
Propagated RSD[a]	7.8	5.8	2.1	5.3	5.8
Propagated SD[b]	7.3	5.8	2.2	5.3	5.7
95% CI[c]	10.1	8.0	3.1	7.3	7.8

[a] Propagated relative standard deviation (RSD)=square root [(RSD of preps)^2 +(RSD of standards)^2]
[b] Propagated standard deviation (SD)=(recovery% × propagated RSD)/100
[c] 95% confidence interval (CI)

Table X. Recovery Determination Using Prednisone Dissolved in Dimethyl Sulfoxide and Dimethylformamide with Procedure B Parameters

	1,1 dichloroethene area (pA s)	1,1,1 trichloroethane/carbon tetrachloride area (pA s)	Benzene area (pA s)	1,2 dichloroethane area (pA s)
Prednisone pamoate dissolved in dimethyl sulfoxide				
100% spiked solution				
Global average	15.8	12.1	8.4	2.5
SD	0.8	0.4	0.2	0.1
RSD	5.0	3.4	2.3	4.7
Class 1 mixture standard solution				
Global average	20.6	14.1	10.5	2.6
SD	3.9	2.2	0.4	0.3
RSD	19.1	15.4	4.2	10.2
Recovery (%)	76.6	85.8	80.8	93.9
Propagated RSD[a]	19.7	15.8	4.8	11.2
Propagated SD[b]	15.1	13.6	3.8	10.5
95% CI[c]	20.9	18.8	5.3	14.6
Prednisone dissolved in dimethylformamide				
100% spiked solution				
Global average	10.2	12.1	5.6	2.0
SD	1.3	1.2	0.1	0.1
RSD	12.4	9.6	1.7	3.0
Class 1 mixture standard solution				
Global average	12.4	13.2	6.7	2.6
SD	0.6	0.2	0.2	0.1
RSD	4.6	1.5	3.4	5.6
Recovery (%)	82.6	91.3	83.9	78.9
Propagated RSD[a]	13.2	9.7	3.8	6.3
Propagated SD[b]	10.9	8.9	3.2	5.0
95% CI[c]	15.1	12.3	4.4	6.9

[a] Propagated relative standard deviation (RSD)=square root [(RSD of preps)^2 +(RSD of standards)^2]
[b] Propagated standard deviation (SD)=(recovery% ×propagated RSD)/100
[c] 95% confidence interval (CI)

component recoveries ranged from 93% to 104% for Procedure A and 79% to 91% for Procedure B (Tables IX and X).

Based on the recovery data, the more polar API (hydroxyzine pamoate) seemed to have higher Class 1 component recoveries in both Procedures A and B when the API was dissolved in DMSO. In contrast, the less polar compound (prednisone), in general, had higher Class 1 component recoveries in DMF for both Procedures A and B. This observation illustrates the importance of the solvent used to dissolve the sample and may provide a guide regarding which solvent should be used to dissolve a polar water-insoluble material *vs* a less polar material.

Insights Gained during Experimentation

Several replicate injections of a Class 1 mixture solution prepared using DMF as the solvent and both Procedures A and B were made (see METHODS section for sample details). The results for Procedure A are shown in Table XI. With Procedure A, the overall RSD for all of the components ranged from 3.5% to 20.0%, and for Procedure B the range was 1.9% to 7.7% (results not shown). A rise in the RSD was often observed with increasing numbers of preparations. In this case, the RSD of the six injections from the fourth preparation showed the greatest amount of variance. The RSDs for the first three preparations (six injections per preparation) were typically 15% or below. One possible explanation was the buildup of DMF in the headspace lines

Table XI. Precision of Components of the Class 1 Mixture Standard Solution for Procedure A for Two Separate Trials of Four Preparations with Six Injections Each

1,1 dichloroethene	Overall range
Average area	9.76–14.37 pA s
Standard deviation	0.64–2.00
RSD	5.40–20.03
Carbon tetrachloride	Overall range
Average area	1.40–2.09 pA s
Standard deviation	0.13–0.33
RSD	6.44–17.01
1,2 dichloroethane	Overall range
Average area	1.83–2.94 pA s
Standard deviation	0.10–0.37
RSD	4.91–17.71
1,1,1 trichloroethane	Overall range
Average area	9.58–15.02 pA s
Standard deviation	0.50–2.10
RSD	3.48–17.39
Benzene	Overall range
Average area	5.53–7.78 pA s
Standard deviation	0.26–1.10
RSD	3.46–15.90

RSD relative standard deviation

because the temperatures are well below the boiling point of DMF (153°). To remedy this problem, baking out the transfer line, loop, and oven before each experiment is recommended (1).

During the course of testing we gained insights into sample preparation that helped improve results (i.e., peak responses). During sample dilutions, to allow the system to come to equilibrium we allowed sample flasks to sit a minute or two before we took an aliquot for the next dilution. We also observed that different grades/brands of solvents used to prepare solutions had different components or impurities, which could complicate the chromatograms. HPLC-grade DMF was found to give the fewest extraneous peaks. For this work we used a brand of DMSO that had less than 0.001% residue on evaporation.

Syringe Headspace

Data were obtained not only on a loop injection headspace instrument but also on a syringe headspace instrument. A Class 1 mixture solution prepared in DMF and DMSO using Procedure A parameters was examined (Table IV). It met the system suitability requirement of a signal-to-noise ratio of not less than 5 for 1,1,1-trichloroethane using the Class 1 mixture solution. It also met the requirement of a resolution between acetonitrile and methylene chloride of not less than 1 using a Class 2 Mixture A solution. In addition, all of the Class 1 mixture solution component peaks had signal-to-noise ratios greater than 3. Also, two different agitator (headspace oven) temperatures were investigated in conjunction with syringe temperatures. In one case the agitator temperature was 105° and the syringe temperature was 110°, and in the other case the agitator temperature was 80° and the syringe temperature 85°. In both cases the Class 1 mixture solution system suitability requirement of a signal-to-noise ratio for 1,1,1-trichloroethane of not less than 5 could be met, but peak heights, areas, and signal-to-noise ratios were generally lower when the agitator temperature was 105° compared to an agitator temperature of 80°.

CONCLUSION

The optimized and modified water-insoluble procedure presented in *USP* General Chapter <467> appears to be sensitive, specific, and accurate for the detection and quantitation of residual solvents. Some of the key changes include using DMSO or DMF as the solvent, adding 5 mL of water and 1 mL of sample (dissolved in DMSO or DMF) to the headspace vial, using a 3:1 GC split ratio, and utilizing new matrix-matched system suitability solutions.

The optimized procedure was verified using two APIs—hydroxyzine pamoate and prednisone. In the investigation, the more polar material (hydroxyzine pamoate) showed greater recoveries for both Procedures A and B when prepared in DMSO. The less polar material (prednisone) typically had greater recoveries in DMF for both Procedures A and B.

These tests provided several insights. A periodic column bake out is recommended to maintain instrument performance. Sample preparation is a key factor in identifying residual solvents, and the grade/purity of solvent chosen can affect the results of the analysis. In addition, system suitability was met using a second style of headspace equipment.

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