Evaluation of a Headspace-GC Method for Residual Solvents in a Serotonin 5-HT3 Antagonist Compound

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Abstract

A static headspace gas chromatographic method is developed and evaluated for the quantitation of residual 2-propanol, methanol, and toluene in bulk (2α, 6α, 8α, 9α β)-octahydro-3-oxo-2,6-methanon-2H-quinolizin-8-yl-1H-indole-3-caboxylate methanesufonate hydrate, a serotonin 5-HT3 receptor antagonist drug compound. This method is accurate and precise, and it includes the use of 1-propanol as an internal standard. The gas chromatographic conditions utilize a dimethylpolysiloxane phase (SPB-1) capillary column and a flame ionization detector. Validation of this test method includes a recovery study of known levels of the three target analyte solvents to verify the accuracy of this method, because these solvents were used in the recrystallization and synthesis of all current and future lots of the bulk drug. The tested range is 0.05% to 1.0% (w/w) for 2-propanol and methanol and 0.01% to 0.10% (w/w) for toluene. Mean recovery of all spikes is 107% (w/w) of theory for methanol (*n* = 15) and 101% (w/w) for 2-propanol. Toluene mean recovery of all spikes is 98% (w/w) of theory within the tested range (*n* = 6). These data and other facets of the development of this headspace method are discussed.

Introduction

Residual solvents in pharmaceutical products have to be monitored by the manufacturer, and residual solvent analysis and testing has been extensively reviewed and discussed in the literature (1–5). Analysis of residual solvents has been described as one of the most challenging analytical and control tasks in the production of pharmaceuticals (6). Residual solvents are organic volatile impurities which can cause numerous problems in the production of pharmaceutical products. These residual volatiles are the remains from the processing agents during synthesis and production of the drug substance, or active pharmaceutical ingredient, which can be retained after incomplete drying. Also, excipients used in a pharmaceutical product may be a source of residual solvents. Residual solvents have had official limits in the United States as set in USP XXV (7) and by the FDA guidance in 1997 (8,9). This FDA guidance (8,9) lists acceptable amounts for specific residual solvents derived only for patient safety considerations. Other possible problems derived from high levels of residual solvents include color or odor changes in the finished pharmaceutical product, and the fact that residual organic solvents can play a role in the physicochemical properties of a bulk drug substance, including its crystalline structure. Variations in crystalline structure, including the possibility of inclusion of a solvent within the crystalline structure, can lead to changes in dissolution properties and create problems with the formulation of a finished drug product. This could lead to bioavailability or delivery problems for the active drug. Residual solvent testing and monitoring is a necessary final check for all bulk drug compounds and finished pharmaceutical products.

The drug compound (2α, 6α, 8α, 9α β)-octahydro-3-oxo-2,6-methanon-2H-quinolizin-8-yl-1H-indole-3-caboxylate methanesufonate hydrate is a serotonin (5-HT3) antagonist and has been under study for its pharmaceutical properties (10,11). This class of drugs has extensive potential in the treatment of cancer therapy-induced nausea (11). Headspace sampling coupled with gas chromatographic (GC) analysis is a fairly common analysis technique for bulk drug substance analysis (2,3,12) and has been widely reported in the literature (13–15). As the purity of any pharmaceutical, including the level of residual solvents, is important, the monitoring of bulk pharmaceutical product is commonly performed using headspace analysis.

There are two main types of headspace analysis techniques available: dynamic and static; both have been described in detail in the literature (1–5,16). Briefly, in dynamic headspace analysis, a continuous flow of gas is swept over the surface of a sample matrix. Volatiles from the sample are conveyed into a trap and are accumulated prior to introduction into the GC. A thermal desorption cycle of the trap is initiated, and a carrier gas takes the analytes into the GC. The trap is usually composed of a column containing a sorbent such as Tenex, Chromosorb, Porapak, or Amberlite XAD resins. Cold trapping followed by thermal vaporization is another variation of dynamic headspace analysis. In static headspace, no trap is used; a liquid or sometimes a solid sample is placed into a sealed vial. This vial is heated until a thermodynamic equilibrium between

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the sample and the gas phase in the vial is reached. A volume of the headspace gas is sampled and injected into the gas chromatograph for analysis. Although dynamic headspace sampling and multiple headspace extraction techniques have been reported in pharmaceutical testing (16,17), static headspace analysis has also been frequently used (1–4,12, 13,18,19). The serotonin antagonist drug substance in this study was known to be thermally unstable above 150°C; therefore, headspace sampling was the preferred analysis technique over direct injection of a drug substance solution into the GC to quantitate the residual solvents. Static headspace analysis was chosen for a number of reasons. Static headspace sampling generally has the advantage of ease of use, especially when using an automated sampler system. It also has the advantage of avoiding the possibility of any artifact peaks (2,3,12) which could produce interferences with analyte peaks, or at least in static headspace the artifact peaks are reduced to insignificant levels. In the developed GC-headspace procedure reported in this manuscript, an SPB-1 capillary column (dimethylpolysiloxane phase) was used for the separation of the known potential residual solvents used in the synthesis and recrystallization of the drug substance. The flame ionization detector (FID) was used for this procedure because of its advantage of high sensitivity with a wide linear range (20).

Residual 2-propanol was the most likely solvent to be encountered, because it was used as the final recrystallization solvent for the serotonin antagonist drug substance used in this study. Methanol and toluene were used in the last synthetic step of this drug. Because the 1997 FDA guidance (8) classifies solvents according to toxicity, the recovery levels chosen for method validation were consistent with the guidance. Although the standard addition technique has been stated as the most frequently used quantitation approach in residual solvent analysis, owing to sample matrix effects in headspace sampling (21,22), higher sample through-put, by not spiking numerous sample solutions while maintaining good accuracy, was desired for this procedure. A spiked recovery study of 2-propanol (Class 3) and methanol (Class 2) over a range of 0.05% to 1.0% (w/w) was used to verify the accuracy of this test method compared to an aqueous-only standard solution containing the equivalent of 0.5% or those two solvents. Toluene (Class 2) was studied in the range of 0.01% to 0.10% (w/w) against an aqueous-only standard solution containing the equivalent level of 0.1% toluene. These recovery data, as well as several of the other aspects about the evaluation of this test method, will be discussed in more depth.

Experimental

Chromatographic conditions and apparatus

The headspace sampling was conducted using a Tekmar Model 7000 HT headspace sampler (Teledyne-Tekmar, Mason, OH). The headspace sampling conditions are summarized in Table I. The chromatographic analysis was conducted using an Agilent Technologies model 5890 gas chromatograph (Palo Alto, CA) equipped with an FID and a Supelco SPB-1 capillary column (Bellefonte, PA). The optimized chromatographic conditions used for this test procedure are summarized in detail in Table I; the GC sampling interval was approximately 33 min. This included the 20 min analysis time, the post run, and the column re-equilibration period.

Chromatographic procedure

The standard and sample vials were placed in the headspace sampler and equilibrated under the conditions described previously (See Table I). After the GC was equilibrated, the headspace from the standard vial was injected and the chromatogram recorded for approximately 20 min. After the post-temperature run (see Table I), the GC was recycled back to the initial conditions and allowed to equilibrate. The drug substance sample or spiked drug substance sample was then injected and its chromatogram recorded. The peak areas for each solvent peak were then determined in each chromatogram.

Reagents

The 2-propanol, methanol, toluene, and 1-propanol were all ACS reagent grade. All water used for dilutions and preparations was doubly deionized (Barnstead NANOpure, Dubuque, IA). Dimethyl sulfoxide (DMSO) and the other common reagents used in this study were ACS reagent grade and commercially available. The (2α, 6α, 8α, 9α β)-octahydro-3-oxo-2,6-methanon-2H-quinolizin-8-yl-1H-indole-3-caboxylate methanesufonate hydrate, the drug substance, was obtained "in-house."

Table I. Optimized Headspace-GC Conditions

Headspace Conditions
- Loop size: 2 mL
- Sample temperature: 70°C
- Sample equilibrium time: 40 min
- Vial size: 20 mL
- Mixer: off
- Vial pressurization time: 0.20 min
- Vial pressurization equilibrium time: 0.05 min
- Loop fill time: 0.15 min
- Loop equilibrium time: 0.05 min
- Sample loop temperature: 80°C
- Transfer line temperature: 105°C

GC Conditions
- Injection type: Split, 40:1 ratio
- Injector temperature: 150°C
- Split flow: 40 mL/min
- Column: Supelco SPB-1 (dimethylpolysiloxane) 60 m x 0.32 mm i.d., 1 µm film
- Column program:
- Initial temperature 45°C isothermal for 10 min, then 10°C/min to 145°C. A post-run step to 190°C for 5 min was used.
- Flow rate: 1.0 mL/min helium (approximately 12 psig head pressure)
- FID temperature: 250°C

FID gas flows
- Nitrogen (make-up): 30 mL/min
- Hydrogen: 30 mL/min
- Air: 400 mL/min

Electrometer—Attn 2 (0) = 1 millivolt/picoamp

Standard solution preparation

Approximately 100 mg of methanol, 2-propanol, and 1-propanol were accurately weighed into separate 50-mL volumetric flasks containing water. Each was diluted to volume with water. Flasks were kept capped as much as possible to avoid loss of the volatile solvents. A final standard solution at a concentration of 0.1 mg/mL for each solvent was prepared by pipetting 10.0 mL of each stock standard solution into a 200-mL flask and diluting to volume with water. Ten milliliter portions of this mixed standard solution were placed into the 20-mL headspace sampler vials and sealed with Teflon-backed septa and crimp caps. These standards were equivalent to a 200 mg drug sample containing 0.5% (w/w) of the two solvents of interest and the 1-propanol internal standard. A toluene standard solution was prepared by accurately weighing 50 mg of toluene into a 50-mL volumetric flask and diluting the solution to volume with DMSO. Two milliliters of this solution, along with 5.0 mL of the 1-propanol stock solution, were mixed and diluted to 100 mL with water. This solution was equivalent to a 200 mg sample of the drug containing 0.10% (w/w) of toluene. Ten milliliter portions of this toluene standard solution were placed into the 20-mL headspace sampler vials and sealed with Teflon-backed septa and crimp caps.

Sample solution preparation

Approximately 200 mg of the drug substance were accurately weighed into a 20-mL headspace sampler vial. For a regular sample, 10 mL of a 0.1 mg/mL concentration of the internal standard solution, 1-propanol, were added. The vial was quickly sealed with a septum and crimp cap. Spiked samples containing the methanol or 2-propanol were prepared at the 0.05, 0.1, 0.3, 0.6, and 1.0% (w/w) equivalent levels for the evaluation of a spike recovery study. Toluene-fortified sample solutions were prepared at 0.01, 0.05, and 0.1% (w/w) equivalent levels for a toluene spike recovery study.

Calculations

The peak areas of all the solvent peaks found in each chromatogram were determined using common instrumental integration. Peak area ratios were calculated for each of the solvent peaks as follows: Area ratio for solvent, R = peak area of the solvent/peak area of the 1-propanol. For methanol and 2-propanol, the weight percent (% w/w) for each individual solvent "S" was calculated as follows:

$$\% \text{ (w/w) } S = (R_U/R_S) \cdot (10 \text{ mL}/W_U) \cdot (W_S/50 \text{ mL}) \cdot (10 \text{ mL}/200 \text{ mL}) \cdot (100\%)$$

or simplified to

$$\% \text{ (w/w) } S = (R_U/R_S) \cdot (W_S/W_U)$$

where R_U equals the area of the solvent in the sample chromatogram, R_S equals the area ratio of the solvent in the standard chromatogram, W_S equals the weight of the appropriate standard solvent in mg, and W_U equals the weight of the drug substance in mg. Toluene was calculated similarly to the weight percent (%w/w) using the appropriate standard solution weight and dilution factors, and simplified as follows:

$$\% \text{ (w/w) Toluene} = (R_U/R_S) \cdot (W_S/W_U)$$

where R and W represent the same area ratios and weight of toluene and drug substance as outlined previously.

Results and Discussion

Chromatographic and headspace conditions

Good chromatographic separation was obtained using the 60 m SPB-1 column and the temperature program as described. The chromatographic conditions developed for this test procedure appeared to have no apparent interferences with the analyte peaks. Typical chromatograms are displayed in Figure 1. This figure shows chromatograms of spiked drug substance samples at the 0%, 0.1%, and 0.3% (w/w) levels of methanol and 2-propanol. Again, chromatographic resolution of all peaks was very large; the resolution between the methanol and 2-propanol peaks was generally the lowest of any two peaks in any chromatogram generated during this study. Of the two SPB-1 columns evaluated during this study, both gave a calculated resolution, R_s, in excess of 6.0 for the methanol and 2-propanol peaks. Also, all solvent peaks were separated by more than a 1 min retention time, which was very desirable within the chromatographic run time. It should be noted that this chromatographic system was devised with the added advantage of being readily modified to include additional solvents should the synthesis or recrystallization process of the drug substance be changed in the near future. This made it a very adaptable method for any possible changes in the target residual solvent analytes. If the need for a new solvent determination occurred, the method could be quickly re-validated for the new solvent using few modifications in the chromatographic or headspace conditions. The use of more generic chromatographic procedures for the

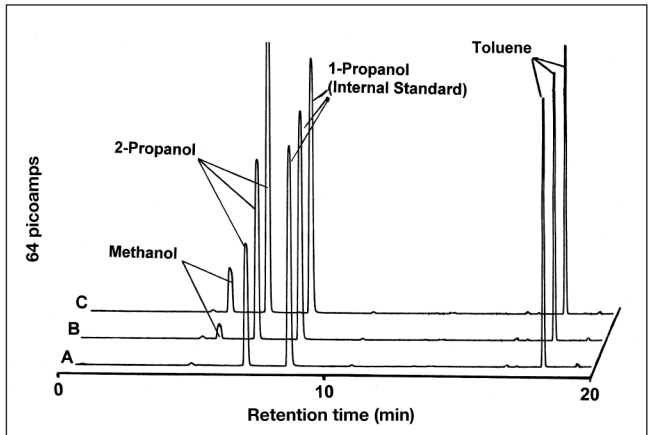

Figure 1. Chromatograms of one non-spiked drug substance sample solution (A) and two methanol and 2-propanol spiked drug substance solutions at 0.1% (w/w) (B) and 0.3% (w/w) (C). The toluene peak is from the trace level present in this specific lot of drug, which is less than 0.01% (w/w). The lot of drug used appeared to contain 0.26% (w/w) 2-propanol before adding the spikes.

headspace analysis of pharmaceuticals to minimize re-validation efforts has been widely recognized and stated in the literature (21,23). Also, although a shorter chromatographic run time and less well-resolved chromatography could have been used, the FID detector is a general or universal detector and lacks specificity; excellent chromatographic resolution was more of an emphasis. Finally, the non-spiked sample solution chromatograms containing only the 1-propanol internal standard (Figure 1A) with the drug substance showed baselines devoid of any interferences for the analyte peaks, supporting the specificity of these chromatographic conditions.

A headspace equilibrium time of 40 min was chosen, as this has been demonstrated to be an optimal time for headspace equilibrium for organic solvents in an aqueous medium (24–26). Polar alcohols tend to require additional equilibrium time over non-polar solvents from an aqueous matrix. The peak area ratios did not change significantly when using longer equilibrium times. Also, it should be noted that no headspace vial agitation was used; therefore, the 40 min equilibrium time for the alcohols was necessary. The headspace temperature of 70°C was found to give acceptable peak response for methanol at the 40:1 spilt ratio and sample concentration range used in this method. The use of a low headspace temperature was done to minimize possible septum bleed; Fliszar et al. (6) described the same strategy in choosing an 80°C headspace equilibrium temperature for a pharmaceutical static headspace analysis method. Higher equilibrium temperatures were of course possible, but a detection limit of slightly below 0.05% (w/w) for the alcohols was all that was required for the test method in the current work. Toluene had a very high response and 0.01% (w/w) could be readily quantitated. Alcohol solvent levels in excess of 0.5% would be indicative of the need for further drying or treatment of the drug substance, and ultimately, excessively low detection limits were not required for this test procedure.

Headspace analysis requires careful consideration of the matrix media, especially for the analysis of pharmaceuticals (3,27). Water is usually the matrix medium of pharmaceutical analysis when using the FID; pure water has no interferences or artifact peaks to be detected by the FID (3,27). The serotonin antagonist drug substance was readily soluble in either water or DMSO; however, DMSO had small interference impurity peaks with toluene under the chromatographic conditions used. Water was selected as the matrix medium for the drug substance, but toluene was not soluble enough to prepare the stock standard in water, although it was soluble in DMSO. It was found that upon a 50 to 1 dilution of DMSO with water, the DMSO impurity peaks were insignificant and did not interfere with the toluene peak. Therefore, the final toluene standard solution was diluted with water and appeared to work well for this method.

Choice of the internal standard

The activity coefficient or partitioning of the analytes into the gas phase from the liquid phase is reasonably matched for the analyte solvents and the 1-propanol internal standard. Although methanol has the lowest partitioning into the gas phase above the aqueous liquid phase in the headspace vial, the 70°C equilibrium temperature produced a reasonable headspace concentration and response for methanol. 1-Propanol also had the advantage of having a suitable retention time and was well resolved from the analyte peaks in this chromatographic system. Toluene has a very high activity or partitioning into the gas phase of the headspace vial, and thus, had a very high response for this headspace/chromatographic system. Despite this apparently poor match of activity coefficients, 1-propanol was found to be a reasonable internal standard for the range of toluene in this study (the recovery data for toluene will be described in more detail in a following section of the discussion). The use of only one internal standard also simplified the operation of this headspace method. It was decided early during the development of this method to have a limit test of 0.1% (w/w) for toluene, and use of 1-propanol was demonstrated to be both accurate and precise for the target analytes.

Method validation criteria

Linearity and limit of detection

The response of the alcohol solvents using this test method was linear over the narrow range studied, that was 0.05% to 1.0% (w/w) equivalent concentrations levels for the drug substance. Correlation coefficients for peak areas and heights were 0.99 or greater for the solvents in the stated range. The limit of detection, defined by traditional procedure as three times the average height noise level divided by the slope of peak height calibration curves (28) was determined for each of the analyte solvents. The average short-term noise level was determined by obtaining the mean response of the chromatographic baseline using 100 data points measured at a rate of 20 points per second. Several areas of the baseline were checked to get an accurate measurement of average baseline height noise. Methanol had the highest limit of detection, which was determined to be equivalent to a 0.002% (w/w) (20 ppm) level when using 200 mg samples of the drug substance. The detection limit for 2-propanol was found to be 0.001% (w/w) (10 ppm). Toluene had the lowest detection limit; it was calculated at 0.0002% (w/w) (2 ppm) equivalent level in the drug substance. These results are comparable to those reported in the literature for static headspace analysis; Carmarasu (29) reported detection limits under 10 ppm for various class I, II, and III pharmaceutical solvents using static headspace analysis. The methanol, 2-propanol, and toluene method described here was not designed for maximum sensitivity; the GC split ratio was 40:1 and the 70°C HS equilibrium temperature could both have been adjusted to lower detection limits. This developed test method, therefore, gives reasonable and accurate solvent determinations at 0.05% (w/w) for both alcohols and 0.01% (w/w) for the toluene, which are adequate for testing drug substance batches by the FDA residual solvent classification guidance (8).

Analyte recovery studies and method reproducibility

Methanol and 2-propanol. A recovery study of alcohol-fortified solutions containing the drug substance was performed over three separate experimental run days using two SPB-1 columns of different lot numbers to demonstrate accuracy and precision. This was necessary, as the objective of this study was the creation of a validated test method (28,30–32). The batch of drug substance used for this study appeared to have a low level of 0.26 (w/w) 2-propanol and a trace level of toluene of much less than

0.01% (w/w) when no spikes were added (see Figure 1). The recovery study indicates that the method was accurate and precise; the data are displayed in Tables II and III. The mean calculated recovery of fortified samples was within 10% of the known spike level at the 0.1% to 1% (w/w) levels for the two alcohols. The lower fortified level, 0.05% (w/w), was somewhat less accurate on a relative basis for methanol and 2-propanol, but these results were acceptable for a test of this nature for a pharmaceutical bulk drug. Calculating the mean percent recovery of all the spiked samples from Table II gave good results. Methanol had a mean recovery of 107% (n = 15) with a relative standard deviation (RSD) of 5.9%. Subtracting the 0.26% 2-propanol level from the spikes in Table II, the mean percent recovery for 2-propanol was 101% (n = 15) with an RSD of 5.3%. Camarusu (29) reported an RSD of 5.5% and less for a static headspace method for pharmaceutical solvents, therefore, this developed method gave comparable performance. Also, as the data in Table II show, there is a slightly high bias for the lower level spikes. In actual use for pharmaceutical testing, a limit of approximately 0.5% would be normal to disqualify a lot of bulk drugs for release, and would require further drying or processing. 2-Propanol is classified as a class 3 solvent by the FDA guidance (3,8) and would normally have a 0.5% (w/w) limit for a finished pharmaceutical product. Methanol is a class 2 solvent, and the FDA guidance limit is 3000 ppm (0.3% w/w) (3,8). The FDA guidance is aimed at finished product limits, and the bulk drug would represent only a portion of the final product after the addition of binders and other formulation excepients. Therefore, the chosen range of analysis for the developed test procedure was appropriate. The relative accuracy of a residual solvent much below the testing limit would not be as significant as the accuracy needed for higher levels more near the limit. The chromatograms displayed in Figure 1 are from part of this recovery study and have been previously described.

Table II. Multilevel Recovery Study of Methanol and 2-Propanol Solvents from Fortified Drug Sample Solutions*

Spike Level (% w/w)	Solvent	Level Found by Headspace (% w/w) Day 1	Day 2	Day 3	Mean (n = 3)
0	Methanol	0	0	0	0
	2-Propanol	0.26	0.26	0.25	0.26
0.05	Methanol	0.06	0.05	0.06	0.05
	2-Propanol	0.32	0.31	0.31	0.31
0.1	Methanol	0.11	0.11	0.11	0.11
	2-Propanol	0.36	0.36	0.36	0.36
0.3	Methanol	0.31	0.31	0.33	0.32
	2-Propanol	0.56	0.56	0.55	0.56
0.6	Methanol	0.61	0.61	0.63	0.62
	2-Propanol	0.86	0.86	0.85	0.86
1.0	Methanol	1.02	1.03	1.04	1.03
	2-Propanol	1.25	1.26	1.26	1.26

* All results are stated as % w/w based on the weight amount of individual solvent added to a weight amount of drug substance. The lot of drug substance had a non-spiked level of 0.26% (w/w) 2-propanol. Day 1 and 2 experimental recoveries were obtained on the same chromatographic column; a second column of different manufacturing lot was used on day 3.

Table III. Multilevel Recovery Study of Toluene from Fortified Drug Sample Solutions

Spike Level (%/w/w)	Level Found by Headspace (% w/w) Column 1	Column 2	Mean (n = 2)
0	< 0.01	< 0.01	< 0.01
0.01	0.01	0.01	0.01
0.05	0.05	0.05	0.05
0.10	0.11	0.08	0.10

Toluene. The toluene recovery study was only conducted over a two-day period on the two different lot SPB-1 columns. The data in Table III show that the test procedure is reasonably accurate and precise for toluene. The mean percent recovery for the six spikes listed in Table III would be 98% with an RSD of 10%. In a static headspace method developed by Otero et al. (21), toluene recovery was 117% (n = 9) with an RSD of 5.6%. The levels tested were not the same for both of these methods, so accuracy and precision did differ slightly. The apparent high bias in the Otero et al. (21) study is not clear; both the Otero study (21) and the current one used 1-propanol as an internal standard. In actual pharmaceutical batch testing, toluene would be limited to less than 0.1% (w/w); the batch of drug would require further drying if levels were higher. Toluene is an FDA class 2 solvent, and has a guidance limit of 890 ppm (0.089% w/w) in finished pharmaceutical products (3,8). As far as quantitation bias, none can be seen for this test procedure by the recovery results obtained; therefore, the method can be considered accurate for the tested concentration ranges for the residual alcohols and toluene.

Other considerations

The robustness of the procedure, that is, its characteristic of remaining unaffected by small changes, was demonstrated by the use of the two different production lots of SPB-1 columns. Both columns gave similar results and excellent chromatographic separation and performance. As for future work, the method will be applied to new synthetic lots of the drug as they are produced and become available, as the method appears to be applicable for residual solvent monitoring.

Conclusions

A static headspace test procedure was evaluated and validated to determine the level of methanol, 2-propanol and toluene in (2α, 6α, 8α, 9α β)-octahydro-3-oxo-2,6-methanon-2H-quinolizin-8-yl-1H-indole-3-caboxylate methanesufonate hydrate drug substance. Those solvents were resolved from each other using a 60-m SPB-1 (dimethylpolysiloxane phase) and a temperature gradient starting at 45ºC and ending at 145ºC. An aqueous-only solution containing the known levels of the target analytes and internal standard was used in the standard sample vials,

which was less labor-intensive than quantiation by multiple spikes by standard addition. A multilevel spiked drug solution recovery study ranging from 0.05% to 1% (w/w) for the alcohols and ranging 0.01% to 0.1% (w/w) for the toluene demonstrated good accuracy for the target solvents. The mean calculated recovery of spiked samples as always within 10% of the known spike at the 0.1% to 1% (w/w) levels for the alcohols. The 0.05 (w/w) spike level for the methanol was somewhat less accurate on a relative basis. The batch of drug substance used for this study appeared to have 2-propanol present at a level of 0.26% (w/w) and a trace level of toluene of less than 0.01% (w/w).

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