Development of a Residual Solvent Test for Bulk α-Phenyl-1-(2-phenylethyl)-piperine Methanol Using Headspace Sampling

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Abstract

An automated static headspace gas chromatographic method for the determination of residual solvents in the bulk drug substance α-phenyl-1-(2-phenylethyl)-piperine methanol, a serotonin 5-HT2 receptor antagonist, is evaluated. The method 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 acetone, ethyl acetate, methanol, and methyl ethyl ketone in the range of 0.05% to 1.0% (weight-per-weight or w/w) to verify the accuracy of this method; these four solvents are the most likely residual volatiles used in the production of the drug substance. These data and other aspects of the development of this test method are discussed.

Introduction

Residual solvents are organic volatile impurities and are a potential problem for pharmaceutical products. They have been monitored by pharmaceutical manufacturers for over two decades. These residual volatiles are the remains from processing agents from the synthesis and production of bulk pharmaceutical compounds. Often in the case of bulk drug substance, residual solvents from recrystallization or synthesis may remain from incomplete drying of the material. Residual solvent testing of pharmaceuticals has been reviewed in the literature (1,2). Residual solvents have had official limits in the United States as set in United States Pharmacopeia XXV (3) and by the United Stated Food and Drug Administration guidance in 1997 (4,5). Residual solvent analysis of bulk drug substance, as well as finished pharmaceutical product, is necessary for several reasons. High levels of residual organic solvent may represent a risk to human health because of their toxicity. Residual organic solvents can also play a role in the physicochemical properties of bulk drug substance; residual solvents can be incorporated within a crystalline structure. Variations in crystalline structure may lead to changes in the dissolution properties and create problems with formulation of finished drug product. Odor and color changes in the finished product may relate back to the level of residual solvents in a batch of bulk drug. Therefore, residual solvent monitoring as a final check is a necessary test for all pharmaceutical products.

The drug compound α-phenyl-1-(2-phenylethyl)-piperine methanol is a serotonin (5-HT2) antagonist and has been under study for its pharmaceutical properties (6–8). The level of impurities of any pharmaceutical is important, including the level of volatile ones. Headspace sampling coupled with gas chromatographic (GC) analysis is a fairly common analysis technique for bulk drug substance analysis (1,2,9) and has been widely reported in the literature (10–13). Although dynamic headspace sampling and multiple headspace extraction techniques have been frequently used in pharmaceutical testing (14,15); static headspace analysis has also been used frequently (1,2,9,13,16–18), and it has the advantage of ease of use, especially when having an automated sampling system. Static headspace sampling also has the advantage of minimizing artifact peaks (1,2,9) or, at least, lowering artifact or carryover peaks to insignificant levels. This, in turn, can avoid interferences with analyte peaks, which is why static headspace analysis was chosen for this work. In the developed headspace–GC method reported in this paper, 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. Residual acetone was the most likely solvent to be encountered because it was used as the final recrystallization solvent for the drug substance. Methyl ethyl ketone was used in the last synthetic step, and methanol and ethyl acetate were used in the recrystallization and last synthetic step of the immediate precursor 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 (19). A spiked recovery study of methanol, acetone, methyl ethyl ketone, and ethyl acetate over a range of 0.05% to 1.0% (w/w) was used to verify the accuracy of this test method. These data, as well as sev-

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eral of the other facets of the development and validation of this test method will be discussed in further detail.

Experimental

Reagents

The acetone, ethyl acetate, methanol, methyl ethyl ketone (2-butanone), and 1-propanol were all ACS reagent grade and commercially available. All water used for dilutions and preparations was doubly deionized (Barnstead NANOpure, Dubuque, IO). All other common reagents used in this study were commercially available American Chemical Society reagent grade. The α-phenyl-1-(2-phenylethyl)-piperine methanol, the drug substance, was obtained "in-house" as a finished product. As the synthesis and development of this drug is outside the scope of this analytical chemistry manuscript, this information will be described in detail elsewhere in the near future.

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 gas chromatographic sampling interval was approximately 20 min. This included a 12 min analysis time, a post run, and column re-equilibration.

Table I. Optimized Headspace–GC Conditions

Headspace conditions

Loop size: 1 mL
Sample temperature: 80°C
Sample equilibrium time: 40 min
Vial size: 20 mL
Mixer: off
Vial pressurization time: 0.25 min
Vial pressurization equilibrium time: 0.05 min
Loop fill time: 0.2 min
Loop equilibrium time: 0.15 min
Sample Loop temperature: 95°C
Transfer line temperature: 110°C

GC conditions

Injection type: Split, 50:1 ratio
Injector temperature: 175°C
Split flow: 50 mL/min
Column: Supelco SPB-1 (dimethylpolysiloxane) 60 × 0.32 mm i.d., 1-µm film
Column program: Initial temperature 50°C isothermal for 12 min, then a post run at 100°C for 3 min
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, acetone, methyl ethyl ketone, and ethyl acetate were accurately weighed into separate 50-mL volumetric flasks containing water. Each was diluted to volume with water. Approximately 125 mg of 1-propanol was accurately weighted into a 50-mL volumetric flask containing water and 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 pipeting 10.0 mL of each stock standard solution and 8.0 mL of the stock 1-propanol internal standard solution into a 200-mL flask and diluting to volume with water. Portions (10.0 mL) of this mixed 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 was accurately weighed into a 20-mL headspace sampler vial. Eight milliliters of a 0.0625M sulfuric acid solution containing 0.125 mg/mL of 1-propanol was added, and the solution was sonicated to dissolve the drug substance. Two milliliters of a 0.5M sodium bicarbonate solution was added to neutralize the sample solution. The vial was quickly sealed with a septum and crimp cap. Spiked samples containing the four solvents of interest were prepared at the 0.05%, 0.1%, 0.3%, 0.6% and 1.0% (w/w) equivalent levels for evaluation of a spike recovery study.

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 12 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 the chromatogram was recorded. The peak areas for each solvent peak was then determined in each chromatogram.

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 four solvent peaks as follows: Area ratio for solvent = R = peak area of the solvent/peak area of the 1-propanol. The weight percent (% w/w) for each individual solvent "S" was calculated as follows:

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

or simplified to:

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

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.

Results and Discussion

Chromatographic and headspace conditions

The chromatographic conditions developed for this test procedure proved to be appropriate and had no apparent interferences with the analytes. Good chromatographic separation was obtained using the 60-m SPB-1 column and the isothermal temperature of 50°C. This column was chosen because of previously reported work of residual solvent testing methods for pharmaceuticals (13). Typical chromatograms are displayed in Figures 1 and 2. Figure 1 shows a chromatogram of the standard solution; all analyte peaks are readily resolved. Figure 2 shows chromatograms of spiked drug substance samples at the 0%, 0.1%, and 0.3% (w/w) levels. Again, chromatographic resolution of all peaks was very large; the resolution between the methyl ethyl ketone and ethyl acetate peaks was generally the lowest of any two peaks in any chromatogram generated during this study, but were highly resolved. This chromatographic system was devised with the added advantage of being easily modified to include additional solvent should the synthesis or recrystallization of the drug substance be changed. The wide separation of the peaks would allow for the possible separation of additional solvents. Of the two SPB-1 columns evaluated during this study, both gave a calculated resolution (Rs) in excess of 7.0 for the MEK and ethyl acetate peaks. Also, all solvent peaks were separated by at least a 1 min retention time, which was very desirable within a relatively (short 12 min) chromatographic run time. Finally, the unspiked sample solution chromatograms containing only the 1-propanol internal standard (Figure 2A) showed baselines without any significant level of interferences for the analytes.

A headspace equilibration 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 (13,20). The peak area ratios did not change significantly using longer equilibrium times. It should be noted that this headspace equilibration time was established for a non-agitated solution. Agitated or mixed solutions would have a shorter equilibration time, but this option was not used on the Tekmar headspace sampler used in this study. The headspace temperature of 80°C was found to give acceptable peak response for methanol at the 50:1 split ratio and sample concentration range used in this method. Higher bath temperatures were, of course, possible, but a detection limit of slightly below 0.05% was all that was required for this test method. Solvent levels in excess of 1% 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 the test.

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 four 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 80°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.

The drug substance sample solutions had slightly greater partitioning of both analytes and internal standard over the aqueous standard solution. This was due in part to a "salting out" effect of the sample solutions from the drug substance itself and the low concentration of sodium sulfate in the drug substance sample medium. Sulfuric acid was necessary to dissolve the drug followed by neutralization with sodium bicarbonate. This was done to insure that complete dissolution of the drug had been achieved and that no residual solvent remained incorporated within an undissolved crystal structure of the drug substance. Complete dissolution in the aqueous medium would mean any residual solvent would have to be within the aqueous matrix.

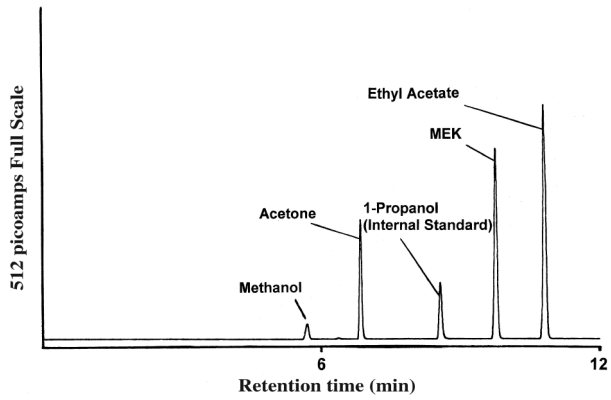

Figure 1. A chromatogram of a standard solution containing equal levels of methanol, acetone, 1-propanol (internal standard), methyl ethyl ketone (MEK), and ethyl acetate. The concentration of the standard solution is 0.1 mg/mL for each solvent. The headspace vial contains 10 mL of the solution meaning 1 mg of each solvent is present in the headspace vial, and this is equivalent to 0.5% (w/w) for a 200 mg sample of bulk drug.

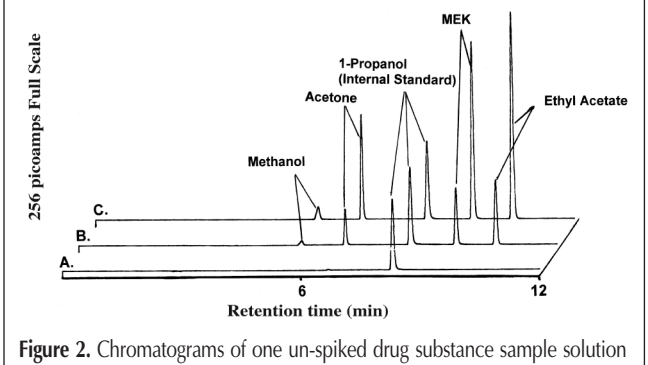

Figure 2. Chromatograms of one un-spiked drug substance sample solution (chromatogram A) and two spiked drug substance solutions at 0.1% (w/w) (chromatogram B), and 0.3% (w/w) (chromatogram C). The un-spiked drug substance contained a low level of acetone of less than 0.01% (w/w).

Although Dennis et al. (21) has shown significant changes in sensitivity between polar and non-polar solvents in an aqueous dissolution medium, the concentration of sodium sulfate in this procedure was very low; thus, the peak area ratios of analytes to internal standard was significantly different from a pure aqueous system. This was demonstrated during the accuracy verification phase of the recovery study discussed in the following section and further validates the use of 1-propanol as an internal standard for all four analytes.

Method validation criteria

Linearity and limit of detection

The response of the four solvents using this test method was linear over the narrow range studied [that was 0.05% to 1.0% (w/w) equivalent concentration levels for the drug substance]. Correlation coefficients for peak areas and heights were 0.98 or greater for the solvents in the stated range. The limit of detection, as defined by traditional procedure as three times the average height noise level divided by the slope of peak height calibration curves (22), was determined for each of the four 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/s. 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) level when using 200 mg samples of the drug substance. Ethyl acetate had the lowest detection limit; it was calculated at a 0.0001% (w/w) level. The resulting test method, therefore, could be assumed to give reasonably accurate solvent determinations at 0.05% (w/w), which was the lowest spike level used in the recovery study discussed in the next section.

Analyte recovery studies and method reproducibility

A recovery study of 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 it was the objective of this study to create a validated test method (22–25). The batch of drug substance used for this study appeared to have a trace level of acetone (less than 0.01%) when no spikes were added. The recovery study indicates that the method was accurate and precise; the data are displayed in Table II. The mean calculated recovery of fortified samples was within 10% of the known spike level at the 0.1% to 1% (w/w) levels. The lower fortified level, 0.05% (w/w), was somewhat less accurate on a relative basis for methanol and acetone, but these results were acceptable for a test of this nature for a pharmaceutical bulk drug. In actual use for pharmaceutical testing, a limit of 0.5% to 1% would be normal to disqualify a lot for release and require returning it for further drying or processing. The relative accuracy of a residual solvent at a level significantly below the testing limit would not be as important as the accuracy needed for higher solvent levels near the limit. The chromatograms displayed in Figure 2 are from part of this recovery study and have been previously described. As can be seen from the higher spike levels in the data from Table II, the mean recoveries for acetone, MEK, and ethyl acetate were slightly low, and the recovery of methanol was slightly higher than theory. If there was any bias for this test method, it is minimal and less than 10% of theory. Therefore, the method can be considered accurate for the tested concentration range.

The precision demonstrated by the data listed in Table II was acceptable. The 0.05 solvent level had a range of calculated percent relative standard deviation (%RSD) of 0% to 10% (n = 3 for each solvent). The %RSD of the solvents at the high levels were all 7.8% and less. The %RSD for solvents at the 1% (w/w) spike level ranged from 0.6% for ethyl acetate to 3.4% for methanol; this indicated an acceptable level of precision for this test method. In view of the International Conference on Harmonization (ICH) considerations (2,26), acetone, MEK, and ethyl acetate are class 3 solvents, which are less toxic and have less stringent restrictions. Also, this is a test for a bulk drug substance. A finished pharmaceutical product would have the most strict restrictions as it would be the form delivered to a patient.

Table II. Multilevel Recovery Study of Four 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	Acetone	< 0.01	< 0.01	< 0.01	< 0.01
0.05	Methanol	0.06	0.05	0.06	0.06
	Acetone	0.05	0.06	0.06	0.06
	MEK	0.05	0.05	0.05	0.05
	Ethyl acetate	0.05	0.05	0.05	0.05
0.1	Methanol	0.12	0.11	0.11	0.11
	Acetone	0.11	0.11	0.10	0.11
	MEK	0.10	0.10	0.09	0.10
	Ethyl acetate	0.09	0.09	0.09	0.09
0.3	Methanol	0.32	0.31	0.31	0.31
	Acetone	0.29	0.29	0.29	0.29
	MEK	0.28	0.28	0.29	0.28
	Ethyl acetate	0.25	0.26	0.29	0.28
0.6	Methanol	0.65	0.62	0.63	0.63
	Acetone	0.57	0.59	0.55	0.57
	MEK	0.56	0.57	0.53	0.55
	Ethyl acetate	0.54	0.55	0.52	0.54
1.0	Methanol	1.09	1.04	1.02	1.05
	Acetone	0.95	0.97	0.92	0.95
	MEK	0.91	0.93	0.93	0.92
	Ethyl acetate	0.90	0.90	0.89	0.90

* Notes: All results are stated as percent weight-to-weight (% w/w) based on the weight amount of individual solvent added to a weight amount of drug substance. 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.

Methanol is a class 2 solvent by the ICH and the Food and Drug Administration classifications (2,26). Methanol has a limit of 3,000 ppm (part per million) or 0.3% (w/w) for finished pharmaceutical formulation products. The recovery for methanol in Table II was 0.31% (w/w) for the 0.3% level spike, and the %RSD was 1.8% (n = 3). The precision of the method is acceptable for the testing of a bulk drug substance.

Other considerations

The robustness of the procedure (i.e., its ability or characteristic to remain 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. Based upon these results, the method appears to be applicable for residual solvent monitoring.

Conclusion

A static headspace test procedure to determine the level of methanol, acetone, MEK, and ethyl acetate in α-phenyl-1-(2-phenylethyl)-piperine methanol drug substance was evaluated and validated. Those four solvents are resolved from each other using a 60-m SPB-1 (dimethylpolysiloxane phase) and an isothermal temperature setting at 50°C. A multilevel spiked recovery study ranging from 0.05% to 1% (w/w) demonstrated good accuracy for the four target solvents. Calculated recovery of spiked samples were always within 10% of the known spike at the 0.1% to 1% (w/w) levels. The 0.05 (w/w) spike was somewhat less accurate on a relative basis for methanol and acetone. The batch of drug substance used for this study appeared to have only acetone present at a level less than 0.01%.

References

1. C. Witschi and E. Doelker. Residual solvents in pharmaceutical products: Acceptable limits, influences on physicochemical properties, analytical methods and documented values. *Eur. J. Pharm. Biopharm.* **43:** 215–42 (1997).
2. C. B'Hymer. Residual solvent testing: a review of gas-chromatographic and alternative techniques. *Pharm. Res.* **20:** 337–44 (2003).
3. Organic volatile impurities. In *The 2002 United States Pharmacopeia XXV and National Formulary XX*. The United States Pharmcopeial Convention, Inc., Rockville, Maryland, 2001, pp. 1943–45.
4. International Conference on Harmonisation. Guidance on Impurities: Residual Solvents. *Fed. Regist.* **62:** 67377–88 (1997).
5. *Guidance for Industry, Q3C Impurities: Residual Solvents*. U.S. Food and Drug Administration, Rockville, Maryland, December 1997.
6. E. Edwards, C.R. Ashby, and R.Y. Wang. (+/-)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and alpha-methyl-5-HT: T-HT2 receptor antagonistic action on phosphatidylinositol metabolism in the rat fronto-cingulate and entorhinal cortex. *Neuropharmacology* **31:** 615–21 (1992).
7. C.J. Schmidt, G.M. Abbate, C.K. Black, and V.L. Taylor. Selective 5-hydroxytryptamine2 receptor antagonists protection against the neurotoxicity of methylenedioxymethampheramine in rats. *J. Pharmacol. Exp. Ther.* **255:** 478–83 (1992).
8. C.J. Schmidt, V.L. Taylor, G.M. Abate, and T.R. Nieduzak. 5-HT2 antagonists steroselectively present the neurotoxicity of 3,4-methylenedioxymethamphetamine by blocking the acute stimulation of dopamine synthesis: reversal by L-dopa. *Pharmacology* **256:** 230–35 (1991).
9. C. B'Hymer. Headspace sampling in gas chromatography. *Encyclopedia of Chromatography On-line Supplement*. www.dekker.com, (date accessed November 28, 2005).
10. T.K. Natishan and Y. Wu. Residual solvents determination in the antibiotic L-749,345 by static headspace gas chromatography. *J. Chromatogr. A* **800:** 275–81 (1998).
11. Q.C Li, K.A. Cohen, and G. Zhuang. A capillary gas chromatographic procedure for the analysis of nine common residual solvents in water-insoluble bulk pharmaceuticals. *J. Chromatogr. Sci.* **36:** 119–24 (1998).
12. I.D. Smith and D.G. Waters. Determination of residual solvent levels in bulk pharmaceuticals by capillary gas chromatography. *Analyst* **116:** 1327–31 (1991).
13. C. B'Hymer. Static headspace gas chromatographic method for the determination of residual solvents in vigabatrin drug substance. *J. Chromatogr.* **438:** 103–107 (1988).
14. F.P. Wampler, W.A. Bowe, and E.J. Levy. Dynamic headspace analysis of residual volatiles in pharmaceuticals. *J. Chromatogr. Sci.* **23:** 64–67 (1985).
15. B. Kolb, P. Pospisil, and M. Auer. Quantitative headspace analysis of solid samples—a classification of various sample types. *Chromatographia* **19:** 113–22 (1984).
16. T.K. Natishan and Y. Wu. Residual solvent determination in the antibiotic L-749,345 by static headspace gas chromatography. *J. Chromatogr. A* **800:** 275–81 (1998).
17. J.P. Guimbard, M. Person, and J.P. Vergnaud. Determination of residual solvents in pharmaceutical products by gas chromatography coupled to a head-space injection system and using an external standard. *J. Chromatogr.* **403:** 109–21 (1987).
18. W.C. Kidd. Evaluation of the proposed automated headspace method for organic volatile impurities. *Pharm. Forum.* **19:** 5063–66 (1993).
19. D.G. Westmoreland and G.R. Rhodes. Analytical techniques for trace organic compounds–detectors for gas chromatography. *Pure Appl. Chem.* **61:** 1148–60 (1989).
20. C. B'Hymer and K.L. Cheever. Development of a headspace gas chromatographic test for the quantification of 1- and 2-bromopropane in human urine. *J. Chromatogr. B.* **814:** 185–89 (2005).
21. K.J. Dennis, P.A. Josephs, and J. Kokladalova. Proposed automated headspace method for organic volatile impurities (467) and other residual solvents. *Pharm. Forum.* **18:** 2964–72 (1992).
22. J.M. Green. A practical guide to analytical method validation. *Anal. Chem.* **68:** 305A–309A (1996).
23. R. Brown, M. Caphart, P. Faustino, R. Frankewich, J. Gibbs, E. Leutzinger, G. Lunn, L. Ng, R. Rajagopalan, Y. Chiu, and E. Sheinin. Analytical procedures and method validation: highlights of the FDA's draft guidance. LC/GC **29:** 74–84 (2001).
24. M.E. Swartz and I.S. Krull. *Handbook of Analytical Method Validation*, Marcel Dekker, New York, 2003.
25. International Conference on Harmonization. Draft guideline on validation of analytical procedures: definitions and terminology. *Fed Regist.* **60:** 11260–65 (1995).
26. International Conference on Harmonisation. Guidance on impurities: residual solvents. *Fed Regist.* **62:** 67377–82 (1997).

Manuscript received July 14, 2006;
revision received November 24, 2006.