# Determination of 1,4-Dioxane Impurity Levels in Triton X-100 Raw Material by Gas Chromatography with Mass Spectrometric Detection

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## Abstract

**Triton X-100 (octoxynol 9) is a commercially available surfactant used as a solvent detergent in numerous pharmaceutical applications including virus inactivation. A byproduct formed during its synthesis is 1,4-dioxane, the cyclic dimer of ethylene oxide and a possible carcinogen to humans. The United States Pharmacopoeia (USP) contains a labor-intensive 1,4-dioxane test for Triton X-100. The method couples vacuum distillation to extract the 1,4-dioxane from the Triton X-100 matrix followed by gas chromatography (GC) using a packed column with flame-ionization detecton. In order to provide a more automated and specific test methodology, a headspace GC–mass spectrometry (MS) method has been developed for this application. Analyte quantitation is accomplished by the method of standard additions. The automated sample preparation, coupled with the specificity inherent in high-efficiency capillary column separations together with single-ion MS detection, results in an assay that is more efficient, accurate, and precise than the USP procedure. Performance characteristics of the headspace GC–MS method are contrasted with those characteristics of the USP methodology.**

## Introduction

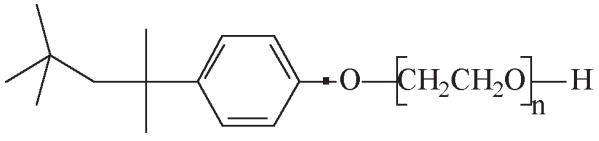
Triton X-100 [octoxynol 9, poly(oxy-1,2-ethanediyl), α-(octylphenyl)-ω-hydroxy-polyethylene glycol mono(octylphenyl) ether, CAS RN [9002-93-1] is a commercially available surfactant used as a solvent detergent in numerous pharmaceutical applications including virus inactivation. Triton X-100 is synthesized from octylphenol polymerized with ethylene oxide by way of a base catalyst (1,2). The average molecular weight of the compound is 625, and the average number of ethylene oxide units per molecule on the side chain is approximately 9.5. The chemical structure is given in Figure 1.

A byproduct formed during this synthesis is 1,4-dioxane [CAS RN (123-91-1)], the cyclic dimer of ethylene oxide (3). 1,4-Dioxane has been shown to be carcinogenic to animals and has been considered as a possible carcinogen to humans (4–8). Target organs effects have been documented for the liver (9), lungs and kidneys (10), and central nervous system (11). Thus, the level of the 1,4-dioxane impurity in Triton X-100 for pharmaceutical applications is limited to 10 µg/g (12).

The United States Pharmacopoeia (USP) contains a labor-intensive 1,4-dioxane test for Triton X-100 (3,12). The method is a two-step procedure with the initial step being a vacuum distillation to extract the 1,4-dioxane from the Triton X-100 matrix. The resultant extract is then analyzed by gas chromatography (GC) using a flame-ionization detector (FID).

Utilization of more modern analytical procedures may result in a more viable assay, particularly with respect to specificity. The coupling of headspace sampling methods with GC has been used for such applications with either FID (13) or mass spectrometric (MS) detection (11). Although both methods reported excellent results, the specificity of the FID procedure is not optimal, and the MS methodology utilized an isotope-dilution approach.

The purpose of this study was to develop and evaluate a GC–MS headspace method to determine the 1,4-dioxane levels in the Triton X-100 matrix based on quantitation via the method of standard additions. This technique aggressively addresses




$n$ = ~ 9.5

Triton X-100, octoxynol 9 (CAS RN [9002-93-1])

1,4-Dioxane [CAS RN (123-91-1)]

**Figure 1.** Trition X-100 and its impurity, 1,4-dioxane.

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matrix interferences and eliminates matrix effects by performing standardization in the sample itself. The performance of the developed method is compared with that of the USP distillation, GC–FID approach.

## Experimental

### Test articles

The test article for this study was one lot of Triton X-100, obtained from Union Carbide (Danbury, CT). A stripped test article (from which the volatile impurities, including 1,4-dioxane, were removed) was generated from this lot of Triton X-100. The stripped sample was prepared by vacuum distillation via a USP procedure for preparing stripped polyethylene glycol (14). Approximately 1005 g of Triton X-100 was placed in a flask fitted with a nitrogen purge fritted tube and a ground-glass valve to which the vacuum line was attached. The sample was first degassed by vacuum until the evolution of gas stopped. A slow nitrogen purge, introduced with a constant vacuum, was performed overnight. The resulting stripped material was tested for 1,4-dioxane by the GC–MS headspace procedure by monitoring the ion at $m/z$ = 88 amu. The absence of this ion in the stripped sample confirmed that all of the 1,4-dioxane had been removed.

### Reference materials, reagents, and instrumentation

The reagents used in the USP assay included 1,4-dioxane (99.9% purity) as the standard reference material, 4-methyl-1,3-dioxane as the internal standard, and *N*,*N*-dimethylformamide (DMF) as the sample diluent (all from Sigma-Aldrich, St. Louis, MO). Research-grade water was used in the USP method.

The vacuum apparatus used in the USP method is shown in Figure 2. The instrumentation used for the USP method included a Hewlett-Packard GC with FID (model 5890), autosampler (model 7673A), and controller (model 7673A) (Hewlett-Packard, Palo Alto, CA). The operating conditions for this method are listed in Table I.

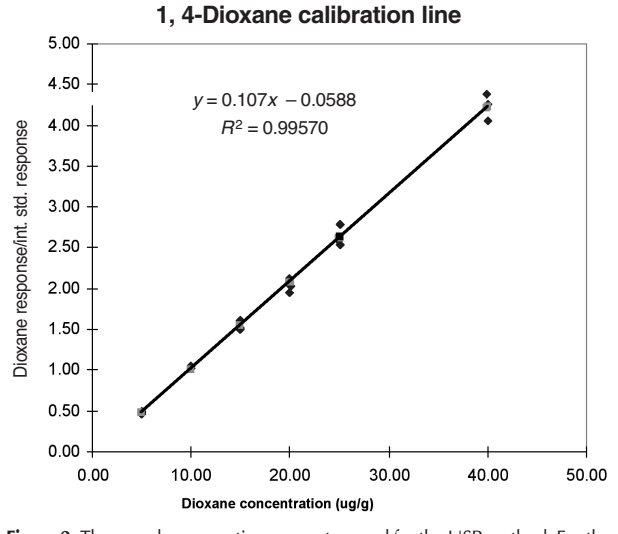


**Figure 2.** The sample preparation apparatus used for the USP method. For the figure, (◆) *Y*, (■) predicted *Y*, and (—) linear (predicted *Y*).

Test articles were analyzed by a GC–MS headspace method with the following analytical instrumentation: PerkinElmer (Norwalk, CT) HS 40XL headspace sampler, and a Hewlett Packard 5890 GC and 5970 MS for the detector. The carrier gas (helium) was set at 13.8 to 14.0 psi on the HS40XL headspace sampler, resulting in a carrier gas linear velocity of approximately 30 cm/s (~ 3.9 mL/min). The carrier gas was pumped directly from the HS 40XL headspace sampler through a sample transfer line into the chromatographic column. The transfer line was a J&W Scientific (Folsom, CA) DB-1 fused-silica capillary (1.5-m × 0.32-mm i.d. × 0.25-µm film). The transfer line was

**Table I. Operating Conditions for the USP Method**

| Operating Parameter | Operating Condition |
|---|---|
| Column | Supelco GC column, 6-ft × 2-mm glass column with Haycep C support (USP support 10), 80/100 mesh size, serial number 2961051-01. |
| GC operating conditions | Oven temperature, 180°C; carrier gas, helium, 32 mL/min; injection port, 200°C; detector, 250°C; and injection volume, 3 µL. |

**Table II. Operating Conditions for the GC–MS Headspace Method**

| Operating parameter | Operating condition |
|---|---|
| Column | J&W Scientific, DB-WAX, 30 m × 0.53 mm × 1 µm (film). |
| GC oven program | Initial temperature, 30°C; initial time, 2.0 min. Ramp at 3°C/min to 50°C, hold for 0 min. Ramp at 25°C/min to 190°C, hold for 0 min. Total time 14.27 min. |
| Carrier gas flow rate | ~ 30 cm/s (~ 3.9 mL/min) |
| Carrier gas pressure | 13.8–14.0 psi |
| MS information | Solvent delay: 2.50 min<br>Low mass: 28<br>High mass: 250<br>Mode: $EI^+$<br>Transfer line: 225°C<br>Filament turned off at 10.5 min<br>Quantitating ion for 1,4-dioxane, $m/z$ = 88 amu<br>Quantitating ion for 4-methyl-1,3-dioxane, $m/z$ = 101 amu |
| Headspace conditions | Oven temperature: 80°C<br>Thermostat (heating) time: 40 min<br>Needle temperature: 110°C<br>Transfer line: 120°C<br>Pressurization time: 0.3 min<br>Injection time: 0.3 min<br>Withdrawal: 0.2 min<br>Cycle time: 30 min<br>Shaker: on |

connected to the chromatographic column by means of a Supeltex M-2B ferrule and a Supleco capillary butt connector (Supelco, Bellefonte, PA). There was no other contribution to the carrier gas other than that which was supplied by the headspace sampler.

Additional operating conditions for this method are listed in Table II. In order to address the mismatch between the effluent flow from the megabore column used and the pumping capacity of the MS detection system, an MS transfer line was threaded into the end of the megabore column to limit its effluent flow into the MS. The MS transfer line consisted of 1.3-m × 0.15-mm-i.d. × 0.30-mm-o.d. tubing (Chrompack eactivated fused silica) (Varian, Palo Alto, CA).

### Sample/standard additions preparations for the GC–MS headspace procedure

The method of standard additions was used to characterize the test articles and was implemented as follows. Four test samples of each test article were prepared by placing 1.00-g aliquots of the test article into four separate GC headspace vials. A 100-µL aliquot containing approximately 15 µg of 4-methyl-1,3-dioxane (utilized as an internal standard) was delivered into the first vial (unspiked level). Three 100-µL aliquots with increasing concentrations (~ 5, 10, and 15 µg) of 1,4-dioxane and approximately 15 µg of 4-methyl-1,3-dioxane were delivered into the remaining three vials, respectively, producing the three spiked samples. Triplicate preparations were made at each level and the diluent in all cases was DMF. The sample preparations were thoroughly mixed prior to analysis and were processed once via the GC–MS headspace analyzer. To verify the linearity of the assay up to 25 ppm (in the test sample), two runs were completed with the stripped Triton X-100 sample. The stripped test article was fortified to contain 1,4-dioxane and analyzed to generate the calibration curve. Forty micrograms per gram is equivalent to a test article originally containing 25 µg/g of 1,4-dioxane plus the fortification amount of 15 µg/g for the calibration curve.

### Sample preparations for the USP procedure

Twenty grams of Triton X-100 were weighed into a 50-mL round-bottom flask and 1 mL of water was volumetrically added to the flask. The flask was connected to the U-tube vacuum apparatus, similar to that as shown in Figure 3, and was wrapped with heating tape to which a voltage of 10 V was applied. The flask was frozen in a methanol–dry ice bath for 10 min, after which a 0.03-mm vacuum was drawn on the flask for 20 s. The flask was placed in a water bath having a temperature of 20–25°C for 5 min and then warmed to a temperature of 35–40°C. The test article was completely thawed at this temperature, and degassing of the sample began to take place as evidenced by the appearance of bubbles in the flask. The thawed solution was slowly stirred with a magnetic stirring bar that was added to the flask before it was attached to the vacuum apparatus.

The flask was placed into a room temperature water bath and ice was added to the bath to chill the flask for approximately 2 min. The freezing step, as outlined previously, was again performed, as was the heating step with the exception that the final temperature was between 45° and 50°C. A liquid nitrogen bath was raised slowly up onto the concentrator tube to initiate the liquid distillation process, and the liquid nitrogen bath was raised higher with respect to the distillate until approximately the 2-mL mark on the concentrator tube. The distillate in the concentrator tube was thawed to determine the quantity of distillate present, and the distillation process was continued until a minimum of 0.9 mL of distillate was collected. The distillate was frozen again to release the vacuum and diluted to the 2-mL mark on the concentrator tube after it had thawed. The diluted distillate was gently mixed with the aid of a Pasteur pipette, transferred to a GC vial, and sealed.

This process of preparing a sample effectively concentrates 1,4-dioxane by tenfold. Therefore, the responses obtained from the diluted distillate were compared with those generated from an external 100 ppm 1,4-dioxane standard prepared in water.

### Linearity assessment and GC–MS headspace method

The range of 5–40 µg/g 1,4-dioxane was assessed by fortifying the stripped Triton X-100 material with 1,4-dioxane at six levels (5, 10, 15, 20, 25, and 40 µg/g). Each of these standards was analyzed in triplicate by the GC–MS headspace method in order to generate a calibration curve.

### Performance evaluation, USP, and GC–MS headspace methods

Accuracy and precision were assessed by taking the stripped Triton X-100 material and adding a known amount of 1,4-dioxane (10 µg/g). Fortified samples for the GC–MS headspace assay were spiked with a 1,4-dioxane–DMF solution, and samples prepared for the USP assay included a 1,4-dioxane–water spike followed by continuous stirring to ensure that the samples were homogeneous.

Specificity was addressed by a direct comparison of the two methodologies. Each method was used to analyze three replicate preparations of the unstripped Triton X-100 test article. The results from the three replicates from the GC–MS method were then compared with results obtained by the USP method to demonstrate specificity. The purpose of this comparison was to determine whether unknown analytes were coeluting with the 1,4-dioxane in the USP method.

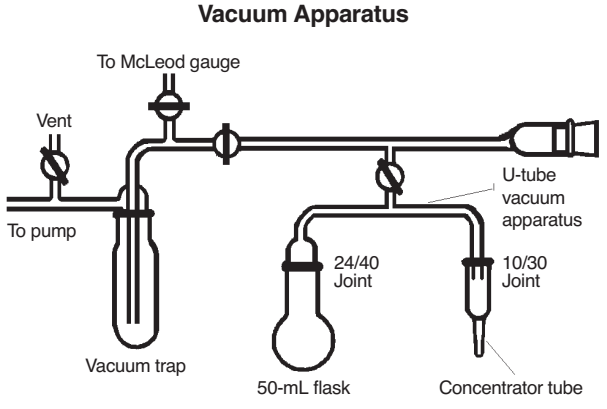


**Figure 3.** Standard calibration curve for the GC–MS headspace assay demonstrating linearity of the sample response up to a concentration of 40 µg/g. Although the $r^2$ value obtained meets the acceptance criterion for this parameter (and thus the assay is judged to be linear up to 40 µg/g), an increase in assay variability is noted at the higher analyte levels.

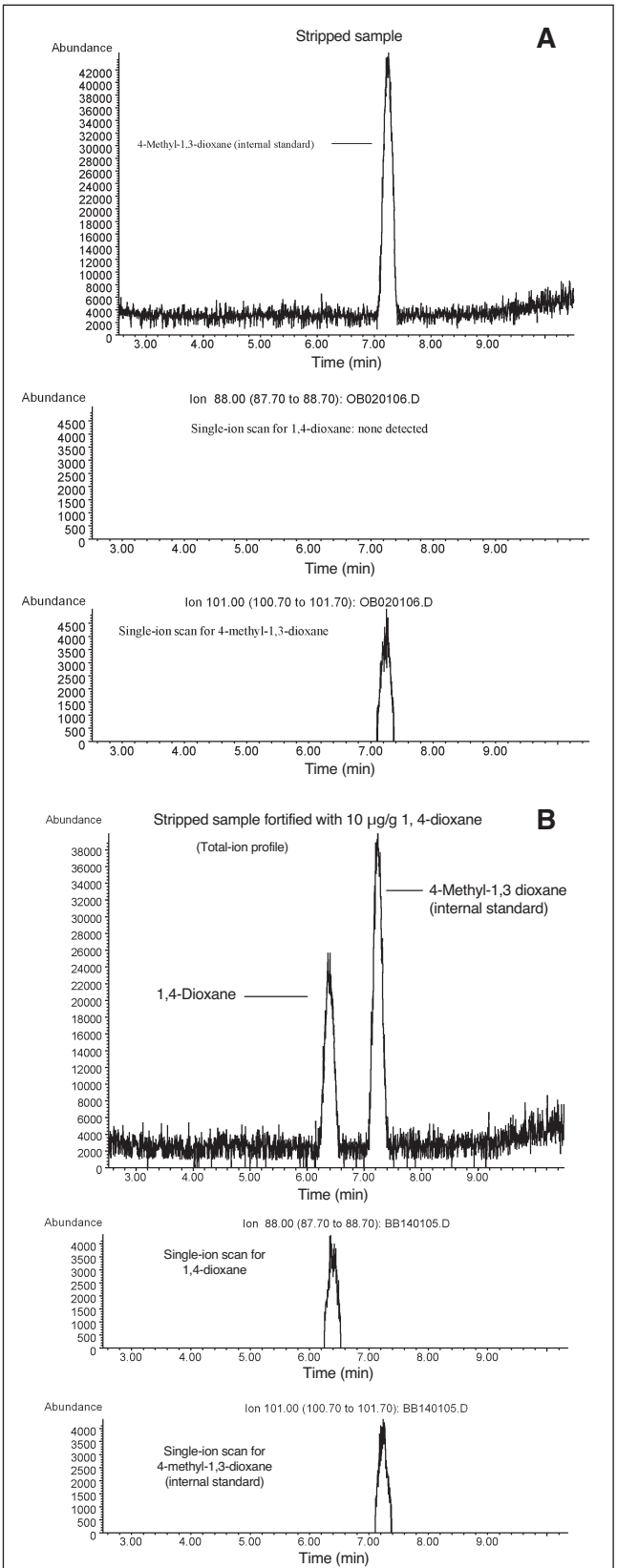


**Figure 4.** Typical chromatograms of the GC–MS headspace method. The chromatograms shown are the stripped sample (A), unstripped sample (B), and stripped sample spiked to contain 10 µg/g 1,4-dioxane (C). The combination of separation via high-efficiency capillary columns with highly specific single-ion MS detection results in chromatograms that are devoid of any interferences.

To ensure that the GC portion of the USP method was operating properly, system precision was evaluated for each run by performing five replicate injections of the standard 1,4-dioxane preparation prior to any sample analysis. Peak height was used for the quantitation as required by the USP. The five replicate injections were followed by injections of the samples bracketed by two standard injections. Carry over from one injection to another was evaluated by placing blank water injections between the samples and the standards before and after them. Each sample was injected twice, and the value reported for each sample was the average of the duplicate injections.

## Results and Discussion

### Linearity assessment and GC–MS headspace method

Linearity was assessed by fortifying the stripped test article with 1,4-dioxane at six different levels (5, 10, 15, 20, 25, and 40 µg/g). The upper limit of 40 µg/g corresponds to a test article originally containing 25 µg/g of 1,4-dioxane and a 15-µg spike for the sample preparation. The results of this experiment are illustrated in Figure 2. Although the $r^2$ value obtained is acceptable and thus linearity is established up to 40 µg/g, it is observed that the assay variation increases at the higher spike levels.

The effect of the increased assay variation at the higher analyte levels was also observed during the analysis of the unstripped raw material. The results for the triplicate standard addition analysis of the unstripped sample resulted in $r^2$ values

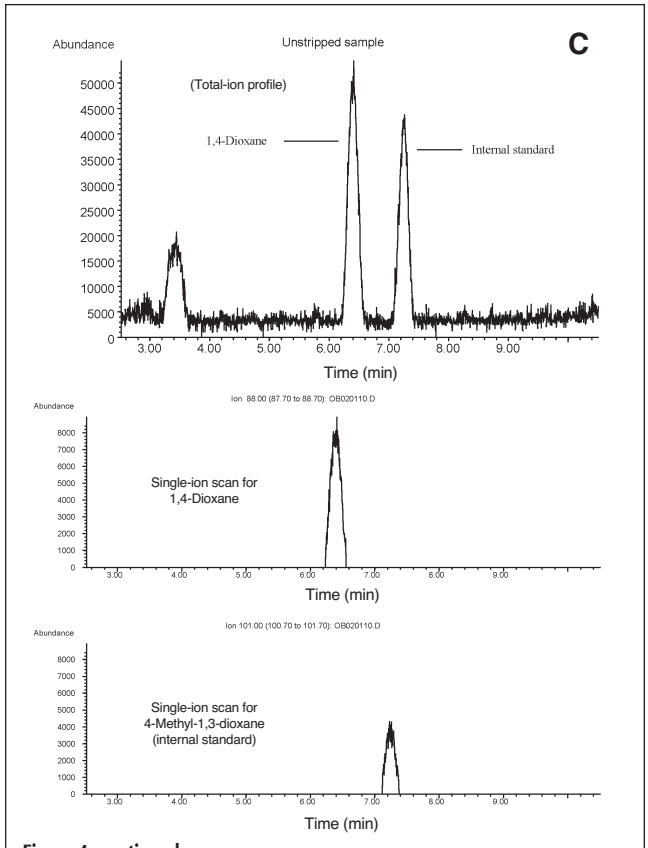


**Figure 4. continued.**

of 0.97, 0.98, and 0.99. This behavior does not reflect the lack of response linearity because visual inspection of the best fit lines suggest a good linear fit. Rather, the $r^2$ values obtained at the higher sample 1,4-dioxane levels is a reflection of the fact that there is more variability in the assay as the concentration of 1,4-dioxane increases above a total concentration of 30 µg/g. The impact of this effect is small at the 10 µg/g USP limit, and the imprecision at the higher levels is partially offset by the fact that replicate preparations were used. Therefore, linearity was deemed acceptable for test articles that contain levels of 1,4-dioxane up to 25 µg/g, which is well above the USP assay limit of 10 µg/g.

**Performance evaluation**

The primary focus of this study was to determine and contrast the performance characteristics of the USP and GC–MS headspace methods. The net goal of this study was to perform such a comparision utilizing the same test design implemented in both methods. Performance characteristics that were considered included accuracy, precision, and specificity.

Accuracy and precision were evaluated at the 10-µg/g level of 1,4-dioxane via the analysis of six spiked test articles. The 10-µg/g level of 1,4-dioxane was chosen because it is the upper limit that a sample can contain per the USP. The test articles were obtained by spiking a stripped portion of Triton X-100 with the appropriate amount of the analyte. All the spiked test articles for each assay were generated from one lot of stripped Trition X-100. The precision of these six spiked test articles is represented as the percent relative standard deviation (%RSD). The percent recovery for accuracy was calculated as follows:

$$\text{Percent recovery} = \frac{\text{Experimental concentration}}{\text{Known concentration of the spike}} \times 100 \quad \text{Eq. 1}$$

Specificity was addressed by preparing the unstripped article in triplicate and evaluating the results obtained by both test procedures. Sources of differences between the two separate assays include potential sample-to-sample variation and the presence of potential interferences

The specificity of the GC–MS headspace method is achieved via both the GC separation and by the ion-specific MS detection. Specificity was also demonstrated in this study as the ion at *m/z* = 88 amu, representative of the 1,4-dioxane not present in the stripped test article (Figure 4). MS detection could not be used for specificity for the USP method because of the incompatibility between the packed column used in that procedure and conventional MS equipment.

In considering the specificity of the USP method, both the sample preparation and analysis portions of the entire method are pertinent. Although the USP sample preparation method can separate the analyte of interest from the bulk of the sample matrix, the procedure used is relatively nonspecific in terms of separating the analyte from other chemically similar impurities in the tested material. In addition to being highly labor-intensive, the multistep sample preparation procedure could be prone to contamination issues and may even be sufficiently aggressive so that it produces potential interferents such as sample decomposition products (sample preparation artifacts).

The GC analysis used in the USP procedure couples packed column technology with FID detection. The packed column technology offers relatively low separation efficiencies, and the FID detection is universal in the sense that it responds well to a large number of organic compounds. Thus, the inherent specificity of the USP analytical methodology is limited.

The practical manifestation of this limited specificity is the appearance of an interferent peak in the sample chromatograms obtained for the materials tested in this study. This interferent peak directly but variably coeluted with the analyte peak. As shown in Figures 5 and 6, the nature of the coelution varies from direct coelution (a single, superimposed peak) to, at best, the appearance of a discernible shoulder on the analyte peak.

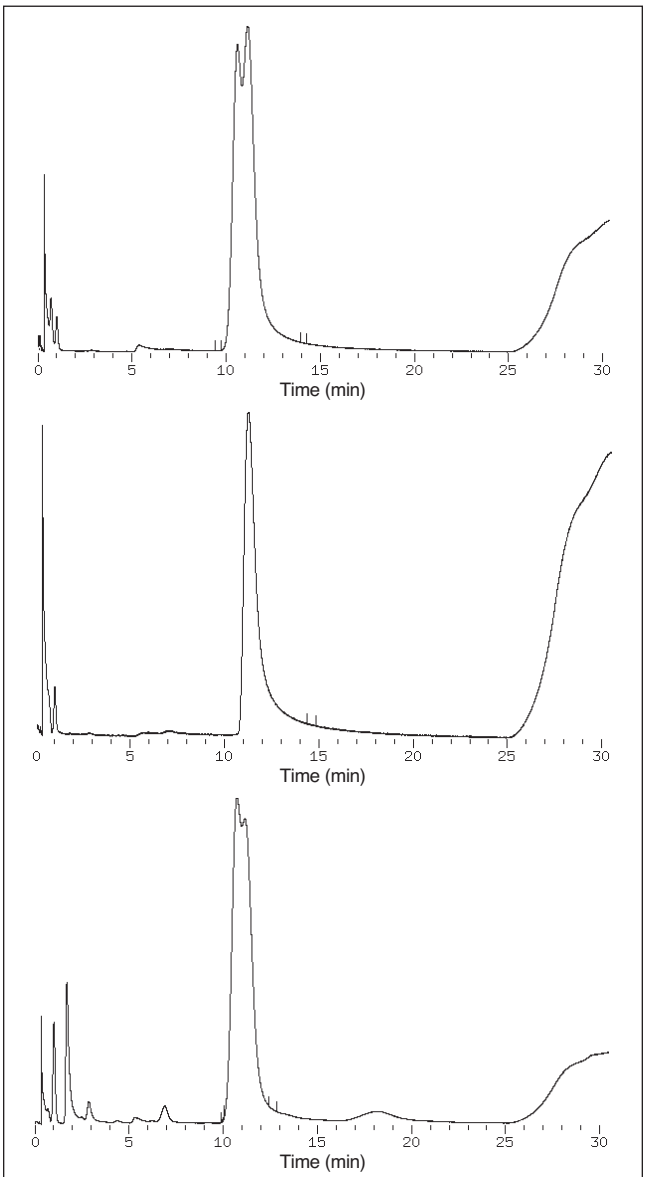


**Figure 5.** Typical chromatograms of the USP method. The chromatograms shown are (from top to bottom): the stripped sample spiked to contain 10 µg/g 1,4-dioxane, stripped sample, and unstripped sample. The presence of an interfering, later-eluting entity is clearly illustrated as the only peak observed in the stripped sample chromatogram. The presence of this peak in the stripped sample indicates that the unknown responsible for the peak is nonvolatile.

Because the peak shapes observed in these figures were reproducible over replicate sample runs, it was clear that the peak shapes were not a manifestation of declining column performance, but rather a true reflection of the composition of the samples. Although the various peak situations could be delineated in this study (i.e., peaks could be assigned to the analyte and interferent), the ability to do so is not implicit in the USP procedure, but rather results from the experiments as they were performed in this study. Though the use of a stripped sample (blank) and the availability of GC–MS results in this study facilitated the delineation of analyte and interferent peaks, such supporting information would not be available in routine applications of the USP method.

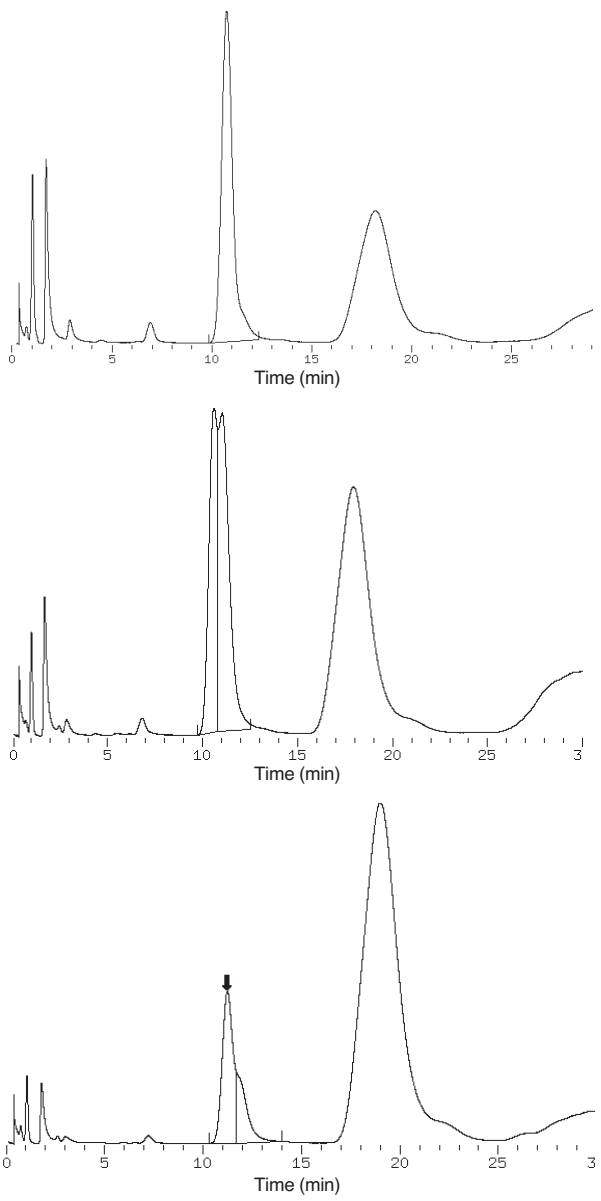


**Figure 6.** Typical chromatograms of the USP method. The three chromatograms were the results of the GC–FID analysis of the three separate sample preparations of the nonstripped Trition X-100 test article per the USP method. The peak profile of each sample is substantially different with respect to the ability to resolve the analyte (peak denoted by the arrow) from an interferent. The variable ability to resolve the analyte from the interferent results in a situation that ranges from no significant bias (bottom chromatogram, analyte peak height can be fully distinguished from the interferent response) to a large bias (top chromatogram, analyte and interferent peak heights are superimposed). The arrow on the bottom chromatogram denotes the 1,4-dioxane peak.

To amplify this scenario somewhat, Figure 5, which compares chromatograms generated from the stripped and unstripped samples, clearly shows the presence of the interfering entity. The fact that this interferent is not the primary analyte is confirmed by the GC–MS headspace analysis, which demonstrated that the stripped sample contained no 1,4-dioxane.

The results of the analysis of the spiked stripped test material and actual unstripped TritonX-100 raw material are summarized in Tables III and IV. Armed with the information made available by the experiments performed in this study, it was possible for the analyst to interpret the complex peaks produced by the USP method. This capability greatly improved the apparent performance of the USP method. Even so, the precision of the USP methodology is poor, as reflected in a 43% RSD for the analysis of the spiked stripped sample and a 28.8% RSD for the analysis of the unstripped raw material. One reason for this poor precision is the difficulty that the analyte and interferent peaks can be visually resolved so that integration can be accomplished. Even though a

**Table III. Analysis Results and Triton X-100 Raw Material**

| | Measured level of 1,4-dioxane (ppm) | |
|---|---|---|
| Replicate | From USP method | From GC–MS method* |
| 1 | 20.4 | 23.54 |
| 2 | 19.1 | 23.60 |
| 3 | 31.5 | 22.20 |
| Mean | 23.7 | 23.11 |
| %RSD | 28.8 | 3.42 |
| | | Specific bias: 1.03 |

* Data related to the performance characteristics of the analytical runs in which these results were generated are summarized in Table V.

**Table IV. Analysis Results and Triton-X Raw Material, Stripped and Spiked to Contain 10 ppm 1,4-Dioxane**

| | Measured level of 1,4-dioxane (ppm)* | |
|---|---|---|
| Replicate | From USP method | From GC–MS method† |
| 1 | 7.78 | 9.65 |
| 2 | 12.98 | 10.33 |
| 3 | 21.87 | 9.35 |
| 4 | 9.80 | 10.10 |
| 5 | 10.01 | 9.75 |
| 6 | 9.4 | 10.04 |
| Mean | 12.0 | 9.87 |
| %Recovery | 115.9% | 98.7% |
| %RSD | 42.9 | 3.59 |
| | | Analytical bias: 1.22 |

* A blank (stripped TritonX-100 material) was analyzed and found to contain no detectable 1,4-dioxane.
† Data related to the performance characteristics of the analytical runs in which these results were generated are summarized in Table V.

baseline can be drawn and a peak height assigned to the analyte peak, ascertaining the analyte peak apex is difficult and nonreproducible. This difficulty is highlighted in the analysis of the spiked stripped sample, wherein one of the six USP replicates generated a result that was discernibly different than the other five. The imprecision of the USP method was clearly not inherent to the GC methodology because replicate injections of the standard ($n$ = 6, analyzed at the front of the test sample runs) generated precisions that were no greater than 1.5%. The GC–MS methodology, with no interference issues, produced information that had a high level of precision (%RSD of 4 or less).

The percent recovery results for the six analyses of the spiked stripped samples reflect the inherent accuracy of both methodologies. The inherent accuracy of both the USP and GC–MS methods was good, with a mean recovery of the spike of 116% for the USP method and 99% for the GC–MS method. The actual mean concentrations obtained for the spiked stripped material were 12.0 µg/g for the USP method and 9.87 µg/g for the GC–MS method. The ratio of the mean result obtained from the USP method versus that obtained from the GC–MS method is termed the analytical bias. The value obtained for this ratio was 1.22 and potentially indicates that the USP method has a positive bias versus the GC–MS method. However, it was observed that this value of 1.22 may also reflect the high degree of imprecision in the USP methodology.

The results obtained for the three analyses of the unstripped raw material reflect the true bias of the two analytical methods. The mean results obtained for both methods were similar, 23.7 µg/g for the USP method and 23.1 µg/g for the GC–MS method. The ratio of the mean result obtained from the USP method versus that obtained from the GC–MS method is termed the specific bias. The value obtained for the specific bias was 1.03.

As the analytical bias and specific bias were of similar magnitude, it was concluded that the USP method, when fortified with information that allows for the delineation of analyte and interferent responses, and the GC–MS method are similar in accuracy. However, if the analyst cannot effectively distinguish between the analyte and interferent in the USP method, this method will produce results for 1,4-dioxane that potentially have a large positive bias (i.e., that overestimate the level of 1,4-dioxane). This bias is illustrated by the analysis of the stripped sample. The GC–MS methodology confirms that the stripping process was complete and that the stripped sample contained no 1,4-dioxane. With the USP method, however, a quite different result was obtained. If the analyst was unaware of the small retention time difference between the analyte and the interferent, one might mistakenly report 1,4-dioxane as being present in the stripped sample when in fact the sample contained no 1,4-dioxane. Had this scenario been realized in this study, the USP method would have erroneously given the stripped sample a 1,4-dioxane content of nearly 7 ppm.

Results obtained via the GC–MS headspace method are summarized in Table V.

## Conclusion

The USP method, consisting of sample preparation and instrumental analysis steps, was found in this investigation to be susceptible to matrix-related assay interferences. Neither the sample preparation nor analysis procedures are sufficiently specific, alone or combined, to allow an analyst to routinely and confidently distinguish between a clean analytical peak and one whose peak shape is influenced by a coeluting interferent. The interference observed in this study is direct but somewhat variable in its manifestation. The elution properties of the analyte and interferent are such that the coelution varies from direct overlap (exact retention time matched with the appearance of a single well-shaped peak) to partial overlap (interferent shoulder on the main analyte peak). In the absence of the diagnostic information available in this study, the inability to differentiate between a pure peak and one compromised by a coeluting interferent can lead to a potential positive analytical bias and a greater degree of imprecision.

Utilizing a more efficient separation technology, a reproducible and selective sample preparation method, and a highly specific single-ion monitoring MS detection, the GC–MS headspace method was found to be a highly accurate and selective means for performing the desired analysis.

**Table V. Standard Addition Curve Results and GC–MS Headspace Method**

| | | Calibration line | | | Concentration (µg/g, ppm) | | |
|---|---|---|---|---|---|---|---|
| Sample | Rep. | Intercept | Slope | $r^2$ | Estimated | 95%, Low* | 95%, High* |
| Spiked stripped | 1 | 1.02 | 0.11 | 0.9863 | 9.65 | 8.26 | 11.28 |
| | 2 | 1.06 | 0.10 | 0.9934 | 10.33 | 9.31 | 11.46 |
| | 3 | 1.03 | 0.11 | 0.9874 | 9.35 | 8.04 | 10.88 |
| | 4 | 1.07 | 0.11 | 0.9888 | 10.10 | 8.81 | 11.59 |
| | 5 | 1.09 | 0.11 | 0.9853 | 9.75 | 8.30 | 11.44 |
| | 6 | 1.05 | 0.10 | 0.9937 | 10.04 | 9.06 | 11.13 |
| Unstripped | 1 | 2.40 | 0.10 | 0.9873 | 23.54 | 21.21 | 26.26 |
| | 2 | 2.43 | 0.10 | 0.9700 | 23.60 | 20.12 | 28.04 |
| | 3 | 2.42 | 0.11 | 0.9784 | 22.20 | 19.35 | 25.72 |

* High and low refer to the upper and lower boundaries of the 95% two-sided confidence interval for the estimate of the concentration from the standard addition regression model.

## References


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