A biotechnology company requested analysis of residual 1,4-dioxane in a polymer. The polymer also contained residual amounts of ethylene oxide, propylene oxide and isopropanol. The specification for residual 1,4-dioxane in the polymer was to be no more than 0.5 parts per million (ppm).

The company required the test method be qualified for on-going non-GMP testing activities.

The polymer was soluble in water. A method for quantitation of 1,4-dioxane was developed, ensuring adequate resolution from the other three known components.

Data was collected for specificity, linearity, detection limit, quantitation limit, range, precision (repeatability and intermediate precision), accuracy/recovery, robustness, and solution stability. The qualification activities were performed based on the requirements for a test method validation (outlined in FDA guidance and ICH Q2 (R1) requirements).

Headspace Gas Chromatography – Flame Ionization Detector (HS GC-FID)

Headspace GC-FID ANALYSIS

The polymer samples were prepared by dissolving the samples 1:1 (w:w) with water.

Stock standards of 1,4-dioxane were prepared in DMSO. Linearity standards were prepared by serial dilution in water with resulting concentrations ranging from 0.5 ppm to 15 ppm. The presence of 1,4-dioxane in the prepared sample solutions was confirmed by retention time comparison with the reference standards. The concentration was determined by comparing the instrument response of the linearity curve and the 1,4-dioxane in the sample solution.

RESULTS

The qualification activities yielded the following:

- Linearity: The calibration curve was linear with an $R^2 \geq 0.99$. 
System Suitability: The system suitability concentrations were ± 30% of the theoretical concentrations.

Specificity: The peak corresponding to 1,4-dioxane was visually distinguishable from adjacent peaks in all chromatograms. The resolution, R, between 1,4-dioxane peak and any adjacent peaks was at least 0.5.

Detection Limit: The 1,4-dioxane at a concentration of 0.25 ppm had a signal to noise ratio of not less than 3.

Quantitation Limit: The 1,4-dioxane at a concentration of 0.5 ppm had a signal to noise ratio of not less than 10.

Accuracy: The percent recovery of 1,4-dioxane was ± 30% of the anticipated concentration throughout the range of the method.

Precision (Repeatability): The % RSD of the percent recoveries of 1,4-dioxane was not more than 15.0 % throughout the range of the method.

Precision (Intermediate Precision): The % RSD of the percent recoveries of 1,4-dioxane at the middle level spiked concentration was not more than 15.0 %. The combined % RSD for the percent recoveries determined by two different analysts was not more than 20%.

Robustness: The headspace oven and the GC inlet temperatures were increased and decreased by 5°C from the original method. The percent recovery of 1,4-dioxane was ± 15% of the anticipated concentration with the method variations.

Solution Stability - Headspace vial: The 1.25 ppm spiked polymer sample solution and a 1 ppm standard solution were considered stable when stored in a sealed headspace vial under ambient conditions for no more than 5 days.

Solution Stability – bench top (ambient room temperature): The 1.25 pm spiked polymer sample solution was considered stable when stored at ambient conditions in a screw top vial for no more than 2 days. The 1 ppm standard solution was considered stable when stored at ambient conditions in a screw top vial for no more than 4 days.
• Solution Stability – refrigerated (2-8°C) conditions: The 1.25 ppm spiked polymer sample solution and a 1 ppm standard solution were considered stable when stored in a screw top vial under refrigerated (2-8°C) conditions for no more than 5 days.

Representative chromatograms are outlined below:

Figure 1. Representative chromatogram of a water blank.

Figure 2. Representative chromatogram of a 1-ppm mixed standard.
**CASE STUDY:**
**ANALYSIS OF 1,4-DIOXANE IN POLYMER**

Figure 3. Representative chromatogram of an unspiked polymer sample.

Figure 4. Representative chromatogram of a 0.25-ppm spiked polymer sample.

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