The Analysis of Residual Solvents in Pharmaceutical Products Using GC-VUV and Static Headspace

Introducing a New Method for Faster Sample Throughput and Shorter GC Runtimes

APPLICATION NOTE

Introduction

Volatile organic compounds are used in pharmaceutical manufacturing during the production of drug substances, pharmaceutical additives, and drug products. Known also as residual solvents, they account for 50-90% of mass in typical pharmaceutical operations and represent most of the process toxicity. These organic solvents can contaminate the drug product during its packaging, storage, and transportation. Testing for the presence of these solvents in Active Pharmaceutical Ingredients (APIs) is critical for patient safety and commonly follows Unites States Pharmacopeia (USP) Method <467> guidelines, or more broadly, International Council for Harmonization (ICH) Guideline Q3C(R6). The toxicity limits analyzed by USP Method <467> are harmonized to be equivalent to the concentration recommendations of ICH Q3C(R6) guidelines.

The gas chromatography (GC) runtime suggested by USP Method 467 is 60 min. Each solvent class is injected separately for analysis to ensure complete separation of solvent compounds. In addition, it is recommended that Class 1 and 2 solvents be identified and quantitated by complementary methods utilizing different stationary phase selectivity when solvents are found to meet or exceed the permitted daily exposure (PDE) concentration limits.

A Gas Chromatography – Vacuum Ultraviolet (GC-VUV) method was developed to significantly reduce the analytical bottleneck involved in the testing of residual solvents in pharmaceutical excipients and products. The GC-VUV method has been shown to result in >5X reduction of GC runtime while allowing the combination of residual solvent classes into a single run. It utilizes the unique capability of VUV spectroscopy to produce spectral "fingerprints" of most compounds, as well as the ability to easily deconvolve co-eluting species through VUV software algorithms.

GC-VUV spectral data is inherently three dimensional (time, absorbance, wavelength) and specific to compound chemical structure. Most compounds absorb strongly in the VUV region (120 – 240 nm) of the UV spectrum measured by VUV detection. Photons in this wavelength range are capable of producing electronic transitions in virtually all chemical bonds, especially in ground state to excited state transitions such as $\sigma \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$. The result is spectral signatures that are specific to each compound and can be readily identified by the VUV library. This characteristic of VUV spectroscopy lends itself to intentional chromatographic compression due to the ability to deconvolve overlapping spectral responses.

The GC-VUV method for residual solvent analysis described below offers a few key advantages over USP Method 467 experimental conditions. First, VUV absorbance spectra provide authoritative solvent identification while providing compound quantitation. A separate method is not required to verify compound identity or to provide quantitative analysis. Second, chromatographic compression is possible without any loss of data quality. Runtimes of 8 minutes or less have been shown to be sufficient for the analysis of Class 1 and 2 residual solvents. Third, different residual solvent classes can be analyzed in a single run. The VUV detector delivers a wide linear range, detection limits that

VUV ANALYTICS

extend beyond PDE concentration boundaries, and the ability to deconvolve analyte co-elution.

Experimental

- Instrumentation

GC-VUV analysis was completed with the following setup:

Detector: VUV Analytics VGA-100

Static Headspace Sampler: GERSTEL MPS2

Gas Chromatograph: Agilent 6890

Column: Restek 30m x 0.25mm x 1.40µm Rxi-624Sil MS

Experimental conditions for the gas chromatograph, static headspace sampler, and detector are provided below.

- Gas Chromatograph

An Agilent 6890 gas chromatograph was used with its injection port set to 250°C. A helium carrier gas flow rate of 4 mL / min was used throughout the experiment. The oven profile for the residual solvent analysis began at 35°C (held for 1 min), followed by an increase to 245°C at a rate of 30°C/min. A split ratio of 2.5 was utilized to help maximize sensitivity. A total GC runtime of 8 minutes was programmed for each sample analyzed.

- Static Headspace Sampler

A GERSTEL Multi-Purpose Sampler (MPS) was used to provide optimal temperature and injection setting automation and the most efficient delivery of residual solvent samples to the GC. The syringe temperature was held constant at 90°C. An incubation temperature of 80°C was set to best volatilize residual solvents while not going above the boiling point of water. The incubation time was set to 10 min to closely match the GC run time of 8 min. An injection volume of 250 µL was selected to ensure good peak shape. The agitator was 250 rpm (10 sec on, 1 sec off), and an injection speed of 200 µL/sec was used for sample introduction.

- VUV Detector

A VGA-100 gas chromatography detector manufactured by VUV Analytics was used in this experiment. The transfer line & flow cell temperatures were set at 275°C. The makeup gas pressure used was approximately 0.36 psi. An acquisition range of 120 to 240 nm was selected with an acquisition speed of 4.5 spectra/sec.

Results and Discussion

- Class 2 Residual Solvent Analysis

Initial experiments undertaken to demonstrate the chromatographic capabilities of GC-VUV when applied to residual solvent analysis used citric acid as a model excipient. Samples were prepared by mixing 2-mL of water with 100 mg of citric acid and spiking Class 2 Solvent Mix A and B (Restek) to meet the desired concentration limits. The total GC runtime was set to 8 minutes as described in the Experimental section. Figure 1 shows the GC-VUV chromatogram of these Class 2 solvents. Tetralin was the last analyte to elute in the chromatogram and had a retention time of less than 7 minutes. All Class 2 solvent analytes were identified in the condensed chromatogram despite the occurrence of co-elution.

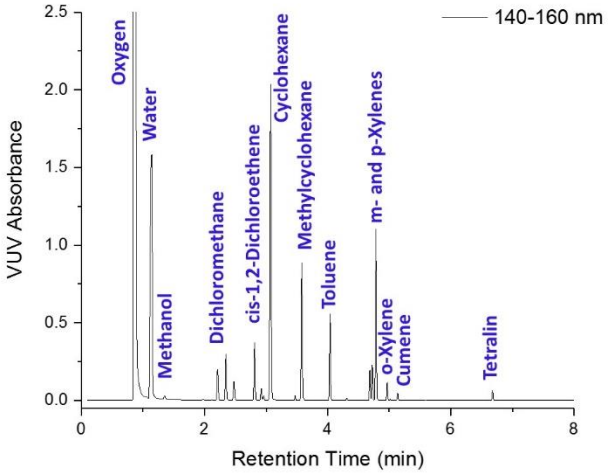

Figure 1: Compressed gas chromatogram showing Class 2 residual solvents separated and identified in less than eight minutes. The typical runtime for the USP 467 method is 60 min.

Residual solvents, as is true for most compounds, have unique spectral features in the VUV wavelength range. This characteristic is demonstrated in the spectral overlay of select Class 2 solvents shown in Figure 2. The co-eluting isomers m- and p-xylene have distinct spectra that provide for straightforward identification and chromatographic peak deconvolution. VUVision software matches analyte spectra with compounds in the VUV library. Goodness of fit information is provided to ensure that correct compound identification takes place during data analysis.

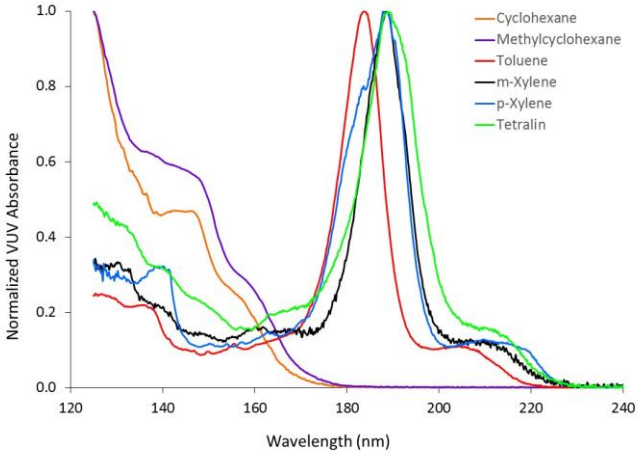

Figure 2: VUV spectral overlay of Class 2 residual solvents. Spectra are distinct despite chemical similarities between compounds and can be reproducibly identified by the VUV library.

Once it was demonstrated that the 8-minute GC-VUV method provided adequate separation of solvent analytes for identification and quantitation purposes, the experiment was repeated to test the VUV detector linearity at concentrations ranging from 2X to 0.1X their ICH permitted daily exposure (PDE) concentration limits. All toxicity concentration range experiments are shown in terms of their ICH limits due to their equivalency with USP <467> guidelines. Figure 3 shows the chromatographic linearity of chlorobenzene, ethylbenzene, m- and p-xylene, and o-xylene. The predictable changes in response held true even at low ppm concentrations.

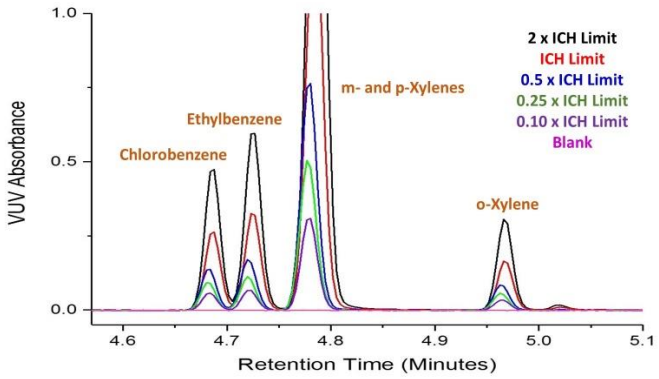

Figure 3: Chromatographic view of Class 2 residual solvents shown at concentrations ranging from 2X to 0.1X their ICH permitted daily exposure (PDE) concentration limits. VUV detection demonstrates a good linear response across the ranges shown, even at low ppm detection limits.

The same linear behavior can be observed in the zoomed in GC-VUV chromatogram of Figure 4 showing cumene analyzed from 140 to 7 ppm. Excellent linearity of 0.9992 R^2 across this range can be seen in Figure 5.

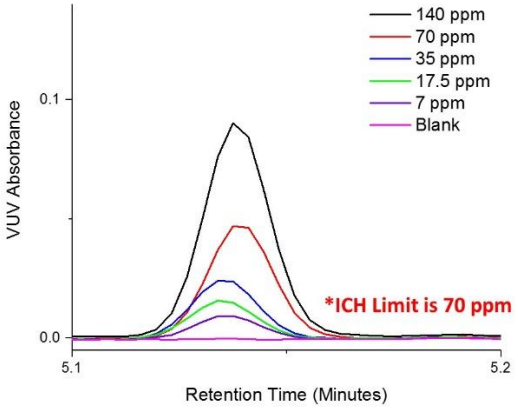

Figure 4: Chromatographic comparison of cumene ranging from 2X to 0.1X its ICH concentration limit.

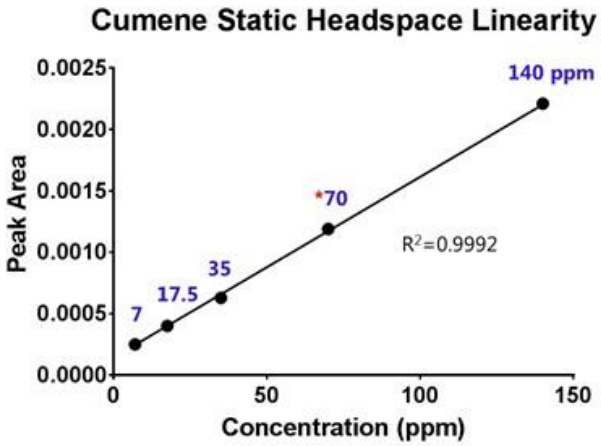

Figure 5: Linearity plot of cumene analyzed across a 2X to 0.1X ICH concentration limit range.

Comparable results were observed when dichloromethane was analyzed from 1200 to 60 ppm. Figure 6 shows a chromatographic comparison of concentrations ranging from 1200 to 60 ppm and Figure 7 reports the R^2 linearity of 0.9969.

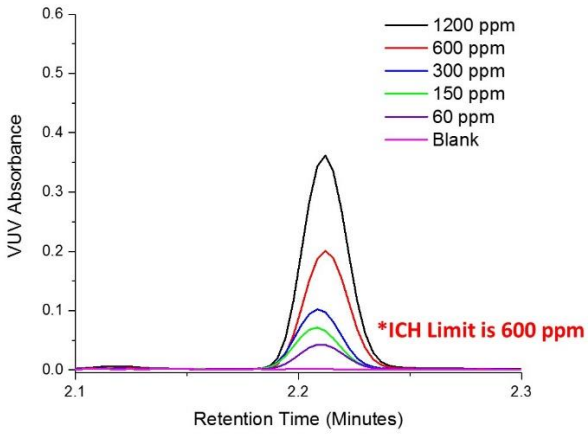

Figure 6: Chromatographic comparison of dichloromethane ranging from 2X to 0.1X its ICH concentration limit.

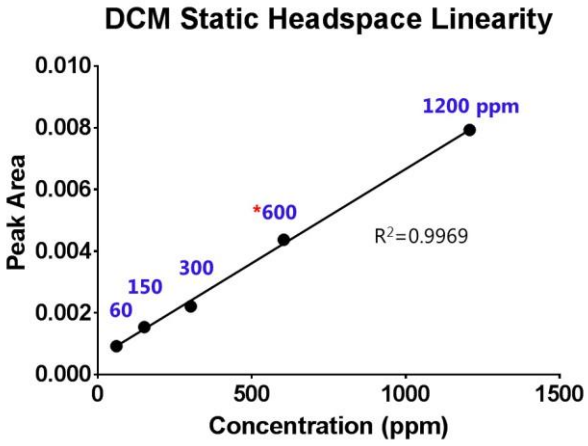

Figure 7: Chromatographic linearity of dichloromethane across a 2X to 0.1X ICH concentration limit range.

- Class 1 & 2 Combined Analysis

The next step in optimizing the GC-VUV method for residual solvents analysis was to characterize them within the context of a pharmaceutical product matrix. Generic over the counter throat spray was selected as the test case, and a standard mixture of Class 1 and 2 solvents was spiked into 2 mL of solution at concentrations ranging from 2X to 0.1X the ICH limits of target analytes.

Figure 8 shows a chromatographic representation of the linearity of benzene from 2X to 0.1X its ICH concentration limit. The plot in Figure 9 demonstrates the ability of the VGA-100 detector to provide an acceptable linear response (R^2 = 0.9938) at low to sub-ppm concentration ranges (4 ppm – 0.2 ppm).

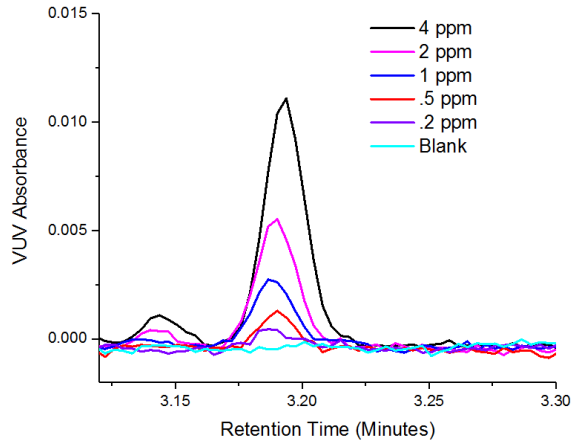

Figure 8: Chromatographic comparison of benzene shown at concentrations ranging from 2X to 0.1X its ICH concentration limit of 2 ppm.

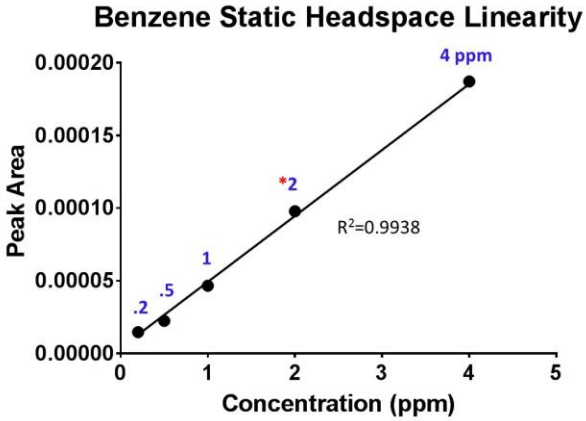

Figure 9: Chromatographic linearity of benzene across a 2X to 0.1X ICH concentration limit range.

Another important aspect of assessing GC-VUV capabilities in residual solvent analysis is to determine its reproducibility in a pharmaceutical product matrix. Class 1 and 2 residual solvent standards were spiked into generic throat spray at 0.5X the ICH concentration limit of the targeted analytes. Figure 10 shows a chromatographic comparison of Class 2 solvent cumene with five replicate injections. VUV detection resulted in an impressive RSD of 2.8% at 35 ppm. Reproducibility performance remained consistent at even at the lowest of ppm concentrations. An RSD of 6.6% was observed when Class 1 solvent benzene was injected five times at 1 ppm, as demonstrated in Figure 11.

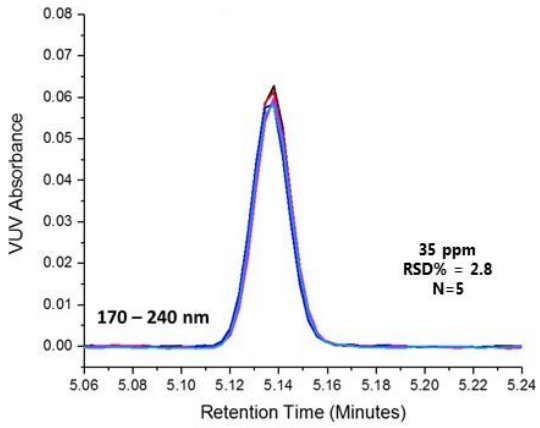

Figure 10: Cumene chromatographic reproducibility shown at 0.5X its ICH concentration limit.

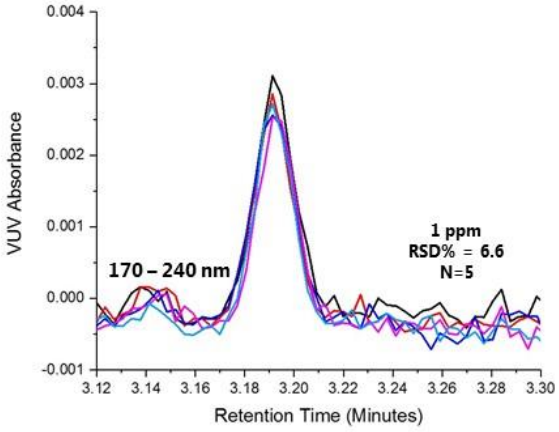

Figure 11: Benzene chromatographic reproducibility shown at 0.5X its ICH concentration limit.

As noted previously, one the primary strengths of the GC-VUV method for residual solvents analysis is the ability to compress chromatography and utilize spectral deconvolution during post-run analysis. An example of a forced co-elution can be seen in Figure 12. In the full spectrum view (125 – 240 nm) benzene appears as a shoulder of 1,2-dichloroethane. Spectral filters have been applied during post-run analysis to visualize the relative absorbance of each compound at different

wavelength ranges. When viewing the spectral profiles of 1,2-dichloroethane and benzene in Figure 13 it is apparent that both compounds absorb strongly in different regions of the VUV spectrum. 1,2-Dichloroethane absorbs most strongly between 125 – 160 nm whereas benzene has its peak absorption between 170 – 240 nm. The application of spectral filters can be an effective quantitation tool when there is no spectral overlap of competing analytes. In the case of co-eluting compounds like 1,2-dichloroethane and benzene, the most effective means of quantitating their relative concentrations is to take advantage of VUVision software deconvolution. Because co-eluting analyte spectra can be reliably matched with compounds in the VUV library, VUV software algorithms can reconstruct their chromatographic peaks based on the wavelength, absorption, and time data collected during the GC run. Figure 14 shows the reconstructed chromatogram peaks of 1,2-dichloroethane and benzene that reflect their relative abundance in the sample.

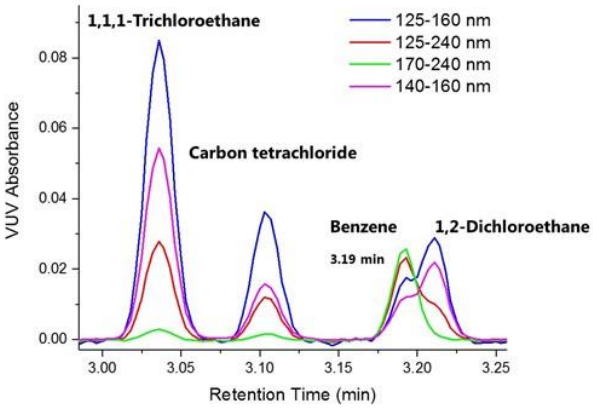

Figure 12: Co-elution of 1,2-Dichloroethane and Benzene. Spectral filter overlays show how the peak profiles of co-eluting compounds are influenced by the wavelength ranges where they absorb.

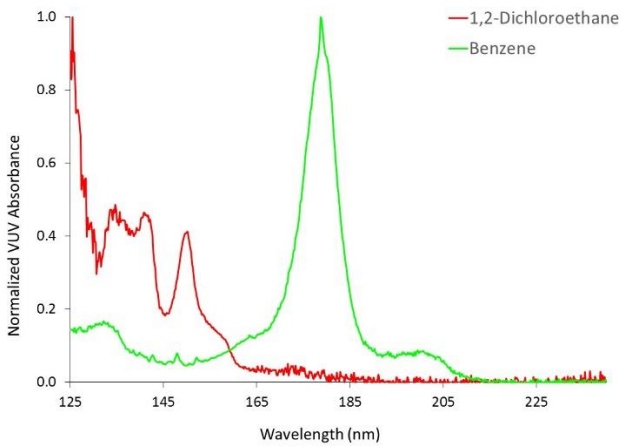

Figure 13: Spectral comparison of 1,2-Dichloroethane and Benzene. The differences in spectral features of the co-eluting compounds enable spectral matching with the VUV library and VUVision software deconvolution.

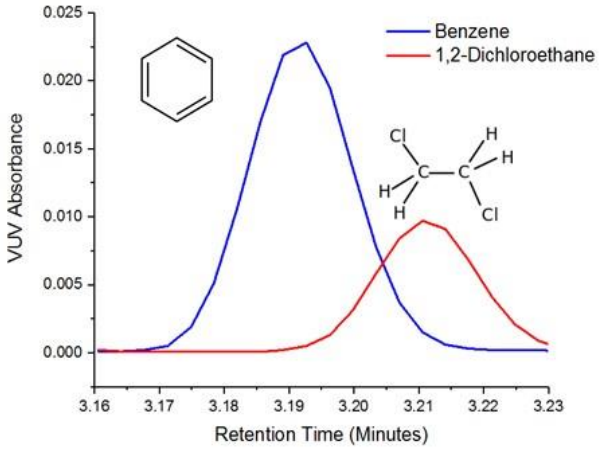

Figure 14: Deconvolution of 1,2-Dichloroethane and Benzene. Co-elution is completely resolved using VUVision software deconvolution, and the relative amounts of each compound are represented in the reconstructed chromatogram.

Conclusion

A novel GC-VUV method for the analysis of residual solvents in pharmaceutical products has been demonstrated to significantly reduce GC runtimes and increase sample throughput as compared with USP 467 experimental conditions. GC runtimes can be reduced by 5X or greater, and different solvent classes can be combined into the same run for increased sample throughput. Chromatographic compression is made possible by the unique spectral signatures of compounds in the VUV range that enable straightforward deconvolution of co-eluting analytes. The GC-VUV method was shown to provide a linear, highly reproducible response at concentrations ranging from 2X to 0.1X the Class 1 and 2 PDE detection limits. The analysis of residual solvents by GC-VUV delivers a single method for the identification and quantitation of toxic compounds in pharmaceutical products.

For more detailed information please visit our website at www.vuvanalytics.com, or contact us at info@vuvanalytics.com.

Acknowledgements

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