TECHNOLOGY BRIEF

MEASURING MOISTURE IN ACETONITRILE USING GAS CHROMATOGRAPHY AND THE LUMA™ MULTI-CHANNEL VACUUM ULTRAVIOLET (VUV) DETECTOR: TRACE LEVELS AND BEYOND

GOAL

Demonstrate reliable measurement of water at trace levels and beyond in acetonitrile using gas chromatography (GC) combined with the LUMA Multi-Channel Vacuum Ultraviolet (VUV) Absorbance detector. Additionally, we will demonstrate the ability of LUMA to detect water and multiple solvents in a single analysis.

BACKGROUND

The water content of organic solvents is one of the crucial properties that affect the quality of the products and the efficiency of the manufacturing processes. In many organic solvents, water is generally considered an impurity, and therefore its measurement in solvents is of importance for many industries and technologies. This is especially true, for example, when synthesizing oligonucleotides where excess moisture can increase the rate of hydrolysis of phosphodiester bonds, which can lead to reduced product yield and degraded product quality.

Traditionally, Karl Fisher (KF) titration has been the leading method for moisture analysis. While this technique has several useful characteristics including a wide dynamic range and a potentiometric end point. It has several drawbacks as well. Most notably KF titration is susceptible to side reactions that interfere with results, it is not well suited for measuring trace levels of moisture, it is labor intensive, does not lend itself to high throughput environments and requires harsh solvents. Therefore, a rapid, simple, and sensitive alternative approach for the determination of trace amounts of water in organic solvents is very appealing.

Gas Chromatography (GC) is a powerful analytical technique used to separate, identify, and quantify volatile compounds. GC is routinely used to analyze acetonitrile, a volatile and reactive solvent commonly used in many laboratory applications. While GC is often used to analyze residual solvents the ability to measure moisture reliably and at trace levels has been challenging due to existing detection technologies.

Barrier Discharge Ionization [BDI] detectors are highly sensitive and can measure moisture down to sub-ppm levels. Unfortunately, they have reduced dynamic range and nonlinear response.

LUMA Multi-Channel Vacuum Ultraviolet Absorbance Detector

MEASURING MOISTURE IN ACETONITRILE USING GAS CHROMATOGRAPHY AND THE LUMA™ MULTI-CHANNEL VACUUM ULTRAVIOLET (VUV) DETECTOR: TRACE LEVELS AND BEYOND

Thermal conductivity detectors [TCD] require high volumes of helium (which is in short supply), have low sensitivity and suffer from interferences caused by carrier gas impurities and stationary phase bleeding. Flame Ionization detectors [FID] show little response to water, requiring indirect methods involving complex reactions. While they can get to <10 ppm levels, methods using FID are destructive, and cannot detect several functional groups, such as carbonyl, alcohol, halogen, and amine. Other indirect methods, such as fluorine NMR (19F-NMR), are highly sensitive but require complicated methods and access to expensive, high-end instrumentation.

LUMA, a new multi-channel vacuum ultraviolet absorbance detector overcomes the issues described with existing GC detection technologies. LUMA is unique in that it is the first absorbance detector for GC that works from 118nm to 1050nm which includes the vacuum ultraviolet range (118nm – 240nm) of the electromagnetic spectrum where nearly all compounds absorb, except for the carrier gases Helium, Hydrogen, and Argon – making it a universal detector. LUMA is sensitive to sub picogram levels – making it ideal for trace and ultra-trace level analysis. Additionally, LUMA acquires up to 12 channels of data providing for channel selectivity. This channel selectivity allows for a wide dynamic range and the ability to look for many different compounds in a single analysis. Finally, LUMA is easy-to-use and operate. Its compact design installs on top of the GC, does not require separate gases, works with existing chromatography data systems, and does not require a vacuum pump.

In this work, we present a GC method using a LUMA Multi-Channel VUV Absorbance detector to analyze moisture in acetonitrile from 250ppb through to percent levels. This approach has several advantages over traditional techniques like Karl Fischer titration including superior sensitivity for the analysis of trace levels of water in acetonitrile as well as percent levels. The achievable limit of detection for water in acetonitrile is 250ppb, with method linearity over 0.25ppm – 100ppm producing R^2 ≥.999.

SOLUTION

SENSITIVITY, LINEARITY, AND REPEATABILITY

To demonstrate feasibility, the GC-VUV separation using the LUMA Multi-Channel Vacuum Ultraviolet Absorbance detector was based on a previously described method[1]. The method was changed by modifying the GC conditions, including the choice of column, to incorporate the LUMA detector. Method conditions for this experiment are described in Table 1.

GC Conditions	
Injection Volume	1µL
Inlet Temperature	200°C
Split Ratio	5:1
Column	RTX-VolatileAmine (60m x 0.32, 5 µm)
Carrier Gas	Hydrogen @ 3mL/min
Oven Program	40°C, hold 0.1 min; 35°C/min to 250°C (8 min)
Run Time	14 minutes
LUMA Conditions	
Makeup Gas / Pressure	N_2 / 5 PSI
System Gas / Pressure	N_2 / 50 PSI
Flow Cell Temperature	275°C
Transfer Line Temperature	275°C
Acquisition Frequency	5 Hz

Table 1 Instrument conditions for analysis of water in acetonitrile.

To highlight sensitivity, linearity, and repeatability it is normal to prepare a standard mixture of varying concentrations. However, to minimize the impact of environmental moisture in this experiment a single commercially available KF water standard (100 ppm water in xylene) was used as the sensitivity mixture. Replicate injections of the standard (5 at each level) were made with varying injection volumes and split ratios to simulate the following water concentrations: 0.25, 0.5, 1, 2, 5, 10, and 100 ppm (Table 2).

Standard Injection Parameters		
Injection Volume	**Split Ratio**	**Simulated Concentration**
1µL	**5:1**	**100 ppm***
1µL	**50:1**	**10 ppm**
1µL	**100:1**	**5 ppm**
0.2µL	**50:1**	**2 ppm**
0.2µL	**100:1**	**1 ppm**
0.2µL	**200:1**	**0.5 ppm**
0.2µL	**400:1**	**0.25 ppm**

Table 2 Simulated concentrations based on varying injection volumes and split ratios.

The resulting chromatogram at the default concentration (100 ppm) provides good separation of water and oxygen from the matrix, as well as good separation of xylene and its impurities (Figure 1). Figure 2 shows an overlay of the water peaks at each concentration level (0.25 ppm – 100 ppm) with excellent peak shapes.

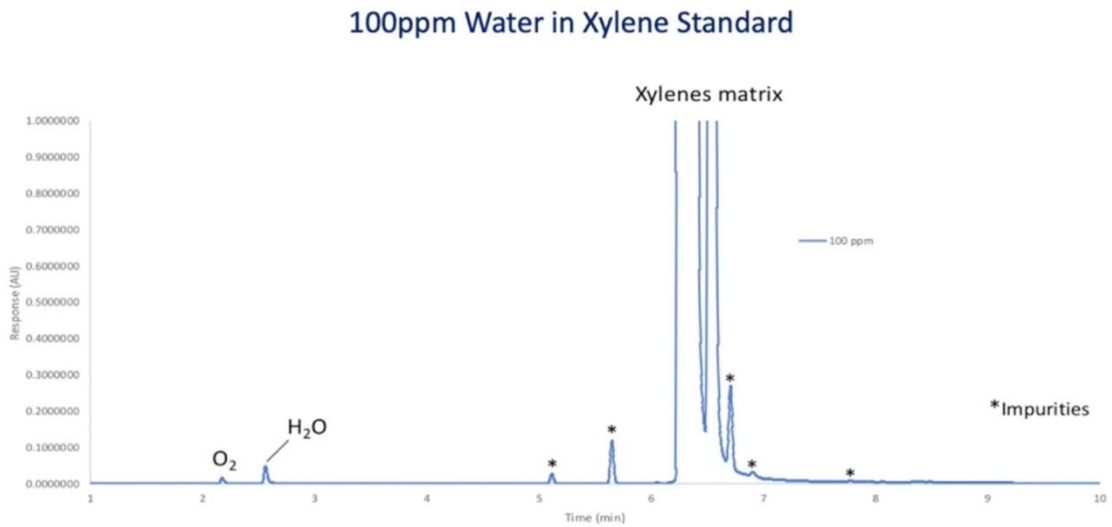

Figure 1 - 100ppm Water Standard in Xylene

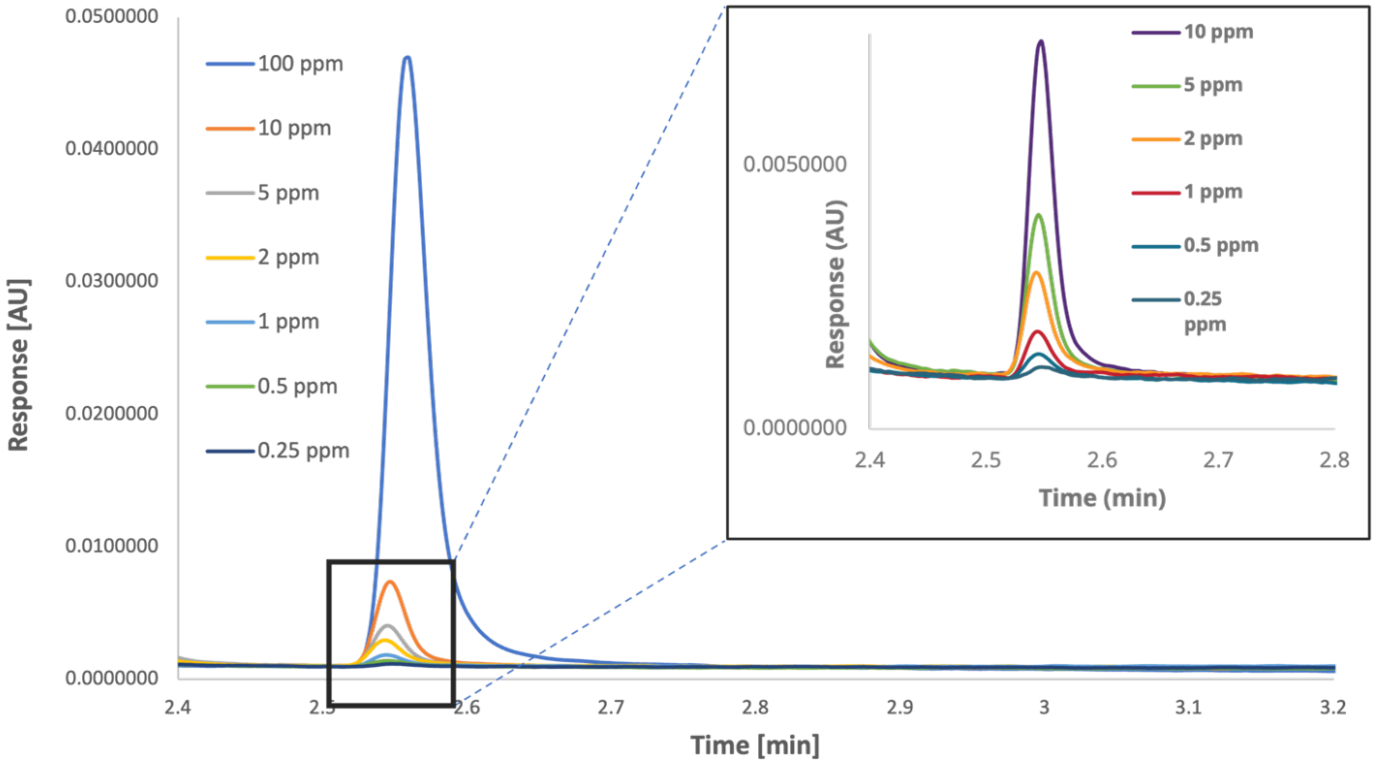

Figure 2 - Water Peak at varying concentrations from .25 ppm to 100 ppm.

Method linearity is excellent (R2 ▯.999) and was determined by plotting the peak areas of each replicate injection across all seven (7) concentration ranges (Figure 3). While this is not a strict linearity test using a set of standards, it does show the method's potential for this application.

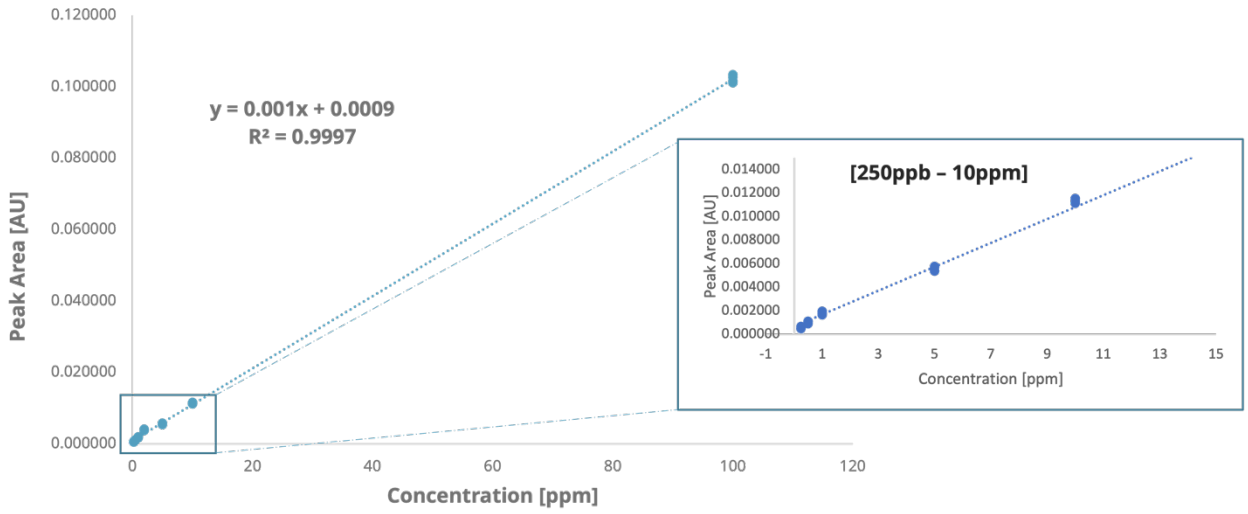

Figure 3 - Linearity curve from .25ppm - 100ppm

Good repeatability was obtained which, as expected, decreased toward the lower concentrations due to reduced sensitivity. %RSDs are displayed in Table 3. With further optimization, this method could potentially be extended to sub-100 ppb for quantitative methods.

Peak Areas								
Concentration	Inj. 1	Inj. 2	Inj. 3	Inj. 4	Inj. 5	MEAN	STDEV	% RSD
100 ppm	0.103342	0.101531	0.101111	0.102659	0.101657	0.102060	9.14E-04	0.90
10 ppm	0.011377	0.011215	0.011546	0.011130	0.011542	0.011362	1.88E-04	1.66
5 ppm	0.005732	0.005690	0.005391	0.005794	0.005380	0.005597	1.97E-04	3.52
2 ppm	0.003786	0.004103	0.003810	0.003703	0.003791	0.003839	1.53E-04	4.00
1 ppm	0.001743	0.001942	0.001965	0.001660	0.001801	0.001822	1.30E-04	7.14
0.5 ppm	0.001100	0.001002	0.000908	0.001075	0.001103	0.001038	8.31E-05	8.01
0.25 ppm	0.000557	0.000502	0.000510	0.000690	0.000500	0.000552	8.07E-05	14.62

Table 3 - Linearity data for trace level analysis.

BEYOND TRACE LEVELS

Driving efficiency and productivity in analytical science is of paramount importance to the pharmaceutical industry. With its high sensitivity, selectivity, and ease of use, LUMA would be an ideal detector for many pharmaceutical and high throughput applications. For example, it could have utility as a replacement for KF titrations for routine testing of higher levels of moisture in active pharmaceutical ingredients [APIs] and formulated products and by combining channel selectivity it's possible to perform multiple analyses in a single injection.

To test the dynamic range of the detector, samples of acetonitrile containing from 0.5% to 5% water were tested using this approach. While the λ_{max} for water occurs in Band 5 (165-172nm) on LUMA which is ideal for trace level analysis, however, linearity in this band is not optimal for higher concentration as evidenced by the data in Table 3. LUMA's unique channel selectivity makes it is possible to extend the Linear Dynamic Range of the detector by analyzing higher concentration samples in a different wavelength band. For this analysis, we switched the detector to utilize Band 3 (143-145nm) where water also absorbs. Using this wavelength band reduces sensitivity while demonstrating significantly better linearity for higher concentration samples (Figure 4).

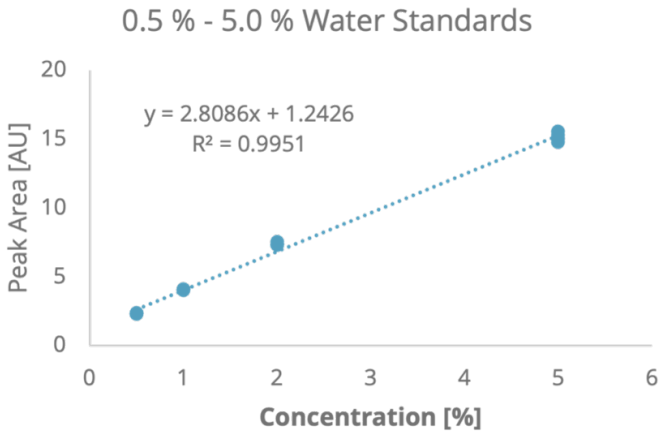

Figure 4 - Linearity from 0.5% - 5%, Water Standards.

Additionally, using the full range of LUMA bands, it was possible to demonstrate that LUMA could be used to not only determine the concentration of water in a sample but also to simultaneously determine the levels of a range of other solvents present at the 500ppm level (figure 5).

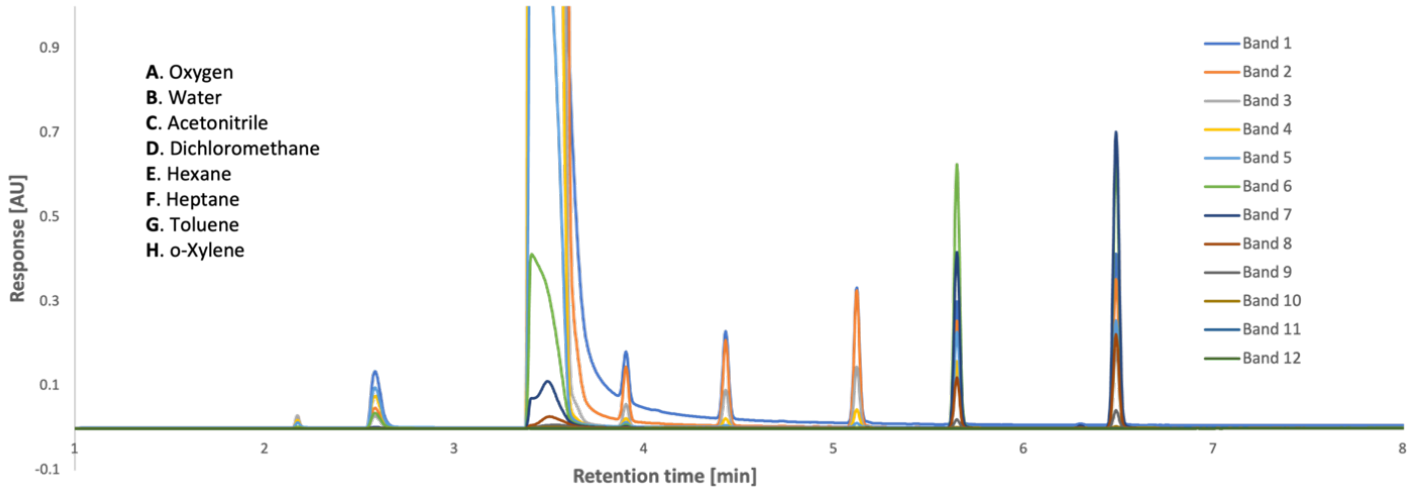

Figure 5 - Separation of Water from other organic solvents in a single analysis.

This demonstrates that instead of analyzing APIs for solvents using GC and for water using KF titration in separate tests, analysts could perform both tests in a single GC analysis which can help to drive significant efficiency and productivity in today's laboratories especially as all batches of API produced are routinely tested for moisture and residual solvents. Given the sensitivity and selectivity of the detector it is possible to build on the water and solvent experiments to demonstrate the detector's ability to determine multiple analytes in each sample. For example, water, solvents, and a range of impurities including potential genotoxic impurities (PGI's).

SUMMARY

LUMA, an innovative multi-channel VUV absorbance detector is capable of accurately determining moisture content in solvents from ultra-trace amounts to levels as high as 5%. In addition, LUMA offers the possibility of measuring water and residual solvents in a single GC analysis, eliminating the need for separate KF titrations. Additional work involving preparation of acetonitrile standards with ultra-trace moisture levels is underway to fully validate its utility. The detector operates in the 125 nm to 1050nm region of the spectrum, where nearly all compounds absorb, making it a universal GC detector. In addition to having high sensitivity and selectivity, it is easy to use, fits into existing laboratory workflows, and does not require a vacuum pump or separate special carrier gases.