

NIR Spectroscopy for Managing Water Content in the Pharmaceutical Industry



Water is vital to life. It covers 70% of the planet we inhabit and accounts for more than half our body composition. Water's unique physical properties, especially those related to intermolecular hydrogen bonding, make life on earth possible.

At the same time, these properties make retaining solvent purity difficult in some cases. The ubiquity of water therefore creates significant challenges for industries where water can interfere with product stability, chemical reactivity, and analytical accuracy. Even trace amounts of water at concentrations less than 0.01% (100 ppm) can impact the results of industrial processes.

The presence of water in solvents is tightly regulated in the pharmaceutical industry, not just for the potential to impact processes but also due to its role in supporting microbial growth. Pharmaceutical-grade solvents labeled as “anhydrous” must meet very tight moisture specifications (as low as 50 ppm depending on the solvent). Water concentration must be controlled in pharmaceutical processes to avoid a variety of undesirable consequences, including:

- Hydrolysis of pharmaceutical ingredients
- Degradation of sensitive functional groups
- Introduction of impurities from side reactions
- Unpredictable changes in crystallization behavior
- Failed Good Manufacturing Practice (GMP) validations due to residual water

Near-Infrared (NIR) spectroscopy is an important tool for industrial processes where precise, real-time quantification of moisture content is critical. By leveraging the absorbance properties of water in the near-infrared region (900 – 2500 nm), NIR spectroscopy enables real-time monitoring of water levels even at trace concentrations. This capability is crucial in industries such as pharmaceuticals, where stringent control of water content is essential to prevent degradation of active ingredients, maintain the efficacy of formulations, and ensure compliance with regulatory standards. NIR spectroscopy's rapid, non-destructive analysis makes it useful in the pharmaceutical industry but also in industries like semiconductors and energy, where controlling water content is vital for maintaining product quality and optimizing processes.



Solvents in the Pharmaceutical Industry

Solvents play a role in nearly all stages of drug development and manufacturing in the pharmaceutical industry. A list of common pharmaceutical solvents with strict water content requirements along with the specific pharmaceutical processes where they are used is shown in the table below.

Common Pharmaceutical Solvents with Water Content Requirements

Solvent	Typical Processes	Water Sensitivity
Acetonitrile	HPLC analysis (as mobile phase)	Yes – hygroscopic
	API crystallization	
	Reaction solvent in synthesis	
Methanol	API synthesis	Moderate
	Extraction of natural products	
	Mobile phase in chromatography	
Ethanol (absolute)	Solubilizing poorly soluble APIs	Yes – hygroscopic Must be dry for API stability or synthesis
	Granulation solvent in oral dosage forms	
	Herbal extract preparation	
Toluene	Solvent in API synthesis	Yes – water can cause phase separation issues
	Recrystallization of intermediates	
	Extraction	
Tetrahydrofuran (THF)	Polymer synthesis	Very – hygroscopic Forms peroxides
	Ring-opening reactions	
	Grignard and organometallic reactions	

Water content requirements for pharmaceutical solvents are set and controlled by regulatory agencies. Key regulations for the pharmaceutical industry are summarized in the table below. These regulations specify the standards and testing methodologies required for determining water content. Karl Fischer (KF) titration is widely recognized across regulatory documents as the primary method for accurate, trace-level moisture detection in solvents. NIR spectroscopy, also widely accepted, is categorized as a secondary or alternative technique qualified for routine, high throughput testing when calibrated and validated against KF titration.

Key Water Content Regulations for the Pharmaceutical Industry

Regulation	Focus	Specified Standards Sensitivity	Allowed Water Content (ppm or %)
USP <921>	Official water determination methods (Karl Fischer preferred)	Water content determination using validated methods	Typically, below 0.1% (<1000 ppm)
Ph. Eur. 2.5.12	Micro-determination of water in "anhydrous" labeled solvents	Precise measurement of water content to ensure compliance with the "anhydrous" claim	Typically, below 0.05% (<500 ppm)
ICH Q6A	Requires water content specification and validated test methods	Specification for water levels in solvents with mandatory method validation	Product-dependent Stringent control required
ICH Q3C	Addresses residual solvents, implying control of solvent quality	Water content managed within the broader framework of solvent quality control	General compliance within acceptable limits (<1000 ppm)
GMP (EU/FDA)	Requires specification, validation, and control of water in solvents for manufacturing	Strict guidelines for water content to ensure manufacturing consistency and product stability	Product-dependent Often <0.1% (<1000 ppm)

Measuring Water Content in Solvents

Both Karl Fischer (KF) titration and near-infrared (NIR) absorbance spectroscopy are used to measure water content in pharmaceutical solvents. These techniques differ in terms of accuracy, sensitivity, speed and regulatory acceptance.

KF titration is a wet chemical method that quantifies water through a chemical reaction. It is the gold standard for detecting water content in pharmaceutical solvents. It is highly accurate, sensitive, and specific, with the ability to detect trace water levels down to low ppm. The KF titration method is widely accepted and specified in regulatory guidelines. This method is best suited for batch release and stability testing with stringent water content specifications. Online Karl Fischer titration is possible for continuous or high-frequency monitoring using specialized, automated setups, but is less real-time than other techniques like NIR spectroscopy. KF titration is generally more complex, reagent-dependent, and better suited for critical process steps where extremely accurate water measurement is required.

NIR spectroscopy is a direct detection method that measures absorbance of water’s O–H overtones and combinations. It is complementary to KF titration offering a simplified, direct measurement that can be implemented on the process line for continuous water content monitoring. NIR spectroscopy is a rapid, non-destructive method, making it ideal for in-line, on-line, or at-line monitoring during process development and manufacturing. Unlike KF titration, NIR spectroscopy does not require chemical reagents. This results in reduced operational costs and lessened environmental impact. Furthermore, NIR is highly automatable, enabling high throughput testing and real-time analysis to support Process Analytical Technology (PAT) initiatives. While KF titration is often the specified method for precise trace-level water quantification and compliance, NIR’s speed, ease of use, and suitability for higher water content make it an efficient, complementary tool. These properties make NIR important when real-time, rapid screening and process insights are needed.

It is important to note that while KF titration is considered the gold standard for highly accurate determination of water in pharmaceutical solvents, NIR spectroscopy is acceptable as a validated alternative, offering faster, non-destructive analysis. Once NIR spectroscopy is shown to correlate reliably with KF titration results, a hybrid approach can be used. This approach would use KF titration combined with NIR spectroscopy to build validated calibration models. NIR spectroscopy could then take over routine, inline/online analysis with periodic validation using KF titration to ensure accuracy. The table below highlights the key differences between online water measurements with KF titration and NIR spectroscopy.

Comparison of KF Titration and NIR Spectroscopy for Online Measurements

Feature	Karl Fischer	NIR
Measurement Time	Minutes	Seconds
Consumables	Yes (titrants, solvents)	No
Complexity	High	Moderate
Multi-Parameter	No (water only)	Yes
Use for Continuous Monitoring	Moderate	Excellent

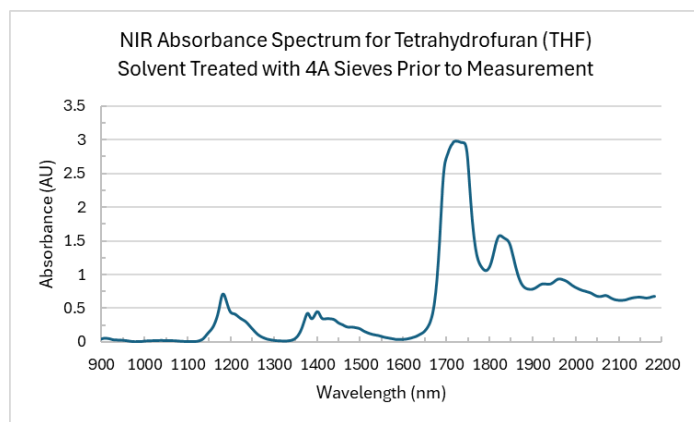
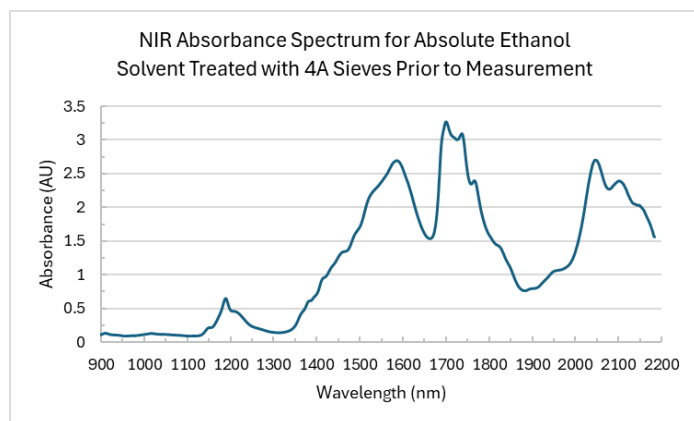
NR2.2 for Measuring Water in Pharmaceutical Solvents

The Ocean Optics NR2.2 was used to monitor absorbance for important pharmaceutical solvents tetrahydrofuran (THF) and absolute ethanol (defined as ≥99.5% ethanol by volume) to demonstrate the power that NIR spectroscopy brings to this critical measurement. These are highly hygroscopic solvents that require tight control of water content in pharmaceutical applications. Both solvents are used widely for synthesis, crystallization, and formulation processes. Stringent control of water content is critical for active pharmaceutical ingredient (API) production, where even trace levels of water may disrupt chemical reactions or compromise stability. Regulatory standards specify a maximum allowable water content of ≤0.05% w/w (500 ppm) for pharmaceutical grade THF and ≤0.3% w/w (3000 ppm) for absolute ethanol. Strict water content control is required for these solvents to ensure that even minimal traces of water do not adversely impact solubility, extraction efficiency, or formulation stability.

NIR absorbance spectra were measured across the 900 – 2200 nm wavelength range using the Ocean Optics NR2.2 spectrometer. Fresh bottles of each solvent were opened, and 40 mL of solvent was transferred to a 50 mL conical tube containing 4A molecular sieves. Molecular sieves are synthetic zeolites with pores that selectively absorb water molecules. They are widely used for drying solvents, gases, and other materials to maintain low moisture levels and prevent water-sensitive reactions.

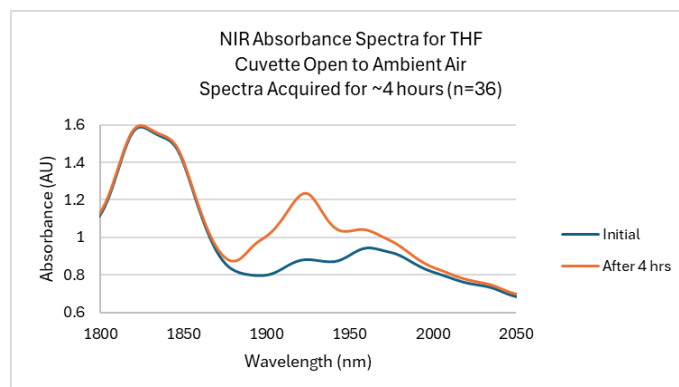
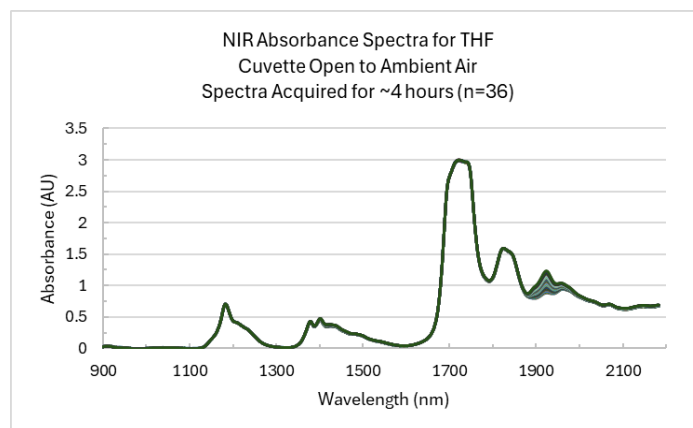
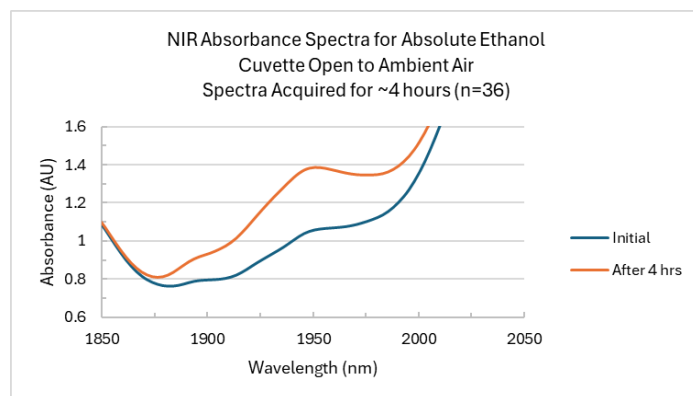
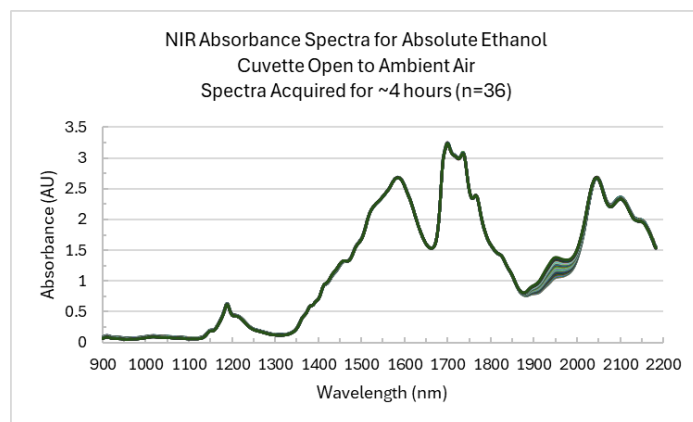
NIR absorbance was measured for each solvent relative to ambient air (50% humidity, 24°C) in an empty, quartz cuvette (3 mL solvent volume). The spectra shown in Figure 1 were the first spectra collected for the freshly opened solvents. The NIR absorbance spectra measured for the solvents had distinct spectral features across the NIR region. Both solvents also had lower absorbance in the 1900 – 1950 nm region where water has strong absorbance. This makes the 1900 – 1950 nm region ideal for monitoring water content in THF and absolute ethanol. Absorbance in this region is primarily associated with a combination band resulting from the O-H stretching and H–O–H bending vibrations of water molecules. This water absorption band is widely used in NIR spectroscopy to detect and quantify water content in various matrices, including pharmaceuticals, food, and solvents.

Figure 1: NIR absorbance spectra for Absolute Ethanol and THF measured with the NR2.2 spectrometer



A series of time-based measurements were made for each pure solvent with the quartz cuvette capped and then left open to ambient air to observe water uptake by the strongly hygroscopic solvents. NIR absorbance spectra were saved every 5 minutes for 75 minutes with the capped cuvette and for 4 hours with the cuvette open to ambient air. The measurements with the capped cuvette (data not shown) had relatively small changes in absorbance over 75 minutes. The spectra collected during the open cuvette measurements are shown in Figure 2. When the cuvette cap was removed, both solvents showed a significant increase of at least 0.3 AU during the 4-hour trial. The increase in absorbance observed between 1900 and 1950 nm was the result of increasing water content as the solvent took up water from the ambient air. Note that the impact of solvent evaporation was minimized by using enough pure solvent to maintain a filled pathlength throughout the 4-hour open cuvette trial. Decreases in absorbance outside the range where water absorbed (solvent absorbance only) were very small (less than 0.02 AU decrease) suggesting that evaporation did not have a significant impact on the results.

Figure 2: Water uptake in THF and absolute ethanol from ambient air measured with the Ocean Optics NR2.2 spectrometer



NIR absorbance measurements for THF and absolute ethanol were also made with a series of solvent samples with known water content. These measurements were made to evaluate the performance of the NR spectrometer for detecting ppm water content in solvents. Solutions for the highest water content samples - 2500 ppm/0.25% THF and 9000 ppm/0.9% absolute ethanol - were prepared by adding deionized water to the solvents. Dilutions to achieve lower water content were done directly in the cuvette by removing a volume of sample from the cuvette and replacing it with pure solvent. The cuvette was not removed from the cuvette holder during the measurements to reduce variability. This mimicked the increased stability of inline/online measurements where the flow cell is not moved during the measurements. The water/solvent samples measured for the standard curves are shown in the table below. All samples were measured relative to a dry, empty quartz cuvette. Note that according to the certificate of analysis (COA) received with the solvents, absolute ethanol contained no more than 1000 ppm/0.1% water content. THF had a much lower initial water content of 130 ppm/0.013%. All concentrations are reported relative to the baseline water content for the solvents.

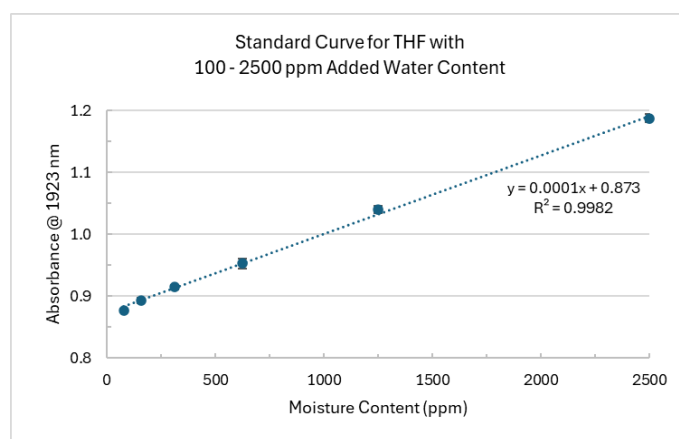
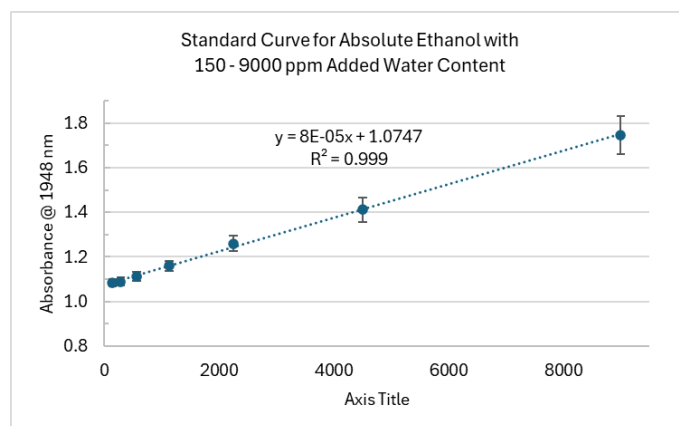
Water Content Samples Used for the Measurements

THF Concentrations	Absolute Ethanol Concentrations
2500 ppm / 0.250%	9000 ppm / 0.900%
1250 ppm / 0.125%	4500 ppm / 0.450%
625 ppm / 0.063%	2250 ppm / 0.225%
313 ppm / 0.031%	1125 ppm / 0.113%
156 ppm / 0.016%	563 ppm / 0.056%
78 ppm / 0.008%	281 ppm / 0.028%
	141 ppm / 0.014%
	70 ppm / 0.007%

Standard curves for absorbance at 1948 nm for absolute ethanol and 1923 nm for THF are shown in Figure 3. Error bars represent the standard deviation calculated for 4 replicate measurements of each absolute ethanol/water concentration and 3 replicate measurements for THF/water concentrations. The data show outstanding absorbance linearity for both solvents with strong correlation between absorbance and water content. THF samples above ~100 ppm water content were separated by at least 3 times the standard deviation giving high confidence in the NR2.2's ability to distinguish these concentrations from one another.

For absolute ethanol, concentrations above ~1000 ppm were separated by at least 3 times the standard deviation. Lower water content samples were more difficult to distinguish from one another due to increased replicate variability. Note that baseline correction at 1070 nm did not have a significant impact on the linearity results.

Figure 3: Standard Curves for Absolute Ethanol and THF measured with the Ocean Optics NR2.2 spectrometer.



Increased variability was observed in the solvent replicates for absolute ethanol compared to THF. Error bars for the THF replicates are approximately the same size as the data point markers in the graph, indicating very low variability for the replicates. This difference in variability is attributed to the intrinsic properties of the solvents. THF is less polar, less hydrophilic, and absorbs atmospheric moisture more slowly than absolute ethanol. In contrast, absolute ethanol is highly polar, strongly hydrophilic, and readily absorbs water from the environment. In addition, absolute ethanol samples contained higher initial residual water content (baseline water content) compared to THF and showed greater spectral overlap with water absorption bands, which could also contribute to measurement variability.

Summary

These results demonstrate the effectiveness of the Ocean Optics NR2.2 for measuring ppm level water concentration in pharmaceutical solvents. The measurements show high linearity between water content and absorbance down to ppm levels for both solvents. For THF, concentrations above ~100 ppm were distinguishable with high confidence due to low variability for the replicate measurements. For absolute ethanol, concentrations above ~1000 ppm were well-separated with high confidence. Lower water concentrations in ethanol showed increased variability. This increased variability is likely the result of a higher residual/baseline water content and pronounced overlap of spectral features between water and absolute ethanol. With a detection limit of 100 ppm for THF and 1000 ppm for absolute ethanol, the Ocean Optics NR2.2 is a great option to confirm solvents meet the regulatory standards - $\leq 0.05\%$ w/w (500 ppm) for THF and $\leq 0.3\%$ w/w (3000 ppm) for absolute ethanol – in real-time.

Conclusions

These results highlight the potential of NIR spectroscopy for rapid and effective monitoring of water in solvents. NIR spectroscopy is particularly useful in production environments where spectroscopy complements the Karl Fischer titration

method. These results show excellent linearity between ppm level water content in solvents and absorbance. While KF titration is recommended to ensure compliance with strict quality standards, NIR spectroscopy serves as a complementary technique for in-line monitoring during production. NIR spectroscopy offers a rapid, non-destructive means for in-process control, making it more suitable for production environments and continuous monitoring than the highly precise trace-level detection of KF titration.

Regulatory References:

USP: Official compendium for water determination techniques, with Karl Fischer titration being the preferred method for precise moisture analysis.

Ph. Eur. 2.5.12: European Pharmacopoeia monograph focused specifically on micro-determination of water content, particularly in “anhydrous” solvents.

ICH Guidelines (Q6A & Q3C): International Conference on Harmonisation documents addressing water content specifications (Q6A) and broader solvent quality and residual solvents (Q3C).

GMP (EU/FDA): Current Good Manufacturing Practices that enforce strict control, specification, and validation processes for water content in manufacturing solvents.

