Application Note
Pharmaceuticals

Fast, Easy, and Reliable Measurements of Liquid Samples Using Transmission FTIR

Comparison of the DialPath Module and a traditional liquid cell for the quantification of simethicone using the Agilent Cary 630 FTIR

Authors

Fabian Zieschang
Wesam Alwan
Agilent Technologies, Inc.

Introduction

Fourier transform infrared (FTIR) spectroscopy is a commonly used technique in the pharmaceutical industry, where it plays an important role in the quality control of final pharmaceutical products. However, achieving accurate and precise measurements of liquid pharmaceutical samples is often difficult by transmission FTIR if demountable or flow cells are used. There are several challenges that make the use of these cells for liquid analysis complicated, error-prone, and time-consuming:

- The cells are fragile, and spacers and windows can be difficult to assemble
- The cell design makes it hard to achieve a reproducible pathlength
- Cells tend to leak
- Air bubbles can interfere with the analysis
- Cleaning and assembling the cells are time-consuming tasks
- Sticky and viscous samples are difficult to measure
- Significant amounts of sample volume and rinsing solvent are required

The unique Agilent DialPath sampling module for the Agilent Cary 630 FTIR spectrometer eliminates the need for traditional flow or demountable liquid cells, simplifying the analysis of liquid samples. A small drop of liquid sample is placed between two horizontally positioned windows of the DialPath module, as shown in Figure 1 (middle) and Figure 2. The distance between the two windows defines the optical pathlength.

Figure 1. Three simple steps allow the analysis of liquid samples using an Agilent Cary 630 FTIR spectrometer equipped with the DialPath module.

The DialPath module provides instant selection of three preset pathlengths that can be selected ("dialed-in") by turning the module head. The Agilent TumblIR module, which uses the same technology as the DialPath module, has one pathlength available rather than three. Both modules are permanently aligned and are easily attached to the front of the Cary 630 FTIR engine. The Agilent MicroLab software automatically detects which module is fitted to the Cary 630 FTIR engine and applies the correct settings, allowing even inexperienced users to swap modules within seconds.

In this study, a Cary 630 FTIR engine fitted with a DialPath module was used for the quantification of simethicone in a commercially available anti-acid medicine. For comparison purposes, data was also acquired using the traditional FTIR approach with a demountable cell in a transmission sample compartment. The quantification of simethicone is described in USP-NF monograph, USP43-NF38, page 4044 (*1*). According to the monograph, the amount of simethicone in a drug formulation is determined as the percentage concentration of the sample compared to a 2 mg/mL simethicone standard solution (2 mg/mL equals 100% percentage concentration).

Experimental

Preparation of blank, standard, and control sample solutions

USP43-NF38 describes simethicone quantification as a percentage concentration relative to a known simethicone standard. A simethicone standard solution containing 2 mg/mL (100%) simethicone was prepared using a simethicone USP reference standard. Around 25 mg of simethicone USP (CAS 8050-81-5) was accurately weighed and transferred into a 50 mL glass screw-capped centrifuge tube. 12.5 mL of spectroscopy grade toluene was added to the tube followed by 25 mL of 4.8 M HCl. The tube was shaken by hand for 5 minutes followed by 5 minutes shaking in a vortex mixer. Next, the tube was centrifuged at 1500 rpm for 30 minutes. About 5 mL of the upper organic layer was transferred to a 50 mL screw-capped centrifuge tube containing about 1 g of anhydrous sodium sulfate to remove any residual water in the organic layer. The tube was shaken vigorously by hand for 1 minute and centrifuged for 10 minutes until a clear layer was obtained. A blank was prepared following the same procedure but without adding simethicone USP. To test the method using high, low, and within range samples, three control samples containing 81.2, 114.0, and 100.7% percentage concentration simethicone were also prepared.

Preparation of capsules containing simethicone

One type of commercially available simethicone capsules was purchased from a local pharmacy. The label indicated that each capsule contained 100 mg simethicone. Each capsule was dissolved in 100 mL of 4.8 M HCl and 50 mL of toluene to obtain a solution with a nominal concentration of 2 mg/mL (100% percentage concentration). The extraction followed the same procedure as described for the simethicone standard solution.

Figure 2. The liquid sample is placed onto the sample window of the DialPath module fitted to the Agilent Cary 630 FTIR spectrometer.

Instrumentation

Two Agilent Cary 630 FTIR spectrometers were used in this study. The first Cary 630 FTIR was equipped with a transmission module designed for a traditional demountable liquid cell with calcium fluoride windows and a 500 micron spacer (Omni-cell, Specac Ltd., UK). The second Cary 630 FTIR was equipped with an Agilent DialPath module with a pathlength of 500 microns (Figure 2).

The samples were divided into two lots for parallel analysis by the two FTIR spectrometers using the liquid cell and DialPath module. To minimize any residue or carryover between samples, the liquid cell was washed with large amounts of toluene. Only a few drops of isopropanol on a cloth were needed to wipe clean the two sample windows of the DialPath. The MicroLab software was used to collect the data and to create a method for the direct calculation of the sample concentration. To compensate for the absorbance of the sample solvents, the background was measured against the blank in the liquid cell and on the DialPath, respectively. The data collection parameters are summarized in Table 1.

Table 1. FTIR data collection parameters used for quantification measurements using both the traditional liquid cell and the DialPath module.

Parameter	Setting
Spectral range (cm^{-1})	4000 to 650
Background scans	64
Sample scans	64
Spectral resolution (cm^{-1})	2
Background collection	Blank was used
Pathlength (microns)	500*

**Specified in USP43-NF38*

Results and discussion

Quantitative analysis: traditional demountable transmission cell vs DialPath

The quantitative analysis of the simethicone samples was performed using the two Cary 630 FTIR spectrometers, one with a traditional demountable liquid cell and one with a DialPath module. The maximum absorbance of the band at approximately 1260 cm^{-1} was used for quantification. According to the USP43-NF38 monograph, the absorbance values for the standard and the samples can be used to calculate the percentage concentration of simethicone per equation 1 (*1*):

Equation 1:

$$\text{Percentage concentration} = \frac{A_U}{A_S} \cdot \frac{C_S}{C_U} \cdot 100\%$$

In more detail:

$$\text{Percentage concentration} = A_U \cdot \frac{1}{A_S} \cdot \frac{C_S}{C_U} \cdot 100\% \equiv A_U \cdot \left(\frac{1}{A_S} \cdot \frac{C_S}{C_U} \cdot 100\%\right)$$

$$\equiv A_U \cdot \text{(multiplication factor)}$$

A_U = absorbance of the sample solution
A_S = absorbance of the standard solution
C_S = concentration of simethicone in the standard solution
C_U = nominal concentration of simethicone in the sample solution

Automatic calculation of percentage concentration

The MicroLab software guides users through the entire analytical workflow using instructive pictures and an easy-to-navigate design. Both the FTIR methods for the routine analysis of simethicone solutions using the DialPath and liquid cell were created in the MicroLab software. The methods automatically determine the maximum absorbance at ~1260 cm^{-1} (using peak height with a single baseline component) and the software automatically applies the calculation per equation 1 (see Figure 3).

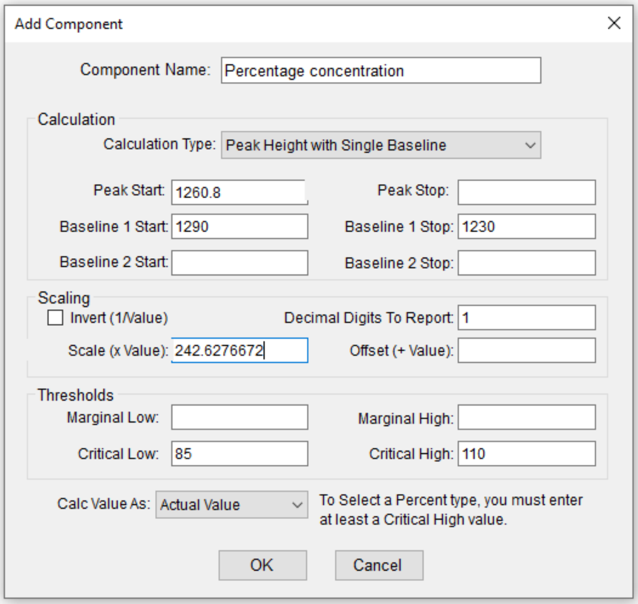

Figure 3. In the MicroLab software, "components" are used to calculate quantitative results from the measured spectra. The component shown above automatically determines the absorbance value (A_u) at the maximum of the band, in this example at around 1260.8 cm^{-1}. The scale (x value) then applies a multiplication factor to the absorbance value to give a final result: Percentage concentration = absorbance value (A_u) x multiplication factor—as explained in equation 1. In this study, the multiplication factor for the DialPath is 242.6276672.

The percentage concentration of simethicone in a sample is reported directly after data acquisition. If any results are outside the USP-specified acceptance criteria of 85–110%, they are flagged in red, as shown in Figure 4 for control sample 1 which contains 81.2% simethicone.

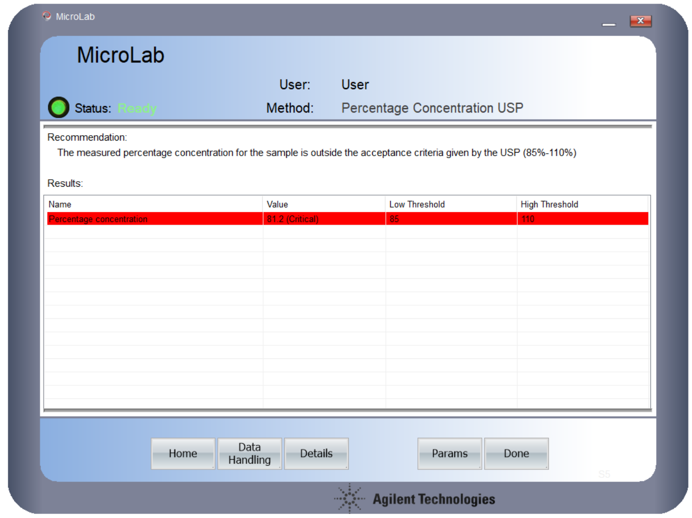

Figure 4. The MicroLab software was used to determine the percentage concentration for the control samples and commercial capsule. Color-coded, actionable results are reported directly after data acquisition in line with the threshold settings of the method.

The two Cary 630 FTIR methods were used to determine the percentage concentration of the control samples 1, 2, and 3 using the liquid cell and the DialPath module. The results in Table 2 show that more accurate results were obtained using the DialPath accessory than with the liquid cell.

Table 2. Percentage concentration of simethicone in the three control samples measured using a Cary 630 FTIR with DialPath and a Cary 630 FTIR with a transmission cell.

		Cary 630 FTIR with DialPath		Cary 630 FTIR with Transmission Cell	
Control Sample	Theoretical Percentage Concentration of Simethicone (%)	Measured Percentage Concentration (%)	Accuracy (%)	Measured Percentage Concentration (%)	Accuracy (%)
1	81.2	81.2	100	80.3	98.8
2	114.0	113.1	99.2	111.1	97.4
3	100.7	102.6	98.1	103.1	97.7

Percentage concentrations of simethicone in the commercial capsules measured by the two Cary 630 FTIR fitted with the DialPath and with the liquid cell were within the acceptance range of 85.0–110.0% that is specified in the USP (Table 3) (*1*). The results suggest that the DialPath is a suitable sampling technique for the determination of simethicone percentage concentrations in liquid drug formulations in accordance with USP requirements.

Table 3. Percentage concentrations of commercial simethicone capsules obtained using FTIR with a DialPath module and a transmission cell.

	DialPath Measured Percentage Concentration (%)	Transmission Cell Measured Percentage Concentration (%)
Commercial Sample 1	101.3	103.8
Commercial Sample 2	101.7	101.8
Commercial Sample 3	102.4	102.6

The performance of the Cary 630 FTIR with the DialPath module was also evaluated in terms of accuracy, precision, linearity, and concentration range.

Measurement accuracy

A standard addition method test was carried out to check the accuracy of the DialPath measurements. Three aliquots of a sample with a nominal percentage concentration of 97.3% simethicone were spiked with 5, 10, and 15% simethicone by adding accurate volumes of a high concentration simethicone standard solution. Each spiked sample was measured three times and the mean recovery was calculated according to the following equation:

Equation 2: $\% \text{ Recovery} = (C_1/C_2) \times 100$

C_1 = measured percentage concentration

C_2 = calculated percentage concentration

The recovery results in Table 4 were all within 100.5%, suggesting that highly accurate measurements can be achieved with the Cary 630 FTIR equipped with the DialPath module.

Table 4. Analysis of a simethicone sample spiked with simethicone at three percentage concentrations using the Cary 630 FTIR with DialPath module to determine the accuracy of the method, n=3.

Sample Percentage Concentration (%)	Spiked Percentage Concentration (%)	Calculated Percentage Concentration (%)	Measured Percentage Concentration (%)	Mean Recovery (%)
97.3	5.0	102.3	102.4	100.1 ± 0.2
97.3	10.0	107.3	107.8	100.5 ± 0.4
97.3	15.0	112.3	112.7	100.4 ± 0.1

Measurement precision

The precision of the Cary 630 FTIR with DialPath measurements was evaluated by running a repeatability study. A simethicone solution was split into six portions and each portion was analyzed separately. The repeatability of the results was assessed by calculating the average and the standard deviation of the absorbance and percentage concentration of simethicone (Table 5). The Cary 630 FTIR with DialPath module showed excellent precision with a standard deviation of only 0.0013 in absorbance and 0.34% of concentration measurements.

Table 5. Cary 630 FTIR with DialPath repeatability study over six measurements of the same sample.

	Absorbance	Sample Percentage Concentration (%)
Sample portion 1	0.4154	100.8
Sample portion 2	0.4144	100.5
Sample portion 3	0.4137	100.4
Sample portion 4	0.4167	101.1
Sample portion 5	0.4158	100.9
Sample portion 6	0.4178	101.4
Average	0.4156	100.9
Standard Deviation	0.0013	0.34

Calibration linearity and concentration range

Linearity between the analyte concentration and the absorbance reading using the DialPath module was demonstrated by creating a calibration curve and evaluating the curve using least squares regression. Calibration standards for five different simethicone concentrations were prepared in the range of 0–190% percentage concentration through appropriate dilutions. Peak height corresponding to simethicone (1260.8 cm^{-1}) was used to evaluate the linearity of the DialPath's spectral response. These measurements were used to obtain the calibration curve as well as to calculate the correlation coefficient (R^2) from the calibration curve. The calibration curve was created, and all statistical calculations were carried out using MicroLab Quant software.

The plot of the peak height of simethicone (at 1260.8 cm^{-1}) as a function of concentration indicates that the DialPath spectral response has an excellent linearity with a correlation coefficient of R^2 =0.9997 (Figure 5).

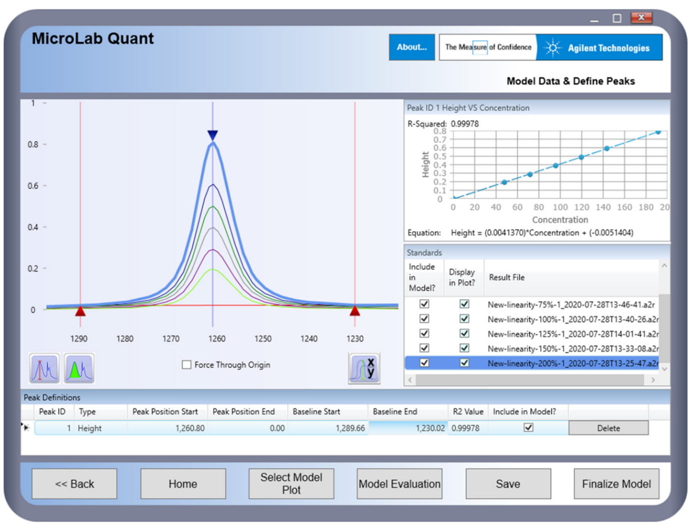

Figure 5. Linearity evaluation of the DialPath using MicroLab Quant software. Calibration curves and correlation coefficient calculations are performed automatically in the software. Users can report the obtained results for documentation purposes.

Sample throughput and use of resources

Quantification of simethicone using the Cary 630 FTIR with the DialPath module provides significantly higher sample throughput, minimizes sample volume, and reduces solvent waste compared to the analysis using the liquid transmission cell (see Figure 6).

In one hour of analysis time, the DialPath enabled the analysis of 40 sample extracts—twice as many samples as the liquid cell. Data acquisition time was approximately 1 min for both sampling techniques. In addition, the DialPath required only 20 µL of simethicone sample solution while the liquid cell required 5 mL of each sample.

To avoid carryover, the liquid cell needs to be flushed multiple times before injecting the next sample. The DialPath can be cleaned with a cloth and a few drops of solvent, leading to a reduction in solvent waste and cost savings for solvent and waste disposal. The DialPath is a simple and easy to use sampling technique that overcomes the complexity associated with liquid transmission cell (e.g. cleaning between each sample introduction, ensuring no gas bubbles are trapped, and proper handling of the aperture plates). The DialPath offers considerable sample, time, and cost savings.

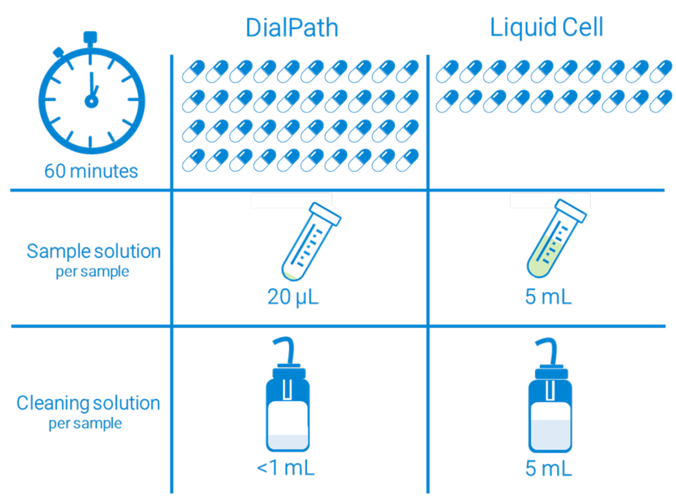

Figure 6. Scoreboard after 60 min of analyzing simethicone extracts using the Cary 630 FTIR with the DialPath module and the Cary 630 FTIR with a traditional liquid transmission cell.

Conclusion

In this application note, the Cary 630 FTIR fitted with highly efficient transmission sampling accessory (DialPath) was used for the rapid quantitative analysis of liquid pharmaceutical samples.

The performance, speed of analysis, and innovative sampling of the Agilent Cary 630 FTIR with the DialPath module enabled the quick development and deployment of a quantitative method for the measurement of simethicone.

The quality of the quantitative data generated by the Cary 630 FTIR with DialPath was comparable or better to the results provided by a Cary 630 FTIR with a traditional cell. However, the usability of the DialPath offered considerable time and cost savings compared to the use of traditional liquid cells, making it ideal for pharmaceutical applications.

The Cary 630 FTIR with the DialPath provided highly linear calibration, up to 190% simethicone, with excellent repeatability. The excellent accuracy and precision of the analytical data obtained for the measurement of simethicone demonstrated the effectiveness of the instrumentation, method, and analytical results.

The Cary 630 FTIR meets the performance requirements of global pharmacopoeia such as the European, US, Indian, and Japanese Pharmacopoeia. The optional MicroLab Pharma software facilitates compliance as defined by US FDA 21 CFR Part 11, EU Annex 11, and similar national electronic record regulations.

Reference

1. Simethicone Emulsion. United States Pharmacopeia and National Formulary (USP43-NF38-4044). DocID: GUID-4965A93F-3617-485C-87B4-1DFBDF33EC3F_4_en-US.

www.agilent.com/chem

DE44297.9470717593

Printed in the USA, April 14, 2021
5994-3046EN

Agilent
Trusted Answers