

Application Note: Binary Solvent Mixtures for HPLC

Binary solvent mixtures are used as carriers in production scale pharmaceutical HPLC separations. Analysis of the mixture ratios is required prior to injection into the HPLC column. The correct mixture ratio ensures efficient and correct separation of the chemical targeted for separation. Concentrations of individual solvents can be measured over the entire range from 0 to 100%.

As an example of the ability of Near Infrared (NIR) Spectroscopy to measure the concentration ratios of binary mixtures, we undertook the experiments outlined below using two different mixtures:

- 1) Heptane / Ethyl Acetate
- 2) Methylene Chloride / Ethyl Acetate

For more than two decades, NIR spectroscopy has been used to measure composition in a wide variety of chemical, petrochemical and agricultural applications. NIR technology, equipment, software, and methods are quite mature and sophisticated. Guided Wave has been a supplier to the chemical industry for more than 20 years, pioneering the use of fiber optic coupled spectrometers for in-line process spectroscopy.

Introduction

Binary solvent mixtures are used as carriers in HPLC columns to extract drugs and drug precursors during the manufacturing process. Accurate control of the composition of the mixture is crucial for accurate and efficient separation of the desired pharmaceutical compounds. Online measurement of the composition of solvent mixtures is made possible using NIR spectroscopy. NIR spectroscopy is ideal for measuring clear liquids online since the analyzer can be remotely located and connected to a flow cell or in-line probe via fiber optic cables. This remote measurement capability makes NIR an excellent choice for measurements on flammable or explosive liquids,

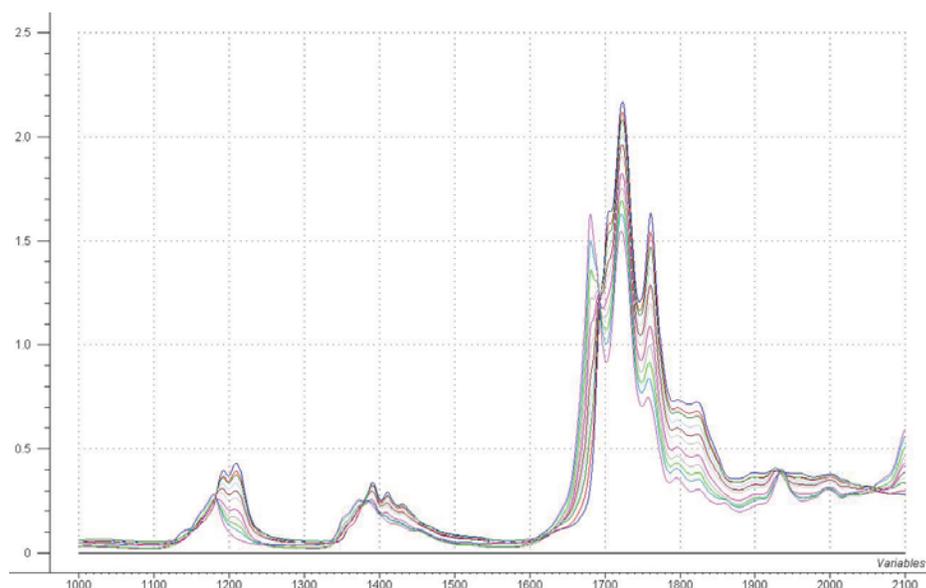


Figure 1. Heptane / Ethyl Acetate Mixture Spectra

minimizing both exposure and safety risks. Guided Wave NIR spectrometers have excellent signal-to-noise ratios, superb long term stability, multi-channel capability and provide fast and reliable results.

The feasibility experiments described below involve binary mixtures of heptane / ethyl acetate and methylene chloride / ethyl acetate. These are just two examples of binary solvent mixtures frequently used in preparative scale HPLC separation separations.

Experimental

The binary mixtures were prepared by weight in the laboratory. NIR spectral scans were collected with an extended range (1000 nm to 2100 nm) Guided Wave Model 412 Dual Beam NIR Spectrophotometer. The sample pathlength was 5 mm. The sample cell was connected to the spectrometer by two lengths of 20 m long, 500 μ m diameter low-OH optical fiber. Each scan consisted of an average of 4 scans for a total data collection time of 12 seconds.

Analysis and Results

Part 1 - Heptane and Ethyl Acetate

The concentrations of the calibration samples range from 0 – 100% in both Ethyl Acetate and Heptane. The raw spectral data are shown in figure 1. There are many regions that show changes in the spectral data as a result of the concentration differences. The absorbance region in the first overtone (1600 – 1800 nm) is greater than 1AU for several of the samples. Focusing the data on the region between 1100 and 1600 nm would allow the sample pathlength to be expanded to a 10 mm gap with no decrease in performance.



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The correlation coefficients of the spectral data to Heptane and Ethyl Acetate concentrations are shown in Figures 2 and 3. There are many regions of the spectral data with very high correlation to the concentration. This is a good indication that the calibration will predict the component concentrations accurately. It is also an indication that either PLS or MLR calibration techniques will work for this application. PLS calibration provides a robust model that can detect unusual samples or samples with contaminants and warn the user of the situation. MLR provides simple, easy to understand calibration models that could even be used in conjunction with a photometer measurement.

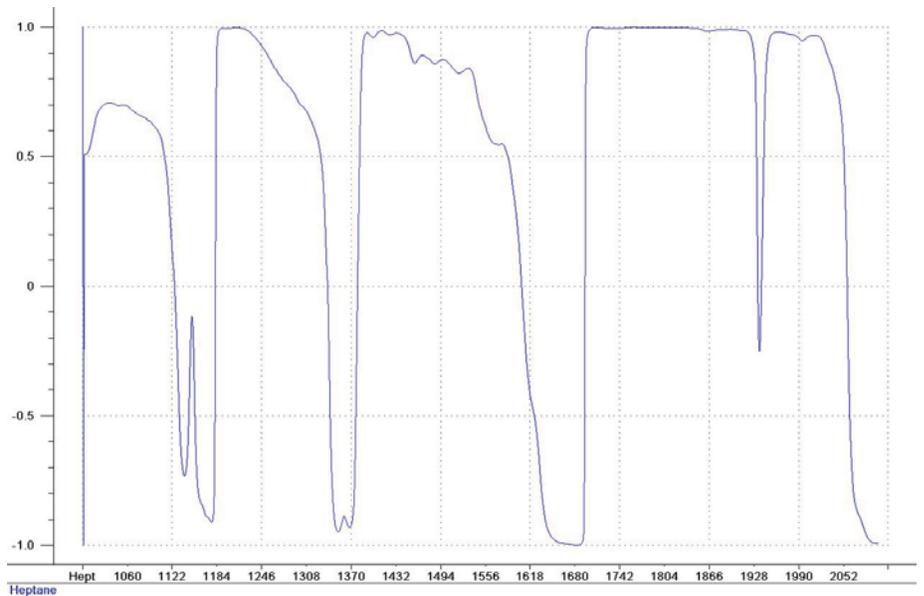


Figure 2: Correlation - Heptane

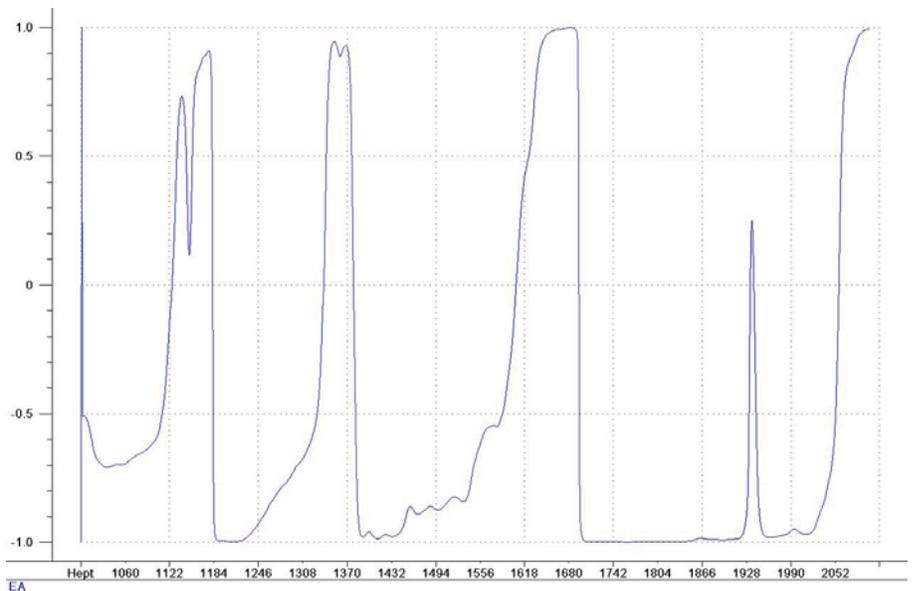


Figure 3: Correlation Ethyl Acetate

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For this example, a first derivative was applied to the spectral data. This removes irrelevant spectral changes and helps to focus on the spectral changes that result only from the chemical concentration differences. PLS calibrations were generated for both components using the full range of spectral data. For Heptane, the calibration results are shown in Figure 4 and for Ethyl Acetate in Figure 5. The estimated prediction uncertainty is 0.1% for both the Heptane and the Ethyl Acetate measurements.

There are ways to potentially improve this accuracy depending on the actual need. Often times a model will provide more accurate predictions if it is focused on a narrower range of concentrations. Also, improvements in calibration sample preparation will always be of value.

All Guided Wave sample probes allow for easy interaction with the sample. The probe can be placed directly in a vessel or reactor or it can be placed on a flow loop. This configuration will allow the direct measurement of Heptane and Ethyl Acetate with one simple scan.



Insertion Probes with Flanges and Fiber Optic Cables with Connectors

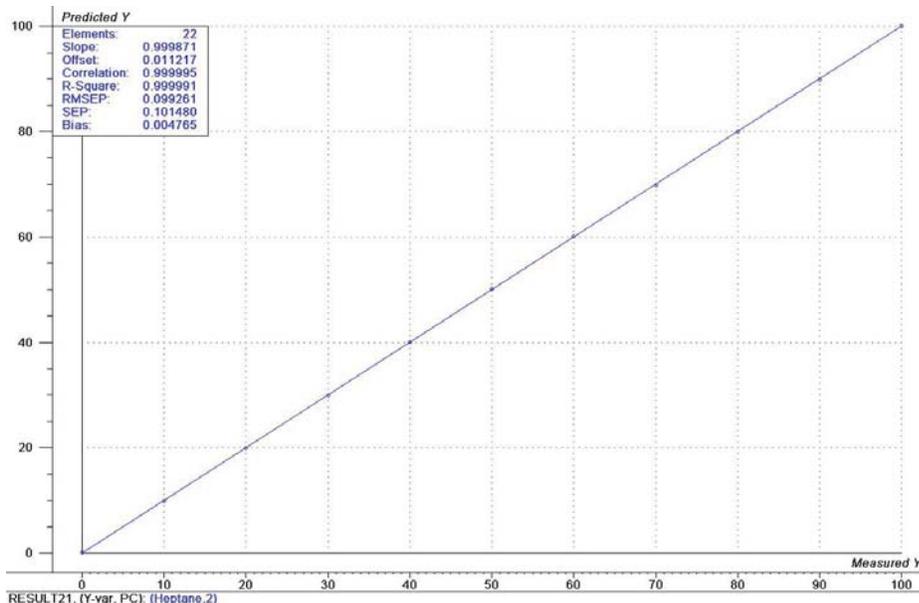


Figure 4. Results Heptane

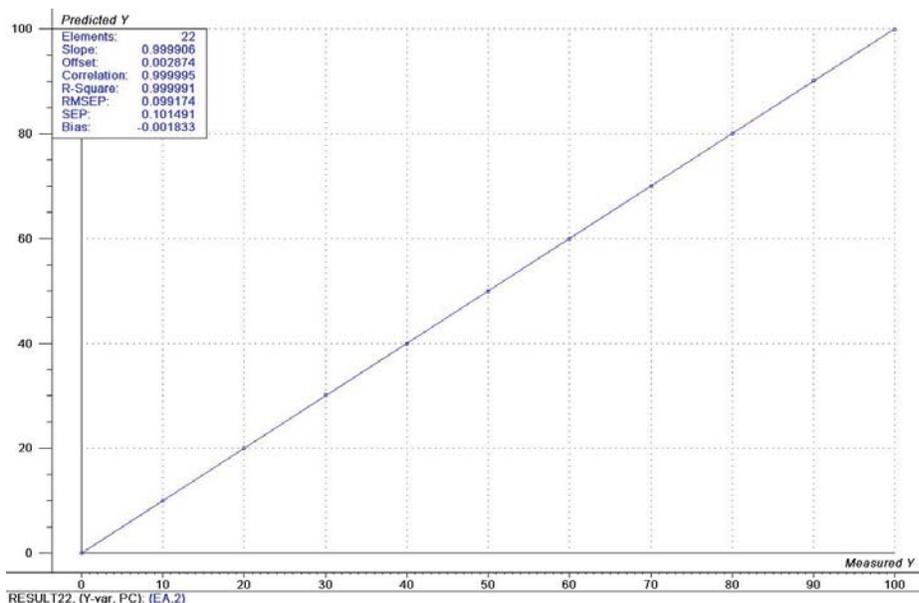


Figure 5. Results Ethyl Acetate

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Part 2 –

Methylene Chloride and Ethyl Acetate

The concentrations of the calibration samples range from 0 – 100% in both Ethyl Acetate and Methylene Chloride. The raw spectral data are shown in Figure 6. There are many regions that show changes in the spectral data as a result of the concentration differences. The absorbance region in the first overtone (1600 – 1800 nm) is greater than 1AU for several of the samples. Again, a sample pathlength of 10 mm can be used if the wavelength region is narrowed to 1100 – 1600 nm.

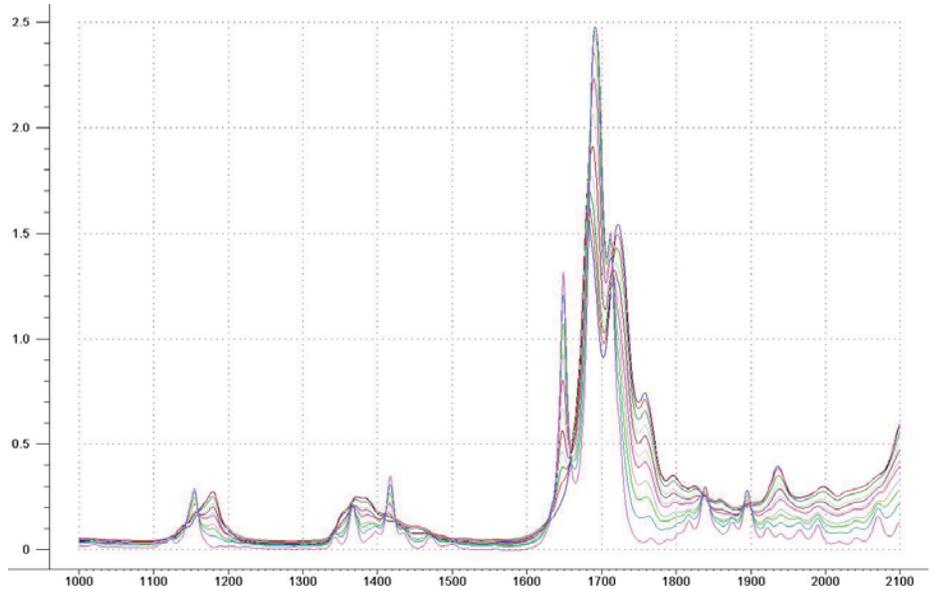


Figure 6: Methylene Chloride / Ethyl Acetate Mixtures



Guided Wave Model 412 NIR Spectrometer



ClearView db Photometer Enclosure Options



Guided Wave Assorted Probes and Flow Cells