Application note

In situ chemotherapy drug analysis using Raman spectroscopy

Application to intravenous 5-fluorouracil bag preparations diluted in 0.9% sodium chloride and 5% dextrose matrix

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Keywords

Chemotherapy, Infusion Bags, 5-fluorouracil, Raman, spectroscopy, DXR3 SmartRaman, Omnic Suite

Industry/Application

Medical, Chemotherapeutics

Products used:

Thermo Scientific™ DXR3 SmartRaman Spectrometer, Thermo Scientific™ DXR™ Universal Platform Sampling Accessory, Thermo Scientific™ OMNIC™ Software Suite

Goals

Prior to intravenous administration, chemotherapeutic drugs must undergo quality assurance, traditionally involving laboratory analysis. We propose using Raman spectroscopy as a cost-effective and time-effective alternative. This technique enables rapid, non-invasive sample analysis through containers, minimizing exposure risks for healthcare professionals. In our study, we utilized the Thermo Scientific DXR3 SmartRaman Spectrometer to quantify 5-fluorouracil (5-FU) infusions in polyethylene bags and classified into different groups based on the formulation matrix. The findings confirmed Raman spectroscopy's strong potential for accurate drug analysis and identification directly through containers, ensuring precise dosing and reducing mismatch errors.

Key Analytes

Chemotherapy treatment: 5-fluorouracil diluted in 0.9% sodium chloride or 5% dextrose solutions.

Key Benefits

- No sample preparation is required, which simplifies workflows and saves time.
- Non-invasive analysis can be performed directly through containers, minimizing chances of contamination and reducing exposure risks for healthcare professionals.
- The technique is rapid and easy-to-use, making it ideal for clinical environments. It also has the potential for full automation of measurement, quantification, and report generation using macros.

Introduction

Chemotherapy is a widely used treatment for various types of cancer. The therapy involves the use of high-risk drugs with narrow therapeutic windows and significant toxicity. Each treatment schedule is individualized, tailored to a single patient based on a physician's prescription and validated by a pharmacist. Given the vulnerability of cancer patients, medication errors in oncology can have serious consequences. To mitigate these risks, the preparation of chemotherapeutic agents is centralized in specialized reconstitution units under strict pharmaceutical oversight. In accordance with Good Preparation Practices, each preparation must undergo quality control before administration.

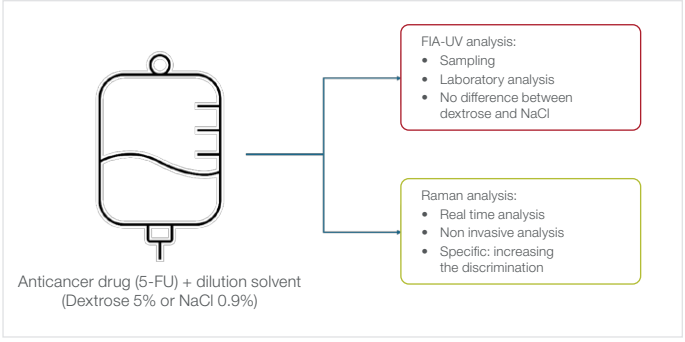
Raman spectroscopy presents a promising solution for this critical quality control step. Its speed, ease of use, and ability to perform non-invasive measurements make it particularly well-suited for verifying drug preparations in pharmacy units. Previous studies have demonstrated the potential of Raman spectroscopy to analyze preparations directly through their containers—such as infusion bags, elastomeric pumps, and syringes—without the need for sampling. This approach enhances safety by reducing healthcare professionals' exposure to hazardous drugs and ensures that patients receive the correct medication at the correct dose. Moreover, it enables analytical control of all preparations, not just a subset.

In this study, we evaluated the performance of the Thermo Scientific™ DXR3 SmartRaman Spectrometer for verifying infusion preparations of 5-fluorouracil (5-FU), one of the most frequently prescribed intravenous anticancer drugs in hospitals. The 5-FU preparations were compounded under aseptic conditions in an isolator by pharmacy staff, using either 0.9% sodium chloride (NaCl) or 5% dextrose as diluents.

Our approach involved two key steps:

1. Developing quantitative models to measure 5-FU concentrations in either solvent directly through the polyethylene infusion bags.
2. Creating a discriminative model to distinguish between the two diluents, enabling the positive identification before delivery to the patient.

This work highlights the potential of Raman spectroscopy to enhance safety, accuracy, and efficiency in the chemotherapy preparation process.



Experimental

For the two dilution solvents, three series of infusion bag were prepared at the therapeutic range from 1.0 to 13.0 $mg.mL^{-1}$. All samples were analyzed using DXR3 SMARTRaman spectrometer equipped with a 785 nm monochromatic laser and a Thermo Scientific™ DXR™ Universal Platform Sampling Accessory (Figure 1). Laser power at sample was set at 150 mW, and the spectral range studied was from 50 cm^{-1} to 3250 cm^{-1} with a spectral resolution better than 5 cm^{-1}. The exposure time was 5 seconds with 2 coadds. Each measurement was repeated 3 times per sample to evaluate the repeatability of the measurement.

Figure 1. Infusion Bag placed in the DXR Universal Platform Sampling Accessory of the DXR3 SmartRaman spectrometer.

Model development can be performed with Thermo Scientific™ TQ Analyst™ Software. Spectra can also be converted in several formats to develop models with other software. In this case, data analysis was performed using Matlab® 2024b software. Quantitative analyses were performed using partial least square regression (PLS-r). Two data sets were determined: the calibration data set to develop the model, and the validation data set to assess the performance of the model. The optimal number of latent variables was determined by leave-one-out cross validation (LOOCV) to reduce the risk of over-fitting the model. For the selected model, the root mean square error of cross validation (RMSECV), the root mean square error of prediction (RMSEP), and the regression coefficient (R^2) between theoretical and predicted concentrations were calculated. For each quantitative model, the accuracy profile was calculated based on the predicted concentrations from the calibration set and the figures of merit were calculated with the validation set, including repeatability, accuracy and intermediate fidelity. The acceptable thresholds for the developed model, in terms of both the figure of merit and the accuracy profile, were defined based on the methodology routinely applied in the laboratory for the analytical control of the preparation.

Discriminant analysis between the two dilution solvents was performed using partial least square discriminant analysis (PLS-DA).

Results

A total of 179 spectra were acquired including 90 spectra with 5% dextrose and 79 spectra in 0.9% NaCl solution. 5-FU spectra in 0.9% NaCl are shown in Figure 2 while Figure 3 represents a zoomed in view of the specific spectral zone.

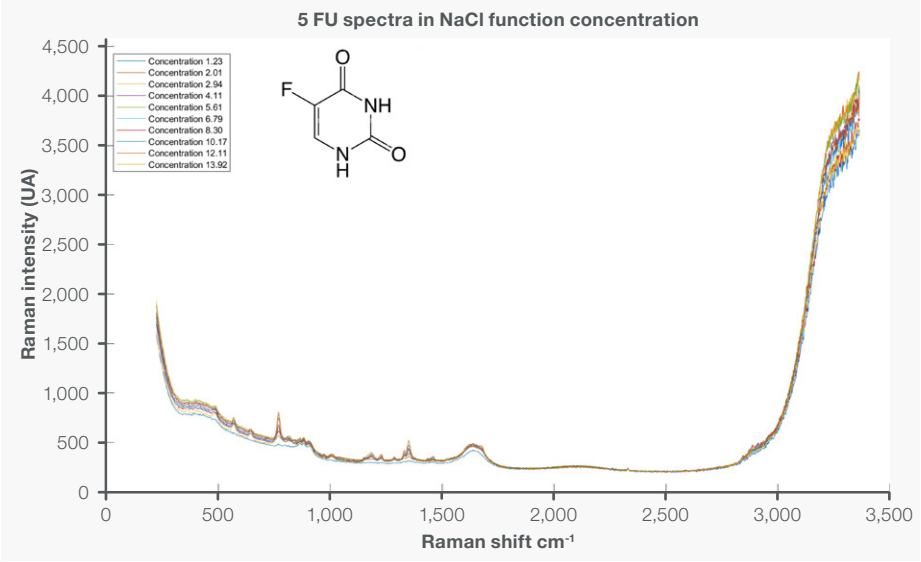


Figure 2. 5-FU spectra in NaCl function of different concentration.

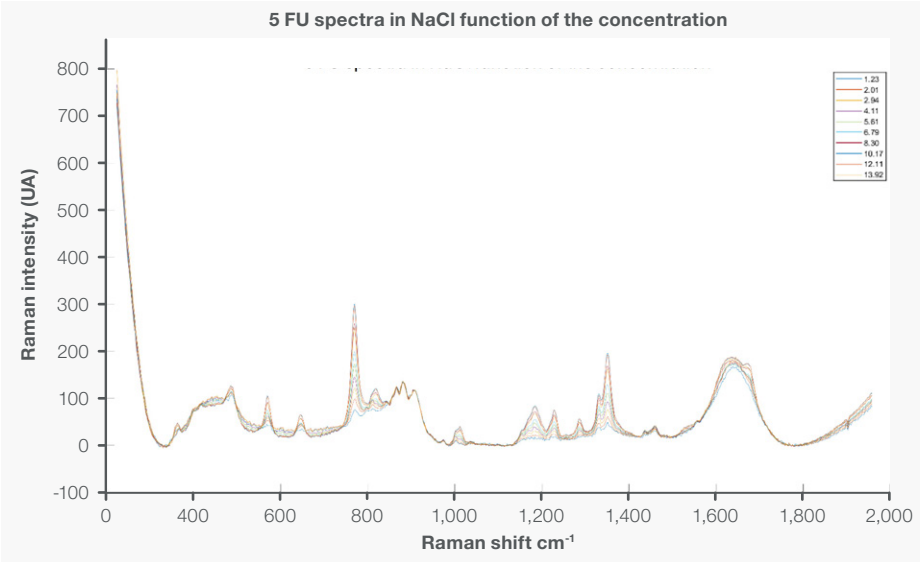


Figure 3. Specific spectral region of interest for 5-FU in NaCl with a baseline correction. The 1350 cm^{-1} peak is highlighted by red arrow to demonstrate the correlation of intensities with concentrations that forms the basis of the quantitative models discussed below.

Based on the publication of Pavel *et al.*[6], specific bands could be attributed to the 5-FU in solution. The intense band at 785 cm^{-1} is characteristic of out plane deformation of the ring and of the double bond between the C4 and the O8. Another intense band around 1350 cm^{-1} corresponds to the stretching of the ring and the in plane deformation of the bond between N and H. Due to the good spectral resolution of the DXR3 SmartRaman spectrometer, other small characteristic bands of 5-FU can be seen and used for model development.

1. Quantitative analysis PLS-r

Quantitative models were developed to predict 5-FU concentration both in 5% dextrose and 0.9% NaCl matrix. The regression lines between the theoretical and the predictive concentrations and the accuracy profile are shown in Figure 4 for 5-FU in 0.9% NaCl and Figure 5 for 5FU in 5% dextrose matrix.

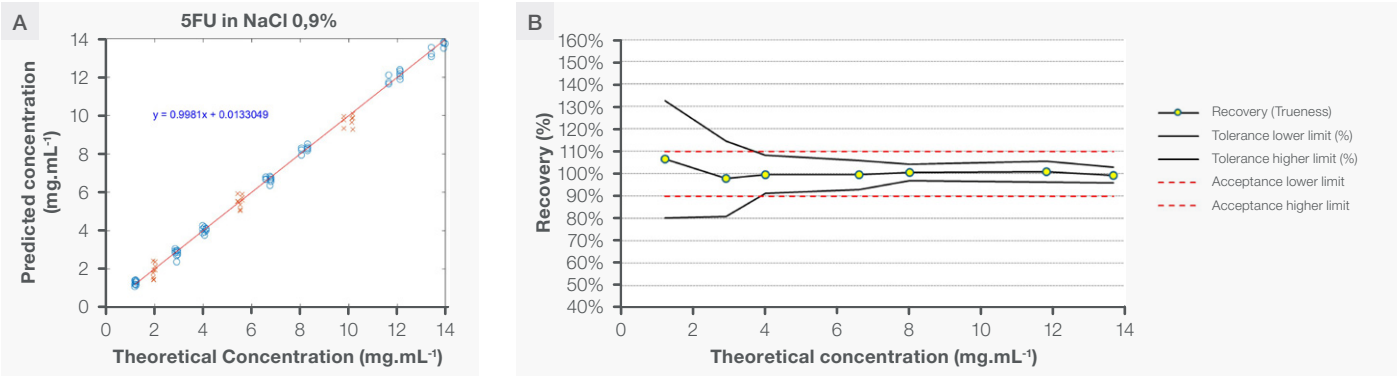


Figure 4. 5-FU in 0.9% NaCl matrix. A) Regression line between predicted and theoretical concentrations. The blue points represent the calibration set and the orange crosses represent the validation set. B) Accuracy profile for the calibration set concentrations predicted by the LOOCV model calculated with the predictive concentrations of the calibration set with acceptance limits fixed at 10% and a ß-tolerance level at 90% (according to our validation method in the laboratory).

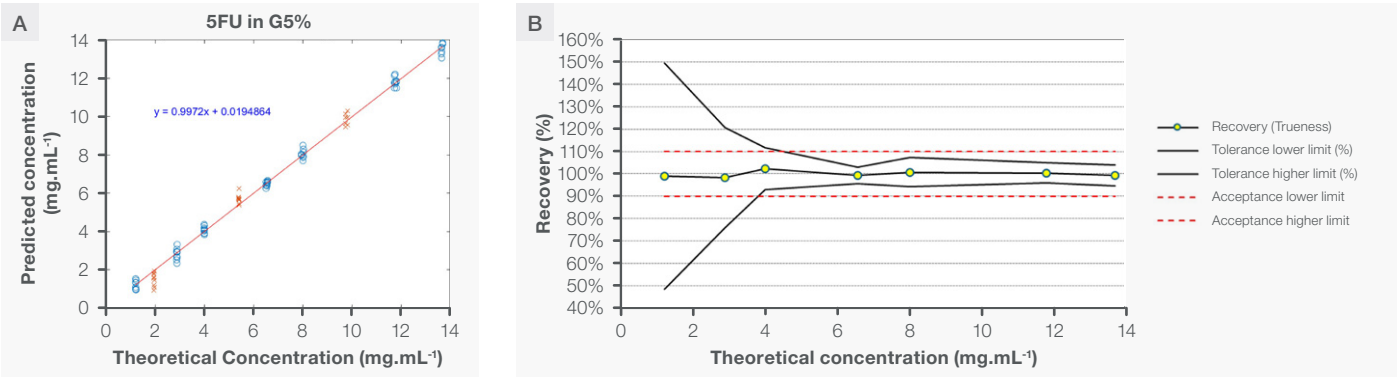


Figure 5. 5-FU in 5% dextrose matrix. A) Regression line between predicted and theoretical concentrations. The blue points represent the calibration set and the orange crosses represent the validation set. B) Accuracy profile for the calibration set concentrations predicted by the LOOCV model calculated with the predictive concentrations of the calibration set with acceptance limits fixed at 10% and a ß-tolerance level at 90%.

For the two solvents (NaCl and Dextrose), the PLS quantitative model was obtained using multivariate analysis over the entire spectral range without any spectral preprocessing, with five latent variables. As there is no pre-processing, this number of latent variables allows the integration of all spectral variations such as the baseline linked to the various components of the infusion bag.

For the NaCl model, the RMSECV, RMSEP and R^2 were 0.1898 mg.mL^{-1}, 0.3663 mg·mL^{-1} and 0.9999 respectively. Based on the accuracy profile, the limit of quantification was calculated at 3.69 mg·mL^{-1}. The linearity range was validated between 3.69 mg·mL^{-1}and 14 mg·mL^{-1}. The figures of merit were calculated with the predictive concentrations from the validation set and are presented in Table 1. The calculated values are lower than 5% for the concentrations included in the linearity range which falls within the acceptable limit for our laboratory.

In 5% dextrose matrix, the RMSECV, RMSEP and R^2 were 0.228 mg·mL^{-1}, 0.430 mg·mL^{-1} and 0.9999 respectively. The regression line between the theoretical and the predictive concentrations was presented in Figure 5A. The accuracy profile (Figure 5B) was calculated with the predictive concentrations of the calibration set with acceptation limit fixed at 10% and a ß-tolerance established at 90%. Based on the accuracy profile, the limit of quantification was calculated at 4.48 mg·mL^{-1}. The linearity range was validated between 4.5 and 14.0 mg·mL^{-1}. The figures of merit were calculated with the predictive concentrations for the validation set and were presented in Table 1. The calculated values were lower than 5% for the concentrations included in the linearity range, which is the acceptable limit for our laboratory.

Matrix	Dextrose 5%		NaCl 0.9%	
Concentration of 5FU (mg.mL^{-1})	**5.38**	**9.76**	**5.52**	**10.02**
Mean relative error (%)	5.3	2.9	4.4	3.2
Repeatability (CV %)	4.7	3.3	3.5	3.3
Accuracy (%)	5.3	1.4	-0.05	-3.1
Intermediate fidelity (CV %)	4.7	3.8	7.1	2.7

Table 1. Figure of merit (FOM) for the two developed quantitative models.

In the hospital, 5-FU is currently controlled by direct injection into the flow coupled with UV detection (FIA-UV). The repeatability (CV%), the intermediate fidelity (CV %) and the accuracy (%) are lower than 5% for this range of concentrations with FIA-UV and are similar to the FOM calculated with the Raman spectrometer method that does not require direct injection.

2. Discriminant analysis : PLS-DA

In addition to the quantitative model that was developed, it was deemed essential to create a complementary model to distinguish the two formulations, to ensure that the nature of the dilution bag had not been reversed. As presented in Figure 6, the 5-FU spectra differed depending on the dilution solvent used in the infusion bag. To obtain the best predictive model, spectra were mean-centered and preprocessed with a first derivate. A cross validation by venetian blinds with 10 groups was performed to calibrate the model. For the prediction, a confusion matrix was constructed (Table 2), 100% of samples analyzed were correctly assigned using this model.

Matrix assigned / Real matrix	0.9% NaCl	5% dextrose	Not Assigned
0.9% NaCl	30	0	0
5% dextrose	0	27	0

Table 2. Confusion matrix for the predicted class by PLS-DA.

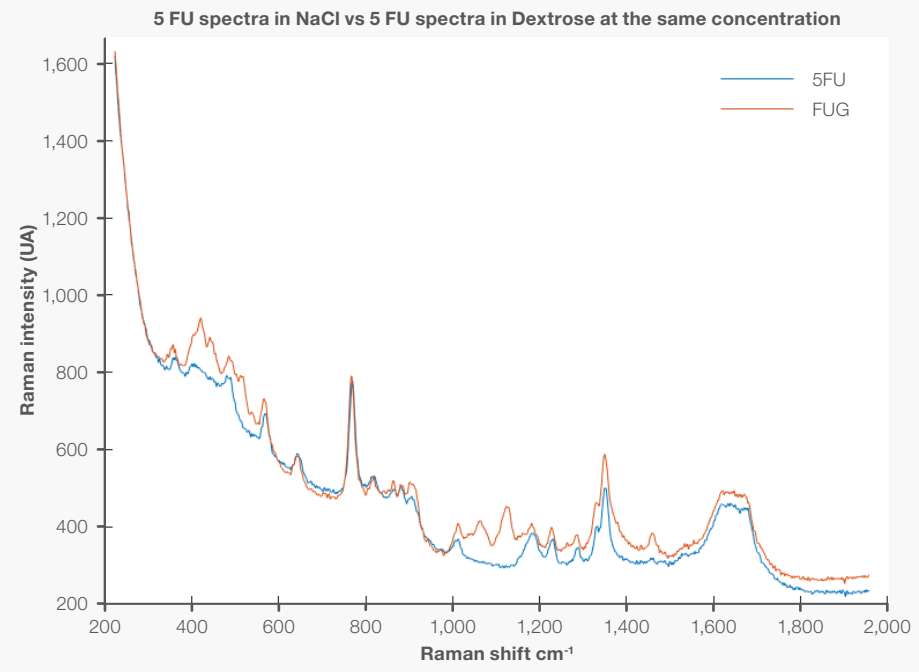


Figure 6. 5-FU spectrum in NaCl and in dextrose for the same concentration.

Conclusion

In conclusion, the DXR3 SmartRaman spectrometer demonstrates significant potential for monitoring chemotherapy preparation directly through containers, such as infusion bags, while also effectively distinguishing between different dilution solvents. The spectral acquisition is straightforward, achieved by directly placing the infusion bag on the measuring device. Moreover, the repeatability calculated for the Raman spectrometer is close to the repeatability calculated for the FIA-UV method. The analysis is rapid, non-invasive and compatible with routine quality control methods for preparations in hospital settings.

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