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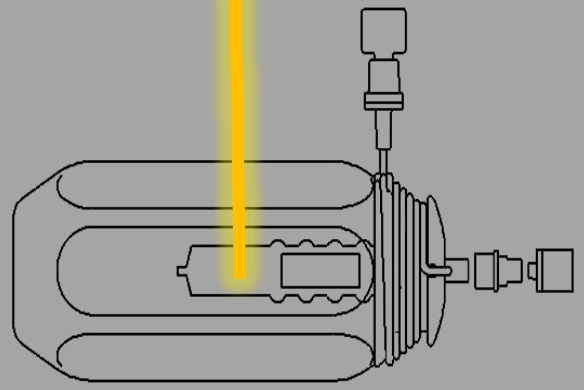
*Graphical Abstract

laser source

CDD detector

Raman spectroscopy

Probe

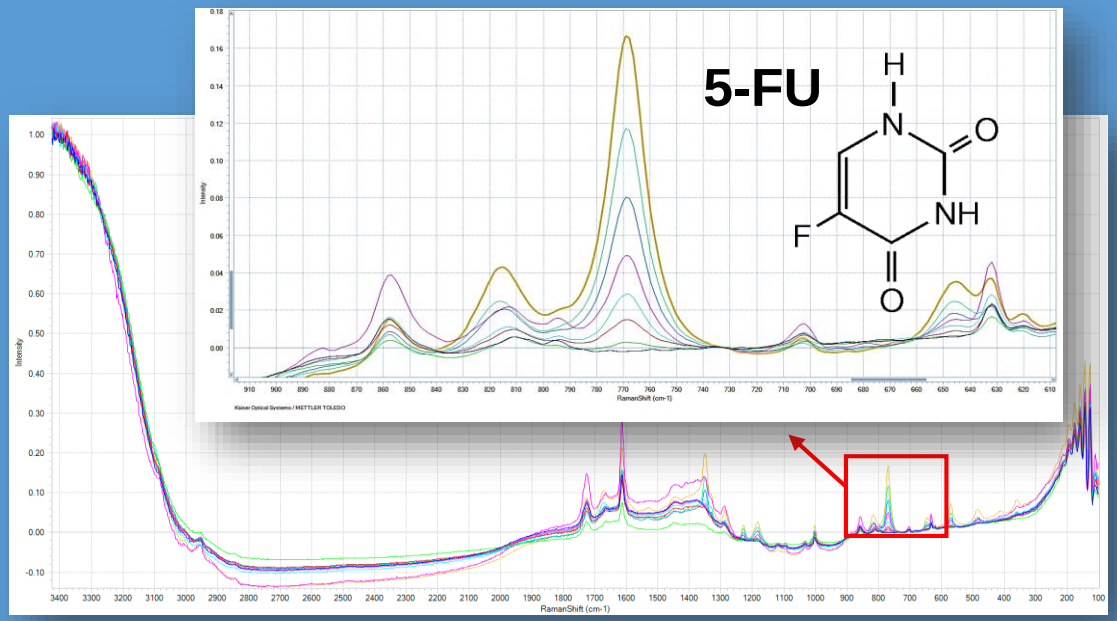


Raman illumination chamber

Fluorouracil compounded in portable infusion pump

acquisition

Raman spectra



treatment of the signals

non-intrusive analytical quality control

Highlights

- We compare the performance of HPLC to Raman spectroscopy.
- The two methods were validated for trueness, precision and accuracy.
- There is a statistical correlation between Raman Spectroscopy and HPLC.
- Raman Spectroscopy is effective for analytical quality control of 5-fluorouracil.
- Raman Spectroscopy contribute to protect caregivers and their working environment.

Comparison of Raman spectroscopy vs. high performance liquid chromatography for quality control of complex therapeutic objects: model of elastomeric portable pumps filled with a fluorouracil solution

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ABSTRACT

This study compares the performance of a reference method of HPLC to Raman spectroscopy (RS) for the analytical quality control (AQC) of complex therapeutic objects. We assessed a model consisting of a widely used anticancer drug, i.e., 5-fluorouracil, which was compounded in a complex medical device, i.e., an elastomeric portable infusion pump. In view of the main objective, the two methods provided excellent results for the analytical validation key criteria, i.e., trueness, precision and accuracy, ranging from 7.5 to 50 mg/mL and in either isotonic sodium or 5% dextrose. The Spearman and Kendall correlation tests (p-value < 1.10^{-15}) and the statistical studies performed on the graphs confirm a strong correlation in the results between RS and the standard HPLC under the experimental conditions. The selection of a spectral interval between 700 and 1400 cm^{-1} for both the characterization and quantification by RS was the result of a gradual process optimization, combining matrix and packaging responses. In this new application, we demonstrate at least eight benefits of RS: a) operator safety, b) elimination of disposables, c) elimination of analysis waste, which contributes to the protection of the environment, d) a fast analytical response of less than two minutes, e) the ability to identify the solubilizing phase, f) reduction of the risk of errors because no intrusion or dilution are needed, g) negligible maintenance costs and h) a reduction in the budget dedicated to technician training. Overall, we indicate the potential of non-intrusive AQC performed by RS, especially when the analysis is not possible using the usual techniques, and the technique's high potential as a contributor to the safety of medication.

Introduction

1.1. Background and purpose

In France, central IV admixtures of chemotherapy treatments (CT) are required by law [1]. This process is currently performed under pharmaceutical liability, especially at hospitals. This requirement represents an important step forward in terms of both the quality and safety of care [2], as well as a) a strong contribution to the standardization of prescribing practices, b) a lower exposure of caregivers to chemicals, c) an improved organization of caregiver workloads and d) a substantial cost savings [3]. However, we see a) an increasing number of combined therapies, b) an increasing number of patients and c) more individualized and more complex therapeutic regimens. In this multifactorial context, the development of effective tools for the quality control of therapeutic objects (TO)* is highly relevant [3]. Our goal is to ensure a high and stable quality in our pharmaceutical preparations for the benefit of patients, caregivers and the environment. Furthermore, a systematic analysis of the production process reveals several critical points; these abnormalities may dangerously weaken the validity of the process. In the course of pioneering studies started 12 years ago at the Gustave-Roussy Institute (Villejuif 94805 cedex, France), we demonstrated the importance of linking the physical product resulting from a compounding process, which we call TO, to the flow of information [4-8]. This idea occurred to us long before the recent and serious health accidents that were highly publicized in the French media. We designed an analytical quality control (AQC) process that we applied, as systematically as possible, to the three following key parameters: identity, purity and nominal concentration of an active pharmaceutical ingredient (API) in solution or in suspension in a sterile medium. However, experience indicates that a TO cannot be reduced to the API. Under these conditions, the following question is important: is it possible to offer an analytical solution, ideally non-intrusive, that could manage the entire TO, i.e., API, solubilizing matrix and its container?

In this context, the purpose of this study was to develop and validate a Raman spectroscopy (RS) method as an effective tool for the non-intrusive AQC of geometrically complex TO(s). We studied the model of elastomeric portable infusion pumps filled with fluorouracil (5-FU) either in a normal saline solution or in a 5% dextrose solution. This protocol was compared to a reference HPLC method. We also examined how the use of one analytical method vs. another contributes to the security and safety of the administration of medication at the hospital.

* We call Therapeutic Object(s) (TO(s)) the product resulting from a compounding process, performed by specialized staff, i.e., a) an active principle in solution or in suspension in an appropriate medium, usually normal saline or 5% dextrose solution, and b) an immediately labeled package, possibly pre-connected to an infusion set. The presence of secondary packaging may complete the definition.

1.2. Analytical quality control and Raman Spectroscopy

Ideally, the purpose of AQC is to allow the analytical certification of the TO prior to its administration to a patient. In terms of hospital organization, the AQC should be fast, reliable, and fully integrated into the production process and treatment. This is particularly relevant for day care units. However, the need to withdraw a fraction of a TO for analysis purposes should also be considered in terms of security and safety, for both operators and their working environment. For some TOs, withdrawal is difficult, even impossible, e.g., small syringes, autonomous infusion devices (elastomeric portable infusion pumps), and PCA devices. The most frequently used analytical techniques are: a) chromatographic methods coupled with appropriate detection systems, b) HPTLC (high performance thin layer chromatography) methods, and c) coupling of UV/visible light spectroscopic techniques to a Fourier transform infrared spectroscopy detector. Chromatographic methods are powerful, but their implementation is costly and sometimes tedious. They also require specialized skills. We will not

detail the strengths and weaknesses of this reference option. According to our criteria, and despite substantial technical improvements, chromatographic methods are not suitable to high-throughput AQC. RS allows for the qualitative and quantitative characterization of an API and its solubilization matrix, without any risk of alteration. However, among the characterization parameters, both the specificity and reliability of the technique must be demonstrated through experimentation; furthermore, some molecules are structurally similar [9]. Finally, we must systematically study the spectral behavior of packaging layers (of varying number and thickness), their contents, and the possible interferences of their respective signatures. For these reasons, the term “contextual analysis” by RS will be used. It is worth to note that quantitative Raman studies of APIs in injectable are very rare [10,11].

2. Material and Methods

2.1. Choosing a suitable API and working conditions

In brief, fluorouracil is a pyrimidine analogue that has been used for more than 40 years. This drug is widely used in the treatment of many forms of cancer and often in combination with other anticancer drugs. Some of its main indications are in colorectal and pancreatic cancer. It is also used in the treatment of inflammatory breast cancer, an aggressive form of breast cancer [12]. Most of the protocols involving 5-FU are listed in Table 1. 5-FU is administered at therapeutic concentrations from 12.5 to 50 mg/mL, either in saline or 5% dextrose; the analytical comparison was performed within these two values and in both solvents. To test the analytical performance of RS vs. HPLC and to build robust calibration models, we expanded the range of concentrations from 1.5 mg/L, leading to the production of a large number of calibration TOs and external validation TOs.

2.2. Choosing the medical device

5-FU is usually administered by IV, by means of either an infusion bag (in this case, gravity is used), or a more or less sophisticated electromechanical pump or, finally, by autonomous elastomeric infusion systems that use a combination of the elastomeric material pressure and Hagen-Poiseuille's law. This last option is of particular interest because: a) these systems are disposable medical devices, b) their physical structure and their geometry are quite complex, c) they are commonly used in both hospitals and in homecare, as well as for ambulatory patients. We selected a pump model widely used in protocols, such as FOLFOX and FOLFIRI (Table 1), i.e., the Infusor® SV2 System (Baxter ref 2C1702KD, batch 09C044 2011-12-31); Fig. 1 is a diagram of the device.

2.3. HPLC analysis

Chromatographic separation was performed on a Dionex Ultimate® 3000 series liquid chromatographic system equipped with a quaternary pump, a variable UV/visible detector, and an autosampler (Dionex, 78960 Voisins le Bretonneux, France). Chromatographic separation was performed on Lichrospher® C18 column (125 mm x 4 mm, *dp* = 5 μm (Merck, 69008 Lyon, France). The mobile phase consisting of a mixture of water for injection and KH_2PO_4 (40 mmol/L) adjusted to pH 7.0 with NaOH (10% solution in water), was delivered at rate of 0.8 mL per min. The mobile phase was filtered through a 0.45 μm membrane (Millipore, 67120 Molsheim, France) and degassed prior to use. Separation was performed at ambient temperature, i.e., 21°C; detection was performed at 260 nm. The injection volume was 5 μL with a run time of 6 min. Data were recorded; the Dionex Chromeleon® (version 6.80) software was employed for data collection and processing. The chromatographic conditions were based from a HPLC method coupled with UV detection developed by Alanazi *et al.* [13].

2.4. RS analysis

The RS analysis was performed between 100 and 3400 cm^{-1} using an RXN1 bench (Kaiser Optical System, Ann Arbor, USA); Fig. 2 is a diagram of the bench. This industrial machine is equipped with a user-safe laser source emitting in the near infrared at 785 nm. According to the manufacturer's specifications, the rated power of the laser source is 345 mW vs. 210 mW to the end of the probe. The acquisition of signals was performed using a charge-coupled device (CDD detector) through the iC Raman® Instrument software (Mettler-Toledo AutoChem Inc., Columbia, MD, Version 4.1). The treatment of the signals and the partial least square regression (PLS), first derivative, and mean center were then calculated with the iC Quant® software (Mettler-Toledo AutoChem Inc., Columbia, MD, Version 4.1). We developed a Raman illumination chamber (RIC) that allows examining examination of TOs of any geometry. This light-tight chamber is equipped with a micrometer focal adjustment system used for accurate fixing and excellent repeatability of the distance between the laser source (the extremity of the probe) and the object, e.g., syringe, pouch, bottle, or portable pump.

2.5. Validation protocol

Analytical validation of the methods was conducted in accordance with the recommendations of the Commission of the French Society of Pharmaceutical Science and Technology (SFSTP, 2003) [14, 15]. This guide provides a consensus on the various existing international standards, e.g., the International Conference on Harmonization: Validation of Analytical Procedures Q2 (R1) [16, 17], FDA guidelines: Guidance for Industry, Bioanalytical Method Validation [18]. It also incorporates ISO terminology. Calculation of both the accuracy profiles and the validation parameters based on the Quality Control (QC) results was performed using the e.noval® (version 3.0) software (Arlenda, Liège, Belgium). Because the focus of the present study is the AQC of complex therapeutic objects, the acceptance limits were set at ± 10% for the validation, whereas the probability of obtaining results within the tolerance interval was set at 95%.

The validation protocol was strictly identical for the 2 techniques comparing HPLC and RS. Three campaigns were conducted on 3 consecutive days, either in saline or 5% dextrose. To

calculate the calibration and validation parameters of the methods, external validation TOs and calibration TOs were produced and analyzed 6 and 3 times per campaign, respectively.

2.6. External validation

An accurately weighed quantity of 5-FU was dissolved in isotonic saline or 5% dextrose. The pH was adjusted to 9.0 by adding a solution of sodium hydroxide at 14.7 mg/mL (VWR, Fontenay-sous-Bois, France, ref. 97064-634) to obtain two reference solutions of known concentrations of approximately 50 mg/mL. Two groups of five external validation solutions, i.e., 2.5, 7.5, 15, 30 and 40 mg of 5-FU per mL, were prepared each day in saline and 5% dextrose and transferred into portable infusion pumps (Infusor® SV2) to obtain the daily external validation set.

2.7. Calibration sets

Three independent calibration sets were produced with nine points, 0, 1.5, 2.5, 5, 10, 17.5, 25, 35, and 50 mg/mL, using a ready-to-use commercial solution available at the theoretical nominal concentration of 50 mg of 5-FU per mL (Mylan, 69792 Saint Priest, France) either in isotonic sodium or 5% dextrose according to our compounding protocols. The commercial solution consisted of 5 g of 5-FU in 100 mL of water for injection and sodium hydroxide (pH 8.5 to 9.5).

2.8. PLS parameters

Partial least squares (PLS) is the most popular multivariate model for quantitative Raman analysis. PLS uses factor analysis to compress the size of the spectra and to remove redundant information. PLS uses the analyte concentration information along with sample variance in compression process to create factors that are correlated with analyte concentration. More the number of factors increases and more the model will take into account the variance of the set of

spectra. However, an excessive number of factors will result in overtraining of the model at the expense of future predictions. The number of factors is therefore crucial for the quality of the calibration model and is determined by cross-validation which allow the calculation of RMSECV (root-mean-square error of cross-validation) whose value must to be minimal.

2.9. Validation parameters

The analytical validation steps included the following parameters: a) Linearity, the ability of a method, within a certain concentration range, to give linear and proportional results to the analyte concentration. The parameter was verified by the study of three independent calibration sets and tested on three consecutive days (3 x 3/day) for a total of nine curves per method. After fitting the linear regression model, i.e., the calculated concentrations vs. actual concentrations, the linearity was verified by the calculation of a correlation coefficient (R^2). To demonstrate the linearity of the method, the approach based on the expected tolerance level β, expressed in absolute values, was also used. b) The lower and upper limits of quantification (LLOQ and ULOQ) define the range where an analytical method is able to quantify accurately. These limits are, respectively, the smallest and the highest concentration levels where the expectation tolerance intervals are included within the acceptance limit. c) Accuracy refers to the closeness of agreement between the test result and the accepted reference value, namely the conventionally true value. It is assessed from the accuracy profile and the relative β–expectation tolerance limit. The parameter was calculated from each determination of the external validation TOs, i.e., six measurements per day for three days. d) Precision is the closeness of agreement among measurements from multiple samplings of a homogeneous sample. It gives information about the average value of the results and variances (expressed in relative standard deviation, RSD). The intra-series variance leads to repeatability variance, whereas the intra- and inter-series variances sum leads to the intermediate precision variance. The precision was obtained from the six replicate determinations under the prescribed conditions for each external validation TO per day for three days. e) Trueness refers to the

closeness of agreement between a conventionally accepted value or a reference value vs. a mean experimental value. It provides information on systematic error. The parameter is expressed in terms of relative bias (%) at each concentration level of the external validation TOs. f) Uncertainty is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurement [19, 20]. The relative expanded uncertainties lead to a relative interval around the results where the unknown true value can be observed with a confidence level of 95% [21, 22].

2.9. Methodological progression

The portable pumps were first analyzed by RS. In a second step, samples were analyzed by HPLC. The need for repeated withdrawals (250 µL per sample) from sealed and closed systems forced us to follow this methodological progression. For HPLC analysis, samples were systematically diluted in the corresponding medium, i.e., 1/50 in normal saline or in 5% dextrose. After characterizing the performance of each instrument, RS was compared to the reference method.

2.10. Statistics and correlation study

To study the strength of the relationship between a series of measurements obtained from the two techniques, we used the statistical methodology proposed by Bland and Altman [23]. Data analysis was primarily graphic and based on following the H1 hypothesis: concentrations measured for each sample by HPLC and by RS are strictly identical. According to H1, the measurements displayed on a graph are distributed according to the concentrations of 5-FU measured by HPLC vs. those measured by RS; they should be positioned on the line of y = x, with y and x the concentrations determined, respectively, by HPLC and RS. According to H1, a second graph will show the differences in concentrations of 5-FU measured, either by HPLC or by RS for each matched sample (y = [HPLC - RS]) vs. the concentration average for the same sample determined via the two methods ([HPLC + RS]/2). These differences are

theoretically equal to zero and should be positioned on y-axis (y = 0). Taking into account the repeatability of the two analytical methods, this approach is interesting. However, to give a strong statistical conclusion, and considering that data obtained from the two sequences do not allow for a hypothesis about their underlying distribution, we applied two convergent nonparametric tests of rank, i.e., the Rho (ρ) of Spearman and the Tho (τ) of Kendall. To be applicable, these various tests must be conducted by the two techniques on the same samples. In this way, the values obtained from each of the three daily independent calibration sets and the external validation set (either in saline or 5% dextrose) were averaged. Finally, statistical analysis was performed on two homogeneous groups of 36 physically and statistically comparable results.

3. Results and Discussion

3.1. Spectral signatures generated by RS

Exposure of objects to the laser beam was both direct and through the primary packaging (Fig. 1 and 2); this is safe for both operators and their working environment. Mastering the tool allowed us to systematically maintain a total acquisition time of 1 min; this value is dependent on the type and geometry of the device and its content. The RIC allows for the optimization of the focal length by repeating the measures applied to a device, filled, for example, with a hydro-ethanolic solution (5:95, v/v). The setting results in a focal length of 0.5 mm between the extremity of the probe and secondary packaging, i.e., a polycarbonate envelope in the present case. This focal length reflects the optimal ratio (signal of the therapeutic solution/signal of the device). Figure 3 depicts the Raman signatures of the portable pump studied under four conditions and without 5-FU. The polyisoprene chamber was filled either by normal saline, 5% dextrose, sterile water, or ambient air. The Raman signatures were obtained by scanning between 100 and 3400 cm^{-1} at 21°C. Figures 4 and 5 show the signatures of the portable pumps filled with 5-FU (concentrations from 1.5 to 50 mg/mL) in normal saline or 5% dextrose. The comparison of the RS signatures generated with and without the API allows for the definition of a PLS (Partial Least Square) regression interval between 700 and 1400 cm^{-1}. Within this interval, there is minimal variation in response to the matrix. In contrast, the signature of 5-FU is intense between 740 and 800 cm^{-1}. RS signatures are highly specific of a TO and are an assembly of rigid layers (the container) and liquid layers (the content). This assembly is both the main limiting factor of the RS performance (see LOQs in Table 2) and one of the main assets of the method. Analysis can be applied to an object while maintaining its integrity. This illustrates the major importance of a rigorous selection of spectral bands for both the characterization and quantification of an API. Thus, the quantification step is the result of an adjustment process; it is also an empirical compromise between many parameters, including

the matrix and packaging. PLS regression was performed with the calibration set. Cross-validation based on random subsets was performed to select the model number of PLS factors. An optimal number of 12 PLS factors were chosen for both matrixes in order to minimize the RMSECV. As seen in Table 3, the RMSEC (root mean square error of calibration), RMSECV and RMSEP (root mean square error of prediction) values are low and thus confirm the quality of the models. However, those criteria do not allow the assessment of the ability of the model to accurately quantify over the entire concentration range. Therefore, the performance of the predictive model was evaluated with accuracy profiles computed on the external validation results.

3.2. Method validations

Figure 6 displays the accuracy profiles computed with the external validation set results. For the reference method, the expectation tolerance limits are fully included within the ±10% acceptance limits. For the RS in both saline and 5% dextrose, the expectation tolerance limits are included between the range from 7.5 to 40 mg/mL within the ±10% acceptance limits.

The ICH Q2 (R1) validation criteria obtained for both methods (HPLC and RS) are summarized in Table 2. As shown on the accuracy profiles, the bias is less than 3% except for RS in dextrose, where it is 7.3% at a concentration of 2.5 mg of 5-FU per mL. The precision of each method was estimated by measuring the repeatability and intermediate precision at the 5 concentrations levels. The dispersion of the results was below 4% from 7.5 to 40 mg/mL (Fig. 6). The precision of the RS is less satisfactory at the 2.5 mg/L concentration level, particularly in 5% dextrose. This leads to larger LLOQs, i.e., 3.8 and 6.1 mg/L, respectively, in saline and in 5% dextrose vs. 2.5 mg/L for HPLC in both cases. To verify the linearity, each model was fitted on the back calculated concentrations of the validation standards for all series as a function of the introduced theoretical concentration. The slope and intercept are close to 1 and 0, respectively, confirming the absence of a proportional and constant systematic error in each model. The linearity of the models were between the range from 7.5 to 40 mg/mL for RS and in

the entire range of concentration levels tested for HPLC because the absolute β-expectation tolerance limits were within the absolute acceptance limits (Fig. 6 and table 2). The range of linearity was smaller by RS compared to HPLC; however, it covers the entire range of concentrations encountered in clinical practice, especially when using a portable pump. This is also true for the FOLFOX and FOLFIRI protocols, which are commonly prescribed to children presenting with a BSA of approximately 1 m² and with a dose reduction of 50% (Table 1). Considering the ranges of linearity, the relative expanded uncertainties are not higher than 6% and 7%, respectively, for HPLC and RS (Table 2). This means that for both techniques, and with a confidence level of 95%, the unknown true value is located within a maximum of ±7% around the measured results. The two methods are satisfactory for the key criteria of trueness, precision and accuracy in the entire range of concentrations encountered in current clinical practice. The chromatographic method remains more powerful; both the precision RSD and the accuracy relative to the β-expectation tolerance limit are lower with the HPLC method than with the vibrational spectroscopic method. Analysis conducted by RS can be described as contextual, i.e., the combined effects of matrixes and packaging (Fig. 1 and 3) in addition to the vibrational spectroscopic response of the API.

3.3. Correlation study

Considering the 36 averaged samples, i.e., means of each concentration level (from 1.5 to 50 mg/mL) by day and for three consecutive days, the values of ρ (Spearman test) and τ (Kendall test) were all greater than 0.93. Thus, the hypothesis H0, by which the ranks are independent, was rejected with a lower risk than $2.2.10^{-16}$ with both Spearman and Kendall tests and in both matrices (lowest p-value provided by the statistical software). According to Bland and Altman [20], we found in both matrices a) that points are homogeneously distributed along the line of equality; this finding indicates that the two methods are well correlated; b) that the dispersion on both sides of the line of equality is homogeneous and there is no measurement bias; and c) that the dispersion is greater on the x axis (analysis by RS); this result

358 demonstrates higher variability of the measurements performed by RS. The variability of
359 measurements was also greater when 5-FU was in 5% dextrose. The average of the differences
360 in the concentrations [CLHP – RS] (mg/mL ± SD), limits of acceptability (LoA, mg/mL) and limits
361 of acceptability weighted by the repeatability of the analytical techniques (LoAP, mg/mL) were,
362 respectively, 0.15 ± 0.42, [-0.9 – 1.2] (LoA), [-2.5 – 2.8] (LoAP) in normal saline and, 0.07 ±
363 0.50, [-1.2 – 1.3] (LoA), LoAP [-3.2 – 3.3] in 5% dextrose (Fig. 7 and 8).

364 To simplify the development of the method, we did not take into account the range of
365 concentrations. Thus, the difference in the concentrations [HPLC - RS] examined side-by-side
366 vs. the average value increased with the same average concentration. Whatever the mixture,
367 clouds of points were homogeneously distributed in terms of LoAP, whereas differences of LoAs
368 were reasonably low in the considered range of concentrations, i.e., from 1.5 to 50 mg 5-FU per
369 mL. The dispersion of the differences for both measurements and the LoAPs (and their
370 variability) were higher in 5% dextrose. Considering a) the highly statistical significance of the
371 two rank tests (Spearman and Kendall tests), b) the strong graphic correlations and c) the
372 homogeneous distribution of the differences for both the LoAs and LoAPs, there was a strong
373 correlation in the results (5% statistic risk) between the two methods under the experimental
374 conditions used. The correlation tests and the statistical studies performed on the graphs
375 confirmed that the RS gave satisfactory results vs. the standard HPLC method. We also
376 identified the functional limit of RS, which becomes less accurate and less powerful when the
377 matrix is dextrose in water. The spectral signature of this matrix is much more intense than that
378 obtained in saline. In the context of AQC, this leads to the following recommendation: unless the
379 use of dextrose is necessary (which is rare to our knowledge), it is preferable to use a saline
380 matrix.

381

382 *3.4. RS as a tool for routine control of TOs*

383

384 We now examine where RS has potential for routine AQC. The main advantage of RS is
385 to allow rapid and safe contextual analysis of the TO. In fact, for routine control of TOs, it's not

necessary to recalibrate the instrument and the established calibrations are sufficient to proceed to the AQC of the TO in a unique spectroscopic analysis.

This major benefit could be considered as a relative disadvantage by some professionals. We proceed with the simultaneous analysis of at least four entities: a) an API (5-FU in the present case), b) a dilution matrix, c) an immediate packaging whose geometry may vary over time, i.e., a flexible elastomeric (polyisoprene) shape memory reservoir, d) a secondary packaging, i.e., a rigid polycarbonate shell in the present case, and sometimes, e) a plastic overwrapping, i.e., a plastic film or a polyethylene soft bag. This contextual variability might require new analytical validations if one of the four entities change. Such work might also be necessary in the case of a change of supplier, either for the medical device or for the drugs it contains. Conversely, this relative disadvantage could be another benefit of RS; in the near future, the gradual construction of a spectral database could help us to optimize the choice of the products that we use.

In view of our objectives, and including the AQC of a therapeutic solution of 5-FU in portables infusion systems, Raman technology presents a number of attractive benefits. First, we showed that with the addition of an RIC, it was possible to qualitatively and quantitatively explore TOs of various geometries, without any intrusion or destruction to the material. In addition, we identified multiple benefits: a) operator safety is guaranteed at all times, i.e., during the production step as well as in the laboratory, b) the complete elimination of disposables, c) the elimination of analysis waste is a precious guarantee of protection to the working environment, d) a fast analytical response of less than 2 minutes (compatible with the rejection of a non-compliant and a fast reshaping of the TO), e) the ability of RS to discriminate and identify the solubilizing phase of an API, f) a lower risk of errors vs. HPLC, which requires stages for withdrawal and often dilution of samples, g) negligible maintenance costs, which is not surprising because the machine does not contain any mechanical moving parts, and h) reduction in the budget dedicated to technician training. In total, we demonstrated that contextual analysis by RS allows for efficient AQC, especially for complex and non-withdrawal therapeutic objects, e.g., autonomous infusion systems or small syringes.

4. Conclusions

We demonstrated the potential of RS as an effective tool to perform non-intrusive AQC of complex TOs, especially its non-inferiority vs. HPLC, to determine the concentration of a widely used anticancer drug, such as 5-FU. From a practical point of view, the selection of bands for the characterization and quantification by RS was the result of a gradual adjustment process combining the matrix effects. Our results confirmed the potential of this option for future applications, owing to its capability to explore therapeutic objects with any type of geometry and its contributions to the safety of the medication circuit. This method also contributes to the protection of caregivers and their working environment.

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496

Legends for tables (n=3)

Table 1.

List of the major chemotherapy protocols that include a continuous infusion of 5-FU and their main parameters of use. LV2, SV2, and LV5 are brand names of Baxter Healthcare.

Table 2.

Trueness, precision, accuracy, lower and upper limit of quantification, and uncertainty, obtained with HPLC vs. RS in saline and 5% dextrose.

Table 3.

Spectral range, spectral pre-treatment, number of PLS factors, r^2 of validation, RMSEC, RMSECV and RMSEP of the Raman spectroscopy model.

Legends for figures (n=8)

Fig. 1. Elastomeric portable pump diagram (Infusor® SV2 system, ref 2C1702KD, Baxter Laboratories). Courtesy of Baxter.

Fig. 2. Configuration of the Raman bench used for the AQC of the TOs. The laser source is connected via an optic fiber to the Raman Illumination Chamber (RIC) that allows examining examination of TOs of any geometry. The acquisition of signals is performed using a charge-coupled device (CDD detector) through the acquisition interface.

Fig. 3. Raman signatures of the portable pump (Infusor® SV2 system) studied in different circumstances and without 5-FU. The polyisoprene chamber was filled either by normal saline, 5% dextrose, sterile water, or ambient air. Raman signatures were obtained after scanning between 100 and 3400 cm^{-1} at 21°C. Insert: the research interval, between 300 and 1850 cm^{-1}.

Fig. 4. Raman signatures obtained after scanning between 100 and 3400 cm^{-1} of an elastomeric portable pump (Infusor® SV2 system) filled with 5-FU in saline (concentration range between 1.5 and 50 mg/mL). Insert a: selected band of PLS regression, i.e., 700-1400 cm^{-1}. Insert b: chemical structure of fluorouracil (5-FU), i.e., 5-fluoro-1H-pyrimidine-2,4-dione.

Fig. 5. Raman signatures obtained after scanning between 100 and 3400 cm^{-1} of an elastomeric portable pump (Infusor® SV2 system) filled with 5-FU in 5% dextrose; the concentration range is between 1.5 and 50 mg/mL). Insert: selected band of PLS regression, i.e., 700-1400 cm^{-1}.

Fig. 6. Accuracy profiles from both techniques, HPLC vs. Raman spectroscopy (5-FU in saline and 5% dextrose). The plain red line is the relative bias, the blue dashed lines and the black dotted lines are, respectively, the β-expectation tolerance limits and the acceptance limits. The

538 green dots represent the relative error of the back-calculated concentrations; they are plotted
539 with respect to the targeted concentrations.
540
541 **Fig. 7.** Average values for the measurements obtained by both techniques vs. the differences in
542 the 5-FU concentrations (mg/mL) in saline, determined for each pair of values [HPLC - RS]: the
543 equality of measures (black solid line), the mean of the differences determined between the 36
544 pairs of values (solid line), the limits of acceptability for a statistical risk of 5% (dotted lines), and
545 the acceptability limits weighed by the repeatability of the measurements (red solid lines).
546
547 **Fig. 8.** Average values for the measurements obtained by both techniques vs. the differences in
548 the 5-FU concentrations (mg/mL) in 5% dextrose, determined for each pair of values [HPLC -
549 RS]: the equality of measures (black solid line), the mean of the differences determined
550 between the 36 pairs of values (solid line), the limits of acceptability for a statistical risk of 5%
551 (dotted lines), and the acceptability limits weighed by the repeatability of the measurements (red
552 solid lines).

Table 1.

Protocol name	Dose (mg/m²)	Time of infusion (h)	Volume (mL)	Infusion system brand name	Ct° max[a] (mg/mL)	Ct° min[b] (mg/mL)
TPF	3750	120	240	LV2	31.3	10.1
GORTEC	2400	96	192	LV2	25.0	8.1
VOKES	4000	120	240	LV2	33.3	10.8
CDDP-5-FU C225	4000	96	192	LV2	41.7	13.5
LOHP 5-FU	5000	120	240	LV2	41.7	13.5
FOLFOX or FOLIRI	2400	46	96 230	SV2 LV5	50.0 20.9	16.3 6.8
FOLFOX or FOLIRI pediatric use[c]	2400	46	96	SV2	50.0	12.5

[a] Ct° max (recommended): upper limit of concentration into the infusion system for a BSA (Body Surface Area) of 2 m².
[b] Ct° min (recommended): lower limit of concentration into the infusion system for a BSA of 1.3 m² and a dose reduction of 50%.
[c] Ct° max and Ct° min: recommended values for a BSA of 1 m² and a dose reduction of 50%.

Table 2.

		Relative bias (%)			
Trueness	Concentration	NaCl 0.9%		G5%	
	level (mg/mL)	HPLC	RS	HPLC	RS
	2.5	1.07	0.97	-0.73	7.29
	7.5	1.51	1.14	-0.14	-0.43
	15	2.27	0.59	-0.64	-1.17
	30	1.33	-0.03	2.51	0.04
	40	0.54	-0.23	0.73	0.23
		Repeatability (RSD %)			
Precision	Concentration	NaCl 0.9%		G5%	
	level (mg/mL)	HPLC	RS	HPLC	RS
	2.5	0.92	7.43	1.60	7.23
	7.5	1.02	2.56	1.50	2.66
	15	1.01	2.21	1.77	3.34
	30	1.12	1.40	1.60	2.32
	40	0.85	1.42	1.94	2.38
		Intermediate precision (RSD %)			
Precision	Concentration	NaCl 0.9%		G5%	
	level (mg/mL)	HPLC	RS	HPLC	RS
	2.5	1.39	7.65	2.24	11.2
	7.5	1.18	2.56	1.97	2.66
	15	1.52	2.36	1.94	3.34
	30	1.35	1.58	1.62	2.32
	40	0.85	1.58	2.60	2.38
		Relative β-expectation tolerance limit (%)			
Accuracy	Concentration	NaCl 0.9%		G5%	
	level (mg/mL)	HPLC	RS	HPLC	RS
	2.5	[-2.9, 5.0]	[-15.8, 17.8]	[-6.8, 5.3]	[-25.5, 40.1]
	7.5	[-1.3, 4.2]	[-4.4, 6.67]	[-5.3, 5.0]	[-6.2, 5.4]
	15	[-2.1, 6.6]	[-4.7, 5.9]	[-5.0, 3.8]	[-8.5, 6.1]
	30	[-1.9, 4.6]	[-3.7, 3.6]	[-1.0, 6.0]	[-5.0, 5.1]
	40	[-1.3, 2.4]	[-3.8, 3.4]	[-6.1, 7.6]	[-5.1, 5.6]
		Lower LOQ (mg/mL)			
Limits of quantification (LOQ)		NaCl 0.9%		G5%	
		HPLC	RS	HPLC	RS
		2.5	6.0	2.5	6.8
		Upper LOQ (mg/mL)			
Limits of quantification (LOQ)		NaCl 0.9%		G5%	
		HPLC	RS	HPLC	RS
		50.0	50.0	50.0	50.0
		Relative Expanded Uncertainty (%)			
Uncertainty	Concentration	NaCl 0.9%		G5%	
	level (mg/mL)	HPLC	RS	HPLC	RS
	2.5	3.05	15.8	4.90	24.7
	7.5	2.49	5.26	4.28	5.47
	15	3.34	4.92	4.06	6.88
	30	2.87	3.32	3.33	4.78
	40	1.75	3.32	5.66	5.02

Table 3

RS model	Selected parameter NaCl 0.9%	Selected parameter G5%
Spectral range selected (cm−1)	700-1400	700-1400
Spectral pre-treatment	First derivative + Mean Center	First derivative + Mean Center
Number of PLS factors	12	12
R2	0.995	0.999
RMSEC (mg/mL)	0.001	0.001
RMSECV (mg/mL)	0.787	0.482
RMSEP (mg/mL)	1.13	0.397

Figure 1

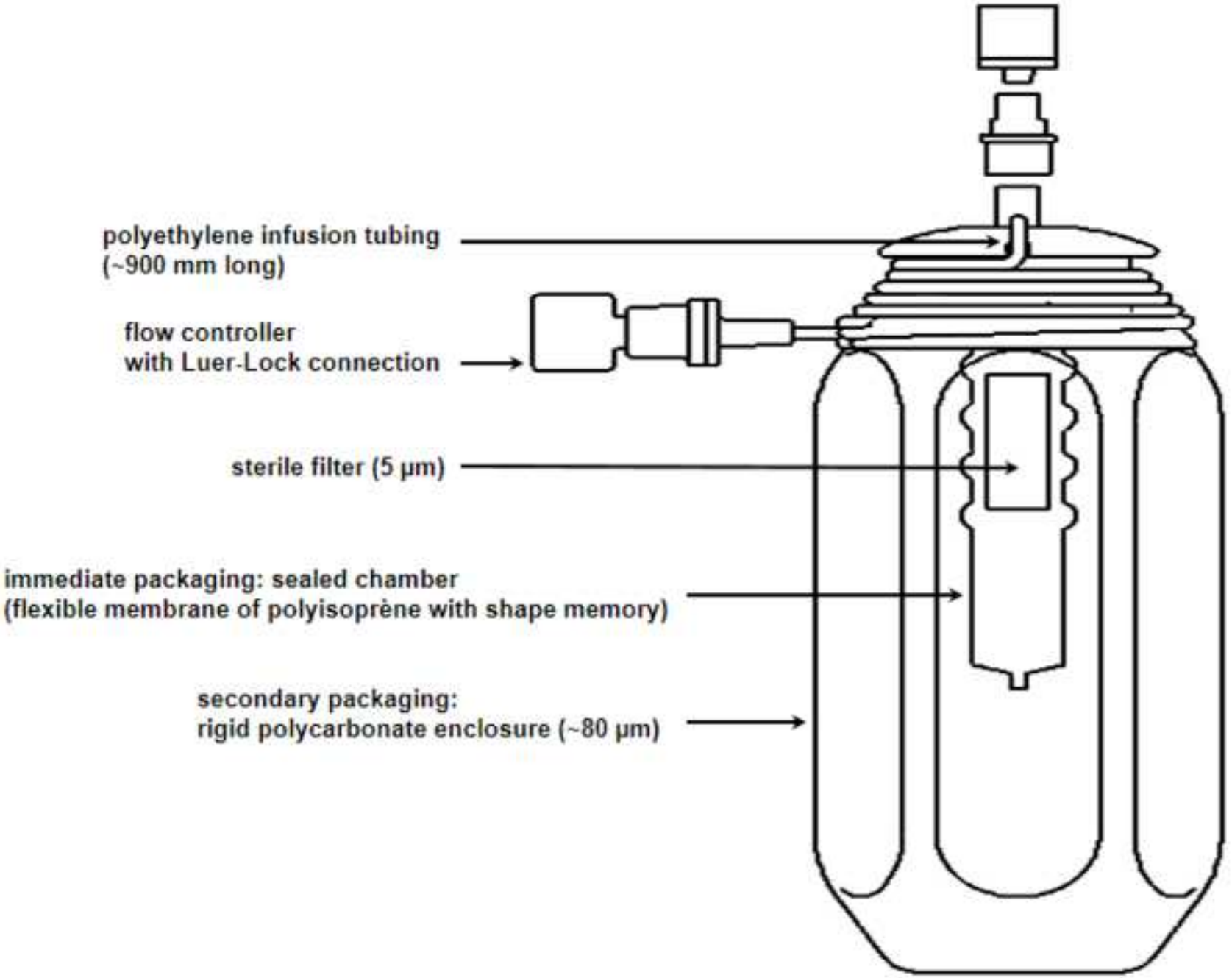

polyethylene infusion tubing
(~900 mm long)
flow controller
with Luer-Lock connection
sterile filter (5 µm)
immediate packaging: sealed chamber
(flexible membrane of polyisoprène with shape memory)
secondary packaging:
rigid polycarbonate enclosure (~80 µm)

Figure 2

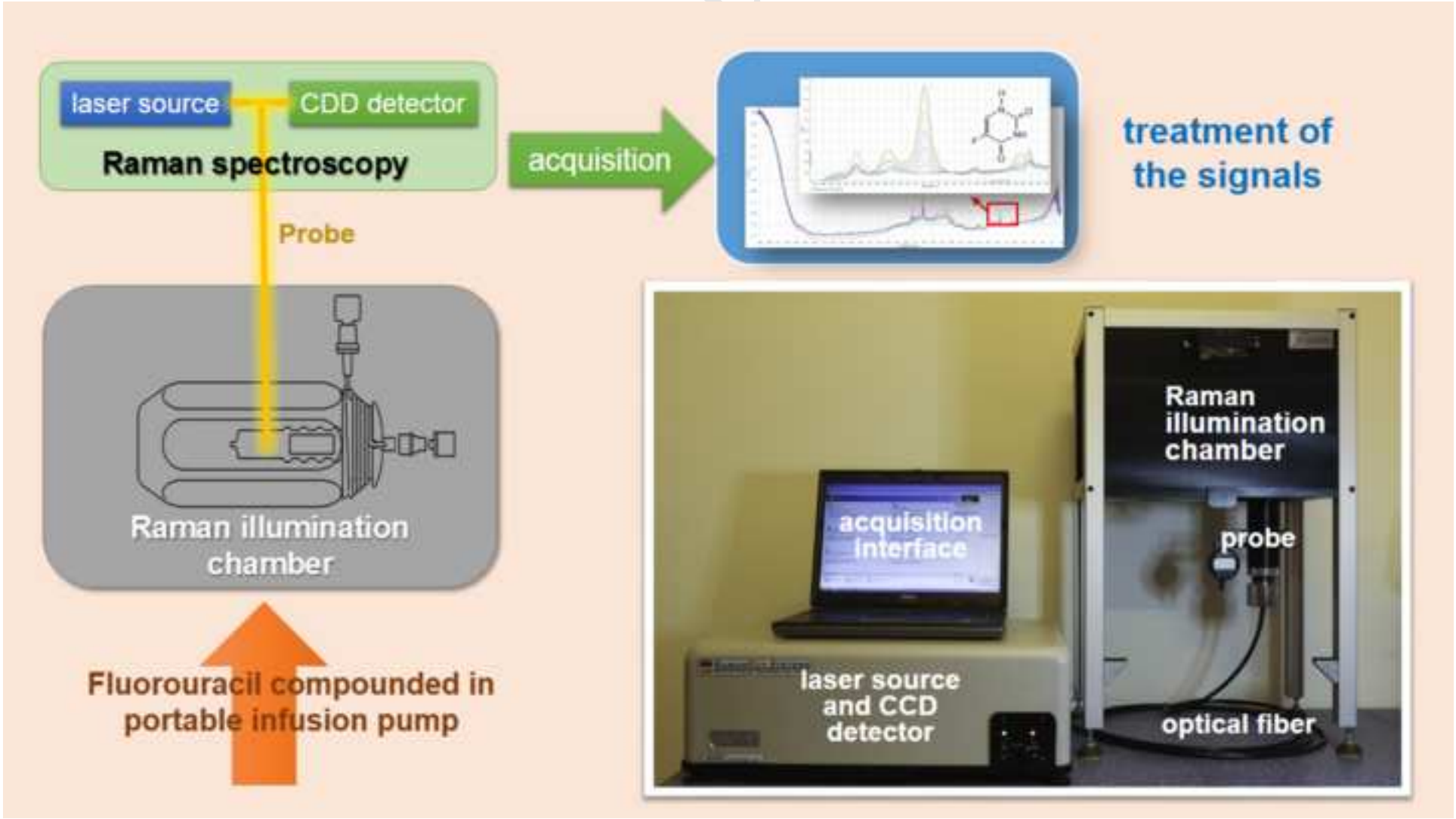
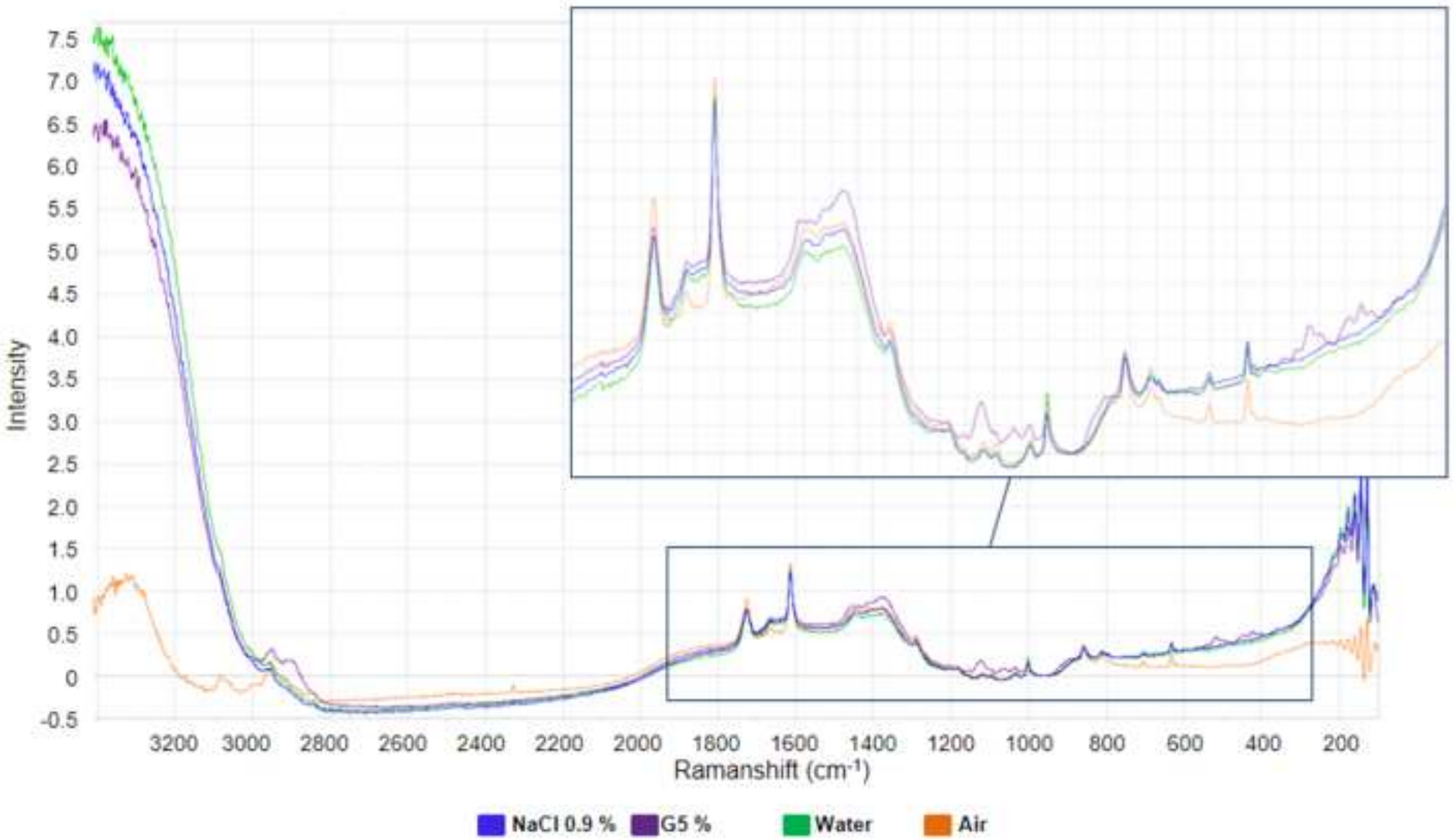

laser source
CDD detector
Raman spectroscopy
Probe
acquisition
treatment of the signals
Raman illumination chamber
Fluorouracil compounded in portable infusion pump
acquisition interface
laser source and CCD detector
Raman illumination chamber
probe
optical fiber

Figure 3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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
Ramanshift (cm-1)
NaCl 0.9 %
G5 %
Water
Air

Figure 4

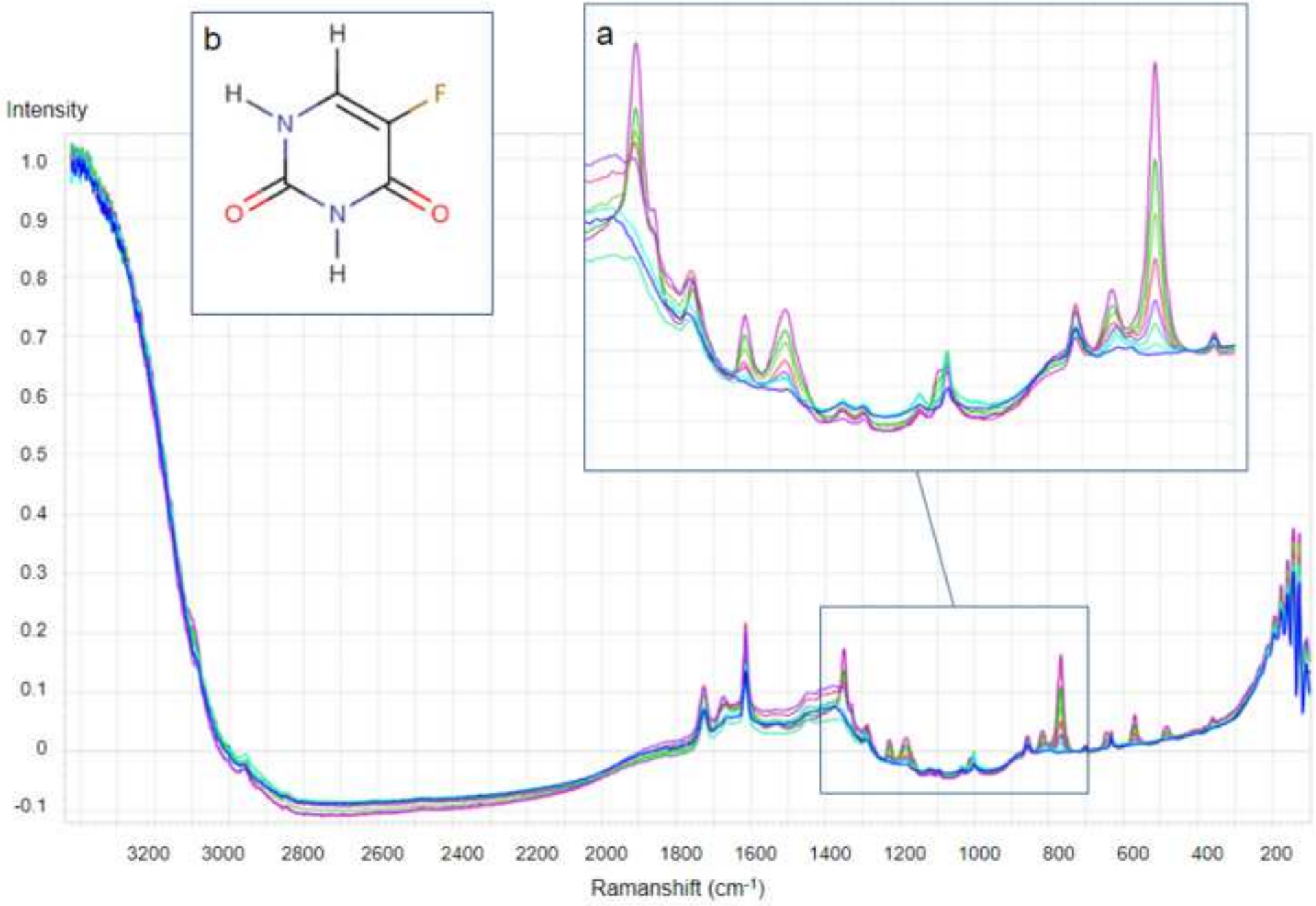

b
a
Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
Ramanshift (cm-1)

Figure 5

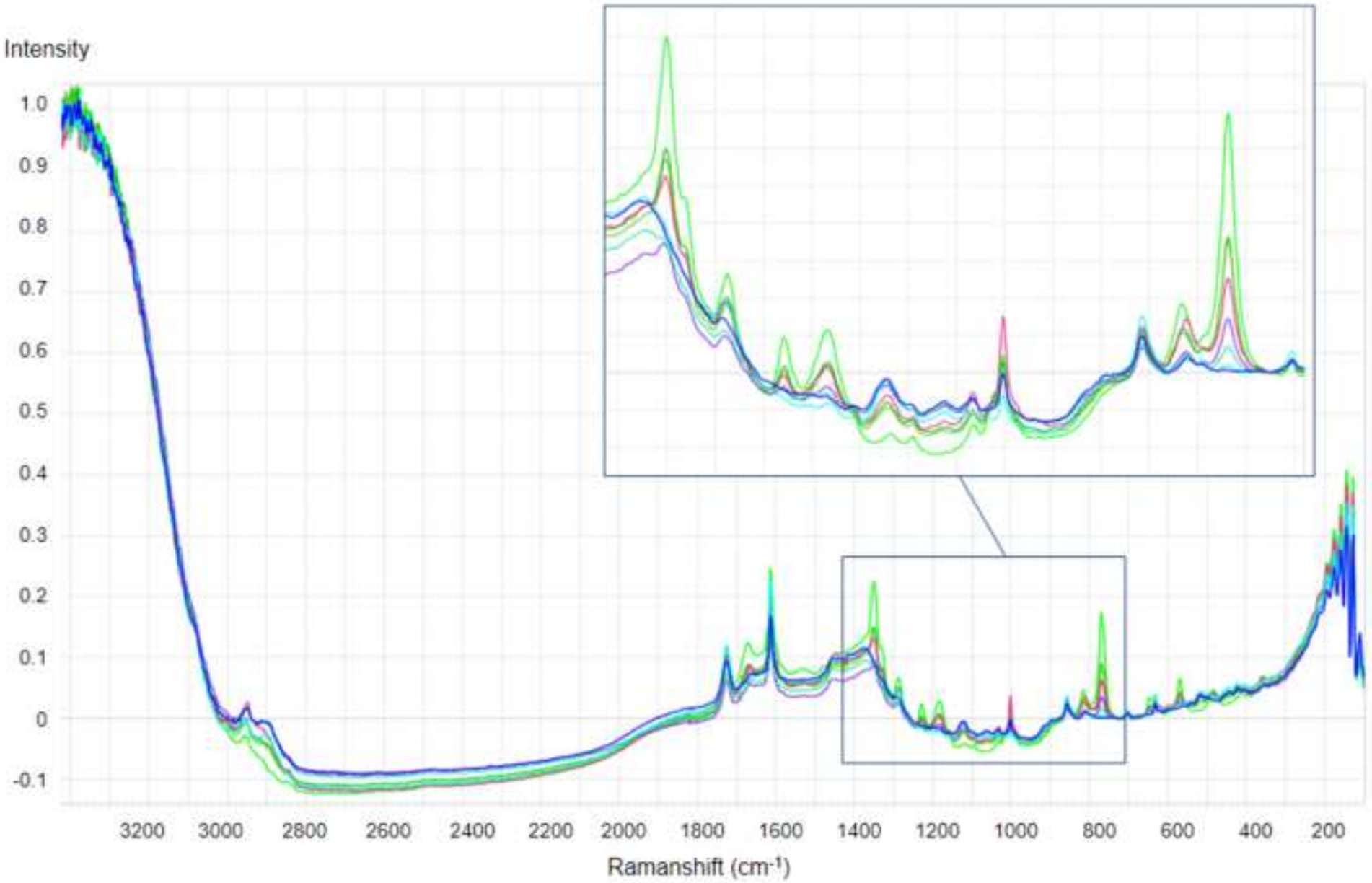

Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
Ramanshift (cm-1)

Figure 6

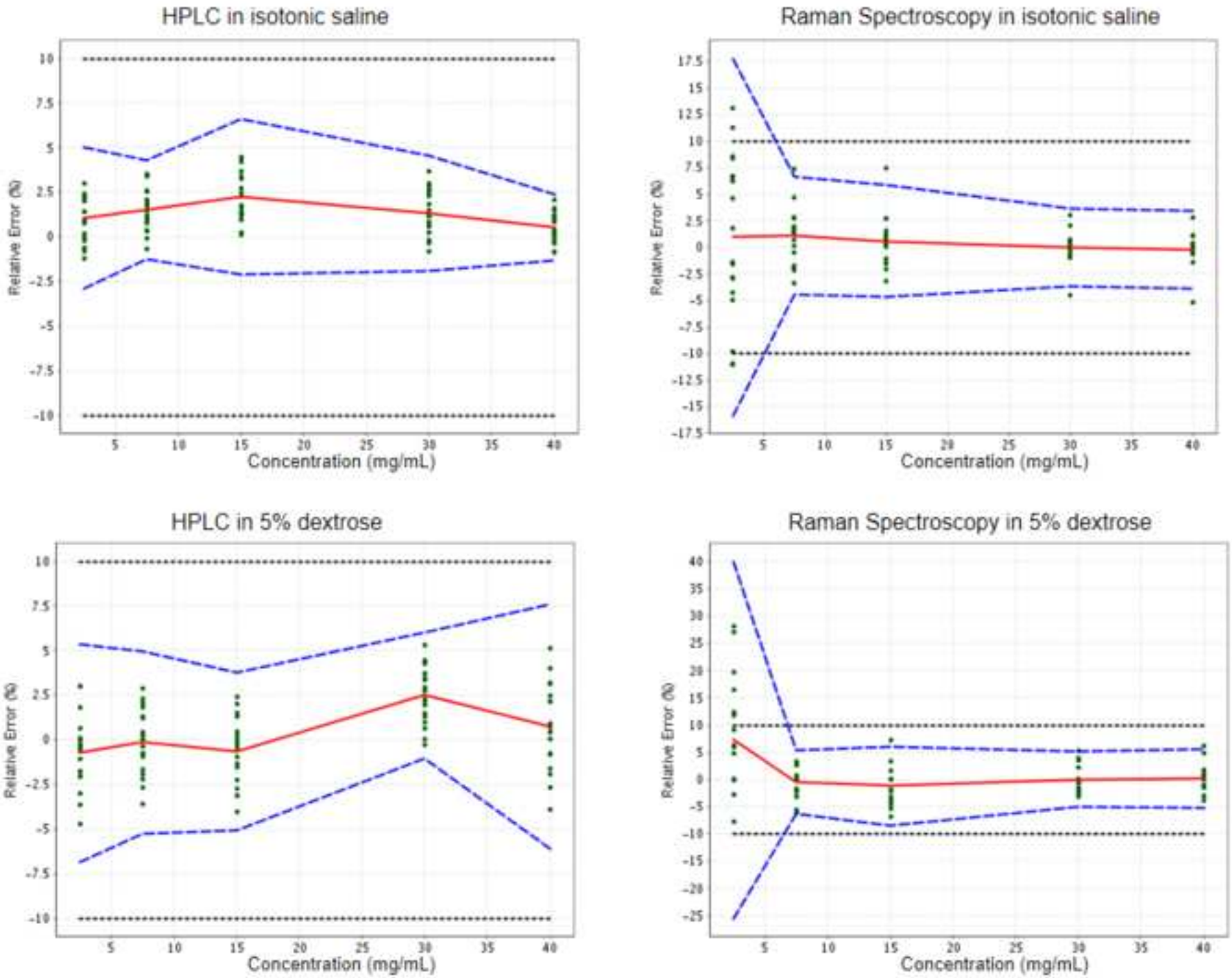

HPLC in isotonic saline
Raman Spectroscopy in isotonic saline
HPLC in 5% dextrose
Raman Spectroscopy in 5% dextrose
Relative Error (%)
Concentration (mg/mL)

Figure 7

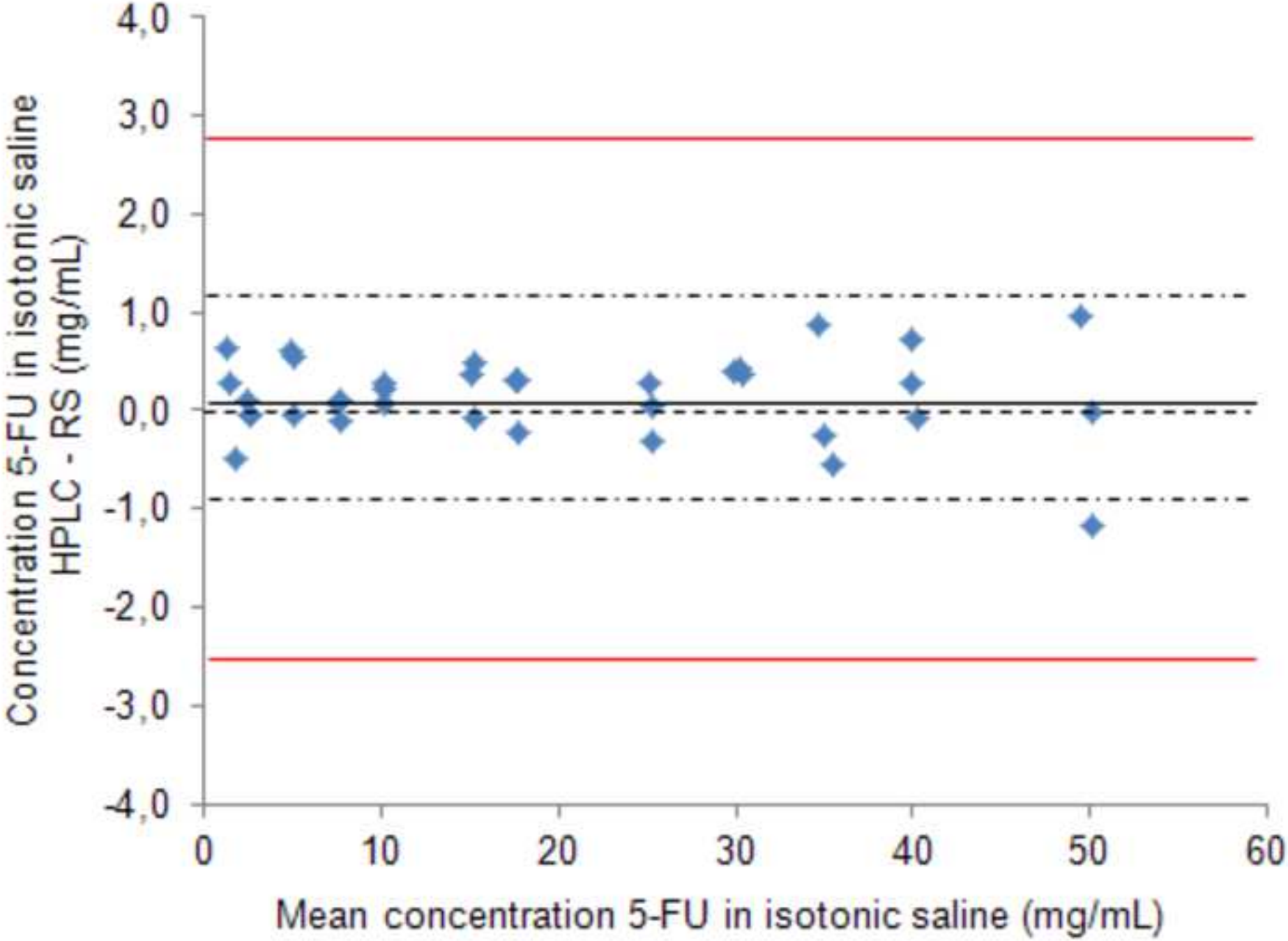
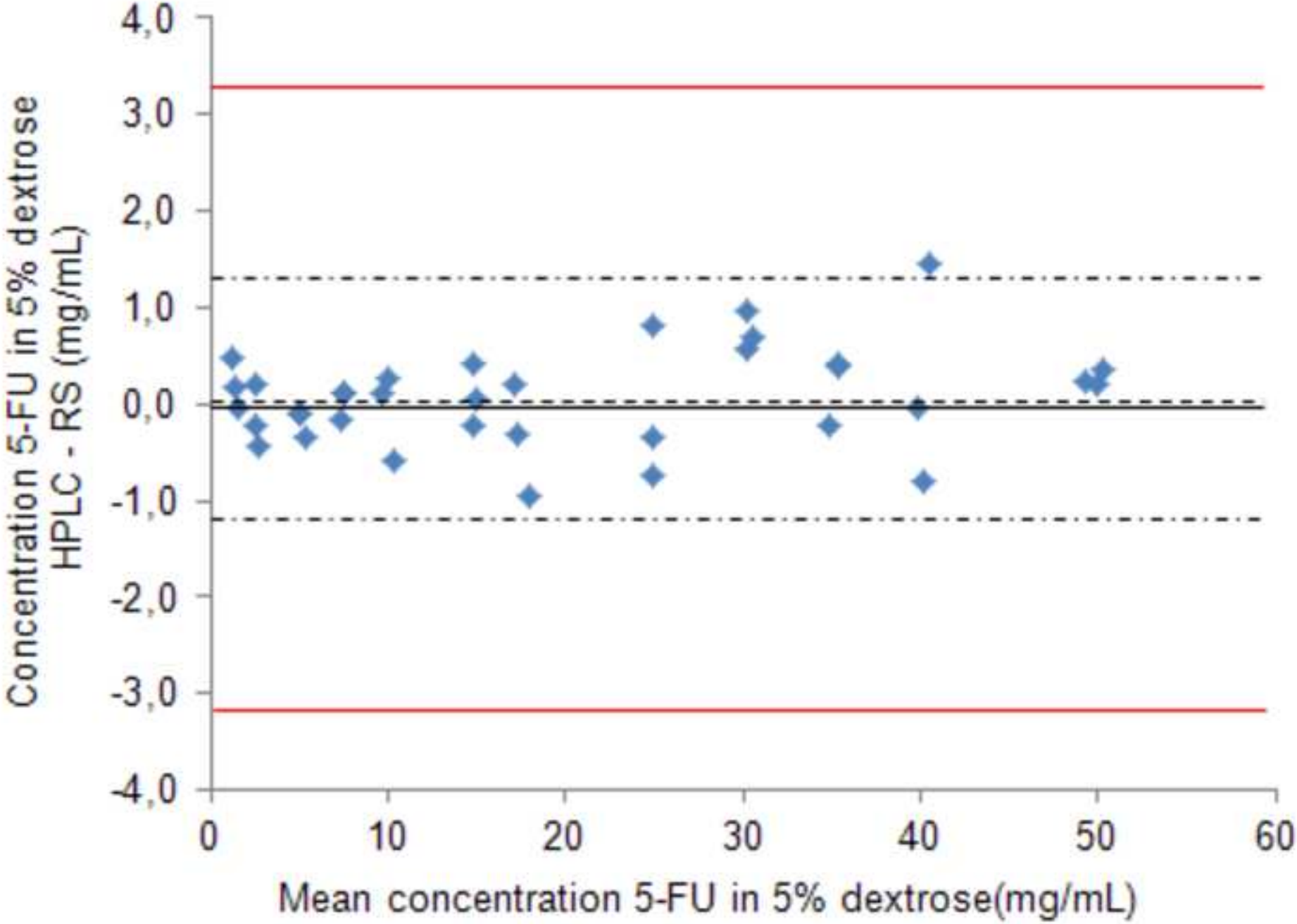

4,0
3,0
2,0
1,0
0,0
-1,0
-2,0
-3,0
-4,0
0
10
20
30
40
50
60
Concentration 5-FU in isotonic saline HPLC - RS (mg/mL)
Mean concentration 5-FU in isotonic saline (mg/mL)

Figure 8


Concentration 5-FU in 5% dextrose HPLC - RS (mg/mL)
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3,0
2,0
1,0
0,0
-1,0
-2,0
-3,0
-4,0
0
10
20
30
40
50
60
Mean concentration 5-FU in 5% dextrose(mg/mL)