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Ensuring quality control in HME, a quantitative analysis of hydrocortisone filament using a portable Raman spectrometer

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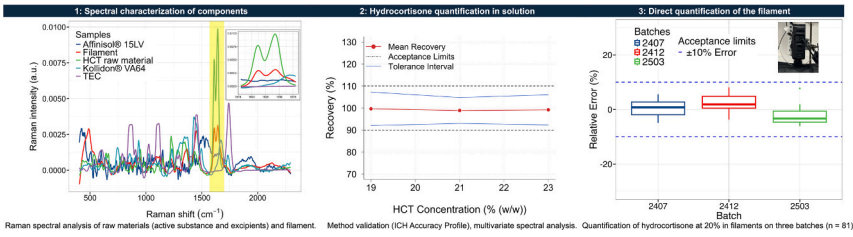
HIGHLIGHTS

- Non-destructive direct Raman analysis for quantifying hydrocortisone in 3D printing filaments.
- A validated methodology with performance assessed against pharmaceutical regulatory standards.
- Performance verified through direct comparison with HPLC-UV method and Bland-Altman test.
- Real-world validation of a filament developed to treat adrenal insufficiency in pediatric patients.

GRAPHICAL ABSTRACT



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ABSTRACT

3D printing is a breakthrough in drug development, offering advantages like personalized medication and the ability to create complex drug formulations. Ensuring safety and efficacy of these printed medications requires rigorous quality control, for which Raman spectroscopy is a powerful tool. This technique can be integrated into the hot melt extrusion (HME) process or the printing process itself, analyzing the drug-content in filament after its production and before it is melted and formed into the final dosage form.

This project focused on developing a quantitative analytical method using a portable Raman spectrometer to measure the concentration of hydrocortisone (HCT) in a filament. This filament acts as a pharmaceutical ink, designed for printing solid oral forms for individualized dosing.

Following ICH guidelines, a validated method for HCT quantification in solution was established. This method was then successfully adapted for direct quantification of HCT within the filament composed of 20 % HCT and excipients. The initial step involved defining a specific spectral region unique to HCT. Preprocessing methods were optimized, including smoothing, baseline correction, derivatives and Extended Multiplicative Signal Correction, used to mitigate unwanted spectral variations.

The method proved highly accurate for the target HCT concentration across three filament batches, achieving a mean absolute error of 2.96 %. This project highlights the value of using a portable Raman probe to control the

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quality of the filament either at the output of HME or directly at the point of care, in order to verify the quality of the received filament.

1. Introduction

Three-dimensional (3D) printing has the potential to revolutionize the drug development process and enable personalized medicine by adapting the drug's characteristics to the specific needs of the patients. It includes facilitating the production process, changes in drug release rate, composition, and shape of the formulations (printed forms) [1].

In Parulski's work [2], the 3D printing of hydrocortisone (HCT), a drug used to treat patients with adrenal insufficiency (AI) by increasing cortisol levels in plasma, was adapted to meet the specific needs of pediatric patients. Authors successfully formulated a filament to produce mini-3D-printed hydrocortisone tablet (*i.e.* printlet) with a concentration of active pharmaceutical ingredient (API) suitable for pediatric use, having child-friendly sizes and shapes while achieving an *in vitro* release of 80 % within 45 min.

The production of hydrocortisone filaments is based on a HME process which is a technique where API and polymers are mixed and melted together to form desired shapes of filaments. This process is followed by a fused deposition modeling step (FDM), where these filaments are melted at high temperatures to build objects layer by layer with an FDM 3D printer [2].

As medicines, it is mandatory to ensure the pharmaceutical quality throughout the process of all steps according to Process Analytical Technology (PAT) approach: filaments printing considered as pharmaceutical ink as well as the quality of produced pharmaceutical products.

The filaments must meet various quality criteria, such as mechanical and rheological properties suitable for FDM printing, a precise and consistent diameter, and a compliant and homogeneous active pharmaceutical ingredient (API) content. The competent authorities (EMA and the EDQM) are currently working on defining quality criteria or a monograph to regulate the status and quality of inks used for 3D printing of medicines.

A significant challenge in this evolving field is to develop non-destructive methods to determine API content, which is crucial for quality control in the context of small batches within hospital/academic manufacturing units. As interest in 3D printing grows, there's a pressing need for methodologies to control these new intermediate inks and printed formulations at various stages of drug production [3].

Both Raman and infrared spectroscopies are good options for characterizing pharmaceutical 3D printing filaments. They offer non-destructive, rapid, and low-cost analysis with portable devices. These techniques provide complementary insights into the filaments' molecular structure and composition.

For example, Yang and al. [4] explored the use of a low-cost, portable Near-Infrared (NIR) spectroscopy as a PAT for monitoring drug content in 3D-printed tablets, using FDM at the POC. The research highlights the potential for real-time quality control, which is a significant challenge for 3D printing. They highlighted the necessity of controlling the quality of the raw material (filament) even before the printing process begins to ensure the quality of the final product. For such applications, the same probe could be used during all the process.

Raman spectroscopy, was reported to be highly beneficial in pharmaceutical field [5]. It enables both rapid identification of chemical composition and quantification of component concentrations. Its principle relies on the inelastic scattering of light after interaction with the sample [6]. Furthermore, it requires minimal sample preparation and allows for non-destructive analysis of various drug forms, including liquids, powders, and 3D-printed formulations.

Despite these advantages, few studies have explored Raman spectroscopy for analytical quality control (AQC) in 3D printing [7–11]. Tumuluri et al. investigated the use of a Raman probe to analyze hot-melt extruded films containing APIs (ketoprofen and clotrimazole) [7]. They acquired both on-line and off-line measurements to characterize the films, and did not fully validate the concentration of API by pharmaceutical standard methodology. Saerens et al. also investigated the Raman probe as a PAT tool for in-line determination of API concentration and polymer–drug solid state during hot-melt extrusion of metoprolol tartrate [9]. Finally, the same team validated a calibration model for in-line API determination using Raman spectra during hot-melt extrusion, implementing the probe in the die for PAT analysis [8]. These studies emphasized the benefits of on-line monitoring to ensure consistent API content and physical state during extrusion. Developing a quantitative analysis of filaments that meets pharmaceutical standards is a major challenge due to the difficulty of producing the necessary materials. Therefore, this work first focused on validating HCT quantification in a solution, where Raman spectroscopy is a more practical option than infrared. We then transferred this validated method to the direct analysis of the filament by selecting the appropriate spectral region.

In our work, we directly analyzed the final filament produced containing hydrocortisone. This control at the filament stage is important to prevent quality issues, as it directly impacts the final product's characteristics and provides valuable insights into the process and product quality. We then developed a quantitative analysis according to pharmaceutical standards and compared the results with reference HPLC/UV analysis.

The primary objective of this study was to accurately determine the HCT content in filaments developed by HME. This filament was specifically designed for 3D printing into printlet™ to serve pediatric patients, ensuring compliance with rigorous pharmaceutical quality recommendations. The main novelty of this article lies in its direct, quantitative analysis of these drug-loaded filaments before they are printed. By following The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) recommendations [12,13], this approach allows for immediate estimation and control of API (HCT) content right after the HME process [14]. Consequently, filaments can be quality-checked after production, minimizing waste, reducing analysis time, and preventing the.

The SFSTP framework [12,15] is designed to guarantee that every measurement performed in subsequent analyses will be sufficiently close to the unknown true value, with the difference consistently remaining below a preset acceptance limit.

In this study, we developed and validated quantitative methodology based on ICH Q2 guidelines and following accuracy profile methodology for quantifying HCT in solution. We then meticulously analyzed the Raman spectra of filaments, API, and excipients to define a specific, interference-free analytical region. Crucially, we successfully transposed this strategy to the direct analysis of filaments, including multiple production batches. This new methodology was then compared against a HPLC-UV reference method, confirming its accuracy.

2. Materials & methods

2.1. Chemicals

The study utilized HCT, purchased from Pharma Chemicals (Wauthier-Braine, Belgium), along with a range of excipients and solvents. Excipients included Kollidon® VA64 (vinylpyrrolidone-vinyl acetate copolymer) from BASF (Ludwigshafen, Germany), Affinisol® 15LV HPMC from Dupont (Wilmington, Delaware, United States), while tri (ethyl)citrate (TEC) was sourced from Vertellus (Greensboro, North

Carolina, United States). For chromatographic analysis, HPLC-grade acetonitrile (ACN) and methanol were purchased from J.T. Baker (Deventer, The Netherlands). Water purification involved a Millipore system (18.2 MΩ/cm resistivity, Milli-Q) followed by filtration through 0.22 µm Millipore Millipak – 40 disposable filter units (Millipore Corporation, USA). [2]

Dimethyl sulfoxide HPLC-grade was purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

2.2. Instrumentation

A Mira P Portable Raman Spectrometer (Metrohm, Herisau Switzerland) was used for all spectral acquisitions. This instrument has a 785 nm excitation wavelength, a spectral resolution of 8–10 cm^{-1} and a wavenumber range of 400–2300 cm^{-1}. The laser's output power was up to 100 mW, but it was set to 50 mW (level 5 on a scale of 1 to 10). It features two point-to-contact sampling attachments, enabling analysis with or without packaging. To construct the calibration of HCT in solution, a vial holder attachment was employed to analyze samples within glass vials with the accumulation time automatically set by the device (0.40 and 0.41 s). For direct analysis of the filament, a point-and-shoot attachment was used. This attachment was fixed on position one, which allowed for direct contact measurement with a focal point at 1 mm. For FLT measurements the accumulation time was set at 10 s.

Prior to measurements, the device underwent calibration using a special Calibration/Verify attachment containing Toluene-Acetonitrile and NIST-traceable polystyrene sites for verification. This spectrometer utilizes Orbital Raster Scan (ORS) technology, which captures large surface areas (1 mm beam diameter, 42 µm spot size) and analyzes dispersed surfaces. The device's 8000 rpm rotation speed during measurement enhances resolution, sensitivity, and representativeness by reducing fluctuations between replicates. A notable advantage is its lower laser power compared to previous Mira generations, preventing heating of flammable samples. Spectra were transferred for further analysis by connecting the Mira P device to a laptop and using Mira P Cal software.

2.3. Methods

2.3.1. Production of hydrocortisone filaments

The filament of 20 % HCT were produced according to Parulski et al. [2]. To prepare the formulations containing 18 %, 19 %, and 21 % HCT, the proportions of the two main polymers were adapted from the initial formulation (Table 1). HCT and the excipients were manually homogenized using a mortar and pestle. The resulting powder blend was then introduced into a gravimetric feeder (Pharma 11 MT20 Gravimetric Twin-Screw, ThermoFisher Scientific, Waltham, MA, USA), which delivered the material at a controlled feed rate of 0.174 kg/h into the barrel of a Pharma 11 twin-screw extruder (ThermoFisher Scientific, Germany). The extrusion was performed at 140 °C and 50 rpm using a screw configuration with two kneading zones, and the process was completed with a die diameter of 1.75 mm. An air-cooled conveyor belt was used to cool the extrudate, with a haul-off speed adjusted to maintain a filament diameter of 1.75 ± 0.05 mm. Filament diameter was continuously monitored throughout the process using a digital caliper (VWR International, Radnor, PA, USA). The 20 % formulation was extruded in triplicate to produce three independent batches.

Table 1
Filament composition at various hydrocortisone concentrations.

HCT	Affinisiol® 15LV	Kollidon® VA64	Tri(ethyl) citrate
18 %	55 %	24 %	3 %
19 %	54.5 %	23.5 %	3 %
20 %	54 %	23 %	3 %
21 %	53.5 %	22.5 %	3 %

2.3.2. Determination of HCT concentration in the filament by HPLC

The quantification drug content into the filament was evaluated by a validated high-performance liquid chromatography (HPLC) method. The HPLC system used was an Agilent® 1100 series (Agilent Technologies, Santa Clara, CA, USA). A solution at a concentration of 20 µg/mL HCT was prepared by dissolving a section of the filament in a water/acetonitrile mixture (60:40 V/V) using an ultrasonic bath for 15 min. The resulting solution was then filtered through a 0.45 µm cellulose mixed ester (CME) membrane filter (25 mm diameter, Sartorius®, Göttingen, Germany). The analytical column was a Symmetry Shield® RP 18 with particles of 5 µm and a dimension 150 mm × 3.9 mm ID. The mobile phase was also composed of mixture water and ACN (60,40 V/V). The flow rate was set to 1.0 mL/min, and the column temperature was maintained at 30 °C. An injection volume of 100 µL was used, with samples introduced at room temperature. The chromatographic run time was set to 6 min, and HCT detection was performed at a wavelength of 246 nm [2].

2.3.3. Validation of quantitative analysis of HCT in solution

All measurements were performed in accordance with the ICH Q2 (R2) guideline for the validation of analytical procedures, thereby ensuring robust compliance with established regulatory standards for method validation [16]. The target concentration of HCT in the filaments was 20 % *w*/w. Recognizing hydrocortisone's limited solubility (practically insoluble in water, slightly soluble in ethanol according to European Pharmacopeia 11.8 [17]), DMSO was selected as the solvent. This decision was made despite DMSO's inherent strong Raman signal, which presented a potential for interference with the HCT analysis.

For sample preparation, a consistent volume of 1 mL of DMSO solution was added to each vial. These solutions were then heated in a water bath at 65–70 °C for 15–20 min. This heating ensured the complete dissolution of all powder. The prepared samples were subsequently measured using the vial holder attachment on the Mira P device, with each measurement recorded using an automatic integration time of 0.41 s.

To accurately quantify the hydrocortisone concentration in DMSO, a calibration curve was established. This involved preparing and measuring five distinct concentrations of HCT in DMSO: 16 %, 18 %, 20 %, 22 %, and 24 % *w*/w, which effectively covered the range from 80 % to 120 % of the target concentration. A total of 15 calibration samples were analyzed, comprising three series, each prepared by a different operator. For the assessment of accuracy and trueness, 27 validation standards were generated by obtaining three replicates for each of three concentrations (19 %, 21 %, and 23 %), with preparation distributed among three different operators.

The final validation of the method was performed using the accuracy profile methodology. The principle of this validation is to ensure that the absolute difference between any individual routine measurement (x_i) and the unknown true value of the sample (μ_T) will always be less than an acceptable limit (λ) [12]. This relationship is mathematically expressed in equation below (Eq. 1):

$$|x_i - \mu_T| < \lambda \tag{1}$$

In this study, we steed λ at 10 %. Therefore, the procedure is considered acceptable if the probability that measurements will fall outside these established acceptance limits is equal to or less than the acceptable risk for routine analytical use (Eq. 2):

$$P(|x_i - \mu_T| < \lambda) \geq \beta \tag{2}$$

In this context, β represents the proportion of measurements that do fall within the acceptance limits. The expected proportion of measurements outside these limits serves to quantify the inherent risk associated with the analytical procedure. For this study, a 95 % proportion of measurements to fall within the acceptance limits was targeted.

2.3.4. Measurements of filaments with Raman probe

Raman measurement reproducibility was improved by using a setup that precisely fixed the handheld Raman spectrometer and maintained the filament at a consistent height for optimal laser focus.

To acquire Raman spectra from the filaments, direct laser analysis was performed in a light-controlled environment. Each sample underwent a rigorous measurement protocol: three individual filaments were analyzed, with three distinct zones targeted per filament, and each filament was rotated three times to ensure comprehensive data collection. A fixed integration time of 10 s was used for all measurements. Reproducibility was assessed for the 20 % target concentration by analyzing three separate production batches. Three filaments were also prepared at HCT concentrations of 18 %, 19 %, and 21 %.

For the several excipients contained in 3D filaments, such as Affinisol® 15LV, Kollidon® VA64 and TEC, and we recorded their Raman Spectra by using the same Mira device. This step was performed by using a specific vial holder attachment of the device, where excipients were analyzed. The measurements were conducted with 3 repetitions.

Given that the predictive model was built using HCT in DMSO solution, the first step involved a thorough examination of spectra obtained from both HCT in solution and HCT within the filament. This detailed observation was necessary to pinpoint a specific spectral region solely attributable to HCT, ensuring it was free from any interference from excipients. It also allowed for the correction of any Raman band shifts that might have occurred due to the different physical forms of the sample for the calibration and the prediction (from liquid solution to solid filament).

2.3.5. Chemometrics

For chemometric analyses, R software, version 4.3.2 (released October 31, 2023), was employed. Various preprocessing methods were evaluated *via* the baseline package (version 1.3–5), including asymmetric least square (ALS, second derivative constraint equal to 7) baseline correction, Savitzky-Golay (SG, first order polynomic and a window size equal to 11) smoothing, and the application of first (d1, window size equal to 11) and second (d2, window size equal to 11) derivatives. Extended Multiplicative Signal Correction (EMSC), a technique used in Raman spectroscopy to mitigate unwanted spectral variations, particularly physical artifacts encountered when analyzing complex solid forms like filaments, was also investigated [18]. Quantitative analysis, encompassing Partial Least Squares Regression (PLS-R) was carried out with the mdatools package [19].

PLS-R, involves the construction of a regression model, as depicted in (Eq. (3)). In this model, Y denotes the response matrix (*e.g.*, concentrations), X represents the matrix of predictors, B is the matrix of coefficients, and E signifies the residual matrix.

$$Y = XB + E \tag{3}$$

The primary aim of PLS is to construct new variables, termed latent variables (LVs). These LVs are linear combinations of the original variables, optimized to encompass the maximum variance while exhibiting the strongest correlation with the response variable under investigation. This technique is highly advantageous for the analysis of spectral data due to its ability to mitigate collinearity phenomena. Furthermore, PLS facilitates the rapid and concise visualization of the most influential spectral regions, thereby indicating areas that hold the most weight in the model's development.

Following this, a mathematical model was established using HCT in DMSO solution and validated in accordance with ICH Q2(R2) guidelines. The optimal number of latent variables (LVs) was determined by calculating the root mean square error (RMSE, Eq. (4)), derived from validation standards. Following these recommendations, the calibration and validation sets was established. We then determined the optimal number of LVs by minimizing the RMSEP of the independent validation set. The accuracy profile was then constructed with a β tolerance interval of 95 % and a limit of acceptability of ±10 %.

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\hat{y}_i - y_i)^2} \tag{4}$$

Following its construction and validation, the model was directly applied to the spectra obtained from the filaments. The identical preprocessing methodology was maintained, and the prediction's performance was assessed using the mean absolute relative error (MARE, Eq. (5)).

$$MARE = \frac{1}{N} \times \frac{|\hat{y}_i - y_i|}{y_i} \tag{5}$$

Finally, the prediction performance of the new direct Raman analysis methodology was compared to results obtained with the reference HPLC method using Bland-Altman analysis. The Bland-Altman plot is a widely used graphical method to assess the agreement between two different methods of measurement. In this work, two different techniques (Raman *vs.* HPLC) were compared. It provides a visual representation of the agreement and helps to identify any systematic bias and random errors between the two methods. The limits of agreement (LoA) were set at 95 %. These limits define the interval within which 95 % of the differences between the two methods are expected to lie, assuming a normal distribution of these differences. They were calculated as the mean difference ±1.96 times the standard deviation of the differences.

The final methodology used in this work is fully recap in Fig. 1:

3. Results

3.1. Spectral analysis of raw materials

The initial step involved examining the spectral data, specifically the raw spectra of the API (HCT), excipients, and solvents, to identify an HCT-specific spectral area.

Fig. 2 illustrates the raw spectra of HCT, Affinisol® 15LV, Kollidon® VA64, TEC, and the filament, all preprocessed with total area normalization and ALS baseline correction. Total area normalization is a common technique used to compare Raman spectra from different samples, such as excipients and a filament, by minimizing the impact of experimental variations. By normalizing each spectrum to the same total area, differences in signal intensity caused by factors like laser power fluctuations or sample size variations are reduced. While helpful, this method can sometimes amplify background noise. If a spectrum has a low signal-to-noise ratio to begin with, normalizing it can disproportionately increase the background noise along with the intended signal, making the spectrum appear noisier.

While TEC displayed a considerable Raman signal, its overall impact on the filament (FLT) spectrum was limited, as it accounted for only 3 % of the formulation. Based on this analysis, a specific spectral area attributed to HCT was definitively established within the 1550 to 1660 cm^{-1} range, marked by two key Raman shifts located at 1611 cm^{-1} and 1641 cm^{-1}. Both Raman shifts can be attributed to stretching vibrations of C═C double bond present in the cyclic structure of hydrocortisone [20–22]. Furthermore, in this specific selected zone there was no contribution of DMSO.

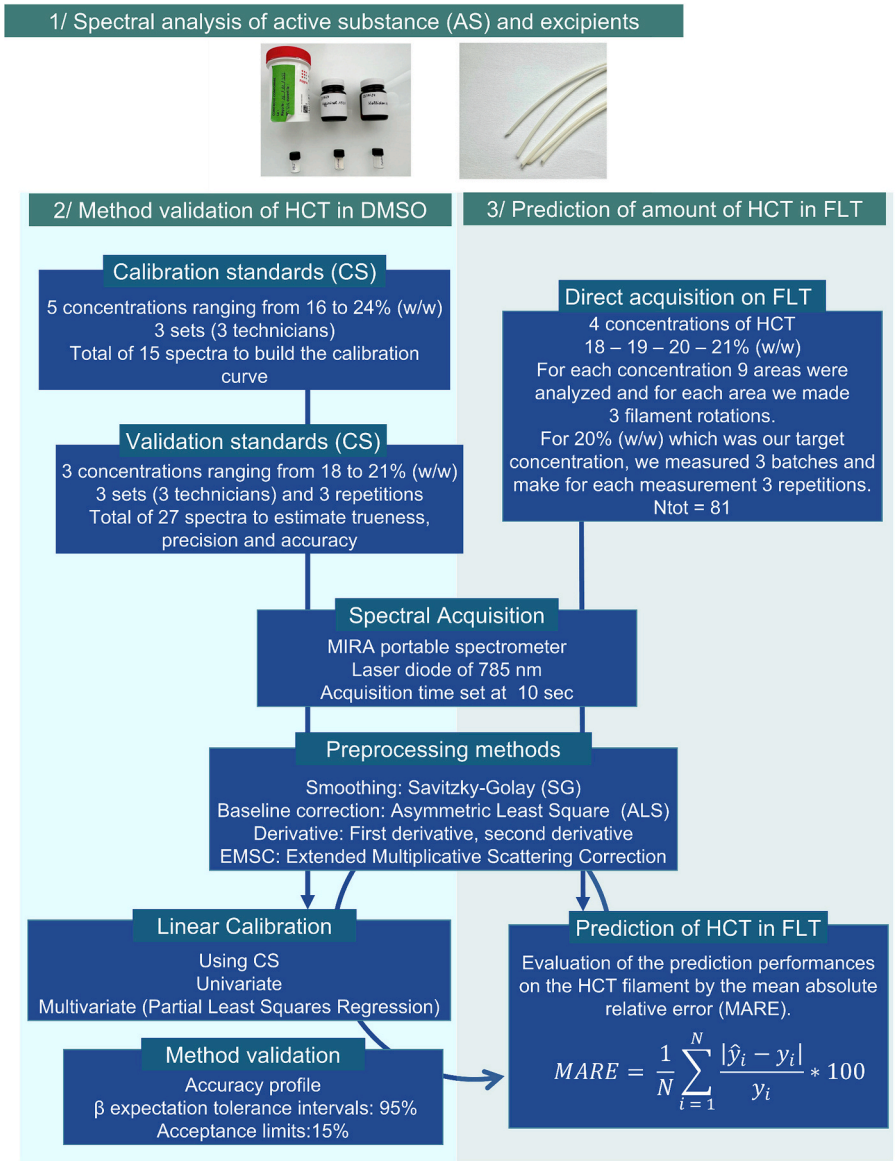

Fig. 1. Flowchart of the entire methodology developed to directly predict the amount of hydrocortisone in a filament using a model initially developed in solution, ensuring compliance with pharmaceutical standards.

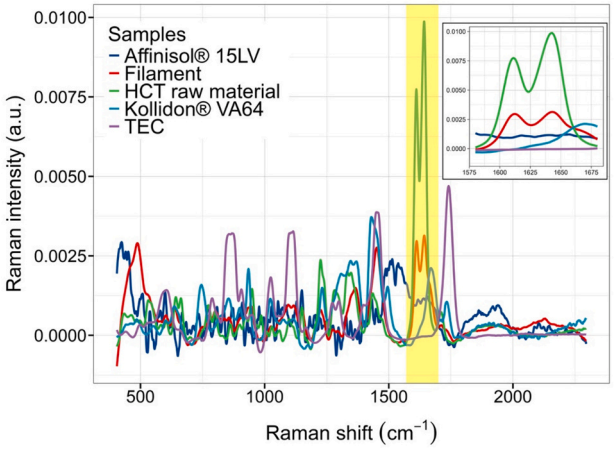

Fig. 2. Raman spectra of Affinisol® 15LV, Kollidon® VA64 tri(ethyl)citrate (TEC), Hydrocortisone raw material and filament of 20 % hydrocortisone. Preprocessing: total area normalization, asymmetric least squares baseline correction.

3.2. *Quantitative method of HCT in DMSO*

Upon selecting an appropriate spectral area, a quantitative method was developed in line according to ICH and SFSTP recommendations, using the accuracy profile methodology. The most favorable results were obtained by focusing on the 1552–1636 cm^{-1} spectral area, combined with a preprocessing sequence of Savitzky-Golay (SG) smoothing, Asymmetric Least Squares (ALS) baseline correction, EMSC, and the second derivative (d2), followed by a multivariate Partial Least Squares Regression (PLS-R) model. Table 2 sum up main statistic of the validation of the quantification model.

As shown in Fig. 3, the accuracy profile of the quantitative model was validated within a range of 80 % to 120 % of the target concentrations. The optimal number of latent variables (LVs) was determined by minimizing the root mean square error (RMSE) on the validation dataset, resulting in the selection of two LVs (RMSE = 0.813 %). All final spectral selections and preprocessing methods were specifically optimized to achieve the best performance on the filament (FLT) dataset.

Table 2
Trueness and precision for the quantification of hydrocortisone in solution [] represents the confidence interval à 95 % for mean recovery.

Theoretical Conc (%)	Mean Recovery (%)	Intermediate Precision SD	Adjusted Precision SD
19	99.68 [98.94–100.41]	0.73	0.77
21	98.91[98.32–99.50]	0.63	0.66
23	99.21 [98.51–99.91]	0.80	0.84

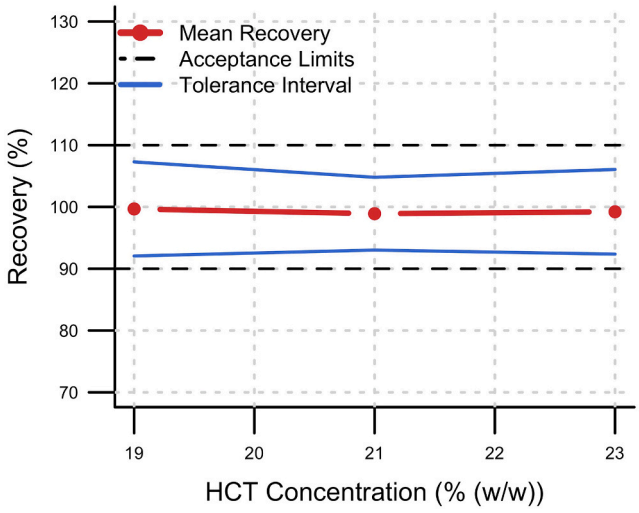

Fig. 3. Accuracy profile of hydrocortisone concentration in DMSO. Black dotted lines represent the limits of acceptability (± 10 %) blue lines represent the limits of the β interval (95 %).

3.3. Prediction performance on the filament

During this work 162 measurements from the filaments (27 for each of the 18 %, 19 %, and 21 % concentrations, and 81 for the 20 % target concentration) were acquired. The pre-established model was then applied to predict HCT concentrations in these filament samples, with optimization efforts focused on minimizing the Mean Absolute Relative Error (MARE).

A critical step for optimal performance was correcting for the Raman band shift attributed to the liquid-to-solid phase change, specifically centered at 1611 cm^{-1} (Fig. 4). This result is aligned with the observations made by Tumuluri (7) and Saerens (8) regarding the capabilities of Raman spectroscopy to distinguish crystalline from amorphous forms of API in the filament. The most accurate results were achieved by applying Raman shifting correction, then SG smoothing, ALS baseline correction, EMSC and d2 preprocessing within the PLS-R model.

The mean spectra of each concentration of HCT in FLT with final pretreatments are represented in Fig. 5. Using the second derivative can be interesting by converting the broad, overlapping peaks of the original spectrum into sharper, more distinct negative peaks, which could explain the improvement of predictive quantification performance in a complex mixture. This approach yielded a total MARE of 3.31 % across all concentrations and 2.96 % for the target 20 % concentration. The dispersion of relative error (RE) across concentration levels of HCT in the filament is represented in Fig. 6.

Table 3 shows the main quantitative performance of the model based on the preprocessing methods used. The results demonstrate that each successive preprocessing step significantly improves prediction performance. While we tested numerous methods and spectral regions, it is not possible to present all of them in detail.

A notable increase in prediction performance was observed when moving from the first derivative to the second derivative. It's important

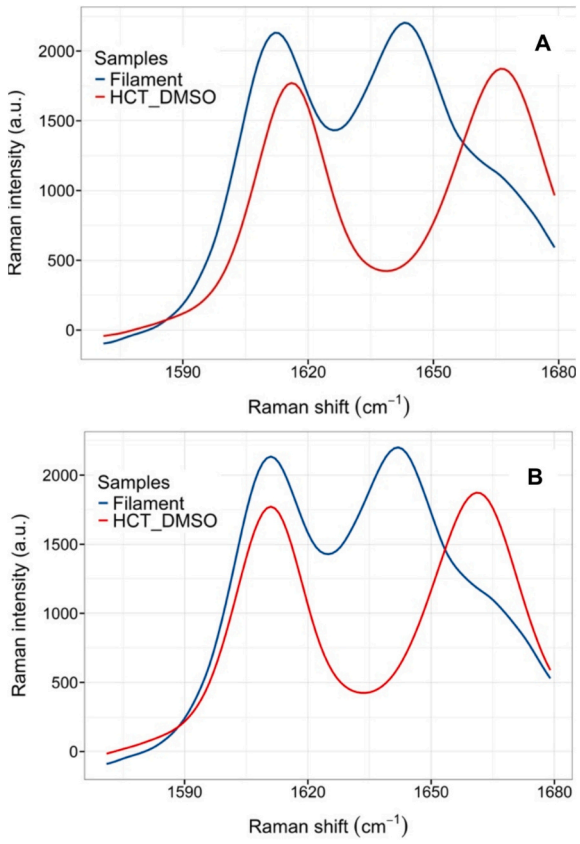

Fig. 4. Mean spectra of filament (HCT 20 %, in blue) and HCT 20 % un DMSO (red). Before (A) and after (B) shifting correction (center in 1611 cm^{-1}). Preprocessing: SG, ALS, EMSC.

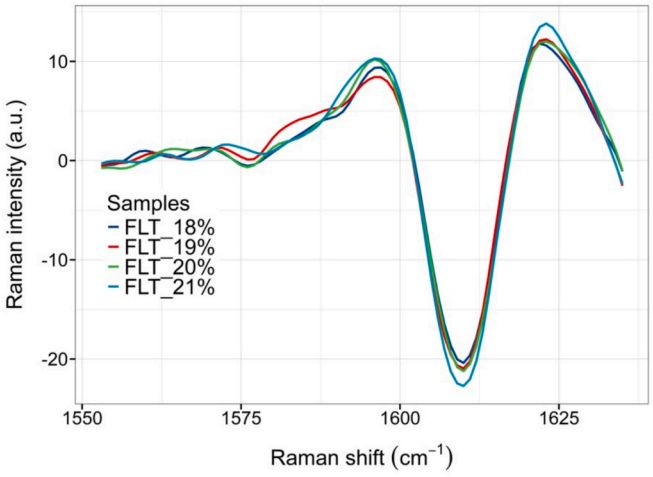

Fig. 5. Mean spectra of selected Raman shift area, with final preprocessing for quantification of hydrocortisone within the filament.

to note that, in the result presented, the spectral region selection was specifically optimized for the second derivative. Performance for the first derivative could potentially be improved by using a slightly different spectral region.

The application of EMSC also resulted in a significant improvement in performance. This highlights its effectiveness in addressing challenges in Raman spectral analysis, particularly when dealing with different material types (solutions *vs.* filaments).

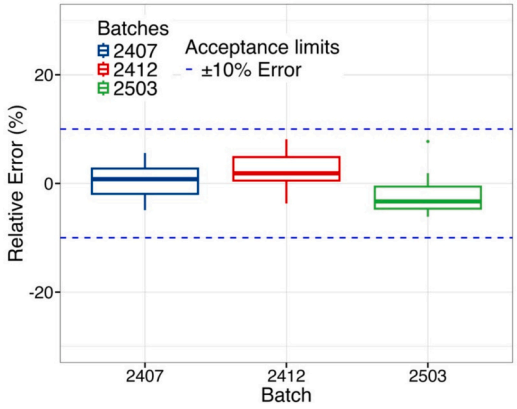

Fig. 6. Relative error plotted against batch of production of hydrocortisone filament (concentration of 20 %, $n = 27$ for each batch, ntotal = 81).

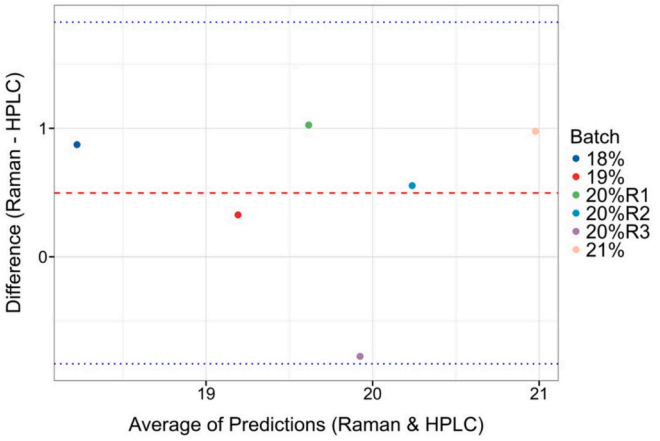

Fig. 7. Bland-Altman plot to compare HPLC-UV and Raman analysis. The red dashed line represents the mean difference. The blue dotted lines represent the limit of agreement.

Table 3
main quantitative performances according to the preprocessing methods used. The table should be read with an increment at each row, except for the derivative. For example, "SG + ALS" is in row 3, while "SG + ALS + shifting correction" is in row 4. SG: Savitzky-Golay, ALS: asymmetric least squares, EMSC: extended multiplicative scattering correction, RMSEP: root mean squared error of prediction, nLV: number of latent variables, MARE: mean absolute relative error.

Preprocessing methods	RMSEP (%)	nLV	Total MARE (%)	Target concentration (20 %) MARE (%)
Raw spectra	28.2	6	134	124
Spectral selection	21.3	5	96.4	96.0
SG + ALS	9.54	1	39.4	44.2
Shifting correction	7.42	1	34.4	30.4
EMSC	6.76	2	31.4	28.5
First derivative	3.05	2	11.91	15.5
Second derivative	0.813	2	3.31	2.96

3.4. Comparison of Raman prediction to HPLC

Prediction performances were compared with standard HPLC-UV method. All prediction results are sum up in Table 4. The Bland-Altman plot to compare our two techniques is represented on Fig. 7.

The x-axis represents the average of the predicted values from the Raman and HPLC methods for each group (averaged by batch, $n = 6$ for each group in HPLC measurements, $n = 27$ for Raman measurements).

The red dashed line represents the mean difference and indicates the average systematic bias between the two methods. On average, the Raman predictions were 0.50 units higher than the HPLC predictions (*i.e.* 2.5 %). In the current plot, all points fell within the LoA, suggesting no extreme outliers from the 95 % agreement range. Although a systematic bias of 2.5 % was observed, its significance must be evaluated within the context of established pharmaceutical standards. Regulatory guidelines such as the ICH and the United States Pharmacopeia (USP) typically permit drug content to fall within a range, often between 90 % and 110 % of the labeled amount. Within this established tolerance, a consistent systematic bias of 2.5 % is well within acceptable limits. The consistency of this bias further demonstrates the reliability of the Raman method for quality control purposes.

Therefore, the new *in situ* measurement without destroying sample was equal to standard methodology. The new method was fully validated.

In this work, we developed and validated a method for the quantitative analysis of an API in a filament. This method was specifically designed for HCT filaments intended for use in 3D printing for pediatric patients suffering from adrenal insufficiency.

The method was first validated by analyzing the API in a solution, using the accuracy profile methodology as recommended by the ICH and the SFSTP. This approach allowed us to ensure the method's accuracy and precision for the targeted concentration. We first validated the method in a solution, which allowed us to more conveniently analyze the raw API spectra and establish a robust basis. This validated technique was then successfully adapted for the direct analysis of the solid filament by carefully selecting the optimal spectral region. The key contribution of this work was providing a comprehensive methodology to guarantee the quantity of the API in the filament. This method can be easily reused for other HCT printlet formulations by simply adjusting the analysis for new excipients and identifying the correct spectral zones. Another option could be to apply a chemometric methodology, like Independent Component Analysis (ICA), which is specifically designed to isolate and identify underlying signal sources [23]. It could also be

Table 4
Comparison of HPLC and Raman analysis. Summary of trueness and precision for each concentration.

	HCT 18 %	HCT 19 %	HCT 20 %R1	HCT 20 %R2	HCT 20 %R3	HCT 21 %
			High pressure liquid chromatography			
n			6			
Mean recovery	98.83	100.15	95.52	99.80	101.56	97.57
SD	0.52	0.29	3.88	0.65	0.21	0.05
CI95 %	98.41–99.24	99.92–100.38	92.41 - 98.63	99.29–100.32	101.39–101.73	97.53–97.61
			Raman spectroscopy			
n			27			
Mean recovery	103.68	101.87	100.64	102.57	97.68	102.22
SD	3.04	4.21	2.85	3.03	3.03	4.24
CI95 %	103.09–104.26	101.05–102.68	100.10–101.19	101.99–103.15	97.10–98.26	101.40–103.04

reused to other API following the same methodology.

To further confirm these results and ensure viability of the process, a few steps are necessary. Additional batches of the filament will be needed to confirm the results and create quality control maps for each new batch to monitor for any deviations in API concentration. The current study focused on the target concentration, which is suitable for the intended therapeutic use. For wider applications, the good linearity of the method must be confirmed over a broader concentration range. It is also crucial to implement a control chart to track the method's performance and predict any potential deviations.

The validated method could also be applied to the final printlets produced from the filament to confirm that the API content is consistent and correct in the final dosage form.

4. Conclusion

In this study, we developed a direct and non-invasive analytical method using Raman spectroscopy to quantify HCT content in filaments intended for FDM printing of medicinal dosage forms. We accurately quantified 20 % hydrocortisone in a complex formulation.

This work unequivocally demonstrated the significant potential of Raman spectroscopy, coupled with robust chemometric analysis, for enhancing the quality control of pharmaceutical filaments intended for 3D printing, particularly in hospital manufacturing settings.

While the current focus was on the filament, extending this methodology to the final printed product represents a highly impactful area for future investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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