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Rapid discrimination and quantification analysis of five antineoplastic drugs in aqueous solutions using Raman spectroscopy

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ABSTRACT

The aim of this study was to assess the ability of Raman spectroscopy to discriminate and quantify five antineoplastic drugs in an aqueous matrix at low concentrations before patient administration.

Five antineoplastic drugs were studied at therapeutic concentrations in aqueous 0.9% sodium chloride: 5-fluorouracil (5FU), gemcitabine (GEM), cyclophophamide (CYCLO), ifosfamide (IFOS) and doxorubicin (DOXO). All samples were packaged in glass vials and analyzed using Raman spectrometry from 400 to 4000 cm^{-1}. Discriminant analyses were performed using Partial Least Squares Discriminant Analysis (PLS-DA) and quantitative analyses using PLS regression.

The best discrimination model was obtained using hierarchical PLS-DA models including three successive models for concentrations higher than the lower limit of quantification (0% of fitting and cross-validation error rate with an excellent accuracy of 100%). According to these hierarchical discriminative models, 90.8% (n = 433) of external validation samples were correctly predicted, 2.5% (n = 12) were misclassified and 6.7% (n = 32) of the external validation set were not assigned. The quantitative analysis was characterized by the RMSEP that ranged from 0.23 mg/mL for DOXO to 3.05 mg/mL for 5FU. The determination coefficient (R^2) was higher than 0.9994 for all drugs evaluated except for 5FU (R^2 = 0.9986).

This study provides additional information about the potential value of Raman spectroscopy for real-time quality control of cytotoxic drugs in hospitals. In some situations, this technique therefore constitutes a powerful alternative to usual methods with ultraviolet (UV) detection to ensure the correct drug and the correct dose in solutions before administration to patients and to limit exposure of healthcare workers during the analytical control process.

1. Introduction

In the last decade, Raman spectroscopy has become an important tool in the pharmaceutical industry. It is a rapid, noninvasive and nondestructive analysis and this molecular vibrational spectroscopy, as in the case of near infrared spectroscopy, is widely used in process analytical technology (PAT) (André et al., 2015; Brouckaert et al., 2016; Esmonde-White et al., 2016). It is defined by the United States Food and Drug Administration as a system to design, analyze and control pharmaceutical manufacturing processes by the measurement of critical process parameters which affect the quality of the final product (FDA, 2004). In the case of PAT, Raman spectroscopy is also suited for the characterization and quantification of starting materials, pharmaceutical ingredients and polymer materials (Caudron et al., 2011; Eliasson and Matousek, 2007; Lê et al., 2016).

Raman spectroscopy measures vibrational, rotational and other low frequencies of an analyte. In contrast to infrared spectroscopy (IR) based on focusing a broad spectrum of light on a sample and measuring the absorbance of IR wavelengths, Raman spectroscopy uses a single wavelength of light. The scattered light is collected to obtain Raman spectra which depend on the bond strength of the analyte, the mass of bound atoms and intermolecular interactions.

In the pharmaceutical industry, analytical procedures are required to ensure the identity, quality, purity and potency of drug substances and drug products (FDA, 2015). Many hazardous drugs designed for parental administration such as antineoplastic drugs, however, require aseptic reconstitution or dilution to yield a final sterile preparation. Doses of these drugs are individually adapted by the physician and are prepared in a specific unit of hospital pharmacies by a pharmacy technician before administration by a nurse. Concentrated commercial

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solutions are often diluted in aqueous 0.9% sodium chloride or 5% glucose solutions to obtain the required dose of the medication prescribed. These preparations are regulated as pharmaceutical compounding products by the Good Preparation Practices (BPP, 2007) and the United States Pharmacopeia (USP) (USP < 797 >).

In the pharmaceutical industry and according to FDA regulations, each final product must be controlled in order to ensure its quality. Despite the continuous increased use of cytotoxic drugs in hospitals (increase of > 50% of compounding cytotoxic drugs from 2010 to 2016 in our hospital), this control is not required by pharmaceutical regulations. Regarding the risk of adverse effects due to under- or overdosing cytotoxic preparations but also risk linked to the preparation, for example use of the wrong solvent that can dramatically alter the drug stability and security (*i.e.* sodium chloride instead of glucose/dextrose solutions for platine based drugs), implementing the analytical control of these drugs compounded in hospitals is essential for ensuring the safety of the medication process. The challenge is therefore to develop an analytical tool for the discrimination and quantification of all compounded drugs over the entire therapeutic concentration range without slowing the compounding process.

Numerous strategies have been developed in hospitals. Most of the time, antineoplastic drugs are controlled using their UV properties by flow injection analysis (FIA). For some drugs, generally of the same family with similar chemical structures, UV spectral data are often non-discriminant and these drugs are discriminated with respect to other parameters by combining UV detection with other analytical technique: on their retention times after separation by liquid chromatography or other vibrational spectral signatures obtained by Fourier transform infrared spectrometry (UV/vis-FTIR) or Raman spectroscopy(Bazin et al., 2010; Bourget et al., 2014a; Dziopa et al., 2013; Nardella et al., 2016). Despite good analytical performances, these controls still require the final product to be sampled. Consequently, only antineoplastic drugs packaged in plastic bags are analytically controlled in our hospital; elastomeric pumps and syringes are excluded to control.

Raman spectroscopy has the potential for non-invasive and non-destructive analysis by direct measurement through containers and satisfying quantitative and discriminative results previously obtained in glass vials for two isomeric antineoplastic drugs (Amin et al., 2014; Bazin et al., 2014, 2015; Bourget et al., 2014a, 2014b; Lê et al., 2014, 2016).

We decided to extend this approach to other antineoplastic drugs among the most often used and listed on the World Health Organization's list of essential medicines. The aim of this study was first to assess the ability of Raman spectroscopy (RS) to discriminate and quantify five antineoplastic drugs in an aqueous matrix at low concentrations in order to consider its potentiality to control drugs directly in the preparation before patient administration and secondly, Raman spectroscopy and FIA or HPLC/UV methods were compared.

2. Material and methods

2.1. Drugs

In order to assess the feasibility of the RS, this study focused on several drugs largely produced in our unit and corresponding to 25% of our chemotherapy activity (over approximately 60 drugs). Five antineoplastic drugs were studied.

Concentrated solutions of 5-fluorouracil at 50 mg/mL (5FU, PubChem CID: 3385) and gemcitabine at 40 mg/mL (GEM, Pubchem CID: 6075) were obtained from Teva (La Defense, France) and Mylan (Saint Priest, France). Cyclophophamide at 20 mg/mL (CYCLO, PubChem CID: 2907) and ifosfamide at 40 mg/mL (IFOS, PubChem CID: 3690) were obtained from Baxter (Guyancourt, France). Doxorubicin at 2 mg/mL (DOXO, PubChem CID: 31703) was obtained from Accord (Lille, France).

2.2. Sample preparation

In order to develop robust models, three sources of variability linked to batches of active ingredient and batches of diluents and containers from the same manufacturer were introduced to prepare sample sets. As a result of the absence of specific guidelines to control pharmaceutical products by Raman spectroscopy, the study was performed in accordance with the guidelines for the use of near infrared spectroscopy by the pharmaceutical industry published by the European Medicines Agency (EMA, 2014). Three sets of samples representative of production were prepared: a calibration set to construct the calibration models, a validation test set to optimize and validate the calibration model and an external validation test set that included independent samples to evaluate predictive performances of the selected calibration model. All samples were packaged in glass vials from Interchim® (Montluçon, France) and analyzed using Raman spectrometry.

2.2.1. Calibration and validation test set

For each drug, three series of solutions were prepared by dilution of the concentrated commercial solution with aqueous 0.9% sodium chloride (FreeFlex® Fresenus Kabi, Sèvres, France) to obtain diluted solutions in the range of therapeutic concentrations. Each solution was divided into three aliquots and packaged in three different Interchim®glass vials. At least 90 samples of each drug were analyzed at 10 concentrations in accordance with therapeutic dosage ranges: from 1.0 to 50.0 mg/mL for 5FU, from 1.0 to 40.0 mg/mL for GEM, from 0.5 to 20.0 mg/mL for CYCLO, from 1.0 to 40.0 mg/mL for IFOS and from 0.01 to 2.00 mg/mL for DOXO.

2.2.2. External validation test set

In order to validate the predictive capacity and the robustness of calibration models, 477 independent samples of antineoplastic compounding drugs were prepared at different concentrations with batches of active ingredient and 0.9% sodium chloride different from those we used for calibration and validation test sets: 95 5FU samples from 6.5 to 45.0 mg/mL, 93 GEM samples from 3.5 to 35.0 mg/mL, 95 DOXO samples from 0.35 to 1.75 mg/mL, 97 CYCLO samples from 3.5 to 15.0 mg/mL and 97 samples IFOS from 3.5 to 35.0 mg/mL.

2.3. Instrumentation

2.3.1. Raman spectroscopy

Raman spectra were acquired with a Labram HR Evolution microspectrometer (Horiba Jobin Yvon, Lille, France). The excitation source was a 633 nm single-mode diode laser (Toptica Photonics, Germany) generating power of 35 mW on the sample. The microspectrometer was equipped with an Olympus microscope and measurements were recorded using a 10 × objective (Olympus, Japan). Light scattered by the sample was collected through the same objective. Rayleigh elastic scattering was intercepted by an edge filter. A Peltier cooled (− 70 °C) multichannel CCD detector (1024 × 256 pixels) detected the Raman Stokes signal dispersed with a 300 µm slit width and 300 grooves/mm holographic grating. Spectral resolution calculated from the full width at half maximum of the silica wafer band at 521 cm^{-1} was 2 cm^{-1}. The spectral region studied was 400–4000 cm^{-1}. The acquisition time of each spectrum was optimized for each drug at 2 × 15 s per acquired spectrum for CYCLO, IFOS, DOXO and 2 × 5 s for GEM and 5FU. Spectral acquisition and data pre-processing were conducted with LabSpec6 software (Horiba Jobin Yvon SAS, Lille, France).

Sample analyses were performed directly through the glass vial. The sample compartment was not suited for analyzing vials and was therefore adapted in order to standardize the location of the vial and secure the position of the sample on the base plate. In order to correct spectral variations due to a change of focusing, normalization based on total area was applied to all Raman spectra before chemometric analysis using LabSpec6 software.