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Non-invasive quantification of 5 fluorouracil and gemcitabine in aqueous matrix by direct measurement through glass vials using near-infrared spectroscopy

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ABSTRACT

Fourier transform near infrared spectroscopy (NIRS) was used for quantitative analysis of two cytotoxic drugs used in pharmaceutical infusion, 5-fluorouracil (5FU) and gemcitabine (GEM), at therapeutic concentrations in aqueous matrix.

Spectra were collected from 4000 cm^{-1} to 13,000 cm^{-1} by direct measurement through standard glass vials and calibration models were developed for 5FU and GEM using partial least-squares regression. NIR determination coefficient (R^2) greater than 0.9992, root-mean-square-error of cross-validation (RMESCV) of 0.483 mg/ml for 5FU and 0.139 mg/ml for GEM and the root mean square error of prediction (RMSEP) of 0.519 for 5FU and 0.108 mg/ml for GEM show a good prediction ability of NIR spectroscopy to predict 5FU and GEM concentrations directly through a glass packaging. According to accuracy profile, the linearity was validated from 7 to 50 mg/ml and 2 to 40 mg/ml for 5-fluorouracil and gemcitabine respectively.

This new approach for cytotoxic drugs control at hospital has shown the feasibility of near infrared spectroscopy to quantify antineoplastic drugs in aqueous matrix by a direct measurement through glass vial in less than 1 min and by non-invasive measurement perfect to limit exposure of operator to cytotoxic drugs.

1. Introduction

According to World Health Organization, cancers represented the first cause of death with more than 12.7 million of peoples new cases worldwide diagnosed in 2008 [1]. Cytotoxic drugs represent the most often used in anticancer chemotherapy treatment.

The treatment is adapted for each patient and concentrated formulations have to be diluted by nurses or pharmacy technicians with chloride sodium 0.9% or glucose 5% to obtain individualized treatment in accordance to prescriptions. Even if final control of cytotoxic preparations is not required by pharmaceutical regulations, analytical control reduces medication errors and thus, consequences on patient health. By identification and quantification, analysis control can ensure correct molecule and concentration and contributes to improve the security of the antineoplastic drugs process at hospital. Numerous analytical methods such as HPLC/UV, LC/MS/MS, GC/MS have been developed to quantify cytotoxic drugs in pharmaceutical formulations [2].

At hospital, cytotoxic drugs are identified but also quantified using flow injection analysis coupled with a diode array detector (FIA-UV) using UV absorption properties [3,4]. Thus, at the end of the cytotoxic preparation process, a sample of each preparation was collected for analytical control.

However, exposure to antineoplastic drugs can cause short-term toxicity such as nausea, rush but also long term effects with fecundity troubles and organ toxicity because of potential genotoxic, carcinogenic, teratogenic properties. Despite guidelines for good handling of cytotoxic drugs [5], cytotoxic drugs have been identified in urine samples of health care workers [6–11]. Thus, handling those drugs presents a risk of occupational exposure for health care workers during preparation, administration but also control of those chemotherapy drugs.

In this context, non-invasive techniques have to be preferred to control antineoplastic preparations to minimize this occupational exposure. Due to its rapidity, non-invasive and non-destructive properties, near infrared spectroscopy (NIRS)

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represent an interesting method. This method does not require any sample preparation, and thanks to a short acquisition time, allows a high measurement throughput for a large number of molecules which can be quantified [12].

Numerous methods have been developed with NIRS to determine active content such as active drugs, excipients or moistures in various types of pharmaceutical formulations (*i.e.* powder, granulate, tablet, gel, lyophilized vials and liquid) [12–15].

Because of the possibility of rapid, non-destructive and non-invasive analysis, the use of NIRS has recently increased in industry and extended at hospital to control pediatric capsules [16]. NIRS is now currently used for process analytical technology (PAT) in accordance to Pharmaceutical Current Good Manufacturing Practices to control raw materials, intermediate but also final products [17].

However, due to the high absorption of water in the NIR region, the determination or the quantification of chemical molecule in aqueous environment seems to be very difficult. This explains the non-popularity of NIRS to quantify chemical component in aqueous liquid formulations. In fact, the NIR absorption is due to combination bands of the chemical component, the vials and the vector. Whereas specific wavelengths are proportional to the concentration of chemical components, NIR spectra are very complex and complicated to interpret [18]. Broad *et al.* have shown the possibility to quantify in a multi-component pharmaceutical oral liquid by direct measurement through amber plastic bottles using Fourier transform near-infrared spectroscopy (FT-NIRS) [19].

Thus, the aim of this pilot study was to assess the feasibility of near infrared spectroscopy as a non-invasive analytical method to quantify cytotoxic drugs at therapeutic concentrations in aqueous solution by direct measurement through glass vials.

2. Materials and methods

2.1. Reagents

Because 5-fluorouracil (5FU) and gemcitabine (GEM) (Fig. 1) are ones of the most often used cytotoxic drugs, those two molecules have been selected for this feasibility study. 50 mg/ml 5FU vials were obtained from Teva (La defense, France) and containing water with hydrochloric acid and sodium hydroxide. 40 mg/ml GEM vials with ethanol and water with hydrochloric acid and hydroxide sodium as excipients were obtained from Mylan (Saint Priest, France).

2.2. Experimental design

2.2.1. Calibration and validation sample sets

Solutions containing drug concentrations in the range from 1 to 40 mg/ml for GEM and 1 to 50 mg/ml for 5FU were independently produced by dilution of the respective commercialized solution into chloride sodium 0.9% (v/v) FreeFlex® (Fresenus Kabi, Louviers, France).

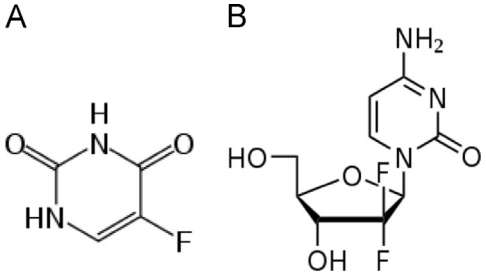

Fig. 1. The chemical structure of 5-fluorouracil (A) and gemcitabine (B).

To develop a robust calibration model, different sources of variability have been introduced into the models. For each drug, 5 series of solutions were prepared using 5 vials of cytotoxic drugs from the same batch by 5 operators. Each series included 10 and 11 levels of concentrations for GEM and 5FU respectively. Dilutions were produced using 5 batches of chloride sodium 0.9%. Due to direct measurements through the glass vial, the variability of the packaging had also been taking into account by introducing each solution into 3 different glass vials Interchim® of 2 ml (Montluçon, France). Thus, the sample set comprised 150 samples for GEM and 165 samples for 5FU.

All solutions were analyzed by FT-NIRS and the spectra were split into two groups: first, a calibration set including 3 series (90 samples for GEM and 99 samples for 5FU) to develop the prediction model and second, a validation set including 2 series (60 samples for GEM and 66 samples for 5FU) to evaluate the best prediction model in accordance to Guidelines on the use of NIRS [20].

2.2.2. Pharmaceutical preparation sample

In October 2012, a total of 58 pharmaceutical preparation samples from 2 to 7 mg/ml of 5FU and 40 samples from 2 to 6 mg/ml for GEM were collected from the production at the end of the cytotoxic preparation process. All samples were conditioned in glass vials Interchim®.

2.3. Instrumentation

2.3.1. NIR spectroscopy

NIR transmission spectra were analyzed using a Bruker Vector 33 SI001400 FT-NIR spectrophotometer (Bruker Optics®,Ettlingen, Germany) configured with a tungsten lamp source, a helium–neon 632.8 nm laser and a Ge diode detector. Spectral data were collected and analyzed using Opus software version 6.5 (Bruker Optics®, Ettlingen, Germany).

All spectra were collected by accumulation of 64 scans. Samples were scanned with a resolution of 8 cm^{-1} over the range from 4000 cm^{-1} to 13,000 cm^{-1}. An adaptation of the FT-NIR sample compartment has been done to align the vial and secure the position of the sample on the base plate. A glass vial Interchim® with 0.9% chloride sodium was used as a background reference.

2.3.2. Flow injection analysis with UV detector (FIA-UV)

FIA was performed using on Varian Pro Star HPLC system (Agilent technologies®, Les Ulis, France) equipped with automatic sample Prostar 410, a pump Prostar 230, a column valve module Prostar 500 and a diode array detector Prostar 330. All analysis were performed using Galaxie® software (Varian®, Les Ulis, France). 5 µl of 5-fluorouracil sample and 6 µl of gemcitabine sample were injected at room temperature across the chromatographic system without column in isocratic condition. The mobile phase was ultra-pure water from Milli-Q® integral water purification system (Millipore Guyancourt, France), with a flow rate of 1.5 ml/min. The DAD detector was used to monitor spectral data using a spectral range from 200 to 400 nm. The quantification was carried out at 269 nm and 268 nm for 5FU and GEM respectively. Each collected ultraviolet spectrum was compared with reference library for identification. Both for 5FU and GEM, the analytical method was validated from 1 to 10 mg/ml with a R^2 of 0.9981 and 0.9998 respectively.