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Discrimination and quantification of two isomeric antineoplastic drugs by rapid and non-invasive analytical control using a handheld Raman spectrometer

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ABSTRACT

Raman spectroscopy is a rapid, non-destructive and non-invasive method that is a promising tool for real-time analytical control of drug concentrations. This study evaluated a handheld Raman device to discriminate and quantify two isomeric drugs used to treat cancer. Doxorubicin (DOXO) and epirubicin (EPIR) samples were analyzed at therapeutic concentrations from 0.1 to 2 mg/mL (n=90) and 0.08–2 mg/mL (n=90) by non-invasive measurements using a portable Raman spectrometer.

The discrimination of these two molecules was demonstrated for all concentrations (n=180) by qualitative analysis using partial least square discriminant analysis (PLS-DA) with 100% classification accuracy, sensitivity and specificity and 0% error rate. For each molecule, quantitative analyses were performed using PLS regression. The validity of the model was evaluated using root mean square error of cross validation (RMSECV) and prediction (RMSEP) that furnished 0.05 and 0.02 mg/mL for DOXO and 0.17 and 0.16 mg/mL for EPIR after pretreatment optimization. Based on the accuracy profile, the linearity range was from 1.256 to 2.000 mg/mL for DOXO (R^2=0.9988) and from 0.553 to 2.000 mg/Ml for EPIR (R^2=0.9240) and repeatability (CV% max of 1.8% for DOXO and 3.2% for EPIR) and intermediate precision (CV% max of 2.8% for DOXO and 4.5% for EPIR) were both acceptable.

Despite the narrow validated concentration range for quantitative analysis, this study shows the potential of a handheld Raman spectrometer coupled to chemometric approaches for real-time quantification of cytotoxic drugs, as well for discriminating between two drugs with similar UV absorption profiles. Finally, the use of a handheld spectrometer with the possibility of a direct measurement of substances in containers is a potentially valuable tool for combining patient safety with security of healthcare workers.

1. Introduction

Chemotherapy is widely used to treat cancers and other diseases. The therapeutic agents used include numerous anticancer drugs classified as antimetabolites, DNA-interactive agents and antitubulin agents, according to their mechanism of action.

Treatments are individually adapted by physicians and specifically prepared by pharmacists. Commercialized formulations are highly concentrated and so formulations are dissolved and/or diluted in isotonic saline or 5% aqueous glucose solution before administration in order to obtain a final personalized product at the desired concentration. Preparation is one of the most critical steps just after administration [1].

The prevention of medication errors represents a major public health challenge. Current pharmaceutical regulations do not require the analytical control of each individualized final product administered. Nevertheless the implementation of quality control is a major healthcare preoccupation to verify the right drug at the right dose before administration to patients.

Different strategies including flow injection analysis with UV detection (FIA/UV) [2], high performance liquid chromatography with UV detection (HPLC/UV) [3], Fourier transform infrared (FTIR) or UV spectrometry [4–7], have been developed and conducted by hospital pharmacies to control cytotoxic drug preparations. These analyses require a sample of the anticancer preparation and constitute a risk of occupational exposure for healthcare workers.

As a consequence, some studies have explored noninvasive analytical control methods using near infrared spectroscopy (NIRS) [8] or Raman spectroscopy [9–11] to control cytotoxic drugs with

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higher security for operators. Samples can be analyzed by direct measurements through glass and plastic containers [12,13]. These vibrational spectroscopies are suitable tools in the design of the pharmaceutical product because of the possibility of rapid, non-destructive and non-invasive measurements without sample preparation [14] and process analytical technology with on at-line and on-line measurement to control the manufacturing process [15]. In contrast to Raman spectroscopy, water has considerable absorption in the NIR region which limits the analysis of molecules in an aqueous environment and explains the small number of applications for quantifying chemicals in aqueous formulations.

This explains why we investigated Raman spectroscopy. Even if its feasibility has been demonstrated to control cytotoxic drugs in syringes or elastomeric infusion pumps [9–11], this analytical method is not often used in hospitals. Raman spectra provide data on the multiple contributions from the drug substance, excipients and container. Raman data are therefore often difficult to interpret. A chemometric approach is necessary to extract relevant information from spectral data. Familiarity with chemometric methods and availability of instrumentation are the main obstacles to the development and use of this method in hospitals.

The miniaturization of this technology now offers a multitude of possibilities. A handheld Raman spectrometer is of considerable interest for the real-time control of pharmaceutical drugs produced in hospitals administration to patients by reducing medication errors in hospitals. This study involved a worst-case scenario and explored the feasibility of a compact handheld Raman spectrometer with direct measurements to discriminate and quantify two isomeric drugs at therapeutic concentrations in aqueous solutions.

2. Experimental

2.1. Choice of molecules

This study focused on two isomeric chemotherapy drugs that cannot be discriminated by UV analysis: epirubicin (EPIR) and doxorubicin (DOXO) (Fig. 1). These drugs are anthracyclines, antitumor antibiotics consisting of a planar anthraquinone nucleus attached to an amino-containing sugar. Doxorubicin is a natural product extracted from *Streptomyces galilaeus* while epirubicin is its semi-synthetic analog which differing only by its stereochemistry. These molecules act by intercalating DNA strands and inducing the formation of complexes that inhibit DNA and RNA synthesis.

2.2. Sample preparation

Commercial aqueous formulations of doxorubicin and epirubicin at 2 mg/mL, both with hydrochloric acid and sodium chloride as excipients were obtained from Accord® and Mylan®, respectively. Solutions were diluted with FreeFlex® isotonic saline (0.9% NaCl) (FresenusKabi, France) at various concentrations which covered the entire therapeutic range. Three sets (one batch of sodium chloride solution per set) of 10 solutions from 0.01 to 2.00 mg/mL for doxorubicin and 0.08–2.00 mg/mL for epirubicin were prepared and packaged in glass vials (Interchim®, Montluçon, France).

2.3. Raman spectroscopy

Metrohm Instant Raman Analyzer (MIRA, Metrohm, France) was used to obtain Raman spectral acquisitions. The excitation source was a 785 nm single-mode diode laser generating a maximum of 75 mW on the sample. Analyses were conducted using

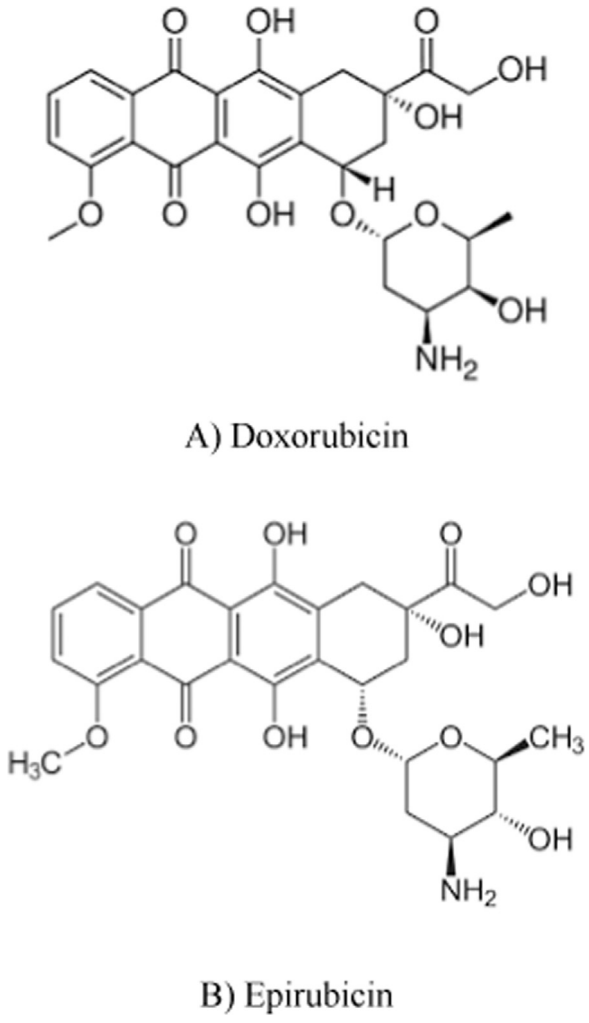

Fig. 1. The chemical structure of doxorubicin (A) and epirubicin (B).

the vial module at a focal distance of 1.0 mm with the orbital raster scan (ORS) system. This technology extends the area of the sample surface, taking measurements at several points and averaging them, significantly increasing accuracy, reproducibility and reliability of measurements. The spectral region studied was 400–2300 cm^{-1} with a spectral resolution from 12 to 14 cm^{-1}. Acquisition time of each spectrum was 20 s. Spectral acquisition and data pre-processing were conducted with Metrohm Mitra software (Metrohm, France).

Samples were analyzed in glass vials in triplicate in order to include container-induced variability. Ninety spectra were therefore acquired for each molecule.

2.4. Chemometric analysis

Chemometric analyses were performed using Matlab® 7.12.0 (R21011a) software.

There exist no specific guidelines for Raman spectroscopy. This is why calibration models for qualitative and quantitative analyses were developed, optimized and validated according to Guidelines of the use of NIRS by the pharmaceutical industry published by the European Medicines Agency [16]. The Guidelines require two sets of samples representative of production: a calibration set to construct the calibration model and a calibration test set for validation.

In order to develop and validate the calibration model with the highest variability, leave one out cross validation (LOOCV) was