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Short communication

Near-infrared spectrometric determination of dipyrone in closed ampoules

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ABSTRACT

The present paper proposes an analytical method for fast near-infrared (NIR) determination of dipyrone in injectable formulations with a nominal content of 50.0% m v^{-1} without violation of the ampoule. For this purpose, two multivariate calibration methods are evaluated, namely Partial-Least-Squares (PLS) and Multiple Linear Regression (MLR) with variable selection by the Successive Projections Algorithm (SPA). The resulting models comprised four latent variables (PLS) and five spectral variables (MLR-SPA). Appropriate predictions were obtained in both cases, with RMSEP values of 0.39 (PLS) and 0.35% m v^{-1} (MLR-SPA) and correlation coefficients of 0.9970 (PLS) and 0.9975 (MLR-SPA) for a calibration range of 40–60% m v^{-1}. No systematic error was observed and no significant differences were found between the predicted and reference values, according to a paired *t*-test at 95% confidence level.

1. Introduction

Dipyrone (metamizole sodium; (sodium [(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazole-4-yl) methylamine] methanesulfonate) is an analgesic and antipyretic drug with peripheral, central and anti-inflammatory actions related to the inhibition of the cyclooxygenase enzyme system (COX-1 and COX-2) [1]. Sodium dipyrone in injectable form is routinely used in hospital practice and postoperative care in Brazil and other countries. The injectable formulation has the advantage of providing faster therapeutic effects when compared to other delivery forms, as the active principle is directly deployed in the bloodstream [2]. However, quality control is a major concern, which motivates the development of fast, low-cost, and selective methods for routine analysis.

Iodometric titration is indicated in the Brazilian pharmacopeia as the official method for determination of dipyrone in injectable formulations [3]. This method involves a time-consuming chemical reaction, which must be carried out at a controlled temperature below 15 °C. An additional difficulty is related to the instability of the iodine solution. These inconveniences have motivated the development of methods based on instrumental techniques, such as spectrophotometry [4], fluorimetry [5], electrochemistry [6], chemiluminescence [7] and high performance liquid chromatography (HPLC) [8]. However, such methods still generate chemical waste and require the violation of the sample ampoule, which must be subsequently discarded. To overcome these drawbacks, near-infrared (NIR) spectrometry may be an advantageous alternative.

NIR spectrometry has been employed in several applications to carry out fast and non-destructive analyses without the need for sample treatment or chemical reagents [9,10]. Within the scope of pharmaceutical applications, NIR spectrometry has been widely used for determination of quality parameters in drug samples. Most investigations in this field have been concerned with solid formulations [11,12], although some works have also dealt with creams [13] and injectables [14]. An interesting feature of NIR spectrometry consists of the possibility of carrying out non-invasive analyses of drug samples in closed packages, such as powders in USP vials [15]. A recent study reported the discrimination of genuine and counterfeit samples of injectable dexamethasone on the basis of NIR spectra measured through the closed ampoules [16].

However, the application of NIR spectrometry for quantitative analysis of injectable drugs in closed ampoules still merits investigation. In this context, the present paper proposes a novel analytical method for fast and accurate determination of sodium dipyrone without violation of the ampoule. For this purpose, NIR spectrometry is employed with two multivariate calibration techniques, namely Partial Least Squares (PLS) [17] and Multiple Linear Regression (MLR) with variable selection by the Successive Projections Algorithm (MLR-SPA) [18–20].

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2. Experimental

2.1. Samples

The investigation involved a total of 1340 commercial ampoules from 71 different batches with nominal value of 50.0% m v^{-1} of sodium dipyrone. Chemical analysis reports with actual sodium dipyrone content were provided by the manufacturers, following the iodometric titration method indicated in the Brazilian pharmacopeia [3]. In addition, 15 synthetic samples with concentrations 40.0, 44.0, 44.5, 45.0, 45.5, 46.0, 48.0, 50.0, 52.0, 54.0, 54.5, 55.0, 55.5, 56.0, 60.0% m v^{-1} were prepared in the laboratory by dissolving sodium dipyrone (analytical grade) in water for injectable preparations. This range of concentrations is in agreement with the recommendation of the European Medicines Agency for the Evaluation of Medicinal Products (EMEA) [21], which states that the calibration interval should extend from 80% to 120% of the label claim (50.0% m v^{-1} in the present case). This range also comprises the interval of concentrations accepted by the Brazilian National Health Authority, namely 45.0–55.0% m v^{-1} [3].

2.2. Spectrum acquisition

The sample spectra were acquired by using an FT-NIR spectrophotometer (Perkin Elmer, Spectrum GX). A lab-made teflon holder (Fig. 1) was used to support the ampoules in the spectrophotometer sample compartment. The external diameter of the ampoules was approximately 1 cm. Each spectrum was obtained as the average of 16 scans in the range 9110–8200 cm^{-1} with a resolution of 1 cm^{-1}. Temperature and relative humidity were controlled around 26 °C and 45%, respectively.

In order to account for small manufacturing differences among the ampoules, four different ampoules were used as cells for recording the spectra of the 15 synthetic samples. Therefore, 60 spectra were obtained. The spectra of the commercial samples were acquired with the original closed ampoules. The blank spectrum was obtained by using water for injectable preparations. Each spectrum was acquired in triplicate and the average was then used in the subsequent stages of the study. Moreover, the spectra of all samples of the same batch were coaveraged. The resulting 131 spectra (60 for the synthetic samples and 71 for the commercial samples) are shown in Fig. 2a.

2.3. Chemometric procedures and software

A Savitzky–Golay derivative filter (second-order polynomial and 81-point window) was employed to eliminate undesirable baseline features in the NIR spectra. The resulting derivative spectra, which are presented in Fig. 2b, comprised 831 variables. In addition, the variables were mean-centered before the modeling procedures.

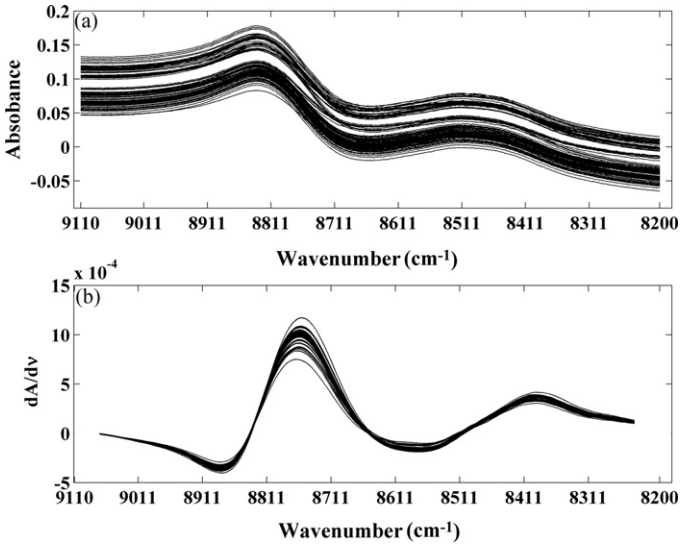

Fig. 2. (a) Raw and (b) derivative NIR absorbance spectra.

The sample set was divided into calibration (65), validation (33) and prediction (33) subsets by applying the SPXY (sample set partitioning based on joint *x*–*y* distances) algorithm [22] to the derivative spectra. The calibration and validation samples were used in the model-building process. The prediction samples were only employed in the final evaluation and comparison of the resulting models.

PLS regression was carried out in the Unscrambler® X.1 software (CAMO S.A.), whereas data pre-treatment and MLR-SPA were implemented in Matlab 2010b (Mathworks). The MLR-SPA routine was implemented as described elsewhere [18]. The validation set was employed to guide the selection of latent variables in PLS and the individual spectral variables in MLR-SPA. The default settings of the computational routines were employed throughout.

The resulting models were compared in terms of the root-mean-square error (RMSEP) and correlation coefficient (r_{pred}) in the prediction set.

3. Results and discussion

Fig. 3a and b present the plots of predicted versus reference values of dipyrone concentration for PLS (4 latent variables) and

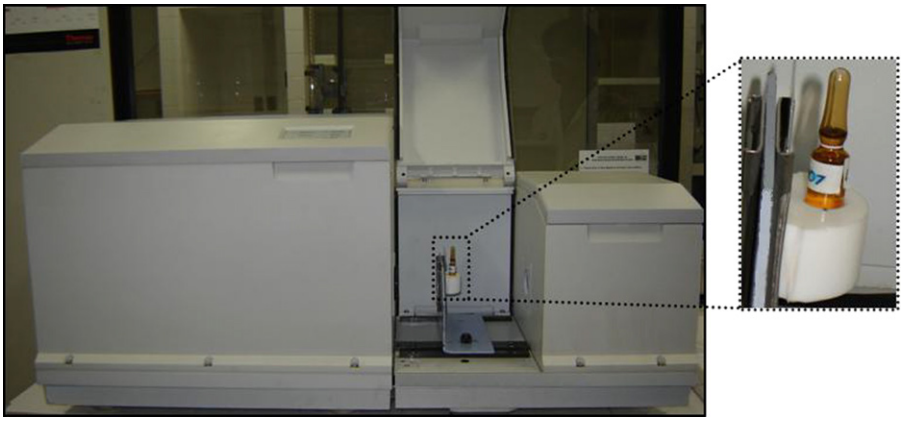

Fig. 1. NIR spectrophotometer with the lab-made teflon holder which was used to support the ampoules in the spectrophotometer sample compartment.

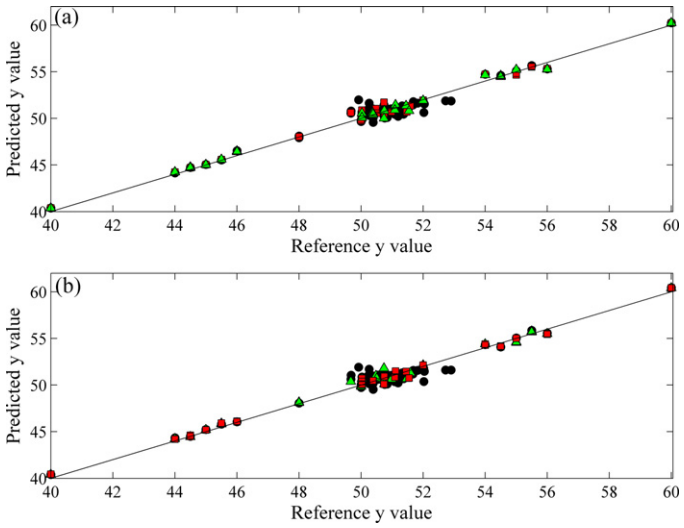

Fig. 3. Predicted versus reference plots obtained with: (a) PLS (full-spectrum) and (b) MLR-SPA models for determination of sodium dipyrone in the calibration (●), validation (■) and prediction (▲) sets.

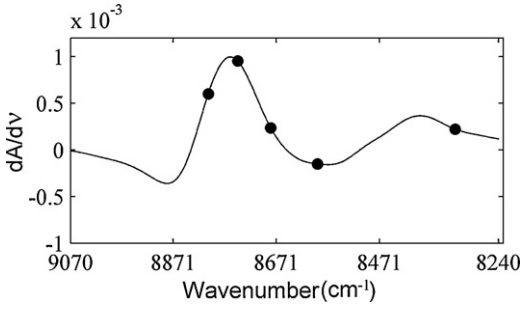

Fig. 4. Mean NIR spectrum with wavenumbers selected by MLR-SPA.

MLR-SPA (5 spectral variables), respectively. As can be seen, there is good agreement between predicted and reference values over the calibration, validation and prediction sets. Moreover, no systematic error is present, as the points are distributed on both sides of the bisectrix line along the entire range of *y*-values.

In terms of performance metrics for the prediction set, appropriate results were obtained by using either PLS (RMSEP = 0.39% m v^{-1}, r_{pred} = 0.9970) or MLR-SPA (RMSEP = 0.35% m v^{-1}, r_{pred} = 0.9975). Moreover, in both cases, a paired *t*-test for the prediction set did not indicate significant differences between the predicted and reference values at 95% confidence level. These results suggest that the proposed analytical method is a suitable strategy for determination of dipyrone in closed ampoules.

Finally, it is worth noting that an *F*-test at 95% confidence level did not indicate significant RMSEP differences between the PLS and MLR-SPA models. However, the possibility of obtaining suitable results by using a small number of variables in MLR-SPA (five variables, as illustrated in Fig. 4), offers good perspectives for the design of dedicated, less costly equipment employing LEDs in the NIR range [23,24].

4. Conclusion

The present paper proposed a fast and accurate analytical method for determination of sodium dipyrone in injectable formulations without violation of the ampoule. For this purpose, NIR spectrometry was employed and two multivariate calibration methods (PLS and MLR-SPA) were evaluated. Appropriate predictions were obtained, with RMSEP values of 0.39 (PLS) and 0.35% m v^{-1} (MLR-SPA), which can be deemed small in view of the calibration range (40–60% m v^{-1}). The good agreement between predicted and reference values for dipyrone concentration is also corroborated by the high correlation coefficients observed in the PLS (0.9970) and MLR-SPA (0.9975) results. Furthermore, in both cases no systematic error was observed and no significant differences were found between the predicted and reference values, according to a paired *t*-test at 95% confidence level.

Although the prediction performance of MLR-SPA was not significantly superior to PLS, the possibility of obtaining suitable results by using few spectral variables (five in this case) may be useful for other reasons. The variable selection result could be used, for instance, to guide the design of dedicated, less costly equipment for use in routine analyses.

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