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Short communication

FT-NIR spectroscopy for rapid and simple determination of nimesulide in rabbit plasma for pharmacokinetic analysis

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ABSTRACT

High-throughput analysis of a large number of samples for pharmacokinetic study is necessary in drug development and pharmacovigilance. Usually, drug quantification for pharmacokinetics and bio-availability is achieved through matrix extraction and HPLC analysis, which is time, labour and cost intensive method. A prompt and solvent free method is the quest for such analysis in the present times. Pharmacokinetic analysis of nimesulide from plasma samples of rabbits through Fourier transform near infrared (FT-NIR) spectroscopy analysis combined with partial least squares (PLS) regression model was undertaken with validation through HPLC analysis. Pharmacokinetic parameters obtained through FT-NIR and HPLC were found to be statistically similar with errors below the acceptable limits. The study demonstrates the use of FT-NIR for pharmacokinetics and bio-availability studies. This high throughput method analyses more than 50 samples in an hour without solvents usage and provide ample scope for automation and commercial utilization.

1. Introduction

Near infrared (NIR) spectroscopy is a noninvasive, relatively low cost optical technique, portable, useful for real-time measurement of changes in tissues in relation to oxygenation and perfusion [1], body fat [2], qualitative and quantitative measurements of various chemicals in foods, pharmaceuticals, materials, medical, agricultural produce etc. [3]. In addition, Fourier transform near infrared (FT-NIR) is extensively used in different fields of the pharmaceutical industry like prediction of tablet properties of raw-mixed powders before compression [4]; quantitative measurement of components in intact tablets [5,6]; raw materials, intermediate and finished product forms including gels [7] and development of solid dosage forms [8] besides others. Recently, FT-NIR has extensively being explored for studies in urology [1,9]; sports medicine [10], real-time study of brain vascular and metabolic activities [11]. Much of the appeal of NIR technique is because of the fact that wealth of chemical and physical information can be obtained with in seconds often without the need for any sample preparation.

Although Fourier transform infrared spectroscopy (FT-IR) has been used for determining molecular concentrations of different bio-active analytes in various biological matrices like blood, serum, plasma and urine [12], the use of FT-NIR in the bio-analysis of pharmacophore in biological samples like blood, urine, plasma or serum samples has not yet been reported. There is a tremendous need and scope for novel and high throughput analytical method for bioanalysis in the course of drug discovery and development [13]. Hence in the present experiment, we have done pharmacokinetics study of nimesulide in rabbit through FT-NIR analysis of plasma samples and validated the same through HPLC analysis.

Nimesulide (4-nitro-2-phenoxymethanesulfonanide) was chosen because of its continuing use in India and other developing countries as a prominent, selective cyclooxygenase (COX-2) inhibitor in inflammatory conditions as well as the easiest availability of high-performance liquid chromatography (HPLC) methods and IR data for the purpose of cross validation. The pharmacokinetics aspects of nimesulide have been documented in human [14] and some animal species using traditional methods [15,16], but none has explored the novel method of calculating kinetic parameters from FT-NIR generated data. Rabbits were preferred to other rodents because its heart and cardiac circulation are closely similar to those of human [17].

2. Materials and methods

2.1. Animals

Adult male and female New Zealand white rabbits (2.5 kg body weight), six in numbers, being maintained at the in vivo testing

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facility, CIMAP, Lucknow, India were used for the study. The animals were acclimatized to the experimental environment for 7 days before the actual experimentation. The animals were re-used after a wash period of 21 days. The protocols used were duly approved by Institutional Animal Ethics Committee (IAEC) through CPCSEA, Government of India.

2.2. Chemicals

Nimesulide and Cremophore EL were obtained from Sigma Chemicals, India. HPLC grade solvents were obtained from Merck India Ltd., India

2.3. Treatment of animals with nimesulide and collection of samples

A clear aqueous solution of nimesulide was prepared in 10% cremophore EL in 0.9% sodium chloride (NaCl) in water. Nimesulide was injected at 5 mg kg^{-1} body weight intraperitonially (i.p.) to the rabbits. Blood samples were collected in heparinised tubes, in duplicate, at 0, 5, 15, 30, 60, 120, 240, 360 and 480 min after the administration of the drug. Clear plasma samples were collected from the blood samples after centrifugation at 5000 × *g* for 5 min at 4 °C.

2.4. FT-NIR analysis

The plasma samples thus obtained were used directly for the Fourier transform near infrared (FT-NIR) analysis. The FT-NIR absorbance spectra from 10,000 cm^{-1} to 4000 cm^{-1} at a resolution of 4.0 cm^{-1} (using Antaris II Analyzer, Thermo Fisher Scientific, USA) in transmission mode, using indium gallium arsenide (InGaAs) detector were recorded for each sample. To reduce sampling error, the three vials (0.5 mm cell with 170 μl rabbit plasma) of samples from each plasma sample prepared in the previous step were analysed individually. The plasma obtained from the blood collected at zero (0) minute (just before the administration of the drug) was considered as the blank and the background measurements were taken using the blank before every spectral measurement.

Data acquisition, spectral mathematical treatments and partial least squares (PLS) regression were done using TQ Analyst Software (Thermo Fisher Scientific, USA). The second derivative of absorbance spectra smoothed with Norris derivative (segment length 19, gap between points 2) was used for all regression work. For path length corrections, standard normal variate (SNV) path length was chosen.

2.5. Sample preparation for nimesulide assay

Plasma samples (275 μl) were vigorously vortexed with the addition of acetonitrile (1000 μl) in a microcentrifuge tube for the extraction of nimesulide; the tubes were then centrifuged at 1000 × *g* for 5 min and the solvent phases were separated in fresh microcentrifuge tubes. After the evaporation of the solvent phase, the samples were reconstituted in 100 μl of acetonitrile which were then subjected to high-performance liquid chromatography (HPLC) analysis. A standard solution of nimesulide, in acetonitrile, was used for calibration of the HPLC peaks.

2.6. HPLC analysis

For the calibration, as well as cross validation of PLS model developed using FT-NIR data, HPLC analysis were done as described by Toutain, et al. [15] and Rao, et al. [16], using an HPLC system with UV detector (LC 10A with SPD 10AT) from Shimadzu, Japan connected with a SymmetryShield (Waters) RP-18, column. The UV detector was set at 210 nm and the analyses were carried out using the same series of the extracted plasma samples used in the FT-NIR analysis.

2.7. Kinetic analysis

Pharmacokinetic parameters were calculated following the procedure as reported earlier [18] using the software PK87.

The whole experiments were conducted three times in order to verify the reproducibility of the method. The spectra obtained from first and second rounds of experiments were used for calibration and validation of the PLS model. The spectra from the third round of the experiment were used only for prediction, and the predicted values were compared with the results from the HPLC analysis.

2.8. Calibration in raw plasma samples

A stock solution of nimesulide (5 mg ml^{-1}) was prepared in double distilled water containing 0.9% NaCl and 12.5% cremophore EL. Two fold serial dilution of this nimesulide stock solution ranging from 2.5 mg ml^{-1} to 0.02 mg ml^{-1} was prepared in freshly collected rabbit plasma. Four replicates of the eight dilutions were subjected to FT-NIR analysis using plasma as blank.

3. Results and discussion

The raw absorbance spectra recorded for nimesulide in rabbit plasma, taken at different time intervals after the i.p. injection, are shown in Fig. 1. The FT-NIR data from first and second rounds of the experiment were randomly split into calibration set (64 samples, 4 rabbits) and validation set (32 samples, 2 rabbits). All the data from the third round of the experiment were used as prediction set (48 samples, 6 rabbits). The two validation animals were selected randomly in each time interval.

The visual analysis of the second derivative spectra gave peaks and dips with an indication that at wave number ranges 6430–6360 cm^{-1} (Fig. 2), 5364–5290 cm^{-1} and 4849–4833 cm^{-1} the *y*-axis values change in the same way as the concentration of the drug, quantified through HPLC analysis, in the collected blood samples. At wave numbers ranging between 5364 and 5290 cm^{-1}, the spectra showed a dip. The FT-IR spectrum of the pure drug showed prominent peaks at wave numbers 3284, 1590, 1488, 1342, 1282 and 1247 cm^{-1} [19]. So the significant contribution of the FT-NIR band obtained at 6430–6360 cm^{-1} could be from the combination of overtones arised from N–H stretching and N–H deformation. Similarly, the major contribution for the band at 4849–4833 cm^{-1} might be influenced strongly by the combination of overtone from asymmetric C–O stretching and asymmetric N–O stretching.

PLS regression models were built using these three wavelength ranges viz., 6430–6360 cm^{-1}, 5364–5290 cm^{-1} and 4849–4833 cm^{-1}. Two latent variables were used to make the PLS models. This number was used because of two reasons. One reason is that the TQ Analyst Software selected the two variables in its calculations, and the other reason is that it gave lower root mean squares error of cross validation (RMSECV). In order to find the closeness between reference value (value obtained through HPLC analysis) and the value found by the calibration model, RMSEP (root mean square error of prediction) was also calculated using the formula:

$$\mathrm{RMSEP} = \frac{\sum_i (Yi_{\mathrm{pre}} - Yi_{\mathrm{HPLC}})^2}{n}$$

where *n* is the number of samples used for prediction. Similarly, root mean square error of calibration (RMSEC) and root mean square error of validation (RMSEV) were also calculated.