Applications of FT-Raman Spectroscopy in the Quality Assurance/Quality Control (QA/QC) of Drugs

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Abstract: FT-Raman spectroscopic technique was applied to identify and quantify the active pharmaceutical ingredient (API) in the raw material and commercial forms of a drug model. As it is a common anti-inflammatory drug and usually used as a drug model in the area of drug research, diclofenac-sodium was used as a drug model in this work. Series of different concentrations ranging from 10% to 100% (with an increment of 10%) of pure diclofenac-Na were prepared in a matrix of pure starch. Heights and areas of some characteristic peaks in the FT-Raman spectra of the diclofenac-Na samples were calculated for each concentration to obtain calibration curves. These curves were used to predict the concentration of the diclofenac-Na in some prepared samples and some commercial formulae. The accuracy of these calibration curves was found to be in the range 98.3-99.8% and was comparable to that obtained by the HPLC technique, the standard official method used in QA/QC of drugs. The predictive ability of the calibration models based on the peaks' heights and areas were also tested by calculation of the relative standard error of prediction (RSEP). The RSEP values were 2.2%-4.1% and 2.9%-3.4% for peaks' heights and areas models, respectively. In conclusion, the results of this work suggest the FT-Raman spectroscopy as a new accurate, relatively quick and economic method, in comparison with the HPLC method, in QA/QC in the field of drug industry.

Key words: FT-Raman spectroscopy, Diclofenac-sodium, quality assurance/quality control.

INTRODUCTION

Pharmaceutical industry regulations imposed by regulatory bodies such as FDA are becoming much stricter and encompass not only released products but also incoming materials and products shipped between different sites of a single company[1]. HPLC is the most widely accepted technique for product testing because of the current state of automation and high degree of precision and accuracy that can be achieved[2, 3].

In recent years, the concept of parametric release and the evolution of in-process analysis have required the development and application of rapid and reliable non-destructive analytical tools. As spectroscopic techniques offer these possibilities, methods such as infrared analysis are already well established within the pharmaceutical industry[4-5]. FT-Raman spectroscopy is also becoming increasingly popular as a tool to analyze the solid state of pharmaceuticals or for quantitative analysis including quality assurance and quality control[6-8]. It also exists as a powerful method for the molecular analysis of polymers and biological molecules. The fundamental chemical information available from FT-Raman spectroscopy is being used for raw material identification or incoming testing, often by comparison with a single reference spectrum. Extensive libraries can be built and new compounds added at any time[9].

Raman spectroscopy provides information about the structure of a molecule complimentary to the data obtained from infrared analysis and both techniques combined provide complete vibrational information about a molecule[10]. This technique is based on the detection of scattered light following irradiation of a molecule by laser light and excitation of electrons to a higher vibrational or rotational state. The acquisition of data is rapid and there are no special requirements for sample preparation and it allows optically imperfect samples to be analyzed, which is an important criterion for solid state samples[10]. It also allows analysis in aqueous environments as water is a weak Raman scatter, whereas water often overwhelms the spectral features of interest in an IR spectrum.

Diclofenac is a common nonsteroidal anti-inflammatory drug from the group of the arylalacnoic acid derivatives. It has large therapeutic applications in symptomatic standard treatment of the rheumatic affections. It is common in the research field to use the diclofenac as a drug model[11-13].

Jedvert et al[2] developed multivariate calibrator models to quantify the active substance, isosorbide-5-mononitrate in Imdur 120 mg tablets by NIR diffuse reflectance and Raman spectroscopy in combination with liquid chromatography (LC) as a reference method. They concluded that both Raman and NIR are possible alternatives to the LC method since the accuracy for...
spectroscopic techniques were equal in merit to the LC method with a 95% confidence interval.

Vergote et al[8] used FT-Raman spectroscopy for quantitative determination of diltiazem hydrochloride in commercial tablets within its blister package and experimental tablets prepared at lab-scale. They used the total peak area of the Raman spectral band between 1625 and 1560 cm⁻¹ (after vector normalization) to determine the percent of diltiazem hydrochloride in each tablet. They found that the drug dosage per tablet obtained from the Raman spectroscopy method was correlated well with the results obtained using HPLC analysis for both the commercial and the experimental tablets.

Auer et al[14] applied FT-Raman spectroscopy for determination of polymorphic forms in a number of common drug products, qualitatively and quantitatively, relying on the position, intensity and shape of characteristic bands. They used the partial least square model, PLS, for multivariate calibration for quantification and tested the predictive ability of the models by standard error of cross validation. They found the method perfectly suited to meet the regulatory requirements of monitoring crystal forms in drug products during processing and storage even when the excipients are not known.

Mazurek and Szostak[15] performed FT-Raman quantification of diclofenac-sodium and aminophylline commercial injection solutions (2.4%) using three different procedures for spectra treatment including classical univariate intensity ratio, multivariate partial least squares, PLS, and principal component regression. Their results revealed that the chemometric treatment of FT-Raman spectra could be a fast and convenient alternative to the standard pharmacopoeial procedures of API quantification even in relatively diluted injection solutions.

The aim of this study was the investigation of the potential of FT-Raman spectroscopy technique for identification and quantitative assessment analysis of diclofenac-Na in experimental samples and commercial tablets using direct univariate calibration methods.

**MATERIALS AND METHODS**

**Materials:** Diclofenac-Sodium ([2-(2,6 dichloroanilino)-phenyl] acetic acid) obtained from Sigma, U.K. was used as a drug model. Extra pure starch supplied by Sisco Research Lab., PVT-LTD was used as a matrix to spread the drug in it. Series of different concentrations of the pure diclofenac-Na (10 to 100% with an increment of 10%) were prepared in a matrix of the pure starch. These series of the homogenous dry powder mixtures (batch size 400 mg and particle size 90-125 µm) were hand-blended and then mixed in a vibratory mixer, twice, for 5 minutes (to avoid heating of the sample). Diclofenac-Na is commercially available in two tablet strengths, Voltaren manufactured by Novartis pharma, 25 mg and 75 mg per tablet is containing approximately 16.67% and 32.75% drug (w/w), average of ten tablets, were also used in this study to check the validity of the method.

**FT-Raman Measurements:** The powder samples were filled in NMR tubes for FT-Raman measurements. The spectra were collected from three different points on the tube for each sample to avoid errors due to any inhomogeneous distribution of the drug. All the FT-Raman measurements were carried out by a FTIR-FT-Raman Spectrometer, Nexus 670, Nicolet, USA. The excitation source in the FT-Raman module is Nd-YAG laser, which emits continuous-wave laser energy at a wavelength of 1064 nm (9398 cm⁻¹). The installed air-cooled detector is InGaAs. The beam splitter is XT-KBr. The used sample configuration is 180° reflective with fully motorized sample position adjustment, with an NMR-tube sample holder. All the FT-Raman spectra were collected in the spectral range from 3701 to 98 cm⁻¹ at resolution 8 cm⁻¹ and 132 scans. The laser power was fixed at 0.7 W. The spectra were processed using the computer program “Omnic ESP.5.2a” provided by Nicolet, USA.

The predictive ability of the calibration curves obtained from Raman data analysis were determined by calculating the relative standard error of prediction (RSEP) for each univariate calibration curve (peak height or area) according to the following equation[15]:

\[
\text{RSEP(\%)} = \sqrt{\frac{\sum_{i=1}^{n} (C_i - C_i^A)^2}{\sum_{i=1}^{n} (C_i)^2} \times 100}
\]

Where \(C_i^A\) is the actual concentration, \(C_i\) is the predicted concentration and \(n\) is the number of samples.

**HPLC Measurements:** After FT-Raman measurements, the drug content was determined in each sample by HPLC as a reference method. The HPLC system used was a Waters alliance HPLC consisting of 2695 separation module and 2996 photodiode array detector with Empower software. Determination of diclofenac-Na by HPLC was carried out according to the official method B.P[16]. The chromatographic procedure was carried out using (a) a symmetry column (25cm x 4.6 mm) packed with octylsily silica gel for chromatography (5µm), (b) as a mobile phase with a flow rate of 1 ml per minute a mixture of 34 volumes of a mixture of equal volumes of a 0.1% w/v solution of orthophosphoric acid and a 0.16% w/v solution of sodium dihydrogen orthophosphate, adjusted to pH 2.5, and 66 volumes of methanol and (c) a detection wavelength of 254 nm.

**RESULTS AND DISCUSSION**

The Raman spectra of the pure diclofenac-Na and starch are shown in figure (1). The Raman spectrum of the drug is consistent with that reported in literature[10]. It exhibits intense bands in the frequency range 1560-1620 cm⁻¹ characteristic of aromatic ring stretching. The specific bands of both aromatic C-H and aliphatic =CH₂ stretching modes appear in the region 3100-3000 cm⁻¹. The bands in the region 2970-2850 cm⁻¹ are attributable to...
aliphatic CH, and CH₂ stretching.

Figure (2) shows spectral series of different concentrations of diclofenac-Na diluted with extra pure starch (which is commonly used as an excipient). The intensity of the scattering peaks corresponding to the drug molecules shows a concentration-dependent change in the spectra. In order to quantify these changes in the Raman spectra, the peaks’ heights and areas of two scattering bands specific to the drug at 606 cm⁻¹ and 1162 cm⁻¹ were calculated at each concentration. These two scattering bands have medium intensities and do not interfere with the starch bands, which recommend them to be sensitive to any change in the concentration.

Four calibration curves (Figures 3 and 4) were drawn from the relationships between the concentration of the drug and the peaks’ heights and areas calculated using corrected 2-points baseline method. These figures exhibit linear relationships with
Fig. 3: Raman calibration curves for the diclofenac-Na using peaks height against the drug concentration.

Fig. 4: Raman calibration curves for the diclofenac-Na using area under the peaks against the concentration.

excellent mathematical properties. The coefficients of determination ($R^2$) were 0.97 and 0.98 for the peaks’ height curves and 0.98 for the peaks’ area curves. The correlation coefficients were 0.98 and 0.99 for the peaks’ height curves and 0.99 for the peaks’ area curves.

The accuracy of the Raman quantitative analysis was validated by cross-referencing the actual and the predicted concentration of diclofenac-Na (using the calibration curves) after analysis of samples containing known amounts of the drug. Two test concentrations of the drug, 25% and 75% (w/w), were prepared in starch matrices. Also, two known concentrations of diclofenac-Na in commercial tablet dosage form, 16.67% and 32.75% (w/w), were used for the same purpose. Each concentration was prepared and analyzed six times using
the Raman technique. The recorded spectra of the commercial samples are presented in figure (5). It appears from this figure that the characteristic Raman bands of the diclofenac-Na under investigation are existed.

By using the calibration curves presented in figures (3 and 4), the concentration of the drug content was calculated. Each value of the predicted concentration (an average of six) together with its standard deviation and percentage error are given in table (1). The percentage error ranges of the predicted concentration values were 1.68%-0.40% and 1.56%-0.24% using the calibration curves of figures (3 and 4), respectively. These results prove that the accuracy ranges of the technique to predict an unknown concentration, using the peaks’ heights and areas calibration curves were 98.32-99.60% and 98.44-99.76%, respectively.
The relative standard error of prediction (RSEP%) determined for the testing data set (prepared and commercial samples) mounted for 4.1% and 2.15% based on the peaks' height calibration curves of the peaks 606 cm$^{-1}$ and 1162 cm$^{-1}$, respectively. The corresponding values based on the peaks' area calibration curves were 2.91% and 3.4%, respectively.

All of the known concentration samples underwent the standard HPLC analysis technique (figure 6). The measured concentration values and their standard deviations and percentage errors are given in table (1). This table reveals a comparison between the Raman and the HPLC methods in determining the drug concentration present within other environment. The minimum and maximum errors (%) in HPLC results were 0.03% and 0.96% respectively which gave an accuracy range 99.04%-99.97%. The results obtained by the Raman method are comparable to those obtained by the reference HPLC method. Our results are also in agreement with previous authors' work who concluded that Raman spectroscopy is a useful method to determine the solid state form of a drug present in tablets and even to determine if a mixture of polymorphic forms are present<sup>[5, 17]</sup>. Hence, Raman spectroscopy can be used for identification of an API within solid dosage formulae qualitatively and quantitatively using a simple univariate calibration model with an accepted error level.

**Conclusions**: FT-Raman spectroscopy applied to the analysis of individual pure drug, drug-matrix preparations and commercial formulae of the drug revealed spectra qualitatively fingerprints of the molecular structure of the drug. The quantitative analysis results confirm an agreement with previous authors' work who concluded on the peaks' heights calibration curves of the peaks 606 cm$^{-1}$ and 1162 cm$^{-1}$, respectively. The corresponding values based on the peaks' area calibration curves were 2.91% and 3.4%, respectively.

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**REFERENCES**


