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Short communication

Dissolution testing of isoniazid, rifampicin, pyrazinamide and ethambutol tablets using near-infrared spectroscopy (NIRS) and multivariate calibration

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ABSTRACT

This work utilized the near-infrared spectroscopy (NIRS) and multivariate calibration to measure the percentage drug dissolution of four active pharmaceutical ingredients (APIs) (isoniazid, rifampicin, pyrazinamide and ethambutol) in finished pharmaceutical products produced in the Federal University of Rio Grande do Norte (Brazil). The conventional analytical method employed in quality control tests of the dissolution by the pharmaceutical industry is high-performance liquid chromatography (HPLC). The NIRS is a reliable method that offers important advantages for the large-scale production of tablets and for non-destructive analysis. NIR spectra of 38 samples (in triplicate) were measured using a Bomen FT-NIR 160 MB in the range 1100–2500 nm. Each spectrum was the average of 50 scans obtained in the diffuse reflectance mode. The dissolution test, which was initially carried out in 900 mL of 0.1 N hydrochloric acid at 37 ± 0.5 °C, was used to determine the percentage a drug that dissolved from each tablet measured at the same time interval (45 min) at pH 6.8. The measurement of the four API was performed by HPLC (Shimadzu, Japan) in the gradiente mode. The influence of various spectral pretreatments (Savitzky-Golay smoothing, Multiplicative Scatter Correction (MSC), and Savitzky-Golay derivatives) and multivariate analysis using the partial least squares (PLS) regression algorithm was calculated by the Unscrambler 9.8 (Camo) software. The correlation coefficient (R^2) for the HPLC determination versus predicted values (NIRS) ranged from 0.88 to 0.98. The root-mean-square error of prediction (RMSEP) obtained from PLS models were 9.99%, 8.63%, 8.57% and 9.97% for isoniazid, rifampicin, ethambutol and pyrazinamide, respectively, indicating that the NIR method is an effective and non-destructive tool for measurement of drug dissolution from tablets.

1. Introduction

Tuberculosis is a major global health problem because it is easily spread, primarily through the air. Although the disease can strike any organ of the body, the bacillus reproduces and grows quickly in areas with high concentrations of oxygen, which explains the more frequent attacks to the lungs. In 1944, the investigator Selman Waksman discovered streptomycin, the first effective antibiotic action against tuberculosis. Since then, several other drugs have been quite successfully used in the treatment of the disease, including isoniazid (1952), rifampicin (1965), pyrazinamide (synthesized in 1936, but used only since 1970) and ethambutol (1960), which are shown in Fig. 1 [1].

One methods used to evaluate drug delivery is the study of the dissolution testing, because the dissolution of the active ingredient in solid dosage forms is considered a key variable in the process of absorption in the gastrointestinal tract [2]. Dissolution is a dynamic process, strongly dependent on both the medium composition and hydrodynamics. Because the environment of the luminal gastrointestinal tract varies considerably, it is necessary to measure different variables to arrive at a complete picture of the behavior of the drug from its active versions.

The procedures for the dissolution test vary according to the number of active ingredients in the formulations. The official methods United States Pharmacopeia (USP) for rifampicin, isoniazid and rifampicin capsule, where the dissolution medium was 0.1 mol L^{-1} HCl. Procedures for three-drug fixed dose combinations (FDC) became official in USP 24 Supplement 2, whereas four drug (FDC) became official in USP 26 (2003) [3].

Different studies, such as dissolution tests, make use of the technique of high-performance liquid chromatography (HPLC) for quantification of drugs, as regulated by the United States Pharmacopeia (USP). Currently, the use of HPLC methods in studies of dissolution has been increasing because the in vitro dissolution of

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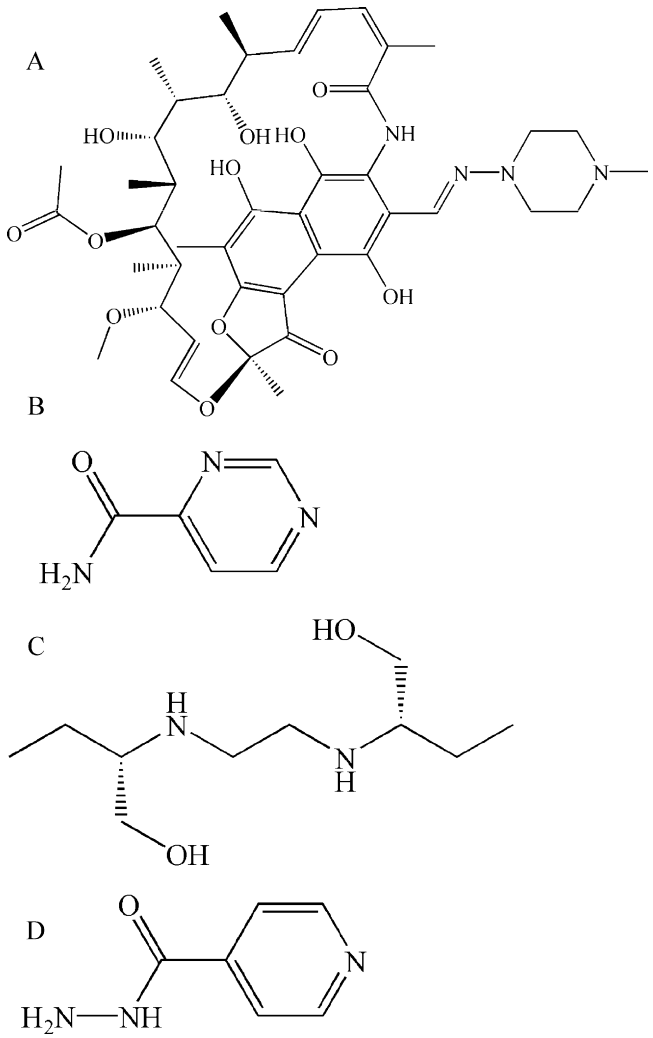

Fig. 1. Molecular structures of the compounds: (a) rifampicin; (b) pyrazinamide; (c) ethambutol and (d) isoniazid.

the active principle is an important parameter for the pharmaceutical industries in different stages of the development and production of a drug. Agrawal et al. [4] developed a study that aimed to compare the bioavailability of rifampicin, isoniazid and pyrazinamide in the formulation of FDC, with the values obtained in separate formulations of the same dose levels. The results demonstrated the bioequivalence of rifampicin, isoniazid and pyrazinamide accurately and reliably [4]. Khuhawar and Rind [5] used the HPLC technique for the determination of rifampicin, isoniazid and pyrazinamide in pharmaceutical formulations and in blood that was collected from patients who were suffering from tuberculosis and who had undergone chemotherapy with these three drugs. The results were satisfactory, and the separation of the three drugs was achieved. The presence of common additives and other drugs do not interfere with the determination [5].

The cited examples indicate the effectiveness of HPLC, owing to its high precision and accuracy in analysis and, reproducibility of results, among other advantages. However, this is a costly technique because it requires the use of many reagents for sample preparation. The time factor can also be unfavorable, because, in general, the readings of both the samples and the standard are made in triplicate, and, depending on the analyzed material, each reading can take several minutes. The preparation of solutions also contributes to making the method time-consuming. Additionally, HPLC requires specialized personnel for handling the equipment and for interpreting the results. For all of these reasons, the search for new analytical techniques, which have a fundamental importance in quality control of production processes, that lower both analysis time and cost has grown.

Near infrared spectroscopy (NIRS) uses the region of the electromagnetic spectrum that lies between the visible and mid-infrared bands. The NIR spectrum is formed from the signals due to the molecular vibrations of C–H, N–H and O–H bonds [6,7]. Currently, NIR spectroscopy is widely accepted by the pharmaceutical industry as a method of both qualitative analysis and quantitative control of the physicochemical parameters of the samples, providing direct measurement of solid samples with rapid and accurate analysis, while requiring little or no sample manipulation [8]. Use of the new spectroscopic method was beneficial for the pharmaceutical industries because it eliminated the analyte dilution step, both saving time and increasing productivity. In addition, NIRS can be used to determine various physical and chemical properties of drugs, for example, content, density, dissolution testing, hardness and dispersion of molecules, among others. One of the most important advantages of the method is its non-destructive nature, as it avoids important steps that are responsible for the introduction of errors and allows the tablet to be reused after the measurement [9].

Tabasi et al. [10] used NIR spectroscopy and multivariate calibration models to predict the dissolution behavior of theophylline. They sampled 117 tablets separated into five lots of different proportions of Eudragit NE 30D. For all readings, the standard error of calibration was less than the standard error of prediction. Therefore, the results were considered successful and showed that NIRS, combined with multivariate modeling [10]. Li et al. [11] used an NIR method based on principal component analysis (PCA) to predict the content uniformity of low-dose pills that had been manufactured by a process of direct compression. The model was able to predict the content uniformity in tablets that were prepared with varying degrees of lactose [11]. Although the technique is old, NIRS has only recently come into use as a result of computerization. NIRS requires advanced technology that involves sophisticated techniques and multivariate calibration due to the advent of chemometrics tools (PCR, PLS, MLR and others) for data processing of highly overlapping of absorption bands.

Freitas [12] conducted a study of the dissolution testing of clonazepam tablets using diffuse reflectance spectroscopy. The chemometric method PLS was used, which proved to be a suitable regression method in constructing the calibration models, without recourse to pre-treatment data. All 21 curves of predicted versus measured dissolution rates were consistent and showed satisfactory correlation [12], demonstrating the potential of this method in assisting in the development of appropriate dissolution testing formulations.

Therefore, this study aims to investigate the possibility of using the NIRS technique and multivariate calibration tools simultaneously to measure the percentage drug dissolution of isoniazid, rifampicin, pyrazinamide and ethambutol in pharmaceutical preparations produced at UFRN towards the treatment of pulmonary tuberculosis.

2. Experimental

2.1. Materials

For the determination of the four active pharmaceutical ingredients through the dissolution method, the following reagents and solutions were used: Sodium phosphate, dibasic, anhydrous (99.0%, Labsynth), phosphoric acid (85.0%, analytical reagent), acetonitrile (0.7791 g m L^{-1}, JT Baker Solusorb), triethylamine (Vetec), methanol (JT Baker Solusorb), isoniazid (98.21%), rifampicin

(93.09%), pyrazinamide (99.10%), and ethambutol (99.87%). The pharmaceutical formulation used to study the dissolution consists of isoniazid (7.9%), rifampicin (15.8%), pyrazinamide (42.2%) and ethambutol (29.0%) as active ingredients and aerosil (0.14%), explocel (2.80%), talc (1.4%) and magnesium stearate (0.7%) as excipients in tablet form.

Three mobile phases (A, B and C) for the analysis of pharmaceuticals were prepared using HPLC. Mobile phases A and B consisted of phosphate buffer and acetonitrile in a 96:4 and a 55:45 ratio, respectively. For mobile phase C, triethylamine buffer was first mixed with water at a 1:1000 ratio. The pH was adjusted to 7.0 ± 0.1 using phosphoric acid, and the solution was vacuum filtered and degassed for 10 min. The buffer solution was then mixed with acetonitrile at a ratio of 50:50. All mobile phases were vacuum filtered and degassed for 10 min before use.

2.2. NIRS

NIR reflectance spectra were measured using a Bomem model MB 160 coupled to a diffuse reflectance accessory. Each measured spectrum (in triplicate) was the average of 50 scans obtained with a resolution of 8 cm^{-1} and over the range of 1100–2500 nm. The spectrum of a polytetrafluoroethylene (PTFE) sample was used as background.

2.3. HPLC and dissolution apparatus

The Erweka Model BT-80 automatic dissolutor was used to perform the dissolution of sample tablets containing the active ingredients. The HPLC equipment (Shimadzu) used to obtain the chromatograms consists of the following modules: degasser (DGU-20A5), pump (LC-20AT), auto-injector (SIL-20A), column oven (CTO-20A), iodine array detector (SPD-M20A), and communication (CBM-20A). To weigh the samples and standards, an Ohaus Adventure high precision analytical balance was used. A FANEM Model 257 stirrer was used for homogenization of prepared solutions. A Tecnal Tec-3MP pH meter was used to control the pH of the buffer solutions. An Eletrolab Model 504 vacuum pump was used to assist the filtration of prepared solutions. The automatic dissolutor was set up with the following specifications: a length of 45 min at 37 °C, a paddle apparatus, 900 mL of phosphate buffer at pH 6.8 as dissolution medium and a rotation rate of 100 rpm. The tablets dissolved automatically under the reported conditions. Aliquots of each dissolved sample were collected and filtered using filter paper and transferred to a 1.5 mL vial for chromatography. The values calculated for the dissolution of tablets analyzed ranged from 5% to 90%.

2.4. Software and data analysis

The import and pretreatment of data and the construction of the chemometric models were performed using the Unscrambler 9.8 software from Camo. The data were pretreated by Savitzky-Golay smoothing, derivatives and multiplicative scatter correction (MSC). Subsets of calibration (24 samples), validation (7 samples) and prediction (7 samples) were chosen based on the scores from principal component analysis. The PLS multivariate regression model was used.

3. Results and discussion

The objective of this work was to develop a quantitative methodology to simultaneously determine the dissolution testing of four active pharmaceutical ingredients (API) (isoniazid, rifampicin, pyrazinamide and ethambutol) in tablets using a simple, rapid and nondestructive method. The raw NIR spectra (38 samples), shown in Fig. 2, are the averages of triplicate measurements for each tablet and were recorded in the region from 1100 to 2500 nm. The spectra are highly overlapping, and it is impossible to identify the constituents of the samples, both active ingredients and excipients, or to distinguish similar features between them. However, it is possible to assign some overtones and combination bands evidenced in the spectrum, including the following: the 1450–1460 nm region is assigned to N–H (amides) for the first overtone the 1600–1800 nm region is assigned to C–H for the first overtone; the 1910–1980 nm region is assigned to C=O (amide) for the second overtone, and the 2110–2205 nm and 2250–2260 nm regions are assigned to N–H (amines) and O–H (water), and are related to a combination of vibrations.

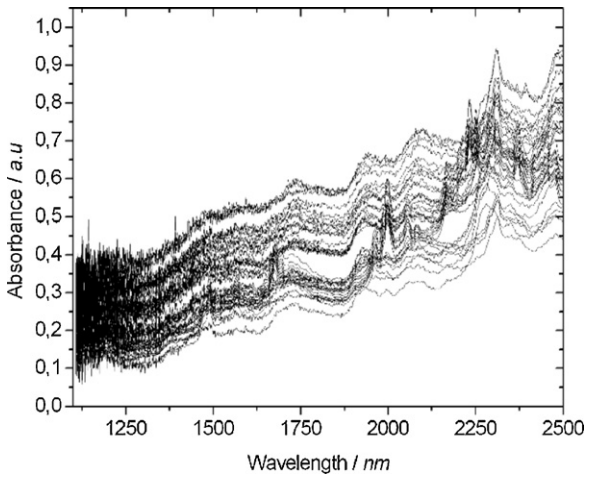

Fig. 2. Spectra of the original 38 samples.

The raw spectra are affected by noise, instrumental errors due to the scattering of light, which caused the displacement of the spectrum vertically, and multiplicative scattering. It was necessary to apply the pretreatments in order to facilitate the interpretation of the properties related to the analytical signals. Savitzky-Golay smoothing, with a window of (1–9) was used to remove random instrumental noise from the spectra, thus increasing the signal-to-noise ratio. Multiplicative scatter correction, MSC, was also applied to the spectra because granulation and the geometry of the particles in the sample dispersed light in all directions, which led to changes in the relationship between the intensity of reflectance measurements and the concentration of the species analyzed. Finally, the calculation of the first- and second-order derivative spectra employed windows ranging from 1 to 31 points. The best models obtained during the pretreatment stage utilized Savitzky-Golay smoothing (with a window of 7 points), MSC and the first derivative of the Savitzky-Golay polynomial (with a window of 7 points), as can be seen in Fig. 3.

For the construction of multivariate models that are needed to quantify the four API in tablets for the treatment of pulmonary tuberculosis, the principal component analysis (PCA) was applied to the data set, as shown in Fig. 4. It is observed in this figure that PC1 (53%) is defined in the direction of maximum variance in the data from the entire set of samples. PC2 (29%) is defined in the direction that describes the maximum variation in the subspace orthogonal to PC1. In the process of data compression, four principal components were necessary to explain 90% of all the variability information. As can be seen in Fig. 4, there was the formation of three clusters of samples due probably to differences between the values of dissolution of tablets. Based on this plot, and looking at the scores of PC1 and PC2, 24 samples from the calibration set, 7 samples from the validation set and 7 samples from the prediction set were chosen at random.

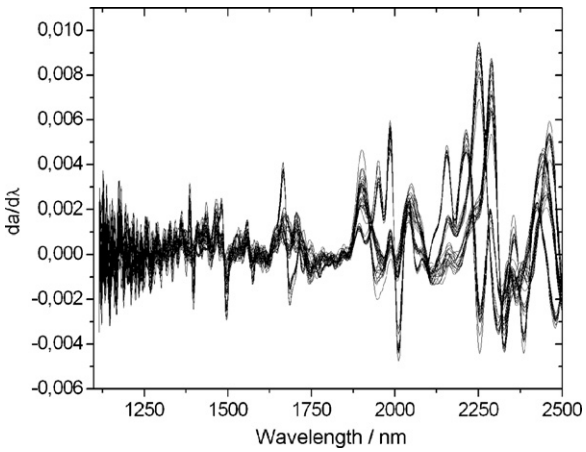

Fig. 3. Derivative spectra of the original 38 samples of tablets after pretreatment (Savitzky-Golay smoothing, MSC and a Savitzky-Golay derivatives).

A parameter needed to build a useful calibration model using the PLS method is the number the latent variables to be used. PCA was conducted on the spectral data to determine the optimal number of latent variables to be included in the chemometric PLS model. For model optimization the leave-one-out cross-validation method was used. In such a method, a calibration model is built with the entire set of samples except one. The procedure is repeated iteratively, so that all samples are predicted once. All deviations to the reference concentration values are computed to give a parameter called root mean square error of cross validation (RMSECV) and root mean square error of prediction (RMSEP). According to the above mentioned optimized pretreatment, Savitzky-Golay smoothing (with a window of 7 points), MSC and the first derivative of the Savitzky-Golay polynomial (with a window of 7 points) was chosen to establish the PLS quantitative analysis model because the value of RMSECV/RMSEP was found minimal.

Fig. 5 shows curves constructed from multivariate PLS models for the four API in tablets for the treatment of pulmonary tuberculosis. The correlation coefficients were 0.90, 0.98, 0.88 and 0.90, and the RMSEP values were 9.99%, 8.63%, 9.97% and 8.57% for isoniazid, rifampicin, pyrazinamide and ethambutol, respectively. Using the prediction set (7 samples), a good correlation and lows RMSEP were found between the dissolution percentage predicted by NIR and the

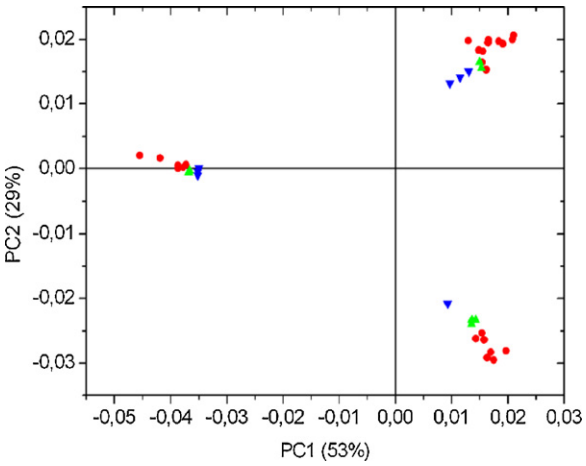

Fig. 4. First principal component against second principal component for dissolution testing determination: (●) calibration samples; (▲) validation samples; (▼) predicted samples.

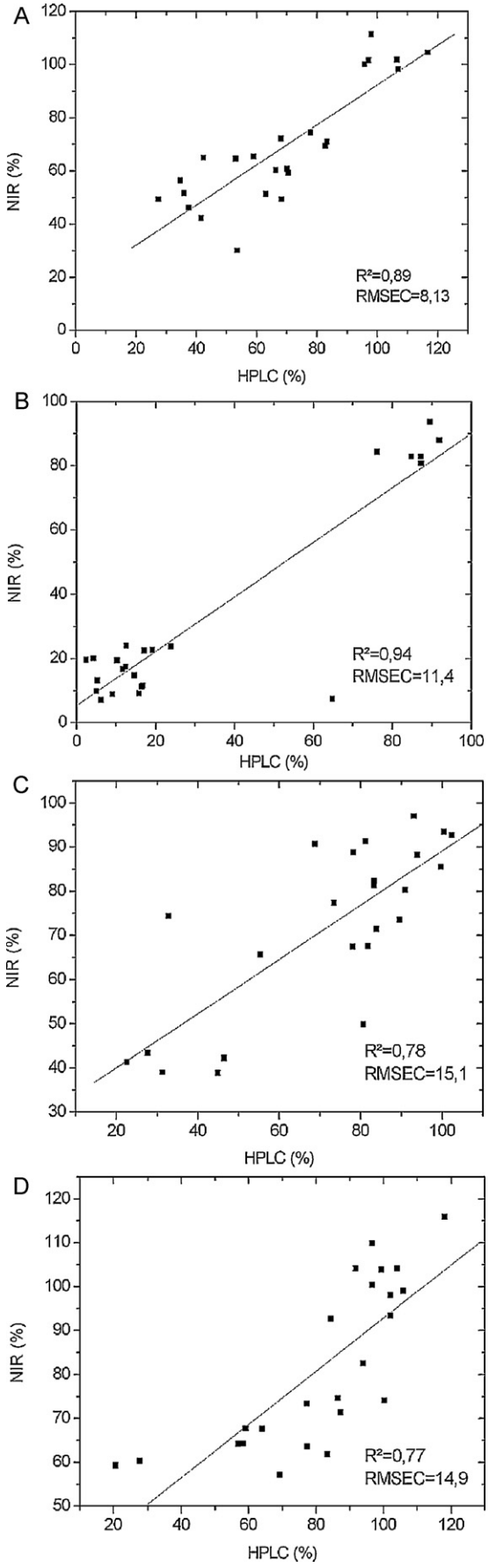

Fig. 5. Graphs of the calibration models illustrating the correlation between values obtained by HPLC and NIRS: (a) isoniazid; (b) rifampicin; (c) pyrazinamide and (d) ethambutol.

Table 1
Results obtained for model calibration and prediction.

API	R^2 (calibration set)	RMSECV (%)	R^2 (prediction set)	RMSEP (%)	LV
Isoniazid	0.89	8.13	0.90	9.99	4
Rifampicin	0.94	11.47	0.98	8.63	4
Pyrazinamide	0.78	15.1	0.88	9.97	3
Ethambutol	0.77	14.9	0.90	8.57	3

LV, suggested number of latent variable; R^2, coefficient of regression; RMSECV, root mean square error of cross calibration (%); RMSEP, root mean square error of prediction (%).

dissolution percentage determined by laboratory equipment (dissolution apparatus and HPLC determination), as demonstrated in Table 1. These results demonstrate a good prediction quality for the dissolution testing of four API tablets for PLS models.

4. Conclusions

Near infrared spectroscopy by diffuse reflectance, coupled with chemometric methods, was shown to be a satisfactory and efficient analytical technique, especially when used in the study of the dissolution testing of tablets, when compared to HPLC, the current reference technique, which is widely used in industry for such analyses. According to the results, PLS is presented as a good regression method to be used together with pretreatment steps that must be performed initially on the sample spectra, ensuring the construction of good calibration models and consistent prediction results. It should also be noted that the NIRS method offers advantages over the current method in that it does not consume reagents during analysis, it has no need for specialized personnel to perform the experiments, it is a low-cost technique and it non-destructive. Using the above advantages and the results obtained during this study, it is concluded that NIRS and multivariate calibration are efficient and reliable to be applied at various stages of quality control of drugs, mainly to study the dissolution testing of drugs in tablets.

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References

[1] M.V.N. de Souza, T.R.A. Vasconcelos, Fármacos no combate à tuberculose: passado, presente e futuro, Quim. Nova 28 (2005) 678–682.
[2] A. Dévay, K. Mayer, S. Pál, I. Antal, Investigation on drug dissolution and particle characteristics of pellets related to manufacturing process variables of high-shear granulation, J. Biochem. Biophys. Methods 69 (2006) 197–205.
[3] S. Agrawal, R. Panchagnula, Dissolution test as a surrogate for quality evaluation of rifampicin containing fixed dose combination formulations, Int. J. Pharm. 287 (2004) 97–112.
[4] S. Agrawal, I. Singh, K.J. Kaur, S.R. Bhade, C.L. Kaul, R. Panchagnula, Comparative bioavailability of rifampicin, isoniazid and pyrazinamide from a four drug fixed dose combination with separate formulations at the same dose levels, Int. J. Pharm. 276 (2004) 41–49.
[5] M.Y. Khuhawar, F.M.A. Rind, Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood, J. Chromatogr. B 766 (2002) 357–363.
[6] J. Luypaert, D.L. Massart, Y.V. Heyden, Near-infrared spectroscopy applications in pharmaceutical analysis, Talanta 72 (2007) 865–883.
[7] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. de La èzuela, Near-infrared spectroscopy in the pharmaceutical industry, Analyst (Cambridge, U.K.) 123 (1998) 135–150.
[8] M. Blanco, J. Coello, A. Eustaquio, H. Iturriaga, S. Maspoch, Analytical control of pharmaceutical production steps by near infrared reflectance spectroscopy, Anal. Chim. Acta 392 (1999) 237–246.
[9] M. Blanco, M.A. Romero, Near-infrared libraries in the pharmaceutical industry: a solution for identify confirmation, Analyst (Cambridge, U.K.) 126 (2001) 2212–2217.
[10] S.H. Tabasi, V. Moolchandani, R. Fahmy, S.W. Hoag, Sustained release dosage forms dissolution behavior prediction: a study of matrix tablets using NIR spectroscopy, Int. J. Pharm. 382 (2009) 1–6.
[11] W. Li, L. Bagnol, M. Berman, R.A. Chiarella, M. Gerber, Applications of NIR in early stage formulation development. Part II. Content uniformity evaluation of low dose tablets by principal component analysis, Int. J. Pharm. 380 (2009) 49–54.
[12] M.P. Freitas, A. Sabadin, L.M. Silva, F.M. gianotti, D.A. do Couto, E. Tonhi, R.S. Medeiros, G.L. Coco, V.F.T. Russo, J.A. Martins, Prediction of drug dissolution profiles from tablets using NIR diffuse reflectance spectroscopy: a rapid and nondestructive method, J. Pharm. Biomed. Anal. 39 (2005) 17–21.