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MIR AND NIR SPECTROSCOPY AS RAPID METHOD TO QUANTIFY MOLSIDOMIN IN GENERIC TABLET

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ABSTRACT: This work investigates the potential of Partial Least Square (PLS) regression using two vibrational spectroscopies as mid infrared (MIR) and near infrared (NIR) spectroscopies for the quantification of molsidomin in tablets. Two very simple, rapid, inexpensive and green strategies were applied for this determination. Powder blends containing molsidomin and excipients were prepared. The excipients correspond to those in Inverter® tablets. Two experimental design approaches were used in generating 9-levels % calibration and validation samples sets. Fourier transforms of mid-infrared (MIR) and near-infrared (NIR) spectra were measured by transmission and diffuse reflection techniques respectively. The calibration models were generated by PLS models without spectral treatment and were used to predict the drug content in the powder blends made for validation models and for commercial tablets. The mean percentages of recoveries for the analysis of molsidomin in generic Inverter® 4 mg tablet are 101.5 and 99.6 % for FT-MIR and FT-NIR respectively.

INTRODUCTION: Molsidomin (i.e. N-carboxy-3-morpholino-sydnonime ethylester) is an active Pharmaceutical Ingredient (API) for different pharmaceutical formulations like Corvasal and generics. These antianginal drugs represent an alternative to the organic nitrates, such as isosorbide dinitrate and nitroglycerine in the treatment of ischemic coronary artery disease [1, 2].

This API is metabolized in the liver to its active hepatic metabolite 3- morpholino sydnonimin (SIN-1) which is a best-known compound because it releases NO via a radical process following reaction with molecular oxygen [3, 4]. The beneficial effects of molsidomin were recognized before endogenous NO was identified. Then this double efficacy becomes the drugs relevant to this API very used.

Most of the published assays for drugs relevant were suggested for their determination in biological fluids rather than in pharmaceutical preparations. The typical oral dose of molsidomine per tablet is 2-4 mg: thus a sensitive analytical method is necessary to collect API concentrations in solid drugs.

The principal methods involve extraction by ion-pair complex [5] liquid chromatography (LC) coupled to UV spectrophotometry [6], and mass spectrometric [7, 8]. In the few last years, high performance liquid chromatography (HPLC) methods coupled with photodiode-array UV detection have been widely utilized in biomedical fields for both identification and quantification of drugs and metabolites at low concentrations [9]. A lot of assays were also attempted, like NMR spectroscopic and crystallographic investigations or modeling investigation of the inclusion of this API in cyclodextrins [10]. All these techniques require time consuming and not eco-friendly procedures contributing towards high cost and generating waste.

In opposite, FT-MIR is a method which simplifies handling, avoids the use of chemical solvents and thus save time and chemical reagents. The main drawback of spectral data is the overlapped information contained in the signal for chemical mixtures. Furthermore, the relationship between the properties of interest may result in non-linear correlation. In order to take advantage of spectroscopic techniques, chemometric approaches such as Partial Least Squares (PLS) regression have frequently been used in quantitative spectral analysis to obtain very selective information from unselective data. Since two decades, tremendous quantitative works, applied efficiently on quantification of pharmaceutical formulations, have been published [11, 12].

As for near–infrared spectroscopy (NIRS), this vibrational technique can be performed directly on intact samples without any sample preparation or solvents utilization. It is a simple and non-destructive technique. When it is coupled with multivariate analysis, these combined techniques are rapidly becoming unavoidable in the pharmaceutical industry [13], and have been applied to determine drug contents [14], polymorphic contents of pharmaceuticals [15], particle sizes of powders [16], and determination of rheological behavior [17].

The objective of this work is to investigate the potential of the chemometric PLS technique associated to solvent free methods which are vibrational FT-MIR and FT-NIR spectroscopies in order to quantify molsidomin amount in tablet containing low amount of API.

No reference method was used and the validation of the developed PLS models were performed in powder blends. Blanco *et al* [18] successfully used NIRS as an absolute technique to determine the API in tablets avoiding the use of a reference method, the reference values were obtained by weighing formulation components. Our methods were evaluated for API content determination in commercial products.

MATERIALS AND METHODS:

Apparatus: FT-MIR spectra were obtained with a Mattson model Genesis II FTIR [TM] spectrometer controlled by Winfirst software from Mattson Instruments Inc. (Win. Lab. Instruments, Bagnolet, France). Chemometric analysis was done with Simca-P (version 9.0 of Umetrics, Umea, Sweden). FT-NIR spectra were acquired on a ThermoScientific FT-NIR analyzer model Antaris II (ThermoScientific Instrument, Madison, USA) controlled by Result and Omnic softwares. This instrument is equipped with a Michelson interferometer, a matched InGaAs detector, a quartz halogen light source (50W) and an Antaris II Integrating sphere for measuring solids by diffuse reflectance. The spectrophotometer was equipped with the software package from ThermoScientific Instrument, including OMNIC version 6.1a for spectral acquisition and TQ ANALYST version 6.21 for spectral processing and chemometric analysis.

Chemicals: Inverter 4mg, a Corvasal generic tablet, contains 12.5 mg/g (w/w) of molsidomin in a mixture of hypromellose, magnesium stearate, an anhydrous colloidal silica, monohydrate lactose and mannitol for a tablet. The pharmaceutical specialty of Inverter was kindly donated by Saïdal laboratories (Algiers, Algeria). Pharmaceutical excipients, namely colloidal silica from Cooper (France), cornstarch, hypromellose, monohydrate lactose and mannitol from Prolabo (France) were of pharmaceutical grade. Infrared quality potassium bromide (Merck, Darmstadt, Germany) was used for the KBr pellet technique.

Samples preparation: For calibration and validation purpose power blends of Inverter 4 mg tablet were prepared. The mixture composition was designed for a tablet of a total mass of 320mg and for 4 mg of molsidomin. This formulation will be further considered as the 100 % active content formulation.

Six series of reconstituted pharmaceutical forms were prepared by weighting and mixing together hypromellose, magnesium stearate, colloidal silica, monohydrate lactose, mannitol, corn starch and molsidomine to reach the required percentage of molsidomine from 80 to 120 % in 5% increments of the labeled concentration of the commercial product **(Table 1).**

TABLE 1: QUANTITATIVE AND QUALITATIVE COMPOSITION OF POWDER BLEND OF INVERTER®

Molsidomine Content (%)	80%	85%	90%	95%	100%	105%	110%	115%	120%
Molsidomine (mg)	**3.20**	**3.40**	**3.60**	**3.80**	**4.00**	**4.20**	**4.40**	**4.60**	**4.80**
Corn starch (mg)	45.80	46.60	48.40	49.20	50.00	50.80	51.60	52.40	53.20
Monohydrate lactose (mg)	88.00	86.00	84.00	82.00	80.00	78.00	76.00	74.00	72.00
Mannitol (mg)	166.50	167.50	167.50	168.50	169.50	170.50	171.50	172.50	173.50
Magnésium stéarate (mg)	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20
Colloidal silica (mg)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Hydomellose (mg)	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Total mass (mg)	307.00	307.00	307.00	307.00	307.00	307.00	307.00	307.00	307.00

These 54 samples were then divided into 2 parts: the calibration set was composed with 27 samples (i.e. 3 series with nine levels of concentration of API) and the validation set was composed with 27 samples (i.e. 3 series with nine levels of concentration of API).

For FT-MIR spectral acquisition, each power blend was mixed in KBr at 1% sample/KBr ratio, pulverizing into a finely ground homogenous mixture, placing the mixture into a stainless steel, and subjecting it to 8000 p.s.i. of pressure with an 12 ton Press (International Crystal Laboratories, New Jersey, USA) for a period of 3 minutes to produce a glass pellet (13 mm in diameter). Each pellet was then placed into the FT-IR spectrophotometer and spectra were acquired in transmission mode.

For FT-NIR spectral acquisition, each power blend was aliquoted in a 1.5 mL disposable glass vial (10 mm in diameter) to reach 5 mm height of powder, put on the sampling sapphire window of the reflectance module and scanned directly through the bottom of the vial. A special holder made to fit the round suitable shape kept the vial or the tablet in centered position over the window.

ANALYTICAL METHOD:

FT-MIR method: Generated by Fourier transform of interferogram measured between 4000 and 400 cm^{-1} in the transmission mode, each spectrum was the average of 32 scans with a resolution of 4 cm^{-1}. Later, all transmittance data were transformed in absorbance data. Then spectra were normalized by setting the minimum data value to zero (the difference between the minimum signal and zero was then subtracted from all other data values). Data obtained from Winfirst software were exported in ASCII format to Microsoft Excel. Calibration and quantification were done using the PLS model as provided in Simca P software (version 9.0 of Umetrics, Umea, Sweden). Variables were centered and unscaled over the whole range. The number of the factors was chosen to minimize the Standard Error of Cross Validation (SECV) by the leave-one-out method.

FT-NIR Method: Each spectrum was the average of 32 co-added scans, obtained with a resolution of 8 cm^{-1} over the range 4000-9999 cm^{-1} and corrected against the background spectrum of room environment which was performed routinely. Each spectrum was collected in the diffuse reflection mode and then converted in absorbance units. A PLS model as provided in TQ Analyst software was used for the quantification of API in Inverter generic tablet. All data were mean centered before analysis.

The best calibration model was determined to minimize the Standard Error of Cross Validation (SECV) by the leave-one-out method in the PLS software. The predictability of the model was tested by applying the validation set that was not used during its development.

RESULTS AND DISCUSSION:

Number of PLS factors: For FT-MIR data, the totality was used to build PLS model. API concentrations constitute the Y observation matrix and the normalized data of absorbance spectra constitute the X variable matrix. The goodness of fit of the PLS model was assessed by minimizing the SECV [19, 20]. In order to maximize the analysis, the optimal number of 4 latent variables was preferred [21] **(Table 2).**

For FT-NIR data, the totality was mean centered before analysis. By minimizing the SECV, the optimal number of latent variables was fixed at 6 **(Table 2).** Validation of the PLS models was then performed.

TABLE 2: SECV VALUES ACCORDING TO NUMBER OF FACTORS.

Number of factors	FT-MIR SECV	FT-NIR SECV
1	0.0201	2.64
2	0.0154	2.75
3	0.014	2.7
4	**0.0132**	2.43
5	0.0132	2.33
6	0.0142	**2.24**
7	-	2.26
8	-	2.26

Validation of the PLS models

Linearity: For FT-MIR (eq 1) and FT-NIR (eq 2), the respective linear regression equation obtained with the partial least square method applied to predict the 27 samples percentage of the validation set, are:

Predicted $_{FT-IR}$ % = 0.9871 (±0.0138) introduced % + 1.7085 (± 1.3888) (eq 1) with R = 0.9952 and F_1 = 5135.51 **(Figure 1A)**

Predicted $_{FT-NIR}$ % = 0.9900 (±0.0123) introduced

% + 1.1389 (± 1.2438) (eq 2) with R = 0.9961 and

F_2 = 6440.63 **(Figure 1B)**

As the R values are very close to 1 and F_1 and F_2 higher to F (0.05, 1, 25) = 4.24, the two methods are linear.
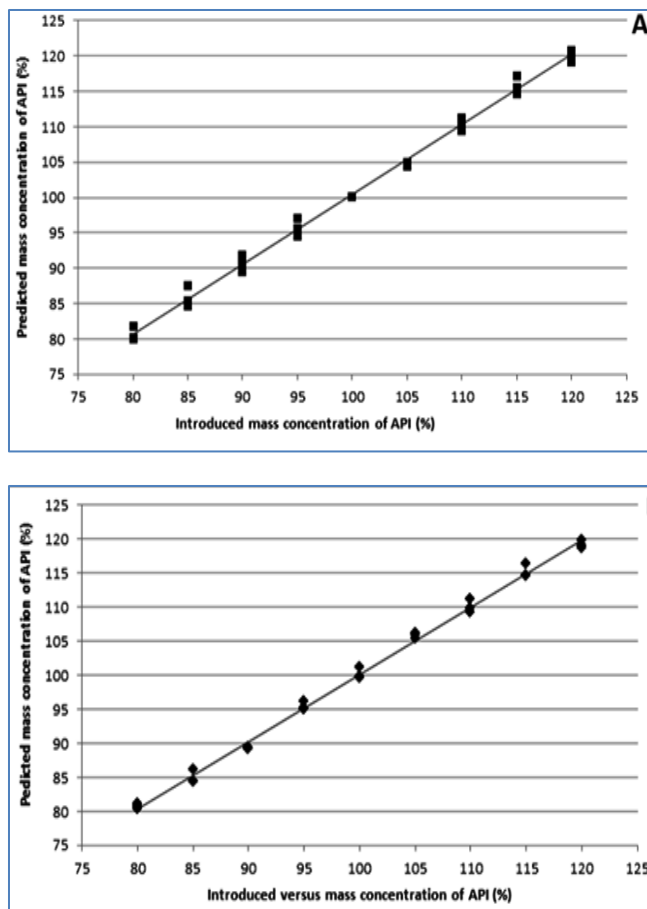
FIG. 1: PREDICTED VERSUS INTRODUCED MASS CONCENTRATION OF API (%) IN FT-MIR PLS MODEL (A) AND FT-NIR PLS MODEL (B).

Specificity: For the two methods, Student's t tests show that the y-intercept of the two regression equations (eq 1 and eq 2) are not significantly different from zero:

t = 1.23 < t° (0.05, 25) = 2.06 for FT-MIR
t = 0.92 < t° (0.05, 25) = 2.06 for FT-NIR
More the slope of the two regression equations were not significantly different from one: the specificity of the two methods was confirmed.
t = 0.94 < t° (0.05, 25) = 2.06 for FT-MIR
t = 0.81 < t° (0.05, 25) = 2.06 for FT-NIR

Accuracy: For FT-MIR and FT-NIR, the mean percentages of recoveries were respectively found to be 100.45 and 100.16 % with a respective coefficient of variation of 0.18 % and 0.82 %, indicating a very good accuracy for the two methods.

Repeatability and reproducibility: For FT-MIR and FT-NIR, the repeatability (or Intra-day Precision) and the reproducibility (or Inter-Day Precision) were expressed as the coefficient of variation of determinations of six synthetic mixtures (with 100 % of the label claim of API). The first one, carried the same day, indicate with 0.41 and 0.77 % a good repeatability **(Table 3)** and the second, carried out on three different days, indicate with 1.26 and 0.85 % a good reproducibility (Table 3).

TABLE 3: REPEATABILITY AND REPRODUCIBILITY FOR FT-MIR AND FT-NIR MODELS

	FT-MIR	FT-NIR
	Intra-day precision	Intra-day precision
Average	99.84	101.13
RSD, %	0.41	0.77
	Inter-day precision	Inter-day precision
Average	100.82	100.03
RSD, %	1.26	0.85

Application to API determination in commercial tablets of Inverter: These optimized PLS calibration models were used to determine the API amount of five commercial samples of INVERTER 4mg. Twenty tablets of INVERTER 4mg were pulverized to prepare the samples for content determination. Therefore, five different pellets were prepared for FT-MIR containing each 1% of this powder and five different vials were filled with this powder until to reach 5 mm of height for FT-NIR. The results showed the absence of significant difference between the theoretical and calculated concentration for each tested sample **(Table 4).**

The mean percentages of recoveries of FT-MIR and FT-NIR were 101.5 and 99.6 % respectively. The quantitative determination of these tablets was checked by HPLC. As the percentage of mean recovery by HPLC was found equal to 99.3% that confirmed the suitability of transmission FT-MIR and diffuse reflectance FT-NIR spectroscopies for assessment of exact quantity of molsidomin to control the quality of tablets.

Tablets API content was found between 95 and 105 % of the labeled content as required in pharmaceutical industry [22]. Furthermore, we demonstrated that the chemometric methods developed for FT-MIR and FT-NIR can be totally developed by a free-solvent approach.

TABLE 4: FT-MIR, FT-NIR AND HPLC QUANTITATIVE DETERMINATION OF API IN COMMERCIAL TABLETS OF INVERTER (N=5).

	FT-IR	FT-NIR	HPLC
Mean recovery, %	101.5	99.6	99.3
RSD	1.1	3.9	1.4

CONCLUSION: The two vibrational techniques presented in this work and operated with PLS regression were validated for the determination of content of molsidomine in generic pharmaceutical tablet form. First, a FT-MIR chemometric method, based on transmission spectra of a KBr pellet of API and PLS regression, was developed and validated in terms of trueness, precision and accuracy. The result demonstrated that the developed method is appropriate for direct active content assay in tablet even if the drug content in tablets is low (1.25 %).

The use of FT-MIR with KBr pellet preparation could be simplified with an Attenuated Total Reflectance accessory which makes instantaneous the sample preparation. Secondly, FT-NIR-chemometric method, based on NIR reflection spectra of powder blends and PLS regression, has been developed and validated with the same way. The results are also an efficient determination of molsidomine in tablets. In both cases, PLS calibration models were designed and appeared rapid and very suitable. Considering the results presented in this work, the two vibrational techniques coupled with PLS regression were suitable for rapid determination of API in tablet saving reactive, time and money.

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