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Identification of pharmaceutical tablets by Raman spectroscopy and chemometrics

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ABSTRACT

Raman spectroscopy has become an attractive tool for the analysis of pharmaceutical solid dosage forms. In the present study it is used to ensure the identity of tablets. The two main applications of this method are release of final products in quality control and detection of counterfeits. Twenty-five product families of tablets have been included in the spectral library and a non-linear classification method, the Support Vector Machines (SVMs), has been employed. Two calibrations have been developed in cascade: the first one identifies the product family while the second one specifies the formulation. A product family comprises different formulations that have the same active pharmaceutical ingredient (API) but in a different amount. Once the tablets have been classified by the SVM model, API peaks detection and correlation are applied in order to have a specific method for the identification and allow in the future to discriminate counterfeits from genuine products. This calibration strategy enables the identification of 25 product families without error and in the absence of prior information about the sample. Raman spectroscopy coupled with chemometrics is therefore a fast and accurate tool for the identification of pharmaceutical tablets.

1. Introduction

Vibrational spectroscopy encompasses near-infrared (NIR), mid-infrared (MIR) and Raman spectroscopy. Aided by relentless instrumentation advances, the Raman techniques have now entered common use for the study of solid-state samples [1,2]. Raman spectroscopy has thus found many applications in the pharmaceutical industry such as polymorphism screening, solid-state analysis, quantitative determination of active pharmaceutical ingredient (API) content [3] and distribution of API in tablets by Raman mapping [4,5]. Moreover Raman spectroscopy can be used in a laboratory for quality control application but also for on-line analysis [6] and for process analytical technology projects [7].

In this paper a method is proposed to identify pharmaceutical tablets by Raman spectroscopy. The objective of the study is, in case of release analyses of products in quality control, to ensure the identity of the samples. Classical analytical tools have so far been used for the identification of pharmaceutical drugs and counterfeits: liquid chromatography [8], high performance liquid chromatography [9], nuclear magnetic resonance [10] or recently near-infrared (NIR) spectroscopy [11–14] and near-infrared imaging [15]. Raman spectroscopy is a fast technique compared to other techniques such as classical chromatography. Indeed, in less than 1 min, a sample can be analyzed. Contrary to NIR spectra, Raman spectra of tablets have the advantage of revealing API specific peaks.

The present paper describes the calibration strategy developed for an automatic identification of the tablets produced by the firm in Basel, which represents 25 product families. The classification determines the product family. Moreover acceptance criteria like API peak detection and correlation with the database spectra are applied. No prior information about the sample is needed to identify correctly its product family as long as the product type is part of the database. For some of the products, the formulation can even be automatically identified.

2. Materials and methods

2.1. Pharmaceutical samples

In this study, 25 different product families have been analyzed and initially 44 formulations have been measured. A product family comprises different formulations that have the same API in different amounts. Depending on the concentration of API in the tablets, the excipients may have different concentrations within the same product family. The chemical composition of excipients can also slightly differ from one formulation to another.

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For each formulation, five batches were selected and for each batch, one tablet was measured three times. So that a complete range of tablets can be identified by the same method, samples from different origins or different stability and release testing were measured and included in the spectral library. In this way, variability induced by differences in stability conditions is part of the database. Three of the five batches were used for the calibration while the two other batches were included in the validation set, in order to have two independent data sets, according to the requirements of the European Medicines Agency (EMEA) guideline for near-infrared spectroscopy [16].

2.2. Raman measurements

The measurements were performed with a dispersive spectrometer, the RXN1 from Kaiser Optical Systems (Kaiser Optical Systems Inc., Lyon, France). A PhAT probe was used, generating a 6 mm diameter spot that covers the main part of the tablets. This allows the analysis of a representative surface of the samples and results for each measurement in a mean Raman spectrum. The principles and utilization of Raman spectroscopy are described in the United States Pharmacopeia [17] and European Pharmacopeia [18].

The laser excitation wavelength used was 785 nm. The spectral coverage was 150–1890 cm^{-1} and the spectral resolution 5 cm^{-1}. The exposure time was 3 s and 10 accumulations were performed at a laser power of 260 mW on the samples. Dark subtraction (electric noise), intensity correction and cosmic ray filter were applied. The samples were analyzed directly at an approximate distance of 4 cm from the probe. The coating of all the film-coated tablets was systematically removed with a scalpel while the uncoated tablets remained intact.

2.3. Chemometric tools

2.3.1. Data pretreatments

The spectra of the tablets were normalized with the Standard Normal Variate (SNV) method, i.e. the spectra were mean centered and scaled to unit variance by spectrum using the following calculation:

$$\mathrm{SNV}_i = \frac{x_i - \bar{x}}{\sqrt{\sum_i (x_i - \bar{x})^2/(w-1)}}$$

where x_i is the Raman intensity value at the wavenumber i, w is the number of wavenumbers, $\bar{x}$ is the mean of the Raman intensity values and SNV_i is the corrected Raman intensity value at the wavenumber i.

The second pretreatment applied to the method was the Savitzky–Golay first derivative (filter length: 11 points and filter order: 2) which corrects the baseline shift after the normalization and compensates for the influence of fluorescence.

2.3.2. SVM classification method

The objective of the study being the identification of tablets, a classification method had first to be applied to the calibration spectra. Identifying a sample first implies to determine its product family and secondly if possible its formulation. Two classification models have therefore been developed in cascade in order to identify the product family name (one name attributed per class) and formulation name of the tablets.

The Support Vector Machines (SVMs) is a powerful classification method in which the training data are attributed a class label. The aim is to predict the class labels for samples that are tested, it means in this case the calibration and validation sets and then unknown samples.

The SVM algorithm is based on the concept of maximal margin hyperplane [19]. For samples that cannot be linearly (in 2D dimension) separated, this method first increases the dimension of the data. Samples that were not separable in the previous space may then be distinguished in the newly created hyperplane. A feature mapping is realized, it means a non-linear mapping of the input data in the new feature space, and the dimension-increasing technique used here is the kernel function K [20]. Several kernel functions can be applied such as the linear, polynomial, radial basis or sigmoid functions. In this study the radial basis function (RBF) has been used, its main advantage being that it can handle the case when the relation between the class labels (the target values) and the attributes (the features of the training set) is non-linear.

The general RBF equation is the following one:

$$K(\mathbf{x}_i\mathbf{x}_j) = \exp(-\gamma||\mathbf{x}_i - \mathbf{x}_j||^2), \quad \gamma > 0$$

with γ being a parameter controlling the width of the kernel function and $\mathbf{x}_i$, $\mathbf{x}_j$ the vectors of the ith and jth training samples.

Once the dimension of the data has been increased, the concept of the SVM is the construction of an Optimal Separating Hyperplane (OSH) in the feature space which maximizes the margin between the classes of products. The support vectors are then the training vectors at the boundary that determine the maximal margin hyperplane.

The regularization parameter, c (>0), offsets the maximization of the margin width and the minimization of the training error [21]. The two main parameters, γ and c, can be optimized so that the SVM model gives the best calibration and the best prediction for the calibration set. For this purpose the Matlab "grid-search" uses a cross-validation where pairs of (c, γ) are tested. The best cross-validation accuracy is finally chosen and a suitable SVM model can be computed with the corresponding c and γ parameters [22]. Detailed explanation of the SVM method can be found in other publications [19–21].

2.3.3. Software

The software used for the data acquisition was HoloGRAMS (Kaiser Optical Systems Inc., Lyon, France). All the Raman data were exported and computed with Matlab R12 (The Mathworks, Natick, USA) and the PLS toolbox (Eigenvector, Manson, USA). The SVM toolbox [20] and the Matlab peak detection method (local maxima detection [23]) have also been used for the development of the method. For the calculation of the correlations, the corrcoef function of Matlab has been applied [22].

2.4. Method validation

According to the European Pharmacopoeia for NIR and Raman spectroscopy and the ICH requirements, the identification method had to be validated using specificity and robustness tests. An independent validation set has been used to test and validate the model and the classification method.

"The specificity of the database which makes it possible to identify positively a given material and distinguish it adequately from other materials in the database is to be established during the validation procedure" [18]. In this case the specificity consisted in testing all the spectra of calibration and validation sets with the method.

"The robustness should show the reliability of an analysis with respect to deliberate variations in method parameters" [24]. "The robustness of the qualitative procedure must also be challenged to test the effect of minor changes to normal operating conditions on the analysis" [25]. It has been decided to test the influence of the laser power by comparing the results obtained with an input power of 200 mW and then 400 mW. The effect of photobleach-

ing was then estimated by measuring ten times the same tablet. Finally tablets were measured at two different distances between the probe and the sample, which permitted to evaluate the influence of the laser focus.

3. Results and discussion

3.1. Spectral interpretation

API specific peaks can be detected in Raman spectra of tablets observed in Fig. 1. The spectra of two tablets of the same product family have been displayed together with the spectrum of their common API, the first tablet containing 24% of API and the second one a higher dosage (32%). The API can clearly be observed on the spectra of both tablets.

The API content of some tablets is very low. In Fig. 2A the detection of API by Raman can be evaluated by observing the spectra of increasing percentages of API in a given type of tablet. Differences between the heights of the peaks can indeed be noticed on the spectra. In order to estimate the limit of detection of API, a semi-quantitative analysis was tested for the same samples (Fig. 2B). A linear relationship between the area under the curve (AUC) and the concentration of API in the tablet can be observed in Fig. 2B. The limit of detection [25] obtained was 0.59% API (Fig. 2B). In general, the detection limit was between 1 and 2% of API for the analyzed solid dosage forms. The limit of detection depends on the strength of the Raman signal and the nature of the excipients.

The main challenge to this classification is the presence of different products with similar spectra, i.e. products with the same excipients or products with API having close chemical properties. Different product families containing APIs with close chemical properties and formula are indeed difficult to identify with classification methods. Even if tablets are actually mixtures of several compounds, resulting in complex spectra, differences can clearly be detected between these products. The tablets can therefore be specifically identified as observed in Fig. 3. This is all the more interesting as the present samples are both benzodiazepines with close chemical structures.

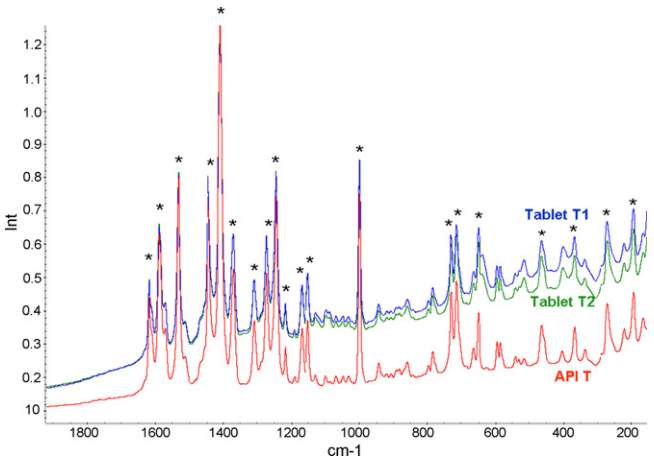

Fig. 1. API specificity of Raman spectroscopy. API peaks (*) are clearly detected in tablet T1 containing 24% of API and in tablet T2 containing 32% of API [1617.8; 1589.3; 1532.1; 1444.0; 1408.0; 1370.6; 1309.7; 1273.1; 1245.1; 1217.9; 1169.6; 1151.0; 1000.0; 729.4; 714.4; 650.3; 463.6; 367.6; 269.6; 193.3].

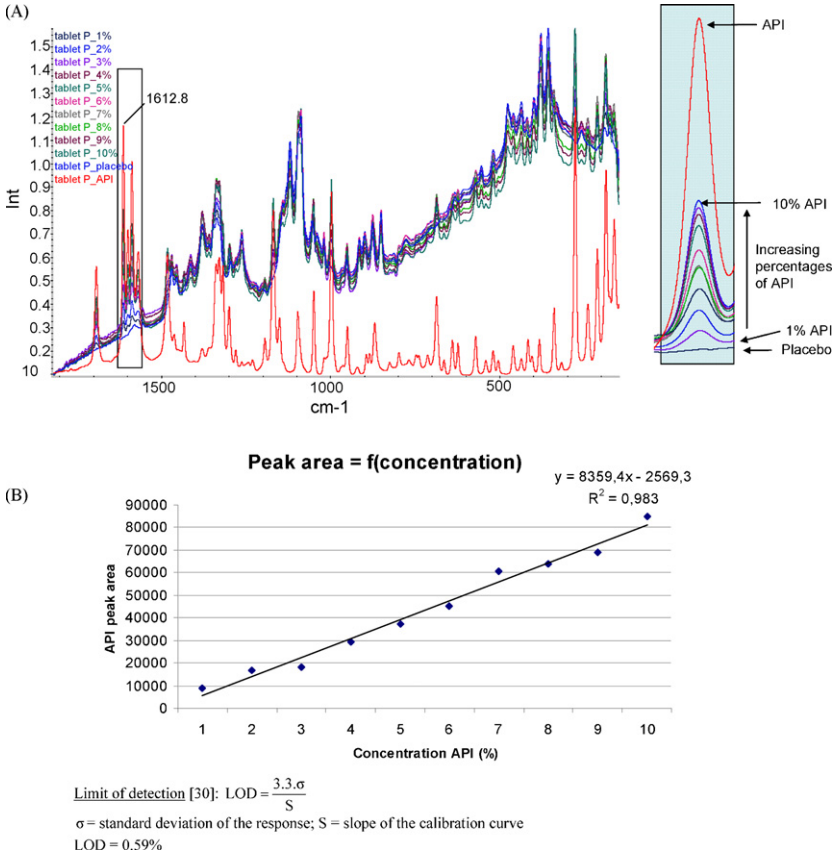

Fig. 2. (A) Spectra of tablet P containing increasing percentages of API (0–10%). The API of the tablet A can be observed from very low concentrations. The height of the peaks of API is increasing proportionally to the concentration. The integration of an API specific peak (1612.8 cm^{-1}) in the tablets was done to perform semi-quantitative analysis. (B) Estimation of the detection limit of API (1–10%) in the tablet P. For an API specific peak of tablet P (1612.8 cm^{-1}), the values of API peak areas under curve (AUC) were plotted against the API concentrations. The limit of detection (LOD) of API was calculated with the calibration results and is of 0.59% API.

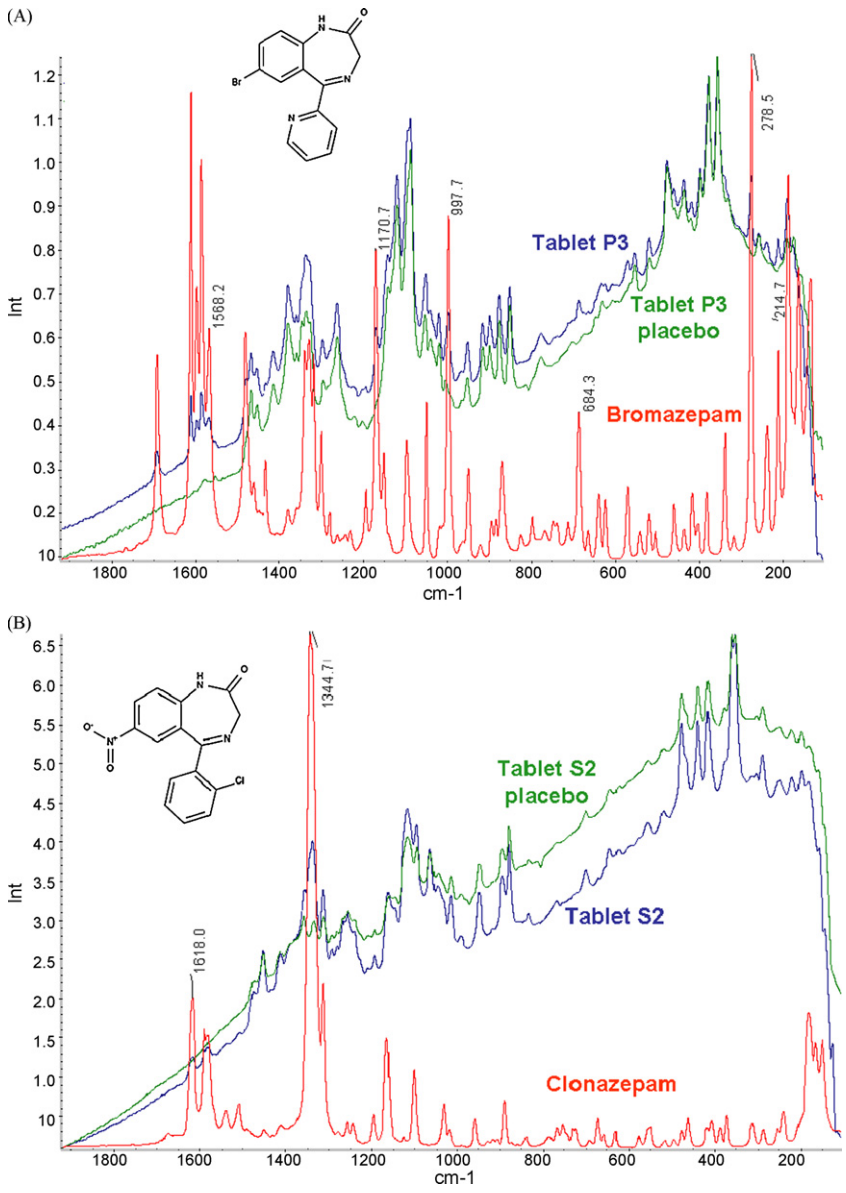

Fig. 3. Comparison between tablets, placebo and API spectra of two formulations of benzodiazepine. The differences observed between the APIs can be found in the tablets of 3% of bromazepam (tablet P3) (A) and 1% of clonazepam (tablet S2) (B).

A second challenge for the classification is to deal with heterogeneous classes and classes with similar spectra. Sometimes samples of the same product family can generate heterogeneous spectra. All the formulations of one product family have indeed the same API but the excipients might be different as observed in Fig. 4. On the contrary, formulations containing a low API content (1–2%) can have very similar spectra if they have the same excipients (Fig. 5). In fact tablet spectra are the weighted sum of the components (mixtures spectra), so low dosage tablets will look similar. This is why difficulties were encountered to distinguish between the formulations of the same product family.

Another difficulty encountered during the analysis of tablets is that the coating can prevent the laser from reaching the core and detecting the API in the sample (Fig. 6A). To keep the advantage of the API specificity of Raman spectroscopy, the coating of the film-coated tablets was removed with a scalpel. This was done for all coated tablets so that we could follow a systematic procedure, but for some tablets the coating did not prevent the detection of API peaks (Fig. 6B). It appeared that the coating was actually a problem when the API content was under approximately 2% or the coating too thick or containing metal compounds like titanium dioxide.

Compared to other fast identification methods like near-infrared spectroscopy, Raman is more specific (i.e. API peaks can generally be detected). However, the limit of detection will depend on the intensity of the API peaks and the similarity between the API and the excipients.

3.2. Calibration and method validation

3.2.1. Calibration strategy for automatic identification with a large database

The strategy adopted for the identification of tablets consists in three steps: first the identification of the product family, then the identification of the formulation when possible and finally the validation of the best match with acceptance criteria. The correlation with the reference spectra and the control of the API peak positions in the tablets spectra have been used as acceptance criteria in order to confirm the results provided by the SVM supervised classification.

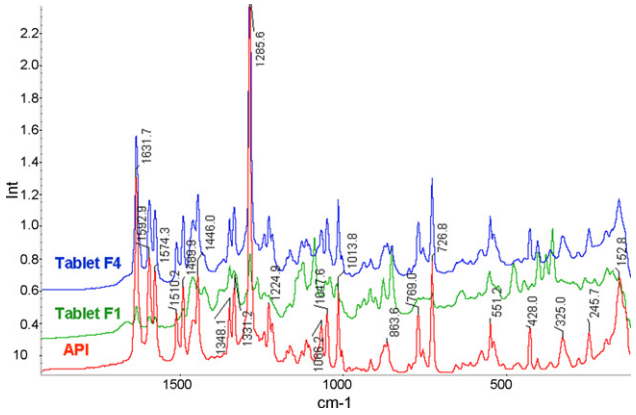

Fig. 4. Comparison between the spectra of two formulations of the same product. Differences in the composition of excipients can be found that lead to unlike spectra for tablets of the same product family.

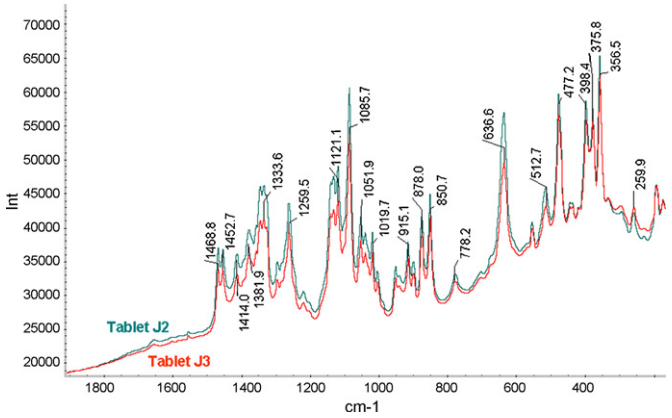

Fig. 5. Similar spectra of two tablets of the same product family. The low concentration of API (0.8% for tablet J2 and 1.3% for tablet J3) and the presence of the same excipients prevent to differentiate between the formulations.

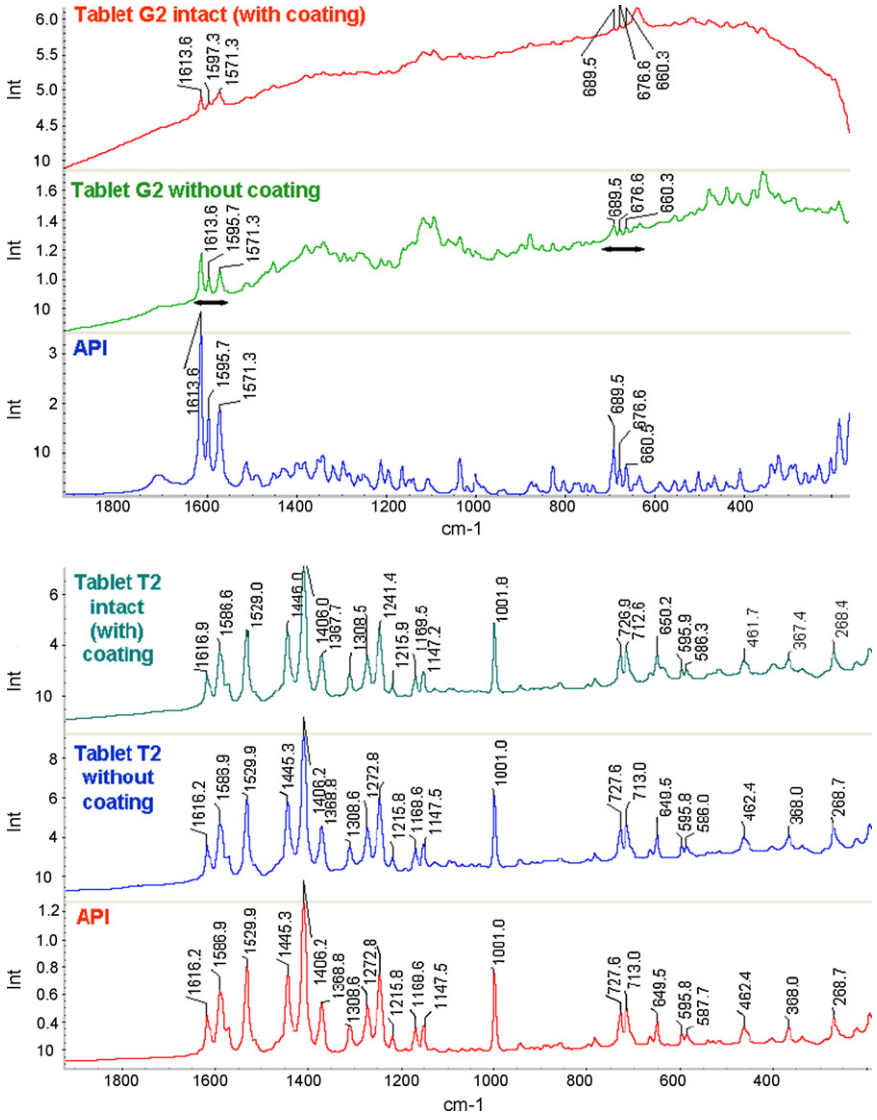

Fig. 6. Spectra of coated tablets and spectra of tablets with their coating removed together with their API. (A) The little amount of API (7% in the coated tablet) is more observable when the coating has been removed. The arrows highlight peaks that have been revealed or enhanced when removing the coating of the tablet G2. (B) No significant improvement regarding the API peaks revealing has been observed when removing the coating of the tablet T2 (32% API). Even if not necessary in all cases, it was decided to remove systematically the coating in order to standardize the analytical workflow.

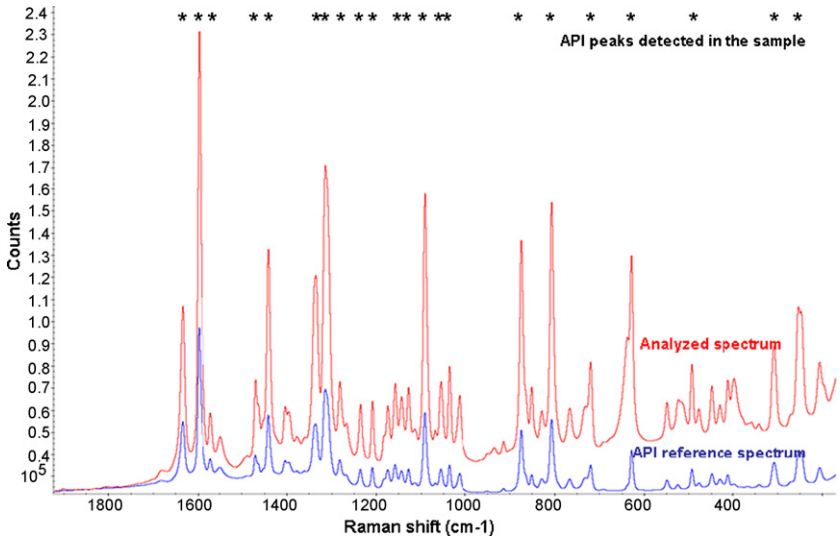

Fig. 7. Example of API peak detection. The tablet A2, part of the validation set, has been correctly identified and the following API peaks have been detected: [1634.0; 1597.0; 1570.7; 1469.6; 1440.9; 1335.2; 1313.9; 1280.4; 1235.3; 1208.6; 1174.4; 1157.9; 1143.7; 1128.1; 1091.5; 1055.8; 1036.5; 873.9; 850.7; 806.3; 766.1; 719.3; 627.7; 493.1; 448.6; 307.3; 252.9; 205.8] cm^{-1}.

The first step deals with the identification of the product family of the sample. For this purpose an SVM model was created with all the 25 product families [22]. The SVM gives a best match answer (product name) while analyzing an unknown sample.

The second step allows the determination of the formulation based on the product family best match answer. Calibrations are developed for each product family. The formulations are predicted either by SVM or by correlation. SVM models were computed separately to determine formulations for 2 product families. For 5 product families, the best correlation value obtained is retained and determines the formulation name. 13 product families contain only one formulation and consequently require neither the SVM nor the correlation.

The SVM model created for the determination of the product family is a hard classification, it implies that one class is necessarily attributed to the newly analyzed spectrum. It provides a best answer even if the analyzed tablet is not included in the calibration database spectra. Consequently, specificity criteria have to be applied to the best match. This third step consists in two successive specificity tests. The correlation value between the unknown spectrum and the database mean spectra has to be superior to 85% otherwise the analyzed sample will be automatically identified as unknown. This limit was set after testing all the calibration spectra against the method. Correlation allows the detection of outliers when an error of measurement occurs for example. If the correlation obtained with an unknown sample is above this value, a second criterion is then applied: an API peak detection (Fig. 7). API specific peak values for each tablet of the calibration have been consequently stored in the database. The algorithm compares the peaks of the unknown spectrum with the peaks of API. If one API peak cannot be detected, the tablet cannot be identified. If all API peaks are present in the tablet spectrum, the best match proposed by the SVM is accepted.

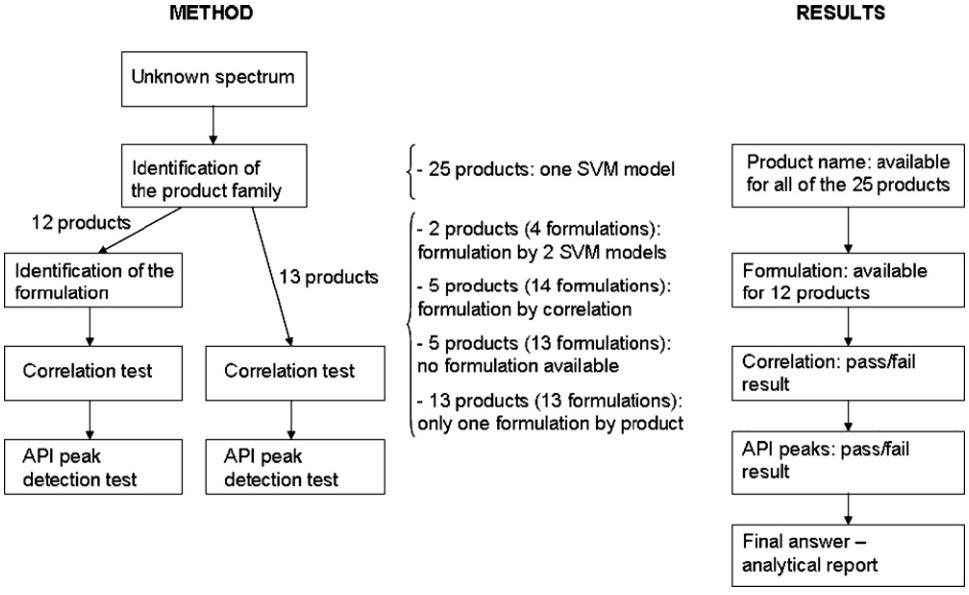

Fig. 8. Calibration method and results obtained when measuring an unknown sample. For all the products present in the library, the product family is determined. The additional information of the formulation of the sample is defined for 12 of the product families. For all the samples, a correlation test and an API peak detection are applied as acceptance criteria for the identification.

3.2.2. Calibration results

Regarding the product family identification, all the samples (i.e. the 25 product families) from the calibration sets have been correctly classified by the SVM model. 267 vectors have been used on the whole for this model, which represents between 2 and 20 vectors per class. The radial basis function (RBF) was used as a kernel function for this SVM model and the parameters were 0.002 for γ and 1 for c.

Concerning the determination of the formulation of the product, one calibration was carried out separately for the 12 product families containing more than one formulation. Among the 25 product families, 13 enclosed only one formulation, 7 contained several formulations that were identified and 5 contained formulations that were not differentiated (Fig. 8). This was due to low API contents in tablets or very close compositions within the same product families. Chemometrics consequently allowed the distinction between the different concentrations of API for 7 products out of 12, which represents approximately 60% of the products that contain more than one formulation. However, even if the formulation could not be determined, the right product family was predicted (Table 1).

One can deplore the complexity of the main SVM model that uses in this case 267 support vectors. However, it was not possible in this study to identify the products with linear classification methods.

Table 1
General results obtained with the Raman calibration. For five product families, the product family name could be identified and for the other 20 product families, the formulation name could also be determined.

Product	Formulation	Final answer by Raman spectroscopy
A	A.1	A.1
	A.2	A.2
B	B.1	B.1
C	C.1	C
	C.2	
D	D.1	D.1
E	E.1	E.1
F	F.1	F.1
	F.2	F.2
	F.3	F.3
	F.4	F.4
G	G.1	G.1
	G.2	G.2
H	H.1	H.1
I	I.1	I.1
J	J.1	J
	J.2	
	J.3	
	J.4	
K	K.1	K.1
L	L.1	L.1
M	M.1	M
	M.2	
N	N.1	N.1
O	O.1	O.1
P	P.1	P.1
	P.2	P.2
	P.3	P.3
Q	Q.1	Q.1
	Q.2	Q.2
R	R.1	R.1
S	S.1	S.1
	S.2	S.2
T	T.1	T
	T.2	
	T.3	
U	U.1	U.1
V	V.1	V.1
W	W.1	W.1
	W.2	W.2
	W.3	W.3
X	X.1	X.1
Y	Y.1	Y
	Y.2	

3.2.3. Validation of the method

The following paragraphs describe how the whole calibration strategy has been validated, including the determination of the product family and formulation names and the specificity tests of correlation and API peak detection.

3.2.3.1. Specificity. Once the calibration of the method had been created, it had to be validated. The specificity was checked by testing the calibration against each of the 396 calibration set spectra and each of the 264 spectra of an independent validation set. 100% of the calibration samples and 100% of the validation samples have been properly identified. It means that 100% of the samples have been correctly classified by the SVM models for the product family identification and have passed the correlation and API peaks test (Fig. 8). Moreover the formulation could be additionally predicted for 7 products out of 12 containing more than one formulation, i.e. for about 60% of the products with several formulations and 40% of all the calibration and validation samples.

3.2.3.2. Robustness. Different tests have been performed to validate the robustness, such as the laser power. Two initial powers of the laser have been tested: 200 and 400 mW. Both turned out to give the same results, it means 100% of the samples were correctly identified.

The effect of photobleaching was also taken into account by measuring ten times an uncoated tablet. No differences have been detected, therefore the API detection passed and the correlation value was 100% for all the measurements. The laser beam was consequently considered not to be degrading the tablets.

The focus of the laser on the sample was modified by testing two different heights of the probe of the spectrometer. Two distances between the probe and the sample have been tested: 1.5 and 9 cm, 9 cm corresponding to the maximal possible height of the probe in the chamber. These measurements revealed that this distance had no influence on the identification of the sample.

4. Conclusions

A new scheme for the identification of tablets by Raman spectroscopy has been proposed in this paper. The presented method is specific and fast regarding the measurement and the automatic generation of a result. Even close chemically related structures like benzodiazepines could be distinguished. The method is also robust and effective. This innovative calibration strategy first consists in the determination of the product family name and then of the formulation of the samples when available. The SVM method used for the determination of the product family is a hard classifier meaning that a class will be predicted even if the unknown sample is not present in the calibration library. Therefore correlation and API peak tests have been implemented as acceptance criteria of the classification. The identification is possible even when a small amount of API, until around 1% for some samples, is present in the tablet. The whole calibration strategy has been successfully validated by an independent set of data. It has therefore been demonstrated in this study and for the given samples that Raman

spectroscopy coupled with the appropriate chemometrics allows the fast and specific identification of pharmaceutical tablets. The method enables to check the identity of release samples in quality control and can moreover be applied to counterfeit detection.

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