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Short communication

Non-destructive quantitative analysis of risperidone in film-coated tablets

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Abstract

A simple, non-destructive, methodology based on FT-Raman spectroscopy was developed for the quantitative analysis of risperidone in commercially available film-coated tablets. A simple linear regression model was constructed based on standard tablets, prepared using the same manufacturing process as the commercially available. The tablets contained 0.27, 0.54, 1.08, 1.62, 2.16, 3.24 and 4.32 wt% risperidone. The most prominent Raman vibration of the active pharmaceutical ingredient at 1533 cm^{-1}, recorded using a home-made rotating system, was plotted against concentration. The model was tested on commercial film-coated tablets. The results were compared against those obtained by application of HPLC on the same samples.

Keywords: Raman spectroscopy; Risperidone; Quantitative analysis; Film-coated tablets

1. Introduction

Risperidone, a benzisoxazole derivative, is a novel antipsychotic drug that binds with high affinity to the serotonin type 2 (5-HT2), dopamine D2 and alpha1-adrenergic receptors. The chemical designation is 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a] pyrimidin-4-one. Its molecular formula is $C_{23}H_{27}FN_4O_2$ and its molecular weight is 410.49. Risperidone is also known to exhibit polymorphism.

Several methods have been developed for the determination of risperidone in bulk powder, pharmaceutical formulations and biological fluids. HPLC methods have been applied for the determination of risperidone in bulk powder [1,2], in urine [3], in plasma and serum [3–5] in postmortem fluids [6] and in pharmaceutical formulations [7–9]. Risperidone was also determined together with its major metabolite 9-hydroxy risperidone, by HPLC, in serum and plasma using different columns and different mobile phase mixtures [10–23]. Electrospray ionization tandem MS methods for the detection of risperidone were reported [24,25]. Risperidone was also determined in blood by LC-ionspray tandem-MS [26]. Risperidone and the major degradation products in bulk drug and pharmaceutical dosage forms were determined after isolation by preparative LC plasma and use of IR, MS and NMR [27]. Methods like dual-plate overpressured layer chromatography [28], capillary gas chromatography [29], negative ion chemical ionization GC–MS [30], capillary zone electrophoresis [31,32], sheathless capillary electrophoresis [33], voltammetric techniques for determination in tablets [34], HPLC and thin layer densitometric methods on powder and tablets in the presence of its degradation products [35], as well as chemiluminescence assay for pharmaceutical preparations [36] have also been published. All these techniques and methodologies are rather complicated and time consuming since dissolution of the tablet and separation by chromatography is usually required.

Recently, FT-Raman spectroscopy, FT-IR and XRD were applied for the identification of risperidone polymorphs in commercial tablets [37]. It was found that Raman spectroscopy exhibited two advantages over the other two techniques: (a) lower detection limit and (b) it was not destructive for the tableted samples.

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Raman spectroscopy has been used in the recent past in pharmaceutical analysis [38,39] mainly for identification purposes but there are also a few reports of its use for the quantitative determination of polymorphs in mixtures, e.g. Ref. [40] or active ingredients in solid formulations [41–50]. In these bibliographic reports, a plethora of methodologies have been applied including the ratio of two analytes in the same spectrum, setting the area of the spectral segment to 1, multiplicative scatter correction, standard normal variant transformation and the use of internal or external standards [40,41]. There are also reports where no intensity normalization was attempted, e.g. Refs. [47–50]. In most of these cases, a more sophisticated data handling technique, based on chemometrics such as PLS, was applied.

The reason of using rather complex quantitative methodologies lies with both the technique and the sample. The laser excites a very small portion (usually 1 mm in diameter) of a very difficult to homogenize sample. The presence of numerous excipients with very different and sometimes rather large particle sizes, e.g. 50–100 μm, complicates the problem. Since the Raman intensity is proportional to the number of molecules in the sample volume probed, the packing of the sample is an equally important factor. In addition to the above, the tablet shape, the measurement temperature and the distance between the laser and the tablet can influence the analysis. Recently, an attempt to overcome some of these problems by increasing the illumination area appeared in the literature [51]. The shortcoming of this methodology is that an external standard, in the form of a thin tablet, with non-overlapping vibrations to the sample is necessary. Due to the presence of the external standard tablet some attenuation of the Raman signal is also expected.

In this work, an attempt to establish a simple methodology for a direct, non-destructive, quantitative determination of risperidone in intact uncoated and film-coated tablets, using FT-Raman spectroscopy will be made. For reasons of comparison, the commercial tablets were also analyzed using the HPLC methodology provided by the manufacturer.

2. Experimental

2.1. Materials used

Film-coated risperidone tablets as well as placebo tablets and pure risperidone powder were kindly provided by Specifar Pharmaceuticals SA, Athens, Greece. The risperidone in all cases was of polymorph A, which is known to remain stable through time and during the manufacturing process [37]. Standard tablets were prepared as described in Section 2.2 and contained the following excipients: lactose, maize starch, microcrystalline cellulose, hypromellose 2910, magnesium stearate, colloidal anhydrous silica, sodium lauryl sulphate and propylene glycol. Each tablet weighted 185 mg. The tablets had a disk shape with 8.1 mm diameter and 3.0 mm thickness at the center of the disk. The thickness at the outer ridge of the disk was 2.6 mm. The film coating was carefully removed from the tablets, when necessary, with a scalpel.

2.2. Experimental steps to improve Raman performance

In order to circumvent the problems described earlier, two steps were taken: (a) a home-made device, capable of rotating the sample with variable speed, was constructed and placed in the acquisition compartment. The rotation speed was adjusted to 60 rpm although rotation speeds of up to 200 rpm were also tested and no influence on the Raman intensity was noticed. By rotating the sample: (i) overheating of the irradiated spot was avoided, (ii) the recorded spectrum was obtained from the perimeter of a circle, securing a better averaged Raman signal since the sampling volume was increased; (b) standard film-coated tablets containing 0.5, 1, 2, 3, 4, 6 and 8 mg of the active ingredient or 0.27, 0.54, 1.08, 1.62, 2.16, 3.24 and 4.32 wt%, respectively, were manufactured in collaboration with Specifar Pharmaceuticals SA, using the same pressure as the commercially available tablets, and the same coloring agent identical for all standards, too. Thus, it was secured that the packing as well as the attenuation of the Raman signal due the presence of film were similar for all standards and commercial tabs. The small difference in risperidone content between the minimum 0.5 mg (0.27 wt%) and the maximum 8 mg (4.32 wt%) strength tablets which is approximately 4 wt% was not expected to have any significant influence on tablet packing. Since the ingredients were provided by the manufacturer their particle size in commercial and standard tablets was the same and a possible influence in the signal collected was avoided. With these steps, effects such as inhomogeneity, packing and particle size were minimized.

2.3. Spectroscopic conditions

The Raman spectra were recorded using a FRA-106/S FT-Raman (Bruker) with the following characteristics: the laser excitation line used, was the 1064 nm of a Nd:YAG laser. A secondary filter was used to remove the Rayleigh line. The scattered light was collected at an angle of 180°. The system was equipped with a liquid N_2 cooled Ge detector (D 418). The power of the incident laser beam was set at the maximum output and yielded about 370 mW on the sample's surface. The distance of the sample from the lens was 2 cm. The sample compartment of the FT-Raman model was equipped with a motorized XYZ sample stage which permitted accurate sample positioning. Typical spectral line width was 0.5 cm^{-1} while the recorded spectra were the average of 300 scans. Typical time necessary for recording a spectrum was approximately 10 min. All Raman spectra were recorded at ambient temperature.

Spectra of the uncoated and the as-received intact film-coated tablets were recorded by placing directly into laser's beam path using the rotating home-made system described earlier.

In order to be able to compare spectra collected at different days, five spectra from one tablet, used as reference, were recorded every day. The average absolute intensity of the strong vibration at 1533 cm^{-1}, after background subtraction, was used for normalization of all intensities collected at different days. In this way, signal variations between-days were automatically

incorporated in the analytical model as well as errors introduced from the sample positioning.

2.4. HPLC instrumentation and chromatographic conditions

A Dionex Ultimate 3000 HPLC system was used for the analysis. The injection volume was 20 μl. The method was carried out on a Dionex Acclain 120 C8, 3 μm (1.5 mm × 150 mm) column. The mobile phase (flow rate 1.5 ml/min) was composed of MeOH/ammonium acetate solution 5 g/l = 80%/20%. The mobile phase was degassed by filtering through a 0.45-μm PVDF filter. The detector was operated at 260 nm. The analysis was carried out at room temperature.

2.5. Preparation of standard solution and sample for the HPLC system

The standard solution was prepared by accurately weighing 50 mg risperidone powder which was dissolved in 50 ml methanol. The solution was sonicated for 5 min and then diluted 1:10 with methanol. Then it was filtered through a 0.45-μm PVDF filter and injected to HPLC. The standard solution was injected six times and the R.S.D. of the replicates was 1.3%.

Each commercial tablet that was analyzed using HPLC was grounded and dissolved in 4 ml HCl 0.01N. The solution was sonicated for 5 min and then it was allowed to cool to room temperature. It was diluted to 20 ml with methanol and filtered through nitrocellulose paper filter. Before injection, it was filtered through a 0.45-μm PVDF syringe filter. Each sample was analyzed three times.

3. Results and discussion

3.1. Development of the Raman analytical methodology

It is well known that the intensity of a Raman line is analogous to the concentration of the analyte [52]. The Raman spectrum of a film-coated and an uncoated 4.32 wt% risperidone standard tablet along with the spectrum of the placebo and the pure active pharmaceutical ingredient (API) can be seen in Fig. 1. It is apparent that the most intense vibration at 1533 cm^{-1} of risperidone, marked with an arrow, should be used for the analysis.

Quantitative analysis was done using the absolute Raman intensity of the analyte. Usage of either peak areas or heights of the 1533 cm^{-1} vibration yielded similar results and thus, for reasons of simplicity, the proposed calibration model was based on the intensity of the peak maximum. A simple linear regression model was constructed from a series of standard tablets containing 0.27–4.32 wt% risperidone prepared in the way previously described.

3.2. Raman calibration model for film-coated risperidone tablets

The film-coated standards were prepared, as described above, using the Opandry Orange as coloring agent. Their Raman spectra were recorded and a plot of the band's intensity at 1533 cm^{-1} against the risperidone concentration yielded a straight line (see Table 1). The vibration could not be observed in the spectra obtained from the 0.27 and 0.54 wt% standards. Details, including the standard deviation around regression (Sr) and the standard deviations (Sc) in wt% obtained through the calibration line using as input the Raman signal of the standard 2 mg strength tablet can be found in Table 1. The limit-of-detection (LOD), for the applied laser power, was found to be 0.61 wt% and the respective limit-of-quantitation (LOQ) 1.85 wt% [53].

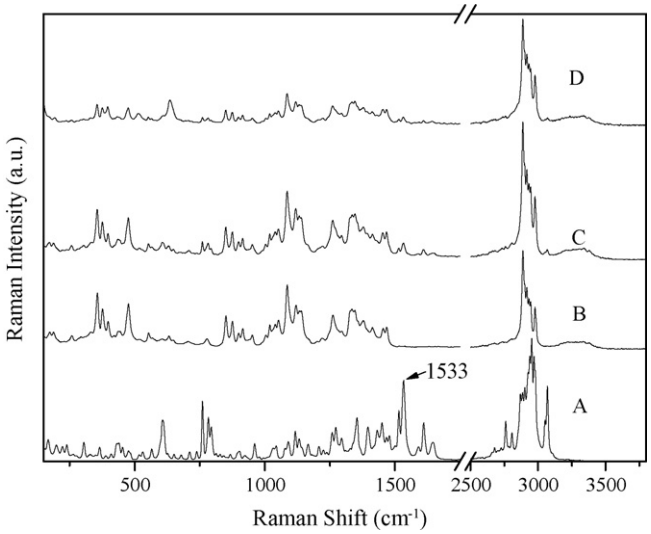

Fig. 1. Raman spectra of: (A) risperidone powder, (B) placebo tablet and (C) uncoated tablet with 4.32 wt% risperidone. (D) 4.32 wt% risperidone film-coated tablet (Opandry Orange).

In order to check the applicability of the model, three commercial tablets with 2 mg strength (1.08 wt%), T1, T2 and T3, coated with Opandry Orange, were analyzed. Tablets with this strength were chosen because they were coated with the same coloring agent as the standard tablets. Five spectra from each tablet were recorded and their average Raman intensity was introduced in the linear regression model of Table 1. The calculated concentrations can be found in Table 2. It is worth noting that the calibration model seems to work quite efficiently for

Table 1
Regression analysis data of risperidone in calibration standards curve

Parameter	Uncoated tablets	Coated tablets
Concentration range	0.54–4.32 wt%	1.08–4.32 wt%
Slope	$1.19\ (\pm 0.17) \times 10^{-3}$ [a]	$6.88\ (\pm 1.06) \times 10^{-4}$ [a]
Intercept	$-2.9\ (\pm 4.5) \times 10^{-4}$ [a]	$-3.0\ (\pm 2.9) \times 10^{-4}$ [a]
Sr [b]	1.53×10^{-4}	8.67×10^{-5}
Sc [b] (for the 1.08 wt% standard tablet)	0.10 wt%	0.10 wt%
R	$r = 0.9971$	$r = 0.9966$
LOD [c]	0.33 wt%	0.61 wt%
LOQ [c]	1.0 wt%	1.85 wt%

[a] 95% confidence limit.
[b] Standard deviation about regression (Sr) and standard deviation obtained through the calibration line (Sc) were calculated according to Ref [54].
[c] Limit-of-detection and limit-of-quantitation were calculated according to 6.3 and 7.3 of Ref [53].

Table 2
Results from the analysis of three commercial tablets (1.08 wt% risperidone strength) with Raman and HPLC

Tablet 1.08 wt%	Raman results (coated tablets)		Raman results (uncoated tablets)		HPLC results	
	Strength (wt%)	Recovery (%)	Strength (wt%)	Recovery (%)	Strength (wt%)	Recovery (%)
T1	1.07 ± 0.021	99.1	1.07 ± 0.016	99.0	1.06 ± 0.013	98.1
T2	1.06 ± 0.026	97.9	1.05 ± 0.020	97.2	1.04 ± 0.018	96.3
T3	1.07 ± 0.027	98.8	1.07 ± 0.019	97.2	1.06 ± 0.011	98.1

tablets having lower risperidone quantities than those expected based on the theoretical calculated LOQ.

At this point it should be noted that the described linear regression model is only applicable to tablets coated with Opandry Orange coloring agent. This is because the Raman intensity is attenuated due to partial absorption of the incident radiation by the film. The extent of signal attenuation depends on the color of the film. Therefore, a new calibration model should be constructed, for tablets of different coloring agent based on standard tablets covered with the specific agent.

3.3. Raman calibration model for uncoated risperidone tablets

In order to study the influence of the film on Raman intensity, construction of a calibration line in the absence of film was decided. Coating from the standard and commercially available tablets was removed with a scalpel. The plot of the risperidone's more intense band at 1533 cm^{-1} against the analyte concentration yielded, as expected, a straight line. No risperidone Raman signal could be observed in the spectrum recorded from the 0.27 wt% standard. Details, including the standard deviation around regression (Sr) and the standard deviations (Sc) in wt% obtained through the calibration line using as input the Raman signal of the standard 2 mg strength tablet are quoted in Table 1. The LOD was found to be 0.33 wt% and the respective LOQ 1.0 wt%. It should be noted that comparison between the linear regression data quoted in Table 1, shows that the slope in the case of the coated tablets was lower than the respective for the uncoated. This is reasonable as the Raman signal was attenuated due to the presence of the coloring agent (Fig. 1). Furthermore, the LOD and LOQ for the uncoated tablets were respectively lower.

The three commercial tablets with 1.08 wt% risperidone T1, T2 and T3 were also analyzed after removal of the coating. The average Raman intensity at 1533 cm^{-1} from five spectra was introduced into the derived linear regression model. The calculated concentrations can be found in Table 2.

3.4. Comparison with HPLC

The T1, T2 and T3 1.08 wt% strength tablets were grounded and analyzed using the HPLC methodology described in Section 2.5. As can be seen in Table 2, the results from both techniques studied are in sufficient agreement. Raman spectroscopy exhibits the advantage of being non-destructive for the formulation, applicable even in cases of film-coated tablets, easier, less time and solvent consuming. The drawback of the rather high detection limit can be weathered through employment of a more powerful laser.

4. Conclusions

A simple calibration model for the analysis of risperidone in pharmaceutical tablets based on the API's most intense Raman vibration gave results with adequate accuracy when it was applied on intact film-coated tablets. The necessary steps prior to the analysis included manufacturing of standard tablets at the same conditions as the commercial, application of the same coloring agent for the formation of the film, and the rotation of all samples during Raman recording in order to obtain better averaged spectra. The main disadvantage of the described method is the relatively high detection limit, stemming from the rather low laser power available.

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