Application of micro-Raman Spectroscopy for Automatic Chemical Identification and Size Distribution Measurement of API Particles in Pharmaceutical Suspensions. The case of Fluticasone Propionate in Suspension Nasal Sprays.

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Key message

Micro-Raman spectrometer and ParticleFinder™ software were employed successfully in order to chemically identify the solid particles of nasal spray samples and simultaneously measure their size distribution.

Introduction

Establishing equivalence of locally applied, locally-acting (LALA) drug products is rather challenging. Competent authorities are now pointing towards the possibility of approval of a "generic" (hybrid) application for a LALA nasal spray suspension based solely on *in vitro* data[1],[2],[3], with a battery of tests and appropriate acceptance criteria for each parameter that show equivalence between the test and reference drug product[4]. The PSD of the Active Ingredient in nasal suspension formulations has the potential to influence the rate and extent of drug availability to nasal sites of action and to systemic circulation[5]. Consequently, the goal of achieving the same particle size distribution of the active substance in the test and reference drug product with a method that is suitable to discriminate between the active substance and other solid particles in the product is a pre-requisite for establishing equivalence. Such an advanced approach is presented in this work which can be used as an alternative to Morphology Directed Raman Spectroscopy (MDRS)[6].

Experimental methods

The LabRam HR Evolution micro-Raman spectrometer (Horiba Scientific, Palaiseau, France) equipped with the specialized ParticleFinder™ software (Horiba Scientific, Palaiseau, France), was employed for the identification of the particles in nasal spray suspensions and the determination of their associated PSDs. A 532 nm laser source was used and a 50x long working distance Olympus lens was employed to view the particles.
Three azelastine hydrochloride/fluticasone propionate nasal spray formulations were tested. A "generic" nasal spray, a batch of Dymista® nasal spray within its expiration period and an already expired batch of Dymista® (10 months after its expiry date) were used.
An actuation of each nasal suspension was sprayed on a gold coated glass slide, it was left to dry so that the particles were stabilized and then a mosaic image was captured. About 1000 particles were recognized automatically in each mosaic image and their Raman spectra were recorded and automatically compared against the reference materials. The agglomerated particles, which could not be distinguished as separate particles, were excluded from the size distributions. The mean and median diameters, their standard deviations and the D10, D50 and D90 values of each cumulative size distribution were automatically exported through the ParticleFinder™ software.
The surface-weighted PSDs of raw materials used in the "generic" formulation were measured using a Mastersizer 3000 (Malvern Panalytical, Malvern, UK) laser diffraction apparatus.

Results and discussion

The Raman spectra of fluticasone propionate API, azelastine HCl API and Avicel® CL-611 (excipient) were acquired. Characteristic peaks were identified and used to distinguish fluticasone propionate from the other materials (**Figure 1A**). In all three Nasal Spray Suspensions, only particles of fluticasone propionate and Avicel CL-611 were recognized automatically by the software (**Figure 1**). The Raman chemical signature of azelastine HCl was found in some Avicel® particles suggesting a possible complex formation. No individual particles of this API were identified probably due to a superfine distribution (below the optical microscopy size limit, approximately 0.7 μm). Particles with mixed Raman spectra were excluded from the particle size distribution due to their co-crystal character.
Fluticasone propionate API (**Table 1**) and Avicel® CL-611 excipient particle size distributions were subsequently determined automatically by the ParticleFinder™ software for all three Nasal Spray Suspensions. Their distribution in the reference (Dymista®) and the "generic" nasal spray suspension are very close. However, aggregated particles of fluticasone propionate API were detected in the expired Dymista® nasal spray suspension and thus particles of larger diameter were recorded (**Figure 1B**). It is worth noting that no significant differences were observed concerning Avicel® CL-611 in the expired reference product.

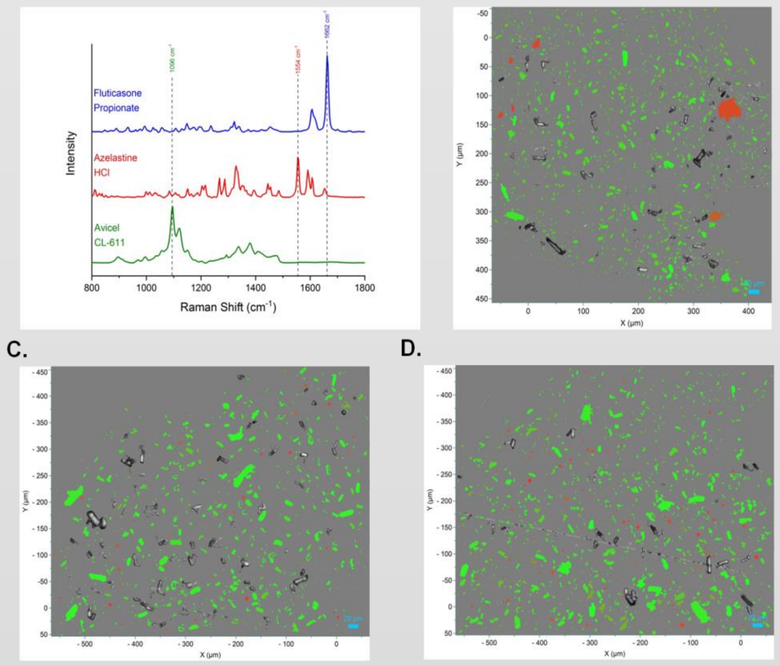

Figure 1 - A. The Raman spectra of Fluticasone Propionate API, Azelastine HCl API and Avicel® CL-611. Particles identified by the ParticleFinder™ software B. Dymista® Nasal Spray expired 10 months before analysis, C. Dymista® Nasal Spray and D. "Generic" Azelastine HCl/Fluticasone Propionate Nasal Spray (red particles: Fluticasone Propionate API, green particles: Avicel® CL-611, black: particles excluded from the analysis as aggregates)

	Expired Dymista® Nasal Spray	Dymista® Nasal Spray	"Generic" Azelastine HCl/Fluticasone Propionate Nasal Spray
Count (number)	9	42	116
Mean (μm)	11.2	4.0	3.5
Standard Deviation (μm)	10.8	1.5	1.6
Median (μm)	8.9	4.1	3.3
D_s10 (μm)	1.7	2.4	1.7
D_s50 (μm)	9.4	4.0	3.3
D_s90 (μm)	37.3	5.9	5.9
Maximum (μm)	37.3	7.3	9.0

Table 1 - Particle size surface distribution of Fluticasone Propionate API in Dymista® Nasal Spray Expired 10 months before analysis, Dymista® Nasal Spray and "Generic' Azelastine HCl/Fluticasone Propionate Nasal Spray.

Ageing of suspensions can lead to crystal growth and/or aggregation. Both changes were observed in the expired Dymista® nasal spray in which fluticasone propionate API particles were increased in size and reduced in number (**Table 1)**. The aggregated particles were not excluded from the distributions, on the contrary with the agglomerated ones. Similar PSD data for fluticasone propionate API particles were calculated in both the reference and the "generic" Azelastine HCl/fluticasone propionate nasal spray suspension that was within its expiration period.

The surface particle size distribution of the raw fluticasone propionate and Avicel® CL-611 particles that were used in the "generic" formulations were determined using: a) laser diffraction (Mastersizer 3000; Malvern, UK), b) the ParticleFinder™ software of the micro-Raman spectrometer (**Table 2**).

	Fluticasone Propionate Laser Diffraction	Avicel® CL-611 Laser Diffraction	Fluticasone Propionate ParticleFinder™	Avicel ®CL-611 ParticleFinder™
D_s10 (μm)	0.8	1.3	0.8	1.9
D_s50 (μm)	1.6	3.0	2.0	3.7
D_s90 (μm)	3.7	9.1	4.3	9.3
Maximum (μm)	6.6	29.2	6.0	32.3

Table 2 - Particle Size Surface Distributions of the Raw Fluticasone Propionate and Avicel® CL-611 measured by Micro-Raman-ParticleFinder™ and Laser Diffraction

The surface-weighted PSDs of the raw materials (**Table 2**) were found in good agreement with the Particle Size Surface Distributions of the Dymista® and "generic" formulation **(Table 1**), thus revealing that the manufacturing process does not have an impact on raw materials' particle size.

The above mentioned results show that the correlation between the proposed methodology and the well-established technique of laser diffraction for particle size distribution measurements provided consistent results. The relative standard deviations (RSD) between the Laser Diffraction and ParticleFinder™ of derived PSD values were inside the acceptance limits set by the European Pharmacopoeia. This outcome, along with the specificity of micro-Raman in chemical identification and discrimination of the APIs and excipient tested, suggest that validation of the methodology is possible for this type of analysis for the formulations tested.

Conclusion

The combination of micro-Raman spectroscopy and the ParticleFinder™ software offers a fast, reliable, automated and validatable method for identification of particles of nasal spray suspensions and simultaneous measurement of the size distribution of each solid constituent. Fluticasone propionate and Avicel® CL-611 were found to have similar particle size distributions in both reference and test formulations. However, fluticasone propionate particles in the expired nasal suspension were found to form aggregates.

References

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