Method Validation Study for the Preparation and Analysis of Ingredient-Specific Particle Sizing by Raman Chemical Imaging as it Relates to the Generic Nasal Spray Suspensions

Oksana Olkhovyk and Ryan Priore - Gateway Analytical, 5316 William Flynn Highway, Gibsonia, PA 15044

INTRODUCTION

Accurate, objective and precise drug particle size distribution (PSD) assessment is basis for safely expediting the approval of pharmaceuticals and reducing their development cost. The FDA's Critical Path Initiative has identified a specific need to determine in vitro drug PSD in generic nasal spray formulations, indicating if such information were obtained in an accurate and precise manner, in vivo biostudies would be waived. Gateway Analytical has developed a cGMP-compliant, automated method of ingredient-specific particle sizing (ISPS) measurements utilizing wide-field Raman Chemical Imaging (RCI).

The purpose of this study was to challenge and demonstrate the reliability, suitability, accuracy and precision of Ingredient-Specific Particle Sizing (ISPS) data produced by the Falcon II™ Raman Chemical Imaging System as it relates to particle sizing analysis for the nasal spray suspension. The study was aimed to address the FDA's Critical Path Opportunity for Generic manufacturers of OINDP for BE submissions, specifically for in vitro drug PSD evaluation in generic nasal spray formulations.

METHODS

The validation elements of this study were selected in order to demonstrate validity and suitability of the Falcon II for the intended purpose (ISPS).

This validation study was divided into three (3) parts:

- **PART 1- Validation/verification of the performance of the Falcon II**
  The Falcon II System was evaluated and challenged for Raman dispersive spectroscopy, Raman LCTF imaging and particle sizing capability based upon suitability, accuracy, precision, detection limit and linearity criteria.
- **PART 2- Validation of the sample preparation and ISPS analysis procedures of the nasal spray suspension manufactured by generic company**
  Procedures for the preparation and Raman chemical imaging analysis of the generic nasal spray suspension was challenged and evaluated based upon suitability, accuracy, precision and specificity of the method.
- **PART 3- Comparison of the nasal spray suspension manufactured by generic company with the innovator product.**
  A comparison of the PSD based on equivalent circle diameter was compared at the D10, D50 and D90 values for the generic and innovator nasal spray suspension products.

Validation Elements selected for this study included:

- **System Suitability**- Expressed the instrument's suitability for use.
- **Precision**- Expressed the closeness of agreement between a series of measurements obtained from repeat sampling or samples under prescribed conditions. Particle sizing and Raman dispersive spectroscopy/LCTF imaging precision was challenged in three (3) ways:

1. *Instrument Precision*- expressed precision of consecutively repeated measurements under the same operating conditions on the same instrument.
2. *Day-to-Day Precision*- expressed precision of repeated measurements on the same instrument over a period of days.
3. *Analyst-to-Analyst*- expressed precision between scientists and/or analytical techniques.

- **Accuracy**- Expressed the closeness of a test result obtained to the true value. Accuracy of particle sizing and LCTF Raman was performed using certified PSMS standards.
- **Specificity**- Expressed the ability of the analytical method to unequivocally detect and analyze the compound(s) of interest in the presence of other product components without interference.
- **Linearity of Sizing**- Expressed the ability to generate proportional sizing data over a selected range.
- **Sizing Detection Limit**- Expressed the lowest particle size that could be detected.

RESULTS

PART 1 – Evaluation of Falcon II RCI System

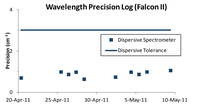
System Suitability Performance Verification (Wavelength Accuracy and Intensity Validation)

A performance verification (PV) was performed daily prior to data collection to verify and document that the Falcon II system was functioning according to specifications based on wavelength accuracy and signal throughput. Wavelength accuracy was measured using a validated Raman wavelength standard, acetaminophen (APAP). Signal throughput was measured on a silicon wafer at 520 and 580 cm^{-1}, for signal and background intensity values, respectively.

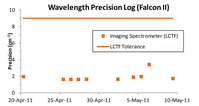
Wavelength precision was measured for both dispersive and imaging spectrometers using a certified USP APAP wavelength standard. Twelve (12) Raman peaks for the precision study were chosen to span the entire Raman spectrum. The peak positions were calculated using a center-of-mass equation:

$$\lambda_{COM} = \frac{\sum_{i}^{N} I_i \times \lambda_i}{\sum_{i}^{N} I_i}$$

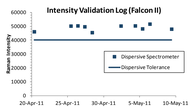
Where λ_{COM} is the wavenumber calculated, λ is the wavenumber(s) around the peak, I is the measured intensity, and i is the index of spectral position from 1 to N points which encompass the peak.



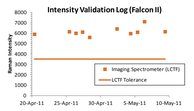
AC:	RMSE of twelve (12) selected APAP peaks must be ≤ to 3.0 cm^{-1}.
Result:	Pass
Deviations:	N/A



AC:	RMSE of twelve (12) selected APAP peaks must be ≤ to 9.0 cm^{-1}.
Result:	Pass
Deviations:	N/A



AC:	Signal minus baseline must be ≥ to 40,000
Result:	Pass
Deviations:	N/A



AC:	Signal minus baseline must be ≥ to 3,500
Result:	Pass
Deviations:	N/A

Analyst-to-Analyst Accuracy & Precision (Dispersive & LCTF Spectrometers)

Ten (10) consecutive Raman LCTF spectra of APAP were collected to determine the instrument precision. Ten (10) consecutive Raman dispersive spectra of APAP were collected over three (3) days to determine the day-to-day precision. Ten (10) consecutive Raman dispersive spectra of APAP were collected by a 2nd analyst to determine the analyst-to-analyst precision.

Dispersive				LCTF			
Wavenumber	Average- Analyst #1	Average- Analyst #2	Cumulative Std. Dev. (cm^{-1})	Wavenumber	Average- Analyst #1	Average- Analyst #2	Cumulative Std. Dev. (cm^{-1})
390.9	397.26	394.14	0.8	390.9	392.99	392.75	0.5
797.2	798.53	797.57	0.8	797.2	798.01	796.64	0.4
1168.5	1167.9	1168.02	0.9	1168.5	1167.14	1167	0.2
1278.5	1278.89	1278.78	0.4	1278.5	1279.4	1278.14	0.2
1323.9	1325.12	1325.4	0.8	1323.9	1325.38	1324.47	0.2
1561.6	1561.19	1561.56	0.6	1561.6	1561.03	1561.31	0.2
1648.4	1650.48	1651.01	0.6	1648.4	1652.28	1651.94	0.3
2931.1	2930.75	2930.24	0.6	2931.1	2929.34	2929.28	0.2
3064.6	3063.85	3062.7	0.4	3064.6	3063.53	3063.4	0.2
3102.4	3103.22	3102.54	0.2	3102.4	3103.78	3103.65	0.2
3326.6	3327.18	3327.32	0.4	3326.6	3327.31	3326.9	0.5

AC:	The cumulative standard deviation of the collected spectra must be < 1 cm^{-1}
Result:	Pass
Deviations:	N/A

AC:	Root Mean Squared Error (RMSE) for the selected APAP peaks must be ≤ 3 cm^{-1} and ≤ 9 cm^{-1} for the dispersive and imaging (LCTF) spectrometers, respectively.
Result:	Dispersive: Pass LCTF: Pass
Deviations:	N/A

Particle Sizing Limit of Detection and Linearity

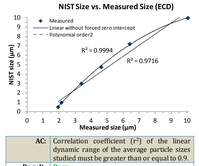
NIST-traceable PSMS of various sizes spanning the size range of API in the final nasal spray formulation were measured. Bias parameters were calculated as difference between stated by Vendor PSMS size and Measured Average PSMS size. The specific sizes measured were 1, 5 and 10 μm.

Limit of Detection

NIST Mean (µm)	NIST Std. Dev. (µm)	# Particles	Mean (µm)	Std. Dev. (µm)	RSD	Bias (µm)
0.50	0.01	136	1.93	0.24	12%	1.43
0.99	0.03	606	2.14	0.15	7%	1.15
3.00	0.07	566	3.39	0.32	9%	0.39
4.76	0.20	582	4.62	0.41	9%	-0.14
7.22	0.26	284	6.48	0.55	9%	-0.82
9.98	0.41	403	10.04	0.76	8%	0.06

AC:	Limit of detection must be ≤ 1 µm
Result:	Pass
Deviations:	N/A

Linearity



AC:	Correlation coefficient (r^2) of the linear dynamic range of the average particle sizes studied must be greater than or equal to 0.9.
Result:	Pass
Deviations:	N/A

PART 2 – RCI Analysis of Generic Nasal Spray

Nasal Spray Suspension Sample Preparation Precision

Precision of the automated actuator was determined gravimetrically using a calibrated analytical balance. Two (2) different sample numbers (i.e. lot numbers) were used for this study.

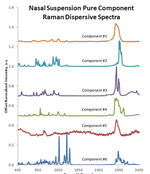
	Sample #1		Sample #2	
Measurement #	Bottle Mass (mg)	Spray Mass (mg)	Bottle Mass (mg)	Spray Mass (mg)
Initial	57027.6	0	42739.2	0
1	56975.4	52.2	42686.9	52.3
2	56923.6	51.8	42634.0	52.9
3	56869.6	54.0	42578.5	55.5
4	56815.0	54.6	42526.1	52.4
5	56759.9	55.1	42473.6	52.5
6	56705.7	54.2	42421.0	52.6
7	56651.1	54.6	42368.9	52.1
8	56596.9	54.2	42316.6	52.3
9	56541.4	55.5	42264.2	52.4
10	56487.7	53.7	42212.5	51.7
Average (mg)	---	54.0	---	52.7
Std. Dev. (mg)	---	1.2	---	1.0
% RSD	---	2.2%	---	2.0%

AC:	%RSD of the gravimetric (no fewer than 3) mass results must be ≤ 10%
Result:	Pass
Deviations:	The sample container was weighed initially and then after each actuation in order to calculate actuated mass. The VP plan indicated that the sample would be actuated onto a pre-weighed microscope slide, which was determined to be a less accurate method.

Method Development for Nasal Spray Suspension

Pure Component Spectral Library

Raman dispersive spectra were collected in order to determine the optimal region for ISPS. No fewer than ten (10) repeat measurements of no fewer than three (3) fields of view were measured for each pure component sample. Final spectral range evaluated was 450 – 3450 cm^{-1}.



RCI Analysis of Generic Nasal Spray

Analyst-to-Analyst API Particle Sizing Precision

Ten (10) consecutive RCI measurements of a single sample preparation of three (3) particles were collected and tabulated. Ten (10) consecutive RCI measurements of a single sample preparation of three (3) particles were collected and tabulated over the course of three (3) days. Ten (10) consecutive Raman images for each of the three (3) PSMS sizes were collected by a second analyst to determine the analyst-to-analyst precision.

10 particles				≥100 particles			
NIST Mean (µm)	Average- Analyst #1	Average- Analyst #2	Cumulative %RSD	NIST Mean (µm)	Average- Analyst #1	Average- Analyst #2	Cumulative %RSD
0.99	2.14	2.01	11%	0.99	2.15	2.11	14%
4.76	4.34	4.54	7%	4.76	4.61	4.68	18%
9.98	9.74	9.91	5%	9.98	10.16	9.69	14%

AC:	The cumulative percent relative standard deviation (%RSD) of the measurements for all sizes must be ≤ 20%.
Result:	Pass
Deviations:	Laser power was 20 mW instead of 150 mW. Exposure time was 10 seconds instead of 2 seconds. EMCCD readout averages were set to 1 frame instead of 3 frames.

PART 3 – Comparison of Innovator & Generic Nasal Spray Suspensions

Samples were prepared and analyzed as received using an automated actuator. Data collection was conducted on approximately 1,000 FOVs (1.7mm^2 area sampled). A two-sample Kolmogorov-Smirnov statistical test was utilized to determine whether the API particles of the generic suspension are equivalent to the innovator suspension. Based on the results of the population study (data not shown), no fewer than 750 particles were retained for statistical analysis from each sample.

	Innovator	Generic	Relative % Difference
D10 (µm)	2.1	2.0	4.8%
D50 (µm)	2.9	2.9	0.0%
D90 (µm)	4.9	5.0	-2.0%
Average. (µm)	3.3	3.2	3.0%
Std. Dev. (µm)	1.3	1.3	0.0%

CONCLUSION

Based on the results of this study, it was demonstrated that Raman Chemical Imaging for ISPS offers an objective evaluation of API particle size in complex matrices like nasal spray suspensions to support the BE submissions, and in vivo biostudies waiver. Direct comparison of the drug PSD in the generic and innovator product was obtained with sufficient accuracy and precision and evaluated for equivalence based on statistical methods.

The following was demonstrated:

- Suitability and reliability of the Falcon II Raman Chemical Imaging System
- Ability to accurately and precisely prepare and analyze the Generic Nasal Spray Suspension for Ingredient Specific Particle Sizing (ISPS)
- Based on results of the K-S test, the ISPS of three (3) Generic Nasal Spray Suspensions were statistically similar to three (3) samples of the Innovator product

Performed in a GMP environment by skilled analysts who develop validated methods for each individual formulation, the RCI technique has the potential to address the FDA's Critical Path Opportunity (CPO) for direct measurement of particle size equivalence in nasal spray suspensions, possibly allowing for a waiver of in vivo biostudies and savings of millions of dollars in development costs and up to half of the time required for clinical studies.