Raman spectroscopy for the process analysis of the manufacturing of a suspension metered dose inhaler

James Butz, Luis de la Cruz, Jason DiTonno, Kevin DeBoyace,
Gary Ewing, Brent Donovan, Joseph Medendorp*

Merck Respiratory Product Development, 556 Morris Avenue, S7-A2-2123, Summit, NJ 07901, United States

ABSTRACT

The purpose of this research was to demonstrate the utility of Raman spectroscopy for process analysis of a suspension metered dose inhaler manufacturing process. Chemometric models were constructed for the quantification of ethanol and active pharmaceutical ingredient such that both could be monitored in real-time during the compounding and filling operations via tank measurements and recirculation line flow-cell measurements. Different spectral preprocessing techniques were used to delineate the effects of mixing speed and temperature changes from actual concentration effects. Raman spectroscopy offers advantages in time savings and quality of information over the standard methods of analysis for respiratory formulations, such as a drug content assay via HPLC and ethanol testing via GC. The successful implementation of this work will allow formulation scientists to quantitatively assess both the formulation (e.g., the concentration of active pharmaceutical ingredient (API) and ethanol), as well as the manufacturing process (e.g., determination of mixing endpoints) in real-time.

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1. Introduction

Current pressurized metered dose inhalers (pMDIs) consist minimally of a hydrofluoralkane (HFA) propellant and an active pharmaceutical ingredient (API). In many cases, co-solvents and/or surfactants are necessary to give the pMDI adequate and sustainable performance characteristics. Since the API frequently is present as suspended micronized particles, an inhomogeneous suspension during manufacturing can lead to inconsistency during product filling, thus causing inconsistencies in the API content in individual pMDI units. A good pMDI suspension is one in which the API remains suspended in the propellant for as long as possible without sedimenting, creaming, or aggregating. As such, most suspension formulations require constant agitation to maintain the API in suspension during both compounding and filling operations. It is not inherently difficult to devise a formulation that remains suspended, but the addition of excipients to the base formulation often leads to other undesired effects, such as increased solubility and/or reduced product stability. For example, while many excipients are insoluble in HFA, ethanol (EtOH) has been explored as a co-solvent to improve the suspension characteristics of pMDIs. Typically this increases the API solubility in the formulation, creating the potential for particle growth and particle degradation over time. As a result, it is necessary that drug makers understand and quantitatively control the co-solvent concentration and API suspension characteristics of their formulation.

A typical pMDI 1-step filling manufacturing process consists of initially dispersing the API in the bulk formulation to make a homogeneous suspension. Once the compounding process is completed, the formulation is recirculated through the filling equipment in order to fill individual pMDI units. Drug particles in the bulk formulation are kept in suspension by mixing the bulk formulation in the compounding tank and recirculation through the filling equipment. In propellant based systems, the tank is typically maintained at a constant temperature to ensure a consistent fill weight during filling operations. Changes to manufacturing parameters such as mixing speed, homogenization time and speed, and product temperature can greatly affect the suspension characteristics of the resulting batch.

During development work, formulators first rely on a qualitative visual inspection of suspension quality by filling experimental formulations into transparent glass bottles. Separation ratios may also be calculated from the suspension height at t_0 (where t_0 is time immediately after shaking) compared to suspension height at some time in the future, t_x [1]. Using these as the basis for evaluation, one can assess the time required for the suspension to settle or cream, thus the duration that the pMDI is dispersed and appropriate for dosing. After the initial visual screening techniques, formulators rely on much more sophisticated, but highly labor intensive

analytical methods, such as cascade impaction and through-life dose content uniformity to assess product performance [2]. Historically, the standard analytical method for measuring drug content in respiratory drug products has been a long and tedious process, consisting of many demanding analytical steps in terms of both labor and instrument hours.

In contrast to the standard respiratory analytical techniques, online Raman provides a wealth of information beyond the glass bottle visualization technique and arduous HPLC drug content methods. With rapid sample analysis times and its nondestructive nature, the pMDI formulation can be inspected in real-time during filling operations rather than end-point batch testing a representative fraction of units. With this in mind, the primary purposes of this research were (1) to demonstrate the ability to monitor the concentrations of EtOH and API in both the compounding tank and the filling lines using Raman spectroscopy, and (2) to use this information to investigate the process effects on product quality throughout the manufacturing process. In terms of process effects on product quality, it is important to demonstrate: (1) that the bulk formulation was sufficiently blended during compounding to form a homogeneous product; and (2) that the formulation was adequately mixed during filling operations to remain properly suspended. Collectively, this information can be used to confirm that individual MDI units are filled in a uniform manner. Successful implementation of this research will set the stage for the pharmaceutical scientist to answer fundamental questions about pMDI manufacturing processes and suspension characteristics of new formulations.

Raman spectroscopy has received much attention as a viable approach to process analytical technology (PAT). It is a noninvasive, nondestructive, and rapid method of analysis, capable of identifying and quantifying APIs and excipients in pharmaceutical formulations, even at relatively low concentrations [3]. Raman fiber probes are readily available and adaptable to many manufacturing processes, from lab scale through commercial scale manufacturing. For example, Raman has been used to monitor the solid state conversion between anhydrous and monohydrate API forms during high-shear wet granulation [4]. It has been used to monitor the freeze drying process in real time, confirming the onset of nucleation and crystallization and the release of hydrate water during storage [5]. Raman has been used to measure API homogeneity during a powder blending process [6], and to measure tablet active coating thickness and uniformity [7]. It has also received some attention for its ability to monitor pharmaceutical suspensions, such as gels and emulsions [8], homogenization of aqueous suspensions [9], and deposition characteristics of pMDIs [10]. The research presented here provides an extension to current scientific scholarship by applying dispersive Raman spectroscopy to the simultaneous quantification of API and co-solvent in a pressurized compounding tank and the loop of a recirculation line used during filling operations. This capability allows for the quantitative assessment of mixing speed changes and flow stoppages throughout the manufacturing process, providing insight into the final drug product otherwise unavailable without extensive offline testing. Implementation of this work is consistent with the goal of PAT, setting the stage for risk minimization through process understanding.

2. Materials and methods

2.1. Materials

To develop the Raman analytical method, a model MDI suspension product comprises an HFA-based propellant, the co-solvent EtOH and micronized API was studied. The role of the ingredients is as follows. The HFA propellant is a compressed liquid, which boils

at ambient conditions to form the resulting aerosol. The presence of EtOH improves the suspension characteristics of the formulation. Finally, the API is milled to a narrow particle size suitable for inhalation, such as 1–5 μm. The manufacturing process for this particular formulation, which is described elsewhere, had been previously optimized to prevent particle growth [11].

Two batch schemes were used for the construction of the chemometric calibration models for API and for EtOH. The first scheme for EtOH was a wide-range calibration designed to hold API constant while varying EtOH from 25 to 550% of the target concentration, and a wide-range calibration designed to hold EtOH constant at the target concentration while varying API at five different levels from 0 to 400% of the target concentration. This design was to establish the proof-of-concept and an acceptable model for concentration predictions beyond the practical range of interest. Even across this wide range, univariate models were adequate for the prediction of both API and EtOH, demonstrating the strength of Raman for this application.

The second scheme was to vary both API and EtOH simultaneously around a narrower range for ethanol (70–130% EtOH, 50–450% API) to confirm linearity around the concentrations relevant to multiple drug product strengths. To accomplish this goal five different batches were made, each with a fixed concentration of API (50%, 150%, 250%, 350% or 450% LC) and with a varying concentration of EtOH (70%, 85%, 100%, 115% and 130%). This set the stage for a much more robust and predictive calibration model around the target API and co-solvent concentrations. Note that the API concentrations bracket potential product strengths of interest, therefore, the API range was kept similar for both batch schemes. However, the wide-range EtOH calibration was extended far beyond the 70–130% target to investigate potential matrix effects at extreme concentrations.

2.2. Instrumentation and integration parameters

Table 1 shows the Raman instrument and collection parameters used for the flow-cell and compounding tank measurements. Please refer to Fig. 1 for the T-configuration flow-cell. A flow cell was placed in the recirculation line such that the drug product passed from the metering chamber through the cell to the fill head, making it possible to monitor the contents of units as they were filled. The cell was designed with a 6 in. immersion optic fiber probe plumbed into the line perpendicular to the direction of flow. The probe was configured as a short focus probe; therefore, the focal point was just outside of the sapphire window at the tip. In this configuration, as long as material continued to flow freely past the window, the depth of penetration into the T-cell had no impact on the measurement.

A series of experiments (shown in Table 2) were conducted with the low-strength and high-strength products to determine the universal optimum integration parameters to obtain an acceptable signal-to-noise ratio over the full range of concentrations. As a result of these experiments, the Raman instrument was set to collect 10 accumulations at 5 s exposures, resulting in a 50 s total integration time (1 scan = 10 accumulations × 5 s). Based on

Table 1
Instrument details and collection parameters.

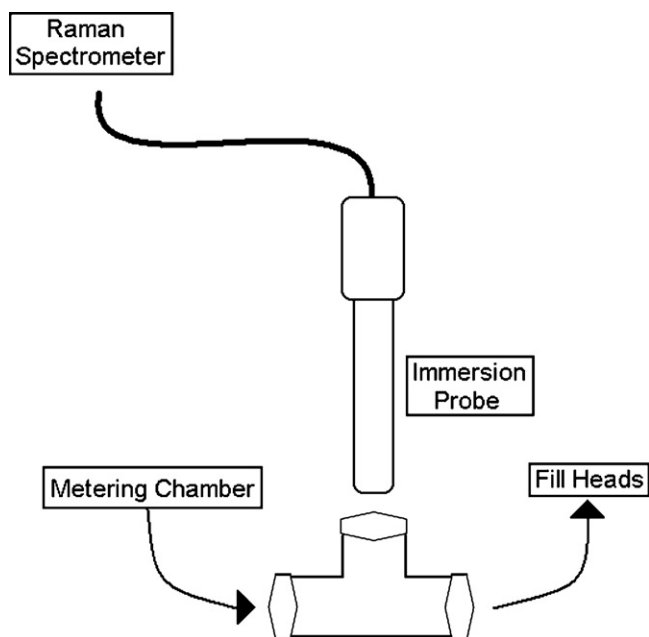|	
Instrument	Raman RXN2, 785 nm laser
Channels	4 channels
Probes	6 in. immersible length (flow cell)
	24 in. immersible length (tank)
Exposure time	5 s
Number of scans	10 accumulations
Laser power	400 mW
Operating range	100–3425 cm^{-1}

Fig. 1. In-line T-configuration flow cell used for monitoring ethanol and API in real-time.

the speed of the filling mechanism, each Raman scan theoretically corresponded to the filling of approximately 25 pMDI units.

3. Chemometric models for API and ethanol

3.1. Preprocessing and chemometric model for ethanol wide-range univariate calibration

Preprocessing and wavelength ratio for ethanol wide-range univariate calibration are given in Table 3.

3.2. Preprocessing and chemometric model for ethanol narrow-range multivariate calibration

Preprocessing and PLS model for ethanol narrow-range multivariate calibration are given in Table 4.

3.3. Preprocessing and chemometric model for API univariate calibration

Preprocessing and wavelength ratio for API univariate calibration are given in Table 5.

3.4. Preprocessing and chemometric model for API multivariate calibration

Preprocessing and PLS model for API multivariate calibration are given in Table 6.

3.5. Qualitative homogeneity

To assess the qualitative effects of EtOH addition, API addition, low-speed mixer speed changes, and filling stoppages on suspension homogeneity, principal component scores were calculated from the raw spectra and displayed against time. This made it possible to determine the exact points of steady state after manufacturing changes, such as mixing speed, addition of API and/or EtOH, the start of recirculation, etc.

4. Results and discussion

Careful examination of the Raman spectra from the compounding tank provided two initial observations: (1) Raman spectral intensity increased with an increase in mixing speed, and (2) spectral intensity decreased with a decrease in temperature. Recall that one of the aims of this work was to study batch homogeneity in the compounding tank and in the flow-cell. Fundamental questions about the manufacturing process needed to be addressed in order to delineate the effects of mixing versus actual concentration effects.

For example, if the suspension was prepared at 100% LC and was homogenously dispersed at 400 rpm, the integrated Raman signal would correctly read at 100% LC. Clearly, if the same suspension was non-homogeneously dispersed or if the flocculation (particle aggregation) size is altered, the Raman would read pockets of higher and lower concentrations and a principal component plot would exhibit more variability than one with a homogeneously distributed material at steady state. However, depending upon the chemometric model selected, the same suspension homogeneously dispersed at 200 rpm could be incorrectly measured at 50% LC. The adopted chemometric model needed to be robust enough to differentiate between mixing effects and concentration effects, even in the presence of an increased number of scattering events and the corresponding effect on Raman intensity.

During development of this analytical method, a number of preprocessing techniques were tested, including normalization to unit area, multiplicative scatter correction, Savitzky-Golay second derivative smoothing, and mean centering. To eliminate the effects of mixing speeds for API quantification, normalization and second derivatives were the most effective for the wavelength ratio regression and second derivatives and mean centering were the most effective for PLS modelling. Second derivatives eliminated the

Table 2
Exposure times and accumulations tested for signal-to-noise optimization.

Strength (%)	Interval (s)	Accumulations (#)	Exposure (s)	S/N
	15	5	2	1.65
	45	15	2	3.25
	60	25	2	3.97
50	60	10	5	3.77
	10	1	5	0.86
	15	2	5	1.47
	60	3	15	3.08
	15	3	2	5.01
	30	10	2	8.67
	15	5	2	6.67
400	60	25	2	13.16
	60	10	5	18.46
	15	2	5	7.25
	60	3	15	18.13

Table 3
Preprocessing and wavelength ratio for ethanol wide-range univariate calibration.

Preprocessing	Second derivative	Savitzky Golay, 15-point window for spectral data
	Wavelength selection	883 cm^{-1}, 919 cm^{-1}
Cross validation	Leave-one-out	–
	Factors included	Not applicable (N/A), wavelength ratio used
Univariate model	Calibration set	5 batches, 25–550% LC
	Validation set	N/A – cross validated only
		r^2: 0.998
Performance statistics	Model performance	RMSEC: 0.016
		RMSECV: 0.016

Table 4
Preprocessing and PLS model for ethanol narrow-range multivariate calibration.

	Normalization	1-Norm (area = 1)
Preprocessing	Mean centering	Spectral data and concentration vector
	Wavelength selection	490–1800 cm^{-1}
Cross validation	Random subsets	10 data splits, 20 iterations
	Factors included	3 latent variables
PLS model	Calibration set	5 batches, 70–130%
	Validation set	1 batch
		RMSEC: 0.054
Performance statistics	Model performance	RMSECV: 0.055
		RMSEP: 0.034

Table 5
Preprocessing and wavelength ratio for API univariate calibration.

	Normalization	1-Norm (area = 1)
Preprocessing	Second derivative	Savitzky Golay, 15-point window for spectral data
	Wavelength selection	σ_1 (cm^{-1}), σ_2 (cm^{-1})[a]
Cross validation	Leave-one-out	–
	Factors included	NA, wavelength ratio used
Univariate model	Calibration set	5 batches, 0–400% LC
	Validation set	NA – cross validated only
		r^2: 0.997
Performance statistics	Model performance	RMSEC: 0.018
		RMSECV: 0.020

[a] Information blinded for proprietary reasons.

baseline offset due to mixing speed changes, and normalization eliminated any spectral intensity differences due to temperature variations. For PLS modelling, mean centering was also used to correct for any changes in sampling or scattering efficiency, resulting in stronger calibration and validation statistics. EtOH however, could be adequately quantified using any of these preprocessing methods because EtOH was fully miscible at the given concentrations. As is consistent with the literature, temperature also appeared to be a factor in the Raman spectra [12,13]. Specifically, for this formulation and process when the bulk formulation was cooled from ambient temperature to −15 °C, the signal intensity of the main features decreased. These changes in spectral features are attributed directly to changes in formulation temperature since the formulation was insulated from the effects of environmental fluctuations through various means (e.g., the tank was sealed and pressurized and the formulation temperature was tightly controlled using an external chiller).

4.1. Ethanol quantification

Quantification of EtOH and API is essential to demonstrate the correct amounts are dispensed into the compounding tank when generating the bulk formulation and into individual units during filling operations. The exact target concentrations of EtOH and API are not imperative in principle for this manuscript; however, it should suffice to mention that both are typical of respiratory products. As such, the integration parameters were adjusted such that the quantification limits were necessarily below 1.0% (w/w) of the total formulation. The wide-range univariate EtOH calibration was based on the use of wavenumber ratios and a linear regression as listed in Table 3. Fig. 2 illustrates the wavenumbers most highly correlated to the presence of EtOH (CC stretch at 883 cm^{-1}, and CH$_2$ and CH$_3$ stretch at 2878–2972 cm^{-1}, CH$_3$ bending at 1454 and 1479 cm^{-1}) [14]. The surface plot in Fig. 3 illustrates a clear increase in Raman signal in response to an increase in EtOH concentration. The feature at 883 cm^{-1} was chosen as the primary peak
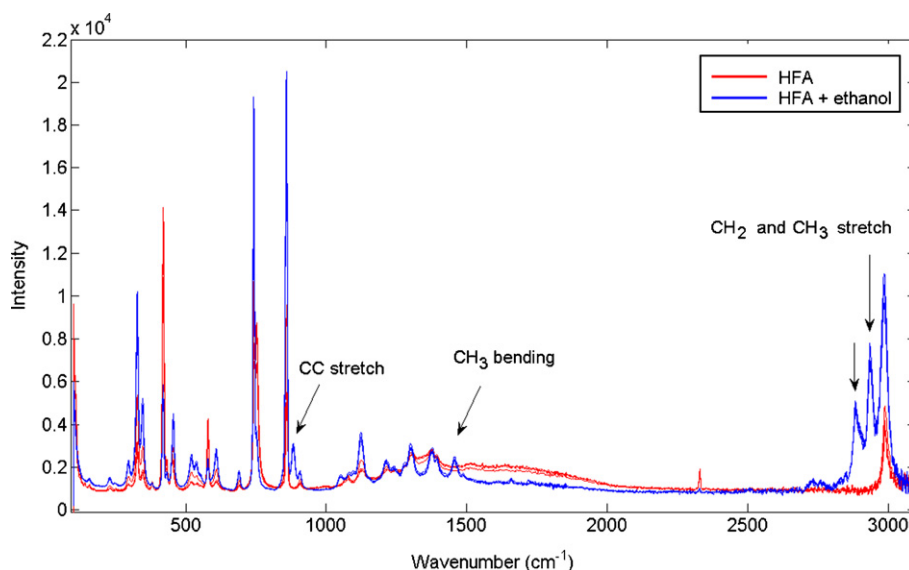
Table 6
Preprocessing and PLS model for API multivariate calibration.

	Second derivative	Savitzky Golay, 15-point window for spectral data
Preprocessing	Mean centering	Spectral data and concentration vector
	Wavelength selection	1500–1800 cm^{-1}
Cross validation	Random subsets	10 data splits, 20 iterations
	Factors included	2 latent variables
PLS model	Calibration set	5 batches, 50–450% LC
	Validation set	1 batch
		RMSEC: 0.139
Performance statistics	Model performance	RMSECV: 0.144
		RMSEP: 0.133

Fig. 2. Regions of interest for the quantification of ethanol. These regions are sufficiently distinct from the propellant and API.
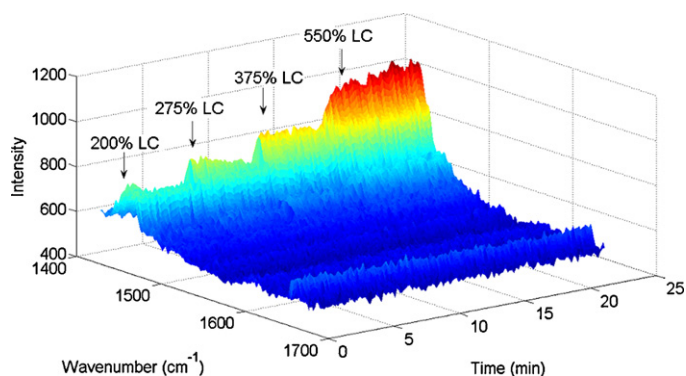

Fig. 3. Representative surface plot demonstrating the linear increase in peak height as ethanol concentration increases.

Fig. 5. Predicted values project on top of the formulated values for the quantification of EtOH collected in the flow cell in real-time.

of interest because there was no competing contribution from the propellant or from the API, and 919 cm^{-1} was chosen as a reference peak because it was constant even as EtOH and API concentrations were varied.

Fig. 4 shows the calibration line for EtOH and Fig. 5 shows the predictions from an actual batch. To generate the EtOH calibration curve, EtOH was added to the compounding tank in bolus shots to pre-mixed formulations consisting of API and propellant. The
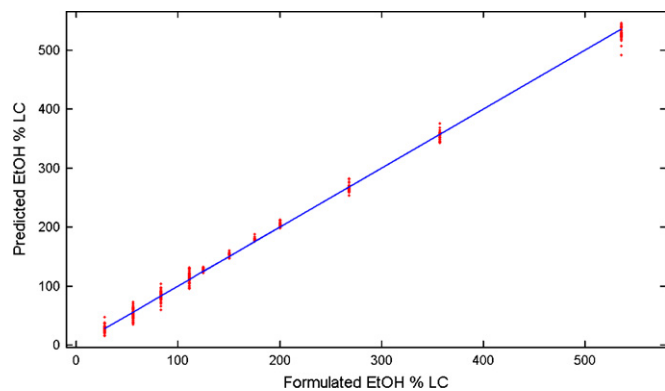

Fig. 4. EtOH univariate linear calibration line, formulated values versus Raman predicted values.
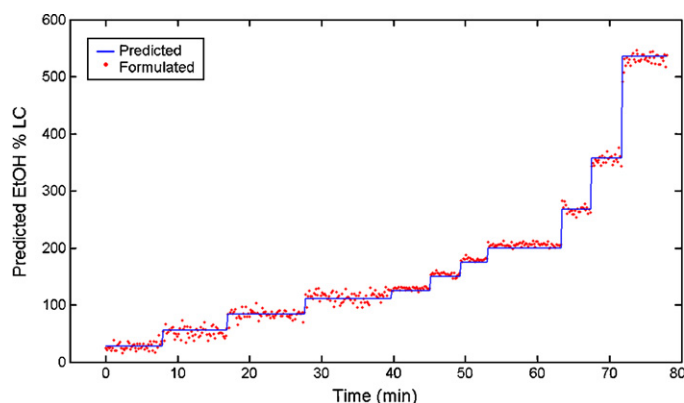
predicted values project on top of the formulated values, demonstrating the high degree of predictive ability with the selected chemometric model. The calibration model used for the wide-range univariate quantification of EtOH spanned the range from 25 to 550% LC, and resulted in a root mean squared error of calibration (RMSEC) of 0.016, root mean squared error of cross validation (RMSECV) of 0.016, and r^2 of 0.998.

The multivariate calibration for the narrow-range of ethanol levels (70–130% target concentration) was based on the use of a three latent variable partial least-squares model as described in Table 4. The performance statistics for this model were RMSEC = 0.054, RMSECV = 0.055, and RMSEP = 0.034. As described in Section 2, this model was calibrated on five independent batches.

4.2. API quantification

The spectra in Fig. 6 show the Raman features most strongly correlated to the presence of API. For the quantification of API, the univariate calibration model resulted in an RMSEC = 0.018, RMSECV = 0.020, and $r^2 = 0.997$, which spanned the concentration range from 0 to 400% LC. Different preprocessing techniques were examined for the purposes of API quantification, and normalization and second derivatives were determined to be the most effective, as shown in Table 5. Multiplicative scatter correction and
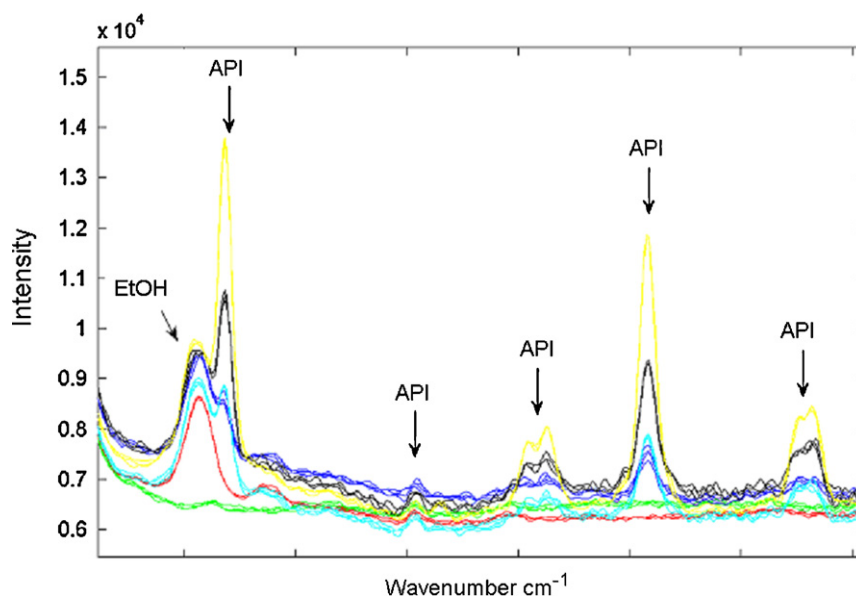
Fig. 6. Raman spectra of the drug product in regions specific for API and EtOH.

other baseline correction methods were also effective for the calibration set, but did not hold up as well on independent model validation.

The multivariate API calibration (50–450% target concentration) was based on the use of a two latent variable partial least-squares model as described in Table 6. The performance statistics for this model were RMSEC = 0.139, RMSECV = 0.144, and RMSEP = 0.133. This model was calibrated on five independent batches, each with different concentrations of API and all five different levels of EtOH (70–130% target concentration).

4.3. Effects of mixing speed and filling stoppages on the bulk formulation

During filling operations for suspension MDI products, the bulk formulation must be continually mixed to maintain a homogeneous suspension. The ability to monitor in real-time the concentrations of API and EtOH provided the opportunity to study the effects of different mixing speeds on the batch suspension. Examination of

Fig. 7 illustrates the advantages of having this Raman method. After the formulation was fully dispersed, the mixing speed was systematically increased from 0 to 500 rpm. During this study, the measured API concentration decreased until it reached a steady state reading. The degree of variability at 0 and 50 rpm is much higher than the higher mixing speeds, suggesting that the suspension is no longer homogeneous as it breaks and creams on the surface of the window of the probe. The difference in measured API concentration between 100 and 500 rpm is statistically negligible, indicating that a minimum of 100 rpm is required to maintain the suspension during the filling operation. API adhesion to the probe surface and fouling effects were not observed given that the measured concentrations returned to 100% LC upon resuming mixing at speeds greater than 100 rpm. Recall that the multivariate API calibration model extended to 450% LC. Therefore, the measured concentrations at 0 rpm fell outside of the validated range. However, for the purposes of this research, the high concentrations are taken to be representative of the suspension creaming effect.
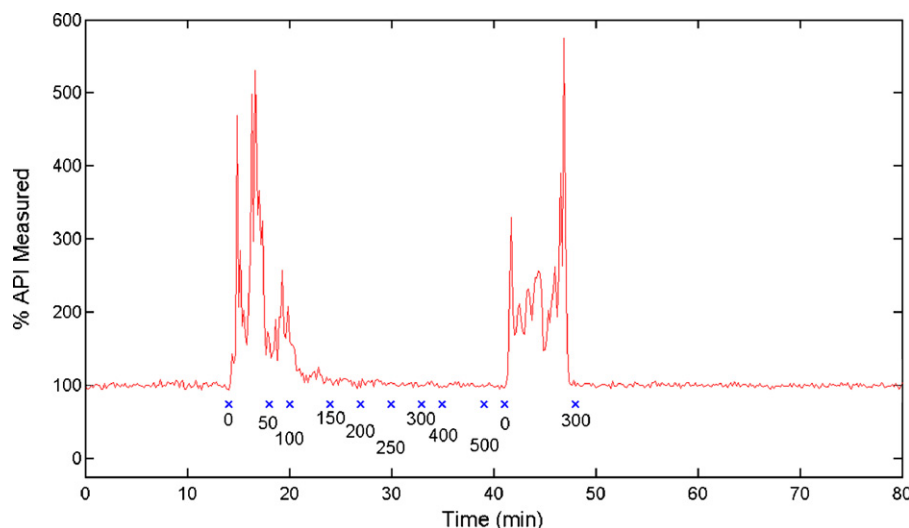

Fig. 7. The effects of mixing speeds (rpm) on the predicted API concentration.
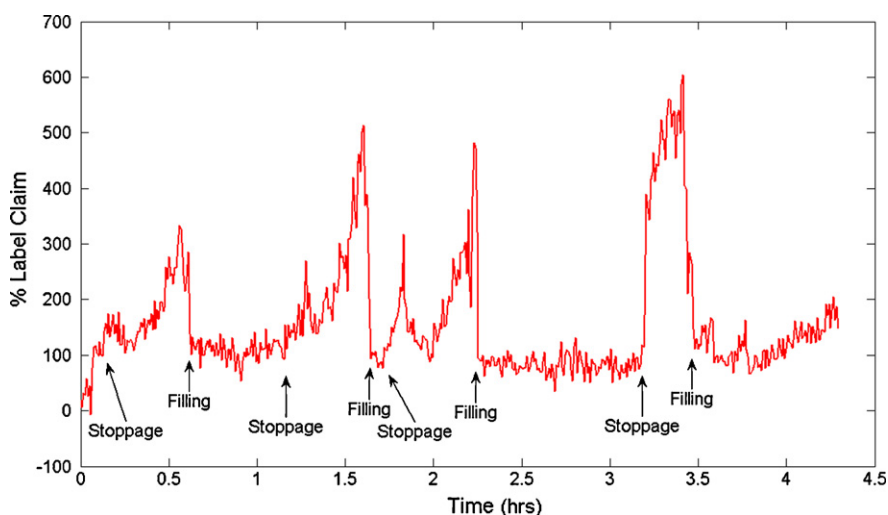
Fig. 8. Effect of filling stoppages in the flow-cell on API concentration.

It is also important to note that the probe position plays an important role in the predicted concentration. For example, when probes are placed at different depths throughout the compounding tank, it was possible to see the time-resolved effects of creaming at different depths (data not shown). During manufacturing, the effects of any mixing inconsistencies can be detected by this method.

The same technique was applied in the flow-cell, as illustrated in Fig. 8. As with low mixing speeds in the compounding tank, when filling is interrupted, the flow-cell probe detects an increase in product concentration at the recirculation lines as the suspension breaks. When filling operations are resumed, the turbulent mixing generated during filling operations helps to restore the proper suspension characteristics.

5. Conclusion

Raman spectroscopy has proven to be a valuable tool for process development, analysis, and control in a pressurized metered dose inhaler manufacturing process, offering dramatic advantages over conventional off-line methods of analysis. By monitoring API and co-solvent concentrations in real-time, process parameters such as the mixing speeds and times required to fully disperse ethanol and API during compounding operations can now be scientifically justified. Furthermore, manufacturing parameters such as changes in mixing speeds or disruption of filling operations can be quantitatively assessed and corrected prior to unit filling. This approach sheds light on formulation details previously unavailable to formulation scientists, enabling the manufacturing process to be developed in a more systematic manner with greater scientific rigor.

Declaration of interest

The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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