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ORIGINAL ARTICLE

Development of a high-throughput UHPLC–MS-based content uniformity method as a tool for assessing dry powder inhalers

Joseph Medendorp, Brent Donovan and Justin Pennington

Schering-Plough Research Institute, Oral and Specialty Product Development, Summit, NJ, USA

Abstract

Background: Dry powder inhaler (DPI) product manufacturing requires the assessment of uniformity at various stages of the manufacturing process. *Results*: To efficiently and precisely determine the uniformity of the small doses inherent to DPI technology, an ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS)-based content uniformity method was developed. Using mathematical modeling and proper selection of bracketing standards, a volumetric approximation of sample weight was utilized, eliminating the need for accurate sample weights and reducing sample preparation time. *Conclusion*: UHPLC–MS coupled with mathematical modeling makes high-throughput CU testing of DPI drug products possible which allows for an enhanced understanding of the manufacturing process.

Key words: *Content uniformity; dry powder inhaler; inhalation; mass spectrometry; quality-by-design*

Introduction

Content uniformity (CU) is a ubiquitous critical quality attribute for drug products and delivery systems, including tablets, transdermals, suspensions, emulsions, gels, solids, suppositories, and inhalation products[1,2]. Traditionally, CU was assessed on a minimal number of samples as a release test to ensure that the drug product was uniform at various locations in a production batch. This was partially because of the difficulty in obtaining precise data in a timely fashion with large numbers of samples. However, the pharmaceutical industry is transitioning to a quality-by-design (QbD) approach to pharmaceutical development that requires an enhanced understanding of process parameters and quality attributes as they relate to efficacy and safety. To gain the enhanced understanding of this quality attribute and its associated influential parameters, it has become necessary to explore analytical techniques that can assess CU on a large number of replicates in minimal time.

The current CU method of analysis for dry powder inhalers (DPIs) and other inhalation products is assay by high-performance liquid chromatography (HPLC). HPLC requires extensive sample preparation and analysis time because of its reliance on accurate measurement of sample weights. FDA guidance suggests that CU tests be performed on sample sizes of one to three times a unit dose[2–4]. For oral solid dosage forms, a single-unit dose equates to sample sizes ranging from 200 to 500 mg with both sample preparation and analysis posing few limitations in terms of difficulty of preparation. However, in the case of inhalation products, a single-unit dose can be in the order of 1 mg. In circumstances where large numbers of samples must be analyzed quickly, it is not practical to accurately weigh, prepare, and analyze such small unit doses.

In keeping with the need for real-time or near real-time information for vast amounts of data generated during the drug development process, researchers have long-sought replacements for the HPLC for uniformity testing. Near-infrared and Raman spectroscopy have

Address for correspondence: Dr. Justin Pennington, Schering-Plough Research Institute, Oral and Specialty Product Development, 556 Morris Avenue, Summit, NJ 07901, USA. Tel: 908-473-6605. E-mail: justin.pennington@spcorp.com

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been used with much success, largely because of their potential for online and real-time analysis[5,6]. Spectroscopic techniques require the construction of multivariate calibration models for the prediction of new samples. Success of these models is dependent on whether each excipient or analyte exhibits its own unique contribution to the spectroscopic signal. Although spectroscopic methods typically demonstrate the selectivity for the analytes of interest, variability at the single milligram dose can be high. In the case of uniformity testing, any variability in the performance of the instrument will erroneously be read as variability in the uniformity of the analytes of interest, posing questions related to the quality of the resulting data. Additionally, spectroscopic measurements may not always have the necessary sample-to-sample reproducibility to provide unequivocal uniformity results. For drug products with low strengths (e.g., 1–3% of a 3 mg sample), as are typical in inhalation therapy, the instrument may lack adequate sensitivity for the components of interest.

The research presented in this work describes the implementation of an ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS) method into DPI drug product development as an alternative method of analysis for CU testing. Traditional chromatography-based uniformity methods require a unique ultraviolet (UV) response for each component, which is achieved by chromatographic resolution, and detection is typically accomplished by UV detection at a fixed wavelength. However, if the drug substance (DS) or excipients do not contain chromophores, a detector such as mass spectrometer may be better suited for the quantification of all components in a formulation.

Four distinct advantages of UHPLC–MS have been realized throughout the implementation of this method: (i) it offers a substantial gain in efficiency over standard methods of analysis; (ii) the MS detector monitors all components in the DPI formulation; (iii) small sample sizes of 1–3 mg are sufficient for accurate analysis; and (iv) the error associated with analyst variability is no longer paramount to the success of the experiment. These facts taken together suggested that the new UHPLC–MS method was a viable approach for CU analysis.

Methods

Materials

The samples utilized for this study were chosen to bracket the current formulation development activities for the drug product. The three components of the drug product used in this study will be referred to as carrier, drug substance A (DS-A), and drug substance B (DS-B). The carrier concentration ranged from 61% to 82%, DS-A ranged from 3% to 24%, and DS-B was held constant at 15%. Drug product components were manufactured and formulated in-house, the details of which will not be provided in this manuscript as they are not necessary for this discussion. Acetonitrile (ACN) (optima grade), methanol (optima grade), formic acid (FA), trifluoroacetic acid (TFA), and glacial acetic acid (HPLC grade) were obtained from Fisher Scientific (Fisher Scientific, Pittsburgh, PA, USA). In-house water was purified using a Milli-Q water purification system, or bottled water was purchased from Fisher Scientific.

Instrumentation and chromatography

The separations were conducted utilizing a Waters (Waters Corporation, Milford, MA, USA) Acquity™ BEH C8 (1.7 μm particle size, 2.1 cm × 50 mm ID) column. The mobile phase consisted of 0.05% FA in 95% water : 5% ACN (solvent A) and 0.05% FA in 95% ACN : 5% water (solvent B). A Waters Acquity™ UHPLC–MS system consisting of the following components was utilized for data collection: Acquity™ Binary Solvent Manager, Acquity™ Sample Manager, Acquity™ UPD Photodiode Array Detector, and a MicroMass Quattro Micro™ mass spectrometer fitted with a Z-Spray Electro-Spray source.

UHPLC–MS analysis

The chromatographic separation consisted of a gradient with an initial isocratic hold at 30% solvent B for 0.66 minutes, followed by a linear rise to 70% solvent B over 0.94 minutes, a final isocratic hold at 70% solvent B for 0.40 minutes, and a 1-minute allowance for re-equilibration to starting conditions. A constant 0.5 mL/min flow rate with an isothermal column temperature of 35°C and a 10 μL full loop injection was utilized. The photodiode array detector was set to scan from 210 to 400 nm for UV-based detection. The MicroMass Quattro Micro mass spectrometer was coupled in-line with the chromatography system with a 50:50 flow split at the MS source. MS detection was accomplished through segmented selected-ion recording (SIR) to only detect the ion of interest at specified times in the chromatographic run.

Results and discussion

Method development strategy

To assess the impact of the systematic manipulation of process parameters on final product attributes, DPI formulation development studies required a significant amount of CU testing. Traditional HPLC–UV-based

methods were impractical for the hundreds of samples to be analyzed because of time-consuming sample preparation and length of HPLC analysis. The method development process was focused on developing a high-throughput method capable of analyzing hundreds of samples in a single day. To accomplish this goal, sample preparation, data generation, and data processing were each evaluated for potential efficiency gains. The following three sections ('Chromatography', 'Mass Spectrometry', and 'Mass Spectrometry Detection Mode') address control of the analytical method factors, and the next three sections ('Accurate Sample Weight', 'Mathematical Modeling (Elimination of Sample Weight)', and 'Mathematical Modeling (Drift Cancellation)') discuss a creative approach to the suppression of the noise factors through a second-order mathematical drift correction.

Chromatography

Traditional HPLC–UV-based CU methods rely heavily on chromatographic resolution to ensure accurate final results. Consequently, HPLC methods are developed to optimize chromatographic resolution between peaks at the expense of long run times. In the case of the DPI product, this applies only to the chromatography for DS-A and DS-B, as the carrier exhibited no UV chromophore. This makes the carrier undetectable, despite the fact that it is present in higher concentrations than in either DS. In HPLC–UV, the percent composition is calculated from direct determination of the recovery of the individual DS's with no regard to calculation of the percentage of the carrier.

For this work, a UHPLC method was developed to achieve suitable separation between the carrier and the DSs. Because of the high degree of selectivity provided through use of the mass spectrometer, a rapid fit-for-purpose method was developed. Although baseline resolution is required for nonselective detectors such as UV, it is typically not required for MS-based methods. However, baseline resolution was necessary between the actives and the carrier to allow for the switch from electrospray-negative ESI (–) for the carrier to electrospray-positive ESI (+) for DS-A and DS-B.

DS-A is a salt of a weak base and was found to tail significantly in reversed-phase separations because of secondary silica interactions. TFA was initially screened as a potential combination pH modifier and ion-pairing agent to alleviate the associated poor peak shape, despite the fact that TFA is well known to cause significant ion suppression and variability in detector sensitivity[7–9]. The chromatographic benefits of TFA did not overcome its deleterious effects on sensitivity; therefore, FA was chosen as the pH modifier. Because SIR–MS detection is utilized for the method, additional selectivity is achieved by the mass spectrometer, eliminating the negative effects of peak shape on the ability to accurately quantify instrument response.

By utilizing UHPLC for the chromatographic separation, a total analysis time of 3 minutes per sample was achieved, a significant reduction from typical HPLC methods. In addition, UHPLC offers greater chromatographic efficiency and resolution. Without consideration of the savings generated by reduced sample preparation time, the conversion to UHPLC alone offers significant time savings over traditional HPLC.

Mass spectrometry

Mass spectrometry was chosen as a detection method for the current development project for two reasons: its high level of selectivity and the ability to accurately quantify the carrier. The carrier has not been quantified previously in uniformity studies because it is invisible to UV detection. In addition, the highly polar nature of the carrier makes it elute on the solvent front of reversed-phase separations, necessitating the use of a selective mode of detection such as MS to obtain reliable results, free from chromatographic interference.

The selection of the MS instrumental parameters was accomplished through tuning by direct infusion into the MS and optimizing the parameters for the highest molecular ion peak intensity for each of the three components. The same MS parameters were found suitable for all three components. However, the carrier was found to ionize far better in ESI (–) mode while DS-A and DS-B required ESI (+) mode, necessitating the negative to positive mid-run switch. The ions of interest were selected by scan mode direct infusions and correlated with $[M - H]^-$ for the carrier and $[M + H]^+$ for DS-A and DS-B.

Mass spectrometry detection mode

For samples that contain only three components, such as the DPI CU samples, SIR–MS is a suitable mode of detection. Selectivity is obtained through chromatographic resolution and mass-to-charge ratio. To increase sensitivity, the chromatographic run was divided into segments where the MS detected only the peak of interest, thus eliminating the need to alternate between ions of interest. There is one fundamental limitation of the SIR approach: only the selected ions of interest are detected. Any impurity, contamination, or addition of another molecule will not be detected. The mathematical model is built upon the fundamental assumption that only the carrier, DS-A, and DS-B are present in the sample. This is an acceptable compromise because the method is intended for formulation and process development

purposes only and runs in conjunction with an assay and impurities method.

Accurate sample weight

The most significant limitation of CU testing for formulation development is caused by the sampling size of one to three times the unit dose (~2 mg) for DPI products. Accurate sample weight and dilution not only require considerable amounts of preparative time but are also the primary sources of error in this conventional method of analysis. By eliminating the need for accurate sample weight and dilution from the sample preparation steps of this high-throughput method, it became possible to analyze several hundred samples in a single day. Sample preparation involves volumetric approximation of 2 mg of sample into a scintillation vial followed by addition of 10 mL of sample solvent through a volumetric pipette. To remove accurate sample weight from CU analysis, all components of the product must be precisely quantified in relation to the other components. Although the carrier can be detected by refractive index or charge aerosol detectors, their lack of selectivity coupled with the carrier's poor chromatographic retention make bulk property detectors unsuitable for the current project.

The calculation of percent deviation cannot be achieved simply by dividing the peak area of each component by the total peak area and determining the deviation from the average. Ionization efficiency dramatically affects the peak response on the detector. For example, although the carrier is present in the highest concentration of any of the three components, it exhibits the lowest instrumental response. For this reason, more complicated mathematical modeling is required for the method and will be described fully in subsequent sections.

Mathematical modeling (elimination of sample weight)

Typical HPLC methods involve the calculation of a standard response factor, calculated as peak area divided by concentration. When operating within the linear range of the instrument, this response factor is consistent across the entire range, independent of the concentration of the analytes. As clearly shown in Figure 1, peak area has a nonlinear relationship with standard concentration in MS. To accurately determine a concentration from peak area, it is necessary to quantify how the instrument responds at each level.

Despite the nonlinear relationship with MS, it is possible to model with a second-order equation, therefore, making quantitative use of peak areas achievable. A stock standard solution was prepared at 200% of the nominal target assay concentration by weighing the individual components at the targeted formulation composition and volumetrically diluting to the following levels: 150%, 100%, 50%, and 25%. Standard injections

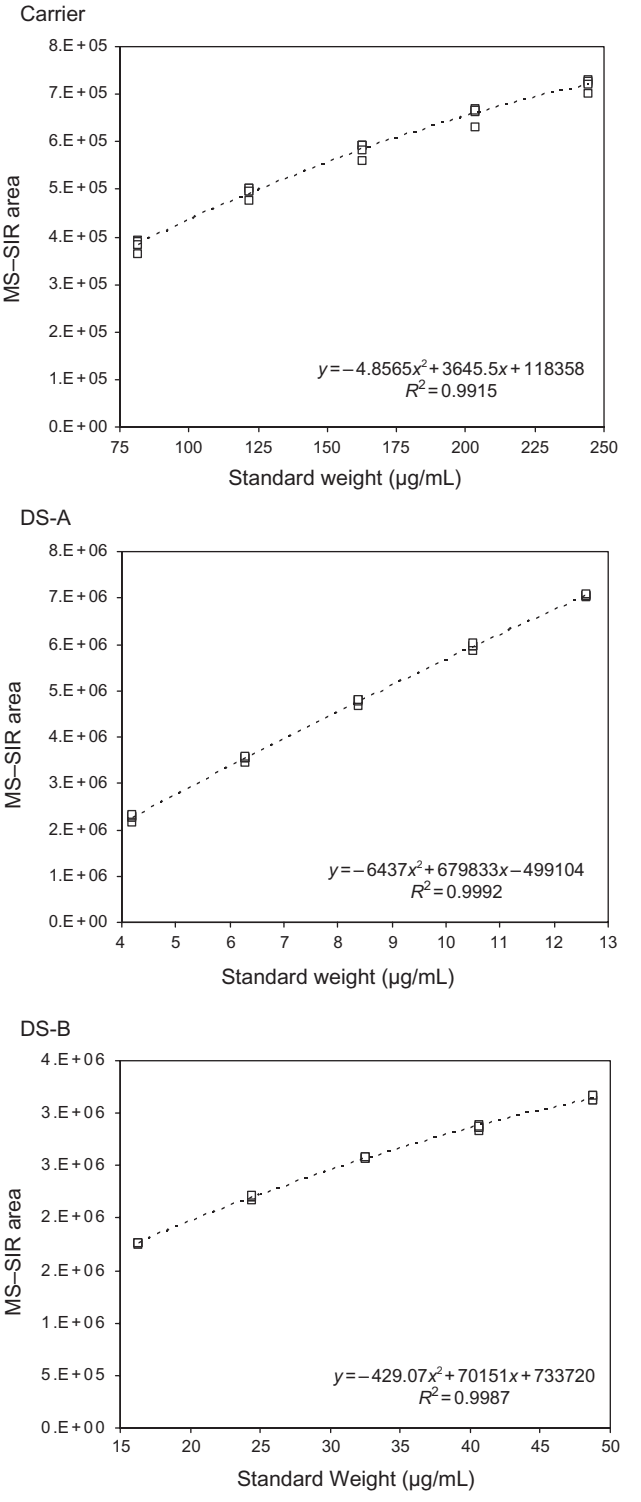

Figure 1. Batch 1 LC–MS standard calibration plots from 25% to 200% of the nominal 200 µg/mL sample concentration, demonstrating the nonlinear relationship between concentration and MS response.

at each of these concentration levels were used to bracket the sample injections, with no more than 40 sample injections (~120 minutes) between standards. Standard area was plotted on the *x*-axis against standard weight on the *y*-axis. The direction of this plotting upon first inspection seems backward; however, to use peak areas (x) to predict standard weight (y) with a regression equation, the predictor variable must be used as the independent variable. A second-order polynomial is fit through the data points to model the relationship between peak area and standard weight. All models were constructed separately for each component: carrier, DS-A, and DS-B, according to Equation (1):

$$\begin{aligned} y_{\text{Cweight}} &= a_{\text{C1}}(x_{\text{C}})^2 + b_{\text{C1}}(x_{\text{C}}) + c_{\text{C1}} \\ y_{\text{AA weight}} &= a_{\text{AA1}}(x_{\text{AA}})^2 + b_{\text{AA1}}(x_{\text{AA}}) + c_{\text{AA1}}, \\ y_{\text{AB weight}} &= a_{\text{AB1}}(x_{\text{AB}})^2 + b_{\text{AB1}}(x_{\text{AB}}) + c_{\text{AB1}} \end{aligned} \quad (1)$$

where a_{C1} and b_{C1} are scalar regression coefficients, x_{C} is the SIR–MS peak area of the standard, $y_{\text{C weight}}$ is the standard weight, and the subscripts in all equations are C for carrier, AA for DS-A, and AB for DS-B. This equation is a quadratic least-squares polynomial fit between peak area and standard weight, where calculation of the regression coefficients is designed to minimize the sum of squares of the residuals. Typically, the regression equation shown above has a constant term, the bias term. For the purposes of this application, this bias term can be eliminated, forcing the equation through zero. Therefore, if no MS peaks are detected, the standard weights will correctly read as 0. Sample peak areas for each component are substituted into the *x*-variable of their respective equations to obtain their component weights. The conversion from milligram weight to percent composition is calculated with Equation (2). The relationship in Equation (2) requires that all three components taken together sum to 100%, which limits the percent composition of each component to be interpreted as a semiquantitative approximation. However, this relationship does not influence the desired outcome of CU analysis, which is a precise measure of the deviation around the average value. Implementation of this model simply requires the analyst to input the standard and sample peak areas into a spreadsheet or Matlab® algorithm. The model is automatically generated from the standards, and the approximate percent compositions of the samples along with deviations from the average are calculated as follows:

$$\frac{y_{\text{Cweight}}}{\text{total weight}} + \frac{y_{\text{AA weight}}}{\text{total weight}} + \frac{y_{\text{AB weight}}}{\text{total weight}} = 100\%, \quad (2)$$

where $\text{total weight} = y_{\text{Cweight}} + y_{\text{AA weight}} + y_{\text{AB weight}}$.

The MS response varies predictably with the different analyte concentrations, thereby eliminating the need for sample weights in the calculation of percent deviation. To verify that sample weight was superfluous upon implementation of this new approach, total predicted sample weights were back-calculated. As shown in Figure 2, the calculated weights versus the analyst-reported weights are nearly identical for the two batches tested. Note that any small deviation between the two weights is equally likely to be a product of analyst error as it is to be a product of MS measurement error, therefore, does not serve as an indication of MS inconsistency. Batch 1 (82% Carrier, 3% DS-A, 15% DS-B) and Batch 2 (61% Carrier, 24% DS-A, 15% DS-B) were selected because they bracketed the extremes of the samples tested.

Mathematical modeling (drift cancellation)

Mass spectrometers have a few limitations that are not present in other chromatographic detection methods. The MS signal can significantly drift because of electronics and changes in ionization efficiency. Electronic drift can largely be alleviated by allowing suitable time for the instrument to warm up to operating temperature. Changes in ionization efficiency can be caused by ion suppression related to the chromatographic mobile phase, sample matrix effects, or the amount of contamination collected in the source. The sample matrix does not present any issues for this application because it contains three fully chromatographically resolved components. Even with proper warm up, selection of mobile phases, and general instrument maintenance, drift will occur.

To demonstrate the amount of drift possible, a DPI sample at 0.2 mg/mL (100% nominal standard level) was injected 300 times consecutively, utilizing the UHPLC method described previously (Figure 3). The instrument was not allowed to warm up or equilibrate, therefore, injection 1 was made within 2 minutes of instrument initialization. Although greater than 40% of the total drift was observed within the first hour of the experiment, the instrument continued to drift throughout all 300 injections. For this reason, it was necessary to account for system drift through either bracketing standards, internal standards, or mathematical drift cancellation. Although an internal standard would correct instrument-related drift, all three components were found to vary independently, necessitating the use of three separate internal standards. A method using three separate internal standards would greatly increase the complexity of the analysis and significantly reduce the usefulness of the method. It was for this reason that bracketing standards and a mathematical drift cancellation algorithm were explored.

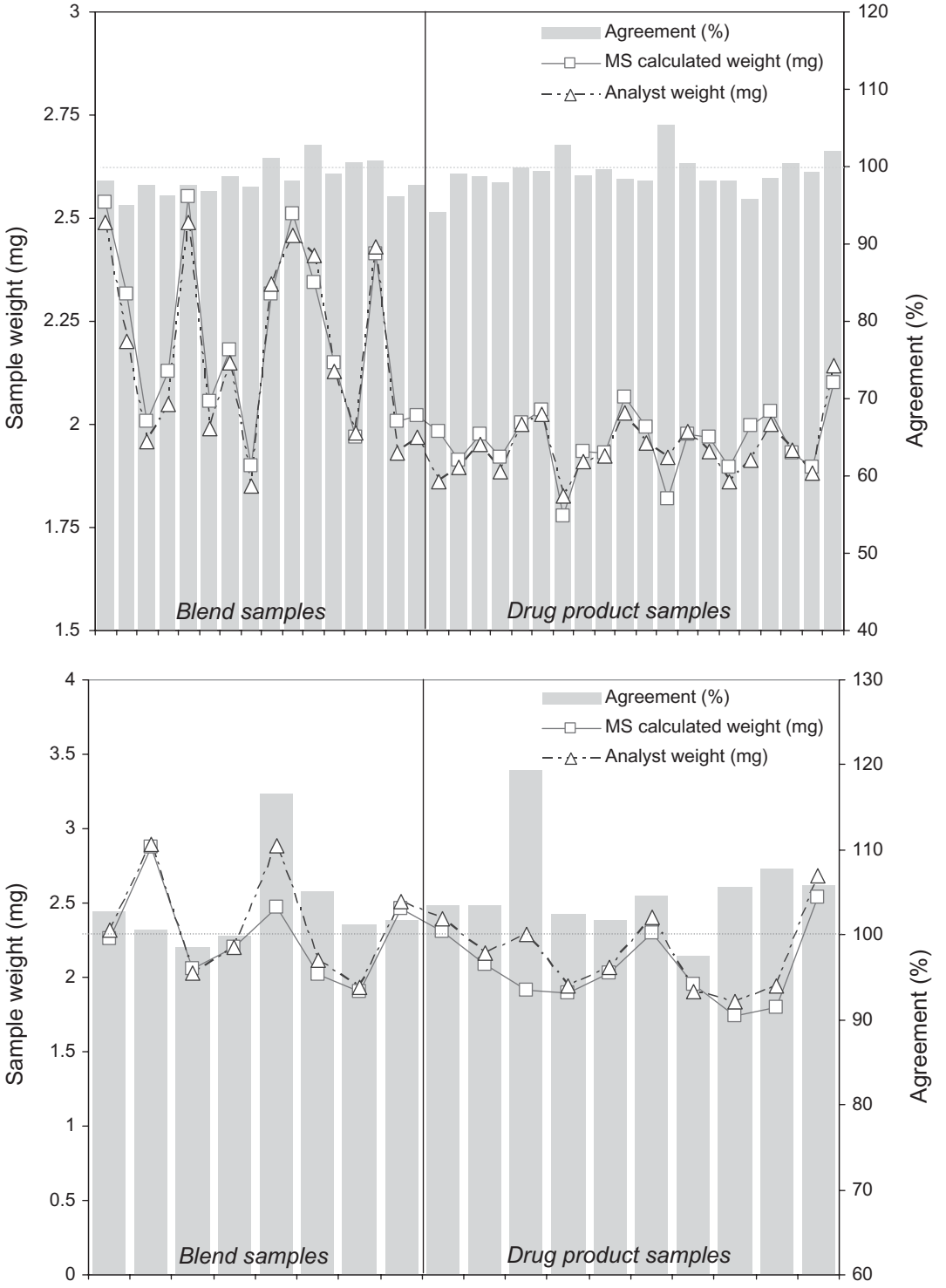

Figure 2. Analyst versus MS-calculated sample weight correlation: Top: Batch 1, Bottom: Batch 2. The histograms represent the percent agreement between MS-calculated weight (mg) and the analyst weight (mg).

To correct for instrumental drift, replicate injections of the bracketing standards were used as the metric by which the instrumental noise is quantified. A second-order mathematical model is constructed for each component according to Equation (3):

$$\begin{aligned} y_{\text{C area}} &= a_{\text{C2}}(x_{\text{C2}})^2 + b_{\text{C2}}(x_{\text{C2}}) + c_{\text{C2}} \\ y_{\text{AA area}} &= a_{\text{AA2}}(x_{\text{AA2}})^2 + b_{\text{AA2}}(x_{\text{AA2}}) + c_{\text{AA2}}, \\ y_{\text{AB area}} &= a_{\text{AB2}}(x_{\text{AB2}})^2 + b_{\text{AB2}}(x_{\text{AB2}}) + c_{\text{AB2}} \end{aligned} \tag{3}$$

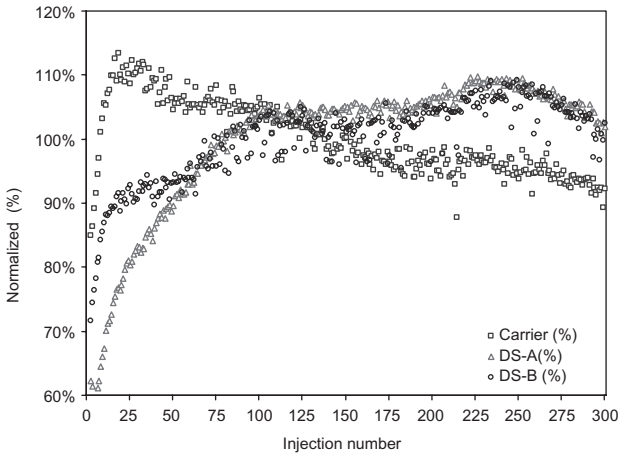

Figure 3. Injection of 300 Batch 1 (0.2 mg/mL) standards in a row without allowing time for equilibration, demonstrating the time-dependent drift associated with the mass spectrometer. The total chromatographic run time is 3 minutes. Ionization by ESI (–) for the carrier and ESI (+) for DS-A and DS-B.

where a_{C2}, b_{C2}, and c_{C2} are scalar regression coefficients for the carrier, x_{C2} is the run number of the standard injection, $y_{C\ area}$ is the SIR–MS peak area of the carrier, and the subscripts in all equations are C for carrier, AA for DS-A, and AB for DS-B. In this case, run number is synonymous with injection time; therefore, this equation models instrumental drift versus time. The quadratic model is trained on all standard injections taken throughout the run. By substituting the sample injection run number into Equation (3), the analyst obtains the theoretical standard SIR–MS peak areas at every time point for each component and standard level. As the peak areas vary over time, standard injections at the start of a bracket are slightly different than replicate injections of the same samples at the end of the bracket. Therefore, it is a valid approximation to use the theoretical standard peak areas by which to quantify the sample injection.

A second-order polynomial is fit between the new theoretical standard areas (e.g., $y_{C\ area}$) from Equation (3) and known standard weights for every run. This creates a new second-order equation for every run as shown in Equation (4):

$$\begin{aligned} y_{C\ weight} &= f(y_{C\ area}) = a_{C3}(x_{C3})^2 + b_{C3}(x_{C3}) + c_{C3} \\ y_{AA\ weight} &= f(y_{AA\ area}) = a_{AA3}(x_{AA3})^2 + b_{AA3}(x_{AA3}) + c_{AA3}, \\ y_{AB\ weight} &= f(y_{AB\ area}) = a_{AB3}(x_{AB3})^2 + b_{AB3}(x_{AB3}) + c_{AB3} \end{aligned} \quad (4)$$

where a_{C3} and b_{C3} are scalar regression coefficients for the carrier, x_{C3} is the theoretical peak area of the carrier in the standard injections, and $y_{C\ weight}$ is the known component mass of the carrier standards. Notice that this step is the same as Equation (1) with no drift correction, using the standard peak areas to calculate the sample weights. The last step of the drift correction process is to convert the component weights into percentage deviation as described previously.

This process can be visualized graphically as demonstrated in Figure 4. When the two variables (run number and peak area) have linear relationships to the variable of interest (sample weight), the response surface in three-dimensional space is a flat plane. If one variable has a quadratic relationship and the other has a linear relationship with the variable of interest, the response surface becomes a curved plane across one of the axes. In the case of mass spectrometric mathematical drift correction, both predictor variables exhibit quadratic relationships to the final variable of interest. In effect, the combination of these two quadratic relationships creates a response surface in which both predictor variables vary independently from each other. The surface plots for the carrier, DS-A, and DS-B in Figure 4 illustrate this phenomenon. The [*X*,*Y*] Cartesian coordinates (injection time, SIR peak area) are used as the predictor variables for *Z*, the sample weights, which are subsequently used to calculate the percentage of each component. The response surfaces are different for each component, but each contains the information necessary to calculate its respective component weight. The equations in the plots shown in Figure 4 correspond to the *i*th sample injection, as indicated by the arrows in the response surface.

Note that the last step of the drift correction process is the same as the first step in the nondrift correction approach. Comparison of Figure 1 (the conventional approach) and Figure 4 demonstrates the enhanced ability of the drift model to calculate the sample weight. In place of the single quadratic fit used in the conventional approach, the drift-corrected approach makes use of a new set of theoretical standards for each injection.

Characterization experiments

Several method characterization experiments were conducted to ensure the reliability of the results obtained from the newly developed MS-based CU method. Results are presented with and without drift cancellation.

To gain confidence that the new UHPLC–MS-based CU method was providing reliable results, a series of test cases were analyzed at the extremes of formulation compositions requiring testing. Batch 1 (82% Carrier, 3% DS-A, 15% DS-B) and Batch 2 (61% Carrier, 24% DS-A, 15% DS-B) were analyzed by both traditional UV-based detection and the MS method. As shown earlier,

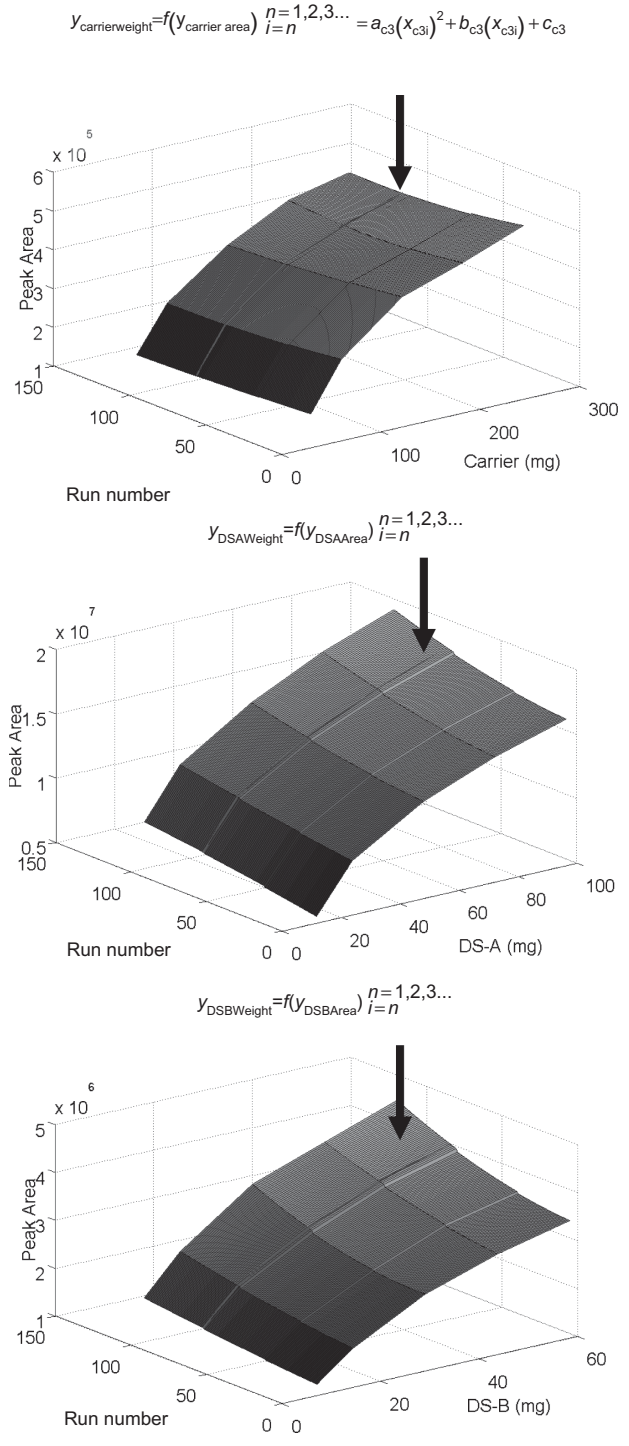

Figure 4. Curved response surfaces that demonstrate the MS mathematical drift cancellation design space. The equations represent the *i*th sample and the theoretical standards used to for the calculation of results.

the predicted sample weights and the actual analyst recorded weights are in close percent agreement (Figure 2). These results support the claim that with the UHPLC–MS method, it is unnecessary to collect accurate sample weights to accurately measure CU.

The data obtained from both the UV- and the MS-based methods are shown in Figure 5, demonstrating the close agreement between the two methods. Because sample weight plays a significant role in the UV-based method and has no impact on the MS-based method, small variations in the results are both expected and acceptable. Note that the carrier plot in Figure 5 does not contain diode data, as it exhibits no UV chromophore.

Example: representative scale batch

A representative scale batch of the DPI product was evaluated to test the performance of the MS method for uniformity testing and contained 73% Carrier, 12% DS-A, and 15% DS-B. The batch consisted of 115 samples, 35 blend samples, and 80 spherical drug product samples. This experiment presented an opportunity to determine whether the high-throughput goal of the method development process had been attained. Analysts prepared each of the samples utilizing a volumetric approximation. With this approach, a single analyst was able to prepare 115 samples in a matter of 2–3 hours. Compared with the conventional sample preparation, this is a time savings of several days. With the analytical run time for the UHPLC–MS method of only 3 minutes, a single batch including bracketing standards was run overnight, allowing for near-continuous operation. A graphical representation of the results of the representative scale batch is shown in Figure 6.

Instrumental drift was present in the representative scale batch tested. By utilizing the mathematical drift cancellation previously described, a direct comparison of both corrected and noncorrected data can be generated (Figure 6). This graph represents each sample run sequentially from left to right with the vertical division lines demonstrating the locations of the bracketing standards. The data clearly display that as the instrument was allowed more time to warm up, less drift cancellation was required. For nearly every point, the drift-corrected data were closer to the average values than the uncorrected data. Although drift cancellation did remove percent composition trends from the data, the drift was relatively insignificant in comparison to overall variability.

System suitability

The system suitability requirements for mass spectrometry methods differ from other chromatographic detection methods because of the variability associated with ionization efficiency. With mass spectrometers, it is important to ensure adequate time for the electronics to warm up and for stable ionization to occur. Even when taking proper precautions, drift can

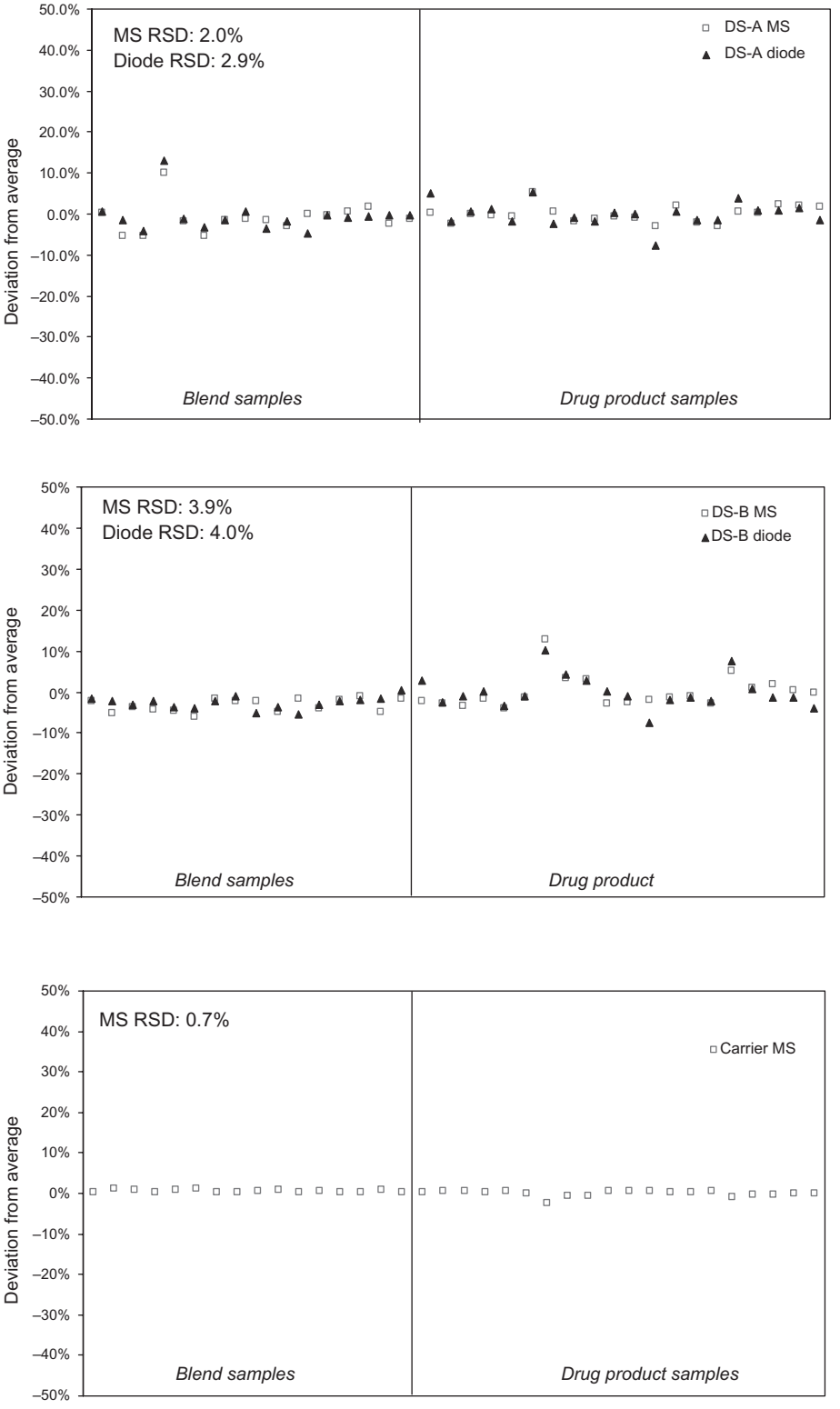

Figure 5. Direct comparison of traditional UV versus MS content uniformity results for Batch 1 DS-A and DS-B obtained from the same samples and chromatographic runs. The carrier lacks a chromophore allowing for only MS content uniformity results.

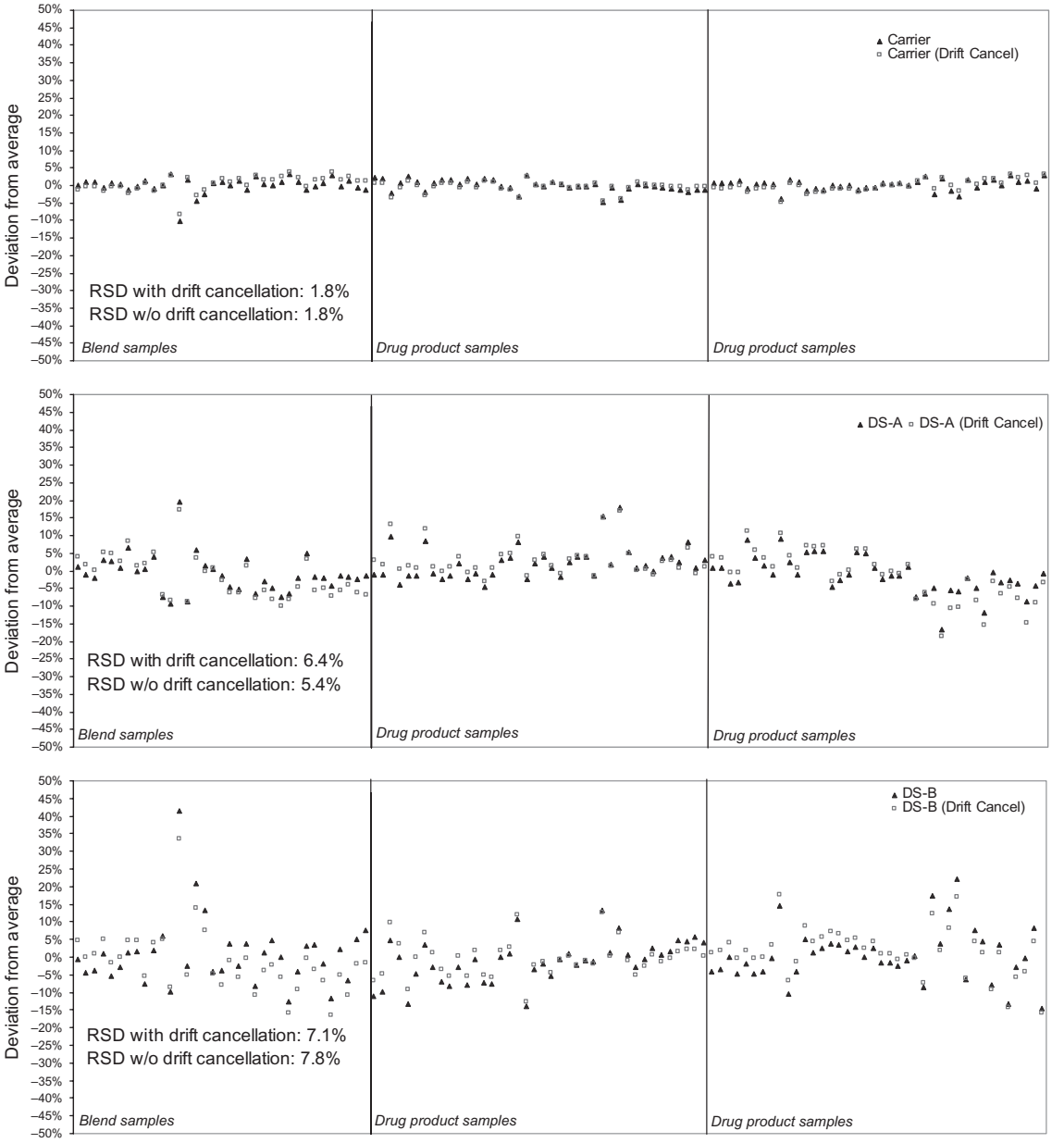

Figure 6. Demonstration of the capability to determine 115 content uniformity samples in a single day by utilizing the UHPLC–MS-based method on a representative batch. The vertical lines represent when the bracketing standards were injected. The results are presented with and without mathematical drift cancellation.

and will occur. The drift cancellation algorithm provides a potential option to alleviate issues associated with instrumental drift.

Of greater importance to reliable data is that the sample weight is properly bracketed by standards. Polynomial fit equations are not valid beyond their respective end points. Although the method does not require accurate sample weight, an approximation is required. To ensure that the sample is within the proper standard bracketing range, the predicted sample weight can be plotted as shown in Figure 7. If the predicted sample weight falls outside of this range, it also fell outside of the standard calibration range, and therefore may not be valid.

Conclusion

UHPLC–MS coupled with mathematical modeling provides a framework for high-throughput CU testing of DPI drug products. This innovation allows for an

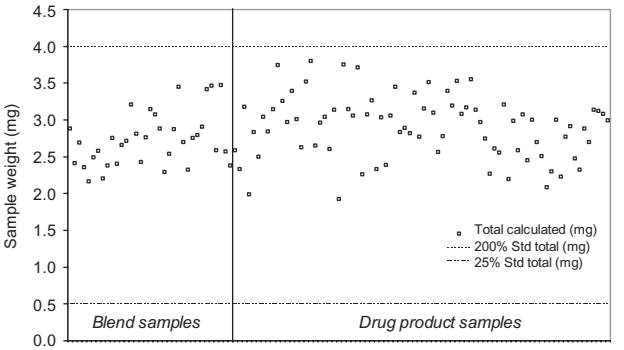

Figure 7. The calibrated range of standards from 25% to 200% of the nominal 2-mg sample from the representative batch. The UHPLC–MS-calculated sample weight is plotted to ensure that all samples are contained within the calibrated design space.

enhanced understanding of the manufacturing process by efficiently analyzing a greater number of representatively sampled replicates. The method has been demonstrated as a suitable method for high-throughput sample analysis and offers a significant efficiency gain over the conventional HPLC method of analysis. Furthermore, UHPLC–MS allows for quantification of all components of the DPI formulation and eliminates errors associated with sample weight and dilution. The method described provides a valuable tool for assessing CU of DPI drug products for use during formulation development.

Declaration of interest

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

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