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Development of Molecular Laser-Induced Breakdown Spectroscopy (MO-LIBS) using Chemometrics: An Innovative Spectroscopic Approach

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Abstract. In the present work, Laser-Induced Breakdown Spectroscopy (LIBS) and chemometrics have been successfully applied to the analysis of several molecular compounds in a pharmaceutical formulation. Traditional application of LIBS for analysis of pharmaceutical materials has been limited to the active pharmaceutical ingredient (API) or material that can be tagged by an atomic compound which is not present in the matrix bearing the material of interest. Typically these elements are present only in the active pharmaceutical ingredient (API). However, using the emission signal originating from small diatomic fragments like C2, CN and CH, we have successfully developed a spectroscopic approach capable of monitoring several ingredients present in a formulation. Additionally, we have successfully applied chemometrics to accurately predict the formulation ingredients. Consequently, the concentration for the API and formulation excipients: Avicel® (microcrystalline cellulose), lactose and magnesium stearate have been analyzed using the molecular, atomic and ionic species (i.e. C2, CN, CH, , H, C, Ca and Mg) generated by a laser-induced plasma. Using such an approach, we have been able to accurately quantitate the API and magnesium stearate (< 4% relative bias). For the other formulation ingredients such as excipients (i.e. Avicel®, lactose), we have been able to accurately predict the composition of these compounds with accuracy better than 15% relative. Combining LIBS and chemometrics has provided a novel approach for the quantitative analysis of several molecules that was not technically possible with the traditional approach using a target specific element (API, excipients).

Laser-Induced Breakdown Spectroscopy, LIBS, MO-LIBS, Pharmaceutical, Molecular band, Emission spectroscopy, chemometrics, Monitoring, Process Analytical Technology, PAT

Introduction. Laser-Induced Breakdown Spectroscopy (LIBS) is known as an elemental analysis technique based on the detection of atomic and ionic emission produced by laser-induced plasma of a gas, a liquid or a solid sample. The LIBS technique appeared soon after the introduction of the first ruby laser[1] in 1960. Brech and Cross were the first to report the detection of a spectrum from a ruby laser-induced plasma[2]. In 1964, Runge et al. reported the first spectrochemical analysis using a laser-induced plasma as a single emission source[3]. Interest in LIBS declined in the 1970's because of the expensive nature of the instrumentation and the poor analytical performance resulting from the lack of reliable technology for time-resolved measurements. Additionally, during this period of rapid development of optical emission spectroscopy LIBS could not compete with more established analytical techniques like Atomic Emission and Optical Emission spectroscopy as well as more parallel multi-element emission techniques such as Graphite Furnace Atomic spectroscopy and Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES).

The interest in LIBS reemerged in the mid 1980's with the availability of cost efficient, robust Nd-YAG lasers and, more importantly, with the development of sensitive optical detectors such as the intensified charge-coupled device (ICCD). The emergence of these optical emission detectors enabled reliable time-resolved measurements, which are essential to the successful spectrochemical measurements using LIBS. The unique advantages of LIBS such as the ability for rapid, in-situ, multi-elemental qualitative and quantitative analysis were immediately recognized. The broad spectroscopic and capability initiated a wide range of LIBS applications; this wave of scientific interest is still increasing in 2007[4-7]. General interest can be monitored by recent review papers[8-13], and LIBS has also been recently described as "a future super star" in a 2004 review article[14].

The use of Laser-Induced Breakdown Spectroscopy for direct detection of organic material is currently a subject of great interest in the LIBS community. The topic that receives the most attention is the use of LIBS for standoff analysis of explosive residues and biologic material for homeland security applications[7, 15-25]. Many approaches have been proposed for qualitative analysis of explosive residues[7, 15-19]. In particular, the use of atomic ratio of neutral lines of nitrogen, oxygen and carbon[7, 18-21] have been

used for the differentiation of explosives residues. However, theses approaches have been hindered by the atmospheric gases which are composed mainly of nitrogen and oxygen elements commonly found in non-metallic organic materials. This has been a challenging technical problem for standoff detection of organic material in air. To overcome these issues some researchers have proposed to include emission bands of CN and C_2 to the atomic ratio criterion for discrimination of explosive residue spectra under atmospheric conditions[7, 18-21]. These approaches along with the use of clustering techniques[23-25] and spectral database searchs[7, 18, 19] have been evaluated and suggest promise. Recently, Schade and Bohling have proposed to follow the temporal emission decay of molecular bands of CN along with an artificial neural network approach to differentiate different explosive residues[15, 16]. The multiplicity of research approaches and multivariate analysis techniques show the fundamental interest in utilizing LIBS as an analytical technique for the stand-off detection of organic compounds.

In the pharmaceutical arena, the story begins in 1998 with the patent of Sabsabi and Bussière that describes a spectroscopic method and apparatus for pharmaceutical analysis[26]. Soon after, a wide range of pharmaceutical applications for LIBS began to emerge[4, 27-34]. These applications include the monitoring of the active pharmaceutical ingredients (API) and a formulation lubricant such magnesium stearate[31] as well as the possibility of generating the first results capable of globally mapping pharmaceutical solid dosage forms[28]. As is typical in NIR and Raman reflectance spectroscopy, pure components are analysed before their preparation in a solid dosage form. Although, for these baseline spectral techniques, it has been shown that physical properties such as particle size will result in spectral diffusion that limits the applicability of these baseline spectra to facilitate the differentiation of physical changes from chemical or compositional changes that may result in combination of these components into a formulation[35-37]. Additionally, matrix effects and excipient interactions may limit the use of these reflectance baseline spectra and affect overall spectral information[35-37]. With MO-LIBS spectroscopic analysis have shown good selectivity for those pharmaceutical formulation constituents without the need of baseline spectra[32]. Well established reflective spectroscopic techniques such as NIR and Raman are also hindered by the penetration depth at the solid dosage form surface[38-40]. Whereas atomic LIBS or

MO-LIBS have the distinct ability to selectively obtain information at the tablet surface and throughout the entire tablet without spectral diffusion that is usual with NIR and Raman transmission techniques.

Another important application of LIBS for pharmaceutical materials is the analysis of coating thickness and uniformity on the tablet[28] for rapid at-line analysis for enhanced process control. LIBS has also been applied to the on-line monitoring of liquid pharmaceutical formulations[30], which have demonstrated the on-line/in-line process monitoring capability of LIBS. It is only as recently as 2006, that LIBS applications for pharmaceutical materials was first reviewed in a book chapter[4]. The technique has also been compared to traditional analytical techniques such as scanning electron microscope coupled with energy dispersive X-ray emission (SEM-EDX)[33] and with near-infrared (NIR) spectroscopy for determination of magnesium stearate a critical formulation ingredient necessary for ensuring product performance[34]. The efforts of these early LIBS researchers have highlighted the presence of the "matrix effect". To overcome the matrix effect resulting primarily from physical property variations induced by the manufacturing changes on solid dosage forms, efforts have been made to understand the influence of many parameters on the LIBS signals[27, 29, 41]. These atomic LIBS studies agree that the use of matrix-matched calibration standards are necessary to overcome the matrix effect to produce accurate quantitative results[27, 29, 41, 42]. Since atomic LIBS signals can be affected by many physical parameters in the manufacturing process, traditionally known as matrix-effects, two research studies[27, 29] have demonstrated that the LIBS technology is a valuable tool for process analytical technologies period. The matrix effect is sensitive enough to be utilized for a in process monitoring approach for pharmaceutical manufacturing[41, 42].

Since the Process Analytical Technologies (PAT) initiative of the Food and Drug Administration (FDA) was formally introduced in 2004[43], the FDA has encouraged the use of PAT to promote real time process understanding to facilitate innovation and risk-based regulatory decisions. In many applications, LIBS makes possible at-line rapid measurements of many formulation ingredients as well as the possibility of stand-off, in-situ, in-line and on-line process monitoring capability. These capabilities make LIBS an attractive PAT sensor technology that can be introduced virtually everywhere in the

pharmaceutical manufacturing process. This enables the process engineers to track critical parameters in order to optimize many aspects of the process performance often related to efficiency, cost effectiveness and manufacturing success. PAT is a scientific framework promoting a set of scientific principles and tools supporting innovation. PAT represents a paradigm shift in pharmaceutical manufacturing that will dramatically enhance our technical understanding of pharmaceutical preparation through the application of novel analytical and mathematical tools such as LIBS and chemometrics to provide real-time process understanding for enhanced quality assurance. Atomic LIBS has laid the foundation for the unique spectroscopic assessment and accurate quantitation of pharmaceutical API in solid and liquid dosage forms through discrete atomic spectral lines. Atomic LIBS is limited by the inherent requirement of target elements typically absent in formulation excipients, but the application of a novel spectroscopic approach such as monitoring molecular bands in complex matrices can extend the capability of LIBS, thought previously to have been inadequate and at best confounding for complex matrices.

Traditionally, molecular bands emitted by small diatomic fragment in conventional emission sources for spectroscopy (e.g. flame Atomic Emission Spectroscopy, AES) were considered as ancillary or secondary albeit interfering signals[44]. It has been argued that, compared to flame-AES, an Inductively Coupled Plasma (ICP-AES) source is a more energetic source with a better atomization/ionization capability that produces significantly less molecular fragments into the plasma[45]. Therefore, the broad spectral band emission in the UV-Vis spectral region contributes less to the spectral interference of atomic or ionic lines in this region of the spectrum[45]. These molecular bands have been studied intensively; for pure spectroscopic understanding and for applied uses in other fields[20, 32, 46-49]. The groundbreaking work of Herzberg[50], a 1971 Nobel laureate in chemistry, developed the important part of the theory that clarified our understanding of the emission of the molecular bands[50].

The basic hypothesis of the MO-LIBS approach developed here, is that the emission from small molecular fragments coming from the laser-induced plasma are characteristic of the parent molecules fragmented in the creation of the latter. It is well known that the fragmentation of a molecule is a function of the chemical bonding and the functional groups present in the molecule. The fundamental

molecular structure, the nature of the bonds, the conformation, and the isomers contribute to the specific nature of the molecular emission. We propose to use the emission signal from the small molecular fragments coupled with chemometrics to evaluate the primary chemical structures in pharmaceutical formulations. Using favorable experimental conditions, the fragmentation will produce selective daughter molecular fragments from the primary or parent molecule. Molecular band emission will be used for the first time to the best of our knowledge for the determination of the chemical moieties and the evaluation of the chemical structure of both the API and excipients in a complex pharmaceutical formulation. MO-LIBS is an information rich multiplex spectral technique which contains both atomic and molecular emission spectra that, following analysis by multivariate approaches (i.e. chemometrics), will extract this unique combination (molecular, atomic, ionic) of spectral information from MO-LIBS. Additionally, MO-LIBS is able to avoid many of the classical spectroscopic limitations such as light scattering, diffusion, absorption, penetration depth, sample preparation, material type, etc. These inherent advantages provide a broader range of problem solving capability (e.g. accurate depth profiling, 3D chemical mapping, direct solid, liquid and gas analysis) and can be used for in-process control (raw materials), process control (blending, mixing, tableting), process understanding (PAT, quality by design (QbD), risk management and quality assurance). Although this work focuses primarily on pharmaceutical applications, the potential for MO-LIBS transcends pharmaceuticals and extends analytical science, opening the door to many useful applications.

This study investigated the laser-induced molecular bands emission coupled with chemometrics for the qualitative and quantitative analysis of molecular compounds found in a model pharmaceutical formulation. Our goal is to establish laser-induced plasma conditions that enhance selective emission of molecular fragments from the sample. By doing so, we can analyze the photons emitted by small diatomic fragments with a conventional optical spectrometer that will be mathematically processed with chemometrics to study the parent compounds such as API and excipients simultaneously. To the best of our knowledge, this spectroscopic approach is the first documented effort to conduct qualitative and

quantitative analysis of both API and excipients simultaneously using Laser-Induced Breakdown Spectroscopy or with any other atomic emission spectroscopy techniques.

As discussed, the use of atomic LIBS for pharmaceutical applications was traditionally limited to a target molecular compound (i.e. Cl, Br, P, etc) by tagging an atom characteristic of this ingredient, which is absent from the bulk formulation. This has also been a limitation for the broad application of atomic LIBS for pharmaceutical, since 70% of the API drug substances possesses these tag elements. In the present work, we carried out a chemometric study of the plasma emission from different model pharmaceutical formulations, which has resulted in the unprecedented ability to detect and accurately monitor the API in pharmaceutical formulations simultaneously a significant advance over the conventional univariate atomic LIBS approach. With the traditional univariate approach, the analysis of molecular compounds composed only of C, H, O or N atoms was nearly impossible, eliminating the possibility to detect pharmaceutical excipients. Our recent results show that the combination of LIBS and chemometrics permits the analysis of a broad range of molecular species that was not previously possible to be analyzed with the traditional univariate atomic spectroscopic speciation approach. Additionally, our recent results suggest the considerable potential for LIBS to be more widely applied to process monitoring and quality control of pharmaceuticals ingredients, and to provide information previously unavailable through other PAT sensor technology or analytical techniques: the excipient content.

Experimental section.

Apparatus and Materials. A Nd:YAG laser operating at 1064 nm (Surelite II-10, Continuum, Santa Clara, CA, USA) producing pulses of 6 ns duration (full width at half maximum) was used. The pulse energy at the laser exit was 100 mJ. The laser beam was focused on the tablet surface using a plano-convex lens (515-mm focal length), producing crater diameters of approximately 500 μm. The horizontal beam was incident on the vertical tablet surface at 90° as represented in Scheme 1.

The tablet was held in a custom-made sample holder which could accommodate 12 mm tablet. The sample holder was mounted on a motorized X–Y stage, allowing programmable analysis at several sites

on a given tablet. The experiments were performed in argon at atmospheric pressure using a 1 L/min argon flow on the target. The light given off by the laser-induced plasma was collected head-on by mirror and then directed to a 0.66-m Czerny-Turner spectrometer (McPherson, Acton, MA, USA). Using a lens, the plasma was imaged with 1:2 magnification on the entrance slit of the spectrometer, which is equipped with a 150 grooves/mm grating. The dispersed light was detected at the exit slit of the spectrograph with an intensified photo-diode array (IPDA) (Princeton Instruments, Trenton, NJ, USA) detector. The emission signal was time-resolved using a pulse generator (BNC, Model 565, San Rafael, CA, USA) (itself synchronized with the laser pulse) by sending a gating signal to the intensifier with a delay of 4 μs and width 750 ns, these conditions were found to be optimal regarding signal to noise ratio and reproducibility of the analytical signals. The pulse repetition rate was 2 Hz, allowing 99 measurements on a given tablet to provide a representative average compositional analysis in less than 50 seconds for the 3 x 3 raster (i.e. 9 sampling sites x 11 shots/site = 99 laser shots/sample). The resulting spectra were stored and processed using a personal computer.

The experimental set-up and spectral data were controlled using a custom application developed in LabVIEW 6 (National Instruments, Austin, TX, USA). Spectral data post-treatment with chemometrics was performed using a custom algorithm under Matlab 6.5 environment (The MathWorks Inc., Natick, MA, USA). The Matlab build-in singular value decomposition (SVD) function was used in order to extract the principal components used for the construction of principal component regression (PCR). For the construction of partial least square (PLS) regression a PLS2 model was used.

Calibration standards. For each standard, 6 replicate tablets were prepared. The powder mixing was done by mortar and pestle mixing for 5 minutes using the formulation quantities presented in Table 1. The solid dosage form tablets were prepared by compressing 320 mg of powder with 2,000 psi (Enerpac, P112, Whaley Bridge, High Peak, UK) for 15 sec. The furosemide API used in the model formulation was (Lot 36H0944) (Sigma-Aldrich, St-Louis, MO, USA). Avicel PH 101 (Lot 6108C), (FMC BioPolymer, Philadelphia, PA, USA) was used with Lactose 200M monohydrate (Lot M00448),

(Mallinckrodt, St. Louis, MO, USA) as excipients. Magnesium Stearate (Lot M00295, Mallinckrodt) was used in the formulation as lubricant.

Safety considerations. It is important to note that there are some safety considerations before trying to reproduce the present experimental conditions using a standard spectroscopic experimental instrument set-up. The analysis of solid dosage form by laser-induced breakdown spectroscopy will generated particulate that may be inhaled by the person exposed to the air near the experimental setup. Therefore, in order to reduce exposure to these particles that result from laser ablation of the sample, the experimental set-up should employ an appropriate aspiration system to avoid the contamination of the ambient air with potentially harmful particulates. The reader is invited to consult these references for more information[51-53].

Results and discussion.

Selectivity of the laser-induced plasma. The chemical structures of the molecules studied with the model formulation are presented in Scheme 2. It should be quickly noted that the furosemide molecular structure is the only one that contains target sulfur and chlorine atoms when compared to the other molecular structures of the excipients present in the model formulation, such as magnesium stearate, Avicel® and lactose.

The traditional approach for the analysis of the Active Pharmaceutical Ingredient (API) was to monitor the emission signal of a target element[31]. Therefore, the peak height or peak area of the signal resulting from sulfur or chlorine atomic lines, in the case of furosemide, can be plotted against the concentration to produce a calibration curve for quantification of furosemide. The advantage of such an univariate approach is that it uses simple mathematics for calibration and therefore a quantitation approach that is generally well understood by the analyst in the laboratory. However, this approach requires matrix-matched calibration standards to produce an accurate prediction[27, 29].

Following the set-up of a favorable argon atmosphere that will minimize the contribution of air to the laser-induced plasma and by the same occasion enhance the molecular band emission signal, we performed the MO-LIBS spectroscopic experiments on a series of model formulations composed of

furosemide, Avicel®, lactose and magnesium stearate. Avicel® and lactose are pharmaceutical excipients (inactive pharmaceutical ingredients) that represent typically 80 percent of the total mass of a pharmaceutical solid dosage form. In analytical chemistry, these excipients are named the matrix since they are the major constituents of the sample other than the analytes. Characteristic spectra produced from a laser-induced plasma from a furosemide pharmaceutical formulation is presented in Figure 1. The spectra presented in Figure 1 show the emission of many small diatomic fragments such as CN, CH and C_2 for a constant matrix composed of pure lactose as the excipient. Furthermore, it is also possible to observe the presence of atomic lines of carbon, hydrogen and magnesium and two ionic lines of calcium. The laser-induced spectra presented in Figure 1 shows the LIBS response for 80, 100 and 120% of the label claim for furosemide while the other excipients remain constant with 100% lactose. It is possible to observe that the tree series of molecular bands of C_2 (roughly between 455-475 nm, 500-520 nm and 540-565 nm) vary with the increase of the API concentration. The emission of the C_2 bands is in agreement with previous univariate work which associate the C_2 emission with the presence of the benzene ring in the molecule[32]. On the other hand, the two ionic lines of calcium at 393 and 397 nm in Figure 1 remain constant. The other molecular bands of CN and CH in addition with the atomic lines of H and C in figure 1 are less affected by the increase of the API concentration. These spectral behaviors indicate a correlation between variables for which chemometrics models, are presently uniquely capable of processing; which clearly demonstrate the information rich nature of MO-LIBS spectra. This is a result of the emission of the molecular bands which originate from the emission of the major mass (80%w/w of the solid dosage form) of the formulation which is pure lactose in the case of Figure 1. These data would not have been obtained with atomic LIBS usual experimental conditions.

We present in Figure 2 the mean emission spectra obtained for 6 replicate spectra of our model pharmaceutical formulations containing 3 different Avicel®/lactose ratios while maintaining the API (at 100% of the nominal content in furosemide) and lubricant concentration constant. It is possible to observe that the molecular bands present between 390 to 520 nm are selective to changes in the matrix composition. The two calcium ionic emission lines observed as the net intensity at 393 and 397 nm are

inversely proportional with the Avicel® content. This indicates that lactose contains more calcium than the Avicel® which seems obvious since lactose is generally extracted from milk. Interestingly, the presence of Avicel® seems to contribute globally to the emission bands and atomic lines in this spectroscopic window. In fact, the presence of Avicel® impacts the entire spectrum presented in Figure 2 in the same spectroscopic pattern as lactose. The later observation can be quite obvious when considering the characteristics of the molecular structures of Avicel® and lactose presented in Scheme 2. The chemical building block of Avicel® (cellulose) is nearly identical to the structure of lactose, the differences consist of two fewer hydrogen, and that Avicel® is a polymer containing between 500 and 5,000 units whereas lactose is a dimer. On the other hand, this does not explain the significant signal intensity difference observed for these two materials. A reasonable explanation for this phenomenon may be that since Avicel® is a polymer, the fragmentation might be less effective, producing more molecular fragments. In other words, the fragmentation for lactose leads to the greater production of atoms and ions and less molecular fragments than in the case of Avicel®.

Construction of chemometrics model. The MO-LIBS spectroscopic data presented in Figure 1 and Figure 2 highlight the physico-chemical properties and the statistical requirements required for the building of a reliable chemometrics model as recommended by Gemperline[54], The MO-LIBS emission spectra obtained for the calibration set and the validation set are presented in Figure 3.

It is possible to observe each distinct individual mean spectrum (n=6) in Figure 3 which reveals the selectivity of the MO-LIBS emission spectra for the different standards using this spectral window. The individual spectra were used for constructing the chemometric calibration (formulation A to O) and validation (formulation P to R) sets. The evaluation of various data pre-treatments (i.e. raw data, mean-centering, range scaling and auto-scaling) for principal component regression (PCR) and partial least square (PLS) is presented in Table 2. This table reports the corresponding root mean square error of calibration (RMSEC), the root mean square error of prediction (RMSEP) for furosemide, and correlation coefficient (R^2) obtained for the different possibilities. It is observed in Table 2 that the best combination based on our conditions is PLS coupled with auto-scaling. This combination presents the

least number of latent variables with the lowest RMSEC and RMSEP (i.e. prediction errors) for furosemide. It should be noted that the same data pre-treatments minimized also the errors for the other constituents (data not shown).

The determination of an optimal number of latent variables is considered fundamentally essential for PCR or PLS, and can be evaluated by examining the plot of the RMSEC and RMSEP against the number of latent variables shown in Figure 4 for the PLS model with auto-scaling. It is then possible to observe that the calibration error (RMSEC) and the prediction error (RMSEP) drops significantly after four latent variables which is consistent with the number of compounds in our formulation (i.e. four). The RMSEP, which corresponds to prediction error of the validation set, passes through a minimum at five latent variables before it rises again, as is often observed when building PLS or PCR models. It is important to note that additional latent variables will start to include non-significant variation in the spectra, which will then increase the prediction error (i.e. RMSEP) since the regression model will start to model random noise and other non-correlated spectral data; this is referred as over fitting in chemometrics terms.

In theory, the number of latent variables expected should be the same as the number of independent variables which is four in this case (i.e. furosemide, Avicel®, lactose and magnesium stearate). However, in practice, it is often a few more than what the theory suggest, such that non-linear behavior of the signal and uncontrolled parameters in the calibration set can require additional latent variables to build a PCR or PLS model. Since manufacturing changes influence the LIBS signal, it should be possible to identify some "buried" variables using a more complex calibration set using a design of experiment (DOE) approach with an additional independent variable such as manufacturing changes (e.g. compression strength). The fact that the number of latent variables is five compared to four independent variables is a good indication of the validity of the developed PLS model with auto-scaling. Researchers from other spectroscopic fields have shown that near-infrared spectroscopy is influenced by the compression strength[55]. Hence, this could also be the case with MO-LIBS; consequently, this

additional latent variable can be attributed to the compression strength. Present work on MO-LIBS show that the compression strength is a significant independent variable that influences MO-LIBS signals.

Validation of the PLS model for five latent variables is presented in Figure 5 for the comparison of the predicted mass in the formulation against the weighted mass for the prepared standard. The 1:1 correspondence line shows the precise agreement between the predicted values and the weighted mass. For the calibration set the predicted values are in good agreement with the weighted mass for the calibration standards, the R^2 corresponding to all formulation ingredients for the calibration set is good with a noted value of 0.964. The worst cases are observed for the prediction of Avicel® and lactose (red and green points in Figure 5), where bias between the predicted values and the weighted mass in formulation is less than 15% relative. Overall, these results are a breakthrough considering that the pharmaceutical manufacturers do not possess a process analytical sensor technology that enable the fast monitoring of excipients like Avicel® and lactose. For the other pharmaceutical ingredients furosemide and magnesium stearate (blue and cyan points in figure 5), the relative accuracy for the prediction of the validation standards, expressed in percent is less than 4%. This is an excellent result showing that it is possible to predict accurately an API without the use or requirement of a traditional tag element in atomic LIBS. Collectively the statistics for RMSEC and RMSEP are shown in Table 3.

Nevertheless, the standard prediction for lactose and Avicel® reveals more scatter than for the API and lubricant compared to the weighted mass in the formulation. Considering the fact that the standards prepared in this study where made entirely manually using mortar and pestle, weighting and non-automated compression methods; all these physical manipulations might incorporate additional random variations in the data sets. It may also indicate that several physical parameters may influence the LIBS signal as previously shown by other studies[27, 29]. This places a greater importance on the use of standard calibration sets prepared under current good manufacturing practices (cGMP) conditions. The latter may have shown less spectral bias. Alternate studies in our laboratory using cGMP prepared formulation sample sets have provided preliminary data that seem to support this hypothesis. Future work will utilize cGMP sample sets for pharmaceutical studies which will require extensive validation and robustness

testing to verify this hypothesis. Furthermore, the new calibration set should consider the potential manufacturing change/s that may influence the MO-LIBS signals using a DOE approach that will spread the variance of these independent variables.

Preliminary pharmaceutical applications with atomic LIBS focused on the determination of the API or excipients in drug formulations with tag elements. Typically, it was difficult to accurately determine the API without matrix-matched standards due to the matrix effect. The analytical requirement for standard preparation offsets LIBS intrinsic analytical advantages of efficient direct determinations without sample preparation. Additionally, the direct determination of major excipients such as Avicel®, lactose, etc was not readily accessible through traditional atomic spectroscopy techniques that were limited by sensitivity and selectivity issues. These spectroscopic and sensitivity limitations mostly confined LIBS to laboratory based confirmatory measurements, generally in a research setting. Applications such as the 3D-chemical mapping or accurate determination of coating thickness could provide important *in vitro* quality control tools to better assess *in vivo* drug bioavailability by predicting and or verifying drug dissolution. Our results indicate that it is now fully feasible to implement LIBS as an on-line or near-line PAT setting for in-process monitoring of manufacturing unit operations such as mixing or blending or even in process control. With the development of MO-LIBS and the potential for the simultaneous determination of API and excipients in complex matrices, the potential pharmaceutical applications should increase dramatically. Currently no other PAT sensor technology or analytical technique can duplicate the broad applicability of this methodology. This suggests that MO-LIBS can actively monitor and provide efficient data for real time process monitoring over a wide range of manufacturing operations. MO-LIBS could compliment existing PAT sensor technologies such as Near-Infrared spectroscopy, chemical imaging, raman spectroscopy. When coupled with stand-off capability MO-LIBS has the potential to dramatically enhance analytical capabilities in a PAT environment and evolve process understanding to a currently unimagined level with efficient real time global (surface and interior) analysis of drug blends and final solid dosage forms.

MO-LIBS may also provide a powerful pharmaceutical research tool that can enhance excipient screening, formulation development through novel dosage form assessment of excipient-API interactions, migration, tablet preparation, and ultimately formulation understanding that will provide a novel *in vitro* approach to *in vivo* performance.

Conclusions. We have successfully demonstrated that LIBS coupled with chemometrics can provide the qualitative and quantitative prediction of all ingredients present in a pharmaceutical formulation. MO-LIBS possess the distinctive capability to produce a combination of selective molecular, atomic and ionic emission signals that can differentiate between various molecules composing a pharmaceutical formulation. This novel spectroscopic approach, MO-LIBS, has significant potential for opening up new area in analytical chemistry research. There is also enormous potential when MO-LIBS is instrumentally configured in a stand-off design (for distance measurements e.g. 10 meters away from the samples) for an innovative technological advance for chemical stand-off analysis, as well as a new wide range of pharmaceutical applications. MO-LIBS instruments could be deployed across a broad range of pharmaceutical manufacturing processes.

The ability to analyze all the pharmaceutical ingredients simultaneously with MO-LIBS is a novel approach for pharmaceutical applications, since most analytical techniques or PAT sensor technologies are unable to detect in-situ formulation API or excipients like Avicel® or lactose as efficiently, specifically and globally as MO-LIBS. Additionally, this may provide new useful opportunities for improved process understanding and process optimization to better ensure the quality of the final products in a regulatory setting.

This study has clearly demonstrated that the direct detection of pharmaceutical or formulation excipients although impacted by matrix effects mitigated by MO-LIBS still required the use of high quality standard like those produced in a cGMP setting. The use of such standard will reduce spectral variability and enhance the accurate chemometrics prediction of the formulation ingredients. Yet, even this small sample-matrix based spectral variability may be minimized by instrumental and chemometric advances.

In conclusion, the development of MO-LIBS may lay the foundation for analytical and spectroscopic advances. The broad range of current LIBS applications, instrumental configurations, and novel analytical applications that result from the unique selectivity of atomic and molecular emission and adaptively made possible by LIBS. This spectroscopic technique may open up new fields in analytical chemistry.

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FIGURES.

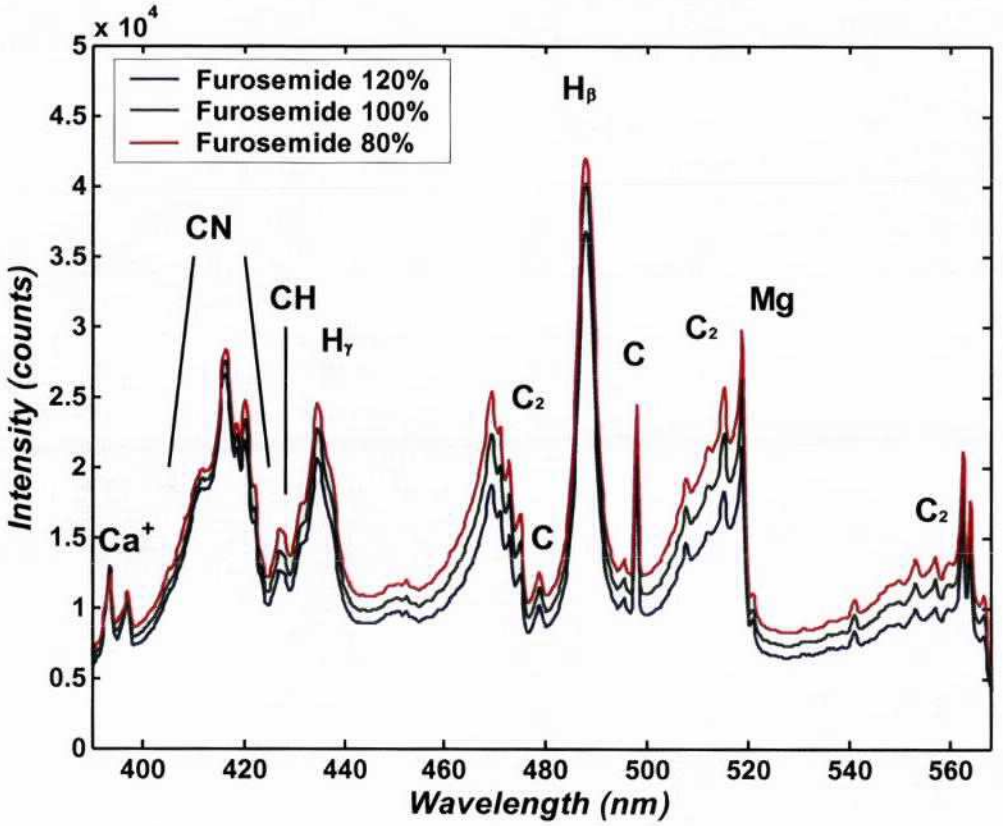


Figure 1. MO-LIBS spectra obtained for different active pharmaceutical ingredient concentrations in the furosemide formulation (lots G, H and I).

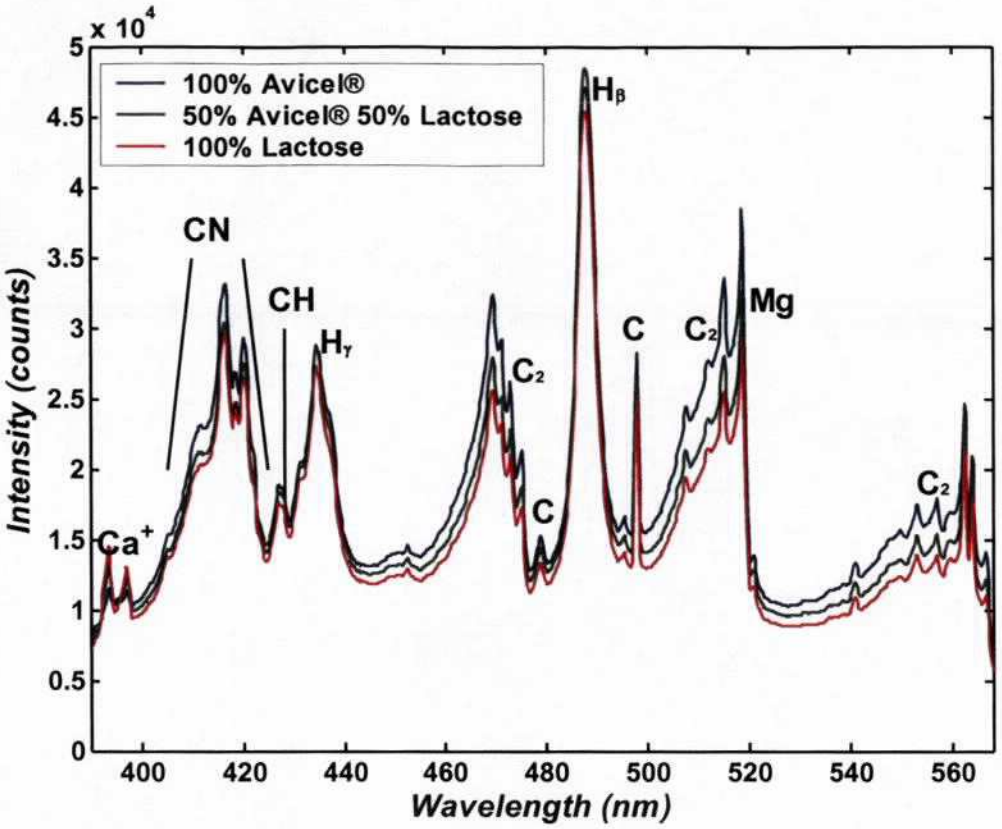


Figure 2. MO-LIBS spectra obtain for different major excipients composition in the furosemide (nominal 100%, lots B, E and H) formulation.

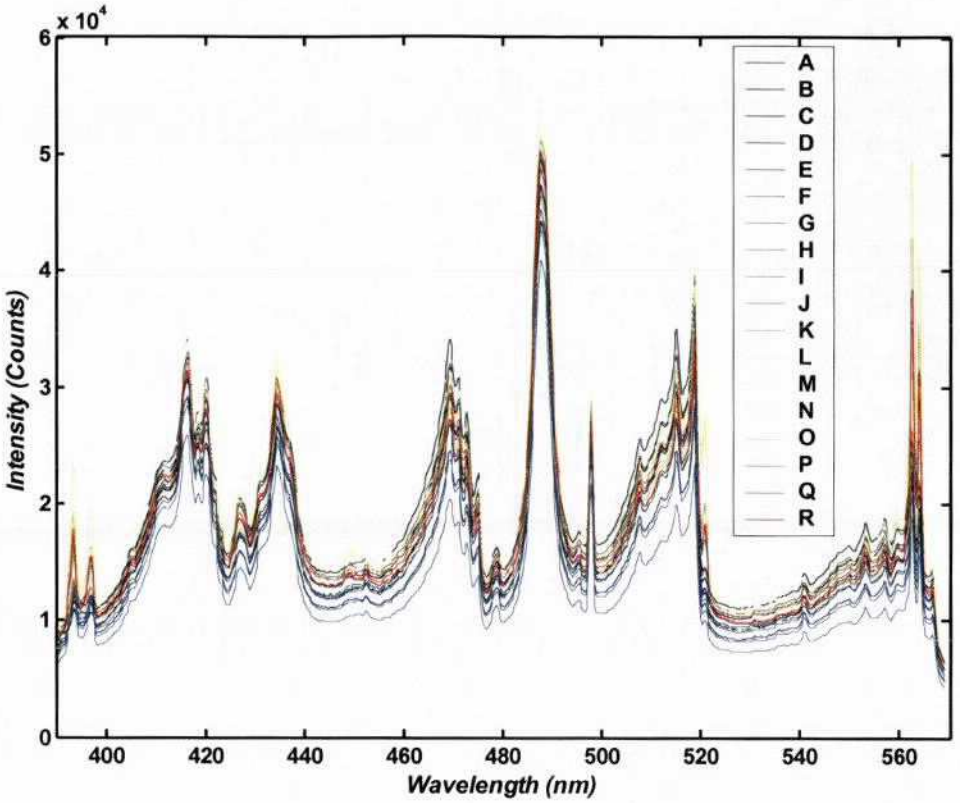


Figure 3. Mean MO-LIBS spectra obtained for the calibration and validation standard sets average spectra (n=6) for the model formulations presented in Table 1. The individual spectra were used for constructing the chemometric calibration and validation sets.

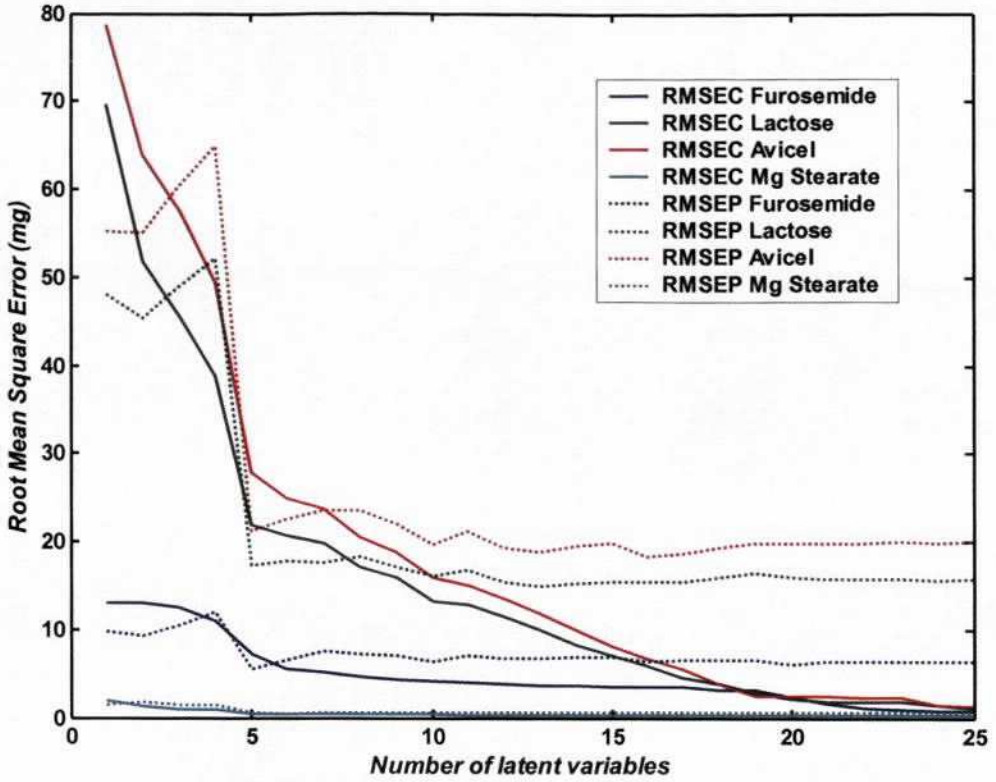


Figure 4. Plot of the root mean square error of calibration and prediction respectively for the calibration and the validation set as a function of the number of latent variable considered in the partial least square model.

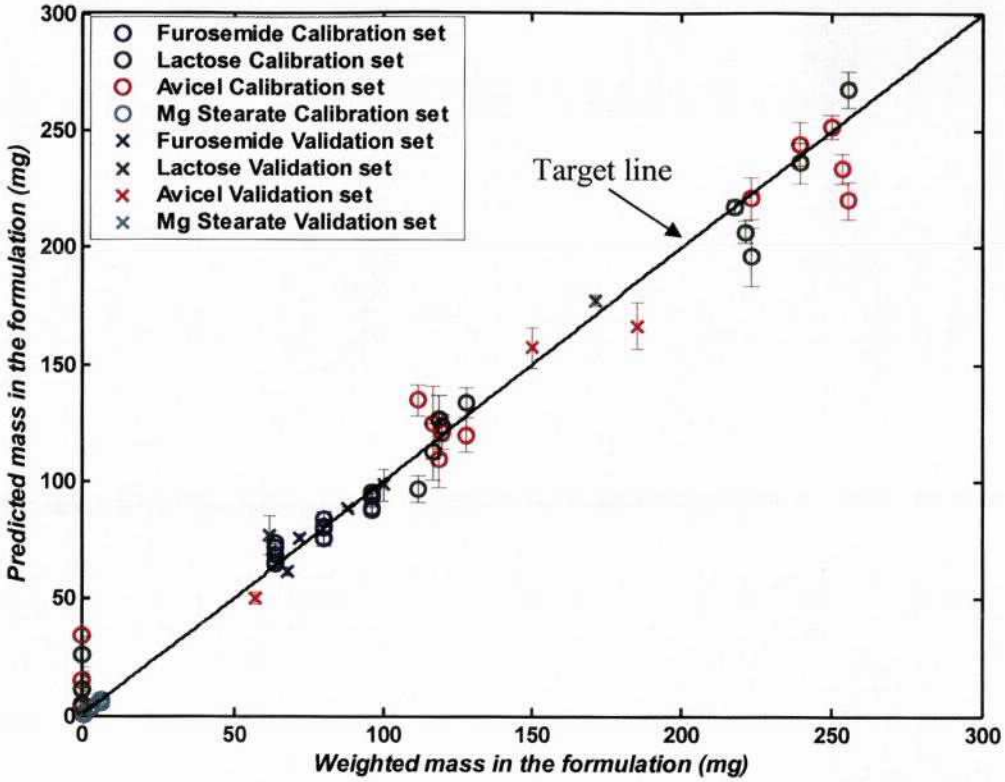
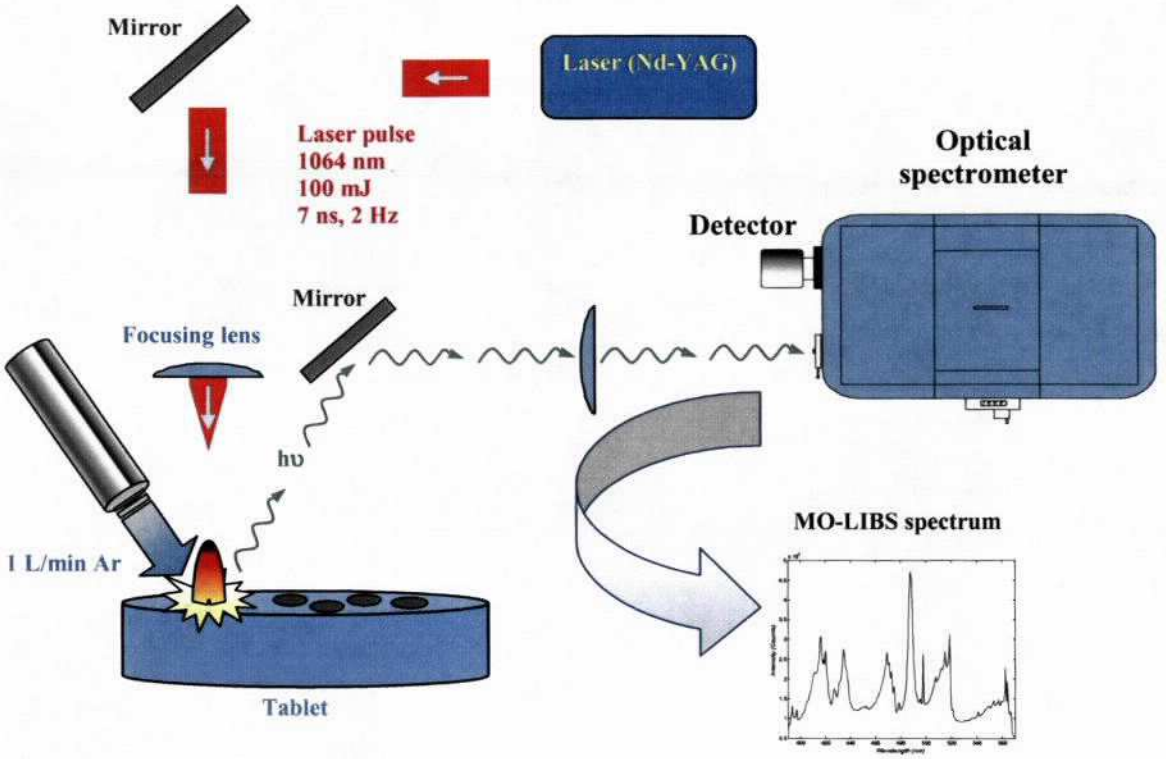
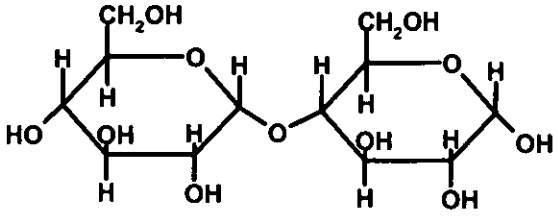


Figure 5. Comparison between the predicted mass and the weighted mass for the different compounds entering in the studied furosemide formulation for the calibration set and the validation set for the PLS model using five latent variables. Points represent the mean of the six standard replicates and error bars represent the corresponding standard error.

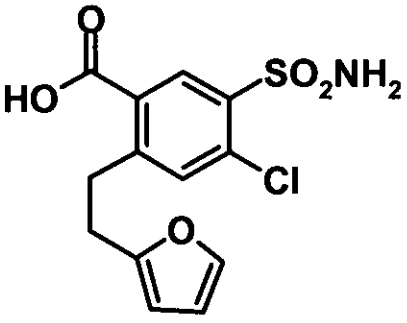
SCHEME TITLE.



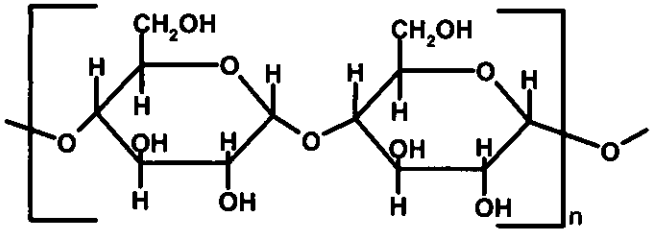
Scheme 1. Simplified representation of the experimental set-up.



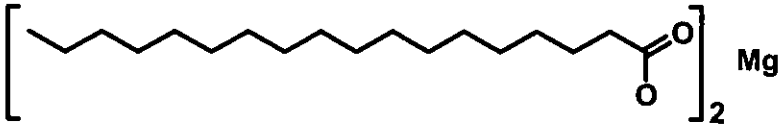
lactose



furosemide



cellulose (Avicel®)
n = 500 to 5,000 units



magnesium stearate

Scheme 2. Chemical structures of the different molecular compounds present in the model furosemide formulations.

TABLES.

Table 1. Composition of the model formulation

Standard Name	Lactose in matrix (%)	Furosemide (% of nominal)	Furosemide (mg)	Lactose (mg)	Avicel® (mg)	Mg Stearate (mg)
A	0	80	64	0	255.2	0.8
B	0	100	80	0	239.2	0.8
C	0	120	96	0	223.2	0.8
D	50	80	64	127.6	127.6	0.8
E	50	100	80	119.6	119.6	0.8
F	50	120	96	111.6	111.6	0.8
G	100	80	64	255.2	0	0.8
H	100	100	80	239.2	0	0.8
I	100	120	96	223.2	0	0.8
J	0	80	64	0	253.2	2.8
K	50	100	80	118.6	118.6	2.8
L	100	120	96	221.2	0	2.8
M	0	80	64	0	249.6	6.4
N	50	100	80	116.8	116.8	6.4
O	100	120	96	217.6	0	6.4
P[a]	25	90	72	61.75	185.25	1
Q[a]	75	110	88	171	57	4
R[a]	40	85	68	100	150	2

[a] Standard used as validation standard

Table 2. Evaluation of PCR and PLS for different data pre-treatments

Data pre-treatment	nLV [a]	Furosemide RMSEC [b] (mg)	Furosemide RMSEP [c] (mg)	R^2 [d]
PCR				
Raw data	10	6.36	6.64	0.956
Mean centering	9	7.32	5.98	0.935
Range scaling	8	6.56	6.71	0.954
Auto-scaling	7	5.99	6.88	0.959
PLS				
Raw data	5	11.0	5.94	0.952
Mean centering	5	5.93	9.99	0.929
Range scaling	5	7.30	5.88	0.957
Auto-scaling	5	7.18	5.54	0.964

[a] Number of latent variables considered in the model

[b] Root mean square error of calibration

[c] Root mean square error of prediction

[d] R-square calculated on the calibration set

Table 3. Statistics for PLS model using 5 latent variables for auto-scale data

	RMSEC [a] (mg)	RMSEP [b] (mg)
Furosemide	7.2	5.5
Lactose	22	17
Avicel®	28	21
MgSt [c]	0.50	0.53

[a] Root mean square error of calibration

[b] Root mean square error of prediction

[c] Magnesium Stearate

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