The Effects of Physical Parameters on Laser-Induced Breakdown Spectroscopy Analysis of Intact Tablets

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ABSTRACT

With the advent of the Food and Drug Administration initiatives to investigate and encourage the use of process analytical technologies, laser-induced breakdown spectroscopy (LIBS) is considered an excellent analytical tool to understand the processability of solid dosage form. In this article, the feasibility of the LIBS system for quantitation of active drug within a solid dosage form, as well as the effects of various physical parameters on its signal, is investigated. A model drug containing chlorine and sulfur was used. The examination of the specificity and reproducibility of the measurements led to the use of chlorine and carbon as the internal standard. An overall relative SD of 1.1% for the signal was found. For quantitation purposes, calibration curves using compound-X in formulated tablets were generated. It was found that curves generated from roller-compaction tablets generally gave higher LIBS signal than those generated using direct-compressed (DC) process. To investigate these differences, effect of LIBS signals from several physical properties of the tablets were examined. It was found that unmilled compound-X used in the manufacture of the tablets gave a LIBS signal 30% lower than when milled compound-X was used. However, by using multiple crushing-recompression DC process of the milled compound-X, the LIBS results were comparable with those found from both processed tablets using milled compound-X. Other physical parameters, such as wide ranges of granule size and tablet hardness found in the typical manufacturing process, had limited effect on the LIBS signal. From these results, it was noted that for accurate quantitation, it is necessary to use the same physical properties of compound-X and the same manufacturing process in the calibration standards as the actual samples.

KEYWORDS: laser-induced breakdown spectroscopy, solid sample, active pharmaceutical ingredients, physical properties, process analytical technologies

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INTRODUCTION

Laser-induced breakdown spectroscopy (LIBS) is an elemental analysis technique based on the detection of atomic or ionic emission produced by a plasma generated after the ablation of a gas, liquid, or solid sample. The plasma energy is such that the molecules are vaporized and dissociated into atoms, which are then excited and ionized and finally relaxed by emitting photons, which can be detected and quantified.[1-3]

More recently, a draft process analytical technology guidance was distributed by the Food and Drug Administration to describe a new approach encouraging the industry to develop and implement innovative tools to maintain or improve the current level of pharmaceutical manufacturing and quality assurance. This draft contributed to the growing interest in LIBS technology in the pharmaceutical industry. LIBS technology is mostly applied as an identification technique for ore[4,5] and for trace analysis in metallurgy.[4,6,7] However, in the pharmaceutical industry, the interest in LIBS started when Sabsabi and Bussière[8] patented its feasibility for rapid in situ analysis of an active pharmaceutical ingredient (API) in a solid-dosage form. They also showed that LIBS could be applied for the determination of pharmaceutical excipients used in the formulation. In line with the Food and Drug Administration goal, several articles explored different applications in pharmaceuticals. Mowery et al[9] used LIBS to determine coating thickness uniformity on film-coated tablet (FCT) using titanium atom as a marker. Good et al[10] examined the magnesium stearate distribution in powder blends. St-Onge et al[11] demonstrated the possibility of using quantitative analysis of the API in a solid-dosage form using halogen atom as a marker.

The growing interest in LIBS is attributable in part to the advantages that this technique can offer for process monitoring,[12] especially when used as a rapid at-line elemental analysis technique. Some of these advantages are the requirement for minimal or no sample preparation[9,13,14] and short analysis time ($\leq$1 minute). Furthermore, because of the ability of LIBS to provide spatial resolution (200 μm),[2,15,16] the mapping of the drug in a tablet is possible. This rapid and "real-time" feedback will allow for monitoring of dynamic changes in the manufacturing process, which will control the quality of the end product.

Although most of the studies published were qualitative analyses, some of the quantitative work did not examine the significance of the physical attributes of the samples on the LIBS signal.

To use LIBS for drug analysis, the drug must contain an element that is unique to the active ingredient and is absent from the matrix so that it can be used for identification and quantitation purposes. In addition, this element should be free from spectral interferences from the excipient or the drug itself. Because LIBS is a surface analysis technique, the energy transferred to the sample must be high enough to break the intimately bound matter that is attributable to compression. Therefore, it is anticipated that different processes used for the solid-dosage form might influence ablation, hence the necessity of investigating the effects of some tablet physical attributes on the LIBS signal. In this article, the use of LIBS for quantitation of analyte is explored. However, to develop an accurate method, the interferences and effects of physical properties of the sample, such as particle size and hardness of the tablets, had to be investigated. In addition, understanding of effects related to the roller-compaction (RC) and direct-compression (DC) processes were also investigated. The main goal was to determine and understand the physical parameters that influence the LIBS signal so that drug quantitation in solid intact tablets can be improved.

MATERIALS AND METHODS

Instrumentation

LIBS

The bench-top LIBS-based instrument (PharmaLIBS, prototype) dedicated to pharmaceutical applications was acquired from PharmaLaser Inc (Montreal, Quebec, Canada) and is illustrated in Figure 1. The equipment included a Nd:YAG (neodymium doped yttrium aluminium garnet) laser (New Wave Research Inc, Fremont, CA) operated at 1064 nm with a pulse width of approximately 7 ns. The pulse energy delivered to the samples was attenuated by means of a half waveplate and polarizing beamsplitter. The laser beam was focused slightly below (approximately 5 mm) the sample surface by a 30-cm focal length plano-convex lens, resulting in a spot size of approximately 150 μm. The spectrograph (Princeton Instrument PI320, Roper Instruments Inc, Trenton, NJ) was equipped with 3 gratings (1,200 g/mm blazed 750 nm, 1,200 g/mm blazed visible, and 600 g/mm blazed 1,000 nm). An interline readout CCD array detector (MicroMax, Roper Instruments Inc) allowed gated detection of the spectrum.

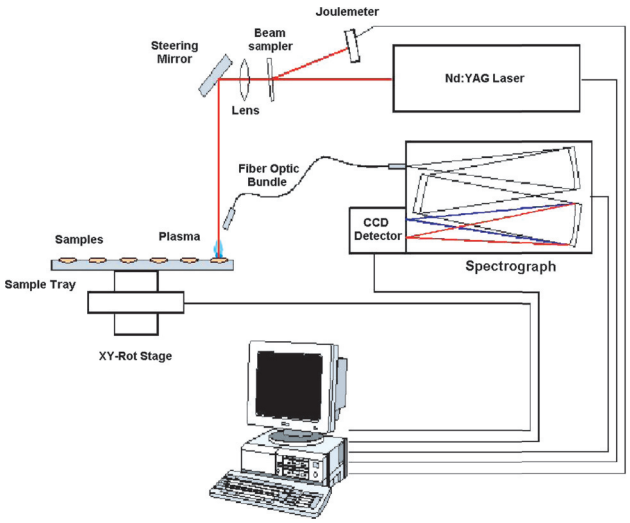


Figure 1. Illustration of the PharmaLIBS prototype.

Although the spectrograph had an operating range from 200 to 1,100 nm, the best sensitivity of the CCD camera was defined between 300 and 1,000 nm. The chlorine and sulfur spectroscopic atomic lines were chosen for compound-X. According to the National Institute of Standards and Technology (NIST) Atomic Spectra Database (http://physics.nist.gov/cgi-bin/AtData/main_asd), the most sensitive atomic lines for chlorine and sulfur were 837.6 nm and the triplet from 921.3 to 923.8 nm, respectively.

The camera delay, the camera exposure time, and the laser energy were optimized for the sulfur and chlorine using compound-X FCT. The optimized conditions are defined in Table 1.

High-Performance Liquid Chromatography Analysis

The high-performance liquid chromatography (HPLC) ultraviolet (UV) system (HP1100, Agilent Technologies, Palo Alto, CA) was composed of a dual pump, an autosampler, and a photograph-diode array detector. The data were collected using Multichrom software v.2.1.1 from Thermo Electron Corp (Woburn, MA). The HPLC conditions consisted of a 10 × 0.3 cm Inertsil-ODS-2 column (Metachem Technologies Inc, Torrance, CA) with 5-μm particle size. The column was controlled at a temperature of 40°C with flow rate of 0.6 mL/min. A 60:40 (acetonitrile-to-water) mobile phase was used for the separation in an isocratic mode. The compound-X was detected using a UV detector at a 280-nm wavelength. This procedure was able to separate the drug from the excipients.

Sample and Standard Preparation

Although the parameter optimization was performed using compound-X FCT, for simplicity, the studies described on the effect of LIBS signal used core tablets. All of the tablets were prepared with US Pharmacopeia-grade pharmaceutical excipients. RC and DC standards were compressed with a dwell time (±SD) of 5 ± 1 seconds using

Table 1. Listing of the LIBS Prototype Operating Parameter Conditions for Chlorine and Sulphur Analyses in Compound-X Tablets

Variable	Chlorine	Sulphur
Laser		
Neodynium:yttrium aluminium garnet, 1,064 nm	150 mJ per pulse	200 mJ per pulse
Q-switched 7ns pulses	2 Hz	2 Hz
Focusing optics	320 mm focal length lens (600–700 μm in diameter craters)	
	1,200 g/mm grating	600 g/mm grating
	Resolution 0.1 nm	Resolution 0.2 nm
Detection	1.0 μs delay	0.5 μs delay
	3 μs gate width	5 μs gate width
	Binning group: 300	Binning group: 300
Analytical line	Cl (I) 837.57 nm	S (I) 922.81 nm

the Enerpac manual press (GlobePharma, New Brunswick, NJ) with the same image as those of the samples. The compression pressure ranged from 500 (380 N) to 8,000 psi (6.1 kN). If not stated otherwise, the compression pressure applied on the standards was 2,000 psi (1.5 kN). All of the tablets used as standards and samples weighed 300 ± 5 mg and were prepared using the techniques described below.

RC Tablets

The samples used as unknowns were part of an engineering run and, therefore, were manufactured on an automatic press. These 20% drug load tablets were prepared from milled compound-X.

Calibration standards used to establish the calibration curves (see Figure 5) and for physical parameters studies were prepared from 30% drug load granules and were diluted to the desired drug concentration with RC placebo granules.

For the experiment on the effect of breaking strengths on LIBS signal, 20% drug load tablets were prepared using 54-μm sieved 30% drug-load granules and were diluted with 58-μm sieved placebo granules. Fifteen tablets from each breaking strength were prepared. Five tablets were used to determine the tablet breaking strength using a hardness tester (Key International Inc, Englishtown, NJ), and the rest was first analyzed by LIBS followed by HPLC analysis.

For the experiment on the effect of granule size on LIBS signal, nominal concentration tablets of 20% drug load were prepared using 30% drug load granules. The granules were initially sieved and separated. The 188-μm sieved placebo granules were then used to dilute the separated 30% drug load-sized granule to make the 20% drug load tablets with specific granule size.

DC Tablets

Standards of 30% drug load were prepared by geometric mixing of pharmaceutical materials. Appropriate pharmaceutical excipients and compound-X were weighed and mixed with a mortar and pestle without putting any pressure to avoid attrition. For standards with drug loads <30% (w/w), a placebo blend was used for dilution. For the experiment on the effect of API particle size on the LIBS signal, 25 DC tablets using milled or unmilled compound-X (Table 2) were crushed and recompressed. For each compression, 10 tablets were isolated before the next recompression. DC tablets and tablets after a second compression were collected and compared.

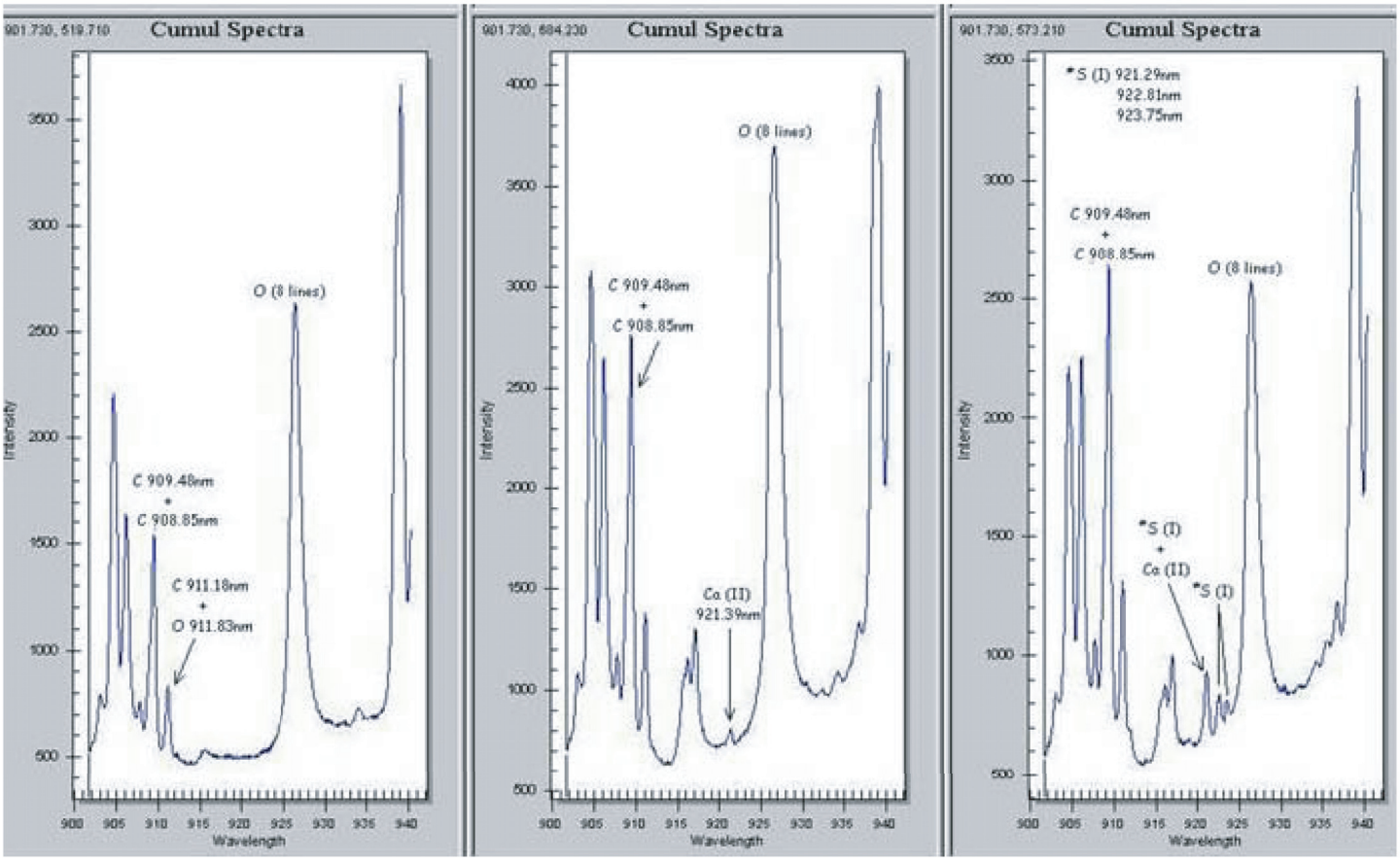
Tablet markings were used to differentiate the upper and lower side of the tablets. For manually pressed tablets, the markings were on the lower side, whereas for the tablets compressed using the automatic feeder, the same markings corresponded with the upper side.

Statistics

Treatments were compared using either the single-factor analysis of variance (ANOVA) test or the *t* test for 2 samples assuming unequal variances. The ANOVA test was applied when multiple groups are compared. The *t* test was applied to compare the mean of 2 samples that were prepared differently. These 2 statistical functions were

Table 2. Particle Size Characteristic of Milled and Unmilled Compound-X

Variable	Milled	Unmilled
Mean of average	7.3 μm	52 μm
95% of the material is less than	21.3 μm	114 μm



Sample holder | Compound-X placebo tablet | Compound-X 20% drug load tablet

Figure 2. LIBS spectra using a 600 gooves/mm grating for sulfur line identification after laser ablation (150 mJ laser energy, 1 μs camera delay, 3 μs camera exposure).

available in Microsoft Excel from MS Office 97. Calibration curves were fitted using the linear or second-order polynomial equation found in PharmaLIBS software version 1.2.

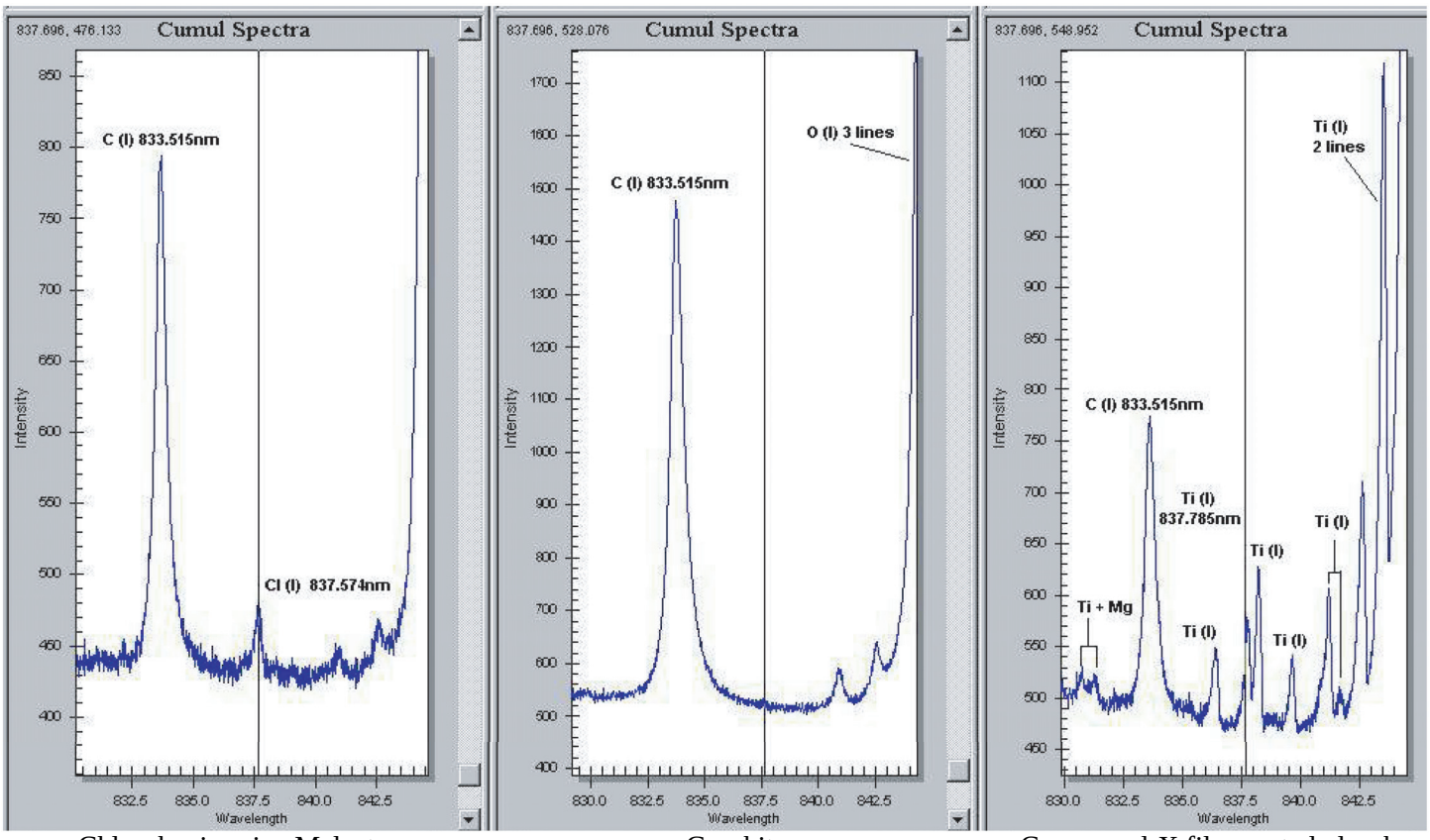
RESULTS AND DISCUSSION

Specificity of Measurement and Reproducibility

The specificity of measurement for compound-X was investigated using sulfur and chlorine atoms to determine whether they can be used as markers. According to the NIST Atomic Spectra Database, the most sensitive atomic line for chlorine is 837.6 nm and the triplet from 921.3 to 923.8 nm for sulfur. To ensure that the theoretical and instrumental chlorine wavelength was comparable, chlorpheniramine maleate tablets containing cellulose were used. Because these tablets had a more simple matrix, the determination of the chlorine line was easier than using compound-X core tablets. This type of confirmation was not necessary for the sulfur lines, because the presence of triplet lines was unique enough.

Sulfur

For sulfur, 3 spectral lines could be used: 921.3 nm (the most sensitive), followed by 922.8 nm and 923.8 nm (the least sensitive). Sulfur spectral interferences using LIBS were investigated at 600 gooves/mm grating with compound-X tablets, to allow for the coverage of a wider wavelength range. To determine the possible interferences of the sulfur lines, several control samples were used. In Figure 2, the first panel shows the LIBS spectrum after the ablation of the sample holder, made of Delrin. This allowed for the oxygen, nitrogen, and carbon lines from the ambient air to be detected. In the second and third panel of Figure 2, spectra after the ablation of compound-X placebo tablet and 20% drug load core tablet are illustrated, respectively. A placebo tablet was used to verify the spectral interferences contributed by the pharmaceutical excipients. For instance, in panel 3, the calcium line from the placebo interfered with the sulfur line at 921.3 nm. Alternatively, the sulfur spectral line at 922.8 nm was used. However, because this line was located at the ascending slope of the oxygen line (927.5 nm), accurate integration



Chlorpheniramine Maleate | Graphite | Compound-X film coated placebo

Figure 3. LIBS spectra using a 1,200 gooves/mm grating blazed at 750 nm for chlorine line identification after laser ablation (150 mJ laser energy, 1 μs camera delay, 3 μs camera exposure).

of this line may be affected. Because of these pitfalls, the use of the sulfur line may not be appropriate for the accurate quantitation of compound-X. Because compound-X also contained chlorine that could be used as a marker, the specificity of the chlorine line at 837.6 nm was also investigated.

Chlorine

Chlorpheniramine maleate core tablet (Figure 3, panel 1) was used to confirm the chlorine line at 837.6 nm, which is marked in the different panels by a vertical line. Compound-X placebo FCTs (Figure 3, panel 3) were also analyzed to determine spectral interferences from the matrix and coating. Titanium contained in the film coating was not well resolved from the chlorine line, which would contribute to increase variability in the measurement. This problem was alleviated by either manually removing the film coating before analysis or by ablating the coating with the LIBS laser as was done when measuring film coating thickness.[9] As a result, subsequent investigation in the quantitation of compound-X was done using the chlorine line.

Reproducibility

The use of an internal standard was found to be necessary to normalize the signal intensities so that the variation found in energy distribution from shot to shot could be overcome.[11] This normalization increased the reproducibility by subjecting both the analyte and the internal standard to the same energy distribution.

Internal standardization required the presence of an element that emitted in the same spectral region of the analyte and should contain an excitation energy similar to the analyte. This technique corrected the changes in the energy distribution within the plasma for both the analyte and the standard. It was found that in pharmaceutical tablets, carbon can be used as the internal standard, because this is present in all organic molecules. The carbon line at 833.5 nm and the chlorine line at 837.6 nm were found to have similar excitation energies, which were 9.17 eV and 10.41 eV, respectively, and were chosen for the accurate quantitation of compound-X.

To ensure that the carbon line was appropriately chosen, pure graphite disks (Figure 3, panel 2) were used to locate the carbon emission lines seen in the spectral window

Table 3. Statistical Single-Factor ANOVA of the Chlorine Concentration on Carbon 833 nm Integration

Drug Content	Count	Sum	Average	Variance	F	F crit
20%	5	1039965	207993	18970454	1.05	3.24
25%	5	1024732	204946.4	8100810.8		
30%	5	1020285	204057	25118776		
35%	5	1017971	203594.2	22105155		

including the emission coming from ambient air. According to the NIST spectral database, the carbon line at 833.5 nm may contain a small chlorine contribution resulting in a potential interference. To verify this, an evaluation was performed to determine the carbon line intensity variation with respect to compound-X concentration. The statistical ANOVA showed in Table 3 revealed that the observed F value was a factor 3 lower than the F critical value. This indicated that the variation was randomized and that these differences between the groups, that is, the carbon mean signal values at different levels of compound-X, were not statistically significant. Therefore, the 833.5-nm carbon line was appropriately selected as internal standard.

To test the selected lines for reproducibility of measurement, 5 compound-X core tablets were analyzed on 10 sites with 12 shots per site and the chlorine-to-carbon ratio measured. Relative standard deviation (RSD) improved from 7.5% to 1.1% with chlorine standardization. Furthermore, normalization with the carbon line also improved the day-to-day reproducibility, which is illustrated in Figure 4.

Calibration Samples

The 2 common processes used for manufacturing tablets at our facility are DC and RC. To develop a quantitative method for compound-X, the calibration standards were prepared using both processes. Figure 5 shows the difference in the calibration curves from the 2 processes. The plots showed that the LIBS response curve for RC tablets was higher than for DC tablets. To understand the differences in these responses, it is important to determine the factors affecting the LIBS signal. Because LIBS is based on a surface analysis, the response will be highly affected by the physical properties of the sample. As a result, the physical property differences between these processes were examined.

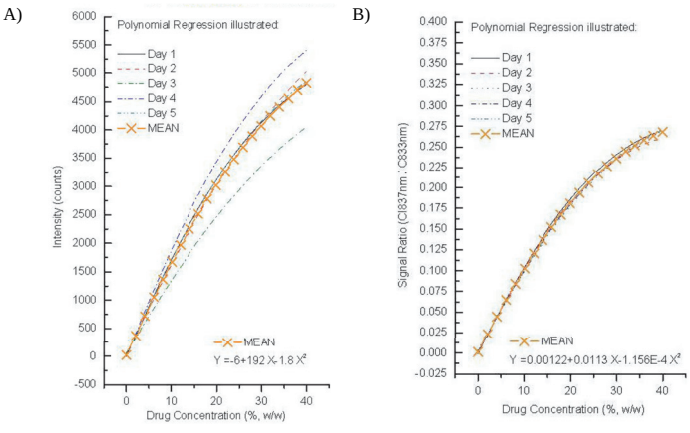


Figure 4. Effect of chlorine standardization with carbon line on calibration curve for day-to-day reproducibility. A, chlorine (837.5 nm) calibration curves without standardization; B, chlorine (837.5 nm) standardization with carbon line.

Physical Attributes of Tablets

Effect of Tablet Breaking Strength on LIBS Signal

RC tablets of different hardness were prepared using granules of similar diameter where the drug and placebo granule average diameters were 54 and 58 μm, respectively. The samples were prepared by using different compression pressures (Figure 6). In this figure, the hardness values found for each compression pressure is indicated for each point. The LIBS signal, as represented by a hollow triangle with its SD, was shown for each compression pressure. The dark circle symbol illustrates the HPLC results measured on the same tablets used for LIBS analysis.

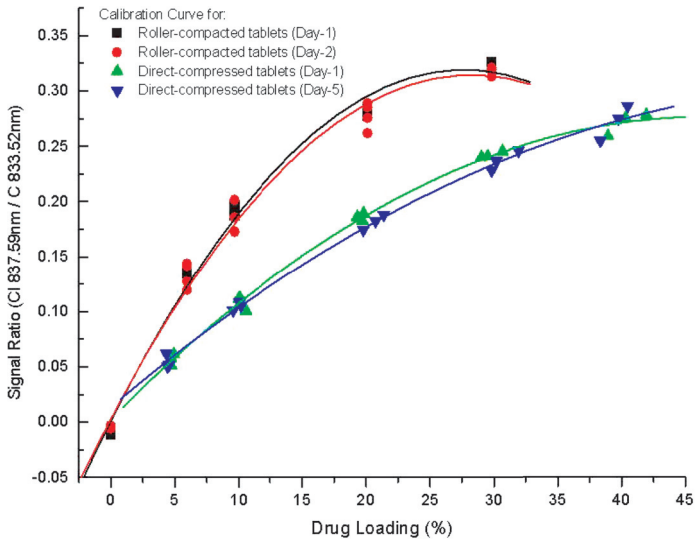


Figure 5. Compound-X calibration curve monitoring chlorine from RC tablets of milled compound-X (circles and squares) and DC tablets of unmilled compound-X (triangles).

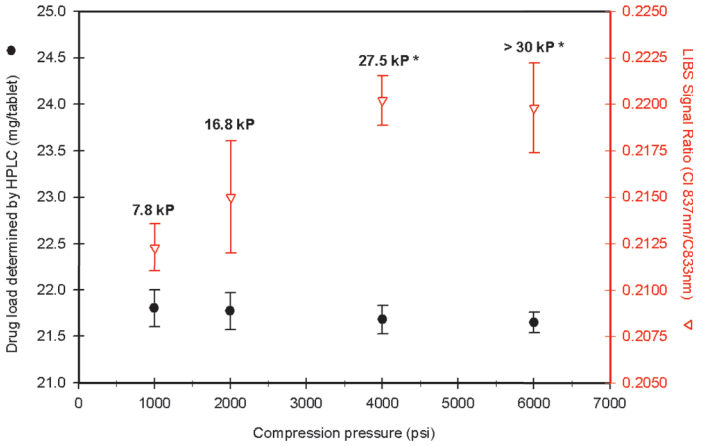


Figure 6. Effect of hardness on LIBS signal from tablets made of 54-μm compound-X granule diameter and 58-μm placebo granules. LIBS signal (▽) and HPLC signal (●). Bars, ±SD. ANOVA for LIBS signal: $F_{1,8\ (P\ =\ 0.05)\ (7\text{-}17\ kP)} = 3.64$, $F_{1,8\ (P\ =\ 0.05)\ (17\text{-}27\ kP)} = 12.7$, $F_{1,8\ (P\ =\ 0.05)\ (27\text{-}30\ kP)} = 0.05$.

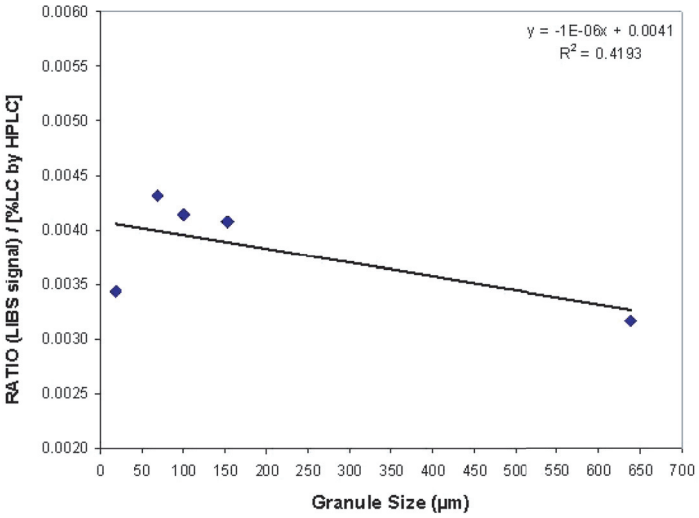


Figure 7. Ratio of LIBS signal over HPLC percentage label claim of compound-X relative to granule size. The label claim was 20% (w/w).

Figure 6 shows that hardness influences the overall LIBS signal in the 7 to 30 kP range. However, the LIBS response from 7 to 17 kP, which is in the specified range used for manufacturing tablets, was not statistically different based on the ANOVA single-factor test. It is noteworthy that for this formulation using the same dwell time, the density of the compacts was similar in the 9 to 17 kP hardness range. The similarity of the LIBS response in this hardness range was additionally confirmed by the low RSD values for both LIBS and HPLC (Table 4).

At the higher tablet breaking strength (>17 kP), the LIBS signal was considerably higher than those found at the lower hardness. This difference in LIBS signal was not attributable to changes in the drug content, because the HPLC values remained constant throughout the range. This indicated that the much harder surface of the samples affected the penetration of the laser beam, which, in turn, could reduce the quantity of ablated materials. This decrease in the ablated material could create a less dense cloud, which affected the distribution of the plasma energy required for the excitation of the atoms. On the other hand, a less-dense cloud caused by the harder surface could increase the signal, because the diffraction of emitted photons was less significant. However, at the high-breaking strength, the LIBS signal reached a plateau when no additional ablation of the surface and no additional excitation of the analytes can be achieved.

Table 4. Relative Standard Deviation (RSD) Data Appearing in Figure 6 (n = 5)

	RSD (%)		
Hardness	**Hardness**	**HPLC**	**LIBS**
7.8 kP	4.7	0.9	0.6
16.8 kP	5.1	0.9	1.4
27.5 kP	2.6	0.7	0.6
> 30 kP	N/A	0.5	1.1

Effect of Granules Diameter on LIBS Signal

The study of the effect of granule diameter on LIBS signal was investigated using RC tablets, which were prepared as described in the experimental section. These tablets were first analyzed by LIBS and then by HPLC to determine the exact drug concentration. The ratio of the LIBS signal to the percentage label claim was used to take into account the differences in compound-X concentration. The correlation of the ratio with the different granule sizes, as shown in Figure 7, was fitted to a straight line. The linear regression gave a low R^2 (0.4193) and a small slope (-1×10^{-6} μm^{-1}). The data showed no correlation between the LIBS signal and the granule sizes.

From the data on Figures 6 and 7, the LIBS signal differences observed in Figure 5 between the RC tablets and the DC tablets were not related to the tablet hardness nor the granules size used to prepare the tablets. Therefore, other physical attributes between the DC and RC had to be additionally examined.

Effect of the Number of Compression and the Compound-X Milling Status on LIBS Signal

The difference between the DC and RC processes used in Figure 5 is that the DC tablets were compressed from blends of all of the pharmaceutical ingredients, whereas the RC tablets were prepared from granules of milled compound-X, which were made by breaking up a thin ribbon

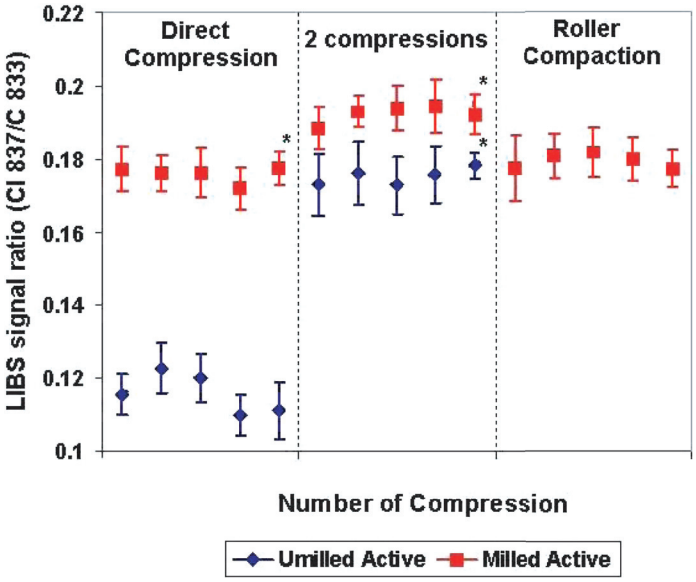


Figure 8. LIBS signal of RC tablets of milled compound-X and tablets prepared from milled or unmilled compound-X after different number of compression. (◆), unmilled compound-X; (■), milled compound-X; *, statistically different when compared with RC tablets.

prepared previously from the compression of all of the pharmaceutical ingredients and followed by compression of the granules. In other words, in DC tablets, the compound-X was subjected to 1 compression, whereas in RC tablets, it was subjected to 2 compressions. Generally, the RC process is used to eliminate segregation as a result of an increase in the homogeneity of the flow of the excipients and compound-X, on the other hand, in the DC process, an additional milling step is incorporated to give the same effect. Beside the compression differences, it is also worth noting that the tablets used in the RC calibration curves were made using milled compound-X, whereas the DC calibration curves were prepared from unmilled compound-X.

As a consequence, the effects of the milling of compound-X and the number of compressions of compound-X tablets on the LIBS signal were pursued. Milled and unmilled lots of 20% (w/w) of compound-X-loaded tablets manufactured by DC with a compression pressure of 2,000 psi were initially used in this investigation. In Figure 8, 5 tablets were analyzed for each compression and were compared with the RC tablets. Note that each point on the plot represents the mean of 120 ablations (10 sites × 12 shots per site). The results showed that signals from milled and unmilled compound-X tablets gave a significantly different LIBS signal on the first compression (DC). Milled compound-X gave a 30% higher LIBS signal than the unmilled compound-X tablets. These data tend to suggest that a smaller particles size resulted in an increased LIBS signal.

To additionally pursue the differences in the 2 processes, an additional crushing-recompression cycle for both the milled and unmilled compound-X was investigated. In Figure 8, the DC tablets using unmilled compound-X showed a significant increase in the LIBS signal after a second compression cycle, as determined by the two-tailed *t* test. Similarly, on the second compression, the LIBS signals from milled compound-X were also significantly different from the unmilled compound-X, however, to a lesser extent than on the first compression. In both cases, the milled compound-X tablets gave higher LIBS signal than the unmilled tablets. Moreover, the unmilled compound-X made from the second compression of DC showed a comparable LIBS signal to that found for the RC tablets. Although the LIBS signal was close, this was not statistically the same. In all of the cases of the milled tablets (DC versus RC), the DC tablets on first compression gave a lower signal than the RC tablets, whereas the second compression gave a higher signal than the RC tablets. These differences in LIBS signal could be attributable to the additional reduction of the particle size of compound-X that was produced during the compression/recompression of the granules.

To demonstrate the closeness of the LIBS signal of milled materials made from DC and RC, new calibration curves prepared manually using different processes are summarized in Figure 9. In this plot, an overall 14% lower signal was found for DC tablets when compared with RC tablets, whereas in Figure 5, a 38% signal difference was found. On the other hand, when milled compound-X is subjected to a second compression, the calibration curve is almost superimposable to that from the RC tablets with a 3% difference in signal. Therefore, it is important to note that for accurate quantitation calibration, curves need to be processed the same way as that of the samples.

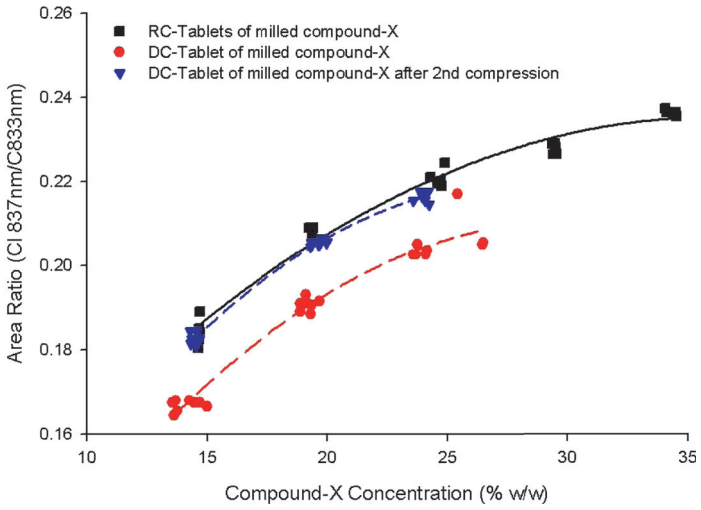


Figure 9. Comparison of calibration curves from milled compound-X tablets prepared by DC (●) and by RC (■).

CONCLUSION

This article illustrated several important factors when using LIBS for quantitation. It was found that when choosing your marker for quantitation, it is important to verify the specificity of the chosen spectral line so that it is free of any spectral interference. The use of the carbon line as internal standard also improved the shot-to-shot and the day-to-day reproducibility for high-energy excited atoms. In this study, the overall chlorine signal RSD improved from 7.5% to 1.1%.

It was also shown that before developing the quantitative method, it is important to understand the effect of tablet physical attributes on the measurement. LIBS signal was mostly affected by the milling of the API and the number of compressions. These affected the particle size of the analyte and, thus, the energy transfer for ablation, as well as the energy distribution for volatilization, atomization, and excitation. Furthermore, it was shown that manufactured tablet hardness in the 7 to 17 kP range does not affect the LIBS signal. Moreover, the tablet breaking strength studies showed that at extreme hardnesses, the LIBS signal was influenced by the amount of ablated material, which affected the cloud of matter after the ablation, which, in turn, affected the transfer of emitted photons from the plasma to the detector. Therefore, it is important to consider that for quantitation, the standards must have the same physical attributes as the samples so that accurate quantitation can be achieved.

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