International Journal of Pharmaceutics 391 (2010) 13–20

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Light induced fluorescence for predicting API content in tablets: Sampling and error

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ARTICLE INFO

Article history:
Received 9 October 2009
Received in revised form 23 January 2010
Accepted 8 February 2010
Available online 13 February 2010

Keywords:
Tablets
Surface analysis
Fluorescence
PAT
Content uniformity

ABSTRACT

The use of a light induced fluorescence (LIF) instrument to estimate the total content of fluorescent active pharmaceutical ingredient in a tablet from surface sampling was demonstrated. Different LIF sampling strategies were compared to a total tablet ultraviolet (UV) absorbance test for each tablet. Testing was completed on tablets with triamterene as the active ingredient and on tablets with caffeine as the active ingredient, each with a range of concentrations. The LIF instrument accurately estimated the active ingredient within 10% of total tablet test greater than 95% of the time. The largest error amongst all of the tablets tested was 13%. The RMSEP between the techniques was in the range of 4.4–7.9%. Theory of the error associated with the surface sampling was developed and found to accurately predict the experimental error. This theory uses one empirically determined parameter: the deviation of estimations at different locations on the tablet surface. As this empirical parameter can be found rapidly, correct use of this prediction of error may reduce the effort required for calibration and validation studies of non-destructive surface measurement techniques, and thereby rapidly determine appropriate analytical techniques for estimating content uniformity in tablets.

1. Introduction

1.1. Quality control of tablets

Ensuring that the tablets manufactured are within their active pharmaceutical ingredient (API) content specification is critical to producing safe, economic tablets. Current methods of demonstrating that tablet batches meet specifications involve destructively assaying a small fraction of the tablets in each batch. When inconsistency in API content between sampled tablets is found, it causes entire batches of tablets to fail and be discarded. In potentially more serious cases where inconsistency is not found, tablets that appear to meet specifications, but actually do not, are released to the public and may cause harm to patients.

It would be ideal to have a rapid, non-destructive, accurate estimation of the API content in every tablet as it is manufactured. This study is working toward that goal by further demonstrating light induced fluorescence (LIF) as a surface measurement to predict the total content of a fluorescent API in a tablet. This technique has been demonstrated in pharmaceutical blending applications (Lai and Cooney, 2004; Lai et al., 2001) and a preliminary study on tablets has been completed (Lai et al., 2004). The applicability of the LIF technique is dependent upon the strength of fluorescence of the component to be tracked (e.g. the API) relative to the fluorescence of other components within the tablet (e.g. excipients). As described in prior work, an estimated 60% of the top two hundred API molecules fluoresce while most common excipients (lactose, microcrystalline cellulose, starch, etc.) do not significantly fluoresce (Lai et al., 2004). Fluorescence has also been successfully used previously to monitor the content of a drug in urine and serum (Ocana, 2000). This study demonstrates a number of LIF sampling strategies and the theoretical error associated with the surface measurement and compares this error to the experiments.

1.2. Methods for rapidly estimating tablet concentration

A significant amount of recent research has been designing and investigating suitable methods for quickly estimating API concentration of tablets without destroying the tablets. Most of these techniques rely upon spectroscopic analysis. All of these methods are significantly faster than the current quality control practice of dissolving the entire tablet and then assaying with HPLC or UV absorption. LIF has the potential to be advantageous in the use of monitoring fluorescent compounds due to its speed, univariate data for straightforward analysis, relatively low cost, and sensitivity to differentiate active content at low concentrations (Lai et al., 2004).

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doi:10.1016/j.ijpharm.2010.02.009

1.2.1. Raman spectroscopy

Near-infrared FT-Raman spectroscopy was used by Vergote et al. (2002) to determine content of an active ingredient (diltiazem hydrochloride) in both commercial and experimental tablets with good accuracy and precision. The technique that was used took ten minutes per tablet and involved averaging spectra from five different locations on each tablet. Raman spectroscopy can be utilized through some packaging material. The instrument used was calibrated for each session (a start-up time was required).

Similarly, FT-Raman was used by Szostak and Mazurek (2002) to determine content of acetylsalicylic acid and acetaminophen in tablets with very good agreement to UV–vis results. Each spectra required about 90 s to collect. Bell et al. (2000) were able to detect ecstasy and related compounds. Niemczyk et al. (1998) were able to analyze content through gel capsules. Breitenbach et al. (1999) used Raman to map drug content on solid surfaces. More recent work by Wikstom et al. (2006) have utilized low-resolution Raman to accurately determine tablet content while taking approximately five spectra per second.

Raman spectroscopy has also been used to determine the solid state form of some drugs in tablets by Taylor and Langkilde (2000). The solid state form of some components was found to change after Raman sampling because of local overheating by the laser. This change in solid state form could alter the drug dissolution or bioactivity.

1.2.2. Near infrared

Both reflectance and transmittance near infrared (NIR) have been employed to estimate total tablet content. One recent study by Thosar et al. (2001) compares the two NIR methods for a range of active (1–20%) in tablets. Both methods had difficulty with low concentration determination (1% and 2%).

Work by Maik et al. (2001) analyzed a set of aspirin tablets packaged in blister packaging for salicylic acid and water content. The set contained 1300 tablets and the 43 tablets with the best NIR readings were compared to HPLC results and the standard error of prediction was 0.06%. A standard was used for calibration before use of the instrument on new days or under new conditions. This technique required minutes of time to collect the data, but was able to capture information about a large number of packaged tablets.

Excellent agreement between transmission NIR and HPLC for content of steroid tablets was found in a complete study by Broad et al. (2001). The sample time was about 35 s per tablet monitored. NIR was used by Chen et al. (2001) in the successful prediction of both content of theophylline in tablets and tablet hardness. Study by Ritchie et al. (2002) and Mark et al. (2002) of NIR transmission spectroscopy for determining active content in both tablets and capsules was successful in its ability to estimate content compared with HPLC. A similar recent study by Ito et al. (2008) on individual caffeine tablets of approximately 30% caffeine demonstrated agreement between NIR spectroscopy (both reflective and transmission) and HPLC. Other work with NIR has shown that it is a valuable technique with a wide range of applications (Blanco et al., 2000a,b; Scafi et al., 2001; Neubert et al., 1997; Buchanan et al., 1996; Moffat et al., 2000; Herkert et al., 2001; Gowen et al., 2008; Sulub et al., 2008; Moes et al., 2008).

1.2.3. Laser-induced breakdown

Laser-induced breakdown is able to analyze heteroatoms in a section of the tablet by vaporization and a spectrograph (St-Onge et al., 2002). The vaporization results in a crater approximately ten microns deep and four hundred microns in diameter. After each laser event, an extraction hose removes aerosol particles. Each tablet was fully analyzed with about one hundred laser events in about one minute. The accuracy and precision were good (RSD 1–4%) but are semi-destructive. The technique can also be used to monitor magnesium stearate with high sensitivity.

1.2.4. Micro-thermal analysis

Micro-thermal analysis uses a modified atomic force microscope (AFM) to measure the local heat resistance of a substrate (Royall et al., 1999). The method is slow and highly dependent upon local topography and has therefore not been used widely.

2. Materials and methods

This study demonstrates the use of a LIF instrument to estimate API content in tablets of two different sets of ingredients. One set of tablets contained triamterene (GlaxoSmithKline, Philadelphia, PA) as the API with lactose and colloidal silicon dioxide as excipients. The other set of tablets contained caffeine (Bristol Meyers Squibb, New Brunswick, NJ) as the API with microcrystalline cellulose, lactose, and magnesium stearate as excipients. After all of the LIF sampling on the tablets was complete, UV spectroscopy analysis was performed on each tablet to determine the total amount of API. UV spectroscopy was chosen for its high reproducibility and acceptance as a standard analytical technique for content analysis in the pharmaceutical industry. Separate calibration and validation tablet sets were used for each set of tablets. The accuracy of the LIF estimation was determined by comparison to the UV analysis results for each tablet with the validation sets.

In addition to demonstrating the use of the LIF instrument, a theoretical description of the accuracy of the estimation of API content in tablets was developed. This theory allows for the prediction of the accuracy of the method in other cases and using different sampling techniques.

The tablets, instruments, and statistical approaches used in this study are presented in the following sections.

2.1. Pharmaceutical tablets used

The triamterene tablets were made in four batches in a Carver hydraulic tablet press (Carver Laboratory Equipment, Wabash, IN) with compression of approximately two-tenths of a ton compression load as described in previous work (Lai et al., 2004). The triamterene tablets averaged 320 mg in weight and were cylindrically shaped. The average height was 4.2 mm and the diameter was 8.8 mm. The first three batches contained 1.64%, 3.22%, and 4.75% triamterene by weight respectively. The other ingredients in the triamterene tablets were anhydrous lactose and syloid (silicon dioxide, 0.1% by weight). The fourth batch was designed to make less homogeneous tablets by simultaneously placing some of each of the powder blends from the first three batches into the tablet press hopper with no additional mixing as described in previous work (Lai et al., 2004).

The caffeine tablets were produced in three batches on a 16-station beta press with three-ton compression loads. The three batches contained 5%, 10%, and 20% caffeine respectively. The other ingredients in the caffeine tablets were microcrystalline cellulose (40%, w/w), lactose (59.5–39.5%, w/w), and magnesium stearate (0.5%, w/w). The caffeine tablets averaged 500 mg in weight and were cylindrically shaped with an average height of 3.4 mm and diameter of 12.9 mm.

2.2. LIF instrument and filters

The light induced fluorescence (LIF) instrument used in this study was developed at MIT and the most recent version was manufactured by Honeywell primarily for use in blend uniformity monitoring. It has been described in previous publications (Lai and

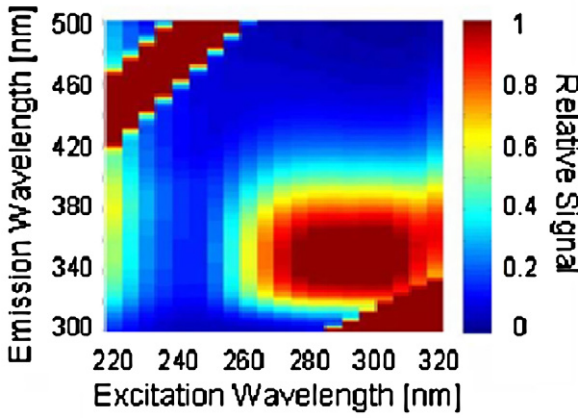

Fig. 1. Relative absorbance spectra of caffeine.

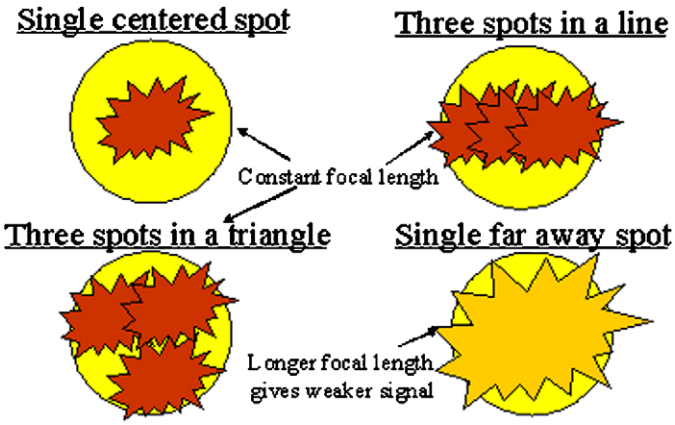

Fig. 3. LIF tablet sampling strategies.

Cooney, 2004; Lai et al., 2004). The instrument monitors fluorescence of a sample by illuminating it with light at an excitation wavelength and collecting the resulting emission light at a second, higher wavelength. The specific wavelengths used are dependent on the fluorescence spectra of the sample and are selected with a set of optic filters.

The current instrument is able to collect fluorescence samples as fast as 100 Hz. A power setting comparable to normal artificial light is used to limit photo-bleaching damage to the tablet. The data is transmitted from the instrument to a computer by radio frequency. The data is processed and displayed on the computer using instrument-specific software provided by Honeywell.

The filters used in the optic block of the LIF instrument need to be selected for the API of interest. The fluorescence properties of triamterene have been described previously (Lai and Cooney, 2004; Lai et al., 2001; Lai et al., 2004). The filter set used in this study was the same as in previous work, the "XF22" set from Omega Optics Inc. (Brattleboro, VT). None of the other tablet ingredients were significantly fluorescent near the excitation wavelengths of triamterene.

Caffeine is naturally fluorescent, absorbing light in the range of 260–320 nm and emitting in the range of 320–400 nm. The fluorescence spectra of caffeine are shown in Fig. 1. None of the other tablet ingredients were significantly fluorescent near this wavelength. From the known deep UV light absorbance of caffeine, optical filters were selected for use in the LIF instrument. A filter set named "XF01" from Omega Optical, Inc. (Brattleboro, VT) was used. This filter set allowed for excitation centered at 254 nm (bandwidth of 25 nm) and emission centered at 330 nm (bandwidth of 60 nm).

2.3. Sampling of tablets with LIF

In order to sample the tablets, the LIF instrument was positioned above the tablets as demonstrated in Fig. 2. The position of the tablet under the instrument could be adjusted with a rotating dish. The distance between the tablet and the instrument could be adjusted by a support jack (pictured as the large green rectangle on the left).

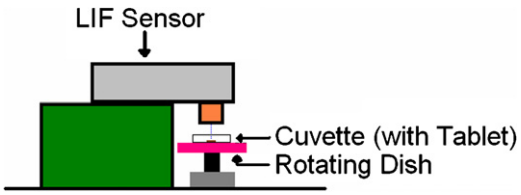

Fig. 2. Alignment of tablets relative to LIF instrument.

The tablets were examined in four different sampling strategies as presented in Fig. 3. For the first three sampling strategies (all but the Far Away spot), the distance from the LIF lens to the surface of the sample ("focal length") was kept constant at 13 mm for the caffeine tablets and 30 mm for the triamterene tablets. The single "Far Away spot" had a focal length corresponding to the strongest signal for that focal length: 26 mm for the caffeine tablets and 60 mm for the triamterene tablets. In Fig. 3, the LIF sample spot is depicted as an irregularly shaped beam because the beam is not perfectly circular. Depending on the focal length used, some of the sampling strategies may have overlapping spots. For each of the positions, ten consecutive LIF readings were collected. These ten readings were then averaged to give a mean LIF reading for each position. The instrument has previously been shown to not significantly penetrate the depth of the tablet by more than 1 mm from the surface (Lai et al., 2004).

The size of the light spot on the tablet surface is dependent upon the focal length. To test the spot size, an adjustable photographical shutter was placed on top of a tablet. By adjusting the circular opening of the shutter, the diameter of light that passed through onto the tablet was controlled. The LIF signal demonstrated a first-order relationship with the size of the incident beam and, for a focal length of 13 mm, reached 100% of the maximum signal when the spot area was approximately 4.0 mm^2. For a circle sample area, this corresponds to a diameter of 2.24 mm. Considering the large caffeine tablet size (12.9 mm) and the intent to position the spots such that overlap was avoided, it is likely that the spots in a triangle and in a line did not overlap for the caffeine tablets.

The same experiment was performed with a focal length of 30 mm (the operating length for the triamterene tablets). For this longer focal length, the maximum signal occurs with a sample area of 40 mm^2, corresponding to a diameter of 7.1 mm. This beam size is large relative to the tablet diameter (8.8 mm), and the sample areas in the triangle and line sampling strategies overlap.

2.4. Spectroscopic analysis of total tablets

The triamterene content was measured in a UV microplate reader. The caffeine content in each tablet was measured with a UV reader.

2.4.1. Microplate reader for triamterene tablets

For total tablet content analysis, each triamterene tablet was dissolved in 10 mL of formic acid. The solution was filtered to remove undissolved excipients. The resulting solution was diluted with 100 mL of 10% formic acid in water. A second dilution of 10 mL of the solution was made with 400 mL of 10% formic acid in water. Three

100 μL wells were filled with the diluted solution for each tablet. Ninety-six well plates were used in a microplate reader with the absorbance wavelength of 340 nm monitored for each well. Each plate contained eight standard solutions (each run in triplicate) made from known amounts of pure triamterene. The calibration curve for the UV microplate reader was both linear (with R^2 of 0.9994) and reproducible.

2.4.2. UV reader for caffeine tablets

Each caffeine tablet was weighed before being dissolved in 200 mL of de-ionized water. The tablet was then broken with a spatula and the flask shaken. To allow settling of the excipients that did not dissolve, the flask was left to sit overnight. After sitting, 70 μL of the clear supernatant was diluted with 930 μL of water and the solution was pipetted into a quartz vial. The caffeine content of this solution was then quantified by measuring its absorbance at 280 nm using a Hewlett Packard 8452A Diode Array Spectrophotometer. Pure caffeine powder (Sigma Anhydrous Caffeine Powder C-0750, Sigma–Aldrich, St. Louis, MO) was used to generate a UV absorbance calibration curve.

2.5. Statistics for estimation accuracy

The analysis of estimation accuracy of a non-destructive technique (such as LIF or NIR) compared to the complete and destructive technique (such as dissolution followed by UV or HPLC) can be completed on an individual tablet basis and/or a population basis. Statistics of both are used in this analysis. Estimations of prediction of individual tablet contents would be of most practical value if acceptance or rejection of tablets evolved to occur at an individual level. As the current regulations around tablet content uniformity focus on population statistics, non-destructive predictive techniques have mostly been evaluated on that basis (Ito et al., 2008; Sulub et al., 2008; Moes et al., 2008; Martins et al., 1998).

Regarding the predictive accuracy of the LIF technique for individual tables: the LIF instrument samples a fraction of the total tablet. If different sections of the same tablet have different concentrations of API, then different intensities of fluorescence will be found at different locations (Lai et al., 2004). The theoretical ability of a tablet section to accurately estimate the total tablet content can be estimated from the variation in concentration measured at different locations by assuming a distribution of API concentration throughout the tablet and using a model of reliability (Cochran, 1977; Levy and Lemeshow, 1991; Scheaffer et al., 1996).

To develop a description of the accuracy of LIF samples, it is assumed that the within a single tablet, any variation in API concentration is normally distributed. With this assumption, the determination of the dependence of confidence or accuracy intervals on the number of samples required to estimate a population mean can be completed.

The resulting reliability associated with an estimation of content of an individual tablet is dependent upon the number of independent samples taken on the tablet, n, the maximum relative error associated with the estimate, ε, and the relative standard deviation of the independent samples, RSD, and the normal reliability coefficient for the selected confidence interval, z. This relationship is presented in Eq. (1). The selected maximum relative error to use for individual tablets varies depending on the research as there is no regulated or practical current usage. Recent research, for example, considers maximum relative error in the range of 5–25% (Martins et al., 1998). For simplicity, this research considers a maximum relative error of 10% between the LIF and UV.

$$z^2 \leq \frac{n\varepsilon^2}{\mathrm{RSD}^2} \quad (1)$$

Reliability of an estimate

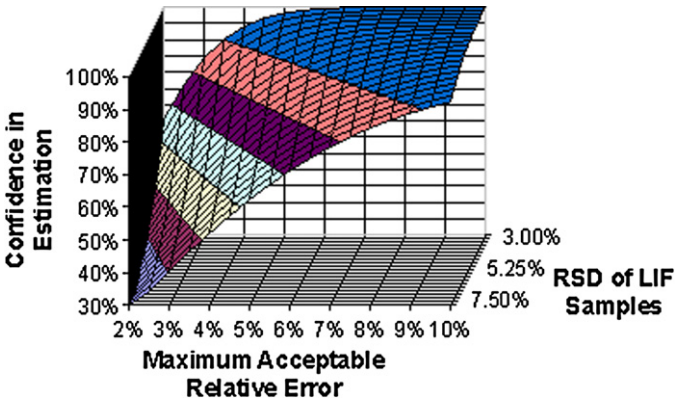

Fig. 4. Confidence associated with an estimate.

With this model, the confidence associated with an individual tablet content estimate can be found if the maximum acceptable relative error is defined and the number of samples and relative standard deviation between samples are known. The relationship of confidence level for a single sample from a population is presented in Fig. 4 for a range of acceptable errors and relative deviations. Logically, as the maximum acceptable error increases, the confidence in the estimate increases. As the relative deviation between samples increases, the confidence in the estimate decreases.

Common metrics for comparison of population statistics across analytical techniques include comparison of content averages, deviations between tablets within a batch, and standard errors of prediction between the non-destructive technique and the established destructive technique (Ito et al., 2008; Sulub et al., 2008; Moes et al., 2008). All of these are used in this research. The root mean standard error of prediction between two techniques is presented in Eq. (2) where y is the API content by UV, Y is the API content by LIF, and n is the number of tablets:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - Y_i)^2}{n}} \quad (2)$$

Root mean standard error of prediction.

3. Results and discussion

The calibration of the LIF signal to API content in the tablet was completed for each set of tablets separately. The validation of the ability of the LIF to accurately estimate the API content was validated with a separate set of tablets of each API.

LIF sampling and UV total tablet assay were performed on twenty tablets per batch from three batches of triamterene tablets at concentrations of 1.64%, 3.22%, and 4.75% of the triamterene. Half of the tablets from each batch were used for calibration and half for validation. Additionally, one hundred tablets from the fourth batch of tablets pressed from poorly mixed powder were also analyzed to represent a “worst case scenario” in which heterogeneity on a tablet surface and within a population would be relatively high.

Similarly, LIF sampling and UV total tablet assay were performed on twenty caffeine tablets for each of three batches at higher concentrations than that of triamterene (5%, 10%, 20% caffeine concentration). Again, half from each batch were used for calibration and half for validation.

3.1. Calibration

A calibration curve between LIF output signal and the tablet concentration was established using ten tablets from each of the first three triamterene tablet batches (1.64%, 3.22%, 4.75%). For this calibration set, the total tablet concentration was assumed to be that

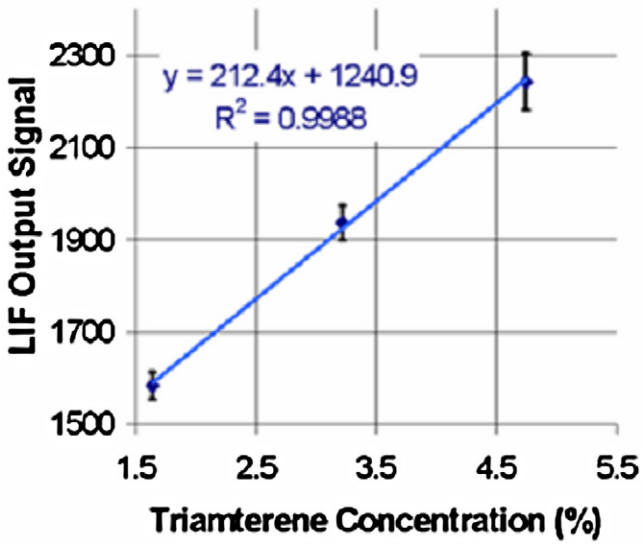

Fig. 5. Calibration curve between LIF signal and triamterene concentration for single point sampling strategy.

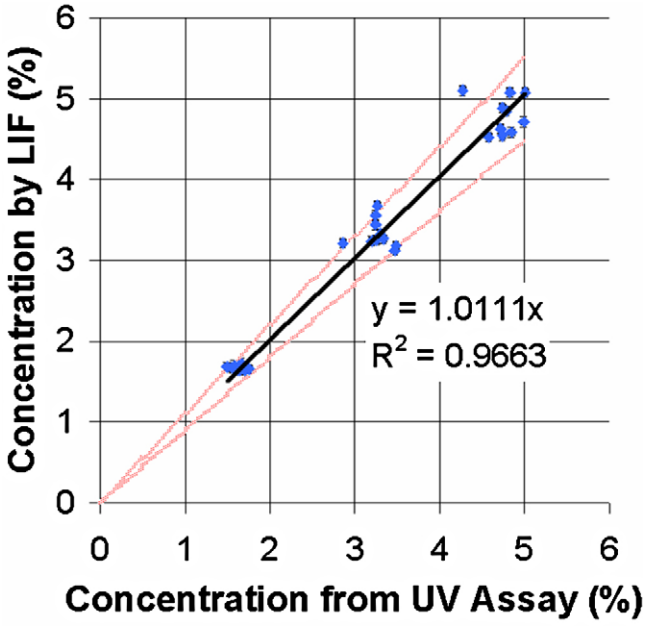

Fig. 6. Parity chart of triamterene concentration for LIF and UV.

of the bulk batch. Calibration curves were determined for each LIF sampling strategy (sampling strategies shown in Fig. 3) and all calibration curves were found to be linear. The calibration curve for single point sampling strategy is demonstrated in Fig. 5. The error bars indicate ±1 standard deviation of the LIF signal values across the tablets in each batch.

Similar steps were taken to generate LIF calibration curves for caffeine for the different LIF sampling strategies. As the caffeine tablets covered such a wide range of concentrations (from 5% to 20%), the best-fit calibration curves were logarithmic functions with respect to caffeine concentrations in tablets. This non-linearity was likely due to partial saturation of the PMT detector in the LIF unit or to insufficient power from the light source to excite all of the caffeine present on the tablet surface and may negatively impact resolution of the LIF results at high concentrations.

3.2. Individual tablet content statistics

The concentration of triamterene estimated by the LIF samples was compared to UV analysis of the dissolved entire tablets. Ten tablets from each concentration batch (1.64%, 3.22%, 4.75%) were used in this validation and the results for the linear sampling strategy are presented in Fig. 6. The data points that fall within the dashed lines are estimated by LIF within 10% of the measured UV concentration. In this case, twenty-six of the thirty estimations from LIF measurements fall within ten percent of the UV concentration. For the linear strategy on both sides of the tablet, all of the tablet concentrations were accurately predicted within 10% of the spectroscopic analysis. The success of the different strategies is presented in Table 1.

Similarly, the concentrations of caffeine estimated by the LIF samples were compared to the UV analysis of the dissolved entire tablets. Ten tablets for each of the three caffeine concentrations (5%, 10% and 20%) were used in this validation and the results are summarized in Table 2, which shows the percent of tablets whose LIF-estimated concentrations fall within 10% of the total tablet concentration measured by UV analysis. As with the triamterene tablets, the best LIF prediction was achieved by using the linear strategies on both sides of the tablets, predicting 97% of the tablets to within 10% of the UV-measured concentrations. This strategy was able to predict the caffeine concentrations of all thirty validation tablets within 11% of the UV values. The Far Away strategy gave unsatisfactory caffeine content predictions, being able to predict only two-thirds of the tablets within 10% of the UV-measured concentrations.

Overall, sampling strategies that obtained more samples per tablet (linear or triangle both sides of the tablet) gave better predictions than strategies with fewer samples (single, Far Away, one side only). This was expected, as there was more information about the tablet when it was more broadly sampled. Although the Far Away sampling did cover most of the exposed tablet, its suboptimal predictive performance is likely due to significant loss of focused light to the environment at the extended focal lengths.

3.2.1. Accuracy of LIF in "Worst Case Scenario"

A fourth batch of triamterene tablets was made by simultaneously placing leftover powders from the first three batches (1.64%, w/w; 3.22%, w/w; 4.75%, w/w) into the tablet press hopper with no premixing. The tablets made from the fourth batch were expected to have greater heterogeneity of triamterene within the tablets and the local sampling of the LIF instrument was expected to give less accurate estimation of total tablet content.

Table 1
LIF estimation of triamterene concentration within 10% by sampling strategy.

Method	Sampling one side only		Sampling both sides	
	RSD of LIF samples	Tablets estimated within 10%	RSD of LIF samples	Tablets estimated within 10%
Sinqle:	6.74%	82%	4.74%	93%
Linear:	5.84%	88%	3.98%	100%
Trianqle:	4.88%	90%	3.85%	100%
Far Away:	6.87%	88%	5.07%	90%

Table 2
LIF estimation of caffeine concentration within 10% by sampling strategy.

Sampling strategy	Tablets estimated within 10% of UV	
	One side	Both sides
Single	72%	73%
Linear	87%	97%
Triangle	85%	87%
Far Away	63%	67%

Table 3
Accuracy of LIF estimation for tablets from an unmixed blend.

Sampling strategy	Tablets estimated within 10% of UV	
	One side	Both sides
Single	83%	88%
Linear	85%	90%
Triangle	82%	86%
Far Away	85%	88%

Using the UV measurement of triamterene content as the true value, the ability of the four LIF sampling methods to estimate the triamterene concentration within 10% of the true value is presented in Table 3. For this "worst case scenario" of poor mixing, all of the sampling methods produced similar levels of accuracy in estimation, with an average of 83% of the tablets estimated within 10% from sampling one side and 88% from sampling of both sides. The linear strategy was slightly better than the other sampling strategies. The resulting parity chart for the linear spots sampling method on both sides of the one hundred tablets is presented in Fig. 7. The error bars represent one standard deviation of the repeatability of each measurement technique.

3.2.2. *Accuracy of statistical estimation of error*

In an attempt to test the reliability model described by Eq. (1) and depicted in Fig. 4, the relative standard deviation of LIF samples at different locations on the same tablet was determined.

Given the empirically determined average RSD, the distribution of expected extent of error associated with the LIF estimation could be calculated and compared to the experimental error. An example of this is presented in Fig. 8 for the linear strategy on one side of all triamterene tablets. The results shown are from the linear strategy applied on both sides of the tablets. The solid pink line is the theoretical cumulative percentage of tablets that are predicted within a threshold of accuracy by the LIF Linear strategy. The experimental values were consistent with the theoretically predicted values and similar agreement between theory and experiment was observed for all other sampling strategies and the caffeine tablets as well.

The agreement between theory and observation suggests that the assumption of API being normally distributed across the surfaces of the tablet is reasonable. It also provides confidence in the applicability of the reliability model which, if used in place of full experiments such as this, could provide significant time savings in estimating success of other sampling strategies. For example, those strategies or techniques that demonstrate low point-to-point sample RSD and provide sufficient resolution across concentrations would be most likely to have accurate predictions of total content in individual tablets.

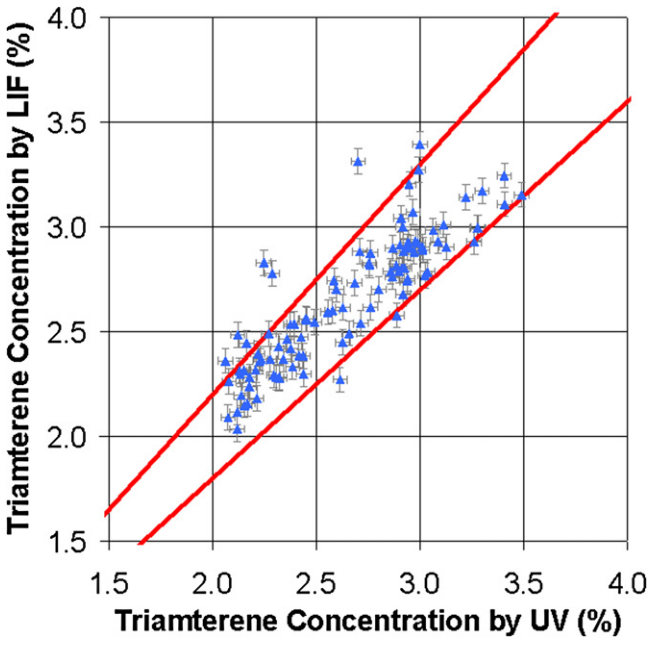

Fig. 7. Parity chart for concentration estimation of tablets from unmixed blend.

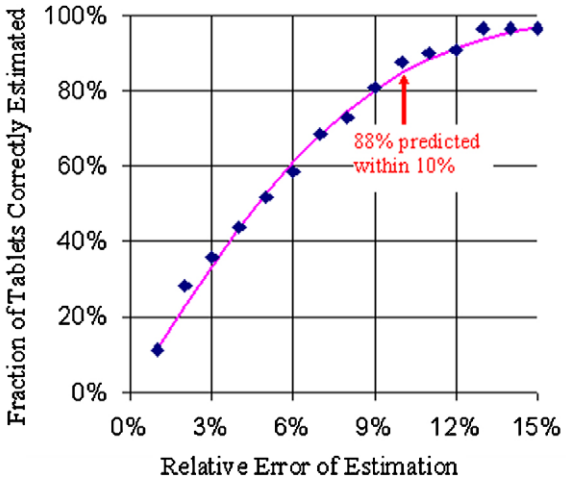

Fig. 8. Theoretical and experimental error in LIF estimation of total tablet concentration of triamterene.

Table 4
Population statistics for all validation tablets.

API	Target API Conc. [%]	Measured Avg. API Conc.		RSD API Conc.		RMSEP [%]
		By UV [%]	By LIF [%]	By UV [%]	By LIF [%]	
Tnamterene	1.64	1.64	1.67	4.88	1.80	5.33
Triamterene	3.22	3.31	3.33	5.14	5.41	7.93
Triamterene	4.75	4.75	4.80	4.42	4.79	6.71
Triamterene	2.6 (unmixed)	2.66	2.65	14.30	12.30	7.06
Caffeine	5	4.94	5.10	2.04	6.91	6.23
Caffeine	10	9.96	9.77	2.97	3.68	4.40
Caffeine	20	19.27	18.56	4.91	7.14	5.50

3.3. Population statistics for the tablets

The population statistics compared across the LIF and the UV techniques are presented in Table 4. The metrics displayed that compare the two techniques are average API concentration for all validation tablets within a batch, relative standard deviation between validation tablets of a batch, and the root mean standard error of prediction (RMSEP) between the two techniques. The use of these summary population statistics to compare analytical techniques of pharmaceutical tablets is consistent with recent research on applying NIR techniques (Ito et al., 2008; Sulub et al., 2008; Moes et al., 2008). Both techniques clearly identified significantly higher population variability in the unmixed triamterene tablets and the LIF surface technique was able to maintain a comparable agreement with UV (as measured by RMSEP) for this unmixed batch as with other batches. This suggests that the LIF technique could be used for batch trending to identify batches or segments of batches that have abnormal absolute concentration or concentration variations.

The most direct metric for comparison of the current LIF technique to the NIR techniques is RMSEP as it is a relative measure of difference between a new non-destructive technique (LIF or NIR) and established destructive technique (dissolution followed by UV or HPLC). The RMSEP for LIF was found to be in the range of 4.40–7.93% which is higher than recently reported values for NIR, which recently report RMSEP around 2% (Ito et al., 2008; Sulub et al., 2008; Moes et al., 2008). Given that the LIF instrument and technique are relatively early in development (this research uses the first generation LIF instrument of its type) compared to a large body of NIR research (Blanco et al., 2000a,b; Scafi et al., 2001; Neubert et al., 1997; Buchanan et al., 1996; Moffat et al., 2000; Herkert et al., 2001; Gowen et al., 2008; Sulub et al., 2008; Moes et al., 2008) and given that the LIF instrument used was designed for monitoring blending, improvements in the accuracy of the LIF technique are likely to be achieved through further research. Rationale for furthering the LIF technique includes its speed, simplicity, lower production cost compared to NIR, and sensitivity to low concentrations of fluorescent APIs.

4. Conclusions

Light induced fluorescence (LIF) analysis was done on two sets of tablets, each with a different API. The triamterene tablets ranged in concentration from 1.64% to 4.75%. The caffeine tablets represented higher concentration sets in which the API concentration was as high as 20%. For both sets of tablets, the LIF instrument was able to accurately estimate the API concentration in the total tablet (determined by dissolving the entire tablets and UV absorbance) within 10% more than 90% of the time. An unmixed set of triamterene tablets ("worst-case" of mixing) was made and the LIF instrument was able to provide slightly less accurate estimations of the concentration of triamterene in the total tablet.

A theoretical description of the surface sampling statistics was developed and provided agreement with experimental data for both the caffeine tablets and the triamterene tablets. The use of this theory requires one empirically determined parameter: the relative standard deviation (RSD) of concentration estimations at different locations on the tablet surface. Correct use of this theory will allow for an estimation of the expected accuracy of the LIF, or another surface measurement technique, to predict total tablet concentration by simply determining the RSD parameter. This would reduce the dependence on full scale studies, such as this one, that require time consuming dissolution and spectroscopic analysis of each tablet.

Tablet population statistics were also analyzed that showed a root mean standard error of prediction of the LIF instrument to be in the range of 4.40–7.93% which is higher than the 2% benchmark recently reported for NIR instruments. However, the LIF instrument and technique are relatively untested and are expected to improve in accuracy.

The light induced fluorescence (LIF) instrument was demonstrated as a rapid, non-destructive method of estimating drug concentration in low dose tablets. Its ability to sample the surface of a single tablet at rapid speeds gives hope that the technique may be used to monitor the tablets inline on a tablet press.

Acknowledgements

The authors acknowledge Professor Garnet Peck and Mr. Mark Alkire at the School of Pharmacy and Pharmaceutical Sciences of Purdue University for help producing the triamterene tablets. The authors acknowledge John Peragine and Ron Miller of Bristol-Meyers Squibb for help producing the caffeine tablets. The authors acknowledge Weil Lai and Chee Kong Lai of the Department of Chemical Engineering of the Massachusetts Institute of Technology for help obtaining and analyzing the triamterene tablets. The authors thank the Consortium for the Advancement of Manufacturing of Pharmaceuticals (CAMP) for providing funding for this work.

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