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Selective Imaging of APIs in Powdered Blends with Common Excipients Utilizing TPE-UVF and UV-SONICC

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Abstract

Second order nonlinear optical imaging of chiral crystals (SONICC) and two-photon excited fluorescence measurements [both autofluorescence and two-photon excited UV-fluorescence (TPE-UVF)] were assessed for the selective detection of APIs relative to common pharmaceutical excipients. Active pharmaceutical ingredients (APIs) compose only a small percentage of most tabulated formulations, yet the API distribution within the tablet can affect drug release and tablet stability. Complementary measurements using either UV-SONICC (266 nm detection) or TPE-UVF were shown to generate signals >50-fold more intense for a model API (griseofulvin) than those produced by common pharmaceutical excipients. The combined product of the measurements produced signals >10⁴-fold greater than the excipients studied. UV-SONICC or TPE-UVF produced greater selectivity than analogous measurements with visible-light detection, attributed to the presence of aromatic moieties within the API exhibiting strong one and two photon absorption at ~266 nm. Complementary SONICC and fluorescence measurements allowed for the sensitive detection of the three-dimensional distribution of tadalafil within a Cialis[®] tablet to a depth of >140 μm.

Introduction

The uniformity of an API within the final dosage form, as well as any changes in those properties (e.g., crystal size and/or spatial distribution) can influence bioavailability and efficacy. Quality control standards for the pharmaceutical industry include process monitoring during the preparation of APIs, process monitoring during the mixing of formulations, examination of the spatial homogeneity of the resulting tablets, as well as the API dissolution kinetics. Numerous formulations strategies have emerged to address the broad diversity of physicochemical stability and bioavailability of emerging APIs. In tablets, APIs in crystalline form generally exhibit enhanced physico-chemical stability. Tablet and

Supporting Information:

The supporting information contains two detailed sections on the preparation of the pharmaceutical ingredients and excipients, as well as data analysis details. Also included are supplementary figures. **Figure SI-1** is a comparison of TPE-UVF and UV-SONICC signals acquired within griseofulvin powder, with each subsequent image probing 10 μm deeper. **Figure SI-2** includes 3D renderings of UV-SONICC signals produced by anhydrous lactose, and lactose monohydrate. **Figure SI-3** is a 3D rendering of the TPE-UVF signals produced from Opadry[®] white. **Figure SI-4** is a 3D rendering of the UV-SONICC signals generated from placebo mixtures 1 and 2. **Figure SI-5** is an image comparing signals from conventional SONICC and fluorescence produced from tadalafil, along with its corresponding brightfield image. **Figure SI-6** is a compilation of planes acquired with 20 μm steps thorough a Cialis[®] tablet, utilizing the TPE-UVF signal generated to produce the corresponding 3D rendering. **Figure SI-7** is a line trace of griseofulvin, comparing the UV-SONICC signal with TPE-UVF. **Figure SI-8** is a line trace of tadalafil, comparing the conventional SONICC signal with TPEF.

powdered mixture analysis techniques designed to quantify these properties are extensively utilized in various pharmaceutical applications, including: stability assessments,¹ counterfeit detection,² process monitoring,³ and potential litigation/patent issues.⁴

Current methods of assessing the spatially averaged composition of APIs within powdered solid state formulations include X-ray powder diffraction (XRPD),^{5–9} scanning electron microscopy (SEM),^{10–12} Raman^{10, 13–15} infrared spectroscopy,¹⁰ NMR,¹⁶ and differential scanning calorimetry.^{17, 18} Each technique has its own strengths and limitations, including: a relatively high limit of detection for XRPD (>~1% typically),^{5, 19} long analysis times for NMR,¹⁶ interference by excipients and subsampling with Raman,^{13, 20, 21} relatively small fields of view and the inability to probe sub-surface details for SEM.^{1, 7, 10, 22} Consequently, sensitive detection of crystallinity at low loadings deep within tablets and powders represents an important niche not easily addressed by existing common methodologies.

Raman imaging is one potentially promising method of probing the spatial distribution of API within final dosage forms (as opposed to the averaged composition) by providing rich vibrational spectral information within each voxel.²³ However, practical complications still restrict its broader utility. Specifically, conventional Raman imaging requires long integration times on each pixel (>~5s)²⁴ given the relative inefficiency of the Raman process, corresponding to long image acquisition times (up to several hours per frame).²⁴ Interferences from non-API excipients can complicate isolation of the API response, which can be addressed through multivariate methods for dimension reduction, such as multivariate curve resolution (MCR) or direct classical least squares (DCLS).²⁵ However, reliable quantitation from these methods typically requires high signal to noise spectral measurements, further exacerbating limitations from long acquisition times.²⁴ Improvements in signal intensities by increasing laser fluence is often limited by sample photodamage from local heating, with the upper intensity limit depending on the nature of the sample, the tightness of the focus, integration time, etc.²⁶ Finally, optical scattering in turbid matrices routinely encountered in final dosage forms quickly degrades the spatial resolution of Raman imaging at planes more than 10 microns below the surface.²⁴

Another technique utilized in the probing of final dosage forms is X-ray computed tomography (X-ray CT).^{27, 28} The technique utilizes the transmission of X-ray beams through the analyte of interest, and the intensity of the transmitted beam is utilized to construct a 3D rendering.²⁷ The maximum spatial resolution of this technique is only 10 μm , utilizing a micro-focus X-ray source.²⁷ This technique is useful in providing the physical density distribution of final dosage forms, but provides limited chemical information on API distribution. For example, X-ray CT cannot easily discriminate between crystalline vs. amorphous forms.

More recently, second order nonlinear optical imaging of chiral crystals (SONICC) has been suggested as a new method to address this unfilled niche.^{19, 29, 30} Initial investigations of APIs, specifically griseofulvin, produced high signal to noise (on the order of 10,000:1) with low excitation powers (25–75 mW), with both kinetics and trace crystallinity probed.¹⁹ SONICC, utilizing second harmonic generation (SHG), relies on the frequency doubling of light upon the interaction with non-centrosymmetric crystals of specific classes, which include most chiral crystals.³¹ Previously, ensemble-averaged SHG was utilized as a tool for monitoring API distributions.^{32–35} The recent extension of spatially resolved measurements by SONICC to the analysis of powders suggests potential applications in characterizing complex mixtures and tablets.¹⁹ SONICC allows for the acquisition of both particle size and location information, clearly distinguishing APIs from non-crystalline excipients. In previous studies with pure APIs in both thin films and in powders, SONICC has been shown to successfully allow determination of crystal nucleation and growth kinetics with several

decades improvement in both the lower limit of detection and linear dynamic range for crystallinity when compared to common alternative methods.³⁰ The detection limits of SONICC were recently compared to that of XRPD, utilizing crystalline griseofulvin, which was cryomilled for time increments of 15 to 180 minutes, resulting in a distribution of crystal sizes.¹⁹ The results suggested more than a three decade improvement in the lower limits of detection for % crystallinity in powders, and a six decade improvement in thin films for SONICC compared to XRPD.¹⁹ In addition, the utilization of a resonant mirror (8 kHz) significantly minimizes the likelihood of laser-induced photodamage.¹⁹

An extension of nonlinear optical imaging to more complex powdered formulations that include both the API and biologically inert excipients would expand the scope of SONICC analysis to powdered mixtures and tablets. However, many common excipients are also both chiral and crystalline, potentially producing a nonzero background for conventional SONICC imaging. This preliminary study is designed to provide an initial foundation for assessing the capabilities and limitations of SONICC for discriminating APIs from commonly used excipients. Since over 75% of APIs contain aromatic groups,³⁶ two-photon excited ultraviolet fluorescence (TPE-UVF) was explored as a complement to SONICC, providing additional imaging selectivity through largely independent light-matter interactions. Whereas most APIs are aromatic, most common excipients are comprised of purely aliphatic building blocks and can reasonably be expected to produce little UV fluorescence. In addition, UV-SONICC was explored as a mechanism for improved contrast taking advantage of resonance enhancement of the APIs at the second harmonic generated frequency for greater selectivity relative to non-aromatic excipients of chiral crystals.³⁶ Figures of merit were assessed for conventional SONICC, UV-SONICC, and TPE-UVF for API analysis in systematic measurements of isolated excipients and placebo mixtures. Initial measurements were also performed with commercial tablets.

Experimental

Instrumental Design

SONICC images were captured using two different custom-designed microscopes. Both microscopes incorporated beam scanning units to reduce both the sample damage and the sample acquisition times, utilizing a galvanometer-driven mirror (Cambridge Technologies) for the slow-scan and a resonantly-driven mirror (EOPC) at approximately 8 kHz for the fast-scan axis. For the studies with IR illumination, the fundamental light was generated by a Spectra-Physics Mai Tai Laser, operating at 80 MHz, 100 fs, 800 nm, and an average power of 25–75 mW at the sample, and was focused onto the sample with a 10x objective (Nikon, 0.3 NA). The resulting SHG signal was collected in both epi and transmission, with dichroic mirrors and filters (Chroma) centered around 400 nm being used to reject the excitation light and pass the SHG signal. Autofluorescence (460–780 nm) and bright field images were also collected in the epi direction. Acquisition times for a field of view of 600 × 600 μm ranged from 8 to 10 seconds per frame, with a resolution of 140 × 280 pixels for the fast and slow-scan axis, respectively. Three different fields of view per sample were investigated, with signal intensities averaged over 11 Z-planes per sample at 20 μm increments per plane. For both TPE-UVF and UV-SONICC studies, the fundamental beam was generated by a mode-locked, free-space coupled Fianium fiber laser at 1064 nm, doubled to 532 nm through focusing onto and through a lithium triborate crystal (LBO, 5 × 5 × 2 mm). The 532 nm incident light was directed through the beam scanning unit and then focused onto the sample with a dichroic mirror centered at 532 nm with a 10× objective (Nikon, 0.3 NA). The SHG signal at 266 nm was collected in transmission by a 10× near-UV objective (Thorlabs, 0.25 NA), with the signal being collected by a solar blind PMT (Hamamatsu R2078) following reflection and stray-light rejection by a dichroic and narrow bandpass filter, respectively, centered around 266 nm (Semrock FF01-260/16-25). The TPE-UVF signal was collected

back through the excitation objective and directed towards a PMT (Hamamatsu R6094) by reflection off of a broadband fluorescence filter (Semrock FF310-Di01-25.4-D). Bright field images were also collected in the epi-direction. Three different fields of view were also investigated in these studies with 11 Z-planes collected, 5 above and 5 below the focus, at 10 μm steps. The final images generated from this instrument had a similar resolution as described above but for a $370 \times 370 \mu\text{m}$ field of view and an acquisition time that ranged from 2 to 4 seconds per frame.

Results

Powder Imaging of Griseofulvin and Excipients

Table I contains a compiled summary of the average excipient signal intensities relative to that of griseofulvin, with 95% confidence intervals. Most excipients generated less than 5% of the signal produced by griseofulvin for the conventional SONICC measurements. Only conventional SONICC measurements were performed on the excipients shown in the last row of Table I (vitamin E TPGS, HPC, SLS, PVP, HPMCAS-LF, magnesium stearate, A-TAB, Kollidon VA64, and colloidal SiO_2). These excipients exhibited an average conventional SONICC signal intensity that was less than 0.020% that of griseofulvin, less than the lowest values on the table. There were a couple of notable exceptions that generated a significant amount of signal relative to that of griseofulvin (e.g., anhydrous lactose, which resulted in signal intensities as much as 12% relative to griseofulvin for conventional SONICC).

The measurement signal to noise and specificity for the API increased substantially for both TPE-UVF and UV-SONICC compared to their infrared counterparts. Measurements by UV-SONICC in the investigation of griseofulvin led to bright signal intensities observed over multiple planes within a single field of view (Figure 1). The relative signal intensities of the pharmaceutical excipients compared to griseofulvin for UV-SONICC measurements followed a similar trend as that from the conventional SONICC measurements, with anhydrous lactose producing the largest UV-SHG signal (Table I). Arguably, most significantly, UV-SONICC and TPE-UVF both substantially improved image contrast between APIs and excipients relative to 1064 nm incident light. Whereas conventional SONICC measurements of anhydrous lactose generated a signal intensity of 12% of that from griseofulvin the UV-SONICC intensity of this excipient relative to griseofulvin was only 2.1%.

SONICC images of griseofulvin, to which all measurements are compared, are shown in Figure 1. Both the bright field and the corresponding 3D UV-SONICC and TPE-UVF overlay are shown. A full 3D rendering as a function of depth-profiling is provided in the Supporting Information. A brighter color corresponds to greater signal intensity. UV-SONICC ImageJ 3D renderings of both anhydrous lactose and lactose monohydrate are displayed in Figure SI-2 (sup. info). Conventional SONICC measurements displayed similar trends, with significantly higher relative values for both anhydrous lactose and lactose monohydrate. Within the TPE-UVF measurements, there was a substantial amount of fluorescence from the Opadry[®] white, which is typically used as a thin film moisture barrier on tablets, but all other excipients were TPE-UVF inactive. Figure SI-7 (sup. info) is a line trace of griseofulvin comparing the spatial overlap of the UV-SONICC signal with that of TPE-UVF.

An ImageJ 3D rendering of the TPE-UVF signal from Opadry[®] white is shown in Figure SI-3 (sup. info). The two placebo mixtures were similar in composition, but yielded differences in average signal intensity, with placebo mixture 1 being about three times

greater than that of placebo mixture 2. ImageJ 3D renderings of UV-SONICC threshold images of both mixtures are shown in Figure SI-4 (sup. info).

Tablet Imaging of Cialis®

A commercial tablet form of Cialis®, with the active ingredient tadalafil, was probed with conventional SONICC, TPEF, as well as UV-SONICC and TPE-UVF. The conventional SONICC and TPEF comparison is found in Figure SI-5B (sup. info). as a threshold image, with blue representing the conventional SONICC signal, green representing the fluorescence signal, and red representing the overlap of both. TPE-UVF images of the Cialis® tablet is shown in Figure SI-6 (sup. info) as a stack of individual Z-slices spanning a total of 140 μm along with a 3D rendering of the composite data set. A line trace of tadalafil is provided in Figure SI-8 (sup. info), with a comparison of the spatial overlap of both the conventional SONICC signal and TPEF signal.

Discussion

The conventional SONICC data acquired with the infrared incident beam summarized in Table I. are in good qualitative agreement with expectations based on previous SONICC analyses.^{19, 29, 30} The excipients with the greatest SHG-activity were from anhydrous lactose and lactose monohydrate chiral crystals, producing signals as much as 12% of those from griseofulvin. Consistent with expectations from symmetry arguments, the amorphous excipients produced little or no detectable SHG. Although mannitol is also chiral and crystalline, the intrinsic SHG activity was found to be substantially weaker, representing signals of only 0.5%. The difference is attributed primarily to the intrinsic variability of the SHG efficiencies across materials, which can arise from a combination of differences in the intrinsic molecular nonlinear polarizability, the molecular orientation within the lattice, and the symmetry operations present in the lattice. Most significantly, all of the excipients tested, including the placebo mixtures, exhibited SHG intensities less than 1% of griseofulvin except lactose crystal forms.

In general, the TPEF intensities for both griseofulvin and the excipients were relatively weak. As seen in Figure SI-8 (sup. info), the TPEF signal was three-fold lower than that of the corresponding conventional SONICC signals. However, the TPEF measurements of the excipients were characterized by much lower signals compared to the API. Unlike conventional SONICC measurements where the signal varied among the excipients, the same trend was not exhibited for TPEF measurements, where the fluorescence signals were similar among the excipients.

Compared to conventional SONICC and TPEF, the increased selectivity for APIs observed for UV-SONICC and TPE-UVF relative to common excipients is postulated to be due to resonant enhancement from the aromatic groups within the APIs. Griseofulvin exhibits strong overlapping one-photon absorption with bands spanning from 320 nm through at least 240 nm.³⁷ Using an incident beam of 532 nm, the two-photon resonance-enhancement corresponds to 266 nm, falling within this absorption window. Although two-photon and one-photon absorption spectra can be difficult to directly compare, conjugated chromophores typically exhibit numerous overlapping excited states in this spectral range capable of resonance-enhancement. This explanation suggests that similar resonance-enhancement can be reasonably expected in API's comprising aromatic groups, which includes ~75% of emerging candidates.³⁶ However, contrast in TPE-UVF depends on both absorption and fluorescence quantum yield, which may differ substantially for different aromatic APIs. Since UV-SONICC is a parametric process (i.e., no energy is deposited into the molecules from the process of SHG), there is no equivalent dependence on quantum yield.

The origin of easily detectable TPEF autofluorescence signals from griseofulvin with 1064 nm incident light is not as obvious. The API investigated contains no intrinsic fluorophore obviously responsible for two-photon absorption at an energy corresponding to 532 nm light and subsequent fluorescence emission. However, intrinsic emission of visible light has been reported in previous studies of seemingly innocuous organic assemblies lacking obvious chromophores, including triethylamine,³⁸ poly(amido amine) dendrimers,^{38–43} aliphatic polyamides,⁴⁴ poly(ether amide)s,^{43, 45} poly(propylene imine),⁴⁶ and poly(amine-amide)s.⁴⁷ In those studies, a combination of fluorescence and phosphorescence was observed, with oxygen implicated as a critical component, possibly through oxygen exciplex formation. Recent quantum chemical calculations also support the viability of visible light emission from oxygen exciplex formation.⁴⁸ Alternatively, the emission may be arising from trace impurities or oxidation products. Irrespective of the underlying mechanism, TPEF autofluorescence is significantly greater in griseofulvin than in the common excipients and can be used as a mechanism for image contrast.

Two interesting phenomena with UV-SONICC and TPE-UVF measurements developed from the excitation of Opadry® white and the two placebo mixtures. Despite generating no statistically significant UV-SONICC signals, excitation of Opadry® white using TPE-UVF produced significantly higher signals generating signals 11% that of griseofulvin (Figure SI-3 (sup. info)). One of the main ingredients of the Opadry® white sample used in the current study was TiO₂, which is commonly used for photovoltaic applications, such as solar cell construction, due to its ability to efficiently absorb UV light.^{49–51} The bright fluorescence signal of Opadry® white is tentatively attributed to two-photon absorption by titanium dioxide, followed by electron-hole recombination and fluorescence emission.⁵²

UV-SONICC measurements of the placebo mixtures produced a 10-fold signal difference between the two samples (Figure SI-4 (sup. info)). Although the composition of each placebo was different, analysis of these ingredients did not seem to correlate with the difference in the SHG activities. The difference in crystal size between the two placebo formulations was believed to be the origin in the variations in SHG signals, where the UV-photons generated from larger crystals would experience a lesser degree of scatter. However, the relative signal percentage, as compared to griseofulvin, was still well below 1% and could be readily distinguished from an API generating bright SONICC signals.

Although both UV-SONICC and TPE-UVF individually exhibited the potential for false positives from particular excipients, the mechanism of action responsible for the interferences was distinct in each case, such that the combination of both independent measurement techniques results in high overall selectivity. When combining TPE-UVF with UV-SONICC, the product intensity for the excipient that generated the highest signal, anhydrous lactose, was only 0.008% (i.e., 80 ppm), with further analysis of the other excipients illustrating comparable or much lower relative signal intensities. However, this higher selectivity is limited to APIs that both are chiral and contain an aromatic moiety.

Both conventional and UV-SONICC techniques are effective in their detection of the APIs tadalafil and griseofulvin. Conventional SONICC imaging of a Cialis® tablet, containing the API tadalafil, led to the generation of bright SONICC and TPEF signals for image planes exceeding a 300 µm depth of penetration into the sample (Figure SI-5 (sup. info)). However, shorter penetration depths in API powders were observed for UV-SONICC compared to conventional SONICC measurements, which can be attributed to a combination of increased scatter and absorption at shorter wavelengths. Three-dimensional renderings of Cialis® illustrated the ability to generate strong signals from both TPE-UVF as deep as 140 microns into the sample (Figure SI-6 (sup. info)), despite the short wavelength of the detected SHG signal. Intense signals from both UV-SONICC and TPE-UVF measurements were also

evident during the imaging of griseofulvin, as seen in Figures SI-1 and SI-7 (sup. info), with high resolution image contrast being generated at a depth exceeding 100 microns. Even though there was significant overlap between UV-SONICC and TPE-UVF signals of griseofulvin, the intensity and distribution of the SHG and fluorescence signals were not identical across the sample. The differences in the intensities were attributed to scatter of the incident light and signal photons. Although the TPE-UVF signal was uniform across the sample, as compared to UV-SONICC signals, both a further reduction in the sample thickness to reduce scatter or, more simply, an increase in the incident power to amplify the generated SHG, could lead to a further increase in the detectable SHG signal, helping to extend further the achievable imaging depths through tablets.

It is interesting to compare these results to conventional Raman hyperspectral imaging. The per-pixel signal integration times of SONICC/TPE_UVF were $\sim 250 \mu\text{s}$ in all of the images reported here, compared to a typical Raman integration time of $\sim 5 \text{ s}$ per pixel, corresponding to a $>10^4$ -fold difference. Furthermore, UV-SONICC and TPE-UVF can be acquired simultaneously. Raman imaging and UV-SONICC/TPE-UVF exhibit comparable spatial resolution for analysis of APIs at the interface of a tablet. However, no substantial loss in spatial resolution was observed down to depths of $>120 \mu\text{m}$ in TPE-UVF, consistent with the general insensitivity of multi-photon fluorescence to optical scattering.⁵³ In comparison, Raman imaging suffers resolution losses for depths of only $\sim 10 \mu\text{m}$ due to scattering effects.²⁴

These collective results demonstrate that the combination of UV-SONICC and TPE-UVF, or under favorable conditions conventional SONICC and TPEF, can enable highly selective detection of the APIs investigated relative to the excipients investigated. However, extrapolation of these results to a broad diversity of APIs and new, emerging excipient cocktails should still be made cautiously at this early stage. Only ~ 75 – 80% of emerging APIs contain aromatic constituents,³⁶ and would be expected to produce the increased selectivity afforded by UV-SONICC and TPE-UVF relative to their longer wavelength counterparts. Furthermore, considerable diversity may exist in both the cross section for two-photon excitation and in the subsequent quantum yield for fluorescence. SHG can only arise from non-centrosymmetric crystals, such that APIs prepared from achiral precursors or as racemic co-crystals will most commonly adopt SHG-inactive space groups. Additional in-depth studies designed to characterize the variability in signal strength produced from different APIs would provide additional insights into the sensitivity and selectivity of complementary SONICC and fluorescence measurements to detect broad ranges of APIs in diverse excipient mixtures. With those caveats appropriately in place, the combination of UV-SONICC and TPE-UVF in particular was found to enable selective imaging of model homochiral aromatic APIs in mixtures containing the common excipients studied herein.

Conclusions

SONICC and multi-photon fluorescence were explored for selective detection of model APIs in mixtures with common excipients. The most common complicating excipients for SONICC were forms of crystalline lactose, representing $\sim 12\%$ of the SHG activity of griseofulvin for conventional SONICC and $\sim 2\%$ for UV-SONICC. The enhanced selectivity of UV-SONICC was attributed to resonance-enhanced SHG from the aromatic moiety in griseofulvin. The simultaneously acquired TPE-UVF exhibited negligible interference from lactose crystal forms, but significant UV fluorescence background from Opadry[®] white, attributed to titania nanoparticle fluorescence. The absence of overlapping interferences between SONICC and TPE-UV provides high selectivity for aromatic and homochiral APIs such as griseofulvin through the combined measurements. Investigations on cleaved Cialis

tablets allowed measurements of tadalafil distributions to depths of 140 μm while preserving high S/N and image quality.

The ability to selectively detect and image APIs helps lay the foundation for applications involving API distribution and homogeneity studies within tablets and formulations. Assessing API distribution is a key step in maintain homogeneity of the drug within powdered blends through the dosage form (e.g., within a tablet) and across forms (e.g., between tablets). The distribution of APIs could potentially serve as an intrinsic “signature” for identification of counterfeit dosage forms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Khan H, Ali M, Ahuja A, Ali J. *Curr. Pharm. Anal.* 2010; 6:142–150. (Copyright (C) 2011 American Chemical Society (ACS). All Rights Reserved.).
2. Deisingh AK. *Analyst* (Cambridge, U.K.). 2005; 130:271–279. (Copyright (C) 2011 American Chemical Society (ACS). All Rights Reserved.).
3. Argekar AP, Kapadia SU, Raj SV, Kunjir SS. *Indian Drugs.* 1996; 33:261–266. (Copyright (C) 2011 American Chemical Society (ACS). All Rights Reserved.).
4. Balto DA. *Food and Drug Law Journal.* 2000; 55(3):321–341. [PubMed: 11824464]
5. Bates S, Zografi G, Engers D, Morris K, Crowley K, Newman A. *Pharmaceutical Research.* 2006; 23(10):2333–2349. [PubMed: 17021963]
6. Nunes C, Mahendrasingam A, Suryanarayanan R. *Pharmaceutical Research.* 2005; 22(11):1942–1953. [PubMed: 16132342]
7. Tozuka Y, Ito A, Seki H, Oguchi T, Yamamoto K. *Chemical & Pharmaceutical Bulletin.* 2002; 50(8):1128–1130.
8. Kitahara S, Ishizuka T, Kikkoji T, Matsuda R, Hayashi Y. *International Journal of Pharmaceutics.* 2004; 283(1–2):63–69. [PubMed: 15363502]
9. Yamada H, Masuda K, Ishige T, Fujii K, Uekusa H, Miura K, Yonemochi E, Terada K. *Journal of Pharmaceutical and Biomedical Analysis.* 2011; 56(2):448–453. [PubMed: 21703798]
10. Meyer JD, Jun Bai S, Rani M, Suryanarayanan R, Nayar R, Carpenter JF, Manning MC. *Journal of Pharmaceutical Sciences.* 2004; 93(5):1359–1366. [PubMed: 15067711]
11. Mhraryan A, Llagostera AP, Karmhag R, Stromme M, Ek R. *International Journal of Pharmaceutics.* 2004; 269(2):433–442. [PubMed: 14706254]
12. Bernardi L, Oliveira P, Murakami F, Silva M, Borgmann S, Cardoso S. *Journal of Thermal Analysis and Calorimetry.* 2009; 97(2):729–733.
13. Kojima T, Onoue S, Murase N, Katoh F, Mano T, Matsuda Y. *Pharmaceutical Research.* 2006; 23(4):806–812. [PubMed: 16575500]
14. Taylor LS, Zografi G. *Pharmaceutical Research.* 1998; 15(5):755–761. [PubMed: 9619786]
15. Black DB, Lovering EG. *Journal of Pharmacy and Pharmacology.* 1977; 29(1):684–687. [PubMed: 22603]

16. Berendt RT, Sperger DM, Munson EJ, Isbester PK. *TrAC Trends in Analytical Chemistry*. 2006; 25(10):977–984.
17. Johari GP, Ram S, Astl G, Mayer E. *Journal of Non-Crystalline Solids*. 1990; 116(2–3):282–285.
18. Feng T, Pinal R, Carvajal MT. *Journal of Pharmaceutical Sciences*. 2008; 97(8):3207–3221. [PubMed: 17990307]
19. Wanapun D, Kestur US, Taylor LS, Simpson GJ. *Analytical Chemistry*. 2011; 83(12):4745–4751. [PubMed: 21598920]
20. Davies MC, Binns JS, Melia CD, Bourgeois D. *Spectrochimica Acta Part A: Molecular Spectroscopy*. 1990; 46(2):277–283.
21. Taylor LS, Langkilde FW. *Journal of Pharmaceutical Sciences*. 2000; 89(10):1342–1353. [PubMed: 10980509]
22. Rogers JA, Paul KE, Jackman RJ, Whitesides GM. Using an elastomeric phase mask for sub-100 nm photolithography in the optical near field. 1997; Vol. 70:2658–2660. AIP:
23. McGoverin CM, Rades T, Gordon KC. *Journal of Pharmaceutical Sciences*. 2008; 97(11):4598–4621. [PubMed: 18306273]
24. Gordon KC, McGoverin CM. *International Journal of Pharmaceutics*. 2011; 417(1–2):151–162. [PubMed: 21194560]
25. Zhang L, Henson MJ, Sekulic SS. *Analytica Chimica Acta*. 2005; 545(2):262–278.
26. Fu Y, Wang HF, Shi RY, Cheng JX. *Opt. Express*. 2006; 14(9):3942–3951. [PubMed: 19516542]
27. Sinka IC, Burch SF, Tweed JH, Cunningham JC. *International Journal of Pharmaceutics*. 2004; 271(1–2):215–224. [PubMed: 15129988]
28. Miguélez-Morán AM, Wu C-Y, Dong H, Seville JPK. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009; 72(1):173–182. [PubMed: 19130881]
29. Wanapun D, Kestur US, Kissick DJ, Simpson GJ, Taylor LS. *Analytical Chemistry*. 2010; 82(13):5425–5432. [PubMed: 20515064]
30. Kissick DJ, Wanapun D, Simpson GJ. *Annu. Rev. Anal. Chem.* 2011; 4:419–437. (Copyright (C) 2011 American Chemical Society (ACS). All Rights Reserved.).
31. Wampler RD, Kissick DJ, Dehen CJ, Gualtieri EJ, Grey JL, Wang HF, Thompson DH, Cheng JX, Simpson GJ. *Journal of the American Chemical Society*. 2008; 130(43):14076–+. [PubMed: 18831587]
32. Strachan CJ, Windbergs M, Offerhaus HL. *International Journal of Pharmaceutics*. 2011; 417(1–2):163–172. [PubMed: 21182913]
33. Lee CJ, Strachan CJ, Manson PJ, Rades T. *Journal of Pharmacy and Pharmacology*. 2007; 59(2):241–250. [PubMed: 17270077]
34. Rawle CB, Lee CJ, Strachan CJ, Payne K, Manson PJ, Rades T. *Journal of Pharmaceutical Sciences*. 2006; 95(4):761–768. [PubMed: 16498571]
35. Strachan CJ, Rades T, Lee CJ. *Optics and Lasers in Engineering*. 2005; 43(2):209–220.
36. Constable DJC, Dunn PJ, Hayler JD, Humphrey GR, Leazer JL, Linderman RJ, Lorenz K, Manley J, Pearlman BA, Wells A, Zaks A, Zhang TY. *Green Chemistry*. 2007; 9(5):411–420.
37. Knoblauch J, Zimmermann I. *European Journal of Pharmaceutics and Biopharmaceutics*. 2007; 67(3):743–751. [PubMed: 17540547]
38. Chu CC, Imae T. *Macromolecular Rapid Communications*. 2009; 30(2):89–93. [PubMed: 21706580]
39. Lee WI, Bae YJ, Bard AJ. *Journal of the American Chemical Society*. 2004; 126(27):8358–8359. [PubMed: 15237975]
40. Wang DJ, Imae T. *Journal of the American Chemical Society*. 2004; 126(41):13204–13205. [PubMed: 15479057]
41. Wu DC, Liu Y, He CB, Goh SH. *Macromolecules*. 2005; 38(24):9906–9909.
42. Onoshima D, Imae T. *Soft Matter*. 2006; 2(2):141–148.
43. Antharjanam PKS, Jaseer M, Ragi KN, Prasad E. *Journal of Photochemistry and Photobiology a-Chemistry*. 2009; 203(1):50–55.

44. Jeong S, Kwak G, Takagi A, Fujiki M, Lee DH, Park LS, Yoon KB. *European Polymer Journal*. 2008; 44(4):1149–1156.
45. Lin Y, Gao JW, Liu HW, Li YS. *Macromolecules*. 2009; 42(9):3237–3246.
46. Tamano K, Imae T. *Journal of Nanoscience and Nanotechnology*. 2008; 8(9):4329–4334. [PubMed: 19049021]
47. Mohamed NA, Fahmy MM. *Journal of Applied Polymer Science*. 2009; 113(2):767–776.
48. Hauptert LM, Simpson GJ, Slipchenko LV. *The Journal of Physical Chemistry A*. 2011; 115(36): 10159–10165. [PubMed: 21812447]
49. Yang H, Zhu S, Pan N. *Journal of Applied Polymer Science*. 2004; 92(5):3201–3210.
50. Kim JY, Kim SH, Lee HH, Lee K, Ma W, Gong X, Heeger AJ. *Advanced Materials*. 2006; 18(5): 572–576.
51. Gratzel M. *Nature*. 2001; 414(6861):338–344. [PubMed: 11713540]
52. Murakami S-Y, Kominami H, Kera Y, Ikeda S, Noguchi H, Uosaki K, Ohtani B. *Research on Chemical Intermediates*. 2007; 33(3–5):285–296.
53. Centonze VE, White JG. *Biophysical Journal*. 1998; 75(4):2015–2024. [PubMed: 9746543]

Figure 1.

UV-SONICC and TPE-UVF comparison of griseofulvin, with (A) the bright field image and (B) the 3D threshold image overlay, with UV-SONICC signal (blue), TPE-UVF signal (green), and the overlap (red). The UV-SONICC threshold was set to 2× that of TPE-UVF for better spatial overlap. 2D threshold images of (C) the UV-SONICC signal, (D) TPE-UVF signal, and (E) the combination of UV-SONICC and TPE-UVF where the spatial overlap of both signals is shown in red. Scale bars are 100 μm .

Table I

Compiled summary of average excipient signal intensities for UV-SONICC, conventional SONICC, TPE-UVF, and TPEF relative to the active pharmaceutical ingredient, griseofulvin, as well as their 95% confidence intervals. The results are displayed in the order of decreasing signal intensities for UV-SONICC. Also displayed are the combined UV and IR values. HPC = hydroxypropylcellulose, HPMCAS = hydroxypropylmethylcellulose acetate succinate, TPGS = d- α -tocopheryl polyethylene glycol 1000 succinate, SLS = sodium lauryl sulfate, A-TAB = dibasic calcium phosphate, PVP = polyvinylpyrrolidone. The combined UV column is the product of the UV-SONICC and TPE-UVF columns, and the combined IR column is the product of the conventional SONICC and TPEF columns.

Excipient	Percentages relative to Griseofulvin					Combined UV	Combined IR
	UV-SONICC	Conventional SONICC	TPE-UVF	TPEF			
<i>Griseofulvin (API)</i>	100	100	100	100		100	100
Anhydrous lactose	2.1 $\pm$ 0.9	12.0 $\pm$ 0.2	0 $\pm$ 4.2	4 $\pm$ 1		8.2 $\times$ 10 ⁻³	0.48
Lactose Monohydrate	1.0 $\pm$ 0.3	8.53 $\pm$ 0.07	0 $\pm$ 0.7	1.45 $\pm$ 0.05		4.2 $\times$ 10 ⁻⁵	0.12
Placebo Mixture 1	0 $\pm$ 1.5	0 $\pm$ 1.3	0 $\pm$ 2.0	1.8 $\pm$ 0.4		1.4 $\times$ 10 ⁻⁴	9.0 $\times$ 10 ⁻³
Placebo Mixture 2	0 $\pm$ 1.3	0.5 $\pm$ 0.3	0 $\pm$ 0.1	2.8 $\pm$ 0.2		2.0 $\times$ 10 ⁻⁵	0.013
Mannitol	0 $\pm$ 0.7	0.6 $\pm$ 0.1	0 $\pm$ 0.9	1.28 $\pm$ 0.05		7.8 $\times$ 10 ⁻⁶	7.2 $\times$ 10 ⁻³
Avicel	0 $\pm$ 0.4	0 $\pm$ 0.2	0 $\pm$ 0.7	2.57 $\pm$ 0.03		7.3 $\times$ 10 ⁻⁶	2.0 $\times$ 10 ⁻³
Opadry® White	0 $\pm$ 1.3	0.11 $\pm$ 0.02	11 $\pm$ 3	4.45 $\pm$ 0.05		2.3 $\times$ 10 ⁻⁴	4.8 $\times$ 10 ⁻³
Vitamin E TPGS, HPC, SLS, PVP, HPMCAS-LF, magnesium stearate, A-TAB, Kolldon VA64, colloidal SiO ₂	--	0.020	--	--		--	--