Detection of Fluorescent Brightening Agents Tinopal CBS-X and FB28 in food matrices using UPLC with tandem PDA/FLR detection

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ABSTRACT
A method was developed and validated for the detection of Fluorescent Brightening Agents Tinopal CBS-X and FB28 in food matrices, based on existing extraction and analysis methods from peer-reviewed literature. The method utilizes a simple extraction procedure followed by Ultra Performance Liquid Chromatography (UPLC) analysis using tandem photodiode array (PDA) and fluorescence (FLR) detectors. A qualitative validation was performed using frozen shrimp, rice flour, and dry rice noodles for the test matrices, three food products that have previously been reported to contain these adulterants. Quantitative use of the method was also examined as part of the validation process. The method performed reliably on a qualitative basis down to an estimated Method Detection Limit of 50 ppb for Tinopal CBS-X and 260 ppb for FB28, with no False Positive or False Negative errors detected in the matrices tested.

Keywords: Color additive, Fluorescent Brightener/Whitener Agents (FBA/FWA), Optical Brightener, Liquid Chromatography, Photodiode Array, Fluorescence, UV Spectra.

INTRODUCTION
Numerous reports in the scientific literature describe the adulteration of foods and other products with Fluorescent Brightening Agents (FBAs), also known as Fluorescent Whitening Agents (FWAs), to enhance their appearance. FBAs are legally used in products such as bleach-free laundry brighteners, but are not permitted in food matrices in the United States or many countries throughout the world. FBAs are part of a class of chemicals commonly called Optical Brighteners because they are typically synthetic molecules designed to make textiles and other materials appear whiter and brighter, and psychologically, cleaner. This is a modern version of the 'blueing' practice that was popular in the early 20th century, which had a similar effect. FBAs are also commonly used in paper products, such as anti-counterfeiting stripes in currency bills that are easily illuminated under UV light. There are nearly 400 Fluorescent Brightener compound designations currently listed in the International Colour Index, utilizing a variety of different chemical mechanisms. The majority of FBAs are fluorescent compounds that absorb energy (excite) at ultraviolet (UV) (non-visible) wavelengths but emit light in the visible region (approximately 400 – 700 nm). This results in a brightness perceived by the human eye from non-visible illumination. This is most noticeable when an FBA-treated fabric is observed under UV illumination such as a 'black' light, resulting in a bright white or colored appearance compared to untreated materials.

Two commonly used FBAs are Tinopal CBS-X [FB351, CAS# 27344-41-8, 4,4'-bis(2-sulfonatostyryl)biphenyl disodium and Fluorescent Brightener 28 [FB28, CAS# 4404-43-7, 4,4'-bis(6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino)stilbene-2,2'-disulfonate disodium, as they both demonstrate good stability and are highly water-soluble (Figure 1). Both

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compounds are of the stilbene chemical class, which contains many different fluorescent brightener compounds.

Various methods have been developed to detect FBAs in consumer products, including the use of Thin Layer Chromatography (TLC) coupled with fluorescence detection, HPLC/UPLC with PDA detection, and LC/MS (Liquid Chromatography/Mass Spectrometry) technologies. Ko et al. (2014) describes a method to specifically detect Tinopal CBS-X in rice papers and rice noodles using HPLC with fluorescence detection and confirmation by LC-MS/MS. According to the article, there had been noted concern among Korean consumers about processed rice products from Vietnam that had been adulterated with this compound.

Liao (2016) describes a similar method using UHPLC with diode array detection (DAD) to detect Tinopal CBS-X and FB28, as well as two other similar FBA compounds in facial masks.

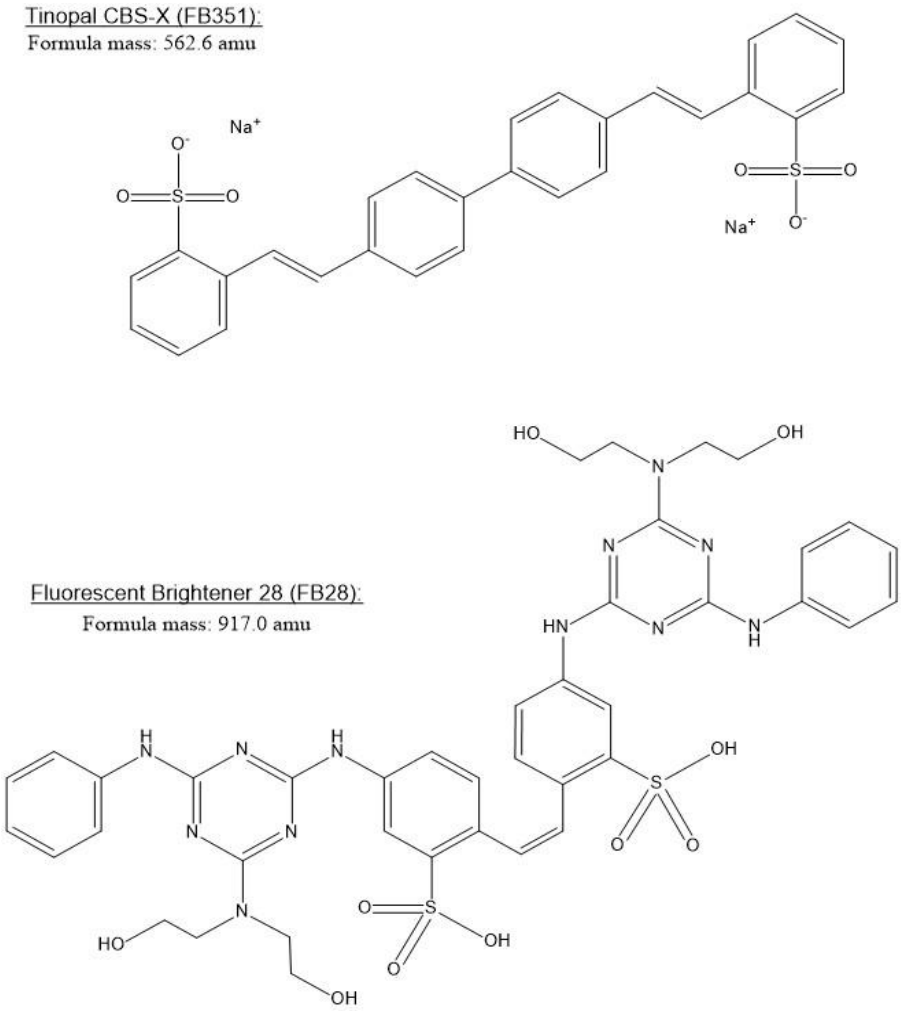

Figure 1: Chemical Structures of Tinopal CBS-X and FB28

Because there is no legal tolerance for FBA chemicals in food products, this method was developed and validated on a qualitative basis. If analysis positively determines the presence of an FBA in a food product, it is considered adulterated.

There have also been some concerns raised in the literature concerning the potential transfer of FBAs from product packaging to the food products contained within. Detection of these compounds in food matrices, regardless of the mechanism of adulteration, is needed to protect consumer health, as their potential toxicological effects have not been well characterized. The current method was designed with a simple extraction process and subsequent analysis using relatively inexpensive technology (LC with PDA/FLR detection).

EXPERIMENTAL

Equipment & Consumables

(Equivalents may be substituted as necessary)

- Acquity H-Class UPLC running Empower 3 (FR2 Build 3471) with tandem Photodiode Array (PDA) and Fluorescence (FLR) detectors (Waters, Milford, MA)
- Column: Acquity BEH Shield RP18 (2.1 x 50 mm; 1.7 µm particle size) (Waters)
- Vials: Tapered 12x32mm glass Total Recovery UPLC vials with pre-slit caps (Waters)
- Centrifuge: Sorvall Lynx 6000 (Thermo Scientific, Waltham, MA)
- Pipettor: Biohit 1-10 mL (Biohit, Helsinki, Finland)
- Balances used: (Standards) Mettler-Toledo XS205 (Mettler, Greifensee, Switzerland)
    - (Sample material) Ohaus Pioneer PA3102 (Ohaus, Parsippany, NJ)
- Multi-tube vortexer: Fisher (Fisher Scientific, Pittsburg, PA)
- Tubes: 50 mL Polypropylene Falcon conical centrifuge tubes (Fisher Scientific)
- BD 1 mL plastic syringes (BD, Franklin Lakes, NJ)
- Filters: Acrodisc PTFE, 13mm diameter, 0.2 µm pore size (Pall, Port Washington, NY)
- Water bath: Boekel-Grant ORS200 at 80 °C (Boekel Scientific, Bucks County, PA)
- Milli-Q water purification system (Millipore, Burlington, MA)

Reagents

- Methanol (Optima LC/MS Grade, Fisher Scientific)
- Mobile Phase A: 10 mM Ammonium acetate (ACS Grade, JT Baker, Radnor, PA) in Milli-Q water
- Mobile Phase B: Acetonitrile (Optima LC/MS Grade, Fisher Scientific)

Procedure

1. Add 20 mL 75% methanol to each 50 mL conical tube containing sample/blank/control. Cap tightly and vortex for 1 min.
2. Heat tubes for 30 min in an ~ 80 °C water bath, preferably with shaking at ~ 60-120 cycles/min. It is critical to keep tubes upright during incubation, as they will likely leak if they begin to float horizontally.
3. Carefully remove the tubes from the water bath. Be aware that the solvent will likely be boiling, and ensure that tubes are pointed away from face and body. Note any tubes that have lost significant volume, as these tubes may be leaking and cannot be analyzed quantitatively (if that is desired).
4. Vortex warm tubes for ~1 min, then centrifuge tubes at 6,000 rpm for 5 min (refrigeration is not necessary).
5. Filter 0.5-1 mL of supernatant through a 0.22 µm PTFE syringe filter into a UPLC vial. Note that some matrices may have increased backpressure when filtering due to matrix components, and may require multiple filters to get ~ 0.5-1 mL of filtered material. Do not use excessive pressure to force material through filter or it may become compromised and burst under pressure. Each UPLC vial must be checked, if it has a conical bottom, to ensure that no air

bubbles persist in the bottom of the tube prior to UPLC injection. Slight tapping of the vial can remove any lingering air bubbles.

Instrument Parameters

- Flow rate of 0.4 mL/min, with gradient:
  - Initial: 90% A, 10% B
  - 8 min: 0% A,100% B, hold 4 min
  - 13 min: 90% A, 10% B, hold 4 min; 17 min total run time.
- Column temperature of 40 °C, with sample compartment temperature set to 25 °C.
- Fluorescence data collected at 350 nm excitation and 430 nm emission wavelengths.
- 3D (Max Plot) PDA data (210-400 nm) collected to obtain spectra for observed peaks.
- 2D UV-Visible data collected at 350 nm as described in Liao *et al*.
- Gradient scheme based on Liao *et al*., but simplified and shortened. Overall run time selected as 17 min to reduce carryover, ensure matrix components elute fully from column, to have sufficient chromatographic space to allow addition of other FBA analytes in the future, and to ensure system is re-equilibrated prior to the next injection.

Standard Preparations

- Tinopal CBS-X (CAS# 27344-41-8) was obtained from Toronto Research Chemicals (Toronto, Canada)
- Fluorescent Brightener 28 (FB28) (CAS# 4404-43-7) was obtained from Sigma-Aldrich (Burlington, MA)
- Approx. 100 mg of each FBA powder were weighed analytically and added to individual 100.0 mL colored glass volumetric flasks, then brought to volume with 5% methanol in Milli-Q water to make ~ 1 mg/mL stock solutions. Solutions were mixed then sonicated for 5 min at room temp. All standard solutions were stored at ~ 4 °C when not in use.
- A ~ 100 ppm mixed standard solution was made by dispensing 5.00 mL Tinopal CBS-X and 25.00 mL FB28 stock solutions into a 50.00 mL glass volumetric flask, bringing it to volume with Milli-Q water. This solution was diluted 1:10 with Milli-Q water to make the ~ 10 ppm mixed standard spiking solution. (Note that the name "100 ppm solution" refers only to the concentration of CBS-X. The FB28 concentration would be approx. 5x higher.)

Sample Preparations

- Weigh at least ~ 3 g (typically no more than 15 g) of shrimp tissue (with or without shell, as presented) or rice-based product matrix into a 50 mL conical, plastic centrifuge tube. (Can use a whole shrimp, though it may need to be cut into pieces.) When analyzing shrimp tissue, try to include soft portions that are exposed (i.e., not underneath the shell), as brighteners are typically applied externally. Record weight of material. Product may be cut into smaller pieces or extracted whole.
- Spike positive control shrimp or other material with approx. 3 mL of the mixed standard spiking solution (3 mL/~ 3 g material) (see Standard Preparations section). Typical spike level will be at ~ 5-10 ppm Tinopal CBS-X, but the level may vary depending on the fluorescent properties of the FBAs under study. FB28 spikes would be at ~ 25-50 ppm, as FB28 is approx. 5x less fluorescent (i.e., has a lower emission efficiency) than CBS-X at the emission detection wavelength of 430 nm. Let matrix incubate at room temperature in spiking solution for a minimum of 10 min.

Data

For purposes of the level II single lab validation, three spike levels (2, 5, 10 ppm CBS-X and 10, 25, 50 ppm FB28) were analyzed in three different matrices: frozen partially shelled

shrimp, rice flour, and dry rice noodles. From in-house observations and results published in Ko *et al.*, 2014 and Liao, 2016, Tinopal CBS-X is highly effective at 1-10 ppm, whereas FB28 is approx. 5x less fluorescent. Thus, a reference concentration of 5 ppm CBS-X (25 ppm FB28) was selected for the validation.

There was a significant difference in apparent detection capability (sensitivity) using the PDA and fluorescence detectors. The signal from the FLR detector was approx. 100x stronger than that of the PDA absorbance at 350 nm. Initial feasibility tests suggested CBS-X can be detected down as low as ~ 1 ppb using the FLR detector, but only down to ~ 0.1 ppm using the PDA detector. Furthermore, a test curve of CBS-X standard (alone) indicated that the FLR detector shows evidence of self-quenching when the signal is too high (> ~ 15 ppm) for a single component reference standard solution, resulting in a slightly quadratic calibration curve. The PDA signal was linear at all concentrations tested.

Use of a lower range of standard concentrations and mixed standard solutions (as opposed to individual standard solutions) can reduce the likelihood of self-quenching (which can result in underestimated peak areas). When self-quenching occurs, it dramatically affects the visible peak shapes, creating portions of the peak with negative area counts, but fortunately this is quite apparent in the chromatogram. If self-quenching is observed for standard injections as part of a calibration curve, the affected points cannot be used in the curve. If this is observed, the curve should be constructed at a lower concentration range where the FLR signal remains linear and no quenching is observed.

Given the information above, the method was designed to be performed with tandem PDA and FLR detection. FLR detection was tested down to 1 ppb CBS-X (to determine the instrument Limit of Detection (iLOD)) and will be used as a screen to detect the presence of low levels of FBAs. Quantification, if desired, appears to be possible using the PDA detector as well as the FLR detector as long as no self-quenching is observed in the datapoints. An evaluation of the LOQ was also made using the data collected as part of the validation, however, the validation was performed with the intent of it being a qualitative method.

<u>Peak Detection Requirements</u>
The qualitative determination of whether an analyte in a given injection is *detected* or *not detected* was based on the criteria listed below.

As described in FDA CPGM 7309.006 (Oct 2019), for the analysis of food and color additives, qualitative confirmation of an analyte requires “at least two lines of characterizing data (e.g., UV-Vis spectra, TLC Rf-value, HPLC retention times, etc.)”.

For this method, confirmation of identity utilized fluorescence, UV absorbance, and retention time, as follows:

- The analyte of interest produced a peak that demonstrated fluorescence (emission) at 430 nm when excited at 350 nm, similar to the reference standard.
- The analyte of interest produced a UV absorbance peak at 350 nm, similar to the reference standard.
- The retention times for both FLR and UV chromatographic peaks are within 5% of the retention times observed for the reference standard.
- In addition to the three criteria above, the signal to noise ratio of the chromatographic peak must be at or above 3:1.

Controls/samples with a chromatographic peak meeting the above criteria are determined to be *detected*.

Controls/samples without a chromatographic peak (< MDL) or with a peak that does not meet the above criteria are determined to be *not detected*.

Further confirmation of identity (if desired) may be obtained by comparing the full UV absorbance spectra of the reference standard and the corresponding sample peak:

- The UV spectrum of the peak has a maximum (or maxima, if multiple are used for comparison) absorbance within 5 nm of that of the reference standard, and/or:
- The instrument-determined match calculation between the spectra for the reference standard and the peak of interest demonstrates a high level of certainty. For the Waters instrument (Empower) software, this would represent a calculated Match Angle less than or equal to 4.0.

However, at low analyte concentrations (below ~ 100 ppb), the full UV spectra (labeled in data as "PDA MaxPlot" or the "PDA Spectrum" channel) tend to be of poor quality (i.e., low signal to noise), and can be difficult to compare to reference spectra. In these cases, point smoothing can be applied (though it may slightly shift the annotated maxima) or extracts can be concentrated to improve the spectral quality, if a full spectral comparison is desired. In addition, matrices often contain compounds which absorb highly in the far-UV region (wavelengths < 230 nm) and can thereby distort a spectral comparison. Narrowing the comparison window, such as 220- or 230- to 400 nm can help reduce this interference, when overlaying with reference spectra.

In addition, for low concentrations, the (Waters) MaxPlot algorithm (which mathematically selects the greatest absorbance wavelength (lambda max) for a peak across a range of wavelengths) will sometimes list/annotate a peak based on a large UV absorbance < 230 nm. Thus, in the tabular report for the MaxPlot data, it will sometimes annotate peaks incorrectly and should not be relied upon as part of the detection criteria.

Additional confirmation of identity beyond these criteria can be obtained, when needed, through further analyses such as mass spectrometry.

Statistical Evaluation

Mean, range, minima and maxima, standard deviation, RSD, Student's t-test, and other statistical calculations were performed using Microsoft Excel 365 MSO (32-bit). The false positive/negative rate evaluation was completed based on the Laplace method for estimating uncertainty (Laplace, 1814). Evaluation of sensitivity, selectivity, estimates of MDL and iLOD, LOQ, measurement uncertainty, etc., were performed using the FDA *Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products*.

RESULTS AND DISCUSSION

Initial injections of individual FBA standards indicated that Tinopal CBS-X and FB28 produce well-shaped chromatographic peaks, detectable using the FLR excitation/emission (350 nm/430 nm) wavelengths used in Ko *et al.*, 2014 and PDA detection at 350 nm. Tinopal CBS-X produced a single peak at approximately 3.30 min retention time (RT) while FB28 produced a primary peak at approximately 2.97 min, with secondary peaks at ~ 2.87 min and ~ 3.23 min, all of which were individually integrable. However, only the primary peak for each compound was used for detection purposes. Each primary and secondary peak gave a unique UV spectrum, and the primary Tinopal CBS-X and FB28 peaks corresponded well with published spectra of the *trans* form of these compounds (Liao, 2016).

Retention times of the reference standard injections were stable (highest RSD was 0.18%) across the approx. 33 hour run time for the sequence. Repeated injections had area counts within 1% for both FLR and PDA detector types, except the RSD for FLR detection of FB28, which was slightly higher at 2.7%.

The Instrument Limit of Detection (iLOD), the lowest concentration of analyte that can be detected (i.e., distinguished from noise) by the instrument, was estimated to be 1 ppb for CBS-X and 5 ppb for FB28 using the fluorescence (FLR) detector, and 50 ppb for CBS-X and 260 ppb for FB28 using the UV detector. However, since the detection parameters defined for the method require analyte detection on both detectors, the higher (UV) values will be used.

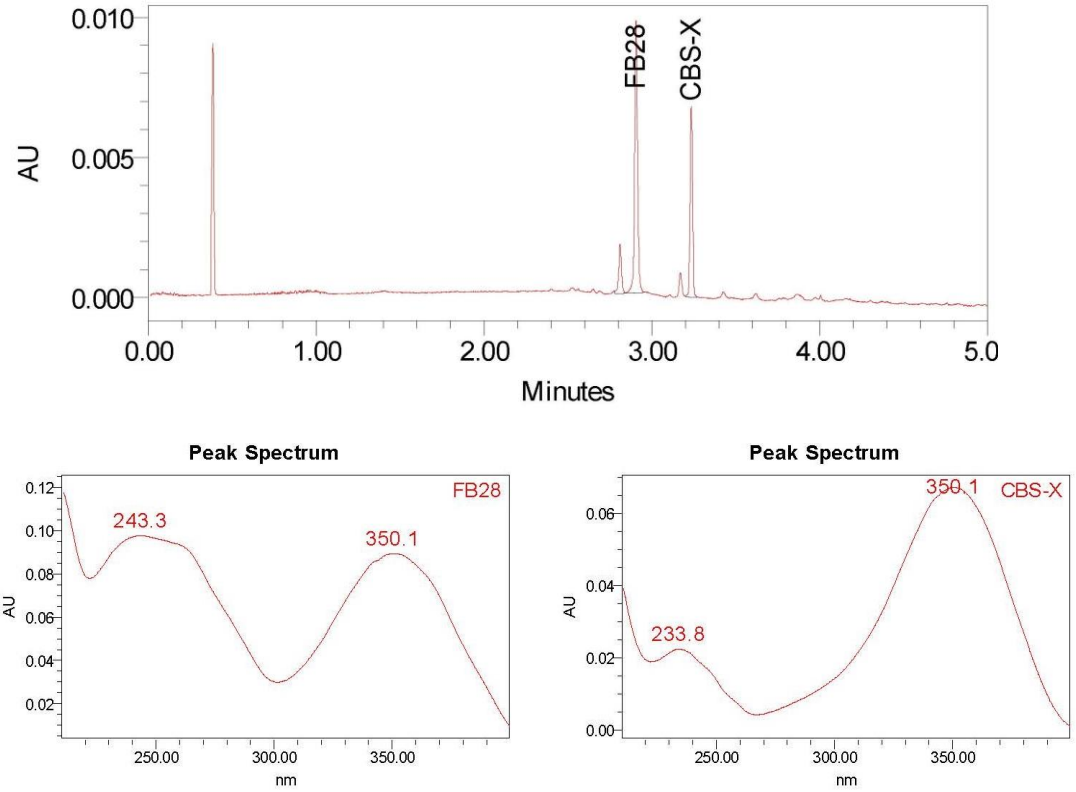

Figure 2: Representative chromatogram showing primary FB28 and CBS-X peaks (top). UV Spectra of FB28 (left) and Tinopal CBS-X (right) from the validation runs (5 ppm CBS-X, 25 ppm FB28) (bottom).

The Method Detection Limit (MDL) takes the entire method into account rather than just the instrument detection. The MDL was estimated using the standard deviation value observed between seven spikes at 10% of the reference concentration (0.5 ppm CBS-X), using the higher value between the FLR and UV detectors. This resulted in estimated MDL values of 18 ppb for CBS-X and 63 ppb for FB28. However, these values were below the iLOD estimations based on the criteria for detection, so the MDL values were estimated at the iLOD values: 50 ppb for CBS-X and 260 ppb for FB28.

The method selectivity was evaluated by examining its performance across all of the injections made as part of the validation study. All blanks, matrix, and negative controls tested (36) were determined to be *Not Detected* using the detection criteria described above. In all but two cases, the positive controls (matrix spikes), reference standard, CCV, and ICV injections (70) were determined to be *Detected*. The only exceptions were one injection attempt where there was a bubble trapped in the bottom of the vial, and low-end injections which were below the determined method detection limit for one or both of the detectors. Following the procedure as described will help mitigate the presence of bubbles in the bottom of the vial, and reference standard injections should routinely be made at concentrations at or above the limit of quantitation (LOQ), estimated to be 0.50 ppm for CBS-X and 2.5 ppm for FB28.

Since the method is intended for use primarily as a qualitative method, the false positive/false negative rates were examined based on the Laplace method of uncertainty estimation (Laplace, 1814). In this case, 19 injections were made of a low level (10% of the reference level) spike as well as 19 injections of a method blank. Using this approach, the probability of the occurrence of either a false positive or false negative outcome was estimated to be less than 5%. Estimation of the measurement uncertainty was also performed, both on a mid-level reference standard and for low level spikes (10% of the reference concentration). The maximum measurement uncertainty values for the reference standard were calculated to be 2.7% for CBS-X and 6.7% for FB28. The maximum measurement uncertainty values for the low shrimp spike were observed to be 21.3% for CBS-X using UV detection (13.1% using FLR detection) and 14.6% for FB28, regardless of detector used.

The method was also evaluated for the potential of matrix interferences. Due to the high sensitivity of the fluorescence detector and the occasional presence of naturally fluorescing compounds in biological matrices, it was not uncommon to see small peaks (< 10 EU) in the FLR spectra of the matrices tested. However, in most cases they were significantly smaller than the lowest (1 ppb CBS-X) standard level injected. Furthermore, there were no corresponding peaks in the UV chromatogram that absorbed at 350 nm (which is a requirement for positive detection). Similarly, some matrices produced UV-350 chromatograms with observable peaks but none of these contained peaks near the same RT that also appeared in the FLR chromatogram. This is the primary reason for utilizing tandem UV-FLR detection in the method. Even if a matrix were to contain peaks at approximately the correct RT for one of the target FBA analytes in both the FLR and UV-350 chromatograms, one could use the software to extract and compare the full UV spectrum for that peak to the spectrum for the FBA of interest for confirmation. All of the interferences observed in the testing and validation of this method could be readily discriminated from the analytes of interest using this tandem UV-FLR screen and confirmatory UV spectral comparison.

It is possible, however, that the full UV spectral comparison to the reference standard could be affected by the nature of the sample matrix. Biological matrices such as foods frequently contain compounds that absorb strongly in the UV region (200-260 nm), though this is less common in the 320-360 nm region (where FBAs typically have an absorbance maximum). Thus, if the extract of interest happens to contain a compound that absorbs in the far-UV region and has a retention time near the analyte of interest, the resulting UV spectrum observed may be skewed in the lower wavelength portion. For example, one spiked shrimp matrix used in the validation showed absorbance at ~ 230 nm that skewed the shape of the curve near the expected 243 nm maximum of FB28. However, the maximum near 350 nm (and hence, the UV-based chromatogram) was unaffected. Matrices such as these may require additional confirmation via another technology such as mass spectrometry, if the presence of an FBA is suspected from the UV-FLR screening run but cannot be confirmed even with the full UV spectral comparison.

The rice flour matrix tested was exceptionally challenging, because of an apparent interaction of the analytes with the matrix, resulting in low overall spike recoveries (performed only as part of the quantitative evaluation), that differed in severity depending on the FBA. In addition, this matrix contained a compound with an RT very near CBS-X that absorbed strongly at 287 nm (though it did not fluoresce at the emission wavelength). It produced such a strong absorbance signal that it dwarfed the absorbance maximum of the CBS-X, making full UV spectral comparison impossible for this matrix. Thus, additional confirmation of identity (if desired) would need to be performed using a different technology. Note; however, that this interferent did not absorb at 350 nm, and therefore did not impact the UV-350 nm chromatogram.

Another potential interference is the possibility of carryover detection from previous injections with the extremely sensitive FLR detector. Although small carryover peaks were sometimes observed with blank injections, they were below the integration threshold and often amounted to less than 0.2 EU, whereas the lowest (1 ppb) CBS-X standard tested resulted in a signal on the order of 1 EU. This level is significantly below the MDL and should not impact detection ability.

One other potential interference of note, more relevant to the use of the method on a quantitative basis, is that one of the subsidiary peaks of FB28 is near the RT for CBS-X. Thus, it is possible that the auto-integration of a CBS-X peak could mistakenly integrate across the FB28(c) peak as well. Though somewhat unlikely due to the near baseline resolution of the two peaks, this would merely result in a small over-estimation of the CBS-X area and would only occur if there was a significant (> 5x) amount of FB28 present in a sample as compared to the amount of CBS-X, given the size of the subsidiary peak (~ 5% of the primary FB28 peak area). Furthermore, the two analytes have distinct UV spectra such that the peaks could be easily distinguished on that basis. This issue should have no impact on the qualitative detection of either FBA analyte tested.

Although the method was intended for qualitative screening, some quantitative data was also collected for the method and matrices of interest. The quantitative evaluation of the method revealed some additional concerns for consideration. First, as mentioned previously, the analyst must watch for self-quenching within the fluorescence signal, which significantly affects the integrated area calculations as it causes the peaks to be partially negative. However, this is easily noticed upon examination of the chromatographic data. Second, due to the dilution factors involved, spike recovery calculations can involve the extrapolation of very small areas (at the very low end of the calibration curve when centered around the 5 ppm reference concentration). Even though all concentrations tested created linear curves, the regression equations across the full 1 ppb to 15 ppm (5 orders of magnitude) curve resulted in large y-intercept values. Thus, when calibration curves are used as part of quantitative analysis, an appropriate range/spiking level should be selected that is close to the concentration of interest, even though detection is possible across a wide range of concentrations. This will result in more accurate concentration extrapolations when calculating recoveries. Finally, spike recoveries clearly depended on the characteristics of the matrix. The method was tested with three of the matrices reported as most commonly adulterated with FBAs: shrimp, rice flour, and rice noodle products. While all were detectable on a qualitative basis, the spike recoveries varied widely across these matrices, and appeared to depend on the hygroscopic nature of each matrix. Shrimp performed well (89-125% recoveries for CBS-X), whereas rice noodles gave intermediate results that varied between the two analytes. Rice flour (the most hygroscopic) performed poorly at the spike levels tested (mean recoveries were ~10 – 20%). Thus, the hygroscopic nature of the matrix to be spiked may need to be considered when selecting the reference and spike concentrations. Alternate extraction methods (such as the use of extraction solutions with low aqueous content) may be more appropriate for flour and other extremely hygroscopic matrices.

CONCLUSION

The FBA detection method described here, based on literature methods from Ko *et al*. (2014) and Liao (2016), was found to perform adequately in our laboratory. Since the U.S. currently has no tolerance limits set for the presence of FBAs in food products, the method is intended to be used qualitatively as a screen for the presence of these compounds above their determined detection limits. The only concern of note for qualitative use is that the analyst needs to ensure that there are no bubbles in the bottom of the LC vials which may interfere with the sampling needle during injection.

The Level II validation data collected across multiple matrices and analyte levels demonstrated specific and reliable detection of the analytes tested on a qualitative basis, with estimated MDL values of 50 ppb for Tinopal CBS-X and 260 ppb for FB28, two prominent FBA compounds. Some data were also collected to evaluate whether the method could be used quantitatively, in the event that tolerance limits are eventually established and to examine the necessary spiking levels for qualitative recovery across the matrices tested.

It is worth noting that there are a vast number of FBAs currently in use, but the two tested here have a significant presence in the scientific literature and have readily available reference standards. Detection of other compounds which readily fluoresce with excitation in the near-UV and emission in the visible region warrant further investigation, and may also work with this analytical method. Although many foods contain natural ingredients that are capable of fluorescence, these are unlikely to be extracted using this procedure at ppm-level concentrations. Compounds with these properties may represent other FBA molecules added as adulterants and should be subjects of further study.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Michael McLaughlin (ORA/ORS) and Michael McLendon (PNL, *ret.*) for their support of the method testing, validation, and implementation, as well as Steven Bloodgood (DSS/CFSAN), Dr. Yinqing Ma (OC/CFSAN), and Dr. Michael Wichman for their assistance in creating and executing tinopal-related sampling assignments, and also to Dr. Wichman for his suggestions on manuscript edits.

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