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Determination of Tinopal CBS-X in rice papers and rice noodles using HPLC with fluorescence detection and LC-MS/MS

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To date there have been no reports of methods to determine Tinopal CBS-X. We developed a rapid and simple method to determine the Tinopal CBS-X content in rice noodles and rice papers using HPLC equipped with fluorescence detection. Heating the rice noodles and rice papers to 80°C after adding 75% methanol solution induced the release of Tinopal CBS-X from processed rice products. Tinopal CBS-X was separated using an isocratic mobile phase comprising 50% acetonitrile/water containing 0.4% tetrabutyl ammonium hydrogen sulphate at pH 8.0. The samples suspected to be positive by HPLC analysis were then confirmed by LC-MS/MS analysis. This study also investigated the Tinopal CBS-X content of three rice noodle products and two rice papers. The limits of quantification for rice papers and rice noodles were 1.58 and 1.51 μg kg^{-1}, respectively, and their correlation curves showed good linearity with $r^2 \geq 0.9997$ and ≥ 0.9998, respectively. Moreover, rice papers had recoveries of 70.3–83.3% with precision ranging from 5.0% to 7.9%, whereas rice noodles had slightly lower recoveries of 63.4–78.7% and precisions of 8.5–11.5%. Only one rice noodle product contained Tinopal CBS-X, at around 2.1 mg kg^{-1}, whereas it was not detected in four other samples. Consequently, Tinopal CBS-X from rice noodles and rice papers can be successfully detected using the developed pre-treatment and ion-pairing HPLC system coupled with fluorescence detection.

Keywords: Tinopal CBS-X; rice noodles; rice papers; adulteration; HPLC

Introduction

Public concern related to processed rice products adulterated with Tinopal CBS-X has recently emerged in Vietnam (see http://news.dbv.vn/detecting-toxic-acids-in-foods-from-rice-166623.html,2013). People in Korea frequently consume rice noodles and rice papers imported from Vietnam. Tinopal CBS-X might be intentionally used to increase the whiteness of rice papers and rice noodles. Therefore, consumers in Korea want to know whether processed rice products imported from Vietnam are safe because adulteration of these products might threaten consumer health.

Generally, fluorescent whitening agents known as optical brighteners are used as supplementary agents to increase colour, temperature, whiteness and brightness during the manufacturing processes. They are known to absorb energy in the ultraviolet (UV) light region and emit it in the visible spectral region (Leaver & Milligan 1984; Kramer et al. 1996; Canonica et al. 1997). In addition, fluorescent organic compounds have natural affinities with various substrates and the amount of fluorescent emission is affected by the type of resin matrix (Shu & Ding 2009). Tinopal CBS-X (IUPAC name: disodium 2-[(*Z*)-2-[4-[4-[(*Z*)-2-2(2-sulfonatophenyl)ethenyl]phenyl]phenlyl]ethenyl] benzenesulfonate, $C_{28}H_{20}O_6S_2Na_2$; Figure 1) has a molecular weight of about 562.56 and is known as a fluorescent whitening agent (FBZ 351) or STILBENE 3 (Pubchem; Stana et al. 1995; Blanco et al. 2001). Because Tinopal CBS-X has very good stability and solubility, it is suitable for incorporation in conventional or compact washing powders, liquid detergents, industrial detergents and washing creams (Iamazaki & Atvars 2006). Moreover, it is widely used for dyes, paper, paints, cellulose fibre, plastics, textiles, etc., to increase their whiteness and brightness (Damant & Castle 1999; Shu & Ding 2005; Yamaki et al. 2005; Iamazaki & Atvars 2006; Iamazaki & Atvars 2007).

A number of methods have been reported to detect fluorescent whitening agents (FWAs), including Tinopal, in commercial products: spectrophotometric methods were used for the determination of FWAs in textile and paper products (Burg et al. 1977; Lepri et al. 1985); florescence spectroscopy was employed for the quantification of Tinopal CBS on fibre surfaces (Iamazaki & Atvars 2006); thin layer chromatography coupled with fluorescence spectrophotometry was used for the separation of optical brighteners in laundry products (Schulze et al. 1974); HPLC equipped with fluorescence detection (FLD) has been one of the frequently employed procedures for the separation and determination of FWAs in detergents and environmental samples such as lake water (Kirkpatrick 1977; McPherson & Omelczenko 1980;

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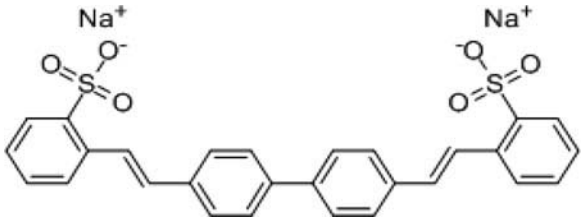

Figure 1. Chemical structure of Tinopal CBS-X.

Micali et al. 1984; Jasperse & Steiger 1992; Poiger et al. 1996; Stoll & Giger 1997; Shu & Ding 2005; Shu & Ding 2009; Vindogopal & Peller 2010). In the research employing HPLC analysis, hot water extraction and/or C_{18}-SPE cartridges were used as the major pre-treatment methods to separate FWAs from clothes and papers (Shu & Ding 2009; Vindogopal & Peller 2010). In addition, the combination of HPLC and LC/ESI-MS or LC/ESI-MS/MS seems to be a more powerful method for determining FWAs in detergents, paper materials and clothes rather than by HPLC analysis alone (Ogura et al. 1995; Chen & Ding 2006).

Tinopal CBS-X is reported to have mildly toxicological properties; however, the compound possesses neither teratogenic nor mutagenic and does not reproduction toxicity (Keplinger et al. 1974; Lyman et al. 1975). However, Tinopal CBS-X is one of the substances banned from use in foodstuffs or food processing in Korea. Based on the recent concern related to the adulteration of rice noodles and rice papers with Tinopal CBS-X, it became important for the Department of Food Safety Evaluation, Korea Ministry of Food and Drug Safety (KMFDS), to determine whether Tinopal CBS-X was illegally added to imported processed rice products. Until now, many methods have been reported for the determination of FWAs in various matrices such as paper, textiles and water. However, there is no reported method that can determine the presence of Tinopal CBS-X in foodstuffs. Therefore, it became necessary for the MFDS to develop a novel method for separating Tinopal CBS-X from foodstuffs such as processed rice products. In the present study, a novel pre-treatment method was developed using methanol extraction with heating to extract Tinopal CBS-X from processed rice products prior to HPLC analysis.

Materials and method

Materials

Tinopal CBS-X (CAS No. 27344-41-8), known as a chemical reagent for detergents and fibres, was purchased from Toronto Research Chemicals (Toronto, ON, Canada). A rice noodle product adulterated with Tinopal CBS-X was imported from Vietnam to Korea. We acquired the sample from the Seoul regional MFDS that conducts various examinations of imported foods to investigate whether processed rice products contained Tinopal CBS-X. Two rice noodles and two rice papers used for the quantification of Tinopal CBS-X were purchased from local markets in Chungcheongbuk-do, Korea. Methanol and acetonitrile were of HPLC grade and purchased from Sigma. Tetrabutylammonium bisulphate (TBA) used as the mobile phase was a Sigma product.

Ultraviolet (UV) radiation

As a preliminary test, it was investigated whether rice noodles or rice papers have fluorescent properties prior to quantification analysis using radiation from a UV lamp with a main wavelength 365 nm.

Sample and standard solution preparation

Rice papers and rice noodles were cut into pieces and ground with a mixer (Philips blender HR 2094, China). Then about 3 g of each sample were weighed and put into a glass bottle. Methanol (30 ml) of 50%, 75% or 87% concentration was added to the bottle and it was heated in a water bath (Daeki Science, DK-SAWO10, Seoul, Korea) at 60, 70, 80 and 90°C for 30 min. The methanol solution has a relatively low boiling point and could easily be evaporated during heating; therefore, the bottles were closed with caps but not tightly closed to release the gas formed during heating. The heated samples were transferred to tubes; after cooling, they were centrifuged (Hanil Corp. Supra-22K, Anyang, Korea) at 5000 rpm for 10 min. The supernatant was collected and the same concentration of methanol (10 ml) was added to the precipitate. The precipitate solution was sonicated (OMAX, AJC-4020, Buchun, Korea) for 10 min at RT and the solution was then centrifuged under the same conditions as described above. After separating the second supernatant, re-extraction was conducted by adding the same concentration of methanol (10 ml) to the precipitate and then centrifugation was achieved after sonication. Each supernatant was collected into a volumetric flask and then the solution was massed up to 50 ml with the same concentration of methanol solution. After the aqueous solution was filtered using a 0.45 µm membrane filter, it was used for HPLC analysis. In addition, for the LC-MS/MS analysis, the final solution was evaporated to dryness by a nitrogen stream and then dissolved in 100 µl of 50% acetonitrile.

HPLC conditions

The HPLC-FLD analysis was carried out in an HPLC system (Nanospace SI-2, Shiseido, Tokyo, Japan) consisting of a quaternary pump, an auto-sampler, a column oven and a fluorescence detector. Chromatographic separation was performed with a CAPCELL PAK SP C_{18} (5 mm, 4.6 mm i.d. × 250 mm; Shiseido) column. The mobile

phase consisted of 0.4% tetrabutylammonium hydrogen sulphate in a 50% acetonitrile–water mixture adjusted to pH 8.0, and the analysis was performed with isocratic elution (Vindogopal & Peller 2010). The flow rate was set at 1 ml min^{-1}, with an oven temperature of 40°C and an injection volume of 20 µl. The fluorescence detector was operated at an excitation wavelength of 350 nm and an emission wavelength of 430 nm.

LC-MS/MS conditions

Samples that had certain peaks with the same retention time as that of the standard solution in the HPLC analysis were confirmed using an LC-MS/MS system comprising an micro-inert HPLC system (SPˆLC, Shiseido) with a binary pump, an auto-sampler and a column oven, equipped with a Thermo TSQ Quantum Ultra detector (Thermo, Pttsburgh, PA, USA). The LC-MS/MS analytical conditions for Tinopal CBS-X were modified by the method of Chen and Ding (2006). The column used in the LC-MS/MS analysis was ZORBAX Extend C_{18} 15 cm × 2.1 mm id, with a 5 µm particle size (Agilent, Santa Clara, CA, USA); the flow rate was 0.2 ml min^{-1}, and the injection volume was set at 10 µl. The mobile phase consisted of 5 mM di-*n*-hexylammonium acetate in deionised water (A) and acetonitrile (B). The analysis was performed under gradient conditions as follows: initial A:B (85:15); change to A:B (55:45) in 10 min; change to A:B (50:50) in 8 min; run to only B in 7 min and hold for 4 min; return to the initial conditions (A:B = 85:15) in 3 min; and re-equilibrate the column for 3 min. The full and product-ion scans were performed by direct infusion (10 µl min^{-1}) of Tinopal-CBX solutions (1000 ng ml^{-1}) into the MS. The data were acquired in the single-reaction monitoring mode (Table 1) and ESI was performed in negative mode. The precursor and product ions were monitored and then the operating ion source parameters for MS/MS were optimised as follows: capillary temperature of 330°C, ion spray voltage of 4300 V, sheath gas pressure of 40 in arbitrary units, and collision gas (argon) at 1.5 m Torr.

Method validation

The quantification method was validated for linearity, accuracy, precision and sensitivity. To estimate the LOD, LOQ and linearity of the method developed in the present study, the stock solution of standard Tinopal CBS-X was homogenously distributed into the final extract solutions obtained from negative rice noodles and papers, which subsequently had concentrations of 2.5, 5, 10, 20 and 40 µg kg^{-1}. Each solution was injected into the HPLC system and the intensity of emission was measured by the FLD detector. This experiment was repeated four times. The linearity of the method was determined by the analysis of standard plots associated with a five-point standard calibration curve. LOD was calculated by 3.3 × SD of the intercept/slope of the calibration curve, whereas LOQ was calculated by 10 × SD of the intercept/slope of the calibration curve. Accuracy and precision were estimated by calculating the recovery rate (%) and coefficient of variation (CV, %), respectively. The recovery rate was determined by the experiment conducted as follows: 0.5 ml of each stock solution of Tinopal CBS-X (1, 2 or 10 mg kg^{-1}) was spiked into 3 g of ground rice papers and rice noodle samples, which were considered to be negative samples, and subsequently the final spiked concentrations corresponded to be 10, 20 or 100 µg kg^{-1}. The spiked samples were kept for 10 min at RT and then the sample solution was pre-treated and analysed by the same procedure as described above. This test was performed with three replicates. Some of the final solutions obtained from the samples spiked with high concentrations of the standard solution were adequately diluted with 75% methanol prior to HPLC analysis to bring them into the acceptable concentration range of the calibration curve. The concentrations of Tinopal CBS-X in each solution were calculated using the calibration curve equation.

Determination of Tinopal CBS-X in five processed rice products

The contents of Tinopal CBS-X in five processed rice products, including positive rice noodles, were determined by HPLC analysis and sample pre-treatment developed for the present study. The samples assumed to be positive or suspected by the HPLC analysis were confirmed using LC-MS/MS to identify whether the analyte was identical to the standard substance. The analysis was performed in triplicate on each sample and methanol blanks and standards were run at an interval of every five samples.

Results and discussion

UV radiation

Tinopal CBS-X clearly exhibits blue fluorescence when irradiated with UV light because it absorbs energy in that

Table 1. Single reaction monitoring (SRM) parameters for LC-MS/MS analysis of Tinopal CBS-X.

RT (min)	Average mass (Da)	Parent ion *m/z*	Product ion *m/z* (%)[a]	Tube lens offset energy (V)	Collision energy (eV)
17.73	562.58	517.2	348.3 (45)	203	42
	562.58	517.2	437.2(100)	203	39

Note: [a]Relative abundance.

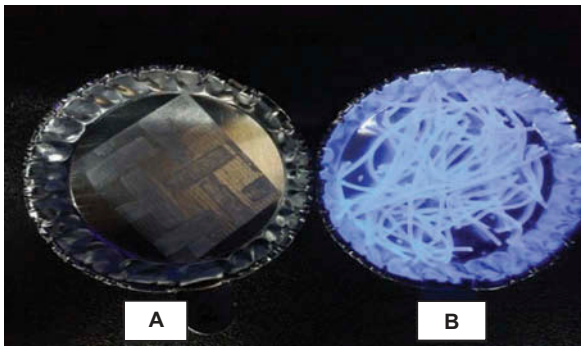

Figure 2. Negative rice paper (A) and positive rice noodles (B, containing Tinopal at 2.1 mg kg^{-1}) when radiated by ultraviolet (UV) light.

region. Therefore, this study conducted UV radiation tests of processed rice products suspected or issued publicly and imported from Vietnam. As a result, a rice noodle product indicated strong fluorescent light, whereas rice papers did not show fluorescent properties (Figure 2). In addition, after preparing 0.1% Tinopal CBS-X solution, the solution was diluted 100, 1000, or 10 000 times to make standard solutions of 0.1, 1.0, or 10 mg kg^{-1}, respectively. Rice papers were cut to a constant size, dipped into each standard solution, and then dried at 60°C in an oven and radiated by a UV lamp set at a main wavelength of 365 nm. As a result, a piece of rice paper dipped into a 10 mg kg^{-1} Tinopal CBS-X solution radiated strong fluorescent light, whereas the pieces of rice paper dipped into the solution of 0.1 mg kg^{-1} indicated little fluorescent light (Figure 3). The UV radiation employed as a preliminary test was considered to be a useful tool for easily predicting whether Tinopal CBS-X exists in processed rice products when its adulteration degree is sufficiently large; however, it is difficult to distinguish visually when small amounts of FWA were illegally used in foodstuffs. Therefore, we focused on the development of a new method that can objectively quantify the content of Tinopal CBS-X used to adulterate foodstuffs.

Optimal temperature and MeOH concentration for extracting Tinopal CBS-X

Given that no studies related to the quantification of FWAs in foodstuffs have been reported until now, the establishment of a pre-treatment procedure to separate the target substance from the food matrix is very important in the development of an analytical method. This study used positive rice noodles as indicated by the strong fluorescent light from UV radiation (Figure 2) to afford constant conditions. First, the positive rice noodles were dipped in 100% methanol without heating to extract Tinopal CBS-X; however, we found that Tinopal CBS-X was not extracted. We used the analysis method suggested for the determination of FWAs in the standards and specifications for equipment, containers and packages (KMFDS 2013); however, there was no available method that could separate Tinopal CBS-X from processed rice products.

Therefore, 75% MeOH solution was added to adulterated rice noodles, which were then heated for 30 min in a water bath to induce the release of Tinopal from the rice noodles. We discovered that the target substance could be separated by heating the rice noodles. To determine which temperature was optimal for the extraction of Tinopal CBS-X, the sample bottles were heated for 30 min at 60, 70, 80, and 90°C after the addition of 75% methanol. The heating temperature significantly influenced the recovery of Tinopal CBS-X because the emission intensities in HPLC system are proportional to the concentrations of Tinopal CBS-X. As shown in Figure 4, a small amount of Tinopal CBS-X contained in rice noodles was released from the matrixes after heating at 60°C for 30 min; the amount decreased after heating at 90°C, as expected.

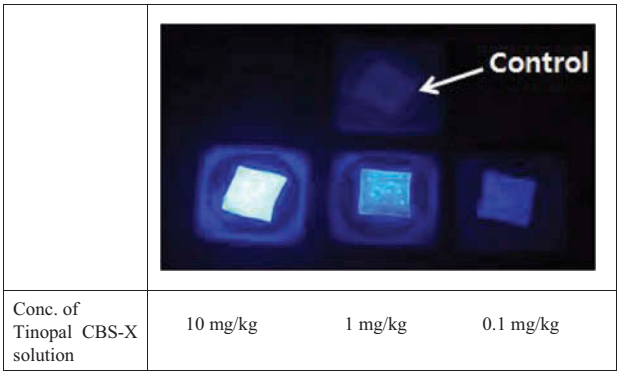

Figure 3. Difference in the fluorescent intensity in each Tinopal CBS-X solution: after dipping rice paper into the Tinopal CBS-X solution, the sample was radiated with UV; and *control represents the rice paper sample without dipping treatment.

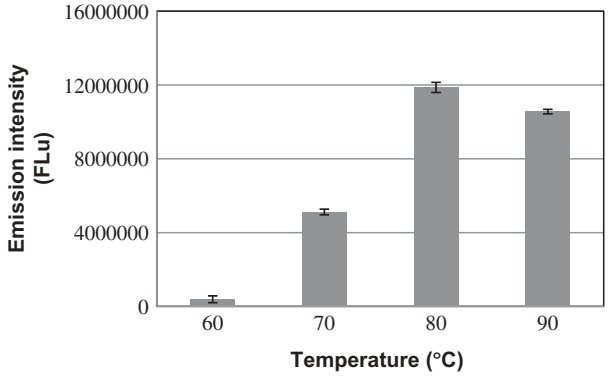

Figure 4. Optimal heating temperature for the extraction of Tinopal CBS-X from positive rice noodles: after addition of 75% methanol the sample solution was heated at each temperature for 30 min.

Methanol, which has a low boiling point, can be easily evaporated during heating in a water bath. Even though the bottles were capped to prevent evaporation of the solvent, a part of the methanol was considered to be volatile when it was heated to 90°C. Thus, the optimal heating temperature was determined to be 80°C for the extraction of Tinopal CBS-X from rice noodles in 75% methanol.

Moreover, because the sample solution was heated after the addition of a methanol solution, this study used methanol concentrations of 50%, 75% and 87% as solvents to extract Tinopal CBS-X from rice noodles, instead of 100% methanol. That is, 30 ml of each methanol solution were distributed into the sample bottles with caps and then the samples were heated for 30 min at 80°C in water baths. After heating, the samples were centrifuged and the supernatant was analysed under the sample HPLC conditions described above. As shown in Figure 5, the 75% methanol solution exhibited the highest emission intensity, whereas the 50% methanol solution indicated the lowest emission intensity. The reason that the 87% methanol solution had a much lower emission intensity than the 75% methanol seemed to be related to the evaporation of methanol. That is, the 87% methanol solution could be more easily evaporated than the 75% methanol when the sample solution was heated at 80°C for 30 min. To conclude, the combination comprising 75% methanol and heating at 80°C provided the optimal conditions for separating Tinopal CBS-X from rice noodles.

HPLC and LC-MS/MS analyses

The present study employed HPLC analysis using isocratic elution with a 50% acetonitrile solution containing 0.4% (w/v) of TBA at pH 8.0 to separate Tinopal CBS-X

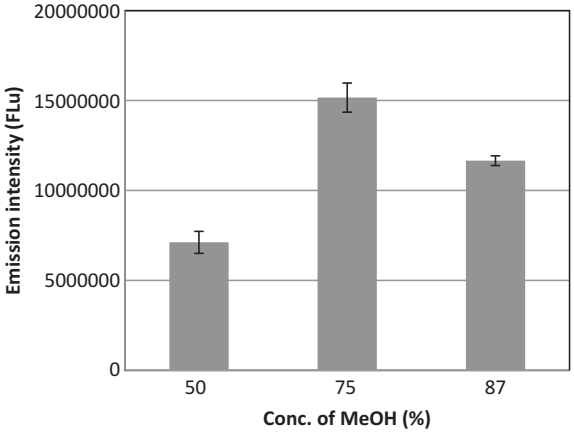

Figure 5. Optimal MeOH concentration for the extraction of Tinopal CBS-X from positive rice noodles: after addition of each methanol solution the sample solution was heated at 80°C for 30 min.

effectively from food matrix substances. The protocol was the same as the method reported by Shu and Ding (2009) and Vindogopal and Peller (2010). When the sample solutions prepared by the pre-treatment procedure described above were analysed by ion-pair chromatography, an apparent peak was observed at the same retention time as that of the standard solution of Tinopal CBS-X (Figure 6). We found that the emission intensity of the FLD did not increase proportionally as the concentration of Tinopal CBS-X increased and the sample peak did not draw a Gaussian curve when the target substance existed at relatively high concentrations. In the HPLC analysis coupled with an FLD, adequate dilution was necessary for accurate quantification because the FLD can react sensitively only within a specified range of concentrations.

The anionic form of Tinopal CBS-X is doubly charged and its pK_a (acid dissociation constant) is –0.92; thus, the analyte cannot be separated by a simple HPLC method (Shu & Ding 2009; Licha et al. 2013). Ion-pair chromatography using a reversed-phase HPLC column and an ion-pair reagent has been frequently employed in the separation of anionic organic compounds because the ion-pair reagent can generate neutral ion pairs with the anionic analytes. Shu and Ding (2009) report that the separation of FWAs, including Tinopal CBS-X, in an HPLC system is significantly improved when TBA is used as an ion-pair reagent because TBA salts have longer alkyl groups that can promote the interaction between the stationary phase and the analyte. They claimed that the selectivity of the analyte seemed to increase in a C_{18} reversed-phase HPLC column. Moreover, isocratic elution is primarily recommended in ion-pairing chromatography (IPC) separation rather than gradient elution because the equilibration reaction among the analyte, stationary phase, and ion-pair reagent is more complex and slow (Harris 2003). Moreover, one of the critical points in IPC analysis is the pH adjustment of the mobile phase because it can affect the analyte charge as well as the retention behaviour of ion-paired analytes and the stationary phase (Chen & Ding 2006).

The samples suspected as being positive by the HPLC analysis should be confirmed by LC-MS/MS analysis because some interference in the HPLC analysis might elute at the same retention time as the analyte. Therefore, the present study performed the LC-MS/MS analysis using a procedure modified from the method of Chen and Ding (2006). They chose the parent ion and product ion of Tinopal CBS-X as *m*/*z* 517 and 348.3, respectively. In the present study, the most intense parent ion of Tinopal CBS-X was determined to be *m*/*z* 517 and two product ions were chosen, *m*/*z* 348.3 and 437.2, to identify more accurately the analyte in a qualitative confirmation by knowing the ratio of fragments between the two product ions. The collision energy was chosen when the product ion appeared to have the highest intensity (Table 2). The mobile phase consisted of water–acetonitrile containing volatile 5 mM di-*n*-hexylammonium acetate as an ion-pairing

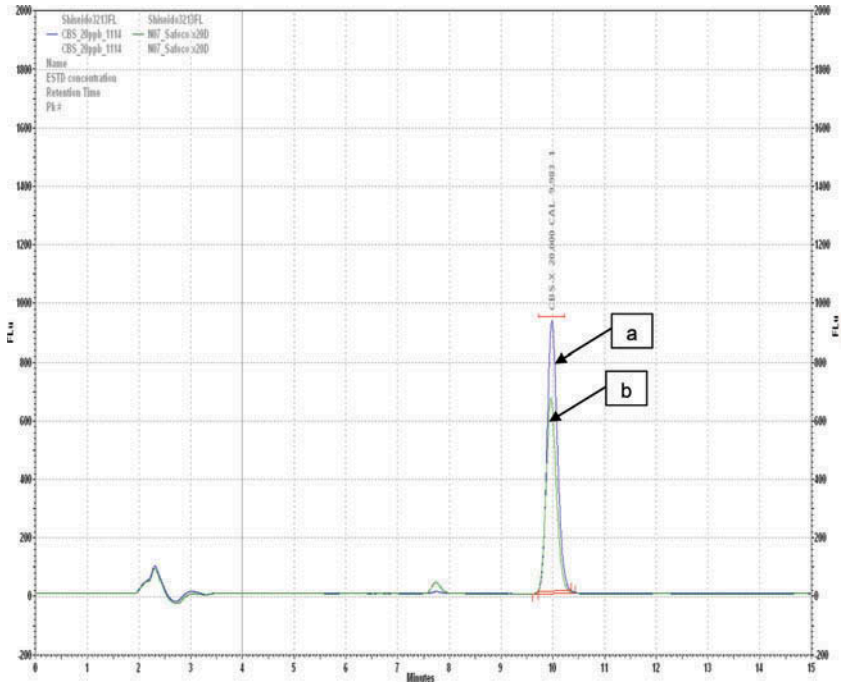

Figure 6. (colour online) Chromatogram of the standard solution of Tinopal CBS-X (a) and positive rice noodle sample (b) obtained by HPLC analysis coupled with a florescent detector.

Table 2. Recovery and precision (CV) obtained from the final extraction solution of rice papers and rice noodles spiked with each stock solution of Tinopal CBS-X.

		Spiked concentration of Tinopal CBS-X (n = 3)		
Sample		10 μg kg^{-1}	20 μg kg^{-1}	100 μg kg^{-1}
Rice papers	Recovery (%)	83.35	70.35	76.17
	CV (%)	7.89	5.08	7.88
Rice noodles	Recovery (%)	78.67	66.85	63.43
	CV (%)	11.51	10.21	8.55

reagent because it was necessary for the ionisation of Tinopal CBS-X. Ion-pair reagents are known to reduce the relative abundances of the Na^+ adducts that interfere with the ionisation of the analyte in LC-MS/MS analysis and subsequently to decrease the suppressing effect (Kreisselmeier & Dürbeck, 1997; Holčapek et al. 2004; Chen et al. 2006). As a result, the chromatogram and spectrum of positive sample obtained through LC-MS/MS analysis corresponded well with those of standard solution of Tinopal CBS-X. Also, we confirmed that the fragmentation ratio of the two product ions in positive samples was identical to that of the standard solution (Figure 7).

Method validation

The linearity of the method was evaluated at concentrations ranging from 2.5 to 40 μg kg^{-1} (five levels). The external standard calibration curves for rice paper and rice noodles had linearity with coefficients of determination (r^2) of ≥ 0.9997 and ≥ 0.9998, respectively. The LODs in rice papers and rice noodles were 0.52 and 0.50 μg kg^{-1}, respectively, whereas the LOQs of rice paper sand rice noodles were determined to be 1.58 and 1.51 μg kg^{-1}, respectively. The sensitivity of the developed procedure was evaluated by HPLC coupled with FLD. As shown in Figure 6, the rice noodles adulterated with Tinopal CBS-X had a peak with the same retention time as the standard solution and its appearance was distinguished apparently from the base line. Moreover, in the LC-MS/MS analysis, the chromatogram and spectrum obtained from the positive samples correspond with those of the standard solution of Tinopal CBS-X (Figure 7).

The recoveries of Tinopal CBS-X from spiked rice papers were indicated to be in the range 70.4–83.4% and their CV values were less than 8%; for the spiked rice noodles, the method produced recoveries in the range

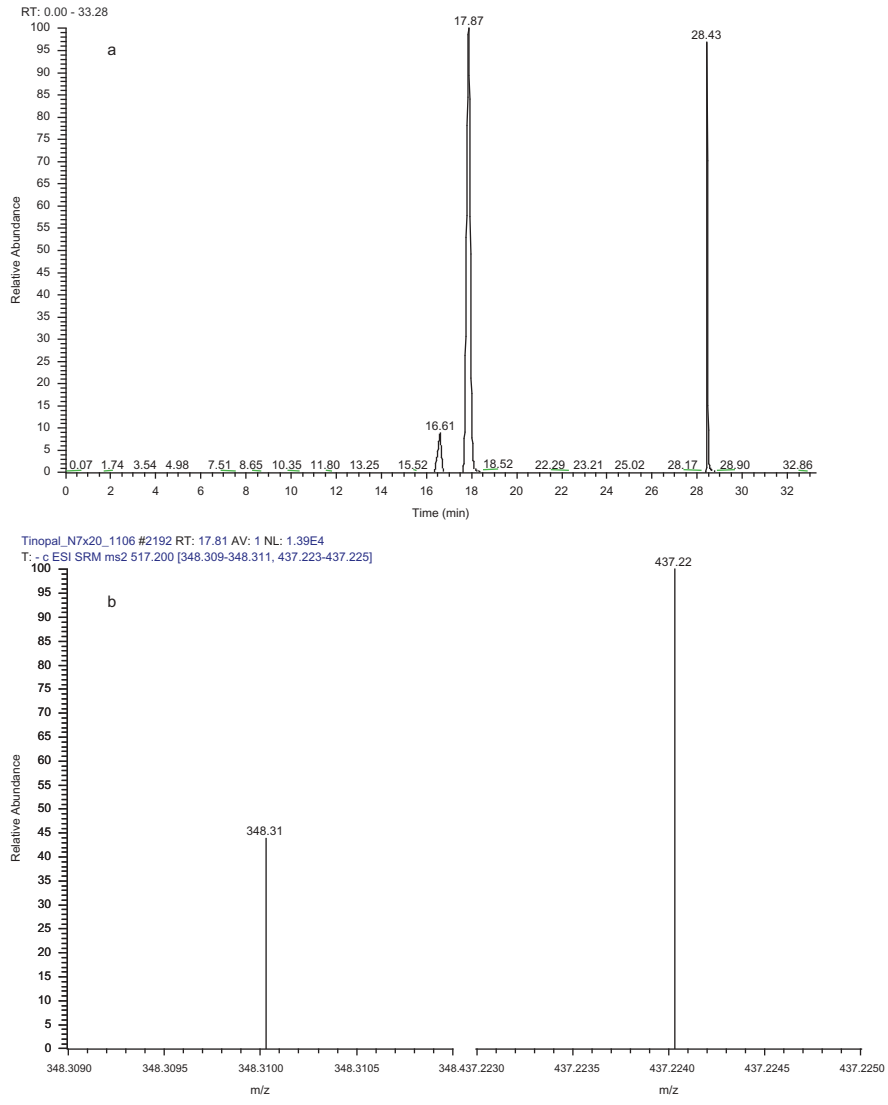

Figure 7. Mass chromatogram (a) and spectrum (b) of the positive rice noodles obtained after filtering by SRM mode of LC-MS/MS.

63.4–78.7% and CV values of 8.6–11.5%. Rice noodles showed entirely lower recovery and higher CV values than rice papers in all samples spiked with low, medium and high concentrations. Moreover, samples spiked with high concentrations seemed to have relatively lower recovery rates than those spiked with low concentrations. To increase the recovery rate, the present study repeated extractions in triplicate with 75% methanol. The second and third extractions were performed without heating and slightly increased the recovery values that corresponded to around less than 10% of the recovery obtained by the first extraction. Tinopal CBS-X is reported to have a low solubility because, owing to divalent permanent organic anions with two sulfonic groups, it is prone to form low-solubility salts with di- and trivalent cations (Licha et al. 2013). Because of this characteristic of Tinopal CBS-X, it is assumed to be difficult to obtain sufficiently high recovery from processed rice products. Considering that the analyte might escape with solvent evaporation, heating with a cooling system was considered to enhance the recovery rate, although this study did not conduct such a test.

Contents of Tinopal CBS-X in five rice process products

The contents of Tinopal CBS-X in five processed rice products, including the positive rice noodles, were measured using the novel procedure developed in the present study. Only one sample contained Tinopal CBS-X (Table 3). The samples suspected as positive by HPLC analysis were analysed by the LC-MS/MS system to confirm whether the analyte was identical to the target substance after concentrating the final extract solutions under a nitrogen stream and dissolving them with 100 µl of 50% acetonitrile. The positive rice noodles that had strong fluorescent light emission

Table 3. Content of Tinopal CBS-X in rice noodles and rice papers purchased from a local market in Korea.

Sample	Mean (mg kg^{-1}) ± STD
Vietnam rice paper A	n.d.
Thai noodle	n.d.
Vietnam rice paper B	n.d.
Korea rice noodle	n.d.
Vietnam rice noodle[a]	2.1 ± 0.11

Note: n.d., Not detected (< LOD).
[a]Vietnam rice noodle was identical to the positive sample represented in Figure 2, and provided from Seoul regional MFDS.

by UV radiation (Figure 2) were determined to have a substance identical to that of the standard solution through the LC-MS/MS analysis. The content of Tinopal CBS-X in the positive rice noodles was about 2.1 mg kg^{-1}. Two samples of the four other processed rice products appeared to have trace amounts of Tinopal CBS-X, corresponding to less than the LOD. However, qualitative analysis using LC-MS/MS revealed that the peaks were not identical to Tinopal CBS-X; therefore, we concluded that the four samples did not contain the FWA.

Conclusions

UV irradiation conducted as a preliminary test seemed to be relatively subjective because it was evaluated by visual observation. It is assumed to be difficult to distinguish the FWA using UV radiation when certain processed rice products were adulterated with extremely low concentrations of Tinopal CBS-X. This study focused on the development of a procedure to separate and more determine objectively and accurately the Tinopal CBS-X content in processed rice products. A simple and economical pre-treatment procedure was developed that induced the release of Tinopal CBS-X from processed rice products by using 75% methanol in a water bath at 80°C for 30 min. In the HPLC analysis, ion-pair chromatography coupled with an FLD was employed for the quantification of Tinopal CBS-X. The developed method consisted of a novel pre-treatment process and IPC, which resulted in recovery rates of 63.4–83.3% and CV values of less than 12%. Moreover, the samples suspected as being positive by the HPLC analysis were then confirmed by LC-MS/MS analysis for identifying the parent ion and the fragmentation ratio of two product ions. This study shows that the developed method can adequately separate and quantify Tinopal CBS-X from processed rice products by considering the recovery rate and the CV value. Even though the recovery is not sufficiently high, this method can be used to determine the Tinopal CBS-X content in processed rice products. This method becomes all the more important because no method to separate Tinopal CBS-X from foodstuffs has been reported until now.

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