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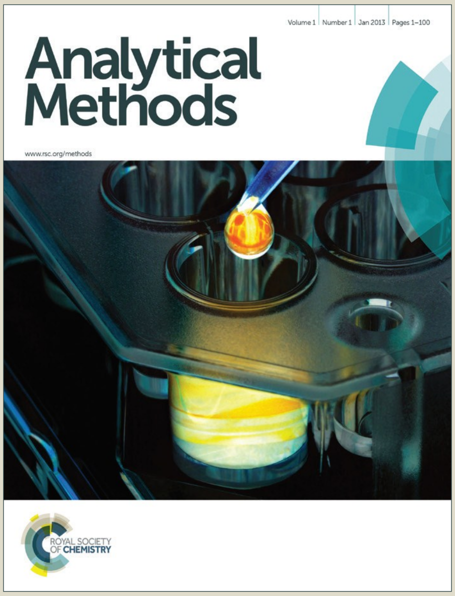

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Simultaneous determination of fluorescent whitening agents (FWAs) and photoinitiators (PIs) in food packaging coated paper products by UPLC-MS/MS method using ESI positive and negative switching mode

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ABSTRACT

A sensitive ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was established for the simultaneous determination of fluorescent whitening agents (FWAs) and photoinitiators (PIs) in food packaging coated paper products. Samples were firstly soaked by water and then ultrasonic extracted by methanol-trichloromethane. The extracting solution were subsequently separated by a Phenomenex Luna C18 (2) chromatographic column (50 mm×2.00 mm, 3 μm) using methanol-5 mmol/L ammonium acetate as the mobile phase. The target analytes were ionized by the ESI positive and negative switching mode and detected using multiple-reaction monitoring (MRM) mode. The method was validated for linearity and range, accuracy, precision and sensitivity. Under the optimized condition, the calibration curves were linear over the selected concentration ranges of 10-1000 μg/L and 0.5-50 μg/L for four FWAs (FWA28, FWA85, FWA71 and FWA351) and the rest ten compounds, respectively, with calculated coefficients of determination (R^2) of greater than 0.99. The instrument limit of quantitation (ILOQ), and the corresponding method limit of quantitation (MLOQ) of fourteen target analytes were in the range of 0.5-10 μg/L and 6-125 μg/kg, respectively. Recoveries were calculated at three levels of concentration spiked in negative samples and the values were found between 79.2% and 115% with relative standard deviation (RSD) values of 3.2%-12.3% for intra-day precision (*n* = 6) and 4.5%-11.5% for inter-day precision (*n* = 5) 2.5-7.2%. The method was successfully applied to analyse twenty-five food packaging coated paper products samples and FWA184 and 4-MBP were detected in only two samples with the concentrations of 151 μg/kg and 32 μg/kg, respectively.

Keywords: Fluorescent whitening agent (FWA); Photoinitiator (PI); Food packaging coated paper products; Ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

Introduction

Fluorescent whitening agents (FWAs), colorless or weakly colored organic compounds, which can absorb light in the UV range (290-400 nm) and emit visible blue light (400-480 nm), thus enhancing the optical impression of “brightness” and “whiteness”, are widely used in household detergents to eliminate the yellowish cast of white fabrics and increase the whiteness and brightness of products. In recent years, with the rapid development of industries, FWAs are also extensively applied in textiles, coatings, plastics and even paper manufacturing.[1-4] Although thousands of FWAs are available, only approximately 400 of them are used widely and most of FWAs used in paper and board manufacturing are based upon stilbene derivatives.[5] Toxic effects of FWAs have not yet been observed, indicating that the contact with FWAs or even FWAs that migrate into food from food contact products does not represent a risk to human health. However, the chemical stability of FWAs make them hard to degrade, in addition, the over-use of them can also lead to environmental pollution, which has the potential to transfer to human beings through the food chain and accumulate in the bodies and threaten our health.[3, 5] In China[6] and the western countries,[7-8] FWAs are authorized to be used in materials in contact with food as food additives, for example, FWA184, FWA393 and FWA236 can be used in the EU and China according to the regulations of 2002/72/EC and GB 9685-2008.[9] But not all of them are authorized for use by the US Food and Drug Administration and their inclusion in paper and board (P&B) intended for food packaging in the European Union (EU) is under consideration due to their potential migration to the packaged products.[5] Moreover, the usage limitation, even the specific migration limits (SPL) are clearly described in relevant food regulations. For example, according to GB 9685-2008,[9] the SPL of FWA184 is 0.6 mg/kg and the maximum usages in polystyrene (PS) and polyvinyl chloride (PVC) materials are 0.02% and 0.015%, respectively. In these circumstances, appropriate methods which a simultaneously determine the concentrations of FWAs in food contact products are required.

Photoinitiators (PIs), which are low molecular weight compounds and can be used to utilize activity substance produced by photosensitive groups to initiate polymerization during the process of optical absorption, have been widely used in packaging materials as a main component of UV inks.[10] The withdrawal from the market of more than 30 million liters of milk by Italian authorities hit the headlines for food contamination in 2005. It was mainly because that a kind of photoinitiator (2-isopropylthioxanthone (2-ITX)) migrated into infant milk was detected by Italian Food Control Authority with the concentrations of 120 μg/L to 300 μg/L. Recent studies have found that after the curing completion of the printing ink, the remaining PIs can migrate through chemical or physical contact under certain conditions, resulting in contamination of the food inside the packaging, and so as have the potential harm to human body health.[11-13] On account of this circumstance, the foreign countries successively promulgated the regulations and licensing list to restrict the use of PIs, for example, the SPLs of benzophenone (BP), 4-methyl benzophenone (4-MBP) and 4, 4’-Bis(dimethylamino)-benzophenone (MK) were regulated by EU as 0.6, 0.6 and 0.01 mg/kg, respectively. Therefore, appropriate methods to determine

PIs in food contact products are also extremely required.

It has been reported that FWAs and PIs can be determined by ultraviolet light observation method, gas chromatography-mass spectrometry, high-performance liquid chromatography, and ion-pair high-performance liquid chromatography/tandem mass spectrometry.[1-5] Chen, et al. adopted solid-phase extraction and ion pair liquid chromatography-tandem mass spectrometry to determine FWAs in environmental waters and favorable limits of quantitation (LOQs, 4 to 18 ng/L) were obtained, but mean recoveries were between 68 and 97%. In another study, a novel, single step method for the determination of seven ink photoinitiators in carton packed milk samples was developed, The LOQs of the optimized method were between 0.2 and 1 μg/L and a good linearity in the range between 1 and 250 μg/L was obtained.[14] However, these methods mentioned above are available only in the matrix of water, laundry detergents, and infant clothes for FWAs determination and beverages, milk, and other packaged food for PIs determination, to our best knowledge, few reports have been focused on the determination of FWAs and PIs in food packaging coated paper products. In the present work, fourteen FWAs and PIs in food packaging coated paper products were simultaneously determined by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) using the ESI positive and negative switching mode. More satisfactory validation parameters were obtained, including linearity, accuracy, precision, and ILOQs and MLOQs, comparing with the method mentioned in the above references. The developed method can provide reference for the detection and specific migration study of related compounds in food packaging coated paper products.

Materials and methods

Chemicals and reagents

Methanol (HPLC grade) was provided by Merck Chemicals Co., Ltd (Darmstadt, Germany). Ammonium acetate (HPLC grade) was supplied by CNW Technologies GmbH (Düsseldorf, Germany). Chloroform (Analytical Reagent grade) was purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

Disodium 4,4'-bis (2-sulfonatostyryl)biphenyl (FWA351), 4-(2-Benzoxazolyl)-4'-(5-methyl-2-benzoxazolyl) stilbene (FWA368), 1,4-Bis(2-benzoxazolyl) napthalene (FWA367), 2,5-Bis(5'-tert-butyl-2-benzoxazolyl) thiophene (FWA184), 7-Diethylamino-4-methylcoumarin (FWA52), 4,4'-Bis(2-benzoxazolyl) stilbene (FWA393) and benzophenone (BP) (Purity ≥ 95.0%) were supplied from TCI (Shanghai, China). Disodium 4,4'-bis[(4-anilino-6 -hydroxyethylamino-1,3,5-triazin-2-yl)amino] stilbene-2,2' -disulphonate (FWA85), disodium 4,4'-bis[(4-anilino-6 -morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (FWA71), 2,5-Bis(2-benzoxazolyl) thiophene (FWA185) and 1,2-Bis(5-methyl-2-benzoxazole)ethylene (FWA135) (Purity ≥ 98.0%) were purchased from Internatioanal Laboratory USA and disodium 4,4'-bis[6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate (FWA28), 4-Methyl benzophenone (4-MBP) and 4,4’-Bis(dimethylamino)-benzophenone (MK) were obtained from Sigma Co. (St.

Louis, USA). Their chemical structures of the three FWAs are shown in **Fig. 1**.

Instrumentation

The ACQUITY^{TM} ultra high performance liquid chromatography and Waters Xevo^{TM} TQ tandem triple quadrupole mass spectrometer (UPLC-MS/MS, Waters Co., USA) were used for sample analysis. The samples were vortex mixed with a MS3 basic vortex mixer (IKA GmbH, Germany), ultrasonicated by the KQ-250DV numerically-controlled ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd, China) and centrifuged by a LD5-2A centrifuge (Beijing Jingli Centrifuge Co., Ltd, China). The Milli-Q A10 (Millipore Co., USA) was used to offer Milli-Q water (18.2 MΩ·cm).

Standard solutions

Appropriate amounts of FWA28, FWA85, FWA71, FWA351, FWA52, FWA184, BP, 4-MBP and MK were dissolved in methanol respectively to obtain the individual standard stocking solutions (100 mg/L). Individual standard stocking solutions of the rest five analytes were prepared at the concentration of 100 mg/L in trichloromethane. The mixed standard stocking solution with the concentrations of FWA28, FWA85, FWA71 and FWA351 of 10 mg/L and the concentrations of the rest analytes of 5 mg/L was prepared by diluting the each individual standard stocking solution with methanol, which was then stored in refrigerator at -20°C.

Before use, the mixed standard working solutions were obtained by further stepwise diluted the mixed standard stocking solution to the required concentrations (Concentrations of FWA28, FWA85, FWA71 and FWA351 were 10, 25, 50, 250 and 1000 μg/L, and the rest ten analytes were 0.5, 1.0, 5.0, 20 and 50 μg/L, respectively) with 60% methanol-water solution (*v*/*v*). All of the standard working solutions were preserved in refrigerator at 4°C.

Moreover, the matrix calibration working solutions of required concentrations were prepared using the negative sample extracting solution which was obtained according to the next section.

Sample and sample preparation

Two kinds of food packaging coated paper products were provided by National centre for quality supervision and testing of packaging products (Guangzhou), one of which (A) was confirmed to have FWA351 and FWA184, the other (B) was confirmed to contain 4-MBP. Additionally, twenty-five coated paper products samples, including paper cup, paper bowl, paper bag, and so on, were purchased from local market.

All paper products were cut into 5 mm × 5 mm in size and mixed evenly. Then, 0.5 g of as-prepared sample, 3 mL of water were orderly added into a 25 mL of stoppered colorimetric tube and the mixture was ultrasonicated for 20 min. 20 mL methanol-trichloromethane (7 : 3, *v*/*v*) was added into the colorimetric tube and subsequently ultrasonicated for 30 min. After that, the extracting solution was diluted to 25 ml with methanol, then 10 mL of which was withdrawn into glass centrifuge tube and centrifuged at 3000 rpm for 5 min. Subsequently, 5 mL of supernatant was withdrawn into nitrogen blowpipe and concentrated to dry in water bath at 40°C under nitrogen blowing. Finally, the dried analyte was dissolved with 1.0 mL 60% methanol-water solution (*v*/*v*) and the solution was transferred into a sample tube for UPLC-MS/MS analysis.

UPLC-MS/MS conditions

Chromatographic separation was performed on the Acquity^{TM} UPLC system with a

Phenomenex Luna C18 (2) chromatographic column (50 mm×2.00 mm, 3 μm). Separation of target analytes was achieved by a gradient elution program with the mobile phase of a mixture of 5 mmol/L ammonium acetate (A) and methanol (B). The gradient elution program was optimized as follows: started from 60% A and a linear gradient to 40% A in 2 min, then decreased to 5% A in 3 min and maintained for 1.5 min, subsequently, 60% A was obtained from 5% A in 1.5 min and maintained for 2 min, the total run time was 9.0 min. The flow-rate was set as 0.4 mL/min and the column temperature was kept in constant of 40°C. An injection volume of 5 μL was performed using an auto-sampler.

MS/MS detection was performed on a triple quadrupole mass spectrometer detector equipped with a jet stream electro spray ionization (ESI) source. ESI positive and negative subsection acquisition mode (0-3.2 min was in ESI^- mode and 3-9 min was in ESI^+ mode) was used for quantification with the capillary voltage of 1.5 kV. The switching time from negative ESI mode to positive ESI mode was 20 ms. The ion source temperature and desolvation temperature were optimized at 150 and 400°C. The flow rates of the desolvation gas (N_2), cone gas (N_2) and collision gas (Ar) were set at 800 L/h, 50 L/h and 0.20 mL/min, respectively. The MS detector was operated in multiple reaction monitoring (MRM) mode for all analytes and the cone voltage, collision energy and the other MS parameters of fourteen target analytes are shown in **Table 1**.

Results and discussion

Optimization of extraction solvent

According to the principle that the similar substance is more likely to be dissolved by each other, the extraction solvent should sufficiently extract target compounds and decrease the extraction of the other interfering components as far as possible. Among the fourteen target compounds, the solubility of FWA367, FWA135, FWA185, FWA368 and FWA393 in trichloromethane is bigger than that in methanol and acetonitrile, while FWA28, FWA85, FWA71, FWA351, FWA52, FWA184, BP, 4-MBP and MK are soluble in methanol. Therefore, methanol-trichloromethane extraction solvent was selected and the effects of different ratios of methanol versus trichloromethane (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6) on the recoveries of target analytes were investigated. In the present work, 20 μL of mixed standard stocking solution was added into a negative sample and the sample was subsequently stored in dark for 4 h to absorb the standard solution sufficiently. Then the sample was extracted using the methanol-trichloromethane extracting solvent of different ratios. Relying on obtained results (**Table 2**), it can be assumed that as trichloromethane was increased, there were increases in the recoveries of FWA367, FWA135, FWA185, FWA368 and FWA393. It was worth mentioning that the recovery of FWA393 was observably increased with the increase of trichloromethane. However, the recoveries of these five analytes reached stationary values when the ratio of trichloromethane was 40%. On the contrary, the recoveries of FWA28, FWA85, FWA71 and FWA351 decreased along with the increase of trichloromethane. As for FWA52, FWA184, BP, 4-MBP and MK, there were no obvious changes in recoveries with increasing trichloromethane. It was noticeable that the recoveries of all analytes were relatively favorable when the ratio of methanol versus trichloromethane was 7 : 3 (*v*/*v*). In addition, in consideration of the circumstance that FWA28, FWA85, FWA71 and FWA351 are always added in the form of sodium salt in the industrial production, and with regard to dried samples, soaking with water can accelerate the dispersion of sample matrix and enhance the extracting recoveries. Thus, the effects of different extracting ways, methanol-trichloromethane (7 : 3, *v*/*v*) extraction with and without water soaking, on the recoveries of samples A and B were also studied. One obvious feature presented in **Fig. 2** was that favorable contents of target analytes were obtained using methanol-trichloromethane (7 : 3, *v*/*v*) extraction with water soaking pretreatment. For example, the content of FWA351 increased by 12.2% with water soaking pretreatment. Hence, all sample were firstly soaked with water and then extracted by methanol-trichloromethane (7 : 3, *v*/*v*).

Optimization of chromatography and mass spectrometry conditions

FWA28, FWA85, FWA71 and FWA351 are stilbene FWAs whose structures contain sulfonic acid group ($-SO_3^-$), which have the highest responses of precursor ions ($[M-2H]^{2-}$) under the ESI negative mode. The remaining seven FWAs, which contain tertiary nitrogen atoms, can generate $[M+H]^+$ under the ESI positive mode, likewise, BP, 4-MBP and MK can also generate $[M+H]^+$ under the ESI positive mode. Therefore, the present work adopted the ESI positive and negative switching mode to analyze fourteen target analytes. The collision energy, cone voltage and the other parameters were optimized to obtain the best response of the analytes. The optimized parameters were presented in **Table 1**.

The effects of three kinds of mobile phase system, including acetonitrile-water system, acetonitrile-10 mmol/L ammonium acetate system and methanol-10 mmol/L ammonium acetate system, on the chromatographic separation were investigated. The results found that four kinds of toluylene FWAs had unfavorable peak shape in the acetonitrile-water system. By adding ammonium acetate solution, which provides a certain number of ionic strength, can effectively improve the peak shape and achieve well separation for these FWAs. When methanol was used as the organic phase, it could provide proton for the compounds ionized under ESI+ mode, that is why the response is apparently higher that that with acetonitrile as the organic phase. Moreover, the concentration of ammonium acetate could suppress the ionization of analytes, thereby influence the response of target analytes. So, in the present work, effects of ammonium acetate with the concentrations of 2 mmol/L, 5 mmol/L and 10 mmol/L on the chromatographic behavior of fourteen target analytes were also studied. The results indicated that as the concentration of ammonium acetate was increased, there was a decrease in peak area of fourteen target analytes and longer retention time. The peak shape of FWA28 was bifurcate under the concentration of 2 mmol/L, while in the concentrations of 5 mmol/L, the peak shape of all compounds were favorable and the stability of retention time and peak area were good. Therefore, methanol-5 mmol/L ammonium acetate system was chosen as the mobile phase for the following experiment. The selected ion chromatograms of fourteen target analytes under the optimized instrumental conditions were shown in **Fig. 4**.

Method validation

Method specificity

The developed method was applied to determine target analytes in twenty negative samples. In the meantime, sample solutions added with the fourteen target analytes were also determined by this method. The results found that impurity compositions in the samples had no interference effects on the determination of all the fourteen target analytes because of the preferable chromatographic separation of fourteen compounds and high selectivity of the triple quadrupole mass spectrometry, indicating that the specificity of the developed method was favorable.

Linearity range, ILOQs, MLOQs and matrix effects

The method developed to determine fourteen FWAs and PIs in food packaging coated paper products samples was validated by studying the linear ranges, instrument limit of quantitation (ILOQ), method limit of quantitation (MLOQ) and the repeatability (expressed as relative standard deviation (RSD)) for all the compounds, under optimized instrumental conditions. The results obtained are summarized in **Table 3**. The standard curves were obtained by plotting the ratios of each target analyte quantitative ion peak area (on the ordinate (y)) versus the corresponding mass concentrations (on the abscissa (x, μg/L)) using five concentration levels in duplicate. In the mean time, a series of matrix calibration solutions were prepared by using negative matrix extracting solutions, and the corresponding matrix calibration curves were obtained likewise. The matrix effects (ME) were investigated according to the slopes' ratio of calibration curve of the matrix versus standard working curve of pure solvent (ME > 1 and ME < 1 represent matrix enhancement and matrix suppression, respectively).

The instrument limit of detection (ILOD), ILOQ, which refers the triple signal-to-noise ratio (*S*/*N* = 3) and tenfold signal-to-noise ratio (*S*/*N* = 10), were measured by gradually diluting the standard solution with pure solvent. The results found that the ILOQs of fourteen target analytes were in the range of 0.5-10 μg/L. By taking the pretreatment processes and the recoveries of various samples into consideration, the corresponding MLOQ were between 6 μg/kg and 125 μg/kg (**Table 3**), indicating the high sensitivity of the developed method. Additionally, in the concentration ranges of 10-1000 μg/L and 0.5-50 μg/L for four FWAs (FWA28, FWA85, FWA71 and FWA351) and the remaining ten compounds, respectively, all of the correlation coefficients were more than 0.99, indicating the good linear relationship between the quantitative ion peak areas and analyte concentrations. For these compounds, the slope ratios between matrix calibration curves and pure standard solution curves were in range of 0.82-1.33, manifesting that the matrix effects were very small and thus could be ignored.

Accuracy, precision and stability

Negative samples at three spiked levels of analytes with 1 × MLOQ, 5 × MLOQ, 50 × MLOQ of mixed standard substances were used to test the recoveries and inter-day precision of analytes according to the proposed method, with six identical samples (*n* = 6) tested at each concentration, in addition, the intro-day precision was also investigated by analyzing five spiked replicates (*n* = 5) for 5 × MLOQ level. The results indicated that the recoveries of the fourteen target analytes were satisfactory with values in the range of 79.2%-115% (**Table 4**). Moreover, relative standard deviations (RSDs) of 3.2%-12.3% for intra-day precision (*n* = 6) and 4.5%-11.5% for inter-day precision (*n* = 5) were observed, meaning that the accuracy, precision and stability can meet the requirements for such an analysis.

Analysis of practical samples

The method established in this work was adopted to determine a total of twenty-five food packaging coated paper products samples collected from local markets. Among these samples, two samples were found to contain FWA184 and 4-MBP, and the contents were determined to be 151 μg/kg and 32 μg/kg, respectively. No FWAs and PIs were detected in the remaining samples.

Conclusions

A simple and sensitive analytical method, using UPLC-MS/MS technique, was developed for the simultaneous determination of eleven FWAs and three PIs in food packaging coated paper products samples. The ESI positive and negative switching mode and MRM way were adopted to detect the target analytes. Satisfactory validation parameters were obtained, including linearity, accuracy, precision, and ILOQs and MLOQs. In addition, the method is simple in pretreatment and low in reagent consumption. The results obtained confirm the suitability of the method proposed for FWAs and PIs determination in food packaging coated paper products samples.

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Table 1

MS parameters for the analyse of the fourteen target analytes.

Compounds	Abbreviations	Cas No.	Precursor ion (*m/z*)	Daughter ion (*m/z*)	Cone voltage/V	Collision energy/eV	RT (min) / Polarity of ionization
disodium 4,4'-bis[(4-anilino-6-hydroxyethylamino-1,3,5-triazin-2-yl)amino] stilbene-2,2'-disulphonate	FWA85	17958-73-5	413.1	311 [a]	40	25	1.35
				271.1	40	25	ESI^-
disodium 4,4'-bis[6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate	FWA28	4404-43-7	457.3	293.1 [a]	45	25	1.75
				333.2	45	30	ESI^-
disodium 4,4'-bis (2-sulfonatostyryl)biphenyl	FWA351	27344-41-8	258	79.9 [a]	35	25	2.77
				194.1	35	20	ESI^-
disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate	FWA71	16090-02-1	439.1	284 [a]	40	30	2.82
				324.1	40	25	ESI^-
7-Diethylamino-4-methylcoumarin	FWA52	91-44-1	232	188 [a]	30	25	3.42
				203	30	17	ESI^+
1,2-Bis(5-methyl-2-benzoxazole) ethylene	FWA135	1041-00-5	291.2	106.9 [a]	50	35	4.88
				157. 9	50	30	ESI^+
2,5-Bis(2-benzoxazolyl) thiophene	FWA185	2866-43-5	319.1	200 [a]	55	35	4.89
				226	55	30	ESI^+
4,4'-Bis(2-benzoxazolyl) stilbene	FWA393	1533-45-5	415.3	207.2 [a]	60	40	5.50
				321.2	60	40	ESI^+
1,4-Bis(2-benzoxazolyl) napthalene	FWA367	5089-22-5	363.2	270.1 [a]	60	35	5.56
				244.1	60	37	ESI^+
4-(2-Benzoxazolyl)-4'-(5-methyl-2-benzoxazolyl) stilbene	FWA368	5242-49-9	429.2	321.2 [a]	60	40	5.67
				221	60	40	ESI^+
2,5-Bis(5'-tert-butyl-2-benzoxazolyl) thiophene	FWA184	7128-64-5	431	399 [a]	60	45	6.16
				415.1	60	40	ESI^+
Benzophenone	BP	119-61-9	183	105.1 [a]	20	15	3.27
				77.1	20	25	ESI^+
4-Methyl Benzophenone	4-MBP	134-84-9	197.1	105.1 [a]	20	15	4.35
				119.1	20	12	ESI^+
4,4'-Bis(dimethylamino)-benzophenone	MK	90-94-8	269.2	148 [a]	30	22	3.74
				254.2	30	20	ESI^+

[a] Transitions for quantification

Table 2

Effects of ratios of methanol to trichloromethane on the recoveries of the target analytes (*n* = 6).

Target analytes	Recoveries ± SD					
	9:1	8:2	7:3	6:4	5:5	4:6
FWA28	85.2 ± 5.0	84.3 ± 6.2	84.0 ± 4.3	80.0 ± 5.2	73.0 ± 7.8	65.0 ± 8.9
FWA85	86.3 ± 3.5	86.0 ± 5.1	84.0 ± 5.7	81.0 ± 7.3	77.0 ± 6.4	70.0 ± 8.1
FWA71	84.5 ± 4.1	84.0 ± 5.1	80.0 ± 6.6	76.0 ± 8.1	70.0 ± 7.7	68.0 ± 7.2
FWA351	87.6 ± 5.3	86.0 ± 7.1	82.0 ± 7.5	77.0 ± 8.6	72.0 ± 7.4	63.0 ± 6.6
FWA52	91.4 ± 6.2	93.7 ± 5.5	92.6 ± 4.3	93.1 ± 5.3	92.1 ± 3.7	93.5 ± 6.5
FWA135	65.3 ± 11.5	87.5 ± 7.5	89.2 ± 5.5	90.1 ± 6.7	90.5 ± 4.9	90.2 ± 6.1
FWA185	72.6 ±10.7	89.1 ± 7.9	91.3 ± 4.7	91.1 ± 5.6	92.2 ± 4.4	91.5 ± 5.9
FWA393	53.7 ± 12.4	67.8 ± 9.2	80.1 ± 6.3	83.2 ± 5.5	84.1 ± 7.1	84.9 ± 4.6
FWA367	67.2 ± 9.8	87.3 ± 8.1	92.2 ± 5.2	92.5 ± 4.4	91.7 ± 5.6	92.3 ± 6.5
FWA368	57.2 ± 11.9	70.5 ± 8.3	82.9 ± 5.6	85.7 ± 6.1	87.1 ± 7.4	87.6 ± 4.9
FWA184	93.4 ± 8.1	92.5 ± 6.6	93.1 ± 4.5	93.7 ± 5.9	92.9 ± 6.3	92.1 ± 5.1
BP	91.7 ± 7.5	93.1 ± 4.9	92.6 ± 3.3	92.2 ± 6.5	92.8 ± 5.9	91.2 ± 4.2
4-MBP	96.8 ± 4.5	94.9 ± 5.2	96.3 ± 3.7	95.5 ± 4.8	95.1 ± 5.7	96.5 ± 5.9
MK	94.1 ± 4.3	94.4 ± 5.7	95.2 ± 3.9	95.7 ± 4.4	94.8 ± 6.2	95.1 ± 4.9

Table 3

Linear equations of calibration curve and standard working curve, correlation coefficients, matrix effects, MLODs and MLOQs of the fourteen target analytes.

Analytes	Linear equation, r (solvent)	Linear equation , r (matrix)	Matrix effect	ILOQ (μg/L)	MLOQ (μg/kg)
FWA28	y=205.28x-58.262, 0.9989	y=168.33x-103.07, 0.9964	0.82±0.03	10	125
FWA85	y=218.14x-38.477, 0.9966	y=189.78x-76.966, 0.9983	0.87±0.05	10	125
FWA71	y=241.68x+49.508, 0.9991	y=205.43x+89.231, 0.9987	0.85±0.05	10	125
FWA351	y=249.06x-41.615, 0.9990	y=224.15x-85.433, 0.9991	0.90±0.07	10	125
FWA52	y=354.41x-110.89, 0.9987	y=365.04x-117.35, 0.9975	1.03±0.04	0.5	6
FWA135	y=2963.9x+17.434, 0.9994	y=3704.9x-56.806, 0.9990	1.25±0.09	0.5	6
FWA185	y=646.98x+23.551, 0.9991	y=763.44x+62.829, 0.9955	1.18±0.10	0.5	6
FWA393	y=5610.9x+577.29, 0.9982	y=5442.6x+330.19, 0.9977	0.97±0.07	0.5	6
FWA367	y=3028.2x+828.56, 0.9980	y=2816.2x+600.49, 0.9962	0.93±0.05	0.5	6
FWA368	y=3199.1x-213.54, 0.9985	y=3902.9x-167.23, 0.9959	1.22±0.10	0.5	6
FWA184	y=8928.6x+791.14, 0.9974	y=11875x+1469.4, 0.9981	1.33±0.13	0.5	6
BP	y=370.93x-211.89, 0.9995	y=378.35x-247.14, 0.9990	1.02±0.06	0.5	6
4-MBP	y=907.24x-397.66, 0.9993	y=1052.4x-434.48, 0.9993	1.16±0.08	0.5	6
MK	y=680.55x-189.03, 0.9990	y=660.13x-169.14, 0.9988	0.97±0.05	0.5	6

Table 4

The recoveries and precision for the fourteen target analytes.

Compounds	Added (μg/kg)	Recovery (%, *n* = 6)	Intra-day precision (%, *n* = 6)	Inter-day precision (%, *n* = 5)[a]
FWA28	125, 625, 6250	108, 87.2, 82.3	7.5, 7.8, 8.6	8.5
FWA85	125, 625, 6250	94.7, 85.5, 81.9	6.3, 5.2, 5.7	7.7
FWA71	125, 625, 6250	112, 83.9, 79.2	10.4, 6.7, 10.1	7.0
FWA351	125, 625, 6250	107, 85.9, 80.7	8.9, 4.6, 5.2	5.9
FWA52	6, 30, 300	97.8, 95.4, 95.6	5.9, 6.3, 4.2	5.4
FWA135	6, 30, 300	90.4, 92.1, 89.7	6.8, 4.2, 7.1	6.2
FWA185	6, 30, 300	104, 96.1, 95.0	10.6, 5.7, 4.5	6.3
FWA393	6, 30, 300	90.0, 88.7, 85.6	5.4, 6.9, 5.5	7.5
FWA367	6, 30, 300	93.8, 91.4, 84.1	7.2, 8.6, 5.9	6.9
FWA368	6, 30, 300	97.2, 89.7, 92.1	8.1, 9.3, 4.9	9.2
FWA184	6, 30, 300	115, 98.1, 93.3	12.3, 8.3, 6.5	11.5
BP	6, 30, 300	98.4, 107, 94.3	11.8, 8.4, 6.5	8.1
4-MBP	6, 30, 300	92.8, 94.5, 89.2	8.5, 5.6, 4.7	6.0
MK	6, 30, 300	88.7, 93.4, 93.8	4.2, 3.9, 3.2	4.5

[a] spiked level was 5×MLOQ.

Fig. 1

Chemical structures of the eleven fluorescent whitening agents (FWAs) and three photoinitiators (PIs).

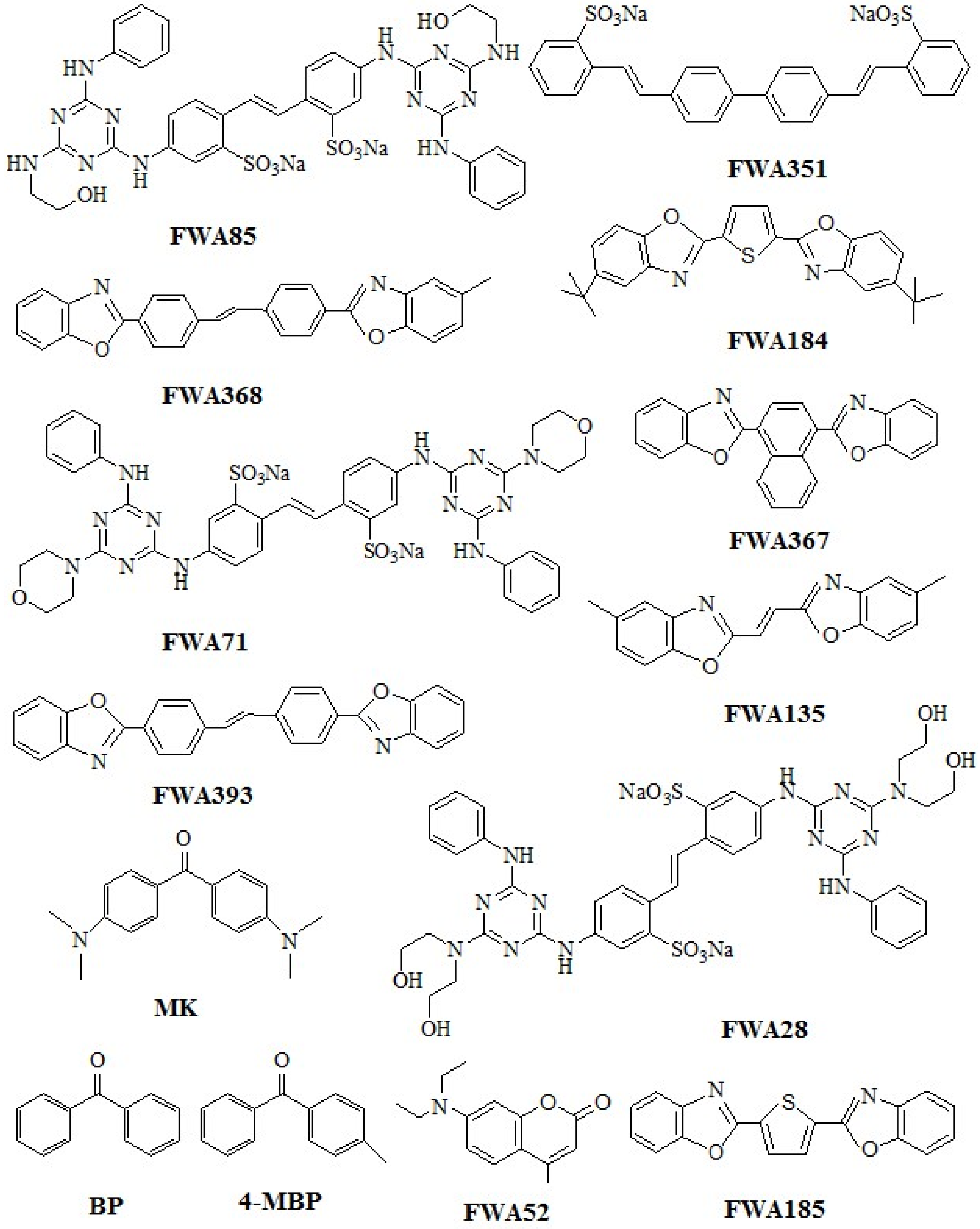

Fig. 2

Effect of water soaked pretreatment on the extraction results of the selected compounds.

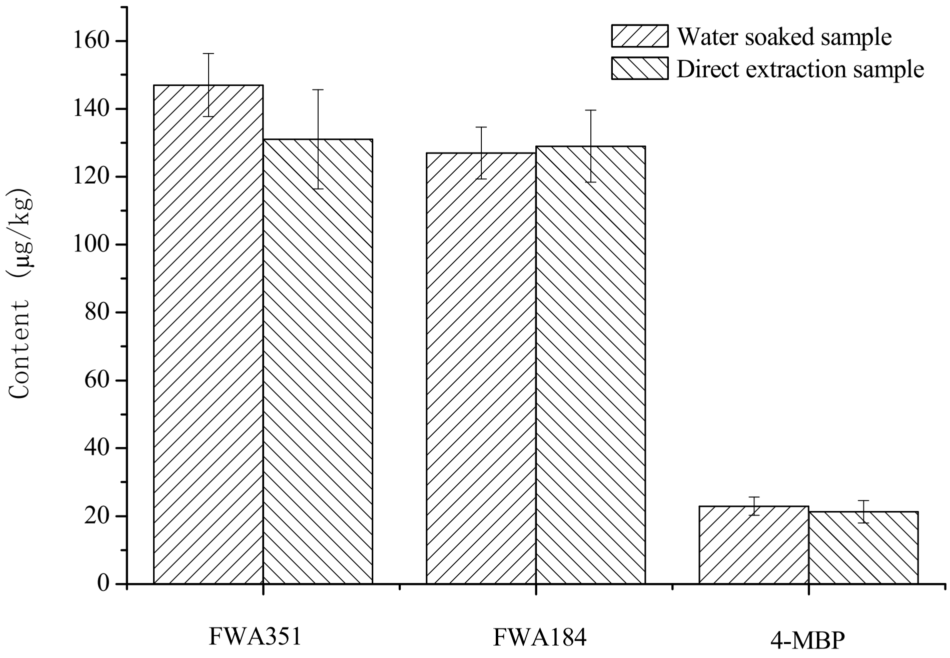

Fig. 3

Effects of extraction time and different extraction methods on the extraction results of the selected compounds.

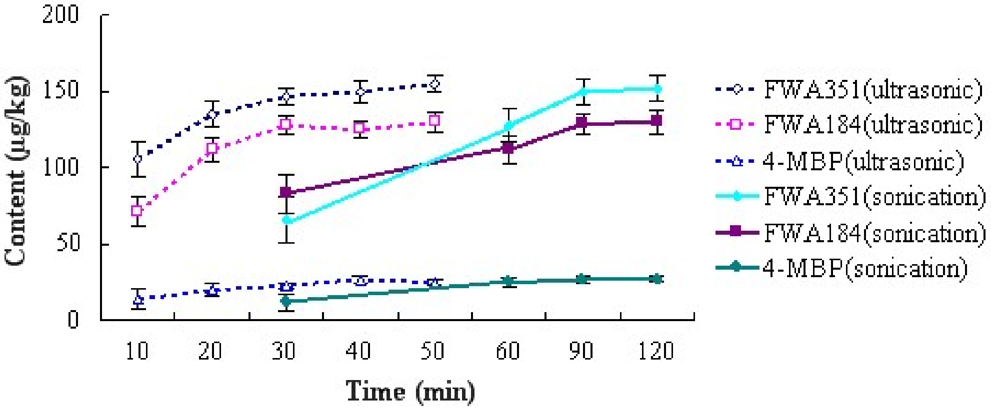

Fig. 4

Selected ion chromatograms of the fourteen target analytes under the optimized conditions.