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Determination of three fluorescent whitening agents (FWAs) and their migration research in food contact plastic packaging container and food stimulants by UPLC-MS/MS method

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ABSTRACT

In order to determine three fluorescent whitening agents (FWAs), including FWA184, FWA368 and FWA 393 in migration solutions of food contact plastic packaging container, a sensitive UPLC-MS/MS method was developed based on the migration tests using food simulants. Under the optimized condition, the calibration curves were linear over the selected concentration ranges of 0.03-200 μg/L for all the three analytes, with calculated coefficients of determination (R^2) of greater than 0.999. The limits of detection (LODs) and the limits of quantitation (LOQs) of the method were 0.01-0.03 μg/L and 0.03-0.09 μg/L, respectively. Recoveries were calculated at three levels of concentration spiked in negative sample. The values were found between 96.1% and 111.4% with relative standard deviation (RSD) values of 3.0%-6.1% for intra-day precision (n = 6) and 2.6%-6.4% for inter-day precision (n = 5). The developed method was applied to study the migration trend of target analytes in different migration temperatures and time, and corresponding migration equations with correlation coefficients more than 0.991 were obtained. Finally, the method was successfully applied to analyze migration solutions of twenty samples and FWA184 was detected in one sample with the concentration of 0.57 and 0.21 μg/L for the storage temperatures of 25°C and 5°C, respectively.

Introduction

Fluorescent whitening agents (FWAs), which can enhance the "whiteness" and "brightness" characteristics and compensate the yellowish shade of washed fabrics, are frequently used in laundry detergents.[1-4] In recent years, with the rapid development of industries, FWAs has now been widely used in not only textiles, detergents, paper, but also coatings, plastic products, even food contact plastic packaging containers (FCPPC). For example, in the United Kingdom, a survey has reported that high FWA concentrations (430-1160 mg/kg) were detected in some napkins and paper materials used for "take-out" food.[5] In China, high FWA concentrations were also detected in popcorn container in 2011. The widespread use of FWAs and the increasing public concern over food safety have stimulated our interest to investigate the content of FWAs in FCPPC and some other materials which have the probability to contact with food. In fact, although the limited toxicological information available on specific types of FWAs has indicated that the contact with FWAs or even FWAs that migrate into food from FCPPC does not represent a risk to human health, FWAs are hard to degrade due to their chemical stability, and the over-use of FWAs leads 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.[6-9] That is why FWAs are authorized to be used in materials in contact with food as food additives in China,[10] the USA,[11] and the European Union,[12,13] and most importantly, the FWAs used and their usage limitation, even the specific migration limits (SPL) are clearly described in relevant food regulations. Three FWAs, which

are FWA184, FWA393 and FWA236, can be used in the EU and China according to the regulations of 2002/72/EC and GB 9685-2008. However, the maximum usages and the SPL of these FWAs are specified, for example, as for FWA184, the SPL is 0.6 mg/kg and the maximum usages in polystyrene (PS) and polyvinyl chloride (PVC) materials are 0.02% and 0.015%, respectively, according to GB 9685-2008.[14] Under this kind of circumstance, appropriate methods which can simultaneously determine the concentrations of FWAs in FCPPC are extremely required.

It has been reported that FWAs can be determined by ultraviolet light observation method,[15] high-performance liquid chromatography,[16] and ion-pair high-performance liquid chromatography/tandem mass spectrometry.[1-3] However, ultraviolet light observation method can only detect the total content of FWAs, while the kind of which cannot be identified. The other methods are available only in detecting FWAs in the matrix of paper, water, laundry detergents, and infant clothes, and few reports have been focused on the determination and migration research of FWAs in FCPPC. In the present study, we developed a sensitive method to routinely determine three selected FWAs (FWA184, FWA393 and FWA368) in FCPPC. In addition, the method was applied to the determination of FWAs in the food simulants, the accuracy and precision of the established method were validated, and the migration research of these FWAs from FCPPC to food simulants was also illustrated. The results obtained in this work can be used for the prediction of FWAs migration trend from FCPPC to food.

Materials and methods

Chemicals and reagents

Methanol of HPLC grade was obtained from Merck (Darmstadt, Germany). Formic acid (HPLC grade, purity 98-100% for analysis) and chloroform (analytical reagent grade) were purchased from Fluka (Buchs, Switzerland) and Guangzhou Chemical Reagent Factory (China), respectively. Ultrapure water (18.2 MΩ) was obtained from a Milli-Q system (Millipore, Bedford, USA). FWA368, FWA184 and FWA393 (purity ≥ 95.0%) were purchased from TCI (Shanghai, China). Their chemical structures of the three FWAs are shown in Fig. 1.

Stock standard solutions containing 100 mg L^{-1} of the individual FWAs and stock standard mixed solution (100 mg L^{-1}) were prepared using methanol as solvent. Then appropriate proportions of working standard analyte mixtures were obtained by diluting the stock standard mixed solution with methanol. All stock standard solutions, working standard solutions and samples were stored at −20°C in the darkness to prevent light-induced conversion of trans-FWA isomers.

Instrumentation

Chromatographic separation was performed on an Acquity™ ultra performance liquid chromatography (UPLC) system (Waters Technologies, Milford, MA, USA) with a PHENOMENEX KINETEX C18 (100 mm × 2.1 mm, 2.6 μm). Separation of target analytes was achieved by a gradient elution program with the mobile phase of a mixture of methanol (A) and 0.1% formic acid in ultrapure water (B). The gradient elution program was optimized as follows: started from 60% A and a linear gradient to 100% A in 4 min and maintained for 6 min, then decreased to 60% A over 0.1 minute

and subsequently maintained for 4 min, the total run time was 14 min. The flow-rate was set as 0.3 mL min^{-1} and the column temperature was keep in constant with of 40°C. The injection (20 μL) was performed using an auto-sampler and vials of 2 mL capacity.

MS/MS detection was performed on a triple quadrupole mass spectrometer detector (Waters Technologies, Milford, MA, USA) equipped with a jet stream electro spray ionization (ESI) source. Positive ESI with the multiple reaction monitoring (MRM) mode was used for quantification. Nitrogen gas which is generated by a N_2 generator (Peak scientific, Billerica, MA) was used for the collision gas. The auxiliary heater temperature was set at 550°C with an ionization voltage of 5500 V. The nebulizer gas and auxiliary gas were all 50 psi. The instrumental conditions and method parameters are shown in Table 1.

Sample preparation and migration conditions

In order to investigate the migration characters of the three FWAs, a positive FCPPM sample which was determined by our previous established method [9] with the concentrations of 69.1mg/L, 45.5 mg/L and 66.2 mg/L for FWA393, FWA184 and FWA368, respectively, was used. Four kinds of food simulants, which were distilled water, 3% acetic acid, 10% ethanol and 95% ethanol were selected to represent water food, acidic food, wine food and fat food, respectively to perform the migration experiment according to GB/T 23296.1-2009[17] and EN 13130-1:2004.[18] The migration experiment was conducted in a constant temperature oven with the temperatures of 5, 15, 25, 35 and 45°C for 1, 3, 5, 7, 9, 12, 15, 20, 25, 30 days

respectively. All the pieces of samples were soaked in the solution and the soak area and simulants volume were approximately 2 dm^2 and 250 mL. The soak solutions were then filtered through a 0.22 μm filter membrane and finally transferred into sample bottles for UPLC-MS/MS analysis.

Statistical analysis

Data were analyzed by using SPSS (SPSS Inc., Chicago, IL, USA) and presented as mean ± SD with triplicates. Significance was determined at $P < 0.01$ by analysis of variance (ANOVA) followed by Duncan's least significant test.

Results and discussion

Optimization of UPLC-MS/MS conditions

The three target analytes, which contain tertiary nitrogen atoms, generated precursor ions [M+H]+ under ESI positive mode.[9,19] At the optimized cone voltage, collision energy (Table 1), considerable signals for the [M+H]+ peaks were obtained. In addition, the precursor ion and daughter ions of the three target FWAs were also obtained and presented in Table 1. Under the conditions optimized, the strength of the molecular ion and characteristic fragment ion pair of each compound can reach the maximum.

Under the ESI positive mode, the formic acid which added in the mobile phase can provide the H^+ that is required for ionization, thus can increase the response value. In the present work, the effects of two mobile phase systems, including acetonitrile-0.1% formic acid water and methyl alcohol-0.1% formic acid water, on the separation and detection sensitivity of all target analytes were investigated. The results found that the

detection sensitivity of all target analytes using methyl alcohol as organic phase was apparently higher than that using acetonitrile as organic phase, especially, the response value of FWA184 increased more than 30 times with methyl alcohol-0.1% formic acid water mobile phase system. That is why the optimal system, methyl alcohol-0.1% formic acid water mobile phase system, was chosen for the separation of target compounds. In addition, the elution gradient was also optimized, in the first 4 min, the proportion of organic phase gradually increased from 60% to 100%, which can elute the interfering substances of high polarity and reduce the matrix interference. Then the three target analytes were eluted with 100% organic phase and favorable separation was obtained. The 100% organic phase was maintained for 6 min to completely elute the impurities of low polarity that remained on the chromatographic column, thus can prolong the service life of chromatographic column. Fig. 2 shows the typical quantitative daughter ion chromatograms of these three FWAs under the optimized instrument condition.

Optimization of food simulants

FCPPC can be used to store food in the low temperature and room temperature condition. So in the present work, distilled water, 3% acetic acid, 10% ethanol and 95% ethanol were selected to represent water food simulant, acidic food simulant, wine food simulant and fat food simulant, respectively to perform the migration experiment under migration temperatures of 5^{o}C (low temperature) and 25^{o}C (room temperature) for 30 days. The results found that three target analytes could be only detected in fat food simulant, the migration values of FWA 184,

FWA393 and FWA368 were 27.5, 95.5 and 67.5 μg/L in 25°C and 9.3, 28.5 and 21.4 μg/L in 5°C (Fig. 3). It is probably because the big log K_{ow} of these FWAs makes them soluble in fat food stimulant, while the solubility of these FWAs in distilled water, 3% acetic acid, 10% ethanol are too low and thereby without migration in these kinds of food. So, in the following migration research experiment, 95% ethanol was selected as the migration stimulant of these three FWAs.

Linearity range, LODs and LOQs

The analytical characteristics of the developed method, such as linearity range, linear equations, LODs and LOQs, were investigated to evaluate the efficiency of the method and the possibility of the method application to real samples. A series of mixed standard solutions with the concentrations from 0.03 to 200.0 μg/L of three FWAs were prepared. Under the UPLC-MS/MS conditions optimized in this work, the linear equations were obtained by plotting the peak areas of quantification ion pair of each target compound (on the ordinate (y)) *versus* the corresponding concentrations (on the abscissa (x)) using five concentration levels in duplicate. The LODs and LOQs were calculated by analyzing the spiked sample solution that underwent pretreatment and yielded a signal-to-noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively. [20] The linear equations, linearity range, correlation coefficients, the LODs and LOQs of the target analytes are shown in Table 2. R^2 values for the three FWAs were all greater than 0.999, demonstrating excellent linearity for the range studied in this work. The correlation coefficients obtained in this work using the developed method are even more favorable than those ($R^2 \geq 0.995$) in a previous

study published by Guo, et al. (2013).[9] The LODs and LOQs were ranging from 0.01 to 0.03 μg/L and 0.03-0.09 μg/L, respectively, indicating high sensitivity of the developed method.

Recoveries, accuracy and precision

Negative samples at three spiked levels of three target analytes with 1 × LOQ, 10 × LOQ, 100 × LOQ of mixed standard solutions were used to test the recoveries and intro-day precision of analytes according to the proposed method, with six identical samples tested at each concentration. In addition, the inter-day precision was also investigated by analyzing five spiked replicates for 10 × LOQ level. The results indicated that the recoveries of the three target analytes were satisfactory with values in the range of 96.1%-111.4% (Table 3). Moreover, relative standard deviations (RSDs) of 3.0%-6.1% for intra-day precision (n = 6) and 2.6%-6.4% for inter-day precision (n = 5) were observed, meaning that the accuracy, precision and stability can meet the requirements for such an analysis.

Migration research of three FWAs

The effects of different temperatures on the migration values of the target FWAs (Migration time: 30 days) were investigated using a positive sample and the results were shown in Fig. 4. It can be seen from Fig. 4 that sharp increases in migration values of three FWAs were observed with the migration temperatures increasing from 5^{o}C to 45^{o}C (P < 0.01). In the meantime, the effects of migration time on the migration values of three FWAs under 5^{o}C and 25^{o}C were also studied. Changes in migration values of these FWAs under 5^{o}C and 25^{o}C for different migration time were

shown in Fig. 5 and Fig. 6. From the results, it can be seen that with the increase of migration time, the migration values of all FWAs increases accordingly. Moreover, the migration rates for the three FWAs in the first ten days were very fast, while those in the following ten days were slow and finally changed to be steady with the migration time more than twenty days. These results were in accordance with a previous study conducted by Xian et al. (2014) who also found that the migration levels of FWA 184 and FWA 393 were increased with the increase of storage temperature and storage time. [21] The migration equations (Table 4) for the FWAs in 5^{o}C and 25^{o}C were obtained with the data further processed. It can be seen from Table 5 that the migration equations were all quartic equations with the correlation coefficients ranging from 0.9912 to 0.9977. The results indicated that FWA393, FWA184 and FWA368 in FCPPC could be migrated to food when fat kind food was stored. The migration values and risk levels were closely related to the concentration of FWAs in plastic containers, storage time and even storage temperature. Fortunately, the trend of migration of FWAs from FCPPC to food could be predicted by the migration equations obtained in this work.

Analysis of practical samples

The method established in this work was adopted to determine a total of twenty migration solutions of FCPPC samples collected from local markets conducted under 5^{o}C and 25^{o}C for thirty days, using fat food stimulant as soak solution. FWA184 was detected in migration solutions of only one sample with the concentration of 0.57 μg/L for 25^{o}C and 0.21 μg/L for 5^{o}C, and no other FWAs were detected in the other

migration solutions. The selected ion chromatogram of FWA184 in the migration solution of the typical sample with the migration condition of 25^{o}C was shown in Fig. 7.

Conclusions

A simple and sensitive analytical method, using UPLC-MS/MS technique, was developed for the simultaneous determination of three FWAs in migration solutions of FCPPC samples. Satisfactory validation parameters were obtained, for example, the calibration curves were linear over the selected concentration ranges of 0.03-200 μg/L with R^2 greater than 0.999 for all the three analytes. LODs and LOQs of the method were 0.01-0.03 μg/L and 0.03-0.09 μg/L, respectively. Favorable recoveries (96.1-111.4%) were obtained with RSDs of 2.6%-6.4%. The migration trends of target analytes were analyzed and the corresponding migration equations with correlation coefficients more than 0.991 were obtained. The results obtained can also confirm the suitability of the method proposed for FWAs determination and monitoring from FCPPC to food.

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296 **Table 1**

297 LC-MS/MS conditions for the three target analytes by MRM in positive ion mode
298 with cone voltage of 60 V.

Compound	Precursor ion (m/z)	Product ion [a] (m/z)	Collision energy (eV)	Retention time (min)
FWA393	415.2	321.2	55	2.26
		207.1	55	
FWA368	429.2	321.2	40	3.15
		221.0	40	
FWA184	431.2	415.2	60	4.62
		401.2	60	

299 [a] The first product ion was used for quantification, whereas the second one was used for
300 identification.

301

302 **Table 2**

303 Linear equations and R^2, LODs and LOQs of the three target analytes.

Compound	Linear equation	R^2	Linear range (μg/L)	LOD (μg/kg)	LOQ (μg/kg)
FWA393	y = 29620 x+1081.0	0.9995	0.03-200.0	0.01	0.03
FWA368	y = 18968 x+2325.2	0.9996	0.09-200.0	0.03	0.09
FWA184	y = 22023 x+1982.2	0.9991	0.03-200.0	0.01	0.03

304

305 **Table 3**

306 The recoveries and precision for the three target analytes.

Compound	Added (μg/L)	Intra-day(n = 6), Recovery (%RSD)		Inter-day (n = 5), Recovery (%RSD)[a]	
		5	25	5	25
FWA393	0.03, 0.3, 3.0	103.0 (5.2), 102.8 (4.7), 101.4 (6.1)	98.7 (4.1), 98.5 (3.2), 94.2 (2.9)	105.8 (6.4)	106.6 (4.1)
FWA368	0.09, 0.9, 9.0	98.3 (4.6), 105.2 (4.0), 98.1 (4.5)	99.3 (4.3), 96.1 (3.7), 99.0 (3.0)	98.3 (4.7)	111.4 (2.6)
FWA184	0.03, 0.3, 3.0	103.5 (4.1), 107.2 (3.8), 99.6 (4.0)	101.6 (5.5), 97.5 (5.1), 96.7 (4.9)	99.4 (4.2)	104.3 (5.9)

307 [a] spiked level was 10 × LOQ.

308

309 **Table 4**

310 Migration equations and correlation coefficients of the FWAs.

Anaytes	5°C		25°C	
	Migration equation	Correlation coefficients	Migration equation	Correlation coefficients
FWA184	$y = -2E\text{-}05x^4 + 0.0015x^3 - 0.0497x^2 + 0.7223x + 2.4141$	0.9977	$y = -0.0002x^4 + 0.0128x^3 - 0.3491x^2 + 3.9825x + 10.014$	0.9928
FWA393	$y = -0.0002x^4 + 0.017x^3 - 0.4448x^2 + 4.9171x + 11.554$	0.9932	$y = -0.0007x^4 + 0.0525x^3 - 1.3799x^2 + 15.385x + 30.134$	0.9912
FWA368	$y = -8E\text{-}05x^4 + 0.0063x^3 - 0.1729x^2 + 2.0629x + 5.5752$	0.9920	$y = -0.0003x^4 + 0.0261x^3 - 0.7373x^2 + 9.0391x + 23.887$	0.9962

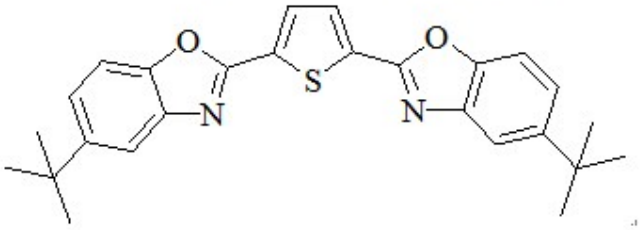
311

312

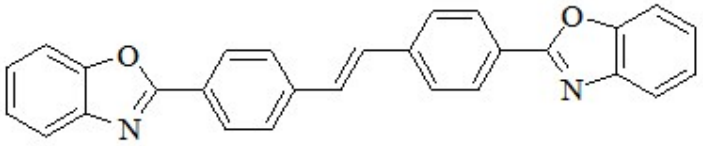
313 **Fig. 1**

314 Chemical structures of the three fluorescent whitening agents (FWAs).

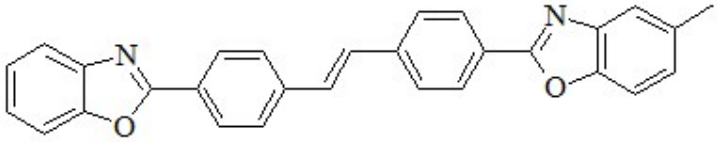
2,5-Bis(5′-tert-butyl-2-benzoxazolyl)thiophene (FWA184)



4,4′-Bis(2-benzoxazolyl)stilbene (FWA393)



4-(2-Benzoxazolyl)-4′-(5-methyl-2-benzoxazolyl)stilbene (FWA368)



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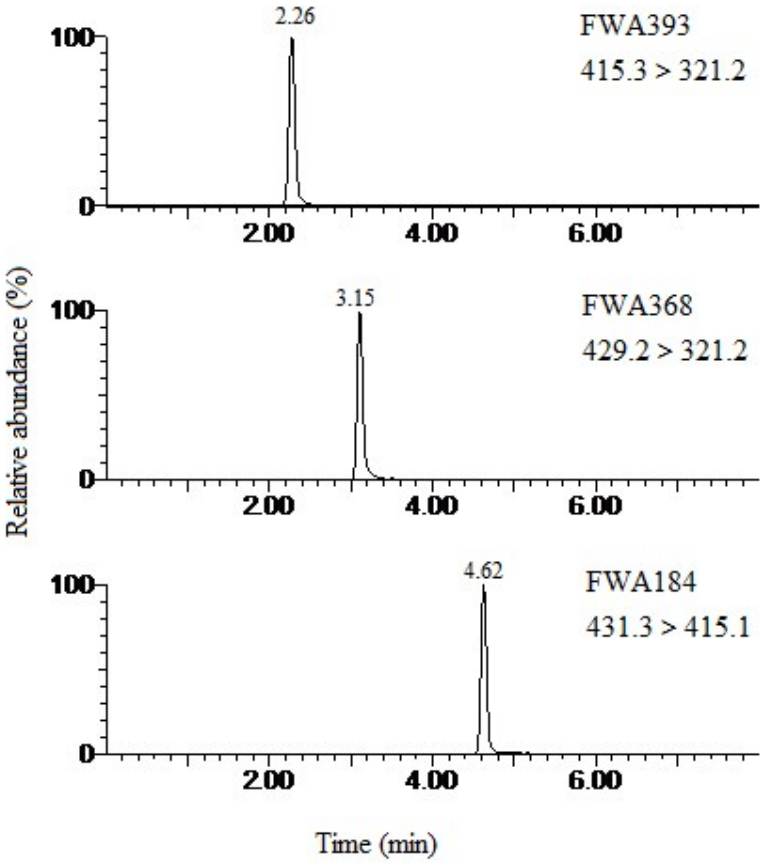
316

317 **Fig. 2**

318 MRM chromatograms of the three FWAs.



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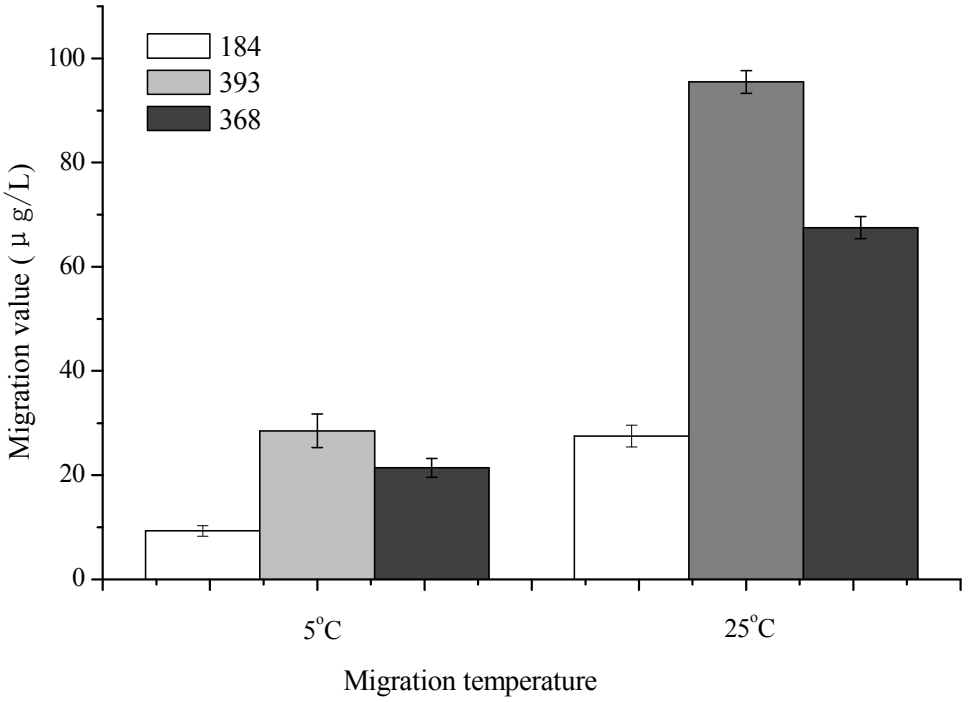
320

Fig. 3

Migration values of the three target analytes detected in fat kind food (95% ethanol) under 5°C and 25°C, respectively.[a]



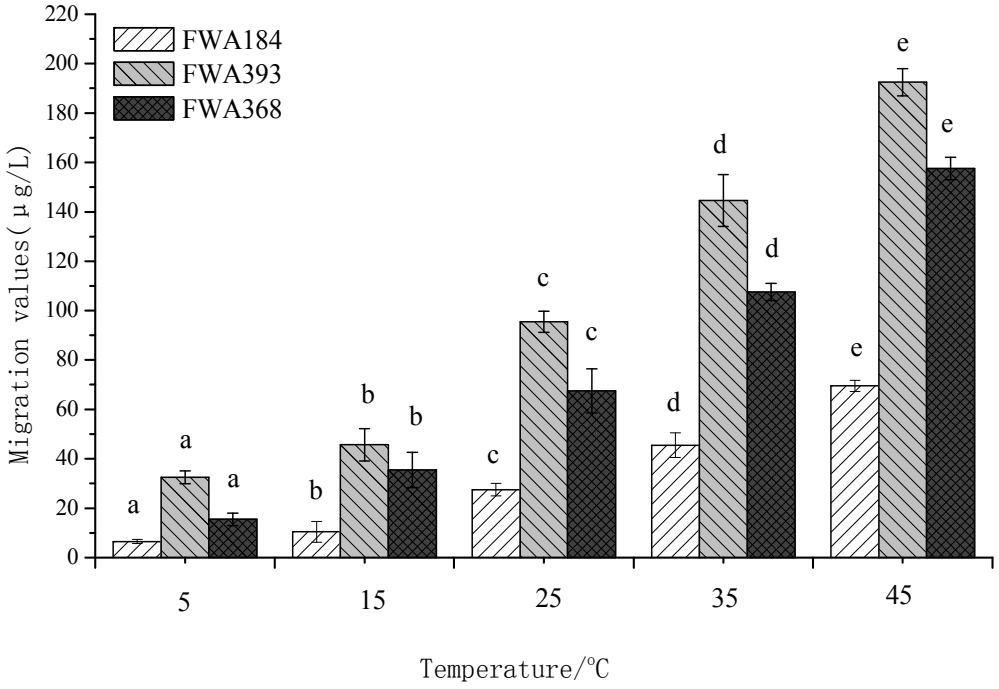
[a] Four food stimulants were selected to perform the migration experiment, where A, B, C and D represent water kind food (distilled water), acidic kind food (3% acetic acid), wine kind food (10% ethanol) and fat kind food (95% ethanol). Only fat kind food (95% ethanol) was detected with these three target analytes, migration to A-C was below the detection limits.

331 **Fig. 4**

332 Effects of different temperatures on the migration values of the three FWAs.

333 The data are expressed as means ± SD. Values within the same mullion with different

334 letters above are significantly different at $P < 0.01$.



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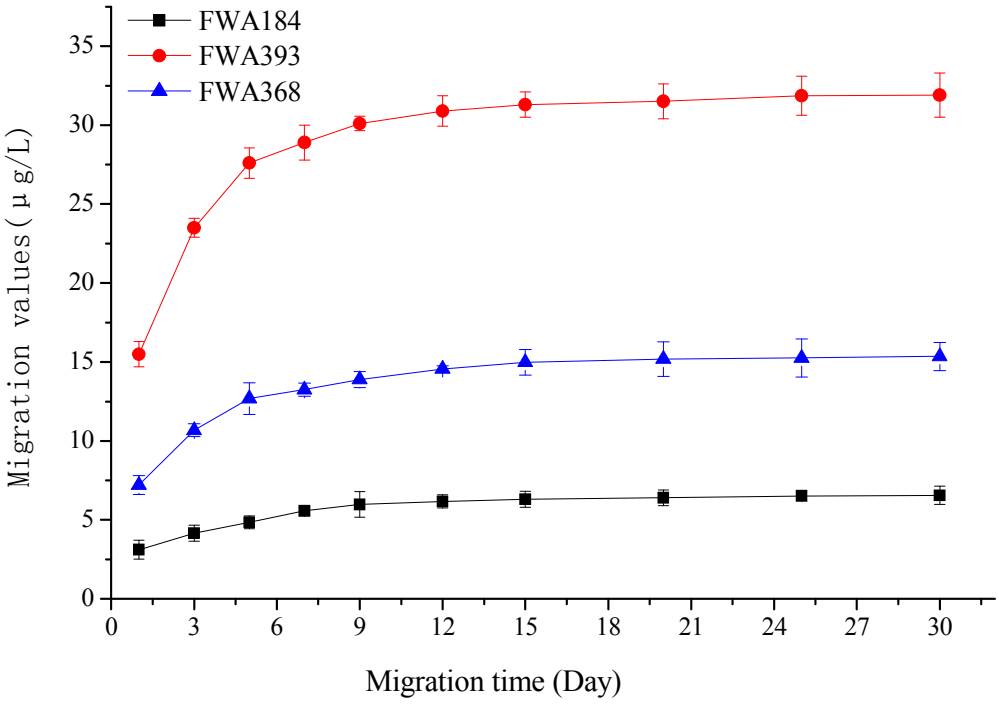
337 **Fig. 5**

338 Effects of migration time on the migration values of the three FWAs at 5°C.



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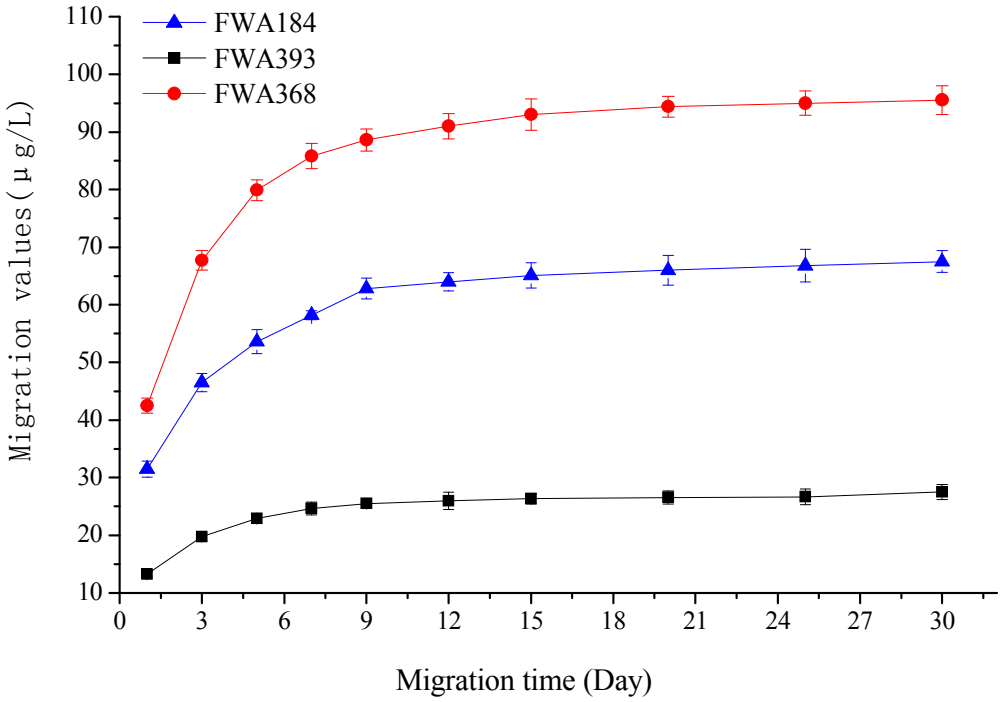
342 **Fig. 6**

343 Effects of migration time on the migration values of the three FWAs at 25°C.



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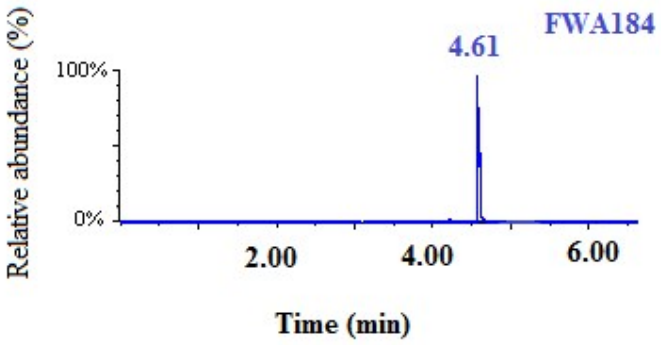
347 **Fig. 7**

348 Selected ion chromatograms of FWA184 in the migration solution of positive sample

349 under 25°C.



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