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A novel method for trace aldehyde determination in foodstuffs based on fluorescence labeling by HPLC with fluorescence detection and mass spectrometric identification

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Abstract A stable, effective, sensitive and selective method for simultaneous determination of 11 aldehydes in foodstuffs using a novel fluorescence-labeling reagent 2-(12-benzo[*b*]-acridin-5-(12*H*)-yl)-acetohydrazide (BAAH) has been developed by HPLC with fluorescence detection and mass spectrometric identification. Response surface methodology was employed to optimize the derivatization reaction between BAAH and aldehydes. The completed separation of the 11 aldehydes was achieved in as little as 18 min on a reversed-phase Hypersil BDS C_8 column with aqueous acetonitrile as mobile phase in conjunction with a binary gradient elution. Excellent linear coefficients were found to be of >0.9994. This method also showed excellent reproducibility and offered the low detection limits of 0.21–0.58 nM (at a signal-to-noise ratio of 3). The developed method was successfully applied to analyze aldehydes in various foodstuffs and exhibited satisfactory applicability.

Keywords Aldehydes · HPLC-FLD-MS/MS · Pre-column derivatization · Response surface methodology · Foodstuffs

Introduction

Alarm and awareness of possible health hazards associated with food is recently increasing (Sjaastad et al. 2010). Aldehydes can be generated during the food processing, especially frying and fermentation processes, and their concentration increases at high temperatures due to the degradation of sugars, proteins, and fats present in food (Gosetti et al. 2011; Huang et al. 2011). In recent years, aldehyde determination in foodstuffs has received much attention. And many of them have been identified in foodstuffs such as wines (Sáenz-Navajas et al. 2010; Verzera et al. 2008), cooked food (Gosetti et al. 2011), fruits (Verzera et al. 2011), and honey (Spano et al. 2009). Most aldehydes are acknowledged to have corrosive, irritant, and carcinogenic effects on biological tissues (Moskovitz et al. 2002; Stafiej et al. 2006). Formaldehyde is a highly toxic volatile carcinogen and can result in asthma, watery eyes, dermatitis, respiratory irritation, and pulmonary edema (Ahmadi et al. 2012). Acetaldehyde in the presence of alcohols reacts with the amino groups in nucleosides to yield mixed acetals which are claimed to increase the risk of breast cancer in women (Nascimento et al. 1997). Furaldehyde (FA) produced from pentose degradation is toxic to organism (Zaldivar et al. 1999). Hexanal and propanal were the dominating toxic compounds emitted from linseed oil paint (Fjällström et al. 2002). In addition, the existence of aldehydes can directly influence the quality of foodstuffs. For example, aldehydes are considered to play an important role in the flavor of many alcoholic beverages (Gonçalves et al. 2010; López-Vázquez et al. 2012). Therefore, the development of sensitive and selective analytical methods for monitoring trace aldehyde in foodstuffs targeted in health-related studies is of great importance.

However, it is very difficult to detect aldehydes directly in complex matrices due to the lack of intrinsic chromophores or fluorophores as well as their volatility and activity. To overcome this problem, chemical derivatization of aldehydes is preferred prior to analysis. Various derivatization schemes for the determination of aldehydes have been reported (Kato et al. 2002; Pal and Kim 2007; Shibamoto 2006). The common

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analytical method is gas chromatography (GC) (Mandić et al. 2013), which was made based on adsorptive enrichment on solid sorbents followed by thermal desorption. Several gas chromatographic methods have been described for the determination of aldehydes in water or exhaust gases as their 2,4-dinitrophenylhydrazine (DNPH) derivatives (Dong and Moldoveanu 2004; Nishikawa and Sakai 1995). In fact, GC separation of aldehyde derivatives can be unsatisfactory due to the derivatives' low volatility and decomposition at high temperature required by the GC operation (Kim and Pal 2010). In contrast with GC, use of HPLC allows the aldehydes to be directly converted to a large number of different derivatives. Derivatization by the formation of less polar compounds can overcome some problems, such as tailing peaks and low detector sensitivity, which can be more easily analyzed by LC (Andreoli et al. 2003; Lin et al. 2009). Thus, HPLC coupled with pre-column derivatization has been widely used to the accurate determination of aldehydes in complex matrices (foodstuffs, environmental samples, etc.). Fluorescence detection is the best choice in numerous determinations of target materials. The recent upsurge of interest in this area has resulted in many fluorescence-labeling reagents, such as fluoren-9-yl-methoxycarbonylhydrazine (Zhang et al. 1991), O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (López-Vázquez et al. 2012), 2,4-dinitrophenylhydrazine (Kim and Pal 2010; Lin et al. 2009), *O*-phenylenediamine (Barros et al. 1999) and 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-*s*-indacene (Xiong et al. 2010). Many reagents for the labeling of aldehydes possess a hydrazine group ($-NHNH_2$) as the reactive site. Although some of them are sensitive enough for the determination of aldehydes or ketones in air, water, exhaled breath condensate, and wines, some shortcomings have also been found in their applications, such as short detection wavelengths, relatively low sensitivity, poor stability, and serious interference for the determination of real samples (Basheer et al. 2010; Santa 2011). Therefore, it is necessary and significant to develop a novel method for aldehyde determination that offers high sensitivity and selectivity, and good stabilities of the reagents and the derivatives. At present, a series of reagents based on benzoacridin (BA) were synthesized in our research group because BA is a well-known fluorophore characterized by valuable properties such as excellent stability, independence of solvent and pH, high-fluorescence quantum yields, and large molar absorption coefficients. 2-(12-benzo[*b*]acridin-5-(12*H*)-yl)-acetohydrazide (BAAH) as one of these reagents was proved to be highly sensitive and selective and has been used for fatty acid determination (Xie et al. 2012). In this study, this reagent was applied to the aldehyde determination, and 11 aldehydes including furaldehyde and ten aliphatic aldehydes from formaldehyde to decanal were determined from several kinds of foodstuffs using HPLC-fluorescence detection (FLD) method on a Hypersil BDS-C_8 column. To the best of our knowledge, this is the first trail of exploring BAAH fluorescent probe for the determination of aldehydes.

It is of great importance for the sufficient derivatization labeling of the analyzed components. Traditionally, derivatization optimization in analytical chemistry involves changing one independent variable while keeping the other factors constant at a time (López-Vázquez et al. 2012; Lin et al. 2009). This optimization procedure is troublesome, reagents- and time-consuming as well as ignoring the interaction effect of parameters. In order to overcome these problems, response surface methodology (RSM) has been carried out by using multivariate statistic techniques. Box-Behnken design (BBD), one of the RSMs, only has three-levels and needs fewer experiments. It is more efficient and easier to arrange and interpret experiments in comparison with others (Ferreira et al. 2007). Therefore, BBD of RSM was applied to optimize the main derivatization parameters of aldehydes in the present study.

Materials and Methods

Instrumentation

Experiments were performed using a LC/MSD-Trap-SL electrospray ion trap liquid chromatography/mass spectrometry (1100 Series LC/MSD Trap, a complete LC/MS/MS). All the HPLC system devices were from the HP 1100 series and consisted of a vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostatic column compartment (model G1316A), and a fluorescence detector (model G1321A). Derivatives were separated on Hypersil BDS-C_8 column (200 mm × 4.6 mm, 5 μM, Dalian Yilite Co., China). The HPLC system was controlled by HP Chemstation software. The mass spectrometer from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization. Fluorescence excitation and emission spectra were obtained at a 650-10S fluorescence spectrophotometer (Hitachi). The mobile phase was filtered through a 0.22-μm nylon membrane filter (Alltech, Deerfield, IL).

Chemicals

Aldehyde standards including furaldehyde, formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonaldehyde, and decanal were purchased from Sigma Co (St. Louis, MO). HPLC-grade acetonitrile was purchased from Yucheng Chemical Reagent Co. (Shandong Province, China). Trichloroacetic acid was analytical grade from Shanghai Chemical Reagent Co. Water was purified on a Milli-Q ultrapure system (Millipore, Bedford, MA). The standard trichloroacetic acid (TCA, catalyst) for derivatization

reaction at concentrations of 1.0 % (*m*/*m*) was prepared by dissolving the trichloroacetic acid crystals with anhydrous acetonitrile. BAAH was synthesized in our laboratory (Xie et al. 2012). All other reagents used were also of analytical grade unless otherwise stated.

Preparation of Standard Solutions

The labeling reagent solution (1.0×10^{-3} mol/L) was prepared by dissolving 31.7 mg of BAAH in 10 mL anhydrous acetonitrile. Individual stocked solution of the aldehyde (1.0×10^{-2} mol/L) was prepared in acetonitrile. The standard aldehyde for HPLC analysis at individual concentration of 1.0×10^{-4} mol/L was prepared by diluting the corresponding stock solution (1.0×10^{-2} mol/L) of each aldehyde with acetonitrile. When not in use, all standards were stored at 4 °C in a refrigerator.

Pretreatment of Samples

All foodstuffs were purchased from local supermarkets or retailers in Qufu (China) including wines (red wine, white wine and rice wine), yoghurt, potato crisps, fried dough sticks, dried shrimps, brined fish, and honey. Each sample of wines was filtered through 0.22-μm nylon filters prior to derivatization. Aldehydes were extracted from potato crisps, fried dough sticks, dried small shrimps, and brined fish by adding 5 mL acetonitrile to 2 g sample, respectively. After 20 min extraction in an ultrasonic bath, the supernatant was prepared for the next analysis. The yoghurt samples were centrifuged at 4,500 rpm (4 °C) for 15 min and the supernatant was further deproteinized by mixing it with acetonitrile at the volume ratio of 1:4 (200 μL of supernatant was placed in a vial filled with 800 μL acetonitrile). And then, the resultant was centrifuged at 4500 rpm (4 °C) for 15 min (Xiong et al. 2010). The supernatant was collected for the derivatization directly. The honey solutions were prepared by dissolving 2 g homogenized sample with ultra pure water and then filling to the mark in a 10-mL volumetric flask (Spano et al. 2009). All derivatives of samples were filtered through 0.45-μm filters before injection onto the HPLC column.

Derivatization Procedure

The BAAH-aldehydes derivatization proceeded in acetonitrile solution in the presence of trichloroacetic acid catalyst. A 20-μL volume of mixed aldehydes in acetonitrile(1.0×10^{-4} mol/L each) (or 120 μL sample extracted solutions) was added into a vial (2.0 mL), and then 20 μL of 1.0 % TCA acetonitrile solution and 100 μL of BAAH acetonitrile solution (1.0×10^{-3} mol/L) were successively added. The vial was then sealed and the mixture was heated at 52 °C for 12 min in a thermostatic water-bath, and the reaction solution was cooled in ice-water to stop the reaction. An aliquot (10 μL) of the derivatization solution was injected to HPLC. The derivatization scheme of BAAH with aldehydes is shown in Fig. 1.

HPLC Conditions

HPLC separation of BAAH derivatives was carried out by Hypersil BDS-C_8 column with binary gradient elution. Eluent A was 5 % of aqueous acetonitrile; B was acetonitrile (100 %). During conditioning of the column prior to injection, the mobile phase composition was 60 % (A) and 40 % (B). The gradient elution program was as follows: 40–55 % (B) from 0 to 10 min; 55–100 % (B) from 10 to 25 min. The flow rate was constant at 1.0 mL/min and the column temperature was set at 30 °C. The fluorescence excitation and emission wavelengths were set to λ_{ex}=280 and λ_{em}=510 nm, respectively.

The ionization and fragmentation of the aldehyde derivatives were identified by online mass spectrometry with atmospheric pressure chemical ionization (APCI) in positive-ion detection mode. The mixed aldehydes in acetonitrile were derivatized under the optimized derivatization procedure. An aliquot (10 μL) of the derivatization solution was injected to LC-APCI-MS system. The APCI probe was heated to 400 °C to ensure complete vaporization of the column effluent. Other mass spectra conditions were as follows: nebulizer pressure 60 psi; dry gas temperature, 350 °C; dry gas flow, 5.0 L/min. APCI Vap temperature 450 °C; corona current (nanoamperes) 4,000 (pos); capillary voltage 3,500 V. To obtain the stable and sensitive MS ion current responses, the spray chamber and the tip of the corona needle (APCI) were daily cleaned with 30 % aqueous isopropanol.

Box-Behnken Design for Pre-column Derivatization Optimization

The software Design Expert (Trial Version 7.1.3, Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, data analysis and model building. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and the significances of the regression coefficient were checked by *F* test and *p* value. BBD with three variables was used to determine the response pattern and then to establish a model. Three factors including reaction temperature (X_1), the molar ratio of BAAH to total aldehydes(X_2), and reaction time (X_3) were chosen based on single-factor designs for further optimization. Table 1 lists BBD matrix and the response values that were carried out for developing the model. Pentanal was chosen as the tested compound, and the peak area was taken as the response. For predicting the optimal point, an empirical second-order polynomial model was fitted to correlate relationship between independent variables and

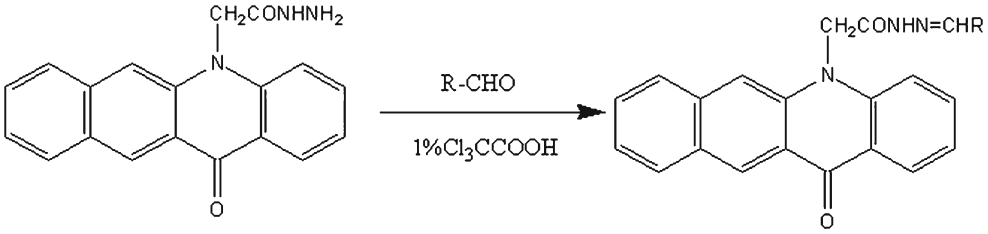

Fig. 1 Derivatization scheme of 2-(12-benzo[*b*]acridin-5-(12*H*)-yl)-acetohydrazide (BAAH) with aldehydes in the presence of trichloroacetic acid catalyst

response. For the three factors, the behavior was explained by the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Where Y represents the response variable, β_0 is a constant, β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interactive coefficients, respectively.

Results and discussion

Optimization of Pre-column Derivatization Parameters by RSM

By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$Y = 3652 - 261.1 X_1 + 98.98 X_2 + 136.9 X_3 - 336 X_1 X_2 - 361.9 X_1 X_3 - 110.7 X_2 X_3 - 589.0 X_1^2 - 240.1 X_2^2 - 56.95 X_3^2$$

Table 1 The Box-Behnken design matrix of three test variables in coded and natural units along with the observed responses (peak area)

Run	Independent variable			Peak area ($\times 10^6$)
	X_1 (Temperature, °C)	X_2 (BAAH, ratio)	X_3 (Time, min)	
1	20(−1)	6(+1)	12.5(0)	2.3613
2	45(0)	2(−1)	20(+1)	3.2762
3	20(−1)	2(−1)	12.5(0)	0.6584
4	70(+1)	6(+1)	12.5(0)	3.2147
5	45(0)	2(−1)	5(−1)	2.6228
6	20(−1)	4(0)	5(−1)	0.7773
7	70(+1)	4(0)	5(−1)	3.1006
8	45(0)	4(0)	12.5(0)	3.5692
9	45(0)	4(0)	12.5(0)	3.5526
10	20(−1)	4(0)	20(+1)	2.8834
11	45(0)	6(+1)	20(+1)	3.5956
12	45(0)	4(0)	12.5(0)	3.5526
13	45(0)	4(0)	12.5(0)	3.5574
14	45(0)	6(+1)	5(−1)	3.3849
15	70(+1)	4(0)	20(+1)	3.3971
16	45(0)	4(0)	12.5(0)	3.5526
17	70(+1)	2(−1)	12.5(0)	3.1916

The ANOVA for the experimental results indicated that all the linear parameters and quadratic parameters were significant at the level of $p<0.01$. F value for the lack of fit was insignificant ($p>0.05$), meaning that this model was sufficiently accurate for predicting the relevant responses. Coefficient of variation (C.V.%) of 8.30 % indicated that the model was reproducible.

The 3D response surfaces are shown in Fig. 2, which illustrate the relationship between independent and dependent variables. Figure 2a shows interaction between reaction temperature and amount of BAAH (mol (BAAH)/mol (total aldehydes)) on the peak area. As shown in Fig. 2a, with a definite derivatization temperature, the peak area increased with the increasing amount of BAAH and reached a maximum value, and then no obvious variation for peak areas were observed with the further increases. It can be seen from Fig. 2b, reaction temperature and reaction time had remarkable interaction. With a given reaction time, the peak area increased with the increase of temperature and reached the highest value at 52 °C, followed by a decline with its further increase. Similarly, Fig. 2c describes the effect of amount of BAAH and reaction time on peak area.

By employing the software Design Expert, the solved optimum values of the tested variables were reaction temperature 51.77 °C, ratio of BAAH to aldehydes 4.34, reaction time 11.24 min. Under the optimal conditions, the maximum predicted peak area of pentanal was 37,500. Taking account of the operating convenience, the optimal parameters were determined as following: temperature 52 °C, the ratio of BAAH to aldehydes 4.5, and time 12 min. By these parameters, the predicted peak area was about 37,385, slightly less than that of the maximum predicted value.

In order to validate the adequacy of the model equation, five verification experiments were carried out under the modified optimal conditions. A mean value of 37,327±96 (n=5) was gained, which was in agreement with the predicted value significantly ($p>0.05$). The correlation coefficient (R^2) between the experimental and predicted values was 0.988. The

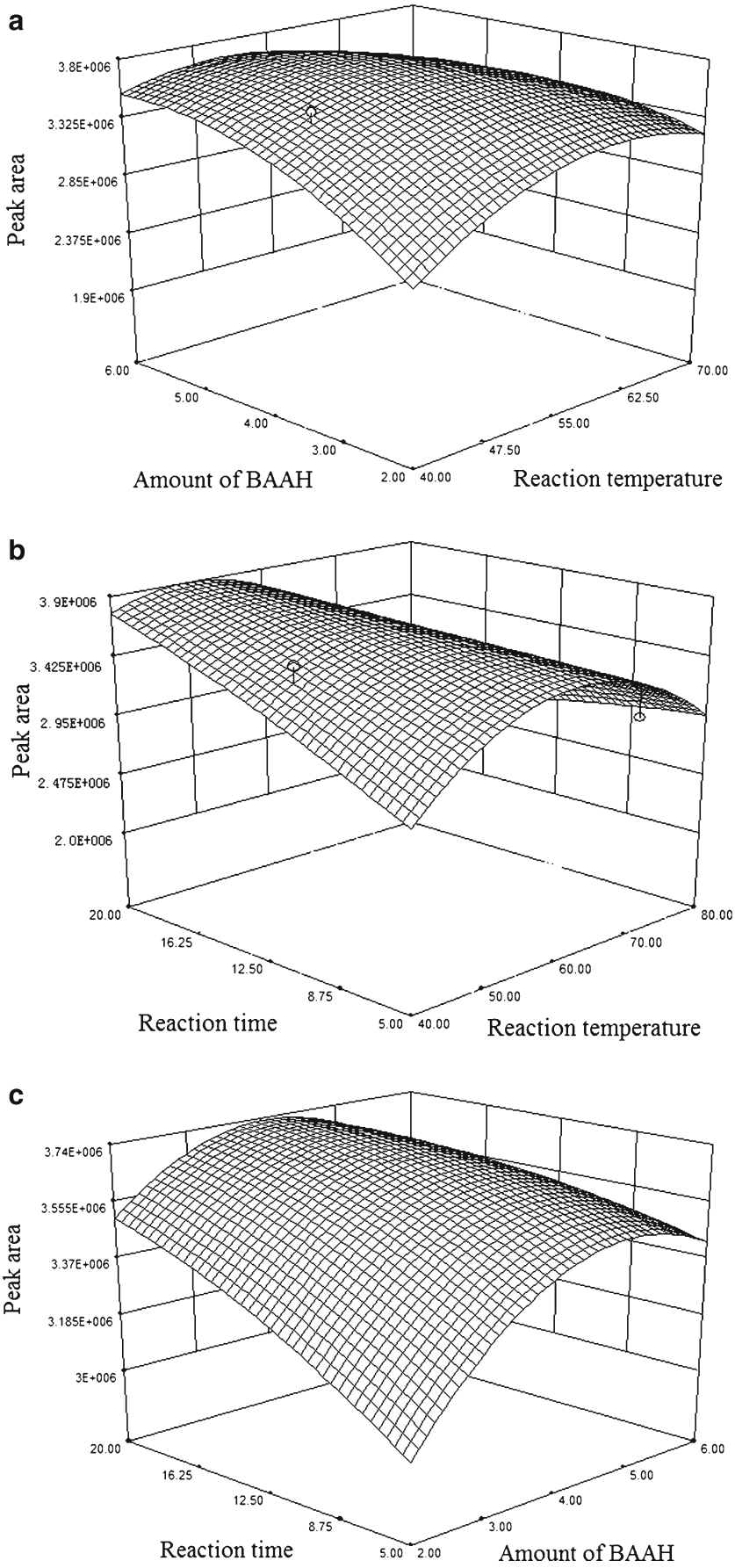

Fig. 2 The 3D response surface of the derivatization yield (expressed in terms of peak area) affected by the varying derivatization temperature and molar ratio of BAAH to aldehydes (**a**), reaction temperature and reaction time (**b**), and molar ratio of BAAH to aldehydes, and reaction time (**c**)

results of analysis indicated that the response model was accurate and adequate for the derivatization of aldehydes.

Chromatographic Separation

A reversed-phase mode is usually used in HPLC for the separation of aldehyde derivatives. In the present study, methanol-water and acetonitrile-water were tested as the mobile phase for the separation of 11 aldehyde derivatives. Considering a relatively shorter analytical time, a higher sensitivity and a better resolution of target peaks, acetonitrile-water system was used as the mobile phase. Several gradient programs were examined starting with acetonitrile-water mixtures and ending with pure acetonitrile. The most efficient separation of BAAH derivatives was achieved following the gradient profile as presented in HPLC Condition section. The derivatives were tested and compared with different analytical columns such as Eclipse XDB-C_8 column (150 mm×4.6 mm, 5 μm), Hypersil C_{18} (250 mm×4.6 mm, 5 μm), and Hypersil BDS C_8 column

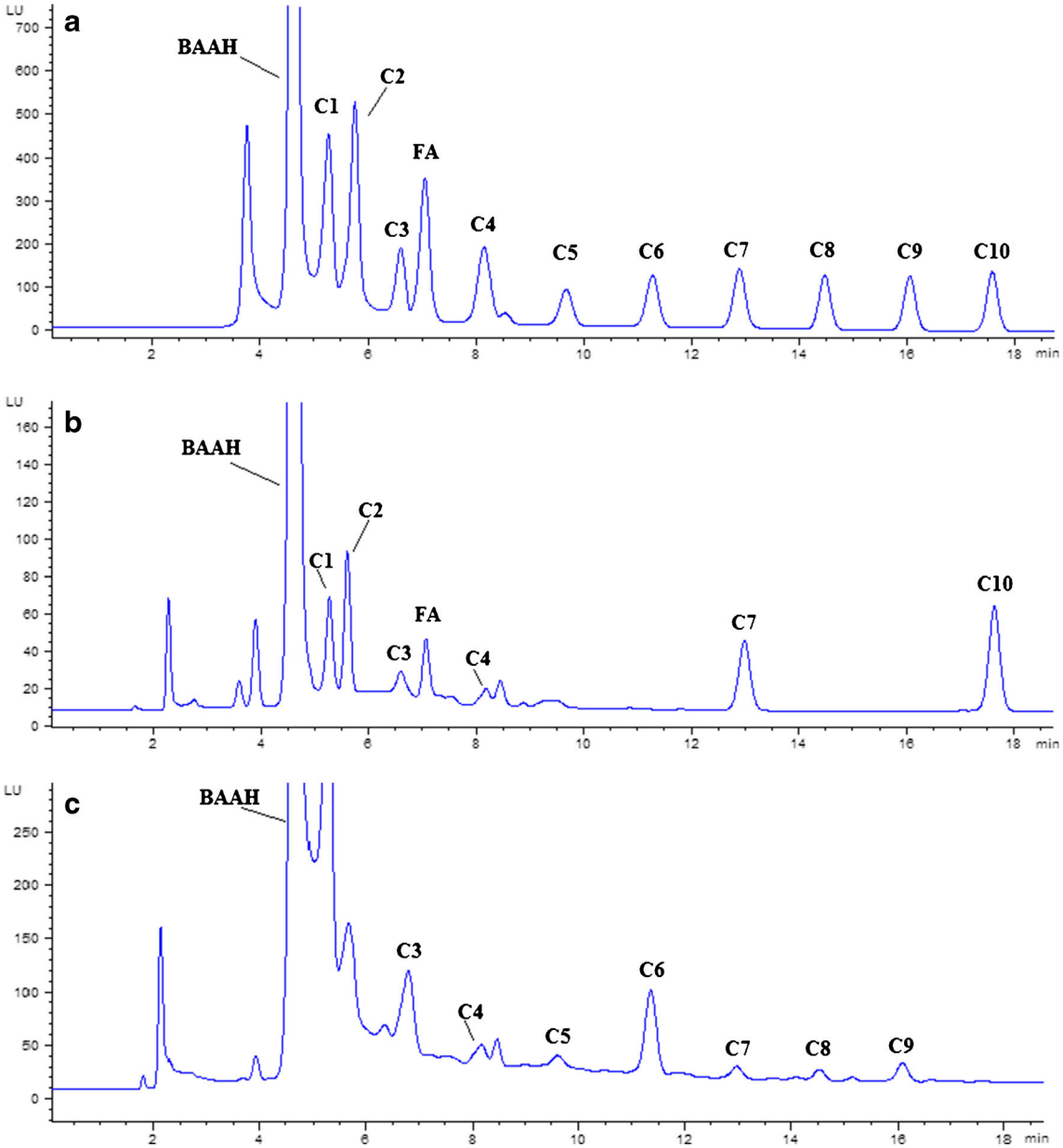

Fig. 3 The typical chromatograms for aldehydes standards (**a**), white wine (**b**) and brined fish (**c**); Chromatographic conditions: column temperature at 30 °C; excitation wavelength λex 280 nm, emission wavelength λem 510 nm; Hypersil BDS C8 column (200 mm×4.6 mm, 5 μm); flow rate=1.0 mL/min; Peak labels: *C1* (formaldehyde), *C2* (acetaldehyde), *C3* (propanal), *FA* (furaldehyde), *C4* (butanal), *C5* (pentanal), *C6* (hexanal), *C7* (heptanal), *C8* (octanal), *C9* (nonaldehyde), *C10* (decanal)

Table 2 MS and MS/MS data, linear regression equation, limit of detection and quantification, repeatability, precision and recovery of aldehyde derivatives

Aldehyde derivatives	MS $M+H]^+$	MS/MS	Regression equation		LOD[b] (nM)	LOQ[c] (nM)	Repeatability R.S.D. (%) (n=6)		Precision R.S.D. (%) (n=6)		Recovery (%)	
			$Y=AX+B$[a]	r			Retention time	Peak area	Intra-day	Inter-day	Mean	RSD (n=5)
Formaldehyde	330.5	246.2, 286.4, 301.6	$Y=2.71X-4.2$	0.9995	0.58	1.95	0.04	1.41	1.89	2.13	95.8	1.6
Acetaldehyde	344.5	246.3, 286.3, 301.7	$Y=5.73X+8.26$	0.9997	0.58	1.95	0.03	1.35	2.08	2.23	102.7	2.1
Propanal	358.6	246.3, 286.4, 301.5	$Y=19.27X-66.36$	0.9994	0.52	1.76	0.01	1.16	3.56	3.80	98.9	1.8
Butanal	372.5	246.2, 286.4, 301.6	$Y=37.99X+5.07$	0.9997	0.49	1.65	0.02	1.44	1.75	3.42	101.6	2.2
Pentanal	386.7	246.3, 286.2, 301.7	$Y=15.63X-15.49$	0.9997	0.32	1.15	0.01	1.52	2.73	2.75	97.4	1.9
Hexanal	400.4	246.2, 286.3, 301.7	$Y=20.79X-0.77$	0.9999	0.29	1.02	0.02	1.46	2.69	2.65	98.0	1.7
Heptanal	414.3	246.2, 286.2, 301.5	$Y=22.72X+4.58$	0.9998	0.47	1.56	0.02	1.35	2.43	2.21	99.1	1.5
Octanal	428.5	246.2, 286.3, 301.6	$Y=22.15X+0.84$	0.9999	0.40	1.35	0.03	1.25	2.87	3.26	98.2	1.6
Nonaldehyde	442.6	246.4, 286.5, 301.4	$Y=23.09X+5.54$	0.9999	0.47	1.56	0.01	1.19	3.18	3.09	100.3	1.3
Decanal	456.4	246.3, 286.2, 301.6	$Y=24.31X+5.52$	0.9997	0.45	1.50	0.02	1.24	3.25	2.93	99.7	1.5
Furaldehyde	396.6	246.3, 286.3, 301.6	$Y=90.69X-1.17$	0.9999	0.21	0.65	0.05	1.38	2.14	2.06	96.8	2.0

[a] Y peak area; X injected amount of each aldehyde (picomoles), 10 μL injection volume

[b] Signal-to-noise ratio=3

[c] Signal-to-noise ratio=10

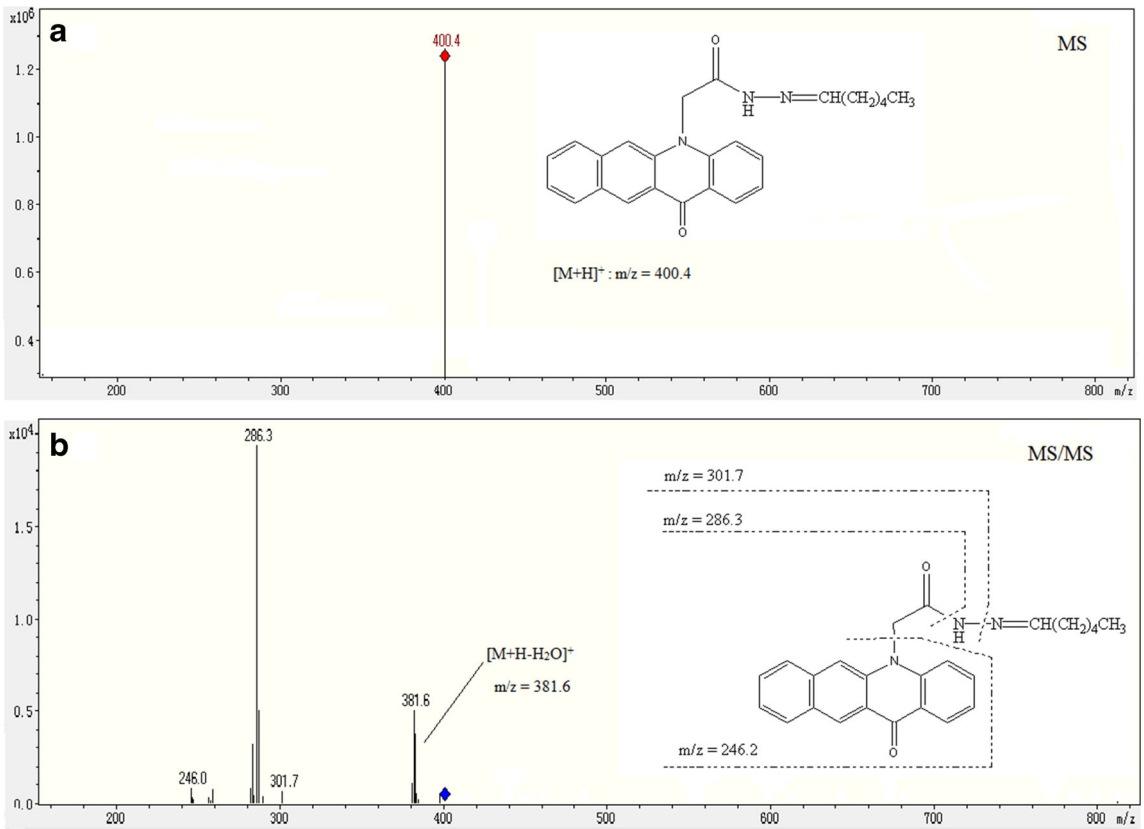

Fig. 4 MS spectra of the representative hexanal derivative (**a**) and the cleavage mode of protonated molecular ion (**b**)

(200 mm×4.6 mm, 5 μm). It was also proved that the result was better when the flow rate was 1 mL/min and the column temperature was kept at 30 °C. Finally, a Hypersil BDS C_8 column (200 mm×4.6 mm, 5 μm) with acetonitrile-water (5/95, *v*/*v*) as mobile phase A and 100 % of acetonitrile as mobile phase B was chosen as the preferred chromatographic conditions. With the chromatographic conditions described above, a satisfactory resolution for 11 derivatives was obtained within 18 min. Representative chromatograms for the standard analytes and the samples are shown in Fig. 3.

Characterization of BAAH-aldehyde Derivative by MS/APCI

The MS and MS/MS data of all aldehyde derivatives are shown in Table 2. The MS, MS/MS spectra, and cleave mode of the representative hexanal derivative are shown in Fig. 4.

Table 3 Comparison of the developed method with the traditional methods

Method	Separation condition	Detection	LOD (nM)	LOQ (nM)	Reference
GC–MS	Agilent 6890/5973 GC–MS system equipped with a Supelco Equity-5 column (30 m×0.32 mm i.d., 0.25 μm), GC oven was 100 °C held for 0.1 min, increased at 18 °C/min to 330 °C, injection temperature: 230 °C, a constant flow of 3.8 mL/min	EI/MS	14–25	47–84	(Dong and Moldoveanu 2004)
HPLC	Kromasil C_{18} column (5 μm, 250 mm×4.6 mm i.d.) with a binary gradient. Eluent A was 30 mM formic acid/ammonia buffer (pH 7.50) and eluent B was THF–acetonitrile (2:8, v/v); flow rate : 1.0 mL/min; column temperature: 20 °C	FLD at $\lambda_{Ex/Em}$=495/505 nm	0.43–0.69	NM[a]	(Xiong et al. 2010)
GC–MS	DB-WAX column (60 m×0.25 mm i.d., 0.25 μm); carrier gas He at 1 mL/min; chromatographic oven was held at 40 °C for 5 min, then raised to 210 °C at 2 °C min^{-1}, and finally was held at 210 °C; 1-μL extract was injected in the splitless mode, with a pulse pressure of 40 psi for 1.5 min	EI/MS	1.07–5.82	NM[a]	(Culleré et al. 2004)
HPLC	A guard column and a Beckman Ultrasphere C_{18} column (150 mm×4.6 mm;5 μm particle size); mobile phases: A: acetonitrile and B: acidified water with H_2SO_4 at pH 3.0; flow rate: 1.0 mL/min; column temperature: ambient temperature	UV detector at λ=365 nm	7–28	NM[a]	(Lin et al. 2009)
CE	Fused-silica capillary (57 cm (50 cm to detector)×50 μm i.d); electrophoresis temperature: 25.0±0.1 °C; voltage: 20 kV; buffer: 60 mM sodium borate adjusted to pH 10 and 10 μM of Triton X-100	LIF detector at $\lambda_{Ex/Em}$=488/520 nm	102–155	341–516	(Bianchi et al. 2007)
UHPLC	Ultimate 3000 UHPLC; Acquity UPLC HSS T3 column (2.1×150 mm, 1.8 μm); mobile phase C: mixture of 10.0 mM ammonium acetate solution, D: ACN/H_2O (85/15, *v*/*v*) solution and E: ACN/CH_3OH/$(CH_3)_2CHOH$ (49/49/2, v/v/v); flow rate: 0.250 mL/min; column temperature: 40 °C	NM[a]	0.26–11.8	0.77–35	(Gosetti et al. 2011)
CZE	Fused-silica capillaries (Beckman): 50 mm I.D. and 50 cm effective length (total length of 60.0 cm); BGE: 60 mM sodium borate adjusted to pH 10 and 10 mM of Triton X-100; electrophoresis temperature: 25.0±0.1 °C; voltage: 20 kV	LIF detector at λ=520 nm	2.08–6.03	14–34	(Baños and Silva 2010)
HPLC	Hypersil BDS-C_8 column (200 mm×4.6 mm, 5 μM); mobile phases: A: 5 % of aqueous acetonitrile and B:100 % acetonitrile; flow rate: 1 mL/min; column temperature: 30 °C	FLD at $\lambda_{Ex/Em}$=280/510 nm	0.21–0.58	0.65–1.95	The present work

[a] *NM* not mentioned

As a result, the collision-induced dissociation spectra of molecular ions produced intense and stable fragment ions at *m/z* 246.2, 286.3, and 301.7 (Fig. 4b), which were the specific fragment ions for BAAH-labeled derivatives. In most cases, the collision-induced dissociation spectra of *m/z* $[M+H]^+$ for the aldehyde derivatives produced a specific fragment ions by losing H_2O molecules, giving the ion at *m/z* $[MH\text{-}H_2O]^+$, which was a specific fragment ion for the identification of aldehyde derivatives. There was no detectable signal from the blank deionized water sample using this transition.

Method Validation

Validation of the analytical method was evaluated by linearity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, accuracy and precision according to United States Food and Drug Administration (FDA) guidelines (FDA 2001). For linearity validation, aldehyde standards at eight concentration levels (for C1–C10, 0.156–20 nmol/mL; for furaldehyde, 0.142–18.176 nmol/mL) were prepared and analyzed under the optimized derivatization procedure and separation conditions. Multi-point calibration curves were constructed by linear regression analysis of the peak area of each analyte, versus injected amount. All aldehydes were found to give excellent linearity with correlation coefficients of >0.9994. The LOD and LOQ for each derivatized aldehyde with fluorescence detection were achieved at the signal-to-noise (*S/N*) ratio of 3 and 10, respectively. The LOD and LOQ were in the range of 0.21–0.58 nM and 0.65–1.95 nM, respectively (Table 2).

The repeatability of the developed method was investigated by measuring the relative standard deviations (RSD) for peak area and retention time. A 10-μL standard sample was injected into the chromatograph by means of an automatic sampler (n=6). The RSD of the retention times and peak areas varied from 0.01 to 0.05 % and from 1.16 to 1.52 %, respectively (Table 2). The precision test was carried out by the inter- and intra-day variability and expressed as relative standard deviations (RSD). Standard solutions at three different concentrations (2, 5 and 10 μmol/mL) were added into wine samples, respectively. Then, the spiked samples were derivatized and analyzed under the optimal conditions by the proposed method. As shown in Table 2, intra- and inter-day precisions were found to be in the range of 1.75–3.56 % and 2.06–3.80 %, respectively. The small values of RSD confirmed the high precision of the developed method.

To further evaluate the accuracy of the method, recovery experiments were performed by a standard addition method. Two representative samples including potato crisps and white wine were chosen to the recovery experiments. In five identical real samples, a known amount of 11 standard aldehydes was added. The concentration range was from 0.05 to 10.0 μmol/L. The samples or the extracting solutions were derivatized and analyzed under the optimal conditions. The percentage of recovery was obtained by comparing the results from the original samples. The average recovery of the aldehydes was within the range of 95.8–102.7 % with their RSDs of 1.3–2.2 % (Table 2), which indicated that the proposed method was sufficiently accurate for the simultaneous determination of the 11 aldehydes.

Comparison with the Traditional Methods

To comment on the attributes of the proposed method, the separation condition, LOD, LOQ,sssss and other details were compared with several reported methods including GC–MS, capillary zone electrophoresis and HPLC with UV or MS detection (Table 3). As a result, our method showed a lot of

Table 4 Aldehydes content in food samples

Food samples	Aldehydes (μg/mL[b] or μg/g[c], n=3)										
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	FA
White wine	3.40	4.26	0.99	0.21	ND[a]	ND[a]	4.85	ND[a]	ND[a]	6.76	0.52
Red wine	2.32	5.02	0.38	0.05	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	0.03
Rice wine	5.44	7.04	0.06	ND[a]	ND[a]	0.20	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Yoghurt	ND[a]	ND[a]	0.63	0.05	0.33	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	0.03
Potato crisps	ND[a]	ND[a]	1.85	0.16	ND[a]	ND[a]	ND[a]	2.76	ND[a]	ND[a]	2.01
Fried dough sticks	ND[a]	ND[a]	0.28	0.10	0.93	0.11	ND[a]	ND[a]	1.08	ND[a]	ND[a]
Dried shrimps	4.68	ND[a]	12.5	0.18	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Brined fish	ND[a]	ND[a]	1.21	0.14	1.32	2.97	0.26	0.28	0.52	ND[a]	ND[a]
Honey	1.82	ND[a]	8.06	ND[a]	5.37	1.23	ND[a]	ND[a]	ND[a]	ND[a]	0.89

[a] Not detected

[b] Micrograms per milliliter for liquid samples

[c] Micrograms per gram for solid or viscous liquid samples

advantages. For example, the LOD of the methods for aldehyde analysis mentioned in Table 3 remained at the micromolar level, while the developed method in this study offered the exciting LOD and LOQ of 0.21–0.58 nM and 0.65–1.95 nM, respectively. For separation time, the completed separation of 11 aldehydes can be achieved in as little as 18 min. HPLC separation conditions of our method including mobile phase and elution program were more facile. The fluorescence detection for derivatives at specific excitation and emission wavelengths significantly improved the analytical selectivity. In addition, FLD can be easily available in common analytical laboratories compared to LIF detector with an air-cooled argon-ion laser (Baños and Silva 2011).

Application to Foodstuffs

In order to evaluate the feasibility of the proposed method, it was applied for the determination of aldehydes in the different food matrices as presented in "Pretreatment of Samples" section. The representative chromatograms for the analysis of aldehydes from white wine and brined fish with fluorescence detection are shown in Fig. 3b–c. The peaks were doubly identified by chromatographic retention time and online MS identification. As expected, 11 aldehydes in food samples could be simultaneously separated with a good baseline resolution. The contents of aldehydes in all food samples are summarized in Table 4. As can be seen from Table 4, the content of the 11 relevant aldehydes in food samples exhibits significant difference. Propanal presented in all tested samples. It is probably due to the fact that propanal is usually used for food aroma (Bianchi et al. 2007; Verzera et al. 2011). However, the content of propanal in dried shrimps was significantly high. C1–C3 aldehydes were detected in all wines—white wine, red wine, and rice wine. Besides, the wines except rice wine were confirmed to contain furaldehyde, which was not mentioned in previously reported literatures (Culleré et al. 2011; Toshimasa and Yi-Ming 1995). Fried food such as potato crisps and fried dough sticks showed butanal content as well as propanal. On the other hand, furaldehyde in potato crisps was obviously higher. Fermented food such as yoghurt was detected to contain C3–C5 aldehydes and furaldehyde. In addition, the result indicated that honey contained furaldehyde, which was in good agreement with previous report (Spano et al. 2009).

Conclusions

In the present study, a novel method for aldehyde determination using BAAH as labeling reagent has been successfully developed by HPLC with FLD coupled with online MS identification. This method was proven to be inexpensive, simple, selective, sensitive, accurate, and reliable for trace aldehyde determination. Furthermore, this developed method exhibits powerful potential for the analysis of aldehydes in other liquid or solid foodstuffs.

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Conflict of Interest Wenli Wang declares that she has no conflict of interest. Guoliang Li declares that he has no conflict of interest. Zhongyin Ji declares that he has no conflict of interest. Na Hu declares that she has no conflict of interest. Jinmao You declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

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