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Identification and determination of carboxylic acids in food samples using 2-(2-(anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS) as labeling reagent by HPLC with FLD and APCI/MS

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ABSTRACT

A new labeling reagent for carboxylic acids, 2-(2-(anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS) has been designed and synthesized. It was used to label eight fatty acids (lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, oleic acid, linoleic acid and linolenic acid) and four hydroxy pentacyclic triterpene acids (oleanolic acid, ursolic acid, betulinic acid and maslinic acid), successfully. APIETS could easily and quickly label carboxylic acids in the presence of K_2CO_3 catalyst at 85 °C for 35 min in *N*,*N*-dimethylformamide solvent. The carboxylic acids derivatives were separated on a C_8 reversed-phase column with gradient elution and fluorescence detection at $\lambda_{ex}/\lambda_{em}$ = 315/435 nm. Identification of these derivatives was carried out by online mass spectrometry with atmospheric pressure chemical ionization in positive ion mode. The detection limits obtained were 13.37–30.26 fmol (signal-to-noise ratio of 3). The proposed method has been applied to the quantification of carboxylic acids in sultana raisin (Thompson seedless), hawthorn flake (Crataegus pinnatifida Bge.), *Lycium barbarum* seed oil and *Microula sikkimensis* seed oil with recoveries over 95.3%. It has been demonstrated that APIETS is a prominent labeling reagent for determining carboxylic acids with high performance liquid chromatography.

1. Introduction

Carboxylic acids are widely distributed in nature and important as nutritional substances. Various carboxylic acids including fatty acids, bile acids, ascorbic acid, prostaglandins, etc., play important roles in living bodies. Among them, fatty acids (FAs) are of the particular interest, since they are the essential nutrimental substances existing in food and the metabolic products of life activity existing in blood. FAs are obtained mainly from dietary sources and so their role as nutrients is paramount. Therefore, the determination of FAs profiles is critical in the lipid analysis of food samples [1]. In addition, hydroxy pentacyclic triterpene acids (TAs) are another group of compounds having carboxyl, which are often reported in many food samples, such as grapes [2,3], hawthorn [4], olive oil [5], and so on. In recent years, TAs held attraction in the scientific field because of their anti-inflammatory [6], antimicrobial [7], antioxidant [8] and anticarcinogenic activities [9], which makes them effective in healthcare food products as functional compounds [10]. Thus, the quantitative determination of TAs is also very important in the scope of food analysis.

Gas chromatography (GC) coupled with various kinds of detectors provides a powerful technology to analyze multi-components with high sensitivity and selectivity. It is difficult to analyze carboxylic acids directly by GC, because of high polarity, low volatility, and those that form hydrogen bonds. To overcome these difficulties, volatile derivatives can be prepared. The most frequently used derivatives are alkyl derivatives and silylation [11]. GC methods have been widely used for carboxylic acid analyses, however the use of elevated temperatures in GC is a disadvantage for thermally labile compounds such as mono- and polyunsaturated FAs, and hydroxy FAs [11,12]. A major advantage of high performance liquid chromatography (HPLC) over GC is the lower temperature used during analysis, which reduces the risk of damage of heat-liable compounds. Applications for HPLC have expanded dramatically into almost every area of chemical and biochemical research as

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well as food analysis [13]. A difficulty in the HPLC analysis of carboxylic acids is related to the absence of efficient detection properties in these molecules to facilitate the detection by ultraviolet absorption (UV) or direct fluorescence (FL). Furthermore, absorbance near 205 nm cannot be recommended, because the solvents added to the mobile phase absorb at the detection wavelength. Hence, sophisticated methods possessing suitable detection properties are required for sensitive and selective quantification for carboxylic acids. An increasingly popular way to enhance the sensitivity and selectivity of detection for carboxylic acids is chemical derivatization of the carboxyl moiety with a suitable chromophore or fluorophore. And a number of ultraviolet and fluorescent labeling reagents for carboxyl group have been developed in the last decades [13–15]. Many researchers have described the application of those labeling reagents in HPLC analysis of FAs from a variety of sources; however, as far as our knowledge, few people have employed the derivatization procedure in HPLC analysis of TAs.

Typical labeling reagents for carboxyl include 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone (BrDMEQ) [16], 9-anthryldiazomethane (ADAM) [17], 6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone-3-propionylcarboxylic acid hydrazide (DMEQ-Hz) [18], 4-aminomethyl-6,7-dimethoxycoumarin (ADC) [19], 6-oxy-(acetyl piperazine) fluorescein (APF) [20], 4-[2-(*N*,*N*-dimethylamino) ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DAABD-AE) [21], 1-[2-(*p*-toluenesulfonate)-ethyl]-2-phenylimidazole-[4,5-f]-9,10-phenanthrene (TSPP) [22], and so on. However, it has also been reported that these reagents have various shortcomings in their application, such as poor stability, tedious operation, and short detection wavelengths [13–15]. In this work, we report the synthesis of a novel fluorescence reagent 2-(2-(anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS). With APIETS as labeling reagent, a HPLC method for the simultaneous determination of eight FAs and four TAs has been developed and validated. At the same time, the proposed method has also been applied for the determination of FAs in edible oils and TAs in dry fruit samples. The results demonstrate that the method is suitable for quantitative analysis of FAs and TAs from food samples.

2. Experimental

2.1. Instruments

Experiments were performed using Agilent 1100 Series high-performance liquid chromatography (Agilent, USA). The HPLC system consisted of an online vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostated column compartment (model G1316A), and a fluorescence detector (FLD) (model G1321A). The mass spectrometer (MSD Trap SL, model G2445D) from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source (model G1947A). The HPLC–MSD system was controlled by Agilent Chemstation software (version B.01.01). Derivatives were separated on Hypersil BDS C_8 column (200 mm × 4.6 mm, 5 μm i.d., Dalian Elite Co., China).

The semi-preparative HPLC system was Waters Delta 600 (Waters, Japan) and consisted of an online degasser, a Waters 600 controller with Waters 2489 UV/visible detector and an auto-fraction collector. Reverse-phase semi-preparative HPLC-separation was performed on a SunFire™ Prep-C_{18} column (10 mm × 150 mm, 10 μm, Made in Ireland) with Zorbax PrepHT guard cartridge columns. Fluorescence excitation and emission spectra were obtained on a F7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

2.2. Chemicals

2-(2-(Anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS) was synthesized in authors' laboratory as described in Section 2.3 and Fig. 1. Fatty acids standards including dodecoic acid (C12), tetradecoic acid (C14), hexadecanoic acid (C16), octadecanoic acid (C18), eicosanoic acid (C20), 12-octadecenoic acid (C18:1), 9,12-octadecadienoic acid (C18:2), 9,12,15-octadecatrienoic acid (C18:3) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Triterpene acids standards including oleanolic acid (Ole), ursolic acid (Urs), betulinic acid (Bet) and maslinic acid (Mas) were purchased from Sigma Co. (St. Louis, MO, USA). HPLC grade acetonitrile (CH_3CN) was purchased from Yucheng Chemical Reagent Co. (Shandong Province, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents like methanol (MeOH), tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF), were of analytical grade unless otherwise stated.

2.3. Synthesis of 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS)

2.3.1. Synthesis of 2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazole (API)

2-(Anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazole (API) was synthesized as follow: 9,10-phenanthraquinone (8 g), anthracene-9-carbaldehyde (10 g), ammonium acetate (60 g), and glacial acetic acid (150 mL) were fully mixed in a 250-mL round-bottom flask. The contents of the flask were rapidly heated to 90 °C with stirring for 3 h. After cooling, pH of solution was adjusted to 7–8 with ammonium hydroxide. The precipitated solid was recovered by filtration, washed with water and dried at room temperature for 48 h. The crude product was recrystallized twice from MeOH/DMF mixed solvent (5:1, *v*/*v*) to afford yellow crystals (17.3 g), yield 90.0%.

2.3.2. Synthesis of 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazol-1-yl)ethanol (APIE)

API (16 g), ethylene carbonate (4.5 g), and KOH (0.2 g) were dissolved together in 150 mL DMF in a 250-mL round-bottom flask and rapidly heated to reflux for 6 h with vigorous stirring. After cooling, the contents were poured into 300 mL water with vigorous stirring. The precipitated solid was recovered by filtration, washed successively with water, aqueous ethanol solution (ethanol/water 3:2, *v*/*v*). The crude product was dried at room temperature and recrystallized twice from CH_3CN/DMF mixed solvent (5:1, *v*/*v*) to afford yellow crystals (14.4 g); yield 82.8%.

2.3.3. Preparation of 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS)

To a solution of APIE ethanol (2.0 g) in 50 mL pyridine (0 °C) in a 100-mL round-bottom flask, p-toluenesulfonyl chloride (2.5 g) was added in batches with vigorous stirring. After stirring at 0 °C for 6 h, the contents were kept at ambient temperature for another 4 h with stirring. The contents were transferred into ice water with vigorous stirring for 0.5 h; the precipitated solid was filtrated, washed with water and dried at ambient temperature for 48 h. The crude product was re-crystallized twice from CH_3CN to give slightly yellow crystals (2.1 g); yield 78.5%. Found, C 77.02, H 4.76, N 4.74, O 8.08, S 5.40; Calculated, C 77.00, H 4.76, N 4.73, O 8.10, S 5.41; APCI-MS (positive mode), *m*/*z*: 593.5; MS/MS: *m*/*z*: 439.5, 421.4, 395.3.

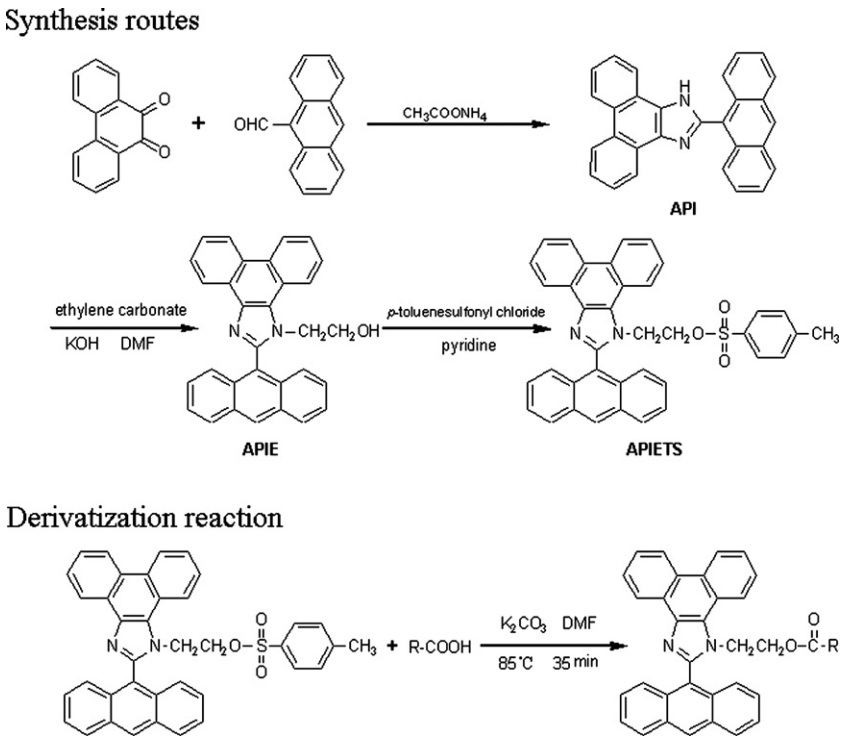

Fig. 1. Synthesis routes of APIETS and derivatization scheme of APIETS with carboxylic acids.

2.4. Preparation of solutions

The standard carboxylic acids for HPLC analysis at individual concentration of 1.0×10^{-4} mol/L were prepared by dilution of the corresponding stock solution (1.0×10^{-2} mol/L) with the mixed solvent of CH_3CN/DMF (9:1, *v*/*v*). APIETS solution (5.0×10^{-2} mol/L) was prepared by dissolving 296.8 mg APIETS in 10 mL DMF. The corresponding low concentration of derivatization reagent solution (5.0×10^{-3} mol/L) was obtained by diluting the stock solution with DMF. When not in use, all solutions were stored at 4 °C in a refrigerator until HPLC analysis.

2.5. Sample preparation

2.5.1. Extraction of TAs from raisin and hawthorn flake

Sultana raisins (Thompson seedless) and hawthorn flakes (Crataegus pinnatifida Bge.) samples were purchased from drysaltery stores. A sample of the pulverized samples and methanol (5.0 mL) were placed in a 10-mL round-bottomed flask. The flask was immersed in an ultrasonic water-bath and sonicated for 20 min. The samples were each extracted three times and the extracts were combined. The final solution was evaporated to dryness by a stream of nitrogen gas at ambient temperature. The residue was re-dissolved in 1.0 mL DMF and stored at 4 °C until derivatization and HPLC analysis.

2.5.2. Treatment of the oil sample

Lycium barbarum seed oil and *Microula sikkimensis* seed oil were provided by Prof. Yourui Suo (Northwest Plateau Institute of Biology, Chinese Academy of Science). To a 10-mL test tube, 0.1 g seed oil and 2.0 mL potassium hydroxide/methanol solution (2 mol/L) were added. After being sealed, the test tube was immersed in a water bath at 90 °C for 2 h. After cooling to ambient temperature, the contents were transferred into a centrifugal test tube, to which 2 mL water was added, and pH was adjusted to 7.0 with 6 mol/L hydrochloric acid solution. This solution was extracted with chloroform three times (3 mL × 3). The combined chloroform was filtered and evaporated under a stream of nitrogen. The residue was re-dissolved in 50 mL DMF, filtered through a 0.2 mm nylon membrane filter, and stored at 4 °C until derivatization.

2.6. Derivatization procedure

To a solution containing 50 μL of a standard FAs mixture in a 2-mL vial, 140 μL derivatization reagent solution, 10 mg K_2CO_3 and 210 μL DMF were added. The vial was sealed and allowed to react in a water bath at 85 °C for 35 min with shaking in 5-min intervals. The derivatization procedure is shown in Fig. 1. After the reaction was completed, the mixture was taken to cool at room temperature. A 600 μL volume of CH_3CN/DMF solution (1:1, *v*/*v*) was added to dilute the derivatization solution. The diluted solution was injected directly into the chromatography (10 μL).

2.7. HPLC–FLD and APCI/MS conditions

HPLC separation of carboxylic acid derivatives was carried out on a Hypersil BDS C_8 column by gradient elution. Eluent A was 30% aqueous acetonitrile solution (H_2O/CH_3CN, 70/30, *v*/*v*) containing 30 mmol/L formic acid buffer (pH 5.5); Eluent B was acetonitrile. Mobile phases were filtered through a 0.2 μm nylon membrane filter (Alltech, Deerfiled, IL, USA). The linear gradient conditions: initial = 70% A and 30% B, 40 min = 100% B, then maintained 100% B for 10 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 at λ_{ex} 315 nm and λ_{em} 435 nm, respectively.

Chromatographic peaks were identified by spiking the working standard with each individual carboxylic acid in turn, and simultaneously confirmed by mass spectrometry. Ion source conditions: APCI in positive ion detection mode; nebulizer pressure 60 psi; dry gas temperature, 350 °C; dry gas flow, 5.0 L/min. APCI Vap tem-

Table 1
Experimental data for the average peak area of APIETS-FA and APIETS-TA obtained from Box–Behnken design ($n = 3$).

Run number	Independent variables						$\mathbf{R}_1$ (average peak area of APIETS-FA derivatives)	$\mathbf{R}_2$ (average peak area of APIETS-TA derivatives)
	Coded levels			Actual levels				
	C	**T**	**t**	**C** (molar ratio)	**T** (°C)	**t** (min)		
1	1	−1	0	10.00	70	30	1789.3	1929.5
2	−1	0	1	5.00	85	45	2335.6	2476.3
3	0	0	0	7.50	85	30	3169.2	3387.8
4	−1	0	−1	5.00	85	15	2014.3	2134.3
5	0	−1	−1	7.50	70	15	1430.8	1533.1
6	0	0	0	7.50	85	30	3203.4	3369.4
7	0	1	1	7.50	100	45	1844.8	1973.8
8	0	0	0	7.50	85	30	3173.4	3354.2
9	0	1	−1	7.50	100	15	1236.5	1326.2
10	1	0	−1	10.00	85	15	2639.4	2799.6
11	1	0	1	10.00	85	45	3176.1	3380.4
12	−1	1	0	5.00	100	30	1178.3	1262.2
13	1	1	0	10.00	100	30	1950.3	2048.8
14	0	−1	1	7.50	70	45	1648.1	1744.5
15	0	0	0	7.50	85	30	3194.6	3403.7
16	0	0	0	7.50	85	30	3193.4	3389.3
17	−1	−1	0	5.00	70	30	1184.7	1299.1

perature 350 °C; corona current 4000 nA (pos); capillary voltage 3500 V.

2.8. Measurement of fluorescence properties

Semi-preparative HPLC separation was used to obtain the single APIETS-C_{16} derivative which was used to test the fluorescence properties. The derivatized C16 solution (1000 μL, 1.0×10^{-3} mol/L) was injected into the semi-preparative HPLC system. An isocratic elution with acetonitrile at 2 mL/min was carried out, and the APIETS-C16 derivative fraction was eluted within the chromatographic window of 9–12 min. The collected APIETS-C_{16} fraction was made up to total volume of 25 mL with acetonitrile, and the corresponding APIETS-C_{16} concentration was 4.0×10^{-5} mol/L. This solution was diluted to 1.0×10^{-7} mol/L with various solvents, and the obtained solutions were used to evaluate the fluorescence properties. The fluorescence excitation and emission were recorded on F-7000 fluorescence spectrophotometer and the slits were both set at 5 nm.

2.9. Experimental design for optimization of derivatization and statistical analysis

APIETS plays the same esterification with carboxylic acids as do of TSPP [28], because they have the same active functional group of *p*-toluenesulfonate. Based on previous studies on conditions of this esterification reaction [28], DMF was chosen as co-solvent for the derivatization procedure, and K_2CO_3 (10 mg) was used as basic catalyst. Three factors including the amount of APIETS (**C**: molar ratio of APIETS to total carboxylic acids, expressed as [APIETS]/[carboxylic acids]$^{\mathbf{T}}$), reaction temperature (**T**) and reaction time (**t**), were optimized by employing a three-level, three-variable Box–Behnken design (BBD) from response surface methodology (RSM). The coded and uncoded independent variables used in the RSM design and their respective levels were listed in Table 1. The average peak area of carboxylic acids derivatives was taken as the responses (see Table 1; specifically, $\mathbf{R}_1$ denoted the average response of the mentioned eight FAs, and $\mathbf{R}_2$ symbolized the average response of the four TAs). The surface response models were obtained by fitting the data to a polynomial model. Eq. (1) shows the most general function for BBD.

$$\mathbf{R} = \beta_0 + \beta_1\mathbf{C} + \beta_2\mathbf{T} + \beta_3\mathbf{t} + \beta_{12}\mathbf{CT} + \beta_{13}\mathbf{Ct} + \beta_{23}\mathbf{Tt} + \beta_{11}\mathbf{C}^2 + \beta_{22}\mathbf{T}^2 + \beta_{33}\mathbf{t}^2 \quad (1)$$

A software Design-Expert 7.1.3 (State-Ease, Inc., Minneapolis, MN, USA) was used to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the determined coefficient (R^2), and its statistical significance was checked by an *F*-test.

3. Results and discussion

3.1. Optimization of derivatization conditions

The derivatization parameters were traditionally optimized by single factor design experiments, which was troublesome and time-consuming. Contrastively, RSM is more efficient, requires fewer data and provides interaction effects on the response. In this work, a three-level, three-variable BBD was employed to optimize the derivatization conditions, and obtained data are shown in Table 1. The experimental data were performed polynomial fitting, and the model coefficients for each response are presented in Table 2.

The analysis of variance for the experimental results of BBD (Table 2) indicated that the proposed mathematical models were significant ($p < 0.001$); the derivatization temperature showed insignificant effects on the responses, and other linear parameters, the interaction parameters and quadratic parameters were significant ($p < 0.05$ or $p < 0.01$). The value of R^2 (>0.99) revealed that the experimental data were in good agreement with the predicted values of peak area. F-value for the lack of fit was insignificant ($p > 0.05$), meaning that these models were sufficiently accurate for predicting the relevant responses. Coefficient of variation (C.V.%) of less than 1.17% indicates that the model was reproducible.

The 3D response surface plots (Fig. 2) provide a method to visualize the relationship between responses and each variable, the interactions between two test variables, and the optimum derivatization conditions. The optimal conditions obtained by calculating the regression equations were as follows: for FAs, [APIETS]/[carboxylic acids]$^{\mathbf{T}}$ = 8.93, 85.46 °C reaction temperature and 35.81 min reaction time; for TAs, [APIETS]/[carboxylic

Table 2
Estimates of the model coefficients for the responses.

Coefficients	Response surface quadratic model of APIETS-FAs		Response surface quadratic model of APIETS-TAs	
	Estimates	*p*-value	Estimates	*p*-value
β_0	−43247.1		−45408.7	
β_1	796.0	<0.0001	884.3	<0.0001
β_2	985.7	0.0738*	1036.0	0.2218*
β_3	50.5	<0.0001	51.5	<0.0001
β_{12}	1.1	0.0158	1.04	0.0255
β_{13}	1.4	0.0047	1.59	0.0035
β_{23}	0.4	0.0001	0.48	<0.0001
β_1^2	−52.8	<0.0001	−55.4	<0.0001
β_2^2	−5.9	<0.0001	−6.22	<0.0001
β_3^2	−1.4	<0.0001	−1.49	<0.0001
Lack of fit	$p = 0.0557$*		$p = 0.1302$*	
p-value of model	<0.0001		<0.0001	
R^2	0.9936		0.9944	

* Non-significant ($p > 0.05$).

acids]^T = 8.89, 85.54 °C reaction temperature and 35.69 min reaction time. As a comprise, the overall optimum conditions for the derivatization of carboxylic acids were defined to be a 9-fold molar excess of APIETS and heating at 85 °C for 35 min.

3.2. Fluorescence properties of APIETS carboxylic acids derivatives

Since the separation of the reagent and its derivatives maybe performed in the mobile phase containing H_2O, MeOH, CH_3CN, THF or DMF in HPLC, the fluorescence spectra of carboxylic acid derivatives in these five solvents have been measured. In this paper, APIETS-C16 derivative was chosen as the model to investigate, and the obtained spectra are shown in Fig. 3(a). It is found that APIETS-C16 derivative exhibits its maximal emission at about 410 nm in MeOH and THF, and at about 430 nm in H_2O, DMF and CH_3CN. As also can be seen from Fig. 3(a), the fluorescence of APIETS-C16 derivative is strongest in CH_3CN, which indicated that CH_3CN/H_2O was preferable as the mobile phase composition instead of others.

In order to evaluate the effect of media pH on fluorescence properties of the derivatives, the fluorescence spectra of APIETS-C16 in aqueous acetonitrile solutions at different pH values (Britton–Robinson buffers/CH_3CN, 50/50, *v*/*v*) were also determined, and the obtained spectra are shown in Fig. 3(b). It is found that the maximum $\lambda_{ex}/\lambda_{em}$ of APIETS-C16 are at 315/435 nm, exhibiting no obvious red-shift or blue-shift with varying media pH. The fluorescence intensity of APIETS-C16 presents a persistent decrease with the increasing media acidity. These results are probably due to the fact that the lone pairs of nitrogen atom in the core structure of API undergoes protonization in acidic solutions, and the protonization leads to the quench of fluorescence. Considering the detailed trends in the suitable pH range for reversed-phase HPLC (pH 8–3), the fluorescence intensity of APIETS-C16 initially

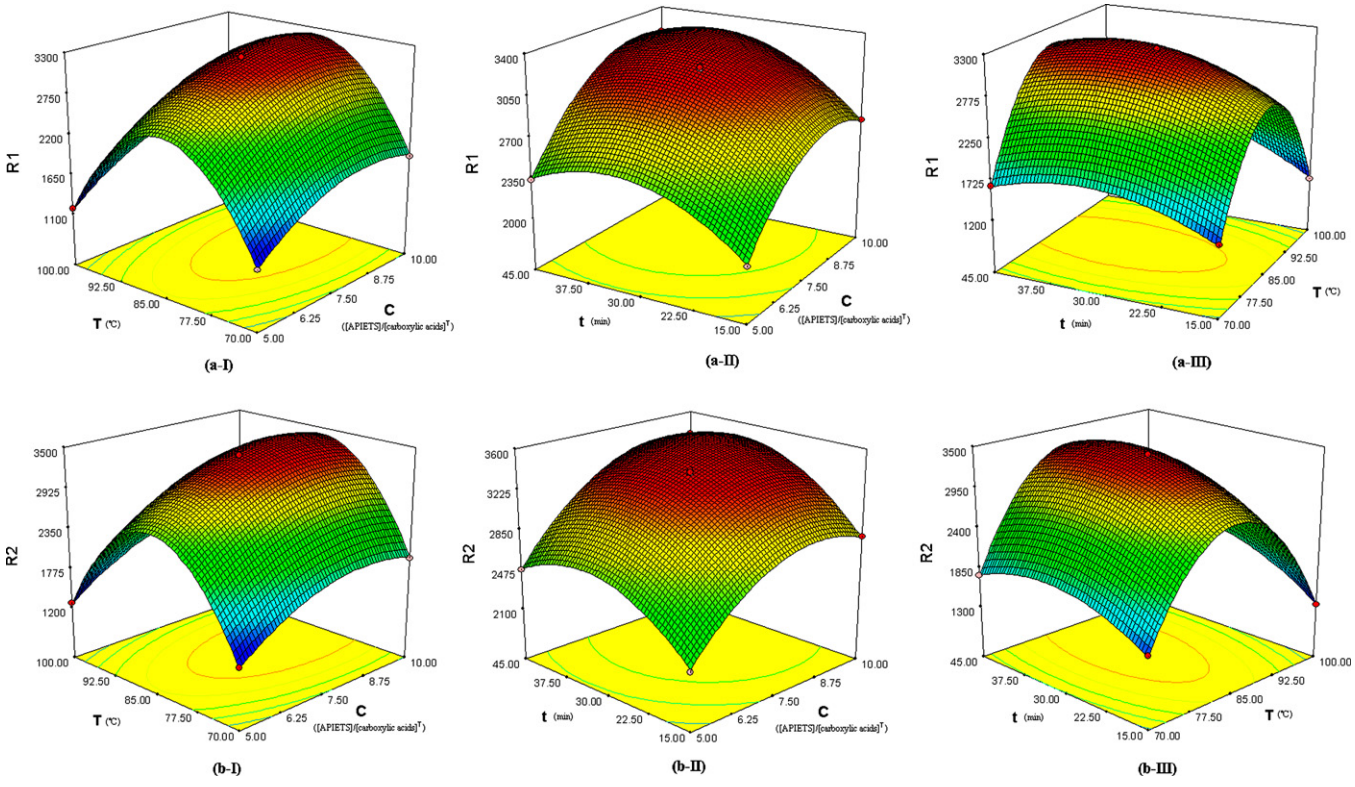

Fig. 2. The 3D response surface plots of APIETS-FA (a) and APIETS-TA (b) affected by derivatization temperature, reaction time and APIETS amounts. The effect of derivatization temperature and time on the peak area (I); the effect of derivatization time and APIETS concentration on the peak area (II) and the effect of derivatization pH and APIETS concentration on the peak area (III).

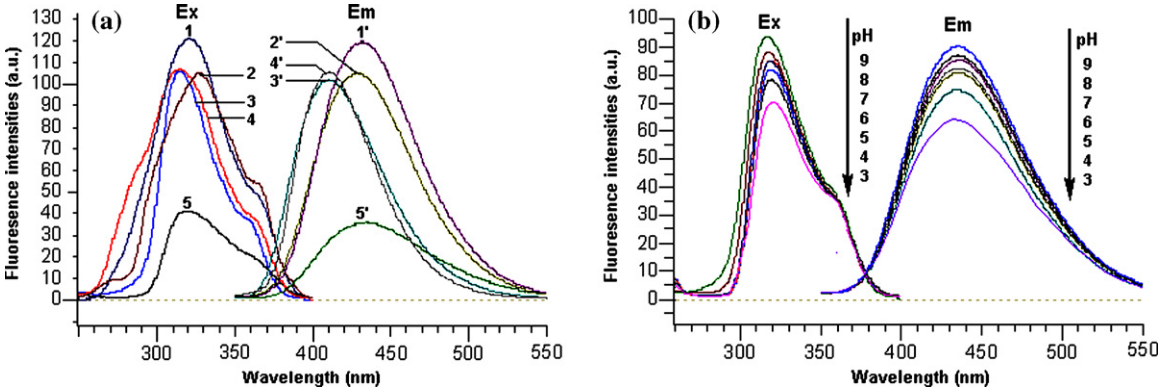

Fig. 3. Fluorescence spectra for APIETS-C16 in various solvents (a) and aqueous acetonitrile at different pH values (b). (1 and 1′) Excitation and emission spectra of APIETS-C16 in CH_3CN, respectively; (2 and 2′) excitation and emission spectra of APIETS-C16 in DMF, respectively; (3 and 3′) excitation and emission spectra of APIETS-C16 in THF, respectively. (4 and 4′) Excitation and emission spectra of APIETS-C16 in MeOH, respectively; (5 and 5′) excitation and emission spectra of APIETS-C16 in H_2O, respectively. Aqueous acetonitrile solutions at different pH values were prepared by equivalently mixing Britton–Robinson buffers with acetonitrile. Britton–Robinson buffer was prepared by mixing 0.04 mol/L mixed acids (H_3PO_4, H_3BO_4 and acetic acid) and 0.2 mol/L NaOH solutions and adjusting to the required pH value.

exhibits a slight decrease (about 5.8%, with media pH decreasing from 8 to 5), and then a sharp fall (about 25.7%, with media pH decreasing from 5 to 3). Accordingly, the preferable mobile phase used for HPLC should be adjusted to the pH range of 8–5.

3.3. Chromatographic separation

As described in Section 3.2, to obtain an intense fluorescence response, the mobile phases for HPLC should be controlled in the pH range of 8–5. In our experiments, the mobile phases containing 30 mmol/L formic ammonium buffer (pH 5.5) were used. The slightly acidic mobile phases were not only suitable for the fluorescence detection but also favorable for the online post-column APCI/MS identification in positive mode. The separation of 12 carboxylic acid derivatives could be achieved on different columns such as ODS, BDS-C_8, XDB-C_8, and so on; however, the separation on the BDS-C_8 column gave the best results. Therefore, a Hypersil BDS C_8 column was selected in conjunction with gradient elution. The gradient elution was carried out as described in Section 2.7, and the elution gave a goodish separation with the shortest retention values and the sharpest peaks. The chromatogram of all carboxylic acid derivatives is shown in Fig. 4. The chromatogram indicates that 12 carboxylic acid derivatives are separated with satisfactory resolution, and the excess reagent and by-products do not produce any interference.

3.4. Mass spectrometry identification

Chromatographic peaks were identified by spiking the working standard with each individual carboxylic acid derivative in turn, and simultaneously confirmed by online post-column APCI/MS in positive mode. Data from the MS and MS/MS spectra for all of the derivatized carboxylic acids are shown in Table 3. The MS data indicate that both APIETS-FA and APIETS-TA derivatives exhibit intense quasi-molecular ion peak of $[M+H]^+$. For the MS/MS of APIETS-FA derivatives, the main fragment ions are $[MH-API]^+$ and $[API+H]^+$ which are formed by cleavage of N–CH_2 bond; and fragment ions resulted from cleavages of carbon chains are also found in the MS/MS of unsaturated FAs derivatives. For the MS/MS of APIETS-TA derivatives, the main fragment ions were $[MH-API]^+$ and $[MH-API-62]^+$; and there was also a small quantity of fragment ion at m/z 669, which was resulted from cleavages of carbon ring in TAs moiety. The MS, MS/MS and cleavage mode of APIETS-C12 and APIETS-Ole, as representatives of APIETS-FA and APIETS-TA, are shown in Fig. 5(a) and (b), respectively.

A close comparison study indicates that MS/MS spectra of APIETS-TA are quite different from these of APIETS-FA. Firstly, $[API+H]^+$ at m/z 395 is the most abundant fragment ion in the MS/MS of APIETS-FA, while it cannot be found in the MS/MS of APIETS-TA. This differentia can be explained as follow. For the $[M+H]^+$ of APEITS-FA, the prontonization position were probably

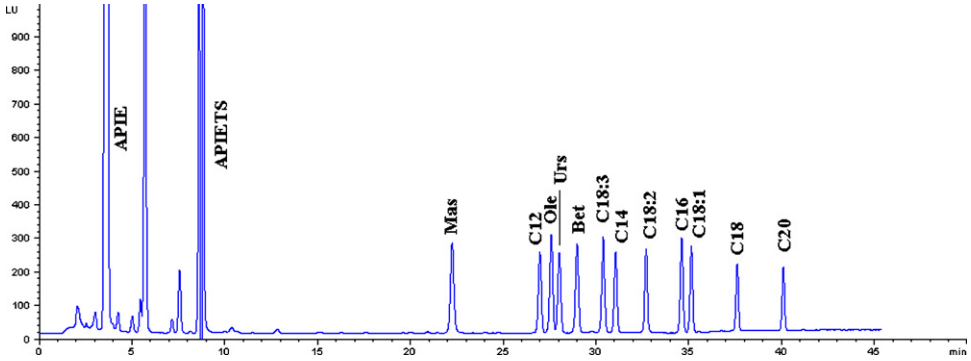

Fig. 4. Chromatogram for standard carboxylic acids derivatives. Chromatographic conditions: Hypersil BDS C_8 column (4.6 mm × 200 mm, 5 μm), column temperature 30 °C; flow rate 1.0 mL/min; excitation and emission: $\lambda_{ex}/\lambda_{em}$ = 315/435 nm. Peaks are labeled with abbreviations for all carboxylic acids: C12 (dodecoic acid); C14 (tetradecoic acid); C16 (hexadecanoic acid); C18 (octadecanoic acid); C20 (eicosanoic acid); C18:1 (12-octadecenoic acid); C18:2 (9,12-octadecadienoic acid); C18:3 (9,12,15-octadecatrienoic acid); Mas (maslinic acid); Urs (ursolic acid); Ole (oleanolic acid); Bet (betulinic acid); APIE (2-(2-(anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethanol); APIETS (2-(2-(anthracen-10-yl)-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethyl 4-methylbenzenesulfonate).

Table 3
The retention times, MS and MS/MS data for 8 APIETS-FAs and 4 APIETS-TAs.

Analytes	Retention time (min)	APCI-MS identification				
		MS	MS/MS (relative abundance %)			
		$[M+H]^+$ and $[MH-H_2O]^+$[a]	$[API+H]^+$	$[MH-API]^+$	$[MH-API-62]^+$	Fragment ions resulted from cleavages of carbon chain or ring (relative abundance < 5%)
C12	26.96	621.4	395.3 (100)	227.4 (25.2)	–	–
C14	31.04	649.4	395.4 (100)	255.5 (29.3)	–	–
C16	34.60	677.5	395.4 (100)	283.5 (35.4)	–	–
C18	37.59	705.5	395.4 (100)	311.5 (33.5)	–	–
C20	40.08	733.5	395.5 (100)	339.6 (36.8)	–	–
C18:1	35.12	703.5	395.4 (100)	309.4 (26.5)	–	496.0; 557.6
C18:2	32.68	701.5	395.4 (100)	307.4 (13.7)	–	506.4; 565.1; 657.9
C18:3	30.37	699.4	395.4 (100)	305.4 (7.2)	–	480.4; 508.1; 578.1
Mas	22.23	893.8	–	499.5 (100)	437.4 (4.7)	668.9
Ole	27.58	877.7	–	483.5 (100)	421.3 (7.5)	668.9
		859.3[a]		465.5 (100)[b]	403.4 (6.7)[b]	669.2[b]
Urs	28.01	877.8	–	483.5 (100)	421.4 (6.9)	668.7
		859.6[a]		465.4 (100)[b]	403.3 (6.6)[b]	
Bet	28.96	877.8	–	483.6 (100)	421.5 (5.3)	668.6
		859.7[a]		465.6 (100)[b]	403.7 (5.5)[b]	

[a] $[MH-H_2O]^+$ the quasi-molecular ion losing a moiety of water.
[b] The fragment ions in the MS/MS spectra of $[MH-H_2O]^+$.

at the lone pair of nitrogen in API moiety. As a result of heterolytic cleavage of N–CH_2 bond during collision, the $[M+H]^+$ would produce the API molecule which is ready to accept H^+ to form $[API+H]^+$. So an abundance of $[API+H]^+$ can be found in the MS/MS spectra of APIETS-FA (see Fig. 5(a)). For the $[M+H]^+$ of APEITS-TA, the proton maybe attach to the TA moiety, and the cleavage of N–CH_2 bond would give the anion of $[API-H]^-$. $[API-H]^-$, even if transformed to API by accepting H^+, cannot be detected by APCI/MS in positive mode. Therefore there is few fragment ions at m/z 395 in the MS/MS of APIETS-TA (see Fig. 5(b)). Secondly, APIETS-FA did not give the fragment of $[MH-API-62]^+$, and $[MH-API-62]^+$ was unique to the MS/MS of APIETS-TA. This phenomenon can be explained as follow: The deduction of 62 Da may be resulted from losing one moiety of $HOCH_2CH_2OH$. In the $[MH-API]^+$ of APIETS-TA, there are three γ-C atoms around the positive charge center, which are ready to provide γ-H atoms to the oxygen atoms, facilitating loss of $HOCH_2CH_2OH$ (see Fig. 5(b)). However, there is only one γ-C atom in the $[MH-API]^+$ of APIETS-FA, and further fragmentation could not cause neutral loss of $HOCH_2CH_2OH$ because of lack of γ-H (see Fig. 5(a)).

3.5. Method valuation

The validation parameters studied were response linearity, detection limits (LOD), precision (including instrumental precision, derivatization precision and method precision) and accuracy (derivatization accuracy and method recovery).

A series of mixtures of standard carboxylic acids at different concentrations were prepared and analyzed to determine the linearity under the optimal derivatization and separation conditions. Linearity data were generated by plotting the peak area versus the injection amounts of analyte in the range of 0.1–50 pmol. The results (regression equations and correlation coefficients) obtained are reported in Table 4. All of the derivatized carboxylic acids were found to give excellent linear responses in this range with correlation coefficient >0.9993. The calculated LODs with fluorescence detection (at a signal-to-noise ratio of 3) were from 13.37–30.26 fmol, which indicate APIETS a sensitive fluorescence labeling reagent for HPLC determining carboxylic acids.

Instrumental precision was examined by measuring peak areas and retention times of six replicative injections of 50 pmol standard

Table 4
The linearity, LODs, precision and accuracy for the established derivatization method.

Analyte	Linearity		LOD (fmol)	Derivatization precision (%)		Derivatization accuracy (%) ($n=3$)
	$Y=AX+B$[a]	r		Intra-day RSD ($n=3$)	Inter-day RSD ($n=3$)	
C12	$Y=94.83X+5.03$	0.9998	14.45	1.6	2.3	101.5
C14	$Y=97.61X+4.76$	0.9997	17.56	1.5	1.9	97.3
C16	$Y=96.45X+6.36$	0.9997	13.37	1.7	1.7	98.4
C18	$Y=94.26X+4.41$	0.9998	22.94	1.8	1.8	99.0
C20	$Y=89.64X+5.55$	0.9999	26.18	1.6	2.5	103.1
C18:1	$Y=95.21X+7.70$	0.9997	23.77	1.5	1.9	97.6
C18:2	$Y=92.32X+3.73$	0.9998	27.47	1.9	1.8	96.3
C18:3	$Y=99.42X+5.28$	0.9997	24.41	2.4	1.9	96.2
Mas	$Y=104.71X+4.17$	0.9998	30.26	1.8	2.3	98.6
Ole	$Y=103.16X+4.79$	0.9996	21.06	2.0	2.1	103.5
Urs	$Y=92.74X+3.92$	0.9995	25.75	1.9	2.6	104.1
Bet	$Y=93.32X+4.17$	0.9997	23.74	2.1	2.4	96.8

[a] X: the injected amounts (pmol); Y: the peak area detected with fluorescence detector.

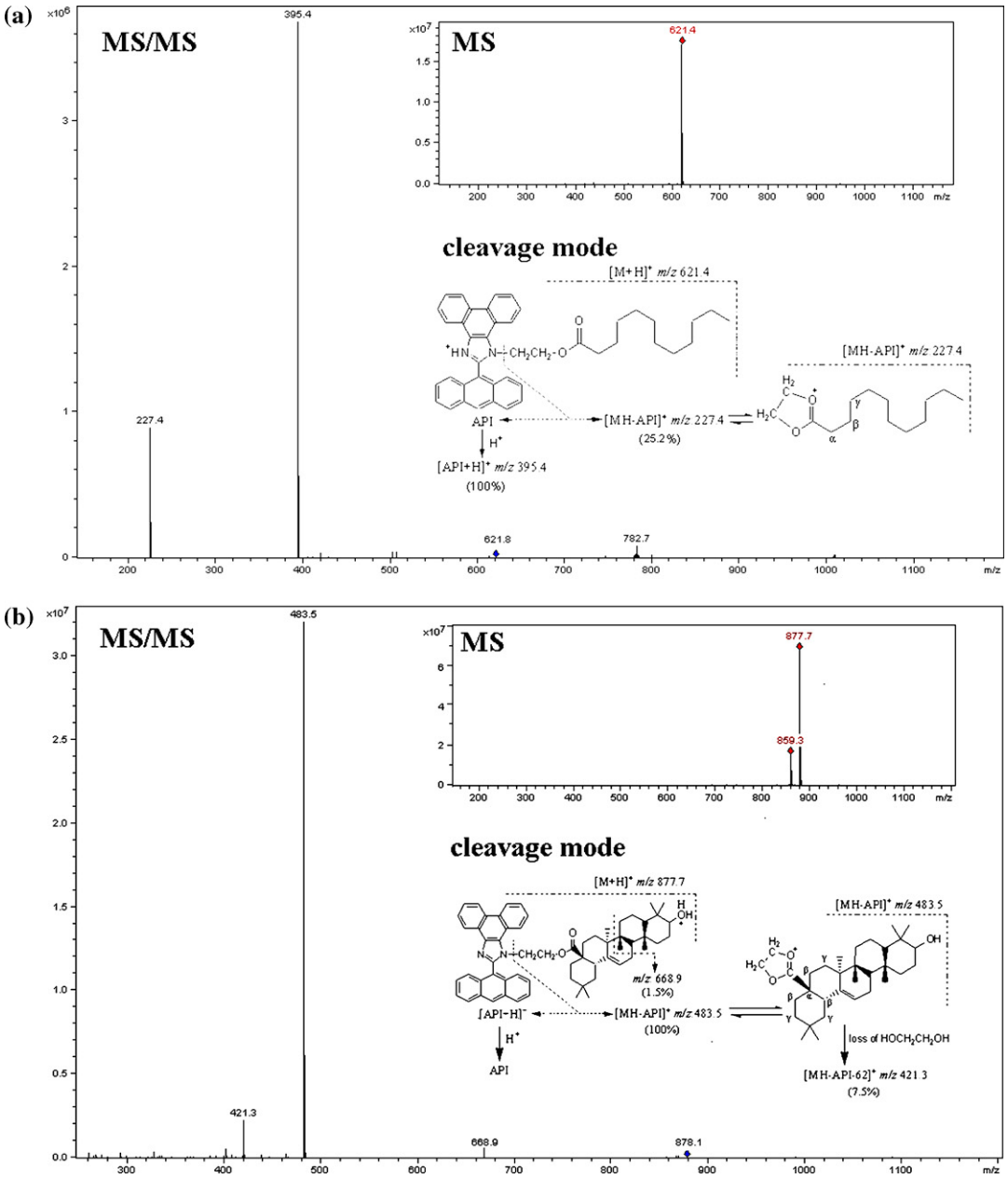

Fig. 5. The MS, MS/MS and cleavage mode for APIETS-C12 (a) and APIETS-Ole (b).

carboxylic acids derivatives. The obtained relative standard deviations (RSDs) of the peak areas and retention times were from 0.94 to 2.04% and from 0.009 to 0.048%, respectively. The precision of the derivatization procedure was checked from injections of a standard that had been derivatized five times over 3 days and three times on 1 day. And the intra-day and inter-day precisions were in the range of 1.5–2.4% and 1.7–2.6%, respectively (see Table 4). The precision of the method was finally estimated by applying the whole procedure to real samples in triplicate, and the obtained RSDs were ≤5.1%. The results indicated that the described method was precise enough for routine analysis of carboxylic acids from food samples.

The accuracy of the derivatization step was evaluated by analyzing standards in triplicate at three levels (1, 10 and 50 pmol), and comparing the analytical results to the known value. The mean accuracies of carboxylic acids from derivatization step were in the range of 96.3–104.1% (see Table 4). To evaluate the accuracy of the method, the recovery experiments were also conducted with real samples. Food samples were spiked with each carboxylic acid standards at a closed concentration to the original values, and the spiked samples were subjected the whole procedure, including extraction, hydrolysis, derivatization and injection. The recoveries were calculated based on the formula of (measured value − endogenous value)/added value × 100. All analyses were carried out in triplicate, and the results are listed in Table 5. The results show that the recoveries of all carboxylic acid ranged 95.3–103.9%. Such results further demonstrated that this method is accurate and practical for the analysis of carboxylic acids from food samples.

3.6. Analysis of carboxylic acids in real food samples

The proposed method was applied to the determination of TAs extracted from sultana raisins and hawthorn flakes. The obtained chromatograms are shown in Fig. 6(a and b), and the analytical results were listed in Table 5. The results indicated that sultana has high content of Ole, and hawthorn flakes contains an abundance of Urs and also small quantities of Ole and Mas. The proposed

Table 5
Analytical results of food samples ($n = 3$).

Samples	Hawthorn flake				Sultana raisin			
	Original (μg/g)	Add (μg/g)	Found (μg/g)	Recovery (%)	Original (μg/g)	Add (μg/g)	Found (μg/g)	Recovery (%)
Mas	46.6 ± 2.36	59.1	102.5 ± 5.18	97.0	ND	118.2	114.7 ± 3.33	96.2
Ole	117.1 ± 3.16	114.2	226.4 ± 7.43	97.9	762.8 ± 30.5	799.4	1527.8 ± 52.6	97.8
Urs	623.5 ± 25.6	628.1	1270.7 ± 43.8	101.5	ND	114.2	114.6 ± 3.09	100.5
Bet	ND	114.2	112.8 ± 3.05	98.8	ND	114.2	113.6 ± 3.41	99.4
	Lycium barbarum seed oil				*Microula sikkimensis* seed oil			
C12	ND	10.0	9.8 ± 0.26	98.0	ND	10.0	9.6 ± 0.28	96.0
C14	8.5 ± 0.23	6.7	15.4 ± 0.39	101.3	5.7 ± 0.23	6.7	12.2 ± 0.34	98.4
C16	57.4 ± 1.49	51.2	104.5 ± 2.51	96.2	71.0 ± 2.06	76.8	143.6 ± 3.73	97.1
C18	25.7 ± 0.67	28.2	53.6 ± 1.55	99.4	18.4 ± 0.48	21.3	40.5 ± 0.93	102.0
C20	8.4 ± 0.23	7.8	15.5 ± 0.48	95.7	9.3 ± 0.24	7.8	16.9 ± 0.47	98.8
C18:1	113.0 ± 3.84	141	246.0 ± 8.86	96.8	256.1 ± 8.71	282	559.4 ± 19.6	103.9
C18:2	459.6 ± 19.6	420	838.3 ± 32.7	95.3	208.9 ± 7.73	210	409.7 ± 16.4	97.8
C18:3	15.1 ± 0.43	13.9	28.2 ± 0.76	97.2	197.6 ± 6.72	208.5	393.5 ± 11.9	96.9

ND: not detected.

method was also applied to the determination of FAs in *L. barbarum* seed oil and *M. sikkimensis*. The obtained chromatograms are shown in Fig. 6(c and d), and the analytical results were summarized in Table 5. It could be concluded that the *L. barbarum* seed oil has high content of C18:2 and C18:1, and *M. sikkimensis* seed oil has high content of C18:3, C18:2 and C18:1. These two kinds of oils have abundant unsaturated fatty acids that are healthy to human body.

3.7. Comparisons of the proposed method with the reported methods

3.7.1. Comparisons of the proposed method with other methods for labeling FAs

In order to evaluate APIETS further, the proposed method are compared with those established with other labeling reagents in term of derivatization condition, detection mode and detection limit (see Table 6). Derivatization of FAs for GC analysis is often performed to increase the volatility of the substances, to improve separation, and to reduce tailing [11,12], and the most frequently used derivatives are alkyl derivatives [23–25] and silylation [26]. Although GC methods are extensively used for FA analyses, the use of elevated temperatures in GC is a disadvantage for the separation of thermally labile compounds, especially for polyunsaturated FAs [11,12]. Derivatization of FAs for HPLC analysis is often employed to make UV or FL detector available, enhancing the detector sensitivity and selectivity [22–28]. BrDMEQ is one of the most used bromoalkyl reagents for HPLC–FLD analysis of carboxylic acids. However, the derivatization with BrDMEQ is carried out in acetone or toluene solvent in the presence of phase transfer agent; consequently, the derivatization solution must be pretreated to remove the toluene and the phase transfer agent before injection [22]. ADAM, as a diazomethane reagent, cannot be stored for long periods as a solution, or even as a solid. The reagent often requires purification just before use with a suitable method like column chromatography [23]. Hydrazine reagents and amine reagents, such as DMEQ-Hz, ADC, DAABD-AE, and APF, can easily label carboxylic acids under mild conditions in presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as condensation reagent. However, these derivatization often need accelerating with expensive or virulent activators, such as 1-hydroxy-1*H*-benzotriazole (HOBt), 4-(dimethylamino) pyridine (DMAP), and pyridine [24,25,27]; otherwise the derivatization can only be completed with a long reaction time over 60 min [26]. For the sulfonate reagents (like APIETS and TSPP [28]), the derivatization with carboxylic acids can be accomplished within 35 min at about 90 °C in DMF with K_2CO_3 as catalyst, free of condensation reagent, phase transfer agent and activators; and the obtained derivatization solutions can be directly injected without pretreatment prior to HPLC. An obvious advantage of APIETS over TSPP is that APIETS derivatives exhibit the superior fluorescence properties. Both maximal λ_{ex} and maximal λ_{em} of APIETS derivatives exhibit about 55 nm red-shift comparing with those of TSPP derivatives, which facilitate diminishing interference from backgrounds when determining trace amount of analytes. As also can be seen from Table 6, the LODs obtained with APIETS are comparable or lower than those obtained with other reagents. Overall, APIETS is prospectively significant as a labeling reagent for FAs in terms of stability, simplicity and sensitivity. The convenient derivatiztion as well as low detection makes APIETS superior to the other methods.

3.7.2. Comparisons of the proposed method with reported methods for determining TAs

The comparisons of the proposed method with reported methods for determining TAs are shown in Table 7. TAs are a group of difunction carboxylic acids, and their hydroxyl groups should also be derivatized before GC analysis. TAs may be adsorbed on the column during GC analysis if the hydroxyl group is not derivatized, resulting in non-symmetric peaks [11]. The reported GC methods included methylation with diazomethane [27,28] and silylation [29–31]. Diazomethane is undoubtedly toxic, carcinogenic, corrosive, and explosive, and this has discouraged its wider use. Furthermore, there are many articles in the literature describing the artefacts of derivatization with diazomethane. During the methylation of carboxylic acid with diazomethane, other functional groups can be affected if present, such as phenolicorenolic-, carbonylic-, or olefinic bonds [11]. Silylation of TAs was usually a tedious procedure requiring reaction times about 2 h [29,31], and less time would result in uncompleted derivatives impeding accurate analysis of TAs in samples [30]. In most cases, TAs are directly determined by HPLC coupled to various detectors, without employing derivatization procedures [32–36]. Because of no chromophores in the analyte, the most popular UV detection can only be carried out at about 205 nm, which often result in high LODs and fluctuant baselines [32]. The HPLC methods involving evaporative light scattering detection (ELSD), mass spectrometry (MSD) or nuclear magnetic resonance (NMR) have also been reported to determine TAs, and the obtained LODs were at the level of pmol/μL [33–36]. To our best knowledge, only J. Sun and co-workers have ever employed derivatization procedure in HPLC analysis of TAs. Using paranitrobenzoyl chloride (PNBC) as labeling reagent, they introduced

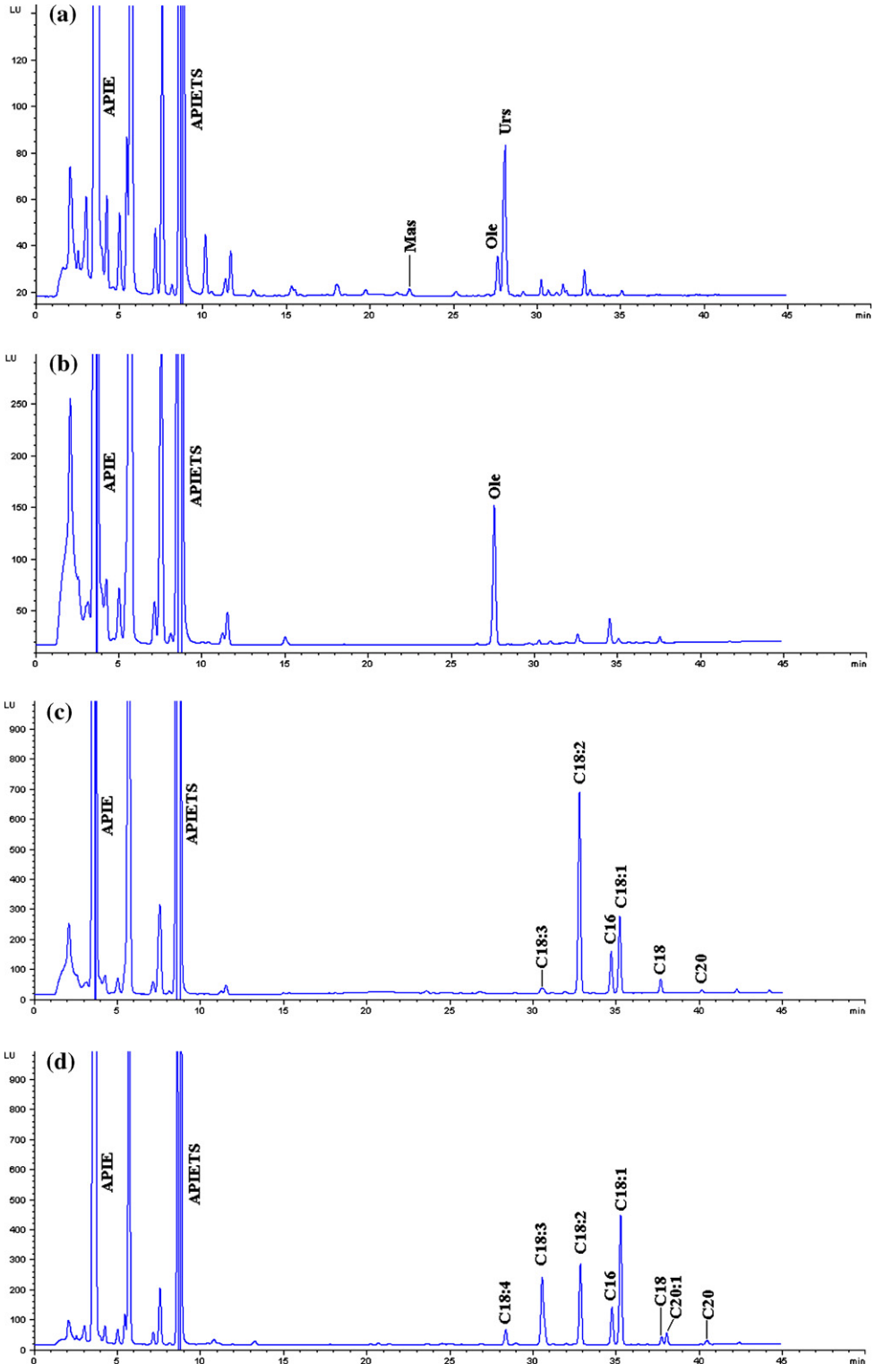

Fig. 6. The representative chromatograms for carboxylic acids in food samples (hawthorn flake (a), sultana raisin (b), *Lycium barbarum* seed oil (c) and *Microula sikkimensis* seed oil (d)). Chromatographic conditions and peaks labels are the same with Fig. 4.

paranitrobenzoyl to Ole molecule at hydroxyl group, and achieved the UV detection of Ole at 254 nm [37]. However, the PNBC is not a specific labeling reagent for carboxyl group. When PNBC is used to derivatize Ole in real samples, the co-extracted phenols, amines and alcohols would also be labeled, which would complicate the chromatogram and bring some interference. In this work, the TAs are labeled with APIETS at carboxyl group, and the derivatives are sensitively detected at fmol/μL level with fluorescence detection at $\lambda_{ex}/\lambda_{em}$ = 315/435 nm. In addition, this chromophoric derivatization is also favor of excellent chromatographic resolution of the isomeric TAs, which may be attributed to the fact that the introduction of API greatly changed the polarity of the isomers as a whole. Thus it can be seen that the proposed method had higher sensitivity and better selectivity than those reported methods.

Table 6
Comparison of the proposed method with reported methods for FAs from food samples.

Analytes	Labeling reagents	Derivatization conditions	Separation and detection	LODs (original values in literatures)	Ref.
C14–C18 (even); C18:1; C18:2	MeOH–[OH^-]	MeOH containing NaOH, 65 °C, 2 h	GC–FID (oven temperature up to 310 °C)	NA[a]	[23]
37 kind of saturated and unsaturated FAs	MeOH–[H^+]	MeOH containing 1% H_2SO_4, toluene, refluxed for 3 h	GC–MS (oven temperature up to 240 °C)	NA[a]	[24]
C6–C18 (even); C15; C16:1; C18:1; C18:2; C18:3	Boron trichloride (BCl_3)–MeOH	In MeOH containing 0.5 M NaOH, boiling for 30 min; After adding BF_3, boiling for another 20 min	GC–FID (oven temperature up to 230 °C)	7–25 ng/10 μL (0.7–2.5 ng/μL)	[25]
C12–C24 (even); C18:1; C18:2	BSTFA/1% TMCS[b]	*n*-Hexane, 70 °C, 30 min	GC–MS (oven temperature up to 320 °C)	1–12 ng/10 μL (0.1–1.2 μg/mL)	[26]
C3–C20	Br-DMEQ	Acetone or toluene, K_2CO_3, 18-crown-6, 60 °C, 30–60 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 370/455 nm)	10–15 fmol/10 μL	[16]
Okadaic acid	ADAM	THF, 37 °C, 2–2.5 h	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 255/415 nm)	~100 pg/10 μL	[17]
C5–C20	DMEQ-Hz	333 mM EDC, 1.7% pyridine, room temperature, 15 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 365/447 nm)	3–6 fmol/10 μL	[18]
C12–C19	ADC	250 mM EDC, 5.7 mM HOBt, 25 °C, 5 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 353/451 nm)	20–50 fmol/10 μL	[19]
C4–C20	DAABD-AE	8 mM EDC, 20 mM DMAP, 60 °C, 30 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 430/570 nm)	110–660 fmol/10 μL (55–330 fmol/5 μL)	[21]
C12–C18	APF	3 mM EDC, 60 °C, 60 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 467/512 nm)	1–64 fmol/10 μL (0.1–6.4 nmol/L)	[20]
C20–C30	TSPP	K_2CO_3, DMF, 90 °C, 30 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 260/380 nm)	26–77 fmol/10 μL	[22]
C12–C20 (even)	APIETS	K_2CO_3, DMF, 85 °C, 35 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 315/435 nm)	13–27 fmol/10 μL	This work

[a] NA: not available.
[b] BSTFA/1% TMCS: N,O-bis-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane.

Table 7
Comparison of the proposed method with reported methods for TAs from food samples.

Analytes	Labeling reagents	Derivatization conditions	Separation and detection	LODs (original values in literatures)	Ref.
Ole, Urs, Bet	Diazomethane	Diethyl ether	GC–FID	NA[a]	[27]
Ole, Urs, Bet	Diazomethane	Diethyl ether	GC–FID	NA[a]	[28]
Ole, Urs, Bet	N-methyl-N-trimethylsilyl trifluoroacetamide	Pyridine, 70 °C, 2 h	GC–MS	NA[a]	[29]
Ole, Urs, Mas	Hexamethyldisilazane, trimethylchlorosilane, pyridine	Diethyl ether, room temperature 10 min.	GC–FID	NA[a]	[30]
Ole, Urs	BSTFA/1% TMCS[b]	Pyridine, 80 °C, 2 h	GC–FID	20 ng/10 μL (2 ng/μL)	[31]
Ole, Urs, etc.	–	–	HPLC–UV (210 nm)	25.4–32.8 pmol/10 μL (1.16–1.5 μg/mL)	[32]
Ole, Urs, Bet, Mas, etc.	–	–	HPLC–ELSD	68–111 pmol/10 μL (3.22–5.08 μg/mL)	[33]
Ole	–	–	HPLC–APCI/MS	5.24 pmol/20 μL (0.12 mg/L)	[34]
Ole, Urs, etc.	–	–	HPLC–ESI/MS (MRM)	0.57–2 pmol/10 μL (0.26–0.91 ng/10 μL)	[35]
Ole, Urs	–	–	HPLC–NMR	88 pmol/10 μL (8.8 μmol/L)	[36]
Ole	Paranitrobenzoyl chloride (PNBC)	Room temperature, ultrasonic, 60 min	HPLC–UV (254 nm)	~2 pmol/10 μL (2 ng/20 μL)	[37]
Ole, Urs, Bet, Mas	APIETS	K_2CO_3, DMF, 85 °C, 35 min	HPLC–FL ($\lambda_{ex}/\lambda_{em}$ = 315/435 nm)	21–30 fmol/10 μL	This work

[a] NA: not available.
[b] BSTFA/1% TMCS: N,O-bis-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane.

4. Conclusion

APIETS, a novel carboxylic acid-reactive fluorescent labeling reagent, has been well designed and developed. The APIETS reagent contains the fluorescence core structure of API. The API moiety has a large planar n–π conjugation system, which was favorable for achieving sensitive determination of carboxylic acids with fluorescence detection. In addition, the API moiety contains one weakly basic nitrogen atoms, which makes APIETS derivatives easy to form $[M+H]^+$ in MS ionization chamber, thereby exhibiting intense

responses under APCI/MS in positive ion detection mode. Comparing with other fluorescent reagents for carboxylic acids, APIETS has advantages of moderate stability, convenient derivatization procedure and high sensitivity as well. Using APIETS as labeling reagent, a HPLC–FL method for simultaneously determining eight FAs and four TAs has been established. The proposed method shows high sensitivity, good precision and accuracy. The proposed method has been successfully applied to the determination of FAs in edible oils, and TAs in roughly processed fruits samples. And this method should also have powerful potential in the analysis of carboxylic acids in other complex samples.

Acknowledgements

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