Food Chemistry 146 (2014) 264–269

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of triterpenic acids in fruits by a novel high performance liquid chromatography method with high sensitivity and specificity

Shijuan Zhang [a,c], Yuanpeng Sun [b], Zhiwei Sun [b], Xiaoyan Wang [a], Jinmao You [a,b,*], Yourui Suo [a]

[a] Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Science, Xining, PR China
[b] Shandong Province Key Laboratory of Life-Organic Analysis, Qufu Normal University, Qufu, PR China
[c] University of Chinese Academy of Science, Beijing, PR China

ARTICLE INFO

Article history:
Received 2 March 2013
Received in revised form 8 August 2013
Accepted 8 September 2013
Available online 18 September 2013

Keywords:
Triterpenic acids
Pre-column derivatisation
HPLC
CPMS

ABSTRACT

A novel and interesting pre-column derivatisation method was developed for the analysis of triterpenic acids by high-performance liquid chromatography (HPLC) with fluorescence detection. Each triterpenic acid produced two HPLC peaks with similar peak areas after derivatising with chiral 1-(9H-carbazol-9-yl) propan-2-yl-methanesulfonate (CPMS), while the fatty acid derivative of CPMS had only one peak. This phenomenon greatly increased the confidence in analyte confirmation. Compound with only one peak or two peaks differing greatly in their peak areas could be excluded from the target compound list. CPMS was compared with five other derivatising reagents, four of which produced only one peak for one triterpenic acid, to study the possible mechanism. Analytes with different behaviours were also studied to better interpret the mechanism. The proposed method also showed the merits of high sensitivity and less sample consumption. It was successfully applied to the analysis of triterpenic acids in fruit peels and flesh. There is no prior report on the two peak phenomenon of triterpenic acids. The information provided in this study will be helpful for those who are also engaged in derivatisation study.

1. Introduction

Fruits are preferred by people all over the world because of their effectiveness in reducing the risks of developing chronic diseases. Recent studies indicate that the peels of fruits are rich in triterpenic acids (Frighetto, Welendorf, Nigro, Frighetto, & Siani, 2008; He & Liu, 2007; Jäger, Trojan, Kopp, Laszczyk, & Scheffler, 2009). Triterpenic acids possess anti-inflammatory, antioxidant, antiatherogenic, antihyperlipidemic, antitumor, antidiabetic and hepatoprotective effects (Allouche, Beltrán, Gaforio, Uceda, & Mesa, 2010; Hichri, Jannet, Cheriaa, Jegham, & Mighri, 2003; Kazmi et al., 2012; Li et al., 2011; Somova, Nadar, Rammanan, & Shode, 2003; Xia, Wei, Si, & Liu, 2011). Therefore, the analysis of triterpenic acids including ursolic acid (UA), oleanolic acid (OA), maslinic acid (MA), betulinic acid (BIA), corosolic acid (CA) and betulonic acid (BOA) has attracted increasing attention now (Caligiani et al., 2013; Chen, Li, Song, Suo, & You, 2012; Gbaguidi, Accrombessi, Moudachirou, & Quetin-Leclercq, 2005; Guo et al., 2011; Lesellier, Destandau, Grigoras, Fougère, & Elfakir, 2012; Martelanc, Vovk, & Simonovska, 2009; Sánchez-Ávila, Priego-Capote, Ruiz-Jiménez, & Luque de Castro, 2009). Because of the lack of strong chromophores in triterpenic acid molecules, the HPLC sensitivity of these compounds was low. Derivatisation of the carboxyl moiety with a suitable chromophore or fluorophore can greatly enhance the HPLC sensitivity of triterpenic acid and becomes popular in recent years. For example, 2-(5-benzoacridine)ethyl-p-toluenesulfonate (BAETS) (Li & et al., 2011) and 2-(2-(pyren-1-yl)-1H-benzo[d]imidazol-1-yl)-ethyl-p-toluenesul-fonate (PBIOTs) (Chen et al., 2012) had been successfully applied to the analysis of triterpenic acids in different samples. However, interferences might often occur during the analysis process, and online MS confirmation was therefore often needed to get an accurate result (Chen et al., 2012; Li et al., 2011). This greatly hampered the application of these methods since the LC–MS instrument was still too expensive for normal researchers. It is desirable if the accuracy of HPLC method could be enhanced.

In this paper, we report a sensitive HPLC method for the determination of triterpenic acids with high accuracy and no need of LC–MS confirmation. In our previous study, we have reported the synthesis of 1-(9H-carbazol-9-yl) propan-2-yl-methanesulfonate (CPMS) and its application in fatty acid analysis (Zhang, You, Zhou, Li, & Suo, 2012). Recently, we found an interesting phenomenon concerning the derivatisation of CPMS with triterpenic acids. After derivatisation, each triterpenic acid produced two HPLC peaks with different retention times but similar peak areas, while the fatty acid derivative of CPMS remained only one peak. Two different retention times and two similar peak areas for a compound double ensured the accuracy of HPLC identification. Compounds lacking

* Corresponding author. Address: Chinese Academy of Sciences, Northwest Institute of Plateau Biology, Xining 810001, PR China. Tel./fax: +86 537 4456305.
E-mail address: jmyou6304@163.com (J. You).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.09.056

any one of the above factors could be excluded from the target compound list. The HPLC confidence in confirming a target compound was therefore greatly increased, and LC–MS confirmation was no longer indispensable. The proposed method was successfully applied to the analysis of triterpenic acids in the peels and flesh of various fruits.

2. Materials and methods

2.1. Instruments

The HPLC analysis was performed using an Agilent 1290 Series HPLC system, equipped with an on-line-degasser, a binary pump, an autosampler and a thermostated column compartment. A fluorescence detector (model G1321B, Agilent, USA) was adjusted at wavelengths of 293 and 360 nm for excitation and emission. Chromatographic separation was achieved on a SB C18 column (2.1 × 50 mm, 1.8 μm i.d., Agilent, USA). Solvent A was 5% acetonitrile in water and B was acetonitrile. The flow rate was constant at 0.25 mL min^{-1} and the column temperature was kept at 30 °C. The gradient condition of mobile phase was as follows: 80–86% B from 0 to 30 min; 86–100% B from 30 to 32 min. The column was equilibrated with the initial mobile phase for 5 min before the next injection. The injection volume was 5 μL. An Agilent 6460 Triple Quadrupole MS/MS system (Agilent, USA) equipped with an Agilent Jet Stream electrospray ionisation source (ESI source) was used in Scan mode to characterise the derivatives. Except that 0.1% formic acid was added in the mobile phase to enhance the mass response, the other HPLC conditions were exactly the same.

2.2. Reagents and chemicals

OA and UA acid were obtained from national institute for the control of pharmaceutical and biological products (China). BIA, MA, CA and BOA were purchased from Sigma–Aldrich (USA). Acetonitrile, methanol and ethanol were of HPLC grade (Sigma–Aldrich, USA). Dimethylformamide (DMF) and potassium carbonate (K_2CO_3) were of analytical grade and obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Pure distilled water was purchased from Watsons (Guangzhou, China). All other reagents used were also of analytical grade unless otherwise stated. CPMS was synthesised in authors' laboratory as described in our previous study (Zhang et al., 2012). CPMS solution was analysed by an automatic polarimeter at a concentration of 0.05 g/mL, and the determined value was zero ($[\alpha]_D^{30} = 0$).

2.3. Samples

Seasonal fruits were all purchased from a fruit market in east of Qufu normal university in September 2012. Peels and flesh were separated and dried at 45 °C. They were then milled and stored at 4 °C until analysis.

2.4. Preparation of solutions

Stock solutions of the analytes were initially prepared at 1000 mg L^{-1} by dissolving 10 mg of the compound in 10 mL of DMF and stored at 4 °C in the dark. The corresponding working standard solutions were obtained by dilution of the stock solutions with acetonitrile. The working solutions were stored in a refrigerator and used within 3–4 weeks. CPMS solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving 3.03 mg CPMS in 10 mL of acetonitrile.

2.5. Sample preparation

The prepared sample (50 mg) was weighed in a 5 mL glass centrifuge tube and then mixed with 3 mL of ethanol. Extraction was performed with ultrasonication for 30 min. Sample was centrifuged at 4000 r/min for 10 min, then the supernatant was collected, and 2 mL of ethanol was added into the residue for further extraction. The twice supernatants were united, then 100 μL of the extracted solution was evaporated to dryness for later derivatisation.

2.6. Derivatisation procedure

To a 2-mL vial containing certain volume of standard solution or dried sample, 100 μL derivatising reagent solution, 10 mg K_2CO_3 and 100 μL acetonitrile were added. The vial was sealed and allowed to react in a water bath at 90 °C for 30 min. The derivatisation procedure is shown in Fig. 1. After the reaction was completed, the mixture was cooled to room temperature, then an appropriate volume of acetonitrile solution was added to dilute the derivatisation solution to 1.0 mL. The diluted solution was syringe filtered using a 0.22 μm nylon filter and injected directly for HPLC analysis.

3. Results and discussion

3.1. Extraction method selection

Soxhlet extraction and ultrasonic extraction were compared for their extraction efficiency. The recoveries obtained by both methods were higher than 85%. Considering the simplicity of ultrasonic extraction, it was applied in this study. Ethanol and methanol have been traditionally used in the extraction of triterpenic acids, and they were also compared in our study. Ethanol was environmental friendly and showed better extraction efficiency in real sample analysis. Therefore, it was applied in subsequent experiments.

3.2. Optimisation of derivatisation conditions

The concentration of derivatising reagent plays an important role in pre-column derivatisation. The effects of CPMS concentrations on derivatisation were studied in detail to ensure sufficient reaction of the analytes. Constant fluorescence intensity was achieved with the addition of an eightfold molar reagent excess

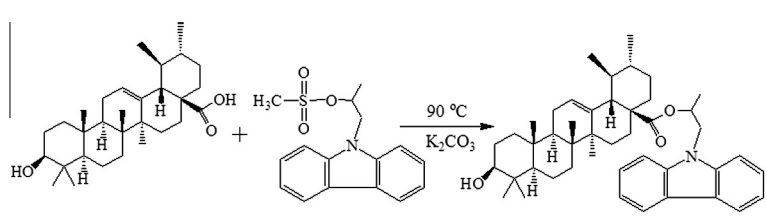

Fig. 1. Derivatisation scheme of UA with CPMS.

to total molar triterpenic acids. Further increasing the excess of reagent beyond this level had no improvement on the yields. For the convenience of operation, 1.0×10^{-3} mol L^{-1} of CPMS was applied in later experiments since most of the triterpenic acids in real samples were far below this level. The other derivatising parameters such as reaction temperature and time were optimised according to the method described before (Zhang et al., 2012). Finally, derivatisation at 90 °C for 30 min with CPMS concentration of 1.0×10^{-3} mol L^{-1} were employed in later experiment.

3.3. Chromatographic separation

Among the six triterpenic acids studied in this paper, CA and MA are isomers, so are UA and OA. It is quite challenging to separate the two pairs of isomers by a normal reversed phase column. Derivatisation can enhance the differences between isomers and lead to complete separation of them. For example, UA and OA could be separated through derivatisation (Li et al., 2011). When CPMS was applied to the analysis of triterpenic acids, the isomers were completely separated. Interestingly, there were two peaks for each triterpenic acid derivative. The two peaks were several minutes away from each other, but their peak areas were similar with peak area deviations of less than 5%. Therefore, as long as one of the two peaks could be integrated, quantification could be carried out. In this study, the HPLC separation of the derivatives could be accomplished by using a SB C18 column in combination with a gradient elution with water and acetonitrile as mobile phase (see Fig. 2). This chromatographic separation is sufficient enough for the samples analysed in this study. Better HPLC separation could be achieved by using longer separation time and buffer solutions.

3.4. Study of the possible reason of the two peak phenomenon

Normally, there is one peak for one analyte. In this study, two peaks were observed for each triterpenic acid derivative. At first, we thought it was derived from the decomposition of the standard solutions. Therefore, standard solutions were newly prepared and analysed immediately. However, two peak phenomenon was still observed for each analyte. Thus, the decomposition of the standard could be excluded. The effectiveness of CPMS was also studied by applying it to the analysis of fatty acids. Only one peak was observed for each fatty acid derivative. Therefore, the decomposition of CPMS could also be excluded.

The contribution of derivatising reagents to the two peak phenomenon was first studied by comparing the structure of CPMS with five other derivatising reagents which had been previously synthesised in our laboratory and were still available now (see Fig. 3). CPMS and 1-(11H-benzo[a]carbazol-11-yl) propan-2-yl 4-methylbenzenesulfonate (BCPMS) produced two peaks for each triterpenic acid derivative, while the other derivatising reagents

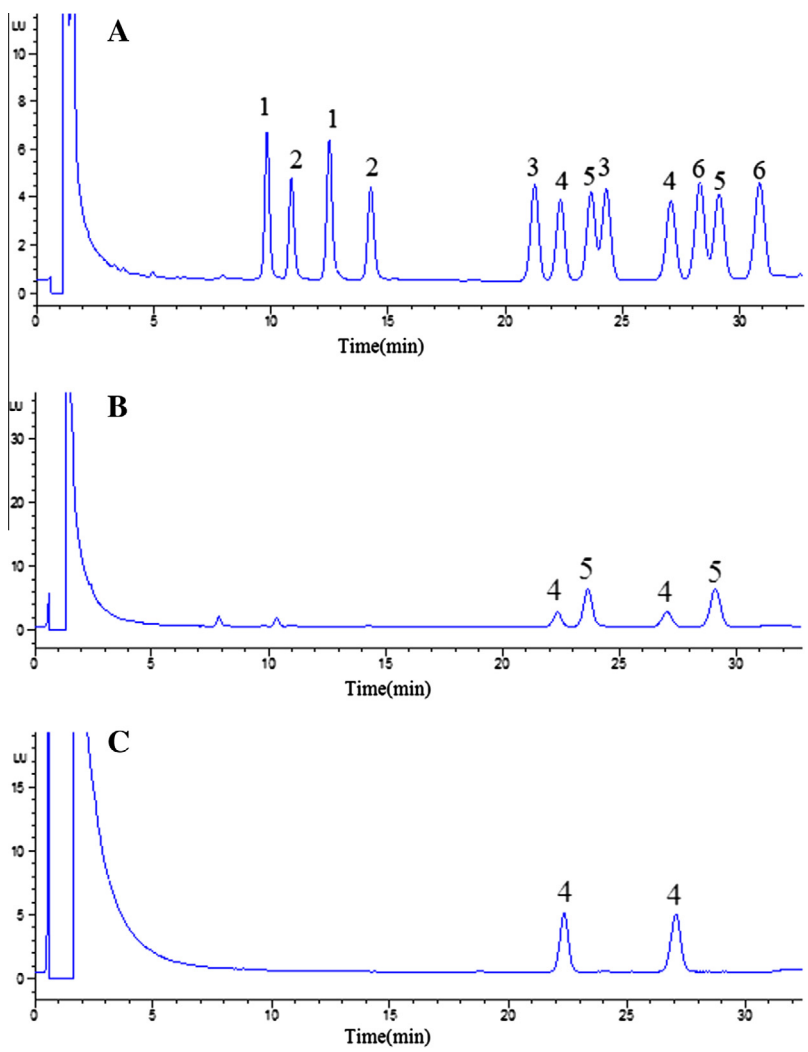

Fig. 2. Chromatograms of triterpenic acids from (A) standard solution (1: MA, 2: CA, 3: BIA, 4: OA, 5: UA, 6: BOA), (B) peels of persimmon and (C) peels of grape.

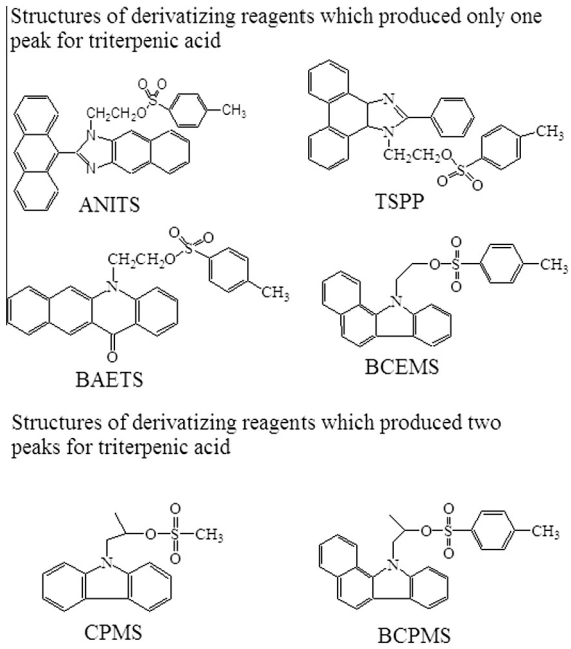

Fig. 3. Structures of derivatising reagents which produce either one or two HPLC peaks for one triterpenic acid.

produced only one peak. The main differences between the two kinds of labelling reagents lied in the existence of a chiral carbon atom. A clear example was BCPMS and 2-(11H-benzo[a]carbazol-11-yl)ethyl 4-methylbenzenesulfonate (BCEMS), the structures of which were similar. Derivatising reagents with no chiral carbon atom produced only one peak for one triterpenic acid derivative, while derivatising reagents containing a chiral carbon atom produced two peaks. This phenomenon also made clear a fact that triterpenic acids themselves did not contribute to the two peak phenomenon.

However, chiral carbon atom itself did not give rise to the appearance of two peaks for one compound since this phenomenon was not observed for all CPMS derivatives, and the chiral carbon atoms in triterpenic acids also did not lead to the appearance of two HPLC peaks. The two peak phenomenon might derive from the comprehensive effects of the chiral carbon atom of CPMS and the complex structure of the analytes. Bible acids (BA), the structures of which were similar to triterpenic acids, together with triterpenic acids and fatty acids were then studied carefully to interpret the mechanism. As shown in Fig. 4, two peak phenomenon was observed for the CPMS derivatives of cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and triterpenic acids, while only one peak was observed for the derivatives of fatty acid, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA) and lithocholic acid (LCA). Unlike the two peaks of triterpenic acid which were several minutes away from each other (Fig. 2), the two peaks of CA were separated with only baseline overlapped, while the resolutions of the two peaks of DCA and CDCA were <1 (Fig. S1).

MS confirmation indicated that the two peaks of each triterpenic acid derivative represented two compounds with identical molecular weight, which was the same as those of their theoretical derivatives (Fig. S2). The two peak phenomenon might come from the separation of the isomers caused by the chiral carbon atom in CPMS. When the analytes were fatty acids or other compounds with small molecular size, the differences between isomers were not sufficient enough for the HPLC separation of them, and therefore one peak phenomenon was observed. When CPMS was applied to the analysis of compounds with larger molecular size (at least four-ring molecules), the chiral carbon atom was connected to two large moieties. One was tricyclic carbazol, and the other was tetracyclic or pentacyclic analytes. The differences between the isomers increased and complete HPLC separation of them achieved. Any parameters which were helpful to increase the differences between the isomers were beneficial for the appearance of the two peak phenomenon. This was confirmed in the analysis of triterpenic acid and bible acids. Analytes with lager molecular size were inclined to produce two peaks for their CPMS derivatives (Fig. 4). For example, DCA and LCA are bile acids with similar structure, but DCA has one more hydroxyl group and its molecular size is larger. This difference made DCA had two HPLC peaks for its CPMS derivative, while LCA had only one peak.

However, HPLC separation is a complex process during which many factors function. Large structure difference is just one necessary condition for the two peak phenomenon. Another important factor is the ample separation time of the isomers. To achieve this, the analytes should possess some hydrophobicity to make sure that they can be better retained on the column to achieve complete separation of the isomers. GDCA, the molecular size of which is a bit larger than that of DCA can be used as an example. DCA had two peaks, while GDCA with larger molecular size had only one peak. This might be caused by the hydrophilic amino group in the GDCA molecule which led to the quick elution of the isomers. Under this condition, the interaction time between the isomers and the column was greatly reduced and it was not sufficient enough to separate the isomers.

The mechanism of the two peak phenomenon is complicated and needs to be further studied. One conclusion we can drawn from the current study is that chiral carbon atom connected to two large molecules which are also of certain hydrophobicity is prone to produce two HPLC peaks on a normal reversed phase column. This information can be applied in derivatisation to enhance the HPLC accuracy.

3.5. Comparison of CPMS with other derivatising reagents reported before

The derivatisation and separation behaviours of CPMS were then compared with those of 1-[2-(p-toluenesulfonate)-ethyl]-2-phenylimidazole-[4,5-f]-9,10-phenanthrene (TSPP) (Zhao, Wang, You, & Suo, 2007), 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) (Zhu et al., 2007) and BAETS which had been previously synthesised in our laboratory. All of the four target compounds showed good sensitivity for triterpenic acids but their separation behaviours were different. UA and OA could be separated when ANITS was used as labelling reagent, but CA and MA were completely overlapped. When applying BAETS or TSPP as derivatising reagents, the isomers of CA and MA could be separated, but UA and OA became overlapped. Besides, since fatty acids could also react with the labelling reagents due to the carboxyl groups in their molecules, the peaks of triterpenic acids had very high incidence to overlap with fatty acid peaks, especially for samples which were rich in fatty acids. For example, decoic acid (C10) and MA had identical retention times when ANITS was used as derivatising reagent, while myristic acid (C14) and BOA were overlapped when TSPP was used as derivatising reagent. Overestimation might occur if MS identification was not available. Therefore, MS identification was applied by most of the previous methods concerning HPLC analysis of triterpenic acids.

When CPMS was used as derivatising reagent, the two pairs of isomers were completely separated. More importantly, the derivatisation strategy was free of interferences. Interferences from fatty acids were greatly reduced because each fatty acid derivative had

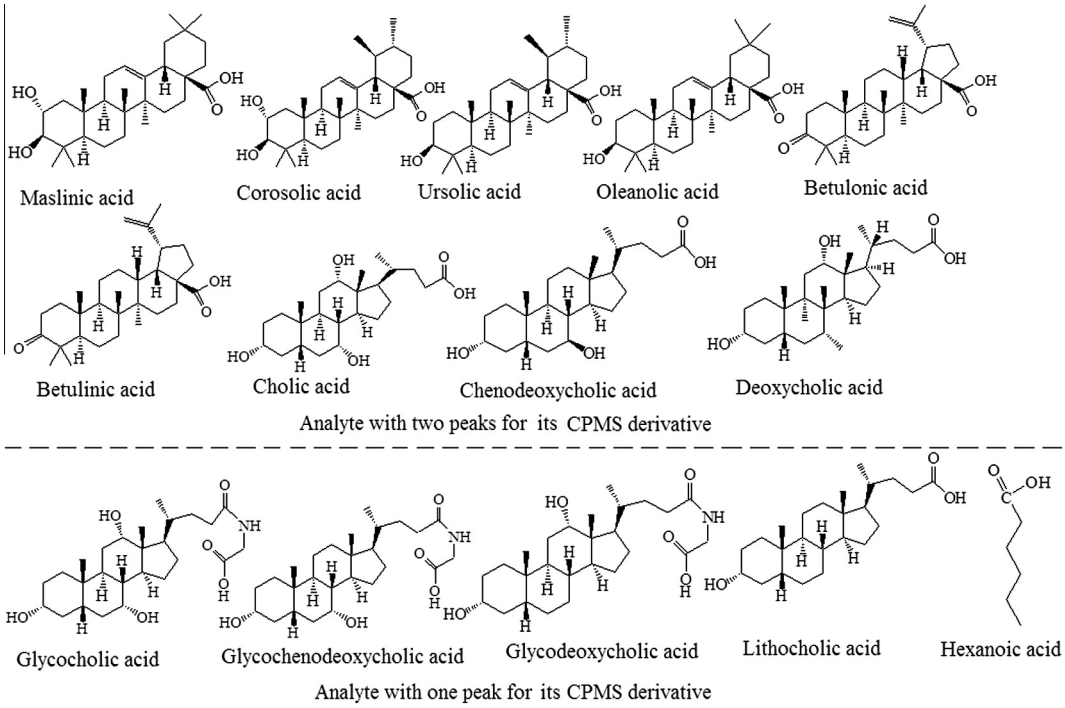

Fig. 4. Structures of analytes possessing one or two HPLC peaks for their CPMS derivatives.

only one peak while the triterpenic acid derivative had two peaks. Interferences introduced by several compounds which happened to have identical retention times with the two peaks of the target compound could also be excluded because the peak areas of the two peaks of target compound were similar, while the chances for interferences to have both identical retention times and similar peak areas were very small. The accuracy of the HPLC analysis of triterpenic acids was therefore greatly enhanced. This is especially valuable if MS confirmation was not easily available. Moreover, the synthesis of CPMS contains only two simple steps, much easier than the synthesis of other labelling reagents.

3.6. Method valuation

The method was validated for linearity, limits of detection (LOD), precision and accuracy. Linearity data was generated by plotting the peak areas versus concentrations in the range of 2.0–100 ng mL^{-1}. The sum of the two peak areas of each triterpenic acid derivative was applied for calculation. The correlation coefficients were found to be >0.997, indicating excellent linearity of the analytes. Instrument LODs calculated at a signal to-noise ratio (S/N) of three were in the range of 0.5–1.0 ng mL^{-1} (see Table 1), while method LODs were in the range of 0.5–1.0 μg g^{-1}. Lower method LODs could be obtained by using lager sample amount or applying the whole extracted solution to derivatisation. Since the sensitivity of the method was sufficient enough for daily analysis, only 100 μL of the 5 mL extracted solution was applied to derivatisation to reduce the waste of chemical reagents and interferences introduced into the column. Precision of the method was estimated by applying the whole procedure to real sample analysis in triplicate, and the obtained RSDs were ⩽6.0%. Accuracy of the method was measured by analysing samples spiked at 3.0, 6.0 and 10 μg g^{-1} of triterpenic acids, respectively. All analyses were carried out in triplicate. The results indicated that the recoveries of all triterpenic acids were in the range of 85.5–94.7% (Table S1).

The stability of CPMS and its triterpenic acid derivatives were also studied. Solid CPMS could be stored at room temperature for 1 year without any decomposition. Acetonitrile solution of CPMS was stable at room temperature for at least 2 weeks with no obvious decrease in derivatisation yields. Triterpenic acid derivatives of CPMS were placed at 4 °C for 1 week with peak area deviations of less than 5%. Therefore, the stability of CPMS and its derivatives were sufficient enough for daily analysis.

Table 1
Linearity, instrument LODs and LOQs of triterpenic acids.

Analyte	Calibration equation	R	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
OA	$Y = 0.3187X + 0.3716$[a]	0.99985	1.0	3.0
UA	$Y = 0.4329X + 0.4955$	0.99968	0.8	2.4
BIA	$Y = 0.4506X + 0.3213$	0.99908	0.8	2.4
MA	$Y = 0.5140X + 0.5992$	0.99715	0.5	1.5
BOA	$Y = 0.4629X + 0.3716$	0.99973	0.7	2.1
CA	$Y = 0.4029X + 0.1651$	0.99892	1.0	3.0

[a] Y = peak area, X = theoretical concentration of triterpenic acids.

3.7. Analysis of triterpenic acid in fruits

The proposed method was applied to the analysis of triterpenic acid in the peels and flesh of various fruits. The compositional data of triterpenic acids is shown in Table 2. Fig. 2 shows the chromatograms of triterpenic acids in the peels of pear and persimmon. The results indicated that the peels of fruits were rich in triterpenic acids. The flesh of fruits might also contain some amount of triterpenic acids, but their concentrations were much lower than those in peels. Therefore, triterpenic acids were not detected in some flesh samples when 50 mg of sample was used for analysis. It is quite a waste to discard the peels of fruits, and alternative ways can be applied to utilise the active ingredient of peels. For example, it is a good choice to drink water containing some dried peels of fruits which are inedible when they are fresh. This has already been done by ancient Chinese doctor. For example, *Chenpi*, the dried peels of orange, is used as a traditional medicine. For the

Table 2
Concentrations of triterpenic acids in different fruits ($n = 3$).

Sample	OA (mg kg^{-1})	UA (mg kg^{-1})	BIA (mg kg^{-1})	MA (mg kg^{-1})	BOA (mg kg^{-1})	CA (mg kg^{-1})
Apple peel	17.2 ± 0.7[a]	60.3 ± 2.8	–[b]	–	–	–
Apple flesh	–	–	–	–	–	–
Pear peel	102.5 ± 4.5	358.4 ± 12.4	65.3 ± 3.1	–	–	–
Pear flesh	–	–	–	–	–	–
Persimmon peel	367.7 ± 17.5	879.7 ± 30.6	–	–	–	–
Persimmon flesh	17.2 ± 0.6	25.8 ± 0.9	–	–	–	–
Grape peel	176.2 ± 6.6	–	–	–	–	–

[a] Data are expressed as mean value ± S.D.
[b] –, not detected.

peels such as the peels of persimmon, which can not be eaten because of the high content of tannic acid in its peels, triterpenic acids could be extracted first and then provided for people.

4. Conclusion

In this paper, a sensitive and accurate HPLC method was developed for the analysis of triterpenic acids in fruits. Each analyte produced two HPLC peaks after derivatising with CPMS. This phenomenon greatly reduced the risks of overestimation due to the double insurance of two retention times and two peak areas. It will be especially useful for the analysis of triterpenic acids in complex samples which also contain some other carboxylic acids such as fatty acids. The mechanism needs to be further studied in our later study, and we hope it will be helpful for those who are also engaged in derivatisation or triterpenic acid analysis.

Acknowledgements

The work was supported by 100 Talents Program of the Chinese Academy of Sciences (328), National Science Foundation of China (No. 21275089) and the Natural Science Foundation of Shandong Province (ZR2012BQ022).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.09.056.

References

Allouche, Y., Beltrán, G., Gaforio, J. J., Uceda, M., & Mesa, M. D. (2010). Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. *Food and Chemical Toxicology, 48*, 2885–2890.
Caligiani, A., Malavasi, G., Palla, G., Marseglia, A., Tognolini, M., & Bruni, R. (2013). A simple GC–MS method for the screening of betulinic, corosolic, maslinic, oleanolic and ursolic acid contents in commercial botanicals used as food supplement ingredients. *Food Chemistry, 136*, 735–741.
Chen, G., Li, J., Song, C., Suo, Y., & You, J. (2012). A sensitive and efficient method for simultaneous trace detection and identification of triterpene acids and its application to pharmacokinetic study. *Talanta, 98*, 101–111.
Frighetto, R. T. S., Welendorf, R. M., Nigro, E. N., Frighetto, N., & Siani, A. C. (2008). Isolation of ursolic acid from apple peels by high speed counter-current chromatography. *Food Chemistry, 10*, 767–771.
Gbaguidi, F., Accrombessi, G., Moudachirou, M., & Quetin-Leclercq, J. (2005). HPLC quantification of two isomeric triterpenic acids isolated from *Mitracarpus scaber* and antimicrobial activity on *Dermatophilus congolensis*. *Journal of Pharmaceutical and Biomedical Analysis, 39*, 990–995.
Guo, S., Duan, J., Tang, Y., Qian, Y., Zhao, J., Qian, D., et al. (2011). Simultaneous qualitative and quantitative analysis of triterpenic acids, saponins and flavonoids in the leaves of two Ziziphus species by HPLC–PDA–MS/ELSD. *Journal of Pharmaceutical and Biomedical Analysis, 56*, 264–270.
He, X., & Liu, R. H. (2007). Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. *Journal of Agricultural and Food Chemistry, 55*, 4366–4370.
Hichri, F., Jannet, H. B., Cheriaa, J., Jegham, S., & Mighri, Z. (2003). Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds. *Comptes Rendus Chimie, 6*, 473–483.
Jäger, S., Trojan, H., Kopp, T., Laszczyk, M. N., & Scheffler, A. (2009). Pentacyclic triterpene distribution in various plants – Rich sources for a new group of multi-potent plant extracts. *Molecules, 14*, 2016–2031.
Kazmi, I., Rahman, M., Afzal, M., Gupta, G., Saleem, S., Afzal, O., et al. (2012). Anti-diabetic potential of ursolic acid stearoyl glucoside: A new triterpenic gycosidic ester from Lantana camara. *Fitoterapia, 83*, 142–146.
Lesellier, E., Destandau, E., Grigoras, C., Fougère, L., & Elfakir, C. (2012). Fast separation of triterpenoids by supercritical fluid chromatography/evaporative light scattering detector. *Journal of Chromatography A, 1268*, 157–165.
Li, G., Zhang, X., You, J., Song, C., Sun, Z., Xia, L., et al. (2011). Highly sensitive and selective pre-column derivatization high-performance liquid chromatography approach for rapid determination of triterpenes oleanolic and ursolic acids and application to Swertia species: Optimization of triterpenic acids extraction and pre-column derivatization using response surface methodology. *Analytica Chimica Acta, 688*, 208–218.
Martelanc, M., Vovk, I., & Simonovska, B. (2009). Separation and identification of some common isomeric plant triterpenoids by thin-layer chromatography and high-performance liquid chromatography. *Journal of Chromatography A, 1216*, 6662–6670.
Sánchez-Ávila, N., Priego-Capote, F., Ruiz-Jiménez, J., & Luque de Castro, M. (2009). Fast and selective determination of triterpenic compounds in olive leaves by liquid chromatography–tandem mass spectrometry with multiple reaction monitoring after microwave-assisted extraction. *Talanta, 78*, 40–48.
Somova, L. O., Nadar, A., Rammanan, P., & Shode, F. O. (2003). Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine, 10*, 115–121.
Xia, Y., Wei, G., Si, D., & Liu, C. (2011). Quantitation of ursolic acid in human plasma by ultra performance liquid chromatography tandem mass spectrometry and its pharmacokinetic study. *Journal of Chromatography B, 879*, 219–224.
Zhang, S., You, J., Zhou, G., Li, C., & Suo, Y. (2012). Analysis of free fatty acids in *Notopterygium forbesii* Boiss by a novel HPLC method with fluorescence detection. *Talanta, 98*, 95–100.
Zhao, X., Wang, H., You, J., & Suo, Y. (2007). Determination of free fatty acids in bryophyte plants and soil by HPLC with fluorescence detection and identification by online MS. *Chromatographia, 66*, 197–206.
Zhu, F., Zhao, W., Zhao, X., Suo, Y., Liu, S., & You, J. (2007). Determination of free fatty acids by high performance liquid chromatography with fluorescence detection and identification with mass spectrometry. *Chinese Journal of Analytical Chemistry, 35*, 489–494.