Journal of Pharmaceutical Analysis 14 (2024) 295–307

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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

Review paper

Chemical derivatization strategies for enhancing the HPLC analytical performance of natural active triterpenoids

Xiao-Feng Huang [a, d, 1], Ying Xue [a, c, 1], Li Yong [c], Tian-Tian Wang [a, b], Pei Luo [b, **], Lin-Sen Qing [a, *]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, 610041, China
[b] State Key Laboratory for Quality Research in Chinese Medicines, Macau University of Science and Technology, Macao, China
[c] Sichuan Center for Disease Control and Prevention, Chengdu, 610041, China
[d] University of Chinese Academy of Sciences, Beijing, 100049, China

ARTICLE INFO

Article history:
Received 10 April 2023
Received in revised form
6 July 2023
Accepted 6 July 2023
Available online 8 July 2023

Keywords:
Triterpenoid
HPLC
Derivatization
Quantification

ABSTRACT

Triterpenoids widely exist in nature, displaying a variety of pharmacological activities. Determining triterpenoids in different matrices, especially in biological samples holds great significance. High-performance liquid chromatography (HPLC) has become the predominant method for triterpenoids analysis due to its exceptional analytical performance. However, due to the structural similarities among botanical samples, achieving effective separation of each triterpenoid proves challenging, necessitating significant improvements in analytical methods. Additionally, triterpenoids are characterized by a lack of ultraviolet (UV) absorption groups and chromophores, along with low ionization efficiency in mass spectrometry. Consequently, routine HPLC analysis suffers from poor sensitivity. Chemical derivatization emerges as an indispensable technique in HPLC analysis to enhance its performance. Considering the structural characteristics of triterpenoids, various derivatization reagents such as acid chlorides, rhodamines, isocyanates, sulfonic esters, and amines have been employed for the derivatization analysis of triterpenoids. This review comprehensively summarized the research progress made in derivatization strategies for HPLC detection of triterpenoids. Moreover, the limitations and challenges encountered in previous studies are discussed, and future research directions are proposed to develop more effective derivatization methods.

1. Introduction

Triterpenoids are a class of botanical secondary metabolites that are condensed from six isoprene units. They are widely existing in nature in the form of aglycones or glycosides. According to the type of carbon skeleton, they can be divided into tetracyclic triterpenoids (including dammarane-type, lanostane-type, cycloartane-type, tirucallane-type and cucurbitane-type triterpenoids) and pentacyclic triterpenoids (including oleanane-type, ursane-type, lupane-type, and friedelane-type triterpenoids), as shown in Fig. 1. The chemical structures of triterpenoids are very similar, with the characteristic of no conjugated system or low conjugation degree. It is noteworthy that C-3, C-28, and other positions of the carbon skeleton contain free hydroxyl and carboxyl groups (for aglycones), which are also the glycoxidation sites (for glycosides). Studies have shown that triterpenoids have significant anti-HIV [1], anti-cancer [2], antiviral [3], anti-inflammatory [4], and metabolic regulation [5] activities with amazing applications in medicine, food, and cosmetics industries.

Given the medicinal importance of triterpenoids, the detection of triterpenoids has always been a research hotspot in the field of analytical chemistry. A variety of methods have been reported for triterpenoid analysis, such as colorimetry [6], capillary electrophoresis (CE) [7], infrared spectroscopy (IR) [8], thin-layer chromatography (TLC) [9], gas chromatography (GC) [10], high-performance liquid chromatography (HPLC) [11–13] and immunoassay [14]. Among them, HPLC has become the predominant technology for triterpenoid analysis with fast analysis speed, high sensitivity, high selectivity, good repeatability, and high sample universality. Unfortunately, achieving effective separation of triterpenoids by conventional HPLC methods is challenging due to the similar structure and polarity, necessitating significant

Peer review under responsibility of Xi'an Jiaotong University.
* Corresponding author.
** Corresponding author.
E-mail addresses: pluo@must.edu.mo (P. Luo), qingls@cib.ac.cn (L.-S. Qing).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.jpha.2023.07.004

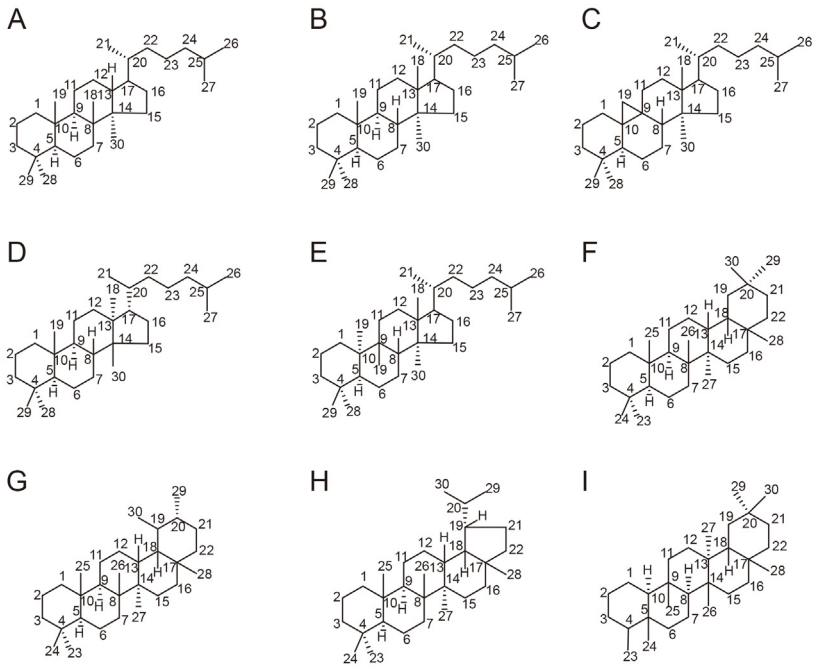

Fig. 1. Distinct skeletons of triterpenoids. (A) Dammarane-type, (B) lanostane-type, (C) cycloartane-type, (D) tirucallane-type, (E) cucurbitane-type, (F) oleanane-type, (G) ursane-type, (H) lupane-type, and (I) friedelane-type.

improvements in analytical methods. Moreover, triterpenoids often lack characteristic ultraviolet (UV) absorption groups and chromophores, along with low ionization efficiency in mass spectrometry, resulting in an extremely limited detection response. Especially for the low abundance of triterpenoids in biological samples, accurate quantitative detection is more difficult.

To address these issues, derivatization techniques have been introduced to HPLC-based detection of triterpenoids. Chemical derivatization, as an indispensable technique in the field of chromatographic analysis, has the advantages of improving separation, sensitivity, and selectivity. The combination of derivatization technology with GC [15] and LC [16] plays a vital role in the fields of pharmaceutical analysis [17], environmental monitoring [18], metabolomics [19], and food safety [20]. Appropriate derivatization of triterpenoids not only improves the chromatographic behavior but also significantly enhances the instrument response, thereby greatly enhancing its application potential in trace analysis.

To date, there have been many research articles but only three reviews on the analysis of triterpenoids [21–23]. However, the published reviews lacked a comprehensive description of the derivatization technology and were rather general in nature. This review focuses on various derivatization strategies that can improve chromatographic performance and detection sensitivity during high performance liquid chromatography-ultraviolet-fluorescence detector-mass spectromatry (HPLC-UV/FLD/MS) analysis of triterpenoids. It should be noted that chemical derivatization is not applicable in prep-HPLC purification and HPLC-evaporative light scattering detection (ELSD) analysis. Although ELSD is a common detector for weak-UV-absorbing analytes under equal or gradient elution modes [24], chemical derivatization cannot change its inherent disadvantages, such as low sensitivity, poor baseline stability, cumbersome parameter setting, and nonlinear response behavior [25]. Thus, neither prep-HPLC purification nor HPLC-ELSD analysis of triterpenoids has been discussed in this review. The derivatization strategies are based on the following three principles: (1) introduce chromophores or conjugate units to meet the requirements of HPLC-UV detection, (2) introduce fluorophore units for HPLC-FLD detection, and (3) introduce ammonia nitrogen fragments for HPLC-MS detection (Table S1) [26–68]. Detailed examples are systematically listed and commented according to different sites of derivatization reactions, including hydroxyl, carboxyl, and other functional groups (e.g., double bond and carbonyl group) at positions C-3, C-28, etc., as shown in Fig. 2. The principles, advantages, and limitations of each method are thoroughly discussed, hoping to inspire further research into the development of superior derivatization reagents and procedures.

2. Derivatization of hydroxyl groups in triterpenoids

Due to the weak UV absorption and electrospray ionization efficiency of C-3 hydroxyl group, it is difficult to effectively detect triterpenoids by conventional chromatography. Thus, it has to be determined after chemical derivatization. The nucleophilicity of hydroxyl group is still too weak to be derivatized, especially in the aqueous phase. Therefore, it needs to be labeled with strong electrophile reagents. The derivatization of hydroxyl groups in triterpenoids reported in the literature mainly includes acyl chlorides [27,31,32], rhodamines [37–39], isocyanates [40], and other strong acylation reagents.

2.1. Acyl chloride-type derivatization reagents

Acyl chlorine-derivatized reagents, such as benzoyl chloride (BC), *p*-nitrobenzoyl chloride (PNBC), 3,5-dinitrobenzoyl chloride (3,5-DNB), etc., contain benzoyl chromophores, which can significantly improve the instrument response at specific wavelength. With the increase in the p-π conjugate system of the derivatization

reagent, the instrument response of the derivative increases gradually. In addition, the stronger electron-withdrawing of the group linked to the carbonyl group in the derivatization reagent will reduce the electron cloud density of the carbonyl group more, thus enhancing the reaction activity with the nucleophile and finally obtaining a higher derivative yield. The reactions are usually carried out in pyridine solution. On the one hand, the esterification reaction requires a weak alkaline environment, and on the other hand, the derivatization reagent can be avoided from hydrolysis in aqueous solution. In practice, it is necessary to add excessive derivatization reagent to obtain a higher yield.

2.1.1. Benzoyl chloride-type derivatization reagents

Ginsenosides are dammarane-type tetracyclic triterpenoid saponins that play an important role in immune regulation, central nervous system disease, and cerebrovascular diseases [69–71]. Panaxadiol (PD), as the hydrolysate of ginsenoside, is often determined as an indicator of total ginsenosides. It was reported that PD could be fully derivatized by BC in a water bath at 80 °C for 2 h [26]. In addition to the derivatization of the hydroxyl group of the aglycone moiety, the hydroxyl group of the sugar chain can also directly participate in the reaction. By comparing the derivatization reagents BC, 4-nitrobenzoyl chloride, 3,5-DNB and β-naphthyl chloride, BC was selected as the best derivatization reagent for ginsenoside to generate a single derivative [27]. More than 10-fold amounts of BC were used in the pyridine solution. There was no hydroxyl characteristic peak observed in the infrared spectrum, indicating that all the hydroxyl groups of ginsenoside were benzoylated. The method is simple to operate and can be used to distinguish white ginseng from the root hairs of ginseng by HPLC pattern analysis.

Astragaloside IV (AS-IV) is a cycloartane-type triterpenoid saponin that is considered the primary active ingredient of *Astragali Radix* [72]. Due to the lack of characteristic absorption groups in its molecular structure, it is difficult to analyze directly by UV detection. Based on this, the use of excessive BC to derivatize AS-IV not only improves the sensitivity of AS-IV but also significantly improves the chromatographic behavior [28]. A comparative detection of AS-IV was performed between HPLC-ELSD and derivative HPLC-UV. After derivatization by BC, the results of the derivative HPLC-UV method were consistent with those of the HPLC-ELSD method, which overcame the limitations of equipment [29]. It is worth noting that certain sample pretreatment is needed due to the complexity of natural products. For example, liquid-liquid extraction (LLE), macroporous adsorption resin column chromatography, and solid-phase extraction were successively used to purify the AS-IV extract to reduce the influence of the complex matrix [30].

Other benzoyl chloride-type reagents containing more chromophores can further enhance the sensitivity of triterpenoid detection, including PNBC, 3,5-DNB and 4-(4,5-diphenyl-1H-imidazole-2-yl) benzoyl chloride (DIB-Cl). Some derivative HPLC-UV studies have been reported with effective diminishment of matrix interference and great improvement of detection sensitivity, such as the determination of eruboside B at 260 nm in garlic after derivatization by PNBC [31], the determination of oleanolic acid at 254 nm in *Achyranthes bidentata Bl* after derivatization by PNBC [32], the determination of PD and panaxatriol (PT) at 230 nm in red ginseng and Shengmai injection after derivatization by 3,5-DNB [33], and the determination of AS-IV at 230 nm in *Astragali Radix* after derivatization by 3,5-DNB [34]. What is particularly interesting is that DIB-Cl has been used as a derivatization reagent for the determination of betulinic acid, ursolic acid, and oleanolic acid in rosemary extract [35]. The derivatization reaction was completed after standing for 5 min at room temperature. The larger conjugated structure originating from DIB-Cl made the detection sensitivity of the derivative HFLC-FLD method more than 2000 times higher than that of the nonderivative HPLC-UV method [73], reaching a low detection limit comparable to that of liquid chromatography tandem mass spectometry (LC-MS/MS).

2.1.2. Dansyl chloride

In addition to the benzoyl chlorides mentioned above, dansyl chloride (DC) is commonly used in amine derivatization and plays an important role in protein detection [74]. It is often used for the derivatization of phenolic hydroxyl and alcohol hydroxyl groups [75]. A novel HPLC-FLD method for the detection of triterpenoids was developed [36]. DC was used in pyridine solution to derivatize 12 triterpenoids in plant secretions. The linear ranges of triterpenoids were 0.12–2500 μg/mL ($r = 0.9969$–0.9995), and the limit of detections (LOD) was less than 0.45 ng/mL.

2.2. Rhodamine-type derivatization reagents

Since the conjugated system is introduced by the acyl chloride derivatization reagent, the LODs of the analytes are greatly improved by UV or FLD detectors [35,73]. However, the derivative is still not suitable for MS detection. This is due to the low ionization efficiency of the derivative without the ammonia nitrogen fragment, resulting in limited signal enhancement on the MS detector. To achieve lower detection limits by LC-MS, especially for trace analysis in biological samples, the use of rhodamine-type reagents for derivatization came into being.

Rhodamine is a synthetic dye with high fluorescence and photostability that was originally used as a fluorescent stain for cells [76,77]. As a quaternary ammonium, rhodamine has high ionization efficiency in electrospray ionization (ESI) positive ion mode. Thus, it can be used as a derivatization reagent for amine and hydroxyl detection with a clear signal improvement in LC-MS analysis [78,79]. To enhance the reactivity of the derivatization reagent, the carboxyl group of rhodamine is often first converted into a more electrophilic amide with N, *N*-carbonyl diimidazole (CDI) in the presence of catalyst 4-dimethylaminopyridine (DMAP). In addition, microwave-assisted derivatization has been adopted to obtain a higher yield. For example, a ultra high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS) method was reported for PD detection after derivatization by rhodamine B (RB). The derivatization reaction was carried out in two steps (Fig. 3A) [37]. RB was first activated by CDI/DMAP and then reacted with the ginseng powder extract pretreated by ultrasonic assisted dispersive liquid-liquid microextraction (UA-DLLME). The derivatization reaction could be completed in 30 min with the assistance of microwaves, which greatly increased the yield rate and shortened the reaction time compared with conventional water bath heating. The quantitative analysis of PD could be completed within 3 min after chromatographic separation. The LOD was as low as 4.0 ng/L, which improved the sensitivity by at least 250 times compared to that without derivatization [80].

There were two kinds of cleavage pathways to produce different fragment ions of rhodamine derivative. One was the cleavage of the bond outside the oxygen atom in the ester bond, resulting in the rhodamine main structure fragment ion; the other was the cleavage of the bond between the benzene ring and the carbonyl group (Fig. 3B) [39].

RB not only has a slow reaction rate due to the steric hindrance of the 2′-carboxyl group but is also easy to convert into a lactone structure by the loss of a positive intramolecular charge, resulting

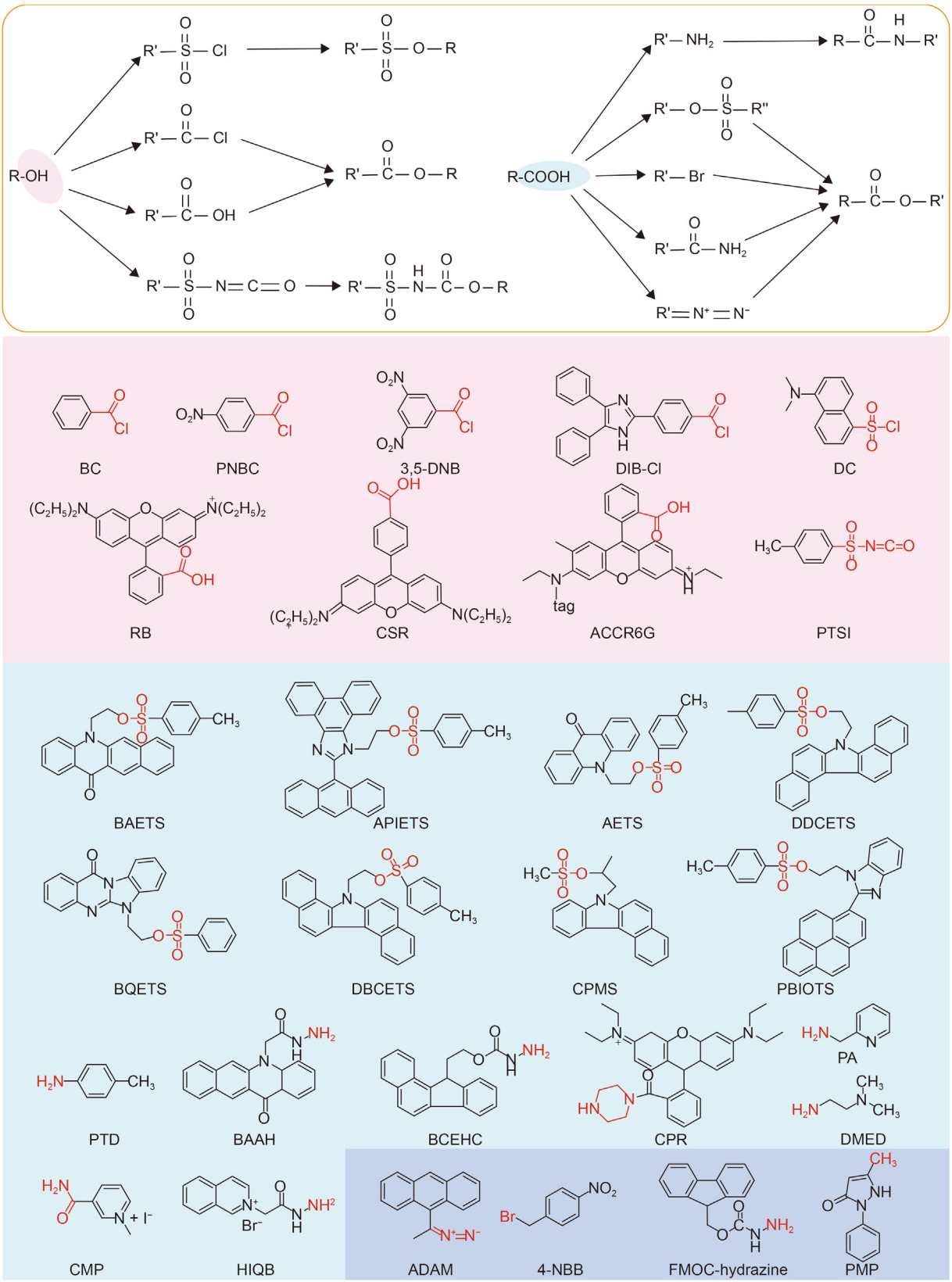

Fig. 2. Common derivatization strategies based on hydroxyl and carboxyl reactions and corresponding derivatization reagents. BC: benzoyl chloride; PNBC: *p*-nitrobenzoyl chloride; 3,5-DNB: 3,5-dinitrobenzoyl chloride; DIB-Cl: 4-(4,5-diphenyl-1H-imidazole-2-yl) benzoyl chloride; DC: dansyl chloride; RB: rhodamine B; CSR: 4′-carboxy-substituted rosamine; ACCR6G: 3-*N*-(D_0-/D_3-methyl-, and D_0-/D_5-ethyl-)-2′-carboxyl chloride rhodamine 6 G; PTSI: *p*-toluenesulfonyl isocyanate; BAETS: 2-(5-benzoacridine) ethyl-*p*-toluenesulfonate; AETS: acridone-9-ethyl-*p*-toluenesulfonate; APIETS: 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d] imidazole-1yl) ethyl 4-methylbenzenesulfonate; DDCETS: 2-(12,13-dihydro-7H-dibenzo [a, g] carbazol7-yl) ethyl4-methylbenzenesulfonate; BQETS: benzimidazo-[2,1-b] quinazolin-12(6H)-one-5-ethyl-*p*-toluenesulfonate; DBCETS: 2-(7H-dibenzo [a, g] carbazole-7-yl) ethyl-4-methylbenzenesulfonate; PBIOTs: 2-(2-(pyren-1-yl)-1H-benzo[*d*]imidazole-1-yl)-ethyl-*p*-toluenesulfonate; CPMS: 1-(9H-carbazol-9yl) propan-2-yl-

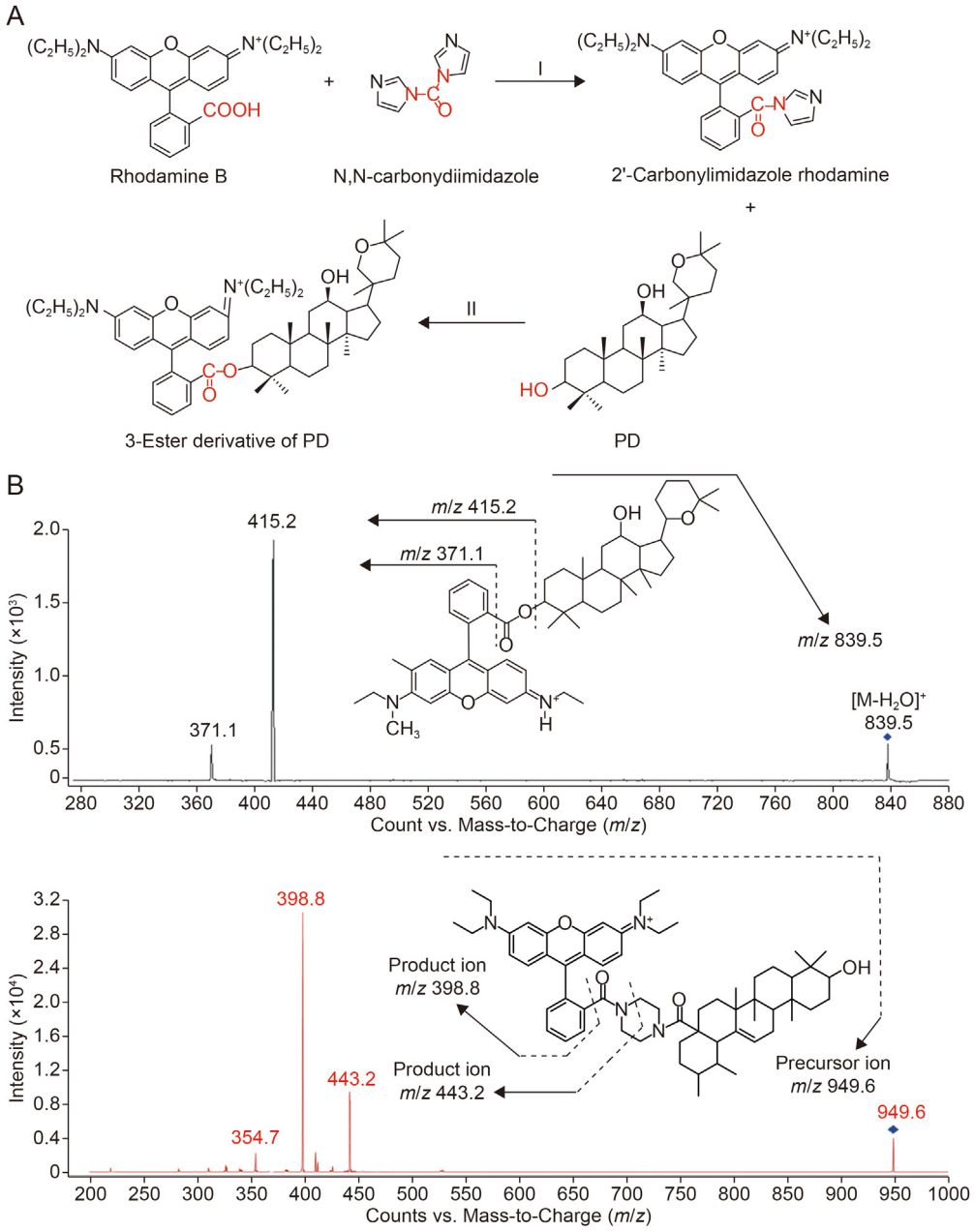

Fig. 3. Derivatization of triterpenoids with rhodamine. (A) Derivatization of panaxadiol (PD) by rhodamine B. (B) Fragmentation pathway of rhodamine derivatives in electrospray ionization (ESI) positive ion mode. Reprinted from Refs. [37,39] with permission.

in decreased reactivity. 4′-Carboxy-substituted rosamine (CSR), another rhodamine derivatization reagent, is a good substitute for RB. A simple and environmentally friendly UHPLC-MS/MS method was developed to determine 20(S)-protopanaxadiol (PPD) and 20(S)-protopanaxatriol (PPT) in plasma after derivatization by CSR [38]. This reaction had two obvious advantages: (1) it could be quickly completed in 5 min with the assistance of microwaves; (2) it was a one-step reaction, which no longer required the participation of CDI and DMAP.

In recent years, sample derivatization based on stable isotope labelling has emerged for LC–MS analysis, showing great potential in pharmaceutical analysis and food safety monitoring [81–83]. A series of quadruple stable isotopes, 3-*N*-(D_0-/D_3-methyl-, and D_0-/D_5-ethyl-)-2′-carboxyl chloride rhodamine 6 G (ACCR6G), were designed and synthesized to improve the chromatographic performance and MS response of PD and PT. This strategy first introduced an isotopic methyl or ethyl tag to PD and PT from samples and an analogue propyl tag to PD and PT from the standards, respectively. Then two categories of derivatives were mixed equally for the subsequent magnetic dispersive solid-phase extraction before UPLC-MS/MS determination (Fig. 4) [39]. In this method, the detection sensitivity of PD and PT derivatives was increased by more than 200 times compared with that of unlabeled forms.

methanesulfonate; PTD: *p*-toluidine; BAAH: 2-(12–benzo[b]acridin-5-(12H)-yl)-acetohydrazide; BCEHC: 2-(5H-benzo[a]-carbazol-11(6H)-yl)-ethyl-hydrazine-carboxylate; CPR: 2′-carbonyl-piperazine rhodamine B; PA: 2-picolylamine; DMED: *N*,N-dimethylethylenediamine; CMP: 3-carbinol-1-methylpyridinium iodide; HIQB: (2-(2-hydrazinyl-2-oxoethyl) isoquinolin-2-ium bromide; ADAM: 9-anthryldiazomethane; 4-NBB: 4-nitrobenzyl bromide; PMP: 1-phenyl-3-methyl-5-pyrazolone.

2.3. Isocyanate-type derivatization reagents

Isocyanates are another derivatization reagent that can be used for MS detection. Different from the positive ion detection mode of rhodamine derivatives, isocyanate derivatives are usually detected in negative ion mode. *P*-toluene sulfonyl isocyanate (PTSI) was reported to label hydroxyl groups in triterpenoids, and the resulting esters can improve ionization. The derivatization reaction was rapid and quantitative, completed within a few minutes at room temperature, followed by the addition of ammonium acetate to terminate the reaction [40,41].

Betulin is a neutral pentacyclic triterpene with two hydroxyl groups. It is difficult to directly detect because of its low ionization efficiency in ESI/MS. The researchers attempted a direct analysis without derivatization, and unfortunately no detection signal was observed. After comparing several different derivatization reagents, including acetic anhydride, N, O-bis (trimethysilyl) trifluoroacetamide, *o*-phthalic anhydride, perfluoropropionic anhydride, and PTSI, PTSI was selected as the best one to establish an LC-MS/MS method for the determination of betulin in plasma, since it could generate a derivative with higher ionization efficiency [40]. Similarly, another derivatization determination of lupeol was also performed by PTSI to monitor its dynamic change in rat plasma [41].

3. Derivatization of carboxyl groups in triterpenoids

Most triterpenoids also have carboxyl groups in addition to hydroxyl groups. Thus, it is a good choice to prepare carboxylic ester derivatives of triterpenoids by introducing high response units for FLD and MS detection. Sulfonate derivatization design is most commonly reported [43,44,51]. This method can not only enhance sensitivity but also improve chromatographic separation, making it more advantageous for simultaneous quantification of multiple triterpenoids with similar structure. In addition, aromatic amines, hydrazides, and quaternary ammonium salts are also used for carboxyl derivatization [58,60,64]. Due to the introduction of ammonia nitrogen, the signal of derivatives in MS analysis is also greatly improved, which is particularly beneficial for the detection of trace analytes in biological samples.

3.1. Sulfonic ester-type derivatization reagents

Sulfonic esters are common alkylation reagents [84]. The common structural feature of these reagents is that an aromatic ring planar group is connected to a large conjugate structure through an ethyl sulfonate link chain. The sulfonate part, as a good leaving group, can generate a carbocation nucleophilic substitution reaction with the carboxyl group, thus introducing a high fluorophore. In other words, the reaction of the sulfonates with the carboxylic acid is actually the alkylation of the carboxyl group. Due to the introduction of high fluorophores, sulfonate derivatives can be easily detected by FLD with improved sensitivity. This is a simple and quick derivatization reaction, which can be completed by heating at 90 °C for approximately 30 min. The derivatization reaction is usually carried out in the cosolvent DMF, which can not only yield a higher fluorescence response but also avoid the precipitation of lower polarity analytes. To obtain higher reaction efficiency, K_2CO_3 is usually added as a catalyst.

There are abundant triterpene acids with similar structures in plants, especially some isomers that appear in pairs [85]. Due to the small structural differences, these compounds are not well separated under conventional chromatographic conditions. Sulfonation derivatization provides an excellent solution to improve sensitivity and chromatographic separation performance. For example, oleanolic acid/ursolic acid and maslinic acid/corosolic acid are both very common pairs of epimers with low chromatographic resolution. After derivatization by 2-(5-benzoacridine) ethyl-*p*-toluenesulfonate (BAETS), not only was the response of the detector greatly improved, but their chromatographic behaviors were also significantly improved [42]. Another HPLC-FLD method was reported for the determination of five triterpene acids (betulinic acid, betulonic acid, maslinic acid, ursolic acid, and oleanolic acid) within 16 min after labelling with the fluorescent derivatization reagent acridone-9-ethyl-*p*-toluenesulfonate (AETS) [43]. Similarly, a large number of studies have been reported using other derivatization reagents to achieve simultaneous detection of multiple triterpene acids, such as 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d] imidazole-1yl) ethyl 4-methylbenzenesulfonate (APIETS) [44], 2-(12,13-dihydro-7H-dibenzo [a, g] carbazol7-yl) ethyl4-methylbenzenesulfonate (DDCETS) [45], benzimidazo-[2,1-b] quinazolin-12(6H)-one-5-ethyl-*p*-toluenesulfonate (BQETS) [46], BAETS [47,48] and 2-(7H-

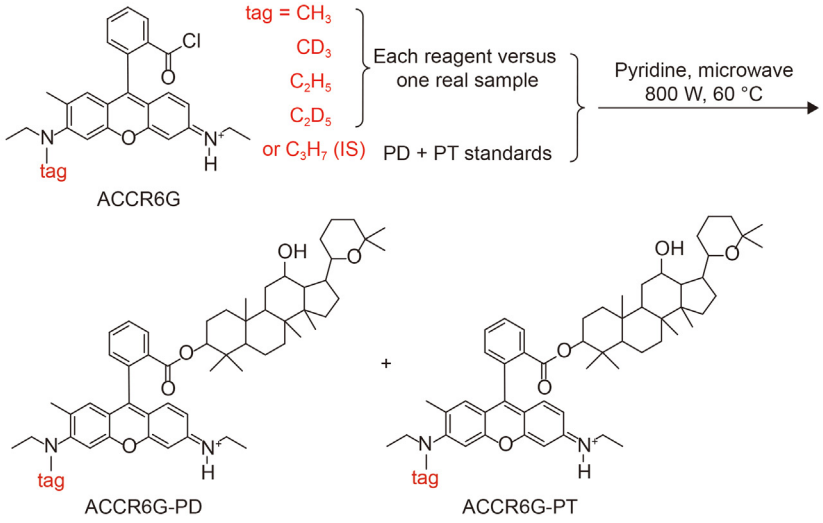

Fig. 4. Derivatization of panaxadiol (PD) and panaxatriol (PT) with 3-*N*-(D_0-/D_3-methyl-, and D_0-/D_5-ethyl-)-2′-carboxyl chloride rhodamine 6 G (ACCR6G) based on stable isotope labelling. Reprinted from Ref. [39] with permission.

dibenzo [a, g] carbazole-7-yl) ethyl-4-methylbenzenesulfonate (DBCETS) [49,50]. These works fully demonstrated the great advantages of the sulfonic ester derivatization strategy in improving the chromatographic separation, sensitivity, and universality of triterpene acids.

In addition to the above analysis of plant extracts, semiautomatic extraction derivatization has been used to study the pharmacokinetics of seven triterpene acids (tormentic acid, ursolic acid, oleanolic acid, betulonic acid, betulinic acid, corosolic acid, maslinic acid) with only 20 μL plasma required [51]. The protocol of this method was that the blood was pumped into a modified micro-syringe with cosolvent, catalyst and derivatization reagent through a tail vein puncture. The circulating water was passed through the outer cannula of the syringe to provide heat for the reaction. After the reaction, acetonitrile was added to precipitate the protein. The triterpene acids were derivatized by 2-(2-(pyren-1-yl)-1H-benzo[*d*]imidazole-1-yl)-ethyl-*p*-toluenesulfonate (PBIOTs). The whole pretreatment procedure was more conveniently finished within 26 min. Seven analytes were rapidly separated on an RP-18 analytical column within 30 min and quantified by a fluorescence detector. An artificial neural network combined with a genetic algorithm was applied to optimize the derivatization conditions, achieving high sensitivity (LODs: 0.67–1.08 ng/mL). This semiautomatic extraction derivatization method overcame the shortcomings of a large blood sample volume and strong injury to animals in the traditional pharmacokinetic blood collection model [86,87], which was especially suitable for a large number of subbiosample analyses.

According to chemical reaction principles, other types of natural products containing carboxyl groups (e.g., fatty acids and phenolic acids) can also react with derivatization reagents. To avoid interference, an appropriate identification capacity is needed. The most accurate identification is mediated via comparison to retention time and MS^n fragment with standards [88–90]. However, the penetration rate of high-end instruments is not high, such as high-resolution MS (e.g., Orbitrap, quadrupole time-of-flight (Q-TOF), and Fourier transform ion cyclotron resonance (FT-ICR)), which to a certain extent increases the difficulty of analysis. To solve this problem, a chiral derivatization reagent, 1-(9H-carbazol-9yl) propan-2-yl-methanesulfonate (CPMS), was easily synthesized and applied to the detection of six triterpenoid acids (maslinic acid, corosolic acid, betulinic acid, oleanolic acid, ursolic acid, and betulonic acid), which could realize the effective separation of isomers and avoid the interference of chromatographic peaks of fatty acid derivatives. It was revealed that each triterpene acid produced two chromatographic derivative peaks with similar peak areas (Fig. 5) [52], while the fatty acid produced only one peak. The paired peaks confirmed each other to ensure the accuracy of analytes. The quantitative analysis using the bimodal phenomenon needs to meet the following three elements, none of which is dispensable: (1) the derivatization reagent contains chiral carbon atoms, (2) the analyte has chiral carbon atoms (tetracyclic and above), and (3) the analyte has good hydrophobicity.

3.2. Amine-type derivatization reagents

The reaction using sulfonic esters to derivatize triterpenoid acids must occur in an organic environment. However, it is not suitable for the direct derivatization of aqueous biological samples (plasma, urine, bile, etc.). Thus, the amine derivatization method based on carbodiimide-mediated condensation is a good alternative to directly react in the aqueous phase, greatly simplifying the sample preparation process. Highly visible derivatization reagents with amino groups, including hydrazine and amine reagents, are often used to label triterpenoid acids from amide bonds for LC-UV or LC-FLD detection. Furthermore, the introduction of ammonia nitrogen makes it particularly suitable for detecting trace metabolites in biological samples by LC-MS.

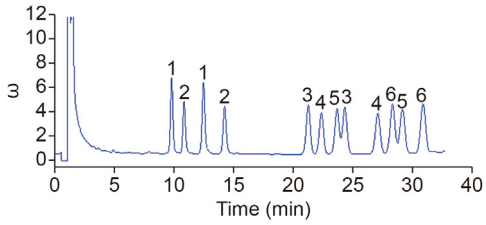

Fig. 5. Chromatogram of triterpene acids derivatized by chiral derivatization reagent. Peaks 1, 2, 3, 4, 5 and 6 are maslinic acid, corosolic acid, betulinic acid, oleanolic acid, ursolic acid, and betulonic acid, respectively. Reprinted from Ref. [52] with permission.

Carbodiimide-mediated condensation reaction is a simple method to synthesize amide in aqueous solution [91–94]. Derivatization reagents can rapidly label carboxylic acids under mild conditions by carbodiimide (i.e., EDC and DCC) in the presence of acylation activators, (i.e., DMAP [95] and HOBt [96]). Otherwise, the derivatization reaction will need to be assisted by heating or more than 1 h to complete without acylation activators. For example, *p*-toluidine is often used to derivatize triterpene acids without DMAP or HOBt, yielding derivatives with aromatic UV absorption for HPLC-UV detection [53–57]. The derivatization reaction was usually performed in a water bath heated at 30 °C for 3 h or 80 °C for 1 h.

In addition to derivative HPLC-UV detection [53–57], an HPLC-FLD method for the determination of four triterpenoid acids (oleanolic acid, ursolic acid, maslinic acid, and betulinic acid) was established by using 2-(12-benzo[b]acridin-5-(12H)-yl)-acetohydrazide (BAAH) as the derivatization reagent [58]. Plasma samples could be directly reacted with BAAH without additional extraction or precipitation pretreatment. This method overcame the limitation of 'anhydrous conditions' and greatly simplified the experimental operation, making it suitable for high-throughput serum sample screening. After that, a comparison HPLC-FLD test for the determination of five triterpenoid acids (asiatic acid, betulinic acid, corosolic acid, maslinic acid and oleanolic acid) was reported by precolumn derivatization with two different labelling reagents, 2-(5H-benzo[a]-carbazol-11(6H)-yl)-ethyl-hydrazine-carboxylate (BCEHC) and BAAH [59]. The results revealed that both labelling reagents could favorably satisfy the analytical requirements with their own advantages. BCEHC had lower limits of detection (0.42–1.35 μg/L vs 0.81–3.03 μg/L), while BAAH had a shorter retention time (8 min vs 11.5 min).

LC-MS is the most sensitive detection method for ammonia derivatives. Accurate and sensitive LC-MS/MS determination of oleanolic acid and maslinic acid in human urine and serum was achieved after derivatization with 2-picolylamine (PA) [60]. Another UPLC-MS/MS determination of oleanolic acid and ursolic acid in a pharmacokinetic study was also reported using the labelling reagent 2′-carbonyl-piperazine rhodamine B (CPR) [61].

When LC-MS analysis is performed, it is usually necessary to add an appropriate internal standard to correct errors in sample preparation and overcome possible ion suppression in MS detection. The use of isotope labeled derivatization reagent to generate internal standard is the optimal method. For example,

an LC-MS/MS method was developed to determine two pentacyclic triterpenoids (oleanolic acid and ursolic acid) using deuterated-N, N-dimethylethylenediamine (DMED-d_4)-derivatized standards as internal standards [62]. Similarly, 3-carbinol-1-methylpyridinium iodide (CMP) and CMP-d_3 were used to label triterpene acid in another study using stable isotope coding techniques [63]. Isotope labeling method has unparalleled advantages in correcting matrix effects, eliminating experimental errors, and improving accuracy and precision. However, the drawbacks are also evident: the isotope labeled derivatization reagents are expensive and even not commercially available, which limits its application.

Reference standards are necessary for quantitative analysis. However, in practical work, some reference standards often cannot be obtained or are expensive. Thus, the development of analytical methods free of reference standards has always been a hot topic. Quantitative analysis of multiple components via a single marker method provides one solution [97,98], while the detection of equivalent ions after derivatization provides another solution. Based on the ion equivalence strategy, a quaternary ammonium cationic derivatization reagent, (2-(2-hydrazinyl-2-oxoethyl) isoquinolin-2-ium bromide (HIQB), was synthesized to derivatize seven available triterpenoid acids (pachymic acid, polyporenic acid C, dehydrotumulosic acid, 16α-hydroxytrametenolic acid, dehydrotrametenolic acid, 3-o-acetyl-16α-hydroxydehydrotrametenolic acid, and poricoic acid B). Then, a novel LC-MS/MS method was developed using oleanolic acid as an internal standard (Fig. 6) [64]. Two kinds of fragment ions, i.e., *m/z* 130 and 170, were generated by the derivatives and were thus used as the quantitation ion and qualitative ion, respectively. The results showed that the sensitivity of each of the seven triterpene acids after derivatization was significantly increased by 20–160 times. This derivatization method could determine a total of 53 and 19 triterpene acids in the *Poria cocos* extracts and madin-darby canine kidney (MDCK) cell bidirectional transport samples with an limit of quantification (LOQ) of 0.1 nM, respectively. It can be expected that this novel ion equivalence strategy has great potential for analyzing other analogues of triterpenoid acids under the following two preconditions: one is to ensure the same MS quantitative ion fragments produced by internal standard and analytes; the other is to prove that the ionization efficiency of derivatives is only related to the concentration of analytes.

3.3. Other reagents for derivatizing carboxylic acids

Benzyl bromide as a widely used alkylation reagent, it can generate electrophilic carbocations that react with the nucleophile carboxylic acid to form esters [99]. 4-Nitrobenzyl bromide (4-NBB) was reported to label carboxyl groups in triterpenoids. The electrophilicity of carbocation can be further enhanced by the electron-absorbing action of nitro group. For example, an HPLC-UV method was established by using 4-NBB to derivatize glycyrrhizic acid [65].

Diazomethane is another class of alkylating reagents for carboxylic acid derivatization. Under acidic conditions, the methylene group of diazomethanes is protonated and reacts with carboxylic acids to form esters [100,101]. Based on this, a fluorescent derivatization reagent, 9-anthraquinone diazomethane (ADAM), was synthesized to label triterpene acids (ursolic acid, oleanolic acid, and betulinic acid) in toluene medium [66]. The LOD measured by HPLC-FLD after derivatization was nearly 100 times lower than that measured by HPLC-UV before derivatization.

4. Derivatization of other functional groups

Triterpenoids containing carbonyl groups in the structure can directly react with quaternary ammonium reagents (i.e., Girard's reagent T and Girard's reagent P) to form oximes and hydrazones, thus enhancing the detection signal intensity [102,103]. Due to the influence of the spatial structure of the derivatization reagents, small molecules and monocarboxylic compounds are easier to be derivatized [104]. $NH_2OH·HCl$ is a nucleophile that can react rapidly with carbonyl groups to form easily ionized hydroxylase oxime under mild conditions [105]. The carbonyl group in triterpenoids was derivatized using $NH_2OH·HCl$ for ESI/MS detection in our team's previous work [106]. This method enhances the MS sensitivity of triterpenoids with low polarity, poor ionization and even no ionization. It can be used not only to detect carbonyl groups but also to detect hydroxyl groups, provided that the primary and secondary hydroxyl groups are oxidized to carbonyl groups in advance. If there is no carbonyl group in the analyte itself, it is also possible to obtain its carbonyl intermediate for derivatization to achieve quantitative analysis. For example, the double bond on the side chain of ginsenosides at C-17 could be oxidized to a carbonyl group by ozone and then reacted with FMOC-hydrazine to form the corresponding hydrazone under weakly acidic conditions (Fig. 7) [67].

In addition to the functional groups of the aglycone part, some special sugar groups of saponins can also be used as derivatization sites. 1-Phenyl-3-methyl-5-pyrazolone (PMP) is a typical reducing sugar derivatization reagent for LC-UV and LC-MS detection [107,108]. The advantage of the PMP-derivatized reaction is that it can be quantitatively reacted with reducing sugar under mild conditions to obtain a unique detectable product. For example, *D*-quinovose (6-deoxyglucose) is a specific monosaccharide in the triterpenoid glycosides of sea cucumber. Using *D*-quinovose as the measurement standard, triterpenoid glycosides were indirectly determined by HPLC-UV detection after PMP derivation in NaOH solution [68].

5. Sample pretreatment

Appropriate sample pretreatment of botanical extracts can not only enrich trace analytes, but also reduce matrix interference, thereby achieving the goal of improving the analytical performance. On the other hand, the derivatization process may generate new impurities. Moreover, excessive derivatization reagents and catalysts will not only decrease chromatographic performance but also pollute the analytical column. Therefore, it is very important to preprocess the samples in an appropriate way before and after derivatization.

The basic requirement of chromatographic analysis is to remove possible interfering impurities and simplify the chromatogram as much as possible. LLE, as a classic method, is widely used for sample pretreatment, such as the extraction of ginsenosides and astragaloside IV from crude extract using *n*-butanol [27,28]. LLE is highly universal and free of any special equipment. However, it requires a large number of toxic chemical solvents and is tedious, time-consuming and laborious to perform. Solid-phase extraction (SPE) is another commonly used sample pretreatment before derivatization using various fillers, including octadecyl-silylated silica gel, polystyrene/divinyl benzene polymer, alumina, hydrophilic adsorbents, and silica gel bonded with quaternary ammonium ions. For example, the use of a C_{18} column to purify saponins in garlic [31] and ginsenosides in plasma [67] and the use of Supelclean LC-SAX to purify triterpene acids in raspberry [66] have the advantages of

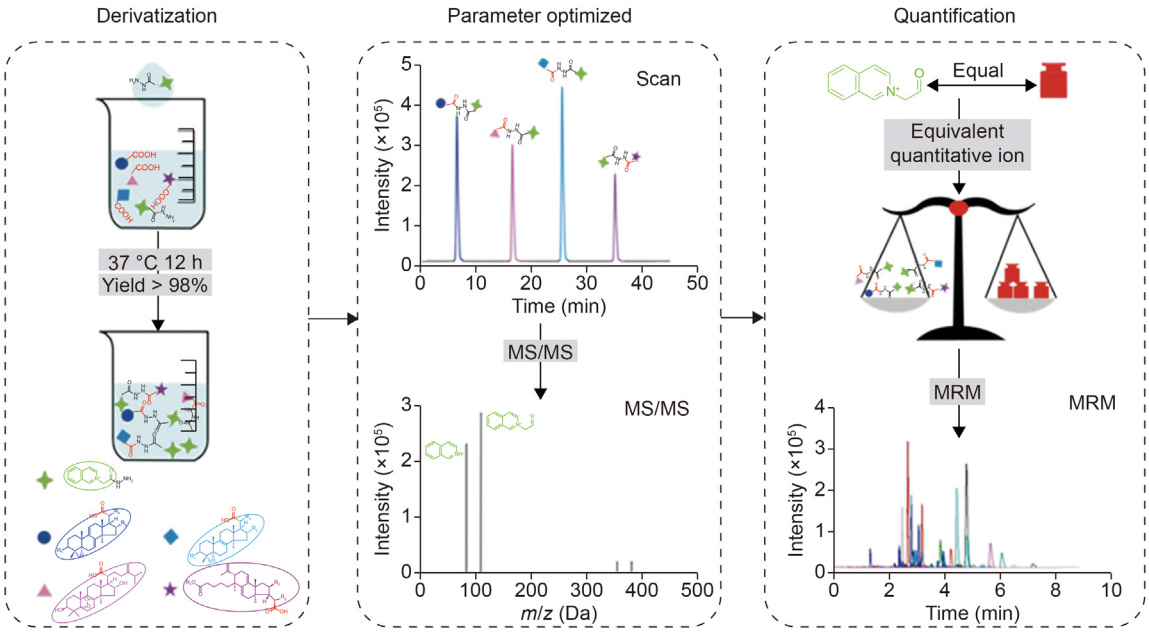

Fig. 6. Equivalent quantitative ion strategy with quaternary ammonium cation derivatization. Reprinted from Ref. [64] with permission. MS: mass spectromatry; MRM: multiple reaction monitoring.

organic solvent savings and convenient operation. However, the expensive price of SPE cartridges hinders their extensive use. In recent years, the application of dispersive liquid-liquid microextraction (DLLME) has overcome the problems of LLE and SPE in sample preparation [109,110]. A large amount of triterpenoids can be enriched in a short time with a small amount of organic solvent through a simple operation. In addition, the ultrasonic assistance technique is often used to improve the extraction efficiency of DLLME, for example, the extract of panaxadiol [37,39] and several other triterpene acids [45,46] from some Chinese medicinal herbs.

Given the excessive derivatization reagents and catalysts used, the chemicals have a serious impact on the detection results. Especially for mass spectrometry detection, excessive derivatization reagents would strongly suppress the signal intensity of the analytes [20]. Therefore, the pretreatment of triterpenoid derivatives is also crucial for HPLC analysis. The interference is significantly weakened by LLE treatment, such as the ginsenoside derivative extracted by chloroform [27]. Dual-ultrasonic-assisted dispersive liquid-liquid microextraction (Dual-UADLLME) was used for sample treatment before and after the derivatization of PPD and PPT. In view of the disadvantages of chlorine solvent used in traditional DLLME with high toxicity and low degradability, brominated solvent was used as a good substitute for sample extraction in this new Dual-UADLLME. Furthermore, a back-UADLLME procedure was designed to remove excess derivatization reagents and catalysts to avoid ion inhibition effects as much as possible (Fig. 8A) [38]. In recent years, dispersed solid-phase extraction (DSPE) has attracted extensive attention due to its easy operation, time savings, and environmental friendliness. Magnetic DSPE has been applied to extract PD derivative and PT derivative [39] and oleanolic acid derivative and ursolic acid derivative [61]. The principle of the method is to use magnetic functional nanomaterials (e.g., Fe_3O_4/graphene oxide) as the carrier of the derivatization reaction. After the reaction, the derivative is separated by an external magnet accompanying solvent cleaning for LC analysis (Fig. 8B) [111]. The use of magnetic dispersants can improve the specificity and selectivity of the separation.

6. Conclusions and outlook

Effective monitoring of triterpenoids is of great significance to guide natural medicine R&D and their clinical application. HPLC is

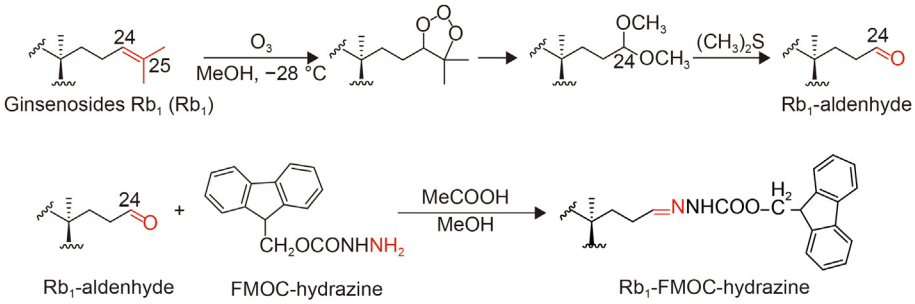

Fig. 7. Derivatization of ginsenoside Rb_1 by N-(9-Fluorenylmethoxycarbonyl)-hydrazine (FMOC-hydrazine). Reprinted from Ref. [67] with permission.

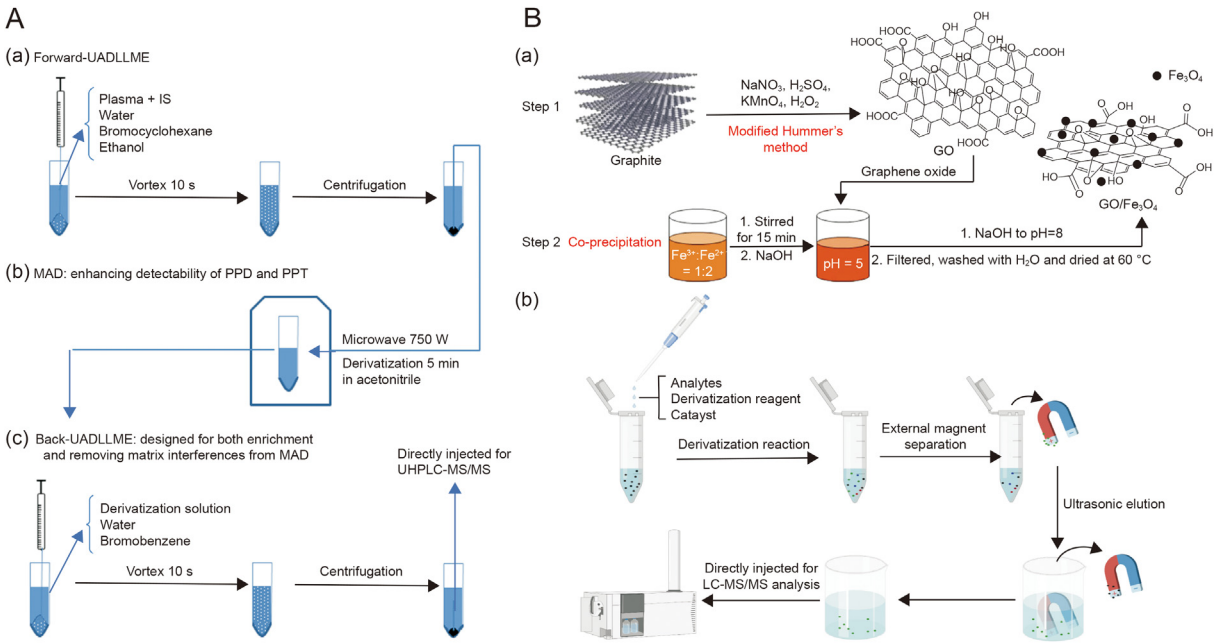

Fig. 8. Sample pretreatment operations. (A) Dual-ultrasonic-assisted dispersive liquid-liquid microextraction (Dual-UADLLME) used for sample pretreatment. (B) Magnetic dispersive solid-phase extraction (MDSPE) for sample pretreatment. (a) Preparation of magnetic graphene oxide (Fe_3O_4/GO) from graphene oxide (GO). (b) MDSPE sample processing process (By Figdraw, www.figdraw.com). Reprinted from Refs. [38,111] with permission. IS: internal standard; PPD: 20(S)-protopanaxadiol; PPT: 20(S)-protopanaxatriol; UHPLC-MS/MS: ultra high performance liquid chromatography coupled with mass spectrometry; LC-MS/MS: liquid chromatography tandem mass spectometry; MAD: microwave-assisted derivatization.

the preferred method for the analysis of triterpenoids. This review comprehensively summarizes multiple derivatization methods of triterpenoids for HPLC-UV/FLD/MS detection.

The specific derivatization reaction process is described in this review. It can be clearly observed that there are significant differences in reaction conditions among different studies. For example, for the derivatization of triterpenoid saponins, AS-IV could be completed at 4 °C for 12 h [29], while ginsenoside needed to be reacted at room temperature for 3 days [27]. Besides, the structures of some derivatives are not detailed. For example, when AS-IV was derivatized by excessive BC, there have been report of one hydroxyl group being derivatized [28] and there have also been reports of all hydroxyl groups being derivatized [30]. When using BC to derivatize ginseng triterpenes, all hydroxyl groups were derivatized [26,33]. In the derivatization by rhodamine, it was reported that only 3-OH other than 6-OH or 12-OH was involved in the reaction [37,39]. However, another work reported that only 20-OH participated in the reaction, while 3-OH did not, unless the amount of derivatization reagent was far excessive [38]. These phenomena indicate that the reactive sites and derivatives vary greatly with multiple factors, including the structures of derivatization reagent, the types of triterpenoids, the actual dosages, and the reaction time, etc. Relevant researchers have also noticed this aspect and usually optimize the reaction conditions through reasonable experimental design, mainly including single-factor experiments and response surface methodology (RSM). For example, the reaction parameters, including derivatization temperature, reaction time and reagent dosage, were optimized by RSM for the determination of four triterpene acids [44].

In recent years, stable isotope labelling derivatization has been developing increasing rapidly. It is a new type of sample pretreatment technique for LC-MS/MS with powerful capabilities in terms of sensitivity, accuracy, and qualitative and quantitative performance. However, limited by the synthesis and commercial sales of reagents, their application is still in a bottleneck period. It shows great potential applications in complex triterpenoids analysis and will be an interesting research field and direction of exploration. In addition, although a derivatization program has been developed for the analysis of triterpenoids by virtue of the 'bimodal phenomenon' of chromatography [52], mainstream derivatization methods still need to rely on mass spectrometry to ensure the accuracy of experimental results. A suggestion can be made in the selection of derivatization reagents: derivatization reagents containing ammonia nitrogen in the molecule should be selected as much as possible. Overall, with the continuous progress of basic research, more effective derivatization strategies are expected to be developed in the near future to improve the performance of triterpenoids in HPLC analysis.

CRediT author statement

Xiao-Feng Huang: Methodology, Validation, Investigation, Data curation, Writing - Original draft preparation; **Ying Xue**: Methodology, Validation, Data curation; **Li Yong**: Validation; **Tian-Tian Wang**: Data curation; **Pei Luo**: Writing - Reviewing and Editing, Supervision, Funding acquisition; **Lin-Sen Qing**: Writing - Reviewing and Editing, Investigation, Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was financially supported by Sichuan Science and Technology Program (Grant No.: 2022ZYD0026), Biological

Resources Program, Chinese Academy of Sciences (Grant No.: KFJ-BRP-008-007) and the Macao Science and Technology Development Fund (Grant No.: 0028/2019/AGJ).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.07.004.

References

[1] H. Wu, S.L. Morris-Natschke, X. Xu, et al., Recent advances in natural anti-HIV triterpenoids and analogs, Med. Res. Rev. 40 (2020) 2339–2385.
[2] C. Şoica, M. Voicu, R. Ghiulai, et al., Natural compounds in sex hormone-dependent cancers: The role of triterpenes as therapeutic agents, Front. Endocrinol. 11 (2021), 612396.
[3] P. Darshani, S.S. Sarma, A.K. Srivastava, et al., Anti-viral triterpenes: A review, Phytochem. Rev. 21 (2022) 1761–1842.
[4] R.S. Miranda, B.D.S.M. de Jesus, S.R. da Silva Luiz, et al., Antiinflammatory activity of natural triterpenes-An overview from 2006 to 2021, Phytother. Res. 36 (2022) 1459–1506.
[5] P. Yao, Y. Liu, Terpenoids: Natural compounds for non-alcoholic fatty liver disease (NAFLD) therapy, Molecules 28 (2022), 272.
[6] J. Luo, W. Cai, T. Wu, et al., Phytochemical distribution in hull and cotyledon of adzuki bean (*Vigna angularis* L.) and mung bean (*Vigna* radiate L.), and their contribution to antioxidant, anti-inflammatory and anti-diabetic activities, Food Chem. 201 (2016) 350–360.
[7] T. Ren, Z. Xu, Study of isomeric pentacyclic triterpene acids in traditional Chinese medicine of Forsythiae Fructus and their binding constants with β-cyclodextrin by capillary electrophoresis, Electrophoresis 39 (2018) 1006–1013.
[8] Q. Wang, H. Huang, Y. Wang, FTIR and UV spectra for the prediction of triterpene acids in *Macrohyporia cocos*, Microchem. J. 158 (2020), 105167.
[9] K. Naumoska, I. Vovk, Analysis of triterpenoids and phytosterols in vegetables by thin-layer chromatography coupled to tandem mass spectrometry, J. Chromatogr. A 1381 (2015) 229–238.
[10] L. Gadouche, A.S.M. Alsoufi, D. Pacholska, et al., Triterpenoid and steroid content of lipophilic extracts of selected medicinal plants of the mediterranean region, Molecules 28 (2023), 697.
[11] Y. Jaiswal, Z. Liang, A. Ho, et al., Tissue-based metabolite profiling and qualitative comparison of two species of *Achyranthes* roots by use of UHPLC-QTOF MS and laser micro-dissection, J. Pharm. Anal. 8 (2018) 10–19.
[12] Y. Li, S. Guo, Y. Zhu, et al., Comparative analysis of twenty-five compounds in different parts of *Astragalus membranaceus* var. *mongholicus* and *Astragalus membranaceus* by UPLC-MS/MS, J. Pharm. Anal. 9 (2019) 392–399.
[13] J. Xie, J. Xiong, L. Ding, et al., A efficient method to identify cardioprotective components of Astragali *Radix* using a combination of molecularly imprinted polymers-based knockout extract and activity evaluation, J. Chromatogr. A 1576 (2018) 10–18.
[14] C. Chen, L. Dai, H. Feng, et al., A new strategy for the preparation of antibody against natural glycoside: With astragaloside IV as an example, Fitoterapia 142 (2020), 104488.
[15] M. Ali Farajzadeh, N. Nouri, P. Khorram, Derivatization and microextraction methods for determination of organic compounds by gas chromatography, Trac Trends Anal. Chem. 55 (2014) 14–23.
[16] R. Dogra, M. Kumar, A. Kumar, et al., Derivatization, an applicable asset for conventional HPLC systems without MS detection in food and miscellaneous analysis, Crit. Rev. Anal. Chem. (2022) 1–21.
[17] R. Dogra, U.K. Mandal, Recent applications of derivatization techniques for pharmaceutical and bioanalytical analysis through high-performance liquid chromatogra-phy, Curr. Anal. Chem. 18 (2022) 217–243.
[18] S.N. Atapattu, J.M. Rosenfeld, Analytical derivatizations in environmental analysis, J. Chromatogr. A 1678 (2022), 463348.
[19] S. Zhao, L. Li, Chemical derivatization in LC-MS-based metabolomics study, Trac Trends Anal. Chem. 131 (2020), 115988.
[20] B. Qi, P. Liu, Q. Wang, et al., Derivatization for liquid chromatography-mass spectrometry, Trac Trends Anal. Chem. 59 (2014) 121–132.
[21] I.S. Azenha, M.M.Q. Simões, A. Mendes, et al., Adsorbents, mobile phases, and strategies for the chromatographic separation of betulinic, oleanolic, and ursolic acids, Biomass Convers. Biorefin. (2023) 1–32.
[22] Y. Wang, Y. Ma, L. Tao, et al., Recent advances in separation and analysis of saponins in natural products, Separations 9 (2022), 163.
[23] C. Xu, B. Wang, Y. Pu, et al., Techniques for the analysis of pentacyclic triterpenoids in medicinal plants, J. Sep. Sci. 41 (2018) 6–19.
[24] I. D'Acquarica, M.C. Di Giovanni, F. Gasparrini, et al., Isolation and structure elucidation of four new triterpenoid estersaponins from fruits of *Pittosporum tobira* ait, Tetrahedron 58 (2002) 10127–10136.
[25] N. Vervoort, D. Daemen, G. Török, Performance evaluation of evaporative light scattering detection and charged aerosol detection in reversed phase liquid chromatography, J. Chromatogr. A 1189 (2008) 92–100.
[26] L. Shi, W. Qin, Z. Zhu, et al., RP-HPLC determination of panaxadiol in ginseng with pre-column derivatization, Phys. Test. Chem. Anal. 46 (2010) 482–484.
[27] H. Besso, Y. Saruwatari, K. Futamura, et al., High performance liquid chromatographie determination of ginseng saponin by ultraviolet derivatisation, Planta Med. 37 (1979) 226–333.
[28] M. Yao, Y. Qi, K. Bi, et al., A precolumn derivatization high-performance liquid chromatographic method with improved sensitivity and specificity for the determination of astragaloside IV in *Radix* Astragali, J. Chromatogr. Sci. 38 (2000) 325–328.
[29] W. Liu, D. Cai, L. Liu, et al., HPLC determination of astragaloside IV in *Danggui Buxue* tablets, Chin. J. Pharm. Anal. 29 (2009) 1553–1555.
[30] L. Zhu, Z. Wang, D. Yang, Study on the determination method of the astragaloside IV in health food, J. Hyg. Res. 38 (2009) 203–206.
[31] E. Mochizuki, T. Yamamoto, Y. Mimaki, et al., Ultraviolet derivatization of steroidal saponin in garlic and commercial garlic products as *p*-nitrobenzoate for liquid chromatographic determination, J. AOAC Int. 87 (2004) 1063–1069.
[32] J. Sun, J. Zhang, X. Qin, et al., A pre-column derivatization HPLC-UV method for the determination of oleanolic acid in *achyranthes bidentata Bl*, Chin. J. Mod. Appl. Pharm. 27 (2010) 49–53.
[33] Z. Li, X. Xu, C. Feng, et al., Determination of ginsenediol and ginsentriol in Red ginseng and *Shengmai* Injection by high performance liquid chromatography derivatization, West China, J. Pharm. Sci. 14 (1999) 271–273.
[34] G. Wang, Z. Zhu, M. Lin, et al., A pre-column derivation HPLC method for the determination of astragaloside IV in *Radix* Astragali, Chin. J. Anal. Lab. 28 (2009) 108–111.
[35] M. Wada, Y. Tojoh, S. Nakamura, et al., Quantification of three triterpenic acids in dried rosemary using HPLC-fluorescence detection and 4-(4, 5-diphenyl-1H-imidazole-2-yl) benzoyl chloride derivatization, Lumin. 34 (2019) 130–132.
[36] A. Aksamija, C. Mathe, C. Vieillescazes, Liquid chromatography of triterpenic resins after derivatization with dansyl chloride, J. Liq. Chromatogr. Relat. Technol. 35 (2012) 1222–1237.
[37] X. Zhao, T. Lü, N. Wei, et al., Sensitive determination of panaxadiol using rhodamine B as sensitizing derivatization reagent by ultrahigh performance liquid chromatography triple quadrupole mass spectrometry, Chin. J. Anal. Chem. 42 (2014) 1629–1633.
[38] X. Zhao, T. Lv, S. Zhu, et al., Dual ultrasonic-assisted dispersive liquid-liquid microextraction coupled with microwave-assisted derivatization for simultaneous determination of 20(S)-protopanaxadiol and 20(S)-protopanaxatriol by ultra high performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1437 (2016) 49–57.
[39] S. Zhu, Z. Zheng, H. Peng, et al., Quadruplex stable isotope derivatization strategy for the determination of panaxadiol and panaxatriol in foodstuffs and medicinal materials using ultra high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1616 (2020), 460794.
[40] Z. Hu, N. Guo, Z. Wang, et al., Development and validation of an LC-ESI/MS/MS method with precolumn derivatization for the determination of betulin in rat plasma, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 939 (2013) 38–44.
[41] Z. Wang, Y. Wang, T. Yu, et al., An LC-ESI/MS/MS method for the determination of lupeol via precolumn derivatization and its application to pharmacokinetic studies in rat plasma, Biomed. Chromatogr. 35 (2021), e5005.
[42] G. Li, X. Zhang, J. You, et al., 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, Anal. Chim. Acta 688 (2011) 208–218.
[43] G. Li, J. You, C. Song, et al., Development of a new HPLC method with pre-column fluorescent derivatization for rapid, selective and sensitive detection of triterpenic acids in fruits, J. Agric. Food Chem. 59 (2011) 2972–2979.
[44] Z. Sun, J. You, C. Song, et al., Identification and determination of carboxylic acids in food samples using 2-(2-(anthracen-10-yl)-1H-phenanthro [9, 10-d] imidazol-1-yl) ethyl 4-methylbenzenesulfonate (APIETS) as labeling reagent by HPLC with FLD and APCI/MS, Talanta 85 (2011) 1088–1099.
[45] H. Wu, G. Li, S. Liu, et al., Simultaneous determination of six triterpenic acids in some Chinese medicinal herbs using ultrasound-assisted dispersive liquid-liquid microextraction and high-performance liquid chromatography with fluorescence detection, J. Pharm. Biomed. Anal. 107 (2015) 98–107.
[46] Y. Wang, Y. Suo, Y. Sun, et al., Determination of triterpene acids from 37 different varieties of raspberry using pre-column derivatization and HPLC fluorescence detection, Chromatographia 79 (2016) 1515–1525.
[47] T. Ma, N. Hu, Q. Zhang, et al., Simultaneous determination of five triterpenic acids in four *Corydalis*herb medicines by reversed-phase high performance liquid chromatography–fluorescence-mass spectrometer (RP-HPLC–FLD-MS) based on pre-column derivatization, J. Liq. Chromatogr. Relat.Technol. 41 (2018) 49–57.
[48] N. Hu, Y. Suo, Q. Zhang, et al., Rapid, selective, and sensitive analysis of triterpenic acids in *Hippophae rhamnoides* L. using HPLC with pre-column

fluorescent derivatization and identification with post-column APCI-MS, J. Liq. Chromatogr. Relat. Technol. 38 (2015) 451–458.
[49] W. Zhou, N. Hu, Y. Wang, et al., Determination of five kinds of triterpene acid in the fruits of seabuckthorn in Qinghai-Tibet plateau by HPLC-FLD-APCI/MS, West China, J. Pharm. Sci. 33 (2018) 535–538.
[50] E. Yuan, G. Li, H. Wu, et al., Determination of 4 tritepenoidic acids in pomegranate by precolumn fluorescent derivatization coupled with HPLC-FLD, Nat. Pro. Res. Dev. 27 (2015) 214–220.
[51] G. Chen, J. Li, C. Song, et al., A sensitive and efficient method for simultaneous trace detection and identification of triterpene acids and its application to pharmacokinetic study, Talanta 98 (2012) 101–111.
[52] S. Zhang, Y. Sun, Z. Sun, et al., Determination of triterpenic acids in fruits by a novel high performance liquid chromatography method with high sensitivity and specificity, Food Chem. 146 (2014) 264–269.
[53] W. Ma, H. Wang, H. Zhang, Determination of ursolic acid in Xiasangju by HPLC after pre-derivazation with *p*-toluidine, Can. J. Anal. Sci. Spectros. 20 (2004) 284–286.
[54] X. Zheng, S. Wang, Determination of Asiatic acid in beagle dog plasma after oral administration of *Centella asiatica* extract by precolumn derivatization RP-HPLC, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 477–481.
[55] Y. Zhang, L. Yin, Z. Liang, et al., Study on pharmacokinetics of asiatic acid in rats, Chin. J. Mod. Appl. Pharm. 32 (2015) 314–317.
[56] X. Huang, Y. Zhang, L. Yin, et al., Oral absorption of asiatic acid lipid nanoparticles in rats, Chin. J. Mod. Appl. Pharm. 33 (2016) 444–448.
[57] L. Yin, Y. Zhang, Y. Zhou, et al., Preparation and pharmacokinetic study of asiatic acid loaded chitosan-deoxycholic acid polymeric micelles in rats, Chin. Tradit. Herb. Drugs 48 (2017) 4891–4896.
[58] J. You, D. Wu, M. Zhao, et al., Development of a facile and sensitive HPLC-FLD method via fluorescence labeling for triterpenic acid bioavailability investigation, Biomed. Chromatogr. 31 (2017), e3894.
[59] Z. Zeng, W. Zhou, N. Hu, et al., Comparison of using two different labeling reagents for rapid analysis of triterpenic acids by pre-column derivatization with RP-HPLC-FLD and application to plant samples, Anal. Methods 11 (2019) 4354–4361.
[60] O.J. Pozo, M. Pujadas, S.B. Gleeson, et al., Liquid chromatography tandem mass spectrometric determination of triterpenes in human fluids: Evaluation of markers of dietary intake of olive oil and metabolic disposition of oleanolic acid and maslinic acid in humans, Anal. Chim. Acta 990 (2017) 84–95.
[61] Z. Zheng, X. Zhao, S. Zhu, et al., Simultaneous determination of oleanolic acid and ursolic acid by *in vivo* microdialysis via UHPLC-MS/MS using magnetic dispersive solid phase extraction coupling with microwave-assisted derivatization and its application to a pharmacokinetic study of arctiumlappa L. root extract in rats, J. Agric. Food Chem. 66 (2018) 3975–3982.
[62] D. Luo, Y. Feng, J. Yao, et al., Determination of oleanolic acid and ursolic acid in loquat leaf extract by chemical derivatization coupled with liquid chromatography-tandem mass spectrometry, Chin. J. Chromatogr. 35 (2017), 27.
[63] J. Adamec, A. Jannasch, S. Dudhgaonkar, et al., Development of a new method for improved identification and relative quantification of unknown metabolites in complex samples: Determination of a triterpenoid metabolic fingerprint for the *in situ* characterization of *Ganoderma* bioactive compounds, J. Sep. Sci. 32 (2009) 4052–4058.
[64] Y. Zheng, S. Liu, J. Xing, et al., Equivalently quantitative ion strategy with quaternary ammonium cation derivatization for highly sensitive quantification of lanostane-type triterpene acids without standards by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), Anal. Chem. 90 (2018) 13946–13952.
[65] H. Zhang, Z. Zhu, Y. Zhang, et al., Determination of glycyrrhizic acid in *Radix Glycyrrhizae* by high performance liquid chromatography with pre-column derivatization, J. Anal. Sci. 29 (2013) 265–267.
[66] Ł. Woźniak, K. Marszałek, S. Skąpska, et al., Novel method for HPLC analysis of triterpenic acids using 9-anthryldiazomethane derivatization and fluorescence detection, Chromatographia 80 (2017) 1527–1533.
[67] D. Shangguan, H. Han, R. Zhao, et al., New method for high-performance liquid chromatographic separation and fluorescence detection of ginsenosides, J. Chromatogr. A 910 (2001) 367–372.
[68] P. Dong, C. Xue, L. Yu, et al., Determination of triterpene glycosides in sea cucumber (*Stichopus japonicus*) and its related products by high-performance liquid chromatography, J. Agric. Food Chem. 56 (2008) 4937–4942.
[69] J. Cai, K. Huang, S. Han, et al., A comprehensive system review of pharmacological effects and relative mechanisms of Ginsenoside Re: Recent advances and future perspectives, Phytomedicine 102 (2022), 154119.
[70] Y. Sun, Y. Yang, S. Liu, et al., New therapeutic approaches to and mechanisms of ginsenoside Rg1 against neurological diseases, Cells 11 (2022), 2529.
[71] H.J. Kim, P. Kim, C.Y. Shin, A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system, J. Ginseng Res. 37 (2013) 8–29.
[72] Y. Wu, Z. Li, J. Li, et al., Elucidation of the binding mechanism of astragaloside IV derivative with human serum albumin and its cardiotoxicity in zebrafish embryos, Front. Pharmacol. 13 (2022), 987882.
[73] H.L. Alvarado, G. Abrego, M.L. Garduño-Ramirez, et al., Development and validation of a high-performance liquid chromatography method for the quantification of ursolic/oleanic acids mixture isolated from *Plumeria obtusa*, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 983-984 (2015) 111–116.
[74] S. Liu, J. Xu, C. Ma, et al., A comparative analysis of derivatization strategies for the determination of biogenic amines in sausage and cheese by HPLC, Food Chem. 266 (2018) 275–283.
[75] R.E. Nelson, S.K. Grebe, D.J. OKane, et al., Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma, Clin. Chem. 50 (2004) 373–384.
[76] H. Lv, S. Huang, B. Zhao, et al., A new rhodamine B-based lysosomal pH fluorescent indicator, Anal. Chim. Acta 788 (2013) 177–182.
[77] L. Qi, J. Zhang, Y. Gao, et al., Peptide-RNA complexation-induced fluorescence "turn on" displacement assay for the recognition of small ligands targeting HIV-1 RNA, J. Pharm. Anal. 12 (2022) 923–928.
[78] N. Wei, X. Zhao, S. Zhu, et al., Determination of dopamine, serotonin, biosynthesis precursors and metabolites in rat brain microdialysates by ultrasonic-assisted *in situ* derivatization-dispersive liquid-liquid microextraction coupled with UHPLC-MS/MS, Talanta 161 (2016) 253–264.
[79] X. Wang, J. Sun, X. Zhao, et al., Stable isotope labeling derivatization coupled with magnetic dispersive solid phase extraction for the determination of hydroxyl-containing cholesterol and metabolites by *in vivo* microdialysis and ultra-high performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1594 (2019) 23–33.
[80] L. Yang, Y. Liu, X. Zeng, et al., HPLC-MS/MS determination ginsenoside Rg_1 in *Shenmai injection* and in human plasma, Chin. J. Pharm. Anal. 25 (2005) 905–908.
[81] T.K. Davis, M.E. Jennings 2nd, Site-specific conjugation quantitation of a cysteine-conjugated antibody-drug conjugate using stable isotope labeling peptide mapping LC-MS/MS analysis, Anal. Chem. 94 (2022) 2772–2778.
[82] R. Gajula, R. Maddela, V.B. Ravi, et al., A rapid and sensitive liquid chromatography-tandem mass spectrometric assay for duloxetine in human plasma: Its pharmacokinetic application, J. Pharm. Anal. 3 (2013) 36–44.
[83] S.K. Gudlawar, N.R. Pilli, S. Siddiraju, et al., Highly sensitive assay for the determination of therapeutic peptide desmopressin in human plasma by UPLC-MS/MS, J. Pharm. Anal. 7 (2017) 196–202.
[84] S. Glowienke, W. Frieauff, T. Allmendinger, et al., Structure-activity considerations and *in vitro* approaches to assess the genotoxicity of 19 methane-, benzene- and toluenesulfonic acid esters, Mutat. Res. 581 (2005) 23–34.
[85] S. Song, R. Wang, Z. Li, et al., Role of simulated *in vitro* gastrointestinal digestion on biotransformation and bioactivity of astragalosides from *Radix* Astragali, J. Pharm. Biomed. Anal. 231 (2023), 115414.
[86] L. Qing, T. Chen, W. Sun, et al., Pharmacokinetics comparison, intestinal absorption and acute toxicity assessment of a novel water-soluble astragaloside IV derivative (astragalosidic acid, LS-102), Eur. J. Drug Metab. Pharmacokinet. 44 (2019) 251–259.
[87] W. Sun, Z. Zhang, J. Xie, et al., Determination of a astragaloside IV derivative LS-102 in plasma by ultra-performance liquid chromatography-tandem mass spectrometry in dog plasma and its application in a pharmacokinetic study, Phytomed. 53 (2019) 243–251.
[88] C. Chen, Y. Xue, Q. Li, et al., Neutral loss scan-based strategy for integrated identification of amorfrutin derivatives, new peroxisome proliferator-activated receptor gamma agonists, from *Amorpha fruticosa* by UPLC-QqQ-MS/MS and UPLC-Q-TOF-MS, J. Am. Soc. Mass Spectrom. 29 (2018) 685–693.
[89] L. Qing, Y. Xue, J. Zhang, et al., Identification of flavonoid glycosides in *Rosa chinensis* flowers by liquid chromatography-tandem mass spectrometry in combination with ^{13}C nuclear magnetic resonance, J. Chromatogr. A 1249 (2012) 130–137.
[90] S. Kumar, A. Singh, B. Kumar, Identification and characterization of phenolics and terpenoids from ethanolic extracts of *Phyllanthus* species by HPLC-ESI-QTOF-MS/MS, J. Pharm. Anal. 7 (2017) 214–222.
[91] Q. Wang, J. Li, X. Li, et al., A simple nano-SiO_2-based ELISA method for residue detection of 2, 4-dichlorophenoxyacetic acid in bean sprouts, Food Anal. Meth. 10 (2017) 1500–1506.
[92] Q. Wang, J. Li, X. Li, et al., An efficient direct competitive nano-ELISA for residual BSA determination in vaccines, Anal. Bioanal. Chem. 409 (2017) 4607–4614.
[93] Q. Wang, J. Xie, X. Li, et al., Development of a nano-SiO_2 based enzyme-linked ligand binding assay for the determination of ibuprofen in human urine, Talanta 167 (2017) 617–622.
[94] Y. Yi, J. Hu, S. Ding, et al., A preparation strategy for protein-oriented immobilized silica magnetic beads with Spy chemistry for ligand fishing, J. Pharm. Anal. 12 (2022) 415–423.
[95] Y.L. Tain, L.C. Jheng, S.K.C. Chang, et al., Synthesis and characterization of novel resveratrol butyrate esters that have the ability to prevent fat accumulation in a liver cell culture model, Molecules 25 (2020), 4199.
[96] A.K. Ghosh, D. Shahabi, Synthesis of amide derivatives for electron deficient amines and functionalized carboxylic acids using EDC and DMAP and a catalytic amount of HOBt as the coupling reagents, Tetrahedron Lett. 63 (2021), 152719.
[97] J. Xie, R. Wang, L. Yong, et al., Determination of nine nucleosides in Rhizoma Paridis by quantitative analysis of multi-components via a single marker method, J. Sep. Sci. 44 (2021) 1866–1874.
[98] J. Xie, J. Li, J. Liang, et al., Determination of contents of catechins in oolong teas by quantitative analysis of multi-components via a single marker (QAMS) method, Food Anal. Meth. 10 (2017) 363–368.
[99] G.Y. Wittman, H. van Langenhove, J. Dewulf, Determination of acetic acid in aqueous samples, by water-phase derivatisation, solid-phase

microextraction and gas chromatography, J. Chromatogr. A 874 (2000) 225–234.
[100] J. Salimon, T.A. Omar, N. Salih, An accurate and reliable method for identification and quantification of fatty acids and trans fatty acids in food fats samples using gas chromatography, Arab. J. Chem. 10 (2017) S1875–S1882.
[101] S. Yu, C. Cai, Y. Wang, et al., Quantification of phytic acid in baby foods by derivatization with (trimethylsilyl)diazomethane and liquid chromatography-mass spectrometry analysis, Rapid Commun. Mass Spectrom. 35 (2021), e9194.
[102] W.J. Griffiths, G. Alvelius, S. Liu, et al., High-energy collision-induced dissociation of oxosteroids derivatised to Girard hydrazones, Eur. J. Mass Spectrom. Chichester Engl. 10 (2004) 63–88.
[103] W.J. Griffiths, Y. Wang, G. Alvelius, et al., Analysis of oxysterols by electrospray tandem mass spectrometry, J. Am. Soc. Mass Spectrom. 17 (2006) 341–362.
[104] S. Gao, Z. Zhang, H.T. Karnes, Sensitivity enhancement in liquid chromatography/atmospheric pressure ionization mass spectrometry using derivatization and mobile phase additives, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 825 (2005) 98–110.
[105] J. Li, X. Li, T. Li, Synthesis of oximes under ultrasound irradiation, Ultrason. Sonochem. 13 (2006) 200–202.
[106] X. Liu, Y. Zhou, H. Chen, et al., Detection of carbonyl groups in triterpenoids by hydroxylamine hydrochloride derivatization using electrospray ionization mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 1981–1992.
[107] W. Wang, F. Chen, Y. Wang, et al., Optimization of reactions between reducing sugars and 1-phenyl-3-methyl-5-pyrazolone (PMP) by response surface methodology, Food Chem. 254 (2018) 158–164.
[108] X. Wu, W. Jiang, J. Lu, et al., Analysis of the monosaccharide composition of water-soluble polysaccharides from *Sargassum fusiforme* by high performance liquid chromatography/electrospray ionisation mass spectrometry, Food Chem. 145 (2014) 976–983.
[109] Y. Xue, X. Xu, L. Yong, et al., Optimization of *Vortex*-assisted dispersive liquid-liquid microextraction for the simultaneous quantitation of eleven non-anthocyanin polyphenols in commercial blueberry using the multi-objective response surface methodology and desirability function approach, Molecules 23 (2018), 2921.
[110] M. Sajid, Dispersive liquid-liquid microextraction: Evolution in design, application areas, and green aspects, Trac Trends Anal. Chem. 152 (2022), 116636.
[111] M.D. Dung, T.T.V. Nga, N.T. Lan, et al., Adsorption behavior and mechanism of As (V) on magnetic Fe_3O_4-graphene oxide (GO) nanohybrid composite material, Anal. Sci. 38 (2022) 427–436.