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Review

Recent Advances in Separation and Analysis of Saponins in Natural Products

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Abstract: To better control the quality of saponins, ensure their biological activity and clinical therapeutic effect, and expand the development and application of saponins, this paper systematically and comprehensively reviews the separation and analytical methods of saponins in the past decade. Since 2010, the electronic databases of PubMed, Google Scholar, ISI Web of Science, Science Direct, Wiley, Springer, CNKI (National Knowledge Infrastructure, CNKI), Wanfang Med online, and other databases have been searched systematically. As a result, it is found that ionic liquids and high-performance countercurrent chromatography are the most popular extraction and separation techniques for saponins, and the combined chromatography technique is the most widely used method for the analysis of saponins. Liquid chromatography can be used in combination with different detectors to achieve qualitative or quantitative analysis and quality control of saponin compounds in medicinal materials and their preparations. This paper provides the latest valuable insights and references for the analytical methods and continued development and application of saponins.

Keywords: saponin; ionic liquid; HPCCC; HPLC-MS; QAMS; metabolomics; qualitative and quantitative; quality control

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1. Introduction

Saponin is a kind of natural secondary metabolite, which is composed of sapogenin and a sugar chain [1]. They can be divided into triterpenoid or steroidal types according to the sapogenin differences: The triterpenoid saponin's sapogenin is triterpene, whereas the steroidal saponin's is spirostane.

The sapogenin of triterpenoid saponins is a triterpenoid derivative composed of 30 carbon atoms and a basic skeleton of six isoprene units. Again, distinguished by sapogenins differences, the triterpenoid saponins are divided into tetracyclic and pentacyclic triterpenoids. The tetracyclic triterpenoid types include lanostane, euphane, dadamane type [2], cucurbitacin alkanes, cycloxylane-type, meliacanes, and more; the pentacyclic triterpenoid types include oleanane type [3], ursane, lupane, friedelanes, and others. The sapogenins of steroidal saponins are steroidal derivatives that generally contain 27 carbon atoms and have the basic skeleton of spirostane. Steroidal saponins are divided into spirostanol, isospirostanol, furostanol, and deformed spirostanol types. Saponins are widely distributed in nature and are commonly found in roots, stems, leaves, flowers, and seeds of terrestrial higher plants. Triterpenoid saponins are mainly distributed in the Leguminosae, Araliaceae, Umbelliferae, Compositae, Polygalaceae, and other plant families. Steroidal saponins are mostly found in monocotyledons, such as Dioscoreaceae, Liliaceae, and Scrophulariaceae plant families. Saponins represent a main effective component in many kinds of Chinese

herbal medicines, such as ginseng, astragalus, bupleurum, *Ophiopogon japonicus*, notoginseng [4], *Anemarrhena*, *Polygala*, *Platycodon grandiflorus*, and licorice. In addition, saponins exist in sea creatures, such as the sea cucumber.

Pharmacological studies have shown that saponins have many important biological activities and pharmacological actions [5–13], such as immunity enhancement, antitumor, anti-inflammatory, antifungal, and anti-viral actions, blood glucose and lipid reduction, antioxidation, cardiovascular function improvement [14,15], hemolysis [16], and more. In recent years, saponins have been widely used in medicines, health foods, animal feed, cosmetics, and other items. In addition, saponins are used as plant growth regulators and insect repellents in agriculture. Therefore, saponins have great research value and broad developmental prospects (Figures 1–3).

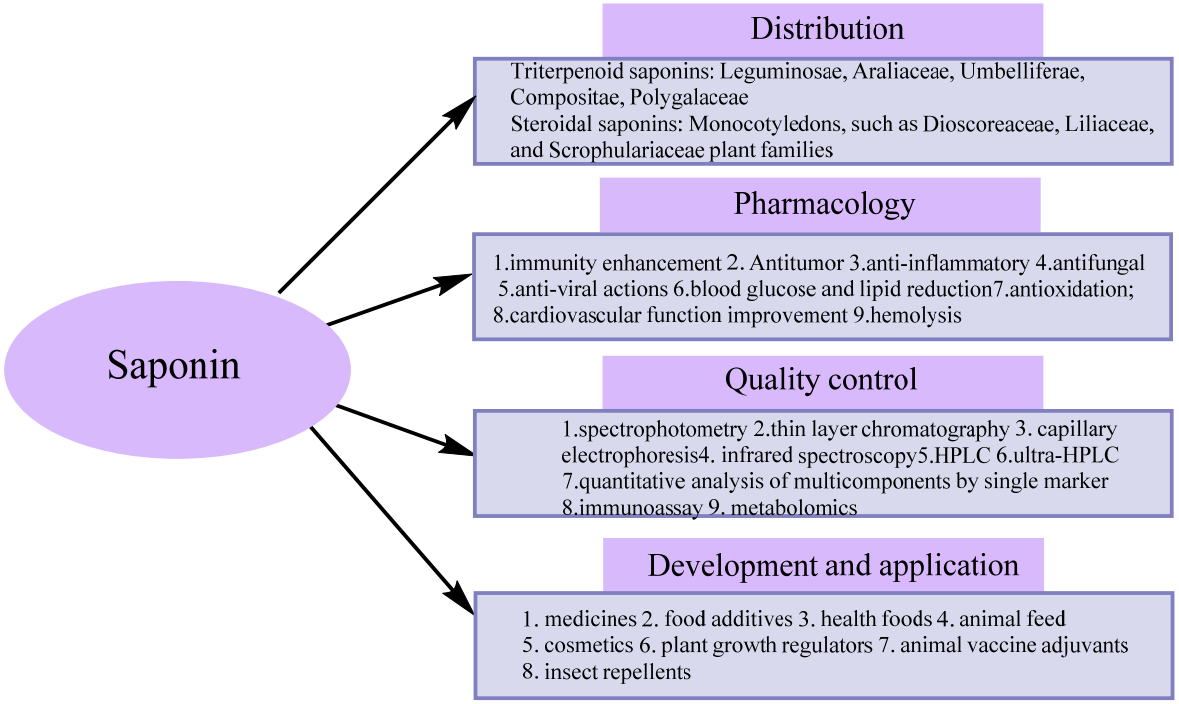

Figure 1. Saponins: distribution, pharmacology, quality control, development and application [5–17].

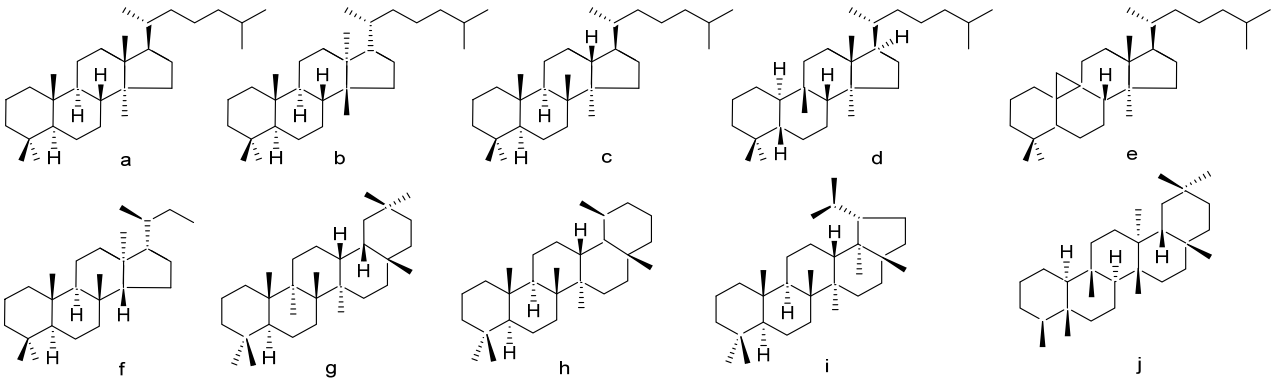

Figure 2. Chemical structures of ten kinds of triterpenoid saponins, (**a**) lanostane, (**b**) euphane (**c**) dadamane type, (**d**) cucurbitacin alkanes, (**e**) cycloxylane-type, (**f**) meliacanes (**g**) oleanane type, (**h**) ursane, (**i**) lupine, (**j**) friedelanes.

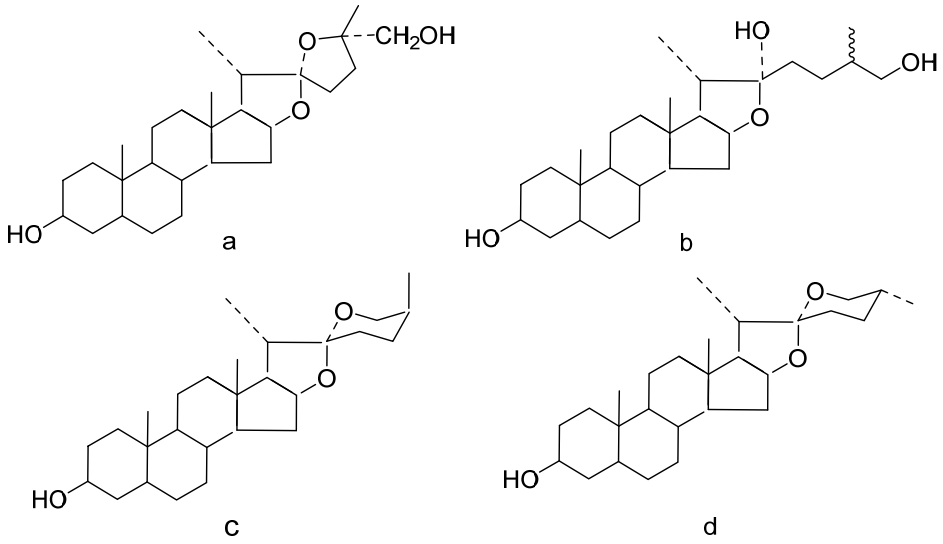

Figure 3. Chemical structures of four kinds of steroidal saponins, (**a**) deformed spirostanol types, (**b**) furostanol, (**c**) spirostanol, (**d**) isospirostanol.

However, most saponins have a similar chemical structure and have no UV absorption or terminal absorption. In addition, the content and proportion of saponins are easily affected by geographical location, cultivation technology, harvest times, differences in batches from different manufacturers, and other factors, which all lead to unqualified quality, reduced biological activity, and limited clinical applications. Given the determination challenges, researchers around the globe have proposed different analytical methods for saponins (Figures 1–3). These methods include spectrophotometry, thin-layer chromatography (TLC), capillary electrophoresis (CE), infrared spectroscopy, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), quantitative analysis of multicomponents by single marker (QAMS), immunoassay, and metabolomics [17].

In recent years, as significant pharmacological action of saponins has become well known and their general application in various fields has increased, the quality control of saponins has become a global concern (Table 1). Therefore, based on the qualitative and quantitative perspectives, this article discusses the problems and future development trends of these analytical methods by reviewing the literature of the past ten years and provides a valuable reference for choosing appropriate analytical methods to control the quality of saponins (Table 2).

Table 1. Efficacy table of Chinese medicinal materials mainly containing saponins.

No.	Drug Name	Medication Site	Main Ingredient	Efficacy	Ref.
1	*Panax ginseng* C. A. Mey.	Dry roots and rhizomes	Triterpenoid saponins, ginseng polysaccharides, ginseng alkynols, amino acid proteins, sugars, vitamins, organic acids, trace elements, flavonoids and peptides	It can strengthen the vitality, strengthen the body, nourish blood and blood, nourish the spleen and benefit the lungs, calm the heart, calm the mind and promote wisdom	[14,18,19]

Table 1. *Cont.*

No.	Drug Name	Medication Site	Main Ingredient	Efficacy	Ref.
2	*Panax pseudoginseng* Wall. Var. *notoginseng*(Burkill) Hoo et Tseng	Roots and rhizomes, leaves, flowers	Dammar type tetracyclic triterpene saponins, flavonoids, notoginsenosides (amino acids), proteins, volatile oils, acetylenes, alcohols, polysaccharides, polyols, polyacetylene alcohols, organic acids, trace elements, etc.	Diffuse stasis to stop bleeding, reduce swelling and relieve pain	[15,20,21]
3	*Platycodon grandiflorus* (Jacq.) A. DC.	Dry root	Oleanane-type pentacyclic triterpene saponins, flavonoids, phenols, sterols, polysaccharides, polyacetylenes, steroids, phenolic acids, polysaccharides, fatty acids, fatty acids and trace elements, inorganic elements, etc.	Promoting lung, relieving asthma, dispelling cold, benefiting pharynx, expectorating phlegm, discharging pus, inducing drugs to increase	[5–7,22]
4	Astragali Radix	Dry root	Triterpene saponins, polysaccharides, flavonoids, amino acids	Fill the air to raise Yang, fixed surface antiperspirant, diuretic to poison, discharge pus, collect sore muscle, benefit water to reduce swelling	[8,23,24]
5	*Anemarrhena asphodeloides* Bunge	Dry rhizome	Steroid saponins diphenpyrone, flavonoids, lignin, polysaccharides, alkaloids, amino acids, volatile oils, organic acids and trace elements, inorganic elements, etc.	Clearing heat and purging fire, nourishing Yin and moistening dryness, quenching thirst and eliminating annoyance	[9,25–27]
6	Bupleuri Radix	Dried roots, whole grasses	Bupleurum saponins, flavonoids, volatile oils, polysaccharides, sterols, polyols, coumarins, lignans, fatty acids (oleic acid, linolenic acid, palmitic acid, stearic acid, etc.), tryptophan, wood sugar alcohol, uridine, adenosine and trace elements, etc.	Antipyretic, anti-inflammatory, lowering blood cholesterol, reconciling the inside and outside, soothing the liver and stagnating depression, raising Yang, lifting depression, protecting liver and boldness, cooling down, relieving the stasis of the liver qi, relieving qi, relieving pain and reducing inflammation, anticancer, resisting liver fibrosis, evacuating fever, soothing liver and relieving depression, raising Yang, lifting qi	[10–12,28,29]

Table 1. *Cont.*

No.	Drug Name	Medication Site	Main Ingredient	Efficacy	Ref.
7	*Polygala tenuifolia* Willd	Dry roots	Triterpene saponins, ketones, oligosaccharides, alkaloids, phenylpropanoid flavonoids, lactones, coumarins, lignin, etc.	Dispelling phlegm, reducing swelling, calming the mind and improving intelligence	[13,30]
8	*Glycyrrhiza ur alensis* Fisch.	Dried roots and rhizomes	Triterpenoid saponins flavonoids and polysaccharides	Replenishing spleen and qi, clearing away heat and detoxifies, expelling phlegm and cough, relieving pain, reconciling all drugs	[31–33]

Table 2. Quality standard table of important medicinal materials mainly containing saponins in the 2020 Chinese Pharmacopoeia.

No.	Drug Name	Content Determination
1	*Panax ginseng* C. A. Mey.	Total amount of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$) and ginsenoside Re ($C_{48}H_{82}O_{18}$) should not be less than 0.27%, and ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$) should not be less than 0.18%
2	*Panax pseudoginseng* Wall. var. *notoginseng* (Burkill) Hoo et Tseng	Total amount of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$), ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$) and notoginseng R_1 ($C_{47}H_{80}O_{18}$) should not be less than 5.0%
3	*Platycodon grandifloras* (Jacq.) A. DC.	Platycodon grandiflorum saponin D ($C_{57}H_{92}O_{28}$) shall not be less than 0.10%
4	Astragali Radix	Astragaloside IV ($C_{41}H_{68}O_{14}$) shall not be less than 0.080%, and calycoflavone glucoside ($C_{22}H_{22}O_{10}$) shall not be less than 0.020%
5	*Anemarrhena asphodeloides* Bunge	Mangiferin ($C_{19}H_{18}O_{11}$) shall not be less than 0.70%, and Anemarrhena saponin BII ($C_{45}H_{76}O_{19}$) shall not be less than 3.0%
6	Bupleuri Radix	Total content of saponin a ($C_{42}H_{68}O_{13}$) and saponin d ($C_{42}H_{68}O_{13}$) should not be less than 0.30%
7	*Polygala tenuifolia* Willd.	Polygala tenuifolia saponins ($C_{36}H_{56}O_{12}$), not less than 2.0%, Polygala ketone III ($C_{25}H_{28}O_{15}$) not less than 0.15%, containing 3,6′-dierucyl sucrose ($C_{36}H_{46}O_{17}$) not less than 0.50%
8	*Glycyrrhiza uralensis* Fisch.	Glycyrrhizin ($C_{21}H_{22}O_9$) shall not be less than 0.50%, glycyrrhizic acid ($C_{42}H_{62}O_{16}$) shall not be less than 2.0%

2. Extraction and Separation Methods

Since saponins are complex mixtures with very similar structures and polarities, their extraction and separation are challenging. Therefore, based on this problem, researchers at home and abroad have explored green, efficient, solvent-saving and time-saving methods for the extraction and separation of saponins.

2.1. Extraction of Saponins

2.1.1. ILs

Traditionally, saponins are mainly extracted by decoction, recrystallization and other methods. However, these methods have many disadvantages, such as time-consuming, low extraction efficiency, use of toxic and harmful organic solvents, and environmental pollution. In recent years, with the development of green chemistry, environment-friendly technologies have attracted more and more attention. Ionic liquids (ILs) are liquid salts

composed of organic cations and inorganic or organic anions with negligible volatility, low flammability, chemical stability, good environmental friendliness, and good solubility for organic compounds and extraction ability. By fine-tuning its chemical structure and properties, selectively distinguishing one compound from other compounds and other advantages, it has shown great potential to replace traditional organic solvents in many fields and has been widely used in the extraction and separation of saponins. Some researchers have explored the use of IL-ATPS to extract ginsenosides (Rg1, Re, Rd and Rb1) from the crude extract of ginseng root, which has high extraction efficiency and good selectivity [34]. Other researchers have explored the determination of seven rare ginsenosides (ginsenosides) in Xuesaitong injection by ILATPE based on imidazolium ionic liquid (1-butyl-3-methylimidazolium bromide (Bmim)Br) and salt (K_2HPO_4). Rg6, F4, 20(S)-Rg3, 20(R)-Rg3, Rk3, Rk1, Rg5) potential applications, studies have shown that this method has a higher extraction rate [35].

2.1.2. UAE

There are currently some auxiliary techniques combined with ILs, such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), which show significant improvements in the field of extraction and separation. Ionic liquid-based ultrasonic-assisted extraction (IL-UAE) has been shown to be the most efficient method for the extraction of saponins from natural plants. Some researchers have established an ionic liquid ultrasonic extraction of licorice root liquiritigenin (LQ), liquiritigenin apigenin (LA), isoliquiritigenin (ILQ), isoliquiritigenin apigenin (ILA) and glycyrrhizin (GA), and compared with the traditional UAE method, the IL-UAE method has higher extraction efficiency and significantly shortens the extraction time [36]. There are studies using the method of coupling IL-UAE and ABS to extract eight kinds of ginsenosides from ginseng flower buds (ginsenoside-Rg1, ginsenoside-Rg2, ginsenoside-Rc, ginsenoside-Rd, ginsenoside-Re, ginsenoside- Rf, ginsenoside-Rb1 and ginsenoside-Rb2) [37].

2.1.3. MAE

The use of microwave energy enables fast dissolution, drying, acidic digestion, and extraction of organic compounds from complex matrices. The microwave heats the solvent or solvent mixture directly, and the direct interaction of microwaves with the free water molecules present in the glands and vascular systems results in subsequent rupture of the plant tissue and release of components into the organic solvent. Its main advantages are reduced solvent volume and time consumption and increased sample throughput. Thus, MAE provides an alternative method to conventional extraction methods in plants.

2.2. Isolation of Saponins

2.2.1. SFC

SFC is a green chromatographic separation technology. By using supercritical fluid with low viscosity and high diffusivity, such as supercritical carbon dioxide as the mobile phase, SFC shows some remarkable features, such as high separation efficiency, high flow rate, short analysis time, environmental friendliness and low cost, etc. In addition to its achievements in chiral separations, SFC has also shown great potential in the separation of saponins from natural products, becoming a complementary technique to gas chromatography and liquid chromatography, offering a wide range of adaptability and selectivity for chromatographic analysis. Some researchers used UHPSFC technology to successfully separate five saikosaponins, including SSa, SSb1, SSb2, SSc and saikosaponin f (SSf) within 22 minutes, of which SSa, SSb1 and SSb2 are a group of isomers. Furanosterol saponins have the same aglycone but different sugar chains. SFC is sensitive to the amount and type of sugars. Therefore, SFC is suitable for separating hydrophilic furosterol saponins and analyzing traditional Chinese medicines mainly containing steroid saponins [38]. Some researchers used supercritical fluid chromatography-single quadrupole mass spectrometry technology to establish a fast and effective method for the separation of matrine saponins

and ginsenosides. Compared with reversed-phase liquid chromatography, the SFC method shows higher resolution and shorter run time [39]. Other researchers found that UHPSFC can effectively separate spirosterol saponins with the same aglycone and different sugar chains, while ultra-high pressure liquid chromatography (UHPLC) can well separate spirosterol saponins with the same sugar group and different aglycones. UHPLC and UHPSFC are complementary in the separation of spirosterol saponins. Considering that the naturally occurring spirosterol saponins in Chinese herbal medicine are different in both aglycones and sugar chains, the combination of UHPLC and UHPSFC can achieve better separation [40].

2.2.2. HSCCC

As an all-liquid partition chromatography technique, it eliminates the irreversible adsorption loss of samples on solid support matrix columns, has high sample loading compared with traditional liquid–solid separation methods, and has good reproducible sample recovery after scale-up, which is a unique advantage over other devices. Therefore, HSCCC has been widely used to prepare saponin-like active ingredients from natural products (Figure 4). Nine new triterpenoid saponins (1–9), namely camoreoside A-I, were extracted and isolated by high-performance countercurrent chromatography and preparative reversed-phase high-performance liquid chromatography [41]. Some researchers have used high-speed countercurrent chromatography to successfully separate four minor saponins from Panax notoginseng leaves, namely Gynostemma saponin XVII, ginsenoside Rd2, Panax notoginsenoside Fe and Panax notoginsenoside Fd [42]. Some researchers have used high-speed countercurrent chromatography (HSCCC) and preparative RP-HPLC to separate and purify 300-O-acetylplatycoside D and polygalactoside D with a purity of more than 98.9%. Studies have shown that this method can be used for crude extract of Platycodon grandiflorum Preparation and rapid separation of medium and trace saponins [43]. Some researchers used high-speed countercurrent chromatography (HSCCC) combined with evaporative light scattering detection to separate three furosterols and four spirosterol saponins (parvifloside; methyl protodeltonin; trigofoenoside A-1; zingiberensis saponin I; deltonin; dioscin; prosapogenin A of dioscin) from Dioscorea, and studies have shown that HSCCC is an effective method for the separation and purification of two different steroidal saponins from plant extracts [44]. Some studies have used a linear gradient elution method to separate four triterpenoid saponins (hederasaponin B, hederacolchiside E, cernuoside A, cernuoside B) from Pulsatilla officinalis [45]. Some researchers have isolated two saponins with cytotoxicity to cancer cells from the root of A. chinensis by high-speed countercurrent chromatography (HSCCC) [46].

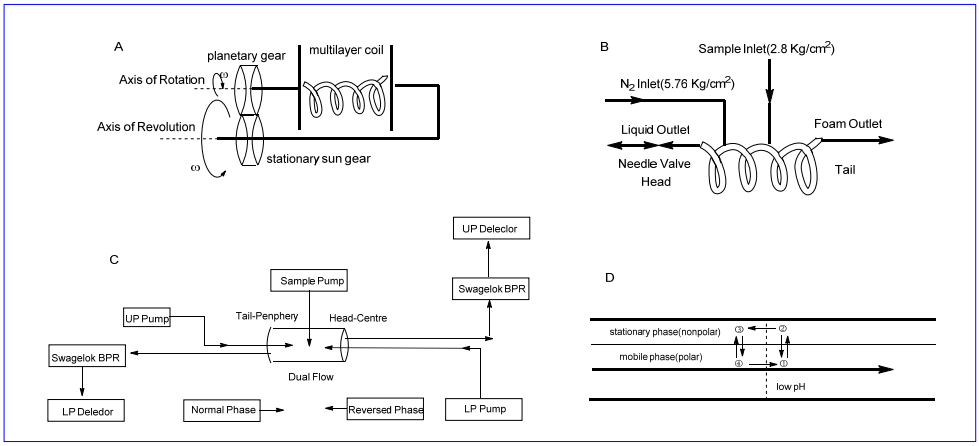

Figure 4. Schematic diagram of different types of HSCCC [47]. (**A**) The synchronous planetary motion of a multilayer coil separation column; (**B**) design of the coiled column for dual HSCCC; (**C**) Design of the coiled column for foam HSCCC; (**D**) mechanism of pH-zone-refining HSCCC.

2.2.3. Foam Fractionation

It is a physical adsorption and separation technology that concentrates surface-active substances according to their different surface activities. This technology has the advantages of simple equipment, small investment, low energy consumption, and strong environmental adaptability, and is a "solvent-free" substitute for solvent extraction (Figure 5). Since saponins are typical biosurfactants, they have good foaming properties. Therefore, saponins can be enriched from the leachate by foam fractionation. Some researchers have developed a new process for the separation of Achyranthes saponins combined with extraction and foam fractionation. The main compounds in the concentrated and purified foam salts obtained by the developed technology are triterpenoid saponins [48]. Some researchers use foam separation and resin adsorption technology to separate soybean saponins in soybean meal [49].

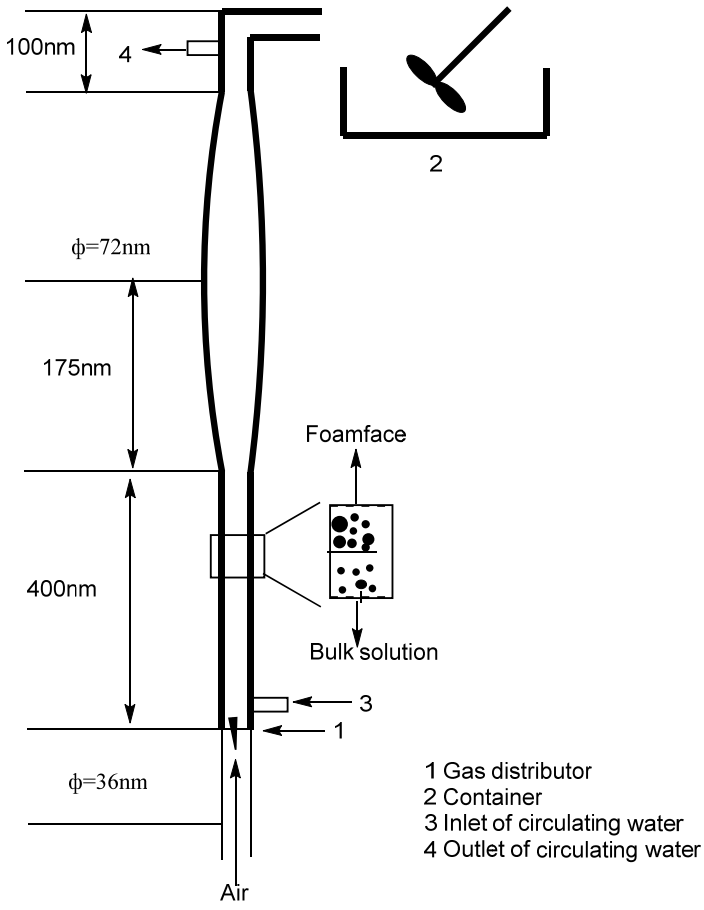

Figure 5. Experimental apparatus of foam fractionation [48].

3. Analytical Methods

In addition to the traditional analytical methods, such as spectrophotometry, TLC and HPLC, this paper also describes the analytical methods of QAMS, UPLC, immunoassay, CE, infrared spectroscopy, and metabolomics. This paper reviews the research status of different analytical methods of saponins during the past 10 years to provide the latest valuable insights and references for quality control and clinical application of saponins (Figure 6).

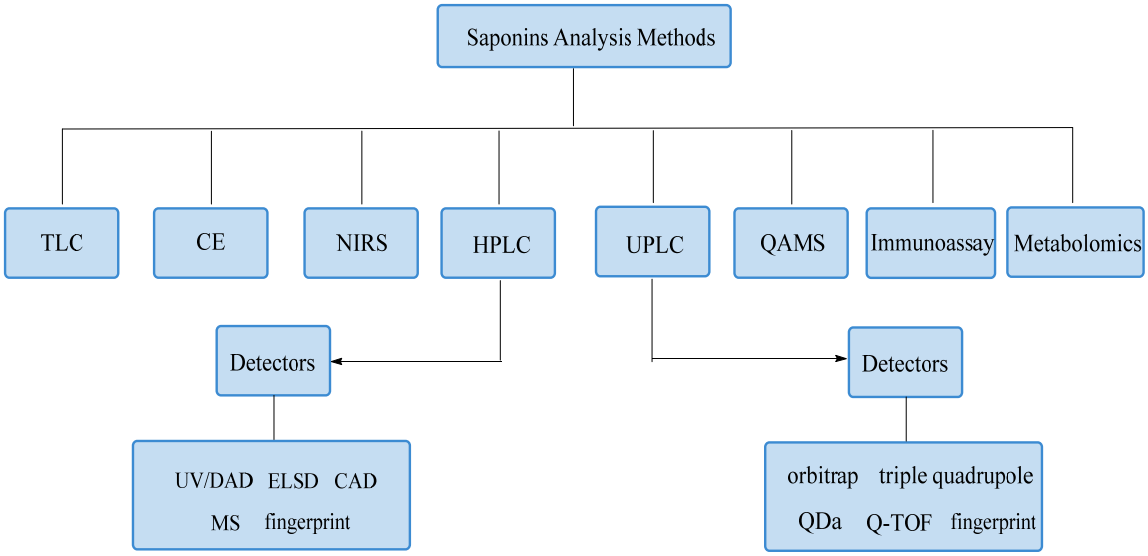

Figure 6. Structural diagram of analytical methods for Saponins.

3.1. TLC

TLC is a commonly used method for the analysis of saponins; it has the advantages of simple operation, strong separation ability, low cost, and fast detection speed. A study on the quantitative analysis and comparison of diosgenin in Rhizoma Paridis by reversed-phase HPLC and TLC showed that the two methods had good separation effects on diosgenin in Rhizoma Paridis. There was no significant difference in the determination results [50]. Another study examined the simultaneous detection and quantitative analysis of diosgenin and sea buckthorn diosgenin in the extract of *Cornus officinalis*. This study established a sensitive, fast, and effective test (TLC). The results showed that the retention coefficients of diosgenin and sea buckthorn diosgenin on TLC plates were 0.49 and 0.6, respectively [51]. TLC can also be used for simultaneous quantitative analysis and identification of several saponins in medicinal preparations. One study carried out a quantitative analysis of ginsenoside Rb1 and Rg1 in Sanqi shangyao capsules by a TLC scanning method; it combined this assessment with the determination of naringin to jointly control the quality of the preparation [52]. Another study carried out a qualitative analysis of saikosaponin in Hugan tablets, as identified by TLC. The TLC method was fast, simple, easy to operate, and low cost [53]. TLC has the characteristics of simple operation as well as rapid qualitative and quantitative determination of various compounds, so it has become one of the main methods for the determination of saponins in natural drugs and preparations.

3.2. CE

CE is a powerful separation and quantitative analysis technology, which has become a standard tool for the analysis of saponins in many plant extracts. A study screened licorice extracts by affinity CE to identify active anti-HIV components. Then, solid-phase extraction technology was used to separate and purify the effective parts. The research explored a simple, fast, and effective method for combining CE-electrospray MS and liquid chromatography (LC)-electrospray MS. The results showed that glycyrrhizin and glycyrrhizin G2 were the main components providing anti-HIV activity [54]. In another study, rapid separation and quantitative determination of bupleurum saponins a, c, and d in Chinese herbal extracts from different regions (Nacalai Tesque, Kyoto, Japan; Toray, Siga, Japan) were performed by capillary zone electrophoresis. This method has become a powerful technology for the analysis of complex extracts in Chinese herbal medicines [55]. In addition, another study achieved simultaneous separation and quantitative determination of diastereomers of triterpene saponins in *Alexandrium* algae (soybean saponin I methyl ester and azukisaponin V methyl ester or bersimoside I methyl ester and bersimoside II methyl

ester). In this study, β-cyclodextrin was selected as a stereoselective reagent, underwent borate complexation, and was assessed by CE. The method had the characteristics of high resolution, high sensitivity, good repeatability, and high detection limit [56]. CE has become an ideal analytical method for the separation and quantitative analysis of saponins because of its advantages of high selectivity, short analysis time, low sample consumption, high efficiency, and high resolution. Of course, CE also has some disadvantages, such as low detection sensitivity, poor reproducibility, and small injection volume, etc.

3.3. NIRS

Near-infrared spectroscopy (NIRS) is a rapid analytical technique developed recently and is widely used to detect saponins in medicinal materials and preparations (Figure 7). One study described the quantitative analysis of shengmaxinside I in the process of honey-frying of *Cimicifuga foetida* and established near-infrared diffuse reflectance spectroscopy as a simple and effective analytical method. The study also used the partial least square method to establish the near-infrared quantitative model. The research showed that the experimental model had better prediction ability [57]. In another study, the content of total steroidal saponins in different species of *Paris* from Yunnan Province were analyzed, and a fast qualitative analysis method combining Fourier transform infrared spectroscopy and UPLC was adopted. The results showed that linear partial least square regression was more suitable than nonlinear support vector machine regression for the determination of total steroidal saponins in different species of Paris [58]. In addition, another study focused on three saponins (notoginsenoside R_1, ginsenoside Rg_1, and ginsenoside Rb_1) in *Panax notoginseng* and established NIRS technology as fast and simple for quantitative analysis. The results showed that this method could accurately predict the total content of three saponins in *Panax notoginseng* [59]. NIRS has many advantages in the analysis of saponins from natural products such as plants, including its simplicity, its fast analytical speed, its ability to not damage samples, its lack of chemical pollution, and more.

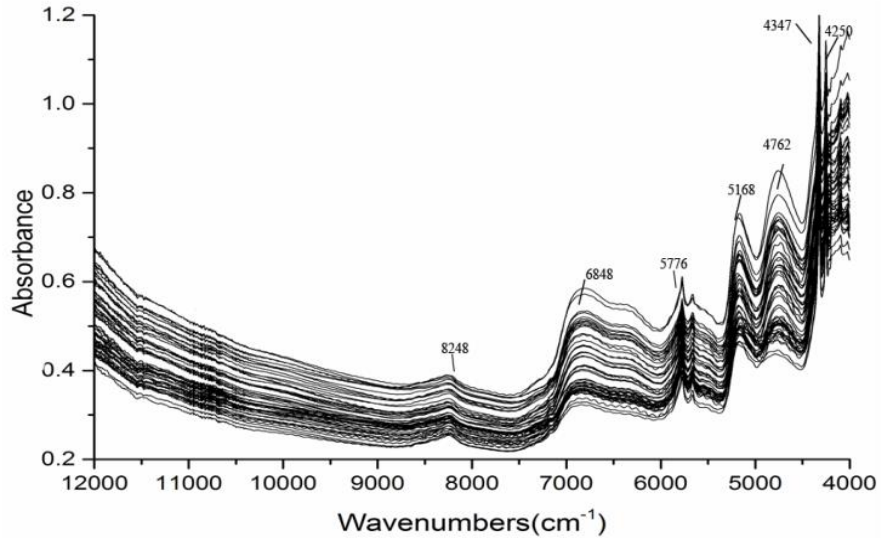

Figure 7. NIRS spectra of 150 batches of Rhizoma Cimicifugae [57].

3.4. HPLC

In recent years, HPLC has been widely used to identify and quantitatively analyze saponins and their preparations because of its high resolution, high selectivity, and high sensitivity. HPLC combined with various detectors has become the mainstream method for saponin analysis; potential combinations include the UV/diode array detector, evaporative light scattering detector (ELSD), charged aerosol detector (CAD), chromatographic fingerprint, and mass spectrometer detector (MS). LC is connected with these detectors to

qualitatively or quantitatively analyze multiple saponins in complex medicinal materials and their preparations.

3.4.1. HPLC-UV/DAD

The UV detector is the detector most commonly paired with HPLC. It has the advantages of wide application range, high sensitivity, wide linear range, and compatibility with gradient elution. In addition, HPLC diode array detection is a classic method of natural product analysis. The diode array approach is widely used to analyze complex samples of natural products. Its repeatability and, similar to UV detection, its high linearity in the determination of saponins are positive features. Compared with the UV detector, the diode array detector can detect many saponins simultaneously through a segmented monitoring strategy based on variable wavelength detection. Although the diode array detector provides a multi-wavelength spectrum, its sensitivity is lower than that of the UV detector.

HPLC diode array detection provides a potential analytical platform for the quality control and pharmacodynamic evaluation of various saponins with medicinal potential [60]. Compared with the colorimetric method, this analytical method can provide more information about the chemical composition of herbal extracts and their preparations [61]. One Study used *ilexoside II* as the external standard to verify the validity of the determination of total saponins in the immature fruits of *Ilex paraguariensis* by HPLC-UV spectrophotometry. This method had a high saponin yield and good reproducibility [62]. Kwon and Park separated astragalin and astragaloside from the head, main root, and lateral root of 4-, 5-, and 6-year-old astragalus by reversed-phase chromatography and detected them with high sensitivity by pulsed amperometric detection under alkaline conditions. The research showed that standardized cultivation and an appropriate storage technology are key to producing high-quality astragalus extracts rich in bioactive substances [63]. Extending this research, Lee realized the simultaneous quantitative analysis of isoflavones (calyx glycosides, formalonin) and triterpenoid saponins (AST-I-IV) in astragalus by increasing the detection potential [64]. In addition, another study used HPLC diode array detection technology to rapidly and simultaneously determine the four effective components of a *Panax notoginseng* injection: notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, and ginsenoside Rb1 [65]. HPLC combined with photodiode array detection can provide a three-dimensional fingerprint, making this method not only easy to understand but also effective for obtaining quantitative information about target components and accurate results. Therefore, this method has broad application prospects in the rapid determination of complex samples.

3.4.2. HPLC-ELSD

As a general detection method for saponin compounds, ELSD has been successfully applied to the quantitative analysis of saponins. Many studies have explored a fast and accurate HPLC approach combined with ELSD to simultaneously and quantitatively determine the content of saponins in traditional Chinese medicines from different origins and harvest periods [66]. Combining this approach with the methods of principal component analysis (PCA) and cluster analysis to classify and identify saponins of different samples evaluates the quality of medicinal materials even more thoroughly [67–69]. To compare the differences between medicinal materials from different areas, one study established a simple and reliable HPLC method combined with ELSD to compare the main saponins in the samples of *Platycodon grandiflorus* from southern and northern China [70] and to compare the contents of Ophiopogonin D′, Ophiopogonin D, Ophiopogonin B in the tubers and fibrous roots of *Ophiopogonin japonicas* in Cixi City, Zhejiang Province, and Santai County, Sichuan Province [71]. In addition, a study used HPLC diode array detection ELSD to determine the content of seven kinds of flavonoids and five kinds of saponins in the roots of *Astragalus membranaceus* var. mongholicus from Shanxi, Hunyuan, with different specifications and grades. The study found obvious differences in the concentration dis-

tribution law of flavonoids and saponins in astragalus, with different specifications and different grades [72]. Thus, ELSD can avoid the interference from terminal absorption wavelengths of saponins and overcome the difficulty of traditional analytical methods in the determination of saponins.

3.4.3. HPLC-CAD

As a quality detector, the charged aerosol detector is based on the principle of an aerosol detector, the sample solution is atomized by the atomizing gas (nitrogen) in the atomizer and then hits the collision baffle at a higher flow rate to form solute particles of different sizes. The larger particles are discharged from the waste pipe under the influence of gravity, and the smaller particles flow into the drying pipe with nitrogen; at the same time, the other flow path of the inlet nitrogen passes through the corona device (containing high-voltage platinum wire electrode) to form positively charged nitrogen particles, which collide with the dried solute particles in the collision cell. The charge is then transferred to the particles—the larger the solute particle, the more charge. The solute particles transfer their charge to the collector, and the charge amount of the solute particles is measured by a highly sensitive electrostatic detector. The resulting signal current is proportional to the content of the solute (Figure 8) [73].

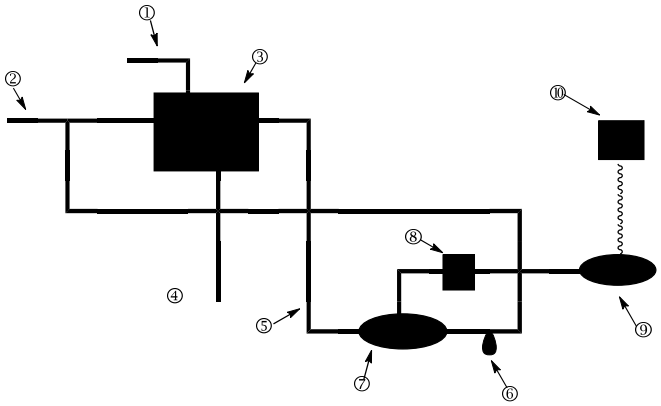

Figure 8. The structure and working diagram of the CAD [74]. ① HPLC eluent inlet. ② Nitrogen inlet. ③ Spray chamber: droplets form a spray. ④ Waste pipe: It attaches to large droplets. ⑤ Drying tube: dry particles are left after evaporation. ⑥ Corona electrodes: the gas is positively charged after ionization. ⑦ Collision cell: positive charges migrate to the surface of the particle. ⑧ Ion trap: it removes charged particles with high mobility. ⑨ Collector: the electrometer measures the surface charge. ⑩ Electrometer: signal strength is proportional to the amount of the analyte.

The CAD detector is based on a unique new design principle, which solves some limitations of other detector design principles. Its biggest advantage is that the detection does not depend on the molecular structure of the analyte or ionize the analyte, which achieves the purpose of versatility. It has the same response to different compounds and is not sensitive to external influences. It can carry out gradient elution. At the same time, it can achieve higher sensitivity and lower detection limit, good reproducibility, and wide dynamic detection range. The CAD detector combined with a high-performance liquid chromatography system is simple and convenient to operate and has good stability. It is widely used to detect most semi-volatile and non-volatile organic compounds, especially suitable for the analysis and detection of saponin compounds. One study simultaneously detected and quantitatively analyzed 15 triterpenoid saponins in the leaves, stems, root bark, and fruits of *Acanthopanax senticosus*. High-performance liquid chromatography-charged aerosol detection-electric spray mass spectrometry technology proved to be a simple and accurate method for the detection of triterpenoid saponins. The results showed that the baseline was stable, the sensitivity was high, and the reproducibility was good, which was significantly better than HPLC-UV [75]. Another study combined UPLC with

CAD to quantitatively analyze marker composition in ginseng. The results showed that the contents of ginsenoside Re, Rd, and Rg1 as well as compound K comprise approximately 22% of the ginseng plant. The total saponin content was determined by vanillin sulfuric acid system colorimetry and computer-aided design reaction [76].

3.4.4. Chromatographic Fingerprint

The chromatographic fingerprint can comprehensively measure the complex components of traditional Chinese medicine and provide an effective means for quality control of this medicine. The purposes of this method are to identify species, evaluate quality, and develop the consistency and stability of traditional Chinese medicine. One study used a simple method of HPLC with a UV light scattering detector to quantitatively analyze 10 batches of saponins from a pair of medicines—Anemarrhena and Phellodendron—and combined the method with the chromatographic fingerprint, for the first time in 2014 [77]. Tian et al. used HPLC-ELSD and high-performance TLC simultaneously to analyze the fingerprint of saikosaponin in bupleurum; the study found that the roots of different species of bupleurum can be easily distinguished with this technology, making it convenient for the classification of commercial samples [78]. Another study used HPLC with a UV fingerprint to analyze the Xuesaitong injection. The results showed that the fingerprints of 10 batches of samples had 27 characteristic peaks [79]. Qi et al. compared the changes of the liquid phase characteristic map of saponins in 13 batches of samples of astragalus before and after alkalization and determined that astragalus saponins I, II, III, and IV were the characteristic components. This method monitored the content and proportion of the four components at the same time, which could more truly reflect the quality of the original medicinal material [23]. In addition, another study combined HPLC-MS and nuclear magnetic resonance (NMR) techniques to characterize the structure of two isoflavones and four saponins. The study found that the combination of HPLC-MS and nuclear magnetic resonance (NMR) techniques could achieve an accurate description of the structure of these compounds [80]. Thus, chromatographic fingerprint analysis is a comprehensive qualitative method that simultaneously provides determination of multiple components of traditional Chinese medicine and comprehensive measurement of the complex component information of traditional Chinese medicine (Tables 3 and 4).

Table 3. Detailed conditions for the analysis of saponins in natural medicines and foods by high-performance liquid chromatography.

Detection Compound	Stationary Phase	Mobile Phase	The Detector	Ref.
4 triterpenoid saponins from Sophora flavescens	Luna C_{18} (2) column (150 × 4.6 mm; 5 μ particle size)	water (0.1% acetic acid) (A) and acetonitrile (0.1% acetic acid) (B)	996 Photodiode array detector (Waters Corp.), 75 ELS detector Sedex (SEDERE) And MS: ESI: TOF	[66]
3 steroidal saponins	Tigerkin C_{18} column	water (0.02% formic) (A) and acetonitrile (0.02% formic) (B)	Mass spectrometry	[81]
6 steroidal saponins	RP-18e monolithic column (50 × 2 mm)	acetonitrile (A) and formic acid aqueous solution (0.1%, *v/v*) (B)	Mass spectrometry	[82]
11 saponins of Achyranthes bidentate	Inertsil PREP-ODS column (20 × 250 mm)	volatile ion pair reagent (dihexyl ammonium acetate)	SPDM10AVP Photodiode Array Detector and Shimadzu LC-MS-2020 Mass spectrometry	[83]
12 diosgenin in six batches of polygala samples	Rich Alorich Ascentis C_8 column (100 mm × 4.6 mm, 3 μm)	diosgenin methyl: water (A) and methanol (B) 11 other saponins: water (A) and acetonitrile (B)	High Resolution Mass Spectrometry: (-):HESI(+/−)	[84]

Table 3. *Cont.*

Detection Compound	Stationary Phase	Mobile Phase	The Detector	Ref.
4 triterpene saponins in the Asparagus leaves	Dikma Diamonsil C_{18} column (4.6 mm × 250 mm, 5 μm)	acetonitrile (A) and water (B)	2000ES Diode array detector	[67]
9 oleic acid saponins	Kromasil 100-5 C_{18} column (250 mm × 4.6 mm, 5 μm)	water (0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B)	SPD-M20A DAD detector and LTQ Orbitrap Velos Pro mass spectrometer: (-): electrospray	[60]
5 triterpenoidal saponins In *Pulsatilla koreana*	Shiseido CapCell PAK C_{18} analytical column (4.6 mm × 150 mm, 5 μm)	water (A) and acetonitrile (B)	MS: (-): ESI	[68]
15 triterpenoid saponins from the leaves, stems, root skins and fruits of Acanthopanax quiculata	Kinetex XB-C_{18} column (100 mm × 4.6 mm, 2.6 μm)	acetonitril (A) and water (B)	Charged Aerosol Detection and Agilent 6530q-TOF mass spectrometry: (+)	[75]
4 steroidal saponins	Diamonsil C_{18} column (4.6 mm × 250 mm, 5 μm)	acetonitrile (A) and water (B)	UV detector, Sedex75 ELSD system and DAD	[77]
6 components in extract of ivy leaf	YMC Hydrosphere C_{18} analytical column (150 × 4.6 mm, 5 μm)	acetonitrile (A) and 0.1% phosphoric acid (B)	G4212A UV-Visible Diode Array Detector	[61]
triterpene saponins in Camellia plants	Inertsil ODS-3 column (2.1 mm × 100 mm)	methanol (A) and 5 mM trifluoroacetic acid (B)	UV-visible light detector	[85]
Arachnoside F in rat plasma	Reverse phase Zorbax SB-C_{18} column (150 × 4.6 mm, 5 μm)	ammonium acetate (A) and acetonitrile (B)	Agilent 6460 Triple Quadrupole Mass Spectrometer: Electrospray (+)	[86]
5 saponins in 10 batches of Panax notoginseng	Agilent Zorbax SB-AQ analytical column (4.6 mm × 50 mm, 3.5 μm)	deionized water (A) and acetonitrile (B)	Diode array detector	[87]
Panax notoginseng saponin Fc and Ginsenoside Rc in Notoginseng leaf	Zorbax ODS C_8 column (250 mm × 4.6 mm, 5 μm)	water (A) and acetonitrile (B)	Waters 2996 photodiode array detector	[88]

3.4.5. HPLC-MS Detector

HPLC-MS is a powerful tool to identify and quantitatively analyze saponins. Compared with other detectors, it has higher sensitivity and offers more complete structural information, and it has the advantages of short analysis time, low sample consumption, and online access. For this method, common ion sources include electron bombardment ionization sources and chemical ionization sources for gas samples; electrospray ionization sources, an atmospheric pressure chemical ionization source, and an atmospheric pressure photoionization source for liquid samples; and a matrix-assisted laser desorption ionization source for solid sample analysis.

HPLC-MS has been used widely to identify and quantitatively analyze saponins and to assess the quality control of medicinal extracts [69,75,84,89–96]. One study has carried out qualitative and quantitative analyses of bitterness from fresh and processed asparagus components, specifically the *Bidens* saponin 1*a*/*β*-6, using a sensitive method of HPLC-MS/MS. The study found that the single bridged saponin 5*a*/*β* was the main factor causing the bitterness of fresh asparagus, whereas the saponins 1*a*/*β* and 2*a*/*β* were the main factors contributing to the bitter taste of processed asparagus [97]. Another study used the multiple reaction monitoring of positive and negative ion switching in a quantitative analysis of 15 active ingredients of Chaihu Guizhi decoction. The study in rats after oral administration of the decoction established a simple, sensitive, and selective HPLC-electrospray tandem

MS approach [98]. In another study, qualitative and quantitative analyses of the effective components of raw and processed licorice were used to explore the in vitro metabolism of the two decoctions in the gastrointestinal tract. The study used two HPLC methods, one paired with a diode array detector and one paired with an electrospray mass spectrometer. The results showed that the processing of licorice could change the content of the main components and affect its GI metabolism (Table 4) [99].

Table 4. Application of high-performance liquid chromatography-mass spectrometry in the determination of medicinal materials and their preparations containing saponin.

Name	Qualitative/ Quantitative	Analytical Method	Chromatographic Conditions	Test Results	Ref.
Polygonti Rhizome	Qualitative and quantitative	UHPLC-Q-Exactive Orbitrap HRMS	stationary phase: Alorich Ascentis C_8 column (10 cm × 4.6 mm, 3 μm), mobile phases: water (A) and acetonitrile (B), column temperature: 25 °C	12 diosgenin:Dioscin, Gracillin, Deltonin, Trillin, Prosapogenin A, zingiberensis New Saponin, Protodioscin, Protogracillin, Protodeltonin, Pseudoprotodioscin, Pseudoprotogracillin, Methyl protodioscin	[84]
Soybeans	Qualitative and quantitative	SPE-HPLC-MALDI-TOF-MS	stationary phase: Gemini C_{18} column (150 × 4.6 mm, 5 μm), mobile phase: water (0.25% acetic acid) (A) and methanol (0.25% acetic acid) (B)	soyasaponins I and βg	[95]
Achyranthes	Quantitative	LC-MS	stationary phase:Inertsil PREP-ODS column (20 × 250 mm), mobile phase: UPW (5 mM DHAA) (A) and MeCN (5 mM DHAA) (B)	chikuset-susaponins IVa and V, achyranthosides B, C, D, E and G, sulfachyranthosides B and D, and betavulgarosides II and IV	[83]
Paris and *Trillium*	Qualitative	HPLC-ESI (+/−)-MS^n	stationary phase: Kromasil RP-C_{18} column (4.6 mm × 250 mm, 5 μm), mobile phase: water (A) and acetonitrile (B)	12 steroidal saponins: Dichotomin, Protosaponin3Glc-Rha-Ara, Methyldichotomin, Methyl protodioscin, Methylprotosaponin2Glc-2Rha-Ara, Diosgenin 2Glc-3Rha, PolyPhyllin H, Methylprotogracillin, Diosgenin2Glc-Rha-Ara, PennogeninGlc-2Rha, Pennogenin 2Glc-Rha, Diosgenin2Ara-Rha-Glc	[69]
Ophiopogon japonicus	Quantitative	HPLC-MS	stationary phase: Tigerkin C_{18} column, mobile phase: water (0.02% formic acid) (A) and acetonitrile (0.02% formic acid) (B)	three steroidal saponins: cixi-ophiopogon A, cixi-ophiopogon B, cixi-ophiopogon C	[81]
Chaihu	Quantitative	anionic adducts-based liquid chromatography tandem mass spectrometry method	stationary phase: Agilent Zorbax SB-C_{18} column (100 × 3.0 mm, 3.0 μm), mobile phase: water (0.06% formic acid) (A), acetonitrile (B) and methanol (C)	saikosaponin a, saikosaponin c, saikosaponin d and saikosaponin b_2	[94]
Chaihu-Guizhi decoction	Quantitative	HPLC-ESI- MS/MS	stationary phase: Halo® C_{18} column (2.1 × 100 mm, 2.7 μm), mobile phase: water (0.1% formic acid) (A) and acetonitrile (B), flow rate: 0.3 mL/min	15 active compounds: Saikosaponin A, Baicalin, Wogonin, Glycyrrhizic acid, Glycyrrhetinic acid, Albiflorin, Paeoniflorin, Liquiritin, Isoliquiritin, Liquiritigenin, Isoliquiritigenin, Cinnamic acid, Gallic acid, Wogonoside and Oroxylin A	[98]

Table 4. *Cont.*

Name	Qualitative/ Quantitative	Analytical Method	Chromatographic Conditions	Test Results	Ref.
Zhimu-Baihe herb-pair	Qualitative	high-performance liquid chromatography and time-of-flight mass spectrometry	stationary phase: Zorbax XDB-C_{18} analytical column (2.1 × 50 mm, 1.8 μm), mobile phase: water (0.1% formic acid) (A) and acetonitrile (B), flow rate: 0.2 mL/min	24 saponins, 3 xanthones, 1 anthraquinone and 2 alkaloids: Neomangiferin, Mangiferin, Isomangiferin, Timosaponin B-V, Timosaponin B-VI, Timosaponin H1, Timosaponin I1, Timosaponin B-IV, Timosaponin I2; Timosaponin H2, Neohyacinthoside, Timosaponin E1, Timosaponin E, Timosaponin N, Timosaponin E2, Macrostemonoside K, Timosaponin B-II, Timosaponin D, Timosaponin B-I, Timosaponin B-III, Brownioside 1, Brownioside 2, Timosaponin F, Anemarrhenasaponin I, Anemarrhenasaponin Ia, Timosaponin G, Timosaponin AIII, Timosaponin A-I, Colchicine, Emodin	[100]
Radix Astragali	Qualitative	HPLC-Q- TOF/MS	stationary phase: Gemini C_{18} column (4.6 mm × 250 mm, 5 μm), mobile phase: water (0.3% formic acid) (A) and ACN (B)	22 types of astragaloside IV	[90]
Ophiopogon Japonicus Ker-Gawler	Qualitative	High-Performance Liquid Chromatography with Ion Trap Mass Spectrometry	stationary phase: Tigerkin C-$_{18}$ column (4.6 × 250 mm, 5.0 μm), mobile phase: water (0.05% formic acid) (A) and acetonitrile (0.05% formic acid) (B), flow rate: 0.5 mL/min, detection wavelength: 203 nm	8 steroidal saponins: ophiogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-xylose-(1→3)-*β*-D-glucose-(1→4)-*β*-D-glucose, ophiogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-glucose-*β*-D-glucose, ophiogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-xylose-*β*-D-glucose, pennogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-xylose-(1→3)-*β*-D-glucose, ruscogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-xylose-(1→3)-*α*-L-ara*β*inose, ruscogenin 3-*O*-*α*-L-rhanose-(1→2)-*β*-D-xylose-(1→3)-*β*-D-fucose, pennogenin 3-*O*-*α*-L-Rha-(1→2)-*O*-*β*-D-Xyl-(1→3)-*O*-*β*-D-Xyl-(1→4)-*O*-*β*-D-Glc, pennogenin 3-*O*-*α*-L-Rha-(1→2)-*O*-*β*-D-Glc-(1→3)-*O*-*β*-D-Glc or ruscogenin 3-*O*-*α*-L-Rha-(1→2) -*O*-*β*-D-Glc-(1→3)-*O*-*β*-D-Glc	[91]
Glycyrrhiza uralensis	Qualitative	rapid-resolution liquid chromatography with time-of-flight mass spectrometry (RRLC/TOF-MS)	stationary phase: Agilent ZorBax SB-C_{18} column (4.6 × 50 mm, 1.8 μm), mobile phase: water (0.2% formic acid) (A) and acetonitrile (B)	19 oleic acid alkanestype triterpene saponins: uralsaponin C, uralsaponin D, uralsaponin F, uralsaponin E, 24-hydroxyl-licorice E_2, licorice-saponin A_3, 22-acetoxyl-glycyrrhizin, licorice-saponin E_2, 22-acetoxyl-Glycyrrhaldehyde, licorice-saponin G_2, glycyrrhizin, 18a-glycyrrhizin and uralsaponin B	[93]
Shaoyao-Gancao-Decoction	Quantitative	HPLC–MS/MS	stationary phase: Zorbax XDB-C_{18} column (2.1 mm × 50 mm, 3.5 μm), mobile phase: water (0.1% formic acid) (A) and methanol (0.1% formic acid) (B)	Albiflorin, oxypaeoniflorin, paeoniflorin, liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, ononin, glycyrrhizin andglycyrrhetinic acid	[101]

Table 4. *Cont.*

Name	Qualitative/ Quantitative	Analytical Method	Chromatographic Conditions	Test Results	Ref.
Glycyrrhiza yunnanensis	Qualitative	HPLC–MS/MS	stationary phase: YMC-Pack ODS-A column (4.6 mm × 250 mm, 5 μm), mobile phase: acetonitrile (A) and water (0.1% formic acid) (B), column temperature: 35 °C, flow rate: 1 mL/min	glyyunnansapogenin I, yunganosides E_3, L, M, N_1,O, P and N_2	[92]
Dioscorea panthaica Prain et Burk	Quantitative	high-performance liquid chromatography-electrospray tandem mass spectrometry	stationary phase: RP-18e monolithic column (50 mm × 2 mm), mobile phase: acetonitrile (A) and water (0.1% formic acid) (B)	six steroid saponins: HSY-14, HSY-10, dioscin (DS), gracillin (GC), pseudoprotodioscin (PDD), pseudoprotogracillin (PDG)	[82]
Ardisia Crenata	Qualitative and quantitative	Ultra fast liquid chromatography-electrospray quadrupole mass spectrometry (UFLC-MS)	stationary phase: Zorbax Eclipse Plus C_{18} column (100 mm × 2.1 mm, 1.8 μm), mobile phase: water (0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B), flow rate: 0.2 mL/min	13,28-epoxy-oleanane-type triterpenoid saponins	[96]
Acanthopanax henryi	Qualitative	HPLC-ESI-TOF-MS	stationary phase: Kinetex XB-C_{18} column (100 mm × 4.6 mm, 2.6 μm), mobile phase: acetonitrile (A) and water (B)	15 triterpenoid saponins	[75]
Panax notoginseng	Qualitative	HPLC-QTOF/MS	stationary phase: agilent Eclipse XDB-C_{18} column (250 mm × 4.6 mm, 5 μm) mobile phase: water (0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B), flow rate: 0.8 mL/min	234 ginsenosides	[89]
Triguero asparagus	Qualitative and quantitative	HPLC-MS	stationary phase: reversephase analytical column (25 cm × 4.6 mm, 5 μm), mobile phase: water (0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B)	saponins (HTSAP1 to HTSAP8) and protodioscin	[102]
fresh and cooked white asparagus	Qualitative and quantitative	HPLC-MS/MS	stationary phase: Zorbax Eclipse XDB-C_{18} column (150 × 2.1 mm, 5 μm), mobile phase: acetonitrile (0.1% formic acid) (A) and water (0.1% formic acid) (B)	the monodesmosidic saponins 5a/b, bidesmosides 1a/b and 2a/b	[97]
crude *Glycyrrhizae radix* and processed *Glycyrrhizae radix*	Qualitative	HPLC-ESI/MS	stationary phase: Kromasil 100-5 C_{18} column (4.6 × 250 mm, 5 μm), mobile phase: water (0.1% formic acid) (A) and acetonitrile (B), detection wavelength: 254 nm	eleven constituents: liquiritin apioside, liquiritin, licuraside, isoliquiritin, ononin, glycyrrhizin, liquiritigenin-7,4′-diglucoside, licorice saponin A_3, 22β-acetoxylglycyrrhizic acid, licorice saponin G_2, and yunganoside E_2	[99]

3.5. UPLC

In 1996, the Waters Corporation launched Alliance HPLC. With the progress of science and technology and the development of industry, the requirements for LC in various fields are increasing day by day. In 2004, the Waters Corporation launched the world's first ultra-high-performance liquid chromatograph, Acquity UPLC, which uses a small-particle-packed column (less than 2 μm) and an ultra-high pressure system (more than 105 kpa) and is suitable for the separation of trace complex mixtures and high-throughput research [103]. Compared with the HPLC system, UPLC can significantly improve the separation degree of the chromatographic peaks and the detection sensitivity and, at the same time, greatly shorten the analysis time and reduce the solvent consumption. However, there are some limitations to UPLC, such as the lengthy sample pretreatment time and the system's ultra-high pressure resulting from the small particle size packing. Because of these limitations, higher requirements are placed on the sealing of the instrument, the injection of the injection valve, the infusion of the pump, and the performance of the detector.

UPLC and its combination technology have developed rapidly, especially with MS. Examples of its use include analysis of components of traditional Chinese medicine (identification of traditional Chinese medicine components [88], determination of the components' content [104,105], fingerprint study of traditional Chinese medicine [106]) and metabonomics. In one study, UPLC-quadrupole time-of-flight (Q-TOF)-MS was used to qualitatively and quantitatively analyze the chemical structure of the main saponins in quinoa seeds and assessed the contents of two quinolone saponin components, FQ70 and FQ90. The study found that both quinolone saponin components significantly improved the humoral and cellular immune responses to ovalbumin (OVA) in mice, with obvious immune adjuvant properties [107]. In another study, the sources of 12 kinds of ginseng were assessed to analyze the development trend of ginseng varieties; assessments were completed with fast and accurate UPLC-tandem MS [108]. Another study identified and determined the content of six steroidal glycosides and one aglycone in pangolin and yam using the efficient and reliable UPLC-Q-TOF-MS. Then, the researchers compared the chemical composition of pangolin and yam by chromatographic fingerprint similarity evaluation, using a significance test (*t* test) and PCA. The study results demonstrated that the chemical composition of all samples of pangolin and yam showed a high degree of overall similarity [109]. An additional study on the distribution and quantitative analysis of the main active saponins in different tissues of *Panax notoginseng* (cork, cortex, phloem, and xylem) used a simple, sensitive, and accurate UPLC-Q-TOF-MS combined with a fluorescence microscope and laser microdissection technology. The research revealed the distribution of the main saponins of *Panax notoginseng* in tissues [110].

For UPLC techniques, the differentiation among the type of detector and ionization could be better described, and so this technique could be used in the rapid separation, structure identification, and content determination of several saponins in complex natural products. In one study, astragalus was used as an example to explore simple, economic, and effective quality control methods. The astragaloside content was determined by UPLC, and the fingerprints of astragaloside and total flavonoids of astragalus were established by full scan mode, which met the requirements of product quality supervision in the production process [111]. In addition, the contents of 25 compounds in different parts (roots, rhizomes, stems, leaves, and flowers) of two species of astragalus have been analyzed and compared in another study [112], and 14 main chemical components (five flavonoids and nine triterpenoid saponins) in 94 batches of astragalus from different places (China, Korea, and Germany) had been determined simultaneously [113]. Astragaloside III was not only an important chemical marker for the identification of astragalus and membranous astragalus, but it was also a potential chemical marker for the classification of cultivated astragalus and semi-wild astragalus, as determined with UPLC. Many studies focus on the identification and quantitative analysis of triterpenoid saponins in *Glycyrrhiza* plants by UPLC-MS with the simultaneous determination and rapid screening of several effective components, including saponins and flavonoids in licorice [114–117]. Some studies determined the

eight triterpenoid saponins in dog plasma after oral administration of total saponins in *Glycyrrhiza* [118], and another performed preliminary identification of the active ingredients of licorice in Wutou decoction [119]. Another study applied a UPLC-MS method based on a standard addition to quantitative analysis of 14 compounds in *Glycyrrhiza*. Using this method, *G. glabra*, *G. uralensis* and *G. inflata* in a variety of forms, including root powders and extracts, as well as complex dietary supplements, could be differentiated and chemically standardized [120].

3.6. QAMS

QAMS was first proposed by Zhimin Wang et al. in 2006 [121]. By studying the internal functional proportional relationship between the active ingredients of traditional Chinese medicine and introducing a relative correction factor on the basis of the internal standard method, this study achieved for the first time the simultaneous determination of the contents of multiple components tested from traditional Chinese medicine and the preparations with a reference substance. The basic research supporting this method relies on the principle that the amount of component (mass or concentration) in a certain linear range is proportional to the response of the detector, which is represented by $W = f \times A$. In the multi-index quality evaluation, a representative component of the medicinal material (the stable and easily obtained reference substance) is used as an internal reference, and the relative correction factor (RCF) between this internal reference and other components is established, without providing reference products of other components, by the RCF to calculate the number of other components.

Suppose a sample contains i components, and $f_i = [(W_i) \div (A_i)]$ (i = 1, 2...k,...m), in which W_i is the component concentration and A_i is the component peak area. Select one of the components k as the internal reference, and establish the RCF between the component k and the other components m. The quantitative calculation formula $W_m = [(W_k \div f_{km}) \times (A_m \div A_k)]$, in which f_{km} is the RCF of the internal reference and other components to be measured and f_k and f_m are the absolute correction factors of the internal reference substance and other components to be measured, respectively. A_k and A_m are the peak areas of the internal reference and other components to be tested, respectively, and W_k and W_m are the concentration (or mass) of the internal reference and other components to be tested, respectively [87]. This method is suitable for the simultaneous qualitative and quantitative determination of the same kind of multiple components when a reference substance is rare [122] and preparation cost is high [123].

QAMS uses a single index to quantitatively analyze multiple components [124,125]; this approach not only reduces the analysis time and cost but also improves the analysis efficiency to provide a more comprehensive quality evaluation of medicinal materials and prescription preparations. One study established a QAMS method for the determination of the active components of treatments for rheumatoid arthritis (using astragaloside IV as the internal standard). There was no significant difference between the content of active components in Astragalus membranaceus and the content determined by the external standard method (RSD < 0.05), and the RCF established had good reliability [106]. The latest research evaluated and discussed the volatility and stability of RCFs by using 19 kinds of ginsenosides as reference standards under different MS conditions (different HPLC-MS instruments and different de-aggregation potentials). This study found that the RCF had enough reproducibility under a wide range of changes to verify the rationality of simultaneous determination of 19 ginsenosides using a single test and multiple evaluation methods [126]. Another study used a relative response factor method and a UV-MS gradient elution method to determine the content of ginsenosides in ginseng extract and ginseng products. Compared with the external standard method, the QAMS method that was based on the relative response factor has a smaller difference [125]. In addition, another study established a QAMS method for the simultaneous determination of 11 saponins in *Panax notoginseng* and identification of the effects of the chemical structure of the internal standard, the concentration of the quantitative components, and the purity

of the reference substance. The study assessed the accuracy of the QAMS method and showed that the concentration of the analyte in the sample solution was the main parameter affecting the accuracy of the QAMS method. By calculating and controlling the applicable concentration range of the analyte in the sample, the high accuracy of the QAMS method was ensured [127].

3.7. Immunoassay

The immunological analysis method has high sensitivity and specificity, which can be used to analyze saponins. One study established a time-resolved fluorescence immunoassay system to determine the content of saikosaponin a (SSa) in 10 commercial samples of bupleurum. The bupleurum methanol extract and a mouse anti-SSa monoclonal antibody were used as materials, and the Eu^{3+}-labeled SSa-human serum albumin conjugate was used as a tracer (Figure 9). This technology had the advantages of high sensitivity, convenience, and speed [128].

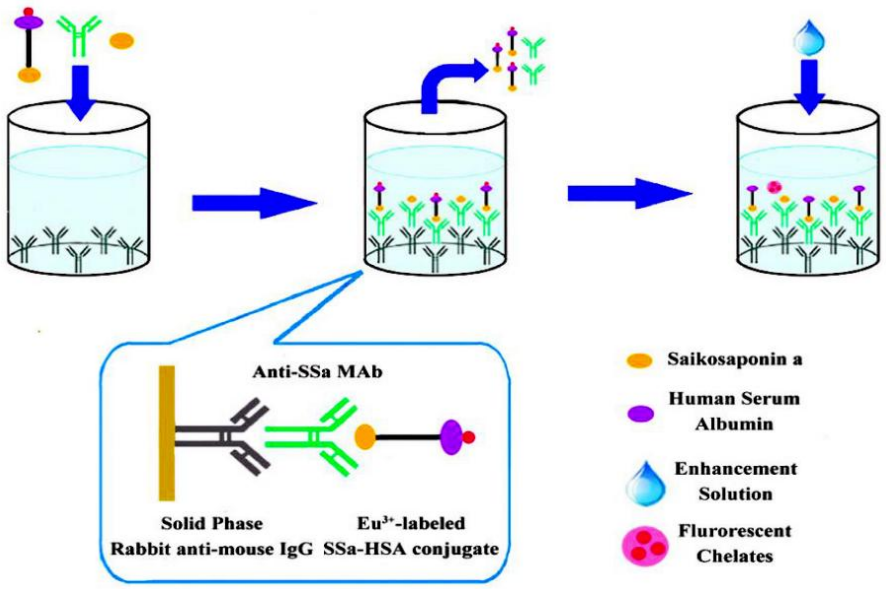

Figure 9. Schematic diagram of the TRFIA system for SSa detection [128].

3.8. Metabolomics

Metabolomics is a new discipline developed in the mid-1990s to analyze all low-molecular-weight metabolites of a certain organism or cell qualitatively and quantitatively. Its core approach is to take the physiological and pathological process of the human body as a dynamic system and study the types, quantity, and changes in endogenous metabolites after the organism is disturbed by internal and external environmental factors. Metabolomics can be divided into nontargeted and targeted metabolomics according to different research purposes. Nontargeted metabolomics is a systematic and comprehensive analysis of endogenous metabolites, whereas targeted metabolomics is the analysis of specific metabolites. Targeted metabolomics is accurate in qualitative and quantitative analyses, but its coverage of substances is limited. Although the coverage of nontargeted metabolomics across substances is extensive, this approach lacks absolute qualitative and quantitative data (Figure 10).

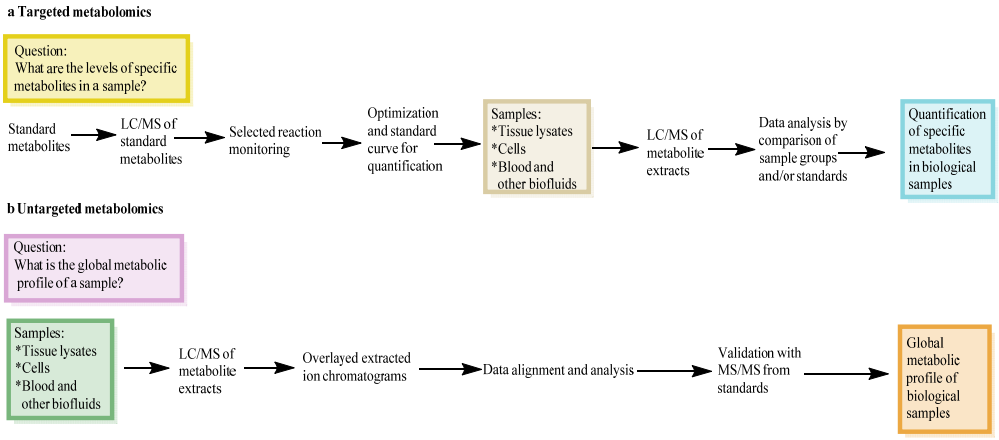

Figure 10. The targeted and untargeted workflow for LC/MS-based metabolomics [129].

In the analysis of saponins, metabolomics techniques are mainly combined with MS, liquid chromatography, gas chromatography, and NMR spectroscopy. NMR spectroscopy provides valuable details about metabolite characteristics of complex plant extracts and has the advantages of fast detection speed, easy absolute quantification, and clear compound identification; these positive attributes make the method an appropriate technique for analyzing large amounts of metabolites. High-performance TLC has the advantages of universal availability of derivatization reagents and a short running time. In addition, the simplicity of preparing HPTLC provides great potential for enhancing metabolomic studies of saponin-rich medicinal materials. The combination of UPLC and electrospray ionization-Q-TOF-MS can shorten the analysis time, provide accurate quality measurements and more molecular formula information, and facilitate the analysis of known compounds and the interpretation of unknown compounds in complex matrixes. Therefore, in recent years, the combination of UPLC with electrospray ionization-Q-TOF-MS has become the preferred technology for the analysis and identification of chemical components and metabolites in vivo after oral administration. Compared with LC-MS, gas chromatography-MS has better peak resolution and sensitivity. However, this technology also has some disadvantages, such as relatively low signal reproducibility and measurement of volatile compounds, and thus gas chromatography is not suitable for the determination of saponins.

The metabolic spectrum analysis technology can help researchers understand the biochemical composition of an organism in more detail. Many medicinal plants use saponins as their main metabolites, and the changes in metabolic components are significantly related to genetics (per species or in varieties within species) and environmental factors (geographic location and planting time). One study revealed the composition differences of primary and secondary metabolites in *Glycyrrhiza* through the combination of NMR and MS technologies combined with multivariable data analysis. It was found that the glycoside conjugates of glycyrrhizic glycoside, 4-hydroxyphenylacetic acid, and glycyrrhizin/isoglycyrrhizin are the main spectral peaks to distinguish species in the ^{1}H NMR and MS spectra [130]. In another study, a fast and sensitive HPLC-electrospray ionization-tandem MS was used to identify the structure of a novel composite bellflower glycoside metabolite transformed by human intestinal bacteria. The study showed that under chromatographic conditions, eleven main peaks were detected in the metabolites of Platycodon grandiflorum. Through the comparison of spectra in positive and negative ion modes, clear information about the molecular weight of metabolites was found [131]. Another study identified and studied the whole metabolic process of *Ophiopogon japonicus* roots at different ages (1 to 3 years old) collected from two producing areas (Zhejiang Province and Sichuan Province, China) by coupling ^{1}H NMR and high-performance TLC. It was found that Ophiopogon saponin, Ophiopogon saponin C and Ophiopogon saponin D were the marker metabolites in *Ophiopogon japonicus* roots [132]. In view of the significant differences in chemical components in different parts of

Panax notoginseng, one study carried out quantitative and qualitative analyses and a comparison of different parts of *Panax notoginseng* (rhizome, main root, lateral root, and fibrous root) in its main production area of Wenshan City, Yunnan Province, through the proven UPLC-Q-TOF-MS method and nontargeted metabolomics. The study showed significant differences between rhizome and other parts, and it identified the content of monomer saponins and total saponins as the highest in the rhizome. The results showed that this part was suitable to use as the raw material for ginsenoside products [133]. A study used UPLC-Q-TOF-MS metabolomics to identify and quantitatively analyze the different chemical constituents of the roots, stems, leaves, and seeds in *Polygala*. A total of 22 markers were detected, and seven triterpene saponins were significantly different in different tissues [134].

4. Discussion

Traditional extraction and separation techniques, such as thermal reflux extraction, Soxhlet extraction and liquid chromatography, have many shortcomings, such as long pretreatment time, cumbersome operation steps, and large consumption of organic solvents. At present, both ionic liquids and supercritical fluid chromatography (SFC) are green chromatographic extraction and separation technologies, which show great potential to replace traditional organic solvents in many fields. High-speed countercurrent chromatography (HSCCC) is an all-liquid-partition chromatography method that eliminates the irreversible adsorption loss of samples on solid support matrix columns and has been widely used for the separation of saponins from natural products due to its superior separation ability.

Currently, the main analytical methods of saponins are thin-layer chromatography, CE, NIRS, HPLC, UPLC, QAMS, immunoassay, and metabonomics. HPLC has been combined with a variety of detectors, such as UV/diode array detector, ELSD, CAD, MS, and chromatographic fingerprint.

These analytical methods have their own advantages and disadvantages; for example, spectrophotometry is a simple and reliable operation but can only provide the content of total saponins. TLC has the advantages of simple operation, strong separation ability, low cost, and fast detection speed, so it can be used for the analysis of several saponins. CE is an effective analytical technique with a short analysis time, high resolution, small sample size needs, and high selectivity, but it has disadvantages of low sensitivity and poor reproducibility. NIRS has many significant advantages in the analysis of saponins in natural products, including its simple use, fast analysis speed, lack of damage to samples, lack of chemical pollution, and more. The immunoassay approach has high sensitivity and specificity, which can rapidly determine saponin content to support quality control assessments of drugs and their preparations; this method also contributes to the exploration of mechanisms of action in saponin-rich drugs and helps identify the active substances. However, the disadvantage of metabolomics is the need for a large number of samples. This analysis also must be combined with other analytical instruments. A single-test multi-evaluation method comprehensively evaluates the quality of medicinal materials on the basis of several indexes. It is not only easy to operate but also can reduce the cost of detection, so it has been widely used. UPLC has many advantages, such as a fast analysis speed, high resolution, and less solvent consumption. However, because the particle size of the packing in the UPLC column is small, the sample must be pretreated carefully. UPLC-electrospray ionization-tandem MS has the advantages of high sensitivity, high resolution, and high-quality measurement accuracy. It is a powerful tool to comprehensively determine a variety of saponins in complex Chinese medicinal materials, but it has numerous requirements regarding the types and acquisition of reference substances.

HPLC combined with a variety of detectors also has obvious advantages and inevitable limitations. For example, a UV detector has the advantages of a wide application range, high repeatability, wide linear range, and compatibility with the gradient elution. Because UV detection is limited to analytes with suitable chromophores, some problems occur in the determination of saponins—namely, low sensitivity and low accuracy—so the utilization

rate is gradually reduced. As a general detection method of saponins, ELSD overcomes the difficulty of determination and avoids the interference of a terminal absorption wavelength. Even when it is used in gradient elution analysis of nonchromophores and nonvolatile compounds, ELSD has a stable baseline and has been successfully applied in saponin extraction and quantitative analysis. However, ELSD has the disadvantages of its complex sample pretreatment, narrow linear range, low sensitivity, and inability to quantitatively determine tracesaponins. CAD is a new detection technology, which is suitable for the analysis of weak or non-UV absorption compounds. It has the advantages of a suitable gradient elution, a stable baseline, high sensitivity, and simple operation. The chromatographic fingerprint can comprehensively reflect the complex components of traditional Chinese medicine, and this method effectively assesses the quality control of traditional Chinese medicines. The ion trap mass spectrometer can be used for multi-stage tandem MS to provide chemical structure information. Q-TOF-MS has the advantages of accurate quality information and high sensitivity, but it also has the limitation of great expense.

The combination of multiple analytical methods to achieve high sensitivity, high selectivity, and high accuracy for simultaneous qualitative and quantitative analyses of multiple saponins in medicinal materials and their preparations has become a trend. HPLC and MS detectors combine the rapid separation ability of LC with the high sensitivity, high specificity, and good selectivity of MS detectors. This combination can produce more accurate and specific analysis results. Therefore, HPLC-MS has become the preferred method for the rapid determination of complex saponins in medicinal materials. As a new, efficient, and low-cost method for evaluating the overall quality of traditional Chinese medicine, QAMS can effectively overcome the difficulties in preparing reference materials with complex structures and the instability of saponins under acidic conditions. These obvious advantages will increase the role of QAMS in saponin analysis, and QAMS may become a powerful tool for assessing the quality control of saponin compounds. Conversely, the combination of metabolomics and gene expression analysis has become a hot research topic. The combination of these two analyses can clarify the mechanism of saponin biosynthesis, find key enzyme genes, improve the yield of saponin-rich medicinal materials by controlling gene expression, and guide the cultivation of excellent plant varieties through the best aspects of biosynthesis to enhance the development and use of saponin components (Figure 11).

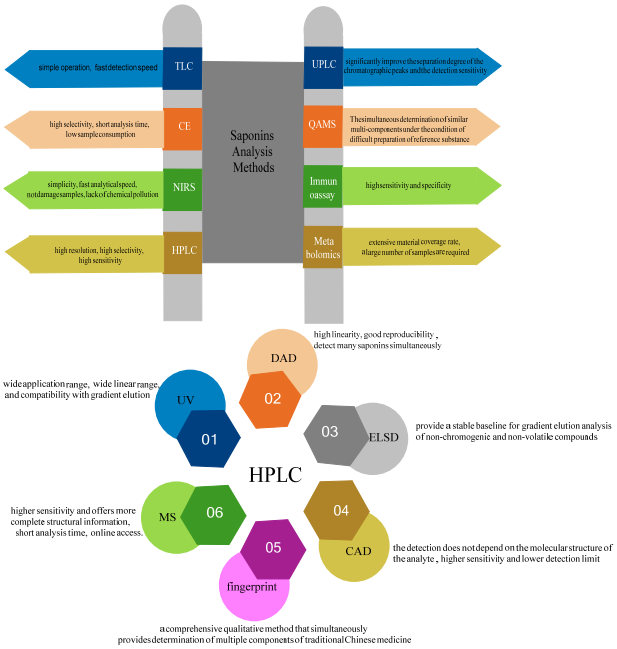

Figure 11. Characteristic diagram of the saponin analysis method.

5. Conclusions

In conclusion, this article provided a systematic and comprehensive review of methods for the separation and analysis of saponins over the past 10 years. The collected data provide the latest valuable insights and references for separation, quality control and for continued development and application of saponins.

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Abbreviations

ILs	ionic liquids
SFC	supercritical fluid chromatography
UAE	ultrasonic-assisted extraction
MAE	microwave-assisted extraction
HSCCC	high-speed counter-current chromatography
DAD	diode array detector
MSD	mass spectrometry detector
ELSD	evaporative light-scattering detector
CAD	charged aerosol detector
QAMS	quantitative analysis of multi-components by single-marker
NIRS	near-infrared spectroscopy
TLC	thin-layer chromatography
CE	capillary electrophoresis
HPLC	high-performance liquid chromatography
UPLC	ultra-high-performance liquid chromatography
UV/DAD	ultraviolet/diode array detection
SMD	standard method difference
PLS	partial least square
PCA	principal component analysis
RSD	relative standard deviation
CG	calycosin-7-*o*-*β*-d-glucoside
NMR	nuclear magnetic resonance
HPTLC	high-performance thin-layer chromatography
IT	ion-trap
PR	polygala radi

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