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Review

Research Progress on Quality Control Methods for Xiaochaihu Preparations

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Abstract: Xiaochaihu (XCH) is a classic Chinese medicine formula. XCH tablet, XCH granule, XCH capsule, and XCH effervescent tablet are included in the Chinese Pharmacopoeia. In this review, the formula and quality standards of XCH preparations at home and abroad were compared. The differences in manufacturing process of XCH preparations are discussed. The progress of research on the qualitative identification, quantitative detection and fingerprint chromatogram/specific chromatogram of XCH preparations was reviewed. The characteristic components of Pinelliae Rhizoma Praeparatum Cum Zingibere Et Alumine and Jujubae Fructus was rarely analyzed for XCH preparations. It is suggested that the specificity of drug quality detection methods should be improved. Considering drug safety and drug efficacy, it is suggested to set the upper and lower limits of the content of saikosaponins. The standards for heavy metals and other limited items for XCH preparations are also suggested to be set.

Keywords: Xiaochaihu (XCH) formula; preparations; quality control; standard

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1. Introduction to XCH Formula

Xiaochaihu (XCH) formula, which was created by Zhang Zhongjing in the East Han Dynasty, is capable of inducing sweat to dispel heat, channeling the liver, regulating the spleen, soothing the stomach [1], etc. Traditionally, the recipe is composed of *Bupleuri Radix*, *Scutellariae Radix*, *Ginseng Radix Et Rhizoma* (*Ginseng Radix*), *Glycyrrhizae Radix Et Rhizoma Praeparata Cum Melle* (*Glycyrrhizae Radix*), *Zingiberis Rhizoma Recens*, *Jujubae Fructus*, and *Pinelliae Rhizoma* [2]. According to the principle of JUN-CHEN-ZUO-SHI (emperor-minister-assistant-courier in English), in this formula, *Bupleuri Radix* is JUN, *Scutellariae Radix* is CHEN, *Glycyrrhizae Radix* is SHI, and the others are ZUO. Modern research has verified that XCH has anti-inflammatory [3] and antitumor [4] functions and regulates the endocrine system [5]. Clinically, the formula is applied to treat various diseases of the respiratory system [6], digestive system [7], urogenital system [8], immune system [9], circulatory system [10], etc. The mechanism of XCH acting on the human body can be preliminarily explored by means of liquid chromatography-mass spectrometry, network pharmacology, and animal experiments. For fever, the widest application of XCH, potential antipyretic mechanism includes the reduction of inflammation level, inhibition of endogenous pyrogen and COX-2 [11]. Some active ingredients of XCH including quercetin, baicalein, and hanbaicalein can significantly inhibit the growth of hepatocellular carcinoma and induce apoptosis of hepatocellular carcinoma cells [12]. In recent years, many novel applications have been reported, including the prevention and treatment of methicillin-resistant *Staphylococcus aureus* [13], syncytial virus, and adenovirus [14], as well as the inhibition of influenza A virus [15], etc. For the period from 2000 to 2020, an overall trend of a steady rise in the numbers of publications in the field of XCH could be found. In the

database of www.cnki.net, the number has grown annually and ranged from about 150 to nearly 400 works [16].

2. Formula Differences of Existing XCH Preparations

Capsules, granules, pills, tablets, and other XCH preparations are all on the Chinese domestic market. Among those, XCH tablets, XCH effervescent tablets, XCH capsules, and XCH granules were included in the 2020 edition of the Chinese Pharmacopoeia [17]. The Pharmaceuticals and Medical Devices Agency in Japan [18] has published more than 10 kinds of XCH preparations, which are mainly granules or tablets. The Japanese Pharmacopoeia includes two different specifications of the Shosaikoto extract [19]. In Korea, Soshiho-Tang is widely used as a classic recommendation, which is mainly sold in granules [20].

The raw materials of the Japanese XCH preparations are Pinelliae Rhizoma, Ginseng Radix, Bupleuri Radix, Scutellariae Radix, Glycyrrhizae Radix, Zingiberis Rhizoma Recens, and Jujubae Fructus. However, the main XCH preparations on the Chinese market use Pinelliae Rhizoma Praeparatum Cum Zingibere Et Alumine (Jiangbanxia), Codonopsis Radix, Bupleuri Radix, Scutellariae Radix, Glycyrrhizae Radix, Zingiberis Rhizoma Recens, and Jujubae Fructus as raw materials. Table 1 shows four XCH preparations that are listed in the Chinese Pharmacopoeia. The JUN material Bupleuri Radix is the highest in mass ratio among the four dosage forms included in Chinese Pharmacopoeia, accounting for approximately 30%. The raw material mass ratio of XCH tablets and XCH capsules is exactly the same, and the mass ratio of Jiangbanxia is higher than those of XCH effervescent tablets and XCH granules. Regarding the materials of Japanese XCH preparations, the mass ratio of Pinelliae Rhizoma is lower than that of Bupleuri Radix but higher than that of any other herb. The mass ratio values of Glycyrrhizae Radix and Zingiberis Rhizoma Recens are both lower than 10%.

Pinelliae Rhizoma can cause adverse reactions, such as mucosal irritation [21], hepatorenal toxicity [22], and pregnancy toxicity [23,24]. It has been reported that the needle crystals of calcium oxalate and its lectin protein contained in *Pinelliae Rhizoma* are the main irritant toxic substances [25,26]. In China, there is a long history to use *Zingiberis Rhizoma Recens* to alleviate the toxicity of *Pinellia ternata.* The processing standards for preparing Jiangbanxia have been established [27]. Therefore, the use of Jiangbanxia in XCH preparations in China is conducive to improving drug safety [28].

In Table 1, we compared the amount and mass ratio of raw materials in different XCH preparations which were included in the Chinese and Japanese Pharmacopoeia [17,19]. By having materials divided by the total weight, the mass ratios are calculated and listed.

Table 1. Formula amount of raw materials, their mass ratio and preparation amount in different XCH preparations in pharmacopoeias.

Raw Materials	**XCH Tablets & XCH Capsules**		**XCH Effervescent Tablets**		**XCH Granules**		**Shosaikoto Extract (Japanese)**			
—	**Amount (g)**	**Mass Ratio (%)**	**Amount (g)**	**Mass Ratio (%)**	**Amount (g)**	**Mass Ratio (%)**	**Amount (g)**	**Mass Ratio (%)**	**Amount (g)**	**Mass Ratio (%)**
Bupleuri Radix	445	29.6	1550	31.0	150	31.0	7	29.2	6	26.1
Jiangbanxia	222	14.8	575	11.5	56	11.5	-	-	-	-
Pinelliae Rhizoma	-	-	-	-	-	-	5	20.8	5	21.7
Scutellariae Radix	167	11.1	575	11.5	56	11.5	3	12.5	3	13.0
Codonopsis Radix	167	11.1	575	11.5	56	11.5	-	-	-	-
Ginseng Radix	-	-	-	-	-	-	3	12.5	3	13.0
Glycyrrhizae Radix	167	11.1	575	11.5	56	11.5	2	8.33	2	8.70
Zingiberis Rhizoma Recens	167	11.1	575	11.5	56	11.5	1	4.17	1	4.35
Jujubae Fructus	167	11.1	575	11.5	56	11.5	3	12.5	3	13.0
Preparation amount	XCH tablets: 1000 tablets, 0.4 g each; XCH capsules: 1000 capsules, 0.4 g each;		XCH effervescent tablets: 1000 tablets, 2.5 g each		XCH granules: 1000 g (combined with sucrose); 400 g (combined with mannitol); 250 g (combined with lactose)		Not specified			

3. Differences in XCH Preparation Methods

There are different manufacturing processes for preparing XCH [29–32] preparations. Manufacturing processes included in the Chinese Pharmacopoeia [17] are shown in Figures 1–4. For Codonopsis Radix, Glycyrrhizae Radix, Bupleuri Radix, Scutellariae Radix, and Jujubae Fructus, the plants are extracted with water decoction. Jiangbanxia and Zingiberis Rhizoma Recens are extracted with ethanol solution with percolation. Compared with water decoction process, the percolation process is time consuming and solvent consuming. However, the volatilization or degradation of active components can be effectively decreased with the percolation process because it is operated at a low temperature. It has been reported that gingerols are easily degraded at a high temperature [33]. Therefore, it is reasonable to extract active components from Jiangbanxia and Zingiberis Rhizoma Recens with a percolation process [34]. Gingerol and other components in Zingiberis Rhizoma Recens have low solubility in water [35]. Therefore, ethanol solution is generally used as the percolation solvent [36].

In the production process for XCH capsules and XCH tablets, part of Codonopsis Radix and Glycyrrhizae Radix are directly crushed and added, which is significantly different from the process of XCH effervescent tablets and XCH granules. Codonopsis Radix and Glycyrrhizae Radix powder can play a role similar as excipients [37]. The excipients in XCH preparations vary depending on the formulation forms.

Apart from the manufacturing processes included in the Chinese Pharmacopoeia mentioned above, there are several other manufacturing processes for different XCH preparations, such as XCH sustained release tablets [38], nano XCH preparations [39], and others [40,41].

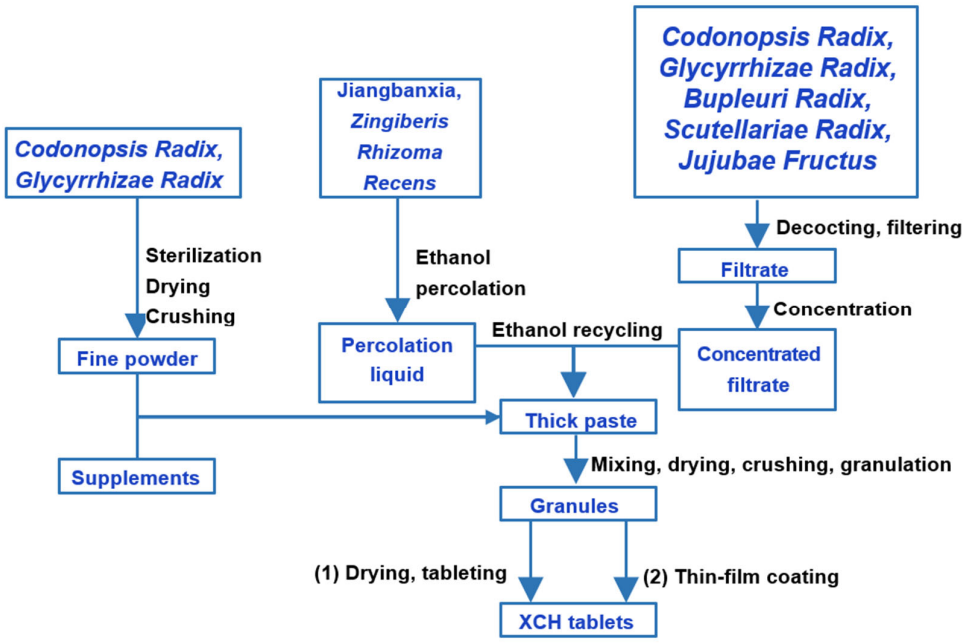

Figure 1. XCH tablets Production Process.

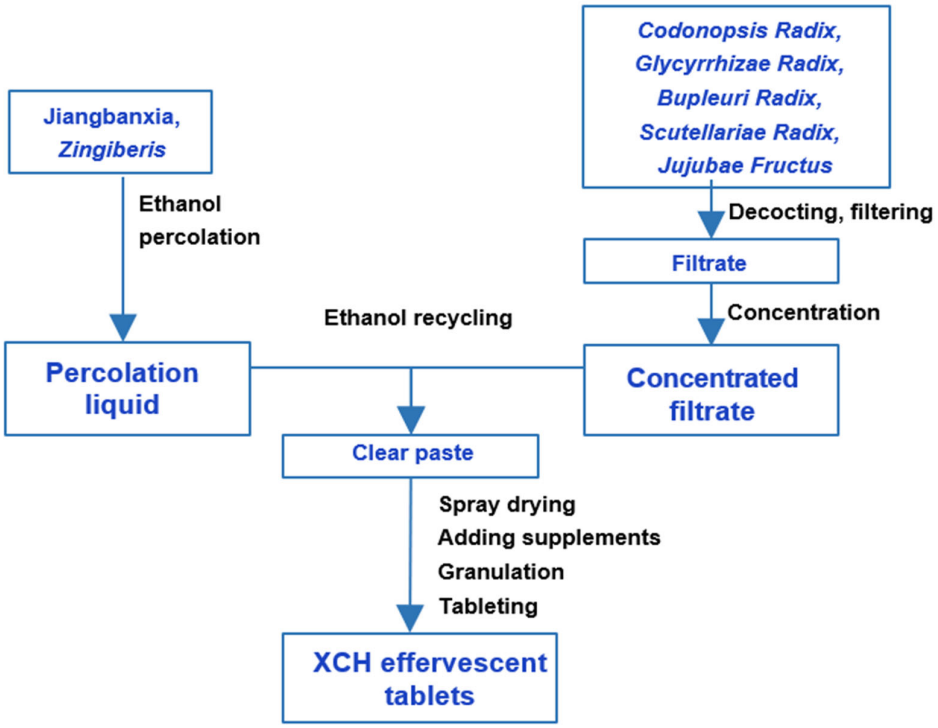

Figure 2. XCH effervescent tablets Production Process.

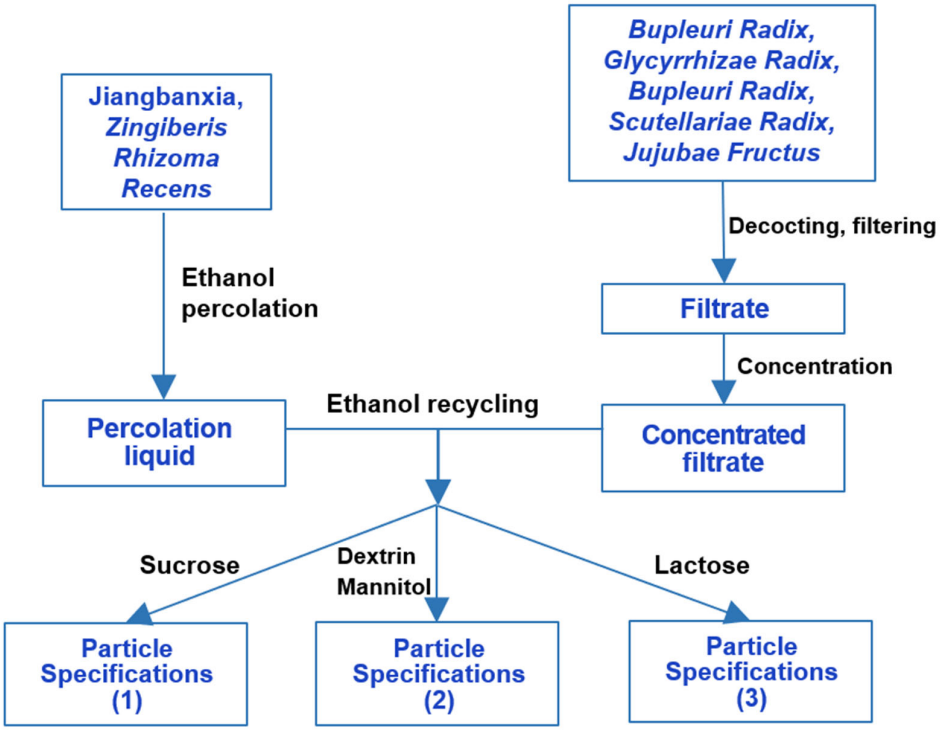

Figure 3. XCH granules Production Process.

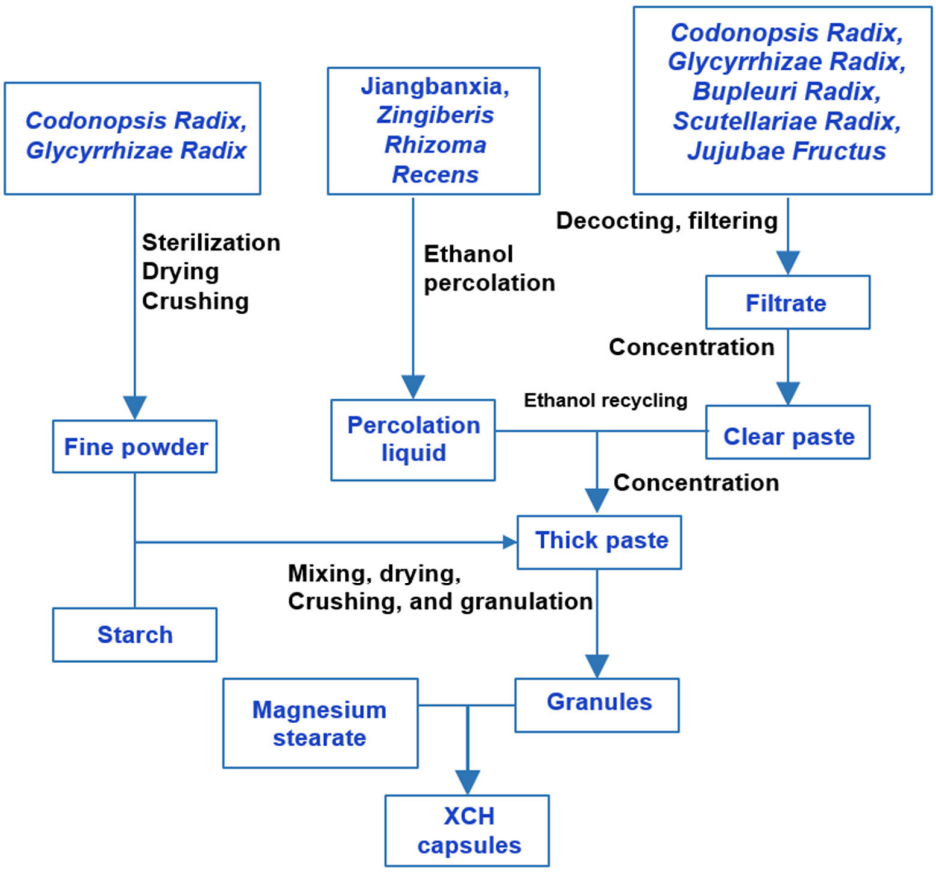

Figure 4. XCH capsules Production Process.

4. Differences in Quality Test Indices and Limits of Different XCH Preparations

4.1. Indicators for Qualitative Identification

There are two kinds of qualitative methods for the preparations of XCH in production: microscopic identification and thin-layer chromatography (TLC) identification. The identification methods and reference materials from the Chinese Pharmacopoeia and the Japanese Pharmacopoeia are summarized in Table 2.

The method involving the microscopic identification of medicinal materials is suitable for fragmentary medicinal materials or powdered medicinal materials. Raw powdered medicinal materials of *Glycyrrhizae Radix* and *Codonopsis Radix* are used in the manufacturing of XCH tablets and XCH capsules, which is suitable to be analyzed with microscopic identification. Both Chinese and Japanese XCH preparations adopt thin-layer identification, but there are obvious differences. First, in Chinese Pharmacopoeia, thin-layer identification uses reference medicinal materials, including *Glycyrrhizae Radix*, *Bupleuri Radix*, and *Codonopsis Radix*. However, thin-layer identification uses only reference substances in Japanese Pharmacopoeia. Second, Japanese thin-layer identification uses more reference substances, including the index components from *Bupleuri Radix*, *Zingiberis Rhizoma Recens*, *Scutellariae Radix*, *Glycyrrhizae Radix* and *Ginseng Radix*. In contrast, the use of reference medicinal materials in thin-layer identification can provide more information than using reference substances, which is conducive to assessing the authenticity of the medicinal materials used. Both Chinese and Japanese XCH preparations quantitatively analyze baicalin contents. Therefore, it seems unnecessary to use baicalin as the reference substance in thin-layer identification.

Silica gel G thin layer plate is mostly widely used in TLC identification. A mixed solvent of ethyl acetate-butanone-formic acid-water is usually used for baicalin identification. A mixed solvent of chloroform-methanol-water is usually used for the identification of Glycyrrhizae Radix.

Table 2. Qualitative identification method and comparison of XCH preparations in the Chinese and Japanese Pharmacopoeias.

Preparations	Identification Method	TLC Reference Substance	TLC Control Crude Drug
XCH tablets	Microscopic Identification, Thin-Layer Chromatography Identification	Baicalin	*Glycyrrhizae Radix*
XCH effervescent tablets	Thin-Layer Chromatography Identification	Baicalin	*Glycyrrhizae Radix*, *Bupleuri Radix*, *Codonopsis Radix*
XCH capsules	Microscopic Identification, Thin-Layer Chromatography Identification	-	*Glycyrrhizae Radix*, *Bupleuri Radix*, *Codonopsis Radix*
XCH granules	Thin-Layer Chromatography Identification	Baicalin	*Glycyrrhizae Radix*, *Bupleuri Radix*
Shosaikoto Extract (Japanese)	Thin-Layer Chromatography Identification	Saikosaponin B2, 6-Gingerol, Wogonin, Ginsenoside Rb1, Liquiritin	-

4.2. Quantitative Determination

Table 3 lists the quantitative detection methods for XCH preparations in Chinese Pharmacopoeia and Japan Pharmacopoeia. According to the Chinese Pharmacopoeia, the XCH tablets weight 0.4 g per tablet, the XCH capsules weight 0.4 g per capsule, and the XCH effervescent tablets weight 2.5 g per tablet. The XCH granules have three specifications (10 g/4 g/2.5 g per bag) due to the various preparation methods. The only quantitative determined index component for XCH preparations mentioned in the 2020 edition of the Chinese Pharmacopoeia is baicalin, while the determination of baicalin, saikosaponin B2, and glycyrrhizic acid are required in Japan Pharmacopoeia. Considering that there are seven medicinal materials in the formula for XCH preparations, more index components should be determined to control drug quality. The Chinese Pharmacopoeia specifies a lower limit for baicalin, while the Japanese Pharmacopoeia specifies both the upper and lower limits for the contents of saikosaponin B2, baicalin, and glycyrrhizic acid. Herbal materials of XCH preparations were often decocted before quantitative analysis. Methanol is a common solvent for sample preparation.

Table 3. Comparison of quantitative detection methods for XCH preparations in the Chinese and Japanese Pharmacopoeias.

Preparations	Detection Component	Prescribed Limit
XCH tablets	Baicalin	Not lower than 2.0 mg per tablet/capsule
XCH capsules		
XCH effervescent tablets		Not lower than 20.0 mg per tablet/package
XCH granules		
Shosaikoto Extract (Japanese)	Saikosaponin B2, baicalin and glycyrrhizic acid	Saikosaponin B2: 2–8 mg

Table 4 lists the published works on the quantitative detection of XCH preparations. In such work, the raw materials often went under decoction treatment before they were put into use. In addition, methanol is a common solvent in the procedure. HPLC technology is used to separate the components of XCH preparations. The detectors stated in the literature are mostly ultraviolet detectors, and a few are diode array detectors (DAD) and mass spectrometer detectors. Since the content of a saikosaponin is low, mass spectrometer detectors are used more often to analyze it. In some papers, the method of quantitative analysis of multiple components by a single marker (QAMS) was used, which can reduce

the cost of testing. There are more reports on the detection of index components of *Bupleuri Radix* and *Scutellariae Radix*, which reflects the emphasis on JUN and CHEN drugs. Some literature has detected gingerol, liquiritin, glycyrrhizic acid, lobetyolin, and other substances, which can help control the contents of chemical components in *Zingiberis Rhizoma Recens*, *Glycyrrhizae Radix*, and *Codonopsis Radix*. However, there are still few detections of index components in *Jujubae Fructus* and Jiangbanxia.

Table 4. Quantitative detection methods for the XCH formula.

	Year	Apparatus	Quantified Components	Other Instructions	Reference
1	2020	HPLC	Baicalin, Wogonoside, Baicalein, Wogonin, Ammonium Glycyrrhizinate, Saikosaponin B2, Saikosaponin B1	QAMS	[42]
2	2018	HPLC	6-Gingerol	-	[43]
3	2018	HPLC	Ginsenoside Rg1, Ginsenoside Re, Ginsenoside Rb1, Saikosaponin A, Saikosaponin D	QAMS	[44]
4	2018	HPLC	Baicalin, Baicalein, Wogonin	-	[45]
5	2017	HPLC	Saikosaponin A, Saikosaponin D, Saikosaponin B1, Baicalin, Ginsenoside Rb1, Ginsenoside Re, 6-Gingerol, Liquiritin, Ammonium Glycyrrhizinate	-	[29]
6	2017	HPLC	Lobetyolin, Liquiritin, Baicalin, Baicalein	Detection wavelength switched	[46]
7	2016	HPLC	Baicalin	-	[47]
8	2016	UPLC	Baicalin	-	[48]
9	2015	HPLC-MSMS	Saikosaponin A, Saikosaponin D	-	[49]
10	2015	HPLC	Liquiritin, Baicalin, Wogonoside, Baicalein, Wogonin	-	[50]
11	2015	HPLC	Baicalin	-	[51]
12	2015	HPLC	Saikosaponin, Baicalin, Ginsenoside Rg1, Liquiritin, Ephedrine, 6-Gingerol	-	[52]
13	2014	HPLC	Saikosaponin A, Baicalin	-	[53]
14	2014	HPLC	Liquiritin, Baicalin, Wogonoside, Baicalein, Ammonium Glycyrrhizinate, Saikosaponin A, Wogonin	-	[54]
15	2014	HPLC	Saikosaponin B2	-	[55]
16	2013	HPLC	Saikosaponin, Baicalin, Glycyrrhizic acid	-	[56]
17	2012	Capillary electrophoresis	Saikosaponin A, Saikosaponin D	-	[57]
18	2012	HPLC	Baicalin	-	[58]
19	2012	HPLC	Baicalin	-	[59]
20	2010	HPLC	Baicalin, Wogonoside	-	[60]
21	2010	HPLC	Baicalin, Baicalein, Wogonoside, Wogonin, Glycyrrhizic acid	-	[61]
22	2010	HPLC	Saikosaponin A	-	[62]
23	2007	HPLC-DAD-MS	Saikosaponin A, Baicalin, Glycyrrhizic acid	-	[63]
24	2007	HPLC	Baicalin	-	[64]
25	2006	HPLC/DAD	Baicalin, Glycyrrhizic acid	-	[65]
26	2006	HPLC-MSMS	Cytidine, Tyrosine, Uridine, Adenine, Guanosine, Phenylalanine, Adenosine, Tryptophan	-	[66]
27	2004	HPLC-MSMS	Saikosaponin A, B1, B2, C, D, G, H, I	-	[67]

"-" means there is no special instruction that is necessary to be presented.

4.3. Fingerprint and Specific Chromatogram

Fingerprint and specific chromatogram detection methods can reflect the overall characteristics of Chinese medicines and are widely used in drug quality analysis. At present, the application of these two methods represents a significant research progress. Both qualitative identification and quantitative detection can be carried out on the basis of fingerprint and specific chromatograms. A summary of the fingerprint and specific chromatogram detection methods for XCH preparations is shown in Table 5. Compared with the quantitative methods listed in Table 4, fingerprint and specific chromatogram detection can identify more components in chromatographic peaks, therefore providing more information. At present, the most identified components are the saponins in *Bupleuri Radix*, flavonoids of *Scutellariae Radix*, gingerol in *Zingiberis Rhizoma Recens*, liquiritin, and glycyrrhizic acid in *Glycyrrhizae Radix*, etc. However, the characteristic components of *Jujubae Fructus* and Jiangbanxia have not been identified.

In some of the research works, a quantitative fingerprint or specific chromatogram of the XCH preparation was obtained. The quantitatively determined components are mainly from *Scutellariae Radix*, *Glycyrrhizae Radix*, *Bupleuri Radix*, and *Codonopsis Radix*. Wang et al. [68] compared the HPLC spectra of XCH granules at different wavelengths and concluded that spectral analysis at a single ultraviolet absorption wavelength is not suitable for quality detection. Liu et al. used charged aerosol detector (CAD) to analyze saikosaponins [69]. Compared with using evaporative light scattering detector (ELSD), lower detection limit and wider detection range can be realized with CAD.

In some studies, the active substances in *Bupleuri Radix* such as saikosaponin A and saikosaponin D were not detected in XCH granules, which may be due to the hydrolysis of saikosaponin during decoction [70]. There are also reports that the existing detection methods often add an acid to the mobile phase, and saikosaponin A and saikosaponin D are prone to degrade under acidic conditions, which makes them difficult to detect [71].

Table 5. Fingerprint/specific chromatogram detection methods for XCH preparation.

	Year	Apparatus	Quantitative Determined Components	Qualitatively Identified Components	Detection Method	Reference
1	2021	HPLC-CAD	Saikosaponin A, B1, B2, C, G, H, I	Saikosaponin A, B1, B2, C, G, H, I	Fingerprint chromatogram	[69]
2	2021	UHPLC	Liquiritin, Baicalin, Wogonin, Baicalein, Glycyrrhizin G2, Glycyrrhizic acid, Saikosaponin B2, Saikosaponin B1	-	Fingerprint chromatogram	[71]
3	2018	UPLC	Liquiritin, Baicalin, Berberine, Wogonoside, Baicalein, Ammonium Glycyrrhizinate	Liquiritin, Baicalin, Berberine, Wogonoside, Baicalein, Ammonium Glycyrrhizinate	Specific chromatogram	[72]
4	2017	HPLC	-	-	Fingerprint chromatogram	[73]
5	2017	HPLC	-	-	Fingerprint chromatogram	[74]
6	2016	HPLC	Lobetyolin, Saikosaponin A	Lobetyolin, Saikosaponin A	Specific chromatogram	[75]
7	2016	HPLC	Baicalin, Ammonium Glycyrrhizinate	Baicalin, Ammonium Glycyrrhizinate	Specific chromatogram	[68]
8	2015	HPLC-ELSD	-	Liquiritin, Ginsenoside Re, Baicalin, Wogonoside, Baicalein, Ginsenoside Rb1	Fingerprint chromatogram	[76]

Table 5. *Cont.*

	Year	Apparatus	Quantitative Determined Components	Qualitatively Identified Components	Detection Method	Reference
9	2014	HPLC	Liquiritin, Baicalin, Wogonoside, Baicalein, Ammonium Glycyrrhizinate, Saikosaponin A, Wogonin	Liquiritin, Baicalin, Wogonoside, Baicalein, Ammonium Glycyrrhizinate, Saikosaponin A, Wogonin	Specific chromatogram	[54]
10	2013	HPLC	-	Liquiritin, Baicalin, Wogonoside, Baicalein, Ammonium Glycyrrhizinate, Saikosaponin A, Wogonin	Fingerprint chromatogram	[77]
11	2013	HPLC	Baicalin, Glycyrrhizic acid	Baicalin, Glycyrrhizic acid	Fingerprint chromatogram	[78]
12	2013	HPLC	-	Liquiritin, Baicalin, Ononin, Wogonoside, Saikosaponin A, Skullcapflavone II, etc.	Fingerprint chromatogram	[79]
13	2012	HPLC-DAD-ESI-MS	Homogentisic acid, Baicalin, Glycyrrhizic acid, Saikosaponin A, 6-Gingerol, Ginsenoside Rg3	-	Fingerprint chromatogram	[80]
14	2012	HPLC	Baicalin, Wogonoside, Baicalein, Wogonin, Glycyrrhetic acid	Baicalin, Wogonoside, Baicalein, Wogonin, Glycyrrhetic acid	Specific chromatogram	[81]
15	2012	UPLC	-	Glycyrrhizin, Ginsenoside Rg1, Baicalin, Isowogonin, Baicalein, Saikosaponin A	Fingerprint chromatogram	[82]
16	2012	HPLC	Baicalin, Wogonoside, Baicalein, Wogonin	Baicalin, Wogonoside, Baicalein, Wogonin	Specific chromatogram	[83]
17	2011	HPLC-DAD	-	-	Fingerprint chromatogram	[84]
18	2009	HPLC-TOF/MS	-	Liquiritin, Baicalin, Wogonoside, Ginsenoside Rg1, Glycyrrhizic acid	Fingerprint chromatogram	[85]
19	2009	HPLC	-	-	Fingerprint chromatogram	[86]

"-" means there is no quantitative determined or qualitatively identified component.

In conclusion, a fingerprint/specific chromatogram can be used to characterize multiple chemical component information of XCH preparations. However, fingerprint/specific chromatogram is not included in Chinese Pharmacopoeia. Further development of quality control technology with use of the fingerprint/specific chromatogram is required for XCH preparations.

5. Prospect on the Development Direction of Quality Control of XCH Preparations

5.1. Improvement in the Specificity of Quality Testing

According to Chinese Pharmacopoeia, *Glycyrrhizae Radix, Bupleuri Radix* and *Codonopsis Radix* are used as TLC reference materials, and baicalin is used as a TLC reference substance in qualitative identification. However, less attention has been given to *Zingiberis Rhizoma Recens*, *Jujubae Fructus*, and Jiangbanxia. The specific components of Jiangbanxia and *Jujubae Fructus* are not quantitatively analyzed in literature. Recently, guanosine, uridine, hypoxanthine and several other components were analyzed [87], which do not especially belong to Jiangbanxia, but it still suggests a way to improve the specificity of HPLC detection by detecting these compositions with strong polarity.

It is essential to distinguish the authenticity of *Bupleuri Radix*. There are 36 species, 17 varieties, and seven forms distributed all over China [88]. Among them, *Bupleurum marginatum var. stenophyllum* and even poisonous *Bupleurum longiradiatum* are common varieties that are all easy to mix up [89]. To confirm whether or not *Bupleurum marginatum var. stenophyllum* had been added, Liu et al. using the retention time and peak area of the specific ion detected in the mass spectrum as standards [90]. Liang et al. tried to establish near-infrared spectrum models to distinguish products of different factories, which provided a practical technology for low-cost and rapid detection [91]. Lai et al. used a polymerase chain reaction (PCR) method based on the site specificity of the Internal Transcribed Spacer (ITS) sequence to identify *Bupleurum marginatum var. stenophyllum* from *Bupleurum chinense* DC [92]. These new technologies provide ideas for improving the specificity of analytical methods. *Bupleurum scorzonerifolium Willd* and *Bupleurum chinense* DC are both included in the Chinese Pharmacopoeia, but National Institutes for Food and Drug Control can provide only the reference material of *Bupleurum chinense* DC. Hence, the lack of reference material of *Bupleurum scorzonerifolium Willd* is a problem for quality control of *Bupleuri Radix*.

5.2. Setting Reasonable Content Range of Index Components from Bupleuri Radix

Bupleuri Radix is the JUN of XCH formula. Thus far, qualitative identification using the reference material of *Bupleuri Radix* was adopted in Chinese Pharmacopoeia. However, considering drug safety and efficacy, the contents of saikosaponins should be controlled in specific ranges. Studies have indicated that saikosaponins are important active ingredients of *Bupleuri Radix*, which has antipyretic, anti-inflammatory and antitumor activities. Therefore, it is necessary to set up lower limits for their contents [93,94]. Moreover, some reference materials have reported that *Bupleuri Radix* has a certain degree of toxicity when taken in a large dose for a long period of time, and its toxic side effects are often caused by its saponins and volatile substances, which mainly affect the liver [95]. Therefore, from the perspective of drug safety, it is necessary to set up upper limits for saikosaponins. At present, the upper and lower limits of the saikosaponin B2 content are set up in the Japanese Pharmacopoeia, which is worth referencing. When setting up the lower limit, companies can consider collecting big data from clinical practice. Accordingly, the needs of drug quality control indicators can be taken into consideration, such as drug interactions and medications for special populations.

5.3. Strengthening the Standard of Limited Detected Items

In recent years, great progress in the control of heavy metals, pesticides, and biological toxins in Chinese medicines and extracts was achieved. The Chinese Pharmacopoeia has specially listed items General Principle for Inspection of Crude Drugs and Decoction Pieces and Guidelines for Establishment of Limit for Harmful Residue of Traditional Chinese Medicine, which have provided guidance for controlling heavy metals, pesticides and biological toxins for medicinal materials. The Chinese Pharmacopoeia stipulates that *Jujubae Fructus* needs to be tested for aflatoxin, *Glycyrrhizae Radix* needs to be tested for heavy metals, harmful elements and pesticide residues, and *Codonopsis Radix* needs to be tested for sulfur dioxide residues, all of which help to guarantee the safety of XCH preparations. However, the current guidelines for XCH preparations still require more relevant limiting items for heavy metals, pesticides and biological toxins, and other toxic ingredients. The Japanese Pharmacopoeia stipulates the limits of heavy metals and arsenic in XCH preparations. It takes the increase in heavy metals during the production process into account, which is more rigorous and improves the level of quality control.

Therefore, from the perspective of drug safety, XCH preparations require an upper limit for the amounts of certain active ingredients, heavy metals, pesticides, biotoxins, and other toxic components. Similar quality control problems exist for many other Chinese medicines. Therefore, the development direction of quality control presented in this work can also be referenced for that of other Chinese medicines.

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References

1. Liu, T.T.; Yao, K.W.; Duan, J.L.; Yu, Z.; Zhang, L.D. Efficacy of Xiaochaihu Decoction on Treating Coronary Heart Disease. *Lishizhen Med. Mater. Med. Res.* **2019**, *30*, 2216–2218.
2. Zhang, W.Z.; Nie, H.; Wang, Z.T.; Mao, D.X. Discussion of ginseng used in Xiaochaihu Decoction. *Lishizhen Med. Mater. Med. Res.* **2013**, *24*, 2480–2481.
3. Zhang, Y.; Zhou, X.L.; Shao, Q.; Fang, Z.E.; Wang, S.S. Anti-inflammatory effect of Xiaochaihutang in rats with collagen-induced arthritis and the mechanism. *Immunol. J.* **2015**, *31*, 781–785.
4. Zhai, Y.X.; Zhao, Y.; Xiang, R.W.; Zhai, F.; Zeng, Y.; Liang, J.K. Study on anti-heatoma mechanism of Xiaochaihu decoction based on weighted network pharmacology. *Chin. J. Med. Chem.* **2020**, *30*, 658–668.
5. Lu, X.W.; Zhu, L.H.; Huang, H.; He, K. Clinical Observations on Modified Xiao Chaihutang in Treatment of Subacute Thyroiditis. *Chin. J. Exp. Tradit. Med. Formulae* **2018**, *22*, 153–158.
6. Zhang, W.Z.; Tian, H.Z.; Zhang, L.J.; Yang, S.J. Clinical Study and Analysis of Modified Xiaochaihu Decoction in Treating Cough Caused by Variant Asthma. *World J. Integr. Tradit. Western Med.* **2020**, *15*, 790–793.
7. Liu, L.; Wang, C.X.; Cui, S.Y.; Zhang, H.X.; Wu, X.X.; Zhang, W. Observation of curative effect of lamivudine combined with compound glycyrrhizin and Xiaochaihu Decoction in the treatment of chronic hepatitis B. *Modern J. Integr. Tradit. Chin. Western Med.* **2020**, *29*, 4042–4045.
8. Luo, X.M. Clinical observation on treating chronic renal failure with the Xiaochaihu decoction. *Clin. J. Chin. Med.* **2012**, *4*, 99.
9. Wang, D.X.; Liu, X.Z.; Lv, C.Y.; Fan, H.; Sun, Y.F.; Yang, S.L. Changes of Platelet- Associated Parameters in Patients with Refractory Immune Thrombocytopenia Treated with Xiaochaihu Decoction and Erzhi Pill. *J. Hebei North Univ. Nat. Sci. Ed.* **2020**, *36*, 5–9.
10. Jiang, M. Effect observation of Minor Decoction of Bupleurum treating acute viral myocarditis. *World Latest Med. Inf.* **2015**, *15*, 1–14.
11. Ma, R.; Liu, H.; Zheng, R.W.; Xu, Z.T.; Jiang, Z.Q.; Zhang, J.H.; Jiang, Y.F.Y.; Bi, C. Pharmacological analysis and confirmation of antipyretic mechanism of Xiaochaihu Granules. *J. Pharm. Res.* **2021**, *8*, 497–503.
12. Li, X.; Li, Y.Y.; Wen, C.X.; Liang, Y.Y.; Zhou, Z.L. Study on the mechanism of Xiaochaihu Decoction in treatment of hepatocellular carcinoma based on network pharmacology and bioinformatics. *Anti-Tumor Pharm.* **2021**, *4*, 456–462.
13. Ma, R.; Liu, H.; Jiang, Y.F.Y.; Zheng, R.; Jiang, Z.Q.; Bi, C.; Zhang, J.H.; He, Y.X.; Du, H.Y. A New Use of Xiaochaihu Granules Combined with Antibiotics. Guangdong Province Patent CN111529671A, 14 August 2020.
14. Liu, H.; Huang, W.; Cheng, X.Y.; Bi, C.; Zheng, R.W.; Jiang, Z.Q.; Zhang, J.H.; He, Y.X.; Du, H.Y. New Application of Xiaochaihu Granules in the Control of Syncytial Virus and Adenovirus. Patent CN111467466A, 31 July 2020.
15. Zheng, R.W.; Liu, H.; Situ, W.H.; Jiang, Z.Q.; Bi, C.; Zhang, J.; He, Y.X.; Du, H.Y. New Application of Xiaochaihu Granules in Inhibiting Influenza a Virus. Patent CN111358929A, 3 July 2020.
16. China National Knowledge Infrastructure. Available online: https://kns.cnki.net/kns8/defaultresult/index (accessed on 9 October 2021).
17. Chinese Pharmacopoeia Commission. *The Chinese Pharmacopoeia*; China Medical Science Press: Beijing, China, 2020; pp. 602–606.
18. Pharmaceuticals and Medical Devices Agency. Available online: https://www.pmda.go.jp/PmdaSearch/iyakuSearch/ (accessed on 22 September 2021).
19. Japanese Pharmacopoeia Committee. *The Japanese Pharmacopoeia*, 17th ed.; The Ministry of Health, Labour and Welfare: Tokyo, Japan, 2016; pp. 1988–1990.
20. Kim, H.M.; Kim, Y.Y.; Jang, H.Y.; Moon, S.J.; An, N.H. Action of Sosiho-Tang on systemic and local anaphylaxis by anal administration. *Immunopharm. Immunot.* **1999**, *21*, 635–643. [CrossRef]
21. Zhong, L.Y.; Wu, H. Current researching situation of mucosal irritant components in Acacae family plants. *China J. Tradit. Chin. Med. Pharm.* **2006**, *18*, 1561–1563.
22. Zhang, L.M.; Bao, Z.Y.; Huang, Y.Y.; Huang, W.; Zhang, Y.N.; Sun, R. Experimental Study on the "Dosage-Time-Toxicity" Relationship of Acute Hepatotoxicity induced by Water Extraction from *Rhizoma Pinelliae* in Mice. *Chin. J. Pharmacovigil.* **2011**, *8*, 11–15.
23. Jin, X.Q.; Huang, C.Q.; Zhang, G. Toxic Components and Processing Mechanism of *Rhizoma Pinelliae*. *Lishizhen Med. Mater. Med. Res.* **2019**, *30*, 1717–1720.

24. Zhang, K.; Shan, J.J.; Xu, J.Y.; Wang, M.M. Research progress and prospects of pregnancy toxicity of pinellia. *China J. Tradit. Chin. Med. Pharm.* **2016**, *31*, 938–941.
25. Zhu, F.G.; Yu, H.L.; Wu, H.; Shi, R.J.; Tao, W.T.; Qiu, Y.Y. Correlation of *Pinellia ternata agglutinin* and *Pinellia ternata raphides*' toxicity. *China J. Chin. Mater. Med.* **2012**, *37*, 1007–1011.
26. Zhong, L.Y.; Wu, H.; Zhang, K.W.; Wang, Q.R. Study on irritation of calcium oxalate crystal in raw Pinellia ternate. *China J. Chin. Mater. Med.* **2006**, *20*, 1706–1710.
27. Su, T.; Zhang, W.W.; Zhang, Y.M.; Cheng, C.Y.B.; Fu, X.Q.; Li, T.; Guo, H.; Li, Y.X.; Zhu, P.L.; Cao, H.; et al. Standardization of the manufacturing procedure for *Pinelliae Rhizoma Praeparatum cum Zingibere et Alumine*. *J. Ethnopharmacol.* **2016**, *193*, 663–669. [CrossRef]
28. Shi, R.J.; Wu, H.; Yu, H.L.; Chen, L. The advance of the research that zingiber officinale rosc detoxify the tuber of pinellia. *Chin. J. Inf. Tradit. Chin. Med.* **2010**, *17*, 108–110.
29. Jiang, X.; Yan, F.J.; Jiang, L.; Si, G.M. Different Decocting Methods Influence 9 Kinds of Ingredients of Xiao Chaihutang by HPLC. *Chin. J. Exp. Tradit. Med. Formulae* **2017**, *23*, 98–103.
30. Megumi, S.; Yuko, S.; Fumio, I.; Yoshiro, H.; Takao, N. Extraction Efficiency of Shosaikoto (Xiaochaihu Tang) and Investigation of the Major Constituents in the Residual Crude Drugs. *Evid.-Based Compl. Altern. Med.* **2012**, *2012*, 7.
31. Sakata, K.; Kim, S.J.; Yamada, H. Effects of commercially available mineral waters on decoction of Kampo Medicines. *Kampo Med.* **2000**, *51*, 225–232. [CrossRef]
32. Honma, S.; Ogawa, A.; Kobayashi, D.; Ueda, H.; Fang, L.; Numajiri, S.; Kimura, S.; Teresawa, S.; Morimoto, Y. Effect of hardness on decoction of Chinese medicine. *J. Tradit. Med.* **2003**, *20*, 208–215.
33. Jiang, S.Z.; Fu, S.Q.; Wang, N.S. Overview of chemical constituents of gingerol. *Tradit. Chin. Drug Res. Clin. Pharmacol.* **2006**, *05*, 386–389.
34. Yu, N.; Zeng, H.Y.; Deng, X.; Zeng, M.X.; Feng, B. Study on Extraction, Identification and Antioxidation of Gingerol. *Food Sci.* **2007**, *8*, 201–204.
35. Zhao, W.Z.; Zhang, R.X.; Yu, Z.P.; Wang, X.K.; Li, J.R.; Liu, J.B. Research process in ginger chemical composition and biological activity. *Sci. Technol. Food Ind.* **2016**, *37*, 383–389.
36. Liu, D.; Zhang, C.H.; An, R.H.; Feng, X.Q.; Zhou, F.; Deng, Y.J.; Zhang, J.L.; Liu, H. Advances on extraction and application of ginger bioactive ingredient. *Sci. Technol. Food Ind.* **2016**, *37*, 391–400.
37. Zhang, D.K.; Fu, C.M.; Lin, J.Z.; Ke, X.M.; Zou, W.Q.; Xu, R.C.; Han, L.; Yang, M. Study on theory and application value of "unification of medicines andexcipients" in Chinese materia medica preparations. *Chin. Tradit. Herb. Drugs* **2017**, *48*, 1921–1929.
38. Du, Y.; Xue, J.L.; Zhang, L.P.; Liang, G.X.; Ding, H. A Xiaochaihu Sustained Release Tablet along with Its Preparation Methods. Patent CN105106914B, 12 March 2019.
39. Yang, M.J. A Nanoscale Xiaochaihu Pharmaceutical Preparation along with Its Preparation Method. Patent CN1362246, 7 August 2002.
40. Li, C.H. A Fast Classification and Screening Device for Xiaochaihu Granules. Patent CN208960363U, 11 June 2019.
41. Xu, D.J.; Chi, H.C.; Feng, Z.W.; Li, W.Z. A Preparation Method of Xiaochaihu Granules. Patent CN108853027A, 23 November 2018.
42. Liu, A.X.; Xu, T.T.; Yan, Y.; Wu, Q.Y.; Zhou, D.D.; Sha, Y.W. Study on determination of seven components in Xiaochaihu Granules by QAMS. *Drug Eval. Res.* **2020**, *43*, 2217–2221.
43. Qu, N.N. Determination of 6-Gingerol Content in Minor Bupleurum Tablets by HPLC Method. *Heilongjiang Med. J.* **2018**, *31*, 718–720.
44. Shao, J.X.; Lin, L.F.; Liu, Y.L.; Wang, C.M.; Li, H.; Yang, Y.J. Determination of five saponins in *Ginseng Radix et Rhizoma-Bupleuri Radix* herb pair and its preparations by QAMS method. *Chin. Tradit. Herb. Drugs* **2018**, *49*, 2873–2877.
45. Chen, L.L.; Lin, R.X.; Li, R.M. Effects of Different Decoction Methods of Xiaochaihu Decoction on Active Chemical Constituents of Scutellaria Baicalensis Georgi. *World Chin. Med.* **2018**, *13*, 743–745+750.
46. Li, C.D.; Wang, X.; Pan, X.B.; Zhang, W. Detection of Four Kinds of Ingredients in Modified Xiao ChaiHu Capsules by HPLC Wavelength Switching Method Simultaneously. *Western J. Tradit. Chin. Med.* **2017**, *30*, 26–28.
47. Tang, F.S.; Zhang, X.H.; Lan, X.; Meng, C.; Wang, Y.H. Study on the Dissolution of Xiaocaihu Pill from Different Manufacturers. *China Pharm.* **2016**, *27*, 4272–4274.
48. Li, J.; Yang, P.P.; Tang, W.X.; Li, H.J.; Xiang, W.; Jiang, H.; Yang, S.H.; Li, W.; Hou, Y. NIR Calibration Model to Detect Baicalin Content of Xiaochaihu Granules by Using Characteristic Spectrum Selection. *Chin. J. Exp. Tradit. Med. Formulae* **2016**, *22*, 72–77.
49. Wang, J.L.; Wang, S.T. Simultaneous determination the saikosaponin a and saikosaponin d in Sho-Saiko Granule with HPLC-MSMS. *Anhui Med. Pharm. J.* **2015**, *19*, 453–456.
50. Yuan, H.X.; Wang, T.T.; Yan, Y. Simultaneous Determination of Five Components in Ethyl Acetate Extract of Xiao Chaihu Tang by HPLC. *Chin. J. Exp. Tradit. Med. Formulae* **2015**, *21*, 64–66.
51. Lan, X.; Tang, F.H.; Chen, Z.N.; Zhang, Y.; Wang, W.; Zhou, X.M. Determination of baicalin in Xiaochaihu decoction by HPLC. *J. Zunyi Med. Univ.* **2015**, *38*, 435–438.
52. Xu, C.C. Determination of active components in Xiaochaihu Decoction by HPLC. *Asia-Pac. Tradit. Med.* **2015**, *11*, 39–40.
53. Jiang, S.Z.; Huang, X.B.; Chen, C.L.; Wu, H.X.; Xiang, Y.Y.; Qiu, Z.J.; Qiu, J.T. Effect of Ginger on Dissolution of Saikosaponin a and Baicalin in Xiaochaihu Decoction Determined by HPLC. *Chin. Arch. Tradit. Chin. Med.* **2014**, *32*, 1652–1654.

54. Zhang, H.; Yan, L.; Lin, L.F.; Dong, X.X.; Shen, M.R.; Qu, C.H.; Ni, J. Determination of seven index components in Xiaochaihu Granules by HPLC. *Drugs Clin.* **2014**, *29*, 162–165.
55. Li, P.T.; Lin, L.R.; Lin, L.H.; Wang, S.L.; Liu, S.Q.; Gao, X.L. Determination of Saikosaponin b2 in Xiaochaihu Granules by HPLC. *Tradit. Chin. Drug Res. Clin. Pharm.* **2014**, *25*, 356–359.
56. Zhang, S.Y. Determination of Saikosaponin, Baicalin and Glycyrrhizic Acid Content in Xiaochaihu Granule by HPLC. *J. Shanxi Univ. Chin. Med.* **2013**, *14*, 20–22.
57. Yi, R.Q.; Song, F.Y. Determination of Saikosaponin a and Saikosaponin d in Xiaochaihu Granules by Capillary Electrophoresis. *Chin. J. Pharm.* **2012**, *43*, 47–50.
58. Zhang, Y.J.; Mai, Y.M.; Mo, Z.J. Investigation of Determination and Evaluation for in vitro Dissolution of Chinese Medicine Preparations by Co-Chromatography and UV. *Chin. J. Exp. Tradit. Med. Formulae* **2013**, *19*, 17–21.
59. Liu, L.N.; Qi, Z.H.; Guo, Z.W.; Zhang, Y.; Zhang, H.F.; Zhang, W.J. Study on quality standard of xiaochaihutang dripping pill. *Pract. Pharm. Clin. Remedies* **2012**, *15*, 742–744.
60. Ji, S.G.; Liu, X.F.; Zhu, Z.Y.; Liang, S.S.; Zhao, L.; Zhang, H.; Chai, Y.F. High performance liquid chromatography in determination of baicalin and wogonoside contents in Xiaochaihu decoction. *Acad. J. Sec. Mil. Med. Univ.* **2010**, *31*, 1010–1013. [CrossRef]
61. Liu, Q.C.; Zhao, J.N.; Yan, L.C.; Yi, J.H.; Song, J. Simultaneous determination in Xiaochaihu Tang by HPLC. *China J. Chin. Mater. Med.* **2010**, *35*, 708–710.
62. Zhang, J.Y.; Xie, H.; Pan, S.L. Determination of saikosaponin a in four common bupleurum preparations by HPLC. *Chin. Tradit. Pat. Med.* **2010**, *32*, 81–83.
63. Zhou, B.Y.; Zhu, Z.Y.; Wang, B.; Zhao, L.; Zhang, H.; Xu, Q.; Chai, Y.F. HPLC-DAD combined with TOF/MS technique in determination of saikosaponin a, baicalin and glycyrrhizic acid in Xiaochaihu Decoction. *Acad. J. Sec. Mil. Med. Univ.* **2007**, *5*, 527–530.
64. Shi, Y. Determination of baicalin in Xiaochaihu Decoction pill by HPLC. *Chin. Tradit. Pat. Med.* **2007**, *7*, 1115–1117.
65. Zhu, Z.Y.; Liu, Y.; Zhao, B.Y.; Zhang, H.; Li, X.; Chai, Y.F. Simultaneous determination of two effective components in Xiaochaihu decoction by HPLC/DAD. *J. Pharm. Pract.* **2006**, *6*, 331–334.
66. Chen, P.; Li, C.; Liang, S.P.; Song, G.Q.; Sun, Y.; Shi, Y.H.; Xu, S.L.; Zhang, J.W.; Sheng, S.Q.; Yang, Y.M. Characterization and quantification of eight water-soluble constituents in tubers of *Pinellia ternata* and in tea granules from the Chinese multiherb remedy Xiaochaihu-tang. *J. Chrom. Banalytical Technol. Biomed. Life Sci.* **2006**, *843*, 83–193. [CrossRef] [PubMed]
67. Bao, Y.W.; Li, C.; Shen, H.W.; Nan, F.J. Determination of Saikosaponin Derivatives in *Radix bupleuri* and in Pharmaceuticals of the Chinese Multiherb Remedy Xiaochaihu-tang Using Liquid Chromatographic Tandem Mass Spectrometry. *Anal. Chem.* **2004**, *76*, 4208–4216. [CrossRef]
68. Wang, D.Q.; Chang, F.M.; Wang, Y.H.; Zhao, P.; Wang, Y.G. Multi-wavelength HPLC analysis of Xiaochaihu Granules and medicinal materials-preparation peak pattern matching analysis. *J. Chin. Med. Mater.* **2016**, *39*, 810–812.
69. Liu, A.X.; Xu, T.T.; Yang, W.N.; Zhou, D.D.; Sha, Y.W. Quantitative Determination of 7 Saikosaponins in Xiaochaihu Granules Using High-Performance Liquid Chromatography with Charged Aerosol Detection. *J. Anal. Methods Chem.* **2021**, *2021*, 6616854. [CrossRef] [PubMed]
70. Guo, Z.; Peng, B.; Li, Z.Y.; Pan, R.L. Study on Transformation Rule of Saikosaponin d Under Different Extraction Conditions by UPLC-QTof-MS. *Nat. Prod. Res. Dev.* **2014**, *26*, 716–720.
71. Zhang, X.; Wu, H.W.; Lin, L.N.; Tang, S.H.; Liu, H.H.; Yang, H.J. The development and application of a quality evaluation method for Xiaochaihu Granules granules which is based on "benchmark sample". *China J. Chin. Mater. Med.* **2021**, *7*, 1–11. [CrossRef]
72. Yang, M.L.; Wu, H.H.; Zhou, A.J.; Chen, S.H.; Liu, Y.W. Study on the Quality Standard for Modified Xiaochaihu Granules. *China Pharmacist.* **2018**, *21*, 1299–1303.
73. Zheng, R.W.; Jiang, Z.Q.; Cheng, G.H.; Hu, W.L.; Zhang, J.H. Study on HPLC Fingerprint for Xiaochaihu Granules. *Eval. Anal. Drug-Use Hosp. China.* **2017**, *17*, 1089–1093.
74. Li, X.D.; Gong, W. Multiwavelength fingerprints of Xiaochaihu Granules preparation and analysis of medicinal materials-preparation peak pattern matching analysis. *China Health Ind.* **2017**, *14*, 42–43.
75. Wang, X.; Li, C.D.; Pan, X.B.; Zhang, W. Quality Standard for Flavored Xiaochaihu Yigan Capsules by HPLC with Wavelength Switch. *China Pharmacist.* **2016**, *19*, 1597–1599.
76. Yuan, H.X.; Liu, Y.Q.; Li, H.F.; Pei, M.R. Fingerprint of n-butanol effective fraction of Xiao Chaihu Tang by HPLC-ELSD. *J. Shenyang Pharm. Univ.* **2015**, *32*, 623–632.
77. Yan, L.; Lin, L.F.; Zhang, H.; Dang, X.F.; Ni, J. Study on fingerprint of Xiaochaihu granules sold in the market. *China J. Chin. Mater. Med.* **2013**, *38*, 3498–3501.
78. Chen, W.X.; Wu, J.L.; Ma, D.; Zhang, P.; Gao, X.; Hu, F.D. Fingerprint study on Xiaochaihu Granules-medicinal materials based on peak pattern matching. *Chin. Tradit. Herb. Drugs* **2013**, *44*, 3154–3161.
79. Jin, Y.; Sun, L.; Qiao, S.Y. Establishment of HPLC fingerprint of Xiaochaihu Decoction. *J. Inter. Pharm. Res.* **2013**, *40*, 224–232.
80. Yang, H.J.; Ma, J.Y.; Weon, J.B.; Lee, B.Y.; Ma, C.J. Qualitative and quantitative simultaneous determination of six marker compounds in soshiho-tang by HPLC-DAD-ESI-MS. *Arch. Pharm. Res.* **2012**, *35*, 1785–1791. [CrossRef]
81. Zhuang, Y.H.; Cai, H.; Liu, X.; Cai, B.C. Simultaneous determination of five main index components and specific chromatograms analysis in Xiaochaihu granules. *Acta Pharm. Sin.* **2012**, *47*, 84–87.

82. Yang, J.; Huang, D.X.; Lu, X.M.; Wang, F.; Li, F.M. Analysis on chemical constituents in Xiaochaihu Decoction and their in vivo metabolites in depressed rats. *Chin. Tradit. Herb. Drugs* **2012**, *43*, 1691–1698.
83. Li, C.H.; Mei, Z.Q.; He, B.; Tian, J. Simultaneous determination of 4 components in Xiaochaihu granules by HPLC. *J. Southwest Med. Univ.* **2012**, *35*, 399–401.
84. Wu, Y.Q.; Gou, Y.Q.; Han, J.; Bi, Y.Y.; Feng, S.L.; Hu, F.D.; Wang, C.M. Evaluation preparation technology of Xiaochaihu granules using fingerprint-peak pattern matching. *J. Pharm. Anal.* **2011**, *1*, 119–124. [CrossRef]
85. Liu, X.F.; Lou, Z.Y.; Zhu, Z.Y.; Zhang, H.; Zhao, L.; Chai, Y.F. HPLC-TOF/MS in rapid identification of chemical compositions in Xiaochaihu decoction. *Acad. J. Sec. Mil. Med. Univ.* **2009**, *30*, 941–946. [CrossRef]
86. Li, J.; Jiang, H.; Yin, W.P. Study on the HPLC Fingerprint of Saikosaponins in *Radix Bupleuri* and Its Water Extract. *Lishizhen Med. Mater. Med. Res.* **2009**, *20*, 2205–2206.
87. Chen, W.; Zhang, C.; Sun, L.; Xue, M. Combination of fingerprint and chemometrics for identification of four processed products of *Pinelliae Rhizoma* based on nucleosides and nucleobases. *Chin. J. Pharm. Anal.* **2021**, *41*, 919–928.
88. Chinese Academy of Sciences. Flora of China. Available online: http://www.iplant.cn/info/Bupleurum?t=z (accessed on 28 July 2021).
89. Zhao, J.F.; Guo, Y.Z.; Meng, X.S. The toxic principles of *Bupleurum longiradiatum*. *Acta Pharm. Sin.* **1987**, *7*, 507–511.
90. Liu, X.X.; Luo, Z.Y.; Ji, Z.Z.; Li, H.; Zhang, W.Q.; Chen, F. A Detection Method of Adulteration of B. Marginatum var.Stenophyllum in Xiaochaihu Granules Preparation and Its Chinese Herbal Medicine Raw Materials. Patent CN110779993A, 11 February 2020.
91. Liang, H.L.; Tan, C.C.; Jiang, X.J.; Xiao, X.Y.; Xu, W.B. Study on Rapid Identification on Xiaochaihu Granules from Different Manufacturers by Near Infrared Spectroscopy. *Guiding J. Tradit. Chin. Med. Pharm.* **2021**, *27*, 62–64.
92. Lai, J.; Shi, Z.F.; Song, P.S.; Wang, Z.X.; Ni, L.; Teng, B.X. Identification of *Bupleurum marginatum var.stenophyllum* Adulterated *Bupleurum chinese* by PCR Amplification of Based on ITS Sequences. *Res. Pract. Chin. Med.* **2021**, *35*, 18–21.
93. Xie, D.H.; Cai, B.C.; An, Y.Q.; Li, X.; Jia, X.B. The research progerss in saikosaponin and its pharmacology action. *J. Nanjing Univ. Tradit. Chin. Med.* **2007**, *1*, 63–65.
94. Lv, X.H.; Sun, Z.X.; Su, R.Q.; Fan, J.W.; Zhao, Z.Q. The pharmacology research progress in bupleurum and its active constituents. *Chin. J. Inf. Tradit. Chin. Med.* **2012**, *29*, 105–107.
95. Liu, Y.M.; Liu, X.M.; Pan, R.L. The research progress in toxic effect of bupleurum. *Chin. Tradit. Pat. Med.* **2012**, *34*, 1148–1151.