Pharmacology and Safety Review of Qili Qiangxin Capsules

Qili Qiangxin capsules received a Chinese new drug certificate (China Food and Drug Administration Approval No. Z20040141) in 2004 and a patent under the title “A kind of pharmaceutical composition and a preparation method for treating chronic cardiac failure” in 2005 (Patent No. ZL02146573.8). Qili Qiangxin capsules consist of 11 types of herbs: Astragali Radix, Ginseng Radix et Rhizoma, Aconiti Lateralis Radix Preparata, Salvia Miltiorrhiza Radix et Rhizoma, Descurainiae Semen Lepidii Semen, Alismatis Rhizoma, Polygonati Odorati Rhizoma, Cinnamomi Ramulus, Carthami Flos, Periplocae Cortex and Citri Reticulatae Pericarpium. All of these ingredients are included in the Chinese pharmacopoeia.

According to traditional Chinese medicine (TCM) theory, Chinese herbs are generally prescribed in formulas that contain “king” medicines, which provide the strongest therapeutic action; “minister” medicines, which assist the “king” medicine in its therapeutic actions; “assistant” medicines, which aid the “minister” medicine in treating a lesser aspect of the disease; and “ambassador” medicines intended to reduce the toxicity of the other medicines in the formula or guide the formula to the targeted organ or region of the body. In the Qili Qiangxin formula,

the “king” medicines are Astragali Radix and Aconiti Lateralis Radix Preparata. To guarantee the quality and consistency of Qili Qiangxin capsules, the raw medicinal materials were of a certain variety, and their areas of origin, medicinal parts and processing methods were kept consistent. The ingredients of the Qili Qiangxin capsules are shown in Table 1.

1. Quality control and chemical component study

The quality control of the production process of Qili Qiangxin capsules strictly adhered to the GMP of national drug production. To verify the stability of the product, we analyzed 10 batches of Qili Qiangxin capsules and fingerprints using the software “Chinese medicine chromatographic fingerprint similarity evaluation system version 2004 A”, which was developed by a committee of the national pharmacopoeia. The multipoint correction and automating matching were performed for each chromatographic peak of the fingerprint with the average correlation coefficient method, and reference fingerprint chromatograms (R) of the common mode of Qili Qiangxin capsules were obtained. Then, the similarity calculation was performed. The similarity of 10 batches of Qili Qiangxin capsules and the reference fingerprint is shown in Table 2. The results showed that the fingerprint similarity of these

batches was greater than 95%, which indicates that the product was stable and controllable.

As the chemical components of this medicine are complex and varied, we adopted a chromatographic process to separate and analyze the chemical components to study the material basis of Qili Qiangxin capsules. LC-MS/MS (AB SCIEX Triple TOF™ 5600) analysis was performed for the Qili Qiangxin Capsule compound components, and nearly 200 compounds were identified (Figures 2, 3). The component compounds of Qili Qiangxin identified with liquid chromatography mass spectrometry (LC-MS/MS) that exhibited physiological activity are listed below.

1.1 Ginseng saponin compounds: Ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Rd, ginsenoside Rf, ginsenoside Ro, ginsenoside Rb2, ginsenoside F3, ginsenoside F7, ginsenoside F5, 20(S)-ginsenoside F1, 20(R)-ginsenoside F1, ginsenoside F2, ginsenoside Rg7, 20(S)-ginsenoside Rg2, 20(R)-ginsenoside Rg2, ginsenoside Ra1, ginsenoside Ra2, ginsenoside Ra3, 20(S)-ginsenoside Rh1, 20(R)-ginsenoside Rh1, ginsenoside A, ginsenoside P, ginsenoside La, ginsenoside F4, ginsenoside Rh4, 20(S)-ginsenoside Rg3, 20(R)-ginsenoside Rg3 and others. The majority of ginseng saponin compounds have been shown to improve hypoxia tolerance, slow the heart rate,

increase cardiac output and coronary blood flow and enhance myocardial contractility (1-9).

1.2 Astragalus saponin compounds: Astragaloside V, astragaloside VII, astragaloside VI, astragaloside VIII, astragaloside I, isoastragaloside I, astragaloside IV and others. Astragalus saponin compounds protect myocardial cells and blood vessel endothelium and improve blood flow (10-13).

1.3 Phenolic acid compounds: Salvianolic acid A, salvianolic acid B, salvianolic acid C, salvianolic acid D, salvianolic acid E, salvianolic acid F, salvianolic acid H and others. Phenolic acid compounds possess antioxidant, anti-inflammatory and anti-platelet aggregation activity (14-18).

1.4 Flavonoid compounds: Isoquercitrin, rutin, quercimeritrin, quercetin-3-O-β-D-glucopyranoside, quercetin, kaempferol-7-O-β-D-glucopyranoside, quercetin-3-O-α-L-rhamnopyranoside, isorhamnetin-3-O-glucoside, calycosin-7-O-β-D-Glucopyranoside-6''-O-malonate, isomucronulatol-7-O-glucoside, isomucronulatol-7,2'-di-O-glucoside, calycosin, 7-hydroxy-5,6,8,4'-tetramethoxyflavone and others. Flavonoid

compounds possess anti-oxidant and anti-inflammatory activity (19-24).

1.5 Cardenolide compounds: Periplocoside, periplocoside G, russelioside D, S-4a, S-4b, S-5, S-6 and others. These compounds demonstrate excellent activity in strengthening the heart (25, 26).

2. Toxicological study of Qili Qiangxin capsules

The clinical dosage of Qili Qiangxin capsules was 0.056 g/kg/d. The acute toxicity test in rats showed that the maximum administration dosage of rat was 32.73 g/kg/d, which was 584.27 times the clinical dosage. No deaths in rats were observed.

3 Mechanisms of action for Qili Qiangxin capsules in heart disease treatment

Zou et al. compared the effects of Qili Qiangxin capsules on the development of cardiac hypertrophy to those of losartan in mice undergoing transverse aortic constriction. Cardiac hypertrophy, function and remodeling were evaluated by echocardiography, catheterization, histology and examination of specific gene expression and ERK phosphorylation. The results showed that Qili Qiangxin capsules inhibited myocardial inflammation and cardiomyocyte death and promoted cardiomyocyte proliferation, leading to amelioration in cardiac remodeling and function in a mouse model of pressure overload. The potential mechanisms

responsible for these effects may involve the inhibition of the angiotensin II type 1 receptor and activation of ErbB receptors (27).

Xiao et al. investigated the effects of Qili Qiangxin capsules on cardiac function and expression of the pro-inflammatory cytokine TNF α and the anti-inflammatory cytokine IL-10 in rats with myocardial infarction. The results indicated that Qili Qiangxin treatment significantly improved cardiac function and histopathologic changes with down-regulated ratio of TNF-α/IL-10. These data suggest that Qili Qiangxin may improve the cardiac function of rats with myocardial infarction by regulating the balance between TNF-α and IL-10. Thus, one potential immunopharmacologic mechanism underlying the beneficial effects of Qili Qiangxin may involve the balance between pro-inflammatory and anti-inflammatory cytokines in cardiomyocytes (28).

Liu et al. evaluated the effects of oral high-dose (4 g/kg/d) and low-dose (1 g/kg/d) Qili Qiangxin capsules on cardiac function in spontaneously hypertensive rats (SHRs). Echocardiography was performed to evaluate cardiac function and hemodynamic parameters. In addition, hematoxylin and eosin (HE) and Masson's trichrome staining were performed, and the expression levels of

myocardial angiotensin (Ang)-converting enzyme, chymase, TGF-β and collagen-type I and III were evaluated with real-time reverse transcription-PCR. The results showed that in SHRs, the number of chymase enzyme-positive mast cells increased in the left ventricle in comparison to WKY rats. Moreover, Qili Qiangxin capsules significantly decreased mast cell density and cardiac chymase levels and improved the ejection fraction and cardiac systolic function as compared to vehicle treatment, and Qili Qiangxin capsules also decreased left atrial diameters and improved the E/A ratio. Treatment with Qili Qiangxin capsules was also shown to suppress the mRNA levels of collagen type I and III and TGF-β, as well as AngII activity, in a dose-dependent manner. Whereas no difference in ACE activity was found between SHRs, chymase expression and activity were significantly decreased following treatment with Qili Qiangxin capsules. These data suggest that Qili Qiangxin capsules improve both systolic and diastolic cardiac function in SHRs by down-regulating the cardiac chymase signaling pathway and chymase-mediated AngII production (29).

Wei et al. compared the effects of different doses of Qili Qiangxin capsules on L-type Ca^{2+} current ($I_{Ca\text{-}L}$) between normal and hypertrophied myocytes. A total of 40 healthy SD rats were randomly divided into 2 groups (control group and hypertrophy

group), and cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta. The whole-cell patch-clamp technique was used to study the effects of Qili Qiangxin on $I_{Ca\text{-}L}$. The results showed that the inhibition of $I_{Ca\text{-}L}$ resulting from Qili Qiangxin treatment was lower in the hypertrophy group than in the control group. Thus, the underlying mechanism may be that Qili Qiangxin affects L-type Ca^{2+} channels and blocks $I_{Ca\text{-}L}$, to affect cardiac function. Qili Qiangxin also demonstrates diphasic action, which makes it act as either a class IV antiarrhythmic agent or an agent that improves cardiac function (30).

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Figure 1. UPLC fingerprints of 10 batches of Qili Qiangxin capsules.

Figure 2. Ion-current chromatograms obtained in negative-ion mode. This figure shows ion-current chromatograms of the chemical component extract screened using negative-ion mode.

Figure 3. Ion-current chromatograms collected in positive-ion mode. This figure shows the ion-current chromatograms of the chemical component extract screened using positive-ion mode.

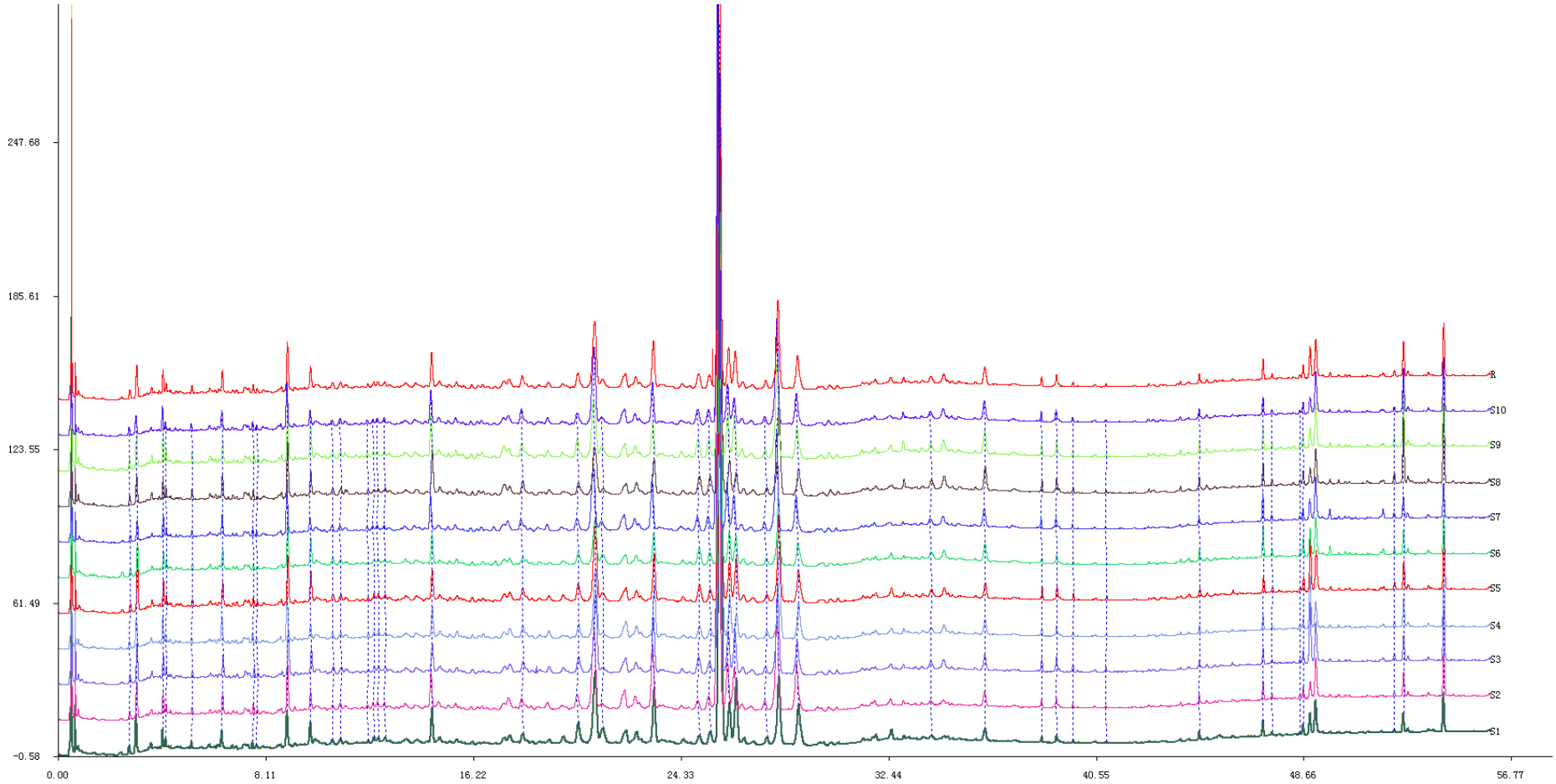
Table 1. Complex compounds contained in Qili Qiangxin capsules.

Material	Species	Combination Principle	Origin	Medicinal Parts	Concocted
Astragali Radix	*Astragalus membranaceus* (Fisch) Bge. Var. *mongholicus* (Bge.) Hsiao.	King	Longxi, Gansu	Roots	Drying
Ginseng Radix et Rhizoma	*Panax ginseng* C. A. Mey.	Minister	Fusong, Jilin	Roots and Rhizomes	Drying
Aconiti Lateralis Radix Preparata	*Aconitum carmichaeli* Debx.	King	Jiangyou, Sichuan	Lateral roots	Boil, Steam, and Drying
Salvia Miltiorrhiza Radix et Rhizoma	*Salvia miltiorrhiza* Bge.	Minister	Yiyuan, Shangdong	Roots	Drying
Descurainiae Semen Lepidii Semen	*Lepidium apetalum* Willd.	Minister	Jinzhou, Hebei	Seeds	Drying
Alismatis Rhizoma	*Alisma orientalis* (Sam.) Juzep.	Assistant	Sichuan	Tubers	Drying
Polygonati Odorati Rhizoma	*Polygonatum odoratum* (Mill.) Druce	Assistant	Hunan	Rhizomes	Drying
Carthami Flos	*Carthamus tinctorius* L.	Assistant	Xinjiang	Flower	Drying
Periplocae Cortex	*Periploca sepium* Bge.	Assistant	Shanxi	Root barks	Drying

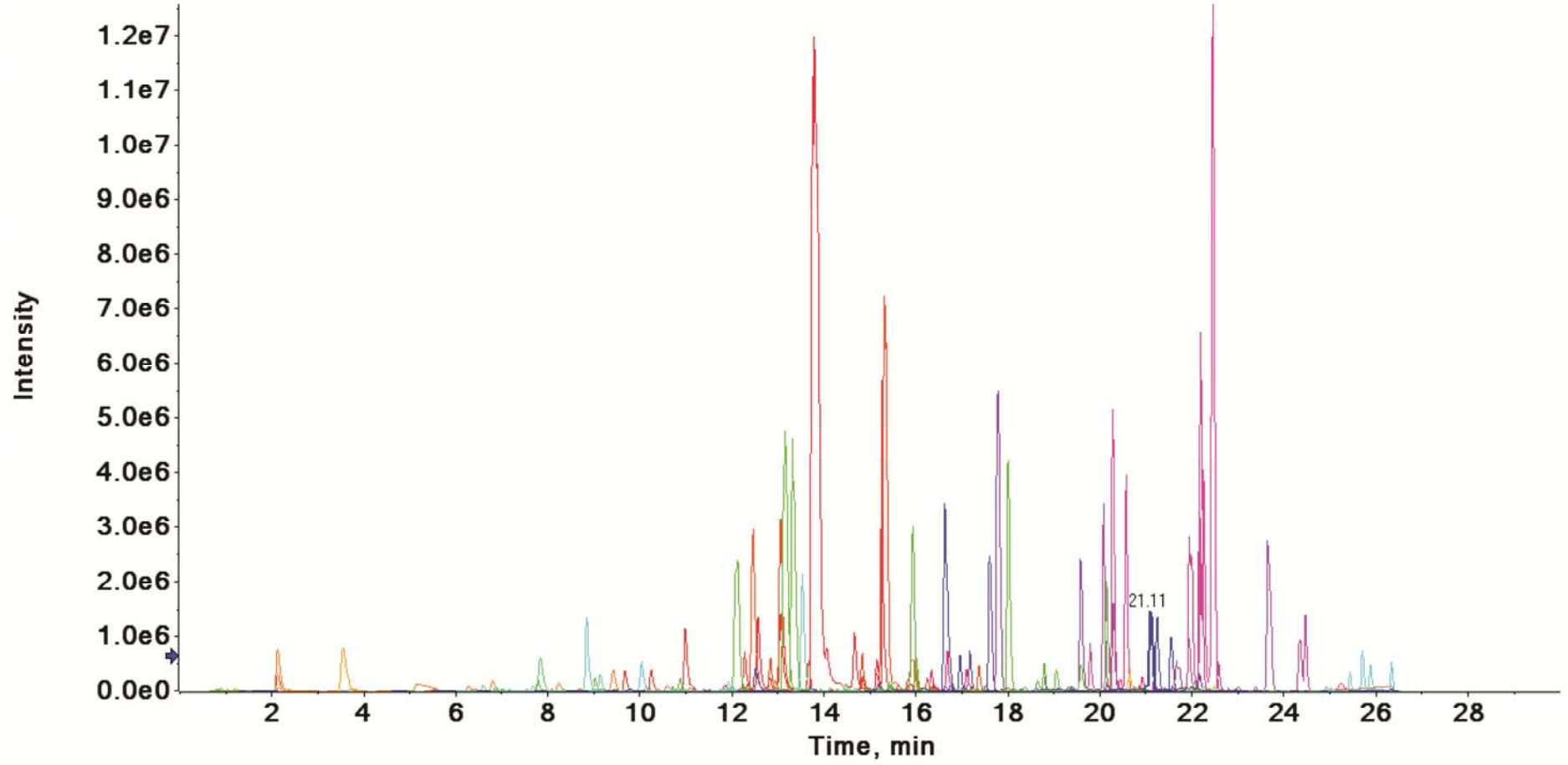
Cinnamomi Ramulus	*Cinnamomum cassia* Presl	Assistant	Guangxi	Twigs	Drying
Citri Reticulatae Pericarpium	*Citrus reticulata* Blanco	Ambassador	Zhejiang	Peels	Drying

Table 2. The similarity of UPLC fingerprints from the 10 batches Qili Qiangxin capsules.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**R**
S1	1.000	0.995	0.991	0.995	0.997	0.996	0.997	0.992	0.997	0.998	0.998
S2	0.995	1.000	0.979	0.989	0.990	0.996	0.999	0.983	0.991	0.992	0.993
S3	0.991	0.979	1.000	0.996	0.995	0.987	0.985	0.998	0.995	0.995	0.996
S4	0.995	0.989	0.996	1.000	0.999	0.995	0.991	0.999	0.999	0.998	0.999
S5	0.997	0.990	0.995	0.999	1.000	0.996	0.992	0.998	0.999	0.997	0.999
S6	0.996	0.996	0.987	0.995	0.996	1.000	0.997	0.991	0.997	0.997	0.997
S7	0.997	0.999	0.985	0.991	0.992	0.997	1.000	0.986	0.994	0.995	0.995
S8	0.992	0.983	0.998	0.999	0.998	0.991	0.986	1.000	0.997	0.996	0.997
S9	0.997	0.991	0.995	0.999	0.999	0.997	0.994	0.997	1.000	1.000	1.000
S10	0.998	0.992	0.995	0.998	0.997	0.997	0.995	0.996	1.000	1.000	0.999
R	0.998	0.993	0.996	0.999	0.999	0.997	0.995	0.997	1.000	0.999	1.000



supplementary file-Fig1



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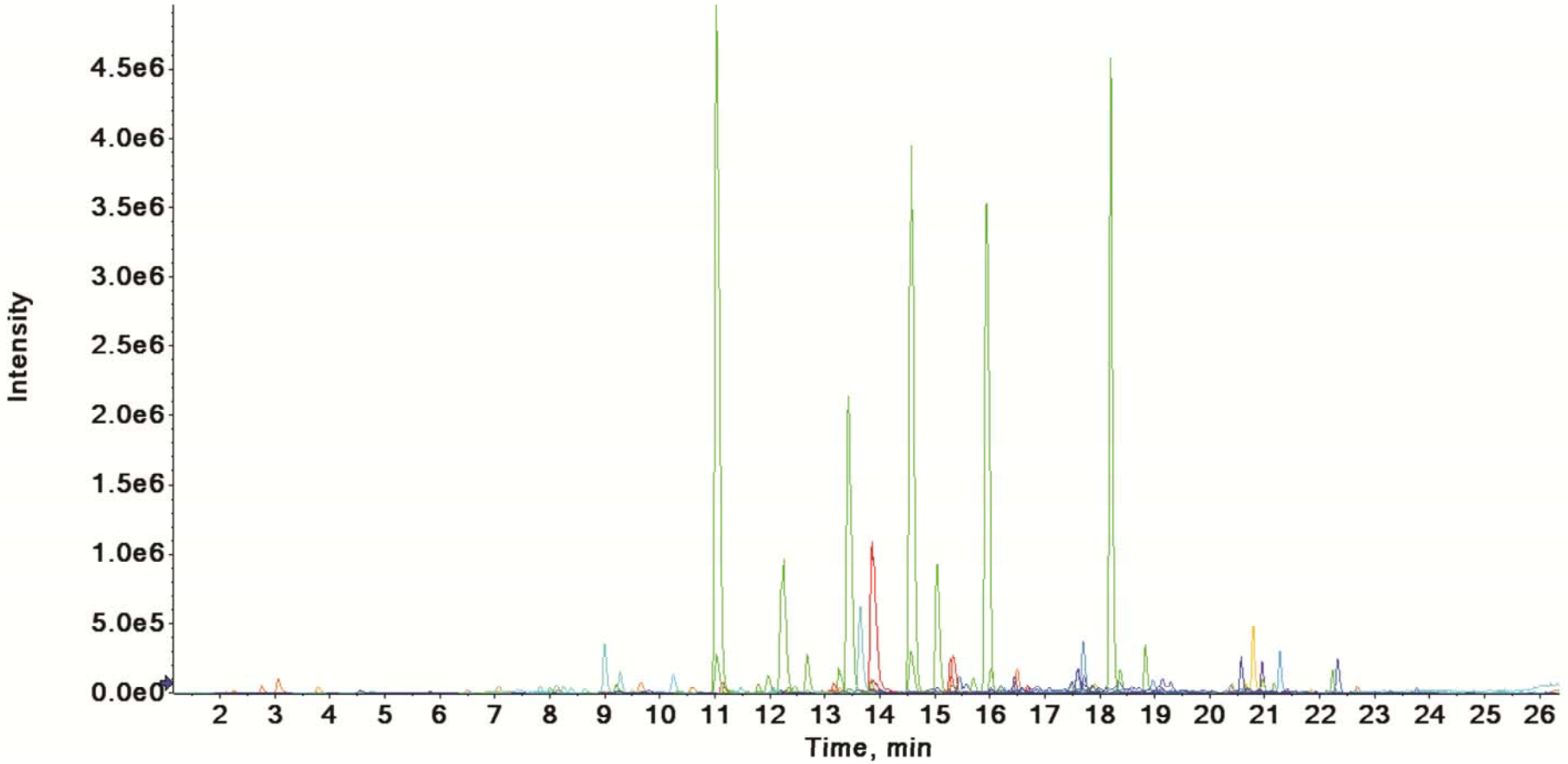

supplementary file-Fig3