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Preventive effects of turmeric on the high-fat diet-induced hyperlipidaemia in mice associated with a targeted metabolomic approach for the analysis of serum lysophosphatidylcholine using LC-MS/MS

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ABSTRACT

Consumption of turmeric diets demonstrated preventive effects on high-fat diet (HFD) induced hyperlipidaemia. However, the preventive effects on serum lysophosphatidylcholines (Lyso PCs) have not yet been studied. For this study, an approach for characterization of Lyso PCs in mice serum using LC-QTOF-MS/MS was developed. The preventive effects of turmeric diets were evaluated on quantitative changes of individual Lyso PCs in mice serum by LC-QTRAP-MS/MS. As a result, 74 Lyso PCs were characterized, among which 59 Lyso PCs were quantified. The results indicated that significant changes occurred for the Lyso PCs with higher degree of unsaturation in the fatty acid chain. This is the first report regarding a targeted metabolomic approach for analyses of serum Lyso PCs and its application to interpretation of preventive effects of turmeric on mice hyperlipidaemia. These metabolic changes and potential biomarkers might serve as scientific evidence for future diagnosis and therapeutic intervention of hyperlipidaemia.

Abbreviations: Lyso PC, Lysophosphatidylcholine; HFD, high-fat diet; HDL-C, high-density lipoprotein cholesterol; MRM, multiple reaction monitoring; TC, total cholesterol; AI, atherogenic index; IS, internal standard; TIC, total ion chromatogram

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1. Introduction

Phospholipids are the major constituents of cellular membranes (Pereto, Lopez-Garcia, & Moreira, 2004). They can be divided into 20 distinct molecular classes based on the glycerophospholipid classification system proposed by the LIPID MAPS consortium (Fahy, Cotter, Sud, & Subramaniam, 2011). Lyso PCs are often considered as a single species, which belongs to glycerophosphocholine-derived lipids distinguished by the linkage of one carbon chain to the phosphoglyceride backbone with different length and degree of unsaturation, and another carbon chain to a hydroxyl group (Fig. 1).

As second messengers in biology, Lyso PCs are not merely transient metabolites of structural glycerophospholipids. They are also immediate-response molecules that elicit physiological and pathophysiological responses independently or through activation of specific G-protein-coupled receptors (Asaoka, Oka, Yoshida, Sasaki, & Nishizuka, 1992; Liebisch, Drobnik, Lieser, & Schmitz, 2002; Liebisch & Schmitz, 2009; Meyer zu Heringdorf & Jakobs, 2007). Hyperlipidaemia refers to a condition where there is an imbalance of lipid profiling. A low level of high-density lipoprotein cholesterol (HDL-C) is an indicative risk factor for cardiovascular disease (Chirovsky, Fedirko, Cui, Sazonov, & Barter, 2009; Cooney, et al, 2009; Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977). However, accumulating evidence suggests that HDL-C alone may not be an adequate and reliable marker of atheroprotection (Yetukuri et al., 2010). Since Lyso PCs are known to be associated with proatherogenic conditions such as hyperlipidaemia, (Glass & Witztum, 2001) serum Lyso PC levels may provide more precise markers for specific metabolic phenotypes than total lipid class concentrations.

Metabolomic approach is generally unbiased for the identification and quantification of all metabolites within an organism. The technique can generate substantial amounts of metabolic data that can give surprisingly detailed insights into the changes in metabolic processes in whole organisms (Dettmer, Aronov, & Hammock, 2007; Nicholson & Wilson, 2003). There are two different strategies for metabolites profiling: (1) untargeted analysis based on full-scan analysis using high resolution mass spectrometers [e.g., LC-QTOF-MS/MS)]; (2) targeted analysis of a subgroup of metabolites using the most suitable chromatography and MS scan mode [e.g., LC-QTRAP-MS/MS with a multiple reaction monitoring (MRM) mode]. They represent the breadth-first (qualitative) and depth-first (quantitative) screening approach, respectively. Class-targeted metabolomics of certain subsets of metabolome were recently demonstrated to be a powerful tool in better understanding the focused molecular profiles in complex systems. Particularly, LC-QTRAP-MS/MS with a MRM mode is recently undergoing a renaissance within the metabolomics community for its superior sensitivity, selectivity, and wide linear dynamic range (Liu et al., 2013; Lutz, Lutz, & Lutz, 2006; Strassburg et al., 2012; Yan, Lin, Ye, Wang, & Yan, 2014). The most important bottleneck in MRM-based metabolomics is generally the limited analytes coverage and throughput capacity, which is complementary using LC-QTOF-MS/MS with a full-scan mode. Therefore, a targeted metabolomic approach integrating the two complementary LC-MS/MS platforms could enable the better discovery of molecular biomarkers, especially the identification and quantification of the low-abundance metabolites.

The hypolipidaemic effect of dietary turmeric was previously reported (Manjunatha & Srinivasan, 2007a, 2007b; Piyachaturawat, Teeratagolpisal, Toskulkao, & Suksamrarn, 1997). However, little is known about its hypolipidaemic effect on serum Lyso PCs. In the present study, a targeted metabolomic approach regarding the analysis of Lyso PCs in mice serum was established using LC-QTOF-MS/MS for the initial identification of the Lyso PCs, while using LC-QTRAP-MS/MS for the in-depth Lyso PC profiling. The approach was applied to systematically investigate the changes of Lyso PCs in hyperlipidaemic mice and the hypolipidaemic effect of dietary turmeric on serum Lyso PCs. These metabolic changes and potential biomarkers might serve as scientific evidence for future diagnosis and for therapeutic intervention of hyperlipidaemia. Meanwhile, the present study provides a deeper insight into the possible mechanism of the preventive effect of turmeric on HFD-induced hyperlipidaemia.

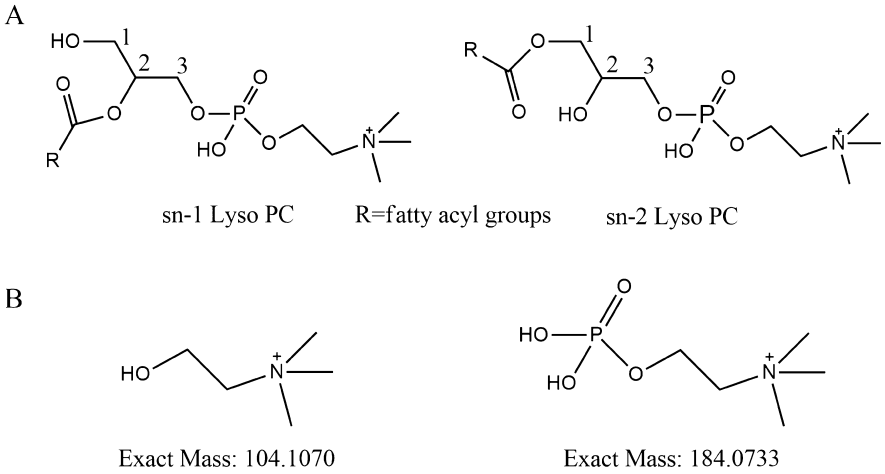

Fig. 1 – The basic chemical structures (A) and characteristic fragments and neutral losses of Lyso PCs (B).

2. Materials and methods

2.1. Drugs and chemicals

The plant of *Curcuma longa* L. was grown in Suining, Sichuan Province, China. Dried Rhizome of *C. longa* L. were collected in the local area. The herb was identified and authenticated by Professor Keli Chen of Key Laboratory of Chinese Medicine Resource and Compound Prescription (Hubei University of Chinese Medicine), Ministry of Education. The voucher specimens were deposited at the herbarium of Huazhong University of Science and Technology.

Simvastatin was purchased from Sanchine Pharmaceutical Co., Ltd. (Heilongjiang, China). Total cholesterol (TC) and HDL-C assay kits were purchased from Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, China). HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was produced by a Milli-Q water system (Millipore, Bedford, MA, USA). Formic acid (≥98%) of analytical grade was purchased from Sinopharm Chemical Reagent Co., LTD (Shanghai, China).

2.2. Experimental diets and animals

Male Kunming mice (20–25 g) were purchased from the Experimental Animal Centre of Huazhong University of Science and Technology. The mice were acclimatized under standard conditions of 12 h light/dark cycle at 22–26 °C with a humidity of 55–65%. The mice were fed with standard diet, or HFD, which was made of standard diet (78.8%), egg yolk (10%), lard (10%), cholesterol (1%) and cholate (0.2%) or HFD supplemented with turmeric powder (0.5% or 2.0% w/w).

After acclimation for 3 days, all mice were randomly divided into five groups of eight animals each: normal control (**C**) group, hyperlipidaemic group (**H**), positive group (**P**), turmeric low ($\mathbf{T_L}$) and high ($\mathbf{T_H}$) dose groups. The mice in **C** group were fed with standard diet, and the mice in **H** and **P** groups were fed with HFD, whereas $\mathbf{T_L}$ and $\mathbf{T_H}$ groups were fed with HFD+0.5% and HFD+2.0% turmeric powder during the experimental period. The mice in **P** group were treated with 5 mg/kg·day of simvastatin. Animals from all groups were fed and treated for 4 weeks. The mice were allowed free access to water and food during the experiment. Food and water intake was monitored every 4 days. Body weights were measured before and after the treatments. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology.

Blood samples were collected by ophthalmectomy after being anaesthetized with pentobarbital injection from each mouse with fasting diets for 12 hours but free access to water on the 28th day. The samples were taken into sterile tubes without anticoagulant. Serum was separated from blood samples by centrifugation and stored at –80 °C until analyses.

2.3. Biochemical analysis

Serum TC and HDL-C levels were measured by enzymatic colorimetric method using commercial kits. Values were measured with a semi-automatic biochemical analyzer (RT-9600, Shenzhen, China). The atherogenic index (AI) was calculated as AI = (TC – HDL-C)/HDL-C.

2.4. Sample preparation

For the sample preparation, a pool was made by combining serum from eight mice per group (10 μL/mouse). Next, the pooled serum (80 μL) was combined with 800 μL of 100% acetonitrile (final acetonitrile concentration is 90.9%) and vortexed vigorously for 15 min followed by sonication in ice water for 15 min. The mixture was centrifuged at relative centrifuge force 14,000 g for 30 min. Then 450 μL supernatant were removed carefully to a sample vial for injection. Fifty microlitres of fexofenadine at 500 ng/mL was added to each extract as internal standard (IS) and mixed well for LC-MS/MS.

2.5. LC-QTOF-MS/MS analysis

The LC analyses were conducted on a Shimadzu UHPLC system (Kyoto, Japan) consisting of a solvent delivery system LC-30AD, an autosampler SIL-30AC, a CTO-30A column oven, a degasser DGU-20A_3 and a controller CBM-20A. A Welch Ultimate UHPLC C18 column (100 mm × 2.1 mm, 1.8 μm) was used at a flow rate of 0.3 mL/min. The injection volume was 2 μL. Solvent A was water/formic acid (1000:1, v/v) and solvent B was acetonitrile/formic acid (1000:1, v/v). The following binary gradient with linear interpolation was used: 0.01 min, 20% B; 3 min, 57% B; 12 min, 57% B; 17 min, 95% B; 19 min, 95% B; 19.1 min, 20% B; 21.5 min, 20% B. The column oven temperature was set at 40 °C.

The LC-QTOF-MS/MS analysis was carried out using a Triple TOF™ 5600 system with a Duo Spray source in the positive electrospray ion mode (AB SCIEX, Foster City, CA, USA). The MS operating conditions were as follows: an ion spray voltage of 4500 V, an ion source temperature of 500 °C, a curtain gas of 25 psi, a nebulizer gas (GS 1) of 40 psi, a heater gas (GS 2) of 50 psi, a declustering potential of 90 V. The mass ranges were set at *m/z* 100–1000 for TOF MS scan and 50–1000 for TOF MS/MS experiments. The collision energy was set at 35 eV and the collision energy spread was 10 eV for MS/MS. The most intensive eight ions from each full MS scan were selected as precursor ions for MS/MS fragmentation. Dynamic background subtraction was used to match the information dependent acquisition criteria. The data were analyzed by the Peak View Software™ 1.2 (AB SCIEX).

2.6. LC-QTRAP-MS/MS analysis

The LC operating conditions were the same as described in the previous paragraph. The LC-QTRAP-MS/MS analysis was performed on an AB Sciex Qtrap 4000 system in the positive electrospray ion mode (AB SCIEX). The MS operating conditions were optimized with an ion spray voltage of 4500 V, a turbo ion spray temp of 500 °C, a curtain gas of 25 psi, a nebulizer gas (GS 1) of 40 psi, a heater gas (GS 2) of 50 psi, a declustering potential of 90 V, a collision energy of 35 eV, a collision cell exit potential of 10 V, and a dwell time of 10 ms. Quantification was carried out using MRM mode to monitor precursor–product ion transitions at each precursor ion (Table 1) of Lyso PCs-184.0 and

Table 1 – Lyso PCs identified in mice serum using LC-QTOF-MS/MS.

No.	RT (min)	Name	Formula	$[M+H]^+$		
				Observed *m/z*	Theoretical *m/z*	ppm
1	4.10	lyso/16:0-OH	$C_{24}H_{51}NO_8P$	512.3361	512.3347	2.73
2	4.26	lyso/18:2-OH	$C_{26}H_{51}NO_8P$	536.3360	536.3347	2.42
3	4.37	OH-18:2/lyso	$C_{26}H_{51}NO_8P$	536.3367	536.3347	3.73
4	4.54	lyso/18:3-OH	$C_{26}H_{49}NO_8P$	534.3200	534.3190	1.87
5	4.55	lyso/19:6-OH	$C_{27}H_{45}NO_8P$	542.2899	542.2877	4.06
6	4.62	lyso/20:4-OH	$C_{28}H_{51}NO_8P$	560.3354	560.3347	1.25
7	4.68	OH-19:6/lyso	$C_{27}H_{45}NO_8P$	542.2903	542.2877	4.79
8	4.74	OH-20:4/lyso	$C_{28}H_{51}NO_8P$	560.3377	560.3347	5.35
9	4.85	lyso/20:3-OH	$C_{28}H_{53}NO_8P$	562.3529	562.3503	4.62
10	4.92	lyso/16:3	$C_{24}H_{45}NO_7P$	490.2953	490.2928	5.10
11	4.97	lyso/14:1	$C_{22}H_{45}NO_7P$	466.2932	466.2928	0.86
12	5.02	OH-20:3/lyso	$C_{28}H_{53}NO_8P$	562.3535	562.3503	5.69
13	5.10	18:3-OH/lyso	$C_{26}H_{49}NO_8P$	534.3208	534.3190	3.37
14	5.12	14:1/lyso	$C_{22}H_{45}NO_7P$	466.2941	466.2928	2.79
15	5.14	16:3/lyso	$C_{24}H_{45}NO_7P$	490.2942	490.2928	2.86
16	5.65	OH-18:2/lyso	$C_{26}H_{51}NO_8P$	536.3363	536.3347	2.98
17	5.66	lyso/16:2	$C_{24}H_{47}NO_7P$	492.3114	492.3085	5.89
18	5.73	lyso/14:0	$C_{22}H_{47}NO_7P$	468.3108	468.3085	4.91
19	6.12	lyso/20:5	$C_{28}H_{49}NO_7P$	542.3272	542.3241	5.72
20	6.17	14:0/lyso	$C_{22}H_{47}NO_7P$	468.3101	468.3085	3.42
21	6.20	lyso/17:2	$C_{25}H_{49}NO_7P$	506.3272	506.3241	6.12
22	6.21	16:2/lyso	$C_{24}H_{47}NO_7P$	492.3095	492.3085	2.03
23	6.27	lyso/18:3	$C_{26}H_{49}NO_7P$	518.3256	518.3241	2.89
24	6.32	OH-16:0/lyso	$C_{24}H_{51}NO_8P$	512.3390	512.3347	8.39
25	6.37	lyso/22:7	$C_{30}H_{49}NO_7P$	566.3262	566.3241	3.71
26	6.43	lyso/16:1	$C_{24}H_{49}NO_7P$	494.3256	494.3241	3.03
27	6.50	20:5/lyso	$C_{28}H_{49}NO_7P$	542.3260	542.3241	3.50
28	6.63	17:2/lyso	$C_{25}H_{49}NO_7P$	506.3269	506.3241	5.53
29	6.64	lyso/19:4	$C_{27}H_{49}NO_7P$	530.3253	530.3241	2.26
30	6.69	OH-16:0/lyso	$C_{24}H_{51}NO_8P$	512.3377	512.3347	5.86
31	6.70	18:3/lyso	$C_{26}H_{49}NO_7P$	518.3267	518.3241	5.02
32	6.76	22:7/lyso	$C_{30}H_{49}NO_7P$	566.3279	566.3241	6.71
33	6.97	16:1/lyso	$C_{24}H_{49}NO_7P$	494.3265	494.3241	4.86
34	7.01	lyso/15:0	$C_{23}H_{49}NO_7P$	482.3246	482.3241	1.04
35	7.09	19:4/lyso	$C_{27}H_{49}NO_7P$	530.3264	530.3241	4.34
36	7.56	lyso/18:2	$C_{26}H_{51}NO_7P$	520.3425	520.3398	5.19
37	7.62	lyso/22:6	$C_{30}H_{51}NO_7P$	568.3428	568.3398	5.28
38	7.67	lyso/20:4	$C_{28}H_{51}NO_7P$	544.3423	544.3398	4.59
39	7.70	15:0/lyso	$C_{23}H_{49}NO_7P$	482.3262	482.3241	4.35
40	8.10	lyso/17:1	$C_{25}H_{51}NO_7P$	508.3403	508.3398	0.98
41	8.17	22:6/lyso	$C_{30}H_{51}NO_7P$	568.3436	568.3398	6.69
42	8.25	18:2/lyso	$C_{26}H_{51}NO_7P$	520.3424	520.3398	5.00
43	8.31	20:4/lyso	$C_{28}H_{51}NO_7P$	544.3428	544.3398	5.51
44	8.67	lyso/22:5	$C_{30}H_{53}NO_7P$	570.3576	570.3554	3.86
45	8.92	17:1/lyso	$C_{25}H_{51}NO_7P$	508.3411	508.3398	2.56
46	9.03	lyso/19:2	$C_{27}H_{53}NO_7P$	534.3574	534.3554	3.74
47	9.05	lyso/16:0	$C_{24}H_{51}NO_7P$	496.3414	496.3398	3.22
48	9.27	lyso/20:3	$C_{28}H_{53}NO_7P$	546.3576	546.3554	4.03
49	9.45	22:5/lyso	$C_{30}H_{53}NO_7P$	570.3578	570.3554	4.21
50	10.12	lyso/22:5	$C_{30}H_{53}NO_7P$	570.3588	570.3554	5.96
51	10.14	16:0/lyso	$C_{24}H_{51}NO_7P$	496.3429	496.3398	6.25
52	10.23	20:3/lyso	$C_{28}H_{53}NO_7P$	546.3577	546.3554	4.21
53	10.53	lyso/18:0-OH	$C_{26}H_{55}NO_8P$	540.3676	540.3659	3.15
54	10.66	lyso/18:1	$C_{26}H_{53}NO_7P$	522.3578	522.3554	4.59
55	10.70	19:2/lyso	$C_{27}H_{53}NO_7P$	534.3561	534.3554	1.31
56	11.06	22:5/lyso	$C_{30}H_{53}NO_7P$	570.3583	570.3554	5.08
57	11.45	lyso/17:0	$C_{25}H_{53}NO_7P$	510.3565	510.3554	2.16
58	11.64	OH-18:0/lyso	$C_{26}H_{55}NO_8P$	540.3683	540.3659	4.44
59	11.87	lyso/22:4	$C_{30}H_{55}NO_7P$	572.3717	572.3711	1.05
60	11.93	18:1/lyso	$C_{26}H_{53}NO_7P$	522.3581	522.3554	5.17
61	12.27	17:0/lyso	$C_{25}H_{53}NO_7P$	510.3577	510.3554	4.51
62	12.75	lyso/20:2	$C_{28}H_{55}NO_7P$	548.3736	548.3711	4.56

(*continued on next page*)

Table 1 – (*continued*)

No.	RT (min)	Name	Formula	$[M+H]^+$		
				Observed *m/z*	Theoretical *m/z*	ppm
63	13.01	lyso/17:0	$C_{25}H_{53}NO_7P$	510.3581	510.3554	5.29
64	13.16	22:4/lyso	$C_{30}H_{55}NO_7P$	572.3718	572.3711	1.22
65	13.87	17:0/lyso	$C_{25}H_{53}NO_7P$	510.3576	510.3554	4.31
66	14.10	20:2/lyso	$C_{28}H_{55}NO_7P$	548.3739	548.3711	5.11
67	14.27	lyso/19:1	$C_{27}H_{55}NO_7P$	536.3727	536.3711	2.98
68	14.88	19:1/lyso	$C_{27}H_{55}NO_7P$	536.3732	536.3711	3.92
69	15.23	lyso/18:0	$C_{26}H_{55}NO_7P$	524.3739	524.3711	5.34
70	15.85	lyso/20:1	$C_{28}H_{57}NO_7P$	550.3883	550.3867	2.91
71	15.86	18:0/lyso	$C_{26}H_{55}NO_7P$	524.3735	524.3711	4.58
72	16.37	20:1/lyso	$C_{28}H_{57}NO_7P$	550.3887	550.3867	3.63
73	16.92	lyso/19:0	$C_{27}H_{57}NO_7P$	538.3897	538.3867	5.57
74	17.22	19:0/lyso	$C_{27}H_{57}NO_7P$	538.3893	538.3867	4.83

at *m/z* 502.5-466.2 for fexofenadine. Peak areas were integrated for each MRM transition.

2.7. *Statistics*

Results were presented as the means ± *SD*. The data were analyzed by *t* test to compare treatments *vs* the hyperlipidaemic group **(H)** using GraphPad Prism 6 software. *P* values < 0.05 were considered statistically significant.

3. Results and discussion

In the present study, a targeted metabolomic approach using the two LC-MS/MS platforms was established for systematic analysis of Lyso PCs in the mice serum, especially for the identification and quantification of the low-abundance Lyso PC biomarkers.

With the rising trend in the global incidence of various metabolic diseases, particularly hyperlipidaemia and its related health problems, there is a growing public interest in functional foods that could improve the lipid profiles. Using the targeted metabolomic approach established in the present study, a variety of serum Lyso PCs were found dysregulated in hyperlipidaemic mice and many of these Lyso PCs were changed towards normal levels after consumption of turmeric diet for 4 weeks.

3.1. *Physiological and biochemical parameters*

All animals used in this study were in good health throughout the experiment. No side effects, such as abnormal behaviour or diarrhoea, were observed. The differences in body weight among the five groups were not statistically significant at the beginning of the experiment. The intake of food and water were unaffected by treatments during the experiment (data not shown). As shown in Table 2, 4 week treatments with simvastatin induced a significant reduction of body weight, which was 13.4% lower than that of hyperlipidaemic mice in the **H** group. The body weight of mice fed HFD+2.0% turmeric powder ($\mathbf{T_H}$ group) was slightly lower than that of mice in **H** group at the end of the experiment. However, the difference was not significant. In addition, the liver weights of mice in **H** group were significantly higher than those of mice in the **C** group. The hyperlipidaemic mice in **P** and $\mathbf{T_H}$ groups showed significant decreases in the mean liver weight, which were 16% and 14%, respectively, lower than that of hyperlipidaemic controls.

At the end of the experiment, the marked increase in serum TC level indicated a successful establishment of a mice model of hyperlipidaemia induced by HFD (Table 2). The serum TC levels in all treatment groups were significantly decreased, whereas the serum HDL-C levels in $\mathbf{T_L}$ and $\mathbf{T_H}$ groups were significantly elevated compared with the hyperlipidaemic mice in **H** group. In particular, the serum HDL-C levels of

Table 2 – Physiological and biochemical parameters of mice fed with turmeric for 4 weeks (mean ± *SD*, n = 8).

	C[a]	H[a]	P[a]	T_L[a]	T_H[a]
Physiological parameters[b]					
BW (g) at 0 day	26.91 ± 3.71	26.16 ± 3.37	25.92 ± 3.29	25.33 ± 3.42	26.53 ± 4.16
BW (g) at 28th day	43.81 ± 3.49	45.44 ± 3.66	39.35 ± 1.87*	44.23 ± 3.48	41.62 ± 4.77
Liver weight (g)	1.90 ± 0.13	2.48 ± 0.36	2.08 ± 0.18**	2.45 ± 0.48	2.14 ± 0.11**
Biochemical parameters[b]					
TC (mmol/L)	1.88 ± 0.26	5.40 ± 0.58	3.07 ± 0.67*	4.04 ± 0.55**	3.51 ± 0.64*
HDL-C (mmol/L)	1.03 ± 0.15	0.94 ± 0.14	0.96 ± 0.16	1.13 ± 0.15**	1.36 ± 0.12*
TC/HDL-C ratio	1.85 ± 0.28*	5.85 ± 1.18	3.20 ± 0.67*	3.60 ± 0.54*	2.58 ± 0.46*
AI	0.85 ± 0.28*	4.85 ± 1.18*	2.20 ± 0.67*	2.60 ± 0.54*	1.58 ± 0.46*

* $P < 0.01$, **$P < 0.05$ significantly different from group H.

[a] C, normal control group; H, hyperlipidaemic group; P, positive group; T_L, turmeric low dose group; T_H, turmeric high dose group.

[b] BW, body weight; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; AI, atherogenic index, AI = (TC – HDL-C)/HDL-C.

hyperlipidaemic mice in group $\mathbf{T_H}$ was 1.36 units, which was the highest in all treatment groups. Meanwhile, the TC/HDL-C ratios of hyperlipidaemic mice in groups **P**, $\mathbf{T_L}$ and $\mathbf{T_H}$ were 3.20, 3.60 and 2.58, which were significantly lower than those in **H** group. For AI, the value was 2.20, 2.60 and 1.58 units in groups **P**, $\mathbf{T_L}$ and $\mathbf{T_H}$, respectively, which was 54%, 46% and 67% lower than that in group **H**. Accordingly, the TC/HDL-C ratio and AI were the lowest in TH group.

Dietary cholesterol, fat and fibre can induce abnormal lipid profiles such as a high serum TC and triglycerides concentrations (Anderson, Chen, & Sieling, 1980). In the present study, 4 week turmeric diet significantly reduced the level of serum TC and increased the level of serum HDL-C in hyperlipidaemic mice. Meanwhile, the preventive effect on elevated serum HDL-C concentrations of turmeric powder was better than that of simvastatin. Cholesterol concentration in the blood is affected by both cholesterol content of diet and cholesterol synthesized in liver. The intake of food was unaffected by treatments during the experiment, which eliminated the influence of exogenous cholesterol. Therefore, the decrease of endogenous cholesterol content should be the main cause for the decline of the serum cholesterol concentration. This result was supported in part by the evidence of the increased serum HDL-C level accompanied with the decrease of serum TC level in hyperlipidaemic mice. HDLs are among the most structurally complex and functionally versatile forms of circulating serum lipoproteins. One of the most important anti-atherogenic functions of HDL is the ability to initiate cholesterol efflux and thereby facilitating removal of excess cholesterol from peripheral tissues and delivery to the liver for degradation through reverse cholesterol transport pathways (Fielding & Fielding, 1981; Kashyap, 1989). The level of serum HDL-C is a major indicator to evaluate the ability of HDL in facilitating translocation of cholesterol to the liver for catabolism (Nofer et al., 2002). In the present study, significant decreases of TC level and considerable increases of HDL-C value were observed in hyperlipidaemic mice fed HFD+0.5% and HFD+2.0% turmeric powder, indicating the potential preventive effect of turmeric on the HFD-induced hyperlipidaemia in mice. The results were consistent with previous reports (Manjunatha & Srinivasan, 2007a, 2007b; Piyachaturawat et al., 1997).

3.2. *Simultaneous profiling of Lyso PCs by LC-QTOF-MS/MS*

Chloroform or dichloromethane-containing solvent systems are the common solvent systems for simultaneous extraction of polar and non-polar lipids from serum and plasma in many studies (Cequier-Sanchez, Rodriguez, Ravelo, & Zarate, 2008; Croset, Brossard, Polette, & Lagarde, 2000). However, simultaneous extraction of all lipids from serum is difficult to be efficient and complete due to their different polarities. In the present study, the focus was to analyze lyso PCs, the polar lipids in serum. Thus, the extraction method was designed based on the polarities of lyso PCs in mice serum. Considering the higher efficiency of acetonitrile on protein precipitation, an acetonitrile–aqueous solvent system was used in this study. In order to avoid generation of artificial compounds during extraction, the extraction was conducted at room temperature and completed in 3 hours without addition of any chemicals. Lyso PCs in the crude extract of serum were directly analyzed by LC-QTOF-MS/MS without using multi-step purification and separation processes to avoid potential isomerization and other structural changes.

The basic chemical structures and characteristic fragments of Lyso PCs discussed in this study were shown in Fig. 1. Total ion chromatogram (TIC) + TOF MS profile filtered with *m/z* 184.0733 and 104.1070 of Lyso PCs is shown in Fig. 2. The peak of each component can be obtained by extracted ion chromatogram using their respective accurate masses.

Lyso PCs could yield characteristic fragments and neutral losses during MS/MS experiments, which are helpful for structural elucidation of these components. The major characteristic fragments from collision-induced dissociation in positive mode are the product ions at *m/z* 184.0733 and 104.1070 for Lyso PCs. The ion at *m/z* 184 is an important indicator for the presence of phosphatidylcholine (Welti & Wang, 2004). In the present study, the structures of Lyso PCs were characterized by their retention time, accurate mass measurement, and MS/MS fragmentation patterns. Since the corresponding standards of all endogenous Lyso PCs were not available, no standards were used in this study. Meanwhile, the numbers of double bonds and hydroxyl groups were determined by their MS data, but

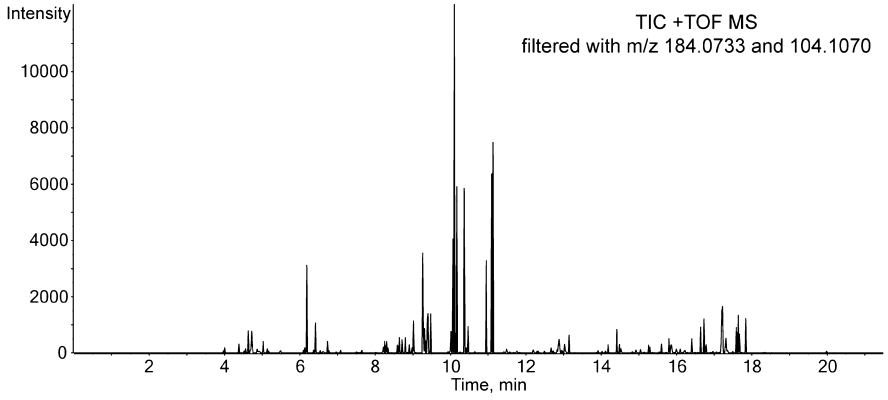

Fig. 2 – Total ion chromatogram +TOF MS profile filtered with *m/z* 184.0733 and 104.1070 of Lyso PCs.

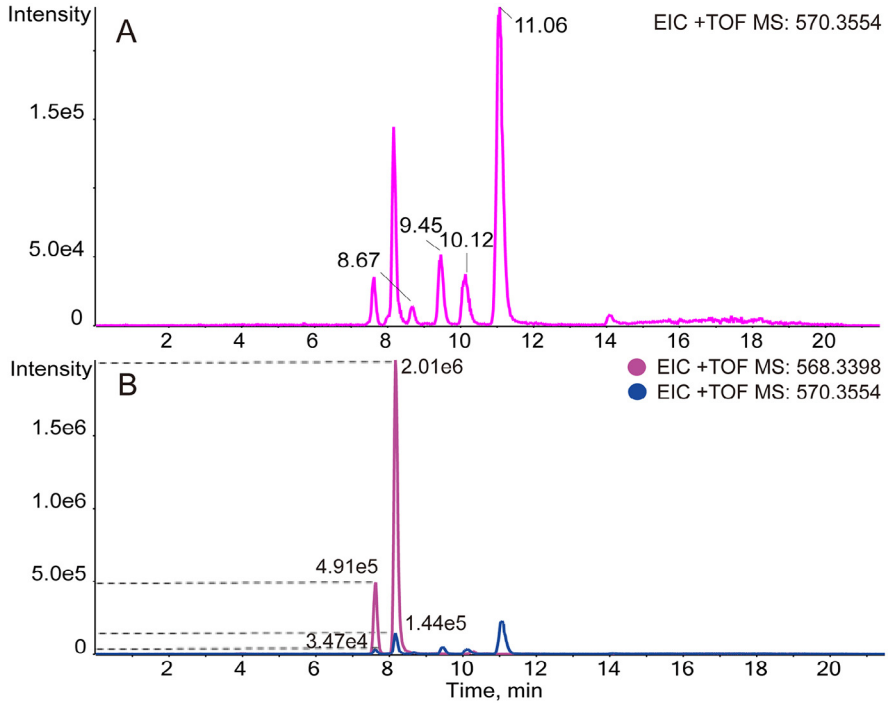

Fig. 3 – Extracted ion chromatogram of TOF-MS at *m/z* 570.3554 (A) and at *m/z* 570.3554 and 568.3398 (B).

their positions could not be determined by LC-MS/MS approach.

As shown in Table 1, 74 Lyso PCs were simultaneously characterized in the extract of serum. A data mining strategy with a precursor ion filter setting at *m/z* 184.0733 and 104.1070 was used to screen and identify more precursor ions of Lyso PC components. However, the various types of structures of fatty acids lead to an extremely complicated Lyso PC species. Isotopic peaks of some Lyso PCs are easily confused with many other low abundant ones. Therefore, it is necessary to exclude false-positive identities by exhaustive manual check of the MS data. For example, six peaks were observed in the extracted ion chromatogram of *m/z* 570.3554 (Fig. 3A). However, two of them were isotopic peaks of *m/z* 568.3398 by the evidence of the peak intensity ratios of the isotopic peaks of the particular molecular formula ($2.01e^6/1.44e^5$ and $4.91e^6/3.47e^4$) and the same retention time depicted in Fig. 3B. The 74 Lyso PCs characterized in this study were checked one by one in order to exclude false-positive identities using the above method.

The discrimination between regioisomers of lyso PC (*sn*-1 or *sn*-2 lyso PCs) is difficult because of their closely related structures. However, cleavage of the monoacyl glycerol part and the polar headgroup part was favoured for *sn*-1 lyso PC regioisomers, resulting in predominant product ion at *m/z* 184 with more than 80% relative intensity. In contrast, the intensity of product ion at *m/z* 184 from sn-2 lyso PC regioisomers was relatively low (Yu, Peng, Ronis, Badger, & Fang, 2010). In addition, sn-2 Lysophospholipids are retained longer on the reversed-phase sorbent compared with their corresponding sn-1 isomers (Fang, Yu, & Badger, 2003). This method was successfully applied to distinguish *sn*-1 and *sn*-2 lyso PCs regioisomers in previous reports and also applied to the present study. A representative example of four regioisomers of lyso PC with the same $[M+H]^+$ at *m/z* 570.35 was shown in Fig. 4. The *sn*-1 Lyso PCs of compound 44 and 50 showed a relative higher peak intensity ratio of product ions at *m/z* 184 compared to that of *sn*-2 Lyso PCs compound 49 and 56. In addition, a product ion at *m/z* 552.35 was more easily obtained by elimination of a H_2O from the protonated molecular ion $[M+H]^+$ at *m/z* 570.35 for the *sn*-2 Lyso PCs. Therefore, the discrimination between regioisomers of *sn*-1 and *sn*-2 lyso PCs was characterized by the consistent MS/MS fragmentation patterns described above.

3.3. *The quantitative analysis of Lyso PCs by LC-QTRAP-MS/MS*

For targeted quantitative analysis of Lyso PCs in mice serum, a LC-QTRAP-MS/MS method using MRM mode was selected due to its high sensitivity and selectivity. The normalization of Lyso PCs peak areas based on IS peak areas was applied for LC-MS/MS analyses. The TIC of 25 MRM transitions for 59 targeted Lyso PCs was shown in Fig. 5. Each peak was selected on the basis of a reasonable retention time with high repeatability.

Lyso PCs represent 5–20% of total serum phospholipids depending on the mammalian species (Croset et al., 2000). They are one of the major classes of lipid components in serum. In addition, they are recognized as an important factor underlying signal transduction and play critical signalling roles in various diseases, including atherosclerosis, diabetes, and cancer mediated by Lyso PC specific G-protein-coupled receptors (Asaoka et al., 1992; Liebisch et al., 2002; Liebisch & Schmitz, 2009). Furthermore, the relative concentration between

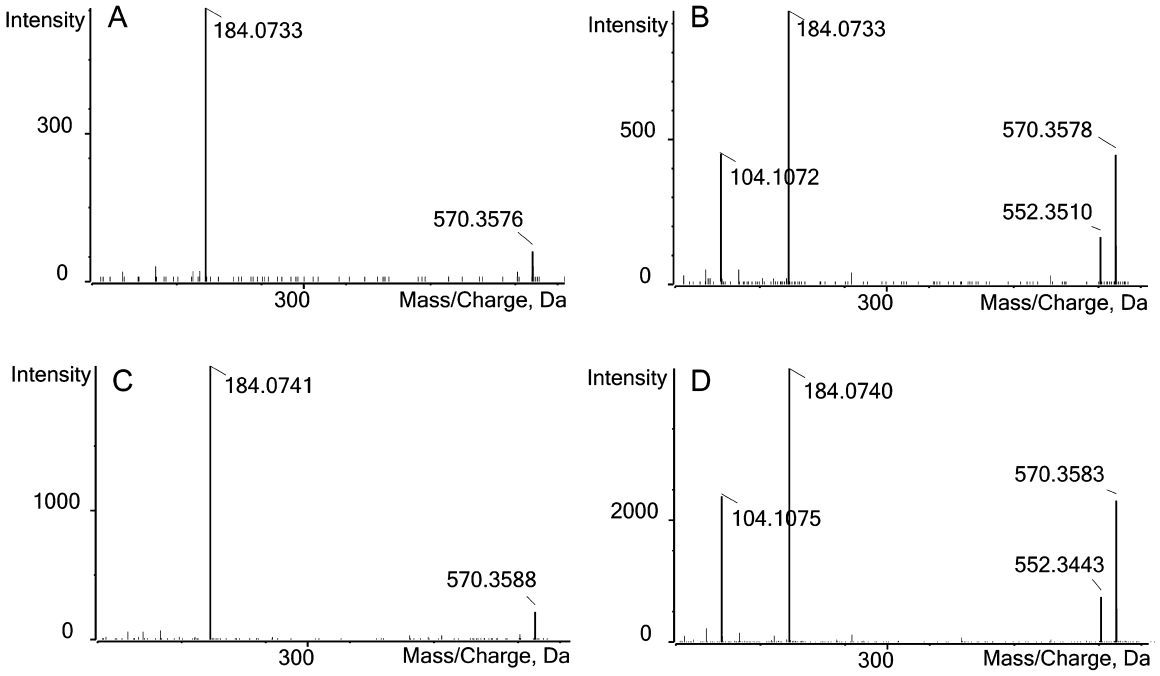

Fig. 4 – Product ion spectra of compound 44 (A), 49 (B), 50 (C) and 56 (D) at *m/z* 570.3576, 570.3578, 570.3588 and 570.3583, respectively.

regioisomers of Lyso PC (*sn*-1 or *sn*-2 subspecies) is also important because generation of these isomers would influence their removal from blood, their uptake and acylation, and/or their catabolism in tissues (Besterman & Domanico, 1992; Morash, Cook, & Spence, 1989). Turmeric was reported to exhibit a hypolipidaemic effect in mice (Besterman & Domanico, 1992; Morash et al., 1989). Therefore, these findings emphasized the need to analyze individual Lyso PC components in serum for evaluating the hypolipidaemic effect of turmeric consumption in hyperlipidaemic mice.

The quantitative changes of 59 targeted Lyso PCs in mice serum were shown in Table 3 and Fig. 6. However, the Lyso PCs of 4, 5, 9–15, 17, 22, 29, 35, 46 and 55 could not be quantified due to the extremely low-abundance of these compounds. The results showed that 26 serum Lyso PCs in mice fed with HFD were down-regulated when compared with that of mice in the control group (the radio ≥ 1.2). In particular, the levels of nine of these Lyso PCs in normal mice were ≥1.5 times higher than those of mice fed with HFD. In contrast, only 13 Lyso PCs were up-regulated in mice fed with HFD compared with those of normal mice (the radio ≤ 0.8). The data also indicated that major serum components of Lyso PCs are compounds 42, 43, 47, 51, 60 and 71. However, the significant changes of individual Lyso PCs including many low-abundance ones, such as compounds 1, 6, 16, 44, 49, 50, 69, 74 and 21, in mice fed with HFD showed significant changes. This suggested that the trace amount ones may also play a crucial role in hypolipidaemic effect and may serve as potential biomarkers.

In the present study, the effects of consumption of turmeric on individual serum Lyso PCs were investigated. A variety of serum Lyso PCs dysregulated in hyperlipidaemic mice were changed towards normal after turmeric consumption for 4 weeks. However, the levels of most Lyso PCs in mice treated with simvastatin did not show significant changes compared with those of hyperlipidaemic mice. As depicted in Fig. 6, the levels of compounds 44 (lyso/22:5), 49 (22:5/lyso), 19 (lyso/20:5) and 27 (20:5/lyso) in mice fed with 2% turmeric were at least doubled when compared with those in hyperlipidaemic mice. The levels of compounds 64 (22:4/lyso), 37 (lyso/22:6), 41 (22:6/lyso), 25 (lyso/22:7), 32 (22:7/lyso), 6 (lyso/20:4-OH), 1 (lyso/16:0-OH) were 1.5–2.0 times higher compared with those of hyperlipidaemic mice. The results indicated that the significant changes more likely occurred on the Lyso PCs of which the fatty acid chain possessed higher degree of unsaturation. Fatty acids are usually derived from triglycerides or phospholipids including Lyso PCs. It is commonly realized that polyunsaturated fatty acids, such as EPA (20:5, n-3) and DHA (22:6, n-3), are essential nutrients for health. A previous study reported that high consumption of fish carries a lower risk of cardiovascular disease as a consequence of consumption of high dietary omega-3 long chain polyunsaturated fatty acid content (Lluis et al., 2013). Therefore, the decreased serum polyunsaturated Lyso PCs in mice fed with HFD in this study may be relevant to the pathogenesis of hyperlipidaemia. These polyunsaturated Lyso PCs showing significant changes may be used as potential biomarkers of hyperlipidaemia, which need further confirmation in future study. In addition, most polyunsaturated Lyso PCs, dramatically decreased in hyperlipidaemic mice, were elevated towards normal after turmeric consumption for 4 weeks, providing scientific evidence in explanation of the preventive effect of turmeric on HFD-induced hyperlipidaemia.

Table 3 – The quantitative changes of 59 targeted Lyso PCs in the serum from hyperlipidaemic mice fed with turmeric for 4 weeks.

Q-Trap MRM (Ion pairs)	No.[a]	RT (min)	Peak area[b]					$A_{(C)}/A_{(H)}$	$A_{(P)}/A_{(H)}$	$A_{(T_L)}/A_{(H)}$	$A_{(T_H)}/A_{(H)}$
			C[c]	H[c]	P[c]	T_L[c]	T_H[c]				
572.3/184.0	59	11.10	0.47 ± 0.035	0.33 ± 0.030	0.29 ± 0.0026	0.43 ± 0.051	0.44 ± 0.030	1.45	0.89	1.33	1.34
	64	12.25	0.88 ± 0.04	0.62 ± 0.025	0.68 ± 0.025	0.85 ± 0.025	1.01 ± 0.035	1.41	1.09	1.36	1.63
570.3/184.0	44	8.15	0.66 ± 0.012	0.35 ± 0.076	0.35 ± 0.081	0.54 ± 0.091	0.87 ± 0.076	1.86	0.98	1.52	2.45
	49	8.83	1.04 ± 0.07	0.66 ± 0.11	0.70 ± 0.091	0.79 ± 0.082	1.51 ± 0.11	1.58	1.06	1.20	2.30
	50	9.49	3.47 ± 0.23	2.15 ± 0.39	2.02 ± 0.15	1.77 ± 0.21	2.58 ± 0.38	1.62	0.94	0.83	1.20
	56	10.32	6.20 ± 0.63	4.55 ± 0.77	4.05 ± 0.017	3.32 ± 0.20	5.70 ± 0.71	1.36	0.89	0.73	1.25
568.3/184.0	37	7.23	16.57 ± 0.41	11.25 ± 0.59	11.77 ± 0.67	15.67 ± 0.85	18.85 ± 1.78	1.47	1.05	1.39	1.68
	41	7.72	35.54 ± 2.77	24.26 ± 2.82	27.70 ± 0.75	32.47 ± 1.93	45.88 ± 4.18	1.46	1.14	1.34	1.89
566.3/184.0	25	6.13	0.08 ± 0.02	0.061 ± 0.020	0.052 ± 0.016	0.076 ± 0.024	0.10 ± 0.0059	1.33	0.85	1.24	1.67
	32	6.45	0.21 ± 0.012	0.17 ± 0.018	0.17 ± 0.0098	0.18 ± 0.010	0.28 ± 0.015	1.30	0.99	1.07	1.71
560.3/184.0	6	4.13	0.43 ± 0.032	0.28 ± 0.032	0.33 ± 0.0038	0.33 ± 0.032	0.52 ± 0.021	1.53	1.15	1.18	1.82
	8	4.64	0.86 ± 0.042	0.66 ± 0.036	0.69 ± 0.036	0.68 ± 0.036	0.93 ± 0.036	1.31	1.05	1.03	1.41
550.3/184.0	70	15.57	0.96 ± 0.050	0.86 ± 0.050	0.81 ± 0.050	0.94 ± 0.05	0.74 ± 0.050	1.12	0.94	1.09	0.86
	72	16.12	6.06 ± 0.12	6.44 ± 0.12	5.90 ± 0.13	6.19 ± 0.13	5.40 ± 0.11	0.94	0.92	0.96	0.84
548.3/184.0	62	11.85	0.38 ± 0.0057	0.55 ± 0.10	0.46 ± 0.090	0.63 ± 0.17	0.59 ± 0.13	0.69	0.84	1.16	1.09
	66	13.31	1.52 ± 0.068	2.29 ± 0.49	1.91 ± 0.054	2.43 ± 0.48	2.29 ± 0.19	0.67	0.83	1.06	1.00
546.3/184.0	48	8.70	4.62 ± 0.69	5.85 ± 0.64	5.23 ± 0.34	9.29 ± 1.73	7.73 ± 1.28	0.79	0.89	1.59	1.32
	52	9.51	11.50 ± 0.93	17.09 ± 3.63	15.61 ± 0.80	22.93 ± 2.68	24.06 ± 1.96	0.67	0.91	1.34	1.41
544.3/184.0	38	7.28	36.23 ± 0.90	32.45 ± 4.68	28.57 ± 1.30	37.62 ± 5.03	42.71 ± 4.58	1.12	0.88	1.16	1.32
	43	7.83	102.84 ± 7.40	98.38 ± 18.13	86.46 ± 3.16	103.50 ± 9.97	134.48 ± 15.23	1.05	0.88	1.05	1.37
542.3/184.0	7	4.64	0.56 ± 0.036	0.40 ± 0.030	0.51 ± 0.036	0.44 ± 0.036	0.54 ± 0.036	1.41	1.29	1.11	1.36
	19	5.89	0.20 ± 0.030	0.19 ± 0.031	0.25 ± 0.030	0.30 ± 0.030	0.46 ± 0.030	1.05	1.32	1.59	2.44
	27	6.22	0.49 ± 0.025	0.55 ± 0.025	0.61 ± 0.030	0.85 ± 0.036	1.20 ± 0.041	0.89	1.10	1.54	2.17
540.3/184.0	53	9.86	0.03 ± 0.0030	0.05 ± 0.0038	0.058 ± 0.0031	0.047 ± 0.0013	0.066 ± 0.0074	0.59	1.15	0.93	1.31
	58	10.67	0.13 ± 0.017	0.22 ± 0.021	0.25 ± 0.024	0.24 ± 0.021	0.28 ± 0.024	0.60	1.10	1.09	1.23
538.3/184.0	73	16.57	1.55 ± 0.26	1.19 ± 0.33	0.93 ± 0.090	1.00 ± 0.15	1.00 ± 0.14	1.31	0.78	0.84	0.84
	74	17.03	6.68 ± 2.48	3.46 ± 1.57	2.93 ± 0.71	3.62 ± 0.95	3.17 ± 0.81	1.93	0.85	1.04	0.91
536.3/184.0	2	4.18	0.035 ± 0.0069	0.031 ± 0.0092	0.034 ± 0.011	0.028 ± 0.0083	0.025 ± 0.0043	1.16	1.12	0.91	0.82
	3	4.30	0.094 ± 0.012	0.093 ± 0.054	0.080 ± 0.013	0.077 ± 0.017	0.075 ± 0.022	1.02	0.86	0.83	0.80
	16	5.47	0.031 ± 0.0093	0.016 ± 0.0061	0.012 ± 0.0028	0.013 ± 0.0031	0.019 ± 0.0049	1.87	0.76	0.78	1.13
	67	13.74	0.07 ± 0.02	0.086 ± 0.0094	0.047 ± 0.0087	0.086 ± 0.014	0.070 ± 0.021	0.80	0.54	1.00	0.81
	68	14.45	0.64 ± 0.070	1.10 ± 0.20	0.84 ± 0.074	0.89 ± 0.21	0.99 ± 0.18	0.58	0.76	0.81	0.90
524.3/184.0	69	14.88	62.21 ± 6.08	37.16 ± 4.66	36.34 ± 6.076	38.04 ± 6.50	34.98 ± 5.92	1.67	0.98	1.02	0.94
	71	15.58	328.20 ± 86.19	209.30 ± 53.95	185.18 ± 26.14	224.78 ± 52.68	199.40 ± 30.74	1.57	0.88	1.07	0.95
522.3/184.0	54	9.98	35.20 ± 1.90	43.97 ± 3.76	40.46 ± 4.69	44.46 ± 6.24	46.94 ± 4.51	0.80	0.92	1.01	1.07
	60	11.10	147.60 ± 8.98	198.90 ± 38.28	172.50 ± 9.78	182.50 ± 24.87	206.97 ± 18.21	0.74	0.87	0.92	1.04
520.3/184.0	36	7.18	51.97 ± 3.06	54.29 ± 11.51	51.14 ± 2.37	64.92 ± 16.85	67.03 ± 9.35	0.96	0.94	1.20	1.23
	42	7.78	184.90 ± 12.92	198.10 ± 32.04	185.70 ± 6.06	207.20 ± 19.98	228.88 ± 31.23	0.93	0.94	1.05	1.16
518.3/184.0	23	6.01	1.75 ± 0.12	1.59 ± 0.16	1.07 ± 0.079	1.63 ± 0.24	1.75 ± 0.22	1.10	0.68	1.03	1.11
	31	6.40	4.10 ± 0.23	4.53 ± 0.75	3.13 ± 0.26	3.60 ± 0.35	5.07 ± 0.21	0.90	0.69	0.79	1.12
512.3/184.0	1	4.04	0.18 ± 0.026	0.11 ± 0.0026	0.17 ± 0.032	0.18 ± 0.016	0.21 ± 0.032	1.60	1.54	1.64	1.90
	24	6.07	0.07 ± 0.0051	0.083 ± 0.0067	0.10 ± 0.023	0.11 ± 0.025	0.11 ± 0.031	0.90	1.23	1.35	1.37
	30	6.38	0.25 ± 0.021	0.30 ± 0.023	0.34 ± 0.026	0.39 ± 0.032	0.38 ± 0.032	0.81	1.12	1.30	1.26
510.3/184.0	57	10.71	0.33 ± 0.038	0.23 ± 0.0049	0.23 ± 0.045	0.20 ± 0.032	0.30 ± 0.056	1.39	0.99	0.87	1.27
	61	11.48	1.44 ± 0.026	1.66 ± 0.10	1.38 ± 0.11	1.42 ± 0.18	1.88 ± 0.22	0.87	0.83	0.85	1.13
	63	12.12	1.72 ± 0.21	1.61 ± 0.30	1.26 ± 0.19	1.22 ± 0.10	1.53 ± 0.048	1.07	0.78	0.76	0.95
	65	13.06	6.18 ± 1.25	5.08 ± 0.95	4.93 ± 0.17	5.73 ± 1.07	5.43 ± 0.54	1.22	0.97	1.13	1.07
508.3/184.0	40	7.66	0.25 ± 0.0035	0.36 ± 0.05	0.27 ± 0.031	0.32 ± 0.073	0.39 ± 0.043	0.70	0.75	0.88	1.08
	45	8.37	1.32 ± 0.15	1.73 ± 0.24	1.29 ± 0.091	1.43 ± 0.16	1.80 ± 0.19	0.76	0.75	0.83	1.04
506.3/184.0	21	5.97	0.0046 ± 0.0010	0.015 ± 0.002	0.016 ± 0.0025	0.027 ± 0.0038	0.017 ± 0.0027	0.30	1.09	1.80	1.10
	28	6.36	0.054 ± 0.0075	0.064 ± 0.0065	0.035 ± 0.0057	0.054 ± 0.0067	0.054 ± 0.0074	0.84	0.54	0.84	0.85
496.3/184.0	47	8.53	139.10 ± 8.43	111.70 ± 13.91	106.84 ± 16.29	103.56 ± 13.72	107.57 ± 8.40	1.25	0.96	0.93	0.96
	51	9.52	554.90 ± 81.8	493.00 ± 102.63	449.90 ± 61.28	470.30 ± 102.57	461.69 ± 85.22	1.13	0.91	0.95	0.94
494.3/184.0	26	6.17	4.60 ± 0.17	3.51 ± 0.65	2.18 ± 0.15	3.22 ± 0.52	3.04 ± 0.47	1.31	0.62	0.92	0.87
	33	6.64	23.49 ± 1.47	18.39 ± 4.06	12.32 ± 0.76	14.14 ± 1.66	16.27 ± 2.42	1.28	0.67	0.77	0.88
482.3/184.0	34	6.69	0.52 ± 0.079	0.55 ± 0.0053	0.45 ± 0.14	0.47 ± 0.10	0.57 ± 0.040	0.95	0.82	0.86	1.04
	39	7.31	2.53 ± 0.23	2.75 ± 0.46	2.22 ± 0.083	2.23 ± 0.15	2.71 ± 0.17	0.92	0.81	0.81	0.99
468.3/184.0	18	5.53	0.38 ± 0.034	0.31 ± 0.0010	0.22 ± 0.025	0.30 ± 0.037	0.26 ± 0.05	1.25	0.73	0.98	0.85
	20	5.92	2.55 ± 0.23	2.02 ± 0.35	1.26 ± 0.017	1.63 ± 0.019	1.39 ± 0.17	1.26	0.62	0.81	0.69

[a] The Lyso PCs of 4, 5, 9–15, 17, 22, 29, 35, 46 and 55 were not quantitative due to their extremely low-abundance of these compounds.
[b] The values of peak area (A), normalized by IS, are mean ± SD (n = 3).
[c] C, normal control group; H, hyperlipidaemic group; P, positive group; T_L, turmeric low dose group; T_H, turmeric high dose group.

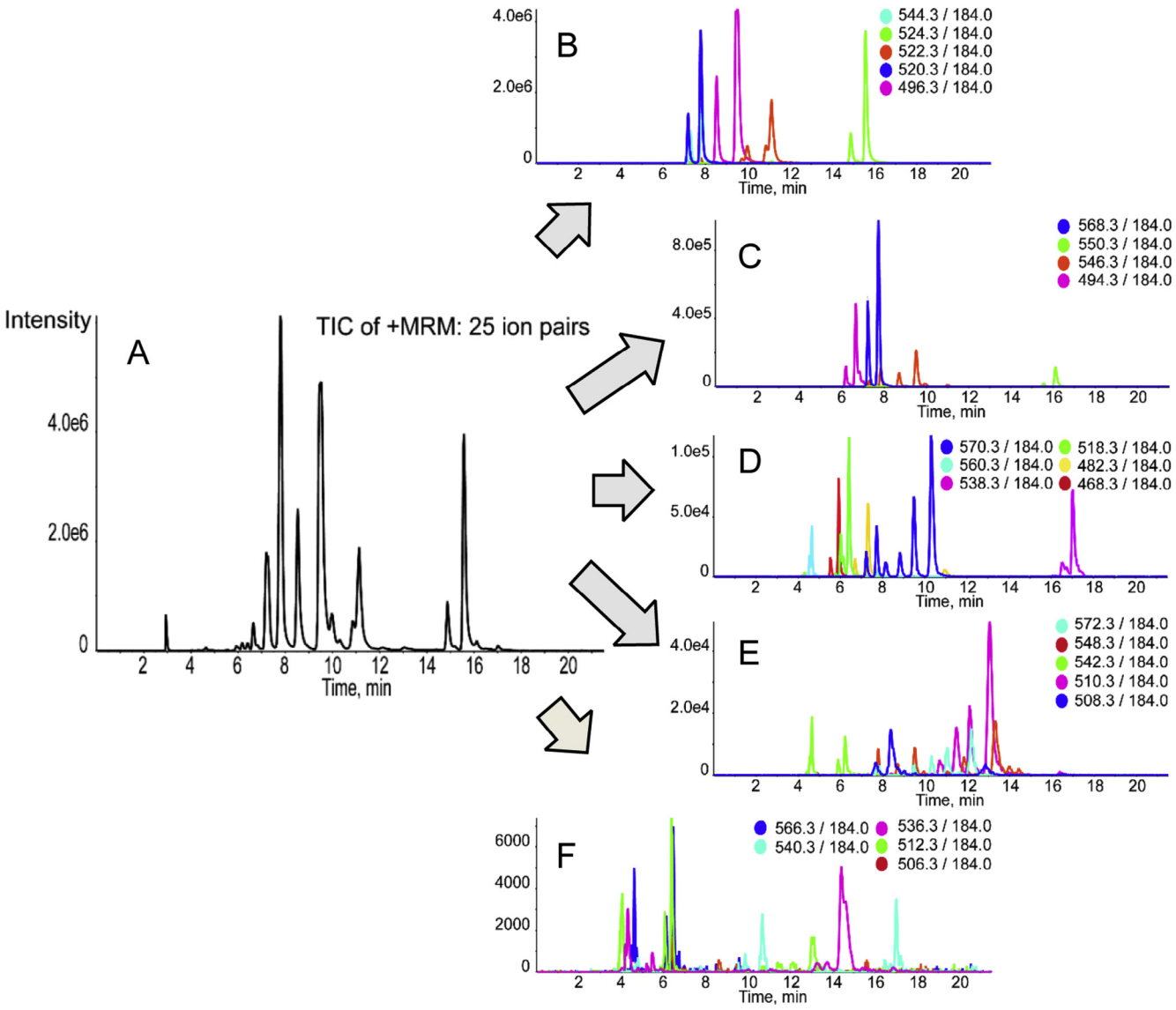

Fig. 5 – Total ion chromatogram (A) and extracted ion chromatogram (B-F) + MRM of 25 transitions for 59 targeted Lyso PCs by Q-TRAP LC-MS/MS.

4. Conclusions

In summary, a targeted metabolomic approach for the analysis of Lyso PCs in mice serum was established by using two LC-MS/MS platforms. This approach was demonstrated to be well suited for the rapid characterization of lyso PCs from complex matrixes. Seventy-four Lyso PCs were characterized and the quantitative changes of 59 targeted Lyso PCs in the serum from hyperlipidaemic mice fed with turmeric were determined. From a lipidomic perspective, this method is expected to be useful for the qualitative and relative quantitative analysis of high-abundance as well as low-abundance Lyso PCs in biological samples. This targeted approach provided valuable insight into the roles of Lyso PCs in the pathogenesis of hyperlipidaemia. However, the potential biomarkers of hyperlipidaemia found in this study will need further validation in future studies before they can be used for diagnosis or therapeutic intervention of hyperlipidaemia.

Conflict of interest

The authors declare no conflicts of interest.

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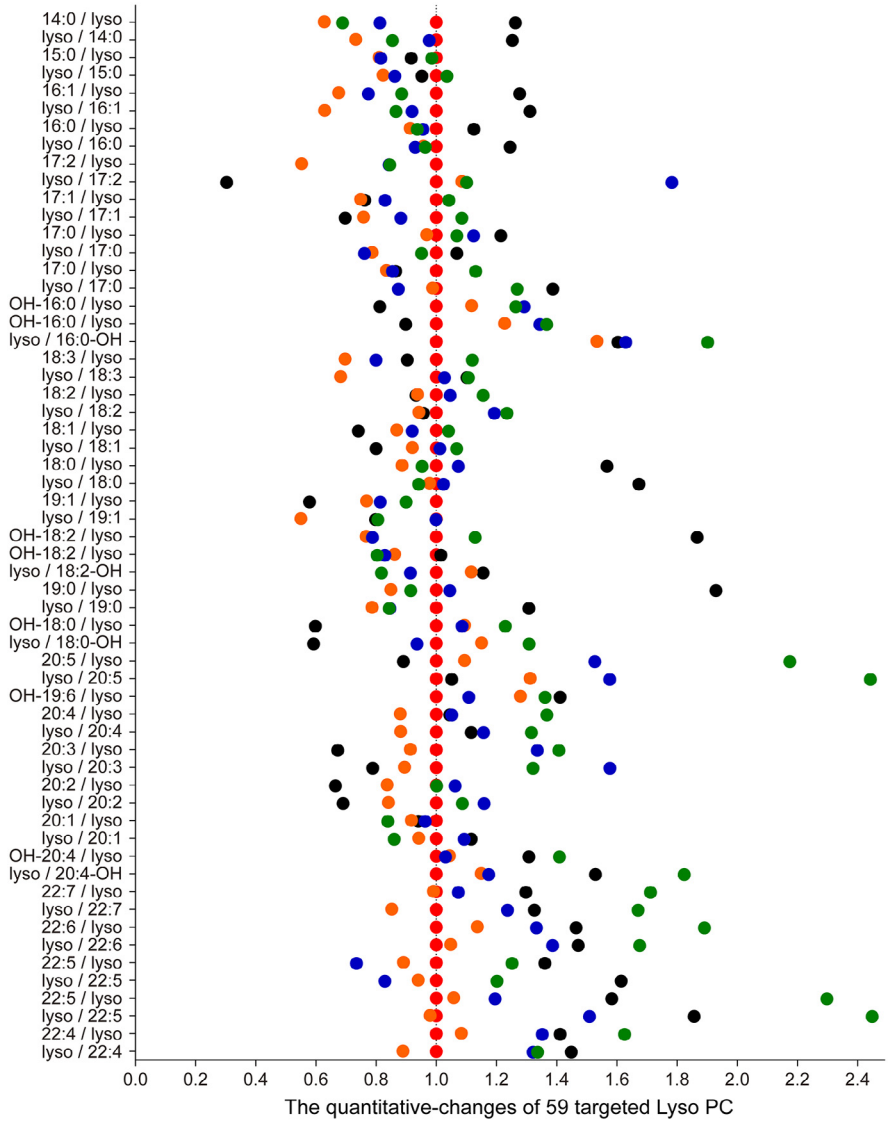

Fig. 6 – The quantitative changes of 59 targeted Lyso PCs in mice serum. Red dot, H group; black dot, C group/H group; orange dot, P group/H group; blue dot, T_L group/H group; green dot, T_H group/H group.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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