Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

A simple and rapid extraction of lipids in plasma using spin column with superabsorbent polymer beads for mass spectrometry

Jung Hoon Choi[1†], Geul Bang[2†], Jeong Ah Kim[3,4] and Young Hwan Kim[1,5*]

Abstract

The efficient extraction of lipid species in plasma was developed for quick sample preparation and accurate lipid analysis, using spin column with superabsorbent polymer beads (SAP), as the improvement of previous SAP method reported before. The modified SAP (mSAP) method is composed of simple steps, including sample loading, swelling, solvent addition, centrifugation, and drying of the resulting lipid solution. The mSAP method offers several advantages over the conventional and the previous SAP methods for lipid analysis in plasma sample. The mSAP method was approximately 10 times faster than the modified Folch protocol (Matyash method), methyl-*tert*-butyl ether based extraction method. The mSAP method offered an excellent recovery rate for most all major classes in the lipid standard mixture, outperforming the Matyash method. The limit of detection (LOD) of 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine (PC 17:0/17:0) spiking in plasma by the mSAP method is about seven times lower than those of the conventional methods. The relative standard deviation (RSD) values for inter- and intra-day variability of the mSAP method over a 5-day period were significantly lower than those of the previous SAP and Matyash methods. The mSAP method has been shown to be more time-saving, sensitive, reproducible and reliable, as evidence by its faster processing time, lower LOD, lower RSD values and high recovery rate for different lipid classes. Overall, these findings suggest that this method may be a promising approach for the application of total lipid analysis in lipidomic research in future.

Keywords Lipid extraction, Superabsorbent polymers, Spin column, Liquid chromatography, Mass spectrometry

Introduction

Most biofluids contain a wide range of biological components, including lipids, metabolites and proteins, which can provide valuable information for diagnosis and research (Siebes and Ventikos 2010). For example, lipids play a critical role in energy storage, cell signaling as second messengers, and major constituents of the cell plasma membrane (Welte and Gould 2017; Wu et al. 2016; Lingwood and Simons 2010). Therefore, dysregulation of lipid metabolism has been associated with a variety of diseases, including diabetes, cardiovascular disease, and cancer (McGarry 2002; Deprince et al. 2020; Bian et al. 2021; Kim et al. 2013). Analysis of lipids in

†Jung Hoon Choi and Geul Bang these authors contributed equally to this work.

*Correspondence:
Young Hwan Kim
yhkim@kbsi.re.kr
[1] Bio-Chemical Analysis Team, Korea Basic Science Institute, Chungbuk 28119, Republic of Korea
[2] Research Center for Bioconvergence Analysis, Korea Basic Science Institute, Chungbuk 28119, Republic of Korea
[3] Center for Scientific Instrumentation, Korea Basic Science Institute, Chungbuk 28119, Republic of Korea
[4] Department of Bio-Analytical Science, University of Science and Technology, Daejeon 34113, Republic of Korea
[5] Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 34134, Republic of Korea

biofluids such as plasma, serum, and cerebrospinal fluid can provide valuable insights into disease pathogenesis and potential biomarkers (Burla et al. 2018; Lewinska et al. 2021; Seyer et al. 2016). Especially, plasma is one of the most widely used biofluids in the development and validation of analytical methods for lipidomic studies. Plasma is relatively easy to collect and generally stable after collection. Also, plasma contains a wide range of lipid classes, including triglycerides, phospholipids, and cholesterol esters, making it an ideal sample for comprehensive lipidomic analysis (Quehenberger and Dennis 2011). Recently, a liquid chromatography-mass spectrometry (LC–MS) has become a widely used technique for the identification and quantification of lipid species in plasma, because LC–MS can provide detailed information on the chemical structures of lipids, detect a wide range of lipid classes and be used in high throughput lipid analysis (Cajka and Fiehn 2014; Bang et al. 2014). Thus, the development of efficient and reliable methods for the extraction of plasma lipids suitable to LC–MS analysis has important implications for disease diagnosis, disease pathogenesis and new therapeutic targets.

Two of most commonly used methods for extracting and purifying lipids in animal tissue are the Folch and Bligh-Dyer protocols, which use the chloroform/methanol-based liquid–liquid extraction (LLE) (Folch et al. 1957; Bligh and Dyer 1959). Both of these methods have also been shown to be effective for the extraction and purification of lipids from plasma and biological samples (Ulmer et al. 2018; Limb et al. 1999). However, they do have some drawbacks, such as being time-consuming and requiring large volumes of solvents. In addition, there are concerns regarding the use of chloroform, which is a toxic and potentially carcinogenic solvent. Chloroform also forms a lipid-rich lower layer in the two-phase partitioning system, which can lead to contamination of the extract and loss of lipids during the extraction process. Alternative lipid extraction by methyl-*tert*-butyl ether (MTBE)/methanol-based LLE protocol, called as Matyash method (Matyash et al. 2008), and butanol/methanol-based one-phase extraction protocol, called as BUME method (Löfgren et al. 2012), have been developed to address these disadvantages mentioned above (Ulmer et al. 2018; Sostare et al. 2018). Matyash and BUME methods greatly simplifies sample handling due to the formation of lipid-containing upper phase and enables automated processing of minute amounts of plasma and other biological samples (Matyash et al. 2008; Ulmer et al. 2018; Löfgren et al. 2016). Recently, a solid-phase extraction (SPE) is a commonly used sample preparation technique in target lipidomics due to its ability to selectively isolate and concentrate specific lipid classes based on their chemical properties, such as polarity and hydrophobicity (Saini et al. 2021). Additionally, SPE allows for the removal of interfering compounds and matrix effects and do not require solvent/water partitions, resulting in improved sensitivity and accuracy of lipid analysis (Aldana et al. 2020). However, to overcome the limited use of the previous SPE methods for the isolation and purification of selected lipid class, we recently reported a new approach to lipid extraction for untargeted lipidomics using superabsorbent polymer powders (SAP) (Bang et al. 2017). The method simplified remarkably lipid extraction process and improved the extraction efficiency and reduced the loss of lipid extracts in various samples, including plasma, mouse brain and liver, and *E. coli.*

The previous SAP method was found to cause the fluctuation of analytical results throughout the experimental process. In this study, a modified SAP method (mSAP), which uses a spin column filled with SAP beads instead of powder was developed to improve further efficiency and convenience of lipid extraction. The efficiency of the mSAP method was compared to the Matyash and previous SAP methods by measuring the recovery rates, the limit of detection (LOD) and precisions of standard lipids spiking in plasma. In comparison, mSAP method was shown to be the more time-saving, sensitive, reproducible, and reliable than the Matyash and the SAP methods for lipid extraction. Overall, the mSAP method demonstrated superior performance in lipid extraction from plasma sample.

Materials and methods

The superabsorbent polymer beads (diameter 1 mm) and powder, which were made of cross-linked polyacrylates were purchased from LG Chem, Ltd. (Seoul, Republic of Korea). The particle size of SAP powder ranges from 100 to 300 μm, based on the SEM image obtained in the previous work (Bang et al. 2017). The lipid standard mixture such as 1,2-diheptadecanoyl-*sn*-glycero-3-phophocholine (PC 17:0/17:0), 1,2-diheptadecanoyl-*sn*-glycero-3-phosphoglycerol (PG 17:0/17:0), 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine(LPC17:0),1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine (PC 19:0/19:0), 1,2-diheptadecanoyl-*sn*-glycero-3-phosphoethanolamine (PE 17:0/17:0), N-heptadecanoyl-D-erythro-sphingosine (Cer d18:1/17:0), N-lauroyl-D-erythro-sphingosylphosphorylcholine (SM d18:1/12:0) and 1,2,3-tripentadecanoylglycerol (TG 15:0/15:0/15:0) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA), and human plasma from Sigma-Aldrich (St. Louis, USA). The same lipid standard mixture was used for recovery tests of Matyash and mSAP extraction methods in both positive-ion and negative-ion modes of LC–MS. Especially, PC (17:0/17:0) and PG (17:0/17:0) were used as standards

for evaluating the sensitivity and precision of three lipid extraction methods in the positive-ion and negative-ion modes, respectively. Each lipid standard and human plasma were dissolved in organic mixture of methanol (MeOH) and chloroform (1:1 v/v) and in MS-grade deionized water (DW), respectively.

Lipid extraction using spin column filled with SAP beads

For SAP bead-based lipid extraction from plasma, 12–15 SAP beads were placed into a upper reservoir with 0.2 μm polytetrafluoroethylene (PTFE) membrane of a spin column from Thermo Fisher Scientific (Rockford, IL, USA). Then, the 10–50 μl of the plasma sample were dropped onto SAP beads and left for 1 min to complete the swelling of beads for absorbing the aqueous solution. 200 μl of the organic mixture (2:1 v/v) of methyl-*tert*-butyl ether (MTBE) and MeOH were loaded onto the swelled SAP. After 1 min, the sample in the reservoir was spun down by centrifugation at 6000 rpm for 1 min, and the lipid extracts contained in a collection tube were dried with gentle N_2 gas. Then, they were redissolved in 200 μl mixture of 2-propanol (IPA), acetonitrile (ACN), and DW (65:30:5 v/v/v) and analyzed using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS).

Lipid extraction using SAP powder

Lipid extraction with SAP powder was conducted as described previously (Bang et al. 2017). Briefly, 2–15 mg of SAP powder was placed into a microcentrifuge tube, and then 10–50 μl of the plasma samples were dropped onto SAP powder and left for 30 s to complete the gelation. 600 μl of the organic solvent, such as a mixture of MTBE/MeOH (2:1 v/v) was loaded onto the gel and the samples were incubated for 2–3 min. The organic phase containing the solubilized lipids was collected and dried with nitrogen gas and resulting lipid extracts were stored at 4 °C. Prior to UPLC-MS analysis, the dried lipid extracts were resuspended in 200 μl of IPA/ACN/DW (65:30:5 v/v/v).

Lipid extraction by Matyash method

For comparison with mSAP method, the lipids from the plasma sample were also extracted by using the Matyash method, which is a modification of the Folch method, as described in the previous literature (Matyash et al. 2008). The 200 μl of MeOH and 600 μl of MTBE were added into 10–50 μl plasma sample. After mixing for 1 h, 180 μl DW was added for phase separation followed by centrifugation at 13,000 rpm for 10 min. The upper organic phase, MTBE solution containing the lipid extracts, was collected and dried with nitrogen gas. The dried lipid extracts were stored at 4 °C and then resuspended in 200 μl of IPA/ACN/DW (65:30:5 v/v/v) for UPLC-MS analysis.

Profiling and quantification of lipids by UPLC-MS analysis

For lipid analysis of plasma, high-resolution mass spectrometric analysis was performed using the AQUITY UPLC™ system coupled to a Synapt G2 HD mass spectrometer (Waters, Manchester, U.K.) with MassLynx 4.1 software. For UPLC, a CSH C18 shield column (1.8 μm, 2.1 mm × 100 mm) from Waters (Milford, MA, USA) was utilized at a flow rate of 0.4 mL/min, with an injection of 5 μl of the plasma lipid samples extracted by the different methods. The mobile phase A and B consisted of ACN/H_2O (60:40, v/v) with 10 mM ammonium formate and IPA/ACN (90:10, v/v) with 0.1% formic acid, respectively. The gradient of mobile phase B was as follows: 0 min, 40%; 2 min, 43%; 2.1 min, 50%; 12 min 54%; 12.1 min, 70%; 18 min, 98%; 18.1 min, 40% and 20 min, 40% (Bang et al. 2017). The total run time for chromatographic separation was 20 min. The extracts were analyzed in positive-ion and negative-ion modes of electrospray ionization (ESI) source within the acquisition mass range of *m/z* 50 to 1200. The other MS parameters were optimized with a capillary voltage of 2.5 kV, cone voltage of 40 V, source temperature of 140 °C, and desolvation gas flow rate of 500 L/h. For lipid profiling, the lipid samples were analyzed in high-resolution mode (R = 35,000), and then the lipid species were identified with the accuracy of 10 ppm mass tolerance using an in-house iLipid searching software (Ver. 1.2) developed at the Korea Basic Science Institute (KBSI). In order to compare the sensitivity and precision of the three different extraction methods, the extracted ion chromatogram (XIC) peak area of each lipid standard spiked in plasma was measured using MS analysis, and the ratio of the peak area obtained from each method was compared.

Results and discussion

Strategy for lipid extraction using modified SAP method

The workflow of total lipid extraction with superabsorbent polymer (SAP) beads is shown in Fig. 1a. The plasma samples were applied onto SAP beads in the spin column with PTFE membrane for lipid extraction. Instead of liquid–liquid extraction (LLE) by the hydrophobic and hydrophilic properties, SAP beads can rapidly absorbed aqueous solution with hydrophilic and ionic components in plasma sample and are converted into the swelling gel. The gel polymerization of SAPs was explained in detail before (Bang et al. 2017). The hydrophobic lipids captured into fibrous SAP gel were solubilized and eluted directly by the organic solvent, MTBE. Because the spin column with the SAP beads pass through quickly and easily lipid-containing

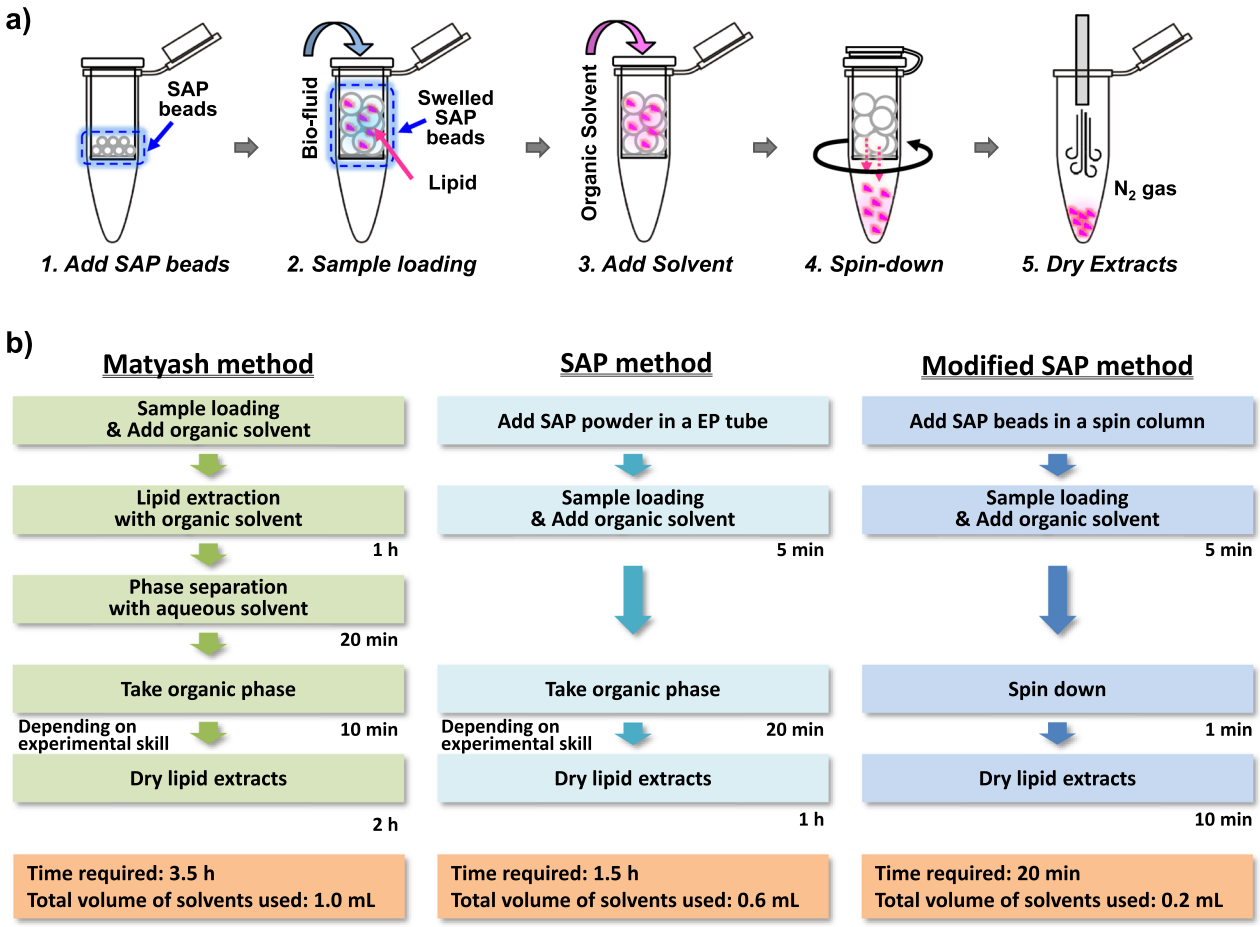


Fig. 1 **a** Schematic scheme of the steps involved in the modified SAP (mSAP) method for total lipid extraction using a spin column filled with SAP beads. **b** A chart showing the comparison of the procedures for Matyash, SAP, and the mSAP protocols

organic solution into the bottom of collection tube during the centrifugation, the mSAP method can minimize the liquid handling and sample loss while maximizing recovery yield without the non-reproducible withdrawing of lipid-containing organic phase in the LLE methods. The mSAP method is merely composed of sample loading, gel swelling, MTBE addition, pass-through by centrifugation, and drying of resulting lipid solution. A comparison of the extraction times and solvent volumes used by Matyash, SAP, and mSAP methods showed that the mSAP method was approximately 10 times faster than the Matyash method, which involves phase separation and dryness of lipid extract with a high solvent-to-sample ratio and takes more than 3 h. Thus, the mSAP method significantly reduces the extraction time for total lipid extraction by about 90%, as compared to the conventional LLE methods. This is because the mSAP method requires a smaller volume of solvent, and the lipid extraction process is carried out more efficiently due to the rapid absorption of the aqueous components of the plasma sample by the SAP beads, as shown in Fig. 1.

Lipid identification from plasma by using the three extraction methods

The comparison of the lipid species identified by using the mSAP, previous SAP, and Matyash methods involved extracting lipid species from 50 µl of human plasma using each method and identifying them through UPLC-MS analysis in both positive- and negative-ion modes. As shown in Fig. 2, the base peak ion chromatograms (BPIs) of the lipid extracts obtained from Matyash (Fig. 2a), SAP (Fig. 2b), and mSAP (Fig. 2c) methods were well compared in both positive-ion and negative-ion modes, and the results showed that lipid species of various classes were well separated within the runtime for chromatographic separation. The BPI chromatograms of lipids extracted by three different methods show a similar pattern for all the major lipid classes, including phosphatidylcholine (PC), phosphatidylethanolamine

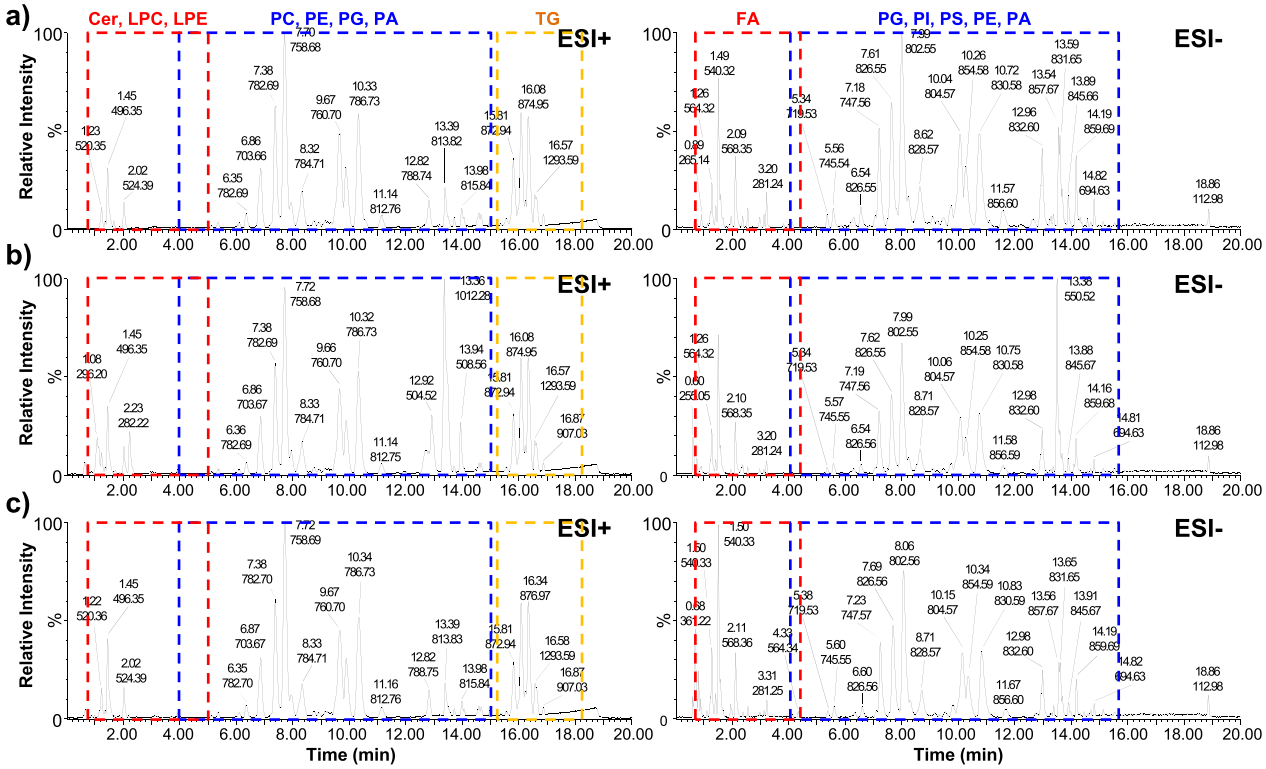


Fig. 2 Base peak ion chromatograms (BPIs) of the lipids extracted from 50 µl of plasma by **a** Matyash, **b** SAP, and **c** mSAP methods using UPLC-MS in the positive-ion (left) and negative-ion (right) modes

(PE), lysophosphatidylcholine (LPC), triacylglycerol (TAG), and ceramide (Cer) in the positive-ion mode and phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS), lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), triacylglycerol (TAG), glucosyl cerimide (GlcCer) in the negative-ion mode, respectively. The similarity in the chromatogram patterns between Matyash and mSAP methods suggests that the two methods have comparable performance in identifying the major lipid classes. However, the presence of an unidentified ion of *m/z* 361.2 at 0.68 min in the negative-ion chromatogram of mSAP method suggests that further investigation is needed to determine its identity. On the other hand, the differences observed in the SAP chromatogram, such as the intense peaks of *m/z* 504.52 and 1012.28 observed at 12.92 and 13.36 min in positive-ion mode, respectively, and *m/z* 550.52 at 13.38 min in negative-ion mode, indicate that the SAP method may not be able to accurately identify specific lipid species within certain classes. As listed in Additional file 1: Table S1, a total 240 lipid species (64 PC, 31 PE, 6 PS, 7 PG, 2 PI, 7 PA, 14 LPC, 4 LPE, 1 LPS, 1 LPG, 3 LPI, 3 LPA, 62 TG, 18 Cer and 17 GlcCer) by the mSAP method, 242 species (58 PC, 40 PE, 6 PS, 7 PG, 3 PI, 8 PA, 10 LPC, 6 LPE, 2 LPS, 1 LPG, 66 TG, 18 Cer and 17 GlcCer) by the SAP method, and 242 species (53 PC, 39 PE, 7 PS, 6 PG, 2 PI, 6 PA, 10 LPC, 7 LPE, 3 LPS, 3 LPG, 3 LPI, 3 LPA, 63 TG, 18 Cer and 19 GlcCer) by the Matyash method were identified in each lipid extract, respectively. The use of different extraction techniques such as LLE and SPE, as well as the particle size differences in the SAP beads and powder, may result in these variations in selectivity and sensitivity, leading to differences in the number and types of lipids that are extracted and detected by the UPLC-MS. However, the overall comparable identification of lipid species indicates that each method is effective in lipid analysis.

Comparison of efficiency between lipid extraction methods by recovery rate

The efficiency of different lipid extraction methods can be evaluated by comparing their recovery rates (Patterson et al. 2015). In this study, the efficiency of the SAP bead-based lipid extraction method was compared to the Matyash method by evaluating the recovery rates of 12 lipid standards spiked in human plasma. The lipid standard mixture was spiked into the plasma before and after extraction with the same amount of each lipid standard, and the amount of each standard spiked in plasma was

varied between 5 and 1 pmol. The lipid standard mixture was then analyzed using UPLC-MS, and the extracted ion chromatogram (XIC) peak area was used to calculate the recovery of each lipid molecule. The recovery rates were calculated by comparing the XIC peak areas obtained before and after lipid extraction using the formula, % Recovery = XIC peak area after extraction / XIC peak area before extraction × 100. The recoveries were then averaged (n = 3) and plotted in Fig. 3. The value for each measurement was listed in Additional file 1: Table S2. The results showed that the mSAP method had higher recovery rates than the Matyash method, with increase of 1.03–1.40 times for LPC, Cer, SM, PC, PG and PE classes except for TG in the positive–ion mode and 1.06–1.37 times for PG, PE, LPC, Cer, SM and PC classes in negative-ion mode. However, some classes of lipids such as PA, PS, PI, Cholesterol, and MAG were rarely detected, possibly due to their insufficient ionization efficiency and high limit of detection (LOD) above the standard concentrations. Furthermore, based on the comparable recovery results of SAP and Matyash method which were reported in previous work (Bang et al. 2017), mSAP and previous SAP methods had similar efficiency for lipid extraction. These results suggest that the mSAP is an efficient method for lipid extraction, with comparable efficiency to previous SAP method and improved efficiency compared to the Matyash method.

Comparison of sensitivity between lipid extraction methods by LOD

The limit of detection (LOD) is often used to compare the sensitivity of different lipid extraction methods (Khoomrung et al. 2013). Thus, the sensitivity of three lipid extraction methods, mSAP, SAP and Matyash, were compared by calculating their LOD for PC (17:0/17:0) standard in positive-ion mode and PG (17:0/17:0) standard in negative-ion mode. The XIC peaks of $[M+H]^+$ ion (*m/z* 762.6) of PC (17:0/17:0) species and $[M-H]^-$ ion (*m/z* 749.50) of PG (17:0/17:0) species spiked in human plasma were well resolved, as shown in Additional file 1: Fig. S1. The XIC peak areas of lipid standards at different concentrations such as 0.2, 0.4, 1, 2, 4, 10, 20, and 40 pmol of PC and 0.4, 1, 2, 4, and 10 pmol of PG were measured as shown in Fig. 4, and the LOD was determined using the slope (S) of the each calibration curve and standard deviation (SD) of the peak areas, based on the formula, LOD = 3.3 × SD / S (Armbruster et al. 1994). The LOD for the mSAP method was significantly lower (0.0351 pmol) than those of the Matyash (0.2431 pmol) and SAP (0.3096 pmol) methods, indicating that it is about 7 times more sensitive than the Matyash method and about 9 times sensitive than the SAP method in positive-ion mode. Similarly, the mSAP method is about 2 times more sensitive than the Matyash and SAP methods in negative-ion mode. The linear regression analysis showed that the correlation coefficient value of mSAP method ($R^2 = 0.9997$) was higher than those of SAP ($R^2 = 0.9955$) and Matyash ($R^2 = 0.9955$) methods in positive-ion mode, suggesting that the mSAP method provides more accurate quantification. These results indicate that the mSAP method is more sensitive and quantitative than the Matyash and SAP methods.

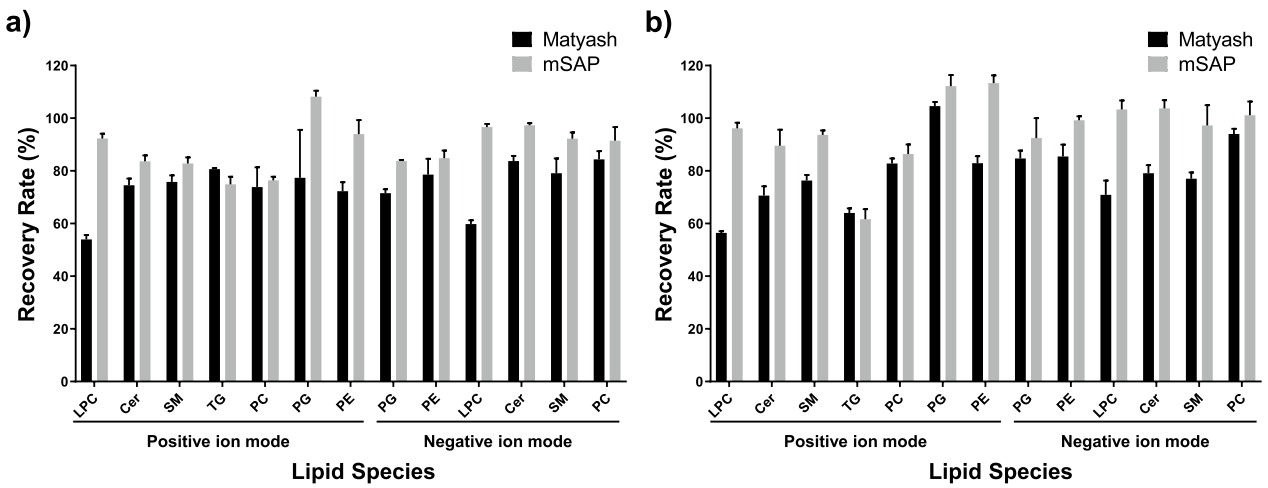
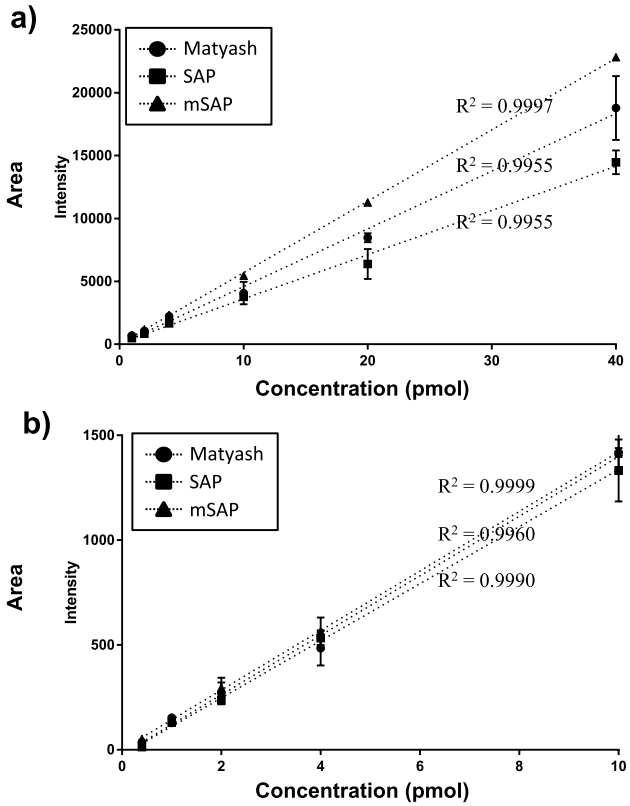


Fig. 3 Comparison of lipid recovery from lipid standard mixture by Matyash (black) and mSAP (gray) extraction methods using UPLC-MS in both positive-ion and negative-ion modes. The lipid standards were spiked before and after extraction of lipids from human plasma at the two different concentrations of **a** 5 pmol and **b** 1 pmol



	Matyash	SAP	mSAP
LOD (pmol)	0.2431	0.3096	0.0351
Slope	457.48	351.66	566.50
SD	33.71	33.05	6.03
Intercept	31.26	87.28	49.98
R^2	0.9955	0.9955	0.9997

	Matyash	SAP	mSAP
LOD (pmol)	0.2340	0.2359	0.1280
Slope	141.06	134.10	142.02
SD	2.00	3.06	1.15
Intercept	-15.25	-9.73	0.92
R^2	0.9960	0.9994	0.9999

Fig. 4 Comparison of the limit of detection (LOD) of Matyash, SAP, and mSAP methods based on the calibration curve of XIC peak areas of the target lipid standards spiked in human plasma. The target lipids used for comparison were (**a**) PC (17:0/17:0) in the positive-ion mode and (**b**) PG (17:0/17:0) in the negative-ion mode. The error bars on the calibration curves represent the standard deviation of three replicates at each point

Comparison of repeatability and reproducibility between lipid extraction methods

In this study, the repeatability and reproducibility of three different lipid extraction methods were evaluated using human plasma spiked with 10 pmol of PC (17:0/17:0) and PG (17:0/17:0) in positive-ion and negative-ion modes, respectively, of MS polarity. The XIC peak areas and their relative standard deviation (RSD) were measured for 25 PC (or PG) samples spiked in plasma over a 5-day period, with 5 extractions performed each day (Ståhlman et al 2009). Intra-day area variation refers to the variation in measured peak areas of PC standard extracted 5 times daily during the 5 consecutive days by each method, as shown in Fig. 5a–c. The data in Fig. 5 show that the mSAP method had the smallest intra-day area variation. Average value of XIC peak areas calculated for 25 samples over a 5-day period, as indicated by dotted lines in Fig. 5a–c and intra-day RSD (%) value for each day were summarized in Table 1. The RSD (%) were calculated using the equation: RSD (%) = SD × 100 / Average XIC area of 5 measurements for each day. The results showed that the mSAP method had a higher average peak area of PC standard than the SAP and Matyash methods, indicating that it was more efficient in extracting the lipids. In addition, the intra-day RSD (%) values for the mSAP method were significantly lower than those of the SAP and Matyash methods, indicating that it was more repeatable. The distribution of intra-day RSD values refers to the variation in RSD values of daily 5 extractions of each method during 5 days. The scatter dot plots in Fig. 5d demonstrate a narrow range of mean ± SD of intra-day RSD (%) values for the mSAP, indicating a high level of repeatability and precision. On the other hand, the inter-day RSD (%) value for the mSAP method was also lower than those for the SAP and Matyash methods, as listed in Table 1. The mSAP method was found to be about 3.3 times more reproducible than the SAP method and about 2.2 times more reproducible than the Matyash method. These results mean that the measurements obtained using the mSAP method are highly consistent and reproducible over time. This is an important factor to consider when selecting a lipid extraction method, as it affects

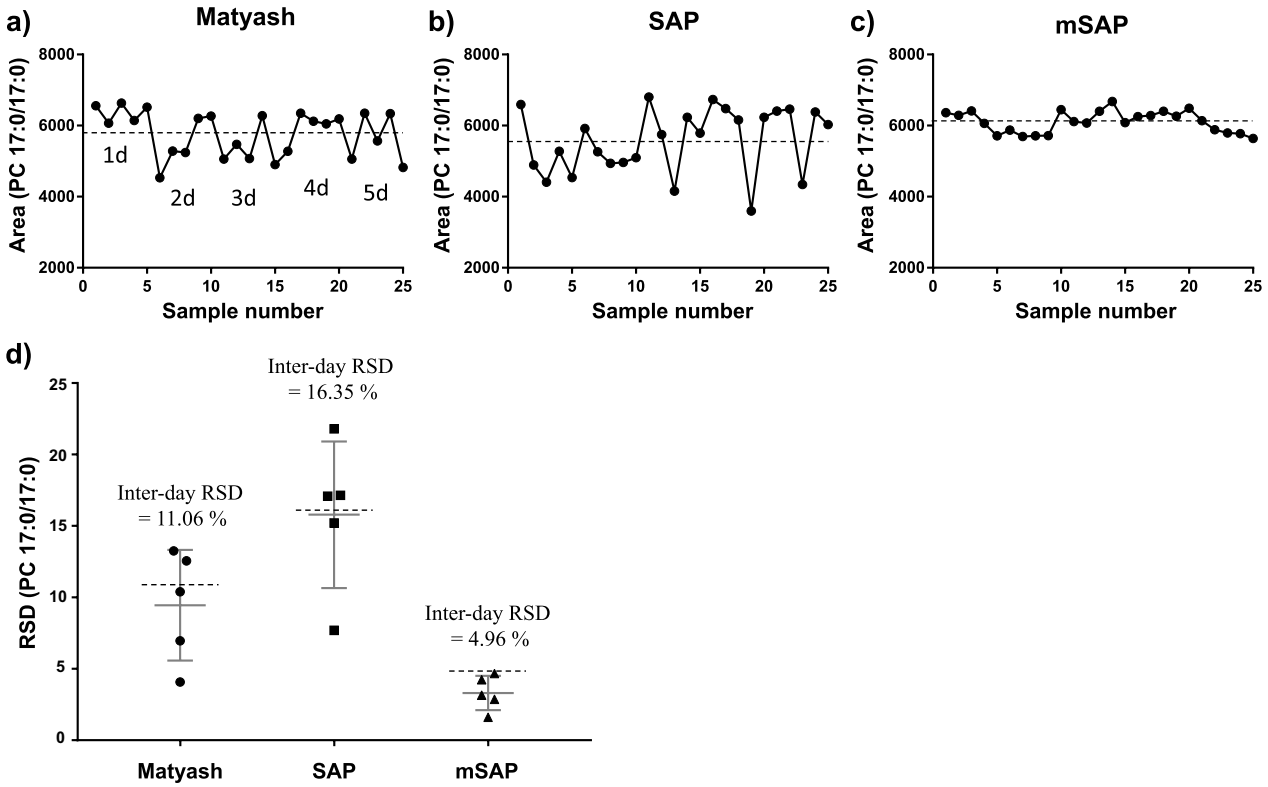


Fig. 5 Comparison of the repeatability and reproducibility of total lipid analysis with the three different extraction methods for PC standard spiked in plasma in the positive-ion mode. The intra-day variation of XIC peak areas and average area (dotted lines) of PC extracted 5 times daily during the 5 consecutive days by **a** Matyash, **b** SAP, and **c** mSAP methods. **d** The scatter dot plots show the intra-day RSD (%) variation and inter-day RSD (%) (dotted lines) of XIC peak areas of PC over a period of 5 days. The middle lines of the scatter dot plots display the mean value of intra-day RSD (%) values over 5 days. The upper and lower lines display mean + SD and mean-SD values, respectively

Table 1 Intra- and inter-day precision values for PC spiked in human plasma for the three extraction methods using positive-ion UPLC-MS

		Matyash		SAP		mSAP	
		Measured peak area	**RSD (%)**	**Measured peak area**	**RSD (%)**	**Measured peak area**	**RSD (%)**
Intra-day	Day 1	6382.97 ± 259.99	4.07	5143.58 ± 878.99	17.09	6167.90 ± 287.13	4.66
	Day 2	5504.57 ± 729.44	13.25	5235.33 ± 402.89	7.70	5888.58 ± 321.31	5.46
	Day 3	5355.43 ± 556.56	10.39	5747.02 ± 985.05	17.14	6269.25 ± 265.23	4.24
	Day 4	5995.99 ± 417.61	6.96	5839.61 ± 1272.82	21.80	6337.83 ± 102.23	1.61
	Day 5	5625.93 ± 706.49	12.56	5924.22 ± 900.96	15.21	5845.24 ± 184.58	3.16
	Ave	–	9.45	–	15.79	–	3.82
Inter-day		5772.98 ± 638.39	11.06	5577.95 ± 912.03	16.35	6101.76 ± 302.76	4.96

the reliability and accuracy of the results obtained. Similar trends were observed for the negative-ion mode, as shown in Additional file 1: Fig. S2 and listed in Additional file 1: Table S3. Overall, these findings indicates that the SAP bead-based extraction method is more repeatable and reproducible than the previous methods for total lipid analysis.

Conclusions

In summary, recent developments in mass spectrometry have greatly enhanced our ability to perform comprehensive lipidomic analysis. However, lipid extraction remains a critical step in the lipidomics workflow, and the choice of lipid extraction method can greatly impact the accuracy and reproducibility of the results. Despite this, there has been relatively little attention to the development of new lipid extraction methods, and many researchers continue to rely on the traditional Folch, Bligh-Dyer, or Matyash protocols. There is a need for continued innovation in lipid extraction methods to keep up with the demands of high-throughput, automated lipidomics pipelines. In this study, the SAP bead-based mSAP method for lipid extraction from plasma has several advantages over conventional methods, including convenience, rapidity, reproducibility, reliability, and efficiency. The method is also applicable to other biofluids such as urine, saliva and cerebrospinal fluid and has potential applications in biomarker discovery, rapid disease diagnosis, and other lipidomic research. The ongoing development of this method aims to improve its automation and increase the throughput of lipid analysis for clinical samples using liquid handling system and UPCL-MS. Overall, advancements in lipidomics analysis can lead a better understanding of lipid metabolism and its critical roles in various biological processes and diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-023-00388-z.

Additional file 1. Supplementary Tables and Figures.

Author contributions

YHK designed the study. JHC and GB performed material and sample preparation, and UPLC-MS experiments. JHC, GB, JAK and YHK analyzed the data and wrote the manuscript with input from all authors. All authors read and approved the final manuscript. JHC and GB equally contributed to this study.

Funding

This work was supported by grants from Korea Basic Science Institute (C330222) and (C380200).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

This authors declare that they have no competing interests.

Received: 5 April 2023 Accepted: 18 May 2023
Published online: 25 May 2023

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