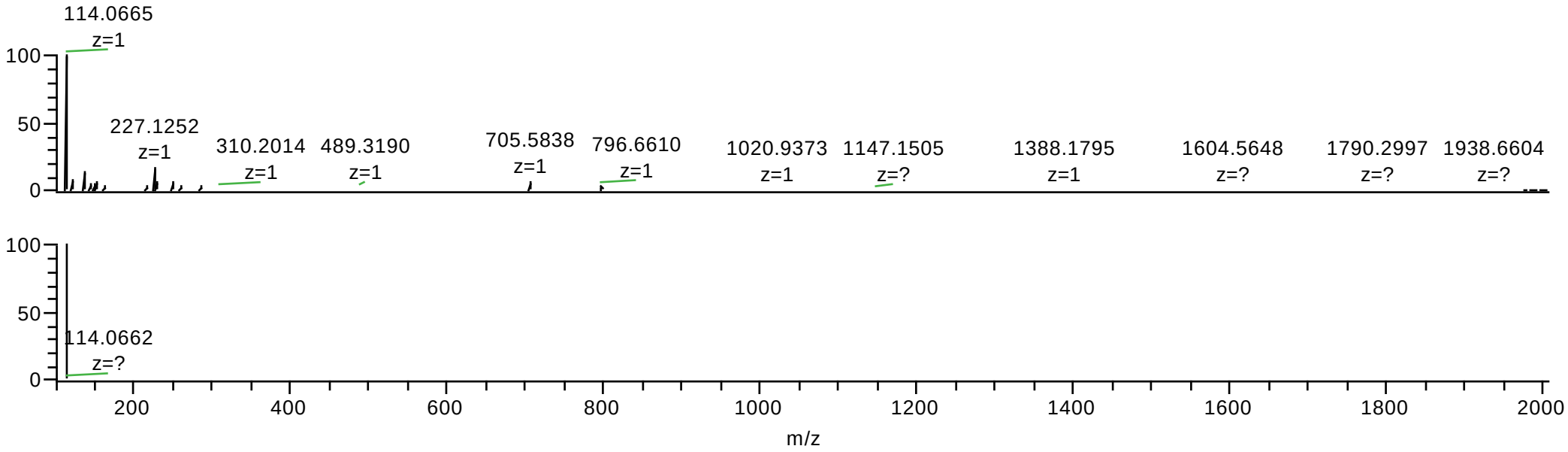
尿液檢體截至目前共收到62個樣品,

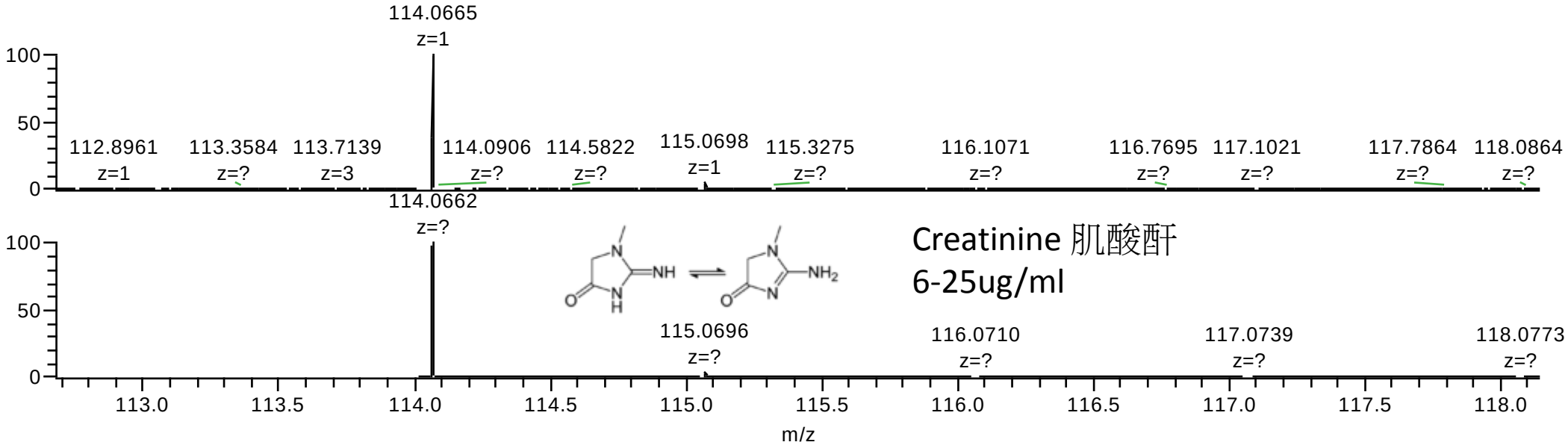
12個no-VUR, 50個VUR, 預期目標是各50個,

目前樣品前處理已做到第40個

前瞻-尿液樣品檢驗

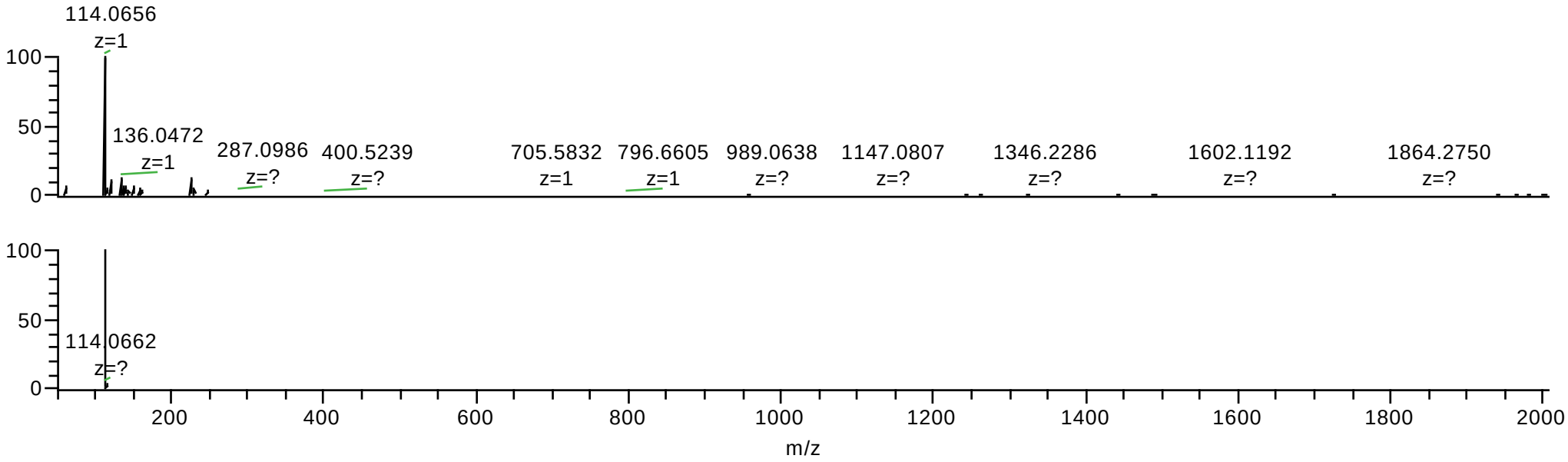
前處理:
取2ul 尿液加入198ul buffer B (80% ACN, 0.1%FA), 高速離心(16000xg, 15min),之後取上清液上機





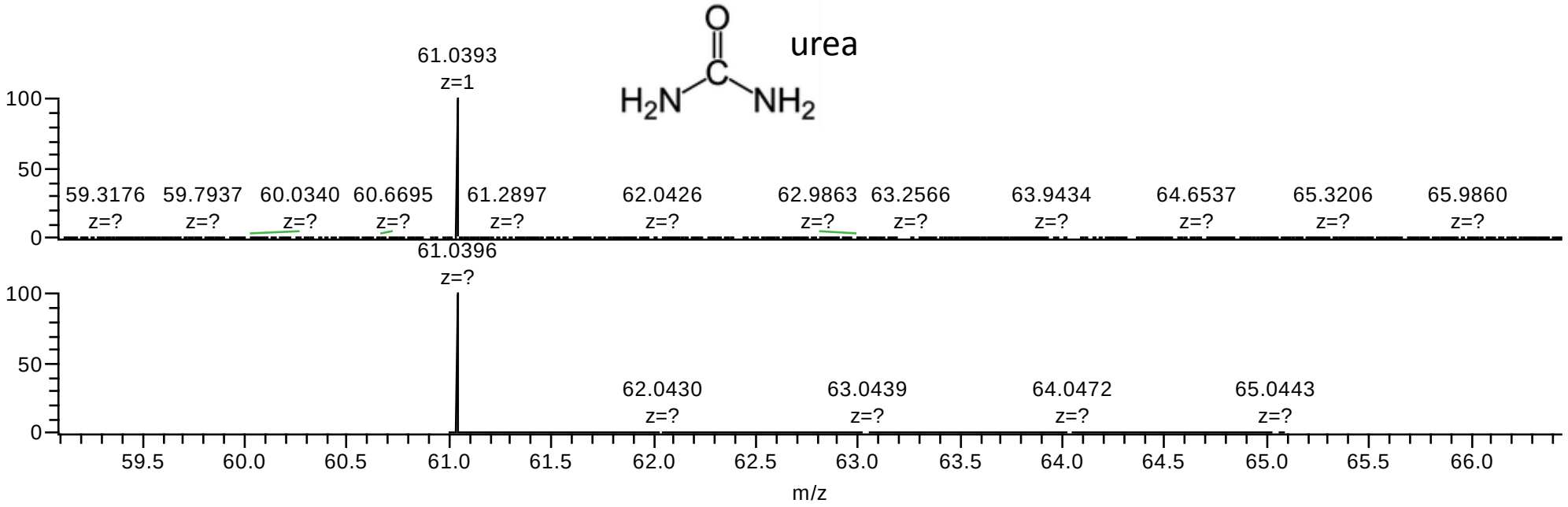
Creatinine 肌酸酐
6-25ug/ml

http://www.accuspeedy.com.tw/A16_search_items/024_urine_analysis/120_Urine_creatinine.htm



NL:
1.79E6
1_180417100004#38-68 RT: 0.83-1.41 AV: 31 SB: 84 0.01-0.61 , 1.73-2.99 T: FTMS + p ESI Full ms [50.00-2000.00]

NL:
2.22E4
$C_4H_7N_3O$ +H:
$C_4H_8N_3O_1$
p (gss, s/p:40) Chrg 1
R: 40000 Res.Pwr. @FWHM



NL:
1.23E5
1_180417100004#38-68 RT: 0.83-1.41 AV: 31 SB: 84 0.01-0.61 , 1.73-2.99 T: FTMS + p ESI Full ms [50.00-2000.00]

NL:
2.30E4
CH_4N_2O +H:
$C_1H_5N_2O_1$
p (gss, s/p:40) Chrg 1
R: 40000 Res.Pwr. @FWHM

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Long-Chain Free Fatty Acid Profiling Analysis by Liquid Chromatography–Mass Spectrometry in Mouse Treated with Peroxisome Proliferator-Activated Receptor α Agonist

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A change in the free fatty acid (FFA) profile reflects an alteration in the lipid metabolism of peripheral tissue. A high-throughput quantitative analysis method for individual FFAs therefore needs to be established. **We report here an optimized LC-MS assay for a high-throughput and high-sensitivity analysis of the 10 major long-chain FFAs in mouse plasma and liver.** This assay enables quantification of individual FFAs by using trace amounts of samples (2L of plasma and 10 mg of liver tissue). We apply this method to analyze the FFA profile of plasma and liver samples from an obese mouse model treated with bezafibrate, the peroxisome proliferator activated receptor (PPAR) agonist, and show a change in the FFA profile, particularly in the palmitoleic and oleic acid contents. This assay is useful for quantifying individual FFAs and helpful for monitoring the condition of lipid metabolism.

FFAs are frequently analyzed by **GC-MS or an enzymatic assay**.

The GC-MS method is a **conventional routine procedure** for an FFA analysis **after methylation.**

This method has **high resolution** and can **separate structural isomers** on the basis of the position of the double bonds.

A new metabolic profiling method using GC-MS was established in a previous study to **quantify 15 fatty acid species in human plasma** and was applied for **screening potential biomarkers of diabetic mellitus and diabetic nephropathy.**

However, fatty acid **conversion (methylation)** was necessary with this method which significantly increased the time for sample preparation.

Enzyme-based assays, i.e., based on **acyl-CoA synthase (ACS) and acyl-CoA oxidase (ACOD)**, are **simple and highly sensitive** methods for measuring the **plasma FFA** concentration.

Such assays require **no conversion treatment** of the sample and do not require a **large sample amount**; for instance, only **2 mL of mouse plasma** is needed to quantify the FFA concentration when using an enzyme-based assay.

However, **only total FFAs in plasma can be measured** with these assays, it being **impossible to quantify individual FFAs.**

Extraction of the **mouse plasma and liver** samples. Mouse blood samples were centrifuged at **15,000 rpm for 10 min at 4 C**, and resulting plasma samples (**2 mL**) were mixed with **98 mL of an FFA extraction solvent** (**99.5% ethanol containing 1 mg/mL of HDA as an internal standard**) or a **bezafibrate extraction solvent (80% methanol containing 0.1 mg/mL HMF as an internal standard).**

Mouse liver samples (10 mg) were homogenized with a Mill MM 300 mixer (Qiagen, Hilden, Germany) at 25Hz for 10 min in 1mL of the FFA extraction solvent. The supernatant was collected as the tissue extract after centrifugation (15,000 rpm for 10 min at 4 C). The extract was filtered through a 0.2 mm-pore PVDF membrane (Whatman, Brentford, UK), and the resulting filtrate was used for LC-MS.

LC-MS was performed with an Acquity UPLC system coupled to a Xevo Quadrupole Time-of-Flight (QTOF)-MS system (Waters, Milford, MA), equipped with an electrospray source operated in the **negative-ion mode** with a lockspray interface for accurate mass measurement.

It was **infused** straight into the MS system at a flow rate of **20 uL/min** at a concentration of **200 pg/mL in 50% acetonitrile and 0.1% formic acid**.

An aliquot of the extracted sample (3 uL) was injected into an Acquity UPLC **BEH-C18** reversed-phase column (**2.1 x 100 mm column size, 1.7 um particle size**). The column temperature was set at **40˚C**. The amount of FFA or bezafibrate was estimated from calibration curves obtained by using analytical-grade standard compounds. The peak area of **[M-H]±0.05 Da** was divided by the area of the internal standard, this value being used to generate the calibration curves.

Mobile phases A (**90% acetonitrile, 10mM ammonium formate, and 0.1% formic acid**) and B (**98% acetonitrile and 0.1% formic acid**) were used for FFA separation. The buffer gradient consisted of 0.1% B for 0–5 min, 0.1 to 99.9% B for 5–6 min, 99.9% B for 6–11 min, 99.9% to 0.1% B for 11–12 min, and 0.1% B for 3 min before the next injection, at a flow rate of 400 uL/min.

Mobile phases A (**H2O and 0.1% formic acid**) and B (**acetonitrile and 0.1% formic acid**) were used for bezafibrate separation. The column temperature was set at 40 C. The buffer gradient consisted of 5.0% to 99.0% B for 0–10 min, 99.0% B for 10–15 min, 99.0% to 5.0% B for 15–15.5 min, and 5.0% B for 4.5 min before the next injection, at a flow rate of 300 uL/min.

Method optimization of for the FFA analysis

Approximately **94% of FFAs in human plasma** are composed of the **10 major FFA species, MA, PA, POA, SA, OA, LIA, LNA, AA, EPA, and DHA**. We therefore analyzed individual FFAs, focusing on the major FFA species, by using an LC-MS-based method. We first investigated the optimum analytical conditions for LC-MS to improve the FFA peak sensitivity and high-throughput analysis, including optimizing the peak separation and quantification. The influence of ion suppression on the FFA peak detection and recovery rate was also investigated. We used different LC elution buffer conditions and MS ionization parameters to improve the peak sensitivity of individual FFAs by LC.

We initially analyzed FFAs based on **acetonitrile/H2O including 0.1% formic acid** in the gradient mode (an acetonitrile rate from **30% to 90%). It proved difficult to detect the unfragmented deprotonated molecular ion ($[M-H]^-$) under this condition (data not shown).**

We next adopted **90% acetonitrile with 0.1% formic acid as the solvent under an isocratic condition** and detected **weak FFA-specific peaks** (data not shown). To further improve the peak sensitivity, the LC elution buffer was supplemented with **10mM ammonium formate as well as 0.1% formic acid**. This condition enabled sensitive detection of FFAs as unfragmented deprotonated molecular ions ($[M-H]^-$) (Table 1 and Fig. 1). The resolution of the FFA peaks was significantly improved by adding ammonium formate to the LC buffer, indicating that ammonium formate would be useful for configuring FFA unfragmented deprotonated molecular ions.

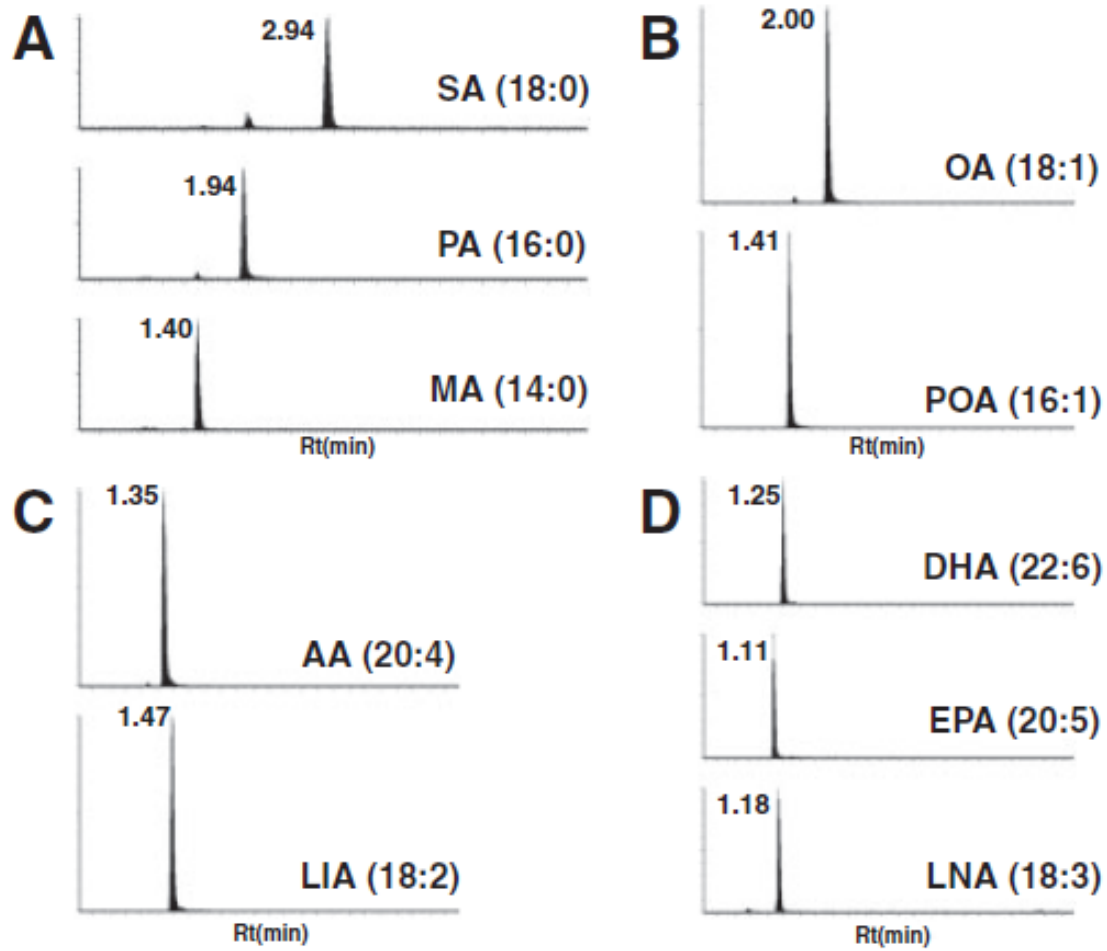


Fig. 1. Elution Peaks of Standard FFAs.

A, saturated fatty acids: MA (lower panel), PA (center panel), and SA (upper panel). B, monounsaturated fatty acids: POA (lower panel) and OA (upper panel). C, n-6 polyunsaturated fatty acids: LIA (lower panel) and AA (upper panel). D, n-3 polyunsaturated fatty acids: LNA (lower panel), EPA (center panel), and DHA (upper panel).

Table 2. LC-MS Calibration Curve ($y = ax + b$) Parameters and Validation Results for the Quantification of FFA
y is the ratio of the analyte peak area and x is the calculated concentration in ng/mL.

Name	Calibration curve	R^2 value	Plasma extraction efficiency (%) (±SD, n = 3)	Liver extraction efficiency (%) (±SD, n = 3)	Linear range (ng/mL)
MA	$y = 0.0006x - 0.0274$	0.9993	60.6 ± 2.6	33.8 ± 4.3	200–5000
PA	$y = 0.0007x + 0.0008$ (plasma)	0.9993	97.0 ± 14.5	—	200–2000
	$y = 0.0006x + 0.1061$ (liver)	0.9936	—	75.8 ± 1.5	200–5000
POA	$y = 0.0010x - 0.0138$	0.9991	119.4 ± 13.3	53.2 ± 6.9	20–5000
SA	$y = 0.0005x + 0.1428$	0.9996	73.7 ± 8.7	83.9 ± 17.5	20–5000
OA	$y = 0.0027x + 0.1307$	0.9986	105.94 ± 12.1	73.5 ± 4.3	20–5000
LIA	$y = 0.0020x + 0.0232$	0.9993	107.6 ± 5.7	95.7 ± 8.0	20–5000
LNA	$y = 0.0003x$	0.9859	91.2 ± 6.1	85.4 ± 7.4	20–2000
AA	$y = 0.0014x$	0.9976	119.3 ± 21.0	90.1 ± 5.2	20–5000
EPA	$y = 0.0061x$	0.9959	112.9 ± 18.7	82.4 ± 4.8	2–200
DHA	$y = 0.0077x$	0.9994	104.0 ± 9.0	70.3 ± 4.7	20–10000

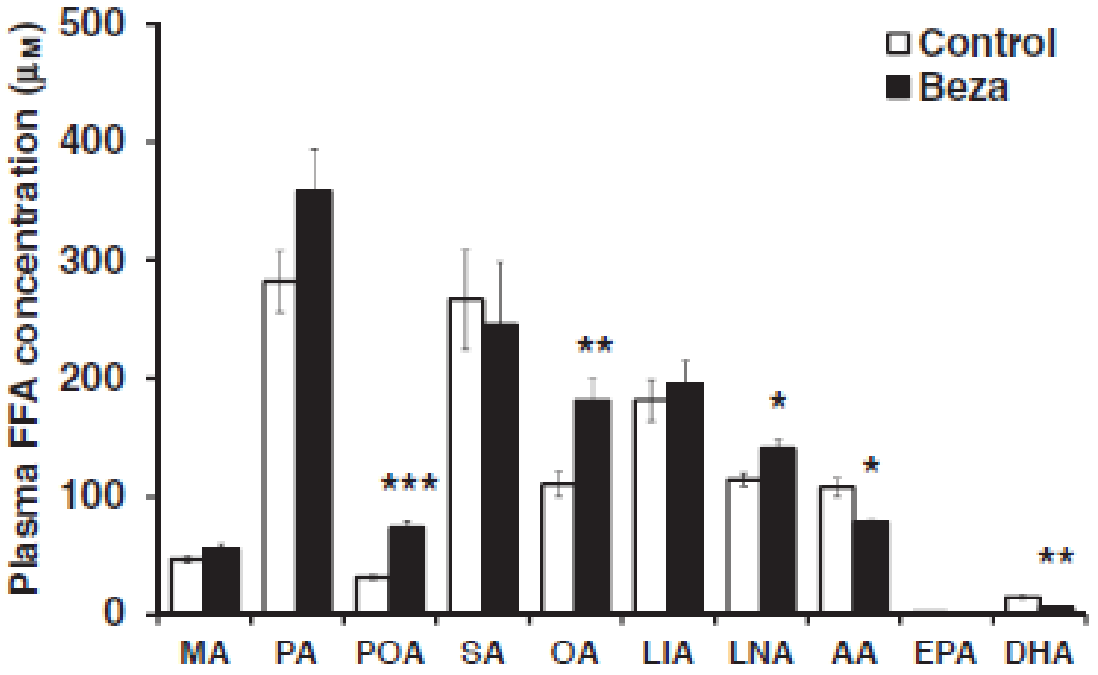
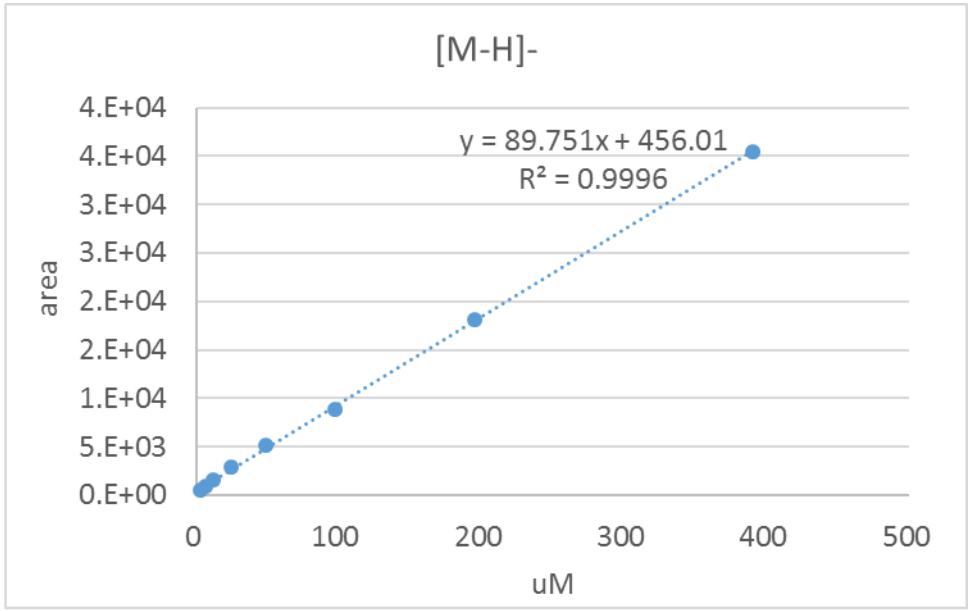
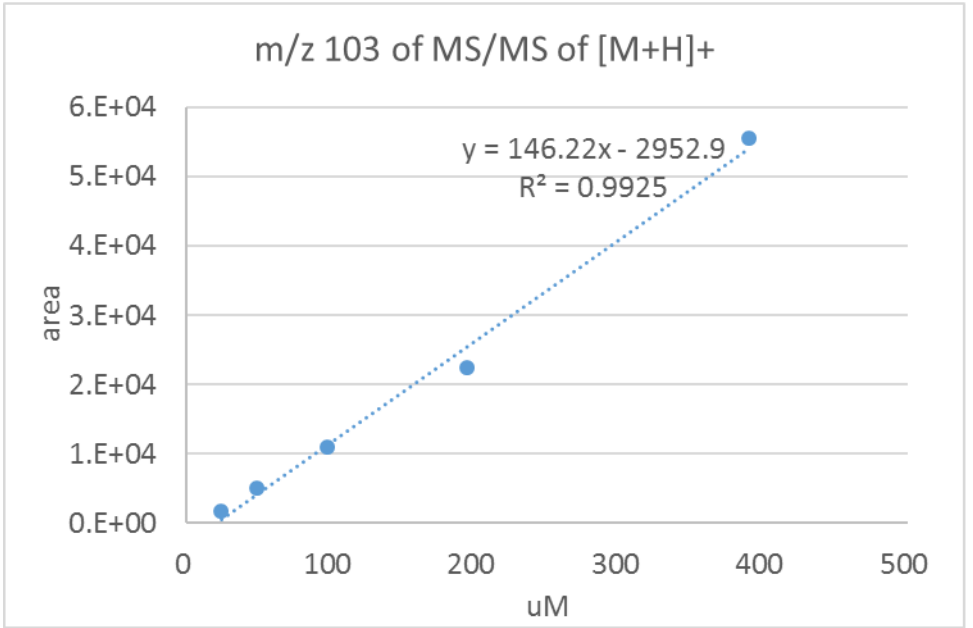
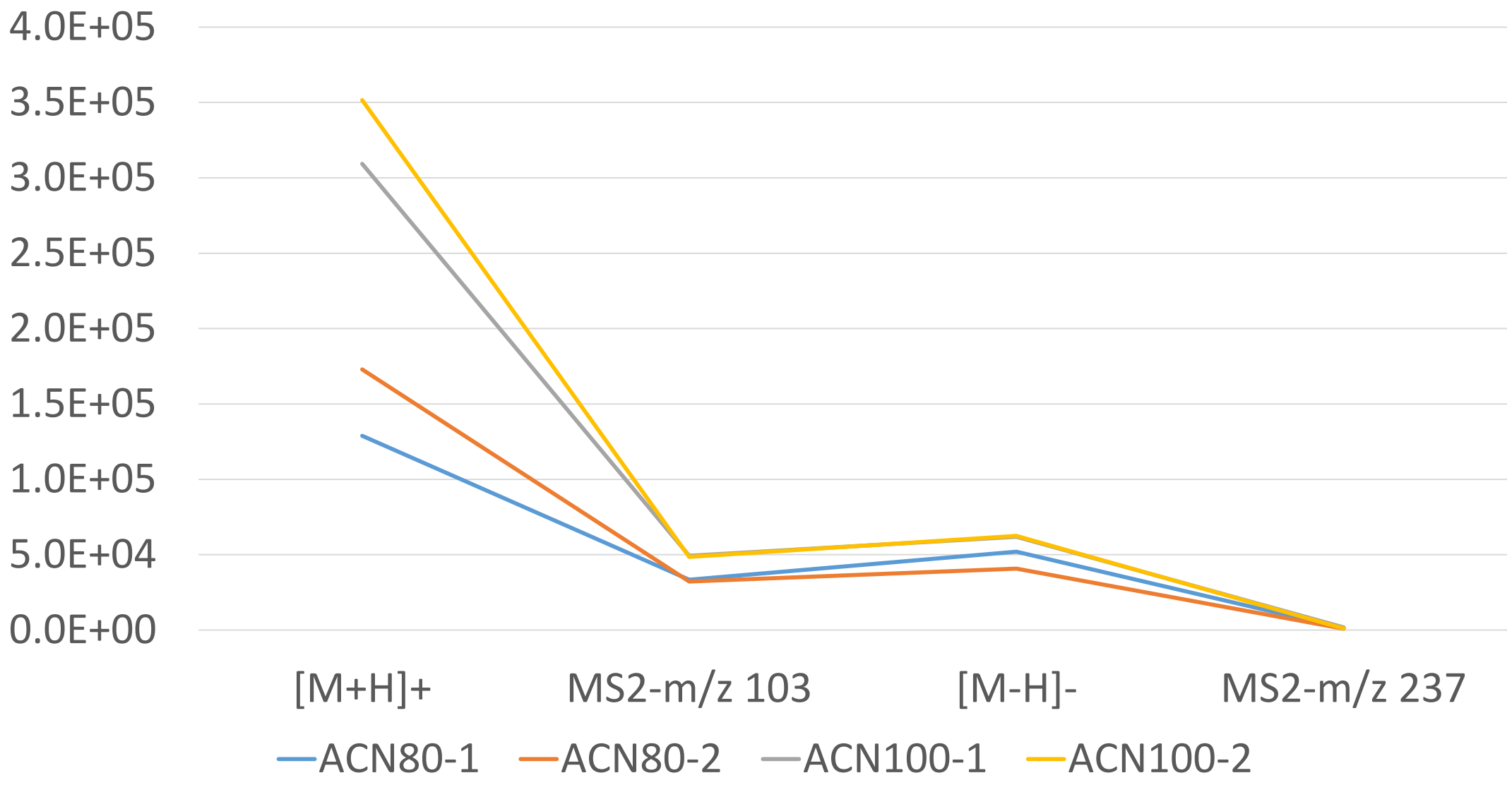


Fig. 2. Effect of Bezafibrate on the Plasma FFA Concentrations. Data shown are the mean ± SEM (n = 4–7). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *vs.* control. Cont, control; Beza, treated with bezafibrate.

Sample 15ul	1	2	3	4	5	6
EIC-Area	53920	69134	58948	57763	46648	47097
ppm	17.7889135	21.898806	19.1471716	18.8270571	15.8244638	15.9457561






4.0E+05
3.5E+05
3.0E+05
2.5E+05
2.0E+05
1.5E+05
1.0E+05
5.0E+04
0.0E+00
[M+H]+
MS2-m/z 103
[M-H]-
MS2-m/z 237
ACN80-1
ACN80-2
ACN100-1
ACN100-2