行政院國家科學委員會專題研究計畫　成果報告

液相層析/串聯質譜儀連線偵測極長鏈脂肪酸於新生兒之先天性過氧化體失調之篩選應用(2/2)

計畫類別：個別型計畫
計畫編號：NSC92-2113-M-039-003-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：中國醫藥大學醫學系

計畫主持人：賴建成
共同主持人：鄔哲源，蔡輔仁，蔡長海

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中　華　民　國 93 年 12 月 6 日

行政院國家科學委員會補助專題研究計畫 ☑ 成 果 報 告 □期中進度報告

液相層析/串聯質譜儀連線偵測極長鏈脂肪酸於新生兒之先天性過氧化酶體失調之篩選應用(2/2)

計畫類別：☑ 個別型計畫　□ 整合型計畫
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執行期間：2003 年 08 月 01 日 至 2004 年 07 月　31 日

計畫主持人：賴建成
共同主持人：蔡輔仁、蔡長海、鄔哲源
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：□精簡報告　☑完整報告

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執行單位：

中　華　民　國　　年　　月　　日

目錄：

（一） 計畫中文摘要：

關鍵詞：過氧化酶體失調、液相層析-電灑游離/質譜/質譜、新生兒篩檢、極長鏈脂肪酸

本研究的目的為國內首次利用液相層析-電灑游離/質譜/質譜技術來大量及專一性地檢測新生兒採血濾紙樣品中 (約含 3.6 μl 血樣) 是否帶有過氧化酶體失調疾病 (如: 腦白質腎上腺營養不良症 (Adrenoleukodystrophy, ALD), 髓磷質腎上腺神經病變 (adrenomyelinoneuropathy, AMN), 過氧化酶體生成缺陷 (peroxisomal biogenesis defects, PBD), 雷弗素姆氏病變 (Refsum disease))。血液中某些極長鏈脂肪酸的濃度是診斷先天性過氧化酶體失調症重要的參數，如: 二十、二十二、二十四及二十六烷酸 (eicosanoic, docosanoic, tetracosanoic and hexacosanoic acids) 等等。目前過氧化酶體失調的的檢測方式大都利用氣相層析或氣相層析質譜連線技術偵測極長鏈脂肪酸，雖然具有靈敏度及專一性高之優點，但常會有步驟緩慢及自動化困難之情形。大量分析、高專一性、低偵測極限與富含分析物結構物訊息是液相層析-電灑游離/質譜/質譜連線技術的優點，因此選擇此技術開發應用於新生兒極長鏈脂肪酸的偵測，而不同型式的先天性過氧化酶體失調症也將可在一次分析中同時篩檢出來。因此相對於傳統方法，新的先天性過氧化酶體失調症篩檢方法將會更準確,快速,經濟及簡便。目前本實驗室發展液相層析/質譜/質譜連線偵測氨基酸、醯基肉毒鹼及 17 羥孕酮已成功應用於新生兒遺傳代謝疾病之偵測。

（二）計畫英文摘要：

Keywords: peroxisomal disorder, high performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS), newborn screening, very long chain fatty acid

In this study, our most important goal was to provide a high throughput and specific method with potential to screen for many of the peroxisomal disorders (ex. adrenleukodystrophy (ALD), adrenomyelinoneuropathy (AMN), peroxisomal biogenesis defects (PBD), Refsum disease and etc.) with a 3-mm blood spot (~3.6 μl blood impregnated on filter paper) from newborns using high performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS). Several very long chain fatty acids (VLCFA) - eicosanoic (C20:0), docosanoic (C22:0), tetracosanoic (C24:0) and hexacosanoic (C26:0) acids are the most important plasma parameters for the diagnosis and monitoring of the peroximal disorders in newborns. Currently, neonatal screening procedures for the peroximal disorders are based on its determination by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Analyses of VLCFA by GC and GC/MS methods, while highly sensitive and specific, is still slow and difficult to automate. LC-ESI/MS/MS is known for its high throughput, high specificity, low detection limit and abundant structure information and is applied to the determination of VLCFA of infants in this study. It is practicable to detect all VLCFA at a time related to peroxisomal disorders in blood using LC/MS/MS. Compare with traditional screening method, this new method should be fast, reliable, reproducible and relatively cheap. Currently, the amino acids, acylcarnitine and 17-hydroxyprogesterone profiling of dry blood specimens using LC/MS/MS has been developed and recognized as a useful tool for screening inherited metabolic defects of newborns in our laboratory.

(三) 報告內容：

1. 前言

中國醫藥大學附設醫院 (中國附醫) 於2000年初安裝液相層析/串聯式質譜儀工作平台 (liquid chromatography/tandem mass spectrometry;LC/MS/MS)，期望利用先進的質譜技術提升國內的遺傳醫學水平，初期針對胺基酸、脂肪酸及有機酸代謝疾病進行篩檢，同年 10 月完成本院 2100 例新生兒篩檢分析，建立國內第一套濃度標準範圍。其中於六月篩檢出一例罕見疾病：異戊酸血症，並及早進行治療，在優生保健部、醫學研究部、兒科部、護理部及婦產部共同努力下，中國附醫於 2000 年 12 月 20 日正式對外宣佈提供台灣第一個第二代新生兒篩檢服務，同時與罕見疾病基金會簽約合作共同推動第二代新生兒篩檢。中國附醫為國內第一個利用液相層析/串聯式質譜儀技術完成正式報告及臨床應用並首度提供此項技術於正式之新生兒篩檢服務，於國內居於領先地位。

目前中國附醫之新生兒篩檢一次 2 分鐘的分析可篩檢 30 種以上之遺傳疾病，平均一天一位技術員可篩檢上百位之新生兒，若能應用至其他各種遺傳疾病篩檢分析，則相對於傳統方法，新的檢驗方法將會更準確、快速、經濟、大量自動化及簡便。

2. 研究目的

Currently, the amino acids, acylcarnitine and 17-hydroxyprogesterone profiling of dry blood specimens using LC/MS/MS has been developed and recognized as a useful tool for screening inherited metabolic defects of newborns in our laboratory. In this study, our most important goal was to provide a high throughput and specific method with potential to screen for many of the peroxisomal disorders (ex. adrenleukodystrophy (ALD), adrenomyelinoneuropathy (AMN), peroxisomal biogenesis defects (PBD), Refsum disease and etc.) with a 3-mm blood spot (~3.6 μl blood impregnated on filter paper) from newborns using high performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS).

3. 文獻探討

There is considerable pressure to expand universal newborn screening programmes to cover a broader range of inborn errors of metabolism. Peroxisomes catalyze the β-oxidation of fatty acids and related substrates. The specificity of peroximal β-oxidation overlaps that of mitochondrial β-oxidation. Substrates that are preferentially or exclusively oxidized in peroxisomes include very long chain fatty acids (VLCFA), polyunsatured fatty acids, dicarboxylic fatty acid, prostaglandins and the side chain of cholesterol. Peroxisomal disorders are a heterogeneous collection of inherited disorders characterized by impaired, reduced or total absence of peroxisomes in cells[1]. Patients with these disorders can be detected by an

accumulation of VLCFA such as eicosanoic (C20:0), docosanoic (C22:0), tetracosanoic (C24:0) and hexacosanoic (C26:0) acids in their red blood cells, plasma or cultured skin fibroblasts. Zellweger syndrome[2] is the most severe of this group of disorders. It is apparent at birth and results in death within the first year. Neonatal adrenoleukodystrophy (ALD)[3] and infantile Refsum disease[4] and hyperpipecolic acidemia[5] are less severe, and some patients are in stable condition in the third or forth decade, albeit with deficits in vision, hearing, and cognitive function. They demonstrate similar, although generally milder, symptoms and biochemical abnormalities. Death often occurs in childhood. Other inborn errors appear to be caused by single peroximal enzyme defects. Some them mimic the disorders of peroxisome biogenesis to a considerable extent but the underlying cause is entirely different. Rhizomelic chondrodysplasia punctata appears to be an intermediate case with deficiency of a subset of peroximal enzymes.

Currently, neonatal screening procedures for the peroximal disorders are based on its determination of VLCFA by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Quantification of VLCFA by GC[6,7] or GC/MS[8,9] takes 1-2 days. Elevated ratios of C26:0/C22:0 and C24:0/C22:0 indicate a peroximal disorder. The GC/MS method has been applied to the analysis of large blood spots containing 50-100 μl of blood[10-12]. An elevated C26:0/C22:0 ratio has been observed in blood spots obtained from Guthrie cards collected at birth from Zellweger syndrome patients[11] and in cord blood of X-linked ALD[12,13]. This gives justtification to developing a rapid, reliable method for screening for peroximal disorders on a sample size as small as that contained in a 3-mm neonatal blood spot. Analyses of VLCFA by GC and GC/MS methods, while highly sensitive and specific, is still slow and difficult to automate.

Recently, electrospray ionization (ESI)[14] has rapidly emerged as a very promising technique for the analysis of compounds with medium or high polarity. Similar to atmospheric pressure chemical ionization (APCI), ESI produces ions at atmospheric pressure, but without the need of high temperature that could decompose labile compounds. Because of its low detection limit, high specificity, soft ionization and more importantly, abundant structural information, electrospray mass spectrometry (ESI-MS) and its related techniques have been considered one of the ideal devices for drug and screening analysis.

Today, tandem mass spectrometry (MS/MS) has already emerged as a powerful analytical tool in clinical biochemical genetics[15]. MS/MS was developed as a technique for expanding the scope and efficiency of newborn screening for inherited metabolic disorders[16-18]. Liquid chromatography coupled with electrospray/multidimensional mass spectrometry (LC-ESI/MSn)[19-21] represents a powerful alternative combining rapidly, easy process, high throughput, accuracy, specificity and sensitivity. One advantage of using MS instead of UV as

the detector is that baseline separation may not be needed for a clear identification, and the relative concentration of each compound can be calculated based on their peak areas. A few publications have proposed the detection of very long chain fatty acids using liquid chromatography/tandem mass spectrometry (LC/MS/MS)[22,23]. An faster LC/MS/MS can be used to replace the slow GC and GC/MS assays. The strategy developed was to liberate all of the VLCFA from the dried blood sample and to derivatize them if possible. An LC/MS/MS assay was suitable for measuring VLCFA in a 3-mm blood spot (~3.6 μl blood). The amino acid, acylcarnitine and 17-hydroxyprogesterone profiling of dry blood specimens using LC/MS/MS has been recognized as a useful tool for screening inherited metabolic defects of newborns in our laboratory[24-26]. A LC/MS/MS coupled with the microtiter plate technology to perform lower-sample volume screening for peroximal disorders in the newborn filter-paper blood specimens was designed in this study. We have sought the first development of an alternative method for the determination of VLCFA in newborn that takes advantage of the analytical versatility, specificity, and high throughput unique to the combination of HPLC and MS/MS.

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5. 研究方法

Chemicals

Glacial acetic acid, very long chain fatty acids (VLCFA) and related compounds were purchased from Sigma (St. Louis, MO, U.S.A.). HPLC grade methanol and acetonitrile were obtained from LAB-SCAN Analytical Science (Labscan Ltd. Dublin, Ireland). Deionized (18MΩ) water (Milli-Q water system, Millipore Inc., Bedford, MA, U.S.A.) was used in the preparation of the samples and buffer solution. Prior to use, the mobile phase was filtered through a 0.45 μm membrane filter (Gelman Sciences, Michigan, U.S.A.). Blank human whole blood samples were obtained from China Medical College Hospital, Taichung, Taiwan.

Standard solutions

Standard stock solutions were prepared at the concentration of 0.1 mg/ml in ethanol and kept

in the dark at -20℃ when not in use. For the calibration, standard samples containing VLCFA were prepared at 500, 300, 250, 200, 150, 100, 50 and 30 ng/ml. 30 μl of a blank sample (mobile phase), and of each standard, were loaded on to newborn screening cards. All newborn screening cards for calibration were dried at room temperature for at least three hours, and then stored in polypropylene bags at room temperature until analyzed.

Collection of filter-paper blood specimens

Standardized filter-paper forms (Standardized Schleicher & Schull filter-paper S&S 903; Dassel, Germany), impregnated with whole capillary blood from peroximal disorder patients or 2-5-day-old infants, were collected from the Department of Genetics, China Medical College Hospital, Taichung, Taiwan. We collected blood spot samples from these patients in order to establish corresponding abnormal VLCFA profiles. All filter papers containing blood samples were dried at room temperature for at least three hours, and then stored in polypropylene bags at room temperature until analyzed.

Sample extraction

One 1/8-inch circles from each blood spot (equivalent to 3.6 μl of whole blood) were excised from a 0.5-inch (12.7mm)-diameter dried blood spot and placed into a flat bottom 96 well block (individual 250μl wells, Corning Incorporated, USA) using an automated Wallac DELFIA DBS puncher (Turku, Finland). A stock solution containing a known concentration of internal standard (100 μmol/L, margaric acid) in ethanol was prepared and added to each well (3.6 μl). Internal standard margaric acid was only used in this extraction step. Acetonitrile (180 μl) and 5N hydrochloric acid (20 μl) were added to each well. The wells were sealed, shaked and heated at 85 ℃ for 50 minutes. Subsequently, using a multi-channel pipette, the extracts were transferred into a clean V-bottomed 96 well microplate (individual 220μl wells, Corning Incorporated, USA). Each 96-well microplate was placed in an evaporator (SPEDry-96, JONES CHROMATOGRAPHY Ltd, U.K.) and the solutions evaporated to dryness under a gentle stream of dry nitrogen.

Derivatization of VLCFA

Oxalyl chloride (50 μl) was added to the residue, then it was sealed and heated at 50℃ for 5 min. The volatile material was evaporated to dryness under a gentle stream of dry nitrogen and several derivatizing agents (dimethylaminoethanol, 2-amino-2-methyl-1-propanol and choline, 50 μl) were added for testing. After 5 min at 20℃, the volatile material was evaporated under a gentle stream of dry nitrogen. The derivatized VLCFA were reconstituted in 50μl of acetonitrile/ water/ acetic acid (70:30:0.2) solution. The plate was covered with aluminum foil and placed on an autosampler tray for HPLC/ESI-MS/MS or other analysis.

HPLC system

The HPLC separation was performed on a Surveyor LC system (Thermo-Finnigan, San Jose, CA). HPLC analysis was performed on a 5 μm C-18 microbore column (50╳2.0 mm I.D.), operated at ambient temperature. A guard column (C-8 cartridge) was used to prolong the life of the HPLC column. The mobile phase was acetonitrile/ water/ acetic acid=70:30:0.2 (v/v) and the flow rate was 200 μl/min. At the end of each series, the column was thoroughly rinsed with a mixture of acetonitrile-deionized water (30:70, v/v) at a flow rate of 200 μl/min for 2 hours, and stored. The autosampler was fitted with a 10 μl loop and equipped with a 96 well sample plate stack. The HPLC and autosampler systems were all synchronized via the PC P4 workstation (Xcalibrate 1.3 software).

Electrospray and Mass Spectrometry (ESI-MS)

A Finnigan LCQ Deca XP quadrupole ion trap mass spectrometer (Finnigan Corp., San Jose, CA, U.S.A.), equipped with a pneumatically assisted electrospray ionization source, was used. The mass spectrometer was operated in the positive ion mode by applying a voltage of 4.5 kV to the ESI needle. The temperature of the heated capillary in the ESI source was set at 280 ℃. To avoid space charge effects, the number of ions stored in the trap was regulated by the automatic gain control, which was set at 1×10^8 ions for full scan mode, 5×10^7 for MS/MS mode, and 2×10^7 for ZoomScan mode. The flow rate of the sheath gas of nitrogen was set at 45 (arbitrary units). Helium was used as the damping gas at a pressure of 10^{-3} torr. Voltages across the capillary and the octapole lenses were tuned by an automated procedure to maximize signal for the ion of interest.

In MS/MS analysis, typical values for the relative collision energy (peak-to-peak amplitude of the resonance excitation) ranged from 0.4 to 0.8 eV. Mass spectra collected in full-mass scan mode were obtained by scanning over the range m/z 95 to 500. The maximum ion collection time was 0.3 s for each step and 3 scans were added for each spectrum.

Matrix assisted laser desorption ionization-quadrupole-time of flight (MALDI-Q-TOF) mass spectrometry

MALDI-Q-TOF was performed with a hybrid quadrupole-time of flight (Q-TOF) mass spectrometer (Qstar XL hybrid Quadrupole TOF system, Applied Biosystems-MDS Sciex).

6. 結果與討論

過氧化酶體失調疾病的種類如表一所示(如：腦白質腎上腺營養不良症 (Adrenoleukodystrophy, ALD), 髓磷質腎上腺神經病變 (adrenomyelinoneuropathy, AMN), 過氧化酶體生成缺陷 (peroxisomal biogenesis defects, PBD), 雷弗素姆氏病變 (Refsum

disease))。如表一中所列，血液中某些極長鏈脂肪酸的濃度是診斷先天性過氧化酶體失調症重要的參數，如: 二十、二十二、二十四及二十六烷酸 (eicosanoic, docosanoic, tetracosanoic and hexacosanoic acids) 等等。

目前過氧化酶體失調的的檢測方式大都利用氣相層析或氣相層析質譜連線技術偵測極長鏈脂肪酸，雖然具有靈敏度及專一性高之優點，但常會有步驟緩慢及自動化困難之情形。大量分析、高專一性、低偵測極限與富含分析物結構物訊息是液相層析-電灑游離/質譜/質譜連線技術的優點，因此選擇此技術開發應用於新生兒極長鏈脂肪酸的偵測，而不同型式的先天性過氧化酶體失調症也將可在一次分析中同時篩檢出來。因此相對於傳統方法，新的先天性過氧化酶體失調症篩檢方法將會更準確,快速,經濟及簡便。目前本實驗室發展液相層析/質譜/質譜連線偵測氨基酸、醯基肉毒鹼及 17 羥孕酮已成功應用於新生兒遺傳代謝疾病之偵測。

為萃取並釋放出血卡中血球及血液中等等所含之所有極長鏈脂肪酸，如: 十四、十六、十八、二十、二十二、二十四及二十六烷酸等，需實驗條件較劇烈之前處理方式，於此利用打孔機將含有樣品之濾紙打下一點 (直徑 1/8-inch、約 3.6 μl 之血液) 置於 96 孔樣品盤中，加入含內標準品之試劑後，我們採用了酸化後加熱將樣品中含極長鏈脂肪酸之脂質全轉換成極長鏈脂肪酸，避免傳統複雜之鹼化與多次萃取方式。

針對長鏈脂肪酸之質譜偵測方式，我們採取電灑法與介質輔助雷射脫附法兩種方式去嘗試，希望能得到較適當且快速之分析方法。

電灑法-串聯式質譜儀：

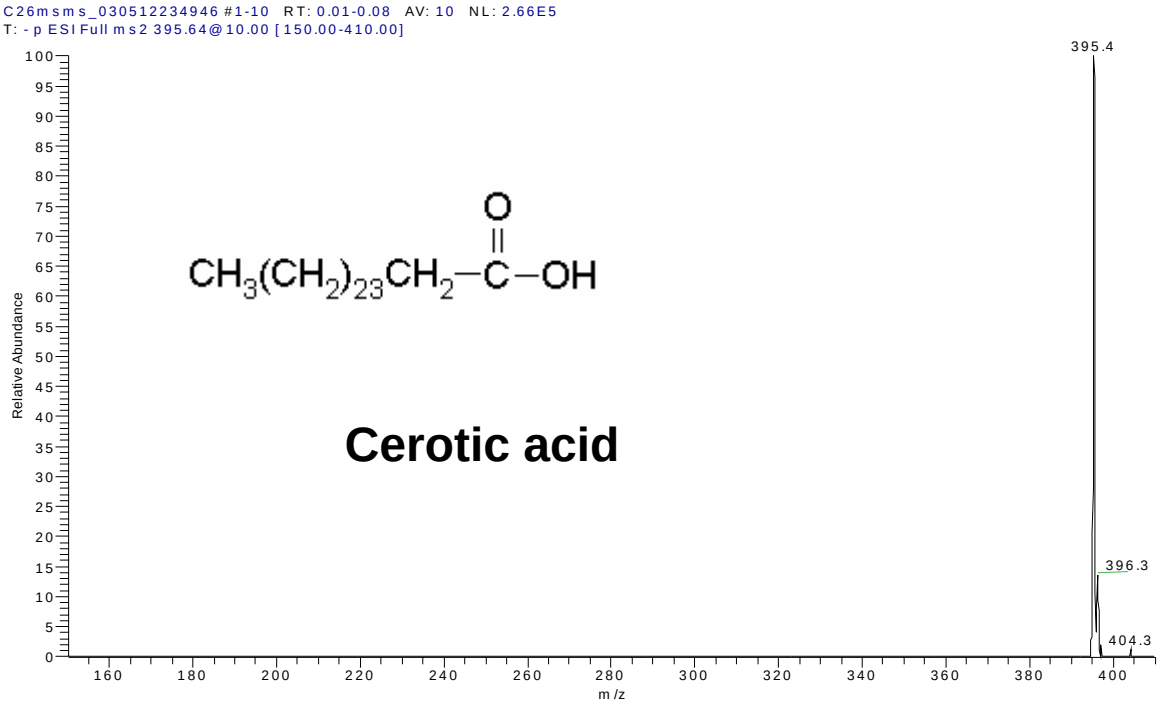
因長鏈脂肪酸本身帶有一羧基，初步以直接進樣電灑分析來測試,如圖 1 所示，於質譜/質譜圖譜中可見到清楚的$[M-H]^-$母離子，嘗試逐步提高碰撞能量，仍未可得到相關之特徵裂解離子，圖 1a 的 26 烷酸與圖 1b 的 22 烷酸質譜/質譜圖中顯示於負離子模式下直接進樣並無法得到裂解圖譜。

在缺乏特徵裂解離子下並無法利用靈敏度及專一性高之選擇反應偵測(SRM)模式，因此嘗試以衍生化的方式來獲得具有特徵裂解離子之母離子，利用不同之醇類與極長鏈脂肪酸進行酯化反應，如 dimethylaminoethanol[23]、2-amino-2-methyl-1-propanol、choline 等等，如圖 2 所示為利用文獻 23 中所用之 dimethylaminoethanol (DMAE)酯化之極長鏈脂肪酸 hexacosanoic acids 之質譜/質譜圖，但為了增加靈敏度，我們仍嘗試了其他之衍生化方式，目前以 DMAE 衍生化後之效果最好。一次 2 分鐘的分析可篩檢所有過氧化酶體之遺傳疾病，平均一天一位技術員可篩檢上百位之新生兒，則相對於傳統方法，新的檢驗方法更準確、快速、經濟、大量自動化及簡便。

表二中列出利用 LC/MS/MS 測量台灣正常孩童與過氧化酶體失調病患採血濾紙樣品中極長鏈脂肪酸濃度，因為過氧化酶體疾病患者中的 phytanic, tetracosanoic (C24) 及

hexacosanoic (C26) acids 都會出現堆積過高之臨床症狀，因此可用來做為診斷疾病之依據，如表二所示，peroxisomal biogenesis defects (PBD)患者之 phytanic acid 及 C24/C22 與 C26/C22 比值都遠高於正常者；而 ALD 及 AMN 患者則是 C24/C22 及 C26/C22 比值高 2~3 倍，遠超過標準偏差，因此利用 LC/MS/MS 可有效篩檢過氧化酶體失調之病童。

(a)



(b)

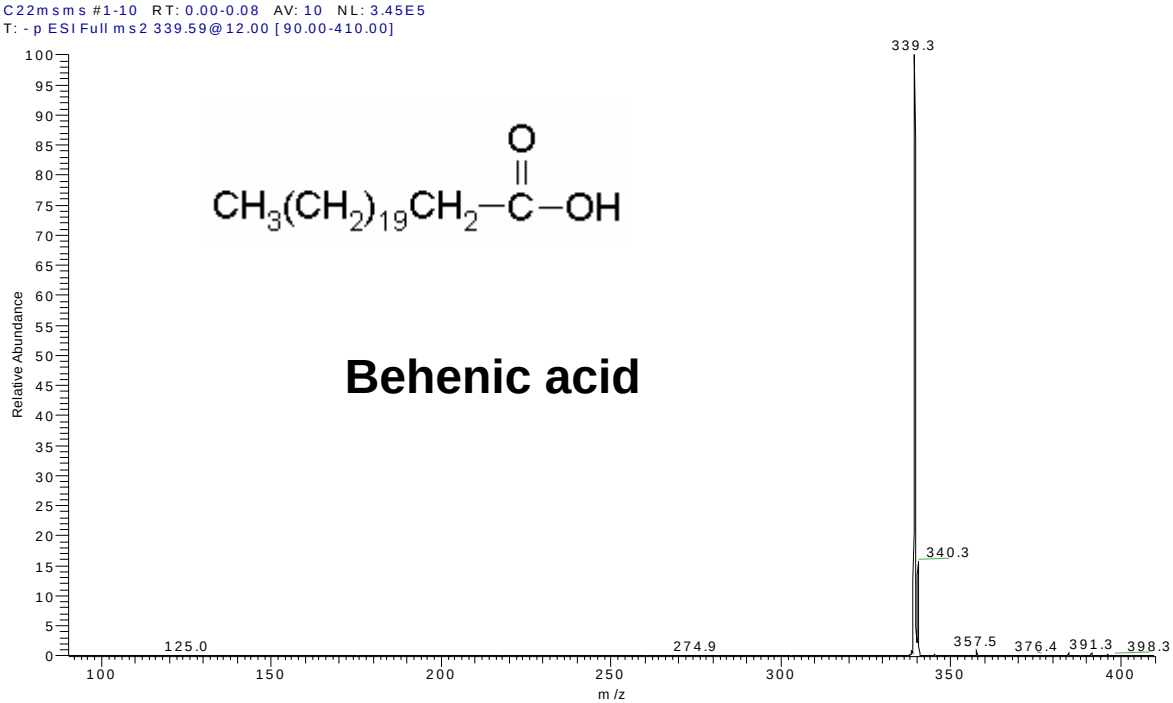


圖 1.利用直接進樣電灑法分析，以負離子-質譜/質譜模式偵測 (a)26 烷酸 cerotic acid (b)22 烷酸 behenic acid (濃度 5 ppm、流速 5 μl/min)

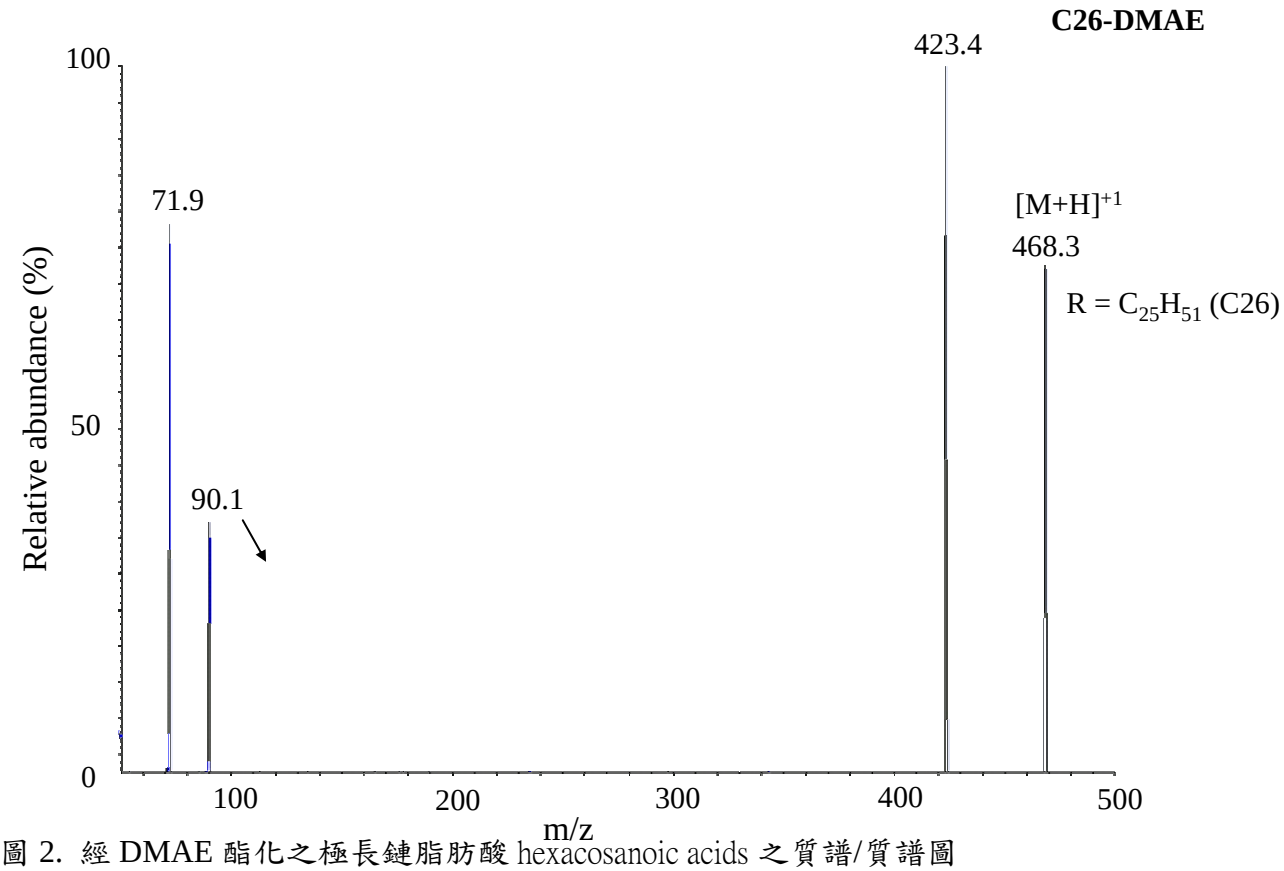


圖 2. 經 DMAE 酯化之極長鏈脂肪酸 hexacosanoic acids 之質譜/質譜圖

表一、過氧化酶體失調病症種類與疾病相關代謝物指標

Disease	Type of assay
Classical ZS Neonatal ALD Infantile Refsum disease Zellweger-like syndrome Pseudo-infantile Refsum disease	VLCFA, bile acids, phytanic acid, pristanic acid…..
RCDP (classical/atypical phenotypes)	Phytanic acid
Isolated peroximal β-oxidation defects	VLCFA, bile acids
Classical Refsum	Phytanic acid

ZS : Zellweger syndrome

ALD : Adrenoleukodystrophy

RCDP : Rhizomelic chondrodysplasia punctata

VLCFA : very long chain fatty acids

表二、利用 LC/MS/MS 測量台灣正常孩童與過氧化酶體失調病患採血濾紙樣品中極長鏈脂肪酸濃度

	Phytanic acid (μmol/l)	C24/C22	C26/C22
PBD patients Mean ± S.D.	24.8 ± 15.9	1.95 ± 0.32	0.33 ± 0.21
ALD/AMN patients	3.55 ± 2.02	1.63 ± 0.27	0.102 ± 0.03
Normal controls	3.21 ± 3.98	0.79 ± 0.12	0.027 ± 0.006

介質輔助雷射脫附游離-四極式-飛行時間式串聯質譜儀 (MALDI-TOF)：

為了比較不同游離化方式得到的好處，目前正持續進行下列幾種不同方式之分析方式：

- 選擇適當介質以直接分析極長鏈脂肪酸，如 DHB、9-aminoacridine、porphyrin。
- 以醋酸鈉對極長鏈脂肪酸進行皂化後，選擇適當介質分析。

首先我們嘗試利用 9-aminoacridine (9-AA)作為負離子游離之介質，9-AA 為鹼性之介質文獻上曾用來測量酸性小分子及核苷酸等，經測試後之效果不佳，VLCFA 之負離子訊號不易偵測，測試其他之 matrix (DHB, CHCA…) 所得之結果亦同；而在正離子模式下以醋酸鈉對極長鏈脂肪酸進行皂化後，選擇適當介質 (DHB) 分析帶有鈉離子之 VLCFA，可偵測到 VLCFA 之訊號，但離子訊號不持續穩定及靈敏度不高為其缺點。因此我們改以正離子模式下偵測經 DMAE 衍生化之 VLCFA，希望如同 LC/MS/MS 實驗所得之結果，增強離子化效率。這也是文獻中首次利用此種衍生化過程進行 MALDI-TOF 之分析，如圖三所示為 500 ppm 之 phytanic acid 經 DMAE 衍生化之 MALDI-TOF 質譜圖，所用之 matrix 為 DHB，此時所得之訊號穩定持續，S/N 比為 11364；當濃度降至 50 ppm 時之 S/N 比仍有 1394(圖 4)，而當 VLCFA 之碳鏈增加到 22 個碳時，如圖 5 所示為 200 ppm 之 docosanoic acid 經 DMAE 衍生化之 MALDI-TOF 質譜圖，此時 S/N 比為 2257，而於 24 個碳的 tetracosanoic acid 於 50 ppm 時之 MALDI-TOF 質譜圖如圖 6 所示，S/N 比為 232，碳鏈增加造成靈敏度下降的原因可能是 VLCFA 揮發性降低造成，也造成所需之雷射能量隨分析物碳數遞增，如圖 7 與圖 8 分別為 50 與 5 ppm 之 hexacosanoic acid (C26) 經 DMAE 衍生化之 MALDI-TOF 質譜圖，於 5 ppm 時之 S/N 比仍有 128，於 500 ppb 時仍可偵測到 C26 之訊號 (未顯示)，這樣的靈敏度比之前我們所試過之任何方式皆來得高，也比文獻上利用 MALDI-TOF 量測類似化合物之靈敏度高，依人體血清中 VLCFA 之平均濃度皆高於 1000ppm 之數據，我們所建立之方法可確定有效測定人體血清中 VLCFA 之濃度並可篩檢過氧化酶體失調症患者，目前我們已應用到實際樣品之篩檢並得到與 LC/MS/MS 相似之結果，所有之數據已在撰寫文章，待投稿接受後將另行附上。

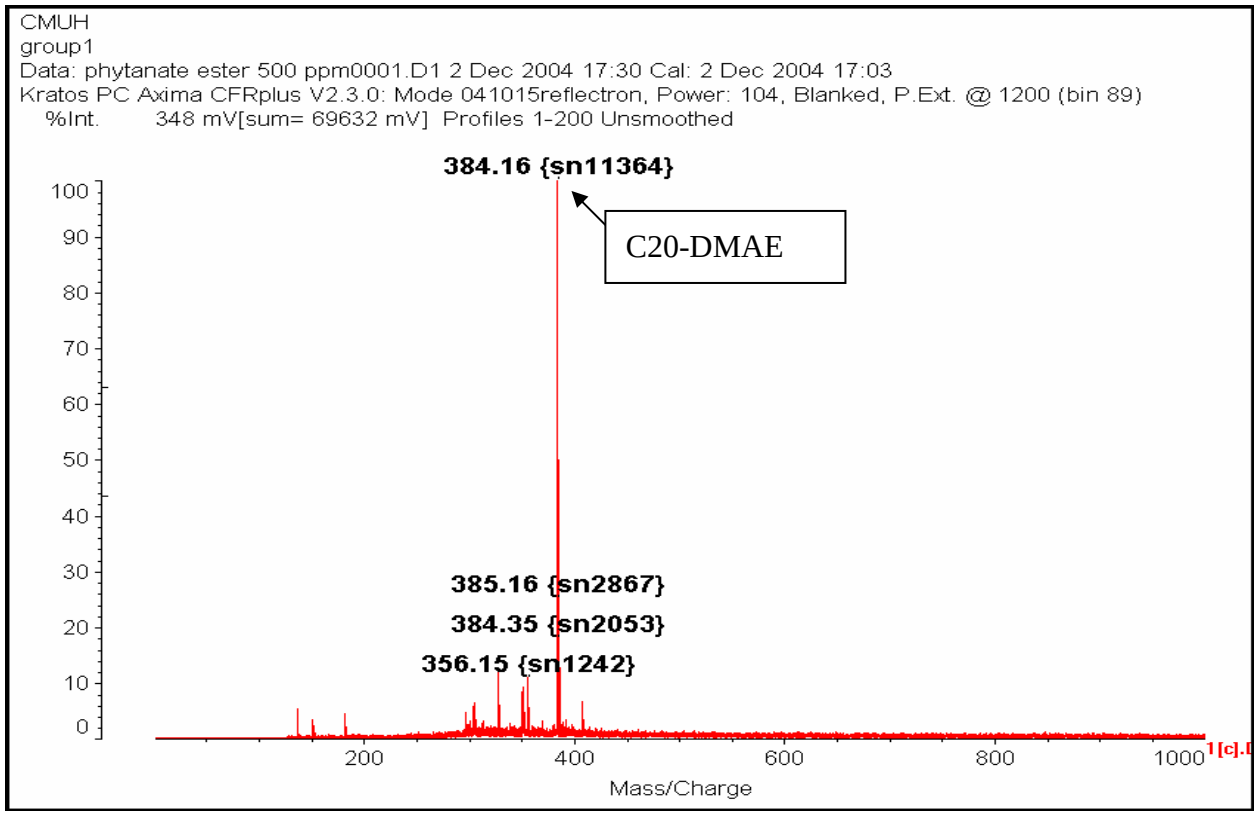


圖 3. 經 DMAE 酯化之極長鏈脂肪酸 phytanic acid 之 MALDI-TOF 質譜圖，濃度：500 ppm，介質：DHB。

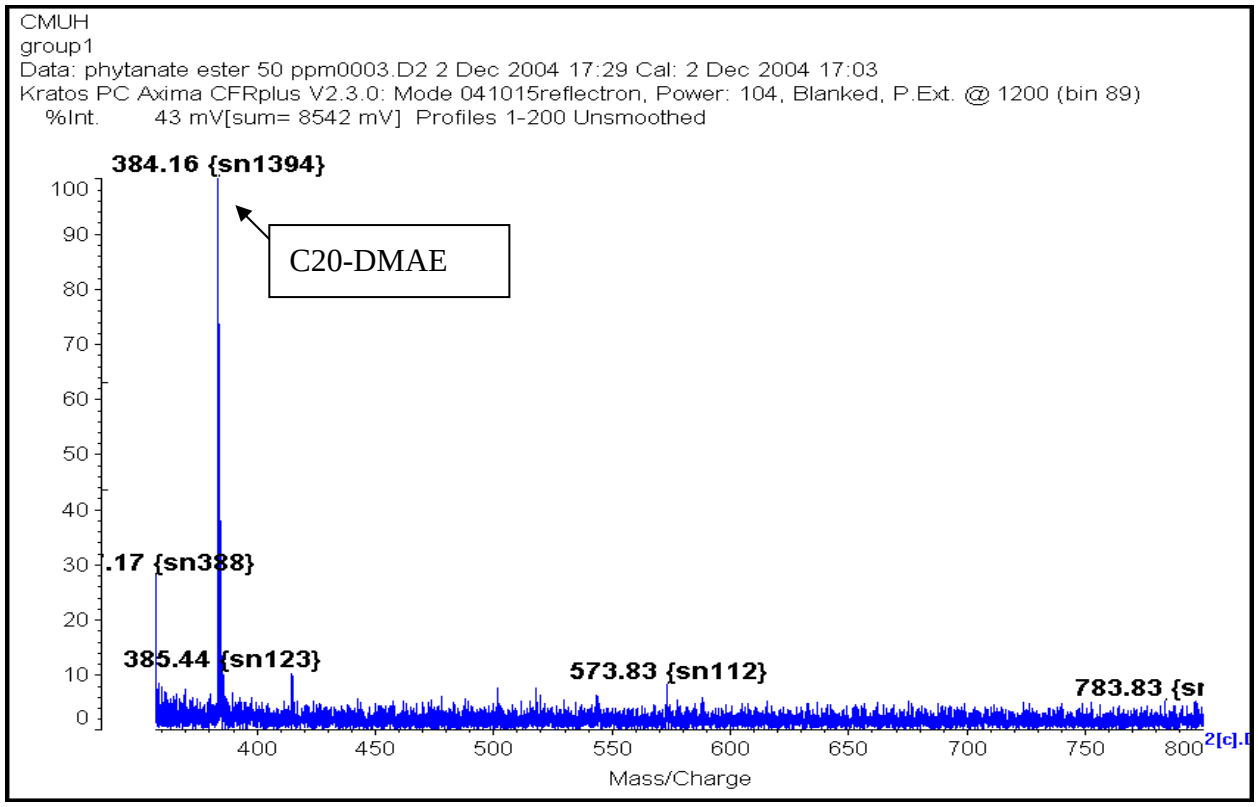


圖 4. 經 DMAE 酯化之極長鏈脂肪酸 phytanic acid 之 MALDI-TOF 質譜圖，濃度：50 ppm，介質：DHB。

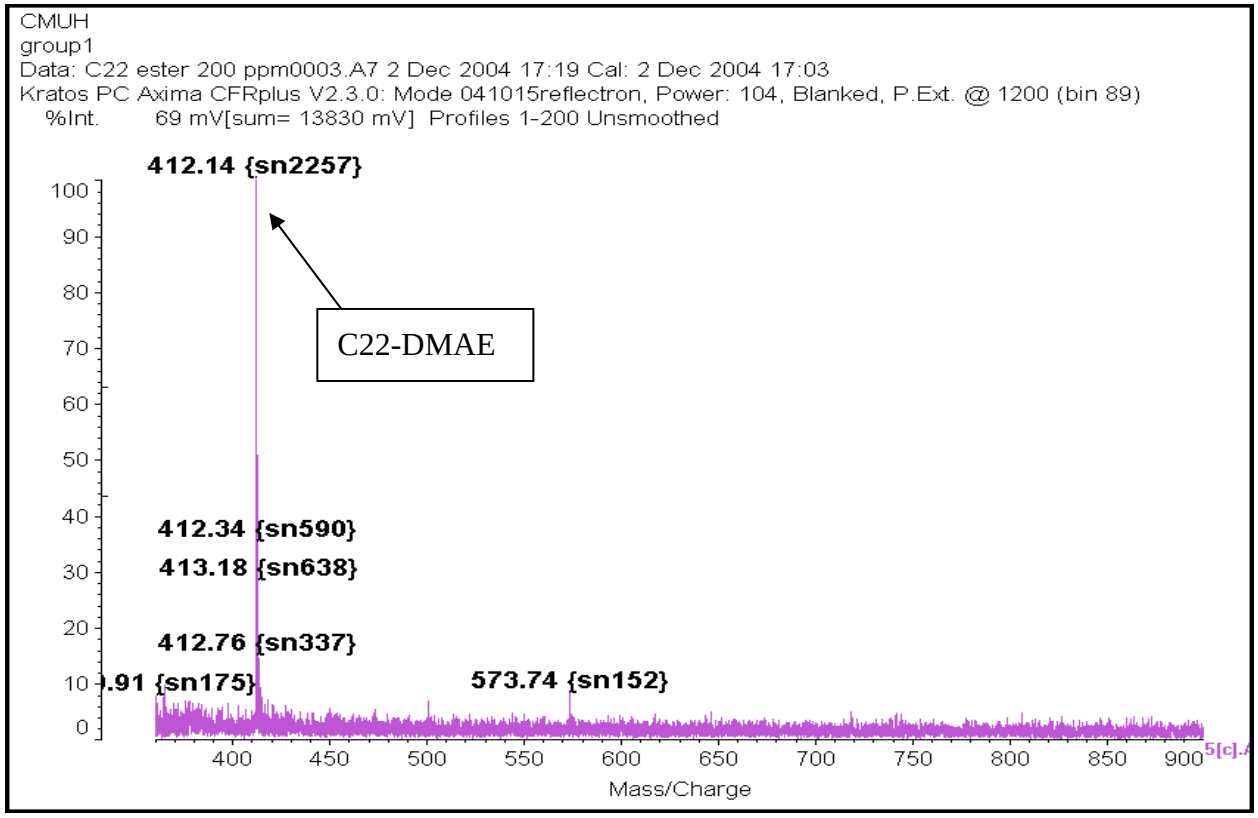


圖 5. 經 DMAE 酯化之極長鏈脂肪酸 docosanoic acid 之 MALDI-TOF 質譜圖，濃度：200 ppm，介質：DHB。

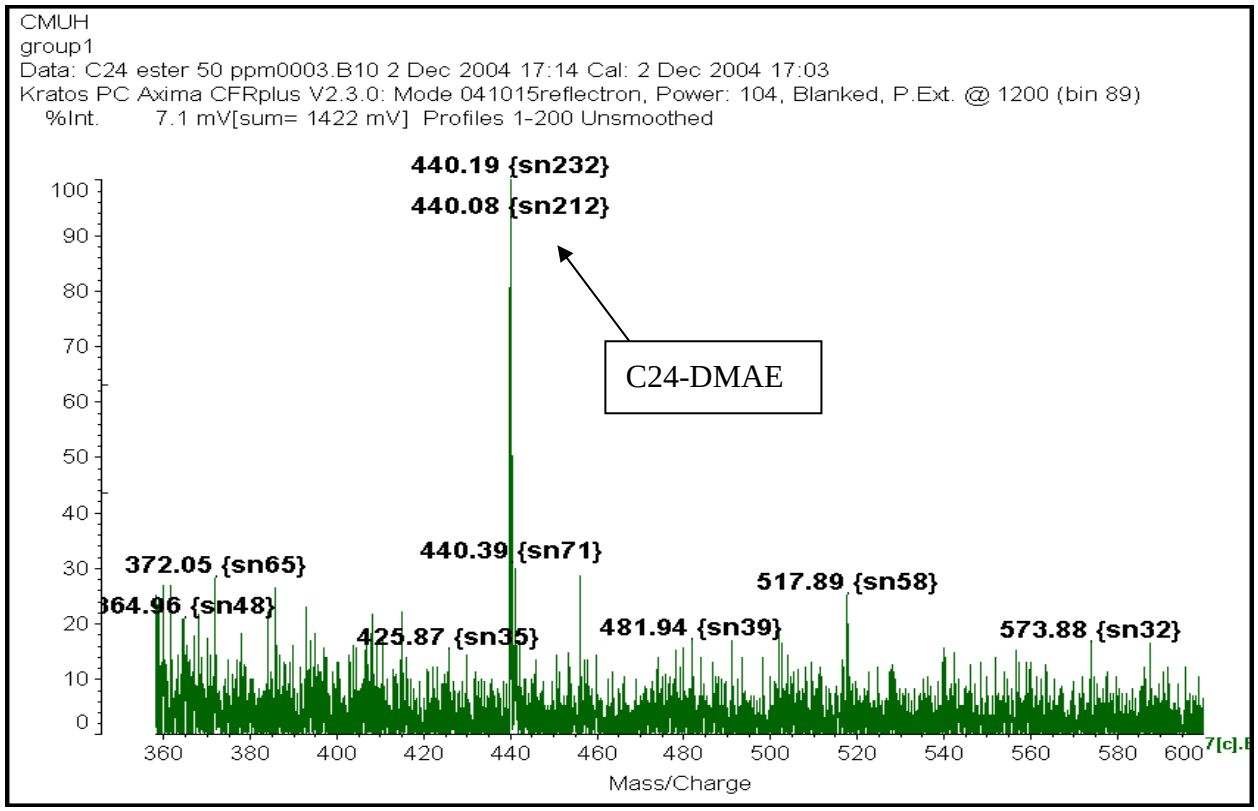


圖 6. 經 DMAE 酯化之極長鏈脂肪酸 tetracosanoic acid 之 MALDI-TOF 質譜圖，濃度：50 ppm，介質：DHB。

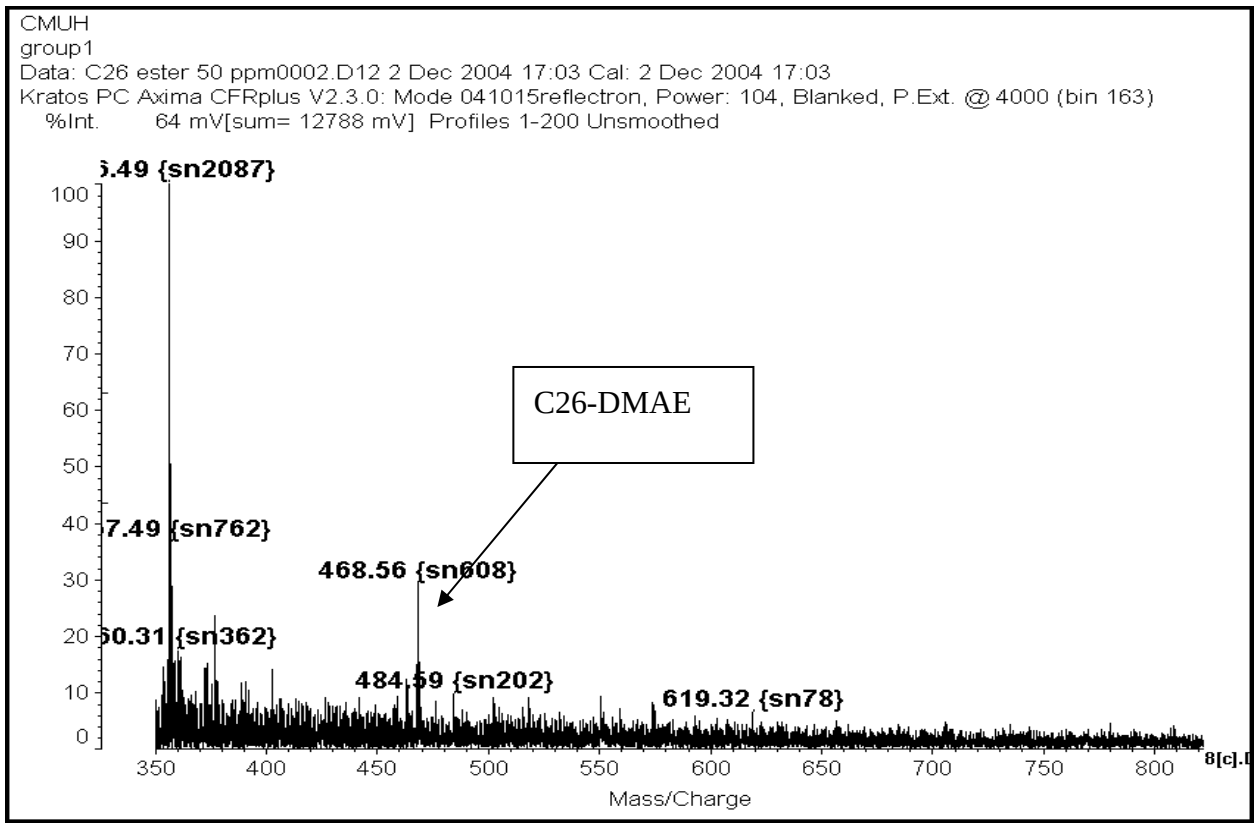


圖 7. 經 DMAE 酯化之極長鏈脂肪酸 hexacosanoic acids 之 MALDI-TOF 質譜圖，濃度：50 ppm，介質：DHB。

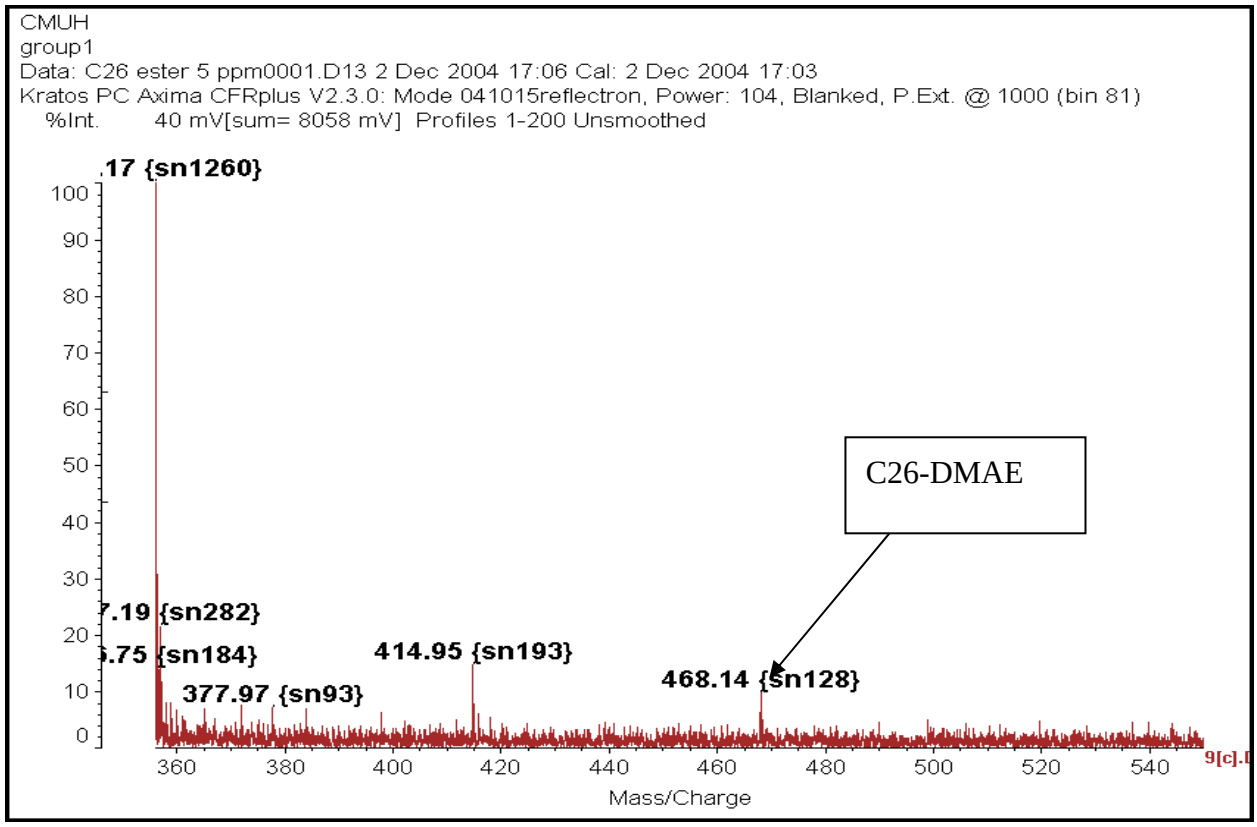


圖 8. 經 DMAE 酯化之極長鏈脂肪酸 hexacosanoic acids 之 MALDI-TOF 質譜圖，濃度：5 ppm，介質：DHB。

(四) 計畫成果自評：

目前過氧化酶體失調的的檢測方式大都利用氣相層析或氣相層析質譜連線技術偵測極長鏈脂肪酸，雖然具有靈敏度及專一性高之優點，但常會有步驟緩慢及自動化困難之情形。大量分析、高專一性、低偵測極限與富含分析物結構物訊息是液相層析-電灑游離/質譜/質譜連線技術的優點，我們成功將此技術應用於國內新生兒極長鏈脂肪酸的偵測，而不同型式的先天性過氧化酶體失調症也將可在一次分析中同時篩檢出來。因此相對於傳統方法，新的先天性過氧化酶體失調症篩檢方法將會更準確,快速,經濟及簡便。目前已達到計畫目標並已應用之實際上線偵測，並與目前本實驗室利用液相層析/質譜/質譜連線偵測氨基酸、醯基肉毒鹼及 17 羥孕酮合併並成功應用於新生兒遺傳代謝疾病之偵測。

第二部份則希望藉由 MALDI-TOF 之快速大量分析為萃取並釋放出血卡中血球及血液中等等所含之所有極長鏈脂肪酸，如: 十四、十六、十八、二十、二十二、二十四及二十六烷酸等，需實驗條件較劇烈之前處理方式，於此利用打孔機將含有樣品之濾紙打下一點(直徑 1/8-inch、約 3.6 µl 之血液) 置於 96 孔樣品盤中，加入含內標準品之試劑後，我們採用了酸化後加熱將樣品中含極長鏈脂肪酸之脂質全轉換成極長鏈脂肪酸，避免傳統複雜之鹼化與多次萃取方式。

針對長鏈脂肪酸之質譜偵測方式，我們採取電灑法與介質輔助雷射脫附法兩種方式去嘗試，希望能得到較適當且快速之分析方法。

附件二

可供推廣之研發成果資料表

□ 可申請專利　□ 可技術移轉　　日期：__年__月__日

國科會補助計畫	計畫名稱：液相層析/串聯質譜儀連線偵測極長鏈脂肪酸於新生兒之先天性過氧化酶體失調之篩選應用 計畫主持人：賴建成 計畫編號：NSC　92－2113－M－039 －003－ 學門領域：M4
技術/創作名稱	質譜儀偵測極長鏈脂肪酸於新生兒之先天性過氧化酶體失調之篩選應用
發明人/創作人	賴建成
技術說明	中文： 本研究為國內首次利用 LC/MS/MS 及 MALDI-TOF 技術來大量及專一性地檢測新生兒採血濾紙樣品中 (約含3.6 μl 血樣) 是否帶有過氧化酶體失調疾病 (如：腦白質腎上腺營養不良症 (Adrenoleukodystrophy, ALD), 髓磷質腎上腺神經病變 (adrenomyelinoneuropathy, AMN), 過氧化酶體生成缺陷 (peroxisomal biogenesis defects, PBD), 雷弗素姆氏病變 (Refsum disease))。不同型式的先天性過氧化酶體失調症也將可在一次分析中同時篩檢出來。因此相對於傳統方法，新的先天性過氧化酶體失調症篩檢方法更準確,快速,經濟及簡便。
	英文：In this study, we provide a high throughput and specific method with potential to screen for many of the peroxisomal disorders (ex. adrenleukodystrophy (ALD), adrenomyelinoneuropathy (AMN), peroxisomal biogenesis defects (PBD), Refsum disease and etc.) with a 3-mm blood spot (~3.6 μl blood impregnated on filter paper) from newborns using LC/MS/MS and MALDI-TOF. It is practicable to detect all VLCFA at a time related to peroxisomal disorders in blood using MS. Compare with traditional screening method, this new method should be fast, reliable, reproducible and relatively cheap. Currently, the amino acids, acylcarnitine and 17-hydroxyprogesterone profiling of dry blood specimens using LC/MS/MS has been developed and recognized as a useful tool for screening inherited metabolic defects of newborns in our laboratory.
可利用之產業及可開發之產品	國內各大醫學中心之新生兒篩檢項目新增

技術特點	因此相對於傳統方法，新的先天性過氧化酶體失調症篩檢方法更準確,快速,經濟及簡便。
推廣及運用的價值	可大量、準確、快速、經濟及簡便的篩檢國內所有新生兒是否患有先天性過氧化酶體失調症。

※ 1.每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

※ **2.本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

※ 3.本表若不敷使用，請自行影印使用。