

出國報告

(出國類別：其他)

參加「第五屆亞太核磁共振雙年會暨澳洲核磁共振年會」報告

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內容摘要

亞太核磁共振雙年會為兩年舉辦一次，今年為第 5 次在澳洲布里斯班 (Brisbane, Australia, 10/27~10/30) 舉辦，本次活動共有約 500 人參加並分：蛋白質結構、生物分子應用、藥物開發設計、小分子應用及核磁影像等 5 大主題發表(口頭及海報)論文約 250 篇。

本局發表的海報論文，論文題目為「Identifying Artificial Flavor Compositions through NMR Mixture Analysis」，本研究利用液相層析時間飛行式高解析度質譜儀串聯固相萃取裝置及核磁共振設備(LC-ToF-SPE-NMR)，同時鑑別人工香料中不特定未知成分分子式及化學結構，預計該項技術可以補強法定檢驗中特定目標檢測中不能檢出未知成分的漏洞，有效的壓制目前層出不窮的惡意商品摻偽。

本次雙年會中發表的約 250 篇論文，針對本局未來核磁共振相關的題目，概分為六大項目：多變數分析、固相核磁共振技術、電磁式低磁場設備、非固定時間取樣技術、殘留磁矩偶合的應用及中國大陸新興基因體研究中心，其中前三項和本局目前研究主題較相關，接下來的兩項為核磁共振的最新發展技術，最後一項為兩岸競合的議題，分別報告。

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壹、緣起及目的

本次出國參加亞太核磁共振雙年會係依 102 年度「參與重點發展科技之國際標準化活動」科發基金計劃第 6 項辦理，原先預定參加歐美地區美國質譜學會年會活動發表「以基質輔助雷射游離時間飛行式質譜儀鑑別人造纖維組成」海報論文(如附件一)，因作業不及奉准改參加亞洲地區亞太核磁共振雙年會並展示海報論文「以核磁共振混合物分析技術鑑別人工香精成分」(如附件二)，意外地，有機會在同一年度分別籌劃兩項重要化學分析技術的活動，並不容易，在本局同仁的協助下還是完成了。

持續參與重點發展科技之國際活動是本局近年來努力的目標，直接參加國際學術團體年會並和學者專家面對面交流研究成果，更是相關計畫目標的具體實踐，核磁共振是一門博大精深的學問，被應用於廣泛的領域，筆者因為接觸到這項技術的時日尚短，不懂的比懂的多得多，在此僅就個人能力所及為各位報告。

本次雙年會中發表的論文，針對本局未來核磁共振相關的題目，概分為六大項目：多變數分析、固相核磁共振技術、電磁式低磁場設備、非固定時間取樣技術、殘留磁矩耦合的應用及中國大陸新興基因體研究中心，其中前三項和本局目前研究主題較相關，接下來的兩項為核磁共振的最新發展技術，最後一項為兩岸競合的議題，逐項報告如後。

貳、過程

雖然實際參加年會的時間只有 3 天半的時間(10/27~10/30)，行程表詳如表一，但所需要準備的時間及同仁的協助遠遠超過預計的數量，僅就個人記憶所及報告如下：

一、研討會活動摘要

亞太核磁共振雙年會今年為第 5 次在澳洲布里斯班(Brisbane, Australia)舉辦，本次活動共有約 500 人參加並分：蛋白質結構、生物分子應用、藥物開發設計、小分子應用及核磁影像等 5 大主題發表(口頭及海報)論文約 250 篇，本局本次所發表的人工香料是屬於小分子應用，雖然是亞太雙年會但除了中國、日本、韓國、香港、台灣、新加坡、印度、土耳其等亞太地區國家參加以外，也有美國及瑞士等非亞太區研究人員參加。

本次活動行程由 102 年 10 月 25 日至 31 日共 7 天，扣除交通往返時間外仍是相當緊湊，一路同行的還有國內相關研究人員分別來自中研院生醫所、台灣大學、清華大學、成功大學及華梵大學約 3~40 人，國內的參加學者以研究生物分子及蛋白質應用較多。

二、年會重要議題摘要

本次雙年會中發表的論文，針對本局未來核磁共振相關的題目，概分為六大項目：多變數分析、固相核磁共振技術、電磁式低磁場設備、非固定時間取樣技術、殘留磁矩耦合的應用及中國大陸新興基因體研究中心，其中前三項和本局目前研究主題較相關，接下來的兩項為核磁共振的最新發展技術，最後一項為兩岸競合的議題，逐項報告如下：

多變數分析(Multivariate Analysis)統計方法中的主要成分分析(Principal Component Analysis)及部分最小平方差迴歸法(Partial Least Square Regression)，運用上述統計方法分析配合核磁共振技術，辨別物料摻偽、區分物品來源、篩選代謝物(或污染物)等相關應用，不但是跨統計及化學分析兩大學門的新興研究，也是目前國際關心的議題，傳統的核磁共振技術以鑑定純物質為主，混合物的圖譜多半過於複雜難以分析，借助統計分析方法可以由大量的相關混合物圖譜中分析比較其異同，建立起混合物的分析技術，表二為多變數法和傳統分析方法的比較。本局預定於 103 年自行研究計劃「以多變數分析配合核磁共振技術調查市售檜木精油來源」中首次利本項技術調查市售檜木精油的來源，由初步可行性試驗中已經可以利用氫核核磁共振圖譜(如圖一)及主要成分分析法(如圖二)區分出不同種類的精油(檜木、玫瑰及迷蝶香精油)，並區分出部分異常的檜木精油產品，預訂於 103 年完成相關研究提供相關單位參考。 本次雙年會中也有日本及韓國的學者利本項技術調查海洋四周底泥的成分分布(Chemical Profiling with NMR-Based Chemical Diversity Index in Deep-Sea, Neritic and Estuarine sediments abs#114)、區分不同採收季節的綠茶(NMR Metabolomics Study for Quality Control of the Hadong Green Tea Classification by the Plucking Season abs#231)及篩選前列腺病理組織的代謝物(Aim for the Cup: non-

Invasive Diagnosis of Prostate Cancer through NMR-based Metabolomics abs#193)等論文，雖然數量較少，但相關技術相當適合本局主管的商品檢驗領域，預料相關技術將可以在未來幾年間成為本局有特色的檢驗技術。

固相核磁共振(solid state NMR)技術也是本局目前積極切入的核磁共振技術，目前大眾化核磁共振設備及相關應用仍是以液相核磁共振(liquid state NMR)技術為主，固相核磁共振設備較特殊而使用技術門檻較高，因此目前為止還未普遍化應用，但對本局主管的商品檢驗領域而言，仍是固相樣品為主，液態樣品較少，對於固相樣品以往常需要先設法溶解轉置為液態之後才能分析，在液態化的過程中不但破壞了固態樣品的固體特性也會增加試驗的複雜性，直接應用固相核磁共振技術可以簡化試驗並量測出樣品的固體特性(如結晶、方向性等特性)，較液態技術更適合本局主管的商品檢驗領域。本局曾於101年自行研究計畫「以核磁共振技術鑑別巧克力食品中的成分組成」中利用膠態進樣探頭成功地分辨出市售巧克力商品中可可脂成分的不同(如圖三)並預定於103年採購固相進樣探頭設備並預訂於104年開始有關塑膠材質的核磁共振研究，屆時將可進入此項技術。本次雙年會中有關固相核磁共振應用的論文相當多無法一一列舉，其中和本局未來業務較相關臚列如下:生質材料熱裂解產品(NMR Characterization of Biomass-PET Pyrolysis Product)、天然氣水合物的結構研究(Effect of Kinetic Hydrate Inhibitors on Natural Gas Hydrate Formation and Structure Analysis by Solid State NMR)、鋰電池電極複合材料研究(Solid State NMR of Transition Metal Alloy-Modified LiMn_2O_4 for Composite Lithium-Ion Battery Cathode)、奈米材料(Solid-State NMR Study of Surface Structure of CdSe Magic-Size Nanoparticles abs#181)等。

電磁式低磁場設備為核磁共振應用的一大突破，電磁式低磁場設備雖然因為磁場較小(60~200 MHz)解析能力不如低溫超導體為主的脈衝式核磁共振儀，但因為不需要低溫環境，所以價格較低且可以在一般環境下作業，大大的提昇了核磁共振技術的工業應用，國內的石化大廠都已經使用這樣的設備做為生產品管，預料再未來將可以取代許多傳統的工業檢測設備，表三為核磁共振製程分析和傳統工業分析技術之比較。本次除會場上有3家廠商做實機展示，也有一篇報告(Applications of a Benchtop NMR Spectrometer in a University Chemistry Laboratory abs#203)，顯示出廠商對這項設備的重視。

非固定時間取樣技術(non-Uniform Sampling)應用於脈衝式核磁共振儀，由於脈衝式核磁共振儀的工作原理是以固定時間取樣的方式紀錄待測樣品，受脈衝激發後的自由衰退時域訊號，再以傅立葉轉換而得到受測核種自旋頻率訊號，但所測得頻率的精確度和核種自旋頻率、自由衰退速度以及取樣的頻率有關，適當的變化取樣的頻率可以提高核磁共振對微細結構的解析能力，目前這樣的技術被有效的應用於解析生物分子的結構。

殘留磁矩耦合(Residual Dipolar Coupling)的應用，在液態樣品可以利用加入液晶結構或者其它的方式，使得待測的化學結構局部性呈現特殊排列，待測的原子核因為這樣的特殊排列關係而產生局部類似固相樣品的方向性關係，通常利用不同的方式產生殘留磁矩耦合，可以測知巨大分子特殊部分的微細結構。以上兩種技術都被應用於解析生物分子的結構。

中國大陸新興基因體研究中心預定設立於上海，有鑑於相關領域的快速成長與市場價值，預計將有更多國家會投入這個已經十分競爭的領域，國內的研究者必需建立自己的特色與利基市場才能留在贏者圈。

三、標檢局核磁共振研究紀要

本局核磁共振設備於 98 年採購，自 99 年起陸續執行各項研究計畫(詳如表四)，充分利用所採購的三個探頭，逐年建立相關技術能力，由較廣泛使用的液相探頭開始，一路摸索至國內較少使用的膠態及液相層析串聯探頭，所應用的範圍也由深層海洋水、食用油(如圖四)，逐漸深入至生質柴油、橡膠、巧克力及未知成分的人工香料等問題，並計劃自 103 年增購固相探頭，更進一步擴充相關應用範圍。

四、參展海報論文摘要

本次發表的海報論文，論文題目為「Identifying Artificial Flavor Compositions through NMR Mixture Analysis」，海報原稿詳附件 1，本研究不使用特定目標成分標準品比對的方式而開發不特定目標檢測的方法，利用液相層析時間飛行式高解析度質譜儀串聯固相萃取裝置及核磁共振設備(LC-ToF-SPE-NMR)，同時鑑別人工香料中不特定未知成分的分子式及化學結構，以確認未知成分，預計該項技術可以補強法定檢驗中特定目標檢測中不能檢出未知成分的漏洞，可有效的壓制目前層出不窮的惡意商品摻偽，另於本次會議收集有關 NMR 儀器在化工商品之檢測應用資訊。

圖四為本研究所使用之 LC-ToF-SPE-NMR 儀表系統，本系統結合液相層析時間飛行式高解析度質譜儀串聯固相萃取裝置及核磁共振設備，本系統雖然功能強大但因為儀器系統串接複雜，使用的門檻很高，在國內使用單位屈指可數，未知人工香料樣品稀釋後，先以液相層析(LC)分離後，再經過分流，5%進入飛行時間式高解析度質譜儀(ToF)測精確分子量，95%進入自動固相萃取設備純化並置換溶液成為重氫乙晴後，再進行核磁共振分析。

圖五為本研究所使用的市售人工香料樣品，包括了牛奶、鳳梨、蘋果、栗子、藍莓、玉米、起司、香草等式人工香類 20 種，多數的市售香料只標示其香味而未標示其化學成分，利用本研究所建立的方法，在不知道特定目標分析物且沒有標準品的情形下，可以鑑定出這些商品中未標示的香精或其它未知成分。

圖六為牛奶香料成分之精確分子量及同位素分布，利用飛行時間式高解析度質譜儀所測得的精確分子量配合線上校正(in-line Calibration)的方式，

在小分子量的範圍內(< 400 amu)，已經可以測到誤差在 2 mDa 內，利用所得到較精確的分子量，可以提昇對未知成分小分子結構分析能力推定未知成分的可能分子式，以做為鑑定未知成分的化學結構的依據。可以提昇對未知成分小分子結構分析能力。

圖七為牛奶香料之氫核核磁共振圖譜，上圖為香料混合物之圖譜，未經液相層析分離前，直接以重氫氣仿為溶劑分析，因為香料商品本身是不明成分的混合物，所含的成分較複雜，其核磁共振圖譜複雜難以解析其化學結構，B10_1 至 B10_4 為牛奶香精經液相層析分離後的各波峯成分，再經過自動固相萃取設備純化並置換成重氫溶劑(重氫乙晴)後，所得到的個別成分較單純可解析化學成分的氫譜圖，由混合物圖譜中可以發現各波峯成分圖譜訊號，其中 2 ppm 左右的兩個信號分別為殘留的液相層析流動相成分(乙晴及水)可不予理會，B10_1 為溶劑峯無法解析出其它成分牛奶香料成分之精確分子量及同位素分布，B10_2~B10_4 各波峯成分經解析後分別鑑定為乙基麥芽酚(Ethyl Maltol $C_7H_8O_3$, MW 140)、香草素(Vanillin $C_8H_8O_3$, MW 152) 及乙基香草素(Ethyl Vanillin $C_9H_{10}O_3$, MW 166)等香料成分，以上鑑定的結果均符合圖六精確分子量所推定的分子式，合併高解度質譜儀及核磁共振設備可以得到更豐富的化學結構資訊。

人工香精以及許多其它的不明添加物是現今國內外消費者關注的焦點，以牛奶香料研究為例，類似的產品推定被廣泛地使用在各類的產品中，但相關產品的資訊揭露卻相對欠缺。以往的法定檢驗以特定目標檢驗為主，必需取得特定的化學標準品才能執行，不是所指定的特定成分(未知成分)或者是沒有標準品就無法檢測，整合液相層析串聯時間飛行式質譜儀及核磁共振儀，可以鑑定未知物化學成分，符合未來檢驗的需求。

參、心得及建議

參加國際學術團體主辦的亞太核磁共振年會，直接和學者專家面對面交流核磁共振研究成果，無疑是快速跨入相關技術的有效方法之一，以下僅提供三點個人心得及建議供各位長官參考：

- 一、核磁共振是一門博大精深的學問，被廣泛地被應用於各種領域，對於任何化學應用工作都是一項不可或缺的工具，隨著相關產業的普及，預料在未來有充分的發揮空間，除了傳統的結構鑑定及反應監測外，配合多變數分析應用於製程監控和來源鑑別等新興的研究領域相當值得投入。
- 二、標檢局的目前業務現況，在核磁共振技術才剛起步，在設備及人才技術方面都無法和國內的學術機構相比，但在研究主題上，特別是摻偽防制及來源鑑別等方向，在國內已經有相當特色，未來應思考相關技術的開發與應用。
- 三、參加國際學術團體年會並和學者專家面對面交流研究成果是一項需要持續努力及投注的工作，有意願參與未來類似國際活動的同仁應及早規劃努力。

表二、比較傳統分析及多變數分析

傳統分析	知識背景分析	多變數分析
可適用一般樣品及各種目的	介於兩者之間	僅適用特定的目的
須背景(樣品及 NMR)知識輔助才能判斷	須具備特定基本圖譜資料	對背景知識要求較低
對統計分析能力要求較低	介於兩者之間	對統計分析能力要求較高
對於圖譜的穩定性要求較低	介於兩者之間	須要多筆高穩定性樣品資料，才能統計分析
無樣品數目的限制	介於兩者之間	須要多筆同種類樣品資料，才能統計分析

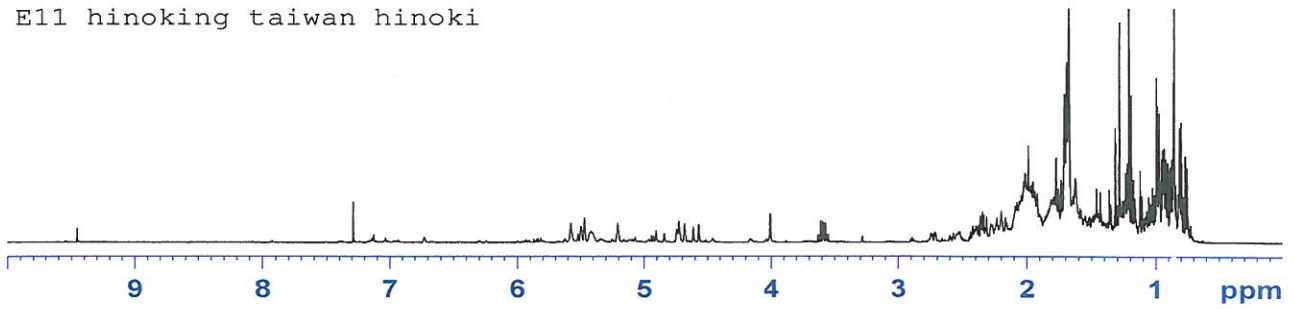
表三、核磁共振製程分析和傳統工業分析技術之比較

分析技術	核磁共振	傳統工業分析
使用設備	製程共核磁共振儀	液相層析儀(HPLC), 氣相層析儀(GC)(DSC), (TGA) 紅外光譜儀(IR) 等
原理	測化學組成結構回估特定工業性質	測工業標準所需特定工業性質
優點	單一儀器可測多種工業性質 不需各別參考物質 缺少工業標準 前處理簡單, 不受含水量及透明度干擾 檢驗速度較快, 但零敏度差	需要配合使用多種儀器 需要各別參考物質 可配合傳統工業標準 前處理較複雜, 受含水量及透明度干擾 檢驗速度較慢, 零敏度較優

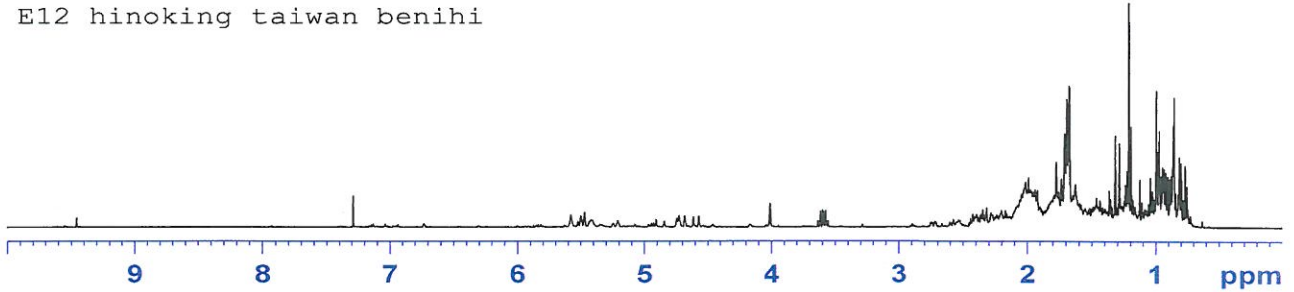
表四、本局歷年執行核磁共振儀相關計劃一覽表

	液相探頭(BBFO)	膠態探頭(HRMAS)	液相析串聯探頭(LC-NMR)
FY103	檜木精油種類鑑別		檜木精油未知成分結構.
FY102			人工香料中未知成分結構
FY101		巧克力食品中的成分組成	
	環保柴油中生質柴油添加量	橡膠製品中加工油多環芳香煙	
FY100	食用油種類鑑別		
	生質柴油脂肪酸分布		
FY 99	¹⁷ O 峰半高寬深層海洋水		

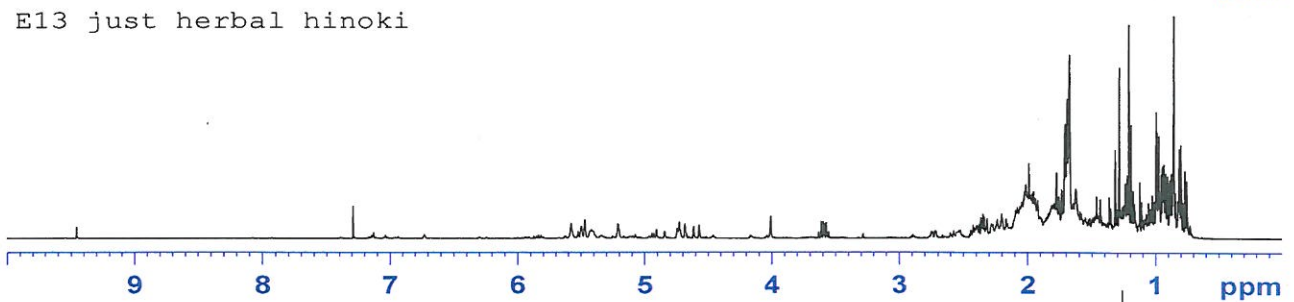
E11 hinoking taiwan hinoki



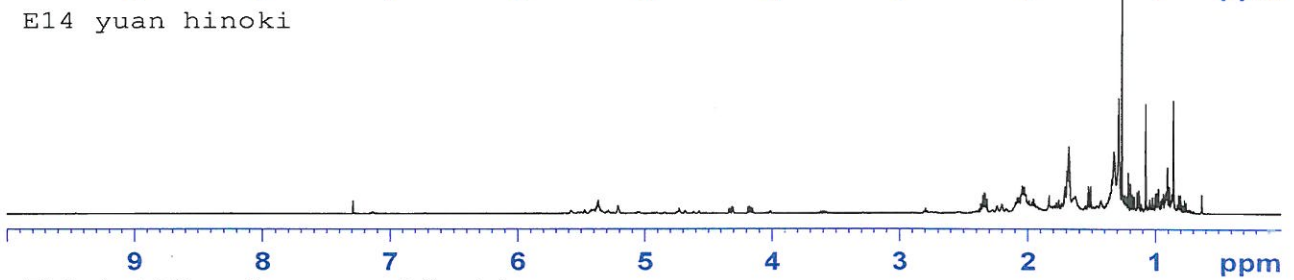
E12 hinoking taiwan benihi



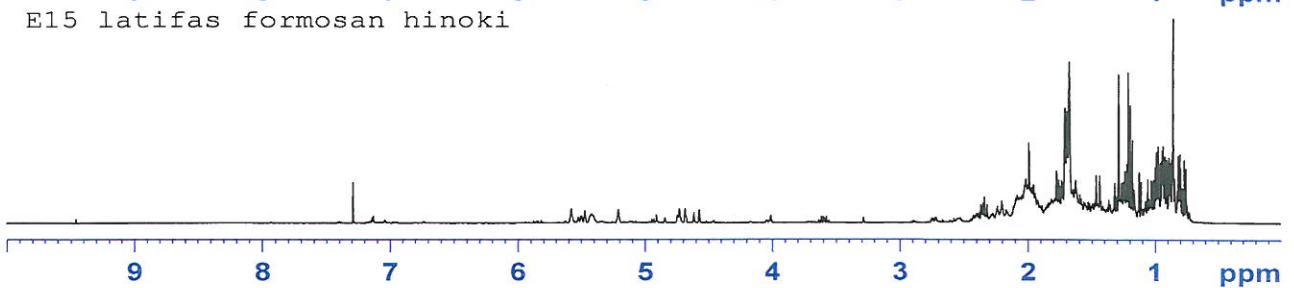
E13 just herbal hinoki



E14 yuan hinoki



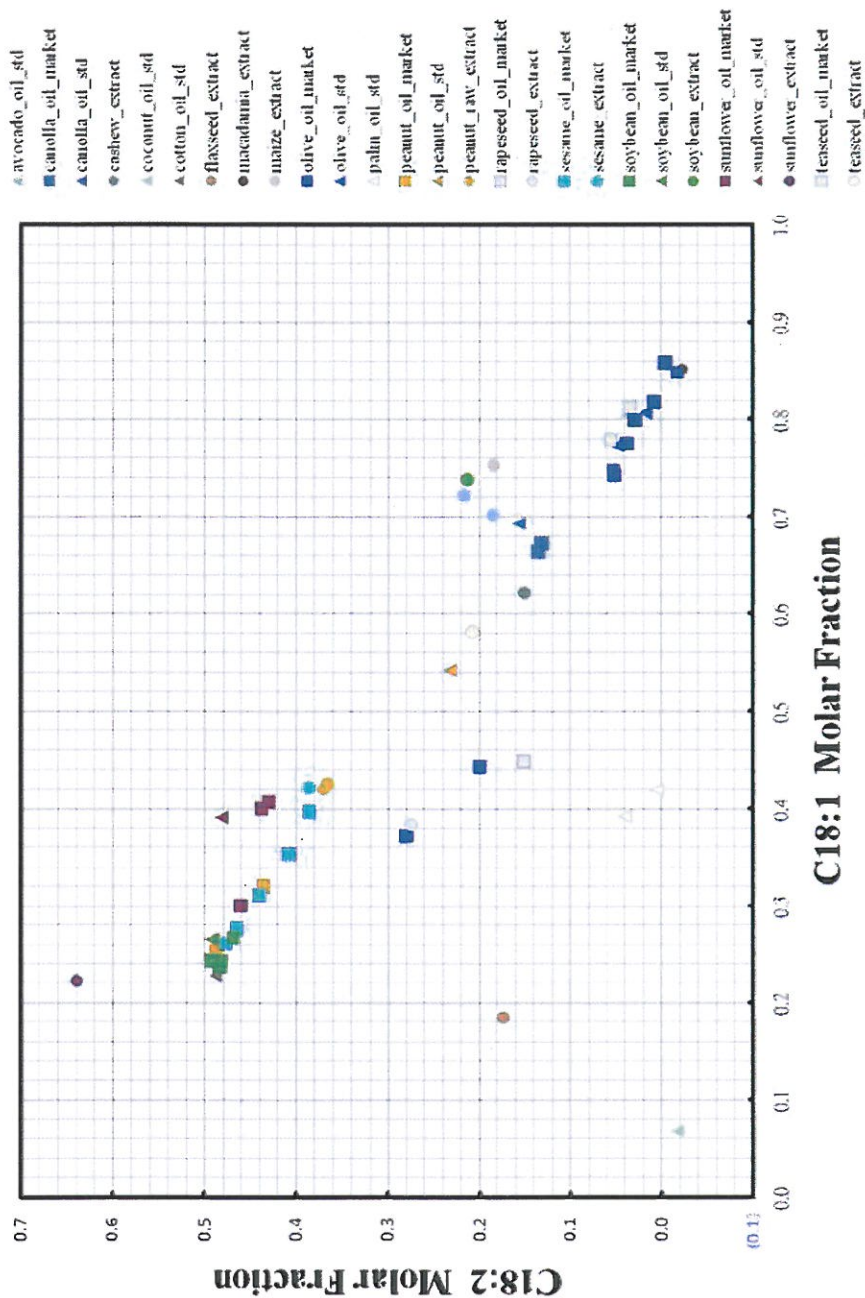
E15 latifas formosan hinoki



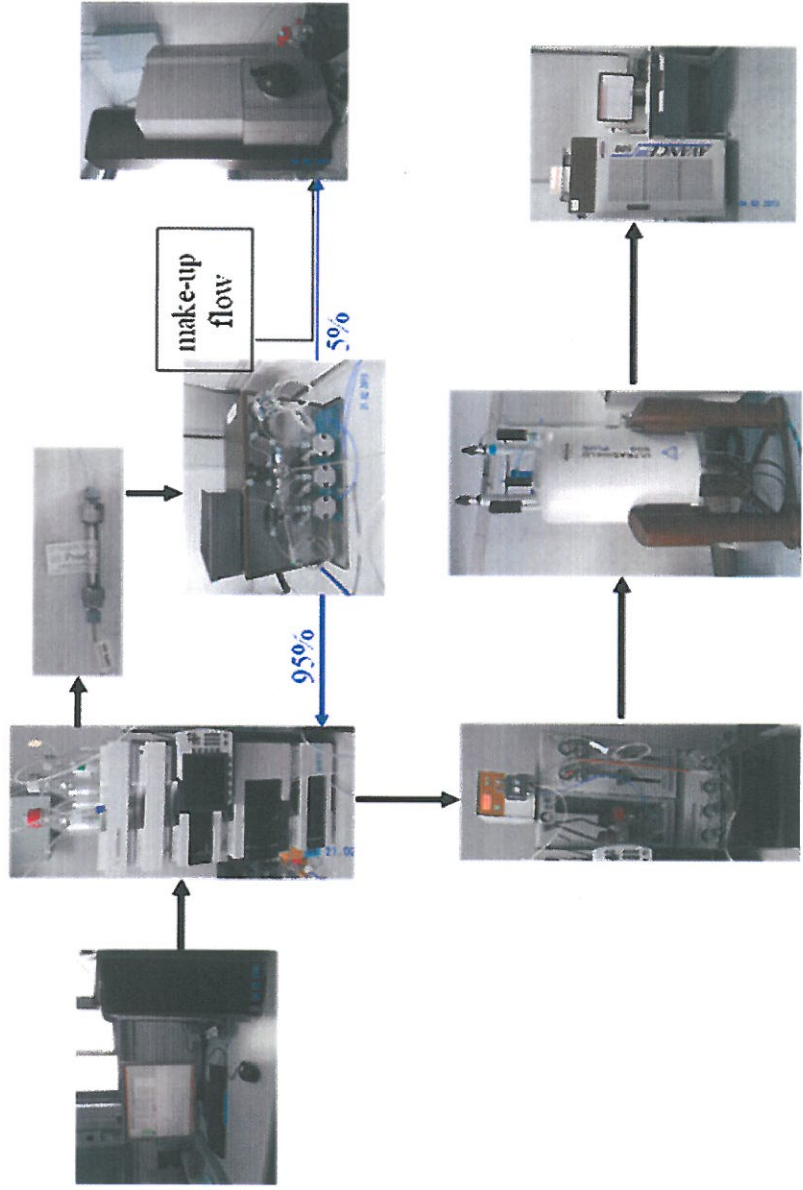
圖一、檜木精油樣品氫核核磁共振圖譜 (E11-15)

其中 E11-13 及 E15 非常接近，反應在多變數分析上自成一個群組。

E14 圖譜和其它樣品不同，由圖譜判斷可能添加植物油成分



圖三、以核磁共振儀檢測食用油研究結果：56種市售植物油之油酸(C18:1)及亞麻仁酸(C18:2)分布，黃豆油(綠色)及葵花油(紫色)油酸量低，檢出結果標準品和市售樣品相當一致；橄欖油(紅色)及茶油(灰色)含油酸\較高，其中部分市售橄欖油樣品脂肪脂肪酸分布情形和標準品差異大

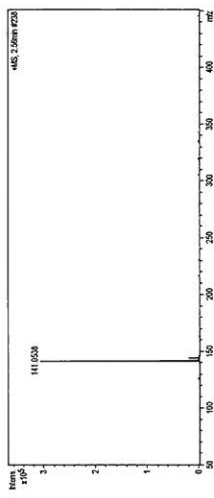


圖四、LC-ToF-SPE-NMR 儀表系統 (海報上半部背景)，未知人工香料樣品稀釋後，先以液相層析(LC)分離後，再經過分流，5%進入飛行時間式高解析度質譜儀(ToF)測精確分子量，95%進入自動固相萃取設備純化並置換溶液成為重氫乙晴後，再進行核磁共振分析

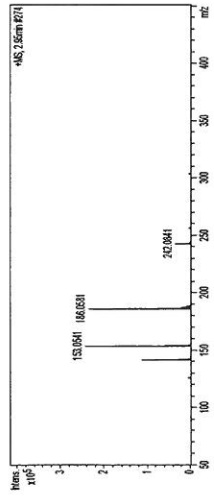


圖五、海報圖 1 人工香精(部分樣品)照片

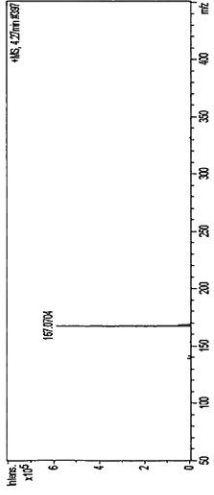
B10_2



B10_3

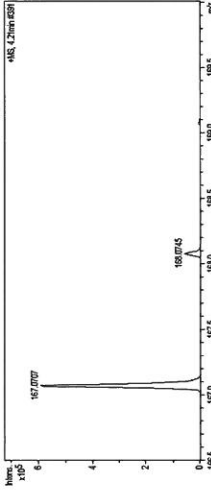
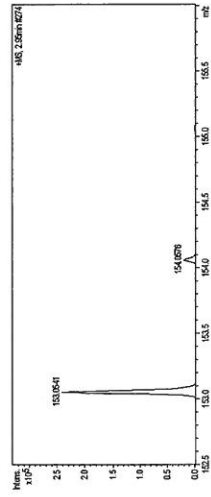
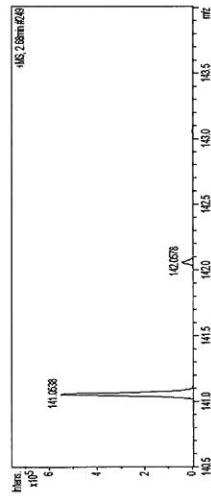


B0_4

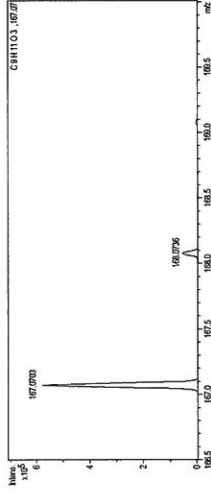
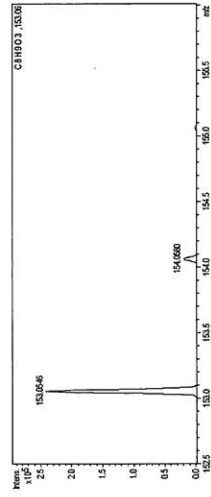
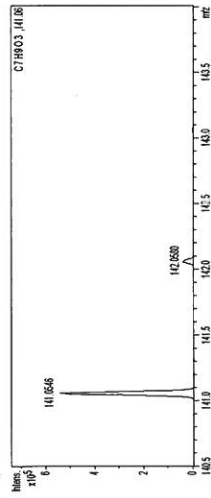


Full Spectra
50~450 Da

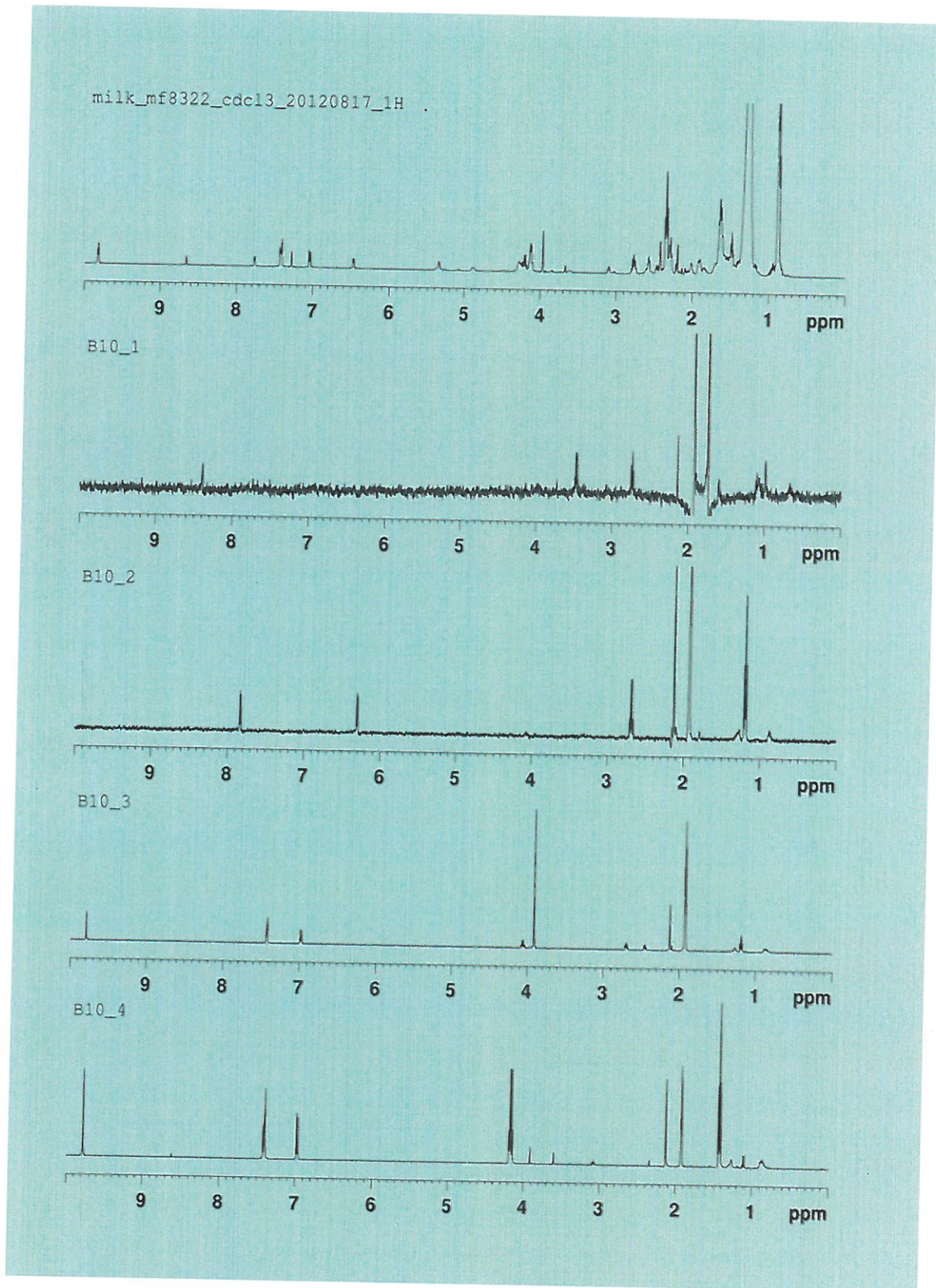
Measured Spectra



Calculated Spectra



圖六、牛奶香料成分之精確分子量及同位素分布(海報圖2)，以時間飛行式高解析度質譜儀測得之牛奶香料中未知成分精確分子量(質量誤差小於 2 mDa)及同位素分布([M+H+1]及[M+H+2])資料推測未知人工香料成可能的分子式，第一行三個未知成分的全範圍譜圖，第二行為第一行的局部放大，由第二行可以看出未知物[M+H]⁺的精確分子量及同位素分布；



圖七、牛奶香料之氫核核磁共振圖譜，上圖為香料混合物之圖譜，因為所含的成分較複雜難以解析其化學結構，B10_1 至 B10_4 為牛奶香精經液相層析分離後，再經過自動固相萃取設備純化並置換成重氫溶劑(重氫乙晴)後，所得到的個別成分較單純可解析化學成分的氫譜圖，其中 2 ppm 左右的兩個信號分別為殘留的液相層析流動相成分(乙晴及水)，B10_1 為溶劑峰無法解析出其它成分



Quantitative Polymer Analysis of Fiber Mixture by Matrix Assistance Laser Desorption Ionization Coupled Time of Flight Mass Spectrometer (MALDI-ToF MS)

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Abstract
 Labeling of textile products is legally required for textile management purposes and the utilization of traditional advanced traditional methods, such as dye reaction method and FT-IR method [1]. These two methods can only provide overall properties of sample polymers which is difficult to differentiate subtle differences of commercial products. MALDI-ToF technology provides a new tool to investigate complex polymer systems in detail and is expected to help establish the market linkage between polymer composition and chemical properties [2]. Despite MALDI-ToF technology has been one of the main tools in polymer analysis, there is one limitation reported in applied MALDI-ToF analysis to commercial products for market management purposes. In this study, a matrix sample labeled as pure PET was subjected to various fiber mixtures. MALDI-ToF mass spectrometry technology of polymer mixtures was attempted to evaluate the fiber mixtures of a textile sample with microscopic elastic properties in this study.

Keywords
 Textile sample, The matrix sample in question was first dissolved into fibers according to weaving directions. The fibers were then dissolved in alcohol according to BSMI (BSMI). The main reason of fiber to fiber labeled high content of polymer in fibers. Collect the solution for MALDI-ToF MS analysis. Sample Preparation: A dried sample sample preparation method with 2,5-dihydroxybenzoic acid (DHB) matrix was chosen in this study. MALDI-ToF instrument: Bruker microflex MALDI-ToF spectrometer in Linear Positive Mode. A nitrogen laser (337 nm) was used to excite the sample.

Results and Discussion
 The mass spectra of five fiber samples were from different weaving directions of the same textile which performs microscopic elastic properties are presented in Fig. 1. Fig. 1 shows that fiber (1) contains repeating units of polyethylene terephthalate (PET) 192 Da only but fiber (2) contains both PET and polyethylene terephthalate (PTT) repeating units (both 192 and 266 Da sequences). Fig. 1 includes data within the same textile product, fibers in different polymer composition are using in different weaving directions to give the microscopic elastic properties. Consequently, labeling this product as PET only may not be appropriate. The above result has been further confirmed before by related samples provided by local fiber manufacturers.

The mass spectra of three selected fiber samples with corresponding pictures and FT-IR spectra are presented in Fig. 2. These samples are provided with polymer composition information by local fiber manufacturers. Among them, S1 is mixed PET and PTT (fiber 32 is pure PET fiber and 23 is pure PTT fiber). From pictures, 1 is appearance are indistinguishable. However, from FT-IR spectra (traditional method), these samples show an significant difference. It reflects that all three samples have similar functional groups. The relative difference in the length of methylene chain (PET has two methylene in repeat unit and PTT has three methylene units) is negligible in FT-IR spectra but always obvious effect as appearance. From MALDI-ToF spectra, the polymer composition of these three samples can be related to their characteristic mass difference sequences (266 Da for PTT, and 192 Da for PET). Fig. 2 shows that the MALDI-ToF method does provide relative information in detail than traditional FT-IR method to help clearly subtle difference in polymer structure. In addition, the proposed method can be applied to investigate commercial products directly which makes possible a retrospective study, the study to investigate the origin of artificial fiber from market surveillance, in the future.

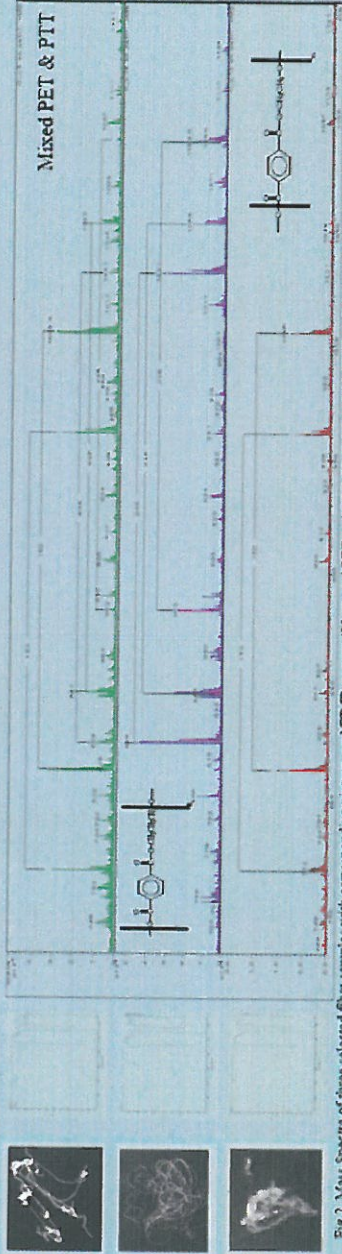


Fig 2. Mass Spectra of three selected fiber samples with corresponding pictures and FT-IR spectra (S1: mixed PET and PTT, S2: PTT, and S3: PET). Different appearance was observed from pictures. However, from FT-IR spectra (traditional method), no significant difference was found (same functional groups). From the MALDI-ToF Spectra (proposed method), different polymers can be identified by their characteristic mass difference sequences (PTT: 266 Da, and PET: 192 Da.)

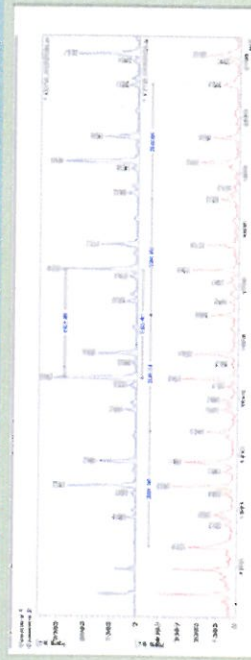


Fig 3. Mass spectra of two fiber samples taken from different weaving directions of the same textile which performs microscopic elastic properties. These spectra reveal the existence of different polymer contents in fibers of the same textile, explained in Fig. 2.

Conclusions

This study successfully applied MALDI-ToF mass spectrometry to verify the polymer content in a market management case and further confirmed the result by identified fiber samples, which are indistinguishable by traditional methods.

Acknowledgments: The authors would like to express their deepest thanks to local textile manufacturers for providing fiber samples and to experts from Bruker Daltonics (Taiwan) for their technical support in this study.

References

1. JST J1830-2/04, "Testing Methods for Quantitative Analysis of Fiber Mixtures of Section - Part 2: Testing Methods for Quantitative Analysis of Fiber Mixtures"
2. Hultin, S.D., "Mass Spectrometry of Polymers and Polymer Surfaces", Chem. Rev. 2004, 104, 537-569.

17. 2007 Institute for Textile Management (BSMI), www.bsmi.gov.tw



Identifying Artificial Flavor Compositions through NMR Mixture Analysis

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¹ Bureau of Standards, Metrology and Inspection, Chung Yuan, Taipei, Taiwan

Introduction

NMR mixture analysis is applied to identify artificial flavor products in this study. 32 samples were collected from market surveillance action for public administration purposes and most of them do not label the composition of artificial flavor. NMR mixture analysis combining with lately developing hyphenated technology and multi dimension experimental skills was chosen to identify the chemical structures of these artificial flavor samples and hopefully to initiate a non target structure elucidation method which is applicable to variant types of sample matrix.

Artificial Flavor Samples

32 artificial flavor samples used in this study were collected during market surveillance plan, see figure 1. These samples were supposed to be used as food additive. A wide varieties of artificial flavors were included, namely, almond, cheese, glassware, corn, lemon, melon, milk, pudding, and so on. Most of these samples were poorly labeled, especially provided no information about ingredients required for food safety management consequently non targeted structure identifying method is needed.

Instrument

A relatively complicated instrument system is chose for the proposed non target analysis to identify the chemical structures of unknown artificial flavors, illustrated as back ground picture. Liquid Chromatography, high resolution Time of Flight (ToF) mass spectrometer operated in Electrospray Positive (ESI+) mode, automated on-line nonpolar Solid Phase Extraction (LK-SPE) and 500 MHz Nuclear Magnetic Resonance with an inverted type flow probe were included. Accurate mass and NMR informations are used interactively to identify unknown chemical structures. Elementary molecular formula obtained from ToF may have many possible chemical structures. NMR information help identify the correct stereoisomers and further confirm the molecular formula proposed by accurate mass information.

Illustration Example

The analysis of an artificial milk flavor was selected to demonstrate the concept of mixture NMR Analysis, because the studied milk flavor came from most different sources.

Figure 2 depicts the high resolution mass spectra of three (2-4) non resolved peaks, discussed later. The upper 2 rows present ToF spectra of a wider mass range (50-500 Da) and smaller range (± 5 Da) to illustrate both the ToF accurate mass and relative isotope information, the 3rd row presents the calculated mass spectra based on theoretical accurate mass and relative isotope information of the highest ranked possible elementary molecular formula of unknown chemical ions $[M-H]^+$, the most common signals of Electrospray Positive (ESI+) mass spectra signals. Figure 2 indicates that the calculated mass spectra (3rd row) of all three peaks agree well with the experimental ToF spectra (upper 2 rows) in both accurate mass and

relative isotope ratios, which reflects that the proposed elementary molecular formula have relatively good chance to be correct, especially for low mass range (< 500 Da). In addition, all three proposed possible elementary molecular formula were later confirmed by ¹H NMR from LC-SPE-NMR spectra. In the wider spectra of Peak 3 (1st row, 2nd column), mass signal 153 was selected among other three mass signals (141, 186 and 204), with the help from NMR results. The other 3 mass signals indicated the possible factors such as co-elutants, fragmentation or different charge states, which required extra cautions during analyzing mass spectra and will not included in NMR discussion. The similar phenomena should be taken into consider during proposing possible elementary molecular formula candidate using accurate mass spectra.

Figure 3 includes ¹H NMR spectra of milk flavor mixture and 4 separated peaks. The mixture spectrum (1st spectrum) was collected separately using a common solution NMR probe and deuterated chloroform solvent, which are too complicated to resolve its ingredients, like spectra of most mixture samples. Spectra of 4 peaks were collected in LC-SPE-NMR system with a double solvent suppression pulse and deuterated acetonitrile solvent. Peak 1 spectrum shows no significant signals to resolve. However, peak 2-4 spectra can be resolved and identified in cooperation with accurate mass information as ethyl methyl, vanillin and ethyl vanillin, individually, and later confirmed with manufacturer's spectra. All three compounds are listed in common artificial flavor. Among them, ethyl vanillin is considered as an artificial substitute of vanillin, which is not derived from any natural identical source. Figure 3 also indicates that peak 2-4 spectra, in combination, resemble most resonances in the milk flavor mixture spectrum, which means that the resolved peak 2-4 spectra represent most ingredients in the spectrum of mixture. This result demonstrates that complicated NMR spectra of mixture can be decomposed to simpler, more resolvable separated spectra with the help of more complicated LC-ToF-SPE NMR instrument system.

Facts and Thoughts

Facts and thoughts benefited from the experience of this study are briefly list below.

Multiple-disciplines involving domestic expert, NMR, mass spectrometry, chromatography and instrumentation are required to operate and maintain this complicated instrument system (LC-ToF-SPE-NMR).

Mostly, only ¹H NMR spectra are practical, and ¹³C spectra are rarely possible, due to the limitations of inverted flow probe and relatively low concentration sample.

Possible interactions between sample ingredients and chemicals of instrument system are worth notice when more complicated instrument system and longer time are required to obtain final analysis results.

LC-ToF-SPE-NMR proved to be a powerful and promising technique for identifying unknown chemical structures from mixture samples.

2013. 10. 27-30



Figure 1. Artificial Flavors Selected from 32 Studied Samples

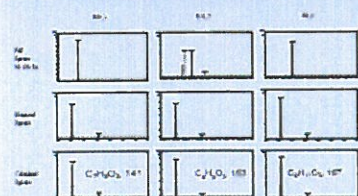


Figure 2. High Resolution Mass Spectra of Resolved Peak 2-4

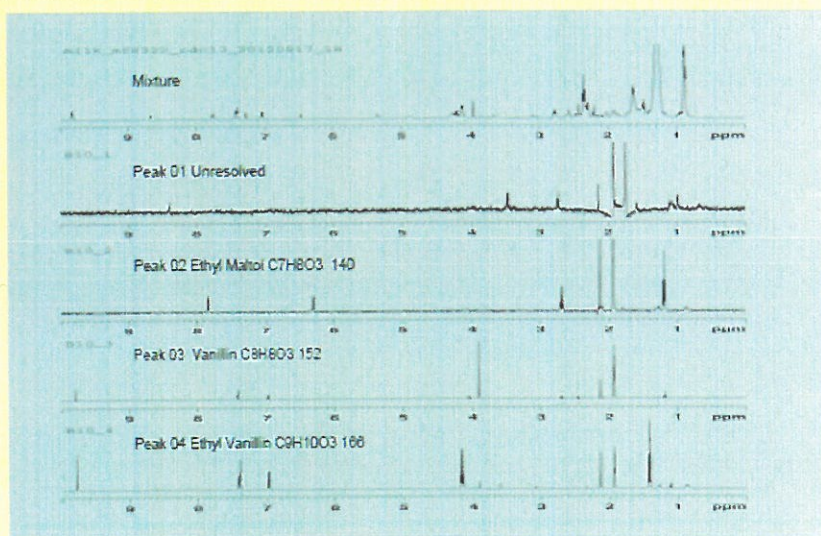


Figure 3. ¹H NMR Spectra of Mixture and Separated Peaks of Milk Flavor

附件三、議程 (原文)

APNMR5/ANZMAG2013 Timetable

Sunday	27 October
14:00 – 17:00	Registration
17:00 – 17:15	Conference Opening Welcome from APNMR. Weontae Lee (Boulevard Auditorium Foyer)
17:15 – 17:55	Plenary Lecture 1: Ad Bax (Boulevard Auditorium)
17:55 – 18:35	Plenary Lecture 2: Ichio Shimada (Boulevard Auditorium)
18:35 – 19:35	Welcome Reception (Boulevard Auditorium Foyer)
Monday	28 October
09:00 – 09:40	Plenary Lecture 3: Gottfried Otting (Boulevard Auditorium)
09:40 – 10:10	Keynote Lecture 1: Yangmee Kim (Boulevard Auditorium)
10:10 – 10:30	Photo of all APNMR Attendees (Boulevard Auditorium Foyer)
10:30 – 11:00	Morning Tea (Boulevard Auditorium Foyer)
11:00 – 12:30	Parallel Session 1: Protein Structure & Dynamics (Boulevard Auditorium) Parallel Session 2: Drug Design & Development (Room B1) Parallel Session 3: Solids & Membranes (Room B2)
12:30 – 13:30	Lunch (Boulevard Auditorium Foyer) APNMR Steering Committee Meeting Presentations by Oxford Instruments (12:30–12:50), JEOL (12:50–13:10, Room B2), and Bruker (13:10–13:30) (Room B2)
13:30 – 15:00	Poster Session 1: Odd Numbered Posters (Boulevard Auditorium Foyer)
14:30 – 15:00	Afternoon Tea (Boulevard Auditorium Foyer)
15:00 – 17:00	Parallel Session 4: Biomolecules I (Boulevard Auditorium) Parallel Session 5: Methods & Small Molecules (Room B1) Parallel Session 6: Imaging & MRS I (Room B2)
17:00 – 17:20	Short break with sponsors (Boulevard Auditorium Foyer)
17:20 – 18:00	Plenary Lecture 4: Gerhard Wagner (Boulevard Auditorium)
18:00 – 19:30	Poster Session 2: Even Numbered Posters (Boulevard Auditorium Foyer)
Tuesday	29 October
09:00 – 09:40	Plenary Lecture 5: Barbara Messerle (Boulevard Auditorium)
09:40 – 10:10	Keynote Lecture 2: Yun-Wei Chiang (Boulevard Auditorium)
10:10 – 10:40	Morning Tea (Boulevard Auditorium Foyer)
10:40 – 12:15	Parallel Session 7: Protein Structure & Interactions (Boulevard Auditorium) Parallel Session 8: Solids, Membranes & Complexes (Room B1) Parallel Session 9: Imaging & MRS II (Room B2)
12:15 – 12:45	Keynote Lecture 3: Peter Lujtjen (Boulevard Auditorium)
12:45 – 13:30	Lunch (Boulevard Auditorium Foyer) ANZMAGnet Meeting (Room B1)
13:30 – 17:00	RDC Workshop (Boulevard Auditorium) Imaging Satellite (Room B1) Trip to Koala Park
15:00 – 15:30	Afternoon Tea for RDC Workshop/Imaging Satellite (Boulevard Auditorium Foyer)
18:30 – 19:00	Pre-dinner drinks (Boulevard Auditorium Foyer)
19:00 – 23:00	Bruker Conference Dinner (Boulevard Room)
Wednesday	30 October
	Note late start due to unavoidable program change
09:40 – 10:10	Keynote Lecture 4 (Sir Paul Callaghan Lecture): Mehdi Mobil (Boulevard Auditorium)
10:10 – 10:40	Morning Tea (Boulevard Auditorium Foyer)
10:40 – 12:40	Parallel Session 10: Biomolecules II (Boulevard Auditorium) Parallel Session 11: Metals & Spin Labels in Structural Biology (Room B1) Parallel Session 12: Metabolomics & Small Molecules (Room B2)
12:40 – 13:30	Lunch (Boulevard Auditorium) Presentation by HTS-110 (13:00–13:20, Room B2) ANZMAG Annual General Meeting (Room B1)
13:30 – 15:30	Parallel Session 13: Biomolecules III (Boulevard Auditorium) Parallel Session 14: Biomolecular Methods (Room B1) Parallel Session 15: Diffusion & Materials (Room B2)
15:30 – 16:00	Afternoon Tea
16:00 – 16:40	Plenary Lecture 7: James Chou (Boulevard Auditorium)
16:40 – 17:00	Closing Ceremony Invitation to APNMR6: Mallik Liu (Boulevard Auditorium Foyer)