行政院所屬各機關出國報告
(出國類別:研究)

參加「國際公定分析化學家協會第 123 屆年會」及訓練課程

服務機關: 行政院衛生署藥物食品檢驗局

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出國期間: **98** 年 **9** 月 **11** 日至 **9** 月 **18** 日

報告日期: **98** 年 **12** 月 **10** 日

目　次

摘 要

第 123 屆國際公定分析化學家協會 (AOAC International) 年會於 2009 年 9 月 13 至 16 日於美國費城 Philadelphia Marriott Downtown 旅館舉行，年會期間舉辦數場 AOAC 之事務會議、座談會、研討會、儀器展覽會以及壁報論文發表會。本局在這次會議中發表了 2 篇壁報論文，題目分別爲「Simultaneous Determination of β-Agonists in Poultry and Livestock by Liquid Chromatography/Tandem Mass Spectrometry」及「Determination of Residual Benzylpenicillin and Procaine Benzylpenicillin in Foods」，同時參觀其他業務相關之壁報論文，與相關檢驗科技之專家等國際人士互相交流。年會期間參加多場不同主題之座談會及研討會，了解國際間檢驗科技之發展現況與趨勢，有利於本局未來工作之推展。並參加國際公定分析化學家協會舉辦之會前訓練課程「化學方法之共同合作研究 (Collaborative Study of Chemical Methods)」，瞭解共同合作研究之目的、內容設計、執行方式、計畫之撰寫與結果統計分析之方法。

壹、 目的

國際公定分析化學家協會 (AOAC International) 之宗旨爲促進科學分析方法之確效及實驗室品質保證，任務包括從事食品及藥物安全相關科學分析方法之開發與確效，並作爲學術交流、網路聯繫及高品質實驗室等資訊之主要交流平台，隨時提供會員相關之諮詢、輔導及訓練等服務。本局派員參加 AOAC 第123屆年會，藉由觀摩其他國家檢驗研究單位之研究成果，可了解國際間最新檢驗科技之應用與發展趨勢；與各國專家學者溝通討論，相互交換意見，以建立本局與國際溝通及聯絡之管道；藉由壁報論文展示本局之檢驗研究成果，以提升本局檢驗技能及國際間知名度。藉由參加訓練課程，吸取相關知識，瞭解共同合作研究 (Collaborative Study) 之執行方式，提升本局專業識能。

貳、行程及會議經過

一、行程與工作記要

9 月 11 至 12 日	啓程 台北-舊金山-費城
9 月 13 日	參加化學方法之共同合作研究訓練課程
9 月 14 至 16 日	參加國際公定分析化學家協會第 123 屆年會
9 月 17 至 18 日	回程 費城-洛杉磯-台北

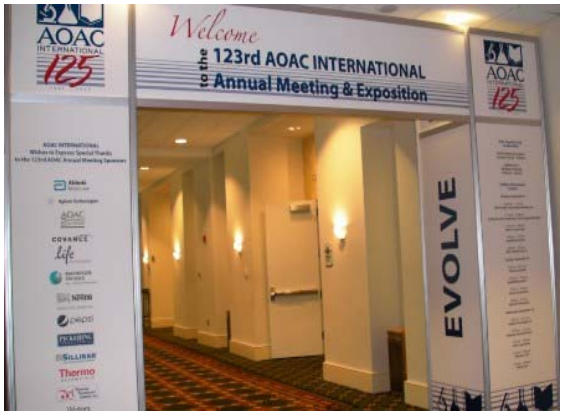
二、內容概要

本次 AOAC 年會於美國費城的 Philadelphia Mariott Downtown 旅館舉行第123屆年會，會期由9月13日至16日共計4天，9月13日參加訓練課程及完成報到手續後，即開始參加各項會議活動，費城是第一屆 AOAC 大會的開會地點，今年也是 AOAC 成立的125週年慶，因此今年的年會特別有紀念性，大會並於晚間舉辦開幕歡迎會及125週年慶祝會。今年共有820餘位來自世界各地的學者專家參加年會，其中來自台灣的有8位，分別是高雄市政府衛生局蔡龍居副局長、蔡佑志股長、林育正股長及許祝榮技士，AOAC 台灣分會理事長孫璐西教授及潘志寬秘書長、本局施養志組長及許雅鈞薦任技士。

在歡迎晚宴中，AOAC Chief Scientific Officer Dr. Al Pohland 舉辦了 “The Great Collaboration: 125 Years of Change” 的新書簽名會。大會亦以放映幻燈片方式回顧了 AOAC 在這125年中的演變，從一個由美國政府分析化學家組成的協會，擴展到目前擁有3000多位來自全球各分會會員的國際知名社團。這個協會對實驗室在化學與微生物檢驗方法的開發與確效，以及食品分析品質之改進，不僅奠定了基礎，更建立了權威的地位。

AOAC 第123屆年會開幕式於9月14日上午8點30分舉行，由會長 Darryl M. Sullivan 主持，並安排頒獎典禮及開幕演講，今年的 Harvey W. Wiley Award 得獎者爲美國 General Mills 公司的 Dr. Jonathan W. DeVries，表彰他40年來在食品化學成分分析的卓越表現。大會演講者爲 Nestle Research Centre 的 Dr. John David Marugg，演講題目爲 ”Validation Requirements for Rapid Methods: Perspective from an International Food Manufacturers” ，以國際食品製造業者的觀點來看有關快速檢

測方法之確效規範。由於食品之流通快速且數量龐大，業者應從原料來源、產品製造流程及產品特性，建立快速有效之品質檢測系統，以確實維護食品安全，因此檢測方法必須兼具快速、簡單及準確性。近年來微生物快速檢測方法的發展蓬勃，許多商業化套組被開發使用，然而新的檢測方法之評估及確效過程是耗時及費錢。依據 ISO16140 (2003)，快速微生物檢測方法之評估及確效必須與參考方法或標準方法比對，例如國際標準組織 (International Standards Organization, ISO)、歐洲標準委員會 (European Committee for Standardization, CEN)、美國 FDA 之微生物檢測方法 (Bacteriological Analytical Manual, BAM)；或是使用經過 Mircoval、AFNOR 和 AOAC 等單位所確效之檢測方法。ISO16140或 AOAC 認可之快速檢測方法，可有效証明其方法準確性，但是認可的過程確是困難的，必須花費許多金錢及時間。因此建議 AOAC 和 ISO16140之確效步驟能進一步調和，以幫助食品製造業者能更有效應用快速檢測方法。於開幕式結束後，隨即參加大會所舉辦之學術研討會、壁報論文展示及儀器廠商、試藥供應商及代檢驗公司舉辦之展示會。



圖一、AOAC 第123屆年會會場入口

圖二、AOAC 第123屆年會開幕式

9月14日上午10點30分至下午1點舉行一場全球各分會之委員會會議，由 AOAC 國際活動組的 Director Mark Coleman 主持，各國代表介紹該分會的近況及經驗，我國則由施養志組長代表台灣分會理事長孫璐西教授參加，會中並介紹台灣分會如何吸引新會員加入之活動。AOAC 台灣分會會議則於9月14日下午6時舉行，由理事長孫璐西博士主持，潘志寬秘書長並準備月餅與鳳梨酥招待與會者。孫理事長介紹台灣分會過去一年來的活動及未來規劃，並請與會者自我介紹，促進交流。與會者除來自台灣的8位代表外，尚有美國與中國大陸代表，共23人參加。AOAC 國際活動組的 Director Mark Coleman 及 Ms. Liz Cribbin 亦出席本會議，十分讚許

本會之表現。大會今年並首次安排 Joint AOAC Asian Section Meeting，於9月14日下午7時舉行，邀請亞洲國家的與會代表出席此會議，孫理事長應邀主持該會議。AOAC International 會長 Dr. Darryl Sullivan，秘書長 Dr. Jim Bradford 及 AOAC 年會籌備單位的 Ms. Dawn Frazier 及 Ms. Liz Cribbin 皆出席此會議，顯見對此會議之重視。孫理事長代表 AOAC International 致辭，期許亞洲國家的會員們藉此會議彼此認識，加強聯繫。本會議共有台灣、日本、香港、新加坡、泰國、韓國及中國大陸等地代表出席。會議中與會者自我介紹並對未來可以合作的項目熱烈發言後，孫理事長彙總大家意見，作出以下結論：(1)未來將廣邀亞洲各國代表出席此會議；(2)亞洲各國可就各種分析方法進行合作研究；(3)亞洲各國分析方法之調和及確效。會議圓滿結束，各國期望未來能有更多合作機會。

圖三、全球各分會之委員會會議

圖四、孫理事長主持台灣分會會議

圖五、AOAC 台灣分會與會者合影

三、研討會及專題演講

大會於同一時間安排多場不同主題研討會，供與會者選擇，本次所參與之研討會摘要如下：

(一) Official methods board training session

AOAC INTERNATIONAL 對於公定分析方法之制定，原本即有一套嚴謹之程序，惟爲進一步提升制訂公定分析方法之嚴謹度，規劃以公定方法委員會 (Official methods board, OMB) 取代 General Referee 統籌負責公定分析方法制訂之整體執行程序，預計於兩年內正式上路。OMB 之職責爲評選所有提出申請的方法，包括新增、修訂或撤銷。申請成爲公定方法 (Official methods) 之程序摘述如下: 首先由專案利害關係人 (stakeholder) 或團體 (communities) 備妥方法申請書等相關文件向 AOAC 提出申請，AOAC 確認申請資料完整後送交 OMB，OMB 針對該申請案成立專責的方法委員會 (method committees) 並指派 study director (SD)，派選相關的 general referee (GR)、topic advisor (TA)、安全及統計學專家作爲顧問。申請者有需要時，可向 AOAC 申請成立專家評審小組 (expert review panels, ERP)，由 AOAC 研擬 ERP 成員名單，經 OMB 審核同意。ERP 成員就收集到的方法進行評估後，開會討論選擇最合適的方法 (一個或複合) 進行後續的確效實驗或共同實驗 (collaborative study)。由方法委員會之 SD 研擬確效實驗計畫或共同實驗計畫，並收集及評估實驗結果，向 OMB 建議成爲 first action method，之後 2 年內，由 TA 收集該草案使用者之意見，若無問題則成爲 final action method。

(二) Fraud in Food and Drug Safety

食品攙僞涵蓋範圍極廣，主要爲不法業者以低價成分混充高價成分謀取暴利之摻假情形，不僅擾亂市場交易安全，並損害消費者權益，若使用不良成分攙混，更有危害消費者健康之虞。美國早有相關法令規範食品攙僞情形，從 1897 年 the Imported Tea Act，1906 年 the Original Pure Food and Drug Act 及 1938 年 Federal Food Drug and Cosmetic Act，近年來 FDA 更邀集各界參與 economically motivated adulteration (EMA) 會議，討論有關攙僞的案例，從中學習經驗。要證明食品攙僞必須克服許多問題，早期食品攙僞檢驗係針對食品原料物種加以鑑別、判定，主要利用型態鑑識，例如觀察顏色與形狀等表徵以及組織結構之顯微觀測等；或是

針對專屬成分或特定成分含量比例進行檢驗，然而市售食品大多經過加工處理，型態表徵已喪失，而特定蛋白質等成分亦已變性或降解，以致無法有效運用傳統方法進行鑑別。近年來檢驗技術發展與應用有重大突破，讓過去無法檢驗之攙僞食品無所遁形，例如 DNA 條碼(DNA barcode)及同位素比質譜儀(Isotope ratio mass spectrometry, IRMS)的應用等。

會中並提出多篇有關攙僞檢驗技術之分享，包括 FDA 以 DNA 條碼解決魚類產品攙僞問題，因魚類經過加工後會造成型態特徵辨識上的困難，因此有許多以低經濟價值魚種取代高經濟價值魚種之摻假情形以及包裝上標示不實之案例，學者因此提出以粒線體細胞色素c氧化酶I (COI) 片段基因作爲鑑定物種之指標，FDA 目前也將此方法進行共同試驗，發展魚類 DNA 條碼標準化方法並進行方法確效，以實際應用於加工食品，做爲行政管理之依據。英國 Eurofins Scientific 公司利用同位素比質譜儀和 SNIF-NMR (site-specific natural isotopic fractionation-nuclear magnetic resonance) 進行 aspirin 及 ibuprofen 之僞藥鑑定，具有精準、快速、非破壞性及多組成份分析等優點。石榴 (Pomegranate) 含有石榴多酚、鞣花酸、花青素、蛋白質與植物雌激素，是近年盛行的保健水果之一，但是產量少及價格高因此發生摻假情形，Krueger Food Laboratories 分析檢體中花青素、多酚類、寡糖含量、甜味劑、人工色素及防腐劑等項目，結果發現市售石榴汁大多經過稀釋或由其他果汁取代，添加了天然或人工色素、酸化劑及防腐劑。另有多篇演講分享利用不同檢驗方法，例如 liquid chromatography-tandem mass spectrometry (LC-MS/MS)、gas chromatography mass spectrometry (GC-MS) 及 ELISA 等方法分析乳製品中三聚氰胺之經驗。

圖六、研討會會場(1)

圖七、研討會會場(2)

(三) What do you do when CRMs are not available? Certifying nothingness-blank matrix reference materials

根據 ISO「度量學辭彙 (the International Vocabulary of Metrology)」的定義，參考物質 (reference material, RM) 係一種物質或材料，其一種或數種特性已被完整充分地建立，可用來校正儀器、評估量測方法，或給予待測物一精確的量測值；驗證參考物質 (certified reference material, CRM)，係一種參考物質，經由特定而有效的技術程序，驗證其具有的一種或數種特性數值，同時出具經由驗證機構對鑑定數值認可之追溯報告證明文件。CRM 用於發展新的分析方法，驗證方法確效、分析步驟準確度、量測結果之追溯性；RM 則大量用於例行分析品質管制，例如確認分析方法之長期再現性、品管圖製作與實驗室間的比對研究。另外在建立分析方法之偵測極限時，空白參考物質 (blank RMs) 的使用亦十分重要，可用以確保實驗過程中未遭受任何汙染。

目前國際二大 CRM 系統，分別是美國國家標準與技術研究所 (National Institute for Standards and Technology, NIST) 生產的 SRM®，與歐盟 (European Community, EC) 生產的 ERM® (European Reference Material，ERM)。歐盟位於比利時的參考物質與量測研究所 (Institute for Reference Materials and Measurements, IRMM) 係隸屬於歐盟 Joint Research centre (JRC) 的機構，任務是提昇、推廣及支持歐盟政策的量測系統，研究領域包括參考物質研製、同位素量測、放射核種度量、食品安全與品質等。本次會中由 NIST 及 IRMM 人員提出許多有關建立參考物質及 blank matrix materials 的經驗及注意事項。製備參考物質之一般程序如下：(1) 樣品收集: 樣品數量足以長時間使用，而且具有分析代表性，(2)前處理與分裝: 必須注意均質性、安定性、避免化學性與生物性污染及儲存容器，(3) 均質性試驗，(4) 短期與長期穩定度測試，(5)確認驗證值及不確定度。

CRM 從製造、認證、到保存所費不貲，再依據保存與運送條件之不同、認證項目多寡、技術難度等因素，也決定 CRMs 之售價高低。食品多樣性及成分複雜，要發展有用的 CRMs 則更加困難，檢驗方法開發時會常面臨到沒有 CRMs 可供利用的情形，因此會中由美國伊利諾州 Silliker 公司提出 4 個建議的替代方案：(1)

spike approach，(2) use of a substituted reference material，(3) collaborative study approach，(4) reference method approach，於沒有 CRMs 可供利用時，可藉由以上方案進行方法開發。

(四) The threat of the unknown contaminants: When you don't know and what you don't know? Finding non-target chemical contaminants in food stuff

受到中國乳製品含三聚氰胺事件的影響，目前食品安全之重要挑戰爲不明污染物，由於科技進步，將有更多新的化學物質及產品被生產，食品中亦可能不斷有其他非預期的污染物被發現，因此食品中不明污染物之檢測成爲各界重視之議題。這些突發案件中，往往沒有公定方法或是國際認可方法可供使用，因此實驗室必須快速開發方法來因應，不論是自行開發或是參考相關文獻所建立的方法，都必須經過確效程序，評估方法可行性及準確性。一般食品安全監測大多針對特定的目標物質進行檢測，無法及時發現食品是否污染了其他化學物質，也許是黑心添加物，也許是無心的工業污染物質，都有可能是影響民眾健康的潛在危機，因此食品安全監測，除分析特定目標物質外，亦應對未知污染物進行篩檢。爲達到全面性監測，必須要有新的檢驗技術來因應，飛行時間式質譜儀 (time-of-flight mass spectrometry, TOF-MS) 則是目前相當強而有力的分析工具，本次研討會中有多篇演講以 TOF-MS 搭配不同的儀器組合進行檢測，只要選擇適當的管柱、飛行時間、溫度等分析條件，搭配資料庫及軟體處理技術，即可展現分析物組成特性與樣品指紋特徵 (fingerprinting)。

美國 FDA 於菠菜及人蔘中添加40~50種農藥，檢體經 QuEChERS 或 GPC 及 SPE 二階段淨化製備所得之檢液，同時以 gas chromatography high-resolution time-of-flight mass spectrometry (GC-HR/TOF-MS) 和 multi-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC LR/TOF-MS) 二種方法進行全面性篩檢，檢驗結果與傳統針對目標物質之 single quadrupole MS SIMs 分析方法進行比較。結果發現 GC-HR/TOF-MS 在低濃度時無法有效的篩選農藥種類，而 GC×GC LR/TOF-MS 則可有效篩選出90%以上的農藥種類。

美國農業部農業研究服務中心 (USDA Agricultural Research Service)，以 gel permeation chromatography 進行樣品前處理，使用 direct sample introduction 進樣系統，搭配 two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/TOF-MS) 進行魚油中持久性有機污染物 (persistent organic pollutants, POPs) 之分析。此方法可減少樣品量及增加分析項目範圍，除可檢測魚油中已知的 POPs 外，亦可同時偵測多種 halogenated natural products (HNPs) 及其他有機化合物。

食品容器使用之金屬罐內壁一般會塗佈塗料阻隔與食物接觸，提高容器耐蝕特性及防鏽，塗料爲高分子成分，如環氧樹脂 (epoxy) 及聚酯樹脂 (polyester) 等，但塗料與食品接觸時，可能會有原料成分溶出之疑慮，危害人體健康。環氧樹脂原料可能會有丙二酚A (bisphenol A, BPA)，或丙二酚A二環氧甘油醚 (bisphenol A diglycidyl ether, BADGE) 單體溶出，BPA 被視爲荷爾蒙干擾物質，而 BADGE 有致癌性疑慮；聚酯樹脂之聚合物也曾被報導有溶出之情形。爲瞭解金屬罐容器中此類化合物可能溶出情形，英國食品與環境研究局 (Food and Environment Research Agency)，應用 liquid chromatography with time-of-flight mass spectrometry (LC/TOF-MS)，以上述2種罐頭塗料爲例建立一套鑑定罐頭食品中不明污染物之檢測法。會中並由美國 FDA 人員分享面臨突發案件的應對方式及利用 LC-MS/MS、GC-MS 及 ELISA 等方法分析乳製品中三聚氰胺之優缺點。

(五) Dealing with the unknown-risk assessment and risk management strategies of industry and regulators

受到中國乳製品中含三聚氰胺事件影響後，當前食品安全之重要挑戰爲不明汙染物，以風險評估、風險管理、風險溝通爲核心的食品安全風險分析成爲保障食品安全的重要工具，會中同時邀請政府單位及食品業者分享相關經驗。美國 FDA 面對新興風險污染事件之處理策略，包括 (1) 以科學爲基礎，進行風險評估及風險溝通，確保民眾能接獲迅速、可靠、客觀及全面的資訊；(2) 建立污染物檢驗方法；(3) 建立食品中污染物之 guidance levels，監控食品工廠必須符合 guidance levels 之規範；(4) 與州政府和其他聯邦機構共同合作處理食品安

全，例如由 FSIS 與 FDA 共同合作成立的食品緊急事件因應網路 (Food Emergency Response Network, FERN)，整合地方與中央之國家食品實驗室，規劃緊急應變措施，防止國際污染事件造成威脅。英國食品標準局 (Food Standards Agency) 爲了有效管理及預防新興風險事件，特別成立新興風險組 (The Emerging Risks unit, ERU)，負責制定監測程序，收集相關資料和數據並進行分析，評估風險危害程度，使能適時提供各界準確、客觀、一致及全面的資訊，確保食品之安全性，建立消費者對政府單位之信心。另由 Mars Incorporated 及麥當勞兩大國際食品公司分享業界如何進行原料及產品生產鏈的風險評估及管理，面臨突發事件的處理方法，以減少問題產品回收等後續處理問題影響公司信譽及造成金錢損失。Mars Incorporated 應用原料品質管理 (material quality management, MQM) 程序爲架構來管理食品安全，進行原料風險評估 (material risk assessment, MRA)，以確保產品品質符合規範。

四、壁報論文展示與閱覽

本次大會之壁報論文分 3 天舉行，每天均有不同研究主題進行展示，9 月 14 日展示主題有 (1) 植物性藥物及膳食營養補充劑 (botanicals and dietary supplements)；(2) 一般分析方法、品質保證與認證 (general methods, quality assurance and accreditation)；(3) 微生物參考方法 (microbiological reference methods)；(4) 績效評估方法 (performance tested methods^{SM})。9 月 15 日展示主題有 (1) 食因性污染物及殘留物之分析 (analysis of foodborne contaminants and residues)；(2) 非食因性污染物及殘留物之分析 (analysis of non-foodborne contaminants and residues)；(3) 食品安全之突發案件 (emerging issues in food safety and security)；(4) 飼料、肥料及相關的農業議題 (feeds, fertilizers and related agricultural topics)。9 月 16 日展示主題有 (1) 天然毒素之檢測 (detection and measurement of natural toxins)；(2) 食品營養及食物過敏原 (food nutrition and food allergens)；(3) 藥物分析之準確性及安全性 (pharmaceutical analysis, authenticity and safety)。本局在會議中發表了 2 篇壁報論文，題目分別爲「Simultaneous Determination of β-Agonists in Poultry and Livestock by Liquid Chromatography/Tandem Mass Spectrometry」及「Determination of Residual Benzylpenicillin and Procain

Benzylpenicillin in Foods」，國內另有台灣大學食品科技研究所孫璐西教授發表之「Investigation on the Potential Anti-aging Edible Plant Materials Using *Caenorhabditis elegans* Model System」，期間並閱覽與業務相關之論文，以了解最新研究情形，參閱之壁報論文擇要整理如下:

(一) Multiresidue determination of quinolone antibiotics in swine muscle using liquid chromatography with tandem mass spectrometry

南韓國家獸醫研究及檢疫部門 (National Veterinary Research and Quarantine Service) 利用逆相高效液相層析串聯質譜方法 (reversed-phase high-performance liquid chromatography tandem mass spectrometry)，以正離子偵測，配合多重反應偵測模式 (multiple reaction monitoring，MRM)，進行豬肉中 quinolone 類抗生素多重殘留分析。包括 sarafloxacin、enrofloxacin、danofloxacin、piromidic acid、oxolinic acid、flumequine、nalidixic acid、ofloxacin、ciprofloxacin、orbifloxacin、pefloxacin、marbofloxacin 及 norfloxacin 等 13 種 quinolone 類抗生素。豬肉檢體經 2% trichloracetic acid/acetonitrile 萃取，以正己烷去除油脂後進行分析。本研究分別就不同之抗生素選定一個母離子與兩個特定子離子，以符合歐盟 2002/657/EC 規定之 identification points。標準品溶液濃度介於 6.25~150 ng/mL 之間，有良好之線性關係，檢體中添加標準品濃度爲 10 ng/g 之 13 種 quinolone 類抗生素，其平均回收率爲 73.8~110.6%，本方法之 limits of quantitation (LOQs) 低於 1 ng/g。

(二) Simultaneous determination of 14 sulfonamides in milk by liquid chromatography with tandem mass spectrometry

南韓國家獸醫研究及檢疫部門利用逆相高效液相層析串聯質譜方法，以正離子偵測，配合多重反應偵測模式，進行牛乳中 14 種磺胺劑多重殘留分析。分別就不同磺胺劑選定一個母離子與兩個特定子離子，以符合歐盟 2002/657/EC 規定之 identification points。牛乳檢體經 acetonitrile 去除蛋白質，以正己烷去除油脂後進行分析。標準品溶液濃度介於 2.5~100 ng/mL 之間，有良好之線性關係，檢體中添加標準品濃度爲 2 ng/mL 之 14 種磺胺劑，其平均回收率爲 76.5~95.6%，本方法之 LOQs 介於 0.2~2.5 ng/ml。

(三) Measurement of chloramphenicol in honey using an online sample extraction with LC-MS/MS

氯黴素屬抑制細菌蛋白質合成的抗生素，爲一廣效性的抗生素，早期可由 *Streptomyces venezuelae* 產生，現今主要以合成方法生產。主要抑制革蘭氏陰性菌、立克次體及部分革蘭氏陽性菌，由於價格便宜及廣效性抗菌範圍，過去常被大量用於肉類動物及水產品的傳染性疾病之治療，所造成的副作用，除了產生抗藥性外，亦會抑制骨髓造血功能，導致顆粒細胞缺乏症、再生障礙貧血症 (fatal aplastic anemia) 等疾病。由於氯黴素對人體健康影響極大，已被歐盟、美國及加拿大等國家公告禁止使用於產食動物及蜂蜜產品。目前利用 LC-MS/MS 分析蜂蜜中氯黴素，其樣品前處理有固相萃取 (solid phase extraction)、QuEChERS 或液/液相萃取等離線萃取 (off-line exaction) 方法，步驟繁複且費時，樣品必須經過濃縮再以適當溶劑回溶後才能進行分析。因此 Thermo Fisher 公司研究開發自動化線上萃取 (on-line exaction) 方法，減少樣品前處理時間，該方法只需將樣品以水稀釋，降低黏度後即可上機分析，方法之 LOQ 爲 0.047 μg/kg，分析時間爲 5 分鐘，可有效縮短檢驗時間。

(四) Analysis of trace residues of fluoroquinolones in honeys by HPLC using polymetric cartridge and metal chelate affinity chromatography

日本食品研究實驗室 (Japan Food Research Laboratories) 利用高效率液相層析儀配合螢光偵測器 (激發波長 285 nm，放射波長 400 nm)，同時分析蜂蜜中包括 norfloxacin、ciprofloxacin、danofloxacin、enrofloxacin、orbifloxacin、sarafloxacin 及 difloxacin 等 7 種 quinolone 類抗生素。蜂蜜檢體經含有 Na_2EDTA 之 macllvaine buffer (pH 4.0) 萃取，再以 polymetric cartridge 和 metal chelate affinity column 純化後進行 HPLC 分析，方法之平均回收率爲 70~120%，LOQ 低於 0.01 μg/g。

(五) Monitoring of carbadox and olaquindox in meat products by liquid chromatography tandem mass spectrometry in Korea

卡巴得 (carbadox) 及歐來金得 (olaquindox) 添加於動物飼料作爲抗菌劑，可促進豬隻生長，增加重量及改善飼料利用率，因具有潛在之基因毒性及致癌性，可能對消費者健康造成危害，已陸續被許多國家禁用，南韓亦於 2009 年禁止使用卡巴得及歐來金得。爲了解市售產品中卡巴得及歐來金得殘留情形，南韓食品藥物管理局建立肉品中卡巴得及歐來金得之殘留量檢驗方法，用以監測市售產品。利用液相層析串聯質譜法，以電灑法正離子偵測模式，可同時檢測卡巴得及歐來金得之主要指標代謝物 quinoxaline-2-carboxylic acid (QCA)及 methyl-3-quinoxaline-2-carboxylic acid (MQCA)。QCA 及 MQCA 之母離子分別爲 m/z 175 及 m/z 189，QCA 之主要子離子爲 m/z 129 及 m/z 102；MQCA 之主要子離子爲 m/z 145 及 m/z 102。此方法分析卡巴得及歐來金得之方法 LOQ 均爲 4 μg/kg，應用此方法檢測市售畜禽產品中卡巴得及歐來金得，結果於牛肉 50 件、豬肉 55 件及雞肉 40 件中均未檢出不符規定情形。

化學方法之共同合作研究訓練課程

(Collaborative study of chemical methods training courses)

日期：2009年9月13日

講師：Mark C. Roman。

內容：包括共同合作研究之目的、內容設計原則、計畫之撰寫與結果之統計分析方法。

一、共同合作研究之目的

藉由多個實驗室之共同合作研究，提供實驗室間之變異性 (variability)、準確性 (accuracy)、再現性 (reproducibility)、回收率及重複性 (repeatability) 等資訊，客觀地評估方法之適用性。

二、共同合作研究之設計

包括確認樣品基質 (matrix)、分析物 (analyte) 之濃度範圍、樣品數目、添加試驗、參與實驗室數目及結果資料蒐集與計算。

(一) 試驗材料 (materials) 之選擇原則

試驗材料係由分析物、基質及濃度等 3 項因子所組成。

(1) 基質相同但分析物之濃度相差 5%以上者，視爲兩種試驗材料；相同濃度之同一分析物，存於不同基質中，也視爲兩種不同試驗材料。

(2) 進行共同合作研究時，至少需要 5 種試驗材料，當只有單一特定基質時，僅需要 3 種。

(3) 對於基質中分析物之濃度範圍，必須具代表性。濃度範圍依法規、標準、標示或其他特定情況而訂。

(4) 考慮試驗材料之穩定性及均質性，非均質性之試驗材料會造成高變異性之結果；亦須注意試驗材料運送與儲存過程之溫度、濕度等條件控制。

(二) 決定樣品數目 (determining number of samples)

進行共同合作研究至少需 5 種試驗材料，並考慮是否需做添加回收試驗及空白基質試驗。利用重複盲樣 (blind replicates) 或 youden pairs 等方式計算重複性 (repeatability)。樣品數目爲試驗材料數及重複次數 (replicates) 之乘積。

(三) 試驗材料添加 (spiked materials) 原則

(1) 添加已知濃度之分析物於試驗材料或空白檢體中，其添加量必須具有代表性，一般採用兩種或以上之添加濃度，添加方式包括大批 (bulk) 及個別 (individual) 添加。大批添加方式時，需避免沉澱或分離現象發生，尤其於低濃度添加時，必須再確認其均質性。

(2) 添加試驗必須確認空白基質並無待測分析物存在，添加試驗回收率之結果接近 100%，並不一定代表方法之準確性，因爲添加試驗檢體及真實檢體中之萃取效率不同，加上基質干擾，也可能增加回收率。

(四) 決定測試實驗室數目

一般化學分析定量方法 (quantitative method) 至少需 8 家實驗室以上參與，定性試驗 (qualitative method) 則需 10 家以上，爲避免有些實驗室未依計畫書之規定執行，或未於指定時間內完成工作，因此建議實驗室家數要大於上述最小值，以免最後結果不足以進行統計分析。

(五) 空白樣品 (blanks)

(1) 基質空白 (matrix blank)，指其除待測分析物外，所有組成皆與陽性樣品相同，進行殘留量分析時，須先驗證基質爲空白樣品，確實不含待測分析物。

(2) 試劑空白 (reagent blank) 對於測定低濃度樣品時，尤爲重要。

(六) 結果數據之收集

數據總數量爲實驗室數量、試驗材料數量及重複數量之乘積。例如當測試實驗室有 8 家，試驗材料有 5 種，進行 2 重複測試，則會產出 80 個數據結果。

三、撰寫共同合作研究計畫書 (collaborative study protocol)

計畫書內容包括 10 部份：標題、作者、前言、共同合作者、研究設計、方法、試驗樣品之準備、原始數據報告、原始數據分析及附件。

(一) 標題：明確詳述方法、分析物及基質等，一般寫法爲「以某種方法檢測某種基質中之某分析物」，例如「Determination of ephedrine alkaloids in botanicals and dietary supplements by HPLC-UV」。

(二) 作者：包括全名及通訊方式。

(三) 前言：包括方法背景、研究目標/目的、方法描述、方法應用範圍及方法原理。方法背景說明此方法之重要性及進行共同合作研究之必要性。研究目的期能藉由評估再現性、重複性、準確性 (accuracy)、僞陽性及僞陰性等項目後，建立一公定方法 (official method)。方法描述包括樣品萃取技術、儀器設備及內部標準品、外部標準品或標準品添加等定量方法。方法應用範圍包括分析物及其濃度範圍、基質種類、定性或定量之說明等。方法原理說明包括萃取、分離及定量等原理。

(四) 共同合作者 (collaborators)：選擇有意願參與、對此研究主題有興趣、有足夠儀器設備及專業能力執行之實驗室。以附錄表列所有共同合作者之通訊方式。所需實驗室數目，定量方法至少需要8家 (一般建議12家)，定性方法至少需要10家 (一般建議14家)。一般由 AOAC 會員或主辦單位等協尋合適實驗室參與。並視情況提供相關設備或耗材，如層析管柱及參考物質 (reference material)等。

(五) 研究設計：說明需要多少試驗材料，包括基質、分析物及其濃度，重複性測試方式，是否使用空白材料及陰性或陽性對照，待測分析物爲自然存在或是外添加於基質中。使用何種方式決定分析物濃度，例如使用對照參考物質、參考方法或添加回收試驗等，並描述如何進行樣品均質、確保運送及儲存過程之穩定性。

(六) 方法：需詳細描述方法流程，避免分析者誤解內容造成結果差異。說明包括分析物及基質種類等方法適用範圍。一般使用同等級設備即可，若爲特殊儀器設備，需詳細描述。詳細描述包括取樣、標準品配置、萃取步驟及分析條件等檢測步驟，關鍵步驟及注意事項須特別強調，並提供實驗中之必需計算式。

(七) 試驗材料之取得及製備：試驗材料必須足量，品質需求依分析方法而訂，可以向廠商或 NIST、NRC、EC JRC 或 IRMM 價購試驗材料，或由實驗室自行合成。樣品分送前要先隨機抽樣進行分析，確認樣品之均質性。樣品應包裝完整，並確保其運送過程中物理化學性質之安定性。在共同合作研究正式實施之前，亦可提供練習樣品 (practice samples) 進行檢測，結果滿意後再進行正式共同試驗，若發現有問題時，則探討解決辦法。

(八) 原始數據報告及分析：提供包括圖表及單位等原始數據紀錄。將各實驗室之原始數據彙整後，先以 Cochran 偏離性測試 (代表同一實驗室內的重覆性) 及 Grubbs 偏離性測試 (代表不同實驗室間的再現性) 剔除偏離之數據，剩餘之數據分別計算出平均數、Sr (重覆性標準偏差)、S_R (再現性標準偏差)、RSDr (實驗室內之相對標準偏差)、RSD_R (實驗室間之相對標準偏差)、$PRSD_R$ (預期相對標準偏差=$2C^{-0.1505}$，C 爲濃度)，並計算出 HORRAT 值，HORRAT 值爲 RSD_R 與 $PRSD_R$ 之比值，計算公式爲 $HORRAT = RSD_R/2C^{-0.1505}$。HORRAT 值是用來測量方法可接受性的指標，可接受 HORRAT 值範圍爲 0.5~2。若 HORRAT 值介於 0.5~1.5，表示此方法的再現性符合標準；若 HORRAT 值 ≤ 0.5 或>2，表示此方法的再現性可能有問題，應要放棄此次研究結果；若HORRAT值 ≥ 1.5，表示此方法的再現性高於期望值，應探討樣品均質性、分析物穩定度或其他原因。

參、心得與建議

一、 國際公定分析化學家協會每年皆吸引來自全世界檢驗研究單位專業人士參加，提供各界一個資訊交流及教育訓練平台，此次有幸能參加此大規模之國際學術會議，增廣見聞，受益良多，對拓展視野及研究工作有很大的激勵。本次年會所發表之壁報論文約220篇，涵蓋各種生物性及化學性檢驗方法之研究。隨著檢驗科技之快速發展，氣相及高效(超高效)液相層析串聯質譜儀等精密儀器已成爲主流，本次年會以質譜儀搭配其他設備進行研究之主題約有50篇，占有相當高之比例，本局今年發表之壁報論文2篇，亦係利用液相層析串聯質譜儀建立食品中抗生素等動物用藥殘留之檢測方法，顯示本局之檢驗技術水準符合國際潮流。

二、受到中國乳製品中三聚氰胺污染事件之影響，食品中不明污染物之分析成爲亟待解決之重要議題，因此食品安全之監測，除分析特定目標物質外，亦應對未知污染物進行篩檢。本次研討會中有多篇演講以飛行時間式質譜儀搭配不同的儀器組合進行檢測，只要選擇適當分析條件，搭配資料庫及軟體處理技術，即可展現分析物組成特性與樣品指紋特徵，達到較廣範圍之監測。

三、食品攙僞涵蓋範圍極廣，主要爲不法業者以低價成分混充高價成分謀取暴利之摻假情形，若使用不良成分攙混，更有危害消費者健康之虞。市售食品大多經過加工處理，型態表徵已喪失，而特定蛋白質等成分亦已變性或降解，要證明食品攙僞必須克服許多問題。近年來檢驗技術快速發展，讓過去無法檢驗之攙僞食品無所遁形，會中並提出多篇有關攙僞檢驗技術之分享，例如 DNA 條碼及同位素比質譜儀的應用等，可供業者建立品質管制之程序，並提供主管機關執法時之所需，共同維護食品安全。

四、爲維護檢驗結果之品質，使用參考物質及空白檢體進行方法確效爲重要程序。NIST 及 IRMM 人員於會中提出許多有關建立參考物質及空白檢體之一般程序、注意事項及經驗。由於食品多樣性及成分複雜，檢驗方法開發時常會面臨到沒有參考物質可供利用之情形，因此美國伊利諾州 Silliker 公司提出包括進行共同試驗、添加試驗、使用替代參考物質及與參考方法進行比對等 4 個替代方案，可作爲本局開發檢驗方法之參考。

五、AOAC INTERNATIONAL 對於公定分析方法之制定原本即有一套嚴謹之程序，爲進一步提升其嚴謹度，正規劃以 Official Method Board 取代 General Referee 統籌負責公定分析方法制訂之整體執行程序，預計於兩年內正式上路，此流程或可作爲本局公告檢驗方法審議之參考。我國亦可擴大參與 AOAC INTERNATIONAL 之各項活動，藉以提升各相關領域之水準。

六、面對新興風險污染事件之處理策略，以風險評估、風險管理、風險溝通爲核心的食品安全風險分析成爲保障食品安全的重要工具。爲了有效管理及預防新興風險事件，在美國有 FSIS 與 FDA 共同合作成立的食品緊急事件因應網路，英國食品標準局亦特別成立新興風險組。未來我國成立之食品藥物管理局或可參考此模式，規劃緊急應變措施，確保民眾能迅速接獲、可靠、客觀及全面的資訊，建立消費者對政府單位之信心。

七、藉由參加訓練課程，學習如何進行實驗室間共同合作研究，瞭解其目的、內容設計、執行方式、計畫之撰寫與結果統計分析之方法，期能作爲本局未來參加共同合作研究之參考。

八、此研討會是極佳的學習與交流平台，除吸收相關領域之新知外，亦可與會中專家學者彼此交換相關資訊，有利於工作之發展，建議讓更多同仁有機會參與，以掌握最新資訊，擴大交流網絡。

PART 8

HOW TO WRITE DOCUMENTS IN AOAC STYLE

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FORMAT OF A COLLABORATIVE STUDY PROTOCOL

- Introduction explaining the purpose of the study and a brief description of the method.
- Design of the collaborative study, e.g., number of collaborators, type and number of test samples to be tested, levels of spiking, etc.
- The method written in AOAC style, i.e., the way methods are published in the *Official Methods of Analysis of AOAC INTERNATIONAL*.
- Letter to collaborators that would include the equipment and reagents requirements, the intended date of beginning of the study, length of time of the study, time frame for reporting results of analyses, etc.
- Forms to be used by collaborators to report results of analyses.
- Basic Format is as follows:

TITLE
AUTHOR(S)
INTRODUCTION
COLLABORATORS
STUDY DESIGN
TEST SAMPLE PREPARATION
METHOD
REPORTING RAW DATA
ANALYZING RAW DATA
APPENDICES (or TABLES AND FIGURES)

Collaborative Study protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

FORMAT OF A COLLABORATIVE STUDY PROTOCOL

The following format should be followed when writing a collaborative study protocol:

(1) **TITLE:** Choose a title. Be explicit and descriptive enough to give an idea of your approach. For example, *Cholesterol Analysis in Foods by Direct Saponification Gas Chromatographic Method.*

(2) **AUTHOR(S):** Include your name, affiliation, mailing address, phone number, fax number and email address.

(3) **INTRODUCTION:** Include the following:

- (a) Background: describe work on the current method or other methods available. If there is a reference method it should be discussed.
- (b) Goals of the Study.
- (c) The needs and purpose of the study.
- (d) A description of your approach to the problem.
- (e) Intended scope/applicability of method, regarding analyte(s), matrix(ces), and concentration ranges (LOD/LOQ) and whether the method is quantitative or qualitative.
- (f) A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.

(4) **COLLABORATORS:** Qualify the collaborators:

- (a) State whether study is planned in cooperation with any other organization.
- (b) State number of planned collaborators.
- (c) Tell how collaborators/laboratories will be selected.
- (d) List the collaborators who have already agreed to participate with their full contact information (name, title, affiliation, mailing address, phone number, fax number and email addresses) when available

(5) **STUDY DESIGN:** Outline the design of the study: number of materials, number of blind duplicates or Youden pairs, number of blanks, number of positive and negative controls, where applicable, and number of analyte levels.

(6) **TEST SAMPLE PREPARATION:** Describe preparation of test samples:

- (a) What will be the individual materials? What is the matrix, what is the analyte, and at what concentration?
- (b) How will analyte/matrix combinations be prepared? By spiking or as naturally occurring materials?
- (c) How will actual content be determined? If using a reference method to determine actual content, how will the reference method be determined to be in control? How will the reference method be selected?
- (d) What other analytes or contents of interest to this test (interferences, etc.) will be included in materials?
- (e) Comment on Homogeneity of the samples (see Appendix E of this manual)
- (f) What will be the blanks, if appropriate?
- (g) What quality control measures will be followed to assure content of samples?
- (h) What stability data are available and how is that information used?
- (i) How will test samples be packaged and how will collaborators handle them upon receipt?

(7) **METHOD:** Write the method in AOAC style (see Format of AOAC® *Official Method*℠, example of the method, Part 8 and AOAC website):

- (a) Include applicability statement (matrices, analyte concentrations
- (b) Write the method as a series of commands. For example, "add 10 mL," "stir the solution."

(c) List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that don't need special preparation are listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the method. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official Methods℠* (see Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.

(d) If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.

(e) Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. (Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy method; etc.)

(f) Provide calculations with SI units if applicable.

(g) Include any necessary alerts to critical steps, precautions, or warnings.

(8) **REPORTING RAW DATA:** Provide instructions on how to report the data from the analysis. Give draft data reporting sheet (see Part 7 for example of the reporting form.)

(9) **ANALYZING RAW DATA:** Indicate how the data will be analyzed. Indicate what method performance statistics will be determined from study design (recovery, RSD_r, RSD_R, s_r, s_R, HORRAT, etc.). See Appendix D of the OMA (Part 6) for additional information.

(10) **APPENDICES** or **FIGURES AND TABLES:** Provide examples of the the draft letter to collaborators that would include the information on the study, e.g., type of analysis, equipment and reagents requirements, the intended date of beginning of the study, length of the study, time frame for reporting results of analyses, etc. See Part 7 for the example of a letter to collaborators, solicitation postcard, etc.

(11) **SUPPORTING DATA:** See Checklist for Protocol Design of Collaborative Studies (Appendices Q and R).

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FORMAT OF A COLLABORATIVE STUDY MANUSCRIPT

Refer to the AOAC website for examples of collaborative studies (www.aoac.org).

- *Title*-Title of manuscript that ends with ":Collaborative Study."
- *Author(s)*- provides authors' full (e.g. no initials) names and contact information.
- *Abstract*-Specific information on the method and study.
- *Introduction*-Information on why collaborative study was conducted, how many collaborators participated in the study, previous work done, and information on compound or process that was studied.
- *Collaborative study*-Information on matrices and number of test samples tested, test sample preparations, instructions for collaborators, etc.
- *Method*-Written in AOAC style.
- *Collaborators' comments*-Any comments and suggestions received from collaborators and information on how they were addressed by the Study Director, e.g., incorporating instructions into the method, etc.
- *Results and Discussion*-Information on type of statistical analyses performed on raw data, reasons for rejecting some of the data, discussion of results with references to tables and figures, discussion of the method performance, etc.
- *Recommendation*-Study Director's recommendation to adopt method First Action.
- *Acknowledgments*-Full names and addresses of all collaborators that participated in the study.
- *References*-All references cited in the text.

Collaborative Study manuscripts and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org). Examples of collaborative studies for the 10 methods committees are located on our website (www.aoac.org).

FORMAT OF A COLLABORATIVE STUDY MANUSCRIPT

Your manuscript may be in either of two categories: (1) a preliminary or first manuscript in which you give the background of the problem, describe a method that you either devised yourself or selected for study, and present data obtained in your own laboratory (such as optimization studies, ruggedness testing); or (2) a manuscript of a collaborative study, either successful or unsuccessful. If your manuscript does not seem to be in either of these two categories, you may still be able to adapt these guidelines for your particular situation. Alternatively, contact AOAC OMA/PVM department for special instructions.

1. PRELIMINARY OR FIRST MANUSCRIPT (OPTIONAL)

Choose a title. Be explicit and descriptive enough to give an idea of your approach. Include matrix, analyte and technique. For example, *Determination of Ochratoxin A in Baby Food by Immunoaffinity Column Cleanup and HPLC*.

Include your complete name, address, and phone number. Add a footnote identifying the manuscript as your Study Director manuscript. Include the exact title of your study and, if appropriate, the year(s) of poster presentation at the Annual Meeting in the footnote.

Include the following in your introduction:

a. The purpose of the study.

b. A brief summary of previously published work directly related to your problem and a statement of how your work is related to previous work. Include pertinent literature references. This may be a "why I did this study and why the proposed method is better than the existing method" statement.

c. A description of your approach to the problem and a brief statement of whether your work was successful.

d. A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.

e. Why the method is important to the scientific community and needed.

f. If a reference method is used, it should be described.

Write the method in AOAC style, as follows:

a. Write the method as a series of commands, e.g., Add 10 mL or Stir solution.

b. List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that don't need special preparation are not listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the manuscript and must be included. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official Methods℠* (See Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.

c. If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.

d. Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. [Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy

method; etc.]

e. Provide calculations if applicable.
f. Include any necessary alerts to critical steps, precautions, or warnings.

Describe your experimental plan, for example, the number and types of commodities studied. Present experimental results, preferably in tables or figures. Tables and figures should have detailed titles or legends.

Discuss or clarify your data wherever appropriate and draw suitable conclusions.

Make a recommendation of some type. For example: "Collaborative study of the method is recommended."

Provide complete and accurate bibliographic references for any literature citations in the manuscript.

Collaborative Study manuscripts, protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

2. COLLABORATIVE STUDY MANUSCRIPTS

The following format should be followed when writing collaborative study manuscripts:

Title—Use title from protocol or choose a title based on your study. Be explicit and descriptive enough to give an idea of your approach. Include matrix, analyte and technique. For example, *Cholesterol Analysis in Foods by Direct Saponification-Gas Chromatographic Method: Collaborative Study*. The title of the collaborative study manuscript, method and interlaboratory study results table must be the same.

Author(s)—Include your complete name, affiliation, mailing address, phone number, fax number and email address. Include in the footnote, if appropriate, the year(s) of poster presentation at the Annual Meeting.

Abstract— Provide an abstract of the paper, however, do not send manuscript to publications at this time. Manuscript will be published after OMA Methods Committee's approval for First Action.

Introduction—Include the following in your introduction:

a. The purpose of the study.

b. A brief summary of previously published work directly related to your problem and a statement of how your work is related to previous work. Include pertinent literature references. This may be a "why I did this study and why the proposed method is better than the existing method" statement.

c. A description of your approach to the problem and a brief statement of whether your work was successful.

d. A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.

e. Why this method is important to the scientific community and needed.

Collaborative Study—Describe the design of your collaborative study. Include the number of collaborators, number and nature of test samples, special instructions to collaborators, etc.

Method—Write the method in AOAC style (see Format of the AOAC® *Official Methods*℠ in Part 8 and example of the method):

a. Write the method as a series of commands. For example, "add 10 mL," "stir the solution."

b. List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that need no special preparation need not be listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the manuscript and must be included. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official Methods℠* (see Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.

c. If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.

d. Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. (Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy method; etc.)

e Provide calculations with SI units if applicable.

f. Include any necessary alerts to critical steps, precautions, or warnings.

Collaborator Comments—Summarize any of the collaborators' comments or experiences that were significant.

Results and Discussion—Present experimental results, preferably in tables or figures. Tables and figures should have detailed titles or legends. Give all collaborator results. Identify outlier results, by outlier test, not included in the statistical calculations, by footnotes. See *AOAC Guidelines for a Collaborative Study*, Part 6.

Discuss and interpret the collaborators' results where applicable.

Recommendation—Make a recommendation to adopt the method as First Action.

Acknowledgements—Acknowledge the help of the collaborators; list each collaborator by name, address and affiliation. Also acknowledge other help you have received. If you wish to recognize anyone whom you feel has made significant and substantial contributions, you may include them as a co-author.

References—Provide complete and accurate bibliographic references for any literature citations in the manuscript.

Collaborative Study manuscripts, protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

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FORMAT OF AOAC® *OFFICIAL METHODS*℠

- *Title*-Includes analyte being determined, type of matrix (matrices), and technique used for analysis.
- *Applicability statement*-Provides range or limits of determination as well as specific matrices.
- *Precaution statement*-Makes an analyst aware of hazardous materials used in analysis.
- *Interlaboratory study results* -Table that presents performance parameters including matrices tested in a collaborative study, levels of analyte(s), % recovery, RSD_r, RSD_R, s_r, s_R, HORRAT, number of observations, etc (see Part 6 for complete list).
- *Principle*-Explains mechanism of the analysis.
- *Apparatus*-Section that lists equipment that requires assembly or that has specifications critical to the method performance. Do not use brand names. Describe equipment in terms of performance characteristics.
- *Reagents*-Section that describes in terms of performance characteristics.
- *Preparation of test sample.*
- *Determination*-Describes the actual analysis.
- *Calculations*-Section that explains how to calculate final results; presented in a form of equation or description.
- *Other sections as needed.*

Examples of methods written in AOAC format for each of the 10 methods committees are located on our website (www.aoac.org).

FORMAT OF AOAC® *OFFICIAL METHODS*℠

Introduction

AOAC® *Official Methods*℠ are designed to be performed by trained scientists who staff the analytical laboratories of regulatory, industrial, and research institutions concerned with agricultural products, food, drugs, environmental media. Because many of the methods are used to define the legal status of regulated materials, it is essential that directions are uniformly interpreted by both the regulating and regulated laboratories. This attribute leads to the basic AOAC requirements of reporting: clarity, completeness, consistency, and brevity.

The AOAC style used for preparing methods for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* includes the following essentials:

(1) Standardized format that follows the order of laboratory operations.
(2) Use of the imperative mode.
(3) Cross-references to identical reagents, apparatus, and operations.
(4) Use of standardized definitions, terminology, and style.
(5) Use of accepted abbreviations and simplifications.
(6) Use of SI units
(7) Methods should be written as complete and self-contained as practical.
(8) Normality should be referred in terms of Molarity.
(9) ppm should be changed to mg/kg or mg/L
ppb should be changed to ng/g or ng/mL
ppt should be changed to pg/g or pg/mL

The following publications will be useful in conjunction with the preparation of methods of analysis:

(1) "Definitions of Terms and Explanatory Notes," in *Official Methods of Analysis of AOAC INTERNATIONAL*. (Includes abbreviations and symbols used in OMA, See Appendix D.)
(2) *Handbook for Authors of Papers in Journals of the American Chemical Society*, American Chemical Society Publications, 1155 6th St, NW, Washington, DC 20036, USA.
(3) *Reagent Chemicals*, American Chemical Society Specifications, American Chemical Society Publications, 1155 16th St, NW, Washington, DC 20036, USA.

EDITING METHODS IN AOAC STYLE

This editing section is for the method author. Before submitting your method to AOAC INTERNATIONAL, you should do the following:

The language of the method should be concise and completely free from ambiguity (see example of method edited in AOAC style). Conciseness is desirable, both to ensure clarity and to save space. Whenever there is a conflict between clarity and style, clarity is more important. Points that should be considered in editing are listed below:

1. Present Tense and Imperative Mode

Check sentences that do not begin with a verb and change them, if feasible, to the imperative mode (e.g. Pipet 10 mL...., Stir...., etc.). Exceptions are: use of adverb modifier ("Accurately weigh...."), prepositional clause ("For refined sugars, use..."), permissive statements ("Ferric hydroxide may be used..."), and statements in the "Principle" section.

2. Abbreviations

Most abbreviations are the same as those used by Chemical Abstracts. Do not use abbreviations in titles and headings. See the *Definitions of Terms and Explanatory Notes* in Appendix D.

3. Repetition and Redundancy

Eliminate repetition and redundancy as far as possible; use only for emphasis. Do not use "distilled" with water, "concentrated" with common acids, "95%" with alcohol, or "ACS" with reagents covered by ACS specifications. These are understood by definition.

4. Formulas and Chemical Names

In general, use the chemical formula when the compound is easily recognizable. Use the chemical name at the beginning of a sentence (rare) for more complicated organic structures, and where the formula is longer than the name. Follow Chemical Abstracts nomenclature, in general. Check the spelling of all chemical names. Be sure that the correct number of molecules of water of hydration is used.

5. Consistency

Watch for internal contradictions in the text: volumes that do not add up or that exceed the capacity of the container; too abrupt a transition from one operation to another (a line may be omitted); and impractical or impossible numbers (e.g., 100 g NaCl will not dissolve in 100 mL water).

6. Cross-references

All new AOAC methods should be written as complete and self-contained as practical. Do not refer to other AOAC methods. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

7. Definitions

The section "Definition of Terms and Explanatory Notes," *Official Methods of Analysis of AOAC INTERNATIONAL*, is the basic guide to conventions and consistency (see Appendix D).

8. Illustrations

If symbols are used on the figure, include an explanation in the caption or text.

9. Tables

Provide descriptive titles for tables. Explain any obscure headings in a footnote.

10. Bibliographic References

Check all references for accuracy. Use standard Chemical Abstracts abbreviations for *Journal* titles. In general avoid references in method. Cite background references in the "Introduction" or "Discussion" section of the collaborative study manuscript -- not in the method. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

11. Terminology

For names of chemical compounds, use the spelling, hyphenation, and word division given in Chemical Abstracts. Use a national pharmacopeia for names for drugs. Use ISO nomenclature for pesticides and Codex nomenclature for names of food additives and color additives.

12. Safety

All methods must be reviewed against the *Safety Checklist* (see Appendix H) for potential hazards. Authors and editors should become familiar with the general criteria set forth in the introduction of the chapter on *Laboratory Safety* (see Appendix C). They should automatically incorporate cross-references to the safety statement(s), or bring questioned conditions to the attention of the Committee on Safety for resolution.

Decisions regarding inclusion of safety statements should be practical, recognizing that overuse will be self-defeating.

Methods that create toxic, obnoxious or environmentally hazardous fumes and wastes should contain practical directions for disposal.

13. Checking Edited Copy

The author must review a copy of the original version and edited copy to ensure that there has been no change in meaning, to correct typographical errors, and to answer any questions posed by the editor.

14. Proofreading

The author must review the typeset method for accuracy.

GENERAL STYLE GUIDE

The main sections of a method should flow from one operation to the next as they will be performed. Interruptions in operations for preparing reagents, assembling apparatus, and making standard curves must be kept to a minimum. Similar methods in the *Official Methods of Analysis of AOAC INTERNATIONAL* should be examined for guidance.

The main sections of a method and possible major subdivisions in lengthy methods are as follows:

1. Title

The title of the method should state the substance being determined (analyte) in terms of its common name, with chemical name (as used by Chemical Abstracts), trade names, and/or synonyms given in parenthesis. The placement of a method within the chapter is included in the top, left-hand corner of the method. Title includes analyte being determined, type of matrices, and technique used for analysis. For example,

AOAC Official Method 2001.01

Determination of Trans-galactooligosaccharides in Selected Food Products by Ion-Exchange Chromatography

Proposed First Action 2001

2. Applicability Statement

Applicability statements must be definitive, and must state the matrices for which the collaborative study was conducted and approved. This section is also used to include the scope and sensitivity of the method; its applicability to certain types of test samples and its non-applicability, because of interference, solubility, or other reasons, to other types of test samples. Statement about limit of detection and limit of quantitation must be listed here if given and defined appropriately. (Miller, J.C, and Miller, J.W., Statistics for Analytical Chemistry First Edition, 1984, reprinted 1986; Ellis Horwood, Publishers, Chichester, England. ISBN 0-85312-662-3. Second Edition, 1987. Third Edition, 1993, Ellis Horwood, Publishers, Prentice Hall, London. ISBN 0-13-030990-7. For example,

(Applicable to the detection of fluoride in peanut butter at concentrations of 0.8-200 mg/kg)

3. Caution Statement

Special safety or operational precautions that are generally applicable throughout the method are also placed into this section. Safety statements or cross-references applicable to a single reagent or operation are inserted at the specific point of applicability (see Appendix I, *Safety Checklist*). Remove the repetitious statement of the need for safe handling of solvents, acids, and alkalis and proper disposal of waste solvents. Make sure you include unusual situations that require specific advice, particularly with regard to disposal and destruction. Do not refer to Appendix B, the safety chapter in OMA. For example,

Caution: Listeria monocytogenes infections can cause fetal death. It is recommended that pregnant women avoid handling this organism. Attention should be given to sterilization of contaminated equipment and media before disposal or reuse.

4. Interlaboratory Study Results Table

Immediately following the precaution statement, the method must also contain statistical information if the collaborative study provides sufficient information with regard to the reliability of the method. The required statistical study results are s_r, s_R, RSD_r, RSD_R, HORRAT, % Rec, mean, # of labs retained after eliminating outliers, and # of outlier labs removed (see Part 6) and should be presented in a table. For example:

See Table 2001.xxA for Interlaboratory study results that support acceptance of the method.

Table xxx.xx. Interlaboratory Study Results for...

Material		No. of Labs [a(b)]	Mean (units)	Recovery [c] %	Repeatability	Reproducibility	
Matrix	Level, (units)				RSD_r (%)	RSD_R (%)	HORRAT[c]

[a(b)] a = number of labs retained after eliminating outliers, (b) = number of labs removed as outliers

[c] when applicable

5. Principle

The method should include a statement explaining the purpose of various steps during analysis and the basis of unfamiliar or unusual reactions. For example, the method for the alkaline titration of cyanide with silver nitrate was the subject of many letters that pointed out an apparently incorrect stoichiometric factor. An insertion was made in the method to the effect that 1 Ag is equivalent to 2 CN in that reaction. The principle should establish the scope of the method and purpose. For example,

A. Principle

Trans-galactooligosaccharides (TGOS) and lactose are extracted from a test portion with hot phosphate buffer. The extract is treated with ß-galactosidase to hydrolyze TGOS and lactose. Both the initial and the treated solution are analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). In the first assay, free galactose and lactose are determined in the initial test solution. In the second assay, the total amount of galactose released from TGOS and lactose is determined in the treated solution. TGOS are calculated from concentrations of lactose and galactose.

6. Apparatus

Ordinary apparatus -- beakers, flasks, funnels, etc. -- which are usually part of the standard equipment of the ordinary analytical laboratory, or which are listed in the catalogs of the larger supply houses, are not generally listed in the apparatus section of a method. Common laboratory equipment such as analytical balances and pH meters do not need to be mentioned in a method.

Apparatus that requires assembly and/or specifications, or which may not be readily available, is listed in this section. Descriptions or specifications are preferred for assembled apparatus, however, line drawings or, in rare instances, photographs may be included for clarity. With drawings, the scale should be indicated and the parts should be labeled or coded. The source of commercially available apparatus used in the collaborative study and *Official Method*℠ should be given, with complete (and correct) name, address, city, state, and zip code. A recent letterhead, advertisement, or catalog should always be checked for this information. The annual *Guide to Scientific Instruments* published by Science and the *Laboratory Guide* published by Analytical Chemistry are useful for this purpose. All

proprietary equipment must be described in performance language giving suitability tests where possible. Critical parameters must be identified and defined, and system suitability standards must be established for non-proprietary equipment or reagents so that the product can be defined generically and equivalency can be readily determined. When writing a method, the author must refer and comply with the AOAC policy for *Determination of Equivalency of Products Used in Official Methods*℠ (see Appendix A).

If the equipment must be operated in accordance with the instructions supplied by the manufacturer, this may be stated, as is frequently the case with flame photometers and spectrophotometers. Checking the reliability and accuracy of instruments is an implied function of the operator as is calibration of weights and volumetric apparatus. Convenient calibration standards for both wavelength and absorbance scales of spectrophotometers are given in *Definitions of Terms and Explanatory Notes* (see Appendix D).

Chromatographic columns:

When several columns must be prepared for a single method, a separate section may be used. For example:

(a) *Column 1.* (1) *Lower layer*— Mix 3 g diatomaceous earth and 2 mL citrate buffer; transfer to tube and tamp. (2) *Upper layer* — Add 0.5 mL aliquot of extract. Add 3 g diatomaceous earth, mix, and transfer to tube. Dry-wash beaker with 1 g diatomaceous earth and add wash to column; tamp and add glass wool pad.

(b) Column 2. — Mix 3 g diatomaceous earth and 2 mL 1.0M K_2HPO_4 (17.42 g/100 mL); transfer to tube, tamp, and add glass wool.

Gas and liquid chromatography:

Because of the numerous parameters involved in gas or liquid chromatographic equipment, a separate section may be devoted to this type of apparatus, including preparation of columns. The following items should be included (adapted from J. Agric. Food Chem. 17, 160 (1969)):

(a) *Apparatus* — Performance criteria are preferred over specific manufacturer's makes and models. Summarize, if feasible, performance requirements by specifications as in examples below:

(1) Monitor performance of gas chromatograph by noting separation of campesterol and sitosterol expressed as peak resolution = 2D/(C+B), where D = distance between the 2 peak maxima, C = campesterol peak base width, B = beta-sitosterol peak base width. Peak resolution should be <1:6.

(2) Optimum conditions for gas chromatographic separation are obtained when peaks for solvent, methyl enanthate standard, and formic acid are completely resolved. Conditions vary to some degree from instrument to instrument and should be experimentally reestablished.

(3) Select as operating voltage that voltage at which heptachlor epoxide causes ca 40-50% full-scale recorder deflection. Check linearity of system from 0.2 - 2.0 ng heptachlor epoxide.

Detector type, such as thermal conductivity, flame ionization, electron capture, etc., and associated parameters such as recorder range, detector voltage, and bridge current must be described.

(b) *Column length and diameter* — Inside diameter is preferred, but outside diameter and wall thickness, particularly if this is commercial usage, may be indicated. Materials: glass, copper, stainless steel, etc. Packing: weight percent of liquid phase on support. Give mesh size and pre-treatment of support. Supply sources of materials. Capillary: only liquid phase or support-coated liquid phase. Conditioning: if necessary.

(c) *Conditions* — Temperatures: injection, column, detector isothermal or programmed (give initial and

final temperatures and rate of temperature rise); e.g., "Temperatures (°C) - injection 250, column 135, detector 235." Flow rates: carrier gas (mL/min at exit port), other gases; e.g., "Flow rates (mL/min)-N 25, H 25, air 300."

(d) *Analyte solution* -- Quantity (mL) injected, solvent, if used, retention time (minutes or distance), retention time relative to reference compound and internal standard, if used.

(e) *Chromatogram* -- Recorder response for specific quantity of standard; resolution in terms of separation of specified compounds; peak measurements and baseline correction; calculation.

For example,

B. Apparatus and Materials

(a) *High performance anion-exchange chromatograph (HPAEC).* — Liquid chromatograph gradient pump, de-gas module, microinjection valve, pulsed electrochemical detector (1.0-mm diameter gold working electrode and a pH-Ag|AgCl combination reference electrode; the titanium body of the cell serving as the counter electrode) working in pulsed amperometric detection mode (PAD), automated sampler (Dionex Corporation, P.O. Box 3603, Sunnyvale, CA 94088-3603, USA, USA +1-408-737-0700, Fax: +1-408-730-9403, or equivalent) and data integrator (Shimadzu, 1 Nishinokyo Kuwabaracho, Nakagyou-ku, Kyoto 604-8511, Japan, Phone: +81-75-823-1111, Fax: +81-75-823-1361, or equivalent.)

HPAEC conditions — Column temperature, constant ± 0.5°C between 20-30°C, preferably 20 ± 0.5°C; flow rate, 1.0 mL/min; injection volume, 20 μL; detector sensitivity, analog range 1-3 μC. See Table 2001.01B for eluent gradient and Table 2001.01C for detector time program. Parameters may be varied in order to optimize chromatography.

Table 2001.01B for eluent gradient and Table 2001.01C for detector time program. Parameters may be varied in order to optimize chromatography.

Table 2001.01B Eluent gradient for HPAEC-PAD analysis

Time (min)	(%) Mobile phase A	B	C
0.00	95	5	0
20.10	95	5	0
35.00	0	100	0
36.00	0	100	0
36.10	0	0	100
46.00	0	0	100
46.10	95	5	0
61.00	95	5	0

Table 2001.01C Detector program for HPAEC-PAD analysis

Time (s)	Potential (V)	Integration
0.00	0.05	
0.20	0.05	Begin
0.40	0.05	End
0.41	0.75	
0.60	0.75	
0.61	-0.15	
1.00	-0.15	

(b) Column.— CarboPac PA-1 Pellicular anion exchange resin, 250 x 4 mm id with 50 x 4 mm id guard column of same resin composed of sulfonated ethylvinylbenzene-divinylbenzene particles agglomerated with 350μm of Micro Bead quaternary amine-functionalized latex, or equivalent.
(c) pH-meter. — Temperature compensated, standardized with pH 4.0 and 7.0 buffer solutions.
(d) Plastic vials. — 50 mL, with screw caps, resistant to temperatures up to 100°C.
(e) Water baths.— With shaker, maintaining 60 + 2°C and 80+ 2°C.

7. Reagents

Do not list common reagents that would ordinarily be expected to be available in a well equipped analytical laboratory. As in the apparatus section, describe reagents in terms of critical performance characteristics, rather than brand names.

Reagents without specifications are automatically reagent grade, conforming to the specifications of the American Chemical Society (ACS) when such specifications exist. If there are no ACS specifications, the best available grade is understood. Designate Reference Standard reagents available from NIST, BCR, USP, etc., as such, e.g., "USP Reference Standard Digitoxin."

The "Reagents" section is also used for materials requiring directions for preparation, purification, or standardization and must be included. Standard compounds will often need specifications or a source of supply (see Apparatus section 6 for designation of suppliers).

List reagents in whatever appears to be the most convenient sequence: alphabetically, order of use in method, or systematically: pure compounds, standard solutions, solutions of approximate strength, and indicators.

For completeness, note the following points: indication of stability, particularly of solutions; designation of anhydrous or number of moles of water of hydration for hydratable compounds (particularly important in buffer solutions and media); alternative use of a homologous alcohol, or special denatured alcohol, for pure alcohol (see "*Definitions of Terms and Explanatory Notes*, Appendix D).

Strength of solutions:

(1) Common acids and ammonia are always the concentrated reagents, unless otherwise specified.

(2) Express dilutions as molarity (0.03 M), or by a parenthetical expression [HCl (3 + 2)] where the first number always refers to the volume of reagent and the second to the volume of diluting solvent (which in most cases is understood to be water). For multiple mixtures, use the form: alcohol+acetate+ether (3 + 4 + 1).

(3) Express reagent concentrations in terms of weight/volume percent, unless otherwise specified. An "x%" solution means that x g of material is dissolved in water or other solvent and diluted to 100 mL. However, avoid use of percent in the case of liquids with a density appreciably different from that of water (e.g., sulfuric acid). Because of possible ambiguity, use form (2) above with liquids or specify whether weight or volume is meant, e.g., (w/w), (w/v), or (v/v). Note that when a compound like hydrochloric acid, sulfuric acid, alcohol, etc., is used in a non-reagent sense (i.e., the material being determined), it means the compound itself, unless otherwise specified [e.g., formalin = 37% (w/w) HCHO in water].

(4) Although "alcohol" is understood to be the 95% azeotrope, express all other dilutions in terms of the amount of the actual compound present as prepared by the following dilution rule:

An "x%" alcohol solution is prepared by diluting x mL 95% alcohol to 95 mL with water. When anhydrous alcohol is meant, specify as such.

Standard solutions:

Indicate the material first. If dilutions are required, designate them as stock (if storable), intermediate, and working solutions. Indicate concentration immediately following the title. Note these examples:

(a) *Manganese standard solution, 50 mg/mL*-- Dissolve 50.0 g pure Mn metal powder in 20 mL 0.1 M H_2SO_4 and dilute to 1 L with water.

(b) *Riboflavin standard solutions*-- (1) *Stock solution, 100 mg/mL*-- Dissolve 50 mg USP Reference Standard Riboflavin, previously dried and stored in dark in desiccator over P_2O_5, in 0.02 M acetic acid to make 500 mL. Store under toluene at ca 10°. (2) *Intermediate solution, 1.0 mg/mL*-- Dilute 100 mL stock solution to 1 L with 0.02 M acetic acid. Store under toluene at ca 10°. (3) *Working solution 1, 1.0 mg/mL*-- Dilute 10 mL intermediate solution to 100 mL with water. Prepare fresh for each assay. (4) *Working solution 2, 0.1 mg/mL*-- Dilute 10 mL intermediate solution to 1 L with water. Prepare fresh for each assay.

When a number of components are measured in the same method and a separate standard solution must be prepared for each, or if the preparation of the standard solutions occupies a fairly large portion of the reagent section, a separate section on "Standard Solutions" may be used. Multiple component standard solutions could be written as follows:

Methyl 9,10-dibromostearate (DBS) and methyl 9,10,12,13-tetrabromostearate (TBS) standard solutions -- Pipet 3, 5, and 10 mL (6, 10, and 20 mg) DBS standard solution into 3 separate dry conical flasks and add 3, 5, and 10 mL (3, 5, and 10 mg) methyl pentadecanoate (MPD) standard solution to each. Similarly prepare TBS-MPD solutions. Treat each solution as follows: Evaporate solvent with N at 40°C. Add 25 mL 1% Na in methanol and 12 mL anhydrous benzene, and reflux 1 h. Cool, and transfer to 125 mL separator containing 50 mL water. Acidify with 1.0 M H_2SO_4 and extract with three 30 mL portions of ether, using first 30 mL to rinse flask. Combine ether extracts in second separator, wash with two 10 mL portions of water, dry over anhydrous Na_2SO_4, filter, and evaporate solvent on rotary evaporator at 40°C. Dissolve residue in 3 mL ether.

For natural product reference standards, e.g., mycotoxins, supply the following information: the source of the compound and the method of isolation, the method of purification and criteria of purity, stability data under conditions of dispensing and use, and the method for checking concentration and purity.

Buffers:

Indicate the nature and pH of the solution. Always indicate the hydration state of the salts used, e.g.:

Phosphate buffer solution, pH 8-- Dissolve 16.73 g anhydrous K_2HPO_4 and 0.523 g anhydrous K_2HPO_4 in water and dilute to 1 L.

Water:

"Water" unqualified refers to distilled water, except where it does not mix with the determination, as in a water bath. If deionized water may be used, insert a statement to that effect in the "Reagents" section or in an introductory or parenthetical statement at the beginning of the method or determination.

Purification:

When solids are to be purified by recrystallization, specify the preferred solvent, temperature, and initial solid-to-volume ratio. When liquids are purified by distillation, specify the characteristics of the starting material and the boiling point range of the desired fraction, and state the fraction of the first distillate and residue that should be discarded.

If pre-purified material is available, indicate the source and any physical constants that are important in checking purity.

Media:

Microbiological media are often not well characterized chemically. A particular effort should be made to obtain the exact formula prepared and used by the originator or user. Media designated by an investigator's name may often be found to have several different compositions. When acceptable, use the formulas already given in *Official Methods of*

Analysis of AOAC INTERNATIONAL, under disinfectants, antibiotics (in feeds), or microbiological methods. Prepared or dehydrated media may be used, provided directions for preparation by the analyst from the basic ingredients are incorporated.

In the media statement, specify the hydration state of the salts used and the pH of the medium (before or after sterilization), and how to dispense and sterilize.

In a series of methods using the same buffers and media, or with minor modifications, use a combined "Reagents and Media" section for all the methods as is done for the microbiological methods in feeds.

For example,

C. Reagents

(a) Phosphate buffer. — 0.2 M, pH 6.0. Dissolve 22.0 g KH_2PO_4 and 6.0 g $K_2HPO_4 \cdot 3H_2O$ in water and dilute to 1 L. Sterilize 30 minutes at 120°C in the autoclave.
(b) Hydrochloric acid. — 1 M. Dilute 8.3 mL HCl to 1 L with water.
(c) Sodium hydroxide solution. — 50%, carbonate-free, density 1.54 kg/L. To 100 g NaOH, containing ±1% Na_2CO_3, add 100 mL water. Stopper and swirl until solution is complete. Let stand until Na_2CO_3 has settled, leaving a clear liquid (about 10 days). Keep tightly closed when not in use.
(d) Sodium hydroxide solution. — 1 M. Dilute 54 mL NaOH solution, c, to 1 L with CO_2-free water.

Apparatus and Reagents:

If a method contains either a single apparatus entry or a single reagent entry, they may be combined into a joint section titled "Apparatus and Reagents" (or "Reagents and Apparatus").

8. Preparation of Calibration Curve

If a calibration curve is prepared by conducting a series of standards throughout the entire determination, place this section at the beginning or end of the method with directions to treat the specified standards as in the determination.

If the calibration curve is prepared at only the final determinative step, place the section before the determination as a special section. With this arrangement, most of the conditions of the final measurement can be placed here and need not be repeated for the determination. Some of the points to be checked, with special reference to spectrophotometry, are:

(1) Preparation of a series of standard solutions in the concentration range of interest;

(2) Volumes of reagents to be added and the order, waiting periods, temperatures, and dilution to final volume, if necessary;

(3) Optimum instrument settings for gain, slit width, response, speed, drum drive settings, cell length, etc., or instructions on how to obtain these settings;

(4) Directions for preparation of positive and negative control solutions, blanks, and reference solutions;

(5) Spectral range of interest;

(6) Procedure for making background corrections;

(7) Plotting of absorbance (A) or transmittance (T) against concentration or absolute quantities; and

(8) Frequency that the calibration curve should be repeated.

9. Preparation of Test Sample

This section contains the directions for preparing a homogeneous analytical sample; dissolving, dispersing, or diluting the test portion; and preparing a solution that is ready for separations or determinations. It may also contain several alternatives in accordance with the nature of the starting material or in the concentration of the active ingredient.

For example,

D. Preparation of Test Sample

Homogenize liquid laboratory samples immediately before analysis. Cut or shatter hard materials to pass through a 1 mm 2 sieve (No 18).

When a method is applicable to a wide range of component concentrations where different test portion weights and dilutions are required to bring the final concentrations into the measurable range, a table of weights, volumes, dilutions, and dilution factors applicable to specific concentrations or ranges are convenient. This section need not be included in short methods.

Significant figures:

Maintain consistency in the degree of accuracy required in various measurement steps. There is no need to weigh a test portion to five significant figures in a spectrophotometric method where the final absorbance measurement yields data with only three significant figures.

The phrase, "Accurately weigh approx. x [test portion] and record to the nearest y units" means that the weight taken should be ± 10% of that specified but that the weight should be known to the number of significant figures commensurate with the other determinative steps in the method.

In general, a measurement is understood to utilize the full degree of sensitivity of the instrument, e.g., 0.1 mg for an analytical balance; 0.01 mL for a buret or pipet. The statement, "accurately weigh" or "pipet," needs no further qualification, i.e., "accurately weigh about 5 g (to 5 significant figures)" or "pipet 10 mL" means 5.xxxx g or 10.00 mL, respectively. Alternative expressions, usually for emphasis, that may be used are: "Weigh 5.000 g" or "Transfer 5.00 mL [from buret]." Do not use the redundant forms, "accurately weigh 5.000 g..." and "Pipet 10.00 mL," unless the exact specified quantity is required.

"Recommendations for Preparation of test samples for Collaborative Study Manuscript of Microbiological Methods", *J. Assoc. Off. Anal. Chem.* 70, 931-937 (1987).

"Biological/Pass-Fail Task Force Report", *JAOAC*, 70, 348-349 (1987).

"Validation of modern methods in food microbiology by AOAC INTERNATIONAL collaborative study", *Food Control*, 7, 19-29 (1996)

"Three validation programs for methods used in the microbiological analysis of foods', *Trends in Food Science and Technology*, 7, 147-151 (1996)

10. Determination

If a method is fairly straightforward or consists only of a single major step, place all operations directly under the statistical parameters of the method. If the method is complex, however, divide the determinative step into several parts which may be characterized by the type of operation performed. The chapters on metals, natural poisons, and

pesticide residues contain numerous examples of special section headings that, if properly selected, give an outline of the method.

All critical control points must be identified. Indicate when a determination may be interrupted overnight. Even more important, indicate when a determination may not be interrupted. Indicate limitations of determinative steps that appear to be precise but in practical terms are not. Examples: "Dry to constant weight" should indicate the weight deviation which is considered as constant, e.g., ±1.0 mg or ±0.5 mg; "Color is stable" should indicate for how long; "Read color after 5 min" should indicate the time range which is satisfactory. If the method is empirical, emphasize this fact so that the analyst will make a deliberate effort to maintain constant conditions from test sample to test sample. If steps in various parts of the method are the same, use identical wording throughout the method; do not use synonyms. For example,

E. Determination of Lactose and Galactose

(1) *Preparation of test solutions for HPAEC-PAD analysis.*— Dilute the centrifuged filtrates from F with 3% acetonitrile, g, so that galactose and lactose contents are within the working standards concentration range. It may be necessary to use 3 different dilution factors, depending on the nature of the test materials. Guide values and denotation for dilution factors can be found in Table 2001.01D.

Table 2001.01D Guide values for dilutions for HPAEC-PAD analysis

Laboratory sample category	M_2 (g)	D_1	D_2	D_3
Yogurt drink (4-7% TGOS)	2.5	25	250	100
Fruit syrup (14-18% TGOS)	1.5	10	300	100
Custard (4-7% TGOS)	2.0	4	200	100
Orange juice (4-7% TGOS)	2.5	3	125	30
Biscuits (7-10% TGOS)	2.0	6	200	60
Cereal (4-7% TGOS)	1.0	5	200	200

D_1 = Dilution factor of galactose in assay A_1
D_2 = Dilution factor of galactose in assay A_2
D_3 = Dilution factor of lactose in assay A_1

(2) *Determination.*— Use the same type of integration for the test solutions and for the standard working solutions by choosing the same peak width, threshold settings, and other integration parameters. Carefully control the baseline selection by extending the baseline next to the peak. Use peak area for quantification.

First run the 4 calibration standards of each sugar to establish linearity. Then repeat the 4 standards. Between every two sets of standards, run 9 test solutions (e.g., WS_1, WS_2, WS_3, WS_4, initial test solution $1A_1$ (dilution factor D_1), initial test solution $1A_1$ (dilution factor D_3), treated solution $1A_2$, initial test solution $2A_1$ (dilution factor D_1), initial test solution $2A_1$ (D_3), treated solution $2A_2$, initial test solution $3A_1$ (D_1), initial test solution $3A_1$ (D_3), treated solution $3A_2$, WS_1, WS_2, WS_3, WS_4, initial test solution $4A_1$ (D_1), initial test solution $4A_1$ (D_3), treated solution $4A_2$, etc.). Continue this process until all test solutions have been analyzed. Use average response factor from the standards bracketing the test solutions to calculate sugar concentration for each test solution.

(3) *Possible interferences.*— Galactose is the most important parameter for the assessment of TGOS content in the product. Inaccuracies in the determination of galactose after enzymatic hydrolysis of test samples high in lactose like cereal and powdered milk based products, can influence the measurement of galactose liberated from TGOS. Also non-selective hydrolysis of alpha-galactans (e.g. carob powder) by ß-galactosidase may occur, leading to erratic results. Use fresh enzyme preparations only.

11. Calculations

Calculations are included in a method for convenience to avoid the need for looking up factors and deriving equations, particularly when a series of multiple dilutions or aliquots are used at various steps in the method. In most

cases, editors will transform equations into the single-line form. Particular care must be taken to ensure that there is no ambiguity with regard to the entries in the numerator and the denominator. The following equation, although mathematically clear, is often used incorrectly:

$$y = a/b \times c$$

Mathematically this equation means: $y = a/(b \times c)$. Authors usually intend it to mean:

$$y = (a/b) \times c, \text{ or } (a \times c)/b$$

Write an equation so that the interpretation can be followed easily. Use a separate line for each equation, unless the equation is small and can be incorporated into the paragraph. If the equation is complex, add a simplified final equation in which all of the numerical factors have been combined into a single defined constant.

When the equation includes initial test portion weights or specified aliquots, errors are often made by incorrectly placing modified weights and volumes in the numerator or denominator. Therefore, the preferred form should be similar to the following:

Component = (A of component/g test portion) x (total vol./mL aliquot) x (g std X % purity/A of std)

If numerous or variable dilutions have been made, the volume relationships may be combined into a single dilution factor which may be mathematically defined in a separate equation for simple calculation. A dilution factor is the final volume to which a solution is diluted, V_f, divided by the volume of the original solution, V_o, contained in the final dilution:

$$F=\frac{V_f}{V_o}$$

For serial dilutions:

$$F=\frac{(V_f)}{(V_o)} \times \frac{(V_{f1})}{(V_{fx})} \times \ldots$$

As a rule, use symbols in equations, defining them in a separate entry. Use A only for absorbance, W for weight, and V for volume. To differentiate unknown and standard, use the symbol alone for the unknown (A) and the symbol followed by a prime mark for the standard (A'). Where several components are involved, differentiate the measured values by subscript symbols. Letters of the alphabet are best, especially if they can be related to the component being measured, e.g., use A_P for absorbance of the phenobarbital component and $A_{P'}$ for that of its standard. Where 2 components have the same initial letter, use numbers or other letters. Do not italicize (underline) subscripts, because these are often difficult to read.

For example,

F. Calculations

Calculate the initial (free) galactose, G_b, and (initial = final) lactose, L_b, contents in the buffered extracts assay A_1 and total (final) galactose content, G_t, of the hydrolyzed solution A_2 in g/100g product of test sample using the following formula:

$$G_b=[C_{Gb}\times D_1\times(M_9-M_7)]/(F\times M_8\times 100)$$

where C_{Gb} = mg galactose/kg in initial test solution A_1 and F is a factor calculated as:

$$F=(M_2\times 100)/(M_3-M_1)$$

$$G_t=[C_{Gt}\times D_2\times(M_6-M_4)]/(F\times M_5\times 100)$$

where C_{Gt} = mg galactose/kg treated solution in assay A_2.

$$L_b=[C_{Lb}\times D_3\times(M_9-M_7)]/(F\times M_8\times 100)$$

where C_{Lb} = mg lactose/kg initial test solution in assay A_1.

Calculate galactose released from TGOS (G_g) (g/100g test sample):

$$G_g=G_t-G_b-G_l$$

where G_l = galactose released from lactose = L_b/1.9.

Calculate TGOS content (g/100g test sample):

$$TGOS=k\times G_g$$

where k = (180 + 162 n)/(180 n). n is the average number of galactose moieties in the TGOS molecules. For example, if n = 2, k is 1.4.

12. Notes

In general, avoid use of notes at the end of the method. Incorporate their content at the appropriate place in the method. If the note is applicable to the entire method, place it at the beginning of the method directly under the statistical performance parameters, e.g., "(Rinse all apparatus with dilute nitric acid)." Use the Principle section to establish the scope of the method and the modifications necessary for products outside the scope. If modifications have been tested, place them in separate sections or subsections. Place special precautions, preparations, treatments, storage conditions, stability, sources of supply, tests, purifications, specifications, and similar materials pertaining to apparatus and reagents in their specific sections. If the Determination section includes special treatments for interfering materials or elimination of certain steps in special cases, handle these as parenthetical statements, special sections, or subsections. Place comments about when the method may or may not be interrupted in the Determination section.

Use parentheses sparingly and never for essential information. Commas are often a satisfactory substitute. Avoid use of the double and triple parentheses, except in formulas where they may be essential.

13. Automated Methods

Because of the many special parameters and requirements involved in automated analysis, follow the guidelines given below:

(1) *Principle* — Indicate, in performance language if possible, the products to which the method applies;

give applicable range and statistical information on accuracy and precision. Give reactions involved, determinative technique, and any interferences.

(2) *Apparatus* – List individual components of the automatic analyzer under a single heading when they are part of the basic unit and give instructions for any modifications. List other items separately. Submit a labeled flow diagram and separate diagrams of any specially constructed pieces of equipment. Describe equipment generically, in terms of performance, so that equivalent equipment can be determined.

(3) *Reagents* – Give concentrations of reagents and any necessary directions for purification, preparation, storage, etc. State how long a standard solution or reagent may be kept before a fresh one must be prepared, if this point is critical. Do not list stock items usually found in the laboratory that need no special preparation. Indicate the amount of reagent used for each series of analyses.

(4) *Analytical system* – Refer to the flow diagram and describe air and solution stream flows. Show pump tube rates in mL/min of analytes, reagents, standard solutions, and air, and specify the pump tube material. Give inner diameters of critical interconnecting tubing and any other information that needs to be specified.

(5) *Start-up and shut-down of system* – Give steps for starting and stopping the system, and the procedure for leaving the system in satisfactory condition for the next series. Indicate troubles that may develop and the means to correct them, e.g., irregular baseline, irregular peaks, poor reproducibility.

(6) *Maintenance* – Indicate how to clean and maintain the equipment.

(7) *Checks and calibration* – Indicate how to check for proper operation of the system, including checks for contamination, recoveries, linearity, drift, and carryover. Give settings for various controls (or condition to be maintained, e.g., temperature) and approximate readings that should be obtained for range of concentration to be measured.

(8) *Preparation of calibration curve* – If applicable, give directions, including instructions for handling the blank.

(9) *Isolation of analyte* – Indicate how to prepare test solutions from original materials to be analyzed by the method. Give general directions for the applicable commodities, not just for the particular commodities used in the collaborative study.

(10) *Determination* – State how many and in what order standards and analytes are to be analyzed and whether replication is necessary. Indicate how to draw the baseline and report results. Provide formulas for calculation if applicable.

DEFINITIONS AND CALCULATIONS OF HORRAT VALUES
FROM INTRALABORATORY DATA

1. ***Definitions:***

1.1 ***Replicate data*** are data developed under common conditions in the same laboratory: simultaneous performance, or, if necessary to obtain sufficient values, same series, same analyst, same day. Such data provides "repeatability statistical parameters".

1.2 ***Pooled data*** are replicate data developed in the same laboratory under different conditions but considered sufficiently similar that for the purpose of statistical analysis they may be considered together. These may include different runs, different instruments, different analysts, and different days.

1.3 ***Average*** = $\bar{x}$ = sum of the individual values, x_i , divided by the number of individual values, n.

$$\bar{x} = (\sum x_i)/n$$

1.4 ***Standard deviation*** = $s_i = [\sum(x_i - \bar{x})^2/n]^{0.5}$

1.5 ***Relative standard deviation*** = RSD = $s_i \times 100/\bar{x}$.

1.5.1 ***Repeatability*** *relative standard deviation* = RSD(r) or RSD_r
The relative standard deviation calculated from within-laboratory data.

1.5.2 ***Reproducibility*** *relative standard deviation* = RSD(R) or RSD_R
The relative standard deviation calculated from among-laboratory data.

1.6 ***Mass fraction***. Concentration, C, expressed as a decimal fraction. For calculating and reporting statistical parameters, the data may be expressed in any convenient units (e.g., %, ppm, ppb, mg/g, µg/g; µg/kg; µg/L, µg/µL, etc.). For reporting HORRAT values, the data must be reported as a mass fraction where the units of the numerator and denominator are the same: e.g., for 100% (pure materials), the mass fraction C = 1.00; for 1 µg/g (ppm), C = 0.000001 = (E-6). *See* table, **1.8**, for other examples.

1.7 ***Predicted relative standard deviation*** = PRSD(R) or $PRSD_R$. The reproducibility relative standard deviation calculated from the Horwitz formula:

$$PRSD(R) = 2C^{-0.15}$$

Where C is expressed as a mass fraction. *See* table, **1.8.**

In spreadsheet notation: PRSD(R) = 2 * C ^(-0.15).

1.8 ***HORRAT value.*** The ratio of the reproducibility relative standard deviation calculated from the data to the PRSD(R) calculated from the Horwitz formula:

HORRAT = RSD(R) / PRSD(R)

To differentiate the usual HORRAT value calculated from ***reproducibility*** data from the HORRAT value calculated from ***repeatability*** data, attach an R for the former and an r for the latter. But note that the denominator always uses the PRSDR calculated from reproducibility data because this parameter is more predictable than the parameter calculated from repeatability data:

$$HORRAT(R) = RSD_R / PRSD(R)$$
$$HORRAT(r) = RSD_r / PRSD(R)$$

Some expected, predicted relative standard deviations are given in the following summary table:

Concentration, C	Mass fraction, C	PRSD(R) (%)
100 %	1.0	2
1 %	0.01	4
0.01 %	0.0001	8
1 ppm	0.000001	16
10 ppb	0.00000001	32
1 ppb	0.000000001	45

2.0 *Acceptable HORRAT values*

2.1 ***For interlaboratory studies:***
HORRAT(R): The original data developed from interlaboratory (among-laboratory) studies assigned a HORRAT value of 1.0 with limits of acceptability of 0.5 to 2.0. The corresponding within-laboratory relative standard deviations were found to be typically one half to two thirds the among-laboratory relative standard deviations. See 2.2.1.

2.1.1 *Limitations*

HORRAT values do not apply to method-defined (empirical) analytes (moisture, ash, fiber, carbohydrates by difference, etc.), physical properties or physical methods (pH, viscosity, drained weight, etc.), and ill-defined analytes (polymers, products of enzyme reactions).

2.2 *For intralaboratory studies:*

2.2.1 *Repeatability*

Within-laboratory acceptable predicted target values for repeatability are given in the following table at 1/2 of PRSDR, which represents the best case.

Concentration, C	PRSD(R) (%)	Target RSD(r)(%)
100 %	2	1
1 %	4	2
0.01 %	8	4
1 ppm	16	8
10 ppb	32	16
1 ppb	45	22

2.2.2 *HORRAT(r)*

Based on experience and for the purpose of exploring the extrapolation of HORRAT values to single-laboratory validation (SLV) studies, take as the minimum acceptability one half of the lower limit ($0.5 \times 0.5 \approx 0.3$) and as the maximum acceptability two thirds of the upper limit ($0.67 \times 2.0 \approx 1.3$).

Calculate HORRAT(r) from the SLV data:

$$\text{HORRAT(r)} = \text{RSD(r)} / \text{PRSD(R)}$$

Acceptable HORRAT(r) values are 0.3 – 1.3. Values at the extremes must be interpreted with caution. With a series of low values check for unreported averaging or prior knowledge of the analyte content; with a series of high values, check for method deficiencies such as unrestricted times, temperatures, masses, volumes, and concentrations; unrecognized impurities (detergent residues on glassware, peroxides in ether); incomplete extractions and transfers and uncontrolled parameters in specific instrumental techniques.

2.3 ***Other limitations and extrapolations***

The HORRAT value is a very rough but useful summary of the precision in analytical chemistry. It overestimates the precision at the extremes, predicting more variability than observed at the high end of the scale (C >ca 0.1; i.e., >10%) and at the low end of the scale (C <E-8; i.e., 10 ng/g; 10 ppb).

If the limit of detection is assumed to be three times the standard deviation of the blank, then the lower limit of analytical chemistry is delineated by an interlaboratory relative standard deviation of about 33% (equivalent to an intralaboratory relative standard deviation of 20-25%), as shown by Thompson and Lowthian (J. AOAC Int. (1997) 80, 676). Indeed, the mycotoxins paper of the HORRAT series (J. AOAC Int. (1993) 76, 461) shows the number of false negatives in the interlaboratory environment increases as the concentration decreases below about 10 ng/g. On the other hand, a number of recent interlaboratory studies from the European Union show HORRAT(R) values of 1.0 and below in studies of the mycotoxins methods for numerous analytes and commodities at levels of 1 ng/g and below, reflecting a considerable investment in practice and training.

Another observation of Horwitz that was confirmed by Thompson and Lowthian is that the precision in analytical chemistry at any given concentration does not improve with time, despite the advances in analytical technology.

2.4 ***<u>Example</u> (for reported values of 17-18 ppm):***

	Value (g/g)	Average	Std Dev	RSD(r)	PRSD(R)	HORRAT(r)
Day 1	0.0000185					
	0.0000166					
	0.0000178					
	0.0000167					
	0.0000169	0.0000173	8.216E-7 (0.0000008216)	4.749	10.4	0.46
Day 2	0.0000182					
	0.0000186					
	0.0000185					
	0.0000178					
	0.0000175	0.0000181	4.658E-7	2.571	10.3	0.25
All 10 values:		0.0000177	7.637E-7	4.312	10.3	0.42

<u>Interpretation</u>: The target "best case" values for RSD(r) are ([1/2]*10.3) = 5.2% ([1/2]*PRSD(R)). The actual RSD(r) values are better than this, and falling well within the prescribed acceptable HORRAT(r) region of 0.3-1.3.

NOTE: For those not familiar with spreadsheet notation (Microsoft EXCEL), it uses the following symbols:

* = Multiplication sign (x or center dot in mathematical formulae)

^ = Exponential sign (superscript in mathematical formulae)

The + sign is used for addition, the – sign for subtraction, and the forward slash "/" for division as in ordinary arithmetic.