(出國報告類別:國際會議)

赴美國參加 2013 年美國法醫毒物學者學會聯合年會(SOFT)會議報告

出國人員姓名/服務機關/單位/職稱

林棟樑/法務部法醫研究所/毒物化學組/組長

劉秀娟/法務部法醫研究所/毒物化學組/副研究員

出國期間：民國 102 年 10 月 25 日至民國 102 年 11 月 4 日

出國地點：美國奧蘭多

報告日期：民國一○二年十二月三十一日

出國報告名稱：赴美國參加 2013 年美國法醫毒物學者學會年會(SOFT)會議報告

頁數：21　　含附件：否

出國計畫主辦機關/聯絡人/電話

法務部法醫研究所/陳忠福/22266555

出國人員姓名/服務機關/單位/職稱/電話

林棟樑/法務部法醫研究所/毒物化學組/組長/22266655

劉秀娟/法務部法醫研究所/毒物化學組/副研究員/22266655

出國類別：其他

出國期間：民國一○二年十月二十五日至民國一○二年十一月四日

出國地點：美國奧蘭多

報告日期：民國一○二年十二月三十一日

關鍵詞：法醫毒物、論文發表

內容摘要：

二〇一三年十月二十五日至十一月四日期間赴美國奧蘭多參加 2013 年美國法醫毒物學者學會年會(SOFT)，為期十一日。

參加美國 2013 年美國法醫毒物學者學會年會(SOFT)，會議包括專題演講、研討課程、口頭發表論文、壁報張貼論文及儀器、書籍展示等，來自世界各地與會代表數百人參與，本所並於年會中公開發表有關法醫毒物分析之論文二篇。

與來自全美及世界各地之學者權威、毒藥物檢驗專家齊聚一堂，討論研究內容及研究方向並發表心得，促進國際學術及鑑識技術之交流，並可了解先進國家在毒藥物鑑識科學領域的具體作法，並比較先進國家與本國流行毒藥物之不同，機會非常難得，一方面可增廣見聞，瞭解世界鑑識科學發展的趨勢，除此之外本所派員參加亦可促進學術交流，增加國際曝光度，提升本國及本所國際聲譽。

赴美國參加法醫毒物國際會議報告報告

目　　次

壹、出國目的…… 2

貳、過程…… 3

叁、會議內容…… 4

肆、檢討建議及心得感想…… 6

伍、附件資料(論文壁報及摘要)…… 12

摘　　要

二〇一三年十月二十五日至十一月四日期間赴美國奧蘭多參加2013年美國法醫毒物學者學會年會(SOFT)，為期十一日。

參加美國2013年美國法醫毒物學者學會年會(SOFT)，會議包括專題演講、研討課程、口頭發表論文、壁報張貼論文及儀器、書籍展示等，來自世界各地與會代表數百人參與，本所並於年會中公開發表有關法醫毒物分析之論文二篇。

與來自全美及世界各地之學者權威、毒藥物檢驗專家齊聚一堂，討論研究內容及研究方向並發表心得，促進國際學術及鑑識技術之交流，並可了解先進國家在毒藥物鑑識科學領域的具體作法，並比較先進國家與本國流行毒藥物之不同，機會非常難得，一方面可增廣見聞，瞭解世界鑑識科學發展的趨勢，除此之外本所派員參加亦可促進學術交流，增加國際曝光度，提升本國及本所國際聲譽。

壹、出國目的：

為促進國際學術交流、觀摩學習先進國家在法醫毒物鑑識科學領域之作法及研究概況，並由論文發表提升本所國際學術地位。本所於一〇二年度政府科技計畫內編列預算計劃派員至美國奧蘭多參加美國法醫毒物學者學會年會及於會議中發表政府科技計畫研究成果論文。

經向本屆會議投稿，獲評審委員團審核通過准予本屆年會中公開發表有關法醫毒物分析之論文二篇：「General Unknown Screening in Postmortem Blood Specimens by UHPLC-QTOF/MS and Automated Library Search（以液相層析四極柱飛行時間質譜分析法及自動資料庫比對方式快速篩驗屍體檢體內毒藥物成分）」（林棟樑、劉秀娟、楊筑安、劉瑞厚）；「Direct Injection LC-MS/MS Analysis of Opiates, Methamphetamine, Buprenorphine, Methadone and their Metabolites in Oral Fluid from Substitution Therapy Patients（以液相層析串聯質譜儀直接注射方式同時定量替代療法病人唾液中鴉片類、安非他命類、丁基原啡因、美沙冬及其代謝物成分）」(劉秀娟、李習慈、許雅晴、黃美涵、劉瑞厚、陳泰瑞、林棟樑)。本次出席會議，除職等外尚有本所前顧問美國阿拉巴馬大學鑑識科學研究所退休榮譽教授劉瑞厚教授、前高雄醫學大學毒物學教授蔡錦蓮博士及本部調查局鑑識科學處調查官張琪媛亦出席本次會議。

本所毒物化學組於九十年成立以來，在所長及組長極力推動研究發展業務，並於九十二年間延攬美國阿拉巴馬大學鑑識科學研究所所長劉瑞厚教授返國指導研究工作及研究論文發表，因此自九十二年起在法醫業務或科技計畫項下均編有出席國際會議發表論文之經費預算，本所能繼續赴國外接受專業訓練、發表論文及參與國際會議，是法醫毒物研究發展最大支柱，此要感謝法務部長官的持續鼓勵與支

持，對本所法醫科學學術地位之提升，頗有助益，也藉此機會增加我國國際曝光度並促進本所與各國法醫毒物學界的知名學者與教授在法醫毒藥物分析技術之交流，汲取法醫毒物新知，以充實本所未來研究發展實力。

在這裡要感謝本所周所長章欽之支持與指導，才有此次機會赴美國參加學者年會並了解觀摩國外法醫毒物鑑識之發展。

貳、過程：

10 月 25 日	自桃園機場搭乘中華航空班機前往美國洛杉磯
10 月 26 日	於美國洛杉磯轉機至奧蘭多
10 月 27 日	抵達奧蘭多會議地點 Buena Vista Palace Hotel 赴大會會場辦理報到手續
10 月 28 日	研討課程 Workshop
10 月 29 日	研討課程 Workshop
10 月 30 日	論文口頭報告、論文壁報展示、發表本所論文
10 月 31 日	論文口頭報告、論文壁報展示、發表本所論文
11 月 01 日	美國法醫毒物學者學會年會大會閉幕
11 月 02 日	於奧蘭多整理行旅準備返國
11 月 03 日	於奧蘭多搭機前往洛杉磯 自美國洛杉磯搭乘中華航空班機回國
11 月 04 日	回程

叁、會議內容：

一、 二〇一三年十月二十五日至十一月四日期間赴美國奧蘭多參加美國法醫毒物學者學會年會(SOFT)，今年出席人數估計約數百人，研討課程十三項、口頭論文發表有五十三篇，壁報論文展示共有一百二十七篇。領域橫跨法醫毒物學、臨床毒物學、現場檢測、分析萃取方法探討、合成大麻類、浴鹽、揮發性物質等主題。

二、 除了參加大會並參觀與大會同時進行的廠商展示、書籍販售以及蒐集最新儀器及各式實驗室相關資料，現場並有免費的毒物鑑識領域雜誌可索取。美國各著名檢驗儀器、檢驗耗材廠商及刑事鑑識書籍廠商，利用會場展覽及販售有關毒物分析、法醫病理等鑑識科學相關書籍、儀器及耗材，並展示最新可應用於法醫毒物學之儀器設備。

三、 於大會張貼壁報論文，今年度本所發表在法醫毒物類計有二篇「General Unknown Screening in Postmortem Blood Specimens by UHPLC-QTOF/MS and Automated Library Search（以液相層析四極柱飛行時間質譜分析法及自動資料庫比對方式快速篩驗屍體檢體內毒藥物成分）」（林棟樑、劉秀娟、楊筑安、劉瑞厚）;「Direct Injection LC-MS/MS Analysis of Opiates, Methamphetamine, Buprenorphine, Methadone and their Metabolites in Oral Fluid from Substitution Therapy Patients（以液相層析串聯質譜儀直接注射方式同時定量替代療法病人唾液中鴉片類、安非他命類、丁基原啡因、美沙冬及其代謝物成分）」（劉秀娟、李習慈、許雅晴、黃美涵、劉瑞厚、陳泰瑞、林棟樑）。除了與前往會場閱覽之專家學者進行討論、分享彼此研究

過程所遇到的問題外，也藉此觀摩其他學者所張貼之壁報論文，了解最新研究發展情形。本所發表二篇論文中文摘要如下：

（一）以液相層析四極柱飛行時間質譜分析法及自動資料庫比對方式快速篩驗屍體檢體內毒藥物成分

法醫系統毒物分析是應用於在不知何種毒藥物存在下及案情不明之中毒或死亡案件，進行一般未知毒藥物檢測，此項鑑定工作實有如大海撈針其困難度及複雜性皆非常高。在法醫毒物或臨床毒物實驗室對於生物檢體未知毒藥物之初步篩驗是非常重要且具挑戰性的檢驗項目，傳統毒藥物初步篩驗的方法常用的包括免疫分析法、液相層析二極體陣列檢測法、氣相層析氮磷檢測法及氣相層析質譜檢測法。近年來隨著液相層析質譜分析法的技術進展，已提供了一個發展更具有專一性檢測的機會，亦即使用單一種分析方法來達到篩驗及確認的兩種檢測目的，在法醫毒物或臨床毒物實驗室的應用正快速增加。本研究的目的首先以液相層析四極柱飛行時間質譜儀(UHPLC-QTOF/MS)建立 1000 種以上毒藥物之精確分子量及子離子質譜圖資料庫，藉由結合滯留時間、精確分子量及同位素之分析比對達成檢體內未知毒藥物成分自動資料庫比對分析，並應用於法醫毒物或臨床毒物之生物檢體中未知毒藥物成分。本研究將每一種毒藥物標準品配製濃度為 1-10 μg/mL，分別將 3 μL注入UHPLC-QTOF/MS，液相層析管柱為Zorbax SB-Aq (Agilent 2.1 mm x 100 mm, 1.8 μm particle)，移動相為含 0.1%甲酸之甲醇及水，流速為 0.31 mL/min。經層析管分離、四極柱飛行時間質譜儀選取母離子(M+H)，再以 10、20、40 eV三種不同固定碰撞能量將母離子碰撞為子離子，選取子離子全掃描質譜圖，將其建檔為標準子離子質譜圖資料庫，並同時校正微調每

一種毒藥物及其代謝物成分之滯留時間，供送驗檢體內未知毒藥物分析比對使用。本所目前已建立 1000 種以上毒藥物ESI-TOF/MS之精確分子量資料庫及 600 種以上毒藥物ESI-TOF/MS/MS之標準子離子質譜圖資料庫，包含鴉片類、安非他命類、鎮靜安眠藥、抗憂鬱劑、農藥及一般常見藥物等。以本研究開發之QTOF檢驗技術檢測分析比較死者之血液檢體100 件，先以Toxi-Tubes® A液相-液相萃取後，注入GC/MS以三種市售標準質譜資料庫分析比對；經GC/MS分析後剩餘之萃取物，再注入RRLC-IT/MS/MS及UHPLC-QTOF/MS，以本所建立之RRLC-IT/ MS/MS二次質譜圖譜資料庫及UHPLC-QTOF/MS精確分子量資料庫來分析。分析結果以UHPLC-QTOF/MS建立之標準精確分子量資料庫比對檢驗出 654 種毒藥物成分，以RRLC-IT/MS/MS建立之標準二次質譜資料庫比對檢驗出351種毒藥物成分，以市售之 3 種GC/MS質譜資料庫比對檢驗出 130種毒藥物成分。本研究結果證明UHPLC-QTOF/MS檢驗技術在未知毒藥物成分的檢測能力上有重大突破及明顯提昇。

（二）以液相層析串聯質譜儀直接注射方式同時定量替代療法病人唾液中鴉片類、安非他命類、丁基原啡因、美沙冬及其代謝物成分

唾液與尿液採樣相比最大之優點是可在公開眾人前直接監督以非侵入性及不涉及隱私權之方式採樣，不會有如尿液攙假、外加其他物品或在採尿過程被掉包或與他人混合之情形發生。由於受限於唾液採集之檢體體積較少之因素，因此在檢驗方法上之研發更高靈敏度之檢驗技術有其必要性。本研究以直接注射方式建立簡單、準確與快速之液相層析三段四極柱串聯質譜分析法(LC-MS/MS)，同時定量分析替代療法病人唾液內美

沙冬、丁基原啡因、甲基安非他命、鴉片類及其代謝物等 10 種成分。採集之唾液置於-20 ^{o}C冰箱冷凍，實驗時取出回溫，以 12000 rpm離心 10 分鐘，精確量取 40 μL唾液上清液，加入 10 μL 10 種氘內標之混合液，混合均勻後，再次離心取上清液，注射 10 μL 至 LC-MS/MS 分析。液相層析管柱為 Zorbax SB-Aq (Agilent 2.1 mm x 100 mm, 3.5 μm particle)、管柱溫度 50 °C，移動相為含 0.1%甲酸之甲醇及水，流速為 0.35 mL/min，以正電離子模式電灑游離法(ESI)配合多重反應偵測(MRM)進行定量方法評估分析。因減少檢體前處理及萃取濃縮等步驟，可縮短分析所需之時間和減少檢體的使用量，以梯度沖提逆相層析方式在 10 分鐘內可將所有待測成分分離，每一檢體檢測時間為 17 分鐘。在濃度 1(5)-100 ng/mL範圍內具有良好之線性關係，線性關係(r^2)均在 0.995 以上；9 種成分之偵測極限(LOD)及定量極限(LOQ)分別為 0.1–1.0 ng/mL 及 0.25–1.0 ng/mL，buprenorphine 均為 5 ng/mL。空白唾液配製濃度為 1(5)-100 ng/mL等 5 種不同濃度之日內及日間精密度(CV%)範圍分別為 0.87-12.20%及 1.27-12.80%。以完成之實驗方法已成功應用於 62 位參加毒品減害替代療法病人之唾液定量分析，分析結果 57 位美沙冬治療者唾液中methadone及EDDP濃度範圍分別為 5.83-25566 及 0.49-35.36 ng/mL；5 位丁基原啡因治療者唾液中 buprenorphine及norbuprenorphine濃度範圍分別為 7.39-2784 及 1.28-34.37 ng/mL；另於 62 位中檢出 amphetamine 或 methamphetamine 9 件（佔 14.5%）、檢出morphine或codeine 27 件（佔 43.5%）、檢出 6-acetylmorphine或 6-acetylcodeine 6 件（佔 9.7%）。由分析數據顯示，62 位接受毒品減害替代療法計畫在治療過程中仍持續施用海洛因毒品或甲基安非他命佔 50.0%以上。其中發現有 7 位前往醫療單位服用替代藥物治療前，同時

先行施用海洛因及甲基安非他命毒品之行為，此項發現值得執行毒品減害替代療法計畫相關單位參考。

四、 研討課程內容

本次大會共安排十二項研討課程，課程內容包括法醫毒物學綜論、SWGTOX 在法醫毒物方法確效之標準作業流程、固相萃取法於法醫毒物之應用、酒精與性侵死亡案件探討、鑑識人員在研究發表品質上之探討、毒物案件剖析、法醫毒物學回顧、合成大麻之藥理及毒理作用、不尋常死因由分析到研判、高解析度精確質譜分析法於毒物學之應用、大麻老的毒品新的資料等，茲將與本所毒物化學組在分析方法研發相關之課程簡述如下：

Workshop：Solid Phase Extraction：Application in Forensic Toxicology

此課程主題為探討固相萃取技術在法醫毒物上的應用。課程由固相萃取基本原理開始談起，接著介紹固相萃取在法醫毒物上的應用，及分享應用於實際案例屍體檢體檢驗上之經驗。此外，也分享了使用固相萃取方式萃取血液中合成類大麻素成份，最後經由 LC/MS/MS 分析所得到的研究成果。一個好的萃取不僅可以得到更準確的分析，在法醫毒物領域上有時也會是個破案關鍵，因此如何運用萃取方式來增加毒藥物的萃取效率，進而達到更準確的定性定量結果是一個非常關鍵的步驟。目前本組在法醫檢體前處理上是以液相-液相萃取法為主要萃取方式，因此，藉由此研討課程的學習，不僅可以增進我們在萃取方法上的應用，更可以在日後遇到難以處理的檢體、或是萃取回收率不佳的藥物分析時，提供一個良好的解決方式。

Workshop：High Resolution Accurate Mass Spectrometric Methods for Toxicology

此課程主題為探討高解析度精確質譜分析法於毒物學之應用。此種分析方法具有高靈感度及高專一性之特性，可檢測毒藥物及其代謝物成分。該項儀器具有高於 1 mDa 之精確質譜可與建檔之現有毒藥物資料庫內之成分，進行精確分子量、分子式及同位素含量比值之比對，若能結合每一毒藥物滯留時間(Retention Time)之比對，則該分析法更能提高其比對之準確性。對於疑似中毒案件之未知毒藥物之篩驗提供一個絕佳的分析法。高解析度精確質譜分析法能鑑別合成大麻類毒品在人體內經由肝臟代謝之代謝成分，此項優點係 LC-MS/MS 所欠缺的功能。與具相同感度之 LC-MS/MS 相比，高解析度精確質譜分析法較具有毒藥物成分篩驗之功能，此外，並具有同時能定性及定量低濃度毒藥物及其代謝物之功能。

肆、檢討建議及心得感想：

一、 國際會議部分心得

會議名稱雖為美國法醫毒物學者學會年會,與會人士多為美國學者,但仍有許多來自世界各國的研究學者、鑑識專家共襄盛舉,藉由此會議彼此之間互相進行學術交流、聯絡感情之外,尚且讓我們瞭解當前各國研究之方向、毒藥物鑑驗方法的進展,更重要的是與其他學者分享我們的研究成果,藉由彼此之間的切磋討論,來增進日後我們的研究發展。

二、口頭論文發表及壁報論文閱讀摘要心得

1. 合成類大麻素(Synthetic Cannabinoids)以往為醫療研究或藥廠研發成果,可與人類的大麻受體結合。但近來由於非法製造及青少年濫用,而成為主要新興合成毒品之一,美國及歐洲也已針對這些藥物制訂相關規定以預防濫用情形,但在合成類大麻素檢測發展上仍是一大挑戰。美國 James Clarke 團隊「Development of Synthetic Cannabinoids “SPICE” ELISA Kits for Screening of Human Urine and Blood」,利用 JWH-018 與 JWH-250 兩個半抗原與蛋白質結合,注射至兔子體內製造出多株抗體來製作 ELISA kit,發現以 JWH-018 抗血清所發展的 ELISA 可廣泛的和人類血液及尿液中結構相似的合成類大麻素交叉反應,特別是 JWH-018 及其代謝物。而以 JWH-250 抗血清所發展的 ELISA 也可廣泛的和人類血液及尿液中結構相似的合成類大麻素交叉反應,特別是 JWH-203、RCS-8、JWH-250 及其代謝物。這兩種 kit 都可運用於篩檢美國偵毒署(DEA)所制定列表管制的合成類大麻素。

2. 一氧化碳血紅素檢測主要運用於火災及在汽車內死亡案件分析,而採血管的種類可能會影響COHb濃度。美國Jason

Stibley團隊「Evaluation of the Avoximeter-4000® to Measure the Stability of Carboxyhemoglobin in Different Blood Collection Tubes Over Time」分析包含灰頭採血管(含NaF及potassium oxalate)、紅頭採血管(不含添加物)、紫頭採血管(含EDTA)、綠頭採血管(含heparin)及藍頭採血管中COHb濃度。實驗結果顯示，除了灰頭採血管所測得的COHb濃度明顯較低外，其餘採血管所測得的COHb濃度是沒有差異的。此外，在COHb偵測上目前使用的方法包含UV及FTIR分光光度法、CO-oximetry及毛細管電泳法，但是CO-oximetry在偵測上的限制為無法偵測少於 10%COHb以及無法偵測腐敗的血液。因此中國Hongxia Hao團隊「Microanalysis of Carbon Monoxide in Decomposed Blood and Hepatic Tissues by Headspace Gas Chromatography and Mass Spectrometry」利用兔子吸入CO死亡後，取出血液及組織進行HS/GC/MS分析，同時將樣本放置於 17℃、-4℃及-20℃中 7 天、14 天及 45 天，發現於腐敗血液中的%COHb濃度與新鮮血液中的%COHb濃度相符，顯示可利用HS/GC/MS來檢測腐敗血液中%COHb濃度。此外，於實驗中發現溫度對於%COHb濃度的影響是可忽略的，並且利用HS/GC/MS可偵測到極低的%COHb濃度。

3. 在酒駕的案件中，血中酒精濃度檢測是非常重要的數據。但在案件審理過程，為了證明原始酒精濃度的檢測數據是可信的，因此常需再次分析血液樣本。此時，酒精的濃度就與採血管、樣本體積、樣本開關次數及保存的溫度、時間有著密切關係。美國 Monica Escobar Jacobs 團隊「Investigation Into the Stability of Ethanol in Blood Samples Collected for Suspected Driving Under the Influences (DUI) Cases」收集 50 個疑似酒駕案件的血液樣本，其中 48 個樣本儲存於 2-8℃約

0.5-8 年時間；兩個樣本儲存於室溫約六年以上。這些樣本皆使用灰頭採血管內含 100 mg 氟化鈉及 20 mg 草酸鉀採集，並使用 HS/GC 進行再次分析。實驗結果發現，樣本保存於 2-8℃下 8 年以內血中酒精濃度下降幅度約為每年 2-12%；若血液採檢體積過少會使酒精濃度下降幅度高達每年 38%；若血液檢體須進行多項檢測導致檢體瓶多次開關，則會加速酒精揮發速度使酒精濃度下降幅度增加。另外，英國 Gail Cooper 團隊「Long-Term Stability of Ethanol in Blood Samples Collected from Suspected Drunk-Drivers」收集 412 個疑似酒駕案件的血液樣本將之分為 group A (N=188)儲存於室溫、4℃冷藏及-20℃冷凍等不同的溫度條件，保存時間為 4-8 年；group B (N=224)為先冷藏保存隨後再冷凍保存，保存時間為 8-52 個月。使用 HS-GC-FID 儀器進行原始分析及再次分析。實驗結果顯示，於 group A 長時間室溫保存下，酒精濃度平均下降 33%，中位數為 31%；而長時間冷藏保存下，酒精濃度平均下降 12%，中位數為 3%。group B 樣本先冷藏保存隨後再冷凍保存，酒精濃度平均下降 9%，中位數為 6%。證實了血中酒精濃度於室溫保存下是不穩定的，且最佳的保存方式為先冷藏保存隨後再冷凍保存，其保存時間可長達 8 年。

4. 本次會議中有多篇關於LC-QTOF-MS研究發表。美國 Joshua Z. Seither 團隊「High Resolution MS/MS Spectral Library and Compound Database for the Detection of Designer Drugs by LC-QTOF-MS」利用Agilent 1290 HPLC系列搭配 Agilent 6530 Accurate-Mass A-TOF LC-MS建立了 263 筆 designer drugs資料庫，且大多數藥物的最低偵測極限小於 10 ng/mL。將此資料庫與MS/MS資料庫合併使用，在法醫毒物判別designer drugs上是一極有利之工具。美國Kenneth Ihenetu

團隊「Rapid UPLC-MS^E/TOF Method for Simultaneous Quantitation of Cocaine and Its Metabolites Using Direct Extracts of Pulverized Human Hair」利用均質機將頭髮進行機械式破壞使頭髮之皮質及髓質層露出，再利用超音波震盪方式加速頭髮內藥物及代謝物之釋放，經過酸性甲醇萃取後注入高精密儀器UPLC-MS^E/TOF進行cocaines、benzoylecgonine、cocaethylene及norcocaine之分析，分析範圍為50-4000 pg/mg且具良好線性關係，最低偵測極限及最低定量極限可達25 pg/mg及50 pg/mg。美國Thomas G. Rosano團隊「Drug Screening in Medical Examiner Casework by High Resolution Mass Spectrometry (UPLC-MS^E/TOF)」利用UPLC-MS^E/TOF進行屍體檢體藥物篩檢，可同時篩檢高達950種以上毒藥物。UPLC-MS^E/TOF之mass error平均為1.27 PPM，其最低偵測極限範圍為0.5-100 ng/mL。將300件屍體血液檢體注入UPLC-MS、UPLC-MS/MS及UPLC-MS^E/TOF進行藥物篩檢比較藥物檢出率，發現300件檢體於三種儀器共篩檢出1528個成分，其中UPLC-MS檢出率為57％、UPLC-MS/MS檢出率為72％，而UPLC-MS^E/TOF檢出率高達99％。顯示UPLC-MS^E/TOF相較於其他兩種儀器具有較高的敏感度。

三、建議：

1. 由於對法醫毒藥物數據的品質要求越來越高，分析儀器也從低解析度、低精密度進階至追求高解析度及高精確度，世界各國也多已使用高解析度及高精密度之儀器分析實驗檢測數據，以求最精準之實驗數據，提供司法機構毒藥物檢測之參考。而參加此會議，不僅可以得知目前法醫毒藥物研究發展的進展及現況，也可參閱相關研究來補足或改進研究上所遇

到之困難或問題，也可與學者進行討論來啟發相關之研究發展。因此在加強人員專業訓練方面，對於實驗準確度及鑑驗技術之發展，建議無論是鑑識單位或各執法人員除了參加國際會議進行交流外，也可安排人員至國外進行短期進修，培養專業之法醫鑑識人力。

2. 為了維護毒藥物檢測數據的可靠性與數據追溯性，檢測研究室多採用統一的國際認證標準，提高實驗室產出數據的品質與可信度。為了提昇本組法醫毒物實驗室的專業公信力，本所於 100 年已經通過財團法人全國認證基金會(TAF, Taiwan Accreditation Foundation)評鑑，並取得 ISO/IEC 17025 認證，且在今年 102 年除了通過 TAF 監督評鑑外同時通過六項增項認證項目。以持續積極鼓勵相關鑑驗人員參加各項實驗室認證課程，以期應用於各項鑑驗案件，提供更具品質之數據。

3. 爲了維護鑑驗報告之公信力，在實驗室認證方面由於層面極廣，因此需要龐大人力及心力進行維持及改進。加上本所逐年增加之原有鑑驗案件及函詢案件，以及爲了因應層出不窮的新興毒藥物及日益精進之新型儀器而必須進行相關之研究，以目前法醫研究所毒物化學組編制人力及預算已不堪負荷，難以如國外團隊發展研究之進度迅速且範圍廣泛。因此培育法醫毒物專業人才，實為當務之急。

4. 大會研討課程通常邀請數位法醫毒物、臨床毒物或法律相關的學者專家及檢察官針對當前熱門的研究或新興毒物做一個主題演講，內容豐富且可對演講的主題有更深的了解。演講時間為一個上午或下午時段的課程，雖然課程豐富但課程費用約為 50 至 100 美元上下，且此次赴美國參加國際會議，因

會議研討課程報名費較往年調漲甚多，經費不足支付相關課程費用，因此無法參加許多研討課程，實為可惜。

General Unknown Screening in Postmortem Blood Specimens by UHPLC-QTOF/MS and Automated Library Search

Dong-Liang Lin[*,1], Hsiu-Chuan Liu[1], Chu-An Yang[1], Ray H. Liu[2]

[1]Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan; [2]Department of Justice Sciences, University of Alabama at Birmingham, Birmingham, AL, USA.

Introduction and Objectives: Preliminary screen of drugs and toxic compounds in various matrices is an important and challenging task performed by forensic and clinical laboratories. Traditional methods for preliminary screen include immunoassay (IA), gas chromatography-nitrogen/phosphorous detection (GC/NPD), mass spectrometry (MS), GC/MS, and liquid chromatography-diode array detection (LC/DAD). Recent advances in the LC-MS/MS technology have provided an opportunity for the development of more specific approaches to achieve the "screen" and "confirmation" goals in a single analytical step. The objectives of this study are: (a) the establishment of chromatographic and mass spectrometric database including 1000 plus toxic compounds; and (b) the development of an effective UHPLC-QTOF/MS (ultra high performance liquid chromatography/quadrupole time-of-flight mass spectrometry) protocol for general unknown screen of these compounds for application in forensic and clinical laboratories.

Methods: Liquid-liquid extraction procedure — using Toxi-tubes® A protocol — was coupled to an Agilent 6540 Q-TOF instrument equipped with a Jet Stream interface in combination with an Agilent 1290 Infinity LC instrument. Separation was achieved within 15 minutes, at a 0.31 mL/min flow rate, by gradient chromatography on Agilent Zorbax SB-Aq (2.1 x 100 mm, 1.8 μm) analytical column operated at 50 ^{o}C. Mobile phase consisted of solvent mixture composed of methanol and water containing 0.1% formic acid. Ions were generated in positive electrospray ionization mode. Samples were detected at 2 GHz single MS mode, m/z range 100–1000 with a scan rate of 2.0 spectra/sec. Data were acquired and processed with MassHunter B.05.00 software. An in-house database, comprising more than 1000 drugs and metabolites, was established using data resulting from the analysis of samples prepared from certified standards or other documented reference materials. The

"Find-by-Formula" algorithm was used for data extraction. Matching tolerance parameters were: ±5-ppm mass accuracy; ±0.20-min retention time deviation; and ≥10000-count peak height. These matching parameters and isotope pattern were used to derive identification scores. Established protocol was used for the analysis of postmortem blood specimens for effectiveness assessments.

Result: Current database includes 1043 toxic compounds. The established method was applied to the analysis of 100 postmortem blood samples. The numbers of drug detected, by UHPLC-QTOF/MS, LC-IT/MS (LC/ion trap mass spectrometry) and GC/MS methods were 654, 351, and 130, respectively. The established method was found highly effective when applied to the analyses of postmortem specimens.

Conclusions: The over-all protocol provides a rapid, sensitive approach to isolate, screen, and confirm a broad spectrum of toxic compounds. No significant interference was found at the retention time expected of the targeted compounds. Preliminary data derived from the analysis of postmortem blood specimens are promising, significantly more effective than the RRLC-IT/MS and GC/MS approaches. More specific parameters, such as specificity and accuracy, of this method are currently under evaluation.

Key Words: UHPLC-QTOF/MS, General Unknown Screening, Postmortem Samples

General Unknown Screening in Postmortem Blood Specimens by UHPLC-QTOF/MS and Automated Library Search

Dong-Liang Lin*[1], Hsiu-Chuan Liu*[1], Chu-An Yang[1] and Ray H. Liu[2]

[1]Forensic Toxicology Division, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan

[2]Department of Justice Sciences, University of Alabama at Birmingham, Birmingham, AL, USA

Introduction and Objectives

Preliminary screen of drugs and toxic compounds in various matrices is an important and challenging task performed by forensic and clinical laboratories. Traditional methods for preliminary screen include immunoassay (IA), gas chromatography-nitrogen/ phosphorous detection (GC/NPD), mass spectrometry (MS), GC/MS, and liquid chromatography-diode array detection (LC/DAD). Recent advances in the LC-MS/MS technology have provided an opportunity for the development of more specific approaches to achieve the "screen" and "confirmation" goals in a single analytical step. The objectives of this study are: (a) the establishment of chromatographic and mass spectrometric database including 1000 plus toxic compounds; and (b) the development of an effective UHPLC-QTOF/MS (ultra high performance liquid chromatography/quadrupole time-of-flight mass spectrometry) protocol for general unknown screen of these compounds for application in forensic and clinical laboratories.

METHODS

Liquid-liquid extraction procedure — using Toxi-tubes® A protocol — was coupled to an Agilent 6540 Q-TOF instrument equipped with a Jet Stream interface in combination with an Agilent 1290 Infinity LC instrument. Separation was achieved within 15 minutes, at a 0.31 mL/min flow rate, by gradient chromatography on Agilent Zorbax SB-Aq (2.1 x 100 mm, 1.8 µm) analytical column operated at 50 °C. Mobile phase consisted of solvent mixture composed of methanol and water containing 0.1% formic acid. Ions were generated in positive electrospray ionization mode. Samples were detected at 2 GHz single MS mode, m/z range 100-1000 with a scan rate of 2.0 spectra/sec. Data were acquired and processed with MassHunter B.05.00 software.

Instrument Conditions

Pump

Time (min)	% Water (B) (0.1% formic acid)	% Methanol (A) (0.1% formic acid)	Flow rate (mL/min)
0	88	12	0.31
4	88	12	0.31
12	0	100	0.31
13	0	100	0.31
14	88	12	0.31

Post time:	2 minutes
Total run time:	16 minutes
Injection volume:	3 µL with needle wash
Column: ZORBAX SB-AQ	100 mm x 2.1 mm x 1.8 µm

Q-TOF/MS

Ionization	Dual AJS ESI Positive
Gas temp	325 °C
Drying gas	8 L/min
Neubulizer pressure	35 psig
Sheath gas temp	325 °C
Sheath gas flow	10 L/min
Capillary V (+ve)	4000 V
Acquisition MS Mode	
Scan m/z range	100-1000
Acquisition rate	2 spectra/s
Acquisition time	500 ms/spectrum
Acquisition Auto MS/MS Mode	
MS Scan m/z range	50-1000
MS/MS Scan m/z range	50-800
Acquisition rate	1 spectra/s
Acquisition time	1000 ms/spectrum
Collision energy	use fixed CE: 10, 20, 40 Ev

Reference Masses
m/z: 121.0509
m/z: 922.0098

Sample preparation

Creating the library standard solutions:
The drug and toxic compound standards were made up individually as 1 - 10 µg/mL in solvent A:B 10:90 (v/v) and 3 µL were injected.

Liquid-Liquid extraction:
Using Toxi-Tubes® A (Agilent, Lake Forest, CA).

AUTOMATED LIBRARY SEARCH

An in-house database, comprising more than 1000 drugs and metabolites was established using data resulting from the analysis of samples prepared from certified standards or other documented reference materials. The "Find-by-Formula" algorithm was used for data extraction. Matching tolerance parameters were: ≤5-ppm mass accuracy; ≤0.20-min retention time deviation; and ≥10000-count peak height. These matching parameters and isotope pattern were used to derive identification scores. Established protocol was used for the analysis of postmortem blood specimens for effectiveness assessments.

RESULTS AND DISCUSSION

Table 1. Theoretical accurate mass of 21 opiates in IFM QTOF LC-MS database

Compound	Retention time (min)	Formula	Monoisotopic mass	$[M+H]^+$ accurate mass	Observed mass	Mass error (ppm)
Normorphine	1.351	$C_{16}H_{17}NO_3$	271.12084	272.12832	271.12104	0.74
Dihydromorphine	1.333	$C_{17}H_{21}NO_3$	287.15214	288.15911	287.15198	-0.57
Morphine	1.351	$C_{17}H_{19}NO_3$	285.13649	286.14496	285.13755	3.73
Noroxymorphone	1.405	$C_{16}H_{17}NO_4$	287.11576	288.12312	287.11583	0.24
Oxymorphone	1.494	$C_{17}H_{19}NO_4$	301.13141	302.13885	301.13071	-2.31
Hydromorphone	1.678	$C_{17}H_{19}NO_3$	285.13649	286.14463	285.13726	2.69
Nalorphine	2.377	$C_{19}H_{21}NO_3$	311.45214	312.15999	311.15263	1.57
Norcodeine	2.466	$C_{17}H_{19}NO_3$	285.13649	286.14421	285.13685	1.26
Dihydrocodeine	2.618	$C_{18}H_{23}NO_3$	301.16779	302.17531	301.16809	1.01
Naloxone	2.643	$C_{19}H_{21}NO_4$	327.14706	328.15416	327.14698	-0.25
Codeine	2.645	$C_{18}H_{21}NO_3$	299.15214	300.15204	299.15204	-0.34
Noroxycodone	3.378	$C_{17}H_{19}NO_4$	301.13141	302.13968	301.13240	3.29
Oxycodone	3.095	$C_{18}H_{21}NO_4$	315.14706	316.15488	315.14757	1.61
Naltrexone	3.406	$C_{20}H_{23}NO_4$	341.16271	342.17023	341.16303	0.95
Hydrocodone	3.433	$C_{18}H_{21}NO_3$	299.15214	300.15924	299.15301	2.90
6-Acetylmorphine	3.754	$C_{19}H_{21}NO_4$	327.14706	328.15392	327.14671	-1.07
6β-Naltrexol	4.026	$C_{20}H_{25}NO_4$	343.17836	344.18890	343.17858	0.63
6-Acetylcodeine	7.154	$C_{20}H_{23}NO_4$	341.16271	342.17019	341.16288	0.50
Heroin	7.248	$C_{21}H_{23}NO_5$	369.15762	370.16548	369.15705	-1.54
Norbuprenorphine	8.258	$C_{25}H_{35}NO_4$	413.25661	414.26395	413.25671	0.24
Buprenorphine	8.921	$C_{29}H_{41}NO_4$	467.30356	468.30947	467.30223	-2.79

Current database includes 1043 toxic compounds. The established method was applied to the analysis of 100 postmortem blood samples. The numbers of drug detected, by UHPLC-QTOF/MS, LC-IT/MS (LC/ion trap mass spectrometry) and GC/MS methods were 654, 351, and 130, respectively. The established method was found highly effective when applied to the analyses of postmortem specimens.

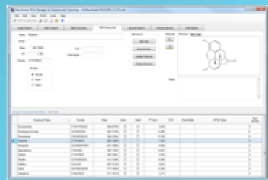

Figure 1. IFM QTOF/MS compound database and library for forensic and toxicology.

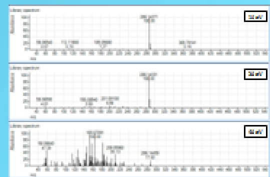

Figure 2. Accurate mass spectra of morphine at CID energy of 10, 20 and 40 eV as stored in the library.

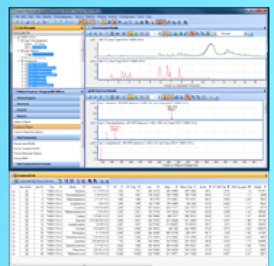

Figure 3. Find Compounds by Molecular Feature (MFE) and Database search results, MassHunter qualitative analysis result.

Table 2. Summary of compounds identified in 10 opiates postmortem cases by automatic searching the in-house LC-QTOF/MS, LC-IT/MSMS database and GC/MS

Case	Sample	LC-QTOF/MS	LC-IT/MS	GC/MS
1	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	[illegible]
2	Blood	[illegible]	[illegible]	[illegible]
	Urine	[illegible]	[illegible]	[illegible]
3	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	[illegible]
4	Blood	[illegible]	[illegible]	[illegible]
	Urine	[illegible]	[illegible]	[illegible]
5	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	ND
6	Blood	[illegible]	[illegible]	[illegible]
7	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	ND
8	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	[illegible]
9	Blood	[illegible]	[illegible]	[illegible]
	Urine	[illegible]	[illegible]	[illegible]
10	Blood	[illegible]	[illegible]	ND
	Urine	[illegible]	[illegible]	[illegible]
Identified substances		146	97	21
6-Acetylmorphine		11	10	6
6-Acetylcodeine		4	5	3
Heroin		1	1	0

ND: not detected

CORRESPONDING AUTHOR

Dong-Liang Lin, Ph.D.
Chief Toxicologist
Institute of Forensic Medicine, Ministry of Justice
Taiwan, R. O. C.
e-mail: dllin@mail.moj.gov.tw

Table 3. Comparison of findings by LC-QTOF/MS, LC-IT/MS/MS and GC/MS methods: results for 100 postmortem bloods.

Drugs	LC-QTOF	LC-IT	GC/MS	Drugs	LC-QTOF	LC-IT	GC/MS
Lidocaine	25	0	5	Omeprazole	4	0	0
Acetaminophen	24	2	12	Oxazepam	4	1	0
Chlorpheniramine	24	24	12	Ambroxol	3	3	1
Codeine	22	17	2	Amitriptyline	3	2	1
Morphine	21	16	3	Chlorpromazine	3	2	1
Zolpidem	20	12	6	Citalopram	3	3	2
7-Aminoflunitrazepam	17	3	0	Domperidone	3	2	0
Diphenhydramine	13	10	3	Doxepin	3	2	0
Hydroxymidazolam	13	7	0	Etodolac	3	0	0
Methylephedrine	13	3	3	Hydroxychloroquine	3	3	0
Trazodone	13	8	0	MDA	3	3	0
Cimetidine	13	8	0	MDMA	3	2	1
Pseudoephedrine	12	8	3	Mephenoxalone	3	1	1
Tramadol	10	4	6	Metoclopramide	3	2	1
Ketamine	10	5	3	Mosapride	3	5	0
Heroin	1	1	0	Diphenidol	0	0	1
Hydroxytramadol	1	1	0	Fluoxetine	0	3	3
[illegible]	1	1	0	Ibuprofen	0	0	1
Ketoconazole	1	0	0	Norsertraline	0	0	1
Lorazepam	1	0	0	Norvenlafaxine	0	1	1
Lormetazepam	1	0	0	Valproic acid	0	0	1
[illegible]	1	0	0	Zotepine	0	0	1
Meprobamate	1	0	0				
Identified substances	654	351	130				

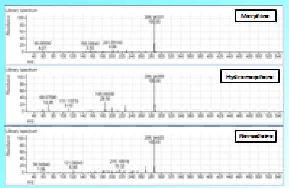

Figure 4. Accurate mass spectra of morphine, hydromorphone and norcodeine at CID energy of 20 eV as stored in the library.

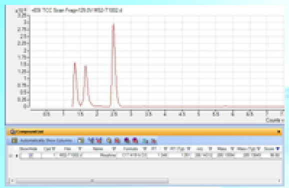

Figure 5. Find Compounds by Molecular Feature (MFE) and Database search results.

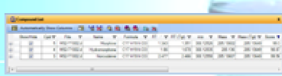

Figure 6. Find Compounds by Formula (FBF) and Database search results.

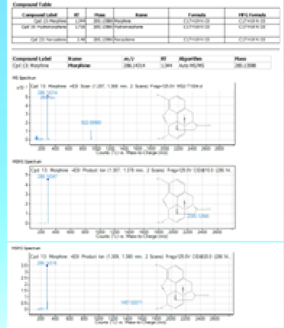

Figure 6. Find Compounds by Auto MS/MS and Database search results.

CONCLUSIONS

The over-all protocol provides a rapid, sensitive approach to isolate, screen, and confirm a broad spectrum of toxic compounds. No significant interference was found at the retention time expected of the targeted compounds. Preliminary data derived from the analysis of postmortem blood specimens are promising, significantly more effective than the RRLC-IT/MS and GC/MS approaches. More specific parameters, such as specificity and accuracy, of this method are currently under evaluation.

REFERENCES

1. S. Broecker, S. Herre, B. Wust, J. Zweigenbaum, F. Pragst. Development and practical application of a library of CID accurate mass spectra of more than 2500 toxic compounds for systematic toxicological analysis by LC-QTOF-MS with data-dependent acquisition. *Anal. Bioanal. Chem.* 400: 101-117 (2011).
2. S. Broecker, F. Pragst, A. Bakdash, S. Herre, M. Tsokos. Combined use liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and high performance liquid chromatography with photodiode array detector (HPLC-DAD) in systematic toxicological analysis. *Forensic Sci. Int.* 212: 215-226 (2011).
3. S. Broecker, S. Herre, F. Pragst. General unknown screening in hair by liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). *Forensic Sci. Int.* 218: 68-81 (2012).
4. H. C. Liu, R. H. Liu, H. O. Ho, and D. L. Lin. Development of an information-rich LC-MS/MS database for the analysis of drugs in postmortem specimens. *Anal. Chem.* 81: 9002-9011 (2009).
5. H. C. Liu, R. H. Liu, H. O. Ho, and D. L. Lin. Rapid screening and confirmation of drugs and toxic compounds in biological specimens using LC/ion trap tandem mass spectrometry and automated library search. *Rapid Commun. Mass Spectrom.* 24: 75-84 (2010).

Direct Injection LC-MS/MS Analysis of Opiates, Methamphetamine, Buprenorphine, Methadone and Their Metabolites in Oral Fluid from Substitution Therapy Patients

Hsiu-Chuan Liu[*,1], Hsi-Tzu Lee[1], Ya-Ching Hsu[2], Mei-Han Huang[2], Ray H. Liu[3], Tai-Jui Chen[4], Dong-Liang Lin[1]

[1]Department of Forensic Toxicology, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan; [2]Department of Medical Laboratory Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan; [3]Department of Justice Sciences, University of Alabama at Birmingham, Birmingham, AL, USA; [4]Department of Psychiatry, E-Da Hospital, Kaohsiung, Taiwan.

Introduction and Objectives: As an alternative matrix for drug testing, oral fluid is relatively cleaner and more accessible to sampling. The sampling process can be readily supervised, reducing risks of adulteration or substitution; while cleaner matrix facilitates direct LC/MSMS analysis. In this study, we explored the LC-MS/MS methodology, with a simple sample preparation step, for simultaneous quantification of heroin, methamphetamine, buprenorphine, methadone and their metabolites (6-acetylmorphine, morphine, codeine, amphetamine, norbuprenorphine, EDDP) in oral fluid.

Methods: Clinical oral fluid specimens were collected upon patients' arrival for their daily dose. In most cases, patients' last doses were taken approximately 23 h earlier. Patients were asked to rinse prior to sample collection and the samples were stored at –20 ^{o}C until use. IRB protocols established by E-Da hospital were followed for sample collection and information processing and usage. For analysis, 40 μL of thawed oral fluid was fortified with 10 μL of 10-internal standard solution (0.1 μg/mL). Samples were briefly vortex-mixed and centrifuged at 12000 rpm for 10 min. Ten microliter of supernatant was injected onto the LC-MS/MS system. Standard oral fluid samples were prepared similarly using oral fluid collected from laboratory personnel. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm x 2.1 mm i.d.; 3.5 μm particle) analytical column operated at 50 °C. The mobile phase consists of 0.1% formic acid (v/v) in water (A) and methanol (B). Under these conditions, all of the analytes eluted in less than 10 minutes with a total run time of 15 minutes. Mass

spectrometric analysis was performed in positive-ion mode; applying multiple reaction monitoring (MRM) using optimized collision energy for each precursor ion designating each analyte of interest.

Result: The overall protocol was evaluated by: (a) applying the method to the analysis of laboratory-prepared standards; and (b) comparing analytical data of clinical samples derived from this and GC-MS methods. When applied to the analysis of oral fluid specimens fortified with 1–100 ng/mL of the 10 analytes of interest, this method achieved the following results: (1) inter-day and intra-day precisions range from 1.3 to 12.8% and 0.9 to 12.2% (percent CV), respectively; (2) method linearity (r^2), detection limit and quantitation limit for all analytes were >0.995, 0.1–1.0 ng/mL and 0.25–1.0 ng/mL (5 ng/mL for buprenorphine), respectively. Analytical data derived from LC-MS/MS and GC-MS analysis of methadone in 13 clinical samples were found compitable. Specifically, 10 out of the 13 sets of data overlap at the ±20% level, with the remaining 3 sets barely outside of overlap range.

Conclusions: Direct injection of freeze-and-thaw samples appears to generate favorable results for LC-MS/MS analysis of oral fluid samples. Peak shape of chromatograms derived from oral fluid containing high concentrations of certain drugs, such as morphine, codeine, and (to a lesser degree) 6-acetylmorephine and buprenorphine, show irregular characteristics, which can be improved by diluting the sample with the mobile phase prior to injection. With heroin exhibiting ideal LC-MS/MS chromatographic characteristics, inclusion of this compound in the analyte list help detect abnormality of the analytical protocol and test specimens. Overall, our data indicate the LC-MS/MS method is more effective than our current GC-MS methods in the screening and analysis of the 10 analytes included in this study. We are currently conducting further studies on: (a) irregular chromatographic behavior of certain analytes and improvement methods; and (b) comparing analytical findings of all analytes derived from LC-MS/MS and GC-MS analysis.

Key Words: Drugs of abuse, Oral fluid, LC-MS/MS

Direct Injection LC-MS/MS Analysis of Opiates, Methamphetamine, Buprenorphine, Methadone and Their Metabolites in Oral Fluid from Substitution Therapy Patients

Hsiu-Chuan Liu*[1], Hsi-Tzu Lee*[1], Ya-Ching Hsu[2], Mei-Han Huang[2], Ray H. Liu[3], Tai-Jui Chen[4], Dong-Liang Lin[1]

[1]Forensic Toxicology Division, Institute of Forensic Medicine, Ministry of Justice, Taipei, Taiwan; [2]Department of Medical Laboratory Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan; [3]Department of Justice Sciences, University of Alabama at Birmingham, Birmingham, AL, USA; [4]Department of Psychiatry, E-Da Hospital, Kaohsiung, Taiwan

Introduction and Objectives

As an alternative matrix for drug testing, oral fluid is relatively cleaner and more accessible to sampling. The sampling process can be readily supervised, reducing risks of adulteration or substitution; while cleaner matrix facilitates direct LC-MS/MS analysis. In this study, we explored the LC-MS/MS methodology, with a simple sample preparation step, for simultaneous quantification of heroin, methamphetamine, buprenorphine, methadone and their metabolites (6-acetylmorphine, morphine, codeine, amphetamine, norbuprenorphine, EDDP) in oral fluid.

METHODS

Clinical oral fluid specimens were collected upon patients' arrival for their daily dose. In most cases, patients' last doses were taken approximately 23 h earlier. Patients were asked to rinse prior to sample collection and the samples were stored at -20 °C until use. IRB protocols established by E-Da hospital were followed for sample collection and information processing and usage. For analysis, 40 µL of thawed oral fluid was fortified with 10 µL of 10-internal standard solution (0.1 µg/mL). Samples were briefly vortex-mixed and centrifuged at 12000 rpm for 10 min. Ten ml aliquot of supernatant was injected onto the LC-MS/MS system. Standard oral fluid samples were prepared similarly using oral fluid collected from laboratory personnel. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm x 2.1 mm i.d.; 3.5 µm particle) analytical column operated at 50 °C. The mobile phase consists of 0.1% formic acid (v/v) in water (A) and methanol (B). Under these conditions, all of the analytes eluted in less than 10 minutes with a total run time of 16 minutes. Mass spectrometric analysis was performed in positive-ion mode; applying multiple reaction monitoring (MRM) using optimized collision energy for each precursor ion designating each analyte of interest.

Instrument Conditions

LC: Agilent 1200 Series

Pump

Time (min)	% Water (0.1% formic acid)	% Methanol (0.1% formic acid)	Flow rate (mL/min)
0	90	10	0.35
2	90	10	0.35
10	0	100	0.35
11	90	10	0.35

Post time:	5 minutes
Total run time:	16 minutes
Injection volume:	10 µL with needle wash
Column temperature:	50 °C
Column:	ZORBAX SB-Aq 100 mm x 2.1 mm x 3.5 µm

Mass: Agilent 6410 Triple Quadrupole

MS Detection

Ionization	ESI Positive
Gas temp	350 °C
Drying gas	10 L/min
Nebulizer pressure	40 psig
Capillary V (+ve)	4000 V
Acquisition mode	Dynamic MRM

Table 1. Retention times, transitions, and MS/MS parameters for each analyte and internal standard

Analyte	RT (min)	Precursor Ion (m/z)	Fragment Ion (m/z)	Target CE (V)	Qualifier Ion (m/z)	Qualifier CE (V)
Morphine-d_6	1.35	292.2	166	153.1	43	181	36
Morphine	1.36	286.2	166	165.1	44	181	36
Amphetamine-d_8	1.93	144.2	92	97.1	16	127.1	4
Amphetamine	1.93	136.1	77	91	16	119	4
Methamphetamine-d_9	2.28	158.2	92	93	20	124.1	8
Methamphetamine	2.29	150.1	87	91	16	119	8
Codeine-d_6	2.61	306.2	171	165	52	213.1	24
Codeine	2.61	300.2	166	165	48	215	24
6-Acetylmorphine-d_6	3.90	334.2	171	165	48	211.1	24
6-Acetylmorphine	3.90	328.2	166	165.1	56	211.1	24
6-Acetylcodeine-d_3	7.37	345.2	162	229.1	28	165.1	80
6-Acetylcodeine	7.38	343.2	161	229	24	165	80
Norbuprenorphine-d_4	8.35	417.4	240	399.3	40		
Norbuprenorphine	8.35	414.4	240	187.2	40	340.4	28
EDDP-d_3	8.61	282.2	186	235.2	32	250.2	24
EDDP	8.61	279.2	186	235.2	32	250.2	24
Buprenorphine-d_4	8.95	472.5	240	400.4	48		
Buprenorphine	8.95	468.4	240	414.4	36	396.1	36
Methadone-d_9	9.18	319.2	97	265.2	12	105.1	32
Methadone	9.18	310.2	109	265.2	12	105.1	32

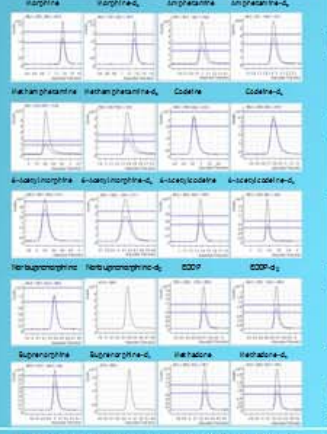

Figure 1. Multiple reaction monitoring chromatograms of the 20 ng/mL spiked standards with drug free oral fluid in LC/MS/MS.

Sample preparation

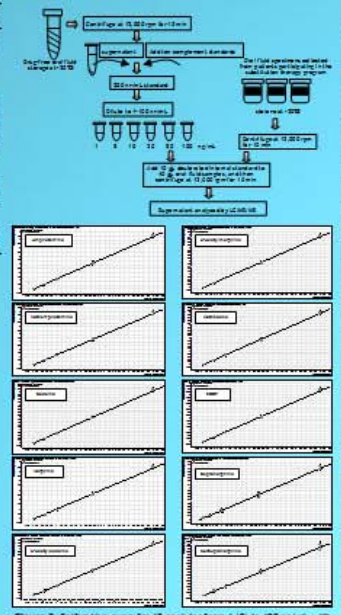

Figure 2. Calibration curve for 10 analytes from 1 (5) to 100 ng/mL with LC-MS/MS.

CORRESPONDING AUTHOR

Dong-Liang Lin, Ph.D.
Chief Toxicologist
Institute of Forensic Medicine, Ministry of Justice
Taiwan, R. O. C.
e-mail: dllin@mail.moj.gov.tw

RESULTS AND DISCUSSION

The overall protocol was evaluated by: (a) applying the method to the analysis of laboratory-prepared standards; and (b) comparing analytical data of clinical samples derived from this and GC-MS methods. When applied to the analysis of oral fluid specimens fortified with 1-100 ng/mL of the 10 analytes of interest, this method achieved the following results: (1) Inter-day and intra-day precisions range from 1.3 to 12.8% and 0.9 to 12.2% (percent CV), respectively; (2) method linearity (r^2), detection limit and quantitation limit for all analytes were >0.995, 0.1-1.0 ng/mL and 0.25-1.0 ng/mL (5 ng/mL for buprenorphine), respectively. Analytical data derived from LC-MS/MS and GC-MS analysis of methadone in 13 clinical samples were found comptrable. Specifically, 10 out of the 13 sets of data overlap at the ±20% level, with the remaining 3 sets barely outside of overlap range.

Table 2. Method calibration

Analyte	Linear range (ng/mL)	Regression equation	Correlation coefficient (r^2)	LOD (ng/mL)	LOQ (ng/mL)
Amphetamine	1-100	y=1.2889x-0.0073	0.99876	0.25	0.25
Methamphetamine	1-100	y=1.3565x-0.0155	0.99891	0.1	0.25
Codeine	1-100	y=0.8204x-0.0051	0.99879	1	1
Morphine	1-100	y=1.0349x-0.0118	0.99803	0.5	0.5
6-Acetylcodeine	1-100	y=0.6577x-0.0070	0.99847	0.25	0.25
6-Acetylmorphine	1-100	y=0.7774x-0.0066	0.99873	0.5	0.5
Methadone	1-100	y=0.7973x-0.0072	0.99900	0.1	0.25
EDDP	1-100	y=0.8791x-0.0068	0.99876	0.25	0.25
Buprenorphine	5-100	y=0.3309x-0.0332	0.99391	5	5
Norbuprenorphine	1-100	y=1.4995x-0.0069	0.99737	1	1

Table 3. Method validation

Analyte	Intraday (n=3) Precision (CV%)	Intraday (n=3) Accuracy (%)	Interday (n=3) Precision (CV%)	Interday (n=3) Accuracy (%)	Matrix effect (%)
Amphetamine	1.54-4.73	94.5-107.4	1.72-5.92	94.9-106.6	70.8
Methamphetamine	2.41-3.52	94.4-107.3	2.67-7.60	95.6-104.4	93.6
Codeine	1.33-7.04	94.0-109.4	5.07-11.6	91.9-104.9	119
Morphine	2.07-4.64	92.4-112.8	5.47-11.0	93.7-110.7	61.3
6-Acetylcodeine	0.87-3.74	91.8-112.4	1.37-6.28	93.1-110.7	64.7
6-Acetylmorphine	1.94-4.12	95.9-109.2	1.60-8.93	96.0-106.3	88.1
Methadone	1.69-5.89	96.0-108.8	4.03-6.86	93.0-103.3	81.3
EDDP	1.34-3.52	93.5-110.0	5.55-10.2	94.5-103.9	109
Buprenorphine	2.42-9.11	94.4-110.0	2.31-11.0	95.4-104.3	87.5
Norbuprenorphine	2.14-12.2	96.3-105.2	2.13-12.8	97.0-104.9	99.5

Table 4. Concentration range and number of detected cases of 62 oral fluid specimens

Analyte	n	conc. range (ng/mL)
Amphetamine (AM)	5 (8.1%)	1.90-444.9
Methamphetamine (MA)	9 (14.5%)	0.58-949.9
Codeine (CO)	27 (43.5%)	1.63-184.9
Morphine (MO)	18 (29.0%)	5.76-515.7
6-Acetylcodeine (6AC)	6 (9.7%)	1.03-26.61
6-Acetylmorphine (6AM)	5 (8.1%)	5.55-152.0
Methadone (MD)	57 (91.9%)	5.83-255.66
EDDP (ED)	50 (80.6%)	0.49-35.36
Buprenorphine (BU)	5 (8.1%)	7.39-278.4
Norbuprenorphine (NB)	2 (3.2%)	1.28-34.57

Methadone cases (n=37) Detected analyte	n
CO	5
CO + MO	10
CO + 6AC	1
CO + MO + 6AC + 6AM	5
MA	1
AM + MA	2
AM + MA + CO + MO	1
AM + MA + CO	2
MA + CO + MO	2
MA + CO	1

CONCLUSIONS

Direct injection of freeze-and-thaw samples appears to generate favorable results for LC-MS/MS analysis of oral fluid samples. Peak shape of chromatograms derived from oral fluid containing high concentrations of certain drugs, such as morphine, codeine, and (to a lesser degree) 6-acetylmorphine and buprenorphine, show irregular characteristics, which can be improved by diluting the sample with the mobile phase prior to injection. With heroin exhibiting ideal LC-MS/MS chromatographic characteristics, inclusion of this compound in the analyte list help detect abnormality of the analytical protocol and test specimens. Overall, our data indicate the LC-MS/MS method is more effective than our current GC-MS methods in the screening and analysis of the 10 analytes included in this study. We are currently conducting further studies on: (a) irregular chromatographic behavior of certain analytes and improvement methods; and (b) comparing analytical findings of all analytes derived from LC-MS/MS and GC-MS analysis.

Table 5. Comparison of LC-MS/MS and GC-MS data (methadone)

Donor no.	Dose (mg/day)	LC-MS/MS (ng/mL) Result	- (or +) 20%	- (or +) 20%	GC-MS (ng/mL) Result	Overlap
34	10	30.5	24.4	36.6	23.4	Yes
17	15	158.5	126.8	190.2	112.3	Yes
9	20	82.0	65.6	98.4	95.6	Yes
12	30	135.2	107.8	162.6	104.8	Yes
36	40	196.4	157.1	235.7	176.6	Yes
11	50	148.7	119	178.4	111.8	Yes
19	55	179.9	143.9	215.9	120.1	Yes
22	60	117.5	94.0	141.0	79.3	Yes
26	65	218.2	174.6	261.8	205.6	Yes
4	70	180.9	144.7	217.1	115.6	No
31	80	237.1	189.7	284.5	223.6	No
28	90	77.9	62.3	93.5	72.4	Yes
1	100	299.7	240	359.7	195.3	No

REFERENCES

1. Cone EJ. Legal, workplace, and treatment drug testing with alternate biological matrices on a global scale. J. Forensic Sci. Int. 2009; 121: 7.
2. Vindenes V, Yttredal B, Gjestad EL, Waal H, Bernard JP, Morland JG, Christophersen AS. Oral fluid is a viable alternative for monitoring drug abuse: detection of drugs in oral fluid by liquid chromatography-tandem mass spectrometry and comparison to the results from urine samples from patients treated with methadone or buprenorphine. J. Anal. Toxicol, 2011; 35: 32-39.
3. Simões SS, Ajanjo AC, Franco JM, Vieira DN, Dias MJ. Liquid chromatography/tandem mass spectrometry for the qualitative and quantitative analysis of illicit drugs and medicines in preserved oral fluid. Rapid Commun. Mass Spectrom, 2009; 23: 1451-1460.
4. Concheiro M, Gray TR, Shakleya DM, Huestis MA. High-throughput simultaneous analysis of buprenorphine, methadone, cocaine, opiates, and metabolites in oral fluid by liquid chromatography, tandem mass spectrometry. Anal Bioanal Chem. 2010; 398(2): 915-924.
5. Dams R, Murphy CM, Lambert WE, Huestis MA. Urine drug testing for opiids, cocaine, and metabolites by direct injection liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom, 2003; 17(14): 1665-1670.