行政院所屬各機關因公出國人員出國報告書
(出國報告類別:其他)

赴義大利參加2015年第53屆國際法醫毒物學者學會年會(TIAFT)會議報告

出國人員服務機關: 法務部法醫研究所
出國人員姓名/單位/職稱:
曹芸甄/毒物化學組/助理研究員
賴詠淳/毒物化學組/技士
出國地點: 義大利佛羅倫斯
會議期間: 民國一〇四年八月三十日
至民國一〇四年九月四日
報告日期: 民國一〇四年十一月三日

出國報告名稱:赴義大利參加2015年第53屆國際法醫毒物學者學會年會(TIAFT)國際會議報告

頁數：17　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/

出國人員姓名/服務機關/單位/職稱/電話

曹芸甄/法務部法醫研究所/毒物化學組/助理研究員/

賴詠淳/法務部法醫研究所/毒物化學組/技士/

出國類別：其他

會議期間：民國一〇四年八月三十日至民國一〇四年九月四日

出國地點：義大利佛羅倫斯

報告日期：民國一〇四年十一月三日

關鍵詞：法醫毒物、論文發表

內容摘要：

二〇一五年八月三十日至九月四日期間赴義大利佛羅倫斯參加2015年第53屆國際法醫毒物學者學會年會（TIAFT）國際會議。

參加2015年第53屆國際法醫毒物學者學會年會（TIAFT）國際會議，會議內容包括專題演講、口頭發表論文及電子壁報論文等，以及與鑑識科學研究與實務操作相關的商業展覽，並提供各級相關學位學程進修的資訊。來自世界各地與會代表約六百多人參與，本所並於國際年會中公開發表有關法醫毒物分析之論文二篇。

與來自世界各地研究鑑識科學的學者權威、實務專家齊聚一堂，討論實務案例、相關議題的研究內容及研究方向並發表心得，可促進國際學術及鑑識技術之交流，並了解先進國家在鑑識科學各領域的具體作法，機會十分難得。一方面拓展視野，增加對鑑識科學領域多元化的認識，亦可體認先進國家與本國鑑識科學發展模式與人才培養途徑的不同，並瞭解世界鑑識科學研究的趨勢與近期關注的議題；另一方面，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。

赴義大利參加2015年第53屆國際法醫毒物學者學會年會（TIAFT）會議報告

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摘　　要

二〇一五年八月三十日至九月四日期間赴義大利參加 2015 年第 53 屆國際法醫毒物學者學會年會（TIAFT），會議採各學門分組及分項形式同時進行，包括專題演講、研討課程、口頭發表論文及壁報張貼論文等，以及與鑑識科學研究與實務操作相關的商業展覽，來自世界各地與會代表六百柒拾玖人參與，分別來自於六十三的國家，本所並於年會中公開發表有關法醫毒物分析之論文二篇。

與來自世界各地研究鑑識科學的學者權威、實務專家齊聚一堂，討論實務案例、相關議題的研究內容及研究方向並發表心得，可促進國際學術及鑑識技術之交流，並了解先進國家在鑑識科學各領域的具體作法，機會十分難得。一方面拓展視野，增加對鑑識科學領域多元化的認識，亦可體認先進國家與本國鑑識科學發展模式與人才培養途徑的不同，並瞭解世界鑑識科學研究的趨勢與近期關注的議題；另一方面，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鑑識科學領域之作法及研究現況，並由論文發表提升本所國際地位。本所於一〇四年度內編列預算計畫派員至義大利參加2015年第53屆國際法醫毒物學者學會年會（TIAFT），並於會議中發表論文。

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：「Simultaneous Determination and Quantitation of Fentanyl, Norfentanyl, Alfentanil, And Sufentanil in Postmortem Blood and Urine By LC-MS/MS（以液相層析串聯質譜分析法同時定量血液及尿液中 Fentanyl、Norfentanyl、Alfentanil 及 Sufentanil 成分）」（曹芸甄*、劉秀娟、劉瑞厚、林棟樑）、「Preparing Postmortem Blood by "QuEChERS" Extraction Methods for LC-MS/MS Analysis of Drugs and Toxic Compounds (應用 QuEChERS 分散式固相萃取法於毒藥物鑑驗之研究」(劉秀娟、李習慈、賴詠淳*、曹芸甄、劉瑞厚、林棟樑）。

本所毒物化學組於每年申請之法務部科技計畫，均編有此項經費預算，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鑑識科學界之法醫毒物學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。

在這裡要感謝本所涂所長達人及毒物化學組林組長棟樑之支持與指導，才有此次機會赴義大利參加年會並了解觀摩國外法醫鑑識之發展。

貳、過程：

08 月 30 日	辦理大會報到手續 年會開幕式 開幕式演講 (Lecture)
08 月 31 日	論文口頭報告、論文壁報展示 研討課程 Workshop (1 堂)
09 月 01 日	論文口頭報告、論文壁報展示 研討課程 Workshop (2 堂)
09 月 02 日	論文口頭報告、論文壁報展示
09 月 03 日	論文口頭報告、論文壁報展示 發表本所論文
09 月 04 日	論文口頭報告、論文壁報展示 發表本所論文 閉幕式演講 (Lecture) 年會結幕式

參、會議內容：

一、 二〇一五年八月三十日至九月四日期間赴義大利佛羅倫斯參加2015 年第 53 屆國際法醫毒物學者學會年會（TIAFT），今年出席人數 679 人，分別來自於 63 的國家，演講(Lecture) 2 堂，大會研討課程(Workshop) 3 門，口頭報告共 122 篇及壁報論文展示共 274 篇。領域橫跨法醫毒物學、毒物代謝動力學、酒精議題、新興毒品濫用之分析鑑定及案例探討、酒後及服藥後駕車之影響、運動禁藥議題、中毒案例探討、頭髮分析方法探討、新技術之研發等主題。

二、 除了參加大會並參觀與大會同時進行的廠商展示，蒐集最新儀器及各式實驗室相關資料。世界各著名檢驗儀器、檢驗耗材廠商及鑑識書籍廠商，利用會場展覽及販售有關儀器及耗材，並展示最新可應用於鑑識科學之科技以及各式自動化及半自動化設備。

三、 於大會張貼壁報論文，今年度本所發表在法醫毒物類共有二篇「Simultaneous Determination and Quantitation of Fentanyl, Norfentanyl, Alfentanil, And Sufentanil in Postmortem Blood and Urine By LC-MS/MS（以液相層析串聯質譜分析法同時定量血液及尿液中 Fentanyl、Norfentanyl、Alfentanil 及 Sufentanil 成分）（曹芸甄*、劉秀娟、劉瑞厚、林棟樑）、「Preparing Postmortem Blood by “QuEChERS” Extraction Methods for LC-MS/MS Analysis of Drugs and Toxic Compounds (應用 QuEChERS 分散式固相萃取法於毒藥物鑑驗之研究」(劉秀娟、李習慈、賴詠

淳*、曹芸甄、劉瑞厚、林棟樑)，期間與前來閱覽之與會學者討論，並與其他壁報論文作者們交換意見，了解最新研究情形。

肆、檢討建議及心得感想：

一、國際會議部分心得

今年的國際法醫毒物學者學會年會（TIAFT）在義大利佛羅倫斯舉辦。另外，本次會議的壁報展示為「Poster」的形式。領域橫跨法醫毒物學、毒物代謝動力學、酒精議題、新興毒品濫用之分析鑑定及案例探討、酒後及服藥後駕車之影響、運動禁藥議題、中毒案例探討、頭髮分析方法探討、新技術之研發等主題。

二、口頭論文、壁報論文閱讀摘要心得

本次會議的口頭發表論文及壁報論文主題分為：(一) 毒物代謝動力學 (Toxicokinetics)；(二) 酒精分析相關議題 (Alcohol Use Markers)；(三) 新興毒品濫用之分析鑑定及案例探討 (New Psychoactive Substances – intoxication cases and identification)；(四) 酒後及服藥後駕車之影響 (Driving Under the Influnence)；(五) 運動禁藥議題 (Antidoping)；(六) 中毒案例探討 (Poisoning Case Reports)；(七) 活體內法醫毒物學 (In vivo Forensic Toxicology)；(八) 頭髮分析方法探討 (Hair Analysis)；(九) 新技術之研發 (New Technologies)；(十)死後法醫毒物學 (Post Mortem Forensic Toxicology)等十大主題，茲就與毒物化學組業務相關摘要如下：

1. PMMA (4-Methoxymethamphetamine)主要代謝是透過多型性的 CYP2D6 酵素，形成活化的代謝產物 4-hydroxymethamphetamine，在進一步變成 PMA。由於

PMMA 在人體中的代謝很多仍是未知的，因此本篇研究的目的主要是探討 PMMA 在人體肝臟微粒體的體外 (in virto)實驗之代謝機制並與 methamphetamine (MA)及 MDMA 相互比較。實驗結果顯示 PMMA 會依序代謝成 OH-MA、PMA、4-hydroxymethamphetamine (4-OH-MA) 及 dihydroxy-methamphetamine (di-OH-MA)；而 MA 依序代謝成 amphetamine、OH-MA、di-OH-MA；MDMA 依序代謝成 di-OH-MA、MDA。在 PMMA、MA、MDMA 實驗過程中顯示 incubation120-360 分鐘後產生 di-OH-MA 的濃度是最高的分別為 0-2.2%、0-0.01%、2.2-5.4%。在基因型分析實驗結果顯示，CYP2D6 基因型(genotype)對於 PMMA 的代謝相較於 MA 及 MDMA 影響是最大的。而 PMMA 在人體肝臟微粒體主要產生的代謝物是 4-OH-MA，其形成的 4-OH-MA 程度和 CYP2D6 基因型有重要的相關性。MDMA 代謝產生具有神經毒性的 di-OH-MA 相較於 PMMA 及 MA 多。

2. 在日本雖然有嚴格的法規來取締人工合成毒品(designer drugs)，但仍然有因濫用 designer drugs 而導致急性中毒案件的產生。在生物檢體中分析 designer drugs 是非常的困難，主要的原因是因為低劑量的 designer drugs 就足以對人體產生影響，或者是毒品在血液中的半衰期很短而導致很快的代謝。因此法醫毒物學家在對於因新興人工合成毒品之急性中毒案件的相關資料收集也更為困難。本篇研究的目的主要是探討因 designer drugs 導致急性中毒的個案。首先我們使用 LC/Q-TOFMS 來篩驗(screening test)法醫生物檢體中的 newly

encountered synthetic cathinone、α-PHP、acetylfentanyl，並定量及探討 designer drugs 在人體中主要的代謝過程。

首先收集法醫解剖後之血液及尿液檢體，血液檢體加入 methanol 去蛋白，離心後取上層吹乾，在以 20% methanol-10mM ammonium acetate buffer 回溶上機分析。LC/Q-TOFMS 部分使用 NexeraX_2 (Shimadzu) 及 TripleTOF5600 SYSTEM(AB SCIEX)，分離管柱為 L-column 2 ODS(3 μm particle；150 x 1.5 mm)，移動相分別為 A: 5% methanol-10mM ammonium acetate buffer(AAc) B: 95% methanol-10mM (AAc) buffer，流速為 0.10 mL/min，電灑游離法以正電離子模式。血液中α-PHP 及 acetylfentanyl 的定量採用標準添加法(standard addition method)。α-PHP 及 acetylfentanyl 的標準品加入血液檢體中並在鹼性環境下使用 ethyl acetate(EA)萃取，離心後使用氮氣吹乾，再使用 EA 回溶後注射至 GC/MS，以 SIM 模式行定量分析。(GC/MS-QP_{2010} Plus)（capillary column Rix-$_5$Sil MS；0.25 x 1.5 mm，0.25 μm)

實驗結果顯示急性中毒個案的血液檢體中分別檢出α-PHP 413 ng/mL 及 acetylfentanyl 69 ng/mL。α-PHP 代謝反應分別為 hydroxylation、reduction of β-keto moiety 及 2”-oxidation，acetylfentanyl 代謝反應分別是 hydroxylation、deacetylation。本研究顯示出受害者(victim)的死亡和使用 α-PHP 及 acetylfentanyl 有相關性。

3. 倫敦大學帝國學院的毒物化學單位承辦了倫敦及倫敦近郊

的法醫毒物案件。mephedrone (4-methylmethcathinone)屬於New Psychoactive Substance (NPS)在英國很常見。在法醫解剖檢體中 mephedrone 易在常規檢驗中的被檢出，而其他的NPS drugs 似乎出現的快消失的也快。本研究總共收集 2010 年至 2015 年 3 月，約 5 年期間，mephedrone 相關致死案例共計 60 案。

收集法醫解剖後之股靜脈血液檢體，來進行實驗室常規檢驗，先以 liquid-liquid extraction(LLE)進行萃取再以 GC/MS 分析，檢出 mephedrone 陽性反應的檢體再以 LLE 萃取及 N-methyl-bis(trifluoroacetamide)(MBTFA) 衍生後注射至 GC/MS 進行定量分析。

2010 至 2015 年 3 月 mephedrone 相關致死案件數依年度統計分別為 2010 年 (1 件, 0.11%)、 2011 年 (5 件, 0.45%) 、2012 年(4 件, 0.30%) 、 2013 年 (16 件, 0.79%) 、 2014 年(22 件, 1%)、2015 年 1-3 月 (12 件, 2.4%)。mephedrone 相關致死案件，平均年齡為 38 歲(範圍為 21-71 歲)，其中以男性占大多數為 92%，而 87%的個案有藥物濫用前科。mephedrone 致死案件中血液檢體檢出 mephedrone 濃度範圍為 0.05-7.0 μg/mL。根據統計有 95%的 mephedrone 致死案件案件有合併使用 1 至 5 種的其他藥物或是酒精。其中 95%的個案有合併使用「club drugs」，像是典型的安非他命類(MDMA、amphetamine、methamphetamine)占了 47%，其它的包括有 GHB 33%、cocaine 27%、ketamine 7%、methiopropamine (MPA) 3% 及其它的 NPS drugs 包括 ethylphenidate、ethylone、methoxetamine、6- (2-aminopropyl) benzofuran (6-APB)。其中

有 28%的個案有合併使用酒精其酒精濃度範圍為 20-201 mg/dL。而 12 % mephedrone 的個案有合併使用海洛因，顯示非只是典型的「club drugs」使用者會使用 mephedrone。雖然 mephedrone 在英國從 2010 年 4 月開始列為管制毒品，但案件數仍是逐年上升。mephedrone 相關致死案件占 2010 至 2015 年 3 月期間總案件數的 0.7%，而其它典型興奮性毒品分別為 amphetamine 1.8%、methamphetamine 1.0%、MDMA 1.1%、cocaine 7.5%。mephedrone 使次者通常會與其它 club drugs 合併使用，比較少是單一使用。經由毒物化學初步篩驗，發現除了可檢出常見的毒品成份，重要的是亦可檢出其它少見的毒品包括 GHB 或是其它的 NPS drugs。

4. GABA 的衍生物，pregabalin 在近年來被廣泛當成醫療用藥使用並且有濫用的趨勢，但 pregabalin 並非實驗室常規檢驗的一部分。本篇研究目的是建立pregabalin的定量分析方法，應用於法醫毒物案件，並且幫助研判法醫案件之毒物分析結果。

以 LC-MS-MS 分析法醫毒物案件之血液檢體中的 pregabalin 濃度，且紀錄各案件中是否有服藥後駕車或是伴隨使用其它藥物的情形。根據實驗結果資料顯示，檢測出 pregabalin 相關案例共計 93 案。其中有 80 案為濫用藥物死亡案件。在血液檢體中檢出 pregabalin 之 median 濃度及範圍分別為濫用藥物死亡案件為 7.0 μg/mL (< 0.6-21.6 mg/L)，而其它原因導致死亡的案件為 2.6 μg/mL (< 0.6-4.9 mg/L)。其中因 pregabalin 而導致死亡案件為 57.0 μg/mL (28-182 mg/L)。服用 pregabalin 後駕車共計 4 案，其濃度及範圍為 2.8 μg/mL

(11-46.7 mg/L)。pregabalin 相關致死案件中發現，93 案皆合併使用其它藥物或飲用酒精。實驗結果顯示使用的藥物種類，依頻率多至少依序分別為 antidepressants、opioids、benzodiazepines、opiates、alcohol、antipsychotics、cocaine、cardiac drugs、amphetamine、cannabis、anticonvulsants、antihistamines。而 New Psychoactive Substances (methxophenidine and synthetic cannabinoids) 只占了其中 2 案。實驗結果顯示，pregabalin 的使用者特別會與其它中樞神經抑制劑合併使用，因此建立pregabalin的定量分析方法，有助於研判法醫案件之毒物分析結果。

5. Novel Psychoactive Substances (NPS) 在現今非法毒品交易販賣有嚴重濫用之趨勢，因此 new cathinones 及其代謝物的鑑別也就更為困難。PV8, synthetic pyrrolidinophenone 在 2013 年於日本非法毒品交易市場被販售 (Uchiyama et al 2014)，接著在荷蘭及德國也相繼出現。而服用 PV8 對人體所導致的影響目前也只有網路的毒品相關論壇有報告指出具有和 α-PVT 及 α-PVP 相似的作用，和其它的許多的 NPS 一樣並沒有藥物動力學及藥物代謝反應的相關研究資料。而最重要的原因是目前並沒有相關文獻研究資料探討PV8的代謝過程，及服用後產生的反應。如果可以在尿液檢體找到專一性鑑別標記，就可有效的讓社會大眾了解服用 PV8 的危險性。因此本篇研究主要目的是探討PV8在人類肝臟微粒體(HLM)代謝的穩定性以及透過 high-resolution mass spectrometry (HRMS) 研究 PV8 在肝臟細胞中的代謝產物。

在半衰期研究方面，先把 PV8 incubation 在 HLM pool 中 1

小時後，再用 mobile phase A (0.1% formic acid in water)稀釋 100 倍後，以 Accucore C18 column (2.6 μm；100 x 2.1 mm) 分離 20 分鐘。PV8 代謝產物分析方面，是把 PV8 以低溫方式 incubation 在人類肝臟細胞 2 小時，接著把肝臟細胞檢體用 mobile phase A 稀釋 5 倍後，再以 Synergi Hydro-RP column (4 μm；150 x 2 mm)分離 30 分鐘。分離出來的檢體在利用 Thermo Scientific Q-Exactive HRMS 以 full-scan 方式和 data-dependent mass spectrometry 方法分析。肝臟細胞利用 silico MetaSite software 分析其 metabolite masses，也利用 all – ion-fragmentation mass 方法去鑑別代謝產物。

實驗結果顯示，PV8 半衰期為 28 分鐘，內生性清除率為 24 μL/min*mg，因此預估 PV8 在人體肝臟的 intermediate 清除率為 23.0-mL/min/kg。其代謝路徑包括 iminium formation、aliphatic hydroxylation、ketone formation、N-delkylation、N-dealkylation、ketone reduction 、aliphatic hydroxylation、glucuronidation。在肝臟細胞 3 條主要的代謝路徑為 di-hydroxylation > ketone reduction > hydroxylation。部分 glucuronidated 代謝物也在肝臟細胞被鑑別出。而 3 個主要的代謝物片斷為 hydroxylated 代謝產物 (aliphatic 及 aromatic)。

這是 PV8 的代謝產物第一次被鑑別出來，且以 high-resolution mass spectrometry 分析其結構式。可進一步應用於法醫及臨床檢驗，利用液相層析質譜儀鑑別出尿液中的 PV8 代謝物。

建議：

1. 國際上大部份的實驗室及研究單位對於法醫毒物分析的品質要求越來越來高，分析儀器也追求至高解析度及高精密度，以求最精準之實驗數據，因此採購高精密度及高解析度之儀器，不僅可應用於未來司法鑑識案件，更有助於提升國內法醫毒物分析之品質，也能與國際法醫毒物分析或毒藥物分析之各研究單位或鑑識單位接軌，有利於提高國內鑑識品質及在國際上的曝光率。

2. 由於法醫毒化鑑識科技日新月異,一日千里,隨社會的變遷，犯罪案件與日俱增，千變萬化，因此專業人員的在職訓練相當重要，除了參加國際會議外，宜安排人員赴國外作短期進修，培養人力，藉此強化本所鑑識技術能力，汲取國外相關之專業知識和技能。

3. 希望以後在參與國際會議時，能順便安排參觀一、兩天的實驗室行程，除了增長見識外，亦可培養人脈，將來若有培訓的需要，才有與國外合作交流的機會。

Simultaneous Determination and Quantitation of Fentanyl, Norfentanyl, Alfentanil, And Sufentanil in Postmortem Blood and Urine By LC-MS/MS

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

Introduction: Fentanyl (F), norfentanyl (NF), alfentanil (AF), and sufentanil (SF) are short-acting and highly potent μ-opioid agonists that are widely used for anesthetic and analgesic purposes. Therapeutic levels of these fentanyl-like compounds are as low as 1 ng/mL in plasma, therefore it's important to develop a sensitive method for detection and quantitation.

Aims: Since these synthetic compounds are often present in postmortem specimens from accidental, suicidal, and homicidal poisoning cases, we have developed a simple and sensitive LC-MS/MS method for their analysis in postmortem samples to assist medical examiners in determining the cause of death.

Methods: 2 mL of 1.5 M Na_2CO_3/$NaHCO_3$ (pH = 9.5) buffer solution was added to 1 mL of blood or urine samples containing the analytes's deuterated analogs, F-d_5, NF-d_5, AF-d_3, and SF-d_5 — quantitatively added to serve as internal standards. Liquid-liquid extraction was performed with 3 mL dichloromethane/1,2-dichloroethane/*n*-heptane/ethyl acetate (1:1:1:1, v/v) mixture. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm × 2.1 mm i.d., 1.8-μm particle) analytical column at 50 °C. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32 mL/min. Mass spectrometric analysis was performed under electrospray ionization in positive-ion multiple reaction monitoring (MRM) mode. The precursor and two transition ions (*m/z*) adopted for F, NF, AF, and SF were 337, 188/105; 233, 150/84; 417, 268/197; and 387, 238/111, respectively. Corresponding precursor and transition ions (*m/z*) for F-d_5, NF-d_5, AF-d_3, and SF-d_5 were 342, 188/105; 238, 155/84; 420, 271/200; and 392, 238/111, respectively.

Results: Drug-free blood and urine samples, fortified with 2–40 ng/mL of the four analytes of interest, used for method validation yielded the following results: (a) average extraction recoveries ranges: 67.04–98.64% for blood, 58.93–98.90% for urine; (b) intraday and interday precision ranges (percent CV): 0.37–3.31% and 0.77–8.55%; (c) intraday and interday accuracy ranges: 88.93–104.6% and 92.37–106.3%; and (d) calibration linearity (r^2), detection limit (LOD), and quantitation limit (LOQ): >0.999, 0.01–0.1 ng/mL, and 0.01–0.1 ng/mL, respectively. LOD was defined as the lowest concentration at which ion ratio pairs monitored for a particular analyte fell within ±20% of that observed in the standard; while LOQ was defined as the lowest concentration at which LOD requirements were met and the observed concentration also fell within ±20% of the expected value. Observed ion suppression was about 30% for F and NF; 35% for AF; and 45% for SF. This phenomenon was closely monitored — and found adequately compensated for — when the analytes' deuterated analogs were used as the internal standards for quantification. Among 3740 toxicological cases during the 2014– Feb. 2015 periods in our institute, 18 blood specimens were found to contain at least one of these four compounds with the following means and concentration ranges (ng/mL): F (6.51, 0.18–29.10); NF (1.80, 0.09–5.79).

Conclusions: The validated protocols are easy and quick to carry out, and have been successfully utilized to the analysis of these fentanyl-like compounds in postmortem samples.

Key Words: Fentanyl, Fentanyl-like drugs, Postmortem, LC-MS/MS

Simultaneous Determination and Quantitation of Fentanyl, Norfentanyl, Alfentanil, and Sufentanil in Postmortem Blood and Urine by LC-MS/MS

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Introduction

Fentanyl, norfentanyl, alfentanil, and sufentanil are short-acting and highly potent μ-opioid agonists that are widely used for anesthetic and analgesic purposes. Therapeutic levels of these fentanyl-like compounds are as low as 1 ng/mL in plasma, therefore it's important to develop a sensitive method for detection and quantitation.

Since these synthetic compounds are often present in postmortem specimens from accidental, suicidal, and homicidal poisoning cases, we have developed a simple and sensitive LC-MS/MS method for their analysis in postmortem samples to assist medical examiners in determining the cause of death.

Methods

1. Instrument conditions

Agilent 1290 Infinity LC Systems and 6460 Triple Quadrupole

Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100 mm × 2.1 mm I.d., 1.8-μm particle) analytical column at 50°C. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32 mL/min. Mass spectrometric analysis was performed under electrospray ionization in positive-ion multiple reaction monitoring (MRM) mode.

Table 1. Transitions and MS/MS conditions for each analyte and internal standard in LC-MS/MS

Analyte	Retention time(min)	Precursor ion (m/z)	Fragment (V)	Target ion(m/z)	CE	Qualifier ion(m/z)	CE	Dwell
Fentanyl	6.02	337	150	188	20	105	45	25
Fentanyl-d_5	6.00	342	150	188	20	105	40	25
Norfentanyl	4.35	233	150	150	16	84	16	100
Norfentanyl-d_5	4.32	238	150	155	16	84	16	100
Alfentanil	6.09	417	119	268	16	197	28	25
Alfentanil-d_3	6.08	420	126	271	16	200	28	25
Sufentanil	6.53	387	126	238	16	111	44	25
Sufentanil-d_4	6.52	392	131	238	20	111	44	25

2. Sample preparation

2, 5, 10, 20, 40 ng/mL spiked standards with 1 mL drug-free blood or urine and 50 μL internal standards (10 ng/mL)

↓

Add 2 mL of 1.5 M Na_2CO_3/$NaHCO_3$ (pH = 9.5) buffer solution and mix

↓

Add 3 mL dichloromethane/1,2-dichloroethane/n-heptane/ethyl acetate (1:1:1:1, v/v) mixture and shake for 15 minutes

↓

Centrifuge at 4000 rpm for 15 minutes and transfer the supernatant to new tubes
Evaporation of the organic extract system

↓

Reconstitution with the LC mobile phase
5 μL of the reconstituted extract was injected to the LC-MS/MS

Results

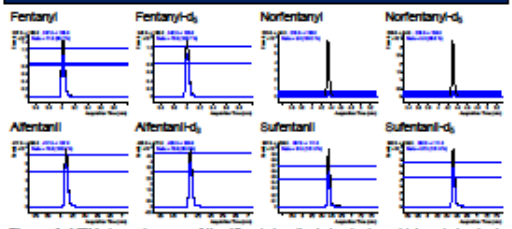

Figure 1. MRM chromatograms of the 10 ng/mL spiked standards and internal standards with drug free urine.

1. Matrix effect and recovery

Table 2. Extraction recovery and matrix effect of fentanyl, norfentanyl, alfentanil and sufentanil in blood and urine, Mean ± S.D. (n=3)

Analyte	Concentration (ng/mL)	Blood		Urine	
		Matrix effect	Recovery (%)	Matrix effect	Recovery (%)
Fentanyl	5	75.10±5.84	96.54±6.01	64.44±8.47	97.22±13.16
	10	75.29±3.60	95.49±5.70	71.30±2.61	93.59±9.67
	20	70.45±7.48	97.66±5.44	70.62±3.56	97.42±7.02
Norfentanyl	5	97.15±6.76	70.52±6.41	68.46±9.37	72.37±7.67
	10	85.55±2.85	67.04±4.30	62.54±5.00	58.93±1.01
	20	86.24±6.28	69.93±0.83	70.79±2.44	70.78±5.34
Alfentanil	5	77.09±5.74	91.42±5.46	63.34±8.26	98.90±7.83
	10	76.25±1.95	94.04±2.57	63.40±1.73	85.57±2.43
	20	73.04±3.41	95.70±1.85	64.78±2.43	92.46±4.03
Sufentanil	5	62.39±6.00	87.30±4.85	51.04±5.61	97.00±13.61
	10	61.46±1.44	93.51±4.37	57.10±2.95	84.89±4.34
	20	56.61±3.94	96.64±4.49	55.60±2.62	95.47±5.93

2. Calibration

Table 3. Calibration results, LOD and LOQ of fentanyl, norfentanyl, alfentanil and sufentanil in blood and urine

Sample	Analyte	Regression equation	Correlation linearity (r^2)	Concentration range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Blood	Fentanyl	y=1.1247x+0.0278	0.99986	2-40	0.01	0.01
	Norfentanyl	y=2.0530x+0.0668	0.99962	2-40	0.1	0.1
	Alfentanil	y=1.3559x+0.0143	0.99993	2-40	0.01	0.01
	Sufentanil	y=1.3071x+0.0217	0.99987	2-40	0.01	0.025
Urine	Fentanyl	y=1.0142x+0.0222	0.99973	2-40	0.01	0.025
	Norfentanyl	y=1.9018x+0.0163	0.99997	2-40	0.1	0.1
	Alfentanil	y=1.2945x+0.0024	0.99998	2-40	0.01	0.01
	Sufentanil	y=1.1561x+0.0084	0.99995	2-40	0.01	0.025

3. Precision and accuracy

Table 4. Summary of intraday and interday precision and accuracy (n=5)

Sample	Analyte	Intraday precision (CV%)	Intraday accuracy (%)	Interday precision (CV%)	Interday accuracy (%)
Blood	Fentanyl	0.56-3.06	91.72-101.79	0.77-7.09	93.57-105.95
	Norfentanyl	0.72-2.10	88.93-102.38	1.61-8.55	92.37-106.31
	Alfentanil	0.37-2.38	99.12-102.12	2.67-7.18	96.88-104.44
	Sufentanil	0.56-2.37	99.42-100.30	2.42-6.75	96.06-104.78
Urine	Fentanyl	1.12-3.16	98.70-102.23	2.89-7.38	99.02-100.60
	Norfentanyl	0.82-1.87	98.46-104.57	2.11-7.31	99.34-100.23
	Alfentanil	0.52-2.08	98.17-102.50	2.14-3.46	97.66-102.35
	Sufentanil	0.53-3.31	98.29-103.03	2.89-5.70	97.89-101.92

4. Applications

After method validation, the procedure was applied to forensic samples. Among 4685 toxicological cases during the 2014- May. 2015 periods in our institute, 23 cases were found to contain at least one of these four compounds.

Table 6. Results of the analysis of fentanyl and norfentanyl in postmortem samples

Analyte	Sample	n	Concentration range (ng/mL)	Mean ±S.D. (ng/mL)
Fentanyl	Blood	22	0.18-33.03	7.84±9.04
	Urine	5	7.83-69.41	22.51±26.29
	Gastric content	6	3.06-55.85	22.23±24.30
	Bile	3	1.61-28.37	12.33±14.15
Norfentanyl	Blood	18	0.10-5.79	1.81±1.71
	Urine	4	24.06-159.41	63.10±64.80
	Gastric content	5	0.23-4.87	2.54±1.92
	Bile	2	2.33-10.92	6.63±6.07

Conclusion

The validated protocols are easy and quick to carry out, and have been successfully utilized to the analysis of these fentanyl-like compounds in postmortem samples.

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Preparing Postmortem Blood by "QuEChERS" Extraction Methods for LC-MS/MS Analysis of Drugs and Toxic Compounds

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Aim: To develop a modified QuEChERS method for analysis of drugs and toxic compounds in postmortem blood samples.

Objective: Analysis of drugs and toxic compounds in postmortem blood is complicated by the presence of hemolyzed blood products and a wide variety of analytes of interest (often at low concentrations) with basic/acidic, hydrophilic/hydrophobic characteristics. Having noted successful applications of the "QuEChERS" (*quick*, *easy*, *cheap*, *effective*, *rugged*, and *safe*) extraction methods to the analysis of pesticide residues in food and agricultural products, we have conducted this study to develop an optimal QuEChERS method for effective extraction of drugs and toxic compounds from postmortem blood samples for LC-MS/MS analysis.

Materials and Methods: The modified QuEChERS approach involved a 2-step process, i.e., extraction/partitioning and dispersive-solid phase extraction (d-SPE). In step 1, 1-mL aliquots of blood sample were extracted by six different QuEChERS methods, each partitioning into three layers by centrifugation. In step 2, each of the six resulting top extract layers was processed with three different d-SPE sorbents, followed by centrifugation. Supernatants derived from these processes (a total of 18 combinations) were analyzed by LC-MS/MS to evaluate the recoveries of the analytes of interest. A mixture of 31 forensically relevant drugs (including opiates, amphetamines, cocaine, benzodiazepines) and 23 case samples were included in this study; results were compared against those derived from the Toxi-tubes® A liquid-liquid extraction (LLE) method, that has been established and routinely used in our laboratory.

Results: The modified QuEChERS method included the use of inorganic salts helpful to blood coagulation and isolation of the organic extract phase. Combination of 1-mL Na_2CO_3/$NaHCO_3$ buffer, 0.8-g anhydrous $MgSO_4$ (as dehydrating agent), 0.2-g NaCl (as salting-out agent), 2-mL acetonitrile (as organic solvent), and the d-SPE cleanup sorbent (containing 25-mg PSA, 25-mg C18EC and 150-mg $MgSO_4$) provided optimal sample pretreatment products. Recoveries of the 31 analytes (each at 0.5 μg/mL) ranged from 56 to 78%, except morphine (40%) and benzoylecgonine (33%). Application of this modified QuEChERS and the LLE methods to the analysis of 23 casework postmortem blood specimens generated a combined total of 168 positive results of 84 compounds; 85.1% and 82.7% of these positives were reported by the modified QuEChERS and the LLE methods, respectively. For drugs that were detected by both methods, their quantitative data were in good agreement.

Conclusion: A modified QuEChERS method, operated under alkaline condition, has been successfully developed to pretreat postmortem blood for LC-MS/MS analysis of drugs and toxic compounds. New abuse drugs, such as 4-chloroamphetamine, 5-MeO-MiPT, and PMMA, can also be detected with this approach. With low cost and easy to use, this approach can potentially become the preferred cleanup method for the analysis of drugs in postmortem blood sample.

Key Words: QuEChERS, dispersive solid-phase extraction, Postmortem blood, LC-MS/MS

Preparing Postmortem Blood by "QuEChERS" Extraction Methods for LC-MS/MS Analysis of Drugs and Toxic Compounds

Hsiu-Chuan Liu[1], Hsi-Tzu Lee[1], Yung-Chun Lai*[1], Ray H. Liu[2], Dong-Liang Lin[1]

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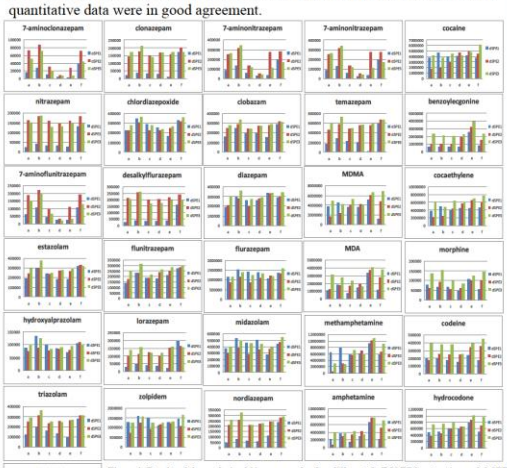

Figure 1. Results of the analysis of 31 compounds after different QuEChERS extraction and d-SPE steps using LC-MS/MS.
(a) EN method (b) Original method (c) AOAC method(d) FA method (e) TFA method (f) IFM method
d-SPE1: 25 mg C18EC,150 mg $MgSO_4$
d-SPE2: 50mg PSA,7.5mg carbon,50mg C18EC,150 mg $MgSO_4$
d-SPE3: 25mg PSA,25 mg C18EC,150 mg $MgSO_4$

Table 1. Recovery of the analysis of 31 compounds after EN method and d-SPE1, IFM method and d-SPE3, and toxi-tubes A extraction.(mean±S.D., n=5)

Recovery(%)	EN/d-SPE1	IFM/d-SPE3	Toxi-tubes® A
7-aminoclonazepam	50.90 ± 4.84	63.94 ± 1.65	66.57 ± 2.23
7-aminoflunitrazepam	53.72 ± 4.37	68.79 ± 1.46	87.18 ± 1.08
7-aminonitrazepam	44.14 ± 4.33	56.81 ± 1.94	61.56 ± 0.91
alprazolam	60.04 ± 1.77	66.68 ± 4.38	80.69 ± 3.54
chlordiazepoxide	57.97 ± 1.20	64.92 ± 0.59	82.57 ± 3.29
clobazam	63.25 ± 2.09	65.96 ± 2.40	85.95± 4.72
clonazepam	58.47 ± 0.92	61.49 ± 2.55	78.07 ± 6.34
desalkylflurazepam	46.89 ± 2.14	57.30 ± 0.94	74.19 ± 4.62
diazepam	56.18 ± 2.41	67.89 ± 2.71	76.01 ± 3.47
estazolam	59.55 ± 0.45	64.88 ± 3.00	83.21 ± 4.02
flunitrazepam	62.76 ± 1.59	66.47 ± 3.36	85.45 ± 4.09
flurazepam	69.79 ± 2.34	70.13 ± 1.87	76.19 ± 5.74
hydroxyalprazolam	64.30 ± 2.26	60.89 ± 4.86	79.60 ± 3.78
lorazepam	58.51 ± 1.58	58.69 ± 2.40	64.08 ± 4.38
midazolam	66.91 ± 3.14	65.62 ± 3.05	83.42 ± 5.19
nitrazepam	46.78 ± 3.37	56.08 ± 1.80	75.74 ± 5.82
nordiazepam	54.58 ± 2.32	65.19 ± 2.00	73.25 ± 3.74
oxazepam	56.99 ± 0.79	58.32 ± 1.71	60.47 ± 2.88
temazepam	61.39 ± 1.48	62.98 ± 2.80	79.65 ± 4.28
triazolam	56.10 ±1.20	61.72 ± 2.53	81.30 ± 4.08
zolpidem	68.06 ± 2.55	70.38 ± 0.76	84.21 ± 3.82
amphetamine	54.04 ± 0.60	55.35 ± 2.25	80.91 ± 3.03
benzoylecgonine	42.18 ± 1.59	32.55 ± 1.92	5.45 ± 0.28
cocaethylene	71.00 ± 1.52	78.07 ± 2.67	86.01 ± 3.25
cocaine	70.37 ± 2.27	74.16 ± 2.50	88.93 ± 2.59
codeine	52.75 ± 0.72	57.84 ± 3.55	87.80 ± 1.22
hydrocodone	61.21 ± 1.19	66.18 ± 4.22	88.29 ± 1.57
MDA	59.00 ± 0.90	56.28 ± 2.73	82.57 ± 1.15
MDMA	63.55 ± 0.45	65.01 ± 2.14	86.17 ± 1.30
methamphetamine	62.33 ± 0.78	64.48 ± 2.73	84.66 ± 3.82
morphine	42.15 ± 1.23	39.93 ± 1.69	42.67 ± 1.49

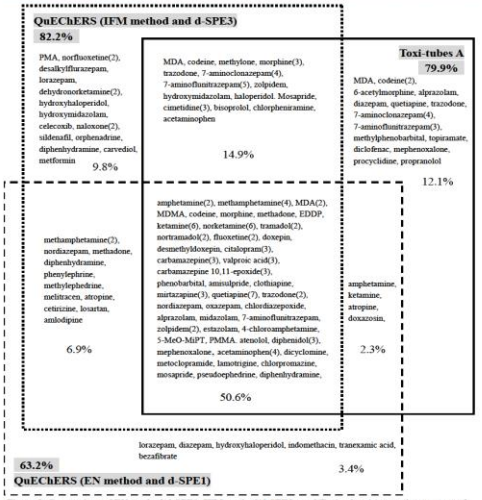

Figure 2. Comparison of EN method/d-SPE1, IFM method/d-SPE3 , and Toxi-tubes A extraction on general unknown screening procedures for the detection of drugs and toxic compounds in 23 postmortem blood samples.

Conclusion

A modified QuEChERS method, operated under alkaline condition, has been successfully developed to pretreat postmortem blood for LC-MS/MS analysis of drugs and toxic compounds. New abuse drugs, such as 4-chloroamphetamine, 5-MeO-MiPT, and PMMA, can also be detected with this approach. With low cost and easy to use, this approach can potentially become the preferred cleanup method for the analysis of drugs in postmortem blood sample.

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