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（出國類別：研究）

研究國外刑事鑑識制度及設施

服務機關：中央警察大學
出國人　職　稱：副教授
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出國地區：美國
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內容摘要: 中央警察大學副教授王勝盟於90年7月2日至8月3日奉派至美國紐澤西州林祕郎博士主持之刑事毒物實驗室及佛羅里達大學病理、免疫學系Goldberger教授實驗室研習刑事鑑識制度、濫用藥物尿液檢驗技術及認證制度，並蒐集相關資料，以提昇國內刑事鑑識技術及濫用藥物尿液檢驗之水準及豐富濫用藥物相關資訊。兩間實驗室主要之任務爲接受各該州內幾個行政區的法醫解剖案件之藥毒物鑑定及從事特殊法醫毒物案件之研究，各該實驗室之主持人在藥毒物鑑定均具有豐富之專業知識與經驗，且建立各該實驗室對於送鑑案件之標準操作流程，使得各個技術人員有標準的規範遵循，從其對實驗室管理井然有序、成員任事熱忱之態度以及對濫用藥物檢驗專業之尊重，使得各該實驗室在濫用藥物檢驗技術具一流水準，深值得個人學習與努力。

本文電子檔已上傳至出國報告資訊網

出國類別：研究

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服務機構及職稱：中央警察大學鑑識科學系副教授

報告人姓名：王勝盟

派赴國家：美國

出國期間：90 年 7 月 2 日至 8 月 3 日

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摘要

中央警察大學副教授王勝盟於 90 年 7 月 2 日至 8 月 3 日奉派至美國紐澤西州林祑郎博士主持之刑事毒物實驗室及佛羅里達大學病理、免疫學系 Goldberger 教授實驗室研習刑事鑑識制度、濫用藥物尿液檢驗技術及認證制度，並蒐集相關資料，以提昇國內刑事鑑識技術及濫用藥物尿液檢驗之水準及豐富濫用藥物相關資訊。兩間實驗室主要之任務為接受各該州內幾個行政區的法醫解剖案件之藥毒物鑑定及從事特殊法醫毒物案件之研究，各該實驗室之主持人在藥毒物鑑定均具有豐富之專業知識與經驗，且建立各該實驗室對於送鑑案件之標準操作流程，使得各個技術人員有標準的規範遵循，從其對實驗室管理井然有序、成員任事熱忱之態度以及對濫用藥物檢驗專業之尊重，使得各該實驗室在濫用藥物檢驗技術具一流水準，深值得個人學習與努力。

一、目的

個人奉派赴美國紐澤西林祑郎博士主持之刑事毒物實驗室及佛羅里達大學病理、免疫學系 Goldberger 教授實驗室研習刑事鑑識制度、濫用藥物尿液檢驗技術及認證制度，並蒐集相關資料，以提昇國內刑事鑑識技術及濫用藥物尿液檢驗之水準及豐富濫用藥物相關資訊。

二、過程(參觀及研習經過)

職於七月二日下午搭長榮航空班機至美國紐澤西州紐瓦克機場，途中於西雅圖停機一小時，由於時差十二小時，到達紐澤西州紐瓦克機場的時候已經是仍是七月二日晚上十二時，在安頓好住宿的問題之後，七月三日隨即拜訪紐澤西州立刑事毒物實驗室主任林祑郎博士，林博士非常熱心的接待，並簡單介紹該實驗室的成員與各項設備，在往後的幾天中，就進行收蒐集資料與詳細的研習、參觀及討論活動。

結束紐澤西州立刑事毒物實驗室的參觀、訪問後，轉搭美國 Continental 航空公司班機至佛羅里達 Jacksonville 機場，隨後轉至位於 Gainsville 的佛羅里達大學，在安頓好住宿問題之後，隔天隨即拜訪佛羅里達大學病理、免疫學系 Goldberger 教授實驗室，在 Dr. Goldberger 很友善與熱心地介紹實驗室成員與各項儀器設備之後，就進行參觀、研習與討論活動，並利用空餘時間蒐集資料。在結束佛羅里達大學的參觀及研習活動後，於八月二日搭長榮航空班機返國。

以下就所參觀的兩個實驗室進行詳細之說明：

(一) 紐澤西州立刑事毒物實驗室

紐澤西州立刑事毒物實驗室的主任是林祑郎博士，下有兩個行政助理，一個管理帳務之行政官員，七個分析技術員等共十一位，該實驗室主要是處理該州部分行政區(約有八個)的法醫中心所解剖案件的檢體，其檢體包括腦、肝臟、血液、膽汁、尿液、胃內容物、眼球液...等等，每天的送鑑數量

約有十多件(但樣品數目不定)，每週的數量約有數十件，該實驗室係州立的，所以對送鑑案件之檢驗是不收任何費用的。該實驗室其主要目的在於分析了解送鑑的檢體中，可能含有的藥毒物，以作為法醫判斷案件的依據，另一方面則須將分析之結果提出於法庭，並且以專家證人之身分，詮釋分析結果，以為法庭釐清案情之依據。該實驗室在林博士的領導下，建立相當不錯的聲譽，從與林博士討論的幾次實際案例中，令我感到一個實驗室領導者之經驗及標準化分析流程的建立，對一個實驗室是相當重要且必須的。

首先針對該實驗室對於濫用藥物的檢驗分析、使用之儀器設備及檢體之前處理技術等，作一簡單介紹：

1. 藥毒物的分類方面

該實驗室依標準分析流程設定檢測的藥物分為下列幾項：

A.揮發性藥毒物：主要檢測一氧化碳、氰氫酸及酒精濃度分析。

B.鴉片類藥物：主要檢測的項目為嗎啡、可待因、6-乙醯嗎啡、Hydromorphone、Nalophine、Oxycodone、Hydrocodone...等。

C.大麻類藥物：主要分析其中 11-nor-9-carboxy-delta-9-THC 成分。

D.古柯鹼及其代謝物：主要分析其中 cocaine、benzoylecgonine 等，而 benzoylecgonine 為古柯鹼主要的代謝產物。

E.安非他命類藥物：主要分析的藥物包括安非他命、甲基安非他命、phenylpropanolamine、phenethylamine、pseudoephedrine、MDA 及 MDMA 等。

F.PCP：主要分析的藥物為 PCP。

G.吩坦尼(Fentanyl)：主要分析的藥物為 Fentanyl。

2. 分析儀器方面

所謂「工欲善其事，必先利其器」，幾乎每一個從事檢驗、分析工作的實驗室，均有相當完整的分析儀器，茲簡單介紹該實驗室之分析儀器架構：

A.全自動藥物濃度篩選儀約有 4 部：該實驗室之藥物篩選儀主要用於篩選應

徵警察工作者及目前從事警察工作而服勤不正常，有吸毒傾向者之尿液檢體及篩選由各個行政區法醫中心送鑑之尿液或其他較清澈之體液樣品。

B.CO 濃度檢測儀一部：對於使用微量擴散法 Conway cell 篩檢呈陽性反應之樣品，再利用此儀器偵測 CO 含量，操作簡單、方便。

C.全自動頂空氣相層析儀兩部：此儀器用於檢測血中揮發性有機物種，包括甲醇、乙醇、丙酮、正丙醇、異丙醇...等，因為是全自動的頂空加熱進樣器，所以可以分析大量樣品，但也節省相當的人力，這是器自動化最主要的優點。

D.高效液相層析儀 4 部：主要用於分析各種由法醫中心送來組織、臟器檢體中之藥毒物，高效液相層析儀適合於分析高分子量、低揮發性及遇熱易分解之藥毒物，而在這些濫用藥物中，有許多必須使用液相層析儀來分析較適當，林博士在這方面相當專精，也研究出許多新的、方便、簡單且好用的方法，但由於液相層析儀僅能利用滯留時間提供定性分析之資料，對於確認分析之質譜資料，尚嫌不足，據林博士表示該實驗乃將有計畫地添購液相層析質譜儀，以加強實驗室在這方面分析的功能。

E.氣相層析質譜儀 4 部：氣相層析質譜儀主要用於確認送鑑檢體中藥物的成分，該儀器集分離、純化與結構鑑定於一體，是目前分析實驗室最常用的儀器，而且鑑別率高，為該實驗室廣泛使用的儀器。

F.其他設備及儀器：包括光譜儀、固相萃取系統、液相-液相萃取系統、檢體均質化設備、離心機、氮氣吹乾系統...等等，均是樣品在前處理及分析過程中重要的周邊設備。

3.萃取方法分類方面

由於每種藥物的特性均不相同，站在分析的角度而言，按照各種藥物之性質加以分類，才是成功完成分析的不二法門，也因為如此，至目前為止並無一個簡單且能夠完全把分析檢體中的藥物一次地萃取出來，以進行儀器分析，該實驗室亦是如此，而將萃取、分離的法分為下列幾項：

A.頂空氣相法：此法僅適用於揮發性藥毒物的萃取上，該實驗室目前利用此法以分析檢體中之甲醇、丙酮、乙醇、異丙醇及正丙醇等。

B.固相萃取法：固相萃取法為目前許多藥物分析實驗室常用的前處理流程，操作過程雖不容易，但也不會太難，該實驗室利用 C-18 的固相萃取管柱，以萃取諸如尿液、血液、膽汁、眼球液中的 THC，經過化學衍生後，進行 GC/MS 分析。由於該實驗室有許多組織檢體，在固相萃取中容易阻塞，所以固相萃取法的應用較有限，而大部分以液相-液相萃取法進行。

C.液相-液相萃取法：液相-液相萃取法較複雜，必須要有熟練的技術，才能有較好的萃取效果，該實驗室的組織檢體經過均質化的處理之後，就利用液相-液相萃取法姜分析物萃取出來，其中包括腦、肝臟、脾臟、胃內容物...等檢體中鴉片類藥物、安非他命類藥物、古柯鹼及其代謝物、PCP 及 Fentanyl 的萃取等，隨後再進行高效液相層析儀分析。

(二) 佛羅里達大學病理、免疫學系 Goldberger 教授實驗室

佛羅里達大學病理、免疫學系 Goldberger 教授實驗室，同時也是協助臨床診斷實驗室(Diagnostic Referral Laboratories)及鑑識毒物實驗室(Forensic Toxicology Laboratory)，其主管為 Dr. Goldberger 教授，另外有一位實驗室經理為 Dr. Chris.，其他有四位分析技術員，一位分析助理等共七位，該室主要協助佛羅里達州的八個行政區的法醫毒物分析，每天約有十多件案件，一週的平均樣品數量約有數十件，由於該實驗室設立於佛羅里達大學病理、免疫學系之下，所以對於送鑑案件的分析檢驗，採取收費的方式以供給必要之支出。其接受之檢體包括腦、肝臟、血液、膽汁、尿液、胃內容物、眼球液...等等，但主要以血液及尿液為主，其目的在於分析送鑑的檢體中，可能含有的藥毒物，以作為法醫判斷案件的依據，另一則將分析之結果提出於法庭，並且以專家證人之身分，詮釋分析結果，以為法庭釐清案情之依據。

該實驗室對於送鑑之檢體，從收件、儲存、取樣、分析等步驟，均有檢體監管流程(chain of custody)，以確保分析流程中導入不必要的錯誤。接著對

於需要確認分析之成分，也都建立其檢量線及品質管制標準品，隨時監控人員的操作及儀器的穩定程度，將系統誤差減至最小。以下針對該實驗室，就其濫用藥物分析之分類、所用之儀器設備及其檢體之前處理技術，作一簡單介紹：

1.藥毒物的分類方面

該實驗室之標準分析流程如下：首先對於接受之檢體，先使用免疫分析法做一初步篩選，對於像安非他命類、古柯鹼類、鴉片類、大麻類、安眠藥類(巴比妥類)...等特定之藥物，若呈陽性反應時，則進行氣相層析質譜儀的確認分析。

另外，對於非此特定之分析物，則廣泛地使用酸性藥毒物與鹼性藥毒物的分類方式區分，先經過固相萃取法進行萃取後，將之分為酸性萃取藥物與鹼性萃取藥物，然後進行氣相層析或薄層層析法篩檢分析，依此方法，大概可將藥物分成酸性藥物與鹼性藥物，若這些藥物已經是該實驗室所設定之分析藥物，則進行定量，若無，則再進行氣相層析質譜分析，利用全質譜掃瞄之方式，進行質譜資料庫之比對，鑑定該分析物，該實驗室所檢測的藥物範圍及分類如附件一所示。

對於揮發性化合物分面，該實驗室則有固定之自動化頂空氣相層析儀分析血液或尿液中之酒精濃度，另外，Goldberger 教授也接了一個配製血液中酒精濃度績效監測樣品的計畫，用以提供該州與檢測血液中酒精濃度相關實驗室的績效監測樣品，一則以做實驗室間之比較分析，另一則以使實驗室隨時能夠保證分析之品質，做好品質管制之工作。

所有送鑑案件經過實驗室轉碼後，按照檢體數量的多寡，編入批次，先使用免疫分析儀進行初步篩檢，之後再使用固相萃取進行酸性及鹼性藥毒物的萃取，同時也對於所有的檢體進行頂空氣相層析儀的體液中酒精濃度分析，當發現送鑑檢體被要求做重金屬檢測或懷疑含有重金屬成分時，也會對檢體進行如砷、汞...等重金屬檢測。因此，該實驗室對於幾個經常被使用之

藥物，均列為篩檢的重點藥物。

A.初步篩選部份：包括

(I)使用酵素免疫分析儀篩選的藥物有安非他命類、古柯鹼類、鴉片類、大麻類、安眠藥類(巴比妥類)...等，其分析流程如附件二。

(II)使用氣相層析法篩選的藥物分為酸性藥毒物與鹼性藥毒物，如附件三。

(III)揮發性藥物篩選的部份包括酒精、丙酮與甲醇等，如附件四。

B.確認分析部份：

提到確認分析，目前幾乎以氣相層析質譜儀為必備之分析工具，該實驗室共有三台氣相層析質譜儀，分別進行各檢體之確認分析，其中有一部特別用來進行研究特殊的案件。有些成分是需要定量的，有些成分並沒有進行定量，在定量的部份，花費相當大的工夫及文書作業，以提昇品質保證與品質管制的要求。各確認分析之項目如下：

(I)安非他命類藥物：主要分析的藥物包括安非他命、甲基安非他命等。由於在確認分析的過程，使用氣相層析質譜儀分析的部份大同小異，由於每種藥物之分析之標準化流程所佔之篇幅相當多，本報告僅用安非他命類藥物為代表，其他藥物部份即可舉一反三，依此建立其標準化之流程，該實驗室分析安非他命類藥物之方法如附件五所示，其中包括前言、原理介紹、儀器設備、使用試劑與藥品、標準品的配製、品質管制標準品的配製、前處理流程、儀器分析條件之建立、濃度計算、結果之詮釋與評估、干擾物之評估以及檢驗報告之撰寫等，因此，每一種被分析之藥物種類，均需涵蓋這些過程與步驟，不論是從檢體收件到報告或是從報告追溯至檢體收件，每個步驟均是環環相扣，節節相連，以達成整個檢體之監管程序。

(II)大麻類藥物：主要分析其中 11-nor-9-carboxy-delta-9-THC 成分。

(III)古柯鹼及其代謝物：主要分析其中 cocaine、benzoylecgonine 等，而 benzoylecgonine 為古柯鹼主要的代謝產物。

(IV)鴉片類藥物：主要檢測的項目為嗎啡、可待因、Hydromorphone、

Nalophine、Oxycodone、Hydrocodone...等。

(V)珈瑪 GHB：此類藥物主要分析 gamma hydroxybutyrate 的存在，此種藥物目前在國內也正開始在被濫用。

(VI) BDP：此類藥物主要分析 Benzodiazepines 等化合物。

(VII)阿普挫他(Alprazolam)：此類藥物主要分析 Alprazolam 等化合物。

2.分析儀器方面

該實驗室之分析儀器如下：

A.全自動藥物濃度分析儀：該實驗室之藥物篩選儀為 HITACHI 717，主要用於篩選由各個行政區法醫中心送鑑之尿液、血液或其他較清澈之體液樣品。

B.CO 濃度檢測儀：該實驗室之 CO 濃度檢測儀為 Radiometer OSM3 Hemoximeter，利用此儀器偵測 CO 含量，操作簡單、方便。

C.全自動頂空氣相層析儀：該實驗室之全自動頂空氣相層析儀為 Hewlett-Packard 6890 Series Integrator 配合 Tekmar 7000 Headspace Autosampler，此儀器用於檢測血中揮發性有機物種，包括甲醇、乙醇、異丙醇、丙酮...等，因為是全自動的頂空加熱進樣器，所以可以分析大量樣品，也節省相當的人力，這是器自動化最主要的優點。

D.氣相層析質譜儀 3 部：該實驗室之氣相層析質譜儀均為 Hewlett-Packard 5890 Gas Chromatograph 配合 Hewlett-Packard 5972 Mass Selective Detector 及 Hewlett-Packard 7673 Automatic Liquid Sampler，氣相層析質譜儀主要用於確認送鑑檢體中藥物的成分，該儀器集分離、純化與結構鑑定於一體，且配合自動進樣器的使用，是目前分析實驗室最常用的儀器，而且鑑別率高，為多家實驗室廣泛使用的儀器。

3.萃取方法分類方面

該實驗室為配合氣相層析儀及氣相層析質譜儀的使用，幾乎所有的前處理方式均為固相萃取法，固相萃取法為目前許多藥物分析實驗室常用的前處理流程，操作過程雖不容易，但也不會太難，該實驗室利用 United

Chemical Technologies,INC.出產之固相萃取管柱，以萃取檢體中(大部份為血液及尿液，偶而有膽汁及眼球液)的待測藥物，經過化學衍生後，進行GC/MS 分析，由於該萃取方式囊括了酸性與鹼性藥物之萃取，而且也有不錯的萃取效率，因此該實驗室幾乎不使用液相-液相萃取法進行藥毒物之萃取。

三、研習心得

綜合本次至美國紐澤西州立刑事毒物實驗室及佛羅里達大學病理、免疫學系 Goldberger 教授實驗室參觀、研習及討論後，對於兩間實驗室之人員之陣容、儀器設備之豐富、成員任事負責之態度與工作之精神，有許多是值得國內學習與效法的。對於兩間實驗室而言，有許多相同的部份，也有各自獨具特色的部份，個人參觀及研習之心得，綜合整理如下：

在實驗室領導人方面：紐澤西州立刑事毒物實驗室的主任林礽朗博士在法醫毒物學方面學有專精，從事法醫毒物學的研究已有多年，擔任該實驗室主任將近二十年，以他為華裔的台灣人，要在美國州政府擔任主管職務，若非有相當之能耐，談何容易，林博士待人親切熱誠、笑容可掬，很多事情他多以樂觀開朗的心情去面對，實驗室工作的氣氛和諧，所以該實驗室在其領導下，除了每天解決送鑑之案件外，並也對特殊的案件做有系統的研究，並改良檢測方法，提昇檢測技術。佛羅里達大學病理、免疫學系 Goldberger 教授實驗室的主管 Dr. Goldberger 專長在刑事毒物學，除了主持實驗室實務的檢測分析工作外，同時也是 Journal of Analytical Toxicology 的主編，可說相當忙碌，雖然該實驗室沒有像紐澤西州立刑事毒物實驗室的規模，但是該具備之各項軟硬體設備一應俱全，Dr. Goldberger 是一個有效率的主管，對於實驗室的管理井然有序，本身對於各項檢測的儀器設備及流程，均相當熟悉，所以該實驗室對於特殊案件總是會下一番功夫徹底研究，以確實瞭解該案件之成因，協助法醫以釐清案情或臨床病理的診斷。

在實驗室成員方面：由於紐澤西州立刑事毒物實驗室州政府的單位，所

以它除了主管、分析技術人員外，尚有行政人員，其中有博士學位者一位、碩士學位者三位，其餘的也都是大學畢業，在該實驗室人各所司、個人克盡職責，而且工作氣氛和諧、神情愉快，同事之間相處融洽。佛羅里達大學病理、免疫學系實驗室有兩位博士、一位碩士、兩位學士、一位臨時助理，大部份的工作是由前五位來做，由於實驗室之空間不大，顯得工作環境較擁擠，實驗室的工作負荷相當重，實驗室成員感覺較吃力，因為該實驗室的經費預算是來自於鑑定案件的收費，所以每個成員都很拼命地工作，大部份的案件也都能如期結案。

在實驗室團隊合作方面：紐澤西州立刑事毒物實驗室與佛羅里達大學病理、免疫學系實驗室在這方面是較一致的，亦即在實驗室內，依據檢體的種類性質，以及實驗進行的步驟，大家分工合作，在各個環節都有專門負責的人員進行操作，譬如初步篩檢、萃取流程、儀器分析，或是揮發性藥物檢測等，均有專人負責，然後連貫起來，完成報告，如此在每個環節之間，由於有不同人員進行兩次以上的實做與監管，可以有效地預防錯誤的產生，同時以批次方式進行檢體之分析，整個分析流程可在有效率的機制下完成。

在實驗方法方面：紐澤西州立刑事毒物實驗室所發展之前處理技術是多方面的，包括液相-液相萃取法、固相-液相萃取法兩種、頂空法等，這兩種萃取法是目前前處理技術較常使用的方法，有些檢體適合固相-液相萃取，而且容易自動化，且使用的有機溶劑較少，這是固相-液相萃取主要的優點，但是本法需花費較多的經費購買固相萃取管柱，往往是實驗室經費一筆很大的支出。而液相-液相萃取法雖然使用較多的有機溶劑，不容易進行自動化操作，但是本法可進行一批次多樣品同時進行萃取，花費不高，而且對於類似組織、器官之固態均質化檢體亦容易調整萃取溶劑以進行萃取，因此，該實驗室對於不同種類之檢體，使用不同之萃取方法及不同之儀器分析技術。而所使用的儀器除了氣相層析儀與氣相層析質譜儀外，也兼用高效能液相層析儀，這兩種儀器對於有機物藥物之分析可以說是相互彌補箇中之不足，一則

分析熱穩定、易揮發、分子量較小之藥毒物，另一則分析熱不穩定、沸點較高、分子量較大知藥毒物，二者相輔相成。而佛羅里達大學病理、免疫學系實驗室在前處理方面僅利用固相-液相萃取法、及頂空氣相層析法，因該實驗室大部份接受的檢體為體液樣品(包括尿液、血液、膽汁及眼球液等)，所以適合使用固相-液相萃取法，而在儀器分析方面則以氣相層析儀及氣相層析質譜儀兩種，幾乎沒有使用高效能液相層析儀，主要因為人手較少，同時也是該實驗室並未購置該儀器。

在儀器設備的使用與維護方面：先進精良之儀器是分析的主要利器，要得到準確之分析結果，除了須有精密之儀器外，尚需良好的維護、保養及優良的技術人員方能發揮儀器之功能，兩間實驗在這方面作得相當好，就個人在該兩間實驗室所見，舉一個例子來說，當儀器分析的結果出來時，發現用來定量的檢量線線性不好時，或是所使用的管制標準品超過分析警戒值的上下限時，該執行分析得人員就會先發現問題、初步排除問題，之後再與實驗室管理人討論問題之成因，如果是屬於儀器的問題，則進行檢視與維持，譬如清洗、更換注射埠的內管、清洗注射埠的 gold seal、切除分析管柱前端約十五公分、或是定期清洗離子源區等，如果品質管制標準品還是無法通過時，則進行該批樣品再一次萃取與衍生的前處理流程，務必把問題找出，並解決該問題，絕不籠統含糊，得過且過。

在文件資料處理方面：紐澤西州立刑事毒物實驗室與佛羅里達大學病理、免疫學系實驗室兩間實驗室對於檢體監管流程相當重視，畢竟這是避免分析過程中導入錯誤一項重要的因素，所以兩間實驗室各有各的管制機制，兩間實驗室負責人也很願意將這些文件資料拿出來與個人討論分享，Dr. Goldberger 甚至也將該實驗室之標準操作流程(standard operation procedure,SOP)交付個人影印參考，同時不影響鑑定案件當事人隱私的情形下，Dr. Goldberger 也同意個人參考這些鑑定檔案。兩間實驗室對於這些鑑定案件之資料，都設有專櫃及專人保管，出陽性鑑定書之案件檔案資料，必須

保存之期間，也都有各自的法規規定。

在鑑定案件收費方面：紐澤西州立刑事毒物實驗室之經費全部由州政府支應，但不收費，佛羅里達大學病理、免疫學系實驗室之經費部份由學校編列預算支應，但對送鑑之案件則須收費以供支出，二者各有其不同之背景及其優缺點，目前國內許多檢驗單位或機構，有的是由政府編列預算支應，也有的必需收費，恰巧與個人所參觀之兩個地方的實驗室可以互相觀摩。在民主法治的國家，或許政府機構成立之鑑定實驗室對於解決關於刑事案件有其功效，但是往往受到「僅此一家，別無分號」的影響，使得鑑定的品質無法提昇，甚至由於鑑定案件太多，造成鑑定人員的負擔過於龐大，因此草草鑑定了事，若是如此，則對於當事人之權利影響至深且鉅，每位鑑定人豈可不慎。不管是鑑定案收費與否、亦不論鑑定案件數量的多寡，一個重要的前提就是要能達到鑑定品質的管制與品質保證。能夠多成立幾家鑑定實驗室，相信會是一種良性的競爭。

四、建議

就個人本次參觀、訪問及研習的感想與心得，提出幾項建議，以供國內相關鑑定實驗室參考：

(一)建立刑事鑑識實驗室之認證制度

目前國內的許多家濫用藥物尿液檢驗機構，均已取得行政院衛生署管制藥品管理局的官方認證許可，方可以執行濫用藥物尿液檢驗的業務，由於在認證的過程中，該實驗室除了人員資格、訓練、學經歷、實驗室的空間規畫、儀器、技術、品質管制與保證、文件記錄資料、結果的解釋...等等，均必須依照實驗室所擬定之標準操作流程(SOP)，以確保結果之無誤。而建立認證制度，即是針對所提出之 SOP 循序漸進完成整個分析流程，參與認證，即是可以藉由專家學者的評鑑過程，找出問題、解決問題，並依照 SOP 之精神，建立品質保證與品質管制，使得整個分析流程中，所有可變因子，均可透過品質管制的機制，發掘問題，進而預防問題於機先，達到品質保證的目的。濫

用藥物尿液檢驗的認證制度，在國內算是相當不錯的了，因此，藉著濫用藥物認證制度在國內成功的經驗，可以推廣至其他與刑事鑑識相關鑑定的實務上。或許與刑事案件有關之證物，有部份在實施認證的過程中會有些許的不便，而不易推廣認證，但為了鑑定的品質保證，各個鑑定實驗室亦應建立SOP，如此可為追溯之依據，亦是另一種品質保證的措施。

(二)定期實施績效監測，維持鑑定實驗室水準

不論實驗室是否已經參加認證，抑或是已經具備實驗室內的標準操作手冊，當然，加入認證體系，主辦認證之機構會定期實施實地查核，可以有效地檢驗該實驗室，若無參加認證之實驗室，則沒有第三者的查核，則品質保證不易落實，因此，必須透過績效監測樣品進行實驗室間之比較分析，方能達到目的，一則維持實驗室之水準，另一則做到品質管制與品質保證。

不管是濫用藥物鑑定也好，或是一般刑事鑑識也好，舉凡指紋鑑定、文書鑑定、槍彈及其殘跡鑑識、DNA 鑑定、縱火劑鑑定、玻璃、纖維、毛髮、油漆片鑑定等，建立績效監測樣品進行實驗室間之比對分析，可以互相參考印證，乃是刑事鑑識的一大步。

附件一

Comprehensive Drug Screen

Volatiles

Analysis by headspace gas chromatography: acetone; ethanol; isopropanol; methanol.

Drugs and Drug Metabolites

Analysis by a combination of techniques including spot tests, immunoassay, thin layer chromatography, gas chromatography, and gas chromatography/mass spectrometry.

Antiarrythmics	diltiazem, flecainide, lidocaine, procainamide, propafenone, quinidine, verapamil
Antidepressants	amitriptyline, amoxapine, buproprion, citalopram, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, loxapine, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, protriptyline, sertraline, trazodone, trimipramine, venlafaxine
Antiepileptics	carbamazepine, gabapentin, phenytoin, primidone
Antihistamines	bromodiphenhydramine, brompheniramine, cetirizine, chlorpheniramine, cyproheptadine, diphenhydramine, doxylamine, hydroxyzine, orphenadrine, pheniramine, phenyltoloxamine, promethazine, pyrilamine, tripelennamine
Antipsychotics	chlorpromazine, clozapine, haloperidol, loxapine, olanzapine, quetiapine, trifluoperazine, thioridazine
Barbiturates	amobarbital, butalbital, butabarbital, pentobarbital, phenobarbital, secobarbital
Benzodiazepines	alprazolam, chlordiazepoxide, diazepam, flurazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, triazolam
Cannabinoids	THC-acid (urine only)
Cocaine	benzoylecgonine, cocaine, cocaethylene
Opioids	6-acetylmorphine, codeine, dextromethorphan, dihydrocodeine, fentanyl (by request only), heroin, hydrocodone, hydromorphone, meperidine, methadone, morphine, oxycodone, pentazocine, propoxyphene, tramadol
Sympathomimetics	amphetamine, ephedrine/pseudoephedrine, fenfluramine, methamphetamine, methylenedioxymethamphetamine, methylenedioxyamphetamine, methylenedioxyethylamphetamine, phenylpropanolamine, phentermine
Others	acetaminophen, amantadine, atenolol, atropine, benztropine, buspirone, bupivacaine, caffeine, chloroquine, cotinine, cyclizine, cyclobenzaprine, doxapram, enalapril, ethchlorvynol, fluconazole, gamma-hydroxybutyrate (by request only), glutethimide, ketamine, levorphanol, metaxalone, methaqualone, methyprylon, metoclopramide, metoprolol, nicotine, phencyclidine, procaine, propranolol, quinine, salicylates, strychnine, trimethobenzamide, trimethoprim, zolpidem

rev. 7/16/00

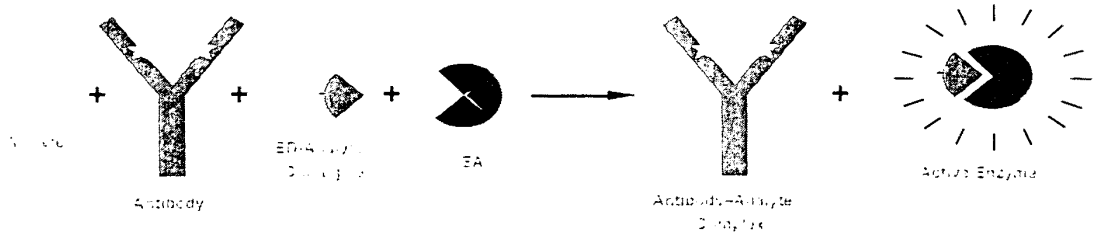
附件二

IMMUNOASSAY SCREENING

Principle of the CEDIA Assay

CEDIA DAU assays are enzyme immunoassays for the qualitative and semiquantitative evaluation of specific compounds in human urine. The assays, which use recombinant DNA technology, are based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically. The drug in the specimen competes with drug conjugated to one inactive fragment of β-galactosidase for antibody binding sites. If drug is present in the specimen, it will bind to antibody, leaving the inactive enzyme fragments free to form active enzyme. If drug is not present in the specimen, antibody will bind to drug conjugated on the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme will be formed. The amount of active enzyme formed and resultant absorbance change are proportional to the amount of drug present in the specimen.

THE ANTIBODY, MAKING ED-ANALYTE CONJUGATE
AVAILABLE FOR ENZYME FORMATION



Overall Summary of Operation

The Hitachi 717 system analyzes for drugs of abuse using an enzyme immunoassay in conjunction with competitive binding immunoassay methodology. Specimen pipetting, measurement and calculations are performed by the Hitachi 717 Analyzer. Specimens are placed in sample rack and assayed with appropriate reagents according to laboratory protocol. The immunologic reaction occurs in reaction cells, and the final enzymatic product is measured spectrophotometrically. Light originating from a tungsten bulb is directed through each cuvette as the reaction disk rotates. Light transmitted by the

reaction mixture is directed to a diffraction grating which separates the light into its constituent wavelengths, and reflects them onto a fixed array of photodiodes. Each photodiode is permanently positioned to detect light at a different wavelength. The computer module processes the data, displays and prints out the concentration or activity for each analyte in the specimen.

SPECIFIC ASSAYS

Amphetamines

Amphetamines and amphetamine' derivatives are sympathomimetic amines with central nervous system stimulant activity. Amphetamine and methamphetamine are classified as Schedule II drugs due to the potential for abuse. The only legitimate medical use for amphetamine is for the treatment of narcolepsy, obesity, and hyperactivity associated with childhood deficit-attention disorders. Amphetamines can be taken orally, injected intravenously, smoked, or snorted. They are psychologically and physiologically addicting; their effects include excitement, alertness, euphoria, loss of appetite, and a reduced sense of fatigue. Side effects at low doses include irritability, anxiety, insomnia, blurred vision, increased blood pressure, and heart palpitations. Chronic, high dose use can lead to psychosis that may be indistinguishable from acute schizophrenia. Amphetamines are rapidly absorbed from the gastrointestinal tract and distributed widely to body tissues. The elimination half-life of amphetamines ranges from 7 to 34 hours and is highly dependent on urine pH. Amphetamines are metabolized to pharmacologically active and inactive metabolites through various metabolic reactions occurring primarily in the liver. Under normal conditions, approximately 30% of a dose is excreted unchanged in the urine in the first 24 hours after administration. Acidification of the urine will enhance the excretion of amphetamines.

The cross-reactivity of the Microgenics CEDIA amphetamines assay is listed below:

Compound	% Cross-Reactivity
d-Amphetamine	101
l-Amphetamine	3.0
d,l-Amphetamine	58
l-Ephedrine	0.4
d-Methamphetamine	100*
d,l-Methamphetamine	65
l-Methamphetamine	12
3,4-Methylenedioxy-amphetamine (MDA)	1.9
3,4-Methylenedioxy-methamphetamine (MDMA)	69
Phentermine	1.9
d,l-Phenylpropanolamine	0.3
d-Pseudoephedrine	0.6

Benzodiazepines

Benzodiazepines are central nervous system depressants utilized for their anxiolytic, sedative-hypnotic, anticonvulsant, and muscle relaxant properties. There are many benzodiazepines which constitute this class of drugs and they are classified as ultra-short, short-, and long-acting. Most benzodiazepines are classified as Schedule IV drugs. Benzodiazepines are commonly given orally or parenterally. Chronic benzodiazepine use may produce physical dependence with withdrawal symptoms of insomnia, agitation, irritability, muscle tension, and in more severe cases, hallucinations, psychosis, and seizures. Benzodiazepines vary greatly, but in general, they are well absorbed from the gastrointestinal tract, distributed widely to body tissues, and, because they are lipid soluble, they readily penetrate the blood-brain barrier and are stored in slow-releasing fatty tissues. Benzodiazepines are extensively metabolized by the liver. Depending on the drug, the resulting metabolites may be active compounds or inactive conjugates. Active metabolites may appear in higher concentrations and have longer half-lives than the parent drug. Half-lives of benzodiazepines range between 5 and 150 hours, whereas half-lives of their active metabolites may be as long as 250 hours.

The cross-reactivity of the Microgenics CEDIA benzodiazepine assay is listed below:

Compound	% Cross-Reactivity	Compound	% Cross-Reactivity
Nitrazepam	100	Halazepam	145
Alprazolam	205	Lorazepam	122
α-OH-Alprazolam	188	Lorazepam glucuronide	1.4
Bromazepam	110	Lormetazepam	165
Chlordiazepoxide	13	Medazepam	135
Clobazam	62	Nordiazepam	211
Clonazepam	140	Oxazepam	107
Clorazepate	84	Oxazepam glucuronide	0.5
Delorazepam	184	Oxaprozin	1.9
Demoxepam	14	Prazepam	184
Desalkyflurazepam	210	Temazepam	144
Diazepam	247	Temazepam glucuronide	1.3
Estazolam	220	Triazolam	191
Flunitrazepam	135	α-OH-Triazolam	193
Flurazepam	189		

Cannabinoids

Marijuana originates from the hemp plant *Cannabis sativa* which grows globally in warmer climates. Drugs of the hemp plant contain at least 61 cannabinoids, of which Δ^9-tetrahydrocannabinol (THC) is the primary psychoactive compound. Having no accepted medical use and high abuse potential, THC is classified as Schedule I drug. Cannabinoids are abused for their euphoriant and relaxation effects. Adverse effects may include tachycardia, respiratory abnormalities, and behavioral and mental impairment. Tolerance and a mild withdrawal syndrome have been observed in chronic users. Principal routes of administration are inhalation (smoking) and oral ingestion.

THC is highly fat soluble, and therefore, readily stored in fatty tissues. It is rapidly metabolized to over 24 metabolites, the primary one being 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid. Approximately 70% of a THC dose is excreted in feces and urine within 72 hours. The concentrations of THC metabolites in urine are influenced by several factors including the amount of THC absorbed in the bloodstream, the frequency of prior use, the timing of urine specimen collection in relation to the last exposure to THC, and the rate of release of stored cannabinoids from fatty tissues.

The cross-reactivity of the Microgenics CEDIA cannabinoid assay is listed below:

Compound	% Cross-Reactivity
11-nor-Δ^9-THC-COOH	100
11-nor-Δ^8-THC-COOH	125
Δ^9-THC	10.4
11-OH-Δ^9-THC	43
8β-OH-Δ^9-THC	2.8
8β,11,di-OH-Δ^9-THC	8.4
1-Δ^9-THC-glucuronide	78
Cannabinol	2.9
Cannabidiol	<0.1

Cocaine Metabolite

Cocaine is a potent central nervous system stimulant used medicinally as a local anesthetic and in surgery of the eye, nose and throat. It is most commonly available as a crystalline water-soluble salt (cocaine hydrochloride), which is sniffed or injected intravenously, or as a cocaine base (commonly called "free base" or "crack"), which is smoked. Cocaine is highly addictive, both psychologically and physically, and is classified as a Scedule II drug. Concomitant use of cocaine with other drugs, particularly sedatives and depressants such as alcohol, is a common occurrence. The vasoconstrictive action of cocaine can lead to myocardial infarcts and strokes while its local anesthetic action can lead to seizure disorders. Cocaine may be associated with withdrawal symptoms including paranoia, insomnia, depression, agitation, and gastrointestinal disturbances. Cocaine is rapidly metabolized, with less than 5% excreted unchanged in the urine. The two major metabolites, which result from enzymatic and nonenzymatic hydrolysis, are benzoylecgonine, accounting for about 45% of cocaine's urine disposition, and ecgonine methyl ester, comprising about 40%, respectively. Cocaine's half-life is approximately 75 minutes.

The cross-reactivity of the Microgenics CEDIA cocaine metabolite assay is listed below:

Compound	% Cross-Reactivity
Benzoylecgonine	100
Cocaethylene	57
Cocaine	54
Ecgonine	1.1
Ecgonine methyl ester	<0.1

Opiates

Opium is obtained from the unripe pods of the opium poppy *Papaver somniferum*. Morphine and codeine are naturally occurring alkaloids of opium. Heroin is synthesized from morphine. Morphine and codeine are classified as Schedule II substances and heroin is classified as a Schedule I substance. Morphine and codeine are analgesics used for the treatment of mild to severe pain. Opiates are abused for their CNS effects including euphoria, sedation, and relaxation. Routes of administration include intravenous, intranasal, oral, and parental. Adverse effects of heroin include hypotension, pulmonary edema, respiratory depression, and coma. Morphine is excreted principally in the urine as conjugated morphine. Codeine is metabolized to morphine and is excreted in urine as free and conjugated forms of codeine and morphine. The plasma half-lives of morphine and codeine are approximately 2-3 hours. Heroin has an extremely short plasma half-life (~5 min) and is rapidly metabolized to 6-acetylmorphine and morphine.

The cross-reactivity of the Microgenics CEDIA opiate assay is listed below:

Compound	% Cross-Reactivity
Morphine	100
Codeine	125
Diacetylmorphine	53
Dihydrocodeine	50
Hydrocodone	48
Hydromorphone	57
Imipramine	1.6
Morphine-3-glucuronide	81
Morphine-6-glucuronide	47
6-Monoacetylmorphine	81
Meperidine	0.2
Oxymorphone	1.9
Oxycodone	3.1

References:

1. Goldberger BA and Jenkins AJ. Testing of abused drugs in urine by immunological techniques. Therapeutic Drug Monitoring and Toxicology In-Service Training and Continuing Education, American Association for Clinical Chemistry 1992; 13(8):5-25.

2. Baselt RC and Cravey RH. Disposition of Toxic Drugs and Chemicals in Man. CTI: Foster City, CA (1995).

3. Liu RH and Goldberger BA. Handbook of Workplace Drug Testing. AACC Press: Washington, DC (1995).

HITACHI 717 CEDIA REAGENTS

A. Reagents

1. Microgenics CEDIA Assay

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.

NOTE 2: The R1 and R2 solutions must be at the reagent compartment storage temperature of the analyzer before performing assays. Solutions which have warmed to room temperature must be cooled to 2-12°C prior to use.

NOTE 3: Avoid cross-contamination of reagents by matching reagent coded stoppers to the proper reagent bottle. The R2 solution should be yellow-orange in color. A dark red or purple-red color indicates that the reagent has been contaminated and must be discarded.

a. EA Reconstitution Buffer: Reagent contains mouse monoclonal antibodies reactive to drug analyte, buffer salts, stabilizer, and preservative.

EA Reagent: Reagent contains Enzyme acceptor (microbial), buffer salts, detergent, bulking agent, and preservative.

R1 Solution: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at room temperature. Mix again. Record the reconstitution and expiration date on the bottle label.

b. ED Reconstitution Buffer: Reagent contains buffer salts and preservative.

ED Reagent: Reagent contains enzyme donor (microbial) conjugated to drug analyte, chlorophenol red-β-D-galactopyranoside, stabilizer, and preservative.

R2 Solution: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at room temperature. Mix again. Record the reconstitution and expiration date on the bottle label.

Storage and Stability of the Microgenics CEDIA Reagents

CEDIA Assay	Stability of R1 and R2 reagents at 2-12°C
Amphetamines	60 days
Benzodiazepines	60 days
Cannabinoids	60 days
Cocaine Metabolite	60 days
Opiates	60 days

B. Calibrators

Microgenics CEDIA calibrators are solutions of human urine containing analytes at concentrations indicated in Appendix 1. Before each use, mix the contents of each bottle by gently inverting several times. After opening, the calibrators are stable at 0-8°C for 60 days or until the expiration date on the bottle label, whichever comes first. Do not freeze.

C. Controls

Negative Urine Control

Negative urine control for use in screening procedures is purchased from Quality Assurance Services (QAS) or prepared by the UFDRL Toxicology Laboratory. No preparation is required. Control material is stored frozen at $\leq$ -20°C until ready for use. Once thawed, the control is stable for 30 days when refrigerated at 0-8°C.

Open Screening Controls

The open screening controls are purchased from Quality Assurance Services and BMC. No preparation is required. The Quality Assurance Services control solution is stored frozen at $\leq$ -20°C until ready for use. Once thawed, the control is stable for 30 days when

refrigerated at 0-8°C. The Microgenics control solution is stored refrigerated at 0-8°C until ready for use. Once opened, the control is stable for 3 months when refrigerated at 0-8°C.

The open control contains the following analytes at the following concentrations*:

Analyte	Manufacturer	Above Cutoff
Methamphetamine	QAS	1250
Benzoylecgonine	QAS	360
Morphine	QAS	360
Oxazepam	QAS	360
THC-acid	BMC	62

RUNNING THE HITACHI ANALYZER

Daily Start-Up

1. Check volume and integrity of reagents, Cell Clean 90 and Hitergent.

2. Complete the daily maintenance.

3. Enter Routine Job Menu, chose Test Selection and clear the data.

Calibration

1. Enter Routine Job menu, then chose Calibrator and Control Test Selection.

2. Chose Start-Up and STD 1. Verify that all drugs to be tested are selected (lit on the keyboard).

3. Press Enter to select S2-S6. Verify that all drugs to be tested are selected (lit on the keyboard).

4. Fill Hitachi specimen cups with the following calibrators and place in the indicated positions:

Position	Calibrator
S-1	Microgenics Negative
S-2	Microgenics Multi-Drug Primary Cut-Off

S-3	Microgenics THC 50
S-4	Microgenics Multi-Drug Secondary Cut-Off

5. Fill Hitachi specimen cups with the following controls and place in indicated position:

Position	Control
C-1	UFDRL/QAS Negative
C-3	QAS Screen Plus
C-5	Microgenics THC High

Program a Batch of Specimens

1. Label Hitachi specimen cups.

2. Transfer an aliquot of urine or vitreous humor into the Hitachi specimen cup

3. Place the Hitachi Specimen cups in appropriate positions.

4. Enter the Routine Job menu, then chose Patient Test Selection.

   - Enter 1, 0 and 1 (this represents sample number, tray number and cup position number, respectively).
   - Enter accession number.
   - Move cursor to test selection. Order requested profile (PM4).
   - Continue with data entry until all accession numbers are entered.

5. Print and Verify Worksheet

   - Move cursor to Worksheet.
   - Enter first sample number, then press enter.
   - Enter last sample number, then press enter.
   - Review worksheet

6. Enter the Routine Job menu, then choose Start Conditions.

   - Enter first sample number, enter.
   - Enter yes for calibration, enter.
   - Enter last sample number for control interval.
   - Move cursor to Wash, choose Air-purge, enter.

7. Verify that no obstructing objects are present and sample and reagent lids are properly positioned.

8. Return to operation monitor screen.

9. Start the Hitachi.

Hitachi 717 Parameters

MICROGENICS CEDIA Assays.

TEMPERATURE: 37 °C

TEST	[*ASSAY NAME*]	
ASSAY CODE	[RATE-A]:[45]-[50]	
SAMPLE VOLUME	[*][*]	*see table below
R1 VOLUME	[130][100][NO]	
R2 VOLUME	[130][100][NO]	
WAVELENGTH	[660][570]	
CALIB. METHOD	[LINEAR][0][0]	
STD. (1) CONC.-POS.	[0]-[**]	**calibrator position
STD. (2) CONC.-POS.	[100]-[**]	
STD. (3) CONC.-POS.	[0]-[0]	
STD. (4) CONC.-POS.	[0]-[0]	
STD. (5) CONC.-POS.	[0]-[0]	
STD. (6) CONC.-POS.	[0]-[0]	
SD LIMIT	[0.1]	
DUPLICATE LIMIT	[200]	
SENSITIVITY LIMIT	[0]	
ABS. LIMIT (INC/DEC)	[32000][INCREASE]	
PROZONE LIMIT	[0][LOWER]	
EXPECTED VALUE	[-50]-[99]	
TECH LIMIT	[-500]-[500]	
INSTRUMENT FACTOR	[1.00]	

Assay	Sample Volume
Amphetamine, Benzodiazepines, Opiates	3 µL
Cannabinoids, Cocaine Metabolite	6 µL

DATA REVIEW

Review of Calibration Data

All MICROGENICS CEDIA assays are calibrated at least once every 24 hours according to the Hitachi 717 Operating Procedures. The assays utilize a two point standard curve. The calibrator information is summarized in Table 1. Immediately following calibration, the Photometric Calibration Monitor is evaluated for precision and shift in absorbance. If a calibration error occurs, a calibration flag will be printed. Complete instructions regarding

operation and troubleshooting are described in the Hitachi 717 Standard Operating Procedures. The following corrective action is necessary:

CALIB flag monitor absorbance values for future shifts; monitor control data

STD & DUPL flag repeat calibration

Review of Control Data

Each batch of specimens is assayed with open quality control samples. In order to accept subject results, results of all quality control data must meet the following criteria:

Control	Acceptable Range (%)
Negative	≤ 25
Above Cutoff	≥ 100

Record the control data in the Toxicology Control Log. If the control is unacceptable, the assay must be recalibrated and specimens retested.

Review of Subject Data

The subject data is reviewed according to the following criteria:

1. Negative Result
   - Amphetamines, Benzodiazepines and Cannabinoids: response < 100%
   - Opiates: response < 75%
   - Cocaine: response < 50%

2. Presumptive Positive Result
   - Amphetamines, Benzodiazepines, Cannabinoids, and Opiates: response $\geq$ 100%
   - Opiates: response $\geq$ 75%
   - Cocaine: response $\geq$ 50%

3. Alarm Flags

   The Hitachi 717 will issue DATA ALARM flags under unusual conditions. DATA ALARM flags appear on the printed reports and on all Screens. Refer to the Hitachi 717 Manual for information regarding alarms.

Appendix 1

Assay	Cutoff Concentration (ng/mL)	Negative	Calibrator	Control
Amphetamines	1000	Microgenics Negative	Microgenics Multi-Drug Primary Cut-Off	UFDRL Negative QAS Above Cutoff
Cannabinoids	50	Microgenics Negative	Microgenics THC Calib	UFDRL Negative Microgenics Above Cutoff
Cocaine Metabolite	300	Microgenics Negative	Microgenics Multi-Drug Primary Cut-Off	UFDRL Negative QAS Above Cutoff
Opiates	300	Microgenics Negative	Microgenics Multi-Drug Secondary Cut-Off	UFDRL Negative QAS Above Cutoff
Benzodiazepines	300	Microgenics Negative	Microgenics Multi-Drug Primary Cut-Off	UFDRL Negative QAS Above Cutoff

附件三

RUG SCREEN BY GC-NPD

Principle of Method

Basic, acidic, and neutral drugs are extracted from a variety of biological fluids and tissues using solid-phase extraction. The acidic and neutral drugs are eluted with hexane:ethyl acetate (1:1) solution, evaporated under nitrogen, and reconstituted in a solution of trimethylanilinium hydroxide (TMAH). The basic drugs are eluted with an 2% ammonium hydroxide in methylene chloride:isopropanol (8:2) solution, evaporated under nitrogen, and reconstituted in methanol. The final extracts are injected into a gas chromatograph equipped with a nitrogen phosphorous detector. All presumptive positive specimens are confirmed by gas chromatography/mass spectrometry operated in the full scan mode.

Specimens

A variety of specimens can be analyzed by this method. The selection of specimens to be assayed by this method is described in the "Specimen Triage" Section of the manual.

All fluids such as blood, urine, bile, and vitreous humor should be vortexed well for 5-10 seconds before aliquoting. Dilutions of stomach contents and tissue homogenates should be prepared as follows:

Note: All dilution factors and specimen amounts should be recorded on the extraction log.

Stomach Content Dilution Preparation

Use Class A volumetric flasks and Eppendorf pipettes to prepare dilutions of stomach contents, *e.g.*, for a 1:10 dilution — 1 mL stomach contents Q.S. to 10 mL with isotonic saline solution.

Tissue Homogenate Preparation

Prepare tissue homogenates as a 1:5 dilution unless otherwise specified in isotonic saline solution.

- Weigh 2.0 g of the specimen in a small clean metal homogenizer.
- Add 8 mL of isotonic saline solution to the specimen and homogenize for 60 seconds.

Other Specimens

Dilutions of all other specimens such as blood and urine should be performed quantitatively using Class A volumetric flasks and Eppendorf pipettes. Specimen dilutions should be in isotonic saline solution.

Equipment

1. Hewlett-Packard 5890 Gas Chromatograph equipped with a Nitrogen Phosphorus Detector
2. Hewlett-Packard Automatic Liquid Sampler
3. Hewlett-Packard Windows-based ChemStation Software
4. Vortex Mixer
5. Zymark TurboVap connected to nitrogen gas
6. Clean Screen Extraction Columns (ZSDAU020), United Chemical Technologies

Reagents

> ***Note:*** Many of these reagents are caustic. Use appropriate personal protective equipment.

1. Acetic Acid, glacial
2. Ammonium Hydroxide, concentrated
3. Ethyl Acetate
4. Hexane
5. Isopropanol
6. Methanol
7. Methylene Chloride
8. Potassium Hydroxide

9. Potassium Phosphate Monobasic

10. Saline, isotonic

11. Trimethylanilinium Hydroxide (TMAH), 0.2 *M* in methanol

12. Water, Millipore

13. 1.0 *M* Acetic Acid

    Add 28.6 mL acetic acid to 400 mL deionized water. Dilute to 500 mL with deionized water. Stable for 1 year at room temperature.

14. Methylene Chloride:Isopropanol:Ammonium Hydroxide Solution (78:20:2; *v/v/v*)

    To 20 mL of isopropanol, add 2 mL of ammonium hydroxide. Add 78 mL of methylene chloride. Mix well. Prepare fresh.

    ***Note:* Methylene chloride is a potential carcinogen. Handle with care.**

15. Hexane:Ethyl Acetate Solution (1:1; *v/v*)

    Mix 250 mL of hexane with 250 mL of ethyl acetate. Stable for 3 months at room temperature.

16. 0.1 *M* Phosphate Buffer, pH 6

    Dissolve 13.61 grams of potassium phosphate monobasic in 900 mL of deionized water. Adjust the pH to 6.0 with 5.0 *M* potassium hydroxide. Q.S. the solution to 1000 mL with deionized water. Stable for 3 months at 0-8°C.

17. 5.0 *M* Potassium Hydroxide

    Dissolve 70.13 grams of potassium hydroxide in 100 mL of deionized water in a 250 mL volumetric flask. Mix well and Q.S. to 250 mL with deionized water. Stable for 6 months at room temperature.

18. Trimethylanilinium Hydroxide Working Solution (0.02 *M*)

    Dilute 1.0 mL of 0.2 *M* TMAH solution to 10 mL with methanol. Stable for 1 year at 0-8°C.

Preparation of Standards

Use Class A Volumetric flasks for preparation of all stock and working standard solutions. The standard stock solutions are prepared using the most readily available form of the drug (analytical purity). Concentration of drug standards are calculated based upon the molecular weight of the free base or acid.

Acidic and Neutral Drugs

A. Barbiturate Stock Standard Solutions (1 mg/mL)

Butalbital – Radian Corporation
Amobarbital – Radian Corporation
Pentobarbital – Radian Corporation
Secobarbital – Radian Corporation
Phenobarbital – Radian Corporation

(Stable for at least 3 years at -10 to 20°C)

B. Barbiturate Working Standard Solution (100 mg/L)

Butalbital	0.1 mL of stock standard solution
Amobarbital	0.1 mL of stock standard solution
Pentobarbital	0.1 mL of stock standard solution
Secobarbital	0.1 mL of stock standard solution
Phenobarbital	0.1 mL of stock standard solution
Methanol, absolute	Q.S. to 10 mL

(Stable for at least 3 years at -10 to 20°C)

C. AED Working Standard Solution (100 mg/L)

Carbamazepine	1 mg
Glutethimide	1 mg
Phenytoin	1 mg
Primidone	1 mg
Methanol, absolute	Q.S. to 10 mL

(Stable for at least 3 years at -10 to 20°C)

C. Hexobarbital Internal Standard Solution - 10 mg/L

Dilute 0.1 mL of the hexobarbital standard solution (1.0 mg/mL) to 10 mL with methanol in a volumetric flask.

(Stable for at least 3 years at -10 to 20°C)

Basic Drugs

	Amount	Concentration (mg/L)	Cf
A. Standard Solution 1			
Meperidine	2 mg	20	0.8
Lidocaine	2 mg	20	0.8
Phencyclidine	2 mg	20	0.8
Methadone Metabolite I	4 mg	40	1.6
Methadone	4 mg	40	1.6
Amitriptyline	5 mg	50	2.0
Nortriptyline	5 mg	50	2.0
Diazepam	10 mg	100	4.0
Nordiazepam	10 mg	100	4.0
Flurazepam	10 mg	100	4.0
Methanol, absolute	Q.S. to 10 mL		

(Stable for at least 3 years at -10 to 20°C)

B. Standard Solution 2			
Ecgonine methyl ester	2 mg	20	0.8
Diphenhydramine	2 mg	20	0.8
Chlorpheniramine	2 mg	20	0.8
Brompheniramine	2 mg	20	0.8
Imipramine	5 mg	50	2.0
Desipramine	5 mg	50	2.0
Codeine	10 mg	100	4.0
Oxycodone	10 mg	100	4.0
Quinine	5 mg	50	2.0
Methanol, absolute	Q.S. to 10 mL		

(Stable for at least 3 years at -10 to 20°C)

C. Standard Solution 3 (A&B)

Solution A:

Amphetamine	0.1 mL of stock standard solution (Radian)
Methamphetamine	0.1 mL of stock standard solution (Radian)
Methanol, absolute	Q.S. to 10 mL

(Stable for at least 3 years at -10 to 20°C)

Solution B:

Doxylamine	2 mg	20	0.8
Dextromethorphan	2 mg	20	0.8
Doxepin	5 mg	50	2.0
Nordoxepin	5 mg	50	2.0
Pentazocine	5 mg	50	2.0
Methanol, absolute	Q.S. to 10 mL		

(Stable for at least 3 years at -10 to 20°C)

D. Basic Drug Screen Internal Standard Solution (100 mg/L)

Mepivicaine	10 mg
Ethyl Morphine	10 mg
Methanol, absolute	Q.S. to 10 mL

(Stable for at least 3 years at -10 to 20°C)

E. Cocaine / Procaine / Cocaethylene Stock Standard Solution (100 mg/L)

Cocaine	1.0 mg
Procaine	1.0 mg
Cocaethylene	1.0 mg
Methanol, absolute	q.s. to 10 mL

(Stable for at least 3 years at -10 to 20°C)

F. Nicotine Stock Standard Solution (670 mg/L)

Nicotine	17.1 µL q.s. to
Methanol, absolute	q.s. to 25 mL

(Stable for at least 3 years at -10 to 20°C)

G. Cotinine Stock Standard Solution (100 mg/L)

Cotinine	2.5 mg q.s. to
Methanol, absolute	q.s. to 25 mL

(Stable for at least 3 years at -10 to 20°C)

H. Caffeine Stock Standard Solution (100 mg/L)

Caffeine	2.5 mg q.s. to
Methanol, absolute	q.s. to 25 mL

(Stable for at least 3 years at -10 to 20°C)

I. Fortified Blank Solution

Nicotine	1.0 mL of stock standard solution
Cotinine	6.0 mL of stock standard solution
Caffeine	6.0 mL of stock standard solution
Methanol, absolute	q.s. to 300 mL

(Stable for at least 3 years at -10 to 20°C)

Procedure

— Specimen Preparation —

1. Set up sufficient culture tubes for:

Tube	Designation	Volume of Standard (μL)
1	Blank (fortified)	
2	Standard 1 (acid/base)	100 μL acid; 40 μL base; 30 μL PPA; 30 μL PE
3	Standard 2 (base)	40 μL
4	Standard 3 (base)	40 μL
5	Cocaine/Procaine Standard	40 μL
6...	Specimens	

2. Add 1.0 mL of the appropriate sample to the corresponding culture tube.

3. Add 20 μL of the base internal standard solution and 100 μL of the acid internal standard solution to all culture tubes.

4. Add 2.0 mL of 0.1 *M* phosphate buffer (pH 6) and vortex well.

5. Centrifuge all tubes at 2000 rpm for 5 minutes.

— SPE Column Conditioning —

6. Place columns into the vacuum manifold, plugging any unused ports.

7. Pass 3.0 mL of methanol through each column.

8. Pass 3.0 mL of water through each column.

9. Pass 1.0 mL of 0.1 *M* phosphate buffer through each column. Do not permit the column to dry.

— Solid-Phase Extraction - *Acidic/Neutral* Drugs —

10. Pour specimen into column. Slowly draw specimen through column (at least 2 minutes) under low vacuum.

11. Pass 3.0 mL of deionized water through each column.

12. Pass 1.0 mL of 1.0 *M* acetic acid through each column.

13. Dry column with full vacuum for 5 minutes.

14. Pass 2.0 mL of hexane though each column.

15. Turn off vacuum. Dry tips. Place labeled disposable culture tubes into column reservoir. Replace vacuum top.

16. Add 3.0 mL of hexane:ethyl acetate solution to each column and collect in disposable culture tubes. Remove disposable culture tubes.

17. Evaporate to dryness at 40°C with a stream of nitrogen.

18. Add 100 µL of TMAH and 100 µL of methanol to each tube and vortex well.

19. Transfer to autosampler vial.

— Solid-Phase Extraction - *Basic Drugs* —

20. Pass 3.0 mL of methanol through each column.

21. Dry column with full vacuum for 5 minutes.

22. Turn off vacuum. Dry tips. Place labeled disposable culture tubes into column reservoir. Replace vacuum top.

23. Add 3.0 mL of Methylene Chloride:Isopropanol:Ammonium Hydroxide solution to each column and collect in disposable culture tubes. Remove disposable culture tubes.

Note: **Methylene chloride is a potential carcinogen. Handle with care.**

24. Evaporate to dryness at 40°C with a stream of nitrogen.

25. Add 100 µL of methanol to each tube and vortex well.

26. Transfer to autosampler vial.

Instrumentation (GC-NPD)

Set-up of the gas chromatograph should include review of the maintenance records and the determination of required preventive maintenance. Routine preventive maintenance may include replacing the septum, liner, and gold seal and clipping of the capillary column. In addition, the voltage to the nitrogen phosphorus detector should be adjusted to obtain a signal of approximately 25.

Set-up of the autosampler should include the exchange of solvent in both autosampler wash bottles with fresh ethyl acetate. If the autosampler waste bottles are filled with solvent, the solvent should be discarded in an appropriate waste bottle. All specimen extracts should be loaded on the autosampler tray in a manner to avoid carryover. This includes analysis of blood prior to analysis of urine or bile. In addition, solvent injections should always follow standard or control injections and injections of specimens with elevated concentrations of analyte.

The capillary column phase used for the drug screen procedure is 5% diphenyl 95% dimethyl polysiloxane. The manufacturer is either Hewlett-Packard. Restek, or J&W.

Note: The GC and Automated Liquid Sampler parameters may be adjusted as needed to meet testing requirements. This includes adjustment of the:

- Injection volume
- Oven Program
- Inlet A Pressure Program

DATA REVIEW AND PEAK ASSIGNMENT

All data is technically reviewed by the Toxicologist performing the analysis, or his/her designee, followed by the Supervising Toxicologist responsible for certification of the

data. This includes a technical review of the raw chromatographic data, as well as a review of the worksheets, for completeness and forensic suitability.

1. Label internal standard peaks on all chromatograms – annotate "IS"

   - Base drug chromatograms - label mepivicaine and ethyl morphine
   - Acid/Neutral drug chromatograms - label hexobarbital

   Note: All internal standards peaks must be clearly present and identifiable. If a peak is missing, the drug screen must be repeated for the specimen in question. If the same result is obtained upon repeat analysis, the specimen may be reported as "Not Suitable for Analysis".

2. Label all peaks present on the fortified blank, standard 1, standard 2, standard 3, and cocaine chromatograms.

3. Review all other chromatograms –

   - Label all identifiable peaks including nicotine, cotinine and nicotine metabolite present on the base chromatograms, and caffeine on the acid/neutral chromatograms.
   - Identify chromatograms that require additional analysis by gas chromatography/mass spectrometry. This should include those chromatograms with peaks of unknown origin. During the review of chromatograms, consultation with another toxicologist to verify peak assignment is desirable. In addition, review of case history including suspected drugs is useful.
   - Review entire batch for potential carry-over of analyte from one specimen extract vial to another. If two consecutive extracts from two *different* cases contain the sample analyte, re-inject the second. Depending on the findings, additional testing including re-extraction may be warranted. Consult with the Laboratory Director for appropriate resolution.

   Note:

   A. The position of all autosampler vials must be verified for correct positioning on the autosampler tray prior to removal of the vials from the trays. The individual performing the check may not be the same person that released the extracts to the autosampler on a given day.

   B. A contaminant is commonly detected in most specimens immediately following the ethyl morphine peak. This contaminant is related to the solid-phase extraction cartridge (tentatively identified as a phthalate). GC/MS confirmation of this peak is not required unless the peak height

exceeds the height of the ethyl morphine peak and/or the peak exhibits an atypical appearance (*e.g.*, presence of a co-eluting peak).

C. Early eluting peaks associated with phenethylamine-related compounds are commonly present in chromatograms of decomposed specimens. GC/MS confirmation of these early eluting peaks is not required.

D. An extract may be re-injected for the following reasons:

- chromatographic overload
- poor chromatography
- possible carryover
- injector error (*e.g.*, missed injection)

When re-injecting the extract, the "miscellaneous data" field should indicate that the data file represents a re-injection. If necessary, extracts may be diluted with reconstitution solvent and/or derivatizing reagent. All initial and re-injection data must be maintained with the data pack.

Confirmation by GC/MS

The identification of peaks of unknown origin is done by gas chromatography/mass spectrometry. The following procedure should be followed:

1. Analyze applicable extract by GC/MS.

   Note: It is not necessary to inject extracts of blood *and* urine if similar peaks are present in both specimen extracts. If the peaks are present in both specimen extracts, the preferred extract for confirmation is blood.

2. Print total ion chromatogram.

3. The spectrum of the peak is retrieved and printed.

4. A library search of the spectrum is performed using one of the following mass spectral databases:

   - UF Toxicology Laboratory Mass Spectral Database - *UF_PMTOX.L*
   - American Academy of Forensic Sciences Toxicology Section Mass Spectral Database - *AAFSLIB.L*
   - Pfleger, Maurer & Weber Mass Spectral Database - *PMW_TOX2.L*
   - National Bureau of Standards 75 K Mass Spectral Database - *NBS75K.L*

Note: The preferred database is the UF Toxicology Laboratory Mass Spectral Database. A listing of the entries in the *UF_PMTOX.L* can be found in the Appendix. If the analyte is not present in the UF database, it is acceptable to use the American Academy of Forensic Sciences Toxicology Section Mass Spectral Database and/or the Pfleger, Maurer & Weber Mass Spectral Database.

5. The best spectral match based upon the following mass spectral characteristics is identified:

- Mass spectral fragmentation pattern including relative abundance of each ion
- Presence of base ion
- Presence of molecular ion
- Presence of characteristic and/or unique ions

In addition, a distinguishing retention time may be used to differentiate drugs with similar mass spectra.

Note: Although the computer-based match quality value for most drugs and drug metabolites is usually greater than 75-80%, the match quality value of some may be less. The lower percentages may be a result of a number of factors including:

- "simple" mass spectrum with abundant, low molecular weight base peak lacking characteristic and/or unique ions
- the concentration of analyte in the specimen extract
- the presence of co-eluting analytes
- GC column bleed

The library match for the unknown must contain at least 5 or more diagnostic ions when the match quality is less than 50%. If fewer than 5 diagnostic ion fragments are present, match criteria will be at the discretion of the Laboratory Director in light of all data derived from the case including other matrices.

A few commonly detected drugs known to produce poor match quality values are:

- Lidocaine
- Amitriptyline
- Ephedrine/Pseudoephedrine
- Phenylpropanolamine

6. Upon identification of an analyte by GC/MS, the GC retention time of the analyte must be verified. This can be done by comparison of the analyte with an analyte present in one of the standards extracted in the batch or an unextracted standard (assayed separate from batch). The retention time of a suspected

analyte in a specimen must be within ± 0.05 minutes (corrected to retention time of internal standard if necessary) of the retention time of the analyte in the standard.

Final Assignment of Analytes

The final assignment of analytes requires appropriate matching of retention time and mass spectrum. In addition, the presence of unique metabolites and the reported case history, which may include a tabulation of suspected drugs, may assist in the assignment of analytes.

1. To complete the task of peak assignment, label all known peaks on the chromatogram. This should include:

   - Peak(s) of known origin - annotate name of analyte
   - Peak(s) of unknown identity and/or origin - annotate "∅ MS"
   - Peaks attributed to decomposition products - annotate "decomp"

2. Transfer the results of the drug screen onto the Toxicology Laboratory Worksheet.

3. Metabolites, identified by GC-NP and GC/MS, when present *in conjunction with* their parent compound or additional metabolites, may be reported *qualitatively* without the need for a calibrator or standard to be available. This option may only be exercised by the Laboratory Director, when appropriate, and may cross specimens for a given case.

   Also, there are some metabolites which are frequently seen in the absence of a parent compound for which standards are not available. Despite this, identification of the metabolite may be important to a case. Under these circumstances, the drug may be reported provided an acceptable GC/MS library match is obtained and the drug is not inconsistent with case history. The laboratory report must indicate that the identification is "tentative".

4. Some compounds may be identified through GC/MS library matches for which a standard is not readily available. The(se) compound(s) may be reported at the discretion of the Laboratory Director. The laboratory report must indicate that the identification is "tentative".

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附件四

VOLATILES BY GAS CHROMATOGRAPHY

Introduction

Alcohol, also commonly referred to as ethyl alcohol and ethanol, is a low molecular weight organic molecule present in alcoholic beverages. Alcohol is readily absorbed by the stomach and gastrointestinal tract within 30 to 60 minutes following oral ingestion. The presence of food in the stomach will delay absorption of alcohol. Upon absorption, alcohol is rapidly distributed throughout the tissues according to water content. Alcohol is a central nervous system depressant that affects all sensory-motor functions. The most prominent and deleterious effects of alcohol use include the deterioration of judgment and self-control, loss of memory, muscle incoordination, and impairment of vision and hearing. Alcohol is principally metabolized in the liver by the enzyme alcohol dehydrogenase to acetaldehyde, followed by metabolism to acetic acid. Acetic acid is converted to carbon dioxide and water. The elimination rate of ethanol in adults is approximately 15–20 mg/dL/h. About 5–10% of the ethanol dose is eliminated unchanged from the body through excretion in the expired breath, sweat, feces, and saliva.

Principle

Biological fluids are diluted with an aqueous solution of n-propanol (used as an internal standard), and placed into a sealed 22 mL vial suitable for headspace analysis. The samples along with appropriate standards and controls are incubated at an elevated temperature allowing volatiles to diffuse out of the liquid layer as vapors into the "headspace" within the sealed vial. Under consistent conditions of temperature, pressure and equilibration time, the vapor phase in each of these vials is sequentially sampled and separated on a suitable gas chromatographic column. The volatile components thus resolved (e.g., ethanol, methanol, isopropanol and acetone) are detected using a flame ionization detector, identified by their characteristic retention times (relative to the internal standard, n-propanol) and quantitated by comparing peak area ratios for unknown concentrations against similar ratios obtained from a known calibrator solution.

The technique minimizes sample handling and eliminates the need for extraction or distillation. Further, it eliminates any solvent front and minimizes column overload or contamination with high boiling or non-volatile materials. Since sampled compounds are already in the vapor phase, better separations are generally achieved than those produced by the more conventional techniques of direct injection gas chromatography.

Specimen Requirements

Blood, urine, and vitreous humor are typically used for analysis. Other samples that can be evaluated for the presence of volatiles include stomach contents, bile, tissue such as liver, kidney, brain and lung among others.

The majority of postmortem blood specimens are stored in containers containing sodium fluoride as a preservative. All other specimens are stored in appropriate containers with sealable tops.

Equipment

1. Hamilton Microlab 500 Series Dilutor-Dispenser equipped with a 2.5 mL reagent/diluent syringe and a 250 µL sample syringe

2. Hewlett-Packard Model 5890 Gas Chromatograph equipped with a Flame Ionization Detector and a 6 foot x 1/8 inch column packed with 60/80 Carbopack B/5% Carbowax® 20M

3. Hewlett-Packard 6890 Series Integrator

4. Tekmar 7000 Headspace Autosampler

5. 22 mL Headspace Vials with gray butyl rubber seals and crimp tops

6. Hand-operated crimper

Reagents

1. Acetaldehyde

2. Acetone
3. Ethanol

4. Methanol

5. 1-Propanol

6. 2-Propanol (isopropanol)

7. Sodium Chloride

8. Water, Millipore

9. Sodium Chloride, 10% Aqueous Solution

Dissolve 200 grams of sodium chloride in 1800 mL of water in a 2 L volumetric flask. Mix well and Q.S. to 2 L with water. Stable for 1 month at room temperature.

Standards

1. 1-Propanol Internal Standard Solution (IS) - 25 mg/dL

   Add 622 µL 1-propanol to a 2 L volumetric flask. Q.S. to 2 L with 10% aqueous sodium chloride solution. Stable for 1 month at room temperature.

2. Ethanol Standard Reference Material – National Institute of Standards & Technology

   SRM 1828a consists of 5 sealed ampoules containing solutions of ethanol-water azeotrope and ethanol in water ranging from 95.60% mass fraction to 0.02309% mass fraction. Store at room temperature. Once opened, the calibrators are stable for 60 days at 0-8°C.

3. Ethanol Calibrators - Sigma

Calibrator	Concentration (mg/dL)
1	50
2	100
3	300

   Once opened, the calibrators are stable for 60 days at 0-8°C.

4. Volatile Standard Solutions (Methanol, Acetone, and Isopropanol) in water

Level	Methanol Concentration (mg/dL)	Amount of Methanol added (µL/mL)	Acetone Concentration (mg/dL)	Amount of Acetone added (µL/mL)	Isopropanol Concentration (mg/dL)	Amount of Isopropanol added (µL/mL)
1	50	31.6 in 50	25	15.9 in 50	50	32.0 in 50
2	100	31.6 in 25	50	15.9 in 25	100	32.0 in 25
3	200	25.3 in 10	100	12.7 in 10	200	25.6 in 10

   The solutions are stable for 6 months at 0-8°C.

5. Acetaldehyde Standard (qualitative)

   Dispense 3 drops of acetaldehyde into 100 mL of water. The standard is stable for 6 months at 0-8°C.

Note: The acetaldehyde standard may require titration with water to obtain a solution of acceptable concentration.

Controls

1. Whole Blood Control (Negative) – blood from Civitan blood bank

2. Sigma Ethanol Control (Low)

   Target Concentration – 71 mg/dL

   Ethanol in human serum base with sodium azide preservative. Once opened, the control is stable for 60 days when refrigerated at 0-8°C.

3. CIBA-Corning Whole Blood Tox 1 and 2 Control

Target Concentration (level 2):	Acetone	~37 mg/dL
	Ethanol	~174 mg/dL
	Methanol	~130 mg/dL
	Isopropanol	~174 mg/dL

   Human blood with bacteriostatic agents added with constituents added to reported concentrations. Once opened, the control is stable for 30 days when refrigerated at 0-8°C.

Procedure - Ethanol Only

Procedure

1. Set-up the GC

   A. Turn on the air, hydrogen, and nitrogen.

   B. Turn the air and hydrogen on with the valves on the front of the GC and press the ignite button. Verify that the flame is lit.

   C. Set the GC parameters as follows:

   - Range 1.0
   - Initial Temp 80°C
   - Ramp Rate 32°C/min
   - Final Time 2.5 min
   - Att 0
   - Initial Time 1.0 min
   - Final Temp 120°C
   - Detector Temp 250°C
   - Injector Temp 200°C

2. Verify the integrator settings. Usual settings are:

- Att 5
- Zero 5
- Chart Speed 1.0
- Stop Time 4.75 min
- Peak Width 0.04
- Threshold 4
- Area Reject 0

To program a parameter, press the button for the parameter to be set, then enter the desired setting, followed by <enter>.

3. Set the Tekmar Autosampler parameters as follows:

- Platen Temperature – 60 °C
- Platen Equilibration Time – 0 min
- Sample Equilibration Time – 14 min
- Vial Size – 22 mL
- Mixer – Off
- Mix – Off
- Mixer Power – No
- Stabilize – No
- Pressurization Time – 0.40 min
- Pressure Equilibration Time – 0.25 min
- Loop – 0.25 min
- Loop Equilibration Time – 0.10 min
- Inject Time – 0.50 min
- Sample Loop Temperature – 120 °C
- Line Temperature – 120 °C
- Injections per Vial – 1
- GC Cycle Time – 5 min
- Transfer Line Back Pressure – 16 psi
- Vial Pressurization Setting – 10 psi

4. Set the Hamilton Microlab 500 Series Dilutor-Dispenser parameters as follows:

- Reagent/Diluent Syringe Volume – 1.0 mL
- Sample Syringe Volume – 250 µL

5. Sample Preparation

A. All calibrators, control samples and subject specimens are prepared in the same manner for analysis. The batch should include:

- three ethanol calibrators of known concentration
- one negative control sample

- one positive control sample for every ten subject specimens
- volatile standard containing methanol, acetone and isopropanol
- acetaldehyde standard
- air blank

B. Label sufficient headspace vials for the calibrators, control samples, and subject specimens.

C. Initially all subject specimens are analyzed in singlicate.

D. Record the UFDRL accession number, subject's name (first initial and last name), and source of specimen (if provided) on the GC Volatile Analysis Log.

E. If appropriate –

- Indicate the aliquot number by placing the digit in parentheses (*e.g.*, (2) designates the second aliquot) in the specimen column on the GC Volatile Analysis Log.

- For hospital and legal blood specimens, indicate date and time of specimen draw (if provided).

- Indicate a dilution of the specimen by placing the dilution factor (*e.g.*, x2 designates a 1:1 dilution) in the specimen column on the GC Volatile Analysis Log.

- If the specimen volume is not adequate to perform a test, indicate by writing "QNS" in the result column on the GC Volatile Analysis Log.

F. Prime the dilutor-dispenser at least 5 times prior to the first sampling step by depressing the toggle switch.

G. Aspirate 250 µL of calibrator, control, or specimen by depressing the toggle switch on the dilutor-dispenser. Wipe off any excess liquid with a clean Kimwipe and depress the toggle switch on the dilutor-dispenser again to dispense the 250 µL aliquot just aspirated, along with 1.0 mL of n-propanol internal standard solution into a pre-labeled headspace sample vial. Immediately cap the vial with a gray butyl rubber septum.

Note:

- Tissue samples are prepared by placing 0.25 g of the tissue sample into a headspace vial and adding 1.0 mL of n-propanol internal standard solution.

- The negative control is prepared by aspirating 250 μL of whole blood (blood bank) and dispensing with 1.0 mL of the working n-propanol internal standard solution.

- The air blank is an empty sealed vial.

J. Clear the dilutor-dispenser at least once between each sampling cycle (for particularly thick samples, additional clearing of the sampling probe may be necessary until the tip is clear).

6. Calibrate the GC

A. Program the Tekmar Autosampler to make a single injection of the Sigma Ethanol-100 mg/dL standard.

- Observe the chromatogram as it's printed to verify that all parameters set into the integrator are correct.

- Press DEL CALIB <enter>.

- Press PREP CALIB <enter>.

- Enter I for internal standard

- Answer the prompts:

  - REF % RTW: 5 <enter>.
  - NON-REF % RTW: 5 <enter>.
  - CAL# 1: Enter the retention time of the ethanol peak, the concentration of the ethanol standard (.100), and type "ETHANOL" <enter>.
  - CAL# 2: Enter the retention time of the internal standard peak, the concentration (.100), and type "I.S." <enter>.
  - CAL# 3: Press <enter> to end requests for peaks.
  - REF PK CAL#: <enter>.
  - ISTD CAL#: 2 <enter>.
  - GROUP PEAKS: N <enter>.
  - RF of uncalibrated peaks: 0.0000E+00 <enter>.
  - Replace calibration fit: N <enter>.

- Disable post-run RT update: N <enter>.
- ISTD AMT: 1.0000E-01 <enter>.
- SAMPLE AMT: 0.1 <enter>.
- MUL FACTOR: 1.0000E00 <enter>.

List Calibration: LIST CALIB <enter>.

B. Edit the sequence on the Tekmar Autosampler to reinject the Sigma Ethanol-100 mg/dL standard. Average the calibration factor by pressing CALIB 1 <enter>.

C. Verify the calibration factor by injecting headspace from the 50 mg/dL, 100 mg/dL, and 300 mg/dL calibration vials. The measured concentration should be within ± 10% of the target concentration.

D. Edit the sequence on the Tekmar Autosampler to inject the samples.

E. All blood, vitreous humor, and tissue specimens with an ethanol result greater than 10 mg/dL are assayed in duplicate.

Procedure - Methanol, Acetone and Isopropanol

1. Set-up the GC

C. Turn on the air, hydrogen, and nitrogen.

D. Turn the air and hydrogen on with the valves on the front of the GC and press the ignite button. Verify that the flame is lit.

C. Set the GC parameters as follows:

- Range 1.0
- Initial Temp 80°C
- Ramp Rate 32°C/min
- Final Time 2.5 min
- Att 0
- Initial Time 1.0 min
- Final Temp 120°C
- Detector Temp 250°C
- Injector Temp 200°C

2. Verify the integrator settings. Usual settings are:

- Att 5
- Zero 5
- Chart Speed 1.0
- Stop Time 4.75 min
- Peak Width 0.04
- Threshold 4
- Area Reject 0

To program a parameter, press the button for the parameter to be set, then enter the desired setting, followed by <enter>.

3. Set the Tekmar Autosampler parameters as follows:

- Platen Temperature – 60 °C
- Platen Equilibration Time – 0 min
- Sample Equilibration Time – 14 min
- Vial Size – 22 mL
- Mixer – Off
- Mix – No
- Mixer Power – No
- Stabilize – No
- Pressurization Time – 0.40 min
- Pressure Equilibration Time – 0.25 min
- Loop – 0.25 min
- Loop Equilibration Time – 0.10 min
- Inject Time – 0.50 min
- Sample Loop Temperature – 120 °C
- Line Temperature – 120 °C
- Injections per Vial – 1
- GC Cycle Time – 5 min
- Transfer Line Back Pressure – 16 psi
- Vial Pressurization Setting – 10 psi

4. Set the Hamilton Microlab 500 Series Dilutor-Dispenser parameters as follows:

- Reagent/Diluent Syringe Volume – 1.0 mL
- Sample Syringe Volume – 250 μL

5. Sample Preparation

A. All calibrators, control samples and subject specimens are prepared in the same manner for analysis. The batch should include:

- ➢ three volatile calibrators of known concentration
- ➢ one negative control sample
- ➢ one positive control sample for every ten subject specimens
- ➢ air blank

B. Label sufficient headspace vials for the calibrators, control samples, and subject specimens.

C. All specimens are analyzed in duplicate.

D. Record the UFDRL accession number and source of specimen (if provided) on the GC Volatile Analysis Log.

E. If appropriate –

- Indicate the aliquot number by placing the digit in parentheses (*e.g.*, (2) designates the second aliquot) in the specimen column on the GC Volatile Analysis Log.
- Indicate a dilution of the specimen by placing the dilution factor (*e.g.*, x2 designates a 1:1 dilution) in the specimen column on the GC Volatile Analysis Log.
- If the specimen volume is not adequate to perform a test, indicate by writing "QNS" in the result column on the GC Volatile Analysis Log.

F. Prime the dilutor-dispenser at least 5 times prior to the first sampling step by depressing the toggle switch.

G. Aspirate 250 µL of calibrator, control, or specimen by depressing the toggle switch on the dilutor-dispenser. Wipe off any excess liquid with a clean Kimwipe and depress the toggle switch on the dilutor-dispenser again to dispense the 250 µL aliquot just aspirated, along with 1.0 mL of n-propanol internal standard solution into a pre-labeled headspace sample vial. Immediately cap the vial with a gray butyl rubber septum.

Note:

- Tissue samples are prepared by placing 0.25 g of the tissue sample into a headspace vial and adding 1.0 mL of n-propanol internal standard solution.
- The negative control is prepared by aspirating 250 µL of whole blood (blood bank) and dispensing with 1.0 mL of the working n-propanol internal standard solution.
- The air blank is an empty sealed vial.

K. Clear the dilutor-dispenser at least once between each sampling cycle (for particularly thick samples, additional clearing of the sampling probe may be necessary until the tip is clear).
Note:

It is not necessary to clear the dilutor-dispenser between replicates of the same specimen, calibrator, or control sample.

5. Calibrate the GC

A. Dispense an aliquot of the volatile standard solution #2 into an autosampler vial using the diluter-dispenser. Cap tightly with a crimp-top seal and place in the Tekmar Autosampler. Program the sequence on the Tekmar Autosampler to make a single injection of the volatile standard solution #2 standard.

B. Observe the plot as it comes off the integrator to verify that all parameters set into the integrator are correct.

C. Press DEL CALIB ENTER.

D. Press PREP CALIB ENTER.

E. Enter I for internal standard.

F. Answer the prompts:

- REF % RTW: 5 <enter>.
- NON-REF % RTW: 5 <enter>.
- CAL# 1: Enter the retention time of the methanol peak, the concentration of the methanol standard (.10), and type "METHANOL" <enter>.
- CAL# 2: Enter the retention time of the acetone peak, the concentration of the acetone standard (.05), and type "ACETONE" <enter>.
- CAL# 3: Enter the retention time of the isopropanol peak, the concentration of the isopropanol standard (.10), and type "ISOPROPANOL" <enter>.
- CAL# 4: Enter the retention time of the internal standard peak, the concentration (.100), and type "I.S." <enter>.
- CAL# 5: Press <enter> to end requests for peaks.
- REF PK CAL#: <enter>.
- ISTD CAL#: 4 <enter>.
- GROUP PEAKS: N <enter>.
- RF of uncalibrated peaks: 0.0000E+00 <enter>.
- Replace calibration fit: N <enter>.
- Disable post-run RT update: N <enter>.
- ISTD AMT: 1.0000E-01 <enter>.
- SAMPLE AMT: 0.1 <enter>.

- MUL FACTOR: 1.0000E00 <enter>.

  List Calibration: LIST CALIB <enter>.

F. Edit the sequence on the Tekmar Autosampler to inject the standards.

- Inject the headspace of the volatile standard solution #2 vial again and average the calibration factor by pressing CALIB 1<enter>.
- Verify the calibration factor by injecting headspace from the volatile standard solution #1, volatile standard solution #2, and volatile standard solution #3 vials. The measured concentration should be within ± 10% of the target concentration.

G. Edit the sequence on the Tekmar Autosampler to inject the samples.

Data Analysis

1. Label the chromatograms with the appropriate accession number and specimen type. Also indicate the aliquot number and dilution factor if appropriate. All sequentially numbers pages are retained.

2. If the specimen is 'unfit for testing', indicate by writing "UFT" in the result column on the GC Volatile Analysis Log.

3. The integrator will quantitate the volatile concentration of the unknown using the calibration factor. At the end of the run, a report will print the retention time of the volatile and IS peaks and their concentrations. Record the concentration on the GC Volatile Analysis Log.

Calibration

Results are based upon single-point standardization. Acceptability of the calibration is based on the results of all three standards and quality control samples analyzed throughout the batch. For subject specimen results to be accepted, they must be bracketed by two acceptable positive control samples.

QC Review

Results of all quality control samples must be within ± 10% of the target concentration. All samples that bracket a failed control sample must be realiquoted and repeated with new control material. The samples may be realiquoted and repeated (with fresh control material) on the same analytical run or they may be rescheduled for the next analytical run. Samples that are bracketed by acceptable controls within the same run may be reported.

Reporting

1. Quantitative results are reported for all specimens.

2. In order to report a quantitative result for ethanol, methanol, isopropanol or acetone, a specimen must meet all of the following criteria. Failure to meet one of the criteria requires that the specimen be either reanalyzed or reported as negative. In some cases it may be necessary for a supervisor to designate the result as unsuitable for analysis.

   - Results of all calibration and quality control material must be acceptable.
   - The retention time of the unknown must be within 2% of the retention time of the calibrator.
   - The height of the acetone peak must be ≥ 0.5 cm.
   - Reproducibility between replicate analyses must be within ±10%. Results of replicate analyses are averaged and truncated.

   Notes:

   - If the concentration of ethanol in the blood is negative and the concentration of ethanol in the urine is ≥ 50 mg/dL, the blood and urine specimens should be repeated.
   - If specimen quantity is insufficient for duplicate analysis, a singlicate result may be reported.
   - If duplicate results are not within ±10%, the sample must be repeated.
   - If, after repeat analysis the results are not within ±10%, the specimen is tested on the next batch.
   - At this point, if they are still not within ±10%, all four values may be averaged if they are within 10% of one another. If not, the specimen may be repeated again, or reported as a qualitative positive only.

3. Chromatographic peaks shapes should be Gaussian and fully resolved from other peaks.

4. The average of the duplicate quantitative value must be at or above the cutoff and at or below the upper limit of linearity (see linearity). Specimens exceeding the upper limit of linearity are diluted and reanalyzed in duplicate.

5. Report any value ≥ 10 mg/dL as "positive".

6. Report any value < 10 mg/dL as "negative".

Additional Information

Chromatographic Elution Order: Methanol, Acetone, Ethanol, Isopropanol, 1-Propanol

Linearity: Ethanol – 10 mg/dL to 400 mg/dL
Methanol and Isopropanol – 10 mg/dL to 200 mg/dL
Acetone – 10 mg/dL to 100 mg/dL

Limit of Quantitation: 10 mg/dL

Specificity:

There are no known interferences to this method. However, it is possible that a volatile substance with a longer retention time than this assay's run time (e.g., toluene) might appear on a successive chromatogram. This type of peak can be recognized since it is unusually broad. In this case it may be necessary to reinject the sample in question if an interference is noted. Unless specifically indicated by case history, the analysis of volatiles other than the alcohols above will not be performed.

Acetaldehyde, used in the manufacture of a number of synthetic chemical products, is also a metabolite of ethanol and paraldehyde. In chronic alcoholics, acetaldehyde concentrations are generally less than 0.025 g%, but may be up to 10 times higher than this if the patient has been taking an inhibitor of acetaldehyde, such as disulfiram. Acetaldehyde does not interfere with the volatile assay. It is expected to elute prior to methanol.

The assay may be used for the identification of additional volatile components, such as benzene and toluene when operating the GC oven at a higher temperature (for example: 110°C) or by extending the run time.

Procedure to limit carry-over:

All specimens except urine are run in duplicate. The SOP requires that sample replicates differing by greater than ±10%, be repeated. This procedure ensures that any variability due to carry-over or sampling errors is resolved before reporting a result.

In addition, since no aqueous specimen is used and the transfer line is purged by a stream of air, the potential for carry-over is further minimized.

References

1. Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed. New York: McGraw-Hill, 1996.

2. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J, eds: Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, 2nd ed. Baltimore: Williams and Wilkins, 1997.

3. Goldberger BA, Watts VW and Simonick TF: Analysis of volatiles by headspace gas chromatography. Therapeutic Drug Monitoring and Toxicology In-Service Training and Continuing Education Program, American Association for Clinical Chemistry 15: 231-236, 1994.

4. NCCLS: Blood Alcohol Testing in the Clinical Laboratory; Approved Guideline (T/DM6-A), September 1997.

CONFIRMATION OF ACETONE POSITIVE SPECIMENS

Principle

Acetest Reagent Tablets are composed of several ingredients, and are primarily used to test for the presence of ketones (acetoacetic acid and acetone) in urine. Serum, plasma, and vitreous humor may also be tested with ACETEST Reagent Tablets for the presence of ketones.

The presence of ketone bodies is important in the evaluation of carbohydrate metabolism. The test is based on the nitroprusside reaction with ketone bodies to give a purple color.

Procedure

1. Remove tablet from bottle and recap promptly. Place tablet on clean, dry, white paper.
2. Put one drop of urine, serum, or plasma directly on top of tablet.
3. For urine testing – compare color of tablet to Color Chart at thirty seconds after application.
4. For serum, plasma or vitreous humor testing – compare color of tablet to Color Chart at two minutes after application.

Note: When a drop of urine is put on a tablet, the drop should be absorbed within 30 seconds. If absorption takes longer tan 30 seconds, the tablets have been exposed to moisture and may not produce acceptable results.

Results

Results with Acetest Reagent Tablets are recorded as negative if no purple color is apparent on the tablet at the appropriate reading time. Disregard any pink, tan or yellow color.

Assay Characteristics

Acetest Reagent Tablets will detect as little as 5 mg of acetoacetic acid/dL in urine. Acetest Reagent Tablets are specific for the detection of acetoacetic acid and acetone. Acetest Reagent Tablets are about 10 times more sensitive to acetoacetic acid than acetone and will not react with betahydroxybutyric acid. In urine, the small color block corresponds to approximately 20 mg acetoacetic acid/dL, the moderate color block to 30-40 mg/dL, and the large color block to approximately 80-100 mg/dL. The lower limit of detection in serum, plasma or whole blood is approximately 10 mg acetoacetic acid per dL.

Reference

Acetest® Reagent Tablets Package Insert, Bayer Corporation, Elkhart, IL (9/95).

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AMPHETAMINES BY GC/MS

Introduction

Amphetamines and amphetamine derivatives are sympathomimetic amines with central nervous system stimulant activity. Amphetamine and methamphetamine are classified as Schedule II drugs due to the potential for abuse. The only legitimate medical use for amphetamine is for the treatment of narcolepsy, obesity, and hyperactivity associated with childhood attention- deficit disorders. Amphetamines can be taken orally, injected intravenously, smoked, or snorted. They are psychologically and physiologically addicting; their effects include excitement, alertness, euphoria, loss of appetite, and a reduced sense of fatigue. Side effects at low doses include irritability, anxiety, insomnia, blurred vision, increased blood pressure, and heart palpitations. Chronic, high dose use can lead to psychosis that may be indistinguishable from acute schizophrenia. Amphetamines are rapidly absorbed from the gastrointestinal tract and distributed widely to body tissues. The elimination half-life of amphetamines ranges from 7 to 34 hours and is highly dependent on urine pH. Amphetamines are metabolized to pharmacologically active and inactive metabolites through various metabolic reactions occurring primarily in the liver. Under normal conditions, approximately 30% of a dose is excreted unchanged in the urine in the first 24 hours after administration. Acidification of the urine will enhance the excretion of amphetamines.

Principle of Method

The method utilizes gas chromatography/mass spectrometry for the analysis of amphetamine, phentermine, methamphetamine, pseudoephedrine, methylenedioxy-amphetamine and methylenedioxymethamphetamine in urine. Specimens are subjected to solid-phase extraction, derivatization with PFPA, evaporation, followed by reconstitution in ethyl acetate.

Equipment

1. Hewlett-Packard 5890 Gas Chromatograph

2. Hewlett-Packard 5972 Mass Selective Detector
3. Hewlett-Packard 7673 Automatic Liquid Sampler

4. Hewlett-Packard ChemStation with DrugQuant Software

5. Heat Block

6. Vortex Mixer

7. Centrifuge

8. Zymark TurboVap connected to nitrogen gas

9. Clean Screen Extraction Columns (ZSDAU020), United Chemical Technologies

Reagents

> ***Note:*** Many of these reagents are caustic. Use appropriate personal protective equipment.

1. Ammonium Hydroxide, concentrated

2. Glacial Acetic Acid

3. Ethyl Acetate

4. Water, Millipore

5. Hydrochloric Acid

6. Isopropanol

7. Methanol

8. Methylene Chloride

9. Potassium Hydroxide
10. Potassium Phosphate Monobasic

11. Methylene Chloride:Isopropanol:Ammonium Hydroxide Solution (78:20:2; *v/v/v*)

    To 20 mL of isopropanol, add 2 mL of ammonium hydroxide. Add 78 mL of methylene chloride. Mix well. Prepare fresh.

> ***Note:*** **Methylene chloride is a potential carcinogen. Handle with care.**

12. 1.0 *M* Acetic Acid

Dissolve 57.5 mL of glacial acetic acid in 500 mL of water in a 1000 mL volumetric flask. Mix well and Q.S. to 1000 mL with water. Stable for 1 year at room temperature.

13. 0.1 *M* Phosphate Buffer, pH 6

Dissolve 13.6 grams of potassium phosphate monobasic in 900 mL of water. Adjust the pH to 6.0 with 5.0 *M* potassium hydroxide. Q.S. the solution to 1000 mL with water. Stable for 3 months at 0-8°C.

14. 5.0 *M* Potassium Hydroxide

Dissolve 70.1 grams of potassium hydroxide in 100 mL of water in a 250 mL volumetric flask. Mix well and Q.S. to 250 mL with water. Stable for 1 year at room temperature.

15. Acidified Methanol

Pipette 100 µL of hydrochloric acid into a 10 mL volumetric flask half filled with methanol. Mix well and Q.S. to 10 mL with methanol. Stable for 1 year at room temperature.

Preparation of Standards

1. Internal Standard Solution – 10 µg/mL

The contents of 100 µg/mL ampoules of D_8-amphetamine, D_8-methamphetamine, D_5-methylenedioxyamphetamine and D_5-methylenedioxymethamphetamine are diluted to 10 mL with methanol in a volumetric flask. Stable for 1 year when stored at $\leq$ -20°C.

2. Amphetamine, Phentermine, Methamphetamine, Methylenedioxyamphetamine and Methylenedioxymethamphetamine Standard Solution – 100 µg/mL

The contents of 1.0 mg/mL ampoules of amphetamine, phentermine, methamphetamine, methylenedioxyamphetamine and methylenedioxymeth-amphetamine standard solutions (1.0 mg/mL; Radian Corporation) are diluted to 10 mL with methanol in a volumetric flask. Stable for 1 year when stored $\leq$ -20°C.

3. Amphetamine, Phentermine, Methamphetamine, Methylenedioxyamphetamine and Methylenedioxymethamphetamine Standard Solution – 10.0 µg/mL

Dilute 1.0 mL of the 100 µg/mL amphetamine, phentermine, methamphetamine, methylenedioxyamphetamine and methylenedioxymethamphetamine standard solution to 10 mL with methanol in a 10 mL volumetric flask. Stable for 1 year when stored ≤ -20°C.

3. Pseudophedrine Standard Solution – 100 µg/mL

Dissolve 1.2 mg of d-pseudoephedrine hydrochloride (Alltech) in methanol in a 10 mL volumetric flask. Stable for 1 year when stored ≤ -20°C.

4. Pseudophedrine Standard Solution – 10 µg/mL

Dilute 1.0 mL of the 100 µg/mL pseudoephedrine standard solution to 10 mL with methanol in a 10 mL volumetric flask. Stable for 1 year when stored ≤ -20°C.

5. Aqueous Amphetamine Standards

Standards are added to 1.0 mL of blank blood according to the following table:

Standard Concentration (mg/L)	Volume of 10 µg/mL standard solution	Volume of 100 µg/mL standard solution
0.5	50 µL	—
1.0	100 µL	—
2.0	—	20 µL
3.0	—	300 µL
5.0	—	500 µL

Preparation of Control Samples

1. Negative Control

2. Amphetamine, Phentermine, Methamphetamine, Methylenedioxyamphetamine and Methylenedioxymethamphetamine Control Solution – 100 µg/mL

The contents of 1.0 mg/mL ampoules of amphetamine, phentermine, methamphetamine, methylenedioxyamphetamine and methylenedioxymeth-amphetamine standard solutions (1.0 mg/mL; Radian Corporation) are diluted to 10 mL with methanol in a volumetric flask. Stable for 1 year when stored ≤ -20°C.

3. Pseudophedrine Control Solution – 100 µg/mL

Dissolve 1.2 mg of d-pseudoephedrine hydrochloride (Alltech) in methanol in a 10 mL volumetric flask. Stable for 1 year when stored ≤ -20°C.

4. Aqueous Amphetamine, Phentermine, Methamphetamine, Methylenedioxy-amphetamine and Methylenedioxymethamphetamine Control

Add the following amount of control solution to 1.0 mL of negative blood:

Standard Concentration (mg/L)	Volume of 100 µg/mL standard solution
2.0	20 µL

Procedure - Amphetamines

1. Set up sufficient culture tubes for:

   A) Blank
   B) Blank with internal standard
   C) Standards
   D) Control samples
   E) Unknown(s)

2. Add 1 mL of the appropriate sample to the corresponding tube.

3. Add 50 µL of the internal standard to all culture tubes except the blank.

4. Add 2.0 mL of 0.1 *M* phosphate buffer (pH 6) and vortex.

5. Centrifuge all tubes at 1800 rpm for 3 minutes.

6. Place columns into the vacuum manifold, plugging any unused ports.

7. Pass 3.0 mL of methanol through each column.

8. Pass 2.0 mL of 0.1 *M* phosphate buffer through each column. Do not permit the column to dry.

9. Pour specimen into column. Slowly draw specimen through column (at least 2 minutes) under low vacuum.

10. Pass 1.0 mL of 1.0 *M* acetic acid through each column.

11. Pass 3 mL of methanol through each column.

12. Dry column with full vacuum for 5 minutes.

13. Turn off vacuum. Dry the tips. Place labeled disposable culture tubes into column reservoir. Replace vacuum top.

14. Add 3.0 mL of methylene chloride:isopropanol:ammonium hydroxide solution to each column and collect in disposable culture tubes. Remove disposable culture tubes.

Note: **Methylene chloride is a potential carcinogen. Handle with care.**

15. Add 30 µL of acidified methanol and vortex.

16. Evaporate to dryness at 40°C with a stream of nitrogen.

Caution: Do not overdry the extracts – this may lead to loss of the drugs.

17. Add 100 µL of PFPA to each tube. Cap tightly and vortex.

18. Place tubes in heating block for 30 minutes at 65°C.

19. Evaporate to dryness at 40°C with a stream of nitrogen.

Caution: Do not overdry the extracts – this may lead to loss of the drugs.

20. Add 100 µL ethyl acetate to each culture tube and vortex.

21. Transfer to injection vial.

Instrumentation

The capillary column phase used for the amphetamines procedure is either 100% methylsiloxane (HP-1, RTx-1 or DB-1) or 5% phenyl-95% methylsiloxane (HP-5, RTx-5 or DB-5). The manufacturer is either Hewlett-Packard, Restek, or J&W. The GC/MS parameters may be adjusted as needed to meet testing requirements.

An autotune must be performed using perfluorotributylamine (PFTBA) prior to each GC/MS run (within 24 hours of the start of a run).

Calculations

Unknown concentrations for any assay are determined by comparing the unknown drug concentration versus a standard curve obtained for that drug. The standard curve, a plot

of the drug concentration (x-axis) versus quantitative ratio (y-axis), is obtained using linear regression. The standard curve should be constructed with a minimum of 3 standards.

Amphetamine Interpretation

The following information is required to properly review the results :

1. Retention times for amphetamine and D_8-amphetamine, if present.

2. Abundances for *m/z* 118, 126, 190 and 193.

3. Ion peak ratios for *m/z* 118/190 and 126/193.

4. Quantitative ion ratios of *m/z* 190/193.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 118, 126, 190 and 193.

Evaluation

To be reported POSITIVE for amphetamine, the following criteria must be satisfied:

1. The retention times of *m/z* 118, 126, 190 and 193 ion peaks must be within $\pm$ 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 118/190 and 126/193 ion peak ratio for the specimen must be within $\pm$ 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 190/193 ion peak ratio is $\geq$ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Phentermine Interpretation

The following information is required to properly review the results :

1. Retention times for phentermine and D_8- amphetamine, if present.

2. Abundances for *m/z* 126, 164, 193 and 204.

3. Ion peak ratios for *m/z* 164/204 and 126/193.

4. Quantitative ion ratios of *m/z* 204/193.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 126, 164, 193 and 204.

Evaluation

To be reported POSITIVE for phentermine, the following criteria must be satisfied:

1. The retention times of *m/z* 126, 164, 193 and 204 ion peaks must be within ± 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 164/204 and 126/193 ion peak ratio for the specimen must be within ± 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 204/193 ion peak ratio is ≥ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Methamphetamine Interpretation

The following information is required to properly review the results :

1. Retention times for methamphetamine and D_8- methamphetamine, if present.

2. Abundances for *m/z* 118, 160, 163, 204 and 211.

3. Ion peak ratios for *m/z* 160/204, 118/204 and 163/211.

4. Quantitative ion ratios of *m/z* 204/211.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 118, 160, 163, 204 and 211.

Evaluation

To be reported POSITIVE for methamphetamine, the following criteria must be satisfied:

1. The retention times of *m/z* 118, 160, 163, 204 and 211 ion peaks must be within ± 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 160/204, 118/204 and 163/211 ion peak ratio for the specimen must be within ± 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 204/211 ion peak ratio is ≥ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Pseudoephedrine Interpretation

The following information is required to properly review the results :

1. Retention times for pseudoephedrine and D_5-methylenedioxyamphetamine, if present.

2. Abundances for *m/z* 160, 167, 194, 204 and 294.

3. Ion peak ratios for *m/z* 160/204, 294/204 and 194/167.

4. Quantitative ion ratios of *m/z* 204/167.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 160, 167, 194, 204 and 294.

Evaluation

To be reported POSITIVE for pseudoephedrine, the following criteria must be satisfied:

1. The retention times of *m/z* 160, 167, 194, 204 and 294 ion peaks must be within ± 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 160/204, 294/204 and 194/167 ion peak ratio for the specimen must be within ± 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 204/167 ion peak ratio is ≥ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Methylenedioxyamphetamine Interpretation

The following information is required to properly review the results :

1. Retention times for methylenedioxyamphetamine and D_5- methylenedioxy-amphetamine, if present.

2. Abundances for *m/z* 162, 167, 135, 190 and 194.

3. Ion peak ratios for *m/z* 135/162, 190/162 and 194/167.

4. Quantitative ion ratios of *m/z* 162/167.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 162, 167, 135, 190 and 194.

Evaluation

To be reported POSITIVE for methylenedioxyamphetamine, the following criteria must be satisfied:

1. The retention times of *m/z* 162, 167, 135, 190 and 194 ion peaks must be within ± 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 135/162, 190/162 and 194/167 ion peak ratio for the specimen must be within ± 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 162/167 ion peak ratio is ≥ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Methylenedioxymethamphetamine Interpretation

The following information is required to properly review the results :

1. Retention times for methylenedioxymethamphetamine and D_5- methylenedioxy-methamphetamine, if present.

2. Abundances for *m/z* 160, 162, 164, 204 and 208.

3. Ion peak ratios for *m/z* 162/204, 160/204 and 164/208.

4. Quantitative ion ratios of *m/z* 204/208.

5. Reconstructed total ion chromatogram.

6. Selected ion chromatograms for *m/z* 160, 162, 164, 204 and 208.

Evaluation

To be reported POSITIVE for methylenedioxymethamphetamine, the following criteria must be satisfied:

1. The retention times of *m/z* 160, 162, 164, 204 and 208 ion peaks must be within ± 1% of the corresponding ions of the intermediate standard.

2. The *m/z* 162/204, 160/204 and 164/208 ion peak ratio for the specimen must be within ± 20% of the corresponding ion peak ratio of the intermediate standard.

3. The correlation coefficient for the standard curve must be 0.985 or greater.

4. The quantitative value for the specimen utilizing the *m/z* 204/208 ion peak ratio is ≥ 0.5 mg/L.

Failure to meet one of the above criteria requires that the specimen be reported NEGATIVE.

Additional Information

Linearity: The method is linear from 0.5 mg/L to 5.0 mg/L

Limit of Quantitation: 0.5 mg/L

References

1. Baselt RC and Cravey RH. Disposition of Toxic Drugs and Chemicals in Man. CTI: Foster City, CA (2000).

2. Liu RH and Goldberger BA. Handbook of Workplace Drug Testing. AACC Press: Washington, DC (1995).

3. Clean Screen Extraction Column Application Manual, United Chemical Technologies.