行政院所屬各機關因公出國人員出國報告書

(出國類別：參加會議)

赴澳洲參加「第四十一屆國際法醫毒理學家協會年會」報告

服務機關：行政院衛生署管制藥品管理局

出 國 人　職　　稱：組長

姓　　名：賴璟賢

出國地點：澳洲

出國期間：自 92 年 11 月 14 日至 92 年 11 月 21 日

報告日期：中華民國 93 年 2 月 13 日

系統識別號:C09300472

公 務 出 國 報 告 提 要

頁數: 11　含附件: 否

報告名稱:

赴澳洲參加「第四十一屆國際法醫毒理學家協會年會」報告

主辦機關:

行政院衛生署管制藥品管理局

聯絡人／電話:

翁銘雄／02-23975006-2332

出國人員:

賴璟賢　行政院衛生署管制藥品管理局　預警宣導組　組長

出國類別: 其他

出國地區: 澳大利亞

出國期間: 民國 92 年 11 月 14 日 -民國 92 年 11 月 21 日

報告日期: 民國 93 年 02 月 13 日

分類號/目: J0／綜合（醫藥類）　J0／綜合（醫藥類）

關鍵詞:　藥物濫用防制,法醫毒理學

內容摘要: 第41屆國際法醫毒理學家協會之年會是在澳洲墨爾本（Melbourne）舉行，有41個國家370餘人報名參加。會議包括兩項特別演講，10場專題研討會及2項講習會，研討內容之主題有：LC-MS 檢測藥物及毒品所產生的技術問題；替代檢體之檢測；死後檢體之檢測；臨床毒理學及治療藥物之監測；毒物、毒素與藥草；普通毒理學；酒精、藥物與駕駛之關係；運動用藥與禁藥管制等專題演講及壁報論文。研討之議題均與法醫毒理學未來研究方向息息相關，也涉及刑事鑑識的鑑定證據、濫用藥物之檢驗技術等。藉由參加此次會議蒐集濫用藥物之相關資訊，以及世界各國有關法醫毒理、鑑識科學之研究趨勢，並與其他國家之會員交換工作經驗，建立聯繫管道，作爲國內推展藥物濫用防制業務之參考。

本文電子檔已上傳至出國報告資訊網

摘　要

第 41 屆國際法醫毒理學家協會之年會是在澳洲墨爾本（Melbourne）舉行，有 41 個國家 370 餘人報名參加。會議包括兩項特別演講，10 場專題研討會及 2 項講習會，研討內容之主題有：LC-MS 檢測藥物及毒品所產生的技術問題；替代檢體之檢測；死後檢體之檢測；臨床毒理學及治療藥物之監測；毒物、毒素與藥草；普通毒理學；酒精、藥物與駕駛之關係；運動用藥與禁藥管制等專題演講及壁報論文。

研討之議題均與法醫毒理學未來研究方向息息相關，也涉及刑事鑑識的鑑定證據、濫用藥物之檢驗技術等。藉由參加此次會議蒐集濫用藥物之相關資訊，以及世界各國有關法醫毒理、鑑識科學之研究趨勢，並與其他國家之會員交換工作經驗，建立聯繫管道，作為國內推展藥物濫用防制業務之參考。

赴澳洲參加「第 41 屆國際法醫毒理學家協會年會」報告

目　　次

第一章　目　的

第 41 屆國際法醫毒理學家協會之年會是於 92 年 11 月 16 日至 20 日，在澳洲墨爾本（Melbourne）之公園希爾頓飯店舉行。該協會是一個歷史悠久的國際性毒理學家組織，已有四十年的歷史，並擁有來自世界各地超過一千四百名會員的機構。該組織的宗旨為促進會員在法醫毒理學方面的合作與協調，並且鼓勵相互之間的研究。會員的組成有來自各國的警察部門、臨床醫學、法醫實驗室、賽馬與運動藥物檢驗實驗室、醫院、鑑識醫學、藥學及藥理學界等。

毒品在世界各地所造成的危害，不論是已開發國家或是貧窮落後國家，都不能倖免於難。毒品與管制藥品為一體之兩面，合法使用者為管制藥品，非法使用者即為毒品。本局掌理管制藥品之管理事項，為瞭解國際間有關濫用藥物毒理科學之發展趨勢，各國有關濫用藥物之檢驗技術及科技新知，藉由參加此次會議瞭解濫用藥物之相關資訊，以及世界各國有關法醫毒理、鑑識科學之趨勢，並與其他國家之會員交換工作經驗，建立聯繫管道，以利國內藥物濫用防制業務之推展。

第二章　過　程

赴澳洲墨爾本參加「第四十一屆國際法醫毒理學家協會年會」行程

日　期	時　間	行　　程　　內　　容
11/14 (Fri)	22:15	搭乘長榮航空 BR315 班機自中正國際機場前往澳洲。
11/15 (Sat)	09:05	澳洲航空 QF617 班機由澳洲布利斯班前往墨爾本。
11/16 (Sun)		參加「第四十一屆國際法醫毒理學家協會年會」
	14:00	大會報到
	17:30	歡迎晚會
11/17 (Mon)	08:30~	開幕典禮及特別演講
	10:30~	論文口頭報告(14 papers)
	15:30~	Oral Fluid Symposium (5 papers)
11/18 (Tue)	08:30~	論文口頭報告(14 papers)
	13:40~	Half Day Excursion
11/19 (Wed)	08:30~	論文口頭報告(28 papers)
11/20 (Thu)	08:30~	論文口頭報告(15 papers)
	13:30~	Great Debate　綜合討論
	19:30~	Closing Banquet　閉幕典禮
11/21 (Fri)	09:00~	由墨爾本經雪梨轉機返回台北
	18:00~	抵達桃園中正國際機場

第三章　參加會議之內容及心得

一、國際法醫學家協會（The International Association of Forensic Toxicologists；簡稱 TIAFT），是一個歷史悠久的國際性毒理學家組織。本次年會之會場在澳洲墨爾本市區內之公園希爾頓飯店(Hilton on the Park)舉行，墨爾本市為位於澳洲南部的大城，全市有四分之一的土地均為公園綠地，是一個文化藝術全面多元性人口眾多、交通便利、商業發達的大城市。由於約有三分之一的居民，為生於海外的移民，使用的語言極多。在街道上，經常可以看到，來自亞洲的留學生或居民，講著不同的華語。本次會議雖然僅有筆者一位來自台灣，會場中尚有來自香港、新加坡、馬來西亞、日本、韓國等東方面孔的與會人士，少數幾位還略懂華語，讓人感覺較不陌生。惟在往返會場途中，偶而也會碰到澳洲之會員，相當親切地打招呼，互相交換意見。

二、特別演講

（一）Unlocking the Significance of Forensic Toxicology Results

由美國國家藥物濫用研究所(National Institute on Drug Abuse；簡稱 NIDA)的 Dr.Marilyn Huestis 主講，說明藥物動力學(pharmacokinetics)在解釋法醫毒理學鑑定的重要性。

在分析毒理學的領域方面，精密儀器的快速發展，不斷的提升檢驗設備的靈敏度及精確度，也改進了準備檢體的技術。作為法醫毒理學家，應該熟練於生物檢體低濃度代謝物的分析，並有義務去決定藥物的檢測濃度。檢驗數據的分析解釋，需要豐富的經驗與知識，必須考慮到檢體

收集的時間、地點，藥物的管理方式，意外事件發生的時間，藥物的使用途徑，現場調查紀錄等，許多方面都要考慮周到。除了從文獻探討完全理解該藥物的藥物動力學外，更要瞭解該種藥物的藥效反應。有許多方法可以建構科學的數據，但必須將藥物的劑量，與對於生理及行為的反應結合起來。從流行病學的資料、犯罪分析、血、尿、唾液和其他案例的組織檢體，可認知的客觀測驗及藥物使用途徑的實驗控制研究，對於制定法醫毒理學的科學數據是有貢獻的。

一種藥物在體內的吸收、分布、代謝和排泄會影響藥物的作用，藥物進入的途徑及藥物吸收的速率，是影響藥物到達腦部速率及是否有明顯副作用的重要因素。藥物以靜脈注射和鼻腔吸食方式，比起口腔、直腸、肌肉或皮下方式進入體內，會更快到達腦部而引起作用。總之，一種藥物在體內的吸收、分布、代謝和排泄作用，是揭開法醫毒理學鑑定以及解釋藥效反應的重要關鍵。

（二）Use of LC-MS in Forensic Toxicology

由義大利 Pavia 大學教授 Aldo Polettini 主講，說明 LC-MS 在法醫毒理學的運用。自從 LC-MS 能有效分離液體色層分離後，廣泛地應用在分析化學，包括法醫毒物分析。目前一些新的儀器設計，利用不同的離子化技術，可以擴展 LC-MS 的分析能力，包括檢測分子量、分子極性，pKa 值及同定的分析物質，並且可從分析的檢體中得到更多相關的數據，特別是一些新型設備已有增加溶解度及提高精準度的趨勢，有助於未知藥物檢測的鑑別能力。無疑的實驗室中 LC-MS 的擴充，將能增進對於藥理學及毒理學

有關機制的理解，並且有助於法醫案例的鑑定。

三、學術研討

本次會議所舉辦之學術討論，計有八個主題，一項專題研討會，一項特殊案例報告及最後的綜合討論。在論文口頭報告方面計有 76 篇，大多與法醫毒物分析及體液分析技術有關，從案例報導方面可以看出一些新興藥物濫用之趨勢。

(一)卡瓦(KAVA)：一種被視為新興濫用藥物的飲料。在法屬南太平洋海島國家新喀里多尼亞(New Caledonia)，由最近一些死亡或昏迷的案例顯示，發現傳統含有鎮靜作用的飲料，一種由 KAVA 葉子煮成，檢測出含有 kavain 成分，可能被當作一種新興濫用藥物。就法醫學的觀點而言，KAVA 似乎是經常與吸食大麻或酒類同時併用。

(二)濫用藥物與駕駛的關係

本項議題可說是本次研討會中頗受重視的問題，不論在論文口頭報告或海報展示方面，均有許多報導。在美國、德國、英國、瑞士、澳洲都有提出關於路邊抽樣調查車輛駕駛濫用藥物的研究，並且大多採用快速篩檢的方法，主要檢測的非法藥物有 Opiates、Cocaine、Cannabis、Amphetamines、Methadone 等，亦有針對 Benzodiazepines 進行檢測。澳洲的 Dr. E. Ogden 提出一套標準現場節制測驗（Standardized Field Sobriety Test；簡稱 SFST），主要在觀察駕駛之頭部運動，要求雙眼向左向右看時眼球之活動，並觀察走路及轉彎、單腳站立等姿勢之反應，此項測試通常對於吸食大麻者很容易被查覺出來。

四、論文海報展示

本次大會共接受 116 篇論文海報展示，但實際上架的有 108 篇，要求作者在旁解說觀眾的疑問，筆者利用研討會空檔瀏覽所有的論文，並索取可提供的影印本，對於其中有興趣的內容，並以數位相機拍攝留存，作為返國後研究之參考。

第四章　建　議

一、香港最近曾發現有人使用 Nimetazepam，可惜找不到標準品比對，據說這種藥物在馬來西亞很流行。而 Nimetazepam（俗稱一粒眠、紅豆、K5）在國內屬第四級管制藥品，為苯二氮平類(Benzodiazepines)之短效型安眠鎮靜藥，具醫療用途；若非合法醫療使用，即為「毒品危害防制條例」所稱之第四級毒品。國外已有出現濫用流行，建議國內各相關機構應加強防範其濫用趨勢。

二、本次參加法醫毒理學家學會之國際性會議，對於會中討論之議題，可以瞭解毒理學研究涉及的範圍極廣，包括生物化學、藥理學、病理學等學科，不但探討毒性物質危害生物系統之機制，並且評估產生危害的機率。而毒物可由自然界產生，也有因人為科技及工業發展而產生，建議國內學者專家能夠多參與有關毒理學方面的國際會議，彼此交換心得，增加瞭解毒物產生的過程、性質，以及其化學檢驗與分析的方法，對於國內有關疾病的預防及治療，一定頗有助益。

三、藥物濫用的預防，應加強流行病學的調查研究。此次看到國外對於路邊駕駛的抽測調查，可以掌握濫用藥物的種類及流行趨勢，採用快速篩檢方式，既能即時監測，亦可適時提供警訊，瞭解藥物濫用的現況及新興濫用藥物的種類，不失為可借鏡的作法。惟為了社會大眾的道路交通安全設想，建議應先考慮以立法方式，制定相關法規，要求道路駕駛者必須配合接受檢測，以利警方之執行。

第五章　附　錄

本次會議攜回許多海報展示之論文影印本，選取與本局業務有關之8篇如下，附於本報告後，提供有興趣之讀者參考。

一、Acetaminophen Serum Level Determination in All Self Poisoned Patients？

二、Application of LC/MS/MS to the Analysis of Benzodiazepines in Blood.

三、An ONLINE^{R} DAT II Immunoassay for the Detection of Cannabinoid Metabolites in Urine.

四、Forensic Intoxication with Clobazam:HPLC/DAD/MSD Analysis.

五、In-vitro Investigation of Amphetamine to Synthetic Melanin Binding in Human Hair.

六、Quantitation of "Liquid Ecstasy"(γ -Hydroxybutyric Acid) by Solid-Phase Dynamic Extraction/GC-MS in Urine.

七、Review of the Development of Workplace & Prison Inmate Drug Testing Programmes in New Zealand over a Ten Year Period.

八、Rapid and Convenient Preparative Method of Phenothiazines Sulfoxides and *N*-oxides of Tricyclic Antidepressants by Hydrogen Peroxide on Titanosilicate Catalyst.

附錄之 1

ACETAMINOPHEN SERUM LEVEL DETERMINATION IN ALL SELF POISONED PATIENTS ?

M. Lhermitte [(1)], **M. Mathieu** [(2)], **R. Grenot** [(3)], **F. Klinzig** [(1)], **N. Houdret** [(3)] **and D. Mathieu** [(3)]

(1) Laboratoire de Toxicologie; (2) Centre antipoison and (3) Urgences Respiratoires et Réanimation Médicale, Hôpital Calmette, Avenue du Professeur J. Leclercq 59037 Lille Cedex, France.

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Aims : Acetaminophen (paracetamol) :

- Important source of poisoning
- Even in acute overdose, it does not cause early neurological or other warning signs of toxicity. If administred early after ingestion, N-acetylcysteine has been shown to be efficient to prevent acetaminophen toxicity
- Antidotal treatment is based on acetaminophen level determination.
- In the U.S.A., recommendation has been issued that acetaminophen level determination has to be performed in every self poisoned patient
- Our study aims were to determine the validity of this recommendation in France

MATERIAL : FPIA test from Abbott

SUBJECTS

- Included period : march to november 2002
- Determination of acetaminophen for 440 patients in two groups :

  - guided group : 270 patients with clinical abnormalities or intake of lesional xenobiotics after clinical interview.

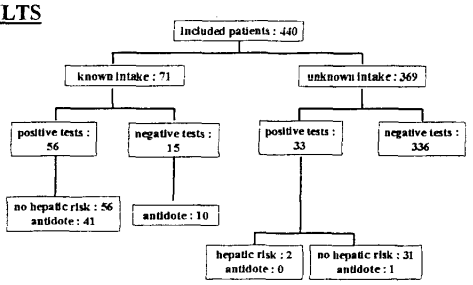
  - systematic group : 170 patients with voluntary xenobiotic ingestion

- Statistical analysis : κ-2 or impaired t-test, according to variable type (statistical significance set at 0.05)

RESULTS

Distribution of patients (440) by sex and age

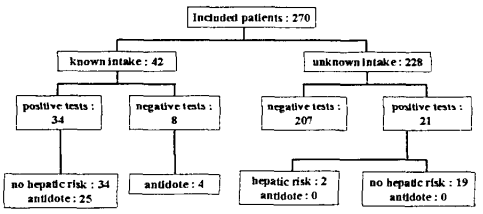
Age (years)	15-20	21-30	31-40	41-50	51-60	> 60
Men	9	69	63	41	11	2
Women	40	65	58	50	15	17
Number of patients	49	134	121	91	26	19



Hepatic risk and treatment by antidote (Nacetyl cysteine) as a function of clinical interview

Distribution of patients in guided group (270) by age

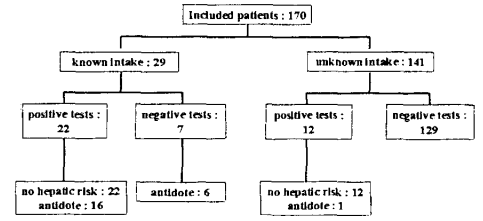
Age (years)	15-20	21-30	31-40	41-50	51-60	> 60
Men	5	45	41	21	7	2
Women	22	42	36	32	9	8
Number of patients	27	87	77	53	16	10



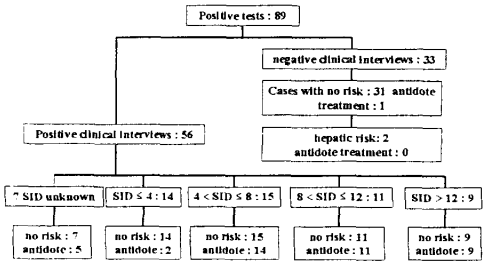
Hepatic risk and treatment by antidote (Nacetyl cysteine) as a result of clinical interview

Distribution of patients in systematic group (170) by age

Age (years)	15-20	21-30	31-40	41-50	51-60	> 60
Men	4	24	22	20	4	0
Women	18	23	22	18	6	9
Number of patients	22	47	44	38	10	9



Hepatic risk and treatment by antidote (Nacetyl cysteine) in the systematic group



POSITIVE ACETAMINOPHEN TESTS
SID = supposed ingested dose
risk = hepatic risk

CONCLUSIONS

- Clinical interview has an unacceptable error rate to predict acetaminophen ingestion
- Although systematic acetaminophen determination is a too large recommendation, it has to be done in all self-poisoned patients when clinical abnormality and/or injurious compound is present

Application of LC/MS/MS to the Analysis of Benzodiazepines in Blood

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Aim

The objective of this study was to develop a sensitive and selective high performance liquid chromatography/tandem mass spectrometry (LC-MS-MS) method for the simultaneous detection of 18 benzodiazepines and metabolites in human blood.

Introduction

There are over 50 different types of benzodiazepines available worldwide, but only 13 are available for medical use in Australia. Benzodiazepines and their metabolites are frequently found in combination with other drugs in drug related fatalities. Some of the benzodiazepines are abused and can be a factor in impaired driving, sexual assault and other crimes. Methods for the analysis of benzodiazepines in blood utilising gas chromatography (GC) have difficulty analysing the more polar benzodiazepines and their polar metabolites. In addition oxazepam and temazepam readily decompose when analysed by GC. These problems with analysis by GC has led to the common use of HPLC with ultraviolet detection. However, this technique lacks both sensitivity and selectivity for benzodiazepines. Recently, methods which overcome these problems by utilising HPLC-MS and HPLC-MS-MS for benzodiazepine analysis have been reported. However, these methods have generally been developed for a very limited number of analytes often for application in therapeutic drug monitoring or clinical toxicology. In forensic toxicology there is more frequently the requirement to screen samples for unknown benzodiazepines and to identify multiple drugs in a blood sample.

Here we report a sensitive and selective HPLC-APCI-MS-MS method for the simultaneous detection of the 13 benzodiazepines available in Australia and 5 of their metabolites in human blood.

Method

- EXTRACTION: 0.5mL blood was extracted with 1.75mL 14% ammonia solution and 10mL 1-chlorobutane. After centrifuging the solvent was drawn off and evaporated to dryness. The residue was dissolved in 0.1mL mobile phase.
- HPLC: Agilent Technologies 1100 series
- COLUMN: Zorbax XDB C8 4.6 mm x 150mm, 5um particle size
- THE MOBILE PHASE: 60:40 v/v mix of methanol and 20mM ammonium formate (pH 9) for 15 minutes, ramped to 100% methanol from 15 to 16 minutes and held for a further 5 minutes. The flow rate was 0.7 mL minute^{-1}.
- INTERFACE: Agilent Technologies G1947A APCI ion source operated in positive ion mode. The APCI vaporiser temperature was 400°C. The nebuliser nitrogen pressure was 60 psi. The drying gas had a flow rate of 5 L minute^{-1} and was heated to 350 °C.
- MASS SPECTROMETER: Agilent Technologies G2445A MSD Trap. The skimmer voltage was set at 24 volts and the capillary exit offset was 69 volts.

Drug identification was based on the combination of retention time and MS/MS spectral characteristics obtained from fragmentation of the pseudomolecular ion. The mass spectrometric analysis during the chromatographic run was divided into several segments, each containing MS/MS parameters for one or more drugs as shown in Table 1.

Segment Number	Benzodiazepine / metabolite	RT (min)	Pseudo molecular ion (M+H$^+$)	Major Product ion (m/z)	Collision energy amplitude (V)	Precursor ion selection window (amu)
1 [1.00-4.00]	7-aminonitrazepam	2.6	252	224	2.00	10
	7-aminoclonazepam	2.8	286	250	2.50	10
	7-aminoflunitrazepam	3.1	284	264	1.88	10
2 [4.00-5.70]	Bromazepam	5.3	316	288	1.92	10
3 [5.70-6.70]	Clonazepam	6.1	316	270	2.00	10
	Nitrazepam	6.2	282	236	1.86	10
	Flunitrazepam	6.3	314	268	1.90	10
4 [6.70-8.80]	Clobazam	7.3	301	259	3.37	40
	Flurazepam	7.6	388	315	2.60	40
	Triazolam	7.8	343	308	3.57	40
	Alprazolam	8.3	309	281	4.67	40
	Lorazepam	8.3	321	275	2.98	40
	Oxazepam	8.6	287	241	3.32	40
5 [8.80-11.10]	Nitrosalkylflurazepam	9.2	289	261	4.57	40
	Temazepam	9.6	301	255	3.72	40
6 [11.10-13.00]	Nordiazepam	12.1	271	243	1.88	10
7 [13.00-17.00]	Diazepam	13.9	285	257	1.90	10
	Midazolam	14.9	326	291	2.05	10
8 [17.00-21.00]	Prazepam	19.4	325	271	1.90	10

TABLE 1. Time segment, retention time, precursor ion, major product ion, collision energy amplitude and precursor ion selection window for each individual drug.

Results

The method reported here provided a reasonable compromise between maintaining a simple chromatographic method and maximising component resolution. The principal product ion chromatograms for the benzodiazepines studied are shown in Figure 1.

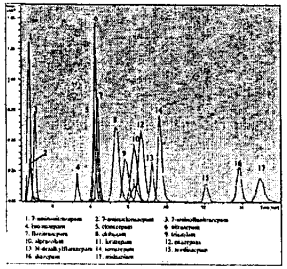

FIGURE 1. The principal product ion chromatograms for the benzodiazepines studied

The MS/MS spectra for selected benzodiazepines with suggested neutral losses, which to our knowledge have not been reported before, are shown in Figure 2. The method produced suitable $(M+H)^+$ pseudomolecular ions in positive ion mode which were ideal as precursor ions for MS/MS. By selecting individual amplitude and width of the collision energy for each drug and metabolite we were able to monitor specific precursor-product transitions. This lead to MS/MS spectral data which was able to provide the specificity for unique identification.

The procedure is able to identify therapeutic and subtherapeutic concentrations of benzodiazepines in casework samples, including cases where multiple benzodiazepines and metabolites are present. Case examples are given in Table 2. Ion chromatograms from case 1 are shown in Figure 3.

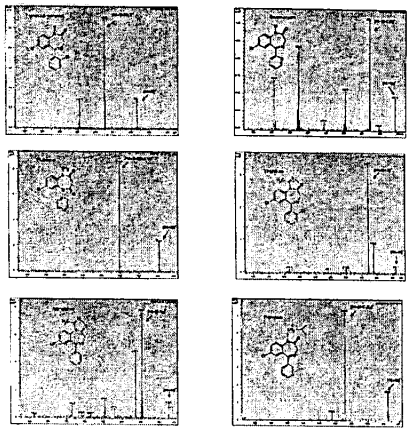

FIGURE 2. The MS/MS spectra for selected benzodiazepines.

Case	Benzodiazepine / metabolite	mg L^{-1}
1	Nitrazepam	0.01
	7-aminonitrazepam	0.08
	Diazepam	0.33
	Nordiazepam	0.32
2	Diazepam	0.06
	Clonazepam	0.009
	7-aminoclonazepam	0.02
3	Bromazepam	0.40
4	Alprazolam	0.006
	Diazepam	0.05
	Nordiazepam	0.01
5	Diazepam	0.58
	Nordiazepam	0.68
	Oxazepam	0.04
	Temazepam	0.12
6	Clobazam	0.10

TABLE 2. Case examples of drugs and their concentrations in blood.

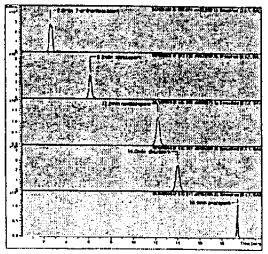

FIGURE 3. The ion chromatograms from the blood sample in case 1 showing the response for 7-aminonitrazepam, nitrazepam, nordiazepam, diazepam and prazepam.

Conclusions

- The HPLC-APCI-MS-MS method provides a single procedure for identification of the wide range of benzodiazepines available for medical use in Australia and their metabolites at their lowest reported therapeutic concentrations using 500uL of blood.
- The MS/MS spectra provide structural information about the drugs.
- Low concentrations of various benzodiazepines have been rapidly and successfully identified in forensic cases.

Government of South Australia

Forensic Science SA

Department for Administrative and Information Services

An ONLINE® DAT II Immunoassay for the Detection of Cannabinoid Metabolites in Urine

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Roche Diagnostics

Abstract

An ONLINE® Generation II immunoassay has been developed for the determination of Cannabinoid Metabolites in urine and has been applied to the Roche/Hitachi and INTEGRA® families of analyzers. This two-reagent system utilizes a THC-specific monoclonal antibody. The extent of the agglutination of antibody-coated microparticles with drug polymer conjugate is monitored. This new assay offers a broad dynamic range of 0-300 ng/mL with 20, 50 and 100 ng/mL cutoff concentrations. Both semi-quantitative and qualitative applications of the assay on the Roche/Hitachi 917 are examined below and are representative of the performance obtained on all Roche/Hitachi analyzers to which this assay has been applied.

Studies demonstrate that the new immunoassay displays a low background, with a clinical sensitivity (mean + 2 S.D.) of 4.8, 2.6 and 0.8 ng/mL for 20, 50 and 100 ng/ml cutoffs respectively. A method comparison of 100 clinical samples that were screened negative by the EMIT® immunoassay yield negative sample results with the ONLINE THC II assay, and EI GC/MS confirmed clinically positive samples result in positive values with the new assay system. A 5-day precision and recovery study of control samples across the range of the curve was performed for all three cutoffs. The median semi-quantitative intra-assay %CV of the 10, 15, 20, 25, and 37.5 ng/mL controls (n=30) for the 20-cutoff assay on Roche/Hitachi 917 are 6.3%, 4.8%, 3.8%, 3.9%, and 3.3%, respectively. The inter-assay precision (n=20) of the same levels ranged from 3.8 to 4.7%. The median semi-quantitative intra-assay %CV of the 25, 37.5, 50, 62.5, and 200 ng/mL controls (n=30) for the 50-cutoff assay on Roche/Hitachi 917 are 5.3%, 4.0%, 4.0%, 3.9%, and 4.4%, respectively. The inter-assay precision of the same levels ranged in value from 4.1 to 5.3%. The median semi-quantitative intra-assay %CV of the 50, 75, 100, 125, and 200 ng/mL controls (n=30) for the 100-cutoff assay are 4.5%, 3.9%, 4.4%, 3.0%, and 3.4%, respectively. The inter-assay precision of the same levels ranged from 3.7 to 5.1%. Comparable results have been obtained on the INTEGRA platform.

The antibody used in this assay was developed against 11-nor-9-carboxy-delta9tetrahydrocannabinol (COOH-THC), however, retains broad cross-reactivity to many cannabinoid metabolites. Studies demonstrate extended reagent stability and higher throughput in comparison to the On-Line Gen I THC assay and come ready to use with no reagent reconstitution required. In summary, the ONLINE DAT II assay for cannabinoids produces reliable results as demonstrated by the above performance characteristics and is well suited for routine drugs-of-abuse screening.

Key Words: THC, Cannabinoid, ONLINE, Immunoassay

Introduction

ONLINE KIMS II

- KIMS - Kinetic Interaction of Microparticles technology
- Liquid, ready to use reagents
- 2-Reagent System:
  - R1: Drug-polymer conjugate
  - R2: Microparticle-antibody
- Monoclonal antibody
- 20, 50 and 100 ng/mL cutoffs
- Semi-quantitative and qualitative modes
- Calibrators:
  - 0, 10, 20, 40, 100 ng/mL
  - 0, 20, 50, 100, 300 ng/mL
  - 0, 50, 100, 200, 300 ng/mL

Results

Precision

20 ng/mL Cutoff on Roche/Hitachi 917

	Within Run		Day-to-Day	
Sample	Mean	CV%	Mean	CV%
10 ng/mL	11.4	6.3	11.6	6.7
15 ng/mL	16.4	4.8	16.5	4.7
20 ng/mL	20.1	3.8	20.4	3.8
25 ng/mL	23.8	3.6	23.4	3.8
37.5 ng/mL	41.2	3.3	41.4	3.6

50 ng/mL Cutoff on Roche/Hitachi 917

	Within Run		Day-to-Day	
Sample	Mean	CV%	Mean	CV%
25 ng/mL	23.1	5.2	22.6	6.3
37.5 ng/mL	38.5	4.0	39.0	4.1
50 ng/mL	50.6	4.0	49.0	4.8
62.5 ng/mL	69.3	3.9	69.2	4.5
200 ng/mL	212.4	4.4	215.8	5.1

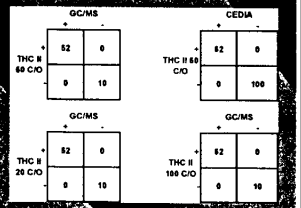
100 ng/mL Cutoff on Roche/Hitachi 917

	Within Run		Day-to-Day	
Sample	Mean	CV%	Mean	CV%
50 ng/mL	55.6	4.5	55.1	5.1
75 ng/mL	77.2	3.9	78.9	4.7
100 ng/mL	95.4	4.4	95.6	5.3
125 ng/mL	139.4	3.0	139.8	3.6
200 ng/mL	222.7	2.4	223.8	2.7

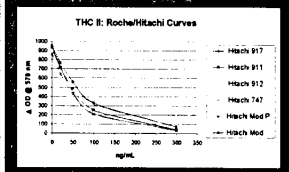
Cross Reactivity

COMPOUND	APPROXIMATE CROSS REACTIVITY
8-β-11-dihydroxy-Δ9 THC	60.8%
8-α-hydroxy-Δ9 THC	14.8%
11-hydroxy-Δ9 THC	13.3%
9-carboxy-11-nor-Δ9-THC-glucuronide	20.3%
9-carboxy-11-nor-Δ9-THC	69.0%

Method Comparison



Curve Comparison: 50 cutoff



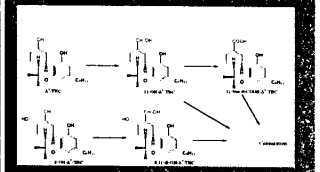
Stability

- 18-month shelf life
- After 10 weeks at 37° C the reagent performance is equivalent to unstressed material
- Exceeds 4-week calibration curve stability and 8-week onboard reagent stability

Sensitivity: Comparison of Cutoffs

	50 ng/mL	20 ng/mL	100 ng/mL
Dynamic Range (ng/mL)	0-300	0-100	0-300
Clinical Sensitivity (mean + 2 SD)	2.6 ng/mL	4.8 ng/mL	0.8 ng/mL
Limit of Detection (ng/mL)	2.6	1.2	3.3

Metabolism of THC



Conclusions

- Inter- and Intra-assay precision ≤ 7% across the range of the curve for all three cutoffs
- Low clinical and analytical sensitivity
- One reagent system for all three cutoffs
- Equivalent curves across analyzer platforms

附錄之 4

FORENSIC INTOXICATION WITH CLOBAZAM: HPLC/DAD/MSD ANALYSIS

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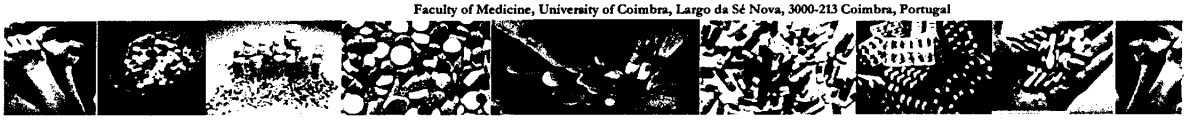

INTRODUCTION

Clobazam (*Castillium®, Urbanil®*) is a new effective antiepileptic drug with most varieties of seizures and epilepsies for both short-term and long-term treatment [2,3], the first 1,5-benzodiazepine having nitrogen atoms in the 1 and 5 positions of the heterocyclic ring, whose chemical structure was designed to give it a different pharmacological profile from that of 1,4-benzodiazepines [4].This benzodiazepine is often used as an anxiolytic, and in the treatment of epilepsy, being considered as a very safe and rarely fatal drug. Several methods for the determination of clobazam have been published. Immunoassays approaches remain extremely useful for a first rapid screening, although gas chromatography is much more sensitive and specific, it requires derivatization procedures and thermolabile benzodiazepines are not suitable to this technique. As consequence high-performance liquid chromatography (HPLC) and more recently, the coupling of HPLC with mass spectrometry (MSD) provides a useful and rugged technique for the analysis of benzodiazepines.
The authors present a fatal case with clobazam and an HPLC/DAD/MSD with electrospray method to detect, confirm and quantify this benzodiazepine in post-mortem samples.

CASE REPORT

A 49-year-old caucasian female was found dead by her husband, at home, lying on the floor of the bathroom. The forensic pathologist was called to perform a scene examination and to verify the time of death. Traces of vomit with no suggestive smell and diarrhoea were present. Many different drugs were found in her bedroom: clobazam, bromazepam, zolpidem, fluoxetin, ranitidine and dissulfiran. She had been undergoing psychiatric treatment for a long time and also had a chronic alcoholism history. Autopsy was performed three days after death in the Forensic Pathology Service of Coimbra's Delegation of the National Institute of Legal Medicine. At autopsy, external examination showed rash, eye congestion, abrasions on the right lower extremity and echymoses on the abdomen, upper and lower extremities. The internal examination revealed visceral congestion signs, hepatic steatosis and part of a blister in the stomach. Microscopic findings showed focus of pneumonia, bronchitis and bronchiolitis, with intra-alveolar hemorrhages and focal mild pulmonary edema. Kidneys presented chronic tubular nephritis.
Samples of blood, stomach, liver and kidney were taken for toxicological analysis.

MATERIALS AND METHODS

Chemicals
Clobazam and prazepam (internal standard) - Sigma-Aldrich
All solvents were analytical or HPLC grade –Merck
Water was purified by a Milli-Q system
Instrumentation (Fig.2)
Waters 2695 Alliance System and a Symmetry® C18 column (2.1mm i.d. x 150mm, 3.5 µm). A methanol/water mobile phase gradient at a flow rate of 0.250 ml/min. The separation was performed at 25°C. The injection volume was 5 µl.
Waters 996 photodiode array detector (DAD) operated in the 210-400 nm, 1.2 nm resolution. UV absorbance at 254 nm.
Waters ZQ 2000 single quadrupole mass spectrometer with an electrospray ionization (ESI) performed in positive mode. Full-scan spectra were recorded from *m/z* 180-380. The mass spectra were represented by centroid mode.
Quantitation employed the selected ion-recording mode (SIR) using the most abundant characteristic ion, *m/z* 301, and the fragment ion, *m/z* 259, for confirmation (Table 1).
Sample preparation
Post-mortem samples (1ml/1g) were spiked with clobazam at the desired concentrations and with 20 µl prazepam (100 µg/ml). 0.5 ml of carbonate buffer (50mM) was added.
Two liquid-liquid extractions were performed, one with 6 ml n-hexane:ethyl acetate (7:3, v/v) and the other with 6 ml n-hexano:2-propanol (99:1, v/v). Supernatants were evaporated to dryness under a slow stream of nitrogen, at 40°C. The dried extracts were reconstituted with 250 µl methanol and an aliquot (5 µl) was injected into HPLC/DAD/MSD system.

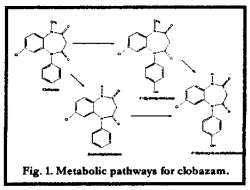

Fig. 1. Metabolic pathways for clobazam.

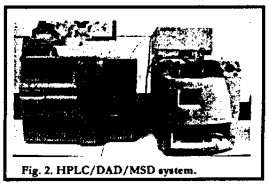

Fig. 2. HPLC/DAD/MSD system.

RESULTS

Table 1- Ions selected, LOD and LOQ and linearity range of clobazam in blood samples

Selected ions (m/z) and cone voltage		Limits (ng/ml)	Linearity	R^2
Quantitation	Confirmation	LOD		
301	259	1	0.01 - 10	0.9988
40 V	60 V	S/N>3		

Table 2- Validation data of recovery and precision for clobazam

Concentration level (µg/ml)	Recovery (%)	Intra-day CV (%)	Inter-day CV (%)	n
0.05	76	8.3	9.7	5
0.1	88	5.7	4.8	5
1	97	3.1	4.9	5
2	92	3.4	5.3	5
5	99	2.2	3.6	5

Table 3- Results of toxicological analysis of our report fatal case

	BLOOD	STOMACH	LIVER	KIDNEY
CLOBAZAM	3.9 (µg/ml	1.1 (µg/g)	2.4 (µg/g)	5.3 (µg/g)

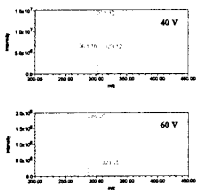

Fig. 2. Positive ESI mass spectrum of clobazam: cone voltage of 40 V and 60 V.

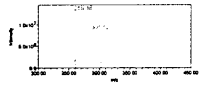

Fig. 3. Positive ESI mass spectrum of desmethylclobazam: cone voltage of 65 V.

DISCUSSION

Ions selected, LOD and LOQ and linearity range of clobazam in blood samples are shown in Table 1. Calibration curves were linear from 0.01 to 10 µg/ml with a r^2 of 0.9988. LOD was 1 ng/ml and LOQ was 10 ng/ml.

Calculated extraction efficiencies for clobazam at 0.05, 0.1, 1, 2 and 5 µg/ml were 76%, 88%, 97%, 92% and 99%, respectively (Table 2). Intra-day and inter-day coefficients of variation (CV) values were determined by replicate analyses (n=5) of blood sample aliquots. The method proved to be precise for clobazam, both in terms of intra-day and inter-day analysis, with coefficients of variation less than 10%.

Regarding the validating data, the procedure is sensitive, selective and reproducible. The developed method was applied to the fatal case presented here.

The toxicological analysis of our report fatal case showed clobazam concentrations of 3.9 µg/ml in blood, 1.1 µg/g in stomach, 2.4 µg/g in liver and 5.3 µg/g in kidney. Blood alcohol analysis gave a negative result. No other drugs were found in post-mortem samples.

Quantitation employed the selected ion-recording mode (SIR) using the most abundant characteristic ion, *m/z* 301, and the fragment ion, *m/z* 259, for confirmation. The fragmentation voltage from 20 V to 70 V was also investigated. The abundance of molecular ion $[M+H]^+$ for this benzodiazepine decreased with higher fragmentation voltages (up of 40 V) and the characteristic ions appeared. Results of positive electrospray ionization mass spectrum of clobazam with a cone voltage of 40 V and 60 V, obtained after injection of extracted blood samples, are shown in Fig. 2. The main pharmacologically active metabolite desmethylclobazam was also detected (Fig. 3).

The blood concentration of clobazam in the fatal case, was 3.9 µg/ml, which is higher than the therapeutic level (0.1 - 0.4 µg/ml). Clobazam was also detected in other organs, which could reveal an ante-mortem and/or post-mortem distribution phenomenon, once authors have published material relating to the phenomenon of post-mortem drug redistribution, but few have assessed if benzodiazepines in general undergo post-mortem redistribution.

CONCLUSIONS

The studied parameters and results achieved allow us to use this methodology in the analytical determination of clobazam in post-mortem samples with a major confidence and liability.
Despite of the scene examination, the psychiatric antecedences of the victim and the autopsy findings, it was definitely the toxicological results that helped concluding that this benzodiazepine was responsible for the death, probably by respiratory depression. Indeed, it is important to notice that the therapeutic index of the benzodiazepines mortality rate associated with poisoning due to benzodiazepines alone is very low. Complicating poisoning, respiratory depression and pneumonia is frequent, as actually presented in our case, where bronchopneumonia, bronchitis and bronchiolitis were diagnosed.

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附錄之 5

In- vitro Investigation of Amphetamine to Synthetic Melanin Binding in Human Hair

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Introduction

Hair analysis is an important tool in forensic and clinical toxicology for determining recent drug use and long-term drug history. In order to fully recognise the implications of a positive hair test result, it is necessary to know how the drugs are incorporated into hair. Melanin, the pigment component of hair, is proposed as one of the factors that influences this process. In this regard, the present *in-vitro* investigation of amphetamine binding to synthetic melanin is intended to characterise the binding mechanism in human hair.

Objectives of the study

The overall aims and objectives of this study are:

- To determine whether or not amphetamine binds to melanin.
- If binding does occur, to analyse the binding data using Scatchard analysis and interpret the resulting plot.
- To determine the association/dissociation constant and the binding capacity of amphetamine for melanin.

Methodology

The binding study was carried out using the indirect method of measuring the decrease in absorbance of drug as the concentration of melanin was increased. Melanin was solubilised in dimethylsulphoxide and diluted in pH 7.4 phosphate buffer. To 4ml aliquots of amphetamine solution (0.939 mg/ml), 20µl of melanin solution (0.3mg/ml) was added. Incubation was carried out at room temperature for 45 minutes with constant mixing using a rocker. The interaction of amphetamine and melanin was monitored using a Genesys 6v, UV/Vis spectrophotometer. A blank was prepared at each step using the same concentration of melanin that was present in each sample. Similarly, positive controls were prepared at each step replacing melanin from sample with buffer to confirm that the decrease in absorbance of the drug is due to binding and not due to dilution only.

Analysis of binding data

To determine the classes of binding sites, association/dissociation constants and the binding capacity of melanin, the binding was analysed by the method of Scatchard *et.al.*,(1949). As the molecular weight and the structure of melanin is still not clear, the number of binding sites can not be calculated directly as an integer. Instead, the ratio of the number of μmoles of drug bound and the dry weight of melanin in μg is considered.

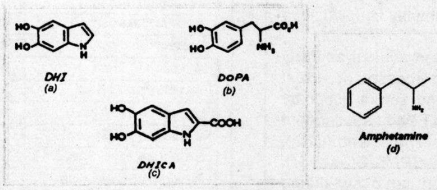

Fig. 1 Structure of the precursors of eumelanin: (a) DHI (5,6-Dihydroxyindole); (b) DOPA (5,6-Dihydroxyphenylalanine); (c) DHICA (5,6-Dihydoxyindole-2-carboxylic acid). Structure of Amphetamine (d)

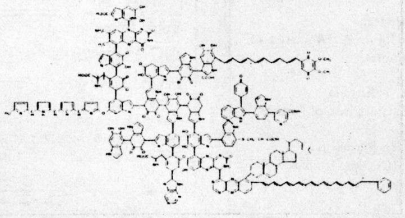

Fig. 2 Structure of melanin polymer

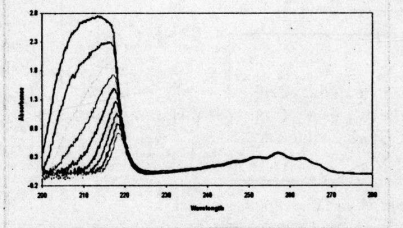

Fig. 3 UV-Vis spectra showing the interaction of amphetamine and melanin

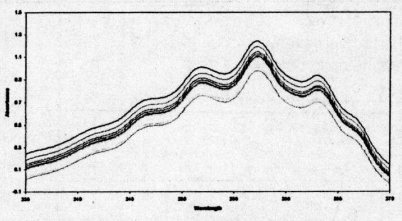

Fig. 4 UV-Vis spectra showing the effect of melanin on the fingerprint region of amphetamine

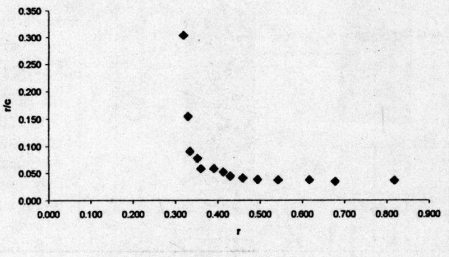

Fig. 5 Scatchard plot of r/c versus r.
(r: µmoles amphetamine bound per µg melanin;
c :µmoles of unbound amphetamine)

Discussion

Figures 3 and 4 show the changes in the spectrum of amphetamine due to the addition of melanin. The shape of the fingerprint region (figure 4) does not show any peak shift whereas the region below 220nm (figure 3) shows a bathochromic shift (shift towards longer wavelength). The latter may be due to the interaction of the amino group of amphetamine and the carboxylic acid group of melanin resulting in peptide bond formation. The detection of several covalent adducts of amphetamine and melanin by Claffey and Ruth (2001) gives additional support to this view.

An example Scatchard plot from the binding data is shown in Figure 5. The curvilinearity of the plot indicates that more than one binding site is present. In addition the upward concavity is indicative of negative cooperativity i.e. the binding of amphetamine to one receptor site in melanin lowers the affinity of binding to additional sites.

This experiment was carried out at physiological pH at which amphetamine exists as a cation. Melanin on the other hand is a polymer containing several states of dopachrome units. It contains many carboxylic acid groups in addition to phenolic and quinoid groups and therefore will exist as an anion at physiological pH. Hence the binding of amphetamine under these conditions is also suggestive of ion exchange. Steric hindrances and physiochemical conditions at the binding sites might result in the carboxylic acid group being differentially available for the binding (Larsson *et. al.*, 1978).

Although the fingerprint region of amphetamine is not structurally changed, the absorbance decreases on addition of melanin (Figure 3). This indicates that Van Der Waal's interactions are occurring between the aromatic indole nuclei of melanin and the aromatic structure of amphetamine.

Conclusion

- Amphetamine binds to melanin *in-vitro*.
- Scatchard analysis yields a curvilinear plot with upward concavity indicating multiple binding sites and negative co-operativity.
- The association and dissociation constants and the binding capacity are currently being determined.

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Larsson B., and H. Tjalve (1979) Studies on the mechanism of drug binding to melanin, *Biochemical Pharmacology. 28: 1181*

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[Online] *Available http://www.rastafarian.net/what_is_melanin.htmarticles/c9710gal.pdf.*

附錄之 6

Quantitation of "Liquid Ecstasy" (γ-Hydroxybutyric Acid) by Solid-Phase Dynamic Extraction/GC-MS in Urine

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Institute of Legal Medicine, Forensic Toxicology, University Hospital Freiburg Germany.

Introduction

Solid-phase dynamic extraction (SPDE) has been introduced by Chromtech (Idstein/Germany) and has been successfully applied for the forensic analysis of amphetamines in hair samples using a CTC/PAL headspace sampler coupled to a GC/MS quadrupole instrument [1].

Gamma-hydroxybutyric acid (GHB) has been abused as party drug ("Liquid Ecstasy") and as a rape drug in cases of sexual assault. GHB can be detected in urine and serum either by precipitation or by liquid/liquid extraction (LLE) and subsequent silylation [2] - or by conversion to gamma-butyrolactone (GBL), LLE and headspace-GC analysis [3] or HS-SPME/GC-MS [4]. GHB and GBL are volatile and evaporation can occur during solvent evaporation steps. **SPDE** like **SPME** offers the possibility to extract GBL directly from urine after acidic lactonisation of GHB.

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[3] M. A. LeBeau, M. A. Montgomery, M.L Miller, S. G. Burmeister.
Analysis of Biofluids for Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) by Headspace GC-FID and GC-MS.
J. Anal. Toxicol. 24, 2000, 421-428.
[4] C. Merckel, V. Auwärter, D. Simmert, F. Pragst: GHB: Bestimmung durch HS-SPME/GC-MS und Fallbeispiele. Toxichem + Krimtech 70/2, 2003, 93-98.

Development of the Method

Pre-Incubation-Time*	[m:ss]	5:00
Syringe-Temp.*	[°C]	60
Incubation-Temp.*	[°C]	60
Agitator-Speed	[rpm]	500
Agitator-On-Time	[m:ss]	0:30
Agitator-Off-Time	[m:ss]	0:05
Sample-Penetration	[mm]	28
Extraction-Strokes*		50
Extraction-Fill-Speed*	[µL/s]	200
Extraction-Eject-Speed*	[µL/s]	200
Desorption-Gas-Volume	[µL]	1000
Injector-Penetration	[mm]	45
Pre-Desorption-Time	[m:ss]	0:10
Desorption-Flow-Speed	[µL/s]	25

Table 1.: optimized SPDE parameters.

A 6890 GC with a 5973 MSD (Agilent) equipped with a CTC Combi PAL autosampler was used to perform the analysis.

Starting with a PAL-autosampler method written by Chromtech (Idstein, Germany) the parameters signed with * were modified step by step to optimize the extraction of GBL.

Preconditioning of needle was used prior to injection of next sample (10 min, 180°C) to avoid carry-over of analyte from preceding sample.

GC-Parameters:

Initial column temp.: 35 °C
Initial hold time: 3 min
Temperature ramp 1: 15 °C/min to 80 °C
Temperature ramp 2: 40 °C/min to 250 °C
Final hold time: 1 min

Column:

Optima δ-3, 0.25 µm 30 m * 0.25 mm (Macherey-Nagel, Düren Germany)

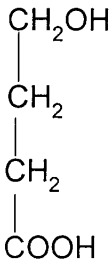
SPDE-Syringe:

PDMS/AC, 50 µm x 56 mm (2.5 mL)

Experimental

1 mL urine was spiked with GHB and hexadeuterated D_6-GHB and treated with 0.15 mL sulfuric acid (98 %) in a 20 mL headspace vial for 5 min at 20 °C. 2 g Na_2SO_4 were added and the vial was placed into the autosampler tray.

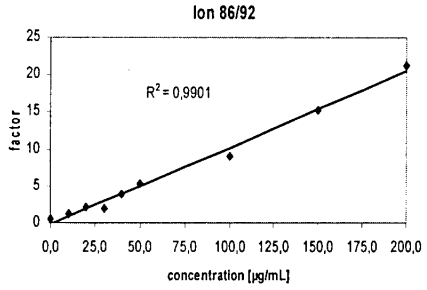
The sample extraction by SPDE and subsequent injection was fully automated by the CTC/PAL-autosampler. A SPDE-syringe with internally coated canula (polydimethylsiloxane/activated charcoal, Chromtech/Idstein, Germany) was used for dynamic headspace extraction using 50 pumping-cycles at a vial temperature of 60 °C. Desorption was performed splitless with helium at 250 °C, SIM-mode was used for the detection of GBL and D_6-GBL (m/z 42/44, 56/60, 86/92 amu).



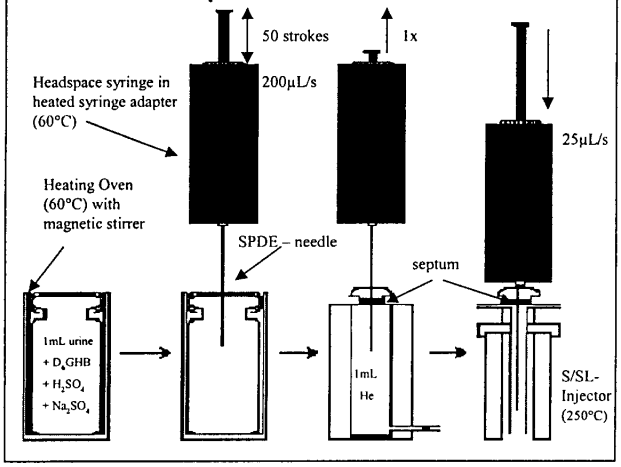
Calibration Curve (Urine)

Urine was spiked with GHB (10 - 200 µg/mL). Ion ratio 86/92 was used for quantification. Ion ratios 42/44 and 56/60 were used as qualifiers.

Linear Range: 10 µg/mL – 200 µg/mL
LLOQ: 10 µg/mL
LOD: < 2 µg/mL
(detection of endogenous GHB-levels possible)



SPDE Technique



Results and Conclusions

HS-**SPDE**-GC/MS – similar to HS-**SPME**-GC/MS - offers a time and labour-saving possibility for the analysis of urine samples for the illegal party and rape drug GHB without derivatisation, without liquid-liquid extraction and without injection port contamination due to the headspace SPDE-technique. The method has been applied for cases of intoxication with GHB (urine-GHB concentrations: 145, 189, 335 and 557 µg/mL, respectively). The SPDE-technique is a promising method for screening of body fluids for volatile compounds. Furthermore, the same SPDE-headspace technique has been used for detection of trichloroethanol (metabolite of chloral hydrate) and propofol in subtherapeutic concentrations in blood in scan-mode.

Review of the development of Workplace & Prison Inmate Drug Testing Programmes in New Zealand Over a ten year period

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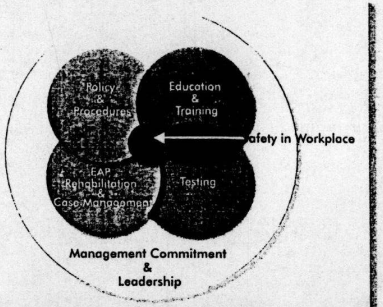
ESR

Introduction

During the 1990s ESR pioneered Workplace Drug Testing (WDT) services in New Zealand (NZ), developing relationships with "Safety-Critical" industries obligated under the Health & Safety in Employment Act 1992, to identify & eliminate workplace hazards. ESR is NZ's leader in developing "Drug & Alcohol Free Workplace Programmes" incorporating the core elements of: policy & procedures, education & training, drug & alcohol testing, results interpretation, expert evidence, medical advice & rehabilitation, and monitoring trends.

Since 1998, ESR has also provided Prison Inmate Drug Testing and Consultancy services for NZ's Public and Private Prisons.

All drug testing follows the strict protocols dictated by the Australian/New Zealand Standard, AS/NZS 4308:2001 "Procedures for the collection, detection and quantitation of drugs of abuse in urine." ESR is currently the only NZ laboratory with IANZ accreditation which has focused on compliance with AS/NZS 4308:2001.
This poster describes the growth and trends of both WDT and Prison Inmate testing.



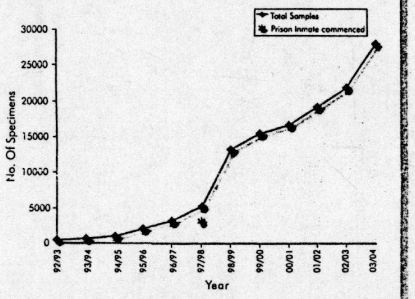
Challenges

- Adulterants/substituted specimens
- Dilute specimens
- Other:
  - Specimens and testing options
  - Oral fluid
  - Hair and sweat
  - "Point of Care" devices
- Legislation
- New drug trends

Acknowledgements

The authors would like to thank the Department of Corrections and ESR's WDT Programme.

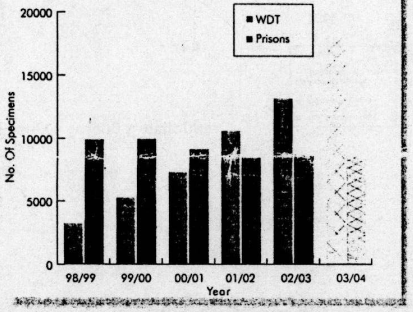
WDT & Prison Inmate - Total Specimen Trends



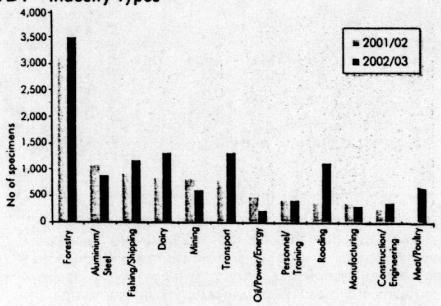
AS/NZS 4308:2001

Procedures for the collection, detection and quantitation of drugs of abuse in urine.

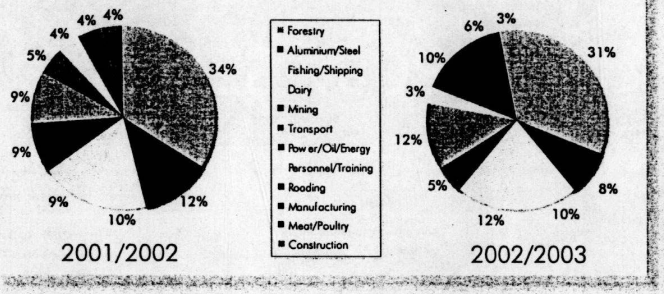
WDT & Prison Inmate - Growth



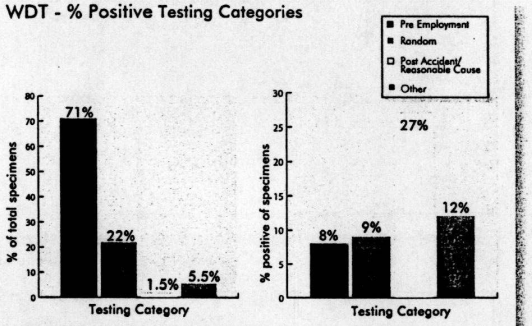
WDT - Industry Types



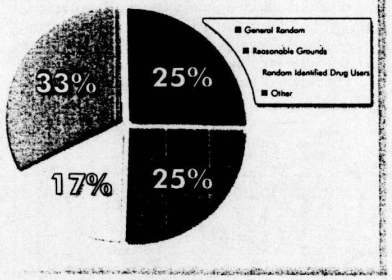
WDT - Comparison: Top 10 Industries: % of Total Specimens



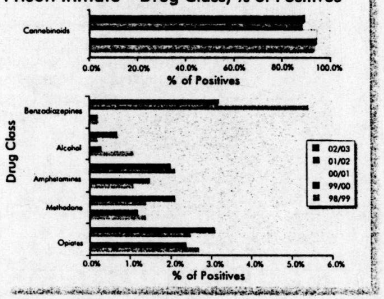
WDT - % Positive Testing Categories



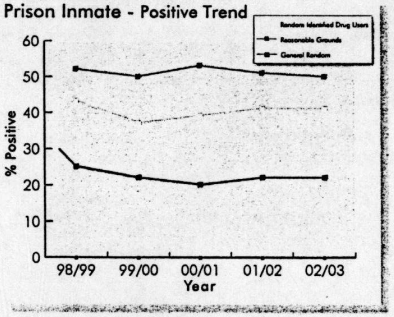
Prison Inmate - Testing Categories, 2002/03



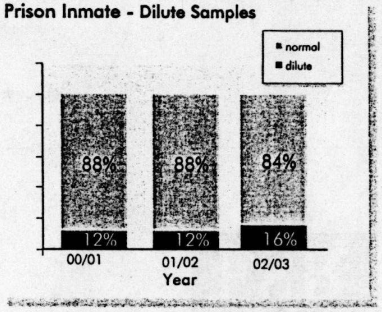
Prison Inmate - Drug Class, % of Positives



Prison Inmate - Positive Trend



Prison Inmate - Dilute Samples



1

Rapid and Convenient Preparative Method of Phenothiazines Sulfoxides and *N*-oxides of Tricyclic Antidepressants by Hydrogen Peroxide on Titanosilicate Catalyst

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2

Aims

We are trying to establish chemical method for preparation of drug metabolites important for forensic toxicology in order to use as analytical standards.

The major metabolites of antipsychotic phenothiazines are each sulfoxide together with ring hydroxylated form. *N*-oxide of tertiary amino cyclic antidepressants is minor metabolite, but useful for identification as it keeps original structure.

We selected titanosilicate as effective catalyst of hydrogen peroxide to obtain oxide from those two drug groups effectively. Titanosilicate has been used as oxidative catalyst in the field of chemical industry. We used TS-1 as available titanosilicates.

3

Flow chart of this study

Small scale reaction (0.5mL)

To determine the best conditions for oxide formation

Drugs: 0.1 mmol / 0.5ml

Temp. & time: at 60℃ for one hour with stirring

⇩ Scale up to 20-times

Preparative scale reaction (10 mL)

To obtain crystals of phanothiazine sulfoxides and *N*-oxide of tertiary amino cyclic antidepressants

Purity: HPLC, Identification: Mass and ^{1}H- ^{13}C-NMR spectrometry.

4

Conclusions

1. The pair of hydrogen peroxide and titanosilicate was found to be efficient oxidative reagents to get phenothiazine sulfoxide and *N*-oxide of tertiary amino cyclic antidepressant.
2. Based on the optimum conditions of small scale reaction, the method consisted of preparative scale one hour reaction followed by extraction and crystallization was established. This method was completed within one or two days.
3. The purity of each obtained crystal of oxides was analyzed by HPLC, and especially tricyclic antidepressants *N*-oxide was found to be pure samples.

5

Methods — Preparation of phenothiazine sulfoxides

Reaction at 60℃ for one hour with stirring,

Additions	0.5 mL scale reaction	10 mL scale reaction
Phenothiazines (1 M in Buf)	100 μL (0.1 mmol)	2 mmol (added directly)*
Acetate buffer (0.2M, pH3.0)	380 μL	7.6 mL
H_2O_2 (30% aq. solution)	20 μL (0.198 mmol)*	400 μL (3.95 mmol)*
Titanosilicate (TS-1)	5 mg	100 mg

*For detail, please refer the Table of Result

After reaction

1) Adjusted to pH 10.0, and extracted with chloroform.

2) Small scale reaction — HPLC analysis of extract

Preparative scale reaction — Crystallization of sulfoxide from extract.

6

Results — Small scale reaction

Sulfoxide formation from antispychotic phenothiazines

Antipsychotic phenpothiazines (100μmol)	30% H_2O_2 (μL)	Measured wavelength (nm)	Formed ratio (%) Sulfoxide	Other Product	Unreacted Drug	Total
Aliphatic amino type						
Chlorpromazine HCl	20	251	92.4	1.1	0.2	93.7
Promazine HCl	20	244	98.4	1.1	0.1	99.6
Promethazine HCl	20	244	94.4	1.4	0.1	95.9
Piperidine base type						
Propericiazine	15	260	87.4	4.5	0.5	92.4
Piperazine base Type						
Fluphenazine HCl	20	247	87.8	2.0	0.3	90.1
Trifluoperazine HCl	15	247	87.3	2.8	1.7	91.8
Perphenazine	20	249	86.2	1.6	1.3	89 2
Prochlorperazine*	20	249	88.1	4.7	0.1	92.9

* Dimaleate salt was converted to free type.

Preparative scale reaction

7

Properties of obtained crystals of phenothiazine sulfoxides

Drugs	Starting amounts	30% H_2O_2 (μL)	Composition of chloroform extract	Crystal of sulfoxide mg (Yield)	mp (°C)	Purity of crystal (HPLC analysis)
Aliphatic amine type						
Chlorpromazine HCl	2 mmol, 710.7 mg	400	Oxide: 77.7 % Other: 15.8 % Drug: 6.2 %	613.3 (82.5 %)	126-130	99.2 % Others: 0.3 % (Rt 3.9) 0.4 % (Rt 4.5) Drug: 0.1 %
Piperidine base type						
Propericiazine	0.8 mmol, 292.4 mg	120	Oxide: 83.9 % Other: 2.7 % Drug: 7.6 %	254.6 (76.2 %)	233-235	93.5 % Others: 3.7 % (Rt 2.2) 1.5 % (Rt 3.8) Drug: 1.3 %
Piperazine base type						
Fluphenazine HCl	2 mmol, 1020.9 mg	300	Oxide: 72.4 % Other: 2.6 % Drug: 2.1 %	583.9 (55.6 %)	183-190	96.9 % Others: 1.6 % Drug: 1.5 %
Trifluoperazine HCl	2 mmol, 990.3mg	300	Oxide: 76.7 % Other: 3.4 % Drug: 2.9 %	504.0 (50.9 %)	198-200	97.7 % Other: 1.1 % Drug: 1.2 %
Perphenazine	2 mmol, 888.0 mg	400	Oxide: 86.3 % Other: 4.4 % Drug: 8.6 %	319.9 (35.2)	140-143	97.7 % Other: 2.2 % Drug: 0.1 %
Prochlorperazine	2 mmol, 747.9 mg	400	Oxide: 78.7 % Other: 9.8 % Drug: 8.4 %	513.7 (66.7)	163-166	99.0 % Others: 0.7 % (Rt 1.8) 0.2 % (Rt 15.3) Drug: 0.07 %

Methods

8

Preparation of *N*-oxide of tertiary amino cyclic antidepressants

Reaction at 60℃ for one hour with stirring.

Additions	0.5 mL scale reaction	10 mL scale reaction
Antidepressant (1 M in MeOH)	100 μ L (0.1 mmol)	2 mmol (added directly)*
Carbonate buffer (1.0 M, pH10.5)	360 μ L	7.2 mL
H_2O_2 (30% aq. solution)	40 μ L (0.395 mmol)*	800 μ L (7.9 mmol)*
Titanosilicate (TS-1)	10 mg	200 mg

*For detail, please refer Table of Results

After reaction

Small scale reaction — HPLC analysis of chloroform extract

Preparative scale reaction — Crystallization of *N*-oxide from extract.

Results

Small scale reaction

9

Formation of *N*-oxide from tertiary amino cyclic antidepressants

Cyclic antidepressants	30% H_2O_2 (μL)	Formed ratio (%)			
		N-oxide	Other product	Unreacted drug	Total
Imipramine HCl	40	93.2	0	0.6	93.8
Clomipramine HC	40	93.8	0	0.7	94.5
Amitriptyline HCl	40	95.7	0	1.0	96.7
Mianserin HCl	100	92.1	6.5	0.1	99.3

Measured wavelength: 215 nm.

Preparative scale reaction

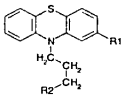
10

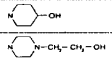
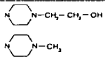
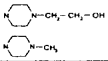
Properties of obtained crystals of antidepressant *N*-oxides

Drugs HCl salts	Starting amounts	30% H_2O_2 (μL)	Composition of chloroform extract	Crystals of N-oxide mg (Yield)	mp (°C)	Purity of crystal (HPLC analysis)
Tricyclic antidepressant						
Imipramine	2 mmol, 633.8 mg	800	Oxide: 85.7 % Other: None Drug: 1.4 %	489.3 (73.5 %)	167-169	100 % Other: None Drug: None
Clomipramine	2 mmol, 702.6 mg	800	Oxide: 85.4 % Other: None Drug: 0.87 %	512.4 (69.8 %)	176-177	100 % Other: None Drug: None
Amitriptyline	2 mmol, 554.8 mg	800	Oxide: 76.8 % Other: None Drug: 0.03 %	260.4 (44.4 %)	92-94	100 % Other: None Drug: None
Tetracyclic antidepressant						
Mianserin	0.4 mmol, 120.3 mg	300	Oxide: 88.6 % (Rt 7.0) Others: 6.4 % (Rt 6.6) 1.5 % (Rt 8.4) Drug: 0.1 % (Rt 6.3)	84.6 (66.0 %)	236-240	99.5 % Other: 0.5 % (Rt 12.2) Drug: None

Structure of antipsychotic phenothiazines

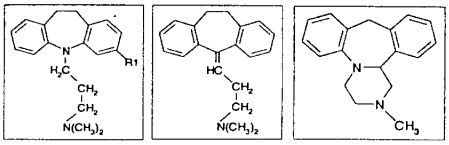
11



Phenothiazines	R_1	R_2
Chlorpromazine HCl	Cl	$N(CH_3)_2$ - $N(CH_3)_2$
Promazine HCl	H	$N(CH_3)_2$ - $N(CH_3)_2$
Promethazine HCl	H	CH_3-$CH(CH_3)$ - $N(CH_3)_2$
Propericiazine	CN	
Fluphenazine HCl	CF_3	
TrifluoperazineHCl	CF_3	
Perphenazine	Cl	
Prochlorperazine	Cl	

Structure of tertiary amino cyclic antidepressants

12



	R_1
Imipramine	H
Clomipramine	Cl

Amitriptyline

Mianserin