6th Annual Meeting of the United Kingdom and Ireland Association of Forensic Toxicologists

Manchester 18th – 19th August 2016

University of Huddersfield

RANDOX TOXICOLOGY

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Dear Friends and Colleagues,

On behalf of the Organising Committee, it is my great pleasure to welcome you to Manchester and to the 6th UKIAFT AGM meeting.

This conference will allow the delegates to see posters and hear presentations on the latest research and concepts in Forensic Toxicology from both the UK and Europe and will hopefully provide new information and stimulate new ideas.

We would like to express our thanks to the sponsors for their generous support and acknowledge that without them, this conference would not take place. We would also like to thank the Manchester Conference Centre and Etihad Stadium for their help with organisation. I would finally like to thank all the people who have helped out behind the scenes - your help has been invaluable.

We hope that you will enjoy the conference and that your interaction with colleagues will be both professionally and personally rewarding. We also hope and trust that you will enjoy your visit to the very beautiful and exciting city of Manchester.

Yours sincerely,

Peter Maskell, PhD, CChem, CSci

Chair

ANNUAL MEETING
18TH-19TH AUGUST 2016

THURSDAY 18TH AUGUST 2016

09:00 – 9:30	*Registration & Coffee with exhibitors*	
09:30 – 11:30	**Annual General Meeting**	
11:30 – 11:45	Coffee Break	
11:45 – 12:45	**Duncan Harding**	**Drug Driving: A UK update**
12:45 – 13:15	**Kirsty Watson**	**The impact of reducing the legal alcohol limit for driving on Driver fatalities in Scotland**
13:15 – 14:00	*Lunch with exhibitors*	
14:00 – 15:00	**Rachel Christie**	**The EU Early Warning System (EWS) and the role of toxicovigilance.**
15:00 – 15:30	**Hilary Hamnett**	**Getting Published in Science**
15:30 – 16:00	*Coffee with exhibitors*	
16:00 – 17:00	**Andy Parrott**	**MDMA (3,4-methylendioxymethamphetaime) or 'Ecstasy': a review of its damaging psychobiological effects**
17:00 – 17:30	**Jane Officer**	**Analytically confirmed exposure to novel psychoactive substances in patients presenting to hospital with severe clinical toxicity in Edinburgh. The Identification of Novel PsychoActive substances (IONA) study.**
19:30 – 23:30	CONFERENCE DINNER ETIHAD STADIUM	

ANNUAL MEETING
18TH-19TH AUGUST 2016

FRIDAY 19TH AUGUST 2016

08:30 – 09:00	*Registration & Coffee with exhibitors*	
09:00 – 10:00	**Philip Lumb**	**Insulin Deaths**
10:00 – 10:45	**Wayne Jones**	**Primer on ADME of Alcohol with Main Focus on Biological Factors that Influence these Processes (Part 1)**
11:00 – 11:15	*Coffee with exhibitors*	
11:15 – 12:00	**Wayne Jones**	**Primer on ADME of Alcohol with Main Focus on Biological Factors that Influence these Processes (Part 2)**
12:00 – 13:00	*Lunch with exhibitors*	
13:00 – 14:00	**John Searle**	**The estimation of uncertainty in alcohol calculations.**
14:00 – 15:00	**Robin Ferner**	**The evidence for acute tolerance to human alcohol intoxication (The Mellanby effect)—a systematic review**
15:00 – 15:15	*Coffee with exhibitors*	
15:15 – 16:15	**Alcohol Uncertainty Discussion**	**(Chaired by Mike Scott-Ham)**
16:15 – 16:30	**Presentation of Poster Prize**	
16:30	MEETING CLOSE	

Presentation Abstracts

Drug Driving: a UK update

D. Harding

Home Office Centre for Applied Science and Technology, Woodcock Hill, Sandridge, St Albans AL4 9HQ

Technology which can screen suspected drug drivers was introduced for the first time in the UK in 2012, after a rigorous evaluation and approval process. Mobile technology which can screen drivers at the roadside was approved for use in 2014, ahead of a new offence being introduced (in March 2015) of driving with a specified drug in the body above a specified limit. Duncan will present an overview of these recent changes, and will discuss the role of preliminary drug testing, the process of type approval, and drug prevalence data.

The impact of reducing the legal alcohol limit for driving on Driver fatalities in Scotland

Kirsty H. Watson*, Fauzia Nurul Izzati, Martha Ilett, Shannah Smith and Hilary J. Hamnett
Forensic Medicine & Science, University of Glasgow

The forensic toxicology laboratory based within the University of Glasgow receives fatal road traffic cases from all regions of Scotland (except the far North). The aim of this study was to examine the toxicological findings in fatally injured drivers before and after the legal blood alcohol concentration (BAC) limit for driving in Scotland was lowered from 80 to 50 mg/100 mL in December 2014.

The laboratory database (for Glasgow cases) and individual case reports (for the rest of Scotland) were examined for driver and motorcyclist fatalities between June 2012 and June 2016, and 173 cases were identified. Of these, 169 cases involved alcohol analysis, and 79 of these cases had occurred since the change in BAC limit. As part of routine casework for these fatalities, post-mortem samples were analysed for alcohol, drugs of abuse and prescription drugs.

Of the 169 cases examined, 78 were negative. Of the 91 positives cases, 16 were positive for alcohol (≥10 mg/100 mL) only and 19 cases were positive for one or more drug(s) in addition to alcohol. Of the 35 cases positive for alcohol, 20 were analysed prior to the BAC limit change and 15 were analysed afterwards.
This presentation will look at the differences in BAC between cases before and after the limit change in Scotland and the effect of the limit change on the prevalence of drug use. The results will be compared including details on the age and gender of the drivers, the most common drugs present in poly drug use cases and the region in which the incidents occurred to try to determine whether the change in the legal BAC limit for driving has had an impact.

Keywords:

Alcohol, Driving Under the Influence, Driver Fatalities.

The EU Early Warning System (EWS) and the role of toxicovigilance

R Christie

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

Since 1997, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has played a central role in Europe's response to new psychoactive substances (NPS). Its main responsibilities in this field are to operate the EU Early Warning System (EWS), with its partner Europol and to undertake risk assessments of new substances when necessary. The EU EWS works by collecting information on the appearance of new substances from the 28 EU Member States, Turkey and Norway, and then monitoring them for signals of harm, allowing the EU to respond rapidly to emerging threats.

A key function of the EU EWS is also to identify signals of serious harms and respond as necessary. This requires monitoring almost 600 substances that have been reported so far. As the market has grown in recent years, the EMCDDA has also had to deal with a growing number of reports of serious harms, often related to acute toxicity leading to hospitalisation and deaths. The EMCDDA has responded to this challenge by working to strengthen the ability of the EU EWS and its network to identify, report, understand and respond to such harms. One of the core activities in this respect is issuing public health alerts, which serve to alert the network on serious and urgent issues.

Getting Published in Science

Dr Hilary J Hamnett*
Department of Forensic Medicine & Science, University of Glasgow

As an insider in scientific journal publishing I have handled several thousand manuscripts from submission to publication. This includes overseeing the peer-review process, doling out rejections and acceptances, and editing and proof-reading. This presentation aims to lift the lid on the editorial process and includes hints and tips for preparing and submitting a paper, persuading editors to publish it, surviving the peer-review process, and developing long-term publishing strategies.

Keywords:

Publishing, manuscript, peer-review

MDMA (3,4-methylendioxymethamphetaime) or 'Ecstasy': a review of its damaging psychobiological effects

Professor Andrew C. Parrott.

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Centre for Human Psychopharmacology, Melbourne, Australia.

MDMA is sometimes presented as a relative benign or safe substance. This review will outline its damaging effects when used as the recreational drug 'Ecstasy'. MDMA is neurochemically messy, and affects several neurotransmitter systems including serotonin and noradrenaline. Acute MDMA increases body temperature, with increased sweating but impaired thermal control. Cortisol levels are increased by 800% in dance clubbers. It can be extremely euphoric, although negative moods are also intensified. The post-MDMA period is typified by feelings of irritability, depression, anger, and behavioural aggression; indeed it has been termed a 'thug drug'. MDMA can cause apoptosis or programmed cell death, and has been assessed for cancer therapy. Recreational users develop chronic tolerance, with increasing self-dosing (up to 25 tablets), accompanied by weaker effects. Regular users show deficits in retrospective memory, prospective memory, problem solving, and social intelligence, while basic cognitive skills remain intact. Neuroimaging studies show reduced serotonin transporters across the cerebral cortex, associated with lifetime MDMA usage, and degree of cognitive impairment. Deficits can also occur in some visual skills and psychomotor abilities. The HPA axis shows changes in cortisol rhythms and homeostasis (e.g. eating, pain perception), with altered sleep architecture, and sleep apnoea in young users. Daily stress levels are increased, while other psychiatric problems include depression and anger/aggression. To summarise, the psychobiological effects of Ecstasy/MDMA can be both damaging and widespread.

Analytically confirmed exposure to novel psychoactive substances in patients presenting to hospital with severe clinical toxicity in Edinburgh. The Identification of Novel PsychoActive substances (IONA) study.

Jane Officer*, Michael Eddleston, Simon Thomas

Scottish Police Authority; National Poisons Information Service; Medical Toxicology Centre, Newcastle University

Objective: The emergence of novel psychoactive substances (NPS) as recreational drugs has challenged emergency departments and poisons centers because of the large numbers of substances involved and the lack of information about their pharmacology and toxicology. The use of branded 'legal high' products is a particular challenge as the chemical constituents may not be known.
The UK Identification Of novel psychoActive substances (IONA) study is collecting biological samples from patients presenting to hospitals with severe toxicity and aims to identify the NPS involved and link these to the clinical features documented. Here we describe results for the first 21 Scottish participants recruited from Edinburgh Royal Infirmary as part of the wider national study.

Methods: With ethical approval, adults (≥16y) presenting to participating hospitals with severe acute toxicity (according to specific definitions) after NPS exposure were recruited with informed consent or, for those individuals without capacity at the time of presentation, with the agreement of an appropriate relative/representative. Clinical features recorded using a structured data collection sheet. Serum and urine samples were collected and analysed by triple quadrupole LC-MS. Additional screening for traditional drugs of abuse and prescribed medication was performed using Orbitrap LC-MS.

Results: Samples were analysed for 21 patients presenting between November 2015 and May 2016. NPS were detected in 11 patients (52%), most often stimulant substances (n=9), which included mephedrone (3), methylpropamine(4), ethylphenidate (2), alpha-PVP (2), 2-AI (2), methoxphenidine (1), N-methyl-2-AI (1) and 3-Fluorophenmetrazine (1). Other NPS identified included designer benzodiazepines such as diclazepam (3) and flubromazepam (3) and synthetic cannabinoid receptor agonists (SCRAs) 5F-MDMB-PINACA (1), AB-CHMINACA (1) and 5-Fluoro-AKB48 and 5-Fluoro-PB-22 (1). Traditional drugs of misuse were also identified in 7 patients (33%) and included MDMA (4), amphetamine (2), methadone (1) and methylamphetamine (1). Prescribed medications were detected in a number of patients. These included trazodone (3), citalopram (2), pregabalin (1), diphenhydramine (1) and sertraline (1). National (UK-wide) clinical features have been collated and the most commonly recorded were confusion (62%), reduced level of consciousness (59%), agitation (57%), tachycardia >140/min (54%), acidosis (35%), paranoia , elevated creatine kinase (each 32%), hypertension, aggression, hallucinations or increased liver transaminases (each 22%). Seizures were reported in 19% of patients and 24% required intubation and ventilation. Comparing SCRA-exposed and other NPS-exposed patients, the only significant differences in clinical features identified were reduced frequencies of tachycardia and pyrexia in SCRA users.

Conclusions: A wide range of substances can be identified in samples from patients presenting with severe toxicity after suspected NPS use. Further data collection is needed to establish a link with any clinical features observed.

Primer on ADME of Alcohol with Main Focus on Biological Factors that Influence these Processes.

A.W. Jones
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According to statistics from WHO (2015) acute alcohol poisoning, alcohol abuse, alcohol-related diseases and alcohol-related trauma resulted in 3.3 million deaths worldwide, which is an astonishing figure for a legally available drug. Not surprisingly, requests to identify and quantitate ethanol in biological specimens dominate the routine duties performed at forensic science and toxicology laboratories worldwide. Over-consumption of alcohol and drunkenness are underlying factors in many crimes, such as drunken driving, drug facilitated sexual assault and drug poisoning deaths. The "gold standard" method used to determine ethanol concentrations in biological specimens from living and deceased persons involves dual-column headspace gas chromatography. When dealing with post-mortem specimens, care is needed to differentiate between ante-mortem ingestion of ethanol and post-mortem synthesis, because ethanol can be produced in the body after death during autolysis and decomposition processes. Interpreting a person's blood-alcohol concentration in relation to the amount of ethanol consumed and the degree of impairment of cognitive and psychomotor functions depends on a host of biological, environmental and genetic factors. These also impact on absorption, distribution, metabolism and excretion (ADME) of ethanol, so-called forensic pharmacokinetics of ethanol. Results from a large number of human alcohol dosing studies will be presented to characterize the disposition and fate of ethanol in the body. When expert statements are made in drink-driving cases, it is important to consider the magnitude of inter- and intra-individual variations in ADME of ethanol. The material presented at this seminar should be useful as a "primer" for use by forensic scientists and the legal profession (barristers, judges and lawyers), when alcohol-related crimes are prosecuted.

Keywords:

ADME, alcohol, ethanol, biological specimens, forensic science, pharmacokinetics, toxicology.

Alcohol Calculations and their Uncertainty

J. Searle

Alcohol calculations are often presented in legal proceedings and in recent years it has become imperative for such calculations to be accompanied by an estimate of the uncertainty. That must be done from the circumstances of the case and papers by Gullberg and by Zuba, suggesting fixed percentages, would appear to be misleading.
The paper presents formulae for calculating the uncertainty in three types of alcohol calculation, those being the Back Calculation, the Forward Widmark and the Reverse Widmark. The same formulae may be applied to calculating the uncertainty of alcohol calculations based on Total Body Water.

The evidence for acute tolerance to human alcohol intoxication (the Mellanby effect)—a systematic review

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Objective: To establish the evidence for acute tolerance to human alcohol intoxication (the Mellanby effect[1]), which has been proposed to diminish the effects of a given blood alcohol concentration (BAC) during the descending part of the BAC–time curve.

Methods: Multiple databases were searched using text words 'tolerance,' 'ascending,' 'descending' or 'Mellanby' with Medline term 'exp *alcohol/' or 'exp *drinking behaviour/' or equivalent. Full text articles were retained for analysis if they dealt with acute (within dose) alcohol tolerance in human subjects and provided quantitative data on both the ascending and descending limbs of the BAC–time curve.

Results: Of 384 unique articles identified and screened, 125 full-text articles were assessed; 19 met criteria for analysis. Most studies were small, median 10 (range 4–28) subjects per group. Doses of alcohol and rates of administration differed. All effects are dependent on drinking history and the degree of intoxication. We distinguished eight major outcome domains (physiological effects, hand-eye co-ordination, perception, decision-making, mental arithmetic and reasoning tasks, verbal skills, memory, subjective alcohol effects), and these were assessed by at least 24 different methods.[2,3] Ratings at a given concentration C were better (closer to sobriety) at C_{down} (descending) than at C_{up} (ascending) for subjects' mean time for maze and peg-board tasks, arithmetic ability, and abstraction. Subjectively, those studied felt less drunk, and were twice as willing to drive at C_{down} as at C_{up}. By contrast, cognitive tasks, error performance, inhibitory control, visual memory and performance in a simulated driving task were worse at $C_{down.}$. [All values $P<0.05$].

Conclusion: The Mellanby effect is most firmly established for subjective intoxication. Confidence in ability to drive increases while measured driving ability falls. Objective measures of impairment are likely to be more robust than a person's own account.

Poster Abstracts

P1 - Comparison of Six Derivatizing Agents for the Determination of Nine Synthetic Cathinones in Gas Chromatography–Mass Spectrometry

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Six acylation reagents have been compared for use as derivatizing agents for the analysis of nine synthetic cathinones by gas chromatography – mass spectrometry (GC – MS). The evaluated reagents were, Pentafluoro-propionic anhydride (PFPA), Trifluoroacetic anhydride (TFA), Chloro di-fluoro acetic anhydride (CL_2FAA), Heptafluorobutyric anhydride (HFBA), Acetic anhydride (AA) and Propionic anhydride (PA). The synthetic cathinones included 4-fluoromethcathinone (Flephedrone or 4-FMC); 4-methylmethcathinone (4-MMC or Mephedrone), Pentedrone (also known as α-methylamino-valerophenone), Methedrone (4-methoxy-N-methcathinone, p-methoxymethcathinone), 3,4-methylenedioxy-N-methylcathinone (Methylone or bk-MDMA), β-keto-N-methylbenzodioxolylbutanamine (Butylone, bk-MBDB), 3,4-methylenedioxy-N-ethylcathinone (MDEC, bk-MDEA, Ethylone), Pyrovalerone and 3,4-methylenedioxypyrovalerone (MDPV). The derivatizing agents were initially optimised for incubation time and temperature then compared with peak area values, accuracy, and relative standard deviations (RSDs), linearities, limits of detection (LODs) and recoveries. The anhydrides studied proved to be suitable for synthetic cathinones – all were below RSD and accuracy of 20 %. PFPA and HFBA followed by TFA are the best choice of derivatising agents based on validation parameters. TFA and AA are the best based on relative ion intensity. HFBA gives more ions and multi-fragmentation patterns.

Keywords:

Derivatizing agents, synthetic cathinones, mephedrone.

P2 - Identification of drug residues in spiked drinks using attenuated total reflectance-Fourier transform infrared (ATR-FT-IR) spectroscopy

Georgina Butler* Sulaf Assi and David Osselton

Department of Archaeology, Anthropology and Forensic Science, Bournemouth University, UK.

Introduction: Drug-facilitated crimes (DFC) are increasingly prevalent both in the UK and internationally. In many cases reporting is often delayed and thus the use of routine toxicology samples, such as blood or urine, is negated (Jones et al. 2008). The testing of drink samples, often used to administer a drug, have therefore been explored. Current techniques, including Gas Chromatography (GC) and Liquid Chromatography (LC), are destructive, time consuming and require extensive sample preparation (Acikkol et al. 2009; Honeychurch 2015), creating the need for rapid and non-destructive methods that can identify the presence of drugs in a variety of drinks.

Objective: The aim of this work was to investigate the feasibility of using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy to identify drug residues in 'spiked' drinks.

Method: Two over-the-counter sleep aids were selected for this study; one herbal 'Wilko SleepAid' and one synthetic 'Kirkland doxylamine succinate'. The ALPHA FTIR spectrometer (Bruker) was initially used to construct spectral libraries using the tablets, their active pharmaceutical ingredients (API) and their excipients. For each sample 16 scans were performed before a spectra was produced and for each substance a total of three different spectra were obtained. The tablets were 'spiked' into three different beverages; water, tea and coffee, and left for 24 hours. Sub-samples were taken from the drink and the residue at numerous time intervals; 15, 30, 60, 180, 360, 480, 600, 720, 840, 1320, 1440 minutes, and were subsequently measured using FTIR. Upon completion, the drink was filtered and the resultant wet residue was measured, left to dry naturally then measured again.

Results: Initially, the raw FTIR spectra of the crushed tablets were compared to the spectra of raw materials mentioned on their labels and those commonly present in formations (figure 1). In this respect, as seen in figure 1, the FTIR spectra of the crushed Wilko Sleep Aid tablet showed corresponding peaks to amylose, hops and maize starch between 3500 and 2800 cm-1. Additional peaks were observed between 1600 and 600 cm-1 and may correspond to microcrystalline cellulose.

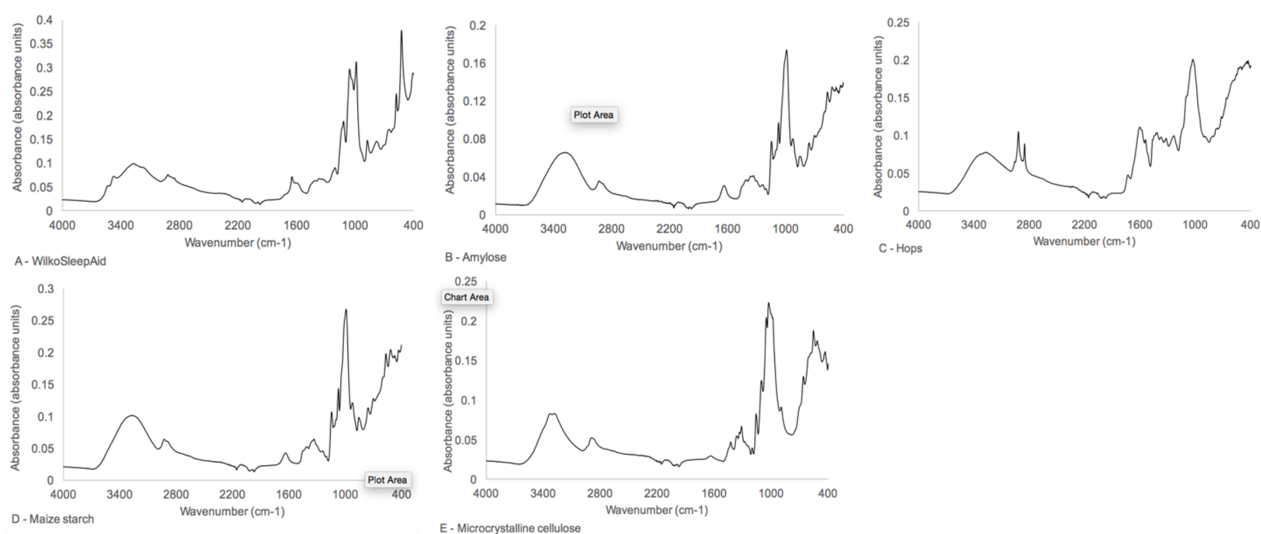

Figure 1 – Raw FTIR spectra of crushed valerian tablets and its excipients measured using the Bruker Alpha FTIR spectrometer equipped with ATR.

During the experiments the FTIR activity of water masked the FTIR activity of the tablets due to low drug concentration, short dissolution time and while the residue remained wet. Between 0 minutes and 24 hours small peaks began to show between 1600 and 600 cm-1 in the residue samples, however the exact excipient in which the peak corresponds to was unknown. Only after 24 hours, once the residue was filtered, were the tablets able to be identified, from both the wet and dry residue

Similarly, when repeated using the synthetic sleep aid, only after 24 hours, once the residue was filtered, were the tablets able to be identified, from both the wet and dry residue.

Discussion/Conclusions: The results show the viability of using ATR-FTIR spectroscopy to identify both herbal and synthetic drug residues in a spiked drink after 24 hours.

References

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P3 - The determination of the cross reactivity of *new* benzodiazepines with commercially available dipstick tests

Marie-Anne Duncan*, Hazel J. Torrance, Lauren C. O'Connor
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New benzodiazepines are categorised as Novel Psychoactive Substances (NPS), and may include benzodiazepines prescribed in other countries *e.g.* etizolam and phenazepam, or designer benzodiazepines synthesised solely for abuse purposes *e.g.* pyrazolam and flubromazepam.
Individuals are using NPS as a way to not only circumvent legislation but also to evade drug testing. The latter is particularly evident with those required to prove abstinence in legal situations. The purpose of this project was to determine the cross reactivity of six *new* benzodiazepines and two metabolites with commercially available dipstick tests. Drug-free urine was spiked at a range of concentrations (7.5–500 ng/mL), which was tested in duplicate with the dipsticks and the concentrations were confirmed using LC-MS.

The dipstick's cross reactivity was assessed on the concentration at which a presumptive positive result was attained for each drug. *New* benzodiazepines with structural similarities to oxazepam – the dipstick's target – exhibited greater cross reactivity. A total of five *new* benzodiazepines (diclazepam, flubromazepam, flubromazepam, phenazepam, and pyrazolam) and one metabolite – delorazepam – were found to cross-react at concentrations well below the cut-off (300 ng/mL).

Etizolam differs significantly in structural comparison to oxazepam, and did not cross-react at any of the concentrations. Moreover, 3-Hydroxyphenazepam appears to be unstable in methanol and therefore, did not cross-react.

There is limited reliable ante-mortem data to compare the concentrations at which cross reactivity was evident. A reliable comparison could be made if data was generated from *e.g.* prison samples, which would firstly be tested with the dipstick and then quantified using LC-MS.
Etizolam is a prominent *new* benzodiazepine in Scotland, as shown by the 2014 drug-related death statistics. This highlights the need to improve the dipstick's ability to detect thienotriazolodiazepines, as this is likely what the majority of individuals are taking, or illicitly produced diazepam has a high etizolam content.

Keywords:

Dipsticks, *new* benzodiazepines, Cross-reactivity

P4 - Quantitative Determination of Paracetamol (Acetaminophen) in Human Whole Blood and Vitreous Humour *in vivo* from Patients undergoing Vitrectomy Surgery.

Stephanie Farrant[1]*, Andrew Morris[2], Nicola Jones[1], Shane McCabe[2], DJ Dagleish[2], Avinash Aswath[2] and M David Osselton[1].

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Introduction

This presentation describes the findings of an investigation to determine the distribution of paracetamol between blood and vitreous humour in living patients undergoing vitrectomy surgical procedures.
Post-mortem toxicology is routinely used to determine the contribution of drugs or poisons to an individual's death however, interpreting the results of post-mortem analysis is often challenging due to uncertainties regarding the disposition of drugs within the body. Post-mortem drug concentrations in blood do not always reflect antemortem drug concentrations as a consequence of post-mortem redistribution. Because drug concentrations can be significantly affected by post mortem change alternative matrices such as the vitreous humour have been investigated to support interpretation. Although blood is currently the preferred matrix for the determination of drug concentrations at the time of death, this may not be truly representative when applied to cases in which there is a time delay between death and sampling. It has been well documented that many drugs enter into the vitreous humour following drug consumption and the ease with which vitreous fluid may be analysed has led toxicologists to investigate vitreous as an alternative matrix for toxicological investigation. This study uniquely provides ratios between blood and vitreous where samples have not been subject to post-mortem variation.

Objective

Although vitreous humour offers many advantages as a specimen for the forensic toxicologist there is a lack of knowledge regarding drug movement and kinetics from the circulating blood into the eye.
In this study, *in vivo* paracetamol concentrations were measured in vitreous humour and venous blood collected from patients undergoing vitrectomy surgery at Bournemouth Hospital (n=17). Administration of paracetamol was either via an oral pre-medication routine approximately 1-2 hours before the procedure or via intravenous (IV) infusion 10-20 minute's pre-operative. Blood and vitreous samples were collected simultaneously in order to determine a ratio between the two samples.

Methods

All samples were extracted using a modified LLE procedure from that outlined by Simonsen et al (2010). Chromatography was undertaken using a Perkin-Elmer series 200 HPLC system with an autosampler, binary pump, fixed wavelength UV detector, vacuum degasser and a C8 column (5 µm). The mobile phase used consisted of HPLC grade acetonitrile: water (1:3, v/v) adjusted to pH 7 using phosphoric acid.

Results

Results showed that paracetamol can be detected in vitreous humour samples following the administration of a 1g dose either orally or via infusion. Table 1 shows the relative concentrations found in both specimens and their respective vitreous: blood (V:B) ratios (n=17 patients). The results show distinct differences in drug ratios depending on the route of administration which may also reflect the time taken for the drug to enter the vitreous.

Route of Administration	Vitreous Concentration	Mean Blood Concentration	V:B ratio
Oral	4.03	10.24	0.39
Oral	3.81	15.64	0.24
Oral	3.42	10.99	0.31
Oral	4.89	20.53	0.24
Oral	6.54	10.89	0.6
Oral	2.37	3.74	0.63
Oral	2.56	12.94	0.2
Mean	**3.95 (2.37-6.54)**	**12.14 (3.74-20.53)**	**0.37 (0.2-0.63)**
IV	0.52	76.07	0.01
IV	0.63	36.2	0.02
IV	0.31	29.31	0.01
IV	2.1	47.08	0.04
IV	2.88	48.72	0.06
IV	1.26	44.66	0.03
IV	2.97	31.13	0.1
IV	2.89	49.03	0.06
IV	1.33	23.03	0.06
IV	2.47	30.62	0.08
Mean	**1.74 (0.31-2.89)**	**41.59(23.03-76.07)**	**0.08 (0.01-0.1)**

Table 1 – Paracetamol concentrations found in blood and vitreous humour samples and their respective V:B ratios.

Conclusion

Paracetamol enters the vitreous humour following oral and IV dosing. Concentrations were significantly higher in blood than in the vitreous humour with an overall mean vitreous: blood ratio of 0.21. The range of paracetamol concentrations were 3.74-76.07 µg/ml and 0.31-6.54 µg/ml in blood and vitreous humour respectively. The route of paracetamol administration greatly influenced the V:B ratios. Patients administered paracetamol orally had a mean V:B ratio of 0.3729 compared to the IV mean V:B ratio of 0.083. It is highly likely that the V:B ratio is greatly affected by many factors, especially the time between drug administration and sample collection.

P5 - Epidemiological study of carbon monoxide poisoning in Scotland

Claudia Forés

University of Glasgow, Department of Forensic Medicine and Science

Carbon monoxide (CO) is a gas generated by the incomplete combustion of hydrocarbons. This gas interacts with haemoglobin reducing its capacity to carry oxygen and leading to hypoxia. The complex formed by the binding of CO to the haemoglobin is called carboxyhaemoglobin (COHb). CO intoxications are quite frequent in forensic toxicology; however, there are very few studies that compare the level of CO among different types of death and examine the demographics of the individual.

Using a total sample population of 209 CO-related deaths in Scotland from the year 2007 until May 2016, this study includes an epidemiological analysis. It also provides ranges of COHb concentrations for each type of case according to the CO source, such as fires and motor vehicle exhausts, and examines the possible role played by alcohol and some pre-existing diseases in the response that the body has to CO intoxication.
Toxicology and police reports were examined for the level of COHb in the blood samples, the presence of other substances and some epidemiological parameters, such as the age, gender and locality. The circumstances of the death and some socio-medical factors, including chronic alcohol abuse, prescribed medications, and the physical and mental health status were also recorded. Statistical analyses were applied in order to examine the potential correlation between findings. Percentages were calculated for the different epidemiological aspects and MS Excel and SPSS software were used to carry out the statistical tests.

Findings of this study show that the proportion of males and individuals over 40 in CO-related deaths is higher than the proportion of females and young people, respectively. Some trends in CO poisoning cases have been determined in different localities of Scotland. The main source of CO poisoning in Scotland is fire, followed by vehicle exhausts, portable barbeques, and generators and gas supply systems. The mean COHb level in firerelated cases is significantly lower than in non-fire-related cases (mean levels of 41.74 and 63.74%, respectively, with a p-value below 0.001). Males accounted for the majority of suspected suicides, with motor vehicle exhausts being the main source of CO in this type of case. Alcohol was detected in a high proportion of the cases (69%), and some prescription drugs, such as benzodiazepines, and some drugs of abuse, such as cannabis, were the most frequently found in CO-related cases. COHb levels were significantly different between cases with and without pre-existing cardiovascular diseases, but not between cases with and without pre-existing respiratory diseases.

This study was approved by the Medical Veterinary & Life Sciences Ethics Committee at the University of Glasgow

Keywords:

Carbon monoxide (CO), carboxyhaemoglobin (COHb), poisoning

P6 - Determination of the stability of duloxetine in solutions and in the autosampler

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The analysis of duloxetine is important for post-mortem toxicology. Literature and experience in our laboratory suggest that duloxetine may be unstable in certain conditions. Here we have assessed the effect of laboratory lighting conditions. Two light boxes were fabricated to measure duloxetine stability when exposed to fluorescent and LED light sources. In addition, the stability of duloxetine was examined in methanol and acetonitrile solvents and using norfluoxetine-d6 and duloxetine-d3 as internal standards. Duloxetine was stable in the autosampler for at least 36 hours in both solutions and with both internal standards. Solutions of duloxetine were generally stable for at least three weeks in ambient temperature, freezer and refrigerator storage conditions. Moreover, Duloxetine in acetonitrile was more stable than in the methanol solution, and it was also stable when measured using IS ratio with duloxetine-d3 (IS) rather than norfluoxetine-d6. Duloxetine exposed to LED and fluorescent light was not significantly affected. That may attribute to the lumens level and the exposure period of time.

Keywords:

Duloxetine, stability, autosampler, Duloxetine-d3, LC-MS/MS, LED, Fluorescent, Solutions.

P7 - Determining the best strain of β-glucuronidase for the hydrolysis of morphine glucuronides in blood, and optimising the temperature of the process.

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Within the UK heroin abuse remains a significant problem. It is a highly unstable drug and rapidly metabolises to morphine within the body. Morphine itself metabolises to form two main metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). For accurate morphine analysis these metabolites must be converted back into morphine through a pre-treatment enzymatic hydrolysis step. Thus far, research has focused almost entirely on the hydrolysis of morphine metabolites within urine. It was the aim of this project to look in detail at the process within blood. Four different strains of β-glucuronidase were investigated. Blank blood was spiked with known concentrations of the metabolites and the hydrolysis pre-treatment process carried out. Morphine was then extracted using solid phase extraction and gas chromatography-mass spectrometry was used to quantify the levels of morphine within each sample. % conversion calculations were carried out to quantify the performance of each strain of β-glucuronidase. The study identified that the *Escherichia coli* strain of β-glucuronidase was the most promising for effective metabolite conversion. It produced a % conversion of M3G and M6G of 25.3 and 32.3%, respectively. In comparison, β-glucuronidase from *Helix pomatia* and *Patella vulgata* both performed to a similar degree and were slightly less effective than *Escherichia coli*. They showed a higher % conversion of M3G compared to M6G. Finally, β-glucuronidase from bovine liver was recognised as a very poor choice for use in the hydrolysis pre-treatment step. Overall, the conversion of both metabolites back to morphine was marginally better within blood than urine. A further preliminary investigation identified 45°C as the optimum temperature for enzyme hydrolysis in blood. This project can now lead to additional studies identifying further optimal conditions for morphine glucuronide hydrolysis within blood, and, hopefully a continuation in improving morphine analysis within forensic toxicology.

Keywords:

β-glucuronidase, hydrolysis, morphine

P8 - The Analysis and Interpretation of Mass Spectrometric Data from Synthetic Cathinones

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Synthetic cathinones have been the main subject of NPS challenges due to their rapid changes. These drugs are a major concern to forensic toxicologists by owing to the problems associated with their detection and analysis.

The aim of this research was to study the fragmentation characteristics in mass spectra of synthetic cathinones. These characteristics were investigated by looking for similarities and differences. The differences in mass spectra were used to differentiate synthetic cathinones by their mass spectra. Whereas the similarities were used to developed a prediction model to determine the structure of unknown synthetic cathinones.

Twenty-two synthetic cathinones were investigated for their mass spectra characteristics. They were methcathinone, ethcathinone, diethylpropion (amfepramone), buphedrone, pentedrone, 4-MMC, 3-MMC, 4-MEC, 3-MEC, 4-FMC, 3-FMC, α-PPP, α-PBP, α-PVP, pyrovalerone, naphyrone, MDPV, pentylone, butylone, ethylone, and methylone. The characterisation was carried out by determining 3 major important peaks in synthetic cathinone EI mass spectra. The molecular ion was fragmented to predict the possible fragmentation mechanism and fragments to match these target peaks. The fragmentation was carried out by applying one or more suitable simple mass fragmentation mechanisms proposed by McLafferty. Characteristic fragments from synthetic cathinone mass spectra were compared by their mass to charge ratio (m/z) to find similarities and differences. These findings were then plotted to illustrate how to differentiate each compound and to develop the prediction model.

The majority of synthetic cathinones can be differentiated through their differences in EI mass spectra. The differentiation was carried out by interpretation of the base peak, which represents the iminium ion, and two characteristic ions originating from the ring. The similarities of mass spectra of synthetic cathinones produced a prediction model which can be used for determination of unknown synthetic cathinones from their spectra. Common features plus differences will be illustration using various fragmentation patterns.

Keywords:

mass spectra, synthetic cathinones, prediction model

P9 - Applicability of Biochip Array Technology to the Multiplex Driving Under the Influence of Drugs (DUID) Screening in Whole Blood

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Introduction. Drug impaired driving is becoming a major problem worldwide. Drug detection involves initial screening of samples for drugs. For legal purposes, the screening procedure eliminates all negatives and positive results require confirmation using confirmatory methods. A multi-analytical approach, enabling the simultaneous screening of drugs, would be advantageous to consolidate testing and increase screening capacity. Biochip array technology enables the simultaneous detection of multiple analytes from a single sample. This study reports the applicability of a biochip array, presenting a broad specificity profile, to the multiplex DUID screening in whole blood. The use of this biochip array allows the semi-quantitative determination of the parent molecule and metabolites of drugs and increases the detection capacity in testing settings.

Methods. Competitive chemiluminescent biochip-based immunoassays were employed. Ligands were immobilized and stabilized on the biochip surface defining an array of discrete test sites. The assays were applied to the fully automated biochip analyser Evidence. The light signal generated from each of the test sites on the biochip was detected using digital imaging technology and compared to that from a stored calibration curve. The signal output is inversely proportional to the concentration of drug in the sample. The system has dedicated software to process, report and archive the data produced.
Assays included were: amphetamine (AMPH), methamphetamine (MAMP), barbiturate (BARB), benzodiazepines (BENZ1, BENZ2), benzoylecgonine/cocaine (BZG), buprenorphine (BUP), cannabinoids (THC), dextromethorphan (DMP), fentanyl (FENT), generic opioids (OPDS), meprobamate (MPB), methadone (MDONE), opiates (OPIAT), oxycodone (OXYC1, OXYC2), phencyclidine (PCP), tramadol (TRM), tricyclic antidepressants (TCAs) and zolpidem (ZOL). The sample volume required was 60µl of whole blood (4-fold dilution).

Results. The biochip array presented broad specificity profile: the AMPH assay detected 7 compounds with cross-reactivity (CR) >24%, the MAMP assay detected 6 compounds with CR>69%, the BARB assay detected 10 compounds with CR>33%, the BENZ assays detected 26 compounds with CR>21%, the BZG assay detected 4 compounds with CR>54%, the BUP assay detected 2 compounds with CR>42%, the THC assay detected 2 compounds with CR>25%, the DMP assay detected 3 compounds with CR>20%, the FENT assay detected 6 compounds with CR≥27%, OPDS assay detected 10 compounds with CR>21%, MPB assay detected 2 compounds with CR≥88%, the MDONE assay detected MDONE, the OPIAT assay detected 10 compounds with CR>38%, The OXYC assays detected 5 compounds with CR≥29%, the PCP assay detected PCP, the TRM assay detected 2 compounds with cross-reactivity>34%, the TCAs assay detected 17 compounds with CR>24%, the ZOL assay detected 2 compounds with CR>47%.
The assays presented the following limits of detection (LOD) for neat samples: AMPH 2.76 ng/mL, MAMP 10.0 ng/mL, BARB 3.67 ng/mL, BENZ1 0.21 ng/mL, BENZ2 0.60 ng/mL, BZG 1.03 ng/mL, BUP 0.11 ng/mL, THC 2.96 ng/mL, DMP 0.07 ng/mL, FENT 0.09 ng/mL, OPDS 1.23 ng/mL, MPB 7.23 ng/mL, MDONE 1.46 ng/mL, OPIAT 0.5 ng/mL, OXYC1 1.01 ng/mL, OXYC2 0.73 ng/mL, PCP 0.27 ng/mL, TRM 0.34 ng/mL, TCA 2.77 ng/mL, ZOL 0.35 ng/mL. Intra and inter-assay precision for different concentration levels expressed as CV (%), was ≤20% for all the assays. Recovery values ranged from 75% to 132%.

Conclusions. The results indicate applicability of biochip array technology to the simultaneous screening of drugs associated with DUID in whole blood. The simultaneous immunoassays arrayed on the biochip surface and applied to the Evidence analyser, allow the multi-analytical screening of samples. The system incorporates dedicated software to process and archive the multiple data generated. This multi-analytical approach leads to test consolidation and increases the screening capacity in test settings.

Keywords:

Drug Impaired Driving, Biochip Array Technology, Whole blood

P10 - Could the Bladder be a site for Post-mortem Redistribution?

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Postmortem redistribution is a process that can have an effect on the alteration of drug concentrations after death. Compared with other sites, less work has been carried out on the possible effect of the bladder in this process. This drug storage organ has the potential to affect drug concentrations in the femoral vein, which is used as the main site for toxicological interpretation. It is unknown how quickly the bladder begins to break down after death and as a result the amount of drug that can diffuse from the bladder into the surrounding tissue. Diffusion studies of porcine bladders were carried out on Franz cells looking into how different temperatures (37, 20 and 5°C), pH (pH 7.4 and 5) and degradation affects the diffusion profile of Rhodamine B. The results show the fastest diffusion was pH 7.4 at 37°C and the slowest diffusion at 5°C for both pH values. Overall, pH 5 displayed slower diffusion than pH 7.4. In relation to post mortem drug movement in humans, as the body cools overtime, these results show that bladder breakdown is unlikely to be a factor in affecting drug concentrations in the femoral vein.

P11 - Characterisation of Pre-Workout Supplements

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Over the years the use of pre-workout supplements (PWS) has increased in the sports industry, amongst both professional and non-elite athletes. The supplements contain a variety of ingredients such as caffeine, creatine and branched chain amino acids (BCAAs) that all work synergistically to produce the associated ergogenic effects.

Although the use of supplements is popular amongst athletes, several studies have analysed different types of PWS and determined inaccuracies in their ingredients labels. Supplements such as *Craze, NOXPUMP, Jack3d* and many more have all been found to contain synthetic harmful substances such as phenethylamines (PEAs) and methylhexaneamine (DMAA). The majority of these substances are not included on the ingredients label, however some of them are listed under misleading names such as "dendrobium" or "geranium" extract, that do not clarify the true nature of the compounds.

Due to the popular status of PWS in the sports industry, it is very important to highlight the issues associated with the majority of supplements, for both legal and health reasons. Because of the inconsistencies in ingredient labelling, some athletes have been banned from competition sports due to positive drug testing, where drugs like PEA derivatives (*N*-ethyl-α-ethyl-phenethylamine and *N,N*-diethyl-phenylethylamine) have been detected. This project reviewed several studies, some of which were carried out in co-operation with the World Anti-Doping Agency (WADA), to screen supplements for the presence of substances that are banned in sports. Many of these substances included analogues of the stimulants amphetamine and methamphetamine as well as DMAA, which was listed under the name geranamine indicating it was extracted from the natural geranium plant. Research into the supplement indicated that this was not the case, with several studies confirming similar results.

Additionally, the project also aimed to use gas chromatography/mass spectrometry (GC-MS) to analyse six PWS (*NO-XPLODE, Shadow-X, The Curse, Gold Standard, Grenade, Xcelr8 Raw*) to determine any inconsistencies in the ingredients' labels. Preliminary procedures were carried out to investigate the solubility properties of each supplement, which was then followed by liquid–liquid extraction and GC-MS analysis. The results indicated the presence of caffeine, and three fatty acids (oleic, stearic and palmitic acid) not listed in the ingredients.

The outcome of the study was to highlight the significance of investigating the components in PWS, in order to avoid future adulteration. Routine analysis of new emerging supplements should be given greater importance to protect athletes from the risk of unintentional administration of harmful compounds that could potentially jeopardise their career in sports.

Keywords:

Pre-Workout Supplements, Phenethylamines, Doping

P12 - Analysis of Ethylene Glycol and related compounds using headspace gas chromatography

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Ethylene Glycol is a common household chemical that has been linked to a number of poisonings, both accidental and deliberate, in the UK. Three rapid headspace-gas chromatography (HS-GC) methods were assessed for their suitability, in-house, in the detection of ethylene glycol in aqueous solutions. The methods employed a single-step derivatisation, using phenylboronic acid, followed by differing extraction temperatures and gas chromatography parameters. A literature method was shown to be the most consistent for the Clarus 580 GC-FID, with an Elite BAC 1 column. This method gave an elution time for ethylene glycol of between 0.613 and 0.616 minutes, showing a range of only 0.003 minutes. Ethylene oxide could not be studied with this method due to problems encountered in the study; however, care will need to be taken in the analysis of ethylene oxide because of similar retention times to that of ethylene glycol. This method had a run time of only 3 minutes per sample, and had the added benefit of having a constant temperature for the gas chromatography oven, allowing the highest throughput of samples in the shortest time.

Keywords:

Ethylene Glycol, Headspace, Gas Chromatography

P13 - Assessing the usefulness of Solid Phase Microextraction (SPME) for drug analysis

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Solid phase microextraction (SPME) is a simple yet convenient sample preparation technique that has been developing for the past two decades. It overcomes some of defects of the conventional sample preparation techniques in terms of time consuming, sample volume, excessive use of solvents and detection limit. This project focuses on the development and application of the new solid phase microextraction (SPME) device, SPME LC Tips, on the optimization of the extraction of amphetamine and methamphetamine. The SPME LC Tips consist of functionally bonded silica particles with a suitable stationary phase coated into a metal fibre using an inert binder and affix in a pipette tip. The extraction mechanism of the SPME LC Tips is based on the distribution constant of the targeted analytes equilibrated between the fibre coating and the sample matrix. The fibres were evaluated for the extraction of amphetamine and methamphetamine from organic solvents and urine. The extracted compounds were analysed by gas chromatography-mass spectrometry (GC-MS) with PFPA derivatisation. In this study, several parameters were optimized in terms of extraction efficiency such as the type of fibre coatings (C18, C18-SCX, and PDMS/DVB), extraction vial, extraction time, desorption vial, desorption solvent, and pH of the buffer. The results show that the SPME LC Tips with C18-SCX coatings performed best for the extraction in organic and urine sample. Methanol produced the best response for the desorption solvent. Preliminary results from spiked urine samples will be presented, with discussion on potential effects of protein binding between the analytes and the urine content, interference by the buffer, or sample loss during evaporation. Further optimization, repetition and validation need to be taken for the SPME LC tips method development.

Keywords:

SPME LC Tips, method development, amphetamine

P14 - Development of a Lateral Flow Immunoassay Capable of Detecting Ochratoxin A

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Mycotoxins are secondary, highly toxic metabolites of several filamentous moulds. These toxins are prevalent in agricultural commodities. Ochratoxin A (OTA) is one such mycotoxin produced by many species of Aspergillus and Penicillium and has been recognised as a common contaminant of cereal crops intended for human and animal consumption. As OTA is genotoxic, hepatotoxic, neurotoxic, immunotoxic, teratogenic and has been recognised by the International Agency for Research on Cancer as a possible group 2B human carcinogen, it is critical to limit the quantity of OTA present in food sources.
The objective of this research was to design and develop a lateral flow immunoassay capable of detecting minute quantities of Ochratoxin A. It is necessary for rapid assays which can be used to rapidly, visually detect OTA contamination under non-laboratory conditions which are in compliance with the European tolerable daily intake levels.

A lateral flow immunoassay has been developed using a monoclonal antibody against OTA labelled with gold nanoparticles as the visual detector. The optimum conditions for conjugation of 40nm colloidal gold to OTA-MAb were established and implemented at the BioHub, Birmingham University, UK in collaboration with Abingdon Health Ltd. (for reasons of commercial confidentiality, further details are not provided within this poster). The reagents were deposited by spraying onto nitrocellulose membrane by spraying using ZX1000B BioDot Dispenser. Ochratoxin A-Conjugated-Bovine Serum Albumin (OTA-BSA) was immobilised as the test line; the control line was sprayed with Sheep-anti-mouse Antibody (SaMAb) and the visual detector was OTA-MAb conjugated to colloidal gold. Three separate concentrations of OTA-BSA (test line) were sprayed onto separate strips for testing for optimum conditions for OTA detection. The strips comprised a nitrocellulose reaction membrane, a glass fibre sample and conjugation pad (optional), together with an absorbent pad, backed with laminate plastic backing. The dipsticks were cut into 5mm strips using BioDot CM4000 guillotine and sealed in foil pouches with desiccant silica beads.

The dipsticks were tested in spiked cereal samples (Extracted with 70:30 methanol/water) with concentrations of Ochratoxin A standard (10µg/ml in acetonitrile; Sigma Aldrich) to establish the visual limit of detection. The limit of detection for this assay was defined as the lowest concentration that gave two red lines (control and test line) for a sample not containing OTA and one red line (control line) for a sample spiked with OTA. The working visual limit detection of this assay currently is 10ng/mL of OTA, this is in accordance with European tolerable daily intake limits.

It is crucial for the development of sensitive lateral flow assays for the detection of a range of food contaminants in order to avoid health implications for humans and animals. Mycotoxins compose a significant percentage of food contaminants so it is necessary to be able to detect and minimise OTA contamination to comply with maximum admissible levels of these mycotoxins. The OTA detection dipsticks provide a visually deterministic device which can distinguish between OTA presence and absence in samples. The dipsticks were manufactured in such a way as to allow for future multiplexing of the device to allow for simultaneous detection of multiple mycotoxins in a singular sample.

Keywords:

Ochratoxin A, Lateral-Flow, Nanoparticle

P15 - Toxicological and Demographic Findings in Suspected Suicide Cases

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Suicide is a global problem, with thousands of people losing their lives to it every year. Owing to various socio-economic factors, it has been determined that Scotland has a higher rate of suicide than other parts of the UK. Previous studies have established various demographic trends as well as correlations of alcohol and drug use with suicidal tendencies. However, much of the research in the UK focuses on England and Wales, whereas reports in Scotland examine data from a mental health perspective. Several studies that have been conducted, while comprehensive in terms of the number of drugs that were analysed, are limited in the geographical area of study and fail to reflect changing populations and drug use.

The research conducted was a retrospective study that examined police and toxicology reports from a total of 488 cases in the West of Scotland, involving 324 males and 164 females, ranging from January 2014 to May 2016. Data was collected from these reports concerning demographics such as age and sex, along with toxicological information about detected drugs and alcohol. Collected raw data was organised and analysed using a series of statistical tests with the aim of establishing both toxicological and demographic trends.

Analysis of the information indicated that the maximum number of suicides occurred in individuals aged between 40 and 60 years, and males composed 66% of the studied population. Among the observed methods of suicide, hanging was the most common for both sexes, followed by substance-related deaths. Women were more than twice as likely as males to die due to intoxication or overdose. These findings were in agreement with previous research. Women were also more likely to suffer from additional mental health issues such as depression and to have made previous attempts at suicide
.
Examination of toxicological findings indicated that both males and females showed similar average alcohol concentrations of around 160 mg/dL. Only a small percentage of the population showed alcohol levels above a pre-determined cut-off. Furthermore, alcohol had no influence in an individual leaving a suicide note. Individuals who left suicide note and those who did not leave them behind had similar percentage of alcohol positive cases. Antidepressants and benzodiazepines were the most frequently appearing drugs in the population, with females showing larger percentages of both classes than males. Opioids in females and stimulants in males were the third most common class of drugs. A comparison of circumstances of the incident and corresponding drugs revealed that prescription medication, including antidepressants, benzodiazepines and opioids, were predominant. The most common illicit drug found post-mortem was THC, which accounted for almost half of all illicit drugs.

Keywords:

suicide, Scotland, toxicology

P16 - Stability Study of Emerging Synthetic Cathinones in Stock Solutions

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Synthetic cathinones are analogues of amphetamine. In the late 1920s, the first synthetic cathinones were developed for therapeutic purposes. In recent times, synthetic cathinones have emerged as novel psychoactive substances. They have become newly available and abused. They are sold as inoffensive everyday products over the Internet and in headshops and are marketed as legal, safer and cheaper alternatives to conventional drugs of abuse.

Synthetic cathinones have been associated with road accidents, assaults and fatalities. As a result, the stability of synthetic cathinones in biological matrices has been investigated but there is no published work describing the drugs' stabilities in stock solutions. In this study, the stabilities of mephedrone (4-MMC), 3-methylmethcathinone (3-MMC) and mexedrone in different solvent media (acetonitrile and methanol) were investigated over 28 days when stored at room temperature, in fridge and in freezer. This study is important, as reference standards for synthetic cathinones are scarce and expensive due to the novelty and constant molecular evolution of NPS available. The study allows insight into the optimum storage temperatures, duration of storage and solvent medium for synthetic cathinones. Analytes losses from stock solutions can be minimised and this provides the leverage for accurate toxicological analysis and interpretations and sustainable laboratory work.

A QTRAP LC/MS/MS system was used to measure the drug response. A decline in the drug levels was observed in both solvent systems and at all three temperatures. In methanolic stock solutions, the drugs were most unstable at room temperature and most stable in freezer. In acetonitrile stock solutions, there were no significant differences observed in the drugs' stabilities, stored at different temperatures. At room temperature, the drugs showed better stability in acetonitrile than in methanol. The structural isomers, mephedrone and 3-MMC had similar stability profiles. In accordance to the general trend, mephedrone was the most stable isomer and mexedrone was the least stable synthetic cathinone. It can be concluded that the use of acetonitrile for making synthetic cathinones' stock solutions and storage in freezer are efficient ways to prevent analytes degradation.

Keywords:

Synthetic cathinones stabilities

P17 - Determination of the Stability of Novel Benzodiazepines in Urine using LC-MS/MS

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Introduction
In recent years the prevalence of Novel Psychoactive Substances (NPS) detected in post-mortem samples has increased. One particular group of drugs under the blanket term of NPS is novel benzodiazepines, also known as research chemical or designer benzodiazepines. These novel benzodiazepines are a particular problem in Scotland where a number of drug seizures have recovered 'fake' diazepam tablets that are suspected to contain these novel drugs. There is a lack of literature surrounding the stability of these drugs which is important to understand when it comes to interpreting the concentrations found in forensic and clinical toxicology cases.

Aim
This study aimed to determine the stability of novel benzodiazepines and some of their metabolites (clonazolam, delorazepam, diclazepam, deschloroetizolam, etizolam, flubromazepam, flubromazoloam, lorazepam, lormetazepam, meclonazepam, phenazepam, pyrazolam and 3-hydroxyphenazepam) in urine under ambient conditions (16 to 24°C) over 24 hours, fridge conditions (2 to 8°C) over 28 days, freezer conditions (-18 to -26°C) over 4 weeks and 3 freeze-thaw cycles over a 2-week period.

Method
Blank urine was spiked at high (0.15 mg/L) and low (0.015 mg/L) concentrations with the 13 benzodiazepines and separated into aliquots of 1.4 mL. Three replicates for each concentration, storage condition and time period were measured. Aliquots were stored under the previously mentioned conditions until required for analysis. Six calibrators (range, 0.005–0.20 mg/L), a blank and a high (0.15 mg/L) and low (0.015 mg/) spike were run with each batch and diazepam-d5 was used as the internal standard. Solid phase extraction was carried out using UCT CleanScreen DAU mixed mode columns and the extracts analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analysis was carried out using an Agilent 1260 Infinity LC system coupled to an AB Sciex 3200 QTRAP MS. Chromatography was achieved using a Phenonmenex Gemini C18 analytical column (150 mm x 2.1 mm, 5 µm) protected with a Gemini C18 SecurityGuard column (4 mmx 2 mm) with de-ionised water as mobile phase A (40%) and methanol as mobile phase B (60%), both supplemented to a concentration of 2 mM ammonium acetate and 0.1% formic acid. The column temperature was maintained at 40°C. Positive electrospray ionisation was used and the MS operated in multiple reaction monitoring mode; monitoring two transitions per drug (quantifier and qualifier).

Results
A mean concentration for each of the three replicates per concentration, storage condition and time period was calculated using the quantifier ion and each mean concentration was plotted versus time. Trend analysis was carried out and the ±20% (compared to time 0) criteria was used to determine the stability of the benzodiazepines. Results will be presented in full and discussed.

Keywords:

Benzodiazepines, LC-MS/MS, Stability Study.

Appendix

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Alcohol calculations and their uncertainty

John Searle[1]

Abstract

A dilution model is widely used to link blood alcohol concentration and the quantity of alcohol consumed. Whilst some authors use the total body water formulation of that model, others use the Widmark Factor formulation. A paper by Forrest gave a table of example values of the Widmark Factor and Barbour, based on Forrest's work and using Forrest's computer program, subsequently presented Forrest's results by way of a chart. Whilst the results of Forrest and Barbour are often used interchangeably, there is a significant difference between them on the factors for women. This paper examines the source of the unexpected discrepancy. It is essential to quote an error range, in blood alcohol concentration calculations, for the results. The extent of that error range was investigated by Gullberg who also employed the Widmark Factor formulation. Gullberg concluded that when reporting a calculated blood alcohol concentration, a coefficient of variation of ±21% should be applied. Similarly, Gullberg concluded that when calculating the volume of drink, a coefficient of variation of 12½% should be applied. The present paper derives and publishes the formulae for calculating this coefficient of variation. It is then shown that Gullberg's conclusions are mistaken: the coefficient of variation is not some fixed percentage but must be calculated in each case.

Keywords

alcohol, calculations, error, uncertainty, Widmark

The basis of alcohol calculations

Blood alcohol calculations originated in the 1920s with the pioneering work of Widmark,[1] who noticed, whilst developing the micro-analysis of alcohol, that the results were always higher than might be expected from a simple dilution calculation. In other words, a dose of m grams of alcohol, in a subject of mass M kilograms would always produce a blood alcohol concentration (BAC) higher than m/M.

Widmark realised that this was due to the proportion of water in the body as a whole being less than the proportion of water in blood. Bones and fat contained little water and so absorbed only a low amount of the alcohol. That was raising the concentration in the blood. To allow for this difference, Widmark proposed to incorporate an empirical factor r, so that the calculated BAC C was given by

$$C = \frac{m}{rM} \times 100 \text{ milligrams per 100 millilitres}$$

The factor r, which has the units of litres per kilogram, became known as the Widmark Factor and has been shown to depend on the gender of the subject, as well as a number of anthropometric factors, of which body mass index (BMI) is perhaps the most important. Many other researchers have similarly given subjects a dose of alcohol and measured the BAC, creating a database of figures from which the Widmark Factor can be estimated for any subject. Zuba and Piekoszewsk[2] commented that the Widmark procedure was the most popular method of making alcohol calculations.

In 1981, Watson et al.[3] suggested an alternative formulation, changing the terminology to bring greater clarity and make the dilution equation easier to understand. Instead of thinking of the body water as a proportion of the body constituents, the volume of body water can be estimated directly. According to Watson:

Men

$$V = 2.447 + 0.3362 \times \text{Weight} + 10.74 \times \text{Height} - 0.09516 \times \text{Age}$$

Women

$$V = 2.097 + 0.2466 \times \text{Weight} + 10.69 \times \text{Height}$$

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If the proportion of water in the blood is P litres per litre, then the dilution equation becomes:

$$C = \frac{mP}{V} \times 100 \text{ milligrams per 100 millilitres}$$

The change is a conceptual one, bringing out the underlying dilution model, but mathematically it is a change of terminology. The Widmark Factor is the total body water divided by the product of the body mass and the proportion of water in blood, both of which are known quantities.

Although total body water is more easily understood, ultimately either formulation can be used. One can write either rM or V/P, because those are equivalent and have the same definition: the mass of alcohol in grams necessary, in the absence of elimination, to create in the subject a BAC of 100 milligrams per 100 millilitres. They have the same value for any subject, a value which can be measured experimentally by giving the subject a dose of alcohol and taking a sample of blood.

The present paper discusses the investigations by Forrest[4] who conducted a large number of such tests. The paper also discusses the uncertainty of blood alcohol calculations, including the examples of uncertainty calculation presented by Gullberg[5] and by Zuba and Piekoszewsk.[2] In all those papers, the authors use the Widmark Factor formulation rather than the total body water formulation. Of necessity therefore, this paper also uses the Widmark Factor formulation.

Scope of paper

Blood alcohol calculations are widely presented in court. Such calculations often rely upon the investigations by Forrest, whose paper[4] tabulates examples of the Widmark factor whilst Barbour,[6] to whom Forrest made available his computer program, presented those results as charts. When such a calculation has been made, the conclusions of Gullberg[5] are then often used to estimate the uncertainty of the calculated result.

The present paper is concerned with two problems which have arisen in this process. First, the factors for women which are given as examples by Forrest ought to coincide with the values given by Barbour's chart for those same examples. There appears to be a significant difference. Second, Gullberg did not publish the derivation of any formula and his method of estimating the uncertainty is based on fixed percentages. That appears to be in contradiction to the estimation of uncertainty suggested by Widmark[1] and Alha.[7]

These two topics form the basis of the present paper.

The dilution model

Using metric units, when the alcohol consumed is diluted in the body then the BAC may be written as:

$$C = \frac{100\,m}{rM} - \beta t \quad \text{milligrams per 100 millilitres}$$

(abbreviated here as mg%)

where

C is the calculated BAC at the relevant time
m is the mass of alcohol consumed during the drinking session, in grams
M is the mass of the subject, in kilograms
r is the subject's Widmark Factor in litres per kilogram.
β is the subject's elimination rate, in mg% per hour
t is the duration in hours from the start of the session to the relevant time.

The 'relevant time' is the time at which an estimation of the BAC is required, for example the time when an accident took place. As an abbreviation, C_o will be used to denote the calculated level of BAC had there been no elimination, that is

$$C_o = \frac{100\,m}{rM}$$

The above formula for BAC is based upon elimination occurring at its full rate from the start of drinking to the relevant time. There are three ways in which that may not be the case:

(a) At the start of the session the rate of drinking may have been so slow that elimination was at less than full rate.
(b) The drinking may have been in two sessions, say lunchtime and evening, and in between the blood alcohol reached zero so that elimination stopped for a while. The calculation must be restricted to the current session.
(c) After drinking ceased, the blood alcohol may have reached zero before the relevant time.

Following Gullberg, the mass of alcohol taken into the body may be expressed in further parameters:

$$m = vzad$$

where

v is the volume of drink consumed in millilitres
z is the strength of the drink as percentage ABV ÷ 100
a is the proportion of the alcohol absorbed
d is the density of alcohol (= 0.789 grams per milli-

One may therefore write:

$$C = \frac{100\,vzad}{rM} - \beta t$$

This formula calculates the BAC from a past history of alcohol consumption, a form which may be called the Forward Widmark calculation. The formula may of course be re-arranged to make v the subject and so calculate, from a measured level of blood alcohol B, the volume of drink consumed. That may be called the Reverse Widmark calculation. Widmark gave examples of both directions of calculation.

The Widmark Factor

The Widmark Factor, denoted by r, is not a simple constant but depends on anthropometric parameters. The influence of such parameters was explored by Forrest, who found that gender and BMI were the most important.

Other parameters such as age and stature[3] have been suggested, and BMI has its limitations in characterising body build.[8] However, Forrest's results are widely used. Forrest published examples of what the average factor would be, for men and for women, at different levels of BMI. Those examples, and interpolations between them, are often used in calculations presented in Court.

Barbour subsequently obtained from Forrest the computer program which had been used to calculate the Widmark Factor from the BMI. Barbour then ran the program to obtain extensive results, which he published in the form of two charts, one for men and one for women. When those charts are applied to the examples Forrest gave, it is found that the two authors agree entirely on the results for men, but for women the results differ:

Widmark Factor for men			Widmark Factor for women		
BMI	Forrest	Barbour	BMI	Forrest	Barbour
17.9	0.80	0.80	15.6	0.74	0.74
21.9	0.75	0.75	20.1	0.69	0.69
24.7	0.72	0.72	22.8	0.61	0.65
27.2	0.69	0.69	25.3	0.58	0.62
29.6	0.66	0.66	27.3	0.53	0.60

This discrepancy for women should not exist. The matter is of practical importance since, for a woman of high BMI, it can lead to a difference of 25% or more in the estimation of BAC.

Figure 1 shows the two sets of results. Forrest[9] gave a mathematical relationship which may be simplified to the following form:

Widmark Factor for men	$r = 1.0181 - 0.01213 \times BMI$
Widmark Factor for women	$r = 0.9367 - 0.01240 \times BMI$

For men, the tabulated examples published by Forrest and the chart by Barbour follow closely this simple relationship. For women, the charts of Barbour follow the relationship but the table by Forrest does not. Forrest's examples, for women, appear to be erroneous.

Zuba et al.[5] comment that the procedure developed by Forrest is practical and appears to encompass the current state of knowledge relating to upgrading Widmark's equation. That appears to be the case, but one must work from the simple mathematical expression of Forrest's results and not from the table of examples he gave.

Uncertainty of the calculated result

With the BAC formula, as with any mathematical formula, errors in the input parameters will produce an error in the calculated result. That error can be estimated, by the method of error propagation, from the contribution of each input parameter.

Suppose in general terms that a result y is to be calculated from a formula

$$y = f(x_1, x_2 \ldots\ldots x_n)$$

where the first input variable x_1 is subject to an error of standard deviation S_1, the second input variable x_2 is subject to an error of standard deviation S_2 and so on. If those input errors are normally distributed then they will, according to the method of error propagation, create in y an error which has a standard deviation of:

$$Sy = \sqrt{[\partial y/\partial x_1]^2 S_1^2 + [\partial y/\partial x_2]^2 S_2^2 + \cdots + [\partial y/\partial x_n]^2 S_n^2}$$

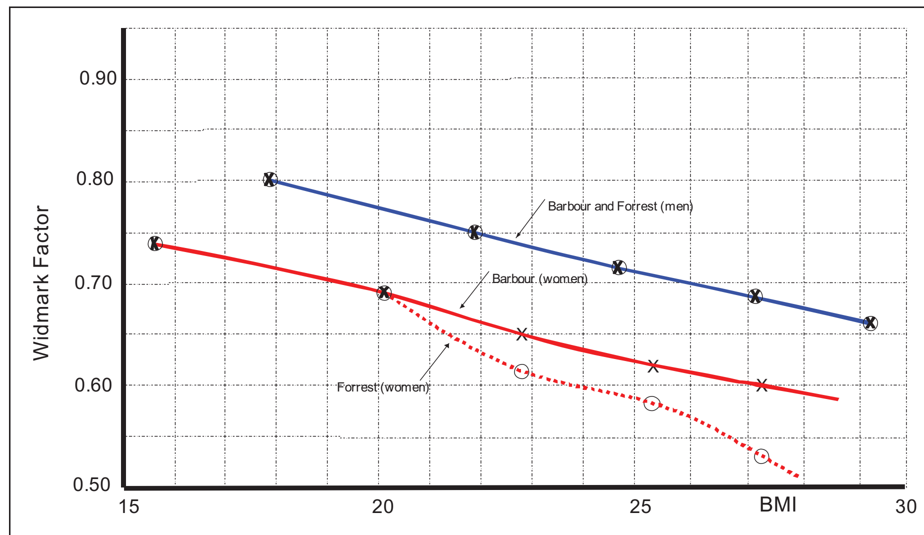

Figure 1. Barbour's and Forrest's results for the Widmark Factor. Note: BMI = Weight in kilograms/Square of height in metres.

The calculation of error propagation is explained in the extensive literature on the subject.[10] The basic principle is that S_1 represents the size of the small errors in x_1, whilst $\partial y/\partial x_1$ represents the change in y which a unit change in x_1 will produce.

The results of applying this formula may often be simplified by expressing the input errors as coefficients of variation rather than standard deviations. The coefficient of variation of each variable is its standard deviation divided by its mean.

Here, the concentration C is a function of eight variables:

$$C = f\,(v, z, a, d, r, M, \beta, t)$$

and each of those input variables will contribute uncertainty to the calculated value of the BAC C. However two of the variables, that is the body mass M and the density of alcohol d (= 0.789), are known with some precision. Their contributions to the uncertainty are ignored in the present paper, although the formula for uncertainty can easily be extended to encompass them.

The remaining variables each have their own uncertainty, which can be expressed as a standard deviation but is more conveniently expressed as a coefficient of variation, that is the standard deviation divided by the mean:

- e_v coefficient of variation of the volume of drink consumed
- e_z coefficient of variation of the alcoholic strength of the drink (ABV)
- e_a coefficient of variation of the proportion of the alcohol absorbed
- e_r coefficient of variation of the Widmark Factor for the individual
- e_β coefficient of variation of the rate of alcohol elimination for the individual
- e_t coefficient of variation of the duration of the drinking session

Error propagation was first applied to alcohol calculations by Widmark himself, later followed by Alha.[7] A shortcoming of their analysis is that they appear to be considering a laboratory environment. They assume that the time when the drink was consumed is known with exactitude whereas in real life there is uncertainty, and often there is uncertainty in the strength of the drink and the proportion absorbed as well. Also, Widmark and Alha did not take into account the negative correlation,[5] that is some –0.135, between the Widmark Factor r and the rate of elimination β. Gullberg took that into account but stated only the general principle of error propagation, without publishing any formula.

Appendix 1 applies the method of error propagation to the uncertainty of the Forward BAC calculation. The coefficient of variation of the calculated level of BAC is found to be:

$$e_c = \frac{C_o}{C}\sqrt{\begin{array}{l}\left[e_v^2 + e_a^2 + e_z^2 + e_r^2\right] + (\beta t/C_o)^2\left[e_\beta^2 + e_t^2\right] \\ -0.27\,(\beta t/C_o)\,e_r e_\beta\end{array}} \tag{1}$$

Some of the coefficients of variation relate to the circumstances of the event under investigation and those coefficients must be estimated from the circumstances. If for example the event is in a laboratory, then the time when the alcohol was consumed will be known exactly, as will be the time at which an estimate of BAC is required, such as the time when a blood sample was drawn. The uncertainty in the duration,

e_v, the uncertainty in the volume drunk, is also likely to be zero in these circumstances. In a real-life event, the duration of drinking and the volume drunk may both have significant uncertainty and estimates of those uncertainties must be made from the circumstances.

Other coefficients of variation concern the parameters relating to alcohol dilution and elimination, that is e_r the accuracy to which Widmark's Factor can be determined, e_z the accuracy of manufacturer's values of ABV and e_β the accuracy of the assumed rate of elimination. Those coefficients of variation have been determined by researchers. Gullberg reviews the published literature and suggests suitable values, that is $e_r = 0.092$, $e_z = 0.03$ and $e_\beta = 0.22$. These will be adopted here, because this paper re-works Gullberg's example and the comparison is made easier by adopting the same input. All the variables are assumed uncorrelated except r and β, where the covariance is –0.135.

Once the coefficients of variation for the input parameters have been established, the uncertainty e_c of the calculated BAC can be obtained from Formula 1.

Appendix 2 presents a similar exercise for the calculation in the other direction, that is the Reverse Widmark calculation of the volume of drink consumed from a measurement of the BAC at a later time. The formula for the volume drunk is:

$$v = \frac{(B + \beta t)}{100\,rM}\,zad \quad \text{where B is the measured level of BAC.}$$

All the variables are assumed uncorrelated except r and β, where the covariance is – 0.135. The coefficient of variation of the calculated value of v, the volume of drink ingested, will be:

$$e_v = \sqrt{\begin{array}{l}[B/B_o]^2 e_B^2 + [\beta t/B_o]^2\left(e_\beta^2 + e_t^2\right) + \left(e_r^2 + e_a^2 + e_z^2\right) \\ -0.27[\beta t/B_o]e_r e_\beta\end{array}} \tag{2}$$

where $B_o = B + \beta t$

Once again the coefficients of variation for the input parameters must be estimated for each of them. The value of e_B, that is the accuracy of blood alcohol analysis, is about 0.0375 in the UK.

Gullberg stated only the general principle of error propagation, without deriving any formula by which the uncertainty could be calculated. He did however give an example and, without showing any working, stated the result he had calculated for it. Gullberg's example is presented here in metric units, but this time giving the formula and calculating through to the result.

In Gullberg's example, a man of mass 81.6 kg having an estimated Widmark Factor of 0.73 ($e_r = 0.092$) drinks 3.55 ± 0.178 litres of beer ($e_v = 0.05$) with an ABV of $4.0 \pm 0.12\%$ ($e_z = 0.03$). It is soon calculated that the mass of alcohol in the drink is $3550 \times 0.040 \times 0.789 = 112$ grams.

It is also soon found that the value of C_o is

$$\frac{100 \times 112}{0.73 \times 81.6} = 188\,\text{mg\%}.$$

The rate of elimination is 14.8 mg%/hour so that after 5 hours, when all the alcohol has been absorbed and some has been eliminated, the calculated BAC will be:

$$C_o - \beta t = 188 - 14.8 \times 5 = 188 - 74 = 114\,\text{mg\%}$$

Hence from Formula 1 the coefficient of variation of the calculated BAC will be:

$$e_c = \sqrt{\begin{array}{l}\frac{188}{114}\left[0.05^2 + 0.03^2 + 0^2 + 0.092^2\right] + \frac{74^2}{188^2} \\ \times\left[0.22^2 + 0^2\right] - 0.27 \times \frac{74}{188} \times 0.092 \times 0.22\end{array}}$$

$$= 0.21.$$

The coefficient of variation of the calculated BAC is therefore 0.21, which is the same result as Gullberg obtained (21%). On the basis of that example, Gullberg concluded that:

> "When reporting an estimated BAC, a 2CV [i.e. twice the coefficient of variation] uncertainty interval should be approximately ±42%".

That is simply not so. The coefficient of variation is not a constant 21% for all circumstances, but must be calculated on a case by case basis. That may be seen from Gullberg's own example, by noting that after a further 7 hours the BAC will have fallen to a calculated 10 mg%. It hardly needs saying that the coefficient of variation of that figure is far greater than the 21% of 10 mg%, which would be only 2.1 mg%. A constant percentage as suggested by Gullberg will not do.

Furthermore, Gullberg has chosen an example where uncertainty in absorption (e_a) can be ignored, as can uncertainty in the duration of the drinking session (e_t).

In that same paper, Gullberg gives an example of the uncertainty of a Reverse Widmark calculation, again without giving any formula. It is based on the same data, except now it is the measured blood alcohol B which is given, as 120 mg%, and the volume of drink is to be calculated. That calculation is straightforward and the result is 3662 millilitres. A back calculation of B_o, the BAC at time zero, gives: $120 + 14.8 \times 5 = 194$ mg%.

By Formula 2 above we have:

$$e_v = \sqrt{\begin{array}{l}[120/194]^2 \times 0.036^2 + [74/194]^2\left[0.22^2 + 0^2\right] \\ +\left[0.092^2 + 0^2 + 0.03^2\right] - 0.27 \times [74/194] \\ \times 0.092 \times 0.22\end{array}}$$
$$= 0.122.$$

In this example, the coefficient of variation of the volume of drink is therefore 0.122, that is about 12½%, the same result that Gullberg obtained. However, again Gullberg generalises that example to all circumstances and says:

> *"A 2CV [i.e. twice the coefficient of variation] uncertainty interval of 25% should be applied when reporting estimates of the number of drinks".*

That is not so at all, as may be seen by considering an example where the measured BAC was 10 mg% but the drinking started 12 hours before. The coefficient of variation of the volume of drink would be about double the value it was in Gullberg's example.

Zuba et al. also give an example of calculating the uncertainty of alcohol calculation, again without giving any formula. Zuba simplified the Widmark Factor to omit elimination and then used a commercially available program on error propagation. Expressed in the nomenclature of the present paper, Zuba's example was:

$v = 250$ millilitres	$e_v = 0.04$	Drink volume
$z = 0.40$	$e_z = 0.0125$	Drink strength ABV
$M = 75$ kg	$e_M = 0.0267$	Body mass
$r = 0.70$	$e_r = 0.0714$	Widmark Factor

With those input values it can soon be calculated that C = 150.3 mg%, the value obtained by Zuba. The coefficient of variation is found, from Formula 1, to be some 0.08708. That corresponds exactly with the result for twice CV quoted by Zuba from his computer program, that being 17.4% which is twice 0.08708 expressed in percentage terms.

Zuba, like Gullberg, generalises the result of the example and says that the uncertainty of blood alcohol calculations is less than 20%. That generalisation is far from correct, especially when elimination has played a large part.

Conclusions

Although the values of the Widmark Factor tabulated by Forrest are derived from the same data as the charts subsequently published earlier by Barbour, for women they differ. Some values in Forrest's table appear erroneous. Those errors may be avoided by using the charts published by Barbour or by using the simple formulae given in this paper.

It is important when reporting calculations of BAC from volume of alcohol, or in the reverse direction the volume of drink from a later BAC, to provide an estimate of the uncertainty of the result. Gullberg appears to be mistaken in suggesting that the coefficients of variation are fixed percentages, that is 21% and 12½%, respectively. Formulae are presented, in the body of the paper, whereby the coefficient of variation can be calculated.

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Appendix 1

Estimation of the uncertainty of a Forward Widmark calculation

Using the nomenclature of the main paper the Forward Widmark calculation is:

$$C = \frac{100vazd}{rM} - \beta t$$

where C is the BAC at the relevant time

v is the volume of drink consumed in millilitres
a is the proportion of the alcohol absorbed
z is the strength of the drink as percentage ABV ÷ 100
d is the density of alcohol (= 0.789 grams per millilitre, constant)
r is the subject's proportion of body water in litres/kilogram, divided by the proportion of water in blood in litres/litre (Widmark Factor)
M is the mass of the subject, in kilograms
β is the subject's elimination rate, in mg% per hour
t is the duration from the start of the session to the relevant time, in hours

Uncertainty in any of the input parameters will add to the uncertainty in C, that is the calculated BAC. Writing S with a suffix to denote the standard deviation of each parameter, the uncertainty (standard deviation) of the BAC is:

$$S_c = \sqrt{\begin{array}{l}[\partial C/\partial v]^2 S_v^2 + [\partial C/\partial a]^2 S_a^2 + [\partial C/\partial z]^2 S_z^2 \\ + [\partial C/\partial r]^2 S_r^2 + [\partial C/\partial \beta]^2 S_\beta^2 + [\partial C/\partial t]^2 S_t^2 \\ + 2[\partial C/\partial r] \cdot [\partial C/\partial \beta] \cdot \mathrm{Cov}(r, \beta)\end{array}}$$

All the variables are assumed uncorrelated except r and β, where the covariance is –0.135 $S_r S_\beta$.

Performing the partial differentiations, we obtain:

$$\frac{\partial C}{\partial v} = \frac{C_o}{v} \quad \frac{\partial C}{\partial a} = \frac{C_o}{a} \quad \frac{\partial C}{\partial z} = \frac{C_o}{z} \quad \frac{\partial C}{\partial r} = \frac{C_o}{r}$$

$$\frac{\partial C}{\partial \beta} = t \quad \frac{\partial C}{\partial t} = \beta$$

Putting those partial derivatives into the formula for the uncertainty of the BAC, and writing $S_v = v e_v$ and so on for the other variables:

$$e_c = \frac{C_o}{C}\sqrt{\begin{array}{l}\left[e_v^2 + e_a^2 + e_z^2 + e_r^2\right] + (\beta t/C_o)^2\left[e_\beta^2 + e_t^2\right] \\ -0.27(\beta t/C_o) e_r e_\beta\end{array}} \tag{1}$$

Appendix 2

Estimation of the uncertainty of a reverse BAC calculation

With the reverse calculation, the volume of drink that has been consumed is to be calculated from a measured level of blood alcohol, denoted by B, obtained from a sample taken at the relevant time. The uncertainty of the blood alcohol measurement is e_B.

Using the nomenclature of the main paper, also presented in Appendix 1, the reverse BAC calculation is:

$$v = (B + \beta t)\frac{zad}{100\,rM}$$

The standard deviation of the calculated value of v, the volume of drink ingested, will be:

$$S_v = \sqrt{\begin{array}{l}[\partial v/\partial C]^2 S_c^2 + [\partial v/\partial \beta]^2 S_\beta^2 + [\partial v/\partial t]^2 S_t^2 \\ + [\partial v/\partial z]^2 S_z^2 + [\partial v/\partial a]^2 S_a^2 \\ + [\partial v/\partial r]^2 S_r^2 + 2[\partial v/\partial r] \cdot [\partial v/\partial \beta] \cdot \mathrm{Cov}(r \cdot \beta)\end{array}}$$

All variables are assumed uncorrelated except r and β, where the covariance is –0.135 $S_r S_\beta$

Performing the partial differentiations we obtain

$$\frac{\partial v}{\partial C} = \frac{v}{(B + \beta t)} \quad \frac{\partial v}{v\beta} = \frac{vt}{(C + \beta t)} \quad \frac{\partial v}{\partial t} = \frac{v\beta}{(B + \beta t)}$$

$$\frac{\partial v}{\partial z} = \frac{v}{z} \quad \frac{\partial v}{\partial a} = \frac{v}{a} \quad \frac{\partial v}{\partial r} = \frac{v}{r}$$

Putting those partial derivatives into the formula for the uncertainty of v and writing $e_v = S_v/v$ and so on for the other variables:

$$e_v = \sqrt{\begin{array}{l}\dfrac{B^2 e_c^2}{(B+\beta t)^2} + \dfrac{B^2 t^2 e_\beta{}^2}{(B+\beta t)^2} + \dfrac{B^2 t^2 e_t{}^2}{(B+\beta t)^2} + e_z^2 + e_a^2 + e_r^2 \\ -2 \times \dfrac{0.135 \beta t\, e_r e_\beta}{(B+\beta t)}\end{array}}$$

Denoting $B + \beta t$ by B_o we have:

$$e_v = \sqrt{\begin{array}{l}[B/B_o]^2 e_B^2 + [\beta t/B_o]^2\left(e_\beta^2 + e_t^2\right) + \left(e_r^2 + e_a^2 + e_z^2\right) \\ -0.27[\beta t/B_o] e_r e_\beta\end{array}} \tag{2}$$

Erratum

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Re: article Alcohol calculations and their uncertainty.

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On page 5 left hand column, the formula should be

$$v = \frac{(B + \beta t)\, rM}{100\, zad}$$

And the same formula in Appendix 2, page 7 right hand column.

$$v = \frac{(B + \beta t)\, rM}{100\, zad}$$

In that same column the symbol C appears three times, and it should be B. Minus signs have been missed in the expressions $\frac{-v}{z}$ and $\frac{-v}{a}$

The expression $\frac{\partial v}{\partial \beta}$ appears incorrectly as $\frac{\partial v}{V\beta}$

THE ESTIMATION OF UNCERTAINTY IN ALCOHOL CALCULATIONS

Abstract

Alcohol calculations are often presented in legal proceedings and in recent years it has become imperative for such calculations to be accompanied by an estimate of the uncertainty. That must be done from the circumstances of the case and papers by Gullberg and by Zuba, suggesting fixed percentages, would appear to be misleading.

The paper presents formulae for calculating the uncertainty in three types of alcohol calculation, those being the Back Calculation, the Forward Widmark and the Reverse Widmark. The same formulae may be applied to calculating the uncertainty of alcohol calculations based on Total Body Water.

Introduction

Alcohol calculations are widely used in forensic work and indeed have been for over 80 years. In the nineteen twenties Erik Widmark, who was one of those developing micro-analysis of blood samples, recognised that the alcohol in the blood was always higher than one would expect from dividing the alcohol ingested by the body mass as if it were all blood. Widmark understood that this was due to some components of the body, such as fat or bone, not helping to dilute the alcohol and he suggested a factor to take account of that.

For all types of forensic calculation, there has been a recent emphasis on providing an estimate of the uncertainty of the result, since without such an estimate the Court would not know what reliance might be placed upon the result. Some alcohol calculations have wide margins of uncertainty and if they were presented without the appropriate 'red flag', it would damage the credibility of this area of work. It is of course much better for the Court to be told the likely range, whatever the margin, rather than the matter being left for inexpert guesses.

It is a truism to say that the uncertainty of the result of an alcohol calculation depends on the uncertainty of the input data. How accurately do we know how much was drunk and its ABV? How well do we know the time from the start of elimination to the 'relevant time', say when an accident has occurred? How sure are we that all the alcohol was absorbed?

The alcohol in the body is the difference between the alcohol absorbed and the alcohol eliminated. That gives rise to another reason why the uncertainty of the calculation varies from case to case. Suppose that a calculation for the blood alcohol two hours after drinking

gave 140mg per 100 millilitres, whilst a similar calculation for ten hours after drinking indicates that it would have fallen to 20. It would be completely wrong to suggest that both figures have the same percentage uncertainty.

Two recent papers dealing with uncertainty have presented conclusions which can only be described as misleading. Gullberg (ref 1) set out the input parameters which contribute to the uncertainty, and gave the general mathematical rule for how to combine uncertainties. Having done so much, Gullberg nevertheless concluded:-

> "Assuming reasonable estimates, a 2CV [twice coefficient of variation] uncertainty of 25% should be applied when reporting estimates of the number of drinks. When reporting an estimated BAC, a 2CV uncertainty interval should be approximately 42%."

For the example just given, applying such percentages would give the uncertainty for two hours after drinking as 81 to 199 mg per 100 ml, and the uncertainty ten hours after drinking as 12 to 28 mg per 100 ml. In fact the latter uncertainty has a wider range than the former, and ± 8 is totally unreasonable. It seems that Mr Gullberg now accepts the conclusion implicit in his own work, that a blanket percentage will not do.

Zuba (ref 2) made a similar generalisation and concluded that "the uncertainty of blood alcohol calculations is generally less than 20%". That generalisation is far from correct, especially when elimination has played a large part.

The purpose of the present work is to provide formulae by which the uncertainty can be calculated for any individual case.

The work of Dr Robert Forrest, linking Widmark's r to gender and BMI, is widely used. Unfortunately Dr Forrest appears to have had the misfortune to state incorrectly, for women of high BMI, what he found. This paper corrects that error, so that Dr Forrest's work can be used with confidence.

Total Body Water

In Widmark's equation the quantity rM, that is the product of Widmark's r and the body mass M, represents the volume of that subject's blood which would have the same diluting effect as the subject's body with its various constituents. Widmark used a fixed value of r but nowadays the value of r can be better estimated from BMI, using a regression equation.

An alternative formulation was suggested by Watson, based upon Total Body Water, by which a regression equation is used to estimate TBW from height and weight. The proportion of water in blood is assumed to be 0.80 for all subjects, and the equivalent diluting volume is then expressed as TBW ÷ 0.80.

Both approaches are estimating the equivalent diluting volume from the height and mass of the subject. Which regression equation is better is determined simply by which gives the more accurate predictions, that is the lower uncertainty. Gullberg suggests that the uncertainty for Widmark's r is 9.2%, whilst Watson suggests 9.04% for men and 10.65% for women as the uncertainty of Total body Water.

There seems no real difference between the two approaches. If the Total Body Water approach is preferred, then in the uncertainty formulae one would simply replace the coefficient of variation of r with that of TBW.

This paper deals with blood alcohol, which is directly related to intoxication. Of course one may deal instead with its proxy measure, breath alcohol, but that is not done here.

<u>Back calculation</u>

To see how to calculate the extent of uncertainty, let us start with the simple back calculations formula:-

$$C = B + \beta t$$

where	C = BAC at the relevant time,	mg%	To be calculated
	B = BAC when sampled later,	mg%	From the case
	β = Subject's rate of elimination,	mg% per hour	From research
	t = Interval between relevant time and sampling,	hours	From the case

We want to calculate the uncertainty of C from the input uncertainties:-

S_B = Uncertainty of BAC measurement

S_β = Uncertainty of rate of elimination

S_t = Uncertainty of the time interval

Now look at how those contribute to the uncertainty in C.

For each unit of uncertainty in B we get one unit of uncertainty in C

For each unit of uncertainty in β we get t units in C

For each unit of uncertainty in t we get β units in C

It would be wrong simply to add those contributions, because they will usually balance one another out to some degree. It would be remarkable if say the error in B happened to be positive and towards the top end, and also the errors in β and t were also positive and towards the top end. All three errors occur independently and one cannot simply add them.

The correct statistical way of combining the contributions is to square them, add, and take the square root.

$$S_C = \sqrt{S_B^2 + t^2 S_\beta^2 + \beta^2 S_t^2}$$

<u>Forward Widmark calculation</u>

Very often an event has occurred, or a crime has been committed, and the intoxication level of one or more of the participants is of interest to the courts. If no breath or blood sample has been taken, but there are accounts of what had been drunk beforehand, then the forensic toxicologist has the responsibility to estimate the likely BAC. It cannot be left to others to make an inexpert guess at the level of intoxication.

The Forward Widmark formula gives the BAC expected from the ingestion of m grams of alcohol by a subject of mass M kilograms, at a time t hours later:-

$$C = \frac{100m}{rM} - \beta t$$ The constant of 100 comes from the mix of units.

Widmark was dealing with the laboratory environment where the mass of alcohol is given to the subject, and the time when it is administered, is known precisely. In the forensic environment that is not the case, which was noted by Jones and Gullberg. Uncertainty therefore arises from the underlying parameters determining the mass of alcohol:-

- v the volume of drink taken
- z the ABV of the drink

Furthermore it may be that absorption was not complete at the 'relevant time' and the proportion absorbed is denoted by a. In these terms Widmark's Equation may be written:-

$$C = \frac{100avzd}{rM} - \beta t$$

In order to apply this formula one needs, for the subject, a value for the ratio r. Forrest conducted extensive research linking Widmark's r to gender and BMI, which will be used as a basis. The work was very valuable but, for obese women, Forrest appears to have had the

bad luck to mis-state what he had found. The error came to light when Barbour re-published Forrest's results, this time in the form of charts.

Taking out that error, Forrest's results (or Barbour's charts) may be represented by two simple expressions:-

For men Widmark's r = 1.0181 – 0.01213 x BMI

For women Widmark's r = 0.9367 – 0.01240 x BMI

The coefficient of variation of r is about 0.092. That uncertainty takes into account not only the variability from person to person, but also any variability due to dilution/elimination not following the Widmark model.

Uncertainty of Forward Widmark calculation

The Forward Widmark formula is more complicated than a simple back calculation and the calculation of the uncertainty is similarly more complicated. It follows however the same principles: each of the input uncertainties is multiplied by the sensitivity of the result to that variable. Those contributions are then squared and added, and the square root taken. It is assumed that the mass M of the subject is known without any significant uncertainty. Similarly the density of alcohol d is known without uncertainty to be 0.789.

The uncertainty is more conveniently written not in terms of the standard deviations of each input variable, but in terms of the coefficient of variation, that is the standard deviation expressed as a proportion. If say the BAC were 150 and had a standard deviation of 15, the coefficient of variation would be 0.10.

The uncertainty formula for the Forward Widmark is

$$e_c = \frac{C_o}{C} \sqrt{[e_v^2 + e_a^2 + e_z^2 + e_r^2] + (\beta t/C_o)^2 [e_\beta^2 + e_t^2] - 0.27\,(\beta t/C_o) e_r e_\beta}$$

where $C_o = C + \beta t$

The formula has multiple components representing the multiple input uncertainties, but is easily calculated on spreadsheet or calculator. Note that as the predicted BAC tends to zero, the uncertainty of the estimate increases without limit.

Reverse Widmark calculation

Sometimes, as Erik Widmark recognised, it is necessary to calculate in the reverse direction, from a measured BAC to the volume of drink consumed. That occurs when say a motorist tells the police after an accident that he had drunk only a modest amount, but the BAC reading is high. Was the motorist misleading the police, or was his account possibly correct?

The approach to that is to calculate the volume of drink by turning the Forward Widmark formula around:-

$$v = (B + \beta t) \frac{r M}{100az}$$

where B the sampled BAC at the later time takes the place of the estimated BAC at the relevant time. The other variables are the same as before. In that way one can calculate, from a sample taken at say 5.00am the next morning, the volume of beer drunk before leaving the public house at 11.00pm.

Uncertainty of Reverse Widmark calculation

A somewhat surprising fact about the Reverse Widmark is that it has a lower uncertainty than the Forward Widmark. One might think that going backwards, calculating say that 6 pints were drunk from being told that the BAC was 130mg% when sampled 4 hours later, would have essentially the same uncertainty as going forwards on the same data, that is calculating a BAC of 130mg% from being told that 6 pints were drunk 4 hours before. That is not so: the reverse calculation has less uncertainty.

The reason is that the Reverse Widmark is additive, in the sense that the alcohol consumed is found by adding the contributions of the measured BAC and of the amount likely to have been eliminated. The result of that calculation has less percentage uncertainty than the other way, when the alcohol remaining is the difference between alcohol consumed and alcohol eliminated.

The formula for the uncertainty of the Reverse Widmark calculation is:-

$$e_v = \sqrt{[B/B_o]^2 e_B^2 + [\beta t/B_o]^2 (e_\beta^2 + e_t^2) + (e_r^2 + e_a^2 + e_z^2) - 0.27[\beta t/ B_o] e_r e_\beta}$$

where $B_o = B + \beta t$

Again this may be used either as a spreadsheet, or simply on the calculator.

<u>The input uncertainty</u>

In Widmark calculations, whether forward or reverse, there are six input variables which may have significant uncertainty.

Starting with absorption, it may be plain that all of the alcohol had been absorbed by the relevant time. However if not, the proportion likely to have been absorbed must be estimated by the toxicologist. That should be done as a range, say 0.60 to 0.90, in which there is a 70% chance that the proportion absorbed will fall. Such a range would be equivalent to saying that the mean absorption is 0.75 and the standard deviation is 0.15. This is an example of an input uncertainty which is due to the uncertainty of an expert's opinion.

The next input uncertainty in the Forward Widmark is the volume of drink consumed, and that needs to be determined from the evidence in the case. When there are many accounts of what was drunk, one may average them and determine the standard deviation from halving the range which contains 70% of the accounts. If there are only one or two accounts it is possible to say "if the evidence of X is accepted ………" and then proceed with zero uncertainty on that variable.

The strength of alcoholic drinks of course varies. For beers, if the brand is known like Timothy Taylor's Landlord bitter or Stella Artois lager, the accompanying list gives the nominal ABV. The actual ABV will deviate from nominal with a coefficient of variation of about 0.03 ABV.

It is however more usual that the drink will be known only in generic terms, such as 'beer' or 'cider' or the like. From published information on the ABV of the different brands, it would seem that the following are reasonable estimates.

Draught ales	$z = 0.0377$	$e_z = 0.064$
Bottled ales	$z = 0.0493$	$e_z = 0.165$
Draught lagers	$z = 0.0447$	$e_z = 0.112$
Bottled lagers	$z = 0.0474$	$e_z = 0.127$

It would appear that the various brands of draught ale have a greater uniformity than the others, whilst bottled ales have a higher average strength and variability, due in part to the

inclusion of ‘barley wine’ type products. The SIBA Beer Report gives the average ABV in the UK as 4.1%.

Normal brands of cider appear to average about 4.96% with a coefficient of variation of 0.135. There is however also ‘white cider’, generally sold in 2 litre bottles, and that has an ABV of 7.5%, where there is a taxation step. The coefficient of variation is low, at about 0.03.

The ABV of ‘wine’ is about 12.6% with a coefficient of variation about 0.10. If the colour of the wine is known then one can be more specific, with ABVs of 13.2% (red), 12.5% (white) and 11.6% (rose). With that extra information, the coefficient of variation falls to about 0.07.

There are a few special wines, such as Prosecco, which have a lower ABV outside the usual range.

The rate at which alcohol is eliminated from the body has been the subject of much research and different experts may use slightly different figures. Unless one has a particular view of the matter, one may follow Gullberg and adopt 14.8 mg per 100ml/hr, with a coefficient of variation of 22%.

The Rearward Widmark calculation is based upon a later measurement of BAC, and that measurement of course has its own standard deviation which, in the UK, is about 3.75%. Note that the measured BAC may be given with two SDs already taken off and if so those must be put back in to obtain the original measurement.

<u>The output uncertainty</u>

By using the uncertainty of the input variables, the standard deviation of the output is obtained. For a back calculation or a Forward Widmark the coefficient of variation of the BAC is obtained as a proportion of the BAC. Similarly for a Reverse Widmark calculation the coefficient of variation of the quantity consumed is obtained as a proportion.

Uncertainty is not a concept well understood by the public, whether in absolute terms as a standard deviation or as a proportion as a coefficient of variation. The uncertainty may be better understood if it is said that there is a 70% likelihood that the BAC was within a certain range of mg per 100 ml (giving the mean ± one standard deviation) and a 90% likelihood that it was within some wider range of mg per 100ml (giving the mean ± two standard deviations).

In the same way, with the Reverse Widmark the range of drink volume with 70% likelihood can be bracketed, as can the range with 90% likelihood.

Validation

The general formula for the propagation of error is too well known to need validation, having been around for 200 years. It is used widely across science and technology.

Erik Widmark was the first to apply this mathematical approach to calculating the uncertainty of alcohol calculations. That appeared to work well, but it was for a controlled environment where the mass of alcohol and the time elapsed after drinking it were both exactly known. Gullberg, relying on Jones and Sundberg, extended the approach to the normal situation in the field, where these factors carry their own uncertainty.

Gullberg did not publish any formula for the uncertainty, but he did quote examples of Forward and Reverse Widmark calculations, giving the standard deviations calculated for those examples. The uncertainty formulae presented in this paper, when applied to Gullberg's examples, give the results he published.

Zuba had a different approach, calculating the output standard deviation by applying a Monte Carlo program to input variables each with their own standard deviation. Zuba, as with Gullberg, published an example result but without an explicit formula. When the uncertainty formula given in this presentation is applied to Zuba's data, Zuba's result is obtained.

Conclusions

1. When giving a back calculation or a Widmark calculation, it is important or indeed imperative to give the uncertainty of the estimate. Just giving the mid-range result is no longer acceptable.
2. This presentation gives uncertainty formulae for the common calculations, allowing the likely range to be specified.
3. The formulae are from a standard mathematical method and are extensions of those originated by Erik Widmark. They have been validated against the examples quoted, with no formulae, by Gullberg and by Zuba.

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Acknowledgements

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Bottled Ales

Brand	ABV%
Old Speckled Hen	5
Hobgoblin	5.2
Newcastle Brown Ale	4.7
Sharp's Doom Bar	4.3
Fuller's London Pride	4.7
Spitfire	4.5
Badger Fursty Ferret	4.4
Bishop Finger	5.4
Black Sheep Ale	4.4
Theakston Old Peculier	5.6
McEwan's No 1 Champion Ale	7.3
Old Crafty Hen	6.5
Tribute	4.2
Old Golden Hen	4.1
Abbot Ale	5
Martons Pedigree	4.5
Punk IPA	5.6
Badger Golden Champion	5
Marstons Old Empire	5.7
Landlord	4.1
Guiness	4.2
Murphy's Stout	4

Bottled Lagers

Brand	ABV%
Budweiser	4.8
Stella Artois	4.8
Becks	4.8
Peroni	5.1
San Miguel	5
Cobra	4.8
Corona	4.6
Heineken	5
Carling	4
Kronenbourg 1664	5
Sol	4.5
Desperados	5.9
Coors	5
Tiger	5
Carslberg Export	4.8
Carlsberg Lager	3.8
Grolsch	5
Foster's Gold	4.8
Amstel	4.1
Carling Zest	2.8
Leffe Blonde	6.6
Kingfisher	4.8
Foster's Ice	5
Foster's Export	4
Miller Genuine Draft	4.7
Bud Light	4.2
Hoegaarden	4.9
Budweiser Budvar	5
Heineken Export	5
Bavaria	5
Asahi	5
Brahma	4.3
Foster's Twist	4.5
Pilsner Urquell	4.4
Bud Ice	5.5
Red Stripe	4.7
Holsten	5
Tennants Lager	4

Draught Ales

Brand	ABV%
Tetley Smoothflow	3.6
Worthington's Creamflow	3.6
John Smiths Smooth	3.8
Boddingtons D/flow	3.5
John Brewers E/Smooth	3.6
Gray's Ex/Smooth Bitter	3.6
Stones Bitter	3.7
Worthington Ale (N.E)	4
Tetley Imperial (N.E.)	4.3
Greenalls Bitter (N.W.)	3.6
Ansells Bitter (Midlands)	3.8
Newcastle Exhibition	4.3
McEwans Best Scotch Bitter	3.6
Magnet	4
Brains Dark Smooth	3.5
Brains SA Smooth	4
Trophy Bitter	3.6
Trophy Special (N.E.)	4
Northumberland IPA	3.6
Brains Smooth Bitter	3.7

Draught Lagers

Brand	ABV%
Carlsberg Lager	3.8
Tuborg	4.2
Castlemaine xxxx	3.9
Export Lager	4.1
Becks Vier	4
Stella 4%	4
Fosters	4
Carling	4.1
Carlsberg Export	5
Kronenbourg	5
Stella	5
Heineken	5
Dortmunder	5
Budweiser	5
Carlsberg Export	5

SALES

2015- 108393000 barrels **sold** in UK-=177764520 hl of beer (UK measurement of '1 Barrel' = 164 L/1.64 hl)
Recorded quarterly (per '000 barrels) - Q1= 27235 Q2= 26843 Q3= 27172 Q4= 27143
Jan-Mar 2016= 27089 = 44425960 hl

2013- Lager sales= 3120000 hl, Ale sales= 885000 hl

Annual sales in UK in excess of £18bn

In 2013- estimated **real ale production** in UK =3637000 hl, total real ale shares in beer market = 8.2% (Source- BBPA)

2014- Average person in the UK drank 7.57 l alcohol

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Conference Dinner - Etihad Stadium

Timings

1930 Arrival at Etihad Stadium

2300 Last Orders

2330 Leave Etihad

2356 Last Tram

Getting to the Etihad Stadium

The Etihad Stadium is easily accessible using all modes of transport and is within easy walking distance from Manchester City Centre.

Car

The Stadium is extremely accessible from all major highways. As you reach Manchester, follow the signs for SportCity. The stadium address for satellite navigation is: Etihad Stadium, Etihad Campus, Manchester M11 3FF. There is parking available on site and guests should enter via Gate 11 on Ashton New Road (A662). Parking is available in the Yellow Car Park and visitors should report to the Colin Bell Stand reception, entrance U, on arrival. Parking is free of charge.

Metrolink

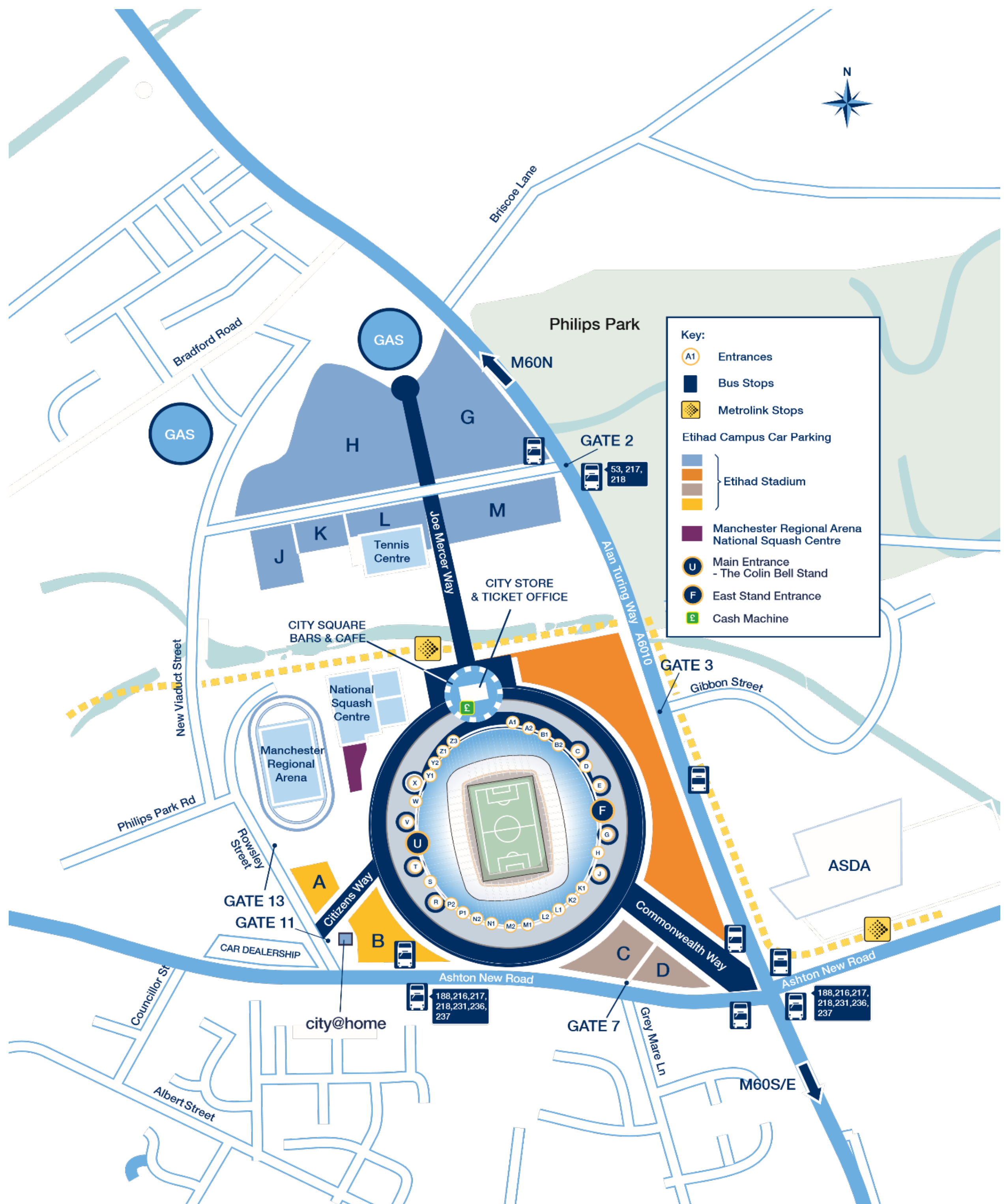
The Metrolink runs from the City Centre, Victoria Train Station and Piccadilly Train Station directly to the Etihad Stadium and on towards Droylsden. The journey time between Piccadilly Train Station and the Etihad Campus stop is 8 minutes. The Etihad Campus stop drops you directly into the City Square Fanzone. **Tickets must be purchased before travel** – ticket machines are located on every Metrolink platform. If you are travelling to Manchester by train, you can usually purchase your Metrolink journey with your train ticket. The consumption of alcohol is not permitted on Metrolink trams. Trams leave approximately every 15 minutes on weekdays. On arrival, visitors should report to the Colin Bell Stand reception, entrance U. **The last tram leaving the Etihad Campus on 18/08/2016 is at 23:56.**

Taxis

Recommend private hire: Mantax +44 (0)161 230 3333. On arrival, visitors should report to the Colin Bell Stand reception, entrance U.

Walking

There is a safe, well-lit and signposted walking route from Manchester Piccadilly Station. Signage will direct you along the route. Please follow the 'City Link' signs. It will take approximately 20-minutes to walk the route. On arrival, visitors should report to the Colin Bell Stand reception, entrance U.


N
Philips Park
GAS
GAS
M60N
Briscoe Lane
Bradford Road
Key:
Entrances
Bus Stops
Metrolink Stops
Etihad Campus Car Parking
Etihad Stadium
Manchester Regional Arena
National Squash Centre
Main Entrance
- The Colin Bell Stand
East Stand Entrance
Cash Machine
G
H
GATE 2
53, 217, 218
J
K
L
M
Tennis Centre
Joe Mercer Way
Alan Turing Way A6010
CITY STORE
& TICKET OFFICE
CITY SQUARE
BARS & CAFE
GATE 3
Gibbon Street
New Viaduct Street
National Squash Centre
Manchester Regional Arena
Philips Park Rd
Rowsley Street
GATE 13
GATE 11
A
B
Citizens Way
CAR DEALERSHIP
ASDA
Commonwealth Way
C
D
Ashton New Road
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