Editorial

Addressing the challenges in forensic drug chemistry

The area of forensic drug chemistry offers exciting opportunities for exploring a multifaceted world and complex problems. Advances made in instrumentation and new applications of analytical methods form an important basis, but the context of work, the questions asked, and how the information is interpreted, are equally important. This special issue captures a diverse range of examples that illustrate these multidisciplinary opportunities of engagement with the world of forensic drug chemistry and toxicology.

The identification and characterization of newly emerging drugs (new psychoactive substances, NPS) form a continuous part of forensic drug chemistry, which can be an especially daunting prospect within the toxicology setting where pre-existing knowledge of a drug’s identity is often needed to implement targeted screening approaches. Difficulties can emerge in the presence of isomers and the fact that mass spectral data alone may not always be sufficient to obtain unambiguous data. At the same time, the study of isomers can go beyond the confines of analytical and synthetic chemistry when pharmacological investigations are included, which can reveal isomer-related differences in pharmacodynamic properties. Equally, some specific isomers may be captured by legislative control measures whereas others may not, so attention to detail is needed because the variations of existing legislation associated with controlled substances around the globe can be quite significant.

Some recently emerging NPS, related to small-scale manufacturing in a traditional clandestine environment back in the 1980s, elicit tragic memories. The difference between then and now, as for example observed with the emergence of fentanyl analogs, is the scale of manufacturing and the ease of global distribution. The identification of misrepresentation, adulteration, or misbranding is a frequent companion of the forensic chemist and the work can facilitate communication between different stakeholders concerned with controlled substances, illicit drugs, medicines, or dietary supplements. As covered in previous special issues in this journal, there exists a striking overlap between those worlds. 

Chemical profiling and analytical fingerprinting efforts remain important tasks, being applied to traditional drugs, NPS, or biological fluids. Knowledge about the quality of street drugs is essential for monitoring and intelligence purposes, and there is a need to strengthen and build on opportunities for data sharing and collaboration.

The presence of isomers when identifying new psychoactive substances (NPS)

This special issue begins with the study presented by McLaughlin et al. who describe the chemical analysis of two powdered products offered by an online retailer as the psychostimulant 4-fluoromethylphenidate (4F-MPH). Interestingly, it was revealed that one powdered product consisted of a diastereomeric mixture represented by racemic (±)-threo and (±)-erythro 4F-MPH whereas the other product consisted of (±)-threo-4F-MPH only, thus, reflecting distinct batches prepared during synthesis and incomplete product clean-up. Furthermore, after chromatographic separation, both forms were subjected to in vitro pharmacological characterization using rat brain synaptosomes, which revealed that the biological activity, i.e., catecholamine selective blockade of monoamine reuptake, was associated with (±)-threo form. 

The NPS mephedrone was advertised as a non-controlled replacement for 4-methylmethcathinone (mephedrone) when it was released to the market. Speculations about the potential identity of mephedrone circulating on the Internet led McLaughlin et al. to attempt a prediction followed by organic synthesis of N-methoxymephedrone. When released to the market, mephedrone was identified as the structural isomer 3-methoxy-2-(methylamino)-1-(4-methylphenyl)propan-1-one that was subsequently also synthesized by the authors. During these studies, it was observed that α-chloromethylmephedrone was identified as a by-product during mephedrone synthesis and that mephedrone was identifiable as a synthesis by-product during the preparation of N-methoxymephedrone, which, as the authors speculate, might have explained why N-methoxymephedrone was not developed and released to the market. Furthermore, pharmacological investigations demonstrated that mephedrone was a weak non-selective uptake blocker without releasing activity at the dopamine transporter (DAT) and norepinephrine transporter (NET), although it displayed weak releasing activity at the serotonin transporter (SERT). Interestingly, N-methoxymephedrone was shown to act as a weak uptake blocker at DAT, NET, and SERT, as well as a fully efficacious substrate-type releasing agent across all three transporters. The synthesis by-product α-chloromethylmephedrone was inactive in all assays.

3-Fluorophenmetrazine (3-FPM), an analog of the controlled psychostimulant phenmetrazine, was obtained from a test purchase by McLaughlin et al. who confirmed its identity by organic synthesis and the preparation of the ortho- and para-substituted positional isomers 2- and 4-FPM, respectively. Under gas chromatography–mass spectrometry (GC–MS) conditions, sufficient separation of the three isomers was obtained by derivatization with heptafluorobutyric anhydride and pentfluoropropionic anhydride. Implementation of liquid chromatography-mass spectrometry (LC–MS) also facilitated adequate separation. Interestingly, derivatization of FPM isomers with trifluoroacetic anhydride yielded diagnostically useful information in the resulting electron ionization mass spectra. 3-FPM could be differentiated from the 2- and 4-FPM isomers, which was considered helpful because of incomplete separation between derivatized 2- and 3-FPM under GC conditions. Several synthetic cannabinoid receptor agonists (SCRAs) contain a 1-adamantyl moiety connected to indazole and indole carboxamide templates. An investigation into the synthesis and
analytical characterization of N-(2-adamantyl)-substituted isomers is presented by Asada et al. who established the ability to differentiate between them, thus, offering predictions of SCRA isomers potentially emerging in the future. An extensive analytical characterization is presented and the authors confirmed that it was possible to differentiate between APINACA, APICA, SF-APINACA, SF-APICA, SCI-APINACA, and adamantyl-THPINACA and their N-(2-adamantyl) counterparts.[10]

Segawa et al. present an exciting application of supercritical fluid chromatography (SFC) and demonstrated that it was feasible to separate positional isomers of ring-substituted amphetamine and methamphetamine analogs. A total number of 30 analogs have been investigated that comprised of ring-substituted analogs of amphetamine and methamphetamine substituted with methyl, methoxy, fluoro, chloro, and bromo groups at the 2-, 3-, and 4-position and most of them were separated within 6 min. The investigations also included recordings of the UV full scan and electrospray ionization tandem mass spectra.[9]

Identification of emerging substances

The controlled substance 3,4-methylenedioxymethamphetamine (MDMA) has always been a popular substance and attempts to develop and disseminate potential candidates of MDMA-like substances either on the NPS or the traditional drug market have always been a matter of interest. The report provided by Collins et al. gives the reader insights into the identification of N-tert.-butoxy carbonyl-MDMA (t-BOC-MDMA), which is a derivatized form of MDMA, obtained from a seizure by the Australian Border Force. As expected with the attachment of such a protecting group, hydrolysis in an acidic medium successfully led to its conversion to MDMA. Furthermore, exposure of t-BOC-MDMA to conditions found in the human stomach (pH 1.5, 37°C) was demonstrated to convert almost completely after 305 min, thus, suggesting that t-BOC-MDMA might potentially serve as a pro-drug in vivo. The authors also demonstrated the preparation of t-BOC derivatives of methamphetamine, pseudoephedrine and mephedrone using di-tert.-butyl dicarbonate.[11] The identification of t-BOC-MDMA was reported in Germany as well,[11] where the seized product also contained silica gel. It was unclear whether the silica gel was added deliberately or whether it was a remainder from a purification procedure using column chromatography[11] but one cannot help but speculate whether a deliberately prepared mixture of t-BOC-MDMA (or any other drug suitable for such a conversion) with silica gel (acidic properties) could give rise to a home production option where a particular pro-drug might be converted to the desired substance, for example in the form of employing an espresso-type machine, thus, potentially resulting in 'ecstasy' type formulations (ecstasy-espresso).

A group of compounds that have particularly caused reasons for concern in several countries are analogs of fentanyl. Over the last few years, a range of analogs has emerged, not only as openly sold ‘research chemicals’, but also in the form of misrepresented products or surreptitiously as hidden admixtures to other substances, such as heroin. The re-emergence of fentanyl invokes tragic memories from the 1980s, where clandestinely produced materials led to pockets of social harm in the USA.[12] Rójkiewicz et al. report on the identification and characterization of 4-fluorobutyrfentanyl (4-FBF) involved in two fatal intoxications that occurred in Poland, which was also identified as a powder and in the form of an electronic cigarette (e-liquid) that were in possession of the deceased. The authors were able to purify the powdered sample and to employ it for quantitative determinations of 4-FBF levels in biofluids and in the e-liquid. In this instance, the authors went one step further when employing purification of the seized powder given that certified material was unavailable.[13] In a related study, Breindahl et al. describe the identification and characterization of acryloylfentanyl (acrylfentanyl) in Denmark that was seized in a psychiatric ward in capsule form.[14]

An extensive analytical characterization of six methylphenidate analogs was provided by Klare et al., who identified 4-methylmethylphenidate, 3,4-dichloromethylphenidate, ethylphenidate, 3,4-dichloroethylphenidate, ethylphenylphthlate and N-benzylethylphenidate. All compounds exhibited the (±)-threo-configuration based on x-ray crystallography studies, which was consistent with biologically active methylphenidate and other analogs.[15]

The need for chemical profiling and fingerprinting

An important element of work in the drug chemistry area includes the study of synthetic routes for forensic purposes. The ring-substituted cathinone methylene, possibly most conveniently prepared from 3,4-methylenedioxypropiophenone, was synthesized from catechol in a four-step procedure by Heather et al. to explore the prospect of using an alternative starting material. The synthetic route was characterized with the help of GC–MS and nuclear magnetic resonance spectroscopy analysis. The authors detected six organic impurities in the 1,3-benzodioxole intermediate, six organic impurities in 3,4-methylenedioxypropiophenone and five organic impurities in methylene, respectively. Several identified impurities were subsequently verified by organic synthesis.[16]

One of the starting materials employed in the synthesis of amphetamine is α-phenylacetocetoxonitrite (APAA) that may be used for the conversion to 1-phenyl-2-propanone (P2P) by hydrolysis under acidic conditions. The hydrolysis of APAA to P2P followed by implementation of the Leuckart procedure was investigated by Power et al. who identified 2,3-diacetyl-2,3-diphenylsuccinonitrile and 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-indene as by-products. An alternative method that could provide access to a non-controlled precursor to P2P is α-methylstereine (AMS) that can undergo transformation into P2P by oxidation using potassium peroxymonosulfate and sodium iodide. When employing this particular approach and exposure of the reaction product to the Leuckart reaction, the impurities 1,1,3-trimethyl-3-phenyl-2,3-dihydro-1H-indene and 1-phenyl-N-(phenylethyl)propan-2-amine were identified by the authors. The presence of the two indenes suggested the potential for differentiation between the two synthetic routes.[17]

Another route that may be used for the preparation of P2P employs phenylactic acid (PAA) as the starting material and one of the reagents used includes the use of lead (II) acetate. Toske et al. investigated the PAA/lead (II) acetate route and subjected the P2P reaction product to reductive amination conditions for the synthesis of methamphetamine. The authors identified the presence of trans-N-methyl-4-methyl-5-phenyl-4-penten-2-amine, called the P-compound, and concluded that it was formed from reductive amination of trans-4-methyl-5-phenyl-4-penten-2-one, a component found in a ketone-cluster detected in a GC–MS trace of crude P2P. The identity of the P-compound was also verified by organic synthesis. Interestingly, the authors report that most
methamphetamine samples obtained from domestic and USA-Mexico border seizures were associated with PAA as the source for P2P and that the PAA/lead (II) acetate reaction may be in decline compared to the route employing PAA/sodium acetate and acetic anhydride.\cite{18}

The analysis of acid/neutral extracts obtained from illicit heroin samples is described by Casale et al. who focus their investigation on the identification and characterization of two particular compounds frequently detected during GC analysis. In this investigation, the authors isolated the alkaloid porphyroxine from the opium poppy (Papaver somniferum) followed by acetylation to yield N\(_2\)-O\(_2\)-diacetylporphyroxine. Acid hydrolysis was then employed to produce N\(_2\)-O\(_2\)-diacetyl-O\(_7\)-desmethyl-epi-porphyrine (called the C compound) and N-acetyl-O\(_7\)-desmethyl-epi-porphyrine (called the B compound), respectively. The authors also confirmed that the prevalence of detection for the B and C compounds differed based on geographical origin: Southwest Asian samples (92–93%) > South American (64–72%) > Southeast Asian (45–49%) > Mexican samples (≤ 3%). In total, five compounds were identified that reflect the acetylation of porphyroxine contaminated-morphine during illicit production of heroin.\cite{19}

Poppy seeds on the other hand are also known to contain several alkaloids that can be determined analytically in illicitly manufactured heroin, which are detectable in human urine samples, thus, giving rise to the ‘poppy seed defence’. Analytical strategies used to target acetylated alkaloids detectable in illicitly manufactured but not in pharmaceutical heroin (and their metabolites) have therefore been of importance when tackling this challenge from a forensic viewpoint. In 2014, Chen et al. identified and characterized the urinary glucuronidated metabolite ATM4G as a potential biomarker for street heroin use, which originated from acetylated thebaine and rearrangement to an acetylated phenanthrene derivative followed by metabolic O-deacetylation (ATM4). An initial study with volunteers revealed that neither ATM4 nor ATM4G were detectable in urine following poppy seed administration whereas ATM4G was detected in 16 out of 22 urine samples obtained from heroin users.\cite{20} In this special issue, Maas et al. follow on from these findings and report their results on the analysis of urine samples for ATM4G, 6-AC (6-acetylcodine), papaverine, noscapine, 6-MAM (6-monoacetylmorphine), morphine, and codeine from subjects following consumption of different German poppy seed products and those with suspicion of preceding heroin consumption. Following poppy seed administration in 25 volunteers and urinalysis, morphine was detected in all samples and codeine was detected in 15 out of 25 subjects, whereas 6-AC, 6-MAM, and ATM4G were not detectable. On the other hand, ATM4G was detected in 9 out of 43 urine samples collected from suspected heroin users, thus, confirming that this phase II metabolite should be included in routine analysis. However, it was also suggested that illicitly manufactured heroin with low thebaine levels might present challenges for ATM4G detection.\cite{21}

Another abundant street drug is cocaine and collecting information about the patterns of adulteration can be crucial for studying how imported drugs are trafficked through territories. Ladróu et al. studied the applicability of GC-combustion/pyrolysis isotope-ratio mass spectrometry to the analysis of cocaine samples containing phenacetin, a frequently encountered cocaine adulterant. Pure phenacetin samples collected from various geographical locations were also characterized by elemental-analysis isotope-ratio mass spectrometry. The authors also inform the reader that phenacetin is present in 28% of cocaine cases dealt with by the laboratories of the French Ministry of the Interior. The authors suggest that phenacetin standards could be differentiated based on \(^{\delta^{13}}C\)/\(^{18}H\) and \(^{\delta^{13}}C\)/\(^{15}N\) data but that the discriminative power was unsatisfactory in the analyzed cocaine samples under the experimental conditions used and that further studies are needed.\cite{22}

An Italian perspective about recent drug seizures is presented by Pichini et al., who convincingly argue that a systematic approach to the qualitative and quantitative analysis of adulterants present in seized street drugs are needed to strengthen monitoring and data sharing procedures across regions and police forces. As an example, the authors present preliminary findings from the analysis of heroin, cocaine, MDMA and amphetamine samples seized by the Carabinieri police between 2013 and 2016. The authors have demonstrated that it is possible to map the differences in drug purity originating in different regions, which highlighted the challenges encountered during data collection and interpretation and that further collaborations with other national police forces are highly desirable.\cite{23}

**Misrepresentation and adulteration of folk medicines and dietary supplements**

An increasing challenge in a globalized and commoditized world involves the ability to assess the quality of drug-containing products offered for human use, for example in the form of traditional folk remedies and dietary supplements. Two striking examples are included in this special issue that serve as a valuable reminder that one cannot be complacent about quality control and effective enforcement of legislation. Gambir, a plant product prepared from Uncaria gambir (Hunter) Roxb., and native to Malaysia, Singapore, Sumatra, and Borneo, has a history in folk medicine. This herbal product can be purchased from local shops and is also offered for sale via the Internet. Samples labelled as ‘Gambir Sarawak’ submitted by law enforcement were analyzed by GC–MS by Lim et al. who show that the detected constituents were inconsistent with the expected plant product and that the samples revealed constituents commonly found in products sold as Chinese medicines known as Chansu and Liu Shen Wan instead. The significance of this misrepresentation was that these particular products originate from glandular secretions collected from Bufo gargarizans Cantor and Bufo melanostictus Schneider. These toad venoms, known for their use in Chinese medicine, contain a variety of compounds with highly toxic properties, thus, exposing the user of the misrepresented Gambir product to potential poisoning by bufadienolides and other components detected by the authors.\cite{24}

The regulation of dietary supplements in the USA is reviewed by Pawar and Grundel from the United States Food and Drug Administration who provide the reader with an overview of legislation and enforcement options available in their country. The authors also place an emphasis on the presence of naturally occurring and/or added synthetic biologically active phenethylamine derivatives present a range of dietary supplement products, which displays the problems faced by consumers exposed to these constituents without their knowledge.\cite{25}

**Analytical challenges: artefact formation during analysis by GC–MS**

The analysis of drug samples by GC–MS constitutes a main tool in the arsenal of chemical analysis and so it is not surprising to find this tool in a laboratory concerned with forensic drug analysis.
However, a well-established, but nevertheless less frequently reported issue is related to GC-induced formation of analytical artefacts and how this impacts on the analysis and identification of drug samples. The study presented by Dowling et al. describes the analytical characterization of modafinil and related substances that have gained increasing interest as wakefulness promoting agents. Similarly to methylphenidate, modafinil is a controlled medicinal product but non-controlled analogs are also available for purchase. These types of substances are marketed as ‘research chemicals’ and NPS but are also attracting consumers interested in health improvement and cognitive enhancement. In the present investigation, artefacts identified during thermal degradation of modafinil, modafinic acid, adrafinil, CRL-40,940, and CRL-40,941 included diphenylmethanol and 1,1,2,2-tetraphenylethane (TPE). This degradation was confirmed by organic synthesis and subjected to analytical characterization including x-ray crystallography. Similarly, the fluorinated TPE analog was also detected during GC–MS analysis of modafinil, CRL-40,940 and CRL-40,941, respectively. GC-induced artefact formation was not limited to this investigation. The aforementioned study reported by Klare et al. on the identification of phenidates also suggested the occurrence of thermal decomposition in the injection port and/or on column to 2-aryl-ethyl-acetates and 2,3,4,5-tetrahydropyridines and the authors suggested this to arise via a 6-membered transition state consistent with results obtained from DFT-computations. Power et al. who shared their investigation on P2P and amphetamine analysis noticed that one of the identified impurities, the APAAN dimer 2,3-diacetyl-2,3-diphenylsuccinonitrile, converted to 2-methyl-1-phenyl-1,3-dicarbonitrile-1H-ene under GC–MS conditions. The same conversion was observed when the former compound was heated with a heat gun. On the other hand, both compounds remained detectable under LC–MS conditions.

Recent years have seen the appearance of a vast array of new psychoactive substances (NPS), novel precursors for traditional drugs and new synthetic routes but we see in this special issue that scientists have made gargantuan efforts to overcome numerous challenges and, by doing so with great success, they have helped usher forensic drug chemistry into a new era of understanding.

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