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Qualitative drug analysis of hair extracts by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry

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ABSTRACT

A technique using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC × GC/TOFMS) is applied to a qualitative analysis of three sample extracts from hair suspected of containing various drug compounds. The samples were also subjected to a quantitative target analysis for codeine, morphine, 6-monoacetylmorphine (6-MAM), amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethylamphetamine (MDMA), methadone, and benzylpiperazine (BZP) by liquid chromatography-tandem mass spectrometry (LC-MS/MS). GC × GC/TOFMS provided a non-specific procedure that identified various drugs, metabolites, and impurities not included in the target analysis. They included cocaine, diazepam, and methaqualone (quaalude). Comprehensive GC × GC separation was achieved using twin-stage cryo-modulation to focus eluant from a DB-5ms (5% phenyl) to a BPX50 (50% phenyl) GC column. The TOF mass spectrometer provided unit mass resolution in the mass range m/z 5–1000 and rapid spectral acquisition (≤500 spectra/s). Clean mass spectra of the individual components were obtained using mass spectral deconvolution software. The 'unknown' components were identified by comparison with mass spectra stored in a library database.

1. Introduction

Recent advances in sensitive analytical techniques have enabled hair testing for drugs of abuse to be routinely applied in clinical and forensic toxicology [1]. Hair is an analytical matrix that can provide information regarding a person's drug use well beyond the time frames of detection afforded with biological fluids such as urine and blood. Cocaine and nicotine have been detected in the hair of ancient South American mummies over 900 years old [2]. Drugs and metabolites are deposited into the hair-shaft from the bloodstream or from sweat and sebaceous glands [3,4]. As the hair grows any drugs or metabolites used are carried along with it and by roughly estimating how quickly the hair grows it is possible to estimate approximately when the drug use had occurred [5].

Confirmation of the presence of drugs and metabolites is usually achieved by a chromatographic technique coupled to mass spectrometry *e.g.* liquid chromatography-mass spectrometry (LC-MS or LC-MS^n) or gas chromatography-mass spectrometry (GC-MS) [6]. Compounds are separated in the chromatographic step and then are further resolved by the mass spectrometer. Two-dimensional GC (GC × GC) provides an added dimension by separating the sample components in two phases (orthogonal separation) prior to entering the mass spectrometer. Deans switching or 'heart cutting' enables a portion of the GC run to be transferred onto a secondary column using a switching device [7,8]. Moore et al. applied this technique with negative ion chemical ionisation MS (NCI-MS) for the detection in hair of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), a major metabolite of cannabis [9]. Comprehensive GC × GC allows the whole chromatogram to be transferred onto a secondary column and incorporates a type of rapid sampling device. It has been recognised as a technique capable of providing improved resolution of complex matrices compared to conventional single dimensional GC (1D-GC) [10–14]. The two-dimensional (2D) separation provides a huge increase in the peak capacity compared to 1D-GC. Therefore, there is the potential to screen for a huge number of compounds in one protocol. This may help to reduce laboratory analysis time and identify compounds not included in routine analyses.

Comprehensive GC × GC/MS has been applied to drug testing procedures. Kueh et al. extracted drugs 'spiked' in horse urine using solid phase extraction (SPE). The extracts were derivatised with acetic anhydride/pyridine. The analysis was performed using quadrupole MS [12]. Song et al. extracted drugs 'spiked'

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in whole blood using liquid–liquid extraction (LLE). The extracts were not derivatised. The analysis was performed using TOFMS [13]. It is evident that this technique may enable large numbers of drugs and metabolites to be identified and quantified in single protocol. However, the sensitivity of the mass analysers used is relatively low compared with high-sensitivity MS platforms *e.g.* triple–quadrupole MS. Also the type of extraction and derivatisation (or non-derivatisation) procedure will determine what drugs can be detected and at what levels of concentration. Therefore, it is not clear whether this technique has the capability to detect the common drugs of abuse at levels expected to be found in users.

There is considerable interest in the development of high-throughput multi-component chromatographic methods to screen for a large range of compounds. Maurer and Peters reported a clean-up method and analysis by GC-MS, in scan mode, to identify >2000 drugs and poisons contained in a library database [15]. Ojanpera et al. applied mass spectrometry with accurate mass measurement to multi-component urinary drug screening [16]. Gergov et al. applied LC-MS/MS multiple reaction monitoring (MRM) to accurately target 238 drug and metabolite compounds [17]. There are certain difficulties associated with these techniques caused by co-eluting matrix constituents. This may result in ion suppression (or enhancement) of the target ions and/or 'cross-talking' between ions of similar mass-to-charge (m/z) [18–20]. This may inhibit the detection or accurate quantitation. Therefore, high-throughput procedures can require a considerable degree of instrument method optimisation [17].

The analysis of hair extracts presents potential difficulties due to the various endogenous compounds that may be present at much higher concentrations than the drugs or metabolites of interest. Extracts may contain thousands of compounds and it is likely that a certain degree of co-elution will occur. Biller–Biemann type algorithms have enabled rapid mass spectral deconvolution of GC-MS data [21,22]. The deconvoluted peaks can be matched with library spectra. Generally, for effective deconvolution, the peak should contain ≥20 data points and a distinct peak apex. However, it is not always possible to obtain sufficient data points across peaks of low signal intensity. The advantage of comprehensive GC × GC separation is that compounds that co-elute in the first chromatographic phase may be resolved in the second phase. In effect the components are resolved across two stationary phases usually of different polarities. The enhanced chromatographic separation reduces the number of interfering peaks. Therefore, effective deconvolution may be achievable with less data points across the peak.

High levels of noise caused by column and septum bleed, and other contaminants can significantly reduce the sensitivity in 1D-GC-MS. Comprehensive GC × GC separation virtually eliminates these problems because these compounds are resolved from other matrix constituents in the second chromatographic phase. Our previous work has demonstrated that 2D separation may provide an increase in sensitivity of up to one order of magnitude compared to conventional 1D-GC analysis using the same system [23]. We demonstrated that low dose; rapidly metabolised benzodiazepines can be clearly detected and quantified at levels expected in users.

Comprehensive GC × GC incorporates a relatively slow separation, in the first phase, based on volatility and polarity, with a very rapid one, in the second phase, based solely on polarity. Twin-stage cryo-modulation provides an effective means of eluant transfer without losing first phase resolution. The raw data consists of a series of short chromatograms or modulations, typically 4–8 s, and very fast eluting, sharp peaks with mid-point peak widths typically 0.1–0.5 s. Rapid second-dimension retention times are achieved by focusing the eluant onto a narrower bore column that results in an increase in the carrier gas velocity. TOFMS with rapid spectral acquisition (≤500 spectra/s) is ideally suited to this application because sufficient data points (10–20) across the peak can easily be obtained. However, faster spectral acquisition requires much larger data files and decreases sensitivity. Song et al. reported a 17-fold increase in signal to noise (S/N) for an acquisition rate of 5 Hz compared with 500 Hz [13].

Data processing software is employed to convert the raw data to 2D chromatographic plots. Components which elute over two or more modulation periods are combined so only the peak apex of the largest peak is represented on the 2D plot and the relative intensity is determined from the combined peaks. Therefore, only one entry is shown in the peak table. Any overlapping peaks may be further resolved using mass spectral deconvolution software.

GC × GC/TOFMS is applied to drug screening of hair with the aim of providing a comprehensive qualitative analysis of the components in the samples. The extraction method was performed, primarily, to enable target analysis by LC-MS/MS and as such deuterated internal standards were added to facilitate the quantitation. The remainder of the extracts were submitted for GC × GC/TOFMS analysis. This was a 'blind' study and no information regarding the constituents in the sample was available with the exception of the internal standards. The extracts were evaporated to dryness and derivatised with N-methyl-N-(tert-butyldimethyl)trifluoracetamide (MTBSTFA) to improve the chromatographic performance. The samples were analysed using splitless and large-volume injection modes. Further analyses of drug standards were performed and a comparison of derivatisation reagents, MTBSTFA and trifluoroacetic anhydride (TFAA), was made to evaluate the sensitivity.

2. Experimental

2.1. Chemicals and reagents

All reagents were analytical grade. N-methyl-N-(tert-butyldimethyl)trifluoracetamide (MTBSTFA), tert-butyldimethychlorosilane (TBMCS), trifluoroacetic anhydride (TFAA) and ethyl acetate were supplied by Sigma–Aldrich (Poole, UK). Dichloromethane, hexane, methanol and acetonitrile were supplied by Fisher Scientific (Loughborough, UK).

2.2. Drug standards

Standards containing dried residues of codeine, morphine, 6-monoacetylmorphine (6-MAM), amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethylamphetamine (MDMA) and benzylpiperazine (BZP), at concentrations of 20, 50, 100, 200, 500 and 1000 ng/vial, were prepared from Cerilliant standards (Round Rock, TX, USA) except BZP (SLN Pharmachem, India).

2.3. Sample extracts

A 20 mg portion of three quality control hair samples was cut into approximate 1-mm sections. The samples were washed three times with methanol (1 mL) to remove any external contamination before extraction. Each sample was then spiked with 50 ng codeine-D3, morphine-D3, 6-MAM-D3, amphetamine-D5, methamphetamine-D5, MDA-D5, MDMA-D5 and BZP-D7. The washed hair samples were extracted with acid (HCl, 0.1 M, 1 mL) for approximately 16 h (overnight) at 37 °C. The samples were then centrifuged (2700 rpm, 5 min) and adjusted to approximately pH 7 using NaOH (100 μL, 1 M). Phosphate buffer was added (0.1 M, pH 7), the tubes vortexed briefly and centrifuged (2700 rpm, 5 min).