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Review

New Applications of Gas Chromatography and Gas Chromatography-Mass Spectrometry for Novel Sample Matrices in the Forensic Sciences: A Literature Review

Oliver Gould, Ngoc Nguyen and Kevin C. Honeychurch *

School of Applied Sciences, University of the West of England, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK
* Correspondence: kevin.honeychurch@uwe.ac.uk

Abstract: The investigation of novel sample matrices in the forensic sciences offers several possible advantages, such as allowing for results to be obtained in cases where common sample types are absent. This review focuses on the application of gas chromatography and gas chromatography–mass spectrometry (GC-MS) for the determination of drugs in alternative sample matrices, including hair, sweat, meconium, breast milk, and vitreous humour. Less common sample types are also reported including air, cerumen, insects, and their larvae and pupae. The application of pyrolysis GC-MS (Py GC-MS) is also reviewed, showing the possibility of determining high molecular weight drugs which would commonly be unattainable by GC-MS. The application of Py GC-MS for the simulation and investigation of the underlying chemistry and the products formed in the smoking of drugs is also reported.

Keywords: gas chromatography; gas chromatography–mass spectrometry; pyrolysis GC-MS; forensic; hair; sweat; meconium; breast milk; vitreous humour

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1. Introduction

Proven as powerful analytical techniques, both gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) have a long application history. As is the case for all chromatographic techniques, GC separates mixtures by exploiting differences in component distribution between two phases; the stationary phase and the moving or more commonly called, carrier gas moving phase. An aliquot of the sample mixture to be separated is introduced to the moving gas phase, just before it encounters the stationary phase. The sample component separation is dictated by differences in their equilibria between the two phases. Those with low affinity for the stationary phase spend more time in the moving carrier gas phase and exit the column more quickly (i.e., have shorter retention times). As there is little interaction between molecules and the carrier gas phase, this phase plays only a small role in separation, acting as only a way to carry the sample components through the column. The main separation mechanism is governed by the sample components' vapor pressures and their sorption to the stationary phase. As a result, chromatographic separation needs to be carried out at temperatures high enough for the components' vapor pressures to be high enough to allow them to exit the column (retention time) in a realistically short analysis time. However, this temperature needs to be at a value such that there are still large enough differences between the individual sample compound's vapor pressure and stationary phase interactions to allow for chromatographic separation. Too high a temperature can result in poor resolution and the risk of column degradation, too low a temperature results in long retention times and poor chromatographic performance. Early applications of GC utilized a single temperature (isothermal) to elute the sample components; an approach which gives satisfactory results for a few sample components, but not for the majority. Consequently, nowadays most GC separations are undertaken

using temperature programming. The analysis is started at a low temperature, suitable for the more volatile sample components. The temperature is then increased in a linear manner as the analysis proceeds, until it reaches a temperature appropriate for the least volatile sample components. Using such an approach, each compound starts to migrate rapidly as the temperature reaches the appropriate level for it. This has been shown to be highly successful, obtaining some of the highest chromatographic efficiencies presently available. Using modern capillary columns, theorical plate values exceeding 100,000 can be obtained [1] compared to only 8000 to 12,000 for high performance liquid chromatography (HPLC) [2]. Notably, the heating of the sample required for GC analys means that the analyte needs to be both sufficiently volatile and thermally stable. This can limit the compounds that can be successfully determined, and derivatization steps are often required to overcome these problems, an issue generally not affecting techniques such as HPLC.

The volatility of a number of commonly encountered analytes is governed by intermolecular hydrogen bonding. The presence of such bonds requires more energy (heat) when changing from a liquid to a vapor. Consequently, the removal of hydrogen bonding effects can markedly increase volatility. Such an approach has made the GC determination of high-boiling point alcohols, phenols, carboxylic acids, and amines a possibility. Removal of hydrogen bonding can also offer improvements in chromatographic performance as bonding between the molecule and polar groups present in the stationary phase is lessened. As a result, poor chromatographic behaviour, such as peak tailing is alleviated. The 'removal' of these offending hydrogen bonding groups is commonly undertaken by forming a suitable derivative of these prior to introduction of the analyte to the GC. Many alcohols, phenols, and amines undergo hydrogen bonding but can be determined by GC as their acetyl derivatives. Such derivatives can be readily formed by reaction with reagents such as ethanoic anhydride in the presence of a catalyst, such as hydrochloric acid, before introduction to the GC. However, probably, one of the most commonly employed derivatization methods is the formation of trimethylsilyl derivatives employing derivatizing agents such as trifluoro-ethanoic anhydride or N-trifluoroacetyl imidazole can be used to give their corresponding trifluoro acetyl derivatives. Many alternative methods now exist for their preparation, such as the reaction of trimethylchlorosilane (TMS) in pyridine or hexamethyldisilazane (HMDS) and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). Notably, the GC analysis of carboxylic acids is also commonly undertaken by converting these compounds to their corresponding methyl esters. There are a number of methods commonly used to obtain this, such as; diazomethane, or methanolic solutions of boron trifluoride. This is an attractive approach for their GC-MS analysis as select ion mode (SIM) can readily be used for the monitoring of the indicative, but the uncommonly encountered m/z 74 ion resulting from the McLafferty rearrangement of the methyl ester.

Helium has proven to be a particularly suitable mobile phase carrier gas for GC applications. It is both inert in the chromatographic system itself and in the ionization sources of mass spectrometric detectors. However, it is not a sustainable, renewable source and as a result there is a growing demand to develop methods that are based on alternative carrier gases, such as; nitrogen or hydrogen. Nevertheless, the elution of sample components from the GC in a gas phase has been an attractive feature for GC, as a wide range of different detector types can be used to give both qualitative and quantitative outputs. A large number of different detectors are commonly utilized, such as flame ionization [3], electron capture [4], and nitrogen–phosphorus [5] detectors, to mention but a few.

One principle draw back with GC is its inability to identify unknown sample components. Identification with such detector systems can only be made by running known standards and then comparing relative retention times. However, one now commonly employed approached, which to large extent overcomes these issues, is the coupling of GC with mass spectrometry (MS). MS is extremely good for allowing for the identification of unknown compounds, be it from user interpretation of mass spectra or via computer-based matching with various libraries of MS spectra. However, MS alone is poor when faced

with mixtures; an issue alleviated when coupled with techniques such as GC, which is particularly good at separating pure compounds out of complex mixtures. Such a coupling makes GC-MS an extremely powerful technique for a number of different disciplines, such as forensic science, where quantification and identification of unknown sample components in complex samples is required.

The first on-line coupling of gas chromatography to a mass spectrometer was reported in the late 1950s [6,7]. The availability of smaller, more powerful computers allowed for the further development of the technique and nowadays GC-MS is commonly seen in most laboratories throughout the world. The ability to handle large volumes of data has allowed for more complex systems such as two-dimensional gas chromatography (GC × GC) [8] to be more routinely applied.

MS as a detector system for GC offers a number of advantages. The detector can be applied in the full scan mode, for near universal detection or in a more selective and sensitive SIM mode. The full scan mode involves scanning the mass range recorded over a predefined range. This is typically from m/z 50 to m/z 500 but is dependent on the mass range anticipated for the sample components of interest. This provides a qualitative and quantitative picture of the sample, allowing for the detection of any compound eluting from the GC that is ionizable and produces fragments with m/z values in the investigated range. The application of lower mass settings can result in interference from nitrogen (m/z 28), carbon dioxide (m/z 44), or Argon (m/z 40) present in relatively large concentrations in air. The approach is less sensitive as, if a very large mass range is selected, sensitivity is adversely affected, as the number of scans per unit time will decrease. Quantitively, the full scan mode offers the advantage of allowing the mass spectrum of individual eluting compounds to be obtained and matched against commercially available library spectra for the identification of unknows. Greater sensitivity and selectivity can be obtained using the SIM approach. In the selected ion monitoring mode, only the m/z of the selected fragments are collected. This is a more targeted approach, used for the determination of known components in the sample. The approach offers greater sensitivity as, if a small mass range is selected, sensitivity is improved, as the number of scans per unit time is increased compared to the full scan mode. This feature also helps eliminate interferences arising due to other ions that can be present in complex sample matrices. Signal-to-noise ratios are also improved, allowing for improvements in detection limits.

Modern tandem mass spectrometry (MS/MS) instruments utilize the technique of multiple reaction monitoring (MRM). Ions are first formed, and a compound indicative predefined selection is made by the first quadrupole. These ions are referred to as precursor or parent ions and are then fragmented in the collision cell to give further fragment ions known as product or daughter ions. A second quadrupole then selects a predefined sample of these. Unlike SIM or full scan mass spectrometry, it is not the actual m/z of the ion that is used as the analytical result. In MRM, it is the value of the ion transition that is utilized. Multiple reaction monitoring allows for increased selectivity, due to the specific collision-induced fragmentations of precursor ions and improved signal-to-noise ratios.

Technology such as pyrolysis GC-MS (Py-GC-MS) has also helped expand the range of samples that can be successfully investigated. Its application for the determination of low-volatility compounds was first described in the early 1960s [9–12] and later in 1967 for Py-GC-MS [13,14]. The addition of mass spectrometry, as a detection system, improves the utility of the approach, as pyrolysis products formed can firstly be separated by GC, then identified from their mass spectra. Early recognition of the power of this approach was demonstrated by its application on the Viking Lander in the search for life on the planet Mars in 1976 [15], and is now common on a number of missions [16]. Present day applications of Py-GC-MS range from research, quality control, and characterization of materials [17] and environmental investigations, including microplastics [18,19], geology [16,20], fuels, conservation [21], as well as medicines [22], and for the identification of bacteria [23]. In forensic science, the technique is commonly employed in the investigation

of condom materials and lubricants [24], photocopier toner [25,26], packaging tapes [27,28], and paint samples [29].

Urine and blood remain the most commonly employed biological samples used for the determination and monitoring of drugs and other substances. However, issues can be associated with these samples. Urine offers a small detection window of usually 2–4 days and is usually favoured for investigating drug use in sport or in drug management programs. The risks of tampering or adulteration during transportation and storage are possible, and issues of privacy in sample collection can also be a problem [30,31]. Blood has a smaller detection window of 2–24 h and is commonly applied to determine current intoxication levels [32]. Collection of the sample can be invasive and expensive, needing trained personnel. Problems can also be associated with locating veins for sampling in certain groups, such as intravenous drug users.

Hair has also been investigated as an alternative less invasive sample allowing for a long detection window, being able to show historic patterns of drug use [33]. Nevertheless, limitations to this as a sample include complex methods of extraction and relatively high analysis costs [34]. Questions regarding external contamination, particularly for smoked substances and the low concentrations they represent, coupled with the often-limited quantities of sample available, can also be an issue [35]. Studies have also shown that when hair is heated concentrations and the nature of the drugs present can be affected. In 2016, Ettlinger and Yeagles [36] reported that levels of Δ9-tetrahydrocannabinol (THC) and cocaine decreased following heat treatment of hair, being converted to cannabinol and benzoylecgonine, respectively.

In this present review we have focused on the discussion of alternative sample matrixes and their analysis by GC and GC-MS for applications in the forensic sciences. The review covers recent advances in both GC and GC-MS applications applicable to the forensic discipline. The applications discussed include sweat and skin [37–52], cerumen [53–56], meconium [57–59], breast milk [60,61], larvae, pupae and insects [62–65], Vitreous humour [66–71], drug paraphernalia [72] cosmetics and fragrances [73–78], and air, gases and vapours [79–84]. A further section is dedicated to the application of pyrolysis GC-MS [85–98].

2. Methods

To compile this paper our primary search engine was Google Scholar; however, we also used Web of Science, and the University of the West of England library. Only papers with full access and English as the published language were included. Since the nature of the paper is to discuss the role of GC/GC-MS in the analysis of novel, niche, or underused sample matrices, we did not set an exclusion date range to enable us to provide a broad overview of the matrices.

The search terms began with GC or GC-MS with the AND Boolean operator followed by one or more of: drugs, illicit substances, forensic, volatile compounds, sweat, fragrance, cosmetics, skin, earwax, meconium, breast milk, larvae, pupae, insects, vitreous humour, air, and/or vapor.

3. Alternative Sample Matrixes

3.1. Sweat and Skin

Sweat is a fluid secreted from the apocrine and eccrine glands distributed over the body. It is used to maintain and regulate a constant body temperature [37]. The application of sweat allows for a possible alternative, non-invasive sample with a large detection window of one to four weeks, offering cumulative information on drug use and an economic alternative to blood sampling. Sweat is readily accessible compared to other bodily fluids [38] and can be obtained via relatively tamper-proof collection methods and avoids problems with privacy and the transport of biohazardous fluids. The large detection window makes sweat an effective approach to determine drug use and is reported to be useful

in drug testing programs. This includes incidences where sweat on clothing has been used to detect drugs [39].

The quantity of sweat produced varies from individual to individual, and depends on the volume of sweat they secrete per gland, and is also affected by changes in their activity, emotions, and heat [40]. Sweat has low tonicity and is more acidic than blood. Basic drugs will therefore preferably accumulate in sweat rather than in the blood [41,42]. The concentration gradient that is set up between blood and sweat allows for diffusion of the free fraction of the drug through the lipid bilayer and its excretion onto the skin in sweat. Although this passive diffusion mechanism is the main route, excretion also occurs via sebum and intracellular diffusion [43]. A number of chemical and biochemical processes can also affect excretions, including possible dissociation constants, molecular mass, lipophilicity, and protein binding [44].

Cone et al. [44] have shown that a dose as low as 1–5 mg of cocaine was sufficient to give detectable levels of cocaine and its metabolites in sweat. Published methods for the detection of substituted amphetamine class drugs such as methamphetamines and MDMA [45,46], nicotine, cocaine [38,47] opiates and heroin [44], THC and other cannabinoids [48] have been given. Barnes et al. [45] have adapted a method to specifically target one or two drug classes to examine sweat following daily doses of methamphetamine using GC-MS and reported that detection of cumulative doses was successful. Others have investigated methods for simultaneous drug detection. Kintz et al. [49] have developed a method for the determination for some of the most commonly abused drug classes; opiates, amphetamine, cocaine, and cannabinoids using both GC-MS or LC-MS in sweat; whilst Cone et al. [44] have adapted a GC-MS technique previously applied to oral fluid and blood, for the detection of heroin, cocaine, and their metabolites in sweat.

One common approach for the collection of sweat is from patches worn for a known time period, such as weeks or as short as just a few hours. Patches can be hidden and are generally non-invasive, making them acceptable to participants in trials or testing. However, due to differences in the density of sweat glands across the body, levels of detection can differ at different sampling sites; a factor that can have a bearing on the results obtained [50]. In one such study [50], an eightfold difference in the concentrations recorded from patches from the lower back and from the upper shoulder were recorded.

Investigations on the effectiveness of sweat patches, compared with urine analysis, for drug screening of cocaine and opiates have previously been conducted by several researchers. Preston et al. [47] determined that the greater quantity of cocaine in sweat patches compared to urine tests was due to different excretion mechanisms, whilst Huestis et al. [51] detected less opiates. Both, however, obtained results indicating that sweat patches generated more favourable and reliable results than urine.

A solid phase extraction and GC-MS method for 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3-hydroxy-4-methoxymethamphetamine (HMMA), 3-hydroxy-4-methoxyamphetamine (HMA), 3,4-methylenedioxyethylamphetamine (MDEA), methamphetamine (MAMP), and amphetamine (AMP) in sweat was reported by De Martinis et al. [46] Drugs were eluted from PharmChek^{TM} sweat patches with a pH 5.0 acetate buffer, extracted by solid phase extraction and determined using GC-MS with selected ion monitoring. Limits of quantification (LOQ) for MDMA, MDEA, MAMP, and AMP were 2.5 ng/patch and 5 ng/patch for MDA and HMA and HMMA.

In addition to the detection of elicit substances from sweat, there is also a slowly emerging field which attempts to identify an individual based on their produced scent profile. In 2014, Choi, and Oh [52] used two dimensional GC-MS to determine the characteristics of human sweat profiles, 574 compounds were identified including compounds associated with age [52]. While the 2014 Choi and Oh study shows promise and significant potential for forensic utility, it is difficult to find other examples of this type of research; this might be due in part to a general lack of forensic research funding and the need for high-resolution mass spectrometers, which generally are not used in forensic laboratories.

3.2. Cerumen

Recently, a number of reports have investigated other possible samples, including cerumen (earwax) [53–56]; bone [99,100]; adipocere, also known as corpse wax, grave wax or mortuary wax; brain tissue; flies; and pupae [58]. Meier et al. reported the detection time window of cerumen, commonly referred as earwax, to be reportedly in excess of that of urine but shorter than that reported for hair [55]. Their study showed that in all cases of the recent use of drugs such as opiates, amphetamine and derivatives, cocaine, methadone and diazepam, investigations of the corresponding cerumen samples were found to be positive. In cases where drugs could only be detected in urine, cerumen samples were also found to be positive. However, where only the hair was positive, drug levels in cerumen were found in only 52.5% of the cases investigated. However, cannabis use was only detected in 31.6% of cerumen samples of deceased cannabis users. Unexpectedly, THC was not detected but its oxidized form, cannabinol, was recorded. Sampling of the cerumen was undertaken using a cotton swab from both ears prior to autopsy, to avoid contamination with blood. These were then dried at room temperature for 24 h and extracted by sonicating for 1 h in 1 mL of ethyl acetate containing internal standard. Samples were centrifuged and evaporated to dryness and reconstituted in the mobile phase for LC-TOF MS and LC–MS/MS analysis.

Gonçalves Barbosa et al. [56] investigated poisoning in cattle by the pyrethroid insecticide, bifenthrin. Via examination of the animal's earwax by headspace/gas chromatography—mass spectrometry (HS/GC–MS) they were able to detect the presence of bifenthrin in the earwax of the exposed animals. Samples of earwax were collected directly using a metallic curette. These were then weighed into 20 mL GC headspace vials, and internal standard was added. The vials were sealed and examined by HS/GC–MS. This was confirmed by comparison of the sample's MS spectrum with a bifenthrin standard, and from its retention time relative to the internal standard, 3-methylcyclohexanone.

Nicotine has been determined in human cerumen by HS/GC–MS [54]. Cerumen samples were collected from three different groups based on their smoking habits (non-smoker, passive, and active smokers). Nicotine cerumen levels were reported to be much lower than its metabolite, cotinine, even for active smokers, which contrasted with nicotine levels reported in scalp hair, where the cotinine levels were much lower than nicotine. The authors concluded that cerumen was potentially a better sample for the measurement of biological levels as it is better protected from external contamination than hair. Nicotine concentration profiles, and the related compounds, o-nicotine, cotinine, and anabasine in the cerumen samples investigated were studied further by data mining and it was shown to be possible to discriminate between the three different groups. These applications are summarized in Table 1.

Table 1. Determination of drugs in sweat, earwax, bone, meconium, breast milk, insects and larvae, and vitreous humour.

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Cocaine and heroin, morphine, 6-acetylmorphine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, benzoylecgonine, norcocaine, norcocaethylene, and benzoylnorecgonine	Sweat	BSTFA with 1% TMCS, N-Methyl-bis-trifluoroacetamide	Sweat patch extracted with internal standard by shaking, centrifuged and filtered, and then purified by SPE, evaporated, and derivatized.	GC-MS/EI	Cocaine, heroin, and metabolites 1.0 ng/patch.	Sweat patch worn for a period of several days to several weeks at a time.	[44]
MAMP and AMP	Sweat	MTBSTFA with 1% TBDMCS and BSTFA with 1% TMCS.	Patches (PharmChek™) were folded and placed in acetate buffer (pH 4.0) for 30 min at room temperature. The solution was then purified by SPE. Analytes eluted and evaporated to dryness under N_2 and derivatized.	GC-MS/EI	Limit of quantification of 2.5 ng/patch.	Weekly sweat patches were applied to participants, one on the back and one on the abdomen, and removed after a week.	[45]
MDMA, MDA, HMMA, HMA, MDEA, methamphetamine and AMP	Sweat	Triethylamine in heptane and HFAA.	Drugs were eluted from PharmChek™ sweat patches with sodium acetate buffer, extracted with disk SPE.	GC-MS/EI	Limits of quantification for MDMA, MDEA, MAMP and AMP were 2.5 ng/patch, and 5 ng/patch for MDA, HMA and HMMA.	A sweat patch was applied for various periods prior to, during, and after MDMA administration. A 1.0 mg/kg of MDMA was administered orally to a participant	[46]
Cocaine, benzoylecgonine, and ecgonine methyl ester.	Sweat and urine	--	The absorbent sweat pad was extracted with 75% methanol/25% 0.2 M sodium acetate, pH 5.0 by shaking for 30 min; eluent was then analysed by ELISA and confirmed by GC-MS by SIM.	GC-MS/EI in SIM	3 ng/mL for cocaine and 2 ng/mL for benzoylecgonine and ecgonine methyl ester. Limits of quantitation were 4 ng/mL for cocaine and 2 ng/mL for benzoylecgonine and ecgonine methyl ester.	Sweat and urine specimens collected from 44 methadone-maintenance patients. ELISA immunoassay compared.	[47]

Table 1. *Cont.*

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Δ9-tetrahydrocannabinol	Sweat	Derivatized with trifluoroacetic anhydride	Patches were extracted with methanol/sodium acetate buffer pH 5, by shaking and isolated by SPE.	GC/MS negative ion CI.	Limit of quantification, 0.4 ng/patch.	In total, 11 daily cannabis users after cessation of drug use. PharmChek® Sweat patches worn for 7 days. Percent recovery from patches was 44–46%.	[48]
Heroin, 6-monoacetylmorphine, morphine, codeine, cocaine, benzoylecgonine, ecgonine methyl ester, Δ9-tetrahydrocannabinol, nordiazepam, oxazepam, AMP, methamphetamine, methylenedioxyam-phetamine, metbylenedioxymetham-phetamine, metbylenedioxyethylam-phetamine and buprenorphine.	Sweat	AMP and related compounds with heptafluorobutyric anhydride. Other drugs by silylation with BSTFA and trimethylchlorosi-lane.	Sweat pads extracted in methanol containing deuterated internal standards by shaking. Methanol solution was divided into 3 for buprenorphine testing, amphetamines testing, and the remainder for the other compounds. Evaporated to dryness. Amphetamine and related compounds were identified after derivatization with heptafluorobutyric anhydride; other drugs were derivatized by sialylation.	GC-MS/EI	From 0.01 to 2.0 ng/patch	Buprenorphine was identified and quantitated by LC/MS.	[49]
Heroin, 6-acetylmorphine, morphine, and codeine	Sweat	---	The absorbent pad was removed and extracted with buffer, 75% methanol/25% 0.2 M sodium acetate (pH 5.0), by shaking. The eluent was then analysed according to package directions by ELISA.	ELISA and GC-MS	GC-MS, 3 ng/mL	The percentage cross-reactivity at 10 ng/mL for each analyte was 100% for morphine, 28% for heroin, 30% for 6-acetylmorphine, 588% for codeine, 143% for hydrocodone, 16% for hydromorphone, and 30% for oxymorphone	[51]

Table 1. *Cont.*

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Cotinine, a major metabolite of nicotine, o-nicotine, and anabasine.	Earwax	---	Earwax samples (20 mg) transferred to GC vials, and 3-methyl cyclohexanone was added as internal standard.	HS/GC–MS by SIM.	---	The monitored ions for quantification were m/z 84 for nicotine, o-nicotine and anabasine, m/z 98 for cotinine, and at m/z 69 for 3-methyl cyclohexanone (IS).	[54]
Bifenthrin	Earwax in cattle	---	Samples were weighed into GC headspace vials, and IS, 3-methylcyclohexanone added.	HS/GC–MS by SIM.	---	m/z ratios of 181 (base peak of bifenthrin) and 112 and 69 (molecular ion and base peak of IS).	[56]
Quetiapine and pregabalin	Human bone		Soft tissues were removed from the bone. Samples were cut in small fragments, dried and pulverized using a ball mill. An aliquot of bone powder and internal standard solution and methanol were vortexed and incubated and sonicated and centrifuged. The supernatants were recovered and evaporated. PBS; 0.1 M, pH 6 was added and samples were subjected to a SPE and eluted with dichloromethane:isopropanol: ammonia and then evaporated. Samples were reconstituted with 100 µL of ethyl acetate, vortexed before introduction to the GC-MS.	GC–MS by SIM	0.1 ng/mg for both.	Pregabalin, m/z; 41-43-55-69-84-141. Quetiapine m/z 144-210-239-321. Sertraline (IS) m/z; 159-262-274-304	[99]

Table 1. *Cont.*

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Fatty acid ethyl esters. MeconiumGC-FIDCocaine, benzoylecgonine, anhydroecgonine methyl ester (metabolite of crack cocaine), nicotine, and cotinine	Meconium	60 µL acetonitrile, 20µL MSTFA, vortexed then heated 60 °C for 20 min.	0.3 g Meconium added to a 10 mL glass tube spiked with ISTD 200 ng/g and methanol added for extraction. An amount of 0.1 M HCL was added prior to the pipette SPE process.	GC-MS in SIM mode, multi-step temperature ramp	2.5 ng/g cocaine, 6 ng/g anhydroecgonine methyl ester, 8 ng/g cocaethylene, 10 ng/g benzoylecgonine, 15 ng/g nicotine, 5 ng/g cotinine	Meconium samples were spiked, there were no non-spiked samples tested.	[58]
Organophosphates: chlorpyrifos, dichlorvos, dimethoate, demeton-smethyl, ethion, malathion, omethoate, pirimiphos-methyl, pyrazophos, and tolclofos-methyl	Meconium		Liquid extraction with acetonitrile and internal standards. Dry residue reconstituted with toluene.	GC-MS, SIM mode	Range from 0.21 to 320.43 µg/g		[59]
20 target analytes, only DMP, DEP, DIBP, DBP, and DEHP detected.	Breast Milk		Liquid/liquid extraction with acetonitrile saturated in *n*-hexane.	GC-MS/MS	Range from 0.5 to 211.2 µg/kg	A very simple extraction method was used, which has the benefit of being easily reproducible, no dangerous levels were found.	[61]
Organochlorine Pesticides (aldrin, dieldrin, endrin, alpha-endosulfan, beta-endosulfan, endosulfan sulphate, heptachlor, heptachlor epoxide isomer B, methoxychlor, endrin ketone, endrin, pp'-DDE, pp'-DDD, pp'-DDT, α−HCH, β−HCH, γ−HCH and δ−HCH)	Breast Milk		Soxhlet extraction	GC-MS	All organopesticides LOD 0.01 ng/mL	A 60 m column with a 54.9 min sample analysis time.	[101]
Heroin and associated metabolites	Flies and larvae		Alkali–acid base extraction method	GC-MS			[63]

Table 1. *Cont.*

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Methamphetamine	Larvae, pupae, spent pupae and adult *Calliphora vomitoria* L.	Trifluoroacetic anhydride.	Washed with DCM, crystalized with liquid nitrogen, homogenised, and extracted with methanol before derivatisation.	GC-MS SIM mode	5 ng/mg	Negative in adult *Calliphora vomitoria* L.	[64]
Opiates, cocaine, barbituates, and antidepressents	Blowfly larvae reared on liver samples which tested positive for the drugs	BSTFA at 70 °C for 15 min for the detection of morphine-2TMS and benzoylecgonine-TMS	Centrifuged homogenate of the larvae and or liver has the supernatant removed prior to derivatisation. SPE or liquid-liquid extraction flash-alkylation with MethElute.	GC-MS SIM mode	Not reported	Methods are difficult to follow.	[65]
Drug and metabolite screening	Vitreous Humour		Internal standards and ascorbic acid are mixed and precipitated with zinc sulphate, then centrifuged. The supernatant is treated with sodium acetate. This is then filtered through an SPE column. Sample extracts are reconstituted with ethyl acetate.	GC-MS Full scan m/z 43–550	Not reported		[66]
11-Nor-9-carboxy-Δ^9 tetrahydrocannabinol	Vitreous Humour	MSTFA heated at 90 °C for 30 min	THC-COOH-d^3 was added to the biological sample. Hydrolysis was performed with sodium hydroxide and hexane, the aqueous layer was transferred, acidified with HCL and extracted with hexane/ethyl acetate.	GC-MS	1 ng/mL		[68]

Table 1. *Cont.*

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Meprobamate, morphine, cyamemazine, caffeine, diazepam, and citalopram	Bile, Vitreous Humour, Whole blood	BSTFA/TMCS morphine analysed as TMS derivitive	Samples mixed with phosphate buffer 0.25 M pH 8.4 along with deuterated analogues of each analyte. For morphine, meprobamate, cyamemazine, and caffeine an automated SPE method was used before phosphate buffer was added.	GC-MS/MS	Not reported		[69]
Antidepressant drugs; amitriptyline, nortriptyline, citalopram, clomipramine, fluoxetine, maprotiline, mirtazapine, sertraline and venlafaxine, and 4 of their metabolites, desmethylmaprotiline, desmethylmirtazapine, desmethylsertraline, O-desmethylvenlafaxine,	Vitreous Humour, Whole Blood	Dry residues were derivatized with HFBA in ethyl acetate at 50 °C 30 min.	SPE using a non-polar C8 sorbent and phosphate buffer 0.1 M pH 6.0, columns are then acidified with acetic acid and washed with methanol. Then, derivatized, and evaporated, dry samples were reconstituted with ethyl acetate.	GC-MS SIM mode	1.5 ng/mL for all analytes	Dynamic range 5-500 ng/mL r^2= 0.99. %RSD less than 10.9% for all analytes.	[71]

AMP amphetamine; BSTFA N,O-bis(trimethylsilyl)trifluoroacetamide; CI chemical ionization; ELISA enzyme-linked immunosorbent assay; GC-MS/EI gas chromatography–mass spectrometry electron impact ionization; HMA 3-hydroxy-4-methoxyamphetamine, HMMA 3-hydroxy-4-methoxymethamphetamine; HS/GC-MS headspace gas chromatography–mass spectrometry; HFAA heptafluorobutyric acid anhydride; IS internal standard; LOD limit of detection; MAMP methamphetamine; MDA 3,4-methylenedioxyamphetamine; MDEA 3,4-methylenedioxyethylamphetamine; MTBSTFA N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide; MDMA 3,4-methylenedioxymethamphetamine; PBS Phosphate buffered saline; SIM select ion mode; SPE solid phase extraction; TBDMCS N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide; TMCS trimethylchlorosilane; DMP dimethyl phthalate; DEP diethyl phthalate; DIEP diisobutyl phthalate; DBP di-n-butyl phthalate; DEHP bis(2-ethylhexyl) phthalate.

3.3. Meconium

Meconium is the earliest stool of a mammalian infant resulting from defecation. Unlike later faeces, meconium is composed of materials ingested during the time the infant spends in the uterus. Consequently, it can be utilized as a sample to investigate in utero exposure.

Recently Mozaner-Bordin et al. used GC-MS [57] to analyse 50 meconium samples, and were able to detect and quantify cocaine, benzoylecgonine, anhydroecgonine methyl ester (metabolite of crack cocaine), nicotine, and cotinine. A solid-phase extraction method was used to extract the analytes, based on the modification of a disposable pipette tip with solid-phase material. The resulting residue was derivatized with acetonitrile and MSTFA. A splitless injection was used with a 30 m × 0.25 mm capillary column (0.25 µm film thickness), these column dimensions are probably the most commonly used in most labs. The temperature ramp was carried out in different stages starting at 70 °C and working up to 320 °C, with the total run taking around 20 min. The MS was set to SIM mode with EI, following method development three ions were selected for each analyte to allow for both identification and quantification. This process can help reduce noise and increase sensitivity, which is beneficial for targeted analysis. This group calculated with standards the linearity, LLOD, and LLOQ, which are common in GC-MS studies. However, this group also took the step of measuring the precision and accuracy over several days using a QC, which would have to be performed in any forensic lab carrying out this type of testing; while many labs carry out this type of procedure it is not always reported in the written article. Cocaine was detected at the 2.5 ng/g level, however, the LOQ was 10 ng/g. Nicotine was the most inconsistent, with recovery percentages ranging from 50.72 to 95.33% [58].

Analysis of meconium is not only limited to the detection of illicit substances. A study of new-borns from Thailand used meconium to determine organophosphate exposure. Eight different organophosphates were found across 98% of the 68 babies analysed. This study showed that there were no statistical differences in exposure to babies whose mothers worked in agriculture and those who lived within 1 km of agricultural sites [58]. Again, this group used an Agilent GC-MS with a 30 m × 0.25 mm capillary column (0.25 µm film thickness), and stepped up the temperature ramp starting at 50 °C and going up to 290 °C. The MS used EI mode and SIM. The total analysis time was 33 min. This paper provides fewer details in terms of the methods and, specifically, the testing of the methods for consistency. There was no derivatization step here, but there was a liquid extraction process. An amount of 0.15 g meconium was added to a phosphate buffer/methanol solution and vortexed prior to internal standard addition. Acetonitrile was added prior to centrifugation, and this process was repeated twice. The evaporated residue was reconstituted with toluene prior to GC-MS injection (using a pulsed splitless injection) [59]. The benefit of standard methods being used by multiple groups and labs around the world is that they are tried and tested and thus are easier to present as forensically valid; as this paper suggests, the future is not necessarily new instruments or modifications but new applications and sample preparations.

3.4. Breast Milk

Similar to meconium, breast milk can serve as an ideal matrix to monitor infants' exposure to medications, drugs of abuse, pesticides, and metals. In a literature review by Drabinska et al., (2021) [59] cataloguing volatile compounds identified from healthy human individuals, the authors cite 290 compounds that have been identified in breast milk. A number of these compounds came from papers investigating exogenous compounds such as: organochlorine pesticides, brominated diphenyl ethers, dioxins, polychlorinated biphenyls, parabens, and flavonoids (along with numerous others). Compounds from garlic-family food stuffs were also identified in breast milk.

Fan et al., 2020 [60] presented the GC-MS/MS analysis of breast milk to detect contamination with phthalates from breast milk storage bags. Through GC-MS/MS analysis, six different phthalates were detected from 40 samples, and while some contamination of breast milk was discovered, the reported intake concentrations were determined to be of no

risk to the infants. Fan, et al., 2020 [60] used an Agilent GC-MS/MS in EI mode. The column was again a 30 m × 0.25 mm with a 0.25 µm stationary phase layer. The temperature ramp used multiple steps starting at 60 °C and moving up to 280 °C. Splitless injection was used; this technique is common for trace level analysis as all the sample enters the column, as opposed to the split method, which has the benefit of removing the solvent but also sweeps out some of the sample (not ideal for trace-level analysis). The samples were extracted with a liquid–liquid extraction, the solvent used was acetonitrile, saturated with *n*-hexane; the samples were reconstituted using *n*-hexane. The concentration range of the detected phthalate esters ranged from 0.5 to 211.2 µg/kg [61].

Similarly, Witczak et al., 2020 [61] use GC-MS to assess the presence of organochlorine pesticides in breast milk. Concentrations of beta-endosulfan showed a positive correlation to maternal fish and poultry consumption during pregnancy. One of the primary limitations of GC-MS is the inability to directly analyse aqueous samples due to column degradation, thus biological samples will, in most cases, require some form of pre-concentration, or solvent extraction, which in a forensic laboratory can be costly in both time and resources. In the case of the Witczak study, Soxhlet extraction was carried out but the authors present limited details as to this process. This group used a 60 m column with a 0.25 mm ID and 2.25 µm film. Using a 60 m column over the more common 30 m can result in better resolution and efficiency, as determined by the calculation of theoretical analytical plates. However, doubling the length of the column will not necessarily double the resolution, but will result in a longer analysis time, which is evidenced by the 54.9 min per sample analysis time. There are no details about the MS detector setting provided in this paper. As a result of the GC being unsuitable to aqueous samples, very often the preferred analysis method for breast milk involves liquid chromatography, usually coupled to MS or a variant thereof.

3.5. Larvae, Pupae and Insects

Different insects are associated with each of the stages of human and animal decomposition. Different populations of flies, and then beetles, followed by moths, are attracted to the body as the process of decomposition progresses. Flies from the family Calliphoridae, especially those from the subfamilies Chrysomyinae, Calliphorinae, and Lucillinae are the first to be attracted to the body for the process of egg laying. Their offspring consume dead tissue and, in the process, chemicals such as drugs that may be contained in the tissue. Consequently, they can be used as an indirect way of assessing the drug levels in the deceased animal or human. However, in the pupal stage, a significant reduction in the concentration of drug is seen, as digestion and metamorphosis occur [101].

The possibility of detecting heroin and its metabolites in flies and their larvae feeding on heroin-fortified meat samples was investigated by Ishak et al. [62]. Flies were collected from a local wet market. Out of this sample, L. cuprina were selected and then reared till their third generation. Their eggs were then placed onto separate minced buffalo meat samples fortified with saline solutions of heroin (0.5 to 10 µg/µL). Every eight hours, ten individual first-instar larvae, ten individual second-instar larvae, and ten migrating third-instar larvae were collected. These were then extracted and examined by GC-MS. Using the toxicology mass spectrum library available with the instrument, the heroin metabolites tryptophan, hydromorphone, and morphine were determined in the analysis of heroin fed *L. cuprina* in the second- and third-instar larvae, but not in the first instar or pupa.

Magni et al. [63] have shown the possibility of determining methamphetamine in the larvae, pupae, spent pupae, and adults of *Calliphora vomitoria* L. (Diptera: Calliphoridae) feeding on liver with doses of methamphetamine matched to human lethal dosages of 5 ng/mg and 10 ng/mg. Larvae, pupae, spent pupae, and adult samples were washed with dichloromethane. These were then crystallized with liquid nitrogen, homogenized, and a 100 mg aliquot extracted with methanol containing the internal standard, diphenylamine, by heating at 55 °C for 15 h. After cooling, the organic phase was acidified with trifluoracetic acid and dried at 70 °C under nitrogen. The resulting sample extract was then derivatized with trifluoroacetic anhydride, dried at 80 °C, and reconstituted in tert-butyl methyl

ether. Methamphetamine was determined by GC–MS in the SIM mode using m/z 154, 118, 110, and 91. Methamphetamine was determined in the immature stages and spent pupae of *C. vomitoria* that had been previously feeding on liver containing 5 ng/mg and 10 ng/mg methamphetamine at 23 °C. Notably no methamphetamine was recorded in in *C. vomitoria* adults as reportedly upon emergence as an adult, the flies rapidly eliminate methamphetamine.

Campobasso et al. [64] have investigated the relationship between drug concentrations present in human liver samples obtained from 18 necropsies previously shown positive for drugs and Blowfly larvae (Diptera: Calliphoridae) reared upon them. Subsequently GC-MS analysis showed that all drugs recorded in the samples of human tissue were also detected in insect specimens. Opiates, cocaine and barbiturates, and antidepressants (clomipramine, amitryptiline, nortryptiline, levomepromezine, and tioridazine) were reported. The larvae of *Lucilia sericata* (Diptera: Calliphoridae) were also reared on fortified liver samples and active feeding mature maggots and post-feeding maggots were collected. Fresh greenbottle (*Lucilia sericata*) eggs were transferred onto fortified liver samples and reared in an incubator. Mature larvae were collected at their peak feeding period, four days after laying, while post-feeding larvae were collected after six days. Once removed from the food source, maggots were washed with deionized water and prior to analysis samples were homogenized in deionized water.

Gas chromatographic mass spectrometry investigations were performed in the SIM mode following solid-phase extraction and derivatization with BSTF, using the ions: m/z 236, 287, 429 for morphine-2TMS and m/z 82, 240, 361 for benzoylecgonine-TMS. Phenobarbital quantification, the internal standard (butalbital) was added followed by liquid–liquid extraction. Extracts were analysed by GC-MS in the SIM mode following flash-alkylation with MethElute using the ions; m/z 232, 260 for methylated phenobarbital and m/z 195, 196 for the methylated-internal standard. Thioridazine (m/z 98, 370), clomipramine, amitriptyline (m/z 58, 202), nortriptyline, and levomepromazine (m/z 58, 328) were determined again, using GC-MS in the SIM mode following liquid–liquid extraction with SKF 525-A as the internal standard.

3.6. Vitreous Humour

The vitreous humour (VH) is a gel-like liquid that fills the eye; as individuals age the VH becomes less viscous. Due to the invasive nature of testing, VH analysis is almost solely reserved for post-mortem sampling. The VH offers long sample stability and a reduced need for pre-treatment versus blood, for example. However, there is a limit to the concentration as any target analyte must cross the blood–brain barrier. In 2016, Metushi et al. [65] compared the analysis of post-mortem whole blood (WB) to VH analysis to assess the effectiveness of the VH in forensic toxicology, using GC-MS. VH samples were extracted from the eye with a syringe (*ca.* 5 mL), 20 mL of WB was added to a glass vial with sodium fluoride and potassium oxalate. SPE extraction was used to extract the analytes from the matrix. In this instance, a 15 m × 0.25 mm column was used with 0.25 µm film thickness. A starting temp of 85 °C and a 40 °C/min ramp was used to 170 °C before a 4 min hold then another 40 °C/min ramp to 190 °C (held for 5 min) and then the final stage of 10 °C/min until an end temperature of 300 °C was held for 7 min. The MS was set to full scan with m/z 43–550. Seventy drugs were identified in this study, all of which are used in medical or clinical treatments but can also be subject to abuse, e.g., diazepam. The group found that while there were more identified analytes from whole blood (209 HB vs. 169 VH) the two matrices were very similar in terms of concentrations, with the exceptions of trazodone and diazepam, which were both significantly higher in WB [65].

In a 2008 brief communication, a gas chromatography–flame ionisation detector (GC-FID) was used to determine the presence of phencyclidine (PCP) and GC-MS to analyse for cannabinoids both in VH [66]. The results of the PCP analysis were promising with 60% of the samples testing positive for the presence of PCP in concentrations ranging from 30–290 ng/mL, however, the group noted that there was no correlation between VH and

WB PCP concentrations. The results with regard to cannabinoids were less encouraging as all screened VH samples tested negative. As this is a brief communication there is only limited information on the GC-MS methods.

Similar findings were also suggested from an earlier study in 2005 in which GC-MS was used to determine cannabinoids levels in VH. A 12 m × 0.2 mm (0.33 µm film thickness) fused silica capillary was used, a simple temperature ramp starting at 160 °C and ending at 280 °C at a rate of 25 °C/min was used. SIM mode detection was performed using *m*/*z* 371, 473, and 488 for THC-COOH and *m*/*z* 374, 476, and 491 for THC-COOH-d_3. Derivatization was conducted with MSTFA heated at 90 °C for 30 min. In this study, 11-nor-9-carboxy-Δ^9tetrahtdrocannabinol-d_3 was used as an internal standard. However, in the few instances that cannabinoids were detected, they were found to be below 10 ng/mL. It was suggested that this is due to the hydrophobic nature of 11-nor-9-carboxy-Δ^9tetrahtdrocannabinol [67].

In 2015, Bévalot et al. [68] compared WB and VH from humans and rabbits for the detection of meprobamate, morphine, cyamemazine, caffeine, diazepam, and citalopram. The group report that in rabbits all six drugs were detected and showed correlation with WB, but the same was not true for cyamemazine and diazepam in the human subject. Even so, all six drugs were detected in humans. Interestingly this group used gas chromatography–tandem mass spectrometry (GC-MS/MS), which differs from conventional GC-MS by adding an additional quadrupole inline separated by a collision cell. The ions from the first quadrupole enter the collision cell where they are fragmented further and then moved onto the second quadrupole for detection to occur. This process eliminates almost all background noise resulting in a superior signal-to-noise ratio versus traditional GC-MS. The net result of this is low limits of detection; thus, for targeted analysis, such as the work by Bévalot et al., 2015, GC-MS/MS has benefits [68]. However, GC-MS/MS is limited by lower mass accuracy and resolution; thus, single quad GC-MS remains the preferred option for untargeted sample analysis [69]. Moreover, GC-MS/MS is not routinely deployed in forensic laboratories and given the substantial outlay, both financial and in terms of space and time, there would need to be multiple routine use cases before we see an uptake by forensic providers.

A new analyte target has been proposed recently: Ntoupa et al., 2020 [70] suggested that antidepressants would be an ideal analyte target for VH analysis. In this instance SPE-GC-MS was employed for analysis. The extraction method involves several steps, including extraction, centrifuging, drying, and derivatization with heptafluorobutyric anhydride (HFBA) and ethyl acetate for 30 min, which of course is all in addition to the GC-MS analysis time itself. This leads to a long analysis time, which can be a significant factor in laboratories with limited resources. The column was a 30 m × 0.25 mm column with a temperature ramp starting at 100 °C and extending up to 300 °C at a rate of 40 °C/min. A splitless injector method was used with an injector temp of 240 °C. However, despite the long sampling times, this method of analysis was able to detect nine antidepressants and four metabolites with a LOD and LOQ of 1.5 and 5.0 ng/mL. The VH analysis matched whole blood analysis for the number of times antidepressants were detected, and in 14 cases yielded a higher concentration in VH compared to whole blood.

3.7. Drug Paraphernalia

Recently, Russell et al. [71] have used direct analysis in real-time mass spectrometry (DART-MS) for the detection of drugs present on swabs taken from drug paraphernalia. Samples were collected in a four-step procedure. Firstly, the drug paraphernalia sample was wiped or swabbed. This was then placed into a small paper envelope and mailed to the laboratory and then extracted and analysed by DART-MS. Samples collected from eight syringe services programs from November 2021 to August 2022. Out of the 364 items of drug paraphernalia investigated, which had shown positive for fentanyl or its analogues, 80% also contained xylazine, a drug commonly used as an animal sedative. Interestingly, heroin was only recorded in 1.9%, and other non-fentanyl opioids, such as tramadol, methadone, and protonitazene, in only 2.5% of the samples. Among the opioid-positive

samples, 98.9% contained fentanyl, with smaller contributions from its analogues of 6.3% fluorofentanyl and 1.6% fentanyl carbamate, with one sample containing fluorofentanyl only. All other fentanyl analogues, such as fluorofentanyl and fentanyl carbamate, were also detected with fentanyl.

3.8. Detection of Cosmetics and Fragrances for Forensic Applications

Since the 1970s, there has been a growing body of research involving the detection of cosmetics for forensic applications. In 2019, a review by Chophi et al. [102] examined the trends in forensic cosmetic analysis. Amongst the numerous techniques described, the group suggest attenuated total reflectance–Fourier transform infrared spectrometry (ATR-FTIR) and Raman spectroscopy as the stand out trends, due to their non-destructive nature. Despite the suggestions toward spectroscopic methods of analysis, there are still numerous papers in which GC (coupled to various detectors) was used for cosmetic analysis. Since there was a 2019 review on the subject and the scope of this paper focuses purely on new trends in forensic GC/GC-MS analysis, this section will highlight some more recent studies from the past four years (since 2019, as of the time of writing).

Recently, Ferrari Junior et al., 2022 [73] utilized headspace (HS) GC-MS to analyse cosmetics seized by the Civil Police of the Federal District in Brazil and, found via derivatization, that formaldehyde concentration levels far exceeded the legal levels (reporting concentrations of 0.33–4.02%). Direct headspace analysis was used for this analysis; however, this can suffer from reduced levels of analyte reaching the instrument compared to preconcentration techniques such as thermal desorption, but it involves far less sample preparation. This group proposed a novel derivatization method and tested several different solutions containing methanol, ethanol, and hydrochloric acid in various ratios. The group determined that the optimal ratio was 25:25:1 (Methanol, ethanol, hydrochloruic acid), incubated for 4 h at 60 °C. An injection volume of 250 µL was used at a split ratio of 15:1 on to a 30 m × 0.25 mm column with 1.4 µm film thickness. The GC oven ramp started at 35 °C and ended at 50 °C with a run time of only 11 min. The cool GC temps and the thick film column allow for analysis of highly volatile analytes.

With a similar application, Nguyen et al., 2022 [74] used both GC-MS, and GC-FID to analyse pharmaceutical tablets: over 56 months, 567 tablets were analysed and 119 identified as being counterfeit. All the pills were analysed in Washington D.C., of the counterfeit pills, fentanyl was identified as the main psychoactive substance, but other opioids and precursors were detected. The GC-MS used splitless injection with 30 m × 0.25 mm columns with 0.25 µm film thickness, and the GC-FID used a 12 m ×0.2 mm column with a 0.33 µm thick film. This research, much like the work carried out in Brazil, emphasizes the need for routine testing of even seemingly legitimate products. GC-MS and GC-FID with easy to automate analytical methods both of samples and backend data are ideally suited to this type of high-throughput work.

A study on the potential of fragrances to be used as a form of trace evidence was carried out in 2019 by Gherghel et al. [72] involving experiments on different variables that may affect the transfer and persistence of fragrances (ageing time of fragrances on the donor fabric, contact time between the donor fabric and the recipient fabric, and fabric type). Solid-phase micro-extraction (SPME) GC-MS techniques were used to extract and analyse the volatile organic compounds (VOCs) in both the primary and secondary fabric. A 30 m × 0.25 mm column with 0.25 µm film thickness was used, the temperature program started at 35 °C, increasing to 300 °C, for the first portion a slow ramp of 5 °C/min was used to 180 °C. There was a 60 min SPME extraction time at 58 °C, with splitless desorption at 250 °C for 3 min. The results showed that all the VOCs from the donor fabric were recovered and detected successfully in the secondary fabric despite the short contact time (10 s) between the two pieces and the up-to-48-hour ageing time of the primary piece. Moreover, the number of low-volatility compounds recovered from secondary fabric was higher than that of high-volatility compounds. Therefore, low-volatility compounds were considered more suitable for forensic analyses than high-volatility compounds. This study

concluded that VOCs within fragrances that stay on clothing are possibly worth analysing in cases such as sexual assault or rape, as they could benefit the establishment whether a contact has occurred between the victim and suspect.

GC-MS was also used in a 2021 study by Raynor et al. [75] to test the persistence of moisturizer products on human skin, regarding the benefits they might have in investigating sexual assault cases. The moisturizer components targeted for analysis were methylparaben, ethylparaben, petrolatum, isopropyl palmitate, glycerol, and cetyl alcohol. Participants were asked to apply either oil-based or glycerol-based moisturizer, and their forearm was swabbed within 24 h after the application. Water pre-wetted swabs were found to be the most effective technique to detect petrolatum compared to those pre-wetted with ethanol and isopropanol. A GC-MS instrument in full scan mode analysing 30–800 m/z was used with a 30 m × 0.25 mm × 0.25 µm column. The moisturizer on human skin can be detected for up to 23.5 h, with the average persistence time of the glycerol-based product of 19.8 ± 1 h and the oil-based product of 13.5 ± 0.7 h. Although this type of evidence holds potential in forensic applications, its time persistence, sample collection, and sampling protocols require further investigation and development.

Recently, a proposed analytical method to measure trace levels of nine banned N-nitrosamines in cosmetic products, which consisted of GC-MS combined with vortex-assisted dispersive liquid–liquid microextraction (VA-DLLME), was successfully developed and validated by Schettino, Benedé and Chisvert (2023) [76]. N-nitrosamines are banned due to their harmful mutagenic, carcinogenic, and teratogenic effects. Therefore, not only N-nitrosamines are prohibited, but also the substances (secondary alkyl and alkanolamines) involved in forming N-nitrosamines. Chloroform was used as the extraction solvent as it allowed the formation of microemulsion by vortexing without the help of a disperser solvent. Three variables of the VA-DLLME (the vortex time, the extractant volume, and the ionic strength of the donor phase) were optimized, and an appropriate way to introduce the sample to the GC was also successfully determined to be a 2 µL injection to a splitless injector at 230 °C. A 30 m × 0.25 mm × 0.25 µm column with a polyethylene glycol stationary phase was used. The ramp began at 60 °C and increased to 240 °C. MS acquisition was carried out in both the SIM and full scan modes. The values achieved from method LODs and LOQs were recorded to be under the threshold value set by the European regulation on cosmetic samples. The lowest LOD was 0.2 ng/L for *N*-nitrosodibutylamine (NDBA) with a LOQ of 0.6 ng/L.

Hemp oil is a beauty product made from hemp seeds obtained from the Cannabis Sativa plant. The application of this type of product was claimed to possibly interfere with the determination of cannabinoid levels in hair samples. To demonstrate the implication, Paul et al., (2019) [77] conducted research involving applying hemp oil directly to participants' head hair, which was then analysed by GC-MS/MS with the multiple reaction monitoring (MRM) acquisition mode carried out. The volunteers all declared that they had neither consumed nor been exposed to cannabis before. They were asked to apply hemp oil to their hair and still carry out their normal hygiene routine for six weeks. Their hair samples were collected before and after the hemp oil application for analysis. All samples were taken through a decontamination process to remove external contamination and sebum. Then, validated extraction methods were utilised to extract cannabinoids and other drug compounds from the samples, where methanol, chloroform, and propan-2-ol were used as solvents. Finally, the extracts underwent an SPE clean-up process using mixed mode cartridges to elute the cannabinoids for GC-MS/MS analysis. GC-MS/MS analysis involved a short 15 m × 0.25 mm column with a 0.25 µm film thickness, the temperature ramp started at 150 °C with an ending temperature of 320 °C. The total run time was only 8 min, due in no small part to the short column. The collision gas was argon, but He was used as the carrier. The MS was run in multiple reaction monitoring modes. The constituents of cannabis targeted were THC, the metabolite [11-nor-Δ(9)–tetrahydrocannabinol-9-carboxylic acid (THC-COOH)], and two cannabinoids: cannabinol (CBN) and cannabidiol (CBD). However, THC and THC-COOH are not likely to be detected in hair since their presence and forma-

tion are obstructed by the characteristics of hair and the pH environment. The LOD for the target analytes ranged from 0.01 pg/mg (THC-OH) to 0.91 pg/mg (CBD), and all the reported RSD% were under 20% with the exception of CBD at 24%. All the volunteers' hair samples provided negative results of Cannabis components before hemp oil application (except for one positive volunteer who was excluded). The post-hemp oil application outcome showed that 89% of participants' hair detected one or more cannabis components, with 33% giving positive results of three major constituents: THC, CBD, and CBN. Despite the fact that cannabis components were detected in volunteers' hair after application, only CBN was detected in the hemp oil, whilst other constituents and metabolites could not be detected. There was controversy regarding identifying the major constituents of cannabis in hair. Some suggested it was because of passive exposure, infrequent use of cannabis or historical exposure, while some opposed it because of chronic and repeated exposure to cannabis. Therefore, it has been suggested that the hair–cannabis results should be treated carefully and cautiously, and the consumption of hemp oil should be taken into consideration prior to interpretation.

GC combined with MS has been successfully applied to analyse fragrance and cream products for forensic purposes. However, because of its destructive nature, GC-MS sometimes is not as prioritised as other non-destructive techniques in analysing trace evidence of cosmetics. Nonetheless, it is worth mentioning that in some cases the contribution of GC-MS is necessary since the information obtained by other techniques is not reliable enough. In research by Gładysz et al., 2021 [78] involving discriminating lipsticks for forensic applications, the ATR-FTIR technique was used first as it is non-destructive; yet the data obtained could not be properly interpreted due to interferences from the substrates. Therefore, GC-MS was proposed as a confirmatory method since it can provide better data with less substrate interferences. Table 2 summarises the key analytes, methods, and limits of detection for the studies relating to the analysis of cosmetic based products.

3.9. Air, Gases and Vapours

The determination of drugs in air has recently been reviewed by Zambas-Adams and Honeychurch [79] An understanding of the levels of drugs and other forensically important compounds in air is important for assessing occupational and environmental exposure. Intelligence on the usage and manufacture of both illicit and licit compounds can also be gained.

Table 2. Summary of key information from the detection of cosmetics.

Analytes	Matrix	Derivatization	Sample Pre-Treatment	Type of GC	LOD	Comments	Ref
Formaldehyde	Cosmetics	Methanol, ethanol, and hydrochloric acid incubated for 4 h at 60 °C	None. Direct headspace analysis	Headspace GC-MS	0.0015% formaldehyde starting concentration	RSD less than 12%	[74]
Screening of pills, fentanyl main substance	Pharmecutical pills	None	The method used is not given.	GC-MS full scan and GC-FID	No quantitative analysis		[72]
Screening of perfume	Fabrics	None	60 min SPME extraction at 58 °C.	GC-MS full scan	Qualitative	SPME analysis testing the persistence of perfumes on fabrics.	[75]
Moisturiser components: petrolatum, glycerol, cetyl alcohol, isopropyl palmitate, ethylparaben, and methylparaben	Swabs from skin		Swabs were extracted into 400 µL of methanol.	GC-MS direct injection full scan	Qualitative	Cetyl alcohol was still detectable from human skin after 23.5 h.	[76]
N-nitrosamines	Costmetic products	None	Some 0.05 g of cosmetic samples were spiked with target analytes. A total of 120 µL of chloroform was used for the extraction, the solution was vortexed and centrifuged. The collected extract was directly injected.	GC-MS both in full scan and SIM mode.	0.2 ng/L	*N*-nitrosodibutylamine.	[77]
Δ^9-tetrahydrocannabiol and associated metabolites	Human hair	20 µL BSTFA (no other details given)	Overnight extraction with methanol dried in a centrifugal concentrator. Hair was then digested in sodium hydroxide and liquid–liquid extraction with chloroform and propan-2-ol. SPE was used to clean up the sample.	GC-MS/MS in multiple reaction monitoring mode	0.12 THC; 0.91 CBD; 0.09 CBN; 0.07 THC-COOH; 0.01 THC-OH (all pg/mg)		[78]

3.10. Cannabis Smoke

Cone et al. [80] have reported on a number of studies focused on investigating the effects of second-hand Marijuana-smoke exposure. In two separate investigations, five drug-free male volunteers with a history of marijuana use were exposed to the side stream smoke of 4 and 16 marijuana cigarettes for an hour a day for six successive days. A further third study was undertaken on two subjects with no previous history of marijuana usage. These were passively exposed to the smoke of 16 marijuana cigarettes in an unventilated room. Vacuum elution through a column containing Gas-Chrom Q adsorbent was undertaken to collect air samples; with exposed tubes eluted with methanol for subsequent determination by gas chromatography with flame-ionisation detection following derivatization with N-methyl-N-trimethylsilyl trifluoroacetamide. Levels of THC were reported to be in the range of 0.9–1.8 μg/L and 3.0–6.7 μg/L, respectively, during the smoking of 4 and 16 marijuana cigarettes. Low-level exposure resulted in urine specimens that tested positive only infrequently. However, high-level exposure studies showed all subjects exhibited significant levels of absorbed THC, with detectable levels of cannabinoid metabolites reported in their urine.

3.11. Synthetic Cannabinoids

The concentrations of synthetic cannabinoid drugs in the air of an English prison were investigated by Paul et al. [81] using two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOF MS). Sample were obtained using both fixed sequential samplers and personal air sampling units worn by prison officers. The air samples were collected onto thermal desorption tubes before examination by GC × GC-TOF MS. Interestingly, analysis of the air samples taken did not reveal any synthetic cannabinoids.

3.12. Anaesthetic Gases Exposure

Commonly referred to as laughing gas, nitrous oxide (N_2O), or nitrous is legally employed as an anaesthetic, especially for surgery, and dentistry. It is also commonly used for pain relief by health care professionals such as midwifes. Its application has been reported to possibly lead to concentrations that can be dangerous, especially in confined spaces. Henderson et al. [82] showed that air levels of N_2O exceeded the legal occupational exposure standards for N_2O in 76% for the midwives they investigated. A passive sampling tube was worn by the midwife for the first four hours of their shift. This contained a steel tube packed with molecular sieve 5A with a diffusive cap on top. This was then removed and the levels of adsorbed N_2O were determined by thermal desorption–gas chromatography with electron capture detection.

In 2015, Giuliani et al. used headspace GC-MS to quantify N_2O and apply their method to a case of N_2O intoxication. To achieve this, two N_2O quantification columns were sequenced in parallel, one with molecular sieve and the other with Porabond Q; hydrogen sulphide was used as an internal standard. This group sought to validate their quantification method over a period of three days, achieving a r^2 mean of 0.98, and a LOD of 5 μM [83]. In the UK in 2019, 9% of 16–24 year-olds reported using N_2O for recreation, these figures make N_2O the second most used controlled substance with only cannabis having more users [84]. However, there is still a lot to learn about the abuse of N_2O, as currently there is limited information on the lethal dosage of N_2O. The Giuliani et al. 2015 [83] paper took samples from the blood and brain of a cadaver, it is hoped that the presented method of analysis can provide new insights into the lethal dosages of N_2O. Moreover, it would be further beneficial to forensic science to develop a breath or blood test that could determine blood concentrations of N_2O in living individuals.

4. Pyrolysis Gas Chromatography for the Investigation of Drugs

As we discussed above, the GC determination of non-volatile compounds that undergo hydrogen bonding can be achieved by their conversion to volatile derivatives via chemical

treatment with agents such as TMS and HMDS. However, a number of compounds are insufficiently volatile due to their relatively high molecular masses, including polymeric materials such as wood and plastic. The formation of derivatives of these cannot readily overcome these issues. One solution is to break these large molecules into smaller fragments which are sufficiently volatile for GC analysis. A number of processes can be used to achieve this, including hydrolysis, oxidation, and pyrolysis. Hydrolysis and oxidation reactions require the addition of chemical reagents and off-line reaction steps which result in the formation of wet reaction products, requiring further processing. However, pyrolysis can be readily undertaken by the application of heat to the sample in an inert atmosphere or a vacuum to give reproducible volatile fragments. Small amounts of sample (mg) are held in contact with a platinum wire or placed in a quartz tube, where a rapid heating to 600–1000 °C can be applied in an inert atmosphere, such as the GC carrier gas. The bonds of the high-molecular weight sample are cleaved at their weakest points, forming smaller, more volatile fragments. These are then thermally focused and introduced directly to the GC, without the need for further clean-up steps. The resulting pyrograms can be used as a fingerprint to identify the specific polymer or from the mass spectra to identify individual fragments to obtain structural information.

Commonly, Py-GC-MS is employed in the forensic sciences for investigation of polymers and macromolecules seen in materials such as condom lubricants, paint, and for the identification of plastics. Alternative applications of Py-GC-MS are summarised in Table 3. However, studies have shown that it is possible to use Py-GC-MS both quantitatively and for exploring the reaction processes occurring during the smoking of various drugs. The first use of Py-GC for the investigation of drugs was described by Janák et al. [9] in 1960, who described the pyrolysis of the sodium salts of fourteen barbiturates and was able to show a number of unique fragments were formed. They showed that the fragments from the drugs were always present and qualitatively and quantitively highly specific. Tsuchihashi, Tatsuno, and Nishikawa [85] have reported the possibility of applying Py-GC-MS for the determination of eight quaternary ammonium drugs (distigmine bromide, bethanechol chloride, pancuronium bromide, methylbenactyzium bromide, propantheline bromide, suxamethonium chloride, neostigmine methylsulphate, and benzethonium chloride) in urine. As with other quaternary ammonium salts, the drugs are very soluble in water and cannot be readily extracted by liquid–liquid extraction with organic solvents, making their extraction from biological fluids difficult. The drugs were first separated by thin-layer chromatography (TLC) and then pyrolyzed using a Curie-point pyrolizer. The resulting fragments were then separated by GC and determined by mass spectrometry utilizing SIM. Detection limits of between 0.01 and 10 μg were reported for each compound. Extraction of quaternary ammonium compounds by using ODS-cartridge was also examined. Nishikawa et al. [86] undertook further investigations utilizing TLC and PyGC with flame ionization detection (FID) for the determination of methylbenactyzium bromide in human urine. In this investigation they showed it possible to use smaller volumes of urine.

Table 3. Pyrolysis gas chromatography–mass spectrometry investigations of drugs.

Analyte	Pyrolysis Conditions	Comments	Ref
Tacrolimus	Platinum crucible using isothermal temperatures of 300 and 400 °C. Pyrolysis analysis was undertaken using isothermal conditions of 300 °C and 400 °C coupled to GC–MS, in full scan mode (m/z 25–900).	Pyrogram obtained at 300 °C predominated by 2,5-dimethyl-3-hexine-2-5-diol.	[87]
Fentanyl	Investigations made under both anaerobic and aerobic conditions coupled to a GC-MS for the modelling of the illicit smoking of fentanyl in transdermal patches.	Propionanilide; pyridine, norfentanyl and despropionyl fentanyl formed along with Cl containing compounds, due to the HCl salt of fentanyl.	[88]
Methamphetamine	Pyrolysis of d-methamphetamine made to identify the products sealed in a glass tube, wrapped in pyrolysis-foil; heated at 200–500 °C. Resulting products extracted in methanol.	Amphetamine and dimethylamphetamine formed. Phenylacetone was reported as an oxidative degradation product. Above 415 °C toluene, styrene, and ethylbenzene were predominated.	[89]
Codeine and morphine	Drugs were pyrolyzed as alkaloid salts. The effect of metal ions on the pyrolysis reactions were investigated. A mixture of metal powder and Na_2CO_3 was added to a piece of pyrolysis foil containing the drug and heated (590 °C). The resulting products were identified by GC-MS.	Morphine, codeine phosphate and dihydrocodeine phosphate, 3,4-dimethyl-1,2-dihydroxybenzene. Based on this compound it was possible to quantify morphine HCl in urine.	[90]
Synthetic cannabinoids (XLR-11, UR-144, A-834735, and PB-22)	Individual drugs were rapidly heated to 800 °C in quartz capillary tubes under an ambient airflow to approximate the burning end of a cigarette whilst being smoked. Products were trapped on a charcoal and passed to the GC-MS.	Analysis of thermolysis products was undertaken by full-GC-MS (m/z 50–550).	[91]
Synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18)	Investigations made to simulate the smoking of drugs.	Above 400 °C, toluene, naphthalene, and 1-naphthalamine were formed. A degradative pathway for the liberation of cyanide was shown.	[92]

Tacrolimus (Figure 1) is a macrolide lactone extracted from *Streptomyces tsukubaensis* with reported potent immunosuppressive activity. Böer et al. [87] have utilized Py-GC-MS and thermal analysis to characterize tacrolimus raw material. Four samples of tacrolimus from different manufacturers were investigated.

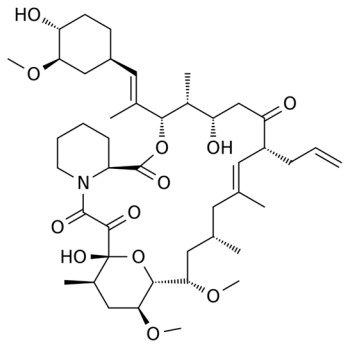

Figure 1. Structure of Tacrolimus.

Powder particles of the immunosuppressive tacrolimus were pyrolysised in a platinum crucible using isothermal temperatures of 300 and 400 °C. Pyrolysis analysis was undertaken using isothermal conditions of 300 ^{0}C and 400 °C coupled to GC–MS, in full scan mode (m/z 25–900). The molecular ion of tacrolimus (m/z 804.01) was not recorded and was concluded to indicate that tacrolimus was totally decomposed. The pyrogram obtained at 300 °C was predominated by a peak with molecular ion of m/z 142 and was identified as 2,5-dimethyl-3-hexine-2-5-diol (Figure 2). The pyrogram of tacrolimus obtained at 400 °C was found to exhibit four main peaks with abundances >50%, with a smaller fifth peak with a relative abundance inferior to 5%.

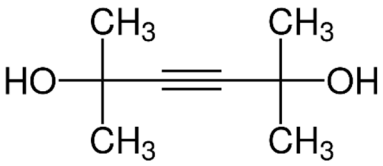

Figure 2. 2,5-dimethyl-3-hexine-2-5-diol.

Fentanyl is a potent opioid analgesic that is increasingly abused. Nishikawa et al. [88] utilized Py-GC-MS to determine the levels of fentanyl present in transdermal patches. The transdermal patches are reportedly easy to obtain and are abused by smoking of the drug-containing reservoir gel or the whole patch itself. Pyrolysis investigations were undertaken using both aerobic and anaerobic conditions utilizing either air of He coupled to the GC-MS. Samples were prepared by sandwiching between 50–100 µg of fentanyl HCl between two separate pieces of quartz wool inside a quartz pyrolysis tube. The pyrolysis tube was then inserted into the platinum coil filament of the pyroprobe and heated in the presence of the reactant gas, which then carries the volatile components formed to a sorbent trap, where they are then focused. Figure 3 summaries the reactions products generated. For the aerobic investigations, during the last minute of flow to the trap, the reactant gas was switched from air to the He carrier gas before transfer to the GC, to avoid damage to the analytical column. Both the anaerobic and aerobic pyrolysis of fentanyl and the transdermal patches were reported to give propionanilide as the major pyrolytic product; pyridine and previously reported metabolites (norfentanyl and despropionyl fentanyl) were also reported. Investigations of fentanyl were also reported to give chlorine-containing products. These were concluded to result from the HCl salt of fentanyl; a result further substantiated by investigations of transdermal patches containing the citrate salt of fentanyl not forming chlorine-containing pyrolytic adducts. The authors concluded that it could be feasible to identify which salt of the drug had been smoked based on the different pyrolytic

products formed. The investigation also reported significant polymeric and hydrocarbon compounds to be formed from the transdermal patch itself.

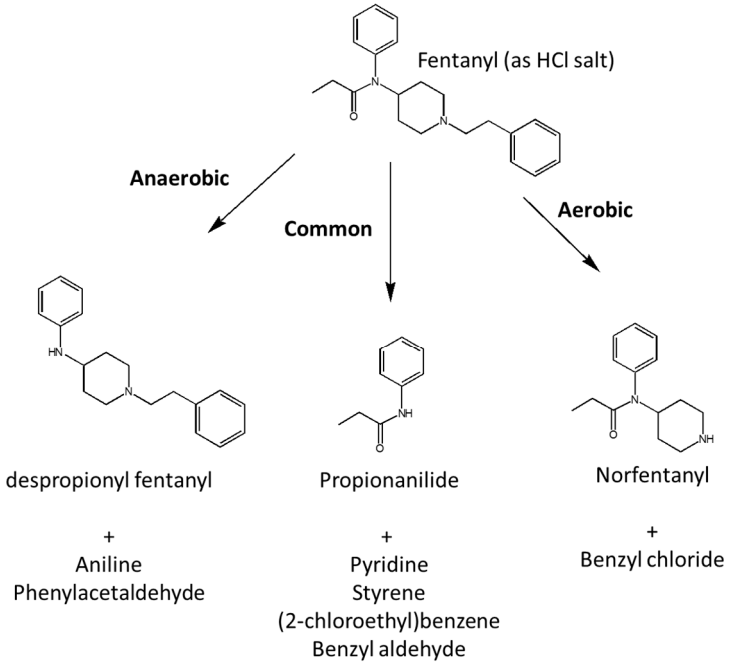

Figure 3. Fentanyl pyrolytic product comparison in anaerobic and aerobic trapping conditions, based on Nishikawa et al. [90].

Illicitly, methamphetamine (MA) is commonly taken by inhalation of the vapor formed by heating the drug on some suitable substrate, such as aluminium foil. Consequently, understanding its behaviour upon heating is of interest for both toxicology and forensic investigations. In light of this, Sato, Hida, and Nagase [89] investigated the mechanism and the products of the pyrolysis of d-methamphetamine (d-MA). Methamphetamine -HCI was sealed in a glass tube, wrapped in pyrolysis-foil which was heated to the Curie point of the foil (200–500 °C). The heated capillary tube was then opened and extracted with methanol. The resulting solution was diluted 1:10 with dichloromethane, for GC-MS or 20 mM of ammonium acetate pH 5 buffer for LC/MS analysis. Investigations of the pyrolysis products of deuterated MA-d3-HCI were also undertaken to explore the transformation of the methyl group of MA. It was found that at temperatures above 315 °C, amphetamine (AM) and dimethylamphetamine (DMA) were produced and were concluded to be formed by demethylation and methylation reactions, respectively. Phenylacetone was reported as an oxidative degradation product and was detected at the same temperature as the demethylation and methylation reactions.

It was concluded that these reactions were the main pyrolysis processes occurring at temperatures below 358 °C. Above 315 °C benzylethyltrimethylammonium (BEMA) was reported to result from reaction of DMA with a methyl group eliminated from the methylamino group of MA. Consequently, it was concluded that this transformation showed that both demethylation and methylation reactions occur in the form of the methyl cation. Above temperatures of 315 °C, thermal degradation of BEMA was reported, occurring via abstraction of a proton at the β-position and elimination of a trimethylamine, giving allylbenzene, cis-β-methylstyrene, and trans-β-methylstyrene. Formation of propylbenzene at temperatures over 423 °C was reported to show that the elimination of a methylamino group of MA occurs without the elimination of a hydrogen atom at the 13-position. At temperatures greater than 445 °C the optical isomers, or l-isomers of AM, MA, and DMA, were reported as the main pyrolysis products formed. Further pyrolysis products of toluene, styrene, and ethylbenzene were also formed, resulting from cleavage of the C-C bond pf the α and β-positions at temperatures. A possible mechanism for the formation of these

products was given and the results were concluded to be important in the field of forensic science, as erroneous identifications of the abused drug could occur from the formation of these pyrolysis products. Mitsui et al. [90] investigated the effects of various metals and inorganic additives on the pyrolysis of codeine and morphine. The drugs were pyrolyzed as alkaloid salts and the pyrolyzed compounds determined by GC. The authors investigated the effects of the addition of metal and inorganic compounds to pyrolysis reactions. A mixture of iron powder and sodium carbonate anhydride (30 mg: 4:1 *w*/*w*) was added to a piece of pyrolysis foil (Curie point of 590 °C). An aqueous solution of the drug under investigation was then added. The foil was dried on a hot plate for 5 min at about 100 °C to evaporate the water. After cooling to room temperature, the pyrolysis foil was inserted into a Curie-point pyrolyzer and the resulting pyrolysis products identified by GC-MS. The pyrolysis products of morphine hydrochloride, codeine phosphate, and dihydrocodeine phosphate were identified by their retention times, as was 3,4-dimethyl-1,2-dihydroxybenzene. Metal powder was added to conduct heat from the pyrolysis foil to the drug under investigation. Aluminium, iron, chromium, zinc, manganese, nickel, or copper, were investigated and iron was found to give the highest sensitivity. Similarly, the effect of the inorganic compounds utilized was also investigated. Sodium carbonate, potassium iodide, or ammonium sulphate were added with the iron powder and the optimum reaction mixture was reported as an iron and sodium carbonate ratio of between 5:1 and 2:1. Gas chromatographic investigations showed a number of peaks, with the largest identified as 3,4-dimethyl-1,2-dihydroxybenzene from its retention time. Using this peak, it was reported to be possible to construct a calibration curve for morphine hydrochloride with a correlation coefficient of 0.9998, and a relative standard deviation for the pyrolysis of 2.5% (n = 10). The authors reported on the possibility of extracting drugs from urine with dichloromethane and the subsequent Py-GC of their salts with reportedly high sensitivity and with good reproducibility.

Synthetic cannabinoids are commonly sold as herbal "spice" for smoking or by vaping. Thomas et al. [91] have shown that heating synthetic cannabinoids which contain a tetramethylcyclopropyl ring, as illustrated for A-834735 in Figure 4, can result in the formation of thermal degradants products which can display pharmacological activity notably different from the parent compound. Using Py GC-MS the authors were able to investigate the effects of heating, similar to that seen whilst smoking or vaping and form these degradants. The synthetic cannabinoids, XLR-11, UR-144, A-834735, and PB-22, were investigated via the addition of 5 µg aliquots of the individual drugs to separate quartz capillary tubes. These were equilibrated at 50 °C, and then rapidly heated to 800 °C (20 °C/s) under an ambient airflow to approximate the burning end of a cigarette being smoked. The resulting degradation products were then trapped on a charcoal desorption tube at 50 °C. The probe was held at 800 °C for 20 s, and then airflow was switched to He while the thermolysis/pyrolysis probe was returned to 50 °C and equilibrated. The charcoal desorption tube was then heated to 300 °C and the He flow diverted to the GC for separation and the analysis of thermolysis products using full-scan mass spectrometry over the mass range, *m*/*z* 50–550.

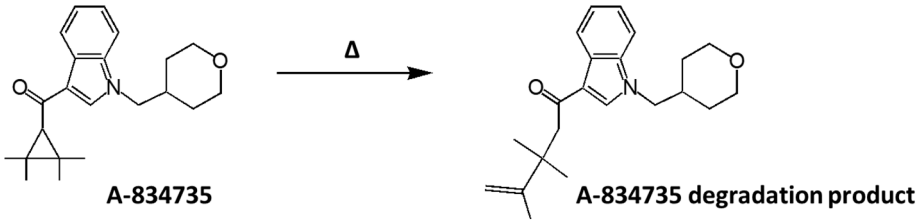

Figure 4. The thermal degradation of the synthetic cannabinoid, A-834735, after Thomas et al. [91].

In a separate study [92], the thermal stability of six carboxamide-type synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA,

NNEI, and MN-18, Figure 5) was undertaken to characterize the possible user exposure to their thermolysis products formed during their smoking. Smoking of these drugs involves heating them to high temperatures via burning in a cigarette or "joint" and can result in temperatures of between 700 °C to 900 °C [93]. As a result of the high temperature that these drugs can be exposed to, their thermal instability can lead to the formation of the carcinogenic compound, naphthalene, resulting from the thermal degradation of the naphthyl group, a common constituent of synthetic cannabinoids. To simulate the smoking of these drugs, samples were heated sequentially to 200, 400, 600, and 800 °C and the resultant pyrolysis products separated and determined by GC-MS. Further analysis quantified the thermolytically generated cyanide and was undertaken by LC–MS/MS.

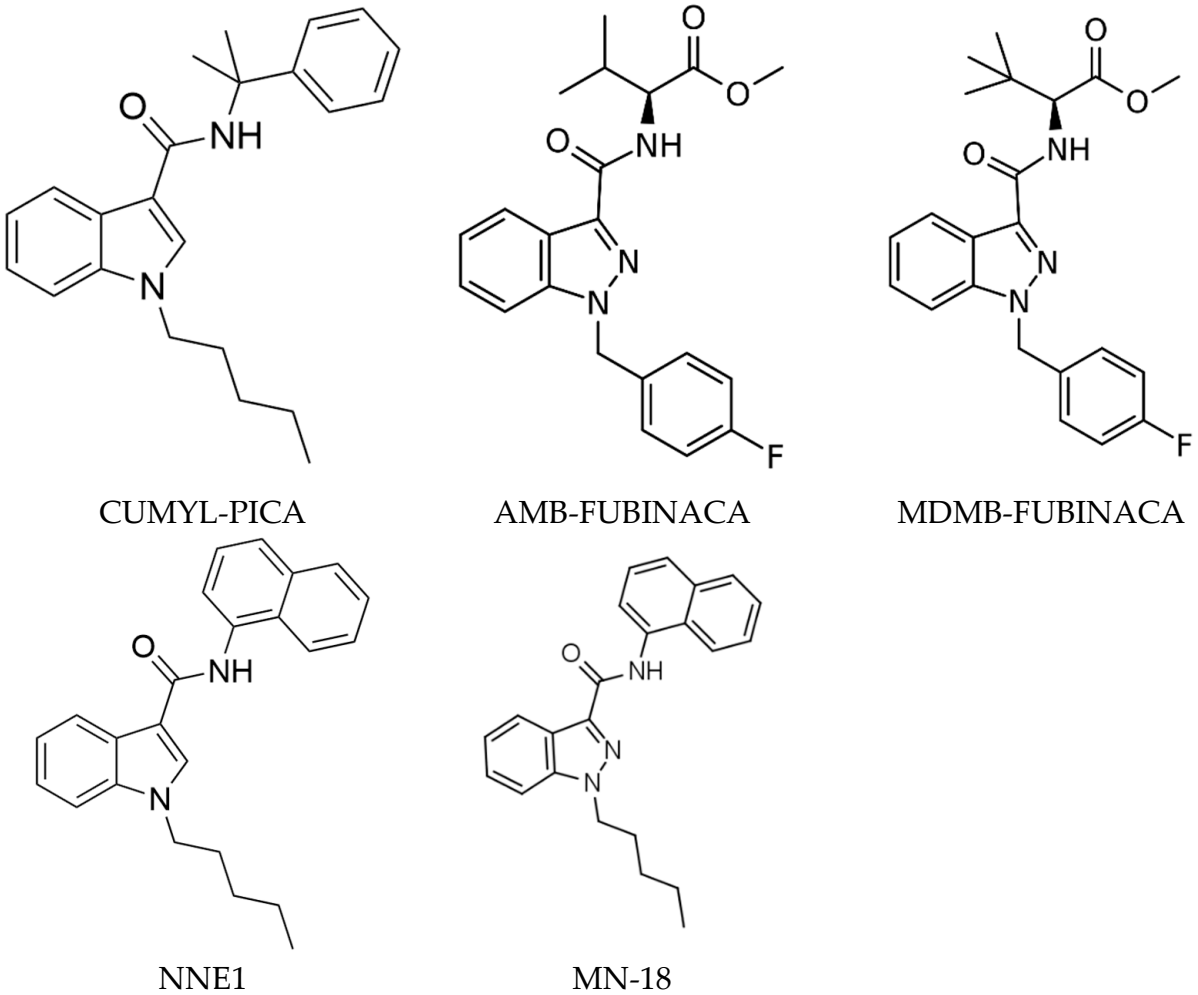

Figure 5. Structures and designations of carboxamide-type synthetic cannabinoids.

Compound-specific pyrolysis products were postulated, and a possible degradative pathway for carboxamide-type synthetic cannabinoids given, which was postulated to result via the formation of an indole or indazole-amide and subsequent dehydration to give an indole or indazole-carbonitrile. Thermolytic liberation of cyanide was also reported with levels up in levels up to 27 µg/mg of the starting drug material. The synthetic cannabinoids investigated were reported to also undergo further thermal degradation above 400 °C, producing toxic products, including toluene, naphthalene, and 1-naphthalamine. However, the drugs were reported to be stable at lower applied temperatures, with 200 °C being generally insufficient to either give degradants or volatilize the parent drug. However, at temperatures between 400–600 °C, CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, and NNEI were reported to undergo extensive degradation while MN-18 and MDMB-FUBINACA reportedly showed greater stability. The authors postulated that this a was

result of the additional nitrogen in the indazole ring of MN-18, compared to NNEI, which conferred improved thermal stability to molecule. The effects of pyrolysis for these drugs was explored by heating sequentially to 200, 400, 600, and 800 °C using methodology similar to that reported previously [92].

A wide range of different chemical stabilities were found for the drugs investigated, with 200 °C being generally insufficient to either give degradants or to volatilize the parent drug. CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, and NNEI underwent extensive degradation at temperatures between 400 and 600 °C, while MN-18 and MDMB-FUBINACA were reportedly more stable. The authors postulated that, compared to NNEI, the additional nitrogen in the indazole ring of MN-18 confers improved thermal stability.

Buscopan® (Figure 6a), is commonly used for the relief of abdominal discomfort, pain, and acute colic. The active ingredient is hyoscine butyl bromide, a quaternary ammonium compound that has low lipid solubility and cannot readily pass the blood–brain barrier. However, under the thermal conditions commonly seen during cigarette smoking, the hyoscine butyl bromide can be converted to scopolamine (Figure 6b). Scopolamine, also known as hyoscine, is a naturally occurring tropane alkaloid and anticholinergic drug that can be used to treat motion sickness, nausea, vomiting, and can decrease saliva production. However, it is also smoked recreationally for its hallucinogenic properties. However, this is not commonly undertaken, as the experiences are reported to be unpleasant, mentally and physically, so repeated recreational use is rare [94]. Nevertheless, in situations such as prisons, the smoking of medications such as Buscopan® to give scopolamine has been reported [95,96].

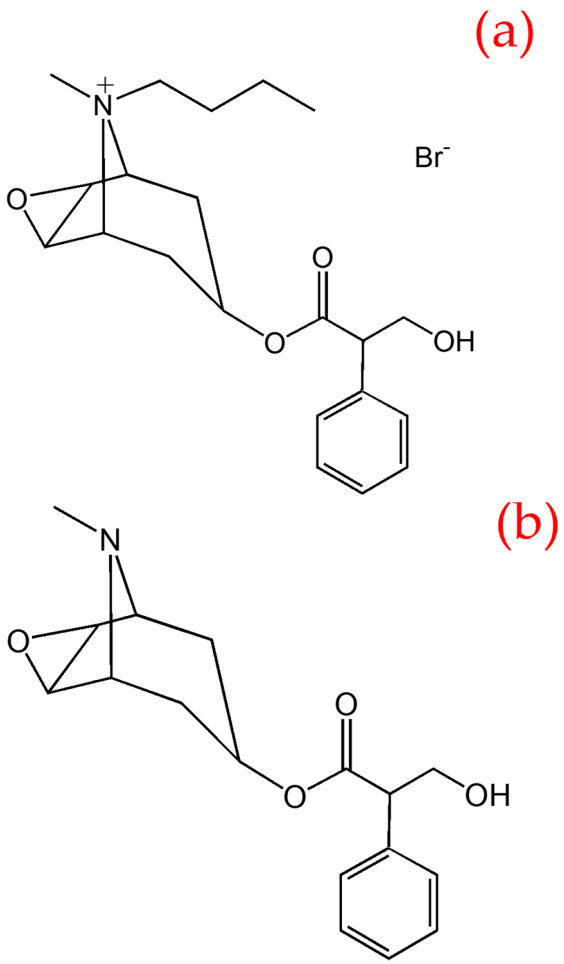

Figure 6. Structure of (**a**) hyoscine butyl bromide and (**b**) scopolamine.

Notably, Deutsch et al. [97] have reported on the problem associated with the determination of scopolamine by GC-MS. They have shown the possibility of its determination following derivatization with HFBA. A base ion of m/z 351 was reported for the mass spectrum of HFBA derivatized scopolamine corresponding to the molecular ion of the HFB derivative. However, further analysis of the mass spectrum showed this to be different from that previously reported. This difference was suggested to result from the HFB scopolamine derivative undergoing a transacylation in the GC-MS inlet system.

Heroin is often inhaled as a vapor via heating on aluminium foil in a process colloquially referred to as "chasing the dragon". Brenneisen and Hasler [98] have studied the possible products formed by simulating these conditions by heating the heroin samples on aluminium foil at 250 to 400 °C. The resulting pyrolysis products then being collected in a condenser trap. Investigation by GC-MS showed 72 pyrolysis products generated from the heating of diacetylmorphine (street heroin) residues and from the aluminium foil itself. Reportedly, however, only half of these compounds could be identified. Diacetylmorphine (base and salt) was found to undergo substitution reactions to complete degradation. Some typical street heroin constituents, such as, morphine, codeine, acetylcodeine, papaverine, and caffeine, were found to be heat-stable, with other adulterants such as, noscapine and paracetamol, being pyrolyzed to a greater extent. The principal chemical reactions leading to the formation of pyrolysis products were reported to be desacetylation, transacetylation, N-demethylation, O-methylation, ring cleavage, and oxidation.

5. Conclusions

Gas chromatography is a mature, robust technique and has been considered the "gold standard" in many analytical forensic investigations. The development of new technologies with matched capabilities and reliability has not emerged in such a way as to reduce the relevance of these techniques in forensic science. The work presented herein, however, demonstrates that rather than looking to new technologies, researchers are finding increasingly creative and non-traditional targets for analysis. This would seem to be partly driven by the increased sensitivity advances in modern GC and GC-MS instrumentation, allowing for smaller sample sizes to be utilized and, hence, opening up the potential for new sample types. The determination of drugs and potentially other compounds in sweat can offer an alternative to the more commonly employed urine, blood, and hair analysis. Similarly, the relatively non-invasive sample cerumen offers advantages, with a detection window longer than that of urine but shorter than that reported of hair and has been shown to be possibly less susceptible to contamination. Both meconium and breast milk can be used to give insights into in utero and infant exposure.

It has been shown to be possible to apply Py-GC-MS beyond its normal application for the determination of polymeric materials to explore the chemistry and products formed during the smoking of a number of common drugs. The determination of drugs in insects, following feeding on meat both fortified and from natural levels found in autopsy-derived samples, has been shown possible. This is an interesting development and it could be possible to use this for drug detection in other application areas. Allowing for the natural abilities of the insect to search out and return with information on the area. Similarly, the possibility of determining drug and explosive residues in plants and other animals and could be an area of future study.

Most GC approaches have a relatively low sample throughput, with typical run times in the order of 15 to 30 min. Additional time is also required for a suitable extraction and concentration step to be made. There is presently interest in fast chromatography, or techniques such as DART MS, which do not require a chromatographic step, which could be applied to overcome this issue. The requirement of the analyte to be both thermally stable and volatile limits the range of compounds that can be successfully determined by GC. A factor that has partly led to the increasing popularity of techniques that can be undertaken at ambient temperatures, such as LC-MS.

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References

1. Stafford, D.T.; Brettell, T.A. *Forensic Gas Chromatography*; CRC Press: Boca Raton, FL, USA, 2019; ISBN 9780203016411/0203016416.
2. Phillips, D.J.; Caparella, M.; El Fallah, Z.; Neue, U.D. Small particle columns for faster high performance liquid chromatography. *Waters Column* **1996**, *6*, 1–7. Available online: https://www.waters.com/webassets/cms/library/docs/wc6-2-1.pdf (accessed on 20 August 2023).
3. Holm, T. Aspects of the mechanism of the flame ionization detector. *J. Chromatogr. A* **1999**, *842*, 221–227. [CrossRef]
4. Pellizzari, E.D. Electron capture detection in gas chromatography. *J. Chromatogr. A* **1974**, *98*, 323–361. [CrossRef]
5. Burgett, C.A.; Smith, D.H.; Bente, H.B. The nitrogen-phosphorus detector and its applications in gas chromatography. *J. Chromatogr. A* **1977**, *134*, 57–64. [CrossRef]
6. Holmes, J.C.; Morrell, F.A. Oscillographic mass spectrometric monitoring of gas chromatography. *Appl. Spectrosc.* **1957**, *11*, 86–87. [CrossRef]
7. Gohlke, R.S. Time-of-flight mass spectrometry and gas-liquid partition chromatography. *Anal. Chem.* **1959**, *31*, 535–541. [CrossRef]
8. Vaye, O.; Ngumbu, R.S.; Xia, D. A review of the application of comprehensive two-dimensional gas chromatography MS-based techniques for the analysis of persistent organic pollutants and ultra-trace level of organic pollutants in environmental samples. *Rev. Anal. Chem.* **2022**, *41*, 63–73. [CrossRef]
9. Janák, J. Identification of the structure of non-volatile organic substances by gas chromatography of pyrolytic products. *Nature* **1960**, *185*, 684–686. [CrossRef]
10. Dhont, J.H. Pyrolysis and gas chromatography for the detection of the benzene ring in organic compounds. *Nature* **1961**, *192*, 747–748. [CrossRef]
11. Hewitt, G.C.; Whitham, B.T. The identification of substances of low volatility by pyrolysis/gas-liquid chromatography. *Analyst* **1961**, *86*, 643–652. [CrossRef]
12. Jones, C.R.; Moyles, A.F. Pyrolysis and gas-liquid chromatography on the microgram scale. *Nature* **1961**, *191*, 663–665. [CrossRef]
13. Oro, J.; Han, J.; Zlatkis, A. Application of high-resolution gas chromatography-mass spectrometry to the analysis of the pyrolysis products of isoprene. *Anal. Chem.* **1967**, *39*, 27–32. [CrossRef]
14. Merritt, C., Jr.; Robertson, D.H. The analysis of proteins, peptides and amino acids by pyrolysis-gas chromatography and mass spectrometry. *J. Chromatogr. Sci.* **1967**, *5*, 96–98. [CrossRef]
15. Biemann, K.; Oro, J.; Toulmin III, P.; Orgel, L.E.; Nier, A.O.; Anderson, D.M.; Simmonds, P.G.; Flory, D.; Diaz, A.V.; Rushneck, D.R.; et al. Search for organic and volatile inorganic compounds in two surface samples from the Chryse Planitia region of Mars. *Science* **1976**, *194*, 72–76. [CrossRef] [PubMed]
16. Reinhardt, M.; Goetz, W.; Thiel, V. Testing flight-like pyrolysis gas chromatography–mass spectrometry as performed by the Mars Organic Molecule Analyzer onboard the ExoMars 2020 rover on Oxia Planum analog samples. *Astrobiology* **2020**, *20*, 415–428. [CrossRef] [PubMed]
17. Murat, P.; Harohalli Puttaswamy, S.; Ferret, P.J.; Coslédan, S.; Simon, V. Identification of Potential Extractables and Leachables in Cosmetic Plastic Packaging by Microchambers-Thermal Extraction and Pyrolysis-Gas Chromatography-Mass Spectrometry. *Molecules* **2020**, *25*, 2115. [CrossRef] [PubMed]
18. Sullivan, G.L.; Gallardo, J.D.; Jones, E.W.; Hollliman, P.J.; Watson, T.M.; Sarp, S. Detection of trace sub-micron (nano) plastics in water samples using pyrolysis-gas chromatography time of flight mass spectrometry (PY-GCToF). *Chemosphere* **2020**, *249*, 126179. [CrossRef]
19. Fischer, M.; Scholz-Böttcher, B.M. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. *Environ. Sci. Technol.* **2017**, *51*, 5052–5060. [CrossRef]

20. Cersoy, S.; Daheur, G.; Zazzo, A.; Zirah, S.; Sablier, M. Pyrolysis comprehensive gas chromatography and mass spectrometry: A new tool to assess the purity of ancient collagen prior to radiocarbon dating. *Anal. Chim. Acta* **2018**, *1041*, 131–145. [CrossRef]
21. Decq, L.; Abatih, E.; Van Keulen, H.; Leyman, V.; Cattersel, V.; Steyaert, D.; Van Binnebeke, E.; Fremout, W.; Saverwyns, S.; Lynen, F. Nontargeted pattern recognition in the search for pyrolysis gas chromatography/mass spectrometry resin markers in historic lacquered objects. *Anal. Chem.* **2019**, *91*, 7131–7138. [CrossRef]
22. Correia, L.P.; Procópio, J.V.V.; de Santana, C.P.; Santos, A.F.O.; de Medeiros Cavalcante, H.M.; Macêdo, R.O. Characterization of herbal medicine with different particle sizes using pyrolysis GC/MS, SEM, and thermal techniques. *J. Therm. Anal. Calorim.* **2013**, *111*, 1691–1698. [CrossRef]
23. Smith, C.S.; Morgan, S.L.; Parks, C.D.; Fox, A.; Pritchard, D.G. Chemical marker for the differentiation of group A and group B streptococci by pyrolysis-gas chromatography-mass spectrometry. *Anal. Chem.* **1987**, *59*, 1410–1413. [CrossRef]
24. Campbell, G.P.; Gordon, A.L. Analysis of condom lubricants for forensic casework. *J. Forensic Sci.* **2007**, *52*, 630–642. [CrossRef]
25. Zimmerman, J.; Kimmett, M.J.; Mooney, D. Preliminary examination of machine copier toners by infrared spectrophotometry and pyrolysis gas chromatography. *J. Forensic Sci.* **1986**, *31*, 489–493. [CrossRef]
26. Munson, T.O. The classification of photocopies by pyrolysis gas chromatography-mass spectrometry. *J. Forensic Sci.* **1989**, *34*, 352–365. [CrossRef]
27. Sakayanagi, M.; Watanabe, K.; Konda, Y.; Harigaya, Y. Identification of pressure-sensitive adhesive polypropylene tape. *J. Forensic Sci.* **2003**, *48*, JFS2002245. [CrossRef]
28. Huttunen, J.; Austin, C.; Dawson, M.; Roux, C.; Robertson, J. Physical evidence in drug intelligence, Part 1: Rationale based on hierarchic distribution of drugs using pyrolysis gas chromatography–mass spectrometry as an example. *Aust. J. Forensic Sci.* **2007**, *39*, 93–106. [CrossRef]
29. Kochanowski, B.K.; Morgan, S.L. Forensic discrimination of automotive paint samples using pyrolysis-gas chromatography–mass spectrometry with multivariate statistics. *J. Chromatogr. Sci.* **2000**, *38*, 100–108. [CrossRef]
30. Schütz, H.; Paine, A.; Erdmann, F.; Weiler, G.; Verhoff, M.A. Immunoassays for drug screening in urine: Chances, challenges, and pitfalls. *Forensic Sci. Med. Pathol.* **2006**, *2*, 75–83. [CrossRef]
31. Gallardo, E.; Barroso, M.; Queiroz, J.A. LC-MS: A powerful tool in workplace drug testing. *Drug Test. Anal.* **2009**, *1*, 109–115. [CrossRef]
32. Centre for Substance Abuse Treatment. Appendix B. Urine Collection and Testing Procedures and Alternative Methods for Monitoring Drug Use. In *Substance Abuse: Clinical Issues in Intensive Outpatient Treatment*; Treatment Improvement Protocol (TIP) Series; Substance Abuse and Mental Health Services Administration (US): Rockville, MD, USA, 2006; No. 47; pp. 237–245.
33. Xu, D.; Ji, J.; Xiang, P.; Yan, H.; Shen, M. Two DFSA cases involving midazolam clarified by the micro-segmental hair analyses. *Forensic Toxicol.* **2022**, *40*, 374–382. [CrossRef] [PubMed]
34. Usman, M.; Naseer, A.; Baig, Y.; Jamshaid, T.; Shahwar, M.; Khurshuid, S. Forensic toxicological analysis of hair: A review. *Egypt. J. Forensic Sci.* **2019**, *9*, 17. [CrossRef]
35. Lendoiro, E.; Quintela, O.; de Castro, A.; Cruz, A.; Lopez-Rivadulla, M.; Concheiro, M. Target screening and confirmation of 35 licit and illicit drugs and metabolites in hair by LC–MSMS. *Forensic Sci. Int.* **2012**, *217*, 207–215. [CrossRef] [PubMed]
36. Ettlinger, J.; Yegles, M. Influence of thermal hair straightening on cannabis and cocaine content in hair. *Forensic Sci. Int.* **2016**, *265*, 13–16. [CrossRef] [PubMed]
37. Gambelunghe, C.; Rossi, R.; Aroni, K.; Bacci, M.; Lazzarini, A.; De Giovanni, N.; Carletti, P.; Fucci, N. Sweat testing to monitor drug exposure. *Ann. Clin. Lab. Sci.* **2013**, *43*, 22–30.
38. Liberty, H.J.; Johnson, B.D.; Fortner, N. Detecting cocaine use through sweat testing: Multilevel modeling of sweat patch length-of-wear data. *J. Anal. Toxicol.* **2004**, *28*, 667–673. [CrossRef]
39. Tracqui, A.; Kintz, P.; Ludes, B.; Jamey, C.; Mangin, P. The detection of opiate drugs in nontraditional specimens (clothing): A report of ten cases. *J. Forensic Sci.* **1995**, *40*, 263–265. [CrossRef]
40. Baker, L.B. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature* **2019**, *6*, 211–259. [CrossRef]
41. Jadoon, S.; Karim, S.; Akram, M.R.; Kalsoom Khan, A.; Zia, M.A.; Siddiqi, A.R.; Murtaza, G. Recent developments in sweat analysis and its applications. *Int. J. Anal. Chem.* **2015**, *2015*, 164974. [CrossRef]
42. Caplan, Y.H.; Goldberger, B.A. Alternative specimens for workplace drug testing. *J. Anal. Toxicol.* **2001**, *25*, 396–399. [CrossRef]
43. Concheiro, M.; Shakleya, D.M.; Huestis, M.A. Simultaneous analysis of buprenorphine, methadone, cocaine, opiates and nicotine metabolites in sweat by liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* **2011**, *400*, 69–78. [CrossRef] [PubMed]
44. Cone, E.J.; Hillsgrove, M.J.; Jenkins, A.J.; Keenan, R.M.; Darwin, W.D. Sweat testing for heroin, cocaine, and metabolites. *J. Anal. Toxicol.* **1994**, *18*, 298–305. [CrossRef] [PubMed]
45. Barnes, A.J.; Smith, M.L.; Kacinko, S.L.; Schwilke, E.W.; Cone, E.J.; Moolchan, E.T.; Huestis, M.A. Excretion of methampheta-mine and amphetamine in human sweat following controlled oral methamphetamine administration. *Clin. Chem.* **2008**, *54*, 172–180. [CrossRef] [PubMed]
46. De Martinis, B.S.; Barnes, A.J.; Scheidweiler, K.B.; Huestis, M.A. Development and validation of a disk solid phase extraction and gas chromatography–mass spectrometry method for MDMA, MDA, HMMA, HMA, MDEA, methamphetamine and amphetamine in sweat. *J. Chromatogr. B Biomed. Appl.* **2007**, *852*, 450–458. [CrossRef] [PubMed]

47. Preston, K.L.; Huestis, M.A.; Wong, C.J.; Umbricht, A.; Goldberger, B.A.; Cone, E.J. Monitoring cocaine use in sub-stance-abuse-treatment patients by sweat and urine testing. *J. Anal. Toxicol.* **1999**, *23*, 313–322. [CrossRef] [PubMed]
48. Huestis, M.A.; Scheidweiler, K.B.; Saito, T.; Fortner, N.; Abraham, T.; Gustafson, R.A.; Smith, M.L. Excretion of Δ9-tetrahydrocannabinol in sweat. *Forensic Sci. Int.* **2008**, *174*, 173–177. [CrossRef] [PubMed]
49. Kintz, P.; Tracqui, A.; Mangin, P.; Edel, Y. Sweat Testing in Opioid Users with a Sweat Patch. *J. Anal. Toxicol.* **1996**, *20*, 393–397. [CrossRef] [PubMed]
50. Uemura, N.; Nath, R.P.; Harkey, M.R.; Henderson, G.L.; Mendelson, J.; Jones, R.T. Cocaine levels in sweat collection patches vary by location of patch placement and decline over time. *J. Anal. Toxicol.* **2004**, *28*, 253–259. [CrossRef]
51. Huestis, M.A.; Cone, E.J.; Wong, C.J.; Umbricht, A.; Preston, K.L. Monitoring opiate use in substance abuse treatment patients with sweat and urine drug testing. *J. Anal. Toxicol.* **2000**, *24*, 509–521. [CrossRef]
52. Choi, M.J.; Oh, C.H. 2nd dimensional GC-MS analysis of sweat volatile organic compounds prepared by solid phase microextraction. *Technol. Health Care* **2014**, *22*, 481–488. [CrossRef]
53. Shokry, E.; Marques, J.G.; Ragazzo, P.C.; Pereira, N.Z.; Filho, N.R.A. Earwax as an alternative specimen for forensic analysis. *Forensic Toxicol.* **2017**, *35*, 348–358. [CrossRef] [PubMed]
54. Shokry, E.; de Oliveira, A.E.; Avelino, M.A.G.; de Deus, M.M.; Pereira, N.Z.; Filho, N.R.A. Earwax: An innovative tool for assessment of tobacco use or exposure. A pilot study in young adults. *Forensic Toxicol.* **2017**, *35*, 389–398. [CrossRef]
55. Meier, S.I.; Koelzer, S.C.; Schubert-Zsilavecz, M.; Toennes, S.W. Analysis of drugs of abuse in Cerumen—Correlation of postmortem analysis results with those for blood, urine and hair. *Drug Test Anal.* **2017**, *9*, 1572–1585. [CrossRef] [PubMed]
56. Gonçalves Barbosa, J.M.; Machado Botelho, A.F.; Santana da Silva, R.H.; Ferreira de Almeida, S.S.; Ferreira, E.R.; Caetano David, L.; Alves Fortuna Lima, D.; Cavalcante ESilva, T.; Jorge da Cunha, P.H.; Roberto Antoniosi Filho, N. Identification of cattle poisoning by Bifenthrin via earwax analysis by HS/GC–MS. *Biomed. Chromatogr.* **2021**, *35*, e5017. [CrossRef] [PubMed]
57. Mozaner Bordin, D.C.; Alves, M.N.; Cabrices, O.G.; de Campos, E.G.; De Martinis, B.S. A rapid assay for the simultaneous determination of nicotine, cocaine and metabolites in meconium using disposable pipette extraction and gas chromatography–mass spectrometry (GC–MS). *J. Anal. Toxicol.* **2014**, *38*, 31–38. [CrossRef] [PubMed]
58. Onchoi, C.; Kongtip, P.; Nankongnab, N.; Chantanakul, S.; Sujirarat, D.; Woskie, S. Organophosphates in meconium of new-born babies whose mothers resided in agricultural areas of Thailand. *Southeast Asian J. Trop. Med. Public Health* **2020**, *51*, 77.
59. Drabińska, N.; Flynn, C.; Ratcliffe, N.; Belluomo, I.; Myridakis, A.; Gould, O.; Fois, M.; Smart, A.; Devine, T.; Costello, B.D.L. A literature survey of all volatiles from healthy human breath and bodily fluids: The human volatilome. *J. Breath Res.* **2021**, *15*, 034001. [CrossRef]
60. Fan, J.C.; Ren, R.; He, H.L.; Jin, Q.; Wang, S.T. Determination of phthalate esters in breast milk before and after frozen storage in milk storage bags. *Food Addit. Contam. Part A Chem.* **2020**, *37*, 1897–1905. [CrossRef]
61. Witczak, A.; Pohoryło, A.; Abdel-Gawad, H. Endocrine-disrupting organochlorine pesticides in human breast milk: Changes during lactation. *Nutrients* **2021**, *13*, 229. [CrossRef]
62. Ishak, N.; Ahmad, A.H.; Mohamad Noor, S.A.; Ahmad, A. Detection of heroin metabolites at different developmental stages of *Lucilia cuprina* (Diptera: Calliphoridae) reared in heroin-treated meat: A preliminary analysis. *Egypt. J. Forensic Sci.* **2019**, *9*, 65. [CrossRef]
63. Magni, P.A.; Pacini, T.; Pazzi, M.; Vincenti, M.; Dadour, I.R. Development of a GC–MS method for methamphetamine detection in *Calliphora vomitoria* L.(Diptera: Calliphoridae). *Forensic Sci. Int.* **2014**, *241*, 96–101. [CrossRef] [PubMed]
64. Campobasso, C.P.; Gherardi, M.; Caligara, M.; Sironi, L.; Introna, F. Drug analysis in blowfly larvae and in human tissues: A comparative study. *Int. J. Legal Med.* **2004**, *118*, 210–214. [CrossRef] [PubMed]
65. Metushi, I.G.; Fitzgerald, R.L.; McIntyre, I.M. Assessment and comparison of vitreous humor as an alternative matrix for forensic toxicology screening by GC–MS. *J. Anal. Toxicol.* **2016**, *40*, 243–247. [CrossRef]
66. Jenkins, A.J.; Oblock, J. Phencyclidine and cannabinoids in vitreous humor. *Leg Med. (Tokyo)* **2008**, *10*, 201–203. [CrossRef]
67. Lin, D.L.; Lin, R.L. Distribution of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in traffic fatality cases. *J. Anal. Toxicol.* **2005**, *29*, 58–61. [CrossRef] [PubMed]
68. Bévalot, F.; Cartiser, N.; Bottinelli, C.; Fanton, L.; Guitton, J. Correlation of bile and vitreous humor concentrations with blood drug concentrations for forensic interpretation: A comparative study between animal experimental and human postmortem data. *Forensic Toxicol.* **2015**, *33*, 131–140. [CrossRef]
69. Gould, O.; Drabińska, N.; Ratcliffe, N.; de Lacy Costello, B. Hyphenated mass spectrometry versus real-time mass spectrometry techniques for the detection of volatile compounds from the human body. *Molecules* **2021**, *26*, 7185. [CrossRef]
70. Ntoupa, P.S.A.; Armaos, K.P.; Athanaselis, S.A.; Spiliopoulou, C.A.; Papoutsis, I.I. Study of the distribution of antidepressant drugs in vitreous humor using a validated GC/MS method. *Forensic Sci. Int.* **2020**, *317*, 110547. [CrossRef]
71. Russell, E. Rapid Analysis of Drugs: A Pilot Surveillance System to Detect Changes in the Illicit Drug Supply to Guide Timely Harm Reduction Responses—Eight Syringe Services Programs, Maryland, November 2021–August 2022. *MMWR Morb. Mortal Wkly Rep.* **2023**, *72*, 458–462. [CrossRef]
72. Gherghel, S.; Morgan, R.M.; Arrebola-Liébanas, J.F.; Blackman, C.S.; Parkin, I.P. Fragrance transfer between fabrics for forensic reconstruction applications. *Sci. Justice* **2019**, *59*, 256–267. [CrossRef] [PubMed]
73. Ferrari Júnior, E.; Salum, L.B.; Damasceno, R.R.; Alves, B.E.P.; Caldas, E.D. Formaldehyde analysis of seized cosmetic products by HS-GC-MS of methylal, ethoxymethoxymethane, and ethylal derivatives. *Braz. J. Pharm. Sci.* **2022**, *58*, e20253. [CrossRef]

74. Nguyen, L.; Evans, A.; Frank, G.; Levitas, M.; Mennella, A.; Short, L.C. Genuine and counterfeit prescription pill surveillance in Washington, DC. *Forensic Sci. Int.* **2022**, *339*, 111414. [CrossRef] [PubMed]
75. Raynor, I.; Coulson, S.A.; Curran, J.M.; Nair, M.V.; Miskelly, G.M.; Rindelaub, J.D. The persistence of moisturizer products on human skin in relation to sexual assault investigations. *Forensic Chem.* **2021**, *25*, 100348. [CrossRef]
76. Schettino, L.; Benedé, J.L.; Chisvert, A. Determination of nine prohibited N-nitrosamines in cosmetic products by vor-tex-assisted dispersive liquid–liquid microextraction prior to gas chromatography-mass spectrometry. *RSC Adv.* **2023**, *13*, 2963–2971. [CrossRef] [PubMed]
77. Paul, R.; Williams, R.; Hodson, V.; Peake, C. Detection of cannabinoids in hair after cosmetic application of hemp oil. *Sci. Rep.* **2019**, *9*, 2582. [CrossRef]
78. Gładysz, M.; Król, M.; Karoly, A.; Szalai, R.; Kościelniak, P. A multitechnique approach for discrimination and identification of lipsticks for forensic purposes. *J. Forensic Sci.* **2022**, *67*, 494–504. [CrossRef] [PubMed]
79. Zambas-Adams, P.; Honeychurch, K.C. Analytical approaches and trends in the determination of psychoactive drugs in air. *Sci* **2022**, *4*, 1. [CrossRef]
80. Cone, E.J.; Johnson, R.E.; Darwin, W.D.; Yousefnejad, D.; Mell, L.D.; Paul, B.D.; Mitchell, J. Passive inhalation of marijuana smoke: Urinalysis and room air levels of delta-9-tetrahydrocannabinol. *J. Anal. Toxicol.* **1987**, *11*, 89–96. [CrossRef]
81. Paul, R.; Smith, S.; Gent, L.; Sutherill, R. Air monitoring for synthetic cannabinoids in a UK prison: Application of personal air sampling and fixed sequential sampling with thermal desorption two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. *Drug Test. Anal.* **2021**, *13*, 1678–1685. [CrossRef]
82. Henderson, K.A.; Matthews, I.P.; Adisesh, A.; Hutchings, A.D. Occupational exposure of midwives to nitrous oxide on delivery suites. *Occup. Environ. Med.* **2003**, *60*, 958–961. [CrossRef]
83. Giuliani, N.; Beyer, J.; Augsburger, M.; Varlet, V. Validation of an analytical method for nitrous oxide (N_2O) laughing gas by headspace gas chromatography coupled to mass spectrometry (HS-GC–MS): Forensic application to a lethal intoxication. *J. Chromatogr. B Biomed. Appl.* **2015**, *983*, 90–93. [CrossRef] [PubMed]
84. Sumnall, H. Recreational use of nitrous oxide. *BMJ* **2022**, *378*, o2297. [CrossRef] [PubMed]
85. Tsuchihashi, H.; Tatsuno, M.; Nishikawa, M. The analysis of quaternary ammonium compounds by pyrolysis gas chroma-tography/mass spectrometry and thin-layer chromatography. *Eisei Kagaku* **1990**, *36*, 28–35. [CrossRef]
86. Nishikawa, M.; Tatsuno, M.; Suzuki, S.; Tsuchihashi, H. Analysis of methylbenactyzium bromide in human urine by thin-layer chromatography and pyrolysis gas chromatography. *Forensic Sci. Int.* **1991**, *49*, 197–203. [CrossRef] [PubMed]
87. Böer, T.M.; Procópio, J.V.V.; do Nascimento, T.G.; Macêdo, R.O. Correlation of thermal analysis and pyrolysis coupled to GC–MS in the characterization of tacrolimus. *J. Pharm. Biomed. Anal.* **2013**, *73*, 18–23. [CrossRef] [PubMed]
88. Nishikawa, R.K.; Bell, S.C.; Kraner, J.C.; Callery, P.S. Potential biomarkers of smoked fentanyl utilizing pyrolysis gas chroma-tographymass spectrometry. *J. Anal. Toxicol.* **2009**, *33*, 418–422. [CrossRef] [PubMed]
89. Sato, M.; Hida, M.; Nagase, H. Analysis of pyrolysis products of methamphetamine. *J. Anal. Toxicol.* **2004**, *28*, 638–643. [CrossRef] [PubMed]
90. Mitsui, T.; Hida, M.; Fujimura, Y. Determination of alkaloidal narcotics by pyrolysis-gas chromatography. *J. Anal. Appl. Pyroly-sis* **1989**, *17*, 83–89. [CrossRef]
91. Thomas, B.F.; Lefever, T.W.; Cortes, R.A.; Grabenauer, M.; Kovach, A.L.; Cox, A.O.; Patel, P.R.; Pollard, G.T.; Marusich, J.A.; Kevin, R.C.; et al. Thermolytic degradation of synthetic cannabinoids: Chemical exposures and pharmacological consequences. *J. Pharmacol. Exp. Ther.* **2017**, *361*, 162–171. [CrossRef]
92. Kevin, R.C.; Kovach, A.L.; Lefever, T.W.; Gamage, T.F.; Wiley, J.L.; McGregor, I.S.; Thomas, B.F. Toxic by design? Formation of thermal degradants and cyanide from carboxamide-type synthetic cannabinoids CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18 during exposure to high temperatures. *Forensic Toxicol.* **2019**, *37*, 17–26. [CrossRef]
93. Baker, R.R. Temperature distribution inside a burning cigarette. *Nature* **1974**, *247*, 405–406. [CrossRef]
94. Freye, E.; Levy, J.V. Pharmacology and abuse of cocaine, amphetamines, ecstasy and related designer drugs. In *Pharmacology of Cocaine*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 49–60. [CrossRef]
95. Strano-Rossi, S.; Mestria, S.; Bolino, G.; Polacco, M.; Grassi, S.; Oliva, A. Scopolamine fatal outcome in an inmate after Buscopan® smoking. *Int. J. Legal Med.* **2021**, *135*, 1455–1460. [CrossRef]
96. Jalali, F.; Afshari, R.; Babaei, A. Smoking crushed hyoscine/scopolamine tablets as drug abuse. Subst. *Use Misuse* **2014**, *49*, 793–797. [CrossRef]
97. Deutsch, J.; Hegedus, L.; Greig, N.H.; Rapoport, S.I.; Soncrant, T.T. Evidence of Transacylation of Scopolamine in the Gas-Chromatograph Inlet. *Anal. Lett.* **1994**, *27*, 671–679. [CrossRef]
98. Brenneisen, R.; Hasler, F. GC/MS determination of pyrolysis products from diacetylmorphine and adulterants of street heroin samples. *J. Forensic Sci.* **2002**, *47*, JFS15456J. [CrossRef]
99. Fernandez-Lopez, L.; Mancini, R.; Pellegrini, M.; Rotolo, M.C.; Luna, A.; Falcon, M. Postmortem analysis of quetiapine and pregabalin in human bone. *Leg. Med.* **2020**, *46*, 101717. [CrossRef]
100. Aly, S.M.; Gish, A.; Hakim, F.; Guelmi, D.; Mesli, V.; Hédouin, V.; Allorge, D.; Gaulier, J.M. In the case of extensively putrefied bodies, the analysis of entomological samples may support and complement the toxicological results obtained with other al-ternative matrices. *Leg. Med.* **2023**, *63*, 102261. [CrossRef]

101. Sadler, D.W.; Fuke, C.; Court, F.; Pounder, D.J. Drug accumulation and elimination in *Calliphora vicina* larvae. *Forensic Sci. Int.* **1995**, *71*, 191–197. [CrossRef]
102. Chophi, R.; Sharma, S.; Sharma, S.; Singh, R. Trends in the forensic analysis of cosmetic evidence. *Forensic Chem.* **2019**, *14*, 100165. [CrossRef]