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Applications of supercritical fluid extraction and chromatography in forensic science

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Abstract

Supercritical fluid technology is a rapidly expanding analytical technique. Here we give a brief insight into the background of supercritical fluid technology and how supercritical fluid extraction and supercritical fluid chromatography work in analysis. The applications of these two techniques in forensic science are known to be important. The main area of forensic use of supercritical fluid technology is in the sample preparation and separation of drugs of abuse particularly opiates, cannabinoids, cocaine and sedatives. Supercritical fluid technology can be used for both time-of-death-related drug analysis and for obtaining information relating to long term drug abuse. We also give a review of the use of supercritical fluids in two other major forensic areas, fingerprinting and the extraction and separation of explosives from both bombing events and gunshot residues. Overall we show that supercritical fluid technology is fast becoming a major part of forensic investigations and that it is an invaluable analysis technique.

Keywords: Supercritical fluid extraction; Supercritical fluid chromatography; Drugs of abuse; Opiates; Cannabinoids; Cocaine; Sedatives

1. Introduction

An ever-increasing demand for improved and more efficient toxicological analyses means that the development and improvement of new techniques is of the utmost importance. Supercritical fluid technology has grown over the past decade and is still growing. Supercritical fluid extraction (SFE) is a sample preparation method that can be automated, whilst supercritical fluid chromatography (SFC) is a separation technique

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which can be easily linked to various detectors to enable identification of a wide range of drugs of abuse from biological material [1]. SFE offers a technique that is rapid and is not environmentally hazardous. SFE also yields results with better precision than when similar extractions are performed using conventional techniques [2].

1.1. Supercritical fluids: properties and advantages

SFC and SFE take advantage of the basic physical properties of supercritical fluids. A supercritical fluid can be defined in terms of its critical temperature and critical pressure [3]. The physical properties are between those of a gas and those of a liquid meaning that SFC is between GC and HPLC; SFC uses both the solvating power of a liquid and the lower viscosities of a gas. The major advantages of supercritical technology include improved efficiency, non-toxicity, cost-effectiveness, speed and convenience of use [4], for instance Edder et al. demonstrate that morphine can be extracted in just 25 min [1]. Staub noted that the diffusion properties of supercritical CO_2 allow the rapid penetration and extraction of samples, and that the low critical temperature allows good stability of thermally labile materials [5]. The analyst should select a fluid that exhibits the best compromise in solubilising the solutes as well as having the mass transfer characteristics needed for extraction [2].

Carbon dioxide (CO_2) is the most used supercritical material for many reasons. These include: ease of manipulation (with critical temperature and pressure being 31°C and 73.8 bar, respectively), good solvent strength, compatibility with solutes, lack of toxicity, and it is non-flammable, non-corrosive, odourless and inexpensive [5]. With proper ventilation CO_2 presents little harm to the analyst [2]. These advantages of CO_2 do not necessarily apply to other solvents, nitrous oxide for example was reported to explode when used for analytical extraction of ground coffee [6]. Also, use of ammonia or hydrocarbons is less convenient and safe than CO_2, as is CO_2 mixed with methanol. The solvating power of supercritical CO_2 may be increased by the addition of modifier substances which are usually organic solvents that are added to the source of compressed fluid before the pump or to the extraction gas after it is compressed [2,3,7], however, gradient elution, as opposed to the use of pre-mixed solvents gives shorter analysis times [7]. Adding an organic solvent to a supercritical fluid can act significantly on the strength; for example, to extract more polar compounds it can be beneficial to add an alcohol to CO_2 [8]. However the use of modifiers does limit the choice of detectors although there is a large number to choose from in the first instance, for example ultraviolet detectors, flame ionisation detectors, nitrogen phosphorous detectors, electron capture detectors and mass spectrometers.

1.2. A brief overview of supercritical fluid extraction

Samples are placed into extraction cells and the chamber temperature is set to the optimum temperature of the compound of interest, or to a temperature that will be able to include as wide a range of compounds as possible, for instance about 70°C. The same applies to the pressure, on average 350 atm is suitable. Density and flow rate of the carbon dioxide are set and an equilibration time of about 10 min, and an extraction time

of about 15 min are also set. After this, modifiers are added and the extraction proceeds. The extraction occurs in the dynamic phase when the extraction vessel is continuously supplied with solute. In the static phase, extraction takes place without the regeneration of solute. The trap temperature is set at much less than extraction temperature (about −25°C) to ensure the compounds elute and are fixed, and the trapped compounds are dissolved in a suitable solvent for analysis [3,8,9]. Thermal trapping is a simple technique where the fluid is depressurised in a cooled recovery chamber; this technique is however limited to non-volatile components. Sorbent trapping is relatively easy and involves the fluid being depressurised and then absorbed onto a solid support, the analyte is then recovered by elution with a small amount of solvent liquid. Finally the simplest method is solvent trapping where the compounds are caught in a liquid solvent, however, choosing a different solvent can alter selectivity [8].

Adjusting parameters such as pressure, temperature and the amount/composition of modifier can readily alter the selectivity of the extraction [5] as can the humidity of the sample, generally a partial dehydration allows for a faster extraction [8]. The density of extracting solvent can also be altered by pressure changes, giving the advantage of being able to alter the solubility parameter (selectivity). Also affecting extraction is the nature of the matrix material. Generally a rapid and complete extraction depends upon the matrix particles being small. An increase in the porosity of the matrix will also lead to improved extraction [8].

1.3. A brief overview of supercritical fluid chromatography

The retention of polar compounds on packed column SFC is principally due to interactions between solutes and the stationary phase, adding a polar modifier to the CO_2 greatly affects these interactions. Capillary SFC has also been employed for the analyses of drugs of abuse. Briefly, samples are injected onto either packed or open columns and separation is obtained by having temperatures of between 100 and 200°C. Analyses are carried out by either density programming or pressure programming [10]. The relatively low viscosity combined with a diffusivity midway between that of a gas and a liquid leads to very favourable column efficiencies [11]. It has been shown that without modifier, SFC cannot compete with GC. This means that high proportions of polar modifier are needed for efficient extraction [1]. SFC in its present state cannot replace GC but it is a good alternative for the analysis of non-volatile, thermolabile or acidic solutes.

Solute solubility is vastly greater than in a gas and approaches that of a normal liquid and the density of the fluid can reach values that exceed those in HPLC. The combination of these parameters forms the basis of a powerful chromatographic technique [11]. The ability to adjust solvent strength and the support material in packed columns improves the versatility of SFC and permits the analysis of a variety of polar drugs [12]. SFC can be performed using binary and ternary mobile phases generated in, and delivered by a single syringe pump [13]. The ease with which relative elution and response characteristics can be altered in SFC make this an ideal method for use in specific screening programmes for target compounds [3]. Overall the speed of analysis in SFC is increased as compared with HPLC, especially for silica columns. Also SFC has a

lower detection limit when compared with LC due to the ready conversion of the supercritical fluid to a gas in the ion source, the more efficient removal of gases by the mass spectrometer (MS) pumping system and narrower chromatographic bands due to efficient operation at higher linear velocities [12].

1.4. Advantages of SFE/SFC over conventional methods

Drugs of forensic interest have traditionally been extracted from solid biological matrices by liquid–solid extraction, this technique is time consuming and the extraction efficiency is often poor [1]. Sample preparation techniques can require the use of toxic solvents, which may be damaging to the results. For instance 6-monoacetylmorphine (6-MAM) presence, which is confirmation of heroin intake, can be hydrolysed to morphine and it becomes impossible to tell if the morphine is there as a result of heroin abuse or due to medical use [9]. One of the main advantages of SFE/SFC over conventional methods is a possibility for direct extraction from complex matrices, in parallel with a reduced risk of sample contamination [4] and the recovery of solvent-free extract [14]. The direct extraction means that analysis time is much reduced, for example Furton et al. found that SFE with an enzyme immunoassay took ~90 min while the conventional method of liquid–liquid extraction followed by gas chromatography takes about 8 h [15]. SFE of pharmaceuticals from solid dosage forms is generally safer, less wasteful, more cost effective and less time consuming than conventional extraction methods [16]. SFE of a number of drugs, such as diosgenin [17] and magnolol [18], has shown higher recoveries than when using conventional methods such as solvent extraction.

One of the attractive features of SFE is that it is relatively simple to couple the extraction technique directly with chromatographic techniques [1], SFE has been successfully coupled to TLC, LC, GC, packed column SFC and capillary SFC [19]. Potential loss of volatile substances can be avoided by using an on-line combination of SFE and capillary SFC where extracts are trapped on a relatively large amount of sorbent before they are re-eluted directly into the capillary column. The major advantages of this dual technique are that sensitivity is very high since 100% of the extract is transferred into the chromatograph, and only very small samples are required for analysis [19]. However on-line SFE requires that the analyst be in control of two techniques simultaneously and therefore the novice in SFE should start with off-line SFE [2]. By changing the extraction conditions, class-selective extractions and fractionation of the extract can be achieved [14]. A major use for SFE has been in analysis of drugs from hair samples; hair can be analysed quickly without the need for lengthy preparation procedures. The technique is particularly useful for hair because of its sensitivity and specificity, that allow for the determination of the very low concentrations of drugs found in hair [5].

A principal advantage of SFC is that it extends the range of compounds that can be eluted as compared to GC. SFC is a possible method of separating traces of involatile compounds and when coupled to MS, SFC can be used for the identification of these involatile traces [13]. SFE/SFC are compatible with a large number of chromatographic detectors, virtually all LC and GC detectors can be used with SFC; both specific

detectors (such as a MS, a Fourier transform infrared spectrometer (FT-IR), an atomic emission detector or a thermal energy analyser) or a variety of non-selective detectors (such as UV absorption, flame ionisation (FID) and electron capture (ECD)) can be coupled with SFE/SFC [19]. SFE/SFC has great potential for forensic laboratories especially as the range of drugs expected to be covered by routine screening is increasing. The incorporation of supercritical technology in forensic laboratories should be seriously considered either as a technique by itself or combined with existing analytical techniques [3]. It seems that modern SFE instrumentation with multiple extraction cells, higher pressure limits and modifier addition systems will provide more versatility for the isolation of target compounds from complex biological samples [14].

2. The use of SFE/SFC in the identification of drugs of abuse

Supercritical fluid technology can be used for both time-of-death-related drug analysis and for obtaining information relating to long term drug abuse [1]. Here we review the uses that SFE/SFC has in the identification of opiates, cannabinoids, cocaine (and other stimulants such as amphetamines), and sedatives, all of which are of interest to the forensic scientist as all are drugs that regularly cause deaths. SFE/SFC can also be used for the identification of steroids such as budesonide, hydrocortisone and dexamethasone which can be legally present in the body [20] but can be abused if used in the wrong circumstances, such as by training athletes. However while these latter analytes are of forensic interest there is not enough literature available to discuss their analysis by SFE/SFC.

2.1. Opiate drugs

Extraction of basic drugs using conventional methods (solid-phase and liquid–liquid extraction) can be tedious, and time-consuming procedures involving losses or contaminations and are not easily automated [1]. Using SFE/SFC it is possible to obtain information on drug related deaths, polydrug abuse, and long-term drug abuse, often with improved efficiency and cost effectiveness, and with reduced risk of sample contamination. When determining optimal conditions for SFE, Edder [21] found that in addition to the solubility of the drug it is necessary to consider the effect of the nature of the matrix. Edder was able to successfully extract morphine (100% recovery), methadone (92%), and codeine (98.2%) from a simulated matrix with a mixture of CO_2/MeOH/Et_3N (90:8.5:1.5 v/v) at 25 MPa; 0.5 ml min^{-1} for 25 min. Methadone (synthetic opiate) and morphine have also been successfully extracted from post-mortem blood and vitreous humor using supercritical CO_2 with 15% MeOH/Et_3N modifier under the following conditions: $P = 3500$ psi, $T = 100$°C and flow rate = 2 ml min^{-1} for 30 min [4].

The extraction of opiates from hair using SFE has also been investigated. Sachs et al. were the first to use SFE to extract opiates from hair using a mixture of CO_2–ethyl acetate, but the recovery and reproducibility of this method was inferior to other conventional techniques [22]. In 1994, Edder et al. demonstrated an improved method

for the quantitative extraction of opiates in hair using CO_2 as the supercritical fluid modified with a polar mixture: MeOH/TEA/H_2O at a volume ratio of 85:6:6:3, respectively [21]. Edder successfully extracted methadone, morphine, codeine, ethylmorphine and 6-MAM using a double syringe pump (SFC 300, Carlo Erba) with optimal conditions set at: $P = 25$ MPa, $T = 40°C$, flow rate = 0.7 ml min^{-1}. The eluted drug collected in MeOH after 30 min was evaporated under nitrogen and then characterised and quantified by GC/MS [8]. Using this method, Edder [21] produced an average SFE yield of 93.5% with a 2.7% coefficient of variation (CV) for morphine. Because morphine is strongly polar it is the most difficult opiate to extract and therefore it can be considered that the SFE is complete for all the opiates. The level of reproducibility ($n = 5$) was satisfactory for this type of analysis, with the CV less than 10% except for codeine (12%) [8].

The use of both capillary and packed column SFC for the analysis of controlled substances, including acetylcodeine, diamorphine and acetylmorphine was investigated by MacKay [13]. Using the capillary column SFC coupled to FID, MacKay was able to chromatograph diamorphine and acetylcodeine using unmodified CO_2, and also phenobarbibitone and methaqualone, which are sometimes used as diluents in heroin. In packed column SFC, it was necessary to add a polar modifier to elute polar compounds from the silica-based column, and 12% MeOH with 1% H_2O was used as this was the only one that eluted diamorphine [13]. This is in agreement with Janicot et al. [23] who reported that large amounts of modifier (MeOH/H_2O/Et_3N) have to be added to CO_2 (15–20%) to obtain a good separation of morphine alkaloids by packed column SFC. This is due to interactions between the solutes and the stationary phase through the silanol groups [1].

2.2. Cannabinoid drugs

Cannabinoid drugs account for a major part of casework in forensic laboratories. The only method presently used for definitive identification is GC-MS, which has a lengthy analysis time and needs prior preparation [24]. It is probable that SFC linked to MS could be beneficially used instead of GC-MS, due to its high resolution and rapid analysis time. Hair samples have been identified as positive for cannabinoids (Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol) by using SFE with the same supercritical phase properties as for the identification of opiates [5]. Illicit preparations of marijuana and hashish contain more than 400 compounds of differing polarities making them a difficult subject for identification. The major cannabinoids tested have been THC and CBD [25].

For the extraction by Veress [25], samples were ground and inserted into the extraction thimbles on filter papers, maximum extraction occurred within 50–70 min, and extraction recovery could be determined exactly for any type of cannabis. Backstrom used SFC coupled to an atmospheric pressure chemical ionisation mass spectrometer (SFC-APCI-MS) and the cannabinoids were separated in less than 8 min, definitive identification was then possible. This separation was not as good as GC-MS but an improvement on HPLC, however derivatization was not required for the SFC procedure [24].

Veress noted that CBD could be extracted much faster than THC, supercritical CO_2 dissolves CBD more effectively than THC. Also cannabinoids from hashish are extracted faster than cannabinoids from marijuana. This is probably due to the nature of the matrix; hashish is a pre-separated material while the cannabinoids in marijuana are embedded within the plant material [25]. These discoveries mean that using SFE it is possible to tell the nature of the cannabinoids and the matrix that they came from depending on their elution time, this information may be useful for prosecution. The only disadvantage of SFE in the identification of cannabinoids seems to be that for very low levels (about 40 μg) recovery from the matrix is only about 90%, and owing to the differences in matrix materials, recovery of cannabinoids from hashish can be nearly double that from marijuana [25]. It seems that at all times matrix materials must be considered when choosing to use SFE for the quantification of cannabis and its metabolites. The SFC-APCI-MS technique has been applied to real casework samples and definitive identification of cannabinoids from the herbal cannabis samples was achieved. This method can be applied without difficulty and is sufficiently sensitive to analyse both bulk samples and trace amounts [24].

2.3. Cocaine and other stimulants

Cocaine is among those drugs of abuse that contain tertiary amino groups while stimulants such as amphetamines have secondary amino groups. Tertiary amines are soluble in CO_2 without solvent modifiers and are particularly suitable to be analysed by SFC. Using SFC it is possible to separate cocaine and its major metabolite benzoylecgonine without any derivatization step, it is not possible to do this using GC [1]. In a study by Staub three models were compared, a piece of Teflon was extracted as a control matrix, hair on which cocaine had been adsorbed for extraction of cocaine from passive uptake and hair which had been soaked in cocaine to simulate a cocaine abuser [5]. The results from this study show that the way in which the drug is incorporated into the matrix determine the conditions for SFE, therefore depending on the parameters used it should be possible to tell whether the sample contains drugs on the surface or whether they are incorporated within the matrix. This finding has also been tested by Morrison and Chesler who confirm that SFE has the ability to selectively recover surface cocaine as opposed to incorporated cocaine [26]. This is important in investigations where the accused protests that they must have been in the presence of the drug but have not actually ingested any.

Morrison and Chesler have reported the development of an SFE method especially for the isolation of cocaine residues from human hair. The method uses CO_2 modified with triethylamine and water. This method means that SFE with its speed and efficiency could be suitable as a sample preparation method for drug screening programmes. SFE coupled with radioimmunoassay has proved to be extremely accurate with all the drug user hair, showing positive results and nine out of 10 non-drug user hair showing negative results in Morrison and Chesler's experiments [26].

Stimulants (including cocaine, amphetamine, methamphetamine and ephedrine) appear to have a wide range of retention on SFC, however changing the concentration of modifier affects retention, selectivity and peak shapes. Altering temperature also has an

effect. Some stimulants require at least 10% modifier to elute at all. Temperature changes cause changes in the selectivity, any temperatures higher than 90°C are unusable. The drug family of stimulants contains a wide variety ranging from weak tertiary amines to strong primary amines; all can be eluted using SFC [27]. The fact that there is no set modifier concentration or temperature is one of the drawbacks of this technology, it means that results depend on the experimental protocol and drugs that are present may be missed. An advantage is that the same procedure can be used to extract cocaine as is used to extract opiates and cannabinoids making this technique potentially valuable as a screening procedure [9].

Altogether the literature shows that SFE is a definite possibility for cocaine screening programmes and that it can be used to distinguish not only the presence of cocaine and other stimulants but also whether they are absorbed from outside the body or from within. A recent publication [28] on SFE of cocaine from human hair suggests the potential for distinguishing between environmental and physiological sources of drugs in the hair.

2.4. Sedatives

Lawrence demonstrated a SFE method for the separation and extraction of benzodiazepines (alprazolam, clorazepate, chlordiazepoxide, diazepam, oxazepam, prazepam, temazepam and triazolam) from the matrix of their solid dosage forms. This involved extracting with CO_2 modified with 2% (v/v) MeOH for 15 min under the following conditions: $P = 100$ atm, $T = 65$°C. The extract was analysed by GC-MS and FT-IR. These results have led to further research using SFE to remove these drugs and metabolites from biological matrixes of forensic interest, such as blood and urine [16]. Extraction of temazepam from whole blood has been carried out by Scott using SFE and HPLC for analysis. Using CO_2 modified with ethyl acetate (95:5 v/v) at 2 ml min^{-1}, 65°C and 3000 psi, temazepam was extracted from dried whole blood with recoveries of 80–100% and reproducibility was good as long as pressure fluctuations were controlled. The results compare favourably with solid phase extraction which is tedious and time-consuming, wasteful of sample and has increased potential for sample contamination [29]. This SFE method also extracts other benzodiazepines and their metabolites of forensic interest, including diazepam, nordiazepam, oxazepam, and chlordiazepoxide.

Barbiturates have been studied in both therapeutic and toxicological studies because of their use in epilepsy treatment [30] and the increasing trend of abuse [29]. The use of SFC to determine barbiturates has been carried out by Smith [30] using two different packed silica columns, UV detector, and CO_2 supercritical fluid modified with MeOH. Initially, when eluting with unmodified CO_2, Smith found that the barbiturates were retained on the octadecylsilane (ODS) column and on the polystyrene-di-vinylbenzene (PS-DVB) column the peak shapes were poor. The addition of MeOH resulted in successful separation of barbiturates with the proportion of the modifier having a marked effect on the retention times. The conditions used in this investigation were: $T = 60$°C, 245 nm UV wavelength and mean column pressure of 2200 psi and 1950 psi for the PS-DVB and ODS columns, respectively. This study did not include analysis of serum

samples. Wong was able to demonstrate the feasibility of using open tubular capillary SFC to analyse phenobarbital, secobarbital, pentobarbital and phenytoin in serum [10].

3. Other uses of SFE/SFC in forensic science

3.1. Fingerprinting

Ninhydrin is the most commonly used enhancement technique for latent fingerprints on paper and other porous surfaces. Along with 1,8-diazafluorene-9-one (DFO) which it is being increasingly combined with, ninhydrin reacts with amino acids present in eccrine sweat to give purple or fluorescent (DFO) fingerprints. The main ingredient of ninhydrin and DFO currently used by British Police Forces is 1,1,2-trichlorotrifluoroethane (CFC113) which must be phased out due to the Montreal Protocol on the Control of Ozone Depletion Substances. Supercritical CO_2 has been presented as a suitable substitute by Hewlett [31]. The equipment and process used by Hewlett is very similar to that of SFE/SFC. The treatment vessel containing the document sample, ninhydrin, acetic acid and water, is heated to 80°C and CO_2 is introduced into the vessel at high pressure (300 bar). At the end of the treatment process the pressure was released slowly over 15 min. This method was successfully used to develop fingerprints in a one-stage process (ninhydrin generally takes two stages), but it had limited diffusion capabilities when articles were treated simultaneously. However, as yet the size of this apparatus limits what can be detected and it is not possible to use at crime scenes [31].

3.2. Identification of explosives

The determination of explosives, propellants, and related compounds are of great interest in forensics. Post-blast debris from bombing events, and gunshot residues from homicides or suicide cases are often analysed by forensic scientists [19]. Many of these compounds are not easily analysed by GC due to their being thermally unstable. Reverse-phase HPLC [32] linked by MS and ECD, GC detectors is used in the analysis of more volatile substances.

SFC is more suitable for the analysis of thermally unstable material than GC because injections are made onto the column without requiring vaporisation [33]. Using supercritical CO_2 and coupling the SFC to a TEA detector, Douse was able to analyse many nitramine, nitrate ester, and nitroaromatic explosives at sub-nanogram levels with the minimum detectable range of 20 to 60 pg [34]. In 1991, Munder characterised several smokeless powders and gunshot residues using simultaneous triple detection (UV absorption, FID, and ECD) following SFE coupled to capillary SFC (SFE-SFC-U^iV/FID/ECD). Using highly purified CO_2 for the mobile phase Munder was able to characterise the following compounds: ethyleneglycol dinitrate (EGDN), triacetin (TA), 2-nitrotoluene (NT), 3-NT, 4-NT, dipropyladipate (DPAd), nitroglycerine (NG), diethylphthalate (DEP), 2,6-dinitrotoluene (DNT), pentaerythritol (PETN), dibutylphthalate (DBP), diethyldiphenylurea (DEDPU), 2,4-DNT, diphenylamine (DPA), *N*-nitrosodiphenylamine (NNDPA), bis(2-ethylhexyl)phthalate (DOP), 2,4,6-trinitrotoluene

(TNT), 2-nitrodiphenylamine (NDPA), 1,3,5-trinitro-1,3,5-triaza-cyclohexane (RDX), *N*-2,4,6-tetranitro-*N*-methylaniline (Tetryl). 1,3,5,7-Tetranitro-1,3,5,7-tetraazacyclooctane (HMX) was not extracted or eluted under the conditions applied. 2,4-DNT, DPA, and NNDPA were not completely resolved chromatographically but were identified through different responses in the three detectors. The FID, with its insensitivity to nitrated compounds, proved to be the least sensitive detector. The UV detector is the most universal detector for the compounds investigated [19].

Munder went on to characterise explosives, propellants, and related compounds in soil using a dual detection mode (UV-ECD) to achieve optimal sensitivity with the ECD [19]. The compounds were extracted for 10 min at a flow rate of 130 ml min^{-1} and then transferred into the chromatographic system. The results showed a relatively low extraction yield from the soil sample (especially with DPA, RDX and Tetryl) which was improved slightly by saturating the liquid CO_2 with water. However, the addition of water resulted in a loss of EGDN and the NTs. A vapour phase treatment of the sample was carried out with ammonia generating a chromatogram almost perfectly matching the original standard chromatogram (with the exception of DPA, EGDN and the NTs).

Smokeless powders, in addition to use as a propellant in firearms, are used in illegitimate explosive devices and the residues of the powder particles can be recovered at the site of an explosion or shooting. Munder [19] investigated the possibility of identifying the type, brand or even lot of powders found during criminal investigations using the triple detection system. Munder found that he was able to easily distinguish between the two basic types of propellants (single/double) as the single is less complex and does not have nitroglycerine. However, he was not able to discriminate between powders of different propellants from different lots as the lot-to-lot variations appeared to be too high, and the differences in their extractable organic constituents were not sufficiently specific to be characteristic of a particular propellant [19].

4. Conclusions

Drugs of forensic interest have conventionally been extracted from solid biological matrices by liquid–solid extraction, which is time consuming and often has poor extraction efficiency [1]. The application of SFE/SFC to many fields of forensics has increased over recent years and will continue to increase due to the major advantages that this technique offers over conventional methods. SFE/SFC is non-toxic and less time-consuming than conventional methods: extraction using acid hydrolysis involves an incubation period of 18 h in HCl [35], enzymatic digestion is expensive due to the use of β-glucuronidase and arylsulfatase [9] and methanolic procedures destroy 6-MAM by hydrolysis to morphine. SFE of opiates is comparable to methanolic extraction and enzymatic digestion [8] and the use of CO_2 with its mild critical parameters allows low extraction temperatures, reducing risk of analyte degradation during the extraction. SFC also enables analysis of thermally labile drugs, metabolites [10] and explosive compounds [19], but a recently published critical appraisal of SFC reported problems still existing in analysis of some classes of drugs of abuse, notably LSD and truxillines of Coca [36].

The development of on-line SFE-SFC reduces potential loss of extract and eliminates sample pre-treatment and offers advantages of short sample extraction and high sensitivity, as 100% of the extract is transferred into the chromatographic system [19]. SFE/SFC is capable of detecting a greater variety of compounds using the same method (cocaine, cannabinoids and opiates [9]) and could be incorporated into drug screening procedures thereby having great potential in the face of increasing demand on toxicology laboratories [3]. SFC in its present state cannot replace GC but it is a good alternative for the analysis of non-volatile, thermolabile or acidic solutes and along with SFE, will become an increasingly important technique in forensic related analysis.

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