FORENSIC SCIENCE JOURNAL SINCE 2002

Forensic Science Journal
2003;2:5-18

Forensic applications of supercritical fluid extraction and chromatography

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Received: November 21, 2002/ Received in revised from: December 23, 2002/ Accepted: March 4, 2003

ABSTRACT

The gas-like mass transfer properties and liquid-like solvation characteristics of supercritical fluid make it a promising medium in performing extractory and chromatographic separations, i.e., supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), respectively. The tunable solvation properties of supercritical carbon dioxide not only improve SFE's efficiency and speed, but also enable SFC to solve niche problems inadequately treated with gas chromatography or high performance liquid chromatography. It is the environmental friendliness of the fluid that accelerates the extensive use of the two methods in forensic science. This review briefly introduces the basic properties of supercritical fluid and how the SFE and SFC instrumentations work in chemical analysis. The applications include the extraction and separation of drugs of abuse, fire debris, and explosives. The invaluable functions of SFE and SFC in forensic investigation are demonstrated.

Keywords: Forensic science, Supercritical fluid extraction, Supercritical fluid chromatography, Drugs of abuse, Fire debris, Explosives

Introduction

Supercritical fluid (SF) is defined as a fluid at a temperature and a pressure exceeding its critical values. The simplistic phase diagram covering the critical point and the SF region is shown in Fig. 1. SF possesses gas-like mass transfer properties and liquid-like solvation characteristics. The solvating power of SF with increasing density approaches that of liquid solvents. Carbon dioxide (CO_2) is the most frequently used SF because of its low critical temperature (31.1℃) and pressure (72.8 atm), being chemically inert and nontoxic, as well as availability in a fairly pure state at a reasonable cost [1-3].

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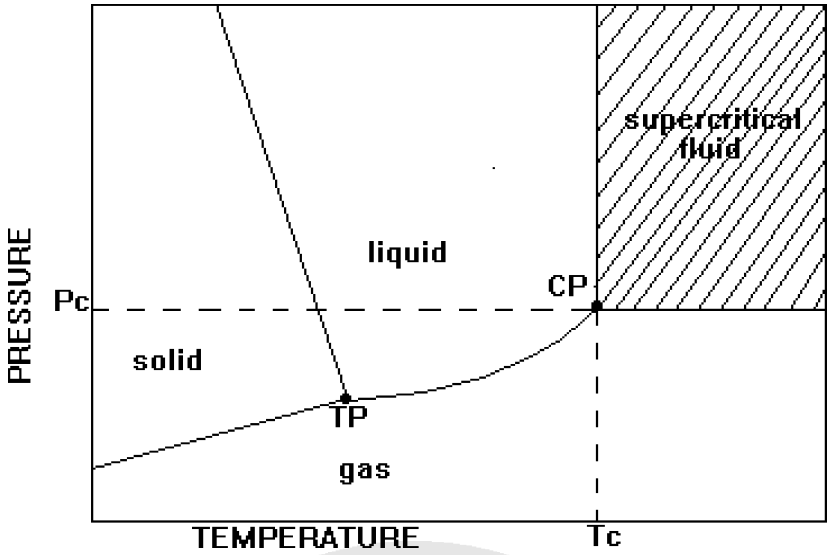

Fig.1 Simplistic phase diagram showing the critical point and the supercritical fluid region.

CO_2-SF near its critical point is a low-polarity solvent, with a polarity comparable to that of hexane. At a constant temperature, the solubility of a non-polar compound in the fluid is favored at lower pressures, whereas at higher pressures, a more polar or higher boiling compound is more soluble. The solvating power of CO_2-SF can be easily controlled by adjusting its temperature and pressure to obtain a desired density. Alternatively, addition of a small percentage of such organic solvent as methanol, called "modifier", to the fluid usually enables it to extract polar compounds. A variety of analytes, ranging from non-polar to polar, can thus be quantitatively extracted. When necessary, the selectivity of supercritical fluid extraction (SFE) can be further improved by depositing the sample onto an analyte-selective sorbent or clean-up sorbent followed by eluting the analytes with SF. This approach simplifies an extraction procedure and a clean-up procedure into an essentially single procedure, saving the analytical time and improving the overall recoveries.

Most of the SF's become gases and dissipate automatically under ambient conditions. Purposely removal of the eluting SF to concentrate the extracts is unnecessary. Therefore, the laborious cost and the potential loss of volatile analytes usually associated with conventional liquid solvent extraction are minimized [4-7]. These make feasible the direct coupling of SFE with gas chromatography (GC) [8-9] or liquid chromatography (LC) [10-11] when good selectivity for tiny samples and/or automation is desired. On the other hand, the gas-like mass transfer properties of SF allow it to penetrate micro-porous solid matrices efficiently. In general, SF has no surface tension and shows low viscosity. While the total analytical time is considerably reduced, the recoveries of analytes achieved by applying appropriate SFE to a solid or semi-solid sample can usually be equal to or better than those by conventional extraction methods.

The increasing use of supercritical fluid chromatography (SFC) in chemical separation also stems from the advantageous properties of SF. Compared to the carrier gas of a GC operated at somewhat elevated temperatures, the cold SF of an SFC can more easily and safely solvate thermally labile and/or non-volatile compounds. The analytes eluding from the column outlet are in vapor phase and ready for detection by common detectors such as flame ionization detector and mass spectrometer. SFC also offers advantages over high performance liquid chromatography (HPLC), mainly because SF shows high efficiency of mass transfer and affords fast speed and fair resolution to routine analysis, particularly to that of a complex mixture. SFC is readily performed either on a packed column with an LC detector or on an open tubular capillary column with a GC detector. SFC solves niche problems inadequately treated with GC or HPLC. Thus far, however, SFC has not yet come into use as a total substitute for GC or HPLC in the separation science community [12-15].

The applications of SFE and SFC in chemical analysis cover a broad spectrum of samples, including food stuffs, natural products, agrochemicals, environmental samples, fuels and lubricants, synthetic polymers and oligomers, organometallic compounds, achiral pharmaceutical agents and biologically important chiral compounds [16]. The drive of the applications is the reduction of solvent use as well as the enhancement of performance, i.e., speed, selectivity, recovery, ease in coupling, etc. McAvoy et al. devoted their critical appraisal to the applications of SFC to the analysis of explosives and drugs of abuse [17]. Barettel et al. in a recent review pointed out that SFE and SFC were both invaluable analytical techniques and should be soon incorporated in most of the analytical protocols for forensic purpose [18].

In addition to drug analysis, the forensic chemists in Taiwan are also largely engaged in the examination of a variety of types of samples, such as paints, fire residues, post-explosion debris, gunshot residues, glass, soil, ink, fingerprints, etc., scrutinizing for any trace evidence of crime. During the course of this manuscript's preparation, another review on similar subject appeared in the literature [19]. While serving as an addendum to the foregoing paper, this relatively short review article lays greater stress on the recent applications of SFE and SFC in the analyses of drugs of abuse, fire debris and explosives, as well as fingerprints enhancement. It is hoped that the present one will somewhat help document the increasingly broadened spectrum of forensic SFE/SFC.

Instrumentation

Supercritical extractor

The basic components of an analytical-scale SFE apparatus includes [4]:

1. SF source: High-purity, i.e., SFE grade, SF contained in aluminum or stainless steel cylinders. Cylinders are usually equipped with a dip tube, a pressurized headspace and a cooling device to ensure stable equilibrium and regular SF delivery.
2. Pump: A high-pressure pump delivers the SF at a regular yet controllable pressure and flow rate. An additional (optional) pump is used to introduce the modifier. An ideal system would provide a wide range of pressure between 1000 and 10,000 psi along with reproducible and non-pulsating flow-rates between 1.0 (l μm/min and 90 ml/min. Most pumps are either syringe or dual piston pumps.
3. Extraction chamber: Also called sample vessel or cell and is used to hold the sample while extraction is in progress. The typical volume is from 0.1 to 50 mL. Fig. 2 shows a typical cell consisting of the extractor body and a couple of frit lids along with threaded seal for tight fit. The frit lids prevent the SF from sweeping the solid sample. The cell will withstand high pressure and is fitted with a safety valve to protect the operator in the event of system malfunction. The temperature of the cell is controlled either by placing the cell in a GC oven or a heating tube.

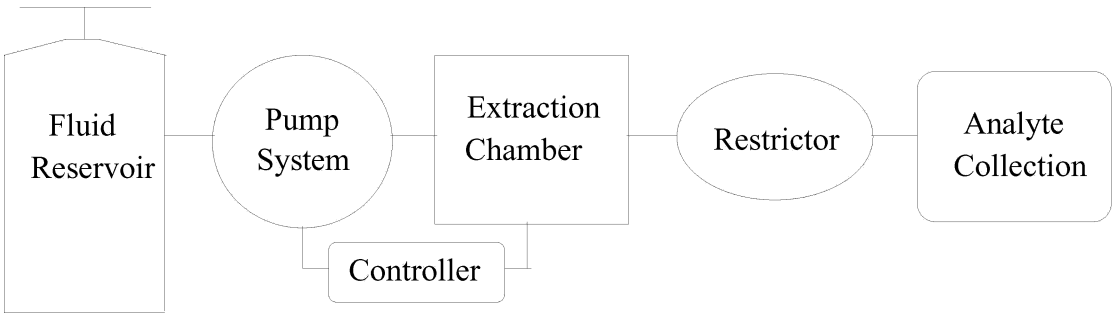

Fig.2 A typical SFE cell consisting of the extractor body and a couple of frit lids along with threaded seal for tight fit.

4. Controller: It controls the flow rate of the SF and the pressure and temperature of the extraction chamber, ensuring the fluid entering the extraction chamber in SF state. More functions, such as the valve switching and extraction time setting, etc., are available in commercial systems, providing automatic operation
5. Collector: A restrictor is connected between the cell outlet and the collector inlet, maintaining the required pressure in the extraction chamber. The extracts are usually collected using an appropriate solvent, a cryogenic trap or a cartridge packed with adsorbent material.

Supercritical chromatograph

The configuration of an SFC is similar to that of an HPLC, except that the former needs additional components to control and maintain the column temperature and pressure. Most of the instruments are currently using packed columns and LC(based detectors. This meets the requirements of higher flow rate and the frequent use of modifiers with packed columns. The foregoing restrictor or a backpressure device also helps convert the eluting SF into vapor phase and makes the eluents ready for detection. The SF source, pumps and controller are similar to those employed in a SFE [13].

The use of SFE and SFC in the analysis of drugs of abuse

Over the past decade the analysis load of illicit and abused drugs has grown tremendously worldwide, partially due to the increasingly stringent legislation and monitoring of controlled substances. Several programs have been implemented internationally to fight against drugs. For instance, the United States federal workplace drug test program initiated in 1988 [20]. Its Taiwanese counterpart, entitled "Essentials of Drug-Testing Laboratory Accreditation and Management", kicked off in 1995 [21], earlier by two years than the year in which an act called "Drug-Free Regulations" was enacted by the Legislative Yuan. The latter allows for collecting urine drug test samples from some particular people forcibly. Both of the USA and ROC programs demonstrate that a forensic drug test involves legal and disciplinary aspects simultaneously. No conflicts should appear between the results derived from the two aspects of consideration. Therefore, the presence of drugs must be confirmed by a gas chromatographic-mass spectrometric (GC-MS) analysis. In practice, the somewhat polar nature of most drugs of abuse necessitates some sort of chemical derivatization (ChD) be done prior to the confirmatory GC-MS test to improve their volatility, chromatographic resolution, instrumental response, and uniqueness of mass spectrum. This section selectively reviews recent advances in applying SFE and SFC to the analyses of opioids, stimulants, hallucinogens, and depressants. Emphases will be placed on the former three drug classes that are currently under mandatory testing in Taiwan, especially opiates, amphetamines, and cannabinoids.

Opiates

The most abused opiates are morphine, codeine, and heroin. Heroin is readily metabolized to 6-acetylmorphine and subsequently to morphine. A review appeared in 1998 that paid considerable attention to the determination of drugs of abuse in blood by SFE/SFC [22]. Shortly, Allen et al. compared the recovery of morphine from blood using SFE with that using solid-phase extraction (SPE), and found both to be nearly quantitative [23]. The SFE conditions were: extraction pressure, 3500 psi; extraction temperature, 100℃; flow rate of CO_2-SF for dynamic SFE, 2 mL/min; modifier, methanol (MeOH)/ethyl acetate (EA) (85:15 v/v). The extracts were collected in MeOH, evaporated to dryness, derivatized, and subjected to GC-MS analysis.

Janicot et al. [24] used an aminopropyl silica column (230 x 4.6 mm, 10 μm film) and a silica gel column (5 μm) at 220 bar and 40.7℃ to analyze morphine, codeine and papaverine. Polar modifiers were added to the CO_2-SF, i.e., CO_2/MeOH/triethylamine (TEA)/H_2O in the w/w ratios of 82.95:16.25:0.50:0.30 (for aminopropyl silica column) and 83.37:16.25:0.15:0.23 (for silica gel column). The separation time with this SFC is only half that with HPLC. Mackay et al. analyzed heroin using an SFC equipped with a capillary column (10 m x 50 μm I.D biphenylpolysiloxane) and interfaced to an MS [25]. A density program was operated to enhance the chromatographic performance. All analytes were sufficiently separated and fully identified.

The merits of hair as a chronological indicator of drug exposure have long been known [26]. Hair drug testing is therefore an ideal complement to urine or blood testing which generally provides information only on recent drug use (e.g., hours to days) [27]. According to the review and book chapter by Staub, it was Sachs and

Uhl in 1992 that demonstrated for the first time the use of SFE in the recovery of drugs from hair [28,29]. In the beginning, however, the recovery of the extraction as well as the reproducibility of the method remained inferior to other conventional techniques. In 1994, Edder et al. demonstrated the quantitative extraction of opiates in hair [30]. Their optimum conditions for the SFE of opiates in hair were: P = 25 Mpa; T = 40℃; flow rate = 0.7 mL/min; t = 30 min; eluent phase = CO_2/MeOH/TEA/H_2O (85:6:6:3 by vol.). Up to date, although isotope-dilution method helps achieve immunity to the complex matrix effects of hair, high and reproducible extraction recoveries of analytes are still crucial to a successful hair drug analysis [31]. Also, to avoid the potential for evidentiary false positives caused by environmental contamination, a wide variety of operationally defined rinsing protocols are employed prior to formal analytical procedure to remove exogenous drugs and/or contaminants from the hair surface [32,33]. Following the rinsing steps, the hair is dried and ground to powder to increase the surface area of the inaccessible domains where the drugs were incorporated. The proteinaceous matrix is then destroyed by acid incubation, alkaline hydrolysis, or enzymatic digestion, and the analytes subsequently extracted with appropriate buffer solutions, organic solvents [34], or, in the present context, modified CO_2-SF [30,35,36]. In a 1996 review entitled "preparation of hair samples for drug analysis" [37], the authors cited three references [30, 35, 38] and gave the following comments: carbon dioxide was used, alone or mixed with solvents, H_2O and TEA to produce recoveries that were comparable with those obtained with enzymatic digestion, thus saving analytical time and maintaining the mild extraction condition required for some drugs.

Stimulants

1. Amphetamines

Allen et al. used SFE followed by GC-MS to determine amphetamines in human hair [39]. The group of amphetamines included 3,4-methylenedioxyamphetamine(MDA), 3,4-methylenedioxymethylamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine(MDEA), and internal standard mephentermine(MP). In addition to authentic fortified case hair samples taken from known amphetamines consumers, to validate information on amphetamine use in hair, powdered hair samples free from drugs were also collected and soaked in a known amphetamine standard solution. The SFE conditions were: P = 3800 psi; T = 70 ℃; flow rate = 2 mL/min; t = 30 min; eluent phase = CO_2/$CHCl_3$/isopropanol (IPA) (90:9:1 by vol.). The ChD was carried out by adding 50 μL of pentafluoropropionic anhydride (PFPA):EA (1:1 v/v). Extraction recoveries better than 84, 71, and 77% were achieved for MDA, MDMA and MDEA. Based on spiked hair samples, the quantitation was found to be linear for a concentration range of 0.02 to 20 ng/mg of hair. The correlation coefficients (r^2) for these three drugs were 0.9703, 0.9773 and 0.9938, respectively. The quality of analysis was reported to be reproducible from sample to sample on a daily basis.

Simultaneous SFE-ChD followed by isotope dilution GC-MS in selective ion monitoring (SIM) mode using either one-point calibration or regression calibration to determine the AP and MA in urine and hair have been successfully experienced in our laboratory [40,41]. Taking urine as an example, the general procedure of the in-situ SFE-ChD was as follow: A 10- μL portion of methanol solution containing appropriate amounts of AP · H_2SO_4 and/or MA · HCl was added to 1 mL of D. I water. A ca. 200- μL portion of ammonium hydroxide was then added to alkalize the aqueous matrix (pH 10-12). The resulting solution was transferred to a 2.5-mL extraction cell that had previously been filled with celite and glass beads. The cell was subjected to vacuum to remove methanol (the solvent used for the standard solutions), water (from urine), and other volatiles or semi-volatiles (from urine). This drying step took about 3 hrs. To save time, it is advisable to run a number of samples at a time. To each dry sample was added 100 μL of trifluoroacetic anhydride (TFA). The mixture was subjected to simultaneous SFE-ChD. This step utilized an ISCO SFX™220 Supercritical Fluid Extraction System equipped with an SFX 220 extractor, an SFX 200 controller and a 260D syringe pump, and was performed at two stages. Stage 1: simultaneous static SFE-ChD under 4000 psi at 90℃ for 5 min. Stage 2: dynamic elution with 12.5 mL of supercritical CO_2 using a variable restrictor to keep the flow-rate at 1 mL/min. The eluent was trapped with 5 mL of EA and concentrated to 200 μL by purging with nitrogen gas. A 1- μL aliquot of this solution was injected for each GC-EIMS analysis. The GC-MS spectra thus obtained and the qualifier/quantifier ions selected thereupon for AP-TFA, AP-d_8-TFA, MA-TFA and MA-d_8-TFA were all in agreement with those previously reported using conventional techniques

[42]; that is, AP, AP-d8, MA, MA-d_8, and their TFA derivatives all underwent the same, yet regiospecific, type of major fragmentation, i.e., β-cleavage.

As mentioned above, isotope-dilution method has often been used in routine drug-of-abuse analysis. Namely, a known amount of the deuterium-labeled internal standard is added to each of the serial calibrators and the unknown sample prior to performing the quantitation. Nevertheless, since a forensic drug urinalysis of unquestionable quality relies on a sound sample pretreatment, an effective GC-MS analytical methodology, and a critical data evaluation process, the important role of the one-pot SFE-ChD with respect to its actual efficiency in the overall analytical scheme cannot be over-emphasized. Consequently, our simultaneous-SFE-ChD study was started with the optimization of its experimental conditions by using Taguchi's method [43]. The conditions stated above for the simultaneous SFE-ChD, i.e., 4000 psi, 90℃, 5 min of static SFE, and 100 μL of TFA, were obtained through this type of evaluation process.

Veuthey et al. developed a packed-column SFC with a UV detector at 269 nm for the quantitative separation of 9-fluorenylmethylchloroformate-derivatized amphetamine (AP), methamphetamine (MA), phenethylamine (PA), ephedrine (ED), and norephedrine (NE) [44]. Using 7.0 % methanol and 4.8 % 2-propanol modifiers in CO_2 mobile phase at 40℃. The columns used were 300 x 0.39 mm I.D Hypersil ODS (10 μm), 300 x 0.39 mm I.D Hypersil aminopropyl silica (5 μm), and 200 x 0.4 mm I.D Nucleosil 100 silica gel (5- μm) columns. The separation was completed in 6 min.

To overcome the co-elution problem of AP- and MA-derivatives with unreacted 9-fluorenylmethylchloroformate, McAvoy et al. used phenylisothiocyanate instead to derivatize AP, MA, MDA, MDMA and MDEA [45]. A dual column system, i.e.,150 x 4.6 mm PSDVB (5 μm) and 125 x 4.6 mm Spherisorb silica (5 μm), connected in series with a zero-volume stainless steel connector and a UV detector at 280 nm was employed to separate the amphetamines and caffeine, a common adulterant and dilatant in amphetamines. Good resolution was achieved via gradient elution using CO_2 mobile phase containing 1 to 12 % methanol modifiers. The SFC technique was compared to high performance liquid chromatography-diode array detection (HPLC-DAD), gas chromatography-flame ionization detection (GC-FID), and capillary zone electrophoresis-diode array detection techniques (CZE-DAD). Although HPLC was advantageous in separation time (7 min vs. 15 min with SFC), SFC-UV achieved the lowest detection limit (0.02 μg compared to 0.1 μg with GC-FID, 0.64 μg with HPLC-DAD, and 13 μg with CZE-DAD). Since the four techniques were based on different principles in separating the analytes, the combined use of them would significantly increase the specificity and were suggested for the identification of amphetamines when GC-MS was not available.

2. Cocaine and other stimulants

While amphetamines have primary or secondary amino groups, cocaine (COC) contains a tertiary amino group. This makes COC soluble in CO_2-SF without solvent modifiers and particularly suitable to be analyzed by SFE/SFC.

Allen et al. developed a CO_2-SFE procedure to extract COC and its major metabolites, benzoylecgonine (BZE) and ecgonine methyl ester (EME), from whole blood and urine, with recoveries better than 70, 40 and 85%, respectively, achieved [46]. The SFE parameters they set were: P = 3500 psi; T = 40℃; flow rate = 2 mL/min; modifier = dichloromethane (DCM)/IPA/NH_3 (78:20:2 v/v, 100-200 μL). The extraction was started with 10 min of static extraction, and then switched to dynamic mode for 20 min. The extracts were collected with 3 mL of MeOH, evaporated under nitrogen at 40℃, and derivatized with 50 μL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) at 70℃ for 20 min, and 1 μL of the resulting sample was injected into the GC-MS. Good run-to-run reproducibility was observed. The limits of detection and quantitation were 1 and 10 ng, respectively, based on 200 μL of blood and urine. A comparative study also showed that a good correlation existed between SPE and SFE for spiked blood and urine samples with percent differences ranging from 0.02 to 0.10.

Both of COC and BZE are also found in significant concentration in hair [28]. Whereas in urine the levels of BZE are typically higher than those of COC, in hair COC is often the major component. Edder et al. used the same SFE apparatus as described for opiates to extract COC and BZE in hair[30]. Morrison and colleagues used a setup which can briefly be described in the following manner: The CO_2-SF was delivered by a syringe pump. The modifier was added to the CO_2 [CO_2/MeOH/TEA/H_2O (85:6:6:3 v/v)] by means of a loop of 100 μL and a Rheodyne valve. The extraction cell was heated to 110℃ and the extracts were then recovered by

trapping the decompressed fluid in a vial containing ca. 3.5 mL of MeOH. The extracts obtained by SFE (P = 25 Mpa, T = 40℃, flow rate = 0.7 mL/min, t = 30 min) were evaporated under a current of nitrogen. BZE, which possesses a carboxylic function, was derivatized, either by the addition of (a) 100 μL of PFPA plus 100 μL of pentafluoropropanol or (b) 50 μL of N,O-bis (trimethylsilyl) acetamide (BSA). A 1- μL aliquot of the final solution was injected onto the GC-MS. The total ion current (TIC) chromatograms thus obtained for COC and BZE demonstrated the satisfactory selectivity of the proposed method.

Morrison et al. also investigated the SFE behavior of COC and BZE, and found it to be highly dependent upon the chemical nature of the matrix and the manner in which the target drug analytes are incorporated into or onto the matrix [47]. The recovery of COC from Teflon wool, filter paper, drug-fortified hair, and drug user hair was studied using a variety of CO_2/modifier mixtures. Incorporation of a TEA/water modifier mixture provided dramatic improvements in the recovery of COC from interactive matrices. The results suggest that the SFE extractability of COC is not limited by analyte solubility; rather, desorption of COC from hair binding sites is a rate-limiting step in the SFE process. A displacement SFE mechanism was hypothesized in which TEA (as triethylammonium cation) competed with COC for negatively charged hair binding sites. The dependence of extractability on hair/drug binding interactions allows the differentiation of COC present at different discrete sites in hair based on differences in SFE behavior. These findings suggest the potential for distinguishing exogenous (i.e., environmental) from endogenous (i.e., physiological) sources of drugs in hair. In contrast to the results observed for COC, SFE recoveries of BZE were poor from all matrices and under all conditions studied. Its increased polarity, the presence of an additional binding site, and the possibility of multiple charged states suggest that poor BZE recoveries may be due to both poor analyte solubility and failure to desorb the analyte from hair binding sites under the conditions employed.

Using SFC it is possible to separate COC, BZE and EME without any ChD step, whereas using GC that is infeasible [19]. A wide range of amino stimulants, including the above discussed AP, MA, ED, MP, COC and other unmentioned amines such as benzphetamine, phenmetrazine, phendimetrazine, methylphenidate, phenylephrine, hydroxyamphetamine, nylidrine, phenypropanolamine, naphazoline, xylometazoline and tetrahydrozoline, have been rapidly and efficiently separated with a tertiary supercritical mobile phase and a cyanopropyl liquid chromatography column [48]. This family of basic drugs ranges from weak to strong and primary to tertiary amines. All of them produced symmetrical peaks, and were generally more retained than antipsycholic drugs. Compared with antidepressant drugs, a few stimulants behaved similarly, but most were more strongly retained. Of the SFC parameters tested, modifier concentration produced the largest changes in both retention and selectivity for the separation of stimulants. The same procedure also applies to the analyses of opiates and cannabinoids making it potentially valuable as a screening protocol.

3. Hallucinogens

Illicit preparations of marijuana and hashish contain more than 400 compounds of differing polarities. They along with their metabolites make the definitive analysis a difficult task. After all, the major cannabinoids tested in forensic laboratories have been cannabinol (CBN), Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).

Veress proposed a mathematical model to calculate the extraction time in dynamic SFE required to reach a predefined level of extraction recovery of the main neutral cannabinoids from marijuana and hashish samples [49]. As a rule of thumb, the extraction of marijuana with CO_2-SF of density 0.9 g/mL at 40℃ for 34 min and of hashish for 18 min was suggested. The proposed parameters ensured at least a 95 % recovery for the main neutral cannabinoids.

Cirimele et al. confirmed the presence of THC, CBD and CBN in hair samples by using the same SFE conditions as for opiates [36]. This was the first report indicating that SFE is able to extract cannabinoids from hair.

Traditionally, cannabinoids are analyzed by GC-MS or HPLC, which more or less suffers from tedious sample preparation, risk of sample contamination while undertaking ChD, lengthy analysis times, limited resolution and short column life. Linking SFC to MS may be advisable since it provides both high resolution and high speed. Backströused SFC coupled to an atmospheric pressure chemical ionization mass spectrometer (SFC-APCI-MS) to separate cannabinoids [50]. The system comprised a 5- μ L injection loop to deliver ethanolic solution into an SFC fitted with a 25 cm × 4.

6 mm I.D cyanopropyl silica column (5- μm particle), with solvent delivery at 2 mL/min, with a methanol modifier gradient (2% methanol in CO_2-SF at the start of analysis, rinsing to 7% methanol in 15 min), at a column temperature of 70℃. The method offered a shorter analysis time (ca. 7 min) than GC-MS, without the need for ChD prior to analysis. It was also faster than HPLC, with better resolution and definitive identification. Linearity of detector response to CBD, Δ -8-THC, Δ -9-THC, and CBN was established, the detection limits being 0.55, 1.20, 0.69 and 2.10 ng respectively.

4. Depressants and other drugs of abuse

Several examples of the SFE/SFC of sedatives have been addressed in the review by Radcliffe et al. [19]. This section gives a few more relevant applications.

Barbiturates are usually analyzed by GC-MS or HPLC. While GC-MS may need flask alkylation to improve chromatographic performance, HPLC often requires a range of solvent system. SFC has offered the third choice of method in terms of a different yet generally better range of selectivities. Smith et al. separated a number of barbiturates by SFC on columns packed with polystyrene-divinylbenzene (PS-DVB) or ODS-silica using CO_2-SF containing methanol as a modifier [51]. The proportion of the modifier showed a marked effect on the selectivity, capacity factors, relative capacity factors, and retention indices. It was because of the changes in relative retentions with the proportion of modifier that the retention indices calculated based on the alkyl aryl ketone scale could not be successfully reproduced in different laboratories.

Smith et al. also successfully separated eleven benzodiazepines using the same SFC system as for barbiturates [52]. Once again, because of the marked effect of the proportion of the modifier in SFC on the relative retentions of compounds containing different functional groups, special care will be needed to reproduce those and similar separations in different laboratories.

In western Scotland in 1994, some 141 deaths were reportedly related to temazepam abuse [53]. Scott et al. developed an SFE procedure to analyze temazepam from whole blood [53]. Quantitative recoveries were verified by HPLC using prazepam as an internal standard with the extraction temperature and pressure carefully monitored. The results were found to correlate well with those obtained by SPE, but SFE also had the advantages of reduced solvent consumption and minimal sample handling.

Methadone has often been used as a tentative substitute for heroin in the detoxification of heroin addicts. Keeping the foregoing remarks on hair drug analysis in mind, hair samples allow for the knowledge of long-term methadone intake. Edder et al. applied the same SFE procedure as for opiates to three hair samples coming from subjects susceptible to having consumed methadone and/or opiates, and found two of them were positive for methadone [30].

The use of SFE in fire debris preparation for detection of accelerants

The most commonly sought physical evidence in arson investigation is the presence of accelerants, usually flammable liquids such as gasoline, kerosene, diesel, charcoal lighter, paint thinner, turpentine, organic solvents, etc., in fire debris samples [54]. Because most structural fires are complex chemical phenomena and because only trace quantities of accelerants are available for analysis, the forensic scientist finds it useful to perform separation and concentration procedures prior to instrumental analysis, mostly GC or GC-MS oriented [55].

Dated back to the 1950s, the first preparation methods relied on (vacuum or steam) distillation and solvent extraction [55]. Later, sampling of (cold or heated) headspace and adsorbed headspace samples (e.g., carbon wire, carbon strip, purge and trap) resulted in easier detectability of smaller accelerant quantities and of pyrolysates from wood and synthetic materials. During the past two decades, the most widely used preparation method has been charcoal adsorption utilized with solvent (carbon disulphide for GC-FID; normal pentane for GC-MS) or thermal desorption followed by GC or GC-MS. This has been chosen because: it gives reasonably high recovery; separates accelerants from some structural pyrolysates; makes all the samples available for analysis (i.e., total-amount or quasi-total-amount analysis); allows repeated analyses; is convenient and virtually complete in terms of clean-up; is sensitive to small amounts of accelerant; and is rapid and cheap. Problems remaining include improving separation of components that are chemically similar to common accelerants but result from the pyrolysis of construction materials such as wood and synthetic polymers; detecting even smaller quantities of accelerants; systemizing [56], standardizing, and automating sample preparation and instrumental analysis.

Lopez-Avila et al. have developed an off-line SFE-infrared (SFE-IR) method for the detection of petroleum hydrocarbons in soils. The extraction of 3 g soil sample was performed at 340 atm and 80℃ using CO_2-SF. The extracts were collected in 3 mL of tetrachloroethene and subsequently cleaned with silica gel prior to IR analysis. This SFE-IR method is rapid (each run takes ca. 40 min) with the extraction efficiency nearly equivalent to that by Soxhlet extraction [57]. Hawthorne et al. further demonstrated that generator-powered SFE-IR shortened the analysis time and was well suited for field surveys [58]. Although SFE method has been validated and approved by the US Environmental Protection Agency for routine extraction of total recoverable petroleum hydrocarbons [59], SFE-IR is virtually a non-specific quantification method and is used mainly for screening purpose. A specific detector such as GC-PID(photon ionization detector) or GC-MS may be employed for quantification. In fact, an SFE-GC based method has been developed for the recovery and analysis of diesel from clays [60].

It becomes clear that SFE and SFC can be promising techniques for the preparation and analysis of fire debris samples. Whereas actual examples of the latter are still lacking, Huang and Hsieh did use SFE to recover accelerants from spiked, simulated and real-case fire debris samples [61]. They even applied Taguchi's orthogonal array experimental design to optimize the SFE parameters. For unleaded gasoline and kerosene in celite, in brief, P = 6000 psi, T = 40℃, t = 10 min for static and dynamic extraction respectively, and eluent phase = pure CO_2-SF; for diesel in celite, P = 3000 psi, other conditions being the same. The extracts were collected in 10 mL of hexane followed by GC-FID and GC-MS analyses. However, the optimal SFE conditions are subject to changes when analyzing real-case samples. For instance, the extraction temperature was raised to 80℃ when processing simulated carpet residues, with recoveries generally better than 80%. Compared to other existing methods, SFE was claimed to be rapid, convenient, efficient, and selective.

The use of SFE and SFC in the analysis of explosives

Chemical explosives are the basic components of the bomber. When bulk evidence captured by law enforcement agencies or post-explosion residues collected from a bomb scene is received in the laboratory, the first priority is to detect and identify the explosive. In many modern forensic laboratories, the initial step in the evidence examination is the screening of the evidence with the aid of an explosives detector [62]. For laboratories not routinely using trace vapor analysis for screening, the analytical approach is dictated by the available staff, time, and experience in the examination of explosives. Traditionally, the optimum situation is to have some discrete particles free from the evidence substrate to examine under a low power microscope for the presence of materials which may be either undetonated explosives or decomposition products characteristic of a particular explosive type. However, because of potential interactions of the substrate and/or explosives with any solvent used to extract the explosive, the separation is best made manually and can be tedious and time consuming. If no identifiable particles are observed during the microscopic examination, a sequential solvent extraction of the evidence is then performed. The evidence is placed in a suitable container and rinsed or covered with solvent. Initially, a solvent is used which will dissolve organic components of the explosive but not inorganic ones. Acetone, chloroform, or carbon tetrachloride is suitable for this step. The solvent is decanted from the evidence, filtered and concentrated by slow evaporation to avoid loss of volatile explosive components. The organic extracts are retained for later testing. The extracted debris is air dried and reextracted with hot water to dissolve inorganic components such as nitrates, chlorates, or chlorides. The water extracts are concentrated by evaporation prior to testing.

The detection and identification of explosives in post-explosion residues is a highly difficult task because: (1) very little of the original material is left on the bombing site; (2) these minute quantities of the unexploded explosives are often seriously contaminated with background compounds or widely dispersed over complex matrices interfering with the analysis [63,64]; (3) the low thermal stability, low volatility, high adsorption activity, and high energy of the explosive can significantly affect the performance of sampling and analysis. The high selectivity, high recovery, high compatibility, and easy handling make SFE an ideal technique for extracting explosives. Engelhardt et al. have developed an off-line coupling of SFE and HPLC to determine 2,4-dinitrotoluene (2,4-DNT), 2,4,6-trinitrotoluene (2,4,6-TNT), ethyleneglycol dinitrate (EGDN) nitroglycerine (NG), pentaerythritol tetranitrate (PETN), N-methylnitro-2,4,6-trinitroaniline (TETRYL), 1,3,5-

trinitro-1,3,5-trizane (RDX), and cyclotetramethylene tetranitramine (HMX) in soil [65]. The SFE conditions were: P = 300 bar; T = 40℃; t = 20 min for dynamic extraction; eluent phase = pure CO_2-SF. The extracts were collected in acetone. The recovery was found to decrease with increasing organic contents of the soil and with increasing polarity of the explosive.

Slack et al. used SFE in combination with off-line GC-ECD and GC-MS to characterize Semtex—a plastic explosive containing RDX in styrene-butadiene copolymer and hydrocarbon oil [66]. The SFE conditions were: P = 5000 psi; T = 50℃; V = 30 mL for dynamic extraction; eluent phase = pure CO_2-SF. The extracts were collected in either methanol or dichloromethane. The results indicated that EGDN was the component responsible for the positive ECD response.

Several works using SFC to analyze explosives have been performed. The SFC approach has to some degree overcome the problems of some analytes being decomposed or over-retained during a GC analysis or inadequately resolved when running an HPLC. Thus, NG, EGDN, PETN, mannitol hexanitrate (MHN), 2-nitrotoluene (2-NT), and 2,4-dinitrotoluene (2,4-DNT) were successfully separated using a 25 m × 1 mm I.D cyanopropyl silica (5 μm) column [67], but the SFC of RDX and HMX only resulted in poor peak shape or failure in elution even if pressure programming had been employed [68]. For the SFC of polar analytes, while the addition of appropriate modifiers to the CO_2-SF could be a feasible choice, trying different columns looked even more inviting. Munder et al. made the use of a biphenylmethylpolysiloxane column (10 m x 0.05 mm I.D) with triple detection, i.e., UV, FID, and ECD [69]. Fancis et al. tried three kinds of SB columns, i.e., octyl-50-superbond, m,p-cyanobiphenyl and p,p-cyanobiphenyl capillary columns [70]. McAvoy et al. utilized a cyanopropyl silica column (250 x 4.6 mm I.D, 5 μm thickness) plus methanol modifier to separate 2, 3-, 2,6-, and 3,4-DNT, 2,4,6-TNT, PETN, RDX, and HMX, with the SFC being coupled with an atmospheric chemical ionization MS [71].

The use of supercritical carbon dioxide in latent fingerprint development

Physical evidence at the crime scene can be present in an infinite variety of forms, materials, dimensions, etc, and its value to the investigation can range from uncertain or of little importance to the identification of an individual, an object, or a fact linked with the crime. Few forms of evidence can permit so unquestionable identification of an individual as fingerprints, dental structure, and genetic code can and only digital patterns possess all the necessary qualities for identification. The ridge patterns on the fingers are the most useful and generalized proof of identity: they are unique, immutable, universal, easy to classify, and leave marks on any object handled with bare hands [72]. However, fingerprints are often minute, invisible and fragile traces that must be properly protected and sensitively detected in an optimal manner on a variety of surfaces after exposure to the environment.

There are three types of fingerprint evidence. Compared to indented and visible fingerprints, the latent fingerprint poses the most problems with its treatment and recording. Ninhydrin is the most commonly used reagent for developing latent fingerprints on paper and other porous surfaces. 1,8-Diazafluorene-9-one (DFO) is being used increasingly in sequence with ninhydrin on exhibits from major crimes. Ninhydrin and DFO react with amino acids present in eccrine sweat to give pale purple or fluorescent fingerprints respectively [73, 74]. Because the old ninhydrin process used many highly flammable solvents including acetone and petroleum ether [72] as the main carrier solvent, the newer ninhydrin and DFO formulations introduced specifically the use of 1,1,2-trichlorotrifluoroethane (CFC113) to reduce the explosion and fire hazards. In addition, CFC provides several advantages; namely, CFC113 is (i) non-flammable; (ii) non-toxic; (iii) volatile; (iv) does not cause diffusion of handwriting. Paper evidence, for instance, is treated by passing the paper through a shallow trough of the reagent solution and allowing the surface to dry completely in the open atmosphere. The solution is also brushed onto cardboard articles and wall paper at the crime scene. The latent fingerprints are then developed by heating the paper in a specifically adapted humidity oven at 80℃ and 65% relative humidity.

With the phasing out of CFC113 under the terms of the Montreal Protocol on the Control of Ozone Depleting Substances, however, the need for a suitable replacement for CFC113 is urgent. As CO_2-SF is non-flammable, non-toxic and volatile, a feasibility study of its use in the ninhydrin process has been undertaken by Hewlett et al. [75]. In the preliminary experiment, the relative solubilities of ninhydrin, DFO, serine, and glycine in CO_2-SF were measured by column

chromatography. Neither of the two amino acids were eluted from the CO_2-SF column at 50℃ and 300 bar. Ninhydrin however was eluted from the column in ca. 1.5 min. DFO could not be eluted in pure CO_2-SF, but could be dissolved by the addition of 5% methanol to the CO_2-SF with an eventual elution time of 3.75 min. For the formal experiments, the prototype reactor and process used by Hewlett was very similar to that described in the foregoing sections for SFE/SFC. The cell or treatment vessel in which the document sample (white photocopying paper, used checks, etc), ninhydrin, acetic acid and water was to be placed had a capacity of 165 cm^3 and was equipped with an electrical heater (set at 80 ℃) surrounded by a ceramic insulator. CO_2-SF was introduced into the vessel from a standard F size cylinder using a high pressure pump (set at 300 bar), the pressure inside the reactor being constantly measured using a pressure gauge. Convection of the CO_2-SF around the vessel was accomplished using an external unheated pipe that connected the top and bottom of the reactor together. At the end of the treatment period, the pressure was released slowly over 15 min by opening a valve to the atmosphere. This method was successfully carried out to develop latent fingerprints in a one-stage process, whereas the currently used ninhydrin formulation takes two stages (impregnation of reagent followed by development in an oven). The performance was dependent on the presence of water and acetic acid, but when too many articles or too closely pinned fingerprints were treated simultaneously the addition of excessive quantities of those compounds did cause significant ridge diffusion (there was no indication of diffusion of handwriting or printing inks on any of the documents treated). Furthermore, the size of the apparatus needed for the use of CO_2-SF would limit the size of article that could be treated. Treating large cardboard boxes, for example, was simply not feasible using the above stated CO_2-SF vessels. This technology probably also shows promise for some sort of SFE of trace amounts of illicit materials from paper evidence or for the application of other types of fingerprint reagent.

Conclusions

SFE is emerging as a valuable analytical technique for use as an alternative to conventional SPE and liquid-liquid extraction (LLE). Due to the increasing need for a simple, rapid, selective, quantitative, automated, environmentally friendly, and GC- or LC-compatible extraction method, CO_2-SFE is particularly attracting most interest as has been demonstrated by its wide applications in the analyses of environmental samples, food, drugs, natural products, and polymers. So far as forensic science is concerned, the tunable solvation properties and environmental friendliness of CO_2-SFE best satisfy the need for an advanced technique to extract, separate, and identify a wide variety of trace amounts of compounds in a wide range of sample matrices

The status of SFC is somewhat different from that of SFE. SFC has been able to solve niche problems inadequately treated with GC and HPLC. However, several instrumental limitations remain to be overcome before it can be widely adopted by the forensic community. Efforts should be made to minimize the chromatographic retention times generally caused by the addition of polar modifiers and to design any robust interface that connects a separatory SFC to a confirmatory MS. In summary, the forensic science oriented research of SFE/SFC is promising.

Acknowledgements

The authors thank the National Science Council of the Republic of China for financial support (NSC 90-2113-M-007-061).

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