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Review

Extraction of pharmaceuticals using pressurised carbon dioxide

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Abstract

This paper reviews the applications of super- and sub-critical carbon dioxide for the extraction of pharmaceuticals from various matrices. The matrices covered are divided into the following types: animal feed, formulations, biological and miscellaneous, with various sub-divisions as appropriate. The polar nature of most pharmaceuticals often precludes the use of carbon dioxide only, so it is common to find the addition of a more polar solvent, as modifier. As the majority of sample types covered are solid, little if any pre-treatment is required, with the exception of grinding, prior to insertion in the sample extraction cell. For liquid-type matrices, sample pre-treatment is the normal. Often this may involve adsorption on an inert support e.g. Celite or diatomaceous earth, or immobilisation on a functionalised silica surface, e.g. C_{18}. The later may take the form of a solid phase extraction cartridge or disk. An attempt has also been made to sample from liquid matrices directly using a modified extraction cell. The variety of sample types, matrices and analyte polarity places stringent requirements on the use of pressurised carbon dioxide. Its potential for effective recovery is examined in this review. © 1997 Elsevier Science B.V.

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1. Introduction

Supercritical fluid extraction (SFE) is widely perceived as a technique for the extraction of low to moderately polar compounds. With increasing concern over the use of chemical solvents in the manufacture of pharmaceuticals, as well as the need for high quality products, alternatives to energy intensive and costly extraction schemes have been sought. Pharmaceutical compounds are usually polar, non-volatile and the matrices may contain co-extractives. The currently used sample preparation techniques for analysis include solid phase extraction, liquid-solid extraction (Soxhlet extraction), and liquid-liquid extraction. Recent studies have shown that the use of supercritical fluids as an extraction media provides a powerful alternative to traditional extraction methods [1,2]. The potential benefits of SFE include: faster analysis; reduced sample handling; protection from degradation by light, heat, or oxygen; high load-

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ability of samples and possibility of trace analysis; elimination of hazardous and/or expensive solvents; and, analysis of aqueous samples (serum, urine, and saline solution) without introducing organic solvents. The gentle extraction conditions used in SFE compared to more traditional extractions such as Soxhlet extraction also provide greater assurance against chemical reaction not taking place during the extraction. This ensures that isolated analytes are representative of the original sample.

Carbon dioxide is the most popular choice of supercritical fluid for extraction of target analytes from solid or liquid pharmaceutical matrices. Although carbon dioxide is a non-polar molecule the addition of polar organic solvents as modifiers or entrainers can aid extraction efficiencies. However, it should be noted that the solubility of analytes within the supercritical fluid does not always ensure the analytes extractibility. The location of the analyte in or on the matrix is critical, and the interaction of the analyte with active sites on the matrix must be disrupted. These features can greatly lengthen the extraction time, require higher extraction pressure/temperature and higher modifier concentration. It is often recommended that recoveries are assessed in terms of recovery from native sample rather than spiked samples. However, the available literature does not often reflect this ideal situation and care should be exercised in selecting appropriate extraction conditions. The following sections represent a review of the current literature available on the use of pressurised carbon dioxide for extraction of pharmaceuticals. No distinction is made in this review between sub- and super-critical carbon dioxide unless suggested in the original article. A summary of pharmaceutical applications, using pressurised CO_2, is shown in Table 1.

2. Animal feed

In the pharmaceutical industry, long-term feeding studies of chemical substances involves fortification of the animals feed with the target analyte prior to administration. Analytical methods are required to monitor the fortification level, to verify uniformity throughout the sample matrix and to test for analyte stability in the feed as part of toxicological investigations. Schneiderman et al. [3]. was the first to report on the applicability of using SFE as a quantitative method for extracting drugs from animal feed matrices. They reported the extraction of menadione (Vitamin K3) from spiked rat chow using supercritical carbon dioxide at 8000 psi and 60°C for 20 min. Collection of the analyte was achieved by trapping the expanding supercritical carbon dioxide into a 6 × 0.25 in i.d. stainless steel tube filled with silica gel. The silica gel was subsequently washed with 10 ml methylene chloride. Menadione was determined in the extract without any cleanup using reversed phase high performance liquid chromatography (HPLC) with reductive mode electrochemical detection. The lowest concentration of menadione extracted quantitatively was 20 μg g^{-1} of feed. An average recovery of 90.5% with 2.2% RSD was reported at the 1 mg g^{-1} level.

In 1991 Euerby et al. [4] reported the SFE of a novel corticosteroid, tipredane, at a level of 10 ppm from rodent diet. A number of collection methodologies were investigated by the authors to obtain maximum recovery of the analyte and it was concluded that the direct collection from a back pressure regulator (BPR) through a short length of tetrafluoroethylene tubing into a calibrated flask containing 1 ml of methanol, stoppered with a small piece of cotton wool to trap any escaped analyte gave the best results. The authors investigated the extraction of the tipredane with and without the presence of ethanol modifier. The flow rate of ethanol was altered, keeping the supercritical CO_2 flow rate constant to optimise the collection efficiency. The optimum extraction conditions employed were as follows: pressure, 200 kg cm^{-2}, temperature 70°C, CO_2 flow rate, 3.0 ml min^{-1}, ethanol flow rate, 0.3 ml min^{-1}, and BPR temperature 40°C and an extraction time of 2 min. A comparison of the optimized SFE extraction conditions with the more conventional extraction procedures of Soxhlet and ultrasonic agitation was reported. The average extraction recovery obtained with SFE was 85% with an RSD of 6% compared with 92% obtained from Ultrasound (RSD 3%) and 94%

Table 1
Summary of pharmaceutical applications of pressurised CO_2

Analyte	Matrix	Extraction conditions	% Recovery	Reference
Menadione	Rat chow	CO_2 only: 8000 psi: 60°C; 20 min	90.5% recovered with 2.2% RSD at the 1 mg g^{-1} level.	[3]
Tipradane	Rodent diet	10% ethanol-modified CO_2: 200 kg cm^{-2}; 70°C; 2 min	85% recovered with 6% RSD	[4]
Hypolipidermic drug (4-trifluoromethyl-2-biphenyl carboxylic acid)	Rat feed	CO_2 only: 0.9 g ml^{-1}; 50°C; 30 min.	84% recovered with RSD 2.1%	[5]
Halogenated aromatic phenoxy derivatives	Dog feed/rodent feed	Methanol–acetonitrile-modified CO_2. Methanol–acetonitrile-modified NO_2. CO_2 and NO_2 only: 0.79 g ml^{-1}	Quantitative	[6]
Atovaquone	Rat feed	CO_2 only: 350 bar; 50°C; 20 min.	>95% recovered with <5% RSD	[7]
Fluconazole	Animal feed	20% methanol-modified CO_2: 250 kg cm^{-2}; 80°C; 40 min.	91.0% (13.2% RSD) $n = 10$ at the 500 mg kg^{-1} level.	[8]
Propanolol, Tamoxifen, ZM 95527 and ZM 169369	Rodent diet	Methanol-modified CO_2: 17.25 MPa; 70°C.	88.4% with an RSD of 12.9% for propanolol; 86.2% with an RSD of 8.1% for tamoxifen; 84.5% with an RSD of 12.1% for ZM95527; and, 81.2% with an RSD of 12.2% for ZM169369.	[9]
Megestrol Acetate	Tablet	10% methanol-modified CO_2: 0.81 g ml^{-1}; 55°C; 20 min	92.8% recovered	[10]
Felodipine	Tablet	8.7% methanol-modified CO_2	98.5% recovered with an RSD of 1.2% ($n = 5$)	[11]
Benzodiapines (7)	Tablet/capsule	2% methanol-modified CO_2: 100 atm; 65°C; 15 min.	Quantitative	[13]
Caffeine and vanillin	Tablet	CO_2 only: 350 bar; 50°C; 25 min.	97.7% recovered for vanillin with RSD of 3.1%; and, 98.5% recovered for caffeine with RSD of 1.4%	[14]
Vitamin A and E	Tablet	CO_2 only: 200 atm; 40°C; 15 min.	>95.6% recovered	[15]
Retinol palmitate and tocopherol acetate	Ointment	10% methanol-modified CO_2: 200 kg cm^{-2}; 40°C; 10 min.	102.0% recovery for retinol palmitate and 101.5% for tocopherol acetate.	[16]
Polymyxin B sulphate	Cream/ointment	5% methanol-modified CO_2. For the cream: 300 atm; 55°C; and, 45 mins. For the ointment: 450 atm; 60°C; and, 45 mins.	108% recovered for the cream with 5% RSD and 136% recovered for the ointment with 1.9% RSD	[17]
Acylvoir	Ointment	2% methanol-modified CO_2: 50°C.	99% recovered with RSD 5.3% at the 50 mg g^{-1} level	[18]
Sulfamethazole and trimethoprim	Septra infusion	From infusion: CO_2 only: 250 atm; 50°C.	Direct from infusion: sulfamethoxazole recovery 5%. After immobilization onto Celite: 113% recovered with 14% RSD for sulfamethoxazole and 85% recovered with 10% RSD for trimethoprim	[19]

Table 1 (continued)

Analyte	Matrix	Extraction conditions	% Recovery	Reference
Triamcinolone	Dermatological patches	Methanol-modified CO_2	10% recovered	[20]
Misoprostol	Hydroxypropyl methylcellulose	CO_2 only: 0.8 g ml^{-1}; 70°C; 4 min.	65% recovered	[21]
Veterinary drugs (4)	Pig-kidney	CO_2 only followed by 20% methanol-modified: 302 bar; 75°C.	Qualitative	[23]
Nitrobenzamide antimicrobial drug residue	Liver	CO_2 only: 680 bar	Quantitative	[24]
Veterinary sulpha drugs (5)	Liver and swine samples	10–20% methanol-modified CO_2: moderate pressure; 1 h.	Recoveries ranged from 27 to 97%	[25]
Codeine, morphine and ethyl morphine	Hair	MeOH, TEA, H_2O modified CO_2: 25 MPa; 44°C; 70 min	93.5%	[26]
Ketorolac and flavone	Plasma	CO_2 only: 30 MPa; 60°C; 20 min.	For flavone: 98% recovered with RSD of 5.2%. For ketorolac: 80% recovered with 11.1% RSD	[27]
Mebervine alcohol	Dog plasma	5% methanol-modified CO_2: 350 atm; 40°C; 10 min.	95% recovered at the 50 ng ml^{-1} spike level	[28]
Morphine	Serum	CO_2 only	96.7% recovered with RSD 3.2%	[29]
Beudesonide	Plasma	CO_2 only: 110°C; 30 min.	>80% recovered	[30]
Caffeine	Kola nuts	THF or methanol: 100 psi: 135°C; 30 min	99.9% recovery	[29]
Taxanes	Needles of yew tree	10% methanol-modified CO_2: 400 atm; 50°C; 100 min.	83% recovered	[31]
Chinese herbal medicines	Plants	CO_2 only: 20 MPa; 50°C; 40 min.	Quantitative	[32]
Diosgenin	Tubers of Dioscorea nipponica	CO_2, range of temperature and pressure studied: 3100 psi; 44°C; 70 min.	Quantitative	[33]
Taxol and baccatin III	Needles of Taxus cuspidata	3% ethanol-modified CO_2: 300 bar; 40°C.	Quantitative	[34]
Zingiber zerumbet rhizomes	Plants	CO_2 only: 200 bar; 60°C; 120 min.	Quantitative	[35]
Mevinolin and its hydroxy acid derivative	Fermentation broth	CO_2 and various modifiers (MeOH, *t*-butyl amine, acetic acid)	Selective extraction investigated	[36]
Phylloquinone	Soy protein and milk based infant formula	CO_2 only: 8000 psi; 60°C; 15 min.	For infant formula: 95.6% recovered with RSD of7.4%. For soy protein: 94.4% recovered with an RSD of 6.5%.	[38]
Triprolidine and pseudoephedrine	Aqueous	CO_2 only	Qualitative	[39]
Steroids	–	CO_2 only and Freon-22	Quantitative	[40]
Ibuprofen	–	CO_2 only	Study of effects of temperature and pressure.	[41]
Steroids (10)	–	CO_2 only	Solubility isotherms over a range of temperatures and pressures.	[42]

Soxhlet extraction (RSD 6%). The authors concluded that the advantage of SFE is that the extraction is rapid and no preconcentration step was required prior to HPLC analysis.

Messer and Taylor [5] reported the extraction of a hypolipidermic drug, 4-trifluoromethyl-2-biphenyl carboxylic acid, from a spiked rat feed matrix at a level of 1%. Extraction was performed with supercritical carbon dioxide off-line and solid phase trapping followed by solvent rinsing was utilized. An optimized method for quantitative extraction of the pure drug was initially developed with high reproducibility. Then triplicate 100 mg samples of spiked animal feed containing 1% drug were extracted using the optimized extraction conditions. However, the average recovery obtained for these extractions was only 68.8% with a RSD of 20.6%. The results obtained led the authors to believe that the problem was with the sample itself rather than the poor solubility or extractibility of the analyte in the supercritical fluid. To investigate whether inhomogeneity in the 'crystalline matrix' caused the poor reproducibility, the animal feed was spiked with the drug in a different manner. Rather than mixing the drug mechanically as a crystalline substance on a large scale, the drug was first dissolved in methylene chloride and a slurry with the pure ground feed was prepared. The slurry was then mixed, allowed to dry and was used as the 'solvent matrix'. The dried solvent matrix was then thoroughly mixed. Triplicate 100 mg samples of the 'solvent matrix' were extracted using the optimized extraction conditions. The average recovery obtained for these extractions was 84.0% and the RSD of 2.09%. This study showed that the 'crystalline matrix' was not homogeneous and since the matrix size was only 100 mg, the sample for each replication was not always representative. The authors concluded that the less than quantitative recoveries may reflect a matrix effect which might be eliminated with the addition of a modifier in the extractant phase, where the modifier function would be to compete with the drug for the active sites of the matrix. In this particular example of extraction of a polar drug from an animal matrix, the use of a modifier such as methanol would probably have resulted in complete recovery.

The potential use of a supercritical fluid other than supercritical carbon dioxide for extraction of pharmaceutical compounds was demonstrated by Sauvage et al. [6]. The comparative behaviour of supercritical nitrous oxide and carbon dioxide was studied for extraction of a halogenated aromatic phenoxy derivative of an aliphatic alkane (HAPA) from a dog feed and a halogenated aromatic phenoxy derivative of urea (HAU) from a rodent feed. The dog and rodent feed were spiked at 5 mg g^{-1} and 2 mg g^{-1}, respectively. Extractions were carried out with pure supercritical fluids with high solvating power (pressure 250 atm and 60°C corresponding to a density of 0.79 g ml^{-1}) and fluids modified with a polar modifier (methanol and acetonitrile). The results indicated that 0.7% (v/v) methanol-modified nitrous oxide gave the best recoveries. The extraction time of HAU from rodent feed was reduced by a factor of 2 with a recovery of 94%. For the extraction of HAPA, the total extraction time required was only a few minutes compared to 70 min extraction time by the classical method.

Messer et al. [7] reported routine extraction of atovaquone [trans-2-[4-(chlorophenyl) cyclohexyl]-3-hydroxyl-1-2-napthoquinon] from rat feed. Initial experiments involved extraction of atovaquone from an inert Celite matrix to establish the extraction profile of the drug in the absence of the matrix. Specifically, after increments of time the dynamic extraction was interrupted, the collection trap was rinsed and the resulting solution was analysed. Spiked test samples were prepared by spiking 0.20 ml of a 1.00 mg ml^{-1} solution of atovaquone in methylene chloride onto a Celite bed inside the extraction cell. The samples were left overnight at ambient conditions to allow solvent evaporation. The atovaquone spiked samples were then extracted using supercritical CO_2 at a pressure of 350 bar, temperature at 50°C and flow rate of 2 ml min^{-1}. The operating conditions chosen corresponded to a supercritical fluid density of 0.9 g ml^{-1}. The trap consisted of stainless steel spheres (100 μm diameter) held at 5°C during extraction and 40°C during rinsing. The total dynamic extraction time was 20 min. The results gave a recovery of 67.1% in the first 2 min and an additional recovery of

16.7% in the subsequent 3 min, giving >80% recovery of atovaquone in the first 5 min of the extraction. The total recovery obtained for the extraction profile after 20 min was 95.4%, indicating high solubility of atovaquone in supercritical carbon dioxide. To study the effect of a modifier, a second set of extractions was performed with 2% methanol modifier. Experimental conditions were kept the same with the exception of the trap temperature which was raised to 70°C. The results obtained under these conditions showed that the extraction recoveries for the combined initial two steps (5 min) was only 61.8% for methanol-modified CO_2, compared with >80% for the extraction with 100% CO_2. The lower recovery was thought to be due to poor collection efficiency on the stainless steel trap. Consequently, pure CO_2 was used for extracting the spiked drug. Atovaquone was studied at six levels ranging from 0.0335 to 1.1208%. Sample sizes for extraction were either 250 or 500 mg depending on the spike level. Extraction were performed in triplicate and >95% recovery with <5% RSD was obtained for all matrices except for the 0.0335% level, which was 89%. The authors [7] concluded that the SFE results were comparable to conventional extraction results.

In 1995 Khundker et al. [8] reported the extraction of fluconazole from a spiked animal feed matrix using supercritical carbon dioxide. Optimum extraction conditions were established using a fractional factorial design. The results of the optimised SFE (pressure 250 kg cm^{-2}; temperature 80°C; extraction time, 40 min; and, 20% methanol as modifier) were compared with a liquid-solid extraction followed by solid phase extraction (SPE) preconcentration/cleanup. The results obtained using SFE were as follows: at a spike level of 10 g kg^{-1} the recovery was 87.0% with an RSD of 8.4% ($n = 10$); while at a spike level of 500 mg kg^{-1} the recovery was 91.0% with an RSD of 13.2% ($n = 10$). These results were compared with the SPE approach. Using SPE the average recovery obtained was 99.1% with an RSD of 1.3% ($n = 5$) at the 40 mg kg^{-1} spike level. It was evident that while comparable recoveries could be obtained by both procedures the precision of SFE was considerably worse (approximately 10 times).

Williams et al. [9] presented a comparison of supercritical, subcritical, hot pressurized and cold solvent extraction of propanolol, tamoxifen and two experimental drugs, ZM 95527 and ZM 169369 from rodent diet. The experimental conditions employed for the supercritical and subcritical fluid extraction were CO_2–MeOH (85:15 v/v or 83:17 v/v) at 70°C and 17.25 MPa. Recoveries obtained by different extraction methods were compared and it was found that hot, pressurised MeOH at 2.0 ml min^{-1} and 70°C consistently gave good recoveries independent of the analyte. The recoveries obtained by the subcritical method were intermediate between the SFE and the hot, pressurised MeOH. The authors concluded that although the efficiency of these methods was similar to conventional extraction with acidified MeOH, the precision obtained by the solvent extraction method was superior to the other methods described.

3. Formulations

This is the largest category of pharmaceutical analysis involving the mostly varied group of complex matrices, for example, ointments, syrups, capsules, infusions, creams and tablets.

3.1. Tablets:

Dean and Lowdon [10] reported extraction of megestrol acetate from a tablet formulation. Preliminary investigations were first performed by extracting the drug from an inert Celite matrix to establish optimized experimental conditions for extraction from the tablet matrix. Optimized extraction conditions were first developed with 100% CO_2 at a temperature of 55°C, density 0.81 g ml^{-1} and a flow rate of 2 ml min^{-1}. Subsequent extraction from a tablet formulation using the optimized extraction conditions gave a poor recovery. The tablets were ground to aid the rate of extraction. A 160 mg tablet was weighed, ground to a powder and 20 mg sub-samples were extracted over increasingly longer periods at the optimum conditions described previously. The results obtained indicated a maximum recovery of

70.4% after 20 min. Further extractions were carried out with 10% modified methanol–CO_2 using the same optimized conditions and a maximum recovery of 92.8% was achieved after 20 min of extraction time. The authors also presented a direct comparison of SFE with a USA Pharmacopoeia (USP) monograph method for the analysis of megestrol acetate from the tablet formulation at two levels (40 and 160 mg) and concluded that the USP method provided the analyst with more precise determinations of megestrol acetate from a tablet matrix.

Howard et al. [11] successfully demonstrated the application of SFE as a sample preparation method for sustained-release felodipine tablets. Static/dynamic mode SFE together with CO_2 with 8.7% MeOH modifier was used for extraction. The average recovery (98.5%; 1.2% RSD; $n = 5$) was comparable to results obtained using a traditional liquid extraction method (99.7%; 2.4% RSD; $n = 5$) with similar levels of drug degradation occurring in either method. The same authors [12] evaluated the SFE of similar tablets using packed-supercritical fluid chromatography with both electron capture and ultraviolet detection. The limit of detection was determined to be 34.4 pg. Lawrence et al. [13] demonstrated the application of SFE for the extraction of several benzodiapines (alprazolam, clorazepate, chlordiazepoxide, diazepam, oxezepam, tamazepam, triazolam) in solid dosage form. One quarter of a ground tablet or capsule samples of various weights were dispersed in 450 mg of Hydromatrix and extractions (5 min static and 10 min dynamic) carried out with 2% MeOH-modified supercritical CO_2 at 100 atm pressure and a temperature of 65°C. The eluate was collected in 500 μl of MeOH which was evaporated to dryness before dissolution of the residue in 50 μl of MeOH. All dosage form extracts were free from matrix components and could be analysed by GC-MS. Extraction yields which were sufficiently high to allow GC-FTIR analysis were only obtained from the dosage forms containing > 1 mg per tablet of benzodiapeine.

Anklam and Mueller [14] reported extraction of caffeine and vanillin from a tablet formulation using supercritical carbon dioxide at 50°C and 350 bar. Tablets were ground to a powder and subsamples of 100 mg were mixed with 1 g of sand and, moistened with 100 μl of water prior to extraction. A 5 min static and 20 min dynamic extraction time was employed. The recovery achieved for vanillin and caffeine was 97.7 and 98.5% with an RSD of 3.1 and 1.4%, respectively. The authors concluded that the SFE results were comparable to those obtained using Soxhlet extraction. Scalia et al. [15] reported the extraction of vitamin A, vitamin E and their esters in tablet preparations using SFE followed by HPLC. Tablets were powdered and subsamples (100–150 mg) were mixed with sea sand (3 ml) and extracted with supercritical CO_2 at 40°C and 200 atm for 15 min. Quantitative extraction data was achieved ($> 95.6\%$) with an RSD of 1.7 and 3.9% for Vitamins A and E, respectively. The authors concluded that the SFE method was superior to the existing standard method.

3.2. Creams/ointments

Masuda et al. [16] determined fat-soluble vitamins (retinol palmitate and tocopherol acetate) in a hydrophobic ointment using a coupled SFE-SFC system. The ointment, triturated with diatomaceous earth, was extracted using supercritical carbon dioxide at 200 kg cm^{-2} pressure, temperature 40°C and 10% methanol-modifier. The extract was collected on a trimethylsilyl silica gel column by reducing the pressure. The analytes were then separated and analysed using a octadecylsilyl silica gel column using ethanol-modified supercritical CO_2. The hyphenated technique allowed retinol palmitate and tocopherol acetate to be determined within 10 min, after placing the sample in the extraction cell. Recoveries were determined to be 102.0 and 101.5% for retinol palmitate and tocopherol acetate, respectively.

Moore and Taylor [17] successfully demonstrated the application of SFE for the extraction of polymyxin B sulphate from cream and ointment matrices. Cream (200 mg) was extracted at 55°C and 300 atm for 45 min with 5% MeOH modified CO_2 (2 ml min^{-1}). For the ointment, a pressure of 450 atm and temperature 60°C was

employed at the same flow rate. Average recoveries reported for the cream and the ointment were 108 and 136% with an RSD of 5 and 1.9%, respectively. The same group [18] investigated the application of inverse analytical SFE and HPLC for the determination of acylvoir from 5% Zovirax ointment. The ointment sample (100 mg) was placed between the frits of a solid phase extraction (SPE) tube, and the tube was subsequently transferred to the extraction cell. The extraction cell was kept at a temperature of 50°C and CO_2 modified with 2% MeOH was used as the extraction fluid at 0.9 ml min^{-1} to extract the hydrocarbon-based ointment base. Two portions of 0.01 M NaOH were used for the ultrasonic extraction of the acylvor active component from the inner vessel. An average recovery of 99% with RSD 5.3% was reported for ointment containing 50 mg g^{-1} of acylvoir.

3.3. Septra infusions

Mulcahey and Taylor [19] reported the application of SFE for direct extraction of polar active ingredients from liquid matrices. The investigation involved the extraction of sulfamethazole and trimethoprim from septra infusion. The active ingredients of septra infusion contained 16 mg trimethoprim and 80 mg sulfamethazole per ml of infusion. The active components are dissolved in 40% propylene glycol, 10% ethyl alcohol, and 0.3% diethanolamine with 1% benzyl alcohol and 0.1% sodium metabisulphite added as preservatives. Extractions were performed in two ways. The first method involved extracting septra infusion liquid directly using a modified extraction vessel designed to bubble the supercritical fluid before exiting to the trap. This method however gave poorer yields as a result of restrictor plugging caused by precipitation of sulphamethoxazole when the solution pH was lowered by the introduction of CO_2. The second method involved spiking the active ingredient onto Celite followed by extraction of the drug with 100% CO_2. In this way quantitative recovery was achieved for both drugs when the active ingredients were spiked at < 1.0 mg. Co-extractives of the drug were observed but did not interfere with the HPLC assay.

3.4. Dermatological patches

Extraction of triamcinolone from dermatological patches was attempted by Edwardson and Gardner [20]. Extractions were carried out with CO_2 modified with 0, 5 or 10% methanol at 80°C for 5 min. Recoveries of only 10% were achieved. However, the authors did not investigate a longer extraction time, different extraction temperature or modifier which may have resulted in the improved extraction of triamcinolone. The SFE of misoprostol, a synthetic prostogladin from a hydroxypropyl methyl cellulose dispersion was reported by Roston [21]. Sample components were extracted and subsequently precipitated in the cryofocussing trap during the decompression step. Experimental conditions used were 70°C, CO_2 density of 0.8 g ml^{-1} and extraction time of 4 min. The maximum extraction efficiency was reported to be only 65%.

4. Biological matrices

The rapid and accurate measurement of ultra-trace levels of drugs and their metabolites in biological matrices plays a major role in the pharmaceutical development process. These measurements of drugs and metabolites provides information for the mechanism of action in pharmacology and toxicology studies, as well as for clinical development [22].

4.1. Human and animal tissues

Ramsey et al. [23] reported using supercritical CO_2 to extract four veterinary drugs, trimethoprim, hexestrol, diethylstilbestrol and dienestrol from freeze dried pig-kidney. The use of on-line SFE-SFC-MS was demonstrated for qualitative analysis of the drugs from the matrix. Portions of powdered kidney (1 g) were mixed with methanol (10 ml), spiked with relevant amounts of each drug, sonicated for 1 h to allow homogenous mixing and then the solvent was dried under a stream of nitrogen. Extractions were carried out, using 100% supercritical CO_2 for 8 min followed by 20% methanol-modified supercritical CO_2 for

0.5 min at a flow rate of 4 ml min^{-1}. During the extraction, drugs were retained in an amino-bonded column while the endogenous material was passed to waste. The eluted drugs were detected with high specificity by tandem MS. The authors concluded that even though the method showed great potential, the detection limits were not sufficient to meet the stringent controls on drug residues in meat for human consumption.

The use of SPE columns as a quantitative trapping device was demonstrated by Maxwell et al. [24]. Three nitrobenzamide antimicrobial drug residues were extracted at 690 bar at expanded gas flow rates of 3–4 l min^{-1} from fortified liver. Residues were eluted from the SPE column by off-line analysis. An integral metering valve-collector assembly reduced the loss of trace analytes in the tissue.

The solubilization of 5 veterinary sulpha drugs in supercritical fluid media and their extraction from spiked chicken liver and swine muscle samples was investigated by Cross et al. [25]. Supercritical CO_2 modified with 10–20% methanol was required and the authors concluded that increasing extraction pressure, time and % modifier all facilitated greater solubilisation, as does the use of small-diameter extraction cells. The effects of temperature varied from drug to drug, but in all cases recoveries were maximized at low temperatures. It was found useful to immobilise the water in the animal tissue by the addition of diatomaceous earth to the extraction vessel. The authors reported recoveries in the mid 90% range at moderate pressures in less than 1 h, with very little variation between the set of related sulphonamides. Greater solubilization of these drugs could probably be achieved with increasing severity of extraction conditions.

Edder et al. [26] demonstrated the application of SFE for extracting codeine, ethylmorphine and morphine in hair of drug addicts followed by GC-MS detection. A sample (50 mg) of pulverised hair was extracted with CO_2:MeOH: triethylamine:H_2O (85/6/6/3) at 0.7 ml min^{-1} and 40°C for 30 min. After extraction, the solvent was evaporated and the samples were treated with 100 μl of pyridine and 100 μl of propionic anhydride at 60°C for 30 min. The solvent was evaporated and the residue was dissolved in ethyl acetate ready for analysis using GC-EIMS. The detection limit of GC-EIMS was determined to be 100 pg mg^{-1}. The recovery obtained for morphine spiked with ^{125}I was 93.5%. The precision of the method was assessed to be between 3–12% RSD based on five replicates. Comparisons of SFE results with those obtained with other extraction techniques were also presented.

4.2. Blood plasma and serum

Liu and Wehmeyer [27] demonstrated the use of SFE for the direct extraction of drugs from plasma prior to analysis. The supercritical fluid was passed directly through the plasma samples spiked with either a neutral (flavone) or an acidic drug (ketorolac). The addition of an antifoam agent to the plasma, prior to extraction, was necessary to avoid restrictor plugging caused by denaturising of the plasma proteins by the supercritical fluid. The effluent from the extraction cell was bubbled through a small volume of methanol or into an empty tube to trap the extracted drug. The effect of extraction pressure and time on absolute recovery was investigated. The optimum extraction conditions used were as follows: pressure 30 MPa, temperature 60°C and an extraction time of 20 min. For a spike level of 25 ng ml^{-1} of [^{14}C]flavone and [^{14}C]ketoflavone recoveries obtained averaged 98% (RSD of 5.2%) and 80% (RSD 11.08%), respectively and for spike levels of 100 and 250 ng ml^{-1} of [^{14}C]flavone the recoveries obtained averaged 85% (RSD 5.8%) and 87% (RSD 3.8%), respectively. The authors concluded that the absolute recovery, selectivity, precision and accuracy of SFE was comparable to that obtained by conventional liquid-liquid extraction.

SFE coupled with SPE using octadecylsilane cartridges has been subjected to selective isolation of ultratrace levels of a drug metabolite, mebervine alcohol, from dog plasma [28]. In these studies dog plasma was applied directly to the extraction cartridge and the cartridge washed to remove protein and then extracted under supercritical conditions using 5% methanol-modified supercritical CO_2. The extracted metabolite of

mebeverine, mebervine alcohol (MEBOH), was trapped by bubbling the effluent from the extraction cell through a small amount of 2-propanol. The effects of extraction pressure and temperature on analyte recovery were examined. [^{14}C]MEBOH was used to examine the absolute recovery of the combined SPE-SFE approach. The addition of amine modifier to the fluid phase was required to aid extraction. For spiked levels of 50 ng ml^{-1}, [^{14}C]MEBOH recoveries averaged 95%. At the 10 ng ml^{-1} spike level, recoveries dropped to 83%. It was reported that these extraction conditions also eluted extraneous material from the SPE cartridges. The accuracy of SPE-SFE was considered to be comparable to conventional SPE methods. So, even though the SPE-SFE procedure was similar to the SPE method, the reduced solvent consumption by utilising the supercritical fluid for elution of the analyte from the cartridge may be beneficial. However, although the SPE-SFE method is also less time-consuming, the SPE method is probably a better choice in terms of cleaner extracts and improved extraction recoveries obtained.

Ndiomu and Simpson [29] reported the extraction of morphine, spiked at 200 μg ml^{-1}, from freeze dried serum samples. Recoveries of 96.7% with an RSD of 3.2% ($n = 10$) were obtained compared to 92.2% for SPE. In the same study, the authors also reported the successful analysis of morphine in placental samples.

Karlsson et al. [30] investigated the application of SFE to the recovery of budesonide from a blood plasma matrix. The plasma was spiked with [^{3}H]budesonide and a 500 μl subsample was deposited onto a filter paper in the extraction thimble. Extractions were with supercritical CO_2 only at 110°C for 30 min. The extracted analytes were collected onto a ODS bonded column. After extraction, the trap was washed with MeOH at 40°C. The budesonide extracts were quantified using a liquid scintillation counter and the average recoveries reported were $> 80\%$.

4.3. Plant material

Ndiomu and Simpson [29] demonstrated the potential of SFE for extracting drugs, such as quinine, from plant material. The level of quinine in lemon grass and dogonyaro leaves (types of herbs) was found to be approximately 10 μg g^{-1}. The same authors [29] also reported the extraction of caffeine from kola nuts. A range of high pressure/temperature extractions were carried out using tetrahydrofuran (THF), MeOH or CO_2 and compared with Soxhlet extractions using THF or MeOH. It was found that the highest recoveries were obtained using THF or MeOH, irrespective of the pressure/temperature. The operating pressure for these two solvents was 100 psi (6.8 atm) and a temperature of 135°C. However, for speed of extraction, high pressure/temperature extraction using MeOH gave the highest recovery (99.9%) in 30 min. This is probably an early example of the so-called accelerated solvent extraction method.

The SFE of taxanes as anticancer drugs from dried needles of the English yew tree *Taxus baccata* was demonstrated by Heaton et al. [31]. The optimum extraction conditions for the extraction of taxicin were 10% methanol-modified supercritical CO_2 at 50°C and 400 atm for 100 min. The extraction efficiency of SFE (666 mg kg^{-1}) was reported to be comparable with liquid solvents (800 mg kg^{-1}); methods were developed for quantitative and qualitative monitoring of the extracts.

Ma et al. [32] demonstrated the extraction of volatile components in Chinese herbal medicines. Three kinds of herbs, frankincense, myrrh and *Evodia rutaecarpa* were extracted using supercritical CO_2. The optimum extraction condition of 20 MPa and 50°C for 40 min extraction time yielded good extraction efficiency and selectivity, especially for the high molecular weight and oxygenated components. SFE was described as a useful alternative to conventional solvents for the study of medicinal plants.

Liu et al. [33] investigated the application of SFE to extract diosgenin, used as a steroid intermediate, following acid hydrolysis from tubers of *Dioscorea nipponica*. Samples of 0.05 g were extracted using supercritical CO_2 only over a range of temperatures and pressures for different periods of time. Diosgenin determinations were carried out using capillary GC of trifluoroacetate

derivatives. The highest recovery was achieved at 3100 psi and 44°C for 70 min, but > 82% of this yield was extracted after 40 min. The yield was 33% lower using conventional light petroleum extraction.

Quantitative extraction of taxol and baccatin III from the needles of *Taxus cuspidata* was demonstrated by Chun et al. [34] Using a continuous flow-through cell, ground needles could be extracted using 3% (w/w) ethanol-modified supercritical CO_2 at 40°C and 300 bar. The crude extract was analysed by HPLC and monitored at 230 nm. Similarly, ground Zingiber zerumbet rhizomes, used in Indonesian traditional medicine, were extracted using supercritical CO_2 at 60°C and 200 bar for 120 min [35].

4.4. Fermentation broths

The potential for selective SFE from a fermentation broth was evaluated by Larson and King [36]. This was demonstrated for the extraction of mevinolin, a metabolite of the fungus *Aspergillus terreus*, from a freeze dried fermentation broth. The solid sample was placed in the extraction cell and extracted using CO_2 and modifiers (MeOH and acetone) at various pressures. A 3% methanol-modified supercritical CO_2 combination was the most effective for mevinolin and its hydroxy acid derivative (L-154 819). In this case, the purity of the sample decreased from 1.83 to 0.3% after extraction, indicating the selectivity of the supercritical fluid to remove mevinolin and its hydroxy acid derivative. Further work, using acid or base co-solvents, indicated that the recovery of mevinolin could be altered. For example, the presence of acetic acid substantially increased the recovery of mevinolin whereas the addition of base (*t*-butylamine) substantially lowered its recovery. These results are consistent with the known interconversion of mevinolin and its derivative. This is further evidence that both selective extraction and simultaneous reaction can occur under supercritical conditions. Reactions under supercritical conditions has been investigated by many authors, see for example [37].

4.5. Milk products

Vitamin K1 (phylloquinone) has been extracted [38] using supercritical CO_2 at 8000 psi and 60°C from commercial soy protein and milk based powered infant formulas. Quantitative extraction required only 15 min and extracts were free of co-extractives from the lipophilic materials. Recovery of vitamin K1 obtained from the milk-based powered formula was 95.6% with an RSD of 7.4%, and those from a soy protein-based product, 94.4%, with an RSD of 6.5%. Application of this method to liquid formulae by using a larger extraction chamber packed with Chromosorb W, to which 7 ml of liquid product was added, proved unsuccessful.

5. Miscellaneous

Hedrick and Taylor [39] reported a qualitative study for the direct extraction of the hydrochloride salts of triprolidine and pseudoephedrine from an aqueous matrix using a modified extraction cell. The analytes were found to be insoluble in supercritical CO_2. A molar excess of tetrabutylammonium hydroxide was added to 3 ml of a 1 mg ml^{-1} solution of both compounds in order to extract the free bases formed using supercritical CO_2 only. Qualitative static extraction results showed that the free bases were extractable direct from water and that subsequent chromatographic separation and analysis could be achieved under supercritical conditions.

The use of Freon-22 in supercritical fluid extraction and chromatography was demonstrated by Li et al. [40], for the extraction of a series of steroids. The extraction efficiency for the seven steroids (estrone, estriol, testosterone, 17, α-methyltestosterone, 17, α-hydroxyprogesterone, cortisone and hydrocortisone) with Freon-22 was found to be significantly better with shorter extraction time compared to supercritical CO_2. However, the impending withdrawal of chlorinated solvents rather negates any advantages that may be offered by Freon-22.

As part of any form of method development in SFE it is often advantageous to assess the solubil-

ity of the analyte in supercritical CO_2. While this does not allow for subsequent matrix effects that might prevent quantitative analysis fundamental solubility data is a useful guide. A limited study on ibuprofen, a non-steroidal anti-inflammatory drug, was reported by Khundker et al. [41], while the same group [42] determined the solubility of ten steroids in supercritical CO_2 over a range of temperatures 35–100°C and pressures (84–231 kg cm^{-2}). The steroids investigated were testosterone, testosterone-17-propionate, beclomethasone-17,21-dipropionate, β-methasone-17,21-dipropionate, megestrol acetate, cortisone acetate, β-methasone, hydrocortisone, prednisolone and prednisone.

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