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The use of supercritical fluid extraction for sample preparation of pharmaceutical formulations

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Abstract

This paper reviews the use of supercritical fluid extraction (SFE) as a sample work-up step in the analysis of drugs in various pharmaceutical formulations. Matrices studies include tablets, animal feed, creams, ointments and infusions. As in other fields of analytical chemistry, SFE has proven to be most suitable for comparatively non-polar compounds in solid matrices. Examples are given however where SFE can also be used, with success, for polar substances or for target compounds present in infusions or other water-based samples. The premise of inverse SFE, i.e. extraction of the matrix instead of the target compound, is discussed.

Keywords: Degradation products; Drugs; Pharmaceutical formulations; Supercritical fluid extraction

1. Introduction

Supercritical fluids offer considerable promise as media for selective isolation of target compounds for complex matrices [1–3]. Carbon dioxide or carbon-dioxide-rich mixtures have been used almost exclusively as the extraction media, due to the inertness and non-toxic properties of the gas. Hence, the use of hazardous organic solvents can be kept to a minimum in the supercritical fluid extraction (SFE) procedure. The technique offers unique advantages since it combines liquid-like solvating capabilities with almost gas-like transport properties. Near-zero surface tension contributes to efficient penetration of porous materials. An attractive property is that solvent power may be tuned by mechanical means, i.e. by varying the temperature or pressure of the media. Most commonly, the pressure is varied, allowing the temperature to be kept low (<40°C), ensuring that no analyte degradation occurs during extraction. The ability to change the selectivity allows SFE to be used in both, for instance, extractions of bulk fat [4,5], as well as for selective recovery of target compounds present in fats [6]. Finally, SFE procedures are easily

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automated and interfaced to both chromatography and spectrometry.

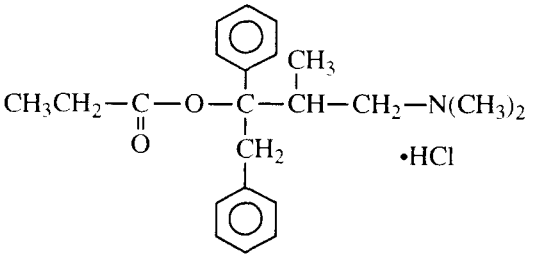
In spite of the obvious advantages of SFE, its use in sample preparation of pharmaceutical formulations has been rather limited. The main reason for this is that the target compounds in these matrices are often very polar, with many being readily soluble in water. Since supercritical carbon dioxide is non-polar in character, recovery of these compounds normally requires somewhat aggressive extraction conditions, in terms of either extreme pressure or temperature or, preferably, additions of a polar component (modifier) to the extraction fluid. The latter action could cause problems for the user, both regarding reproducible deliverance of a certain amount of modifier and in the subsequent analyte trapping process. Currently, however, these problems have been more or less solved and efficient and reproducible systems for the addition of modifier and for solute trapping are offered by SFE equipment vendors, thus enabling time-efficient processing of samples containing reasonably polar drugs and related compounds. For pharmaceutical products containing active compounds that are virtually insoluble in supercritical carbon dioxide or carbon-dioxide-rich mixtures, a better approach has recently been presented, where the matrix is removed from the analyte. This process, inverse SFE [7,8], has created possibilities for widening the field of applications within the pharmaceutical area.

The potential of supercritical fluid technology within the pharmaceutical industry is enormous, especially since the use of hazardous organic solvents will be limited through various world-wide regulations in the future. Some applications, as identified in the literature, are extractions of active compounds from natural materials [9], organic synthesis [10], production of crystalline materials [11,12], and preparative chromatography [13]. In this paper, attention is focused exclusively on the use of SFE as a sample preparation step in the analysis of pharmaceutical products. Early contributions in this particular field were reviewed previously by Messer et al. [14] and these papers will only be discussed briefly here. More specifically, this paper will review applications in four areas: (a) tablets and capsules; (b) animal feeds; (c) creams and ointments; and (d) aqueous matrices and infusions.

2. Applications

2.1. Tablets and capsules

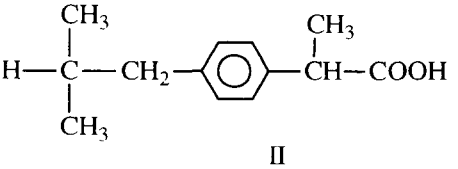
Early work by Andersen et al. [15] describes the use of a coupled SFE–supercritical fluid chromatography (SFC) system for qualitative analysis of propoxyphene hydrochloride (**I**): (Darvon®) tablets. 15 mg of tablet was extracted at 50°C with carbon dioxide at 400 atm. Extracted components were cryotrapped on a capillary SFC column held at −70°C. In the subsequent SFC separation, major peaks were identified as propoxyphene,



I

propoxyphene hydrochloride, caffeine, and aspirin, along with binders and excipients used in the tablet formulation.

Ibuprofen tablets were extracted using carbon dioxide in a sample preparation accessory at 5000 psi and 50°C [16]. A heart-cut from the stream exiting the extraction chamber was injected into an aqueous reversed-phase LC mobile phase for qualitative analysis. The tablets were ground using a mortar and pestle before being placed in the extraction vessel. More recently [17] ibuprofen (**II**)



II

spiked onto Celite was extracted with pure supercritical CO_2. The optimum conditions were stated

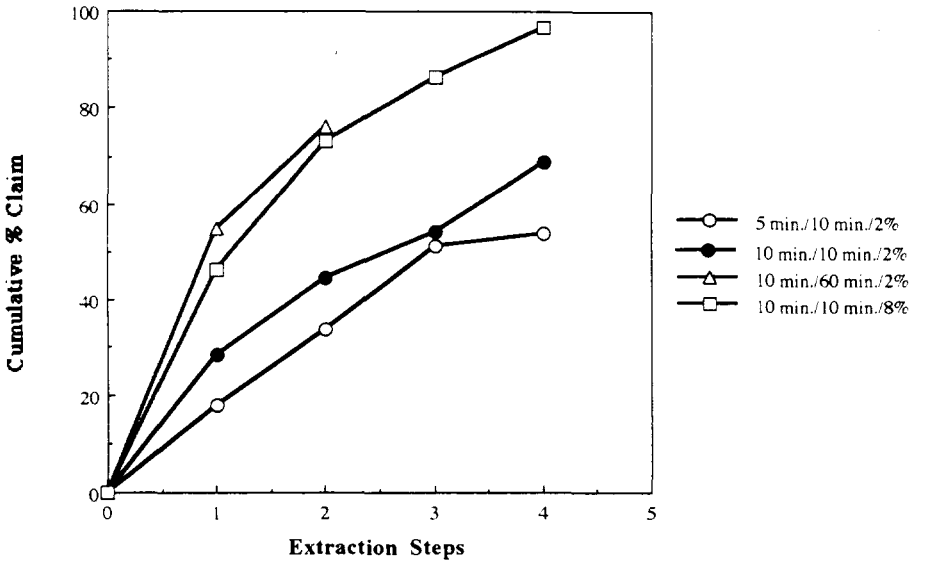
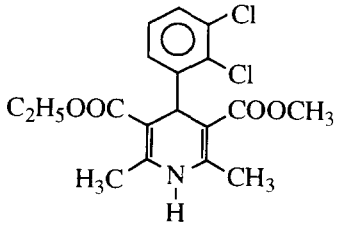


Fig. 1. SFE profile of felodipine tablets using static/dynamic made extraction and methanol-modified CO_2. Profile notation is given in the form static extraction time (min)/dynamic extraction time (min)/percent (w/w) methanol modifier: (A) 5/10/2; (B) 10/10/2; (C) 10/60/2; (D) 10/10/8. Each point on the plot represents a single static/dynamic step.

to be 70°C and 0.7 g ml^{-1}. A very high spike level was used in this study (i.e. 100 μl of 50 000 ppm solution or 5 mg of solute). The authors noted that the extraction of this analyte in different pharmaceutical matrices presented recovery problems.

More recently, Howard et al. [18] used SFE for sample preparation of sustained-release felodipine tablets. The authors identified three criteria for quantitative SFE. The analyte(s) must be (1) soluble in the supercritical media, (2) accessible to the extraction fluid, and (3) "trapable" after the extraction step without losses. To acquire information regarding solubility and trapping characteristics, experiments with felodipine-spiked inert matrices (cotton balls) were performed. This material was extracted at 45°C and 316 atm, but in two steps, each consisting of 2 min static and 20 min dynamic extraction periods, corresponding to a total extraction time of 44 min. In the static mode the system is pressurized, but there is no net flow of extraction fluid through the extraction chamber, whereas fresh supercritical fluid passes through the sample continuously in a dynamic extraction. During collection the trap was set at 0°C. Trap rinsing was done with two 1.4 ml aliquots of methanol at 45°C. SFU-UV was used for quantitative determination of felodipine (**III**)



III

in the extracts [19]. With this procedure, drug recoveries of 99% were reported, indicating that criteria (1)and (3) described above were fulfilled. Poor recoveries were however obtained when the same extraction scheme was applied on felodipine tablets. Only 30% of the target compound was extracted during a 75 min extraction, suggesting that there were problems with criterion (2) described above. The recovery increased to 64% for a crushed tablet. More aggressive conditions, in terms of increasing the pressure to 450 atm or raising the temperature to 80°C failed to produce complete recoveries of the target compound.

Table 1
Solvent usage comparison for analysis of felodipine: packed column SFC vs. HPLC

Parameter	Packed column SFC-UV	Analytical scale HPLC-UV
Mobile phase	6% (v/v) methanol-modified CO_2	Acetonitrile–methanol–50 mM phosphate buffer (pH 3) (40:20:40, v/v/v)
Samples analyzed per h	10 (6 min run time)	4 (15 min run time)
Mobile phase used per h (ml)	120	90
Disposable waster per h (ml)	7.2	90
Mobile phase disposal cost per 55 gallons ($)	48[a]	175[a]

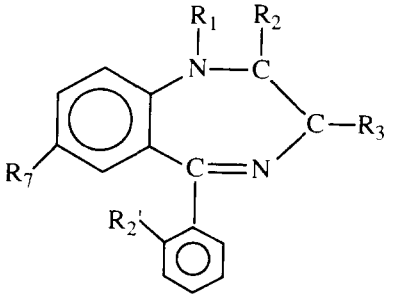
[a] Disposal costs obtained from Solid Waste Management, Merck Research Laboratories (West Point, PA)

Hence, a methanol-modified carbon dioxide phase was used. Good cumulative recoveries (102.1% and 96.3% claim) were reported when using 2.4% (v/v) and 10% methanol-modified carbon dioxide respectively as the extraction fluid at 450 atm and 80°C. The lower recovery for the 10% methanol-modified fluid was assigned to poor solid-phase trapping as a result of either aerosol formation or condensed methanol elution of analytes from the trap column. The extraction profiles, i.e. plots of recovery versus extraction time, obtained for these two extraction fluids were of a similar shape, as was the case for the profile obtained if the 2.4% (v/v) phase was employed at 45°C. Felodipine degradation was expected to be less at 45°C than at 80°C. Although cumulative recoveries were good, the recovery (92.6% claim) for a single 90 min extraction at 45°C was considered not to be quantitative by the authors. Next, extraction strategies using combinations of static and dynamic extraction steps were explored. Four different extraction schemes were investigated, varying the lengths of the extraction periods as well as the amount of modifier in the extraction fluid. Best results were obtained for the strategy using four 10 min static and 10 min dynamic extraction steps, while using a modifier concentration of 8%, as can be seen in Fig. 1.

These conditions were indeed successful in obtaining quantitative felodipine recovery from a tablet. The results compared favorably with data obtained from a liquid extraction method in terms of precision and degradation during work-up. Interestingly, a comparison between the two methods regarding solvent usage and disposal costs was made (Table 1).

As expected, the SFE method was parsimonious in terms of solvent usage. Disposal costs could also be cut considerably due to the fact that no organic/aqueous waste mixtures were used.

Lawrence et al. [20] reported some preliminary



IV

work on the qualitative extraction of several benzodiazepines (**IV**) from solid dosage forms. The SFE extracts were further processed by GC–MS or FT–IR according to Table 2.

Only products with dosage units of over 1 mg per tablet could be analyzed with FT–IR. The SFE step was performed usng pure carbon dioxide at 65°C and 100 atm. The extraction program, of 15 min duration, consisted of a 5 min static step followed by a 10 min dynamic phase. Extracted components were collected in methanol which was evaporated prior to further analysis. The tablets or capsules were ground using a mortar and pestle and dispersed in Hydromatrix™ prior to SFE processing.

Table 2
Dosage forms of manufacturers and instrumental analysis performed on drugs tested[a]

Benzodiazepine	R_1	R_2	R_3	R_7	R_2'	Source	FT-IR	GC–MS
Clorazepate	–H	=O	$-COO^-$	–Cl	–H	Abbott (Tranxene, 7.5 mg)	No	Yes
Diazepam	$-CH_3$	=O	–H	–Cl	–H	Roche (Valium, 5 mg)	Yes	Yes
Oxazepam	–H	=O	–OH	–Cl	–H	Wyeth (Serax, 30 mg)	Yes	Yes
Prazepam		=O	–H	–Cl	–H	Parke-Davis (Centrax, 30 mg)	No	Yes
Temazepam	$-CH_3$	=O	–OH	–Cl	–H	Sandoz (Restoril, 30 mg)	Yes	Yes

[a] See **(IV)**.

Tablet dosage forms containing vitamins A and E usually include among the exipients a protective carbohydrate/protein matrix to reduce degradation of these fat-soluble vitamins [21]. The potential of SFE for the isolation of vitamins A and E and their esters from tablet matrices has been reported [22]. The powdered tablet was loaded into a 3 ml extraction vessel with chromatographic-grade sea sand. Extractions were carried out in the dynamic mode with pure CO_2 for 15 min at a pressure of 250 atm and a temperature of 40°C. Trapping was accomplished with a glass vial that contained 6 ml of tetrahydrofuran maintained at 0°C. The restrictor was held above the liquid solvent and was maintained at 60°C. The decompressed CO_2 flow rate was 190–220 ml min^{-1}. Table 3 summarizes the assay results for the vitamins in four commercial dosage forms extracted by SFE. The data presented confirm the precision of the SFE method and show compliance with the label claim. Frequent column cleanup is required by the previously reported liquid extraction method to remove strongly retained substances interfering with successive analyses. This was not found to be the case with sample preparation by SFE [23] which indicated that the technique afforded enhanced selectivity due to the reduced solubility of interfering formulation excipients in supercritical CO_2 at 250 atm and 40°C.

In a related study One-A-Day™ brand vitamins have been subjected to SFE [24]. Only vitamin E (α-tocopherol) acetate **(V)** was efficiently extracted with CO_2. Vitamin A (retinol) acetate **(VI)** and vitamin D (cholecalciferol); **(VII)** were not effectively extracted even with the addition of a modifier such as ethanol or isopropanol. Using CO_2 alone, only 0–5% vitamin extraction recoveries were obtained. With the addition of 10% methanol as a modifier, recoveries improved to 30–40%. Vitamins A and D are present in the multivitamin tablet as the core constituents of gelatin microbeadlets. For effective extraction, it was necessary for the carrier fluid to permeate the gelatin shell. Maceration of the powdered tablet with dimethyl sulfoxide before placing it into the extraction chamber resulted in an increase in vitamin recoveries to 60%. Preliminary extraction of

Table 3
Assay results for vitamins A and E esters in commercial tablets extracted by SFE

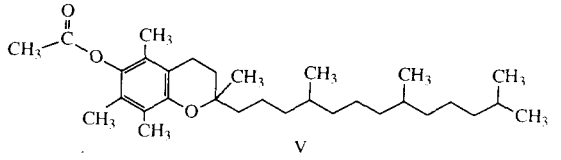
Formulation	Tablet mass (mg)	Label claim	% Found[a]	RSD[b]
Product 1	1000	70 mg of vitamin E acetate	97.8	3.9
		16.5 mg of vitamin A palmitate	110.1	1.7
Product 2	1100	100 mg of vitamin E acetate	97.9	1.5
Product 3	450	17.2 mg of vitamin A acetate	108.2	2.2
Product 4	332	2.2 mg of vitamin A palmitate	102.9	2.9

[a] Each value is the mean of six determinations; percentage of label claim found.
[b] Assays obtained on independent preparations.

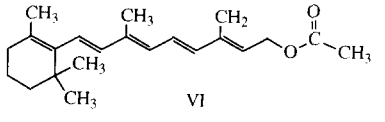
Flintstones™ Complete Children's Chewable Vitamins revealed large quantities of solids attributable to the flavoring agents which led to plugging and/or saturation of the collection trap.

2.2. Animal feed

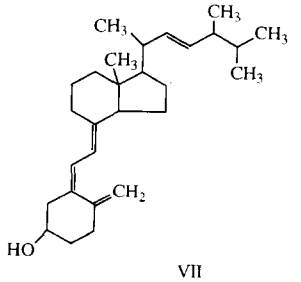
An early contribution to this field was reported by Schneiderman et al. [25] who developed an SFE method for the extraction of menadione (vitamin K_3) from spiked rat chow. The extracts were analyzed using LC with electrochemical detection. Initial studies showed that target compound recoveries above 90% were obtained withpure carbon dioxide at 8000 psi and 60°C. A static extraction step of 20 min duration was employed, followed by trapping on a silica gel column. These parameters were also successfully applied in the extractions of the rat feed, although analyte adsorption effects were observed, causing the relative recovery of small samples to be unsatisfactory. Preliminary work regarding the optimization of a method based on SFE for the extraction of a corticosteroid (tipredane) from rodent diet was reported by Euerby et al. [26]. The diet sample was spiked with the target compound at a level of 10 ppm prior to analysis. Sample (0.7 g) was loaded in the extraction vessel and extracted for 2 min. Collection of the analyte was performed in a flask containing 1 ml of methanol. Investigations were carried out to interpret the influence of type and amount of modifier, extraction temperature, and total flow rate on the extraction recovery in the system. Using an extraction fluid consisting of 10% ethanol in carbon dioxide at pressures ranging from 150–200 bar, with the temperature set to 70°C and the flow rate to 3.0 ml min^{-1}, recoveries in the range 75–95% were found.



V



VI



VII

Sauvage et al. [27] compared the extractability, with or without the addition of modifier, of two compounds of pharmaceutical interest in supercritical carbon dioxide and supercritical nitrous oxide. The samples investigated were rodent feed containing a halogenated aromatic derivative of urea (HAU) and dog feed spiked with a halogenated aromatic phenoxy derivative of an aliphatic alkane (HAPA). Dynamic extractions at 250 atm and 60°C with organic solvent collection were employed. HAU could be extracted using the unmodified extraction fluids, with the more efficient extraction occurring with nitrous oxide as

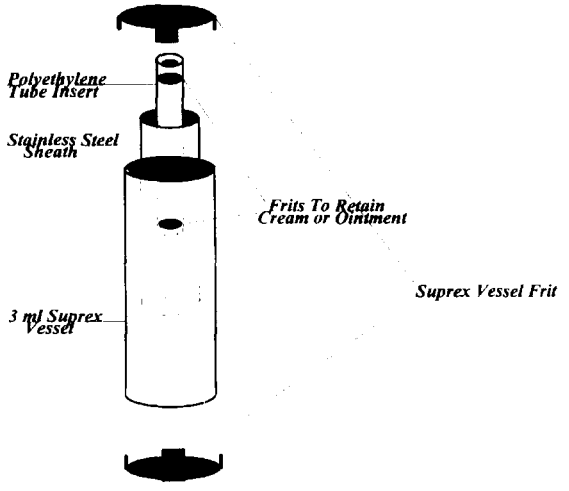
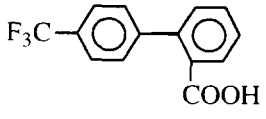


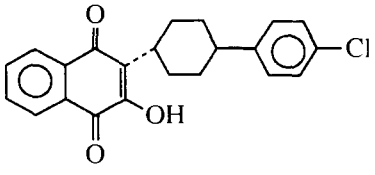
Fig. 2. SFE extraction vessel design for preventing analyte loss through mechanical transfer.

the carrier. Long extraction times (>2 h) forced the authors to add small amounts of modifier, up to 2.4% (v/v) of methanol or acetonitrile. With modified nitrous oxide the total extraction time could be cut to approximately 40 min. The other compound, HAPA, was readily soluble in the unmodified extraction fluids and complete extraction was obtained in 6 and 20 min for nitrous oxide and carbon dioxide respectively.



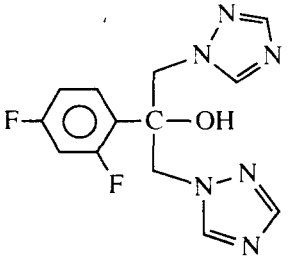
VIII

Messer and Taylor [28] applied SFE for the extraction of 4′-trifluoromethyl-2-biphenyl carboxylic acid (**VIII**) from a rat feed matrix. Pure carbon dioxide at 350 bar and 50°C was pumped at 2.0 ml min^{-1} using a static extraction of 0.5 min duration followed by a 30 min dynamic extraction step. Extracted components were collected on a solid phase trap, eluted with acetonitrile and assayed using an LC method. In initial experiments, the extractability of the pure analyte in supercritical carbon dioxide proved to be sufficient. When the spiked matrices were extracted, however, poor recovery and precision data were obtained (recovery: 68.8%, relative standard deviation: 20.6%). These values could be improved somewhat by modifying the spiking method (recovery: 89.2%, relative standard deviation: 11.2%), but they were still considered unsatisfactory by the authors. Matrix effects, e.g.adsorption of the target compound, were identified as a possible source. Addition of polar modifiers, no doubt, would have improved the situation considerably. In a similar approach,



IX

Messer et al. [29] extracted atovaquone (**IX**) (*trans*-2-[4-(chlorophenyl)cyclohexyl]-3-hydroxyl-1,2-naphthoquinone) from rat feed. A 2 min static extraction step followed by a 25 min dynamic extraction phase, both at 350 bar and 50°C, constituted optimized conditions. The target compound was collected on a trap packed with stainless-steel beads and rinsed out with acetonitrile, prior to determination by LC. The study involved extraction of the drug at six concentration levels from 0.0335% to 1.12% in the rodent feed matrix. Recoveries in the range 89.6%–103.5%, with acceptable precision data, were obtained for the various concentrations.



X

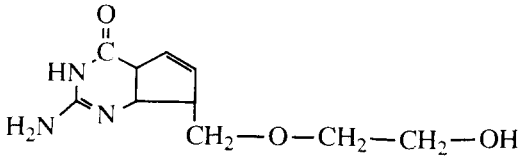
The application of SFE with pure CO_2 and-methanol-modified CO_2 for the determination of fluconazole (**X**) from an animal feed has been studied [30]. A fractional factorial design approach was used to examine the significant experimental variables for quantitative extraction. Modifier level proved to be the most important

factor. The extraction efficiency was determined at two levels: 10 g of drug per kilogram of rodent feed and 500 mg per kilogram of feed. The collection vial contained two cyanopropylpropyl solid phase cartridges in series to prevent any loss of fluconazole. The percentage extraction efficiency repeatability (20% methanol) for the isolation and cleanup of fluconazole from animal feed followed by GC with either flame ionization detection (FID) or mass spectrometric detection (MSD) detection was determined to be 87.0% (RSD = 8.4%) at the higher spike level and 91.0% (RSD = 13.2%) at the lower spike level for 10 replicates.

2.3. Creams and ointments

Masuda et al. [31] designed an SFE–SFC-UV system for quantitative analysis of retinol plamitate and tocopherol acetate in an hydrophobic ointment. The ointment was thoroughly mixed with 9 g of diatomaceous earth powder and 20 mg of this blend was placed in the extraction vessel. Extraction was performed using pure carbon dioxide at 200 atm and 40°C, pumped at 4.0 ml min^{-1} for 4 min. Trapping on a silica-gel material was achieved by reduction of the extraction fluid pressure. Target compounds were eluted from the trap using ethanol-modified carbon dioxide (10% v/v) but with temperature, pressure and flow rate settings remaining the same as in the extraction step. The contents of the analytes were calculated from their peak areas at 284 nm. Experiments on spiked samples resulted in complete recoveries for both compounds of interest. The authors claimed that their 10 min method is applicable to real preparations with an accuracy equivalent to that of the conventional method employing solvent extraction and HPLC.

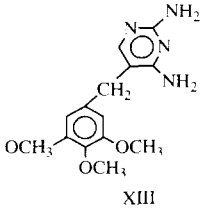
The first report on inverse SFE was published by Messer and Taylor [7] in 1994. In this paper five parameters were identified as being of importance for successful inverse SFE. First, the analyte must be insoluble in the supercritical fluid. Second, matrix components must be readily soluble in the extraction fluid. Third, an efficient method for the recovery of the nonextractable component is desired. Fourth, the authors claimed, the analyte concentration should be relatively high (>2%), allowing small samples to be processed. Fifth, a sensitive assay method for the recovered analyte should be available. In this particular application, a 100 mg Zovirax® ointment 5% sample was placed between the frits of a 1 ml empty solid phase extraction tube, which was used as an extraction thimble insert, as shown in Fig. 2. Extraction of the ointment at 500 atm at 50°C, using methanol-modified carbon dioxide (2% v/v) removed the carrier whereas the active compound, acyclovir (**XI**) remained



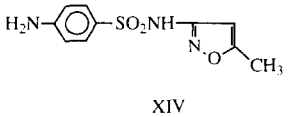
XI

in the extraction vessel. After some optimization of the post-extraction recovery method, the authors settled for sonication of the SPE tube in two 3 ml aliquots of 0.01 N NaOH. The extract was later analyzed using reversed phase LC-UV. Processing of three samples resulted in a mean recovery of 99% with an RSD of 5.3%.

The inverse SFE approach was also applied on Neosporin creams and ointments containing polymyxin B sulfate (**XII**) [8]. This drug repre-

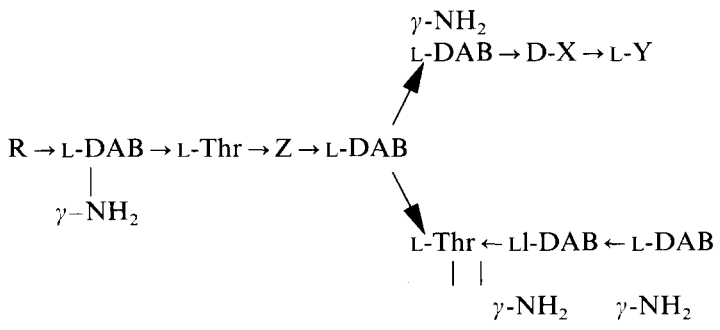


XIII



XIV

sents a class of about eight compounds, each with an individual molecular weight of approximately 1200u. Several polar amino acid functionalities make them virtually insoluble in supercritical carbon dioxide.



R = (+)-6-methyloctanoyl or 6-methylheptanoyl
X = leucine or phenylalanine
Y = threonine or leucine
Z = D-serine or L-DAB
DAB = α,γ-diaminobutyric acid
Thr = threonine

XII

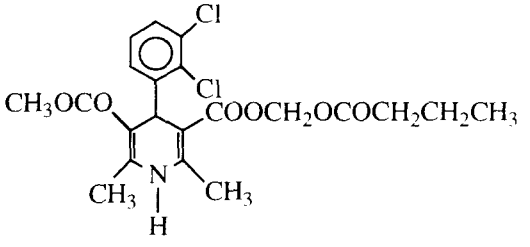
Experiments were designed to inversely extract and determine the amount of active compound in the Neosporin® cream. Cream (200–800 mg) was, as in the previous application [7], placed in an empty SFE tube which was placed in the extraction vessel. Extractions of the matrix were performed using pure carbon dioxide at various temperatures and pressures, whereas the polymyxin compounds were extracted by sonication for 15 min in 0.1 N HCl–methanol (75:25 v/v) with 0.1% Tween. An LC method with UV detection at 215 nm was used for extract analysis. Initial results were discouraging, however, with recoveries around 10%. Also, methyl paraben was not completely extracted along with the other matrix components. This proved to be an obstacle since methyl paraben interfered with one of the peaks of interest in the LC chromatogram. The latter problem was solved by adding 5% of methanol to the mobile phase at 500 atm and 60°C to completely remove methyl paraben. The former problem was eventually attributed to physical entrainment of the analytes in the stream of extraction fluid and could only be solved by making some changes to the sample loading design. A new construction was tested where the sample was squeezed between two layers of 200 mesh silica gel. This construction proved to work better. Using pure carbon dioxide at 300 atm and 55°C the average recovery, after post-extraction work-up, was 108% for six cream samples. However, for exhaustive extraction of the matrix components an extraction step employing 5% methanol-modified carbon dioxide at a density of 0.859 g ml^{-1} was required.

Analysis of the Neosporin® ointment samples was performed in a similar manner. Extractions were carried out at 450 atm and 60°C using 5% methanol-modified carbon dioxide. The authors reported a recovery of 137% relative to a SPE method. The high recoveries were interpreted as an indication that the inverse SFE approach was more efficient in isolating the target compounds.

2.4. Aqueous matrices and infusions

Hedrick and Taylor [32] used a system uniquely designed for aqueous matrices in an attempt to extract triprolidine and pseudoephedrine from water. The two drugs are both hydrochloride salts

with little or no solubility in supercritical carbon dioxide. A molar excess of tetrabutyl-ammonium hydroxide was added to the solutions in order to form the free bases, potentially soluble in the extraction fluid. An SFC analysis confirmed the presence of both drugs in the extracts, but no quantitative data were reported. The same extraction vessel design was also used to extract



XV

trimethoprim and sulfamethoxazole (**XIII** and **XIV** respectively) from Septra® Infusion samples [32]. Extractions were however far from quantitative, probably depending on low distribution coefficients of the polar analytes from water into supercritical carbon dioxide. Instead another approach was used where the infusion samples where first immobilized onto Celite and then placed in the extraction thimbles [33]. A 40 min dynamic extraction step using pure carbon dioxide at 329 bar and 60°C produced recoveries in the range 85–95% for the two compounds of interest.

Recent works by Järemo et al. [34] was devoted to extraction and determination of a dihydropyridine (**XV**) drug in emulsion infusion samples. The analyte was completely extracted using methanol-modified carbon dioxide (5% v/v) at 136 bar and 40°C. With an extraction time of 40 min and with the flow rate set to 4.0 ml min^{-1}, a recovery of 102% was obtained, in comparison with a traditional liquid–liquid extraction method. The repeatability for eight samples was excellent, with an RSD of less than 2%, including also the LC assay. In this application, special focus was put on the trapping and elution characteristics of the collection device. In the optimization of the trapping and elution steps, the authors identified two criteria. First, an efficient trapping in a narrow band at the lower part of the trap column (the stream of extraction fluid entering at the bottom of the trap) is desired. Second, it should be possible to elute trapped components with a small volume of solvent. For convenience, the authors wanted to use the mobile phase of the LC assay as the eluting solvent and with a volume preferably below 1.8 ml, i.e. the volume of the sample vials. To be able to combine adequate trapping with good elution characteristics a trap packed with ODS material and stainless-steel beads in a ratio of 1:4 was used. The smaller ODS plug closest to the trap inlet ensured good trapping of the target compound, whereas elution with 1.5 ml of mobile phase was indeed possible since there was little retention on the stainless-steel part.

3. Conclusions

For routine application in the pharmaceutical industry, there have been no significant advances in sample preparation technology in the last 50 years. Most solid dosage form extractions are still accomplished by liquid–solid extraction techniques. Isolation of the solvated drug and any soluble excipients from the insoluble excipients particles is then achieved by centrifugation, filtration and/or preparative micro-column chromatography. The drug solution from the sample preparation is then assayed by HPLC.

This liquid–liquid extraction procedure has many disadvantages. First, determination of degradate products, of growing concern to regulatory agencies, is significantly hindered by the high volume, low concentration sample solutions obtained by this procedure. Second, large amounts of disposable solvent waste are generated, the disposal of which is very costly. This is particularly true for organic/water mixtures since their value as fuel for combustion is low, thus, making their disposal costs high. Third, the above procedure requires much sample handling which can be both error-prone as well as hazardous to the laboratory worker in terms of contact with the drug substance and organic solvents.

SFE is one innovation in sample preparation that is currently being explored in the pharmaceutical industry. The extracts generated from this procedure are typically low volume, high concentration solutions, ideal for degradate analysis. The procedure is also automatable since both parallel (several samples extracted simultaneously) as well as serial (multiple samples extracted one after another) commercial SFE instrumentation are available. Liquid–solid extractions cannot be automated without robotics since sample preparation equipment (i.e. shaker, centrifuge) is not centralized on one unit as with SFE. In addition, hazards to the laboratory worker are reduced since SFE requires only one step for the laboratory worker (i.e. loading the sample in the vessel). Furthermore, the small amounts of organic waste generated reduces hazards as well as minimizing solvent procurement and disposal costs. The wide variety of pharmaceutical matrices which have been described in this review testifies to the broad application of SFE.

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