Pergamon

0731-7085(94)00071-9

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 10, pp. 1227–1232, 1994

0731-7085/94 $7.00 + 0.00

Analytical inverse supercritical fluid extraction of polar pharmaceutical compounds from cream and ointment matrices

W.N. MOORE and L.T. TAYLOR*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA

Abstract: It has been shown that inverse supercritical fluid extraction (SFE) can be used to analytically isolate a polar analyte from its matrix even at low concentrations (0.016%). Inverse SFE has been shown to be successful not only for the semi-solid Neosporin® ointments, but also for the semi-liquid Neosporin® creams as well. The technique has been shown to be superior to solid-phase extraction in both cases and affords the analytical chemist a quicker and safer method of sample preparation of analytes from both creams and ointments.

Keywords: *Neosporin®; supercritical fluid extraction; creams and ointments.*

Introduction

While sample preparation involving supercritical fluid extraction (SFE) has resided to a large degree in research laboratories, the technique is rapidly gaining recognition in the industrial sector. Supercritical fluids (SFs) offer many desirable properties such as near gas-like diffusivities, low viscosities, and near zero surface tension which allow a variety of matrices to be penetrated quickly affording several advantages including drastically reduced sample preparation time. Perhaps of most interest to industry, however, is the feasibility of the replacement of conventional liquid solvents with SFs. The most common SF, CO_2, possesses safe and nontoxic properties resulting in the elimination of health hazards and associated disposal costs.

As SFE gradually becomes the successor to many of the current methods of analyte separation and isolation that includes for example Soxhlet and liquid–solid extractions, several problems are recognized. Unfortunately, the method has been, and continues to be, limited to nonpolar and moderately polar compounds. While a variety of modifiers, additives, and coanalytes can be added to increase the polarity of the SF or solubility of the analyte in the SF, there still are a large number of compounds which remain insoluble and are, thereby, not extractable with CO_2 as the primary fluid. This problem is especially true in the pharmaceutical industry where many metabolites and water soluble compounds contain numerous polar functional groups and are not CO_2 soluble. Few applications of SFE to polar pharmaceutical compounds can be found in the literature. Some applications include the extraction of transdermal patches [1], the direct extraction of the active ingredient from Septra Infusion® [2], and extraction of drugs from various matrices including animal feeds, animal tissue and plants [1, 3–7].

A recent study involved the use of SFE to isolate a polar drug by extracting the drug carrier, a hydrocarbon based ointment, thereby leaving behind the analyte of interest. This process has been referred to as "inverse SFE" [8–10]. Although still in its early stages of development, the technique promises to have numerous applications in the pharmaceutical industry. Messer and Taylor [4] have identified five parameters which appear to play important roles in the outcome of "inverse SFE". First, the analyte must be totally in-

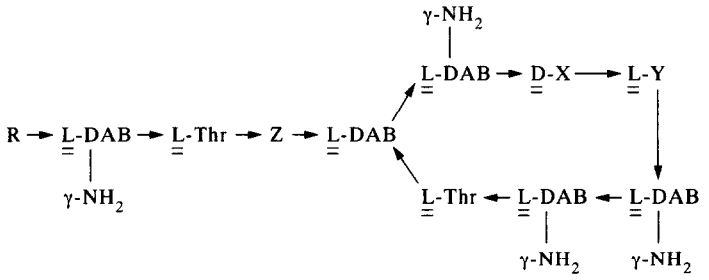
* Author to whom correspondence should be addressed.

soluble in the supercritical fluid. Second, the matrix must be soluble in the SF. Third, a highly efficient washing method must be used to transfer the analyte from the extraction vessel for analysis. Fourth, the analyte concentration in the matrix should be >2%. Fifth, an assay method with low detection limits for the analyte is advantageous. Messer reported quantitative recoveries with good reproducibility for the inverse extraction of acyclovir, the water soluble, active ingredient found in Zovirax® Ointment 5%.

The focus of this work was two-fold. One objective was to try and overcome the concentration limitation as defined by Messer. The second objective was to assess the feasibility of using inverse SFE as a way of isolating a polar drug from a cream matrix, which has near liquid-like properties, compared to the hydrocarbon based ointment which has properties closer to that of a solid. While it is important for quantitative reasons to work with concentrated drug formulations, concentrations of this magnitude are typically not encountered in the pharmaceutical industry. This work focuses on the isolation of an active ingredient polymyxin B sulphate from its cream carrier (Neosporin® Cream) in which the drug is present at 0.17%, and also from its ointment carrier (Neosporin® Ointment) in which the drug is present at 0.8% [10]. Polymyxin B sulphate represents a class of about eight compounds with the general structure shown in Fig. 1. Each individual polymyxin B sulphate compound has a molecular weight of approximately 1200 amu and consists of many polar amino acid functionalities which deem the compounds completely insoluble in supercritical carbon dioxide. The cream matrix consisted of methyl paraben, emulsifying wax, mineral oil, polyoxyethylene polyoxypropylene compound, propylene glycol, purified water and white petrolatum. The ointment is a less complex matrix consisting of a white petrolatum base. Both matrices have proven to have sufficient solubility in pure or modified supercritical carbon dioxide for exhaustive extraction. Supercritical fluid extraction has proven to be superior to the current solid phase extraction (SPE) procedure, with recoveries well over 100% relative to SPE and RSDs of 5% or less for $n = 6$.

Experimental

All extractions were performed on the Suprex Prepmaster (Suprex, Pittsburgh, PA, USA) along with a reciprocating modifier pump (SSI, State College, PA, USA). The extractor employs a dual head reciprocating pump which delivers the CO_2 as a liquid and is capable of achieving 500 atm (7.348 psi). Extraction vessels (Keystone Scientific Inc., Bellefonte, PA, USA) with 3 ml volume were employed for all extractions. An empty polyethylene SPE tube which served as an insert was placed within the vessel and was used to contain the sample to make sonication of the non-CO_2 extractables easier. The vessel configuration that was employed along with the



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

Figure 1
General structure of polymyxin B sulphate taken from *The Merck Index*, ninth Ed. Structures vary only by the R, X, Y or Z constituent.

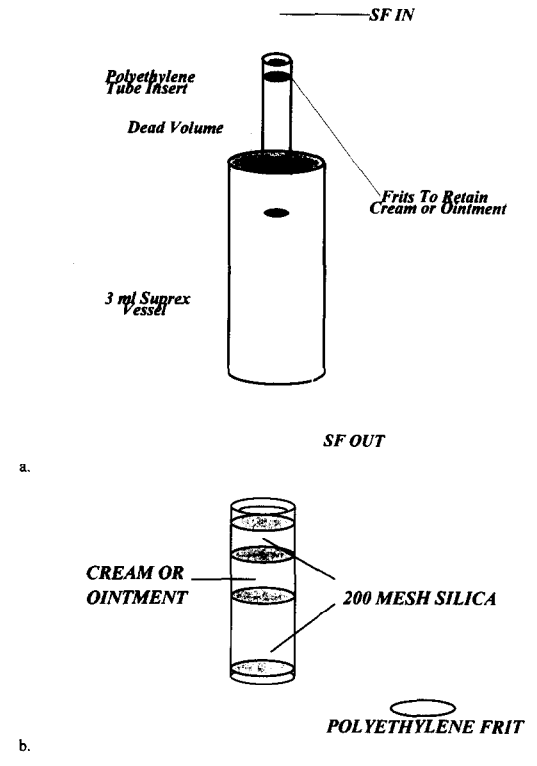


Figure 2
(a) Shows the vessel design which yielded the most efficient extractions; (b) is a diagram of the SPE tube used to contain the sample. As in (a) the flow of SF is downward.

direction of SF flow is shown in Fig. 2. The Suprex Prepmaster was modified slightly by disconnecting the solid phase trap from the flow path of the SF so that the extracted material and compressed fluid were decompressed at the restrictor into the trunk of a hood. The modified restrictor consisted of a plugged zero dead volume union which allowed the SF to be decompressed at a controlled rate. This setup was found to minimize plugging of the restrictor by the two types of matrices. Moreover, the restrictor employed was heated to 300°C by two high temperature heating cartridges to further discourage any plugging of the restrictor. SFE/SFC grade helium headspace CO_2 (Air Products and Chemicals Inc., Allentown, PA, USA) was used for all extractions. HPLC grade methanol (EM Science, Gibbstown, NJ, USA) was employed as the modifier.

Polymyxin B sulphate extracts were assayed by an HPLC method supplied by Burroughs Wellcome Company (Research Triangle Park, NC, USA). Analysis of the extracts were performed with isocratic elution on a 4.6 × 250 mm i.d. Synchropak SCD reversed-phase column (5 μm particle size and 100 Å pore size). The mobile phase consisted of 0.1 M potassium phosphate monobasic with 0.1% trifluoroacetic acid–acetonitrile with 0.1% trifluoroacetic acid (78.5:21.5, v/v). The mobile phase was filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA). The flow rate was 1.5 ml min^{-1}. A Hewlett–Packard (Avondale, PA, USA) 1050 series isocratic pump was used and connected to a Valco (Austin, TX, USA) model EQ-60 LC injector using a 50 μl loop. A Hewlett–Packard (Avondale, PA, USA) 1050 series ultraviolet detector was employed with detection set at 215 nm. A Hewlett–Packard model 3394A integrator was used. All Neosporin® samples and standards were provided by Burroughs Wellcome Company (Research Triangle Park, NC, USA). HPLC quantitation was by cumulative peak area (four peaks) relative to solid-phase extraction. The same tube of cream or ointment was used in comparative extractions as it was stated by Burroughs Wellcome that the tubes could vary in concentration from lot to lot. All samples were filtered prior to HPLC analysis through 0.2 μm filter unit.

The current sample preparation method for polymyxin B sulphate in Neosporin® Cream and Ointment is solid phase extraction on a silica SPE column which uses 1 g of sample and 100 ml of solvent per extraction. Hexane and ethyl acetate are employed as the solvents. In order to elute the polymyxin compounds from the silica stationary phase, 2 ml of 0.1 N HCl followed by 2 ml of a 50/50 mixture of 0.1 N HCl/methanol are employed. The extract is collected in a 5 ml volumetric flask and diluted to the mark. Typical extraction times are approximately 2 h for the solid phase extraction procedure. Following SPE, the compounds are then separated on the proprietary column given above. Typical extraction times can be seen in the chromatogram in Fig. 3.

Results and Discussion

This project began with an attempt to inversely extract and quantitate the amount of polymyxin B sulphate dissolved in Neosporin® Cream. The average concentration reported by Burroughs Wellcome Company for the cream was 1.66 mg of polymyxin B sulphate in 1 g of cream (0.17%). Sample sizes ranging from 200 to 800 mg were weighed into an empty SPE

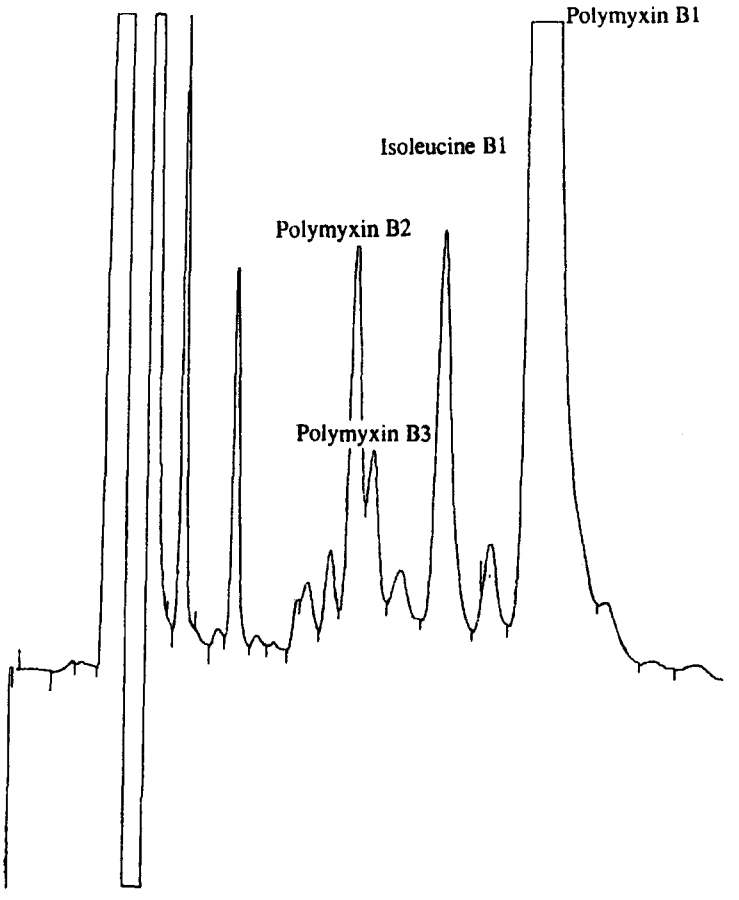


Figure 3
Typical HPLC chromatogram of the polymyxin compounds extracted from either the cream or ointment formulation. The four peaks of interest have been labelled for clarity.

tube and place within the stainless steel extraction vessel. Following extraction, the SPE tube was removed from the vessel, and the SPE frits within the extraction tube were removed and placed with the SPE tube into a 3.7 ml-vial and sonicated with enough solvent to result in a final concentration of 0.2 mg ml^{-1}. The solvent which solvated the polymyxin compounds most successfully was 0.1 N HCl–methanol (75:25, v/v) with 0.1% Tween 80 (polyoxyethylene (20) sorbitan monooleate) (J.T. Baker, Phillipsburg, NJ, USA). Sonication times were varied from 15 to 45 min, and it was found that 15 min was sufficient for an exhaustive washing. All extractions were attempted under a variety of 100% CO_2 pressures and temperatures, however, the results seemed to follow the same trend. In each case, the percentage recoveries of polymyxin B1, B2 and B3 were well below those found with solid phase extraction (≈10%), while the percentage recovery of the third peak of interest, isoleucine B1, was found to be well above that found with SPE (≈300%) (Fig. 4). These initial results were puzzling.

It was known that the cream, which consisted of numerous components, became a free flowing liquid between 65 and 70°C. While all extractions were performed below 65°C (45–60°C), it seemed reasonable that the polymyxin B sulphate compounds which were known to be completely insoluble in supercritical CO_2 could be undergoing physical entrainment or mechanical transfer through or around the SPE frits (50 μm pore size) during extraction of the matrix. This would explain the loss of the three polymyxin compounds, but the question remained as to how 300% recovery of a compound very similar in nature to the other three compounds could be achieved. Further investigation led to the discovery that methyl paraben, a preservative found in the cream, coeluted with the isoleucine B1 in the HPLC assay. The 100% CO_2

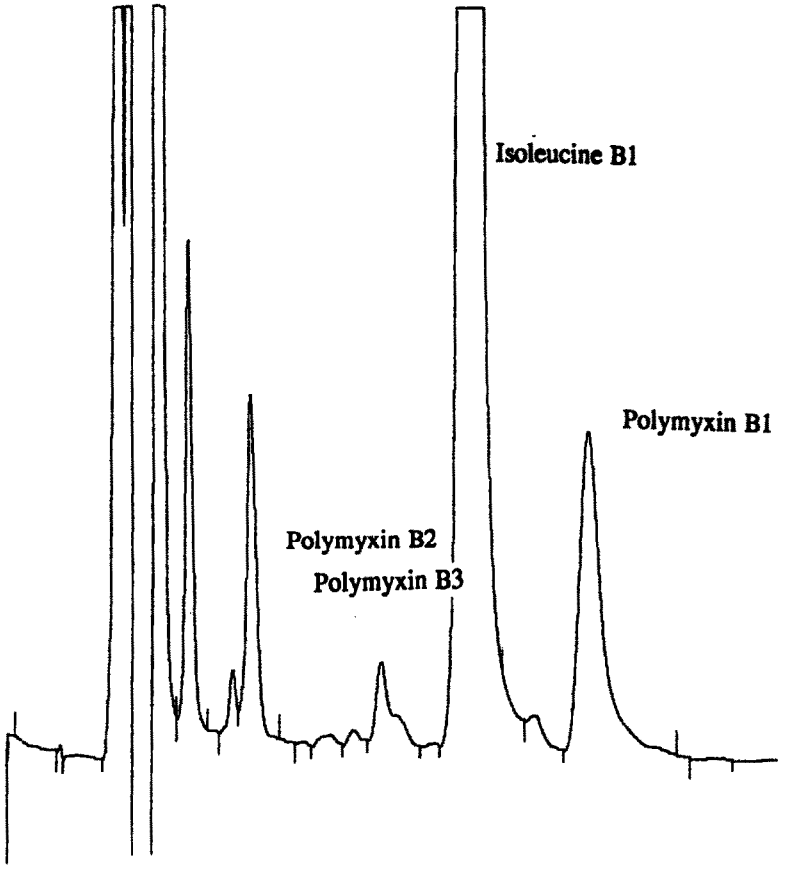


Figure 4
Chromatogram showing the decrease in peaks 1, 2 and 4, and the large increase in peak 3, isoleucine B1.

was then discovered to be unsuccessful at exhaustively extracting the methyl paraben within the extraction time. However, an extraction profile produced from data obtained at 500 atm and 60°C showed that methyl paraben could be removed from the extraction vessel in 30 min with 5% methanol modified CO_2 at a flow of 2 ml min^{-1}. Further extractions of the Neosporin® cream at these conditions proved to remove all of the methyl paraben from the cream, as well as the other cream components. However, recoveries of the four polymyxin B components continued to range from 5 to 15% when compared with the corresponding SPE results.

At this point there were several possible reasons which could explain the loss in recovery of the polymyxins. First, the analytes could still be undergoing some type of physical transfer out of the vessel. Second, the analytes could be reacting with the SF. This premise was considered highly unlikely due to the relative inertness of the CO_2. Third, the analytes could be degraded as a result of the sonication process. It seemed reasonable to assume that possibilities two and three were not responsible for the low recoveries, and in fact, experimental data showed this to be the case. Therefore, the focus was to eliminate the chance for physical entrainment of the analyte in the near-liquid cream.

Several extractions were carried out at relatively low temperatures (40–45°C) in order to prevent any melting of the cream. Also, various types of frit designs were tested. However, in both cases recoveries were still unsatisfactory and RSD approached 100%. Various lower pressures, lower temperatures, and lower flow rates were tested in hopes of preventing mechanical transfer, but to no avail.

It seemed clear at this point that some other vessel design was needed in order to keep the analyte of interest within the vessel while allowing the exhaustive extraction of the matrix components. A new design was tested in which the cream sample was sandwiched in between two layers of 200 mesh silica gel. The

goal of this design was to promote the selective trapping of the analyte of interest prior to supercritical fluid decompression, while allowing the cream to be completely removed from the system. This configuration was tested and found to result in an extremely efficient extraction. For the cream, at a pressure of 300 atm with an extraction temperature of 55°C and a flow rate of 2 ml min^{-1}, average recovery of the analyte of interest was 108% (n = 6) when compared to SPE, and RSDs of 5.0% were generated [10]. The sample size of Neosporin® Cream was approximately 200 mg, which results in 0.033% of polymyxin B sulphate. The extraction time required for exhaustive extraction of each of the matrix components was 75 min with 5% methanol modified CO_2 at a density of 0.859 g ml^{-1}. The restrictor was heated to 300°C, and 58.5 of CO_2 was used (69.8 ml). Following the extraction, the frits, silica gel and SPE tube were placed into the 3.7 ml vial and sonicated for 15 min. The sample was filtered and assayed by HPLC.

It seemed feasible that if 0.033% of the polymyxin B sulphate could be quantitated, then half as much (0.016%) found within the ointment could also possibly be quantitated. Other than the concentration obstacle this extraction should have been relatively simple as the only component of the ointment matrix was a white petrolatum base. This scenario was in fact the case. The extractions were carried out at 450 atm and 60°C. Again the flow rate was 2 ml min^{-1} and the extraction fluid was 5% methanol modified CO_2. The time required for exhaustive extraction was 45 min. The restrictor was kept at 300°C. The density of the SF was 0.913 g ml^{-1} and 71.6 g of SF (78.2 ml) was used. These conditions resulted in an average recovery of 137% (n = 6) for the ointment relative to the SPE method with RSDs of 1.9%. This high recovery indicates that SFE sample preparation is able to isolate the polymyxin components more effectively than the current method of SPE.

It should be noted here that an effort was made to eliminate the amount of dead volume surrounding the SPE tube in the extraction vessel by incorporating a stainless steel sheath. This design can be seen in Fig. 5. However, this modification resulted in decreased recoveries in both cases. It seems that the presence of the dead volume alleviates any physical movement of the analyte through the silica stationary phase by affording multiple pathways for the SF through the vessel and decreasing some of the pressure effects. Furthermore, the flow rate of the SF during the extraction did not seem to affect the recoveries.

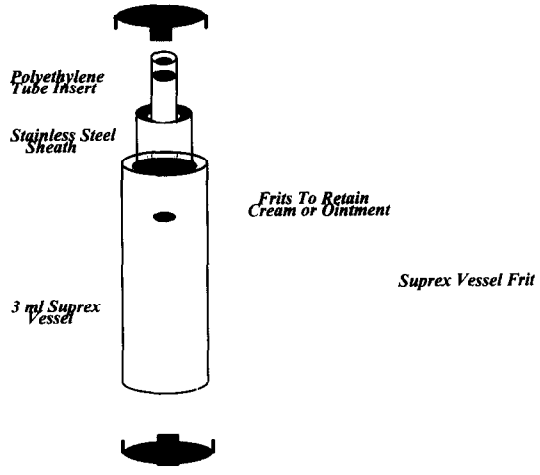


Figure 5
Vessel design in which the void volume surrounding the SPE tube was eliminated, resulting in a low recovery of polymyxin B sulphate.

Acknowledgements — We would like to thank Suprex Corporation for the use of their supercritical fluid extractor and also Air Products and Chemicals, Inc. for supplying the supercritical carbon dioxide. The financial support of Burroughs Wellcome Company is gratefully appreciated.

References

[1] B.E. Richter, A.F. Rynaski and J.L. Ezell, presented at The Fourth International Symposium On Supercritical Fluid Chromatography and Extraction, Paper no. 203, Cincinatti, OH, USA, May 1992.
[2] L.J. Mulcahey and L.T. Taylor, *Anal. Chem.* **64**, 2352–2358 (1992).
[3] D.C. Locke, A.K. Sharma and M.A. Schneiderman, *J. Chrom. Sci.* **26**, 458–462 (1988).
[4] D.C. Messer and L.T. Taylor, *J. High. Resol. Chrom.* **15**, 238–241 (1992).
[5] E. Sauvage, J.L. Rocca and G. Tuossanint, *J. High Resol. Chrom.* **16**, 234–238 (1993).
[6] E.D. Ramsey, J.R. Perkings, D.E. Games and J.R. Startin, *J. Chrom.* **464**, 353–364 (1989).
[7] R.J. Maxwell, O.W. Parks and E.G. Piotrowski, *J. High Resol. Chrom.* **15**, 807–811 (1992).
[8] D.C. Messer and L.T. Taylor. *Anal. Chem.* **66**, 1591–1592 (1994).
[9] D.C. Messer, L.T. Taylor, W.N. Moore and W.E. Weiser, *Therapeutic Drug Monitoring* **15**, 581–587 (1993).
[10] W.N. Moore, D.C. Messer and L.T. Taylor, presented at The Fifth International Symposium On Supercritical Fluid Chromatography And Extraction, Poster, Baltimore, MD, USA, January 1994.

[Received for review 11 March 1994; revised manuscript received 3 May 1994]