DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 18(2), 245-255 (1992)

COMMUNICATION

SUPERCRITICAL FLUID EXTRACTION - SUPERCRITICAL FLUID CHROMATOGRAPHY FOR ANALYSIS OF A PROSTAGLANDIN:HPMC DISPERSION

Daryl A. Roston
Searle Research and Development, 4901 Searle Parkway
Skokie, Illinois 6077

ABSTRACT

An on-line SFE-SFC system was used to extract and chromatograph a synthetic prostaglandin from hydroxypropyl methylcellulose (HPMC), which is a widely used material in controlled-release drug formulations. The only sample preparation for the on-line SFE-SFC analysis of the dispersion was weighing the sample into the extraction vessel insert. The extraction efficiency for a four-minute extraction was approximately 65%.

INTRODUCTION

The present communication portrays preliminary experiments, which demonstrate the potential

utility of on-line supercritical fluid extraction (SFE) -supercritical fluid chromatography (SFC) as an analytical method for pharmaceutical quality control. Quality control procedures for formulated drug substances often involve extensive sample preparation procedures such as liquid-solid extractions, to remove the drug from the formulation matrix. SFE-SFC methodology offers the potential to minimize sample preparation prior to analysis to determine the level of the drug in the matrix. Several previously published reports (1-13) have demonstrated that SFC and SFE have applicability in field of drug analysis; however, the studies have emphasized analysis of drug substances in clinical and biological matrices.

Preliminary efforts in our laboratory have have focused on use of SFE-SFC methodology for the analysis of drug formulations. The intent of studies in this area is the development of simplified, reproducible procedures for pharmaceutical quality control. An on-line SFE-SFC system has been employed to extract and analyze a 1:100 dispersion of the synthetic prostaglandin, misoprostol (methyl (11 ∝,13E)-(+/)-11,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oate) in hydroxypropyl methyl cellulose (HPMC). HPMC is a widely

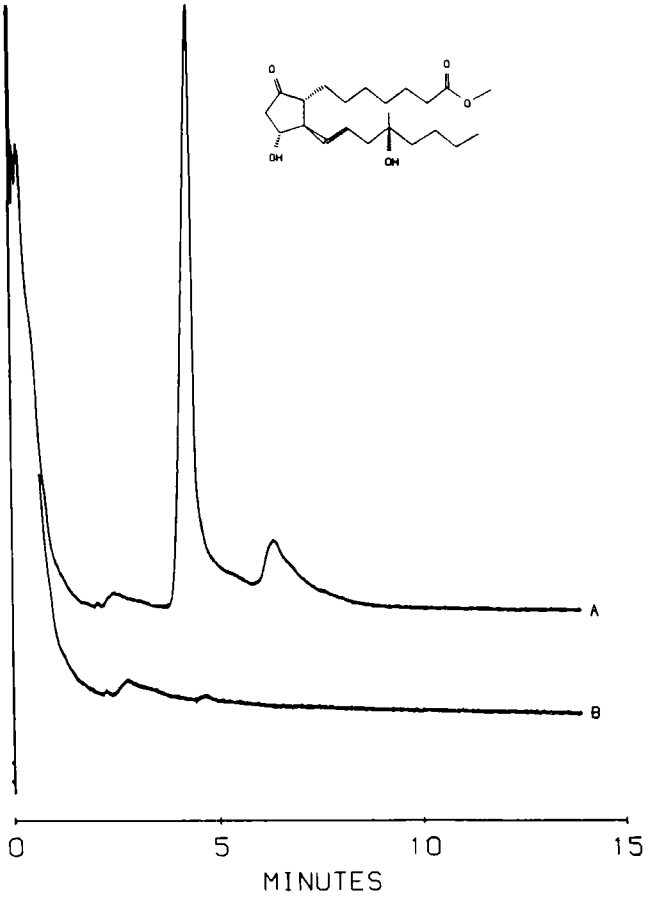

FIGURE 1. SFE-SFC chromatograms for misoprostol and extraction cell blank: A) misoprostol drug substance, B) cell blank.

used material in controlled release formulations. Misoprostol:HPMC dispersions have been characterized previously (14) and are part of the formulation of Cytotec (Searle), an antiulcer medication. The structure of misoprostol is in Figure 1.

EXPERIMENTAL

Instrumentation

All experiments were completed with a Lee Scientific Model 501 Supercritical Fluid Chromatograph and a Lee Scientific Model 501 Extraction System. A Linear Model 204 UVIS detector equipped with an SFC flow cell assembly was also used. The on-line SFE-SFC system used in the present study, including a schematic diagram, was recently described by Anderson et al (15). System restriction was provided at the outlet of the UV detector cell, with approximately 250 mm of 15 micron i.d. fused silica. The extraction cell volume was 0.35 milliliter. Direct injection experiments were completed with a rotary injection valve equipped with a 0.5 microliter loop.

Materials and Reagents

SFC grade carbon dioxide with 5% methanol was purchased from Scott Specialty Gases and used for all experiments. Misoprostol samples, misoprostol:HPMC (1:100) dispersion, and HPMC samples were provided by the Chemical Development Department of Searle Research and Development. The HPMC was originally purchased from

Shin-etsu Chemical. Particle size analysis for the misoprostol:HPMC dispersion yielded the following results: 170 mesh, 6.5%; 325 mesh, 35.9%; 400 mesh, 27.3%.

SFC and SFE-SFC Experiments

The SFE-SFC experiments were completed with a Brownlee Cyano column (200 mm x 1 mm i.d.) and UV absorption detection at 195 nm. Prior to initiation of SFE-SFC experiments, misoprostol drug substance samples were manually loaded into the extraction vessel insert. Drug substance samples were placed in the insert as a dilute solution of acetonitrile that was allowed to evaporate.

SFE-SFC experiments were comprised of three portions: extraction, equilibration for chromatography, and chromatography. During the extraction step, the supercritical fluid flows through the extraction vessel. Sample components are extracted and subsequently precipitated in the cryofocussing trap during decompression of the supercritical fluid. Experimental parameters for the extraction step were as follows: time, 0-4 minutes; selection valve in the extract position; cryogenic coolant (carbon dioxide),

on; cryofocussing trap temperature, approximately -60 degrees C; column oven temperature, 40 degrees C; extraction vessel, 70 degrees C; supercritical fluid density, 0.8 gm/ml. During equilibration, the flow of the supercritical fluid through the extraction vessel is ceased, the column is brought to the temperature required for chromatography, and the cryogenic coolant is turned off. Cessation of the cryogenic coolant flow allows the cryogenic trap chamber to equilibrate to the column oven temperature. Experimental parameters during equilibration were as follows: time, 4-6 minutes, cryogenic coolant, off at 5 minutes; oven temperature increased from 40 to 70 degrees C. Experimental parameters during chromatography were as follows: time, 6 to 20 minutes; the selection valve is switched to the column position (injecting extracted sample components on to the column); supercritical fluid density, 0.8 gm/ml; oven temperature, 70 degrees C.

RESULTS AND DISCUSSION

Figures 1 and 2 show SFE-SFC chromatograms for misoprostol drug substance and misoprostol:HPMC samples, respectively. Figure 1A records SFE-SFC chromatograms for approximately 14 micrograms of a research-grade misoprostol sample. The major peak

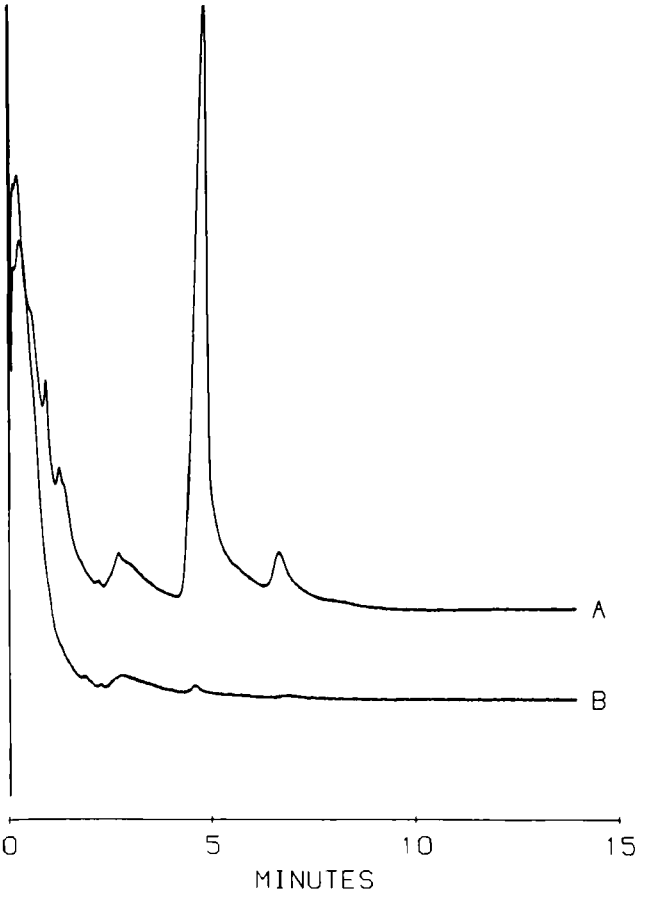

FIGURE 2. SFE-SFC chromatograms for misoprostol:HPMC dispersion and dispersion blank: A) misoprostol:HPMC dispersion, B) HPMC.

evident in Figure 1A was also evident in Figure 2A, which is the chromatogram resulting from an SFE-SFC experiment on 1.2 milligrams of misoprostol:HPMC dispersion. The dispersion sample contained approximately 12 micrograms of misoprostol drug substance. Samples were manually loaded into the

extraction cell insert prior to initiation of the SFE-SFC experiments. The change in baseline during the first two minutes of the chromatograms was due to the sudden pressure change resulting when the column is switched on-line to inject the extracted sample components. Extraction cell blank and dispersion blank (HPMC without misoprostol) SFE-SFC chromatograms are shown in Figures 1B and 2B, respectively.

The chromatograms depicted in Figures 1 and 2 demonstrate that misoprostol in an HPMC dispersion can be selectively analyzed with an on-line SFE-SFC system, using sample weighing as the only sample preparation step. The selectivity is due to the capacity of carbon dioxide-5% methanol to extract the non-polar prostaglandin from HPMC. Comparison of the chromatograms in Figures 1A, 2A, and 2B reveals that HPMC components were extracted during the SFE-SFC experiments; however, coelution of HPMC components with misoprostol was not evident.

Several additional features of the chromatograms in Figures 1 and 2 should be noted. Slightly different chromatographic figures of merit were observed for misoprostol drug substance and misoprostol dispersion peaks. Values are summarized in Table 1. The retention time and asymmetry for the misoprostol:HPMC peak were

TABLE 1. Chromatographic Figures of Merit for Misoprostol Peaks

sample	retention	plate count	asymmetry
misoprostol	4.5	4968	1.2
misoprostol: HPMC	4.9	4136	1.6

slightly greater, while the plate count was less. The differences, which were reproducible, are possibly due to a broadening of the misoprostol precipitate in the cryogenic focussing trap during extraction of misoprostol:HPMC. The extraction efficiency for the misoprostol:HPMC dispersion was estimated at 65%, based on comparison of peak areas for extracted and directly injected sample. The efficiency could undoubtedly be improved by changing parameters such as extraction duration and cryogenic trap temperature (15). It should also be noted that the total analysis time was less than twenty minutes and that the liquid solvent requirements were minimal.

Results in Figures 1 and 2 are preliminary; however, the work suggests that on-line SFE-SFC methodology could be used as part of quality control procedures for formulated drug substances. Our future

efforts will address several areas: 1) optimization of experimental parameters to maximize extraction efficiency; 2) method validation; 3) application of SFE-SFC methodology to other drug formulations.

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