CHAPTER VII

CONCLUSIONS

The focus of this research was to examine the effects of primary and secondary modifiers on the extraction and separation of neutral and ionic pharmaceutical compounds with pure and modified carbon dioxide. Before one can examine the effects of various extraction parameters, one must achieve quantitative analyte trapping. The first part of this research examined several solid-phase traps in hopes of identifying one that could: 1) retain high amounts of analyte, and 2) have the capability of trapping a wide variety of analytes. Therefore, the trapping capacities of three solid-phase traps was determined for a mixture of components varying in polarity and volatility. Three solid-phase traps were investigated including: 1) a 50/50 (w/w) mixture of octadecyl silica (ODS) and glass beads, 2) glass beads, and 3) 50/50 (w/w) mixture of Porapak Q and glass beads. It was seen that analyte breakthrough from the solid-phase varied with trap and analyte composition. The trapping capacity for the ODS/glass beads trap was determined to be less than 8 mg/g of ODS for four analytes and less than 4 mg/g of ODS for naphthalene. The glass beads trap was the least successful for the trapping of the three more volatile analytes resulting in a capacity of less than 0.5 mg/1.5 g. Consequently, the retention of acetophenone, dimethylaniline, and naphthalene on the ODS trap was due to adsorption on the ODS material. However, the glass beads were shown to be an effective trapping material for the two less volatile, higher molecular weight components; 2-naphthol and *n*-tetracosane. A trapping capacity for these two analytes was comparable to the ODS/glass beads trap of > 2 mg/1.5 g of glass beads. In this case, their retention was attributed to cryogenic freezing on the glass beads surface. Finally, the most successful trapping material was shown to be the mixture of Porapak Q® and glass beads resulting in a trapping capacity of at least 10 mg/g of Porapak Q® per analyte.

Now that an optimum solid-phase trap was identified, the extraction of several neutral and ionic pharmaceuticals was investigated via SFE. Although the solubility of a polar analyte may be high in a modified fluid, successful extraction of the analyte from a complicated matrix such as a tablet may be problematic due to large analyte/matrix interactions. The addition of secondary modifiers (i.e. additives) for the extraction of a lovastatin from several complicated matrices such as in-house prepared tablet mixtures and MEVACOR® tablets was then investigated. In this particular case, the analyte being extracted was neutral and contained no ionizable functionalities. Although it was expected that ion-suppression would not play a role in this study, the effect of additive type (acidic, basic, neutral) on its ability to cover active matrix sites and thus displace the polar analyte from the complicated tablet matrix was investigated. In-line methanol-modifier percentage, additive type (acidic, basic, neutral) in the in-line methanol modifier, and the effect of additive concentration on the extraction efficiency were examined. Extractability was shown to be dependent upon modifier concentration and additive type. Due to the lactone ring contributing basic characteristics to lovastatin, isopropylamine was believed to be the most successful additive because of its ability to displace adsorbed lovastatin from the acidic tablet matrix sites, an effect not possible with in-line methanol-modified CO_2 alone. An optimized extraction method was developed, and lovastatin recoveries of 99.5% with a RSD of 1.2% from MEVACOR® tablets with 15% (v/v) (1.0% (v/v) isopropylamine) methanol-modified CO_2 were achieved.

The next goal of this research was to further examine the feasibility of extracting ionic compounds via SFE. Ionic compounds exhibit low solubility and extractability in carbon dioxide because of their high polarity. The recovery of ionic compounds with carbon dioxide may be increased if the analyte is in the presence of an ion-pairing reagent. Through electrostatic interactions, two species of opposite charge may form an ion-pair of reduced polarity, therefore, the extractability of the formed ion-pair complex would be expected to increase. In this part of the research, a screening study consisting of a fractional-factorial design was performed to identify the influential parameters that

significantly affected the recovery of triphenylphosphinetrisulfonate, sodium salt, (TPPTS) from a spiked-sand surface with ion-pairing additives. Several extraction parameters were examined, and four parameters were found to influence the recovery. They were: ion-pairing reagent composition, mole ratio of ion-pairing reagent to TPPTS, static extraction time, and in-cell methanol spike volume. First, the recoveries of the anionic species were shown to be enhanced when in the presence of an ion-pairing additive. Of the two quaternaryalkylammonium salts investigated, the more lipophilic reagent, tetrahexylammonium hydrogen sulfate, was the only ion-pairing reagent that statistically enhanced the recoveries. The increased extractability with the ion-pairing reagent in the non-polar fluid, CO_2, was attributed to reduced analyte polarity. Second, the amount of ion-pairing reagent added was also shown to be influential. By adding an excess of ion-pairing reagent, the equilibrium was shifted towards full and complete ion-pair formation, and an enhancement was observed. Third, static time was shown to negatively affect the recoveries. Over time and in the presence of moisture, it was believed that a mixture of both the neutral and charged trisulfonate species were present, therefore, less ionic species were present to form the ion-pair complex and lower recoveries were observed. Lastly, the recoveries of the polar compound were shown to be enhanced by increasing the polarity of the fluid by a simple increase in the in-cell methanol spike volume.

The extraction of an anionic compound was shown to be feasible with supercritical carbon dioxide, and recovery was enhanced in the presence of an ion-pairing additive. The main goals of this part of the research were to demonstrate that cationic species can be extracted via SFE, and to investigate the effects of modifiers and ion-pairing additives on the recovery of pseudoephedrine hydrochloride from spiked-sand and Suphedrine tablets. First, the feasibility of enhancing the extractability through the use of an ion-pairing reagent was investigated. Quantitative pseudoephedrine recoveries from spiked-sand were shown to be achievable with 1-heptanesulfonic acid, sodium salt, with 10%-methanol-modified carbon dioxide. Second, the effect of the composition and concentration of the ion-pairing reagent was investigated. Recovery was shown to be a

function of ion-pairing reagent composition and concentration. The most successful recovery was obtained in the presence of 1-heptanesulfonic acid, sodium salt, in methanol at a 5:1 mole ratio. The increased extractability in the presence of ion-pairing reagent was attributed to reduced analyte polarity and analyte-matrix displacement. Third, the effectiveness of other in-cell modifiers such as a methanol/water mixture, an acid, and a base were compared for the extraction of pseudoephedrine from spiked-sand. The recoveries obtained with an in-cell modifier of methanol/water and heptanesulfonic acid, sodium salt, in methanol were comparable. Subsequently no further enhancement was observed with heptanesulfonic acid, sodium salt, in methanol/water. It was believed that the solvating power of the extraction fluid was increased in the presence of the methanol/water in-cell modifier, and analyte-matrix displacement from the spiked-sand was greater than in the presence of methanol alone. The addition of an acid and a base as in-cell modifiers as a function of analyte recovery was also examined. The basic additive, tetrabutylammonium hydroxide, was shown to be more successful than the acidic additive, trifluoroacetic acid. Under basic conditions, free base formation was favored of pseudoephedrine, therefore, the recovery of a neutral species in the relatively non-polar fluid was obtained to a greater extent. Likewise, recovery in the presence of tetrabutylammonium hydroxide was shown to be statistically equal to those achieved in the presence of heptanesulfonic acid, sodium salt, in methanol and in the presence of the methanol/water in-cell modifiers. Third, several in-cell and in-line modifiers were examined for the extraction of pseudoephedrine from Suphedrine tablets. As observed in the sand spike-studies, equivalent recoveries in the presence of the in-cell modifiers, heptanesulfonic acid, sodium salt, in methanol and methanol/water, were obtained and were shown to be significantly greater than in the presence of methanol alone with pure and methanol-modified CO_2. The effectiveness of a methanol/water as an in-line modifier versus methanol alone was also examined. Although it was expected that recovery with the more polar modifier (methanol/water) should be greater, no significant differences were noted. Therefore it was hypothesized that the extraction process of the

pseudoephedrine from the tablets was not necessarily solubility limited in the extraction fluid but diffusion limited. The greatest pseudoephedrine recovery from Suphedrine tablets of 82% (7.0%) was achieved with 10% (1% H_2O) methanol-modified CO_2 in the presence of 400 μL of methanol (1% H_2O). Finally AgCl tests and infrared analyses were performed on two tablet extracts. It was confirmed that in the absence of any in-cell modifier, pseudoephedrine hydrochloride was extracted thus disproving the overall assumption that salts cannot be extracted via SFE with a carbon dioxide based fluid.

The last part of this research investigated the use of a carbon dioxide based mobile phase as means of separating a mixture of neutral and ionic phospholipids. Several chromatographic parameters were investigated including: 1) stationary phase composition, 2) addition of an acidic additive to the modified carbon dioxide and its concentration, 3) modifier ramp rate, and 4) column outlet pressure. As in the case of the extraction studies, a secondary modifier was investigated in order to possibly suppress analyte ionization as well as reduce secondary interactions between the analyte and the stationary phase. The results indicated that a normal stationary phase was not effective due to high adsorption of the polar functionality of the phospholipids onto the polar stationary phase, however, a reversed stationary phase proved useful. Retention was attributed to partitioning of the lipholiphilic portions of the phospholipids with the non-polar octyl phase. Elution of each analyte and its peak shape was improved by the addition of an acidic modifier additive, trifluoroacetic acid. Secondary interactions between the phospholipids and the exposed silanol sites on the stationary phase were further reduced by an increase in additive concentration thus altering the column selectivity and improving resolution. The separation of all five phospholipids was achieved by optimizing the modifier gradient and pressure. Several chromatographic parameters used to describe the separation were compared, and good retention and resolution was achieved in a timely matter. Finally, two ELSD detectors were compared in terms of peak response (peak height, area) and peak shape. No significant differences were observed regardless of detector manufacturer.

In summary, both neutral and ionic pharmaceuticals can be extracted and chromatographed with super/subcritical fluid based extraction solvents and mobile phases. Addition of secondary modifiers (i.e. additives) was shown to be critical in improving analyte recovery and separation.