

CHAPTER V

MODIFIER AND ADDITIVE EFFECTS IN THE SUPERCRITICAL FLUID EXTRACTION OF PSEUDOEPHEDRINE HYDROCHLORIDE FROM SPIKED-SAND AND SUPHEDRINE TABLETS

5.1 INTRODUCTION

In this chapter, the feasibility of extracting a cationic species with carbon dioxide was examined as well as determining what factors may improve its extractability and why. Pseudoephedrine hydrochloride (**Figure 5.1**) was chosen as our test analyte. Pseudoephedrine hydrochloride is an active ingredient in many pharmaceutical cold formulations and serves as a nasal decongestant.

Hedrick et al. investigated the feasibility of extracting nitrogenous bases including triprolidine, sulfamethazine, caffeine, 2,5-lutidine, and pseudoephedrine from aqueous solutions using direct SFE.¹ As expected, the greater the lipophilic characteristics of the basic molecule, the more readily it was extracted with the non-polar fluid carbon dioxide from the polar matrix (i.e. water), therefore, the extraction of the larger organic bases, triprolidine and pseudoephedrine from water was successful. The hydrochloride salts of triprolidine and pseudoephedrine were said to be extracted from the water, however, tetramethylammonium hydroxide (TMAOH) was added to the solution to neutralize the salts thus forming the free bases. No quantitation was reported, and the so-called extracted hydrochlorides were not absolutely identified.

Another amine hydrochloride, 3-chloro-*p*-toluidine hydrochloride (PTH), was successfully extracted from spiked avian feed with 10% methanol-modified carbon dioxide.² A two-level, two-factor (2^2) factorial design was used to examine the effect of

¹ J.L. Hedrick, L.T. Taylor, J. High Resolut. Chromatogr., **15** (1992)151.

² M.L. Bicking, J. Chromatogr. Sci., **30** (1992) 358.

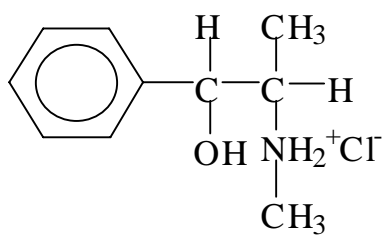


Figure 5.1. Chemical Structure of Pseudoephedrine Hydrochloride

pressure and temperature upon the extraction recoveries. Low temperature (45 °C) and high pressure (320 atm) conditions were shown to result in the highest average recovery (96%). As in the case of Hedrick's work, the extracted amine species was not identified.

More recently, Morrison et al. reported on the supercritical fluid extraction of the free base and hydrochloride salt of cocaine and benzoylecgonine from teflon wool, filter paper, and drug-fortified and drug-user hair with a triethylamine (TEA)/water modifier mixture.³ It was proposed that the drugs were adsorbed strongly to the hair matrix because of a combination of hydrophobic interactions, hydrogen bonding, and ionic interactions, therefore it was expected that the ability of CO₂ alone to remove the strongly held drug from the hair matrix would be unsuccessful. Successful extraction was achieved with CO₂ modified with 100 µL of TEA/H₂O (85:15 (v/v) for the following reasons. First, the H₂O served to swell the hair making the fluid more accessible to the analyte-matrix sites. Second, it was believed that some of the triethylamine modifier existed as the triethylammonium cation due to acidic conditions in the presence of CO₂ and water. Therefore the triethylammonium cation may have displaced the cocaine from the negatively charged hair binding sites through an ion-exchange mechanism. Third, if the TEA existed as neutral in the bulk CO₂, it may serve as a buffer thereby accepting the proton from the displaced cocaine salt so that the cocaine was extractable as the free base.

For these three reported extractions of hydrochloride salts, the strategies of ion-suppression and ion-exchange were shown to be most successful. In these studies ion-exchange represented displacement of an ionic species from any exposed active matrix site with a "stronger" modifier. Ion-suppression involved charge neutralization by the addition of an appropriate acid or base. The strategy of ion-pairing wherein two species of opposite charge electrostatically interact with one another to form an ion-pair-analyte complex was not investigated in any of these studies. Ionic compounds are generally believed to have low solubility and poor extractability in carbon dioxide due to their high polarity; however it was shown in Chapter IV that at least one ionic compound can indeed

³ *J.F. Morrison, S.N. Chesler, W.J. Yoo, C.M. Selavka, Anal. Chem., 70 (1998) 163.*

be extracted. Recall that triphenylphosphinetrisulfonate (TPPTS) was extracted from a spiked-sand surface with supercritical carbon dioxide. Extraction recoveries were shown to be enhanced in the presence of a methanol solution of tetrahexylammonium hydrogen sulfate. It was reasoned that through electrostatic interactions there was a reduction in the polarity of the analyte which increased solubility and thus extractability in carbon dioxide. Other parameters identified as influential to the extraction process were: ion-pair reagent concentration, static extraction time, and methanol-modifier volume. When this work was begun there were no reports on the use of ion-pairing additives as a means of increasing the extractability of cationic compounds with CO₂. Therefore, it was our objective to investigate whether the extraction of pseudoephedrine hydrochloride in the presence of an ion-pairing reagent was indeed feasible (Phase I).

Since the publication of our feasibility study (Phase I), there have been several reports describing the simultaneous ion-pair/supercritical fluid extraction of cationic species.⁴⁻⁶ Jimenez-Carmona investigated the extraction of clenbuterol from spiked diatomaceous earth and food matrices (feedstuff, lyophilized milk and liver).⁴ Three ion-pairing reagents (0.1M in methanol) were investigated: (1S)-(+)-10-camphorsulfonic acid, (1R)-(-)-10-camphorsulfonic acid, and (1R)-(-)-10-camphorsulfonic acid, ammonium salt. In the presence of 0.5 mL of a methanol solution of the ion-pairing reagent, clenbuterol was extracted from spiked diatomaceous earth with CO₂ at 30% and 70% recoveries using camphorsulfonic acid and its ammonium salt respectively. It was believed that camphorsulfonic acid itself could not form the ion-pair, however the ammonium salt could. The influence of the amount of ion-pairing reagent (e.g. ammonium salt) added was also ascertained. Surprisingly, no increase in recovery was observed when the concentration of the ion-pairing reagent was increased from 0.1M to 0.3M. The influence of the extraction fluid was also studied. The recoveries were compared between pure CO₂

⁴ M.M. Jimenez-Carmona, M.T. Tena, M.D. Luque de Castro, *J. Chromatogr. A*, **711** (1995) 269.

⁵ P. Fernandez, A.C. Alder, M.J.F. Suter, W. Giger, *Anal. Chem.*, **68** (1996) 921.

⁶ J.T.B. Strode, L. Karlsson, M. Berglin, "Ion-Pair Supercritical Fluid Extraction of Melagratane from Gelatine Capsules", personal communication.

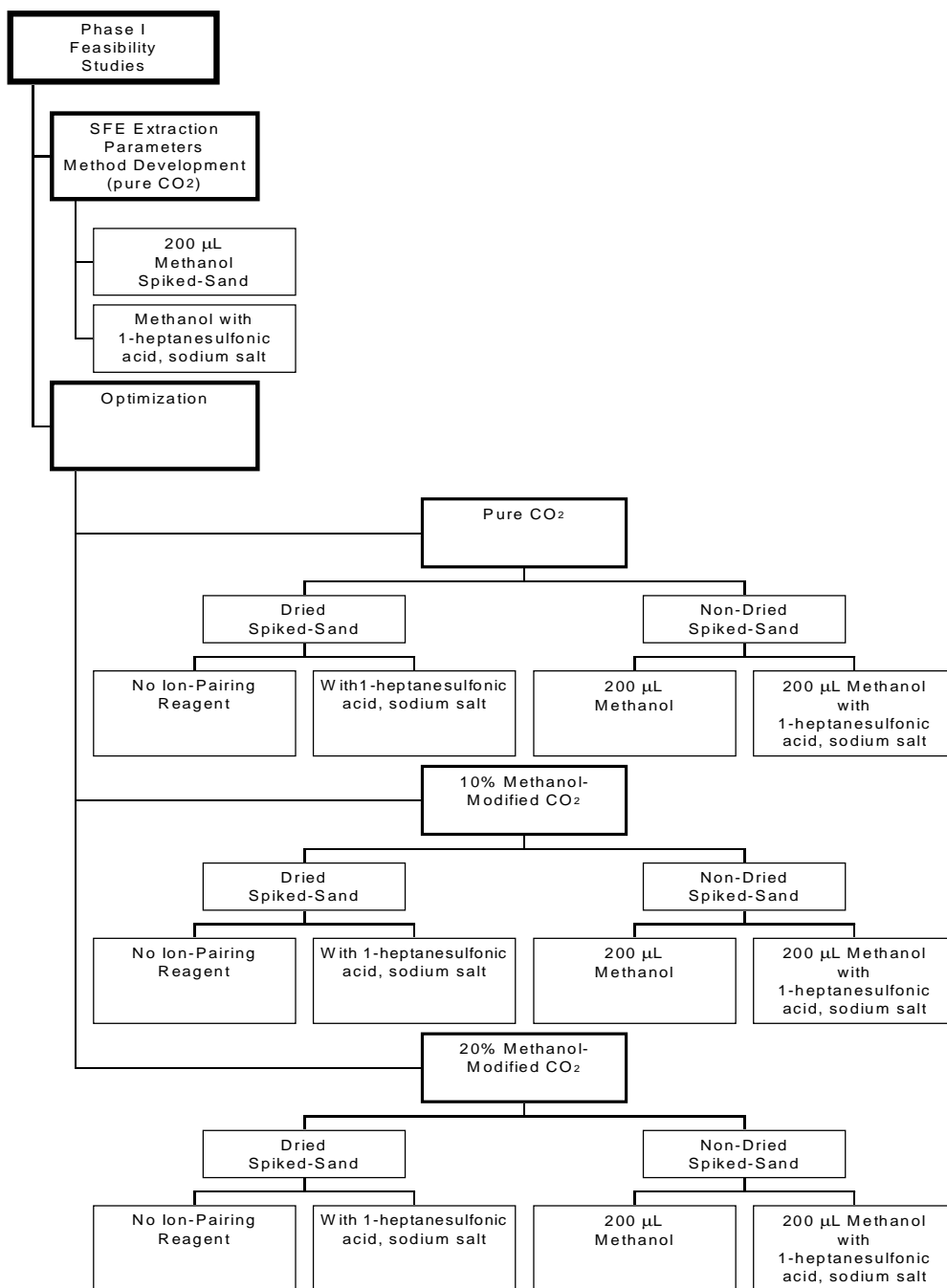
and methanol-modified CO₂ with static addition of the ion-pairing reagent in both cases. Methanol-modified CO₂ had a negative impact on the recoveries (e.g. 80% (pure CO₂), 60% (methanol-modified CO₂)). Once again, decreased recoveries with the methanol-modified fluid were attributed to poor analyte trapping. The influence of the mode of introduction of the ion-pairing reagent was investigated: 1) addition directly to the matrix (static addition), 2) continuous addition by means of in-line methanol-modified CO₂ (dynamic addition), and 3) combination of addition directly to the matrix (static addition) and continuous addition by means of the in-line modified fluid (dynamic addition). Since the extraction system plugged using strategy 2 and 3, only ion-pairing reagent introduction through static addition was explored. Extractions in the presence of the ion-pairing reagent were next performed on the food samples. Recoveries (20 µg clenbuterol) were only 12%, 47%, and 59% for the lyophilized liver, feedstuff, and lyophilized feedstuff respectively. No reasons were given for the observed low recoveries from the food samples. Recovery from the food matrices in the absence of camphorsulfonic acid, ammonium salt was not reported.

Later in 1996 Fernandez et al. used SFE to remove the quaternary ammonium surfactant ditallowdimethylammonium from sewage sludges and marine sediments.⁵ Using a similar approach to that discussed above, 1 mL of toluene-4-sulfonic acid (TSA) in methanol at several concentrations was added to the matrix. The extraction was then performed with pure CO₂. Regardless of ion-pair reagent concentration, the extraction recoveries of the cationic surfactant from the sludge with a methanol-modified matrix alone versus a methanol-modified matrix in the presence of TSA were similar. Quantitative extraction recoveries from the sewage sludge were achieved with 30% methanol-modified CO₂. The usefulness of TSA was more apparent when extracting the marine sediment with 10% methanol-modified CO₂. The presence of the ion-pairing reagent was shown to be beneficial where an enhancement of approximately 35% was observed. However, identical recoveries for the marine sediment were achieved with both 30% methanol-modified CO₂ and 10% methanol-modified CO₂ in the presence of the ion-

pairing reagent. Although smaller amounts of modifier could be used with the ion-pairing reagent, 30% methanol-modified CO₂ was chosen as the extraction fluid because it would have been necessary to remove the ion-pairing reagent prior to HPLC.

Finally, melagatran was quantitatively recovered from an aqueous emulsion (phosphate buffer) of a dissolved gelatine capsule spiked onto Celite by SFE.⁶ Initial drug spike studies on Celite with methanol-modified CO₂ and methanol-modified CO₂ with trifluoroacetic acid were performed. Recoveries were similar. Octylsulfonic acid, sodium salt (OSA) was then added to the modified CO₂ (no trifluoroacetic acid). Again, no enhancement was observed. It was proposed that ion-pair formation with the analyte could not occur with the sodium salt. Consequently, trifluoroacetic acid (trifluoroacetic acid) was added to the ion-pair/methanol modifier system to serve as a proton source. Quantitative recoveries were then observed. Next, extractions were performed on the gelatine capsules. A two-step extraction procedure was employed. First, the gelatine lipids were selectively removed from the emulsion with (1:1:98 (v/v/v) trifluoroacetic acid:OSA:methanol) modifier (4% (v/v)). Second, the drug was subsequently extracted with (1:1:98 (v/v/v) trifluoroacetic acid:OSA:methanol) modifier (25% (v/v)). Although quantitative recoveries were observed, the presence of the trifluoroacetic acid interfered in the analysis of the two degradates of melagatran, therefore phosphoric acid was used. Recoveries in this case (96%) were achieved by first removing the lipids with (1:1:98 (v/v/v) phosphoric acid:OSA:methanol) modifier (4% (v/v)) followed by extraction of melagatran with (1:1:98 (v/v/v) phosphoric acid:OSA:methanol) modifier (20% (v/v)).

The main goals of our work were to demonstrate that cationic species can be extracted via SFE, and to investigate the effects of modifiers and ion-pairing additives on their recovery. This chapter is divided into four phases. Phase I (**Scheme 5.1**) describes the feasibility of extracting an ionic species, pseudoephedrine hydrochloride, from a relatively simple matrix, Ottawa cement testing sand. An initial screening study was performed to qualitatively estimate the effect of CO₂ density, static extraction time, and the addition of an ion-pairing reagent to the spiked-sand surface on recovery. Then under

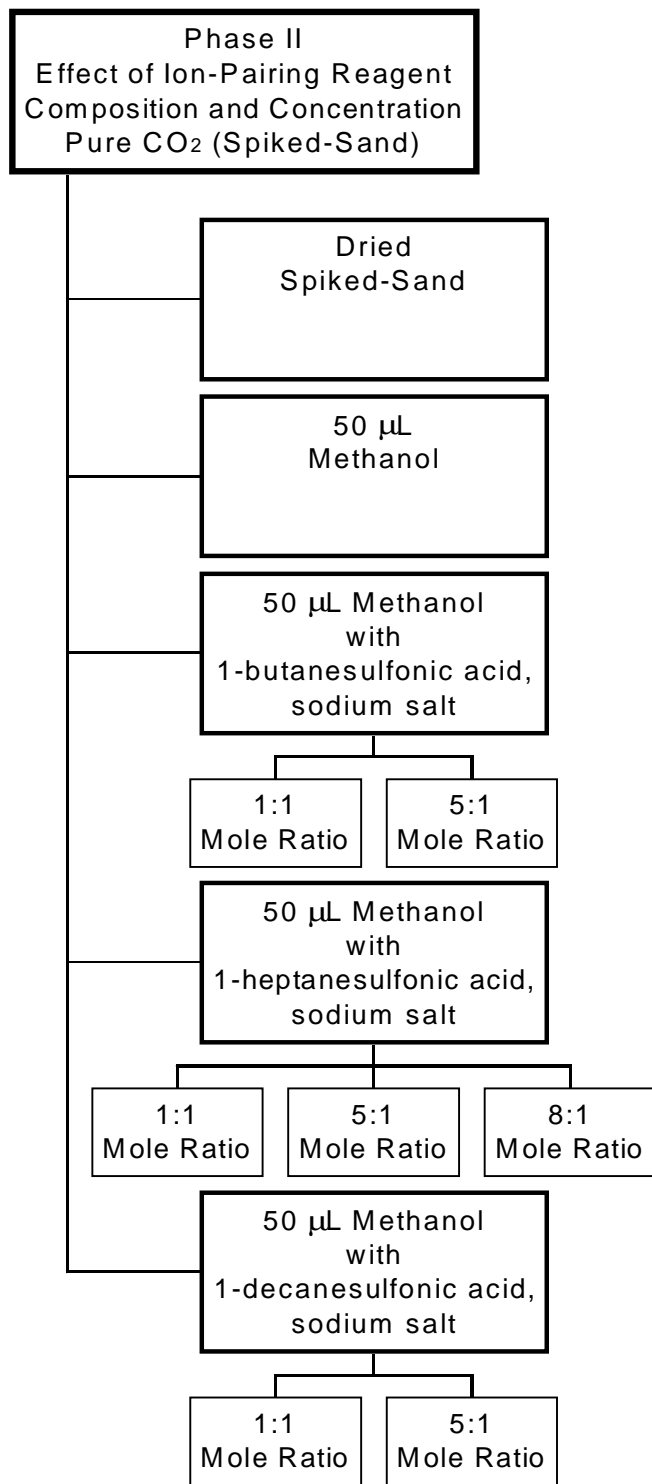


Scheme 5.1. Phase I General Experimental Scheme

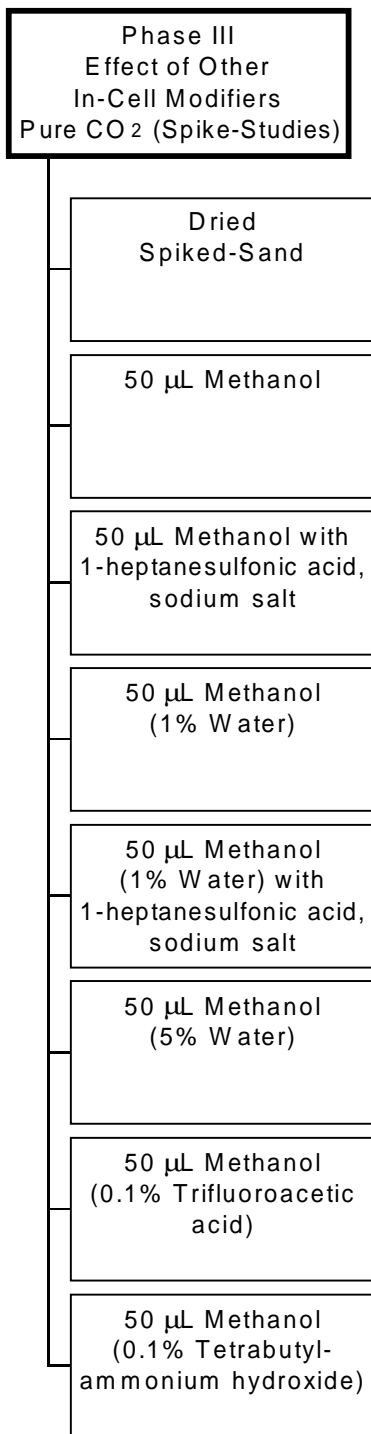
constant SFE conditions, the extraction of pseudoephedrine hydrochloride in the presence and absence of 1-heptanesulfonic acid, sodium salt (from both a previously dried sand surface and from one that had been pre-spiked with methanol) with pure-, 10% methanol-modified-, and 20% methanol-modified-carbon dioxide will be compared. As reported in Chapter IV, the composition and concentration of ion-pairing reagent relative to analyte concentration as well as the amount of modifier in the extraction vessel were shown to influence the extraction process. Therefore in Phase II (**Scheme 5.2**), several alkylsulfonic acid, sodium salts varying in lipophilicity and concentration were investigated. Phase III (**Scheme 5.3**) will consider the influence of acids and bases and other modifier compositions such as a methanol/water mixture on the recovery of pseudoephedrine hydrochloride. Finally the extraction of pseudoephedrine hydrochloride from a commercially available formulation, Suphedrine tablets, will be performed in Phase IV (**Scheme 5.4**). Similar to the sand-spike studies, the effect of the addition of the ion-pairing reagent and modifier composition will be examined. Also, attempts will be made to identify the composition of the extracted analyte.

5.2 EXPERIMENTAL

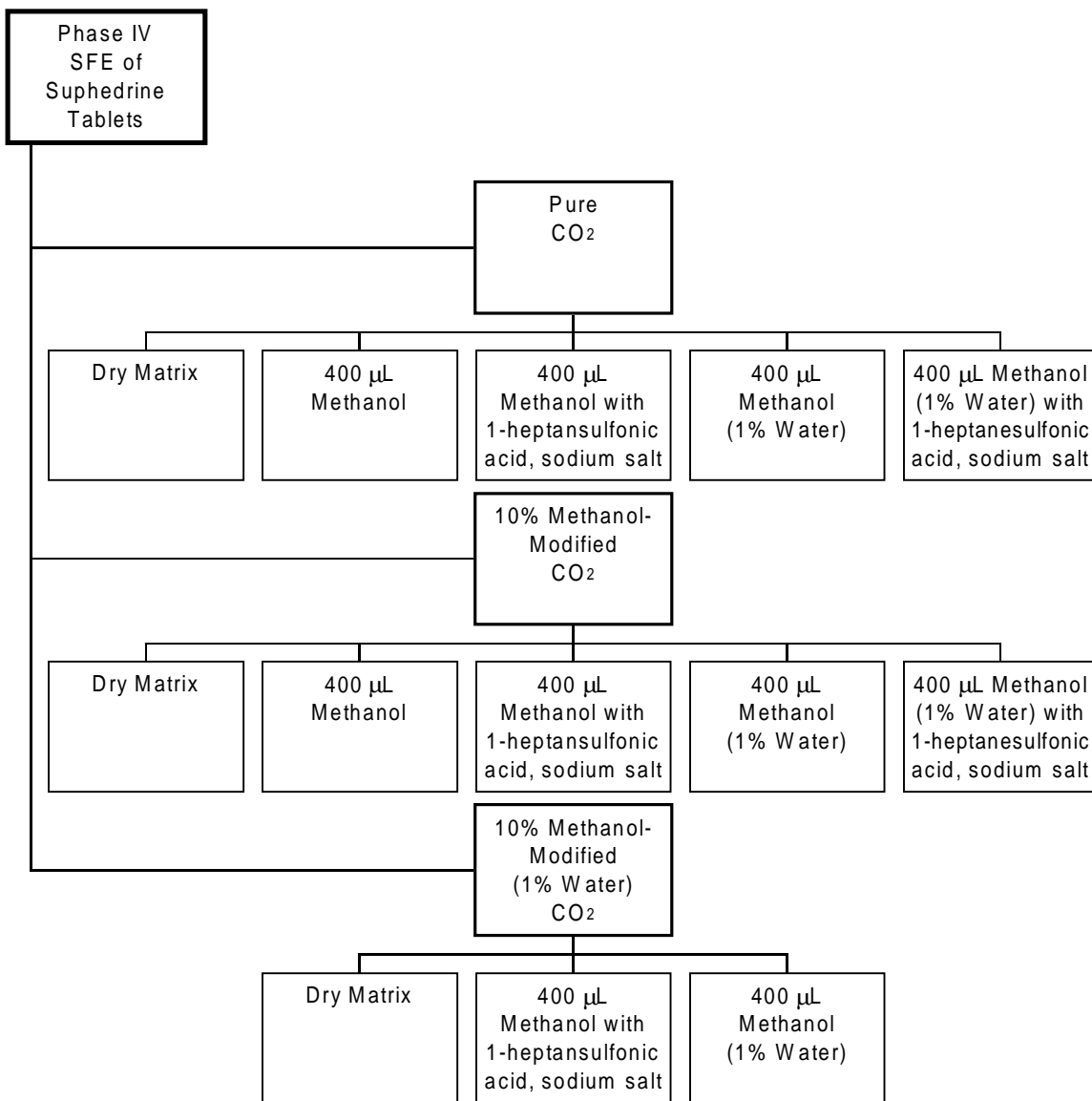
All extractions were performed on the Isco/Suprex Prepmaster (Lincoln, NE) consisting of a dual reciprocating pump, temperature-controlled oven, variable automatic restrictor, Accutrap solid-phase trap collection and rinsing device, and an in-line HPLC micro pump for modifier introduction. Carbon dioxide (SFE/SFC grade) with helium headspace was donated by Air Products and Chemicals, Inc. (Allentown, PA). Pseudoephedrine hydrochloride, 1-butane-, 1-heptane-, 1-decanesulfonic acid, sodium salt and caffeine were obtained from Sigma-Aldrich (St. Louis, MO) respectively. Suphedrine tablets (American Fare, Troy, MI) were purchased at Kmart. HPLC grade methanol and water were obtained from EM Science (Gibbstown, NJ) and Mallickrodt (Paris, KY) respectively. Trifluoroacetic acid and tetrabutylammonium hydroxide (25% in methanol)



Scheme 5.2. Phase II General Experimental Scheme



Scheme 5.3. Phase III General Experimental Scheme



Scheme 5.4. Phase IV General Experimental Scheme

were purchased from Fisher Scientific (Norcross, GA) and JT Baker (Phillisburg, NJ) respectively.

Extract Analysis

Upon completion of the extraction, caffeine was added to the solid-phase trap rinse and tandem-liquid trap as an internal standard. Analysis of the extract solutions was performed by HPLC. Values corresponding to 100% recovery were obtained by direct comparison to the 100% pseudoephedrine hydrochloride standard.

A Hitachi (Danbury, CT) Liquid Chromatograph consisting of a L-7100 pump, a D-7000 interface, a L-7250 autosampler, a L-7400 UV detector, and a D-7000 HPLC system manager was used for all extract analyses in Phase I. All other analyses were performed with a Hewlett Packard (Wilmington, DE) Series 1050 Liquid Chromatograph and a Hewlett Packard 3396 Series II integrator. All separations were isocratically performed on a Deltabond Cyanopropyl (25 cm x 4.6 mm i.d.) column (Keystone Scientific, Bellefonte, PA) with a mobile phase consisting of 89% water, 2% methanol, 9% acetonitrile, and 1.5 grams of 1-heptanesulfonic acid, sodium salt. The pH of the mobile phase was adjusted to 3.0 with phosphoric acid. A flow rate of 1.0 mL/min was used. The injection volume was 10 μ L. Detection was by UV at 205 nm.

Extract Trapping

A solid-phase/tandem-liquid trap was used for analyte collection. The solid-phase consisted of a 50/50 (w/w) mixture of Porapak Q and glass beads. A vial containing 5 mL methanol immediately following the solid-phase trap was used to ensure quantitative trapping. During the extraction, the solid-phase trap was maintained at either 0 °C (pure CO₂) or 70 °C (methanol-modified CO₂). The tandem liquid trap was maintained at room temperature. Upon completion of the extraction, the solid-phase trap temperature was

raised to 25 °C for trap rinsing with 3.2 mL of methanol (Phase I) at a flow rate of 1.0 mL/min. into an empty collection vial. For Phases II-III the solid-phase trap was rinsed twice following the dynamic extraction with 2.0 mL of methanol into an empty collection vial. For Phase IV the solid-phase trap was rinsed with 2.0 mL methanol between dynamic extraction mini-steps directly into the tandem-liquid trap. In this case, the tandem-liquid trap was replaced following each dynamic extraction step.

Procedure - Phase I

Solutions of pseudoephedrine hydrochloride and 1-heptanesulfonic acid, sodium salt were prepared in methanol. A 100- μ L aliquot of pseudoephedrine hydrochloride solution (100 μ g) was spiked into a 3.5 mL Keystone Scientific (Bellefonte, PA) extraction vessel approximately 90% filled with Ottawa Cement Testing sand (Fisher Scientific, Raleigh, NC). Either 100 μ L of pure methanol or 100 μ L of 1-heptanesulfonic acid, sodium salt methanol solution (900 μ g) was added to the drug-spiked sand surface. The mole ratio of ion-pairing reagent to pseudoephedrine hydrochloride was 9:1. The total spike volume of methanol added to the matrix, therefore, was always 200 μ L (e.g. 100 μ L (pseudoephedrine solution) plus 100 μ L (pure methanol or ion-pair methanol solution)). The sand was either extracted immediately or after air drying overnight with pure-, 10%-, or 20%-methanol modified-carbon dioxide. Extraction conditions are found in **Table 5.1**.

Procedure - Phases II-III

Solutions of pseudoephedrine hydrochloride and 1-butane-, 1-heptane-, and 1-decane sulfonic acid, sodium salt were prepared in methanol. A 100- μ L aliquot of pseudoephedrine hydrochloride solution (300 μ g) was spiked into a 3.5 mL Keystone Scientific (Bellefonte, PA) extraction vessel approximately 90% filled with Ottawa

Cement Testing sand (Fisher Scientific, Raleigh, NC), after which the spiked-sand was allowed to dry prior to extraction. Either pure methanol (Phase II-III), methanol with 1% or 5% (v/v) water (Phase III), methanol with 0.1% (v/v) trifluoroacetic acid (Phase III), methanol with 0.1% (v/v) tetrabutylammonium hydroxide (Phase III), or the appropriate ion-pairing reagent in a methanol or methanol-modified solution (Phase II-III) was added to the drug-spiked sand surface. In the case of the addition of the ion-pairing reagent, the reagent was added at either a 1:1 (Phase II) or a 5:1 (Phase II-III) drug to reagent mole ratio unless stated otherwise. The total spike volume of methanol added to the matrix was always 50 μ L (pure methanol, modified-methanol, or ion-pair methanol solution) unless otherwise noted. The sand was extracted immediately with pure carbon dioxide and was not allowed to dry. The extraction conditions used in Phases II-III are found in **Table 5.2**.

Procedure - Phase IV

The extraction of Suphedrine tablets was performed in Phase IV. The mass of pseudoephedrine hydrochloride per tablet was assumed to be 30 mg. Each Suphedrine tablet was placed on top of a piece of weighing paper which was sitting in a mortar cup. A pestle was placed on top of the tablet, and pressure was applied until the tablet particles appeared evenly dispersed as a powder. The weighing paper was carefully removed and the complete crushed tablet was poured into the extraction vessel filled approximately 3/4 with a cotton ball. The weighing paper, mortar, and pestle were wiped clean with an additional small piece of cotton. This particular piece of cotton was then placed on top of the other cotton ball inside the extraction vessel. More cotton was added to fill approximately 90% of the vessel volume. Then either 400 μ L of pure methanol, methanol with 1% (v/v) water, or 1-heptanesulfonic acid, sodium salt in either methanol or methanol with 1% (v/v) water was spiked on top of the cotton ball. A 2:1 mole ratio of drug to reagent was employed. The extraction vessel was then sealed, and the extractions were performed with either pure, 10%-, 20%- methanol-modified CO₂, or 10% (1% (v/v)

Table 5.1. Phase I - Conditions for Ion-Pair/Supercritical Fluid Extraction Screening Study With Pure CO₂

Method #	Pressure (atm)	Oven Temp (°C)	CO ₂ Density (g/mL)	Liquid Flow Rate (mL/min.)	Variable Restrictor Temp (°C)	Static Time* (min.)	Dynamic Time** (min.)
1	300	80	0.75	1.0	60	5	15
2	300	80	0.75	1.0	60	15	15
3	450	80	0.85	1.0	60	15	15
4	400	35	0.97	1.0	60	15	15

In-cell spike volume - 200 µL (i.e. 100 µL pseudoephedrine solution + 100 µL pure methanol or ion-pairing reagent in methanol)

* time allotted for equilibration between spiked-sand surface and supercritical fluid (SF)

** time allotted for SF to pass through extraction vessel at indicated flow rate

Table 5.2. Extraction Conditions Used in Phases II-III

<u>SFE Conditions</u>	
CO ₂ Pressure	450 atm
Temperature	35 °C
Liquid Flow Rate	1.0 mL/min.
Restrictor Temperature	50 °C
Dynamic Mass	25 grams CO ₂
Modifier	Variable
Ion-Pairing Reagent	Variable

water) methanol-modified CO₂. The extraction conditions and profile used in Phase IV are found in **Table 5.3**.

5.3 RESULTS AND DISCUSSION

The goal of this chapter was to extract a cationic compound, pseudoephedrine hydrochloride, via SFE. Due to its ionic and polar characteristics, it is expected that it will exhibit low solubility and poor extractability with a non-polar fluid such as carbon dioxide. Therefore, other means must be devised in order to improve the extractability of pseudoephedrine. Several strategies will be investigated including the addition of modifiers (i.e. methanol and methanol/water) to the matrix, addition of ion-pairing modifiers (alkylsulfonic acid, sodium salts) to the matrix, and modification of the CO₂ extraction fluid (i.e. methanol-, methanol/water-modified CO₂). The first strategy investigated was ion-pairing wherein the extraction from a relatively simple sand matrix was performed with and without an ion-pairing additive. Once this strategy had been shown to be successful, the influence of the ion-pairing reagent composition and concentration was investigated in Phase II. Although ion-pairing seemed beneficial, the extraction recoveries could also have been enhanced by simply modifying the polarity of the in-cell modifier thus favoring the extraction of the polar compound. Therefore, in Phase III, the effect of the in-cell modifier composition (methanol versus methanol/water) was examined. Since ion-suppression may prove worthwhile, a solution of an acid or base was added to the extraction vessel in hopes of shifting the equilibrium between the charged and neutral forms of pseudoephedrine. Correlations between the expected composition (neutral vs charged) and their subsequent recoveries were made. Finally in Phase IV, the extraction of pseudoephedrine from a more complicated matrix, Suphedrine tablets was performed. The effect of the addition of an ion-pairing reagent, the effect of various in-cell modifiers, and the effect of the composition of the fluid (pure vs modified CO₂) were examined to see if similar trends were observed as in the spike-studies. An

Table 5.3. Extraction Conditions and CO₂ Profile Used in Phase IV

<u>SFE Conditions</u>		
CO ₂ Pressure	450 atm	
Temperature	35 °C	
Liquid Flow Rate	2.0 mL/min.	
Restrictor Temperature	60 °C	
Modifier	Variable	
Ion-Pairing Reagent	1-heptanesulfonic acid, sodium salt	
<u>Extraction Profile</u>		
<u>Step</u>	<u>Mode</u>	<u>Time or Mass of CO₂ Used</u>
1	Static	5 min.
2	Dynamic	10 grams
3	Dynamic	10 grams
4	Dynamic	20 grams
5	Dynamic	20 grams
6	Dynamic	20 grams

optimum strategy (i.e. in-cell modifier composition, fluid composition) for the extraction of the cationic species was identified. Finally, the extracted analyte will be identified.

Phase I - Feasibility Studies

The main objective of Phase I was to examine the feasibility of extracting pseudoephedrine hydrochloride with super/subcritical pure and modified-carbon dioxide. The extraction efficiencies were compared with and in the absence of an ion-pairing additive, 1-heptanesulfonic acid, sodium salt. The effect of CO₂ density, static extraction time, and temperature were first examined. Second, the extraction was optimized for quantitative recovery. Third, the extraction efficiencies in the presence of the ion-pairing reagent in methanol from a spiked-sand surface containing a fixed volume of the methanol solution versus a spiked-sand surface that was allowed to dry after it was spiked with the ion-pairing reagent solution were examined. Do the reagent and the drug need to mix in the pressurized CO₂ fluid prior to extraction or can a successful extraction be achieved if the ion-pair spiked-sand surface was dry prior to extraction?

SFE Method Development

A screening study was initially performed to qualitatively estimate the effect of various extraction parameters on pseudoephedrine hydrochloride recovery from a non-dried spiked-sand surface. **Table 5.1** lists the SFE extraction conditions. **Table 5.4** lists the recoveries (n=1) of pseudoephedrine hydrochloride at various temperatures, pressures, and static extraction times from a methanol-spiked sand surface with and without the addition of 1-heptanesulfonic acid, sodium salt. In most cases, methanol containing a small amount (approximately 0.1%) of water was added to the extraction vessel to serve as an in-cell modifier. A number of reactions could take place upon addition of this methanol. For example the hydrochloride salt could dissociate into its ionic components.

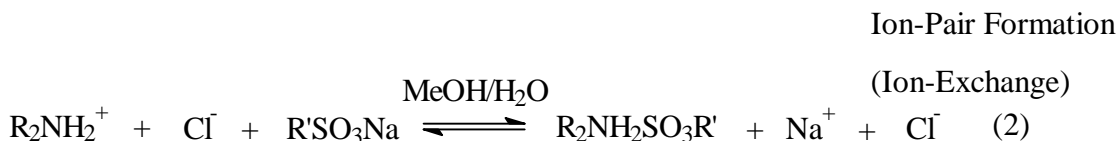
Table 5.4. Percent Recovery (n=1) From Screening Study of Pseudoephedrine Hydrochloride Employing SFE - Phase I
Methods 1-4 with and without Ion-Pairing Agent

Method #	Pseudoephedrine HCl	Pseudoephedrine HCl w/ 1-heptanesulfonic acid, sodium salt
1	7.5	20.5
2	ND*	27.3
3	22.4	45.1
4	17.6	55.2

*ND indicates none detected

Secondly an equilibrium could exist between the ionized drug species and its corresponding free base through hydrolysis. (**Equation 1a, 1b**) Since it is anticipated that ionic species would not be extracted under these conditions, all extracted analyte will be assumed to be present in the free base form in the absence of the ion-pairing reagent. Attempts to identify the composition of the extracted analyte will be made in Phase IV. For the sake of terminology, the extracted analyte in this Chapter will be referred to as pseudoephedrine. Under the conditions listed in Method 1, low density and high temperature, only 7.5% (7.5 ug) of the spiked pseudoephedrine (100 ug) was recovered with pure carbon dioxide from the methanol-spiked sand surface (200 μ L total spike volume).

To examine whether the addition of an ionic additive could enhance recovery of the ionic pseudoephedrine species, an ion-pairing reagent was added to the vessel prior to extraction. Through ion-pairing the pseudoephedrine-sulfonate ion-pair would hopefully form and give enhanced non-polar characteristics to the compound. (**Equation 2**) Under the same SFE conditions and methanol-spike volume, the addition of the ion-pairing reagent, 1-heptanesulfonic acid, sodium salt on the extraction efficiency was then examined. As expected, the extraction recovery increased with the addition of the ion-pairing reagent to 20.5%.



In an attempt to increase the extraction efficiency further, the time allotted for equilibration between the supercritical fluid and the drug-spiked sand surface was increased from 5 to 15 minutes (Method 2) while the density, oven temperature, and dynamic extraction time were held constant as in Method 1. It was believed that a higher recovery would result if more time was allotted for ion-pair formation and for equilibration between the pseudoephedrine-sulfonate ion-pair and the supercritical fluid. Very little pseudoephedrine was extracted without ion-pairing reagent. However the extraction recovery greatly increased to 27.3% with the addition of ion-pairing reagent. If one compares the pseudoephedrine-sulfonate ion-pair extraction recovery via Methods 1 and 2, it appears that the increased static time slightly improved extraction recovery. The true significance of static time cannot be accurately ascertained since each method was run only once.

The next SFE parameter investigated was density. It may be advantageous to increase the density of the fluid in order to increase its solvating power toward more polar analytes. An increase in extraction recovery of pseudoephedrine without the ion-pairing reagent from zero (Method 2) to 22.4% was observed when the CO₂ density was increased from 0.75 g/mL to 0.85 g/mL (Method 3). The added usefulness of the ion-pairing reagent can also be observed at the increased density. The recovery dramatically increased from 22.4% to 45.1% with the addition of 1-heptanesulfonic acid, sodium salt to the matrix.

Due to pump pressure limitations, the carbon dioxide density could only be increased to 0.97 g/mL albeit by decreasing the extraction temperature (Method 4). The recovery of pseudoephedrine with the addition of the ion-pairing reagent was slightly enhanced from 45.1% to 55.2% with this further increase in density; however, the extraction recovery of pseudoephedrine without the ion-pairing reagent appeared to level off or decrease at the greater density.

Ion-Pair/SFE Optimization

The main objectives in part II of Phase I were: 1) investigate the feasibility of increasing the solubility and extractability of pseudoephedrine via charge neutralization with 1-heptanesulfonic acid, sodium salt from a spiked-sand surface; and 2) determine the optimal extraction conditions for full recovery of pseudoephedrine from the spiked-sand surface. High density conditions (0.99 g/mL CO₂, 450 atm, 35 °C) were chosen for part II of this phase. To ensure an exhaustive extraction, the dynamic time was increased to 25 minutes; while the static time was decreased to 5 minutes.

The average percent recoveries of pseudoephedrine with and without the addition of 1-heptanesulfonic acid, sodium salt from a spiked-sand surface (e.g. spiking performed directly into the extraction vessel) with pure-, 10%-, and 20%-methanol-modified carbon dioxide are found in **Figure 5.2**. Although it was previously thought that ionic compounds and salts could not be extracted by SFE, 67.3% (67.3 µg/100 µg) of pseudoephedrine was recovered with pure CO₂ from the methanol enriched spiked-sand surface with no ion-pairing reagent. The in-cell methanol-spike apparently modified the CO₂ making it more polar thus leading to pseudoephedrine's greater solubility in CO₂. Disruption of analyte-matrix interactions may also be another possible explanation for the observed increase in extractability. In-line 10%- and 20%-methanol-modified CO₂ were then utilized to further enhance the extraction of pseudoephedrine from a methanol enriched spiked-sand surface. No significant increase was observed when employing 10%-methanol-modified CO₂ (61.6%); however, a significant increase to 89.3% was observed with 20%-methanol-modified CO₂.

It was believed that the addition of 1-heptanesulfonic acid, sodium salt would further increase the solubility and extractability of pseudoephedrine. This hypothesis was realized with 100% CO₂ (**Figure 5.2**) in that the recovery from the methanol enriched spiked-sand surface was increased from 67.3% (without IP) to 76.5 % (with IP). Another possible explanation for the observed enhancement with the ion-pairing reagent is

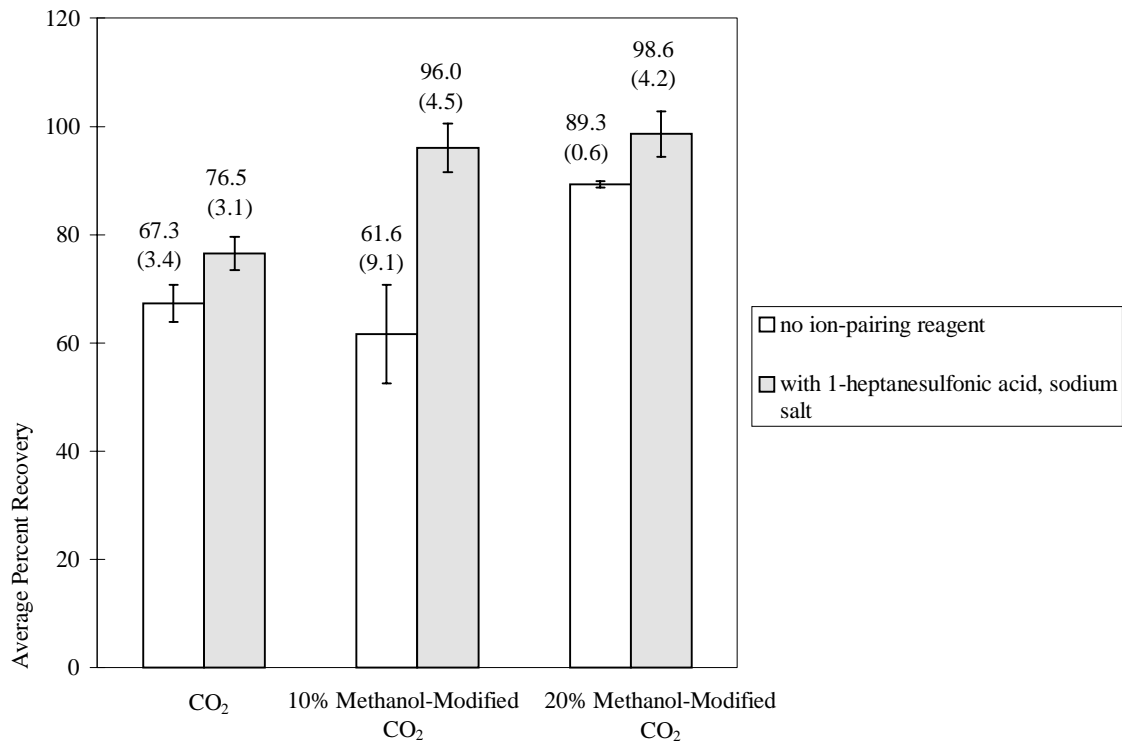


Figure 5.2. Average Percent Recoveries (n=3) of Pseudoephedrine and Pseudoephedrine with 1-Heptanesulfonic Acid, Sodium Salt from a Non-Dried Drug Spiked-Sand Surface. Bars and Numbers in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 1.0 mL/min., 5 min. static time, 25 min. dynamic time. In-cell modifier volume - 200 μL methanol or ion-pairing reagent in methanol.

displacement of the pseudoephedrine from the sand surface by the ion-pairing reagent. Although it was expected that methanol alone should have been able to reduce these interactions, the ion-pairing reagent itself may act as an ionic displacer preferentially adsorbing to these sites thus freeing the ionic pseudoephedrine. Once released into the bulk methanol-modified CO₂ fluid, an equilibrium between the ionic analyte and the free base may ensue such that the free base may be further extracted. This mechanism would presume no electrostatic interaction between the analyte and the ion-pairing reagent existed.

The extraction of pseudoephedrine from the spiked-sand surface in the presence of ion-pairing reagent with CO₂ was shown to be even more successful with in-line-methanol-modified CO₂. Recoveries of 96.0% and 98.6% were achieved with 10%- and 20%-methanol-modified CO₂ respectively relative to 61.6% and 89.3% with no ion-pairing reagent. Paired t-tests were performed to test if the means of the extraction recoveries with the use of the ion-pairing reagent were greater than the recoveries without the ion-pairing reagent with pure-, 10%-, and 20%-methanol-modified CO₂ at a 95% confidence interval ($\alpha=0.05$, $T_{\text{critical}}=2.9$ (one-tailed)). The extraction recoveries of the pseudoephedrine-sulfonate ion-pair as compared to pseudoephedrine alone were shown to be statistically greater ($T_{\text{experimental}} > T_{\text{critical}}$) in the presence of 1-heptanesulfonic acid, sodium salt with pure- ($T_{\text{exp.}}=3.6$), 10%- ($T_{\text{exp.}}=5.9$), and 20%-methanol-modified CO₂ ($T_{\text{exp.}}=3.8$).

The need for the presence of methanol inside the extraction vessel was next examined. For example, if the spiked-sand surface (100- μ L pseudoephedrine solution + 100- μ L pure methanol or ion-pair solution) was allowed to dry overnight prior to extraction, would the recoveries of pseudoephedrine be enhanced by addition of 1-heptanesulfonic acid, sodium salt relative to the situation without ion-pairing reagent with pure-, 10%-, and 20%-methanol-modified CO₂? The percent recoveries of pseudoephedrine with and without the addition of 1-heptanesulfonic acid, sodium salt from a previously dried spiked-sand surface are shown in **Figure 5.3**. The recoveries of

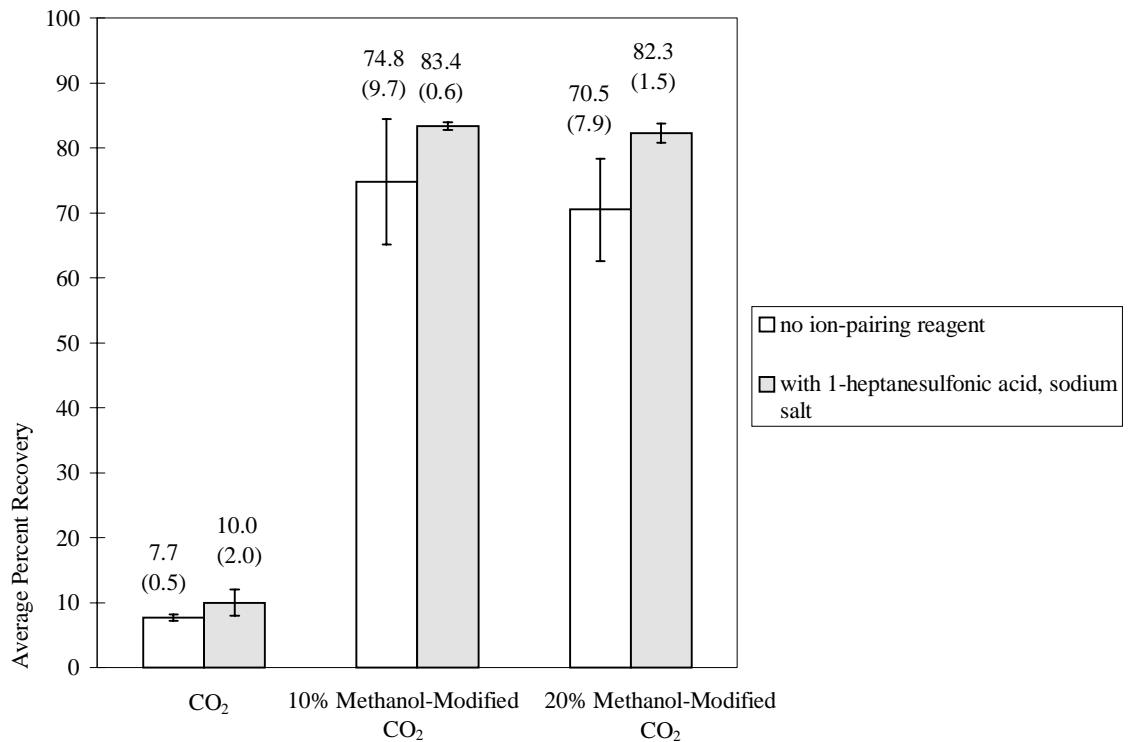


Figure 5.3. Average Percent Recoveries (n=3) of Pseudoephedrine and Pseudoephedrine with 1-Heptanesulfonic Acid, Sodium Salt from a Dried Drug Spiked-Sand Surface. Bars and Numbers in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 1.0 mL/min., 5 min. static time, 25 min. dynamic time.

pseudoephedrine in the absence of the ion-pairing reagent with pure-, 10%-, and 20%-methanol-modified CO₂ were first compared. With pure carbon dioxide, only 7.7% of the spiked pseudoephedrine was recovered. Methanol-modified CO₂ was then utilized to increase the solvating power of the medium. The recovery of pseudoephedrine from the previously dried spiked-surface increased dramatically from 7.7% to 74.8% with 10%-methanol-modified carbon dioxide. This favorable result was expected as the addition of methanol should increase the solvating power of the fluid for the polar compound. Methanol-modified CO₂ at 20% (v/v) was then utilized in an attempt to further increase the extraction efficiency in the absence of the ion-pairing reagent; however, no significant increase (70.5%) was observed.

The usefulness of the addition of the 1-heptanesulfonic acid, sodium salt to the pseudoephedrine hydrochloride spiked-sand surface that was previously dried was then examined with pure- and methanol-modified carbon dioxide. The recoveries obtained in the presence of the ion-pairing reagent as compared to the situation without the ion-pairing reagent were statistically comparable with pure- (7.7% without vs 10.0% with), 10% methanol- (74.8% without vs 83.4% with), and 20% methanol-modified CO₂ (70.5% without vs 82.3% with). In other words, the ion-pairing reagent apparently had no significant effect on extraction efficiency under these conditions.

The presence of in-cell methanol has, therefore, been shown to play a vital role in the extraction process. If indeed an ion-pair complex is being created, the ion-pairing reagent must be allowed to form the pseudoephedrine-sulfonate ion-pair in the pressurized CO₂ fluid during the equilibration period prior to the dynamic extraction. Methanol appears to serve two purposes: 1) increases the solubility of the formed ion-pair in pure-, and methanol-modified carbon dioxide, and 2) support the pseudoephedrine-sulfonate formation.

Phase II - Investigation of Ion-Pairing Reagent Composition and Concentration on the SFE of Pseudoephedrine From A Spiked-Sand Surface

From Phase I it was shown that the extraction of pseudoephedrine from a non-dried spiked-sand surface was feasible with pure and methanol-modified carbon dioxide, and that the recoveries were statistically enhanced when the matrix was in the presence of a methanol solution of 1-heptanesulfonic acid, sodium salt versus methanol alone. It was observed in Chapter IV that an increase in the lipophilicity of the ion-pairing reagent as well as an increase in its concentration relative to the analyte enhanced the extraction efficiencies. Therefore, it was the objective of Phase II to determine if the same trend would hold true when extracting a cationic species. In this case several alkylsulfonic acids varying in chainlength were investigated including 1-butane-, 1-heptane-, and 1-decanesulfonic acid, sodium salt. It was hypothesized that if ion-pair formation between the analyte and reagent occurred, recovery of the pseudoephedrine should increase. Therefore, recoveries of pseudoephedrine in the presence of 1-decane vs 1-butanefulfonic acid, sodium salt should be greater. Also, the amount of ion-pairing reagent added was investigated (i.e. a 1:1 mole ratio and a 5:1 mole ratio of ion-pairing reagent to analyte). Attempts to increase the recovery of cationic analytes by ion-pairing reagent compositional changes have not been found in the literature.

The spike mass of pseudoephedrine hydrochloride was increased from 100 μg to 300 μg ; while, the spike volume of methanol with and without the ion-pairing additive was reduced from 200 μL to 50 μL . The spike mass was increased and the methanol solution volume was decreased in order that changes in recovery due to the ion-pairing reagent could be observed in greater detail. Therefore, the purpose of this study was not necessarily to achieve quantitative recoveries but to examine the extraction process in more fundamental detail. **Figure 5.4** shows the recoveries of pseudoephedrine in the presence and absence of various ion-pairing reagents at variable concentrations. Error bars represent one standard deviation. For baseline purposes, extraction of pseudoephedrine hydrochloride from a previously dried spiked-sand surface were

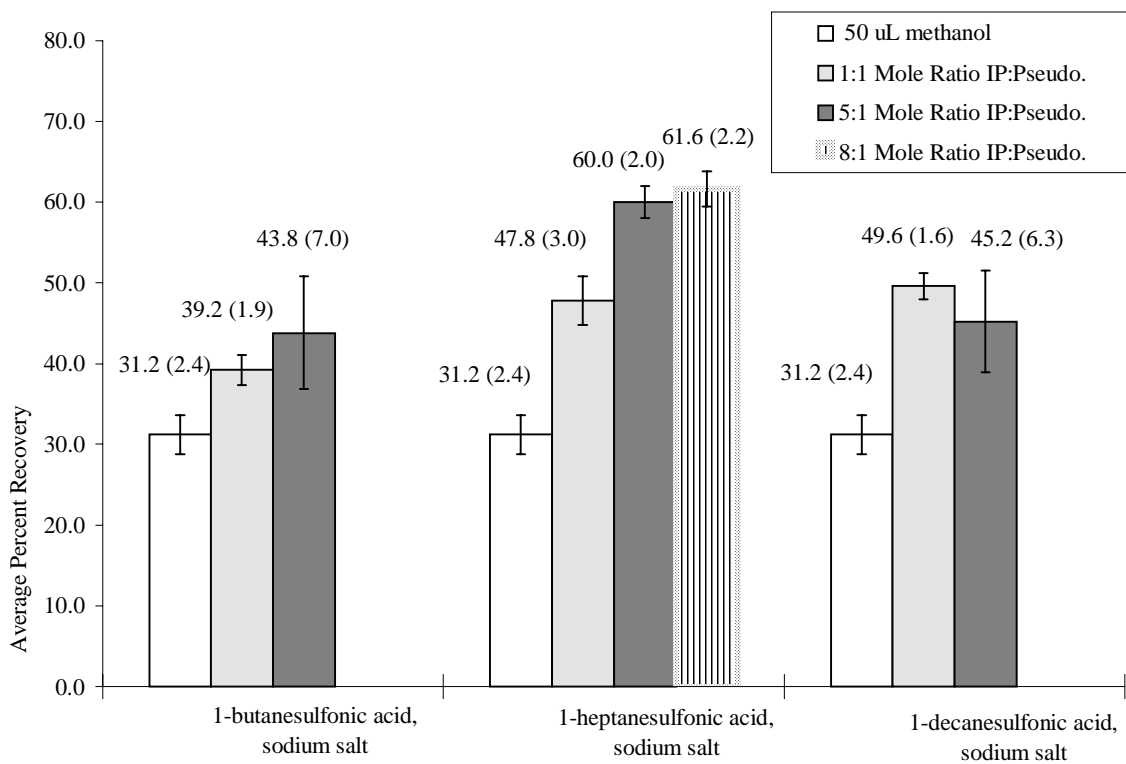


Figure 5.4. Average Percent Recoveries (n=3) of Pseudoephedrine with and without Various Ion-Pairing Additives at Various Concentrations from a Drug Spiked-Sand Surface. Error Bars and Values in Parentheses Represent One Standard Deviation. SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 1.0 mL/min., 5 min. static time, 40 grams CO₂. In-cell modifier volume - 50 μL methanol or ion-pairing reagent in methanol.

performed with pure CO₂, and only 10% (+/- 4.0%) of 300 µg pseudoephedrine was recovered. The ion-pairing reagent must be introduced as a solution of methanol. In order to examine the effectiveness of the ion-pairing reagent, first the sand surface was spiked with only 50 µL of pure methanol. Recovery was enhanced from 10% (no methanol) to 31% (50 µL methanol). Methanol may have served two purposes: 1) disruption of analyte-sand interaction and 2) increased the solvating power of the fluid.

Next the recoveries as a function of ion-pairing reagent composition (1:1 mole ratio of pseudoephedrine:ion-pairing reagent) were examined (**Figure 5.4**). In the presence of 1-butanesulfonic acid, sodium salt 1-heptanesulfonic acid, sodium salt and 1-decanesulfonic acid, sodium salt the recoveries were shown to increase by approximately 8%, 16%, and 18% respectively. Paired t-tests (95% confidence interval, T-critical = 2.9 (one-tailed)) were performed to determine if the recoveries in the presence of the ion-pairing reagents were indeed greater. The T-experimental values were determined to be: 4.5 (no reagent vs 1-butanesulfonic acid, sodium salt), 7.5 (none vs 1-heptanesulfonic acid, sodium salt), and 11.1 (none vs 1-decanesulfonic acid, sodium salt). In all three cases, a statistical enhancement was observed as compared to a purely methanol enriched spiked-surface. When comparing the composition of the ion-pairing reagent, it can be seen that the recoveries in the presence of 1-heptanesulfonic acid, sodium salt were greater than with 1-butanesulfonic acid, sodium salt. As the formed ion-pair became less polar, its solubility in the CO₂ should increase. Surprisingly, a further increase in recovery in the presence of 1-decanesulfonic acid, sodium salt versus 1-heptanesulfonic acid, sodium salt was not observed. The preparation of the 1-decanesulfonic acid, sodium salt spike-solution in methanol was problematic because sonication with heating was needed. It is believed that when in the presence of a less polar medium (CO₂/methanol) versus methanol, precipitation of 1-decanesulfonic acid, sodium salt occurred. Therefore, less ion-pairing reagent would be present to form the ion-pair thus resulting in no further enhancement. Let it be noted that the dissolution of the other ion-pairing reagents in methanol was immediate.

The pseudoephedrine recoveries in the presence of an excess of the various ion-pairing reagents were next compared. As in Chapter IV, it was believed that an excess of ion-pairing reagent would be advantageous because the equilibrium would be shifted thus favoring more complete ion-pair formation. At the lower concentration (5:1), significant increases were observed where 43%, 60%, and 45% pseudoephedrine was recovered with 1-butananesulfonic acid, sodium salt, 1-heptanesulfonic acid, sodium salt, and 1-decanesulfonic acid, sodium salt as compared to 31% with methanol alone (**Figure 5.4**). Of the three ion-pairing reagents investigated, only the addition of an excess of 1-heptanesulfonic acid, sodium salt further increased recovery. An enhancement of 13% was seen with the 5:1 mole ratio versus the 1:1 mole ratio. This was confirmed by a paired t-test ($T\text{-exp.}=5.9$). Next, it was of interest to increase the amount of 1-heptanesulfonic acid, sodium salt added from 5:1 to 8:1 to see if the recoveries could be further increased. However this strategy was not beneficial. In the case of 1-butananesulfonic acid, sodium salt, it was believed that complete ion-pair formation occurred at a 1:1 mole ratio. Also, no further increase was observed when adding an excess of 1-decanesulfonic acid, sodium salt. As stated before, there were problems dissolving 1-decanesulfonic acid, sodium salt in methanol. If the solubility of 1-decanesulfonic acid, sodium salt in the fluid played a role thus causing precipitation, this effect would no doubt be further worsened at a higher concentration.

As described in Phase I, the effectiveness of the ion-pairing reagent may be due to displacement. Here, when added in excess, the reagent may cover more active sites than at the 1:1 mole ratio thus freeing the analyte from the sand to a greater extent.

Phase III - Effect of Various In-Cell Modifiers on the SFE of Pseudoephedrine From A Spiked-Sand Surface

It was shown in Phase II that the extraction efficiencies of pseudoephedrine from spiked-sand with pure CO₂ were increased in the presence of various methanol solutions of ion-pairing additives. Recovery was shown to be a function of ion-pairing reagent

composition and concentration. The most successful recovery of 60% was achieved in the presence of 1-heptanesulfonic acid, sodium salt in methanol at a 5:1 mole ratio. When compared to the purely methanol enriched sand-surface, a 30% enhancement was observed with 1-heptanesulfonic acid, sodium salt.

The objective of Phase III was to examine the effect of other types of in-cell modifiers such as a methanol-water mixture, an acid, and a base. By modifying the methanol-spike with water, an overall increase in the solvating power of the fluid should occur. Thus, the extractability of the polar pseudoephedrine in the presence of methanol/water may increase as compared to methanol alone. Hydrogen bonding interactions between the fluid and the analyte should also increase. Second, the water-methanol mixture would be expected to interact with the sand through hydrogen bonding interactions to a greater extent than methanol alone thus freeing any bound pseudoephedrine. **Figure 5.5** shows the recovery of pseudoephedrine obtained in the presence of several in-cell modifiers. As stated previously, when the spiked-sand surface was allowed to dry prior to extraction and extracted with pure CO₂, 10% pseudoephedrine was recovered. By simply modifying the matrix with 50 μL of methanol, 31% pseudoephedrine was recovered. Next the spiked-sand surface was spiked with 50 μL of methanol containing 1.0% (v/v) water. As expected the pseudoephedrine recovery was further increased by 23% to 59% versus methanol alone. A paired t-test (95% confidence interval, T-critical=2.9) was performed, and a statistical increase was confirmed (T-exp.= 7.6). Also, the recovery with the methanol/water mixture alone versus the recovery with 1-heptanesulfonic acid, sodium salt in methanol was compared, and it was shown that the methanol/water mixture was just as effective as pure methanol containing 1-heptanesulfonic acid, sodium salt. Next a solution of 1-heptanesulfonic acid, sodium salt (5:1 mole ratio) in methanol (1% (v/v) water) was spiked onto the matrix. Surprisingly, the recovery with methanol/H₂O alone versus recovery with 1-heptanesulfonic acid, sodium salt in methanol/H₂O was comparable (**Figure 5.5**).

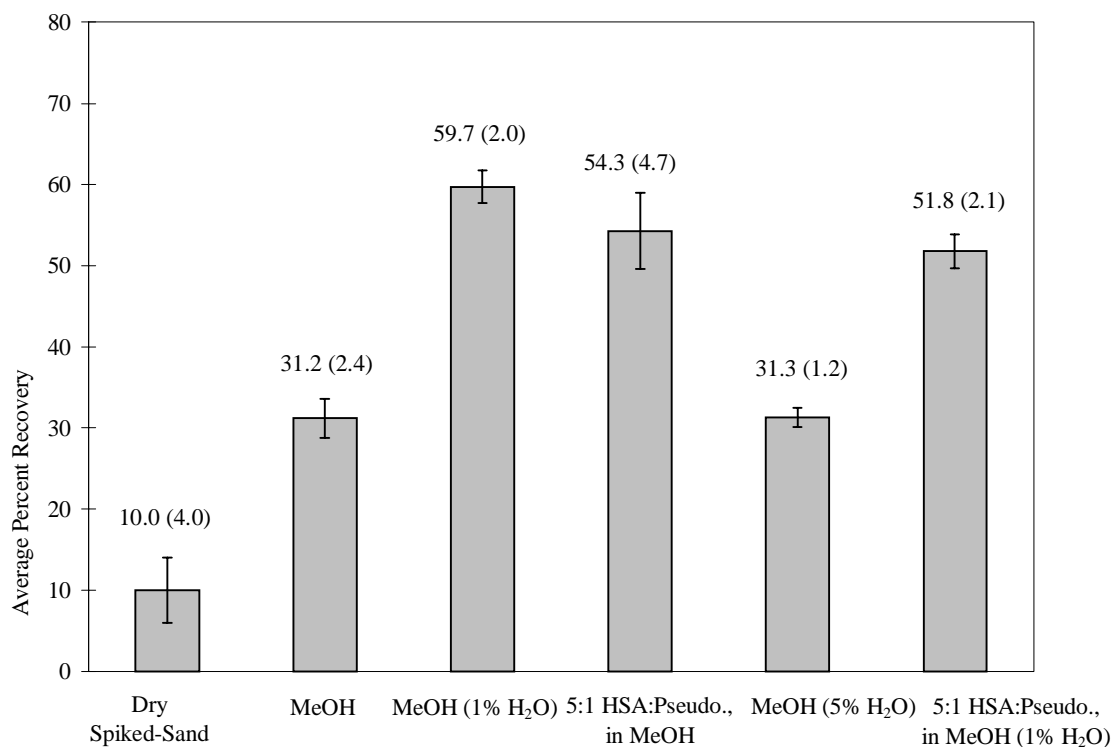


Figure 5.5. Average Percent Recoveries (n=3) of Pseudoephedrine with Various Modifiers and Ion-Pairing Additives from a Drug Spiked-Sand Surface. Error Bars and Values in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 1.0 mL/min., 5 min. static time, 40 grams CO₂, 50 µL in-cell modifier.

Pseudo. - Pseudoephedrine

HSA - 1-heptanesulfonic acid, sodium salt

The effect of water concentration was next examined. For example, the percentage of water in methanol was increased from 1% (v/v) to 5% (v/v), however this strategy did not prove worthwhile. The pseudoephedrine recovery decreased from 59% (1% H₂O) to 31% (5% H₂O) (**Figure 5.5**). In this case it was believed that the solubility of water in the CO₂ was exceeded, therefore, a two-phase system resulted. It would be expected then that a portion of the methanol-water dissolved in the bulk fluid while a plug of methanol-water remained on the sand surface. Consequently a percentage of pseudoephedrine would be solubilized into the bulk CO₂ fluid while a percentage would remain entrapped in the aqueous solution. Due to the high polarity of the aqueous solution, the ability of the bulk fluid to partition into the aqueous solution and thus dissolve and remove the analyte would be reduced.

The effect of acid and base on the pseudoephedrine recovery from a spiked-sand surface was next determined. As stated before, an equilibrium exists between the ionized pseudoephedrine and free base (**Equation 1a and 1b**). By simply adding an acid or a base, the equilibrium should shift thereby favoring the ionic or neutral species. It is expected that in the presence of an acid, more charged species would exist and, therefore, the pseudoephedrine recovery should decrease. Likewise, formation of the free base should be favored under basic conditions thus an increase in the recovery should be observed. Separate solutions of methanol containing 0.1% (v/v) trifluoroacetic acid and 0.1% (v/v) tetrabutylammonium hydroxide were prepared and spiked onto the drug-spiked surface at a volume of 50 μ L. The effect of the addition of the acid and base upon the recovery of pseudoephedrine is shown in **Figure 5.6**. In the presence of trifluoroacetic acid, the recovery increased by approximately 13% from 31% (methanol alone) to 44% (methanol with trifluoroacetic acid). Again, a paired t-test verified a statistical increase (T-exp.=6.2). This increase was unanticipated because under acidic conditions the analyte should exist solely ionized. As an acid, some trifluoroacetic acid will ionize. Ion-pair formation between the ionic pseudoephedrine and the ionized trifluoroacetic acid may have occurred. Also, the negatively charged acid may through electrostatic interactions

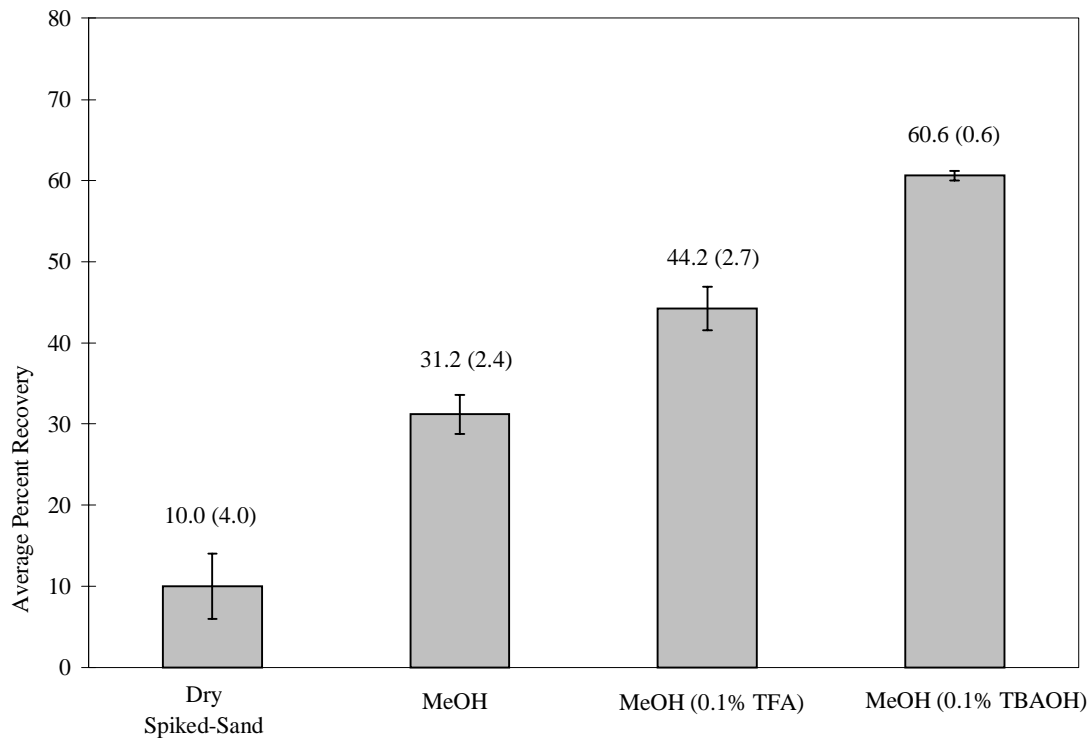


Figure 5.6. Average Percent Recoveries (n=3) of Pseudoephedrine from a Drug Spiked-Sand Surface. Effect of Addition of Acid and Base. Error Bars and Values in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 1.0 mL/min., 5 min. static time, 40 grams CO₂, 50 µL in-cell modifier.

TFA - Trifluoroacetic acid

TBAOH - Tetrabutylammonium hydroxide

adsorb to any exposed positively charged sites on the sand surface thus displacing the ionic pseudoephedrine. Once released into the bulk fluid, a portion of the charged pseudoephedrine will be converted to free base and extracted. Likewise, through hydrophobic interactions, trifluoroacetic acid may adsorb to any neutral sites freeing the free base from the matrix.

The effect of the addition of tetrabutylammonium hydroxide was next examined. As expected, the pseudoephedrine recovery was statistically enhanced by 29% in the presence of tetrabutylammonium hydroxide from 31% (methanol alone) to 60% (methanol with tetrabutylammonium hydroxide) (T-exp.=10.3) (**Figure 5.6**). First, the base may serve as an analyte-matrix displacer. Second, under basic conditions, the pseudoephedrine existed predominantly as the free base thus favoring its extraction in CO₂ as compared to the ionic species.

Phase IV - Extraction Of Suphedrine Tablets

Pseudoephedrine recoveries (from non-dried drug spike studies) achieved with the addition of an excess of 1-heptanesulfonic acid, sodium salt in methanol, methanol (1% water), and methanol (0.1% tetrabutylammonium hydroxide) were shown to be comparable. Quantitative recovery from a spiked-sand was, however, not achieved. The effect of various modifiers and additives was nevertheless examined, and several enhancement strategies were discovered.

The extraction from a tablet matrix may be considered more difficult than extraction from a spiked-sand surface due simply to the tablet's complicated composition. Besides the active drug component, pseudoephedrine, Suphedrine tablets contain: carnauba wax, dicalcium phosphate, FD&C Red No. 40, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, silicon dioxide, and titanium dioxide. Strong interactions between the pseudoephedrine and the matrix were expected.

It was observed in Phase III that pseudoephedrine recovery from spiked-sand can be enhanced by a) adding an ion-pairing additive, 2) varying the composition of the in-cell modifier, and 3) favoring free base formation. It was therefore our objective to apply these same strategies to the extraction of pseudoephedrine from a more complicated matrix, Suphedrine tablets. Besides pure CO₂, modified-carbon dioxide was investigated in this part of the study because the amount of pseudoephedrine in the tablet was 30 mg. It was expected that CO₂ alone would not be able to extract such a large amount in a timely manner regardless of the presence of an in-cell modifier.

100% CO₂

As described in the Experimental, one Suphedrine tablet was crushed and placed into an extraction vessel that contained approximately 50% cotton. More cotton was placed on top of the crushed tablet powder so as to eliminate possible clogging of the extraction vessel frits with tiny tablet particles and to reduce the void volume in the extraction vessel. Then either the extraction was performed immediately or a modifier solution was added directly to the cotton matrix. Extractions were then performed with pure CO₂. The amount of modifier spiked into the vessel was increased from 50 μL (spike-studies) to 400 μL. This spike volume (400 μL) was chosen for the following reasons. From Phase II, it was shown that pseudoephedrine recovery was enhanced if there was an excess of 1-heptanesulfonic acid, sodium salt. In order to achieve this situation, large amounts of methanol dissolved with 1-heptanesulfonic acid, sodium salt must be added to the matrix therein. The addition of 400 μL was the smallest volume that could be added to ensure that the mole ratio of 1-heptanesulfonic acid, sodium salt to pseudoephedrine inside the extraction vessel was 2:1. Larger in-cell modifier spike volumes could have been used, however, slight difference due to the effectiveness of the ion-pairing reagent may be more difficult to ascertain. Also, experimental difficulties such as restrictor plugging occurred at in-cell modifier spike volumes greater than 500 μL.

Other extraction parameters were also altered for the tablets. The CO₂ liquid flow rate was increased from 1.0 mL/min. to 2.0 mL/min. while the dynamic extraction time remained the same as in the spike studies (40 min.). Since much larger amounts of pseudoephedrine needed to be extracted, the amount of fluid was increased; however, a reasonably short extraction time was desired. As in the extraction of the MEVACOR® tablets, dynamic extraction mini-steps were employed so that: 1) the solid-phase trap could be rinsed in short intervals in case that the trapping capacity was exceeded, and 2) to increase the equilibration time between the fluid and the matrix in case of a diffusion limited process.

The extraction with pure CO₂ of pseudoephedrine from Suphedrine tablets with the addition of various modifiers to the matrix was performed (**Figure 5.7**). With pure CO₂ only 0.1% of 30 mg was extracted. This was expected due to the low anticipated solubility of pseudoephedrine in pure CO₂ as well as the possibility of very strong analyte-matrix binding. In order to decrease the analyte-matrix interactions as well as increase the solvating power of the fluid, methanol was added to the extraction vessel as an in-cell modifier. Pseudoephedrine recovery was 8%. Next 400 µL of 1-heptanesulfonic acid, sodium salt (2:1 mole ratio) in methanol was added to the tablet matrix. Heptanesulfonic acid, sodium salt addition with the pure CO₂ fluid was shown to be partially successful since the recovery was doubled to 16% (1-heptanesulfonic acid, sodium salt in methanol).

Next a methanol/water mixture as an in-cell modifier was investigated. The recovery of pseudoephedrine was 15% (methanol/water). As in the spike-studies, the recovery in the presence of the ion-pairing reagent, 1-heptanesulfonic acid, sodium salt in methanol, was comparable to the recovery with only the methanol/water spike and no 1-heptanesulfonic acid, sodium salt. Next, 1-heptanesulfonic acid, sodium salt was added to the methanol/water spike solution but only 16% pseudoephedrine was again recovered. A similar trend was observed in the spike studies where no further enhancement was observed when 1-heptanesulfonic acid, sodium salt was added to the methanol/water spike solution.

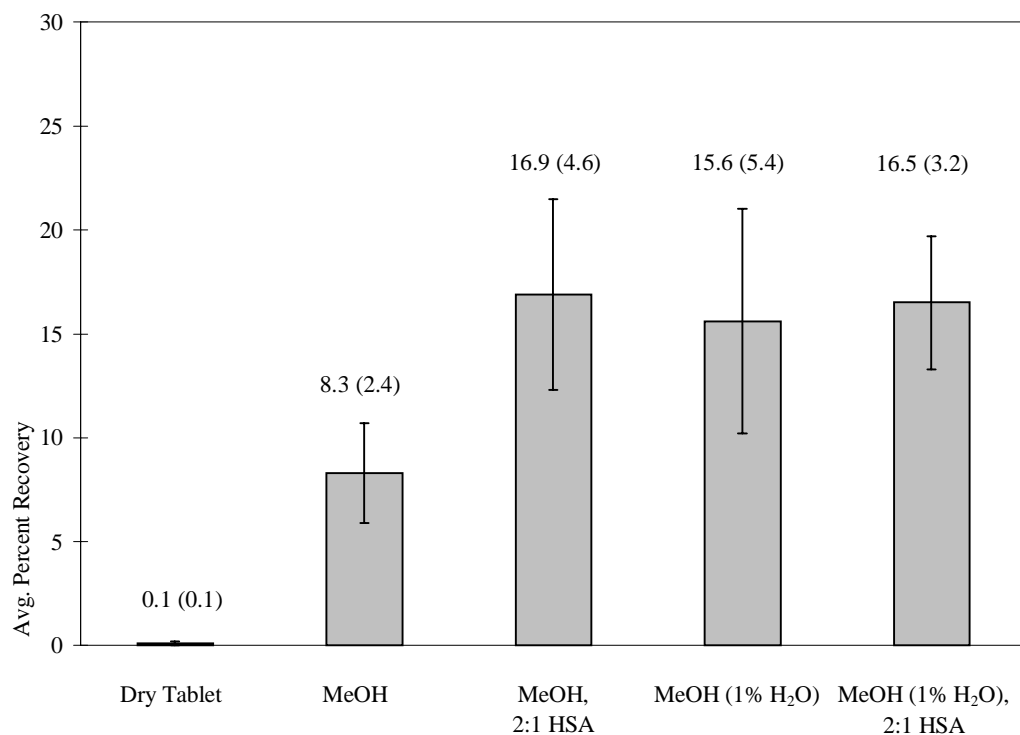


Figure 5.7. Average Percent Recovery (n=3) of Pseudoephedrine from Suphedrine Tablets as a Function of In-Cell Modifier Composition with Pure CO₂. Error Bars and Numbers in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 2.0 mL/min., 5 min. static time, 80 grams CO₂ (5 dynamic mini-steps), 2 min. static time during trap rinsing between dynamic mini-steps, 400 μL in-cell modifier volume.

HSA - 1-heptanesulfonic acid, sodium salt

10% Methanol-Modified CO₂

The extraction efficiencies of pseudoephedrine from Suphedrine tablets were less than desirable with pure CO₂ regardless of in-cell modifier composition. The addition of modifier directly to the CO₂ via a HPLC pump was next investigated as a means of further increasing recovery. **Figure 5.8** examines the effect of various in-cell modifiers with the fluid being methanol-modified CO₂. First a Suphedrine tablet without an in-cell modifier was extracted with 10% methanol-modified CO₂. An increase from 0.1% (pure CO₂) (**Figure 5.7**) to 44% was observed. The addition of an in-line modifier no doubt increased the solvating power of the extraction fluid thus favoring the extraction of the polar pseudoephedrine from the tablet. This was expected because of methanol's ability to hydrogen bond with the analyte. Likewise the methanol could be displacing the pseudoephedrine from the tablet powder.

The addition of methanol directly to the matrix was next investigated. Pseudoephedrine recovery in the presence of 400 µL methanol in-cell modifier increased from 44% to 51%. Attempts were made to further increase recovery by increasing the percentage of in-line methanol from 10% to 20%. It was expected that a subsequent increase in the solvating power of the fluid would result at a higher modifier percentage therefore more pseudoephedrine should be recovered. Surprisingly only 52% pseudoephedrine was recovered from the tablet with 20% methanol-modified CO₂. Since no further increase was observed, it was believed that the extraction of pseudoephedrine was not solubility limited but diffusion limited.

Subsequently the addition of the ion-pairing reagent, 1-heptanesulfonic acid, sodium salt in a 2:1 mole ratio was considered. As expected and previously observed via spike studies, a pseudoephedrine recovery from 51% (methanol-spike alone) to 72% (with 1-heptanesulfonic acid, sodium salt) was observed with 10% methanol-modified CO₂. Next it was of interest to examine if quantitative recovery could be obtained if 1-heptanesulfonic acid, sodium salt in methanol was used as an

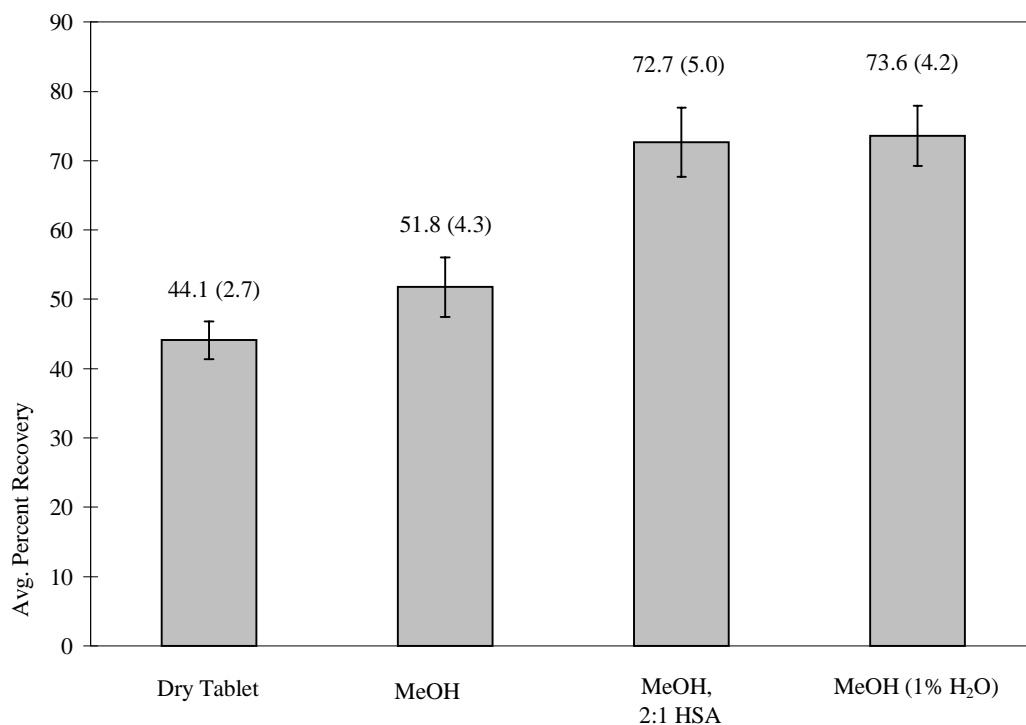


Figure 5.8. Average Percent Recovery (n=3) of Pseudoephedrine from Suphedrine Tablets as a Function of In-Cell Modifier Composition with 10% Methanol-Modified CO₂. Error Bars and Numbers in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 2.0 mL/min., 5 min. static time, 80 grams CO₂ (5 dynamic mini-steps), 2 min. static time during trap rinsing between dynamic mini-steps, 400 µL in-cell modifier volume

HSA - 1-heptanesulfonic acid, sodium salt

in-line modifier. It was expected that further ion-pair formation would occur if the ion-pairing reagent was added continuously. Unfortunately this strategy could not be considered due to experimental difficulties. Problems including modifier pump seal and check valve damage as well as line clogging resulted, therefore, this option was no longer explored.

Finally, a methanol/water mixture was investigated as an in-cell modifier with 10% methanol-modified CO₂. Recoveries increased from 51% (methanol spike alone) to 73% (methanol/water). Once again the methanol/water in-cell modifier proved just as useful the ion-pairing reagent in pure methanol.

Although the addition of tetrabutylammonium hydroxide in methanol to the cell was shown to be beneficial in the extraction of pseudoephedrine from spiked-sand, its role as an in-line modifier was not examined for the tablet extractions. Tetrabutylammonium hydroxide was not explored because: 1) under highly basic conditions, tetrabutylammonium hydroxide may react with the pseudoephedrine causing degradation, 2) immediate flushing of the extractor lines would be needed so as to eliminate possible precipitation, and 3) a methanol/water modifier would be much easier to work with assuming equal effectiveness.

10% (1% H₂O) Methanol-Modified CO₂

The benefits of using the methanol/water mixture as an in-cell modifier were substantial. Next, its ability to serve as an in-line modifier was explored. Extractions were performed with 10% (1% H₂O) methanol-modified CO₂ while the effect of the addition of the ion-pairing reagent and the methanol/water in-cell spike was also examined (**Figure 5.9**). When extracting the tablet with 10% (1% H₂O) methanol in the absence of any in-cell modifier, 51% pseudoephedrine was extracted. When compared to 10% methanol-modified CO₂, a slight enhancement of 7% with the methanol/water in-line modifier was observed.

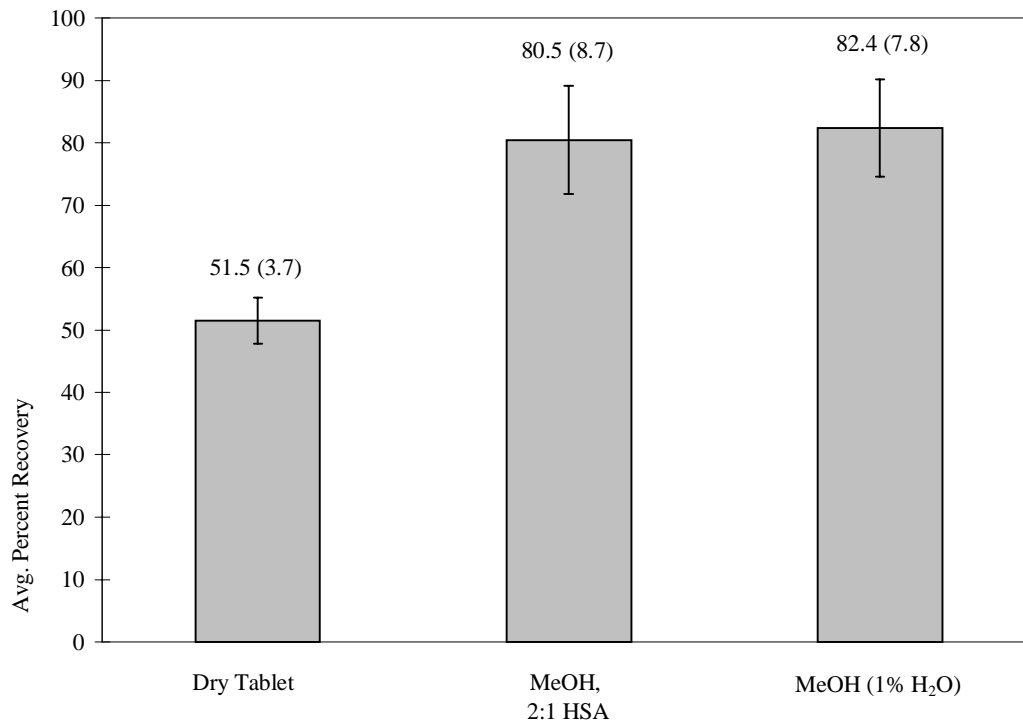


Figure 5.9. Average Percent Recovery (n=3) of Pseudoephedrine from Suphedrine Tablets as a Function of In-Cell Modifier Composition with 10% (1% H₂O) Methanol-Modified CO₂. Error Bars and Numbers in Parentheses Represent One Standard Deviation.

SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 2.0 mL/min., 5 min. static time, 80 grams CO₂ (5 dynamic mini-steps), 2 min. static time during trap rinsing between dynamic mini-steps, 400 μL in-cell modifier volume

HSA - 1-heptanesulfonic acid, sodium salt

The ability of the ion-pairing reagent to serve an extraction aide was also explored using this fluid. Heptanesulfonic acid, sodium salt (2:1 mole ratio) was dissolved in methanol, and the tablet was spiked at a volume of 400 μL . As expected a significant increase versus the dry tablet was observed where 80% was extracted. Extractions in the presence of a methanol in-cell modifier were not performed with this fluid. In-line modifier composition comparisons were then made between the two modifiers, 10% methanol and 10% (1% H_2O) in the presence of 1-heptanesulfonic acid, sodium salt where 72% and 80% were reported respectively. A paired t-test was performed, and the pseudoephedrine recoveries in the presence of a 1-heptanesulfonic acid, sodium salt methanol solution versus the two in-line modifiers were comparable.

Next tablet extractions with 10% (1% H_2O) methanol-modified CO_2 were performed in the presence of a methanol/water in-cell modifier and 82% was recovered. Recoveries in the presence of either methanol/water or 1-heptanesulfonic acid, sodium salt in methanol were similar.

Recovery as a function of in-line modifier composition (methanol, methanol/water) in the presence of the methanol/water spike were then compared where 73% (10% methanol) and 82% (10% methanol (1% H_2O) pseudoephedrine was recovered. A paired t-test was performed, and it was shown that regardless of in-line modifier composition, similar recoveries were obtained.

Regardless of the composition of the in-cell modifier, the results obtained were similar. It was expected that the recoveries with the methanol/water in-line modifier should be greater as compared to methanol alone because the solubility of pseudoephedrine in a more polar fluid should increase. Once again it is postulated that the extraction of pseudoephedrine from the tablet matrix regardless of the in-line modified fluid was not solubility limited but diffusion limited.

Of the various strategies investigated, the extractions with 10% methanol (1% H_2O) in the presence of a 400 μL spike of methanol (1% H_2O) and 1-heptanesulfonic acid, sodium salt in methanol were shown to be equally successful. Of the two in-cell modifiers,

methanol/water would be suggested for further investigation due to the simplicity of its use. When using the ion-pairing reagent in-cell modifier restrictor plugging was observed during the first dynamic mini-step most probably due to the large amounts of both extracted analyte and ion-pairing reagent. However, this occurrence was not observed with the methanol/water in-cell modifier.

Identification of the Extracted Analyte

Previous studies have made attempts to extract amine hydrochlorides with pure and modified carbon dioxide.¹⁻³ Of the three reports, the composition of the extracted analyte was not identified. In all three cases, it was believed that the free base was extracted because salts should not be extractable by SFE due to their ionic characteristics. Therefore it was of interest to identify the form of pseudoephedrine that was being extracted in the absence of any in-cell modifier. Suphedrine tablet extractions were performed with 10% methanol- and 10% (1% H₂O) methanol-modified CO₂. Silver chloride tests were conducted to confirm the presence of chloride in the extracts. Approximately 1-2 mL of 5% (w/v) silver nitrate in water was added to pure methanol and to the methanol extract solutions. When silver nitrate was added to pure methanol, the solution remained clear and colorless. Therefore, it was assured that the amount of chloride in methanol was negligible. Then AgNO₃ was added to the extract solutions (methanol and methanol/water in-line modified), and a white precipitate was observed. Therefore it was concluded that chloride was present in the extracts.

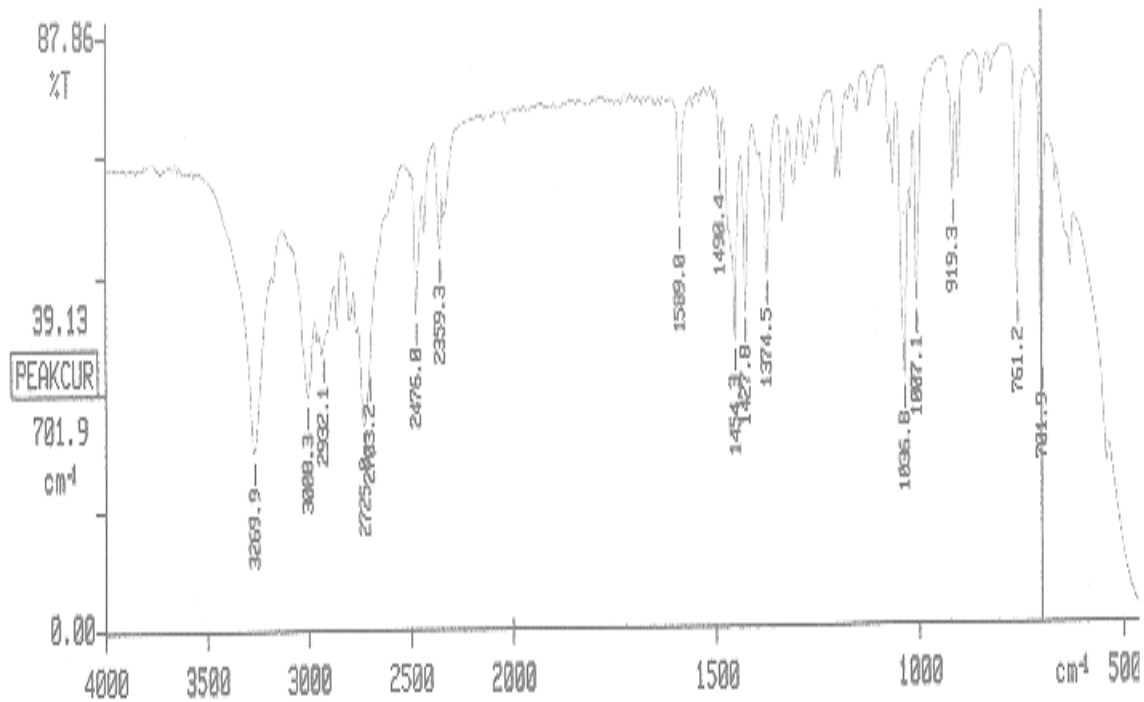
Next infrared spectra of the extracts were obtained with a Perkin Elmer (Norwich, CT) Series 1600 FT-IR spectrophotometer. First a pseudoephedrine hydrochloride solution containing the expected extractant concentration was prepared in methanol. This will be referred to as the pseudoephedrine hydrochloride standard. Via a nitrogen purge, the extract solutions were evaporated to approximately 1.0 mL. Using a disposable pipette, the extract solution was spiked onto a NaCl salt plate and allowed to dry. This

process was repeated until a visual film was observed. Then an IR spectrum of the spiked-salt plate was obtained. The IR of the pseudoephedrine hydrochloride standard is shown in **Figure 5.10**. IR studies of pseudoephedrine hydrochloride from a KBr pellet were previously performed by Benezra et al.⁷ Several spectral assignments were made: 1) OH stretch (3270 cm^{-1}), 2) asymmetric C-H stretch (3010 cm^{-1}), 3) symmetric C-H stretch (2930 cm^{-1}), 4) ^+NH stretch (2700 cm^{-1}), 5) C=C aromatic stretch ($1587, 1490\text{ cm}^{-1}$), OH bend, secondary alcohol (1430 cm^{-1}), and 6) C-H bend, monosubst. benzene ($762, 702\text{ cm}^{-1}$). The spectra of the pseudoephedrine hydrochloride standard and the literature reference were compared and similarities were noted. Bands corresponding to the OH stretch, asym. and sym. C-H stretch, C=C aromatic stretch, OH bend, secondary alcohol, and C-H bend, monosubst. benzene were observed at approximately \pm one wavenumber as compared to the literature reference. The ^+NH stretch for the standard appeared at approximately 2725 cm^{-1} versus 2700 cm^{-1} as referenced; however this difference was felt to be insignificant. Suphedrine tablets were then extracted with 10% methanol- and 10% (1% H_2O) methanol-modified CO_2 , and the infrared spectra were obtained (**Figures 5.11 - 5.12**). The spectra of the extracts and the pseudoephedrine hydrochloride standard were compared and almost identical spectra were observed regardless of modifier composition. Bands were observed at approximately 3270 (OH bend), 3010 (asym. C-H stretch), 1588 (C=C aromatic stretch), 1427 (OH bend, secondary alcohol), 761 , and 702 cm^{-1} (C-H bend, monosubst. benzene). The appearance of the band corresponding to the ^+NH stretch at 2725 cm^{-1} offered the most proof that the extracted pseudoephedrine existed as the hydrochloride salt versus the free base. Secondary amine salts absorb strongly in the $3000\text{-}2700\text{ cm}^{-1}$ with multiple bands extending to 2273 cm^{-1} .⁸ While secondary amines show a single weak band in the $3350\text{-}3310\text{ cm}^{-1}$ corresponding to the asym. and sym. N-H stretching, and medium-to-weak bands in the $1250\text{-}1020\text{ cm}^{-1}$ region corresponding to

⁷ K. Florey, ed., *Analytical Profiles of Drug Substances*, Volume 8, Academic Press, New York (1979) 489.

⁸ R.M. Silverstein, G.C. Bassler, T.C. Morrill, *Spectrometric Identification of Organic Compounds*, Fourth Edition, John Wiley and Sons, New York (1981) 125-129.

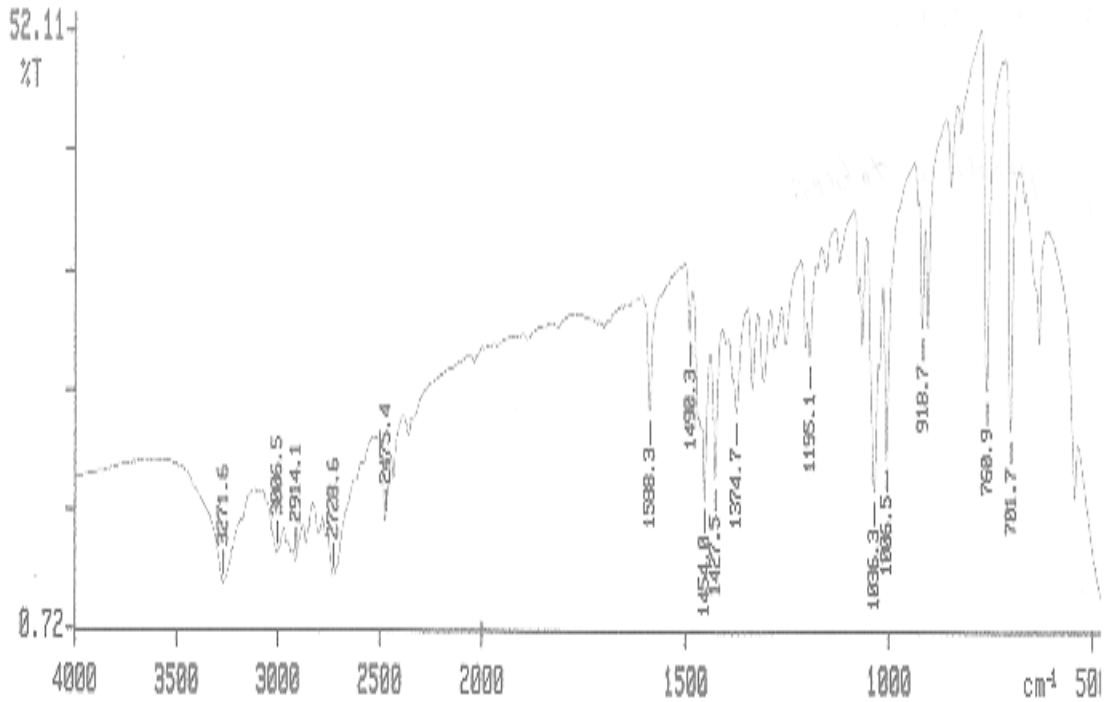
PERKIN ELMER



97/12/12 15:55
X: 16 scans, 4.0cm-1

Figure 5.10. Infrared Spectrum of Pseudoephedrine Hydrochloride Standard. Solution spotted on a NaCl plate

PERKIN ELMER



97/12/16 16:41

X: 16 scans, 4.0cm⁻¹

Figure 5.11. Infrared Spectrum of a Suphedrine Tablet Extract With 10% Methanol-Modified CO₂. Extract spotted on a NaCl plate.
SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 2.0 mL/min., 5 min. static time, 80 grams CO₂ (5 dynamic mini-steps), 2 min. static time during trap rinsing between dynamic mini-steps

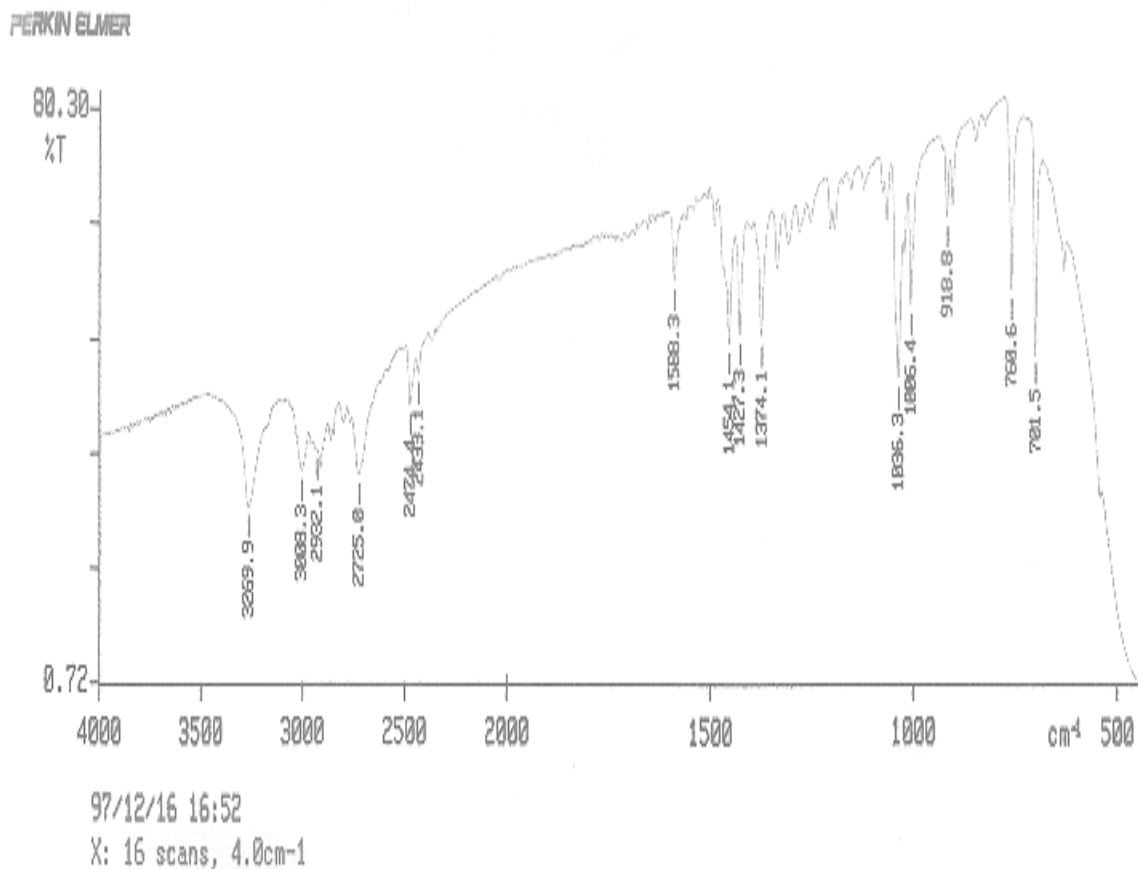


Figure 5.12. Infrared Spectrum of a Suphedrine Tablet Extract With 10% (1% H₂O) Methanol-Modified CO₂. Extract spotted on a NaCl plate.
SFE Conditions: 0.99 g/mL CO₂, 450 atm, 35 °C, 2.0 mL/min., 5 min. static time, 80 grams CO₂ (5 dynamic mini-steps), 2 min. static time during trap rinsing between dynamic mini-steps

C-N vibrations. From these experiments, it has been shown that hydrochloride salts can indeed be extracted with modified CO₂.

5.4 SUMMARY

The effect of modifier and additive composition upon pseudoephedrine recovery from spiked-sand and Suphedrine tablets was examined. First, the feasibility of enhancing the extractability through the use of an ion-pairing reagent was investigated. Recovery was shown to be dependent on CO₂ density as well as the addition of 1-heptanesulfonic acid, sodium salt to the spiked-sand surface. The presence of methanol in the extraction vessel prior to SFE was shown play a significant role in the ion-pair extraction process. The addition of ion-pairing reagent in methanol significantly increased the recovery of pseudoephedrine from a spiked-sand surface with pure-, 10%-, and 20%-methanol-modified CO₂. Quantitative pseudoephedrine recoveries of greater than 95% 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 1-heptanesulfonic acid, sodium salt in methanol were comparable. Subsequently no further enhancement was observed with 1-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. Recovery was shown to be enhanced in the presence of trifluoroacetic acid. Although it was expected that the equilibrium between the ionic and free base species would be shifted favoring the ionic species, a slight enhancement was observed. In this case it was postulated that through hydrophobic and electrostatic interactions, trifluoroacetic acid was preferentially adsorbing to exposed active sites thus displacing the pseudoephedrine from the spiked-sand. Also, ion-pair formation between trifluoroacetic acid and pseudoephedrine may have occurred. The extractability in the presence of tetrabutylammonium hydroxide was significantly enhanced as compared to methanol alone. Under basic conditions, free base formation was favored, 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 1-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, 1-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 CO₂. 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% H₂O) methanol-modified CO₂ in the presence of 400 μL of methanol (1% H₂O).

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.