Feasibility of ion-pair/supercritical fluid extraction of an ionic compound—pseudoephedrine hydrochloride

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Abstract

The supercritical fluid extraction (SFE) of an ionic compound, pseudoephedrine hydrochloride, from a spiked-sand surface was successfully demonstrated. The effect of carbon dioxide density (CO_2), supercritical fluid composition (pure vs. methanol modified), and the addition of a commonly used reversed-phase liquid chromatographic ion-pairing reagent, 1-heptanesulfonic acid, sodium salt, on extraction efficiency was examined. The extraction recoveries of pseudoephedrine hydrochloride with the addition of the ion-pairing reagent from a spiked-sand surface were shown to be statistically greater than the extraction recoveries without the ion-pairing reagent with both pure and methanol-modified carbon dioxide.

Keywords: Supercritical fluid extraction; Carbon dioxide; Pseudoephedrine hydrochloride; Ion-pairing reagent

1. Introduction

In the late 1980s, supercritical fluid extraction (SFE) emerged as a popular alternative to many sample preparation methods that required the use of hazardous organic solvents and laborious procedures. SFE has found applications in many fields including foods, polymers, and environmental matrices, but many analytical SFE applications still remain to be discovered [1–3].

Carbon dioxide is the most commonly used supercritical fluid because it is relatively inert, nontoxic, exhibits readily attainable critical parameters, and has solvent power equivalent to common organic solvents such as hexane and benzene. Compounds of relatively low polarity are generally soluble in pure CO_2 and easily extract assuming minimal analyte/matrix interactions. The addition of small amounts of polar organic solvents (i.e. modifiers), however, may be needed for the successful extraction of more polar compounds.

A number of pharmaceutical matrices have been successfully manipulated with SFE such as the extraction of drugs from animal feed, tablets, and ointments [4–6]. Many pharmaceutical compounds are ionic or ionizable, water soluble, and/or highly polar. The application of SFE in the pharmaceutical field may be considered problematical because it is believed that ionic compounds and/or salts are generally not soluble in supercritical carbon dioxide. One way to enhance the solubility of ionic compounds in supercritical carbon dioxide may be via charge neutralization

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through the use of ion-pairing reagents. The ion-pairing reagent serves two purposes by neutralizing the charge on the ionic species as well as adding lipophilic character to the compound. Both features should increase the solubility of the ionic compound in the relatively non-polar supercritical fluid.

In 1992 Field et al. [7] reported on ion-pair/SFE. Through the addition of tetrabutylammonium hydrogen sulfate to sewage sludge samples, secondary alkanesulfonates and linear alkylbenzene surfactants were quantitatively extracted as tetrabutylammonium-sulfonate ion-pairs with pure carbon dioxide. The extracted ion-pairs were then suggested to have been derivatized to butyl esters in the injection port of the gas chromatograph. Quantification and identification were by mass spectrometry.

Our paper describes a feasibility study of ion-pair/SFE applied to pseudoephedrine hydrochloride with a common reversed-phase liquid chromatographic ion-pairing reagent, 1-heptanesulfonic acid, sodium salt. This study differs markedly from the previous study in that the analyte is cationic rather than anionic. Chemical structure for pseudoephedrine hydrochloride is found in Fig. 1. An initial screening study was performed to qualitatively estimate the effect of CO_2 density, static extraction time, and the addition of the ion-pairing reagent on pseudoephedrine hydrochloride recovery from a sand surface. Then under constant SFE conditions, the extraction recoveries for pseudoephedrine hydrochloride in the presence and absence of 1-heptanesulfonic acid, sodium salt from a sand surface that had been pre-spiked with a methanol solution that either had been dried or non-dried with pure-, 10% methanol-modified-, and 20% methanol-modified-carbon dioxide are compared.
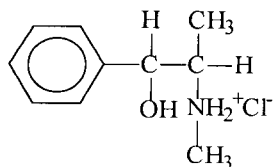

Fig. 1. Chemical Structure of Pseudoephedrine Hydrochloride.

2. Experimental

Extractions were performed on a Suprex Prepmaster (Pittsburgh, PA) 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, (Allentown, PA). Pseudoephedrine hydrochloride, 1-heptanesulfonic acid, sodium salt, and caffeine were obtained from Sigma (St. Louis, MO), respectively.

Solutions of pseudoephedrine hydrochloride and 1-heptanesulfonic acid, sodium salt were prepared in methanol at 1000 and 9000-ppm, respectively. A 100-μl aliquot of pseudoephedrine hydrochloride solution (100 μg) 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-μl of pure methanol or 100-μl of 1-heptanesulfonic acid, sodium salt, methanol solution (900 μg) was added to the drug-spiked sand surface. The total spike volume of methanol added to the matrix, therefore, was always 200-μl (e.g. 100-μl (pseudoephedrine solution) plus 100-μl (pure methanol or ion-pair methanol solution)). The sand was extracted immediately or after air drying overnight with pure-, 10%-, or 20%-methanol modified-carbon dioxide. Extraction and trapping conditions are found in Tables 1 and 2, respectively.

3. Extract analysis

Upon completion of each extraction, caffeine was added to the solid-phase trap rinses and tandem liquid trap (e.g. both contained the extracted analyte) as an internal standard. Analysis of the extract solutions was performed by HPLC. Values corresponding to 100% recovery were obtained by adding 100-μl of the pseudoephedrine hydrochloride spike solution and 50-μl of the caffeine internal standard solution (1000 ppm) to an empty collection vial which was diluted with

Table 1
Conditions for ion-pair/supercritical fluid extraction screening study with pure CO_2

Method no.	Pressure (atm)	Oven temp (°C)	CO_2 density (g ml^{-1})	Liquid flow rate (ml min^{-1})	Variable restrictor temp (°C)	Static time[a] (min)	Dynamic time[b] (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

[a] Time allotted for equilibration between spiked-sand surface and supercritical fluid (SF).
[b] Time allotted for SF to pass through extraction vessel at indicated flow rate.

3.2 ml methanol. Percent recovery values for extracted pseudoephedrine hydrochloride were calculated by direct comparison to the 100% 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. All separations were isocratically performed on a Deltabond Cyanopropyl (25 cm × 4.6 mm i.d.) column (Keystone Scientific, Bellefonte, PA) with a mobile phase consisting of 89% water, 2% methanol, 9% acetonitrile (v/v), and 1.5 g of 1-heptanesulfonic acid, sodium salt. The pH of the mobile phase was then adjusted to 3.0 with phosphoric acid. A flow rate of 1.0 ml min^{-1} was used. The injection volume used 10 µl. Detection was by UV at 205 nm.

4. Results and discussion

4.1. SFE extraction parameter 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. Tables 1 and 2 list the SFE extraction and trapping conditions. Table 3 lists the recoveries ($n = 1$) of pseudoephedrine hydrochloride at various temperatures, pressures, and static extraction times from a spiked-sand surface with and without the addition of 1-heptanesulfonic acid, sodium salt. Under the conditions listed in Method 1, low density and high temperature, only 7.5% (7.5 µg) of the spiked pseudoephedrine hydrochloride (100 µg) was recovered with pure carbon dioxide from the spiked-sand surface (200-µl total spike volume). Under the same SFE conditions and 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%.

Table 2
Trapping conditions for ion-pair extraction with supercritical
fluids

Solid phase trap	50/50 (w/w) porapak Q/glass beads
Liquid tandem trap	Methanol
Liquid tandem trap volume	5 ml
Collection temperature	0°C (CO_2), 70°C (methanol-modified CO_2)
Desorption temperature	25°C
Rinse solvent/volume	Methanol, 3.2 ml per rinse
Rinsing flow rate	1.0 ml min^{-1}.

In an attempt to increase the extraction efficiency further, the static time or the time allotted for equilibration of the supercritical fluid and the drug-spiked-sand surface, was increased from 5 to 15 min (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 from an increased static time because more time would be allotted for ion-pair formation as well as increased time for equilibration between the pseudoephedrine-sulfonate ion-pair and the supercritical fluid. Very little pseudoephedrine hydrochloride was detected without ion-pair reagent; however, as in the case of Method 1, the extraction recovery significantly increased to 27.3% with the addition of the 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 the extraction recovery. Due to time limitations, the effect of each parameter was only qualitatively investigated. The true significance of static time cannot be accurately ascertained since each method was run only once.

The next SFE parameter investigated was density. An increase in extraction recovery of pseudoephedrine hydrochloride without the ion-pairing reagent from none detected (Method 2) to 22.4% was observed when the CO_2 density was increased from 0.75 to 0.85 g ml^{-1}. (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.

The effect of carbon dioxide density was further investigated. Due to pump pressure limitations, however, the carbon dioxide density could only be increased to 0.97 g ml^{-1} albeit by decreasing the extraction temperature (Method 4). The recovery of pseudoephedrine with the addition of the ion-pairing reagent was slightly enhanced with this further increase in density from 45.1 to 55.2%; however, the extraction recovery of pseudoephedrine hydrochloride without the ion-pairing reagent appeared to level off at the increased density.

4.2. Ion-pair/SFE optimization

Our main objectives in part II of this study were: (1) to investigate the feasibility of increasing the solubility and extractability of pseudoephedrine hydrochloride by charge neutralization with 1-heptanesulfonic acid, sodium salt, from a spiked-sand surface with pure- and methanol-modified CO_2; and (2) to determine optimal extraction conditions for full recovery of pseudoephedrine from the spiked-sand surface. High density conditions (0.99 g ml^{-1} CO_2, 450 atm, 35°C) were chosen for part II of this study. To ensure an exhaustive extraction, the dynamic time was increased to 25 min;

Table 3
Percent recovery ($n = 1$) from screening study of pseudoephedrine hydrochloride employing SFE Methods 1–4 with and without ion-pairing agent

Method no.	Pseudoephedrine HCl	Pseudoephedrine HCl w/1-heptanesulfonic acid, sodium salt
1	7.5	20.5
2	ND[a]	27.3
3	22.4	45.1
4	17.6	55.2

[a] ND indicates none detected.

Table 4
Average percent recoveries of pseudoephedrine HCl from a non-dried spiked-sand surface

	CO_2	10% Methanol modified CO_2	20% Methanol modified CO_2
Pseudoephedrine HCl	67.3 (3.4)[a]	61.6 (9.1)	89.3 (0.6)
Pseudoephedrine w/1-heptanesulfonic acid, sodium salt	76.5 (3.1) [12.1][b]	96.0 (4.5) [34.7]	98.6 (4.2) [14.3]

SFE conditions: 0.99 g ml^{-1} CO_2, 450 atm, 35°C, 1.0 ml min^{-1}, 5 min static time 25 min dynamic time.
[a] Numbers in parentheses are standard deviations ($n = 3$).
[b] Numbers in brackets are F experimental values, F critical = 7.7.

while the static time was decreased to 5 min.

Table 4 lists the average percent recoveries of pseudoephedrine hydrochloride 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. A graphical representation of the recoveries is also found in Fig. 2. Although it was previously thought that ionic compounds and salts could not be extracted by SFE with CO_2-based fluids, 67.3% (67.3 µg/100 µg) of pseudoephedrine hydrochloride was recovered from the spiked-sand surface. The in-cell methanol-spike apparently modified the CO_2 making it more polar, and the increased recovery is attributed to pseudoephedrine hydrochloride's increased solubility in the methanol-modified CO_2.

In-line 10%- and 20%-methanol-modified CO_2 was then utilized to further enhance the extraction of pseudoephedrine hydrochloride from a spiked-sand surface. No significant increase was
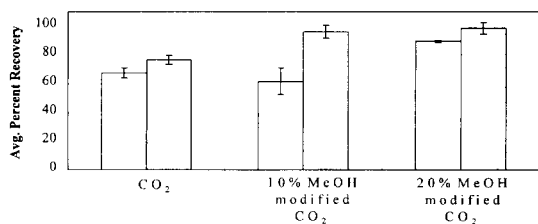

Fig. 2. Average Percent Recoveries ($n = 3$) of □ Pseudoephedrine Hydrochloride and ■ Pseudoephedrine Hydrochloride with 1-Heptanesulfonic Acid, Sodium Salt from a Non-Dried Spiked-Sand Surface. Bars represent standard deviations.

observed when employing 10%-methanol-modified CO_2 where 61.6% pseudoephedrine hydrochloride was extracted; however, a significant increase to 89.3% was observed with 20%-methanol-modified CO_2 vs. pure CO_2.

It was believed that the addition of 1-heptanesulfonic acid, sodium salt would further increase the solubility and extractability of pseudoephedrine hydrochloride, and an increase in recovery relative to recoveries achieved in the absence of the ion-pairing regent would result. This hypothesis was realized (Fig. 2) in that the recovery from the spiked-sand surface was enhanced from 67.3 (without IP) to 76.5% (with IP). The ion-pair formation neutralized the charge on the pseudoephedrine as well as increased the lipophilic character of the compound which in turn increased the solubility of pseudoephedrine in CO_2.

The extraction of pseudoephedrine was shown to be even more successful with in-line-methanol-modified CO_2 from a non-dried spiked-sand surface. The addition of 1-heptanesulfonic acid, sodium salt, had a significant positive effect on the recoveries where 96.0 and 98.6% were achieved with 10%- and 20%-methanol-modified CO_2 relative to 61.6 and 89.3%, respectively with no ion-pairing reagent.

A single-factor ANOVA was then performed to test if the means of the extraction recoveries with and without the use of the ion-pairing reagent with pure-, 10%-, and 20%-methanol-modified CO_2 from a non-dried spiked-sand surface were statistically equal at a 95% confidence

Table 5
Average percent recoveries of pseudoephedrine HCl from a dried spiked-sand surface

	CO_2	10% Methanol modified CO_2	20% Methanol modified CO_2
Pseudoephedrine HCl	7.7 (0.5)[a]	74.8 (9.7)	70.5 (7.9)
Pseudoephedrine w/1-heptanesulfonic acid, sodium salt	10.0 (2.0) [3.9][b]	83.4 (0.6) [2.4]	82.3 (1.5) [6.4]

SFE conditions: 0.99 g ml^{-1} CO_2, 450 atm, 35°C, 1.0 ml min^{-1}, 5 min static time, 25 min dynamic time.
[a] Numbers in parentheses are standard deviations ($n = 3$).
[b] Numbers in brackets are F experimental values, F critical = 7.7.

interval ($\alpha = 0.05$, F-critical $= 7.7$). As can be seen from Table 4, all F-experimental values exceeded a F-critical value of 7.7; therefore, the extraction recoveries of the pseudoephedrine-sulfonate ion-pair as compared to pseudoephedrine hydrochloride alone were statistically greater with pure-, 10%-, and 20%-methanol-modified CO_2.

The nature of the spiked-sand environment was investigated next. For example, if the spiked-sand surface (100-μl pseudoephedrine solution + 100-μl pure methanol or ion-pair solution) was allowed to dry overnight prior to extraction, would the recoveries of pseudoephedrine hydrochloride 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 CO_2? Table 5 lists the average ($n = 3$) percent recoveries of pseudoephedrine hydrochloride with and without the addition of 1-heptanesulfonic acid, sodium salt, from a previously dried spiked-sand surface (Fig. 3). The recoveries of pseudoephedrine hydrochloride in
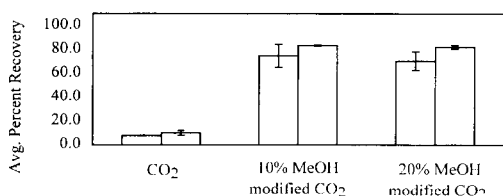

Fig. 3. Average Percent Recoveries ($n = 3$) of □ Pseudoephedrine Hydrochloride and ■ Pseudoephedrine Hydrochloride with 1-Heptanesulfonic Acid, Sodium Salt from a Previously Dried Spiked-Sand Surface. Bars represent standard deviations.

the absence of the ion-pairing reagent with pure-, 10%-, and 20%-methanol-modified CO_2 will be first compared. With pure carbon dioxide, 7.7% of the spiked pseudoephedrine HCl was recovered. The resulting poor recoveries were expected because the solubility of an ionic compound such as pseudoephedrine HCl in pure CO_2 was believed to be small.

Methanol-modified CO_2 was then utilized to increase the solvating power of the extraction medium. The recovery of pseudoephedrine hydrochloride 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 solubility and extractability of a polar compound such as pseudoephedrine hydrochloride in carbon dioxide. Methanol-modified CO_2 at 20% (v/v) was then utilized in an attempt to increase the extraction efficiency in the absence of the ion-pairing reagent further; however, no significant increase (70.5%) was observed from the 10%-methanol-modified conditions (74.8%).

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. A single-factor ANOVA was again performed to test if the means of the extraction recoveries with and without the use of the ion-pairing reagent with pure-, 10%-, and 20%-methanol modified CO2 were statistically equal at a 95% confidence interval ($\alpha = 0.05$, F-critical $= 7.7$). Experimental F values

are found in Table 5. It is seen from Table 5 that the extraction recoveries with and without the ion-pairing reagent from a previously dried methanol-spiked-sand surface were statistically equal (F-experimental < F-critical). The ion-pairing reagent apparently had no significant effect on the extraction efficiencies under these conditions. The presence of in-cell methanol has therefore been shown to play a vital role in the extraction process. It is believed that if the extraction recoveries are to be enhanced by the presence of the ion-pairing reagent, pseudoephedrine hydrochloride and the ion-pairing reagent must be allowed to interact and form the pseudoephedrine-sulfonate ion-pair in the methanol-spike solution prior to extraction. Methanol serves two purposes by increasing the solubility of the formed ion-pair in pure-, and methanol-modified carbon dioxide as well as by supporting the pseudoephedrine-sulfonate formation to a greater extent than a non-polar fluid such as carbon dioxide.

5. Conclusions

The feasibility of Ion-Pair/SFE for the extraction of pseudoephedrine hydrochloride has been examined. Recoveries were shown to be dependent on CO_2 density as well as the addition of 1-heptanesulfonic acid, sodium salt, to the non-dried 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 significantly increased the recovery of

pseudoephedrine from a non-dried spiked-sand surface with pure-, 10%-, and 20%-methanol-modified CO_2. If the sand were dried after spiking, the ion-pair reagent was ineffective. Quantitative pseudoephedrine recoveries of greater than 95% were shown to be achievable with the presence of 1-heptanesulfonic acid, sodium salt, with 10%-methanol-modified carbon dioxide.

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