

Investigation of Ion-Pairing Additives for the Supercritical Fluid Extraction of Triphenylphosphinetrisulfonate, Sodium Salt

Phyllis R. Eckard¹, Gary L. Long¹, Larry T. Taylor^{1,*}, and Gregory C. Slack²

¹Virginia Polytechnic Institute and State University, Department of Chemistry, Blacksburg, VA 24061 and ²The DuPont Merck Pharmaceutical Company, Radiopharmaceuticals Division, N. Billerica, MA 01862

Abstract

A screening study consisting of a fractional-factorial design is performed in order to identify the factors which contribute the most to the recovery of an anionic species, triphenylphosphinetrisulfonate (TPPTS), from a spiked-sand surface employing supercritical fluid extraction with carbon dioxide. The investigated experimental parameters are the type of ion-pairing additive (i.e., tetralkylammonium hydrogen sulfates) and its concentration, CO₂ density, extraction temperature, static extraction time, CO₂ mass used, liquid CO₂ flow rate, and the volume of methanol spiked into the matrix prior to extraction. Of the eight factors investigated, four factors are identified as significantly affecting the recovery of the anionic species: ion-pairing reagent added to the spiked sand surface, ion-pairing reagent concentration, static extraction time, and volume of methanol present in the extraction vessel. The experimental parameters and settings identified as influential by the statistical approach are later shown in concert to yield 100% recovery of TPPTS from the spiked sand.

Introduction

Since the late 1980s, sub- and supercritical fluid extraction (SFE) have become promising sample preparation alternatives to many liquid-liquid and liquid-solid (i.e., Soxhlet) extraction methods. Previous methods requiring large amounts of hazardous organic solvents and many laborious, time-consuming steps can be eliminated by employing SFE. Scientists in the foods, polymers, environmental, and pharmaceutical fields, for example, have developed numerous SFE applications (1-8).

The most commonly used sub- or supercritical fluid is carbon dioxide. Supercritical carbon dioxide's solvating power is comparable to liquid hexane. Carbon dioxide has been shown to be a sufficient solvent for the extraction of non-polar to

moderately polar compounds. The solvation (and subsequent extraction) of more polar compounds, however, can be readily achieved by modifying carbon dioxide with either small volumes of polar organic solvents such as methanol or by adding the organic solvent directly to the analyte matrix (9).

The use of SFE in the pharmaceutical field has been limited by the fact that many pharmaceuticals are ionic or ionizable, water soluble, and highly polar. Because of the low dielectric constant (1.0-1.6) of supercritical carbon dioxide, ionic compounds are considered insoluble in pure CO₂ alone and are therefore unextractable. A technique used in liquid-liquid extractions for the extraction of ionic compounds is ion-pair extraction (10-11). The ionic species of interest is essentially neutralized by combining it with a counter-ion of opposite charge through electrostatic interactions. The resulting ion pair is then extracted.

Numerous reports have examined the role of ion-pairing reagents in the SFE of anionic species (12-18). Most commonly, an ion-pairing reagent such as a tetralkylammonium salt (NR₄⁺) is added along with a certain volume of solvent to the solid matrix to form an ion pair (NR₄A) with the analyte (A⁻). Commonly extracted analytes include phenoxyacid herbicides, alkylsulfonates, and sulphonamides. Several papers have also illustrated the combination of ion pairing and alkylation (12-16). Hawthorne et al. (12) extracted spiked and native phenoxyacid herbicides from soils and sediments and microbial phospholipids and phenols from solid sorbents as methylester derivatives using trimethylphenylammonium hydroxide.

In 1992, Field et al. (13) reported on the extraction of secondary alkylsulfonate and linear alkylbenzenesulfonate surfactants from sewage sludge as their tetrabutylammonium ion pairs and the subsequent quantitation by gas chromatography (GC)-mass spectrometry. The ion pairs were said to form their corresponding butyl esters in the injection port of the GC. The recoveries were shown to be enhanced 2.5-fold in the presence of the ion-pairing reagent.

Further studies describing the SFE of a phenoxyacid herbi-

* Author to whom correspondence should be addressed.

cide (2,4-dichlorophenoxyacetic acid) from soil were described by Rochette et al. (14). Several strategies were studied, including silylation, methyl esterification, ion pairing, and ionic displacement. A solution of (*m*-[trifluoromethyl]phenyl)-trimethylammonium hydroxide in methanol was added as the ionpairing modifier; however, the recoveries were seen to increase from only 6% to 16% in the presence of the ionpairing reagent in comparison with methanol alone. Of the four investigated strategies, ionic displacement with a strong cation-displacing agent ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in methanol yielded the most quantitative recoveries (approximately 87%) from the very active soil matrix.

In 1993, Lopez-Avila et al. (15) investigated SFE as a means of extracting seven chlorophenoxyacid herbicides from soil samples. Several ion-pairing/derivatizing agents were investigated, including trimethylphenylammonium hydroxide (TMPA), benzyltrimethylammonium chloride (BTMAC), benzyltriethylammonium chloride (BTEAC), and tetrabutylammonium hydroxide/methyl iodide (TBA/MI). The main purpose for adding these reagents was to form the methylester derivative during the extraction so that they could subsequently be analyzed by GC. When TMPA, BTEAC, and TBA/MI were compared in a series of spike studies from sand, it was shown that TMPA resulted in the most favorable recoveries (63–96%). Additional experiments were then performed with TMPA, indicating that the conversion from the chlorophenoxyacid herbicides to their corresponding methylesters was indeed occurring in the injection port of the GC rather than during the static SFE step.

The use of trimethylphenylammonium hydroxide in methanol as a static modifier (ion-pairing/derivatizing agent) was compared with methanol-modified CO_2 for the extraction of phenoxyacid herbicides from house dust (16). First, the extraction recoveries with 200 μL methanol and 200 μL TMPA were compared. Similar recoveries were achieved in both cases; therefore, the addition of TMPA as an ion-pairing reagent was shown not to enhance the analyte extraction. Similar to the work of Lopez-Avila et al. (15), the use of methanol-modified CO_2 (20 mol%) was shown to be a better alternative than the use of the static modifiers, resulting in recoveries ranging from 83% to 95%.

The combination of ion pairing with SFE was also investigated as a means of extracting sulphonamides from solid supports and meat tissues (17–18). Tena et al. (17) compared the extraction efficiencies of five sulphonamides from diatomaceous earth with and without the presence of 1 mL of trimethylphenylammonium hydroxide in methanol. The use of TMPA was shown to be quite advantageous; the recoveries were enhanced from an average of 30% to 96%. Comparisons between pure and methanol-modified CO_2 were also made. Surprisingly, less than 20% of each sulphonamide was extractable in the absence of TMPA and less than 5% was extractable in the presence of TMPA with methanol-modified CO_2 .

In 1997, Din et al. (18) investigated the extraction of sulfamethazine and its metabolites from spiked (parts-per-million) "wet" and freeze-dried swine liver and kidney with methanol-modified CO_2 and in situ ion pairing with tetramethylammonium hydroxide (TMA). Recoveries were very matrix-dependent, whereby the lower polarity sulphonamides

were easily extractable from wet kidney with 10% methanol-modified CO_2 . Extraction of the freeze-dried tissues was problematic because of postulated large-sulphonamide-matrix binding. The recoveries of only 3 of the 5 more polar and ionic sulphonamides from ground freeze-dried kidney were enhanced in the presence of TMA. Enhancements ranged from 23 to 72%. The ineffectiveness of TMA for the other matrices was attributed to poor reagent-to-analyte site accessibility.

Previous studies have been mixed, some reporting that the addition of an ion-pairing reagent directly to the matrix significantly enhances the extractability of an ionic species from a matrix. Others have reported no real improvements, postulating high analyte-matrix binding, poor reagent-to-analyte site accessibility, or that the use of modifiers (methanol-modified CO_2 versus pure CO_2) are just as useful or even better than using ion-pairing reagents (12–18). The objective of this study was to fundamentally examine the extraction of an anionic species, triphenylphosphinetrisulfonate (sodium salt), from a relatively uncomplicated matrix, sand, with supercritical CO_2 aided by various ion-pairing additives. It was of interest to examine the ion-pair extraction process in more detail than was studied by others. For example, if tetralkylammonium hydrogen sulfate reagents were used as the ion-pairing reagent, the tetrahexyl ion-pair complex should be more extractable than a tetramethyl ion pair. The recoveries may be improved if the reagent is added in excess. The recoveries would be expected to be enhanced in the presence of a greater amount of modifier. The extraction should be dependent upon the fluid density, extraction temperature, flow rate, equilibration time, or amount of CO_2 used.

Experimental

Instrumentation

All extractions were performed on an Isco/Suprex Pre-master (Lincoln, NE) consisting of a dual reciprocating pump, temperature-controlled oven, Duraflow restrictor, and an Accutrap solid-phase trap collection and rinsing device. A Hewlett-Packard (Wilmington, DE) 1090 liquid chromatographic system was used for all extract analyses.

Reagents

Carbon dioxide (SFE/SFC grade) with approximately 2000 psi helium headspace was obtained from Scott Specialty Gases (Plumsteadville, PA). Triphenylphosphinetrisulfonate (sodium salt) was supplied by the DuPont Merck Pharmaceutical Company radiopharmaceuticals division (N. Billerica, MA) and used as received. Caffeine, tetramethylammonium hydrogen sulfate, and tetrahexylammonium hydrogen sulfate were obtained from Sigma (St. Louis, MO). Ottawa Cement Testing sand was supplied by Fisher Scientific (Raleigh, NC).

Extraction conditions and general procedure

A screening study consisting of a fractional-factorial design was implemented to investigate the effect of several ion-pairing parameters, such as ion-pairing reagent composition and mole ratio of ion-pairing reagent-to-TPPTS, on the extraction effi-

ciency of TPPTS from a spiked-sand surface. Several extraction parameters, including CO₂ density, extraction temperature, static extraction time, CO₂ mass, liquid CO₂ flow rate, and methanol in-cell spike volume, were also investigated. RS 1 Discover software (BBN Software Products, Cambridge, MA) was used for setting up the experimental design and for data analysis. To make the experimental design simple and easy to interpret, two assumptions were made: a linear response existed for each factor investigated and no interactions existed among the various controlled factors which would influence the results. High and low values were chosen for each controlled factor except for ion-pairing reagent composition and concentration (Table I). If a full-factorial design had been performed with 8 controlled factors, 384 experiments would have resulted. Because of time constraints, a fractional-factorial

experimental design was chosen wherein 21 individual experiments ($n = 3$) were randomly investigated. The specific extraction methods that were conducted and their order are found in Table II.

The following general procedure was used for all studies. A 5.0-mL extraction vessel (Keystone Scientific, Bellefonte, PA) was filled approximately 90% with Ottawa Cement Testing sand. Triphenylphosphinetrisulfonate (sodium salt) was dissolved in methanol to prepare a 6000-ppm solution that was used to spike (50 μ L) the sand (300- μ g spike mass) contained in the vessel. Then, either pure methanol or a solution of the appropriate ion-pairing reagent in methanol was spiked onto the previously trisulfonate-spiked-sand surface. The total methanol spike volume in the vessel including TPPTS with or without ion-pairing reagent was 75 or 200 μ L, depending on the extraction method. The extraction vessel was then sealed, vigorously shaken by hand, and immediately extracted. The spiked-sand surface was not allowed to dry prior to extraction.

Table I. High and Low Extraction Parameters Investigated*

	Low	High
Mole ratio (ion pair-to-TPPTS)	3:1	15:1
Carbon dioxide density (g/mL)	0.71	0.85
Extraction temperature (°C)	35	80
Static extraction time (min)	0	30
Dynamic mass (g CO ₂)	15	40
Flow rate (mL/min)	1.0	2.0
Methanol in-cell spike volume (μ L)	75	200

* Ion-pair reagents were either tetramethylammonium hydrogen sulfate or tetrahexylammonium hydrogen sulfate.

Extraction trapping conditions

The solid-phase trap consisted of glass beads (80–100 mesh). To ensure the proper trapping of all extracted analyte, a tandem liquid trap consisting of 2.0 mL methanol immediately following the solid-phase trap was used. The solid-phase trap and tandem liquid trap were maintained at -5°C and room temperature, respectively, during the extraction. Upon completion of the extraction, the solid-phase trap temperature was raised to 30°C , and the trap was rinsed with 2.0 mL methanol into an empty collection vessel. Both the solid-phase trap rinse

Table II. Parameters for Fractional-Factorial Experiments

Extraction order	Ion-pair reagent*	Mole ratio (IP:TPPTS)	CO ₂ Density (g/mL)	Temperature (°C)	Static time (min)	MeOH in-cell spike volume (mL)	Dynamic mass (g CO ₂)	Liquid flow rate (mL/min)
1	hexyl	1:1	0.71	35	30	75	15	2.0
2	hexyl	1:1	0.71	80	0	75	40	1.0
3	none	0:1	0.85	35	0	200	40	1.0
4	none	0:1	0.71	80	30	200	40	2.0
5	methyl	5:1	0.71	35	30	200	15	1.0
6	hexyl	1:1	0.85	80	0	200	15	1.0
7	methyl	1:1	0.71	35	0	75	40	1.0
8	none	0:1	0.85	35	0	75	15	2.0
9	methyl	5:1	0.85	80	30	75	15	2.0
10	methyl	5:1	0.85	80	0	200	40	2.0
11	methyl	1:1	0.85	35	30	200	40	2.0
12	none	0:1	0.85	35	30	75	15	1.0
13	hexyl	5:1	0.71	35	0	200	15	2.0
14	none	0:1	0.71	80	30	75	40	1.0
15	methyl	5:1	0.71	80	30	200	15	1.0
16	none	0:1	0.85	80	0	200	15	1.0
17	methyl	1:1	0.71	35	0	75	40	1.0
18	hexyl	5:1	0.85	80	30	75	40	1.0
19	hexyl	5:1	0.85	35	0	200	40	2.0
20	methyl	1:1	0.85	80	0	75	15	2.0
21	none	0:1	0.71	80	0	75	15	2.0

* Abbreviations: hexyl, tetrahexylammonium hydrogen sulfate; methyl, tetramethylammonium hydrogen sulfate; none, no ion-pairing reagent added.

and the tandem liquid trap were diluted with 3.0 mL of HPLC-grade water and analyzed separately.

Extract analysis

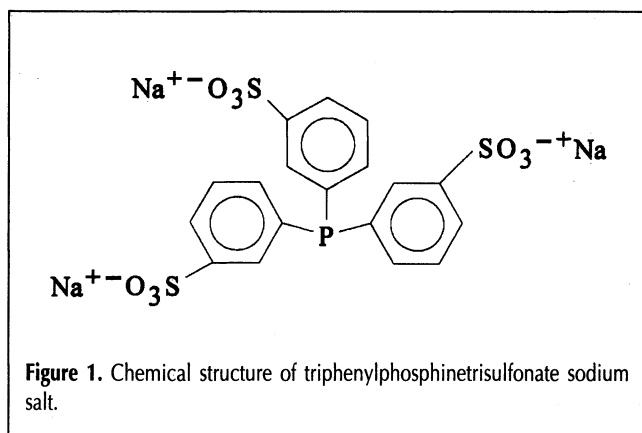
Upon completion of the extraction, 50 μL of 6000-ppm caffeine in methanol (300 μg) was added to the solid-phase trap rinse and tandem liquid trap as an internal standard. The purpose of adding internal standard to the trap rinse was to ensure good quantitation in case there were variations in solid-phase trap rinse volumes or evaporation losses in the tandem liquid trap during the extraction. Analysis of the extract solutions was performed by HPLC. Values corresponding to 100% recovery were obtained by adding 50 μL of the TPPTS spiking solution (6000 ppm) and 50 μL of the caffeine internal standard solution (6000 ppm) to an empty collection vial and diluting with 2.0 mL methanol and 3.0 mL water. Percent recovery values for extracted TPPTS were calculated by direct comparison to the 100% standard.

Chromatographic conditions

All separations were performed on a Cosmosil 5C18-MS (25 cm \times 4.6-mm i.d., 10 μm) column (Waters, Milford, MA) with mobile phase A consisting of 95% 20mM phosphate buffer–5% methanol–0.05% triethylphosphite (v/v) and mobile phase B consisting of 95% methanol–5% water–0.05% triethylphosphite (v/v). The mobile phase gradient program was as follows: 100% mobile phase A for 2 min, ramped to 66% mobile phase A in 13 min, ramped to 33% mobile phase A in 17 min, ramped to 5% mobile phase A in 17.5 min and held for 4.5 min. The system was then allowed to equilibrate for 7 min with 100% mobile phase A prior to the next injection. The column was maintained at 40°C. A flow rate of 1.0 mL/min was used. The injection volume was 10 μL . Ultraviolet detection at 260 nm was used.

Results and Discussion

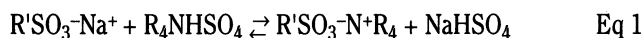
The goal of this research was to identify the main factors (including both ion-pairing additive and SFE parameters) that influenced the extraction of triphenylphosphinetrisulfonate (Figure 1) from a relatively uncomplicated matrix such as sand. This was accomplished by performing a screening study



that investigated ion-pairing reagent composition, the molar ratio of ion-pairing reagent to TPPTS in the extraction vessel, CO_2 density, extraction temperature, static extraction time, CO_2 mass used, and liquid CO_2 flow rate.

It was believed that the ion-pairing reagent composition and the relative amount of reagent added would play a significant role in the extraction process. Two ion-pairing reagents varying in lipophilicity were investigated: tetramethyl- and tetrahexylammonium hydrogen sulfate. It was believed at the outset that ionic compounds, because of their polarity, do not have sufficient solubility in the non-polar fluid CO_2 . In order to enhance an ionic specie's solubility and extractability, an ion-pairing reagent may be added to possibly neutralize the ionic compound's charge and at the same time add sufficient lipophilicity to the compound, making it more non-polar and thus more extractable with a non-polar fluid such as CO_2 . Our hypothesis was that the extraction efficiencies of TPPTS would be greatest with the more lipophilic ion-pairing reagent because of its reduced polarity.

Secondly, the amount of ion-pairing reagent added or the mole ratio of ion-pairing reagent to TPPTS was investigated. Complete ion pair formation may occur at a 1:1 charge neutralization; however, the ion pair formation equilibrium may be more favored when an excess of ion-pairing reagent is present.



Several SFE parameters were also investigated, including CO_2 density, extraction temperature, static extraction time, mass of CO_2 used, and CO_2 liquid flow rate. It is well known that the solvating power of CO_2 is enhanced at a higher density. Extraction temperature may also play a role. If the fluid density is held constant, the extraction efficiencies may be greater at a higher extraction temperature because of the increased solubility and decreased analyte–matrix interactions. Static extraction time was also investigated. If more time is allowed for ion pair formation to occur under supercritical conditions, increased recoveries could be observed. The next parameter investigated was dynamic CO_2 mass. Lastly, flow rate was investigated. In the case where there was no static extraction time, enough time may not be allowed for ion pair formation to occur at a higher flow rate. Therefore, there would be less partitioning of the ion pair into the fluid, giving rise to lower recoveries.

Average percent recovery of TPPTS from a spiked-sand surface versus the various methods is plotted in Figure 2. Error bars representing one standard deviation are also shown. It should be noted that no two methods were alike; therefore, the influence of each particular parameter could not be ascertained from this graph. It can be observed, however, that many methods resulted in very unfavorable recoveries, several methods resulted in adequate recoveries (> 60%), and one method resulted in a recovery of approximately 100%.

In order to evaluate if any of the ion-pairing and extraction parameters had a significant effect on the recovery of TPPTS from a spiked-sand surface, least-square coefficients were determined for the screening study. The assumptions of this model

were that a linear response existed among all the investigated variables and that no interactions existed between the investigated factors, thus attributing to the response (recovery). Least-squares coefficients are used to describe the relationship

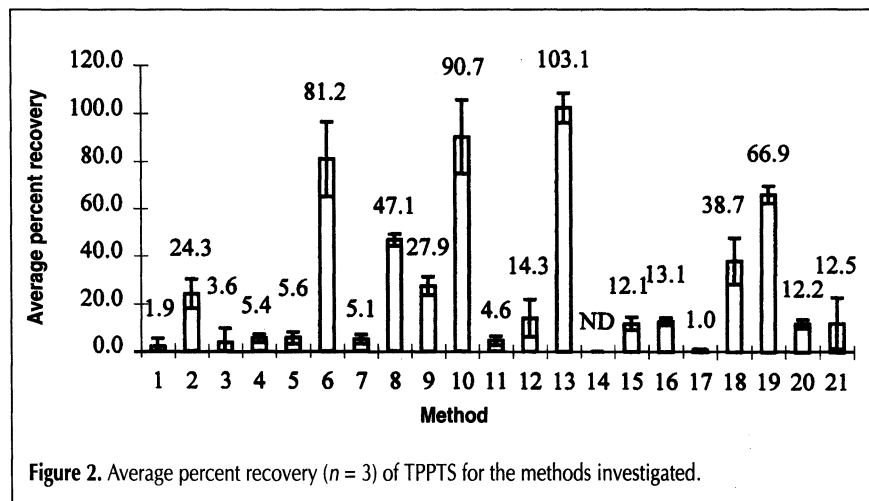


Figure 2. Average percent recovery ($n = 3$) of TPPTS for the methods investigated.

Table III. Least Square Coefficients, Reduced Fit, Response Percent Recovery

Term	Slope	Error of slope	t-Value	p-Value
Constant	27.4	4.9		
Ion pair reagent composition				0.0264
none	-14.1	6.7	-2.11	0.0513
tetramethylAHS*	-7.5	6.7	-1.12	0.2783
tetrahexylAHS†	21.6	7.3	2.98	0.0089
Ion pair-to-TPPTS mole ratio				0.0571
3:1	-9.9	4.8	-2.05	0.0571
15:1	9.9	4.8	2.05	0.0571
Static time	-11.0	4.9	-2.26	0.0384
In-cell methanol spike volume	8.7	4.8	1.81	0.0886

* Tetramethylammonium hydrogen sulfate.

† Tetrahexylammonium hydrogen sulfate.

between the recovery and the settings of the factor so that a mathematical expression can be made. The sign and the magnitude of the least squares coefficients indicate the type of effect the variable has on the response (recovery). Furthermore, by examining the p -values or the significance values, it can be determined if the factor investigated had a significant effect on the response (recovery). For this study, the null hypothesis was that each variable did not contribute to the overall recovery of the anionic species, regardless of its setting. Specifically, the p -value is the probability of making a Type I error, which is rejecting a true null hypothesis. For a p -value of 0.1 (90%-confidence interval), there is a 10% probability that accepting the null hypothesis is incorrect. Therefore, if the controlled factor had a p -value of < 0.1 , the null hypothesis that the variable did not significantly affect the recovery of the anionic species was rejected.

Within a 90%-confidence interval, the ion-pairing reagent composition, mole ratio of ion-pairing reagent to TPPTS, static time, and in-cell methanol spike volume were found to play a significant role in the extraction process ($p < 0.1$) in this study. Therefore, CO_2 density, amount of CO_2 mass used, and flow rate were deemed to be unimportant factors. These factors were removed from the screening study model, and the least-squares coefficients, T -values, and p -values were fitted for the reduced model (Table III). As stated before, the sign and magnitude of the slope of the least-squares coefficients can be examined to visualize how the change in each parameter affects the overall response (recovery). For example, the term having the largest slope was the addition of tetrahexylammonium hydrogen sulfate (21.6 ± 7.3). This indicated that the response was most affected by the addition of this particular ion-pairing reagent. This must be confirmed by examination of the p -value. For this particular term, the p -value was 0.0089. As previously described, by having a p -value of less than 0.1, this effect on the recovery was shown to be statistical. The slope may also be negative. For example, the addition of tetramethylammonium hydrogen sulfate, as well as static extraction time, negatively impacted the response. However, by examining the p -values (< 0.1), only static extraction time was shown to statistically decrease the response (recovery). The relative importance of each of these terms (parameters) will be discussed later.

Next, the significant effects on the recovery were determined (Figure 3). The main effect for each influential parameter is the difference between the means of all the runs at the high setting and low setting for a particular parameter, regardless of the other settings. The most influential parameter controlling the extraction recoveries was determined to be the ion-pairing reagent composition. First, by examining the

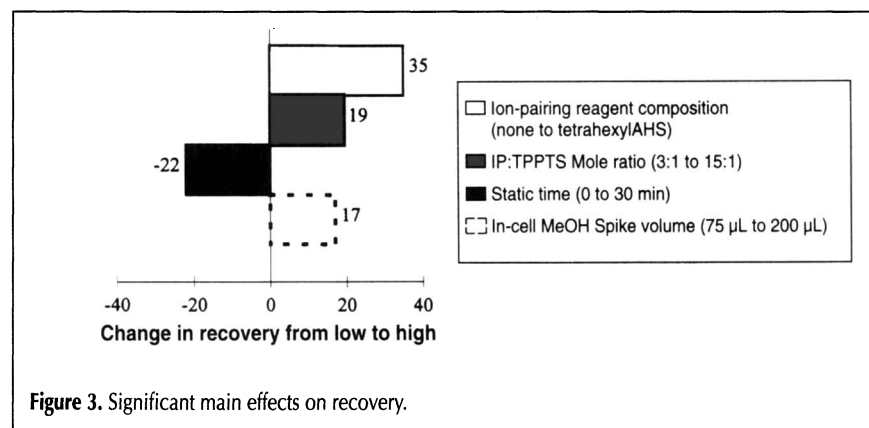


Figure 3. Significant main effects on recovery.

p-values (Table III), the recoveries were shown to be statistically affected by the addition of tetrahexylammonium hydrogen sulfate ($p < 0.1$). The addition of tetramethylammonium hydrogen sulfate apparently had no effect on the recovery of the anionic species (TPPTS) from the spiked sand surface. Once again, it was believed that two factors are operating: charge neutralization and the addition of lipophilic characteristics to the polar ionic compound. Although the charge on TPPTS may be neutralized with the addition of any ion-pairing reagent, the compound may still be too polar to be extractable with methanol-modified CO₂. In the case of the addition of tetrahexylammonium hydrogen sulfate, the ion-pair complex formed would be more nonpolar and thus more soluble and extractable than when forming the tetramethylammonium ion pair. On the average, by simply adding tetrahexylammonium hydrogen sulfate to the sample matrix, the recoveries from the spiked-sand surface were increased by 35%.

In order to better visualize the effectiveness of the addition of the tetrahexylammonium hydrogen sulfate, the recoveries of the seven experimental methods that were performed without any ion-pairing reagent present were averaged. Once again, no two experimental methods were alike. On the average, under the various extraction methods, 14% of the trisulfonate was extracted from the spiked-sand surface. By simply adding the tetrahexyl ion-pairing reagent, the recoveries were increased 3.5-fold on average.

The second parameter found to be influential was the amount of ion-pairing reagent added or the mole ratio of ion-pairing reagent to analyte. For example, TPPTS contains three sulfonate groups; therefore, when placed in solution, the compound has a net charge of -3. Ion-pairing reagent was added in a mole ratio of 3:1 and 15:1, which would correspond to a 1:1 charge neutralization or, in excess, a 5:1 charge neutralization, respectively. It was observed that by adding an excess of ion-pairing reagent (15:1), the recoveries increased by approximately 19% in comparison with the 3:1 mole ratio. The increase in recovery can simply be explained by Le'Chatliers principle. When an excess of ion-pairing reagent was added, the equilibrium shifted towards the right, favoring ion pair formation (Eq 1). In this case, it was assumed that at a 1:1 charge neutralization ion-pair formation was incomplete.

Static extraction time was the third parameter found to be influential. It was originally hypothesized that a static time would be beneficial to the formation of the ion-pair complex; however, this was not the case. The recoveries decreased by approximately 22% when the static time was changed from zero to 30 min. TPPTS, ion-pairing reagent, and a certain volume of methanol were present in the extraction vessel. The methanol was not predried and was assumed to contain about 0.1% (w/w) water. In the presence of CO₂, water will react to form carbonic acid, thus the overall pH of the resulting solution may be decreased to as low as 3.0 (19). The pK_a of triphenylphosphinetrisulfonate is 2-3. If the pH of the resulting solution is decreased to approximately 3.0, the equilibria will consist of an approximately 50:50 mixture of the anionic trisulfonate and the protonated (neutralized) trisulfonate. Therefore, less ionic species would be available to react with the ion-pairing reagent, and lower recoveries would result. This hypothesis

assumes that the protonated (neutralized) trisulfonate is insoluble in methanol-modified CO₂; however, this assumption could not be experimentally confirmed. Because of the trisulfonate's low pK_a , it would be extremely difficult to obtain experimental conditions (pH ~ 1) in which it is assumed that the trisulfonate exists solely in the protonated (neutralized) form. Also, the use of an acid would be necessary, and consequently the analyte-matrix would be modified. Therefore, if enhanced extractability was observed, one could not simply attribute this response to charge neutralization. Decreased analyte-matrix (sand) interactions must also be considered.

The fourth parameter shown to influence the recoveries of TPPTS from a spiked-sand surface was the in-cell methanol spike volume. The purpose of the methanol was threefold. First, the methanol served as a way of introducing a certain amount of the ion-pairing reagent. Second, because ionic compounds are known to exhibit little solubility in CO₂, the methanol offers a medium for the analyte and ion-pairing reagent to mix and form. Third, the methanol could serve as a modifier, thus increasing the solvating power of the fluid towards more polar compounds. For these reasons, the recoveries were enhanced an average of 17% when the volume of methanol was increased from 75 to 200 μ L.

As stated previously, when examining TPPTS recoveries among the various experiments, one particular method (method 13) resulted in 103% recovery (6.0% relative standard deviation). If one compares the parameters for this method with the identified influential parameters, one finds that tetrahexylammonium hydrogen sulfate was used as the ion-pairing reagent and was added at a 15:1 molar excess. Also, no static extraction time was employed, and 200 μ L of methanol was present in the extraction vessel. Experimental method 13 offers proof that the influential parameters identified in this screening study were indeed optimal.

Conclusion

In summary, a screening study consisting of a fractional-factorial design was performed to identify the influential parameters that significantly affected the recovery of triphenylphosphinetrisulfonate (sodium salt) from a spiked-sand surface with ion-pairing additives. The four influential parameters were ion-pairing reagent composition, molar ratio of ion-pairing reagent to TPPTS, static extraction time, and in-cell methanol spike volume. First, the recoveries of the anionic species were shown to be enhanced in the presence of an ion-pairing additive. Of the two quaternaryalkylammonium salts investigated, the more lipophilic reagent, tetrahexylammonium hydrogen sulfate, was the only ion-pairing reagent that statistically enhanced the recoveries. The increased extractability in the non-polar fluid, CO₂, was attributed to charge neutralization and reduced analyte polarity through the formation of the ion-pair complex. Second, the amount of ion-pairing reagent was also shown to be influential. By adding an excess of ion-pairing reagent, the equilibrium was shifted towards full and complete ion pair formation, and an enhance-

ment was observed. Third, static time was shown to negatively affect the recoveries. In the presence of moisture and time, it was believed a mixture of both the neutral and charged trisulfonate species was present; therefore, less ionic species was present to form the ion-pair complex, and lowered recoveries were observed. Lastly, the recoveries of the polar compound were shown to be enhanced by increasing the polarity of the fluid by a simple increase in the in-cell methanol spike volume.

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