

CHAPTER IV

INVESTIGATION OF ION-PAIRING ADDITIVES FOR THE SUPERCRITICAL FLUID EXTRACTION OF TRIPHENYLPHOSPHINETRISULFONATE, SODIUM SALT

4.1 INTRODUCTION

It was demonstrated in Chapter III that the extraction of a highly polar, nonionizable pharmaceutical, lovastatin, from a complicated matrix (i.e. MEVACOR® tablets) was indeed feasible using subcritical fluid extraction. The effect of the addition of methanol to the carbon dioxide was examined, and it was shown that the recoveries could be enhanced due to a subsequent increase in the solvating power of the fluid. Although an enhancement was observed, a secondary modifier (i.e. additive), specifically isopropylamine, was needed to possibly overcome significant analyte-matrix interactions. Due to the mildly basic characteristics of the drug, a stronger base was added to the fluid to facilitate displacement of the drug from any acidic sites on the tablet matrix. In this Chapter, the usefulness of other types of additives will be investigated for the extraction of an ionic compound.

As described previously, the use of SFE in the pharmaceutical field has been limited. Many pharmaceuticals are ionic/ionizable, water soluble, and highly polar. Due to the low dielectric constant (1.0 - 1.6) of supercritical CO₂, 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.¹⁻² The ionic species of interest, through electrostatic interactions, is essentially neutralized by combination with a counter-ion of opposite charge. The resulting ion-pair is then extracted.

Numerous reports have examined the role of ion-pairing reagents in the supercritical fluid extraction of anionic species.³⁻⁹ Most commonly an ion-pairing reagent such as a tetralkylammonium salt $[\text{NR}_4^+]$ is added in a known volume of solvent to the solid matrix to form an ion-pair $[\text{NR}_4\text{A}]$ with the analyte $[\text{A}^-]$. Common extracted analytes include phenoxyacid herbicides, alkylsulfonates, and sulphonamides. Several papers have illustrated the combination of ion-pairing and alkylation.³⁻⁷ Hawthorne et al. extracted a) spiked and native phenoxyacid herbicides from soils and sediments, b) microbial phospholipids, and c) phenols from solid sorbents as methylester derivatives using trimethylphenylammonium hydroxide utilizing this technique.³

In 1992, Field et al. reported on the extraction of secondary alkylsulfonate and linear alkylbenzensulfonate surfactants from sewage sludge as their tetrabutylammonium ion-pairs, followed by subsequent quantification by GC-MS.⁴ The ion-pairs were said to form their corresponding butyl esters in the injection port of the gas chromatograph. The recoveries were shown to be enhanced by 2.5 fold with the presence of the ion-pairing reagent. Further studies describing the supercritical fluid extraction of a phenoxyacid herbicide (2,4-dichlorophenoxyacetic acid) from soil were described by Rochette et al.⁵

¹ R.W. Frei, J.F. Lawrence, Eds., "Chemical Derivatization in Analytical Chemistry, Volume 2: Separation and Continuous Flow Techniques", Plenum Press, New York (1982).

² K. Blau, G.S. King, Eds., "Handbook of Derivatives for Chromatography", Heyden & Son, Ltd., Philadelphia (1978).

³ S.B. Hawthorne, D.J. Miller, D.E Nivens, D.C. White, *Anal. Chem.*, **64** (1992) 405.

⁴ J.A. Field, D.J. Miller, T.M. Field, S.B. Hawthorne, W. Giger, *Anal. Chem.*, **64** (1992) 3161.

⁵ E.A. Rochette, J.B. Harsh, *Talanta*, **40** (1993) 147.

⁶ V. Lopez-Avila, N.S. Dodhiwala, *J. Agric. Food Chem.*, **41** (1993) 2038.

⁷ T.S. Reighard, S.V. Olesik, *Anal. Chem.*, **69** (1997) 566.

⁸ M.T. Tena, M.D. Luque de Castro, M. Valcárcel, *Chromatographia*, **40** (1995) 197.

⁹ N. Din, K.D. Bartle, A.A. Clifford, *J. Chromatogr. Sci.*, **35** (1997) 31.

Several strategies were studied with the herbicide including silylation, methyl esterification, ion-pairing, and ionic displacement. A solution of [*m*-(trifluoromethyl)phenyl]trimethylammonium hydroxide in methanol was added as the ion-pairing modifier; however, the recoveries were only seen to increase from 6% to 16% in the presence of the ion-pairing reagent versus methanol alone. Of the 4 strategies investigated, 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. investigated SFE as a means of extracting seven chlorophenoxyacid herbicides from soil samples.⁶ Several ion-pairing/derivatization 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 subsequently could be analyzed by gas chromatography. When TMPA, BTEAC, and BTMAC 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 which indicated 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 (TMPA) in methanol added as a static modifier (ion-pairing/derivatizing agent) versus methanol-modified CO_2 was compared for the extraction of phenoxyacid herbicides from house dust.⁷ First, the extraction recoveries were compared with 200 μL of methanol vs 200 μL of TMPA. 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, 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 followed by SFE was also investigated as a means of extracting sulphonamides from solid-supports and meat tissues.⁸⁻⁹ Tena et al. compared the extraction efficiencies of 5 sulphonamides from diatomaceous earth within and in the absence of 1 mL of trimethylphenylammonium hydroxide in methanol. The use of TMPA was shown to be quite advantageous wherein the recoveries were enhanced from an average of 30% to 96%. Comparisons between pure and methanol-modified CO₂ were also made. Surprisingly, less than 20% of each sulphonamide was extractable in the absence of TMPA and less than 5% in the presence of TMPA with methanol-modified CO₂.

The extraction of sulphamethazine and its metabolites from parts-per-million spiked “wet” and freeze-dried swine liver and kidney was carried out with both methanol-modified CO₂ and in situ ion-pairing with tetramethylammonium hydroxide (TMA) in 1997 by Din et al.⁹ Recoveries were very matrix dependent, with the lower polarity sulphonamides found to be easily extractable from wet kidney with 10% methanol-modified CO₂. Extraction of the freeze-dried tissues was problematic, this was postulated to be due to 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 results 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 have shown that the use of modifiers (methanol-modified CO₂ versus pure CO₂) are just as useful or even better than results obtained using ion-pairing reagents.³⁻⁹ The objective of our study was to fundamentally examine the extraction of an anionic species,

triphenylphosphinetrisulfonate, sodium salt, from a relatively uncomplicated matrix, sand, with supercritical CO₂ aided by various ion-pairing additives. It was of interest to examine the ion-pair extraction process in more detail than has been previously studied by others. For example, if tetralkylammonium hydrogen sulfate reagents were used as the ion-pairing reagent, would a tetrahexyl versus a tetramethyl ion-pair complex be more extractable? Would the recoveries be improved if the reagent was added in excess? Would the recoveries be enhanced in the presence of a greater amount of modifier? Is the extraction dependent upon the fluid's density, extraction temperature, flow rate, equilibration time, or amount of CO₂ used? It is this line of questioning we wished to investigate.

4.2 EXPERIMENTAL

Instrumentation

All extractions were performed on the Isco/Suprex Prepmaster (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 1090 Liquid Chromatographic System (Wilmington, DE) 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 Chemical Company (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 including ion-pairing reagent composition and mole ratio of ion-pairing reagent to TPPTS upon the extraction efficiency 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. RS1 Discover software (BBN Software Products Corp., Cambridge, MA) was used for setting up the experimental design as well as for data analysis. To simplify experimental design and data interpretation, two assumptions were made: 1) a linear response existed for each factor investigated and 2) no interactions existed among the various controlled factors which influenced the results. A high and a low value were chosen for each controlled factor except for ion-pairing reagent composition and concentration (**Table 4.1**). If a full-factorial design had been performed with 8 controlled factors, 384 experiments would have resulted. Due to time constraints, a fractional-factorial experimental design was chosen wherein 21 individual experiments (n=3) were randomly investigated. The specific extraction methods utilized and the order in which they were conducted are found in **Table 4.2**.

The following general procedure was used for all studies. A 5.0 mL extraction vessel (Keystone Scientific, Bellefonte, PA) was filled to approximately 90% with Ottawa Cement Testing Sand (Fisher Scientific, Raleigh, NC). Triphenylphosphinetrisulfonate, sodium salt, was dissolved in methanol to prepare a 6000-ppm solution which was used to spike (50 µL) the sand (300 µg spike mass) contained in the vessel. Either pure methanol, or a solution of the appropriate ion-pairing

Table 4.1. High/Low Extraction Parameters Investigated*

	Low	High
Mole Ratio (Ion-Pairing Reagent: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 of CO ₂)	15	40
Flow Rate (mL/min.)	1.0	2.0
Methanol in-cell Spike Volume (μL)	75	200

* Ion-Pair Reagents were either Tetramethylammonium hydrogen sulfate or Tetrahexylammonium hydrogen sulfate

Table 4.2. Parameters Used for Fractional-Factorial Experiments

Extraction Order	Ion-Pair Reagent	Mole Ratio		Temp (°C)	Static Time (min.)	MeOH in-cell		Liquid Flow Rate (mL/min.)
		[Ion-Pairing Reagent]: [TPPTS]	CO ₂ Density (g/mL)			Spike Volume (mL)	Dynamic Mass (g CO ₂)	
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

Static time - time allotted for equilibration between analyte and fluid

Dynamic mass - mass of CO₂ flowed at a continuous rate through extraction vessel

* denotes ion-pairing reagent -tetrahexylammonium hydrogen sulfate

** denotes ion-pairing reagent - tetramethylammonium hydrogen sulfate

*** denotes - no ion-pairing reagent added

reagent in methanol was then spiked onto the previously trisulfonate spiked-sand surface. The total methanol-spike volume into the vessel including TPPTS with or without ion-pairing reagent was 75 or 200 μL , depending on the extraction method. The extraction vessel was then sealed, shaken by hand vigorously, and immediately extracted. The spiked-sand surface was not allowed to dry prior to extraction.

Extraction Trapping Conditions

The solid-phase trap used in this study consisted of glass beads (80/100 mesh). To ensure proper trapping of all extracted analyte, a tandem-liquid trap consisting of 2.0 mL methanol immediately followed the solid-phase trap. The solid-phase trap and tandem-liquid trap were maintained at (-) 5 $^{\circ}\text{C}$ and room temperature, respectively during the extraction. Upon completion of the extraction, the solid-phase trap temperature was raised to 30 $^{\circ}\text{C}$, and the trap was rinsed with 2.0 mL of methanol into an empty collection vessel. Both the solid-phase trap rinse and the tandem liquid trap were diluted with 3.0 mL of HPLC grade water and analyzed by HPLC separately.

Extract Analysis

Upon completion of the extraction, 50 μL of 6000-ppm caffeine in methanol (300 μg) was added to both 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 and/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 which was

diluted with 2.0 mL methanol and 3.0 mL of 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 x 4.6 mm i.d., 10 μ m) 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: 100% A for 2 min., ramped down to 66% A in 13 min., ramped down to 33% A in 17 min., ramped down to 5% A in 17.5 min. and held for 4.5 min. The system was then allowed to equilibrate for 7 min. with 100% A prior to the next injection. The column was maintained at 40 °C. A flow rate of 1.0 mL/min and injection volume of 10 μ L was used. Detection was by UV at 260 nm.

4.3 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 (TPPTS) (**Figure 4.1**) from a relatively uncomplicated matrix such as sand. This was accomplished by performing a screening study that investigated ion-pairing reagent composition, the mole ratio of ion-pairing reagent to TPPTS in the extraction vessel, carbon dioxide density, extraction temperature, static extraction time, CO₂ mass used, and liquid CO₂ flow rate.

It was believed that ion-pairing reagent composition and the relative amount of reagent added would play a significant role in the extraction process. Two ion-pairing reagents which varied in lipophilicity were investigated: tetramethyl- and tetrahexylammonium hydrogen sulfate. It was believed at the outset that due to their

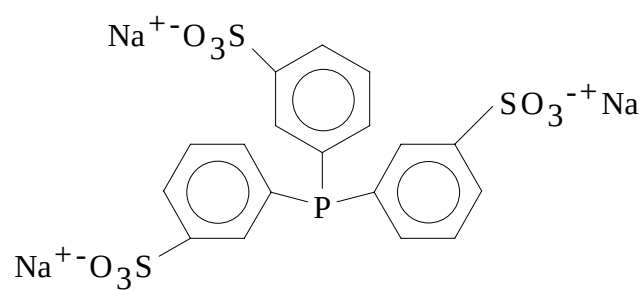


Figure 4.1. Chemical Structure of Triphenylphosphinetrisulfonate, Sodium Salt (TPPTS)

polarity, ionic compounds do not have sufficient solubility in the non-polar fluid carbon dioxide. In order to enhance the ionic compound's solubility and thus 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. This would act to make it more non-polar, and thus more extractable with a nonpolar fluid such as CO₂. 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 mole ratio of ion-pairing reagent to TPPTS was investigated. Complete ion-pair formation should be more favored when an excess of ion-pairing reagent was present.



Several SFE parameters were also investigated including carbon dioxide density, extraction temperature, static extraction time, mass of CO₂ used, and CO₂ liquid flow rate. It is well known that the solvating power of CO₂ is enhanced at a higher density. A higher extraction temperature may also increase the solubility of the analyte. The extraction efficiencies may be greater at a higher extraction temperature due to increased solubility as well as decreased analyte-matrix interactions. Static extraction time was also investigated. Higher extraction recoveries would be expected if more time was allowed for ion-pair formation to occur under supercritical conditions. The next parameter investigated was dynamic CO₂ mass. Larger amounts of CO₂ may be needed to extract all analyte. Lastly, flow rate was investigated. In the case of no static extraction time and at a higher flow rate, not enough time may be allowed for ion-pair formation to occur. Thus 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 investigated is plotted in **Figure 4.2**. Error bars representing one standard

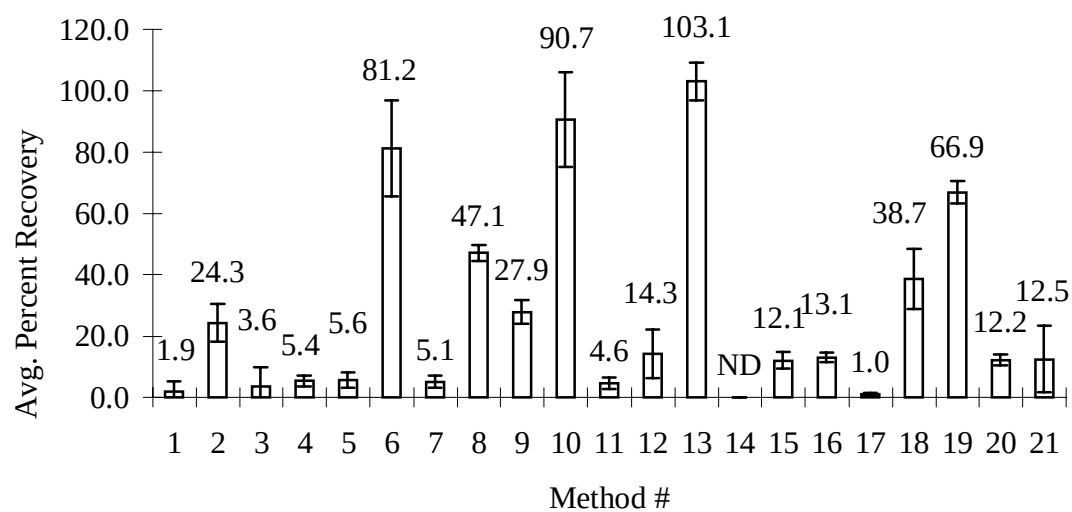


Figure 4.2. Average Percent Recovery (n=3) TPPTS vs Methods Investigated see Table 4.2 for Method # extraction conditions

deviation are also shown. 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 did indeed have 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 variables investigated and that no interactions existed among the factors investigated thus attributing to the response (recovery). Least squares coefficients are used to describe the relationship 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 what type of effect the variable has on the response (recovery). Furthermore, by examining the p-values or the significance values, one can determine 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.

In this study, 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 significantly play a role in the extraction process ($p < 0.1$). Therefore, CO_2 density, 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 4.3**).

Table 4.3. Least Square Coefficients, Reduced Fit, Response Percent Recovery

Term				
Constant	27.4 +/- 4.9			
	Slope	Error of Slope	t-Value	p-Value
IP 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
IP: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

As previously stated, the sign and magnitude of the slope of the least squares coefficients can be used 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), thus indicating that the response was most affected by this addition. 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.

The significant main effects on the recovery were next determined (**Figure 4.3**). 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 on the extraction recoveries was determined to be the ion-pairing reagent composition. First, by examining the p-values (**Table 4.3**), 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 affect 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 be still too polar to be extractable with methanol-modified CO_2 . In the case of the addition of tetrahexylammonium hydrogen sulfate, the formed ion-pair complex would be more non-polar, and thus more soluble and extractable than when forming the tetramethylammonium ion-pair. On the average, it was seen that

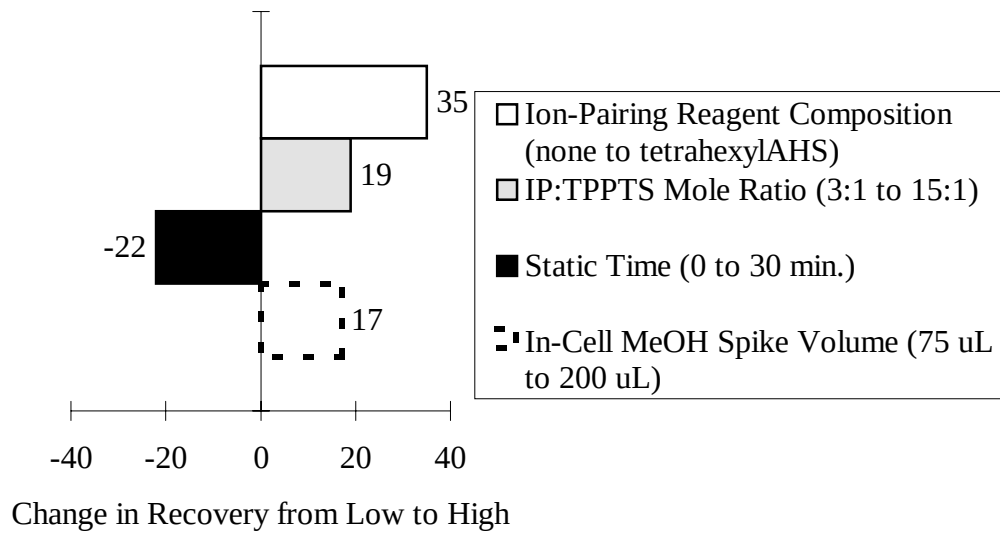


Figure 4.3. Significant Main Effects on Recovery

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 tetrahexylammonium hydrogen sulfate, the recoveries of the 7 experimental methods that were performed without any ion-pairing reagent present were averaged. Once again, no 2 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 on the average were increased by 3.5 fold.

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. It was observed that by adding an excess of ion-pairing reagent (15:1), the recoveries increased by approximately 19% versus the 3:1 mole ratio. The increase in recovery can be simply explained by Le'Chatliers principle. When an excess of ion-pairing reagent was added, the equilibrium shifted towards the right favoring ion-pair formation (see Equation 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 for the formation of the ion-pair complex. However, this was not the case. The recoveries decreased by approximately 22% with a static time of 30 minutes versus no static time. Present in the extraction vessel was TPPTS, ion-pairing reagent, and a certain volume of methanol. The methanol was not predried and was assumed to contain about 0.1% (w/w) water. In the presence of carbon dioxide, water will react to form carbonic acid, thus the overall pH of the resulting solution may be decreased to as low as 3.0.¹⁰ The pKa of

¹⁰ K.L. Toews, R.M. Shroll, C.M. Wai, N.G. Smart, *Anal. Chem.*, **67** (1995) 4040.

triphenylphosphinetrisulfonate is 2-3. If the pH of the resulting solution is decreased to approximately 3.0, the equilibria will consist of approximately a 50/50 mixture of the anionic trisulfonate as well as the protonated (neutralized) trisulfonate. Therefore, less ionic species would have been available to react with the ion-pairing reagent, thus resulting in lower recoveries. This hypothesis would assume that the protonated (neutralized) trisulfonate is insoluble in MeOH-modified CO₂; however, this assumption could not be experimentally confirmed. Due to the trisulfonate's low pKa, it would be extremely difficult to obtain experimental conditions (pH ~ 1) where it is assumed that the trisulfonate exists solely in the protonated (neutralized) form. Also, the use of an acid would be needed and thereby 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 known amount of the ion-pairing reagent to the matrix. Second, since 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 the ion-pair. 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 on the average of 17% when the volume of methanol was increased from 75 µL to 200 µL.

As previously stated, when examining TPPTS recoveries versus the various experiments, one particular method (Method 13) resulted in 103% recovery (6.0% RSD). If one compares the parameters for this method with the identified influential parameters, it is found 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. Experiment 13 offers proof that the influential parameters identified in this screening study were indeed optimal.

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