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Effects of liquid chromatography mobile phases and buffer salts on phosphorus inductively coupled plasma atomic emission and mass spectrometries utilizing ultrasonic nebulization and membrane desolvation

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Abstract

Atomic spectrometry, specifically inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) show promise for heteroatom-based detection of pharmaceutical compounds. The combination of ultrasonic nebulization (USN) with membrane desolvation (MD) greatly enhances detection limits with these approaches. Because pharmaceutical analyses often incorporate liquid chromatography, the study herein was performed to examine the effects of solvent composition on the analytical behaviors of these approaches.

The target analyte was phosphorus, introduced as phosphomycin. AES response was examined at the 253.7 nm atom line and mass 31 ions were monitored for the MS experiments. With pure aqueous solutions, detection limits of 5 ppb (0.5 ng in 0.1 mL injection volumes) were obtained with ICP-MS. The ICP-AES detection limit was 150 ppb. Solvent compositions were varied from 0 to 80% organic (acetonitrile and methanol) with nine buffers at concentrations typically used in liquid chromatography. In general, solvents and buffers had statistically significant, albeit small, effects on ICP-AES sensitivities. A few exceptions occurred in cases where typical liquid chromatography buffer concentrations produced higher mass loadings on the plasma. Indications are that isocratic separations can be reliably performed. Within reasonable accuracy tolerances, it appears that gradient chromatography can be performed without the need for signal response normalization. Organic solvent and buffer effects were more significant with ICP-MS. Sensitivities varied significantly with different buffers and organic solvent content. In these cases, gradient chromatography will require careful analytical calibration as solvent and buffer content is varied. However, for most buffer and solvent combinations, signal and detection limits are only moderately affected. Isocratic separations and detection are feasible.

Keywords: Liquid chromatography; ICP-AES and ICP-MS; Acetronitrile (ACN); Methanol (MeOH)

1. Introduction

As scientists develop medicines to better treat medical conditions and improve drug safety, pharmaceutical compounds are being synthesized that were not imagined a few years ago. Previously, these types of compounds were comprised of small molecules containing carbon, hydrogen, oxygen and nitrogen. The drive for new medications has seen the incorporation of heteroatoms such as sulfur, phosphorous, chlorine and fluorine.

Elemental analysis techniques such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) are utilized primarily for metal determinations. However, due to technological improvements and the incorporation of heteroatoms into drug molecules,

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the potential exists to utilize element selective detection for drug determinations. Coupling liquid chromatography with element selective non-metal detection has the potential to provide a tool complementary to traditional liquid chromatography–mass spectrometry (LC–MS) systems, especially for compounds that will not carry a charge under typical mass spectrometric conditions.

Because many chemists in the pharmaceutical industry approach coupling liquid chromatography to ICP-MS from an LC-MS perspective, there are some prevailing questions as to what mobile phase compositions are compatible with plasma mass spectrometer systems. For example, users of traditional LC–MS systems utilize more volatile mobile phase buffers. Should adding a less volatile salt such as perchlorate to an ICP-based system be an issue? Secondly, possible effects of mobile phase and buffer composition, such as ionization and/or emission quenching, deserve investigation. Lastly, commercial ICP systems were developed with metals detection in mind; due to their higher ionization and excitation energies, the requirements for non-metal signal generation are more stringent than those with metals.

For the pharmaceutical chemist, an important aspect of an analytical approach may be whether the technique can chromatographically resolve and detect the active pharmaceutical ingredient (API) and heteroatom containing impurities at concentrations greater than 0.05% that of the API. Spectroscopists tend to talk in terms of absolute elemental detection limits; however, these limits must correlate to the detectability of the specific drug and associated impurities. A first step towards characterizing the applicability of an LC–ICP-MS system for non-metal detection is to establish detection limits for representative non-metals and determine how the sensitivity is affected by various organic modifiers and mobile phase compositions.

Because of the higher ionization potentials, non-metal sensitivity for ICP-MS is expected to be markedly less than for metals [1]. Additionally, water, nitrogen and organic solvent decomposition products yield low molecular weight polyatomic ions. These ions may produce mass spectral overlaps with lower resolution quadrupole mass analyzers typically utilized in ICP-MS.

One route to improving non-metal detection limits is to enhance analyte transport to the plasma. Plasma-based instruments typically use pneumatic nebulizers for aerosol production. In comparison, ultrasonic nebulizers (USN) are more efficient in that they produce a larger volume of sample mist to increase analyte flux. The USN produces approximately 10 times more sample mist than typical pneumatic nebulizers [2]. They can also accommodate flow rates of a standard analytical chromatographic column. However, enhanced analyte delivery to the plasma is often accompanied by additional solvent droplets and vapor. This is true even in cases where the USN flow stream is directed through a heater–condenser combination to reduce solvent flux. Higher ionization energy elements, such as non-metals, are more susceptible to interferences and ionization quenching caused by solvent effects upon the plasma. This is especially true in the case of organic solvents. Introduction of organic solvent to plasma systems may require either limiting the overall organic solvent content or the utilization of a low liquid flow rate nebulizer so as not to extinguish the plasma or cause large decreases in sensitivity [3–6]. Varying analyte sensitivity over a range of solvent compositions used in gradient chromatography is also a concern. Larsen [7] suggested using isocratic conditions to maintain constant detector response. However, this approach may preclude the use of chromatography or significantly extend the analysis time.

An approach which has seen particular success is removing mobile phase organic containing solvents from the analyte stream before delivery to the plasma. While we have examined a number of methods to accomplish this task, our most successful and practical approach, by far, has been to utilize membrane desolvation (MD) [8–11]. With this technique, the analyte and solvent containing flow stream is heated to evaporate the solvent to the vapor form and produce analyte containing particulates. Solvent vapor is removed by osmotic pressure induced diffusion through a microporous PTFE membrane to a region continuously swept by a dry external countercurrent gas stream. We have been successful in delivering analyte to plasma systems, with minimal analyte loss, while removing essentially all or most of the organic solvents [8,10].

Other studies have been performed utilizing membrane desolvation coupled to plasma systems for HPLC detection [12–14]. These typically begin with a preset separation scheme and subsequent optimization of experimental parameters to achieve the best sensitivity. It would be advantageous to characterize the elemental response over a range of chromatographic mobile phase and ion buffer conditions so that the chromatographer may determine a priori which separation scheme will lead to sensitive non-metal detection. The study presented here is the first to comprehensively address the question “what traditional pharmaceutical mobile phases are compatible with liquid chromatography inductively coupled plasma systems for sensitive non-metal detection?” In doing this study, phosphorus as phosphomycin is examined both by ICP-AES and ICP-MS. Phosphorus was chosen as the model analyte based on the ability to detect it by both AES and MS. These results may also have implications and for predicting the behavior of other pharmaceutical heteroatoms such as sulfur, chlorine and fluorine.

2. Experimental

A schematic diagram of the ICP-MS experimental system is shown in Fig. 1. The ICP-AES system is similar, except that the MS system is replaced with an optical ICP system, as described below. Detailed operating parameters for all experiments are listed in Table 1.

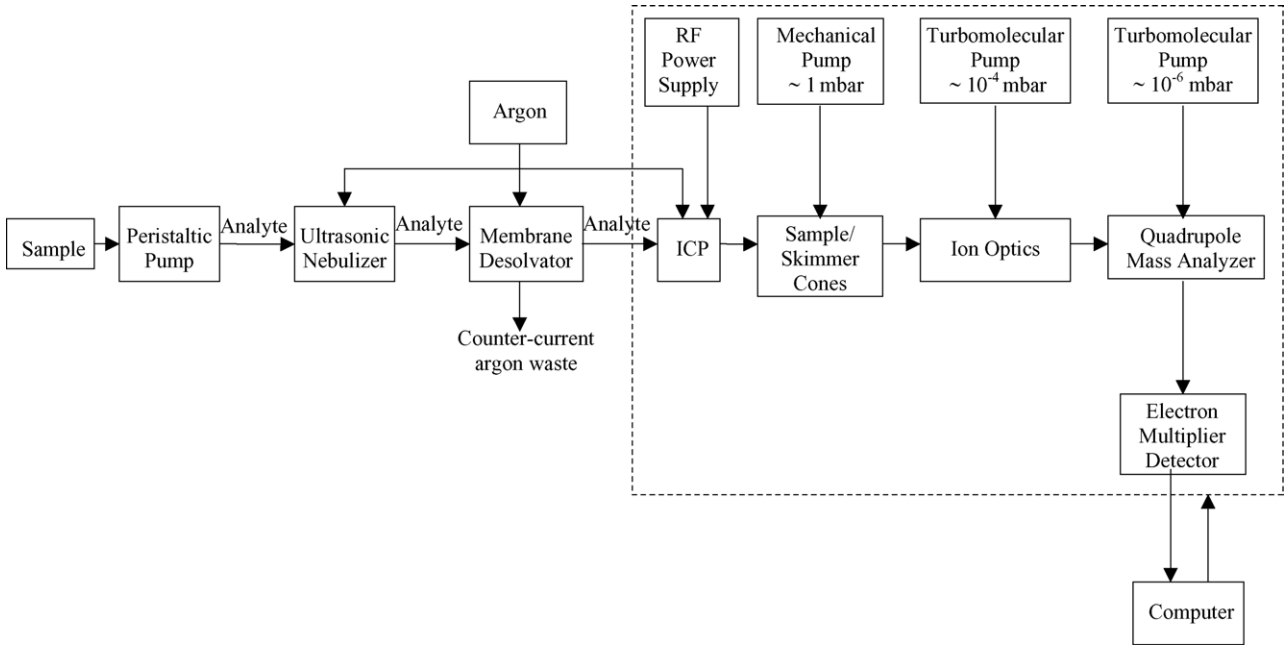

Fig. 1. Schematic diagram of experimental system. Dotted box indicates the ICP-MS unit.

2.1. Sample introduction

Liquid samples were directed to a CETAC U5000 USN (CETAC Technologies, Omaha, NE) with a Gilson Minipuls3 peristaltic pump (Gilson, Inc., Middleton, WI). Upon exiting the USN condensation apparatus, the nebulized mist was directed through a CETAC MDX-100 polytetrafluroethylene membrane desolvator. The desolvated samples were then transported to the plasma.

2.2. ICP-MS instrumentation

A Fisons (Thermo Electron) Instruments PlasmaQuad II 27 MHz ICP-MS was used. Ion signals from a Burle (Sturbridge, MA) Channeltron 4870V were acquired utilizing Thermo Electron PlasmaLab software (Version 1.06.007, Ionflight, Boston, MA). Mass spectra were obtained to confirm the peak position for ^{31}P. Single ion monitoring of peak intensities was used for quantification and the dwell time was set for 250 ms.

Table 1
Instrumentation operating parameters

	ICP-AES	ICP-MS
Peristalic pump flow rate (mL/min)	1.0	0.9
Flow injection volume (mL)	Not applicable	0.1–0.2
Nebulizer flow rate (L/min)	0.78	0.62
Nebulizer heater temperature (°C)	140	140
Nebulizer condenser temperature (°C)	3	3
Membrane desolvator temperature (°C)	140	160
Membrane desolvator countercurrent gas flow rate (L/min)	0.51	1.9
Argon plasma gas flow rate (L/min)	13.0	14.0
Argon intermediate gas flow rate (L/min)	0.1	0.8
Applied plasma power (W)	1250	1000

2.3. ICP-AES instrumentation

Nebulized and desolvated analyte was directed to a Model 2.5 Leeman Laboratories (Hudson, NH) 27 MHz ICP. The image of the ICP was laterally focused upon a Model 1000 SPEX (Edison, NJ) 1 m focal length monochromator equipped with a 1200 groove/mm grating. Entrance and exit slits were set at 25 μm. Phosphorus emission at 253.7 nm was monitored with a Hamamatsu (Middlesex, NJ) R928 photomultiplier tube biased at −850 V and SPEX DataScan2 hardware and software Version 1.5.4.0. Integration time was 250 ms.

2.4. Reagents and sample preparation

Phosphorus containing solutions were prepared by dilution of a stock solution containing phosphomycin calcium salt in 18 MΩ cm deionized water. Solvents included aqueous–organic mixtures containing methanol or acetonitrile. The nine buffer salts, their formulations, applicable acronyms, concentrations and other reagent data are listed in Table 2. It should be noted that it was, for obvious reasons, not possible to examine the behavior of the very common liquid chromatography buffer, phosphate.

3. Results and discussion

3.1. ICP-AES

Solutions containing phosphorus (4 ppm) as phosphomycin were directed to the USN. Phosphorus emission signals, background intensities and background noise were

Table 2
Solvent and buffer components used in this study

Compound (acronym) and aqueous solution concentration	Formula	Grade	Manufacturer
Methanol (MeOH)	CH_3OH	HPLC grade	Fisher Scientific (Pittsburgh, PA)
Acetronitrile (ACN)	CH_3CN	HPLC grade	Fisher Scientific (Pittsburgh, PA)
0.1% formic acid	HCOOH	>99%	Acros Organic (Pittsburgh, PA)
0.1% ammonium formate	$HCOONH_4$	99%	Acros Organic (Pittsburgh, PA)
0.1% acetic acid	CH_3COOH	Analytical grade	Mallinckrodt, Inc. (Paris, KY)
0.1% ammonium acetate	CH_3COONH_4	Reagent grade	Sigma–Aldrich (Milwaukee, WI)
0.1% trifluoroacetic acid (TFAA)	CF_3COOH	99%	Acros Organic (Pittsburgh, PA)
7.5 mM tetrabutylammonium hydroxide (TBAH)	$(C_4H_9)_4NOH$	HPLC grade	Acros Organic (Pittsburgh, PA)
0.2% perchloric acid	$HClO_4$	Reagent grade	J.T. Baker Chemicals (Phillipsburg, NJ)
0.1% triethylamine (TEA)	$(C_2H_5)_3N$	Reagent grade	Fisher Scientific (Pittsburgh, PA)
15 mM 1-heptane sodium sulfonate (HEPS)	$NaSO_3C_7H_{15}$	HPLC grade	Acros Organic (Pittsburgh, PA)

measured for aqueous solutions containing 0, 20, 50 and 80% organic mobile phases and each buffer. All results are reported as averages of triplicates. In cases where apparent anomalies were observed, additional data points were taken to improve the reliability of the averages. Detection limits (DL) and limits of quantification (LQ) were defined as analyte concentrations producing signals 3 and 10 times the standard deviation of the background noise, respectively. Results are summarized in Tables 3 and 4.

Phosphomycin, introduced in deionized water without buffer salts, yielded a phosphorus detection limit in the range of 150 ppb. With the 100% aqueous solutions, the presence of most buffers did not significantly affect the detection limits. Detection limits with six of the buffers remained within 15% of 150 ppb and were not statistically different. For HEPS, TBAH and TEA, the detection limits were degraded slightly more. Because these buffers were made at concentrations normally used in HPLC, the concentrations of the former two buffer salts (15 and 7.5 mM) had mass percentages three and two times that of the other buffers (0.1%). That factor may contribute to the detection limit increases seen with those buffers. In general, the intensities of the background remained constant, regardless of the solution composition.

In most cases, the addition of buffers caused signal depression. This depression is probably caused by a combination of "de-tuning" of the plasma caused by organic loading and effects of analyte nebulization and transport through the system. While membrane desolvation works well for lower boiling point solvents, higher boiling point buffer salts tend to be carried through the desolvator with the analyte. However, the suppression is moderate. Again, suppression was greatest when HEPS, TBAH and TEA were utilized.

To examine the response factors as a function of solvent composition, analytical sensitivity was examined over a range of organic:aqueous solvent concentrations. For the methanol:water solutions, these data are compiled in Table 3

Table 3
Phosphorus ICP-AES detection limits using methanol mobile phases

Buffer	Sensitivity (S/ppm)	DL (ppb)	LQ (ppb)	Buffer	Sensitivity (S/ppm)	DL (ppb)	LQ (ppb)
0% MeOH				50% MeOH			
None	55	150	500	None	53	170	570
0.1% CH_3COOH	53	150	500	0.1% CH_3COOH	53	180	600
0.1% CH_3COONH_4	54	150	500	0.1% CH_3COONH_4	54	190	630
0.1% HCOOH	53	150	500	0.1% HCOOH	52	190	630
0.1% TFAA	54	150	500	0.1% TFAA	54	170	570
0.1% TEA	45	200	670	0.1% TEA	25	410	1400
0.2% $HClO_4$	49	160	530	0.2% $HClO_4$	52	180	600
0.1% $HCOONH_4$	51	160	530	0.1% $HCOONH_4$	54	170	570
7.5 mM TBAH	46	260	870	7.5 mM TBAH	50	200	670
15 mM HEPS	44	190	630	15 mM HEPS	42	230	770
20% MeOH				80% MeOH			
None	59	180	600	None	60	170	570
0.1% CH_3COOH	59	180	600	0.1% CH_3COOH	57	180	600
0.1% CH_3COONH_4	58	200	670	0.1% CH_3COONH_4	56	200	670
0.1% HCOOH	58	180	600	0.1% HCOOH	56	190	630
0.1% TFAAA	60	180	600	0.1% TFAA	58	180	600
0.1% TEA	53	210	700	0.1% TEA	34	300	1000
0.2% $HClO_4$	56	180	600	0.2% $HClO_4$	56	180	600
0.1% $HCOONH_4$	58	180	600	0.1% $HCOONH_4$	59	180	600
7.5 mM TBAH	56	240	800	7.5 mM TBAH	41	390	1300
15 mM HEPS	55	200	670	15 mM HEPS	49	250	830

Table 4
Phosphorus ICP-AES detection limits using acetonitrile mobile phases

Buffer	Sensitivity (S/ppm)	DL (ppb)	LQ (ppb)	Buffer	Sensitivity (S/ppm)	DL (ppb)	LQ (ppb)
0% ACN				50% ACN			
None	55	150	500	None	65	160	530
0.1% CH_3COOH	53	150	500	0.1% CH_3COOH	66	160	530
0.1% CH_3COONH_4	54	150	500	0.1% CH_3COONH_4	66	170	570
0.1% HCOOH	53	150	500	0.1% HCOOH	64	160	530
0.1% TFAA	54	150	500	0.1% TFAA	67	170	570
0.1% TEA	45	200	670	0.1% TEA	43	260	870
0.2% $HClO_4$	49	160	530	0.2% $HClO_4$	71	150	500
0.1% $HCOONH_4$	51	160	530	0.1% $HCOONH_4$	62	170	570
7.5 mM TBAH	46	260	870	7.5 mM TBAH	28	440	1500
15 mM HEPS	44	190	630	15 mM HEPS	62	180	600
20% ACN				80% ACN			
None	40	240	800	None	61	130	430
0.1% CH_3COOH	40	230	770	0.1% CH_3COOH	67	130	430
0.1% CH_3COONH_4	39	260	870	0.1% CH_3COONH_4	13	660	2200
0.1% HCOOH	40	230	770	0.1% HCOOH	65	140	470
0.1% TFAA	40	250	830	0.1% TFAA	65	140	470
0.1% TEA	27	340	1100	0.1% TEA	54	150	500
0.2% $HClO_4$	39	230	770	0.2% $HClO_4$	69	110	370
0.1% $HCOONH_4$	40	240	800	0.1% $HCOONH_4$	54	160	530
7.5 mM TBAH	48	240	800	7.5 mM TBAH	49	170	570
15 mM HEPS	37	260	870	15 mM HEPS	57	150	500

and plotted for selected buffers in Fig. 2. It should be noted that each signal is subject to a 3% or greater standard deviation; credence should be placed on only significant changes in the signals. There is a general trend that compared to the 100% aqueous solutions, a slight depression in sensitivity is seen for the 20% methanol solutions. For most buffers, phosphorus signals increase again with 50% methanol and are enhanced even more greatly with 80% methanol. It is likely that this effect is caused by differences in solution viscosity, surface tension and the ability of the USN to nebulize these solvents with varying methanol compositions. Except for the TEA, ammonium formate and perchloric acid containing solutions, signals remained within 10% of the signal with the buffer and 100% water. The signal with TEA was depressed by 25% with 80% methanol and the signal with perchloric acid was enhanced by 14% with 80% methanol. However, these data indicate that solvent programming should be possible in most cases while retaining the response integrity of the system.

The corresponding study with water:acetonitrile mixtures is listed in Table 4. Fig. 3 shows data from this study with three of the nine buffers. In general, as with the water:methanol solutions there is a trend that the 20% organic:water solutions produces a signal suppression. With 20% acetonitrile, the 23% average suppression is much larger than with the 20% methanol:water solutions. Compared to solutions with no acetonitrile, signals with 50 and 80% acetonitrile exhibit sensitivity enhancements of an average of 18 and 10%, respectively. Within these groups are several outliers. For example, perchloric acid and HEPS produce exceptionally large enhancements (61–73%) with 50 and 80% acetonitrile.

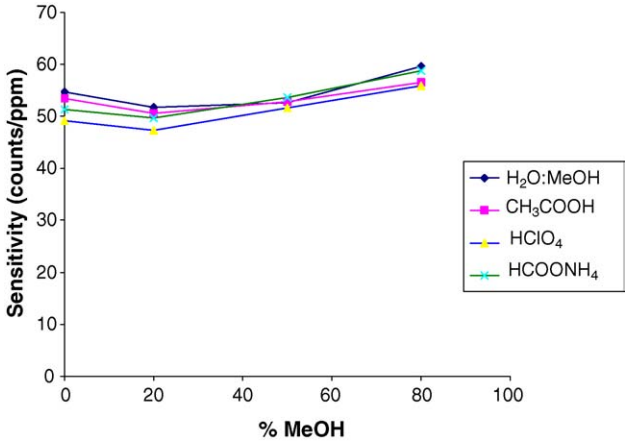

Fig. 2. ICP-AES phosphorus sensitivity as a function of methanol concentration.

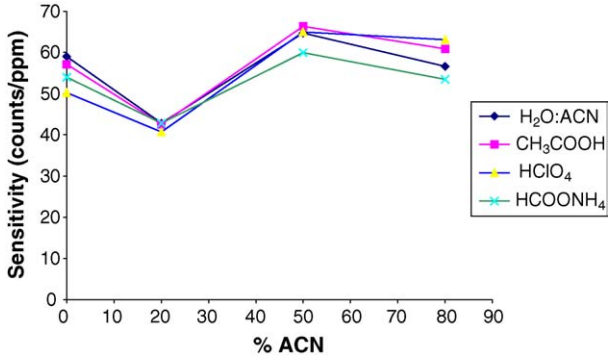

Fig. 3. ICP-AES phosphorus sensitivity as a function of acetonitrile concentration.

It is likely that these prominent differences between methanol and acetonitrile are caused by significant changes in nebulization characteristics, which may be visually seen with the higher acetonitrile solutions.

In summary, detection limits for phosphorus are on the order of 140–200 ppb, with a variety of buffers and solvent compositions. Buffers, in general, have a tendency to produce a depression in sensitivity. Buffers producing the most significant sensitivity effects are TEA, ammonium formate and perchloric acid. Effects of the methanol:water composition upon signal intensity are minimal. Although solvent gradient response varies significantly with the 0.1% TEA buffer, the average standard deviation for the remaining buffers is 6.1% and less than 5% for four of the buffers. It is likely then that LC solvent programming can be done without the need for recalibrating the response of the instrument as a function of the methanol:water ratio. Effects of the acetonitrile:water composition upon signal intensity are more significant. It is unlikely that LC solvent programming can be done reliably without the need for recalibrating the response of the instrument as a function of the acetonitrile:water ratio.

3.2. ICP-MS

Solutions containing the appropriate buffer and aqueous:organic solvent were nebulized. The phosphorus signal at m/z 31 was monitored and recorded to obtain the analyte signal, background and associated background noise. As with the AES experiments, results are reported as averages of triplicates. Using flow injection, 0.1–0.2 mL of phosphomycin containing solutions were introduced. Tables 5 and 6 summarize these results for the methanol and acetonitrile containing mobile phases, respectively. Figs. 4 and 5 display the absolute (mass) detection limits obtained for all buffers in each aqueous–organic solvent mixture for methanol and acetonitrile.

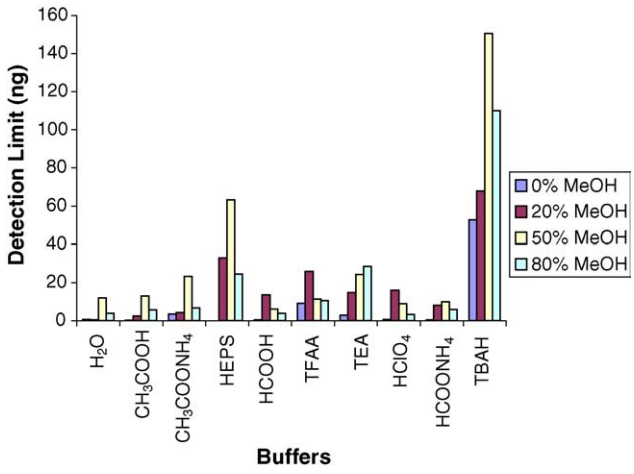

Fig. 4. ICP-MS phosphorus detection limits as a function of methanol concentration with various buffers.

Figs. 6 and 7 demonstrate the effect on sensitivity for methanol and acetonitrile concentration gradients. A large decrease in sensitivity was noted for both organic phases; however, detection limits (Figs. 8 and 9) did not similarly degrade. This behavior is especially true for the acetonitrile mobile phases. These differences are highlighted below and additional discussion is included to explain the signal behavior.

Table 5
Phosphorus ICP-MS detection limits using methanol mobile phases

Buffer	Sensitivity (icps*/ng)	DL (ng)	LQ (ng)	Buffer	Sensitivity (icps*/ng)	DL (ng)	LQ (ng)
0% MeOH				50% MeOH			
None	23000	0.54	1.8	None	220	12	40
0.1% CH_3COOH	77000	0.29	1	0.1% CH_3COOH	210	13	43
0.1% CH_3COONH_4	7300	3.5	12	0.1% CH_3COONH_4	270	23	78
15 mM HEPS	n.d.	n.d.	n.d.	15 mM HEPS	34	63	210
0.1% HCOOH	55000	0.49	1.6	0.1% HCOOH	340	6	20
0.1% TFAA	8200	9.1	30	0.1% TFAA	240	11	38
0.1% TEA	23000	2.9	9.6	0.1% TEA	200	24	81
0.2% $HClO_4$	97000	0.64	2.1	0.2% $HClO_4$	220	8.8	29
0.1% $HCOONH_4$	42000	0.46	1.5	0.1% $HCOONH_4$	330	10	33
7.5 mM TBAH	4600	53	180	7.5 mM TBAH	310	150	500
20% MeOH				80% MeOH			
None	22000	0.36	1.2	None	800	3.8	13
0.1% CH_3COOH	3600	2.4	8.1	0.1% CH_3COOH	910	5.7	19
0.1% CH_3COONH_4	1500	4.2	14	0.1% CH_3COONH_4	710	6.5	22
15 mM HEPS	130	33	110	15 mM HEPS	1000	25	82
0.1% HCOOH	1500	14	45	0.1% HCOOH	800	3.8	13
0.1% TFAA	1000	26	86	0.1% TFAA	750	11	35
0.1% TEA	1500	15	49	0.1% TEA	400	29	95
0.2% $HClO_4$	1300	16	53	0.2% $HClO_4$	650	3.2	11
0.1% $HCOONH_4$	1000	8.1	27	0.1% $HCOONH_4$	630	5.8	19
7.5 mM TBAH	560	68	230	7.5 mM TBAH	480	110	370

n.d.: not detectable under these conditions.
* Ion counts per second.

Table 6
Phosphorus ICP-MS detection limits using acetonitrile mobile phases

Buffer	Sensitivity (icps*/ng)	DL (ng)	LQ (ng)	Buffer	Sensitivity (icps*/ng)	DL (ng)	LQ (ng)
0% ACN				50% ACN			
None	23000	0.54	1.8	None	830	0.88	2.9
0.1% CH_3COOH	77000	0.29	1	0.1% CH_3COOH	1000	1.2	4.1
0.1% CH_3COONH_4	7300	3.5	12	0.1% CH_3COONH_4	1300	1.2	4.1
0.1% HCOOH	55000	0.49	1.6	0.1% HCOOH	1500	0.54	1.8
0.1% TFAA	8200	9.1	30	0.1% TFAA	1400	0.95	3.2
0.1% TEA	23000	2.9	9.6	0.1% TEA	2200	0.48	1.6
0.2% $HClO_4$	97000	0.64	2.1	0.2% $HClO_4$	1500	1.7	5.6
0.1% $HCOONH_4$	42000	0.46	1.5	0.1% $HCOONH_4$	1900	0.63	2.1
7.5 mM TBAH	4600	53	180	7.5 mM TBAH	2800	2.3	7.6
20% ACN				80% ACN			
None	5500	1.2	4.1	None	8900	0.54	1.8
0.1% CH_3COOH	21000	0.69	2.3	0.1% CH_3COOH	4900	0.54	1.8
0.1% CH_3COONH_4	4400	4.6	15	0.1% CH_3COONH_4	6200	0.86	2.9
0.1% HCOOH	3400	1.3	4.2	0.1% HCOOH	9800	0.43	1.4
0.1% TFAA	3700	1.9	6.2	0.1% TFAA	5800	0.72	2.4
0.1% TEA	3400	1	3.5	0.1% TEA	3600	0.41	1.4
0.2% $HClO_4$	49000	1.2	4	0.2% $HClO_4$	6300	0.69	2.3
0.1% $HCOONH_4$	8600	1	3.5	0.1% $HCOONH_4$	6200	0.51	1.7
7.5 mM TBAH	ND	ND	ND	7.5 mM TBAH	2100	3.7	12

ND: not detectable under these conditions.
* Ion counts per second.

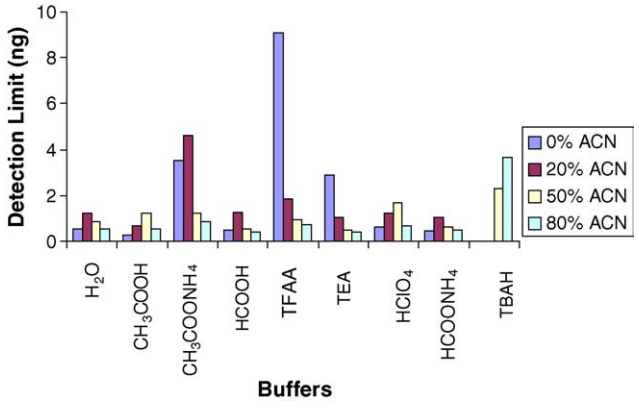

Fig. 5. ICP-MS phosphorus detection limits as a function of acetonitrile concentration with various buffers. The detection limit for phosphorus with tetrabutyl ammonium hydroxide in 0% acetonitrile excluded because of large value. With the same buffer and 20% acetonitrile, phosphorus was not detectable.

Examination of Table 5 indicates a significant loss of sensitivity once methanol is introduced in the solvent system. Fig. 4 illustrates a large range of absolute detection limits for each buffer. Ammonium formate, TFAA, TEA and TBAH most negatively affect detection limits for all mobile phase compositions. The buffers which least affected detection limits were acetic acid, formic acid, perchloric acid and ammonium formate. Detection limits with each of these buffers remained below 20 ng for all mobile phase compositions.

Fig. 6 demonstrates the large drop in sensitivity upon addition of methanol. While signal suppression was associated with an increase in detection limits, the increase was not nearly as significant as might be expected by the sensitivity drop as seen in Fig. 4. Fig. 8 indicates detection limits for a set of buffers for the methanol:water concentration gradient. The combination of these factors indicate that it will

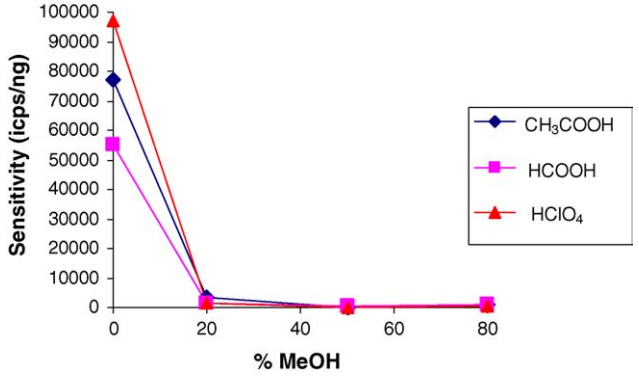

Fig. 6. ICP-MS phosphorus sensitivity as a function of methanol concentration for selected buffers.

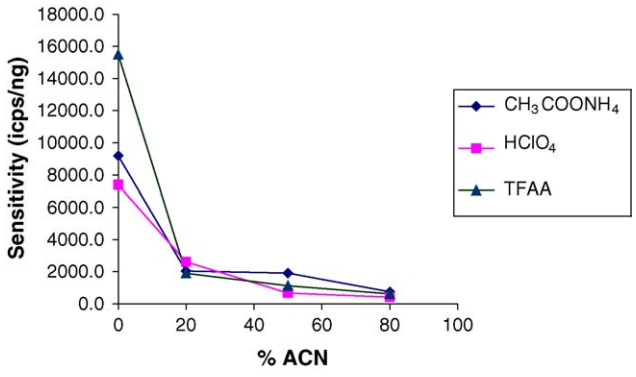

Fig. 7. ICP-MS phosphorus sensitivity as a function of acetonitrile concentration for selected buffers.

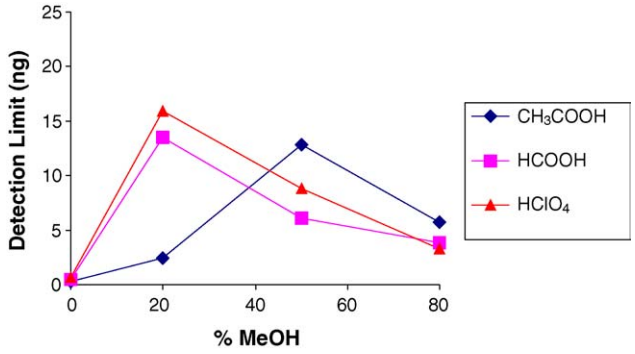

Fig. 8. ICP-MS phosphorus detection limits as a function of methanol concentration for selected buffers.

be difficult, at best, to use methanol:water solvent gradients with USN–MD–ICP-MS.

While there is some loss in sensitivity upon addition of acetonitrile to the aqueous mobile phase solutions, the drop is much less when compared to the methanol mobile phases. Table 6 shows absolute detection limits when using flow injection. Values are in the range of single nanograms or less for all of the solutions except two. Fig. 5 indicates that the buffers, which most negatively affect detection limits, are TFAA, TBAH, TEA and ammonium acetate. The detection limit for aqueous phosphorus with the TBAH buffer was excluded because it is large in comparison to the other buffer mobile phases. Also the phosphorus in the 20% acetonitrile/TBAH buffer solution was not detectable using the nebulized concentration. As with the AES systems, the significant detectability loss with TBAH may be due to the higher mass loading of the higher concentration buffer.

Fig. 7 illustrates the drop in sensitivity upon an increase in solvent acetonitrile content. However, as seen in Fig. 9, detection limits are less affected as the acetonitrile content is increased.

While using acetonitrile, carbon deposits on the sample and skimmer cones became substantial. These deposits were more pronounced with acetonitrile containing mobile phases as compared to methanol mobile phases and required more frequent maintenance. This observation indicates that 100% desolvation of the aerosol stream is not achieved. The slower

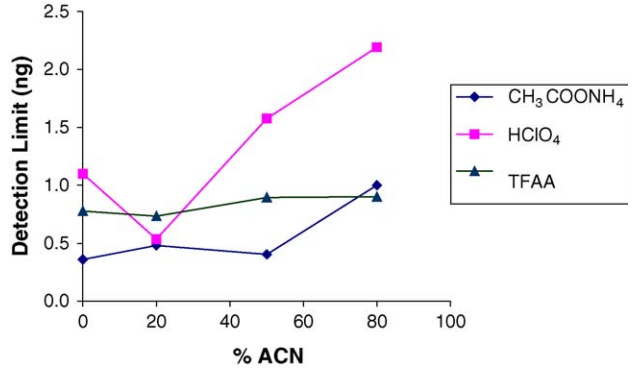

Fig. 9. ICP-MS phosphorus detection limits as a function of acetonitrile concentration for selected buffers.

buildup of carbon with methanol containing mobile phases is likely due to the more oxidizing environment produced by methanol. Adding a small amount of O_2 to the sample gas stream has been shown to reduce carbon deposition when organic solvents are being used [15,16]. This work is currently underway in our laboratory.

With detection limits in the sub-ng/100 μL range (single ppb range), ICP-MS is much more sensitive than AES for phosphorus detection. While this has obvious benefits, the technique is also more susceptible to small changes in sample composition. It is important to identify possible interferences that may be present at the analyte ion mass. Monitoring the ^{31}P isotope, there may be significant interference contributions from $^{14}N^{16}O^{1}H$ and $^{15}N^{16}O$. Matrix elements containing these atoms may have a deleterious effect on the background signal and stability. While several of the buffers in this study contain nitrogen, only TBAH produced a significant increase in the background ion count. Ammonium acetate, ammonium formate and TEA did not produce a substantial increase background signal. A possible explanation for differences as compared to TBAH may be due to the increased mass fraction of the buffer in solution and the lower volatility and, hence, increased transport through the desolvator to the plasma.

The USN nebulization efficiency visibly decreased upon increases in the fraction of both methanol and acetonitrile. These effects may be caused by viscosity and surface tension changes as a function of liquid composition. Of course, any variations in sample nebulization will affect the analytical signal intensity. The nebulization efficiency changes may help explain the loss in sensitivity upon addition of organic solvent. These effects will vary not only with the percent of organic solvent in the solution, but also with the nature of the organic solvent.

Additionally, differences in the physical properties of acetonitrile and methanol-based solvents such as volatility, surface tension, density, thermal conductivity, etc., will alter USN and MD desolvation behavior. For example, the boiling point of methanol is 65.5 °C while that of acetonitrile is 81.6 °C. Lastly, it is possible that the transport efficiency of a semi-volatile analyte such as the phosphomycin used in this study may be influenced by the volatility of the buffer. For example, if the dry analyte containing particulate traverses the desolvation system in the presence of a low volatility buffer, transport efficiency may be positively affect. If, on the other hand, the buffer is of high volatility, it may be volatilized and transported through the desolvator to waste, leaving behind less “protected” and more surface evaporable analyte. These are items, which are beyond the scope of this paper, but deserve fuller characterization.

4. Conclusion

This publication has described experiments meant to aid in characterizing the behavior of ICP-AES and ICP-MS

using USN and MD for heteroatom detection in pharmaceutical compounds. The focus has been to characterize detection behavior as a function of various solvent compositions and buffer combinations, which might be utilized in liquid chromatography. While this manuscript does not specifically report liquid chromatography results, such a combined approach could be used to chromatographically separate and detect phosphorus containing entities in a complex biological sample. The model system of this study examined phosphorus as phosphomycin.

Depending on the solvent–buffer combinations, detection limits in the range of single parts per billion were obtained with ICP-MS. Detection limits were approximately 2 orders of magnitude higher with ICP-AES.

With AES, responses changed minimally as the solvent composition (percent organic) was varied. In fact, it appears that within reasonable accuracy constraints, LC solvent programming can be performed with methanol solvents without instrument response recalibration. Sensitivities varied a bit more with water:acetonitrile mixtures, but still remained within 20% of that of the pure aqueous solvent. However, with ICP-MS, sensitivities decreased significantly as the methanol or acetonitrile content was increased. This behavior may indicate that increased organic solvent may affect phosphorus ionization more significantly than phosphorus atom excitation.

As compared to solutions without buffers added utilizing AES, most buffers only minimally affected signals and detection limits. Exceptions were seen with TEA, TBAH and HEPS. At this point, it is not completely clear why these buffers causes more problems. However, reasons for the deleterious effects with these TBAH and HEPS have been due to higher mass loadings with the concentrations utilized for the chromatography conditions simulated.

Buffer effects were more significant with ICP-MS. Whether in pure aqueous solutions or aqueous–organic mixtures, results were scattered. A number of the buffers produced sensitivity enhancements, but a number produced suppression. While these results are empirical, it is clear that response calibration is closely related to the nature of the solvent–buffer system and that any calibration standards must be closely matched in composition. Further studies are needed to determine the fundamental reasons for these behaviors. As the organic mobile phase concentrations are increased, the phosphorus ion signal decreases. These effects are more pronounced with the methanol-based solvents than with the acetonitrile-based solvents. Ultrasonic nebulizer efficiency may play a role in the signal reduction at higher organic phase concentrations; however, because the signal reduction is less significant, it is more likely that ionization suppression effects caused by higher organic solvent concentrations is the cause.

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