DIONEX

Application Update 172

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Determination of Polyphosphates Using Ion Chromatography

INTRODUCTION

Dionex Application Note 71 (AN 71) demonstrates that ion chromatography (IC) using an IonPac® AS11 column set with a hydroxide eluent gradient and suppressed conductivity detection is a good technique for determining individual polyphosphates in a sample. The same method can also be used for fingerprinting a polyphosphate preparation to determine the distribution of polyphosphate chain lengths (e.g., AN 71, Figure 2) and monitor polyphosphate degradation. Since the publication of AN 71, several publications have reported successful IC methods to analyze a variety of samples for polyphosphates and related compounds.[1–7] Polyphosphates were determined in ham, cheese, and fish paste.[3] A high-capacity AS11 column (IonPac AS11-HC) introduced after the publication of AN 71, was used to determine tripolyphosphate in seafood products.[4] The AS11 column was used to determine polyphosphates and other anions in fountain solutions used in the printing industry.[5] More recent publications measured polyphosphates in fish and cheese to determine if the products contained polyphosphates within allowable limits.[6,7]

In addition to the IonPac AS11-HC column, there have been other significant improvements in IC technology since the publication of AN 71. In 1998, Dionex introduced eluent generation. An eluent generator equipped with the appropriate cartridge produces pure hydroxide eluents with high fidelity. This removes the burden and possible error associated with manual preparation of hydroxide eluents and results in highly reproducible retention times and results. Sekiguchi et al. were the first to apply eluent generation to the determination of polyphosphates, and the results were more precise than when eluents were manually prepared.[3] Dionex also introduced the IonPac AS16 column. Compared to the AS11 column, the AS16 column has almost four times more capacity and greater selectivity for polyvalent anions. These properties make the AS16 column the best choice for polyphosphates, allowing easier elution of larger polyphosphates, determination of lower quantities of larger polyphosphates in the presence of large amounts of smaller polyphosphates, and determination of polyphosphates in more ionic samples without sample dilution compared to the AS11 column. More recent publications that determine polyphosphates by IC use the AS16 column.[6,7]

This application update briefly compares the performance of the IonPac AS16 column with hydroxide eluents prepared by the eluent generator to manually prepared hydroxide eluents and discusses the value of each approach. The method was applied to the determination of polyphosphates in a sausage sample and in a sodium hexametaphosphate powder. The combination of a high-capacity AS16 column, a hydroxide eluent, and suppressed conductivity detection delivers sensitive high-resolution separations and accurate determination of polyphosphates.

EQUIPMENT

Dionex ICS-3000* system consisting of:

- SP or DP Pump
- DC Detector/Chromatography module with single or dual temperature zone equipped with, 2 × 6-port injection valve
- EG Eluent Generator module–only needed for Method B
- EGC II KOH Cartridge (P/N 058900)–only needed for Method B
- AS Autosampler

Chromeleon® 6.8 Chromatography Data System (CDS) software

* Applications requiring only the eluent generation-based method (Method B) can use the ICS-2100 system or any other Dionex RFIC-EG™ system.

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent-grade, 18 MΩ-cm resistivity or better

400 g/L Sodium hydroxide solution (NaOH, KANTO) or 50% NaOH (Fisher)

Trisodium orthophosphate (Na_3PO_4•$12H_2O$, AJAX Finechem)

Tetrasodium pyrophosphate ($Na_4P_2O_7$•$10H_2O$, AJAX Finechem)

Trisodium trimetaphosphate ($Na_3O_9P_3$, Sigma-Aldrich)

Sodium triphosphate pentabasic ($Na_5O_{10}P_3$, Fluka)

Sodium polyphosphate (Sodium hexametaphosphate powder) ($(NaPO_3)_n$•Na_2O, AJAX Finechem)

PREPARATION OF SOLUTIONS AND REAGENTS

200 mM Sodium Hydroxide Eluent Solution

To prepare 1 L, pipette 20 mL of 400 g/L sodium hydroxide solution to the 1 L volumetric flask and bring to volume with DI water. If preparing from a 50% NaOH solution, use 10.4 mL or weigh 8.0 g.

Standard Solutions

Stock Standards

Prepare 1000 mg/L stock standard solutions by dissolving the appropriate weight of the salt listed in Table 1 into separate 100 mL volumetric flasks in DI water.

Secondary Standards

The stock standards are used to prepare the standards for calibration, MDL determination, and the spike recovery test.

Table 1. Mass of Compounds Used for 100 mL of 1000 mg/L Standards

Standard	Salt	Weight (g)
Orthophosphate	Trisodium orthophosphate (Na_3PO_4·$12H_2O$)	0.809
Pyrophosphate	Tetrasodium pyrophosphate ($Na_4P_2O_7$·$10H_2O$)	0.268
Trimetaphosphate	Trisodium trimetaphosphate ($Na_3O_9P_3$)	0.129
Triphosphate	Sodium triphosphate pentabasic ($Na_5O_{10}P_3$)	0.145

Sample Preparation

Sodium hexametaphosphate powder (SHMP) and a sausage sample were analyzed for polyphosphates. The sample preparation is as follows:

SHMP Sample

Weigh and transfer 0.1 g SHMP sample to a 100 mL volumetric flask, bring to volume with DI water, and mix to dissolve.

Sausage Sample

Grind the sausage and transfer 5 g to a 100 mL bottle. Add 95 mL DI water, shake, and place in an ultrasonic bath for 15 min. Filter the sample using a 0.45 µm cellulose filter. Pass the sample through an OnGuard® II RP cartridge (Dionex, P/N 057083) prior to analysis.

CONDITIONS

Method A

Column:	2 mm: IonPac AS16, 2 × 250 mm (P/N 055378) IonPac AG16, 2 × 50 mm (P/N 055379)
Eluents:	A = DI water B = 200 mM NaOH

Gradient:

Time (min)	A (%)	B (%)
–7.00	75	25
0.00	75	25
2.00	75	25
119.90	0	100 (Curve 3)
120.00	75	25

Flow Rate:	0.25 mL/min
Sample Volume:	10 µL
Column Temp:	30 °C
Pressure:	~1200 psi
SRS Current:	124 mA
Suppressor:	ASRS® 300, 2 mm (P/N 064555)
Suppressor Mode:	External water mode
Detection:	Conductivity with CRD 200, 2 mm (P/N 062986)

Method B

Column:	IonPac AS16, 2 × 250 mm IonPac AG16, 2 × 50 mm
Eluent Source:	EGC II KOH
Gradient:	50 mM KOH from 0–2 min, 50–100 mM from 2–80 min
Flow Rate:	0.25 mL/min
Sample Volume:	10 µL
Column Temp.:	30 °C
Pressure:	~2300 psi
SRS Current:	80 mA
Suppressor:	ASRS 300, 2 mm
Suppressor Mode:	External water mode
Detection:	Conductivity with CRD 200, 2 mm

RESULTS AND DISCUSSION

The IonPac AS16 column was designed for fast elution of multivalent anions, such as polyphosphates with: 1) similar hydroxide concentrations to those used with columns having lower capacity, and 2) lower hydroxide concentrations compared to columns with similar capacity. Although the AS16 column has over four times the capacity of an AS11 column (used in AN 71 for polyphosphate separations), the AS16 column is able to elute larger polyphosphates than the AS11 column with the 100 mM maximum hydroxide concentration of the eluent generator. Figure 1 shows a comparison of separations of a sodium hexametaphosphate powder with the AS11 and AS16 columns, and demonstrates that the AS16 should be the column of choice for developing a polyphosphate determination method. Figure 1 also demonstrates that at 100 mM NaOH, there are still polyphosphates eluting from the column at 50 min and the peaks are becoming increasing broad due to the elution being isocratic after 10 min.

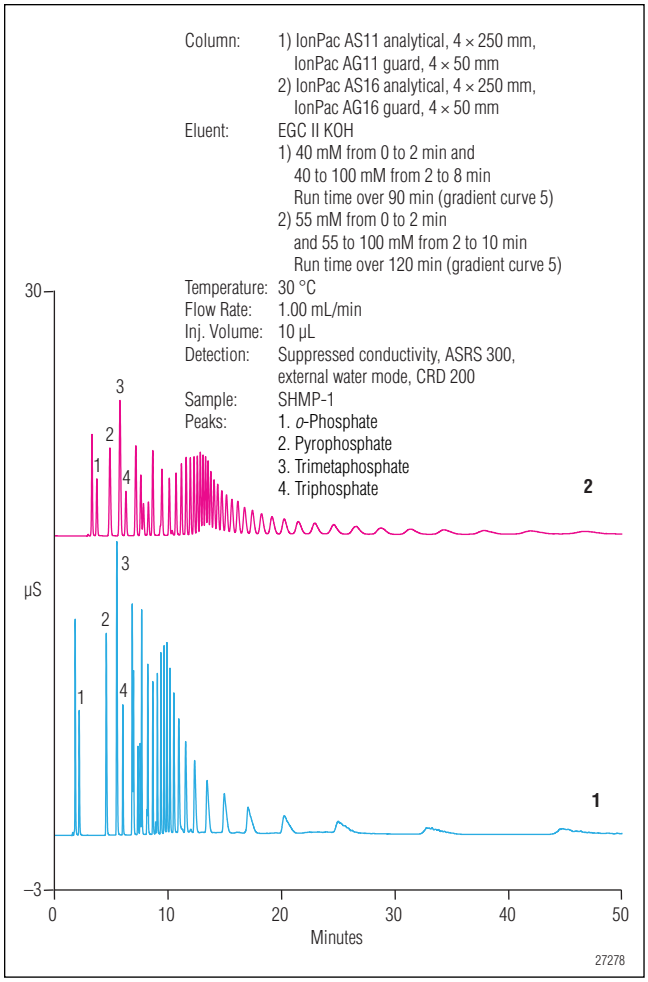

Figure 1. Comparison of a polyphosphate sample separated on an IonPac AS11 column (Panel 1) and an IonPac AS16 column (Panel 2).

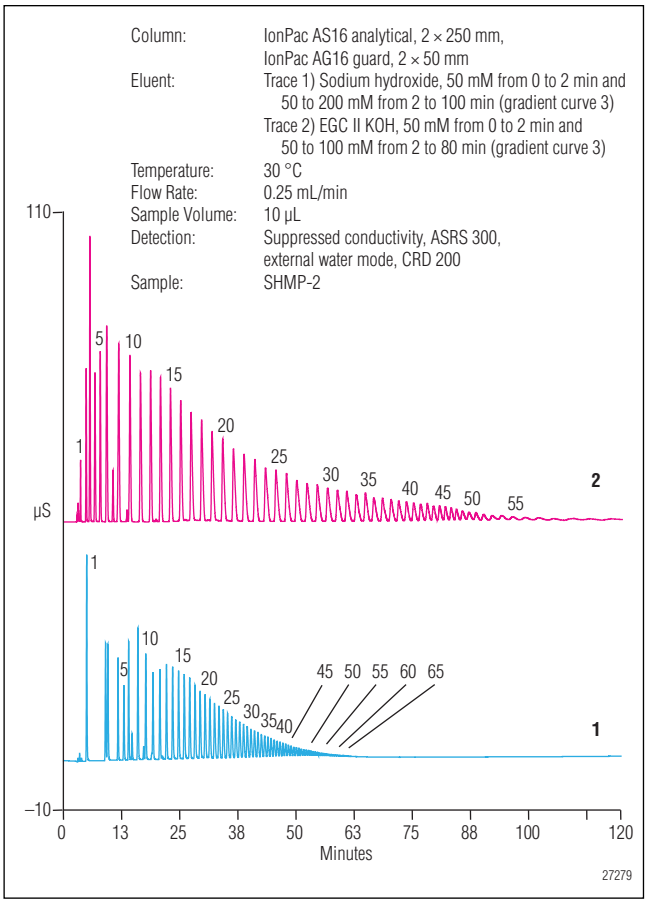

Figure 2. Comparison of the chromatography of the SHMP-2 sample using a 2 mm IonPac AS16 column with either Method A (chromatogram 1) or Method B (chromatogram 2).

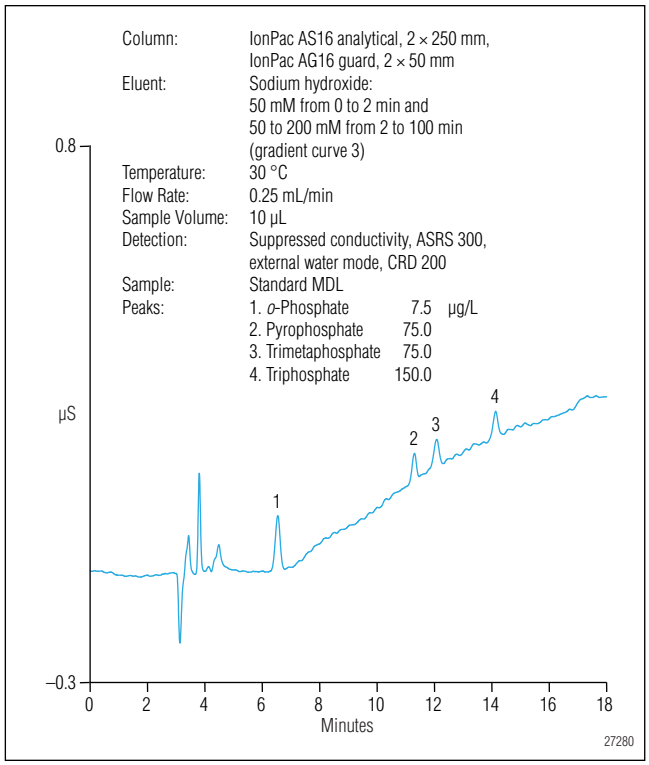

Figure 3. Chromatogram from the MDL study.

Figure 2 shows a comparison of two methods for the separation of a second sodium hexametaphosphate powder: 1) by using an eluent generator to prepare the hydroxide eluent, and 2) by manually preparing hydroxide eluent. Both separations use a 2 mm IonPac AS16 column rather than the 4 mm column used for the comparison in Figure 1. Chromatogram 2 shows the trace generated by the eluent generator. This method is able to elute over 50 peaks but due to the 100 mM KOH upper concentration limit of the eluent generator, it may not be possible to elute all the sample's polyphosphates. This is evident from the small broad peaks eluting between 100 and 120 min when the eluent is 100 mM KOH. Chromatogram 1 uses manually prepared NaOH eluents to achieve a higher final eluent concentration. Using a much steeper eluent slope, we are able to elute greater than 60 peaks in less than 65 min. Though it is possible to shorten this method or reduce the final [NaOH] in the gradient, this method was used by the authors to account for the possibility of samples with larger polyphosphate chains. While a 4 mm column can be used with a flow rate increase to 1 mL/min and suppressor current increase to 495 mA, a 2 mm column uses four times less eluent and therefore, generates four times less waste. A 2 mm column was used for the remainder of the work.

In this study, standards of ortho-phosphate, pyrophosphate, trimetaphosphate, and triphosphate were used to calibrate the method and determine minimum detection limits. Table 2 shows that all four compounds have a linear response over the concentration range chosen. Using seven injections of the MDL standard and the Student's t-test with a 99% confidence limit, the detection limits were determined to be 2.07 µg/L for *o*-phosphate, 16.39 µg/L for pyrophosphate, 22.09 µg/L for trimetaphosphate, and 43.56 µg/L for triphosphate. Figure 3 shows a chromatogram of one of the injections of the MDL standard.

Table 2. Calibration Standard Concentrations and Calibration Results

Analyte	Concentration (mg/L)				r^2 (x 100)
	Level 1	Level 2	Level 3	Level 4	
o-Phosphate	1.25	5.00	20.0	80.0	99.9758
Pyrophosphate	1.25	5.00	20.0	80.0	99.9935
Trimetaphosphate	1.25	5.00	20.0	80.0	99.9868
Triphosphate	1.25	5.00	20.0	80.0	99.9802

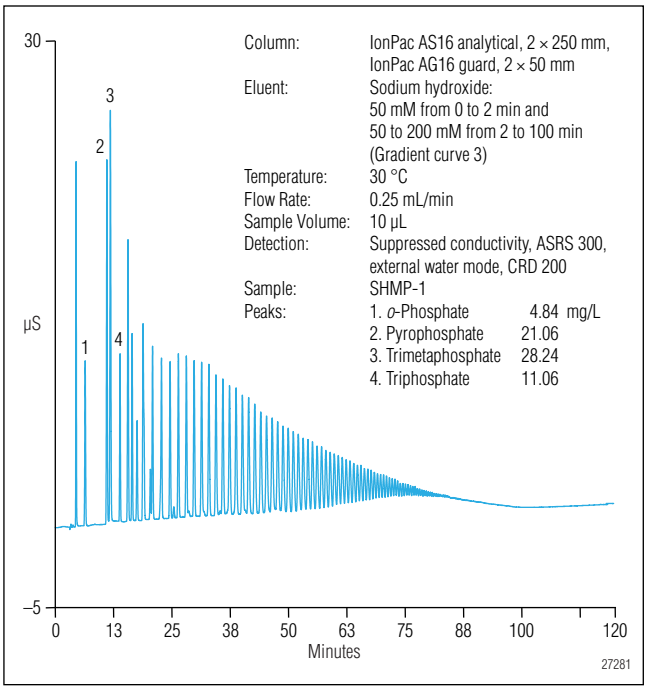

Figure 4. Chromatography of the SHMP-1 sample.

The same method was used to evaluate the two customer-provided hexametaphosphate samples and a sausage sample purchased at a market in Bangkok. Figures 4–6 show chromatography of these samples. Note the difference in the *o*-phosphate peak size between chromatogram 1 in Figures 2 and 5. The sample in Figure 5 was prepared just before analysis while the sample in Figure 2 resided in the autosampler at ambient temperature for a few days and degraded a small amount. See the Precautions section that follows for recommendations on minimizing sample degradation. In addition to determining the amounts of the four calibrated phosphates in each sample, the standards of the same four compounds were also spiked in each sample and

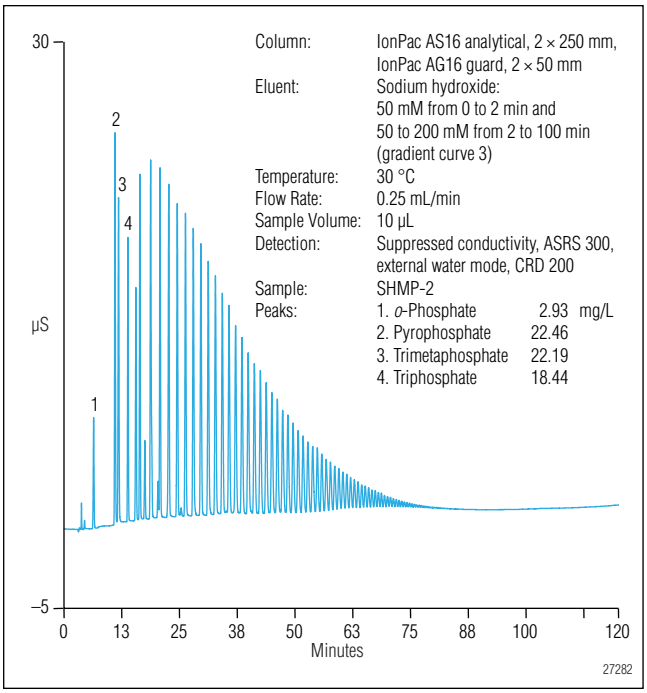

Figure 5. Chromatography of the SHMP-2 sample.

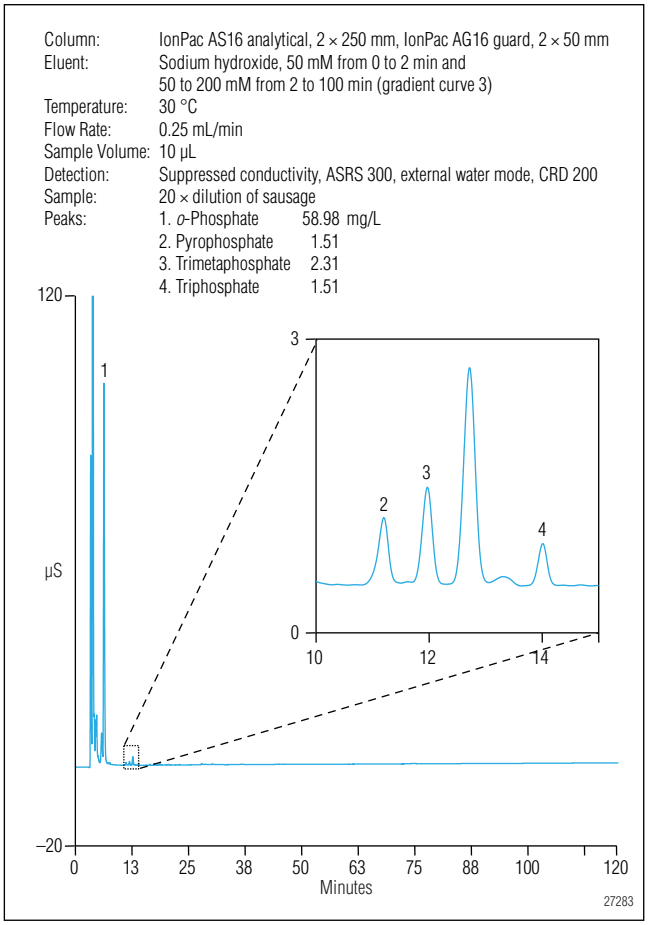

Figure 6. Separation of a 20 × dilution of the sausage sample.

Table 3. Summary of Sample Determinations					
Sample Name		**Amount (mg/L)**			
		***o*-Phosphate**	**Pyrophosphate**	**Trimetaphosphate**	**Triphosphate**
SHMP-1	Sample	4.84	21.1	28.2	11.1
	Spike	0.50	5.00	5.00	5.00
	Sample + Spike	5.58	26.6	34.4	16.2
	%Recovery	105	102	103	101
SHMP-2	Sample	2.93	22.5	22.2	18.4
	Spike	0.50	5.00	5.00	5.00
	Sample + Spike	3.60	27.1	27.3	22.8
	%Recovery	105	98.6	100	97.4
Sausage	Sample	58.98	1.51	2.31	1.51
	Spike	20.00	0.50	0.50	0.50
	Sample + Spike	77.48	1.94	2.61	1.96
	%Recovery	98.1	96.3	92.9	97.5

Table 4. Retention Time Reproducibility of 10 Injections of SHMP-2 with Method A								
Injection #	**Peak #, Retention Time (min)**							
	1	**5**	**10**	**15**	**20**	**25**	**30**	**35**
1	6.540	15.807	23.144	32.044	39.754	46.600	52.717	58.207
2	6.550	15.787	23.137	31.973	39.637	46.470	52.550	Out*
3	6.530	15.803	23.163	31.997	39.617	Out*	Out*	Out*
4	6.520	15.810	23.150	31.990	39.644	46.400	Out*	Out*
5	6.524	15.790	23.147	31.994	39.594	Out*	Out*	Out*
6	6.520	15.807	23.147	31.954	39.554	Out*	Out*	Out*
7	6.524	15.797	23.144	31.984	39.577	Out*	Out*	Out*
8	6.534	15.797	23.154	31.984	39.584	Out*	Out*	Out*
9	6.510	15.790	23.130	31.947	Out*	Out*	Out*	Out*
10	6.523	15.777	23.100	31.910	Out*	Out*	Out*	Out*

* The peak is out of the retention time window established by the first injection.

determined recoveries. The results are displayed in Table 3 and show that recoveries ranged from 92 to 110% for all four compounds in all three samples. These good recovery values suggest that the method accurately determines these polyphosphates in all three samples. The concentrations reported for the sausage sample are the concentrations in the 20 times dilution and not the concentrations in the original extract from 5 g of sausage.

Samples containing polyphosphates may contain larger polymers that are not easily eluted from the column, or other strongly retained compounds that take column capacity.

SHMP-2 is such a sample. Table 4 shows 10 consecutive injections of this sample. Note the later eluting peaks start missing their retention time windows after only a few injections. When a column loses capacity, the multiply charged ions are the first to exhibit retention time loss. Figure 7 shows the 1st injection and the 10th injection. Though difficult to observe, there is loss of retention in the later eluting peaks. For example, peak 30's retention time decreased about 0.8 min between the 1st and 10th injection. Repeating the experiment with a 23 min 200 mM NaOH wash after every injection

Table 5. Retention Time Reproducibility of 10 Injections of SHMP-2 with Method A with 23 min of Cleaning Column by 200 mM NaOH after each Injection								
Injection #	**Peak #, Ret.Time (min)**							
	1	**5**	**10**	**15**	**20**	**25**	**30**	**35**
1	6.427	15.663	21.017	30.010	37.687	44.443	50.520	55.950
2	6.440	15.697	21.084	30.064	37.740	44.477	50.557	55.994
3	6.437	15.704	21.057	30.060	37.740	44.507	50.540	55.900
4	6.450	15.710	21.067	30.053	37.757	44.537	50.557	55.933
5	6.467	15.714	21.070	30.094	37.800	44.584	50.634	56.024
6	6.440	15.714	21.084	30.110	37.830	44.574	50.600	55.990
7	6.443	15.713	21.093	30.117	37.813	44.567	50.650	56.050
8	6.437	15.703	21.070	30.083	37.757	44.517	50.560	55.943
9	6.427	15.687	21.067	30.037	37.690	44.450	50.507	55.840
10	6.440	15.687	21.034	30.014	37.687	44.394	50.387	Out*
Average	6.441	15.699	21.064	30.064	37.750	44.505	50.551	55.958
RSD (%)	0.179	0.103	0.111	0.124	0.139	0.142	0.146	0.115

* The peak is out of the retention time window established by the first injection.

stabilizes the retention times (Table 5). The decision to include a wash depends on the sample and the goals of the analysis. If the goal is to simply identify the number of polyphosphate peaks in a sample, that can be accomplished without a wash for at least 10 injections (Figure 7). It may be more convenient to run a separate column wash after a fixed number of injections.

The sample analyses in this application update used a final eluent concentration of 200 mM NaOH, which was manually prepared. For many polyphosphate determinations, a method using the eluent generator (e.g. Method B) will be appropriate. For example, Method B may have been used to analyze the sausage sample. It is possible that the column may need to be cleaned occasionally with an eluent stronger than 100 mM KOH.

CONCLUSION

The IonPac AS16 column is an ideal column for polyphosphate determinations and can be either paired with an eluent generator or manually prepared eluents. Using a hydroxide eluent gradient, methods can be developed to meet the analytical goals for the determination of small and large polyphosphates in food products and samples for industrial applications.

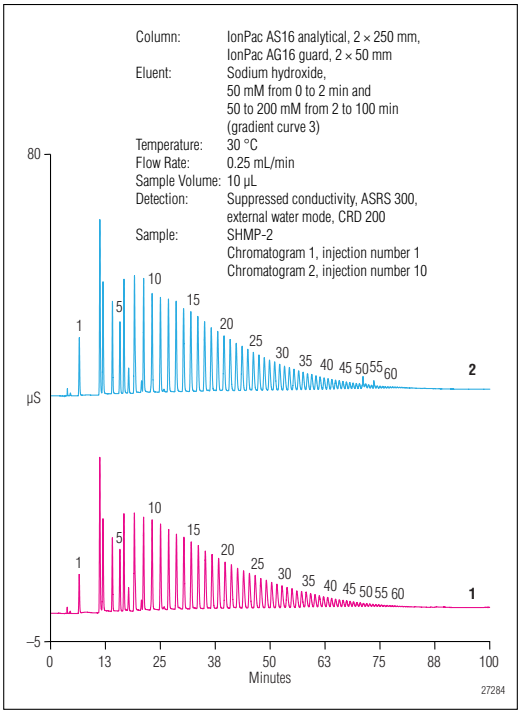

Figure 7. The 1st and 10th injection of SHMP-2 without a column wash (data in Table 4).

PRECAUTIONS

Sample Stability

Cyclic (also known as meta) phosphates are more stable in aqueous solution than linear polyphosphates. High temperatures and low pH will accelerate hydrolysis, so keeping the samples cool will help preserve them. The autosampler can be purchased with a sample cooling option. This will help preserve thermally labile samples. If necessary, the sample pH can be adjusted with sodium hydroxide to further slow degradation. Samples can also be degraded by enzymatic activity. Phosphatases are found on the surface of human skin, so gloves must be worn to avoid sample contamination.

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