Supporting Information

Enigmatic Ion-Exchange Behavior of *myo*-Inositol Phosphates

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Preparation of Inositol Phosphates. 0.994 g of dipotassium phytate (≥95%, www.sigmaaldrich.com) was dissolved in a minimal amount (~10 mL) of 18.2 MΩ deionized (DI) H_2O (MilliQ, www.emdmillipore.com). A 20 mL polypropylene syringe was filled with ~10 mL of Dowex HCR-W2 high capacity strong acid cation exchange resin (www.sigmaaldrich.com) and washed with DI H_2O. The phytate solution was added to the head of the resin bed and allowed to percolate through the resin by gravity. The effluent was collected and an additional 50 mL of DI H_2O, added in 10 mL aliquots, was used to rinse the remaining phytate from the column. The phytate solution was then adjusted to pH 3.93 using 100 mM KOH (GR ACS, www.emdmillipore.com) before being diluted to a final volume of 260 mL. The solution was then transferred to a stoppered 500 mL inert container and kept in a 110 °C convection oven. One milliliter aliquots of the contents were removed every 30 min for the first 14 h, adjusted to pH 7.0 with 0.1 M KOH, and diluted to 10 mL to produce a final concentration of ~0.5 mM total InsP's. This solution was then diluted 10x and 1 mL placed in a glass autosampler for analysis. Subsequent samples were taken at longer intervals (6-12 h) up to 48 h after the sample was put in the oven. Samples were kept refrigerated until all the desired hydrolysates were available for analysis.

Ion Chromatographic Analysis

Ion chromatographic studies were conducted on an ICS-5000 ion chromatograph (all chromatographic components below from www.dionex.com). The eluent (0.300 mL/min) was prepared inline using a KOH EGC-III eluent generator and further purified by a continuously regenerated anion trap column. A 10 µL injection volume was used. IonPac AG/AS-24 and IonPac AG/AS-11HC guard and separator columns sets (www.dionex.com) were used in 2 mm format; we have successfully used these before for phytate measurement (see ref. 6). After suppression (AERS-500 electrodialytic suppressor), the effluent was conductometrically monitored. All tubing prior to detection were $\leq$ 0.25 mm in inner diameter. The electrodialytic devices were operated in the recycle mode.

We examined the following eluents: (a) electrogenerated KOH (b) nominally 100 mM NaOH stock prepared from 50% w/w NaOH (www.fishersci.com) and (c) nominally 100 mM tetramethylammonium hydroxide (NMe_4OH) prepared from 1.0 M solution (ACS grade, www.sigmaaldrich.com). Titration with primary standard grade potassium hydrogen phthalate (≥99.95%, www.mallinckrodt.com) indicated exact concentrations of b and c to be 97.9 and 98.5 mM, respectively. Low pressure mixing capabilities of the gradient pump was used to make various

concentrations of NaOH and NMe_4OH eluents. An optimized gradient was used to carry out separations of the InsP's in under an hour on the AS-24 column as listed in Table S1. A mixed hydroxide gradient using NMe_4OH and NaOH was performed using the AS11HC column as listed in Table S2.

Table S1. Electrogenerated KOH Elution Program for AG/AS24 Columns

Time, min		KOH, mM
0		17.5
5		17.5
15		42
30		42
50		55
55		55
55.5		17.5
60		17.5

Table S2. Mixed Hydroxide Elution Program for AG/AS11HC Columns

Time, min		TMAOH, mM		NaOH, mM
0		6		0
7		6		0
7.5		35		0
14.5		35		0
15		65		0
23		35		30
30		0		65
33		0		65
33.5		6		0
40		6		0

Mass Spectrometry. Mass spectrometric studies were conducted on a TSQ Quantum Discovery Max electrospray ionization (ESI) MS/MS system with a heated ESI probe (www.thermoscientific.com), a different IC system was used with the MS instrument. Instrument parameters were as follows: Electrospray voltage, 500 V; vaporizer temperature, 350 °C; capillary temperature, 250 °C; skimmer offset, 0 V; sheath gas pressure, 50 arbitrary units (AU); auxiliary gas pressure, 50 AU; ion sweep gas pressure, 0 AU; Quadrupole 1 resolution, 0.4 full width half maximum (FWHM); quadrupole 3 resolution 0.5 FWHM. Identical KOH gradient separation was performed on the IC instrument connected to the MS as on the ICS-5000 instrument. This chromatography setup consisted of an IS25 PEEK dual piston isocratic pump, an EG40 eluent generator with an EGC-III KOH cartridge, an LC 30 column oven, and a CD25 conductivity detector with a DS3-1 detector cell, all from Dionex (www.dionex.com). Suppression was performed using an ASRS Ultra II 2mm anion self-regenerating suppressor (www.dionex.com). A 2 µL injection loop was connected to the injection port of a Thermo Finnegan Surveyor autosampler (www.thermoscientific.com). A peristaltic pump delivered ~1 mL/min of DI H_2O for the regeneration of all membrane based devices. The NMe_4OH-NaOH gradient IC analysis required a

pump capable of generating gradients through low pressure mechanical mixing. For this, the aforementioned ICS-5000 system was coupled to the MS.

Molecular ions were measured in the single ion monitoring mode (SIM). $InsP_0^{-1}$, $InsP_1^{-1}$, $InsP_2^{-1}$, $InsP_3^{2-}$, $InsP_4^{2-}$, $InsP_5^{2-}$, $InsP_6^{3-}$ or $InsP_6^{2-}$ were measured at nominal mass to charge ratios of 179, 259, 339, 209, 249, 289, and 219 or 329 m/z respectively. A relatively low voltage of 0.5 kV was used for the ESI as preliminary experiments showed that higher ESI voltages resulted in the loss of a phosphate moiety from $InsP_6$ to $InsP_5$ during tuning. Despite the low ESI voltage, because of the high acidity of the phosphate, ionization was still more than adequate as observed from signal counts and even polyvalent molecular ions for the higher substituted InsP's were abundant.

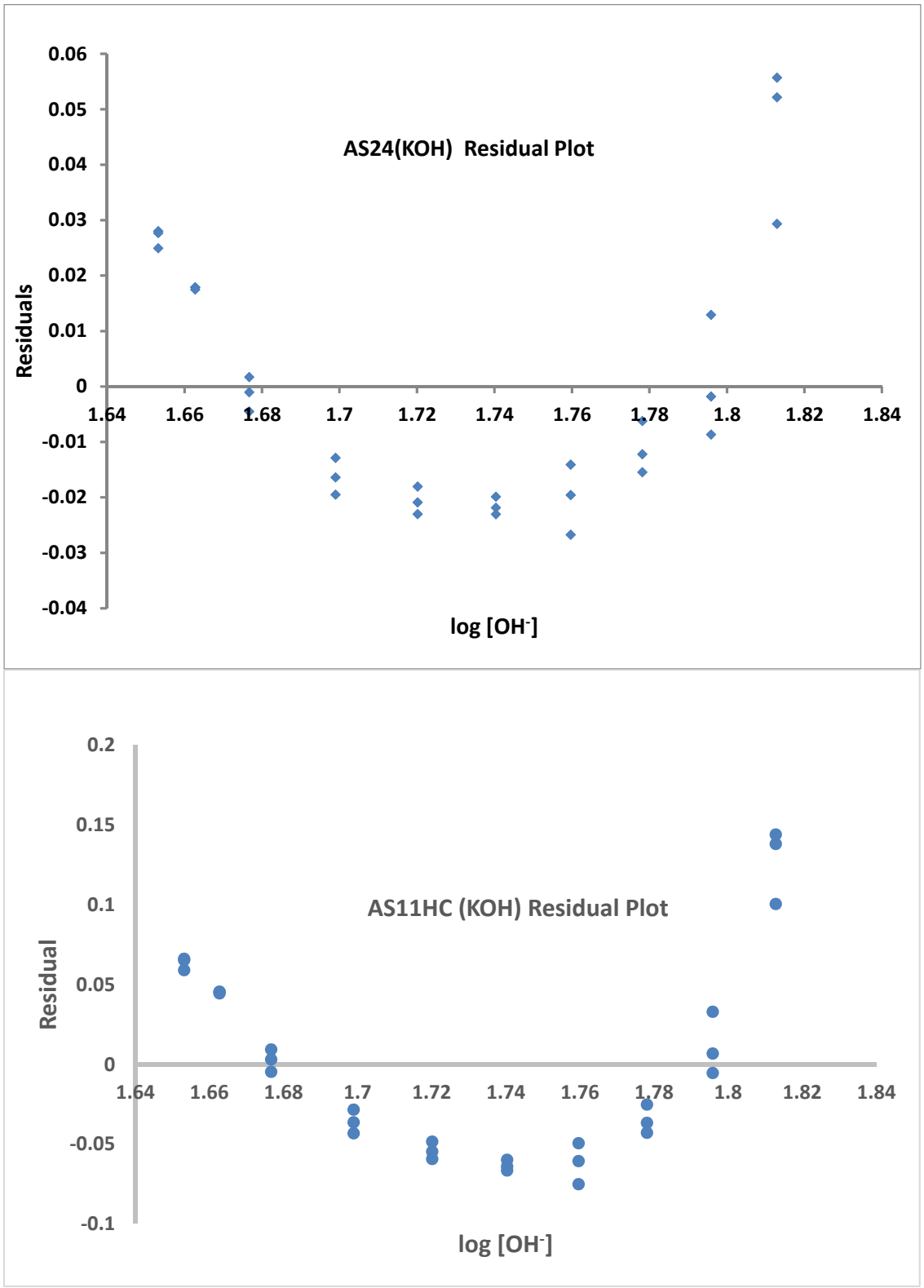

Figure S1. Residual plots, KOH eluent regression plots in Figure 3 (main text)

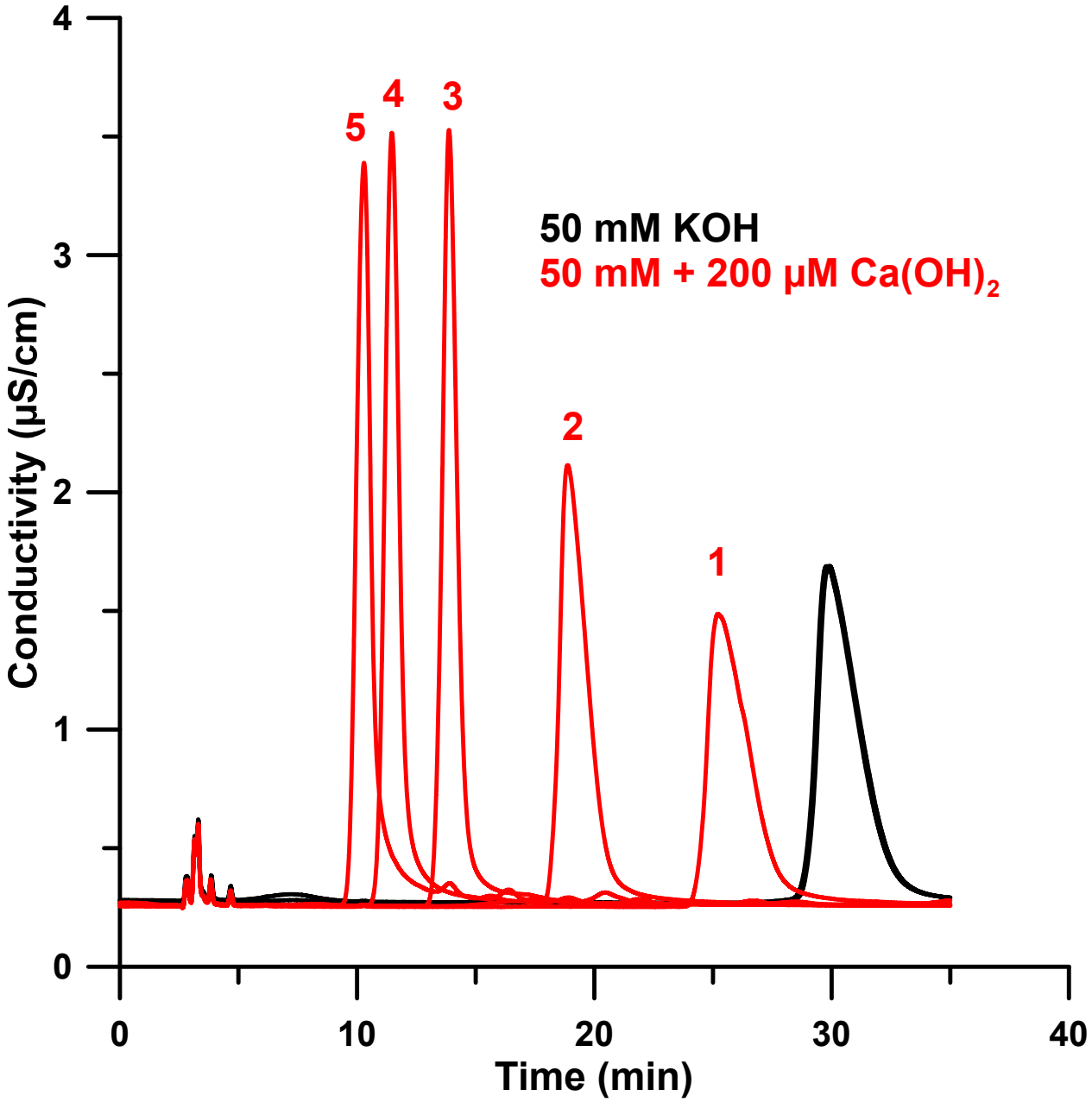

Figure S2. Elution of 25µM phytate using 50 mM KOH (shown in triplicate) and 50 mM KOH + 200 µM $Ca(OH)_2$. Sequential injections are shown of Phytate elution after addition of $Ca(OH)_2$ and numbered accordingly. After addition of $Ca(OH)_2$ the system is slow to equilibrate. Phytate is seen eluting earlier and earlier. Initially an increase in peak height is observed due to the earlier elution, but the peak then begins to tail more heavily after a while.

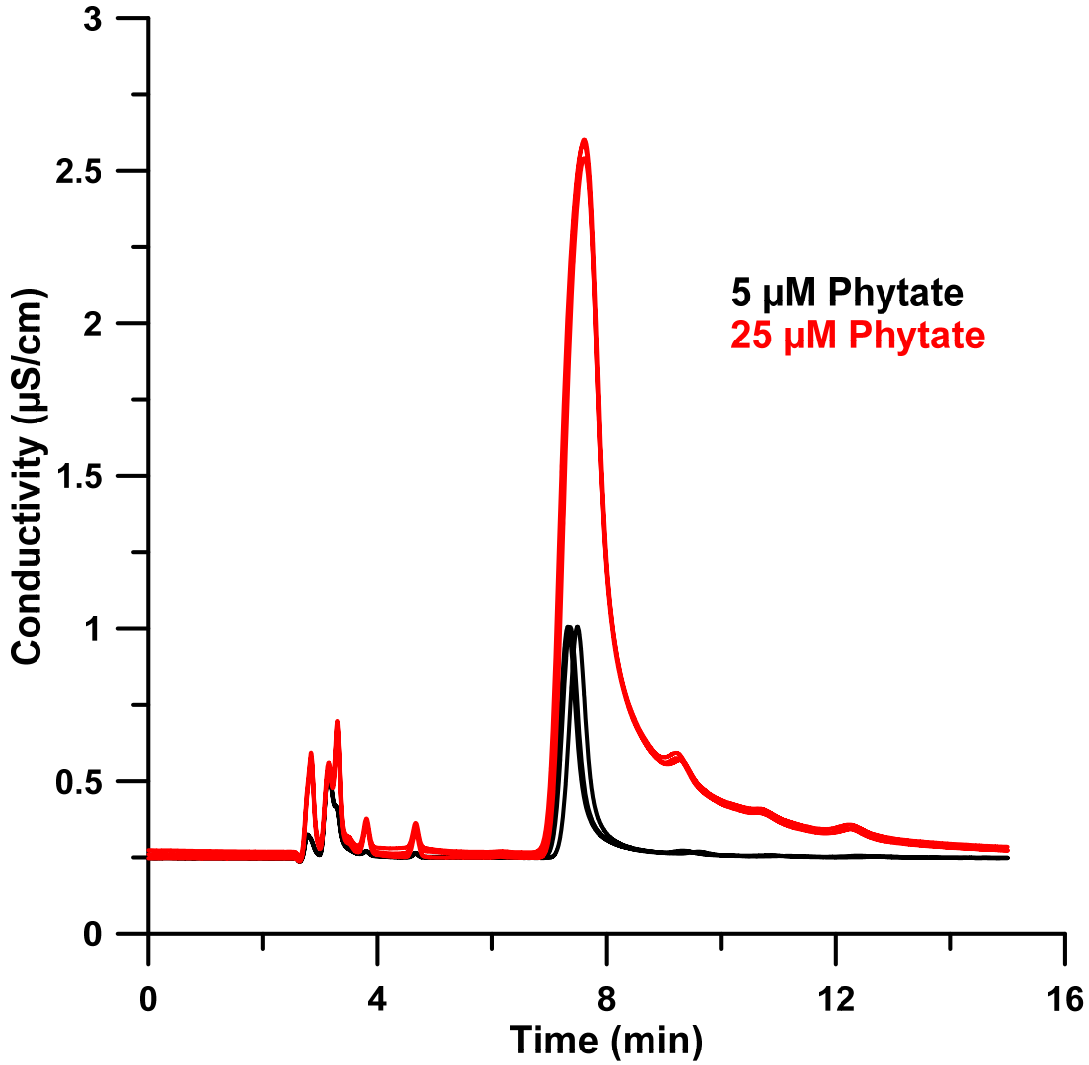

Figure S3. Elution of phytate in 50 mM KOH and 100 µM $Ca(OH)_2$. The system was allowed to equilibrate overnight before testing. Considerably more tailing is observed when 25 µM phytate is injected compared to 5 µM. At 25 µM, phytate is essentially complexing enough calcium to reduce the availability in the solution which results in a higher effective charge and a greater net retention for the less complexed phytate. Lower inositol phosphate impurities are clearly visible on the tail of the 25 µM peak. Such separation was not achieved when just a KOH eluent was used. Repeat runs are shown.

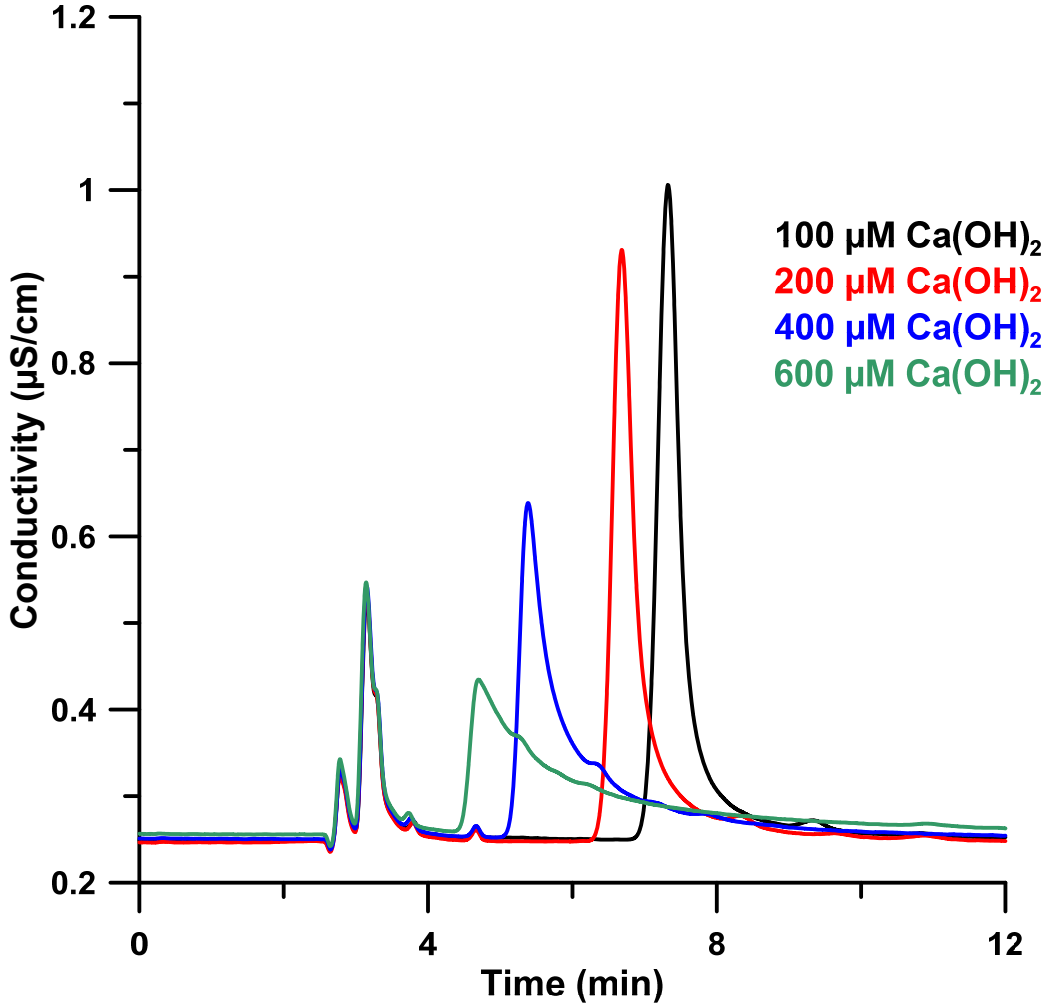

Figure S4. Elution of phytate in 50 mM KOH containing different amounts of $Ca(OH)_2$. The system was allowed to equilibrate for 45 minutes between injections. A loss in efficiency and sensitivity occurs at higher concentrations of $Ca(OH)_2$. Conversely, use of too little $Ca(OH)_2$ limits the range at which phytate can be measured as shown in Figure S3.

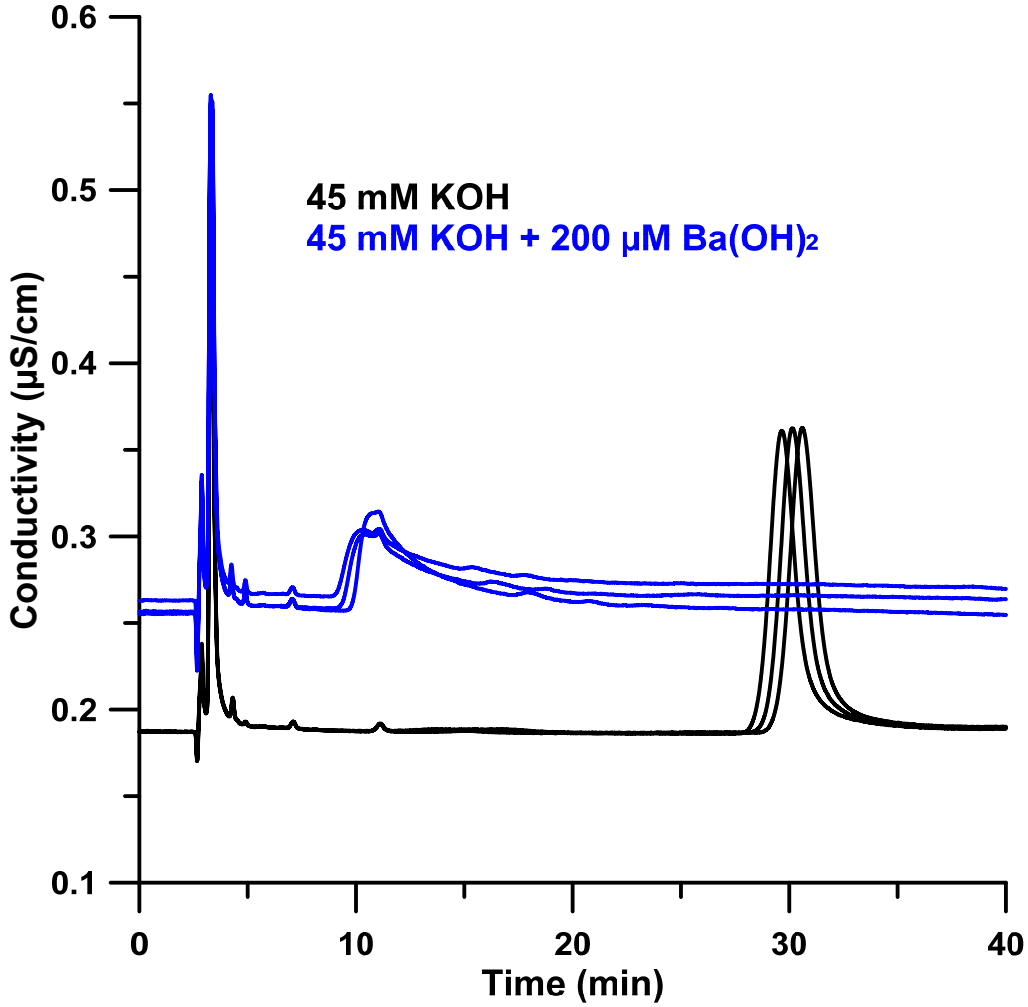

Figure S5. Elution of 5 µM phytate with 45 mM KOH and 45 mM KOH + 200 µM $Ba(OH)_2$. The $Ba(OH)_2$ contains some impurities which accounts for the elevated background. Upon addition of $Ba(OH)_2$ phytate retention decreases but the peak height also decreases and the peak becomes wider. Repeat runs are shown.

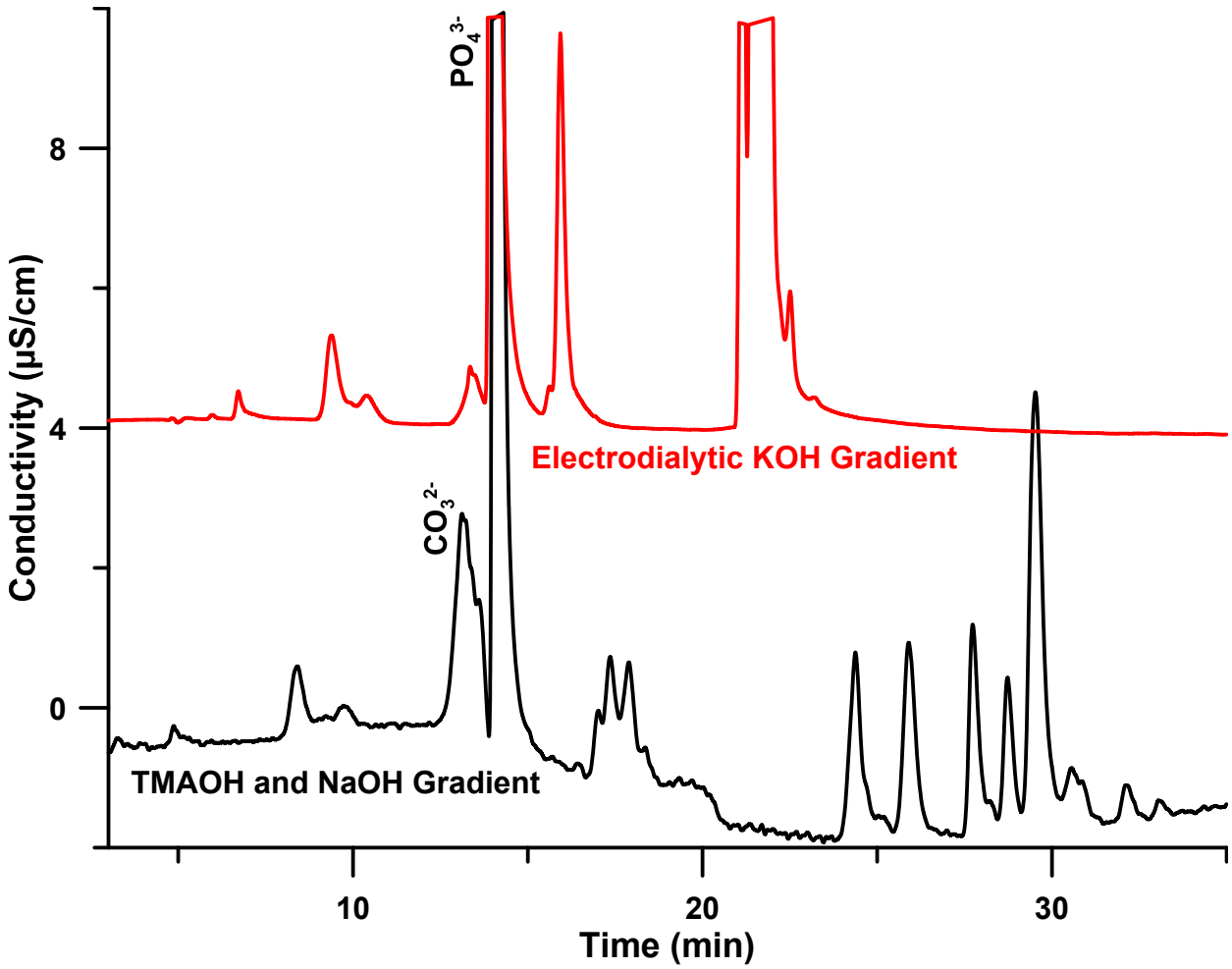

Figure S6. Separation of 12 h phytate hydrolysate. The black traces is the TMAOH and NaOH gradient. The red trace is the same hydroxide based gradient but uses entirely KOH. The red trace has been deliberately delayed by 2.2 minutes to compensate for the volume associated with the gradient mixing chamber. The apparent later elution of $InsP_1$ in the red trace is an artifact of this.

Deconvolution of Peaks. Microsoft Excel Solver was used to fit a series of Gaussian curves to the MS traces. For each InsP SIM-MS trace, the maximum number (*n*) of geometric isomers was fit according to eq s1:

$$F_t = \sum_i^n \alpha_i \, e^{\frac{\left(t_i - t_{ri}\right)^2}{2\sigma_i^2}} \qquad \text{(s1)}$$

F_t is the sum total signal produced from each curve *i* at time *t* in the chromatogram. The constants α, σ, and t_r represent the peak height, width, and retention time respectively. Optimization of these parameters through Excel Solver was carried out using a least squares approach according to eq s2:

$$M = \sum_t \left(F_t - S_t\right)^2 \qquad \text{(s2)}$$

Where S_t is the background subtracted signal of the SIM trace at time *t*. The parameters α, σ, and t_r were iteratively adjusted to minimize *M*. It was necessary to constrain the peak width parameter σ to produce fits with widths no larger than the largest observable peak. Figures S3-S7 show the fits for $InsP_1$ – $InsP_5$.

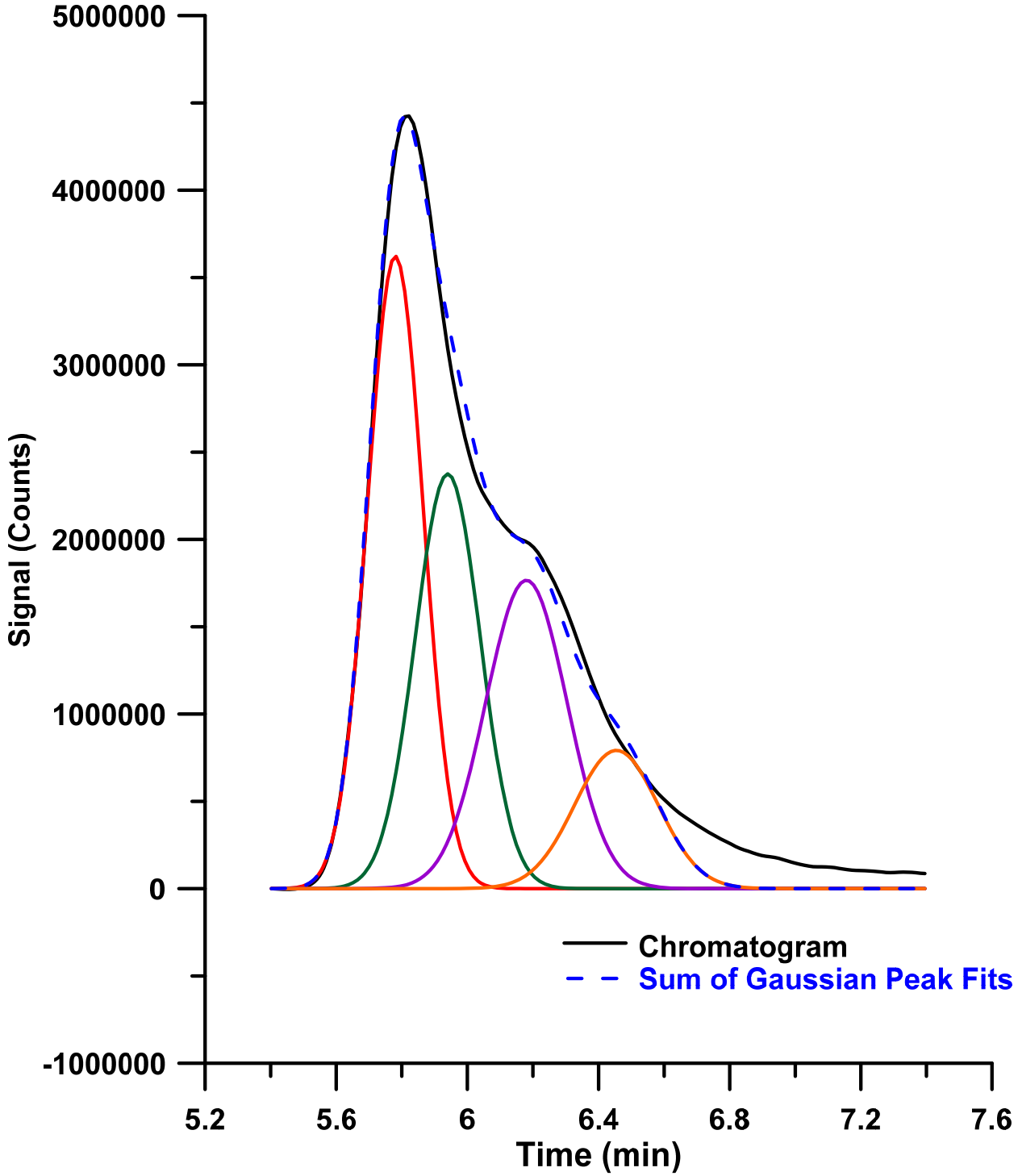

Figure S7. Gaussian peak to $InsP_1$. 4 isomers are possible

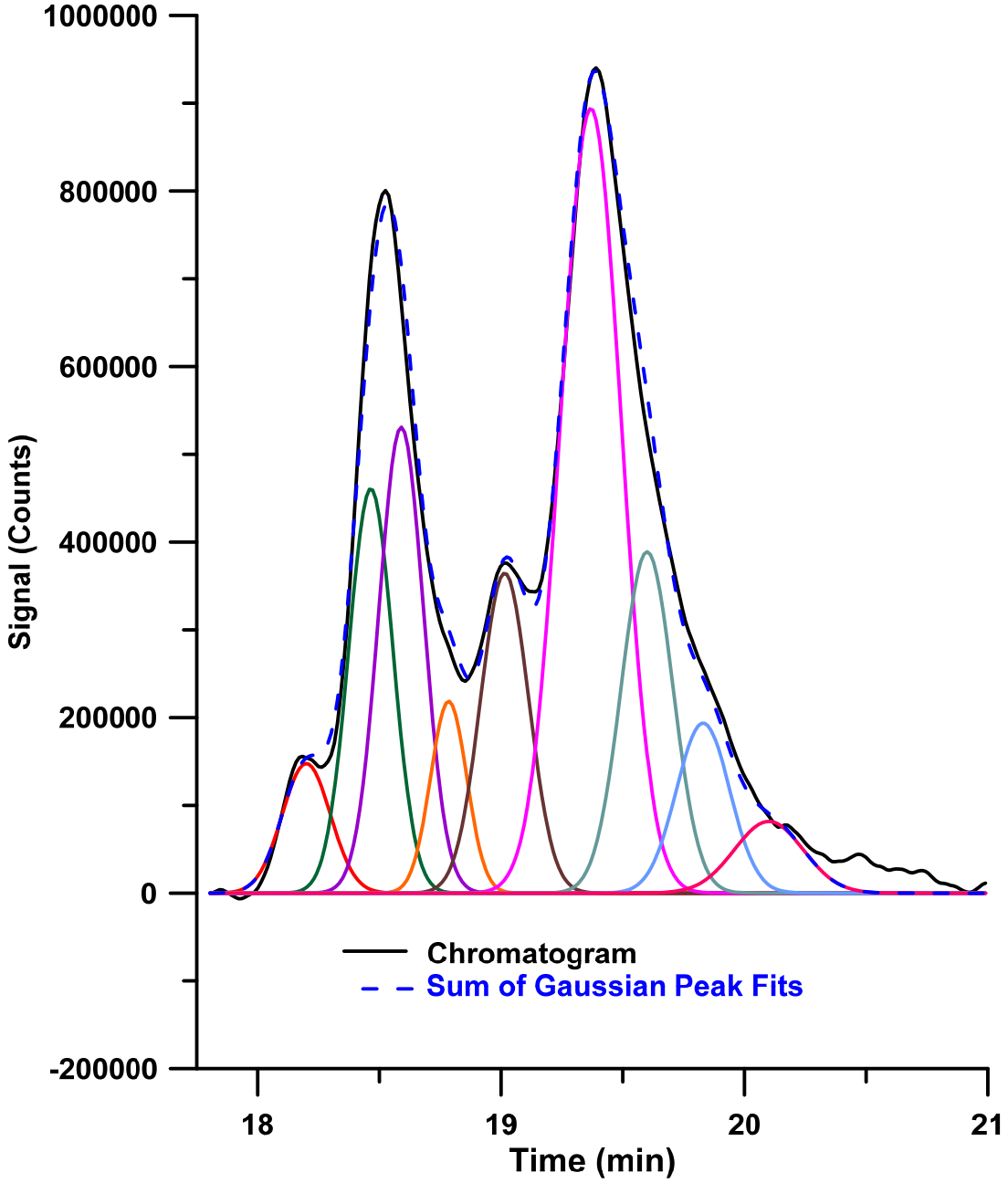

Figure S8. Gaussian peak to $InsP_2$. 9 isomers are possible

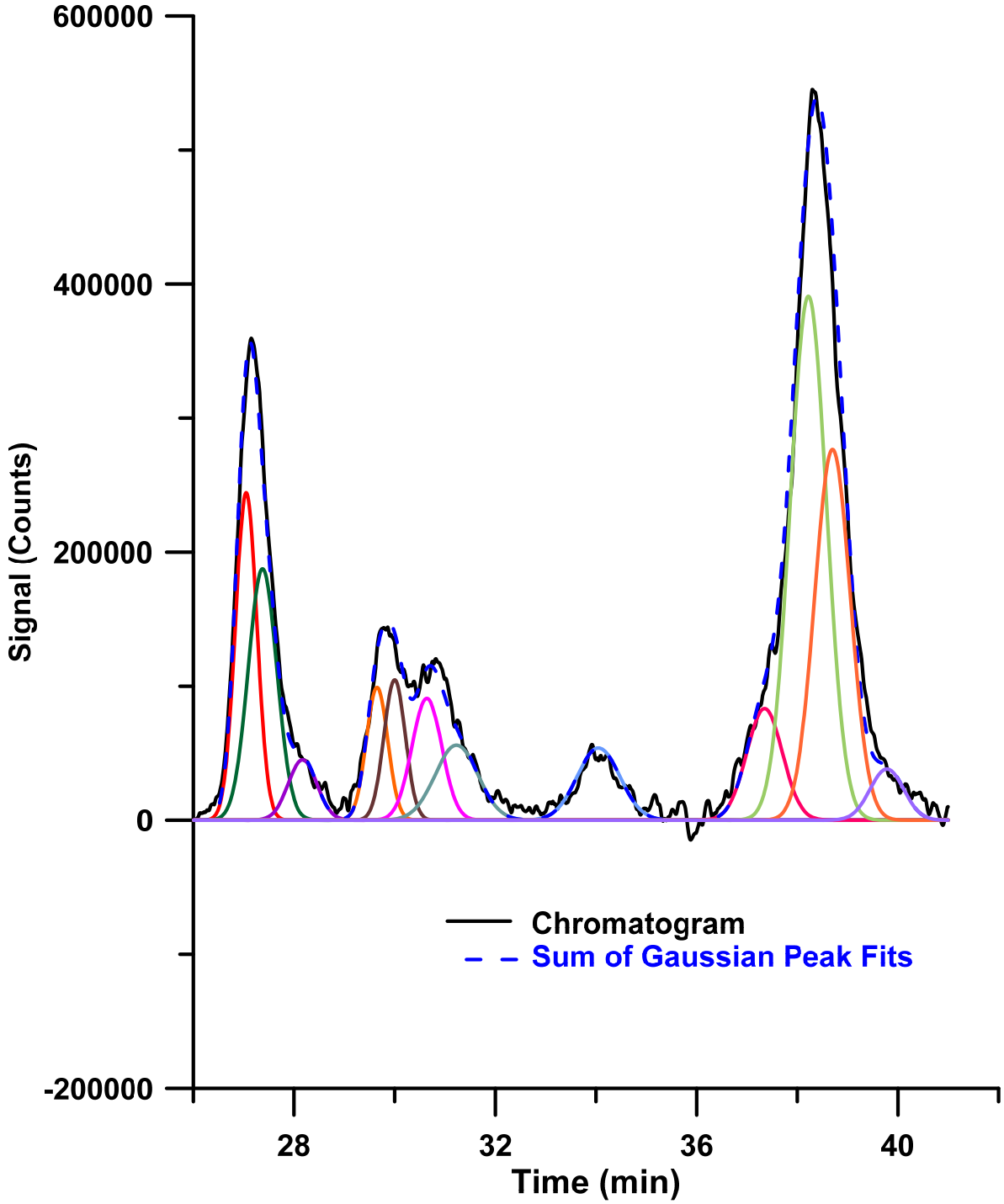

Figure S9. Gaussian peak to $InsP_3$. 12 isomers are possible

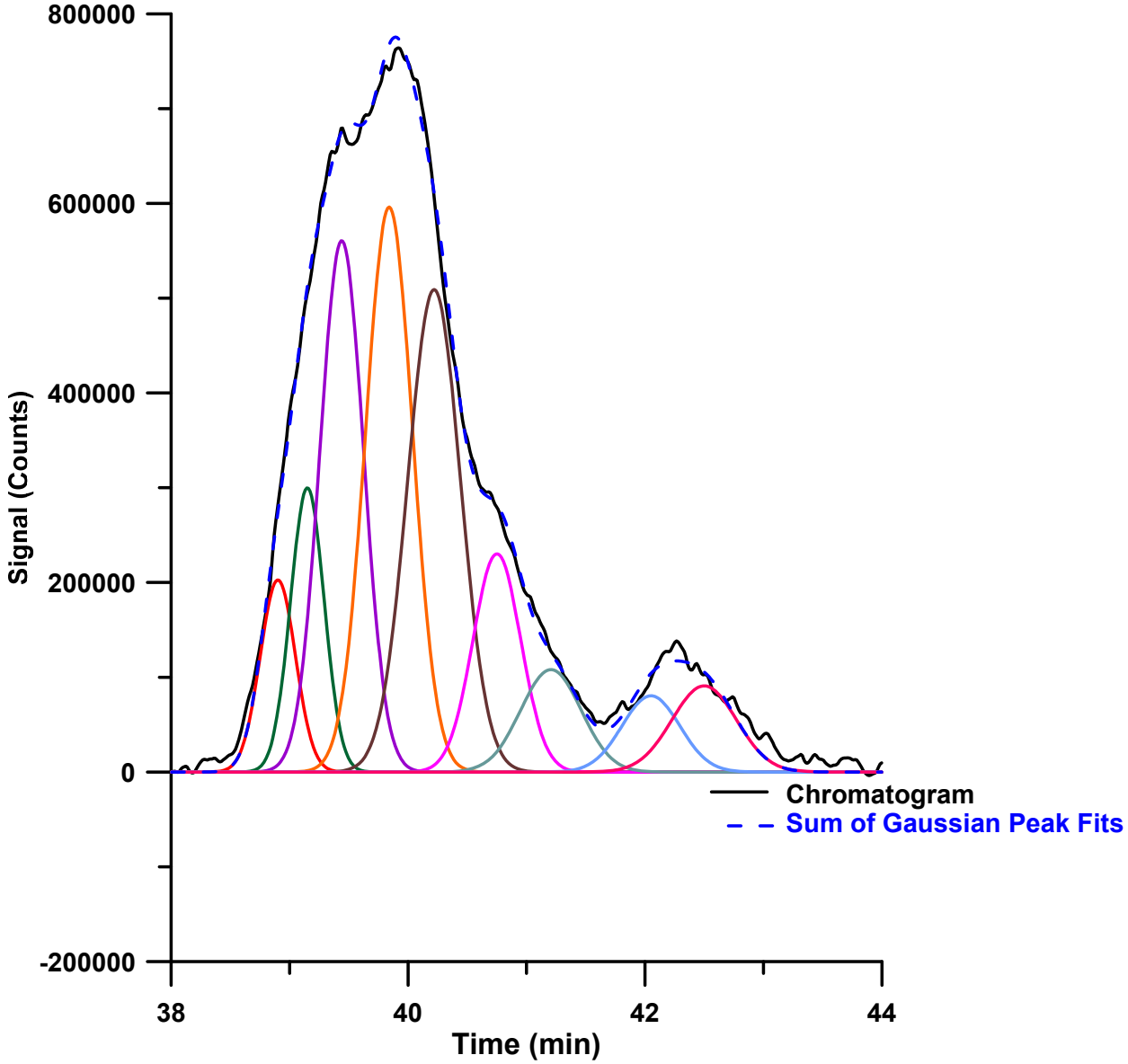

Figure S10. Gaussian peak to $InsP_4$. 9 isomers are possible

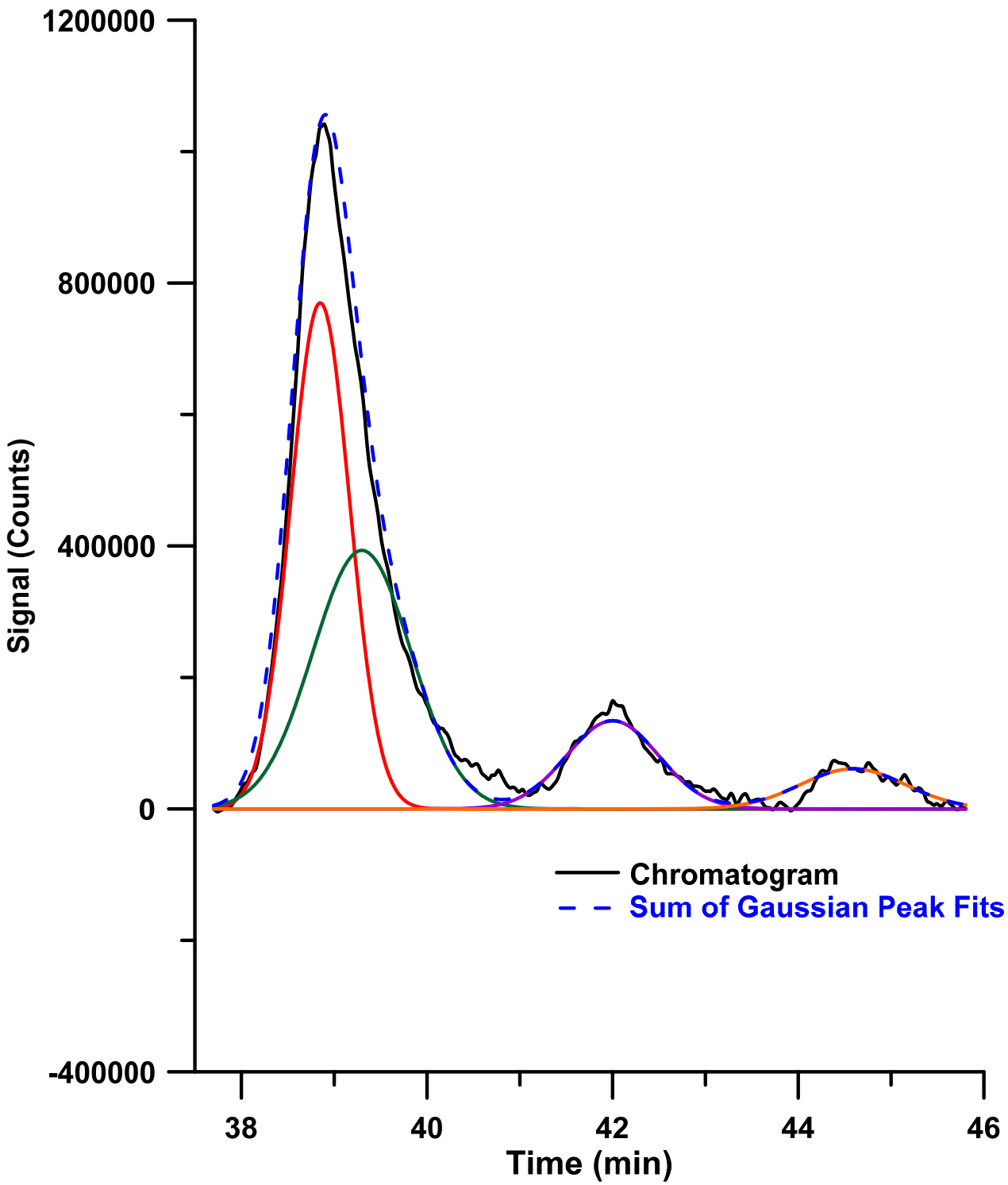

Figure S11. Gaussian peak to $InsP_5$. 4 isomers are possible

We recognize that because *n* isomers are possible, it does not mean that all *n* are produced in significant amounts. An anlaysis was done therefore by fitting

each curve with a reduced number of peaks until there was a significant change in the correlation coefficient (which does take into account the reduced degree of freedom and can actually increase from a reduced number of assumed variables). This is depicted in Table S3.

Table S3. Correlation Coefficients pertaining to deconvoluted fits of the $InsP_{1-5}$ responses (Figures S3-S7) depending on how many contributing isomers are assumed

		r^2 values							
Figure	**No of Peaks Assumed (*n*)**	***N***	***n* - 1**	***n* - 2**	***n* - 3**	***n* - 4**	***n* - 5**	***n* – 6**	***n* - 7**
S3	4	0.9919	0.9980	0.9917	0.8061				
S4	9	0.9961	0.9994	0.9979	0.9979	0.9949	0.9893	0.9574	
S5	12	0.9901	0.9847	0.9647	0.9587				
S6	9	0.9979	0.9994	0.9993	0.9993	0.9992	0.9990	0.9903	0.9813
S7	4	0.9889	0.9585						

It will be apparent that while all 12 $InsP_3$ isomers are present, all possible isomers may not be present in the $InsP_2$ or $InsP_4$ responses. Figure S8 shows how the fitting envelope of Figure S6 ($InsP_5$) will change depending on the number of contributing peaks.

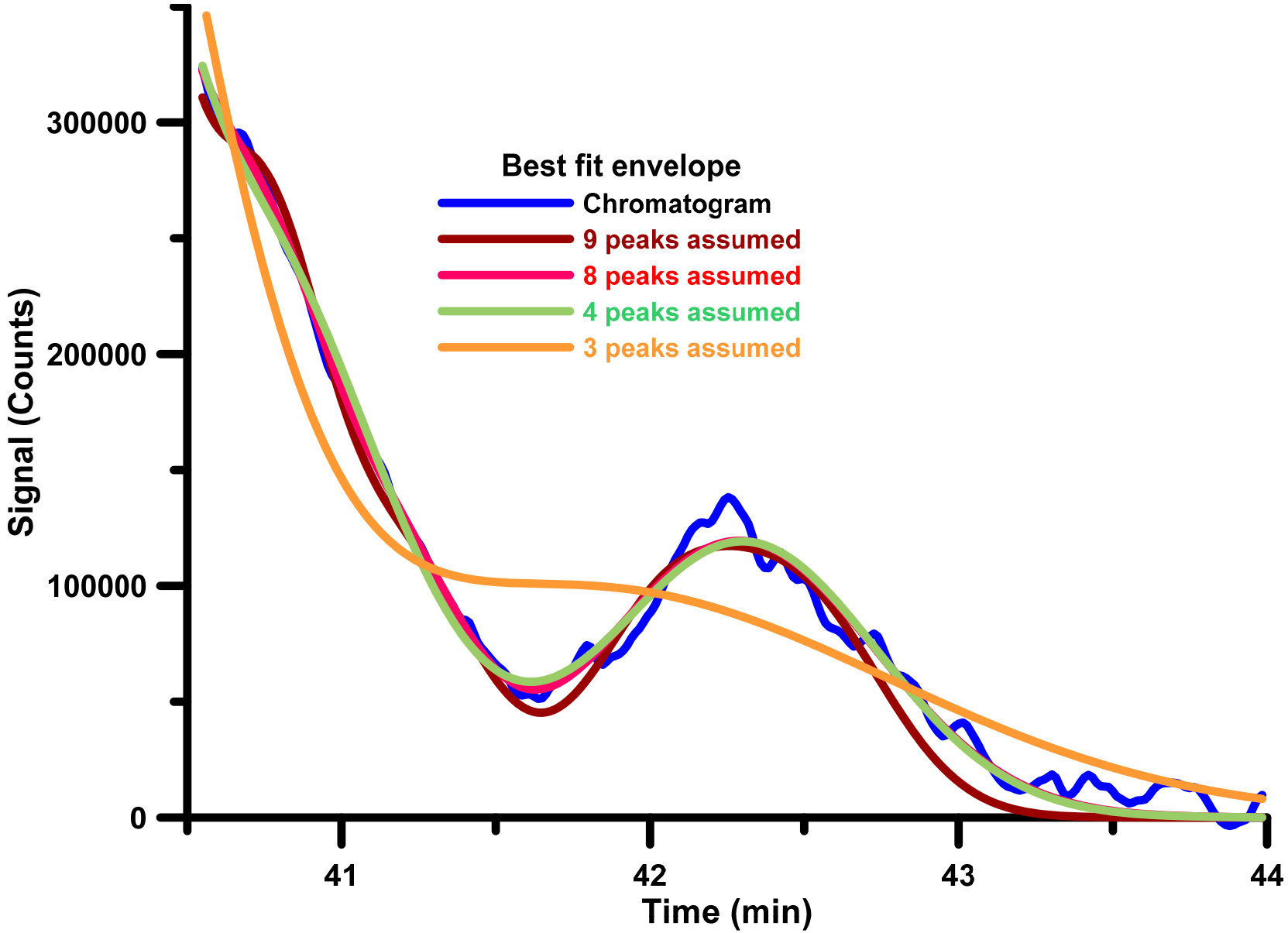

Figure S12. The fit as depicted in Figure S6 with 9, 8, 4, and 3 contributing peaks assumed.

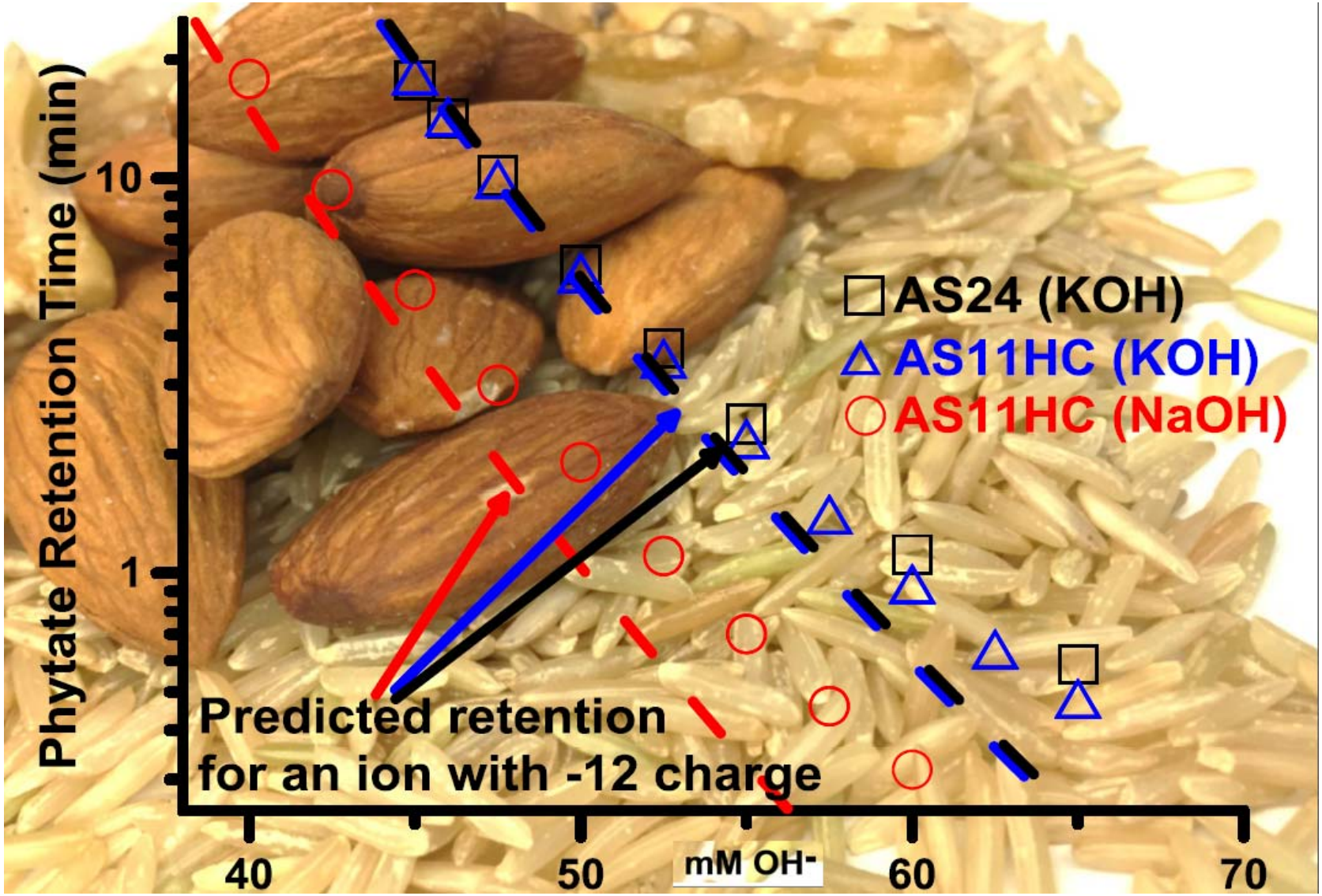

Figure S13. Table of content graphic. $InsP_6$ (phytate) retention time as a function of hydroxide concentration. The dashed lines are for an ideal dodecavalent anion with similar retention to $InsP_6$, calculated using the retention at the 3 lowest eluent concentrations for each case and assuming a -12 charge. Large deviations are clearly visible at higher eluent concentrations due to the lower effective charge on $InsP_6$. The background depicts nuts and brown rice, a rich source of phytate.