DIONEX

Technical Note 55

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Screening of Sample Matrices and Individual Matrix Ingredients for Suitability in *AAA-Direct*™

INTRODUCTION

All chromatographic techniques are susceptible to chemical interferences. Problems associated with chemical interferences include coelution with analytes, column contamination, shifts in retention times, and inhibition of detection. When analytes must first be chemically derivatized for separation or detection purposes, the sample matrix can reduce reaction yields, compromising accurate analyte measurement. Furthermore, some matrix compounds (e.g. reducing agents, oxidizing agents, solvents, and metal chelators) can react with analytes, reducing their concentration and producing nonreactive products. *AAA-Direct*™ is a technique that uses integrated pulsed amperometry (IPAD) to directly detect amino acids. This technique contrasts with the pre- or postcolumn derivatization used to detect amino acids by ultraviolet absorbance (UV), visible (Vis) absorbance, or fluorescence. Unlike UV, Vis, or fluorescence detection, electrochemical detection depends on the ability of the working electrode surface to facilitate an oxidation-reduction reaction that produces an amplified and measured electrical current. Therefore, while IPAD may not be affected by interferences common to other techniques, it can be subject to interferences from ingredients in the sample matrix that interfere with reactions at the electrode surface. It is also possible that these interfering compounds may become trapped at the electrode surface, causing the electrode to be fouled.

It is not always possible to predict the effect of sample ingredients on the separation and detection performance of an analytical system. Therefore, new matrix compounds should be screened. In this technical note, we describe a procedure to screen sample matrices for their effect on peak area, peak height, and retention time. We demonstrate this procedure by testing ethanol for possible interferences in *AAA-Direct*. However, the method described here can also be used to evaluate any matrix component analyzed by chromatography with electrochemical detection. In this technical note, we use the term *test sample (TS)* to describe the specific test compound evaluated for possible interferences.

EQUIPMENT

Dionex BioLC® Chromatography System configured for *AAA-Direct*, consisting of:

- GP50 or GS50 Gradient Pump, microbore, PEEK, with degas option
- ED50 Electrochemical Detector with *AAA-Certified*™ Gold Cell
- AS50 Autosampler and Thermal Compartment with 25-µL injection loop (0.0100 in. i.d.)
- EO1 Eluent Organizer, including three 2-L plastic bottles and pressure regulator

Chromeleon® Chromatography Workstation

Microcentrifuge tubes with detachable caps (sterile polypropylene, 1.5 mL; Sarstedt, P/N 72.692.005 or equivalent)

Nitrogen; 4.8 Grade, 99.998%, <0.5 ppm oxygen (Praxair Specialty Gases or equivalent)

Filter unit, 0.2 µm nylon (Nalgene 90-mm Media-Plus, Nalge Nunc International, P/N 164-0020 or equivalent nylon filter apparatus)

Vial, 0.3 mL, polypropylene, microinjection, 12-32 mm screw thread (Sun International, P/N 500-118)

Septum, pre-slit Teflon®/silicone and polypropylene screw thread cap (for the microinjection vial; Sun International, P/N 500-061)

REAGENTS AND STANDARDS

Reagents

Deionized water, 18 MΩ-cm resistance or higher

Sodium acetate, anhydrous (*AAA-Direct* Certified, Dionex Corp., P/N 059326)

Sodium hydroxide (NaOH), 50%, low carbonate grade (w/w; Fisher Scientific, P/N SS254-500; or equivalent)

Standards

Amino acids in 0.1 M hydrochloric acid; Standard Reference Material 2389 (National Institute of Standards and Technology)

Tryptophan (Trp; Sigma, P/N T-1029)

Test Sample (TS)

Ethanol (Denatured alcohol; EM Science, P/N AX0445-1)

CONDITIONS

Column:	AminoPac® PA10 Analytical (2 × 250 mm, P/N 055406) with AminoPac PA10 Guard (2 × 50 mm, P/N 055407)
Temperature:	30 ºC
Flow Rate:	0.25 mL/min
Inj. Volume:	25 µL
Detection:	Integrated pulsed amperometry (reference electrode in pH mode)
Eluents:	A) Water B) 250 mM NaOH C) 1.0 M sodium acetate

Programmed Method for *AAA-Direct

Time (min)	% A	% B	% C	Curve	Comments
Init.	76	24	0	–	Autosampler fills the sample loop
0.0	76	24	0	–	Valve from load to inject
2.0	76	24	0	1	Begin hydroxide gradient
8.0	64	36	0	8	
11.0	64	36	0	8	Begin acetate gradient
18.0	40	20	40	8	
21.0	44	16	40	5	
23.0	14	16	70	8	
42.0	14	16	70	8	
42.1	20	80	0	5	Column wash with hydroxide
44.1	20	80	0	5	
44.2	76	24	0	5	Equilibrate to starting conditions
75.0	76	24	0	5	End of run

*For the most current programmed method, see the *Installation Instructions and Troubleshooting Guide for the* AAA-Direct *Amino Acid Analysis System.*[1]

Waveform for the ED50*

Time (seconds)	Potential (volts) vs. pH	Integration (begin/end)
0.00	+0.13	
0.04	+0.13	
0.05	+0.33	
0.21	+0.33	Begin
0.22	+0.55	
0.46	+0.55	
0.47	+0.33	
0.56	+0.33	End
0.57	–1.67	
0.58	–1.67	
0.59	+0.93	
0.60	+0.13	

*For the most current programmed waveform, see the *Installation Instructions and Troubleshooting Guide for the* AAA-Direct *Amino Acid Analysis System.*[1]

PREPARATION OF SOLUTIONS AND REAGENTS

Water

Water used in eluent preparation must be qualified for *AAA-Direct* use. Qualification of water is described in Technical Note 50.[2] Water is filtered through a 0.2-µm nylon filter apparatus, free of electrochemically active surfactants or leachable residue. Use of cellulose or polysulfone-based filters for any of the eluents used in *AAA-Direct* may introduce electrochemically active impurities into the eluent, and should be avoided. Other filter brands and types should be qualified prior to use. Water is placed under helium or nitrogen at 4–5 psi to reduce adsorption of carbon dioxide and prevent microbial contamination.

250 mM Sodium Hydroxide

To prepare 2 L of eluent, as per *Installation Instructions and Troubleshooting Guide for the AAA-Direct Amino Acid Analysis System*,[1] combine 26 mL of 50% (w/w) low-carbonate NaOH with 1974 mL of purified and prefiltered water (0.2 µm nylon filter, see comments above). Place this solution immediately under helium or nitrogen at 4–5 psi.

1.0 M Sodium Acetate

To prepare 1 L of eluent, as per *Installation Instructions and Troubleshooting Guide for the AAA-Direct Amino Acid Analysis System*,[1] dissolve 82 g of *AAA-Direct* Certified anhydrous sodium acetate in ~800 mL of purified water. Adjust the total volume to 1000 mL with additional water. Filter the solution through a 0.2-µm nylon filter unit (see comments above) and place it under 4–5 psi helium or nitrogen.

SAMPLE PREPARATION

Preparation of Standards

The amino acid standard mix, obtained from the National Institute of Standards & Technology (SRM 2389), consists of 17 amino acids (but not Trp) at concentrations ranging 2.4–2.9 mM (except cystine, 1.2 mM concentration). Each amino acid concentration is accurately defined on the Certificate of Analysis. We dilute this amino acid standard mix with water to concentrations ranging 240–290 μM (120 μM cystine). Trp is weighed and reconstituted in water to 25 mM concentrations, then diluted to 250 μM in water. These solutions are stored frozen until needed. The 240–290 μM NIST amino acid standard mix (except 120 μM cystine) is combined with the 250 μM Trp standard solutions to make a 9.6–11.6 μM concentration of the amino acid standard mix (4.8 μM cystine) for use as a reference standard to evaluate TS effects.

Preparation of Samples

To determine the effect on the retention times, peak areas, and peak heights of amino acids in the immediate presence of the TS during separation, the 240–290 μM NIST amino acid standard mix (except 120 μM cystine) and 250 μM Trp solution is spiked into the matrix or test sample at a 9.6–11.6 μM concentration of the amino acid standard mix (4.8 μM cystine). The spiked matrix or test sample concentrations are selected based on their expected upper limit. Lower test sample concentrations are selected to demonstrate methods for determination of the lower limit of interference (LLOI). For this technical note, 0.1, 1, 3, 10, and 50% (v/v) of ethanol were spiked with the amino acid mixture.

Screening Procedure

The blanks, analyte (amino acid) standard mix, test sample (TS), and TS spiked with standard mix are sequentially tested in the following manner using an autosampler:

1. Water blank
2. Analyte (amino acid mix) standard (replicate injection 1)
3. Analyte (amino acid mix) standard (replicate injection 2)
4. Analyte (amino acid mix) standard (replicate injection 3)
5. Test sample (TS)
6. Spiked Test Sample (TS spiked with standards)
7. Analyte (amino acid mix) standard (replicate injection 1)
8. Analyte (amino acid mix) standard (replicate injection 2)
9. Analyte (amino acid mix) standard (replicate injection 3)

The first water blank injection assures the chromatography is free of unexpected peaks, and establishes a basis for comparison with the TS chromatogram, from which the elution of the TS or TS-related peaks can be determined. The three injections of the analyte standard (a single solution) establish the expected mean retention times, system stability, and peak areas or heights of the standards. The variance (standard deviation) measured from these replicates can be used to establish confidence intervals for the determination of statistically significant effects due to the TS. The number of replicates can be increased if desired. In this technical note, 99% confidence intervals are the mean, ±3 times the standard deviation of 3 injections. Another method may also be used to define confidence limits (e.g., Student *t* test).

Immediately following the replicate injection of standards, the TS is injected. The presence of extra peaks not found in the water blank is an indication that the TS, or its impurities, elute from the column and are detected by the system.

After the TS injection, the TS spiked with standards is injected. The observed retention times, peak areas, and peak heights of these spiked standards are compared to the upper and lower confidence interval calculated from the standards that precede the TS injection. Significant changes in the retention times of the spiked standards indicate the TS will alter elution time under the conditions tested. Significant increases in peak area or peak height are indications of either coelution by impurity in the TS, or the existence of an endogenous analyte in the TS. Significant decreases in area or height are indications of either electrode fouling, or a chemical reaction of one or more components of the standard with the TS that reduces standard concentration. The presence of new peaks not found in either the TS or the TS spiked with standards suggests a chemical reaction of the TS with the standards. In this technical note, the effects observed for analytes in the TS spiked with standards are referred to as *matrix effects*. It is possible that decreases in peak area could be a cumulative effect of the two TS injections. Only two injections of TS (one alone and one spiked with standard) are included in this procedure to reduce any other effects that would confound interpretation of TS effects.

Immediately after the replicate TS injections, the same solution of standards is injected again in triplicate. These measured parameters are compared with the original standards to determine if any TS-related effects extend

beyond its initial exposure to the chromatography system. Occasionally, the occurrence of a significant effect during TS testing extends beyond the initial injections. For example, the presence of a large TS peak during a gradient may reappear with subsequent injections if the column or autosampler was contaminated. Another post-TS injection effect is the fouling of the electrode surface by the TS, which may persist beyond its initial exposure, resulting in lower peak area or peak heights in the first injection of standard, after the injections of the TS. Trending of electrochemical response (peak area or height) or retention time can suggest the cause of the post-TS injection effect. If the electrochemical detector response decreased in the first injection of standard after the TS, and subsequent replicate injections of standards increase in detector response, this indicates the electrode was fouled and the response is returning through its self-cleaning capability. A downward trend of any newly appearing peaks, or higher than expected analyte peaks, indicates contamination of the column or injector. After injecting the TS, effects observed for standards are referred to in this technical note as *post-TS injection effects*. If no post-TS injection effects occur, the final replicate injections of standards can also serve as the starting standards for the next treatment (e.g. a different TS or next dose level). The development of a suitable report format in Chromeleon can lead to a complete automation of all test-related calculations.

RESULTS AND DISCUSSION

Ethanol

The use of ethanol in commercial products can vary greatly, depending on its purpose.

A broad range of dose levels for ethanol was chosen for this study to demonstrate the effect of TS concentration on the extent of interferences possible. Figure 1 presents (A) chromatograms for the separation of the water blank, (B) the pre-TS amino acid standard, (C) 3% ethanol TS, (D) the 3% ethanol TS spiked with amino acids, and (E) the post-TS amino acid standard. These results for ethanol exemplify a typical early eluting TS. Comparison of the chromatograms for the TS alone (Panel C) with the water blank (Panel A) and the amino acid standards (B) show a large peak belonging to ethanol coeluting with Arg, and tailing into the region of many early eluting amino acids, such as Lys, Ala, Gly, and Val. Figure 1D shows these amino acids eluting on the tail of the ethanol peak. The Arg peak is completely obscured by ethanol and therefore cannot be quantified. The absence of any trace of the TS peak on the following injection of the amino acid standards (Panel E) shows that the TS is completely purged during the gradient used in this study.

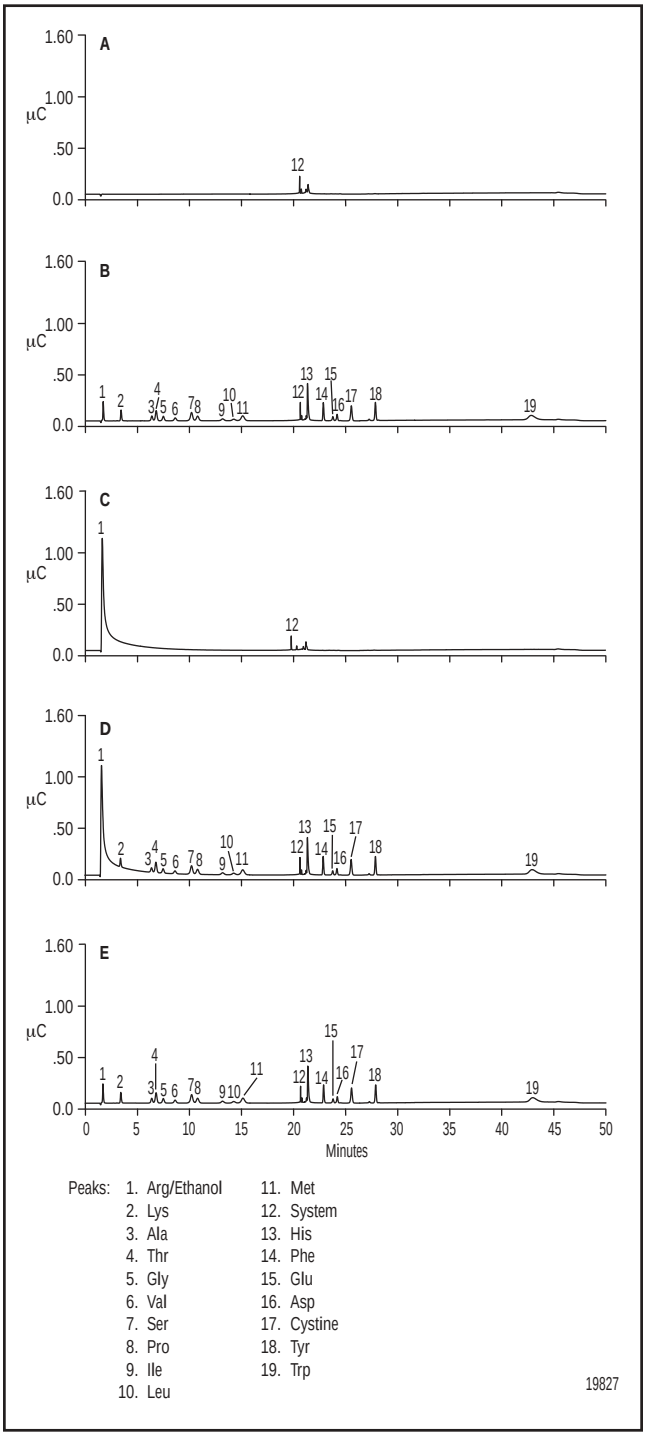

***Figure 1**. AAA-Direct chromatograms of a water blank (A), amino acid standards before exposure to TS (B), 3% ethanol TS (C), amino acid standards spiked into 3% ethanol TS (D), and the post-TS injection of amino acid standards (E).*

Table 1 Data Summary for Lysine in a 3% Ethanol Test Sample			
	Lysine		
Sample Name	Ret. Time (min)	Area (nC*min)	Height (nC)
Water		0.0	0.0
Standard	3.42	12.6	108.2
Standard	3.43	12.6	107.5
Standard	3.41	12.3	105.9
Mean of the Standard:	**3.42**	**12.5**	**107.2**
SD of Standard	**0.01**	**0.2**	**1.2**
RSD:	**0.3%**	**1.4%**	**1.1%**
Upper 99% Confidence Interval:	**3.45**	**13.0**	**110.7**
Lower 99% Confidence Interval:	**3.39**	**12.0**	**103.7**
TS (3% ethanol)	ND	0.0	0.0
Std in TS (AAs Spiked in 3% ethanol)	3.43	9.7	83.3
Matrix Value (Spiked TS-TS):	**3.43**	**9.7**	**83.3**
Adjusted Percent Change for Matrix	**0.3%**	**–22.6%**	**–22.5%**
Standard	3.43	12.3	104.3
Standard	3.42	12.3	104.4
Standard	3.43	12.3	105.4
Mean of Post-TS Standard:	**3.43**	**12.3**	**104.3**
RSD of Post-TS Standard:	**0.2%**	**0.0%**	**0.6%**
Adjusted Percent Post-TS Fouling:	**0.3%**	**–1.6%**	**–2.7%**

ND = Not Deleted

Although an inspection of the chromatograms can reveal much about the effect of the TS, it may not reveal the magnitude of the effect. The calculations performed in Table 1 show the extent of ethanol's effect on Lys.

The mean standard deviation RSD upper and lower 99% confidence interval for retention time, peak area, and peak height are calculated from the triplicate injections of standards. In this example, the Lys retention time confidence interval extends beween 3.45 and 3.39 min. The TS (3% ethanol) did not have any peak corresponding to Lys. However, the Lys peak for TS spiked, with amino acids had a retention time of 3.43 min within the confidence interval, indicating ethanol (3%) did not affect the retention of Lys. Both area and height were affected by the presence of 3% ethanol, where a 23% loss in response was observed for Lys.

Table 1 also represents the post-TS injection effect, which is measured by the change in retention times and for peak area standards before and after injection of the TS. No statistically significant post-TS injection effects were observed for Lys. The post-TS injection RSDs were comparable with the pre-TS standard RSD. No upward trending for the replicate post-TS injection data was observed, indicating that no post-TS injection effect due to electrode fouling or system contamination could be observed at this TS concentration (3% ethanol).

When all the measured percent changes for the two effects (matrix and post-TS injection) are extracted from the calculations and compared across different TS concentrations, it is possible to evaluate the range of TS concentrations suitable for chromatographic analysis. Table 2 presents a summary of matrix effects on all amino acid peak areas, comparing different ethanol levels (0.1, 1, 3, 10, and 50%). Statistically significant differences from expected levels are marked with an asterisk (*) in the tables presented in this technical note. As discussed, Arg is completely obscured by ethanol, thus the high percent differences from expected measurements. Arg cannot be quantified in the range of ethanol concentrations evaluated in this study. At levels greater than 0.1%, peak areas for many of the other early eluting amino acids (Lys, Ala, Thr, Gly, Val) were decreased in a dose-dependant manner. The depressed peak areas indicate a probable interference at the electrode due to its exposure to ethanol. The effects on peak height were generally similar to peak area results (not shown).

The matrix effect on retention time is presented in Table 3. Although significant effects on response (peak area and height) were observed, this was not true for retention time. The presence of ethanol in the sample did not greatly affect amino acid retention times. At 50% ethanol, the retention shift of Ala was –2.2% and Gly was –2.0%. The effect on the other amino acids was less than 2%. With 0.1% ethanol, retention times were unaffected.

The post-TS injection effects were also tabulated across different ethanol TS doses. Table 4 presents the results for the effect on peak area. Statistically significant effects were observed for Phe and Asp at 0.1%, but not at 3 or 10%. At the 1% level, the effect on 11 amino acids was also found to be statistically significant, while at the 3% level, the effect on only 1 amino acid was significant. These inconsistent results suggest that a higher number of replicates might have shown the amino acids in question were unaffected by ethanol at concentrations <3%. It is also important to consider that statistical significance does not always relate to suitability for use. For example, at the 1% dosage level, where 11 amino acids were significantly different from the starting area response, only Glu exceeded a 5% loss, while the remaining amino acids with significant differences ranged from –4.1% to +4.1%. For most practical purposes, losses in response less than 10% are probably acceptable. At the 50% level, the losses in area response exceed 10% for most amino acids. The results and conclusions for the post-TS injection effects

on peak height results (not shown) and peak area are the same (Table 4). Both peak area and height losses at the 50% ethanol level indicate that electrode fouling persists beyond initial exposure, and can result in extended losses in response. The peak area of the post-TS standards for the 50% level showed an increasing trend, indicating the fouled electrode will return to normal response over time.

No post-TS effect on retention time was apparent (Table 5). Even at the 50% ethanol level, the change in retention time ranged from –0.2 to 0.5%, and none of these changes were statistically significant.

The results for the matrix and post-TS injection effects for ethanol show that this organic solvent will foul the electrode during sample analysis, and at high solvent concentrations it will foul the electrode beyond initial exposure. The greater the ethanol concentration, the greater the tailing. The larger the tail, the greater the extent of involvement of early eluting analytes, and thus the greater the number of analytes affected. The suitability of the TS dosage for *AAA-Direct* analysis in this example therefore depends on which amino acids are analytically important. The suitability of the dosage can also be extended with the adoption of correction factors that adjust the measured value of the lower response. In this example however, ethanol could be removed by evaporation, which would likely eliminate any analysis problems.

In cases of similar interference by unretained peaks (e.g., alkylamines), we recommend a recent report that describes the use of mineral acid addition for increasing the retention of Arg.[3]

ADDITIONAL COMMENTS

Sodium nitrite and sodium sulfite were both evaluated at 1 and 10 mM concentrations using the screening procedure (results not presented). No interfering peaks were observed for nitrite; however, sulfite eluted between Asp and cystine at 24 min. Sulfite (1 mM) was not baseline resolved from Asp, and at 10 mM

Table 2
Matrix Effect of Ethanol on Peak Area, Percent Change from Expected

	Ethanol Concentration									
Amino Acids**	**0.1%**		**1%**		**3%**		**10%**		**50%**	
Arginine	102%	*	1012%	*	2420%	*	5400%	*	9520%	*
Lysine	–2.0%		–11.3%	*	–22.6%	*	–67.4%	*	–76.4%	*
Alanine	0.8%		–7.8%	*	–8.7%	*	–39.1%	*	–78.4%	*
Threonine	2.9%		–6.3%	*	0.4%		–9.8%	*	–18.4%	*
Glycine	0.3%		–7.5%		–6.3%		–18.0%	*	–48.8%	*
Valine	2.3%		–4.5%		–3.1%		–8.9%	*	–32.4%	*
Serine	1.7%		–5.2%	*	1.7%	*	1.2%		–13.0%	*
Proline	2.2%		–1.8%	*	3.4%		–1.6%		-11.7%	
Isoleucine	1.4%		–5.7%	*	0.3%		0.9%		–5.8%	
Leucine	2.3%		–6.1%	*	0.3%		0.9%		–2.2%	
Methionine	1.3%		–4.2%	*	–0.8%		–1.6%		–4.4%	
Histidine	6.1%		1.2%		7.4%		–22.2%	*	–4.0%	*
Phenylalanine	4.5%	*	–4.1%	*	2.5%		–2.5%	*	–6.4%	*
Glutamate	7.7%		–7.8%	*	2.1%		6.8%	*	–9.9%	
Aspartate	6.6%		–6.1%	*	1.8%		2.2%	*	–13.6%	*
Cystine	2.2%		–4.4%	*	2.9%	*	0.1%	*	–6.8%	
Tyrosine	2.0%		–4.2%	*	2.1%	*	1.9%		–10.2%	*
Tryptophan	-0.2%		–6.3%	*	1.8%	*	–0.1%		1.3%	

* 99% Confidence Level (±3 standard deviations)
** Amino acids or peaks detected within the amino acid retention time window.

Table 3
Matrix Effect of Ethanol on Retention Time, Percent Change from Expected

	Ethanol Concentration									
Amino Acids**	**0.1%**		**1%**		**3%**		**10%**		**50%**	
Arginine	0.0%	*	–6.4%	*	–5.7%	*	–3.6%	*	–6.4%	*
Lysine	0.3%		–0.2%		0.3%		0.7%		0.0%	*
Alanine	0.1%		0.1%		0.3%		0.2%		–2.2%	*
Threonine	0.1%		0.0%		0.4%		0.3%		–1.7%	*
Glycine	0.0%		0.0%		0.4%		0.3%		–2.0%	*
Valine	0.1%		0.1%		0.3%		–0.2%		–1.6%	*
Serine	0.0%		–0.1%		0.6%		0.2%		–1.8%	*
Proline	0.1%		0.0%		0.5%		–0.1%		–1.4%	*
Isoleucine	0.3%		–0.1%		0.3%		0.0%		–0.5%	
Leucine	0.2%		–0.1%	*	0.2%		0.2%		–0.2%	
Methionine	0.2%		–0.2%	*	0.4%		0.2%		–0.4%	
Histidine	0.7%		0.0%		0.8%		–1.8%	*	0.1%	
Phenylalanine	0.5%		0.0%		0.6%		–1.1%	*	0.2%	
Glutamate	0.2%		0.1%		0.3%		–0.4%	*	0.1%	
Aspartate	0.2%		0.1%		0.3%		–0.3%	*	0.1%	
Cystine	0.2%		0.1%		0.2%		–0.3%	*	0.1%	
Tyrosine	0.0%		0.0%		0.1%		0.1%		–0.1%	
Tryptophan	0.1%		–0.1%	*	0.4%		0.2%		0.2%	

* 99% Confidence Level (±3 standard deviations)
** Amino acids or peaks detected within the amino acid retention time window.

Table 4
Post-TS Injection Effect of Ethanol on Peak Area, Percent Change from Expected

	Ethanol Concentration									
Amino Acids**	**0.1%**		**1%**		**3%**		**10%**		**50%**	
Arginine	0.9%		3.2%	*	0.4%	*	–6.7%		–12.9%	*
Lysine	–0.3%		4.1%	*	–1.6%	*	–6.2%		–13.5%	
Alanine	1.4%		1.9%		–0.7%	*	–5.1%	*	–17.0%	*
Threonine	3.2%		1.0%		0.7%		0.9%		–9.3%	*
Glycine	3.1%		–0.4%		–1.8%		–4.7%	*	–12.7%	
Valine	2.7%		1.9%		–1.4%		–1.4%		–13.2%	
Serine	0.4%		0.9%		1.1%	*	1.5%		–5.7%	
Proline	1.2%		3.4%	*	0.0%		1.7%		–4.0%	
Isoleucine	4.8%		1.6%	*	2.2%		3.0%		–13.0%	
Leucine	5.8%		0.0%		2.4%		3.2%		–6.4%	
Methionine	2.1%		3.1%	*	–1.1%		–2.8%	*	–8.9%	*
Histidine	8.0%		3.6%	*	4.5%		-3.5%		–11.5%	*
Phenylalanine	4.9%	*	2.7%	*	0.3%		0.3%		–12.9%	*
Glutamate	-6.6%		–5.3%		0.6%		7.1%		–21.6%	*
Aspartate	–14.3%	*	–4.1%	*	0.8%		1.3%		–22.3%	*
Cystine	3.2%		2.4%	*	0.8%		–0.4%		–14.4%	*
Tyrosine	3.4%		2.8%	*	–0.3%		–1.4%		–17.6%	*
Tryptophan	1.1%		2.7%	*	0.1%		–3.0%	*	–5.3%	

* 99% Confidence Level (±3 standard deviations)
** Amino acids or peaks detected within the amino acid retention time window.

Table 5
Post-TS Injection Effect of Ethanol on Retention Time, Percent Change from Expected

	Ethanol Concentration									
Amino Acids**	**0.1%**		**1%**		**3%**		**10%**		**50%**	
Arginine	0.0%	*	–1.2%	*	0.8%	*	–0.6%	*	0.1%	*
Lysine	–0.3%		–0.5%		0.3%		–1.4%		0.0%	*
Alanine	–0.3%		–0.1%		0.2%		–1.7%		0.0%	*
Threonine	–0.3%		–0.2%		0.1%		–1.5%		–0.1%	*
Glycine	–0.4%		–0.2%		0.2%		–1.3%		–0.1%	*
Valine	–0.2%		0.0%		0.1%		–1.6%		–0.1%	*
Serine	–0.3%		–0.3%	*	0.4%		–1.2%		–0.2%	*
Proline	–0.2%		–0.2%	*	0.2%		–1.5%		–0.1%	*
Isoleucine	0.1%		–0.3%	*	0.0%		–1.2%		0.2%	
Leucine	0.0%		–0.2%	*	0.1%		–1.0%		0.0%	
Methionine	–0.2%		–0.2%	*	0.3%		–0.7%		0.1%	
Histidine	0.6%		–0.1%	*	0.9%		0.0%		0.1%	
Phenylalanine	0.4%		–0.1%		0.7%		0.1%		0.2%	
Glutamate	0.2%		0.0%		0.4%		0.2%	*	0.1%	
Aspartate	0.1%		0.0%		0.3%		0.2%	*	0.1%	
Cystine	0.2%		–0.1%		0.3%		0.2%	*	0.2%	
Tyrosine	0.1%		0.2%		0.1%		0.0%		0.1%	
Tryptophan	0.0%		-0.3%	*	0.6%		0.3%		0.5%	

* 99% Confidence Level (± 3 standard deviations)
** Amino acids or peaks detected within the amino acid retention time window.

coeluted with Asp. The detection of sulfite was over 10 times less sensitive than the method published in Application Note 54.[4] Both nitrite and sulfite are ionic, and thus at 10 mM cause amino acid retention times to shorten (<5%), but caused no coelutions. Nitrite and sulfite chemically react with some amino acids in the standard mix, resulting in reduced peak area and new peaks.

The use of additional sample preparation techniques, such as acid or base hydrolysis, can produce new interferences or eliminate interferences. Test samples processed by these sample preparation techniques should also be tested using the screening method presented in this technical note. In some cases, acid hydrolysis of protein samples was observed to eliminate interference by additives (e.g., glucosides). A hydrolysate of a TS should thus be screened if protein hydrolysates are being analyzed.

Some sample ingredients may cause contamination of the column or injector. When the increased peak area of the analytes, background level, or additional peak persists for the post-TS injections, the column or injector may be contaminated. Repetitive injections of water blanks often reveal downward trending in the background and areas of unknown peaks when contamination has occurred. Changes in the gradient program to reflect longer column cleanup times or additional injector flushes can often eliminate these contamination problems. We recommend using disposable electrodes (Au from Dionex) when screening types of possible interfering matrix compounds. If significant post-TS injection effects occur, the relatively inexpensive disposable electrodes can be replaced. Laborious and time-consuming electrode regeneration is avoided. Although permanent electrode fouling is a rare event, disposable electrodes can be used to quickly revive high electrochemical response.

SUMMARY

This technical note describes a screening procedure that can be used to evaluate the suitability of a TS for *AAA-Direct*. This screening procedure differentiates between direct matrix interference and post-TS injection effects. Statistically significant changes in retention time, detector response, or a TS coelution with an analyte may be observed. For practical purposes, the extent of an interference is most important and should match the demands of the application.

Although *AAA-Direct* was selected to exemplify this screening procedure, the same procedure can be applied to similar chromatographic methods that use electrochemical detection (e.g., carbohydrate or antibiotic methods).

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