

Optimizing Mass Spectrometric Detection for Ion Chromatographic Analysis of Common Anions and Selected Organic Acids

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INTRODUCTION

Ion chromatography (IC) has been used extensively for environmental, food and bioanalysis for decades. IC is the primary separation technique used for inorganic ion analysis. It has also been widely utilized to separate charged polar molecules such as organic acids and amines.^{1,2} Current developments show the success of using IC to separate large molecules such as proteins, polypeptides, nucleic acids, polynucleotides, and other charged biomolecules.^{3,4,5}

In recent years, mass spectrometry has become a preferred detection technique due to the increasing demands for sensitivity, selectivity, and to obtain structural information.^{6,7,8} Electrospray ionization (ESI) is the preferred interface to couple IC and MS, because the compounds analyzed by IC are generally ionic or highly polar species.

Challenges remain due to the inherent composition of the IC eluent. An eluent generator is used to electrolytically produce high purity potassium hydroxide (KOH) eluent then postcolumn separation an electrolytic suppressor is used to modify the eluent. These suppressors employ the electrolytic reactions of water to generate hydronium $[H_3O]^+$ and hydroxide $[OH]^-$ ions, thus eliminating the need for a separate source of regenerant. The hydronium $[H_3O]^+$ ions replace the eluent potassium cations and neutralize the hydroxide eluent. By using an eluent suppressor, the strongly ionic eluent is converted to water before entering the mass spectrometer, and an additional organic assistant makeup flow is introduced to the main stream to assist the ESI desolvation process.

Although IC/MS has been in use for some time, there is little information available on how to systematically optimize MS detection for IC.

Although each type and model of MS instrumentation differs in achieving optimal MS response, parameters including probe temperature, nebulizer gas, organic assist makeup flow, ESI needle voltage and entrance cone voltage, are among the variables that need to be investigated.

A general approach to optimize the MS response is to isolate and investigate each parameter one at a time while keeping other parameters constant. As a consequence, this approach does not include the interactions of parameters, and thus may yield a false "optimum" response. This one-parameter approach also requires an increased number of experiments to perform the optimization, resulting in increased consumption of time and resources.

Response surface methodology (RSM) is a combination of mathematical and statistical techniques to optimize multiple variables simultaneously with a minimum number of experiments to achieve the best system performance.⁹⁻¹² RSM was applied by using Design-Expert® (DE7) software in this study for experiment design, data treatment, and prediction of optimum instrumentation settings.

In this study, five common anions and three selected organic acids were used as target analytes for optimization. The five previously mentioned MS parameters were screened for significant effects on MS response and to determine the ranges and levels for optimization. The optimization experiments were performed according to the design points from central composite design (CCD), and the MS response was collected, normalized, and treated statistically by DE7. Analyte MS responses were also collected at the predicted optimum conditions from DE7 and compared with experimental MS responses.

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Instrumentation

System:	Dionex ICS-2000 RFIC™ system
Columns:	IonPac® AS20 (2 × 250 mm) with AG20 (2 × 50 mm) CR-ATC Continuously Regenerated Anion-Trap Column
Mobile Phase:	32 mM hydroxide generated from EGC II KOH cartridge
Flow Rate:	0.25 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity (external water at 0.50 mL/min) MSQ Plus™ single quadrupole mass spectrometer
Ionization Source:	Electrospray ionization (ESI)
Desolvation Solvent:	Acetonitrile delivered by an AXP pump
Scan Mode:	Negative Selected Ion Monitoring (SIM)

Scan details shown in Table 1.

Table 1. MS SIM Scans in This Study

Analyte	Adduct	Observed m/z
Fluoride	[F(HF)] ⁻	38.9
Chloride	[Cl] ⁻	34.9
Nitrate	[NO ₃] ⁻	61.9
Sulfate	[HSO ₄] ⁻	97.0
Phosphate	[H ₂ PO ₄] ⁻	97.0
Pyruvate	[CH ₃ COCOO] ⁻	87.0
Tartrate	[HOOC(CH ₂ OH) ₂ COO] ⁻	149.0
α-Ketoglutarate	[HOCCO(CH ₂) ₂ COO] ⁻	145.0

Use 0.5 span and 0.1 s scan time for all SIM scans.

Software

Instrument control, data acquisition and processing, and report generation were achieved through Chromeleon® chromatography data system (version 6.8 SR6).

Design-Expert (DE7, version 7.1.6, Stat-Ease Inc., Minneapolis, MN) was used for experiment design, statistical data analysis, and prediction of optimum conditions and response.

Chemicals and Reagents

Five common anions (fluoride, chloride, nitrate, sulfate and phosphate) were prepared from a mixed five-anion standard solution (Dionex, P/N 37157). Standard solutions of organic acids were prepared by dissolving pure chemicals (in organic or salt form) in deionized (DI) water (pyruvic acid, sodium salt (Sigma, P2256), α-ketoglutaric acid, monopotassium salt (Sigma, K2000), tartaric acid (Aldrich, T400)). Methanol (CH₃OH) and acetonitrile (CH₃CN) were purchased from Burdick & Jackson (HPLC/UV grade, Honeywell, Muskegon, MI).

Working solutions for optimization experiments were prepared by diluting the mixed anion solution and organic acid solutions in DI water to the appropriate level (fluoride: 1 ppm; chloride: 1.5 ppm; nitrate: 5 ppm; phosphate: 7.5 ppm; sulfate: 7.5 ppm; pyruvate: 1 ppm; tartrate: 1 ppm; ketoglutarate: 1 ppm).

RESULTS AND DISCUSSION

Design of Experiment

In this study, MS response is affected by five parameters which include four universal parameters: probe temperature (measured in °C), nebulizer gas (measured in psi), assistant makeup flow (measured in mL/min) and ESI needle voltage; and one customizable parameter which can be specified for each of the individual analytes or individual scan functions: the cone voltage. Among the five parameters, probe temperature, nitrogen gas flow and assistant makeup flow can be categorized as parameters related to the ionization process; needle voltage and cone voltage are recognized as parameters related to ion extraction (or ion transmission).

Optimization of MS response was carried out in two steps: step one is to optimize the ionization process by studying ionization parameters; and step two is to optimize the ion transmission process by studying needle voltage and cone voltage. Each of the two steps includes the optimization of no less than two parameters, and the interactive effects from the parameters may play significant roles in affecting MS response. To overcome this problem, response surface methodology (RSM) is used to simultaneously optimize several parameters.

Screening of MS Parameters

The screening of MS parameters was achieved by changing one parameter at a time while keeping all other parameters constant at their *General Condition* settings (probe temperature: 400 °C; nitrogen nebulizer gas: 80 psi; assistant makeup flow: acetonitrile at 0.20 mL/min; needle voltage: 1.5 kV; cone voltage: 50V).

Assistant Makeup Solvent, Composition, and Flow Rate

Two of the commonly used HPLC organic solvents, methanol and acetonitrile, were compared in their performances as makeup assist solvents to improve MS response. Experiments were performed at the *General Condition* with IC flow rate at 0.10 mL/min; MS response (peak area) was measured with assistant makeup flow (using CH₃OH or CH₃CN) in the range from 0.00 to 0.25 mL/min with 0.05 mL/min increments. At the optimum makeup flow conditions for each analyte, the comparison of MS response of using CH₃CN or CH₃OH as assistant makeup solvent showed that CH₃CN demonstrated better enhancement for each analyte with gains of 114% to 363% in peak area (details will be presented elsewhere).

Experiments were also performed to find the optimum assistant makeup solvent composition, i.e., the percentage of organic solvent in the organic/water mixture, at three conditions: 50%, 75% and 100% CH₃CN. As expected, 100% CH₃CN showed the best enhancement for MS response, because the assistant makeup solvent with 100% CH₃CN assists the desolvation most efficiently and has the least dilution effect for analytes.

An optimum flow rate would be expected due to the compensating effects of desolvation-enhancement and dilution. As shown in Figure 1, the optimum flow rate of assistant makeup flow is ~0.23 mL/min when running the IC flow at 0.25 mL/min. The very closely laid trend lines suggest that the assistant makeup flow has a similar extent of enhancement for the MS response of each analyte.

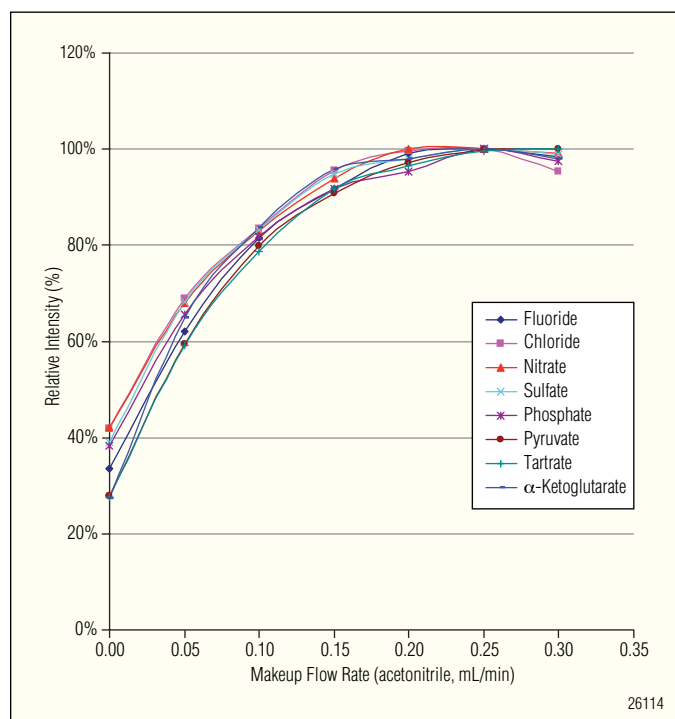


Figure 1. Relative peak intensity vs. assistant makeup flow rate.

Nebulizer Gas

The experiments were performed at the *General Condition* with varying nebulizer gas pressure of 60 to 95 psi. The dependence of MS response on nebulizer gas pressure is shown in Figure 2, and it is obvious that higher nebulizer gas pressure yields a higher MS response for each analyte. The MS responses of most analytes reach a plateau at 80 psi, which is the recommended general condition on the MSQ instrument for ESI applications. However, the MS response for fluoride required a higher pressure of nebulizer gas to reach the optimum response level.

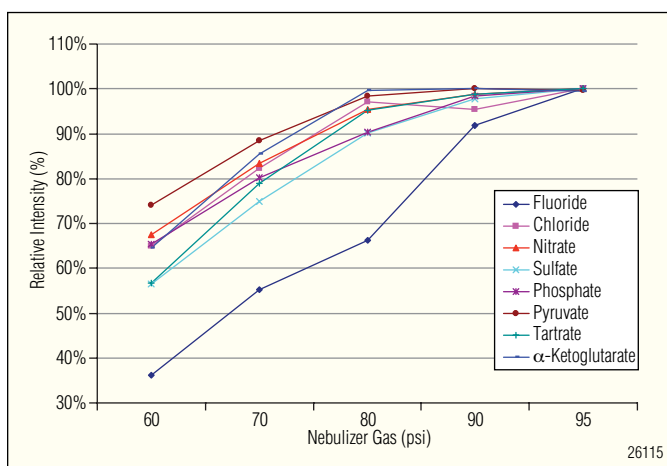


Figure 2. Relative Peak Intensity vs. Nebulizer Gas Pressure.

Probe Temperature

The screening process was carried out at the *General Condition* with the probe temperature varying from 200 °C to 550 °C. As shown in Figure 3, MS response increases with increasing probe temperature, reaches the optimum condition (300 °C to 450 °C dependent on analyte) and decreases with further increased temperature.

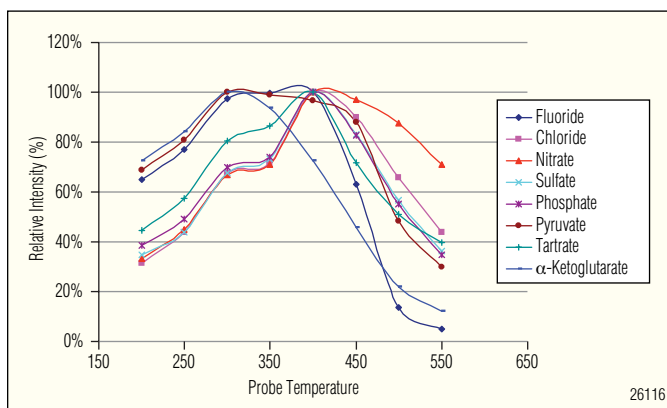


Figure 3. Relative peak intensity vs. probe temperature.

Needle Voltage

The screening of needle voltage was performed from 1.0 kV to 5.0 kV, and the result is shown in Figure 4. The curvatures show that the optimum needle voltage is very specific to individual analytes, e.g. the optimum needle voltage for fluoride and phosphate is near 2.0 kV and 3.5 kV respectively.

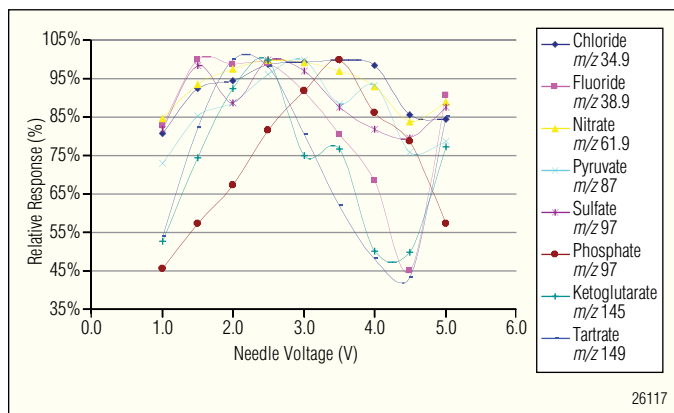


Figure 4. Relative peak intensity vs. needle voltage.

Cone Voltage

Cone voltage is expected to be very analyte dependent and can be customized to different levels for individual analytes or individual scan functions. As shown in Figure 5, optimum cone voltages vary for each of the different analytes.

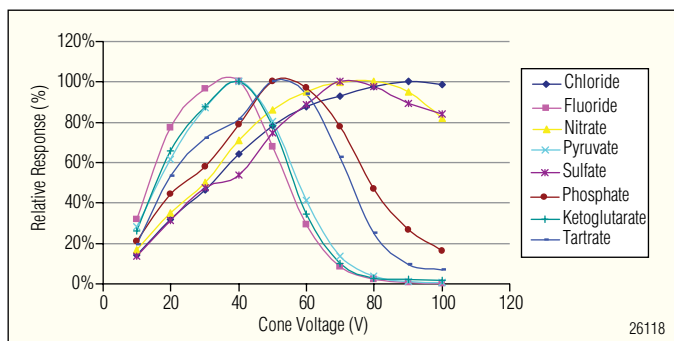


Figure 5. Relative peak intensity vs. cone voltage.

Optimization of Ionization Parameters

Optimization of the ionization parameters is the first step of overall process optimization and includes the simultaneous optimization of probe temperature, nebulizer gas pressure, and assistant makeup flow rate. The experiment points were designed according to CCD, and include eight factorial points, six axial points, and six central points, as shown in Figure 6. Experiments listed in Table 2 were carried out in order of increasing temperature to reduce the equilibration time between experiments reducing the overall time for optimization. MS response was measured as the peak area of each analyte in selected ion monitoring (SIM) operating mode. Peak areas were corrected for time effect and normalized to the fraction of the greatest observed peak area of each analyte at different experimental conditions. The experimental design and the results are shown in Table 2. DE7 was used for statistical analysis of the experimental data, model selection, analysis of variance (ANOVA) and prediction of the optimum conditions and the responses at the optimum conditions. For the MS response of each analyte, different statistical models were evaluated to fit the experiment data, including linear, quadratic and cubic models. Among the evaluated models, the quadratic model showed the best fit for experimental data. ANOVA was performed to assess the validity of fitting the model and the significance of each term in the model. An example of ANOVA assessment for the response surface model of nitrate is shown in Table 3. The Model F-value 57.98 implies the model is significant, and there is less than 0.01% chance this level of fit could occur due to random noise. The Lack of Fit F-value 3.44 indicates the lack of fit is not significant relative to pure error. There is only a 10.08% probability this level of fit could occur due to noise. A p-value less than 0.05 indicates there are significant terms in this model. The MS response of nitrate, probe temperature, nebulizer gas, and assistant makeup flow, as well as the interaction terms *probe temperature* × *assistant makeup flow* and *assistant makeup flow* × *assistant makeup flow*, appear to be the significant terms. The adjusted coefficient of determination 0.9643 showed that the model explained 96.43% of the variability in the data. Model equations, coefficient of determination, and most significant terms in each equation are summarized in Table 4.

Table 2. Experimental Design and the Result of the Central Composite Design for the Optimization of Ionization Processes

Run No.	Design No.	Coded Levels			Actual Levels			Normalized Responses							
		Probe Temp.	Nebulizer Gas Pres.	Makeup Flow Rate	Probe Temp. (°C)	Nebulizer Gas Pres. (psi)	Makeup Flow Rate (mL/min)	Chloride	Fluoride	Nitrate	Pyruvate	Sulfate	Phosphate	α -Ketoglutarate	Tartrate
1	9	-1.68	0	0	249	78	0.20	0.5065	0.3657	0.4424	0.5256	0.5403	0.4628	0.6147	0.5394
2	1	-1	-1	-1	300	70	0.10	0.7595	0.5691	0.7237	0.8264	0.7598	0.6906	0.7510	0.7182
3	5	-1	-1	1	300	70	0.30	0.8142	0.5993	0.7039	0.8610	0.8229	0.7337	0.8934	0.8269
4	3	-1	1	-1	300	85	0.10	0.8692	1.0000	0.8611	1.0000	0.9491	0.8278	1.0000	1.0000
5	7	-1	1	1	300	85	0.30	0.7107	0.4588	0.6396	0.6784	0.6474	0.7068	0.5197	0.5813
6	11	0	-1.68	0	375	65	0.20	0.8158	0.6534	0.7563	0.8610	0.8328	0.7672	0.9277	0.9010
7	13	0	0	-1.68	375	78	0.03	0.4796	0.2702	0.4144	0.5361	0.4900	0.3872	0.5326	0.4805
8	14	0	0	1.68	375	78	0.37	0.7991	0.6517	0.7341	0.8833	0.8181	0.7336	0.9069	0.8729
9	15	0	0	0	375	78	0.20	0.7967	0.6387	0.7523	0.8976	0.8400	0.7459	0.8868	0.8742
10	16	0	0	0	375	78	0.20	0.8089	0.6658	0.7670	0.9158	0.8543	0.7675	0.9197	0.8857
11	17	0	0	0	375	78	0.20	1.0000	0.8492	0.9950	0.8949	1.0000	0.8870	0.5764	0.6728
12	18	0	0	0	375	78	0.20	0.9078	0.5533	0.9227	0.9178	0.9261	0.9433	0.6664	0.7918
13	19	0	0	0	375	78	0.20	0.4453	0.1708	0.5034	0.5405	0.4494	0.4005	0.3798	0.3998
14	20	0	0	0	375	78	0.20	0.5205	0.2344	0.5686	0.5272	0.5439	0.4719	0.3947	0.4379
15	12	0	1.68	0	375	90	0.20	0.4300	0.1541	0.4127	0.5073	0.4378	0.3787	0.5009	0.4603
16	2	1	-1	-1	450	70	0.10	0.7746	0.5693	0.7675	0.8111	0.7983	0.7280	0.6078	0.6483
17	6	1	-1	1	450	70	0.30	0.4398	0.1449	0.4267	0.5271	0.4582	0.3503	0.5255	0.4522
18	4	1	1	-1	450	85	0.10	0.9072	0.3925	1.0000	0.7912	0.9812	0.9413	0.5917	0.8056
19	8	1	1	1	450	85	0.30	0.6749	0.3636	0.6220	0.7666	0.6140	0.6083	0.7135	0.6099
20	10	1.68	0	0	500	78	0.20	0.8988	0.7476	0.7991	0.8077	0.8999	0.8368	0.8409	0.8945

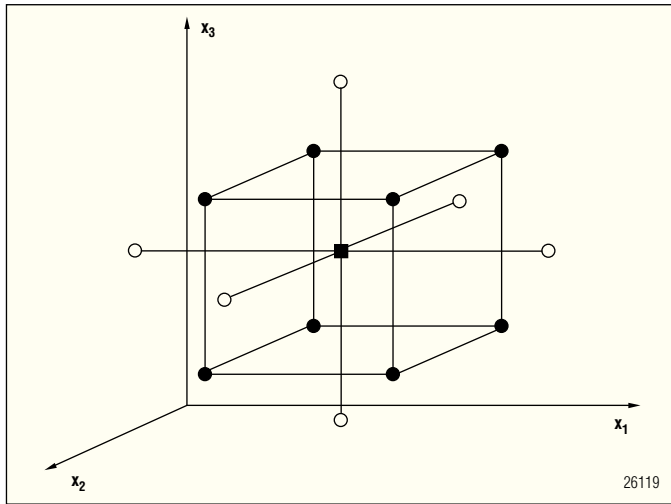


Figure 6. Central Composite Design for three variables. (●) factorial points, (○) axial points, and (■) central points.

Table 3. ANOVA for the Response Quadratic Model of Nitrate

Source of Variance	Sum of Squares	df	Mean Square	F Value	p-value
Model	0.6360	9	0.0707	57.98	< 0.0001
x_1	0.4294	1	0.4294	352.3	< 0.0001
x_2	0.0360	1	0.0360	29.51	0.0003
x_3	0.1182	1	0.1182	97.01	< 0.0001
x_1x_2	0.0034	1	0.0034	2.788	0.1259
x_1x_3	0.0068	1	0.0068	5.616	0.0393
x_2x_3	0.0001	1	6.95E-05	0.0571	0.8160
x_1^2	0.0032	1	0.0032	2.607	0.1375
x_2^2	0.0003	1	0.0003	0.2828	0.6065
x_3^2	0.0407	1	0.0407	33.43	0.0002
Residual	0.0122	10	0.0012		
Lack of fit	0.0094	5	0.0019	3.436	0.1008
Pure error	0.0027	5	0.0005		
Total	0.6482	19			
R^2	0.9812				
Adjusted R^2	0.9643				

Table 4. Response Quadratic Model Equations and Significant Terms

Analyte	Model Equation	Significant Terms*	R ²	Adjusted R ²
Chloride	$Y_1=0.7995+0.1778x_1+0.0536x_2+0.0590x_3+0.0194x_1x_2+0.0303x_1x_3-0.0088x_2x_3-0.0475x_1^2-0.0127x_2^2-0.0592x_3^2$	$x_1, x_2, x_3, x_1^2, x_3^2$	0.963	0.929
Fluoride	$Y_2=0.6308+0.1608x_1+0.1343x_2+0.0380x_3+0.0506x_1x_2+0.0269x_1x_3-0.0057x_2x_3-0.1347x_1^2+0.0114x_2^2-0.0644x_3^2$	x_1, x_2, x_1^2, x_3^2	0.890	0.791
Nitrate	$Y_3=0.6308+0.1608x_1+0.1343x_2+0.0380x_3+0.0506x_1x_2+0.0269x_1x_3-0.0057x_2x_3-0.1347x_1^2+0.0114x_2^2-0.0644x_3^2$	$x_1, x_2, x_3, x_1x_3, x_3^2$	0.981	0.964
Pyruvate	$Y_4=0.8759+0.1195x_1+0.0377x_2+0.0608x_3+0.0114x_1x_2+0.0378x_1x_3-0.0243x_2x_3-0.0871x_1^2-0.0078x_2^2-0.0838x_3^2$	x_1, x_3, x_1^2, x_3^2	0.910	0.830
Sulfate	$Y_5=0.8227+0.1781x_1+0.0759x_2+0.0643x_3+0.0225x_1x_2+0.0392x_1x_3-0.0170x_2x_3-0.0451x_1^2-0.0232x_2^2-0.0629x_3^2$	$x_1, x_2, x_3, x_1^2, x_3^2$	0.958	0.920
Phosphate	$Y_6=0.8227+0.1781x_1+0.0759x_2+0.0643x_3+0.0225x_1x_2+0.0392x_1x_3-0.0170x_2x_3-0.0451x_1^2-0.0232x_2^2-0.0629x_3^2$	$x_1, x_2, x_3, x_1^2, x_3^2$	0.959	0.923
α -Ketoglutarate	$Y_7=0.8845+0.0664x_1+0.0556x_2-0.0284x_3+0.0231x_1x_2+0.0117x_1x_3-0.0535x_2x_3-0.1375x_1^2-0.0321x_2^2-0.1189x_3^2$	x_1, x_1^2, x_3^2	0.832	0.680
Tartrate	$Y_8=0.8489+0.1286x_1+0.0665x_2+0.0050x_3+0.0170x_1x_2+0.01151x_1x_3-0.0518x_2x_3-0.0929x_1^2-0.0307x_2^2-0.1053x_3^2$	x_1, x_1^2, x_3^2	0.848	0.711

*Significant terms are defined as terms having a p-value < 0.05; Terms shown in bold denote the most significant terms with p-value < 0.001.

Table 5. Comparison of Experimental Responses and Predicted Responses at the Optimum Conditions

Analyte	Chloride	Fluoride	Nitrate	Pyruvate	Sulfate	Phosphate	α -Ketoglutarate	Tartrate
Experimental Response	1.033	0.866	0.977	0.899	1.074	0.877	0.868	1.081
Predicted Response	1.029	0.997	1.000	1.038	1.056	0.978	0.889	1.006

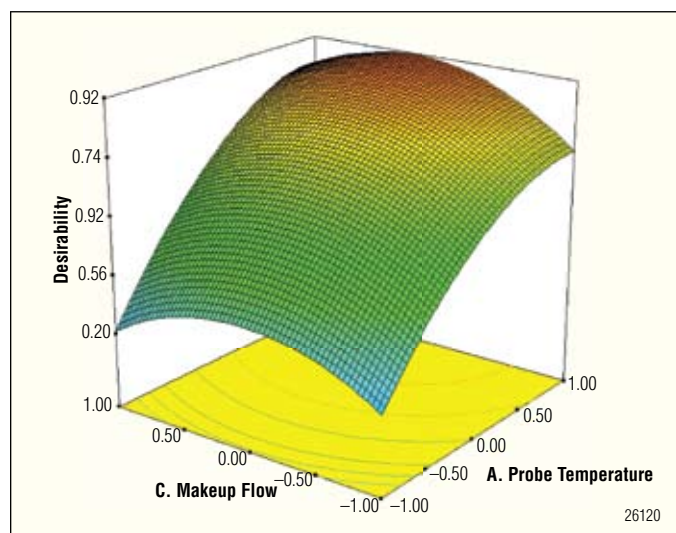


Figure 7. Desirability response surface for system optimization.

The decision of optimum conditions for the maximum system responses of all analytes was carried out using a desirability function with the goal to maximize the response of each analyte. The desirability surface is shown in Figure 7, the maximum desirability for the system is 0.916 which sets probe temperature at 0.96 (447 °C), assistant makeup flow at 0.20 (0.22 mL/min, 100% CH₃CN) and nebulizer gas flow at 1.0 (85 psi). A confirmation experiment was performed at the predicted optimum conditions and the experimental responses were compared with predicted response in Table 5. The comparison showed good agreements between the experimental and predicted responses.

Optimization of Transmission Parameters

The second step to optimize the overall process includes the optimization of two parameters: needle voltage and cone voltage. This step of optimization was performed using CCD (details will be presented elsewhere).

Instead of optimizing the needle voltage for all analytes at the same time, the needle voltage was optimized for fluoride MS response only. This decision was made based on the fact that fluoride showed the lowest MS response factor which makes it the most significant analyte to measure the overall method performance with respect to sensitivity.

For this reason, the needle voltage was set at 1.5 kV for this method. Because the cone voltage can be customized for individual analyte/scan, the optimum cone voltage can be achieved either by graphic estimation from the MS response-cone voltage curve which was generated from the screening process (Figure 5); or use the desirability function to maximize individual response using DE7.

Table 6 summarizes the optimum conditions for each analyte and system optimum conditions. As shown in Table 6, the optimum nebulizer gas pressure was at 85 psi which was the highest achievable value in the range as shown in Figure 2. The probe temperature which was optimized at 450 °C has a slight deviation from the result of the screening process. The deviation can be explained by the increased assistant makeup flow rate and the higher nebulizer gas pressure at the final system optimum settings, which could compensate the response decrease with higher probe temperature. The predictions of the optimum assistant makeup flow agree very well with the results from the screening process which suggests the optimum flow rate ranges from 0.20 – 0.30 mL/min.

A comparison experiment was performed between the MS response at the *General Condition* and optimum condition obtained from the optimization process, and the result is shown in Table 7. Significant MS response improvement was observed for each analyte ranging from +138% (tartrate) to +395% (fluoride).

Table 6. Optimum Conditions for Each Analyte and System Optimum Settings

	Probe Temperature (°C)	Nebulizer Gas (psi)	Assistant Makeup Flow (mL/min)	Needle Voltage (kV)	Cone Voltage (V)	System Optimum Cone Voltage (V)
Chloride	450	85	0.27	2.6	88	88
Fluoride	450	85	0.28	1.5	26	26
Nitrate	450	85	0.30	2	73	73
Pyruvate	450	85	0.28	1.9	45	45
Sulfate	450	85	0.28	2.4	73	69
Phosphate	450	85	0.30	3.4	52	55
α-Ketoglutarate	400	85	0.19	1.5	37	37
Tartrate	450	85	0.22	2.1	50	50
System	450	85	0.22	1.5	varies	—

Table 7. Comparison of MS Responses at General Condition and Optimum Condition

	Chloride	Fluoride	Nitrate	Pyruvate	Sulfate	Phosphate	α-Ketoglutarate	Tartrate
General Condition	18136	796	560505	183163	997398	378367	124517	184992
Optimum Condition	33766	3146	845476	316437	1759253	620670	238669	256148
Improvement*	186%	395%	151%	173%	176%	164%	192%	138%

Data shown in row 1 and row 2 are absolute peak areas from MS SIM detection with *unit counts* × *min*.

* Improvement was calculated by response from optimum condition/response from *general condition* × 100%.

CONCLUSION AND SUGGESTIONS

A systematic method for the optimization of MS response for IC analysis of common anions and organic acids using response surface methodology was demonstrated in this study. This approach has several advantages over the general one-variable-at-a-time approach because: RSM requires substantially fewer number of experiments to reach the optimum system response; RSM includes the interactions between individual parameters which are not studied through the one-variable-at-a-time approach. The optimum conditions for each analyte are suggested as well as the optimum conditions for the best overall system response.

The good agreements between predicted optimum conditions and the general conditions obtained from the authors' experience suggests a rule-of-thumb set of conditions for general IC-MS anion analysis where optimum sensitivity is not crucial or can serve as a starting point to further optimize system performance: when operating IC at 0.25 mL/min, set the probe temperature between 400 – 450 °C; nebulizer gas at 85 psi; and use CH₃CN as assistant makeup flow at 0.20 – 0.30 mL/min. Figure 8 shows the overlaid plots of the contour of individual response, and the high-lighted area indicates the operable ranges (normalized response for each analyte greater than 0.8) for probe temperature and assistant makeup flow when nebulizer gas set at 85 psi.

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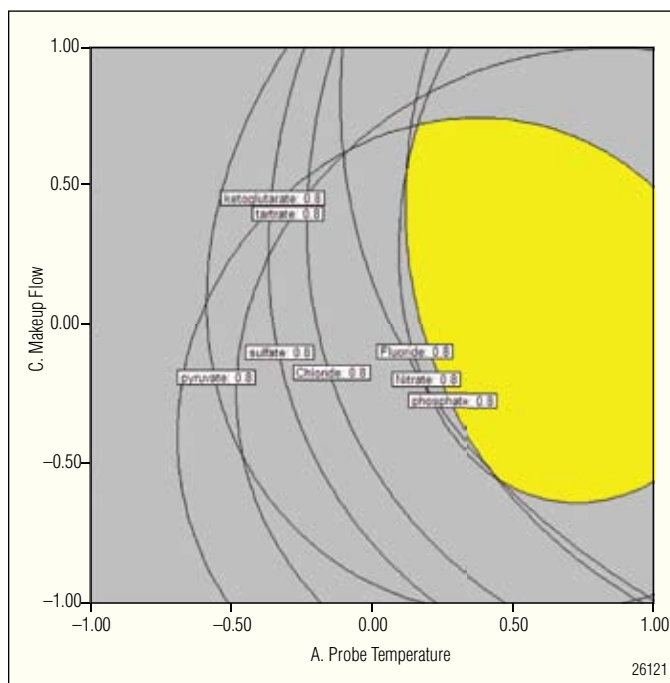


Figure 8. Operable ranges of probe temperature and assistant makeup flow rate with normalized minimum response of each analyte greater than 0.8.

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