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Chemometric study of the influence of instrumental parameters on ESI-MS analyte response using full factorial design

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ABSTRACT

Full factorial experimental design technique was used to study the main effects and the interaction effects between instrumental parameters in two mass spectrometers equipped with conventional electrospray ion sources (Thermo LCQ Deca XP and Shimadzu LCMS 2010). Four major parameters (spray voltage, ion transfer capillary temperature, ion transfer capillary voltage, and tube lens voltage) were investigated in both instruments for their contribution to analyte response, leading to a total of 16 experiments performed for each instrument. Significant parameters were identified by plotting the cumulative probability of each treatment against the estimated effects in normal plots. Analysis of variance (ANOVA) was employed to evaluate the statistical significance of the effects of the parameters on ESI-MS analyte response. The results reveal a number of important interactions in addition to the main effects for each instrument. In all the experiments performed, the tube lens voltage (or Q-array dc voltage in LCMS 2010) was found to have significant effects on analyte response in both instruments. The tube lens voltage was also found to interact with the capillary temperature in the case of the LCQ Deca XP and with the spray voltage in the case of the LCMS 2010. The results of these experiments provide important considerations in the instrumental optimization of ionization response for ESI-MS analysis.

1. Introduction

Variation of analyte response in electrospray ionization mass spectrometry (ESI-MS) constitutes a major concern in the quantitative applications of the technique (e.g., for metabolomics and interactomics) [1–4]. Despite several research efforts in this direction, a comprehensive model for correlating analyte solution concentration with ESI-MS ion abundance remains elusive. Recent works have shown that analyte ESI-MS response may be modeled considering interrelationships of analyte physicochemical properties [5–8]. In order to better understand the way and manner in which analyte signal varies during the electrospray process, it is also important to understand which instrumental factors have the greatest effects on analyte response.

Several instrumental factors are known to influence analyte response in ESI-MS. In performing an ESI-MS experiment, it is important to identify the key parameters that elicit greatest influence on analyte signal intensity and quality. Although most instrument manufacturers provide software packages with their instruments to assist in optimizing instrument conditions, it is still necessary for the user to be familiar with the manner in which these parameters affect the results. More importantly, information on interactions between two or more parameters is often undetectable when using the factory installed optimization software. Such is the case when one or more of the parameters involved in the interaction is a categorical variable, or cannot be varied automatically during the optimization procedure (e.g., the temperature of the transfer capillary between the source and the mass analyzer). As such, a method that facilitates the identification of the main effects due to individual instrument parameters as well as any interaction between them would be useful for guiding optimization.

Chemometrics-based techniques have been employed for optimizing experimental conditions in different fields of science for decades [9]. The most prevalent applications are centered on effective experimental designs for determining important experimental variables, creating mathematical models for experiments, or optimizing selected factors that affect the results of an experiment. The prime advantage of using chemometrics-based experimental design techniques is the ability to gather useful information about the system by conducting a minimal number of experiments. The savings in time and resources realized by using these techniques is a strict consideration in most laboratories as well as in industries.

Commonly employed chemometrics techniques include factorial designs (full factorial designs or fractional factorial designs), response surface designs, central composite designs, and Plackett–Burman designs, among others [9,10]. In this report, we

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Table 1
Design matrix for the factorial experimental design in Yates standard order.

Treatment number	Spray voltage "**A**"	Tube lens voltage "**B**"	Capillary temperature "**C**"	Capillary voltage "**D**"
1	–	–	–	–
3	+	–	–	–
2	–	+	–	–
6	+	+	–	–
4	–	–	+	–
7	+	–	+	–
9	–	+	+	–
11	+	+	+	–
5	–	–	–	+
8	+	–	–	+
10	–	+	–	+
13	+	+	–	+
15	–	–	+	+
12	+	–	+	+
14	–	+	+	+
16	+	+	+	+

Notes: (–) indicates factor at low level while (+) indicates high level (see Table 2).

have employed the full factorial experimental design technique to investigate the effects of four different instrument parameters on the ESI-MS analyte signal intensity of three GXG (Gly-Xxx-Gly; where Xxx represents the amino acids Phe, Glu, and Arg) tripeptides. Effects of spray voltage, capillary voltage, capillary temperature, and Q-array dc voltage (or tube lens voltage) were investigated. The experiments were carried out on two different ESI-MS instruments: LCQ Deca XP (Thermo Electron Corporation) and LCMS 2010 (Shimadzu Scientific Instruments, Inc.). Both instruments have conventional electrospray ionization sources set in an orthogonal configuration to the MS inlet. Yates' algorithm was used to estimate the effect of each parameter as well as combinations of parameters [11]. The most important parameters were identified by plotting the effects against their cumulative probability distribution on a normal probability plot. Statistical significance of each effect was also evaluated by analysis of variance (ANOVA).

2. Experiments

2.1. Materials

The three analytes studied were either synthesized (by a collaborator; GRG), or obtained commercially (John Hopkins University Synthesis and Sequencing Facility, Baltimore, MD; GDG and GFG). Glacial acetic acid, ammonium acetate, HPLC grade methanol, and LC-MS grade water were all obtained from J.T. Baker (Phillipsburg, NJ).

The three analytes were dissolved in equimolar concentrations (25 or 50 μM) in a 90/10 water/methanol solvent system containing 1 mM ammonium acetate and 0.5% acetic acid. Ten percent (10%) methanol was added to aid the electrospray process.

2.2. Instrumentation

All experiments were carried out via direct infusion of the analytes using a syringe pump. In the case of the LCQ Deca XP (Thermo Electron Corporation, West Palm Beach, FL), the built-in syringe pump was utilized for the infusion, whereas an external syringe pump (KD Scientific) was used in the case of the Shimadzu LCMS 2010 (Shimadzu Scientific Instruments, Inc., Columbia, MD). An infusion flow rate of 10 μL/min was employed throughout all the experiments.

The ion sources and transfer optics for the two instruments have some similarities and some differences. Both sources are arranged with a conventional spray capillary orthogonal to the MS inlet. Both sources use pneumatic assistance in the form of unheated N_2, administered coaxially with the solvent spray. Drying or auxiliary gases were not employed. Both sources transfer ions into the high vacuum region through a heated stainless steel capillary. The LCQ uses a straight tube, followed by a tube lens – skimmer arrangement. The LCMS 2010 employs a curved desolvation line (CDL), engineered specifically for maximal analyte solvent declustering, after which the ions enter the Q-array ion optics (set of quadrupole plates), prior to mass analysis.

The parameters investigated were varied according to the design matrix displayed in Table 1. In all, four factors were studied at two levels each (detailed in Table 2), leading to an experimental design which comprised 16 experimental runs (or treatments), or 2^f (where f is the number of factors evaluated). Each treatment was a unique combination of all the factors. As shown in Table 1, negative signs represent low levels of factors while positive signs represent high levels. The same design matrix was used for all the experiments on both instruments. However, the low and high values differed slightly due to inherent differences in instrument design from different manufacturers. For example, whereas the spray voltage in the Thermo Finnigan LCQ Deca XP can be set as high as 8 kV, the maximum on the Shimadzu LCMS 2010 is 5 kV. Also, the dc component of the Q-array voltage in the LCMS 2010 was taken as the equivalent of the tube lens voltage of the LCQ Deca XP.

The main effects identified in these experiments relate to the parameters: **A** (spray voltage), **B** (tube lens or Q-array dc voltage), **C** (capillary temperature), and **D** (capillary voltage). Interactions between parameters can be two-termed, three-termed, or four-termed. The six two-term interaction effects are: **AB** (spray voltage

Table 2
Values of the factors at the two levels investigated.

Parameter	Spray voltage "**A**" (kV)	Tube Lens voltage[a] "**B**" (V)	Capillary temperature "**C**" (°C)	Capillary voltage "**D**" (V)
Low level (–)	3[b]	10	100	10
High level (+)	6[b]	70	250	60

[a] The equivalent of this parameter in the Shimadzu LCMS 2010 is Q-array dc voltage.
[b] These values are 2.5 and 5 kV respectively for the Shimadzu LCMS 2010.

with tube lens/Q-array dc voltage); **AC** (spray voltage with capillary temperature); **AD** (spray voltage with capillary voltage); **BC** (tube lens or Q-array dc voltage with capillary temperature); **BD** (tube lens or Q-array dc voltage with capillary voltage); and **CD** (capillary temperature with capillary voltage). The four three-term interaction effects are: **ABC** (spray voltage, tube lens or Q-array dc voltage, and capillary temperature); **ABD** (spray voltage, tube lens or Q-array dc voltage, and capillary voltage); **ACD** (spray voltage, capillary temperature, and capillary voltage); and **BCD** (tube lens or Q-array dc voltage, capillary temperature, and capillary voltage). The only four-term interaction effect possible is **ABCD**, incorporating all of the tested parameters.

In an additional set of experiments, and in order to ascertain whether the rf component of the Q-array voltage in the LCMS 2010 impacts the analyte response, measurements were carried out which incorporated this factor into the treatments; this led to a design of 32 distinct experiments, the results of which are discussed separately at the end of the discussion below. This was only carried out on the Shimadzu LCMS 2010 as the LCQ Deca XP does not have any equivalent parameter.

2.3. Data analyses

The absolute ion intensities of each analyte were taken directly from the mass spectra. The signal intensity of each analyte was taken as the average of approximately 200 scans in each replicate run per treatment. The average of the signal intensities from all replicates was used as the response for each analyte in each treatment. Once all averages were computed, the treatments, alongside their respective average responses for each analyte, were rearranged according to Yates' standard order according to Table 1. Since no comparison was sought between different analytes intensities, no internal standard was incorporated.

Yates' algorithm was used to compute the effects of each treatment by pairing the responses (from top to bottom of the Yates' table) as they appear in the Yates standard order in such a way that no single value occurred in more than one pair. Successive columns were then generated from the response column as follows: the first entry was the sum of the first pair of values in the response column, the second entry was the sum of the second pair, the third, fourth, fifth, sixth, seventh and eighth entries were obtained similarly by summing the respective pairs of values of responses. Entries 9–16 were obtained by subtracting the first member of each pair from the second member. Thus, entry 9 is (146,641–65,880) and entry 16 is (3052–2757). The next column was derived in exactly the same manner by summing and differencing the pair of values in the preceding column. Columns 3 and 4 were derived using the same method. The resulting values in each row of the last column were then divided by the respective divisor. Since there were 16 treatments in all for each experimental run, the final column for the first row was divided by 16 (this treatment was where all parameters are at low values). The final column of each of the other rows was divided by 8 (since each parameter has half of its treatments at low values and the other half at high values). The procedure was repeated for the response data acquired on both instruments and at the different concentrations.

3. Results and discussion

The ESI-MS response factor of an analyte can be regarded as made up of two components: Analyte-dependent factors and instrumental factors. A considerable number of research studies have been carried out on evaluating the effects of analyte properties on ESI-MS response [12–19], but many of the results in this area are still quite subjective. The effects of instrument parameters on analyte response have also been studied to a fair degree [20,21]. However, most of these works are focused on individual instrument parameters, or when multiple parameters are studied, their effects on analyte signals are interpreted on a case by case basis, with little or no mention made of likely (or sometimes *de facto*) interaction between parameters. The objective of this study was to develop a quick and easy method of evaluating the influence of instrumental parameters on analyte response during ESI-MS experiments, as well as to identify occurrence and extent of interaction between the studied instrumental parameters during ESI-MS experiments.

Table 3
Analyte responses from Thermo LCQ Deca XP (values arranged in Yates order).

Treatment #	GDG	GFG	GRG
1	1,680,596	8,732,094	3,276,638
3	974,415	8,628,939	2,782,437
2	2,185,035	10,570,093	3,962,149
6	1,705,129	11,903,243	3,837,871
4	1,585,208	7,651,542	5,228,588
7	1,114,735	6,166,111	4,879,344
9	204,929	943,993	1,169,601
11	150,653	745,194	1,189,226
5	1,314,400	6,874,246	2,533,553
8	1,140,971	8,529,214	2,802,865
10	1,869,415	9,067,236	3,352,340
13	1,654,943	11,160,180	3,780,775
15	1,077,664	4,607,648	4,167,216
12	716,390	3,866,278	3,739,017
14	277,020	1,182,409	1,716,992
16	201,551	889,617	1,697,960

The responses (in standard order) for the LCQ Deca XP are shown in Table 3 and those for the LCMS 2010 are shown in Table 4 for all three analytes at 50 μM each. An example of the final table obtained from Yates' algorithm is presented in Table 5. The numerical values of the effect of each treatment are presented in the seventh column in Table 5. These are the estimated effects calculated using Yates algorithm. Negative values indicate that increasing the value of this term decreased the analyte response, and positive values indicate an observed increase in analyte response upon increasing the corresponding term. The greater the absolute value of a term, the greater its influence on analyte response. Since all the values are far greater than zero, visual inspection suggests that they all have influence on analyte response. However, several methods can be used to show that not all the factors or treatments are important in determining the analyte response. One method is to use the sum of squares to compute ANOVA and use the variance ratios

Table 4
Analyte responses from Shimadzu LCMS 2010 (values arranged in Yates order).

Treatment #	GDG	GFG	GRG
1	130,859	432,351	272,009
3	276,981	1,037,874	539,197
2	5,261	15,244	37,047
6	8,743	33,174	71,721
4	77,932	247,921	280,562
7	166,447	600,291	595,746
9	2,778	2,665	7,893
11	2,716	3,107	18,761
5	120,553	387,147	262,183
8	265,503	984,912	536,581
10	9,210	29,681	78,780
13	18,265	75,630	162,014
15	58,619	198,332	247,902
12	152,081	536,371	573,505
14	3,006	3,007	23,833
16	3,408	3,913	60,356

Table 5
Results after applying Yates' algorithm to the responses for GDG (LCMS 2010).

GDG	1	2	3	4	Divisor	Effects	SS
65,880	212,520	222,251	361,924	701,463	16	43,841	3.08E+10
146,641	9,731	139,673	339,539	258,673	8	32,334	4.18E+09
3,809	134,484	220,692	130,121	−627,417	8	−78,427	2.46E+10
5,922	5,189	118,847	128,552	−243,955	8	−30,494	3.72E+09
43,632	204,399	82,874	−332,084	−184,423	8	−23,053	2.13E+09
90,852	16,293	47,247	−295,333	−77,314	8	−9,664	3.74E+08
2,581	113,038	85,119	−125,841	154,373	8	19,297	1.49E+09
2,608	5,810	43,433	−118,114	63,883	8	7,985	2.55E+08
62,102	80,761	−202,790	−82,578	−22,385	8	−2,798	3.13E+07
142,297	2,113	−129,294	−101,845	−1,569	8	−196	1.54E+05
5,685	47,220	−188,105	−35,627	36,751	8	4,594	8.44E+07
10,609	27	−107,228	−41,687	7,727	8	966	3.73E+06
34,950	80,195	−78,648	73,495	−19,267	8	−2,408	2.32E+07
78,088	4,924	−47,194	80,877	−6,059	8	−757	2.29E+06
2,757	43,138	−75,271	31,454	7,382	8	923	3.41E+06
3,052	295	−42,843	32,429	975	8	122	5.94E+04

to determine the significance of each effect. An easier and quicker method is the normal probability plot method, which graphically shows the importance of various effects. Here, the cumulative probabilities of the effects are plotted against their numerical values on a normal probability graph. The underlying principle is based on the well-known concept of normal distribution. If the effects are due to chance and random process then they should lie very close to the center of the plot and should fit reasonably well on a straight line. Any effect that does not fit well on a straight line is not due to random error and hence should be treated as important.

The cumulative probabilities are calculated for each effect by first rearranging the effects in ascending order. The probability (P_r) of any effect is then obtained using the formula in Eq. (1):

$$P_r = \frac{100(r - 0.5)}{N - 1} \quad (1)$$

where r is the rank of the effect after rearrangement in ascending order and N is the total number of experiments. The ranks and probabilities used for all the effects in this study are shown in Table 6. Once the effects were ranked, the normal plots could be generated by plotting the probabilities on the vertical axis and the numerical values of the effects on the horizontal axis. Fig. 1 illustrates the application of this method.

The points labeled on the plots with their respective alphabetical notations clearly lie outside the straight line (center). For the LCMS 2010, the most important parameters are the spray voltage (**A**) and Q-array dc voltage (**B**). There appears to be some level of interaction between these two parameters. The treatment corresponding to this interaction is represented by the point labeled (**AB**). The point labeled **AC** on Fig. 1a and b corresponds to the interaction between spray voltage (**A**) and capillary temperature (**C**). For both GDG and GFG, the plots indicate that both capillary temperature (**C**) and an interaction term between capillary temperature and Q-array dc voltage (represented by the treatment labeled (**BC**)) are additional terms that may influence analyte response. It is interesting to note that these two terms (capillary temperature (**C**) and its interaction with Q-array dc (**BC**)) lie very close to the center on the normal plot for GRG, indicating that they are not as important as the other three effects mentioned above. The reason for this slight discrepancy is not very clear. A plausible explanation may be that an analyte property is interacting with an instrumental factor. In other words, the effects of instrumental factors (often taken as constant for all analytes during ESI-MS) likely vary from analyte to analyte. Analyte-dependent response in the

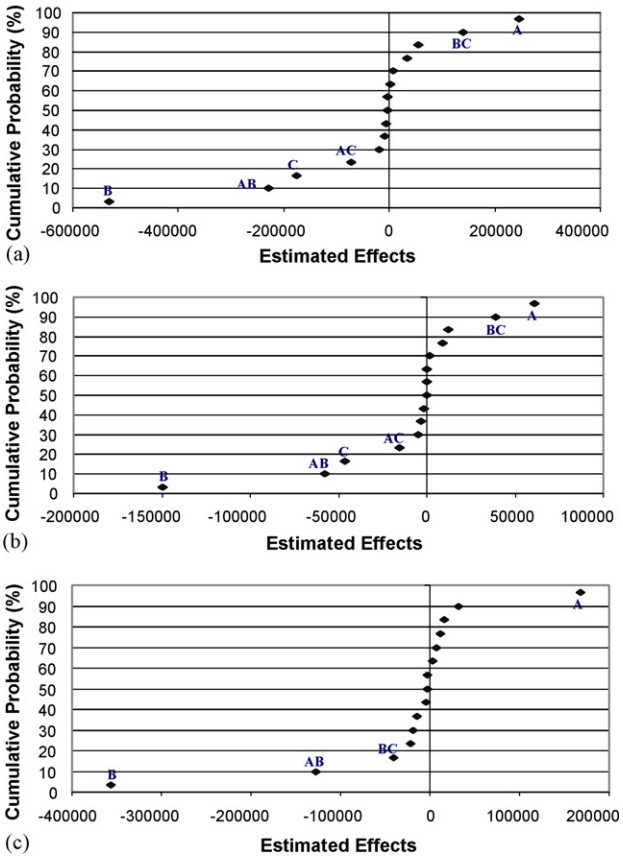

Fig. 1. Normal plot of effects for (a) GFG, (b) GDG, and (c) GRG; **A** (spray voltage), **B** (tube lens or Q-array dc voltage), **C** (capillary temperature), **AB** (interaction term between spray voltage and tube lens), and **BC** (interaction term between tube lens and capillary temperature); (data acquired on Shimadzu LCMS 2010).

Table 6
Cumulative probabilities of the effects based on Eq. (1).

Rank (r)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Probability (%)	3.33	10.00	16.67	23.33	30.00	36.67	43.33	50.00	56.67	63.33	70.00	76.67	83.33	90.00	96.67

presence of a variety of different experimental conditions has been revealed extensively previously [5,8,19], but to our knowledge, not in the context of chemometric interactions. This was indeed one of the features we set out to investigate from the inception of the study.

As a corollary, we deliberately chose the three analytes to be of different characteristics (acidic, neutral, and basic). At the pH of analysis (slightly below 6), GDG exists chiefly as a negatively charged species, GFG as a zwitterion, and GRG as a positively charged species in solution. The positive charge on the GRG at this pH is expected to be on the guanidinium group (on the arginine side chain) which has been reported to have a slightly hydrophobic property [22,23]. This may contribute to its overall responsiveness during the ESI process. In other words, a combination of the essentially permanent positive charge on GRG and the potential hydrophobicity of the guanidinium group likely favors enhanced ionization efficiency for GRG over its two counterparts which do not have these properties.

For the LCQ Deca XP data, the most important parameters are capillary temperature (**C**), tube lens voltage (**B**), and an interaction term (**BC**) between capillary temperature and tube lens voltage. This is interesting in two senses. First is the fact that the instrument manufacturer actually indicates in the instrument manual that changing the capillary temperature during an experiment may require also changing the tube lens voltage in order to obtain optimum signal intensity; another way of saying that both parameters interact. Secondly, the plots indicate that spray voltage (**A**) does not have as much effect on analyte response as it does in the case of the LCMS 2010. This does not underestimate the importance of spray voltage during analysis. What it means is that once the voltage required for the onset of droplet formation is reached, any further variation in spray voltage will have little effect on ionization efficiency, and consequently analyte response, as long as stable spray conditions are maintained. However, knowing fully well that too low a spray voltage may lead to inadequate droplet charging, while too high values may cause arcing or unsuitable spray modes [24], it seems more apt in view of the foregoing results to state that, within the boundary values of the factor space studied for this parameter on this instrument, there is no effect on analyte response.

Another noteworthy observation on the LCQ Deca XP data is the fact that, similar to the case of LCMS 2010, the same factors influence analyte response for GDG and GFG (Fig. 2a and b) while GRG differs in one of the factors (Fig. 2c). All three analytes have **BC** (interaction of tube lens voltage with capillary temperature) in common; both GDG and GFG are also affected by capillary temperature while GRG is affected by tube lens voltage (similar to what was observed in the LCMS 2010). This observation lends further credibility to the above stated assumption that analyte properties (e.g., hydrophobicity) may interact with instrumental parameter (in this case capillary temperature) during ESI-MS.

Particular attention needs to be paid to the interaction effects (**AB** for the LCMS 2010 and **BC** for the LCQ Deca XP). For the LCQ Deca XP, the instrument manual gives information on how both parameters may behave with each other during an ESI-MS experiment. However, such information on factor interaction is not available on the LCMS 2010. The extent of interaction between the spray voltage and the Q-array dc voltage is depicted graphically in Fig. 3. If there is no interaction, the lines should be parallel. The divergence between the two lines clearly indicates that there is considerable interaction.

In an attempt to further justify the statistical significance of each of these effects, an ANOVA was carried out on the sum of squares (last column in Table 5). In order to construct ANOVA, an estimate of the residual error sum of squares is required. One way to obtain this

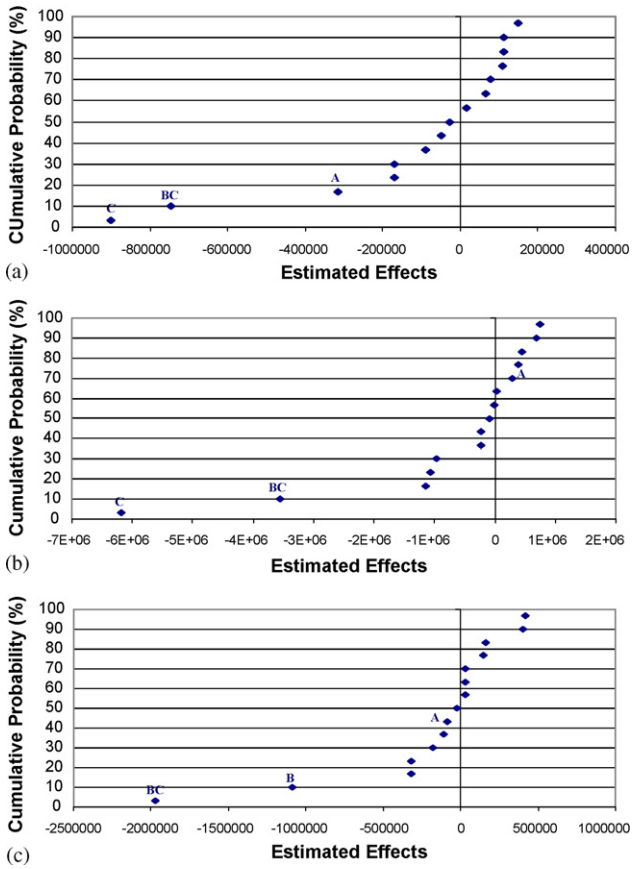

Fig. 2. Normal plot of effects for (a) GDG, (b) GFG, and (c) GRG; **B** (tube lens or Q-array dc voltage), **C** (capillary temperature), and **BC** (interaction term between tube lens and capillary temperature); (data acquired on Thermo LCQ Deca XP).

is to take an average of the squares of residuals (difference between observed responses and predicted responses). Another rough estimate is the average of the sum of squares of the effects that lie mostly on the straight line on the normal plot. The residual sum of squares can also be calculated as the difference between the total sum of squares (a summation of all the squares of replicate measurements of responses) and the treatment sum of squares. Since not all the experiments were carried out in replicates, we have used the first two methods to estimate the ANOVA in this study.

The variance ratios obtained for GDG and GRG are presented in Tables 7 and 8. The results for GFG (data not shown) were similar to those obtained for GDG. Variance ratio 1 is the ratio of the treatment sum of squares to the mean residual sum of squares obtained by pooling the sum of squares for the unimportant effects and averaging them (R1). Variance ratio 2 was calculated in a similar way but

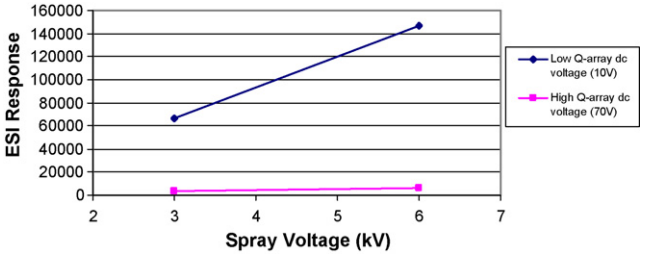

Fig. 3. Interaction between spray voltage and Q-array dc voltage in LCMS 2010 (GDG).

Table 7
Variance ratios of the treatment sum of squares to residual sum of squares (GDG).

Treatment Number	Treatment Sum of squares	Variance ratio 1 (Treatment SS/R1)	Variance ratio 2 (Treatment SS/R2)	Effects
1	30,753,175,501	660.787	633.083	1
3	4,181,993,336	89.858	86.090	**A**
2	24,603,281,885	528.646	506.482	**B**
6	3,719,637,791	79.923	76.572	**AB**
4	2,125,732,499	45.675	43.760	**C**
7	373,590,912	8.027	7.691	**AC**
9	1,489,432,513	32.003	30.661	**BC**
11	255,062,194	5.480	5.251	**ABC**
5	31,317,081	0.673	0.645	**D**
8	153,925	0.003	0.003	**AD**
10	84,413,219	1.814	1.738	**BD**
13	3,731,980	0.080	0.077	**ABD**
15	23,200,278	0.498	0.478	**CD**
12	2,294,720	0.049	0.047	**ACD**
14	3,405,870	0.073	0.070	**BCD**
16	59,373	0.001	0.001	**ABCD**

R1 = 46,540,195 (8 degrees of freedom); R2 = 48,576,847 (15 degrees of freedom).

using the mean residual sum of squares calculated as the average of the squares of residuals. *F*-test with the corresponding degrees of freedom was then used to compare the variance ratios to the tabulated value at $P = 0.05$ level. The degree of freedom for each treatment sum of squares is 1. The degree of freedom for the residual error sum of squares varies depending on the way the residual errors are calculated. If the first method is used, where the sums of squares of all unimportant effects are averaged, then the degrees of freedom will be the number of these effects that are used. Alternatively, if the average of the squares of the residuals is used, then the number of degrees of freedom is $N - 1$ (where N is the number of treatments). The tabulated F value at this probability level, and at (1, 8) degrees of freedom (for variance ratio 1) and (1, 15) degrees of freedom (for variance ratio 2), are 5.317 and 4.543, respectively for GDG. Tabulated F values for GRG for both methods are 4.844 and 4.543, respectively.

Those effects with variance ratios greater than the tabulated values were deemed significant. It is clear that most of the effects identified as important by the normal plots (Figs. 1 and 2) also have variance ratios greater than the tabulated values from *F*-test. For GDG and GFG, the statistically significant effects (in order of importance) are **B > A > AB > C > BC > AC** (the effects have been described earlier). For GRG, the important effects are **B > A > AB > C**.

3.1. Five-variable model incorporating the rf component of Q-array voltage in LCMS 2010

The Q-array voltage on the LCMS 2010 has two voltage components: dc voltage and rf voltage. It is conceivable that both voltages may interact with each other and with other parameters in an ESI-MS experiment. A five-variable model was therefore designed to investigate the possibility of these interactions. It was found that there are several interaction terms resulting from the inclusion of the fifth parameter into the model as shown in the normal plots in Fig. 4a–c.

For all three analytes, Q-array dc voltage, Q-array rf voltage, spray voltage, and the two-term interactions between these parameters seemed to dominate the effects on analyte ESI response. A three-term interaction between all three parameters was also important as it ranks higher than both capillary temperature and capillary voltage. It is observed that in all the experiments performed in both four-variable and five-variable models, capillary voltage (CDL voltage in LCMS 2010) did not have much influence on analyte ESI response.

Another observation in the five-variable model is that the capillary temperature, as well as its interaction terms, lies closer to the center of the normal plots for GRG than for GFG and GDG. This is similar to the scenario in the four-variable model. This trend fur-

Table 8
Variance ratios of the treatment sum of squares to residual sum of squares (GRG).

Treatment Number	Treatment Sum of squares	Variance ratio 1 (Treatment SS/R1)	Variance ratio 2 (Treatment SS/R2)	Effects
1	184,403,826,647	768.676	516.306	1
3	24,790,397,533	103.337	69.410	**A**
2	104,088,503,756	433.887	291.434	**B**
6	13,801,902,842	57.532	38.643	**AB**
4	3,075,682,195	12.821	8.611	**C**
7	1,051,694,090	4.384	2.945	**AC**
9	2,025,878	0.008	0.006	**BC**
1	81,420,544	0.339	0.228	**ABC**
5	117,180,625	0.488	0.328	**D**
8	47,196,900	0.197	0.132	**AD**
10	980,597,910	4.088	2.746	**BD**
13	116,694,006	0.486	0.327	**ABD**
15	188,847,145	0.787	0.529	**CD**
12	28,928,262	0.121	0.081	**ACD**
14	13,722,085	0.057	0.038	**BCD**
16	10,569,001	0.044	0.030	**ABCD**

R1 = 239,897,859 (11 degrees of freedom); R2 = 357,159,915 (15 degrees of freedom).

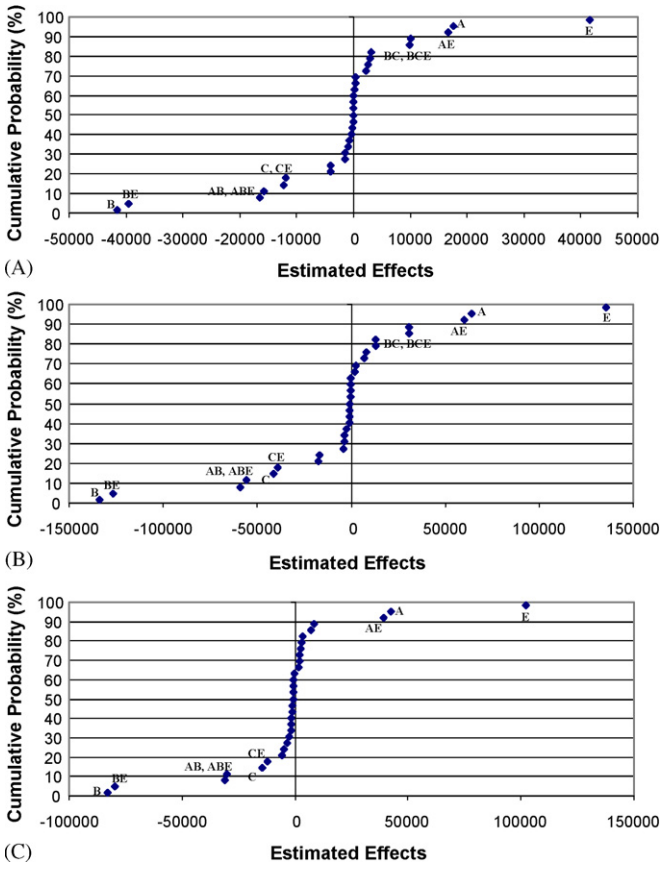

Fig. 4. Normal plots of effects for (a) GDG, (b) GFG, and (c) GRG (five variables on LCMS 2010); **A**, **B**, **C**, and **D** are as defined earlier while **E** is the rf component of Q-array voltage in the LCMS 2010. Two-letter and three-letter terms are interaction terms among the respective parameters.

ther corroborates the conclusion that capillary temperature has less effect on the ESI response of GRG (a basic peptide) than it has on GDG and GFG, which are acidic and neutral, respectively.

4. Conclusion

The previous discussions highlight the benefit of utilizing chemometrics for efficient experimental designs in mass spectrometry. We have demonstrated how simple factorial designs can be used to elucidate the influence of instrumental parameters on analyte ESI response. We have also shown how this basic technique can be used to identify otherwise unnoticeable interactions between two or more parameters. Both graphical and statistical methods were used to justify selection of important parameters. The findings in this study are very important and need to be given considerations in the design and optimization of mass spectrometric experiments on these instruments.

A good understanding of the way and manner in which instrument parameters influence analyte response in ESI-MS is essential in order to obtain optimum response and reproducible data. Particular attention should be paid to how parameters are changed from run to run, as possible interactions between parameters may lead to irreproducible results. The implication of inter-parameter interaction is that it becomes statistically impracticable to interpret the effect of one parameter independently of the other. Although most mass spectrometry instruments come with tuning software that can be used to optimize certain parameters prior to acquiring data, these softwares may not account for interactions between parameters that can only be changed by the user. An example is the case of LCQ Deca XP, where a strong interaction was found between capillary temperature and tube lens voltage. The tuning software will optimize the tube lens voltage at the temperature of the capillary at the time of tuning. However, this value will change (and may become less than optimum) when the capillary temperature is manually varied following tuning.

Having mentioned the remarkable performance of full factorial design in identifying important effects on analyte response, we should also note that this technique is limited to designs with few factors being investigated. The introduction of higher order interactions (e.g., three-term, four-term, etc.) as the number of factors increases renders the procedure cumbersome when used to study many parameters. Fractional factorial designs (that can efficiently reduce the number of experiments) can be used when the number of factors is large. However, important information about interactions may be lost when factors are confounded as is inherent to performing fractional factorial designs. Also, factorial designs are generally best for quick identification of important effects and not necessarily for optimizing the factors as they do not account for curvature in the levels of the factors. Other chemometrics tools such as central composite (or response surface) designs, simplex centroid, and simplex lattice designs are suggested for optimization.

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