MULTIVARIATE OPTIMIZATION OF INSTRUMENTAL PARAMETERS FOCUSED ON THE DETERMINATION OF PESTICIDES, HERBICIDES AND EMERGING POLLUTANTS IN ENVIRONMENTAL SAMPLES

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Abstract *— The persistence of pesticide and herbicide in the environment after the application is potentially harmful. Other compounds such as medication drugs can show the same problems. This persistence can be of some few days, many months or even more. As the knowledge advances, molecules with some special features were developed to be less harmful during its presence in any environment compartment. Recent analytical techniques (LC-MS/MS, for instance) are very interesting for the analysis of environmental samples in determining very low concentration compounds (≤µ.mL*$^{-1}$*). In this concentration level, the parameters optimization for instrumental operation is very important as small variations can mean a successful or failed analysis. In this work, results from a set of multivariate optimization of operational parameters of a liquid chromatograph mass spectrometer used to determine emerging pollutants in environmental samples are presented.*

Index Terms — Multivariate optimization, environment, emerging pollutants, LCMS/MS

INTRODUCTION

After the development of an Atmospheric Pressure Ionization, API, ion source [1] an old dream came true for the analytical chemists. The hyphenation LC-MS (liquid chromatography mass spectrometry) provided by the API device allowed a relatively simple coupling between these two techniques bringing ease in operation, sensitivity and stability in measurements, broader spectrum application, and high sample throughput. Nowadays, Electrospray Ionization, ESI, and Atmospheric Pressure Chemical Ionization, APCI, are the most common atmospheric pressure ionization sources. Despite technical aspects from one to another instrument supplier, inside the APCI, device Figure I, the phenomena that take place are the liquid sample nebulization (gas 1, G1) within a metallic probe and an ionic gas phase formation by applying a Corona discharge generated by a high current (needle current, NC) passing through a metal needle. In order to support the nebulizing process, another gas (auxiliary gas, G2) is blown within the probe surrounding the nebulized sample homogenizing the heat distribution and carrying the sample to the APCI device chamber.

In the path of produced ionic compounds into the interior of the mass spectrometer, a dry gas (curtain gas, CG) is also blown normal to the path completing the drying process. Although many other parameters can be set, these four (G1, NC, G2 and CG) are previously known as the most significant in the ion production in the APCI device. These parameters can be optimized by using software instructions and controls or, as the aim of the present work, applying external multivariate optimization. When the dynamics of a process is governed by several variables or parameters single or univariate sequential adjustment can fail mainly if the complexity of the relationship between them can not be entirely understood and/or controlled. Multivariate optimization is, in this case, a statistical approach that can be used to reach a set of values of optimized conditions.

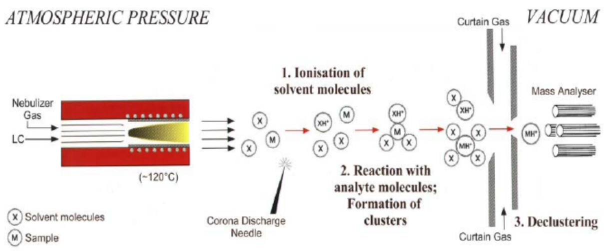

FIGURE I

ATMOSPHERIC PRESSURE CHEMICAL IONIZATION, APCI, MECHANISMS. COURTESY OF [2]

Examples of multivariate optimization application can be found in reducing emissions to the environment [3], in monitoring industrial processes [4], in meteorological

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concerns [4], in, of course, environmental chemometrics [4,6], and many others situations.

Environmental sample analyses are our main interest targeting information that can help the knowledge consolidation, can support the decision action, and can improve attitudes toward a better world. In this way, chemometrics can be seen as a tool to the analytical methodology used to obtain refined results. It is very common in environmental samples the need to measure tiny quantities of some compounds. Frequently, these minimal quantities produce very low signals comparable to background level and almost indistinguishable. In this situation, an improvement of 10 or even 5% in the signal measured by optimized equipment can make the difference in the sample evaluation.

Factorial Design

A proposal to use the multivariate optimization of the instrument conditions (LC-MS/MS) in the analysis of pesticides, herbicides and other compounds, such as those called emerging pollutants, is presented in this work.

A 2^4 factorial design was applied (see in the Experimental) giving a matrix of 16x16 for four factors and two levels. This matrix is also called contrast coefficient table, **X,** and contains the combination of the levels low and high. The effects calculation is performed by the product $\mathbf{X}^t\mathbf{y}$, a vector column, where **y** is the column matrix for the equipment response [7]. A division by 2^{k-1} for the effects and by 2^k for the average, where k is the number of factors, must be consider to obtain the effects.

Normal Graphic Analysis

Normal graphic analysis is used to have a qualitative or a behavior view of the effects. The concept is based on the cumulative probability of a random variable with a normal distribution of a bell shape that follows the function

$$f(x)dx = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma^2}} dx \qquad (1)$$

The discrete values of x are standardized to z values as in (2). An expected graphic z versus effects will give a straight line with a centre distribution in the zero of the absciss (effect), Figure III. Isolated points from this cluster will be evaluated as significant effects.

EXPERIMENTAL

Reagents

Misoprostol (7-[3-hydroxy-2-(4-hydroxy-4-methyl-oct-1-enyl)-5-oxo-cyclopentyl]heptanoic acid, Aldrich, >99%), Figure II, was selected to be used in this optimization as it is used as a medication for ulcer treatment and sometimes used to induce abortion. If water treatment plants are not efficient enough to withdraw this compound from the water supplied to human consumption, it will stay available until decomposition. Misoprostol is well stable in hot climate and under direct light. Of course we consider that, if present, the concentration will be very low. This approach put together misoprostol, other drugs and some pesticides and herbicides in the class of emerging pollutants many of them not already considered in the legislation. Purified water (Barnsted System) was used thoroughly.

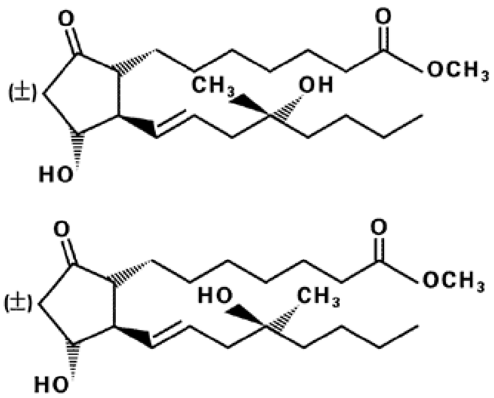

FIGURE II

MISOPROSTOL IS A COMPOSITION OF TWO DIASTEREOMERS OF PROSTAGLANDIN E1 ANALOGUE

Data Treatment

A factorial design with four factors (G1, NC, G2 and CG) and two levels (low and high), 2^4, was used to optimize the HPLC-MS/MS system. Sixteen experiments were carried out in conditions as shown in Table I.

TABLE I

EXPERIMENTAL PARAMETERS, LEVELS AND RESULTS FOR 2^4 FACTORIAL DESIGN IN APCI ION SOURCE OPTIMIZATION

Factors		(-)	(+)		
A – Curtain Gas, psi		15	25		
B – Needle Current, μA		5	6		
C – Gas 1, psi		60	70		
D – Gas 2, psi		20	30		
Run	**A**	**B**	**C**	**D**	**Response, Peak Area**
1	-	-	-	-	9.58×10^4
2	+	-	-	-	9.25×10^4
3	-	+	-	-	9.46×10^4
4	+	+	-	-	8.34×10^4
5	-	-	+	-	9.52×10^4
6	+	-	+	-	8.62×10^4
7	-	+	+	-	1.02×10^4
8	+	+	+	-	8.21×10^4
9	-	-	-	+	9.50×10^4
10	+	-	-	+	8.31×10^4
11	-	+	-	+	9.45×10^4
12	+	+	-	+	9.01×10^4
13	-	-	+	+	9.69×10^4
14	+	-	+	+	8.34×10^4
15	-	+	+	+	9.85×10^4
16	+	+	+	+	8.47×10^4

Peak area was chosen to calculate the effects. A software (Chemomatrix) developed by the Laboratory of Quantic Chemistry and Chemometrics, Chemistry Institute, Campinas University, UNICAMP, São Paulo, Brazil, was used to evaluate the instrumental output. Table I shows the four factors, two levels, number of experiments and the instrumental response for each set of conditions according to optimization achieved for misoprostol measurements in LC-MS/MS.

Instrumental

High Performance Liquid Chromatograph, HPLC, (Agilent 1100 Series, Agilent Technologies, Germany/Japan) associated to a mass spectrometer, MS, (API 2000, Applied Biosystems MDS/SCIEX, Canada), were used in this work. HPLC conditions such as, nature, composition and velocity of mobile phase were kept constant even for equipment software optimization as for external optimization. Reversed Phase chromatographic column C18 (50 mm) was use to enhance the signal intensity. Twenty microlitres were injected into the column. Mass spectrometer ion source temperature and HPLC sample flow were also kept constant at 350 ^{o}C and 0.5 mL.min^{-1}, respectively, in all experiments. The MS measurements were carried out in the positive ion mode having the m/z transition 400.4→365.4 been measured in the multiple ion monitoring (MRM) mode (Precursor ion and at least one fragment measurement/monitoring).

RESULTS AND DISCUSSIONS

Instrumental Optimization

The multivariate optimization is not only a characteristic of modern and complex analytical equipments. If one considers the traditional titration, several parameters (delivering frequency and time, concentration of titrated and the titrating, temperature, ionic force, indicator, interferents, and color change) are acting in same time in this not so simple system [8]. In an instrumental optimization, in addition of these parameters, some electronic ones have also to be considered.

Calculation of Effects

Although factorial design is very interesting to find relationship and interactions between the factors, in this work, the results will be discussed considering the effects from only single factors and not from the interaction of two or more factors. The reason is that the results showed that a single factor discussed in the following is the major one and no other, single or associated, is so significant. The effect like that (-2.60) arising from the ABC factors relationship and interaction, as seen in Table II, must be considered in more detail in another set of experiments.

The calculation showed that the curtain gas, CG, is the parameter that exhibits the most significant effect, Table II.

TABLE II

ESTIMATED EFFECTS AFTER PARAMETERS AND LEVELS INTERACTION

INTERACTION	EFFECT	INTERACTION	EFFECT
I	90500	BC	-75
A	-12125	BD	875
B	1475	CD	-1050
C	1250	ABC	-2600
D	550	ABD	2000
AB	-200	ACD	-825
AC	-1925	BCD	-825
AD	1225	ABCD	650

The negative signal means that curtain gas must be set preferable at low pressures (psi), Figure II. The physical interpretation for the high pressure of CG could be related to an intense disturbance in the ionic gas path and low efficiency in the desolvation process diminishing the desolvated ion transfer into the spectrometer, Figure 1. In the APCI device the process of formation of ionic gas follows the sequence: the first step of the nebulization occurs inside the nebulizer when the nebulized sample is heated; this so generated gas receive a Corona discharge (needle current) ionizing solvent molecules; a fraction of the solvent with the larger droplets is lost; the ionized and non-ionized solvent molecules and the analyte molecules form clusters that are swept into the spectrometer; these clusters are broken just in the first part of the spectrometer being the solvent molecules carried by the curtain gas out of the spectrometer. In the positive ion mode, a charge transfer process from solvent do analyte forms the $M+H^{+}$ charged particles, where M is the analyte. These charged particles enter the ion path, where the m/z discrete character will actuate to filter from undesirable compounds.

With much less pronounced effect, the auxiliary gas 2 has also to be set at low pressure as high values can induce local sample flow disturbance inside the probe. The explanation is that this gas does not directly participate in sample nebulization nor in ion production. One of the effects of high pressure values could be the decrease in the measurements precision, but, unfortunately, it was not registered. In the pressure range (60-70 psi) evaluated for the gas 1 (nebulization) one can observe an increasing response trend as pressure is increased. Although not very effective in this pressure range, a positive effect was expected as nebulization phenomenon is dependant of gas pressure. In decreasing pressure, the nebulization efficiency also decreases because the pneumatic gas expansion is less pronounced and larger droplets are produced. For very high pressures the gas velocity is too high and pneumatic gas expansion also can not nebulize efficiently the liquid sample. In addition, this range could be so close that the extension of the effect is limited or it is already near the optimum value.

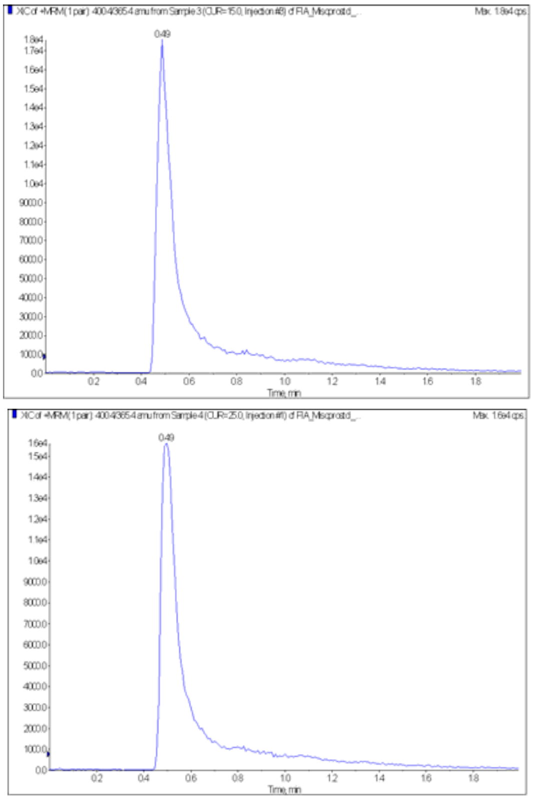

FIGURE II
THE SPECTRA SHOW THE INTENSITY OF THE TRANSITION 400.4→365.4 IN LOW (UPPER) AND HIGH (LOWER) VALUES OF CURTAIN GAS DURIGN THE OPTIMIZATION

As mentioned in Introduction, a current passed through a metal needle will promote the ionization of the solvent. Provided that the nebulization process is efficient, the small solvent droplets will be charged after the Corona discharge by the needle. The estimated effect obtained in the calculation indicates a low correlation towards the optimization. In fact, the effect value, although being the second higher is in same order of magnitude of G1 and G2. This value could be higher if the range (5-6 μA) was set broader.

Accumulated Probability

A graphical mode to represent the effects is to plot z ≈ N(0,1) values (2) versus the accumulated probability or effect, Figure III.

$$z = \frac{x - \mu}{\sigma} \qquad (2)$$

Where,
z = standard normal variable
x = single random sample (effect)
μ = sample mean (effects)
σ = standard deviation

The accumulated probability or effect is the mean of the sum of all effects considering the signal (level - or +) for each of experiment. In this study, the effect range is from -12125 to 2000. Numerically only, these values do not allow to extract a meaning of the effects. However, comparing the whole plots as in Figure III, one can see a behavior or the most significant effects.

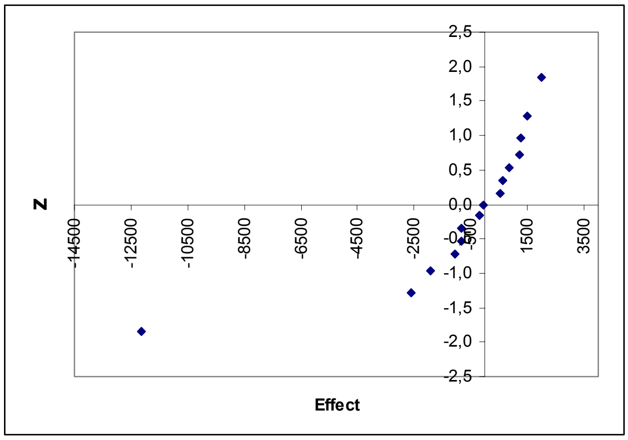

FIGURE III
ACCUMULATED PROBABILITY

As one can see, all but one plots are following a straight line behavior distributed around the zero. The isolated plot at left is due to the Curtain Gas, Table II.

As mentioned before, the straight line cluster around the zero means that all these factors and interactions have not so significant effect comparing to the effect of Curtain Gas. The plots distribution somewhat concentrated in the centre, that is, around zero and scattered in the line extremes means nothing more than a normal distribution in this case. The inclination of this line is roughly 45°. In a 90° line inclination, all the factors that constitute this line could be expected to have no effect to this system. Low angles, on the other hand, can mean experimental errors or the need to change levels, for instance.

Considering the factors used in this study and the

CONCLUSIONS AND REMARKS

Although the instrument optimization can be carried out by the controlling software, external multivariate optimization is a very interesting tool as effects and correlations can be quantitatively explained.

- During planning the schedule of factorial design the entire or at least part of the system has to be known to avoid the possibility of misinterpretation
- Not complex calculations are necessary to be done in order to evaluate the system.
- In order to establish the adequate set of factors (instrumental parameters), one have to have a prior knowledge of the system. In a practical way, trying to correlate any parameters can difficult the optimization.

- Too much parameters in an unique interaction matrix can be a heavy duty calculation.

For the optimization of the APCI device, among the four factors evaluated, this study allowed us to state that:

- The most significant factor was the curtain gas, CG, that influenced positively as its pressure increases. Although not evaluated in this study, the reasons may be related to a mechanism of gas desolvatation and ion transport
- The other three factors, nebulizer gas, auxiliary gas and needle current are not so important at least in those evaluated ranges
- Using the misoprostol, the results showed that even the software performed or the external multivariate optimization can be used to measure emerging pollutants with no significant response difference at intermediate analyte concentration

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