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A short reflection addressed to the analysts about the relationship between mass spectrometry and sample preparation

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Mass spectrometry is one of the most advanced technology applied to qualitative and quantitative analysis. The complexity related to this technique is not the only critical aspect to be considered in the routine of analysis. Actually the quality of analytical results is dependent on previous steps involved to the sample preparation, where well established protocols, conditions, solvents, dilutions, i.e. are crucial to have excellence in the analytical performance. All analysts involved must be qualified and recognize the importance of an integrative view about the sample, from different sources, complex or not, and the protocols to be applied sequentially in order to get prepared for a running through mass spectrometry or hyphenated system (LC-MS). In this work, through a simple and short reflection, the analysts are encouraged to reflect deeply about the importance of these two topics – mass spectrometry and sample preparation – to get excellence during the analytical routine.

Keywords: Mass spectrometry; Sample preparation; LC-MS

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Introduction

The analytical sciences as well as all areas of knowledge have reached an unquestionable advancement in terms of technology, materials and data interpretation. An analytical technique cannot be seen as a simple application of a principle in a sample. The integrative analysis is required for all steps of an experiment previously planned, in order to guarantee that any interference has a negligible influence in the outcomes. The obviousness here raised is not a certainty. If the simplicity involved in whatever the experimental step to be considered is not so important, the main objective will not be reached or partially reached. More than ever, establishing clear procedures and responsibilities can avoid many undesired mistakes, sometimes not detected during an analysis or often underestimated. A central point of reflection is often raised when an analysis is apparently failed in reliability: the analysis was really performed carefully as expected and necessary? Here, the answers would be easy, clear, and reflexive, simply pointing out many aspects concerning the own challenges of analytical sciences. Furthermore, validation expectations with precision and accuracy may lack the quality control the validation is seeking.

In the current days, among the great variety of analytical instruments, mass spectrometry has established a new horizon in terms of identification and quantification, in different fields of expertise, from clinical studies to food sciences, biotherapeutic discovery, pharmaceuticals, toxicology, environmental, among many others. The dynamism found in mass spectrometry has allowed a transformation in the capability for processing different types of samples, with a previous treatment or not. Considering the scenario as a whole, especially the potential of mass spectrometry (MS) to get a profound investigation in different matrices, some points are crucial to be discussed, especially the relationship between mass spectrometry and the sample preparation (SP). Although chromatography (i.e., gas or liquid chromatography) is as critical as SP prior to MS detection, we will focus on the influence of SP on MS based analyses. The complexity of matrices might be not a barrier for an analysis of excellence. Otherwise, a very simple sample (i.e., water) might be processed wrongly and the results are inadequate even applying the major technology available. Careful consideration of the analytical challenges is necessary prior to MS. Perhaps neither the sample preparation nor the instrument conditions are really responsible for the undesired results.

Sample preparation – importance for integrative analysis

The inherent importance of the sample preparation is highlighted, since the selectivity required for analysis is dependent about the types of matrices that will be analyzed. The information about that material has influence in which protocol would be applied, avoiding modification in the real composition and promoting an efficient elimination of interferences that are not welcome. The analytical success is not just related to the ability to measure a sample, but also with the capacity to recognize how eventual undesirable components can interfere in the analytical results, and how we can avoid the interferences from a simple or a complex composition. Modern sample

purification techniques, miniaturized, such as liquid-liquid microextraction (DLME) and microextraction by packed sorbents (MEPS), have been proposed alternatively to the classical liquid-liquid extraction (LLE) or solid phase extraction (SPE) (1,2). The gain in performance with novel materials and advanced instrumentation or automation is a reality in the experimental practices, even though the classical techniques by liquid-liquid extraction (LLE) or solid phase extraction (SPE) continues to be an excellent option, often associated to their low cost and high efficiency (2,3). Dependent on the complexity of the matrix, even dilution could be sufficient. The replacement depends on the real advantage brought up to the whole analysis. In addition, depending on the matrices and the compound characteristics (i.e., aqueous, lipid, protein, viscous, sticky), a simple treatment through a direct relation between solvents, such as LLE procedure, might be enough. Actually, independent of the procedure to be used, all techniques intended to be implemented routinely in a laboratory must be included in a standard operating procedure (SOP), preceded by a methods validation, and eventually tested for each type of matrix. These protocols are charged with being reliable for the measurement of analytes in a complex matrix. Even a simpler matrix may still require employing several steps with intense sample handling. Otherwise, if absent, the analytical procedure may not be acceptable for routine measurements, that would yield inaccurate and/or imprecise results.

In a relation between simple or complex, the final extract to be measured by MS detection must be compatible with the analytical instrument, since the interdependency between them is determinant for the best performance in terms of the analytical response. The instrumental behavior can be predicted from a detailed evaluation of sample solutions, extraction procedures and ultimately tuning the target compounds. Sometimes the complexity involved in an analytical run comes from some details that need to be considered when the samples are collected or initially prepared. Here, the importance of good practices applied to the collection, processing and storage of samples, i.e. biological, must be highlighted. Many pre-analytical aspects should be considered in order to have samples with quality and sufficient quantity. The specimen or biospecimen must be handled carefully by using recommended material and following quality protocols previously established (4). Among a large number of key points to be considered, the sample preservation is here raised due its importance for the integrity of the sample composition. Also related to this, some next steps such as the storage procedure, the initial solubilization, the critical weighing of a biological sample, or even the correct procedure of homogenization (i.e., tissue) has an importance that invariably define the quality of the results obtained from the intended analysis (4,5). Procedures must be put into practice after a critical evaluation (i.e., method validation) in terms of reproducibility and accuracy, not excluding other parameters naturally considered in an analytical performance. As mentioned, SOPs for different types of matrices are essential and must be detailed as much as necessary, since the composition of matrices have variations when undergoing an extraction procedure or a sequential dilution. It can be highlighted here that the analytical performance has a key to get a reliable performance over the analysis: being critical and demanding in all steps, it doesn't matter how simple it is.

Mass spectrometry – a performance dependent on sample preparation

In a search for an integrative way, the analytical instrument comes up as an essential tool to promote the complete knowledge about the sample. Actually, MS-based analysis has become the gold standard for drug discovery and development, diagnostics, investigations, and monitoring. It is important to highlight its versatility, which can be seen by the increase of publications over time. Over the last 25 years, mass spectrometry has gained importance as being an alternative for monitoring different samples from different matrices, even with a different level of complexity. Actually, hyphenated systems GC-MS and LC-MS are traditionally employed, since the chromatographic separation has many advantages that are favorable for MS performance. The increased selectivity, allied to the method sensitivity, allows the simultaneous analysis of many compounds in a single sample. The undesired co-elution is avoided and the spectra are collected individually, becoming viable the analytical response based on peak detection. Consequently, the structural information is specific for each compound to be identified or quantified. Even in a continuous development for getting more sensitivity and accuracy, mass spectrometry, here including the hyphenated systems, is not an isolated technique. It depends on the quality of the previous treatment that was carried out. The results are more valuable if the analytical procedure was set up adequately, considering all variables have influence, even in a non-critical step, like a dilution, a volume measuring, and a choice for an extraction solvent or a homogenizer procedure.

In the current days, the mass spectrometers operate through the more advanced technology available, which means rapid analysis, maximum of sensitivity and reproducibility of response from sequential analysis. Taking into account the principle of compound ionization and ionic mobility in a charged electrically quadrupole, the modern equipment is developed to get a more efficient ionization with precision in the mass-to-charge (m/z) ratio searched (6,7). Being with the more advanced technology do not always means efficiency in the analysis, at least in some cases. Just remind the importance of the quality of sample preparation procedure employed. Qualitatively, the analytical responses desired may have small variations in the full scan profile obtained, having influence on the full view of mass-to-charge (m/z) signals. In these cases, it is usual to expect a more pronounced signal-to-noise relation, which could be avoided with an efficient clean-up procedure or with a simple well established set-up procedure. Instrumental settings usually worked like mass range, energy (voltage), gas desorption temperature and

collision energy are responsible for the fine tuning during the analytical run (7), and can be well explored when the analyst have experience and knows exactly which compounds or matrices are being investigated. Also, aspects related to the analytical concentration (ppm, ppb) or the ionic power employed to the ionization are considered during set up procedure, and can prevent problems over the analysis. Naturally, it is expected a more complexity in the mass spectra from a biological sample, for example, which it does not mean necessarily difficulties to interpret the results. In the same way, simple matrices are interpreted easily if any interference not expected is present. Sometimes, depending on the compound and its reactional profile, variations not expected in the mass spectra are present and can conduct for an erroneous interpretation.

In terms of quantification, the relation between ion abundance and precursor/product ion from a specific m/z signal through MRM acquisition is a pattern well established on QTOF and TripleQuad system. The capability for selecting the exact mass searched on the channels might be considered the key tool for getting good results in terms of precision and accuracy. It is important to take into consideration that the type of matrix is really determinant for having the expected concentration for the investigated substance. A standardized pretreatment on the matrix sample is essential to reproduce their real composition, regardless the contaminants that were intentionally eliminated. Aspects like matrix effect and sample clean-up efficiency are mandatory to get reliable results, since the sensitivity of mass spectrometry can reveal some unexpected signals, which will have a direct influence on the m/z signals pattern from a precursor and fragmentation profile. Considering the quantitative vias, for obvious the validation protocols must be applied, with control samples being analyzed over the analytical run. The comparison between the samples, spiked or not, blank or added of an internal standard will be determinant for getting acceptable analytical results.

In the case of simple matrices, a low interference from sample is expected, and a simple preparation can be considered, sometimes including a single step of solubilization or applying a liquid-liquid extraction. Despite of the great probability for having a good performance in terms of analytical results, it does not mean a total absence of data variation. If the sample preparation is not well conducted or studied, the responses from mass spectrometry loss in data reproducibility, quantitatively and qualitatively. It is important to take into account that mass spectrometry works in a high level of sensitivity, being able to detect any variation that interfere on m/z signal. Here, the analysts need to have attention with the variations source and with the possibility to come from some instrumental settings, which is frequently associated to absence of preliminary testing. When variations come from sample, the signal reproducibility presents variations in the response, such as signal abundance, interference on peak integration, peak area response, unexpected noise, etc. The analytical response is proportional to the real composition of a sample, and this certainty must be used intentionally either for an investigation about undesired compounds (interferences, impurities, contaminants) or a quantitative/qualitative approaching with a focus on a target substance (drug or biomolecule).

In a critical view about the differences between mass analyzers, combined to the properly detection system, the analytical performance must be evaluated according to the goals planned to be reached. Data like mass accuracy and mass resolution have an important role in the quality of the results, and must be continuously reported during the analytical run. The choice for either one must take into account the kind of information to be obtained, such as metabolomic or proteomic, identification or quantification. It is important to consider the versatility naturally existent in mass spectrometry, respecting the limits imposed by sample characteristics. It means that the analysts get have interchangeably between different hybrid systems, which combine ionization, mass analysis and ion detection. The excellence in the performance will be proportional to the whole comprehension about each point that has influence in the results, not restricted to mass spectra, m/z signal and ion abundance. Once more, the concept of integrative analysis can be mentioned. To be more practical, in experiments focused on search for an unknown substance, whose propose is to identify properly, the mass accuracy has an essential role, as much that a QTOF system is recommended, since the mass error tends to be lower. Otherwise, if the main goal is just quantity known compounds in simple or complex matrices, a TripleQuad system has the excellence to perform it with more sensitivity. Actually, the balance between mass accuracy, mass resolution and sensitivity must be considered not just in theory, but through application of a complementary data analysis, either through specific mass spectrometry software or through a statistical processing.

In addition, the type of sample is determinant, since efficiency of ionization system must consider chemical characteristics about the molecules, whether low or high molecular weight, base or acid, polymerization powerful, peptide chain, solid or liquid state, etc. Choices involving chemical solvents, naturally considered for a chromatographic analysis, must be added in capability to promote an efficient ionization, whatever system was selected as the more appropriated to the sample in analysis. A simple transition between positive or negative ionization modes can be useful when the composition is unknown or just to get the more abundance in the m/z signals. The more information about sample composition is available, among them majority compounds, chemical class and ionizable groups, a better prediction can be done previously in order to get good responses from the analytical instrument. Beyond the information about the sample composition, it is desired a previous knowledge about ionization potential from different agents available for a mass spectrometry analysis. Although essential, actually it is something sometimes neglected, and has a deep influence in the quality of the results, either qualitative or quantitative. Another point to consider is the quality of the solvent to be used, which must have a high purity level, compatible to the mass spectrometer and its potential to measure

impurities in the lower level. Repetitive errors or inadequate results can come from the quality of the materials employed or even from an inadequate procedure, which can be exemplified by a glass washing procedure inadequate or by absence of MS grade water and solvents.

In conclusion, our reflection here is raised up for sharing experiences and for contributing with some critical aspects that are present in the routine analysis concerning mass spectrometry and sample preparation. Be complex or simple depends on how we face the challenges. Analytically, the analysts are the own responsible for making simple what seems complex in a first view. The key for that is knowledge, updating and integrative view.

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