Abstract

Mass spectrometry (MS) is a powerful detection technique that has become very important in several chemical disciplines for detection of both small molecules (environmental pollutants, small metabolites) and large biomolecules (proteins, peptides). The "heart" of the mass spectrometer is the analyzer, that uses electrical or magnetic fields, or combination of both, from the region where they are produced, to a detector, where they produce a signal which is amplified. This element separates the gas phase ions according to their m/z (mass to charge ratio) value.

MS is mostly used coupled to high performance liquid chromatography (HPLC) or ultra- high performance liquid chromatography (UHPLC) with an atmospheric pressure ionization (API) interface between the LC and MS. The most important API technique is electrospray ionization (ESI), with a wide range of application from small molecules to large molecules such as proteins or polymers.

Complex matrix, as environmental samples, biological fluids, food or tissue extracts, are not usually compatible with MS detection without extensive sample preparation. In facts, complex matrix could contains substances that can cause the detector signal to decrease or increase for a selected analyte compared to the signal from the same compound in a standard solution. ESI is the ionization technique in which this phenomenon is most common because in the ESI process ions compete for ionization/desorption. Matrix effect is observed in ESI when compounds co-elute with the analyte of interest: this affects sensitivity, linearity, accuracy, precision and the limit of detection (LOD).

Furthermore, the target compound(s) are often present at concentrations lower than their detection limits and require a preliminary concentration step. Therefore, the first step of an analysis is usually some kind of sample pretreatment to improve both the selectivity and sensitivity of the subsequent detection. Many techniques are available for this purpose, the suitability of which depends primarily on the physical state of the sample, e.g. solid-phase extraction (SPE) for liquid and pressurized-liquid extraction for solid samples. Proper sample preparation is critical for MS analysis, because the quality and reproducibility of sample extraction and preparation significantly impact results from MS instruments.

In this Ph.D. research project improvements in the following extraction techniques were evaluated:

Solid-liquid extraction using pH controlled subcritical water to extract very low solubility

Solid phase extraction by molecularly imprinted polymers (MISPE) to selectively extract a class of natural compounds from beverages

Solid phase extraction (SPE) by porous graphitic carbon (PGC) coupled on line with LC/MS detection in order to extract anionic compounds from environmental water samples

For solid-liquid extraction was used pure water at elevated temperature as an extraction fluid for non-polar analytes. Pressurized hot water extraction (PHWE, also known as subcritical water extraction) is currently considered one of the most interesting recent developments in extraction technology. Unfortunately, the applicability of this technique could be limited by the very low water solubility of the target compounds, even at high temperature. In this study the scope of broadening the applicability of PHWE by adjusting the pH of the water used in extraction is dimostrate for the extraction of curcumin (which has very low water solubility) from untreated turmeric rhizomes.

In order to improve the selectivity of solid phase extraction a molecularly imprinted polymer was prepared using (E)-resveratrol as template and was evaluated for multicomponent multiclass analysis of polyphenolic compounds in complex matrices such as natural and alcoholic beverages. Chromatographic evaluation of the polymer exhibited high selectivity for (E)- resveratrol and its structural analogues, quercetin, and other flavonoids. An analytical procedure based on molecularly imprinted solid phase extraction (MISPE) and high HPLC coupled to UV detector was developed and validated for determination of (E)-resveratrol and quercetin in wine and fruit juice samples. The specific binding capacity of the MIP was estimated as 80 μg g^{-1} polymer by the cartridge test. MISPE sample pretreatment allows an excellent sample cleanup, enormously decreasing the number of coextracted potentially interfering compounds. Under the described conditions, by extracting 2 mL samples a clean extract is obtained and (E)-resveratrol and quercetin could be easily identified at concentration levels of, respectively, 1.5 and 7.0 μg L^{-1}.

Finally, the peculiar characteristics of porous graphitic carbon was exploited to develop a method for the determination of perfluoroalkyl acids (PFAS). Because their ability to persist in the environment, bioaccumulate and interfere with the endocrine system these compounds are considered"emerging pollutants". It has been also demonstrated their animal mutagenicity, carcinogenicity and teratogenicity. The feasibility of using PGC for both online solid phase extraction and LC separation of these compounds was evaluated in combination with tamndem mass spectrometry detection in order to obtain structural information and/or achieve better selectivity and sensitivity for quantitative purposes. The optimized analytical procedure was applied to the analysis of real samples, such as drinking and ground waters.