Sonic Spray Ionization (SSI) in LC-MS analysis of Endocrine Disruptors in the Environment

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Introduction: Wildlife and humans are exposed daily to many different compounds that, even in small doses, can disrupt the endocrine system and are often referred to as endocrine disruptors (EDCs). The range of substances reported to cause endocrine disruption is very divers, and includes both natural and synthetic chemicals.

Liquid chromatography coupled to mass spectrometry (LC/MS) is becoming a standard in routine environmental analysis. Recently, sonic spray ionization (SSI), a novel atmospheric pressure ionization technique (API) has been introduced. In SSI, a sonic nitrogen gas flow coaxial to a fused silica capillary gives nebulization and ion formation. The aim of the present work was to develop an optimized LC/SSI-MS method in combination with an off-line solid-phase extraction for the analysis of 20 EDCs in water samples. During method development particular attention was given to the ionization performance of SSI in comparison with traditional ion sources, such as pneumatically assisted electrospray (ESI) and atmospheric-pressure chemical ionization (APCI). The method was validated by determination of the following parameters: recovery, limits of detection and quantitation, linearity, and within-day and between-day reproducibility.

Materials and Methods: Separations were performed on a LaChrom² separation module (Merck, Darmstadt) using a Purospher® Star RP-18 column (55 x 2 mm I.D., particle size 3 µm, Merck, Darmstadt). Mobile phases consisted of water and acetonitrile used in a gradient profile at a flow rate of 250 µL/min. Mass spectral analyses were carried out on an M-8000 ion trap MS (Merck-Hitachi, Darmstadt), equipped with SSI, ESI and APCI interfaces. Extractions were performed manually using cartridges packed with 200 mg of C18 material (Isolute™, Sopachem). SPE columns were conditioned using 6 mL of methanol and HPLC grade water. At a rate of approximately 5 mL/min the sample (50 mL) was passed through the column. The column was washed with 5 mL of water and dried under vacuum for 5 min. Three milliliter of a mixture of dichloromethane/iso-propanol (80:20, v/v) were used to elute the target compounds. The extract was dried under a gentle stream of nitrogen in a TurboVap² LV Evaporator (Zymark, Hopkinton, MA), reconstituted in a 100-µL mixture water-acetonitrile (80:20, v/v), and 50 µL were injected.

Results: The SSI spectra were compared to those obtained by ESI and APCI. All ionization sources gave comparable spectra. However, as can be seen from Figure 1, illustrating estriol as a representative mass spectrum, SSI and ESI spectra were more prone to the formation of solvent cluster-ions as compared to APCI, resulting in more
“grass” or background noise together with occasional spikes in the mass chromatogram. Presumably, this is the result of a difference in ionization phase. SSI, like ESI, is a liquid phase ionization technique. Ionization occurs directly from the solution. The sampling orifice receives sample ions, solvent vapor, and also partially solvated sample ions that have not yet lost the last solvent molecules. APCI shows less cluster ions because chemical ionization occurs in the gas phase. Sample and solvent are fully evaporated prior to ionization.

Despite the difference in amount of chemical background for each of the ion sources, the intensity of the molecular ions was comparable. Consequently, when calculating quantitative results using extracted ion chromatograms, the background ions could be eliminated and signal-to-noise ratios of the peaks ameliorated. This led to corresponding limits of detection (LODs) for all interfaces (3.0 – 11.5 ng/L).

Limits of quantitation (LOQ) were established between 9.9 and 38.0 ng/L. Satisfactory recoveries were obtained ranging from 76.3 up to 113.4 %. Linearity (R²>0.987) was demonstrated using 8 calibration points ranging from 10 to 10000 ng/L. Within-day, between-day reproducibility (n=5), were investigated at three different concentration levels (100, 500 and 1000 ng/L) and ranged from 3.3 to 16.5 % and 7.6 to 19.2 %, respectively.

**Conclusion:** The spectra obtained with SSI are comparable to those obtained with the traditional interfaces like ESI and APCI. The lower level of energy makes the sonic spray ionization process more prone to solvent cluster formation. However, this did not compromise the sensitivity of the method. The SSI interface proved to be applicable to the routine analysis of endocrine disruptors in water samples.

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