

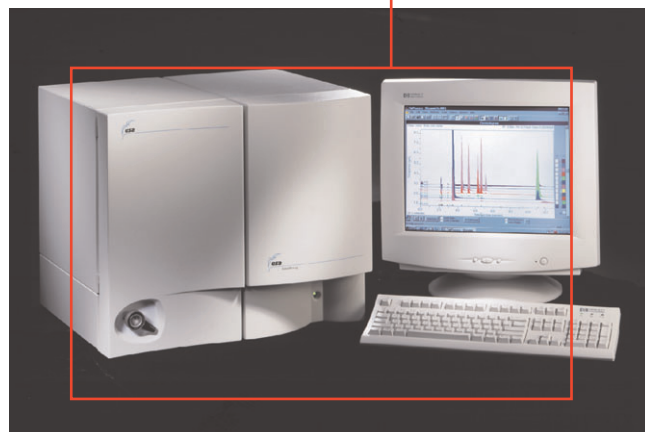
Coulometric Assisted Ionization of Compounds: Expanding the Utility and Applications of Mass Spectrometers

The Widespread Use of Mass Spectrometers

Accurately measuring and identifying small molecules is a key function of many Bioanalytical groups. Quite often, the analytical method of choice for analyzing unlabeled compounds is HPLC, Mass Spectrometer or LC-MS. These techniques offer the capability to detect, quantify, and identify many types of unlabeled compounds in relatively small quantities. Bioanalytical techniques such as LC-MS are most heavily used in pharmaceutical companies in support of Chemistry, Compound Logistics, or ADME-Tox groups to characterize the identity and purity of a given compound or to measure and identify the products after some chemical or biological reaction has occurred on a parent compound.

Today, Bioanalytical groups are under tremendous pressure to provide quick, and accurate assessments of an ever increasing number of samples. This analysis can be complicated by the inherent chemical properties of different compounds. In many cases, compounds may be difficult to ionize for detection by MS, or may be difficult to visualize due to ion suppression effects. While there are several methods to try and overcome these issues, a significant percentage of compounds remain difficult, if not impossible, to analyze by Mass Spectrometry.

To help address some of these issues in bioanalysis, ESA has applied its unique product line and expertise in Coulometric Systems to greatly expand the capabilities of researchers using Mass Spec or LC-MS. ESA systems can provide researchers with additional capabilities to compliment their MS or LCMS operations. ESA systems have been used to oxidize or otherwise react "problem" compounds to enable them to be seen in an MS system. In addition, by using some of the unique properties of the Coulometric System, it is possible to mitigate the ion suppression effects of some compounds.



More Information from Bioanalytical Analyses

In today's Mass Spectrometry laboratory the ability to make quick and accurate measurements and identification of a compound is paramount. To this end, the ESA electrochemistry systems (CoulArray® detector) is capable of:

- *Easy Integration into Almost Any Bioanalytical System*
- *High Throughput Applications*
- *Simple Operation and Instrument Control*
- *Robust, Low Maintenance Performance*

This enables Bioanalytical researchers to:

- *Ionize "Difficult" Compounds to Enable Their Detection by Mass Spectrometry*
- *Mitigate Ion Suppression Effects*
- *More Accurately Identify Parent Compounds and any Resulting Reaction Products or Metabolites*
- *Easily Integrate with Most Existing Bioanalytical and Data Analysis Systems*

ESA *Ionization* Expanding Mass Spec Utility

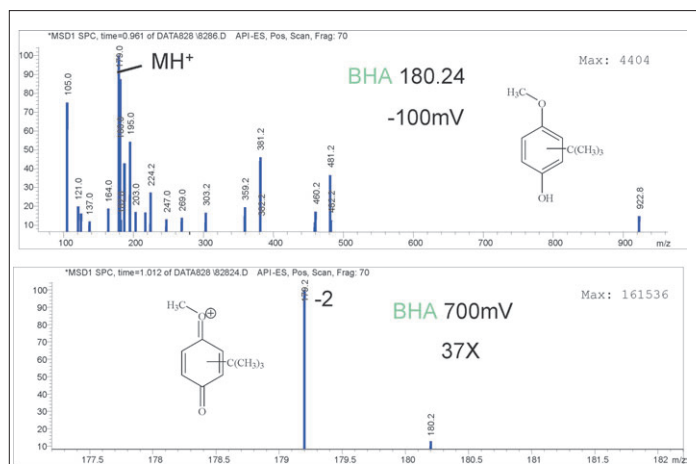


Figure 1: Ionization of BHA with an EC System. BHA was chromatographed on an LCMS system without an integrated EC system (Top Figure). Note the poor signal from BHA in the MS. The bottom panel shows BHA after reaction in an EC system. BHA is oxidized into an ionized species easily detected by MS.

Compound	Abundance Increase*	Ion
Estradiol	3	NH ₄ ⁺ adduct
2-Hydroxyestradiol	8	NH ₄ ⁺ adduct
4-Hydroxyestradiol	7	NH ₄ ⁺ adduct
2-Methoxyestradiol	3	NH ₄ ⁺ adduct
4-Methoxyestradiol	6	NH ₄ ⁺ adduct
1-Naphthol	10	Dimer*
Aniline	74	2M-3 ⁺
Phenol	72	2M-3 ⁺
BHA	37	M-2 ⁻
BHT	4.6	M-2 ⁻
Eugenol	44	M-2 ⁻
o-Toluidine	22	2M-3 ⁺

Figure 2: Mass Abundance Increase of Several Compounds with EC-MS. A series of compounds was reacted with a Coulometric System prior to MS analysis. The relative abundance of many of these compounds was greatly increased by ionizing the compounds through various reactions.

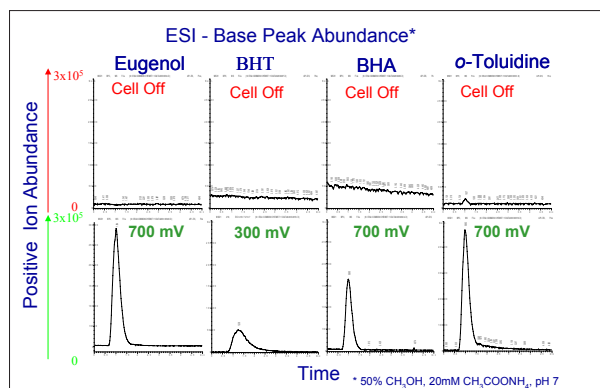


Figure 3: EC Assisted Ionization: Several types of compounds were passed through a Coulchem III equipped LCMS system with the EC cell inactive (top row) or active (bottom row). Note the lack of detection of compound in LCMS without EC assistance.

ESA coulometric systems represent a major leap forward in bioanalytical operations. By using an ESA system in a bioanalytical analysis scheme, researchers can identify a greater number and broader range of compounds with mass spec detection. Contact your local ESA representative to learn more about this and other exciting ESA Applications.

Ordering Information

CoulArray, Model 5600A, sixteen channels
Organizer with Temperature Control
Optional Gradient Upgrade
Pump, Model 582
Autosampler, Model 540 MT

Part No.

70-4335
70-4340T
70-4051
70-4050
70-5110



ESA Inc., 22 Alpha Road,
Chelmsford, MA 01824-4171
T: (978) 250-7000 F: (978) 250-7090
www.esainc.com
An ISO 9001 Company

ESA Analytical, Ltd., Brook Farm
Dorton, Aylesbury, Buckinghamshire
HP18 9NH England
01844 239381
Fax 01844 239382

70-6084P
Rev A