Bioanalysis

Internal extractive electrospray ionization-mass spectrometry: a powerful platform for bioanalysis

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Analytical mass spectrometry (MS) is used for the qualitative and/or quantitative analysis of biological samples (e.g., blood, urine, saliva, plant tissue, animal tissue, etc.) due to its high specificity and high sensitivity [1]. However, conventional MS techniques including LC–MS and GC–MS require time-consuming sample preparation, which reduces the analysis speed.

The throughput of analytical MS has been dramatically enhanced by ambient ionization techniques, which create analyte ions in the open-air environment with no/minimal sample preparation [2]. To date, most efforts have been made to charge neutral molecules on surfaces, resulting in quantitative information after mass analysis but with special units associated with area [3]. Uniquely, internal extractive ESI (iEESI) allows the ultratrace analytes to be quantified with bulk molecular units such as μg/kg [4], which make iEESI–MS a promising method for bioanalytical applications, including clinical analysis, food safety, plant science, environmental analysis, with high accuracy and speed. In fact, analytes in biological fluids can be selectively embedded in a solid substrate to form an artificial bulk sample, which is then analyzed by iEESI–MS with enhanced sensitivity and accuracy [5]. Reducing the matrix effect in this way makes the iEESI–MS a reliable tool for the detection of ultratrace bioactive compounds in real-world biological samples. Herein, the principles of iEESI–MS and the applications of iEESI–MS are discussed, with the emphasis on clinical analysis.

Principle & essential features of iEESI–MS

Since introduced in 2013 [6], iEESI–MS and its variants have been rapidly developed [7]. In principle, neutral analytes distributed inside a bulk volume can be extracted from the bulk sample using a charged solvent to form an ionic mobile phase, which moves forward inside the bulk sample underneath the surface to the ion entrance of the mass spectrometer, producing analyte ions in a nanospray fashion for postmass analysis [6]. Typically, a capillary guiding a charged solution (e.g., methanol, water, acetonitrile, etc.) is directly inserted in the bulk sample, inside which the solvent spreads to extract the analytes distributed in the 3D volume (ca. 20 mm^3). The analyte solution is moved along the electric field gradient inside the bulk sample toward the mass spectrometer, and then forms a stable electrospray plume to produce analyte ions in front of the MS inlet.

Typical features of iEESI–MS are summarized as follows [4,6]:

- Rapidness: Molecular information in the whole-volume sample can be straightforward and obtained within 1 min without either mashing or grinding of the sample;
- Sensitivity: The analytes are extracted directly from a 3D volume of sample with a small volume of charged solution, making the analyte levels in the electrospray solution higher than those for the surface analysis process;
- Selectivity: The analytes can be selectively detected in a simple way by altering the extraction solution;
- Accuracy: iEESI–MS allows for the analysis of the sample in nature to obtain abundant molecular information, which may be lost during sample pretreatment by other analytical methods;

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- Automation: Precision-machined iEESI is also a semiautomatic sampling device that can provide high accuracy and precision.

Applications of iEESI–MS in bioanalysis

By now, iEESI–MS has been applied to fields including, but not limited to, metabolomics analysis [8–13], food analysis [14–16], clinical analysis [17–19], environmental analysis [5], etc. Here, we will focus on the application of iEESI–MS in clinical analysis.

Tissue analysis

Accurate negative margin assessment and complete tumor excision are highly desirable across cancer surgeries. The golden method of surgical margin evaluation is immunohistochemistry protocols. Unfortunately, the golden method takes time (>30 min), which is terrible for surgery. iEESI–MS directly detects the compounds inside tissue, and provides abundant molecular information for tissue assessment and diagnosis. In a typical analytical process [17], mass spectrometric data of normal tissue and cancer tissue identified by immunohistochemistry are collected first by iEESI–MS with high rapidness (<20 s per sample), followed by statistical treatment with statistical software such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to build a calibration model, which is then used for tissue assessment and diagnosis of unknown samples. High diagnosis accuracy above 99% was obtained by iEESI–MS.

Recently, the quantitative performance of iEESI–MS was further improved by developing a disposable iEESI device [4], which allowed precise sampling of volume-defined tissue (e.g., pork lung, plant tissue, etc.) with high reproducibility and accuracy. Various β-agonists, such as salbutamol, tulobuterol, clenbuterol, ractopamine, etc. in pork sample were quantified by iEESI–MS with a lowest detection limit of 0.002 μg/kg. This is the first time for ambient MS to report the analyte levels with the units of μg/kg. Single sample analysis required approximately 1 min with high accuracy (above 90%) and was validated by LC–MS and GC–MS. The improvement of iEESI–MS quantitative performance could further promote the application of iEESI–MS in clinical analysis.

Biological fluids analysis

In addition to tissue analysis, iEESI–MS can also be used for the analysis of biological fluids such as blood [14] and urine [5]. Adsorbing material (e.g., functionalized graphene oxides, molecularly imprinted material, etc.) is first used to enrich analytes in matrix, which then acts as the artificial bulk sample for iEESI–MS analysis. For example, 1-hydroxypyrene (the most widely used biomarker of internal dose of polycyclic aromatic hydrocarbons exposure) in undiluted human urine sample was enriched with polypyrrole-coated Fe_3O_4 nanocomposites and rapidly detected by magnetic solid-phase extraction coupled with iEESI–MS [5]. To further improve the sensitivity, C18 particles were used to capture the 1-hydroxypyrene from a large volume of urine (100 ml), providing an ultrahigh sensitivity (LOD of 0.015 pg/l).

Currently, early diagnosis of ovarian cancer by iEESI–MS is being investigated using blood samples [20]. Whole blood samples from the cancer patients and healthy individuals were analyzed by iEESI–MS employing C18 particles as adsorbing material, resulting in significant information for diagnosis. The findings show iEESI–MS is a useful tool for rapid analysis of whole blood samples for cancer diagnosis.

Conclusion & future perspective

iEESI–MS is a technique elaborately developed for whole-volume sample analysis, which takes advantage of its high sensitivity, selectivity, accuracy and rapidness. Differing in the samples and sampling means, iEESI–MS loaded with targeted analytes [5] has been developed for specific bioanalysis applications, including but not limited to the analysis of tissue, blood and urine.

In the future, iEESI–MS will be further developed in several aspects, including: accuracy: the iEESI device will be further optimized to improve the quantitative performance for both biological tissue and fluid analyses; rapidness: the analysis process will be further enhanced and a standardized analytical protocol will be developed to shorten the analysis time; and field application: by coupling with miniature mass spectrometer, dedicated iEESI–MS instruments will be developed for rapid field analysis in food safety, cancer boundary identification and the early diagnosis of cancer, etc. For example, an instrument is being developed in our group for rapid surgical margin evaluation during surgery.

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