

Method and Apparatus for Detecting an Analyte via Flow Injection Analysis

Technical Field

The present invention relates generally to a method of detecting an analyte via flow injection analysis and an apparatus that carries out the method, and more particularly relates to a method and apparatus for detecting a small molecule that does not make use of chromatographic separation for detection.

Background

Liquid chromatography–mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). MS is an analytical technique where molecules are ionized and the resulting ions are identified by mass-to-charge ratio. Coupling MS to separation techniques (e.g. high-performance liquid chromatography, HPLC; ultra-performance liquid chromatography, UPLC) allows for the characterization of multiple analytes in complex mixtures. Coupled chromatography-MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides spectral information that may help to identify (or confirm the suspected identity of) each separated component. LC-MS is also appropriate for metabolomics because of its good coverage of a wide range of chemicals. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, pharmaceutical, agrochemical, and cosmetic industries. Since the early 2000s, LC-MS (or more specifically LC-MS-MS) has also begun to be used in clinical applications.

However, LC-MS has several limitations, such as lack of flexibility, low throughput and time consuming. Current LC-MS methods require careful selection of the appropriate liquid chromatography column and mobile phases for each different analyte of interest, as well as careful calibration of the mass spectrometer to isolate and identify the analyte of interest. In order to analyze a second different analyte on the same instrument,

one or more of the column, the mobile phases, or the mass spectrometer settings generally need to be changed and optimized by the LC-MS technologist. Because of the time and technical complexity of such changes, random access analysis of individual samples is costly and inefficient. So instead, samples flagged for analysis for the same or similar analytes are generally grouped into large batches and run together. While this arrangement may reduce the number of changes to the LC-MS system set-up from run-to-run, it significantly increases the time to obtain result and decreases assay flexibility for each sample.

An alternative analytical approach is flow-injection mass spectrometry (FI-MS), in which the analytes are directly injected into the mass spectrometer ionization source without prior chromatographic separation. Flow injection analysis (FIA) is an automated method in which a sample (analyte) is injected into a continuous flow of a carrier solution, which mixes with other flowing solutions (reagents) before reaching a detector. This technique is straightforward with very low sample and reagent consumption and high sampling frequencies. FI-MS via modern high-resolution mass spectrometers such as Orbitrap and time-of-flight (ToF) was shown to enable the determination of hundreds to thousands of m/z features in biological samples. However, matrix effects limit the sensitivity of FI-MS.

There are needs to provide an analysis method with high specificity, high throughput, low detection limit and time and cost efficiency.

Summary

To overcome the deficiencies as discussed above, the present invention provides a method for detecting an analyte in a sample via flow injection analysis, wherein the method comprises: a) extracting the analyte from the sample by a selective extraction that
5 targets the analyte to obtain an extracted analyte; b) purifying the extracted analyte by removing impurities to obtain a purified analyte; and c) detecting the purified analyte by injecting the purified analyte into flow stream of an analytical instrument, wherein the detecting of step c) does not include chromatographic separation.

10 Another aspect of the invention provides a method for detecting an analyte in a sample via flow injection analysis, wherein the method includes a) extracting the analyte from the sample by a solid phase conjugated with an adsorbent that targets the analyte; b) purifying the extracted analyte by a solid phase extraction column to remove impurities to
15 obtain a purified analyte; c) detecting the purified analyte by injecting the purified analyte into flow stream of an analytical instrument, wherein the detecting of step c) does not include chromatographic separation, and further wherein the method optionally includes a step of derivatizing the extracted analyte following the step a) but prior to the step b), and the method also optionally includes a step of quantifying the purified analyte by deconvoluting the peaks obtained by the analytical instrument after flow injection of the
20 purified analyte.

Another aspect of the invention provides an apparatus that carries out the method discussed herein.

25 It has been surprisingly discovered that the method according to the present invention allows analysis with high specificity and increased flexibility and enables multiplexing capabilities. Since the method according to the present invention adopts flow injection analysis, throughput is increased as it does not require chromatographic separation and each sample can be quantified in preferably less than 30 seconds. Owing to
30 the exclusion of chromatographic separation in detection (i.e. step c), the amount of time required for detection per sample can be reduced. The present invention also allows the reduction in matrix effect due to the adoption of selective extraction followed by purification, resulting in less suppression and interference of analytes.

Other example embodiments are discussed herein.

Brief Description of Drawings

5 Figure 1 illustrates the workflow of sample detection according to an aspect of the present invention.

 Figure 2 illustrates the analysis of Estradiol (m/z 271.2 > 145.0) by the method according to one aspect of the present invention, represented by a diagram of absolute
10 intensity (CPS) vs. retention time (minutes). Arrow is pointing to estradiol analyte peak as detected by software.

 Figure 3 illustrates the analysis of Testosterone (m/z 289.2 > 96.9) by the method according to one aspect of the present invention, represented by a diagram of absolute
15 intensity (CPS) vs. retention time (minutes). Arrow is pointing to testosterone analyte peak as detected by software.

 Figure 4 illustrates the analysis of Cortisol (m/z 363.2 > 121.1) by the method according to one aspect of the present invention, represented by a diagram of absolute
20 intensity (CPS) vs. retention time (minutes). Arrow is pointing to cortisol analyte peak as detected by software.

 Figure 5 illustrates the peak area ratio and concentration as a log-log calibration curve in HP04 (human plasma).
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 Figure 6 illustrates the peak area ratio and concentration as a log-log calibration curve in HS03 (human serum).

 Figure 7 illustrates the analysis of matrix blank which did not undergo selective
30 extraction (immunoaffinity) but underwent purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Arrow is pointing to a predicted analyte peak as detected by software, due to interferences.

Figure 8 illustrates the analysis of matrix blank which underwent both selective extraction (immunoaffinity) and purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes).

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Figure 9 illustrates the analysis of cortisol in a PBS matrix which underwent selective extraction (immunoaffinity) but did not undergo purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Arrow is pointing to a cortisol analyte peak as detected by software.

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Figure 10 illustrates the analysis of cortisol in a PBS matrix which underwent both selective extraction (immunoaffinity) and purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Arrow is pointing to a cortisol analyte peak as detected by software.

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Figure 11 illustrates the analysis of cortisol in a plasma matrix which did not undergo selective extraction (immunoaffinity) but underwent purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Arrow is pointing to a predicted analyte peak as detected by software, due to interferences.

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Figure 12 illustrates the analysis of cortisol in a plasma matrix which underwent both selective extraction (immunoaffinity) and purification via solid phase extraction using FI-MS, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Arrow is pointing to a cortisol analyte peak as detected by software.

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Figure 13 illustrates the analysis of cortisol in human serum with lower limit measuring interval (LLMI) at a) 100LLMI (50ng/mL), b) 50LLMI (25ng/mL), c) 10LLMI (5ng/mL), d) 5LLMI (2.5ng/mL) and e) LLMI (0.5ng/mL) by the method according to one aspect of the present invention, represented by a diagram of absolute intensity (CPS) vs. retention time (minutes). Shaded area represents the cortisol analyte peak as detected by software.

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Figure 14 illustrates the fitted line plot of e.g. cortisol (peak area against concentration (ng/mL)).

Detailed Description

Reference will now be made in detail to certain embodiments of the disclosed subject matter. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not
5 intended to limit the claims to the disclosed subject matter.

Throughout this document, values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or
10 sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. For example, a range of “about 1-about 100 μ L” or “about 1-100 μ L” should be interpreted to include not just about 1 to about 100 μ L, but also the individual values (e.g., 1, 2, 3, 4, 5...10, 11...98, 99, and 100 μ L) and the sub-ranges (e.g., 2-99, 3-87, 10-90 μ L, etc.) within the indicated range. The statement “about X to Y” has the same
15 meaning as “about X to about Y,” unless indicated otherwise.

As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to "a bead" encompasses a single bead as well as two or more beads, and the like. It is understood that
20 any term in the singular may include its plural counterpart and vice versa.

As used herein, the terms "for example," "for instance," "such as," "e.g.," or "including" are meant to introduce examples that further clarify more general subject matter. Unless otherwise specified, these examples are provided only as an aid for
25 understanding the applications illustrated in the present disclosure, and are not meant to be limiting in any fashion.

The term “about” as used herein can allow for a degree of variability in a value or range, for example, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%,
30 within 4%, within 3%, within 2%, or within 1% of a stated value or of a stated limit of a range, and includes the exact stated value or range.

In one aspect, the present invention provides a method for detecting an analyte in a

sample via flow injection analysis, wherein the method comprises: a) extracting the analyte from the sample by a selective extraction that targets the analyte to obtain an extracted analyte; b) purifying the extracted analyte by removing impurities to obtain a purified analyte; and c) detecting the purified analyte by injecting the purified analyte into
5 flow stream of an analytical instrument, wherein the detecting of step c) does not include chromatographic separation.

Another aspect of the invention provides a method for detecting an analyte in a sample via flow injection analysis, wherein the method includes a) extracting the analyte
10 from the sample by a solid phase conjugated with an adsorbent that targets the analyte; b) purifying the extracted analyte by a solid phase extraction column to remove impurities to obtain a purified analyte; c) detecting the purified analyte by injecting the purified analyte into flow stream of an analytical instrument, wherein the detecting of step c) does not include chromatographic separation, and further wherein the method optionally includes a
15 step of derivatizing the extracted analyte following the step a) but prior to the step b), and the method also optionally includes a step of quantifying the purified analyte by deconvoluting the peaks obtained by the analytical instrument after flow injection of the purified analyte.

20 Matrix refers to the components of a sample other than the analyte. In one aspect, the sample is a biological sample. In a further aspect, the biological sample comprises serum, plasma, blood or body tissue. In a further aspect, the sample is selected from a group consisting of serum, plasma, blood and body tissue. In another aspect, the sample comprises a matrix of phosphate buffered solution. In a further aspect, the sample is a
25 complex sample e.g. serum, plasma, etc.

In one aspect, the sample is divided into more than one aliquot and the extraction is conducted separately for each aliquot. The term “aliquot” refers to a known amount of a homogeneous material, assumed to be taken with negligible sampling error.

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In one aspect, the method according to the present invention requires only a small amount of sample for analysis. In another aspect, the sample required for the analysis is in a volume of less than about 100 μL (e.g. about 1-100 μL); or less than about 50 μL (e.g.

about 10-50 μ L).

In one aspect, the analyte is a small molecule with a molecular weight of less than about 1000 Da, e.g. about 50-900 Da, 100-900 Da, 100-800 Da, 500-900Da, 200-400 Da,
5 100-300 Da. In a further aspect, analyte can be protein, peptide, hormone, compound or other small biochemical molecules.

Step a) - Extraction through selective extraction

In step a) of the method according to the present invention, the analyte is extracted
10 from the sample by a selective extraction that targets the analyte to obtain an extracted analyte. "Selective extraction" refers to a mechanical recycling method in which specific materials or substances are recovered from solutions containing multiple materials and contaminants.

In one aspect, the selective extraction as described in the present invention can be
15 any selective extraction technology that can selectively extract the analyte from the sample. Examples of selective extraction method include solid phase extraction (SPE), electrophoretic separation, microwave accelerated selective Soxhlet extraction (MA-SSE), selective pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE), etc.
20 The selective extraction method is selected based on the physical and chemical properties of the analyte so as to ensure that the analyte is extracted from the sample/matrix.

In one aspect, for step a), the selective extraction is conducted by a solid phase
25 conjugated with an adsorbent that targets the analyte and extracts the analyte from the sample to obtain the extracted analyte. In one aspect, the solid phase is a magnetic, paramagnetic or supra-paramagnetic bead. In one aspect, the adsorbent includes antibody, aptamer or molecularly imprinted polymer. In one aspect, the selective extraction is conducted by a solid phase bead conjugated with an adsorbent that targets the analyte and extracts the analyte from the sample to obtain the extracted analyte. In one aspect, the
30 solid phase bead is a magnetic, paramagnetic or supra-paramagnetic bead.

In one aspect, the analyte is a biomolecule (e.g. protein, peptide, lipids, fatty acids, glycolipids, sterols, monosaccharides, vitamins, hormones, neurotransmitters, etc.), and

immunoaffinity solid phase extraction is preferably adopted for step a). Immunoaffinity solid-phase extraction technique is based upon a molecular recognition mechanism. In one aspect, the high affinity and the high selectivity of the antigen-antibody interactions allow the specific extraction of the analytes of interest even with a low concentration/amount in one step. In pharmaceutical and biological fields, where most often matrices are complex and analytes at trace-levels, this approach constitutes a unique tool for fast and solvent-free sample preparation.

In one aspect, the selective extraction method is immunoaffinity solid-phase extraction, wherein an adsorbent conjugated with a solid phase (such as magnetic beads) is capable of binding one or more biomarker of the analyte.

Step b) - Purification of the extracted analyte

In step b), the extracted analyte is purified by removing impurities to obtain a purified analyte. In one aspect, the impurities can be salt or other compounds that would interfere the detection of the analyte in an analytical instrument. In one aspect, purification can be conducted by any purification method that is suitable for the analyte or the detection of the analyte.

In one aspect, the extracted analyte is purified by a solid phase extraction cartridge. Solid-phase extraction (SPE) is an adsorption and extraction technology that is realized by the affinity between a specific constituent of the components and a solid phase extraction agent in the solid phase extraction cartridge, so as to achieve separation of the specific constituent. SPE uses the affinity of solutes dissolved or suspended in a liquid (known as the mobile phase) for a solid through which the sample is passed (known as the stationary phase) to separate a mixture into desired and undesired components.

In another aspect, the purifying of step b) is conducted with a solid phase extraction cartridge by one loading step, two washing steps, and two eluting steps to obtain an eluate, and the eluate is treated by one drying step and one reconstituting step to obtain a solution containing the purified analyte, and the solution containing the purified analyte is injected directly into the flow stream of the analytical instrument for analysis without liquid chromatographic (LC) separation.

In one aspect, optionally, the method includes a step of derivatizing the extracted analyte between the steps a) and b). Derivatization is a technique used in chemistry which converts a chemical compound into a product (the reaction's derivate) of similar chemical structure, called a derivative. Generally, a specific functional group of the compound participates in the derivatization reaction and transforms the educt to a derivate of deviating reactivity, solubility, boiling point, melting point, aggregate state, or chemical composition. Resulting new chemical properties can be used for quantification or separation of the educt.

The derivatization of the analyte of interest shifts the analyte out of the region where it might potentially overlap with other biological agents found in the sample. Thus, during detection, other biological molecules or contaminants which would ordinarily interfere with the accurate quantification of the analyte of interest are detected at a significantly different range of the spectrum (or eluted completely), allowing the accurate quantification of the analyte of interest.

Chemical derivatization of the analyte can be used to increase the detection sensitivity of some analytes. Using derivatization will change the structure of the analyte such that it is more ionizable in the mass spectrometer, resulting in increased sensitivity and better detection of the analyte.

In one aspect, the derivatization of the analyte is an optional step conducted between the steps a) and b), i.e. derivatization is not required for all analytes. In one aspect, the derivatization method for flow injection analysis includes catalytic methods, photochemical derivatization, electrochemical derivatization, discolorimetry, analyte volatilization, etc.

Step c) - Detection of the purified analyte (Flow injection analysis (FIA))

Flow injection analysis (FIA) is an automated method of chemical analysis in which a sample is injected into a flowing carrier solution that mixes with reagents before reaching a detector. In step c) of the method according to the present invention, detection of the analyte is conducted by injecting the purified analyte into flow stream of an

analytical instrument. In one aspect, examples of the analytical instrument include spectrophotometry, fluorescence spectroscopy, atomic absorption spectroscopy, mass spectrometry, etc.

5 In a preferred aspect, the analytical instrument for the flow injection is mass spectrometer. In one aspect, the detection of the analyte takes a run time of less than about 2 minutes (e.g. 0.05-2 minutes), or less than about 1 minute (e.g. 0.05-1 minute); or about 0.5 minute (e.g. 0.05-0.5 minute). In one aspect, the analyte detected is in a concentration of 0.5-500 ng/mL.

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 In one aspect, the method further includes deconvoluting the peaks obtained by the analytical instrument so that the analyte can be quantified. In one aspect, the peaks obtained by the detection via flow injection analysis are then deconvoluted and quantified using a proprietary software. Post-acquisition data deconvolution is able to differentiate
15 complex compounds and tailor calibration curves for quantification.

 Another aspect of the invention provides an apparatus that carries out the method of the present invention. In a preferred aspect, the apparatus includes a first apparatus for extracting step, a second apparatus for purifying step, and a third apparatus for detecting
20 step. In a further aspect, the apparatus includes an apparatus for other optional steps.

 Advantageously, the present invention unexpectedly provides a method for detecting the analyte by selective extraction method (such as solid phase beads, including magnetic beads conjugated with an antibody that targets the analyte), followed by solid
25 phase extraction (such as solid phase extraction column) and then flow injection analysis (such as directly injecting the solution containing the analyte into the flow stream of MS without LC separation) which allows the detection of analyte in a small sample size in a shorter time and thereby provides increased flexibility and enables multiplexing capabilities.

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 Low sensitivity caused by the matrix effect is well-known as a drawback for FI-MS. Advantageously, the adoption of the selective extraction method (preferably, immunoaffinity solid phase extraction for biomolecule) coupled with purification in the

present invention sufficiently reduces the matrix effect that is generally severe in complex sample matrices (e.g. biological, mineralogical, or environmental samples). The method according to the present invention enables the reduction in the matrix effect and allows a small amount of sample to be used, resulting in less suppression and interference of analytes and allowing for flow injection mass spectrometry analysis of the analyte.

Advantageously, owing to the exclusion of chromatographic separation in the present invention, it allows reducing the amount of time required per sample.

The method of the present invention offers fast detection run time, high specificity, high sensitivity, high flexibility, and high throughput and allows smaller sample size and the detection of small molecules.

EXAMPLES

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

Example 1

Sample Extraction Steps (Immunoaffinity SPE)

- a. Resuspend conjugated magnetic beads via vigorous shaking.
- b. Extract 50 μ l of solution for 0.25 mg of beads. Add 500 μ l of PBS.
- c. Separate beads with a magnet for 1 min. Remove the supernatant
- d. Wash beads with 1mL of PBS. Separate beads magnetically and remove the supernatant. Repeat once.
- e. Mix beads with 50 μ L of sample solution. Add 150 μ L of H₂O. Leave for 10 min with shaking at RT.
- f. Separate beads with a magnet for 1 min. Remove the supernatant. Wash beads with 500 μ L of PBS. Repeat once.
- g. Add 100 μ l elution solution (70% MeOH/H₂O) and leave for 5 min with shaking at RT.
- h. Separate beads with magnet for 1 min and collect the supernatant.
- i. Repeat steps 7 & 8
- j. 200 μ L of supernatant will then go through sample purification.

Sample derivatization step (optional)

Prior to sample purification, the supernatant is derivatized by conventional derivatization method based on the structure and the properties of the analyte.

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Sample Purification Step (Solid Phase Extraction)

- a. Dilute 200 μL of supernatant with 800 μL of H_2O
- b. Condition SPE column with 1 mL MeOH, then 1 mL H_2O
- c. Load resulting sample (1 mL) onto SPE column
- 10 d. Wash with 1 mL H_2O , then 1 mL 40% MeOH
- e. Elute with 100 μL 100% MeOH, then elute again with 100 μL 100% MeOH
 - a. Collect both elutes together
- f. Dry resulting elutes under N_2 , and reconstitute in 50 μL 70% MeOH
- g. Add 10 μL of 10 ng/mL internal standard to the resulting 50 μL of sample
- 15 h. Final solution = 60 μL

Flow injection-mass spectrometry (FI-MS)

- a. Final solution is loaded into autosampler vial and placed into autosampler
- b. Select appropriate chromatography method
- 20 c. Select appropriate mass spectrometry method
- d. Create batch list for acquisition
- e. Run data acquisition on proprietary software

Data Analysis Steps (Post acquisition analysis)

- 25 Simple linear and quadratic fits are not sufficient to plot the calibration curve for the invention.

- a. To determine calibration, extract peak area ratio across 7 concentrations of analyte
- b. To obtain linear calibration, log transform both peak area ratio and concentration
- c. Plot peak area ratio and concentration as a log-log calibration curve
- 30 d. Example calibration curves in HP04 and HS03 are shown in Figure 5 and 6, respectively.

A general schematic of the workflow of the present invention is shown in Figure 1.

Example 2 - Analysis of Estradiol

The analysis of estradiol is conducted using the method according to the present invention as described in Example 1, wherein the sample containing estradiol is extracted
5 by immunoaffinity solid phase extraction using magnetic beads conjugated with anti-estradiol antibody and is purified by SPE column. The analysis of estradiol is shown in Figure 2. It shows that estradiol can be analyzed by the method of the present invention with a retention time of 0.186 minute.

10 Example 3 - Analysis of Testosterone

The analysis of testosterone is conducted using the method according to the present invention as described in Example 1, wherein the sample containing testosterone is extracted by immunoaffinity solid phase extraction as described in Example 1 using magnetic beads conjugated with anti-testosterone antibody and is purified by SPE column.
15 The analysis of testosterone is shown in Figure 3. It shows that testosterone can be analyzed by the method of the present invention with a retention time of 0.201 minute.

Example 4 - Analysis of Cortisol

The analysis of cortisol is conducted using the method according to the present
20 invention as described in Example 1, wherein the sample containing cortisol is extracted by immunoaffinity solid phase extraction as described in Example 1 using magnetic beads conjugated with anti-cortisol antibody and is purified by SPE column. The analysis of cortisol is shown in Figure 4. It shows that cortisol can be analyzed by the method of the present invention with a retention time of 0.211 minute.

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Example 5 – Analysis of matrix blank with and without undergoing selective extraction (immunoaffinity SPE)

A matrix blank solution which is only purified by the sample purification steps as described in Example 1 (SPE) is analyzed by FI-MS/MS. The matrix blank does not go
30 through the sample extraction step. The result of the analysis is shown in Figure 7.

A matrix blank solution which is extracted by the sample extraction step (Immunoaffinity SPE) and purified by the sample purification steps (SPE) as described in

Example 1 is analyzed by FI-MS/MS. The result of the analysis is shown in Figure 8.

By comparing the FI-MS diagram of the matrix blank which did not undergo selective extraction but underwent purification (Figure 7) and the FI-MS diagram of the
5 matrix blank which underwent both selective extraction and purification (Figure 8), it can be seen that high matrix background is observed when selective extraction is not conducted, indicating the importance of selective extraction to the invention in terms of eliminating the matrix effect.

10 **Example 6 - Analysis of sample in simple matrix (PBS) with and without undergoing sample purification (solid phase extraction)**

A sample solution in PBS matrix which is only extracted by the sample extraction steps (immunoaffinity SPE) as described in Example 1 but not purified by the sample purification steps is analyzed by FI-MS/MS. The result of the analysis is shown in Figure
15 9.

A sample solution in PBS matrix which is extracted by the sample extraction steps (immunoaffinity SPE) followed by purification by the sample purification steps (SPE) as described in Example 1 is analyzed by FI-MS/MS. The result of the analysis is shown in
20 Figure 10.

By comparing the FI-MS diagram of the sample solution in PBS which underwent selective extraction but did not undergo purification (Figure 9) and the FI-MS diagram of the sample solution in PBS matrix which underwent both selective extraction and
25 purification (Figure 10), it can be seen that sample purification is essential to the invention as it reduces ion suppression of the analyte that occurs due to interferences present post-extraction.

Furthermore, it is observed that the sample in PBS matrix which has undergone
30 both selective extraction and purification provide FI-MS diagram with less background noise.

Example 7 - Analysis of sample in a complex matrix (plasma) with and without

undergoing selective extraction (immunoaffinity)

A sample solution in plasma matrix which is only purified by the sample purification steps (SPE) as described in Example 1 is analyzed by FI-MS/MS. The sample does not go through the sample extraction step. The result of the analysis is shown in Figure 11.

A sample solution in plasma matrix which is extracted by the sample extraction steps (immunoaffinity SPE) followed by purification by the sample purification steps (SPE) as described in Example 1 is analyzed by FI-MS/MS. The result of the analysis is shown in Figure 12.

By comparing the FI-MS diagram of sample solution in plasma matrix which did not undergo selective extraction but underwent purification (Figure 11) and the FI-MS diagram of the sample solution in plasma matrix which underwent both selective extraction and purification (Figure 12), it can be seen that selective extraction successfully extracts analyte from complex matrix as well as removes matrix background, indicating the importance of selective extraction to the invention in terms of eliminating the matrix effect.

Furthermore, it is observed that the sample in plasma matrix which has undergone both selective extraction and purification provides FI-MS diagram with less background noise.

Example 8 – Comparison between LC-MS and present invention

A sample solution containing cortisol in human serum has been analyzed by the method according to the present invention using FI-MS/MS. The result of the analysis of cortisol in human serum with lower limit measuring interval (LLMI) at 100LLMI (50ng/mL), 50LLMI (25ng/mL), 10LLMI (5ng/mL), 5LLMI (2.5ng/mL) and LLMI (0.5ng/mL) by the method according to the present invention is shown in Figure 13. Deconvolution and quantification has been conducted based on the result obtained by the method according to the present invention. Figure 14 provides the fitted line plot for showing the relationship between the peak areas and the concentration.

The run time for LC-MS analysis takes about 8 minutes while the run time for the Examples of the present invention takes about 0.5 minutes. Thus, to run 45 injections, LC-MS analysis takes about 6 hours while the analysis of the present invention takes
5 about 1.5 hour. It is surprisingly discovered that the sensitivity and specificity of the analysis obtained by the present invention are comparable to that of LC-MS, which is able to extract cortisol from a small amount of sample (50 μ L) and able to quantify range of 0.5 – 500 ng/mL.

CLAIMS

1. A method for detecting an analyte in a sample via flow injection analysis, wherein the method comprises:
 - 5 a) extracting the analyte from the sample by a selective extraction that targets the analyte to obtain an extracted analyte;
 - b) purifying the extracted analyte by removing impurities to obtain a purified analyte; and
 - c) detecting the purified analyte by injecting the purified analyte into flow stream of an analytical instrument,
- 10 wherein the detecting of step c) does not include chromatographic separation.
2. The method of claim 1, wherein for step a), the selective extraction is conducted by a solid phase conjugated with an adsorbent that targets the analyte and extracts the analyte from the sample to obtain the extracted analyte.
- 15 3. The method of claim 2, wherein the solid phase is a magnetic, paramagnetic or supra-paramagnetic bead.
4. The method of claim 1, wherein for step a), the selective extraction is conducted by a solid phase bead conjugated with an adsorbent that targets the analyte and extracts the analyte from the sample to obtain the extracted analyte.
- 20 5. The method of claim 2 or 3 or 4, wherein the adsorbent includes antibody, aptamer or molecularly imprinted polymer.
- 25 6. The method of any one of the preceding claims, wherein for step b), the extracted analyte is purified by a solid phase extraction cartridge.
7. The method of any one of the preceding claims, wherein the method further includes a step of derivatizing the extracted analyte between the steps a) and b).
- 30 8. The method of any one of the preceding claims, wherein the purifying of step b) is conducted with a solid phase extraction column by one loading step, two washing steps,

and two eluting steps to obtain an eluate, and the eluate is treated by one drying step and one reconstituting step to obtain a solution containing the purified analyte, and the solution containing the purified analyte is injected directly into the flow stream of the analytical instrument for analysis without liquid chromatographic separation.

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9. The method of any one of the preceding claims, wherein the analytical instrument is mass spectrometer.

10. The method of any one of the preceding claims, wherein the analyte is a small molecule with a molecular weight of less than about 1000 Da.

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11. The method of any one of the preceding claims, wherein the sample is a biological sample.

12. The method of any one of the preceding claims, wherein the sample is selected from a group consisting of serum, plasma, blood and body tissue.

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13. The method of any one of the preceding claims, wherein the method further includes deconvoluting the peaks obtained by the analytical instrument so that the analyte can be quantified.

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14. The method of any one of the preceding claims, wherein the sample required for the flow injection analysis is in a volume of about 1-100 μL ; or about 10-50 μL .

15. The method of any one of the preceding claims, wherein the analyte detected is in a concentration of 0.01 – 10000 ng/mL.

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16. The method of any one of the preceding claims, wherein the analyte is detected and quantified in about 0.05-2 minutes, or about 0.05-1 minute; or about 0.05-0.5 minute.

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17. An apparatus for carrying out the method of any one of the preceding claims.