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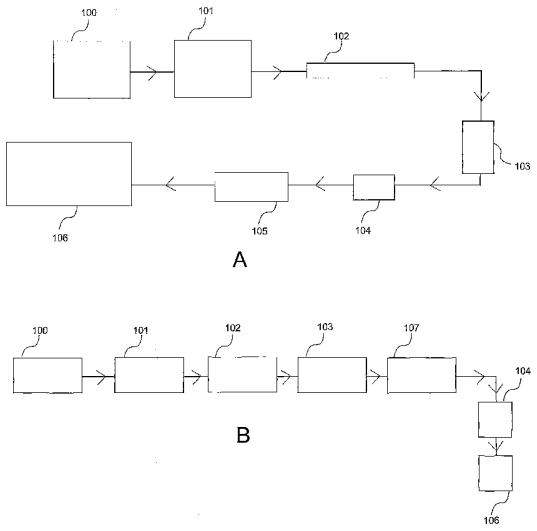
(54) Title: IMPROVEMENTS TO LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY IN THE INVESTIGATION OF SELECTED ANALYTES



(57) Abstract: Apparatus and method for the oxidative or reductive processing of analytes, in particular nitric oxide analytes. The apparatus and method comprises liquid chromatography coupled to mass spectrometry wherein an intermediate redox means is configured to change an oxidation state of the analytes to enhance their detection in the mass spectrometer. In particular, the redox means is configured to oxidise or reduce analytes processed by the liquid chromatograph prior to processing by the mass spectrometer whereby the more detectable analytes can be quantitatively investigated based on a difference in their charge density or polarity. In a specific implementation, the present invention is configured for the processing of nitric oxide derived analytes providing investigation and analysis of enzymic activity, in particular nitric oxide sythase activity.

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IMPROVEMENTS TO LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY IN THE INVESTIGATION OF SELECTED ANALYTES

Field of the Invention

The present invention relates to apparatus and method for the redox processing of analytes, and in particular although not exclusively, to the assay of metabolites of nitric oxide.

Background to the Invention

The investigation and analysis of enzymic activity commonly involves a qualitative and quantitative assessment of the analytes involved during and resulting from the enzyme activity.

Within the art, gas chromatographic coupled to mass spectrometric methods for the processing of metabolites has emerged as a useful tool for the determination of enzymic activity. In particular, Tsikas *et al* [Journal of Chromatography B, 742 (2000) 143-153] assessed nitric oxide synthase activity in vitro and in vivo using gas chromatography coupled to mass spectrometry.

Using the metabolites of nitric oxide synthase as an example involving catalytic oxidation of L-arginine to nitric oxide (NO), the inventors have identified various problems associated with prior art metabolite processing.

Nitric oxide is a small molecule which acts as a signaling molecule in the human body, one of its major roles being the control and specifically the promotion of blood vessel expansion. Nitric oxide is generated in the body from L-arginine, via the enzyme nitric oxide synthase, the free nitric oxide in solution being rapidly oxidised to nitrite (NO_2^-) and then to nitrate (NO_3^-). Moreover, NO may also form complexes with thiol groups of proteins or small molecules to form nitrosothiols.

Because of the number of diseases in which altered NO metabolism is suspected, there is an urgent need to develop methods for its analysis in human body fluids. The short half-life of NO means that direct measurements of NO are only possible with selective electrodes, which are not easy to use or very reliable quantitatively. Accordingly, most measurements of NO are made by assay of the metabolites nitrite and nitrate, and a number of methods for the analysis of these have been developed. A significant problem exists with such methods in that they cannot distinguish between nitrite/nitrate derived from NO and nitrite/nitrate derived from other sources, including dietary intake. In particular, enzymic activity requires a study of both nitrite and nitrate, nitrite being the primary metabolite, due to the ready oxidation of nitrite to nitrate, for example in catalytic degradation. Therefore, quantitative assessment of the metabolites requires processing of nitrite and nitrate at their respective concentrations.

A solution to the problem of distinguishing the target metabolised NO from, for example, nitrite or nitrate generated from dietary intake, is to infuse a labelled form of L-arginine and to follow the rise and fall of the labelled metabolites. The only atom that can be labelled is the nitrogen that is removed from the L-arginine and which is metabolised through NO to nitrite and then nitrate. The established method involves labeling the L-arginine precursor with ^{15}N. The detection method must then be capable not only of determining nitrite and nitrate, but also quantitatively discriminating between the two isotopic forms, which means that the detection method must be mass sensitive.

Horstmann *et al* [NITRIC OXIDE: Biology and Chemistry vol. 6, No. 2, pp. 135-141 (2002)], have shown that laser magnetic resonance is plausible to provide such a distinction however a more popular method is to use mass spectrometry. The well established mass spectrometry method involves gas-isotope ratio mass spectrometry wherein the analytes are converted to gas (first ammonia then nitrogen) and analysed in a specialist isotope ratio mass spectrometer. Numerous disadvantages are associated with this method including primarily the sample processing time which in general, takes seven

days. Additionally, the equipment is expensive and not widely available ultimately providing a technique which is impractical.

Alternative mass spectrometric methods, attempting to reduce sample processing time and using more readily available instruments have been proposed involving conversion to nitrite first, followed by chemical derivatisation to yield volatile nitro-organic compounds which can then be determined using gas chromatography coupled to mass spectrometry. Such methods require the use of an isotopically-labelled internal standard. The major problem associated with chemical derivatisation is the creation of uncertainty surrounding the chemical conversions leading to unwanted side-reactions, impurities, reduced yields and ultimately a decrease in the sensitivity and accuracy of the analytical method. What is required therefore is a coupling of liquid chromatography and mass spectrometry so as to avoid or reduce intermediate apparatus and processing steps.

One problem with electrospay ionisation mass spectrometry is the observed relative insensitivity of the spectrometer to nitrite when compared to nitrate in similar concentrations. At present, nitrite separated from nitrate using the liquid chromatograph is not detectable at high sensitivity, particularly where a conductive suppressor is used to reduce salt concentration and subsequent interference from other adducts.

What is required therefore is apparatus and method for the quantitative analysis of analytes configurable for high sensitivity detection and analysis involving analytes both in their highest, most stable and most detectable oxidation state and less stable analytes of relative lower oxidation state. This represents at least one aim of the present invention.

Summary of the Invention

The inventors have identified a requirement for an accurate, specific and highly sensitive analytical tool for the investigation and determination of analytes

including both stable metabolites in their highest oxidation state and less stable analytes of lower oxidation state. Particularly, the inventors provide coupled liquid chromatography and mass spectrometry configured for the processing of analytes to overcome the problems associated with known methods and apparatus. The inventors provide an analytical tool configured for the separation, detection and quantitative analysis of analytes based on analyte charge density or polarity.

By including an in-line oxidative or reductive treatment, such that previously poorly detected, unstable analytes of low or intermediate oxidation states are oxidised or reduced to their more stable oxidation states, enhanced sensitivity and detection is achieved. In turn, quantitative analysis of a full range of target analytes in serum and other similar matrices is now possible. Additionally, nitric oxide donating drugs can be detected along with the nitric oxide metabolites derived therefrom.

Specifically, the inventors provide apparatus and method for the determination of nitric oxide synthase activity involving a separation of nitrite from nitrate, both being detected at high sensitivity ultimately providing a determination of the ratio of isotopically labelled analyte to non-isotopically labelled analyte (^{14}N:^{15}N) with sufficient precision. In particular, the method allows use of standard mass spectrometers without resort to sophisticated specialist isotope-ratio mass spectrometers involving the disadvantages identified above. For example, quadrupole mass spectrometers or other mass spectrometers, which have reasonably high resolution may be used.

According to a first aspect of the present invention there is provided apparatus for the redox processing of analytes, said apparatus comprising:

a liquid chromatograph being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte redox means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte redox means being configured to oxidise or reduce analytes processed by said liquid chromatograph prior to processing by said mass spectrometer.

Preferably, the apparatus is configured for the redox processing of isotopically labelled analytes.

Preferably, said apparatus is configured for the quantitative discrimination of an isotopically labelled analyte from a non-isotopically labelled analyte.

Preferably, the apparatus further comprises a conductivity suppressor being configured to remove eluting ions prior to said processing of said analytes by said mass spectrometer.

Preferably, the apparatus further comprises a chloride trap, said chloride trap being configured to remove chloride ions from a solution containing said analytes following said processing of said analytes by said chromatograph.

Preferably, said chloride trap comprises a silver-loaded resin.

Preferably, said analyte redox means comprises hydrogen peroxide.

Preferably, said analyte redox means is electrochemical oxidation or reduction means.

Preferably, said liquid chromatograph comprises:

a pump;

a sample introduction device; and

a separation column.

Alternatively , the apparatus further comprises a second chromatography column; wherein said second column is positioned after said electrochemical oxidation means and chloride trap is not used.

Preferably, said liquid chromatograph is a metal free high performance liquid chromatograph or ion chromatograph.

Preferably, said apparatus is configured for the oxidative or reductive processing of analytes generated in vivo.

Preferably, said apparatus is configured for the oxidative or reductive processing of analytes generated in vitro.

Preferably, said apparatus is configured for the determination of nitric oxide synthase activity.

Preferably, said analytes comprise nitrite, nitrate, NO donating or NO carrying compounds.

Preferably, said apparatus is configured to oxidise nitrite to nitrate using said analyte redox means, said nitrite containing an isotopically labelled nitrogen atom.

Preferably, said liquid chromatograph is configured to separate said nitrite from nitrate.

Preferably, said mass spectrometer is configured for detecting an isotopically labelled species from a non-isotopically labelled species.

Preferably, said analytes are generated from L-arginine or NO donating drugs.

According to a second aspect of the present invention there is provided an automated analyte processing apparatus configured for processing and detecting analytes according to the present invention.

According to a third aspect of the present invention there is provided an analyte processing apparatus configured for separation, detection and quantitative analysis of analytes according to the present invention.

According to a fourth aspect of the present invention there is provided a method for the redox processing of analytes, said method comprising:

separating analytes according to their charge density or polarity using a liquid chromatograph;

detecting said analytes processed by said chromatograph using a mass spectrometer;

said method being characterised by:

changing an oxidation state of said analytes using redox processing to enhance detection of said analytes in said mass spectrometer.

Preferably, said step of detecting said analytes using said mass spectrometer includes a detecting of isotopically labelled and non-isotopically labelled analytes.

Preferably, said method is configured for the quantitative discrimination of said isotopically labelled analytes from said non-isotopically labelled analytes.

Preferably, the method further comprises:

prior to said step of detecting said analytes, suppressing eluent counter ions within a sample containing said analytes.

Preferably, the method further comprises:

removing chloride ions from said sample containing said analytes.

Preferably, said step of removing chloride ions involves use of a silver-loaded resin.

Preferably, said step of removing said chloride ions occurs prior to said step of detecting said analytes using said mass spectrometer and after said step of suppressing said eluent counter ions.

Preferably, said step of oxidising said analytes comprises reacting said analytes with hydrogen peroxide.

Preferably, said step of oxidising and reducting said analytes involves electrochemical treatment of said analytes.

Preferably, said step of separating said analytes using said chromatograph comprises:

pumping a sample containing said analytes through a separation column.

Preferably, said analytes are reduced or oxidised prior to said detecting of said analytes using said mass spectrometer.

Preferably, said liquid chromatograph is a metal free high performance liquid chromatograph or ion chromatograph.

Preferably, the method being configured for the oxidative or reductive processing of analytes generated in vitro.

Preferably, said method is configured for the oxidative or reductive processing of analytes generated in vivo.

Preferably, said method is configured for the determination of nitric oxide synthase activity comprising:

oxidising an NO analyte to a nitrate analyte; and

quantifying said nitrate analyte.

Preferably, said analytes are generated from isotopically labelled L-arginine or a NO donating drug.

Preferably, a nitrogen atom of said L-arginine or said NO donating drug is isotopically labelled.

Alternatively, the method comprises:

processing said analytes through a second chromatography column after said oxidation or reduction of said analytes a chloride trap not being required.

According to a fifth aspect of the present invention there is provided apparatus for the oxidative processing of analytes, said apparatus comprising:

a liquid chromatography column being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte oxidation means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte oxidation means being configured to oxidise said analytes processed by said column prior to processing by said mass spectrometer.

According to a sixth aspect of the present invention there is provided apparatus for the reductive processing of analytes, said apparatus comprising:

a liquid chromatography column being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte reduction means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte reduction means being configured to reduce analytes processed by said column prior to processing by said mass spectrometer.

Brief Description of the Drawings

For a better understanding of the invention and to show how the same may be carried into effect, there will now be described by way of example only,

specific embodiments, methods and processes according to the present invention with reference to the accompanying drawings in which:

Figure 1A illustrates schematically the apparatus of the present invention according to a first specific implementation;

Figure 1B illustrates apparatus of the present invention according to a second specific implementation of the present invention;

Figure 2 illustrates chromatograms for the on-line chloride removal using a chloride trap column;

Figure 3 illustrates chromatograms following removal of chloride by the chloride trap;

Figure 4 illustrates chromatograms for nitrite/nitrate measured in urine;

Figure 5 illustrates chromatograms for nitrite/nitrate measured in urine;

Figure 6 illustrates three chromatograms involving the determination of nitrite/nitrate generated by an NO donor;

Figure 7 illustrates chromatograms for nitrite/nitrate measurements with electrochemical oxidation.

Detailed Description

There will now be described by way of example a specific mode contemplated by the inventors. In the following description numerous specific details are set forth in order to provide a thorough understanding. It will be apparent however, to one skilled in the art, that the present invention may be practiced without limitation to these specific details. In other instances, well known methods and structures have not been described in detail so as not to unnecessarily obscure the description.

Figure 1A herein illustrates schematically one arrangement of the coupled liquid chromatographic – mass spectrometric apparatus comprising pump 100; auto sampler 101; separation column 102; redox means 103; suppressor 104; chloride trap 105 and mass spectrometer 106.

Pump 100 is configured to produce a high pressure flow of eluent (e.g. hydroxide). Liquid chromatography column 102 comprises a high exchange capacity ion exchange column found in the art comprising resin beads. Auto sampler 101 provides for automation of the separation process through stages 100, 101 and 102.

Suppressor 104 is configured to remove the eluent/counter ions (e.g. Na^+ and K^+) with replacement of H^+ to provide a low conductivity eluent. This is particular important for the present application where the suppressor acts with the chloride trap to remove sodium chloride which is present at high levels within urine or serum and similar matrices samples so as to avoid clogging of the mass spectrometer with solid particles of sodium chloride. Accordingly, due to the presence of the suppressor, nitrite and nitrate are free negative ions at their expected masses without significant interference from the eluent/counter ions.

Chloride trap 105 is configured to remove excess chloride ions which pass through suppressor 104. The presence in serum of chloride in approximately 10^5-fold excess over nitrite causes problems for liquid chromatography and mass spectrometry wherein nitrite usually exits the separation column shortly after chloride. The nitrite is often masked by the *tail* of the chloride peak. Chloride trap 105, being by way of example a chloride removing silver-loaded resin, is configured to remove chloride in-line without detrimental effect on the mass spectrometer resolution.

It has been observed with electrospray ionisation that the mass spectrometer response to nitrite is lower than that of nitrate in similar

concentrations resulting in reduced sensitivity for nitrite detection. Accordingly, the inventors provide an in-line oxidation step utilising oxidation means 103 whereby after the initial separation of the more easily detected nitrate, (being in its highest oxidation state) from the nitrite, (being in a lower oxidation state), both metabolites may be detected at high sensitivity, being distinguished by their different eluting times through column 102. According to specific implementations of the present invention, oxidation means 103 may be chemical (e.g. hydrogen peroxide) or electrochemical.

According to the specific implementation of the present invention oxidation means 103 is provided between separation column 102 and suppressor 104. This arrangement has been found to be particularly advantageous in that oxidation of the metabolite (nitrite) is more efficient when catalysed by sodium hydroxide present in the eluent prior to removal by suppressor 104.

According to further specific implementations, oxidation means 103 may be positioned at any point within the apparatus arrangement of Figure 1 herein. Obviously, the use of a peroxide as the oxidation means does not introduce unwanted components into the mass spectrometer which would interfere with the metabolite detection, the breakdown product of hydrogen peroxide reduction being water.

Referring to Figure 1B herein there is illustrated schematically a further specific implementations of the present invention comprising: pump 100; sample introduction device (auto sampler) 101; a first chromatography column 102; redox means 103; a second chromatography column 107; a suppressor 104; and mass spectrometer 106. According to this further specific implementation, this apparatus and method employs a second liquid chromatography column after the electrochemical cell so as to separate the nitrate (generated from nitrite) from chloride. This particular specific implementation does not require a chloride trap.

According to specific implementations of the present invention a system is provided for the selected oxidation of intermediate oxidation state analytes which may otherwise form weak acids prior to introduction to the mass spectrometer resulting in ionisation problems and ultimately poor analyte detection and sensitivity. For example, sulphur analytes may also be processed according to the present invention.

Additionally, the present invention is configured for drug development studies, in particular nitric oxide donor/carrier drugs, whereby the relative concentrations of donor drug, nitrite and nitrate may be monitored providing a quantitative assessment of the drugs ability to deliver/carry NO. For example, as the drug peak deminishes the nitrite and nitrate analyte peaks increase as the O-donor is metabolised. As before, isotopically labelled NO may be utilised to allow discrimination between NO originating from the donor/carrier drug and NO metabolised naturally within a subject. As before, this specific implementation includes both in vitro and in vivo applications.

Example 1

Figure 2 herein illustrates chromatograms for the on-line chloride removal by a chloride trap column. Samples comprised 50 µmol/L ^{15}N-nitrite and ^{15}N-nitrate in the presence of 125 mmol/L chloride. Peak 200 corresponds to ^{15}N-nitrate, peak 201 corresponds to ^{15}N-nitrite and peak 202 corresponds to chloride.

As illustrated in Figure 2 herein, the chloride is coeluting with nitrite and effectively suppresses the ionisation or detection of nitrite, resulting in the poor signal intensity, peak 201.

Figure 3 herein illustrates chromatograms following removal of chloride by the chloride trap. As illustrated in Figure 3 herein, the ^{15}N-nitrate peak 300 and ^{15}N-nitrite peak 301 are of similar intensities wherein suppression of the ionisation or detection of nitrite is not observed due to the removal of chloride. The lack of

chloride is seen by the absence of a peak 302. Accordingly, chloride no longer interferes with ionisation of nitrite.

Referring to Figures 4 and 5 herein there is illustrated chromatograms for nitrite/nitrate measurements in urine with oxidation by hydrogen peroxide together with the chloride trap. Figure 4 herein illustrates an unspiked urine sample following clean up by C18 solid phase extraction. The ^{14}N-nitrite (peak 401) occurs at approximately 15.85 mins. and the ^{14}N-nitrate (peak 400) occurs at approximately 20.32 mins. The second and lower chromatogram of Figure 4 herein illustrates endogonous ^{15}N-nitrate (peak 402). Figure 5 herein illustrates a urine sample spiked with 5 µmol/L ^{15}N-nitrite following clean up by C18 solid phase extraction. The ^{14}N-nitrite (peak 501) occurs at approximately 15.88 mins. and the ^{14}N-nitrate (peak 500) occurs at approximately 20.32 mins.

The second and lower chromatogram of Figure 5 herein illustrates the endogonous ^{15}N-nitrate (peak 502) at approximately 20.37 mins. and the spiked ^{15}N-nitrite (peak 503) at approximately 15.63 mins.

Being evident from Figures 4 and 5 herein it is apparent that the ^{15}N-nitrite and ^{15}N-nitrate can be detected independently from the ^{14}N-nitrite and nitrate isoforms. Accordingly the inventors provide method and apparatus configured for the separation and independent determination of analytes of different isoforms and different oxidation states without interference from elements (such as chloride) and other counter ions.

In this case, referring to Figures 4 and 5 herein, the peak area of the ^{15}N-nitrite (peak 503) is almost identical to the peak area of a ^{15}N-nitrite standard solution of the same concentration. Therefore, the inventors provide absolute recovery of 95% $\pm$ 2% of ^{15}N-nitrite. The system is equally configured for absolute recovery of nitrate.

Referring to Figure 6 herein there is illustrated three chromatograms involving the determination of nitrite/nitrate generated by an NO donor, in this case, PAPAnononate in a phosphate buffer.

Chromatogram 600 illustrates peak intensities generated from the apparatus of Figure 1 herein after zero mins incubation where peak 603 corresponds to nitrate, peak 604 is nitrite and peak 605 is PAPAnononate.

Chromatogram 601 details mass chromatogram results after 30 mins incubation with donor. Peaks 606, 607 and 608 correspond to nitrate, nitrite and PAPAnononate, respectively.

The determination after 60 minutes incubation with donor is illustrated by chromatogram 602, peaks 609, 610 and 611 corresponding to nitrate, nitrite and PAPAnononate respectively.

As illustrated with reference to the chromatograms of Figure 6 herein, as the NO is released from the PAPAnononate and is subsequently metabolised, peak 605 decreases through 608 to 611. Conversely, with the release of NO from the NO donor the nitrite and nitrate peak areas increase as the NO is metabolised to nitrite and nitrate.

Referring to Figure 7 herein there is illustrated nitrite/nitrate measurements with electrochemical oxidation without a requirement for the chloride trap, according to the further specific implementation of the present invention. Using the apparatus configuration as detailed previously, involving the use of first and second liquid chromatography columns and an electrochemical cell, chromatograms 700 and 701 illustrate the separation and subsequent determination of nitrite and nitrate even with excess (125 mmol/L) chloride.

Chromatogram 700 illustrates a chloride peak 702 eluting at approximately 21 mins. such that following separation of nitrite, nitrate and chloride via the

second liquid chromatography column, the nitrate peak (approximately 25 mins.) and nitrite peak (approximately 23 mins.) are identified. According to this further specific implementation, an alternative method is provided which does not require a chloride trap whilst providing separation and detection of nitrite/nitrate.

Claims:

1. Apparatus for the redox processing of analytes, said apparatus comprising:

a liquid chromatograph being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte redox means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte redox means being configured to oxidise or reduce analytes processed by said liquid chromatograph prior to processing by said mass spectrometer.

2. The apparatus as claimed in claim 1 being configured for the redox processing of isotopically labelled analytes.

3. The apparatus as claimed in claim 1 or 2 wherein said apparatus is configured for the quantitative discrimination of an isotopically labelled analyte from a non-isotopically labelled analyte.

4. The apparatus as claimed in any preceding claim further comprising a conductivity suppressor being configured to remove eluting ions prior to said processing of said analytes by said mass spectrometer.

5. The apparatus as claimed in any preceding claim further comprising a chloride trap, said chloride trap being configured to remove chloride ions from a solution containing said analytes following said processing of said analytes by said chromatograph.

6. The apparatus as claimed in claim 5 wherein said chloride trap comprises a silver-loaded resin.

7. The apparatus as claimed in any preceding claim wherein said analyte redox means comprises hydrogen peroxide.

8. The apparatus as claimed in any one of claims 1 to 6 wherein said analyte redox means is electrochemical oxidation or reduction means.

9. The apparatus as claimed in any preceding claim wherein said liquid chromatograph comprises:

a pump;

a sample introduction device; and

a separation column.

10. The apparatus as claimed in claims 8 or 9 further comprising a second chromatography column; wherein said second column is positioned after said electrochemical oxidation means.

11. The apparatus as claimed in any preceding claim wherein said liquid chromatograph is a metal free high performance liquid chromatograph or ion chromatograph.

12. The apparatus as claimed in any preceding claim wherein said apparatus is configured for the oxidative or reductive processing of analytes generated in vivo.

13. The apparatus as claimed in any one of claims 1 to 11 wherein said apparatus is configured for the oxidative or reductive processing of analytes generated in vitro.

14. The apparatus as claimed in any preceding claim wherein said apparatus is configured for the determination of nitric oxide synthase activity.

15. The apparatus as claimed in claim 14 wherein said analytes comprise nitrite, nitrate, NO donating or NO carrying compounds.

16. The apparatus as claimed in claim 15 wherein said apparatus is configured to oxidise nitrite to nitrate using said analyte redox means, said nitrite containing an isotopically labelled nitrogen atom.

17. The apparatus as claimed in claim 16 wherein said liquid chromatograph is configured to separate said nitrite from nitrate.

18. The apparatus as claimed in claim 17 wherein said mass spectrometer is configured for detecting an isotopically labelled species from a non-isotopically labelled species.

19. The apparatus as claimed in claim 18 wherein said analytes are generated from L-arginine or NO donating drugs.

20. An automated analyte processing apparatus configured for processing and detecting analytes comprising apparatus according to any one of the preceding claims.

21. An analyte processing apparatus configured for separation, detection and quantitative analysis of analytes comprising apparatus according to any one of the preceding claims.

22. A method for the redox processing of analytes, said method comprising:

separating analytes according to their charge density or polarity using a liquid chromatograph;

detecting said analytes processed by said chromatograph using a mass spectrometer;

said method being characterised by:

changing an oxidation state of said analytes using redox processing to enhance detection of said analytes in said mass spectrometer.

23. The method as claimed in claim 22 wherein said step of detecting said analytes using said mass spectrometer includes a detecting of isotopically labelled and non-isotopically labelled analytes.

24. The method as claimed in claim 23 wherein said method is configured for the quantitative discrimination of said isotopically labelled analytes from said non-isotopically labelled analytes.

25. The method as claimed in any one of claims 22 to 24 further comprising:

prior to said step of detecting said analytes, suppressing eluent counter ions within a sample containing said analytes.

26. The method as claimed in claim 25 further comprising:

removing chloride ions from said sample containing said analytes.

27. The method as claimed in claim 26 wherein said step of removing chloride ions involves use of a silver-loaded resin.

28. The method as claimed in claim 27 wherein said step of removing said chloride ions occurs prior to said step of detecting said analytes using said mass spectrometer and after said step of suppressing said eluent counter ions.

29. The method as claimed in any one of claims 22 to 28 wherein said step of oxidising said analytes comprises reacting said analytes with hydrogen peroxide.

30. The method as claimed in any one of claims 22 to 28 wherein said step of oxidising and reducing said analytes involves electrochemical treatment of said analytes.

31. The method as claimed in any one of claims 22 to 30 wherein said step of separating said analytes using said chromatograph comprises:

pumping a sample containing said analytes through a separation column.

32. The method as claimed in any one of claims 22 to 31 wherein said analytes are reduced or oxidised prior to said detecting of said analytes using said mass spectrometer.

33. The method as claimed in any one of claims 22 to 32 wherein said liquid chromatograph is a metal free high performance liquid chromatograph or ion chromatograph.

34. The method as claimed in any preceding claim being configured for the oxidative or reductive processing of analytes generated in vitro.

35. The method as claimed in any one of claims 22 to 33 wherein said method is configured for the oxidative or reductive processing of analytes generated in vivo.

36. The method as claimed in any one of claims 22 to 35 wherein said method is configured for the determination of nitric oxide synthase activity comprising:

oxidising an NO analyte to a nitrate analyte; and

quantifying said nitrate analyte.

37. The method as claimed in claim 36 wherein said analytes are generated from isotopically labelled L-arginine or a NO donating drug.

38. The method as claimed in claim 37 wherein a nitrogen atom of said L-arginine or said NO donating drug is isotopically labelled.

39. The method as claimed in any one of claims 22 to 25 comprising:

processing said analytes through a second chromatography column after said oxidation or reduction of said analytes.

40. Apparatus for the oxidative processing of analytes, said apparatus comprising:

a liquid chromatography column being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte oxidation means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte oxidation means being configured to oxidise said analytes processed by said column prior to processing by said mass spectrometer.

41. Apparatus for the reductive processing of analytes, said apparatus comprising:

a liquid chromatography column being configured to separate analytes of different charge density or polarity;

a mass spectrometer configured for detecting said analytes processed by said chromatograph;

said apparatus further comprising and being characterised by:

analyte reduction means being configured to change an oxidation state of said analytes to enhance detection of said analytes in said mass spectrometer, said analyte reduction means being configured to reduce analytes processed by said column prior to processing by said mass spectrometer.

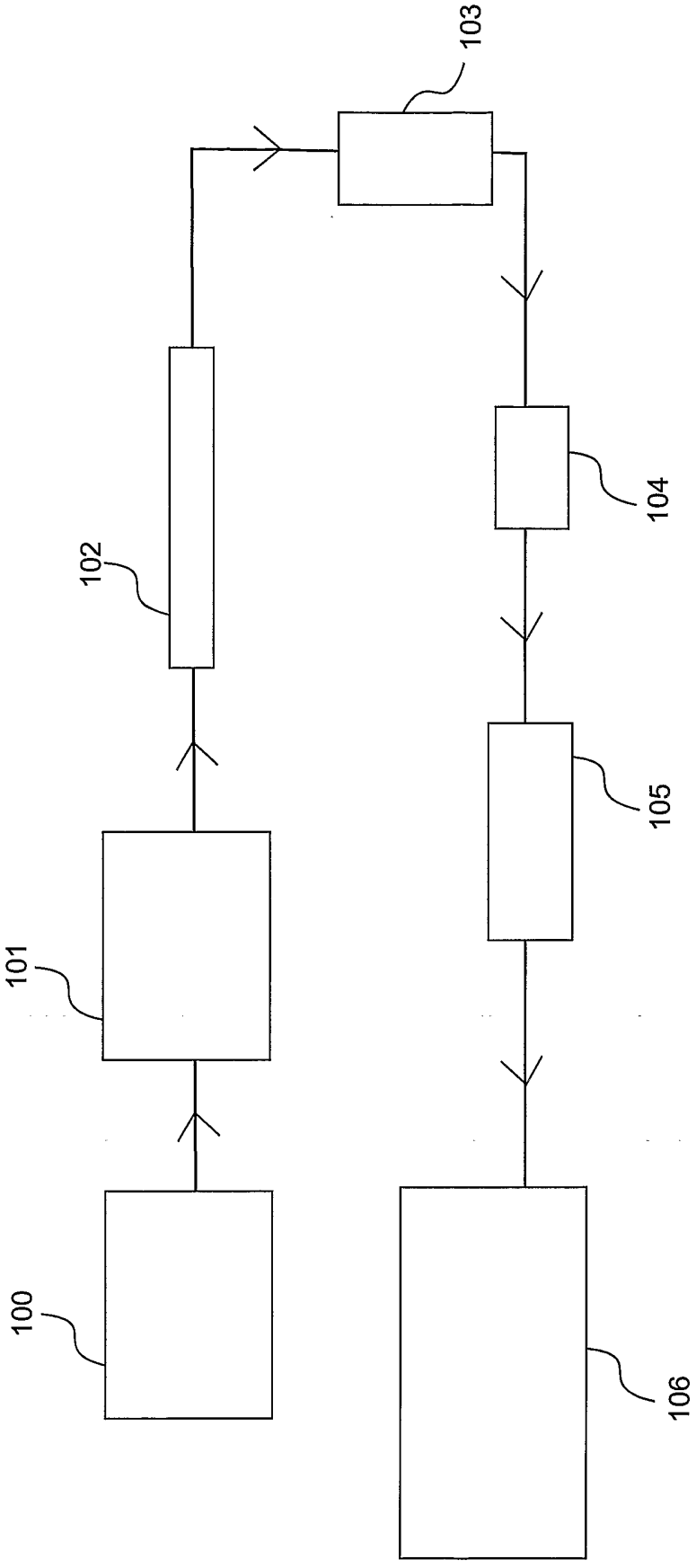

Fig. 1A

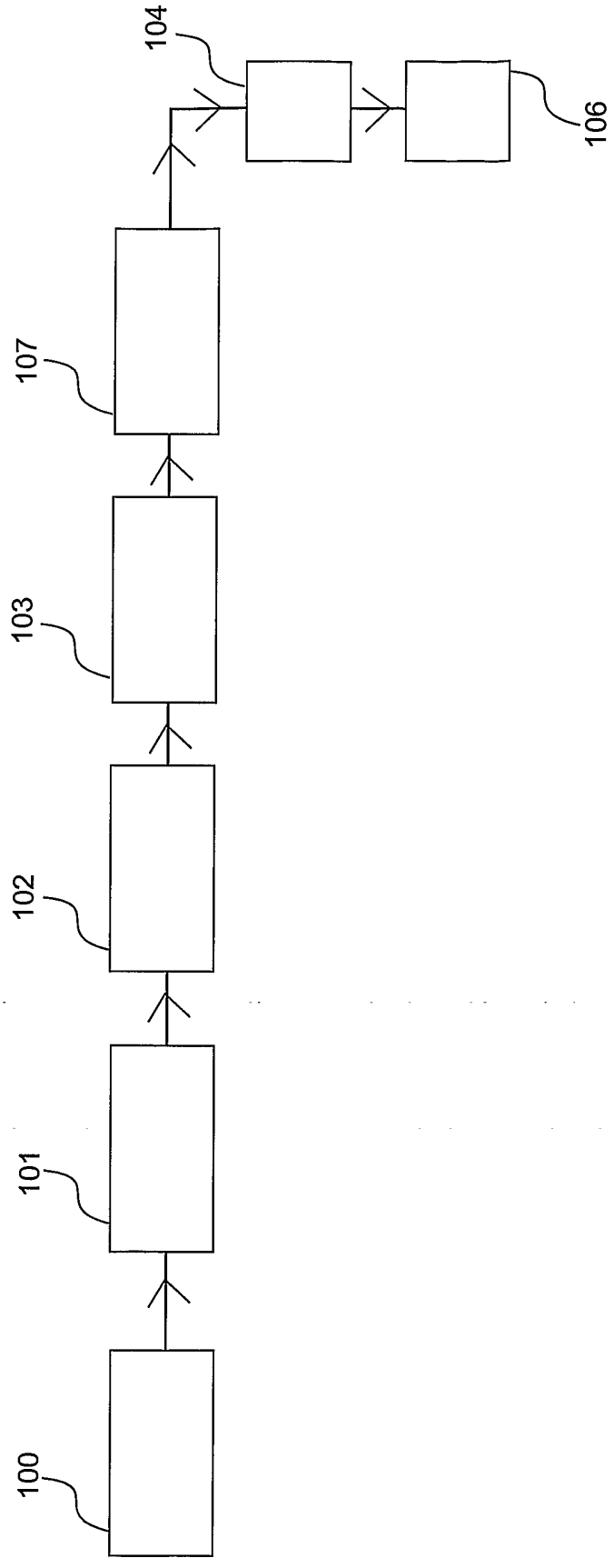

Fig. 1B

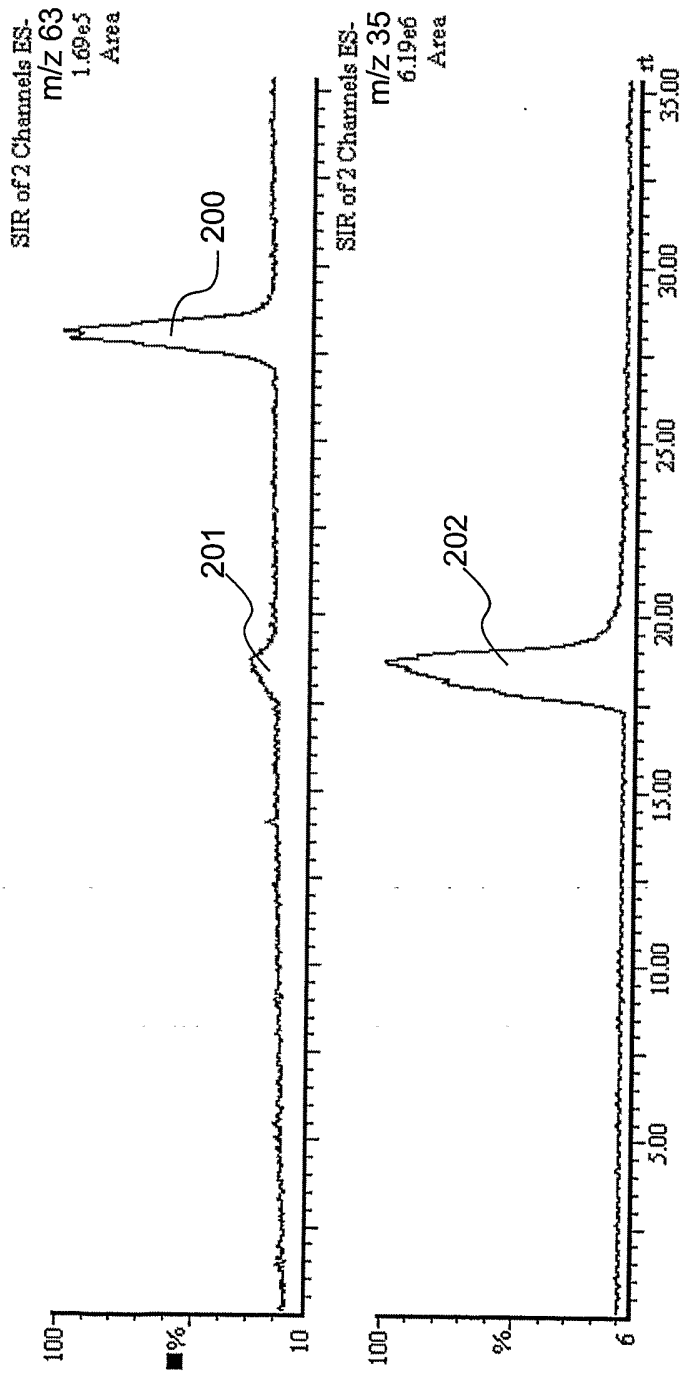
SIR of 2 Channels ES-
m/z 63
1.69e5
Area
200
201
100
%
10
SIR of 2 Channels ES-
m/z 35
6.19e6
Area
202
100
%
6
5.00
10.00
15.00
20.00
25.00
30.00
35.00
rt

Fig. 2

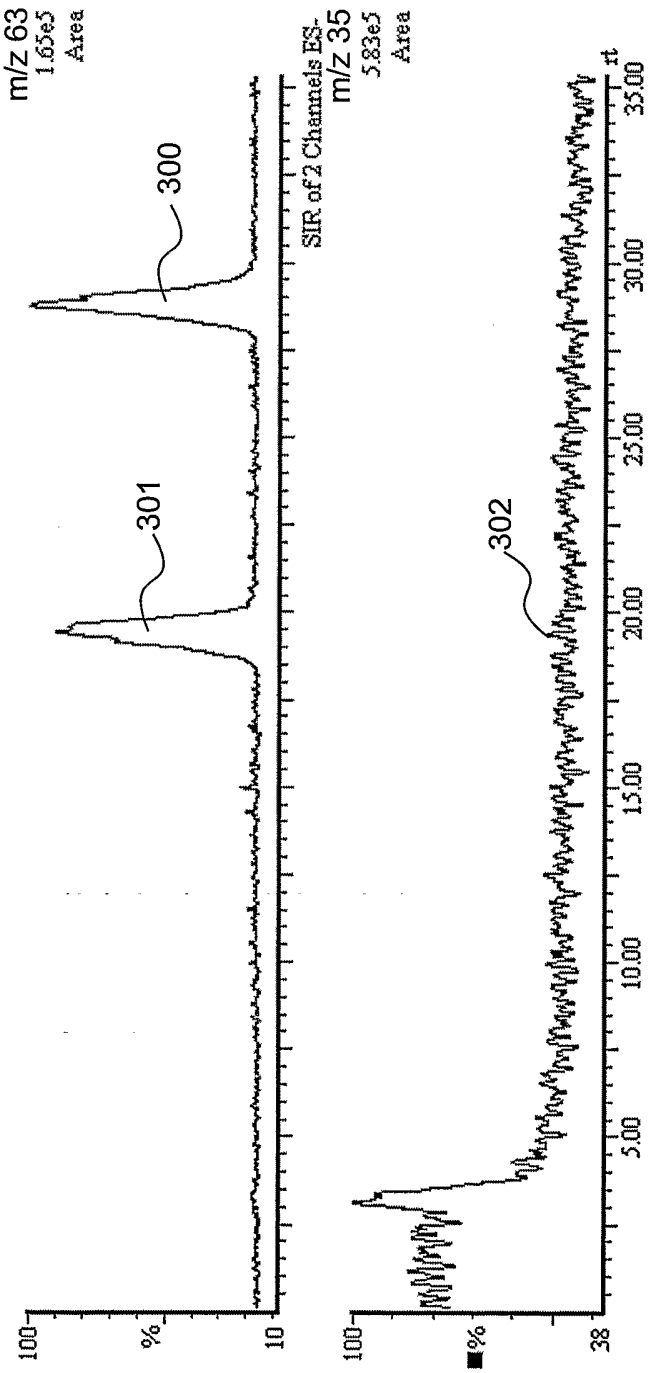
SIR of 2 Channels ES-
m/z 63
1.65e5
Area
300
301
SIR of 2 Channels ES-
m/z 35
5.83e5
Area
302
100
%
10
100
%
38
5.00
10.00
15.00
20.00
25.00
30.00
35.00
rt

Fig. 3

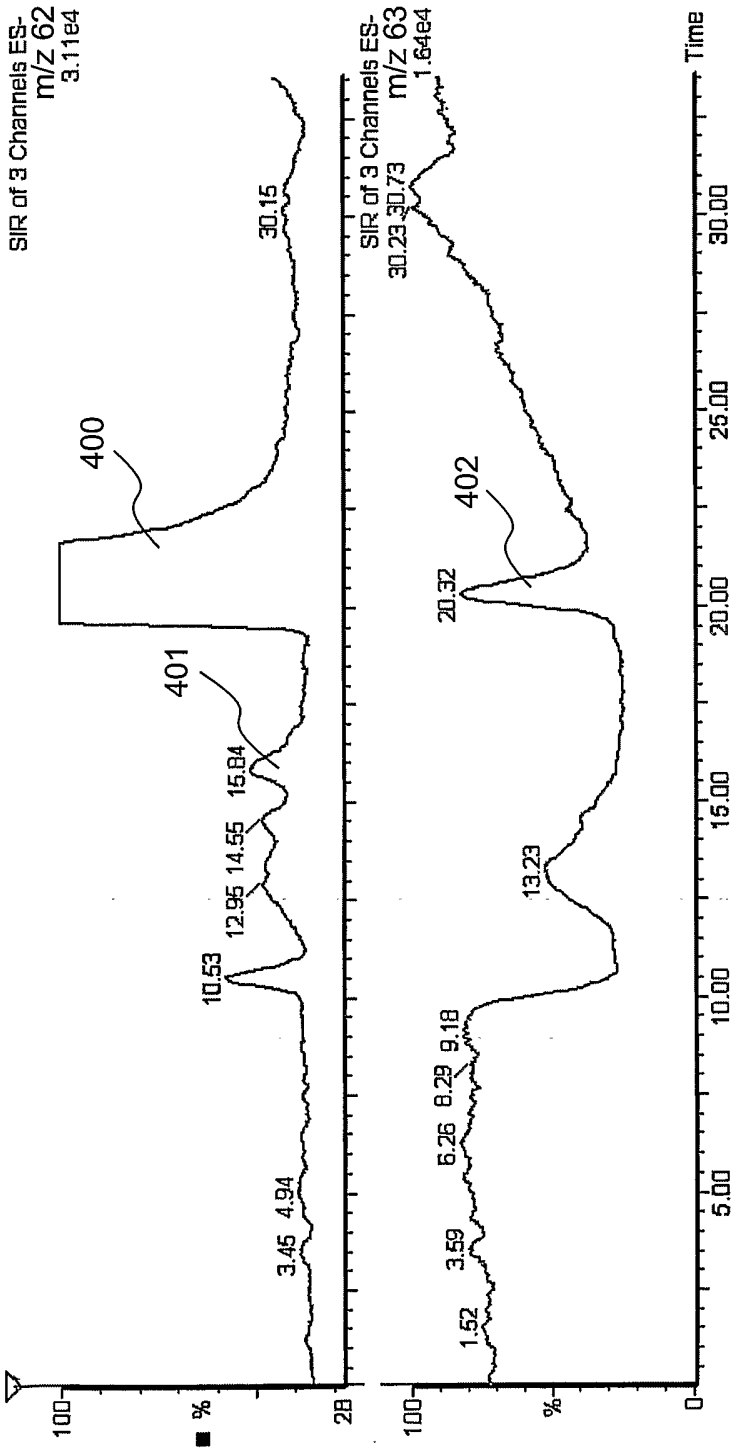
SIR of 3 Channels ES-
m/z 62
3.11e4
400
401
30.15
15.84
14.55
12.95
10.53
4.94
3.45
100
%
28
SIR of 3 Channels ES-
m/z 63
1.64e4
30.23 30.73
402
20.32
13.23
9.18
8.29
6.26
3.59
1.52
100
%
0
5.00
10.00
15.00
20.00
25.00
30.00
Time

Fig. 4

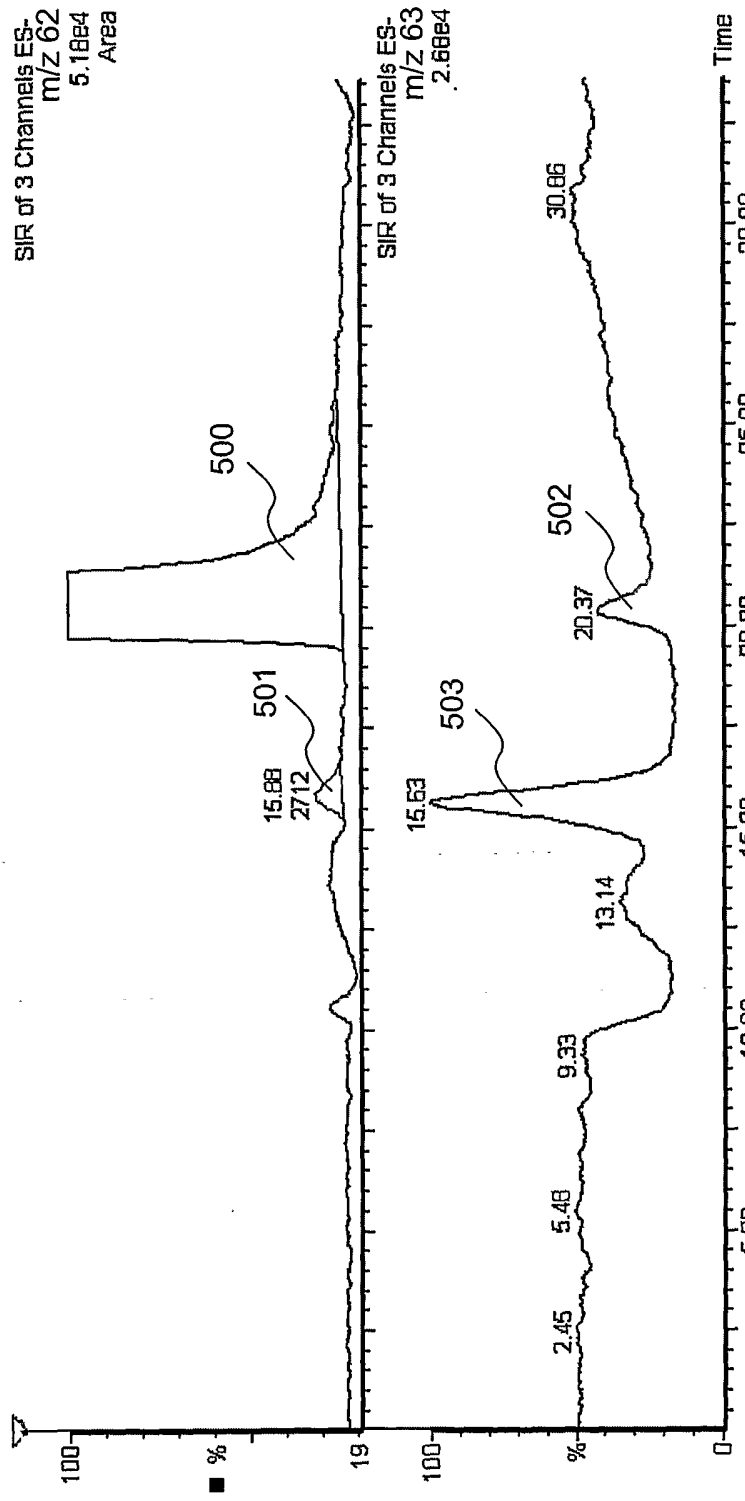
SIR of 3 Channels ES-
m/z 62
5.18e4
Area
SIR of 3 Channels ES-
m/z 63
2.68e4
Time
500
501
15.88
2712
503
15.63
502
20.37
30.86
13.14
9.33
5.48
2.45
100
%
19
100
%
0
5.00
10.00
15.00
20.00
25.00
30.00

Fig. 5

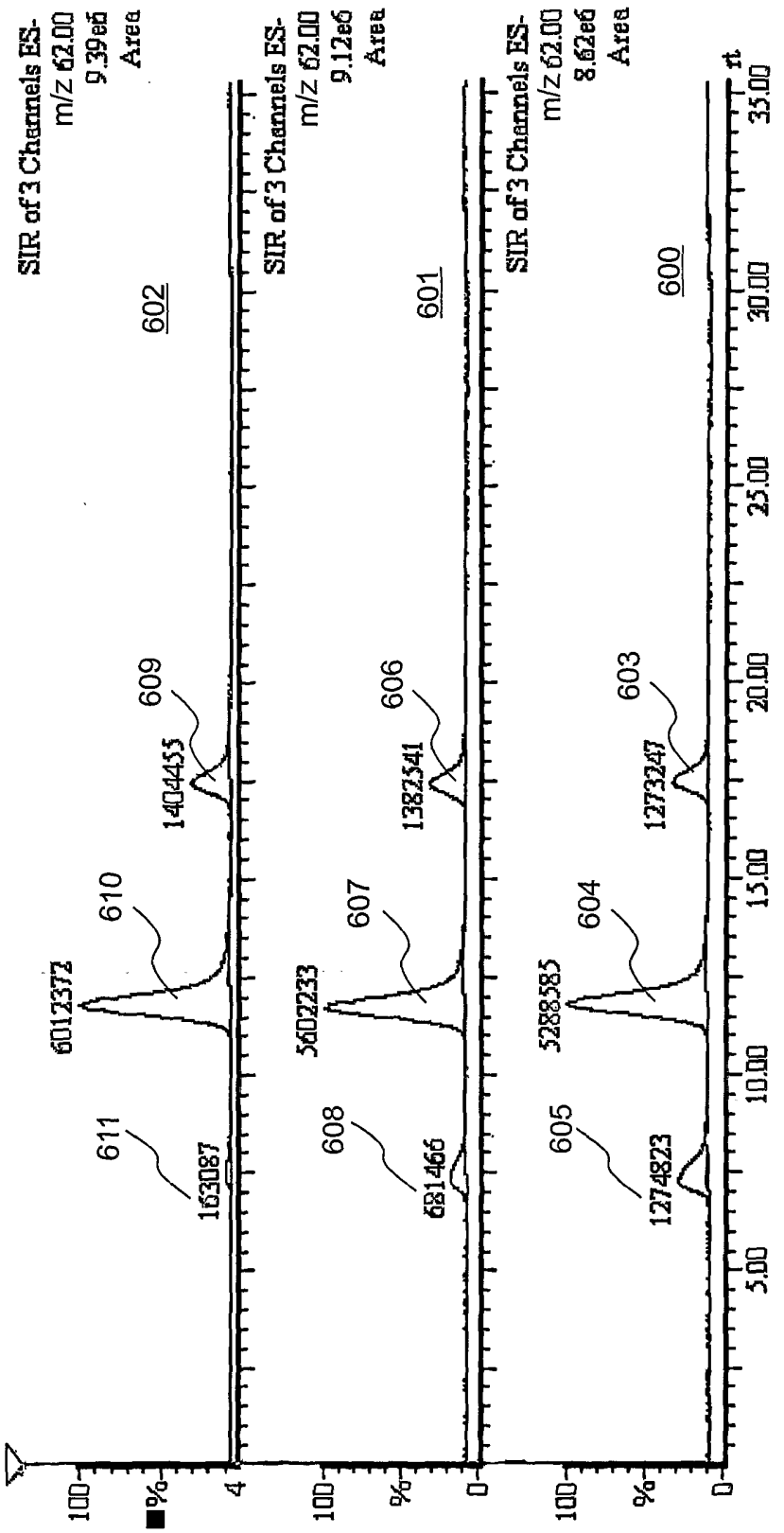
SIR of 3 Channels ES-
m/z 62.00
9.39e6
Area
602
609
1404455
610
6012372
611
163087
SIR of 3 Channels ES-
m/z 62.00
9.12e6
Area
601
606
1382341
607
5602233
608
681466
SIR of 3 Channels ES-
m/z 62.00
8.62e6
Area
600
603
1273247
604
5288585
605
1274823
100
%
0
5.00
10.00
15.00
20.00
25.00
30.00
35.00
rt

Fig. 6

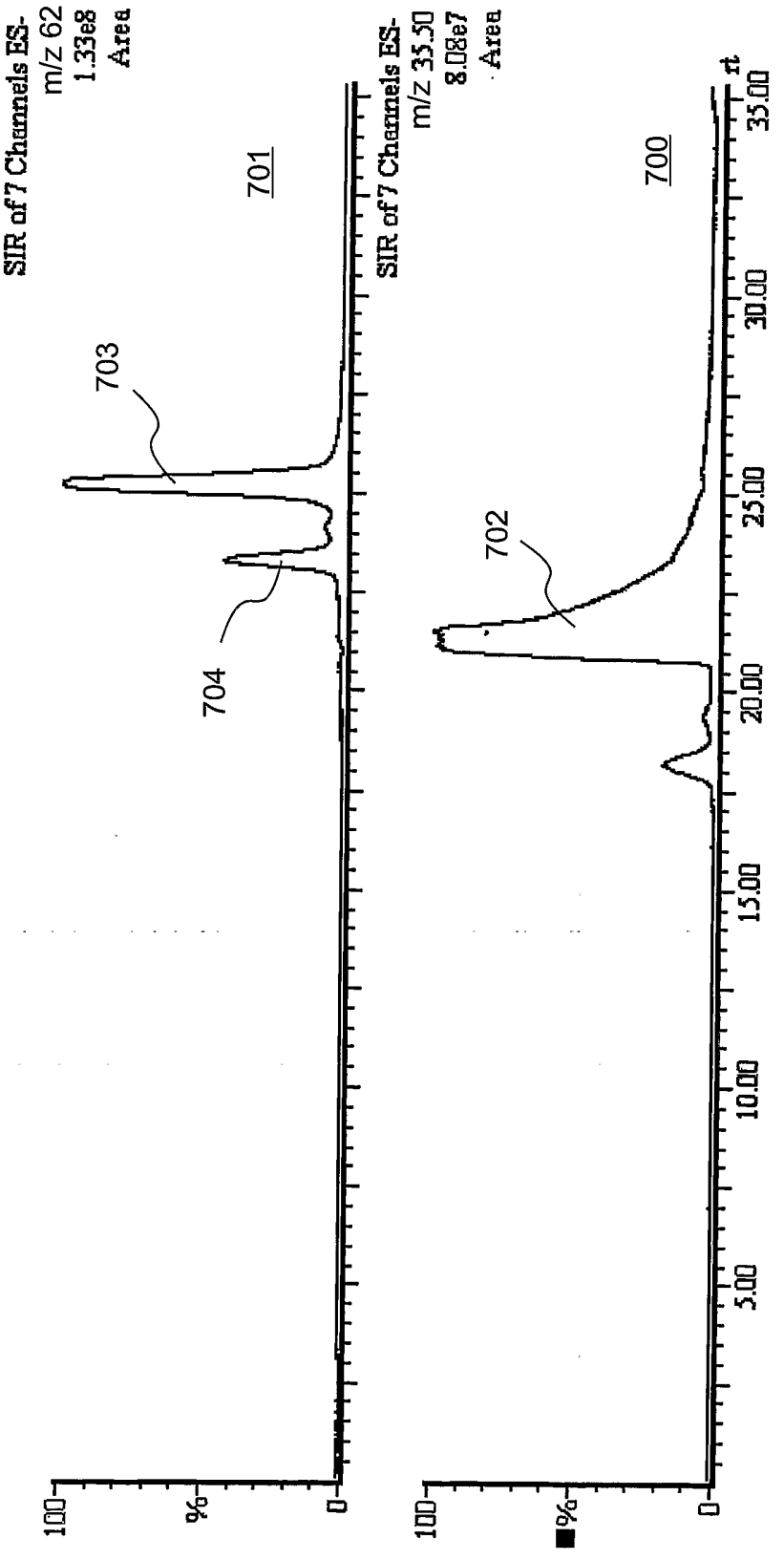
SIR of 7 Channels ES-
m/z 62
1.33e8
Area
701
703
704
100
%
0
SIR of 7 Channels ES-
m/z 35.50
8.08e7
Area
700
702
100
%
0
5.00
10.00
15.00
20.00
25.00
30.00
35.00
rt

Fig. 7