

(51) International Patent Classification:
G01N 31/00 (2006.01)(21) International Application Number:
PCT/US2012/040748(22) International Filing Date:
4 June 2012 (04.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/493,211 3 June 2011 (03.06.2011) US(71) Applicant (for all designated States except US): **DH TECHNOLOGIES DEVELOPMENT PTE. LTD.** [SG/SG]; 80 Raffles Place #25-01, UOB Plaza, Singapore 048624 (SG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **CARAIMAN, Doina** [CA/CA]; 61 Ventana Way, Vaughn, ON L4H1L7 (CA). **LE BLANC, Yves** [CA/CA]; 10 Ballard Cres, Newmarket, ON L3X 1S3 (CA). **LIU, Hua-Fen** [US/US]; 797 Firewood Ct., San Jose, CA 95120 (US).(74) Agent: **BASTIAN, Michael, J.**; AB SCIEX LLC, 500 Old Connecticut Path, Framingham, MA 01701 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: METHOD AND SYSTEM FOR REDUCING INTERFERENCES IN THE SPECTROMETRIC ANALYSIS OF STEROIDS

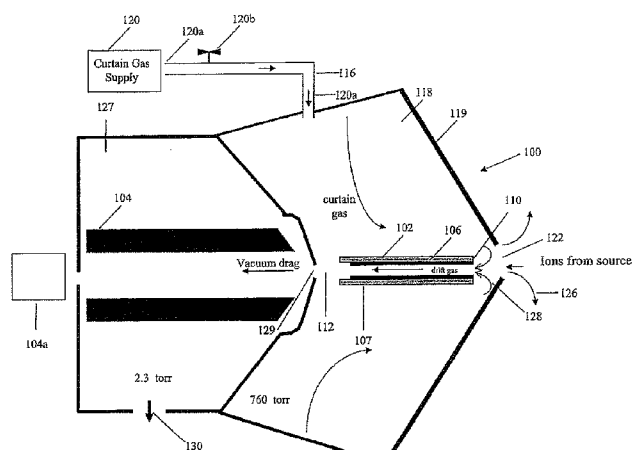


Figure 1

(57) Abstract: A system and method for reducing interferences in the spectrometric analysis of a sample from a blood collection tube comprising an ion source for ionizing a sample extracted from the blood collection tube, the sample containing one or more steroids. The system comprises a differential mobility spectrometer for receiving the ions from the ion source, a boundary member defining a curtain gas chamber containing the differential mobility spectrometer; and a curtain gas supply for providing a curtain gas into an inlet of the differential mobility spectrometer for providing a gas flow through the differential mobility spectrometer and a curtain gas outflow out of a curtain gas chamber inlet. A system and method is disclosed for providing a modifier supply for supplying an actual volumetric flow rate of an organic acid modifier to a drift gas supply for reducing interferences.



- 1 -

**TITLE: METHOD AND SYSTEM FOR REDUCING INTERFERENCES
IN THE SPECTROMETRIC ANALYSIS OF STEROIDS**

RELATED APPLICATION

[0001] This application claims priority to US provisional application no. 61/493,211 filed June 3, 2011, which is incorporated herein by reference in its entirety.

5

FIELD

[0002] The present invention relates generally to methods and systems for reducing interferences in the spectrometric analysis of steroids.

10 **INTRODUCTION**

[0003] Selective detection of steroids by LC-MS/MS can be challenging due to chemical interferences typically observed during the analysis of steroids. The origin of these interferences can vary, but interferences can be from non-steroid endogenous species from matrix and they can also be introduced by blood collection tubes. Plasma and serum are common matrices used in clinical analysis, and they are typically collected using different types of blood collection tubes. The tubes are usually filled with cloth activator as well as gel enabling faster and simpler separation of the plasma and serum from the red blood cells, and additional additives, such as heparin, can also be present in the tubes. These tubes are commonly referred to as Serum Separation Tubes (SST) and Plasma Separation Tubes (PST). The type of collection tube used can cause inaccuracies in the measurement of steroids. To circumvent this problem, a different multiple reaction monitoring (MRM) transition can be used, the preparation of the sample can be modified, the LC conditions can be modified, and an alternate blood sample collection tube can be used. But, each of these current approaches can have drawbacks. For example, using a different MRM transition can lead to increased selectivity, but reduced sensitivity which can require starting with

15
20
25

- 2 -

more samples. Also, modifying the LC conditions can lead to lower sample throughput resulting in longer delay times in supplying information to the requestor. The biggest challenge can be resolving interferences associated with the blood collection tube, especially if a specific test requires the use of a specific blood collection tube since it can impact the overall process. For instance, it can require multiple blood draws from the patient; protocol may be required to educate staff at the collection point, i.e., test for analyte x requires tube y; specific instrumentation may be required to separate plasma or serum from blood cells; it can require protocol to keep track of collection tubes used if pre-processed samples are to be re-analyzed; and physicians may need to be educated on all aspects associated with the special protocols to ensure proper assessment of the results obtained.

SUMMARY

15 [0004] In accordance with an aspect of the applicant's teachings, there is provided a system for reducing interferences in the spectrometric analysis of a sample from a blood collection tube comprising an ion source for ionizing a sample extracted from the blood collection tube, the sample containing one or more steroids. The system comprises a differential mobility spectrometer for receiving the ions from the ion source, a boundary member defining a curtain gas chamber containing the differential mobility spectrometer; and a curtain gas supply for providing a curtain gas into an inlet of the differential mobility spectrometer for providing a gas flow through the differential mobility spectrometer and a curtain gas outflow out of a curtain gas chamber inlet. In various embodiments, the one or more steroids can comprise testosterone. In various aspects, the system further comprises a mass spectrometer in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer. In various embodiments, the system further comprises a gas port having a gas outlet located between the differential mobility spectrometer and the mass spectrometer for drawing a gas outflow from the gas flow to modify the gas flow rate through the

- 3 -

differential mobility spectrometer. In various aspects, the flow rate of the gas outflow is varied to increase the gas flow rate. In various embodiments, the system comprises a vacuum chamber surrounding the mass spectrometer for maintaining the mass spectrometer at a vacuum pressure to draw the gas

5 flow including the ions through the differential mobility spectrometer and into the vacuum chamber. In various aspects, the system further comprises an electrical field generator for providing an electrical field between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber and to prevent ions from being drawn out of the

10 gas outlet. In various embodiments, the differential mobility spectrometer comprises one of straight and curved electrodes. In various aspects, the system further comprises at least one heater to heat at least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of

15 the mass spectrometer. In various aspects, the system further comprises a detector for detecting the ions received by the mass spectrometer. In various embodiments, the sample can be extracted from the blood collection tube by on-line extraction, protein precipitation, liquid-liquid extraction, or solid-phase extraction. In various aspects, the sample can comprise plasma or serum. In

20 various embodiments, the sample can be introduced into a liquid chromatography apparatus comprising a column prior to ionization.

In various embodiments, a method is provided for reducing interferences in the spectrometric analysis of a sample from a blood collection tube. The method comprises providing an ion source for ionizing a sample extracted

25 from the blood collection tube, and the sample can contain one or more steroids. The method further comprises providing a differential mobility spectrometer for receiving the ions from the ion source, providing a boundary member defining a curtain gas chamber containing the differential mobility spectrometer, and providing a curtain gas supply for providing a curtain gas

30 into an inlet of the differential mobility spectrometer for providing a gas flow through the differential mobility spectrometer and a curtain gas outflow out of a curtain gas chamber inlet. In various aspects, the one or more steroids can

- 4 -

- comprise testosterone. In various embodiments, a mass spectrometer can be in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer. In various aspects, a gas outflow between the differential mobility spectrometer and the mass
- 5 spectrometer can be drawn from the gas flow to modify the gas flow rate through the differential mobility spectrometer. In various embodiments, the flow rate of the gas outflow can be varied to increase the gas flow rate. In various embodiments, a vacuum chamber can surround the mass spectrometer for maintaining the mass spectrometer at a vacuum pressure to
- 10 draw the gas flow including the ions through the differential mobility spectrometer and into the vacuum chamber. In various aspects, an electrical field can be provided between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber and to prevent ions from being drawn out of the gas outlet. In various aspects, the
- 15 differential mobility spectrometer can comprise one of straight and curved electrodes. In various embodiments, at least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of the mass spectrometer can be heated. In various embodiments, can be extracted from
- 20 the blood collection tube by on-line extraction, protein precipitation, liquid-liquid extraction, or solid-phase extraction. In various aspects, the sample can comprise plasma or serum. In various embodiments, the method can further comprise introducing the extracted sample into a liquid chromatography apparatus comprising a column prior to ionization.
- 25 **[0005]** In various embodiments, a system is provided for reducing interferences in the spectrometric analysis of steroids. In various aspects, the system can comprise an ion source for ionizing a sample containing one or more steroids, a differential mobility spectrometer for receiving the ions from the ion source, a drift gas supply for providing a drift gas for flowing through
- 30 the differential mobility spectrometer, and a modifier supply for supplying an actual volumetric flow rate of an organic acid modifier to the drift gas supply. In various aspects, one or more steroids can comprise testosterone. In

- 5 -

various aspects, the organic acid modifier can comprise formic acid. In various embodiments, the system can further comprise a meter for setting a selected volumetric flow rate, and the actual volumetric flow rate can be within a percent deviation from the selected volumetric flow rate. In various aspects,

5 the system can further comprise a pump for supplying the actual volumetric flow rate of the modifier liquid to the drift gas supply. In various embodiments, the drift gas supply can be a curtain gas supply for providing a curtain gas, and the spectrometer system can further comprise a boundary member for directing at least a portion of the curtain gas to an inlet of the differential

10 mobility spectrometer to become the drift gas. In various aspects, the system can further comprise a curtain chamber for receiving the curtain gas with the added organic acid modifier, wherein the curtain chamber can comprise a curtain wall surrounding the differential mobility spectrometer, and the boundary member can be a portion of the curtain wall. In various aspects, the

15 drift gas supply can comprise a drift gas conduit, the modifier supply can comprise a modifier conduit, and the system can further comprises a junction for merging the modifier conduit into the drift gas conduit, and the pump can be located in the modifier conduit upstream of the junction to provide the actual volumetric flow rate of the modifier liquid to the drift gas conduit. In

20 various embodiments, the system can further comprise a heater for heating the junction. In various embodiments, the system can further comprise a mass spectrometer in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer. In various aspects, the system can further comprise a detector for detecting

25 the ions received by the mass spectrometer. In various aspects, the system can further comprise an electrical field generator for providing an electrical field between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber. In various embodiments, the differential mobility spectrometer can comprises straight or curved electrodes.

30 [0006] In various aspects, the system can further comprise a heater for heating the curtain chamber. In various aspects, the sample can be

- 6 -

introduced into a liquid chromatography apparatus comprising a column prior to ionization.

[0007] In various embodiments, a method is provided for reducing interferences in the spectrometric analysis of steroids. The method

5 comprises providing an ion source for ionizing a sample containing one or more steroids, providing a differential mobility spectrometer for receiving the ions from the ion source, providing a drift gas supply for providing a drift gas for flowing through the differential mobility spectrometer, and providing a modifier supply for supplying an actual volumetric flow rate of an organic acid

10 modifier to the drift gas supply. In various aspects, the one or more steroids can comprise testosterone. In various aspects, the organic acid modifier can be formic acid. In various embodiments, the method can further comprise providing a meter for setting a selected volumetric flow rate, and the actual volumetric flow rate can be within a percent deviation from the selected

15 volumetric flow rate. In various embodiments, the method further comprises providing a pump for supplying the actual volumetric flow rate of the modifier liquid to the drift gas supply. In various aspects, the drift gas supply can be a curtain gas supply for providing a curtain gas, and a boundary member can be provided for directing at least a portion of the curtain gas to an inlet of the

20 differential mobility spectrometer to become the drift gas. In various aspects, a curtain chamber can be provided for receiving the curtain gas with the added organic acid modifier, wherein the curtain chamber comprises a curtain wall surrounding the differential mobility spectrometer, and the boundary member can be a portion of the curtain wall. In various aspects, the drift gas

25 supply comprises providing a drift gas conduit, the modifier supply comprises providing a modifier conduit, and a junction can be provided for merging the modifier conduit into the drift gas conduit, and the pump can be located in the modifier conduit upstream of the junction to provide the actual volumetric flow rate of the modifier liquid to the drift gas conduit. In various aspects, the

30 method can further comprise heating the junction. In various aspects, the method further comprises providing a mass spectrometer in fluid communication with the differential mobility spectrometer for receiving the

- 7 -

ions from the differential mobility spectrometer. In various embodiments, the method further comprises detecting the ions received by the mass spectrometer. In various embodiments, the method further comprises providing an electrical field between the differential mobility spectrometer and
5 the vacuum chamber for guiding the ions into the vacuum chamber. In various embodiments, the differential mobility spectrometer comprises straight or curved electrodes. In various aspects, the method further comprises heating the curtain chamber. In various aspects, the method further comprises introducing the sample into a liquid chromatography apparatus
10 comprising a column prior to ionization.

[0008] These and other features of the applicant's teachings are set forth herein.

BRIEF DESCRIPTION OF THE DRAWINGS

15 [0009] The skilled person in the art will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the applicant's teachings in any way.

[0010] Figure 1 schematically illustrates a differential mobility spectrometer/mass spectrometer system in accordance with various
20 embodiments of the applicant's teachings (with curtain gas).

[0011] Figure 2 schematically illustrates a differential mobility spectrometer/mass spectrometer system in accordance with various
embodiments of the applicant's teachings (with bleed/throttle gas).

[0012] Figure 3 schematically illustrates a differential mobility
25 spectrometer/mass spectrometer system in accordance with various embodiments of the applicant's teachings (with modifier).

[0013] Figure 4 schematically illustrates a differential mobility spectrometer/mass spectrometer system in accordance with various
embodiments of the applicant's teachings (with modifier/bleed or throttle gas).

30 [0014] Figure 4a shows the types of blood collection tubes that were evaluated.

- 8 -

[0015] Figure 5 shows interferences that are present in MS/MS spectra of a sample extracted from a blood collection tube.

[0016] Figure 6 shows interferences that are present in MS/MS spectra of a sample extracted from a blood collection tube.

5 [0017] Figure 7 shows parameter settings of the DMS-MS system according to various embodiments of the applicant's teachings.

[0018] Figure 7a shows the experimental conditions for Figures 8, 9, 10, and 11 according to various embodiments of the applicant's teachings.

[0019] Figure 8 compares the interferences present with the DMS off and
10 with the DMS on according to various embodiments of the applicant's teachings.

[0020] Figure 9 compares the interferences present with the DMS off and with the DMS on according to various embodiments of the applicant's teachings.

15 [0021] Figure 10 compares the interferences present with the DMS off and with the DMS on according to various embodiments of the applicant's teachings.

[0022] Figure 11 compares the interferences present with the DMS off and with the DMS on according to various embodiments of the applicant's
20 teachings.

[0023] Figures 11a and 11b show interferences from blood collection tubes and the shifting of testosterone from these interferences with the DMS on.

[0024] Figure 12 illustrates the shift in testosterone in the presence of formic acid according to various embodiments of the applicant's teachings.

25 [0025] Figure 13 shows a compensation voltage map displaying the shift in testosterone in the presence of formic acid according to various embodiments of the applicant's teachings.

[0026] Figure 14 shows a compensation voltage map displaying the shift in testosterone in the presence of formic acid according to various embodiments
30 of the applicant's teachings.

- 9 -

[0027] Figure 15a compares the interferences present for a panel of steroids with the DMS off and with the DMS on according to various embodiments of the applicant's teachings.

[0028] Figure 15b lists the MRM monitored for the steroid panel

5 [0029] Figure 16a shows CoV regions for steroid and CoV region for noise introduced by the blood collection tube.

[0030] Figure 16b compares the interferences present for a panel of steroids with the DMS off and the DMS on at CoV 9.0 as representative of noise region for interferences introduced by the blood collection tube and CoV
10 4.5 for the steroid region.

[0031] Figure 17 shows the structure of some steroids.

[0032] Figure 18A shows the CoV shifting towards higher positive values as the SV is increased for testosterone when nitrogen is used as the transporter gas.

15 [0033] Figure 18B shows the CoV shift towards negative values as the SV is increased for testosterone when formic acid is used according to various embodiments of the applicant's teachings.

[0034] Figure 19 shows the CoV values for steroids according to various embodiments of the applicant's teachings.

20 [0035] Figure 20 shows the response for various steroids with the DMS off, DMS with no modifier, and DMS with formic acid.

[0036] Figure 21 shows the analysis of testosterone acquired in single MS mode with the DMS off, DMS with no modifier, and DMS with formic acid.

[0037] Figure 22 shows the analysis of testosterone acquired in MRM mode
25 with the DMS off, DMS with no modifier, and DMS with formic acid.

[0038] Figure 23 shows the detection of isobaric steroids separated by DMS with formic acid according to various embodiments of the applicant's teachings.

- 10 -

[0039] Figures 24A and 24B show a 2-D view and a 3-D view of the detection of isobaric steroids separated by DMS with formic acid according to various embodiments of the applicant's teachings.

[0040] Figure 25 compares the interference caused by the injection of two isobaric steroids detected in MRM mode with the DMS off versus detection in SIM mode with DMS and formic acid.

[0041] In the drawings, like reference numerals indicate like parts.

DESCRIPTION OF VARIOUS EMBODIMENTS

10

[0042] It should be understood that the phrase "a" or "an" used in conjunction with the applicants' teachings with reference to various elements encompasses "one or more" or "at least one" unless the context clearly indicates otherwise. Figure 1 schematically illustrates a system 100 for reducing interferences in the spectrometric analysis of a sample from a blood collection tube in accordance with various embodiments of the applicant's teachings. The system 100 comprises an ion source (not shown) that ionizes a sample, containing one or more steroids, extracted from a blood collection tube. The blood collection tube can separate plasma and serum from the red blood cells in the sample. In various aspects, a sample preparation process, such as online solid-phase extraction, protein precipitation, liquid-liquid extraction, or solid-phase extraction can provide cleaner extracts prior to being introduced into a liquid chromatography apparatus comprising a column before ionization.

[0043] In various embodiments, the differential mobility spectrometer (DMS) 102 can receive the ions from the ion source. In various embodiments, a boundary member 119 defining a curtain gas chamber 118 can contain the DMS 102. A curtain gas supply 120 can provide a curtain gas into an inlet 110 of the DMS 102 for providing a gas flow through the DMS 102 and a curtain gas outflow 126 out of a curtain gas chamber inlet 122. In various

- 11 -

embodiments, the one or more steroids comprises testosterone. In various aspects the system 100 further comprises a mass spectrometer (MS) 104 in fluid communication with the DMS 102 for receiving the ions from the DMS 102. Mass spectrometer 104 can also comprise mass analyzer elements 104a downstream from vacuum chamber 127. Ions can be transported through vacuum chamber 127 and may be transported through one or more additional differentially pumped vacuum stages prior to the mass analyzer 104a. It will be apparent to those of skill in the art that there may be a number of other ion optical elements in the system that have not been described. The differential mobility spectrometer/mass spectrometer coupling described can be applicable to many mass spectrometer systems including, but not limited to, time of flight (TOF), ion trap, quadrupole, or other mass analyzers as known in the art.

[0044] The differential mobility spectrometer 102 comprises plates 106 and an electrical insulator 107 along the outside of plates 106. In various embodiments, the DMS 102 comprises straight or curved electrodes. The plates 106 surround a drift gas 108 that drifts from an inlet 110 of the differential mobility spectrometer to an outlet 112 of the differential mobility spectrometer 102. The insulator 107 supports the electrodes and isolates them from other conductive elements. In various embodiments, the outlet 212 of the DMS 102 is aligned with the inlet of the mass spectrometer 104 to define the ion path of travel between the DMS 102 and the mass spectrometer 104.

[0045] The differential mobility spectrometer 102 can be contained within a curtain chamber 118, defined by curtain plate (boundary member) 119 and supplied with a curtain gas from a curtain gas source 120. The curtain gas source 120 provides the curtain gas to the interior of the curtain chamber 118. Ions 122 are provided from an ion source (not shown) and are emitted into the curtain chamber 118 via curtain chamber inlet 124. The pressure of the curtain gas within the curtain chamber 118 provides both a curtain gas outflow 126 out of curtain gas chamber inlet 124, as well as a curtain gas inflow 128 into the differential mobility spectrometer 102, which inflow 128 becomes the

- 12 -

drift gas 108 that carries the ions 122 through the differential mobility spectrometer 102 and into mass spectrometer 104. The curtain plate 119 may be connected to a power supply to provide an adjustable DC potential to it.

- [0046] As illustrated in Figure 1, the mass spectrometer 104 is contained within a vacuum chamber 127, which can be maintained at a much lower pressure than the curtain chamber 118. The vacuum chamber 127 can be maintained at a pressure of 2.3 Torr by a vacuum pump 130 while the curtain chamber 118 and an internal operating pressure of the differential mobility spectrometer 102 can be maintained at a pressure of 760 Torr. As a result of the significant pressure differential between the curtain chamber 118 and the vacuum chamber 127, the drift gas 108 is drawn through the differential mobility spectrometer 102, and, via vacuum chamber inlet 129, into the vacuum chamber 127. The mass spectrometer 104 can be sealed to (or at least partially sealed), and in fluid communication with the differential mobility spectrometer to receive the ions 122 from the differential mobility spectrometer 102. In various aspects, an electrical field can be provided between the DMS 102 and the vacuum chamber 127 for guiding the ions into the vacuum chamber. In various embodiments, the system can further comprise a detector for detecting the ions received by the mass spectrometer.
- [0047] As known in the art, RF voltages, often referred to as separation voltages (SV), can be applied across an ion transport chamber of a differential mobility spectrometer perpendicular to the direction of drift gas 108 (shown in Figure 1). The RF voltages may be applied to one or both of the DMS electrodes comprising the differential mobility spectrometer. The tendency of ions to migrate toward the walls and leave the path of the DMS can be corrected by a DC potential often referred to as a compensation voltage (CV). The compensation voltage may be generated by applying DC potentials to one or both of the DMS electrodes comprising the differential mobility spectrometer. As is known in the art, a DMS voltage source (not shown) can be provided to provide both the RF SV and the DC CV. Alternatively, multiple voltage sources may be provided.

- 13 -

[0048] In various aspects, at least one heater can be provided to heat at least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of the mass spectrometer.

5

[0049] Referring to Figure 2, there is schematic illustration of a system 300 in accordance with various embodiments of the applicant's teachings. For clarity, the same reference numerals used in Figure 1, with 200 added, are used in Figure 2 to designate elements analogous to the elements of Figure 1.

10 For brevity, the description of Figure 1 is not repeated with respect to Figure 2.

[0050] In the system 300 of Figure 2 drift gas 308 can be drawn through the differential mobility spectrometer 302 and into the vacuum chamber 327 and the mass analyzer 304 by the much lower pressure maintained in the vacuum chamber 327.

[0051] As with the system 100 of Figure 1, the resolution or selectivity of system 300 can be adjusted by adding a throttle gas to a juncture chamber 314 between the differential mobility spectrometer 302 and the vacuum chamber inlet 329. In the system 300 of Figure 2, a common source is provided for both the curtain gas and the throttle gas; however, separate sources may also be provided. For example, this gas could be nitrogen. The throttle gas flows through a conduit branch 320a into the juncture chamber 314. The throttle gas throttles back the flow through the differential mobility spectrometer. In various embodiments, there can be a gas port 332 having a gas outlet located between the differential mobility spectrometer and the mass spectrometer for drawing a gas outflow from the gas flow to modify the gas flow rate through the differential mobility spectrometer. In various aspects, the flow rate of the gas outflow is varied to increase the gas flow rate.

[0052] In various aspects, as shown in Figure 2, gas ports 332 are oriented or inclined such that the throttle gas flows into the juncture chamber 314 at an orientation that is toward the vacuum chamber 327 and away from the

20
25
30

- 14 -

differential mobility spectrometer 302. In various embodiments, the gas ports 332 can be oriented such that the throttle gas flows along the sidewalls of the juncture chamber, somewhat parallel to the ion path of travel. In various embodiments, the juncture chamber can be designed with a much larger diameter than the diameter of the insulator, and the gas port can be oriented such that the gas stream through the inlet is directed along the wall.

[0053] Conduit branch 320a comprises a controllable valve 320b that can be used to control the rate of flow of the throttle gas into the juncture chamber 314. For example, to increase resolution or selectivity, at the price of an acceptable loss in sensitivity, the controllable valve 320b could be opened to admit more throttle gas into the juncture chamber 314 via conduit branch 320a to reduce the gas flow rate within the differential mobility spectrometer 302. This, in turn, can increase the residence time of the ions 322 within the differential mobility spectrometer. The increased residence time can produce narrower mobility peak widths, and therefore, improved selectivity. At the same time, the increased residence time lowers sensitivity somewhat due to increased diffusion losses. At the same time, because of the increased residence time within the differential mobility spectrometer, more of the ions can be lost.

[0054] As shown, Figure 2 also can comprise a valve 320c for controlling the rate of flow of the curtain gas into the curtain chamber 318. As shown in Figure 2, the system can comprise a common gas supply to provide both the curtain gas and the throttle gas flows.

[0055] Referring to Figure 3, there is schematic illustration of a system 200 in accordance with various embodiments of the applicant's teachings. For clarity, the same reference numerals used in Figure 1, with 100 added, are used in Figure 2 to designate elements analogous to the elements of Figure 1. For brevity, the description of Figure 1 is not repeated with respect to Figure 2.

[0056] In Figure 3, the differential mobility spectrometer 202 is contained within a curtain chamber 218, defined by a curtain plate or boundary member

- 15 -

219, and supplied with a curtain gas from a curtain gas supply 220.

Specifically, curtain gas from curtain gas supply 220 can flow through curtain gas conduit 220a at flow rates determined by flow controller 220b. The system 200 also comprises a LC pump and organic acid modifier supply 214 for

5 pumping precise amounts of an organic acid modifier liquid into the curtain gas conduit 220a at T-juncture 216. Specifically, LC pump 214 comprises a meter (not shown), which can be changed to set a selected volumetric flow rate of the modifier liquid into the juncture 216. Based on this adjustment of the meter, the LC pump 214 is then operable to provide an actual volumetric
10 flow rate of the modifier liquid to the juncture 216 and into curtain gas conduit 220a to mix with the curtain gas.

[0057] It will be apparent to those of skill in the art that LC pump 214 could be replaced with a syringe pump or other accurately controllable dispensing devices for dispensing the organic acid modifier liquid. Whatever dispensing
15 device is used, this device can be sufficiently accurate such that the actual volumetric flow rate is within a relatively small percentage deviation of the selected volumetric flow rate.

[0058] Referring to Figure 4, there is schematically illustrated system 700 in accordance with various embodiments of the applicant's teachings. For clarity,
20 the same reference numerals used in Figure 2, with 400 added, are used in Figure 6 to designate elements analogous to the elements of Figure 2. For brevity the descriptions of preceding Figures are not repeated with respect to Figure 6.

[0059] As shown in Figure 4, a curtain gas supply 720 comprises a
25 controllable valve 720b that can be used to control the rate of flow of the throttle gas into the juncture chamber 714 via conduit branch 720a. Conduit or curtain gas supply 720 also comprises a valve 720c for controlling the rate of flow of the curtain gas that will ultimately end up in the curtain chamber 718. The flow of the curtain gas downstream of valve 720c is divided into two
30 branches 720d and 720e. The flow of the curtain gas within branch 720d is

- 16 -

controlled by valve 720f. Similarly, the flow of the curtain gas within branch 720e is controlled by valve 720g.

[0060] The flow of the curtain gas through branch 720d passes into a bubbler 720h, which can be used to add an organic acid modifier liquid to the curtain gas/drift gas, which passes through branch 720d and will ultimately be pumped into the differential mobility spectrometer 702 by the vacuum maintained in the vacuum chamber 727. Similarly, a separate organic acid modifier can be added to the curtain gas flowing through branch 720e in bubbler 720i. The curtain gas outflows from the bubblers 720h and 720i can be controlled by outlet valves 720j and 720k respectively, after which the two branches 720d and 720e merge and then release the curtain gas with the modifiers into the curtain chamber 718. As noted above, the curtain gas and drift gas are one and the same; thus, adding the organic acid modifier to the curtain gas adds simplicity to the system 700. Organic acid modifiers can be vapors that provide selectivity by clustering with ions to different degrees, thereby shifting the differential mobility. Examples of organic acid modifiers can include formic acid, any others to list?. For example, the applicant found unexpected results when adding formic acid as a modifier to a sample containing testosterone. The formic acid modifier or additive to the drift gas shifted the observed compensation voltage for the testosterone peak at a given AC amplitude. The compensation voltage is related to the ratio of high to low field mobility.

[0061] According to various aspects of the applicant's teachings, instead of supplying a throttle gas to a juncture chamber between the differential mobility spectrometer and the mass spectrometer, a bleed gas can be drawn from the gas flow between the differential mobility spectrometer and the mass spectrometer at a bleed gas flow rate to increase a gas flow rate through the differential mobility spectrometer. The bleed gas flow rate can be varied to vary the increase in the gas flow rate. That is, in embodiments in which a bleed gas is drawn from the gas flow between the differential mobility spectrometer and the mass spectrometer, an electrode geometry of the differential mobility spectrometer can initially be selected to provide good

- 17 -

selectivity at the price of poor or very poor sensitivity. Then, sensitivity can be improved, while selectivity is diminished, by increasing the bleed gas flow rate of the bleed gas drawn from the gas flow between the differential mobility spectrometer and the mass spectrometer.

5 [0062] Figure 4a shows the types of BD Vacutainer® blood collection tubes that were evaluated.

[0063] Figure 5 shows interferences that are present in MS/MS spectra of a sample extracted from a BD Vacutainer® PST™ Gel and Lithium Heparin (green cap) blood collection tube. The interferences generate signal as m/z
10 97 and 109 coinciding with the fragment ions of testosterone.

[0064] Figure 6 shows interferences that are present in MS/MS spectra of a sample extracted from a BD Vacutainer® SST™ serum separation tube (gold cap). Two of the major interferences generate signal at m/z 97 and 109 coinciding with the fragment ions of testosterone.

15 [0065] Figure 7 shows parameter settings of the DMS-MS system according to various embodiments of the applicant's teachings.

[0066] Figure 7a shows the experimental conditions for Figures 8, 9, 10, and 11.

[0067] Figures 8, 9, 10, and 11 compare the interferences present in
20 testosterone plasma samples with the DMS off and with the DMS on according to various embodiments of the applicant's teachings. Interferences from the blood collection tube are reduced when the DMS is on. The expected fragment ion ratio between MRM 289-109 and 289-97 is only observed when data is collected with the DMS on.

25 [0068] Figures 11a and 11b show that the BD Vacutainer® PST™ and SST™ blood collection tubes can contribute to interferences in the analysis of testosterone and the shifting of testosterone from these interferences with the DMS on.

[0069] Figure 12 illustrates the compensation voltage (CV) shift for
30 testosterone in the presence of formic acid according to various embodiments

- 18 -

of the applicant's teachings. The shift enables testosterone to be distinguished from the interferences.

[0070] Figure 13 shows a compensation voltage map displaying the shift for testosterone in the presence of formic acid according to various embodiments of the applicant's teachings. In an analysis of water exposed to a serum separation tube and testosterone standard, the CV for the three interferences shifted to positive values at a separation voltage (SV) of 3200 while testosterone shifted to a negative value at SV of 3200 using SIM mode with formic acid as a dopant (1.5% in curtain gas) enabling testosterone to be discernible from the interferences.

[0071] Figure 14 shows a compensation voltage map displaying the shift in testosterone in the presence of formic acid in a urine sample according to various embodiments of the applicant's teachings. In an analysis of a mixture of male and female urine diluted one-to-one in water and spiked with 20 pg/ul of testosterone with a SV of 3200 using SIM mode and formic acid as a dopant (1.5% in curtain gas), the interferences shift to a positive CV while testosterone shifts to a negative CV enabling testosterone to be distinguished from the interferences.

[0072] Figure 15a compares the interferences present for a panel of steroid (10 steroids, 21 MRM pairs – list supplied in Figure 15b) extracted from pooled female and male plasma protein precipitated (PPT) that was obtained from BD Vacutainer® SST™ (Gold Cap) with the DMS off and with the DMS on according to various embodiments of the applicant's teachings. Interferences from the blood collection tube are reduced when the DMS is on. The expected fragment ion ratio between androstenedione (287-97 and 287-109) and progesterone (315-97 and 315-109) are preserved when data is collected with the DMS on. The majority of the other LC interferences are eliminated when the DMS is on. The arrow indicate the retention time for androstenedione and progesterone. Similar results were obtained for other steroids monitored.

- 19 -

[0073] Figure 16a highlights the regions where chemical interferences introduced by the blood collection tube have CoV values ranging from 7.0 to 10.5 when the separation voltage (SV) of the DMS is set at 3200V. Figure 16b shows the TIC for the steroid panel (from Figure 15b) extracted from
5 pooled female and male plasma protein precipitated (PPT) that was obtained from BD Vacutainer® SST™ (Gold Cap) with the DMS off (top panel), the DMS on at CoV 9.0 as representative of the chemical noise introduced from the blood collection tube (middle panel), and the DMS on at CoV 4.5 as representative region of steroid panel (bottom panel). As can be seen, the
10 majority of the interferences originate from the interference region (CoV 9.0 – estimated as > 70% of signal) and can mask the detection of low intensity analytes. The steroid region (as CoV 4.5) represents approximately 25% of the signal in this particular sample. More importantly, low level steroids are now free of interferences which can enable easier detection, lower detection
15 limits, and the possibility to reduce the analysis time by shortening the LC gradient.

[0074] The following describes a general use of the applicants' teachings which is not limited to any particular embodiment, but can be applied to any embodiment. In operation, a sample, containing one or more steroids, from a
20 blood collection tube can be extracted using techniques, such as on-line extraction, protein precipitation, liquid-liquid extraction, or solid-phase extraction, as known in the art. The one or more steroids in the sample can comprise testosterone. The sample can comprise plasma or serum. The extracted sample can be introduced into a liquid chromatography apparatus
25 comprising a column prior to ionization of the sample. An ion source can then ionize the sample and a DMS can receive the ions from the ion source. A boundary member defining a curtain gas chamber containing the DMS can be provided. Furthermore, a curtain gas provided from a curtain gas supply can enter into an inlet of the DMS to provide a gas flow through the DMS and a
30 curtain gas outflow out of a curtain gas chamber inlet. A mass spectrometer can be in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer. A gas outflow

- 20 -

between the differential mobility spectrometer and the mass spectrometer can be drawn from the gas flow to modify the gas flow rate through the differential mobility spectrometer. The flow rate of the gas outflow can be varied to increase the gas flow rate. A vacuum chamber can surround the mass spectrometer for maintaining the mass spectrometer at a vacuum pressure to draw the gas flow including the ions through the differential mobility spectrometer and into the vacuum chamber. An electrical field can be provided between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber and to prevent ions from being drawn out of the gas outlet. The differential mobility spectrometer can comprise one of straight and curved electrodes. At least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of the mass spectrometer can be heated.

[0075] The following describes another general use of the applicants' teachings which is not limited to any particular embodiment, but can be applied to any embodiment. In operation, a sample can be introduced into a liquid chromatography apparatus comprising a column prior to ionization. An ion source is provided for ionizing a sample containing one or more steroids. A differential mobility spectrometer is provided for receiving the ions from the ion source and a drift gas supply is provided for providing a drift gas for flowing through the differential mobility spectrometer, and an organic acid modifier supply can be provided for supplying an actual volumetric flow rate of an organic acid modifier to the drift gas supply. The one or more steroids can comprise testosterone. The organic acid modifier can be formic acid. A meter can be provided for setting a selected volumetric flow rate, and the actual volumetric flow rate can be within a percent deviation from the selected volumetric flow rate. A pump can be provided for supplying the actual volumetric flow rate of the modifier liquid to the drift gas supply. The drift gas supply can be a curtain gas supply for providing a curtain gas, and a boundary member can be provided for directing at least a portion of the curtain gas to an inlet of the differential mobility spectrometer to become the

- 21 -

drift gas. A curtain chamber can be provided for receiving the curtain gas with the added organic acid modifier, wherein the curtain chamber comprises a curtain wall surrounding the differential mobility spectrometer, and the boundary member can be a portion of the curtain wall. The drift gas supply
5 comprises providing a drift gas conduit, the organic acid modifier supply comprises providing a modifier conduit, and a junction can be provided for merging the modifier conduit into the drift gas conduit, and the pump can be located in the modifier conduit upstream of the junction to provide the actual volumetric flow rate of the modifier liquid to the drift gas conduit. The junction
10 can be heated. A mass spectrometer can be in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer. A detector can detect the ions received by the mass spectrometer. An electrical field can be provided between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the
15 vacuum chamber. The differential mobility spectrometer can comprise straight or curved electrodes. The curtain chamber can be heated.

[0076] Example

[0077] Materials and Methods

[0078] Sample Preparation and Chemicals: All steroids were obtained from
20 Steraloids Inc (Newport, RI) and Cerilliant (Round Rock, TX) and prepared as stocks of 1mg/mL in methanol if received in crystal form. Serial dilutions to appropriate levels were made using either sample matrix (urine or acetonitrile protein-precipitated plasma diluted 1:1 in water). Formic acid was obtained from Sigma (St-Louis, MO).

25 [0079] HPLC Conditions: LC was performed using a Shimadzu Prominence UFLC system operated at 400uL/min using a gradient from 90% of mobile phase A (water/acetonitrile (95/5 (v/v)) + 0.1% formic acid) to 80% of B (water/acetonitrile (5/95 (v/v)) + 0.1% formic acid) over 5 minutes. The column oven was operated at 40oC. Two types of HPLC columns were used;
30 1) Luna Kinetex C18 (2x50mm, 2.6u) from Phenomenex (Torrance, CA) and

- 22 -

2) BDS Hypersil Gold (2x50mm, 3u) from Thermo (Ottawa, ON). The injection volume was 10uL.

[0080] DMS and MS/MS Conditions: The SelexION™ Technology is a planar differential mobility separation (DMS) device that attaches between the curtain plate and orifice plate of the 5500 QTRAP® system. Gas draws the ions towards the orifice while an asymmetric waveform applied to the flat plates, which alternates between high field, $K(E)$ and low field, $K(0)$. This moves the charged ion back and forth between plates; an ion will have net drift base on its high and low field mobility. Finally a compensation voltage (CoV, a small DC offset between the plates) is applied at a filtering voltage which is specific for each ion being targeted. The chemical modifier is introduced with a liquid pump directly into the curtain gas stream. Formic acid was introduced directly into the gas stream, and the Turbo V® source was operated with the electrospray ionization (ESI) probe and the source was operated at 650°C with Gas 1 and Gas 2 at 40 and 80 psi, respectively.

[0081] Results: Figure 17 shows the structure of some of steroids used. These were selected to cover a variety of structural features typically encountered in steroids. In all cases, a CoV mapping/optimization was performed under analytical conditions either by using a split-infusion approach post-LC or by injecting on-column and performing monitoring at multiple CoV values (typically ranging from -20 to 20 with increments of 1.25V). Figure 18A shows the CoV shifting towards higher positive values as the SV is increased to 3500V when nitrogen is used as the transporter gas. When the transport gas is doped with 1.5% (v/v) with formic acid, the CoV shift towards negative values under similar conditions (Figure 18B). Similar observations were made with all of the steroids investigated and the CoV values for SV=3500 value are listed in Figure 19. It is not uncommon to observe loss in signal associated with the analyte ion when chemical modifiers are added to the transport region. The losses observed can vary with the compound used and the chemical modifiers used. In the case of steroids, addition of polar alcohols such as 2-propanol (IPA) and methanol (MeOH) which are frequently used to enhance the separation of other compounds, can greatly reduce the intensity

- 23 -

of steroid signals. However, when formic acid is used, a reduction of the signal ranging from 20 to 50% can be observed, depending on the steroid monitored (Figure 20). Testosterone was the species that showed the largest drop in signal (~50%). Though this reduction in signal can be considered

5 large, it can be viewed as similar to losses observed when MSMS is performed to gain selectivity over MS analysis. Figure 21 shows the relative selectivity when the detection testosterone from protein precipitated plasma is performed by single ion monitoring (SIM) in 3 modes of operation for the DMS cell: 1) all DMS voltages turned off, 2) DMS with no chemical modifier and 3)

10 DMS with formic acid in transport gas. Figure 22 shows the relative selectivity when the detection testosterone from protein precipitated plasma is performed in multiple reaction monitoring (MRM) mode in 3 modes of operation for the DMS cell: 1) all DMS voltages turned off, 2) DMS with no chemical modifier and 3) DMS with formic acid in transport gas. Most steroids showed similar

15 optimum CoV values when nitrogen is used without modifier (CoV range of 4 volts). However, the addition of formic acid in the transport gas increased the observed separation by LC between steroids (CoV range of 10V). Though not all of the steroids selected were isobaric and separate, some clearly became separated with formic acid in the transport gas (androstenedione and 1-

20 dehydrotestosterone). The one common behavior observed with formic acid as chemical modifier is that all steroids shifted toward negative CoV values (a-type ions). Of even more interest is the behavior of the majority of endogenous ions generated by ESI. In general, the greater majority of the other ions typically generated by ESI do not seem to be affected by the

25 presence of formic acid in the transport gas, and consequently their CoV value tend to be more positive as SV is increased (c-type ions). These combined effects clearly increase the separation of the steroids from the endogenous species. This is demonstrated in Figure 13 when protein precipitated plasma is monitored in SIM mode for the analysis of testosterone

30 at various CoV values (y-axis) with formic acid in the transport gas. The majority of the isobaric endogenous species have positive CoV values ranging from 5 to 10 volts with formic acid (note that CoV values ranged from 6 to 11

- 24 -

when transport gas is nitrogen only). However, testosterone shifts to a negative CoV value, away from most interferences. Figure 14 shows similar observation when a diluted urine sample is analyzed with formic acid in the transport gas. Again, the majority of the endogenous species that are isobaric (m/z 289) have a positive CoV values, where as testosterone has a negative CoV value.

[0082] Using formic acid in the transport gas enabled separation of androstenedione and 1-dehydrotestosterone which have common precursor masses. Figures 23 and 24 show the CoV map associated with detection of androstenedione and 1-dehydrotestosterone in SIM mode. Figure 24A shows a 2-D view and Figure 24B shows a 3-D view of the same data. Both analytes have unique CoV values with little overlap, thus enabling selective detection even in SIM mode. Though both have unique dominant MRM fragments to ensure selectivity, this was used as a case to compare the selectivity of DMS-SIM with formic acid. Figure 25 shows that DMS-SIM with formic acid gives a level of selectivity that is comparable to MRM alone as similar levels of interference were observed when equimolar levels were indicated. This can emphasize the reliance on the LC separation if using DMS-SIM with formic acid or alternatively relax the LC separation and combine DMS-MRM with formic acid which can obtain ultimate selectivity.

[0083] Conclusions: The addition of formic acid to the transport gas of a DMS cell can show benefit for the analyses of steroids. The steroids can preferentially shift toward negative CoV values when many other endogenous species seem to be essentially unaffected by the presence of formic acid and their CoV typically stay positive. Though the addition of formic acid may reduce the signal intensity by 20-50% when compared to no modifier added in the transport cell, this approach can offer the ability to detect the steroid with a degree of selectivity with SIM detection that can rival MRM detection. The exact nature of the interaction between formic acid and the steroids in the DMS cell can be complex, but it is clearly unique and it can be likely associated with the site of protonation of the analyte. When compared to species where the charge is localized on nitrogen groups (whether primary,

- 25 -

secondary or tertiary amines), the addition of formic acid in the transport gas did not show any shift in CoV values for given SV. For these nitrogen containing species, addition of alcohol in the transport gas causes their CoV to shift towards negative values. The addition of alcohol, even low proton
5 affinity ones such as methanol, can eliminate all of the signal associated with steroids, which is not the case with formic acid. This combination of DMS with formic acid in the transport gas can simplify the MS detection part for the analysis of steroids while still providing a high degree of selectivity.

10 **[0084]** While the Applicant's teachings are described in conjunction with various embodiments, it is not intended that the Applicant's teachings be limited to such embodiments. On the contrary, the Applicant's teachings encompass various alternatives, modifications and equivalents as will be appreciated by those of skill in the art. All such alternatives, modifications and
15 equivalents are believed to be within the sphere and scope of the invention as defined by the claims appended hereto.

[0085] All literature and similar material cited in this application, including, but not limited to, patents, patent applications, articles, books, treatises, and web pages, regardless of the format of such literature and similar materials,
20 are expressly incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

[0086] While the applicants' teachings have been particularly shown and
25 described with reference to specific illustrative embodiments, it should be understood that various changes in form and detail may be made without departing from the spirit and scope of the teachings. Therefore, all embodiments that come within the scope and spirit of the teachings, and equivalents thereto, are claimed. The descriptions and diagrams of the
30 methods of the applicants' teachings should not be read as limited to the described order of elements unless stated to that effect.

- 26 -

[0087] While the applicants' teachings have been described in conjunction with various embodiments and examples, it is not intended that the applicants' teachings be limited to such embodiments or examples. On the contrary, the applicants' teachings encompass various alternatives, modifications, and
5 equivalents, as will be appreciated by those of skill in the art, and all such modifications or variations are believed to be within the sphere and scope of the invention.

CLAIMS

1. A system for reducing interferences in the spectrometric analysis of a sample from a blood collection tube, comprising:
 - 5 a) an ion source for ionizing a sample extracted from the blood collection tube, the sample containing one or more steroids;
 - b) a differential mobility spectrometer for receiving the ions from the ion source;
 - c) a boundary member defining a curtain gas chamber containing the differential mobility spectrometer; and
 - 10 d) a curtain gas supply for providing a curtain gas into an inlet of the differential mobility spectrometer for providing a gas flow through the differential mobility spectrometer and a curtain gas outflow out of a curtain gas chamber inlet.
- 15 2. The system of claim 1 wherein the one or more steroids comprises testosterone.
3. The system of claim 1 further comprising a mass spectrometer in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer.
- 20 4. The system of claim 3 further comprising a gas port having a gas outlet located between the differential mobility spectrometer and the mass spectrometer for drawing a gas outflow from the gas flow to modify the gas flow rate through the differential mobility spectrometer.
5. The system of claim 4 wherein the flow rate of the gas outflow is varied to
- 25 increase the gas flow rate.

- 28 -

6. The system of claim 4 comprising a vacuum chamber surrounding the mass spectrometer for maintaining the mass spectrometer at a vacuum pressure to draw the gas flow including the ions through the differential mobility spectrometer and into the vacuum chamber.
- 5 7. The system of claim 6 further comprising an electrical field generator for providing an electrical field between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber and to prevent ions from being drawn out of the gas outlet.
8. The system of claim 1 wherein the differential mobility spectrometer
10 comprises one of straight and curved electrodes.
9. The system of claim 4 further comprising at least one heater to heat at least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of the mass spectrometer.
- 15 10. The system of claim 3 further comprising a detector for detecting the ions received by the mass spectrometer.
11. The system of claim 1 wherein extracting the sample from the blood collection tube comprises one of on-line extraction, protein precipitation, liquid-liquid extraction and solid-phase extraction.
- 20 12. The system of claim 1 wherein the sample comprises one of plasma and serum.
13. The system of claim 1 wherein the extracted sample is introduced into a liquid chromatography apparatus comprising a column prior to ionization.
14. A method for reducing interferences in the spectrometric analysis of a
25 sample from a blood collection tube, comprising:
 - a) providing an ion source for ionizing a sample extracted from the blood collection tube, the sample containing one or more steroids;

- 29 -

- b) providing a differential mobility spectrometer for receiving the ions from the ion source;
 - c) providing a boundary member defining a curtain gas chamber containing the differential mobility spectrometer; and
 - 5 d) providing a curtain gas supply for providing a curtain gas into an inlet of the differential mobility spectrometer for providing a gas flow through the differential mobility spectrometer and a curtain gas outflow out of a curtain gas chamber inlet.
15. The method of claim 14 wherein the one or more steroids comprises
- 10 testosterone.
16. The method of claim 14 wherein a mass spectrometer is in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer.
17. The method of claim 16 wherein a gas outflow between the differential
- 15 mobility spectrometer and the mass spectrometer is drawn from the gas flow to modify the gas flow rate through the differential mobility spectrometer.
18. The method of claim 17 wherein the flow rate of the gas outflow is varied to increase the gas flow rate.
- 20 19. The method of claim 17 comprising a vacuum chamber surrounding the mass spectrometer for maintaining the mass spectrometer at a vacuum pressure to draw the gas flow including the ions through the differential mobility spectrometer and into the vacuum chamber.
- 25 20. The method of claim 19 wherein an electrical field is provided between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber and to prevent ions from being drawn out of the gas outlet.

- 30 -

21. The method of claim 14 wherein the differential mobility spectrometer comprises one of straight and curved electrodes.
22. The method of claim 17 wherein at least one of the curtain gas, the gas outflow, a region upstream of the inlet of the differential mobility spectrometer, the inlet of the differential mobility spectrometer, and the inlet of the mass spectrometer is heated.
23. The method of claim 14 wherein extracting the sample from the blood collection tube comprises one of on-line extraction, protein precipitation, liquid-liquid extraction and solid-phase extraction.
24. The method of claim 14 wherein the sample comprises one of plasma and serum.
25. The method of claim 14 further comprising introducing the extracted sample into a liquid chromatography apparatus comprising a column prior to ionization.
26. A system for reducing interferences in the spectrometric analysis of steroids, comprising:
- a) an ion source for ionizing a sample containing one or more steroids;
 - b) a differential mobility spectrometer for receiving the ions from the ion source;
 - c) a drift gas supply for providing a drift gas for flowing through the differential mobility spectrometer; and
 - d) a modifier supply for supplying an actual volumetric flow rate of an organic acid modifier to the drift gas supply.
27. The system of claim 26 wherein the one or more steroids comprises testosterone.

- 31 -

28. The system of claim 26 wherein the organic acid modifier is formic acid.
29. The system of claim 26 further comprising a meter for setting a selected volumetric flow rate, and the actual volumetric flow rate is within a percent deviation from the selected volumetric flow rate.
- 5 30. The system of claim 29 further comprising a pump for supplying the actual volumetric flow rate of the modifier liquid to the drift gas supply.
31. The system of claim 29 wherein the drift gas supply is a curtain gas supply for providing a curtain gas, and the spectrometer system further comprises a boundary member for directing at least a portion of the curtain gas to an
10 inlet of the differential mobility spectrometer to become the drift gas.
32. The system of claim 31 further comprising a curtain chamber for receiving the curtain gas with the added organic acid modifier, wherein the curtain chamber comprises a curtain wall surrounding the differential mobility spectrometer, and the boundary member is a portion of the curtain wall.
- 15 33. The system of claim 26 wherein the drift gas supply comprises a drift gas conduit, the modifier supply comprises a modifier conduit, and the system further comprises a junction for merging the modifier conduit into the drift gas conduit, the pump being located in the modifier conduit upstream of the junction to provide the actual volumetric flow rate of the modifier liquid
20 to the drift gas conduit.
34. The system of claim 33 further comprising a heater for heating the junction.
35. The system of claim 26 further comprising a mass spectrometer in fluid communication with the differential mobility spectrometer for receiving the
25 ions from the differential mobility spectrometer.
36. The system of claim 35 further comprising a detector for detecting the ions received by the mass spectrometer.

- 32 -

37. The system of claim 35 further comprising an electrical field generator for providing an electrical field between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber.
38. The system of claim 26 wherein the differential mobility spectrometer
5 comprises one of straight and curved electrodes.
39. The system of claim 32 further comprising a heater for heating the curtain chamber.
40. The system of claim 26 wherein the sample is introduced into a liquid chromatography apparatus comprising a column prior to ionization.
- 10 41. A method for reducing interferences in the spectrometric analysis of steroids, comprising:
- a) providing an ion source for ionizing a sample containing one or more steroids;
 - b) providing a differential mobility spectrometer for receiving the ions
15 from the ion source;
 - c) providing a drift gas supply for providing a drift gas for flowing through the differential mobility spectrometer; and
 - d) providing a modifier supply for supplying an actual volumetric flow rate of an organic acid modifier to the drift gas supply.
- 20 42. The method of claim 41 wherein the one or more steroids comprises testosterone.
43. The method of claim 41 wherein the organic acid modifier is formic acid.
44. The method of claim 41 further comprising providing a meter for setting a selected volumetric flow rate, and the actual volumetric flow rate is within a
25 percent deviation from the selected volumetric flow rate.

- 33 -

45. The method of claim 44 further comprising providing a pump for supplying the actual volumetric flow rate of the modifier liquid to the drift gas supply.
46. The method of claim 44 wherein the drift gas supply is a curtain gas supply for providing a curtain gas, and a boundary member is provided for
5 directing at least a portion of the curtain gas to an inlet of the differential mobility spectrometer to become the drift gas.
47. The method of claim 46 wherein a curtain chamber is provided for receiving the curtain gas with the added organic acid modifier, wherein the curtain chamber comprises a curtain wall surrounding the differential
10 mobility spectrometer, and the boundary member is a portion of the curtain wall.
48. The method of claim 41 wherein the drift gas supply comprises providing a drift gas conduit, the modifier supply comprises providing a modifier conduit, and a junction is provided for merging the modifier conduit into the
15 drift gas conduit, the pump being located in the modifier conduit upstream of the junction to provide the actual volumetric flow rate of the modifier liquid to the drift gas conduit.
49. The method of claim 48 further comprising heating the junction.
50. The method of claim 41 further comprising providing a mass spectrometer
20 in fluid communication with the differential mobility spectrometer for receiving the ions from the differential mobility spectrometer.
51. The method of claim 50 further comprising detecting the ions received by the mass spectrometer.
52. The method of claim 50 further comprising providing an electrical field
25 between the differential mobility spectrometer and the vacuum chamber for guiding the ions into the vacuum chamber.

- 34 -

53. The method of claim 41 wherein the differential mobility spectrometer comprises one of straight and curved electrodes.
54. The method of claim 47 further comprising heating the curtain chamber.
55. The method of claim 41 further comprising introducing the sample into a
5 liquid chromatography apparatus comprising a column prior to ionization.

1/31

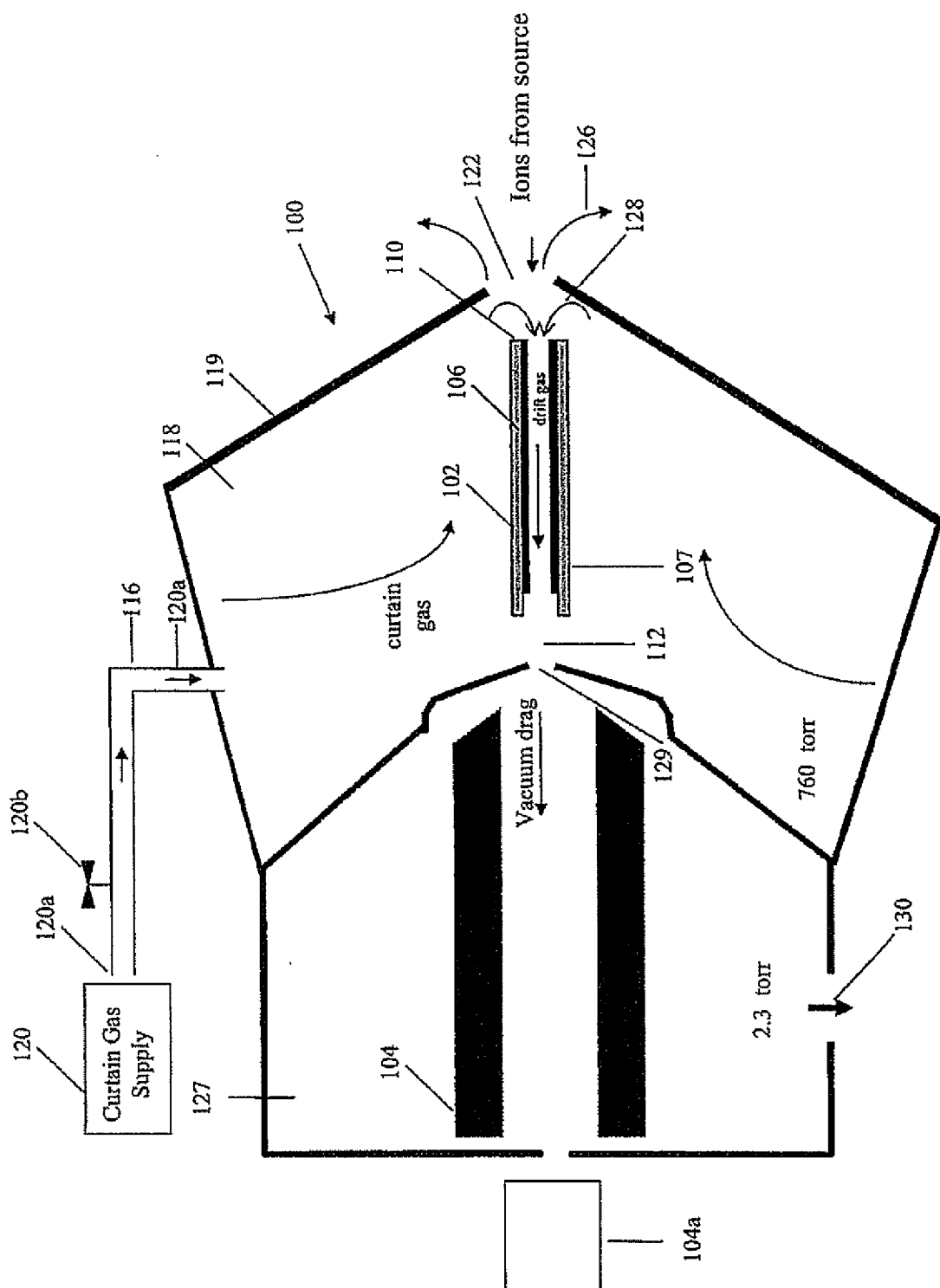


Figure 1

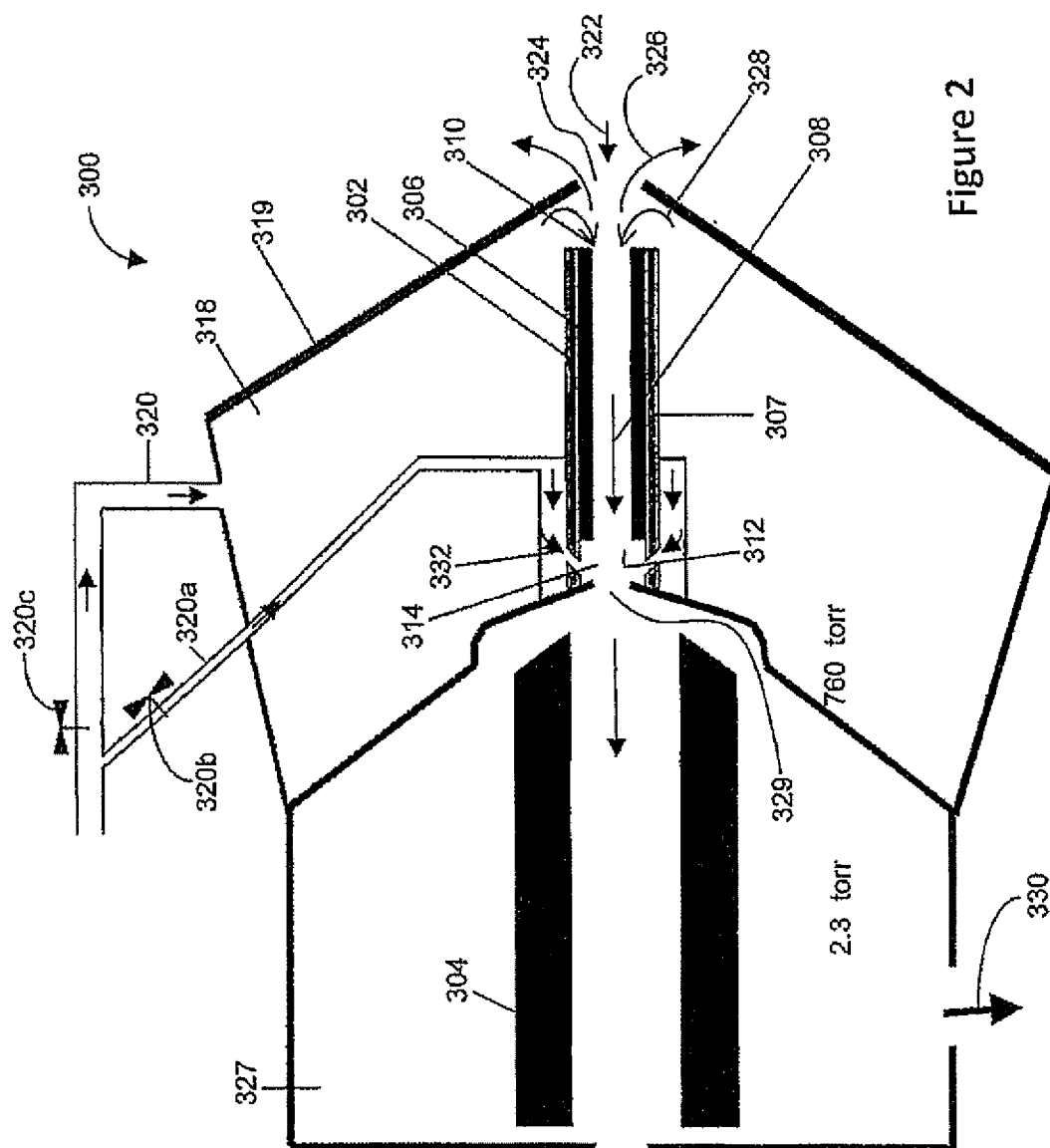


Figure 2

3/31

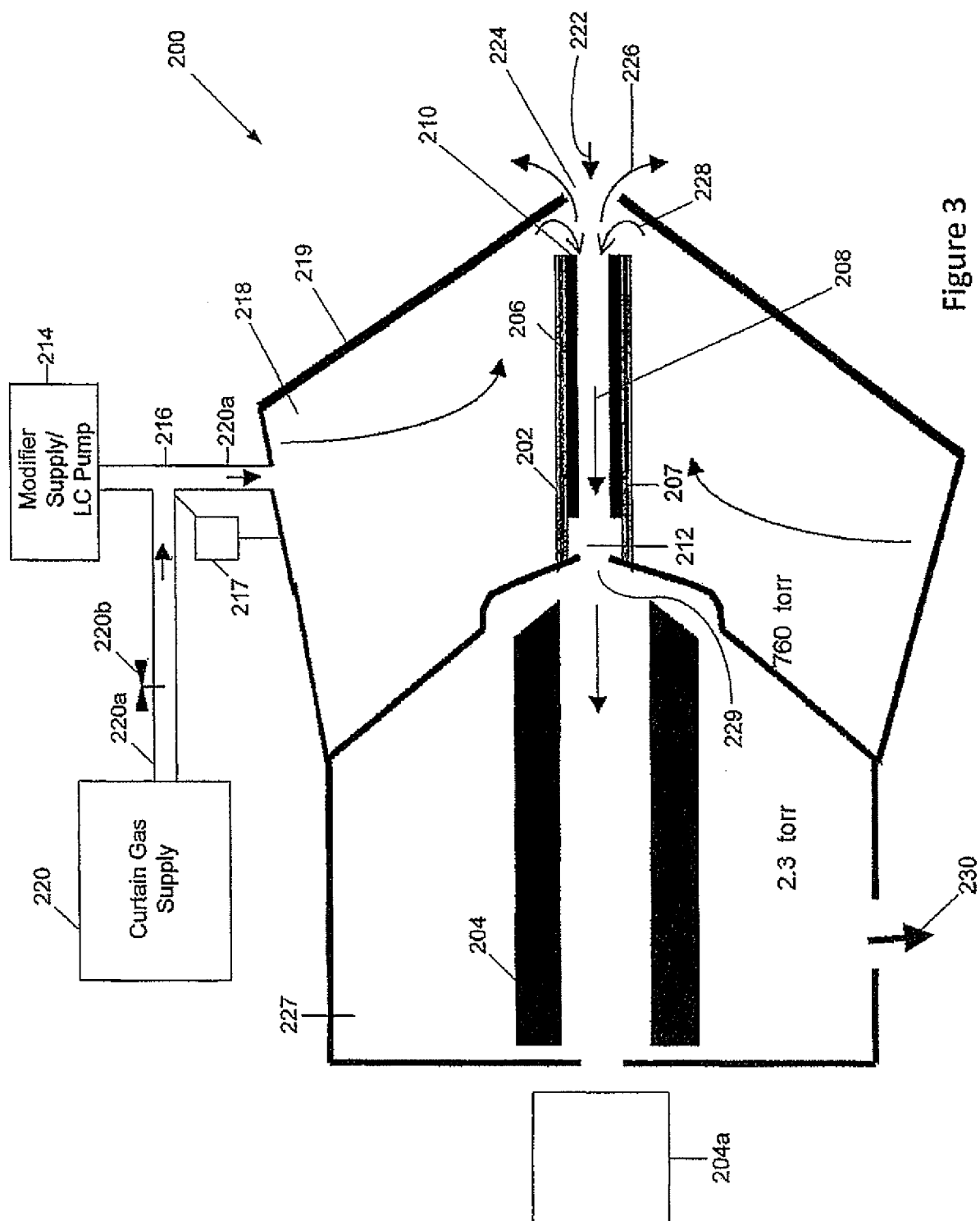


Figure 3

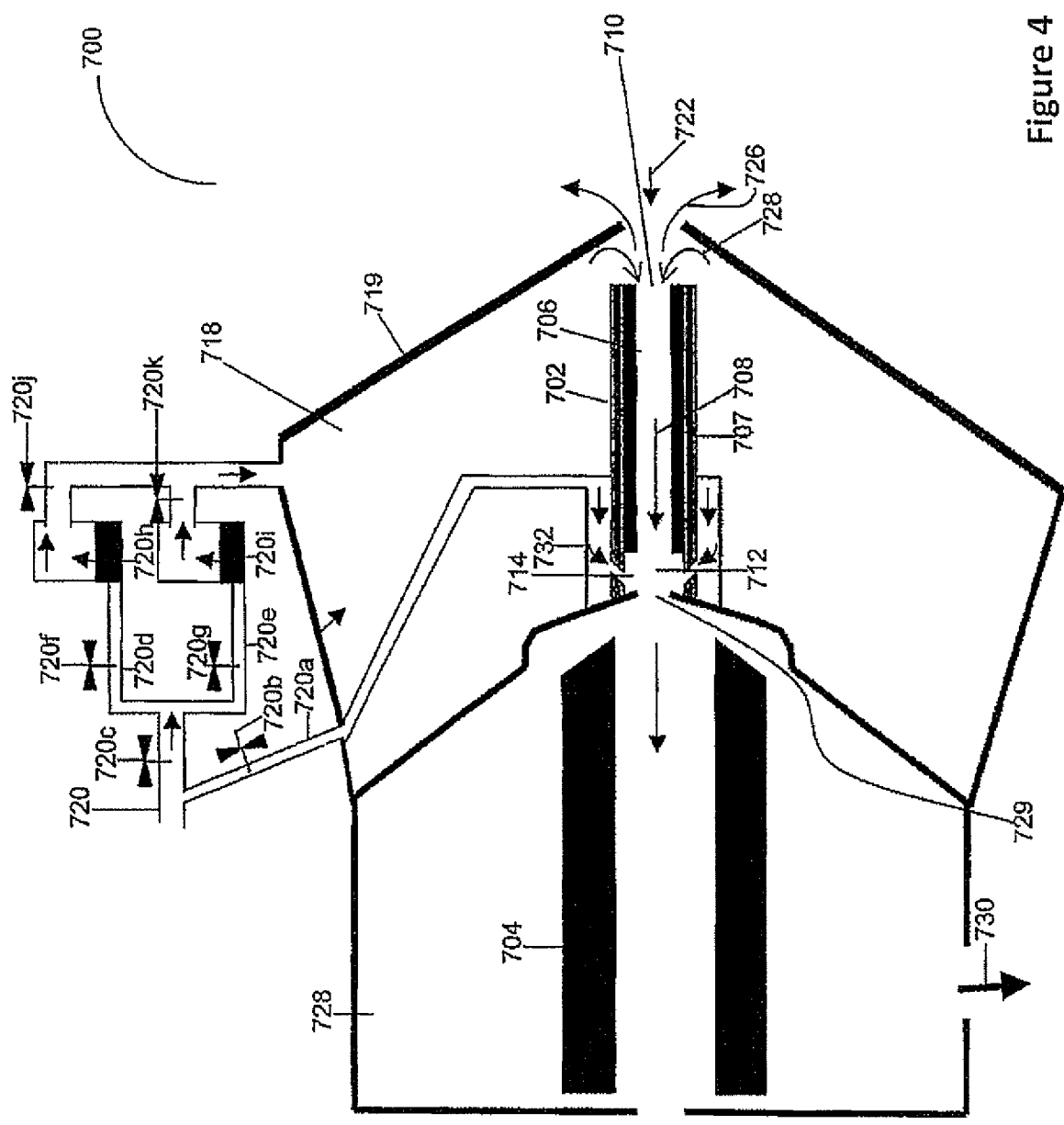


Figure 4

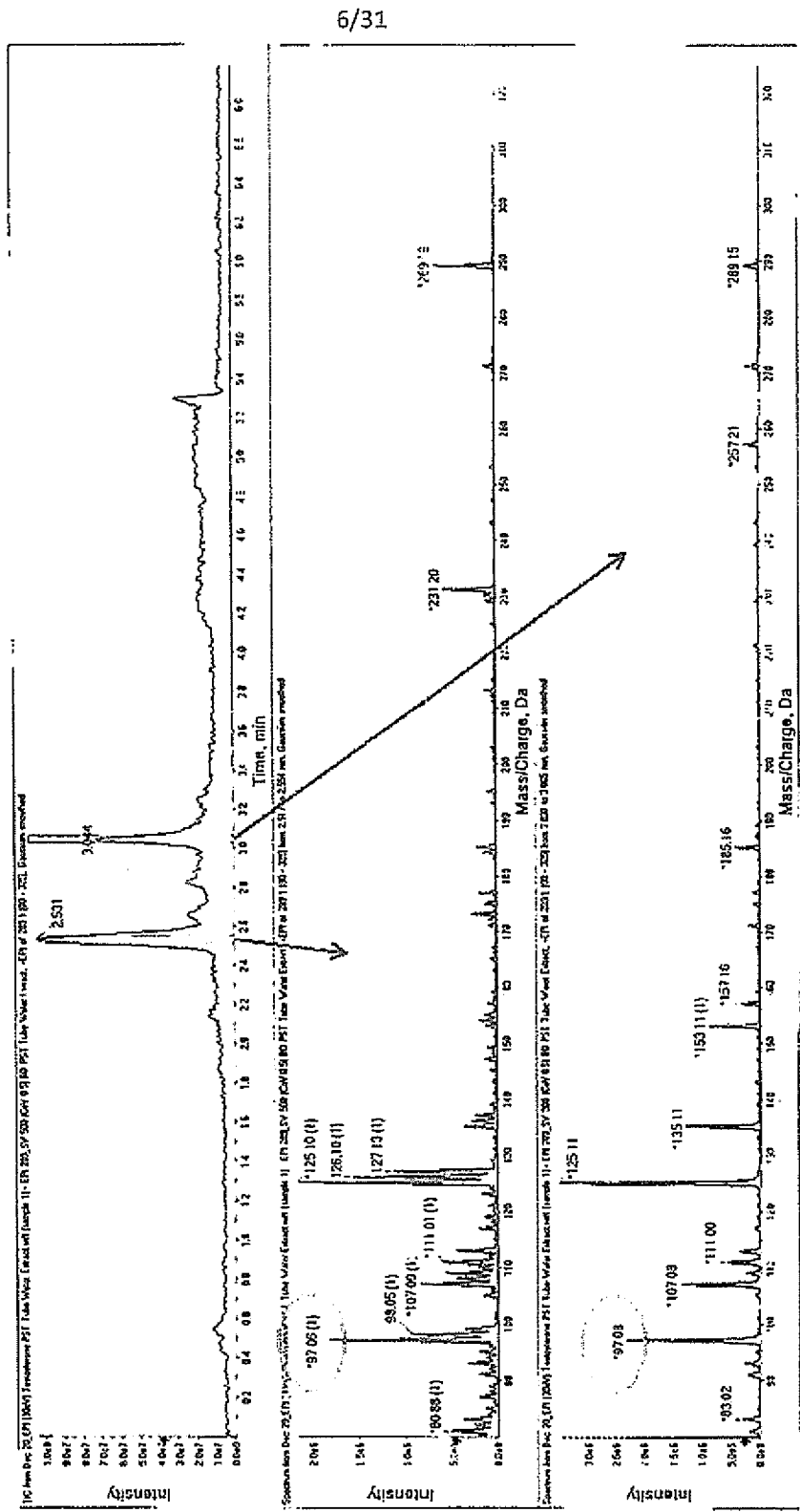
5/31

BD
Vacutainer®
tube tested

Tube Type	Ref #	CAP Color	Vol (mL)		NOTE
SST (serum separation tube)	Ref 367986	GOLD	5	gel	Most commonly used for majority of tests / highest chemical noise observed
PST Gel and Lithium heparin (83 units)	Ref 367962	LIGHT GREEN	4.5	gel	Proposed as alternative for steroid - monitor specific MRM
Serum	Ref 367815	RED	6		
Sodium Fluoride Potassium Oxalate 15mg/12mg	Ref 367925 (grey cap) 6mL Buffer Na Citrate 0.109M Ref 363083 (light blue cap) 2.7mL	GREY	6		
Buffer Na Citrate 0.109M	Ref 363083	LIGHT BLUE	2.7		
K2 EDTA 5.4mg	Ref 367856	LAVENDER	3		

Figure 4a

BD Vacutainer® PST™Gel and Lithium Heparin (green cap)
MSMS of 289 in EPI mode

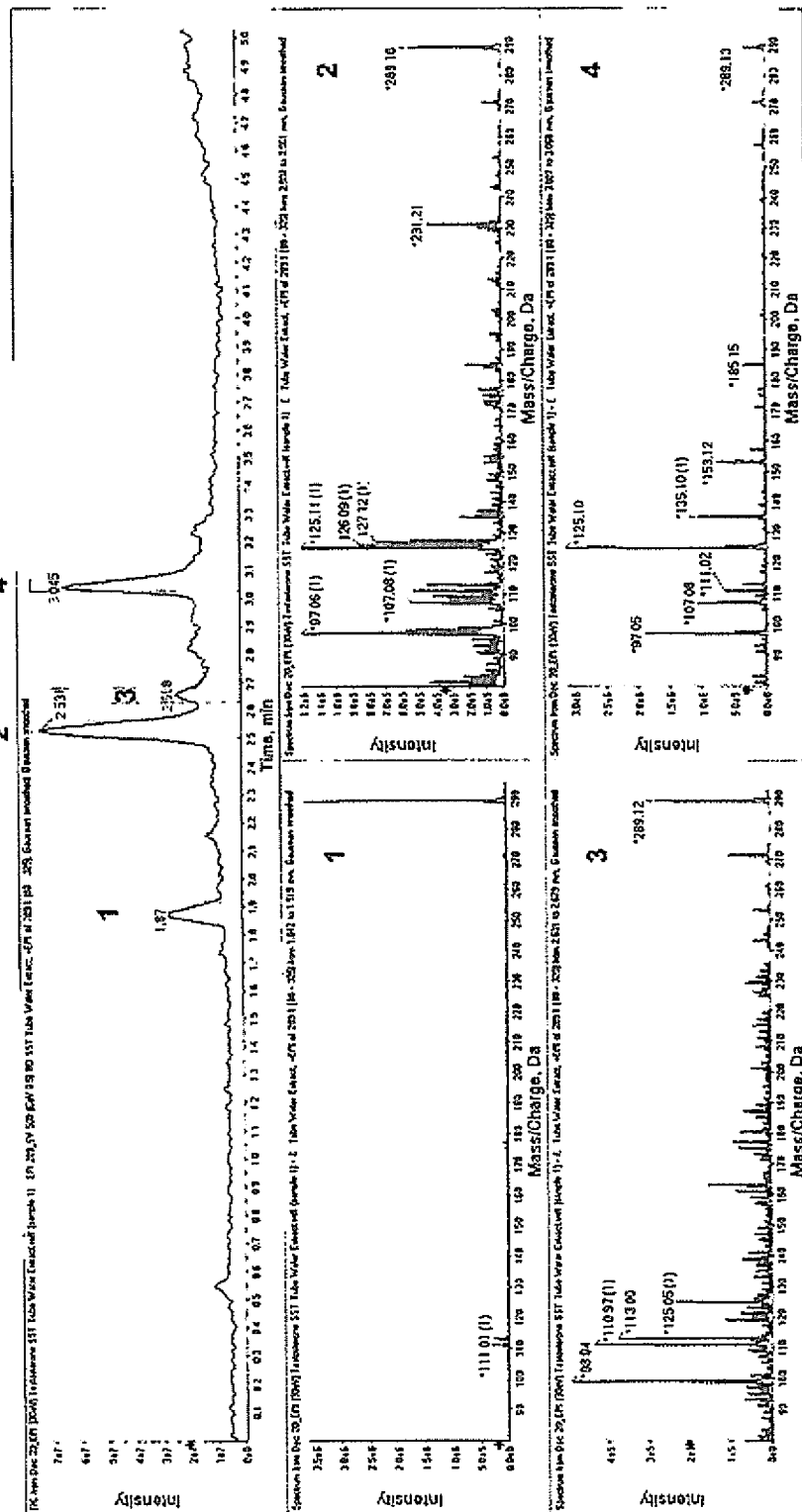


MSMS spectra of both interference that generate signal at m/z 97 and 109

Figure 5

7/31

BD Vacutainer® SST™ serum sep.tube (gold cap) MSMS of 289 in EPI mode



MSMS spectra of major interferences, two of which that generate signal at m/z 97 and 109

Figure 6

8/31

Mass spec parameters settings

☐ Scheduled NRM

	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID
1	289.200	109.200	120.0	Testosterone-109
2	289.200	97.100	120.0	Testosterone-97
3	282.200	97.000	25.0	C13-Testosterone
4				

Period Summary
 Duration: 5.500 (min) Delay Time: 0 (sec)
 Cycle: 1200 (sec) Cycle: 0.3250 (sec)

DMS
 Modifier Gas ☐ On ☒ Off

Period 1 Experiment 1 Parameter Table

Source/Gas Compound

Declustering Potential (DP) 110.0

Entrance Potential (EP) 10.0

Focusing Lens 1 (Q1) -11.0

Focusing Lens 2 (Q2) -20.0

Collision Energy (CE) 30.0

Prefilter (ST1) -18.0

Collision Cell Exit Potential (CXP) 15.0

Compensation Voltage (COV) 4.5

DMS Offset (DMO) 3.0

Separation Voltage (SV) 3200.0

Apply the following parameters to all other experiments of the same polarity:
☐ Source/Gas ☐ Compound

Period 1 Experiment 1 Parameter Table

Source/Gas Compound

Ion Source: Turbo Spray

Curtain Gas (CUR) 20.0

Collision Gas (CAD) Medium

Ion Spray Voltage (IS) 4500.0

Temperature (TEM) 600.0

Ion Source Gas 1 (GS1) 50.0

Ion Source Gas 2 (GS2) 50.0

DMS Resolution (DR) 0.0

DMS Temperature (DT) 150.0

Apply the following parameters to all other experiments of the same polarity:
☐ Source/Gas ☐ Compound

Figure 7

Experiment Conditions

5 Subjects of male and female

STD was prepared in pooled children under age 7 years old

Two sample preparation approach:

LL E with 90/10 hexane/ Ethyl acetate (200 uL serum; 1.2 mL solvent; recon
150 uL of 50/50 MeOH/H2O)

PPT with ACN (200 uL serum; 600 ACN; Dry down and recon 150 uL of
50/50 MeOH/H2O)

Column: Kinetex 2.6 u 50x2.1 mm Run time: 7 min; flow rate 0.3 mL/min
Column oven 40C ; MP A water+0.1% FA; MP B MeOH+0.1% FA

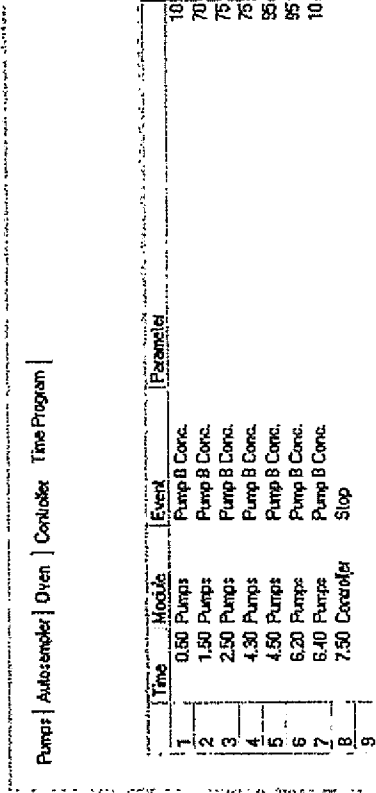


Figure 7a

10/31

Testosterone Female #3 Plasma Sample:LE

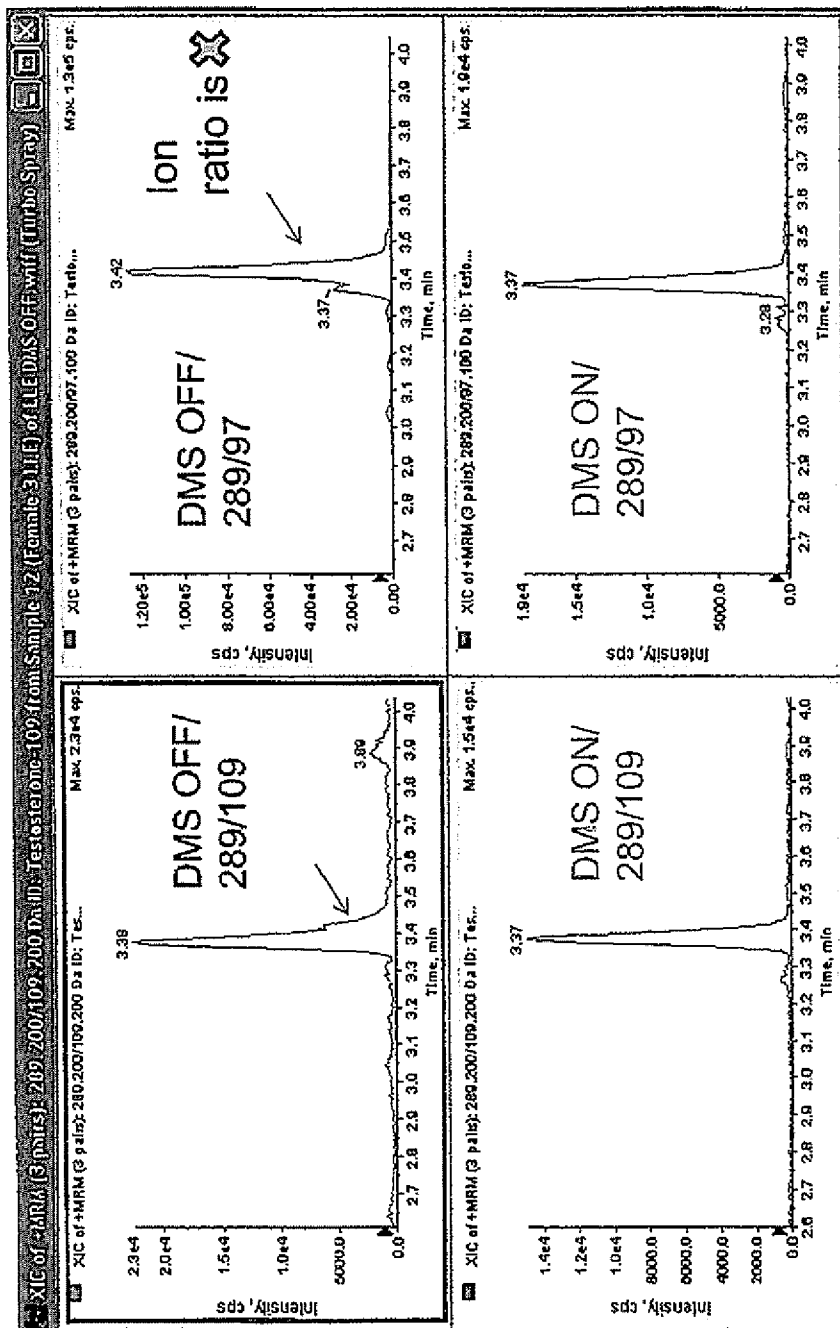


Figure 8

11/31

Testosterone Female #3 LLE / Background

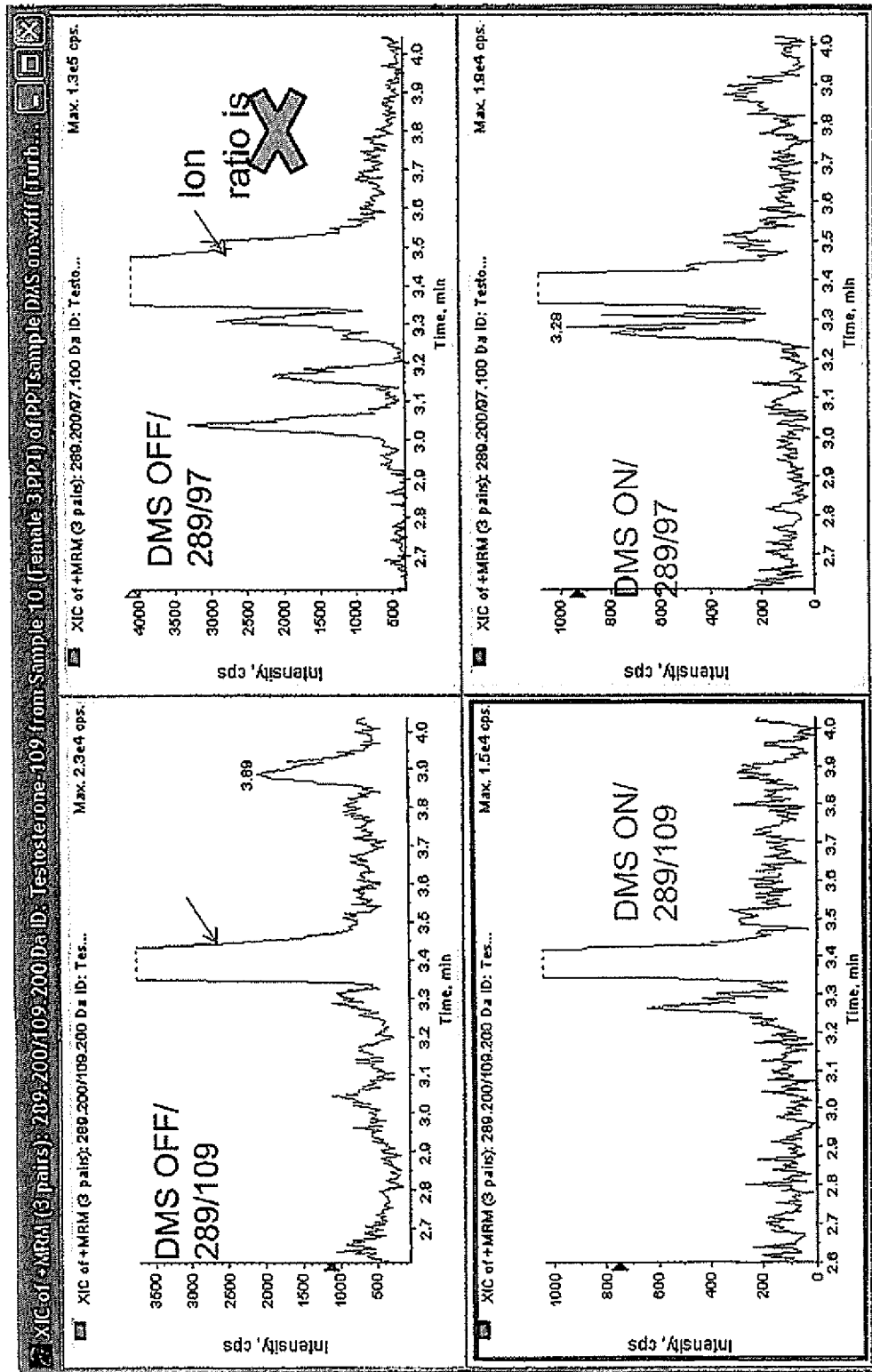


Figure 9

12/31

Testosterone Female #3 PPT

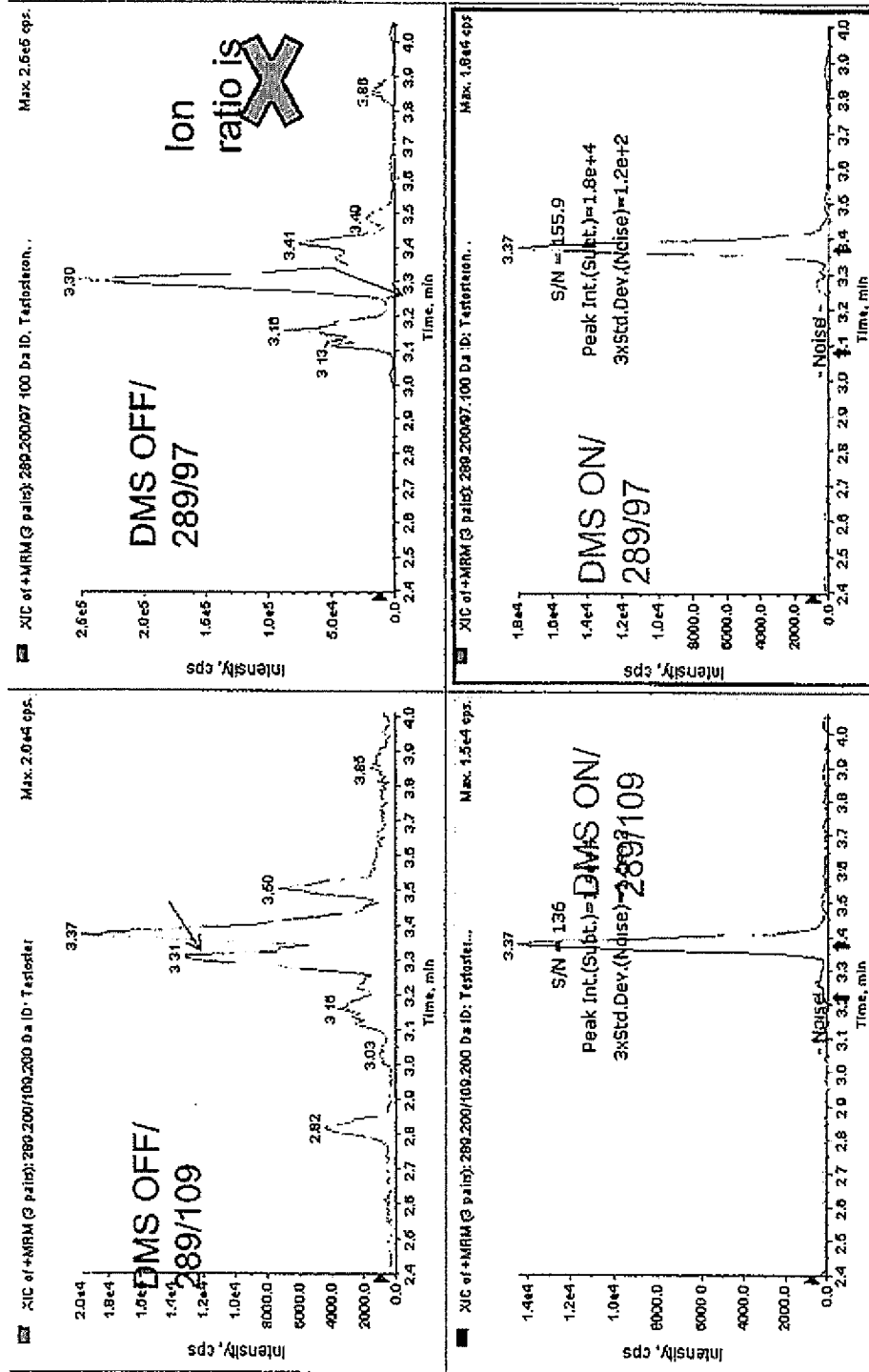


Figure 10

13/31

Testosterone Pooled Children under 7; PPT

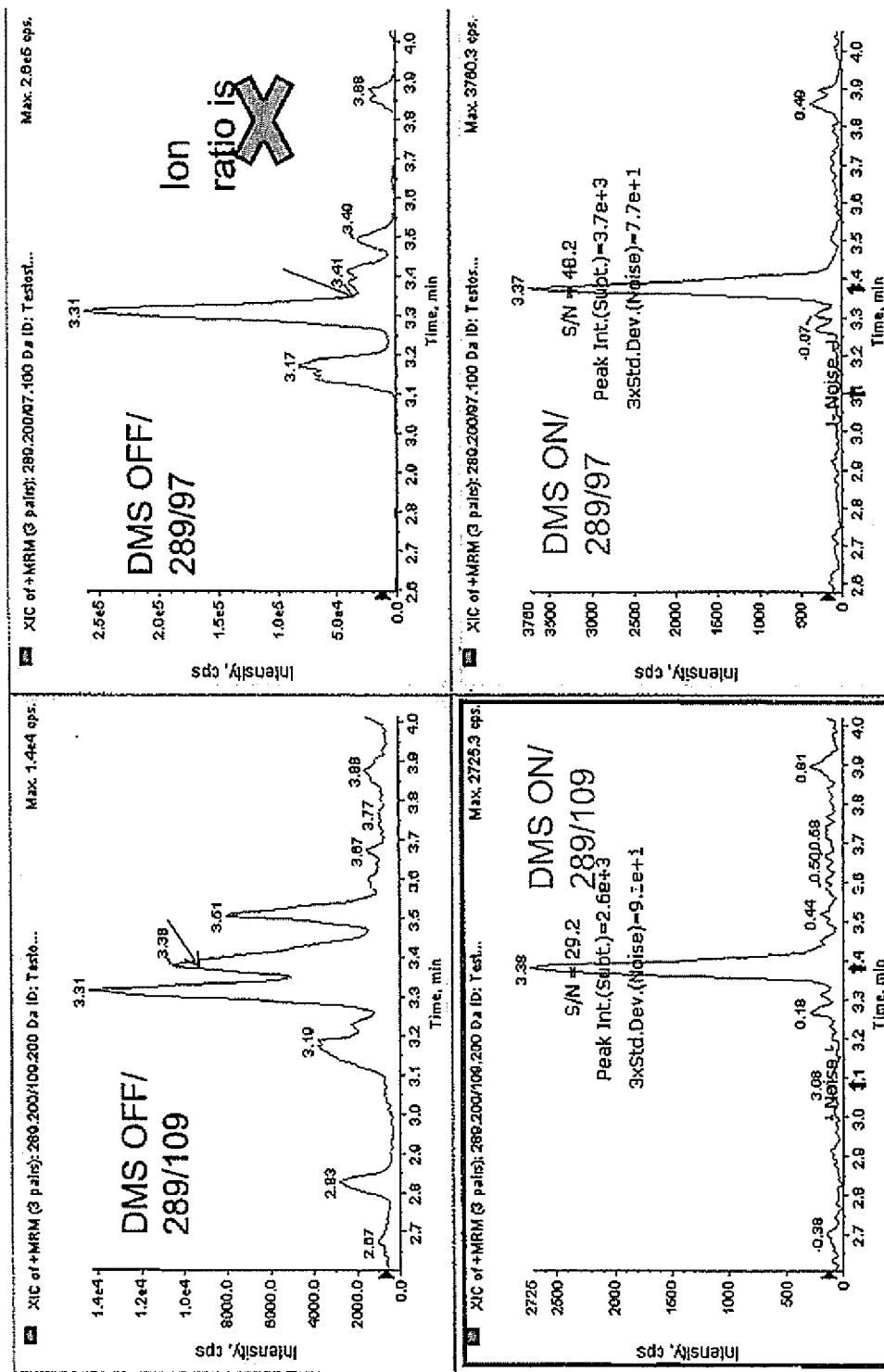


Figure 11

TESTOSTERONE MRM 289 → 109

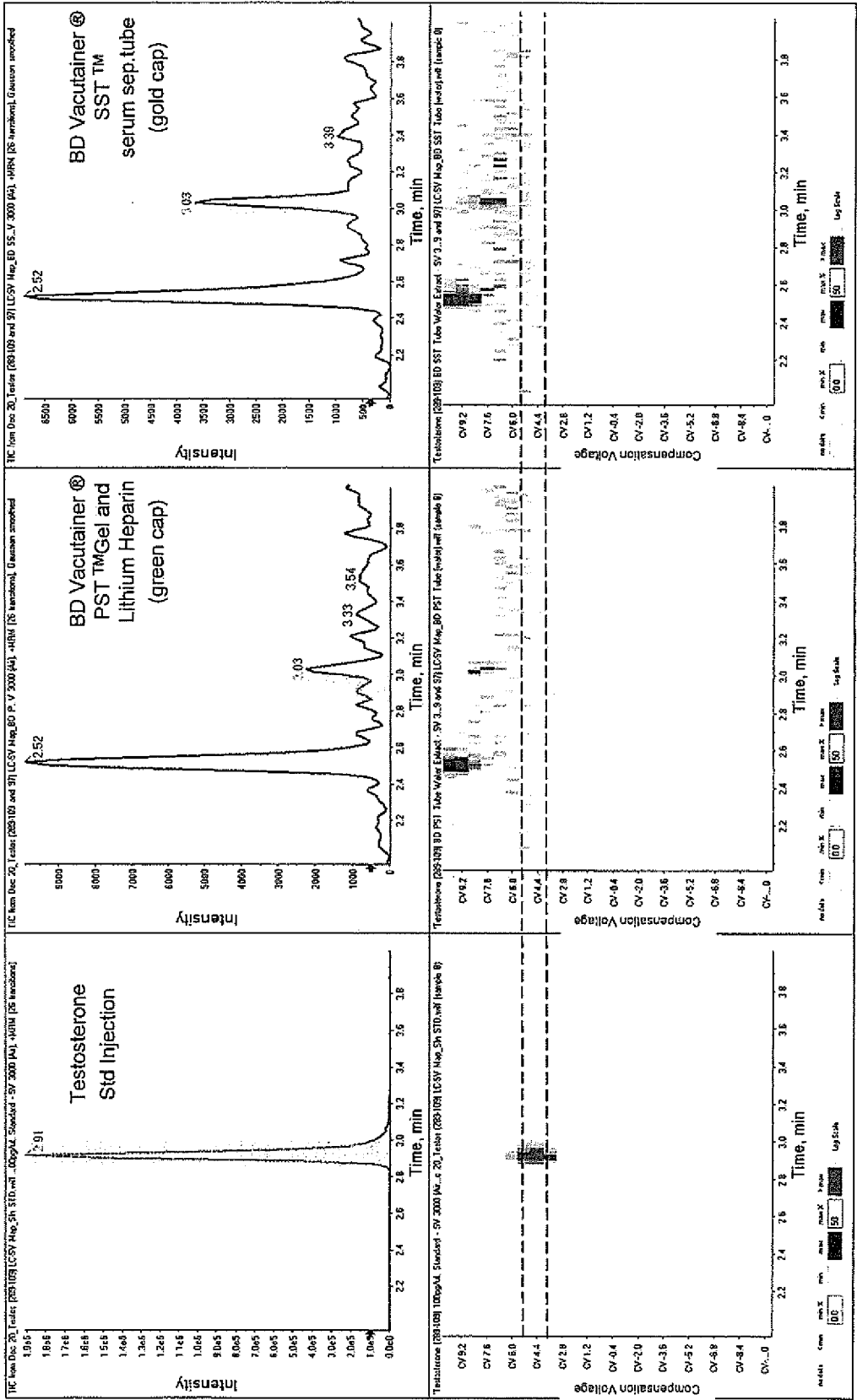


Figure 11a

15/31

TESTOSTERONE MRM 289 → 97

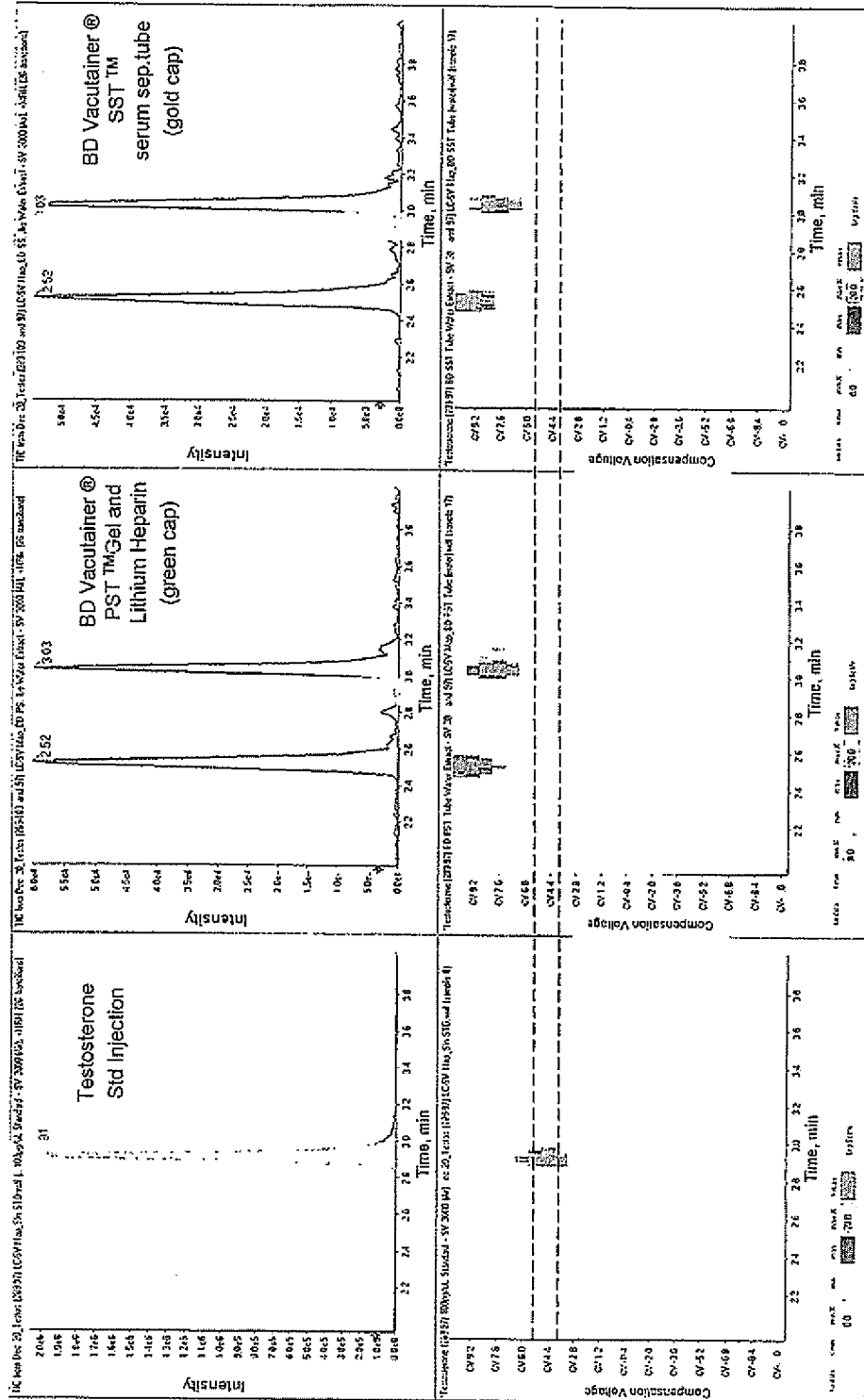


Figure 11b

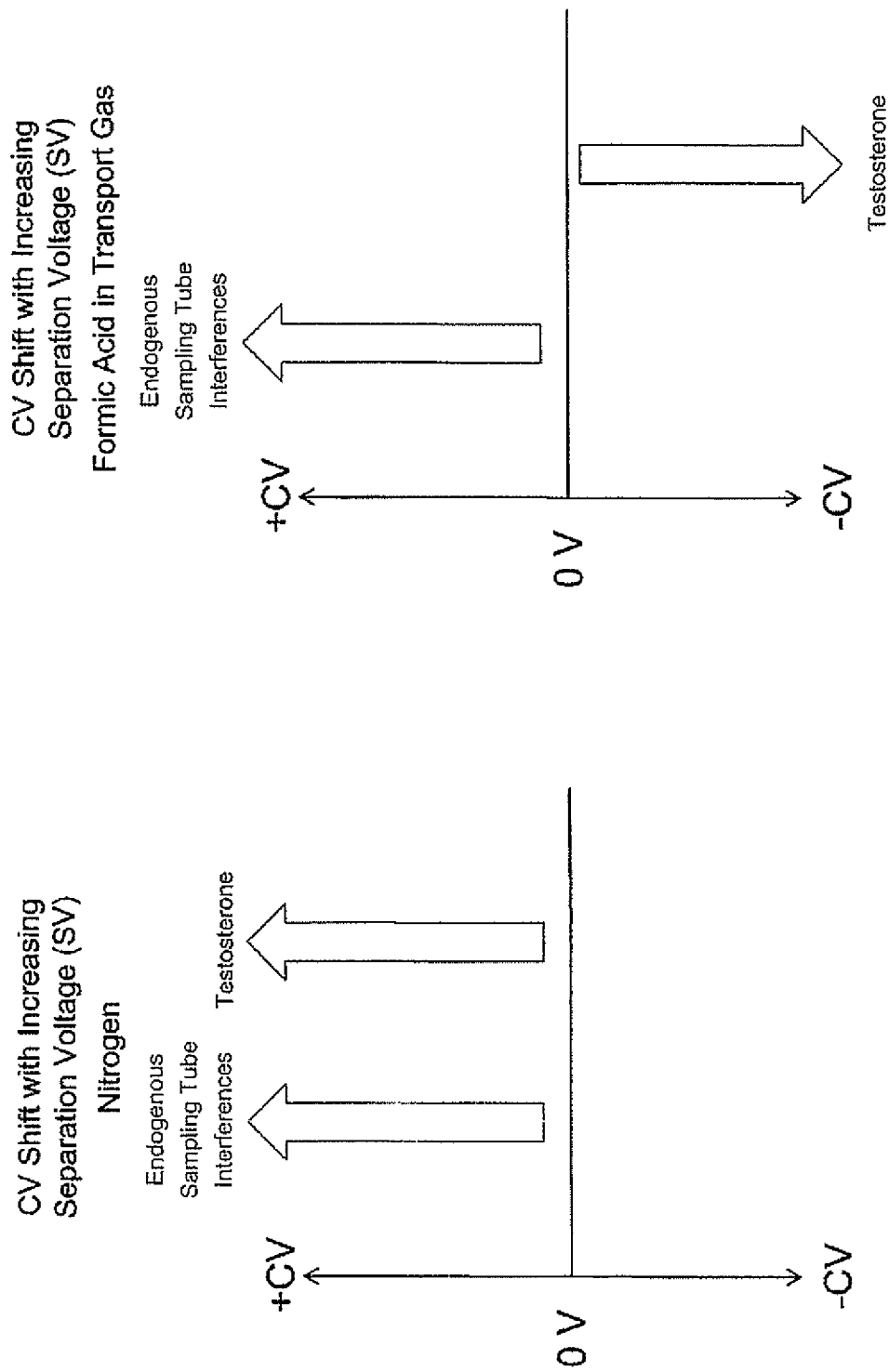


Figure 12

17/31

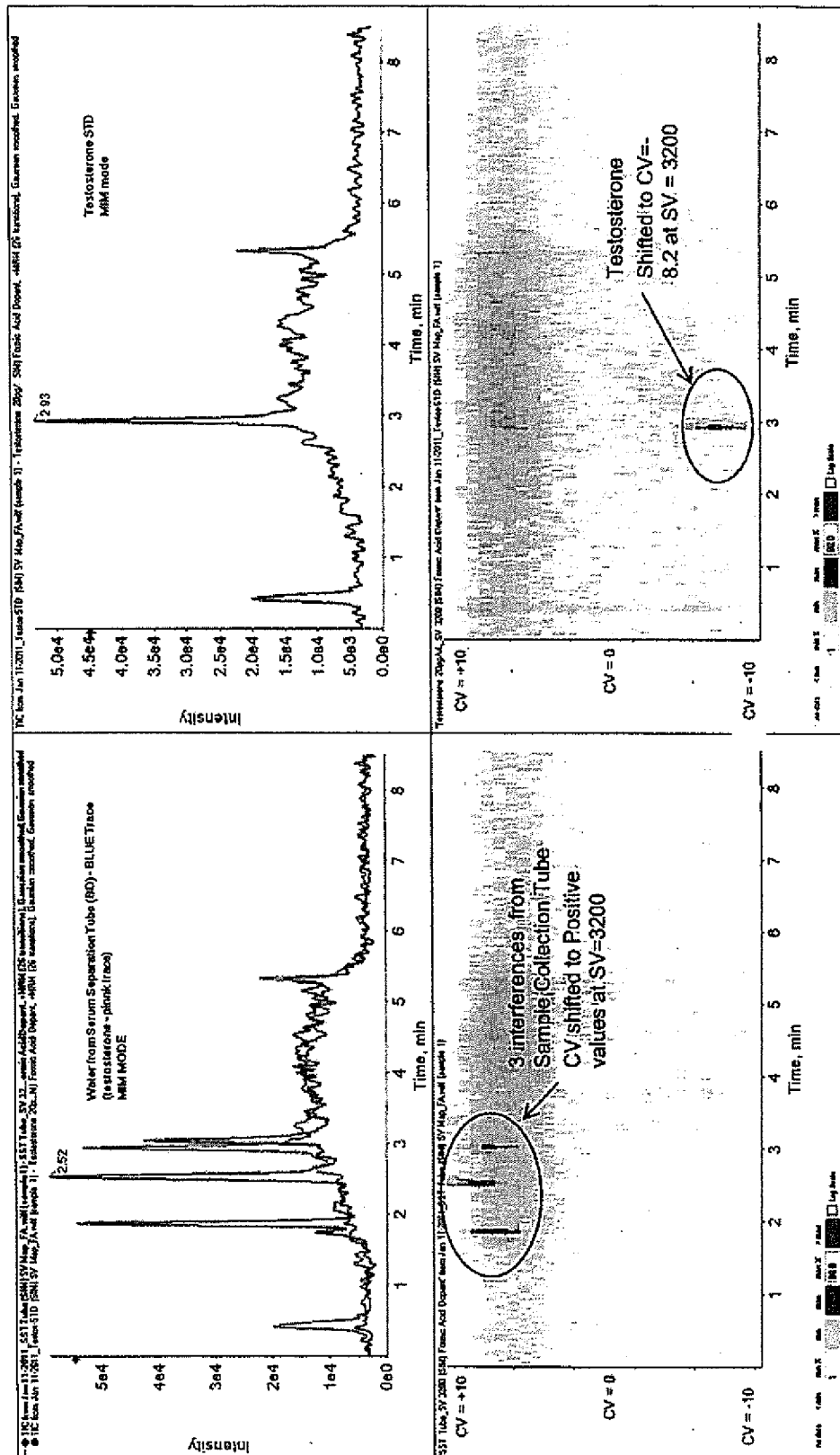


Figure 13

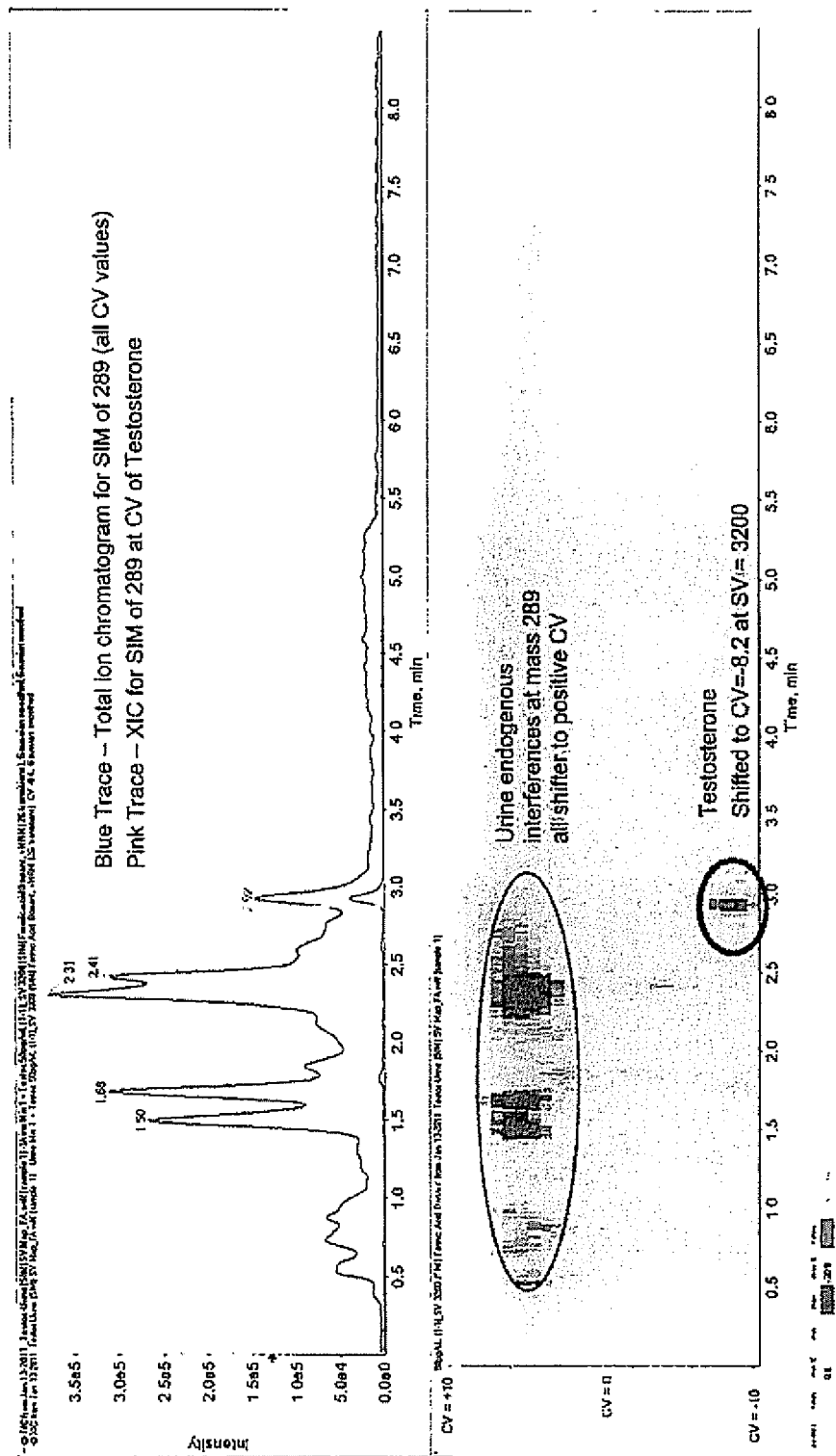
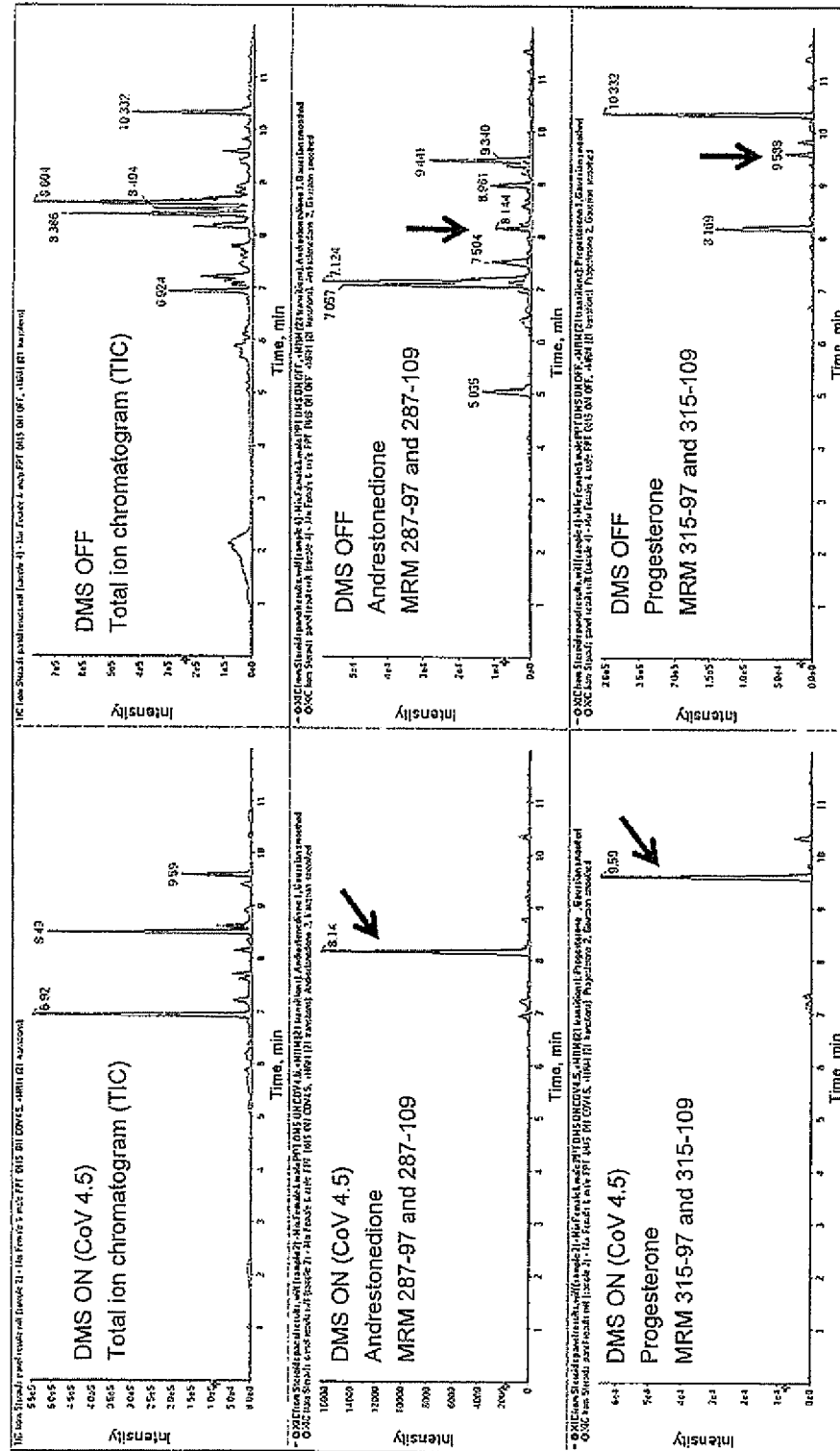


Figure 14

19/31

Panel of 10 steroid analytes (21 MRM monitored)
Combined female and male PPT samples (Blood collected in BD Gold Cap)



20/31

Panel of 10 steroid analytes (21 MRM monitored)

Excalibur MRM			
ID	Q1	Q3	
Androstenedione 1	373.3	97.1	
Androstenedione 2	287.3	109.0	
Cortisol 1	363.3	327.3	
Cortisol 2	363.3	309.2	
Corticosterone 1	347.3	121.0	
Corticosterone 2	347.3	311.3	
11-Deoxycortisol 1	347.3	109.0	
11-Deoxycortisol 2	347.3	97.1	
DHEA 1	271.3	213.1	
DHEA 2	271.3	91.1	
DHEAS 1	271.2	213.3	
DHEAS 2	271.2	81.0	
Estradiol 1	273.2	199.0	
Estradiol 2	273.2	133.0	
Progesterone 1	315.3	97.1	
Progesterone 2	315.3	109.2	
17-OH-Progesterone 1	331.3	97.0	
17-OH-Progesterone 2	331.3	109.1	
Testosterone 1	289.1	97.0	
Testosterone 2	289.1	109.0	
Testosterone-d3	292.3	96.9	

Figure 15b

21/31

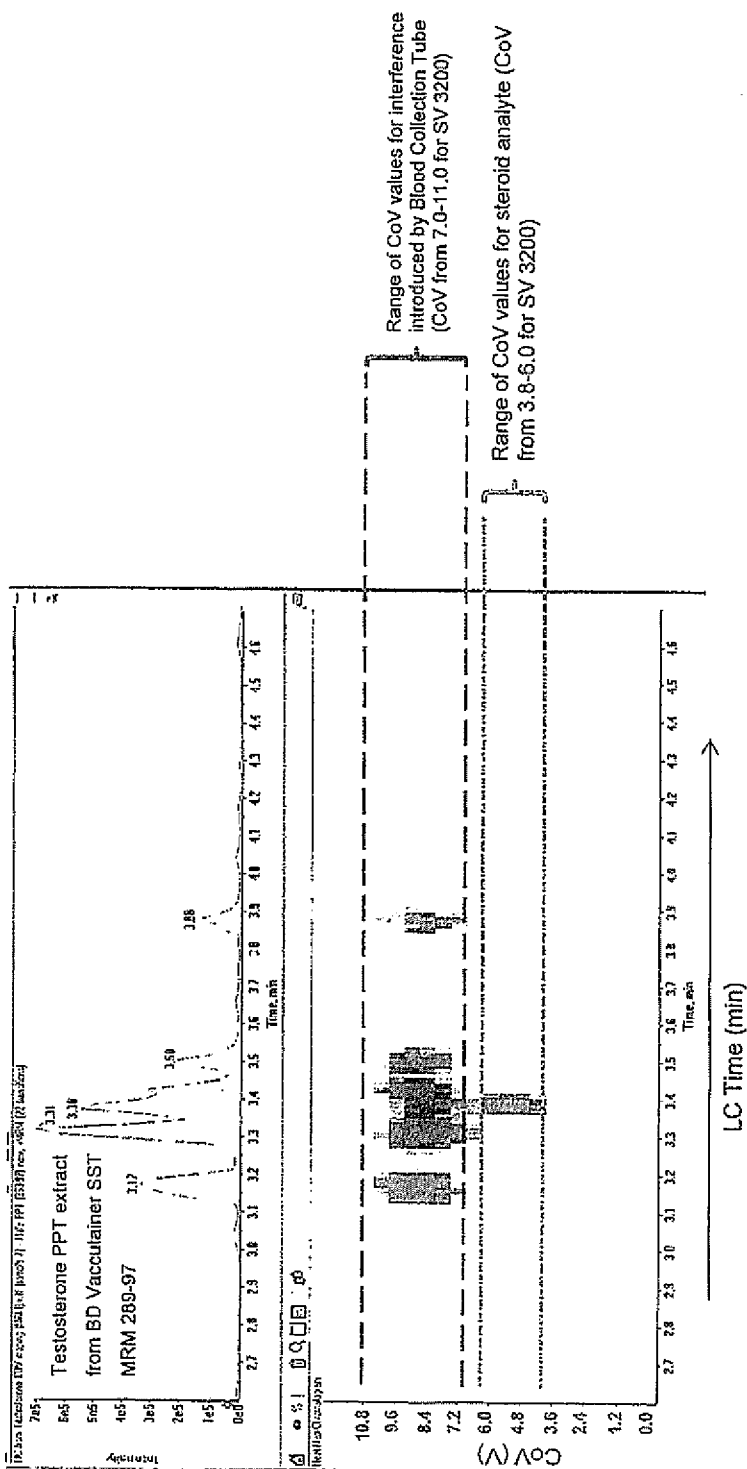


Figure 16a

Panel of 10 steroid analytes (21 MRM monitored)
Combined female and male PPT samples (Blood collected in BD Gold Cap)

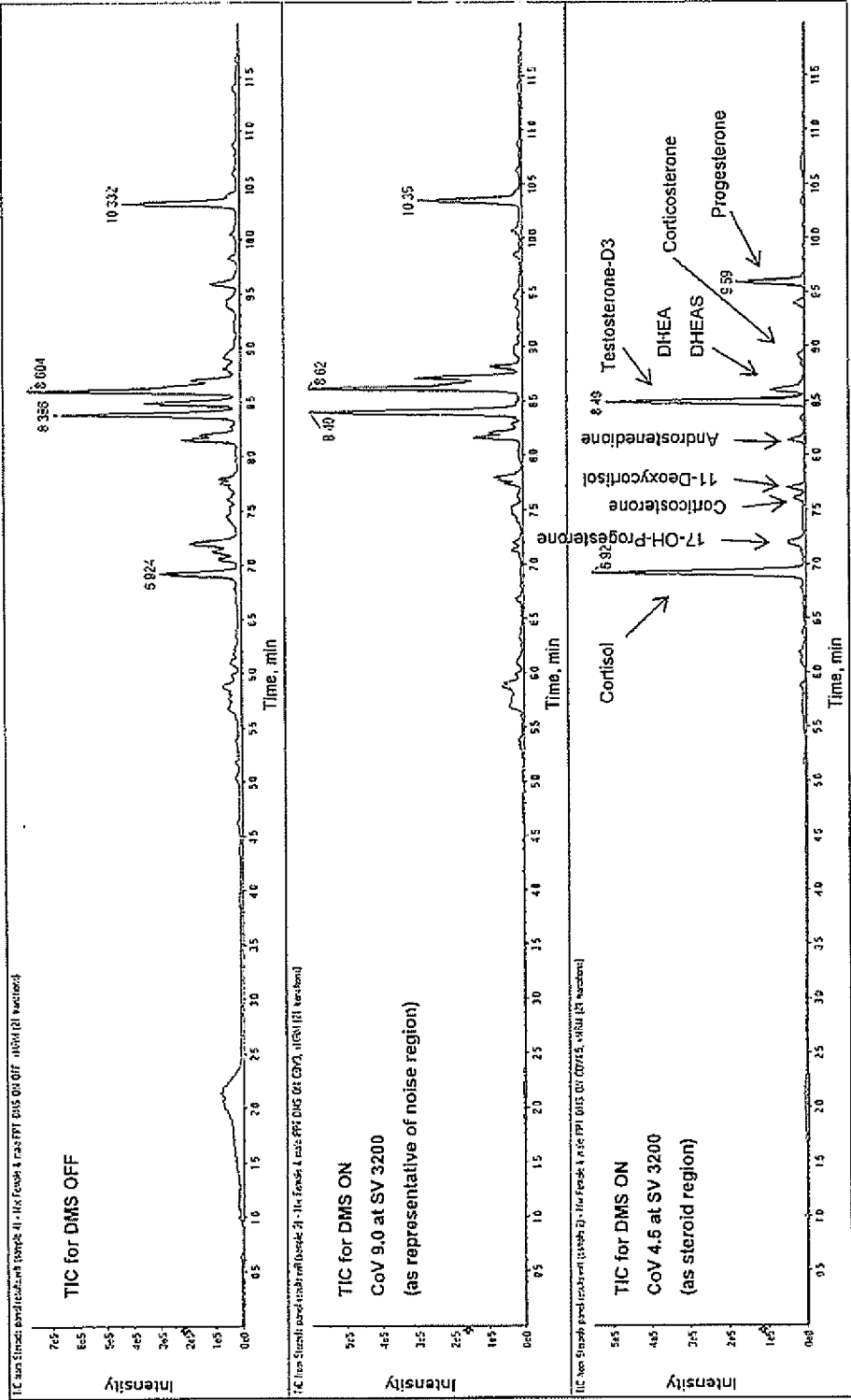


Figure 16b

23/31

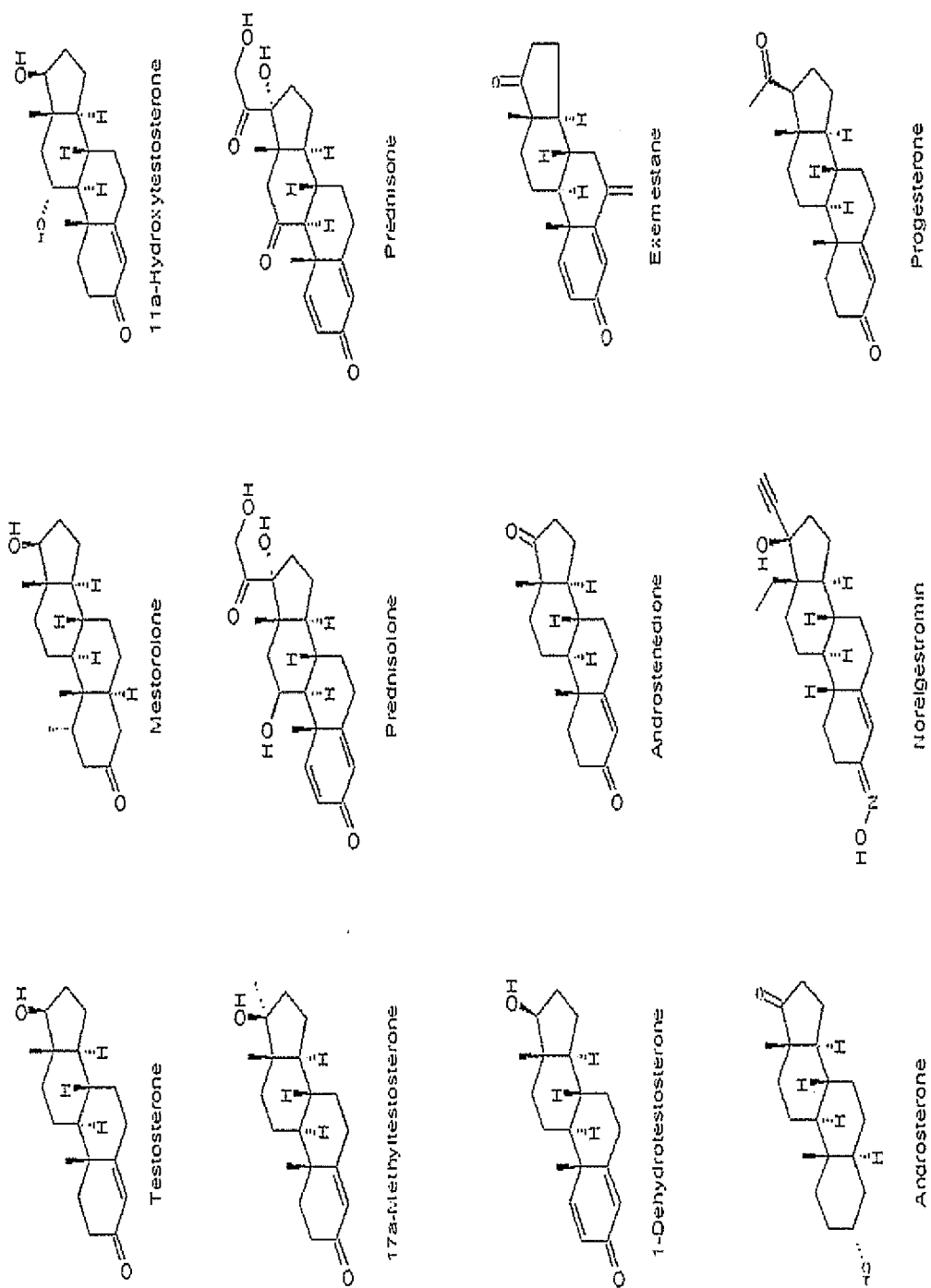


Figure 17

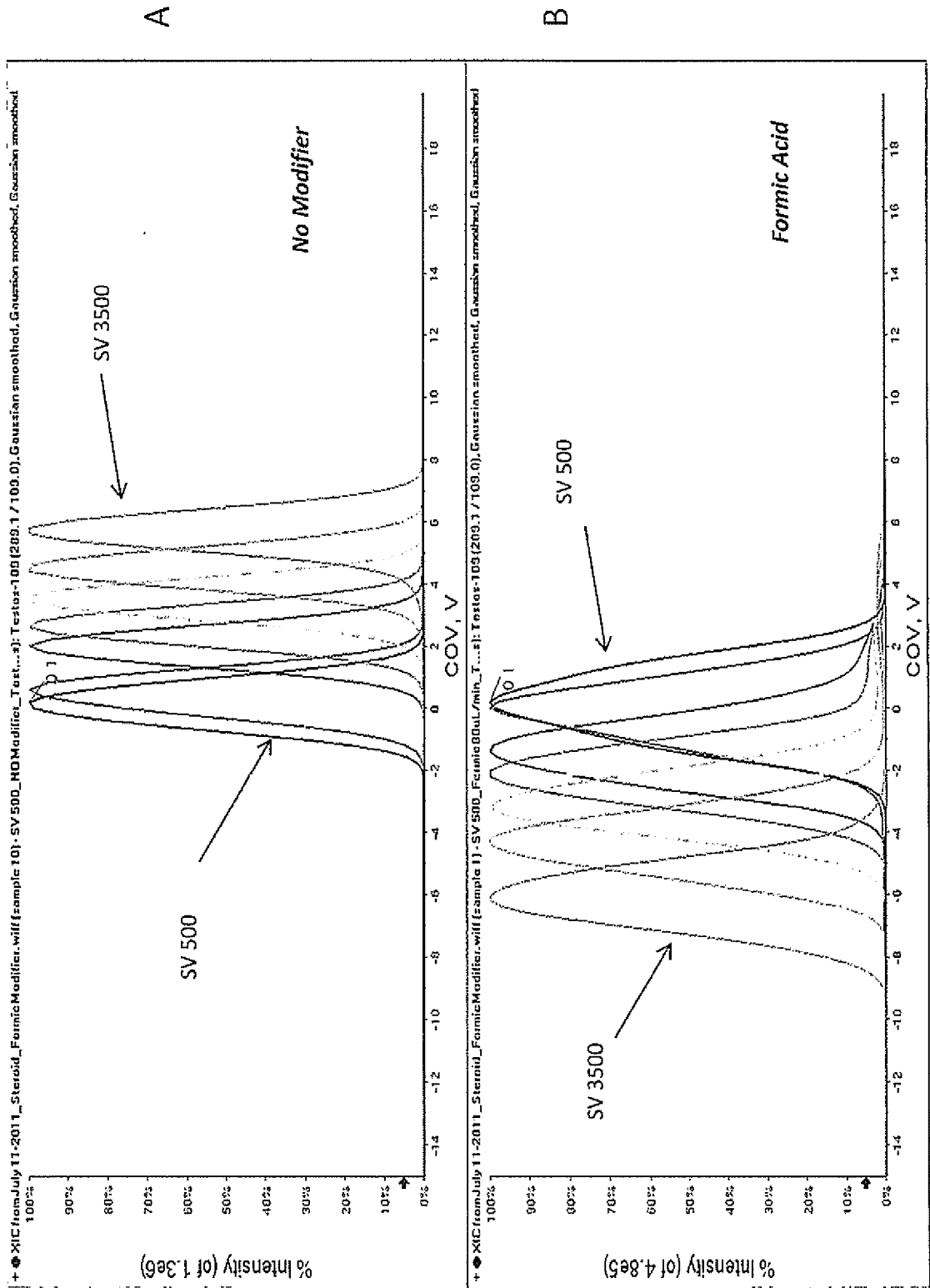


Figure 18

25/31

Name	MH+	Q1	Q3	Q3	Formula	N2	Formic	R.T. (min)
1-Dehydrotestosterone	287.1	287.1	121	135	C19H26O2	7	-11	2.62
Androstenedione	287.1	287.1	109	97	C19H26O2	7	-8.5	2.86
Estrilol	289.1	289.1	107	133	C18H24O3	3.7	n.d.	2.18
Epitestosterone	289.1	289.1	97	109	C19H28O2	5.7	-6.1	2.91
Testosterone	289.2	289.2	97	109	C19H28O2	5.7	-6	2.76
Estrone	271.2	255	159	133	C18H22O2	7.5	-4.5	n.a.
Exemestane	297.2	297.2	121	149	C20H24O2	6.2	-11.4	n.a.
17 α -Methyltestosterone	303.2	303.2	97	109	C20H30O2	6.7	-7.5	2.87
11 α -Hydroxytestosterone	305.2	305.2	121	147	C19H28O3	6.2	-8.8	2.43
Mestrolone	305.2	305.2	173	229	C20H32O2	6.5	-1.5	3.05
Progesterone	315.2	315.2	109	97	C21H30O2	5.4	-5.2	n.a.
Norelgestromin	326.2	326.2	260	124	C21H29NO2	4.6	-10.9	n.a.
Prednisone	359.1	359.1	147	237	C21H26O5	5.2	-8	2.28
Prednisolone	361.2	361.2	343	171	C21H28O5	3.8	-9	2.29
Dexamethasone	393.1	393.1	355	279	C22H29FO5	4.7	-7.5	2.46
Flumethasone	411.1	411.1	253	121	C22H28F2O5	4.5	-5	2.45
Fluticasone propionate	501.2	501.2	293	275	C25H31F3O5S	6.1	-1.3	3.16

Figure 19

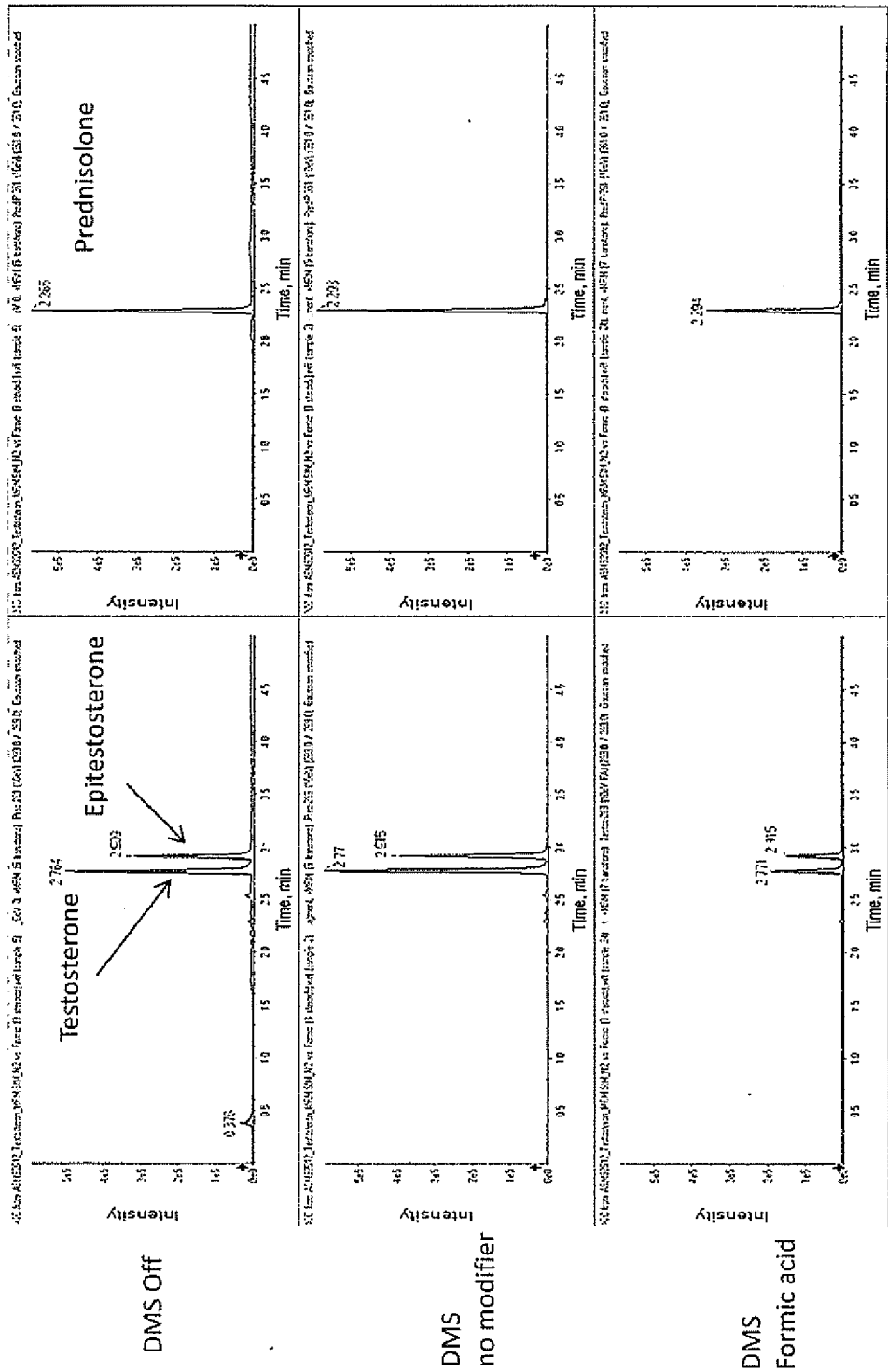


Figure 20

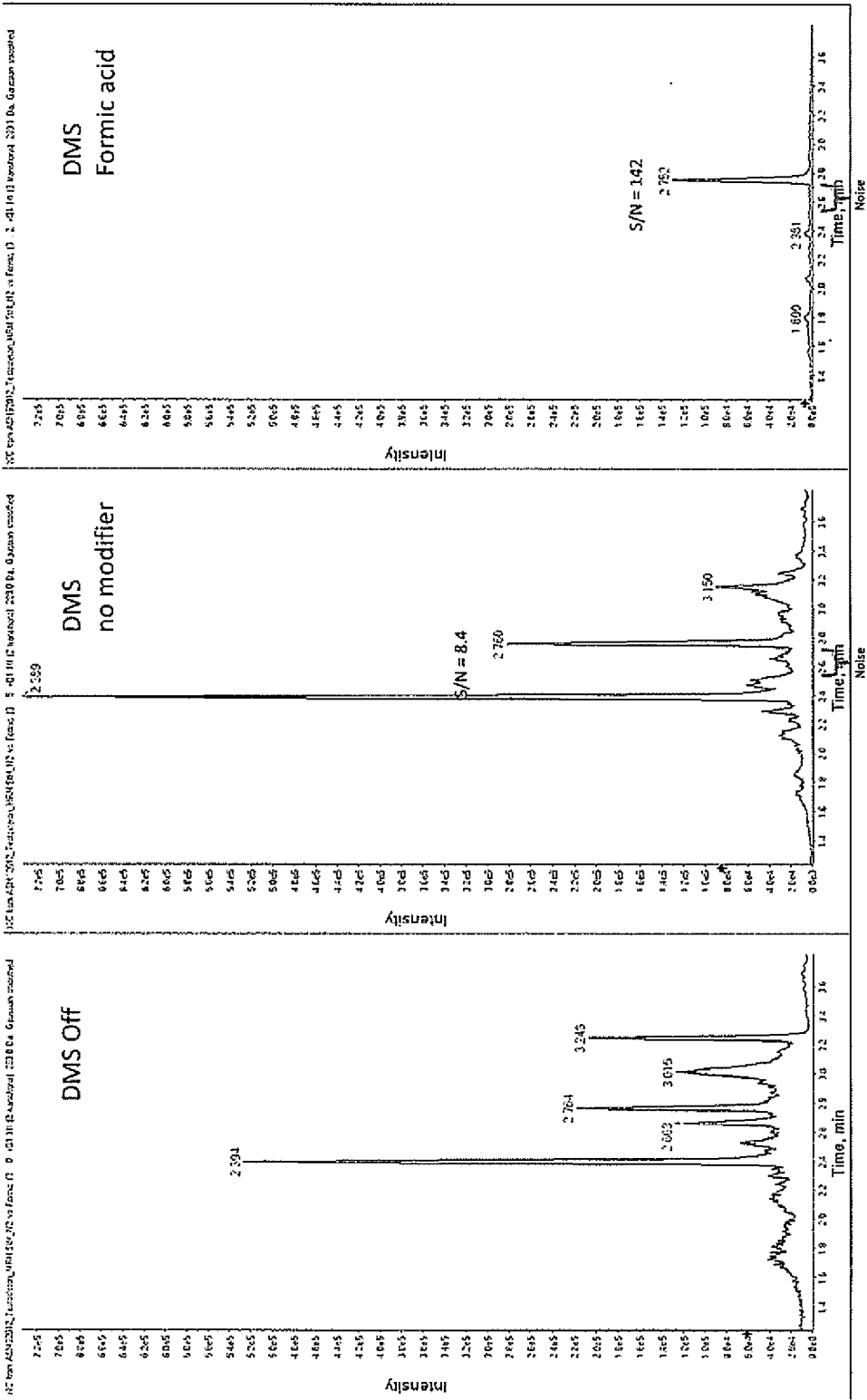


Figure 21

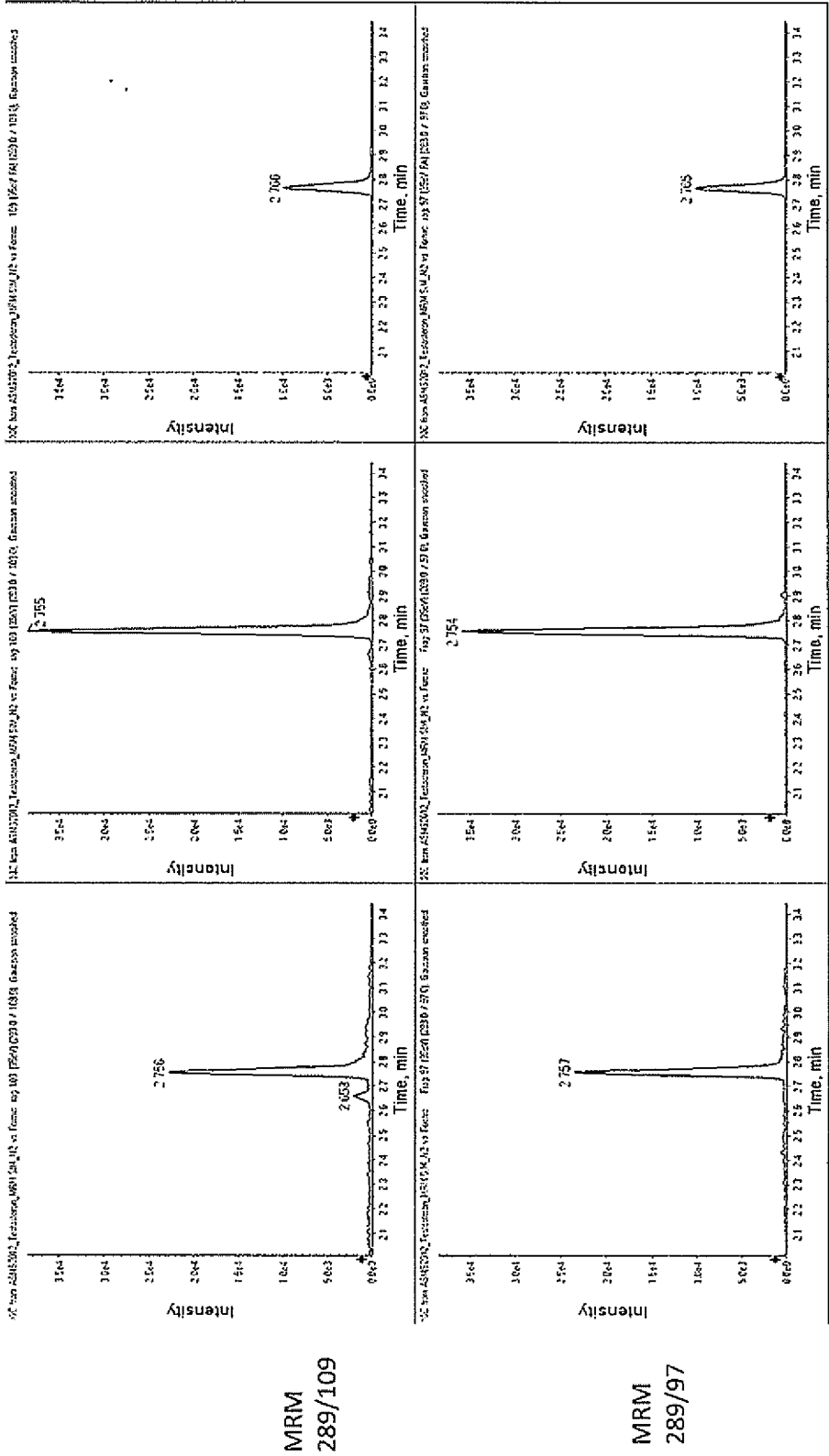


Figure 22

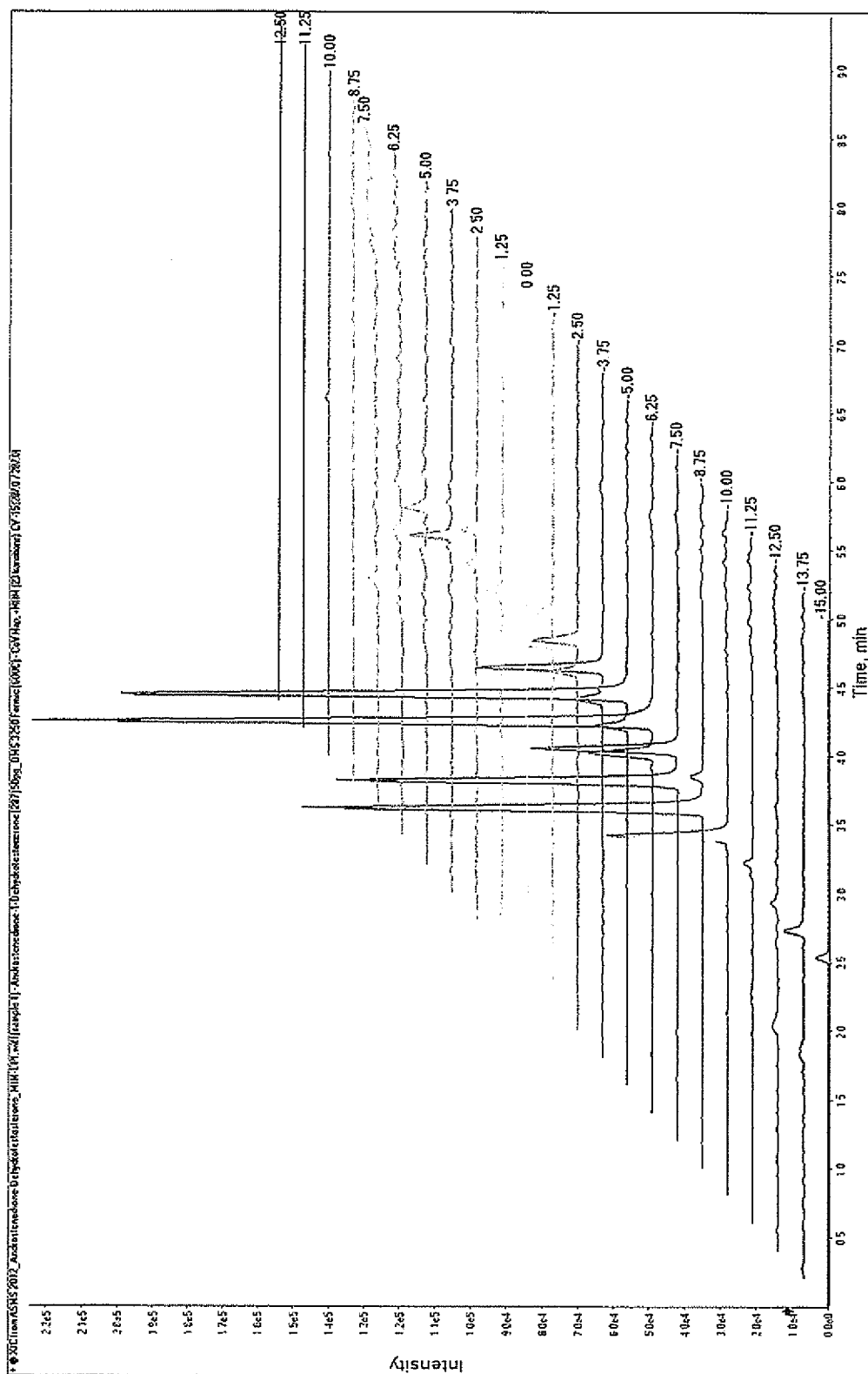


Figure 23

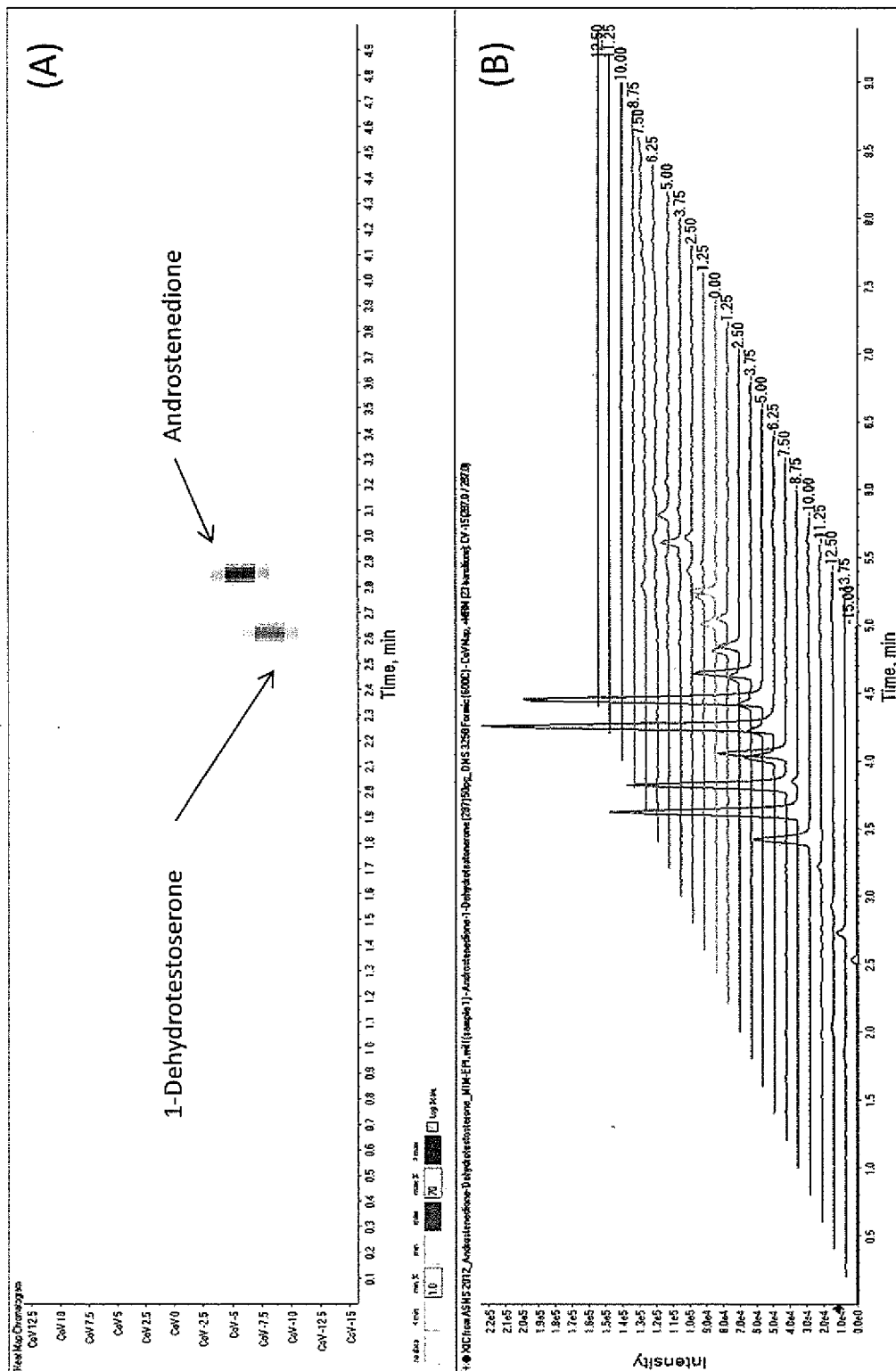


Figure 24

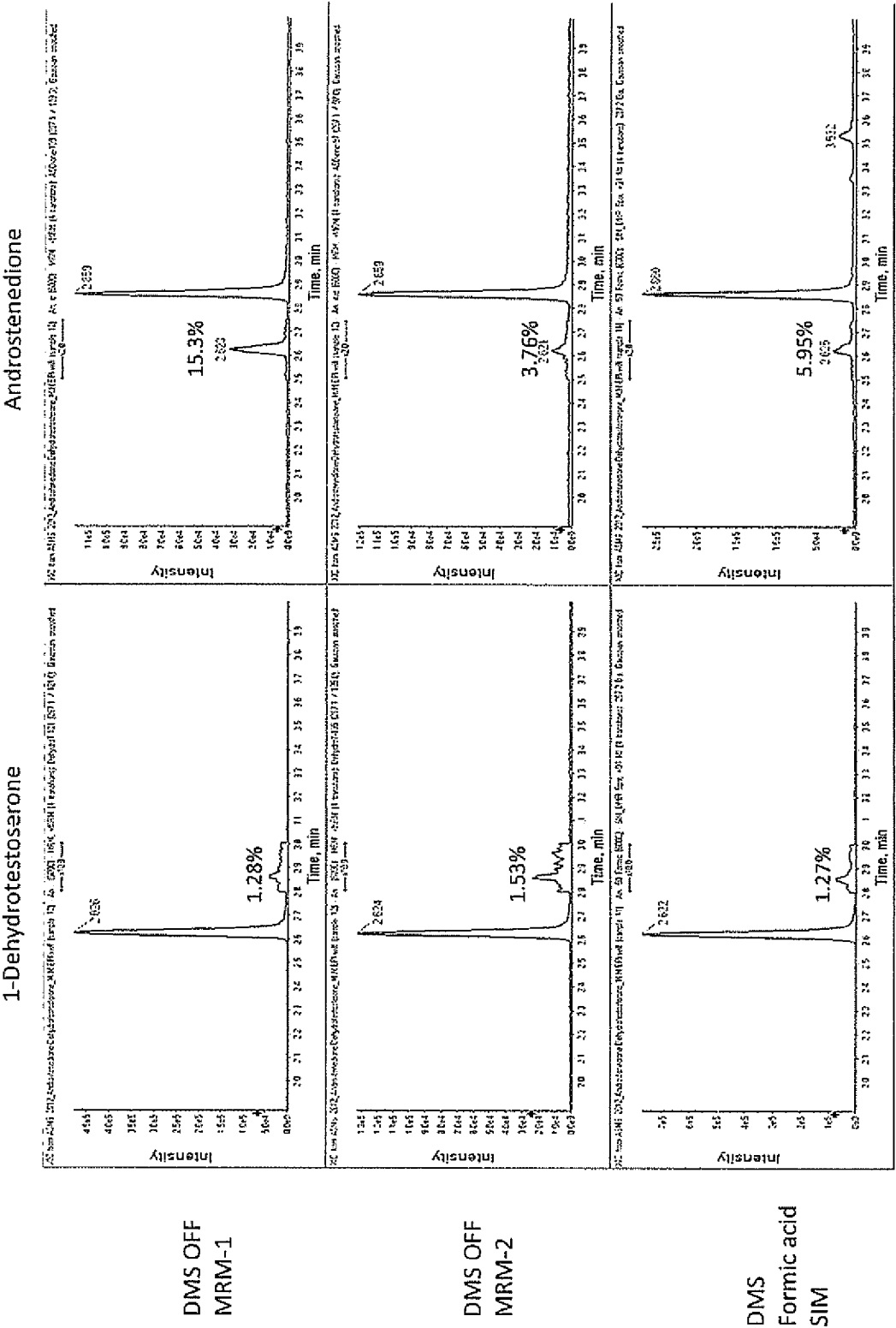


Figure 25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/40748

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 31/00 (2012.01)

USPC - 702/27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8)- G01N 31/00 (2012.01);

USPC- 702/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC- 707/713; 707/E17.017; 706/8;

Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest (US Pat, PgPub, EPO, JPO), GoogleScholar (PL, NPL), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL);
search terms: spectrometer, differential, mobility, ion, ionize, ionisation, steroid, testosterone, curtain, gas, vacuum, serum, blood

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0282966 A1 (SCHNEIDER et al.) 11 November 2010 (11.11.2010), Figs. 1, 2, 5, 6, 9; para [0006], [0008], [0034]-[0040], [0046], [0051], [0054], [0062], [0068], [0069], [0078], [0082], [0087], [0091]-[0094]	1-55
Y	US 2008/0166697 A1 (CAULFIELD et al.) 10 July 2008 (10.07.2008), para [0008], [0015], [0020]-[0022], [0027], [0030], [0032], [0081]	1-55
Y, P	US 2011/0183431 A1 (COVEY et al.) 28 July 2011 (28.07.2011), para [0011]-[0110]	1-55
Y	US 2009/0256073 A1 (GUO et al.) 15 October 2009 (15.10.2009), para [0004]-[0112]	1-55
Y	US 2006/0255261 A1 (WHITEHOUSE et al.) 16 November 2006 (16.11.2006), para [0018]-[0074]	1-55
Y	US 6,703,609 B2 (GUEVREMONT et al.) 09 March 2004 (09.03.2004), col 7-16	1-55

☐ Further documents are listed in the continuation of Box C.


* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 August 2012 (07.08.2012)

Date of mailing of the international search report

21 AUG 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774