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(54) **Title**: METHODS AND MATERIALS FOR REDUCING AMYLOID BETA LEVELS WITHIN A MAMMAL

(57) **Abstract**: This document provides methods and materials for reducing amyloid beta levels within a mammal (e.g. a mammal having Alzheimer's disease). For example, this document provides methods for using compositions containing a potato polysaccharide preparation to reduce one or more symptoms of Alzheimer's disease. In some cases, a composition containing a potato polysaccharide preparation provided herein can be used to increase binding, sequestration, and/or degradation of CNS-derived amyloid beta polypeptides, thereby inhibiting the formation of neurofibrillary plaques.

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METHODS AND MATERIALS FOR REDUCING AMYLOID BETA LEVELS WITHIN A MAMMAL

BACKGROUND

1. Technical Field

This document relates to methods and materials for reducing amyloid beta levels within a mammal having Alzheimer's disease. For example, this document relates to using compositions containing a potato polysaccharide preparation to reduce one or more symptoms of Alzheimer's disease. In some cases, this document relates to using compositions containing a potato polysaccharide preparation to degrade CNS-derived amyloid beta polypeptides.

2. Background Information

Potatoes are starchy, edible tubers obtained from potato plants and form an integral part of much of the world's food supply. In fact, potatoes are the fourth largest food crop in the world. The main potato species worldwide is *Solanum tuberosum*.

SUMMARY

This document provides methods and materials for reducing amyloid beta levels within a mammal (e.g., a mammal having Alzheimer's disease). For example, this document provides methods for using compositions containing a potato polysaccharide preparation to reduce one or more symptoms of Alzheimer's disease. In some cases, a composition containing a potato polysaccharide preparation provided herein can be used to increase binding, sequestration, and/or degradation of CNS-derived amyloid beta polypeptides. In some cases, a composition containing a potato polysaccharide preparation provided herein can be used degrade CNS-derived amyloid beta polypeptides, for example at peripheral sites of action and/or to inhibit the formation of neurofibrillary plaques.

Having the ability to use a composition containing a potato polysaccharide preparation described herein to reduce one or more symptoms of Alzheimer's disease can provide clinicians and patients with an effective treatment regime for improving a patient's quality of life.

This document also provides compositions (e.g., nutritional supplement compositions) that contain a potato polysaccharide preparation. For example, this document provides nutritional supplement compositions containing a potato polysaccharide preparation, methods for obtaining potato polysaccharide preparations, methods for making nutritional supplement compositions containing a potato polysaccharide preparation, and methods for increasing or decreasing expression of polypeptides involved with Alzheimer's disease.

In some cases, the compositions provided herein (e.g., nutritional supplement compositions and potato polysaccharide preparations provided herein) can be used to increase or decrease expression of polypeptides involved with Alzheimer's disease and the metabolism of CNS-derived amyloid beta polypeptides. For example, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of a low density lipoprotein receptor-related protein 1 (LRP1) polypeptide, an amyloid beta (A4) precursor protein binding, family B member 1, (APBB1) polypeptide, an insulin degrading enzyme (IDE) polypeptide, a glutathione peroxidase 3 (GPX3) polypeptide, a glutathione peroxidase 4 (GPX4) polypeptide, or a combination thereof. In some cases, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of an insulin-like growth factor 1 (IGF1), a nitric oxide synthase 2, inducible (NOS2), or a combination thereof.

In some cases, the compositions provided herein (e.g., nutritional supplement compositions and potato polysaccharide preparations provided herein) can be used to increase or decrease expression of polypeptides involved with binding, sequestration, and/or degradation of CNS-derived amyloid beta polypeptides in adipose tissue. For example, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of an LRP1 polypeptide, an APBB1 polypeptide, an IDE polypeptide, a GPX3 polypeptide, or a combination thereof.

In some cases, the compositions provided herein (e.g., nutritional supplement compositions and potato polysaccharide preparations provided herein) can be used to increase or decrease expression of polypeptides involved with binding, sequestration, and/or degradation of CNS-derived amyloid beta polypeptides in blood. For example, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of an APBB1 polypeptide, an IDE polypeptide, a GPX4 polypeptide, or a combination thereof.

In some cases, the compositions provided herein (e.g., nutritional supplement compositions and potato polysaccharide preparations provided herein) can be used to increase or decrease expression of polypeptides involved with oxidative stress and proinflammatory pathways. For example, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of an insulin-like growth factor 1 (IGF1) polypeptide, a nitric oxide synthase 2, inducible (NOS2) polypeptide, or a combination thereof.

In general, one aspect of this document features a method for reducing amyloid beta levels within a mammal having Alzheimer's disease. The method comprises, or consist essentially of, administering to the mammal a composition comprising a potato polysaccharide preparation obtained from raw potatoes, wherein the level of amyloid beta within the mammal is reduced. The mammal can be a human. The level of amyloid beta within the mammal can be reduced in blood. The level of amyloid beta within the mammal can be reduced in adipose tissue. The composition can comprise the potato polysaccharide preparation in an amount that results in between 0.05 mg and 50 mg of the potato polysaccharide component of the potato polysaccharide preparation being administered to the mammal per kg of body weight of the mammal. The composition can comprise between 1 mg and 100 mg of the potato polysaccharide preparation. The composition can comprise between 6 mg and 20 mg of the potato polysaccharide preparation. The composition can comprise between 1 mg and 100 mg of the potato polysaccharide component of the potato polysaccharide preparation. The composition can comprise between 6 mg and 20 mg of the potato polysaccharide component of the potato polysaccharide preparation. The composition can be in the form of a tablet. The composition can comprise alpha lipoic acid. The composition can comprise alpha tocopherol. The potato polysaccharide preparation can be in an amount that results in between 0.075 mg and 0.5 mg of the potato polysaccharide component of the potato polysaccharide preparation being administered to the mammal per kg of body weight of the mammal. At least about 80 percent of the potato polysaccharide preparation can be potato polysaccharide. At least about 90 percent of the potato polysaccharide preparation can be potato polysaccharide. At least about 95 percent of the potato polysaccharide preparation can be potato polysaccharide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is an HPLC chromatogram of a 10% ACN extract of raw potato (Russet Burbank).

Figure 2 is an HPLC chromatogram of collected and re-purified 3.5 minute peak material from a 10% ACN extract of raw potato shown in Figure 1.

Figure 3 is an LC/MS trace of 3.5 minute HPLC peak material.

Figure 4 is a full NMR spectrum of 3.5 minute HPLC peak material.

Figure 5 is an expanded NMR spectrum of 3.5 minute HPLC peak material.

Figure 6 is a total ion chromatogram of derivatized carbohydrate fragments of 3.5 minute HPLC peak material obtained from raw potato Russet Burbank).

Figure 7 is a fragmentation pattern of diacetamide. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 8 is a fragmentation pattern of 3-acetoxy pyridine. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 9 is a fragmentation pattern of 3,4-furan dimethanol, diacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 10 is a fragmentation pattern of 1,2,3-propanetriol diacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 11 is a fragmentation pattern of imidazole, 2-acetamino-5-methyl. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 12 is a fragmentation pattern of 6,7-dihydro-5H-pyrrol[2,1,c][1,2,4] triazole-3-carboxylic acid. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 13 is a fragmentation pattern of acetic acid, 1-(2-methyltetrazol-5-yl) ethenyl ester. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 14 is a fragmentation pattern of 1,2,3,4-butanetriol, tetraacetate (isomer 1). The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 15 is a fragmentation pattern of 1,2,3,4-butanetriol, tetraacetate (isomer 2). The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 16 is a fragmentation pattern of pentaerythritol tetraacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 17 is a fragmentation pattern of 1,2,3,4,5-penta-o-acetyl-D-xylitol (isomer 1). The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 18 is a fragmentation pattern of 1,2,3,4,5-penta-o-acetyl-D-xylitol (isomer 2). The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 19 is a fragmentation pattern of 3,5-diacetoxy benzyl alcohol. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 20 is a fragmentation pattern of β-D-galactopyranose, pentaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 21 is a fragmentation pattern of D-mannitol hexaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 22 is a fragmentation pattern of galacticol, hexaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 23 is a fragmentation pattern of cyclohexane carboxylic acid, 1,2,4,5-tetrakis(acetoxy), (1α,3α,4α,5β)-(-). The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 24 is a fragmentation pattern of muco-inositol, hexaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 25 is a fragmentation pattern of D-glucitol-hexaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 26 is a fragmentation pattern of myo-inositol, hexaacetate. The peak fragmentation pattern is in the top panel, the compound library fragmentation match is in the bottom panel, and an overlay of the two is in the center panel.

Figure 27 is an HPLC chromatogram of a 10% ACN extract of raw Organic Yellow potato.

Figure 28 is an HPLC chromatogram of a 10% ACN extract of raw Purple potato.

Figure 29 is an HPLC chromatogram of a 10% ACN extract of raw Idaho Russet potato.

Figure 30 is an HPLC chromatogram of a 10% ACN extract of raw Yukon Gold potato.

Figure 31 is an HPLC chromatogram of a 10% ACN extract of raw sweet potato.

Figure 32 is an HPLC chromatogram of a 10% ACN extract of boiled Purple potato.

Figure 33 is an HPLC chromatogram of two pooled fraction collections from Idaho Russet potatoes.

Figure 34 is an HPLC chromatogram of fractions collections from 3 g of purple potatoes.

Figure 35 is a real time PCR amplification plot for IDE demonstrating differences in threshold cycle numbers between potato polysaccharide preparation treated ZDF and untreated control ZDF in rat adipose tissue samples. The higher cycle number for the control rat's tissue equates to a lower gene expression.

Figure 36 is a Real time PCR amplification plot for IDE demonstrating differences in threshold cycle numbers between potato polysaccharide preparation treated ZDF and untreated control ZDF in rat blood samples. The higher cycle number for the control rat's tissue equates to a lower gene expression.

Figure 37 is a graph plotting percent change in IDE gene expression, as measured by real time PCR analyses. The enhancements in IDE gene expression were determined to be 44.9 ± 2.8% (n=9, p=0.02) and 51.3 ± 6.2% (n=6, p=0.04), for adipose tissue and blood samples, respectively via Unpaired t-tests.

DETAILED DESCRIPTION

This document provides methods and materials for reducing amyloid beta levels within a mammal (e.g., a mammal having Alzheimer's disease). As described herein, a composition containing a potato polysaccharide preparation can be used to reduce one or more symptoms of Alzheimer's disease. For example, a composition containing a potato polysaccharide preparation provided herein can be administered to any appropriate mammal (e.g., rat, mouse, dog, cat, horse, cow, goat, pig, chicken, duck, rabbit, sheep, monkey, or human) to reduce one or more symptoms of Alzheimer's disease. Examples of Alzheimer's disease symptoms include, without limitation, difficulty in remembering recent events (e.g., short term memory loss), problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self-care, behavioral issues, or combinations thereof.

Any appropriate route of administration (e.g., oral or parenteral administration) can be used to administer a composition containing a potato polysaccharide preparation provided herein (e.g., a nutritional supplement composition provided herein) to a mammal. For example, a composition containing a potato polysaccharide preparation provided herein can be administered orally. In some cases, a composition containing a potato polysaccharide preparation provided herein can be administered by injection.

A composition provided herein (e.g., a nutritional supplement composition) can include one or more potato polysaccharide preparations. A potato polysaccharide preparation can be a preparation that is obtained from a water extract of potato and that contains polysaccharide material having the ability to be eluted from a C18 cartridge (e.g., a Sep-Pak Plus C-18 cartridge) with 10% acetonitrile. In some cases, a potato polysaccharide preparation can be a preparation that is obtained from potato and that contains polysaccharide material having HPLC characteristics of that of the peak eluted at 3.5 minutes as described in Example 1 (see, also, Figures 1, 2, and 27-33). In some cases, a polysaccharide of a potato polysaccharide preparation provided herein can be a polar, water-soluble polysaccharide. In some cases, a polysaccharide of a potato polysaccharide preparation provided herein can be a highly substituted complex xyloglucan material.

In some cases, a potato polysaccharide preparation can be a preparation that is obtained from potato and that contains polysaccharide material that, when derivatized, results in at least the following acylated carbohydrates as assessed using GC/MS: (a) myo-inositol (set to 1X to serve as an internal standard), (b) glucose at about 40X to about 60X the myo-inositol content (e.g., glucose at about 50X the myo-inositol content), (c) xylose at about 10X to about 20X the myo-inositol content (e.g., xylose at about 15X the myo-inositol content), (d) mannose at about 5X to about 15X the myo-inositol content (e.g., mannose at about 10X the myo-inositol content), and (e) galactose at about 3X to about 7X the myo-inositol content (e.g., galactose at about 5X the myo-inositol content). The derivatization procedure can include forming a dry residue of the polysaccharide material that is then hydrolyzed using trifluoroacetic acid. The resulting material is then reduced using sodium borohydride, and after borate removal, the end product is acylated using acetic anhydride and pyridine. The end products of the reaction are then injected directly on GC/MS to identify the acylated carbohydrates.

In some cases, a potato polysaccharide preparation can be a preparation that is obtained from potato and that contains polysaccharide material that, when derivatized and assessed using GC/MS, results in at least four major components (3,4-furan dimethanol, diacetate; 1,2,3,4,5-penta-o-acetyl-D-xylitol (isomer 1); 3,5-diacetoxy-benzyl alcohol; and D-glucitol-hexaacetate). See, e.g., Example 1. In some cases, a potato polysaccharide preparation can be a preparation that is obtained from potato and that contains polysaccharide material that, when derivatized and assessed using GC/MS, results in the compounds listed in Table 1 or results in the profile shown in Figure 6.

In some cases, a potato polysaccharide preparation provided herein can be a substantially pure potato polysaccharide preparation. Typically, a substantially pure potato polysaccharide preparation is a preparation that contains a single peak of material (e.g., a single peak of polysaccharide material) when assessed using, for example, HPLC (see, e.g., Figures 2 and 32). In some cases, greater than 60, 70, 75, 80, 85, 90, 95, or 99 percent of a potato polysaccharide preparation provided herein can be polysaccharide material obtained from a potato.

Any appropriate potato species or variety can be used to obtain a potato polysaccharide preparation provided herein. For example, *Solanum tuberosum*, *Ipomoea batatas*, *S. acaule*, *S. bukasovii*, *S. leptophyes*, *S. megistacrolobum*, *S. commersonii*, or *S. infundibuliforme* can be used to obtain a potato polysaccharide preparation provided herein. In some cases, potato varieties of *S. tunerosum* such as Organic Yellow, Purple or blue varieties, Cream of the Crop, Adirondack Blue, Adirondack Red, Agata, Almond, Andes Gold, Andes Sun, Apline, Alturas, Amandine, Annabelle, Anya, Arran Victory, Atlantic, Avalanche, Bamberg, Bannock Russet, Belle de Fontenay, BF-15, Bildtstar, Bintje, Blazer Russet, Blue Congo, Bonnotte, British Queens, Cabritas, Camota, Canela Russet, Cara, Carola, Chelina, Chiloé, Cielo, Clavela Blanca, Désirée, Estima, Fianna, Fingerling, Flava, German Butterball, Golden Wonder, Goldrush, Home Guard, Innovator, Irish Cobbler, Jersey Royal, Kennebec, Kerr's Pink, Kestrel, Keuka Gold, King Edward, Kipfler, Lady Balfour, Langlade, Linda, Marcy, Marfona, Maris Piper, Marquis, Megachip, Monalisa, Nicola, Pachacoña, Pike, Pink Eye, Pink Fir Apple, Primura, Ranger Russet, Ratte, Record, Red LaSoda, Red Norland, Red Pontiac, Rooster, Russet Burbank, Russet Norkotah, Selma, Shepody, Sieglinde, Silverton Russet, Sirco, Snowden, Spunta, Up to date, Stobrawa, Superior, Vivaldi, Vitelotte, Yellow Finn, or Yukon Gold can be used to obtain a potato polysaccharide preparation provided herein.

Any appropriate method can be used to obtain a potato polysaccharide preparation provided herein. For example, raw potato material can be homogenized (e.g., homogenized with a Polytron homogenizer) in water and maintained at room temperature for a period of time (e.g., about 1 hour) with occasional shaking. The homogenate can be centrifuged (e.g., centrifuged at 4000 g for 10 minutes) to remove any larger solid material. The resulting supernatant can be loaded onto a Solid Phase Extraction cartridge (e.g., a C18 cartridge such as a Sep-Pak Plus C-18 cartridge), and the polysaccharide material eluted with 10 percent acetonitrile. Once eluted, the polysaccharide material can be dried and stored (e.g., stored at about 4°C).

This document also provides nutritional supplement compositions containing one or more potato polysaccharide preparations provided herein. For example, a potato polysaccharide preparation provided herein obtained from Idaho Russet potatoes can be formulated into a nutritional supplement composition.

Any appropriate dose of a potato polysaccharide preparation provided herein can be used to formulate a composition provided herein (e.g., a nutritional supplement composition or potato polysaccharide preparation provided herein). For example, a potato polysaccharide preparation provided herein can be used to formulate a composition for reducing amyloid beta levels within a mammal having Alzheimer's disease. The composition can contain between about 1 mg and about 750 mg (e.g., between about 1 mg and about 500 mg, between about 1 mg and about 250 mg, between about 5 mg and about 40 mg, between about 5 mg and about 30 mg, between about 5 mg and about 20 mg, between about 6 mg and about 50 mg, between about 6 mg and about 20 mg, between about 10 mg and about 25 mg, or between about 15 mg and about 20 mg) of the potato polysaccharide component of the potato polysaccharide preparation. In some cases, a composition (e.g., a nutritional supplement composition) can be formulated to deliver about 0.05 mg of the potato polysaccharide component per kg of body weight to about 0.5 mg of the potato polysaccharide component per kg of body weight to a mammal (e.g., a human) per day. For example, a nutritional supplement composition can be formulated into a single oral composition that a human can swallow once a day to provide between about 0.05 mg of the potato polysaccharide component per kg of body weight to about 0.5 mg of the potato polysaccharide component per kg of body weight.

Any appropriate method can be used to formulate a composition provided herein (e.g., a nutritional supplement composition or potato polysaccharide preparation provided herein). For example, common formulation mixing techniques and preparation techniques can be used to make a composition (e.g., a nutritional supplement composition) having the components described herein. In addition, a composition provided herein (e.g., a nutritional supplement composition or potato polysaccharide preparation provided herein) can be in any form. For example, a composition provided herein (e.g., a nutritional supplement composition or potato polysaccharide preparation provided herein) can be formulated into a pill, capsule, tablet, gel cap, nutritional shake, nutritional bar, rectal suppository, sublingual suppository, nasal spray, inhalant, or injectable ampule. In some cases, a composition provided herein (e.g., a nutritional supplement composition) can include one or more potato polysaccharide preparations provided herein alone or in combination with other ingredients including, without limitation, gelatin, cellulose, starch, sugar, bentonite, lactic acid, mannitol, alpha lipoic acid, alpha tocopherol, L-ascorbate, or combinations thereof.

In some cases, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of a LRP1 polypeptide, an APBB1 polypeptide, an IDE polypeptide, a GPX3 polypeptide, a GPX4 polypeptide, or a combination thereof. In some cases, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of an IGF1 polypeptide, a NOS2 polypeptide, or a combination thereof.

In humans, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of a human LRP1 polypeptide, a human APBB1 polypeptide, a human IDE polypeptide, a human GPX3 polypeptide, a human GPX4 polypeptide, or a combination thereof. In some cases, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of a human IGF1 polypeptide, a human NOS2 polypeptide, or a combination thereof.

A human LRP1 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. NP_002323.2 (GI No. 126012562) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NG_016444 (GI No. 284813599). A human APBB1 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. O00213.2 (GI No. 12229629) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NG_029615.1 (GI No. 342349296). A human IDE polypeptide can have the amino acid sequence set forth in GenBank® Accession No. AAA52712.1 (GI No. 184556) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NG_013012.1 (GI No. 260593646). A human GPX3 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. NP_002075.2 (GI No. 6006001) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NC_000005.10 (GI No. 568815593). A human GPX4 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. AAH22071.1 (GI No. 34784795) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NC_000019.10 (GI No. 568815579). A human IGF1 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. NP_001104753.1 (GI No. 163659899) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NC_000012.12 (GI No. 568815586). A human NOS2 polypeptide can have the amino acid sequence set forth in GenBank® Accession No. AAI30284.1 (GI No. 120660146) and can be encoded by the nucleic acid sequence set forth in GenBank® Accession No. NG_011470.1 (GI No. 22480926).

In some cases, a potato polysaccharide preparation provided herein or a nutritional supplement composition provided herein can be used to increase expression of polypeptides involved with the metabolism of CNS-derived amyloid beta polypeptides within the adipose tissue compartment. For example, a potato polysaccharide preparation provided herein or a nutritional supplement composition provided herein can be used to increase expression of a LRP1 polypeptide, an APBB1 polypeptide, an IDE polypeptide, a GPX3 polypeptide, or a combination thereof. In humans, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of a human LRP1 polypeptide, a human APBB1 polypeptide, a human IDE polypeptide, a human GPX3 polypeptide, or a combination thereof, in human adipocytes.

In some cases, a potato polysaccharide preparation provided herein or a nutritional supplement composition provided herein can be used to increase expression of polypeptides involved with the metabolism of CNS-derived amyloid beta polypeptides within the white blood cell tissue compartment. For example, a potato polysaccharide preparation provided herein or a nutritional supplement composition provided herein can be used to increase expression of an APBB1 polypeptide, an IDE polypeptide, a GPX4 polypeptide, or a combination thereof. In humans, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to increase expression of a human APBB1 polypeptide, a human IDE polypeptide, a human GPX4 polypeptide, or a combination thereof, in human white blood cells.

In some cases, a potato polysaccharide preparation provided herein or a nutritional supplement composition provided herein can be used to increase or decrease expression of polypeptides involved with oxidative stress and proinflammatory pathways. For example, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of an IGF1 polypeptide, a NOS2 polypeptide, or a combination thereof. In humans, a composition containing a potato polysaccharide preparation provided herein or a potato polysaccharide preparation provided herein can be used to decrease expression of an IGF1 polypeptide, a NOS2 polypeptide, or a combination thereof, in human white blood cells.

The document will provide addition description in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Identification and characterization of a potato polysaccharide preparation

6 grams of a Russet potato variety of the *Solanum tuberosum* species were homogenized with a Polytron homogenizer in 20 mL water in a 50 mL centrifuge tube and kept at room temperature for 1 hour. The homogenate was centrifuged at 4000 rpm for 10 minutes. A Sep-Pak Plus C-18 cartridge was activated with 10 mL 100% acetonitrile (ACN) and washed with 10 mL 0.05% trifluoroacetic acid in water (TFA water). 10 mL of the supernatant was loaded onto the cartridge, and all H_2O that passes through cartridge was collected in 1.5 mL Eppendorf tubes. Next, 10 mL of 2% ACN (in 0.05% TFA water) was passed through the column, and the elutriate was collected in 1.5 mL Eppendorf tubes. Next, 10 mL of 5% ACN (in 0.05% TFA water) was used to wash the column, and the elutriate was collected in 1.5 mL Eppendorf tubes. Finally, 10 mL of 10% ACN (in 0.05% TFA water) was collected in 1.5 mL Eppendorf tubes after passing through the column. All of the fractions were dried, and the dried fractions of the same ACN concentration were reconstituted into 1 tube

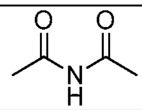
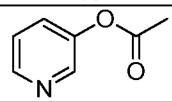
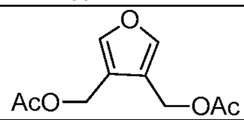
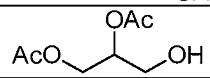
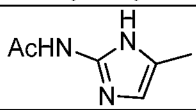
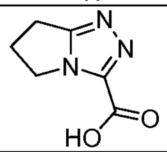
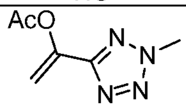
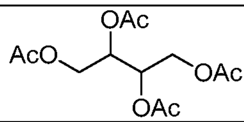
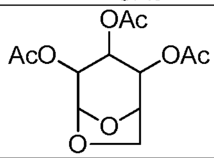
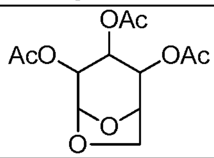
in 1 mL of 0.05% TFA water for further purification via HPLC or reconstituted in 1 mL of phosphate buffered saline for use in cell treatments.

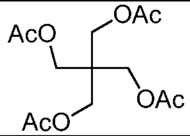
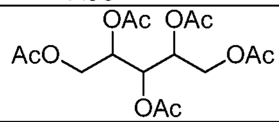
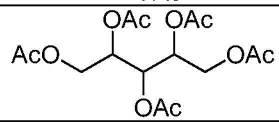
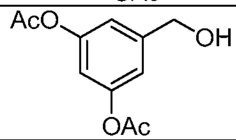
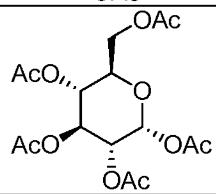
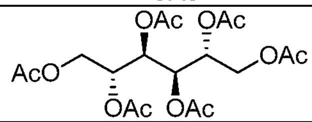
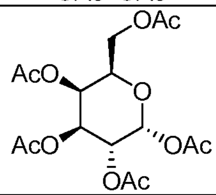
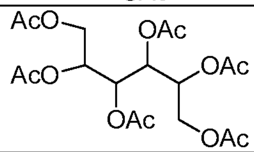
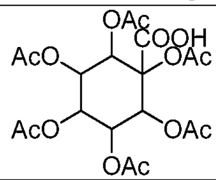
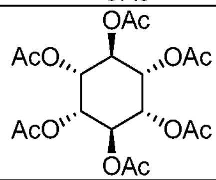
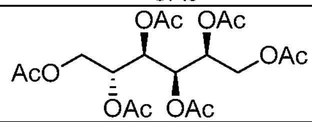
A Waters 2695 separations module with a photodiode array detector was used to purify the 10% ACN extract. An XterraRP C18 column (4.6 X 150 mm) was used for the separation with 0.05% TFA water as the mobile phase. Each HPLC run was a 20 minute gradient ranging from 0 to 2.5% ACN. The injection volume was 100 µL, and the flow rate was 0.5 mL/minute. HPLC fractionation of the 10% ACN extract yielded three major UV absorbing peaks eluted at 3.5, 3.9, and 12.1 minutes (Figure 1). Collection and HPLC re-purification of the 3.5 minute fraction yielded a symmetrical peak displaying a maximum absorbance at 198.3 nm (Figure 2).

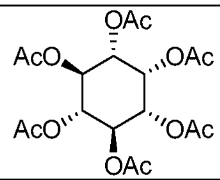
Further chemical characterization of the symmetrical 3.5 minute HPLC peak material was performed. Pooled 3.5 minute HPLC fractions were dried and reconstituted in 1 mL TFA water and subjected to tandem LC/MS/MS (Figure 3) and NMR chemical analyses (Figures 4 and 5). For the NMR analysis, ^{1}H-NMR was run on the sample using deuterium oxide (D_2O) as a solvent to further analyze the sample. The water peak at 4.65 PPM was solvent-suppressed, and the spectrum was acquired for several hours. Acetamide was detected at 3.2 PPM, along with acetonitrile at 1.9 PPM. Minor peaks were detected at 1.05 PPM, 1.17 PPM (broad peak), 1.189 PPM, and 1.864 PPM. One characteristic of polymeric materials in a proton NMR was the broadening of peaks such as the shift at 1.17 PPM. These shifts on the NMR could represent the peak at 4.8 PPM and suggested a polar, water-soluble polymer such as a polysaccharide. Taken together, these results confirmed the presence of high molecular weight polysaccharide material contained in HPLC purified fractions eluting at 3.5 minutes.

Further analysis confirmed that the HPLC purified fraction eluting at 3.5 minutes contains polysaccharide material (e.g., highly substituted complex xyloglucan material). To make the polysaccharide material analyzable by gas chromatography/mass spectroscopy (GC/MS), it was converted into its derivatized carbohydrate fragments. Briefly, the sample was concentrated to a dry residue that was hydrolyzed using trifluoroacetic acid. This was then reduced using sodium borohydride, and after borate removal, the end product was acylated using acetic anhydride and pyridine. The end products of the reaction were injected directly on GC/MS to identify any acylated carbohydrates. Based on the end analysis, a larger carbohydrate existed in the sample. The total ion chromatogram (TIC) is shown below in Figure 6 with appropriate peak labels below in Table 1. The major components identified are indicated in bold (peaks 3, 12, 14, and 21). The corresponding fragmentation for each compound is provided in Figures 7-26. For each fragmentation, the peak fragmentation pattern is on the top, the compound library fragmentation match is on the bottom, and an overlay of the two is in the center. Finally, unlabeled peaks were either column bleed or did not have a sufficient match to the compound library.

Table 1: Summary of GC/MS results.

Peak	Retention Time (min)	Compound Name	Structure
1	10.731	Diacetamide	
2	13.669	3-Acetoxy pyridine	
3	**19.568**	**3,4-Furan dimethanol, diacetate**	
4	19.950	1,2,3-propanetriol diacetate	
5	23.387	Imidazole, 2-acetamino-5-methyl	
6	23.499	6,7-dihydro-5H-pyrrol[2,1,c][1,2,4] triazole-3-carboxylic acid	
7	24.304	Acetic acid, 1-(2-methyltetrazol-5-yl) ethenyl ester	
8	25.538	1,2,3,4-butanetriol, tetraacetate	
9	27.412	(1,5)β(1,3)triacetyl D-galactosan (stereoisomer 1)	
10	28.188	(1,5)β(1,3)triacetyl D-galactosan (stereoisomer 2)	

11	29.210	Pentaerythritol tetraacetate	
12	**29.727**	**1,2,3,4,5-penta-o-acetyl-D-xylitol (isomer 1)**	
13	30.697	1,2,345-penta-o-acetyl-D-xylitol (isomer 2)	
14	**32.477**	**3,5-diacetoxy-benzyl alcohol**	
15	32.677	β-D-glucopyranose, pentaacetate	
16	33.012	D-mannitol hexaacetate	
17	33.106	β-D-galactopyranose, pentaacetate	
18	33.206	Galacticol, hexaacetate	
19	33.364	Cyclohexane carboxylic acid, 1,2,45-tetrakis(acetoxy), (1α,3α,4α,5β)-(-)	
20	33.582	Muco-inositol, hexaacetate	
21	**33.006**	**D-glucitol-hexaacetate**	

22	34.463	Myo-inositol, hexaacetate	

These results demonstrate the presence of sugar monomers that serve as building blocks for a larger carbohydrate. It appeared from these multiple lines of analysis that the potato polysaccharide preparation is a highly substituted complex xyloglucan.

<u>Example 2 – Sweet potatoes and multiple varieties of potatoes exhibit the presence of potato polysaccharide material</u>

Six grams of potato material from multiple varieties of *Solanum tuberosum* (Organic yellow, Purple, Idaho Russet, and Yukon Gold) and six grams of material from sweet potatoes (*Ipomoea batatas*) were extracted in 20 mL of water. 10 mL of that water was then loaded onto a sep-pak cartridge, and the cartridge was then eluted with 10 mL of 10% ACN. The ACN was then dried, and the residue was dissolved in 1 mL of water. A 100 µL injection of this water was assessed using HPLC.

The HPLC chromatograms demonstrated that the amount of the first peak (at 3.5 minutes at 210 nm) was the same for all five types of potatoes tested (Figures 27-31).

In another experiment, material was extracted from a boiled Purple potato and analyzed. The peak at 3.5 minutes was not reduced in the boiled potato (Figure 32).

The 3.5 minute peak from two pooled fraction collections from Idaho Russet potatoes was collected, dried, and reconstituted in 100 µL of water. The material was then injected into the HPLC yielding a single peak at 3.5 minutes (Figure 33). Taken together, these results demonstrate that potatoes within the *Solanum tuberosum* and *Ipomoea batatas* species contain potato polysaccharide material.

<u>Example 3 – Analysis of a potato polysaccharide preparation</u>

A potato polysaccharide preparation was purified using HPLC from 3 g of purple potato. The potato polysaccharide peak was eluted at about 5 minutes (Figure 34). This peak was obtained using a different chromatographic column (10 mm x150 mm) as compared to the column used to obtain the 3.5 minute peak. Since the column was a larger preparative column and the flow rate was 1.5 mL/minute, the elution time of the potato polysaccharide was 5 minutes.

Example 4 – Use of potato polysaccharide preparations to reduce amyloid beta levels in Zucker Diabetic Fatty rats

To assess the ability of potato polysaccharide preparations to reduce amyloid beta levels within a mammal having Alzheimer's disease, blood, the livers, and abdominal fat from rats of four groups of experimental animals were collected, weighed, and examined as described in this Example.

Extraction and purification of a potato polysaccharide preparation

Typically, 6 g of potato were homogenized with a Polytron homogenizer in 20 mL water in a 50 mL centrifuge tube and kept at room temperature for 1 hour. The homogenate was centrifuged at 4000 rpm for 10 minutes and the supernatant fraction was reserved. 10 mL of the supernatant fraction was percolated through a Sep-Pak Plus C-18 cartridge previously activated with 10 mL 100% acetonitrile (ACN) followed by 10 mL 0.05% trifluoroacetic acid in water (TFA water). Following successive low ACN washes, semi-purified potato polysaccharide preparation was eluted in 10 mL 10% ACN in 0.05% TFA water. The eluent fraction was dried and reconstituted in 1 mL 0.05% TFA water for further purification via HPLC.

The reconstituted 10% ACN eluent fraction was subjected to HPLC purification utilizing a Waters Xterra RP C18 column (4.6X150 mm) and Waters 2695 separations module with a photodiode array detector. HPLC purification employed a shallow 20 minute gradient ranging from 0 to 2.5% in 0.05% TFA water at a flow rate of 0.5 mL/min. Collection and HPLC re-purification of a major 198 nm UV absorbing peak at 3.5 minutes yielded a symmetrical HPLC peak containing highly purified potato polysaccharide preparation. The purified HPLC fraction was dried and reconstituted in phosphate buffered saline (PBS) for use in biological experiments.

Potato polysaccharide preparation formulation

Purified potato polysaccharide preparation (10 mL stock solution at 5 mg/mL concentration) was stored at 4°C. The vehicle for the study was sterile water (Catalog number 002488, Butler Schein). Each week, the stock solution was diluted 1:100 in sterile water (0.05 mg/mL) and dispensed into daily aliquots. All vehicle and drug solutions were stored at 4°C and administered at room temperature daily by oral gavage (PO) in a volume of 1 mL/animal (0.15 mg/kg dose based on estimated body weight of 350 g).

In vivo animal model

The Zucker Diabetic Fatty (ZDF) rat model was used (Carley and Severson, *Biochim. Biophys. Acta*, 1734:112-26 (2005)).

Experimental animals

Twenty-two 7-week old, male Zucker Diabetic Fatty rats (ZDF, Code: 370) and twenty-two 7-8 week old, male ZDF Lean rats (Code: 371) were purchased from Charles Rivers Laboratories (Wilmington, MA). The study animals were allowed an acclimation period of 4 days prior to baseline blood collections, at which time two extra animals from each strain were dropped from the study based on baseline body weight. The rats were housed two per cage and maintained in the Innovive caging system (San Diego, CA) upon arrival at PhysioGenix, Inc. Cages were monitored daily to ensure the Innovive system maintained 80 air changes per hour and positive pressure. In accordance with the Guide for Care and Use of Laboratory Animals (Eighth Edition), rat rooms were maintained at temperatures of 66-75 degrees Fahrenheit and relative humidity between 30% and 70%. The rooms were lit by artificial light for 12 hours each day (7:00 AM - 7:00 PM). Animals had free access to water and Purina 5008 rodent food (Waldschimdt's, Madison, WI) for the duration of the study except during fasted experiments.

Dosing and grouping

Two types of rats were used for the study: homozygous obese ZDF/ZDF and heterozygous lean littermates. The rats within the groups were then chosen at random and divided into groups of 10. Group 1 was the homozygous ZDF/ZDF vehicle fed rats, group 2 was the homozygous ZDF/ZDF potato polysaccharide preparation fed, group 3 was the lean vehicle fed rat and group 4 was the lean potato polysaccharide preparation fed rats. The vehicle was distilled water and the potato polysaccharide preparation was given daily each morning via oral gavage at a dosage of 0.05 mg per animal. The dose was usually given in 1 mL of water. Rats were caged in groups and maintained in 12 hours light/12 hours dark (7 am-7pm). The study lasted for 28 days and all animals were euthanized by isoflurane overdose and thoracotomy following the collection of fasted blood glucose data on Day 28 of the Study. Blood was collected via descending vena cava. Liver and abdominal fat were collected, weighed, and a portion of the left lateral liver lobe and abdominal fat were placed into individual histology cassettes and snap frozen in liquid nitrogen. General pathological observations were recorded.

RNA isolation

Total RNA extracted from rat tissue samples was isolated and purified using the RNeasy mini kit (Qiagen, Valencia, CA). Typically, 100 mg of tissue was resuspended in 1.8 mL of RLT lysis buffer (Qiagen) and homogenized with a polytron homogenizer for 30 seconds. Blood RNA was isolated using the PAX RNA kit (Qiagen).

DNA microarray analyses

DNA microarray analyses were performed using a system provided by Agilent. Arrays included four arrays per chip (Agilent 4X44K chip). Total RNA was reverse transcribed (1000 ng) using T7 primers and labeled and transcribed using Cyanine-3 dye. Each array was hybridized with at least 1.65 μg of labeled cRNA at 65°C for 18 hours. Arrays were scanned using an Agilent array scanner. The microarray platform can determine a minimum of a 15% change in gene expression.

Real-time PCR analyses

Real-time PCR analysis of gene expression was performed to validate the DNA microarray data sets. GAPDH was used as a reference gene. The real-time PCR master mix included 25 μL 2x universal master mix, 2.5 μL 20x detector set (with the primer and probe), and 21.5 μL of water. PCR was performed in an Applied Biosystems 7500 sequence detection system. The thermocycler conditions included denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 60 seconds. Forty cycles of PCR were preceded by 95 °C for 10 minutes. Reactions were performed in triplicate. The relative quantities of genes were determined using the formula 2-ΔΔCt using the Applied Biosystems 7500 software.

Results

In vivo administration of purified potato polysaccharide preparation to ZDF rats (n=10) vs. vehicle control ZDF rats engendered a statistically significant enhancement of interactive LRP1, APBB1, IDE, and GPX3 gene expression, normalized as fold changes of 1.8, 1.9, 1.5, and 2.4, respectively, in adipose tissue samples, as depicted in Table 2. In similar fashion, in vivo administration of purified potato polysaccharide preparation to ZDF rats (n=7) vs. vehicle control ZDF rats engendered a statistically significant enhancement of interactive APBB1, IDE and GPX4 gene expression, normalized as fold changes of 1.7, 1.5, and 2.6, respectively, in blood samples, as depicted in Table 3. In vivo administration of purified potato polysaccharide preparation to ZDF rats (n=7) vs. vehicle control ZDF rats engendered a highly dramatic and statistically significant reduction of IGF1 and NOS2 proinflammatory gene expression, normalized as fold changes of -1.7 and -4.6, respectively, in blood samples, as depicted in Table 4.

Table 2. Enhanced gene expression of LRP1, APBB1, IDE, and GPX3 in adipose tissues of Zucker Diabetic Fatty vs. vehicle control ZDF rats (n=10) following in vivo potato polysaccharide preparation administration. Data sets were derived by DNA microarray analyses, as described above. ND=gene expression not detected.

Gene Symbol	Fold Change	Description	p value
LRP1	1.8	Low density lipoprotein receptor-related protein 1	0.02
APBB1	1.9	Amyloid beta precursor protein-binding, family B, member 1	0.0004
IDE	1.5	Insulin degrading enzyme	0.01
GPX3	2.4	Glutathione peroxidase 3	0.0004
GPX4	-1.1	Glutathione peroxidase 4	0.6
IGF1	ND	Insulin like growth factor	-
NOS2	ND	Nitric oxide synthase 2, inducible	-

Table 3. Enhanced gene expression of APBB1, IDE, and GPX4 in blood samples of Zucker Diabetic Fatty vs. vehicle control ZDF rats (n=7) following in vivo potato polysaccharide preparation administration. Data sets were derived by DNA microarray analyses, as described above. ND=gene expression not detected.

Gene Symbol	Fold Change	Description	p value
APBB1	1.7	Amyloid beta precursor protein-binding, family B, member 1	0.04
IDE	1.5	Insulin degrading enzyme	0.04
GPX4	2.6	Glutathione peroxidase 4	0.002
GPX3	ND	Glutathione peroxidase 3	-
LRP1	ND	Low density lipoprotein receptor-related protein 1	-

Table 4. Reduced expression of IGF1, and NOS2 proinflammatory genes in blood samples of Zucker Diabetic Fatty vs. vehicle control ZDF rats (n=7) following in vivo potato polysaccharide preparation administration. Data sets were derived by DNA microarray analyses, as described above.

Gene Symbol	Fold Change	Description	p value
IGF1	-1.7	Insulin-like growth factor 1	0.02
NOS2	-4.8	Nitric oxide synthase 2, inducible	0.003

Real-time PCR analysis of IDE expression was performed to validate the DNA microarray data sets. In particular, the enhancement of IDE gene expression in adipose tissue and blood leukocyte samples, as monitored by DNA microarray analyses, was confirmed by real time PCR analyses. As depicted in Figure 37, the enhancements in IDE gene expression were determined to be 44.9 ± 2.8% (n=9, p=0.02) and 51.3 ± 6.2% (n=6, p=0.04), for adipose tissue and blood samples, respectively. The respective real time PCR traces are depicted in Figures 35 and 36.

These results demonstrate that potato polysaccharide preparations can be used as anti-neurodegenerative agents to reduce amyloid beta levels within a mammal having Alzheimer’s disease.

Example 5 – Use of potato polysaccharide preparations to improve cognition of APP SWE/PSEN1dE9 mice

Dosing and grouping

APP SWE/PSEN1dE9 mice are used as a model for Alzheimer's disease. The mice within the groups are chosen at random and divided into groups of X or Y. Groups are given dosages of potato polysaccharide preparation at a range of 0 mg/kg/day (control) up to 0.5 mg/kg/day. One dose is 0.15 mg/kg/day. The vehicle is distilled water, and the potato polysaccharide preparation is given daily each morning via oral gavage at the dosage to be evaluated. The dose is usually given in 1 mL of water. Mice are caged in groups and maintained in 12 hours light/12 hours dark (7 am-7pm). The study proceeds for several months. A typical protocol starts with potato polysaccharide preparation administration at 20 weeks of age and continues through 30 weeks of age and includes 3 groups (20 animals/group): vehicle, 5 μg/day potato polysaccharide preparation, and 20 μg/day potato polysaccharide preparation.

Data collection

Body weights are recorded weekly. Whole blood, serum, and plasma are collected at day 0 for baseline analysis. Whole blood, serum, and plasma are collected at termination. Brain tissue and vascular are collected and snap frozen in liquid nitrogen at termination. Whole genome microarrays are performed with the frozen tissue samples. Whole blood is preserved in PAX RNA blood tubes for possible gene expression analysis. Histology to determine the amount of plaque development in animal brain tissue is performed at termination. Any appropriate method to determine APP or AP4, amyloid beta polypeptides in blood or tissue is used. Cognition of the study animals is assessed by using the Morris water maze methodology.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for reducing amyloid beta levels within a mammal having Alzheimer's disease, wherein said method comprises administering to said mammal a composition comprising a potato polysaccharide preparation obtained from raw potatoes, wherein the level of amyloid beta within said mammal is reduced.

2. The method of claim 1, wherein said mammal is a human.

3. The method of claim 1, wherein said level of amyloid beta within said mammal is reduced in blood.

4. The method of claim 1, wherein said level of amyloid beta within said mammal is reduced in adipose tissue.

5. The method of claim 1, wherein said composition comprises said potato polysaccharide preparation in an amount that results in between 0.05 mg and 50 mg of the potato polysaccharide component of said potato polysaccharide preparation being administered to said mammal per kg of body weight of said mammal.

6. The method of claim 1, wherein said composition comprises between 1 mg and 100 mg of said potato polysaccharide preparation.

7. The method of claim 1, wherein said composition comprises between 6 mg and 20 mg of said potato polysaccharide preparation.

8. The method of claim 1, wherein said composition comprises between 1 mg and 100 mg of the potato polysaccharide component of said potato polysaccharide preparation.

9. The method of claim 1, wherein said composition comprises between 6 mg and 20 mg of the potato polysaccharide component of said potato polysaccharide preparation.

10. The method of claim 1, wherein said composition is in the form of a tablet.

11. The method of claim 1, wherein said composition comprises alpha lipoic acid.

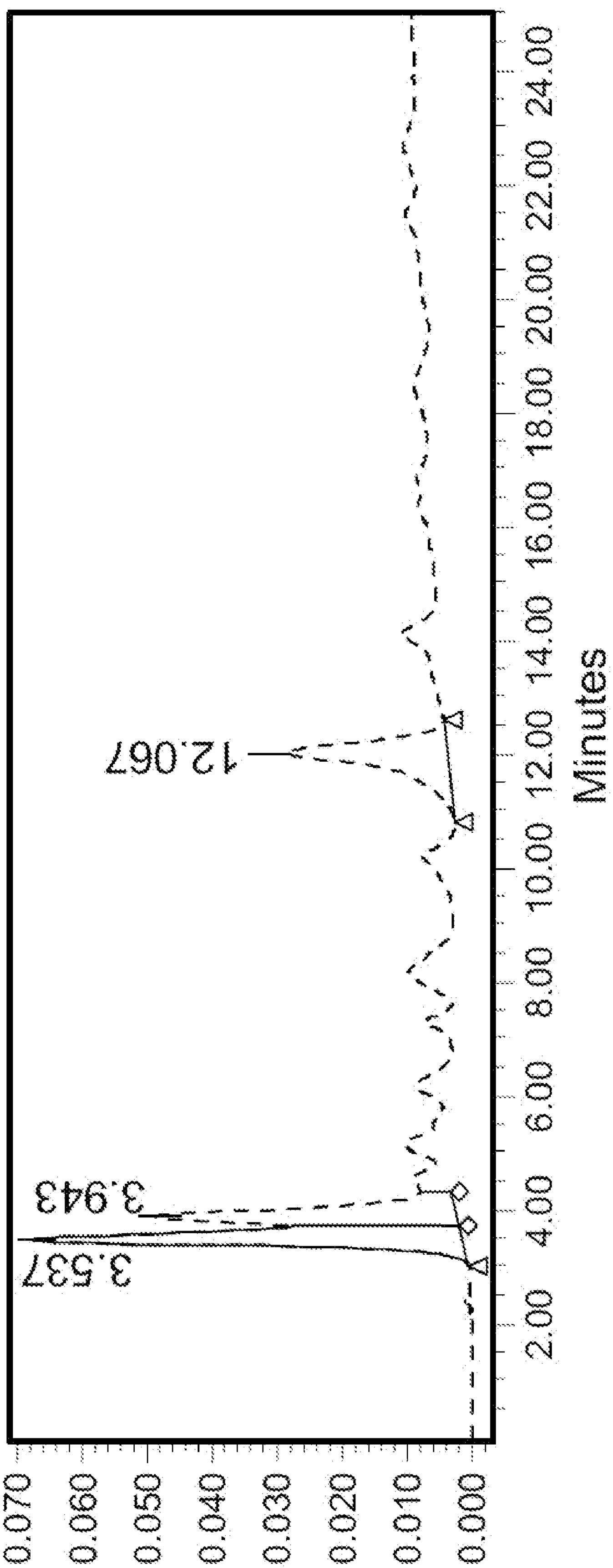
12. The method of claim 1, wherein said composition comprises alpha tocopherol.

13. The method of claim 1, wherein said potato polysaccharide preparation is in an amount that results in between 0.075 mg and 0.5 mg of the potato polysaccharide component of said potato polysaccharide preparation being administered to said mammal per kg of body weight of said mammal.

14. The method of claim 1, wherein at least about 80 percent of said potato polysaccharide preparation is potato polysaccharide.

15. The method of claim 1, wherein at least about 90 percent of said potato polysaccharide preparation is potato polysaccharide.

16. The method of claim 1, wherein at least about 95 percent of said potato polysaccharide preparation is potato polysaccharide.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Minutes
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8.00
10.00
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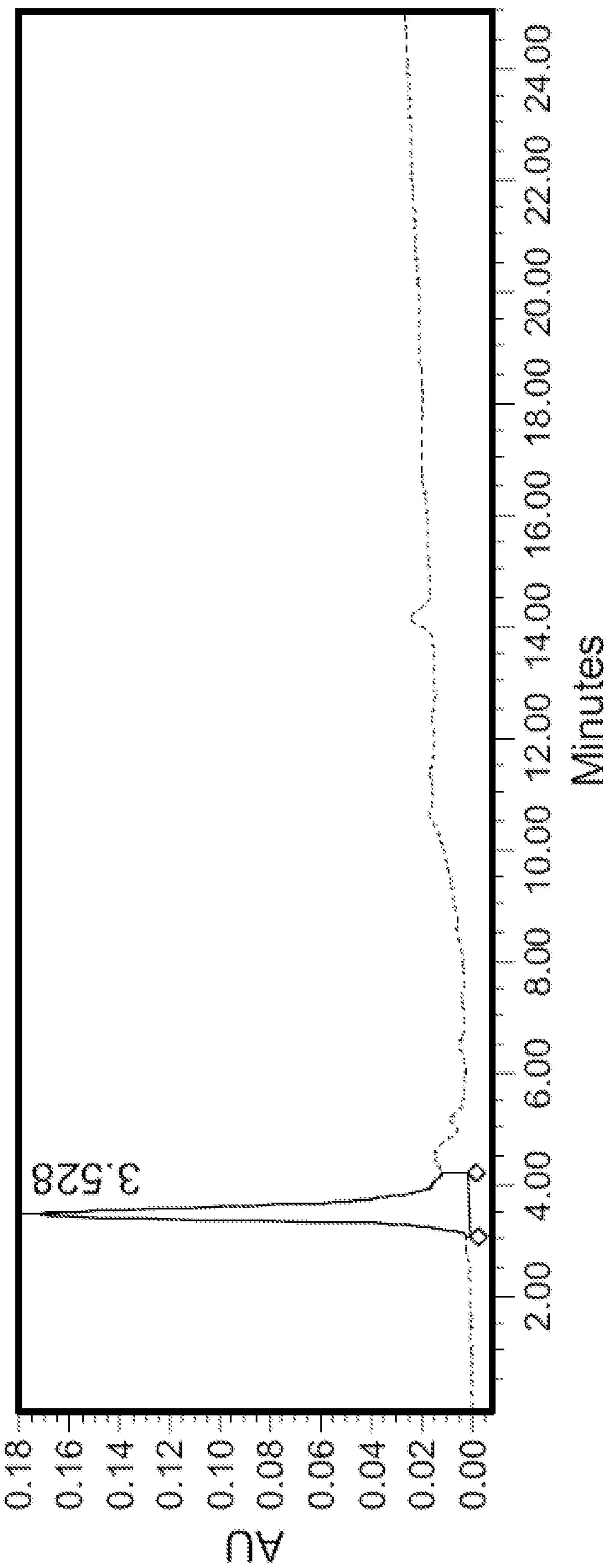
3.943
12.067

FIG. 1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0.16
0.14
0.12
0.10
0.08
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Minutes
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8.00
10.00
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14.00
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18.00
20.00
22.00
24.00

FIG. 2

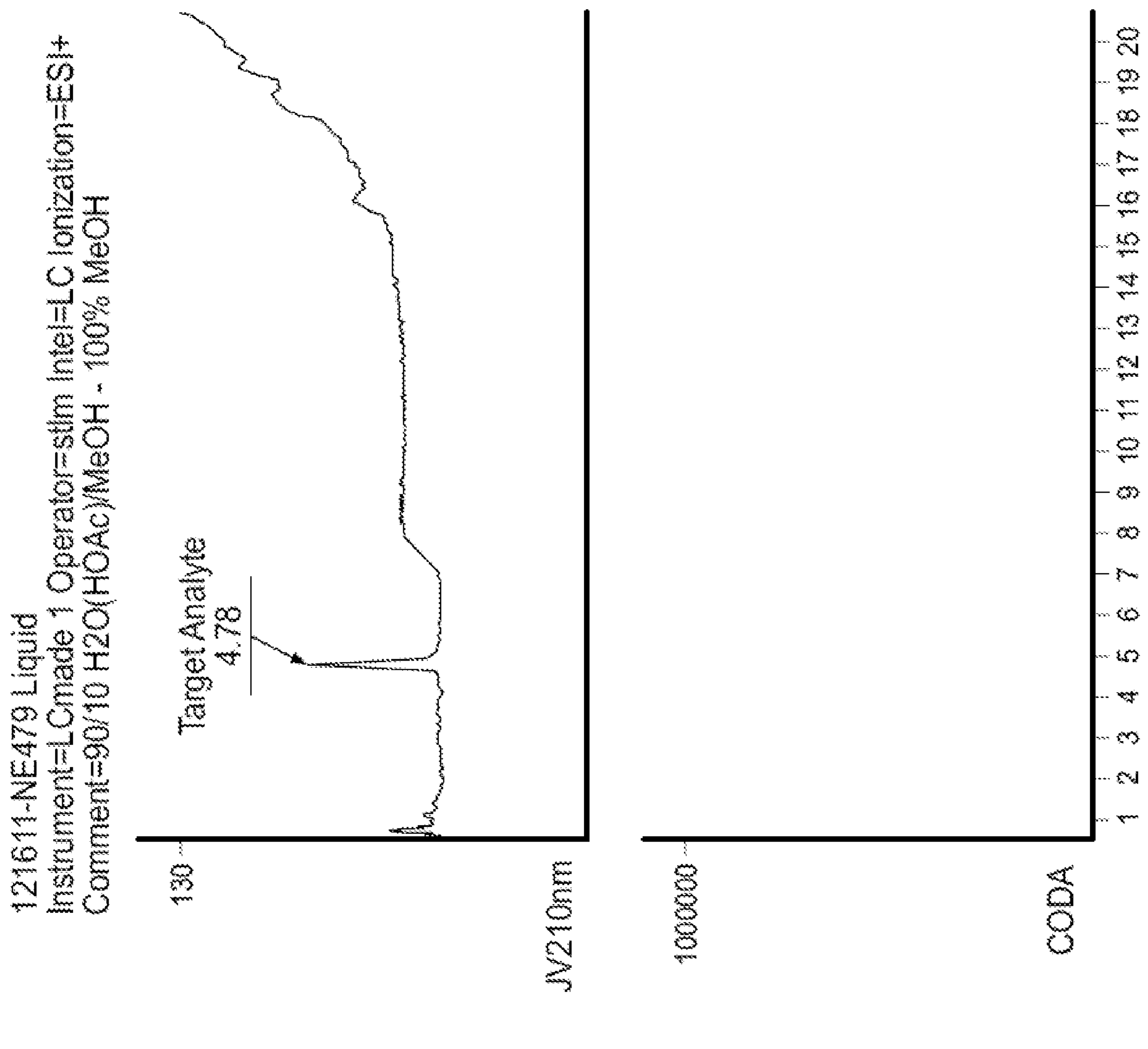
121611-NE479 Liquid
Instrument=LCmade 1 Operator=stlm Intel=LC Ionization=ESI+
Comment=90/10 H2O(HOAc)/MeOH - 100% MeOH
Target Analyte
4.78
130
JV210nm
1000000
CODA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 3

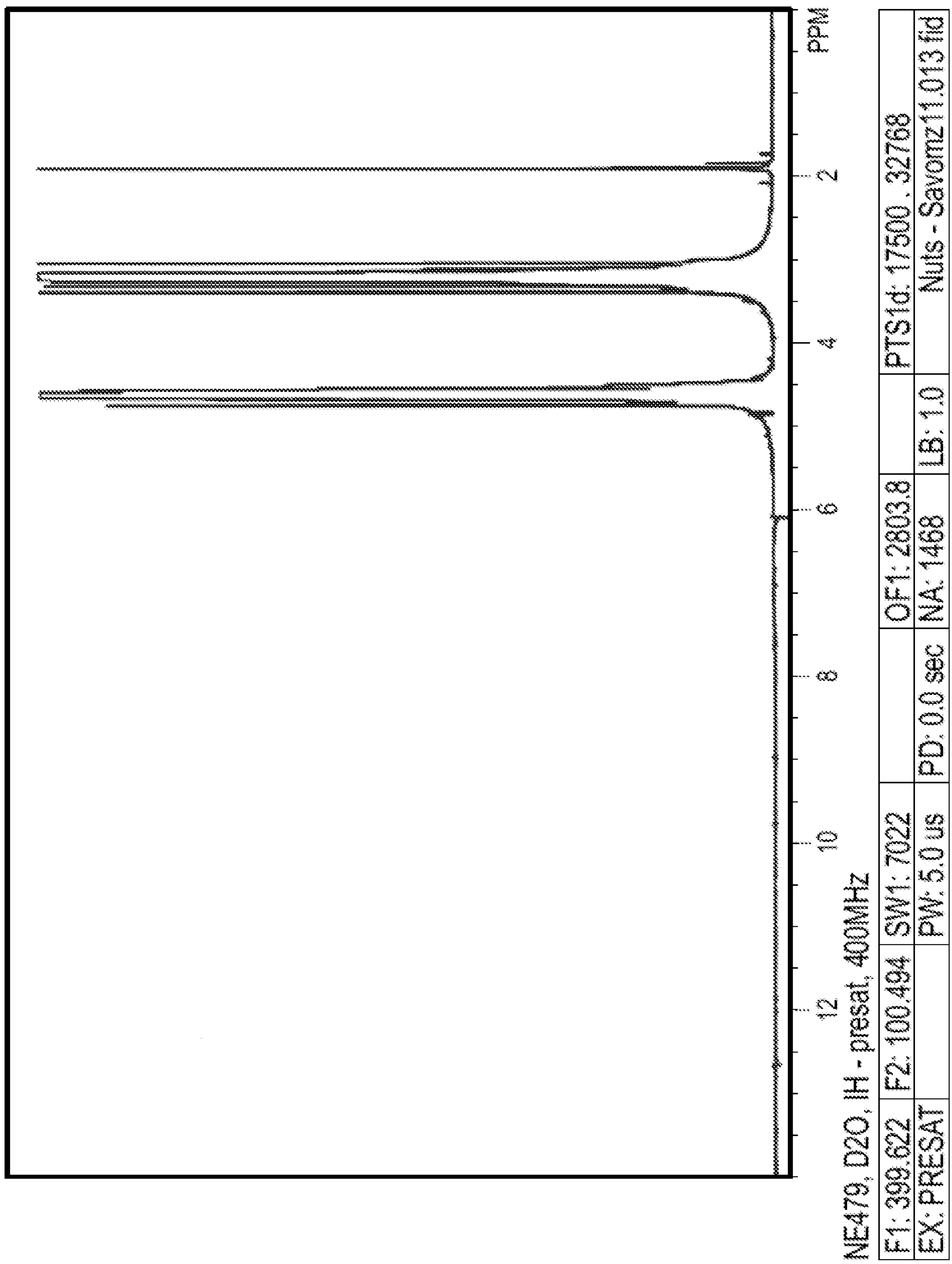
PPM
2
4
6
8
10
12
NE479, D2O, IH - presat, 400MHz
F1: 399.622
F2: 100.494
SW1: 7022
OF1: 2803.8
PTS1d: 17500 . 32768
EX: PRESAT
PW: 5.0 us
PD: 0.0 sec
NA: 1468
LB: 1.0
Nuts - Savomz11.013 fid

FIG. 4

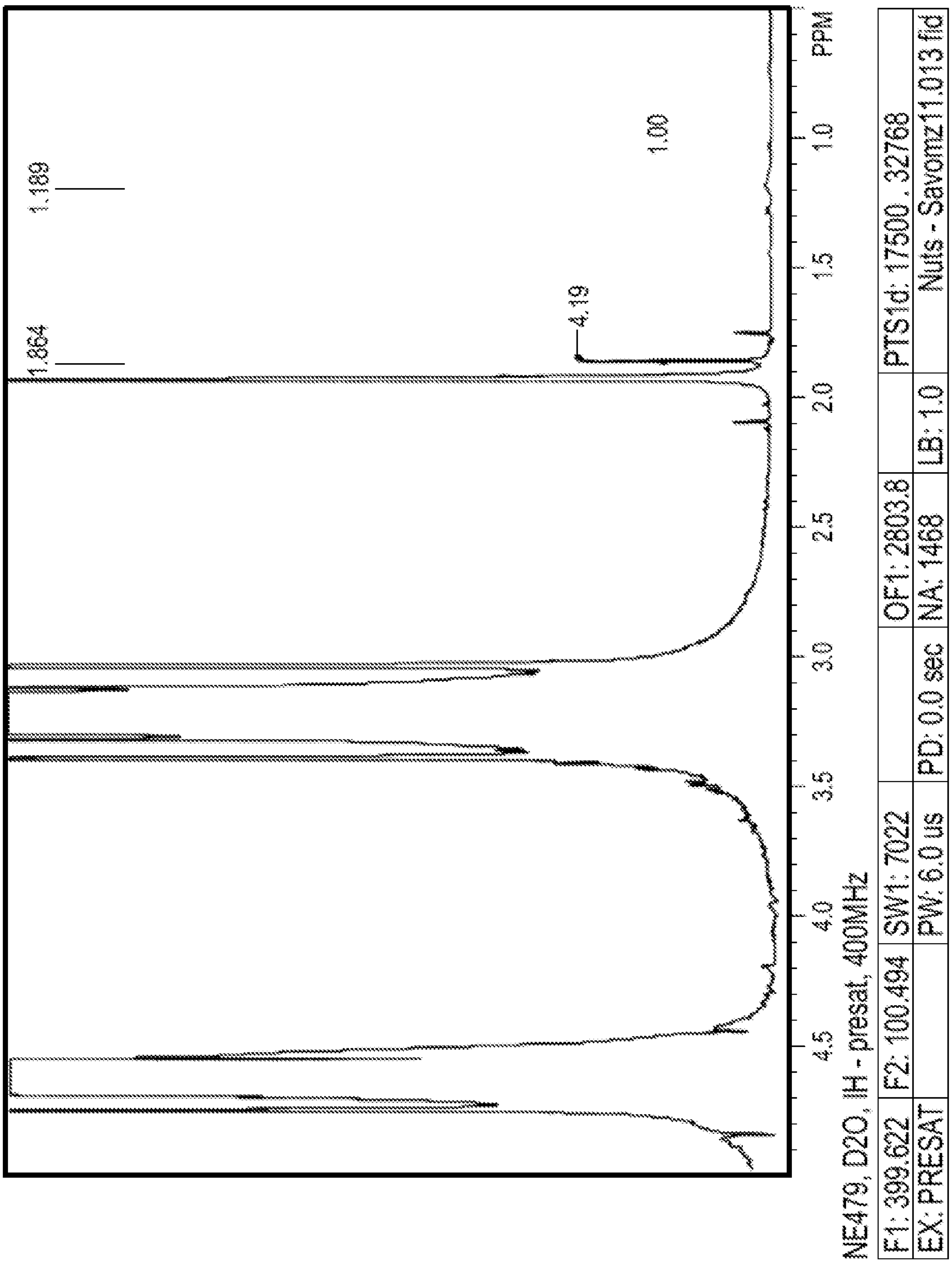
1.864
1.189
4.19
1.00
PPM
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
NE479, D2O, IH - presat, 400MHz
F1: 399.622
F2: 100.494
SW1: 7022
OF1: 2803.8
PTS1d: 17500 . 32768
EX: PRESAT
PW: 6.0 us
PD: 0.0 sec
NA: 1468
LB: 1.0
Nuts - Savomz11.013 fid

FIG. 5

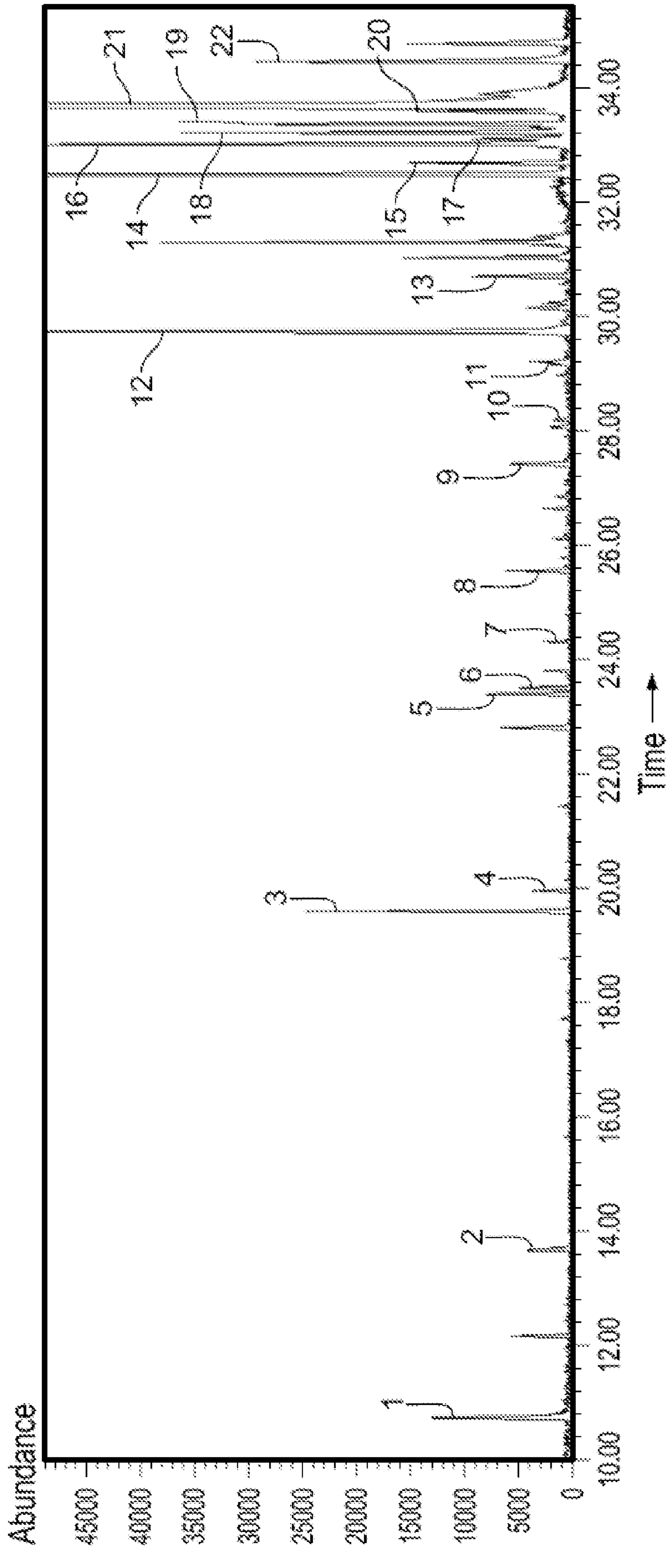
Abundance
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
Time
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG. 6

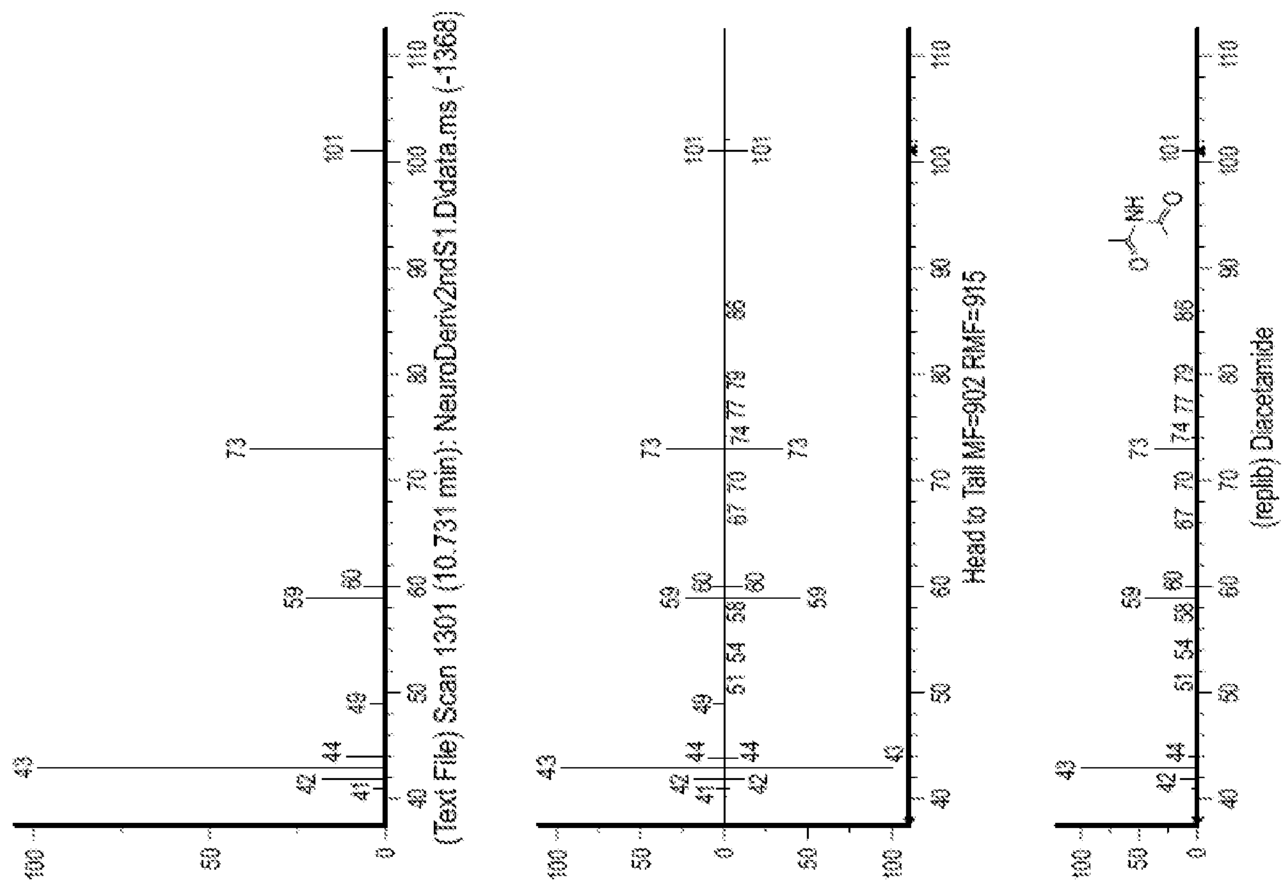
(Text File) Scan 1301 (10.731 min): NeuroDeriv2ndS1.D\data.ms (-1368)
Head to Tail MF=902 RMF=915
(replib) Diacetamide

FIG. 7

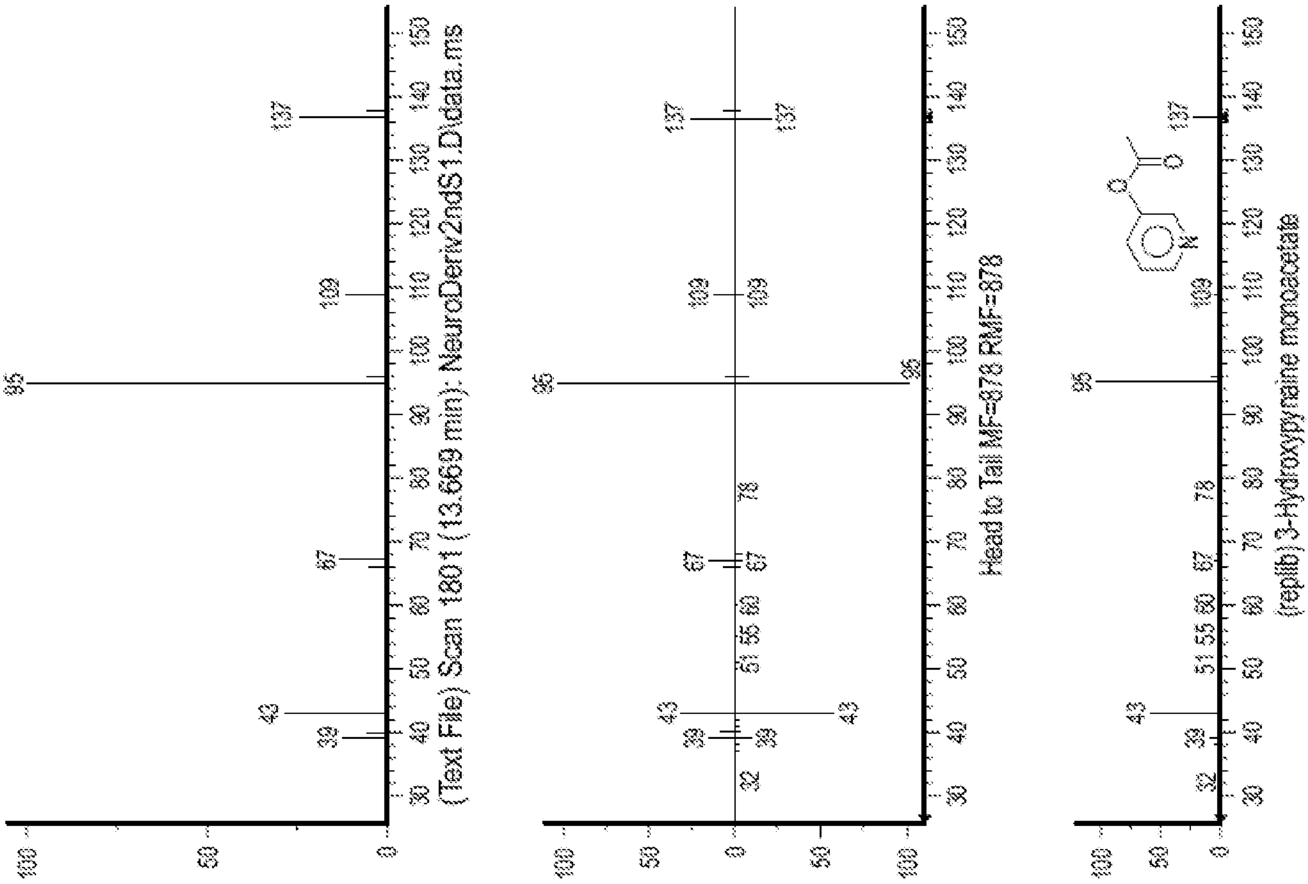
(Text File) Scan 1801 (13.669 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=878 RMF=878
(replib) 3-Hydroxypyridine monoacetate
O
O
N

FIG. 8

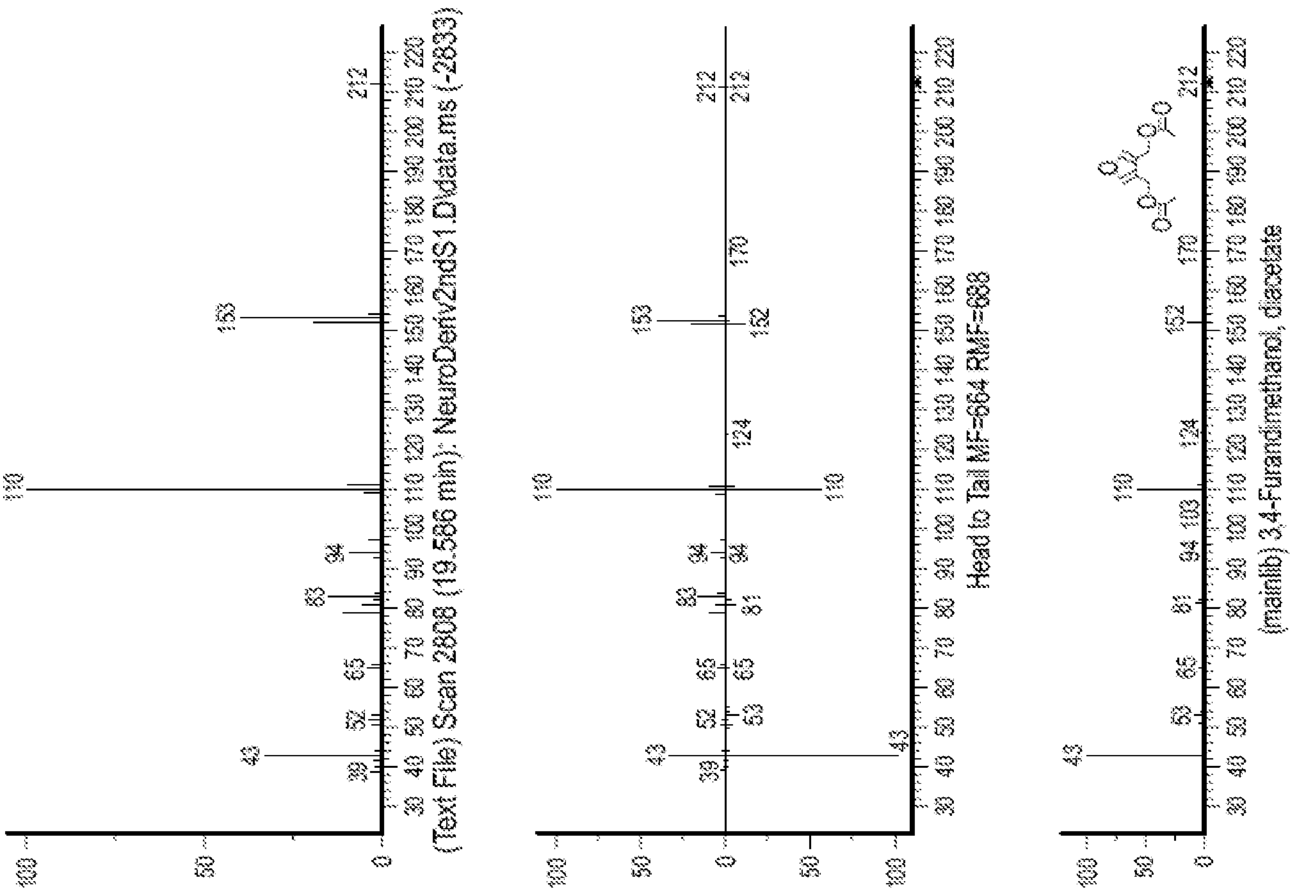
(Text File) Scan 2808 (19.586 min): NeuroDeriv2ndS1.D\data.ms (-2833)
Head to Tail MF=664 RMF=688
(mainlib) 3,4-Furandimethanol, diacetate

FIG. 9

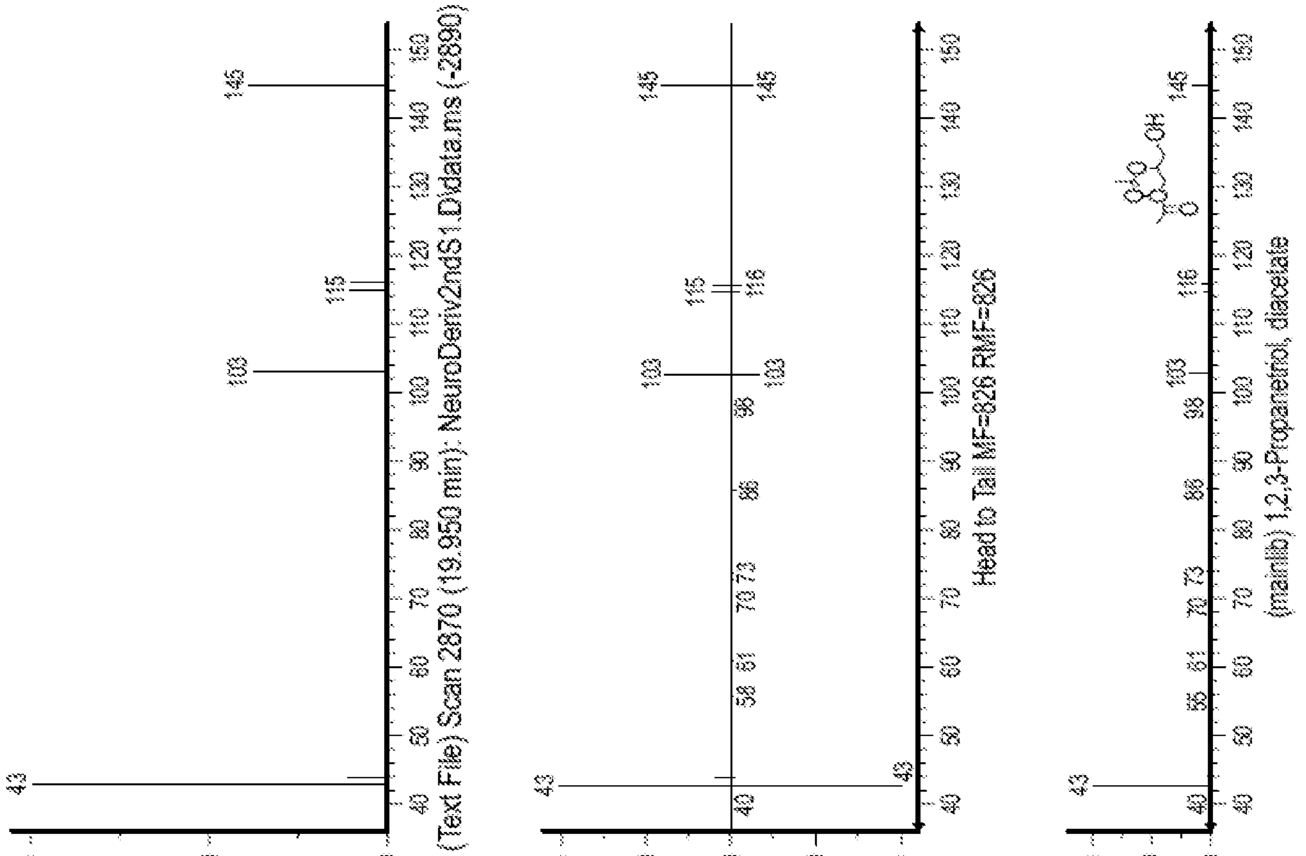
(Text File) Scan 2870 (19.950 min): NeuroDeriv2ndS1.D\data.ms (-2890)
Head to Tail MF=826 RMF=826
(mainlib) 1,2,3-Propanetriol, diacetate

FIG. 10

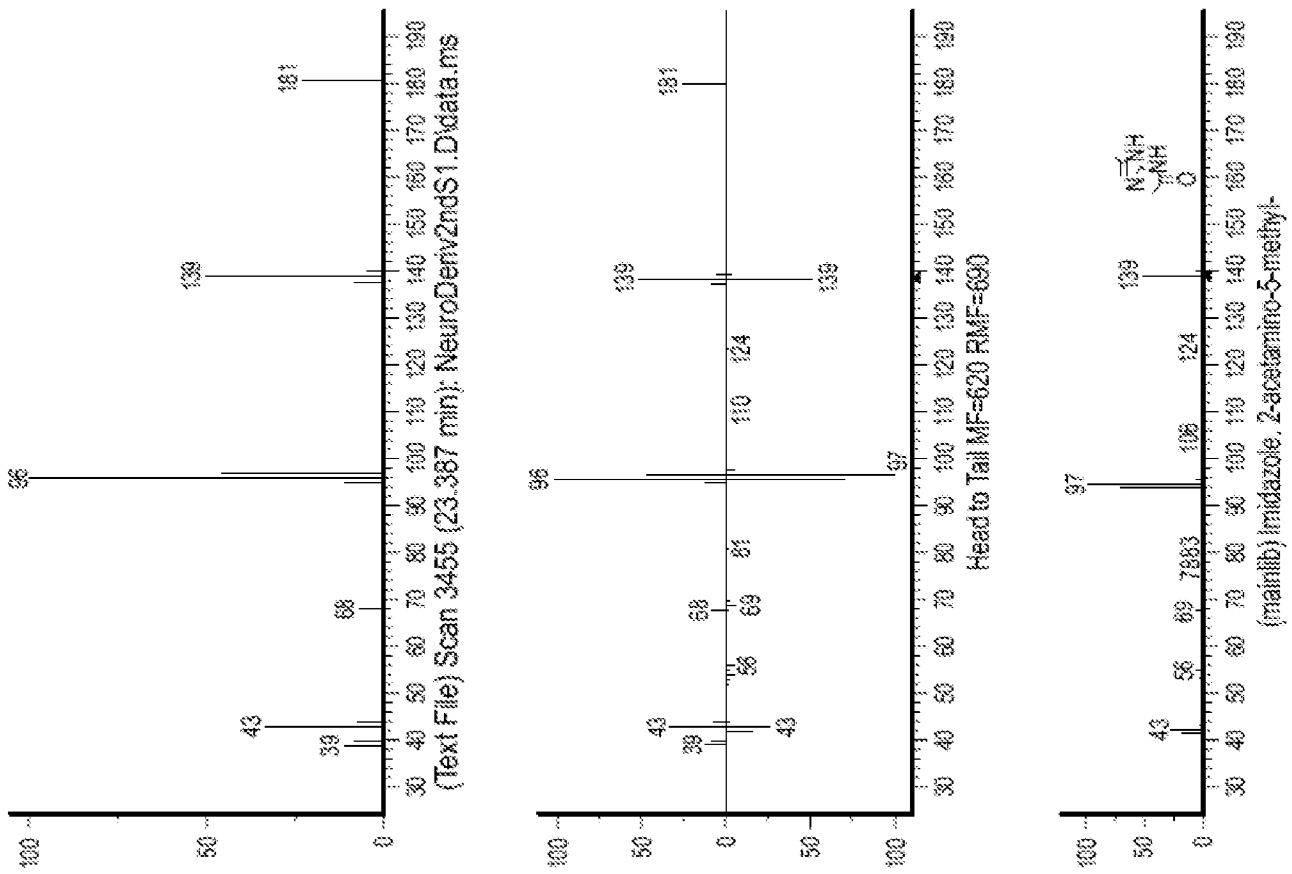
(Text File) Scan 3455 (23.387 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=620 RMF=690
(mainlib) Imidazole, 2-acetamino-5-methyl-

FIG. 11

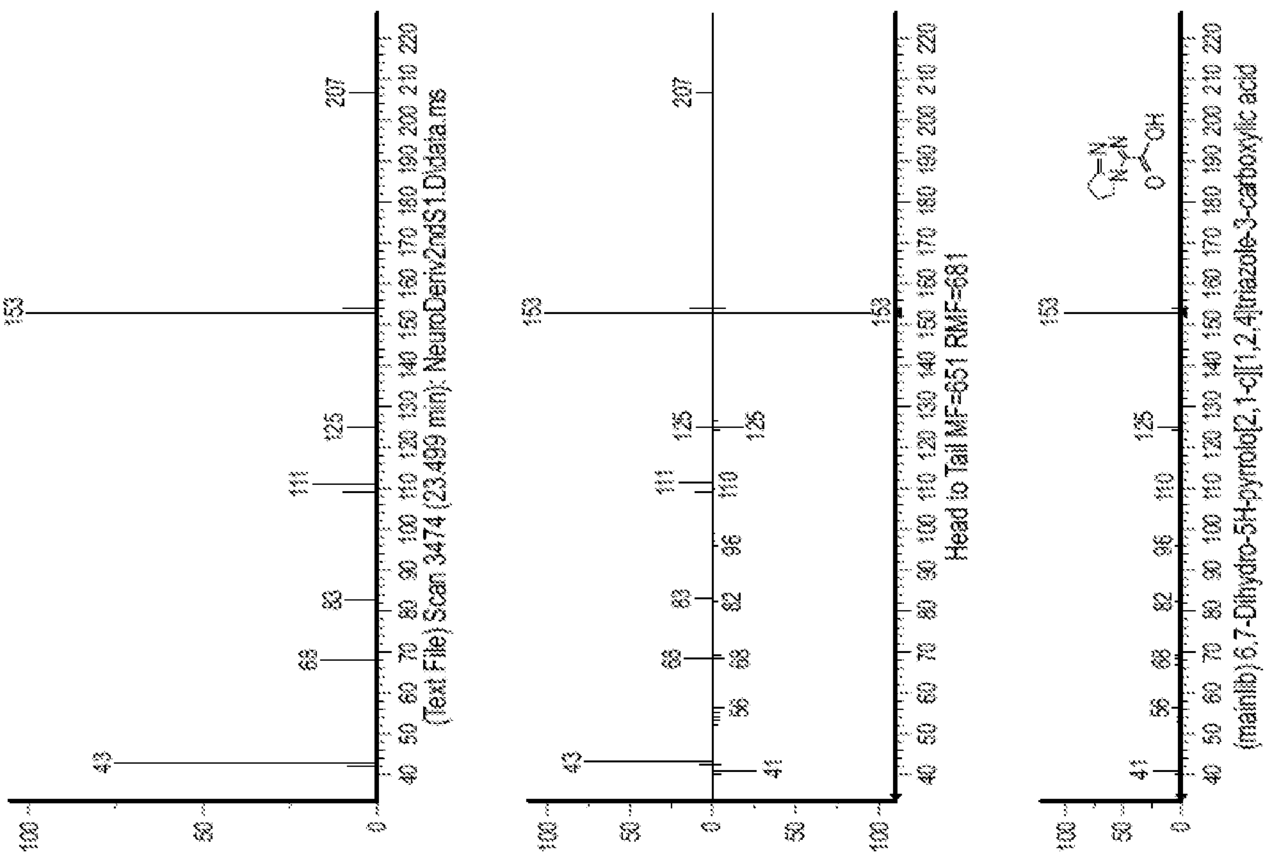
(Text File) Scan 3474 (23.499 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=651 RMF=681
(mainlib) 6,7-Dihydro-5H-pyrrolo[2,1-c][1,2,4]triazole-3-carboxylic acid

FIG. 12

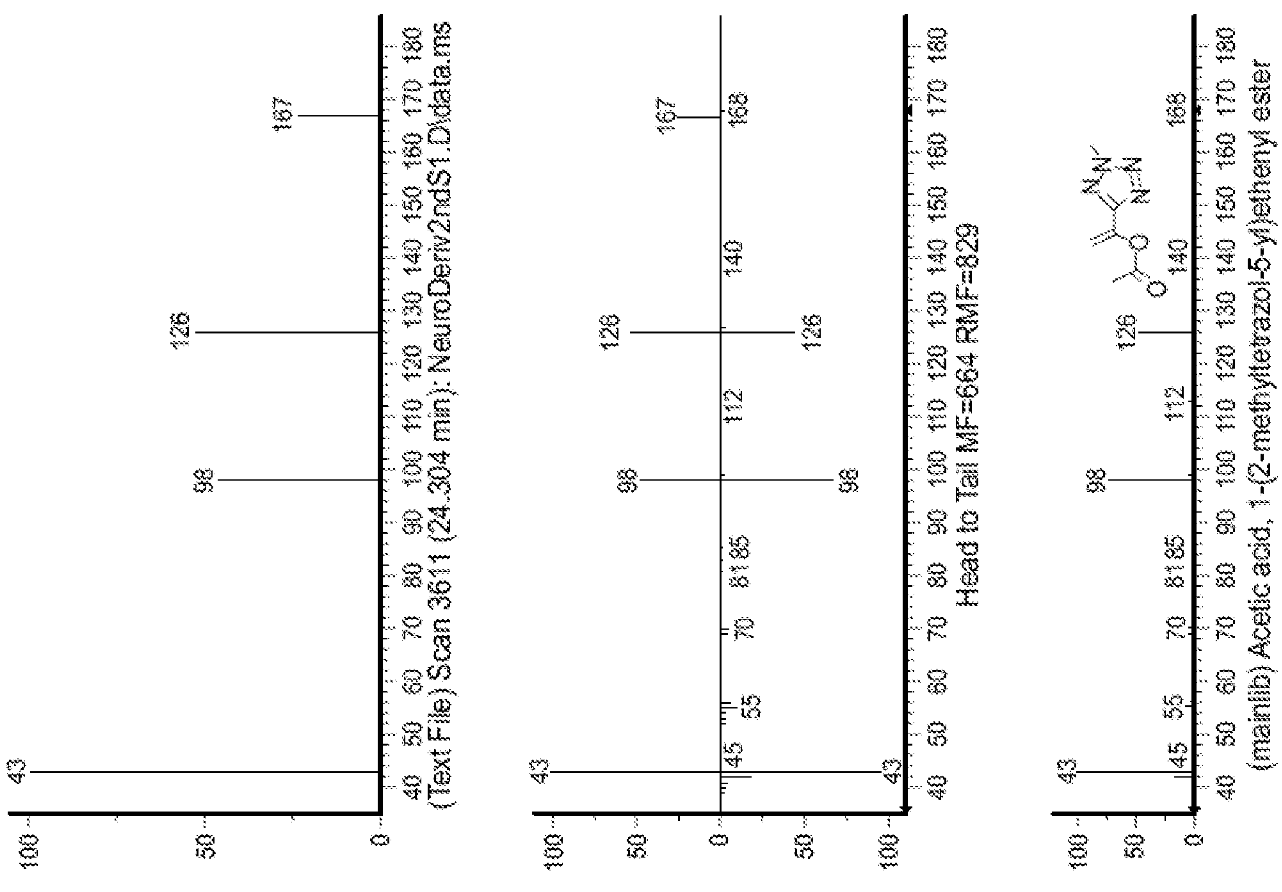
(Text File) Scan 3611 (24.304 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=664 RMF=829
(mainlib) Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester

FIG. 13

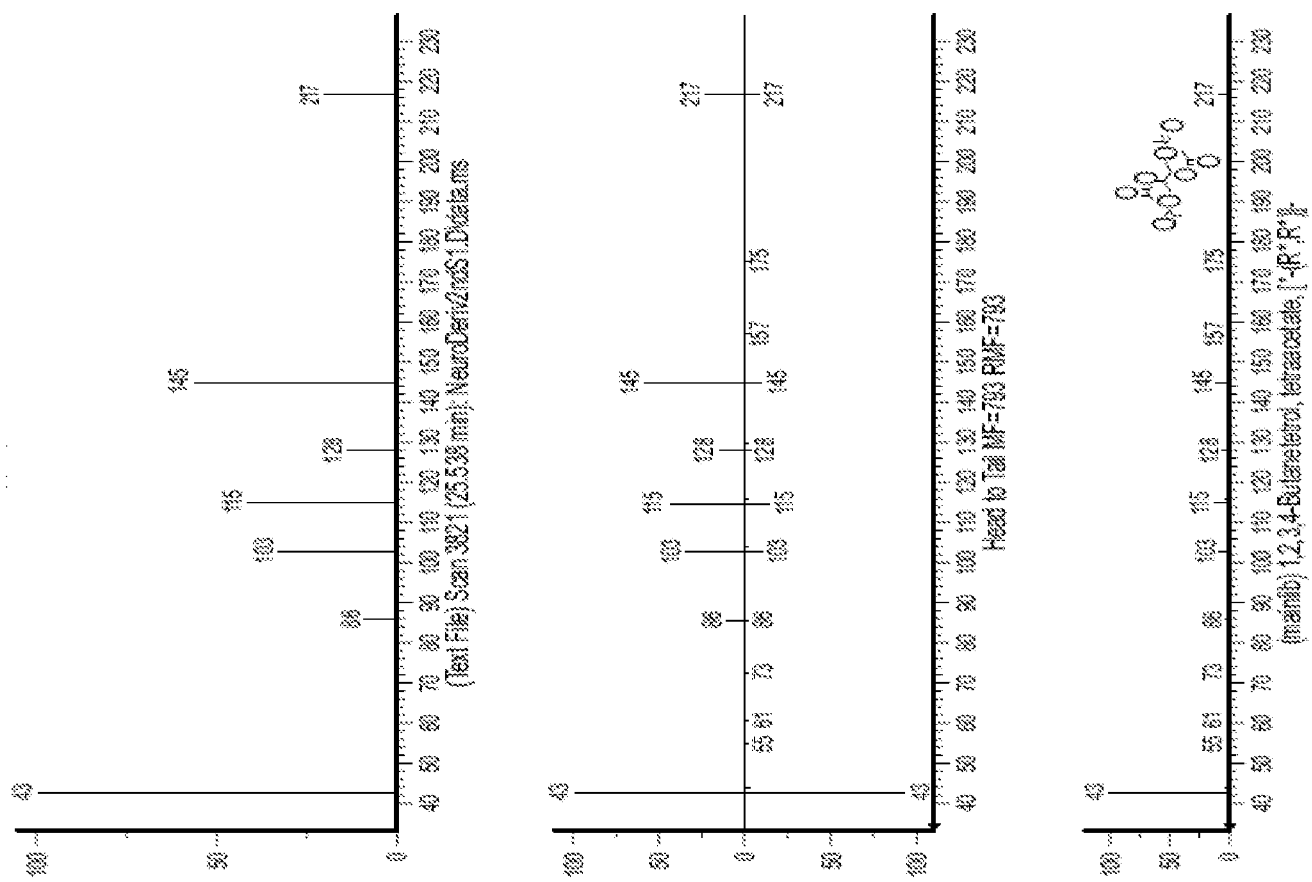
(Text File) Scan 3821 (25.538 min): NeuroDeriv2nd3S1.D\data.ms
Head to Tail MF=793 RMF=793
(mainlib) 1,2,3,4-Butanetetrol, tetraacetate, [R*,R*]-

FIG. 14

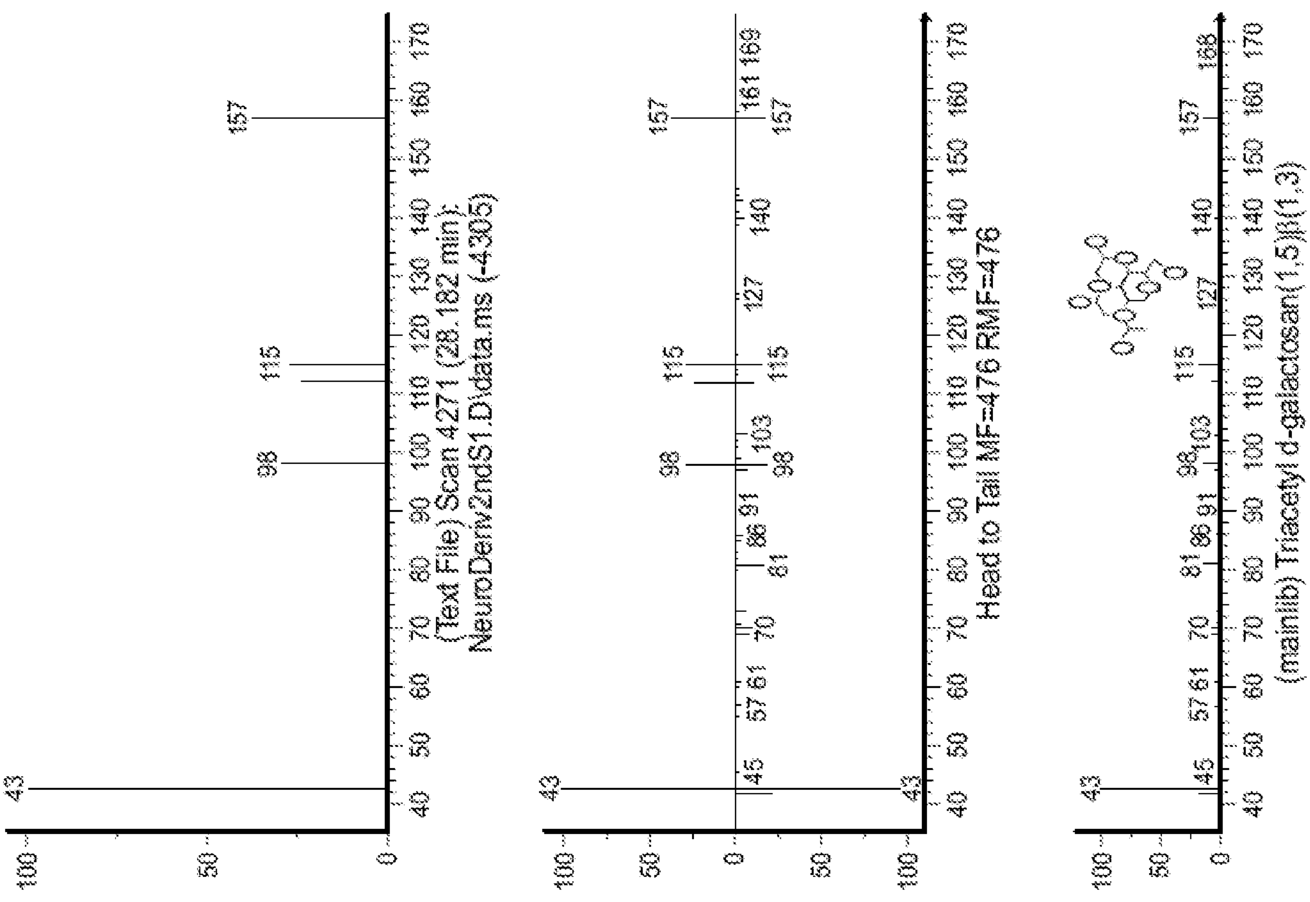
(Text File) Scan 4271 (28.182 min):
NeuroDeriv2ndS1.D\data.ms (-4305)
Head to Tail MF=476 RMF=476
(mainlib) Triacetyl d-galactosan(1,5)β(1,3)

FIG. 15

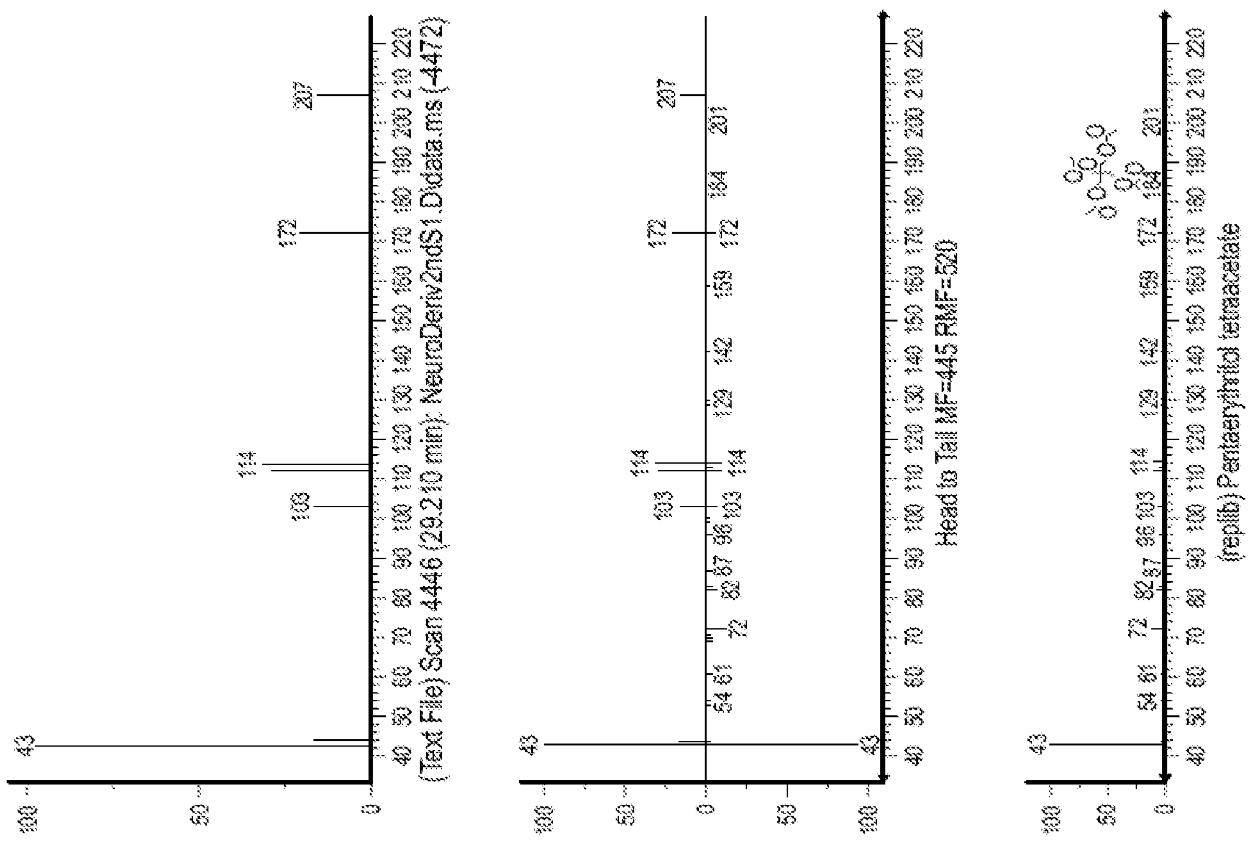
(Text File) Scan 4446 (29.210 min): NeuroDeriv2ndS1.D\data.ms (-4472)
Head to Tail MF=445 RMF=520
(replib) Pentaerythritol tetraacetate

FIG. 16

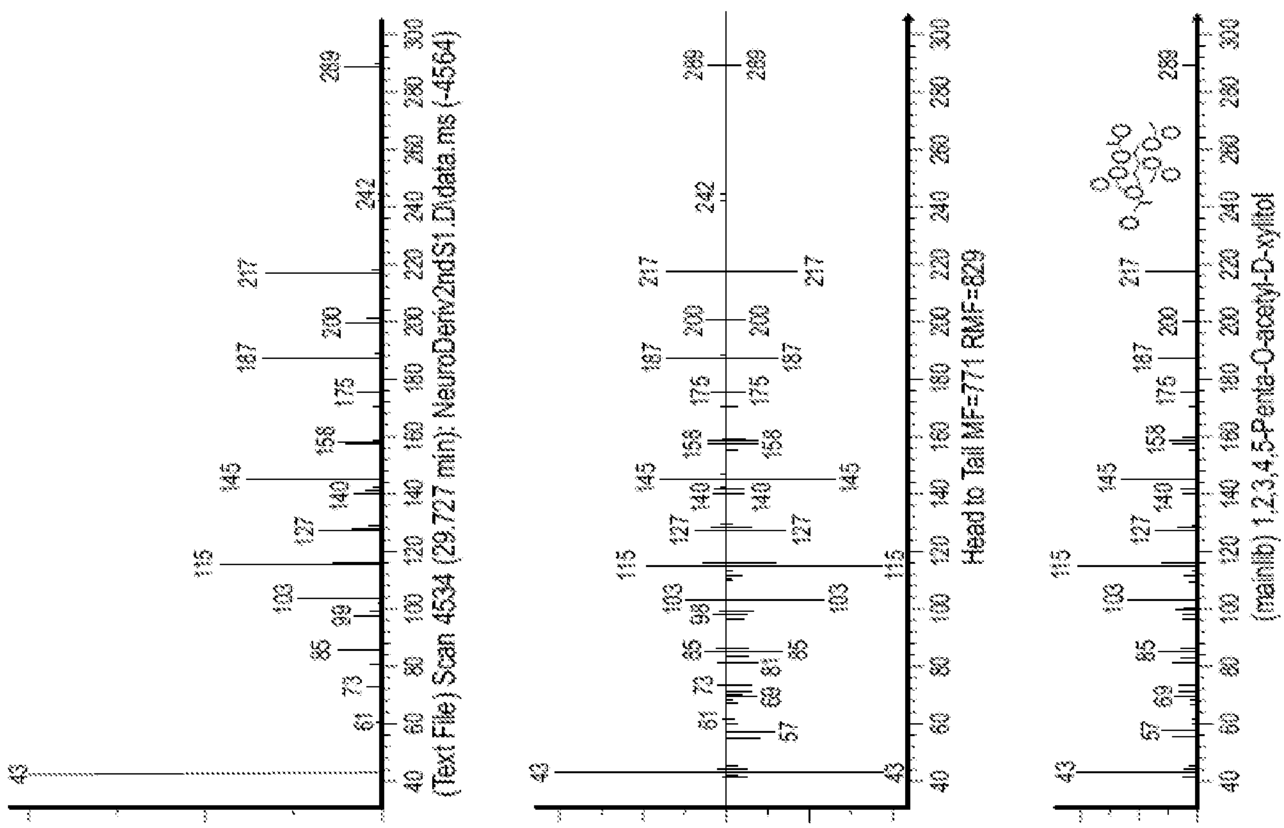
(Text File) Scan 4534 (29.727 min): NeuroDeriv2ndS1.D\data.ms (-4564)
Head to Tail MF=771 RMF=829
(mainlib) 1,2,3,4,5-Penta-O-acetyl-D-xylitol

FIG. 17

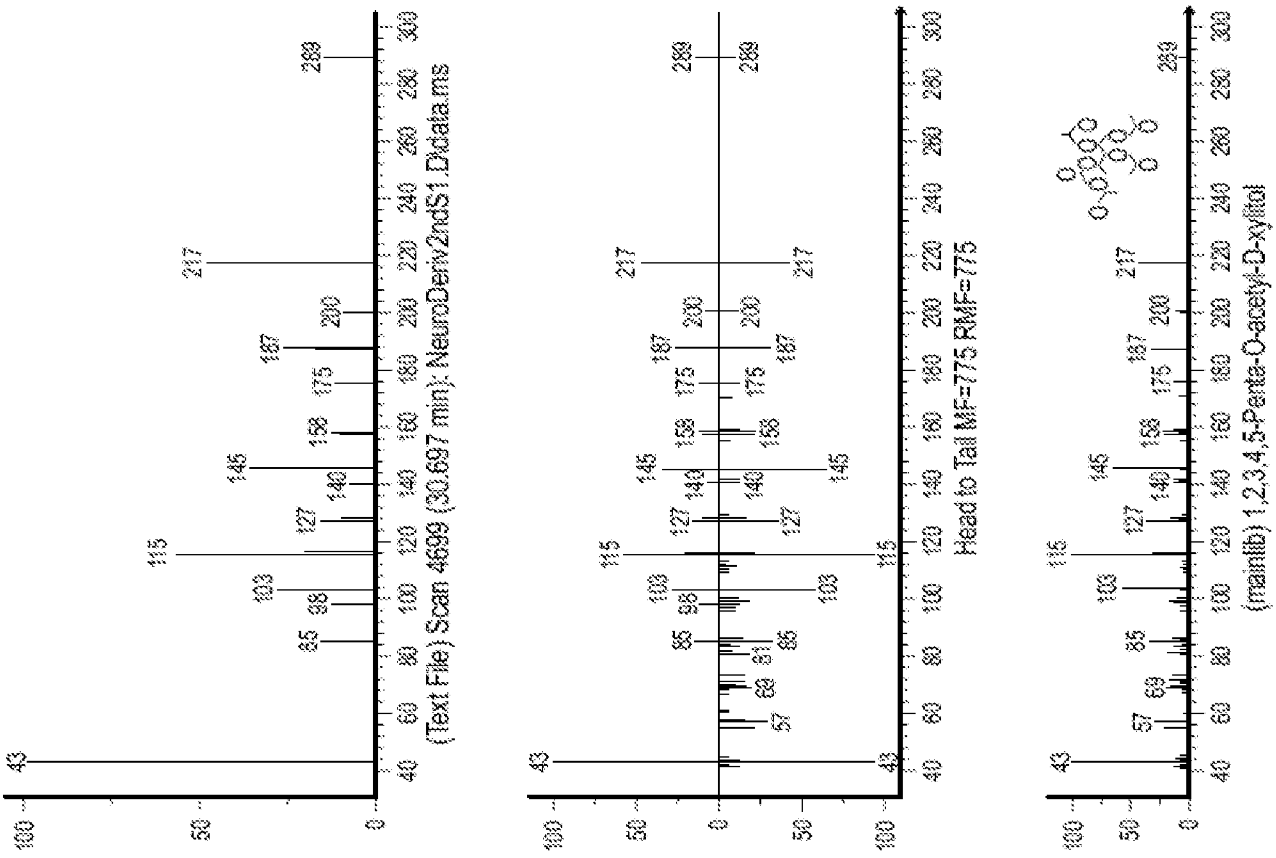
(Text File) Scan 4699 (30.697 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=775 RMF=775
(mainlib) 1,2,3,4,5-Penta-O-acetyl-D-xylitol

FIG. 18

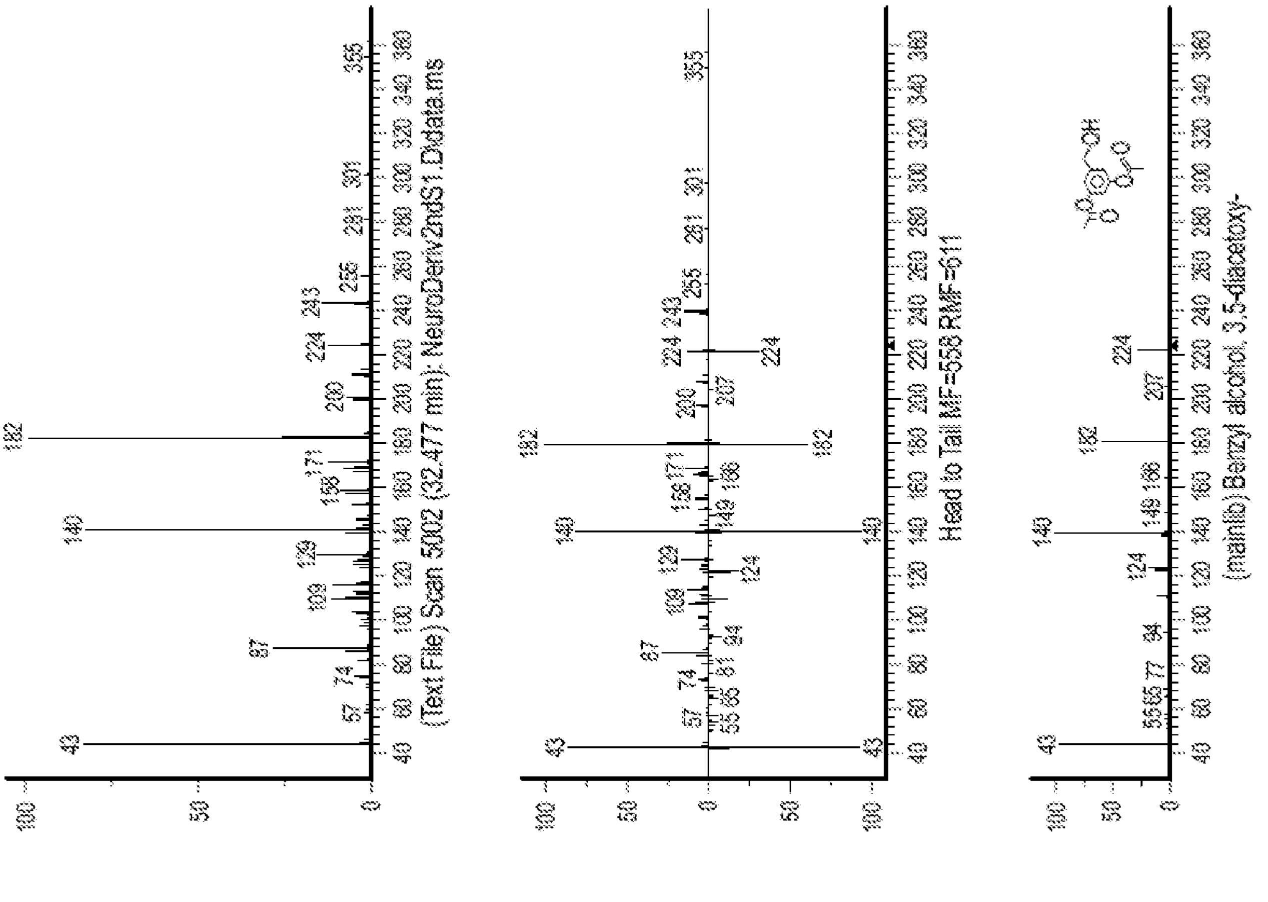
(Text File) Scan 5002 (32.477 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=558 RMF=611
(mainlib) Benzyl alcohol, 3,5-diacetoxy-

FIG. 19

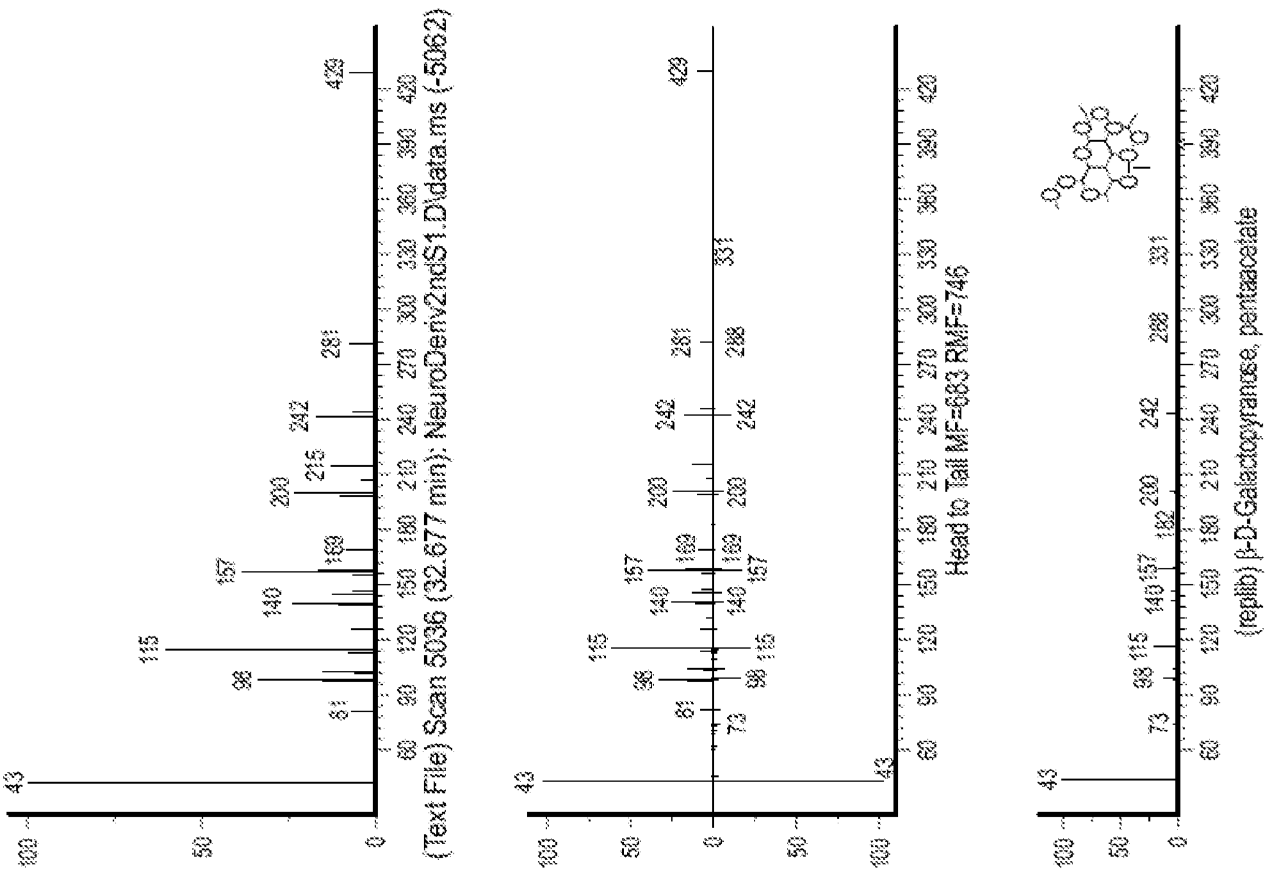
(Text File) Scan 5036 (32.677 min): NeuroDeriv2ndS1.D\data.ms (-5062)
Head to Tail MF=683 RMF=746
(replib) β-D-Galactopyranose, pentaacetate

FIG. 20

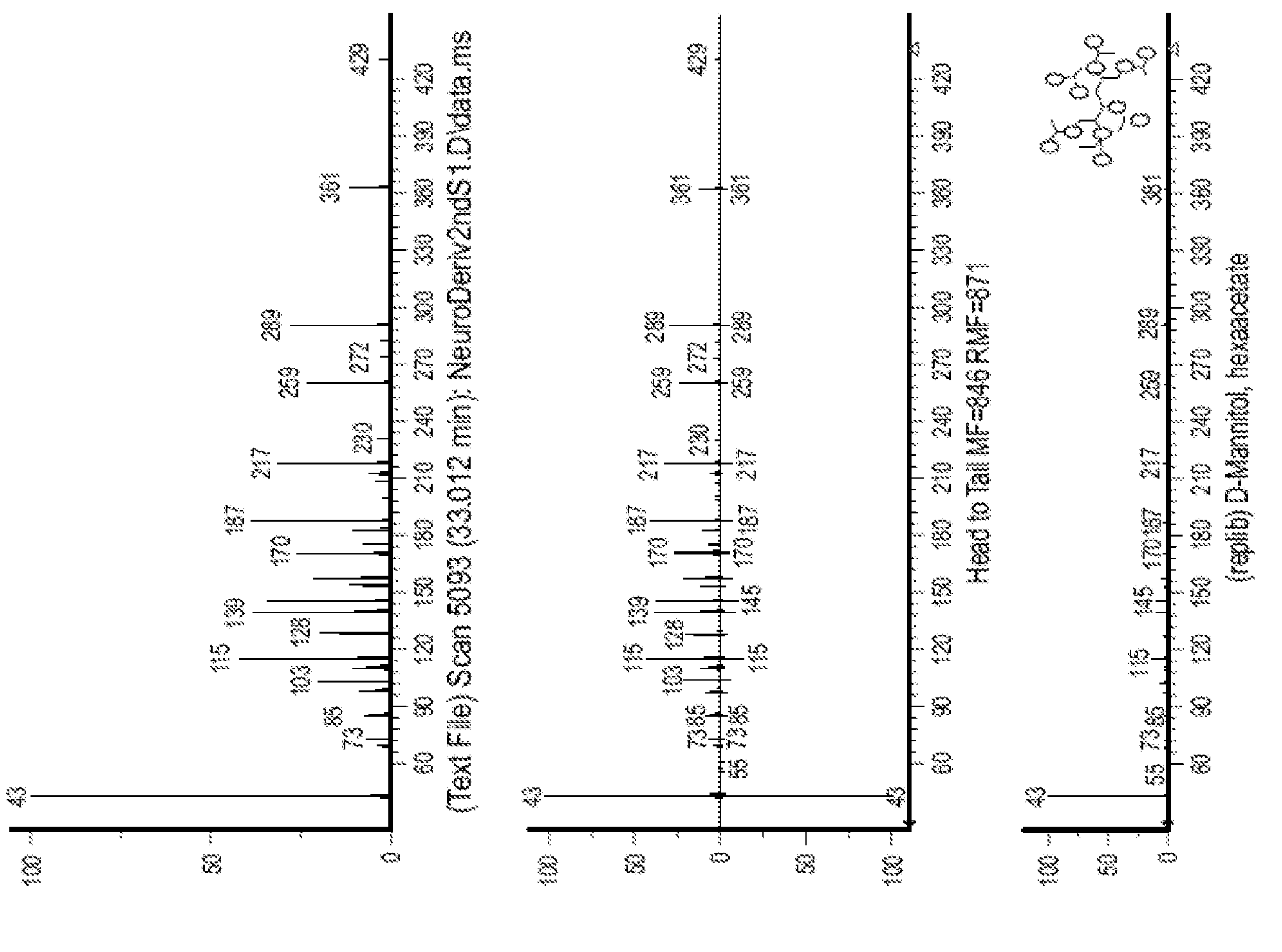
(Text File) Scan 5093 (33.012 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=846 RMF=871
(replib) D-Mannitol, hexaacetate

FIG. 21

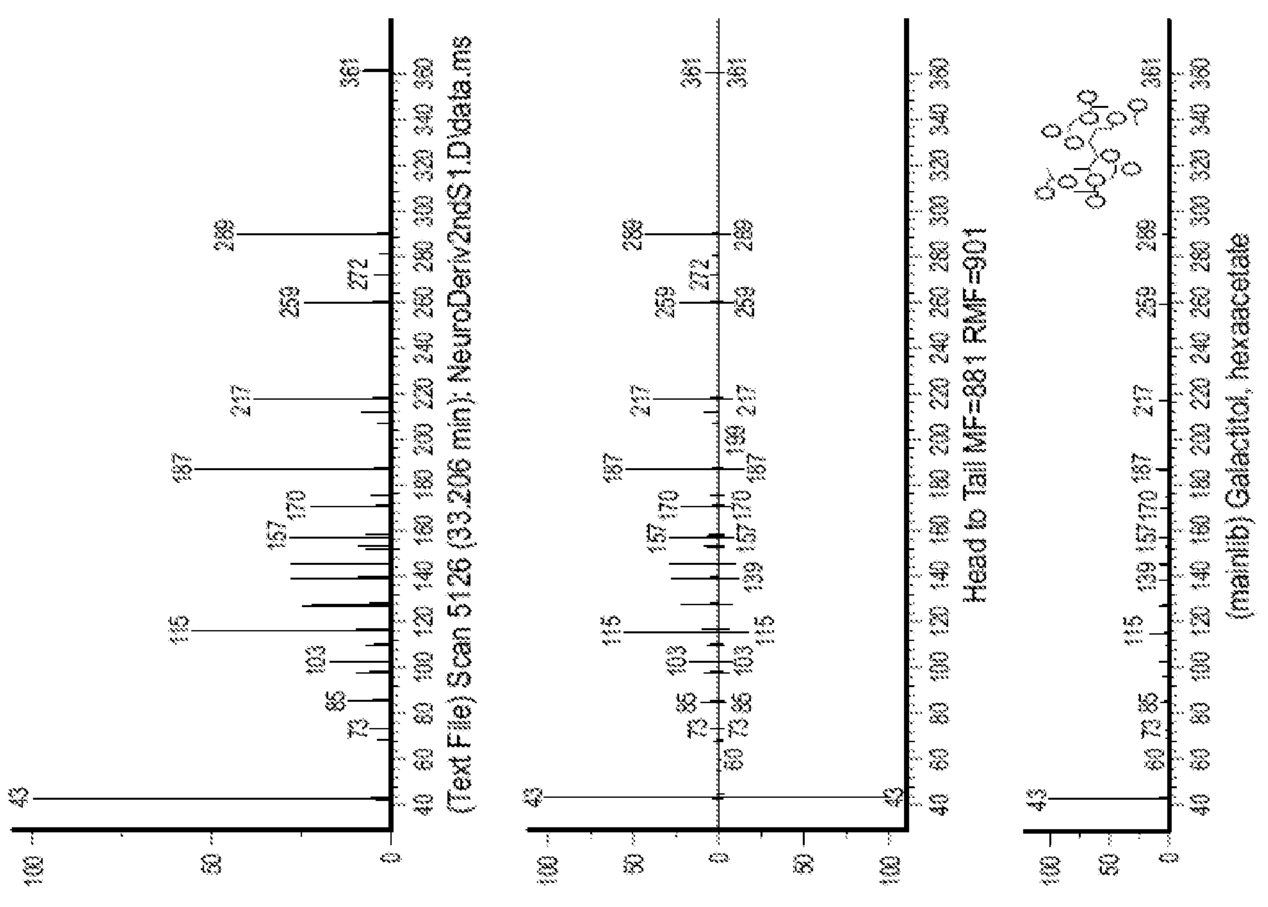
(Text File) Scan 5126 (33.206 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=881 RMF=901
(mainlib) Galactitol, hexaacetate

FIG. 22

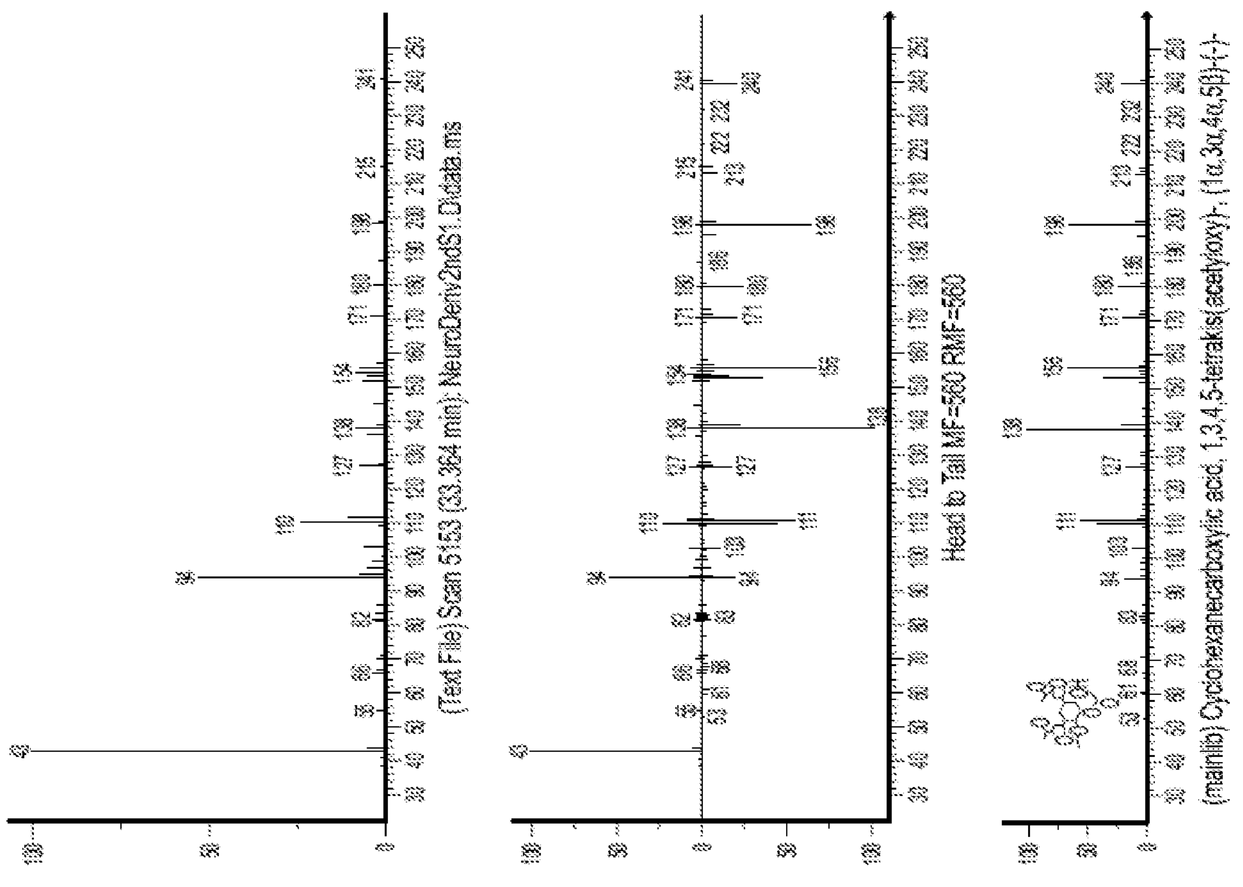
(Text File) Scan 5153 (33.364 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=560 RMF=560
(mainlib) Cyclohexanecarboxylic acid, 1,3,4,5-tetrakis(acetyloxy)-, (1α,3α,4α,5β)-(-)-

FIG. 23

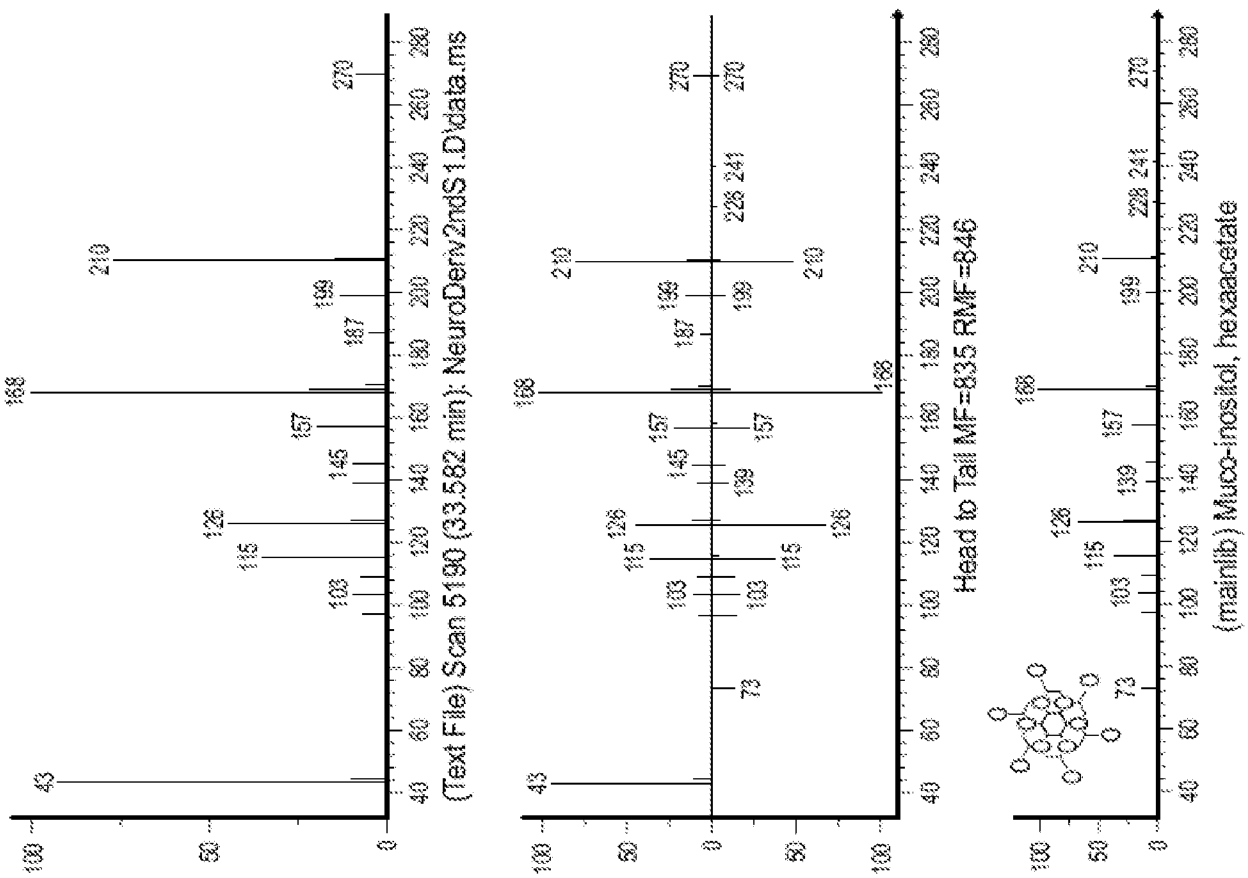
(Text File) Scan 5190 (33.582 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=835 RMF=846
(mainlib) Muco-inositol, hexaacetate

FIG. 24

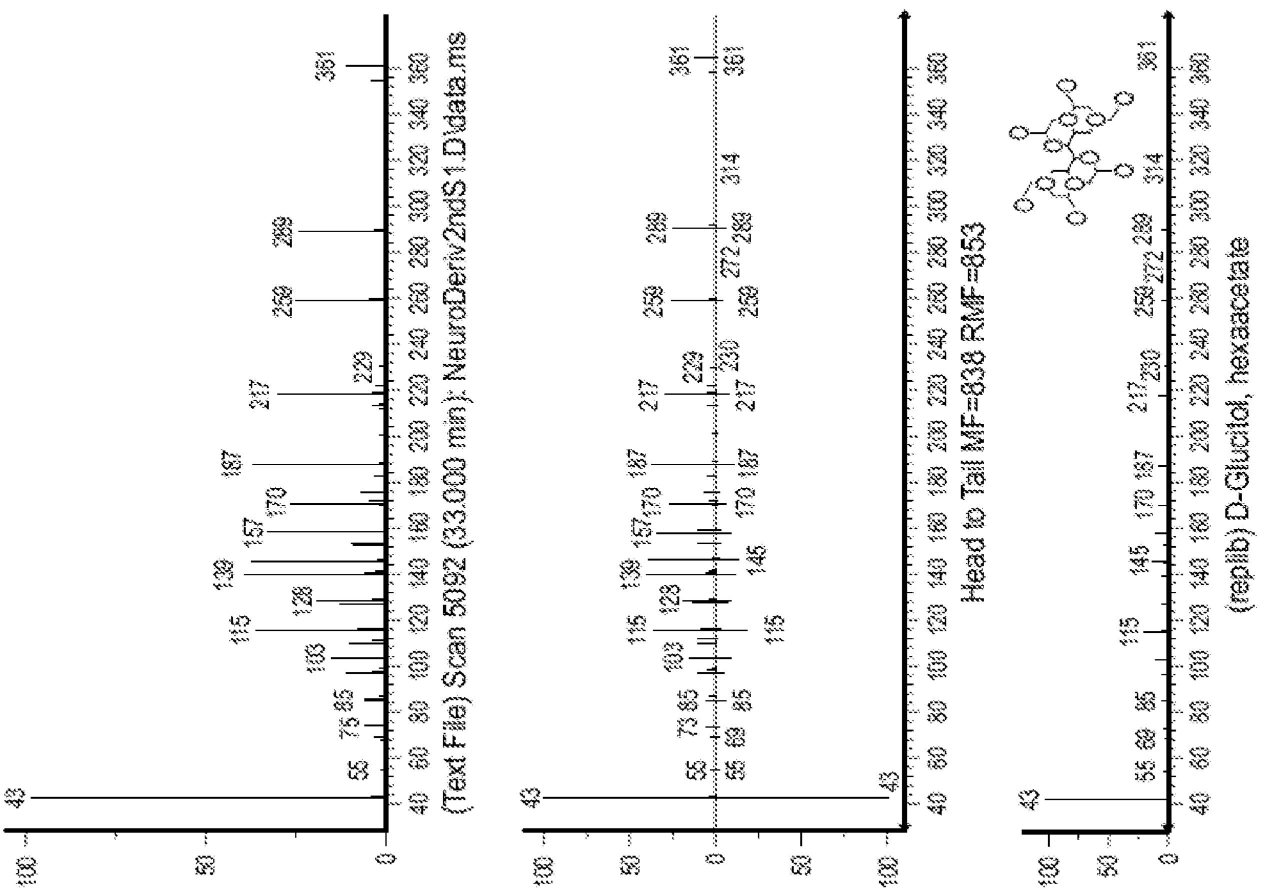
(Text File) Scan 5092 (33.000 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=838 RMF=853
(replib) D-Glucitol, hexaacetate

FIG. 25

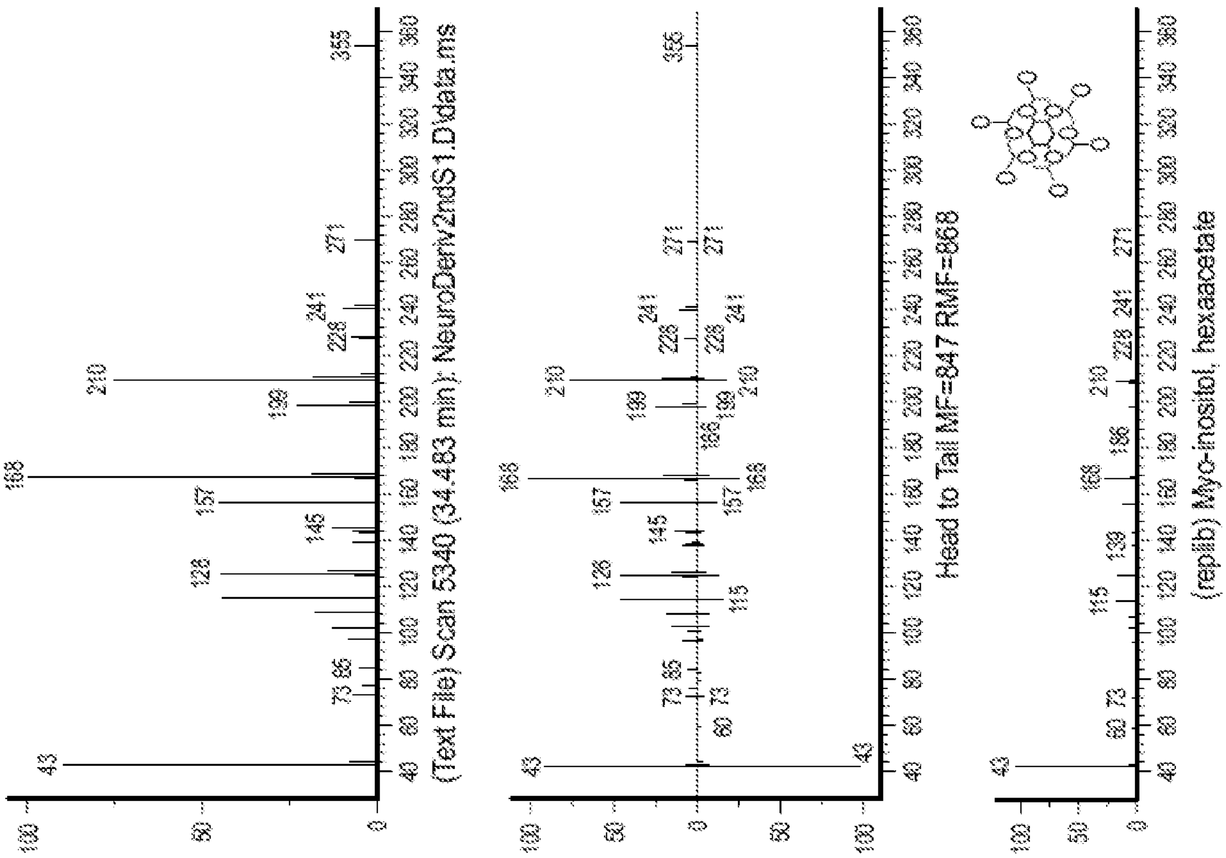
(Text File) Scan 5340 (34.483 min): NeuroDeriv2ndS1.D\data.ms
Head to Tail MF=847 RMF=868
(replib) Myo-inositol, hexaacetate

FIG. 26

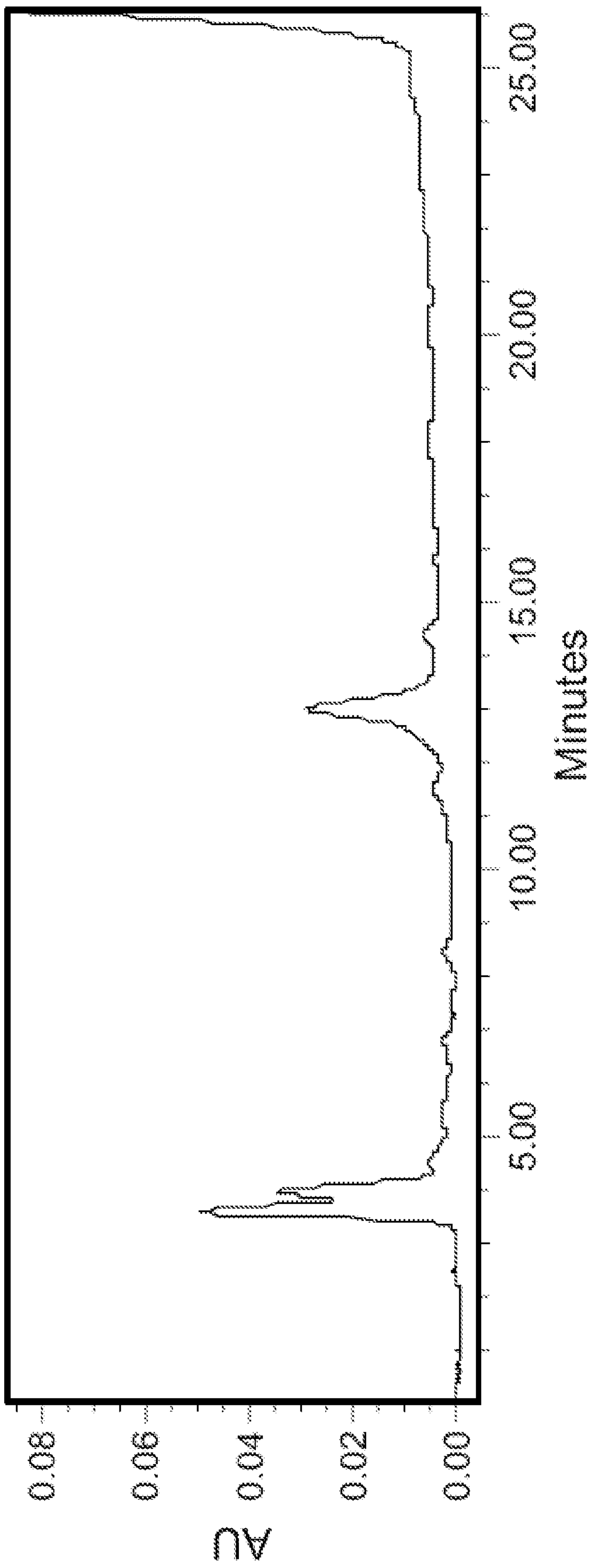

FIG. 27

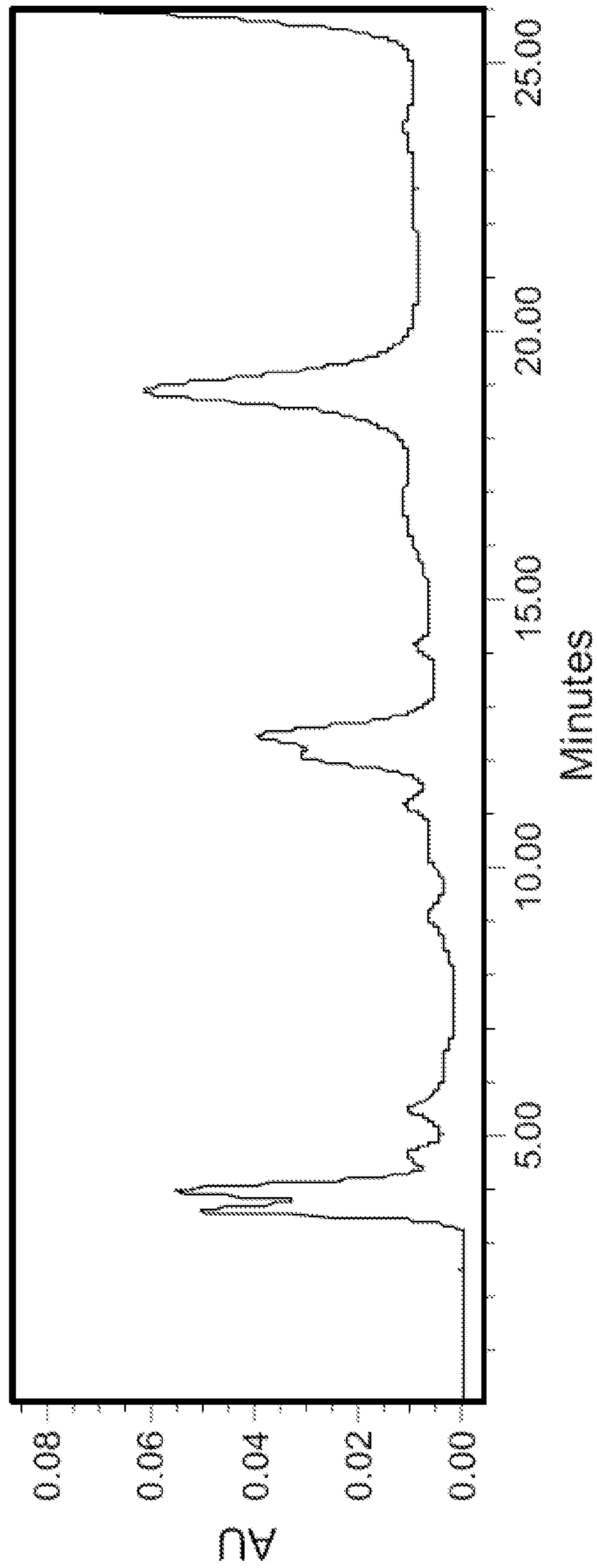

FIG. 28

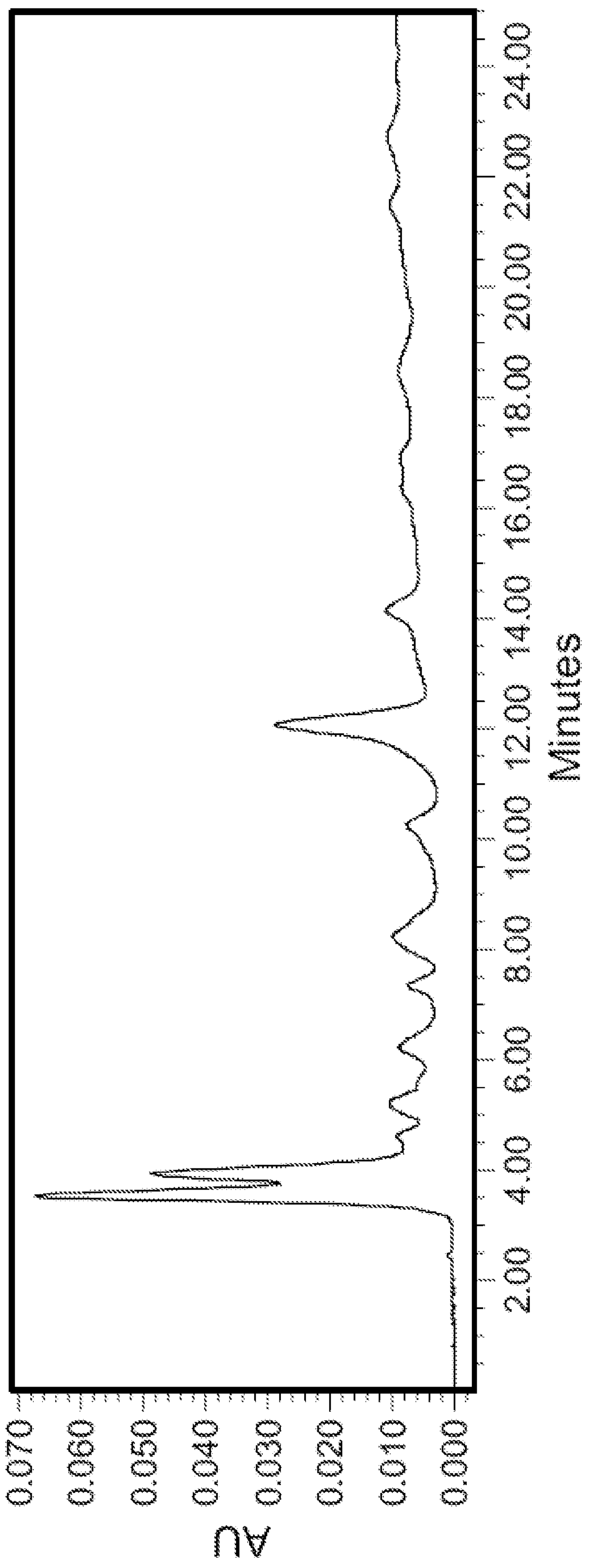
AU
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Minutes
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00

FIG. 29

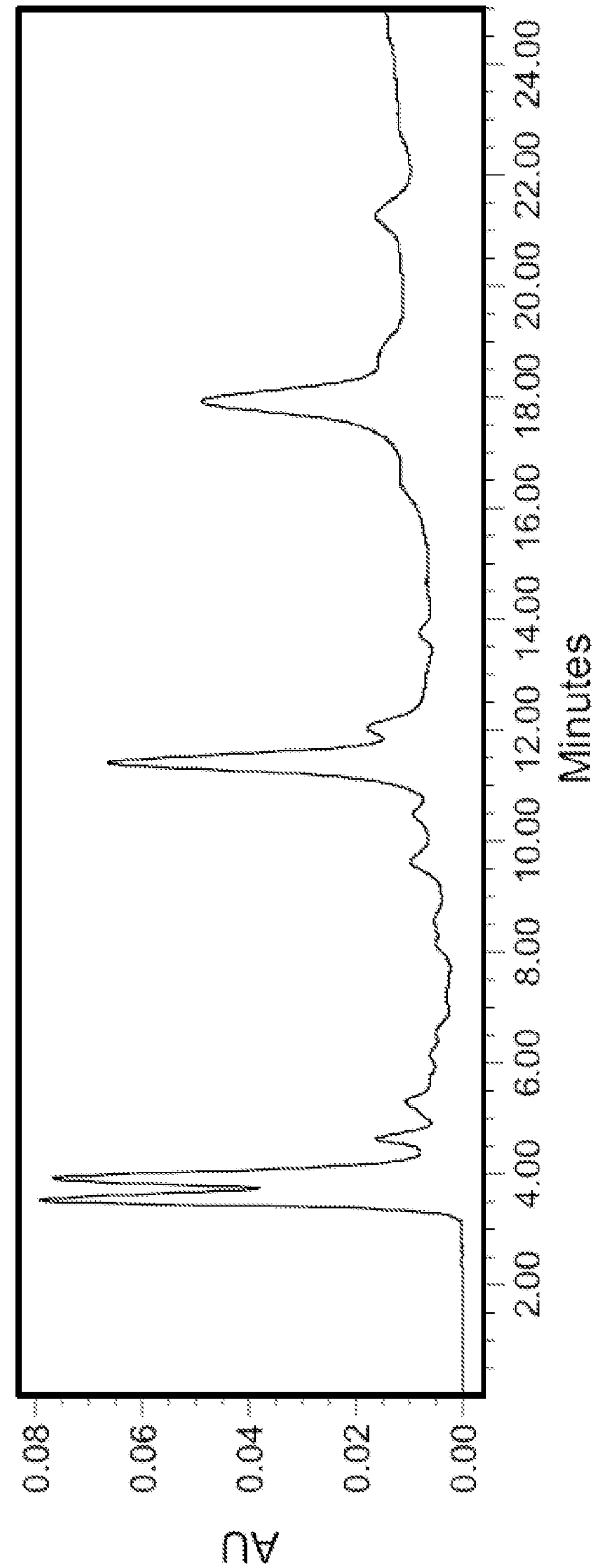
AU
0.08
0.06
0.04
0.02
0.00
Minutes
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00

FIG. 30

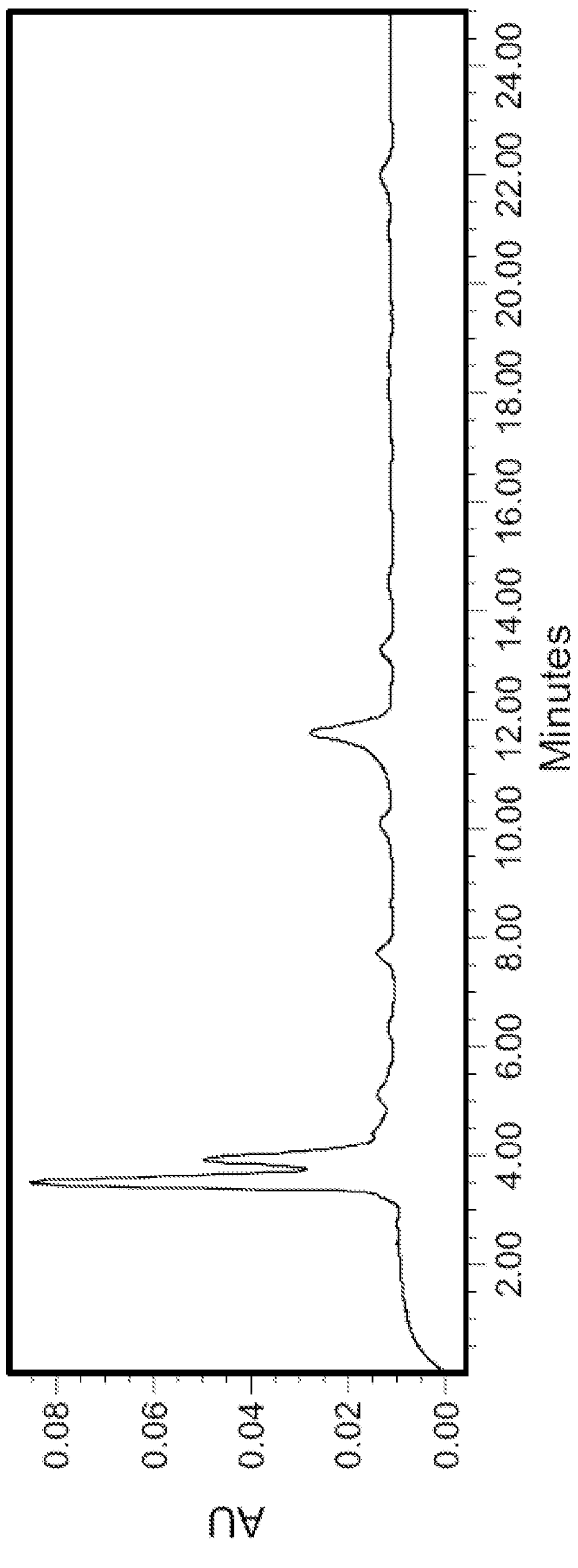
AU
0.08
0.06
0.04
0.02
0.00
Minutes
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00

FIG. 31

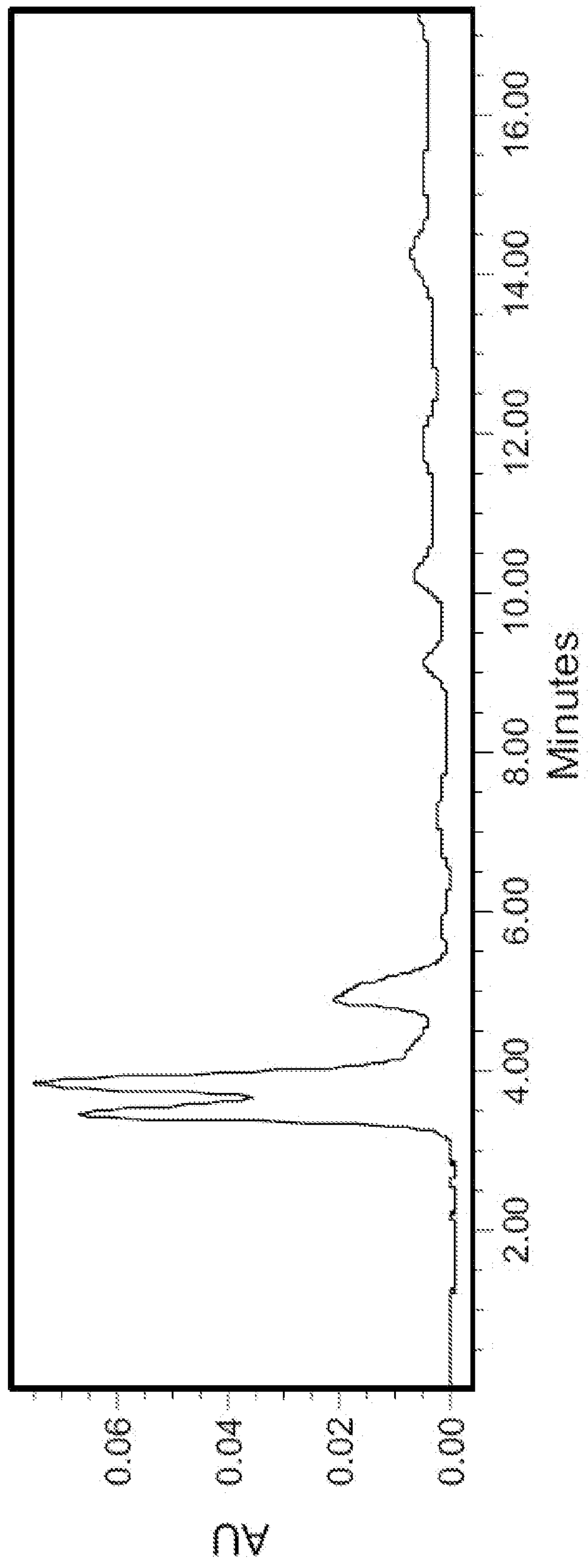

FIG. 32

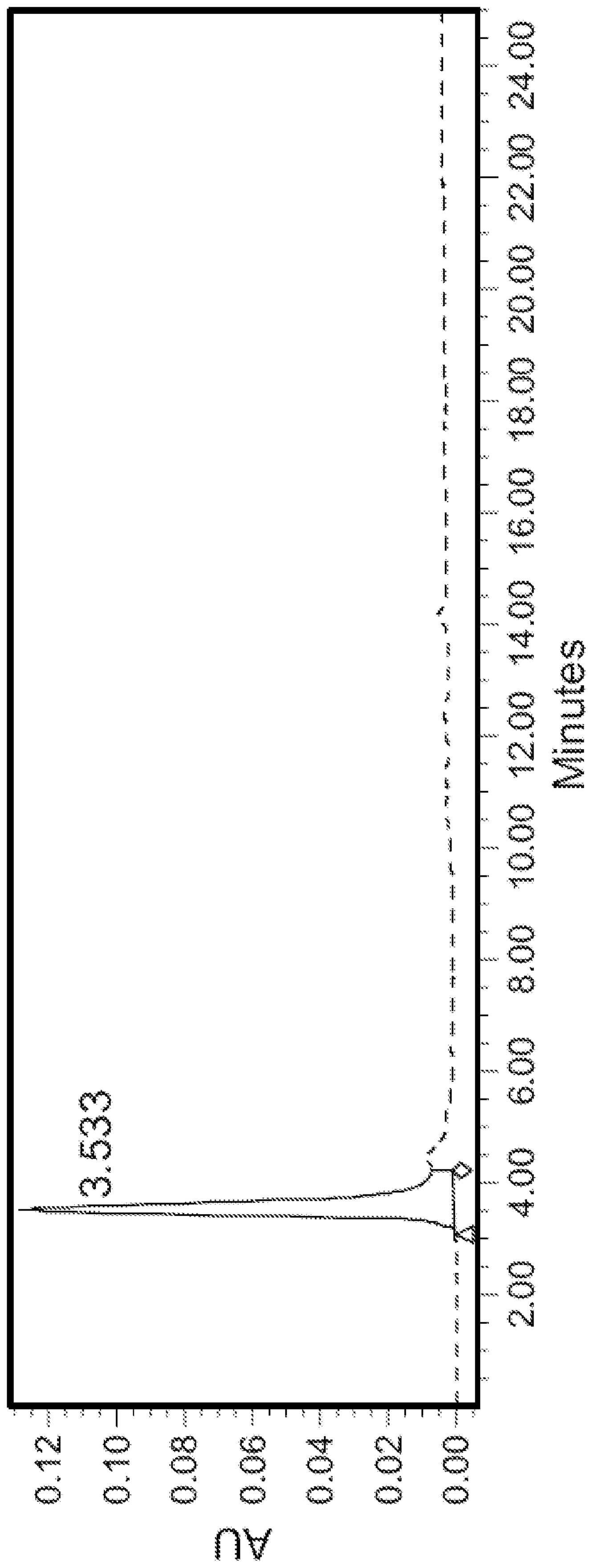
AU
0.12
0.10
0.08
0.06
0.04
0.02
0.00
3.533
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Minutes

FIG. 33

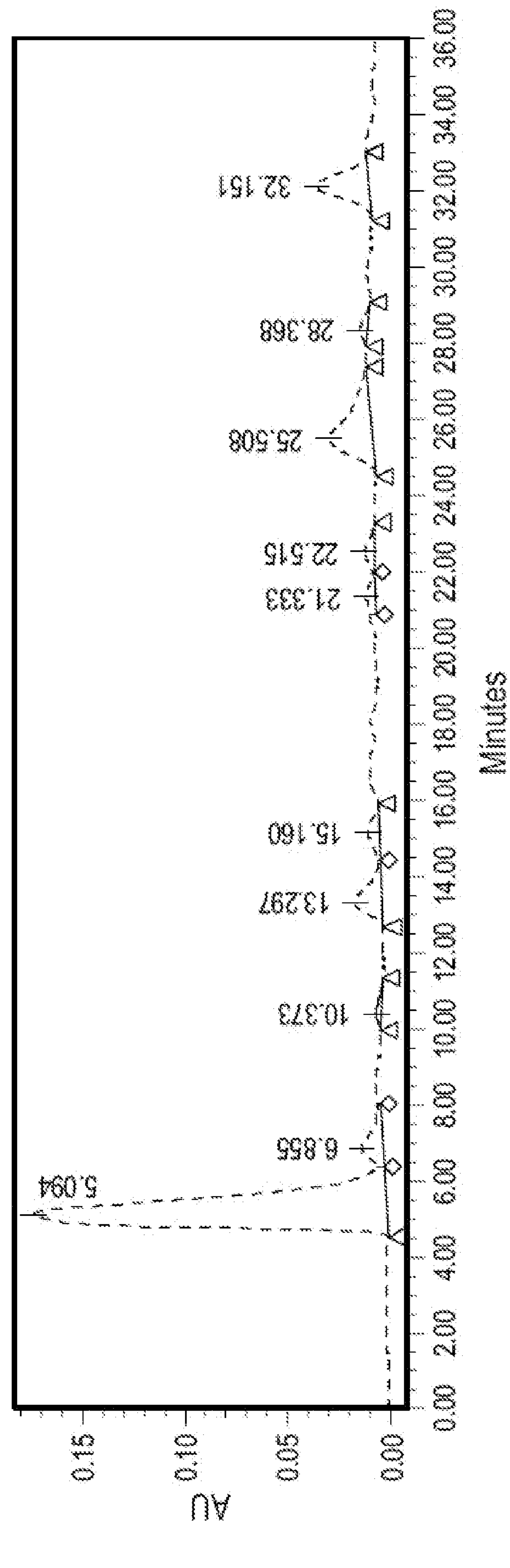
AU
0.00
0.05
0.10
0.15
Minutes
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
5.094
6.855
10.373
13.297
15.160
21.333
22.515
25.508
28.368
32.151

FIG. 34

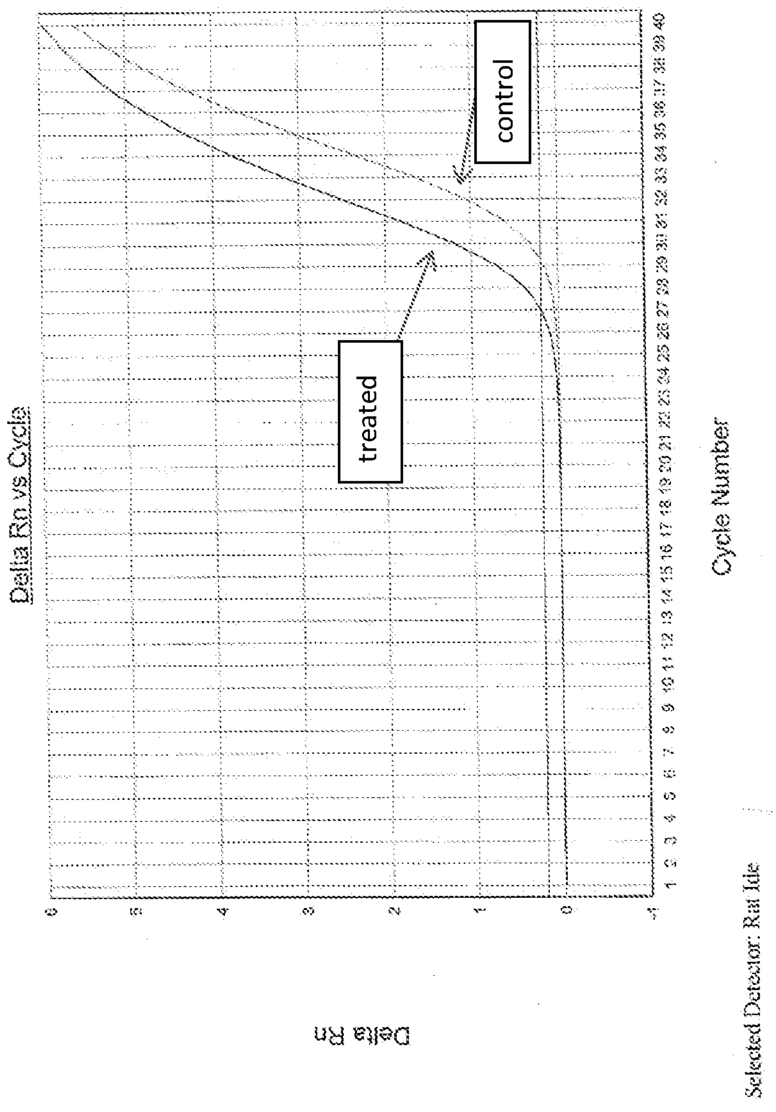

FIG. 35

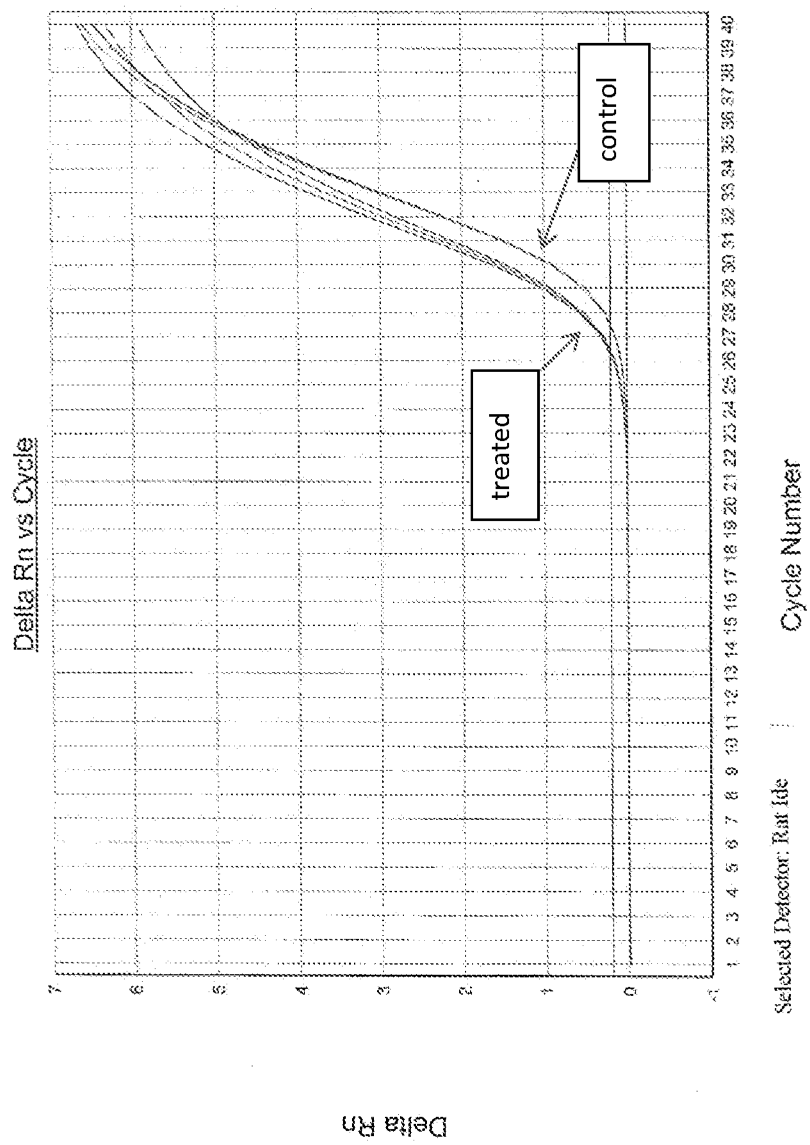
Delta Rn vs Cycle
Delta Rn
Cycle Number
Selected Detector: Rat Ide
treated
control

FIG. 36

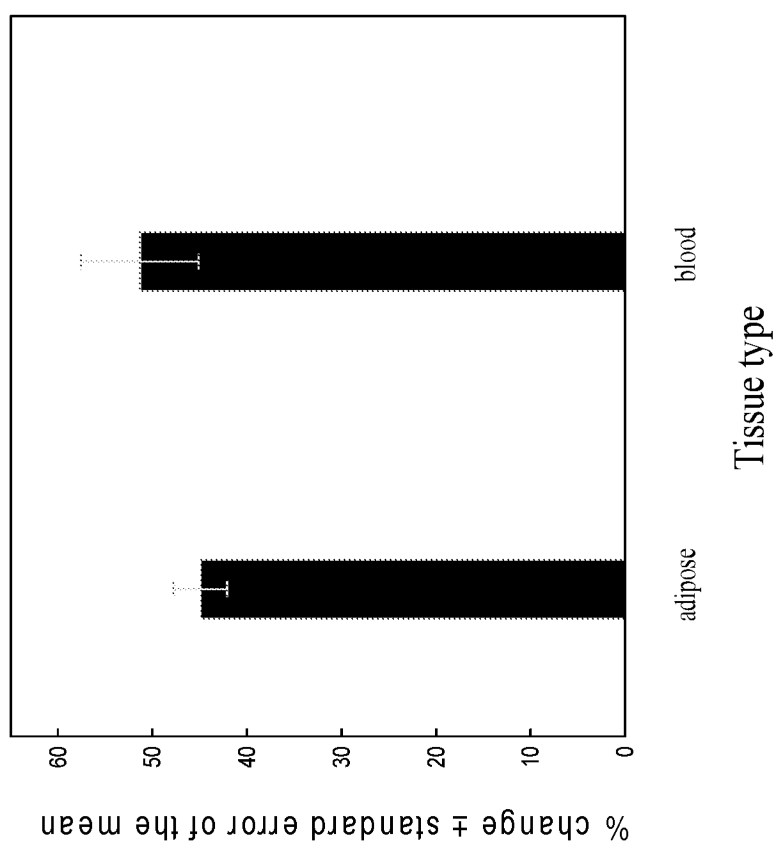
% change ± standard error of the mean
0
10
20
30
40
50
60
adipose
blood
Tissue type

FIG. 37