United States Patent Application Publication	20250258146
Kind Code	A1
Publication Date	August 14, 2025
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QUANTITATIVE DETECTION METHOD OF MULTIPLE METABOLITES IN BIOLOGICAL SAMPLE AND METABOLIC CHIP

Abstract

The present invention discloses a quantitative detection method of multiple metabolic components in a biological sample and a metabolic chip used in the method. The detection method includes performing derivatization treatment on the biological sample and then detecting the derivatized biological sample by liquid chromatography-mass spectrometry. The metabolic chip of the present invention includes a chip carrier microtiter plate and related reagents, and quantitative detection of multiple metabolic components of different magnitudes such as amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid in the biological sample on the same microtiter plate can be achieved.

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Family ID: **1000008615833**

Appl. No.: **19/169027**

Filed: **April 03, 2025**

Foreign Application Priority Data

CN	201811223486.X	Oct. 19, 2018

Related U.S. Application Data

parent US continuation-in-part 17309062 20210717 PENDING WO continuation-in-part PCT/CN2019/112389 20191021 child US 19169027

Publication Classification

Int. Cl.: **G01N30/72** (20060101); **G01N30/02** (20060101); **G01N30/04** (20060101); **G01N30/06** (20060101); **G01N30/14** (20060101); **G01N30/88** (20060101); **G01N33/487** (20060101)

U.S. Cl.:

CPC **G01N30/7266** (20130101); **G01N33/487** (20130101); G01N2030/027 (20130101); G01N2030/045 (20130101); G01N2030/062 (20130101); G01N2030/067 (20130101); G01N2030/146 (20130101); G01N2030/8813 (20130101); G01N2030/8818 (20130101); G01N2458/15 (20130101)

Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] The present application is a Continuation-in-part Application of U.S. patent application Ser. No. 17/309,062 filed on Jul. 17, 2021, which is U.S. national stage application of International Application No. PCT/CN2019/112389, filed on Oct. 21, 2019, which claims the right of the priority of Chinese patent application 201811223486.X filed on Oct. 19, 2018. The contents of the above Chinese patent application are incorporated herein by reference in their entireties.

TECHNICAL FIELD
[0002] The present invention relates to the field of detection of biological samples, specifically relates to a quantitative determination method of multiple metabolic components such as amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid in a biological sample, and more specifically relates to a detection method of a biological sample by using chemical derivatization and tandem mass spectrometry and a metabolic chip used in the method.
BACKGROUND
[0003] Metabolomics involves unbiased analysis of all metabolites (metabolomes) in cells, body fluids and tissues. At present, with a metabolomics platform based on nuclear magnetic resonance (NMR) or mass spectrometry (MS), many small molecule (MW<1500) metabolites are detected, but only relative (non-absolute) concentrations of the metabolites in biological fluids (serum/plasma or urine) and tissues of subjects suffering from metabolic diseases are provided to determine that the concentrations are different from those of a control group. Due to high chemical diversity of the metabolomes, there is a great challenge to full-spectrum quantitative detection of these metabolites. Since a quantitative metabolomics platform for large-scale biological sample analysis is in deficiency, clinical practicality and application of metabolomics have not yet been realized.
[0004] Quantitative metabolomics is used for identifying and quantifying as many metabolites as possible in a biological sample. Compared with traditional targeted and non-targeted methods, quantitative metabolomics has many advantages, including lowest cross-platform variability, improved stability and maintenance of full-spectrum metabolic characteristics and more detailed information about the identity and concentration of specific metabolites.
[0005] With regard to quantification of metabolite concentration, reliable analytical data is a prerequisite for development of metabolic-based clinical trials or a thorough understanding of functions of organisms and biological systems in translational researches.
[0006] One of the technical challenges is that concentrations of metabolites are in a range of more than a dozen of magnitudes. For example, glucose in blood is in some compounds such as

eicosanoids in a millimolar range and a femtomolar range. There are also different platform challenges in size and polarity differences of compounds. In order to overcome these challenges, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) have been applied to a maximum coverage. However, the difficulty in quantitative detection of multiple indexes at the same time has not yet been solved.

[0007] There are only a few major metabolic pathways, such as glycolysis, aerobic respiration, tricarboxylic acid (TCA) cycle, fatty acid oxidation (0-oxidation) and gluconeogenesis. Cells are used to transfer energy and maintain metabolic homeostasis, and due to defects in these key pathways in storage and disposal of major classes of molecules (such as amino acid, carbohydrate, and lipid), metabolic disorders are caused. Therefore, unique characteristics of metabolites in biological systems can be reflected in quantitative detection of these metabolites.

[0008] As important substances involved in physiological metabolism of the human body, amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid are maintained at a certain level in normal metabolism in the human body. Generally, the changes of concentrations of these substances indicate that there are abnormalities in metabolic pathways of the human body. Based on detection of concentrations of these substances and clinical manifestations, judgment of clinical diseases is facilitated.

[0009] In view of the shortcomings of the prior art, an objective of the present invention is to provide a metabolic chip capable of quantitatively detecting multiple components in a biological sample at the same time and a detection method.

BRIEF SUMMARY OF THE INVENTION

[0010] An objective of the present invention is to provide a quantitative detection method capable of quantitatively detecting multiple metabolic components in a biological sample at the same time and a metabolic chip used in the method. The multiple metabolic components include, but are not limited to, multiple amino acids, phenols, phenyl or benzyl derivatives, indoles, organic acids, fatty acids, sugars, and bile acids.

[0011] In the present invention, the quantitative detection method of multiple metabolic components in a biological sample is achieved by using the following solution: derivatization treatment is performed on the biological sample, and then the derivatized biological sample is detected by liquid chromatography-mass spectrometry; during derivatization treatment, 3-nitrophenylhydrazine is used as a derivatization reagent, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is used as a derivatization reaction catalyst; the biological sample is selected from urine, blood, cerebrospinal fluid, tissue, cell, saliva and fecal samples of a mammal; the multiple metabolic components in the biological sample are selected from one or more of amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid and have different magnitudes in content.

[0012] In the present invention, the quantitative detection method of multiple metabolic components in a biological sample is achieved by using the following solution: derivatization treatment is performed on the biological sample, and then the derivatized biological sample is detected by liquid chromatography-mass spectrometry; during derivatization treatment, 3-nitrophenylhydrazine is used as a derivatization reagent, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is used as a derivatization reaction catalyst; the biological sample is selected from urine, blood, cerebrospinal fluid, tissue, cell, saliva and fecal samples of a mammal; the multiple metabolic components in the biological sample detected by the quantitative detection method are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid and have different magnitudes in content.

[0013] In some embodiments, the liquid chromatography adopts a UPLC BEH C18 chromatographic column, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 1-2:1; and the gradient elution conditions: 0-1 min, 5% B; 1-5 min, 5-30% B; 5-9 min, 30-50% B; 9-12 min, 50-75% B;

12-15 min, 75-95% B; 15-16 min, 95-100% B; 16-18 min, 100% B; and 18-20 min, 5% B.
[0014] In some embodiments, the liquid chromatography adopts a UPLC BEH C18 chromatographic column, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 1-2:1; the flow rate is 0.4 mL/min; and the gradient elution conditions: 0-1 min, 5% B; 1-5 min, 5-30% B; 5-9 min, 30-50% B; 9-12 min, 50-75% B; 12-15 min, 75-95% B; 15-16 min, 95-100% B; 16-18 min, 100% B; and 18-20 min, 5% B.
[0015] In some embodiments, the liquid chromatography adopts a UPLC BEH C18 chromatographic column, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 1-2:1; the flow rate is 0.4 mL/min; the injection volume is 5 microliters; and the gradient elution conditions: 0-1 min, 5% B; 1-5 min, 5-30% B; 5-9 min, 30-50% B; 9-12 min, 50-75% B; 12-15 min, 75-95% B; 15-16 min, 95-100% B; 16-18 min, 100% B; and 18-20 min, 5% B.
[0016] In some embodiments, the liquid chromatography adopts a UPLC BEH C18 chromatographic column, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 1-2:1; the column temperature is 40° C.; the flow rate is 0.4 mL/min; the injection volume is 5 microliters; and the gradient elution conditions: 0-1 min, 5% B; 1-5 min, 5-30% B; 5-9 min, 30-50% B; 9-12 min, 50-75% B; 12-15 min, 75-95% B; 15-16 min, 95-100% B; 16-18 min, 100% B; and 18-20 min, 5% B.
[0017] In some embodiments, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 7:3.
[0018] In some embodiments, scan parameters of the mass spectrometry are set as follows: S-lens RF level, 50; mass range, 100 to 1200 m/z; full MS resolution, 70,000; MS/MS resolution, 17,500; and NCE/stepped NCE is set at 10, 20, and 40 eV.
[0019] In some embodiments, ESI ion source parameters of the mass spectrometry are set as follows: spray voltage, 3800 V; sheath gas flow rate, 40 for ESI+ and 5 for ESI−; capillary temperature, 320° C.; probe heater temperature, 350° C.; aux gas flow rate, 10.
[0020] In some embodiments, the liquid chromatography-mass spectrometry is acquired using a Vanquish Flex UHPLC system coupled to a Q Extractive Focus Orbitrap mass spectrometer equipped with a heated electrospray ionization (ESI) source.
[0021] In some embodiments, the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 50 or more, preferably 70 or more, further preferably 90 or more.
[0022] In some embodiments, the number of the metabolic components in a biological sample detected by the quantitative detection method is 80 or more, preferably 90 or more, further preferably 100 or more.
[0023] In some embodiments, the number of the metabolic components in a biological sample detected by the quantitative detection method is 200 or more, preferably 300 or more, further preferably 400 or more.
[0024] In some embodiments, the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 50 or more, preferably 100 or more, more preferably 200 or more, further preferably 600.
[0025] In some embodiments, the number of the metabolic components in a biological sample detected by the quantitative detection method is 1000 or more.
[0026] In some embodiments, the multiple metabolic components of the biological sample detected by the quantitative detection method are selected from the following multiple metabolites: fructose 1,6-diphosphate, 10Z-nonadecenoic acid, trans-li-octadecenoic acid, cis-11-octadecenoic acid, 12-dehydrocholic acid, 12-hydroxystearic acid, 12-ketolithocholic acid, 1-methylhistidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenoic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-

methylhexanoic acid, 2-methylglutaric acid, 2-methylglutamic acid, 2-furoic acid, 2-hydroxy-2-methylbutyric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxybutyric acid, 2-hydroxycaproic acid, 2-hydroxycinnamic acid, 2-hydroxyphenylacetic acid, 2-hydroxyhippuric acid, 2-phenylpropionic acid, 2-phenylglycine, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-(4-hydroxyphenyl)lactic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dehydro-DL-proline, 3,5-diiodo-L-thyroxine, 3β-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxyalanine, 3-aminosalicylic acid, 3-chloro-L-tyrosine, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 3-methylindole, 3-methyladipic acid, 3-methylglutamic acid, 3-nitrotyrosine, sulfate 3-taurolithocholic acid, sulfate 3-lithocholic acid, 3-hydroxy-2-aminobenzoic acid, 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid, 3-hydroxyphenylacetic acid, 3-hydroxyhippuric acid, 3-dehydrocholic acid, 3-phenylbutyric acid, 4-methyl-2-oxovaleric acid, 4-methylhexanoic acid, 4-methoxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, 4-hydroxycinnamic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid, 4-phenylbutyric acid, 5-aminolevulinic acid, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxylysine, 6,7-diketolithocholic acid, 6-phosphogluconic acid, 6-ketolithocholic acid, 7,12-diketolithocholic acid, 7-ketolithocholic acid, 7-ketodeoxycholic acid, 9,11-conjugated linoleic acid, D-2-hydroxyglutaric acid, D-galactose, D-xylose, D-xylulose, D-fructose, D-ribose, D-ribose-5-phosphate, D-ribulose, D-ribulose-5-phosphate, D-mannose, D-glucose, D-maltose, L-2-aminobutyric acid, L-3-phenyllactic acid, L-alanine, L-serine, L-acetylcarnitine, L-lactic acid, L-allothreonine, L-cysteine, L-homoserine, L-homocysteine, L-homocitrulline, L-pipecolic acid, L-aspartic acid, L-asparagine, L-sorbose, L-lignic acid, L-norleucine, L-kynurenine, L-thyronine, L-arginine, L-histidine, L-valine, L-cystine, L-cystathionine, L-proline, L-tryptophan, L-threonine, L-phenylalanine, L-malic acid, L-methionine, L-glutamine, L-glutamic acid, L-lysine, L-tyrosine, L-arabinose, N-(3-phenylpropionyl)glycine, N-acetyl-L-alanine, N-acetyl-L-aspartic acid, N-acetyl-L-phenylalanine, N-acetyl-L-methionine, N-acetyl-L-tyrosine, N-acetylserine, N-acetyl-D-glucosamine, N-acetyl-L-phenylalanine, N-acetylmannosamine, N-acetylneuraminic acid, N-acetylhistidine, N-acetylhydroxytryptamine, N-acetyltryptophan, N-acetylglutamine, N-acetyllysine, N-acetylornithine, N-methylnicotinamide, N-phenylacetyl-glutamine, N-phenylacetylphenylalanine, S-adenosine homocysteine, α-D-glucose, α-lactose, α-linolenic acid, α-hydroxyisobutyric acid, α-ketoglutaric acid, α-muricholic acid, β-D-trehalose, β-alanine, β-ursocholic acid, β-muricholic acid, γ-L-glutamyl-L-alanine, γ-linolenic acid, γ-aminobutyric acid, ω-muricholic acid, butyric acid, trimethylamine nitroxide, adenosine triphosphate, malonic acid, propionic acid, acetoacetic acid, acetylcysteine, acetylglycine, acetylornithine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosenoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosenoic acid, docosanoic acid, docosatrienoic acid, cis-13,16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-docosaenoic acid, cis-13-docosaenoic acid, pentacosanoic acid, octacosanoic acid, hexacosanoic acid, tetracosanoic acid, eicosanoic acid, eicosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, trans-II-eicosenoic acid, cis-ii-eicosenoic acid, cis-5-eicosenoic acid, cis-8-eicosenoic acid, dimethylglycine, adenosine diphosphate, linoleic acid, leucine, alloisoleucine, allolithocholic acid, allocholic acid, undecenoic acid, heptadecanoic acid, tridecanoic acid, nonadecanoic acid, nonadecadienoic acid, pentadecanoic acid, galactonic acid, galactitol, mecysteine, protocatechuic acid, apocholic acid, dehydrolithocholic acid, nordeoxycholic acid, trans-9-tetradecenoic acid, trans-aconitic acid, trans-4-hydroxyproline, trans-9-heptadecenoic acid, trans-9-pentadecenoic acid, trans-9-hexadecenoic acid, trans-linolenic acid, trans-cinnamic acid, elaidic acid, homocysteine, pyrrole-2-carboxylic acid, picolinic acid, indole, indole-3-methyl acetate, indole-3-carboxylic acid, indoleacetic acid, purine, azelaic acid, nonanoic acid, dopa, dopamine, melibiose, fumaric acid, acetaminophen, p-aminohippuric acid, p-cresol sulfate, symmetrical dimethylarginine, p-hydroxymandelic acid, p-hydroxyphenylacetic acid, homogentisic acid, adipic acid, pimelic acid,

isobutyric acid, isoleucine, isovaleric acid, citric acid, isoursodeoxycholic acid, isohyodeoxycholic acid, isolithocholic acid, isocholic acid, isodeoxycholic acid, glutaric acid, glutaconic acid, valeric acid, mandelic acid, lauric acid, fructose-6-phosphate, citraconic acid, citric acid, citramalic acid, ribonolactone, ribonic acid, raffinose, palmitoleic acid, palmitic acid, norvaline, n-hydroxyphenylacetic acid, oxidized glutathione, aminoadipic acid, aminocaproic acid, salicyluric acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, taurolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hyodeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycoursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyl-L-leucine, mannose-6-phosphoric acid, mannitol, methylmalonic acid, methylsuccinic acid, formic acid, capric acid, lithocholic acid, selenomethionine, thiamine, glycolithocholic sulfate, stearic acid, liothyronine, dihydroxyacetone phosphate, phosphoribosyl pyrophosphate, creatine phosphate, hydroxypyruvic acid, glycine hydroxyphenylacetate, cinnamic acid, sarcosine, carnosine, creatine, inositol, epinephrine, choline, cholic acid, dehydrocholic acid, demethylcholic acid, adenosine monophosphate, arachidonic acid, phenylpyruvic acid, phenethylamine, phenylpropionic acid, phenyllactic acid, benzamide, benzoic acid, oxalic acid, shikimic acid, glucaric acid, glucose-6-phosphate, glucose lactone, sebacic acid, ricinoleic acid, sucrose, methionine sulfoxide, itaconic acid, melatonin, glutathione, myristic acid, erythronic acid, erythrose, suberic acid, caprylic acid, phthalic acid, tartaric acid, tyramine, quinic acid, m-aminobenzoic acid, asymmetric dimethylarginine, tannic acid, cis-10,12-octadecadienoic acid, cis-12,15-heneicosadienoic acid, cis-12-tridecenoic acid, cis-15-tetracosenic acid, cis-2-hydroxycinnamic acid, cis-9-tetradecenoic acid, cis-10-heptadecenoic acid, cis-11-dodecenoic acid, cis-4-hydroxyproline, cis-5-dodecenoic acid, cis-7-hexadecenoic acid, cis-9-heptadecenoic acid, cis-aconitic acid, vanillic acid, hippuric acid, maleic acid, homovanillic acid, ornithine, guanosine monophosphate, guanosine triphosphate, anserine, chenodeoxycholic acid, maltotriose, maltitol, rhamnose and murideoxycholic acid.

[0027] The detection method of the present invention specifically includes the following steps:

[0028] a) collecting a biological sample; [0029] b) extracting the biological sample with a mixed solvent of methanol, chloroform, and water, performing centrifugation, and taking a supernatant, namely a biological sample extract; [0030] c) adding the same volume of a 3-nitrophenylhydrazine methanol solution and a 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide pyridine solution into the biological sample extract obtained in b), and performing uniform vortex mixing and heating for derivatization, where the concentration of used 3-nitrophenylhydrazine is 100-320 mmol/L (in this patent application, “mM” represents “mmol/L”), the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 50-200 mmol/L, the reaction temperature is 20-60° C., and the reaction time is 10-120 minutes; [0031] d) adding a carbon-13 labeled isotope internal standard solution obtained from the reaction of 3-nitrophenylhydrazine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide into the derivatized biological sample extract obtained in c); and [0032] e) adding a methanol-water mixed solution into a sample in d) for dilution, and determining amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid by liquid chromatography-mass spectrometry.

[0033] Preferably, in step c), the concentration of used 3-nitrophenylhydrazine is 150-220 mM, the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 80-120 mM, the reaction temperature is 20-40° C., and the reaction time is 30-60 minutes.

[0034] Preferably, the volume ratio of methanol to water in step e) is 1:1.

[0035] When the biological sample is urine, blood, saliva or cerebrospinal fluid, a treatment method of the biological sample in step b) preferably includes: taking an appropriate amount of the

biological sample, extracting the biological sample with a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, shaking the mixture for a few seconds, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at a low temperature for 5-15 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization determination.

[0036] When the biological sample is a fecal sample, a treatment method of the biological sample in step b) preferably includes: freeze-drying the fecal sample; uniformly mixing an appropriate amount of the freeze-dried fecal sample and an appropriate amount of a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at a low temperature for 15-30 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment.

[0037] When the biological sample is a tissue or cell sample, a treatment method of the biological sample in step b) preferably includes: adding an appropriate amount of a mixed solvent of cold methanol, chloroform, and water at a volume ratio of 3:1:1 into the sample for homogenizing, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at 4° C. for 15-30 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment.

[0038] According to the detection method provided in the present invention, 3-nitrophenylhydrazine is used to undergo a derivatization reaction with amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid in the sample in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to produce corresponding derivatives, the detection sensitivity is improved, the detection difficulty is reduced, quantitative detection of multiple substances of different magnitudes in the biological sample can be achieved, defects in the prior art are overcome, and a high-throughput quantitative detection effect is achieved. In addition, in the present invention, a commonly labeled derivatization reagent and a carbon-13 labeled derivatization reagent are used to undergo a reaction with the standard product and the sample solution so that chromatographic behaviors, ionization efficiency of mass spectrometry and matrix effect can be completely consistent, and thus systematic errors are avoided.

[0039] The present invention provides a quantitative detection method of multiple metabolic components of different magnitudes in a biological sample. As understood by a person of ordinary skill, the content of index components in the sample can be calculated by drawing a standard curve in the present invention. On this basis, the applicant further provides a metabolic chip used in the detection method. The metabolic chip includes

[0040] The present invention further provides a metabolic chip used in the detection method. The metabolic chip of the present invention is a device for efficient quantitative detection of multiple metabolic components by using the detection method of the present invention, including a chip carrier, a filter device and dry solid powder of a standard product and a quality control product. The chip carrier is a microtiter plate, and the microtiter plate may be a commercially available 48-well plate, a 96-well plate, and a 384-well microtiter plate suitable for liquid chromatography determination. Each well of the microtiter plate is provided with an independent filter device, and the filter device is a filter membrane made of polyvinylidene fluoride, cellulose acetate, or nylon with a pore size of 0.20-0.45 micron (m). Each well of the microtiter plate is divided into upper and lower parts by the filter device.

[0041] The dry solid powder of the standard product and the quality control product is powder obtained by dehydrating or freeze-drying solutions of the standard product and the quality control product and is placed on the filter device in each well of the microtiter plate. As understood by a person of ordinary skill in the art, when the powder of the standard product is prepared, different standard product solutions are prepared first according to required standard product concentration gradients based on the drawn standard curve, then dehydrating or freeze-drying is performed to obtain the powder, and the powder is placed on the corresponding filter devices in the wells of the

metabolic chip. As understood by a person of ordinary skill in the art, the quality control product is prepared into a corresponding solution and then dehydrated or freeze-dried to obtain the powder, and the powder is placed on the filter device in each well of the metabolic chip.

[0042] The standard product is selected from one or more of amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products. The quality control product is selected from one or more of amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products corresponding to the standard products above.

[0043] The multiple metabolic components in the standard product are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products. The multiple metabolic components in the quality control product are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products corresponding to the standard products above.

[0044] Specifically, the standard product and the quality control product may be selected from the following components: fructose 1,6-diphosphate, 10Z-nonadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, 12-dehydrocholic acid, 12-hydroxystearic acid, 12-ketolithocholic acid, 1-methylhistidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenoic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutaric acid, 2-methylglutamic acid, 2-furoic acid, 2-hydroxy-2-methylbutyric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxybutyric acid, 2-hydroxycaproic acid, 2-hydroxycinnamic acid, 2-hydroxyphenylacetic acid, 2-hydroxyhippuric acid, 2-phenylpropionic acid, 2-phenylglycine, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-(4-hydroxyphenyl)lactic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dehydro-DL-proline, 3,5-diiodo-L-thyroxine, 30-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxyalanine, 3-aminosalicylic acid, 3-chloro-L-tyrosine, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 3-methylindole, 3-methyladipic acid, 3-methylglutamic acid, 3-nitrotyrosine, sulfate 3-taurolithocholic acid, sulfate 3-lithocholic acid, 3-hydroxy-2-aminobenzoic acid, 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid, 3-hydroxyphenylacetic acid, 3-hydroxyhippuric acid, 3-dehydrocholic acid, 3-phenylbutyric acid, 4-methyl-2-oxovaleric acid, 4-methylhexanoic acid, 4-methoxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, 4-hydroxycinnamic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid, 4-phenylbutyric acid, 5-aminolevulinic acid, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxylysine, 6,7-diketolithocholic acid, 6-phosphogluconic acid, 6-ketolithocholic acid, 7,12-diketolithocholic acid, 7-ketolithocholic acid, 7-ketodeoxycholic acid, 9,11-conjugated linoleic acid, D-2-hydroxyglutaric acid, D-galactose, D-xylose, D-xylulose, D-fructose, D-ribose, D-ribose-5-phosphate, D-ribulose, D-ribulose-5-phosphate, D-mannose, D-glucose, D-maltose, L-2-aminobutyric acid, L-3-phenyllactic acid, L-alanine, L-serine, L-acetylcarnitine, L-lactic acid, L-allothreonine, L-cysteine, L-homoserine, L-homocysteine, L-homocitrulline, L-pipecolic acid, L-aspartic acid, L-asparagine, L-sorbose, L-lignic acid, L-norleucine, L-kynurenine, L-thyronine, L-arginine, L-histidine, L-valine, L-cystine, L-cystathionine, L-proline, L-tryptophan, L-threonine, L-phenylalanine, L-malic acid, L-methionine, L-glutamine, L-glutamic acid, L-lysine, L-tyrosine, L-arabinose, N-(3-phenylpropionyl)glycine, N-acetyl-L-alanine, N-acetyl-L-aspartic acid, N-acetyl-L-phenylalanine, N-acetyl-L-methionine, N-acetyl-L-tyrosine, N-acetylserine, N-acetyl-D-glucosamine, N-acetyl-L-phenylalanine, N-acetylmannosamine, N-acetylneuraminic acid, N-acetylhistidine, N-acetylhydroxytryptamine, N-acetyltryptophan, N-acetylglutamine, N-acetyllysine, N-acetylornithine, N-methylnicotinamide, N-phenylacetyl-glutamine, N-phenylacetylphenylalanine, S-adenosine homocysteine, α-D-glucose, α-lactose, α-linolenic acid, α-hydroxyisobutyric acid, α-ketoglutaric acid, α-muricholic acid, β-D-trehalose, β-alanine, β-ursocholic acid, β-muricholic acid,

γ-L-glutamyl-L-alanine, γ-linolenic acid, γ-aminobutyric acid, ω-muricholic acid, butyric acid, trimethylamine nitroxides, adenosine triphosphate, malonic acid, propionic acid, acetoacetic acid, acetylcysteine, acetylglycine, acetylornithine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosenoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosenoic acid, docosanoic acid, docosatrienoic acid, cis-13,16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-docosaenoic acid, cis-13-docosaenoic acid, pentacosanoic acid, octacosanoic acid, hexacosanoic acid, tetracosanoic acid, eicosanoic acid, eicosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, trans-II-eicosenoic acid, cis-ii-eicosenoic acid, cis-5-eicosenoic acid, cis-8-eicosenoic acid, dimethylglycine, adenosine diphosphate, linoleic acid, leucine, alloisoleucine, allolithocholic acid, allocholic acid, undecenoic acid, heptadecanoic acid, tridecanoic acid, nonadecanoic acid, nonadecadienoic acid, pentadecanoic acid, galactonic acid, galactitol, mecysteine, protocatechuic acid, apocholic acid, dehydrolithocholic acid, nordeoxycholic acid, trans-9-tetradecenoic acid, trans-aconitic acid, trans-4-hydroxyproline, trans-9-heptadecenoic acid, trans-9-pentadecenoic acid, trans-9-hexadecenoic acid, trans-linolenic acid, trans-cinnamic acid, elaidic acid, homocysteine, pyrrole-2-carboxylic acid, picolinic acid, indole, indole-3-methyl acetate, indole-3-carboxylic acid, indoleacetic acid, purine, azelaic acid, nonanoic acid, dopa, dopamine, melibiose, fumaric acid, acetaminophen, p-aminohippuric acid, p-cresol sulfate, symmetrical dimethylarginine, p-hydroxymandelic acid, p-hydroxyphenylacetic acid, homogentisic acid, adipic acid, pimelic acid, isobutyric acid, isoleucine, isovaleric acid, citric acid, isoursodeoxycholic acid, isohyodeoxycholic acid, isolithocholic acid, isocholic acid, isodeoxycholic acid, glutaric acid, glutaconic acid, valeric acid, mandelic acid, lauric acid, fructose-6-phosphate, citraconic acid, citric acid, citramalic acid, ribonolactone, ribonic acid, raffinose, palmitoleic acid, palmitic acid, norvaline, n-hydroxyphenylacetic acid, oxidized glutathione, aminoadipic acid, aminocaproic acid, salicyluric acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, taurolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hyodeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycoursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyl-L-leucine, mannose-6-phosphoric acid, mannitol, methylmalonic acid, methylsuccinic acid, formic acid, capric acid, lithocholic acid, selenomethionine, thiamine, glycyllithocholic sulfate, stearic acid, liothyronine, dihydroxyacetone phosphate, phosphoribosyl pyrophosphate, creatine phosphate, hydroxypyruvic acid, glycine hydroxyphenylacetate, cinnamic acid, sarcosine, carnosine, creatine, inositol, epinephrine, choline, cholic acid, dehydrocholic acid, demethylcholic acid, adenosine monophosphate, arachidonic acid, phenylpyruvic acid, phenethylamine, phenylpropionic acid, phenyllactic acid, benzamide, benzoic acid, oxalic acid, shikimic acid, glucaric acid, glucose-6-phosphate, glucose lactone, sebacic acid, ricinoleic acid, sucrose, methionine sulfoxide, itaconic acid, melatonin, glutathione, myristic acid, erythronic acid, erythrose, suberic acid, caprylic acid, phthalic acid, tartaric acid, tyramine, quinic acid, m-aminobenzoic acid, asymmetric dimethylarginine, tannic acid, cis-10,12-octadecadienoic acid, cis-12,15-heneicosadienoic acid, cis-12-tridecenoic acid, cis-15-tetracosenic acid, cis-2-hydroxycinnamic acid, cis-9-tetradecenoic acid, cis-10-heptadecenoic acid, cis-11-dodecenoic acid, cis-4-hydroxyproline, cis-5-dodecenoic acid, cis-7-hexadecenoic acid, cis-9-heptadecenoic acid, cis-aconitic acid, vanillic acid, hippuric acid, maleic acid, homovanillic acid, ornithine, guanosine monophosphate, guanosine triphosphate, anserine, chenodeoxycholic acid, maltotriose, maltitol, rhamnose and murideoxycholic acid.

[0045] Specifically, the multiple metabolic components in the standard product and the quality control product may be selected from: fructose 1,6-diphosphate, 10Z-nonadecenoic acid, trans-11-

octadecenoic acid, cis-11-octadecenoic acid, 12-dehydrocholic acid, 12-hydroxystearic acid, 12-ketolithocholic acid, 1-methylhistidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenoic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutaric acid, 2-methylglutamic acid, 2-furoic acid, 2-hydroxy-2-methylbutyric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxybutyric acid, 2-hydroxycaproic acid, 2-hydroxycinnamic acid, 2-hydroxyphenylacetic acid, 2-hydroxyhippuric acid, 2-phenylpropionic acid, 2-phenylglycine, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-(4-hydroxyphenyl)lactic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dehydro-DL-proline, 3,5-diiodo-L-thyroxine, 30-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxyalanine, 3-aminosalicylic acid, 3-chloro-L-tyrosine, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 3-methylindole, 3-methyladipic acid, 3-methylglutamic acid, 3-nitrotyrosine, sulfate 3-taurolithocholic acid, sulfate 3-lithocholic acid, 3-hydroxy-2-aminobenzoic acid, 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid, 3-hydroxyphenylacetic acid, 3-hydroxyhippuric acid, 3-dehydrocholic acid, 3-phenylbutyric acid, 4-methyl-2-oxovaleric acid, 4-methylhexanoic acid, 4-methoxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, 4-hydroxycinnamic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid, 4-phenylbutyric acid, 5-aminolevulinic acid, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxylysine, 6,7-diketolithocholic acid, 6-phosphogluconic acid, 6-ketolithocholic acid, 7,12-diketolithocholic acid, 7-ketolithocholic acid, 7-ketodeoxycholic acid, 9,11-conjugated linoleic acid, D-2-hydroxyglutaric acid, D-galactose, D-xylose, D-xylulose, D-fructose, D-ribose, D-ribose-5-phosphate, D-ribulose, D-ribulose-5-phosphate, D-mannose, D-glucose, D-maltose, L-2-aminobutyric acid, L-3-phenyllactic acid, L-alanine, L-serine, L-acetylcarnitine, L-lactic acid, L-allothreonine, L-cysteine, L-homoserine, L-homocysteine, L-homocitrulline, L-pipecolic acid, L-aspartic acid, L-asparagine, L-sorbose, L-lignic acid, L-norleucine, L-kynurenine, L-thyronine, L-arginine, L-histidine, L-valine, L-cystine, L-cystathionine, L-proline, L-tryptophan, L-threonine, L-phenylalanine, L-malic acid, L-methionine, L-glutamine, L-glutamic acid, L-lysine, L-tyrosine, L-arabinose, N-(3-phenylpropionyl)glycine, N-acetyl-L-alanine, N-acetyl-L-aspartic acid, N-acetyl-L-phenylalanine, N-acetyl-L-methionine, N-acetyl-L-tyrosine, N-acetylserine, N-acetyl-D-glucosamine, N-acetyl-L-phenylalanine, N-acetylmannosamine, N-acetylneuraminic acid, N-acetylhistidine, N-acetylhydroxytryptamine, N-acetyltryptophan, N-acetylglutamine, N-acetyllysine, N-acetylornithine, N-methylnicotinamide, N-phenylacetyl-glutamine, N-phenylacetylphenylalanine, S-adenosine homocysteine, α-D-glucose, α-lactose, α-linolenic acid, α-hydroxyisobutyric acid, α-ketoglutaric acid, α-muricholic acid, β-D-trehalose, β-alanine, β-ursocholic acid, β-muricholic acid, γ-L-glutamyl-L-alanine, γ-linolenic acid, γ-aminobutyric acid, ω-muricholic acid, butyric acid, trimethylamine nitroxides, adenosine triphosphate, malonic acid, propionic acid, acetoacetic acid, acetylcysteine, acetylglycine, acetylornithine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosenoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosenoic acid, docosanoic acid, docosatrienoic acid, cis-13,16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-docosaenoic acid, cis-13-docosaenoic acid, pentacosanoic acid, octacosanoic acid, hexacosanoic acid, tetracosanoic acid, eicosanoic acid, eicosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, trans-II-eicosenoic acid, cis-ii-eicosenoic acid, cis-5-eicosenoic acid, cis-8-eicosenoic acid, dimethylglycine, adenosine diphosphate, linoleic acid, leucine, alloisoleucine, allolithocholic acid, allocholic acid, undecenoic acid, heptadecanoic acid, tridecanoic acid, nonadecanoic acid, nonadecadienoic acid, pentadecanoic acid, galactonic acid, galactitol, mecysteine, protocatechuic acid, apocholic acid, dehydrolithocholic acid, nordeoxycholic acid, trans-9-tetradecenoic acid, trans-aconitic acid, trans-4-hydroxyproline, trans-9-heptadecenoic acid, trans-9-pentadecenoic acid, trans-9-hexadecenoic acid, trans-linolenic acid, trans-cinnamic acid, elaidic acid, homocysteine,

pyrrole-2-carboxylic acid, picolinic acid, indole, indole-3-methyl acetate, indole-3-carboxylic acid, indoleacetic acid, purine, azelaic acid, nonanoic acid, dopa, dopamine, melibiose, fumaric acid, acetaminophen, p-aminohippuric acid, p-cresol sulfate, symmetrical dimethylarginine, p-hydroxymandelic acid, p-hydroxyphenylacetic acid, homogentisic acid, adipic acid, pimelic acid, isobutyric acid, isoleucine, isovaleric acid, citric acid, isoursodeoxycholic acid, isohyodeoxycholic acid, isolithocholic acid, isocholic acid, isodeoxycholic acid, glutaric acid, glutaconic acid, valeric acid, mandelic acid, lauric acid, fructose-6-phosphate, citraconic acid, citric acid, citramalic acid, ribonolactone, ribonic acid, raffinose, palmitoleic acid, palmitic acid, norvaline, n-hydroxyphenylacetic acid, oxidized glutathione, aminoadipic acid, aminocaproic acid, salicyluric acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, taurolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hyodeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycoursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyl-L-leucine, mannose-6-phosphoric acid, mannitol, methylmalonic acid, methylsuccinic acid, formic acid, capric acid, lithocholic acid, selenomethionine, thiamine, glycyllithocholic sulfate, stearic acid, liothyronine, dihydroxyacetone phosphate, phosphoribosyl pyrophosphate, creatine phosphate, hydroxypyruvic acid, glycine hydroxyphenylacetate, cinnamic acid, sarcosine, carnosine, creatine, inositol, epinephrine, choline, cholic acid, dehydrocholic acid, demethylcholic acid, adenosine monophosphate, arachidonic acid, phenylpyruvic acid, phenethylamine, phenylpropionic acid, phenyllactic acid, benzamide, benzoic acid, oxalic acid, shikimic acid, glucaric acid, glucose-6-phosphate, glucose lactone, sebacic acid, ricinoleic acid, sucrose, methionine sulfoxide, itaconic acid, melatonin, glutathione, myristic acid, erythronic acid, erythrose, suberic acid, caprylic acid, phthalic acid, tartaric acid, tyramine, quinic acid, m-aminobenzoic acid, asymmetric dimethylarginine, tannic acid, cis-10,12-octadecadienoic acid, cis-12,15-heneicosadienoic acid, cis-12-tridecenoic acid, cis-15-tetracosenic acid, cis-2-hydroxycinnamic acid, cis-9-tetradecenoic acid, cis-10-heptadecenoic acid, cis-11-dodecenoic acid, cis-4-hydroxyproline, cis-5-dodecenoic acid, cis-7-hexadecenoic acid, cis-9-heptadecenoic acid, cis-aconitic acid, vanillic acid, hippuric acid, maleic acid, homovanillic acid, ornithine, guanosine monophosphate, guanosine triphosphate, anserine, chenodeoxycholic acid, maltotriose, maltitol, rhamnose and murideoxycholic acid.

[0046] In some embodiments, the number of the multiple metabolic components in the standard product and the quality control product is 50 or more, preferably 70 or more, further preferably 90 or more.

[0047] In some embodiments, the number of the multiple metabolic components in the standard product and the quality control product is 80 or more, preferably 90 or more, further preferably 100 or more.

[0048] In some embodiments, the number of the multiple metabolic components in the standard product and the quality control product is 200 or more, preferably 300 or more, further preferably 400 or more.

[0049] In some embodiments, the number of the multiple metabolic components in the standard product and the quality control product is 50 or more, preferably 100 or more, more preferably 200 or more, further preferably 600.

[0050] In some embodiments, the number of the multiple metabolic components in the standard product and the quality control product is 1000 or more.

[0051] A use method of the metabolic chip in the present invention includes the following steps:

[0052] 1. collecting a biological sample; [0053] 2. according to the sample type, preparing a corresponding biological sample extract by using the corresponding method; [0054] 3. adding the

prepared biological sample extract into each well of the metabolic chip in an equal amount, adding the same volume of a 3-nitrophenylhydrazine methanol solution and a 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide pyridine solution into each well, and performing uniform vortex mixing and heating for derivatization, where the concentration of used 3-nitrophenylhydrazine is 100-320 nM, the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 50-200 nM, the reaction temperature is 20-60° C., and the reaction time is 10-120 minutes; [0055] 4. adding a carbon-13 labeled isotope internal standard solution obtained from the reaction of 3-nitrophenylhydrazine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide into the derivatized biological sample extract obtained in step 3; [0056] 5. adding a methanol-water mixed solution into each well in the metabolic chip in step 4 for dilution, placing the metabolic chip in a tandem mass spectrometer for liquid chromatography-mass spectrometry for determination of amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid by liquid chromatography-mass spectrometry, and calculating concentrations of target metabolites in the sample based on results.

[0057] As understood by a person of ordinary skill in the art, the content of each target detection substance can be calculated based on detection results and the standard curve. The detection results obtained by the metabolic chip can also be obtained by calculation with a metabolite batch quantification software developed by Shenzhen Huiyun Biotechnology Co., Ltd. to quickly obtain the content of each target component. By combining the calculation software with the metabolic chip of the present invention, the work efficiency is greatly improved.

[0058] Traditional targeted metabolomics is constrained by predefined ion pair libraries, limiting its capacity for large-scale, precise metabolite quantification. This study proposes a revolutionary strategy—derivatization-AI synergy—to break the throughput bottleneck of metabolite quantification on triple quadrupole mass spectrometers for the first time. Core innovations include: (1) broad-spectrum functional group modification via derivatization reagents to significantly enhance ionization efficiency and generate predictable fragments; (2) GNN-based retention time prediction for high-confidence metabolite identification; and (3) TQMS-specific fragment ion scanning to improve quantitative specificity. The method overcomes the traditional coverage-precision conflict in metabolomics, enabling simultaneous quantification of >1,000 metabolites. AI-driven retention time calibration eliminates reliance on predefined spectral libraries, ensuring compatibility with any LC-MS platform and adaptability to diverse derivatization reagents. Validation in serum metabolome analysis identified novel low-abundance metabolite biomarkers linked to metabolic syndrome. This framework establishes a new paradigm for large-scale precision metabolomics.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] The accompanying drawings are chromatograms of typical amino acid, organic acid, fatty acid, sugar, and bile acid detected in blood samples in embodiments. It can be seen that multiple target detection components can be effectively separated and detected, and thus high-throughput quantitative detection is achieved.

[0060] FIG. **1** is a chromatogram of typical short-chain fatty acid;

[0061] FIG. **2** is a chromatogram of typical amino acid;

[0062] FIG. **3** is a chromatogram of typical organic acid;

[0063] FIG. **4** is a chromatogram of typical sugar;

[0064] FIG. **5** is a chromatogram of typical bile acid.

DETAILED DESCRIPTION OF THE INVENTION

Example 1

[0065] A metabolic chip is used to detect multiple index components in 10 human blood and fecal samples. FIGS. **1**-**5** are chromatograms of typical amino acid, organic acid, fatty acid, sugar, and bile acid detected in blood samples in this Example.

1. Instrument

[0066] Liquid chromatography-tandem mass spectrometer (LC-MS/MS) equipped with an electrospray ionization source (ESI).

2. Sample Preparation

[0067] Serum sample: Avenous whole blood sample is collected, placed in an anticoagulation tube, then immediately shaken up and down for uniform mixing 5-6 times, and centrifugated within 30 minutes to separate plasma. The sample is placed in a centrifuge tube, extracted with a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, shaken for a few seconds and then centrifugated at a rotation speed of 10000-20000 rpm at 4° C. for 5-15 minutes to obtain a supernatant. The supernatant is transferred into an autosampler glass vial. All water-containing serum or urine sample extracts are used for subsequent derivatization treatment.

[0068] Fecal sample: A fecal sample is freeze-dried. An appropriate amount of the freeze-dried fecal sample and an appropriate amount of a mixed solvent of cold methanol, chloroform, and water at a volume ratio of 3:1:1 are homogenized. The sample is centrifugated at a rotation speed of 10000-20000 rpm at 4° C. for 15-30 minutes to obtain a supernatant. The supernatant is transferred into an autosampler glass vial for subsequent derivatization treatment.

3. Reagent Preparation

[0069] Preparation of standard product solution: Standard products, including amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid, of metabolites in the description are taken, fully dissolved in methanol and uniformly mixed to prepare a 1 mg/ml solution, namely a concentrated stock solution which is prepared into a series of concentrations of solutions to draw a standard curve.

[0070] Preparation of quality control product solution: A corresponding quality control product is taken, fully dissolved in methanol and uniformly mixed to prepare a 1 mg/ml solution, namely a concentrated stock solution, and then the solution is diluted to a certain concentration and reacts with carbon-13 labeled 3-nitrophenylhydrazine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to obtain the quality control product solution.

[0071] Preparation of derivatization reagent (3-nitrophenylhydrazine): A derivatization reagent is uniformly mixed with a 75% methanol aqueous solution to prepare a 200 mM solution, and the solution is sealed and stored at 4° C. for later use.

[0072] Preparation of derivatization reaction catalyst (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide): A reaction catalyst is prepared into a 120 millimolar solution with pyridine, and the solution is sealed and stored at 4° C. for later use.

4. Inspection Method

[0073] 5 μL of a treated biological sample is taken, 20 μl of a derivatization reagent and 20 μl of a derivatization catalyst are added for reaction at 30° C. for 60 minutes, a methanol-water mixed solution is added into a reaction solution for dilution, centrifugation is performed at 13200 rpm for 15 minutes, and 5 μL of a supernatant is taken and introduced for LC-MS/MS analysis.

Mass Spectrometry Conditions:

[0074] Ion source: Multi-reaction detection conditions for detection of multiple substances by tandem mass spectrometry are shown in Table 1. A negative ion scanning mode (ESI−) is adopted for an electrospray ion source, and specific conditions are as follows: The capillary voltage is 1.2 kV, the cone voltage is 55 V, the extraction cone voltage is 4 V, the ion source temperature is 150° C., the desolvent gas temperature is 550° C., the reverse cone gas flow is 50 L/h, the desolvent gas is 650 L/h, the resolution of a low mass zone is 4.7, the resolution of a high mass zone is 15, and a multi-reaction detection mode is used to collect data.

[0075] Gradient elution conditions: A UPLC BEH C18 chromatographic column (100 mm*2.1

mm, 1.7 μm) is used; the column temperature is 40° C.; a mobile phase A includes water (0.1% formic acid), and a mobile phase B includes acetonitrile (0.1% formic acid) and isopropanol at a ratio of (1-2):1; the flow rate is 0.4 mL/min; the injection volume is 5 microliters; and gradient elution conditions: 0-1 min (5% B), 1-5 min (5-30% B), 5-9 min (30-50% B), 9-12 min (50-75% B), 12-15 min (75-95% B), 15-16 min (95-100% B), 16-18 min (100% B), 18-20 min (5% B).

5. Determination Results

[0076] Concentrations of substances in 10 human blood and fecal samples are detected by using the detection method of the present invention (see Table 1 and Table 2 respectively). It can be seen that by using the method of the present invention to detect a single sample, multiple substances of different magnitudes and different properties can be quantitatively detected at one time.

TABLE-US-00001 TABLE 1 Determination results of concentrations of substances in blood of normal people Concentration Concentration Determined target metabolites value unit 2-methylvaleric acid 120.32 ± 5.18 μg/mL 2-hydroxybutyric acid 2.32 ± 0.44 μg/mL 3-(3-hydroxyphenyl)-3- 10.31 ± 0.19 μg/mL hydroxypropionic acid 3-hydroxyphenylacetic acid 6.07 ± 6.12 μg/mL 3-indoleacetonitrile 2.51 ± 0.15 μg/mL 3-methyl-2-oxovaleric acid 4.37 ± 5.08 μg/mL 4-methylhexanoic acid 2.96 ± 0.07 μg/mL Linolenic acid 97.19 ± 33.87 μg/mL Arachidonic acid 5.54 ± 0.45 μg/mL Arachidonic acid 5.62 ± 2.4 μg/mL Docosanoic acid 8.96 ± 0.27 μg/mL β-alanine 10.37 ± 0.44 μg/mL Capric acid 1.22 ± 0.19 μg/mL Caprylic acid 1.39 ± 0.5 μg/mL Citric acid 7.03 ± 1.56 μg/mL Docosahexaenoic acid 15.1 ± 4.36 μg/mL Docosapentaenoic acid n6 15.31 ± 4.16 μg/mL Docosatrienoic acid 8.76 ± 0.95 μg/mL Dodecanoic acid 0.91 ± 0.16 μg/mL Dopamine 23.53 ± 3.93 μg/mL Eicosenoic acid 11.18 ± 3.38 μg/mL Erucic acid 15.25 ± 11.34 μg/mL γ-aminobutyric acid 14.68 ± 2.7 μg/mL Glutathione 6.7 ± 1.62 μg/mL Glycolic acid 159.46 ± 56.76 μg/mL Heptadecanoic acid 5.78 ± 6.5 μg/mL L-α-aminobutyric acid 2.41 ± 0.48 μg/mL L-asparagine 12.32 ± 0.57 μg/mL L-aspartic acid 3.8 ± 0.64 μg/mL L-glutamic acid 8.93 ± 0.53 μg/mL L-histidine 16.76 ± 1.54 μg/mL L-homoserine 12.53 ± 7.81 μg/mL L-isoleucine 5.9 ± 2.18 μg/mL L-leucine 5.88 ± 3.97 μg/mL L-lysine 33.73 ± 4.42 μg/mL L-methionine 6.78 ± 0.73 μg/mL L-norleucine 3.33 ± 1.25 μg/mL L-phenylalanine 8.57 ± 0.84 μg/mL L-proline 11.32 ± 5.94 μg/mL L-serine 9.78 ± 0.68 μg/mL L-tryptophan 30.49 ± 2.93 μg/mL L-tyrosine 20.02 ± 4.37 μg/mL L-valine 28.87 ± 7.87 μg/mL Linoleic acid 162.6 ± 57.21 μg/mL Methylsuccinic acid 34.76 ± 29.31 μg/mL Myristic acid 2.91 ± 0.52 μg/mL Myristic acid 3.39 ± 0.51 μg/mL N-acetyltryptophan 29.5 ± 1.24 μg/mL Nervonic acid 82.71 ± 4.32 μg/mL Dodecanoic acid 5.9 ± 2.13 μg/mL Norvaline 0.86 ± 0.01 μg/mL Ornithine 22.56 ± 5.54 μg/mL Carbonyladipic acid 11.55 ± 2.52 μg/mL Oxoglutaric acid 21.89 ± 5.22 μg/mL Palmitic acid 87.29 ± 30.74 μg/mL Palmitoleic acid 86.36 ± 30.14 μg/mL Pentadecanoic acid 1.81 ± 0.12 μg/mL Pimelic acid 4.47 ± 1.23 μg/mL Propionic acid 0.16 ± 0.03 μg/mL Putrescine 19.96 ± 1.32 μg/mL Pyroglutamic acid 5.59 ± 2 μg/mL Stearic acid 38.06 ± 14.04 μg/mL Succinic acid 36.45 ± 12.11 μg/mL Cis-aconitic acid 2.66 ± 0.11 μg/mL P-hydroxyphenylacetic acid 2.51 ± 0.15 μg/mL Palmitoleic acid 19.32 ± 11.42 uM Nervonic acid 0.25 ± 0.06 uM Cholic acid 67.57 ± 38.23 nM Chenodeoxycholic acid 383 ± 559.28 nM Deoxycholic acid 241.97 ± 197.98 nM Fructose 5.23 ± 1.18 uM Glucose 3.29 ± 0.66 mM Dohomo-g-linoleic acid 2.51 ± 1.01 uM Mannose 34.87 ± 9.22 uM Glycocholic acid 314.89 ± 345.38 nM Glycochenodeoxycholic acid 750.99 ± 574.2 nM Glycohyocholic acid 16.31 ± 10.67 nM Glycolithocholic acid 12.41 ± 10.41 nM Glycoursocholic acid 123.97 ± 124.56 nM Hyocholic acid 26.09 ± 11.26 nM Lithocholic acid 12.61 ± 4.68 nM Oleic acid 239.05 ± 84.26 uM Palmitoleic acid 19.32 ± 11.42 uM Taurocholic acid 88.59 ± 66.4 nM Taurodeoxycholic acid 68.66 ± 47.85 nM Teracosanoic acid 2.28 ± 0.89 uM Taurohyocholic acid 4.06 ± 4.7 nM Tauroursocholic acid 26.16 ± 0.81 nM Ursocholic acid 69.29 ± 44.3 nM g-linoleic acid 20.47 ± 8.36 uM

TABLE-US-00002 TABLE 2 Determination results of concentrations of substances in feces of normal people Concentration Concentration Detected target metabolites value unit (1)-2-methylvaleric acid 0.1 ± 0.19 μg/mL 1H-indole-3-acetamide 0.54 ± 0.07 μg/mL 2-hydroxybutyric

acid 29.5 ± 7.89 ng/mL 3-(3-hydroxyphenyl)-3- 0.19 ± 0.11 μg/mL hydroxypropionic acid 3-hydroxybutyric acid 0.3 ± 0.29 μg/mL 3-hydroxyphenylacetic acid 0.39 ± 0.36 μg/mL 3-indoleacetonitrile 0.17 ± 0.11 μg/mL 3-isopropionic acid 0.32 ± 0.09 μg/mL 3-methyl-2-oxovaleric acid 0.5 ± 0.2 ng/mL 3-methylvaleric acid 0.2 ± 0.3 ng/mL 4-hydroxybenzoic acid 0.42 ± 0.55 μg/mL 4-hydroxycinnamic acid 0.25 ± 0.07 μg/mL 4-methylhexanoic acid 0.7 ± 0.6 ng/mL 5-dodecenoic acid 0.84 ± 2.1 μg/mL Adipic acid 88.6 ± 10.6 ng/mL α-linolenic acid 8.07 ± 9.95 μg/mL Aminoadipic acid 0.14 ± 0.04 μg/mL Arachidonic acid 3.83 ± 2.82 μg/mL Arachidonic acid 0.97 ± 0.88 μg/mL Docosanoic acid 0.33 ± 0.32 μg/mL β-alanine 0.41 ± 0.12 μg/mL Butyric acid 0.88 ± 0.69 μg/mL Capric acid 0.13 ± 0.22 μg/mL Caproic acid 0.53 ± 0.53 μg/mL Caprylic acid 0.37 ± 0.55 μg/mL Citraconic acid 45.8 ± 4.97 ng/mL 2-methylmalic acid 0.18 ± 0.07 μg/mL Citric acid 0.34 ± 0.32 μg/mL D-2-hydroxyglutaric acid 0.14 ± 0.06 μg/mL Docosahexaenoic acid 0.43 ± 0.3 μg/mL Docosapentaenoic acid n6 0.45 ± 0.41 μg/mL Docosatrienoic acid 0.16 ± 0.02 μg/mL Dodecanoic acid 1.07 ± 2.33 μg/mL Eicosenoic acid 8.91 ± 6.15 μg/mL Erucic acid 0.41 ± 0.36 μg/mL Ethylmethylacetic acid 0.73 ± 0.38 μg/mL Fumaric acid 0.12 ± 0.04 μg/mL γ-aminobutyric acid 0.81 ± 0.37 μg/mL Glutaric acid 0.32 ± 0.27 μg/mL Glutathione 3.33 ± 5.08 μg/mL Glyceric acid 4.11 ± 2.07 μg/mL Glycolic acid 0.27 ± 0.21 μg/mL Heptadecanoic acid 1.06 ± 1.15 μg/mL Heptanoic acid 0.12 ± 0.15 μg/mL Homocysteine l.l ± 1.01 μg/mL Hydrocinnamic acid 0.36 ± 0.21 μg/mL Hydroxyphenyllactic acid 0.48 ± 0.27 μg/mL Hydroxypropionic acid 0.17 ± 0.2 μg/mL Indole 0.4 ± 0.34 μg/mL Indoleacetic acid 0.29 ± 0.02 μg/mL Isocitric acid 89.5 ± 5.67 ng/mL Itaconic acid 92.4 ± 15.4 ng/mL L-α-aminobutyric acid 74.7 ± 16.7 ng/mL L-asparagine 0.88 ± 0.59 μg/mL L-aspartic acid 2.44 ± 1.03 μg/mL L-glutamic acid 10.35 ± 8.3 μg/mL L-histidine 0.39 ± 0.07 μg/mL L-homoserine 0.45 ± 0.42 μg/mL L-isoleucine 0.23 ± 0.11 μg/mL L-leucine 0.25 ± 0.42 μg/mL L-lysine 2.29 ± 0.65 μg/mL L-methionine 1.54 ± 0.91 μg/mL L-norleucine 0.13 ± 0.25 μg/mL L-phenylalanine 0.85 ± 0.91 μg/mL L-proline 0.4 ± 0.21 μg/mL L-serine 0.42 ± 0.31 μg/mL L-tryptophan 0.62 ± 0.16 μg/mL L-tyrosine 2.19 ± 1.06 μg/mL L-valine 1.05 ± 0.6 μg/mL Linoleic acid 0.012 ± 0.05 mg/mL Malic acid 0.38 ± 0.21 μg/mL Malonic acid 0.12 ± 0.06 μg/mL Methylsuccinic acid 0.13 ± 0.02 μg/mL Myristic acid 0.54 ± 0.54 μg/mL Myristic acid 0.53 ± 0.53 μg/mL N-acetyl 0.61 ± 0.07 μg/mL Nervonic acid 1.33 ± 0.9 μg/mL Nicotinic acid 0.31 ± 0.09 μg/mL Dodecanoic acid 0.21 ± 0.11 μg/mL Norvaline 29.5 ± 4.56 ng/mL Ornithine 1.29 ± 1.12 μg/mL Oxalic acid 3.49 ± 5.07 μg/mL Carbonyladipic acid 0.72 ± 0.41 μg/mL Oxoglutaric acid 0.17 ± 0.17 μg/mL Palmitic acid 3.79 ± 2.29 μg/mL Palmitoleic acid 3.69 ± 2.19 μg/mL Nonanoic acid 87.4 ± 12.5 ng/mL Pentadecanoic acid 0.31 ± 0.24 μg/mL Phenol 58.7 ± 6.78 ng/mL Phenylacetic acid 1.49 ± 1.14 μg/mL Phenyllactic acid 71.5 ± 8.79 ng/mL Pimelic acid 0.31 ± 0.22 μg/mL Propionic acid 10.09 ± 6.03 μg/mL Pyroglutamic acid 2.6 ± 3.95 μg/mL Stearic acid 1.22 ± 1.63 μg/mL Suberic acid 0.11 ± 0.03 μg/mL Succinic acid 0.42 ± 0.32 μg/mL Tartaric acid 80.3 ± 9.97 ng/mL Thiamine 2.23 ± 4.06 μg/mL Valeric acid 1.39 ± 1.36 μg/mL Vanillic acid 0.17 ± 0.04 μg/mL Cis-aconitic acid 70.05 ± 10.08 ng/mL P-cresol 0.24 ± 0.19 μg/mL P-hydroxyphenylacetic acid 0.3 ± 0.22 μg/mL

Example 2

[0077] A metabolic chip is used to detect multiple index components in 6 human blood and fecal samples.

1. Reagent Preparation

[0078] Preparation of standard product solution: Standard products, including amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid, of metabolites in the description are taken, fully dissolved in methanol and uniformly mixed to prepare a 1 mg/ml solution, namely a concentrated stock solution which is prepared into a series of concentrations of solutions to draw a standard curve.

[0079] Preparation of derivatization reagent (3-nitrophenylhydrazine): A derivatization reagent is uniformly mixed with a 75% methanol aqueous solution to prepare a 200 mM solution, and the solution is sealed and stored at 4° C. for later use.

[0080] Preparation of derivatization reaction catalyst (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide): A reaction catalyst is prepared into a 120 millimolar solution with pyridine, and the solution is sealed and stored at 4° C. for later use.

2. Sample Preparation

[0081] Preparation of serum extraction: An aliquot of standard solution or 20 μL of serum was mixed with 120 μL of methanol. Centrifugation at 13,500 g and 4° C. for 10 min.

[0082] Preparation of feces extraction: Feces samples were freeze-dried, and approximately 5 mg of each sample was weighed. The samples were homogenized with 20 μL of ultrapure water and extracted with 120 μL of methanol. Centrifugation at 13,500 g and 4° C. for 10 min. L of a treated biological supernatant is taken, 20 μl of a derivatization reagent and 20 μl of a derivatization catalyst are added for reaction at 30° C. for 60 minutes, a methanol-water mixed solution is added into a reaction solution for dilution, centrifugation is performed at 13200 rpm for 15 minutes, and 5 μL of a supernatant is taken and introduced for LC-MS/MS analysis.

3. Inspection Method

UHPLC/Q-Orbitrap-MS Analysis

[0083] High-resolution mass spectrometry (HRMS) data for multicomponent characterization and untargeted metabolomics were acquired using a Vanquish Flex UHPLC system coupled to a Q Extractive Focus Orbitrap mass spectrometer equipped with a heated electrospray ionization (ESI) source (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation was performed on a reversed-phase ACQUITY UPLC BEH C18 column (2.1 mm×100 mm, 1.7 m particle size) using a binary gradient elution with mobile phases: 0.1% formic acid in water (solvent A) and acetonitrile containing 0.1% formic acid/isopropanol (70:30, v/v, solvent B). The column temperature is 40° C. The flow rate is 0.4 mL/min; the injection volume is 5 microliters. The gradient program was as follows: 0-1 min (5% B), 1-5 min (5-30% B), 5-9 min (30-50% B), 9-12 min (50-75% B), 12-15 min (75-95% B), 15-16 min (95-100% B), 16-18 min (100% B), 18-20 min (5% B).

[0084] Mass Spectrometry Parameters: The mass spectrometry scan parameters were set as follows: S-lens RF level, 50; mass range, 100 to 1200 m/z; full MS resolution, 70,000; MS/MS resolution, 17,500; NCE/stepped NCE was set at 10, 20, and 40 eV. The ESI ion source parameters were set as follows: spray voltage, 3800 V; sheath gas flow rate, 40 for ESI+ and 5 for ESI−; capillary temperature, 320° C.; probe heater temperature, 350° C.; aux gas flow rate, 10.

4. Determination Results

[0085] Concentrations of substances in 6 human serum and fecal samples are detected by using the detection method of the present invention (Table 3). It can be seen that by using the method of the present invention to detect a single sample, multiple substances of different magnitudes and different properties can be quantitatively detected at one time.

TABLE-US-00003 TABLE 3 Determination results of concentrations of substances in blood and feces of normal people RT Reference Fecal Serum NO. Name m/z (min) Ion (Intensity) (Intensity) 1 Propynoic acid 264.06 6.246 [M − H + HAc] − 1 2510269 ± 841952 ± 92554 28455 2 Acrylic acid 208.07 0.883 [M + H] + 1 13865154 ± 14228426 ± 12993928 23777442 3 Glyoxylic acid 208.04 5.832 [M − H] − 1 12000471 ± 2144384 ± 2000381 228813 4 Isocrotonic acid 239.11 0.434 [M + NH4] + 1 266059225 ± 218886 ± 460019188 43040 5 2-Aminoacrylic acid 221.07 6.247 [M − H] − 1 3464018 ± 6160226 ± 800230 1903288 6 Malonic semialdehyde 222.05 9.361 [M − H] − 1 8953281 ± 1962295 ± 3833020 1202549 7 2-hydroxyacrylic Acid 222.05 7.364 [M − H] − 1 40949997 ± 9472056 ± 4052812 1132094 8 (alpha-D-mannosyl)7-beta-D-mannosyl- 223.08 1.516 [M − H] − 1 458487665 ± 148636884 ± diacetylchitobiosyl-L-asparagine, 63646212 13164304 isoform A (protein) 9 Monoethyl carbonate 224.07 4.177 [M − H] − 1 246941092 ± 71459981 ± 7837139 3414768 10 Methoxyacetic acid 224.07 3.726 [M − H] − 1 5184592776 ± 2172177007 ± 72558533 122437118 11 2-hydroxypropanoic acid 224.07 5.824 [M − H] − 1 157271490 ± 8346423 ± 13475327 299209 12 Senecioic acid 236.10 2.004 [M + H] + 1 76683919 ± 10848175 ±

56264980 10483810 13 Tiglic acid 253.13 0.6 [M + NH4] + 1 1509117642 ± 58052130 ± 2611743446 35789141 14 2-Ethylacrylic acid 253.13 1.254 [M + NH4] + 1 1862909805 ± 2059982 ± 1650227504 695343 15 Angelic acid 253.13 1.784 [M + NH4] + 1 50362708 ± 9238657 ± 86502238 4771737 16 2-Ketobutyric acid 236.07 7.196 [M − H] − 1 1787233 ± 3482001 ± 91095 1733281 17 Succinic acid semialdehyde 236.07 4.368 [M − H] − 1 16202483 ± 4515125 ± 4170048 168205 18 4-Hydroxycrotonic acid 236.07 8.057 [M − H] − 1 142453769 ± 31505827 ± 11877298 3548011 19 (S)-2-Methylbutanoic acid 236.10 6.982 [M − H] − 1 3463810327 ± 7900237 ± 108796706 1585336 20 Pivalic acid 236.10 7.443 [M − H] − 1 6972066992 ± 8738812 ± 105310392 1653469 21 Pyruvatoxime 237.06 2.504 [M − H] − 1 5796134 ± 248976 ± 360009 125681 22 (R)-beta-Aminoisobutyric acid 237.10 3.625 [M − H] − 1 378160 ± 176939 ± 31006 18988 23 N-Ethylglycine 237.10 2.174 [M − H] − 1 12053298 ± 812656 ± 1665099 97973 24 N-Methylalanine 237.10 2.6 [M − H] − 1 7789054 ± 2567964 ± 1095566 546229 25 L-alpha-Aminobutyric acid 237.10 1.67 [M − H] − 1 49720914 ± 3302799 ± 41479142 417895 26 Tartronate semialdehyde 238.05 4.388 [M − H] − 1 3874452 ± 1242695 ± 286591 81426 27 3-Hydroxybutyric acid 238.08 4.952 [M − H] − 1 301806216 ± 103894796 ± 5243093 15933411 28 (S)-3-Hydroxyisobutyric acid 238.08 4.035 [M − H] − 1 102000710 ± 198892671 ± 2677646 6454186 29 (R)-3-Hydroxyisobutyric acid 238.08 4.434 [M − H] − 1 5691133 ± 8508157 ± 4272373 1139426 30 4-Carboxypyrazole 306.08 2.842 [M − H + HAc] − 1 4643029 ± 54817 ± 487793 2815 31 (2E,4E)-2,4-Hexadienoic acid 248.10 1.793 [M + H] + 1 440676 ± 353192 ± 407639 230407 32 1-Pyrroline-2-carboxylic acid 247.08 5.843 [M − H] − 1 510503052 ± 726932 ± 6210566 150549 33 cis-Acetylacrylate 248.07 7.356 [M − H] − 1 474385 ± 15651520 ± 176040 2408852 34 L-3-Cyanoalanine 248.07 9.49 [M − H] − 1 1059622 ± 5370099 ± 22694 434557 35 4-Amino-2-methylenebutanoic acid 249.10 2.188 [M − H] − 1 87350177 ± 56666811 ± 13490729 6935788 36 Levulinic acid 250.08 6.464 [M − H] − 1 178720991 ± 176955 ± 147448038 11387 37 2-Oxovaleric acid 250.08 9.01 [M − H] − 1 9445509 ± 945025 ± 2098125 61720 38 2-Methylacetoacetic acid 250.08 7.484 [M − H] − 1 7951379 ± 8911936 ± 880664 4062180 39 2-Ethylbutanoic acid 250.12 9.023 [M − H] − 1 12672521 ± 4951315 ± 4077090 786439 40 L-2-Amino-3-oxobutanoic acid 253.09 1.836 [M + H] + 1 73838201 ± 320289 ± 63551844 140244 41 L-Aspartate-semialdehyde 251.08 3.026 [M − H] − 1 436917309 ± 4211220 ± 36351677 762056 42 N-Methyl-a-aminoisobutyric acid 251.11 1.92 [M − H] − 1 121887846 ± 61259 ± 11507581 3837 43 5-Aminopentanoic acid 253.13 0.668 [M + H] + 1 3918736 ± 25332320 ± 6415609 20914439 44 4-Hydroxy-2-oxobutanoic acid 252.06 4.852 [M − H] − 1 27476587 ± 343028 ± 1089113 141601 45 xi-3-Hydroxy-2-oxobutanoic acid 312.08 3.66 [M − H + HAc] − 1 29764174 ± 77247104 ± 6501749 9010510 46 3-methoxy-3-oxopropanoic acid 312.08 3.087 [M − H + HAc] − 1 10120248 ± 51191 ± 2417413 3026 47 Fatty acids and conjugates 254.11 2.641 [M + H] + 1 31771483 ± 215413 ± 19499829 173051 48 Fatty acids and conjugates 254.11 2.596 [M + H] + 1 34971729 ± 974769 ± 59722626 785010 49 Fatty acids and conjugates 252.10 6.113 [M − H] − 1 2254633676 ± 72759156 ± 58768592 8909091 50 Fatty acids and conjugates 252.10 4.507 [M − H] − 1 358893190 ± 618874 ± 5309460 77632 51 Fatty acids and conjugates 252.10 5.158 [M − H] − 1 14144656 ± 3494450 ± 3243962 686593 52 Fatty acids and conjugates 252.10 4.92 [M − H] − 1 49093638 ± 7131098 ± 4340581 2311977 53 L-Allothreonine 253.09 1.592 [M − H] − 1 23010815 ± 3858495 ± 3438091 570302 54 3-Methylthiopropionic acid 254.06 6.098 [M − H] − 1 167272734 ± 122956 ± 7300773 12506 55 (S)-3,4-Dihydroxybutyric acid 254.08 2.666 [M − H] − 1 10373529 ± 2146916 ± 1019102 252088 56 2,4-Dihydroxybutanoic acid 254.08 3.154 [M − H] − 1 14557796 ± 1523900 ± 2164021 145535 57 4-Deoxyerythronic acid 254.08 2.641 [M − H] − 1 10369551 ± 2127538 ± 972695 272618 58 4-Deoxythreonic acid 254.08 2.92 [M − H] − 1 809070 ± 3564964 ± 292135 438578 59 A,b-Dihydroxyisobutyric acid 254.08 5.21 [M − H] − 1 23965796 ± 10407027 ± 543134 2942333 60 Isonicotinic acid 259.08 2.651 [M + H] + 1 102305718 ± 105725 ± 82584803 114332 61 5-Methylfuran-2-carboxylic acid 262.08 2.396 [M + H] + 1 188653836 ± 8356972 ± 132888236 9881604 62 Imidazoleacetic acid

262.08 1.284 [M + H] + 1 2506979 ± 69477 ± 1328936 14881 63 2-Heptenoic acid 262.12 8.472 [M − H] − 1 1240023698 ± 284926 ± 112948294 39986 64 1-Pyrroline-4-hydroxy-2-carboxylate 282.12 1.05 [M + NH4] + 1 33383990 ± 392110 ± 33487341 450883 65 Pyrroline hydroxycarboxylic acid 265.09 2.251 [M + H] + 1 384364073 ± 29327068 ± 43796349 48411341 66 (3R,5S)-1-pyrroline-3-hydroxy-5- 263.08 3.694 [M − H] − 1 3855850257 ± 714755282 ± carboxylic Acid 223920707 102960754 67 Vigabatrin 265.13 1.761 [M + H] + 1 37085635 ± 38956215 ± 29307615 33732957 68 Methyl hydrogen fumarate 264.06 4.297 [M − H] − 1 94878164 ± 1018471 ± 6244568 65304 69 2-Hydroxyglutaric acid lactone 264.10 11.6 [M − H] − 1 25655400 ± 22813723 ± 17304663 6117694 70 Adipate semialdehyde 264.10 10.037 [M − H] − 1 20240698 ± 4271382 ± 888382 220403 71 4-Acetylbutyrate 264.10 8.366 [M − H] − 1 1595053 ± 3000545 ± 102771 823663 72 2-Methyl-3-ketovaleric acid 264.10 7.226 [M − H] − 1 2291298 ± 1838653 ± 171975 443476 73 2-Ketohexanoic acid 264.14 9.335 [M − H] − 1 1911246 ± 328137 ± 82034 170919 74 3-Oxohexanoic acid 264.14 9.786 [M − H] − 1 11778657 ± 6661033 ± 265936 1148748 75 2,5-Dioxopentanoate 264.14 9.499 [M − H] − 1 2791835 ± 590708 ± 847837 306700 76 L-Glutamic gamma-semialdehyde 265.09 3.779 [M − H] − 1 1534024418 ± 2205185 ± 48867745 1409406 77 3-Hydroxy-L-proline 265.09 1.517 [M − H] − 1 5432848 ± 14927015 ± 611545 2122487 78 5-Amino-2-oxopentanoic acid 265.09 4.176 [M − H] − 1 593323230 ± 361362 ± 14981948 32619 79 Beta-Guanidinopropionic acid 267.12 0.553 [M + H] + 1 35379585 ± 10444853 ± 58571246 7341921 80 N-methylvaline 265.13 4.995 [M − H] − 1 224420851 ± 58040577 ± 18191555 9624895 81 L-Alloisoleucine 265.13 4.809 [M − H] − 1 29819470 ± 3491846 ± 542615 741341 82 Beta-Leucine 325.15 1.786 [M − H + HAc] − 1 16061225 ± 223094 ± 2695564 69164 83 (S)-Methylbutanethioic acid 268.09 2.947 [M + H] + 1 78649134 ± 50836 ± 12298198 18687 84 2-Hydroxy-4-oxopentanoic acid 268.09 3.054 [M + H] + 1 12626453 ± 12529 ± 1716166 469 85 2-Acetolactate 266.08 5.355 [M − H] − 1 498744073 ± 1437981 ± 31880606 311915 86 N-Carbamoylsarcosine 266.11 7.145 [M − H] − 1 6865075012 ± 3705564 ± 289785550 2438069 87 5-Hydroxyhexanoic acid 266.11 5.321 [M − H] − 1 16277930 ± 262350 ± 290543 129908 88 2-Ethyl-2-Hydroxybutyric acid 266.11 7.407 [M − H] − 1 272231157 ± 546354 ± 18134695 366058 89 L-2-Amino-5-hydroxypentanoic acid 267.11 1.416 [M − H] − 1 1698430 ± 90915 ± 352622 8586 90 2,3-Dihydroxyvaleric acid 268.09 5.419 [M − H] − 1 1061686411 ± 104712 ± 20065806 24588 91 2-Methylbenzoic acid 270.09 7.638 [M − H] − 1 2676986841 ± 10257108 ± 122859059 1274107 92 M-toluic Acid 270.09 10.502 [M − H] − 1 8083724 ± 5066654 ± 500455 742473 93 2-Pyridylacetic acid 271.08 6.732 [M − H] − 1 515650 ± 54619 ± 130925 36348 94 2-Aminonicotinic acid 274.09 1.901 [M + H] + 1 353328922 ± 2232782 ± 301069464 1384730 95 Trans-urocanate 274.09 1.327 [M + H] + 1 97073030 ± 1579413 ± 166209235 861675 96 Methylimidazoleacetic acid 276.11 1.208 [M + H] + 1 299197480 ± 10759423 ± 289848389 961891 97 Pi-Methylimidazoleacetic acid 276.11 0.581 [M + H] + 1 165875087 ± 1786900 ± 286015596 899069 98 2-Propyl-2,4-pentadienoic acid 274.09 1.884 [M − H] − 1 115357069 ± 508197 ± 12378801 352127 99 Arecaidine 275.11 8.359 [M − H] − 1 95267782 ± 24186 ± 6940621 626 100 L-Hypoglycin A 335.14 8.481 [M − H + HAc] − 1 10331411 ± 32943 ± 597912 1634 101 2-Octenoic acid 276.14 9.801 [M − H] − 1 9276052 ± 4416796 ± 374158 736373 102 6-Oxopiperidine-2-carboxylic acid 277.09 6.043 [M − H] − 1 38049394 ± 367162 ± 1546962 53838 103 L-2-Amino-3-methylenehexanoic acid 279.14 2.665 [M + H] + 1 10544073 ± 630369 ± 2839726 1006667 104 3-Hydroxyadipic acid 3,6-lactone 297.12 2.587 [M + NH4] + 1 21236735 ± 78280 ± 21931240 103829 105 4-Hydroxycyclohexylcarboxylic acid 278.11 4.88 [M − H] − 1 157503606 ± 183149 ± 4691297 40988 106 2-Methylheptanoic acid 278.15 10.759 [M − H] − 1 20338229 ± 20473965 ± 1864306 2435501 107 Allysine 279.11 1.108 [M − H] − 1 2807182 ± 314493 ± 696717 20222 108 L-trans-5-Hydroxy-2- 279.11 3.341 [M − H] − 1 1423756 ± 37975 ± piperidinecarboxylic acid 268610 2412 109 N-(2-Carboxymethyl)-morpholine 279.11 4.728 [M − H] − 1 1095546044 ± 407933 ± 71038961 163145 110 N-Propionylalanine 279.11 5.296 [M − H] − 1 497524956 ± 48065 ± 29571905 8053 111 4-Guanidinobutanoic acid 279.12 2.486 [M − H] − 1

1581679 ± 88408 ± 213311 5947 112 2-Aminoheptanoate 281.16 1.415 [M + H] + 1 27567023 ± 178365 ± 23590026 42513 113 Monomethyl glutaric acid 282.11 3.182 [M + H] + 1 26040569 ± 389393 ± 6174601 14572 114 (3S)-3,6-Diaminohexanoate 280.14 0.785 [M − H] − 1 14874828 ± 1136438 ± 3770143 221340 115 (3S,5S)-3,5-Diaminohexanoate 282.16 0.271 [M + H] + 1 3320125 ± 17033 ± 5722971 943 116 L-4-Hydroxyglutamate semialdehyde 283.10 1.575 [M + H] + 1 87944654 ± 8731200 ± 75853946 12166455 117 O-Acetylserine 283.10 1.075 [M + H] + 1 157295107 ± 14428675 ± 269629227 11991693 118 (2R,3R,4R)-2-Amino-4-hydroxy-3- 341.15 1.511 [M − H + HAc] − 1 2434963 ± 12879539 ± methylpentanoic acid 635237 3090466 119 3-phenylprop-2-enoic acid 284.10 3.829 [M + H] + 1 356811 ± 69236 ± 97593 13797 120 trans-Cinnamic acid 282.09 8.72 [M − H] − 1 674959 ± 229561 ± 103340 287646 121 Penicillamine 283.09 4.195 [M − H] − 1 150845352 ± 7917189 ± 705082 696903 122 (\xc2\xb1)-2-Hydroxy-4- 284.07 6.022 [M − H] − 1 251478688 ± 101178 ± (methylthio)butanoic acid 25531414 33234 123 alpha-Fluoro-beta-ureidopropionic acid 286.10 2.635 [M + H] + 1 114904443 ± 41101 ± 68266446 24897 124 2-Deoxypentonic acid 286.10 2.664 [M + H] + 1 43720321 ± 112407 ± 29766910 13529 125 2-Deoxyribonic acid 286.10 2.589 [M + H] + 1 63405399 ± 19779 ± 88251992 4806 126 3-hydroxy-2,2- 284.09 2.71 [M − H] − 1 14187916 ± 298762 ± bis(hydroxymethyl)propanoic acid 2142313 101073 127 3,4-Dimethylbenzoic acid 284.10 8.475 [M − H] − 1 1373693518 ± 4377638 ± 133937468 830103 128 4-Ethylbenzoic acid 285.10 4.733 [M − H] − 1 391105 ± 61527 ± 6989 46564 129 3-Cresotinic acid 286.08 6.152 [M − H] − 1 76223888 ± 214577 ± 2500612 139919 130 4-Hydroxy-3-methylbenzoic acid 286.08 5.692 [M − H] − 1 117011496 ± 1211709 ± 1446748 282448 131 2-Methoxybenzoic acid 286.08 6.66 [M − H] − 1 21130041 ± 603276 ± 921451 485961 132 L-2-Amino-3-(1-pyrazolyl)propanoic 289.11 0.634 [M − H] − 1 17310723 ± 15429847 ± acid 2509618 3088778 133 2-Nonenoic acid 290.15 11.431 [M − H] − 1 938653 ± 16719871 ± 192209 3136814 134 Tranexamic Acid 292.17 11.374 [M − H] − 1 17643544 ± 33307648 ± 2945163 1609740 135 Medicanine 295.14 3.02 [M + H] + 1 114383667 ± 27010 ± 2429148 24846 136 Mono-methyl-adipate 294.11 4.03 [M − H] − 1 164606166 ± 345531 ± 10744545 28815 137 2-Indolecarboxylic acid 297.12 2.517 [M + H] + 1 46182768 ± 731536 ± 3188500 280725 138 trans-S-(1-Propenyl)-L-cysteine 295.10 4.136 [M − H] − 1 139677933 ± 864747 ± 6290835 119490 139 Glutamic acid gamma-methyl ester 295.10 4.243 [M − H] − 1 136680935 ± 23031084 ± 6256752 1842721 140 hydroxybutyrylglycine 295.10 2.966 [M − H] − 1 11551708 ± 215419 ± 2218830 45852 141 Acetylhomoserine 295.10 3.436 [M − H] − 1 15606445 ± 374548 ± 1140507 73028 142 4-Hydroxycinnamic acid 298.08 7.291 [M − H] − 1 405911 ± 1277594 ± 6763 433397 143 Enol-phenylpyruvate 298.08 9.173 [M − H] − 1 1357110 ± 69868 ± 607861 80016 144 2-Phenylbutyric acid 300.13 4.103 [M + H] + 1 39695 ± 17431 ± 944 1723 145 2-methyl-3-phenylpropanoic acid 298.12 9.068 [M − H] − 1 461003 ± 191382 ± 90219 197450 146 Ethiin 299.08 1.487 [M − H] − 1 15453907 ± 1797654 ± 4516907 326811 147 3-Pyridinebutanoic acid 299.11 5.575 [M − H] − 1 247479044 ± 63752454 ± 29561791 14251447 148 xe2\x80\x8bD-\xe2\x80\x8bXylonic 300.08 3.361 [M − H] − 1 2756231 ± 49636 ± acid 298648 3297 149 \xe2\x80\x8bL-\xe2\x80\x8bLyxonic acid 300.08 1.772 [M − H] − 1 104447225 ± 1779717 ± 15622140 282898 150 Arabinonic acid 300.08 2.748 [M − H] − 1 15623924 ± 104679 ± 3130934 5285 151 3-(3-Hydroxyphenyl)propanoic acid 302.11 3.318 [M + H] + 1 118424972 ± 39503 ± 28282143 3265 152 L-3-Phenyllactic acid 302.11 3.093 [M + H] + 1 264477683 ± 129535 ± 5890811 5343 153 4-Methoxyphenylacetic acid 300.10 6.158 [M − H] − 1 736422947 ± 241335 ± 9470657 318345 154 Desaminotyrosine 300.10 6.424 [M − H] − 1 169566041 ± 115990 ± 1708402 7464 155 3-(2-Hydroxyphenyl)propanoic acid 300.10 6.667 [M − H] − 1 1048006863 ± 96793 ± 65341318 24317 156 4-Hydroxyphenyl-2-propionic acid 300.10 7.58 [M − H] − 1 454523791 ± 1456452 ± 7092875 255258 157 2,4,7-Decatrienoic acid 300.14 10.717 [M − H] − 1 2917876 ± 23001139 ± 128098 1659257 158 3-Methoxyanthranilate 303.11 5.247 [M + H] + 1 602388 ± 38221145 ± 520287 5162239 159 3,5-Dihydroxyphenylacetic acid 302.08 4.991 [M − H] − 1 52800191 ± 115985 ± 718656 31839 160 3-hydroxy-5-methoxybenzoic acid 302.08 4.622 [M

− H] − 1 36256751 ± 240292 ± 2482980 137457 161 2-hydroxy-4-methoxybenzoic acid 302.08 8.429 [M − H] − 1 22207132 ± 86833441 ± 2765128 17088956 162 cis-4-Decenoic acid 304.17 11.425 [M − H] − 1 2370592 ± 21248227 ± 152654 574540 163 9-Oxo-nonanoic acid 308.16 4.817 [M + H] + 1 9520814 ± 33059 ± 3588394 14513 164 4-Methylnonanoic acid 306.18 11.823 [M − H] − 1 12847373 ± 14568161 ± 907376 1428350 165 Gly-Norvaline 310.15 1.318 [M + H] + 1 23644773 ± 167234 ± 16247984 23136 166 Argininic acid 311.15 0.344 [M + H] + 1 4320252 ± 281839 ± 6922250 320176 167 2-Keto-3-deoxy-D-gluconic acid 312.08 3.655 [M − H] − 1 29764174 ± 77247104 ± 6501749 9010510 168 4-Methoxycinnamic acid 312.10 10.662 [M − H] − 1 32116255 ± 232400 ± 2003232 98866 169 3-(4-Methylphenyl)oxiranecarboxylic 313.09 11.622 [M − H] − 1 2827472 ± 16637152 ± acid 199823 1965749 170 (Z)-10-Hydroxy-8-decene-4,6-diynoic 313.09 6.476 [M − H] − 1 30002271 ± 10315091 ± acid 138304 1734816 171 3-Deoxyarabinohexonic acid 316.11 2.405 [M + H] + 1 351310931 ± 6215669 ± 231028882 2120326 172 2-Methyl-3-hydroxy-5-formylpyridine-4- 315.07 8.2 [M − H] − 1 101162767 ± 26850547 ± carboxylate 9157274 1483653 173 3,4-Dimethoxybenzoic acid 316.09 5.064 [M − H] − 1 186522028 ± 120696 ± 1420536 33735 174 3,5-dimethoxybenzoic acid 316.09 5.412 [M − H] − 1 133874508 ± 2104614 ± 841918 674483 175 2-(3-hydroxy-5-methoxyphenyl)acetic 316.09 5.974 [M − H] − 1 30487593 ± 45370 ± acid 417392 21447 176 2-hydroxy-2-(3-methoxyphenyl)acetic 316.09 5.809 [M − H] − 1 6557547 ± 112869 ± acid 2121983 99540 177 2-hydroxy-2-(4-methoxyphenyl)acetic 316.09 5.491 [M − H] − 1 96542022 ± 110346 ± acid 65077753 87059 178 3-(sulfooxy)butanoic acid 318.04 1.911 [M − H] − 1 12219282 ± 61577 ± 4023568 3857 179 4-O-Methylgallic acid 318.07 7.051 [M − H] − 1 3039537 ± 38872274 ± 36750 5252002 180 (Z)-3-Methyl-4-decenoic acid 318.18 11.741 [M − H] − 1 2177468 ± 4713008 ± 206122 667305 181 2-Hepteneoylglycine 319.14 6.624 [M − H] − 1 107551942 ± 54158 ± 1217197 3408 182 Pyroglutamylglycine 320.10 3.259 [M − H] − 1 27875922 ± 16318665 ± 3809371 3069977 183 4,6-Dimethylnonanoic acid 320.20 12.19 [M − H] − 1 8577731 ± 2548002 ± 885997 2169931 184 N-Heptanoylglycine 323.17 3.735 [M + H] + 1 7592240 ± 10424 ± 194459 745 185 8-Amino-7-oxononanoic acid 323.17 3.75 [M + H] + 1 21001484 ± 10221 ± 153920 650 186 Glycyl-Isoleucine 322.15 3.35 [M − H] − 1 5635341 ± 52481 ± 1421294 30081 187 5-Hydroxyindoleacetic acid 325.09 6.749 [M − H] − 1 80976425 ± 47719 ± 4891662 3762 188 4-Anilino-4-oxobutanoic acid 327.11 6.873 [M − H] − 1 6392717 ± 235533 ± 140654 172660 189 3-Dehydro-L-gulonate 328.08 1.927 [M − H] − 1 19632441 ± 203936 ± 1938316 8229 190 Monoethyl phthalate 328.09 7.029 [M − H] − 1 14467813 ± 44235 ± 356071 2248 191 5-(4-hydroxyphenyl)pentanoic acid 328.13 8.105 [M − H] − 1 183963030 ± 40100 ± 15285663 3044 192 Leucodopachrome 331.10 3.228 [M + H] + 1 526698 ± 19969 ± 420159 961 193 Dopaquinone 329.09 7.028 [M − H] − 1 1725391 ± 94224 ± 581361 80698 194 L-Dopaquinone 329.09 5.186 [M − H] − 1 1919681 ± 117223 ± 65458 123659 195 Metyrosine 329.09 5.53 [M − H] − 1 1512896 ± 55444 ± 881048 30166 196 Gulonic acid 330.09 4.457 [M − H] − 1 1760105861 ± 8090776 ± 129866114 614995 197 Gluconic acid 330.09 1.694 [M − H] − 1 141707008 ± 13400913 ± 20621354 3618568 198 (S)-2-(4-Methoxyphenoxy)propanoic 332.12 3.182 [M + H] + 1 91293128 ± 448672 ± acid 2062863 16791 199 2-(3,5-dimethoxyphenyl)acetic acid 390.13 6.593 [M − H + HAc] − 1 88547292 ± 54152 ± 2053671 4997 200 (S)-Batatic acid 330.11 6.246 [M − H] − 1 231228281 ± 57698 ± 5392957 4298 201 L-alpha-Amino-1H-pyrrole-1-hexanoic 332.17 2.958 [M + H] + 1 19478437 ± 17172 ± acid 2667138 1147 202 2-methyl-4-(sulfooxy)butanoic acid 332.06 4.804 [M − H] − 1 83155581 ± 39828 ± 3838889 1554 203 5-(sulfooxy)pentanoic acid 332.06 4.37 [M − H] − 1 36907998 ± 67315 ± 1866491 1757 204 trans-Dodec-2-enoic acid 332.20 12.151 [M − H] − 1 3414780 ± 3556575 ± 701029 3855169 205 2-octenoylglycine 335.17 3.801 [M + H] + 1 3815752 ± 13985 ± 56993 831 206 3-octenoylglycine 335.17 3.763 [M + H] + 1 3247986 ± 16512 ± 95378 1154 207 N-Acetylaminooctanoic acid 335.17 8.436 [M − H] − 1 254623752 ± 124045 ± 38033276 7782 208 N-(5-Methyl-3-oxohexyl)alanine 335.17 8.352 [M − H] − 1 32078566 ± 31831 ± 869294 3475 209 R-2-Hydroxy-3-methylbutanoic acid 3- 336.16 6.468 [M − H] − 1 8555654 ±

117204 ± Methylbutanoyl 847309 11661 210 N-Acetylisoputreanine 338.18 3.003 [M + H] + 1 14410756 ± 12787 ± 680847 1274 211 D-Tryptophan 338.13 5.705 [M − H] − 1 22623825 ± 17866954 ± 3536393 3968802 212 Zeanic acid 339.07 7.299 [M − H] − 1 4030088 ± 92055 ± 355673 40593 213 3-Indolehydracrylic acid 339.11 7.3 [M − H] − 1 46977371 ± 2360537 ± 1342344 426453 214 5-Methoxyindoleacetate 339.11 6.338 [M − H] − 1 32803368 ± 31758 ± 1490625 1898 215 N-Propionylmethionine 341.13 3.291 [M + H] + 1 3791811 ± 11764 ± 435019 981 216 2-Amino-4-hydroxy-6- 343.10 2.643 [M + H] + 1 13643172 ± 73043 ± pteridinecarboxylic acid 11605388 89828 217 4-(2-Aminophenyl)-2,4-dioxobutanoic 343.10 3.032 [M + H] + 1 4926716 ± 14291 ± acid 185015 2885 218 triazolopropionic acid 341.10 9.072 [M − H] − 1 2178392 ± 84917 ± 1048846 6258 219 6-Amino-9H-purine-9-propanoic acid 401.13 4.9 [M − H + HAc] − 1 175959193 ± 256652 ± 1 9078502 19357 220 3-Phenylpropionylglycine 343.14 3.686 [M + H] + 1 47607214 ± 36444 ± 1077980 14447 221 Dihydrolipoate 402.12 5.421 [M − H + HAc] − 1 622208098 ± 38010336 ± 38919379 10167513 222 3-(3,4-Dimethoxyphenyl)-2- propenoic 344.12 2.918 [M + H] + 1 17638660 ± 123198 ± acid 859455 156293 223 3,4-Dihydroxyphenylvaleric acid 346.14 3.305 [M + H] + 1 25948519 ± 13913 ± 2294846 1062 224 2-Methoxy-3-(4- 344.13 6.59 [M − H] − 1 205245407 ± 52629 ± methoxyphenyl)propanoic acid 2715599 4856 225 Cucurbic acid 348.19 4.795 [M + H] + 1 15736700 ± 178444 ± 4166507 41304 226 Dihydrojasmonic acid 348.19 4.732 [M + H] + 1 18681975 ± 261157 ± 17465330 57845 227 3-hydroxy-2-methyl-2- 348.05 1.76 [M − H] − 1 112459094 ± 110962 ± [(sulfooxy)methyl]propanoic acid 15500812 5421 228 9-Methyldodecanoic acid 348.23 12.844 [M − H] − 1 277094 ± 2426122 ± 225202 1251444 229 2-hydroxyoct-2-enoylglycine 349.15 10.059 [M − H] − 1 32712097 ± 95923 ± 966406 8313 230 xi-5-Hydroxydodecanoic acid 350.21 11.513 [M − H] − 1 9600303 ± 501983 ± 233980 83437 231 Leucyl-Serine 352.16 5.02 [M − H] − 1 35981421 ± 89528 ± 1015854 24768 232 (2Z)-2-(phenylmethylidene)heptanoic 352.16 5.233 [M − H] − 1 26862526 ± 69676 ± acid 899769 29103 233 Glycyl-Phenylalanine 358.15 3.029 [M + H] + 1 6341464 ± 9407 ± 2542718 1899 234 4-(2-Amino-3-hydroxyphenyl)-2,4- 357.08 3.61 [M − H] − 1 19369899 ± 127600 ± dioxobutanoic acid 1809789 17641 235 Salsolinol 1-carboxylate 357.12 5.318 [M − H] − 1 160824385 ± 77612 ± 5190562 63646 236 2-hydroxyphenylpropionylglycine 357.12 9.578 [M − H] − 1 36220356 ± 72015 ± 8157783 29263 237 Goshuyic acid 358.21 12.391 [M − H] − 1 4858876 ± 5590352 ± 242757 3093250 238 3-(3,4-dihydroxy-5- 360.08 1.919 [M − H] − 1 440578 ± 12277 ± methoxyphenyl)oxirane-2-carboxylic 173758 1054 acid 239 2,3,4,5,6,7-Hexahydroxyheptanoic acid 360.10 4.269 [M − H] − 1 371551509 ± 70261443 ± 26033213 7432650 240 5-(3,4,5-trihydroxyphenyl)pentanoic acid 362.13 1.317 [M + H] + 1 281456 ± 18467 ± 413594 4098 241 5-(3,5-dihydroxyphenyl)-4- 362.13 2.919 [M + H] + 1 106945309 ± 124907 ± hydroxypentanoic acid 3957680 158460 242 b“4-Hydroxy-5-(3,5-dihydroxyphenyl)- 360.12 5.279 [M − H] − 1 287090966 ± 26714 ± valeric acid” 16864207 629 243 b“4-Hydroxy-(3,4-dihydroxyphenyl)- 360.12 5.514 [M − H] − 1 60249031 ± 30572 ± valeric acid” 1804785 1395 244 5-Tetradecenoic acid 360.23 12.667 [M − H] − 1 7452323 ± 5224125 ± 1568864 377259 245 5Z-Tetradecenoic acid 360.23 12.829 [M − H] − 1 496992 ± 3050486 ± 395093 1241632 246 Tsuzuic acid 360.23 12.75 [M − H] − 1 1284972 ± 5741761 ± 605943 1385457 247 Mevalonic acid-5P 362.08 3.137 [M − H] − 1 1116119 ± 35967 ± 860148 2409 248 2-hydroxy-3,4,5-trimethoxybenzoic acid 362.10 5.061 [M − H] − 1 75884655 ± 117575 ± 1386412 32863 249 12-Methyltridecanoic acid 362.24 13.168 [M − H] − 1 73882862 ± 67741975 ± 24830050 11433835 250 2,6,10-Trimethylundecanoic acid 362.25 13.078 [M − H] − 1 19542478 ± 407767 ± 6376340 77424 251 Glycyl-Arginine 365.18 8.09 [M − H] − 1 2358321 ± 36761 ± 81605 1927 252 Salsoline-1-carboxylate 373.15 3.047 [M + H] + 1 6362589 ± 13105 ± 1683811 5140 253 3-(3,4,5-Trimethoxyphenyl)propanoic 374.14 8.104 [M − H] − 1 48924645 ± 38687 ± acid 4581942 2937 254 2-Carboxy-4-dodecanolide 376.19 11.704 [M − H] − 1 9322395 ± 131970 ± 531703 6679 255 13-Methylmyristic acid 376.26 13.381 [M − H] − 1 223486451 ± 1364882 ± 76004756 279573 256 beta-Alanyl-L-arginine 379.20 9.022 [M − H] − 1 6000099 ± 100267 ± 441874 4839 257 3-[3-

(Sulfooxy)phenyl]propanoic acid 380.06 5.557 [M − H] − 1 277496418 ± 88361 ± 5803661 18966 258 3-[4-(sulfooxy)phenyl]propanoic acid 380.06 5.146 [M − H] − 1 155334437 ± 51223 ± 4568783 1624 259 Dihydrowyerone acid 382.15 3.675 [M + H] + 1 18988081 ± 43576 ± 5619119 4298 260 Hydroxynalidixic acid 382.12 9.365 [M − H] − 1 1644804 ± 3354488 ± 59028 457929 261 2-{[hydroxy(4-methoxy-1-benzofuran-5- 383.10 10.939 [M − H] − 1 6323472 ± 118488 ± yl)methylidene]amino}acetic acid 14619 20811 262 Prolyl-Histidine 386.16 5.532 [M − H] − 1 3446983 ± 54918 ± 205990 2734 263 7Z,10Z-Hexadecadienoic acid 386.25 12.789 [M − H] − 1 17375911 ± 2254613 ± 6513568 385728 264 Hypogeic acid 388.26 13.372 [M − H] − 1 2049467 ± 3205581 ± 1062478 4187612 265 3-hydroxy-3-(3,4,5- 390.13 6.59 [M − H] − 1 88547292 ± 53525 ± trimethoxyphenyl)propanoic acid 2053671 4939 266 Isopalmitic acid 390.28 14.227 [M − H] − 1 798566 ± 6387985 ± 908460 970468 267 Homovanillic acid sulfate 396.05 4.572 [M − H] − 1 49914729 ± 240546 ± 1799733 29755 268 Dihydrocaffeic acid 3-sulfate 396.05 5.158 [M − H] − 1 9903409 ± 44531 ± 585484 1412 269 Cinoxacin 396.10 5.28 [M − H] − 1 144214615 ± 28628 ± 17603505 1112 270 Neuraminic acid 401.13 4.405 [M − H] − 1 895204947 ± 756226 ± 22167360 218967 271 N-(1-Deoxy-1-fructosyl)serine 401.13 4.742 [M − H] − 1 247932841 ± 205194 ± 4530101 152930 272 3-Deoxy-D-glycero-D-galacto-2- 404.13 2.649 [M + H] + 1 284073 ± 14630952 ± nonulosonic acid 225961 12664054 273 9E-Heptadecenoic acid 402.28 13.561 [M − H] − 1 6881694 ± 7020470 ± 2783189 639263 274 Cyclohexaneundecanoic acid 402.28 13.66 [M − H] − 1 11737860 ± 6261104 ± 4834761 07290 275 (S)-14-Methylhexadecanoic acid 404.29 14.132 [M − H] − 1 1727447 ± 5804610 ± 777515 1960928 276 16-Hydroxy hexadecanoic acid 406.27 13.431 [M − H] − 1 41800724 ± 6689669 ± 17205726 353350 277 (R)-3-Hydroxy-hexadecanoic acid 406.27 12.08 [M − H] − 1 143844245 ± 7884362 ± 16926271 2287029 278 3-Hydroxyhexadecanoic acid 406.27 13.173 [M − H] − 1 10222183 ± 2508112 ± 3406065 241374 279 5-Hydroxyhexadecanoic acid 406.27 11.916 [M − H] − 1 2615889 ± 12988902 ± 214887 3274590 280 7-Hydroxyhexadecanoic acid 406.27 12.695 [M − H] − 1 197513 ± 5452616 ± 66936 1190164 281 8-Hydroxyhexadecanoic acid 406.27 11.847 [M − H] − 1 2132446 ± 4991423 ± 223916 1237979 282 3,4,5-trihydroxy-6-[(oxolan-2- 414.15 4.637 [M + H] + 1 19217658 ± 90521 ± yl)methoxy]oxane-2-carboxylic acid 4411895 6965 283 3,4,5-trihydroxy-6-[(3- 412.14 9.422 [M − H] − 1 19450281 ± 58025 ± methylbutanoyl)oxy]oxane-2-carboxylic 943617 2795 acid 284 Calendic acid 414.27 8.649 [M + H] + 1 102647771 ± 5100486 ± 27477508 488517 285 Punicic acid 414.27 8.461 [M + H] + 1 48950252 ± 2630668 ± 19169432 1109662 286 Linolenelaidic acid 414.27 7.48 [M + H] + 1 3003039 ± 27593590 ± 923342 4955418 287 3,4,5-trihydroxy-6-[(3- 414.12 8.168 [M − H] − 1 706597767 ± 136155 ± hydroxybutanoyl)oxy]oxane-2- 24989472 54460 carboxylic acid 288 Tyrosyl-Valine 414.18 4.492 [M − H] − 1 1247190 ± 24904 ± 658172 4829 289 Bovinic acid 416.29 9.488 [M + H] + 1 562037780 ± 2307000 ± 83001976 798698 290 (9E,11E)-Octadecadienoic acid 416.29 9.822 [M + H] + 1 174914828 ± 3328836 ± 21737726 4605771 291 Dihomolinoleic acid 416.29 9.712 [M + H] + 1 488647177 ± 1620981 ± 80759240 160741 292 5-Octadecynoic acid 416.29 11.458 [M + H] + 1 5607810 ± 57252158 ± 3308500 8867798 293 6Z,9Z-octadecadienoic acid 416.29 13.896 [M + H] + 1 67451068 ± 665948 ± 30079106 344192 294 Octadecadienoate 416.29 9.615 [M + H] + 1 259619642 ± 1555275 ± 130161609 347600 295 (10E,12Z)-Octadecadienoic acid 414.28 13.498 [M − H] − 1 57038511 ± 33771010 ± 23912024 3873450 296 Mangiferic acid 414.28 13.414 [M − H] − 1 676271339 ± 824087609 ± 266834677 110539718 297 Elaidic acid 416.29 13.89 [M − H] − 1 345423297 ± 589882789 ± 155279070 97391985 298 Vaccenic acid 416.29 13.978 [M − H] − 1 123869005 ± 27280370 ± 54460008 5217242 299 16-Methylheptadecanoic acid 418.31 14.424 [M − H] − 1 10439852 ± 128642288 ± 3930146 34500866 300 xanthurenic acid 8-O-sulfate 419.03 10.048 [M − H] − 1 517087 ± 205207 ± 514753 88643 301 12-hydroxyheptadecanoic acid 420.29 12.39 [M − H] − 1 20657364 ± 171209 ± 2632544 72238 302 Orotidine 422.10 3.158 [M − H] − 1 8172693 ± 30250 ± 6969485 1926 303 Porric acid 422.10 5.508 [M − H] − 1 36755714 ± 67250 ± 3624668 15454 304 3,5-Dihydroxyphenylvaleric acid sulfate 424.08 5.899 [M − H] − 1 72314336 ± 47565

± 3330294 1244 305 3,4,5-trihydroxy-6-[(4-hydroxy-3- 445.16 3.859 [M + NH4] + 1 2047231 ± 96163 ± methylbut-2-enoyl)oxy]oxane-2- 27120 3734 carboxylic acid 306 4-Hydroxyproline galactoside 487.17 5.655 [M − H + HAc] − 1 131390623 ± 426038 ± 5723075 109801 307 17-Hydroxylinolenic acid 428.26 11.762 [M − H] − 1 2567010 ± 5148556 ± 1301141 134212 308 (Z)-13-Oxo-9-octadecenoic acid 430.27 12.372 [M − H] − 1 4527380 ± 8539429 ± 934039 1171168 309 Avenoleic acid 430.27 12.1 [M − H] − 1 29642015 ± 92203928 ± 2317532 33278090 310 12-Hydroxy-8,10-octadecadienoic acid 430.27 12.67 [M − H] − 1 916569 ± 6382616 ± 1164755 1198719 311 (10E,12Z)-9-HODE 430.27 12.474 [M − H] − 1 5124568 ± 11353262 ± 898704 4357069 312 13-HODE 430.27 12.167 [M − H] − 1 26291952 ± 77040779 ± 1780901 12269888 313 alpha-Dimorphecolic acid 430.27 11.929 [M − H] − 1 6069564 ± 21720795 ± 2188196 3055423 314 9,10-Epoxyoctadecenoic acid 430.27 11.858 [M − H] − 1 4797819 ± 11283123 ± 1032732 2319925 315 12,13-EpOME 430.27 12.875 [M − H] − 1 2267111 ± 4829389 ± 2281884 847226 316 Phenethylamine glucuronide 430.27 12.257 [M − H] − 1 9251186 ± 32181745 ± 315903 41126080 317 3-Oxooctadecanoic acid 432.29 12.823 [M − H] − 1 1572760 ± 2572358 ± 174555 511016 318 9-Oxooctadecanoic acid 432.29 12.469 [M − H] − 1 20085576 ± 11604607 ± 4030884 1765007 319 10-Oxooctadecanoic acid 432.29 12.339 [M − H] − 1 510669565 ± 9396774 ± 89374880 8198094 320 11-Oxooctadecanoic acid 432.29 12.277 [M − H] − 1 122641516 ± 22288090 ± 17057886 15318462 321 5-Hexyltetrahydro-2-furanoctanoic acid 432.29 12.883 [M − H] − 1 399730 ± 1123125 ± 64764 334858 322 (R)-3-Hydroxy-Octadecanoic acid 434.30 12.56 [M − H] − 1 1284577242 ± 7545156 ± 305296771 4668860 323 xi-10-Hydroxyoctadecanoic acid 434.30 12.695 [M − H] − 1 27938700 ± 2261349 ± 5715126 91408 324 Pyrogallol-2-O-glucuronide 436.10 11.189 [M − H] − 1 539805 ± 3253620 ± 160230 562979 325 6-(2,4-dihydroxyphenoxy)-3,4,5- 436.10 11.349 [M − H] − 1 2213769 ± 21151840 ± trihydroxyoxane-2-carboxylic acid 719244 2364104 326 6-(2,5-dihydroxyphenoxy)-3,4,5- 436.10 11.63 [M − H] − 1 2068500 ± 22519723 ± trihydroxyoxane-2-carboxylic acid 373176 1015590 327 6-(3,4-dihydroxyphenoxy)-3,4,5- 436.10 11.46 [M − H] − 1 3247448 ± 29391873 ± trihydroxyoxane-2-carboxylic acid 664244 2501473 328 Porric acid A 436.11 5.843 [M − H] − 1 92312578 ± 35725 ± 3795837 431 329 Cis-8,11,14,17-Eicosatetraenoic acid 440.29 11.266 [M + H] + 1 1733568 ± 53280633 ± 489736 41031354 330 2-(3,4-Dihydroxybenzoyloxy)-4,6- 440.07 13.6 [M − H] − 1 356155 ± 82829 ± dihydroxybenzoate 250378 2856 331 5-(3,4-dihydroxyphenyl)-4- 440.08 4.794 [M − H] − 1 176423351 ± 34249 ± (sulfooxy)pentanoic acid 2727594 1355 332 3,4-dihydroxy-2-(4-hydroxy-3,7- 440.18 0.955 [M − H] − 1 21206815 ± 535907 ± dimethylocta-2,6-dien-1-yl)benzoic acid 18068363 30832 333 3,4-dihydroxy-2-[(2Z)-4-hydroxy-3-(4- 440.18 0.91 [M − H] − 1 34747475 ± 1427681 ± methylpent-3-en-1-yl)but-2-en-1-7183172 727074 yl]benzoic acid 334 b“5-Carboxy-gamma-chromanol” 442.23 5.534 [M + H] + 1 39410744 ± 232039 ± 770524 10693 335 5,8,11-Eicosatrienoic acid 440.29 13.577 [M − H] − 1 10228924 ± 16089071 ± 5254138 2598430 336 Corchorifatty acid A 442.23 3.328 [M − H] − 1 39191074 ± 16582274 ± 8328846 2020810 337 Eicosadienoic acid 442.31 13.969 [M − H] − 1 1632999 ± 5330577 ± 1028883 825514 338 3,4,5-trihydroxy-6-{[3-hydroxy-2- 446.14 3.287 [M + H] + 1 14686973 ± 144711 ± (hydroxymethyl)-2- 385197 36494 methylpropanoyl]oxy}oxane-2-carboxylic acid 339 (R)-2-Hydroxysterculic acid 444.29 12.867 [M − H] − 1 2656442 ± 3269807 ± 1286306 212336 340 8(R)-Hydroperoxylinoleic acid 446.27 12.188 [M − H] − 1 12593976 ± 37783556 ± 1999137 11286390 341 (\xc2\xb1)-(E)-13-Hydroxy-10-oxo-11- 446.27 12.051 [M − H] − 1 2831245 ± 8969604 ± octadecenoic acid 905725 2221456 342 Beta-D-Glucopyranuronic acid 448.10 12.379 [M − H] − 1 317677 ± 8922364 ± 165203 674867 343 3,4,5-trihydroxy-6-(3-448.10 11.648 [M − H] − 1 422340 ± 10693456 ± hydroxybenzoyloxy)oxane-2-carboxylic 54500 5702913 acid 344 9,10-Epoxyoctadecanoic acid 448.28 11.641 [M − H] − 1 87004989 ± 1374409 ± 3973815 184155 345 12,13-DHOME 448.28 12.358 [M − H] − 1 1026380 ± 2297055 ± 608068 1494177 346 9,10-DHOME 448.28 12.418 [M − H] − 1 1898753 ± 3003796 ± 312906 1371028 347 (+)-15,16-Dihydroxyoctadecanoic acid 452.31 7.996 [M + H] + 1 182080968 ± 1005144 ±

49497922 477658 348 (9S,10S)-9,10-dihydroxyoctadecanoate 452.31 7.597 [M + H] + 1 33056391 ± 961508 ± 2714072 142134 349 Valproic acid glucuronide 456.20 3.676 [M + H] + 1 32799856 ± 123805 ± 3113321 7280 350 2-Hydroxy-6-tridecylbenzoic acid 454.27 12.179 [M − H] − 1 371798 ± 10320632 ± 232645 7951616 351 19(S)-HETE 454.27 12.35 [M − H] − 1 267167 ± 11165932 ± 38896 1027869 352 10-HETE 454.27 12.615 [M − H] − 1 203539 ± 41648687 ± 48719 2147758 353 13-HETE 454.27 12.428 [M − H] − 1 437565 ± 15692639 ± 96921 3729459 354 12 Hydroxy arachidonic acid 454.27 12.282 [M − H] − 1 222454 ± 11264070 ± 74787 2210161 355 12S-hydroxy-5E,8Z,10Z,14Z- 454.27 12.451 [M − H] − 1 100756 ± 4218983 ± eicosatetraenoic acid 17609 1545730 356 Lepidimoic acid 458.14 3.472 [M + H] + 1 24066028 ± 19662 ± 5739940 1735 357 3,4-Dimethyl-5-pentyl-2-furannonanoic 456.29 12.047 [M − H] − 1 23661841 ± 256504 ± acid 1984650 95569 358 Corchorifatty acid F 463.29 12.897 [M − H] − 1 8172175 ± 135246 ± 2165706 6453 359 9,12,13-TriHOME 464.28 9.733 [M − H] − 1 54386843 ± 592064 ± 3628022 27726 360 9,10,13-TriHOME 464.28 9.812 [M − H] − 1 111662018 ± 1929528 ± 7179078 597563 361 4,8,12,15,19-Docosapentaenoic acid 464.29 13.374 [M − H] − 1 3022443 ± 2386925 ± 1435794 484525 362 9,10,13-Trihydroxystearic acid 466.29 10.34 [M − H] − 1 15563703 ± 109258 ± 377280 6956 363 Prostaglandin B2 468.25 11.539 [M − H] − 1 10813788 ± 184849 ± 1406868 117650 364 3,4,5-trihydroxy-6-{[(2E)-3-(3- 474.12 12.01 [M − H] − 1 285412 ± 10033224 ± hydroxyphenyl)prop-2- 36657 561452 enoyl]oxy}oxane-2-carboxylic acid 365 3,4,5-trihydroxy-6-{[(2E)-3-(4- 474.12 11.83 [M − H] − 1 561451 ± 15328466 ± hydroxyphenyl)prop-2-154877 2978586 enoyl]oxy}oxane-2-carboxylic acid 366 3,4,5-trihydroxy-6-{[3-(3- 474.12 12.27 [M − H] − 1 230347 ± 4180602 ± hydroxyphenyl)prop-2- 28396 2422104 enoyl]oxy}oxane-2-carboxylic acid 367 3,4,5-trihydroxy-6-[3-(4- 478.18 3.776 [M + H] + 1 6144013 ± 8744 ± methoxyphenyl)propoxy]oxane-2- 619614 750 carboxylic acid 368 13-Hydroxy-9-methoxy-10-oxo-11- 476.28 12.356 [M − H] − 1 707597 ± 11725314 ± octadecenoic acid 99637 9946207 369 2-Hydroxy-6-pentadecylbenzoic acid 482.30 11.881 [M − H] − 1 12770057 ± 151740 ± 1591691 2572 370 4-Methylumbelliferone glucuronide 486.12 11.739 [M − H] − 1 749110 ± 27793207 ± 44634 946668 371 Cryptochlorogenic acid 488.13 11.943 [M − H] − 1 1338251 ± 52406067 ± 89179 6324506 372 5-Caffeoylquinic acid 488.13 11.81 [M − H] − 1 1001686 ± 47163602 ± 629003 2046485 373 3,4,5-trihydroxy-6-{[3-(3- 488.13 11.33 [M − H] − 1 764298 ± 65035070 ± methoxyphenyl)prop-2- 193256 4754398 enoyl]oxy}oxane-2-carboxylic acid 374 3,4,5-trihydroxy-6-{[(2E)-3-(3- 488.13 12.013 [M − H] − 1 358182 ± 37143083 ± methoxyphenyl)prop-2- 28055 645807 enoyl]oxy}oxane-2-carboxylic acid 375 3,4,5-trihydroxy-6-{[(2E)-2- 488.13 11.865 [M − H] − 1 3288892 ± 164304226 ± (hydroxymethyl)-3-phenylprop-2- 904240 19453671 enoyl]oxy}oxane-2-carboxylic acid 376 3,4,5-trihydroxy-6-{[(2E)-3-(3- 488.13 11.716 [M − H] − 1 5244846 ± 257754514 ± hydroxyphenyl)-2-methylprop-2- 1462206 29782660 enoyl]oxy}oxane-2-carboxylic acid 377 3,4,5-trihydroxy-6-{[(2E)-3-(4- 488.13 11.641 [M − H] − 1 1183413 ± 110937452 ± hydroxyphenyl)-2-methylprop-2- 344044 11980258 enoyl]oxy}oxane-2-carboxylic acid 378 3,4,5-trihydroxy-6-{[(2E)-3-(2- 488.13 11.572 [M − H] − 1 552018 ± 52501894 ± hydroxyphenyl)-2-methylprop-2- 248208 2188003 enoyl]oxy}oxane-2-carboxylic acid 379 3,4,5-trihydroxy-6-{[3-(4- 488.13 11.208 [M − H] − 1 764298 ± 11449342 ± methoxyphenyl)prop-2-193256 2788799 enoyl]oxy}oxane-2-carboxylic acid 380 Sulindac 490.12 12.5 [M − H] − 1 248999 ± 1067937 ± 124486 44336 381 3,4,5-trihydroxy-6-[5-hydroxy-2- 492.16 7.476 [M + H] + 1 415073 ± 32153032 ± methoxy-4-(prop-2-en-1- 50302 1481907 yl)phenoxy]oxane-2-carboxylic acid 382 3,4,5-trihydroxy-6-[5-hydroxy-4- 492.16 6.333 [M + H] + 1 265877 ± 30343221 ± methoxy-2-(prop-2-en-1- 117576 1013625 yl)phenoxy]oxane-2-carboxylic acid 383 3,4,5-trihydroxy-6-{[3-(3- 492.16 6.393 [M + H] + 1 283221 ± 80304902 ± methoxyphenyl)propanoyl]oxy}oxane-2- 98823 15929303 carboxylic acid 384 3,4,5-trihydroxy-6-{[2-(4- 490.15 11.461 [M − H] − 1 231668 ± 2885292 ± methoxyphenyl)propanoyl]oxy}oxane-2-24742 641625 carboxylic acid 385 Tetracosahexaenoic acid 492.32 7.847 [M + H] + 1 7220101994 ± 1726046 ± 144799958 120207 386 6,9,12,15,18,21-Tetracosahexaenoic acid 492.32 6.284 [M +

H] + 1 171476795 ± 201112 ± 42496040 263735 387 Tetracosahexaenoic acid, n-3 492.32 5.731 [M + H] + 1 275527792 ± 517583 ± 16577519 407316 388 5-hydroxy-2-{[1-hydroxy-3-(4- hydroxy- 493.13 6.238 [M − H] − 1 83824095 ± 53113 ± 3-methoxyphenyl)prop-2-en-1- 5817825 7086 ylidene]amino}-4-methoxybenzoic acid 389 Rosmarinic acid 494.12 6.676 [M − H] − 1 69856616 ± 37698 ± 5550646 3154 390 Feruloyl C1-glucuronide 504.13 11.828 [M − H] − 1 1150529 ± 4030880 ± 12608 859188 391 Isoferuloyl C1-glucuronide 504.13 10.569 [M − H] − 1 761604 ± 11722683 ± 15570 1247781 392 3,4,5-trihydroxy-6-{[3-(4-hydroxy-3- 504.13 10.791 [M − H] − 1 366531 ± 4752867 ± methoxyphenyl)prop-2- 48454 340214 enoyl]oxy}oxane-2-carboxylic acid 393 3,4,5-trihydroxy-6-{[3-(3-hydroxy-5- 504.13 11.361 [M − H] − 1 978673 ± 19126186 ± methoxyphenyl)prop-2- 64639 674346 enoyl]oxy}oxane-2-carboxylic acid 394 3,4,5-trihydroxy-6-{[(2E)-3-(4-hydroxy- 504.13 11.203 [M − H] − 1 541613 ± 10096751 ± 3-methoxyphenyl)prop-2- 25396 2230534 enoyl]oxy}oxane-2-carboxylic acid 395 3,4,5-trihydroxy-6-{[3-(2-hydroxy-4- 504.13 10.956 [M − H] − 1 403501 ± 9765280 ± methoxyphenyl)prop-2- 13037 2718087 enoyl]oxy}oxane-2-carboxylic acid 396 3,4,5-trihydroxy-6-{[3-(4- 504.13 10.69 [M − H] − 1 451786 ± 5949088 ± methoxyphenyl)-2- 31638 1085216 oxopropanoyl]oxy}oxane-2-carboxylic acid 397 (3R)-3-(tetradecanoyloxy)-4- 507.35 10.39 [M + H] + 1 188234605 ± 871188 ± (trimethylazaniumyl)butanoate 73194844 199932 398 Ethyl gallate 3-glucuronide 508.12 8.985 [M − H] − 1 105752943 ± 163314 ± 45846541 7882 399 2-(10-Heptadecenyl)-6-hydroxybenzoic 508.32 13.021 [M − H] − 1 12794494 ± 97035 ± acid 4306336 3888 400 12b-Hydroxy-5b-cholanoic acid 510.33 12.428 [M − H] − 1 641446664 ± 949703 ± 189084164 735439 401 Allolithocholic acid 510.33 12.025 [M − H] − 1 115231454 ± 359177 ± 38319237 362857 402 7a-Hydroxy-5b-cholanic acid 510.33 11.916 [M − H] − 1 1156055 ± 250890 ± 338435 247541 403 6-[(4,7-dihydroxy-2,2-dimethyl-3,4- 520.16 12.006 [M − H] − 1 1870162 ± 11915130 ± dihydro-2H-1-benzopyran-5-yl)oxy]- 475219 2335910 3,4,5-trihydroxyoxane-2-carboxylic acid 404 Treprostinil 524.28 12.31 [M − H] − 1 980525 ± 8520699 ± 523501 451539 405 b“D8-Merulinic acid A” 524.31 11.366 [M − H] − 1 541158745 ± 449488 ± 374961606 279265 406 7-Hydroxy-3-oxocholanoic acid 524.31 11.592 [M − H] − 1 5235781 ± 169193 ± 3448206 8232 407 11-Hydroperoxy-H4-neuroprostane 526.26 11.574 [M − H] − 1 331036 ± 100458 ± 85161 838 408 3a,12b-Dihydroxy-5b-cholanoic acid 526.33 11.944 [M − H] − 1 1812605216 ± 4679585 ± 192418223 378889 409 3b,12a-Dihydroxy-5a-cholanoic acid 526.33 10.534 [M − H] − 1 679106438 ± 388461 ± 40615214 78335 410 3b,7a-Dihydroxy-5b-cholanoic acid 526.33 11.778 [M − H] − 1 548961777 ± 2777820 ± 30873343 251814 411 3a,7a-Dihydroxycholanoic acid 526.33 10.902 [M − H] − 1 660581393 ± 322672 ± 34926669 217922 412 Allodeoxycholic acid 526.33 11.604 [M − H] − 1 44284334 ± 506183 ± 852858 327197 413 Allochenodeoxycholic acid 526.33 11.871 [M − H] − 1 29068727 ± 186069 ± 3682524 5434 414 Murocholic acid 526.33 11.249 [M − H] − 1 148446058 ± 287273 ± 9847095 26392 415 7a,12b-dihydroxy-5b-Cholan-24-oic acid 526.33 11.33 [M − H] − 1 932019319 ± 436568 ± 348382725 286077 416 3beta,12beta-Dihydroxy-5beta-cholanoic 526.33 12.199 [M − H] − 1 232158793 ± 476448 ± acid 10792209 7844 417 Sinapinic acid-O-glucuronide isomer 534.14 11.937 [M − H] − 1 1217503 ± 3553889 ± 81133 730667 418 3,4,5-trihydroxy-6-{[6-(3-hydroxyprop- 534.14 11.325 [M − H] − 1 1832391 ± 142882837 ± 1-en-1-yl)-5-methoxy-2H-1,3- 1084795 7978855 benzodioxol-4-yl]oxy}oxane-2-carboxylic acid 419 3,4,5-trihydroxy-6-[2-hydroxy-3,4- 534.14 11.57 [M − H] − 1 634692 ± 53948195 ± dimethoxy-5-(3-oxoprop-1-en-1- 486133 4992807 yl)phenoxy]oxane-2-carboxylic acid 420 b“5-(3,4,5-Trihydroxyphenyl)-gamma- 534.14 11.654 [M − H] − 1 387193 ± 51877403 ± valerolactone-3-O-glucuronide” 106043 14353027 421 b“5-(3,4,5-Trihydroxyphenyl)-gamma- 536.19 4.204 [M + H] + 1 25890202 ± 146487 ± valerolactone-4-O-glucuronide” 1901296 4740 422 b“4-Hydroxy-5-(3,4-dihydroxyphenyl)- 536.15 11.452 [M − H] − 1 311804 ± 39471028 ± valeric acid-O-glucuronide” 56347 2427874 423 6-{[5-(3,4-dihydroxyphenyl)-4- 536.15 11.878 [M − H] − 1 193855 ± 18955563 ± hydroxypentanoyl]oxy}-3,4,5- 22678 1049628 trihydroxyoxane-2-carboxylic acid 424 3,4,5-trihydroxy-6-[2-hydroxy-3,4- 536.15 11.51 [M − H] − 1 343919 ±

35256359 ± dimethoxy-5-(3- 116260 3190656 oxopropyl)phenoxy]oxane-2-carboxylic acid 425 7-Ketodeoxycholic acid 542.32 4.197 [M + H] + 1 157854029 ± 156172 ± 9462565 5053 426 3,7-Dihydroxy-12-oxocholanoic acid 542.32 3.838 [M + H] + 1 9016731 ± 10773 ± 878392 1084 427 1b,3a,12a-Trihydroxy-5b-cholanoic acid 542.32 10.137 [M − H] − 1 167611063 ± 114235 ± 4327022 4658 428 1b,3a,7a-Trihydroxy-5b-cholanoic acid 542.32 11.148 [M − H] − 1 544907160 ± 3155128 ± 4565684 56662 429 3alpha,7alpha,12beta-Trihydroxy-5beta- 542.32 9.526 [M − H] − 1 1241848325 ± 64094 ± cholanoic acid 21099752 2673 430 3a,4b,7a-Trihydroxy-5b-cholanoic acid 542.32 10.707 [M − H] − 1 3495175556 ± 674030 ± 579880 577596 431 3a,4b,12a-Trihydroxy-5b-cholanoic acid 542.32 9.706 [M − H] − 1 195680988 ± 224977 ± 12-Ketodeoxycholic acid 10657635 9852 432 1,3,12-Trihydroxycholan-24-oic acid 542.32 8.65 [M − H] − 1 925432007 ± 88584 ± 59000243 12829 433 3b,7a,12a-Trihydroxy-5a-Cholanoic acid 542.32 9.97 [M − H] − 1 27588201 ± 429960 ± 4722587 32149 434 3a,7b,12b-Trihydroxy-5b-cholanoic acid 542.32 8.09 [M − H] − 1 35540636 ± 38071 ± 3548529 1995 435 3b,7b,12a-Trihydroxy-5b-cholanoic acid 542.32 8.82 [M − H] − 1 25513288 ± 75397 ± 9016311 3160 436 2b,3a,7a-Trihydroxy-5b-cholanoic acid 542.32 8.973 [M − H] − 1 21080013 ± 118675 ± 14979217 8489 437 Trovafloxacin 552.16 5.011 [M + H] + 1 25987908 ± 16523986 ± 5996264 1033800 438 3,4,5-trihydroxy-6-{4-hydroxy-2-[(2E)- 550.14 10.286 [M − H] − 1 48373249 ± 18790255 ± 3-phenylprop-2-enoyl]phenoxy}oxane-2- 12447522 2443497 carboxylic acid 439 3,4,5-trihydroxy-6-[4-hydroxy-3-(3- 550.14 10.386 [M − H] − 1 44046888 ± 14346146 ± phenylprop-2-enoyl)phenoxy]oxane-2- 12250562 2123539 carboxylic acid 440 7,14-dihydroxy-6-methoxy-4,12-562.14 11.722 [M − H] − 1 199302 ± 8211896 ± dimethyl-5-(3-methylbut-2-enoyl)-10- 25981 372731 oxo-2,9- dioxatricyclo[9.4.0.0\xc2\xb3,\xe2\x81\ xb8]pentadeca-1(11),3(8),4,6,12,14-hexaene-15-carboxylic acid 441 6-[2-(benzoyloxy)-5-(prop-2-en-1- 564.16 10.648 [M − H] − 1 240806 ± 196687 ± yl)phenoxy]-3,4,5-trihydroxyoxane-2- 7836 120183 carboxylic acid 442 3,4,5-trihydroxy-6-{2-[(1E)-3-(4- 564.16 11.847 [M − H] − 1 812681 ± 54894976 ± methoxyphenyl)-3-oxoprop-1-en-1- 311959 17140906 yl]phenoxy}oxane-2-carboxylic acid 443 3,4,5-trihydroxy-6-{5-methoxy-2-[(2E)- 564.16 11.781 [M − H] − 1 879920 ± 46776254 ± 3-phenylprop-2-enoyl]phenoxy}oxane-2- 197511 7914696 carboxylic acid 444 3,4,5-trihydroxy-6-{4-[(1E)-3-(4-564.16 11.532 [M − H] − 1 1396695 ± 54509330 ± methoxyphenyl)-3-oxoprop-1-en-1- 92231 19840841 yl]phenoxy}oxane-2-carboxylic acid 445 3,4,5-trihydroxy-6-{3-[(1E)-3-(4- 564.16 11.953 [M − H] − 1 1383091 ± 10848077 ± methoxyphenyl)-3-oxoprop-1-en-1- 92167 3236227 yl]phenoxy}oxane-2-carboxylic acid 446 Ketoprofen glucuronide 564.16 11.213 [M − H] − 1 2544589 ± 4777722 ± 1554692 1434362 447 3,4,5-trihydroxy-6-{[8-methoxy-6-(3- 570.17 4.615 [M − H] − 1 228973368 ± 600200 ± methylbut-2-en-1-yl)-2-oxo-2H- 15107157 74242 chromen-7-yl]oxy}oxane-2-carboxylic acid 448 simvastatin hydroxy acid 570.32 11.389 [M − H] − 1 34301301 ± 166890 ± 5036658 11744 449 Varanic acid 570.35 11.427 [M − H] − 1 3360794 ± 99464 ± 262300 4778 450 Formononetin 7-glucuronide 580.15 3.987 [M + H] + 1 77327 ± 194116 ± 55313 131885 451 3,4,5-trihydroxy-6-[(8-methoxy-4-oxo-2- 580.15 8.439 [M − H] − 1 115818670 ± 104279 ± phenyl-3,4-dihydro-2H-1-benzopyran-7- 14734095 6542 yl)oxy]oxane-2-carboxylic acid 452 N-Stearoyl tyrosine 581.37 12.27 [M − H] − 1 12635191 ± 120789 ± 2188071 2548 453 3alpha,7alpha,12alpha-trihydroxy-5beta- 584.37 11.903 [M − H] − 1 14617490 ± 96856 ± cholestanate 1062188 3422 454 3,4,5-trihydroxy-6-{6-hydroxy-3-[3-(3- 596.15 8.486 [M − H] − 1 20764642 ± 27169164 ± hydroxyphenyl)-3-oxoprop-1-en-1-yl]-2- 1225036 5774647 methoxyphenoxy}oxane-2-carboxylic acid 455 (3alpha,20R,24Z)-3-Hydroxy-21- 606.39 9.368 [M + H] + 1 98724208 ± 4768874 ± oxoeupha-8,24-dien-26-oic acid 33043801 165596 456 23-Hydroxy-3-oxocycloart-24-en-26-oic 604.38 12.257 [M − H] − 1 7915874 ± 95440 ± acid 2772630 9300 457 Chenodeoxycholic acid 3-sulfate 606.29 9.184 [M − H] − 1 108202813 ± 28217 ± 38912268 632 458 Ursodeoxycholic acid 3-sulfate 606.29 10.816 [M − H] − 1 200146167 ± 350728 ± 64217935 420289 459 Chenodeoxycholic acid sulfate 606.29 9.759 [M − H] − 1 18347698 ± 95308 ± 4837735 4174 460 b“4-O-Methylepicatechin 7-O- 614.16 7.92 [M − H] − 1

65767168 ± 92340 ± glucuronide” 3359931 17237 461 6-{5-[(E)-2-(2,4- 622.20 10.042 [M − H] − 1 1486863 ± 171224 ± dihydroxyphenyl)ethenyl]-3-hydroxy-2- 459795 11362 (3-methylbut-2-en-1-yl)phenoxy}-3,4,5- trihydroxyoxane-2-carboxylic acid 462 7-Sulfocholic acid 622.28 9.981 [M − H] − 1 21115252 ± 339947 ± 1446294 25419 463 Fexofenadine 635.32 10.714 [M − H] − 1 4092130 ± 224486 ± 121573 4692 464 3,4,5-trihydroxy-6-({5,6,14-trimethoxy- 640.18 10.132 [M − H] − 1 83141191 ± 132244 ± 8,17- 16073507 5392 dioxatetracyclo[8.7.0.0\xc2\xb2,\xe2\x81\ xb7.0\xc2\xb9\xc2\xb9,\xc2\xb9\xe2\x81\ xb6]heptadeca-2,4,6,11(16),12,14- hexaen-4-yl}oxy)oxane-2-carboxylic acid 465 4-{7-[(6-carboxy-3,4,5-trihydroxyoxan- 648.12 12.011 [M − H] − 1 337835 ± 6657664 ± 2-yl)oxy]-3-hydroxy-5-sulfino-3,4- 43390 1417613 dihydro-2H-1-benzopyran-2-yl}-2- hydroxybenzen-1-olate 466 3,3-Diiodothyronine 658.93 7.666 [M − H] − 1 778652 ± 66407 ± 28870 38205 467 6-{[6-(3,4-dihydroxy-6-methyl-5- 740.19 3.802 [M + H] + 1 2510636 ± 15222 ± oxooxan-2-yl)-5-hydroxy-2-(3- 93502 1211 methoxyphenyl)-4-oxo-4H-chromen-7- yl]oxy}-3,4,5-trihydroxyoxane-2- carboxylic acid

Claims

1. A quantitative detection method of multiple metabolic components in a biological sample, comprising performing derivatization treatment on the biological sample and then detecting the derivatized biological sample by liquid chromatography-mass spectrometry, wherein during derivatization treatment, 3-nitrophenylhydrazine is used as a derivatization reagent, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is used as a derivatization reaction catalyst; the biological sample is selected from urine, blood, cerebrospinal fluid, tissue, cells, saliva and fecal samples of a mammal; the multiple metabolic components in the biological sample detected by the quantitative detection method are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid and have different magnitudes in content; the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 50 or more; the liquid chromatography adopts a UPLC BEH C18 chromatographic column, the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio of 1-2:1; and the gradient elution conditions: 0-1 min, 5% B; 1-5 min, 5-30% B; 5-9 min, 30-50% B; 9-12 min, 50-75% B; 12-15 min, 75-95% B; 15-16 min, 95-100% B; 16-18 min, 100% B; and 18-20 min, 5% B.
2. The detection method according to claim 1, wherein the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 100 or more.
3. The detection method according to claim 1, wherein the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 200 or more.
4. The detection method according to claim 1, wherein the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 600 or more.
5. The detection method according to claim 1, wherein the number of the multiple metabolic components in a biological sample detected by the quantitative detection method is 1000 or more.
6. The detection method according to claim 1, wherein scan parameters of the mass spectrometry are set as follows: S-lens RF level, 50; mass range, 100 to 1200 m/z; full MS resolution, 70,000; MS/MS resolution, 17,500; and NCE/stepped NCE is set at 10, 20, and 40 eV.
7. The detection method according to claim 1, wherein ESI ion source parameters of the mass spectrometry are set as follows: spray voltage, 3800 V; sheath gas flow rate, 40 for ESI+ and 5 for ESI−; capillary temperature, 320° C.; probe heater temperature, 350° C.; aux gas flow rate, 10.
8. The detection method according to claim 1, wherein the liquid chromatography-mass spectrometry is acquired using a Vanquish Flex UHPLC system coupled to a Q Extractive Focus Orbitrap mass spectrometer equipped with a heated electrospray ionization source.
9. The detection method according to claim 1, wherein the mobile phase includes: A: water containing 0.1% formic acid, B: acetonitrile containing 0.1% formic acid and isopropanol at a ratio

of 7:3.

10. The detection method according to claim 1, comprising the following steps: a) collecting a biological sample; b) extracting the biological sample with a mixed solvent of methanol, chloroform, and water, performing centrifugation, and then taking a supernatant, namely a biological sample extract; c) adding the same volume of a 3-nitrophenylhydrazine methanol solution and a 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide pyridine solution into the biological sample extract obtained in b), and performing uniform vortex mixing and heating for derivatization, wherein the concentration of used 3-nitrophenylhydrazine is 100-320 mmol/L, the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 50-200 mmol/L, the reaction temperature is 20-60° C., and the reaction time is 10-120 minutes; d) adding a carbon-13 labeled isotope internal standard solution obtained from the reaction of 3-nitrophenylhydrazine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide into the derivatized biological sample extract obtained in c); and e) adding a methanol-water mixed solution into the sample in d) for dilution, and determining amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid by liquid chromatography-mass spectrometry.

11. The detection method according to claim 10, wherein in step c), the concentration of used 3-nitrophenylhydrazine is 150-220 mmol/L, the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 80-120 mmol/L, the reaction temperature is 20-40° C., and the reaction time is 30-60 minutes.

12. The detection method according to claim 10, wherein when the biological sample is urine, blood, saliva or cerebrospinal fluid, a treatment method of the biological sample in step b) comprises: taking an appropriate amount of the biological sample, extracting the biological sample with a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, shaking the mixture for a few seconds, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at a low temperature for 5-15 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment.

13. The detection method according to claim 10, wherein when the biological sample is a fecal sample, a treatment method of the biological sample in step b) comprises: freeze-drying the fecal sample; homogenizing an appropriate amount of the freeze-dried fecal sample with an appropriate amount of a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at a low temperature for 15-30 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment.

14. The detection method according to claim 10, wherein when the biological sample is a tissue or cell sample, a treatment method of the biological sample in step b) comprises: adding an appropriate amount of a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1 into the sample for homogenization, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at 4° C. for 15-30 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment.

15. The detection method according to claim 10, wherein the volume ratio of methanol to water in step e) is 1:1.

16. The detection method according to claim 1, wherein the multiple metabolic components of the biological sample detected by the quantitative detection method are selected from the following multiple metabolites: fructose 1,6-diphosphate, 10Z-nonadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, 12-dehydrocholic acid, 12-hydroxystearic acid, 12-ketolithocholic acid, 1-methylhistidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenoic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutaric acid, 2-methylglutamic acid, 2-furoic acid, 2-hydroxy-2-methylbutyric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxybutyric acid, 2-hydroxycaproic acid, 2-hydroxycinnamic acid, 2-hydroxyphenylacetic acid,

2-hydroxyhippuric acid, 2-phenylpropionic acid, 2-phenylglycine, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-(4-hydroxyphenyl)lactic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dehydro-DL-proline, 3,5-diiodo-L-thyroxine, 30-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxyalanine, 3-aminosalicylic acid, 3-chloro-L-tyrosine, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 3-methylindole, 3-methyladipic acid, 3-methylglutamic acid, 3-nitrotyrosine, sulfate 3-taurolithocholic acid, sulfate 3-lithocholic acid, 3-hydroxy-2-aminobenzoic acid, 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid, 3-hydroxyphenylacetic acid, 3-hydroxyhippuric acid, 3-dehydrocholic acid, 3-phenylbutyric acid, 4-methyl-2-oxovaleric acid, 4-methylhexanoic acid, 4-methoxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, 4-hydroxycinnamic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid, 4-phenylbutyric acid, 5-aminolevulinic acid, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxylysine, 6,7-diketolithocholic acid, 6-phosphogluconic acid, 6-ketolithocholic acid, 7,12-diketolithocholic acid, 7-ketolithocholic acid, 7-ketodeoxycholic acid, 9,11-conjugated linoleic acid, D-2-hydroxyglutaric acid, D-galactose, D-xylose, D-xylulose, D-fructose, D-ribose, D-ribose-5-phosphate, D-ribulose, D-ribulose-5-phosphate, D-mannose, D-glucose, D-maltose, L-2-aminobutyric acid, L-3-phenyllactic acid, L-alanine, L-serine, L-acetylcarnitine, L-lactic acid, L-allothreonine, L-cysteine, L-homoserine, L-homocysteine, L-homocitrulline, L-pipecolic acid, L-aspartic acid, L-asparagine, L-sorbose, L-lignic acid, L-norleucine, L-kynurenine, L-thyronine, L-arginine, L-histidine, L-valine, L-cystine, L-cystathionine, L-proline, L-tryptophan, L-threonine, L-phenylalanine, L-malic acid, L-methionine, L-glutamine, L-glutamic acid, L-lysine, L-tyrosine, L-arabinose, N-(3-phenylpropionyl)glycine, N-acetyl-L-alanine, N-acetyl-L-aspartic acid, N-acetyl-L-phenylalanine, N-acetyl-L-methionine, N-acetyl-L-tyrosine, N-acetylserine, N-acetyl-D-glucosamine, N-acetyl-L-phenylalanine, N-acetylmannosamine, N-acetylneuraminic acid, N-acetylhistidine, N-acetylhydroxytryptamine, N-acetyltryptophan, N-acetylglutamine, N-acetyllysine, N-acetylornithine, N-methylnicotinamide, N-phenylacetyl-glutamine, N-phenylacetylphenylalanine, S-adenosine homocysteine, α-D-glucose, α-lactose, α-linolenic acid, α-hydroxyisobutyric acid, α-ketoglutaric acid, α-muricholic acid, β-D-trehalose, β-alanine, β-ursocholic acid, β-muricholic acid, γ-L-glutamyl-L-alanine, γ-linolenic acid, γ-aminobutyric acid, ω-muricholic acid, butyric acid, trimethylamine nitroxide, adenosine triphosphate, malonic acid, propionic acid, acetoacetic acid, acetylcysteine, acetylglycine, acetylornithine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosenoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosenoic acid, docosanoic acid, docosatrienoic acid, cis-13,16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-docosaenoic acid, cis-13-docosaenoic acid, pentacosanoic acid, octacosanoic acid, hexacosanoic acid, tetracosanoic acid, eicosanoic acid, eicosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, trans-11-eicosenoic acid, cis-11-eicosenoic acid, cis-5-eicosenoic acid, cis-8-eicosenoic acid, dimethylglycine, adenosine diphosphate, linoleic acid, leucine, alloisoleucine, allolithocholic acid, allocholic acid, undecenoic acid, heptadecanoic acid, tridecanoic acid, nonadecanoic acid, nonadecadienoic acid, pentadecanoic acid, galactonic acid, galactitol, mecysteine, protocatechuic acid, apocholic acid, dehydrolithocholic acid, nordeoxycholic acid, trans-9-tetradecenoic acid, trans-aconitic acid, trans-4-hydroxyproline, trans-9-heptadecenoic acid, trans-9-pentadecenoic acid, trans-9-hexadecenoic acid, trans-linolenic acid, trans-cinnamic acid, elaidic acid, homocysteine, pyrrole-2-carboxylic acid, picolinic acid, indole, indole-3-methyl acetate, indole-3-carboxylic acid, indoleacetic acid, purine, azelaic acid, nonanoic acid, dopa, dopamine, melibiose, fumaric acid, acetaminophen, p-aminohippuric acid, p-cresol sulfate, symmetrical dimethylarginine, p-hydroxymandelic acid, p-hydroxyphenylacetic acid, homogentisic acid, adipic acid, pimelic acid, isobutyric acid, isoleucine, isovaleric acid, citric acid, isoursodeoxycholic acid, isohyodeoxycholic acid, isolithocholic acid, isocholic acid, isodeoxycholic acid, glutaric acid, glutaconic acid, valeric

acid, mandelic acid, lauric acid, fructose-6-phosphate, citraconic acid, citric acid, citramalic acid, ribonolactone, ribonic acid, raffinose, palmitoleic acid, palmitic acid, norvaline, n-hydroxyphenylacetic acid, oxidized glutathione, aminoadipic acid, aminocaproic acid, salicyluric acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, taurolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hyodeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycoursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyl-L-leucine, mannose-6-phosphoric acid, mannitol, methylmalonic acid, methylsuccinic acid, formic acid, capric acid, lithocholic acid, selenomethionine, thiamine, glycolithocholic sulfate, stearic acid, liothyronine, dihydroxyacetone phosphate, phosphoribosyl pyrophosphate, creatine phosphate, hydroxypyruvic acid, glycine hydroxyphenylacetate, cinnamic acid, sarcosine, carnosine, creatine, inositol, epinephrine, choline, cholic acid, dehydrocholic acid, demethylcholic acid, adenosine monophosphate, arachidonic acid, phenylpyruvic acid, phenethylamine, phenylpropionic acid, phenyllactic acid, benzamide, benzoic acid, oxalic acid, shikimic acid, glucaric acid, glucose-6-phosphate, glucose lactone, sebacic acid, ricinoleic acid, sucrose, methionine sulfoxide, itaconic acid, melatonin, glutathione, myristic acid, erythronic acid, erythrose, suberic acid, caprylic acid, phthalic acid, tartaric acid, tyramine, quinic acid, m-aminobenzoic acid, asymmetric dimethylarginine, tannic acid, cis-10,12-octadecadienoic acid, cis-12,15-heneicosadienoic acid, cis-12-tridecenoic acid, cis-15-tetracosenic acid, cis-2-hydroxycinnamic acid, cis-9-tetradecenoic acid, cis-10-heptadecenoic acid, cis-11-dodecenoic acid, cis-4-hydroxyproline, cis-5-dodecenoic acid, cis-7-hexadecenoic acid, cis-9-heptadecenoic acid, cis-aconitic acid, vanillic acid, hippuric acid, maleic acid, homovanillic acid, ornithine, guanosine monophosphate, guanosine triphosphate, anserine, chenodeoxycholic acid, maltotriose, maltitol, rhamnose and murideoxycholic acid.

17. A metabolic chip used in the detection method according to claim 1, comprising a chip carrier, a filter device and dry solid powder of a standard product and a quality control product, wherein the chip carrier is a microtiter plate, each well of the microtiter plate is provided with an independent filter device, and the powder obtained by dehydrating or freeze-drying solutions of the standard product and the quality control product is placed on the filter device in each well of the microtiter plate; the multiple metabolic components in the standard product are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products; the multiple metabolic components in the quality control product are selected from amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid standard products corresponding to the standard products above; the number of the multiple metabolic components in the standard product and the quality control product is 50 or more.

18. The metabolic chip according to claim 17, wherein the microtiter plate is selected from a 48-well plate, a 96-well plate and a 384-well plate, and the filter device is a filter membrane made of polyvinylidene fluoride, cellulose acetate, or nylon with a pore size of 0.20-0.45 micron.

19. The metabolic chip according to claim 17, wherein the multiple metabolic components in the standard product and the quality control product are selected from: fructose 1,6-diphosphate, 10Z-nonadecenoic acid, trans-11-octadecenoic acid, cis-11-octadecenoic acid, 12-dehydrocholic acid, 12-hydroxystearic acid, 12-ketolithocholic acid, 1-methylhistidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenoic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutaric acid, 2-methylglutamic acid, 2-furoic acid, 2-hydroxy-2-methylbutyric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxybutyric acid, 2-hydroxycaproic acid, 2-hydroxycinnamic acid, 2-hydroxyphenylacetic acid, 2-hydroxyhippuric acid, 2-phenylpropionic acid, 2-

phenylglycine, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-(4-hydroxyphenyl)lactic acid, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dehydro-DL-proline, 3,5-diiodo-L-thyroxine, 30-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxyalanine, 3-aminosalicylic acid, 3-chloro-L-tyrosine, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 3-methylindole, 3-methyladipic acid, 3-methylglutamic acid, 3-nitrotyrosine, sulfate 3-taurolithocholic acid, sulfate 3-lithocholic acid, 3-hydroxy-2-aminobenzoic acid, 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisovaleric acid, 3-hydroxyphenylacetic acid, 3-hydroxyhippuric acid, 3-dehydrocholic acid, 3-phenylbutyric acid, 4-methyl-2-oxyvaleric acid, 4-methylhexanoic acid, 4-methoxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, 4-hydroxycinnamic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid, 4-phenylbutyric acid, 5-aminolevulinic acid, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxylysine, 6,7-diketolithocholic acid, 6-phosphogluconic acid, 6-ketolithocholic acid, 7,12-diketolithocholic acid, 7-ketolithocholic acid, 7-ketodeoxycholic acid, 9,11-conjugated linoleic acid, D-2-hydroxyglutaric acid, D-galactose, D-xylose, D-xylulose, D-fructose, D-ribose, D-ribose-5-phosphate, D-ribulose, D-ribulose-5-phosphate, D-mannose, D-glucose, D-maltose, L-2-aminobutyric acid, L-3-phenyllactic acid, L-alanine, L-serine, L-acetylcarnitine, L-lactic acid, L-allothreonine, L-cysteine, L-homoserine, L-homocysteine, L-homocitrulline, L-pipecolic acid, L-aspartic acid, L-asparagine, L-sorbose, L-lignic acid, L-norleucine, L-kynurenine, L-thyronine, L-arginine, L-histidine, L-valine, L-cystine, L-cystathionine, L-proline, L-tryptophan, L-threonine, L-phenylalanine, L-malic acid, L-methionine, L-glutamine, L-glutamic acid, L-lysine, L-tyrosine, L-arabinose, N-(3-phenylpropionyl)glycine, N-acetyl-L-alanine, N-acetyl-L-aspartic acid, N-acetyl-L-phenylalanine, N-acetyl-L-methionine, N-acetyl-L-tyrosine, N-acetylserine, N-acetyl-D-glucosamine, N-acetyl-L-phenylalanine, N-acetylmannosamine, N-acetylneuraminic acid, N-acetylhistidine, N-acetylhydroxytryptamine, N-acetyltryptophan, N-acetylglutamine, N-acetyllysine, N-acetylornithine, N-methylnicotinamide, N-phenylacetyl-glutamine, N-phenylacetylphenylalanine, S-adenosine homocysteine, α-D-glucose, α-lactose, α-linolenic acid, α-hydroxyisobutyric acid, α-ketoglutaric acid, α-muricholic acid, β-D-trehalose, β-alanine, β-ursocholic acid, β-muricholic acid, γ-L-glutamyl-L-alanine, γ-linolenic acid, γ-aminobutyric acid, ω-muricholic acid, butyric acid, trimethylamine nitroxides, adenosine triphosphate, malonic acid, propionic acid, acetoacetic acid, acetylcysteine, acetylglycine, acetylornithine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosenoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosenoic acid, docosanoic acid, docosatrienoic acid, cis-13,16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-docosaenoic acid, cis-13-docosaenoic acid, pentacosanoic acid, octacosanoic acid, hexacosanoic acid, tetracosanoic acid, eicosanoic acid, eicosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, trans-11-eicosenoic acid, cis-11-eicosenoic acid, cis-5-eicosenoic acid, cis-8-eicosenoic acid, dimethylglycine, adenosine diphosphate, linoleic acid, leucine, alloisoleucine, allolithocholic acid, allocholic acid, undecenoic acid, heptadecanoic acid, tridecanoic acid, nonadecanoic acid, nonadecadienoic acid, pentadecanoic acid, galactonic acid, galactitol, mecysteine, protocatechuic acid, apocholic acid, dehydrolithocholic acid, nordeoxycholic acid, trans-9-tetradecenoic acid, trans-aconitic acid, trans-4-hydroxyproline, trans-9-heptadecenoic acid, trans-9-pentadecenoic acid, trans-9-hexadecenoic acid, trans-linolenic acid, trans-cinnamic acid, elaidic acid, homocysteine, pyrrole-2-carboxylic acid, picolinic acid, indole, indole-3-methyl acetate, indole-3-carboxylic acid, indoleacetic acid, purine, azelaic acid, nonanoic acid, dopa, dopamine, melibiose, fumaric acid, acetaminophen, p-aminohippuric acid, p-cresol sulfate, symmetrical dimethylarginine, p-hydroxymandelic acid, p-hydroxyphenylacetic acid, homogentisic acid, adipic acid, pimelic acid, isobutyric acid, isoleucine, isovaleric acid, citric acid, isoursodeoxycholic acid, isohyodeoxycholic acid, isolithocholic acid, isocholic acid, isodeoxycholic acid, glutaric acid, glutaconic acid, valeric acid, mandelic acid, lauric acid, fructose-6-phosphate, citraconic acid, citric acid, citramalic acid,

ribonolactone, ribonic acid, raffinose, palmitoleic acid, palmitic acid, norvaline, n-hydroxyphenylacetic acid, oxidized glutathione, aminoadipic acid, aminocaproic acid, salicyluric acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, taurolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hyodeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycoursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyl-L-leucine, mannose-6-phosphoric acid, mannitol, methylmalonic acid, methylsuccinic acid, formic acid, capric acid, lithocholic acid, selenomethionine, thiamine, glycolithocholic sulfate, stearic acid, liothyronine, dihydroxyacetone phosphate, phosphoribosyl pyrophosphate, creatine phosphate, hydroxypyruvic acid, glycine hydroxyphenylacetate, cinnamic acid, sarcosine, carnosine, creatine, inositol, epinephrine, choline, cholic acid, dehydrocholic acid, demethylcholic acid, adenosine monophosphate, arachidonic acid, phenylpyruvic acid, phenethylamine, phenylpropionic acid, phenyllactic acid, benzamide, benzoic acid, oxalic acid, shikimic acid, glucaric acid, glucose-6-phosphate, glucose lactone, sebacic acid, ricinoleic acid, sucrose, methionine sulfoxide, itaconic acid, melatonin, glutathione, myristic acid, erythronic acid, erythrose, suberic acid, caprylic acid, phthalic acid, tartaric acid, tyramine, quinic acid, m-aminobenzoic acid, asymmetric dimethylarginine, tannic acid, cis-10,12-octadecadienoic acid, cis-12,15-heneicosadienoic acid, cis-12-tridecenoic acid, cis-15-tetracosenic acid, cis-2-hydroxycinnamic acid, cis-9-tetradecenoic acid, cis-10-heptadecenoic acid, cis-11-dodecenoic acid, cis-4-hydroxyproline, cis-5-dodecenoic acid, cis-7-hexadecenoic acid, cis-9-heptadecenoic acid, cis-aconitic acid, vanillic acid, hippuric acid, maleic acid, homovanillic acid, ornithine, guanosine monophosphate, guanosine triphosphate, anserine, chenodeoxycholic acid, maltotriose, maltitol, rhamnose and murideoxycholic acid.

20. A use method of the metabolic chip according to claim 17, comprising the following steps: a) collecting a biological sample; b) according to the sample type, preparing a corresponding biological sample extract, when the biological sample is urine, blood, saliva or cerebrospinal fluid, a treatment method of the biological sample comprises: taking an appropriate amount of the biological sample, extracting the biological sample with a mixed solvent of cold methanol, chloroform and water at a volume ratio of 3:1:1, shaking the mixture for a few seconds, performing centrifugation on the sample at a rotation speed of 10000-20000 rpm at a low temperature for 5-15 minutes, and transferring a supernatant into an autosampler glass vial for subsequent derivatization treatment; c) adding the prepared biological sample extract into each well of the metabolic chip in an equal amount, adding the same volume of a 3-nitrophenylhydrazine methanol solution and a 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide pyridine solution into each well, and performing uniform vortex mixing and heating for derivatization, wherein the concentration of used 3-nitrophenylhydrazine is 100-320 mmol/L, the concentration of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide is 50-200 mmol/L, the reaction temperature is 20-60° C., and the reaction time is 10-120 minutes; d) adding a carbon-13 labeled isotope internal standard solution obtained from the reaction of 3-nitrophenylhydrazine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide into the derivatized biological sample extract obtained in c); and e) adding a methanol-water mixed solution into each well in the metabolic chip in d) for dilution, placing the metabolic chip in a tandem mass spectrometer for determination of amino acid, phenol, phenyl or benzyl derivative, indole, organic acid, fatty acid, sugar, and bile acid by liquid chromatography-mass spectrometry, and calculating concentrations of target metabolites in the sample based on results.