



US 20250258146A1

(19) **United States**

(12) **Patent Application Publication**

Jia et al.

(10) **Pub. No.: US 2025/0258146 A1**

(43) **Pub. Date: Aug. 14, 2025**

(54) **QUANTITATIVE DETECTION METHOD OF MULTIPLE METABOLITES IN BIOLOGICAL SAMPLE AND METABOLIC CHIP**

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(21) Appl. No.: **19/169,027**

(22) Filed: **Apr. 3, 2025**

Related U.S. Application Data

(63) Continuation-in-part of application No. 17/309,062, filed on Jul. 17, 2021, filed as application No. PCT/CN2019/112389 on Oct. 21, 2019.

(30) **Foreign Application Priority Data**

Oct. 19, 2018 (CN) 201811223486.X

Publication Classification

(51) **Int. Cl.**
G01N 30/72 (2006.01)
G01N 30/02 (2006.01)

G01N 30/04 (2006.01)

G01N 30/06 (2006.01)

G01N 30/14 (2006.01)

G01N 30/88 (2006.01)

G01N 33/487 (2006.01)

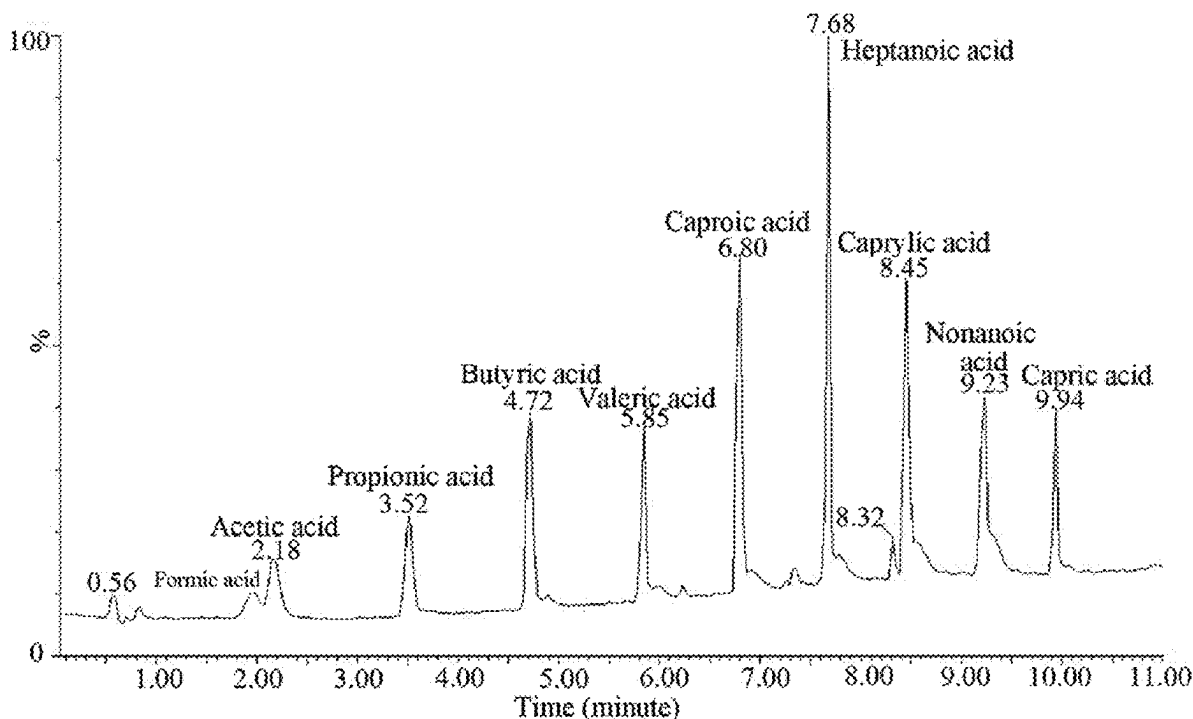
(52) **U.S. Cl.**

CPC **G01N 30/7266** (2013.01); **G01N 33/487** (2013.01); **G01N 2030/027** (2013.01); **G01N 2030/045** (2013.01); **G01N 2030/062** (2013.01); **G01N 2030/067** (2013.01); **G01N 2030/146** (2013.01); **G01N 2030/8813** (2013.01); **G01N 2030/8818** (2013.01); **G01N 2458/15** (2013.01)

(57)

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.



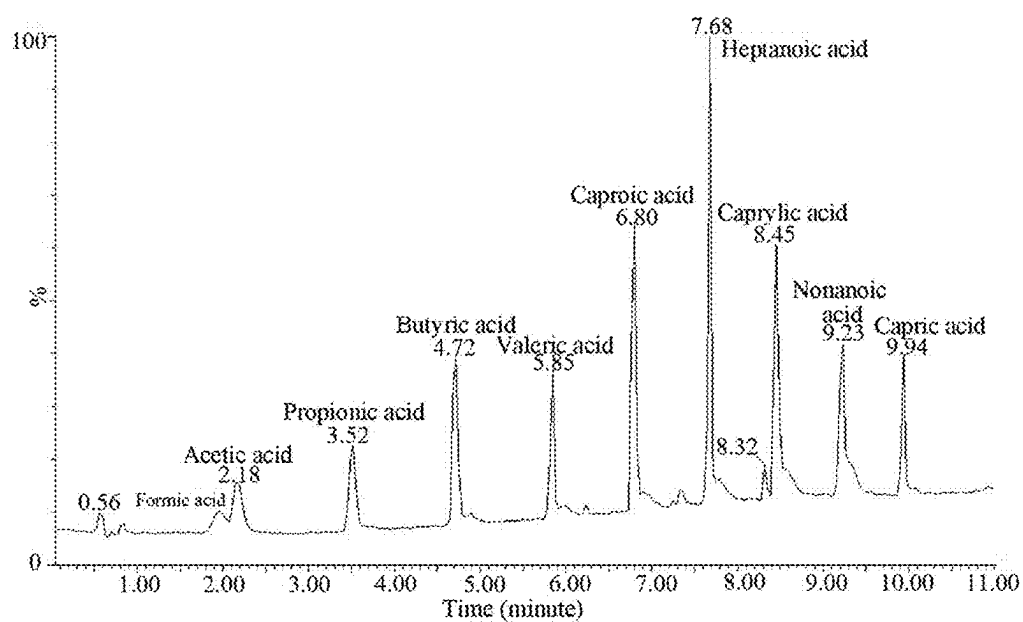


Fig. 1

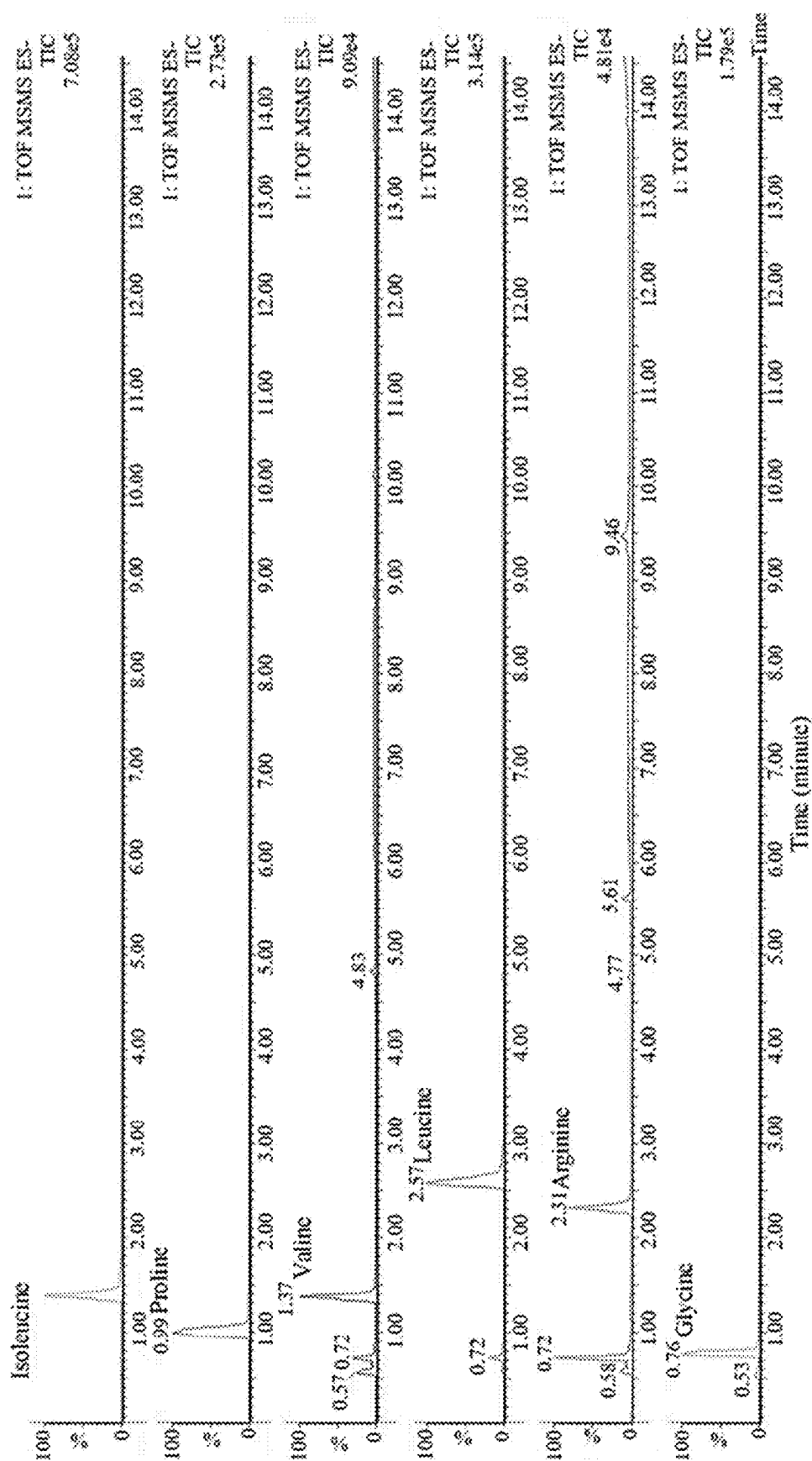


Fig. 2

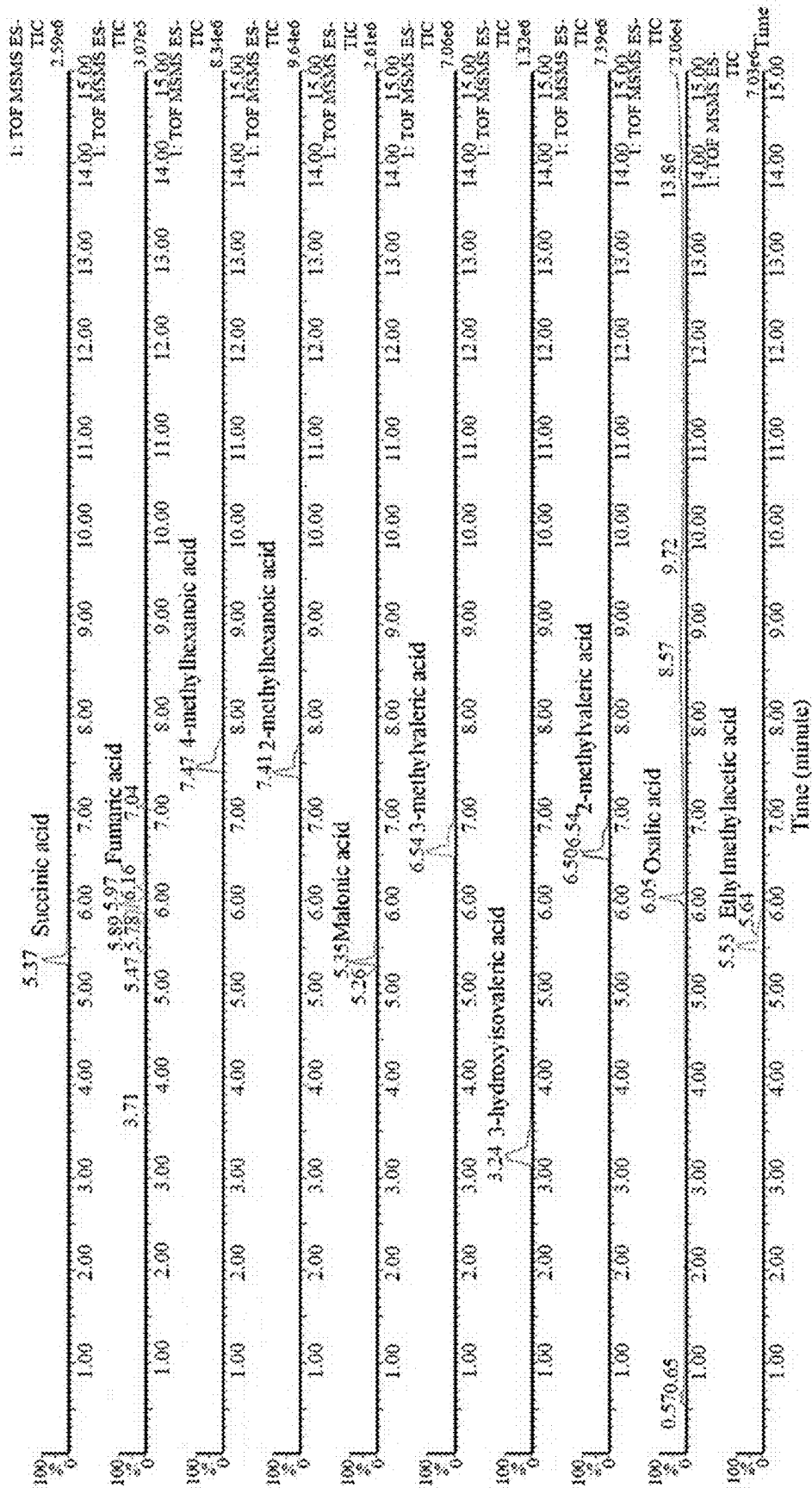


Fig. 3

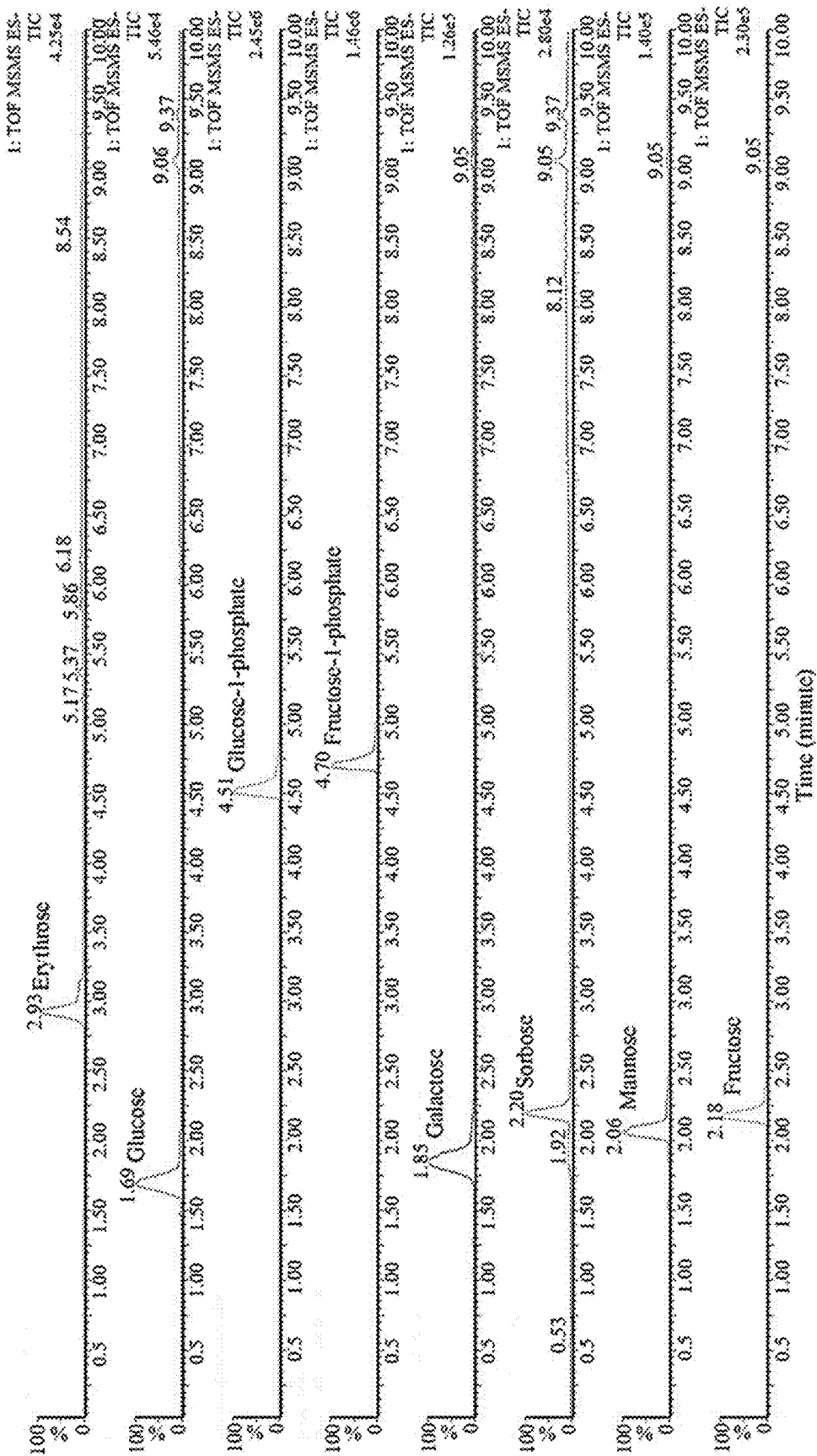


Fig. 4

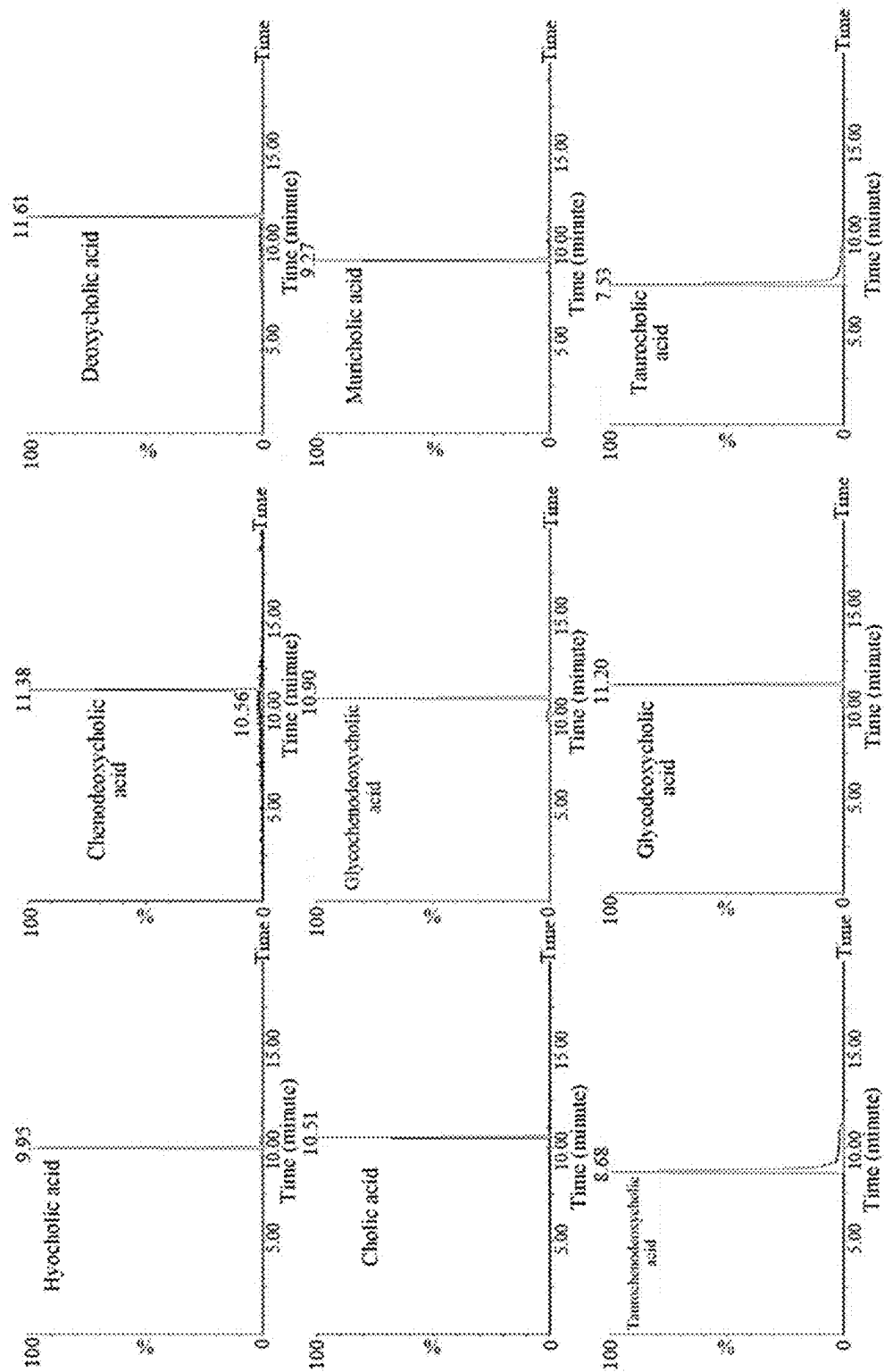


Fig. 5

QUANTITATIVE DETECTION METHOD OF MULTIPLE METABOLITES IN BIOLOGICAL SAMPLE AND METABOLIC CHIP

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

ide 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-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-butenic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutamic 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-oxoalanine, 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-tauroolithocholic 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-homocitrul-

lactone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycooursodeoxycholic 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, lithorinine, 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.

[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 detec-

tion 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-butenic acid, 2-oxoadipic acid, 2-methyl-4-pentenoic acid, 2-methyl-β-alanine, 2-methylbutyric acid, 2-methylhexanoic acid, 2-methylglutamic 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-tauroolithocholic 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-tyrosine, 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-acetylmethionine, N-methylnicotinamide, N-phenylacetylglutamine, 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 -ursodeoxycholic 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, acetylglutamine, acetylmethionine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglycine, heneicosanoic acid, cis-12-heneicosanoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosanoic 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-eicosanoic acid, cis-II-eicosanoic acid, cis-5-eicosanoic acid, cis-8-eicosanoic 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-acetic 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, isohydroxydeoxycholic 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, amino adipic acid, aminocaproic acid, salicylic acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursodeoxycholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, tauroolithocholic 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, glycooursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyll-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-acetic 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-chenodeoxy-

cholic acid, 2-butenic 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-oxoalanine, 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-tauroolithocholic 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-oxoaleric 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-thyroxine, 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-acetylmethionine, N-methylnicotinamide, N-phenylacetylglutamine, 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 -ursodeoxycholic 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, acetylglutamine, acetylmethionine, acetic acid, guanidine acetate, glycolic acid, lactulose, lactoylglutathione, heneicosanoic acid, cis-12-heneicosanoic acid, heptacosanoic acid, tricosanoic acid, cis-14-tricosanoic acid, docosanoic acid, docosatrienoic acid, cis-13, 16-docosadienoic acid, docosapentaenoic acid, docosahexaenoic acid, docosatetraenoic acid, trans-13-do-

cosaenoic 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, isohydroxycholic 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, aminoacetic acid, aminocaproic acid, salicylic acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursodeoxycholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohyodeoxycholic acid, tauroolithocholic 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, glycooursodeoxycholic acid, glycohyocholic acid, glycohyodeoxycholic acid, glycocholic acid, glyproline, glycyll-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.

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 centrifuged 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 centrifuged.

gated 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 μ L of a treated biological sample is taken, 20 μ L of a derivatization reagent and 20 μ L 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 μ L 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 μ m) 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 1

Determination results of concentrations of substances in blood of normal people		
Determined target metabolites	Concentration value	Concentration unit
2-methylvaleric acid	120.32 \pm 5.18	μ g/mL
2-hydroxybutyric acid	2.32 \pm 0.44	μ g/mL
3-(3-hydroxyphenyl)-3-hydroxypropionic acid	10.31 \pm 0.19	μ g/mL
3-hydroxyphenylacetic acid	6.07 \pm 6.12	μ g/mL
3-indoleacetonitrile	2.51 \pm 0.15	μ g/mL
3-methyl-2-oxovaleric acid	4.37 \pm 5.08	μ g/mL
4-methylhexanoic acid	2.96 \pm 0.07	μ g/mL
Linolenic acid	97.19 \pm 33.87	μ g/mL
Arachidonic acid	5.54 \pm 0.45	μ g/mL
Arachidonic acid	5.62 \pm 2.4	μ g/mL
Docosanoic acid	8.96 \pm 0.27	μ g/mL
β -alanine	10.37 \pm 0.44	μ g/mL
Capric acid	1.22 \pm 0.19	μ g/mL
Caprylic acid	1.39 \pm 0.5	μ g/mL
Citric acid	7.03 \pm 1.56	μ g/mL
Docosahexaenoic acid	15.1 \pm 4.36	μ g/mL
Docosapentaenoic acid n6	15.31 \pm 4.16	μ g/mL
Docosatrienoic acid	8.76 \pm 0.95	μ g/mL
Dodecanoic acid	0.91 \pm 0.16	μ g/mL
Dopamine	23.53 \pm 3.93	μ g/mL
Eicosenoic acid	11.18 \pm 3.38	μ g/mL
Erucic acid	15.25 \pm 11.34	μ g/mL
γ -aminobutyric acid	14.68 \pm 2.7	μ g/mL
Glutathione	6.7 \pm 1.62	μ g/mL
Glycolic acid	159.46 \pm 56.76	μ g/mL
Heptadecanoic acid	5.78 \pm 6.5	μ g/mL
L- α -aminobutyric acid	2.41 \pm 0.48	μ g/mL
L-asparagine	12.32 \pm 0.57	μ g/mL
L-aspartic acid	3.8 \pm 0.64	μ g/mL
L-glutamic acid	8.93 \pm 0.53	μ g/mL
L-histidine	16.76 \pm 1.54	μ g/mL
L-homoserine	12.53 \pm 7.81	μ g/mL
L-isoleucine	5.9 \pm 2.18	μ g/mL
L-leucine	5.88 \pm 3.97	μ g/mL
L-lysine	33.73 \pm 4.42	μ g/mL
L-methionine	6.78 \pm 0.73	μ g/mL
L-norleucine	3.33 \pm 1.25	μ g/mL
L-phenylalanine	8.57 \pm 0.84	μ g/mL
L-proline	11.32 \pm 5.94	μ g/mL
L-serine	9.78 \pm 0.68	μ g/mL
L-tryptophan	30.49 \pm 2.93	μ g/mL
L-tyrosine	20.02 \pm 4.37	μ g/mL
L-valine	28.87 \pm 7.87	μ g/mL
Linoleic acid	162.6 \pm 57.21	μ g/mL

TABLE 1-continued

Determination results of concentrations of substances in blood of normal people		
Determined target metabolites	Concentration value	Concentration unit
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
Hyochoic 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 2

Determination results of concentrations of substances in feces of normal people		
Detected target metabolites	Concentration value	Concentration 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-hydroxypropionic acid	0.19 ± 0.11	μg/mL
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

TABLE 2-continued

Determination results of concentrations of substances in feces of normal people		
Detected target metabolites	Concentration value	Concentration unit
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
Docosatienoic 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	1.1 ± 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

TABLE 2-continued

Determination results of concentrations of substances in feces of normal people		
Detected target metabolites	Concentration value	Concentration unit
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 μm 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 3

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
1	Propynoic acid	264.06	6.246	[M - H + HAc] - 1	2510269 ± 92554	841952 ± 28455

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
2	Acrylic acid	208.07	0.883	[M + H] + 1	13865154 ± 12993928	14228426 ± 23777442
3	Glyoxylic acid	208.04	5.832	[M - H] - 1	12000471 ± 2000381	2144384 ± 228813
4	Isocrotonic acid	239.11	0.434	[M + NH4] + 1	266059225 ± 460019188	218886 ± 43040
5	2-Aminoacrylic acid	221.07	6.247	[M - H] - 1	3464018 ± 800230	6160226 ± 1903288
6	Malonic semialdehyde	222.05	9.361	[M - H] - 1	8953281 ± 3833020	1962295 ± 1202549
7	2-hydroxyacrylic Acid	222.05	7.364	[M - H] - 1	40949997 ± 4052812	9472056 ± 1132094
8	(alpha-D-mannosyl)7-beta-D-mannosyl- diacetylchitobiosyl-L-asparagine, isoform A (protein)	223.08	1.516	[M - H] - 1	458487665 ± 63646212	148636884 ± 13164304
9	Monoethyl carbonate	224.07	4.177	[M - H] - 1	246941092 ± 7837139	71459981 ± 3414768
10	Methoxyacetic acid	224.07	3.726	[M - H] - 1	5184592776 ± 72558533	2172177007 ± 122437118
11	2-hydroxypropanoic acid	224.07	5.824	[M - H] - 1	157271490 ± 13475327	8346423 ± 299209
12	Senecioic acid	236.10	2.004	[M + H] + 1	76683919 ± 56264980	10848175 ± 10483810
13	Tiglic acid	253.13	0.6	[M + NH4] + 1	1509117642 ± 2611743446	58052130 ± 35789141
14	2-Ethylacrylic acid	253.13	1.254	[M + NH4] + 1	1862909805 ± 1650227504	2059982 ± 695343
15	Angelic acid	253.13	1.784	[M + NH4] + 1	50362708 ± 86502238	9238657 ± 4771737
16	2-Ketobutyric acid	236.07	7.196	[M - H] - 1	1787233 ± 91095	3482001 ± 1733281
17	Succinic acid semialdehyde	236.07	4.368	[M - H] - 1	16202483 ± 4170048	4515125 ± 168205
18	4-Hydroxycrotonic acid	236.07	8.057	[M - H] - 1	142453769 ± 11877298	31505827 ± 3548011
19	(S)-2-Methylbutanoic acid	236.10	6.982	[M - H] - 1	3463810327 ± 108796706	7900237 ± 1585336
20	Pivalic acid	236.10	7.443	[M - H] - 1	6972066992 ± 105310392	8738812 ± 1653469
21	Pyruvatoxime	237.06	2.504	[M - H] - 1	5796134 ± 360009	248976 ± 125681
22	(R)-beta-Aminoisobutyric acid	237.10	3.625	[M - H] - 1	378160 ± 31006	176939 ± 18988
23	N-Ethylglycine	237.10	2.174	[M - H] - 1	12053298 ± 1665099	812656 ± 97973
24	N-Methylalanine	237.10	2.6	[M - H] - 1	7789054 ± 1095566	2567964 ± 546229
25	L-alpha-Aminobutyric acid	237.10	1.67	[M - H] - 1	49720914 ± 41479142	3302799 ± 417895
26	Tartronate semialdehyde	238.05	4.388	[M - H] - 1	3874452 ± 286591	1242695 ± 81426
27	3-Hydroxybutyric acid	238.08	4.952	[M - H] - 1	301806216 ± 5243093	103894796 ± 15933411
28	(S)-3-Hydroxyisobutyric acid	238.08	4.035	[M - H] - 1	102000710 ± 2677646	198892671 ± 6454186
29	(R)-3-Hydroxyisobutyric acid	238.08	4.434	[M - H] - 1	5691133 ± 4272373	8508157 ± 1139426
30	4-Carboxypyrazole	306.08	2.842	[M - H + HAc] - 1	4643029 ± 487793	54817 ± 2815
31	(2E,4E)-2,4-Hexadienoic acid	248.10	1.793	[M + H] + 1	440676 ± 407639	353192 ± 230407
32	1-Pyrroline-2-carboxylic acid	247.08	5.843	[M - H] - 1	510503052 ± 6210566	726932 ± 150549
33	cis-Acetylacrylate	248.07	7.356	[M - H] - 1	474385 ± 176040	15651520 ± 2408852
34	L-3-Cyanoalanine	248.07	9.49	[M - H] - 1	1059622 ± 22694	5370099 ± 434557
35	4-Amino-2-methylenebutanoic acid	249.10	2.188	[M - H] - 1	87350177 ± 13490729	56666811 ± 6935788
36	Levulinic acid	250.08	6.464	[M - H] - 1	178720991 ± 147448038	176955 ± 11387

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
37	2-Oxovaleric acid	250.08	9.01	[M - H] - 1	9445509 ± 2098125	945025 ± 61720
38	2-Methylacetoacetic acid	250.08	7.484	[M - H] - 1	7951379 ± 880664	8911936 ± 4062180
39	2-Ethylbutanoic acid	250.12	9.023	[M - H] - 1	12672521 ± 4077090	4951315 ± 786439
40	L-2-Amino-3-oxobutanoic acid	253.09	1.836	[M + H] + 1	73838201 ± 63551844	320289 ± 140244
41	L-Aspartate-semialdehyde	251.08	3.026	[M - H] - 1	436917309 ± 36351677	4211220 ± 762056
42	N-Methyl-a-aminoisobutyric acid	251.11	1.92	[M - H] - 1	121887846 ± 11507581	61259 ± 3837
43	5-Aminopentanoic acid	253.13	0.668	[M + H] + 1	3918736 ± 6415609	25332320 ± 20914439
44	4-Hydroxy-2-oxobutanoic acid	252.06	4.852	[M - H] - 1	27476587 ± 1089113	343028 ± 141601
45	xi-3-Hydroxy-2-oxobutanoic acid	312.08	3.66	[M - H + HAc] - 1	29764174 ± 6501749	77247104 ± 9010510
46	3-methoxy-3-oxopropanoic acid	312.08	3.087	[M - H + HAc] - 1	10120248 ± 2417413	51191 ± 3026
47	Fatty acids and conjugates	254.11	2.641	[M + H] + 1	31771483 ± 19499829	215413 ± 173051
48	Fatty acids and conjugates	254.11	2.596	[M + H] + 1	34971729 ± 59722626	974769 ± 785010
49	Fatty acids and conjugates	252.10	6.113	[M - H] - 1	2254633676 ± 58768592	72759156 ± 8909091
50	Fatty acids and conjugates	252.10	4.507	[M - H] - 1	358893190 ± 5309460	618874 ± 77632
51	Fatty acids and conjugates	252.10	5.158	[M - H] - 1	14144656 ± 3243962	3494450 ± 686593
52	Fatty acids and conjugates	252.10	4.92	[M - H] - 1	49093638 ± 4340581	7131098 ± 2311977
53	L-Allothreonine	253.09	1.592	[M - H] - 1	23010815 ± 3438091	3858495 ± 570302
54	3-Methylthiopropionic acid	254.06	6.098	[M - H] - 1	167272734 ± 7300773	122956 ± 12506
55	(S)-3,4-Dihydroxybutyric acid	254.08	2.666	[M - H] - 1	10373529 ± 1019102	2146916 ± 252088
56	2,4-Dihydroxybutanoic acid	254.08	3.154	[M - H] - 1	14557796 ± 2164021	1523900 ± 145535
57	4-Deoxyerythronic acid	254.08	2.641	[M - H] - 1	10369551 ± 972695	2127538 ± 272618
58	4-Deoxythreonic acid	254.08	2.92	[M - H] - 1	809070 ± 292135	3564964 ± 438578
59	A,b-Dihydroxyisobutyric acid	254.08	5.21	[M - H] - 1	23965796 ± 543134	10407027 ± 2942333
60	Isonicotinic acid	259.08	2.651	[M + H] + 1	102305718 ± 82584803	105725 ± 114332
61	5-Methylfuran-2-carboxylic acid	262.08	2.396	[M + H] + 1	188653836 ± 132888236	8356972 ± 9881604
62	Imidazoleacetic acid	262.08	1.284	[M + H] + 1	2506979 ± 1328936	69477 ± 14881
63	2-Heptenoic acid	262.12	8.472	[M - H] - 1	1240023698 ± 112948294	284926 ± 39986
64	1-Pyrroline-4-hydroxy-2-carboxylate	282.12	1.05	[M + NH4] + 1	33383990 ± 33487341	392110 ± 450883
65	Pyrroline hydroxycarboxylic acid	265.09	2.251	[M + H] + 1	384364073 ± 43796349	29327068 ± 48411341
66	(3R,5S)-1-pyrroline-3-hydroxy-5-carboxylic Acid	263.08	3.694	[M - H] - 1	3855850257 ± 223920707	714755282 ± 102960754
67	Vigabatrin	265.13	1.761	[M + H] + 1	37085635 ± 29307615	38956215 ± 33732957
68	Methyl hydrogen fumarate	264.06	4.297	[M - H] - 1	94878164 ± 6244568	1018471 ± 65304
69	2-Hydroxyglutaric acid lactone	264.10	11.6	[M - H] - 1	25655400 ± 17304663	22813723 ± 6117694
70	Adipate semialdehyde	264.10	10.037	[M - H] - 1	20240698 ± 888382	4271382 ± 220403
71	4-Acetylbutyrate	264.10	8.366	[M - H] - 1	1595053 ± 102771	3000545 ± 823663
72	2-Methyl-3-ketovaleric acid	264.10	7.226	[M - H] - 1	2291298 ± 171975	1838653 ± 443476

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
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-uconate	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-piperidinecarboxylic acid	279.11	3.341	[M - H] - 1	1423756 ± 37975 ± 268610 2412

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
109	N-(2-Carboxymethyl)-morpholine	279.11	4.728	[M - H] - 1	1095546044 ± 407933 ±
110	N-Propionylalanine	279.11	5.296	[M - H] - 1	71038961 163145
111	4-Guanidinobutanoic acid	279.12	2.486	[M - H] - 1	497524956 ± 48065 ±
112	2-Aminoheptanoate	281.16	1.415	[M + H] + 1	29571905 8053
113	Monomethyl glutaric acid	282.11	3.182	[M + H] + 1	1581679 ± 88408 ±
114	(3S)-3,6-Diaminohexanoate	280.14	0.785	[M - H] - 1	213311 5947
115	(3S,5S)-3,5-Diaminohexanoate	282.16	0.271	[M + H] + 1	27567023 ± 178365 ±
116	L-4-Hydroxyglutamate semialdehyde	283.10	1.575	[M + H] + 1	23590026 42513
117	O-Acetylserine	283.10	1.075	[M + H] + 1	26040569 ± 389393 ±
118	(2R,3R,4R)-2-Amino-4-hydroxy-3-methylpentanoic acid	341.15	1.511	[M - H + HAc] - 1	6174601 14572
119	3-phenylprop-2-enoic acid	284.10	3.829	[M + H] + 1	14874828 ± 1136438 ±
120	trans-Cinnamic acid	282.09	8.72	[M - H] - 1	3770143 221340
121	Penicillamine	283.09	4.195	[M - H] - 1	3320125 ± 17033 ±
122	(\xc2\xb1)-2-Hydroxy-4-(methylthio)butanoic acid	284.07	6.022	[M - H] - 1	5722971 943
123	alpha-Fluoro-beta-ureidopropionic acid	286.10	2.635	[M + H] + 1	87944654 ± 8731200 ±
124	2-Deoxypentonic acid	286.10	2.664	[M + H] + 1	75853946 12166455
125	2-Deoxyribonic acid	286.10	2.589	[M + H] + 1	157295107 ± 14428675 ±
126	3-hydroxy-2,2-bis(hydroxymethyl)propanoic acid	284.09	2.71	[M - H] - 1	269629227 11991693
127	3,4-Dimethylbenzoic acid	284.10	8.475	[M - H] - 1	2434963 ± 12879539 ±
128	4-Ethylbenzoic acid	285.10	4.733	[M - H] - 1	635237 3090466
129	3-Cresotinic acid	286.08	6.152	[M - H] - 1	356811 ± 69236 ±
130	4-Hydroxy-3-methylbenzoic acid	286.08	5.692	[M - H] - 1	97593 13797
131	2-Methoxybenzoic acid	286.08	6.66	[M - H] - 1	674959 ± 229561 ±
132	L-2-Amino-3-(1-pyrazolyl)propanoic acid	289.11	0.634	[M - H] - 1	103340 287646
133	2-Nonenoic acid	290.15	11.431	[M - H] - 1	150845352 ± 7917189 ±
134	Tranexamic Acid	292.17	11.374	[M - H] - 1	705082 696903
135	Medicanine	295.14	3.02	[M + H] + 1	251478688 ± 101178 ±
136	Mono-methyl-adipate	294.11	4.03	[M - H] - 1	25531414 33234
137	2-Indolecarboxylic acid	297.12	2.517	[M + H] + 1	114904443 ± 41101 ±
138	trans-S-(1-Propenyl)-L-cysteine	295.10	4.136	[M - H] - 1	68266446 24897
139	Glutamic acid gamma-methyl ester	295.10	4.243	[M - H] - 1	43720321 ± 112407 ±
140	hydroxybutyrylglycine	295.10	2.966	[M - H] - 1	29766910 13529
141	Acetylhomoserine	295.10	3.436	[M - H] - 1	63405399 ± 19779 ±
142	4-Hydroxycinnamic acid	298.08	7.291	[M - H] - 1	88251992 4806
143	Enol-phenylpyruvate	298.08	9.173	[M - H] - 1	14187916 ± 298762 ±
144	2-Phenylbutyric acid	300.13	4.103	[M + H] + 1	2142313 101073

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
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	2-(2-hydroxy-3-phenylpropanoic acid)	300.08	3.361	[M - H] - 1	2756231 ± 49636 ± 298648 3297
149	2-(2-hydroxy-3-phenylpropanoic 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 acid	313.09	11.622	[M - H] - 1	2827472 ± 16637152 ± 199823 1965749
170	(Z)-10-Hydroxy-8-decene-4,6-dienoic acid	313.09	6.476	[M - H] - 1	30002271 ± 10315091 ± 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-carboxylate	315.07	8.2	[M - H] - 1	101162767 ± 26850547 ± 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 acid	316.09	5.974	[M - H] - 1	30487593 ± 45370 ± 417392 21447
176	2-hydroxy-2-(3-methoxyphenyl)acetic acid	316.09	5.809	[M - H] - 1	6557547 ± 112869 ± 2121983 99540
177	2-hydroxy-2-(4-methoxyphenyl)acetic acid	316.09	5.491	[M - H] - 1	96542022 ± 110346 ± 65077753 87059
178	3-(sulfoxy)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

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
181	2-Heptenoylglycine	319.14	6.624	[M - H] - 1	107551942 ± 1217197	54158 ± 3408
182	Pyroglutamylglycine	320.10	3.259	[M - H] - 1	27875922 ± 3809371	16318665 ± 3069977
183	4,6-Dimethylnonanoic acid	320.20	12.19	[M - H] - 1	8577731 ± 885997	2548002 ± 2169931
184	N-Heptanoylglycine	323.17	3.735	[M + H] + 1	7592240 ± 194459	10424 ± 745
185	8-Amino-7-oxononanoic acid	323.17	3.75	[M + H] + 1	21001484 ± 153920	10221 ± 650
186	Glycyl-Isoleucine	322.15	3.35	[M - H] - 1	5635341 ± 1421294	52481 ± 30081
187	5-Hydroxyindoleacetic acid	325.09	6.749	[M - H] - 1	80976425 ± 4891662	47719 ± 3762
188	4-Anilino-4-oxobutanoic acid	327.11	6.873	[M - H] - 1	6392717 ± 140654	235533 ± 172660
189	3-Dehydro-L-gulonate	328.08	1.927	[M - H] - 1	19632441 ± 1938316	203936 ± 8229
190	Monoethyl phthalate	328.09	7.029	[M - H] - 1	14467813 ± 356071	44235 ± 2248
191	5-(4-hydroxyphenyl)pentanoic acid	328.13	8.105	[M - H] - 1	183963030 ± 15285663	40100 ± 3044
192	Leucodopachrome	331.10	3.228	[M + H] + 1	526698 ± 420159	19969 ± 961
193	Dopaquinone	329.09	7.028	[M - H] - 1	1725391 ± 581361	94224 ± 80698
194	L-Dopaquinone	329.09	5.186	[M - H] - 1	1919681 ± 65458	117223 ± 123659
195	Metyrosine	329.09	5.53	[M - H] - 1	1512896 ± 881048	55444 ± 30166
196	Gulonic acid	330.09	4.457	[M - H] - 1	1760105861 ± 129866114	8090776 ± 614995
197	Gluconic acid	330.09	1.694	[M - H] - 1	141707008 ± 20621354	13400913 ± 3618568
198	(S)-2-(4-Methoxyphenoxy)propanoic acid	332.12	3.182	[M + H] + 1	91293128 ± 2062863	448672 ± 16791
199	2-(3,5-dimethoxyphenyl)acetic acid	390.13	6.593	[M - H + HAc] - 1	88547292 ± 2053671	54152 ± 4997
200	(S)-Batatic acid	330.11	6.246	[M - H] - 1	231228281 ± 5392957	57698 ± 4298
201	L- α -Amino-1H-pyrrole-1-hexanoic acid	332.17	2.958	[M + H] + 1	19478437 ± 2667138	17172 ± 1147
202	2-methyl-4-(sulfooxy)butanoic acid	332.06	4.804	[M - H] - 1	83155581 ± 3838889	39828 ± 1554
203	5-(sulfooxy)pentanoic acid	332.06	4.37	[M - H] - 1	36907998 ± 1866491	67315 ± 1757
204	trans-Dodec-2-enoic acid	332.20	12.151	[M - H] - 1	3414780 ± 701029	3556575 ± 3855169
205	2-octenoylglycine	335.17	3.801	[M + H] + 1	3815752 ± 56993	13985 ± 831
206	3-octenoylglycine	335.17	3.763	[M + H] + 1	3247986 ± 95378	16512 ± 1154
207	N-Acetylaminooctanoic acid	335.17	8.436	[M - H] - 1	254623752 ± 38033276	124045 ± 7782
208	N-(5-Methyl-3-oxohexyl)alanine	335.17	8.352	[M - H] - 1	32078566 ± 869294	31831 ± 3475
209	R-2-Hydroxy-3-methylbutanoic acid 3-Methylbutanoyl	336.16	6.468	[M - H] - 1	8555654 ± 847309	117204 ± 11661
210	N-Acetylisoputresnine	338.18	3.003	[M + H] + 1	14410756 ± 680847	12787 ± 1274
211	D-Tryptophan	338.13	5.705	[M - H] - 1	22623825 ± 3536393	17866954 ± 3968802
212	Zeanic acid	339.07	7.299	[M - H] - 1	4030088 ± 355673	92055 ± 40593
213	3-Indolehydracrylic acid	339.11	7.3	[M - H] - 1	46977371 ± 1342344	2360537 ± 426453
214	5-Methoxyindoleacetate	339.11	6.338	[M - H] - 1	32803368 ± 1490625	31758 ± 1898
215	N-Propionylmethionine	341.13	3.291	[M + H] + 1	3791811 ± 435019	11764 ± 981
216	2-Amino-4-hydroxy-6-pteridinecarboxylic acid	343.10	2.643	[M + H] + 1	13643172 ± 11605388	73043 ± 89828

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
217	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	343.10	3.032	[M + H] ⁺ + 1	4926716 ± 14291 ±
218	triazolopropionic acid	341.10	9.072	[M - H] ⁻ - 1	185015 2885 2178392 ± 84917 ±
219	6-Amino-9H-purine-9-propanoic acid	401.13	4.9	[M - H + HAc] ⁻ - 1	1048846 6258 175959193 ± 256652 ± 1
220	3-Phenylpropionylglycine	343.14	3.686	[M + H] ⁺ + 1	9078502 19357 47607214 ± 36444 ±
221	Dihydrolipoate	402.12	5.421	[M - H + HAc] ⁻ - 1	1077980 14447 622208098 ± 38010336 ±
222	3-(3,4-Dimethoxyphenyl)-2-propenoic acid	344.12	2.918	[M + H] ⁺ + 1	38919379 10167513 17638660 ± 123198 ±
223	3,4-Dihydroxyphenylvaleric acid	346.14	3.305	[M + H] ⁺ + 1	859455 156293 25948519 ± 13913 ±
224	2-Methoxy-3-(4-methoxyphenyl)propanoic acid	344.13	6.59	[M - H] ⁻ - 1	2294846 1062 205245407 ± 52629 ±
225	Cucurbitic acid	348.19	4.795	[M + H] ⁺ + 1	2715599 4856 15736700 ± 178444 ±
226	Dihydrojasmonic acid	348.19	4.732	[M + H] ⁺ + 1	4166507 41304 18681975 ± 261157 ±
227	3-hydroxy-2-methyl-2-[(sulfooxy)methyl]propanoic acid	348.05	1.76	[M - H] ⁻ - 1	17465330 57845 112459094 ± 110962 ±
228	9-Methyldodecanoic acid	348.23	12.844	[M - H] ⁻ - 1	15500812 5421 277094 ± 2426122 ±
229	2-hydroxyoct-2-enoylglycine	349.15	10.059	[M - H] ⁻ - 1	225202 1251444 32712097 ± 95923 ±
230	xi-5-Hydroxydodecanoic acid	350.21	11.513	[M - H] ⁻ - 1	966406 8313 9600303 ± 501983 ±
231	Leucyl-Serine	352.16	5.02	[M - H] ⁻ - 1	233980 83437 35981421 ± 89528 ±
232	(2Z)-2-(phenylmethylidene)heptanoic acid	352.16	5.233	[M - H] ⁻ - 1	1015854 24768 26862526 ± 69676 ±
233	Glycyl-Phenylalanine	358.15	3.029	[M + H] ⁺ + 1	899769 29103 6341464 ± 9407 ±
234	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	357.08	3.61	[M - H] ⁻ - 1	2542718 1899 19369899 ± 127600 ±
235	Salsolinol 1-carboxylate	357.12	5.318	[M - H] ⁻ - 1	1809789 17641 160824385 ± 77612 ±
236	2-hydroxyphenylpropionylglycine	357.12	9.578	[M - H] ⁻ - 1	5190562 63646 36220356 ± 72015 ±
237	Goshuyic acid	358.21	12.391	[M - H] ⁻ - 1	8157783 29263 4858876 ± 5590352 ±
238	3-(3,4-dihydroxy-5-methoxyphenyl)oxirane-2-carboxylic acid	360.08	1.919	[M - H] ⁻ - 1	242757 3093250 440578 ± 12277 ±
239	2,3,4,5,6,7-Hexahydroxyheptanoic acid	360.10	4.269	[M - H] ⁻ - 1	173758 1054 371551509 ± 70261443 ±
240	5-(3,4,5-trihydroxyphenyl)pentanoic acid	362.13	1.317	[M + H] ⁺ + 1	26033213 7432650 281456 ± 18467 ±
241	5-(3,5-dihydroxyphenyl)-4-hydroxypentanoic acid	362.13	2.919	[M + H] ⁺ + 1	413594 4098 106945309 ± 124907 ±
242	b ⁴ -4-Hydroxy-5-(3,5-dihydroxyphenyl)-valeric acid	360.12	5.279	[M - H] ⁻ - 1	3957680 158460 287090966 ± 26714 ±
243	b ⁴ -4-Hydroxy-(3,4-dihydroxyphenyl)-valeric acid	360.12	5.514	[M - H] ⁻ - 1	16864207 629 60249031 ± 30572 ±
244	5-Tetradecenoic acid	360.23	12.667	[M - H] ⁻ - 1	1804785 1395 7452323 ± 5224125 ±
245	5Z-Tetradecenoic acid	360.23	12.829	[M - H] ⁻ - 1	1568864 377259 496992 ± 3050486 ±
246	Tsuzuic acid	360.23	12.75	[M - H] ⁻ - 1	395093 1241632 1284972 ± 5741761 ±
247	Mevalonic acid-5P	362.08	3.137	[M - H] ⁻ - 1	605943 1385457 1116119 ± 35967 ±
248	2-hydroxy-3,4,5-trimethoxybenzoic acid	362.10	5.061	[M - H] ⁻ - 1	860148 2409 75884655 ± 117575 ±
249	12-Methyltridecanoic acid	362.24	13.168	[M - H] ⁻ - 1	1386412 32863 73882862 ± 67741975 ±
250	2,6,10-Trimethylundecanoic acid	362.25	13.078	[M - H] ⁻ - 1	24830050 11433835 19542478 ± 407767 ±
251	Glycyl-Arginine	365.18	8.09	[M - H] ⁻ - 1	6376340 77424 2358321 ± 36761 ±
					81605 1927

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
252	Salsoline-1-carboxylate	373.15	3.047	[M + H] ⁺ + 1	6362589 ± 13105 ± 1683811 5140
253	3-(3,4,5-Trimethoxyphenyl)propanoic acid	374.14	8.104	[M - H] ⁻ - 1	48924645 ± 38687 ± 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-yl)methylidene]amino]acetic acid	383.10	10.939	[M - H] ⁻ - 1	6323472 ± 118488 ± 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-trimethoxyphenyl)propanoic acid	390.13	6.59	[M - H] ⁻ - 1	88547292 ± 53525 ± 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-nomulosonic acid	404.13	2.649	[M + H] ⁺ + 1	284073 ± 14630952 ± 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-yl)methoxy]oxane-2-carboxylic acid	414.15	4.637	[M + H] ⁺ + 1	19217658 ± 90521 ± 4411895 6965
283	3,4,5-trihydroxy-6-[(3-methylbutanoyl)oxy]oxane-2-carboxylic acid	412.14	9.422	[M - H] ⁻ - 1	19450281 ± 58025 ± 943617 2795
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

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
287	3,4,5-trihydroxy-6-[(3-hydroxybutanoyl)oxy]oxane-2-carboxylic acid	414.12	8.168	[M - H] - 1	706597767 ± 24989472	136155 ± 54460
288	Tyrosyl-Valine	414.18	4.492	[M - H] - 1	1247190 ± 658172	24904 ± 4829
289	Bovinic acid	416.29	9.488	[M + H] + 1	562037780 ± 83001976	2307000 ± 798698
290	(9E,11E)-Octadecadienoic acid	416.29	9.822	[M + H] + 1	174914828 ± 21737726	3328836 ± 4605771
291	Dihomolinoleic acid	416.29	9.712	[M + H] + 1	488647177 ± 80759240	1620981 ± 160741
292	5-Octadecynoic acid	416.29	11.458	[M + H] + 1	5607810 ± 3308500	57252158 ± 8867798
293	6Z,9Z-octadecadienoic acid	416.29	13.896	[M + H] + 1	67451068 ± 30079106	665948 ± 344192
294	Octadecadienoate	416.29	9.615	[M + H] + 1	259619642 ± 130161609	1555275 ± 347600
295	(10E,12Z)-Octadecadienoic acid	414.28	13.498	[M - H] - 1	57038511 ± 23912024	33771010 ± 3873450
296	Mangiferic acid	414.28	13.414	[M - H] - 1	676271339 ± 266834677	824087609 ± 110539718
297	Elaidic acid	416.29	13.89	[M - H] - 1	345423297 ± 155279070	589882789 ± 97391985
298	Vaccenic acid	416.29	13.978	[M - H] - 1	123869005 ± 54460008	27280370 ± 5217242
299	16-Methylheptadecanoic acid	418.31	14.424	[M - H] - 1	10439852 ± 3930146	128642288 ± 34500866
300	xanthurenic acid 8-O-sulfate	419.03	10.048	[M - H] - 1	517087 ± 514753	205207 ± 88643
301	12-hydroxyheptadecanoic acid	420.29	12.39	[M - H] - 1	20657364 ± 2632544	171209 ± 72238
302	Orotidine	422.10	3.158	[M - H] - 1	8172693 ± 6969485	30250 ± 1926
303	Porric acid	422.10	5.508	[M - H] - 1	36755714 ± 3624668	67250 ± 15454
304	3,5-Dihydroxyphenylvaleric acid sulfate	424.08	5.899	[M - H] - 1	72314336 ± 3330294	47565 ± 1244
305	3,4,5-trihydroxy-6-[(4-hydroxy-3-methylbut-2-enoyl)oxy]oxane-2-carboxylic acid	445.16	3.859	[M + NH4] + 1	2047231 ± 27120	96163 ± 3734
306	4-Hydroxyproline galactoside	487.17	5.655	[M - H + HAc] - 1	131390623 ± 5723075	426038 ± 109801
307	17-Hydroxylinolenic acid	428.26	11.762	[M - H] - 1	2567010 ± 1301141	5148556 ± 134212
308	(Z)-13-Oxo-9-octadecenoic acid	430.27	12.372	[M - H] - 1	4527380 ± 934039	8539429 ± 1171168
309	Avenoleic acid	430.27	12.1	[M - H] - 1	29642015 ± 2317532	92203928 ± 33278090
310	12-Hydroxy-8,10-octadecadienoic acid	430.27	12.67	[M - H] - 1	916569 ± 1164755	6382616 ± 1198719
311	(10E,12Z)-9-HODE	430.27	12.474	[M - H] - 1	5124568 ± 898704	11353262 ± 4357069
312	13-HODE	430.27	12.167	[M - H] - 1	26291952 ± 1780901	77040779 ± 12269888
313	alpha-Dimorphocolic acid	430.27	11.929	[M - H] - 1	6069564 ± 2188196	21720795 ± 3055423
314	9,10-Epoxyoctadecenoic acid	430.27	11.858	[M - H] - 1	4797819 ± 1032732	11283123 ± 2319925
315	12,13-EpOME	430.27	12.875	[M - H] - 1	2267111 ± 2281884	4829389 ± 847226
316	Phenethylamine glucuronide	430.27	12.257	[M - H] - 1	9251186 ± 315903	32181745 ± 41126080
317	3-Oxo-octadecanoic acid	432.29	12.823	[M - H] - 1	1572760 ± 174555	2572358 ± 511016
318	9-Oxo-octadecanoic acid	432.29	12.469	[M - H] - 1	20085576 ± 4030884	11604607 ± 1765007
319	10-Oxo-octadecanoic acid	432.29	12.339	[M - H] - 1	510669565 ± 89374880	9396774 ± 8198094
320	11-Oxo-octadecanoic acid	432.29	12.277	[M - H] - 1	122641516 ± 17057886	22288090 ± 15318462
321	5-Hexyltetrahydro-2-furanooctanoic acid	432.29	12.883	[M - H] - 1	399730 ± 64764	1123125 ± 334858

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
322	(R)-3-Hydroxy-Octadecanoic acid	434.30	12.56	[M - H] - 1	1284577242 ± 305296771	7545156 ± 4668860
323	xi-10-Hydroxyoctadecanoic acid	434.30	12.695	[M - H] - 1	27938700 ± 5715126	2261349 ± 91408
324	Pyrogallol-2-O-glucuronide	436.10	11.189	[M - H] - 1	539805 ± 160230	3253620 ± 562979
325	6-(2,4-dihydroxyphenoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	436.10	11.349	[M - H] - 1	2213769 ± 719244	21151840 ± 2364104
326	6-(2,5-dihydroxyphenoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	436.10	11.63	[M - H] - 1	2068500 ± 373176	22519723 ± 1015590
327	6-(3,4-dihydroxyphenoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid	436.10	11.46	[M - H] - 1	3247448 ± 664244	29391873 ± 2501473
328	Porric acid A	436.11	5.843	[M - H] - 1	92312578 ± 3795837	35725 ± 431
329	Cis-8,11,14,17-Eicosatetraenoic acid	440.29	11.266	[M + H] + 1	1733568 ± 489736	53280633 ± 41031354
330	2-(3,4-Dihydroxybenzoyloxy)-4,6-dihydroxybenzoate	440.07	13.6	[M - H] - 1	356155 ± 250378	82829 ± 2856
331	5-(3,4-dihydroxyphenyl)-4-(sulfooxy)pentanoic acid	440.08	4.794	[M - H] - 1	176423351 ± 2727594	34249 ± 1355
332	3,4-dihydroxy-2-(4-hydroxy-3,7-dimethylocta-2,6-dien-1-yl)benzoic acid	440.18	0.955	[M - H] - 1	21206815 ± 18068363	535907 ± 30832
333	3,4-dihydroxy-2-[(2Z)-4-hydroxy-3-(4-methylpent-3-en-1-yl)but-2-en-1-yl]benzoic acid	440.18	0.91	[M - H] - 1	34747475 ± 7183172	1427681 ± 727074
334	b"5-Carboxy-gamma-chromanol"	442.23	5.534	[M + H] + 1	39410744 ± 770524	232039 ± 10693
335	5,8,11-Eicosatrienoic acid	440.29	13.577	[M - H] - 1	10228924 ± 5254138	16089071 ± 2598430
336	Corchorifatty acid A	442.23	3.328	[M - H] - 1	39191074 ± 8328846	16582274 ± 2020810
337	Eicosadienoic acid	442.31	13.969	[M - H] - 1	1632999 ± 1028883	5330577 ± 825514
338	3,4,5-trihydroxy-6-{[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoyl]oxy}oxane-2-carboxylic acid	446.14	3.287	[M + H] + 1	14686973 ± 385197	144711 ± 36494
339	(R)-2-Hydroxysterculic acid	444.29	12.867	[M - H] - 1	2656442 ± 1286306	3269807 ± 212336
340	8(R)-Hydroperoxylinoic acid	446.27	12.188	[M - H] - 1	12593976 ± 1999137	37783556 ± 11286390
341	(\xc2\xb1)-(E)-13-Hydroxy-10-oxo-11-octadecenoic acid	446.27	12.051	[M - H] - 1	2831245 ± 905725	8969604 ± 2221456
342	Beta-D-Glucopyranuronic acid	448.10	12.379	[M - H] - 1	317677 ± 165203	8922364 ± 674867
343	3,4,5-trihydroxy-6-(3-hydroxybenzoyloxy)oxane-2-carboxylic acid	448.10	11.648	[M - H] - 1	422340 ± 54500	10693456 ± 5702913
344	9,10-Epoxyoctadecanoic acid	448.28	11.641	[M - H] - 1	87004989 ± 3973815	1374409 ± 184155
345	12,13-DHOME	448.28	12.358	[M - H] - 1	1026380 ± 608068	2297055 ± 1494177
346	9,10-DHOME	448.28	12.418	[M - H] - 1	1898753 ± 312906	3003796 ± 1371028
347	(+)-15,16-Dihydroxyoctadecanoic acid	452.31	7.996	[M + H] + 1	182080968 ± 49497922	1005144 ± 477658
348	(9S,10S)-9,10-dihydroxyoctadecanoate	452.31	7.597	[M + H] + 1	33056391 ± 2714072	961508 ± 142134
349	Valproic acid glucuronide	456.20	3.676	[M + H] + 1	32799856 ± 3113321	123805 ± 7280
350	2-Hydroxy-6-tridecylbenzoic acid	454.27	12.179	[M - H] - 1	371798 ± 232645	10320632 ± 7951616
351	19(S)-HETE	454.27	12.35	[M - H] - 1	267167 ± 38896	11165932 ± 1027869
352	10-HETE	454.27	12.615	[M - H] - 1	203539 ± 48719	41648687 ± 2147758
353	13-HETE	454.27	12.428	[M - H] - 1	437565 ± 96921	15692639 ± 3729459
354	12 Hydroxy arachidonic acid	454.27	12.282	[M - H] - 1	222454 ± 74787	11264070 ± 2210161
355	12S-hydroxy-5E,8Z,10Z,14Z-eicosatetraenoic acid	454.27	12.451	[M - H] - 1	100756 ± 17609	4218983 ± 1545730

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
356	Lepidimoic acid	458.14	3.472	[M + H] ⁺ + 1	24066028 ± 19662 ± 5739940 1735
357	3,4-Dimethyl-5-pentyl-2-furannonanoic acid	456.29	12.047	[M - H] ⁻ - 1	23661841 ± 256504 ± 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-hydroxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	474.12	12.01	[M - H] ⁻ - 1	285412 ± 10033224 ± 36657 561452
365	3,4,5-trihydroxy-6-[[[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	474.12	11.83	[M - H] ⁻ - 1	561451 ± 15328466 ± 154877 2978586
366	3,4,5-trihydroxy-6-[[[3-(3-hydroxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	474.12	12.27	[M - H] ⁻ - 1	230347 ± 4180602 ± 28396 2422104
367	3,4,5-trihydroxy-6-[3-(4-methoxyphenyl)propoxy]oxane-2-carboxylic acid	478.18	3.776	[M + H] ⁺ + 1	6144013 ± 8744 ± 619614 750
368	13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid	476.28	12.356	[M - H] ⁻ - 1	707597 ± 11725314 ± 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-methoxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.33	[M - H] ⁻ - 1	764298 ± 65035070 ± 193256 4754398
374	3,4,5-trihydroxy-6-[[[(2E)-3-(3-methoxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	12.013	[M - H] ⁻ - 1	358182 ± 37143083 ± 28055 645807
375	3,4,5-trihydroxy-6-[[[(2E)-2-(hydroxymethyl)-3-phenylprop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.865	[M - H] ⁻ - 1	3288892 ± 164304226 ± 904240 19453671
376	3,4,5-trihydroxy-6-[[[(2E)-3-(3-hydroxyphenyl)-2-methylprop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.716	[M - H] ⁻ - 1	5244846 ± 257754514 ± 1462206 29782660
377	3,4,5-trihydroxy-6-[[[(2E)-3-(4-hydroxyphenyl)-2-methylprop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.641	[M - H] ⁻ - 1	1183413 ± 110937452 ± 344044 11980258
378	3,4,5-trihydroxy-6-[[[(2E)-3-(2-hydroxyphenyl)-2-methylprop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.572	[M - H] ⁻ - 1	552018 ± 52501894 ± 248208 2188003
379	3,4,5-trihydroxy-6-[[[3-(4-methoxyphenyl)prop-2-enoyl]oxy]oxane-2-carboxylic acid	488.13	11.208	[M - H] ⁻ - 1	764298 ± 11449342 ± 193256 2788799
380	Sulindac	490.12	12.5	[M - H] ⁻ - 1	248999 ± 1067937 ± 124486 44336
381	3,4,5-trihydroxy-6-[5-hydroxy-2-methoxy-4-(prop-2-en-1-yl)phenoxy]oxane-2-carboxylic acid	492.16	7.476	[M + H] ⁺ + 1	415073 ± 32153032 ± 50302 1481907
382	3,4,5-trihydroxy-6-[5-hydroxy-4-methoxy-2-(prop-2-en-1-yl)phenoxy]oxane-2-carboxylic acid	492.16	6.333	[M + H] ⁺ + 1	265877 ± 30343221 ± 117576 1013625
383	3,4,5-trihydroxy-6-[[[3-(3-methoxyphenyl)propanoyl]oxy]oxane-2-carboxylic acid	492.16	6.393	[M + H] ⁺ + 1	283221 ± 80304902 ± 98823 15929303

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people					
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity) Serum (Intensity)
384	3,4,5-trihydroxy-6-{{[2-(4-methoxyphenyl)propanoyl]oxy}oxane-2-carboxylic acid	490.15	11.461	[M - H] - 1	231668 ± 24742 2885292 ± 641625
385	Tetracosahexaenoic acid	492.32	7.847	[M + H] + 1	7220101994 ± 144799958 1726046 ± 120207
386	6,9,12,15,18,21-Tetracosahexaenoic acid	492.32	6.284	[M + H] + 1	171476795 ± 42496040 201112 ± 263735
387	Tetracosahexaenoic acid, n-3	492.32	5.731	[M + H] + 1	275527792 ± 16577519 517583 ± 407316
388	5-hydroxy-2-{{[1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-ylidene]amino}-4-methoxybenzoic acid	493.13	6.238	[M - H] - 1	83824095 ± 5817825 53113 ± 7086
389	Rosmarinic acid	494.12	6.676	[M - H] - 1	69856616 ± 5550646 37698 ± 3154
390	Fenuloyl C1-glucuronide	504.13	11.828	[M - H] - 1	1150529 ± 12608 4030880 ± 859188
391	Isofenuloyl C1-glucuronide	504.13	10.569	[M - H] - 1	761604 ± 15570 11722683 ± 1247781
392	3,4,5-trihydroxy-6-{{[3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}oxane-2-carboxylic acid	504.13	10.791	[M - H] - 1	366531 ± 48454 4752867 ± 340214
393	3,4,5-trihydroxy-6-{{[3-(3-hydroxy-5-methoxyphenyl)prop-2-enoyl]oxy}oxane-2-carboxylic acid	504.13	11.361	[M - H] - 1	978673 ± 64639 19126186 ± 674346
394	3,4,5-trihydroxy-6-{{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}oxane-2-carboxylic acid	504.13	11.203	[M - H] - 1	541613 ± 25396 10096751 ± 2230534
395	3,4,5-trihydroxy-6-{{[3-(2-hydroxy-4-methoxyphenyl)prop-2-enoyl]oxy}oxane-2-carboxylic acid	504.13	10.956	[M - H] - 1	403501 ± 13037 9765280 ± 2718087
396	3,4,5-trihydroxy-6-{{[3-(4-methoxyphenyl)-2-oxopropanoyl]oxy}oxane-2-carboxylic acid	504.13	10.69	[M - H] - 1	451786 ± 31638 5949088 ± 1085216
397	(3R)-3-(tetradecanoyloxy)-4-(trimethylazaniumyl)butanoate	507.35	10.39	[M + H] + 1	188234605 ± 73194844 871188 ± 199932
398	Ethyl gallate 3-glucuronide	508.12	8.985	[M - H] - 1	105752943 ± 45846541 163314 ± 7882
399	2-(10-Heptadecenyl)-6-hydroxybenzoic acid	508.32	13.021	[M - H] - 1	12794494 ± 4306336 97035 ± 3888
400	12b-Hydroxy-5b-cholanoic acid	510.33	12.428	[M - H] - 1	641446664 ± 189084164 949703 ± 735439
401	Allolithocholic acid	510.33	12.025	[M - H] - 1	115231454 ± 38319237 359177 ± 362857
402	7a-Hydroxy-5b-cholanoic acid	510.33	11.916	[M - H] - 1	1156055 ± 338435 250890 ± 247541
403	6-[[4,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran-5-yl]oxy]-3,4,5-trihydroxyoxane-2-carboxylic acid	520.16	12.006	[M - H] - 1	1870162 ± 475219 11915130 ± 2335910
404	Treprostinil	524.28	12.31	[M - H] - 1	980525 ± 523501 8520699 ± 451539
405	b“D8-Merulinic acid A”	524.31	11.366	[M - H] - 1	541158745 ± 374961606 449488 ± 279265
406	7-Hydroxy-3-oxocholanoic acid	524.31	11.592	[M - H] - 1	5235781 ± 3448206 169193 ± 8232
407	11-Hydroperoxy-H4-neuroprostane	526.26	11.574	[M - H] - 1	331036 ± 85161 100458 ± 838
408	3a,12b-Dihydroxy-5b-cholanoic acid	526.33	11.944	[M - H] - 1	1812605216 ± 192418223 4679585 ± 378889
409	3b,12a-Dihydroxy-5a-cholanoic acid	526.33	10.534	[M - H] - 1	679106438 ± 40615214 388461 ± 78335
410	3b,7a-Dihydroxy-5b-cholanoic acid	526.33	11.778	[M - H] - 1	548961777 ± 30873343 2777820 ± 251814
411	3a,7a-Dihydroxycholanoic acid	526.33	10.902	[M - H] - 1	660581393 ± 34926669 322672 ± 217922
412	Allodeoxycholic acid	526.33	11.604	[M - H] - 1	44284334 ± 852858 506183 ± 327197
413	Allochenodeoxycholic acid	526.33	11.871	[M - H] - 1	29068727 ± 3682524 186069 ± 5434
414	Murocholic acid	526.33	11.249	[M - H] - 1	148446058 ± 9847095 287273 ± 26392

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
415	7a,12b-dihydroxy-5b-Cholan-24-oic acid	526.33	11.33	[M - H] - 1	932019319 ± 348382725	436568 ± 286077
416	3beta,12beta-Dihydroxy-5beta-cholanoic acid	526.33	12.199	[M - H] - 1	232158793 ± 10792209	476448 ± 7844
417	Sinapinic acid-O-glucuronide isomer	534.14	11.937	[M - H] - 1	1217503 ± 81133	3553889 ± 730667
418	3,4,5-trihydroxy-6-{[6-(3-hydroxyprop-1-en-1-yl)-5-methoxy-2H-1,3-benzodioxol-4-yl]oxy}oxane-2-carboxylic acid	534.14	11.325	[M - H] - 1	1832391 ± 1084795	142882837 ± 7978855
419	3,4,5-trihydroxy-6-[2-hydroxy-3,4-dimethoxy-5-(3-oxoprop-1-en-1-yl)phenoxy]oxane-2-carboxylic acid	534.14	11.57	[M - H] - 1	634692 ± 486133	53948195 ± 4992807
420	b''5-(3,4,5-Trihydroxyphenyl)-gamma-valerolactone-3-O-glucuronide"	534.14	11.654	[M - H] - 1	387193 ± 106043	51877403 ± 14353027
421	b''5-(3,4,5-Trihydroxyphenyl)-gamma-valerolactone-4-O-glucuronide"	536.19	4.204	[M + H] + 1	25890202 ± 1901296	146487 ± 4740
422	b''4-Hydroxy-5-(3,4-dihydroxyphenyl)-valeric acid-O-glucuronide"	536.15	11.452	[M - H] - 1	311804 ± 56347	39471028 ± 2427874
423	6-{[5-(3,4-dihydroxyphenyl)-4-hydroxypentanoyl]oxy}-3,4,5-trihydroxyoxane-2-carboxylic acid	536.15	11.878	[M - H] - 1	193855 ± 22678	18955563 ± 1049628
424	3,4,5-trihydroxy-6-[2-hydroxy-3,4-dimethoxy-5-(3-oxopropyl)phenoxy]oxane-2-carboxylic acid	536.15	11.51	[M - H] - 1	343919 ± 116260	35256359 ± 3190656
425	7-Ketodeoxycholic acid	542.32	4.197	[M + H] + 1	157854029 ± 9462565	156172 ± 5053
426	3,7-Dihydroxy-12-oxocholanoic acid	542.32	3.838	[M + H] + 1	9016731 ± 878392	10773 ± 1084
427	1b,3a,12a-Trihydroxy-5b-cholanoic acid	542.32	10.137	[M - H] - 1	167611063 ± 4327022	114235 ± 4658
428	1b,3a,7a-Trihydroxy-5b-cholanoic acid	542.32	11.148	[M - H] - 1	544907160 ± 4565684	3155128 ± 56662
429	3alpha,7alpha,12beta-Trihydroxy-5beta-cholanoic acid	542.32	9.526	[M - H] - 1	1241848325 ± 21099752	64094 ± 2673
430	3a,4b,7a-Trihydroxy-5b-cholanoic acid	542.32	10.707	[M - H] - 1	3495175556 ± 579880	674030 ± 577596
431	3a,4b,12a-Trihydroxy-5b-cholanoic acid	542.32	9.706	[M - H] - 1	195680988 ± 10657635	224977 ± 9852
432	1,3,12-Trihydroxycholan-24-oic acid	542.32	8.65	[M - H] - 1	925432007 ± 59000243	88584 ± 12829
433	3b,7a,12a-Trihydroxy-5a-Cholanoic acid	542.32	9.97	[M - H] - 1	27588201 ± 4722587	429960 ± 32149
434	3a,7b,12b-Trihydroxy-5b-cholanoic acid	542.32	8.09	[M - H] - 1	35540636 ± 3548529	38071 ± 1995
435	3b,7b,12a-Trihydroxy-5b-cholanoic acid	542.32	8.82	[M - H] - 1	25513288 ± 9016311	75397 ± 3160
436	2b,3a,7a-Trihydroxy-5b-cholanoic acid	542.32	8.973	[M - H] - 1	21080013 ± 14979217	118675 ± 8489
437	Trovafl oxacin	552.16	5.011	[M + H] + 1	25987908 ± 5996264	16523986 ± 1033800
438	3,4,5-trihydroxy-6-{4-hydroxy-2-[(2E)-3-phenylprop-2-enoyl]phenoxy}oxane-2-carboxylic acid	550.14	10.286	[M - H] - 1	48373249 ± 12447522	18790255 ± 2443497
439	3,4,5-trihydroxy-6-[4-hydroxy-3-(3-phenylprop-2-enoyl)phenoxy]oxane-2-carboxylic acid	550.14	10.386	[M - H] - 1	44046888 ± 12250562	14346146 ± 2123539
440	7,14-dihydroxy-6-methoxy-4,12-dimethyl-5-(3-methylbut-2-enoyl)-10-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	562.14	11.722	[M - H] - 1	199302 ± 25981	8211896 ± 372731
441	6-[2-(benzoyloxy)-5-(prop-2-en-1-yl)phenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid	564.16	10.648	[M - H] - 1	240806 ± 7836	196687 ± 120183
442	3,4,5-trihydroxy-6-{2-[(1E)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl]phenoxy}oxane-2-carboxylic acid	564.16	11.847	[M - H] - 1	812681 ± 311959	54894976 ± 17140906

TABLE 3-continued

Determination results of concentrations of substances in blood and feces of normal people						
NO.	Name	m/z	RT (min)	Reference Ion	Fecal (Intensity)	Serum (Intensity)
443	3,4,5-trihydroxy-6-{[5-methoxy-2-[(2E)-3-phenylprop-2-enoyl]phenoxy]oxane-2-carboxylic acid	564.16	11.781	[M - H] - 1	879920 ± 197511	46776254 ± 7914696
444	3,4,5-trihydroxy-6-[4-[(1E)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl]phenoxy]oxane-2-carboxylic acid	564.16	11.532	[M - H] - 1	1396695 ± 92231	54509330 ± 19840841
445	3,4,5-trihydroxy-6-[3-[(1E)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl]phenoxy]oxane-2-carboxylic acid	564.16	11.953	[M - H] - 1	1383091 ± 92167	10848077 ± 3236227
446	Ketoprofen glucuronide	564.16	11.213	[M - H] - 1	2544589 ± 1554692	4777722 ± 1434362
447	3,4,5-trihydroxy-6-[[8-methoxy-6-(3-methylbut-2-en-1-yl)-2-oxo-2H-chromen-7-yl]oxy]oxane-2-carboxylic acid	570.17	4.615	[M - H] - 1	228973368 ± 15107157	600200 ± 74242
448	simvastatin hydroxy acid	570.32	11.389	[M - H] - 1	34301301 ± 5036658	166890 ± 11744
449	Varanic acid	570.35	11.427	[M - H] - 1	3360794 ± 262300	99464 ± 4778
450	Formononetin 7-glucuronide	580.15	3.987	[M + H] + 1	77327 ± 55313	194116 ± 131885
451	3,4,5-trihydroxy-6-[(8-methoxy-4-oxo-2-phenyl-3,4-dihydro-2H-1-benzopyran-7-yl)oxy]oxane-2-carboxylic acid	580.15	8.439	[M - H] - 1	115818670 ± 14734095	104279 ± 6542
452	N-Stearoyl tyrosine	581.37	12.27	[M - H] - 1	12635191 ± 2188071	120789 ± 2548
453	3alpha,7alpha,12alpha-trihydroxy-5beta-cholestanate	584.37	11.903	[M - H] - 1	14617490 ± 1062188	96856 ± 3422
454	3,4,5-trihydroxy-6-{6-hydroxy-3-[3-(3-hydroxyphenyl)-3-oxoprop-1-en-1-yl]-2-methoxyphenoxy]oxane-2-carboxylic acid	596.15	8.486	[M - H] - 1	20764642 ± 1225036	27169164 ± 5774647
455	(3alpha,20R,24Z)-3-Hydroxy-21-oxooeupa-8,24-dien-26-oic acid	606.39	9.368	[M + H] + 1	98724208 ± 33043801	4768874 ± 165596
456	23-Hydroxy-3-oxocycloart-24-en-26-oic acid	604.38	12.257	[M - H] - 1	7915874 ± 2772630	95440 ± 9300
457	Chenodeoxycholic acid 3-sulfate	606.29	9.184	[M - H] - 1	108202813 ± 38912268	28217 ± 632
458	Ursodeoxycholic acid 3-sulfate	606.29	10.816	[M - H] - 1	200146167 ± 64217935	350728 ± 420289
459	Chenodeoxycholic acid sulfate	606.29	9.759	[M - H] - 1	18347698 ± 4837735	95308 ± 4174
460	b''4-O-Methylepicatechin 7-O-glucuronide"	614.16	7.92	[M - H] - 1	65767168 ± 3359931	92340 ± 17237
461	6-{5-[(E)-2-(2,4-dihydroxyphenyl)ethenyl]-3-hydroxy-2-(3-methylbut-2-en-1-yl)phenoxy}-3,4,5-trihydroxyoxane-2-carboxylic acid	622.20	10.042	[M - H] - 1	1486863 ± 459795	171224 ± 11362
462	7-Sulfocholic acid	622.28	9.981	[M - H] - 1	21115252 ± 1446294	339947 ± 25419
463	Fexofenadine	635.32	10.714	[M - H] - 1	4092130 ± 121573	224486 ± 4692
464	3,4,5-trihydroxy-6-({5,6,14-trimethoxy-8,17-dioxatetracyclo[8.7.0.0\xc2\x2b2,\xc2\x81\xb7.0\xc2\x2b9\xc2\x2b9,\xc2\x2b9\xc2\x81\xb6]heptadeca-2,4,6,11(16),12,14-hexaen-4-yl}oxy)oxane-2-carboxylic acid	640.18	10.132	[M - H] - 1	83141191 ± 16073507	132244 ± 5392
465	4-{7-[(6-carboxy-3,4,5-trihydroxyoxan-2-yl)oxy]-3-hydroxy-5-sulfinio-3,4-dihydro-2H-1-benzopyran-2-yl}-2-hydroxybenzen-1-olate	648.12	12.011	[M - H] - 1	337835 ± 43390	6657664 ± 1417613
466	3,3-Diiodothyronine	658.93	7.666	[M - H] - 1	778652 ± 28870	66407 ± 38205
467	6-[[6-(3,4-dihydroxy-6-methyl-5-oxooxan-2-yl)-5-hydroxy-2-(3-methoxyphenyl)-4-oxo-4H-chromen-7-yl]oxy]-3,4,5-trihydroxyoxane-2-carboxylic acid	740.19	3.802	[M + H] + 1	2510636 ± 93502	15222 ± 1211

What is claimed is:

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-methyl-histidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenic 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-tyroxine, 30-cholic acid, 3-pyridineacetic acid, 3-indoleacrylic acid, 3-indolepropionic acid, 3-indoleacetamide, 3-oxoalanine, 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-tyrosine, 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-acetylmethionine, N-methylnicotinamide, N-phenylacetyl-L-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, acetylglutamine, acetylmethionine, 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-docosanoic acid, cis-13-docosanoic 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, isohydroxycholic 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, amino adipic acid, aminocaproic acid, salicylic acid, oleic acid, trehalose, nicotinic acid, pyrogutamic acid, ursocholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohydroxycholic acid, tauroolithocholic 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, glycooursodeoxycholic acid, glycohyocholic acid, glycohydroxycholic 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, hydroxyphenylpyruvic 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-tetracosenoic 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-methyl-histidine, 2,2-dimethylsuccinic acid, 2,3-diaminopropionic acid, glucoside 24-chenodeoxycholic acid, 2-butenic 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-oxoalanine, 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-tau-

rolithocholic 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-acetylmethionine, N-methylnicotinamide, N-phenylacetylglutamine, 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, acetylglutamine, acetylmethionine, 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-docosanoic acid, cis-13-docosanoic 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, allosoleucine, 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, isohydroxydeoxycholic 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, salicylic acid, oleic acid, trehalose, nicotinic acid, pyroglutamic acid, ursodeoxycholic acid, ursodeoxycholic acid, tauro-a-muricholic acid, tauro-b-muricholic acid, tauro-w-muricholic acid, tauroursodeoxycholic acid, taurohyocholic acid, taurohydroxydeoxycholic acid, tauroolithocholic acid, taurocholic acid, taurodehydrocholic acid, taurochenodeoxycholic acid, hyocholic acid, hydroxydeoxycholic acid, succinylacetone, succinic acid, citrulline, glycodehydrocholic acid, glycolithocholic acid, glycodeoxycholic acid, glycine, glycochenodeoxycholic acid, glyceraldehyde, glyceraldehyde-3-phosphate, choline glycerophosphate, glycooursodeoxycholic acid, glycohyocholic acid, glycohydroxydeoxycholic 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.

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