



GC-MS Application Note

MetaboAuto[®] - Automated Sample Preparation Platform for on-line GC-MS Metabolomics

Metabolomic screening of more than 250 protic metabolites in biofluids and cell tissues





MetaboAuto[®] - Automated Sample Preparation Platform for on-line GC-MS Metabolomics

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Overview

- Validated automation analytical technology for determination > 90 metabolites and xenobiotics in human serum/plasma, urine and liver tissue.
- The developed sample preparation workflow involves immediate, in-situ derivatization with ethyl chloroformate in an aqueous biological matrix. The derivatized metabolites concomitantly migrate into an immiscible organic phase (simultaneous derivatization + liquid liquid microextraction analycal workflow).
- Metabolite coverage: amino acids, organic acids, small peptides, biogenic amines
- Automated sample preparation in stand-alone or on-line (on-deck) mode integrated with the autosampler function.
- Total analysis time in the on-line mode: < 25 min
- The sample preparation kit contains all necessary reagent media and consumables.
- Metabolite library containing EI mass spectra of > 250 protic metabolite derivatives and their structures that can be uploaded into the NIST® mass spectral library.
- Automated Qual/Quan utility for the Thermo Scientific (Xcalibur, TraceFinder) and Agilent (MassHunter) data processing software
- The sample work-up compatibility with LC-MS pipeline (MetaboAuto® for LC-MS).
- The MetaboAuto® platform extendable for the use of fluoroalkyl chloroformates and reagents containing stable isotopes (D3 and 13C₂).
- The Operation Manual includes a video demonstrating the complete sample preparation workflow.
- Upgrade of the Phenomenex EZ:faast[™] Amino Acid Analysis Kits

Introduction

Despite rapid progress in analysis of polar metabolites, their high-throughput, comprehensive quantitative analysis remains a challenging task. GC-MS represents a cost-effective and well-established technique in routine metabolomic analysis. However, the sample preparation requires derivatization of protic functional groups. Among the current methods, use of alkyl chloroformates (RCFs) such as the ethyl reagent (ECF) have been next to oximation-silylation protocols most established approach.

ECF directly attacks primary; secondary amino-, hydroxy-, thiol- and carboxy groups providing respective carbamate, (thio)carbonate and ester derivatives with high yields in a two-phase aqueousorganic medium. In contrast to the oximationsilvlation approach, the chloroformate reaction media can be applied directly, is-situ, to the aqueous biological matrix. During the reaction, the ECF excess is decomposed to carbon dioxide facilitating the sample mixing and less polar product formation proceeding in seconds. The derivatives are simultaneously transferred into an immiscible organic phase while undesired background sample matrix is thus efficiently eliminated so that even single guadrupole GC-MS instruments can be utilized for the metabolite analyses. Automation of the workflow enables an unattended 24h/7d sample preparation and improves validation parameters of the obtained quantification data. The MetaboAuto® robotic workstation involves a CTC PAL robotic tool exchanger arm and several modules that can be assembled, and their operation programmed according to the customer's needs, Fig.1.

The MetaboAuto[®] platform mounted on a GC-MS system performs the following operations: transfer of liquid samples, sample dilution, separation of two layers of liquids by microcentrifugation, vortexing, and injection of the sample into the GC injector.





Fig. 1 Block diagram of the MetaboAuto[®] unit allowing automated sample preparation and injection into a GC-MS system: on-line (on-deck) version (from left), a microcentrifuge for liqud-liquid microextraction and obtaining an organic phase; a tool area for the storage and changing of sampling and dispensing syringe (volumes 10 μ L, 100 μ L); a tray holder (for 2 x 54 reaction vial; 2 x 54 sample vial and 1 x 15 vials with reagent solutions); a Fast Wash Module for the used solvents (chloroform, isopropanol); a vortex module for mixing of a reaction sample medium.

Methods

Sample preparation

Protic metabolites possessing active hydrogens in various functional groups (carboxy-, amino-, hydroxyand thiol-) can be measured after their ethyl chloroformate (ECF) labeling. **Fig. 2** shows the automated sample preparation workflow for GC-MS metabolomics of protic metabolites in diverse biological matrices. It involves 6 steps: (1) addition of an internal standard, (2) buffer 1, (3) catalytic medium, (4) chloroformate reagent 1, (5) buffer 2 and reagent 2 followed by transfer of organic phase into an autosampler vial and injection into a GC-MS system. Urine (25 μ I) is worked-up online, while plasma/serum (25 μ I) requires a preliminary parallel lipoprotein precipitation, centrifugation of the samples and the supernatant transfer into an autosampler vial. The cell tissue extraction (7 mg) is more complex and comprises a cell structure destruction by homogenization (e.g. with a Tissue-Lyser, Qiagen) at -18 °C, sonification and centrifugation at 4 °C. The arising supernatant is further subjected to the automated sample preparation procedure. The complete process ensures transformation of protic metabolites in well-defined derivatives that are simultaneously extracted into an organic phase. Organelle and membrane residues, (lipo)proteins, sugars and most of the pyridine catalyst are thus efficiently eliminated and background noise minimized providing much cleaner sample extracts and lower GC-MS system contamination. The overall reaction scheme for the derivatization of tyrosine is shown in Fig. 3.



Fig. 2: Automated sample preparation for biological samples and manual presample off-line steps for plasma /serum and cell tissue prior to the developed automated on-line sampler preparation with MetaboAuto[®].



Fig. 3: The ECF – ethanol derivatization scheme demonstrated on the reaction with tyrosine.

Instrumentation

MetaboAuto® enables robust, routine GC-MS metabolomic measurements on every instrument capable to communicate with a CTC PAL RTC equipment. The online sample preparation mode ensures metabolite profiling in less than 22 min and unattended simultaneous preparation of a next sample for another GC-MS run. Fresh sample

extracts are thus prepared in the same time window improving further measurement uncertainties and method validation statistics. The obtained sample extract (1µI) is injected in a splitless mode, the metabolite derivatives are separated on a medium polar capillary column and after electron ionization detected in either a full scan (m/z 40-500 Da) or SIM (MRM) MS scan mode. A typical GC-MS instrument set-up is shown in **Fig. 4**.



Fig 4: A typical GC-MS instrument set-up and temperature programming for profiling of more than 250 protic metabolites used for metabolite analysis after the MetaboAuto[®] sample preparation.

Data processing

For the convenient data handling, the Metabo-Auto[®] platform is equipped with tools for the automatic metabolite identifications and their quantitative analysis. The metabolites (as the ECF-ethanol derivatives) are detected with aid of the Metabo-Auto[®] library retention time (RT) and EI mass spectral libraries which contains RT data and EI mass spectra of > 250 protic metabolites. Moreover, the library can

be uploaded and directly used with a commercial NIST mass spectral library. This feature is illustrated in **Fig.5**, where a routine for the detection of tyrosine in the MetaboAuto[®] library is depicted.

Quantitative analysis of the detected protic metabolites is facilitated by the developed software utility which allows immediate analyte quantifications in the Thermo Scientific (Xcalibur, TraceFinder) or Agilent (MassHunter) data processing environment.



Fig. 5: Inspection of the EI spectrum of the tyrosine metabolite in human urine. The sample was on-line worked-up with the MetaboAuto[®] sample preparation workstation, measured by a single-quadrupole GC-MS and the detected metabolites identified by means of the MetaboAuto[®] EI mass spectral library fully implemented into the NIST mass spectral library environment.

Results

The MetaboAuto[®] platform represents a new, original technology for comprehensive metabolomic GC-MS analysis of analytes in urine, plasma and tissue extracts. On-line (on-deck) use of the MetaboAuto[®] platform enables unattended 24h/7d, automated sample preparation, GC-MS measurement,data processing for more than 250 metabolites and xenobiotics in the complex biological matrices. Systematic profiling in a 25 µl volume of a pooled human urine and serum (a pooled sample, n=10)

revealed the capability to quantify more than 100 and 70 analytes, respectively, using a single quadrupole MS full scan detection. Similarly, presample extraction of 7 mg of pork liver tissue enabled automated profiling of more than 70 protic using the automated MetaboAuto® sample preparation and GC-MS analysis (a pooled tissue extract, n=5). For all the detected metabolites, the relative standard deviation not exceeding 20 % was a criterion for the acceptance in the metabolite quantification list. The metabolite list used for the described MetaboAuto® testing is summarized in **Table 1**.



Fig. 6: Typical clean TIC GC-MS chromatograms obtained by the MetaboAuto[®] sample preparation and single quad GC-MS metabolomic analysis of an examined urine, blood plasma and pork liver extracts.

Table 1: The metabolite list examined for the testing of the MetaboAuto[®] sample preparation workstation hyphenated with a GC-EI/MS instrument (ISQ, ThermoScientific). The profiled metabolites, their KEGG number and method reproducibility expressed as RSD (%) for analytes detected in the examined three biological matrices (human urine, serum and pork liver tissue). A pooled human urine, serum and pork liver sample (n=10 each) was prepared by the robotic workstation (n=45 for each sample matrix) and was accomplished within 60 h.

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2.6-Diaminopimelic acidC00666NANANA2.6-Pyridinedicarboxylic acidNANANANA2-Aminoadipic acidC00956NA542-Aminobutyric acidC023565742-Aminoisobutyric acidC03665NANANA2-Aminoisobutyric acidC03665NANANA2-Aminoisobutyric acidC01546NANANA2-Aminoisobutyric acidC01546NANANA2-Furoic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01546NANANA2-Hydroxy-2-methylbropionic acidC02360NANANA2-Hydroxy-3-methylbutyric acidC05984316232-Hydroxydecanoic acidNANANANANA2-Hydroxysderanoic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidC00109NANANA2-Hydroxysebacic acidC00109NANANA2-Hydroxysebacic acidC00109NANANA <trr>2-Nocoltyric acidC0</trr>	2,4-Diaminobutyric acid	C03283	NA	NA	NA	
2.6-Pyridinedicarboxylic acidNANANANA2-Aminobutyric acidC00956NA542-Aminobutyric acidC023565742-Aminobutyric acidC03665NANANA2-Aminooctanoic acidNANANANA2-Aminopimelic acidNANANANA2-Aminopimelic acidC01546NANANA2-Furcic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01546NANANA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylbutyric acidC02360NANANA2-Hydroxydetanoic acidC018758*14*9*2-Hydroxydetanoic acidC018758*14*9*2-Hydroxydotarcic acidNANANANA2-Hydroxyostocarpric acidC02264NANANA2-Hydroxyostocarpric acidC02504NANANA2-Hydroxyotetaric acidNANANANA2-Isopropylmalic acidC00026NA20*NA2-Oxobutyric acidC00260NANANA2-Oxobatyric acidNANANANA2-Oxobatyric acidC00109NANANA2-Oxobatyric acidNANANANA2-Oxobatyric acidNANANANA2-Oxobatyric acidNANANA	2,6-Diaminopimelic acid	C00666	NA	NA	NA	
2-Aminoadipic acidC00956NA542-Aminoheptanoic acidNANANANA2-Aminoheptanoic acidNANANANA2-Aminobetanoic acidNANANANA2-Aminopimelic acidC03665NANANA2-Aminopimelic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01983NA9NA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-2-methylpropionic acidC02360NANANA2-Hydroxydipic acidC02360NANANA2-Hydroxydipic acidC02364NANANA2-Hydroxydecanoic acidNANANANA2-Hydroxydetric acidC0187S8*14*9*2-Hydroxydetric acidNANANANANA2-Hydroxydetric acidNANANANA2-Hydroxydetric acidNANANANA2-Hydroxydetric acidC00322NANANA2-Oxoadipic acidC00026NA20*NA2-Oxobutyric acidC00109NANANA2-Oxobutyric acidC01090NANANA2-Oxobutyric acidC00109NANANA2-Oxobutyric acidC01019NANANA2-Oxobutyric acidC01026NANANA2-Oxobutyric acidNANANA	2,6-Pyridinedicarboxylic acid	NA	NA	NA	NA	
2-Aminobutyric acidC023565742-Aminoicheptanoic acidNANANANA2-Aminoicheptanoic acidC03665NANANA2-Aminopimelic acidNANANANA2-Aminopimelic acidC01546NANANA2-furoic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01546NANANA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylbutyric acidC02360NANANA2-Hydroxy-3-methylbutyric acidC05984316232-Hydroxydecanoic acidNANANANA2-Hydroxysdepric acidC0108758°14°9°2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidC00254NANANA2-Isopropylmalic acidC00109NANANA2-Oxoadipic acidC00109NANANA2-Oxoadipic acidC00109NANANA2-Oxoadipic acidNANANANA2-Oxoadipic acidNANANANA2-Oxoadipic acidNANANANA <td>2-Aminoadipic acid</td> <td>C00956</td> <td>NA</td> <td>5</td> <td>4</td>	2-Aminoadipic acid	C00956	NA	5	4	
2-Aminoheptanoic acid NA NA NA NA NA 2-Aminojosbutyric acid C03665 NA NA NA NA 2-Aminojosbutyric acid NA NA NA NA NA 2-Furoic acid NA NA NA NA NA 2-Furoic acid C01983 NA 9 NA 2-Hydroxy(phenyl)acetic acid C01983 NA 9 NA 2-Hydroxy-2-methylpropionic acid C21297 NA 13 NA 2-Hydroxy-2-methylpropionic acid C02360 NA NA NA 2-Hydroxybutyric acid C05984 3 16 23 2-Hydroxygbutyric acid C01987 58° 14″ 9° 2-Hydroxygbutyric acid C01087 58° 14″ 9° 2-Hydroxyglutaric acid NA NA NA NA 2-Hydroxyglutaric acid C01087 58° 14″ 9° 2-Hydroxyglutaric acid NA NA NA NA 2-Hydroxyglutaric acid C01087 58° 14″ 9° 2-Hydroxyglutaric acid NA NA NA NA 2-Hydroxyglataric acid NA NA NA NA 2-Hydroxyglataric acid C01087 58° 14″ 9° 2-Hydroxyglataric acid NA NA NA NA 2-Hydroxyglataric acid NA NA NA NA 2-Hydroxyglataric acid NA NA NA NA 2-Hydroxyglataric acid NA NA NA NA 2-Isopropylmalic acid C02504 NA NA NA 2-Sozodipic acid C00322 NA 37 NA 2-Oxodipic acid C00109 NA NA NA 2-Oxoglutaric acid NA NA NA NA 2-Oxoglutaric acid NA NA NA NA 2-Oxoglutaric acid C00026 NA 20° NA 2-Oxobutyric acid C00026 NA 20° NA 2-Oxobutyric acid NA NA NA NA 3-2-DhenylAcetic acid NA NA NA NA 3-4-Dihydroxyphenylacetic acid NA NA NA NA 3-4-Dihydroxyphenylacetic acid NA NA NA NA 3-4-Dihydroxymandelic acid NA NA NA NA 3-4-Dihydroxymandelic acid NA NA NA NA 3-Hydroxydeotecnoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyponoine NA NA NA NA 3-Hydroxyponoine A NA NA NA NA 3-Hydroxyponoine A NA NA NA NA 3-Hydroxyponoine A NA NA N	2-Aminobutyric acid	C02356	5	7	4	
2-Aminoisobutyric acidC03665NANANA2-Aminopimelic acidNANANANA2-Aminopimelic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01981NA92-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylbutyric acidC02360NANANA2-Hydroxy-3-methylbutyric acidC02360NANANA2-Hydroxydopic acidC02360NANANA2-Hydroxydopic acidC05984316232-Hydroxydotacic acidNANANANA2-Hydroxydocanoic acidC0108758°14"9"2-Hydroxydotacic acidNANANANA2-Hydroxydelcic acidC02504NANANA2-Hydroxyaleric acidNANANANA2-Hydroxyaleric acidC00322NANANA2-Isopropylmalic acidC00026NA20"NA2-Oxoadipic acidC00026NANANA2-Oxoadipric acidC00026NANANA2-Oxoadipric acidC07086NANANA2-Oxoadipric acidNANANANA2-Drenylbutyric acidNANANANA2-Oxoadipric acidNANANANA2-Oxoadipric acidNANANANA2-Oxoadipric acidNANANANA <td>2-Aminoheptanoic acid</td> <td>NA</td> <td>NA</td> <td>NA</td> <td>NA</td>	2-Aminoheptanoic acid	NA	NA	NA	NA	
2-Aminooctanoic acidNANANANA2-Aminopimelic acidNANANANA2-Furoic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01983NA9NA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylptotyric acidC02360NANANA2-Hydroxy-decanoic acidC02360NANANA2-Hydroxydecanoic acidC018758*14*9*2-Hydroxydecanoic acidC0108758*14*9*2-Hydroxycotanoic acidNANANANA2-Hydroxycotanoic acidNANANANA2-Hydroxycotanoic acidNANANANA2-Hydroxycotanoic acidNANANANA2-Hydroxycotanoic acidNANANANA2-Hydroxyraleric acidC02504NANANA2-Isopropylmalic acidC00109NANANA2-Oxobutyric acidC00109NANANA2-Oxobutyric acidC00026NA20*NA2-Oxobutyric acidNANANANA2-Oxobutyric acidNANANANA2-Oxobutyric acidNANANANA2-Oxobutyric acidNANANANA2-Oxobutyric acidNANANANA2-Oxobutyric acidNANANANA<	2-Aminoisobutyric acid	C03665	NA	NA	NA	
2-Aminopimelic acidNANANANA2-Furoic acidC01546NANANA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylputyric acidNA822*82-Hydroxy-dipic acidC02360NANANA2-Hydroxydipic acidC02360NANANA2-Hydroxydutyric acidC05984316232-Hydroxydutyric acidC0108758*14*9*2-Hydroxyglutaric acidC0108758*14*9*2-Hydroxyglutaric acidC0108758*14*9*2-Hydroxysecaproic acidC03264NANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Isoprop/malic acidC02504NANANA2-Oxoglutaric acidC00322NANANA2-Oxoglutaric acidC00026NA20*NA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Phenylacetic acidC07086NANANA2-Phenylacetic acidC05580NANANA2-Phenylacetic acidC05580NANANA3-Hydroxydapic acidNANANANA3-Hydroxydapic acidNANANANA <td>2-Aminooctanoic acid</td> <td>NA</td> <td>NA</td> <td>NA</td> <td>NA</td>	2-Aminooctanoic acid	NA	NA	NA	NA	
2-Furoic acidC01546NANANA2-Hydroxy(phenyl)acetic acidC01983NA9NA2-Hydroxy-2-methylborpionic acidC21297NA13NA2-Hydroxy-3-methylbutyric acidC21297NA822*82-Hydroxy-anethylbutyric acidC02360NANANA2-Hydroxydeipic acidC05984316232-Hydroxydgutaric acidC0108758*14*9*2-Hydroxysoctanoic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidC02504NANANA2-Isopropylmalic acidC00109NANANA2-Oxodutyric acidC00109NANANA2-Oxodutyric acidC00109NANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA2-Oxoplutaric acidNANANANA </td <td>2-Aminopimelic acid</td> <td>NA</td> <td>NA</td> <td>NA</td> <td>NA</td>	2-Aminopimelic acid	NA	NA	NA	NA	
2-Hydroxy(phenyl)acetic acidC01983NA9NA2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylptutyric acidNA822*82-Hydroxy-decanoic acidC02360NANANA2-Hydroxydecanoic acidC05984316232-Hydroxydecanoic acidC0108758*14*9*2-Hydroxydecanoic acidC03264NANANA2-Hydroxyoctanoic acidNANANANA2-Hydroxyoctanoic acidNANANANA2-Hydroxyoctanoic acidNANANANA2-Hydroxyoctanoic acidNANANANA2-Hydroxyotetanic acidNANANANA2-Isopropylmalic acidC002504NANANA2-Oxobutyric acidC00109NANANA2-Oxobutyric acidC00109NANANA2-Oxobutyric acidC00026NA20*NA2-Oxobutyric acidNANANANA2-Phenylbutyric acidNANANANA2-Phenylbutyric acidNANANANA2-Phenylbutyric acidNANANANA2-Oxobtxanoic acidNANANANA2-Oxobtyropionic acidNANANANA2-Phenylbutyric acidNANANANA3-Hotroxyphenylacetic acidC0580NANA <td>2-Furoic acid</td> <td>C01546</td> <td>NA</td> <td>NA</td> <td>NA</td>	2-Furoic acid	C01546	NA	NA	NA	
2-Hydroxy-2-methylpropionic acidC21297NA13NA2-Hydroxy-3-methylbutyric acidNA822"82-Hydroxydipic acidC02360NANANA2-Hydroxybutyric acidC02360NANANA2-Hydroxybutyric acidC05984316232-Hydroxybutyric acidC0108758"14"9"2-Hydroxyglutaric acidC0108758"14"9"2-Hydroxyglutaric acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidC02504NANANA2-Mydroxygebacic acidC00322NANANA2-Oxoadipic acidC00026NANANA2-Oxobutyric acidC00026NANANA2-Oxobutyric acidC01081NANANA2-Phenylacetic acidC07086NA17NA2-Phenylacetic acidC05580NANANA3-4-Dihydroxymandelic acidNANANANA3-4-Dihydroxymandelic acidNANANANA3-4-DihydroxypenylocaciaNANANANA3-4-DihydroxypenylocaciaNANANANA3-4-DihydroxypenylocaciaNANA </td <td>2-Hydroxy(phenyl)acetic acid</td> <td>C01983</td> <td>NA</td> <td>9</td> <td>NA</td>	2-Hydroxy(phenyl)acetic acid	C01983	NA	9	NA	
2-Hydroxy-3-methylbutyric acidNA822*82-Hydroxyadipic acidC02360NANANA2-Hydroxydecanoic acidNANANANA2-Hydroxydgutyric acidC05984316232-Hydroxyglutaric acidC0108758*14*9*2-Hydroxyglutaric acidC03264NANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Isopropylmalic acidC002504NANANA2-Oxodutyric acidC00109NANANA2-Oxodutyric acidC00109NANANA2-Oxoplutaric acidC00026NA20*NA2-Oxopexanoic acidNANANANA2-Oxopexanoic acidNANANANA2-Phenylporpionic acidNANANANA2-Phenylputyric acidC0580NANANA3-4-Dihydroxymandelic acidC05145NA8NA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydodecanoic acidNANANANA	2-Hydroxy-2-methylpropionic acid	C21297	NA	13	NA	
2-Hydroxyadipic acidCO2360NANANA2-Hydroxybutyric acidC05984316232-Hydroxyglutaric acidC0108758*14*9*2-Hydroxyglutaric acidC0108758*14*9*2-Hydroxyglotaric acidC0108758*14*9*2-Hydroxyglotaric acidC0108758*14*9*2-Hydroxyglotaric acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Isopropylmalic acidC02504NANANA2-Sovadipic acidC00322NANANA2-Oxobutyric acidC00026NA20*NA2-Oxobutyric acidC00026NANANA2-Oxobexanoic acidC00026NA212-Oxochexanoic acid2-Oxochanoic acidNANANANA2-Phenylbutyric acidNANANA3-4Dihydroxyphenylacetic acidC0580NANA3-4Dihydroxyphenylacetic acidC05145NA83-Hydroxydipic acidNANANANA3-Hydroxydipic acidNANANANA3-Hydroxypropionic acidNANANANA3-Hydroxyphenylacetic acidC0593NANANA3-HydroxyprolineC0593NANANA<	2-Hydroxy-3-methylbutyric acid	NA	8	22*	8	
2-Hydroxybutyric acidC02500NANA2-Hydroxybecanoic acidNANANANA2-Hydroxybutyric acidC0108758*14*9*2-Hydroxyglutaric acidC03264NANANA2-Hydroxyglutaric acidC03264NANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidNANANANA2-Hydroxygebacic acidC02504NANANA2-Methylglutaric acidC00322NANANA2-Oxoebutyric acidC00109NANANA2-Oxoebutyric acidC00026NA20*NA2-Oxoetanoic acidNANANANA2-Phenylacetic acidC07086NA17NA2-Phenylacetic acidC0161NANANA3-4-Dihydroxymandelic acidC0580NANANA3-4-Dihydroxyphenylacetic acidC05145NA8NA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxygebacic acidC05145NA8NA3-Hydroxydecanoic acidNANANANA3-Hydroxygebacic acidC0593NANANA </td <td>2-Hydroxyadinic acid</td> <td>C02360</td> <td>NA</td> <td>NΔ</td> <td>ΝΔ</td>	2-Hydroxyadinic acid	C02360	NA	NΔ	ΝΔ	
2-Hydroxydecanoic acidNANANA2-Hydroxyglutaric acidC0108758°14°9°2-Hydroxyglutaric acidC0108758°14°9°2-Hydroxysctanoic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidNANANANA2-Hydroxysebacic acidC02504NANANA2-Isopropylmalic acidC00322NANANA2-Oxodipic acidC00109NANANA2-Oxoglutaric acidC00026NA20°NA2-Oxoptutric acidC0002619212-Oxoptutric acidC07086NANANA2-Phenylacetic acidC07086NANANA2-Phenylbutyric acidNANANANA3,4-Dihydroxymandelic acidC0580NANANA3-AlanineC00099NA1353-Aminobutyric acidNANANANANA3-Hydroxydodecanoic acidNANANANA3-Hydroxydodecanoic acidNANANANA3-Hydroxydotecanoic acidNANANANA3-Hydroxytopoloic acidNANANANA3-Hydroxytopoloic acidNANANANA3-Hydroxytopoloi	2-Hydroxybutyric acid	C05984	3	16	23	
2-Hydroxydgiutaric acid C01087 58° 14° 9° 2-Hydroxyisocaproic acid C01087 58° 14° 9° 2-Hydroxyisocaproic acid C03264 NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Isopropylmalic acid C02504 NA NA NA NA 2-Methylglutaric acid C02504 NA NA NA 2-Methylglutaric acid C00322 NA 37 NA 2-Oxoglutaric acid C00322 NA 37 NA 2-Oxoglutaric acid C00322 NA 37 NA 2-Oxoglutaric acid C00026 NA 20° NA 2-Oxoglutaric acid C00026 NA 20° NA 2-Oxoplutaric acid C00902 6 19 21 2-Oxobexanoic acid NA NA NA NA NA 2-Phenoxypropionic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 3-Phenylacetic acid C05580 NA NA NA 3-4-Dihydroxymahelic acid C05580 NA NA NA 3-4-Dihydroxymahelic acid NA NA NA NA 3-Alanine C0099 NA 13 5 3-Aminoisobutyric acid NA NA NA NA 3-Hydroxyadipic acid NA NA NA NA 3-Hydroxyadipic acid NA NA NA NA 3-Hydroxyadipic acid C05593 NA NA NA 3-Hydroxyadipic acid C05593 NA NA NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxyphonic acid NA NA NA NA 3-Hydroxyphonic acid NA NA NA NA 3-Hydroxyphonic acid NA NA NA NA 3-Hydroxyphonic acid C05593 NA NA NA 3-Hydroxyphonic acid NA NA NA NA 3-Hydroxyphonic acid MA NA NA NA 3-Hydroxyphonic acid MA NA NA NA NA	2 Hydroxybacynic acid	NA	NIA	NA	2.5	
2-Hydroxysiocaproic acidC01037351432-Hydroxysiocaproic acidNANANANA2-Hydroxysolaci acidNANANANA2-Hydroxyvaleric acidNANANANA2-Hydroxyvaleric acidNANANANA2-Hydroxyvaleric acidNANANANA2-Hydroxyvaleric acidC02504NANANA2-Mydroxyvaleric acidC00322NANANA2-Oxoadipic acidC00109NANANA2-Oxodutyric acidC00026NA20"NA2-Oxoetanoic acidC0026NANANA2-Oxoetanoic acidC007086NANANA2-Phenylacetic acidC0161NANANA2-Phenylacetic acidC0161NANANA3,4-Dihydroxymandelic acidC01161NA8NA3-AlanineC00099NA1353-Aminobutyric acidNANANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-Hydroxydecanoic acidNANANANA3-HydroxysolprineC0514781853-Hydroxypropinic acidC0514781853-Hydr	2-Hydroxydecanoic acid	C01097	E0*	1.4*	0*	
2-Hydroxysoctanoic acid NA NA NA NA 2-Hydroxysoctanoic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Isopropylmalic acid C02504 NA NA NA NA 2-Methylglutaric acid C00322 NA NA NA NA 2-Oxoadipic acid C00322 NA NA NA NA 2-Oxoadipic acid C00109 NA NA NA 2-Oxoglutaric acid C00109 NA NA NA 2-Oxoglutaric acid C00026 NA 20* NA 2-Oxobutyric acid C00902 6 19 21 2-Oxobutyric acid NA NA NA NA 2-Phenoxypropionic acid NA NA NA NA 2-Phenylbutyric acid C07086 NA 17 NA 2-Phenylbutyric acid NA NA NA NA 3-4-Dihydroxymandelic acid C05580 NA NA NA 3-Alanine C0099 NA 13 5 3-Aminoisobutyric acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyponic acid C0593 NA NA 3-Hydroxyponic acid C05147 8 18 5 3-Hydroxyponic acid NA NA NA NA 3-Hydroxyponic acid MA NA NA NA NA 3-Hydroxyponic acid MA NA NA NA NA 3-Hydroxyponic acid MA NA NA NA NA 3-Hydroxyponic acid (dimer) C1013 NA NA NA NA 3-Hydroxyropionic acid (dimer) C1013 NA NA NA 3-Hydroxyropionic acid MA NA NA NA NA NA 3-Hydroxyropionic acid MA NA NA NA NA NA 3-Hydroxyropionic acid MA NA NA NA NA NA	2 Hydroxyglutane acid	C01067	30	14	9	
2-Hydroxysebacic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Hydroxysebacic acid NA NA NA NA NA 2-Isopropylmalic acid C02504 NA NA NA NA 2-Methylglutaric acid C02504 NA NA NA NA 2-Oxoadipic acid C00322 NA 37 NA 2-Oxobutyric acid C00026 NA 20* NA 2-Oxobutyric acid C00902 6 19 21 2-Oxooctanoic acid C00902 6 19 21 2-Oxooctanoic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C07086 NA 17 NA 3-4-Dihydroxymandelic acid C05580 NA NA NA 3-4-Dihydroxymandelic acid C05580 NA NA 3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxysobutyric acid C05593 NA NA NA 3-Hydroxysobutyric acid C05147 NA 8 NA 3-Hydroxysobutyric acid C05147 NA 8 NA 3-Hydroxysobutyric acid C05147 8 18 5 3-Hydroxypropionic acid NA NA NA NA 3-Hydroxysobutyric acid C05147 8 18 5 3-Hydroxypropionic acid NA NA NA NA		003204		IN/A		
2-Hydroxyselacic acidNANANANANA2-Hydroxyselacic acidNANANANA2-Hydroxyselacic acidC02504NANANA2-Mydroxyselacic acidC02504NANANA2-Mydroxycalicic acidC00322NANANA2-Oxoadjuicic acidC00322NANANA2-Oxodjutaric acidC00109NANANA2-Oxobutyric acidC00026NA20"NA2-Oxoctanoic acidC00902619212-Oxoctanoic acidNANANANA2-Phenylacetic acidC07086NA17NA2-Phenylbutyric acidNANANANA3-4-Dihydroxymandelic acidC05800NANANA3-4-Dihydroxyphenylacetic acidC05145NA8NA3-AlanineC00099NA1353-Aminobutyric acidNANANA3-Hydroxydecanoic acidNANANANANANA3-Hydroxydecanoic acidNANANANANA3-Hydroxysobutyric acidC05145NANANA3-Hydroxypropionic acidC05533NANANA3-Hydroxypropionic acid (dimer)C1013NANANA3-Hydroxypropionic acid (dimer)C1013NANANA3-Hydroxyraeric acidNANANANA3-Hydroxyraeric acidNA<	2-Hydroxyoctanoic acid	NA	NA	NA 10	NA	
2-Hydroxyvalenic acid NA NA NA NA NA 2-Isopropylmalic acid C02504 NA NA NA 2-Methylglutaric acid C02504 NA NA NA 2-Oxoadipic acid C00322 NA 37 NA 2-Oxoadipic acid C00109 NA NA NA 2-Oxoglutaric acid C00109 NA NA NA 2-Oxohexanoic acid C00902 6 19 21 2-Oxobutyric acid NA NA NA NA 2-Oxohexanoic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylutyric acid NA NA NA NA 3-4-Dihydroxymenylacetic acid C05580 NA NA NA 3-4-Dihydroxymenylacetic acid C05580 NA NA NA 3-Alanine C00099 NA 13 5 3-Alanine C05145 NA 8 NA 3-Hydroxydecanoic acid NA NA NA 9 NA 3-Hydroxydodecanoic acid NA NA NA 8 3-Hydroxydodecanoic acid NA NA NA 8 3-Hydroxytoole acid C05587 NA NA 8 3-Hydroxydodecanoic acid NA NA NA 8 3-Hydroxytoole acid NA NA NA NA 8 3-Hydroxytoole acid NA NA NA NA 8 3-Hydroxytoole acid NA NA NA NA 8 3-Hydroxyproline C05147 8 18 5 3-Hydroxyprolinic acid NA NA NA NA 8 3-Hydroxyprolinic acid NA NA NA NA 8 3-Hydroxytoole acid NA NA NA 8 3-Hydroxyprolinic acid C10113 NA NA 8 3-Hydroxytorosine NA NA NA 8 3-Hydroxyrosine NA NA NA 8 3-Hydroxyrosine NA NA NA 8 3-Hydroxyrosine NA NA NA 8 3-Hydroxyrosine NA	2-Hydroxysebacic acid	NA	NA	10	NA	
2-Isopropyimalic acid C02504 NA NA NA NA 2-Oxodipic acid NA NA NA NA NA 2-Oxobutyric acid C00322 NA 37 NA 2-Oxobutyric acid C00322 NA 37 NA 2-Oxobutyric acid C00026 NA 20* NA 2-Oxobexanoic acid C00902 6 19 21 2-Oxooctanoic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C07086 NA 17 NA 3-4-Dihydroxymandelic acid C05580 NA NA NA 3-4-Dihydroxymandelic acid C05580 NA NA 3-4-Dihydroxymandelic acid C01161 NA 8 NA 3-4-Dihydroxymandelic acid C05145 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobotutyric acid NA NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid C05145 NA 8 NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxysobutyric acid C06001 35* NA NA 3-Hydroxysobutyric acid C05147 8 18 5 3-Hydroxysobutyric acid C05147 8 18 5 3-Hydroxypropionic acid (C05147 8 1	2-Hydroxyvaleric acid	NA	NA	NA	NA	
2-Methylgiutaric acid NA NA NA NA NA 2-Oxoadjutaric acid C00322 NA 37 NA 2-Oxobutyric acid C00109 NA NA NA 2-Oxodjutaric acid C00026 NA 20* NA 2-Oxodputaric acid C00902 6 19 21 2-Oxocatancic acid NA NA NA NA 2-Oxocatancic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylbutyric acid C01161 NA NA NA 3.4-Dihydroxymandelic acid C05580 NA NA NA 3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminoisobutyric acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxyisovaleric acid C05001 3* NA NA 3-Hydroxyphenylacetic acid C05533 NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Hydroxypropionic acid (dimer) C1013 <	2-Isopropyimalic acid	C02504	NA	NA	NA	
2-Oxoadipic acid C00322 NA 37 NA 2-Oxoadipic acid C00109 NA NA NA 2-Oxobityric acid C00026 NA 20* NA 2-Oxobexanoic acid C00902 6 19 21 2-Oxochoxanoic acid NA NA NA NA 2-Oxochoxanoic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylbutyric acid NA NA NA NA 3.4-Dihydroxymandelic acid C05580 NA NA NA 3.4-Dihydroxymandelic acid C05145 NA 8 NA 3-Alanine C0009 NA 13 5 3-Aminoisobutyric acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C05001 35*	2-Methylglutaric acid	NA	NA	NA	NA	
2-Oxobutyric acid C00109 NA NA NA 2-Oxobutyric acid C00026 NA 20* NA 2-Oxohexanoic acid C00902 6 19 21 2-Oxoctanoic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C05580 NA NA NA 3.4-Dihydroxymandelic acid C01161 NA 8 NA 3.4-Dihydroxymandelic acid C01161 NA 8 NA 3-Aminobotyric acid NA NA NA NA 3-Aminobotyric acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxyisovaleric acid C06001 35* NA NA 3-Hydroxyisovaleric acid C05593 NA NA NA 3-Hydroxypropine C05147 8 18 5 3-Hydroxypropine C05147 8 18 5 3-Hydroxypropine NA NA NA NA 3-Hydroxypropine NA NA NA	2-Oxoadipic acid	C00322	NA	37	NA	
2-Oxoglutaric acidC00026NA20"NA2-Oxoclanoic acidC00902619212-Oxoclanoic acidNANANANA2-Phenylacetic acidC07086NANANA2-Phenylacetic acidC07086NANANA2-Phenylbutyric acidNANANANA3,4-Dihydroxymandelic acidC05580NANANA3,4-Dihydroxyphenylacetic acidC01161NA8NA3-AlanineC00099NA1353-Aminobutyric acidNANANANA3-Alydroxydecanoic acidNANANA3-Hydroxydecanoic acidNANANA3-Hydroxydecanoic acidNANANA3-Hydroxyisovaleric acidC05093NANA3-Hydroxyphenylacetic acidC05593NANA3-Hydroxypropionic acid (dimer)C1013NANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidC051478183-Hydroxypropionic acidC1013NANANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidNANANA3-Hydroxypropionic acidNANANA3-ChlorotyrosineNANA <td>2-Oxobutyric acid</td> <td>C00109</td> <td>NA</td> <td>NA</td> <td>NA</td>	2-Oxobutyric acid	C00109	NA	NA	NA	
2-Oxohexanoic acid C00902 6 19 21 2-Oxooctanoic acid NA NA NA NA 2-Phenoxypropionic acid NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylbutyric acid NA NA NA NA 3-Pinylbutyric acid C05580 NA NA NA 3-4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA NA 3-Hydroxyadipic acid NA NA NA NA 3-Hydroxyadipic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyphonylacetic acid C06001 35" NA NA 3-Hydroxyphonipiacetic acid C05593 NA NA NA 3-Hydroxyphonipiacetic acid C1013 NA NA NA 3-Hydroxyphonipiacetic acid NA NA NA NA 3-Hydroxyphonipiacetic acid NA NA NA NA 3-Hydroxyphonime C05147	2-Oxoglutaric acid	C00026	NA	20*	NA	
2-Oxooctanoic acid NA NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylacetic acid C05580 NA NA NA 3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobottyric acid NA NA NA 17 NA 3-Aminobottyric acid C05145 NA 8 NA 3-Hydroxydecanoic acid NA NA NA 9 NA 3-Hydroxydodecanoic acid NA NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxysoleric acid C05147 8 18 5 3-Hydroxypropine NA NA NA NA 3-Hydroxypropine NA NA NA NA	2-Oxohexanoic acid	C00902	6	19	21	
2-Phenoxypropionic acid NA NA NA NA NA 2-Phenylacetic acid C07086 NA 17 NA 2-Phenylbutyric acid NA NA NA NA 3,4-Dihydroxymandelic acid C05580 NA NA NA 3,4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA 17 NA 3-Aminoisobutyric acid C05145 NA 8 NA 3-Hydroxydecanoic acid NA NA 9 NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C0501 35* NA NA 3-Hydroxysoleric acid C0593 NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxypropionic acid NA NA NA NA 3-Hydroxytorytoric acid NA NA NA NA 3-Hydroxytoaleric acid C0593 NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxyproline NA NA NA NA 3-Hydroxyproline NA NA NA NA 3-Hydroxyproline NA NA NA NA	2-Oxooctanoic acid	NA	NA	NA	NA	
2-Phenylacetic acidC07086NA17NA2-Phenylbutyric acidNANANANA3.4-Dihydroxymandelic acidC05580NANANA3.4-Dihydroxymandelic acidC01161NA8NA3-AlanineC00099NA1353-Aminobutyric acidNANA17NA3-Aminoisobutyric acidNANA17NA3-Aminoisobutyric acidNANA17NA3-Hydroxydalpic acidNANANA93-Hydroxydodecanoic acidNANANANA3-Hydroxyisobutyric acidC0600135*NANA3-Hydroxyphenylacetic acidC0553NANANA3-Hydroxypropionic acid (dimer)C1013NANANA3-Hydroxypropionic acid (dimer)C1013NANANA3-Hydroxypropionic acidNANANANA3-Hydroxypropionic acidNANANANA3-Hydroxypropionic acidNANANANA3-Hydroxypropionic acidNANANANA3-Hydroxypropionic acid (dimer)C1013NANANA3-IndotyrosineNANANANA3-IodotyrosineNANANANA	2-Phenoxypropionic acid	NA	NA	NA	NA	
2-Phenylbutyric acid NA NA NA NA NA 3.4-Dihydroxymandelic acid C05580 NA NA NA 3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA 17 NA 3-Aminoboutyric acid C05145 NA 8 NA 3-Hydroxydecanoic acid NA NA 9 NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C0593 NA NA 3-Hydroxyphenylacetic acid C05593 NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid NA NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Chlorotyrosine NA NA NA NA	2-Phenylacetic acid	C07086	NA	17	NA	
3.4-Dihydroxymandelic acid C05580 NA NA NA 3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA 17 NA 3-Aminoisobutyric acid NA NA NA 9 NA 3-Hydroxydecanoic acid NA NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyphenylacetic acid C05037 NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxyprolinic acid (dimer) C1013 NA NA NA 3-Hydroxyprosine NA NA NA NA	2-Phenylbutyric acid	NA	NA	NA	NA	
3.4-Dihydroxyphenylacetic acid C01161 NA 8 NA 3-Alanine C0009 NA 13 5 3-Aminobutyric acid NA NA 17 NA 3-Aminobutyric acid NA NA 17 NA 3-Aminobutyric acid NA NA 17 NA 3-Aminobutyric acid C05145 NA 8 NA 3-Hydroxyadipic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid C06001 35* NA NA 3-Hydroxyphenylacetic acid C06001 35* NA NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxysine NA NA NA NA 3-Hydroxypropionic acid NA <td>3,4-Dihydroxymandelic acid</td> <td>C05580</td> <td>NA</td> <td>NA</td> <td>NA</td>	3,4-Dihydroxymandelic acid	C05580	NA	NA	NA	
3-Alanine C00099 NA 13 5 3-Aminobutyric acid NA NA NA 17 NA 3-Aminoboutyric acid NA NA NA 17 NA 3-Aminoboutyric acid NA NA NA 9 NA 3-Hydroxydecanoic acid NA NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Chlorotyrosine NA NA NA NA	3,4-Dihydroxyphenylacetic acid	C01161	NA	8	NA	
3-Aminobutyric acid NA NA 17 NA 3-Aminoisobutyric acid C05145 NA 8 NA 3-Hydroxyadipic acid NA NA 9 NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid C06001 35* NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyphenylacetic acid C20827 57 8 NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxyprolinic acid (dimer) C1013 NA NA NA 3-Hydroxyrosproinic acid NA NA NA NA NA 3-Hydroxyroproinic acid (dimer) C1013 NA NA NA NA 3-Hydroxyrosprosine NA NA NA NA NA NA	3-Alanine	C00099	NA	13	5	
3-Aminoisobutyric acid C05145 NA 8 NA 3-Hydroxyadipic acid NA NA NA 9 NA 3-Hydroxydobecanoic acid NA NA NA NA 3-Hydroxydobecanoic acid NA NA NA NA 3-Hydroxydobecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisovaleric acid C05593 NA NA NA 3-Hydroxypropinic acid (dimer) C1013 NA NA NA 3-Hydroxypropinic acid NA NA NA NA 3-Hydroxypropinic acid (dimer) C1013 NA NA NA 3-Chlorotyrosine NA NA NA NA	3-Aminobutvric acid	NA	NA	17	NA	
3-Hydroxyadipic acid NA NA 9 NA 3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodcanoic acid NA NA NA NA 3-Hydroxyidodcanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisovaleric acid C20827 57 8 NA 3-Hydroxypropione C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Hydroxypropionic acid NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Hydroxypropionic acid NA NA NA 3-Hydroxypropionic acid NA NA NA 3-Hydroxypropionic acid NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Chlorotyrosine NA NA NA	3-Aminoisobutvric acid	C05145	NA	8	NA	
3-Hydroxydecanoic acid NA NA NA NA 3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyidodecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C06001 35" NA NA 3-Hydroxyphenylacetic acid C20827 57 8 NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxyraleric acid NA NA NA NA 3-Chlorotyrosine NA NA NA NA	3-Hydroxyadipic acid	NA	NA	9	NA	
3-Hydroxydodecanoic acid NA NA NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C20827 57 8 NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid NA NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) NA NA NA NA 3-Chlorotyrosine NA NA NA NA NA	3-Hydroxydecanoic acid	NA	NA	NA	NA	
3-Hydroxyisobutyric acid C06001 35* NA NA 3-Hydroxyisobutyric acid C20827 57 8 NA 3-Hydroxyisobutyric acid C20827 57 8 NA 3-Hydroxyisovaleric acid C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid NA NA NA NA S 3-Hydroxypropionic acid NA NA NA NA NA S 3-Hydroxypropionic acid NA NA NA NA NA S 3-Chlorotyrosine NA NA NA NA NA NA	3-Hydroxydodecanoic acid	NA	NA	NA	NA	
3-Hydroxyisovaleric acid C00027 57 8 NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Hydroxypropionic acid NA NA NA 3-Idototyrosine NA NA NA	3-Hydroxyisobutyric acid	C06001	35*	NA	NA	
3-Hydroxypotentia add C20021 37 8 NA 3-Hydroxyphenylacetic acid C05593 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxypropionic acid NA NA NA NA 3-Hydroxypropionic acid NA NA NA NA 3-Chlorotyrosine NA NA NA NA	3-Hydroxylsovaleric acid	C20827	57	8	NΔ	
3-Hydroxyproline C05593 NA NA NA 3-Hydroxyproline C05147 8 18 5 3-Hydroxypropionic acid (dimer) C1013 NA NA NA 3-Hydroxyvaleric acid NA NA NA NA 3-Chlorotyrosine NA NA NA NA 3-lodotyrosine NA NA NA	3-Hydroxynbonylacotic acid	C055027	57	NA	NA	
3-Hydroxypropionic acid (dimer) C1013 NA NA 3-Hydroxyvaleric acid NA NA NA 3-Chlorotyrosine NA NA NA 3-lodotyrosine NA NA NA	2-Hydroxyphenyiddelid ddiu	C05147	0	10	5	
3-Hydroxyvaleric acid Off 1 Off 3 NA NA NA 3-Hydroxyvaleric acid NA NA NA NA NA 3-Chlorotyrosine NA NA NA NA NA 3-Iodotyrosine NA NA NA NA NA	2 Hydrovypronionia goid (dimer)	C1012			5	
3-rrycroxyvaleric acid NA NA NA NA 3-Chlorotyrosine NA NA NA NA 3-lodotyrosine NA NA NA NA				NA NA		
3-Chlorotyrosine NA NA NA NA 3-lodotyrosine NA NA NA NA	S-mydroxyvaleric acid	IN/A	INA	INA NIA	NA NA	
3-IODOTYFOSINE NA NA NA NA	3-Uniorotyrosine	NA	NA	NA	NA	
	3-1000lyr0SINe	NА	NA	NA	NA	

		Reproducibility (RSD %)			
The Profiled Analyte	KEGG	Serum	Urine	Liver	
3-Methoxytyramine	C05587	NA	NA	NA	
3-Methylcrotonylglycine	C20828	NA	10	NA	
3-Methylalutaconic acid	NA	NA	NA	NA	
3-Methylalutaric acid	NΔ	11	15	9	
3-Methylbinpuric acid	NA	NA	NA	NA	
3-Nitrotyrosine	NΔ	ΝA	NΔ	NΔ	
3-Nitrotyrosine	NA	NA	NA	NA	
3-Phenyllactic acid	NΔ	NA	6	NΔ	
3-Phenylpropionic acid	C05629	NA	NΔ	NA	
A-Aminobenzoic acid	C00568	NΔ	NΔ	NΔ	
4-Aminobenzoic acid	C00334	NA	22	NΔ	
4-Hydroxybenzoic acid	C00156	NΔ	NΔ	NΔ	
4-Hydroxycinnamic acid	C00811	NΔ	11	NΔ	
4-Hydroxymandelic acid	C11527	NA	8	NΔ	
4-Hydroxynhandelic acid	C00642	Q//*	5	NA	
4-Hydroxyphenylalycino	C12222	54	NA	NA	
4-Hydroxyphenylgiycine	012323 ΝΔ	NΔ	7	NΔ	
	C01170	96*	5	NA	
4-Methoxyphenylacetic acid	NA NA	ΝΔ	NΔ	NΔ	
4 Methylaentensia soid	C21200	NA	NA	NA	
	C21399	N/A N/A	NA NA	NA	
4-Frienyibulyiic acid	C21793		7	INA 4	
5-Aminolevulinic aciu	C00430	N/A	/ NIA	4	
5-Aminovalenciacio	C00431		10	N/A	
5-Hydroxyninocolic acid	C05055	12	NA	15	
5-Hydroxypipecolic acid	COEGEO			15	
9 11 14 Eigenstriangia gold	C03059	22	NA	0	
0.11,14-Elcosaliteriore acid	003242	23	N/A	0	
9,12,15-octadecathenoic acid	C00427	NA NA	INA NA	NA NA	
	000322		7	IN/A	
Aupicaciu	C06104	INA 4	5		
Alapylalycipo	NA	4 25*	NA S	3	
Alloiseleusine	C21006	20	12		
	C21090	14	I J	N/A	
Aminomatonic aciu	C00872	N/A	17*	0	
Arachidic acid	C00425	NA NA	17	NA NA	
	C00219		7	INA 4	
	C000102	4	12	4	
Aspartic acid	C08261	9	12	0	
Azeraic acid	C00201	10*	15	10*	
Cadavorino	C01672	49 NA	NA	NA	
Cattoic acid	C011072	NA		N/A	
Canero acid	C10420	IN/A	NA NA	IN/A	
	C00417	11	INA 6	NA NA	
Citropopio opid	C000417	NA		N/A	
Citramalic acid	C00815	5	12	5	
Citric acid	C00159	ະ ເ	5	0 10	
Custothioning	C02204	NA	5 14	10	
Cystamion	C01670	INA 12*	14	15 7*	
Cysteamine	C01670		14	/	
Cysteine	C00007	INA 1.4*	12	10	
Cysteine	C01410	14" NA	13	19	
Cystemylglycine	C00404	IN/A NIA	INA NA	IN/A NIA	
Cysune Desensis said	C00491	NA 7	INA 22	INA 10*	
Decanoic acid	001571	1		12"	
Ducusanexaenoic acid	C08284	19	NA NA	11 NA	
Ducusanoic acid	000281	NA	INA 10	NA C	
Dodecanoic acid	C02679	6	10	6	



	Reproducibility (RSD, %)						Repro	ducibility (R	SD, %)
The Profiled Analyte	KEGG	Serum	Urine	Liver	The Profiled Analyte	KEGG	Serum	Urine	Liver
Dopamine	C03758	NA	12	NA	N-Acetylmethionine	C02712	NA	NA	NA
Ficosapentaenoic acid	C06428	NA	NA	NA	N-Glutarylglycine	NA	NA	NA	NA
Elaidic acid	C01712	30	NA	6	Nicotinic acid	C00253	NA	NA	9
Ethylmalonic acid	NA	NA	7	NA	N-Isovalerovlglvcine	NA	NA	NA	NA
Ferulic acid	C10470	NA	15	NΔ	N-Laurovlalanine	NA	6	8	4
Fumaric acid	C00122	16	15	8	N-Methylalanine	NA	6	6	3
Fumarylacetone	NA NA	NA	NA	NΔ	N-Methylaspartic acid	NA	NA	NA	7
Contisis acid	C00628	NA	NA	NA	N-Methylisoleucine	NA	NA	NA	NA
Glutamic acid	C00020	8	7	3	N-Methylvaline	NA	NA	NA	NA
Glutamine	C00020	26*	10*	12*	N-Oleovlalvcine	NA	NA	NA	NA
Glutaria acid	C00004	19	0	6	Nonadecanoic acid	C16535	NA	NA	NA
Glycine	C00037	6	5	3	Nonanoic acid	C01601	NA	NA	NA
Glycolic acid	C00037	NA	26*	NΔ	O-Acetylserine	C00979	NA	NA	NA
Glycylproline	NA	NA	10	8	Octacosanoic acid	C21933	NA	NA	NA
Hoptadocanoic acid	NA	NA	0	NA	Octanoic acid	C06423	NA	NA	NA
Hoptanoic acid	C17714	NA	NA		Ornithine	C00077	4	9	7
Hoxacosanoic acid	C21021	NA	NA		Palmitic acid	C00249	8	27	5
Hippurio coid	C01596	01*	7	4	Pentadecanoic acid	C16537	NA	NA NA	NA
	C01360		NIA	4	Pentanoic acid	C00803	NΔ	NΔ	NA
Histidino	C00300	17	0	11	Phenylalanine	C00079	7	5	4
Homogratoine thiologtone	C00135	NA		6*	Phenylethylamine	C02455	NA	NA	NA
Homocysteine thiolacione		N/A	22	NIA	Phenylalvoxylic acid	C02137	NΔ	NΔ	NA
	C00344	N/A	32	N/A N/A	Phenylpropionylalycine	NA	NA	NA	NA
Homovenillie	C00263	NA	NA 6	NA NA	Phenylpropionyigrycine Phenylpyruvic acid	C00166	27	20	NA
Chalastaral	C05582	NA	D NIA	NA NA	Picolinic acid	C10164		NA	10
	C00187	NA NA	INA NA	INA NA	Pimelic acid	C02656	NΔ	8	ΝΔ
	C02835	NA NA	INA NA	INA NA	Pinocolic acid	C00408	NA	NA	NA
Indol-3-propionic acid	NA	NA	INA NA	INA	Prolino	C00408	4	0	1
Indole-2-carboxylic acid		INA 10	INA 22	INA NA	Brolylbydroxyproling	NA		NA	NA NA
	000954	18	23	INA	Protytroscipo	C00134	1//*	NA	0*
ISOCITIC ACIO	C00311	30	10	6	Putrescine Duroquitamia agid	C01970	NIA	N/A	5 NA
Isoleucine	C00407	6	10	4		C01879	NA NA	NA	NA NA
Isovaleric acid	C08262	NA	NA	NA	Salicylic acid	C00805	NA	12	NA
itaconic acid	C00490	NA	8	NA	Sarcosino	C00303	11	10	1
Kainic acid	C12819	NA	NA	NA	S-Bonzy/moreopturic acid	C00213	ΝΛ	NA	4
Kynurenic acid	001717	NA	25	NA	Sobooio opid	000077		N/A	NA
Kynurenine	C00328	NA	NA	NA	Sebacic aciu	C06277	N/A N/A	N/A N/A	N/A
	C00186	2	10	1	Serino	C00065	10	0	1
Laurylamine	NA	NA	NA	NA	S Mothylayotoina	C00005	NA	O NIA	4
Leucine	C00123	6	8	4	Suborio coid	000070	N/A N/A	N/A N/A	N/A
Levulinic acid	NA	NA	NA	NA	Superio acid	C00278	7	7	114
Linoleic acid	C01595	10	NA	5	Succinic acid	C00042		NA NA	4
Lysine	C00047	4	5	4	Succinylacelone	004540	IN/A	N/A	IN/A
Maleic acid	C01384	10	9	5^	Synephine Syripgio opid	C10922	NA NA	NA NA	INA NA
	C00149	14	27^	5	Synnyic acid	C10655	N/A N/A	N/A N/A	N/A
Malonic acid	C00383	NA	NA	NA	Tartanic acid	C00696		N/A N/A	NA NA
Mandelic acid	C01984	NA	NA	NA	Tatroopponoio poid	C02207	N/A N/A	N/A N/A	N/A
wethionine	C00073	5	6	5	Thioproling	C00320	N/A N/A	0	
Methionine_sulfone	NA	13	9	13	Thiopronine	C00199	IN/A	9	4
Methylmalonic acid	C02170	NA	NA	NA	trana Uracania acid	C00188	2 12	15	4
Methylsuccinic acid	NA	NA		NA	Tridoconcio coid	C17076		NA NA	NIA
wuconic acid	002480	NA	NA	NA		C00202		N/A	
Myristic acid	C06424	7	9	10	Tryptamine	C00398	INA 4	INA E	INA 4
N(2)-Acetyllysine	C12989	NA	NA	NA	Typtopnan	C00078	4	5	4
N(6)-Acetyllysine	C02727	NA	12	NA	i yramine	C00483	NA 7	10	
N(6)-Carboxymethyllysine	NA	NA	NA	NA	i yrosine	C00082	/	9	/
N(6)-Methyllysine	NA	NA	NA	NA	Undecanoic acid	01//15	NA	NA 7	INA 4
N-Acetylaspartic acid	NA	6	7	5	valine	C00183	6	/	4
N-AcetyIglutamic acid	NA	NA	NA	NA	vanillic acid	CU6672	8	NA	/
N-Acetylglycine	NA	NA	NA	NA	vaniliyimandelic acid	005584	NA	ŏ	NA

* No internal standard was used; NA: the metabolite data were not acquired; the validated analytes in bold.

Conclusion

- MetaboAuto[®] is a user-friendly cost-effective sample preparation workstation suitable for automated GC-MS profiling of > 250 protic metabolites in animal and human metabolomic studies.
- On line, on-deck sample preparation ensures analysis of a fresh sample extract directly before GC-MS instrumental analysis.
- Quantification of more than 90 metabolites and xenobiotics in the tested urine (25 µL volume) human serum (25 µL) and in the pork liver tissue extract (7 mg).

- MetaboAuto[®] EI mass spectral library covering retention data and EI mass spectra for > 250 metabolites and xenobiotics fully operating in the NIST mass spectral library environment.
- Data processing utility for the Thermo Scientific Xcalibur, TraceFinder and Agilent MassHunter qualitative and quantitative data analysis software is an integral part of the robotic sample preparation package.



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