

## METHODS & TECHNIQUES

# Evaluation of a tandem gas chromatography/time-of-flight mass spectrometry metabolomics platform as a single method to investigate the effect of starvation on whole-animal metabolism in rainbow trout (*Oncorhynchus mykiss*)

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Accepted 1 February 2012

### SUMMARY

This study was conducted to evaluate the use of a two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC/TOF-MS) metabolomic platform to comprehensively analyze the effect of starvation on whole-animal metabolism in rainbow trout (*Oncorhynchus mykiss*). Trout were either fed a commercial diet at 2% body mass twice daily or starved for 4 weeks. Metabolomic analysis was conducted on serum, liver and muscle tissue from each fish. Database searching and statistical analysis revealed that concentrations of more than 50 positively identified molecules changed significantly ( $P<0.05$ ) as a result of starvation. Our results indicate that starving rainbow trout for 4 weeks promotes increased utilization of select tissue fatty acids in liver and muscle. However, starvation did not significantly affect protein catabolism in peripheral tissues, as indicated by reductions in the level of serum amino acids in starved fish. In contrast, starvation appears to promote protein catabolism in liver as the level of methionine, proline and lysine metabolite 2-piperidine carboxylic acid increased significantly. Also, starvation resulted in significant changes in the level of numerous xenobiotics that could indicate the origin of particular feed ingredients and selective retention of these molecules in tissues. We suggest that metabolomic analysis using GC×GC/TOF-MS is an effective tool in studying whole-animal metabolism and the fate of important xenobiotic compounds in rainbow trout as numerous polar and non-polar metabolites were rapidly and accurately profiled using a single method.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/215/10/1627/DC1>

Key words: starvation, rainbow trout, metabolomics, amino acids, fatty acids, xenobiotics, GC×GC/MS.

### INTRODUCTION

The potential for global metabolomic methods to revolutionize research on nutritional metabolism is well documented (Gibney et al., 2005; Kussman et al., 2006; Zeisel, 2007). Metabolite profiles have also been regarded as important indicators of normal phenotype and pathology, and offer the possibility of identifying surrogate biomarkers of disease states that affect metabolism (Whitfield et al., 2004; Wu et al., 2009). Nuclear magnetic resonance (NMR) has been successfully utilized to analyze the effect of starvation and temperature on energy metabolism in teleost fish species (Viant et al., 2003; Kullgren et al., 2010). However, mass spectrometry (MS)-based platforms are more sensitive than NMR at detecting shifts in low abundance metabolites. A gas chromatography (GC)/MS-based platform has significantly improved the ability of investigators to monitor shifts in metabolic fuel utilization as well as low abundance novel metabolites associated with changes in specific dietary components (Noguchi et al., 2003). The advent of two-dimensional chromatography allows a much greater number of compounds to be separated compared with standard GC/MS (Dallüge et al., 2003), providing a more sensitive and effective platform for comprehensive metabolomic analysis (Adahchour et al., 2006).

The purpose of this study was to evaluate the capability of a two-dimensional gas chromatography/time-of-flight mass spectrometry

(GC×GC/TOF-MS) platform to analyze the global metabolome of serum, liver and muscle tissue from fed and starved rainbow trout. Our data reveal significant shifts in the utilization of select tissue fatty acids due to short-term in starvation. Changes in amino acid levels indicate that starvation did not result in increased protein degradation in peripheral tissue but may have resulted in greater protein catabolism in liver of starved trout. In addition, we found significant changes in the levels of numerous xenobiotic compounds due to starvation. Here we discuss the biological significance of these results and highlight the benefits of the analytical technique employed in this study.

### MATERIALS AND METHODS

#### Experimental animals and design

A group of 12 mixed sex rainbow trout (*Oncorhynchus mykiss* Walbaum 1792; 139.2±5.9g, mean ± s.d.) were stocked individually in 30 liter tanks supplied with continuous flow-through well water (16±2°C). All fish were fed a ration of ~2% of their mean total body mass with a 45% protein/16% fat commercial diet (Silver Cup Steelhead Diet, Nelson & Sons Inc., Murray, UT, USA) for 14 days prior to the beginning of the trial. This diet met the NRC (National Research Council, 1993) guidelines for nutritional requirements of rainbow trout. After the end of this acclimation period the mean (±s.d.) mass of all 12 fish was 209.4±21.8g. All

fish were randomly assigned to two treatment groups. Six fish were fed twice daily with same feed and ration as before. The other six fish were starved for the duration of the trial. All fish were maintained on a 12h light/12h dark photoperiod cycle during the acclimation period and during the trial. The experimental trial lasted 4 weeks, at the end of which all fish were killed in a water bath containing 100 p.p.m. tricaine methanesulfonate (Argent Laboratories Inc., Redmond, WA, USA). Each fish was then weighed and sampled for serum, liver and muscle. Approximately 2 ml of blood was drawn from the caudal vein of each fish and allowed to coagulate at room temperature for 10 min, then centrifuged for 15 min at 10,000 *g* for serum collection. Approximately 1 g of white muscle tissue was removed from the left side of each fish, 2.5 cm below the dorsal fin. Whole liver, serum and muscle tissue were flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

#### Metabolite extraction and derivatization

For each fish, 200 mg liver, 200 mg muscle and 200  $\mu\text{l}$  serum were added to three separate 2 ml microcentrifuge tubes. To each tube, 400  $\mu\text{l}$  of methanol was added. Each sample was mechanically homogenized, treated with ultrasonic disruption and mixed for 1 h prior to centrifugation at 12,200 *g* for 10 min at room temperature. Supernatants were removed and added to new 2 ml microcentrifuge tubes. Chloroform (200  $\mu\text{l}$ ) was added to the remaining pellets for each sample. All samples were again sonicated, mixed and centrifuged as before. Supernatants from chloroform extractions were combined with their respective methanol extraction and vacuum centrifuged for  $\sim 90$  min at  $65^{\circ}\text{C}$ . Samples were stored at  $-80^{\circ}\text{C}$  upon completion.

Derivatization was conducted immediately prior to analysis to improve compound volatility. Each sample was reconstituted in 50  $\mu\text{l}$  of anhydrous pyridine, after which a 20  $\mu\text{l}$  aliquot was removed and added to a fresh microcentrifuge tube. Each 20  $\mu\text{l}$  sample was derivatized by adding 20  $\mu\text{l}$  of *N*-tert-butyltrimethylsilyl-*N*-methyltrifluoroacetamide (TBDMS, Sigma-Aldrich, St Louis, MO, USA), followed by heating for 1 h at  $60^{\circ}\text{C}$ , to generate a dimethyl tertbutylsilane (DMTBS) analog. Analysis was performed using a Pegasus 4D GC $\times$ GC/TOF-MS (LECO Corporation, St Joseph, MI, USA), utilizing conditions similar to those reported elsewhere (Ralston-Hooper et al., 2008). Briefly, the first dimension (1D) column was a non-polar DB-5 capillary column (J&W Scientific, 30 m $\times$ 0.25 mm $\times$ 0.25  $\mu\text{m}$ ), coupled to a second dimension (2D) medium DB-17 capillary column (J&W Scientific, 1 m $\times$ 0.10 mm $\times$ 0.10  $\mu\text{m}$ ) by a glass union. High purity helium was used for the carrier gas (1.0 ml min $^{-1}$ ). An injection volume of 2  $\mu\text{l}$  was used, with a 20:1 split ratio. The temperature program for the 1D column began at  $80^{\circ}\text{C}$  with a hold time of 0.2 min and then increased at a rate of  $8^{\circ}\text{C min}^{-1}$  to  $300^{\circ}\text{C}$  with a hold time of 20 min. The 2D column was held in a separate oven and maintained at a  $10^{\circ}\text{C}$  higher temperature than the 1D oven. The 2D separation run time was 4 s and was controlled with an internal cryogenic modulator. The injection inlet temperature and mass spectrometer transfer line were held at  $280^{\circ}\text{C}$ . The electron impact (EI) ion source was held at  $200^{\circ}\text{C}$ , with a filament bias of  $-70$  V. Mass spectra were collected from 60 to 1000 *m/z* at 100 spectra *s* $^{-1}$ .

#### Metabolite identification and peak normalization

Metabolites were identified using the procedure of Oh et al. (Oh et al., 2008). Briefly, raw data from the instrument (Fig. 1) were processed using LECO Corporation ChromaTOF software version 4.0 to generate a peak table. The ChromaTOF software uses the US

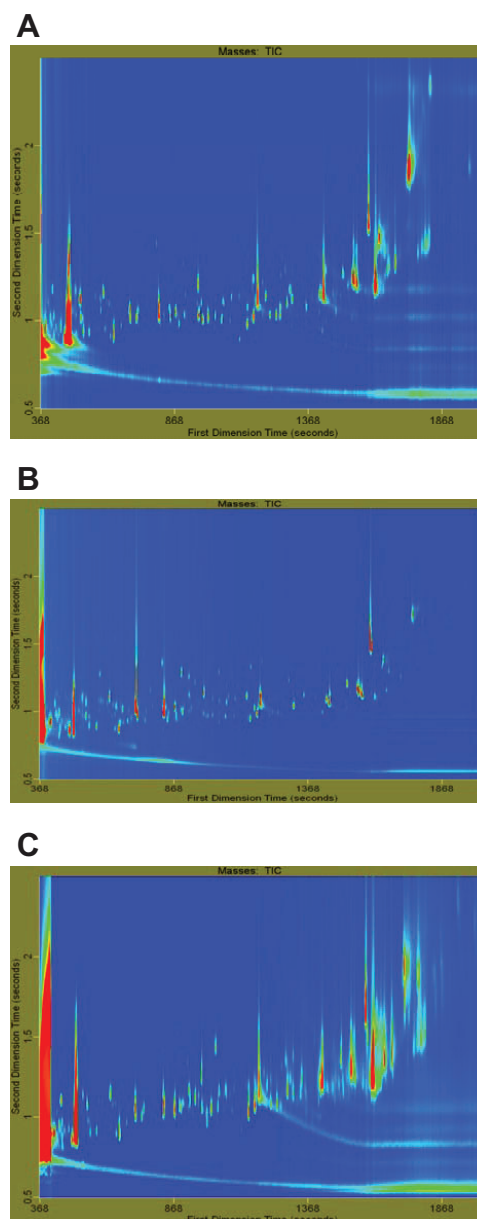


Fig. 1. Representative two-dimensional gas chromatography/time-of-flight mass spectrometry (GC $\times$ GC/TOF-MS) total ion chromatograms of rainbow trout (A) serum, (B) muscle and (C) liver. The first dimension column was a non-polar DB-5 capillary column and the second dimension column was a medium polarity DB-17 capillary column. The blue color represents low signal intensity and the red color represents chromatographic peaks of high intensity. The line across the lower region is the pyridine solvent front in the second dimension.

National Institutes of Standards and Technology (NIST) MS database (NIST MS Search 2.0, NIST/EPA/NIH Mass Spectral Library) for peak compound identification (Fig. 2). The peak table contained the features of each identified peak, such as peak name (if identified), registry number, peak area, 1D retention time, 2D retention time, fragment spectrum and similarity value (SV) for metabolite identification. The SV measures the goodness of fit between a deconvoluted sample peak spectrum and a hit in the NIST chemical database. Scores range from 0 to 999, with a score of 999 being an exact match. Peaks returning a SV of  $\geq 700$  were determined to be positive structural identifications and named with the

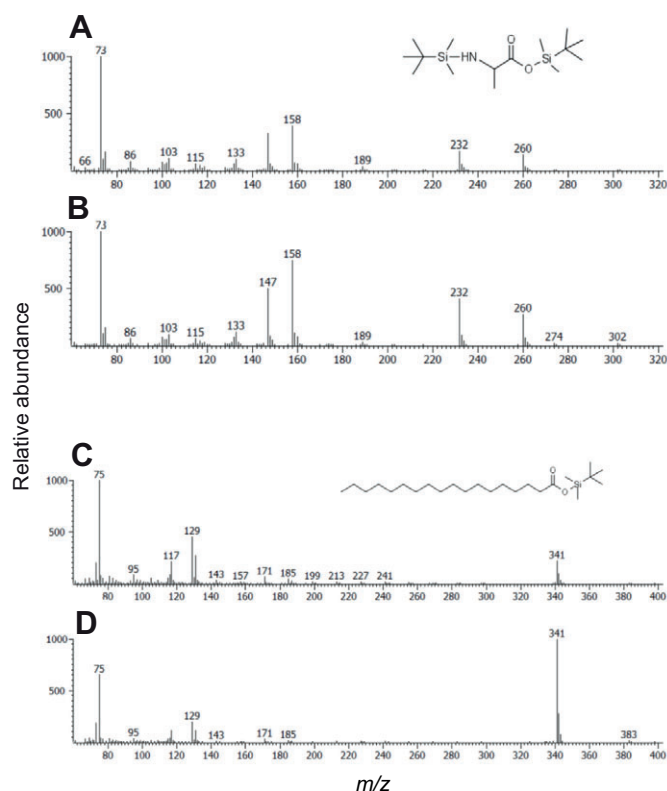


Fig. 2. Example of MS spectra for (A) a deconvoluted serum sample spectrum for the peak identified as alanine, (B) a National Institutes of Standards and Technology (NIST) library reference spectrum for derivatized alanine, (C) a deconvoluted serum sample spectrum for the peak identified as octadecanoic acid and (D) a NIST library reference spectrum for derivatized octadecanoic acid.

corresponding library metabolite (Hope et al., 2005). Peaks were normalized across samples using a constant mean approach where the total ion current was assumed equal across samples (Ralston-Hooper et al., 2008). Normalization was executed using R (v. 2.6.0, www.r-project.org) statistical software. Normalized peaks were then subjected to statistical analysis using software (MSort<sup>®</sup>) to align, match and compare metabolite peaks from various samples and groups (Ralston-Hooper et al., 2008).

#### Data analysis

Mass gain was calculated as a percentage of initial body mass ( $M_b$ ) gained during the experiment (initial  $M_b$ /final  $M_b \times 100$ ). Specific growth rate (SGR) was calculated as  $100 \times (\ln \text{ final mean } M_b - \ln \text{ initial mean } M_b) / \text{trial days}$ . Data analysis using MSort<sup>®</sup> can only be conducted in a pair-wise manner. Therefore, a paired  $t$ -test ( $P < 0.05$ ) was used to analyze differences in mean peak area for all identified metabolites that were present in all sample replicates. Conducting multiple pair-wise comparisons can increase the likelihood of making type I errors (Benjamini and Hochberg, 1995). Therefore, to ensure we did not report potential false positives, false discovery rate (FDR) analysis was conducted (Strimmer et al., 2008). Here, we report only those metabolites that were statistically significant between treatment groups and that were determined as unlikely to be type I errors.

## RESULTS AND DISCUSSION

To determine the effect of short-term starvation on whole-organism metabolism in rainbow trout we conducted metabolite analysis of

multiple tissues from fed and starved rainbow trout using a state of the art GC×GC/TOF-MS platform. Fig. 1 presents GC×GC/TOF-MS chromatograms of rainbow trout serum, liver and muscle. The horizontal axis shows the 1D time separation using a non-polar separation column. The vertical axis is the additional separation obtained by the second GC column, utilizing a mid-polarity separation. The 1D separation is in minutes, whereas the 2D separation occurs in seconds. Within the chromatogram, the blue represents the baseline and the red represents chromatographic peaks of interest, as detected by the mass spectrometer. Note the many instances where multiple compounds would have suffered from co-elution in the first dimension alone, but are separated as a result of employing 2D chromatography. The peak capacity of GC×GC/TOF-MS is far greater than could be obtained by conventional GC/MS. As a result, GC×GC/TOF-MS is a superior technique for the metabolite profiling of complex biological samples. A mass spectrum was generated for each compound observed, two of which are shown in Fig. 2A,C. All empirical spectra were compared with the NIST library for compound identification. Fig. 2B,D shows the library spectra that yielded the highest SV scores for Fig. 2A,C, respectively. As shown by the structures, both library matches corresponded to a DMTBS derivatized compound. Across all tissues, an average of 1857 compounds were detected in each sample. The mean number of positive identifications (SV of  $\geq 700$ ) per sample was 240. Therefore, approximately 13% of the compounds detected were positively identified across all three tissues. A complete list of positively identified metabolites is provided in supplementary material Tables S1–S6. In many instances multiple peaks were identified as the same compound in a given sample. In this scenario each of these peaks was ranked by their SV, with the peak having the highest SV considered the correct identification. The remaining duplicates were removed and not considered for further analysis. This reduced the mean number of positively identified compounds per sample to 143. To reduce the data set to a manageable level and to provide a more robust analysis, we selected only those metabolites that were present in all six sample replicates of both treatment groups for further analysis. This step reduced the mean number of compounds per sample to 23. The remaining compounds were subjected to FDR analysis. Table 1 lists all compounds that were found to be both statistically significant and unlikely to be type I errors.

#### Starvation results in the utilization of select tissue fatty acids

Many teleost fishes must tolerate extended periods of starvation (e.g. a complete absence of dietary intake) as part of their natural life cycle. Because teleost fishes are poikilothermic organisms, their metabolic rate is dependent on the temperature of their aqueous environment. Therefore, the response of a given teleost fish species to extended periods of starvation depends on the temperature of the water in which the animal is being maintained. In this study, we maintained rainbow trout at a temperature of  $16 \pm 2^\circ\text{C}$  with the intention of promoting a high metabolic rate to facilitate anabolic conditions in fed fish and catabolic conditions in starved animals. At the end of the trial, fed fish had gained an average of 78.5% of their initial  $M_b$ , with a final mean mass of  $377.3 \pm 48.9$  g. At the same time, starved fish lost 5% of their initial  $M_b$  resulting in a final mean ( $\pm$ s.d.) mass of  $202.2 \pm 28.6$  g. This reduction in  $M_b$  was most likely attributable to increased utilization of tissue lipid reserves. Serum analysis revealed 13 fatty acids with significantly different concentrations (Table 1). Eleven were significantly reduced in the serum of starved fish. The most

Table 1. List of positively identified metabolites whose concentrations were significantly altered by starvation in serum, liver and muscle of rainbow trout

Tissue	Metabolite	CAS no.	Starved/fed	P-value
Serum	Heptadecanoic acid	506-12-7	26.7	0.038
	3-Penten-2-one, 4-[(dimethyl-2-propenylsilyl)oxy]	88641-47-8	4.80	0.017
	Nonanoic acid	112-05-0	4.40	0.030
	Heptacosane	593-49-7	3.60	0.030
	Oxalate	13425-25-7	2.40	0.017
	2-Octyn-1-ol, 7-[(tetrahydro-2H-pyran-2-yl)oxy]	125483-30-9	2.00	0.017
	Squalene	7683-64-9	2.00	0.004
	<i>N,N,N',N'</i> -Tetraethyl-1,2-di-furan-2-yl-ethane-1,2-diamine	110-18-9	1.70	0.009
	Glycidol stearate	7460-84-6	1.60	0.030
	1-Hydroxycyclohexane-1-carboxylic acid	1123-28-0	0.67	0.004
	2,4-Hexadienoic acid	110-44-1	0.63	0.009
	2-Pentenoic acid	13991-37-2	0.63	0.017
	Decanoic acid	334-48-5	0.59	0.030
	Acetamide, 2,2,2-trifluoro- <i>N</i> -methyl	685-27-8	0.53	0.017
	Tridecane	629-50-5	0.53	0.030
	1-Pentamethyldisilyloxybutane	NA	0.48	0.017
	Pentanoic acid	109-52-4	0.48	0.004
	L-Valine	516-06-3	0.45	0.004
	2-Propenoic acid	79-10-7	0.42	0.030
	L-Leucine	61-90-5	0.40	0.009
	Hexadecane	544-76-3	0.38	0.017
	Glycine	56-40-6	0.37	0.030
	L-Serine	56-45-1	0.33	0.004
	Butanoic acid	107-92-6	0.30	0.017
	4,7,10,13,16,19-Docosahexanoic acid	6217-54-5	0.24	0.017
	L-Glutamine	56-85-9	0.22	0.030
	Propane	74-98-6	0.21	0.009
	Heptadecane, 2,6,10,15-tetramethyl	54833-48-6	0.20	0.004
	Propanoic acid	79-09-4	0.15	0.009
	L-Alanine	56-41-7	0.13	0.004
	Arachidonic acid	506-32-1	0.03	0.009
	Octadecanoic acid	57-11-4	0.01	0.004
Tetradecanoic acid	544-63-8	0.01	0.009	
Liver	L-Methionine	63-68-3	7.80	0.019
	L-Proline	609-36-9	5.40	0.036
	2,2-Dimethyl-5-[1,3]dioxolane-4-carboxaldehyde	5736-03-8	5.40	0.024
	Oleic acid	112-80-1	3.00	0.036
	Tetradecanoic acid	544-63-8	2.80	0.038
	2-Piperidinecarboxylic acid	4043-87-2	1.50	0.038
	Tris(trimethylsilyl)borate	4325-85-3	0.67	0.038
	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	1135-66-6	0.63	0.010
	Pentanoic acid	109-52-4	0.59	0.010
	4-Pyridinecarboxylic acid	55-22-1	0.50	0.010
	Docosanoic anhydride	55726-23-3	0.42	0.010
	1-Octanol, 2-butyl	3913-02-8	0.38	0.024
	L-Threonine	72-19-5	0.31	0.019
	5-Eicosene, (E)	NA	0.31	0.038
Octadecanoic acid	57-11-4	0.11	0.001	
Muscle	Octadecanoic acid	57-11-4	154.4	0.041
	Di- <i>n</i> -octyl phthalate	117-84-0	6.00	0.004
	Carbonimidodithioic acid, methyl-, dimethyl ester	53687-90-4	3.40	0.004
	2-(1,1-Dimethylethyl)-5-oxohexanal	NA	3.00	0.002
	Tetradecanoic acid	544-63-8	2.10	0.026
	Ethanedioic acid, bis(cyclohexylidenehydrazide)	370-81-0	1.80	0.015
	Carbonimidodithioic acid	34318-05-3	1.60	0.026
	Acetamide	60-35-5	1.50	0.015
	Benzenemethanol, $\alpha$ -[1-(ethylmethylamino)ethyl]	NA	0.59	0.026
	4H-Pyran-4-one	29943-42-8	0.42	0.026
	Sarcosine	107-97-1	0.24	0.004
	Oxalate	13425-25-7	0.14	0.002
	L-Aspartic acid	56-84-8	0.11	0.026
	Malonate	141-82-2	0.06	0.002
Hexadecanoic acid	57-10-3	0.04	0.009	

significant were reduced concentrations of the saturated long chain fatty acids (LCFAs) octadecanoic acid and tetradecanoic acid and the polyunsaturated fatty acids (PUFAs) arachidonic acid and 4,7,10,13,16,19-docosahexanoic acid (4,7,10,13,16,19-DHA). In addition to LCFAs, concentrations of numerous short chain fatty acids (SCFAs) were reduced significantly in the serum of starved fish. These included propanoic acid, butanoic acid, 2-propenoic acid, pentanoic acid, decanoic acid and 2-pentenoic acid. The significant reduction in serum concentrations of these molecules may represent increased clearance and utilization of circulating fatty acids for cellular energy metabolism in starved trout. However, starvation did result in an ~27-fold increase in the concentration of the LCFA heptadecanoic acid and a 4-fold increase in the concentration of the SCFA nonanoic acid in the serum of starved trout. This could indicate selective utilization of certain fatty acids early in the starvation process, reflected by their drastic reduction in concentration, and utilization of other fatty acids later in starvation as reflected by their minor reduction or increased concentration in the serum of starved fish. Alternatively, the increased serum concentrations of these two fatty acids may reflect a completely different metabolic origin and potential utilization of these compounds, one that is unrelated to utilizing tissue fatty acid reserves to meet cellular energy demands.

The liver analysis results support the notion that starvation promotes the utilization of select tissue fatty acids, as oleic acid and tetradecanoic acid concentrations increased significantly in livers of starved fish (Table 1). In contrast, levels of octadecanoic acid and pentanoic acid were significantly reduced in livers of starved fish. This may suggest that starved fish rely on hepatic octadecanoic acid and pentanoic acid earlier in the starvation process, while hepatic tetradecanoic acid and oleic acid concentrations may have increased as a result of greater mobilization and utilization of these fatty acids near the end of the 4 week period.

This was also the case for muscle fatty acid profile as hexadecanoic acid concentration was significantly reduced in muscle of starved fish. Notably, however, octadecanoic acid increased by more than 150-fold in muscle of starved fish. This significant increase in octadecanoic acid is notable given the reduced concentration of this fatty acid in serum and liver of starved fish. The results for muscle fatty acid analysis would seem to suggest preservation of octadecanoic acid and perhaps tetradecanoic acid at the cost of depleting hexadecanoic acid levels in starved fish muscle.

#### Starvation promotes increased protein catabolism in liver but not in peripheral tissues

Starvation resulted in a significant reduction in concentrations of circulating amino acids (Table 1). Perhaps most importantly, the gluconeogenic amino acids alanine and glutamine were approximately 8- and 5-fold lower, respectively, in the serum of starved fish. Glycine and serine concentrations were also significantly reduced in the serum of starved fish. This likely indicates that starved fish were not relying significantly on catabolism of peripheral tissues and hepatic gluconeogenesis to maintain an adequate blood glucose level (Aikawa et al., 1973; Odessey et al., 1974). Overall, the profile of serum amino acids seems to indicate that starvation did not result in greater levels of protein catabolism in peripheral tissues of starved fish and agrees with those of previous studies that examined the effect of starvation on energy metabolism in rainbow trout (Covey et al., 1977; Morata et al., 1982).

In contrast, the amino acid profile of liver may be indicative of increased protein catabolism of hepatic tissue in starved trout

(Table 1). Most notably, methionine was nearly 8-times greater in the liver of starved fish. Methionine is an essential amino acid for carnivorous fish species, required for protein synthesis and sulfur metabolism (Espe et al., 2008). Also, concentrations of proline increased by ~5-fold in livers of starved fish. In addition, the concentration of the lysine metabolite 2-piperidine carboxylic acid (2-PCA) was 1.5-times greater in the liver of starved fish. Although 2-PCA is produced during lysine synthesis and degradation, it is unlikely that this increase is related to greater synthesis as lysine is an essential amino acid used for protein synthesis. This finding is consistent with that of Higgins et al. (Higgins et al., 2005), who determined that starving rainbow trout for 2 weeks resulted in a 50% increase in the activity of the lysine degradation enzyme lysine  $\alpha$ -ketoglutarate reductase in livers of starved fish. Therefore, based on the increased methionine, proline and 2-PCA concentrations it seems logical to conclude that, in the current study, starving rainbow trout for 4 weeks promoted increased catabolism of cellular protein in liver.

#### Effect of starvation on biologically relevant xenobiotics

The most abundant group of xenobiotics identified in serum samples was *n*-alkanes (Table 1). Alkanes have been successfully utilized to estimate diet composition and intake of grazing herbivores (Bezabih et al., 2011). The use of *n*-alkanes as markers for determination of intake and digestibility of fish feeds has been reviewed (Gudmundsson and Halldorsdottir, 1995). In this study, short-term starvation resulted in increased concentrations of serum heptacosane, but decreased concentrations of tridecane, hexadecane, propane and 2,6,10,15-tetramethyl-heptadecane in the serum of starved fish. As these molecules are likely indicative of plant-based ingredients in feeds, their overall reduction in the serum of starved fish is not surprising given their absence in the fish diet. However, the increase in serum heptacosane, as well as the varying decreases in other *n*-alkanes in the serum of starved fish may be indicative of retention of these molecules in various tissues and may also indicate increased mobilization of tissue energy reserves. Depending on the selective retention of various *n*-alkanes in specific tissues, serum alkane levels may prove to be useful for determining how lipid, glycogen and other energy stores are mobilized during periods of prolonged food deprivation in rainbow trout.

Starvation also resulted in significant changes in the concentration of xenobiotics that could be of great importance to human health. Most notably, di-*n*-octyl phthalate (DnOP) concentrations increased 6-fold in muscle tissue of starved fish. This molecule has proven to be toxic in rats, primarily affecting the liver, kidneys, thyroid and possibly immune function when administered in longer multiple doses (Lhuguenot, 2009). DnOP is a plasticizer that is commonly used in the commercial production of plastic products, such as plastic tanks and feed containers. Therefore, storage of commercially prepared diets in plastic containers may have resulted in an increased exposure of rainbow trout to DnOP. Further, the increased concentration of DnOP in skeletal muscle of starved trout may indicate that it is not lipid or glycogen soluble and may have been retained in muscle tissue of starved fish. As a result, when an equal mass of tissue was analyzed, there was a greater concentration of DnOP per gram of muscle tissue in starved vs fed trout. Many additional xenobiotic molecules were found to have changed significantly as a result of starvation (Table 1). Thus far, however, little information is available on their toxicity and biological importance. Further studies are needed to determine the impact of these xenobiotics on the health of fish and humans alike.

### CONCLUSIONS

In this study, we have demonstrated the capability of GC×GC/TOF-MS metabolomics to rapidly and accurately detect changes in numerous polar and non-polar molecules. Using this single technique we were able to determine that starving rainbow trout for 4 weeks resulted in the utilization of select tissue fatty acids and increased catabolism of cellular proteins in liver but not in peripheral tissues. Also, we have shown that GC×GC/TOF-MS is useful for analyzing dynamic changes in numerous xenobiotic compounds that may be of great importance to animal and human health. The primary advantage of this metabolomic platform is its sensitivity and accuracy for detecting changes in the concentration of such a dynamic group of chemical compounds from a single sample. Future work should utilize complementary techniques, such as HPLC/MS or NMR, to verify and support the metabolites identified in this screening study.

### ACKNOWLEDGEMENTS

The authors thank Dr Paul B. Brown, Jeff Weber and Robert Rode of the Purdue University Aquaculture Research Laboratory for their technical assistance. The authors also thank Douglas Sweet of the Ohio Department of Natural Resources London Fish Hatchery for kindly providing the animals for this study.

### FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Table S1. Mean similarity value (SV), mass (Da), 1D and 2D retention times (RT) of positively identified (SV $\geq$ 700) metabolites from serum of fed rainbow trout

Name	SV	Mass	1D RT (s)	2D RT(s)
.psi...psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	748	131	1736	1.78
1-Di(tert-butyl)silyloxybutane	706	131	724	1.33
1-Dimethyl(prop-2-enyl)silyloxyoctadecane	720	97	1488	1.06
1-Ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane	701	80	1620	1.10
1-Iodo-2-methylundecane	847	71	1036	1.02
1-Octanol, 2-butyl-	838	71	632	0.98
1-Penten-3-one, 4(R)-t-butyltrimethylsilyloxy-	743	157	552	0.99
1-Propanol, 2,2-dimethyl-	728	284	724	1.04
1-Undecene, 7-methyl-	862	71	648	0.98
1,2-Benzenedicarboxylic acid, diisooctyl ester	893	149	1560	1.43
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	922	149	1584	1.48
1,2-Bis(trimethylsilyloxy)ethane	737	259	848	1.07
1,2-Ethanediamine, N-(2-aminoethyl)-	764	110	852	1.07
1,3-Dioxolane	834	89	956	1.10
1,3-Dioxolane, 2-(1-methylethyl)-	703	147	1572	1.17
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	722	73	1208	0.91
1,8-cis-Undecadien-5-yne 3,7-bis(trimethylsilyl) ether	761	129	1912	1.19
11-Eicosenoic acid, trimethylsilyl ester	724	96	1504	1.14
13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol	814	71	1704	1.25
2-Butenoic acid, 2-[(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	735	147	1004	1.10
2-Cyclopenten-1-one, 2-hydroxy-	721	98	528	1.44
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	856	75	640	0.98
2-Pentadecanol	788	69	640	0.99
2-Pentenoic acid, 2-[(tert-butyltrimethylsilyloxy)-3-methyl-, tert-butyltrimethylsilyl ester	713	301	1804	1.35
2-Pentenoic acid, trimethylsilyl ester	780	157	760	1.36
2-Piperidinecarboxylic acid, tert-butyltrimethylsilyl ester, (DL)-	725	84	1028	1.48
2-Propynamide, N,N-diethyl-3-phenyl-	711	129	1840	1.41
2,2-Dimethyl-5-[2-(2-trimethylsilylethoxymethoxy)-propyl]-[1,3]dioxolane-4-carboxaldehyde	759	70	1620	1.24
2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-	714	174	612	1.11
3-(2-Hydroxyethyl)imidazole-2-thione	738	100	796	1.46
3-Penten-2-one, 4-[(dimethyl-2-propenylsilyloxy)-	769	157	592	1.13
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	911	91	1532	1.41
5-Amino-3-methyl-1,2,4-oxadiazole	719	99	520	1.51
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	769	79	1416	1.33
9-Octadecenamide, (Z)-	771	72	1476	1.43
9-Octadecenoic acid (Z)-, methyl ester	836	87	1300	1.22
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	736	67	2176	0.95
9,12-Octadecadienoic acid, tert-butyltrimethylsilyl ester, (Z,Z)-	794	75	1528	1.19
9,12-Octadecadienoyl chloride, (Z,Z)-	752	98	1544	1.35
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	811	80	1540	1.38
á-Alanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	846	218	944	1.06
Acetaldehyde, O-methylloxime	868	64	700	1.19
Acetic acid, [(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	927	189	820	1.06
Analyte 103	707	160	528	1.07
Analyte 1249	872	117	1112	1.09
Analyte 1458	897	149	1208	1.49
Analyte 153	741	144	560	1.17
Analyte 159	857	71	564	0.96
Analyte 20	780	171	500	0.97
Analyte 229	777	117	596	1.15
Analyte 439	721	152	700	1.23
Analyte 737	730	71	856	1.00
Analyte 766	784	71	864	1.00
Analyte 85	762	143	524	1.11
Arachidonic acid, trimethylsilyl ester	837	91	1484	1.23
Azetidone-2-one, 3-hexyl-3-methyl-	736	70	580	0.97
Azidotrimethylsilane	710	100	604	1.17
Benzaldehyde, 2,4-dimethyl-	809	133	560	1.37
Benzeneacetic acid, 3-methoxy-à,4-bis[(trimethylsilyloxy)-, trimethylsilyl ester	730	297	1200	1.24
Benzoic acid trimethylsilyl ester	841	179	808	1.28
Benzoic acid, 3-amino-, ethyl ester	927	120	852	1.64
Benzoic acid, 4-amino-, tert-butyltrimethylsilyl ester	848	194	1108	1.50
Bis(dimethyl-t-butylsilyl) succinate	848	289	1040	1.12
Butanoic acid, 2-[(tert-butyltrimethylsilyl)amino]-, tert-butyltrimethylsilyl ester, (+.-)-	804	73	912	1.04
Butanoic acid, 4-[(trimethylsilyloxy)-, trimethylsilyl ester	767	147	704	1.06
Carbamodithioic acid, diethyl-, methyl ester	838	60	724	1.54

Carbonimidodithioic acid, methyl-, dimethyl ester	756	88	620	1.64
Cholest-5-en-3-ol (3á)-, carbonochloridate	866	91	2492	2.20
Cholesta-3,5-diene	883	91	1816	2.20
Cholesterol trimethylsilyl ether	757	129	2028	2.53
Cyclooctane, 1,4-dimethyl-, trans-	772	71	848	1.00
Disilane, pentamethyl-	702	147	1436	1.13
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	935	148	492	0.88
Disiloxane, pentamethyl-	713	133	496	0.92
Dodecanamide	884	72	1356	1.40
Dodecane, 4-methyl-	869	71	548	0.96
Dodecane, 4,6-dimethyl-	798	71	580	0.96
Dodecanoic acid, tert-butyldimethylsilyl ester	874	75	1140	1.10
E-9-Tetradecenol,trimethylsilyl ether	712	67	2832	0.96
Eicosane	875	71	812	0.99
Ethanethioic acid S-tert-butyl ester	708	132	512	1.17
Glycidol stearate	719	98	1556	1.31
Glycine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	932	246	872	1.07
Hentriacontane	811	71	1464	1.04
Heptacosane	897	71	1000	1.01
Heptadecane, 2,6,10,14-tetramethyl-	894	71	780	0.99
Heptadecane, 2,6,10,15-tetramethyl-	854	71	980	1.01
Heptadecanoic acid, tert-butyldimethylsilyl ester	861	327	1484	1.12
Hexadecane	914	71	572	0.96
Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	721	98	1432	1.28
Hexadecanoic acid, tert-butyldimethylsilyl ester	829	313	1268	1.40
Hexanedioic acid, bis(2-ethylhexyl) ester	944	129	1492	1.25
Hexasiloxane, tetradecamethyl-	729	221	1108	0.92
Hexatriacontane	808	71	1364	1.06
L-Alanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	935	158	852	1.05
L-Aspartic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	837	147	1336	1.10
L-Cysteine, N,S-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	734	73	1372	1.13
L-Glutamic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	851	272	1416	1.11
L-Leucine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	927	200	992	1.04
L-Lysine, N2,N6-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	815	198	1484	1.07
L-Methionine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	924	218	1208	1.17
L-Phenylalanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	918	234	1296	1.20
L-Proline, 1-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	939	184	1052	1.12
L-Serine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	884	288	1220	1.07
L-Threonine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	869	103	1244	1.06
L-Tryptophan, N,1-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	809	244	1800	1.90
L-Tyrosine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	917	302	1640	1.28
L-Valine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	924	302	956	1.05
Linolenic acid, trimethylsilyl ester	765	91	1524	1.24
Methyl 4-methyl-4-nitroso-2-trimethylsiloxy-pentanoate	704	74	900	1.07
N-Ethylformamide	734	73	1724	1.36
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	944	200	1020	1.05
Nonadecanoic acid, tert-butyldimethylsilyl ester	802	355	1604	1.19
Octadecanoic acid, methyl ester	886	74	1316	1.18
Octadecanoic acid, tert-butyldimethylsilyl ester	828	341	1560	1.13
Oleic Acid	726	67	2824	0.96
Palmitelaidic acid, trimethylsilyl ester	794	60	2156	0.86
Pentadecanoic acid, tert-butyldimethylsilyl ester	858	299	1356	1.12
Pentanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester, (DL)-	774	89	1108	1.17
Pentanoic acid, 3-methyl-, tert-butyldimethylsilyl ester	702	173	616	1.05
Phosphoric acid, tris(tert-butyldimethylsilyl) ester	874	383	992	1.43
Phthalic acid, monocyclohexyl ester	804	179	1588	1.52
Phytanic acid, tert-butyldimethylsilyl ester	748	369	1516	1.10
Picolinic acid, trimethylsilyl ester	824	180	852	1.39
Propane, 1,1-dimethoxy-	718	75	1704	1.59
Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	908	89	1136	1.01
Propanedioic acid, bis(trimethylsilyl) ester	785	233	732	0.99
Propanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester	870	189	804	1.04
Pyridine, 3-trimethylsiloxy-	712	152	588	1.15
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	907	83	1064	2.27
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-	749	146	552	0.94
Silane, (2-methoxyethoxy)trimethyl-	701	89	876	1.05
Silanol, trimethyl-	904	75	700	0.85
Squalene	911	69	1744	1.70
tert-Butylpentamethyldisiloxane	791	147	544	0.85
Tetradecanoic acid, tert-butyldimethylsilyl ester	918	75	1288	1.12
Tetratriacontane	758	71	1592	1.08
Thymol-á-d-glucopyranoside, tetrakis(O-trimethylsilyl)-	706	361	1724	1.20



Tridecane	829	85	504	0.94
Trimethyl(n-pentyl)silane	701	73	1728	1.19
Tris(trimethylsilyl)borate	858	221	640	0.93
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	750	281	968	0.91
Undecane, 2-methyl-	855	71	496	0.95
Undecane, 2,6-dimethyl-	891	71	540	0.96
Urea, N,N'-bis(tert-butyl dimethylsilyl)-	911	231	952	1.23
Z-10-Pentadecenol, trimethylsilyl ether	740	67	2332	0.95
Z-7-Tetradecenol, trimethylsilyl ether	744	67	2260	0.95

'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.

Table S2. Mean similarity value (SV), mass (Da), 1D and 2D retention times (RT) of positively identified (SV≥700) metabolites from serum of starved rainbow trout

Name	SV	Mass	1D RT (s)	2D RT(s)
(Methoxymethyl)trimethylsilane	704	199	808	1.17
1-Butanamine, N-butyl-N-methyl-	748	100	864	1.51
1-Di(tert-butyl)silyloxybutane	701	131	724	1.34
1-Dimethyl(prop-2-enyl)silyloxyoctadecane	743	97	1488	1.11
1-Iodo-2-methylundecane	863	71	1036	1.03
1-Octanol, 2-butyl-	864	71	632	1.00
1-Pentamethylsilyloxydodecane	769	147	744	0.98
1-Pentamethylsilyloxy pentadecane	754	147	1364	1.37
1-Propanol, 2,2-dimethyl-	904	115	952	1.11
1-Undecene, 7-methyl-	827	69	648	1.01
1,1,1,3,5,5,5-Heptamethyltrisiloxane	703	207	812	0.92
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	814	149	1136	1.44
1,2-Benzenedicarboxylic acid, diisooctyl ester	885	149	1560	1.47
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	936	167	1592	1.54
1,2-Dihydroxyanthraquinone, O,O'-bis(trimethylsilyl)-	702	369	1660	1.35
1,3-Dioxolane	775	73	828	1.05
1,3-Dioxolane, 2-(1-bromoethyl)-	714	199	516	1.10
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	720	73	1864	1.53
1,6-Dihydroxy-2-methylanthraquinone, O,O'-bis(trimethylsilyl)-	746	383	1172	1.15
1,8-Naphthalenediamine, N,N,N',N'-tetramethyl-	780	168	1024	1.54
13,13-Dimethyl-3,6,9-trioxo-13-silatetradecan-1-ol	744	71	1712	1.32
2-Cyclopenten-1-one, 2-hydroxy-	774	98	528	1.45
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	860	75	640	0.99
2-Pentenedioic acid, 2-[(tert-butyl dimethylsilyl)oxy]-, bis(tert-butyl dimethylsilyl) ester	774	431	1456	1.04
2-Piperidinecarboxylic acid, tert-butyl dimethylsilyl ester, (DL)-	730	84	1028	1.47
2,2-Dimethyl-5-[2-(2-trimethylsilylethoxymethoxy)-propyl]-[1,3]dioxolane-4-carboxaldehyde	704	79	1524	1.28
2,4-Hexadienoic acid, tert-butyl dimethylsilyl ester, (E,E)-	758	169	736	1.24
2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-	718	174	612	1.12
3-(2-Hydroxyethyl)imidazole-2-thione	760	100	796	1.46
3-Penten-2-one, 4-[(dimethyl-2-propenylsilyl)oxy]-	770	157	592	1.14
3,3-Dichloropropylene	732	67	1616	1.40
4-Pyridinecarboxylic acid, tert-butyl dimethylsilyl ester	851	180	832	1.24
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	923	79	1532	1.46
5-Amino-3-methyl-1,2,4-oxadiazole	797	99	520	1.51

5-Undecene, (E)-	805	70	792	1.01
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	833	79	1416	1.35
6H-Dibenzo(b,d)pyran-1-ol, 3-hexyl-7,8,9,10-tetrahydro-6,6,9-trimethyl-	794	311	1420	1.22
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	751	205	1180	1.45
9-Hexadecenoic acid	708	69	2636	0.98
9-Octadecenoic acid (Z)-, methyl ester	917	87	1300	1.22
9,12-Octadecadienoic acid, methyl ester	722	67	1296	1.26
9,12-Octadecadienoyl chloride, (Z,Z)-	735	82	1544	1.40
̂-Alanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	710	117	944	1.08
Acetic acid, [(tert-butyltrimethylsilyl)oxy]-, tert-butyltrimethylsilyl ester	940	189	820	1.07
Analyte 103	742	160	528	1.08
Analyte 1147	725	140	1060	1.35
Analyte 1249	800	117	1112	1.10
Analyte 1270	859	71	1124	1.04
Analyte 1458	940	149	1208	1.48
Analyte 1619	811	117	1316	1.05
Analyte 1772	879	71	1416	1.07
Analyte 1965	753	87	1536	1.38
Analyte 2024	777	71	1568	1.14
Analyte 229	729	117	596	1.16
Analyte 362	872	71	660	0.99
Analyte 439	755	152	700	1.23
Analyte 476	733	171	716	1.60
Analyte 566	714	73	776	1.04
Analyte 737	807	71	856	1.01
Analyte 766	830	69	864	1.02
Analyte 85	712	143	524	1.11
Analyte 850	758	100	908	1.30
Arachidonic acid, trimethylsilyl ester	830	91	1732	1.36
Benzaldehyde, 2,4-dimethyl-	890	133	560	1.38
Benzeneacetic acid, 3-methoxy-̂,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	726	297	1200	1.23
Benzoic acid trimethylsilyl ester	893	179	808	1.27
Benzoic acid, 3-amino-, ethyl ester	962	120	852	1.65
Benzoic acid, 4-amino-, tert-butyltrimethylsilyl ester	843	194	1108	1.49
Bis(dimethyl-t-butylsilyl) oxalate	712	95	1304	1.13
Butanoic acid, 2-[(tert-butyltrimethylsilyl)amino]-, tert-butyltrimethylsilyl ester, (.+.-)-	787	172	912	1.05
Carbamodithioic acid, diethyl-, methyl ester	865	60	724	1.55
Carbonimidodithioic acid, methyl-, dimethyl ester	753	88	620	1.65
Cholest-5-en-3-ol (3̂)-, carbonochloridate	737	105	1696	2.24
Cholest-5-ene, 3-bromo-, (3̂)-	786	147	1736	2.29
Cholesta-3,5-diene	894	81	1816	2.26
cis-6-Octadecenoic acid, trimethylsilyl ester	800	117	2100	0.95
Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	751	117	1736	1.84
Cyclooctane, 1,4-dimethyl-, trans-	770	69	848	1.02
Decanamide, N-(2-hydroxyethyl)-	750	85	1328	1.28
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	902	148	492	0.90
Dodecanamide	875	72	1360	1.40
Dodecane, 2,7,10-trimethyl-	777	71	720	1.01
Dodecane, 4-methyl-	866	71	548	0.98
Dodecane, 4,6-dimethyl-	846	71	580	0.98
Dodecanoic acid, tert-butyltrimethylsilyl ester	887	75	1140	1.11
Eicosane	874	71	812	1.00
Ethanethioic acid S-tert-butyl ester	743	132	512	1.18
Ethanol, 2-(trimethylsilyl)-	817	89	1532	1.20
Glycidol stearate	715	98	1556	1.36
Glycine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	937	218	872	1.07
Heptacosane	909	71	1112	1.04
Heptadecane, 2,6,10,14-tetramethyl-	867	71	780	1.00
Heptadecanoic acid, tert-butyltrimethylsilyl ester	830	327	1484	1.16
Hexadecane	898	71	604	0.98
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	711	98	1400	1.30
Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	723	98	1432	1.31
Hexadecanoic acid, dimethyl(isopropyl)silyl ester	717	313	2404	0.97
Hexanedioic acid, bis(2-ethylhexyl) ester	951	129	1496	1.27
Hexanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	886	289	1040	1.13
Hexasiloxane, tetradecamethyl-	722	221	1108	0.92
L-Alanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	861	158	852	1.05
L-Aspartic acid, N-(tert-butyltrimethylsilyl)-, bis(tert-butyltrimethylsilyl) ester	723	133	1328	1.14
L-Glutamic acid, N-(tert-butyltrimethylsilyl)-, bis(tert-butyltrimethylsilyl) ester	752	272	1416	1.13

L-Glutamine, N,N2-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	704	74	1512	1.21
L-Leucine, N-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	897	200	992	1.05
L-Lysine, N2,N6-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	769	198	1484	1.11
L-Methionine, N-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	935	61	1208	1.17
L-Phenylalanine, N-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	882	234	1296	1.21
L-Proline, 1-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	941	184	1052	1.13
L-Serine, N,O-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	869	133	1220	1.07
L-Threonine, N,O-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	862	103	1244	1.07
L-Tryptophan, N,1-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	797	244	1800	1.94
L-Tyrosine, N,O-bis(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	918	302	1640	1.34
L-Valine, N-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	925	186	956	1.06
Methyl tetradecanoate	899	74	1012	1.16
N-Ethylformamide	754	73	1836	1.73
n-Hexadecanoic acid	713	69	2644	0.98
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	941	200	1020	1.05
Nonadecane	864	71	772	1.00
Nonadecanoic acid, tert-butylidimethylsilyl ester	740	355	1604	1.24
Nonanoic acid, tert-butylidimethylsilyl ester	827	75	892	1.08
Octacosane	726	71	1592	1.15
Octadecanoic acid, methyl ester	903	74	1316	1.19
Oleic Acid	762	83	2560	0.98
Oleic acid, trimethylsilyl ester	926	117	1380	1.16
Palmitelaidic acid, trimethylsilyl ester	850	129	1248	1.14
Pentadecanoic acid, tert-butylidimethylsilyl ester	861	75	1356	1.14
Pentanoic acid, 2-[(tert-butylidimethylsilyl)oxy]-, tert-butylidimethylsilyl ester, (DL)-	779	89	1108	1.17
Pentanoic acid, 3-methyl-, tert-butylidimethylsilyl ester	791	75	616	1.05
Phosphoric acid, tris(tert-butylidimethylsilyl) ester	947	383	1180	1.11
Phthalic acid, monocyclohexyl ester	836	167	1588	1.59
Phytanic acid, tert-butylidimethylsilyl ester	743	369	1516	1.15
Picolinic acid, trimethylsilyl ester	825	180	852	1.39
Propane, 1,2,3-tris[(tert-butylidimethylsilyl)oxy]-	904	89	1136	1.01
Propanedioic acid, bis(trimethylsilyl) ester	819	117	700	1.10
Propanoic acid, 2-[(tert-butylidimethylsilyl)oxy]-, tert-butylidimethylsilyl ester	830	261	812	1.04
Pyridine, 3-trimethylsiloxy-	722	152	588	1.15
Sarcosine, N-(tert-butylidimethylsilyl)-, tert-butylidimethylsilyl ester	767	158	904	1.07
Silamine, 1,1,1-trimethyl-N-(trimethylsilyl)-	741	146	552	0.96
Silane, (2-methoxyethoxy)trimethyl-	718	89	876	1.06
Silane, (cyclopropylcarbonyl)(1,1-dimethylethyl)dimethyl-	720	61	992	1.07
Silane, tetramethyl-	949	87	1176	1.24
Silane, trimethyl-3-penten-2-yl-, trans	701	256	968	1.12
Silane, trimethylphenoxy-	800	151	612	1.14
Silanol, trimethyl-	878	75	500	0.75
Squalene	858	69	1744	1.75
tert-Butylpentamethyldisiloxane	774	75	668	1.00
Tetradecanoic acid, tert-butylidimethylsilyl ester	918	75	1288	1.13
trans-3-Hexenedioic acid, bis(trimethylsilyl) ester	735	147	1004	1.10
Tridecane	723	85	504	0.96
Tris(trimethylsilyl)borate	827	221	640	0.94
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	759	281	968	0.92
Undecane, 2-methyl-	862	71	492	0.97
Undecane, 2,6-dimethyl-	884	71	540	0.97
Urea, N,N'-bis(tert-butylidimethylsilyl)-	929	231	952	1.23
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	749	98	1420	1.36
Z-7-Tetradecenol,trimethylsilyl ether	872	75	2244	0.96

'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.

Table S3. Mean similarity value (SV), mass (Da), 1D and 2D retention times (RT) of positively identified (SV $\geq$ 700) metabolites from liver of fed rainbow trout

Name	SV	Mass	1D RT (s)	2D RT(s)
(t-Butyldimethylsilyl)[2-methyl-2-(4-methyl-pent-3-enyl)-cyclopropyl]-methanone	712	211	876	1.07
1-Dimethyl(isopropyl)silyloxypropane	764	117	624	1.08
1-Iodo-2-methylundecane	835	71	1060	0.99
1-Octanol, 2-butyl-	823	84	668	0.92
1-Pentamethylsilyloxybutane	741	67	832	0.97
1-Propanol, 2,2-dimethyl-	821	425	1344	1.16
1,1,1,3,5,5,5-Heptamethyltrisiloxane	734	207	832	0.87
1,1'-Methylene-bis(di-2-propenylamine)	711	110	1104	1.14
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	927	149	1216	1.47
1,2-Benzenedicarboxylic acid, diisooctyl ester	904	91	1584	1.88
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	900	149	1580	1.59
1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	770	207	1124	1.37
1,2-Bis(trimethylsilyloxy)cyclohexene	704	258	904	1.05
1,3-Dioxolane	764	73	2328	2.86
1,3-Dioxolane, 2-(1-bromoethyl)-	740	73	2236	2.63
1,3-Dioxolane, 2-(1-methylethyl)-	766	284	748	0.99
1,3-Oxathiane, 2-methyl-2-(1-methylethyl)-	701	117	792	1.23
1,6-Dihydroxy-2-methylanthraquinone, O,O'-bis(trimethylsilyl)-	750	67	1192	1.04
1,8-Dihydroxy-3-methylanthraquinone, O,O'-bis(trimethylsilyl)-	760	172	1192	1.12
1,8-Naphthalenediamine, N,N,N',N'-tetramethyl-	842	168	1044	1.48
13-Heptadecyn-1-ol	711	81	2692	1.05
13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol	712	353	1600	1.27
15-Hydroxypentadecanoic acid	703	98	1432	1.30
2-Benzyl-3-methoxycyclopropanecarboxylic acid, methyl ester	777	129	884	1.34
2-Butenedioic acid (Z)-, bis(trimethylsilyl) ester	789	245	864	0.99
2-Butenedioic acid, bis(tert-butyldimethylsilyl) ester, (E)-	923	287	1076	1.07
2-Butenoic acid, 2-[(tert-butyldimethylsilyloxy)-, tert-butyldimethylsilyl ester	701	273	1264	1.03
2-Butenoic acid, 3-[(tert-butyldimethylsilyloxy)-, tert-butyldimethylsilyl ester	703	273	1036	1.12
2-Butyl(dimethyl)silyloxybutane	706	131	752	1.25
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	818	187	664	0.92
2-Hydroxy-2-methylbutyric acid	717	89	1700	1.40
2-Methyl-1-(trimethylsilylmethyl)dimethylsilyloxypropane	777	203	564	0.85
2-Pentamethylsilyloxybutane	849	231	976	1.11
2-Pentenedioic acid, 2-[(tert-butyldimethylsilyloxy)-, bis(tert-butyldimethylsilyl) ester	753	431	1452	1.03
2-Pentenoic acid, trimethylsilyl ester	723	157	784	1.29
2-Piperidinecarboxylic acid, 1-(trimethylsilyl)-, trimethylsilyl ester	720	156	944	0.94
2-Propenoic acid, (1-methyl-1,2-ethanediyloxy)bis[oxy(methyl-2,1-ethanediyloxy)] ester	718	113	940	0.98
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	773	179	832	1.18
2,4-Hexadienoic acid, tert-butyldimethylsilyl ester, (E,E)-	768	169	764	1.16
2,4,6-tri-tert-Butylaniline	713	246	1092	1.34
3-(2-Hydroxyethyl)imidazole-2-thione	725	100	824	1.38
3-(Cyclopropylamino)propionitrile	851	70	536	0.82
3-Eicosene, (E)-	818	71	860	0.96
3,3-Dichloropropyne	700	282	1076	1.13
3,6,10,14,17-Pentaoxa-2,18-disilanonadecane, 2,2,18,18-tetramethyl-	736	117	1984	1.26
3,6,9-Trioxa-2,10-disilaundecane, 2,2,10,10-tetramethyl-	703	117	1876	1.26
3H-Purin-6-amine, 3-methyl-N-(trimethylsilyl)-	701	206	1252	1.59
4-Pyridinecarboxylic acid, tert-butyldimethylsilyl ester	848	180	876	1.22
4-Undecene, 7-methyl-	756	69	608	0.90
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	769	106	1740	2.30
5-Eicosyne	715	67	2504	1.05
7-Tetradecene, (E)-	766	69	884	0.97
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	764	205	1192	1.43
9-Octadecenoic acid (Z)-, methyl ester	786	87	1304	1.21
9,12-Octadecadienoic acid, tert-butyldimethylsilyl ester, (Z,Z)-	758	75	1524	1.24
9,12-Octadecadienoyl chloride, (Z,Z)-	724	82	2580	1.04
Á-Alanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	911	218	960	1.04
Acetic acid, (acetyloxy)-	705	188	728	1.09
Acetic acid, (trimethylsilyl)-	812	117	960	0.78
Acetic acid, [(tert-butyldimethylsilyloxy)-, tert-butyldimethylsilyl ester	917	247	844	1.02
Analyte 1335	700	70	1064	0.84
Analyte 1574	871	72	1156	1.16

Analyte 518	702	156	740	0.92
Analyte 835	786	165	872	0.91
Arachidonic acid, trimethylsilyl ester	783	91	1624	1.47
Ascorbic acid, 2,3,5,6-tetrakis-O-(tert-butyltrimethylsilyl)-	787	443	1744	1.56
Benzenepropanoic acid, à-oxo-á, á-bis(trimethylsilyl)-	797	293	1188	1.28
Benzoic acid, 2,6-bis(trimethylsiloxy)-, methyl ester	746	297	1112	1.05
Benzothiophene-2-carboxylic acid, 4,5,6,7-tetrahydro-7-hydroximino-3-[2-(4-morpholyl)-1-oxoethylamino]-, ethyl ester	769	96	632	1.06
Bis(dimethyl-t-butylsilyl) adipate	734	317	1208	1.13
Bis(dimethyl-t-butylsilyl) maleate	890	287	1048	1.15
Bis(dimethyl-t-butylsilyl) malonate	704	189	848	1.00
Bis(dimethyl-t-butylsilyl) oxalate	829	261	880	1.07
Bis(dimethyl-t-butylsilyl) succinate	893	289	1056	1.10
Butanedioic acid, 2-[(tert-butyltrimethylsilyloxy)-, bis(tert-butyltrimethylsilyl) ester	911	287	1312	1.10
Butanoic acid, 2-[(tert-butyltrimethylsilylamino)-, tert-butyltrimethylsilyl ester, (.+.-)-	787	172	932	1.00
Butanoic acid, 4-[(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	841	275	984	1.03
Cadaverine, N,N,N',N'-tetrakis(trimethylsilyl)	719	174	640	1.05
Carbamodithioic acid, diethyl-, methyl ester	853	88	752	1.44
Cyclohexane, 1-ethyl-2-propyl-	821	69	1040	1.00
Cyclohexene, 3,3-dimethyl-1-(trimethylsilyloxy)-	708	183	796	1.18
Cyclopentane, 1-butyl-2-propyl-	732	69	1200	1.02
Decane, 2,3,4-trimethyl-	834	71	576	0.89
Decane, 2,6,8-trimethyl-	722	69	604	0.90
Decane, 2,9-dimethyl-	764	71	1068	0.99
Decanoic acid, tert-butyltrimethylsilyl ester	720	229	992	1.05
Di-n-octyl phthalate	727	280	1584	1.68
Diphenylhydramine	731	165	748	0.91
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	948	117	720	0.72
dl-Citrulline	782	83	848	0.97
Docosanoic anhydride	729	98	1552	1.38
Dodecanoic acid, tert-butyltrimethylsilyl ester	886	257	1148	1.08
E-2-Octadecadecen-1-ol	701	110	2020	1.01
Eicosane	876	85	996	0.97
Ethanedioic acid, bis(trimethylsilyl) ester	728	89	948	1.05
Ethanethioic acid S-tert-butyl ester	704	132	544	1.09
Ethanol, 2-(trimethylsilyl)-	707	173	1532	1.26
Glycine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	936	246	888	1.03
Hentriacontane	762	127	1552	1.11
Heptacosane	895	85	1156	0.99
Heptadecane	849	85	936	0.96
Heptadecane, 2,6-dimethyl-	702	71	868	0.96
Heptadecane, 2,6,10,15-tetramethyl-	764	71	1832	1.78
Heptadecanoic acid, tert-butyltrimethylsilyl ester	709	327	1468	1.15
Heptanoic acid, tert-butyltrimethylsilyl ester	758	187	736	1.00
Hexadecane	890	71	632	0.90
Hexadecanoic acid, methyl ester	787	87	1180	1.15
Hexadecanoic acid, tert-butyltrimethylsilyl ester	815	117	1424	1.16
Hexanedioic acid, bis(2-ethylhexyl) ester	950	111	1492	1.30
Hexasiloxane, tetradecamethyl-	712	221	1120	0.88
Hexatriacontane	808	71	1364	1.05
Isotridecyl alcohol, trimethylsilyl derivative	718	71	1396	1.05
L-Alanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	939	70	872	0.99
L-Aspartic acid, N-(tert-butyltrimethylsilyl)-, bis(tert-butyltrimethylsilyl) ester	888	302	1340	1.11
L-Cysteine, N,S-bis(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	811	302	1376	1.13
L-Glutamic acid, N-(tert-butyltrimethylsilyl)-, bis(tert-butyltrimethylsilyl) ester	841	330	1416	1.14
L-Leucine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	931	200	1008	1.01
L-Lysine, N2,N6-bis(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	719	198	1480	1.10
L-Methionine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	941	218	1216	1.14
L-Phenylalanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	918	234	1300	1.19
L-Proline, 1-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	936	258	1064	1.09
L-Serine, N,O-bis(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	754	288	1228	1.04
L-Threonine, N,O-bis(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	910	303	1252	1.03
L-Tyrosine, N,O-bis(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	915	302	1632	1.35
L-Valine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	943	186	972	1.01
n-Hexadecanoic acid	843	256	2100	0.88
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	875	302	1032	1.02
Nonacosane	870	71	1212	1.01
Nonane, 2-methyl-3-methylene-	716	84	676	0.93
Nonane, 2,8-dimethyl-4-methylene-	730	111	868	0.97

Nonanoic acid, tert-butyldimethylsilyl ester	895	215	912	1.04
Octadecanoic acid, methyl ester	844	87	1320	1.17
Octadecanoic acid, tert-butyldimethylsilyl ester	852	343	1540	1.27
Octanoic acid, tert-butyldimethylsilyl ester	856	201	824	1.02
Oleic Acid	856	83	2220	1.01
Pentadecane	759	85	752	0.93
Pentadecanoic acid, tert-butyldimethylsilyl ester	838	299	1356	1.12
Pentanoic acid, 3-methyl-, tert-butyldimethylsilyl ester	823	173	644	0.98
Pentanoic acid, 3-methyl-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	702	159	1052	1.08
Pentanoic acid, 4-oxo-, trimethylsilyl ester	771	173	708	1.16
Phosphoric acid, bis(tert-butyldimethylsilyl) ethyl ester	718	211	960	1.11
Phosphoric acid, tris(tert-butyldimethylsilyl) ester	875	118	1740	0.80
Phytanic acid, tert-butyldimethylsilyl ester	709	369	1512	1.12
Picolinic acid, trimethylsilyl ester	838	180	876	1.33
Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	947	377	1148	0.99
Propane, 2,2'-[methylenebis(oxy)]bis-	703	89	1528	1.19
Propanedioic acid, bis(trimethylsilyl) ester	864	233	740	0.99
Propanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester	836	103	828	1.01
Propanoic acid, 2,3-bis[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester, (DL)-	725	231	1204	1.03
Propanoic acid, 3-[(tert-butyldimethylsilyl)oxy]-2-methyl-, tert-butyldimethylsilyl ester	884	275	920	1.00
Propanoic acid, dimethyl(isopropyl)silyl ester	700	101	900	1.35
Pyridine, 3-methyl-2,6-diphenyl-	760	244	1788	2.01
Pyridine, 3-trimethylsiloxy-	711	152	728	1.17
S-Methyl 2-propenethioate	808	102	692	0.88
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-	722	146	580	0.87
Silanamine, N,1,1,1-tetramethyl-N-[1-methyl-2-phenyl-2-[(trimethylsilyl)oxy]ethyl]-, [S-(R*,R*)]-	724	130	864	1.19
Silane, (2-methoxyethoxy)trimethyl-	700	89	896	1.01
Silane, dimethoxydimethyl-	754	105	576	0.98
Silane, tetramethyl-	789	73	2480	2.70
Squalene	853	81	1732	1.83
t-Butyldimethyl(10-octylundec-10-enyloxy)silane	700	155	1528	1.21
Taurine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	889	296	1176	1.20
tert-Butyldimethylsilanol	706	75	1492	1.25
tert-Butylpentamethyldisiloxane	907	148	536	0.79
Tetradecane	766	71	744	0.93
Tetradecanoic acid, tert-butyldimethylsilyl ester	925	129	1292	1.11
Thiazolidine-2,5-dione	712	117	968	0.99
trans-(2-Chlorovinyl)dimethylethoxysilane	710	149	556	0.92
Trimethyl[4-(1,1,3,3-tetramethylbutyl)phenoxy]silane	742	207	1192	1.60
Trimethylsilyl 3-pyridinecarboximidoate #	769	179	1024	1.37
Tris(trimethylsilyl)borate	843	221	664	0.86
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	735	281	984	0.88
Undecane, 2,6-dimethyl-	864	71	568	0.88
Undecanoic acid, tert-butyldimethylsilyl ester	731	243	1072	1.06
Urea, N,N'-bis(tert-butyldimethylsilyl)-	915	232	972	1.21
Z-10-Pentadecen-1-ol	744	95	2320	1.03

'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.

Table S4. Mean similarity value (SV), mass (Da), 1-and 2-D retention times (RT) of positively identified (SV ≥ 700) metabolites from liver of starved rainbow trout

Name	SV	Mass	1D RT (s)	2D RT (s)
1-[3-(3-Chloro-4-propoxyphenyl)propyl]guanidine	715	173	888	1.01
1-Decanol, 2-hexyl-	775	71	1088	1.00
1-Dimethyl(isopropyl)silyloxypropane	722	117	624	1.09
1-Iodo-2-methylundecane	832	71	676	0.92
1-Octanol, 2-butyl-	850	71	660	0.92
1-Pentamethylsilyloxycyclopentane	781	165	832	1.02
1-Propanol, 2,2-dimethyl-	760	246	960	1.08
1-Propene, 2-(1-methylethoxy)	835	75	632	1.09
1,1,1,3,5,5,5-Heptamethyltrisiloxane	724	207	832	0.87
1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	934	149	1216	1.49
1,2-Benzenedicarboxylic acid, diisooctyl ester	952	72	1584	1.68
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	942	167	1588	1.47
1,2-Bis(trimethylsilyloxy)cyclohexene	706	258	904	1.05
1,3-Dioxolane	792	73	2352	2.86
1,3-Dioxolane, 2-(1-bromoethyl)-	778	70	1184	1.33
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	804	69	652	0.89
1,3-Dioxolane, 2-(6-heptynyl)-	762	314	1204	1.27
1,3-Dioxolane, 2-butyl-	811	85	872	0.99
1,3-Propanediol, 2-hydroxymethyl-2-nitro-, triacetate	741	116	2288	1.20
1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trimethyl-	721	171	752	1.49
1,8-Dihydroxy-3-methylanthraquinone, O,O'-bis(trimethylsilyl)-	842	381	1188	1.15
1,8-Naphthalenediamine, N,N,N',N'-tetramethyl-	782	168	1044	1.49
15-Hydroxypentadecanoic acid	723	98	1432	1.32
1H-Pyrido(4,3-b)indole, 2,3,4,5-tetrahydro-1,1,3,3-tetramethyl-	771	175	724	1.00
2-(3-Chloropropyl)-1,3-dioxolane	736	71	924	0.98
2-Butenedioic acid (Z)-, bis(trimethylsilyl) ester	797	148	864	1.00
2-Butenedioic acid, bis(tert-butyltrimethylsilyl) ester, (E)-	902	287	1076	1.07
2-Butenoic acid, 2-[(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	881	273	944	1.05
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	768	187	664	0.93
2-Pentamethylsilyloxybutane	773	201	824	1.02
2-Pentenoic acid, 2-[(tert-butyltrimethylsilyloxy)-3-methyl-, tert-butyltrimethylsilyl ester	709	143	1776	1.41
2-Piperidinecarboxylic acid, tert-butyltrimethylsilyl ester, (DL)-	710	186	1044	1.44
2-Propen-1-ol, 1-[(1,1-dimethylethyl)dimethylsilyl]-	716	64	832	0.84
2-Propenoic acid, trimethylsilyl ester	711	114	604	1.06
2,2-Dimethyl-5-[2-(2-trimethylsilyloxyethoxy)propyl]-[1,3]dioxolane-4-carboxaldehyde	811	85	1332	1.29
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	777	179	832	1.18
2,4-Hexadienoic acid, tert-butyltrimethylsilyl ester, (E,E)-	782	169	764	1.17
2,6-Dimethyl-6-trifluoroacetoxyoctane	823	71	884	0.97
3-(2-Hydroxyethyl)imidazole-2-thione	724	100	824	1.39
3-Buten-2-one, 3-trimethylsilyloxy-	707	143	552	1.03
3-Cyclopropylcarbonyloxydodecane	712	70	812	0.95
3-Penten-2-one, 4-[(dimethyl-2-propenylsilyloxy)-	759	157	620	1.06
3,6-Dioxa-2,7-disilaoctane, 2,2,4,7,7-pentamethyl-	751	117	928	1.05
3,6,9-Trioxa-2-silaundecane, 2,2-dimethyl-	707	563	1604	1.22
4-Pyridinecarboxylic acid, tert-butyltrimethylsilyl ester	842	180	876	1.34
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	768	91	1724	1.94
5-Eicosene, (E)-	816	69	860	0.97
9-Octadecenoic acid (Z)-, methyl ester	787	87	1304	1.22
α-Alanine, N-(tert-butyltrimethylsilyl)-, tert-butyltrimethylsilyl ester	916	144	960	1.03
Acetic acid, [(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	927	247	844	1.02
Acetic acid, 10-dimethoxymethyl-13-methyl-3-oxo-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3H-cyclopenta[a]phenanthren-17-yl (ester)	729	93	1680	1.65
Acetic acid, 3-[1,3]dioxolan-2-ylpropyl ester	706	73	1916	1.77
Analyte 2289	710	71	1564	1.12
Analyte 981	706	295	924	1.11
Arachidonic acid, trimethylsilyl ester	772	75	1624	1.49
Ascorbic acid, 2,3,5,6-tetrakis-O-(tert-butyltrimethylsilyl)-	744	148	1744	1.59
Benzenepropanoic acid, α-oxo-á, á-bis(trimethylsilyl)-	731	293	1192	1.17
Benzoic acid, 5-methoxy-2-[(trimethylsilyloxy)-, trimethylsilyl ester	723	297	1112	1.06
Bicyclo[6.3.0]undeca-1(8),2-dien-7-one, 5,5-dimethyl-3-(t-butyltrimethylsilyloxy)-	708	263	1092	1.13
Bis(dimethyl-t-butylsilyl) maleate	803	287	1048	1.15
Bis(dimethyl-t-butylsilyl) malonate	833	189	848	1.00
Bis(dimethyl-t-butylsilyl) oxalate	865	261	880	1.08
Bis(dimethyl-t-butylsilyl) succinate	927	289	1056	1.11
Butanal, 2-ethyl-	838	72	1156	1.16
Butanedioic acid, 2-[(tert-butyltrimethylsilyloxy)-, bis(tert-butyltrimethylsilyl)	918	189	1312	1.10

Butanoic acid, 2-[(tert-butyl(dimethylsilyl)amino)-, tert-butyl(dimethylsilyl) ester, (.+)-]	810	246	932	1.01
Butanoic acid, 3-methyl-2-[(trimethylsilyloxy)-, trimethylsilyl ester	707	145	1020	1.05
Butanoic acid, 4-[(tert-butyl(dimethylsilyl)amino)-, tert-butyl(dimethylsilyl) ester	756	274	1056	1.06
Butanoic acid, 4-[(tert-butyl(dimethylsilyl)amino)-, tert-butyl(dimethylsilyl) ester	704	142	1068	1.14
Cadaverine, N,N,N',N'-tetrakis(trimethylsilyl)	721	174	640	1.06
Carbamodithioic acid, diethyl-, methyl ester	852	88	752	1.46
Chloromethyl cyanide	729	75	740	1.05
Cyclopentaneundecanoic acid	726	67	2244	1.03
Dimethyl-(allyl)-silyloxybenzene	777	151	640	1.07
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	879	147	596	0.75
Disiloxane, ethylpentamethyl-	716	190	536	0.78
Dodecanoic acid, tert-butyl(dimethylsilyl) ester	896	257	1148	1.09
E-11-Tetradecenoic acid	710	69	2592	1.03
Eicosane	860	85	616	0.91
Ethanedioic acid, bis(trimethylsilyl) ester	799	110	948	1.05
Ethanol, 2-(trimethylsilyl)-	735	75	808	1.05
Ethyl 2-methylnicotinate	708	137	1228	1.58
Glycine	949	75	836	0.84
Heptacosane	888	71	1180	1.01
Heptadecane	794	71	720	0.92
Heptadecane, 2,6,10,14-tetramethyl-	758	71	1276	1.03
Heptadecanoic acid, tert-butyl(dimethylsilyl) ester	765	83	1484	1.17
Heptanoic acid, tert-butyl(dimethylsilyl) ester	897	187	736	1.00
Hexadecane	891	71	632	0.90
Hexadecanoic acid, methyl ester	905	74	1180	1.16
Hexadecenoic acid, Z-11-	757	67	1844	1.01
Hexane, 2,2,5-trimethyl-	763	71	1484	1.08
Hexane, 4-ethyl-2,2-dimethyl-	756	113	828	0.97
Hexanedioic acid, bis(2-ethylhexyl) ester	957	129	1492	1.31
Hexasiloxane, tetradecamethyl-	722	221	1120	0.89
Hexatriacontane	725	69	1224	1.02
Isopropyl tert-butyl(dimethylsilyl) methylphosphonate	716	153	636	1.11
Isotridecanol-	816	69	1040	1.00
Isotridecyl alcohol, trimethylsilyl derivative	774	71	1396	1.06
L-Alanine, N-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	862	274	872	1.03
L-Aspartic acid, N-(tert-butyl(dimethylsilyl)-, bis(tert-butyl(dimethylsilyl) ester	939	418	1340	1.11
L-Cysteine, N,S-bis(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	882	304	1376	1.13
L-Glutamic acid, N-(tert-butyl(dimethylsilyl)-, bis(tert-butyl(dimethylsilyl) ester	885	432	1416	1.15
L-Glutamine, N,N2-bis(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	797	142	1508	1.21
L-Leucine, N-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	951	200	1008	1.01
L-Methionine, N-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	895	218	1216	1.15
L-Norleucine, N-(trimethylsilyl)-, trimethylsilyl ester	717	158	1024	1.03
L-Phenylalanine, N-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	872	302	1304	1.19
L-Proline, 1-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	795	184	1064	1.10
L-Serine, N,O-bis(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	808	288	1228	1.04
L-Threonine, N,O-bis(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	869	303	1252	1.04
L-Tyrosine, N,O-bis(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	913	221	1632	1.36
L-Valine, N-(tert-butyl(dimethylsilyl)-, tert-butyl(dimethylsilyl) ester	937	186	972	1.02
Malonic acid, bis(2-trimethylsilylethyl) ester	710	73	1700	1.43
Methane, dimethoxy-	747	75	2820	1.17
Methyl 4-methyl-4-nitroso-2-trimethylsilyloxy-pentanoate	939	196	1188	1.24
n-Hexadecanoic acid	768	157	2532	0.90
N-Morpholinomethyl-isopropyl-sulfide	735	127	536	1.06
N,O-Bis-(trimethylsilyl)-2-pyrrolidone carboxylic acid	766	156	944	0.95
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	912	302	1032	1.02
Nonanoic acid, tert-butyl(dimethylsilyl) ester	830	215	912	1.04
Octacosane	850	71	1212	1.02
Octadecanoic acid, methyl ester	854	143	1320	1.19
Octadecanoic acid, tert-butyl(dimethylsilyl) ester	860	81	1540	1.26
Octanamide, N,N-dimethyl-	704	87	1408	1.36
Oleic Acid	778	83	1900	1.01
Oxid. dithiothrietol, bis(t-butyl(dimethylsilyl)- deriv.	761	245	1284	1.19
Pentadecanoic acid, tert-butyl(dimethylsilyl) ester	879	299	1356	1.13
Pentane, 3-methoxy-	791	301	1064	1.08
Pentanoic acid, 3-methyl-, tert-butyl(dimethylsilyl) ester	821	173	644	0.98
Pentasiloxane, dodecamethyl-	725	281	984	0.88
Phosphoric acid, tris(tert-butyl(dimethylsilyl) ester	900	91	1188	1.04
Propane, 1,1-dimethoxy-	741	75	1816	1.96
Propane, 1,2,3-tris[(tert-butyl(dimethylsilyl)oxy]-	863	164	1148	0.98



Propanedioic acid, bis(trimethylsilyl) ester	832	233	724	1.03
Propanoic acid, 2-[(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester	804	233	832	0.99
Propanoic acid, 3-[(tert-butyltrimethylsilyloxy)-, tert-butyltrimethylsilyl ester, (.+.)-	809	218	972	1.03
Pyridine, 3-trimethylsilyloxy-	724	152	728	1.15
S-Methyl 2-propenethioate	757	102	692	0.89
Silane, 1,1,1-trimethyl-N-(trimethylsilyl)-	743	146	580	0.87
Silane, N,1,1,1-tetramethyl-N-[1-methyl-2-phenyl-2-[(trimethylsilyloxy)ethyl]-, [S-(R*,R*)]-	788	130	864	1.20
Silane, (2-methoxyethoxy)trimethyl-	707	89	896	1.01
Silane, tetramethyl-	835	97	1204	1.04
Squalene	701	136	1732	1.87
tert-Butylpentamethyldisiloxane	867	148	540	0.78
Tetradecane, 5-methyl-	715	111	1068	1.00
Tetradecanoic acid, tert-butyltrimethylsilyl ester	930	75	1292	1.12
Thiazolidine-2,5-dione	762	74	732	1.38
Tributylamine	775	142	720	1.22
Tridecane, 2,2,4,10,12,12-hexamethyl-7-(3,5,5-trimethylhexyl)-	762	71	1556	1.12
Trimethylsilyl 3-pyridinecarboximidate #	765	136	1024	1.39
Tris(trimethylsilyl)borate	856	148	664	0.87
Undecane, 2,2-dimethyl-	723	71	1060	0.99
Undecane, 2,6-dimethyl-	886	71	568	0.88
Undecane, 4,8-dimethyl-	812	71	576	0.89
Urea, N,N'-bis(tert-butyltrimethylsilyl)-	925	232	972	1.24
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	756	123	2680	1.04
Z-10-Pentadecen-1-ol	762	111	2372	1.04

'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.

Table S5. Mean similarity value (SV), mass (Da), 1D and 2D retention times (RT) of positively identified (SV $\geq$ 700) metabolites from muscle of fed rainbow trout

Name	SV	Mass	1D RT (s)	2D RT(s)
.psi...psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	748	131	1736	1.78
1-Di(tert-butyl)silyloxybutane	706	131	724	1.33
1-Dimethyl(prop-2-enyl)silyloxyoctadecane	720	97	1488	1.06
1-Ethenyl-3-(1-hexenyl)-4-trimethylsilylcyclopentane	701	80	1620	1.10
1-Iodo-2-methylundecane	847	71	1036	1.02
1-Octanol, 2-butyl-	838	71	632	0.98
1-Penten-3-one, 4(R)-t-butyl dimethylsilyloxy-	743	157	552	0.99
1-Propanol, 2,2-dimethyl-	728	284	724	1.04
1-Undecene, 7-methyl-	862	71	648	0.98
1,2-Benzenedicarboxylic acid, diisooctyl ester	893	149	1560	1.43
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	922	149	1584	1.48
1,2-Bis(trimethylsilyloxy)ethane	737	259	848	1.07
1,2-Ethanediamine, N-(2-aminoethyl)-	764	110	852	1.07
1,3-Dioxolane	834	89	956	1.10
1,3-Dioxolane, 2-(1-methylethyl)-	703	147	1572	1.17
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	722	73	1208	0.91
1,8-cis-Undecadien-5-yne 3,7-bis-trimethylsilyl ether	761	129	1912	1.19
11-Eicosenoic acid, trimethylsilyl ester	724	96	1504	1.14
13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol	814	71	1704	1.25
2-Butenoic acid, 2-[(tert-butyl dimethylsilyloxy)-, tert-butyl dimethylsilyl ester	735	147	1004	1.10
2-Cyclopenten-1-one, 2-hydroxy-	721	98	528	1.44
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	856	75	640	0.98
2-Pentadecanol	788	69	640	0.99
2-Pentenoic acid, 2-[(tert-butyl dimethylsilyloxy)-3-methyl-, tert-butyl dimethylsilyl ester	713	301	1804	1.35
2-Pentenoic acid, trimethylsilyl ester	780	157	760	1.36
2-Piperidinecarboxylic acid, tert-butyl dimethylsilyl ester, (DL)-	725	84	1028	1.48
2-Propynamide, N,N-diethyl-3-phenyl-	711	129	1840	1.41
2,2-Dimethyl-5-[2-(2-trimethylsilylethoxymethoxy)-propyl]-[1,3]dioxolane-4-carboxaldehyde	759	70	1620	1.24
2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-	714	174	612	1.11
3-(2-Hydroxyethyl)imidazole-2-thione	738	100	796	1.46
3-Penten-2-one, 4-[(dimethyl-2-propenylsilyloxy)-	769	157	592	1.13
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	911	91	1532	1.41
5-Amino-3-methyl-1,2,4-oxadiazole	719	99	520	1.51
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	769	79	1416	1.33
9-Octadecenamide, (Z)-	771	72	1476	1.43
9-Octadecenoic acid (Z)-, methyl ester	836	87	1300	1.22
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	736	67	2176	0.95
9,12-Octadecadienoic acid, tert-butyl dimethylsilyl ester, (Z,Z)-	794	75	1528	1.19
9,12-Octadecadienoyl chloride, (Z,Z)-	752	98	1544	1.35
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	811	80	1540	1.38
à-Alanine, N-(tert-butyl dimethylsilyloxy)-, tert-butyl dimethylsilyl ester	846	218	944	1.06
Acetaldehyde, O-methylxime	868	64	700	1.19
Acetic acid, [(tert-butyl dimethylsilyloxy)-, tert-butyl dimethylsilyl ester	927	189	820	1.06
Analyte 103	707	160	528	1.07
Analyte 1249	872	117	1112	1.09
Analyte 1458	897	149	1208	1.49
Analyte 153	741	144	560	1.17
Analyte 159	857	71	564	0.96
Analyte 20	780	171	500	0.97
Analyte 229	777	117	596	1.15
Analyte 439	721	152	700	1.23
Analyte 737	730	71	856	1.00
Analyte 766	784	71	864	1.00
Analyte 85	762	143	524	1.11
Arachidonic acid, trimethylsilyl ester	837	91	1484	1.23
Azetidine-2-one, 3-hexyl-3-methyl-	736	70	580	0.97
Azidotrimethylsilane	710	100	604	1.17
Benzaldehyde, 2,4-dimethyl-	809	133	560	1.37
Benzeneacetic acid, 3-methoxy-à,4-bis[(trimethylsilyloxy)-, trimethylsilyl ester	730	297	1200	1.24
Benzoic acid trimethylsilyl ester	841	179	808	1.28
Benzoic acid, 3-amino-, ethyl ester	927	120	852	1.64
Benzoic acid, 4-amino-, tert-butyl dimethylsilyl ester	848	194	1108	1.50
Bis(dimethyl-t-butylsilyl) succinate	848	289	1040	1.12
Butanoic acid, 2-[(tert-butyl dimethylsilyloxy)-amino]-, tert-butyl dimethylsilyl ester,	804	73	912	1.04
Butanoic acid, 4-[(trimethylsilyloxy)-, trimethylsilyl ester	767	147	704	1.06

Carbamodithioic acid, diethyl-, methyl ester	838	60	724	1.54
Carbonimidodithioic acid, methyl-, dimethyl ester	756	88	620	1.64
Cholest-5-en-3-ol (3á)-, carbonochloridate	866	91	2492	2.20
Cholesta-3,5-diene	883	91	1816	2.20
Cholesterol trimethylsilyl ether	757	129	2028	2.53
Cyclooctane, 1,4-dimethyl-, trans-	772	71	848	1.00
Disilane, pentamethyl-	702	147	1436	1.13
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	935	148	492	0.88
Disiloxane, pentamethyl-	713	133	496	0.92
Dodecanamide	884	72	1356	1.40
Dodecane, 4-methyl-	869	71	548	0.96
Dodecane, 4,6-dimethyl-	798	71	580	0.96
Dodecanoic acid, tert-butyldimethylsilyl ester	874	75	1140	1.10
E-9-Tetradecenol,trimethylsilyl ether	712	67	2832	0.96
Eicosane	875	71	812	0.99
Ethanethioic acid S-tert-butyl ester	708	132	512	1.17
Glycidol stearate	719	98	1556	1.31
Glycine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	932	246	872	1.07
Hentriacontane	811	71	1464	1.04
Heptacosane	897	71	1000	1.01
Heptadecane, 2,6,10,14-tetramethyl-	894	71	780	0.99
Heptadecane, 2,6,10,15-tetramethyl-	854	71	980	1.01
Heptadecanoic acid, tert-butyldimethylsilyl ester	861	327	1484	1.12
Hexadecane	914	71	572	0.96
Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	721	98	1432	1.28
Hexadecanoic acid, tert-butyldimethylsilyl ester	829	313	1268	1.40
Hexanedioic acid, bis(2-ethylhexyl) ester	944	129	1492	1.25
Hexasiloxane, tetradecamethyl-	729	221	1108	0.92
Hexatriacontane	808	71	1364	1.06
L-Alanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	935	158	852	1.05
L-Aspartic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	837	147	1336	1.10
L-Cysteine, N,S-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	734	73	1372	1.13
L-Glutamic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	851	272	1416	1.11
L-Leucine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	927	200	992	1.04
L-Lysine, N2,N6-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	815	198	1484	1.07
L-Methionine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	924	218	1208	1.17
L-Phenylalanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	918	234	1296	1.20
L-Proline, 1-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	939	184	1052	1.12
L-Serine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	884	288	1220	1.07
L-Threonine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	869	103	1244	1.06
L-Tryptophan, N,1-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	809	244	1800	1.90
L-Tyrosine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	917	302	1640	1.28
L-Valine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	924	302	956	1.05
Linolenic acid, trimethylsilyl ester	765	91	1524	1.24
Methyl 4-methyl-4-nitroso-2-trimethylsiloxy-pentanoate	704	74	900	1.07
N-Ethylformamide	734	73	1724	1.36
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	944	200	1020	1.05
Nonadecanoic acid, tert-butyldimethylsilyl ester	802	355	1604	1.19
Octadecanoic acid, methyl ester	886	74	1316	1.18
Octadecanoic acid, tert-butyldimethylsilyl ester	828	341	1560	1.13
Oleic Acid	726	67	2824	0.96
Palmitelaidic acid, trimethylsilyl ester	794	60	2156	0.86
Pentadecanoic acid, tert-butyldimethylsilyl ester	858	299	1356	1.12
Pentanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester, (DL)-	774	89	1108	1.17
Pentanoic acid, 3-methyl-, tert-butyldimethylsilyl ester	702	173	616	1.05
Phosphoric acid, tris(tert-butyldimethylsilyl) ester	874	383	992	1.43
Phthalic acid, monocyclohexyl ester	804	179	1588	1.52
Phytanic acid, tert-butyldimethylsilyl ester	748	369	1516	1.10
Picolinic acid, trimethylsilyl ester	824	180	852	1.39
Propane, 1,1-dimethoxy-	718	75	1704	1.59
Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	908	89	1136	1.01
Propanedioic acid, bis(trimethylsilyl) ester	785	233	732	0.99
Propanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester	870	189	804	1.04
Pyridine, 3-trimethylsiloxy-	712	152	588	1.15
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	907	83	1064	2.27
Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-	749	146	552	0.94
Silane, (2-methoxyethoxy)trimethyl-	701	89	876	1.05
Silanol, trimethyl-	904	75	700	0.85
Squalene	911	69	1744	1.70

tert-Butylpentamethyldisiloxane	791	147	544	0.85
Tetradecanoic acid, tert-butyldimethylsilyl ester	918	75	1288	1.12
Tetratriacontane	758	71	1592	1.08
Thymol- $\alpha$ -D-glucopyranoside, tetrakis(O-trimethylsilyl)-	706	361	1724	1.20
Tridecane	829	85	504	0.94
Trimethyl(n-pentyl)silane	701	73	1728	1.19
Tris(trimethylsilyl)borate	858	221	640	0.93
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	750	281	968	0.91
Undecane, 2-methyl-	855	71	496	0.95
Undecane, 2,6-dimethyl-	891	71	540	0.96
Urea, N,N'-bis(tert-butyldimethylsilyl)-	911	231	952	1.23
Z-10-Pentadecenol,trimethylsilyl ether	740	67	2332	0.95
Z-7-Tetradecenol,trimethylsilyl ether	744	67	2260	0.95

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'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.

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Table S6. Mean similarity value (SV), mass (Da), 1D and 2D retention times (RT) of positively identified (SV $\geq$ 700) metabolites from muscle of starved rainbow trout

Name	SV	Mass	1D RT (s)	2D RT(s)
(Methoxymethyl)trimethylsilane	704	199	808	1.17
1-Butanamine, N-butyl-N-methyl-	748	100	864	1.51
1-Di(tert-butyl)silyloxybutane	701	131	724	1.34
1-Dimethyl(prop-2-enyl)silyloxyoctadecane	743	97	1488	1.11
1-Iodo-2-methylundecane	863	71	1036	1.03
1-Octanol, 2-butyl-	864	71	632	1.00
1-Pentamethyldisilyloxydodecane	769	147	744	0.98
1-Pentamethyldisilyloxy pentadecane	754	147	1364	1.37
1-Propanol, 2,2-dimethyl-	904	115	952	1.11
1-Undecene, 7-methyl-	827	69	648	1.01
1,1,1,3,5,5,5-Heptamethyltrisiloxane	703	207	812	0.92
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	814	149	1136	1.44
1,2-Benzenedicarboxylic acid, diisooctyl ester	885	149	1560	1.47
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	936	167	1592	1.54
1,2-Dihydroxyanthraquinone, O,O'-bis(trimethylsilyl)-	702	369	1660	1.35
1,3-Dioxolane	775	73	828	1.05
1,3-Dioxolane, 2-(1-bromoethyl)-	714	199	516	1.10
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	720	73	1864	1.53
1,6-Dihydroxy-2-methylanthraquinone, O,O'-bis(trimethylsilyl)-	746	383	1172	1.15
1,8-Naphthalenediamine, N,N,N',N'-tetramethyl-	780	168	1024	1.54
13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol	744	71	1712	1.32
2-Cyclopenten-1-one, 2-hydroxy-	774	98	528	1.45
2-Ethyl-1-dimethyl(isopropyl)silyloxyhexane	860	75	640	0.99
2-Pentenedioic acid, 2-[(tert-butyl)dimethylsilyloxy]-, bis(tert-butyl)dimethylsilyl ester	774	431	1456	1.04
2-Piperidinecarboxylic acid, tert-butyl)dimethylsilyl ester, (DL)-	730	84	1028	1.47
2,2-Dimethyl-5-[2-(2-trimethylsilylethoxymethoxy)-propyl]-[1,3]dioxolane-4-carboxaldehyde	704	79	1524	1.28
2,4-Hexadienoic acid, tert-butyl)dimethylsilyl ester, (E,E)-	758	169	736	1.24
2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-	718	174	612	1.12
3-(2-Hydroxyethyl)imidazole-2-thione	760	100	796	1.46
3-Penten-2-one, 4-[(dimethyl-2-propenyl)silyloxy]-	770	157	592	1.14
3,3-Dichloropropyne	732	67	1616	1.40
4-Pyridinecarboxylic acid, tert-butyl)dimethylsilyl ester	851	180	832	1.24
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	923	79	1532	1.46
5-Amino-3-methyl-1,2,4-oxadiazole	797	99	520	1.51
5-Undecene, (E)-	805	70	792	1.01
5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	833	79	1416	1.35
6H-Dibenzo(b,d)pyran-1-ol, 3-hexyl-7,8,9,10-tetrahydro-6,6,9-trimethyl-	794	311	1420	1.22
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	751	205	1180	1.45
9-Hexadecenoic acid	708	69	2636	0.98
9-Octadecenoic acid (Z)-, methyl ester	917	87	1300	1.22
9,12-Octadecadienoic acid, methyl ester	722	67	1296	1.26
9,12-Octadecadienoyl chloride, (Z,Z)-	735	82	1544	1.40
Á-Alanine, N-(tert-butyl)dimethylsilyl)-, tert-butyl)dimethylsilyl ester	710	117	944	1.08
Acetic acid, [(tert-butyl)dimethylsilyloxy]-, tert-butyl)dimethylsilyl ester	940	189	820	1.07
Analyte 103	742	160	528	1.08
Analyte 1147	725	140	1060	1.35
Analyte 1249	800	117	1112	1.10
Analyte 1270	859	71	1124	1.04
Analyte 1458	940	149	1208	1.48
Analyte 1619	811	117	1316	1.05
Analyte 1772	879	71	1416	1.07
Analyte 1965	753	87	1536	1.38
Analyte 2024	777	71	1568	1.14

Analyte 229	729	117	596	1.16
Analyte 362	872	71	660	0.99
Analyte 439	755	152	700	1.23
Analyte 476	733	171	716	1.60
Analyte 566	714	73	776	1.04
Analyte 737	807	71	856	1.01
Analyte 766	830	69	864	1.02
Analyte 85	712	143	524	1.11
Analyte 850	758	100	908	1.30
Arachidonic acid, trimethylsilyl ester	830	91	1732	1.36
Benzaldehyde, 2,4-dimethyl-	890	133	560	1.38
Benzeneacetic acid, 3-methoxy-à,4-bis(trimethylsilyloxy)-, trimethylsilyl ester	726	297	1200	1.23
Benzoic acid trimethylsilyl ester	893	179	808	1.27
Benzoic acid, 3-amino-, ethyl ester	962	120	852	1.65
Benzoic acid, 4-amino-, tert-butyldimethylsilyl ester	843	194	1108	1.49
Bis(dimethyl-t-butylsilyl) oxalate	712	95	1304	1.13
Butanoic acid, 2-[(tert-butyldimethylsilyl)amino]-, tert-butyldimethylsilyl ester, (.+.)-	787	172	912	1.05
Carbamodithioic acid, diethyl-, methyl ester	865	60	724	1.55
Carbonimidodithioic acid, methyl-, dimethyl ester	753	88	620	1.65
Cholest-5-en-3-ol (3á)-, carbonochloridate	737	105	1696	2.24
Cholest-5-ene, 3-bromo-, (3á)-	786	147	1736	2.29
Cholesta-3,5-diene	894	81	1816	2.26
cis, 6-Octadecenoic acid, trimethylsilyl ester	800	117	2100	0.95
Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	751	117	1736	1.84
Cyclooctane, 1,4-dimethyl-, trans-	770	69	848	1.02
Decanamide, N-(2-hydroxyethyl)-	750	85	1328	1.28
Disiloxane, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyl-	902	148	492	0.90
Dodecanamide	875	72	1360	1.40
Dodecane, 2,7,10-trimethyl-	777	71	720	1.01
Dodecane, 4-methyl-	866	71	548	0.98
Dodecane, 4,6-dimethyl-	846	71	580	0.98
Dodecanoic acid, tert-butyldimethylsilyl ester	887	75	1140	1.11
Eicosane	874	71	812	1.00
Ethanethioic acid S-tert-butyl ester	743	132	512	1.18
Ethanol, 2-(trimethylsilyl)-	817	89	1532	1.20
Glycidol stearate	715	98	1556	1.36
Glycine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	937	218	872	1.07
Heptacosane	909	71	1112	1.04
Heptadecane, 2,6,10,14-tetramethyl-	867	71	780	1.00
Heptadecanoic acid, tert-butyldimethylsilyl ester	830	327	1484	1.16
Hexadecane	898	71	604	0.98
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	711	98	1400	1.30
Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	723	98	1432	1.31
Hexadecanoic acid, dimethyl(isopropyl)silyl ester	717	313	2404	0.97
Hexanedioic acid, bis(2-ethylhexyl) ester	951	129	1496	1.27
Hexanoic acid, 2-[(trimethylsilyloxy)-, trimethylsilyl ester	886	289	1040	1.13
Hexasiloxane, tetradecamethyl-	722	221	1108	0.92
L-Alanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	861	158	852	1.05
L-Aspartic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	723	133	1328	1.14
L-Glutamic acid, N-(tert-butyldimethylsilyl)-, bis(tert-butyldimethylsilyl) ester	752	272	1416	1.13
L-Glutamine, N,N2-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	704	74	1512	1.21
L-Leucine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	897	200	992	1.05
L-Lysine, N2,N6-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	769	198	1484	1.11
L-Methionine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	935	61	1208	1.17
L-Phenylalanine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	882	234	1296	1.21

L-Proline, 1-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	941	184	1052	1.13
L-Serine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	869	133	1220	1.07
L-Threonine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	862	103	1244	1.07
L-Tryptophan, N,1-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	797	244	1800	1.94
L-Tyrosine, N,O-bis(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	918	302	1640	1.34
L-Valine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	925	186	956	1.06
Methyl tetradecanoate	899	74	1012	1.16
N-Ethylformamide	754	73	1836	1.73
n-Hexadecanoic acid	713	69	2644	0.98
N,O-Bis(dimethyl-t-butylsilyl)-l-isoleucine	941	200	1020	1.05
Nonadecane	864	71	772	1.00
Nonadecanoic acid, tert-butyldimethylsilyl ester	740	355	1604	1.24
Nonanoic acid, tert-butyldimethylsilyl ester	827	75	892	1.08
Octacosane	726	71	1592	1.15
Octadecanoic acid, methyl ester	903	74	1316	1.19
Oleic Acid	762	83	2560	0.98
Oleic acid, trimethylsilyl ester	926	117	1380	1.16
Palmitelaidic acid, trimethylsilyl ester	850	129	1248	1.14
Pentadecanoic acid, tert-butyldimethylsilyl ester	861	75	1356	1.14
Pentanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester, (DL)-	779	89	1108	1.17
Pentanoic acid, 3-methyl-, tert-butyldimethylsilyl ester	791	75	616	1.05
Phosphoric acid, tris(tert-butyldimethylsilyl) ester	947	383	1180	1.11
Phthalic acid, monocyclohexyl ester	836	167	1588	1.59
Phytanic acid, tert-butyldimethylsilyl ester	743	369	1516	1.15
Picolinic acid, trimethylsilyl ester	825	180	852	1.39
Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	904	89	1136	1.01
Propanedioic acid, bis(trimethylsilyl) ester	819	117	700	1.10
Propanoic acid, 2-[(tert-butyldimethylsilyl)oxy]-, tert-butyldimethylsilyl ester	830	261	812	1.04
Pyridine, 3-trimethylsiloxy-	722	152	588	1.15
Sarcosine, N-(tert-butyldimethylsilyl)-, tert-butyldimethylsilyl ester	767	158	904	1.07
Silamine, 1,1,1-trimethyl-N-(trimethylsilyl)-	741	146	552	0.96
Silane, (2-methoxyethoxy)trimethyl-	718	89	876	1.06
Silane, (cyclopropylcarbonyl)(1,1-dimethylethyl)dimethyl-	720	61	992	1.07
Silane, tetramethyl-	949	87	1176	1.24
Silane, trimethyl-3-penten-2-yl-, trans	701	256	968	1.12
Silane, trimethylphenoxy-	800	151	612	1.14
Silanol, trimethyl-	878	75	500	0.75
Squalene	858	69	1744	1.75
tert-Butylpentamethyldisiloxane	774	75	668	1.00
Tetradecanoic acid, tert-butyldimethylsilyl ester	918	75	1288	1.13
trans-3-Hexenedioic acid, bis(trimethylsilyl) ester	735	147	1004	1.10
Tridecane	723	85	504	0.96
Tris(trimethylsilyl)borate	827	221	640	0.94
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	759	281	968	0.92
Undecane, 2-methyl-	862	71	492	0.97
Undecane, 2,6-dimethyl-	884	71	540	0.97
Urea, N,N'-bis(tert-butyldimethylsilyl)-	929	231	952	1.23
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	749	98	1420	1.36
Z-7-Tetradecenol,trimethylsilyl ether	872	75	2244	0.96

'Analyte #' denotes metabolites whose SV is >700, but whose replicates were not all identified as the same compound. In this scenario the ChromaTOF software assigns a name of 'analyte' plus an accession number.