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Data Article

Dataset for analysis of metabolic pathways and their reversibility associated with anti-proliferative effect of metformin in liver cancer cells

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Dataset link: MTBLS7760: Analysis of glucose-independent metabolic pathways associated with anti-proliferative effect of metformin and their reversibility in liver cancer cells (Original data)

Keywords:
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Glucose- dependence

ABSTRACT

Despite epidemiological indications, utility of metformin in liver cancer remains debated and the understanding of the mechanism underlying its anti-cancer effects remains incomplete. Particularly, whether it operates via similar mechanism under glucose-sufficient and glucose- deficient environments or whether these effects are reversible remains unexplored. This metabolomic dataset was collected from liver cancer (HepG2) cells treated with metformin or placebo over a period of 3 h to 48 h as well as from cells recovering after metformin withdrawal. Cells were exposed to placebo or 2.5 mM metformin with or without glucose (5 mM) supplementation. The cells were harvested at 3, 6, 12, 24, and 48 h post-treatment. Cells were also harvested after 24 h of treatment under one of these conditions followed by reversal of glucose and/or metformin exposure status for 48 h. Metabolites from six biological replicates of each experimental group were extracted using chilled monophasic metabolite extraction solvent (Water: Acetonitrile: Isopropanol= 2:3:3) containing homovanillic acid as an internal standard. Samples were derivatized using MOX reagent followed by MSTFA. Untargeted metabolomic profiling of derivatized samples

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were performed using an Agilent 7890B gas chromatograph coupled to a 5977B single quadrupole mass spectrometer. Analytes were injected through a splitless liner and separated on a HP-5MS ultra-inert column using ultrapure helium as the carrier gas. Peak alignment, annotation, and integration were done using Agilent MassHunter Quantitative analysis software. Multivariate analysis was performed using MetaboAnalyst 5.0. These experiments were performed to unravel the longitudinal evolution of cellular metabolome in response to metformin treatment, its glucose dependence, as well as to examine the reversibility of these changes. The dataset can help to identify glucose-independent pathways involved in anti-cancer effect of metformin. The dataset can be used to design experiments to develop novel therapeutic combinations synergistically acting with metformin to cripple the metabolic fitness of cancer cells. It can also help to develop experiments to test the effect of metformin withdrawal in liver cancer.

Specification Table

Subject	Biological science
Specific subject area	Cancer metabolism research-mechanism underlying anti-cancer effect of metformin in liver cancer.
Type of data	Raw GCMS total ion chromatograms Processed data table (.csv) Illustrations Figure
How the data were acquired	Untargeted metabolic profiling data was acquired in a gas chromatography (7890B, Agilent, USA) system fitted with auto-sampler (G4513A, Agilent, USA) and connected to a single quadruple mass-spectrometer (5977B, Agilent, USA) using HP-5MS UI column (30 m × 0.25 mm × 0.25 µm) and 99.999 % pure Helium as the carrier gas. Metabolites were injected in splitless mode and separated in the gas chromatography with a RTLocked temperature gradient method with constant flow rate of 0.9 ml/min. The oven temperature was set to 70 °C and held for 2 min followed by a ramp to 280°C at a rate of 5°C /min and held for 1 min. The second ramp was set to 295°C at a rate of 10°C/min and held for 5 mins resulting in a run of 52 mins.
Data format	Raw data(.d) Analyzed Filtered Illustrated
Description of data collection	HepG2 cells were treated with 2.5 mM metformin in presence or absence of glucose (5 mM) supplementation and harvested at 3, 6, 12, 24, and 48 hours. Cells were also harvested after exposure to one of these conditions for 24 h followed by 48 h of reversal of metformin and/or glucose exposure status. Metabolites were extracted using chilled metabolite extraction solvent containing 15 µM homovalinic acid as internal standard and MOX-MSTFA derivatized samples were analyzed in GC–MS.
Data source location	Biophysics & Structural Genomics Division,Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata, West Bengal, 700 064, India
Data accessibility	Repository name: MetaboLights Data identification number: MTBLS7760 Direct URL to data: https://www.ebi.ac.uk/metabolights/MTBLS7760 Processed data tables (.csv) Mendeley Data, V1, doi: 10.17632/2nvz3jb8pm.1
Related research article	Islam SR, Manna SK. Identification of glucose-independent and reversible metabolic pathways associated with anti-proliferative effect of metformin in liver cancer cells. Metabolomics. 2024;20(2):29. doi: 10.1007/s11306-024-02096-0 [1]

1. Value of the Data

- These data provide the information about temporal evolution of metabolic signatures of liver cancer cells (HepG2) exposed to sub-lethal dose of metformin in absence or presence of glucose.
- It also provides information on metabolic rewiring after metformin withdrawal in presence or absence of glucose supplementation.
- It provides valuable insight with respect to mechanism underlying effect of metformin on liver cancer cells.
- The dataset would benefit researchers working in the area of cancer metabolism, in general, and liver cancer, in particular. It will also help people looking to work in the area of metabolomics and bioinformatics by providing the detailed dataset to exercise on application of different data mining tools.
- It can be used to design experiments on effects of metformin on cancer cells under varying nutrient availability and to test therapeutic synergy of metformin with other drugs targeting metabolism or dependent on metformin-targeted metabolic pathways for metabolism and efflux or intermittent fasting.
- It may be used to develop experiments to test the effect of metformin withdrawal in liver cancer patients.

Background Despite epidemiological indications [2,3], the utility of metformin in combating liver cancer remains debated. While cancer cells adapt to low glucose, studies on metformin's anti-cancer potential mostly explored its influence on glucose metabolism [4]. Thus, whether metformin is effective irrespective of glucose availability remains unanswered. The impact of withdrawal of metformin treatment in patients with (diagnosed or undiagnosed) liver cancer also demands attention as it may alter the outcome. Metabolic reprogramming plays a pivotal role in determining the behaviour of cancer cells and it spans well beyond glucose metabolism [5,6]. Thus, characterization of the evolution of the metabolic signature upon metformin treatment as well as upon metformin withdrawal is essential to answer these questions. This warrants untargeted metabolomic analysis under these conditions, which motivated generation of this dataset.

Taken together, we aimed to provide a more comprehensive understanding of metformin's role in liver cancer to aid its optimized clinical use. The current dataset will add value to the published article by providing full access to the raw as well as processed data that can be used by interested researchers to comprehend the results better as well as to develop hypothesis and experiments on utility of metformin in liver cancer as indicated above.

2. Data Description

Fig. 1 describes the experimental design from which the metabolomics dataset was generated.

2.5 mM metformin or placebo-treated HepG2 cells with or without glucose supplementation were harvested at five time points as follows- 3 h, 6 h, 12 h,24 h, and 48 h. Samples from six biological replicates for each time point from four experimental groups denoted as *NG* = Control, 5 mM Glucose; *NT*= 5 mM Glucose + 2.5 mM metformin; *WG* = without glucose control; *WT*= without glucose + 2.5 mM metformin were collected in chilled metabolite extraction solvent followed by washing with 150 mM NaCl solution.

The experimental design for investigating the effect of metformin withdrawal in presence or absence of glucose and the corresponding experimental groups is illustrated in Fig. 2. Cells were cultured in NG, NT, WG, and WT conditions for 24 h followed by media change and cultured for another 48 h. The control (NG) group was continued for 48 h with NG media

(1). The NT group was replaced by either NG media (2A) or WG media *(2B)*. For the WG group, the media was replaced with either NG media *(3A)* or WT media *(3B)*. For the WT group,

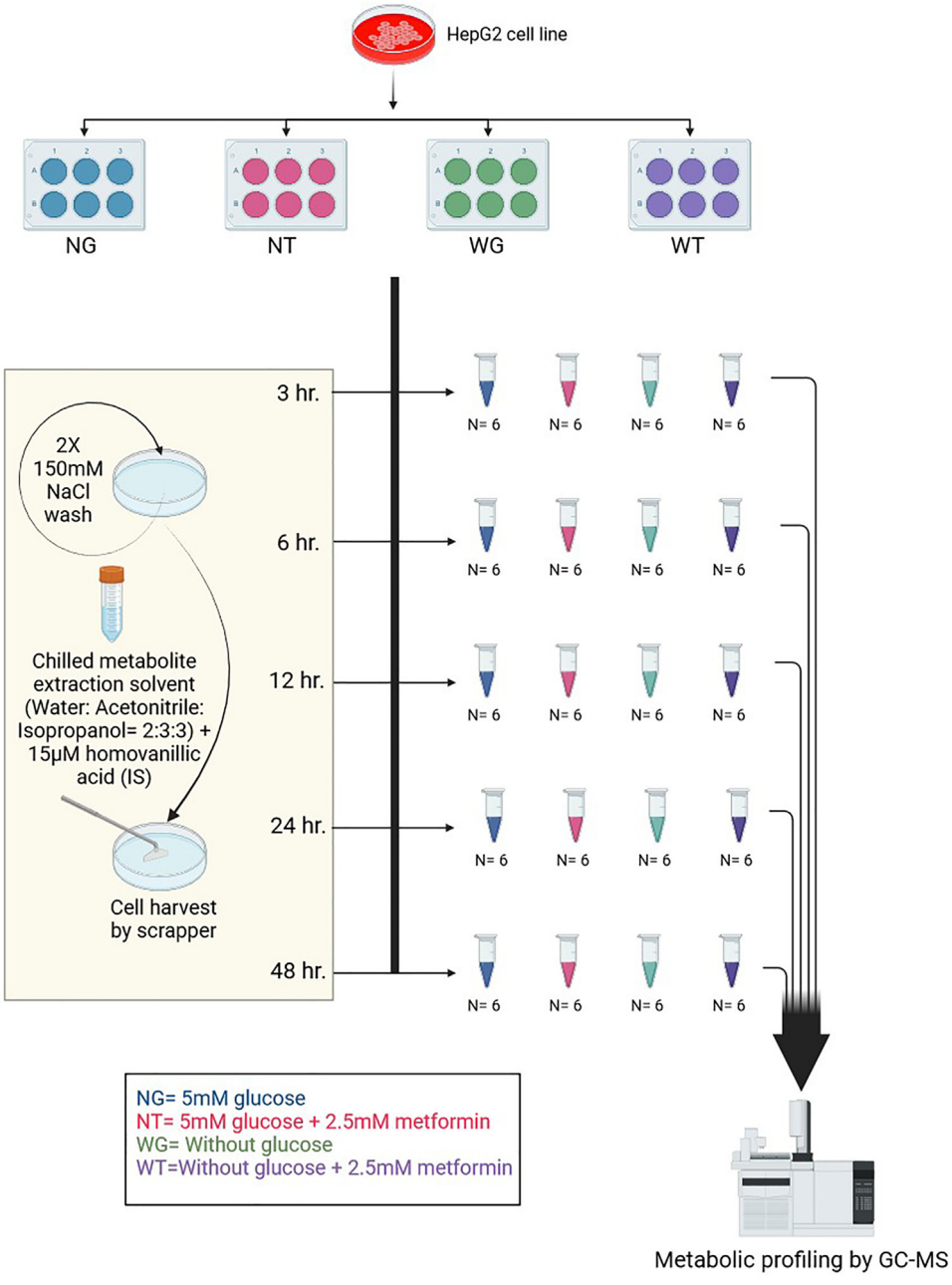

Fig. 1. Experimental workflow for the time-course metabolic profiling of HepG2 cells. A schematic diagram illustrating the experimental procedure for collecting the sample by 150 mM NaCl wash followed by harvesting with metabolite extraction solvent for metabolic profiling of HepG2 cells under control (NG), metformin treatment (NT), glucose deprivation (WG), and metformin treatment under glucose deprivation (WT) conditions at 3, 6, 12, 24, and 48 h.

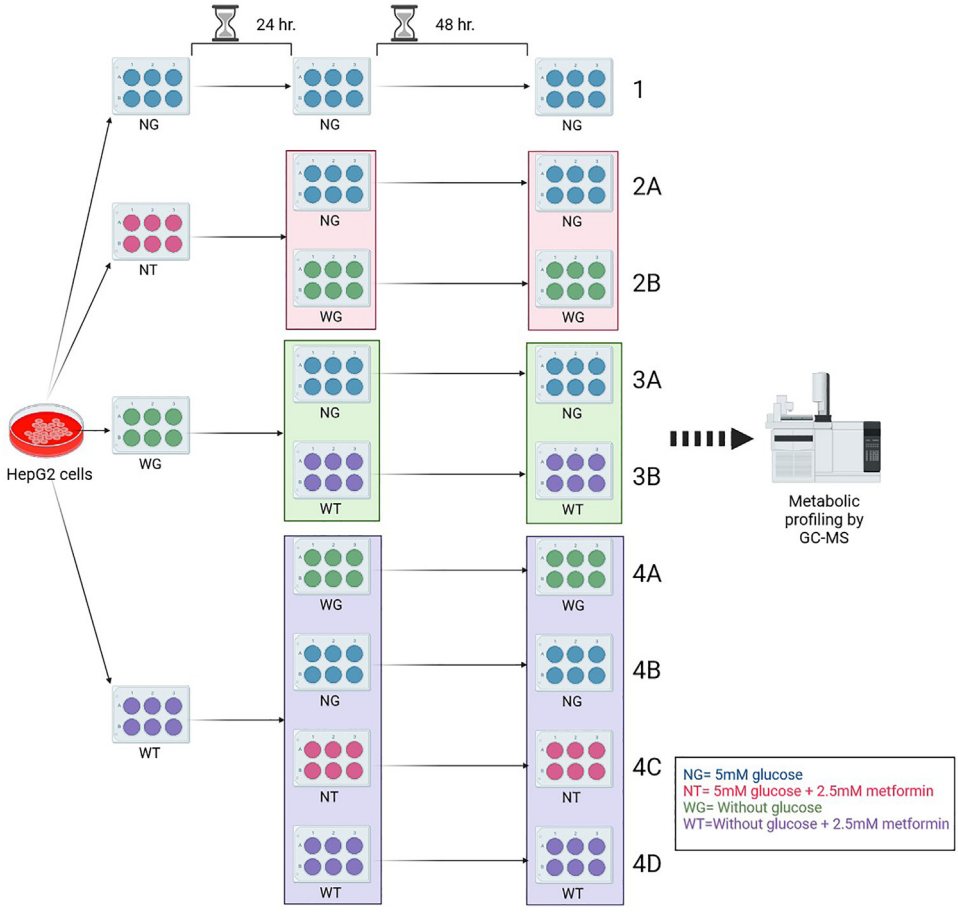

Fig. 2. Experimental design for the rescue experiment. A schematic diagram illustrating the experimental procedure for the treatment of 2.5 mM metformin in presence or absence of 5 mM glucose supplementation for 24 h followed by re-incubation with specified media reversing metformin and/or glucose exposure status for 48 h. Cell samples were collected in chilled metabolite extraction solvent.

the media replaced with WG media *(4A)* or NT media *(4C)* or NG media *(4B)* or continued with WT media *(4D)*. Samples from six biological replicates were collected in similar manner described in previous figure.

Fig. 3 illustrates the pipeline for generating the metabolomic dataset for the study. Three major steps depict sample collection, metabolite extraction and data acquisition in GC–MS, and data processing respectively.

Table 1 describes the Raw data files deposited in the MetaboLights (MTBLS7760) data repository. Samples were arranged in rows as denoted earlier with corresponding treatment nature (Treatment_1, and Treatment_2 for rescue experiment only), duration of each treatment condition (Timepoint_1, and Timepoint_2 for rescue experiment only), and raw spectral data file in each column. Blank and pool samples were denoted as QC sample. Blank samples were extraction solvent without any cell and processed similarly like other samples in each time point. Fig. 4 shows 2D scores plots for principal components analysis (PCA) of the data for each time point and the rescue experiment with pooled QC and actual samples. A total of six processed and filtered data set for statistical analysis were provided in separate .csv file format for each

Table 1
Raw data identifier table of corresponding samples deposited in MetaboLights.

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
A_Blank_1	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_06.D
A_Blank_2	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_16.D
A_Blank_3	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_35.D
A_NG1	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_33.D
A_NG2	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_23.D
A_NG3	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_31.D
A_NG4	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_22.D
A_NG5	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_08.D
A_NG6	NG(Control)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_14.D
A_NT1	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_19.D
A_NT2	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_26.D
A_NT3	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_21.D
A_NT4	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_17.D
A_NT5	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_29.D
A_NT6	NT(Metformin+)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_20.D
A_Pool_1	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_02.D

(*continued on next page*)

Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
A_Pool_2	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_03.D
A_Pool_3	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_04.D
A_Pool_4	QC	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_05.D
A_WG1	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_28.D
A_WG2	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_30.D
A_WG3	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_13.D
A_WG4	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_18.D
A_WG5	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_12.D
A_WG6	WG(Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_07.D
A_WT1	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_09.D
A_WT2	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_11.D
A_WT3	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_27.D
A_WT4	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_10.D
A_WT5	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_24.D
A_WT6	WT(Metformin+, Glucose-)	3	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/A_3hrtreatment/HepG2_A_Cell_MOX-TMS_02062022_32.D
B_Blank_1	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_02.D
B_Blank_2	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_05.D
B_Blank_3	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_19.D

(*continued on next page*)

Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
B_Blank_4	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_26.D
B_Blank_5	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_34.D
B_Blank_6	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_41.D
B_NG1	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_07.D
B_NG2	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_37.D
B_NG3	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_23.D
B_NG4	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_09.D
B_NG5	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_16.D
B_NG6	NG(Control)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_30.D
B_NT1	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_31.D
B_NT2	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_39.D
B_NT3	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_35.D
B_NT4	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_22.D
B_NT5	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_36.D
B_NT6	NT(Metformin+)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_13.D
B_Pool_1	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_03.D
B_Pool_2	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_04.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
B_Pool_3	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_11.D
B_Pool_4	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_18.D
B_Pool_5	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_24.D
B_Pool_6	QC	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_25.D
B_WG1	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_08.D
B_WG2	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_17.D
B_WG3	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_28.D
B_WG4	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_06.D
B_WG5	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_38.D
B_WG6	WG(Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_14.D
B_WT1	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_27.D
B_WT2	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_21.D
B_WT3	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_10.D
B_WT4	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_29.D
B_WT5	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_15.D
B_WT6	WT(Metformin+, Glucose-)	6	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/B_6hrtreatment/HepG2_B_Cell_MOX-TMS_10062022_20.D
C_Blank_1	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_02.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
C_Blank_2	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_05.D
C_Blank_3	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_21.D
C_Blank_4	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_29.D
C_Blank_5	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_40.D
C_NG1	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_25.D
C_NG2	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_15.D
C_NG3	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_33.D
C_NG4	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_26.D
C_NG5	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_07.D
C_NG6	NG(Control)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_30.D
C_NT1	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_14.D
C_NT2	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_16.D
C_NT3	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_08.D
C_NT4	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_24.D
C_NT5	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_35.D
C_NT6	NT(Metformin+)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_06.D
C_Pool_1	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_27.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
C_Pool_2	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_28.D
C_Pool_3	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_37.D
C_Pool_4	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_38.D
C_Pool_5	QC	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_39.D
C_WG1	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_34.D
C_WG2	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_10.D
C_WG3	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_11.D
C_WG4	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_17.D
C_WG5	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_22.D
C_WG6	WG(Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_36.D
C_WT1	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_19.D
C_WT2	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_31.D
C_WT3	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_23.D
C_WT4	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_18.D
C_WT5	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_09.D
C_WT6	WT(Metformin+, Glucose-)	12	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/C_12hrtreatment/HepG2_C_Cell_MOX-TMS_08062022_32.D
D_Blank_1	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_02.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
D_Blank_2	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_05.D
D_Blank_3	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_14.D
D_Blank_4	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_22.D
D_Blank_5	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_29.D
D_NG1	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_17.D
D_NG2	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_08.D
D_NG3	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_20.D
D_NG4	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_19.D
D_NG5	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_26.D
D_NG6	NG(Control)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_10.D
D_NT1	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_15.D
D_NT2	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_36.D
D_NT3	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_30.D
D_NT4	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_11.D
D_NT5	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_31.D
D_NT6	NT(Metformin+)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_16.D
D_Pool_1	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_27.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
D_Pool_2	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_28.D
D_Pool_3	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_38.D
D_Pool_4	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_39.D
D_Pool_5	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_40.D
D_WG1	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_35.D
D_WG2	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_09.D
D_WG3	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_24.D
D_WG4	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_32.D
D_WG5	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_37.D
D_WG6	WG(Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_34.D
D_WT1	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_07.D
D_WT2	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_18.D
D_WT3	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_06.D
D_WT4	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_33.D
D_WT5	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_25.D
D_WT6	WT(Metformin+, Glucose-)	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/D_24hrtreatment/HepG2_D_Cell_MOX-TMS_04062022_23.D
E_Blank_1	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_02.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
E_Blank_2	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_05.D
E_Blank_3	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_19.D
E_Blank_4	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_26.D
E_Blank_5	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_40.D
E_Pool_1	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_24.D
E_Pool_2	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_25.D
E_Pool_3	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_33.D
E_Pool_4	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_34.D
E_Pool_5	QC	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_39.D
E_NG1	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_30.D
E_NG2	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_37.D
E_NG3	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_23.D
E_NG4	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_13.D
E_NG5	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_07.D
E_NG6	NG(Control)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_38.D
E_NT1	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_36.D
E_NT2	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_35.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
E_NT3	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_21.D
E_NT4	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_16.D
E_NT5	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_27.D
E_NT6	NT(Metformin+)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_28.D
E_WG1	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_31.D
E_WG2	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_22.D
E_WG3	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_17.D
E_WG4	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_20.D
E_WG5	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_14.D
E_WG6	WG(Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_12.D
E_WT1	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_29.D
E_WT2	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_15.D
E_WT3	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_08.D
E_WT4	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_06.D
E_WT5	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_32.D
E_WT6	WT(Metformin+, Glucose-)	48	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Timeseriesexperiment/E_48hrtreatment/HepG2_E_Cell_MOX-TMS_06062022_09.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
R_1_1	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_69.D
R_1_2	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_46.D
R_1_3	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_68.D
R_1_4	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_40.D
R_1_5	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_39.D
R_1_6	NG(Control)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_66.D
R_2A1	NT(Metformin+)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_53.D
R_2A3	NT(Metformin+)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_38.D
R_2A4	NT(Metformin+)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_77.D
R_2A5	NT(Metformin+)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_48.D
R_2A6	NT(Metformin+)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_73.D
R_2B1	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_11.D
R_2B2	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_56.D
R_2B3	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_36.D
R_2B4	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_23.D
R_2B5	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_78.D
R_2B6	NT(Metformin+)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_21.D

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Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
R_3A1	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_12.D
R_3A2	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_13.D
R_3A3	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_67.D
R_3A4	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_65.D
R_3A5	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_55.D
R_3A6	WG(Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_58.D
R_3B1	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_59.D
R_3B2	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_10.D
R_3B3	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_75.D
R_3B4	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_20.D
R_3B5	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_74.D
R_3B6	WG(Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_37.D
R_4A1	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_14.D
R_4A2	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_19.D

(*continued on next page*)

Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
R_4A3	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_81.D
R_4A4	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_52.D
R_4A5	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_76.D
R_4A6	WT(Metformin+, Glucose-)	24	hour	WG(Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_35.D
R_4B1	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_85.D
R_4B2	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_18.D
R_4B3	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_45.D
R_4B4	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_44.D
R_4B5	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_32.D
R_4B6	WT(Metformin+, Glucose-)	24	hour	NG(Control)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_29.D
R_4C1	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_63.D
R_4C2	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_54.D
R_4C3	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_62.D
R_4C4	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_64.D
R_4C5	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_30.D

(*continued on next page*)

Table 1 (*continued*)

Sample Name	Treatment_1	Timepoint_1	Unit	Treatment_2	Timepoint_2	Unit	RawSpectralDataFile
R_4C6	WT(Metformin+, Glucose-)	24	hour	NT(Metformin+)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_86.D
R_4D1	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_31.D
R_4D2	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_80.D
R_4D3	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_79.D
R_4D4	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_57.D
R_4D5	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_17.D
R_4D6	WT(Metformin+, Glucose-)	24	hour	WT(Metformin+, Glucose-)	48	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_47.D
R_Pool_1	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_03.D
R_Pool_2	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_04.D
R_Pool_3	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_05.D
R_Pool_4	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_06.D
R_Blank_1	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_28.D
R_Blank_2	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_72.D
R_Blank_3	QC	24	hour	na-	na-	hour	FILES/RAW_FILES/HepG2_Rescueexperiment_72hr/HepG2_Rescue_Cell_MOX-TMS_14062022_84.D

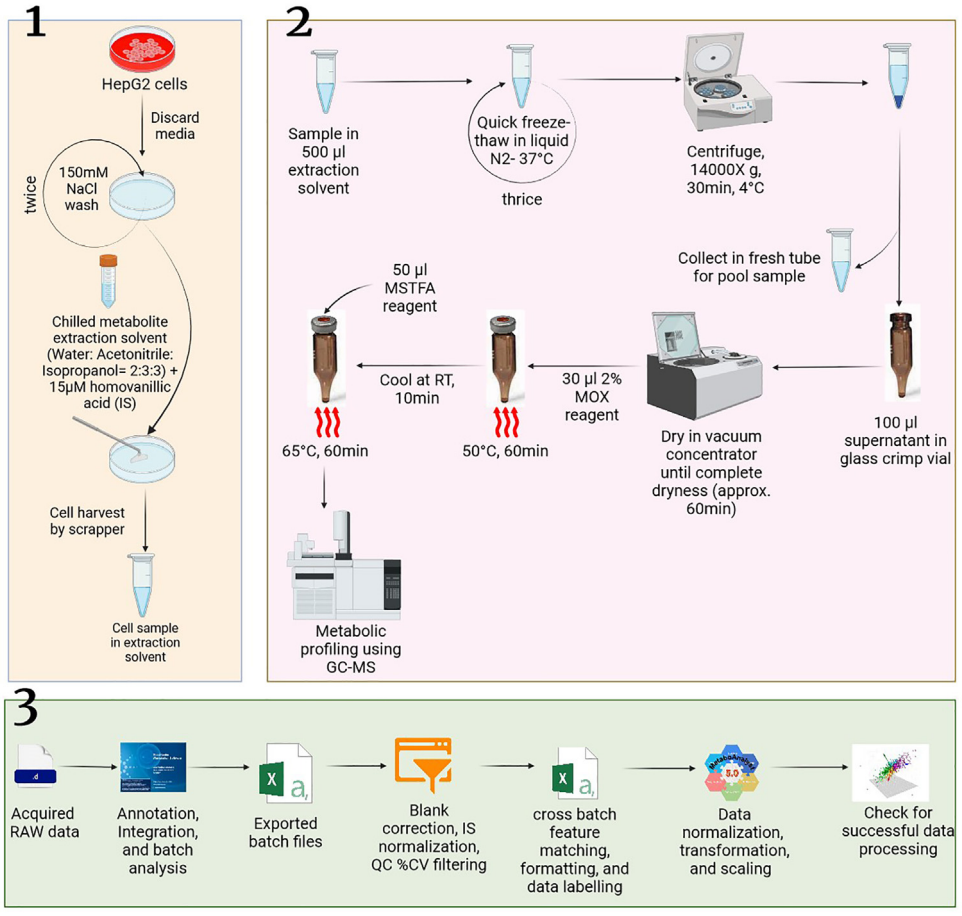

Fig. 3. Experimental workflow for untargeted metabolic profiling of HepG2 cells by gas chromatography mass spectrometry. A schematic diagram entailed three major steps illustrating the experimental procedure for (1) collecting the samples by 150 mM NaCl wash followed by harvesting with metabolite extraction solvent for metabolic profiling. (2) Metabolite extraction and derivatization followed by data acquisition. (3) Analysis of acquired raw data and post-processing for quality check and further statistical analysis.

time point. These data set for each time point were denoted as – ***HepG2_A_cell*** for 3 h and, likewise, ***B, C, D***, and ***E*** for 6, 12, 24, and 48 h, respectively. Dataset for rescue experiment was denoted as ***HepG2_Rescue72_cell***. In these files, samples and metabolite/features were arranged in row and column respectively. Sample name was denoted as - a prefix for time point (e.g.- A, B, C, D, E for 3, 6, 12, 24, 48 h and R_ for rescue samples) followed by experimental group name (e.g.- NG, NT, WG, WT, and for rescue experiment 1, 2A, 2B, etc.) and appended with replicate number (1–6). Pooled QC samples were denoted as 'Pool' in each file.

3. Experimental Design, Materials and Methods

3.1. Cell culture

Liver cancer cells (HepG2) were cultured as described earlier [7] in low glucose DMEM media (AT006, Himedia, India) supplemented with 10 %fetal bovine serum (10,082,147, Gibco^{TM}, USA),

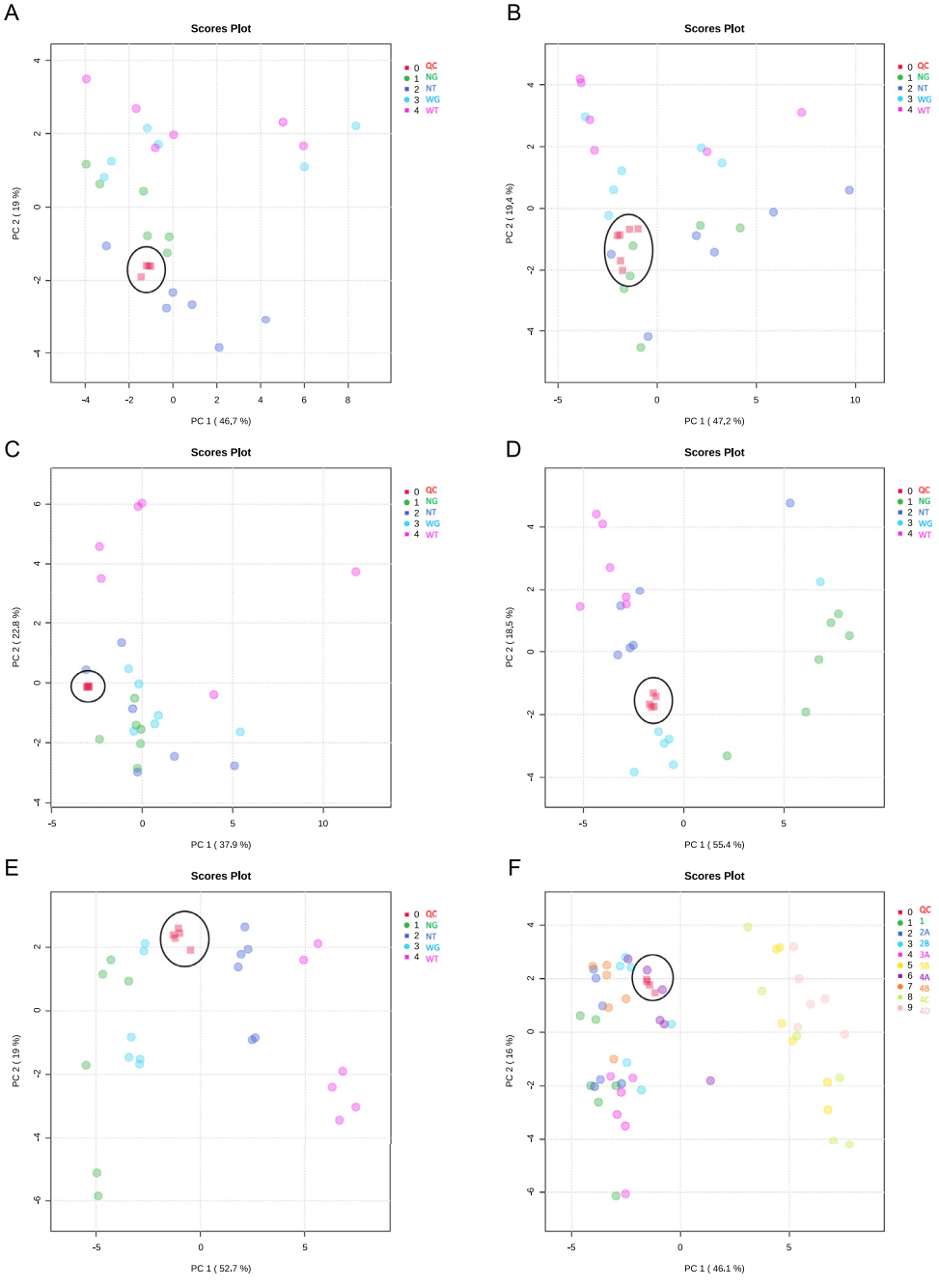

Fig. 4. Principal Components Analysis (PCA) of dataset for each time point for group distribution and QC sample clustering. 2D score plots showing QC data points cluster tightly (encircled red squares) in comparison to the experimental samples (colored circles) in the projection at 3 h (A), 6 h (B), 12 h (C), 24 h (D), 48 h (E), and in rescue experiment (F).

100 u/L penicillin (TC020, Himedia, India), and 50 mg/L streptomycin (TC035, Himedia, India). They were maintained in a humidified 37°C chamber with 5% CO2 supply. Cells between passages number 3 to 10 were used in experiments.

3.2. Experimental design

3×10^5 cells were seeded in 6-well cell culture plates one day before experiment. On the day of experiment, cells were washed with warm PBS and exposed to DMEM media containing 2.5 mM metformin with or without 5 mM glucose along with control groups. Thus, creating four experimental groups, which were denoted as:

NG = Control, 5 mM Glucose; **NT**= 5 mM Glucose + 2.5 mM metformin; **WG** = without glucose control; **WT**= without glucose + 2.5 mM metformin.

The treated cells were incubated for 3, 6, 12, 24, and 48 h and collected for metabolomic analysis.

For the rescue experiment, cells were cultured in NG or NT or WG or WT media as indicated above for 24 h, followed by media change and culture for another 48 h as described below and illustrated in Fig. 2. The NG group continued to be cultured with the same media (1). For NT group, either metformin was removed by replacing with NG (5 mM glucose) media (2A) or exposed to only glucose deprivation using WG (DMEM without glucose) media (2B). For WG group, either glucose was replenished with NG (5 mM glucose) media (3A) or exposed to metformin treatment in absence of glucose using WT (2.5 mM metformin, DMEM without glucose) media (3B). For WT group, either metformin was removed using WG (DMEM without glucose) media (4A) or glucose was replenished using NT (2.5 mM metformin, 5 mM glucose) media (4C) or both were done together using NG (5 mM glucose) media (4B) or continued with WT (2.5 mM metformin, DMEM without glucose) media (4D) as control.

3.3. Sample collection, metabolite extraction, and derivatization

Cells were washed twice with 150 mM NaCl at indicated time points before metabolite extraction and 500 µl of metabolite extraction solvent (Water: Acetonitrile: Isopropanol =2: 3: 3) containing 15 µM of homovanillic acid as internal standard were added to the cell culture plate. Then, cells were scraped with cell scrapper and collected in 1.5 ml Eppendorf tubes and stored in −80 °C freezer until further processing.

Samples from respective time points and rescue experiment were processed together for extraction. Firstly, samples were thawed in ice. Cell lysis was done by three cycles of quick freeze-thaw using liquid nitrogen and 37 °C incubator followed by centrifugation at 14000x g for 30 mins at 4 °C. 100 µl supernatant was taken in 0.6 ml glass crimp-top microvial (27312, Supelco) and dried in a vacuum concentrator for about 1 h. 30 µl of 2% methoxamine (MOX) reagent (TS-45950, ThermoScientific™, USA) was added to each sample and incubated at 50°C for 1 h with the lid closed for methyloxime derivatization. MOX derivatized samples were cooled at room temperature and silylated with 50 µl MSTFA reagent (M-132, Supelco, USA) by heating at 65°C for 1 h in sealed condition in a heat block. Blank samples were also prepared only with extraction solvent following the same procedure. Pooled samples for each time point or the rescue experiment (indicated as batch hereafter) were prepared by mixing equal volume of metabolite extract from each sample from the sample set for that time point or the rescue experiment. These samples were used as the QC sample for the respective batch of samples during GCMS analysis.

3.4. Gas chromatography and mass spectrometry (GC–MS)

Untargeted metabolic profiling was performed using gas-chromatography (7890B, Agilent, USA) equipped with HP-5MS UI column(30 $m \times 0.25$ mm $\times$ 0.25 µm, Agilent, USA) fitted with

auto-sampler (G4513A, Agilent, USA) and connected to a single quadruple mass spectrometer (5977B, Agilent, USA).

A retention time-locked data acquisition method (RTLocking Method) was developed against homovanilic acid for superior chromatographic alignment across the samples. The splitless front inlet transfer liner (5190–2293, Agilent, USA) was set to 300°C. 1 µl of the sample was injected via autosampler into the column in splitless mode. 99.999 % pure Helium was used as the carrier at a constant flow rate of 0.9 ml/min. The oven temperature was set to 70°C and held for 2 min followed by a ramp to 280°C at a rate of 5°C/min and held for 1 min. The second ramp was set to 295°C at a rate of 10°C/min and held for 5 mins resulting in a run of 52 mins. A post-run equilibration was done at 300°C for 5 mins before initialization of the next run. MS source and MS quad temperatures were set to 230°C and 150°C, respectively. Mass spectra were recorded using electron impact ionization (EI) at 70 eV to generate fragments. Spectra were acquired in full scan mode in the *m/z* range of 45–600 with a focus *m/z* of 150 at a speed of 30 Hz/*sec*.

Before the individual sample injections, pooled samples were injected five times for conditioning of the column. All samples were analyzed together in a single batch. Samples from 3, 6, 12, 24, 48 h and rescue experiments are referred to as batch A, B, C, D, E and R, respectively. Samples from each of these batches were analyzed in clusters along with respective pooled QC samples. For example, all 24 samples from 3 h time point (batch A) were run in randomized fashion with intermittent injection of pooled QC samples (pool A) prepared by pooling 24 samples of 3 h time point. Pooled QC samples (4–6) were injected after every 6–8 samples. In addition, sample batches from different time points were analyzed non-sequentially. The order was: B (6 h), E (48 h), A (3 h), D (24 h), C (12 h) and R (rescue).

3.5. Data processing

Acquired raw data were imported in MassHunter Quantitative analysis software (vB.08.00/Build8.0.598.0, Agilent, USA) for feature annotation and integration. A list of features were extracted from pooled samples and vetted by manual feature extraction to define retention time, one quantifier and, at least, one (typically, two) qualifier ions, which are characteristic of the feature of interest. The features were annotated as the compound with highest match score against the NIST14 library and validated with authentic standards wherever available. Unidentified peaks observed across samples, with, at least, two co-eluting ions, were denoted with X_ followed by retention time (e. g., unidentified peak eluting at 20.224 min was denoted as **X_20.224**). The setup method was applied to all the samples for peak integration where the integrated peak area or area under the curve (AUC) represents the relative abundance of that particular feature/metabolite.

The integrated peak area table for each batch (time points or rescue experiment) along with respective batch-specific QC samples was exported as .csv file for further processing in Microsoft Excel. Firstly, the features that were present with comparable intensities in the blank samples were removed from the data matrix. Known contaminants and saturated peaks (e.g., phosphate peak) were removed from the final list. Then, the area of each metabolite from each sample was normalized with the area of internal standard (i.e. homovanillic acid) from the same sample. The features/metabolites with >30 % coefficient of variation (CV) in the pooled QC samples were then excluded from further analysis. Metabolites with multiple derivatives were summed up. The final list from each batch was matched and only common features/metabolites detected across all batches were considered for statistical analysis. Each batch file was formatted and saved in comma-separated values (.csv) format in Excel for analysis in MetaboAnalyst 5.0 [8].

3.6. Statistical analysis

Formatted .csv files were uploaded in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) [8]. The data were sum-normalized, log-transformed, and Pareto-scaled before multivariate analysis.

Unsupervised principal components analysis (PCA) was performed for assessment of the quality and integrity of the processed data. The fitness of the data from a batch for extraction of differential features was confirmed by the tight clustering of the batch-specific pooled QC samples compared to the samples of interest (NG, WG, NT and WT groups) in the principal components analysis (PCA) as shown in Fig. 4. All statistical comparisons involved treatment groups within same batch (time point or rescue experiment). No inter-batch comparison was performed.

Limitations

Due to inherent nature of the technique, several important metabolites (e.g., nucleotide triphosphates, acylCoA, acylcarntines, complex lipids, co-factors, etc.) are not amenable to analysis using GCMS. Thus, the picture of the alteration in metabolic landscape is incomplete. It should also be noted that due to absence of QC samples run across the entire sample set, the utility of the data is limited with respect to comparative changes in metabolite abundances in respective groups across time points.

Ethics Statements

The authors have read and follow the ethical requirements for publication in Data in Brief and confirming that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Data Availability

MTBLS7760: Analysis of glucose-independent metabolic pathways associated with anti-proliferative effect of metformin and their reversibility in liver cancer cells (Original data) (Metabolights).

CRediT Author Statement

Sk Ramiz Islam: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization; **Soumen Kanti Manna:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S.R. Islam, S.K. Manna, Identification of glucose-independent and reversible metabolic pathways associated with anti-proliferative effect of metformin in liver cancer cells, Metabolomics 20 (2024) 1–19, doi:10.1007/S11306-024-02096-0/METRICS.

[2] S.J. Ma, Y.X. Zheng, P.C. Zhou, Y.N. Xiao, H.Z. Tan, Metformin use improves survival of diabetic liver cancer patients: systematic review and meta-analysis, Oncotarget 7 (2016) 66202, doi:10.18632/ONCOTARGET.11033.
[3] Z.J. Zhang, Z.J. Zheng, R. Shi, Q. Su, Q. Jiang, K.E. Kip, Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis, J. Clin. Endocrinol. Metab. 97 (2012) 2347–2353, doi:10.1210/JC.2012-1267.
[4] C. Marini, V. Cossu, M. Bauckneht, F. Lanfranchi, S. Raffa, A.M. Orengo, S. Ravera, S. Bruno, G. Sambuceti, Metformin and cancer glucose metabolism: at the bench or at the bedside? Biomolecules 11 (2021), doi:10.3390/BIOM11081231.
[5] S. Nong, X. Han, Y. Xiang, Y. Qian, Y. Wei, T. Zhang, K. Tian, K. Shen, J. Yang, X. Ma, Metabolic reprogramming in cancer: mechanisms and therapeutics, MedComm 4 (2023) e218, doi:10.1002/MCO2.218.
[6] G.J. Yoshida, Metabolic reprogramming: the emerging concept and associated therapeutic strategies, J. Exp. Clin. Cancer Res. 34 (2015) 1–10, doi:10.1186/S13046-015-0221-Y.
[7] M.T. Donato, L. Tolosa, M.J. Gómez-Lechón, Culture and functional characterization of human hepatoma HepG2 cells, Protocols In Vitro Hepatocyte Res. (2015) 77–93, doi:10.1007/978-1-4939-2074-7_5/COVER.
[8] Z. Pang, J. Chong, G. Zhou, D.A. De Lima Morais, L. Chang, M. Barrette, C. Gauthier, P.É. Jacques, S. Li, J. Xia, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, Nucl. Acids Res. 49 (2021) W388–W396, doi:10.1093/NAR/GKAB382.