Electronic Supplementary Material (ESI) for Analytical Methods

Electronic Supplementary Information

Comparison of extraction conditions and normalization approaches for cellular metabolomics of adherent growing cells by GCMS

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Fig. S1: Principal component analysis displaying the separation of metabolite profiles after application of the four different extraction protocols, i.e. methanolic extraction (methanol), extraction with methanol/chloroform/water (MCW), liquid/liquid extraction (l/l) and extraction using ethanolic potassium hydroxide (KOH). Component 1 includes 64% of the variability, component 2, 14% and component 3, 11%. All detected analytes were included. Missing values were replaced by mean values. n=6

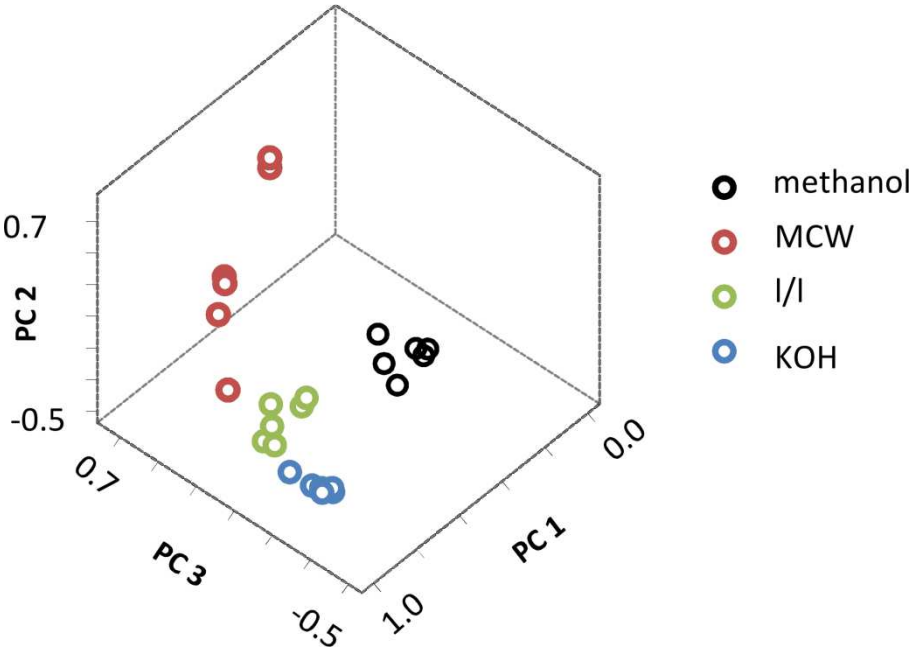


Fig. S2: Principal component analyses of the TIC normalized values of extracted analytes using different extraction protocols. All identified analytes found at least three times were included. Missing values were replaced by the mean value. a: Distribution of compounds displaying extracted component 1 (23% variability) and component 2 (21% variability). b: Separation by polarity; the nonpolar compounds are situated all in the first and fourth quadrant (in the top and bottom right corner) but do not really separate from the polar fraction. c: Separation by basicity/ acidity: basic compounds are found only in the lower quadrants while acidic compounds are mostly situated in the upper part of the graph. However, no separation occurred also if the other extracted components up to number 4 were considered. d: Separation according to compound groups: no separation of a distinct compound group could be observed; AS= amino acid; FS= fatty acid; OA= organic acid.

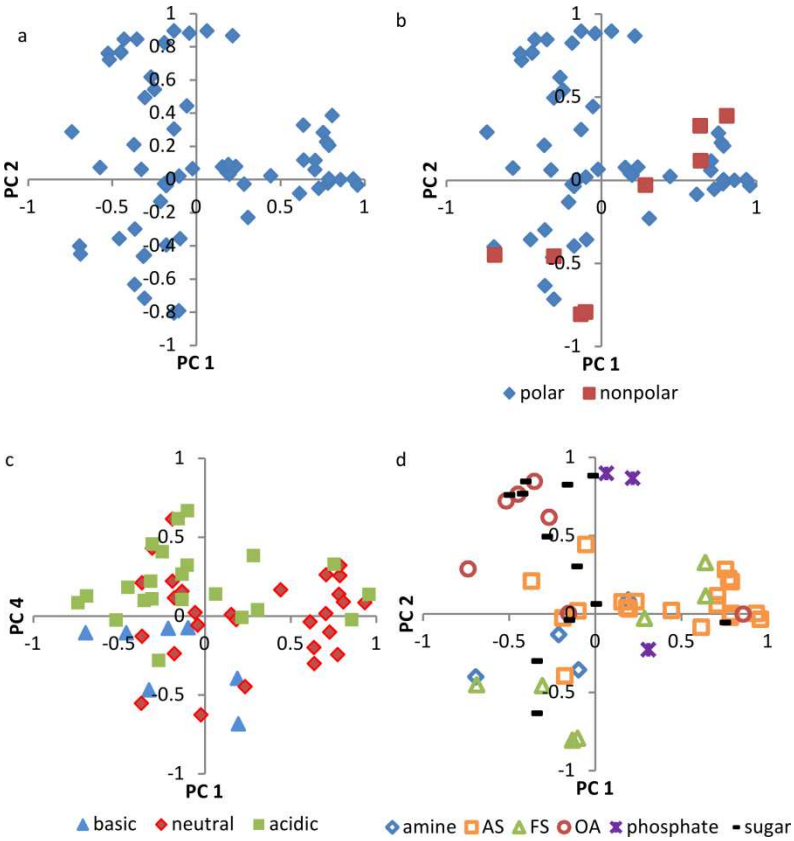
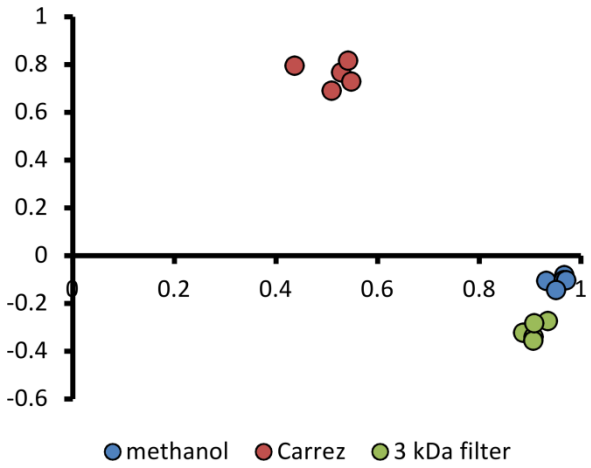


Fig. S3: PCA of samples extracted with methanol without further protein precipitation and samples using Carrez reagent or 3 kDa filters to remove proteins prior to methanol extraction. Protein precipitation with Carrez reagent changed the metabolite pattern while samples further purified with 3kDa filters remained similar to the metabolite pattern after extraction with methanol only. 137 analytes were included in the analysis; missing values were replaced by mean values. PC1 contains 67% of variability, PC2 23%, n=5.



Electronic supplementary material ESI-2: MS Excel sheets providing raw data for deconvoluted peak intensities and peak intensity values normalized to the sum of peak areas (TIC) or the cell count if these data were used in the corresponding experiments. In all data sets, contaminations (identified as pointed out in the experimental section) and analytes that were detected in less than three replicates in one sample set of the corresponding experiment were excluded. The work sheet entitled “extraction” contains raw data and TIC normalized data for the comparison of extraction procedures. Raw data were used for comparison of overall intensity and number of detected analytes, TIC normalized values were subjected to PCA. The work sheet “conc.series_repeated_injection” provides raw data for the repeated injection series used for figure 2. The sheet “conc.series_diff.extractions” contains raw data, normalized data and evaluation of the concentration series conducted with pooled cell material. The specified subset of 42 common metabolites was used to create figure 3. Raw data and normalized data of the different sampling methods are provided in the sheet “sampling methods”. Normalized values of analytes found in at least three replicates of each sample preparation of the dataset “experiment 2” specified as “t-test subset” were used for the evaluation of significant t-test values and mean ratios. Compounds marked with “PCA subset” were included in PC analysis of figure 6. Raw data for assessment of the run order effect are provided in the sheet “run order sensitive analytes”. Analytes were required to be found in each sample replicate set at least three times to qualify for analysis. Potentially run order-sensitive analytes with significant t-test values ($p<0.05$) and mean ratios <0.5 or >2 are marked with “x”.

See attached MS Excel file (ESI-2: supplementary data)