Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Information

Reduction of neutral lipid reservoirs, bioconversion and untargeted metabolomics reveal distinct roles for vitamin K isoforms on lipid metabolism

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Table S 4 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to OH-PhQ vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 5 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K1 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 6 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K2 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 7 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K3 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 8 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to OH-PhQ vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 9 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K1 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 10 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K2 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Table S 11 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K3 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

Appendix A: General Experimental Procedures

One- and two-dimensional (1D and 2D) NMR spectra were acquired with a 600-MHz Bruker Avance III HD (Bruker, Karlsruhe, Germany) equipped with a 5 mm cryoprobe, for samples dissolved in DMSO-*d6* for ^{1}H and ^{13}C experiments. Both chemical shifts (^{1}H and ^{13}C) are expressed in δ (ppm), referenced to the solvent used and the proton coupling constants *J* are given in hertz (Hz). Spectra were assigned using appropriate COSY, HSQC, HMBC and ROESY sequences. LC-ESI-HRMS (liquid chromatography-high resolution electrospray ionization tandem mass spectrometry) data were acquired on a system composed of an UltiMate 3000 HPLC coupled with Q Exactive Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and controlled by Xcalibur 4.1. Then, 5 µL was separated using an ACE Ultracore 2.5 SuperC18 (ACE, United Kingdom), 75 x 2.1 mm inner diameter (id) with the column oven set to 40˚C. The samples were eluted at 0.35 mL/min over a gradient of 99.5 % solvent A (95% H2O, 5% MeOH with 0.1% *v/v* formic acid) to reach 10 % solvent B (95% isopropanol, 5% MeOH with 0.1% *v/v* formic acid) during 0.5 min, followed by an increase to 60% solvent B in 8 min and by another increase to 90% in 1 min, maintaining that isocratic conditions for over 6 min and returning to the initial conditions over 1.5 min, before equilibrating for a final 2 min. The UV absorbance of the eluate was monitored at 206 nm, 248 nm and 254 nm. LC-HR-ESI-MS/MS was obtained in positive ion mode using a capillary voltage of the Heated Electrospray Ionization (HESI) of 3.8 kV, temperature of 300˚C, full MS scan at the resolution of 70,000 FWHM (full width at half maximum; range of 150–2000 *m/z*), and data-dependent MS^2 ($ddMS^2$, Discovery mode) at the resolution of 17,500 FWHM (isolation window used was 3.0 amu and normalized collision energy was 35). The sheath gas and auxiliary gas flow rate were at 35 and 10 (arbitrary unit as provided by the software settings).

UV-Visible (UV-Vis) spectra were acquired on a VWR UV 1600 equipped with a 1 cm path length quartz cuvette. The sample was eluted in methanol (MeOH) at a 0.24 mg/mL and scanned at room temperature from 190 to 800 nm with range of 0.5 nm and analyzed using M Wave software. Fourier Transform Infrared Spectroscopy (FT-IR) spectra was also acquired on a Thermo Scientific Nicolet iS5 (Omnic software), and the sample scanned forty-eight times at a concentration of 0.24 mg/mL, using a real crystal IR card with addition of potassium bromide solution. Optical rotations were measured on a Jasco P2000 polarimeter (10 measurements, 589 nm) using a 1 mL sample cell, at room temperature. The sample was dissolved in MeOH at concentration of 0.5 mg/mL. The electronic circular dichroism (ECD) spectrum was acquired on a J-1500 Spectrometer (JASCO).

Normal-phase HPLC data were obtained with a Merck-Hitachi LaChrom 7000 instrument coupled with a Diode Array Detector L-7450 and Fluorescence Detector L-7480 (Tokyo, Japan) using 254 nm for monitored wavelengths. The software HSM D-7000 LaChrom® was used for data interpretation.

The phase contrast and red fluorescence images of zebrafish bioassays were obtained with a Leica DM6000B microscope.

Appendix B: Isolation and structural elucidation of OH-Phylloquinone

1.1. Cyanobacterial Growth, Extraction and Fractionation

Initial screening assays led to the selection of the cyanobacterial strain, *Tychonema* sp. LEGE 07196 isolated from a Portuguese wastewater treatment plant (Ramos *et al*., 2018) and maintained in the LEGEcc in CIIMAR, Matosinhos, Portugal. Strains were cultured in Z8 medium, at 25 °C, with a photoperiod of 14 h/10 h light and dark, respectively, and at a light intensity of 10–30 µmol photons.m^{-2}.s^{-1}. *Tychonema* sp. LEGE 07196 cultures were grown in 80 l sleeve bag with constant aeration and, at the exponential phase, cells were harvested through centrifugation, before being frozen and freeze-dried. Two rounds of biomass extraction were necessary. A (i) mixture of CH_2Cl_2/MeOH was firstly used and (ii) then based on previous work the biomass (70 g) was extracted by repeated percolation with MeOH, yielding a crude extract of 11.1 g. The crude extract was fractionated by normal-phase (Si gel 60, 0.015–0.040 mm, Merck KGaA, Darmstadt, Germany) VLC (vacuum liquid chromatography) with an increasing polarity grade, from 100% n-Hex to 100% EtOAc and 100% MeOH (Leao *et al*., 2013), giving a total of nine fractions.

1.2. Compound Isolation and Structure Elucidation

The first approach to isolate the compound was based on zebrafish bioassay-guided fractionation. In the re-isolation, the VLC fractions were subjected to an HRMS/MS analysis, with a chromatographic column Luna-C18 (250mm 4.6 mm, 5 m, 100 Å, Phenomenex). Samples were eluted at 0.8 mL/min with a linear gradient starting from 1:1 ACN/H_2O (acetonitrile/water) to 100% ACN in 20 min. Fraction B (9:1 *n*-Hex/EtOAc, *v/v*; ethyl acetate/hexane) showed the monoisotopic mass value 467.3531 *m/z* that appeared to be the major compound with the fragment value 449.3387 *m/z*. Thus, was further sub-fractionated by flash column chromatography using Si gel 60 (0.015–0.040 mm, Merck KGaA, Darmstadt, Germany) as a stationary phase and a gradient of increasing polarity from 1:0 *n*-Hex/ EtOAc to 0:1 EtOAc/ MeOH (*v/v*) (methanol) producing 11 fractions. The sub-fractions B5 and B6, eluted in 9:1 to 4:1 *n*-Hex/ EtOAc (*v/v*), had the previous mass values, so they were pooled. Then, a SPE (solid-phase extraction) separation was performed, using a prepacked normal-phase SiO_2 5g cartridges (Strata SI-1 55 µm, 70 Å, Phenomenex) and a gradient of 1:0 *n*-Hex/ EtOAc to 0:1 EtOAc/ MeOH (*v/v*), yielding 9 fractions. Sub-fractions B5+6C and B5+6D eluted in 19:3 *n*-Hex/ EtOAc (*v/v*) possessed the compound of interest and, thus, were submitted to HPLC using a Luna 5 µm Si (250 x 4.6 mm 100 Å, Phenomenex, Torrance, California, USA) column and using a linear gradient from 19:3 to 9:1 *n*-Hex/ EtOAc (*v/v*) for 5 min. and being maintained at 9:1 *n*-Hex/ EtOAc (*v/v*) for another 22 min. Then there was an increase of the polarity with a linear gradient from 9:1 to 0:1 *n*-Hex/ EtOAc in 1 min. and being maintained at 0:1 *n*-Hex/ EtOAc for another 5 min (1 mL/min. flow). Subfraction B5+6C+D2 needed to be further purified by another round of HPLC with an isocratic mixture of 19:3 *n*-Hex/ EtOAc (*v/v*) for 45 min. (1 ml/min. flow). $5^{1'}$-OH-Phylloquinone was isolated as the major component at 27 min RT (retention time).

The ^{1}H NMR and ^{13}C NMR spectra of the compound (Table S2 and Figure S2 and S3) exhibited all the resonances of the 1,4-naphthoquinone system and the attached phytyl side-chain. All carbons and protons, as well as the functional groups, were assigned to the proposed structure based on 2D NMR spectral data (HMBC and COSY, Figures S5 and S6), as shown in Figure S1. The assignment was corroborated by the reported data for similar moieties (^{1}H NMR, $CDCl_3$: [1, 2]). Additionally, in agreement with Allen *et al*. and Law *et al*. the performance of Fourier Transform Infrared Spectroscopy (FT-IR) and UV-visible (UV-vis) absorption experiments indicated the presence of the hydroxy group in its intermolecular bonded nature at C-5', the double bond at C2'-C3' and the alkane nature of the side-chain, as well as the quinone conjugated system, including the ketones functional groups, depicted by maximum absorption peaks and its shoulders (UV-vis), further confirmed the proposed compound (Figure S8 and S9).[3, 4]

The ROESY spectrum revealed signals of the Overhauser enhancement between OH-$5^{1'}$ at δ 4.16 ppm and methylene H_2-1' at δ 3.32 ppm, and between the latter and H-2' at δ 4.99 ppm. Additionally, signals were observed between the methyl H_3-71' at δ 0.74 ppm and terminal methyl $15^{1'}$ and 16' at δ 0.83 ppm and a very weak one with H-5' at δ 3.59 ppm, in which the latter had with methyl H3-31' at δ 1.74 ppm and with methylene H2-4' at δ 1.93/2.06 ppm (Figure S7). These correlations suggest that the hydroxy group protons are on the same side of the molecule plane as the carbons at C-1' and C-2' positions and on the opposite position of the protons at H_3-$7^{1'}$.

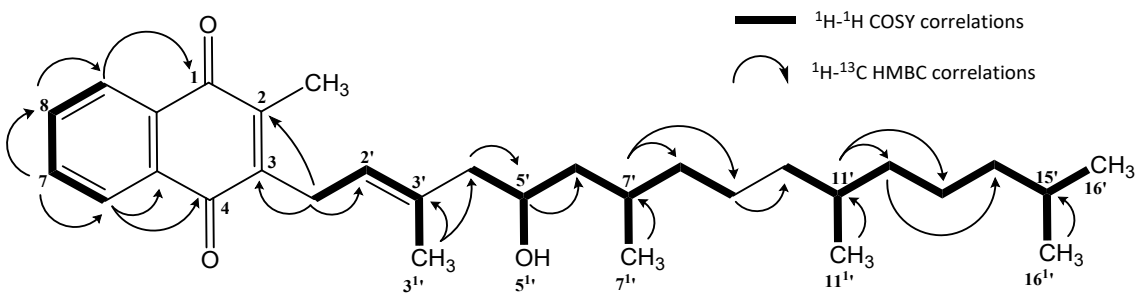

Figure S 1 Key 13C-1H HMBC and 1H-1H COSY correlations for 51'-OH-Phylloquinone.

The OH-PhQ stereochemistry was confirmed by ROESY experiments. Initials experiments performed on isolated natural K1 enlighten the natural 2', 3' -*trans*-(7'*R*, 11'*R*) configurations of the side-chain, along with the natural phytol configuration.[5] Later, Kosugi *et al*. synthesized a mixture of 16 possible stereoisomers of OH-PhQ, confirming the 5'*S*-OH configuration of the natural compound isolated from *Synechococcus* sp. PCC 7942.[6] To further confirm the obtained results and fully uncover the configuration of the isolated natural compound, ECD experiments were performed adjusting the parameters according with the literature data. The simulated spectra were obtained with the weighted (Boltzmann factor) average of the three most stable conformers (data not shown), however the ECD spectrum did not show clear results. Consequently, the 5'*S* stereochemistry configuration was assigned through comparison of ROESY and literature data.

Table S 1 ^{1}H and ^{13}C NMR data (δ, ppm) of $5^{1'}$-OH-Phylloquinone at 600 MHz in DMSO-d6 (deuterated dimethyl sulfoxide).

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1		184.69	**5'**	3.59 ddd (9.2, 6.0, 3.0)	65.84
2		142.89	**$5^{1'}$ - OH**	4.16 d (5.7)	
2^{1}	2.11 s	12.42	**6'**	1.17 m 0.94 m	43.88
3		145.29	**7'**	1.56 m	28.26
4		183.85	**$7^{1'}$**	0.74 d (6.6)	19.17
5		131.56 *	**8'**	0.98 dd (5.1, 1.9)	37.74
6	7.99 ddd (5.9, 3.1, 1.9)	125.89 *	**9'**	1.10 d (1.7)	23.57
7	7.83 dd (5.7, 3.3)	133.85	**10'**	1.18 m	36.57
8	7.83 dd (5.7, 3.3)	133.85	**11'**	1.28 dt (12.2, 5.7)	31.96
9	7.99 ddd (5.9, 3.1, 1.9)	125.81 *	**$11^{1'}$**	0.77 d (6.6)	19.58
10		131.55 *	**12'**	1.01 m	36.66
1'	3.32 d (6.2)	25.49	**13'**	1.20 d (3.1)	24.10
2'	4.99 t (6.7)	121.08	**14'**	1.09 dd (6.1, 3.1)	38.75
3'		134.77	**15'**	1.48 dt (13.3, 6.6)	27.35
$3^{1'}$	1.74 s	16.64	**$15^{1'}$**	0.83 d (6.6)	22.56 *
4'	2.06 dd (13.1, 6.5) 1.93 dd (13.2, 6.4)	48.64	**16'**	0.83 d (6.6)	22.56 *

* - Assignment undetermined due to similarities of chemical surroundings/environment.

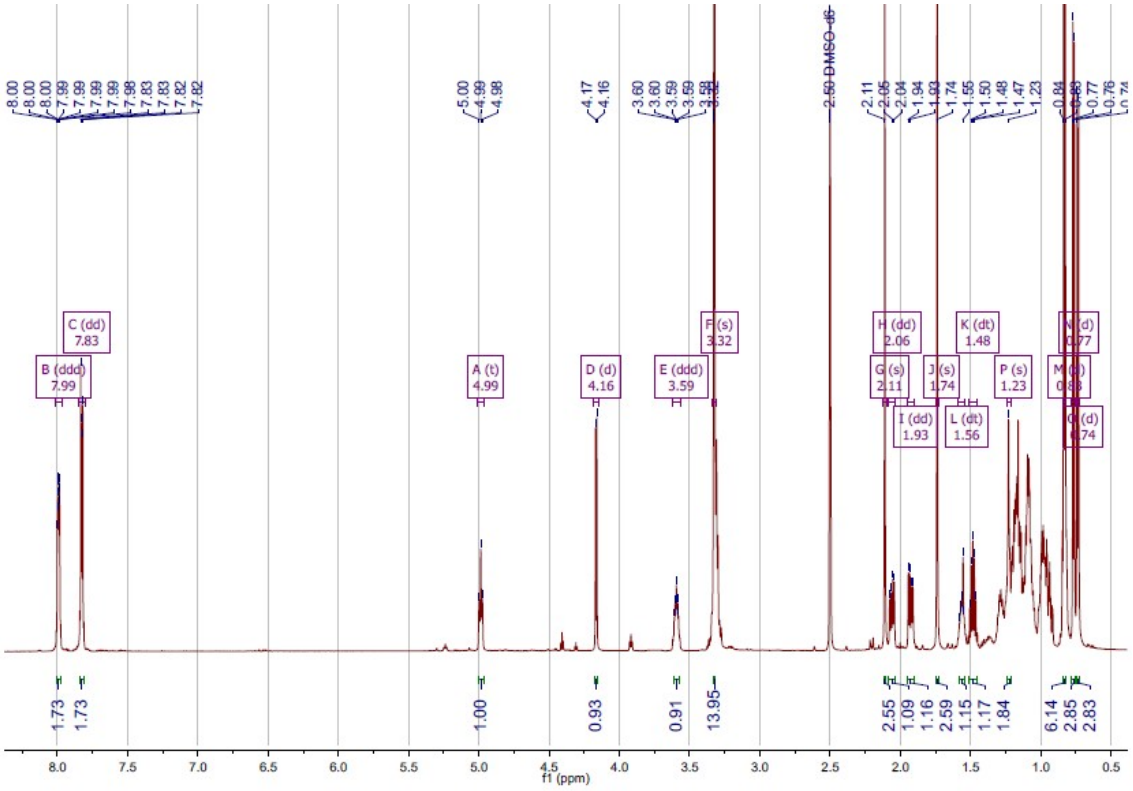

Figure S 2 ^{1}H NMR spectrum of 5$^{1'}$-OH-Phylloquinone in DMSO-*d6* (600 MHz).

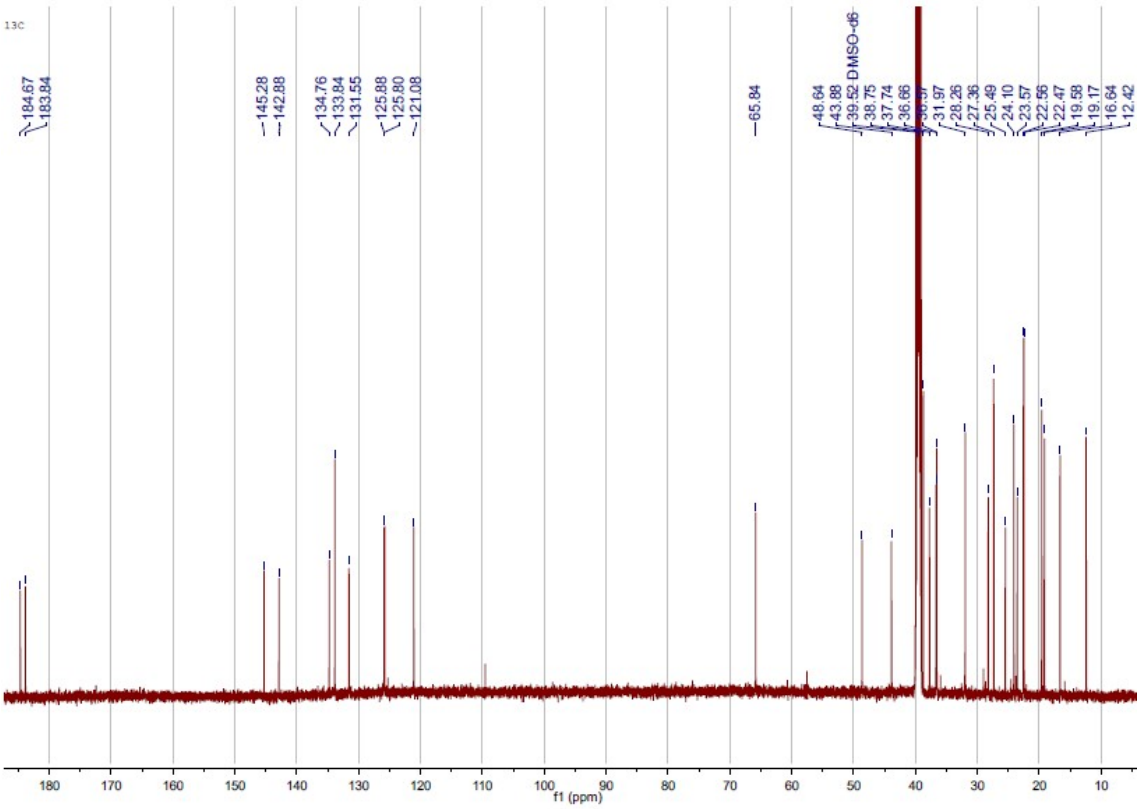

Figure S 3 ^{13}C NMR spectrum of 5$^{1'}$-OH-Phylloquinone in DMSO-*d6* (600 MHz).

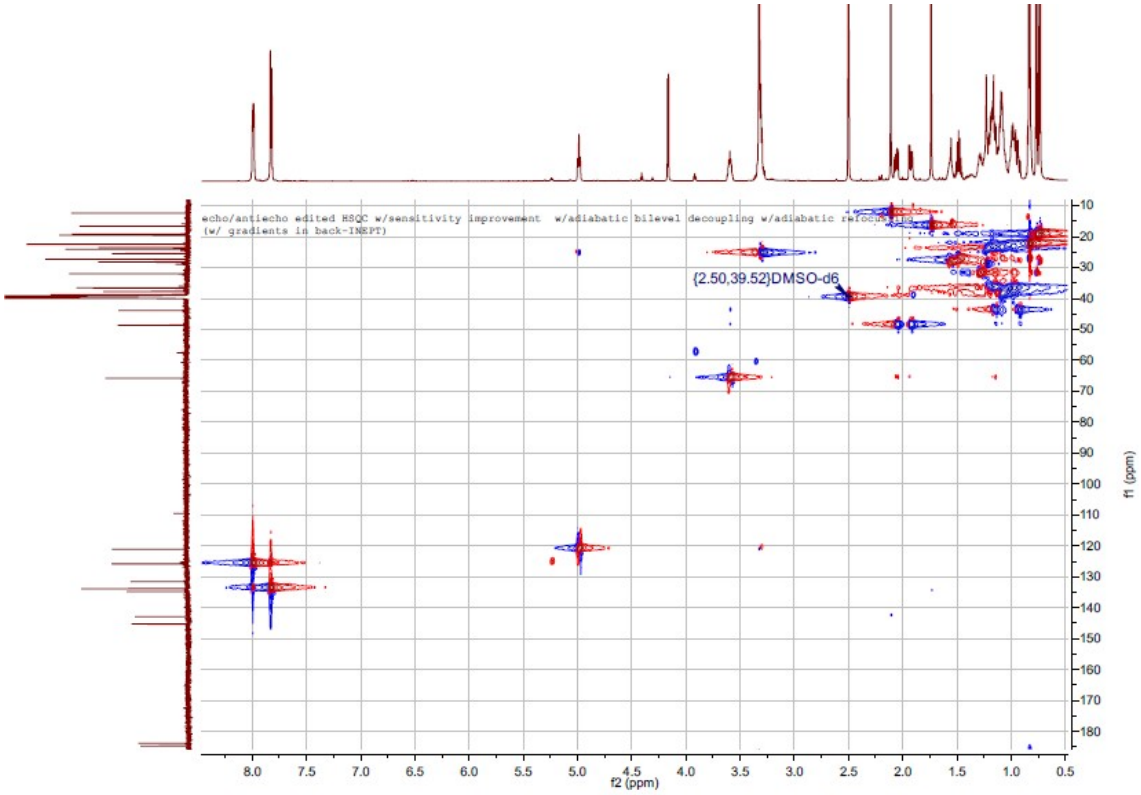

Figure S 4 HSQC spectrum of 5[1']-OH-Phylloquinone in DMSO-*d6* (600 MHz).

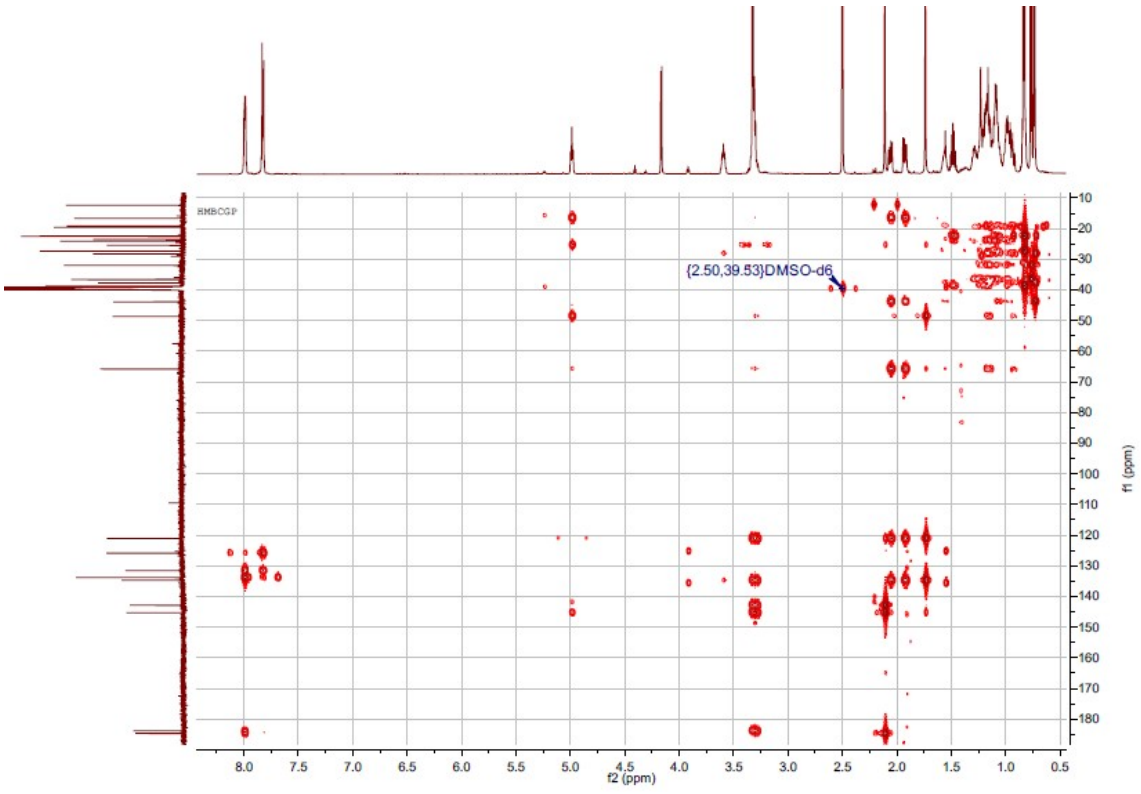

Figure S 5 HMBC spectrum of 5[1']-OH-Phylloquinone in DMSO-*d6* (600 MHz).

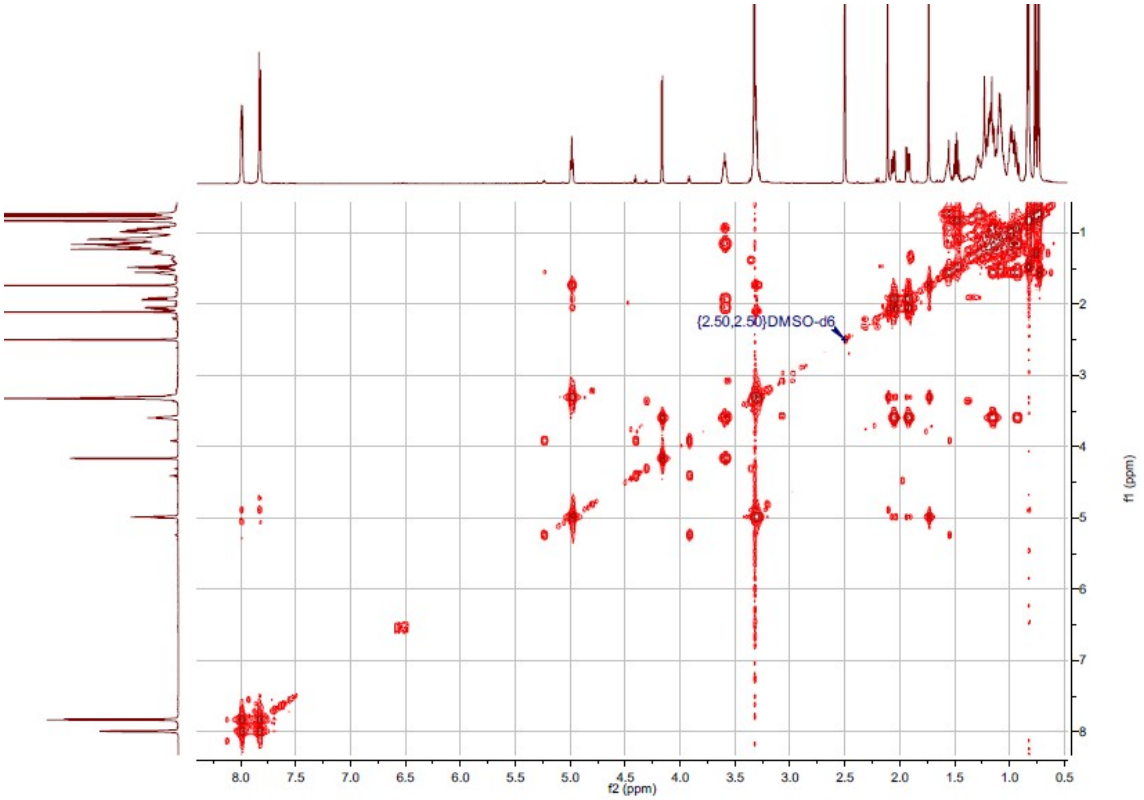

Figure S 6 ^{1}H-^{1}H COSY spectrum of 5$^{1'}$-OH-Phylloquinone in DMSO-*d6* (600 MHz).

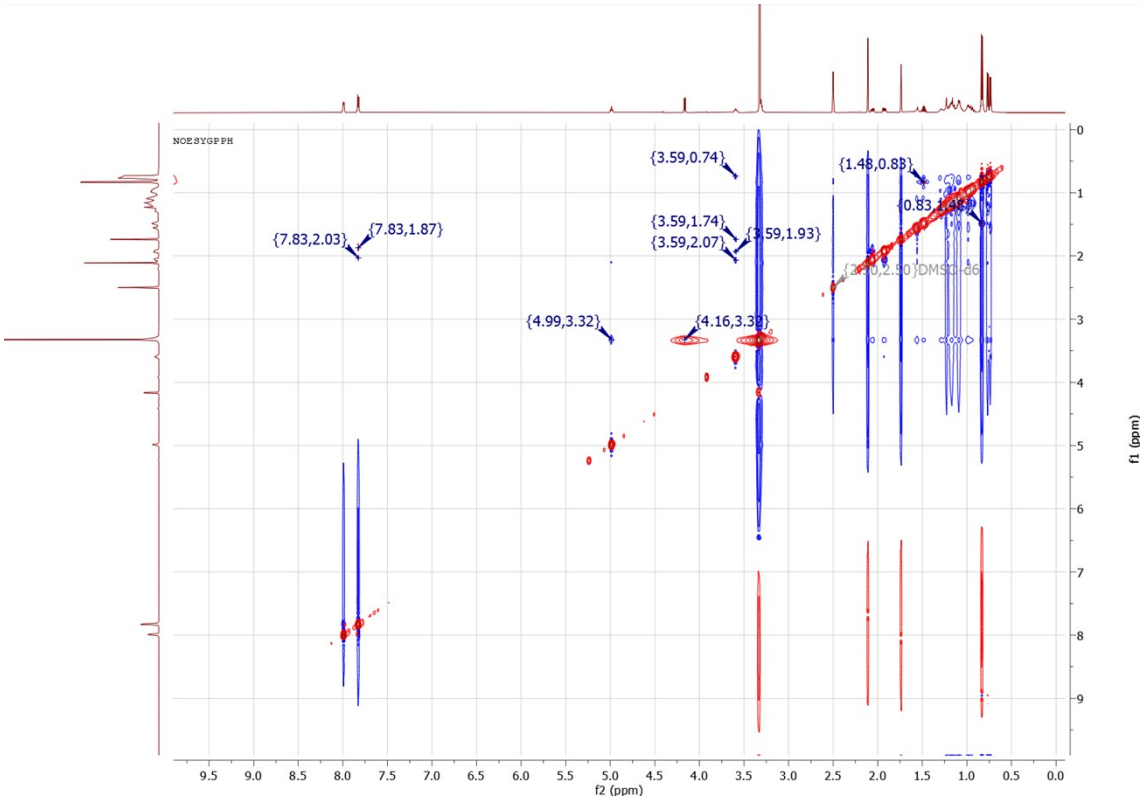

Figure S 7 ROESY spectrum of 5$^{1'}$-OH-Phylloquinone in DMSO-*d6* (600 MHz).

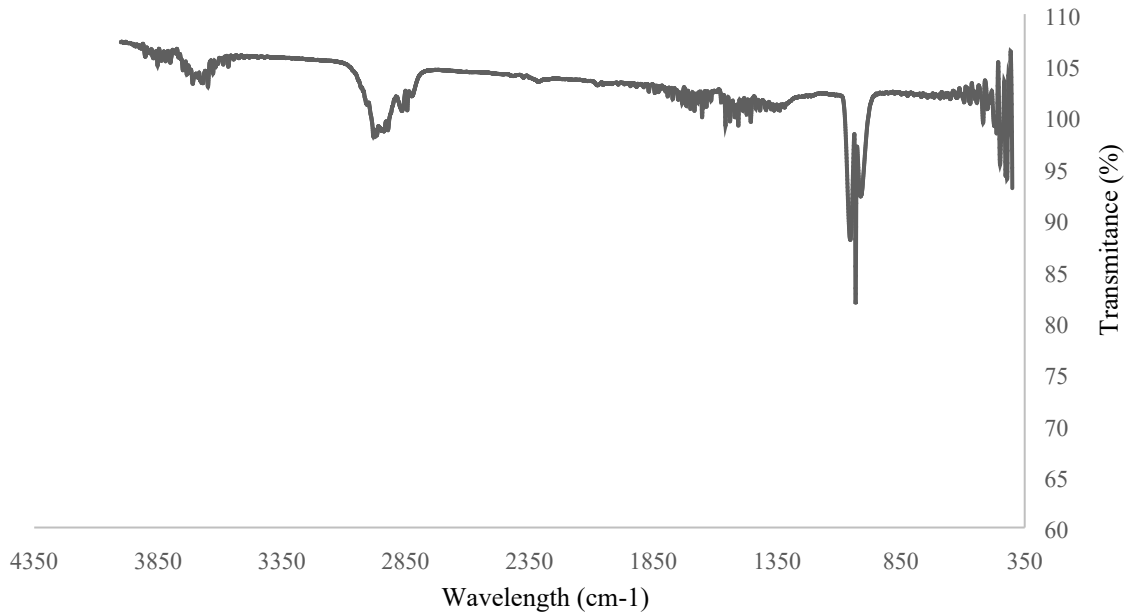

Figure S 8 Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of 5[1']-OH-Phylloquinone (using potassium bromide IR sample card).

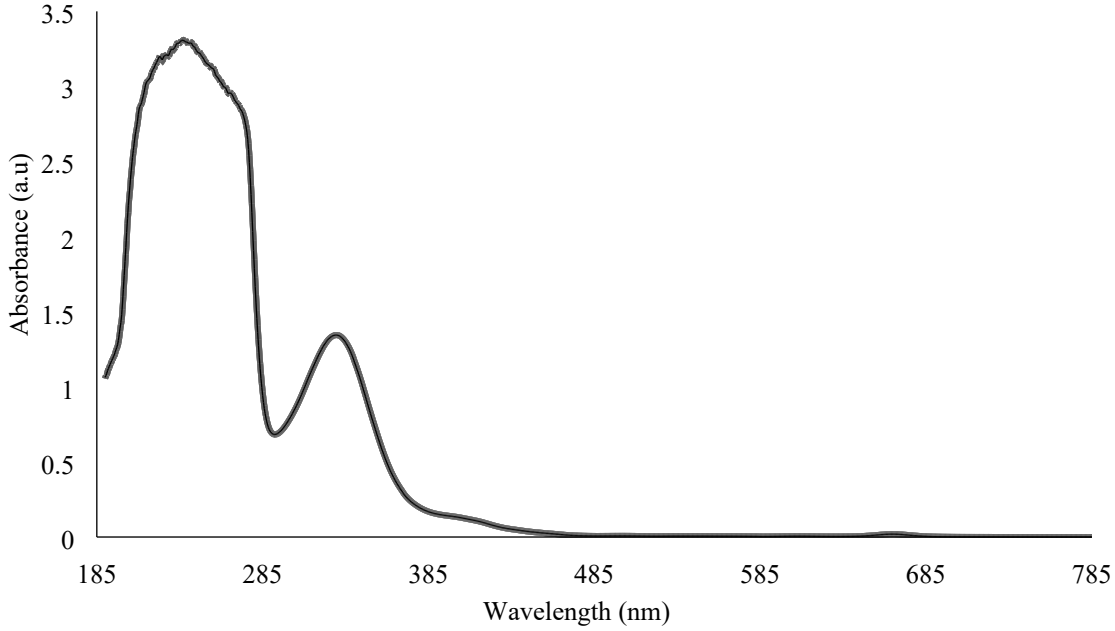

Figure S 9 UV–visible absorption spectrum of 5[1']-OH-Phylloquinone in MeOH.

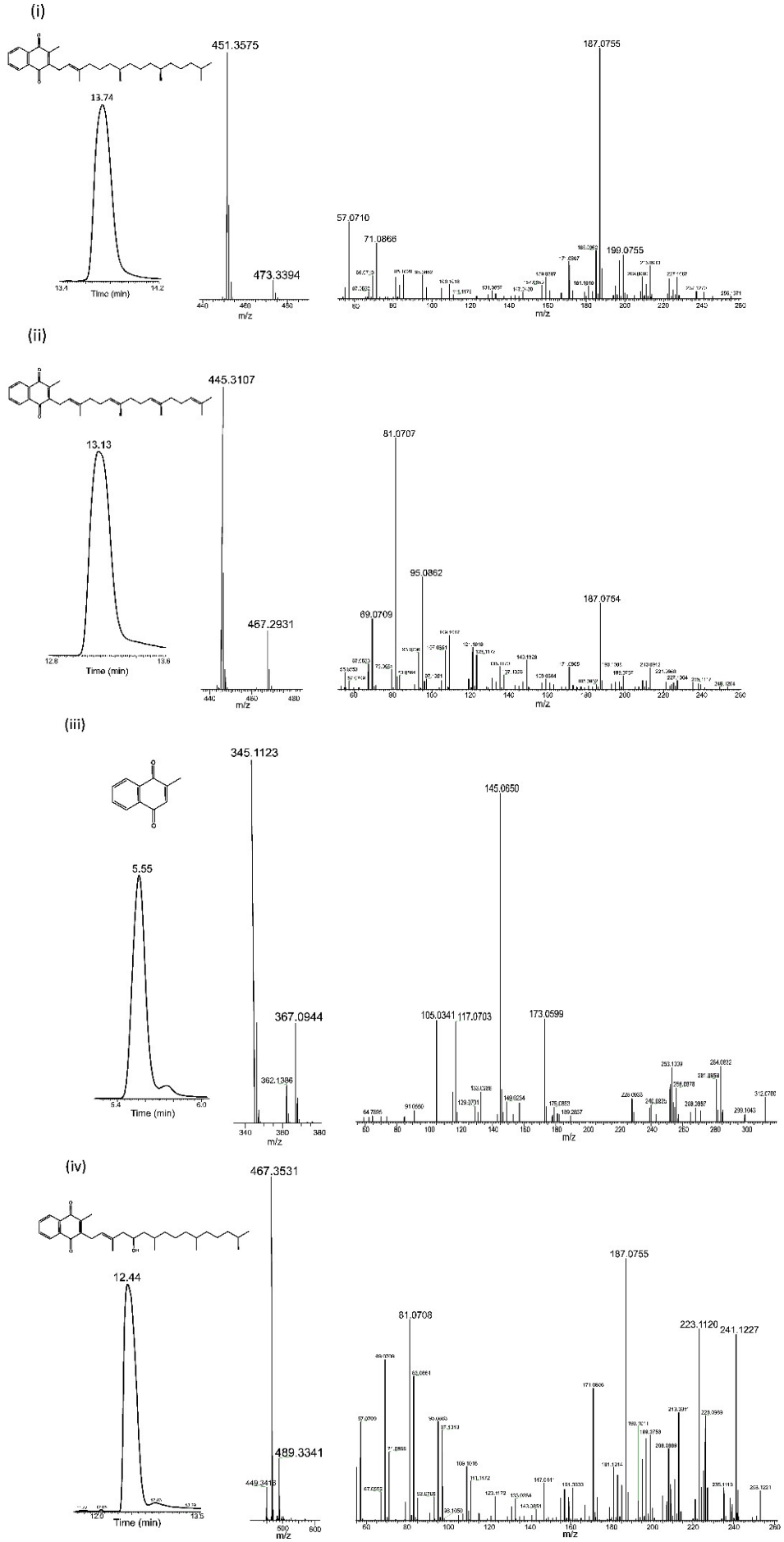

Figure S 10 LC-ESI-HRMS/MS spectra of vitamin K isoforms in the positive ion mode, showing the retention time (RT) and major detected adducts from MS1 data and fragmentation from MS2 data. (i) Phylloquinone, (ii) Menaquinone (MK-4), (iii) Menadione and (iv) OH-PhQ.

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2. M. Vecchi, M. Schmid, W. Walther and F. Gerber, Determination of the diastereomers of Vitamin K1, *Journal of High Resolution Chromatography*, 1981, **4**, 257-259.
3. C. Allen, H. Franke and O. Hirayama, Identification of a plastoquinone and two naphthoquinones in Anacystis nidulans by NMR and mass spectroscopy, *Biochemical and Biophysical Research Communications*, 1967, **26**, 562-568.
4. A. Law, G. Thomas and D. R. Threlfall, 5'-Monohydroxyphylloquinone from Anacystis and Euglena, *Phytochemistry*, 1973, **12**, 1999-2004.
5. L. Jackman, R. Rüegg, G. Ryser, C. von Planta, U. Gloor, H. Mayer, P. Schudel, M. Kofler and O. Isler, On the chemistry of vitamin K. 2. Total synthesis and stereochemistry of trans-and cis-(7'R, 11'R)-phylloquinone and related compounds, *Helvetica Chimica Acta*, 1965, **48**, 1332-1349.
6. M. Kosugi, C. Lee, T. Misaki, Y. Kashino, M. Fujita and T. Sugimura, Stereochemical assignment of the unique electron acceptor 5'-hydroxyphylloquinone, a polar analog of vitamin K1 in photosystem I, *Bioscience, Biotechnology, and Biochemistry*, 2017, **81**, 2244-2252.

Appendix C. *Biological effects of exposed zebrafish larvae to vitamin K isoforms*

1.1. Zebrafish maintenance

Fish were maintained as described in the zebrafish book available at the ZFIN database (https://zfin.org/). Wild-type fish of the AB strain were used and cultured in 30 L aquaria at 28˚C. Adult fish in each tank was fed with dry fish food 2 times per day and occasionally artemia. Embryos spawned under a 14 h on/10 h off light cycle and were collected and cultured in petri dishes containing Egg water (60 µg/mL marine sea salt dissolved in distilled water) at 28˚C.

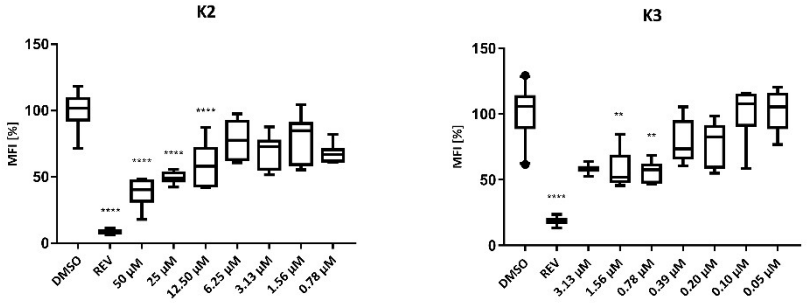


Figure S 11 EC50 values determination in the zebrafish Nile red fat metabolism assay. Seven concentrations of each compound were exposed for 48 h, together with solvent control DMSO (dimethyl sulfoxide 0.1%) and positive control REV (resveratrol 50 µM). Values are expressed as percentage of mean fluorescence intensity (MFI) relative to the DMSO group and are derived from six to eight individual larvae per treatment group. The data are represented as box-whisker plots from the 5th to 95th percentiles. Asterisks highlight significant altered fluorescence intensities that indicate changes of neutral lipid level (**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Table S 2 Parameters used in MZmine 2 for mass feature detection, chromatogram building, feature alignment and filtering for further MetaboAnalyst analyses.

MZmine 2 Workflow	***Parameters used***	
1. Project	Sampler Parameters	DMSO/Hex-EtOH – Solvent control K1/K2/K3/K1-analogue - Treatments
2. Mass detection (centroid)	Noise level for MS1	1E4
	Noise level for MS2	1E3
3. ADAP chromatogram builder	Minimum group size in # of scans	2
	Group intensity threshold	1E4
	Minimum highest intensity	1E4
	m/z tolerance	10.0 ppm
4. Alignment (Join aligner RANSAC)	*m/z* tolerance	10.0 ppm
	Retention time (RT) tolerance	0.3 min
	RT tolerance after correction	0.5 min
	RANSAC iteration	0
	Minimum nº points	80
	Threshold	0.3
	Charge state	Same
5. Filtering (Feature list row filter)	Keep only peaks with MS2 scan and Reset peak number	
6. Filtering (Duplicate peak filter)	MetaboAnalyst	Old Average
	m/z tolerance	10.0 ppm
	RT	0.1 min

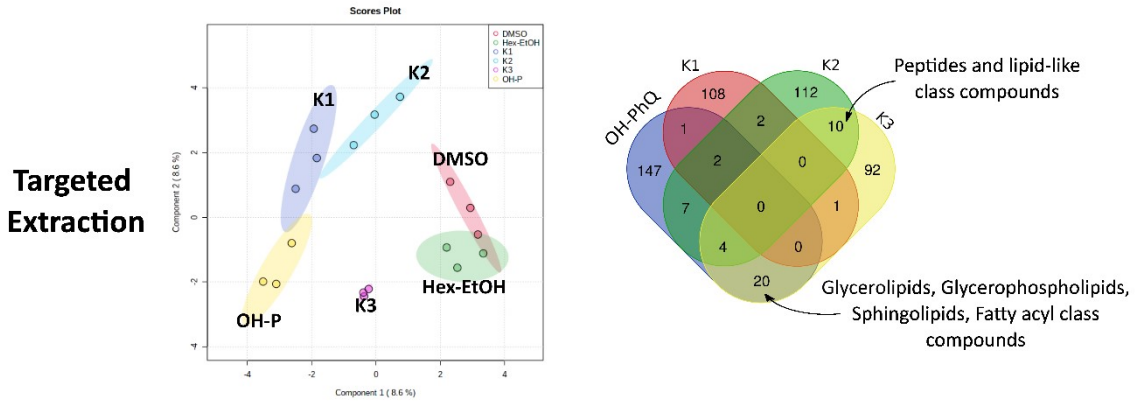


Figure S 12 Statistically significant metabolites from exposed zebrafish larvae to the vitamins K family in comparison with control samples from targeted extraction. Data is presented as Partial Least Squares-Discriminant Analysis (PLS-DA) plot and Venn's diagram with a statistical significance of 0.05 (ANOVA - T-test using Tukey's/Fisher's post hoc test).

Table S 3 Concentrations range (0.03 µM, 0.25 µM, 2.5 µM, 25 µM, 250 µM, 2.5 mM), major adducts, 4 main fragments (relative abundance in which major is in bold), ionization intensities range and RT (retention time) of the different standard compounds detected by ESI source (positive ion mode).

COMPOUND	*CONCENTRATION RANGE*	*ADDUCT*	*m/z*	*FRAGMENTS [RELATIVE ABUNDANCE] (m/z)*	*IONIZATION INTENSITY RANGE*	*RT (min)*
K1	0.03 µM – 2.5 mM	M + H	451.3575	199.0755 [18], **187.0755 [100]**, 71.0865 [22], 57.0710 [31]	8,66E+05 – 4,43E+09	13.74
		M + Na	473.3394			
K2	0.03 µM – 2.5 mM	M + H	445.3107	187.0754 [35], 95.0862 [45], **81.0707 [100]**, 69.0709 [28]	4,01E+06 – 5,31E+09	13.13
		M + Na	467.2931			
K3	0.03 µM – 2.5 mM	M + H	173.0596	–	5,13E+04 – 2,75E+06	5.55
		2M + H	345.1123	173. 0599 [28] **145.0650 [100]**, 117.0703 [30], 105.0341 [27]	3,44E+04 – 4,08E+08	
		2M + Na	367.0944			
OH-PhQ	0.03 µM – 2.5 mM	M + H	467.3531	241.1228 [68], 223.1118 [75], **187.0755 [100]**, 81.0708 [71]	3,23E+06 – 1,21E+09	12.44
		M + Na	489.3341			
		2M + H	933.6982			

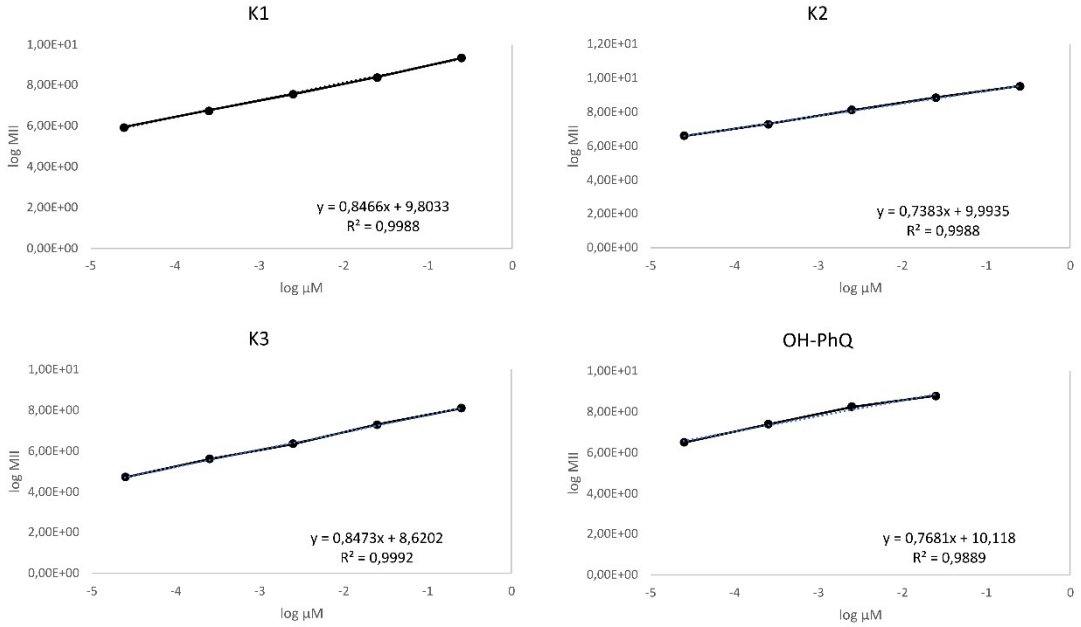
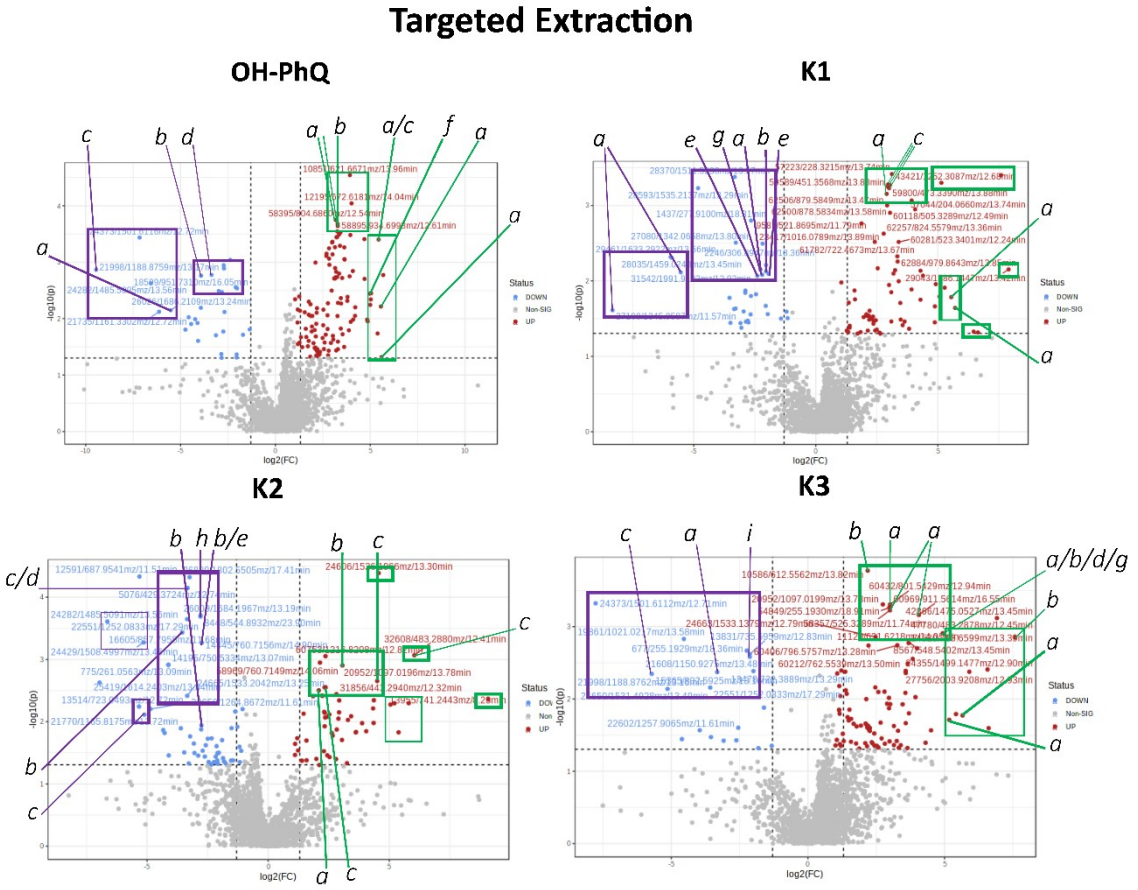


Figure S 13 Standard calibration curves of the vitamins K family (OH-PhQ, K1, K2 and K3) analyzed by LC-ESI-HRMS/MS in the positive ion mode using the same methodology as described under "Material and Methods: General Experimental Procedures". The results are presented as logarithmic concentrations (µM) and logarithmic mean intensity ionizations (MII) of the 4 isoforms.



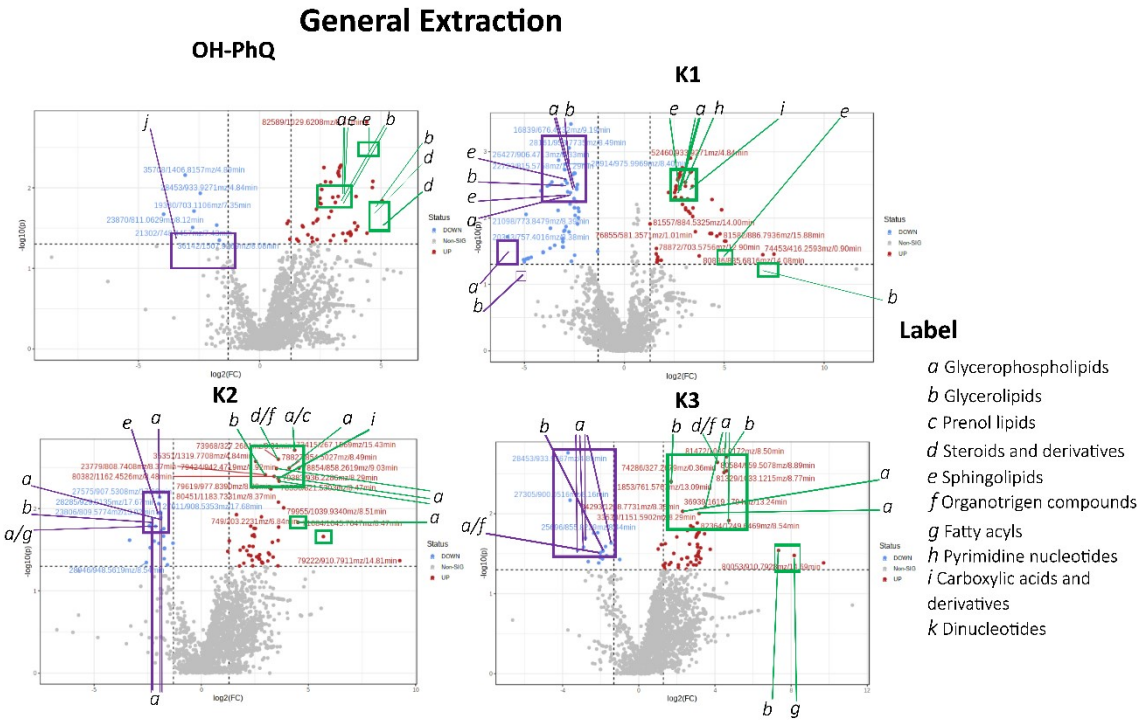


Figure S 14 Statistically significant and enriched metabolites from zebrafish larvae exposed to different vitamins K family, in comparison with control samples, from both extraction procedures. Data presented as volcano plot with a statistical significance of 0.05 (ANOVA - T-test using Tukey's/Fisher's post hoc test) and a threshold of 4-fold difference (2 log2FC) 2.0. In each representation is shown the class of the identified top 20 statistically significant and enriched (2 log2FC ≥ 5) compounds. Boxes green and purple represent the top selected metabolites, up/down, respectively.

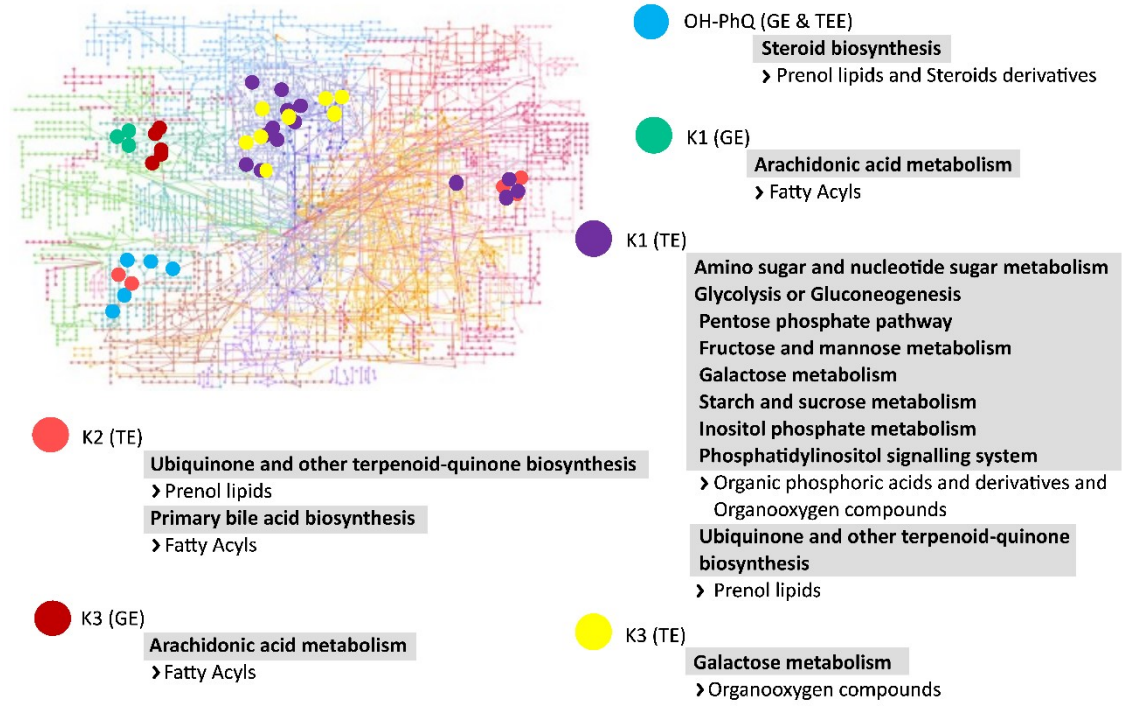


Figure S 15. Statistically significant metabolic pathways resulting from the supplementation of vitamins K family: OH-PhQ, K1, K2 and K3. The two extraction procedures, general (GE) and targeted extraction (TE), were analyzed and compared. In bold and grey shade are listed the pathways whereas below are the class of compounds involved in such pathways. All indicated pathways are up-regulated.

Table S 4 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to OH-PhQ vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

OH-PHQ TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1420.1639	3.25	0.0062	**Several DG**, ex: DG(20:3n6/0:0/22:2n6) (698.5849, 3, [2M+Na]$^+$)	Diacylglycerols	Glycerolipids
1208.7729	2.65	0.0056	**Several CL**, ex: CL(11:0/11:0/11:0/18:2(9Z,11Z)) (1166.7375, 1, [M+ACN+H]$^+$)	Glycerophosphoglycerols	Glycerophospholipids
1029.6208	4.41	0.0015	**NeuAcalpha2-3Galbeta-Cer(d18:1/16:0)** (990.6603, 2, [M+K]$^+$)	Gangliosides	Sphingolipids
989.8166	3.26	0.0069			
977.7994	4.67	0.0083	**TG(22:4(7Z,10Z,13Z,16Z)/18:2(9Z,12Z)/22:5(7Z,10Z,13Z,16Z,19Z))** (994.7989, 3, [M+H-H2O]$^+$)	Triacylglycerol	Glycerolipids
925.5435	3.30	0.0056	**Several PI**, ex: PI(18:1(9Z)-O(12,13)/22:5(7Z,10Z,13Z,16Z,19Z)) (924.5364, 0, [M+H]$^+$)	Glycerophosphoinositols	Glycerophospholipid
894.7038	4.64	0.0099	**27-Nor-5b-cholestane-3a,7a,12a,24,25-pentol** (438.3345, 1, [2M+NH4]$^+$)	Bile acids, alcohols and derivatives	Steroids and derivatives
880.7019	5.05	0.0145	**Sitosterol 3-O-(6'-O-linoleyl-beta-D-glucoside)** or **Isofucosterol 3-O-[6-O-(9-Octadecenoyl)-b-D-glucopyranoside]** (838.6687, 1, [M+ACN+H]$^+$)	Stigmastanes and derivatives	Steroids and derivatives
824.5338	3.30	0.0056	**Several SM**, ex: SM(d16:2(4E,8Z)/22:5(4Z,7Z,10Z,13Z,19Z)-O(16,17)) (760.5155, 3, [M+ACN+Na]$^+$)	Phosphosphingolipids	Sphingolipids
371.2577	3.28	0.0072	**Several DG**, ex: DG(LTE4/0:0/i-14:0) (723.4744, 0, [M+H+NH4]$^+$)	Diacylglycerols	Glycerolipids
1006.5269	-1.77	0.0291	**Several PIP**, ex: PIP(5-iso PGF2VI/18:0) (988.4926, 1, [M+NH4]$^+$)	Glycerophosphoinositol phosphates	Glycerophospholipid
811.0629	-3.96	0.0213	**Diguanosine triphosphate** (788.0718, 2, [M+Na]$^+$)	Dinucleotides	Dinucleotides
748.4457	-2.77	0.0311			
703.1106	-2.71	0.0195			

Table S 5 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K1 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K1 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1071.4167	3.29	0.0021	**(11Z,14Z)-Henicosa-11,14-dienoyl-CoA** or **(8Z,11Z)-Henicosa-8,11-dienoyl-CoA** (1071.3918, 2, $[M+NH4-H2O]^+$)	Peptide	Carboxylic acids and derivatives
994.6264	2.83	0.0020	**Lithocholyltaurine** (483.3018, 0, $[2M+2H+3H2O]^+$)	Bile acids, alcohols and derivatives	Steroids and derivatives
985.5002	2.91	0.0017	**PS(LTE4/18:0)** (946.5353, 2, $[M+K]^+$) or **Several PI**, ex: PI(18:1(12Z)-2OH(9,10)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (940.5313, 2, $[M+2Na-H]^+$)	Glycerophosphoserines Glycerophosphoinositols	Glycerophospholipids
904.6013	2.75	0.0024	**PE(18:1(12Z)-2OH(9,10)/24:1(15Z))** or **PE(24:1(15Z)/18:1(12Z)-2OH(9,10))** (859.6302, 0, $[M+2Na-H]^+$)	Glycero-phosphoethanolamines	Glycerophospholipids
896.6975	2.76	0.0022	**N-[(2S,3S,4R)-3,4-Dihydroxy-1-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoctadecan-2-yl]hexacosanamide** (857.7320, 3, $[M+K]^+$)	Glycosphingolipids	Sphingolipids
835.6816	6.93	0.0356	**Several TG**, ex: TG(15:0/18:3(6Z,9Z,12Z)/18:4(6Z,9Z,12Z,15Z)) (834.6737, 1, $[M+H]^+$)	Triacylglycerols	Glycerolipids
832.2347	3.39	0.0019			
722.4695	2.70	0.0025	**Several CL**, ex: CL(14:0/18:0/18:4(6Z,9Z,12Z,15Z)/20:4(5Z,8Z,11Z,14Z)) (1420.9409, 1, $[M+H+Na]^+$)/(1442.9253, 1, $[M+2H]^+$)	Glycerophosphoglycerols	Glycerophospholipids
718.1740	3.35	0.0020			
703.5756	5.02	0.0222	**SM(d18:1/16:0)** or **SM(d18:0/16:1(9Z))** (702.5676, 1, $[M+H]^+$)	Phosphosphingolipids	Sphingolipids
1250.8136	-2.64	0.0026	**Ganglioside GM3 (d18:1/20:0)** (1208.7758, 3, $[M+ACN+H]^+$)	Glycosphingolipids	Sphingolipids
1009.4497	-2.51	0.0021	**Several PI**, ex: PI(5-iso PGF2VI/20:2(11Z,14Z)) (932.5262, 4, $[M+2K-H]^+$)	Glycerophosphoinositol	Glycerophospholipids
975.9969	-2.94	0.0015	**Ganglioside GT2 (d18:1/14:0)** (1908.9568, 2, $[M+ACN+2H]^+$)	Glycosphingolipids	Sphingolipids
953.7735	-2.76	0.0009	**Several TG**, ex: TG(14:0/O-18:0/22:0) (876.8510, 4, $[M+2K-H]^+$)	Triacylglycerols	Glycerolipids
853.7249	-5.04	0.0424	**Several TG**, ex: TG(16:0/14:0/20:2n6) (830.7363, 1, $[M+Na]^+$)	Triacylglycerols	Glycerolipids
786.5051	-2.46	0.0021	**Several PE**, ex: PE(20:5(6E,8Z,11Z,14Z,17Z)-OH(5)/P-18:1(9Z)) (763.5152, 1, $[M+Na]^+$); **Several PA**(20:3(6,8,11)-OH(5)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (786.4836, 1, $[M+NH4-H2O]^+$); **Several PE-NMe2**, ex: PE-NMe2(22:5(7Z,10Z,13Z,16Z,19Z)/14:1(9Z)) (763.5152, 1, $[M+Na]^+$)	Glycero-phosphoethanolamines Glycerophosphates	Glycerophospholipids
757.4016	-5.85	0.0191	**Several PA**, ex: PA(PGJ2/i-15:0) (712.4315, 1, $[M+2Na-H]^+$)	Glycerophosphate	Glycerophospholipids
676.4232	-2.67	0.0004			
429.2597	-2.95	0.0027	**PA(LTE4/P-16:0)** (815.4771, 1, $[M+ACN+2H]^+$)	Glycerophosphates	Glycerophospholipids
325.2348	-2.98	0.0019	**Several MG**, ex: MG(i-14:0/0:0/0:0) or **1-monomyristoylglycerol** (302.2457, 0, $[M+Na]^+$)	Monoacylglycerols	Glycerolipids

Table S 6 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K2 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K2 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1162.4526	3.40	0.0041	**Several CDP-DG**, ex: CDP-DG(22:6(4Z,7Z,11E,13Z,15E,19Z)-2OH(10S,17)/i-18:0) (1085.5354, 2, $[M+2K-H]^+$)	CDP-diacylglycerol	Glycerophospholipids
1032.8555	5.70	0.0219			
942.4719	3.51	0.0033	**Several PS**, ex: PS(TXB2/20:4(8Z,11Z,14Z,17Z)) (897.5003, 0, $[M+2Na-H]^+$)	Glycerophosphoserines	Glycerophospholipids
936.2286	3.58	0.0043	**Phenylacetohydroximoyl-glutathione** (436.1058, 1, $[2M+ACN+Na]^+$)	Oligopeptide	Carboxylic acids and derivatives
858.2619	4.56	0.0032			
854.5027	4.10	0.0032	**Several PS**, ex: PS(TXB2/14:0) (821.4690, 0, $[M+CH3OH+H]^+$)	Glycerophosphoserines	Glycerophospholipids
821.5303	3.63	0.0046	**Several PA**, ex: PA(20:4(8Z,11Z,14Z,17Z)-2OH(5S,6R)/i-21:0) (798.5411, 0, $[M+Na]^+$)	Glycerophosphate	Glycerophospholipids
808.7408	2.99	0.0039	**Several TG**, ex: TG(15:0/18:0/14:1(9Z)) (790.7050, 2, $[M+NH4]^+$)	Triacylglycerols	Glycerolipids
327.2681	3.59	0.0025	**Becocalcidiol** or **1-Phenyl-1,3-heptadecanedione** (344.2715, 2, $[M+H-H2O]^+$)	Vitamin D and derivatives Carbonyl compounds	Steroids and derivatives Organooxygen compounds
267.1569	4.36	0.0019	**(±)-1,4-Nonanediol diacetate or 1,11-Undecanedicarboxylic acid or Menthyl ethylene glycol carbonate** (244.1675, 1, $[M+Na]^+$)	Fatty alcohol esters/ Fatty acids and conjugates/ Monoterpenoids	Fatty Acyls Prenol lipids
1006.5254	-2.29	0.0133	**PGP(LTE4/i-20:0)** (1041.5377, 0, $[M+H-2H2O]^+$)	Phospho-glycerophosphate	Glycerophospholipids
929.5135	-1.98	0.0087	**Several PI**, ex: PI(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)) (884.5415, 1, $[M+2Na-H]^+$)	Glycerophosphoinositol	Glycerophospholipids
919.7470	-2.43	0.0163	**Several TG**, ex: TG(15:0/20:5(5Z,8Z,11Z,14Z,17Z)/16:1(9Z)) (836.6894, 3, $[M+2ACN+H]^+$)	Triacylglycerols	Glycerolipids
912.5258	-2.13	0.0164	**Several PI**, ex: PI(18:3(9,11,15)-OH(13)/18:3(9Z,12Z,15Z)) (870.4894, 3, $[M+ACN+H]^+$)/(912.50, 3, $[M+NH4-H2O]^+$)/(894.4894, 3, [M+NH4]+); **Several PE-NMe**, ex: PE-NMe(24:1(15Z)/18:4(6Z,9Z,12Z,15Z)) or **PC**, ex: PC(22:5(7Z,10Z,13Z,16Z,19Z)/18:0) (835.6091, 3, $[M+2K-H]^+$)	Glycerophosphoinositol Glycero-phosphoethanolamines Glycerophosphocholines	Glycerophospholipids
907.5308	-1.94	0.0073	**Several PI**, ex: PI(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)) (884.5415, 0, $[M+Na]^+$)	Glycerophosphoinositol	Glycerophospholipids
881.5816	-1.88	0.0186	**N-Eicosapentaenoyl Phenylalanine** (449.2930, 1, [2M+H-H2O]+); **Several PA**, ex: PA(22:5(7Z,10Z,13Z,16Z,19Z)/22:4(7Z,10Z,13Z,16Z)) (798.520, 1, [M+2ACN+H]+); **Several PE-NMe**, ex: PE-NMe2(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (863.5465, 1, [M+NH4]+ / 839.5465, 1, [M+ACN+H]+)	N-acylamides Glycerophosphate/ Glycero-phosphoethanolamine	Fatty Acyls Glycerophospholipids
837.9603	-1.90	0.0114	**Ganglioside GD2 (d18:0/12:0)** (1591.8457, 4, $[M+2ACN+2H]^+$)	Glycosphingolipids	Sphingolipids
780.0296	-2.31	0.0150			
756.0378	-3.34	0.0242			

485.3255	-2.35	0.0165	**Several PC**, ex: PC(18:1(9Z)-O(12,13)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (845.5571, 0, $[M+3ACN+2H]^+$)	Glycerophosphocholines	Glycerophospholipids

Table S 7 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K3 vitamin, in comparison with control samples, from general extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K3 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1249.6469	4.73	0.0121	**Several PC**, ex: PC(20:4(7E,9E,11Z,13E)-3OH(5S,6R,15S)/2:0) (633.3278, 4, $[2M+H-H2O]^+$)	Glycerophosphocholines	Glycerophospholipids
1208.7731	2.31	0.0093	**Several CL**, ex: CL(11:0/11:0/11:0/18:2(9Z,11Z)) (1166.7375, 1, $[M+ACN+H]^+$)	Glycerophosphoglycerols	Glycerophospholipids
1151.5902	3.17	0.0099	**Several CDP-DG**, ex: CDP-DG(TXB2/i-19:0) (1109.5566, 0, $[M+ACN+H]^+$)	CDP-diacylglycerol	Glycerophospholipids
1049.0172	4.60	0.0019	**Several TG**, ex: TG(i-18:0/i-24:0/22:0) (1030.9867, 3, $[M+NH4]^+$)	Triacylglycerols	Glycerolipids
1033.1215	4.48	0.0030			
959.5078	4.61	0.0029	**Several PGP**, ex: PGP(20:3(8Z,11Z,14Z)-2OH(5,6)/22:5(7Z,10Z,13Z,16Z,19Z)) (958.4972, 3, $[M+H]^+$ / 976.5078, 3, $[M+H- H2O]^+$)	Phospho-glycerophosphate	Glycerophospholipids
911.8023	8.17	0.0332	**Arachidyl carnitine** (455.3975, 0, $[2M+H]^+$)	Fatty acid esters	Fatty Acyls
895.7716	7.34	0.0287	**Several TG**, ex: TG(17:0/18:1(9Z)/18:1(9Z)) (872.7833, 1, $[M+Na]^+$)	Triacylglycerols	Glycerolipids
841.4074	3.07	0.0130			
327.2679	4.13	0.0023	**Becocalcidiol** or **1-Phenyl-1,3-heptadecanedione** (344.2715, 2, $[M+H-H2O]^+$)	Vitamin D and derivatives Carbonyl compounds	Steroids and derivatives Organooxygen compounds
1052.7758	-2.16	0.0172			
1009.6254	-2.80	0.0202	**Several PGP**, ex: PGP(18:1(12Z)-O(9S,10R)/i-22:0) (926.5649, 0, $[M+2ACN+H]^+$)	Phospho-glycerophosphate	Glycerophospholipids
987.7356	-2.90	0.0261	**PC(PGF1alpha/24:0)** (945.7034, 2, $[M+ACN+H]^+$)	Glycerophosphocholines	Glycerophospholipids
909.7732	-1.87	0.0291	**Several TG**, ex: TG(15:0/22:4(7Z,10Z,13Z,16Z)/22:4(7Z,10Z,13Z,16Z)) (944.7833, 3, $[M+H-2H2O]^+$)	Triacylglycerols	Glycerolipids
908.3902	-1.45	0.0231	**PA(LTE4/i-16:0)** (831.4720, 1, $[M+2K-H]^+$)	Glycerophosphate	Glycerophospholipids
900.0516	-3.61	0.0068			
873.5255	-1.90	0.0292	**PG(18:1(12Z)-2OH(9,10)/20:1(11Z))**/**PG(20:1(11Z)/18:1(12Z)-2OH(9,10))** (834.5622, 0, $[M+K]^+$); **LysoPA(0:0/18:1(9Z))**/ **DHAP(18:0)** (436.2590, 0, $[2M+H]^+$)	Phosphatidylglycerol/ lysoGlycerophosphate Carbonyl compounds	Glycerophospholipids Organooxygen compounds
855.8229	-2.36	0.0175			
763.4058	-3.22	0.0286	**PA(20:2(11Z,14Z)/15:0)** (686.4887, 2, $[M+2K-H]^+$)	Glycerophosphate	Glycerophospholipids
688.3939	-1.75	0.0257			

Table S 8 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to OH-PhQ vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

OH-PhQ TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
2417.6934	5.39	0.0183			
1656.2165	5.59	0.0481	**Several PC**, ex: PC(20:3(8Z,11Z,14Z)-2OH(5,6)/P-18:0) (827.6040, 1, $[2M+H]^+$)	Glycerophosphocholines	Glycerophospholipids
1325.9446	5.56	0.0061	**Several CL**, ex: CL(14:0/18:0/16:0/14:0) (1324.9409, 3, $[M+H]^+$)	Glycerophosphoglycerols	Glycerophospholipids
934.6998	3.28	0.0002	**Several PE-NMe**, ex: PE-NMe2(22:2(13Z,16Z)/20:2(11Z,14Z)) (851.6404, 1, $[M+2ACN+H]^+$)	Glycero-phosphoethanolamines	Glycerophospholipids
804.6860	3.18	0.0002	**PC(O-18:0/20:0)** (803.6768, 2, $[M+H]^+$)	Glycerophosphocholines	Glycerophospholipids
672.6181	4.02	9.1e-05			
640.5068	5.69	0.0017	**Abietinol** (288.2453, 1, $[2M+ACN+Na]^+$) or **several PE**, ex: PE(P-18:0/14:0) (675.5203, 1, $[M+H-2H2O]^+$)	Diterpenoids Glycero-phosphoethanolamines	Prenol lipids Glycerophospholipids
621.6671	3.93	2.9e-05			
619.6227	5.04	0.0035			
617.5228	5.03	0.0046	**(4E,14Z)-2-Aminooctadeca-4,14-diene-1,3-diol** or **Palmitoleoylethanolamide** (297.2668, 0, $[2M+Na]^+$)	Amines	Organonitrogen compounds
467.3508	3.28	0.0002	Several TG, ex: **TG(15:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/20:5(5Z,8Z,11Z,14Z,17Z))** (910.7050, 0, $[M+H+Na]^+$)	Triacylglycerols	Glycerolipids
2204.9409	-2.02	0.0029			
1686.2109	-5.52	0.0071	**Several PA**, ex: PA(20:3(8Z,11Z,14Z)-2OH(5,6)/i-24:0) (842.6037, 2, $[2M+H]^+$)	Glycerophosphates	Glycerophospholipids
1501.6116	-7.16	0.0004			
1485.5093	-6.57	0.0024			
1188.8759	-9.45	0.0014	**Menaquinone 6** (580.4280, 3, $[2M+2H+3H2O]^+$)	Sesterterpenoids	Prenol lipid
1161.3302	-6.13	0.0075			
1137.0502	-3.37	0.0017	**Several TG**, ex: TG(i-24:0/21:0/i-22:0) (1073.0337, 1, $[M+ACN+Na]^+$)	Triacylglycerols	Glycerolipids
1058.1166	-2.99	0.0032			
733.5906	-2.09	0.0028			
541.1247	-2.71	0.0013	**Estriol-17-glucuronide/ 15-Hydroxynorandrostene-3,17-dione glucuronide/ Estriol-3-glucuronide/ Estriol-16-Glucuronide** (464.2046, 2, $[M+2K-H]^+$)	Steroidal glycosides	Steroids and derivatives

Table S 9 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K1 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K1 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1543.1661	5.14	0.0005			
1492.5972	5.71	0.0228			
1487.5248	6.64	0.0482			
1450.0857	5.07	0.0161			
907.5211	5.55	0.0164	**Several PE**, ex: PE(20:4(8Z,11Z,14Z,17Z)-2OH(5S,6R)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) (843.5050, 0, $[M+ACN+Na]^+$) or **several PGP**, ex: PGP(18:3(9Z,12Z,15Z)/16:0) (824.4605, 0, $[M+2ACN+H]^+$)	Glycero-phosphoethanolamines Glycerophospho-glycerophosphates	Glycerophospholipids
879.5849	2.90	0.0007	**Several PC**, ex: PC(20:4(8Z,11Z,14Z,17Z)-2OH(5S,6R)/DiMe(9,5)) or **PS**, ex: PS(20:4(5Z,7E,11Z,14Z)-OH(9)/22:2(13Z,16Z)) (879.5625, 0, $[M+NH4-H2O]^+$); **Several PA**, ex: PA(24:1(15Z)/22:4(7Z,10Z,13Z,16Z)) (834.6139, 0, $[M+2Na-H]^+$)	Glycerophosphocholines Glycerophosphoserines Glycerophosphates	Glycerophospholipid
489.3337	3.00	0.0006	**Vitamin K1 2,3-epoxide** (466.3447, 0, $[M+Na]^+$)	Quinone and hydroquinone lipids	Prenol lipid
473.3390	3.92	0.0009	**Vitamin K1** (450.3498, 0, $[M+Na]^+$)		
451.3568	2.96	0.0006	**Vitamin K1** (450.3498, 0, $[M+H]^+$)		
228.3215	3.11	0.0004			
1991.9123	-5.53	0.0077	**Several CDP-DG**, ex: CDP-DG(5-iso PGF2VI/i-14:0) (995.4521, 0, $[2M+H]^+$) or **several PIP**, ex: PIP(5-iso PGF2VI/18:2(9Z,12Z)) (984.4613, 0, $[2M+Na]^+$)	CDP-diacylglycerol Glycerophosphoinositol phosphates	Glycerophospholipid
1633.2922	-5.95	0.0048			
1632.1957	-2.39	0.0086	**SM(d18:0/PGF1alpha)** (804.5993, 5, $[2M+Na]^+$)	Phosphosphingolipids	Sphingolipids
1599.1587	-2.89	0.0143	Several SM, ex: **SM(d19:1/20:5(6E,8Z,11Z,14Z,17Z)-OH(5))** (790.5625, 0, $[2M+NH4]^+$)	Phosphosphingolipids	Sphingolipids
1345.9587	-8.32	0.0245	**Several CL**, ex: CL(i-15:0/i-16:0/i-16:0/18:2(9Z,11Z)) (1362.9566, 4, $[M+H-H2O]^+$) **or several PA**, ex: PA(18:1(9Z)-O(12,13)/P-16:0) (672.4730, 4, $[2M+H]^+$)	Glycerophosphoglycerols Glycerophosphates	Glycerophospholipid
1342.0658	-3.28	0.0031			
1056.7334	-2.89	0.0134	**35S-Methylokadaic acid 7-hexadecanoate** (1056.7113, 1, $[M+NH4-H2O]^+$)	Fatty acid esters	Fatty Acyls
1043.7226	-1.93	0.0081	**Several TG**, ex: TG(22:5(4Z,7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/O-18:0) (966.8040, 0, $[M+2K-H]^+$)	Triacylglycerols	Glycerolipids
906.6184	-2.19	0.0083	**Several PC**, ex: PC(18:1(12Z)-2OH(9,10)/22:3(10Z,13Z,16Z)) or **PE**, ex: PE(20:3(8Z,11Z,14Z)-2OH(5,6)/24:1(15Z)) (883.6302, 1, $[M+Na]^+$)/ (861.6459, 1, $[M+2Na-H]^+$)	Glycerophosphocholines Glycerophospho-ethanolamines	Glycerophospholipid
566.8889	-2.04	0.0061			

Table S 10 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K2 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K2 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
1526.1966	4.56	4.1e-05			
1216.8208	2.36	0.0009			
1197.3455	5.05	0.0053			
1097.0196	3.06	0.0012	**Several TG**, ex: TG(20:3(5Z,8Z,11Z)/24:0/24:1(15Z)) (1078.9867, 1, $[M+NH4]^+$)	Triacylglycerols	Glycerolipids
741.2443	9.08	0.0044			
581.3645	2.09	0.0031	**(N-Acetylglucosaminyl)2-diphosphodolichol** (1675.1192, 4, $[M+3Na]^+$)	Polyprenol	Prenol lipid
505.3290	2.33	0.0028	**Several PA**, ex: PA(8:0/i-15:0) (522.3322, 1, $[M+H-H2O]^+$)	Glycerophosphates	Glycerophospholipid
483.2880	6.03	0.0009	**Menatetrenone Epoxide** (460.2977, 2, $[M+Na]^+$)	Quinone and hydroquinone lipids	Prenol lipid
449.3410	2.37	0.0028	**Vitamin K1 2,3-epoxide** (466.3447, 2, $[M+H-H2O]^+$)		
443.2940	4.50	0.0022	**Menatetrenone Epoxide** (460.2977, 2, $[M+H-H2O]^+$)		
1796.5906	-1.36	0.0069	**1-Tetradecanoyl-2-(9Z,12Z-octadecadienoyl)-3-docosanoyl-sn-glycerol** or **several TG**, ex: TG(16:0/18:1(11Z)/20:1(11Z)) (886.7989, 2, $[2M+Na9^+$)	Triacylglycerols	Glycerolipids
1533.2042	-3.34	0.0038			
1508.4997	-5.14	0.0005			
1252.0833	-3.35	0.0002			
1165.8175	-5.11	0.0077	**(3S,3'R,5R,6R)-7',8'-Didehydro-3,6-epoxy-5,6-dihydro-beta,beta-carotene-3',5-diol** (582.4073, 4, $[2M+H]^+$)	Triterpenoids	Prenol lipids
857.7955	-3.55	0.0004	**Several TG**, ex: TG(15:0/O-18:0/20:3(5Z,8Z,11Z)) (856.7884, 0, $[M+H]^+$)	Triacylglycerols	Glycerolipids
760.7156	-2.74	0.0005	**Ganglioside GT1c/GT1b** (d18:0/24:0) (2213.1818, 1, $[M+3Na]^+$) or **Several DG/TG**, ex: DG(22:1n9/0:0/24:1n9) / TG(14:1(9Z)/14:1(9Z)/O-18:0) (760.6945, 2, $[M+NH4-H2O]^+$)	Glycosphingolipids Diacylglycerols/ Triacylglycerols	Sphingolipids Glycerolipids
723.0493	-5.33	0.0057			
544.8932	-2.80	0.0002	**Deoxyuridine triphosphate** (467.9736, 1, $[M+2K-H]^+$)	Pyrimidine deoxyribonucleotides	Pyrimidine nucleotides
429.3724	-3.33	7.1e-05	**Cholesteryl acetate or 3-Hydroxystigmast-5-en-7-one/ 6beta-Hydroxystigmast-4-en-3-one/ 5alpha-Stigmastan-3,6-dione** or **4alpha-Hydroxymethyl-4beta-methyl-5alpha-cholesta-8,24-dien-3beta-ol** (428.3654, 1, $[M+H]^+$)	Steroid esters/ Stigmastanes and derivatives Triterpenoids	Steroids and derivatives Prenol lipids

Table S 11 Identification of statistically significant and enriched metabolites from exposed zebrafish larvae to K3 vitamin, in comparison with control samples, from targeted extraction. Is shown the putative identification (Δ ppm < 5) chemical taxonomy (class and subclass) of the top 20 statistically significant and enriched compounds (p-value ≤ 0.05, with inclusion of all log2FC ≥ 5).

K3 TREATMENT					
m/z	Log2FC	*p-value*	Putative identification (*m/z*, Δ ppm, adduct)	Chemical Taxonomy	
				Sub-Class	Class
2003.9208	5.91	0.0042			
1970.9402	5.66	0.0169	**PGP(LTE4/i-15:0)** (971.4595, 1, $[2M+2H+3H2O]^+$)	Phospho-glycerophosphate	Glycerophospholipid
1463.1588	6.61	0.0254	**Several PC**, ex: PC(O-16:0/16:1(9Z)) (717.5672, 1, $[2M+2H+3H2O]^+$)	Glycerophosphocholines	Glycerophospholipid
911.5614	4.06	0.0007	**Several PG**, ex: PG(20:4(7E,9E,11Z,13E)-3OH(5S,6R,15S)/i-21:0) (888.5728, 1, $[M+Na]^+$)	Phosphatidylglycerol	Glycerophospholipids
801.5429	3.01	0.0005	**Several PA**, ex: PA(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:2(13Z,16Z)) (800.5356, 0, $[M+H]^+$)	Glycerophosphates	Glycerophospholipids
763.6599	7.58	0.0014	**1-Tetradecanoyl-2-(9Z-tetradecenoyl)-3-(8Z,11Z,14Z-eicosatrienoyl)-sn-glycerol** or **several TG**, ex: TG(16:0/14:1(9Z)/18:3(9Z,12Z,15Z)) (798.6737, 1, $[M+H-2H2O]^+$)	Triacylcglycerols	Glycerolipids
612.5562	2.19	0.0002	**Several DG**, ex: DG(18:0/0:0/16:1n7) (594.5223, 0, $[M+NH4]^+$)	Diracylglycerols	Glycerolipids
525.3289	3.00	0.0006	**1-Stearoylglycerophosphoserine** or **LysoPS(18:0/0:0)** (525.3067, 1, $[M+NH4-H2O]^+$)	Glycerophosphoserine lyso-Glycerophosphoserine	Glycerophospholipids
483.2878	4.94	0.0012	**PC(LTE4/20:4(8Z,11Z,14Z,17Z))** (964.5612, 0, $[M+2H]^+$) or **several long-chain FA**, ex: tetradeca-2,4,6-trienoic acid (222.1620, 1, $[2M+K]^+$) or **MG(0:0/24:6(6Z,9Z,12Z,15Z,18Z,21Z)/0:0)** or **5,9-Epidioxyergosta-7,22-diene-3,6-dio**l/ **5,6-Epoxyergosta-8,22-diene-3,7,14-triol**/ **5,9-Epidioxy-3-hydroxyergost-7-en-6-one** (444.3240, 1, $[M+K]^+$)	Glycerophosphocholines Fatty acids and conjugates Monoacylglycerols Ergostane steroids	Glycerophospholipids Acyls Glycerolipids Steroids and derivatives
255.1930	2.99	0.0005			
1531.4928	-5.13	0.0073			
1501.6112	-7.77	0.0005			
1252.0833	-3.57	0.0070			
1188.8762	-5.71	0.0045	**Menaquinone 6** (580.4280, 3, $[2M+2H+3H2O]^+$)	Sesterterpenoids	Prenol lipids
1150.9275	-2.12	0.0026			
1021.0217	-4.54	0.0015			
802.5925	-3.32	0.0042	**Several PC**, ex: PC(18:1(12Z)-2OH(9,10)/P-18:1(9Z)) (801.5884, 4, $[M+H]^+$) or **several PA**, ex: PA(i-21:0/20:3(6,8,11)-OH(5)) (784.5618, 4, $[M+NH4]^+$)	Glycerophosphocholines Glycerophosphates	Glycerophospholipids
735.5959	-2.12	0.0024	**N-Myristoyl Lysine** (356.3039, 2, $[2M+Na]^+$)	Peptide	Carboxylic acids and derivatives
722.3889	-1.99	0.0041			
255.1929	-2.23	0.0021			