Technical Bulletin

Non-Polar Metabolites QC Mix

Catalogue number SBR00073

Product Description

Metabolomics is the profiling study of metabolites that are usually small molecules from biochemical processes and pathways[1,2]. Metabolites are mostly characterized from samples of stool[3], serum/plasma[4], urine[5], cerebrospinal fluid[6], and saliva[7]. Metabolomics deals with diverse areas like microbiome[8], nutrition[9], diseases[10] and agriculture[11]. Generally, metabolites are analyzed by two main approaches: Targeted[12,13] and untargeted[14,15] metabolomics. Targeted metabolomics[12,13] is the analysis of known specific chemical group metabolites like short chain fatty acids[16], bile acids, lipids[17] and amino acids[18]. Whereas untargeted metabolomics[14,15] is analysis of all unknown chemical compounds in one sample.

Liquid chromatography-mass spectrometry (LC-MS) is the main method for metabolite profiling in metabolomics analysis[19,20]. In order to assess any variations in the LC-MS-based metabolomics analysis data, it is recommended to add an additional QC (quality control) sample at the beginning of every analytical experiment batch and should be injected every 4-10 injections in to the workflow steps[19,21,22]. The QC sample evaluates any drifting phenomenon like intensity values, ion suppression or any changes in the retention time of the peaks in the LC-MS data.
Here we offer a ready to use Non Polar Metabolites QC Mix in a solution of ~2:1:1 IPA:ACN:H_2O for mass spectroscopy workflows. By utilizing the QC mix, the user will be able to assess drifting and ion suppression phenomena. The Non-Polar Metabolites QC Mix is comprised of 9 components (see Table 1). The Non-Polar metabolites mix contains hydrophobic type of metabolites such as, cholesterol derivatives, unsaturated fatty acids, phospholipids and ceramides.

Components

Table 1.

Components in the Non-Polar Metabolites QC Mix

No.	Metabolite name	Empirical Formula	Exact mass	Concentration (µg/mL)	Concentration (µM)
1	Progesterone	$C_{21}H_{30}O_2$	314.2246	2	6.4
2	D-Sphingosine	$C_{18}H_{37}NO_2$	299.2824	10	33.4
3	1-Oleoyl-sn-glycero-3-phosphocholine (LysoPC(18:1(9Z)/0:0))	$C_{26}H_{52}NO_7P$	521.3481	2.5	4.8
4	Linolenic acid	$C_{18}H_{30}O_2$	278.2245	20	71.8
5	cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA)	$C_{22}H_{32}O_2$	328.2402	5	15.2
6	Arachidonic acid sodium salt	$C_{20}H_{32}O_2$	304.2402	10	30.6
7	Sodium cholesteryl sulfate	$C_{27}H_{46}O_4S$	466.3116	10	20.5
8	N-palmitoyl-D-sphingosine (C16 ceramide (D18:1/16:0))	$C_{34}H_{67}NO_3$	537.5121	2	3.7
9	1,2-dioleoyl-sn-glycero-3-phosphocholine (18:1 (Δ9-Cis) PC (DOPC)	$C_{44}H_{84}NO_8P$	785.5934	1	1.3

Reagents and Equipment Required but Not Provided

- Column:Agilent® Eclipse plus-C8, 95 Å,RRHD 1.8um 2.1x150mm
- Ammonium formate Cat#70221
- Acetonitrile Cat# 1.00029
- Isopropanol Cat# 1.02781

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the safety data Sheet for information regarding hazards and safe handling practices.
The product is obtained in format of crimp top (Silicone/PTFE liner) amber vial which suitable for use with most of the LC-MS autosamplers.

Storage/Stability

The product is shipped at ambient temperature. Store at -20°C upon receipt.

Preparation Instructions

The Non-Polar Metabolites QC Mix is a ready to inject solution supplied in a crimp top (Silicone/PTFE liner) amber vial 220µl.

Procedure

Mass Spectrometry Conditions: Instrument: Bruker™ Q-Tof Impact II

Source Type: ESI

Method A:

Ion Polarity: Positive

Capillary: 4500 V

Nebulizer: 2.2 Ba0072

Dry gas temperature: 200 °C

Dry gas: 8L/min

Method B: Ion Polarity: Negative

Capillary: 5500 V

Nebulizer: 2.2 Bar

Dry gas temperature: 220 °C

Dry gas: 8L/min

LC Conditions:

Column: Column oven temperature: 55 °C

Flow rate: 0.5 mL/min

Eluent A: 10mM Ammonium formate in water + 0.1% formic acid

Eluent B: 85% ACN, 10% IPA, 5% water + 10mM ammonium formate + 0.1% formic acid.

Injection volume: Method A (ESI+) - 1 µL

Method B (ESI-) - 2 °L

Gradient: See Table 2

Table 2:

Gradient elution time is shown below for Eluent A & B.

Time (min)	A [%]	B [%]
0	47	53
2	47	53
6	35	65
16	35	65
20	0	100
27	0	100
30	47	53
35	47	53

Figure 1:

Method A: Extracted ion chromatogram (EIC) of MS ESI+ of SBR00073.

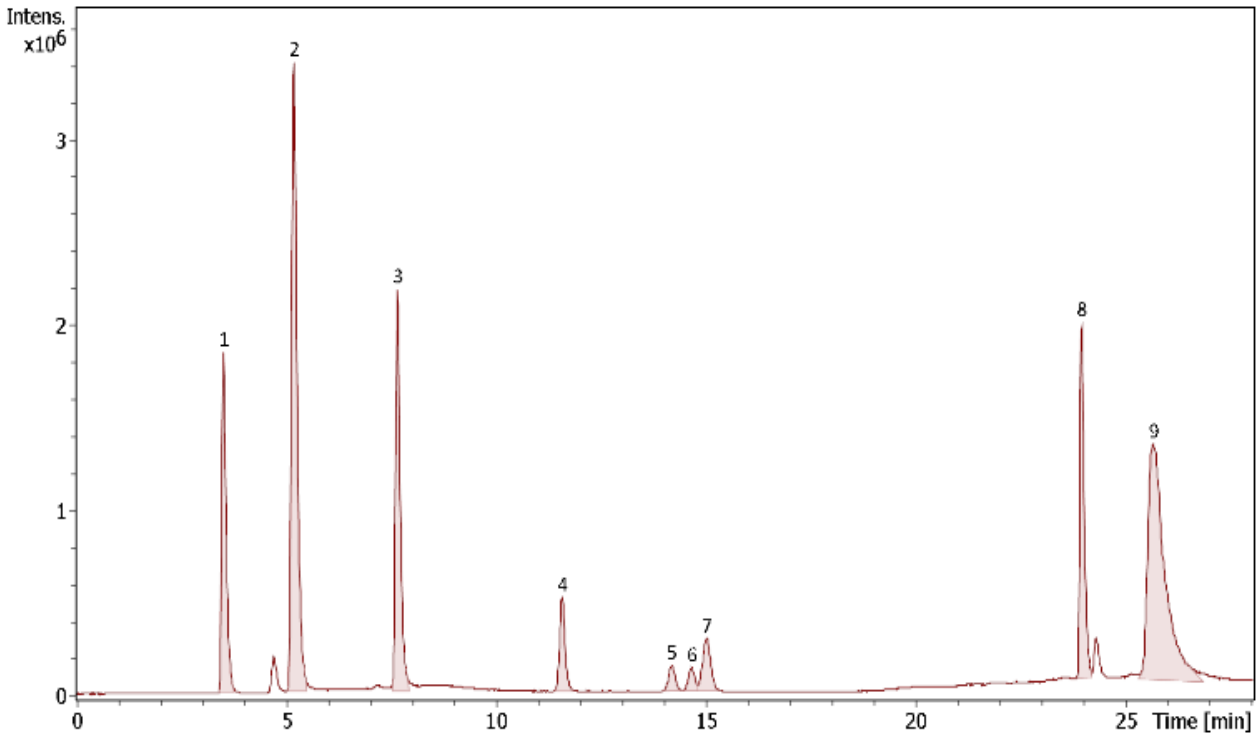
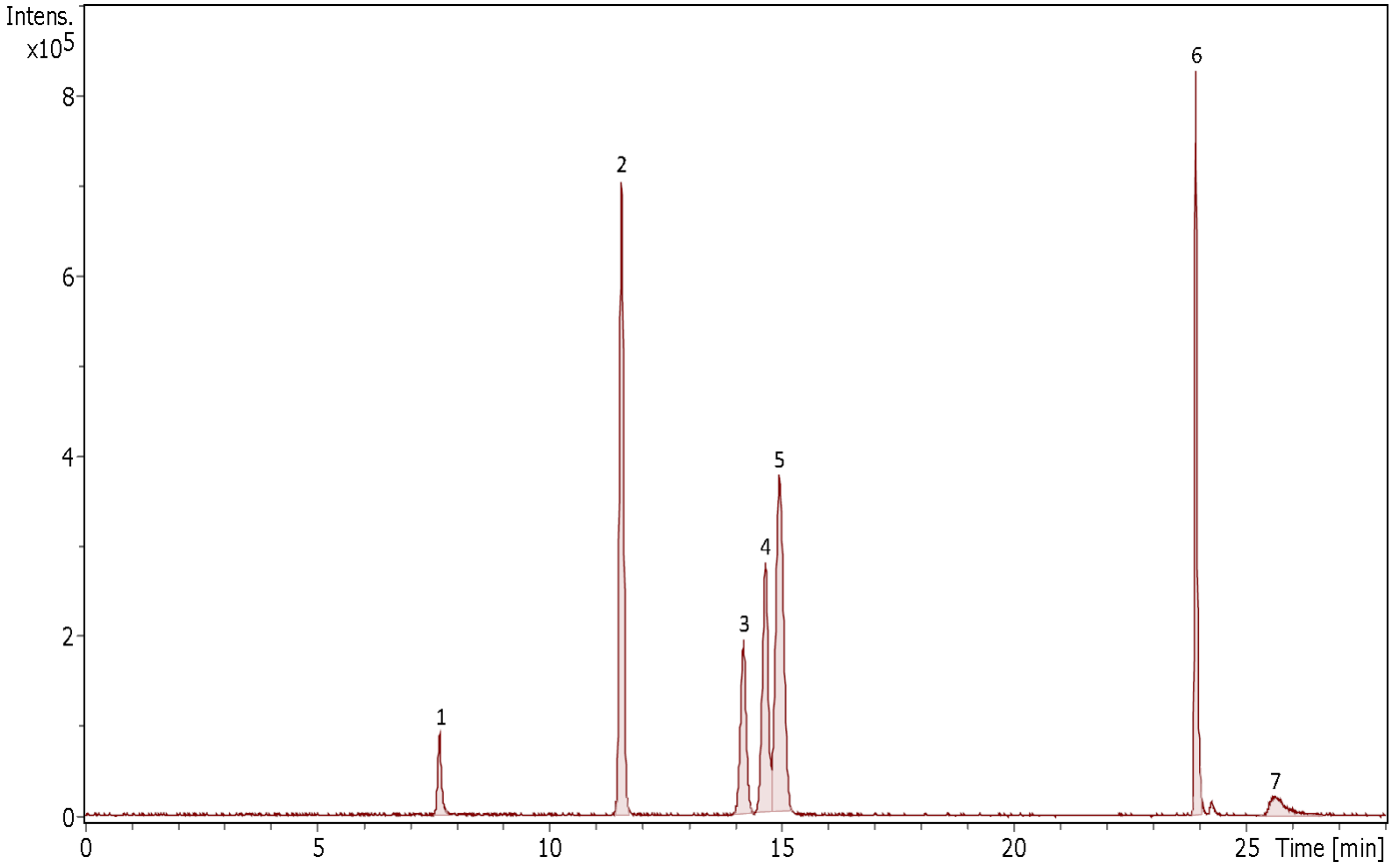


Table 3:

List of metabolites corresponding to the retention times in figure 1 are shown.

Peak no.	Metabolite name	Rt (min)
1	Progesterone	3.5
2	D-Sphingosine	5.2
3	LysoPC(18:1(9Z)/0:0)	7.7
4	Linolenic acid	11.6
5	DHA	14.2
6	Arachidonic acid	14.7
7	Cholesterol sulfate	15.0
8	C16 ceramide (D18:1/16:0)	23.9
9	18:1 (Δ9-Cis) PC (DOPC)	25.7

Figure 2: Method B: Extracted ion chromatogram (EIC) of MS ESI- of SBR00073



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