

1 Supporting Information

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3 **Prebiotic Sugar Formation Under Nonaqueous**
4 **Conditions and Mechanochemical Acceleration**

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25 **1. Experimental Procedures**

26 *1.1 Instrumentations:*

27 1.1.1 Vortex mixer

28 The vortex mixer Lab Dancer by VWR (VWR International GmbH, Darmstadt, Germany) was
29 used for mixing solids.

30 1.1.2. Ball milling

31 For mechanochemically promoted reactions, the oscillatory ball mill *CryoMill* by Retsch (Retsch
32 GmbH, Haan, Germany) was employed equipped with an adapter to fit up to four 5 mL stainless
33 steel ball mill jars each filled with one 7 mm stainless steel ball. In addition, the planetary ball mill
34 *Pulverisette 7* by Fritsch (Fritsch GmbH, Idar-Oberstein, Germany) was used with two 20 mL
35 stainless steel grinding bowls equipped with gassing lids and filled with ten 10 mm stainless steel
36 balls.

37 1.1.3. Infrared temperature sensor

38 Temperatures of reaction mixtures within the ball milling jar were determined immediately
39 after stopping the grinding and usage of the infrared temperature sensor VOLTCRAFT IR 260-8S by
40 Conrad (Hirschau, Germany).

41 1.1.4. Gas chromatography

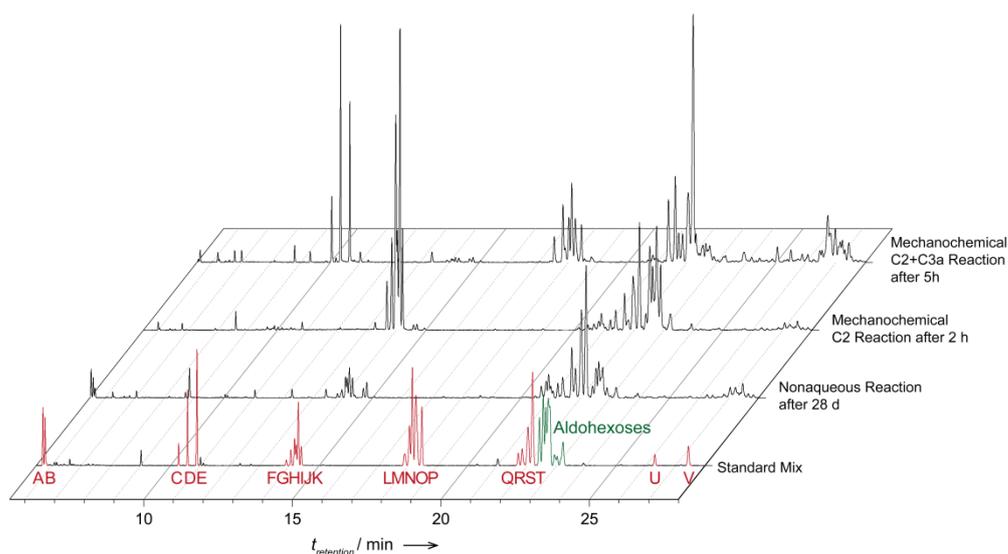
42 GC analysis of carbohydrate mixtures was performed on a TraceGC Ultra system coupled to
43 either a PolarisQ MS (quadrupole-ion mass spectrometer [MS]) (Thermo Scientific, San Jose,
44 California, USA) operated by Xcalibur software 1. SR1. Injections used a split/splitless injector in
45 split mode at 250 °C. Flame ionization detection was co-recorded with MS data and operated under
46 carbon-correction at 250 °C.

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48 2. Data Evaluation

49 2.1 Identification of Carbohydrates

50 Individual carbohydrates and bulks were detected and relatively quantified using the protocol
 51 by Trapp and co-workers.[1] Identification is based on retention times and MS spectra of standard
 52 mixtures. Exemplary chromatograms for each of the presented reactions in comparison to the
 53 standard mix are given in Figure S1. Due to possible *E/Z* isomerism, carbohydrate analytes can show
 54 two signals.
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56 **Figure S1.** Comparative GC chromatograms for the presented reactions and the standard mix. For
 57 method details see experimental section. The given letters denote the following carbohydrates: A/B)
 58 glycolaldehyde (C2), C/D) glyceraldehyde (C3a), E) dihydroxyacetone (C3b), F/H) erythrose, G/I)
 59 threose, I/K) erythrulose, L/M) xylose, L/N) lyxose, N) arabinose, O) xylulose, O) ribulose, P) ribose,
 60 Q/S) tagatose, R/S) psicose, T) sorbose, T) fructose, U/V) galactoheptose. **Ketohexoses (Q,R,S,T)** and
 61 **aldohexoses** can be identified by their characteristic fragments: 319 m/z and 379 m/z for aldoses and
 62 ketoses, respectively.

63 Distinct C3 and C4 constitutional carbohydrate isomers can be identified unambiguously.
 64 Observed C3 carbohydrates are glyceraldehyde (C3a), dihydroxyacetone (C3b); C4 carbohydrates
 65 are erythrose, threose, erythrulose. Due to overlapping peaks, not all isomers of C5, C6 and C7 can
 66 be unequivocally distinguished. From comparison with the unbranched standards, it is likely,
 67 though, that the mixtures contain all known unbranched carbohydrates.

68 2.2 Effective carbon number (ECN)

69 To account for different response factors, the effective carbon numbers of monosaccharides and
 70 the internal standard were calculated from literature values.[2]
 71
 72

Table 1. Calculated ECN values of monosaccharides.

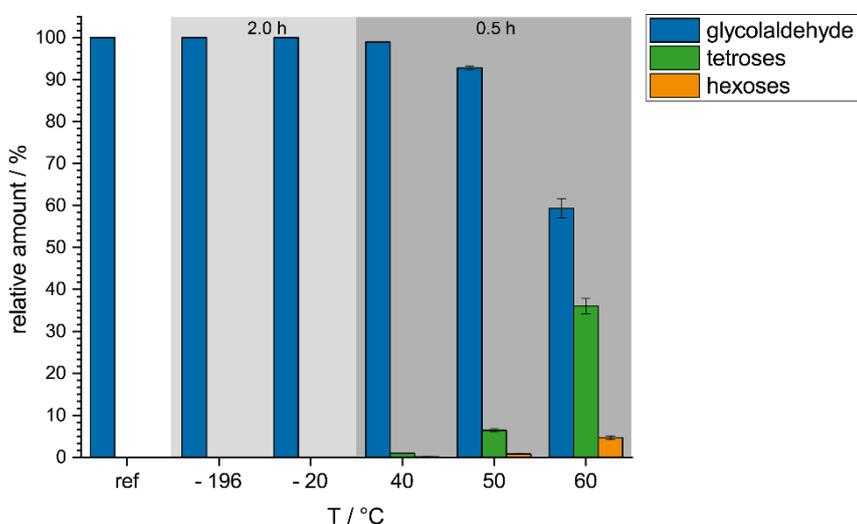
Compound	ECN
glycolaldehyde	5.29
trioses	8.98
tetroses	12.67
pentoses	16.36
hexoses	20.05
phenyl- β -D-glucopyranoside	20.76

73 2.3 Determination of Relative Reaction Rates

74 Using OriginPro 2018G the kinetic data was fitted using linear or cubic polynomial functions.
75 The reaction rates were determined as the first derivative of the molar amount over time for selected
76 time periods.

77 3. Temperature Dependence for Sugar Formation under Nonaqueous Conditions

78 The reaction rate of the sugar formation under nonaqueous conditions is temperature
79 dependent. We investigated the conversion for different temperatures and times. The results are
80 given in Figure S2. Higher temperatures facilitate the successive aldol reactions for the formation of
81 higher carbohydrates in a shorter time. Therefore, it is necessary to freeze samples in liquid nitrogen
82 until they can be derivatized and analyzed by GC-MS. For aldol or formose reactions performed in
83 water, the authors, therefore, want to highlight that bias is introduced to samples when employing
84 lyophilization during work-up procedures. Under such conditions, further reaction is possible due
85 to presence of a basic catalyst.

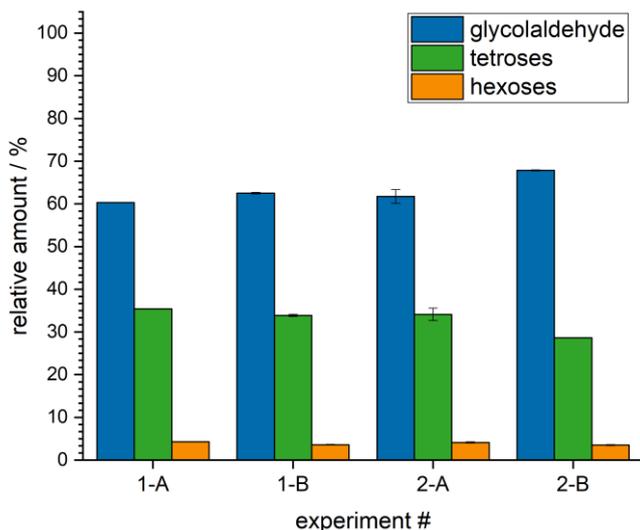


86

87 **Figure S2.** Product distribution of carbohydrate formation under nonaqueous conditions for
88 different temperatures and times. Whereas already after 0.5 h reaction progress can be determined
89 for elevated temperatures, low temperatures do not show any conversion even after 2.0 h. Samples,
90 thus, can be stored for some time under low temperatures. In contrast to the general derivatization
91 protocol described in the experimental procedure section, hydroxylamine hydrochloride was used
92 instead of *O*-ethylhydroxylamine hydrochloride for the experiments of two-hour reaction time.

93 4. Repeatability Study

94 All experiments were performed in parallel repetitions. The following data depicts the results of
95 two sets (1 and 2) of one reaction performed one week apart and analyzed with two separately
96 prepared derivatization solutions (A and B). Each GC measurement was run twice. The deviations of
97 observed areas correspond to the error bars. For reaction conditions see reaction setup #2 of the
98 experimental section. Reaction times were 60 min. Based on the repeatability study, we assess the
99 percentage error of the relative frequency of carbohydrates to be at maximum 5 %.
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Figure S3. Repeatability study for one reaction performed twice each time one week apart (denoted as 1 and 2) and analyzed with two separately prepared derivatization solutions (A and B). Each GC analysis was run twice. Deviations of areas correspond to error bars. Reaction conditions of the experiment studied are described in the experimental section under reaction setup #2. Reaction times were 60 min.

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5. Catalyst Loading

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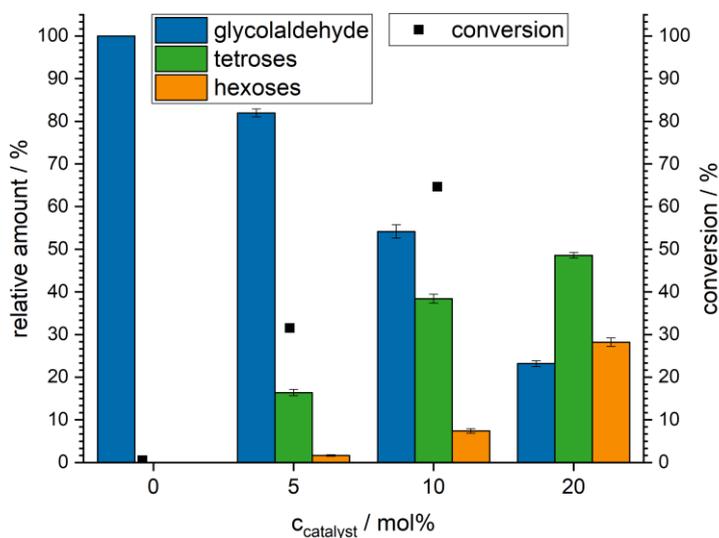
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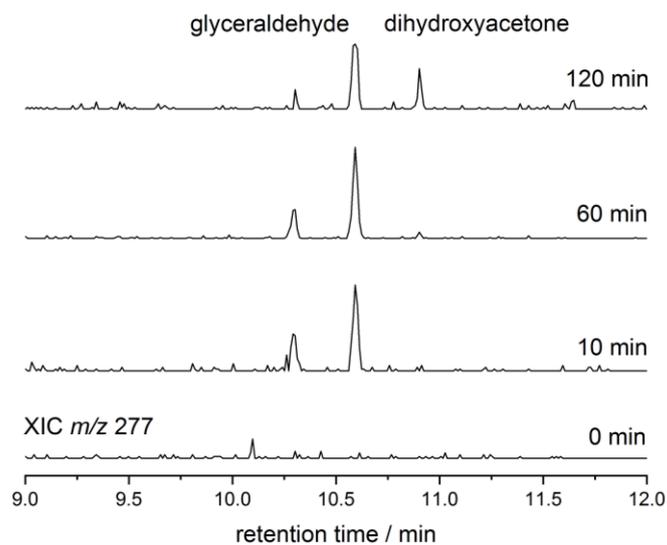
We investigated the catalyst loading in terms of product distribution and substrate consumption (C2 – glycolaldehyde) under conditions given in the experimental section for reaction setup #2. Reaction times were 90 min. Our investigations show that the reaction rate is dependent on the catalyst loading. We, therefore, argue that even small amounts of $\text{Ca}(\text{OH})_2$ are sufficient to trigger the aldose reactions allowing access to the formose reaction network of more complex sugars. It can be envisioned that the slower generation of higher carbohydrates gives way to specific serial reactions with other reactants like amines. In our studies presented here we characteristically employ catalyst loading typical of the formose reaction studied in literature to allow for comparability. Our data, further, implies that with 20 mol% catalyst loading the C2 consumption reaches saturation.



118 **Figure S4.** Dependency of product distribution and substrate consumption (C2 – glycolaldehyde) on
119 catalyst loading. Catalyst loading is in reference to glycolaldehyde monomer.

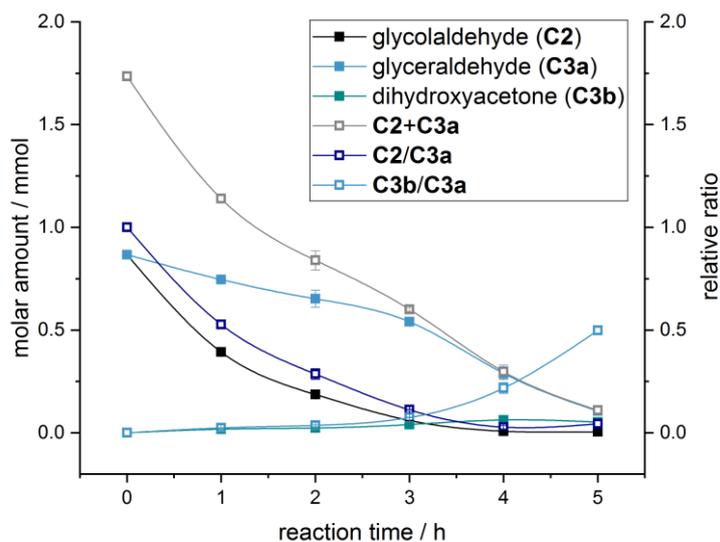
120 6. Occurrence and isomerization of trioses

121 When reactions with the C2 building block were conducted, due to retroaldol reactions of the
122 formed monosaccharides, also trioses were detected in trace amounts. In **figure S5** is the extracted
123 ion chromatogram (M^{+} : m/z 277) in the typical area of trioses depicted. Over time glyceraldehyde
124 was formed and after 120 min the isomerization to dihydroxyacetone was observed. Further
125 reactions of these built up heptoses.



126 **Figure S5:** Extracted ion chromatogram (m/z 277) of the triose area after different reaction times of
127 C2+Ca(OH)₂ reaction in the oscillatory ball mill.
128

129 In **Figure S6** differentiated examination of trioses and the comparable interaction of
130 glycolaldehyde, glyceraldehyde and dihydroxyacetone are depicted.



131 **Figure S6:** Molar amount of glycolaldehyde and trioses as well as the relative amount of
132 glycolaldehyde or dihydroxyacetone with glyceraldehyde up to 5 h of C2+C3a+Ca(OH)₂ reaction in
133 the oscillatory ball mill.
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136 **7. Tabular Data for Depicted Figures**

137 **Table 2.** Relative amounts of sugar mixtures starting from glycolaldehyde with different mineral
 138 catalysts or pure calcium hydroxide as base used in mechanochemical sugar formation (**Figure 1**).¹
 139 Error analysis is based on double determinations.

mineral	glycolaldehyde [%]	tetroses [%]	hexoses [%]
brucite	92.7 ± 1.0	6.8 ± 0.9	0.5 ± 0.1
montmorillonite	85.3 ± 2.6	14.2 ± 2.7	0.6 ± 0.2
portlandite	44.9 ± 1.3	42.9 ± 1.0	12.2 ± 0.4
pure calcium hydroxide	35.8 ± 4.7	50.5 ± 3.0	13.7 ± 1.8

140 ¹ Oscillatory ball mill, 30 Hz, 90 min, 20 mol% catalyst.

141 **Table 3.** Molar amounts of sugar mixtures starting from glycolaldehyde with 20 mol% calcium
 142 hydroxide in mechanochemical sugar formation (**Figure 2**).¹ Error analysis is based on double
 143 determinations.

time [min]	glycolaldehyde [mmol]	tetroses [mmol]	hexoses [mmol]	heptoses [mmol]
0	2.08 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.5	1.88 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	1.66 ± 0.00	0.14 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7.5	1.36 ± 0.01	0.26 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
10	0.99 ± 0.20	0.45 ± 0.06	0.04 ± 0.01	0.00 ± 0.00
20	0.76 ± 0.10	0.54 ± 0.05	0.05 ± 0.01	0.00 ± 0.00
30	0.59 ± 0.04	0.57 ± 0.02	0.07 ± 0.00	0.00 ± 0.00
60	0.41 ± 0.04	0.62 ± 0.00	0.13 ± 0.01	0.00 ± 0.00
90	0.31 ± 0.03	0.59 ± 0.00	0.18 ± 0.01	0.01 ± 0.00
120	0.08 ± 0.01	0.42 ± 0.01	0.27 ± 0.01	0.01 ± 0.00

144 ¹ Oscillatory ball mill, 30 Hz.

145 **Table 4.** Molar amounts of sugar mixtures starting from glycolaldehyde and glyceraldehyde with
 146 20 mol% calcium hydroxide in mechanochemical sugar formation (**Figure 3**).¹ Error analysis is based
 147 on double determinations.

time [h]	glycolaldehyde [mmol]	trioses [mmol]	tetroses [mmol]	pentoses [mmol]	hexoses [mmol]	heptoses [mmol]
0	0.87 ± 0.00	0.87 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	0.39 ± 0.01	0.76 ± 0.00	0.16 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
2	0.19 ± 0.01	0.68 ± 0.04	0.19 ± 0.01	0.09 ± 0.02	0.04 ± 0.01	0.02 ± 0.00
3	0.06 ± 0.00	0.58 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.11 ± 0.00	0.05 ± 0.00
4	0.01 ± 0.00	0.35 ± 0.03	0.03 ± 0.00	0.13 ± 0.00	0.22 ± 0.01	0.08 ± 0.00
5	0.00 ± 0.00	0.16 ± 0.00	0.01 ± 0.00	0.14 ± 0.00	0.29 ± 0.00	0.01 ± 0.00

148 ¹ Oscillatory ball mill, 30 Hz.

149

150 **Table 5.** Molar amounts of ribose and relative ratio of ribose relating to all pentoses during the
 151 reaction starting from glycolaldehyde and glyceraldehyde with 20 mol% calcium hydroxide in
 152 mechanochemical sugar formation (**Figure 4**).¹ Error analysis is based on double determinations.

time [h]	ribose [μmol]	ribose/pentoses [%]
0	0.0 ± 0.0	--
1	4.8 ± 0.2	8.2 ± 0.0
2	6.9 ± 0.5	7.4 ± 0.0
3	10.9 ± 0.2	8.9 ± 0.0
4	14.9 ± 0.3	11.1 ± 0.0
5	16.1 ± 0.1	11.8 ± 0.0

153 ¹ Oscillatory ball mill, 30 Hz.

154 **Table 7.** Relative amounts for the mechanochemical reaction¹ and the reaction in water (140 mM, 40
 155 °C) of glycolaldehyde with 20 mol% Ca(OH)₂ after 30 min (**Figure 5**).

medium	glycolaldehyde [%]	tetroses [%]	hexoses [%]	heptoses [%]
mechanochemical	53	42	5	0
in water	1	12	81	6

156 ¹ Oscillatory ball mill, 30 Hz.

157 **Table 8.** Relative amounts for the mechanochemical aldol reaction in a planetary ball mill at 400 rpm
 158 after 90 min (**Figure 6**).

molecular sieve	glycol- aldehyde [%]	trioses [%]	tetroses [%]	pentoses [%]	hexoses [%]	heptoses [%]
molecular sieves with adsorbed formaldehyde	15	13	42	18	10	1
dry molecular sieves	22	0	57	0	21	0

159 **Table 9.** Product distribution of carbohydrate formation for different storage temperatures and times
 160 starting from glycolaldehyde with 20 mol% calcium hydroxide in a glass vial (**Figure S2**). Error
 161 analysis is based on double determinations.

time [h]	temperature [°C]	glycolaldehyde [%]	tetroses [%]	hexoses [%]
0	23	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	-196	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	-20	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.5	40	98.9 ± 0.1	0.9 ± 0.1	0.1 ± 0.0
0.5	50	92.8 ± 0.5	6.4 ± 0.4	0.8 ± 0.1
0.5	60	59.3 ± 2.3	36.0 ± 1.8	4.6 ± 0.5

162 **Table 10.** Repeatability study for the mechanochemical reaction starting from glycolaldehyde with
 163 20 mol% calcium hydroxide (**Figure S3**).¹ Error analysis is based on double determinations.

experiment #	glycolaldehyde [%]	tetroses [%]	hexoses [%]
1-A	60.3 ± 0.1	35.4 ± 0.1	4.3 ± 0.1
1-B	62.5 ± 0.2	33.9 ± 0.3	3.6 ± 0.1
2-A	61.8 ± 1.6	34.1 ± 1.4	4.1 ± 0.1
2-B	67.9 ± 0.1	28.6 ± 0.1	3.5 ± 0.1

164 ¹ Oscillatory ball mill, 30 Hz, 60 min.

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Table 11. Repeatability study for the mechanochemical reaction starting from glycolaldehyde with 20 mol% calcium hydroxide (**Figure S4**).¹ Error analysis is based on double determinations.

catalyst [mol%]	glycolaldehyde [%]	tetroses [%]	hexoses [%]
0	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	82.0 ± 0.9	16.4 ± 0.7	1.6 ± 0.2
10	54.2 ± 1.6	38.4 ± 1.0	7.4 ± 0.5
20	23.2 ± 0.7	48.6 ± 0.6	28.2 ± 1.0

167

¹ Oscillatory ball mill, 30 Hz, 90 min.

168

169 8. References

- 170 1. Haas, M.; Lamour, S.; Trapp, O. Development of an advanced derivatization protocol for the
171 unambiguous identification of monosaccharides in complex mixtures by gas and liquid
172 chromatography. *J. Chromatogr. A* **2018**, *1568*, 160-167,
173 doi:<https://doi.org/10.1016/j.chroma.2018.07.015>.
- 174 2. Scanlon, J.T.; Willis, D.E. Calculation of Flame Ionization Detector Relative Response Factors
175 Using the Effective Carbon Number Concept. *J. Chromatogr. Sci.* **1985**, *23*, 333-340,
176 doi:10.1093/chromsci/23.8.333.
- 177