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Faculty of Science

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DETERMINATION OF POLYOLS IN *BOMBYX MORI* LARVAE BY GC-MS MEASUREMENT

Jan Tlačil

Bc. Thesis

Supervisor: Ing. Helena Zahradníčková, Ph.D.
Laboratory of analytical biochemistry and metabolomics
Faculty of Science, České Budějovice

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Annotation

Occurrence of various biologically important polyols in the matrix of *Bombyx mori* larvae was examined. After sample derivatization with silyl reagent the GC-MS analyses were performed. The analytical method was partly validated.

Prohlašuji, že svoji bakalářskou práci jsem vypracoval/a samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své bakalářské práce, a to v nezkrácené elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích, 6. 5. 2018

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Last but not least, I would not be able to finish this work without moral support and trust of my family.

Contents

1 Chromatography

1.1 Principle of chromatography

In chromatography, samples are separated between two phases. Mobile phase carries sample through stationary phase, where the separation takes place. During this process, components of the sample can interact with the stationary phase which causes retention of certain components and so their separation from the others. The greater interaction appears the greater detention can be observed. Travel time through the column is recorded [1, 2].

1.2 Gas chromatography

Gas chromatography is one of the most used separation method nowadays. Sample must be evaporated prior to be carried by the mobile phase. Carrier gas must be inert to prevent any interaction and possible changes on the sample. Mobile phase continues through the column up to the detector, where signal recording takes place. Sample is separated according to travelling speed through the column. Some molecules are slowed down by generated interactions between them and the inner surface of the column. This results in different times of reaching the detector, which are recorded and then processed. It is inappropriate to measure macromolecules, organic and inorganic salts because their interactions with stationary phase may affect the measurement. For that reason, derivatization is commonly used to prevent these non-required properties and to prepare sample for chromatography measurement [1].

1.2.1 Gas Chromatograph

In GC carrier gas is provided in gas bottles containing hydrogen, helium or argon. The most important condition for mobile phase is its inertness. Mobile phase must be safe to work with and non-toxic. Right choice of the mobile phase depends on the specific

type of column and detector. To prevent any side effects, the gas undergoes purification from traces of other gasses to prevent any undesirable influence of the measurement. Regulatory system moderates the flow of the mobile phase. Sampler injects the sample in the stream of carrier gas. If liquid sample is inserted, it has to be evaporated in the shortest time interval possible [1].

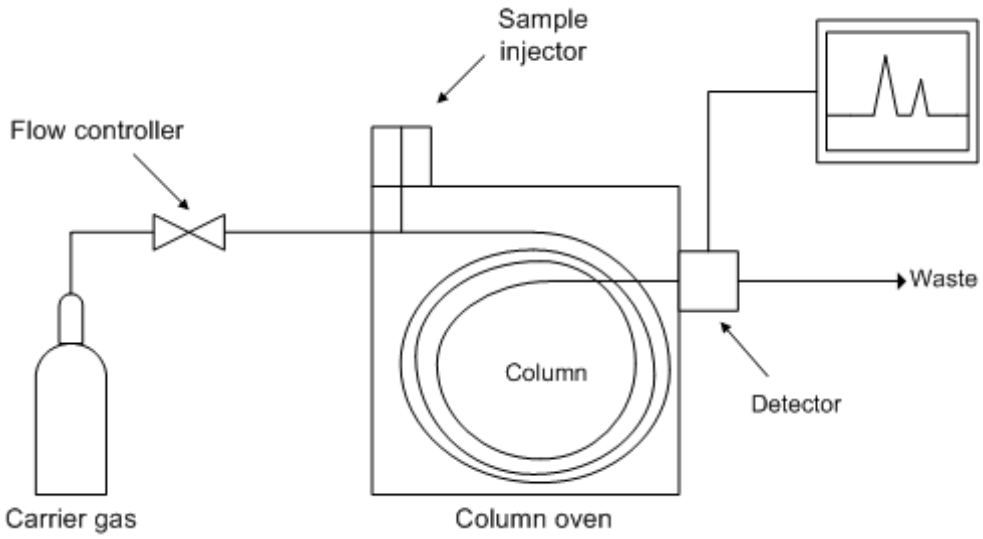

Figure 1: Scheme of gas chromatograph

1.2.1.1 Columns

Two types of columns are recognised. Packed GC columns compose of steel or glass tube filled with sorbents such as silica gel or aluminium oxide. Second type is called capillary GC column. They are usually made of silica and their inner surface is layered with non-volatile liquid acting as stationary phase. Even if they can measure only small amounts of the sample, they provide very precise and high-quality separation [1, 2].

1.3 Mass spectrometry

Mass spectrometry belongs to the group of separation detection. Molecules are cleaved into ions with different mass/charge ration (m/z). Specific fragments appear for different compounds which leads to their identification [2]. Separation takes place under vacuum conditions to prevent collisions of particles of the gas phase which may interfere the results of the measurement [1].

1.3.1 Mass spectrometer

Mass spectrometer consists of three main components. At the beginning analyte molecules undergo ionization by ion source. Consequently, charged species continue towards the analyser, where they are analysed and sorted before sending to the detector. After reaching detector, produced signals are recorded and processed by computer [2].

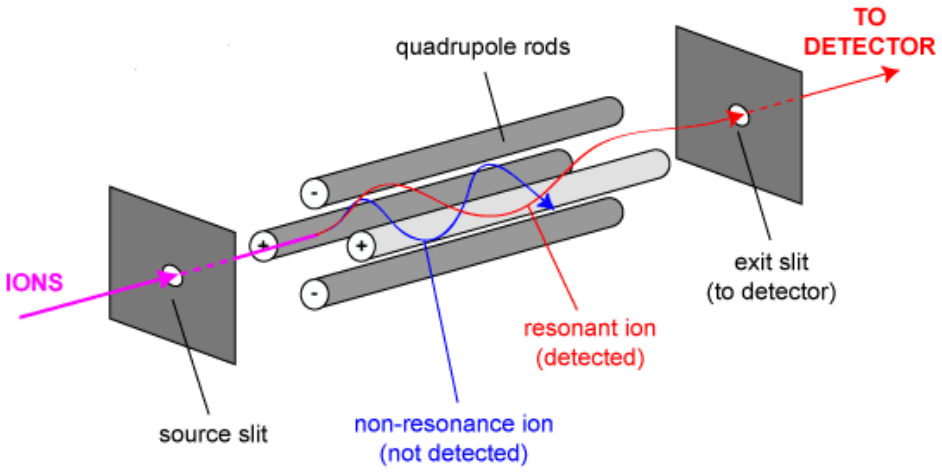

Figure 2: Scheme of quadrupole mass spectrometer

1.3.1.1 Ion source

Ionization process is essential for mass spectrometry. Different types of ion sources exist and their use is determined based on the analyte or aim of the measurement. For combination of gas chromatograph and mass spectrometer, electron ionization or chemical ionization sources are mostly used. Limitations for these types of ionization are the relative volatility and thermal stability of the analyte [3].

1.3.1.1.1 Electron ionization

Widely used ionization techniques in organic chemistry is electron ionization. It is recognized as hard ionization due to the fragmentation of the analyte molecules. Electrons are emitted from heated filament towards the anode. During this process

electrons with high energy collide with molecules of the gaseous analyte and eject electrons out of them. The excess of energy leads to the fragmentation of the molecules which can be useful for identification of unknown analytes [3].

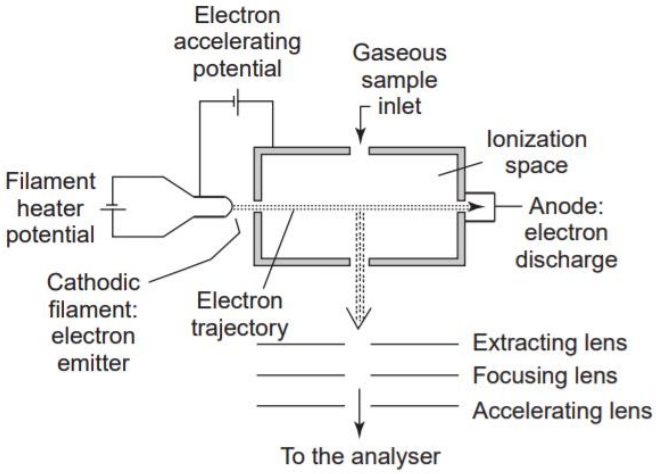

Figure 3: Scheme of an electron ionization source

1.3.1.2 Quadrupole

The quadrupole analyser consists of 4 rods supplied with direct current and radiofrequency field [1]. Induced electric field is oscillating and ions travelling through it follow its trajectory. Entering ions with positive charge are attracted by negatively charged rod. After the swap of rod polarity, the trajectory of the ion will also change. At the given time only ions with certain m/z ratio can pass the analyser towards the multiplier. Ions with other than defined m/z ratios collide with the rod and discharge themselves [3].

2 Carbohydrates

Carbohydrates are compounds essential for every living organism. They play many important roles [4]. Some of them serve as the source of the energy and structural components of bodies of many plants. Certain types of carbohydrates are necessary for synthesis of fats or as a part of the nucleic acid coding genetic information. Thanks to the

attachment of many functional groups, carbohydrates are considered as polyfunctional compounds [5].

In general, carbohydrates are recognised as simple or complex ones. As simple saccharides are classified carbohydrates that cannot be hydrolysed into smaller sugars. Complex carbohydrates consist of more than 2 simple sugars connected via acetal bond [4].

2.1 Glucose

Glucose is a simple sugar consisting of six carbon atoms. It is also called blood sugar as it floats in the bloodstream serving as immediate source of energy. In the metabolism of insect glucose also plays a role in synthesis of chitin, the major component of cuticle. The insects utilize glucose for synthesis of sugar alcohols to adapt to severe conditions as cold or drought [6].

2.2 Trehalose

Trehalose is considered as the main sugar in the hemolymph of insect. It is stored in high concentrations and serves as the instant source of energy for tissues [7]. Molecules of trehalose consist of two molecules of glucose bound in glycosidic linkage [8]. Synthesis of trehalose is driven by two enzymes. Trehalose 6-phosphate synthase transforms molecules of UDP glucose and glucose 6-phosphate into trehalose 6-phosphate. Consequently, phosphate group is removed by trehalose 6-phosphatase and enables trehalose to be released into hemolymph. Before trehalose can be used as a source of energy, it has to be hydrolysed to obtain glucose molecules. This process is driven by enzyme trehalase [9].

2.3 Glycerol and sorbitol

Glycerol is identified as simple polyol and forms a backbone of all triglycerides. It has significant use in food industry as a sweetener. It can be produced by transesterification of saponification of triglycerides obtained from plants or animals.

Sorbitol can be obtained by reduction of one aldehyde group on glucose molecule to hydroxyl group. Sorbitol commonly occurs in animals [10], as well as in humans [11]. Another isomer of this molecule exists, which differs by orientation of hydroxyl group on the second carbon; it is called mannitol.

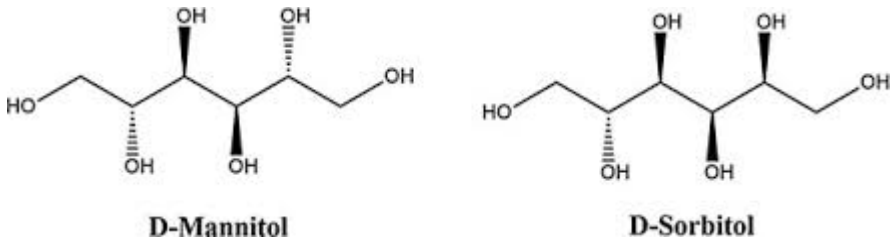

Figure 4: Comparison of molecules of D-mannitol and D-sorbitol.

Presence of both polyols was observed during studies of eggs of Bombyx silkworm. After initiation of diapause glycogen underwent a breakdown process resulting in these two polyols, sorbitol and glycerol. Consequently, at the end of diapause period, glycogen was resynthesized back [10].

2.4 Mannitol

Despite the fact, that mannitol is considered the most abundant straight chained polyol [12], its appearance within animal kingdom is rather rare. It has been observed only in few insect species [13]. Together with sorbitol they are responsible for protection of organisms from cold or osmotic stress [14, 15].

2.5 Saccharose

Saccharose, or also known as sucrose, is disaccharide consisting of two monosaccharide units, fructose and glucose connected via acetal bridge. Because there is no carbonyl group present on the molecule, it is identified as nonreducing sugar [5]. For certain species of insect sucrose serves as a source of energy taken up from plants. Hydrolysis is driven by α-glucosidase during which the saccharose molecule is broken back into monosaccharides [16].

2.6 Arabinitol and ribitol

Both compounds are alcohols and they are formed by reduction of arabinose and ribose, respectively. Position of a two hydroxyl group differentiate between those pentioles. They were suspected to play a role in preventing the insect from freezing [17]. In the study of sugar metabolism in boll weevil intermediate concentrations of polyols were synthesized out of dietary carbohydrates which were fed to boll weevil, among others, arabinitol and ribitol. They were suspected to play a role in preventing the insect from freezing [18].

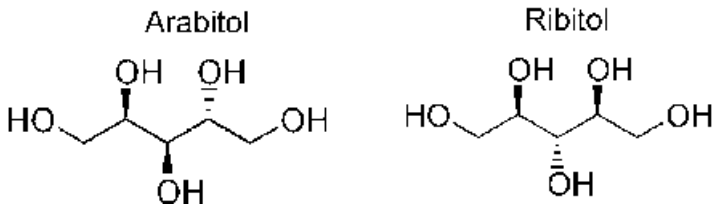

Figure 6: Difference in molecules of arabinitol and ribitol

2.7 Myo-inositol

Myo-inositol is an isomer of inositol. It is can be found in animals and plants where it plays many important roles. For some cells, it is considered as essential substance [19]. Increased concentrations of myo inositol might correlate with brain tumors or neurological disorders [20]. Similarly as discussed earlier, also myo-inositol plays important role in the adaptation and protection against freezing conditions in winter. Increased concentrations of myo-inositol during were observed at D. montana fly as a reaction to inhospitable conditions [21].

3 Derivatization

Derivatization is a chemical process during which the given chemical compound is transformed into its derivative - compound of the same structure. The only change appears on the functional group, which is replaced and results in different physical-chemical properties. Reason for this transformation can be done to enhance the separation by decreasing polarity if the analyte or to stabilize the compound for the measurement.

3.1 Silylation

Silylation became the most commonly used derivatization technique in gas chromatography. Many polar functional groups which cause problem during gas chromatography (such as -OH, NH2, -COOH or -SH) can be sufficiently treated with silylation reagents. The acidic hydrogen on those functional groups is replaced with various alkyl silyl groups, for example, trimethylsilyl (-Si(CH3)3) [22]. Silylated functional groups are protected from the basic or acidic hydrolysis and provides the solubility in nonpolar solvents. Analyte treated with silylation reagents usually becomes more volatile and thermally stable which is advantageous for GC MS measurements [23].

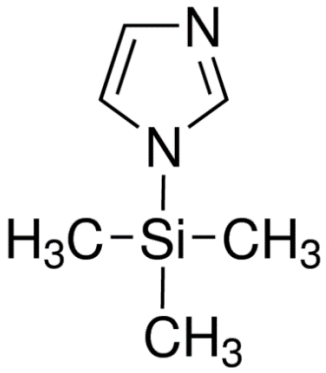

Figure 5: Structure of trimethylsilyl imidazole

4 Experimental part

4.1 Used chemicals and biological materials

Glycerol, ribose, arabinitol, ribitol, fructose, glucose, mannitol, sorbitol, *myo*-inositol, trehalose, dimethylformamide (DMF), pyridine, 1-(trimethylsilyl)-imidazole (TMSI) and methylhydroxylamine (MHA) were purchased from SIGMA-ALDRICH (Czech Republic). Sucrose came from LACHEMA (Czech Republic). QC sample (carbohydrate HPLC Performance check mix) was supplied by RESTEK. Acetonitrile and methanol was purchased from VWR Chemicals (USA).

Extract of larvae of *Bombyx mori* (acetonitrile : methanol : water = 2 : 2 : 1) was a kind gift from prof. V. Košťál from Biology Centre of the Czech Academy of Sciences, Institute of Entomology.

4.2 Calibration

For determination of polyols concentrations standard addition and external calibration method were performed. Samples containing 20 µl of matrix were spiked with 10 µl of internal standard solution. Standard addition samples were prepared at 4 concentration levels as shown in Table 1.

External calibration curve was obtained from polyols standards spiked in water at concentrations of 0.05, 0.1, 0.25, 0.5, 1, 5 and 10 nmol/µl. For sorbitol and trehalose, 20 and 50nmol/µl standards were additionally prepared. Samples were measured using GC-MS instrument. Area ratios between sample and internal standard were plotted against its concentration.

	1st level	2nd level	3rd level	4th level
Glycerol	0.5	1	5	10
Ribose	0.1	0.5	1	5
Arabinitol	0.1	0.5	1	5
Ribitol	0.1	0.5	1	5
Fructose	0.1	0.5	1	5
Glucose	0.5	1	5	10
Mannitol	0.1	0.5	1	5
Sorbitol	5	10	20	50
Myo-inositol	0.1	0.5	1	5

Sucrose	0.1	0.5	1	5
Trehalose	5	10	20	50

Table 1: Specific concentration levels of addition to the sample. All values are described in nmol.

4.3 Procedure

In order to make samples suitable for GC-MS measurement, derivatization reaction had to be performed. This two-step procedure led to volatile and more stable derivates. In the first step, the carbonyl group in polyols was reduced with methylhydroxylamine to form an oxime which was followed by silylation of hydroxyl groups.

Matrix sample (20µl) was placed into reaction vial mixed with internal standard solution. Mixture was evaporated with mild stream of nitrogen gas. For oximation reaction solution of pyridine and MHA was prepared (20mg of MHA per 1ml of pyridine). Evaporated matrix was dissolved in 30 µl of DMF and 25 µl of the mixture of pyridine and MHA. Every vial was vortexed and was allowed to react at 80°C for 30 minutes. After samples cooled down to room temperature, silylation could be performed. Then 30 µl of TMSI with additional 70 µl of DMF were mixed into reaction solution and after mixing, vessels were heated again to 80°C in the oven for another 30 minutes. When samples reached room temperature again, 100 µl of isooctane was injected into reaction mixture, vortexed for approximately 30 seconds and let to stand to form sharp interface between two phases. Approximately 60 µl of upper organic layer was transferred to vial with silylated insert and closed with Teflon cap, and used for GC-MS injection.

QC sample consisting of D-Fructose (2.0mg/ml), D-Glucose (2.1 mg/ml) and Sucrose (4.0 mg/ml) was dissolved in 1 ml of acetonitrile/water mixture (75:25, respectively). 0.5 µl this mixture were injected into reaction vial together with IS solution, while evaporated by nitrogen gas. The rest of the procedure was similar to the one used for sample preparation.

4.4 GC-MS analysis

For sample measurements and characterization Trace Ultra GC gas chromatograph with programmable temperature vaporizing injector and electron ionization ion source

(ThermoFisher Scientific, San Jose, USA). 1µl of sample was injected in splitless mode by injector at temperature of 180°C. GC analysis was performed on Agilent column 30m x 0.25mm, thickness of inner film 0.25µm. Helium was used as a carrier gas with flow rate of 1.1 ml/min. Temperature of ion source was set to 220°C and the transfer line to 180°C. Electron ionization (70eV) was in full scan mode (50-600 Da). Data were processed by Xcalibur software.

4.5 Validation

Lower limit of quantification (LLOQ), accuracy, precision and recovery were processed based on general guidance [24].

LLOQ was determined as the lowest concentration level of the calibration curve having accuracy of 100±20% and precision lower than 20%. Accuracy and precision were obtained by analysing of standards concentrations into matrix and values retrieved from standard addition calibration curves. In the single series 4 concentration levels were analysed (within-run) and over 3-day period (between-run) first 2 levels were measured. Recovery was determined by comparing concentrations of spiked samples with corresponding concentration levels obtained from calibration curve of standards in water, where the value from external calibration was 100%.

5 Results and discussion

5.1 Analysis of polyols in water

Measured values of polyol standards in water were processed by Xcalibur software and used for construction of calibration curves. Area ratios between sample and IS signal were plotted against concentration of the given polyol. Equation of the curve was used to calculate measured concentration.

Lowest calibration level was not the same for all polyols as shown in Table 3. For glycerol, arabinitol, mannitol, myo-inositol and trehalose LLOQ was similar to the lowest prepared concentration, which was 0,05nmol. On the other hand, ribose, ribitol, glucose, sorbitol and saccharose had the lowest level, which fulfils the range of 100±20%, 0.1 nmol. The lower concentration for these polyols was found to be below the detection limit. Special case was with fructose, where the limit of detection reached 0.25 nmol.

5.2 Analysis of matrix samples

Measured data of the pure sample and samples with addition were studied. Area ratios were plotted against added concentrations. Resulting curves had quadratic character. Concentrations of polyols in the sample were then determined by calculation of intercept of the curve with the x-axis, where the concentration was scaled. Determined concentration and equations of the curves can be found in the Table 4. In case of fructose, the matrix contained impurities whose peaks interfered with signals of fructose and caused distortion which influenced mainly lower concentrations. Because of this, determination of fructose concentration in matrix was not possible.

5.3 Accuracy, precision and recovery

Accuracy of the measurement was calculated by comparing values of real concentrations added and the calculated values. For the lowest concentration levels the values should be in the range of 100±20%, otherwise should not exceed 100±15%.

Precision for each concentration of added polyol was determined with the limit of 15%, for the lowest concentration level 20%.

Recovery corresponded to ratio of concentrations obtained from standard addition method and external calibration. Accuracy, precision and recovery of the within-day and between-day measurements are displayed in Table 5.

Increased concentration of glycerol in the sample was probably caused by contamination of one of the solvents with glycerol and was mainly observed at the lowest concentration level also in between day run. This fact is supported by small amount of glycerol found in blank samples. Also in the case of mannitol, the effect of the matrix on added concentration was observed. While the amount of added mannitol was getting higher, the lower the detected concentration was. As was already mentioned, fructose peaks interfered with signals of some impurities present in the matrix causing the distortion of data, mainly the lower concentrations were affected. Higher concentrations were not affected so much and it was possible to use the data to create the curve and calculate concentrations. Greater between-day accuracy in low glucose concentrations was probably caused by accidental mistake during sample preparation.

For the remaining polyols, the analysis was more successful and resulted in determination of their concentrations in the sample matrix with relevant precision and accuracy.

5.4 QC sample analysis

Three measurements of QC samples were performed to ensure the quality of the measurement. Sample containing specific amounts of glucose, fructose and sucrose were prepared and compared to values calculated from calibration curves, as described in Table 2.

	Theoretical amount / nmol	Calculated amount /nmol	Accuracy %	Precision %
Glucose	5.83	5.759	99.29	3.69
Fructose	5.51	5.167	93.78	3.33
Sucrose	5.84	6.143	105.19	2.89

Table 2: Comparison of theoretical amounts of given carbohydrates in QC sample with their calculated values.

6 Conclusion

Analytical method for determination of polyols (glycerol, ribose, arabinitol, ribitol, fructose, glucose, mannitol, sorbitol, *myo*-inositol, sucrose and trehalose) in insect extract using silylderivatization and GC-MS separation and quantification was partly validated. The majority of polyols were determined with relevant precision and accuracy, except glycerol and glucose.

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Figure 1. http://www.se-source.com/gc.htm

Figure 2. https://s-media-cache-k0.pinimg.com/originals/e6/c0/4d/ e6c04db34252250666 0decfe1d 7ac751.gif

Figure 3. HOFFMANN, Edmond de. a Vincent. STROOBANT. Mass spectrometry: principles and applications. 3rd ed. Hoboken, NJ: J. Wiley, c2007. ISBN 978-0-470-03310-4.

Figure 4. Dai, Yiwei & Meng, Qing & Mu, Wanmeng & Zhang, Tao. (2017). Recent advances in the applications and biotechnological production of mannitol. Journal of Functional Foods. 36. 404-409. 10.1016/j.jff.2017.07.022.

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Figure 6. https://www.sigmaaldrich.com/catalog/substance/1trimethylsilylimidazole140261815674611 ?lang=de&region=AT

8 Attachments

Glycerol	0.05	0.1	0.25	0.5	1	5	10	-	-
Ribose	-	0.1	0.25	0.5	1	5	10	-	-
Arabinitol	0.05	0.1	0.25	0.5	1	5	10	-	-
Ribitol	-	0.1	0.25	0.5	1	5	10	-	-
Fructose	-	-	0.25	0.5	1	5	10	-	-
Glucose	-	0.1	0.25	0.5	1	5	10	-	-
Mannitol	0.05	0.1	0.25	0.5	1	5	10	-	-
Sorbitol	-	0.1	0.25	0.5	1	5	10	25	50
Myo-inositol	0.05	0.1	0.25	0.5	1	5	10	-	-
Sucrose	-	0.1	0.25	0.5	1	5	10	-	-
Trehalose	0.05	0.1	0.25	0.5	1	5	10	25	50

Table3: Concentration levels which were used during construction of calibration curves. All numbers in table are in nmol.

	concentration (nmol/µl)	equation of the curve	R^2
Glycerol	3.671±0.035	$y = 0.0121x^2 + 0.6384x + 2.1804$	0.9975
Ribose	0.364±0.015	$y = -0.0794x^2 + 1.2431x + 0.4626$	0.9991
Arabinitol	0.085±0.003	$y = -0.0904x^2 + 1.7739x + 0.1516$	0.9999
Ribitol	0.103±0.019	$y = -0.1x^2 + 2.4905x + 0.2571$	0.9999
Glucose	2.703±0.045	$y = 0.0015x^2 + 0.6989x + 1.8779$	0.9978
Mannitol	0.537±0.143	$y = -0.2958x^2 + 2.6792x + 1.5087$	0.9992
Sorbitol	9.990±0.205	$y = 0.0148x^2 + 1.002x + 8.5332$	0.9996
Myo-inositol	0.379±0.018	$y = 0.1659x^2 + +3.0661x + +1.1409$	0.9999
Sucrose	0.271±0.001	$y = 0.0133x^2 + 0.0679x + +0.0174$	0.9999
Trehalose	20.685±0.071	$y = -0.0002x^2 + +0.0676x + 1.4839$	0.9996

Table 4: Concentrations of polyols in the matrix sample with equations of curves.

	Added amount / nmol	Accuracy / % within-day	Accuracy / % between-day	Precision / % within-day	Precision / % between-day	Recovery / %
Glycerol	**0.5**	158.01	144.41	5.59	7.34	155.88
	1	114.34	110.86	11.37	2.09	102.51
	5	98.15	-	4.06	-	99.33
	10	100.2	-	0.23	-	99.85
Ribose	**0.1**	99.11	110.54	18.36	18.58	84.25
	0.5	110.74	112.9	6.08	5.21	111.53
	1	95.43	-	12.83	-	94.44
	5	100.09	-	3.77	-	99.42
Arabinitol	**0.1**	103.56	93.5	0.32	4.61	108.26
	0.5	97.56	86.83	6.82	2.15	95.52
	1	100.69	-	7.61	-	97.71
	5	100.26	-	8.94	-	100.6
Ribitol	**0.1**	117.09	117.36	7.8	10.1	121.64
	0.5	95.28	98.61	8.85	3.63	93.35
	1	103.04	-	7.33	-	96.85
	5	100.02	-	4.25	-	100.35
Fructose	**0.1**	ND	ND	ND	ND	ND
	0.5	100.02	87.8	ND	5.59	101.69
	1	99.99	-	11.7	-	96.65
	5	100.03	-	2.67	-	100.22
Glucose	**0.5**	119.38	135.61	9.48	1.34	113.61
	1	126.29	140.95	13.55	2.99	110.86
	5	97.74	-	3.32	-	98.97
	10	100.18	-	1.14	-	99.39
Mannitol	**0.1**	112.63	112.14	5.21	18.64	100.23
	0.5	108.18	103.35	11.17	3.57	104.87
	1	97.26	-	7.61	-	90.86
	5	82.61	-	6.38	-	84.35
Sorbitol	**5**	111.91	97.46	5.13	6.85	108.51
	10	102.01	102.68	2.13	2.63	101.73
	25	99.1	-	3.89	-	103.45
	50	100	-	0.73	-	101.44
Myo-inositol	**0.1**	119.93	58.82	6.45	11.5	119.66
	0.5	100.75	87.08	5.92	6.74	104.44
	1	99.49	-	11.33	-	101.07
	5	99.99	-	3.15	-	98.53
Sucrose	**0.1**	109.23	101.28	3.66	0.8	145.15
	0.5	98.66	99.85	8.6	1.5	108.57
	1	94.99	-	2.74	-	115.56
	5	100.03	-	4.7	-	99.51
Trehalose	**5**	97.41	119.32	11.86	12.98	102.43
	10	94.59	96.2	11.65	6.31	94.86
	25	101.1	-	2.37	-	103.42
	50	98.49	-	5.33	-	97.54

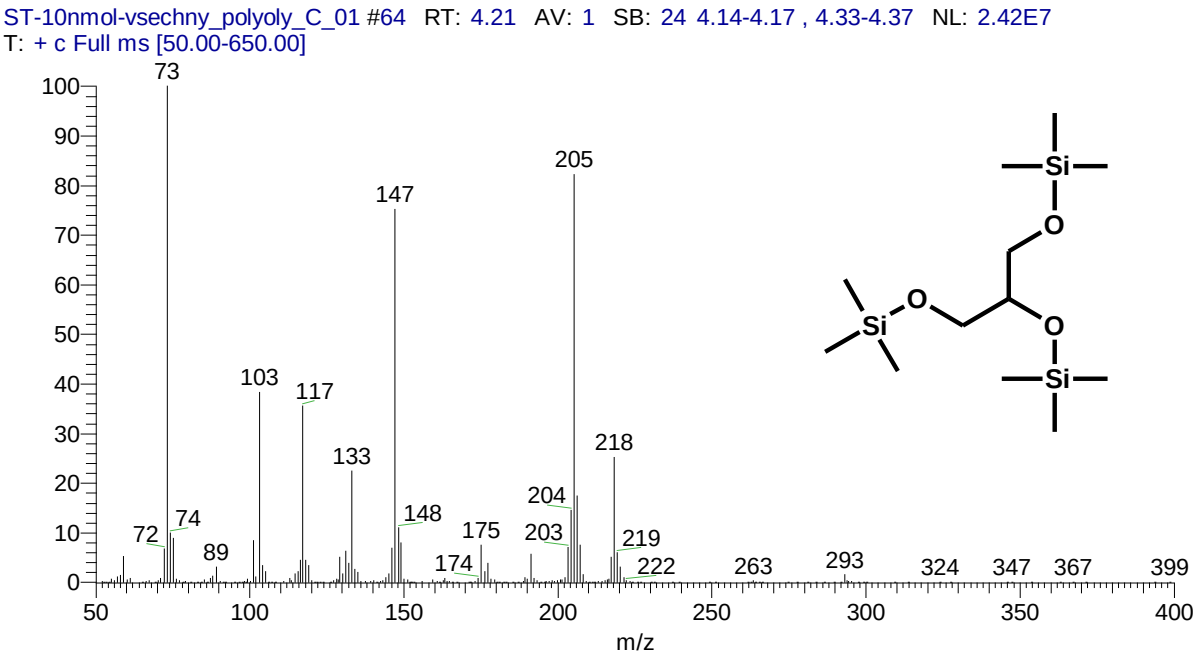
Table 5: Accuracy, precision and recovery of within day and between day runs. *ND stands for not detectable.

Glycerol (RT 4.20)

Name of derivate

Glycerol, tris(trimethylsilyl) ether
Trimethylsilyl ether of glycerol

Molecular Formula = $C_{12}H_{32}O_3Si_3$
Monoisotopic Mass = 308.165923 Da



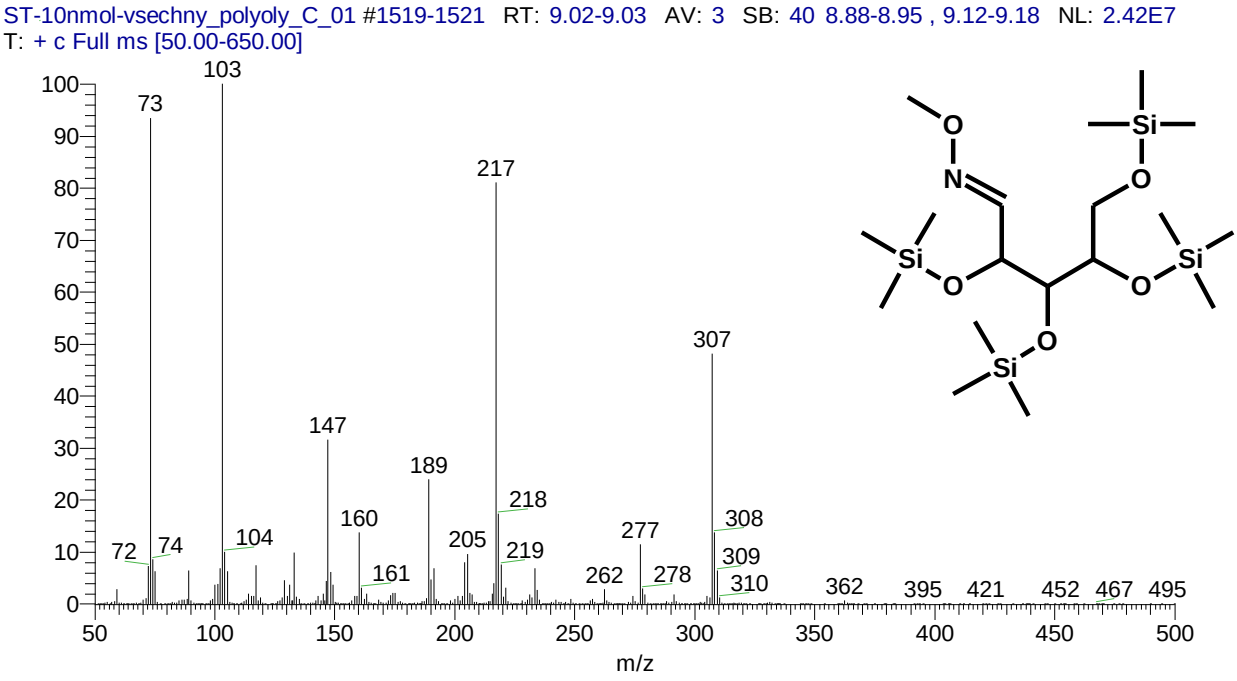
	RT	m/z
Glycerol	4.20	73, 147, 205, 218

Ribose (RT 9.02)

Name of derivative

D-Ribose, 2,3,4,5-tetrakis-O-(trimethylsilyl)-, O-methyloxime
D-Ribose tetra (O-trimethylsilyl)-O-methyloxime

Molecular Formula = $C_{18}H_{45}NO_5Si_4$
Monoisotopic Mass = 467.237478 Da



	RT	m/z
Ribose	9.02	73, 103, 147, 217, 307

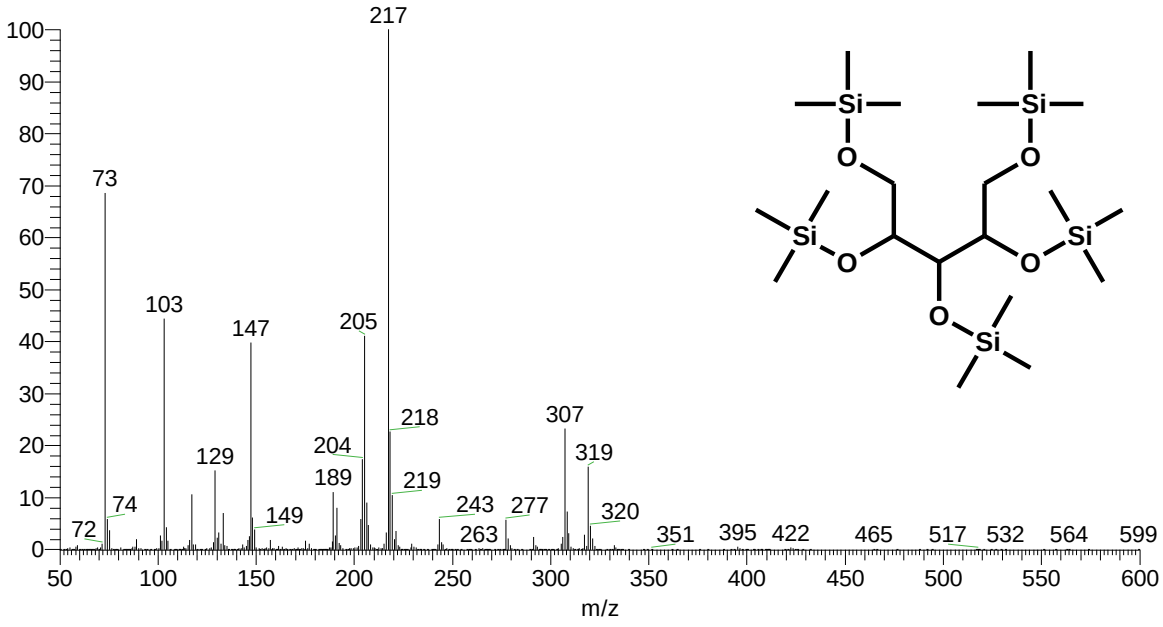
Arabinitol (RT 9.85)

Name of derivative

Arabinitol, pentakis-O-(trimethylsilyl)-
1,2,3,4,5-Pentakis-O-(trimethylsilyl)pentitol

Molecular Formula = $C_{20}H_{52}O_5Si_5$
Monoisotopic Mass = 512.266105 Da

ST-10nmol-vsechny_polyoly_C_01 #1771 RT: 9.85 AV: 1 SB: 47 9.69-9.76 , 10.05-10.13 NL: 4.44E7
T: + c Full ms [50.00-650.00]



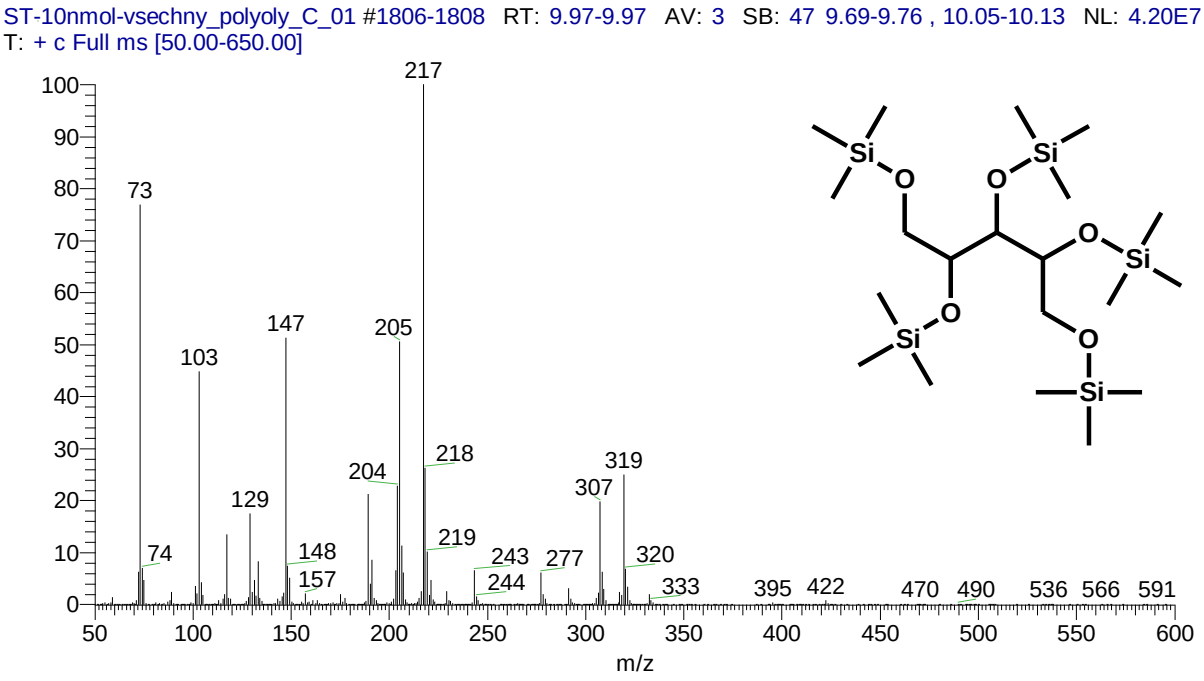
	RT	m/z
Arabinitol	9.85	73, 217, 307, 316

Ribitol (RT 9.97)

Name of derivative

Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-
1,2,3,4,5-Pentakis-O-(trimethylsilyl)pentitol

Molecular Formula = $C_{20}H_{52}O_5Si_5$
Monoisotopic Mass = 512.266105 Da



	RT	m/z
Ribitol	9.97	73, 217, 307, 316

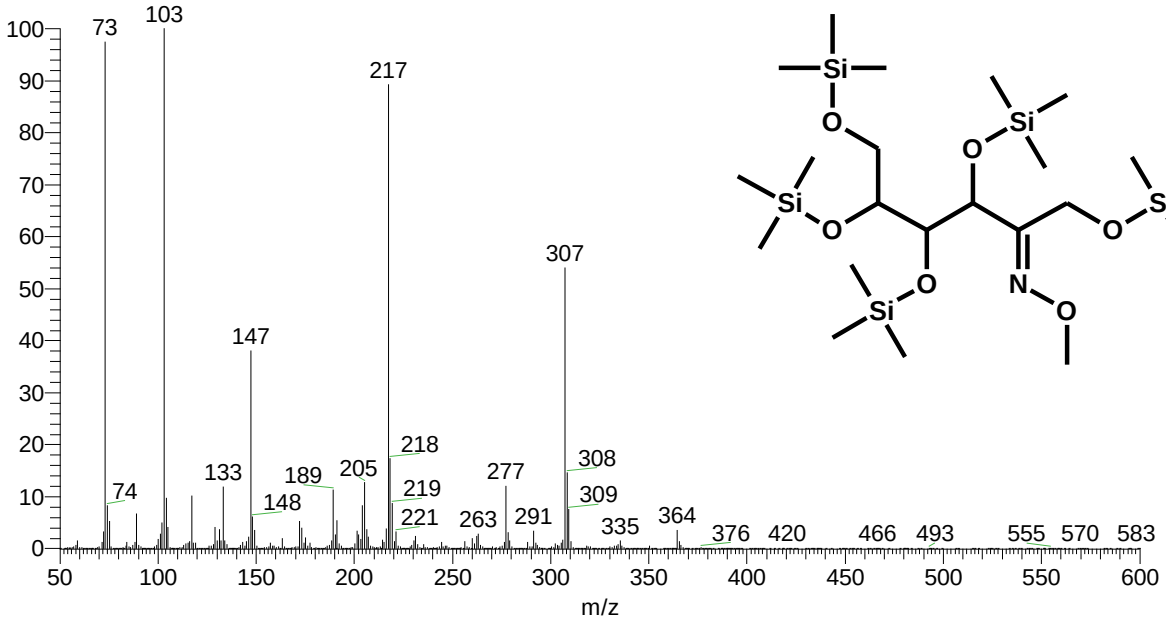
Fructose (RT 12.80)

Name of derivative

D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime

Molecular Formula = $C_{22}H_{55}NO_6Si_5$
Monoisotopic Mass = 569.287568 Da

ST-10nmol-vsechny_polyoly_C_01 #2660-2665 RT: 12.79-12.81 AV: 6 SB: 71 12.54-12.69 , 12.87-12.94 NL:
T: + c Full ms [50.00-650.00]



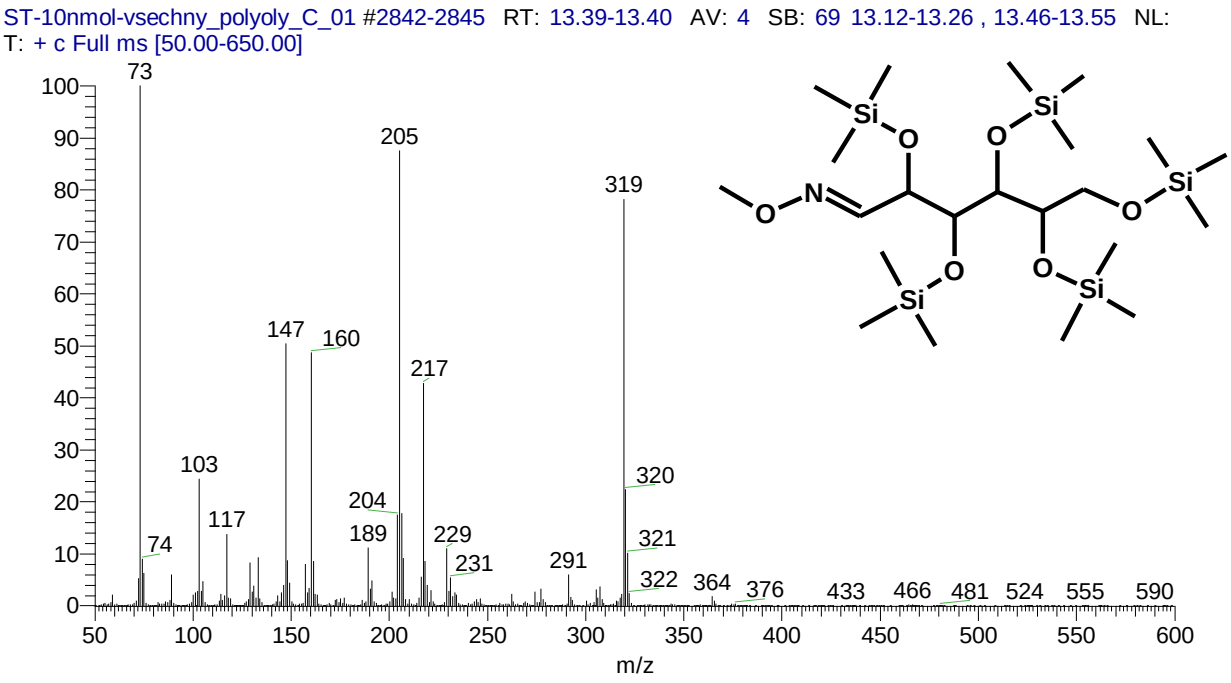
	RT	m/z
Fructose	12.80	73, 103, 147, 217, 307, 364

Glucose (RT 13.40)

Name of derivative

D-Glucose penta (O-trimethylsilyl)-O-methyloxime
D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime

Molecular Formula = $C_{22}H_{55}NO_6Si_5$
Monoisotopic Mass = 569.287568 Da



	RT	m/z
Glucose	13.40	73, 103, 147, 160. 205, 217, 319

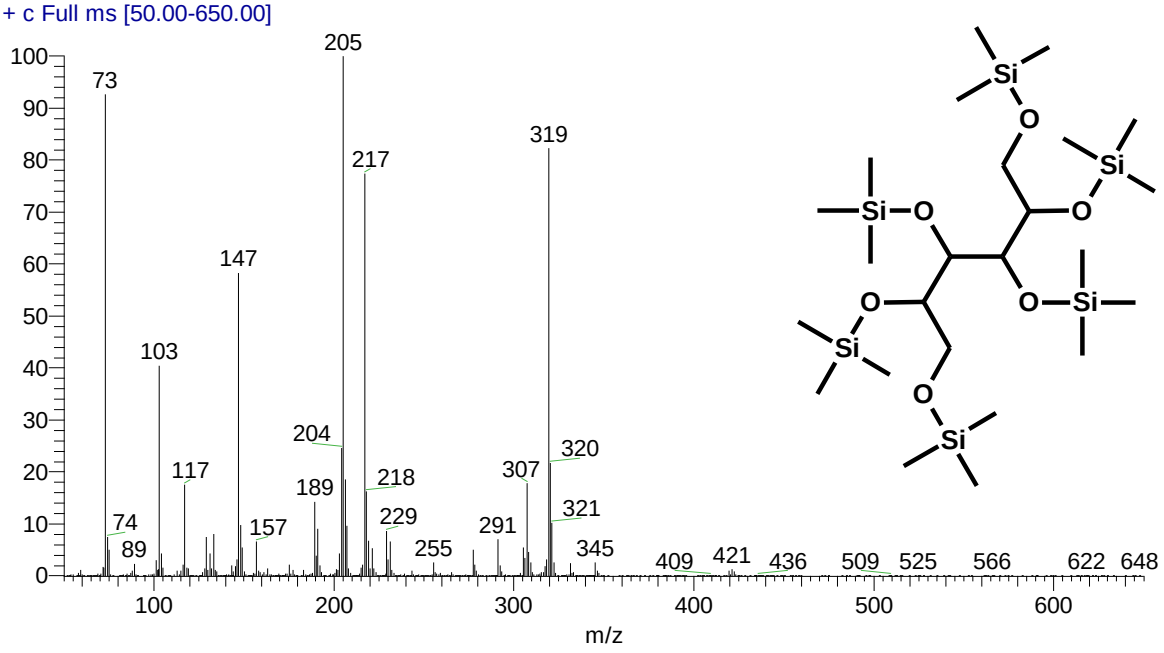
Mannitol (RT 14.40)

Name of derivative

Mannitol, hexakis-O-(trimethylsilyl)-, D-
1,2,3,4,5,6-Hexakis-O-(trimethylsilyl)hexitol

Molecular Formula = $C_{24}H_{62}O_6Si_6$
Monoisotopic Mass = 614.316196 Da

ST-10nmol-vsechny_polyoly_C_01 #3146-3149 RT: 14.40-14.41 AV: 4 SB: 35 14.29-14.34 , 14.60-14.66 NL:
T: + c Full ms [50.00-650.00]



	RT	m/z
Mannitol	14.40	73, 103, 147, 205, 217, 319

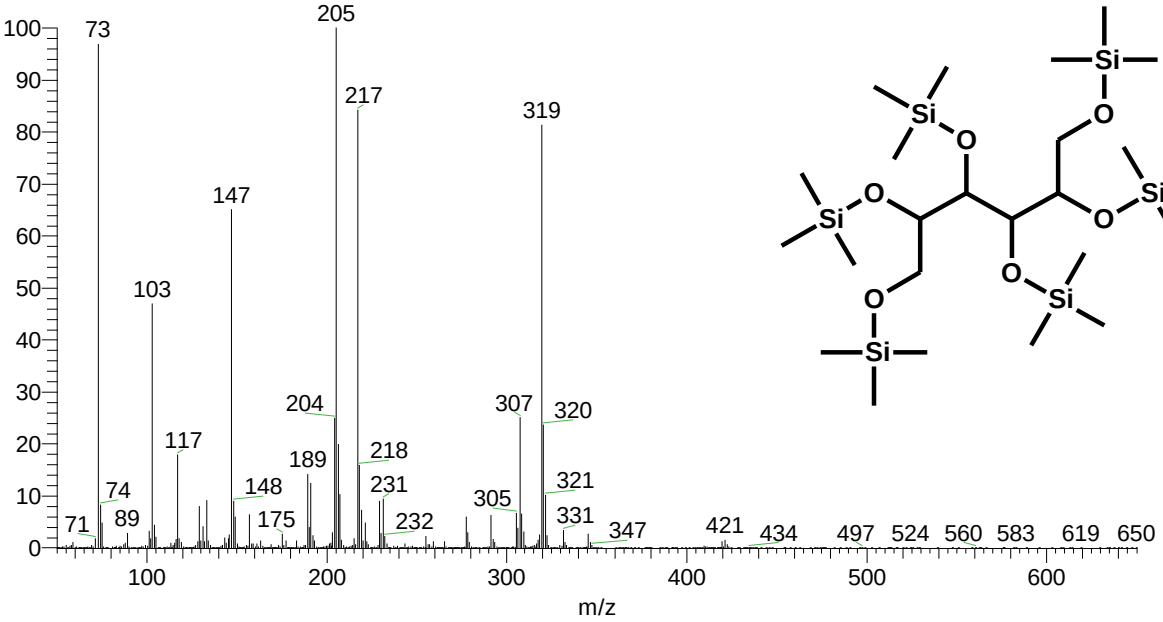
Sorbitol (RT 14.52)

Name of derivative

Trimethylsilyl ether of sorbitol
Sorbitol, hexakis-O-(trimethylsilyl)-ether

Molecular Formula = $C_{24}H_{62}O_6Si_6$
Monoisotopic Mass = 614.316196 Da

ST-10nmol-vsechny_polyoly_C_01 #3181-3182 RT: 14.51-14.52 AV: 2 SB: 27 14.44-14.47 , 14.57-14.62 NL:
T: + c Full ms [50.00-650.00]



	RT	m/z
Sorbitol	14.52	73, 147, 191, 217, 265, 265, 305, 318

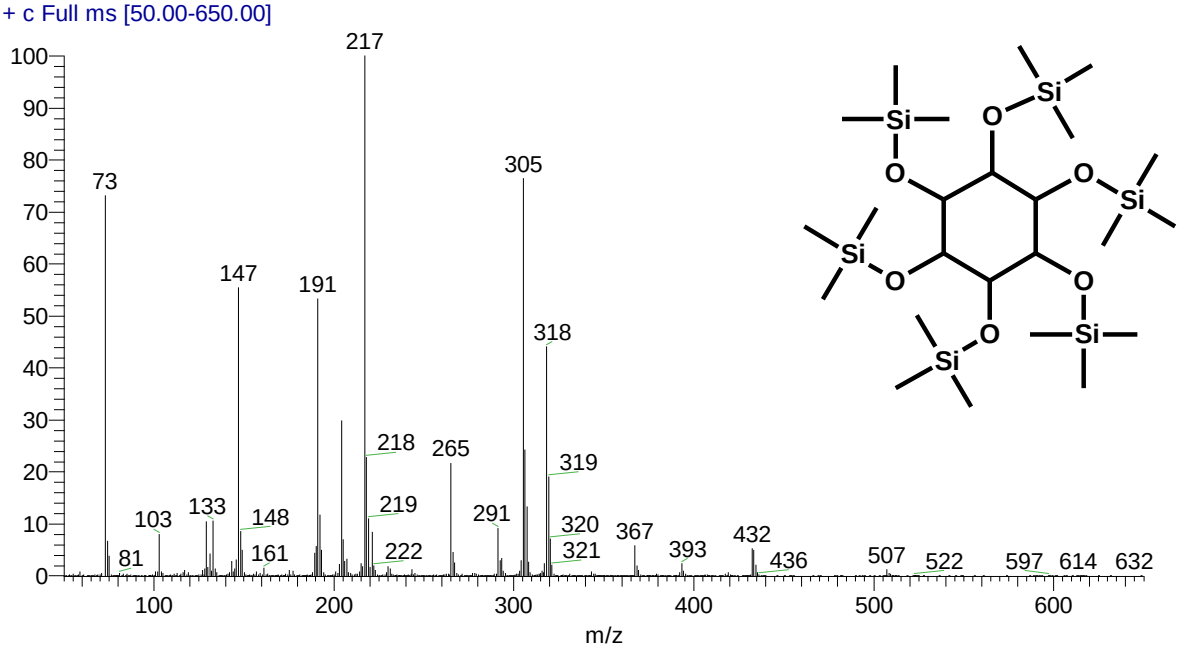
Myo inositol (RT 16.00)

Name of derivative

Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-
Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, myo-

Molecular Formula = $C_{24}H_{60}O_6Si_6$
Monoisotopic Mass = 612.300546 Da

ST-10nmol-vsechny_polyoly_C_01 #3630 RT: 16.01 AV: 1 SB: 15 15.94-15.96 , 16.05-16.07 NL: 1.43E8
T: + c Full ms [50.00-650.00]



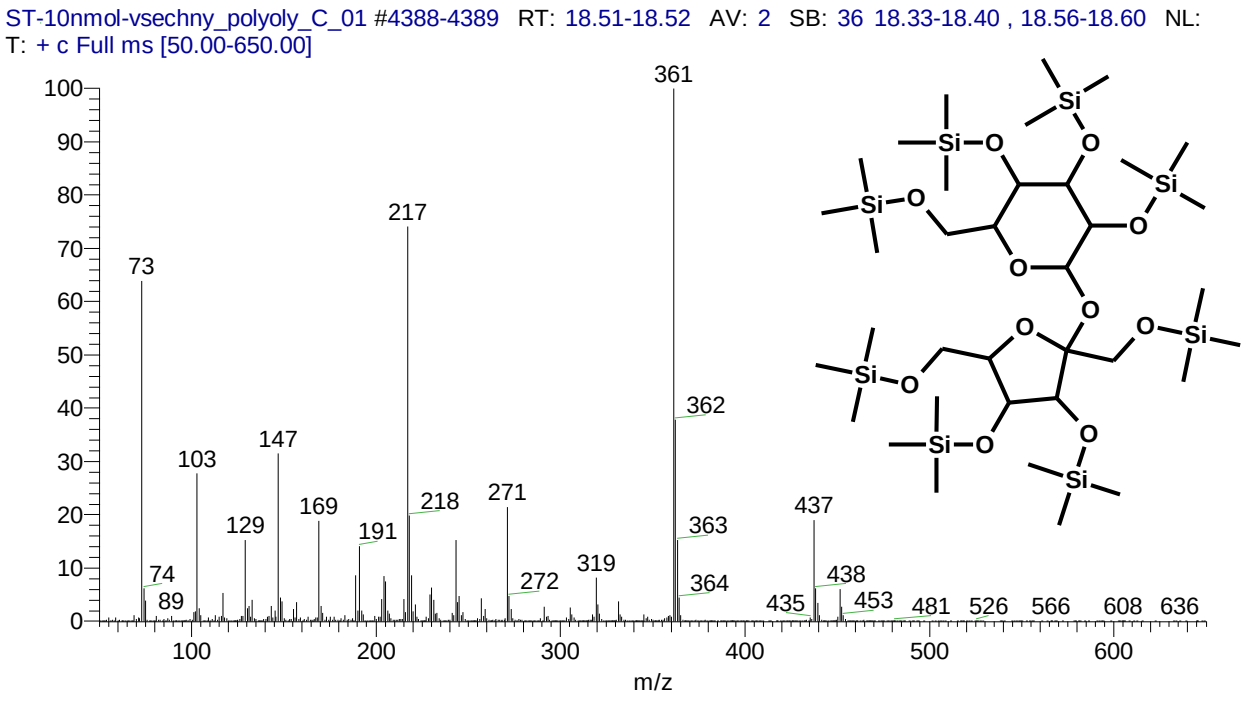
	RT	m/z
Myo-inositol	16.00	73, 147, 191, 217, 265, 265, 305, 318

Sucrose (RT 18.51)

Name of derivative

α-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-
1,3,4,6-Tetrakis-O-(trimethylsilyl)hex-2-ulofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)hexopyranoside

Molecular Formula = $C_{36}H_{86}O_{11}Si_8$
Monoisotopic Mass = 918.432422 Da



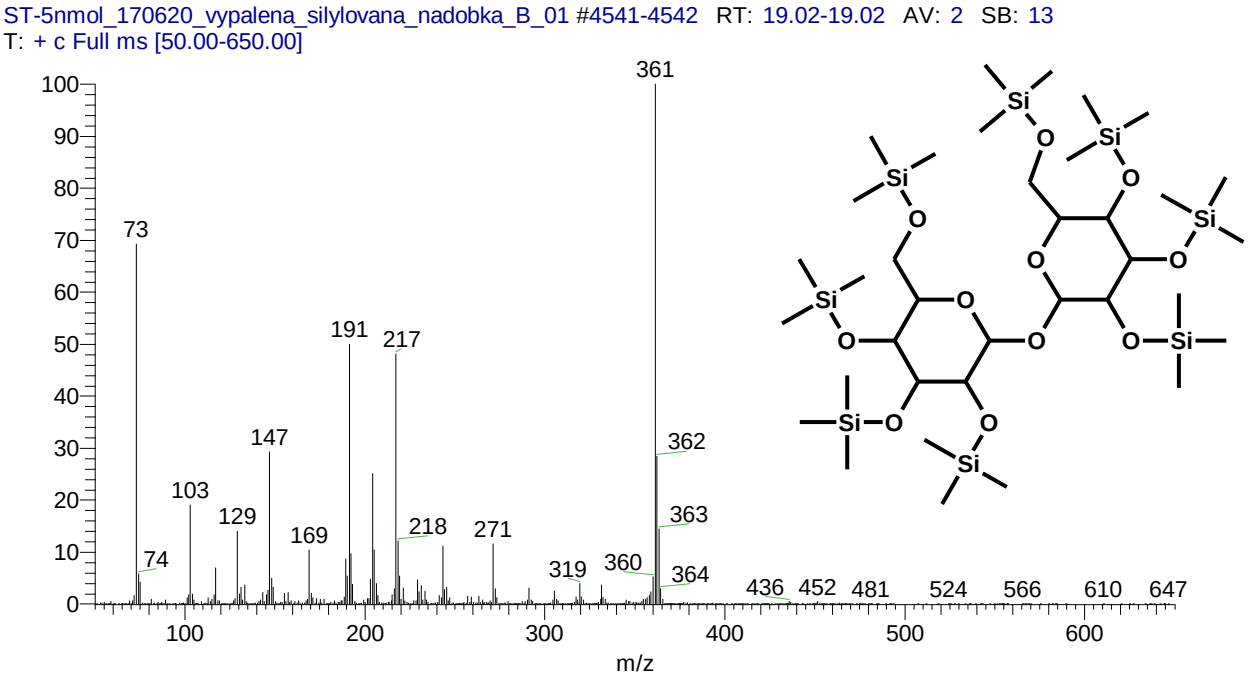
	RT	m/z
Sucrose	18.51	73, 217, 361, 407

Trehalose (RT 19.02)

Name of derivative

Trehalose, octakis(trimethylsilyl)-
α -D-Glucopyranosyl- α -D- glucopyranoside, octakis (trimethylsilyl)-

Molecular Formula = $C_{36}H_{86}O_{11}Si_8$
Monoisotopic Mass = 918.432422 Da



	RT	m/z
Trehalose	19.32	73, 217, 361, 407