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The Use of Various Methods in Assessing the Quality of Honey in Terms of the Presence of 5-Hydroxymethylfurfural (HMF)

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Abstract: The 5-hydroxymethylfurfural (HMF) is a six-carbon heterocyclic organic compound with two substituents an aldehyde group and an alcohol group. It is formed from saccharides as a result of two reactions: the Maillard reaction and the caramelization of saccharides. It has both a negative and a positive effect on the human body, which makes it, even more, an attractive research object. The objective of the study was to determine HMF content in honey samples available on the Polish market using three varied methods and compare the results obtained. The methodology used included: two spectrophotometric methods (by White and Winkler) and the HMF determination method based on the LC-MS/MS technique developed uniquely for this study. The determined HMF content in all tested honey samples did not exceed the applicable standard. All results are within the scope of the European Council Directive 2001/110/EC of 20 December 2001, i.e. they do not exceed HMF content in honey, which is 40 mg/kg.

Keywords: 5-hydroxymethylfurfural; saccharides; spectrophotometric methods; LC-MS/MS methods

1. Introduction

One of the most popular products provided by nature, or more precisely, bees, is honey. The chemical composition of honey is very complex [1]. There have been detected more than 500 chemicals in various honeys. Due to its exceptionally extensive chemical composition, honey has a considerable impact on the human body. However, like every product, it has its advantages and disadvantages. The positives are: it is beneficial for people struggling with anemia, increases immunity; protects plasma (the water part of the blood); has antibacterial properties, which may reduce the occurrence of caries; protects against gastric injury (stomach); prevents, heals some digestive disorders, e.g. ulcers, gastroenteritis; has been used since ancient times to treat eye diseases, e.g. blepharitis, conjunctivitis; reduces the risk factors of cardiovascular and metabolic diseases; natural honey is a powerful antibiotic [2]. Honey, as a whole natural food, is exposed to several pollutants, such as antibiotics, pesticides, heavy metals and other toxic compounds. Their occurrence may result from residual bee treatment, accidental exposure, environmental hazards, or hostile competition practices. These residues of the mentioned compounds harm the human body. Apart from the above-mentioned impurities/ chemical hazards found in honey, is also very common, and

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among them are 5-hydroxymethylfurfural (HMF). All reduce the health benefits and quality of honey.. 5-hydroxymethylfurfural deserves interest due to its specific properties. On the one hand, it belongs to carcinogenic compounds, has a cytotoxic effect, and hinders or prevents DNA replication/ repair [3–5]. On the other hand, it can be used in high mountain diseases, and brain hypoxia and can remove free radicals [5–7]. HMF is a six-carbon heterocyclic organic compound containing both an aldehyde and alcohol (hydroxymethyl) functional group. Figure 1 shows the structure of HMF.

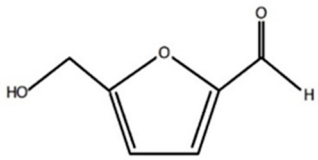

Figure 1. The structure of HMF.

5-Hydroxymethylfurfural is a solid, yellow substance that exhibits a low melting point and high water solubility [8]. It is a natural toxicant, which is formed from hexoses by heating and storing foods containing high amounts of sugar. There are two main ways in which 5-hydroxymethylfurfural is formed. The first is the caramelization of sugars at temperatures exceeding 150°C. The process occurs most easily in sugar reduction reactions, incl. glucose, fructose, or ribose. Also, it is catalysed by an acidic environment. The second method of HMF formation is the Maillard reaction, i.e. a non-enzymatic browning reaction. The reaction takes place as a result of the product's long storage time or its heating. The name "Maillard reaction" comes from the French chemist Louis Maillard, who was the first to describe the reactions between sugars and amino acids in 1912. The aldehyde group of a carbohydrate reacts with the amino group derived from an amino acid or protein to form colorless intermediates, Amadori compounds, which in turn, when heated, form HMF. As a result of further changes taking place, many colored products are created. The products of the Maillard reaction reduce the nutritional value of food by blocking or destroying certain amino acid residues (lysine, cysteine, methionine, tryptophan) and reducing their bioavailability [9]. Natural bee honey, freshly prepared, contains 2-5 mg/kg HMF. After one year, the quantity of HMF changes, and its content increases to 7-10 mg/kg. After two years it is 20-25 mg/kg, while longer storage of honey causes even a further increase in HMF content, up to 50-100 mg/kg [10]. Scientists recommend that honey, regardless of its origin, should be consumed within a year [11]. Permissible HMF content in honey established by the European Council Directive 2001/110/EC of 20 December 2001 [12] relating to honey is up to 40 mg/kg for products from the European Union. In turn, for honey produced in tropical regions, the limit is higher and amounts to 80 mg/kg. The fact that the quantity of HMF has been exceeded indicates that honey is adulterated or overheated [12]. HMF is easily formed at low temperatures, however it must be supported by the second condition, low pH. Additionally, long-term storage and high temperature increase its concentration. In addition to temperature and pH, the rate of HMF formation in the product also depends on the moisture content of honey, so there are frequently taken steps to keep moisture content low [13].Based on the conducted research, numerous scientists have concluded that both temperature and the duration of heating affect the formation of HMF in honey. The work [14] shows the results of honey research collected in Anatolia, Turkey. Honey samples were heated at 135°C for 100s, which produced a similar quantity of HMF as when the samples were heated at 150°C for 40 s. Whereas showed that honey, regardless of its origin, should be consumed within a year [15]. They researched Malaysian honey. In their research, they showed that HMF content in honey changes accordingly with storage time. In honey samples stored for 3-6 months, the level of HMF ranged from 2.80 to 24.87 mg/kg, which was within the limits set by the European Commission (up to 80 mg/kg for honey from tropical regions) [12]. On the other hand, the extension of the storage time from 12 to 24 months resulted in exceeding the permissible standard for HMF levels and ranged from 128.19 to 1131.76 mg/kg. HMF has both harmful and positive effects on the human body. Most of the studies and observations in this regard have been carried out primarily on mice and rats under laboratory conditions. HMF and its derivatives are genotoxic,

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mutagenic, carcinogenic, and destructive to DNA. The compounds also inhibit the work of enzymes in organisms. HMF is an indirect mutagen. It is converted into sulfuric acid ester (VI): 5-sulfoxymethylfurfural (SMF) as a result of activation by sulfotransferases in the liver. SMF is a highly mutagenic compound [3]. Hence, HMF and its derivative SMF are strong carcinogens, a fact proven in a great number of studies. HMF is cytotoxic in high concentrations, it irritates mucous membranes, skin, eyes, and upper respiratory tracts [5]. It is a selective inhibitor of a DNA polymerase enzyme, which disturbs or completely impedes a DNA synthesis during DNA replication or repair [4]. HMF has been shown to have a positive effect on human health. Zhao et al. showed in their studies that HMF can remove free radicals depending on the dose (0.8-6.4 mM) [16]. HMF also increases survival when oxygen levels are low resulting from, for example, hypoxia caused by staying at high altitude (mountain climbing), atherosclerosis, or cancer. Therefore, it can be a therapeutic agent used for treating mountain sickness, cerebral edema, or pulmonary edema at high altitudes [6,7]. HMF measurement is used to assess the quality of honey. It is generally present in trace amounts or does not occur in fresh honey. There are many methods used to determine HMF levels in honey. 5-hydroxymethylfurfural content is determined in a clear, filtered, aqueous solution of honey using a method based on the HPLC technique with a reversed phase and UV detection [6,7,17]. Whereas the International Honey Commission recommends the use of three methods: two spectrophotometric methods White & Winkler, and the HPLC method [18]. In addition, other methods are used to determine HMF in various types of samples, not necessarily honey [19]: HPLC with refractive index detector (HPLC-RID), HPLC coupled with mass spectrometry (LC-MS), gas chromatography coupled with mass spectrometry (GC-MS), [20] electrochemical biosensors [21], micellar electro-kinetic capillary chromatography (MECK) [22].

The objective of this study was to determine 5-hydroxymethylfurfural (HMF) levels in honeys available on the Polish market with three methods: two spectrophotometric by White and Winkler, and one developed for this work based on the LC-MS/MS technique. The indirect aim of the study was to assess the usefulness of all three methods for determining HMF content in honey.

2. Materials and methods

2.1. Samples

Seven honey samples: (1) – honeydew; (2) - honey from forest areas; (3)- nectar phacelia honey; (4) - rapeseed honey; (5) - honeydew honey; (6) - multiflower nectar honey; (7) - multiflower honey collected from local producers (in Poland in the Greater Poland region) and available for general sale in shops and supermarkets were assessed for their HMF content. Each time, three original packages of honey were purchased for analysis.

2.2. Apparatus and reagents

In the work, the following compounds were used for honey determination: potassium hexacyanoferrate (II) trihydrate: purity ≥ 99.5%, POCh, Poland; p-toluidine: high purity grade (99.6%) and barbituric acid: high purity grade (99%) purity, Sigma Aldrich, St. Louis, MO, USA; zinc acetate dihydrate: analytical grade, Merck, Germany, sodium bisulfate: analytical purity, Sigma Aldrich, China, 5-hydroxymethylfurfural: purity ≥ 99%, Sigma Aldrich, China, MS-grade methanol, acetonitrile, and isopropanol were provided by Sigma-Aldrich (St. Louis, MO, USA). The formic acid (90%) used as an addition to the mobile phase was purchased from Baker (UK). Water was prepared by reverse osmosis in a Demiwa system followed by double distillation from a quartz apparatus. Only freshly distilled water was used. HMF was analyzed using high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). LC analysis was performed using the chromatographic system UltiMate3000 RSLC from Dionex (Sunnyvale, CA, USA) connected in series with a 4000 QTRAP Hybrid Triple Quadrupole Linear Ion Trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Spectrophotometric methods for determining HMF content were performed using a Red Tide

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USB650UV spectrometer, Ocean Optics, the USA. Measurements were performed using a 10 mm quartz cuvette.

2.3. Procedures

2.3.1. Preparation of solutions for HMF determination

HMF levels in the analysed honey samples were determined with the LC-MS/MS technique and spectrophotometric methods by White and Winkler, described by the International Honey Commission [18]. Before HMF determinations, the following solutions were prepared for honey samples:

- Carrez I: 15 g of potassium hexacyanoferrate (II) trihydrate was dissolved in distilled water in a 100 mL volumetric flask;
- Carrez II: 30 g of zinc acetate dihydrate was dissolved in distilled water in a 100 mL volumetric flask;
- 0.2% sodium bisulfate (IV) solution: 0.1 g sodium bisulfate (IV) was dissolved in distilled water in a 50 mL volumetric flask (prepared immediately before use);
- p-toluidine solution: 10 g of p-toluidine was dissolved in propan-2-ol in a 100 mL volumetric flask. Preparation of the solution must take place 24 hours before use and can be used up to three days;
- 0.5 % barbituric acid solution: 0.5 g of barbituric acid was dissolved in distilled water in a 100 mL volumetric flask.

2.3.2. The White spectrophotometric method

The spectrophotometric method by White, described by the International Honey Commission, was used to determine HMF content [18]. Five grams of honey were dissolved in 25 mL of distilled water and transferred quantitatively into a 50 mL volumetric flask. Then, the Carrez solution I (0.5 mL) and the Carrez solution II (0.5 mL)were added to the honey solution and made up to 50 mL with water. Such a prepared solution was filtered through a filter paper, rejecting the first 10 mL of the filtrate. The next step aliquots of 5 mL were put in two flasks. One flask was filled with water up to the mark (sample solution), while the other was filled with 5 mL of 0.2% sodium bisulfate (IV) (reference solution).The absorbance of the solutions at 284 and 336 nm was determined. The HMF content in honey was calculated using the following equation [18,23,24]:

$$HMF = (A_{284} - A_{336}) \cdot 149.7 \cdot 5 \cdot D/W \text{ [mg/kg]} \quad (1)$$

where: A_{284} is the absorbance at 284 nm, A_{336} is the absorbance at 336 nm, 149.7 = (126·1000·1000)/(16830·10·5) is constant, 126 is molecular weight of HMF, 16830 is molar absorptivity ε of HMF at λ = 284 nm, 1000 is conversion g into mg, 10 - conversion 5 into 50 ml, 1000 is conversion g of honey into kg, 5 is theoretical nominal sample weight, D is dilution factor in case dilution is necessary and W is weight in g of the honey sample.

2.3.3. The Winkler spectrophotometric method

In Winkler's method, 10 g of honey was weighed into a beaker and 20 mL of distilled water was added. The content was mixed using a magnetic stirrer. The mixed solution was transferred to a 50 mL volumetric flask. Then 1 mL of Carrez solution I and 1 mL of Carrez solution II were added to the honey solution, and filled with distilled water up to the mark. Such a prepared solution was filtered through a filter paper, discarding the first 10 mL. The next step was to collect 2 mL of the filtered honey into two 10 mL flasks. 5 mL of p-toluidine solution was added to both of them. Then, 1 mL of distilled water (reference solution) was added to one flask, and to the second,1 mL of 0.5% barbituric acid solution was added (sample solution). The absorbance of the solutions was determined at 550 nm. HMF content was calculated from equation [15,18,23,24]:

$$HMF = 192 \cdot A \cdot 10/W \text{ [mg/kg]} \quad (2)$$

where: A is absorbance, 192 is factor for dilution and extinction coefficient and W is the weight of honey in grams.

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2.3.4. The LC-MS/MS method

The chromatographic separations were obtained in a temperature 35°C on a 100 x 2.1 mm id, particle size 1.9 µm, Hypersil Gold C18 analytical column with guard column, both supplied by Thermo Scientific (USA). The mobile phase was a mixture of 0.1% aqueous formic acid (A) and methanol (B). The flow rate was 0.2 mL/min, the injection volume was 2 µL. The following gradient used: 0 min 70% B, 2 min 100% B, 2.5 min 100% B. The ESI interface was implemented in positive ion mode. The analyses were performed in multiple reaction monitoring, monitoring two transitions between the protonated precursor ion and the most abundant fragment ions for the compound. HMF was detected using the following settings for the ion source and mass spectrometer: curtain gas 10 psi, nebulizer gas 40 psi, auxiliary gas 40 psi, temperature 350°C, ion spray voltage 5500 V, collision gas set to medium. The MS/MS parameters used for quantitative HMF determination are shown in Table 1, where DP is decluttering potential, CE is collision energy and CXP is collision cell exit potential,

Table 1. MS/MS operating parameters for individual MRM pairs for HMF determination**.**

Pseudo molecular ion [M+1]+	DP [V]	MRM	CE [V]	CPX [V]
127	46	127 → 109*	15	8
127	81	127 → 81	23	6
127	53	127 → 53	31	8

*MRM for quantitative analysis.

2.3.5. Validation of the LC-MS/MS method

For the LC-MS/MS method, there were determined validation parameters, i.e.: limit of detection (LOD), limit of quantification (LOQ) and the linearity range. Also, the measurement of the expanded uncertainty (U) was calculated for the following HMF concentrations 0.001 µg/mL; 0.0025 µg/mL; 0.005 µg/mL; 0.01 µg/mL; 0.025 µg/mL; 0.05 µg/mL; 0.1 µg/mL; 0.25 µg/mL; 0.5 µg/mL. The limit of detection (LOD) for HMF, defined as the concentration that yielded S/N ratio greater than or equal to 5, and the limit of quantification (LOQ), defined as the concentration that yielded S/N ratio greater than or equal to 10, were determined for HMF in methanol (the solvent used for the introduction of the compound into the LC-MS/MS system).Determination of the linearity range. Linearity was determined for a series of standard solutions of the analyzed HMF content in the concentration range from 0.000025 µg/mL to 1 µg/mL.

Calculations regarding the measurement of expanded uncertainty were made based on the Ishikawa diagram (Figure 2), which shows the influence of the uncertainty of individual parameters of the analytical process on the final uncertainty of preparation of the standard solution. The expanded uncertainty was calculated using formulas (3) and (4) [25], where U denotes the expanded uncertainty, k the coverage factor (usually 2), U(c1…c5) the uncertainty of the standard solutions of appropriate concentrations, RSD results the RSD of the results, and the number of independent determinations:

$$U\frac{(c5)}{c5} = \sqrt{[U(c1)^2 + U(c2)^2 + U(c3)^2 + U(c4)^2 + U(c5)^2 + (\frac{RSD^2}{n})]} \quad (3)$$

$$U = k \cdot U(c5) \quad (4)$$

The Statistica program was used to analyze the obtained results.

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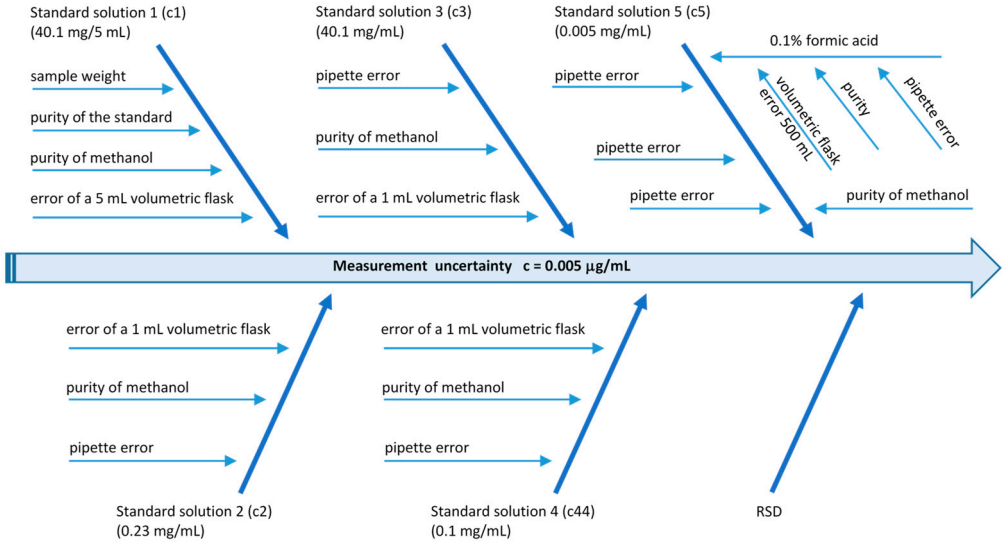

Figure 2. The Ishikawa diagram for a HMF solution with the concentration of c = 0.005 µg/mL.

2.3.6. Preparation of honey for determinations by LC-MS/MS

As in the case of spectrophotometric methods, Carrez I and Carrez II solutions were also used during the preparation of honey samples for determinations by LC-MS/MS. Weights of honey samples were determined based on the results obtained from spectrophotometric measurements, so that 300 µL of the honey sample solution contained approximately 0.05 µg of HMF. After weighing, the honey sample was dissolved in 25 mL of distilled water. The dissolution process was accelerated by stirring with a magnetic stirrer. During the next stage, the mixed solution was poured into a 50 mL flask, where 1 mL of Carrez solution I and 1 mL of Carrez solution II were added and then filled with distilled water up to the mark. Such a prepared solution was filtered through a filter paper, discarding the first 10 mL. Further, the honey sample solution was filtered using PTFE syringe filters with a diameter of 13 mm and a pore size of 0.22 µm to remove solid impurities. Then, 300 µL of the filtered honey solution was taken and 700 µL of methanol was added. The samples thus prepared were subjected to the LC-MS/MS analysis described above.

3. Results and discussion

3.1. Validation of the LC-MS/MS method

The equation of the calibration curve as well as the limit of detection (LOD) and the limit of quantification (LOQ) are shown in Table 2. Linearity was tested by analyzing HMF samples at different concentrations ranging from 0.000025 µg/mL to 1 µg/mL. Good linearity was achieved with correlation coefficients 0.9992. The LOD was 0.0005 µg/mL, while the LOQ was 0.001 µg/mL..

Table 2. Linearity range, limits of detection and quantification for HMF.

Linearity range	Curve equation	Correlation coefficient	LOD [µg/mL]	LOQ [µg/mL]
From LOQ to 0.5 µg/mL	y = 2E+07·x+ 35595	0.9992	0.0005	0.001

The measurement the expanded uncertainty for the method based on the LC/MS-MS technique was calculated for the HMF with the following concentrations from 0.001 to 0.1 µg/mL. The calculations were made on the basis of the dependencies described above. The obtained uncertainties are summarized in Table 3. The measurement the expanded uncertainty increases with the concentration of standard solution of HMF. The lowest concentration was achieved for 0.001 µg/mL.

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Table 3. Measurement the expanded uncertainty of HMF solution with different concentrations.

Concentration [µg/mL]	Measurement uncertainty [µg/mL]
0.001	7.60E-05
0.0025	2.16E-04
0.005	5.50E-04
0.010	7.97E-04
0.025	2.13E-03
0.050	2.59E-03
0.100	5.16E-03
0.250	1.32E-02
0.500	2.72E-02

3.2. Qualitative analysis

A qualitative analysis was performed on the basis of HMF content determinations by LC-MS/MS and spectrophotometric methods. The results of qualitative analysis are presented in Table 4.

Table 4. Qualitative analysis results.

Honey sample number	HMF presence (LC-MS/MS)	HMF presence (White)	HMF presence (Winkler)
1	+	+	-
2	+	-	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+

3.3. Quantitative analysis

Honey samples were prepared following descriptions corresponding to the method used. A quantitative analysis of HMF content in individual honey samples was performed. The standard curve technique was used to determine the content by the method based on the LC-MS/MS technique. In the case of spectrophotometric methods, the quantity of HMF in honey samples was determined based on the equations described above. Each sample was analyzed three times regardless of the method applied. The obtained results are summarized in Table 5.

Table 5. Qualitative analysis results.

Honey sample number	HMF quantity [mg/kg] ± SD (LC-MS/MS)	HMF quantity [mg/kg]± SD (White)	HMF quantity [mg/kg]± SD (Winkler)
1	3.4± 0.2	15.0 ±0.0	0.0 ±0.0
2	2.7± 0.4	0.0±0.0	3.7± 0.1
3	3.7± 0.2	4.5± 0.0	8.8± 1.6
4	2.6± 0.1	1.5± 0.0	3.7± 2.5
5	2.7± 0.2	1.5± 0.0	5.1± 1.3
6	3.0± 0.2	23.5± 1.7	23.4± 0.1
7	3.6± 0.2	28.5± 1.5	20.2± 1.4

Table 4 indicates that the most sensitive method for the determination of HMF is LC-MS/MS. Only in the case of analyzes with this method, HMF was detected in all tested honey samples. Two spectrophotometric methods, White and Winkler, were negative for the presence of HMF in the two honey samples. In the case of the first method, it was sample no. 2, and in the case of the second, sample no. 1. It may result from numerous factors, i.e. the sensitivity of the method, its accuracy or

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repeatability. When comparing the results for honey samples obtained with the method based on the LC-MS/MS technique (Table 5), it was noticed that the highest content of HMF occurred in honey samples number 7 and 3. In none of the tested honey samples, HMF content exceeded the permitted quantity specified in the regulation of the European Commission. The highest HMF content in the tested honey samples determined by the White method was found in honey samples number 6 and 7. HMF was not detected in honey sample number 2, therefore it can be concluded that the value of HMF was below the quantification limit of this method. Similar dependencies were observed for the Winkler method. The highest HMF content was also found in honey samples numbers 6 and 7. However, in honey sample number 1, HMF was not marked, thus it can be concluded that the value of HMF was below the detection limit of this method. When analyzing Table 5, it was noticed that the quantity of HMF differed depending on the method applied. It may result from numerous factors, i.e. the place where the honey sample was taken for testing (external or internal part of the container), the sensitivity of the method, its accuracy or repeatability. It could also be affected by the presence of HMF derivatives (selectivity), which are quantified when testing honey samples using spectrophotometric methods, or by the presence of compounds that facilitate the ionization of HMF in the ion source of the mass spectrometer and overestimate the final result or hinder ionization and underestimate the results obtained by a method based on the LC-MS/MS technique. The obtained results are characterized by high repeatability, which is exhibited through low values of standard deviations.

In [17] emphasized that it is not possible to explain exactly why the methods are not compatible. They suggested that this could be due to the formation of hydroxymethylfurfural precursors. They also looked at the overestimation of the Winkler method compared to the White method and HPLC. In [26] indicated in work that none of the previously used methods of HMF determination in honey reflect its actual content. Thus, it points to the need to develop new methods. Other authors recommend a method based on the HPLC technique with UV detection [11]. Spectrophotometric methods (White and Winkler) are fast but have low specificity and sensitivity. HPLC is more accurate and precise, but time-consuming.

Contrary to other studies, based on our research, it is not possible to unequivocally indicate the best method of determining this compound in honey samples. The method based on the LC-MS / MS technique is supported by its very high sensitivity and selectivity. The latter parameter results from the detection method of fragmentation reaction monitoring. This is a specific example of targeted analysis.

Nevertheless, further research on HMF content in honey should be carried out on a larger sample pool. In addition, it is desirable to develop an LC-MS/MS or other methods to determine not only HMF but also its derivatives, which would give a more complete picture concerning spectrophotometric methods. Furthermore, honey samples should be homogenized before testing, by thorough mixing, which will certainly increase the reliability of HMF content determination. Additionally, it is recommended to take into account the effect of the matrix on the final results of HMF content (overestimation/underestimation) obtained through a method based on the HPLC-MS/MS technique. HMF content in honey samples detected with this method should be determined using a multiple standard addition technique.

4. Conclusions

The validation of 5-hydroxymethylfurfural qualitative and quantitative method based on the HPLC-MS/MS technique was developed and carried out. The developed method was used to determine HMF content in honey samples. The method is distinguished by the linearity range in concentrations from 0.001 μg/mL to 0.5 μg/mL and low values of detection and quantification limits. Both spectrophotometric methods are distinguished by low sensitivity, but high repeatability. HMF contents determined by these three methods are divergent. It may result from numerous factors, i.e. the sensitivity of the method, its accuracy or repeatability, the presence of HMF derivatives, or the presence of compounds that facilitate the ionization of HMF in the ion source and thereby overestimate or lower the results obtained by a method based on the LC-MS/MS technique. The

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determined HMF content in the tested honey samples does not exceed the applicable European Council standard, i.e. they do not exceed the value of HMF content in honey, which is 40 mg/kg. This means that kinds of honey present on the Polish market are of high quality.

Author Contributions: JZ: methodology, formal analysis, investigation, project administration, supervision, writing - original draft; LP: investigation, formal analysis, methodology, validation visualization, writing - original draft; BK: data curation, formal analysis, software, validation, visualization; JB: formal analysis, validation, investigation; EJ-B: formal analysis, funding acquisition, methodology writing - review & editing; JS: formal analysis, supervision, validation, writing - original draft; PK: formal analysis, funding acquisition, project administration writing - review & editing.

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