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DETERMINATION OF IMPURITIES IN MEDICAL PRODUCTS CONTAINING METFORMIN HYDROCHLORIDE

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Abstract: The object of this study was to present a high-performance liquid chromatography (HPLC) method allowing to identify and quantify the impurities in medical products in which the only active substance is metformin hydrochloride. Metformin (dimethylbiguanide) is a biguanide derivative active after oral administration. It reduces the basic and postprandial blood glucose levels in patients with type II diabetes (insulin-independent), with partially maintained insulin secretion. The separation of the impurities was performed using a PARTISPHER SCX column and a spectrophotometric detector (λ = 218 nm). The mobile phase was 1.7% (w/v) ammonium dihydrogen phosphate water solution, with pH adjusted to 3.1 using 85% orthophosphoric acid. The proposed method is rapid, sensitive and selective, and it can be used to evaluate those medical products for which the impurity tests are not currently performed, as well as those for which only cyanoguanidine or cyanoguanidine and melamine assays are performed.

Keywords: metformin hydrochloride, impurities in medical products, HPLC method

Metformin (dimethylbiguanide) is a biguanide derivative in which two hydrogen atoms at the N1 nitrogen have been substituted with methyl groups. It is the only biguanide derivative currently used in the medicine.

The effect of metformin consists in increasing anaerobic glycolysis, leading to reduction of ATP biosynthesis, which in turn leads to glycemia reduction. Therefore, there is a reduction in intestinal absorption of glucose, a reduction in glucose resynthesis in the liver, as well as an increase in tissue glucose consumption as a result of enhancement of the effect of insulin. In addition to the hypoglycemic effect, metformin has very good effects on lipid metabolism, reducing triglycerides, total cholesterol and LDL fraction concentrations. It also has an anti-aggregation effect, reduces tromboglobulin and thromboxane A2 levels. It normalizes the fibrinolytic activity of the vascular endothelium (1, 2).

The medical products containing metformin hydrochloride as the active substance have been used in patients with type II diabetes (insulin-independent), in particular in obese patients who cannot achieve normal blood glucose concentrations despite a diet. In these patients, the pancreas does not produce a sufficient amount of insulin, or the body does not respond normally to insulin, leading to glucose accumulation in the blood. Metformin hydrochloride increases the body's susceptibility to insulin and helps to restore its normal use in the body.

There are many medical products containing metformin as the active substance in the pharmaceutical market. An important element of ensuring their safety of use is to monitor the impurities.

The possible impurities in metformin hydrochloride are the following related substances: cyanoguanidine (impurity No. 1); melamine (impurity No. 2); (4,6-diamino-1,3,5-triazin-2-yl)guanidine (impurity No. 3); N,N-dimethyl-1,3,5-triazine-2,4,6-triamine (impurity No. 4); 1-methylbiguanide (impurity No. 5) and N-methylmethanamine (impurity No. 6).

The objective of our study was to identify and to quantify the first four of the abovementioned impurities (due to the availability of standard substances) in medical products containing metformin hydrochloride, and to determine their limits of detection and quantitation. As the structure of the impurities is similar to that of the active substance, the chromatographic conditions were selected so as to ensure selectivity and sensitivity of the testing method.

EXPERIMENTAL

Equipment

Shimadzu liquid chromatograph with LC-10AT pump, SCL-10A VP control system, SIL-10D VP autosampler, 10AV VP spectrophotometric detector, DGU-14A degasser and a computer with CLASS VP software (version 5.3); Metrohm

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pehameter; Ultron ultrasound bath; Heidolph Unimax shaker.

Standards used

Metformin hydrochloride, 100.3% content; cyanoguanidine, 99% content and, melamine, 99.2% content, all from Aldrich; (4,6-diamino-1,3,5-triazin-2-yl)guanidine, 84.6% content and N,N-dimethyl-1,3,5-triazine-2,4,6-triamine, 99.7% content, from LGC.

Medical products tested

Two medical products (tablets), containing metformin hydrochloride as active substance: Metral – 500 mg/tabl. and Metifor – 850 mg/tabl., produced by two manufactures, were used as tested samples.

Reagents

Ammonium dihydrogen phosphate, analytical grade reagent (FP VI, POCH S.A), water for HPLC and orthophosphoric acid 85% (BDH).

Chromatographic conditions

Column: PARTISPHERE SCX 4.6 × 250 mm (Whatman), column temperature: 40°C; spectrophotometric detector: 218 nm wavelength; mobile phase (phosphate buffer): 1.7% (w/v) ammonium dihydrogen phosphate water solution, with pH adjusted to 3.1 using 85% orthophosphoric acid; mobile phase flow: 1.5 mL/min; injection volume: 40 µL.

Checking chromatographic system performance

In order to: check the resolution between the peaks of cyanoguanidine, melamine and metformin hydrochloride; determine injection repeatability for each standard substance; test the assymetry of peaks of the standard substances tested and to know the number of theoretical plates for the standard substances tested, a checking solution was prepared and injected onto the column five times.

Checking solution

Solution A

Two hundreds fifty mg of metformin hydrochloride standard substance was weighed in a 50 mL calibrated flask. The substance was dissolved in the mobile phase. The mobile phase was added to volume.

Solution B

Ten mg of melamine standard substance and 5 mg of cyanoguanidine standard substance were weighed in a 100 mL calibrated flask. Five mL of solution A was added and water was added to volume.

Table 1. Peak resolution in the solution for chromatographic system checking.

Substances tested	Values of the separation coefficients Rs
Cyanoguanidine Melamine	11.15
Melamine Metformin hydrochloride	25.4
Cyanoguanidine Metformin hydrochloride	29.2

Table 2. Repeatability of injections of the standard substances: cyanoguanidine, melamine and metformin hydrochloride (solution for chromatographic system checking).

Substances tested	Concentration of the tested substance solution in the mobile phase	Peak area	Relative standard deviation RSD [%]
Cyanoguanidine	0.001 mg/mL	402212 395557 400338 398750 399991	0.62
Melamine	0.002 mg/mL	288626 289967 288107 287599 289752	0.36
Metformin hydrochloride	0.005 mg/mL	337119 338545 330527 331752 334585	1.02

Solution C

One mL of solution B was pipetted into a 50 mL calibrated flask and the mobile phase was added to volume. Solution C was injected onto the column five times.

The respective concentrations of standard substances in the prepared solution for chromatographic system checking were: cyanoguanidine: 0.001 mg/mL; melamine: 0.002 mg/mL, metformin hydrochloride: 0.005 mg/mL.

From the chromatograms, the following parameters have been calculated: separation coefficients between the peaks of the tested substances (Rs), number of theoretical plates (N), values of relative standard deviation (RSD) for each peak area and peak asymmetry A (10%). The results are presented in Tables 1–3.

The chromatographic system is considered suitable when it meets the following conditions: – separation coefficient (Rs) between the peaks of melamine and metformin hydrochloride greater or equal 10; – the values of relative standard deviation (RSD) for areas under peaks of the standard substances tested not exceeding 2%; – number of theoretical plates (N) for the standard substances tested equal to 2000 or more; – peak asymmetry A (10%) lower or equal 2.

Determination of impurity content in medical products

Preparation of standard solution C

Five milligrams of melamine, (4,6-diamino-1,3,5-triazin-2-yl)guanidine and N,N-dimethyl-1,3,5-triazine-2,4,6-triamine standard substances were weighed into a 10 mL calibrated flask. The standard substances were dissolved and filled up to volume with water.

Preparation of standard solution D

Ten milligrams of cyanoguanidine standard substance were weighed in a 100 mL calibrated flask. Ten milliliters of solution A were added and water was added to volume.

Preparation of the final standard solution K

Half milliliter of standard solution C and 0.5 mL of standard solution D were transferred into a 50 mL calibrated flask and the mobile phase was added to volume.

The respective concentrations of the tested standards were: – cyanoguanidine: 0.001 mg/mL, corresponding to 0.02% metformin hydrochloride in the prepared sample; – melamine: 0.005 mg/mL, corresponding to 0.1% metformin hydrochloride in the prepared sample; – 4,6-diamino-1,3,5-triazin-2-yl)guanidine: 0.005 mg/mL, corresponding to 0.1% metformin hydrochloride in the prepared sample; – N,N-dimethyl-1,3,5-triazine-2,4,6-triamine: 0.005 mg/mL, corresponding to 0.1% metformin hydrochloride in the prepared sample; – metformin hydrochloride: 0.005 mg/mL corresponding to 0.1% metformin hydrochloride in the prepared sample; (the peak areas for unidentified peaks cannot exceed the peak area of this standard).

Standard solution K was injected onto the column three times. The obtained chromatograms are presented in Figure 1. The values of the separation coefficients Rs are presented in Table 4.

Table 3. Peak asymmetry and number of theoretical plates for the standard substances: cyanoguanidine, melamine and metformin hydrochloride (solution for chromatographic system checking).

Substances tested	Peak asymmetry (10%) (mean value)	Number of theoretical plates (mean value)
Cyanoguanidine	1.13	2922
Melamine	0.97	5060
Metformin hydrochloride	1.11	5169

Table 4. Resolution of the peaks of the impurities tested in standard solution K.

Substances tested	Values of the separation coefficients Rs	Retention times [min]
Cyanoguanidine (peak No. 1) Melamine (peak No. 2)	11.15	1.7 2.9
Melamine (peak No. 2) (4,6-Diamino-1,3,5-triazin-2-yl)guanidine (peak No. 3)	15.9	2.9 6.7
(4,6-Diamino-1,3,5-triazin-2-yl)guanidine (peak No. 3) metformin hydrochloride (peak No. 4)	13.1	6.7 14.7
metformin hydrochloride (peak No. 4) N,N-dimethyl-1,3,5-triazine-2,4,6-triamine (peak No. 4)	3.12	14.7 20.8

Table 5. Percent content of the impurities tested in the selected medical products containing metformin hydrochloride as the active substance.

Product	Impurity content in a tablet [%]				
	No. 1	No. 2	No. 3	No. 4	Unidentified impurities
Metral 500 mg/tabl.	0.003 0.003 0.004 0.004 0.003 0.003	0.009 0.009 0.009 0.009 0.008 0.009	0.025 0.025 0.026 0.026 0.025 0.025	no	sum is not greater than 0.1
	$\bar{x}$ = 0.003 SD = 0.0005	$\bar{x}$ = 0.009 SD = 0.0004	$\bar{x}$ = 0.025 SD = 0.0004 RSD = 1.62%	no	no
Metifor 850 mg/tabl.	0.012 0.011 0.012 0.012 0.011 0.012	0.004 0.004 0.005 0.004 0.004 0.004	0.021 0.020 0.021 0.021 0.021 0.021	no	sum is not greater than 0.1
	$\bar{x}$ = 0.012 SD = 0.0005	$\bar{x}$ = 0.004 SD = 0.0004	$\bar{x}$ = 0.021 SD = 0.0004 RSD = 1.96%	no	no

Table 6. Detection and quantitation limits for the impurities tested.

Substances tested	Limit of detection [µg/mL]	Limit of quantitation [µg/mL]	Allowed concentration in medical products [µg/mL]
Cyanoguanidine	0.01	0.03	1
Melamine	0.04	0.12	5
(4,6-Diamino-1,3,5-triazin-2-yl)guanidine	0.15	0.50	5
N,N-dimethyl-1,3,5-triazine-2,4,6-triamine	0.50	1.6	5

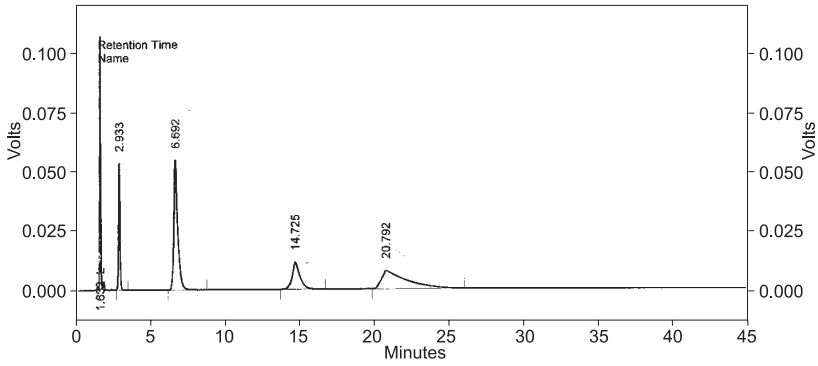

Figure 1. Chromatogram of standard solution K; peak with retention time of ~1.6 min (impurity No. 1); peak with retention time of ~3.0 min (impurity No. 2); peak with retention time of ~6.7 min (impurity No. 3); peak with retention time of ~14.7 min – metformin hydrochloride; peak with retention time of ~20.7 min (impurity No. 4)

Sample preparation

The amount of tablet mass corresponding to 500 mg of the active substance was weighed accurately into a 100 mL calibrated flask. Sixty mL of mobile phase was added and the flask was shaken, then the mobile phase was added to volume. The

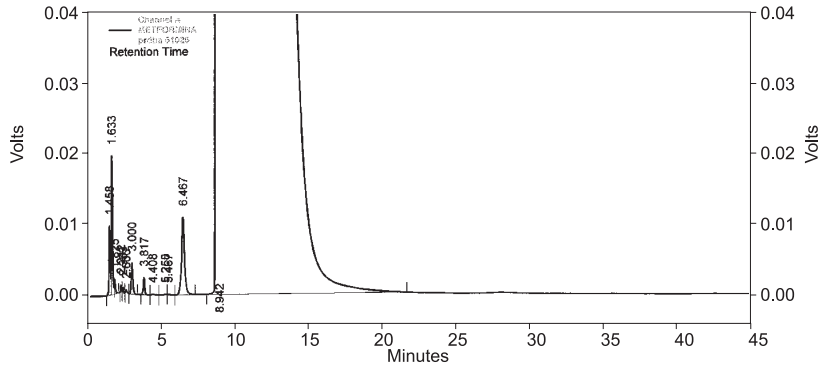

Figure 2. Representative chromatogram of the sample (Metral 500 mg/tabl.); peak with retention time of ~1.6 min (impurity No. 1); peak with retention time of ~3.0 min (impurity No. 2); peak with retention time of ~6.5 min (impurity No. 3); peak with retention time of ~14.7 min - metformin hydrochloride

solution was filtered through a disposable 0.45 µm filter. Metformin hydrochloride concentration in the tested solution was 5 mg/mL.

Six independent samples were prepared for each medical product. Each sample was injected three times.

The analysis of the tested samples was performed in a time twice exceeding the retention time of metformin hydrochloride. The representative chromatogram is presented in Figure 2. The content of the impurities in the sample was calculated and presented in Table 5.

Checking the linearity range for the impurities tested

The following concentrations of the impurities tested were prepared: cyanoguanidine: 0.01, 0.20, 0.50, 0.75, 1.00, 1.25 and 1.50 µg/mL; melamine: 0.04, 0.50, 2.50, 5.00 and 7.50 µg/mL. (4,6-diamino-1,3,5-triazin-2-yl)guanidine: 0.15, 0.50, 2.50, 3.40, 5.00 and 7.50 µg/mL; N,N-dimethyl-1,3,5-triazine-2,4,6-triamine: 0.50, 1.00, 2.50, 3.40, 5.00 and 7.50 µg/mL.. Each solution was injected onto the column three times. The peak areas were recorded. The mean values of peak areas for each concentration were calculated and the relationship between the peak area and the injected concentration was obtained.

Determination of the detection and quantitation limits for the impurities tested

For each impurity, the detection and quantitation limits have been determined. The detection limit was defined as the concentration at which the peak signal was three times higher than the noise signal, and the quantitation limit was defined as the concentration ten times higher than the noise signal. The results are presented in Table 6.

RESULTS AND DISCUSSION

The literature contains many descriptions of methods for determination of metformin hydrochloride using the high-performance liquid chromatography, or liquid chromatography methods in combination with mass spectrometry. These articles describe the determination of metformin hydrochloride both in medical products (3–7) and in biological materials (8–12); however, there are virtually no studies focusing on testing the levels of metformin hydrochloride impurities. Wang et al. performed a separation of metformin hydrochloride impurities using an reversed phase column with a UV detector and two different mobile phases (one for determination of cyanoguanidine, the other for the remaining related substances) (13).

Shahid Ali et al. developed and validated a method for simultaneous determination of metformin hydrochloride and its impurities in tablet formulation (14). They used a Hydrophilic Interaction Liquid Chromatography (HILC) technique, which requires a specific column and special analysts.

Al-Rimawi described the other method of determination of metformin hydrochloride and its single related compound (1-cyanoguanidine) in tablets by HPLC method (15). He used a Nova-Pak silica column and UV detector at a wavelength of 232 nm. Isocratic elution was employed using a mixture of ammonium dihydrogen phosphate buffer and methanol (21:79, v/v).

According to the Ph. Eur. and USP monographs for metformin hydrochloride (substance), only the cyanoguanidine assay (impurity No. 1) and tests for individual unidentified impurities are performed. The maximal content of cyanoguanidine should be 0.001 mg/mL (0.02%), and the content of unidentified impurities should not exceed the value

of 0.005 mg/mL (0.1%), with the sum of all identified impurities not exceeding 0.03 mg/mL (0.6%) at metformin hydrochloride concentration of 5 mg/mL.

However, according to the USP monograph for tablets containing metformin hydrochloride as the active substance, only the level of unidentified impurities is specified. Their amount cannot exceed 0.005 mg/mL (0.1%), and the sum of all impurities cannot exceed the value of 0.03 mg/mL (0.6%) at metformin hydrochloride concentration of 5 mg/mL. The Polish Pharmacopoeia contains no monograph for metformin hydrochloride.

Chromatographic conditions presented in this work allow for selective separation of all four impurities: cyanoguanidine, melamine, (4,6-diamino-1,3,5-triazin-2-yl)guanidine and N,N-dimethyl-1,3,5-triazine-2,4,6-triamine.

The Rs separation coefficients between the peaks of impurities: 1–2, 2–3 and 3–4 are higher than 10. The Rs value for peaks 4–5 is also satisfactory, being equal to 3.12.

The obtained results for the checking solution indicate that the chromatographic system used for the separation of the impurities tested meets the required acceptance criteria.

The dependence between the detector indications and the concentration of the impurity tested is linear in the following range: cyanoguanidine: from 0.01 μg/mL to 1.5 μg/mL; melamine: from 0.1 μg/mL to 10 μg/mL; (4,6-diamino-1,3,5-triazin-2-yl)guanidine: from 0.5 μg/mL to 10 μg/mL; N,N-dimethyl-1,3,5-triazine-2,4,6-triamine: from 1.0 μg/mL to 10 μg/mL.

The determined quantitation limits for the impurities tested are many times lower than the allowed limits in medical products – in the case of cyanoguanidine thirty times, whereas for melamine as much as forty times. High sensitivity of the method allowed a precise determination of the content of impurities in the selected medical products. In the medical products tested, the content of cyanoguanidine, melamine, (4,6-diamino-1,3,5-triazin-2-yl)guanidine were significantly below the accepted limits (0.1%).

N,N-dimethyl-1,3,5-triazine-2,4,6-triamine was not detected. Also the sum of unidentified impurities in the medical products tested has not exceeded the value of 0.1%.

The statistical analysis showed sufficient precision of the proposed method. The RSD values for the tested impurity No. 3 are below 2%. For the other identified impurities (cyanoguanidine, melamine), the values of mean are close to zero, and in such a case it is known that the results concerning relative standard deviation RSD are not reliable; therefore, only the standard deviation SD values are presented in the table.

CONCLUSIONS

The chromatographic conditions with Partisphere SCX column used for impurity separation in medical products containing metformin hydrochloride allow to evaluate the main four impurities of the active substance. The presented method is rapid, sensitive and selective, and it can be used to evaluate the quality of those medical products for which the impurity tests are not currently performed, as well as those for which only cyanoguanidine or cyanoguanidine and melamine assays are performed. The introduction of the above method to quality control shall improve the safety of use of the medical products containing metformin hydrochloride as the active substance.

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