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Analysis Of Different Brands Of Metformin Hydrochloride Tablets Using Ultra Violet Spectrophotometric And High Performance Liquid Chromatographic Methods.

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ABSTRACT

Metformin hydrochloride is an oral antidiabetic from group of the biguanidine with an antihyperglycaemic effect, which reduces basal and postprandial glycaemia. It does not stimulate insulin secretion and does not cause hypoglycemia. It works through three mechanisms: (1) reducing hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis; (2) increasing the sensitivity of the cell to insulin by increasing the peripheral acquisition and utilization of glucose in the muscles; (3) slowing down the intestinal absorption of glucose. In this paper, we conducted a pharmaceutical analysis of products with metformin hydrochloride from the market of Bosnia and Herzegovina. The analysis of metformin hydrochloride in six tested samples of various manufacturers includes the examination of appearance, average weight of the tablet, content uniformity, assay determination, dissolution test and related substances. The results of the study showed that all the tested samples correspond to the specific requirements of the quality of the official pharmacopoeia monographs.

Keywords: metformin hydrochloride; pharmaceutical analysis; spectrophotometric determination; related substance

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INTRODUCTION

Metformin hydrochloride (1,1-Dimethylbiguanide hydrochloride) according to the chemical structure belongs to the bigvanide derivatives (5). This molecule is freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone and in methylene chloride (11).

Our manuscript aimed to show analysis of finished pharmaceutical product on Bosnian market according to ICH 3AQ11a and official Pharmacopoeia (1, 2, 6, 8).

METHOD

Metformin hydrochloride was from LGC, and all other reagents and chemicals were HPLC and analytical grade purity. Water was from Millipore system. To determine appearance, uniformity of mass, uniformity of dosage units (by mass variation), disintegration, content of Metformine Hydrochloride, dissolution and related substances in conventional tablet available in Bosnian market, randomly select products and labeled as sample 1 to sample 6. Samples from 1 to 3 are 500mg strength, and samples from 4 to 6 are 850mg strength.

Appearance

Samples of 20 tablets from each batch were randomly selected and their properties analyzed such as color, shape, shape of the surface, the presence of the described grooves and monograms all on based on visual observation.

Uniformity of mass

Uniformity of mass was tested according to European Pharmacopoeia. Individually point to twenty randomly selected dosage forms, or if each one is one the preparation separately packaged takes up the contents of 20 packs, and calculates the average mass. Only two average masses may deviate more than the permissible percentage deviation, according to European Pharmacopoeia, and no average mass may deviate by more than twice values of permitted percentage of deviation (10).

Uniformity of dosage units

The test for mass variation is applicable for film-coated tablets, containing 25mg or more of an active substance comprising 25 per cent or more, by mass, of the dosage unit and calculate the acceptance value according European Pharmacopoeia 2.9.40 (7).

Disintegration

Disintegration test was performed according to the requirements of European Pharmacopoeia, using water is used as a medium and the test is carried out at a temperature of 37°C (9).

Content of Metformin Hydrochloride

UV-VIS spectrophotometer Shimadzu was used in this study. Standard solution was prepared in water at a concentraion of 0.005 mg/ml. Linearity was determined by six different concentration (0.0025, 0.004, 0.0045, 0.005, 0.0055 and 0.006 mg/ml). Also, linearity was evaluated using the calibration curve. Acceptable value for correlation coefficient in general is (r^2)>0.998. To determine the content of Metformin Hydrochloride in conventional tablets, twenty tablets were weighed, mean weight determined, then finely powedered. The weight of the tablet powered equivalent to 10 mg of Metformin Hydrochloride was transfered into 100 ml flask, added 70 ml of water, sonicated for 15 minutes, and diluted up to 100 ml with water. Solution was filtrated through filter paper blue ribbon, discarding first mililiters of filtarte. The above stock solution was diluted to get sample solution of 0.005 mg/ml. The maximum absorbance was found at 232 nm for all solutions.

Dissolution

Dissolution test was performed according to USP monograph for Metformin Hydrochloride Tablets, Test 1, using phosphate buffer pH 6.8 as a medium, USP apparatus 1 (method of rotating basket), 100 rpm/min, with duration time 45 minutes. Release contetn was determined by UV-VIS spectrophotomer at 233 nm.

Related substances

Related substance was performed according to BP monograph for Metformin Tablets. The HPLC system consisted of Agillent 110. As a stationary phase was column 125 mm x 4.6 mm, 5 µm, ample loop 20 µl, with 17 g/l solution of ammonium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid as a mobile phase. Detection was at 218 nm, and flow rate 1.0 ml/min. Standard solution for 1-cyanoguanidine was performed by dissolving 20 mg of this supbstances into 100 ml water, then diluted 1 ml of the resultaing solution to 200 ml with mobile phase. Standard solution for metformin hydrochloride was performed by dissolving 25 mg of this supbstances into 50 ml water, then diluted 1 ml of the resultaing solution to 100 ml with mobile phase. Sample solutions were prepared by dissolving 0.125 g of fine tablet poweder into 25 ml of mobile phase. The resulting solutions were centrifuged at 4500 rpm/min for 15 minutes.

RESULTS

Appearance

From these visual observations, we can say that all samples are of adequate appearance, without any flaws and damage (Fig. 1).

Fig. 1: Appearance for tested samples

Uniformity of mass

From the below results, all the tested samples satisfy the criteria defined by the European Pharmacopoeia Requirement 2.9.5. (Fig. 2).

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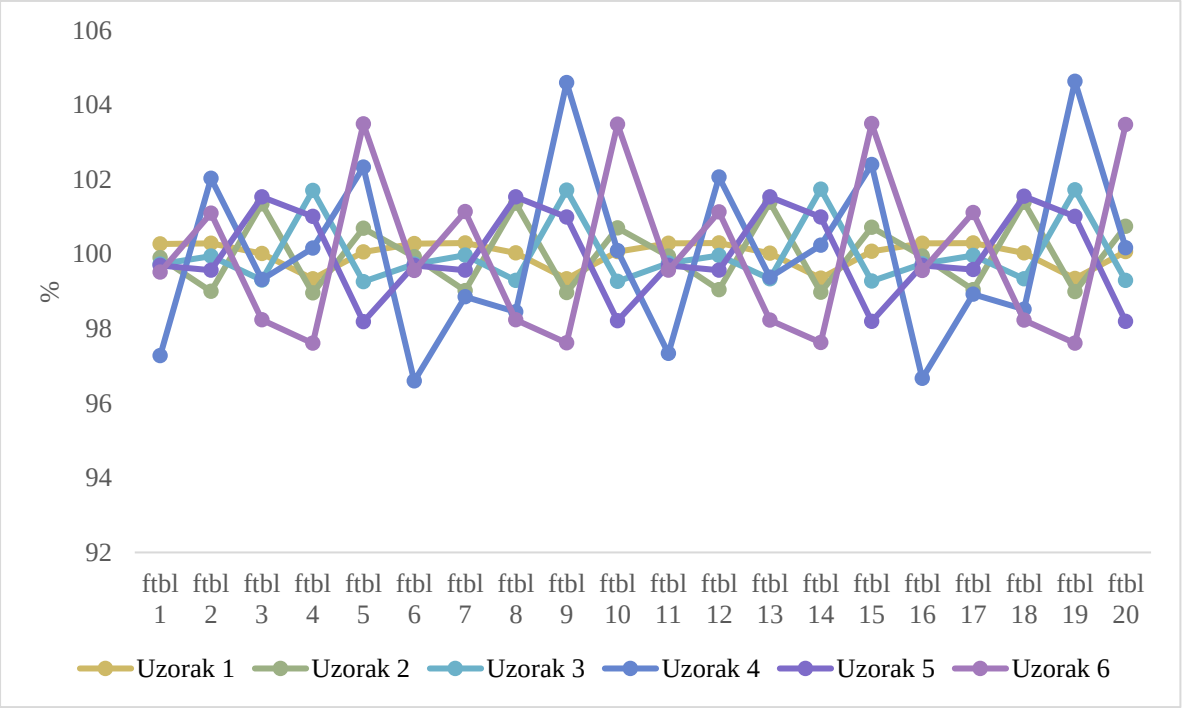

Fig. 2: Uniformity of mass for tested samples

Uniformity of dosage units

From the below results, all tested samples meet the criteria defined by the n European Pharmacopoeia 2.9.40. Acceptable values of parameter L are less than 15 (Fig. 3).

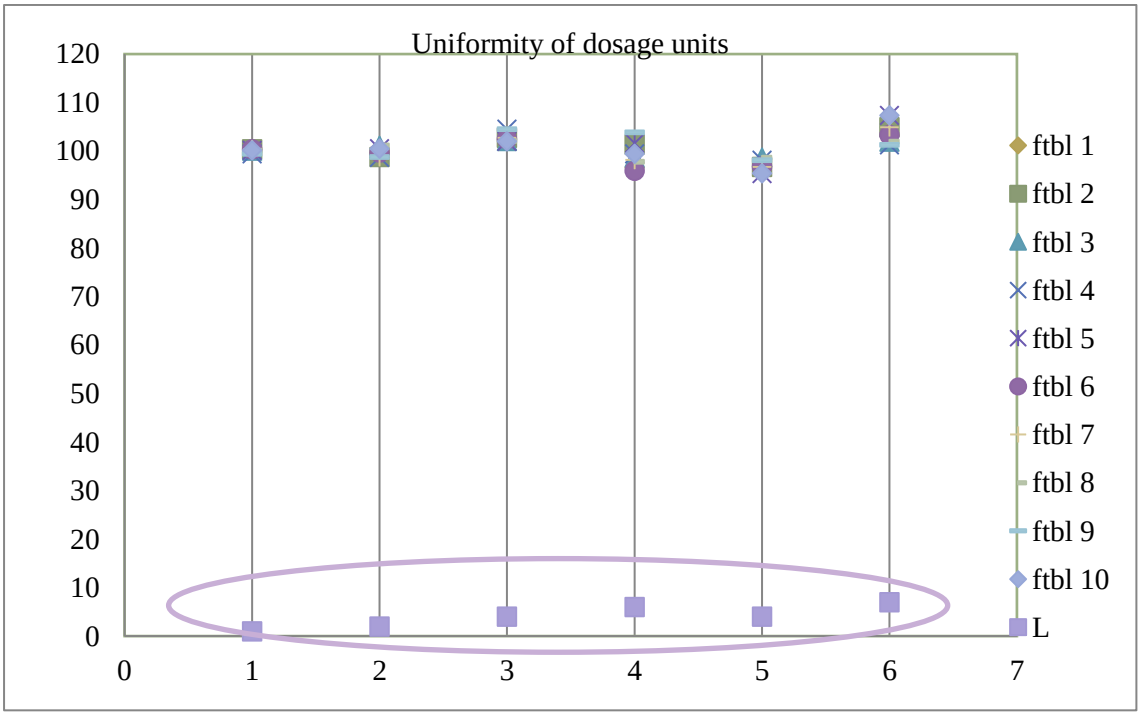

Fig. 3: Uniformity of dosage units for tested samples

Disintegration

From the table results, all tested samples satisfy criteria under the general requirement of Ph.Eur. 2.9.1, for the category of film-coated tablets, maximum 30 minutes, in water at 37ºC (Table 1).

Table 1: Disintegration test results

	1	2	3	4	5	6
max. 30 minutes	02:55-03:23	09:02-15:43	10:48-11:07	07:27-09:32	05:27-06:17	07:30-08:14

Content of Metformin Hydrochloride

Verification of method was tested in the range from 0.0025 to 0.006 mg/ml, with linear relationship r=0.9997 (Fig. 4). From the below results, we see that all samples meet the quality requirements of the ICH 3AQ11a guidelines. Specifications and Control Tests on Finished Products, as well as USP and BP requirements 95.0% -105.0% (Fig. 5).

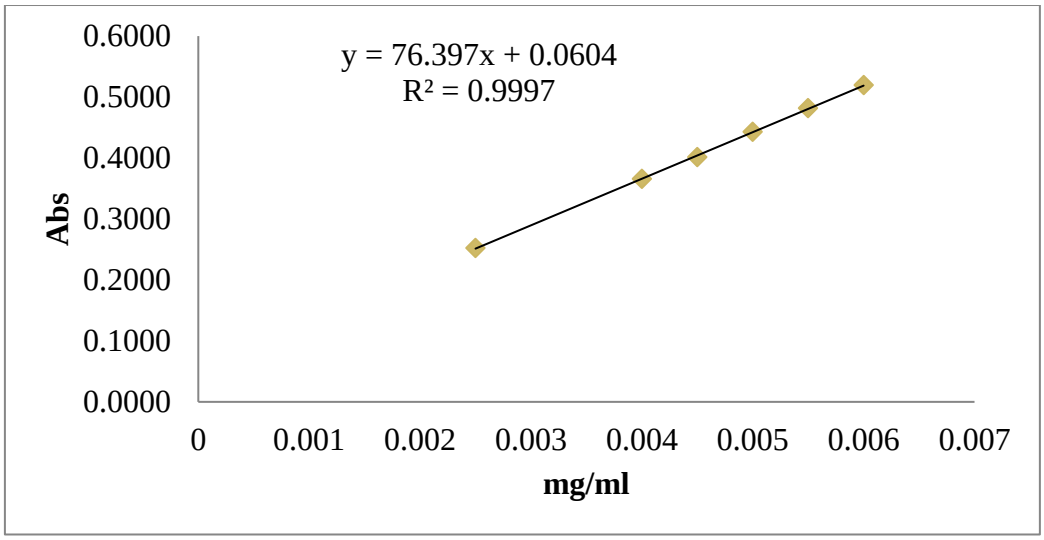

Fig. 4: Standard calibration curve of Metformin Hydrochloride ranging from 0.0025 to 0.006 mg/ml

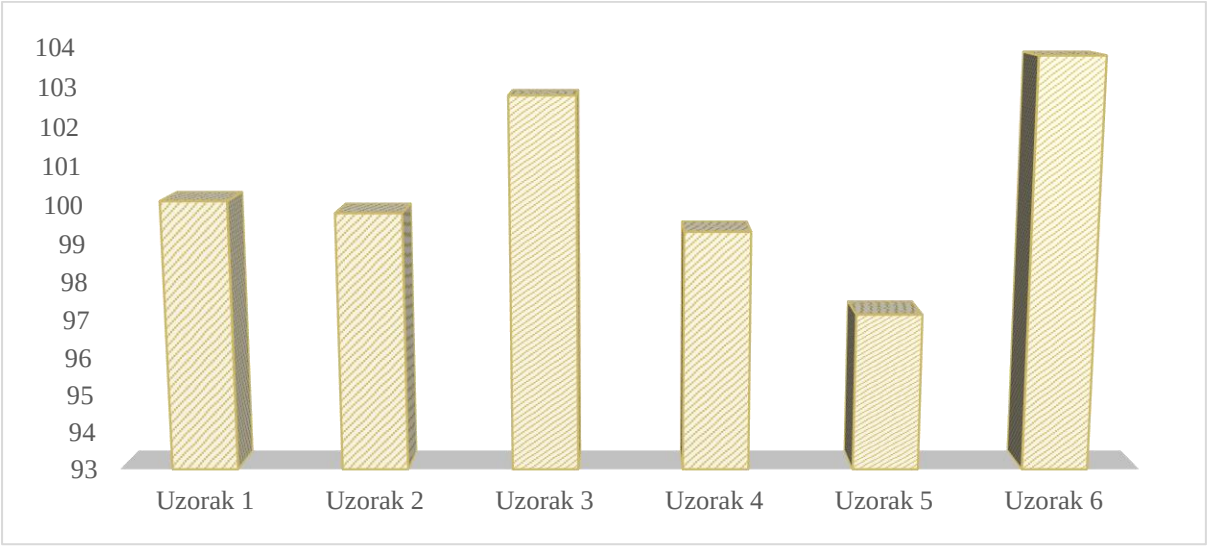

Fig. 5: Content for tested samples

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Dissolution

From the below results, we see that all samples meet the quality requirement of at least 70% (Q), for 45 minutes, which is also the quality requirement of USP, test 1 (Fig. 6).

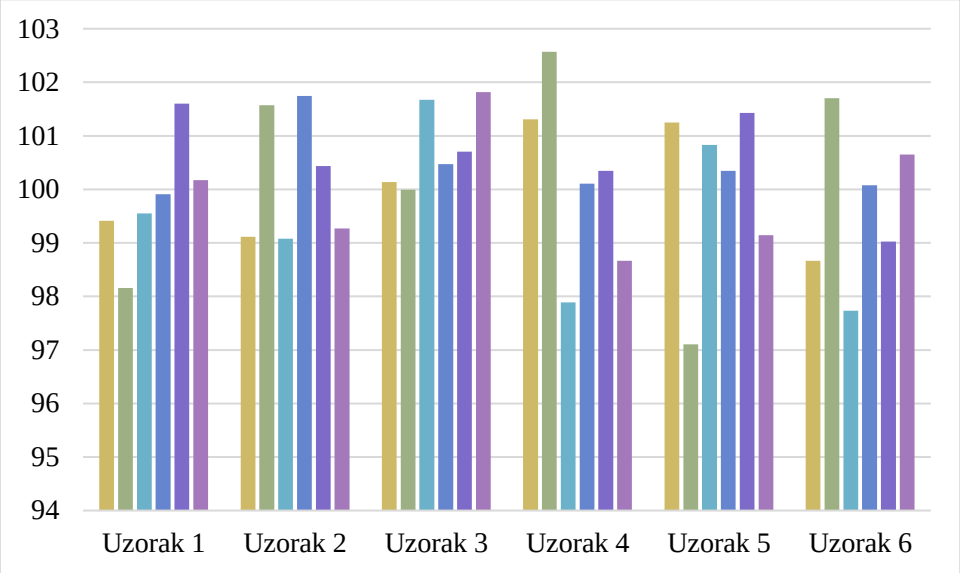

Fig. 6: Dissolution test for tested samples

Related substances

The samples tested were prepared as described in the methods, and in accordance with BP monograph for Metformin tablets. In the stated monograph as a requirement the quality is indicated only by the designated impurity 1-cyanogvanidine. We also set croiteria for individual unknown impurity and total impurities which is consistent with ICH Q3B (R2) (4). The test results are shown in Table 2. In Fig. 7 and Fig. 8. are chromatograms for 1-cyanogvanidine standard solution and for metformin hydrochloride standard solution.

Table 2: Realted substances test results

	1-cyanogvanidine	individual unknown impurity	total impurities
1	0.01%	0.02%	0.09%
2	0.01%	0.02%	0.03%
3	0.01%	0.01%	0.04%
4	0.01%	0.01%	0.04%
5	0.01%	0.02%	0.07%
6	0.01%	0.01%	0.03%

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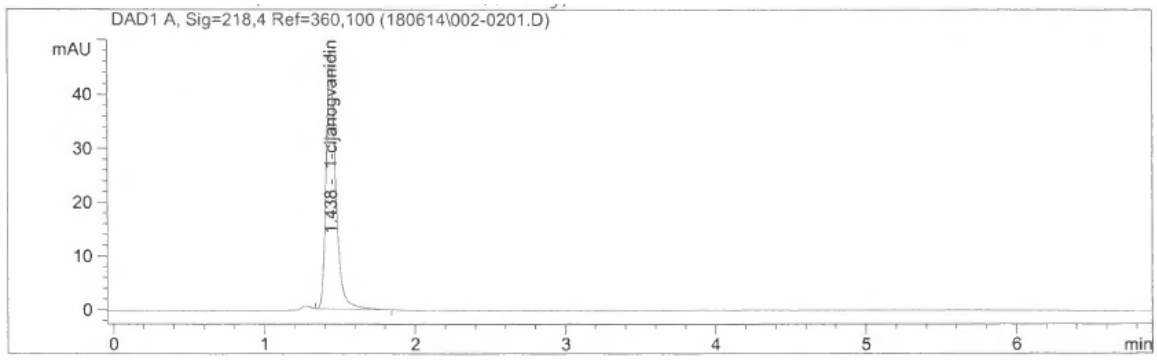

Fig. 7: HPLC chromatogram for 1-cyanogvanidine standard solution

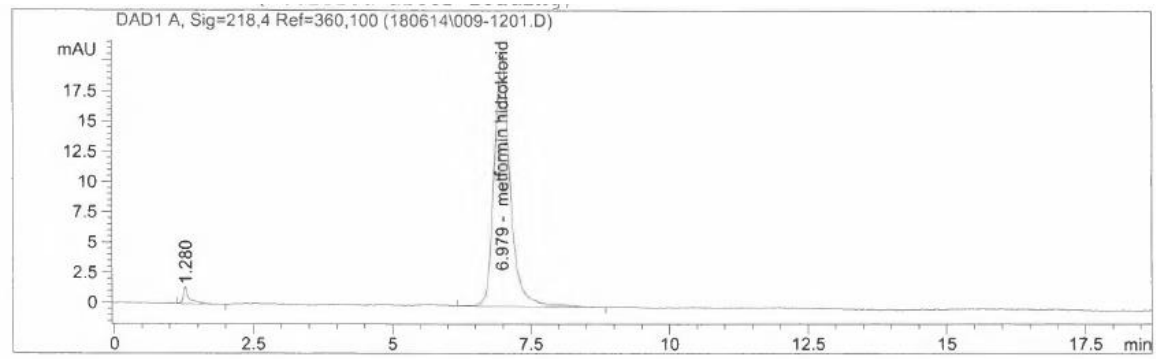

Fig. 8: HPLC chromatogram for metformin hydrochloride standard solution

CONCLUSION

For Metformin Hydrochloride Tablets, official pharmacopoieses provide monographs that make it easy to set up specification requirements. The aim of this study was to demonstrated quality of Metformin hydrochloride tablets on Bosnian market. All of the methods described are very simple, verified and in accordance with ICH requirements for analytical procedure (3).

After the experimental work and the processing of results we can conclude all tested samples of Metformin Hydrochloride tablets correspond to the appearance, uniformity of mass, uniformity of dosage units (by mass variation and L <15), disintegration test with less then 30 minutes. The spectrophotometric method verification was performed by checking the linearity of the method. From the calibration curve results we can conclude that the method is linear and can be used for spectrophotometric determination of the content of Metformin Hydrochloride in tested samples. All examined samples meet the quality requirements of the ICH 3AQ11a guidelines (95.0% -105.0%). Also, dissolution test results showed that all samples corresponded to a valid USP requirement of at least 70% (Q) of the declared content of Metformin Hydrochloride in 45 minutes. Based on the test results of the related substances, we can conclude that all samples meet the requirements for BP, USP as well as according to ICH Q3B (R2).

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