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Uplc method for determination of glibenclamide residual on manufacturing equipment

Larisa Alagić- Džambić, Maja Pašić- Kulenović[1], Maja Ćeranić[2]*

Affiliation of the first author: quality control, analytical chemistry
[1]Affiliation of the second author: quality control, pharmaceutical technology
[2]Affiliation of the third author: quality control, development, registration

Abstract: ***Pharmaceutical products may be contaminated by various substances such as microbiological contaminants, previous products (residues of active pharmaceutical ingredients and excipients), the residuals of cleaning products and residue generated during the cleaning process, using strong acids and bases, and degradation of detergents, acids and bases that can be used as part of the cleaning process. Requirements of Good Manufacturing Practice include prevention of possible contamination and cross-contamination arising from pharmaceutical starting materials, products, cleaning equipment. Appropriate purification techniques play an important role in the prevention of contamination and cross-contamination. Effective cleaning ensure reduction in the risk of contamination to the lowest acceptable level. Validation confirms the efficiency of the cleaning process, which is necessary to achieve adequate cleanliness of equipment to prevent product contamination. The aim of this work is to present ultra performance liquid chromatography method (UPLC) for determining residues of glibenclamide on production equipment. The optimum results were obtained were: column C-18; 2.1 mm x 50 mm; 1.7 µm, kept at 35°C. The mobile phase was 45% V/V acetoniril, contains 1 ml of phosphoric acid. Flow rate was 0.2 ml/min. UV detection was performed at 230 nm. The method has been validated accordance Guidelines ICH Q2 (R1).***

Keywords: cleaning validation; swab sampling; method validation.

INTRODUCTION

Cleaning of production equipment and accessories, as well as cleaning validation is very important for the pharmaceutical industry. Effective cleaning of production equipment actually provides a reduction in the risk of contamination to the lowest possible acceptable level, and in order to protect end user, patient. The objective of cleaning validation is to determine and document that applied cleaning process is effective in remove working residues of active substances, cleaning agents and microbiological residues. Cleaning validation is a multidisciplinary activity. Pharmaceutical industry is often more variety of products produced on the same equipment, and is therefore very important to establish an adequate cleaning process. Validation is considered to be successfully conducted if a visual inspection of equipment does not detect washed residues. To be considered valid, the washing process, it is necessary to spend three consecutive wash cycles, and once a year to do a check in order to confirm cleaning process with the request for validation of cleaning [1, 2, 3, 7, 8, 9, 10].

The aim of this work is to present ultra performance liquid chromatography method (UPLC) for determining residues of glibenclamide on production equipment an validaton of the method according to ICH quidelines [4, 5, 6].

EXPERIMENTAL

Reagents

Working standard of glibenclamide is 100% purity. Chemicals, methanol and acetonitril were from Sigma Aldrich, ethanol 96%, phosphoric acid 85%, hydrochloric acid 37%, sodium hydroxide pellets and hydrogen peroxide 30% were from Merck. All chemicals were lichrosolv and pro analysi grade. Water was purified with a Milli-Q system from Millipore. The UPLC system was Waters Acquity UPLC H-class. All excipients used in our formulation are tested according their monograph and they meet the specifications and quality.

Apparatus and chromatographic conditions

The optimum results were obtained by the use of column Acquity UPLC BEH C-18; 2.1 mm x 50 mm; 1.7 µm, kept at 35°C. The mobile phase was water / acetonitril (550:450) (V:V). On this solution is added 1 ml of 85%. Flow rate was 0.2 ml/min. UV detection was performed at 230 nm. Injection volume was 0.2 µl. Solvent solution for swab was 96% ethanol. Solvent solution for standards and sample preparations was methanol.

Standards and sample preparation

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Standard solution was prepared in concentration 0.825 ppm. Request was to have this system suitability:

1. Coefficient of variation (CV) for glibencalmide peak for five replicate injections in standard solution is not more than 1.8%.
2. Tailing factor (TF) for glibenclamide peak in standard solution is not more than 2.0.
3. Theoretical plates (TP) for glibenclamide peak in standard solution is not less than 1500.

In 50 ml Erlenmeyer flask, containing 3 swabs (two wet and one dry), added 10 ml of the solvent, and than sonicated for 30 minutes.

All solutions were filtered through a nylon filter 0.20 µm, discarding the first ml of the filtrate, and then injected.

Validation

Selectivity was tested by runing solution caontaining all excipents in the same concentrations and conditoins like samples and by running of untreated swabs. Retention time for glibenclamide is about 4 minutes.

Linearity was tested by preparing standard solutions of glibencalmide from 20% to 1000% of the target analyte concentration (0.165 ppm to 8.25 ppm). Accuracy of the method was tested by applaying mixture of glibenclamide and excipients by triplicate in three levels (80, 100 and 120%, correspond from 0.66 pmm to 0.99 ppm). Precision was tested like system repeatibility by runing 6 replication of test solution in target concentration (0.825 ppm). The robustness of the method was tested by changing UV detection, flow rate, column temperature, mobile phase ratio, different apparatus (analyst one and analyst two). We also done recovery of glibenclamide from swab and from stainless steel. The same procedure we repeated with final product. Validation results and recovery results are given in Tables 1, 2 and 3.

RESULTS AND DISCUSSION

Main validations parameters are shown in TABLE I and for robustness of method in TABLE II. Recovery extractions are presented in TABLE III.

TABLE I. Validation results

System Suitability	Glibenclamide
CV	0.84
TF	1.1
TP	8468
Selectivity	
Placebo solution	No interference
Swab solution	No interference
Linearity	
r	0.84
CV	1.1
Accuracy	
Recovery	97 - 103
CV	1.87
System precision	
CV	1.66
CL	±1.76

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TABLE II. Robustness

UV detection	Recovery	Mean Recovery	CV
Low	98		
Target	102	99	2.52
High	97		
Flow rate	Recovery	Mean Recovery	CV
Low	99		
Target	102	100	1.95
High	99		
Column temperature	Recovery	Mean Recovery	CV
Low	98		
Target	102	100	2.13
High	99		
Mobile phase ratio	Recovery	Mean Recovery	CV
Low	98		
Target	102	98	4.54
High	93		
Different laboratory	Recovery	Mean Recovery	CV
Low	102		
Target	99	101	1.48

TABLE III. Recovery results extraction

Extraction	Glibenclamide in ppm	Recovery in %
Active substance from swab	0.825	101
Active substance from stainless steal	0.825	102
Finished product from stainless steal, laboratory I	0.825	102
Finished product from stainless steal, laboratory II	0.825	102

CONCLUSION

The application of UPLC method for analysis of glibenclamide residue on production equipment at first reduce chromatographic time. As a reminder, run time was 6 minutes. Proposed method is selective, becouse there is no interference of mobile phase, solvent and placebo components at the retention time of glibenclamide. Linear relationship between area (response) and concentration of analyt in the range 0.165 to 8.25 ppm was demonstrated with a coefficient of determination 0.99996. Also, limit of detection is 0.08 ppm and limit of quantization is 0.24 ppm. For accuracy, percent recovery, coefficient of variation and confidence limit for nine samples in three concentration are calculated. Since the determined accuracy, precision and recovery are in the expected limits the preposed method is exellent for determination of glibenclamide residual. With smole changing of method parameter condition, we proved robustness of method. On the basis of the obtained results can be concluded that UPLC method for determination of glibenclamide is valid and can be used for the determination of residues of glibenclamide on production equipment.

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