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Research Article

VALIDATED HPLC METHOD FOR THE FAST AND SENSITIVE DETERMINATION OF FEW ANTI-DIABETIC DRUGS RESIDUES IN SUPPORT OF CLEANING VALIDATION IN FORMULATION AREA

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ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed for simultaneous determination of six anti-diabetic (metformin hydrochloride, glipizide, pioglitazone hydrochloride, gliclazide, glibenclamide and glimepiride) active pharmaceutical ingredients (API) residues. A new method is presented, with which it is possible to verify the cleaning process of anti-diabetic drugs producing equipment line used for the production of various pharmaceuticals. The HPLC method was validated using a Thermo Hypersil C18 column with a particle size of 5 µm (250 mm x 4.6 mm) and 0.5% v/v triethylamine buffer-acetonitrile (42:58, v/v) as mobile phase at a flow rate of 1.0 ml/min. Method development and method validation for cleaning control analysis are described. The rapid HPLC method is suitable for cleaning control assays within good manufacturing practices (GMP) of the pharmaceutical industry.

Keywords: Anti-diabetic separation, HPLC, Method validation, Cleaning validation, High speed separation.

INTRODUCTION

In pharmaceutical industry the cleaning procedure is one of the most important tasks to avoid the cross contamination for subsequent batches manufactured in the same equipment. Analytical methods used to determine residuals or contaminants should be specific for the substance or the class of substance to be assayed (e.g., API residue, detergent residue) and be validated prior to cleaning validation.

Guideline recommend thin layer chromatography (TLC), UV-photometric, total organic analysis (TOC), conductivity, gas chromatography (GC), and high performance liquid chromatography (HPLC) methods for cleaning control or validation.

The use of other analytical methods, including capillary gas chromatography, over-pressured layer chromatography (OPLC) or micellar electrokinetic chromatography (MEKC), have also been described. Ion mobility spectrometry (IMS) and TOC have the advantage of speed over the above mentioned methods but TOC is not specific and IMS is usually not available at pharmaceutical manufacturing facilities. Liquid chromatography-mass spectrometry (LC-MS) and ultra performance liquid chromatography-mass spectrometry (UPLC-MS) techniques applied in pharmaceutical cleaning verification have the advantage of improved sensitivity, selectivity and general applicability even for UV-inactive compound. However, these techniques are most expensive than the other techniques mentioned above and not widespread yet in cleaning control analysis. Nowadays HPLC-UV is the most commonly applied technique for cleaning control and validation[1-3].

Cross contamination with active ingredients is a real concern. The Code of Federal Regulation (CFR) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official, or other established requirements". Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic interaction between pharmacologically active chemicals. Since the issuance of the US Food and Drug Administration's "Guide to Inspection of Validation of Cleaning Process" in July 1993, cleaning validations have received increasing attention[4].

The aim of this study was to develop and validate the HPLC method to determine the residues of UV-active anti-diabetic drugs such as metformin hydrochloride, glipizide, pioglitazone hydrochloride, gliclazide, glibenclamide and glimepiride in support of cleaning control and validation for six different pharmaceutical formulation of a manufacturing area. Some of these formulations contain more anti-diabetic drugs in different combinations. A variety of chromatographic methods are described in the literature for the separation and determination of the six anti-diabetic drugs listed above[5-9]. However, no paper can be found in the literature in which the simultaneous determination of these anti-diabetic drugs are described and applied for cleaning control analysis.

MATERIALS AND METHODS

Reagents and materials

The working standards of metformin hydrochloride (99.4%), glipizide (99.3%), pioglitazone hydrochloride (98.9%), gliclazide (99.0%), glibenclamide (99.3%), and glimepiride (99.1%), were obtained from Micro Labs Ltd, India. The triethylamine was of AR grade (Merck, India), ortho-Phosphoric acid was GR grade (Merck, India), ethanol was of GR grade (Changshu Yangyuan Chemicals, China), methanol was of HPLC grade (Merck, India) and acetonitrile was of HPLC grade (Merck, India). Water purified with Millipore water system (Elix 10 C model) was used for the preparation of buffer. Cellulose acetate filter (0.45 micron – Sartorium stedim) was used for the filtration of the mobile phase.

Instrumentation

A Waters HPLC system equipped with a 2695 solvent delivery system, Waters auto injector, thermostatted column compartment and 2996 PDA detector and Empower software was used. Thermo Hypersil C18 column (Hypersil BDS column of 250mm x 4.6mm i.d., 5 µ particle size) was used for the analysis.

Mobile Phase

A mixture of buffer and acetonitrile in the ratio of 42:58 (v/v) was used as the mobile phase. The buffer for mobile phase was prepared by diluting 5 ml of triethylamine to 1000 ml with water, adjusting the pH to 3.5 ± 0.05 using ortho-phosphoric acid. The buffer and acetonitrile mixture was degassed by sonication and filtered through 0.45 µ cellulose acetate membrane filter.

Standard Stock Preparation

About 100 mg of each of working standards of metformin hydrochloride, glipizide, pioglitazone hydrochloride, gliclazide, glibenclamide and glimepiride were weighed and transferred into a 100 ml volumetric flask. About 70 ml of methanol was added and sonicated to dissolve the substances. The volume was made up to 100 ml with methanol and mixed well.

Standard Preparation

Out of standard stock solution, 10 ml of the solution was dilute to 100 ml with mobile phase. The resulting solution (10 ml) was

diluted to 100 ml with mobile phase to obtain a concentration of 10 µg/ml of each of metformin hydrochloride, glipizide, pioglitazone hydrochloride, gliclazide, glibenclamide and glimepiride.

Sample Preparation

The swab sticks were soaked in ethanol and sonicated for 15 minutes. After decantation the ultrasonic wash was repeated for two more times. After the last wash the swab sticks were dried under vacuum. After total drying the swab sticks were stored in a screw cap bottle until usage. The swab sticks were dipped into ethanol before sampling. The surfaces to be sampled were swabbed from top to bottom. Then the sampled swab sticks were placed in a test tubes and 10 ml of mobile phase was added and sonicated for 5 minutes to produce complete dissolution of compound from the swab. Finally each extracted sample solution was poured into a centrifuge tube and was centrifuged for 5 minutes at 4000 rpm.

Chromatographic parameters

For HPLC studies, a flow rate of 1.0 mL/minute and detection wavelength of 230 nm was used. The sample injection volume was 20 µL and the column was maintained at ambient temperature. The run time for each injection was 10 minutes.

Method validation

The method validation was performed in accordance with the current guidelines.

Specificity

The surface of the equipment line consists of mostly (>95%) stainless steel but there are critical surfaces which are made of plexi-glass, teflon and silicone. These specific surfaces are hard to clean so it is necessary to sample these areas during the cleaning verification/ validation process. During the specificity study all types of the sampling surfaces were investigated.

To prove that the determination of active residues is selective and free from any disturbing effects, standard solutions, blank and spiked solutions sampled from stainless steel, plexi-glass, teflon and silicone model surfaces and placebo solutions were injected. Resolution of Rs >2.0 was achieved between the actives, placebo peaks, therefore the method can be considered as a specific method for these six compounds.

Limit of quantitation and detection

Quantitation limits (LOQ) and detection limits (LOD) were determined by the %RSD of five replicated injections of standard solutions. The % RSD of < 10% for LOQ concentration and % RSD of < 30% for LOD concentration criteria are used. The sensitivity of method is proved to be sufficient for each compound.

Linearity of response

For each compound the linearity of response was assessed by injecting standards prepared in mobile phase. The concentration range of compounds was investigated from the quantitation limit (QL) up to the 150% of the median concentration of the method (range: 0.08 – 15.0 µg/ml for metformin hydrochloride, 0.10 – 15.0 µg/ml for glipizide, 0.12 – 15.0 µg/ml for pioglitazone hydrochloride, 0.12 – 15.0 µg/ml for Gliclazide, 0.10 – 15.0 µg/ml for glibenclamide and 0.10 – 15.0 µg/ml for glimepiride). The results were analysed by linear regression. The correlation coefficients, r^2, were found >0.99.

Accuracy

Samples for recovery test were prepared as follows: standard stock solution was prepared in ethanol. This solution was further diluted with ethanol to get concentration of LOQ to 150% of target concentration of each of the compounds.

The above standard solution was dispersed (n=6) in metal plates (10 cm x 10 cm size plates of each of stainless steel, plexi-glass, teflon and silicone) and the plates were dried. Then the plates were swabbed by using the swabs.

Then the sampled swab were placed in test tubes containing the mobile phase and sonicated for 5 minutes to produce complete dissolution of compound from the swab. Finally each extracted sample solution was poured into a centrifuge tube and was centrifuged for 5 minutes at 4000 rpm.

In all cases sample concentrations were determined by reference to a calibration line constructed from standards containing the respective analyte in LOQ to 150% of target concentration. The recovery values are 87.3 – 93.9%, 82.8 – 87.8%, 82.8 – 86.0% and 82.7 – 85.9% from stainless steel, plexi-glass, teflon and silicone surfaces respectively.

Precision

Precision was examined by the relative standard deviation (%RSD) of recovery data (of each compound on different surfaces). Intermediate precision was examined by repeated recovery test by another analyst. The % RSD of recovery results were < 10% which is within the acceptance value (< 15%).

Stability of sample and stock solutions

The stability of standard solution and test (spiked and swabbed from stainless steel) solutions was studied. The solutions were stored in the sample compartment and are chromatographed 12 times within a 24 hours period. The % RSD of peak areas was calculated and % RSD of <2% criterion is used for the method. The standard and test solutions were proved to be stable for each compound with a 24 hours period. There were no detectable degradants on the chromatograms.

Robustness

The robustness was investigated by varying the chromatographic conditions with respect to flow rate, organic modifier and wavelength. The study was conducted at different flow rates of 0.9 ml/min, and 1.1 ml/min (i.e. ± 10% of actual flow of 1.0 ml/min), organic modifier (acetonitrile) concentration was adjusted to 56% and 60% (i.e. ± 2% of actual concentration of 58%) and the wavelength was altered to 225 nm and 235 nm (i.e. ± 5 nm of actual wavelength of 230 nm) for each of the analyte. Standard and sample solutions were injected and the system suitability parameters were evaluated for these modified conditions. The method was found to be robust with respect to small changes in flow rate, small changes in organic modifier percentage and small changes in wavelength. The system suitability parameters such as tailing, resolution and number of theoretical plates were within the specified limits.

RESULTS AND DISCUSSION

The purpose of this study was to develop a fast and sensitive method for the cleaning validation process in the pharmaceutical manufacturing equipment. A fast, isocratic HPLC method has been developed to separate the six anti-diabetic drugs with baseline resolution with in 8 minutes (figure 1), and can be applied for the cleaning control analysis of the anti-diabetic drugs in the manufacturing equipment.

The HPLC method was validated and the data are summarized in Table1. For system precision and suitability, six repetitions of injection from standard solution were used. The acceptance criteria for system suitability is as follows: The minimum resolution between the peaks is at least 2.0, the tailing factor for each peak is not more than 2.0, the number of theoretical plates of the each of peak is not less than 5000 and the %RSD of peak areas generated by five injections is lower than 2.0% for each compound. Specificity, linearity over the range of interest, accuracy (recovery from different types of surfaces) in the range to LOQ -150% of target concentration, precision and limit of quantitation and detection were determined.

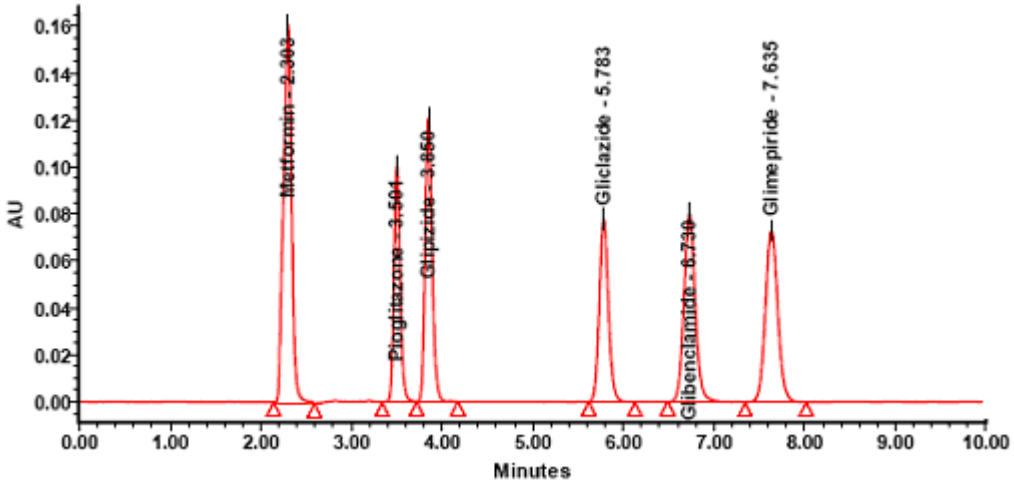

Fig. 1: It shows the retention times of each of the six analytes in standard solution

Table 1: Table shows results of method validation

Parameter	Metformin HCl	Glipizide	Pioglitazone HCl	Gliclazide	Glibenclamide	Glimepiride
Specificity[a]	Passed	Passed	Passed	Passed	Passed	Passed
Linearity[b]						
Correlation	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99
Accuracy[c]						
Steel	93.9%	90.5%	87.3%	88.7%	91.0%	88.7%
Plexi-glass	87.8%	83.9%	85.1%	82.8%	86.1%	85.2%
Teflon	86.0%	84.7%	84.2%	84.1%	83.7%	82.8%
Silicone	85.9%	84.2%	82.7%	83.8%	84.6%	85.0%
Precision[d]						
Steel	2.3%	3.7%	3.5%	3.4%	3.0%	4.2%
Plexi-glass	2.9%	2.2%	2.4%	3.0%	3.5%	2.4%
Teflon	3.5%	3.3%	2.3%	2.2%	3.8%	2.8%
Silicone	3.0%	2.7%	2.7%	3.2%	3.6%	2.4%
Limit of quantitation[e]	0.08µg/ml	0.10µg/ml	0.12µg/ml	0.12µg/ml	0.10µg/ml	0.10µg/ml
Limit of detection[f]	0.03µg/ml	0.03µg/ml	0.04µg/ml	0.04µg/ml	0.03µg/ml	0.03µg/ml

[a] To prove specificity, standard solutions, blank and spiked solutions sampled from stainless steel, plexi-glass, teflon and silicone model surfaces and placebo solutions were injected. The criterion for resolution was Rs> 2.0 between any active, matrix and placebo peaks.

[b] Correlation co-efficient r^2 >0.99.
[c] Mean value of recovery in the range of LOQ to 150% of target concentration (n=6).
[d] Percentage relative standard deviation (% RSD) of recovery data at median concentration level.
[e] Concentration, where % RSD of repeated peak areas (n=6) not exceeded 10%.
[f] Concentration, where % RSD of repeated peak areas (n=6) not exceeded 30%.

The mean accuracy (recovery) from the stainless steel, plexi-glass, teflon and silicone are acceptable for this type of analysis (recovery >80%), they are corrected by a recovery factor during the routine analysis. The results of robustness show that the small changes in the method does not have major impact on the chromatographic parameters such as tailing, resolution and number of theoretical plates. The analytical solutions were found to be stable for a reasonable period of time.

CONCLUSION

The analytical method developed and validated for the estimation of residues of six anti-diabetic drugs (metformin hydrochloride, glipizide, pioglitazone hydrochloride, gliclazide, glibenclamide and glimepiride) from the manufacturing equipment. The validation data were found to be satisfactory. The developed method is sensitive and it is useful for the estimation of these six analytes in the cleaning validation samples.

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