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Original Article

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Determination of Residual Ceftazidime in Pharmaceutical Product line: A Cleaning Validation Study by HPLC

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Abstract

The manufacturing equipment in pharmaceutical industry, could be used in multiple and shared production lines, therefore there is the possibility for the products components and active ingredients to intermix and pollute one another. In this purpose the cleaning methods are used, to reduce the residues levels from the machinery surfaces and decrease the residues to acceptable level but these methods must be validated firstly. This study aimed to validate the cleaning process of ceftazidime using a new method for determination of ceftazidime on equipment surfaces. In this order High Performance Liquid Chromatography (HPLC) has been developed and relative samples have been analyzed. Through this process, type and ratio of the buffer, flow rate and the pH were used as the effective factors in three levels of design for the experiment, and a total of 27 exercises according to Box-Behnken model were designed and implemented. One of these experimental runs was picked and selected by software as the optimum condition in accordance with the separation processes results and the validation of method has been studied and researched. Validation process results proved the accuracy of the analysis method used, demonstrating it could be deployed to determine the level of ceftazidime in low dosages. Therefore: LOQ = 0.63 ppm, LOD = 0.17 ppm, and recovery percentage were reached to 100:71. The results of our study demonstrated the developed method which is appropriate to hold optimal conditions for analysis of cleaning samples containing ceftazidime through HPLC process.

Keywords: Cleaning validation, Ceftazidime, Chromatography, High Performance Liquid Chromatography (HPLC)

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Introduction

According to cGMP rules, cleaning validation is one of the important issues to ensuring products quality, and it plays an extremely important role in all phases of production (Dubey et al. 2013). After making the intended product, every production line undergoes a daily, weekly or monthly cleaning schedule under which, the due cleaning process takes place. After cleaning, samples are taken from critical parts of the equipment that are in high level of exposure to medicinal powders and dispatched for analysis and confirmation. Through this study, we intend to achieve validation of a suitable and economically viable analytical method for ceftazidime powder, a substance for which not much analytical research has been performed. Therefore it is of utmost importance in the pharmaceutical industry and its quality control departments, to develop such new process for multiple deployments and validating it, along with its statistical and chemometrical studies, and also to design necessary experimental methods (Senem et al. 2008; Rana et al. 2013). In pharmaceutical reliable sources, direct measurement of ceftazidime, is performed through HPLC using electrochemical detectors (Senem et al. 2011; Rana et al. 2013). But when it comes down to measuring the residues of ceftazidime, a new process, capable of performing the task properly ought to be designed and validated. The process of validation and cleaning for ceftazidime production line can be carried out with ease (Stoimenova et al. 2011). "One at a time" is one of the most common procedures in the design and development, where all parameters are kept intact except for one substitute, and then results studied. This way, a conclusion can be reached as of the effectiveness of one factor without the intrusion of other factors (Davis & Davis, 2010). Nevertheless throughout the process of this research the method of "factorial" has been deployed for the reasons described below (Kasai et al. 2002). The design of experiment, where the outcome variable for all possible compositions is measured against selected levels of factors, is known as one complete factorial method (Holten & Onusko, 2000). This kind of procedure is in contrast to the common method where results are studied in sequence for each factor as other factors in the same level are kept intact (Rambla-Alegrea et al. 2009).

Two convincing reasons for deploying the factorial process instead of "one at a time" method are:

- Factorial process can estimate any kind of interaction whereas the "one at a time" process lacks such ability (Esser et al. 2006).
- To achieve results of equal precision, the factorial process requires fewer measurements should the effects of factors be combinable. There is no sufficient information available for dosage therapy or toxicities and the selection of restrictions based upon the effects on the next product pollution index in compliance with GMP rules is advised (Sucher et al. 2009).

An experimental process must be developed for the analysis of cleaning method, in order to ensure the adopted procedure's accuracy.

Material and methods

In designing the experiment, 4 parameters of: flow rate, ratio of mobile phase, type of buffer and pH, in three levels were conducted by STATGRAPHICS (plus 5.1) software, based on the Box-Behnken measuring method. Based on this process, 27 runs were selected by the software, standard solutions and equipment conditions were adjusted according to the 27 runs and the standard sample was injected into the machinery (table1).

The information of the separation results were added to table 1 after analysis, and based on this information, the software eventually picked the best run, run no.3 was selected which included the following conditions:

Flow rate: 0.5 ml/min , Ratio of Mobile phase: 85:15 , Buffer: acetate and pH=3
Then the information for process' validation for the exercise was carried out based on ICH Q2B and validated, and the process was selected for analysis of the cleaning samples.

Table 1: Result of experiment design

Average	Resolution2	Resolution1	buffer	Ratio	Flow rate	pH	Number of Test
1	4	1	75.25	Citrate	0	0	0
2	4	1	85.15	Acetate	2.75	2.75	2.75
3	3	0.5	85.15	Acetate	3.85	3.97	3.91
4	5	1.5	85.15	Acetate	0	0	0
5	5	0.5	85.15	Acetate	0	0	0
6	4	1	75.25	Phosphate	0	0	0
7	4	1	95.5	Citrate	0	0	0
8	3	1.5	85.15	Acetate	2.86	2.86	2.86
9	4	1	95.5	Phosphate	8.10	8.10	8.10
10	4	1.5	75.25	Acetate	1.71	1.71	1.71
11	3	1	85.15	Phosphate	2.84	3.08	2.96
12	5	1	85.15	Phosphate	0	0	0
13	3	1	85.15	Citrate	1.84	1.84	1.84
14	4	0.5	95.5	Acetate	3.33	3.33	3.33
15	4	0.5	75.25	Acetate	2.16	2.17	2.165
16	4	1.5	95.5	Acetate	3.71	3.71	3.71
17	4	1	85.15	Acetate	2.73	2.75	2.74
18	5	1	85.15	Citrate	1.19	1.19	1.19
19	5	1	75.25	Acetate	0	0	0
20	4	0.5	85.15	Phosphate	1.21	1.21	1.21
21	3	1	95.5	Acetate	11.45	11.45	11.45
22	4	0.5	85.15	Citrate	0	0	0
23	4	1.5	85.15	Citrate	1.97	1.39	1.68
24	5	1	95.5	Acetate	0	0	0
25	3	1	75.25	Acetate	1.28	1.28	1.28
26	4	1.5	85.15	Phosphate	1.1	1.2	1.15
27	4	1	85.15	Acetate	2.75	2.75	2.75

Result and Discussion

To validate a certain method, necessary parameters have been defined by various domestic and international workgroups along with articles and publications. The following parameters are among those agreed upon by ICH or have been defined by other international centers for pharmaceutical analysis that a single analysis method must be used for validation (Barnes, 1995; Ermer, 2011).

Selectivity

The method in use for analyzing and identifying ceftazidime is a selective, because no peaks in it are observed as disturbing (figure 1, 2).

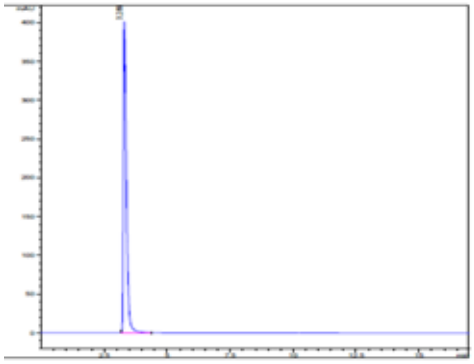

Figure 1: Chromatogram of Ceftazidime

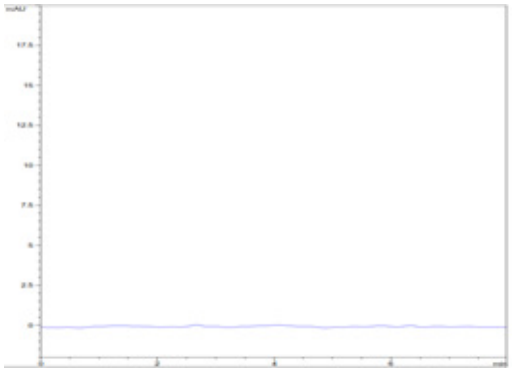

Figure 2: Chromatogram of Blank

Linearity and Range

For being linear of this method within a determined range, 100 ppm original standard solution with 40, 60, 80, 100, 120 and 150 ppm concentration were prepared and injected to the apparatus three times for each concentration. Average of each result pointed on the calibration curve (figure3). Within this criterion, analytics (RSD ≤ 1) is traceable plainly and accurately. Hence this criterion is chosen as the domain (range) of method.

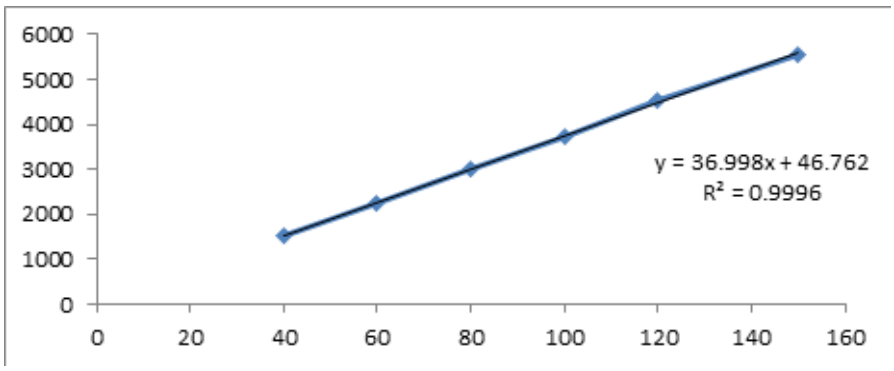

Figure 3: Curve of Calibration

(Horizontal axis shows concentration and Vertical axis shows peak area)

Table 2: Being Linear of domain and concentration and determination of under peak level

Concentration (ppm)	Area
40	1529.23
60	2246.38
80	3013.61
100	3734.55
120	4541.82
150	5564.01

Table 3: Linearity and amplitude determination

Equation	(ppm) concentration range	R^2
x=Y 36.998 46.762+	150-40	0.9996

Related graph shows that this method, within determined concentration range in the ceftazidime related table, is linear and each concentration is plainly and accurately traceable. Hence this range is determined as domain (range) of method (tables 2, 3).

Accuracy

Accuracy of a test is a standard for the proximity of results to true value. Estimating the recovery percentage is a way to assess the accuracy of results. Recovery percentage: could be executed through different methods. One of the most common methods is to add a certain amount of the standard sample to the blank (table 4).

Table 4: Accuracy

	Concentration expected (ppm)	Added concentration (ppm)	The resulting concentration (ppm)	Percent recovery
ceftazidime	2	1	2	98.89
ceftazidime	3	1	3	100.86
ceftazidime	4	1	4	100.05

Precision

To check for precision, ppm 100 has been chosen as the base and injected 6 times over to HPLC and was analyzed according to the mentioned analysis method then RSD was calculated on foundation of under-peaks (figure 4). Results show acceptable RSD (table 5).

Table 5: Precision

No.	Area
1	3693.89
2	3693.87
3	3699.77
4	3702.66
5	3696.81
6	3699.02
Mean	3697.67
SD	3.48
RSD	0.094

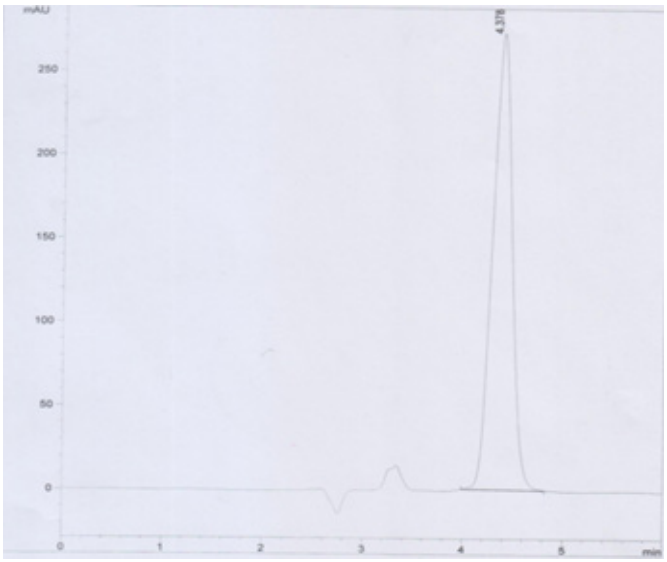

Figure 4: Acceptable criteria: relative Standard deviation not more than 1%

Table 6: Precision on different days

First day	3697.67
Second day	3697.62
Third day	3697.46
Average	3697.58
SD	0.109
RSD	0.0029

LOD and LOQ

To obtain the minimum detection limit, low concentration of analyte were injected and the minimum diagnosable drug by detector as LOD was calculated. There for, by multiple dilution of standard solution, 0.17 ppm was determined as the limit of detection (LOD).
LOQ of method is the minimum concentration of analyze which could be measured plainly and accurately. As it's obvious in the calibration curve, 0.63 ppm concentration is detectable for Ceftazidime determination.

Conclusion

In cleaning validation studies, small amounts of residual products in common surface area of equipment should be measured. Most of the times elder methods are not accurate and new analytical methods should be developed. These methods should be more accurate and could be low cost and fast.
Therefore necessary studying and assessments of HPLC results in this paper resulted that HPLC is an effective method for an accurate, quick and cheap determination of Ceftazidime. In other word, by re- designing and developing of this method and its validation, residuals of Antibiotic on surfaces and equipment which are important for validation and cleaning could be traced.

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