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Research Article

CLEANING VALIDATION FOR RESIDUAL DETERMINATION OF VALGANCICLOVIR HYDROCHLORIDE ON STAINLESS STEEL SURFACE OF PHARMACEUTICAL MANUFACTURING EQUIPMENTS USING HPLC-DAD METHOD

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ABSTRACT

Cleaning validation is designed to demonstrate the effectiveness of cleaning procedure to remove residue of active drug substances, cleaning agents and microbial attributes to predetermined level. A simple, sensitive, rapid and accurate RPHPLC method has been developed and validated for determination of valganciclovir residues on the surface of manufacturing equipments. A Hypersil BDS C18 (150x4.6mm,5µ) column was used as stationary phase by using mobile phase of pH 3.0 of 2.5% triethylemine: Methanol(93:7)v/v. Method was developed in isocratic mode with 8 minutes run time, at floor rate of 1.5 ml/min. valganciclovir was detected at 254 nm. Optimized method was linear in the concentration range of 0.031-2µg/ml. The swabbing procedure was optimized to achieve suitable recovery of valgancyclovir from stainless steel surfaces. RSD of the 6 replicate precision results was found to be 0.8 . The limits of detection and limits of quantification were evaluated to be 0.008 µg/ml. and 0.031 µg/ml. Recovery of the method was found > 90%. This method can be used to detect trace levels of valgancyclovir residue in production equipment area to confirm the efficiency of the cleaning procedure in pharmaceutical industries to avoid cross contamination.

Keywords: Cleaning validation, Valganciclovir hydrochloride, Cytomegalovirus (CMV), HPLC.

INTRODUCTION

Cleaning validation is required in pharmaceuticals to avoid contamination of the following pharmaceutical product in the subsequently manufacturing product. Also to prevent potential clinically, significant synergetic interaction between pharmacologically active materials. Adequate cleaning procedure plays an important role in contamination of the materials and equipments. Residues have a significant cross contamination potential. To monitor the effectiveness of the cleaning operation, the analytical methods should be selective for the substance considered and must of sufficient sensitivity, because residue concentration is usually low. There are many acceptance criteria depends on scientific and logical rationale i.e., mathematical formula, therapeutic dose and toxicity profile. The two types of acceptance criteria are widely used in pharmaceutical field are 10 ppm criteria: NMT

10 ppm of any product should appear in another product.
Milligrams of API in product A permitted/4 inch2 swab area = **RxSxU/T**
where
R= 10 mg of active ingredient in product A/ kg of product B.
S= Batch size in kilogram of product B.
U= 4 inch2 /swab (swab surface area)
T= equipment surface area in common between products A and B expressed as square inches.

Dose criteria
NMT 0.1% of the minimum therapeutic dose of any product should appear in the maximum daily dose of the subsequent product.
Milligrams of API in product A permitted/4 inch2 swab = **IxKxM/JxL.**
Where
I = 0.01x minimum daily dose of product A.
K = No. of dosage units per batch of final mixture of product B.
M = 4 inch2 /swab (swab surface area)
J = maximum no. of dosage units of product B taken per day.
L = Equipment surface area in common between products A and B expressed in square inches.
Regulatory agencies suggest two methods of sampling are generally admitted for performing a cleaning control. a) Swab sample method) b) Rinse sample method with solutions used for analysis.
Valganciclovir Hydrochloride is a prodrug of ganciclovir. Valganciclovir hydrochloride is soluble in water and very soluble in 2-propanol, freely soluble in alcohol. It is Hydrochloride salt of the L-valyl ester of Ganciclovir which is a synthetic guanine derivative active againest cytomegalovirus (CMV) and is indicated for the treatment of CMV retinitis in patients with acquire immuno deficiency syndrome. It exists as a mixture of two diastereomers.After oral administration both diasteromers are rapidly converted to ganciclovir by intestinal and hepatic esterases. Ganciclovir inhibits replication of human CMV in cell culture and invivo.In CMV infected cells ganiclovir is initially phosphorylated to ganciclovir monophosphate by the viral protein kinase, PVL97.Further phosphorylation occurs by cellular kinase to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly.
Literature survey reveals that numerous methods have been published for Quantitative analysis of Valganciclovir Hydrochloride ,such as UV,HPLC e.t.c.A literature revealed that no validated cleaning method is available for valganciclovir residue analysis in pharmaceutical equipment,Taking this consideration into account that the aim of this study was to develop and validate a rapid and sensitive analytical method for determination of trace levels of valganciclovir hydrochloride residues in production area equipment to confirm the effeciency of the cleaning procedure. The analytical method was validated in the terms of precission, accuracy, linearity, selectivity and limit of detection and limit of quantification and solution stability. This method is helpful in pharmaceutical industry for determination of residues on the surface of manufacturing equipment to avoid potential cross contamination.

MATERIALS AND METHODS

Instruments and apparatus

Waters HPLC system connected with PDA detector and Empower 2 software for data acquisition, XS205 Dual range balance (Make: Mettler Toledo), Bandelin Sonorex sonicator, Heraeus Biofuge Stratos Centrifuge and Stainless steel plates (4 cm × 4 cm) were used during development study. Class A Volumetric flasks, pipettes, beakers, measuring cylinders and centrifuge tubes of Borosil glass were used.

CHEMICALS AND REAGENTS

Valganciclovir hydrochloride standard was provided by Dr. Reddy's Laboratories, Hyderabad, India. Methanol-HPLC grade (SDFCL, India), Triethylemine, Hydrochloric acid (Merck, India), Water-HPLC grade and 0.45µ PVDF membrane filters.

METHOD

Chromatographic parameters

All chromatographic experiments were performed in the isocratic mode. Separation was achieved on Hypersil BDS C18 (150x4.6mm,5µ) column by using waters HPLC with PDA detector and controlled by Empower 2 software. Mobile phase was filtered and degassed mixture of (pH 3.0 of 2.5% tri-ethyl amine: Methanol (93: 7), % v/v). Other parameters such as flow rate of 1.5 ml/minute, detection at 254 nm, column temperature of 25‹C, injection volume of 50µl and run time of 8 minutes were finalized during development. Diluent was used as 0.001 N Hydrochloride.

Standard solution preparation

Standard stock solution was prepared by weighing about 50.0 mg of Valganciclovir hydrochloride standard into 50 ml of volumetric flask, added 40 ml of diluent followed by

sonication for 5 minutes in ultrasonic bath to dissolve it. Then cooled at room temperature and made up to volume with diluent and mix (Standard stock of Valganciclovir hydrochloride: 1000 µg/ml.). Transferred 5.0 ml of this stock solution into 250 ml of volumetric flask and made up to volume with diluent. Further dilute 5.0 ml of this solution to 10 ml with diluent. (Standard solution of valganciclovir hydrochloride: 10 µg/ml.). Further dilute 5.0 ml of this solution to 50 ml with diluent (Standard solution of valganciclovir hydrochloride: 1 µg/ml.). The standard stock solution was subsequently diluted with diluent to furnish calibration curve (Linearity) in the range of 0.031-2µg/ml.

Recovery of sample from stainless plates

Selected stainless-steel surfaces (4cm× 4 cm), previously cleaned and dried, were sprayed with 0.1ml Valganciclovir Hydrochloride standard stock solution for positive swab control and then solvent was allowed to evaporate (approximate time was 10 minutes).Swab was passed on the surfaces of Stainless steel in zigzag manner from right to left, returning from left to right, from top to bottom and returning upwards. For recovery of residues removed from plate, the sampling swab was immersed in 25 ml test tubes containing 0.001 N Hydrochloric acid (Diluent). The negative swab control was prepared in the same way as the sample, using swabs, which had not been in contact with the test surface and the tubes were shaked for 10 min on rotary shaker followed by sonication for 10 minutes in ultrasonic bath for 10 min. The resulting solutions were filtered through 0.45µm PVDF filter and solutions were injected into the chromatographic system.

Rinse method

Selected stainless-steel surfaces (4 cm × 4 cm), previously cleaned and dried, were sprayed with 0.1mL Valganciclovir Hcl standard stock solution for positive swab control and then solvent was allowed to evaporate Then rinsed effective area of the stainless steel plate with 10mL of diluent and collected the rinsing solution into beaker. Sonicated the beaker for 10 minutes in ultrasonic bath and filtered the solutions through 0.45 µm PVDF filter and solutions were injected into the chromatographic system.

Swab samples from different locations within the Manufacturing equipment: Swab samples from different locations within the manufacturing equipment and relavant area were submitted for analysis of Valganciclovir hydrochloride residues. These samples were prepared and analyzed as described above.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To find the best chromatographic conditions, wavelength for detection, column and mobile phase were adequately selected. The main objective was to develop an HPLC method enabling determination of Valganciclovir Hydrochloride residues collected by swabs without interference from excipients or impurities present in formulation or active pharmaceutical ingredient. Wavelength for detection was selected by scanned known concentration of Valganciclovir hydrochloride solution in UV Visible spectrophotometer. Valganciclovir hydrochloride spectra showed wavelength maxima at 254 nm.

Stationary phase and Mobile phase selection were tried simultaneously. Different columns such as Inertsil-C18, Zorbax- C18 were assessed with a mixture of pH 1.5 to 3.0 Triethyl amine buffer and methanol in different proportions as mobile phase to achieve good symmetrical peak of Valganciclovir hydrochloride. But problems such as tailing of Valganciclovir hydrochloride, baseline drift at Valganciclovir hydrochloride peak, increase retention of Valganciclovir hydrochloride peak etc. were observed. At last on column Hypersil BDS C18 (150x4.6mm,5µ) and Mobile phase mixture of pH 3.0 of 2.5% triethylemine: Methanol(93:7)v/v with isocratic mode were preferred to improve the peak symmetry, appropriate retention time of Valganciclovir hydrochloride peak. Other parameters such as flow rate of 1.5 ml/minute, detection at 254 nm, column temperature of 25°C, injection volume of 50 µl and run time of 8 minutes were finalized during development. Diluent was used as 0.01N Hydrochloric acid. Valganciclovir hydrochloride was eluted about 5.07 and 5.75 minutes respectively by using developed chromatographic conditions.

Fig 3 and 4 represent the chromatograms of standard, negative swab control of valganciclovir.

Optimization of sample treatment

Cotton swabs were treated with different quantities of Valganciclovir hydrochloride and Placed in glass tubes. After addition of different solvents and their mixtures (water, 0.001 NHydrochloric acid and Acetonitrile), the tubes were sonicated for different times (5, 10, 15 and 30 minutes) and the solutions were analyzed by HPLC. The optimum conditions

were achieved with 0.001 N Hydrochloric acid as diluent and a sonication time of 10 minutes. In all the cases, the best results were obtained using two cotton swabs (the first wetted with diluent and the second dry). Hence this technique was applied in the subsequent work. Some of the results obtained for swab samples from different locations within the manufacturing equipement are summarized in table I.

Validation of analytical method

Once the chromatographic conditions had been optimized, the method was validated for linearity, precision, accuracy, LOD and LOQ, selectivity, solution stability.

System suitability

System suitability test is essential for the assurance of the quality performance of a chromatographic system. Standard of 10 µg/ml was injected five times into HPLC as per test method. % RSD for the sum of two valganciclovir peaks for five standard injections was found 0.8 %. Theoretical plates for the 2 nd valganciclovir hydrochloride peak were found to be 5750. USP tailing of valganciclovir peak was found to be 1.1.The results are summarized in table II.

Linearity

Linearity of the test method was conducted by injecting Valganciclovir hydrochloride solution in the concentration range of 0.031- 2 µg/ml. Plotted a linearity graph of concentration versus area for Valganciclovir hydrochloride. The correlation co-efficient, slope, intercept and bias at 100% response are summarized in table III and **See Fig 5** for linearity plot.

Precision

The precision of the test method was performed by spiking Valganciclovir hydrochloride solution on to stainless steel plate to achieve final solution containing 10 µg/ml. Performed swabbing and analyzed as per test method. This study was assessed by comparing the amount of analyte determined versus the known amount spiked for six replicates. The RSD of % recovery of six sample preparations was found 0.11%. The results are summarized in table IV.

Accuracy

The recovery was assessed by comparing the amount of analyte determined versus the known amount spiked at three different concentration levels (5.0 µg/ml, 10 µg/ml and 15 µg/ml) with 3 replicates (n=3). The accuracy was determined by spiking Valganciclovir hydrochloride on stainless steel plates, performed swabbing and analyzed as per test method. The recovery at three different concentrations was found more than 90%. The results are summarized in table V.

Selectivity

During the sample preparation, some potential contaminant substances extracted from the Absorbond swabs could interfere with the quantitation of Valganciclovir hydrochloride. The selectivity was studied by comparing a blank solution and an Valganciclovir test solution. The test solution was prepared by wiping a plate spiked with valganciclovir hydrochloride, according to the optimized swabbing technique. The blank solution was prepared in the same way without sampling. No sources of interference were observed at the retention time of the analyte. Peak purity of Valganciclovir hydrochloride peak was passed in standard and test sample.

Solution stability

Standard solution of valganciclovir hydrochloride and positive swab control sample of valganciclovir hydrochloride were prepared as per test method and kept on bench top at room temperature. Injected standard and sample solutions into HPLC as per test method at initial, 12 hours, 24 hours and after 2 days. Calculated % assay of standard and test solution against freshly prepared standard of valganciclovir hydrochloride. The assay of standard and sample was found satisfactory from initial, 12 hours,24 hours and to 2 days. This indicates that standard and sample preparation (Positive swab samples) is stable for 2 days at room temperature. The results are summarized in table VI.

LOD and LOQ

LOD **and** LOQ were determined based on the standard deviation of the response and the slope of the calibration curve at low concentration levels according to ICH guidelines. The LOD and LOQ for Valganciclovir hydrochloride were found to be 0.008µg/ml and 0.031 µg/ml respectively. The results are summarized in table VII.

CONCLUSION

A rapid, simple, sensitive and accurate isocratic HPLC method has been developed for determination of Valganciclovir hydrochloride residues on the surface of manufacturing equipment. Validation of the method revealed that method is selective, precise, linear and accurate. The recovery of

Valganciclovir hydrochloride obtained from swabs was found > 90% for three different concentrations. The solution stability data revealed that standard and swab samples are stable for 2 days at room temperature. The overall procedure can be used to determine trace levels of Valganciclovir hydrochloride residues in production equipment area to confirm the efficiency of the cleaning procedure in pharmaceutical industries.

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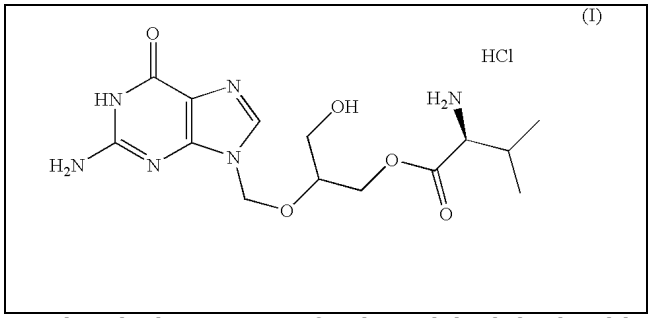

Fig. 1: Chemical Structure of Valganciclovir hydrochloride

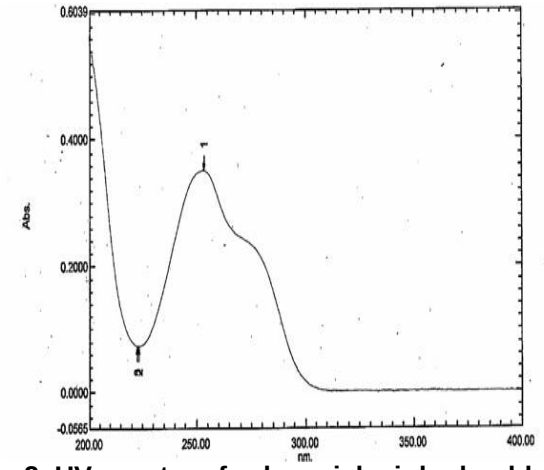

Fig. 2: UV spectra of valganciclovir hydrochloride

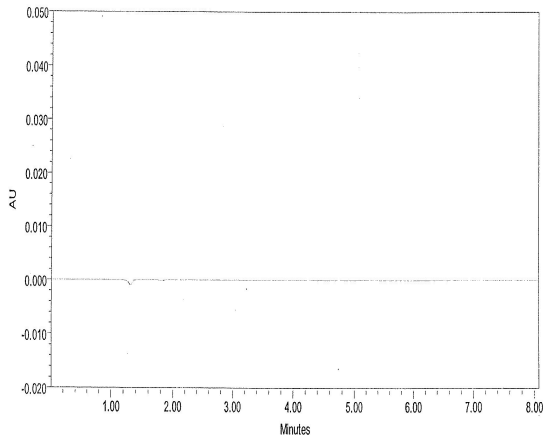

Fig. 3: Negative swab control chromatogram

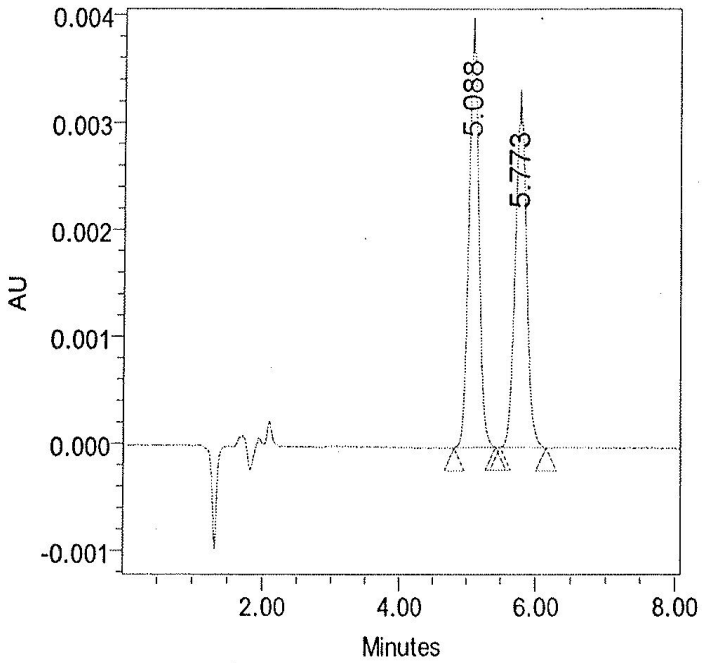

Fig. 4: Standard chromatogram

Table I: Results obtained from analysis of Valganciclovir hydrochloride in swab samples collected from different locations within the equipment area

Equipments	Swab Sample Location	Valganciclovir hydrochloride detected (µg per swab)
API Dispensed scoops	Internal surface	< LOQ
	External surface	< LOQ
Weighing Balance	Balance Platform	< LOQ
Sifter with seive	Mesh surface	< LOQ
Stirrer	Inside surface of stirrer	< LOQ
	Stirrer Assembly	< LOQ
RMG-50 L	Inside bowl surface	< LOQ
	Inside surface of lid	< LOQ
	Impeller Blade	< LOQ
	Chopper surface	< LOQ
	Discharge port	< LOQ
	Bottom of bowl	< LOQ
Double cone Blender	Baffles	< LOQ
	Inside surface of cone(both charging and discharging side)	< LOQ
	Discharge port	< LOQ
20 station Compression machine	Turret surface	< LOQ
	Hopper surface	< LOQ
	Feed frame	< LOQ
	Exhit chute	< LOQ

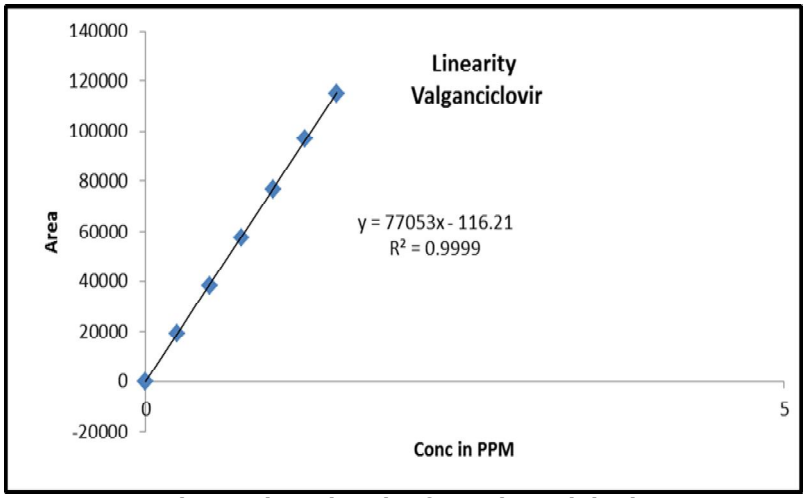

Fig. 5: Linearity plot for Valganciclovir

Table II: Results of System suitability

Parameters	Acceptance criteria	Results
USP Tailing Factor	NMT 2.0	1.1
USP Plate count	NLT 2000	5750
% RSD for the sum of two valganciclovir peaks for five standard injections	NMT 2.0	0.8
Resolution between two valganciclovir peaks	NMT 2.0	0.9

Table III: Results of Linearity

Parameters	Results
Correlation coefficient (NLT 0.999)	0.999
Linearity equation	y = 77053x + 116.2.
Bias at 100% response (NMT 2.0)	0.18

Table IV: Results of Precision

Parameters	Results
Mean % Recovery (n=6) (>90%)	95.6
% of RSD (n=6) **(NMT 5.0%)**	0.15

Table V: Results of Accuracy

Amount added µg/mL	Amount found µg/mL	Recovery (%) (>90%)	% RSD (n=3)
4.99	4.83	96.8	0.11
9.88	9.77	99.0	0.08
14.96	14.87	99.4	0.09

Table VI: Results of solution stability

	Time	% Assay	% Assay difference (3.0%)
Standard	Initial	100.0	0.0
	12 hours	100.0	0.0
	24 hours	100.0	0.0
	48 hours	100.0	0.0
Test Sample	Initial	99.2	0.0
	12 hours	99.2	0.0
	24 hours	99.1	0.0
	48 hours	98.7	0.02

Table VII: LOD and LOQ Values

LOD Value	0.008 µg/mL
LOQ Value	0.031 µg/mL

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