Determination Of Assay And Validation Of Stability Indicating RP-HPLC Method For Ganciclovir In nciclovir Drug Substance

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Abstract: A simple, reliable, touchy and isocratic stability indicating reversed segment excessive performance liquid chromatography (RP-HPLC) technique have become advanced and showed for the willpower of assay of Ganciclovir in Ganciclovir drug substance. The paper describes method development, optimization and validation of an isocratic HPLC approach for the assay of Ganciclovir. The separation become completed on Hypersil BDS C18, 150mm X four.6mm, 5µm particle diameter column. The cell segment consisted of phosphate buffer (zero.01M, pH: 5.Three±zero.05) and acetonitrile 70:30 (v/v); with go with the flow price of 1.Zero mL min-1. The examine was monitored with the aid of manner of photograph diode array detector at 245 nm. The drug substance become subjected to strain situations of hydrolysis, oxidation, photosynthesis, thermal and humidity degradation. The height purity grow to be decided by way of PDA detector using waters empower software program application. A linear response changed into observed over the notice variety from 20 - 30 µg mL-1 with correlation coefficient price 0.9999. The commonplace recuperation is 99.Nine%. The relative significant deviation (R.S.D) for intro-day and inter-day emerge as 0.2% and method is strong in all varied conditions. The results proves that method changed into suitable for the dedication of assay of Ganciclovir and correctly carried out for routine assessment of Ganciclovir drug substance.

Keywords: Ganciclovir; Development; Assay; Validation; Chromatography.

1. INTRODUCTION

Ganciclovir (GNC), chemically identified as 2-amino-9-[(1, 3-dihydroxypropan-2-l)oxy]methyl-6,9-dihydro-3H- purin-6-one(Figure1),isanucleo facet analogue extensively used inside the treatment of cytomegalovirus contagions. It has proved to be effective in competition to cytomegalovirus in immune compromised patients, mainly in people with the obtained immunodeficiency syndrome (AIDS), congenital immunodeficiency, or in people behind organ transplantation[1,2].Several strategies have been developed for the willpower of GNC in prescription drugs. It is bureaucratic inside the United States Pharmacopoeia [3], which describes an HPLC technique for its dedication in injections and in oral suspension. The literature is enriched with numerous techniques for the fortitude of GNC in pharmaceutical dosage bureaucracy including frame fluids. The most importantly used method for the quantification of ganciclovir is HPLC, however most of the strategies the use of this performance are devoted to frame fluids like plasma[4–14], plas maand tissues[15],serum[16], and blood samples [17]. There is handiest one file [18] handling the software of HPLC for the determination of pharmaceutical formulations, this is eye drops. GNC in majority drug and in its preparations has been assayed via UV spectrometry through measuring the absorbance of 0.1 M HCL and 0.1 M NaOH at 253 and 266 nm, respectively [19]. The techniques said are pretty touchy with molar absorptivity values of 2.0 × 103.

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Further stated strategies on behalf of prescription drugs encompass seen spectro photometry [20– 22], go together with the go with the flow injection luminescence spectrometry [23], and radio immunology [24–26]. Use of this test become to boom an investigative HPLC technique for sensitive, specific, and fast dedication of Ganciclovir. Consequently, an alternative clean, touchy RP_H.P.L.C technique with picture diode array finder modified into evolved and augmented to determine the assay of Ganciclovir in Ganciclovir drug element.

2. METHODS AND MATERIALS

2.1 CHEMICALS, REAGENTS AND SAMPLES

The standard and sections of Ganciclovir were procured from Dr.Reddy laboratories. (Hyderabad). Analytical reagent (AR grade) potassium di-hydrogen ortho phosphate, o-phosphoric acid (88 %w/w), hydrochloric acid, sodium hydroxide, hydrogen peroxide (30 %w/v), potassium hydroxide and HPLC grade acetonitrile, 3-ethylamine were got from E Merck India. Vastly distilled H_2O received from Millipore purification gadget.

2.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Chromatographic separation was performed on HPLC, alliance 2695 separations module system well-appointed with 2996 photodiode collection sensor with Empower Pro data control scheme was used [Waters Corporation, USA]. The itinerant section involved of phosphate buffer (zero.01M, ^{pH}:5.3±0.05) and acetonitrile 70:30 (v/v). To prepare the phosphate buffer (zero.01M, pH:5.0), 1.36g of dibasic potassium dihydro-gen orthophosphate salt end up weighed and dissolved in a thousand mL of purified liquid. To this 2.0 mL of $C_6H_{15}N$ introduced, mixed well then pH became adjusted to 5.3 ±zero.05 with ortho-phosphoric acid then filtered thru the 0.Forty five µ porous membrane. The evaluation become carried out on Hypersil BDS C18, a hundred fifty mm prolonged, four.6 mm i.D., 5 µm bit diameter column (Thermolab) at ambient temperature. The cellular segment modified into brought in an isocratic mode at a go together with

the glide amount of 1.0 mL min-1. The inoculation quantity became 10 µ.L. The acquisition stint for the typical and sample end up 10 min. The analytic became monitored with photodiode array detector at 245 nm. Water used for diluting all of the answers of Ganciclovir. The retention time of Ganciclovir is set five minutes. The column performance as decided from the Ganciclovir pinnacle isn't always plenty less than 3000 U.S.P plate count and USP shadowing for the equal height is not extra than 2.Zero. Relative widespread deviation for sum of the height areas of Ganciclovir for five injections of the standard solution isn't always extra than1.0%.

2.3 STANDARD AND SAMPLE SOLUTIONS PREPARATION OF STANDARD SOLUTION

Exactly weigh and transferal about 50 mille grams of Ganciclovir brand new right into a a hundred m.L smooth, dry volumetric flask, upload 70 mL of water and sonicate to dissolve. Make up to amount with water. Dilute 5mL of this technique to one hundred mille liter with water. Filter thru 0.45 ml or better permeability membrane filter out.

2.4 PREPARATION SAMPLE SOLUTION

Precisely mass and allocation about 50 mg of Ganciclovir pattern into a one hundred mL easy, dry volumetric flask, upload 70 m.l of H_2O and sonic ate toward dissolve. Make upto extent with water. Dilute 5 ml of this feature to 100 ml with H_2O. Strainer via 0.45 ml or finer porosity membrane filter.

3. RESULTS AND DISCUSSION

3.1 METHOD DEVELOPMENT AND OPTIMIZATION

The goal of this artwork is, to expand a easy and rapid technique for the willpower of assay of Ganciclovir in Ganciclovir drug substance via way of using HPLC system. Method development become initiated with Ganciclovir drug substance solubility look at, primarily based mostly on that water turned into selected as diluent for diluting all of the answers of Ganciclovir. From the molecular components of Ganciclovir, it was observed that Ganciclovir is polar in nature, based on that non- polar Hypersil BDS C18 table certain section column changed into decided on for developing RP-HPLC technique. Ganciclovir answer pH become 6.7 (C is 1 %weight/volume, in water, at 25°C), primarily based on that buffer end up selected pH 5.3 (pH about ± 2.0 w.r.t determined pH 6.7). BDS C18, 150 mm x four.6mm, 5.0µm particle diameter column with dibasic potassium dihydrogen ortho phosphate buffer (0.01M, pH:5.Three) as As there's chromophore located in Ganciclovir, there may be opportunity for UV-Visible detection, based totally on the colour absorbance take a look at 245 nm changed into decided on for monitoring the response of Ganciclovir. Preliminary take a look at became performed via the usage of Hypersil mobile section, added in an iso-caratic approach with a glide rate of one mL min-1 at ambient temperature and analytes had been monitored with PDA detector, no any top became eluted upto forty mins. Elution of analyte became finished, with the combination of phosphate buffer (0.01M, pH:5.Three) and acetonitrile within the ratio of 60:forty% (v/v). In this trial Ganciclovir height became eluted at about 2 or 3 minutes and interfering with unknown height eluted at about 2 min and additionally sizeable top shapes were decided. For higher selection, trial turned into made with PO_4 buffer (0.01M, pH:5.3) with acetonitrile in the ratio of 70:30% (v/v), peaks had been well resolved from every exceptional and Ganciclovir height changed into eluted at approximately 5.Zero min and unknown peak eluted at about 2.Zero min. For higher height shapes, over again trial became made with 1000mL of phosphate buffer (zero.01M), 2.Zero mL of C6H15N brought after which pH turn out to be adjusted to 5.3. This PO_4 buffer with acetonitrile within the ratio of 70:30% (v/v), notable topmost figure turn out to be accomplished. Finally, excellent separation with higher height form became done, on chromatographic conditions which have been noted in excessive overall performance liquid chromatography (HPLC), became used for validation have a study to assess its standard performance characteristics..

4. METHOD VALIDATION

The approach turned into confirmed as in step with the ICH tips [16], in phrases of specificity, pressured degradation studies (stability indicating nature), linearity, stability of pattern solution, robustness, precision (machine precision, technique precision and intermediate precision or ruggedness) and accuracy.

4.1 SPECIFICITY

For chromatography techniques, growing a separation includes demonstrating specificity, which is the capability of the approach to appropriately diploma the analyte reaction within the existence of all capacity sample additives. Hence comeback of the analyte in take a look at combos containing the analyse and all capability pattern components (synthesis intermediates, degradation merchandise, system impurities) is in comparison with the reaction of an answer containing simplest the examine..

Table 1: *data stastical analysis*

Sample	Purity Angle	Purity Threshold	Mean assay (%w/w)	% Difference
Control Sample	0.055	0.287	99.3	0.3
Spiked Sample	0.076	0.293	99.6	

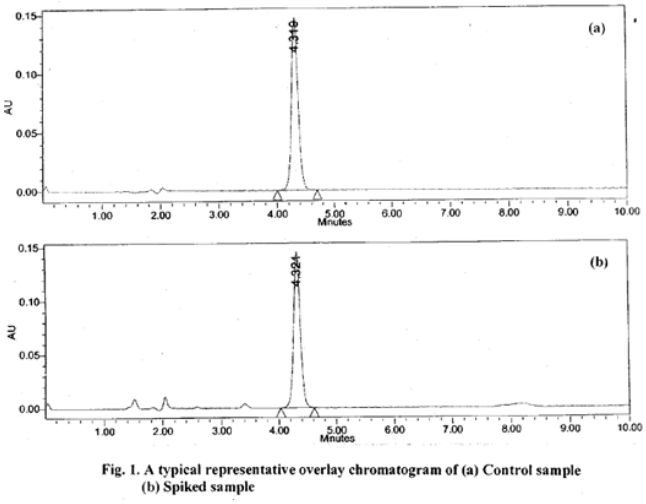

Fig. 1. A typical representative overlay chromatogram of (a) Control sample (b) Spiked sample

For specificity self-discipline, diluent, all related substances of Ganciclovir answers have been prepared for my part as in keeping with technique and inoculated into H_P_L_C to verify the retaining instances. Subsequently that answers of Ganciclovir drug Ingredient arranged in triplicate (consider as manipulate samples) and Ganciclovir drug substance spiked with all associated materials prepared in triplicate (considered

as spiked samples) as consistent with method and injected into HPLC to verify any co-evolution with Ganciclovir Top from any of related substance peak and diluent. The height homogeneousness come to be demonstrated for Ganciclovir top on pinnacle of factors pattern and spiked sample the usage of waters empower software program software and determined to be natural (purity attitude need to be less than purity threshold). The specificity outcomes are shown in Table 1 and an overlay chrome to gram of controls adequate and spiked pattern chromate grams is shown Fig.1. The stability indicating nature of the technique was further evaluated by appearing the pressured degradation research. As in keeping with International Conference on Harmonization (ICH), stress finding out is to be finished to come to be privy to the in all likelihood degradation merchandise or to explain the inherent balance traits of the energetic substance. The drug substance become subjected to strain conditions of hydrolysis, oxidation, photolysis, thermal and humidity degradation and determined that there was no interference decided for Ganciclovir height. The stress study check consequences are shown in Table2.

4.2 Linearity and Range

The linearity is the capacity of the technique to elicit test results which are straight away proportional to analyte concentration internal a given range. Range is the c application language duration between the upper and reduce ranges of analytic been mounted to be decided with accuracy and linearity. The variety is usually expressed within the same devices as the take a look at outcomes obtained by means of the approach. The ICH hints specify a minimum of 5 interest degrees, in conjunction with positive minimal targeted degrees. For assay approach, linearity special variety is from eighty% to 100 and twenty% of the test interest (25.Zero µg mL-1). Linearity emerge as estimated founded on the outstanding famous deviance of a regression line and slope end up accompanied. Standard answers had been injected into HPLC chromatograph, five interest ranges shape 20.0 µ.g m.L-1- 30.Zero µg mL-1(80% to one hundred twenty% of take a look at awareness). A plot of height region (µV*sec) versus cognizance (µg mL-1) emerge as drawn and statistics have become subjected to statistical evaluation the use of a linear-regression model. The statistical parameters slope, intercept, residual preferred on deviation reaction and correlation coefficient values are calculated and proven in Table 3.

Table 2: *Evaluation of forced degradation studies*

Type of Degradation	Degradation Condition	Ganciclovir assay (% w/w)	Degradation (% w/w)	Purity angle	Purity threshold
Undegraded sample	-	99.8	Nil	0.087	0.315
Acid degradation	5.0M HCL Initial	25.1	74.8	-	-
	0.5M HCL Initial	82.8	17.0		
	0.5M HCL R.T/ 10min*	82.1	17.7	0.185	0.558
	0.5M HCL R.T/ 20min	73.6	26.2	-	
	0.05M HCL R.T/ 20min	97.4	2.4	-	-
Alkaline degradation	5.0M NaOH Initial	0.0	99.9	-	-
	0.05M NaOH Initial*	77.5	22.3	0.124	0.421
	0.005M NaOH Initial	98.0	1.8	-	-
	0.005M NaOH R.T 5min	69.2	30.6	-	-
Peroxide degradation	30% H_2O_2 Initial	99.9	-0.1	-	-
	30% H_2O_2 90°C/ 10min	89.8	10.0	-	-
	30% H_2O_2 90°C/ 20min*	74.8	25.0	0.695	1.123
	30% H_2O_2 90°C/ 30min	65.2	34.6	-	-
Thermal degradation	80°C/ 120 Hrs*	99.2	0.6	0.122	0.308
Photolytic degradation	10 K Lux/ 120 Hrs*	99.6	0.2	0.121	0.321
Humidity degradation	92% RH/ 25°C/ 120 Hrs*	99.5	0.3	0.127	0.351

** Upto 25% w/w degradation is considered for reporting in all types of degradation conditions.*

Table 3: Arithmetic data of linearity

Statistical parameters	Ganciclovir
Concentration range (µg mL-1)	20 - 30
Slope	52341
Intercept	-1356
Residual standard on deviation response	1523
Correlation coefficient	0.9999

4.3 Accuracy

Accuracy is the confidence of the check effects acquired by the systematic technique to the actual price. Precision of the method emerge as performed thru recuperation experiments the usage of stylish addition approach. Accuracy standards for an assay technique (FDA) is that the recommend recuperation is probably one hundred ± 2% at each interest over the range of 80 - a hundred and twenty% of the take a look at attention. To document accuracy the ICH guiding principle on method recommends gathering records form not less than 9 determinations over no much less than three Concentration stages masking of desired range triplicate and the percentage recoveries were calculated. The common restoration values ranged from 99.7% - 100.0% and the average healing of 3 degrees (nine determinations) became 99.9%. The virtually confirmed accuracy results are shown in Table4. NThe healing test for the assay approach changed into evaluated in triplicate at 3 one among a kind concentration ranges starting from 80% to 100 and twenty% i.E eighty%, 100% and 100 twenty% of the check attention (25.0µg mL-1). These samples have been prepared as in step with take a look at method, analyzed in[5].

Table 4: *Statistical data of Accuracy*

Sample Identification	Ganciclovir				
	Amount Added (mg)	Amout Found (mg)	Recovery (%)	Statistical analysis	
80% Level sample-1	40.26	40.21	99.9	Mean*	100.0
80% Level sample-2	40.32	40.34	100.0	SD*	0.1
80% Level sample-3	40.40	40.45	100.1	%RSD*	0.1
100% Level sample-1	50.03	49.99	99.9	Mean*	99.9
100% Level sample-2	50.07	49.97	99.8	SD*	0.10
100% Level sample-3	50.01	50.03	100.0	%RSD*	0.1
120% Level sample-1	60.54	60.61	100.1	Mean*	99.7
120% Level sample-2	60.29	60.13	99.7	SD*	0.40
120% Level sample-3	59.63	59.21	99.3	%RSD*	0.4
Overall statistical analysis	Mean*	99.9			
	SD*	0.25			
	%RSD*	0.3			

Table 5: *Statistical data of precision*

Precision (System precision, Method precision and Ruggedness)			
Injection Identification	System precision Area (µV*sec)	Method precision Assay (%w/w)	Ruggedness Assay (%w/w)
1	1125415	100.0	100.3
2	1114519	100.1	100.0
3	1118688	100.2	100.2
4	1115236	99.9	99.9
5	1112837	100.1	99.8
6	1119936	100.0	100.0
Mean	1081113	100.0	100.0
SD	3787	0.10	0.17
%RSD	0.4	0.1	0.2

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5. PRECISION

Precision is the diploma of the diploma of repeat ability of an methodical approach beneath regular process and is usually

expressed because the proportion relative popular deviance (RSD) for a statistically big range of models. The typical overall performance of the method come to be appraised with replicate injections of famous and sample results. Typical answer come to be analyzed six times for checking the performance of the H.P.L.C device under the chromatographic conditions on the day examined (System precision). The transitional exactness have become the Inter-day version (Ruggedness), become defined due to the fact the diploma of The relative widespread deviation for Ganciclovir widespread is zero. Four%. Repeat ability and re-productiveness of the technique grow to be studied with the aid of the usage of reading six pattern answers one at a time. Repeat capacity turned into the infra-day variant (Method precision), confirmed with the aid of manner of having equipped six pattern solutions individually the use of a unmarried batch of Ganciclovir drug substance as consistent with approach and assay modified into decided. The relative massive deviation for the assay of Ganciclovir is zero.1%. Test. The assessment of the same sample (that is used within the Method precision) beneath a diffusion of Reproducibility received with the aid of following the equal gadget as referred to for technique precision conditions the usage of exquisite tool, column, with specific analyst on unique day by way of making prepared new enormous, new deviation for the assay of Ganciclovir is zero.2%. The honestly installed precision (System precision, Method precision and Ruggedness) Results are shown in Table five.

6. ROBUSTNESS

To verify the robustness of the method, experimental conditions were deliberately altered. The study turned into accomplished with respect to pH ± 0.2, wavelength ± five nm, natural model in cell segment ± 2% and column glide ± 10%. In each robustness scenario final chromatography conditions are identical as in line with take a look at approach. In every robustness circumstance, massive solution become organized as in step with technique and injected unmarried time in HPLC system as system suitability and once more trendy solution become injected 5 replicates in HPLC machine. From the device suitability it became determined that there may be no much model in retention time, USP plate count and USP tailing for Ganciclovir peak, received at unique intentionally severa robustness situations from the check method. Hence the take a look at approach is robust for all numerous conditions. The without a doubt robustness effects are proven in Table 6.

7.SOLUTION STABILITY

The pattern solution was organized as in line with check method. The balance of sample answer changed into examined thru recording the chromatograms freshly organized and at unique intervals upto 24 hours through retaining, pattern cooler temperature at 25°C. The % distinction inside the top regions of Ganciclovir from freshly to specific time c application language period changed into decided 0.2. From the effects, it's far concluded that pattern answer end up robust for twenty-four hours at ambient temperature (25°C).

8. CONCLUSION

A clean isocratic stability indicating opposite segment liquid chromatography (RP-HPLC) technique became superior and tested for the self-discipline of assay of Ganciclovir in Ganciclovir drug substance. The consequences of diverse validation parameters showed that the approach is precise, stability indicating, linear, unique, answer balance, strong and accurate. Hence the proposed method is straightforward, dependable and person-excellent, for the determination of assay of Ganciclovir in Ganciclovir drug substance.

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