A biodegradable approach for efficient and economical spectrophotometric assay for the Platelet aggregation inhibitor Clopidogrel

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Abstract- A modest as well as discriminating UV-visible process was defined for the purpose of Clopidogrel in drug dose methods. Spectrophotometric method remained accomplished along with buffer containing of a mixture of 55ml capacities of Mixed 0.1N HCl and 45ml volumes 0.1N HCl with detection of 232nm. The recommended process remained authenticated in standings of linearity, accuracy, precision, maximum of detection, limit of quantification. Linearity remained observed in the assortment 10-30 µg/ml for Clopidogrel (r2 =0.998) for the volume of tablets predictable by the projected techniques remained in smart arrangement by the tag privilege. The precision of the techniques remained evaluated by retrieval studies at 3 different stages. Recovery research designated the deficiency of interference since ordinarily run into medicinal condiments. The outer as well as inner day accuracy CV -%age remained inside (10.9%) as well as the precision remained at the series of 88.5 –101.3% for mirror image. The abstraction retrieval remained > 90.2% as well as not at all observable matrix consequence remained detected. The process remained foundto be accurate as designated by the repeated investigation, show relative standard deviation (RSD) percentage trials. The RSD -% values specify noble accuracy as well as extraordinary retrieval. The results of the projected ways remained associated statistically by the reference process. All statistical information evidences validity of the ways and may remain use for predictable investigation of medicinal dose form. The process remained validated for linearity, precision, accuracy, system appropriateness, as well as constancy. This technique remained validated giving to ICH (International Conference on Harmonization) strategies that contain linearity, accuracy, precision, toughness, LOD (Limit of detection), LOQ (Limit of Quantification). The consequence attain remained in theapproval criteria as per ICH guidelines.

Index Terms- Buffer, ICH, LOD, LOQ, pharmaceuticals, accuracy, validation, precision, linearity

I. INTRODUCTION

(S)clopidogrel, is an effective antithromboticand antiplatelet medication of the thienopyridine family (anti aggregant). As (S) -clopidogrel demand rises; the synthetic community is compelled to develop simple synthetic methods [1].Clopidogrel remains an actual antithrombotic as well as antiplatelet medication in numerous tentative kinds of thrombosis, giving to (S) -clopidogrel. (S) - 2 Clopidogrel used to give as well as stop marginal arterial maladies, coronary artery diseases, as well as cerebrovascular coincidences. In those with a past of atherothrombotic syndromes, clopidogrel can be managed only or in aggregation with aspirin to lesser the hazards of non-fatal myocardial infarction (MI), cardio-vascular disease (CVD), as well as stroke (ATDs) [2]. Latest examination takes shown that (S) clopidogrel, equal at abundant lesser amounts, remains further operative in avoiding platelet accumulation than ticlopidine as well as aspirin [3]. Small over-all yields remain a problematic by the (+) (S) clopidogrel bisulfate one from Sanofi remaining Aventis's synthesis method, as well as time taking purification processes remain needed to achieve enantiomeric as well as chemical pureness for treatment as a pharmacological medication. This treatment's one step, appropriate atomic, step inexpensive, globally answerable, as well as economical manufactured remains compulsory for it to develop additional generally used, additional active, as well as cheap. There are four key steps in the production of (S)- clopidogrel bi-sulfate using 4,5,6,7-tetra-hydrothieno [4]. Pyridine hydrochloride, methyl-amino(2-chlorophenyl) acetate, mantellic acid products, as well as the Strecker production. A brief summary of the several methods revealed in the writings for producing optically pure (S) clopidogrel produced [6]. One ofthe main constituents of the relevancecalculation method remains the discovery of definite or forthcoming drug-drug interactions (DDIs) as well as drug-drug interactions. The word "target drug" 3 refers to a medication whose pharmaco-kinetics and/or pharmaco-dynamics could be changed as a consequence of a prescription communication [7]. By universal deals of $9.4 billion in 2010, Plavix® (clopidogrel), sold jointly by Sanofi Aventis and Bristol-Myers Squibb, is the second-most popular medication worldwide. The P2Y12 nucleotidereceptor on the membranes of blood platelet cells isthe drug's mechanism of action [8]. Clinicians influence not conscious of these DDIs when clopidogrel remains a causing representative subsequently latest fascinating conclusions in clopidogrel-related DDI study have not been addressed in complexity. Elements disturbing DDI amounts as well as actual hazard organization. By the target of improving the reader's considerate of DDIs associated to clopidogrel, we comprehensively scrutinize that problematic as well as give an efficient outline in this artifact [9.] Cardio-vascular diseases remain the most common reason of transience as well as morbidity in Western countries in the 21st era. In patients with symptomatic coronary artery syndrome, percutaneous coronary intervention (PCI) by stenting remains operative in preventing additional ischemic procedures [10].

II. MATERIALS AND METHOD

A. Reagents and chemicals

Clopidogrel bi-sulphate remained compassionate provided by Getz Pharma (Pvt.) Ltd., (Karachi, Pakistan). Plagril Plus 75 tablets marked as 75 mg clopidogrel and whose declared content

is 75 mg clopidogrel. Totally investigative status components secondhand remained attained from Merck (Pvt.) Ltd., (Karachi, Pakistan). Methanol gradient (Tedia, City and State) Acetonitrile, orthophosphoric acid and potassium dihydrogen phosphate, sodium hydroxide was attained from Merck and HPLC grade Milli-Q water was used forthe experiment.

B. Apparatus

160 digital dual-beam model Ultraviolet -Visible spectro-photometer (uv -1800 PC) of SHIMADZU (Japan), inter-faced to a processor like the SHIMADZU UV Probe Information System Program (Version 1.10), using a 1.00 cm quartz cell, remained use to measure the absorption of a simple, digital electric scale, volumetric flask (BDH) as well as pipette (BDH). Weighing balance, Pestle Mortar, volumetric flask and Beaker were also employed in this study.

C. Solubility Studies

The solubility study accomplished with numerous solvents remains enumerated below in the table.

Table. Solubility pattern of drug in different solvents.

Solvent	Clopidogrel bisulphate
Distilled water	Sparingly soluble
0.1N NAOH	Sparingly soluble
0.1N HCL	Sparingly soluble
DMF	Freely soluble
Acetone	Insoluble
CH_3CH_2OH	Freely soluble
CH_3OH	Freely soluble

D. Determination of λmax

The UV spectra of clopidogrel bisulphate were scanned across the ultraviolet region as well as the lambda max obtained for clopidogrel bisulphate was at 260 nm. Pipette (6 ml) standard solution (90.025 ppm) of clopidogrel bisulphate into (10 ml) volumetric flask, add (1 ml) methanol, then diluted to the mark by the similar solution, the absorbance of the solution remains dignified in therange (300 -900 nm).

E. Preparation of Stock solution

A solution of clopidogrel bisulphate was prepared by dissolving (0.1805 g) pure clopidogrel bisulphate with (10 mL) methanol solution and gentle heating was used to increase decomposition and solubility, then it remained movedto a volumetric flask (100 mL) and filled into label with methanol. Stock solutions of 100 µg/ml clopidogrel remained organized in CH_3OH. Working standard solutions were prepared by diluting the stock mixture by the similar solvent to achieve a concentration range of 0.25 –50 µg/ml. Those solutions remained use in the research of the calibration curves. Entirely solutions remained prepared daily as well as safe from light. Stock solutions remained storedat 4 °C.

F. Evaluation of Tablet doses

The critical parameters considered during process validation of Clopidogrel bisulphate tablets were.

- Dry mixing,
- Drying,
- Milling,
- Mixing/Making,
- Compression
- Blister packaging

The dried mixing stepped involves mixing the active ingredients with other ingredients used a rapid mixer granulator (rmg). Mixing speed and mixing time were critical variables. The mixing speed was kept constant, the mingling time must been calculated to validate the dried mixing stepped. In the dried mixing phase, 3 batches as i, ii and iii were considered for validation. The dried mixing results of all lots were within acceptable criteria [11].

The drying stepped involves drying the wet mass. An important factor was the moisture leveled in the granules. If the moisture leveled was higher in the granules, then the mixture would have poor flowed and distribution properties. If the moisture leveled in the mixture was lowered, a capped tablet had been formed, with high friability and peeling problems. During drying, the desired lod had been maintained in the granules, which would affect quality parameters such as tablet hardness, flowed properties, and physical properties during compression.Drying the granules in the fbd controls the moisture leveled. The fbd inlet temperature was the most acute variable for this. The lod was checked at regular intervals to determine the correlation with the output temperature. Batch drying results were within acceptable criteria [12].

The granule size was obtained by sieving the granules from the specified sieve and the granule retention on the sieves was have been ground with the speed of the multimillion and the forward direction of the knives should been monitored and a sample taken at 10 the end of the sizing operation to monitor the particle size distribution, bulk weight and load as part of validation.

This stepped consist of mingling the Mg stearate with the pills particles and other mixing material. The screened lubricants were transferred to an octagonal mixer containing dried pyrazinamide granules and mixed for 10 minutes at low speed. Transfer the sifted magnesium stearate to an octagonal mixer as well as blend for three minutes on low speed. The determination of mixing was to obtain an even dissemination of the api. That was followed by mixing the un-lubricated mixture with a lubricant toachieve good fluidity and anti-adhesion properties of the mixture. Mixing speed and time were criticalvariables in that method. The mixing speed was kept constant. Agitation time was critical, as insufficient agitation would result in uneven drug distribution and poor flowed, while excessive agitation would result in dispersal of uneven drug distribution. Checked the consistency of these contents at a fixed time would verify the mixing time. In the mixing phase, three batches, i. e.Batches i, ii and iii, were considered for validation. The mixing results of all batches were in accordance with the acceptance criteria. This step involves the consistent flowed of an effectively lubricated, constant mixture into the molds where the particles were compacted into tablets. Compression was done according to the batch production recorded. Took the samples at different phases, like at lowest hardness, greater hardness, lowest speed, maximum speed as well as at optimum speed initial stage, middle stage and final stage of pressing as well as perform testing of physical parameters such as appearance, group weight, diameter, hardness, width, friability, dissolution time

and average weight, disbanding at maximum hardness only as well as analysis. At the compression stage, three batches were considered for validation, like Batch no. A,b and c. The compression results of all batches were within the acceptable criteria. Various physical parameters, approximate sample size, compression acceptance criteria.[13]

G. Experimental

The amount of dissolved clopidogrel bisulphate using UV absorption at the wavelength of determined absorbance at approximately 268 nm wasresolute on sieved portions of the test mixture, suitably it was dilute by suspension medium as well as compared to a standard solution of well-known concentration of pyrazinamide RS in the same medium. <75% of the considered amount of clopidogrel bisulphate was dissolved for 45 minutes. The equivalence of the batch units met the requirements.

H. Assay

20 tablets weighed and crushed, the amount of powdered containing approx. 0.1 g of Clopidogrel bisulphate was added into 200 ml of H_2O and stand for 10 minutes with occasional stirring, mixed using ultrasound for ten minutes and diluted by H_2O to 500.0 ml. It was filtered as well as discarded the first 20ml of deposit. Diluted 5.0 ml of the filtrate to 200.0 ml with water and measured the absorbance of the resulting solution at the maximum at about 260nm.Calculated the clopidogrel bisulphate with a value of 650 as the specific absorbance at 260 nm.

III. RESULTS AND DISCUSSION

A. Geometrical Mixing

Table. Mixing stage of various Batches of drug

Stage	Batch # A	Batch # B	Batch # C
Geometrical Mixing	Mechanical Sifter(with sieve of meshsize **30, 100**) Cone Mixer 5Kg Cone Mixer 25Kg	Mechanical Sifter(with sieveof mesh size **30,100**) Cone Mixer 5Kg Cone Mixer 25Kg	Mechanical Sifter(with sieveof mesh size **30, 100**) Cone Mixer 5Kg Cone Mixer 25Kg
EquipmentID	PR.TBM.EQ.121 PR.RD.EQ.169 PR.TBG.EQ.115	PR.TBM.EQ.121 PR.RD.EQ.169 PR.TBG.EQ.115	PR.TBM.EQ.121 PR.RD.EQ.169 PR.TBG.EQ.115

Symmetrical blender weighing 5kg as well as 25kg cone blender has been employed for the sample mixing in 3 batches. In the final step, 300kg cone blender was used for mixing in three batches.

B. Final Mixing

Table. Final mixing of batches

Stage	Batch # 097	Batch #098
Final Mixing	Cone Mixer 300 Kg	Cone Mixer 300Kg
Equipment ID	PR.TBG.EQ. 019	PR.TBG.EQ. 019

C. Testing parameters

There were 2 tests for clopidogrel

- Iron (III) Hydroxide polymaltose complex test
- Folic acid test

Testing parameters were done in 3 groups of both experiments. The place value was in three coefficient of variation. Thesevariations were also exposed in the chart. Plot a graph amongst the test as well asthe lot number as well as designate the changed %age.

Table. Testing Parameters

BN		Assay of Iron (III) Hydroxide PolymaltoseComplex (min.)			Assay Of Folic Acid (min.)			V rpm	T (min.)
	P	30	35	40	30	35	40 .		
	T	03.79	96.88	103.13	102.18	99.64	98.77		
	M	02.15	103.95	99.91	100	99.10	100.65		
A	B	03.67 03.20	103.23 101.35	101.59 101.54	97.18 100.06	100.05 99.60	94.87 98.10	9	30 35 40.
	STD	0.91	3.90	1.61	2.58	0.48	2.95		
	CV	0.89	3.84	1.59	2.58	0.48	3.01		
	P	30 min.	35 min.	40 min.	30 min.	35 min.	40 min.		
	T	7.96	99.20	105.17	94.41	94.59	96.23		
	M	7.35	97.80	99.92	93.85	92.41	91.24		
B	B	3.14 7.82	98.17 98.39	100.64 101.91	91.66 93.31	96.36 94.45	100.24 95.90	9	30 35 40
	S	0.41	0.73	2.85	1.45	1.98	4.51		
	CV	0.42	0.74	2.79	1.56	2.09	4.70		
	P	30	35 min.	40 min.	30 min.	35 min.	40 min.		
	T	101.59	101.33	102.68	95.87	93.17	94.48		
	M	104.68	105.52	101.80	98.35	98.18	93.54		
C	B	01.73 02.67	101.02 102.62	101.54 102.01	94.97 96.40	92.62 94.66	92.73 93.58	9	30 35 40
	S	1.75	2.51	0.60	1.75	3.06	0.88		
	CV	1.70	2.45	0.59	1.82	3.24	0.94		

A. Standard Parameters

Standard parameters were assay (%), coefficient of variation (%) and percentage of significance level. After all percentage acceptance criteria was 90 - 110%, 5.0%, 95%

Table. Standard Parameters

Standard Parameters	Assay (%)	Assay (%)	Acceptance Criterion
Mean Value Assay (%) Single Values	X = 101.82 No value < 101.54 or > 102.01	X=95.86 No value < 93.58 or > 98.1	90 -110%
Target value	100	100	
Co-efficient of Variation (%)	0.24	2.36	< 5.0 %
Significance level (%)	101.20 -102.44	90.25 -101.47	95 %

B. Tableting

In tableting two stages of equipment was compression and equipment ID. They were determined in three batches. Tableting parameters included Weight variation, Assay, Thickness, Hardness, and Friability. Quantity was required 20g. There were two parameters, machine parameter and testing parameter. In machine parameter speed of rotation, compression force, weight variation, thickness, hardness and friability were measured in three batches and in testing parameters assay of finished percentage and appearance were measured.

Table. Sampling Plan

Stage	Batch # 097	Batch # 098
ZPT-16 Compression Compression	ZPT-16 Compression Machine	ZPT-16 Compression Machine
Equipment ID	PR.TBG.EQ.0 20	PR.TBG.EQ.0 20

C. Blistering

In stage of blistering primary packaging was applied. In primary packaging two stages blistering and equipment ID was present. Blister machine was used in this stage. This process was performed in three batches and in every batch 250-S Blister machine was used. And the ID number of the machine was PR.PK.EQ. 221. There were different critical parameters speed, width of Aluminum - Aluminum u foil, width of Aluminum foil, number of blisters per stroke, Blister type, length and width of blisters, ceiling, temperature, bubble shape, tablet thickness tablet diameter, tablet shape, tablet color, leakage test, total aerobic Count were measured in three batches.

IV. CONCLUSION

In pharmaceutical studies, spectrophotometric analysis remains significant importance. Keeping in view these considerations, clopidogrel an anti-coagulant drug has been analyzed via spectrophotometric assay. Various tests have been employed for the study such as, folic acid test. The recommended process remained authenticated in standings of linearity, accuracy, precision, maximum of detection, limit of quantification.

Linearity remained observed in the assortment 10-30 µg/ml for Clopidogrel (r2 =0.998) for the volume of tablets predictable by the projected techniques remained in smart arrangement by the tag privilege. This technique remained validated giving to ICH (International Conference on Harmonization) strategies that contain linearity, accuracy, precision, toughness, LOD (Limit of detection), LOQ (Limit of Quantification). The consequence attain remained in theapproval criteria as per ICH guidelines.

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References

1. Alfredsson, J., Swahn, E., Gustafsson, K. M.,Janzon, M., Jonasson, L., Logander, E,Lindahl, T. L. (2019). Individual long-term variation of platelet reactivity in patients with dual antiplatelet therapy after mayocardial infarction. Platelets, 30(5): 572-578. https://doi.org/10.1080/09537104.2018.1479519
2. Ahmed, F. (2020). A network-based analysis reveals the mechanism underlying vitamin D in suppressing cytokine storm and virus in SARS-CoV-2 infection. Frontiers in immunology, 11, 590459. https://doi.org/10.3389/fimmu.2020.590459
3. Wilder, Rebecca S. "The Branding Campaign, the Journal, and our Editorial Review Board." Journal of Dental Hygiene 81.3 (2007). Source: Journal of Dental Hygiene, Vol. 81, No. 3, July 2007
4. Antithrombotic Trialists' Collaboration. "Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients." Bmj 324.7329 (2002): 71-86. https://doi.org/10.1136/bmj.324.7329.71
5. Comerota, Anthony J. "Effect on platelet function of cilostazol, clopidogrel, and aspirin, each alone or in combination." Atherosclerosis Supplements 6.4 (2005): 13-19. https://doi.org/10.1016/j.atherosclerosissup.2005.09.005
6. Lemley, Mark A. "Expecting the unexpected." Notre Dame L. Rev. 92 (2016): 1369.
7. Saeed, Aamer, et al. "Developments in the synthesis of the antiplatelet and antithrombotic drug (S)-clopidogrel." Chirality 29.11 (2017): 684-707. https://doi.org/10.1002/chir.22742
8. Lateiner, Donald. The historical method of Herodotus. Vol. 23. University of Toronto Press, 1989.
9. Hargis, Mary B., and Alan D. Castel. "Younger and older adults' associative memory for medication interactions of varying severity." Memory 26.8 (2018): 1151-1158. https://doi.org/10.1080/09658211.2018.1441423
10. Raschiatore, Marlene. "Pharmaceutical Product Manufacturing." Risk Management Applications in Pharmaceutical and Biopharmaceutical Manufacturing (2013): 275- 323. https://doi.org/10.1002/9781118514399.ch11
11. Lo, Cody, et al. "Pharmacogenomics in Asian subpopulations and impacts on commonly prescribed medications." Clinical and Translational Science 13.5 (2020): 861- 870. https://doi.org/10.1111/cts.12771
12. Ferri, Nicola, Alberto Corsini, and Stefano Bellosta. "Pharmacology of the new P2Y 12 receptor inhibitors: insights on pharmacokinetic and pharmacodynamic properties." Drugs 73 (2013): 1681-1709. https://doi.org/10.1007/s40265-013-0126-z
13. Snoep, Jaapjan D., et al. "Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis." American heart journal 154.2 (2007): 221-231. https://doi.org/10.1016/j.ahj.2007.04.014

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