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DEVELOPMENT AND VALIDATION OF THE DISSOLUTION METHOD FOR FIXED DOSE COMBINATIONS OF AMLODIPINE AND HYDROCHLOROTHIAZIDE ACCORDING TO ICH GUIDELINES

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ABSTRACT

The goal of this work is development & validation of dissolution method for Amlodipine and Hydrochlorothiazide in different conditions, such as dissolution medium type, volume, rotation speed, apparatus, and filter appropriateness. Apparatus II (paddle) and 1000 mL of phosphate buffer pH 6.8 & 0.1N HCL as the dissolution medium were used to achieve the most discriminative release profile for Amlodipine and Hydrochlorothiazide which was kept at 37 0.5°C with a rotation speed of 75 rpm. UV/Vis spectrophotometry at 239 & 271nm was used to quantify the emitted active ingredient. The dissolution method was validated using the following parameters: specificity, accuracy, precision, linearity, robustness, and stability of the solutions, which were found to match the preset acceptance requirements. A developed dissolution method has the ability to distinguish any changes related to quantitative formulation and can be used for routine batch testing.

Keywords: Dissolution apparatus, Amlodipine, Hydrochlorothiazide, fixed dose combinations, phosphate buffer pH 6.8 & 0.1N HCL

1. INTRODUCTION

Solid dosage forms for oral administration are frequently used in clinical practise because they are convenient, stable, cost-effective, and generally safe. They, on the other hand, present bioavailability issues connected to the absorption process. The release of the drug substance from the drug product, the solubilisation of the drug under physiological conditions, and the permeability throughout the gastrointestinal system all influence drug absorption from a solid dosage form following oral administration. As a result, the significance of dissolving tests and dissolution profiles in establishing pharmacological equivalency must be emphasised (Dressman *et al*., 1998; Ansari *et al*., 2004).

The dissolving stability (i.e., the retention of a solid oral dosage form's dissolving characteristics from the time of manufacture to the expiration date) is a critical metric in terms of quality control, regulatory compliance, and impact on product bioavailability. Significant changes in a medication product's in vitro release patterns during storage may affect its bioavailability. Formulation components (active drug, excipients, and coating materials), processing parameters, storage circumstances, and packaging all have an impact on a product's dissolving stability during ageing (Murthy and Ghebre- Sellassie, 1993). The role of each of these elements in promoting changes in dissolution in both immediate-release and modified-release products varies by product and must be studied on an individual basis.

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Although data obtained under accelerated storage conditions are not useful in predicting the product's dissolution shelf-life under ambient conditions, they are useful in assessing the product's "ruggedness" and ability to withstand the varied climatic conditions during transport, shipping, and storage (Zhang *et al*., 2022). This study deals with Development and validation of the dissolution method for fixed dose combinations of Aliskiren, Amlodipine and Hydrochlorothiazide according to ICH guidelines.

Amlodipine is often used to treat high blood pressure and angina. Amlodipine has antioxidant characteristics as well as the potential to increase the generation of nitric oxide (NO), a vasodilator that lowers blood pressure. Amlodipine's single daily dose option is an appealing aspect of this medication (Naylerm, 1997).

Also, the most commonly given thiazide diuretic is hydrochlorothiazide. It is used to treat edoema and high blood pressure. The usage of hydrochlorothiazide is frequent, however it is diminishing in favour of angiotensin-converting enzyme inhibitors. Many combination medications with hydrochlorothiazide and angiotensin converting enzyme inhibitors or angiotensin II receptor blockers are available (Beermann *et al*., 1976).

2. MATERIALS & METHODS

Chemicals

Distilled water, HCL, Phosphate buffer (ph 6.8), Acetate buffer were obtained from Fine chemicals, Mumbai. All reagents used were of standard laboratory grade.

Solubility of drug

Solubility of the drugs was determined in different media by using orbital shaking method.

Wavelength selection

The standard solution of amlodipine and hydrochlorthiazide were prepared. From these stock appropriate dilutions to obtained solution 10 µg/ml and 25µg/ml concentration of amlodipine and hydrochlorthiazide respectively. Then both the solution were scanned in the spectrum mode over the range of 400-200nm and oerlay spectrum was taken . from overlay spectrum it was observed that amlodipine is interfering with hydrochlorthiazide at the maxima but the hydrochlorthiazide not interfering with amlodipine above 250nm range. Therefore both the drug can be estimated by these method. Two such a wavelengths were selected where the maximum absorbance of amlodipine and hydrochlorthiazide is observed that is 239 & 271nm respectively (Sugano *et al*., 2007).

In vitro dissolution study

In vitro study was performed taken 12 tablet of each brand in two media 0.1N HCL and phosphate buffer pH 6.8. The sample withdrawn at 5, 15, 30, 45, 60 minute time point and analysed by uv spectrophotometer. The dissolution profile was compared by ANOVA, similarity factor (f_2) and difference factor (f_1) analysis (Yuksel *et al*., 2000).

3. VALIDATION FOR DISSOLUTION STUDY

Linearity

Linearity confirms the ability of the dissolution method to provide results that are directly proportional to the concentration of the API(s) within the specified range. FDC dissolution methods must demonstrate linearity for each individual API and their combinations.

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Accuracy

Accuracy assesses the closeness of the measured values to the true or known values. In the context of dissolution method validation for FDCs, it is crucial to ensure that the method accurately determines the amount of each active pharmaceutical ingredient (API) in the presence of other components in the formulation.

Precision

Precision evaluates the repeatability and reproducibility of the dissolution method. It includes both intra-day (within the same day) and inter-day (between different days) precision. For FDCs, precision is particularly important to demonstrate that the method can consistently measure the dissolution of individual APIs and their combinations.

System Suitability

System suitability tests are performed before actual dissolution testing to ensure the proper functioning and suitability of the dissolution apparatus. It includes parameters such as flow rate, paddle/basket speed, temperature, and other relevant instrumental factors.

4. RESULTS & DISCUSSION

The results of solubility study revealed that both the drug are soluble in 0.1 N HCL. Stastical data of linearity for amlodipine indicate that r^2 value of 0.999 with 0.028 as slope. The linearity range was observed to be 10-50 µg/ml. In case of hydrochlorthiazide the r^2 value & slope value was seen to be 0.9998 & 0.063 with linearity range of 5-25 µg/ml. Further % Recovery, SD& %RSD of both the drug was analyzed.

The accuracy, interday & intraday precision for amlodipine was observed to be 100.026, 99.85 & 100.32 respectively. In case of hydrchlorthiazide, interday & intraday precision was observed to be 99.73 & 99.86 respectively. While accuracy of hydrchlorthiazide was seen to be 99.7.

The % cumulative drug release in release profile of amlodipine in pH 6.8 phosphate buffer for ARB, MNB & MAB at 60 min was seen to be 98.23±1.97, 97.65±2.31, & 92.25±1.51 respectively. The Similarity factor (f2) & Difference factor (f1) for MNB as 33.88 & 2.26 & for MAB was observed to be -32.15 & 10.50 respectively.

The release profiles of hydrochlorthiazide in buffer suggested the % cumulative drug release in 60min for ARB, MNB & MAB observed to be 75.23, 91.25 & 74.21 respectively. Here, The Similarity factor (f2) & Difference factor (f1) for ARB was seen to be -1.0223 & 19.38. While for MAB it was observed to be -79.588 & 37.35.

Further the drug release profile of both the drug was checked by using medium of 0.1N HCL. The % cumulative drug release for hydrochlortzide at 60 min was noted to be 93.56, 59.72 & 75.51 respectively for ARB, MNB & MAB.

In case of amlodipine in 0.1 N HCL medium the % cumulative drug release for ARB, MNB & MAB was observed to be 93.43, 81.32 & 90.66 respectively. The Similarity factor (f2) & Difference factor (f1) for MNB was observed to be 107.74& 60.36 while for MAB it was estimated to be 42.34 & -92.38.

Table 1: Solubility of the both the drug in different media

S. No.	Medium	Solubility (µg/ml)	
1	Distilled water	342	132
2	0.1 N HCL	609	139

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3	Phosphate buffer(ph 6.8)	465	109
4	Acetate buffer	232	105

Table 2: Stastical data of linearity for amlodipine

Data for linearity value	Values
Correlation coefficient(r^2)	0.999
Slope(m)	0.028
Interept	0
Linearity range(µg/ml)	10-50 µg/ml

Table 3: Stastical data of linearity for hydrochlorthiazide.

Data for Linearity value	Values
Correlation coefficient(r^2)	0.9998
Slope(m)	0.063
Interept	0
Linearity range(µg/ml)	5-25 µg/ml

Table 4: Stastical data of accuracy and precision for simultaneous estimation method

Method parameter	**Stastical parameter**					
	% Recovery		**SD**		**%RSD**	
	AMLO	**HCTZ**	**AMLO**	**HCTZ**	**AMLO**	**HCTZ**
Accuracy	100.026	99.7	0.8845	0.034486	0.8842	0.03458
Precision						
Interday precision	99.85	99.73	0.2325	0.2001	0.2385	0.2006
Intraday Precision	100.32	99.86	0.232	0.4076	0.231	0.4081

Table 5: Data of linearity

Data for linearity	Values
Correlation coefficient(r^2)	0.9998
Slope (m)	0.063
Intercept	0
Linearity range(µg/ml)	5-25 µg/ml

Table 6: Response ratio of hydrchlorthiazide

S. No.	Concentration (µg/ml)	Response ratio
1	5	0.06891
2	10	0.06371
3	15	0.06442
4	20	0.07001
5	25	0.06721

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Table 7: Drug release data for similarity actor analysis of amlodipine in buffer

Time in min.	% cumulative drug release(mean ± SD, n=12)		
	ARB	MNB	MAB
5	78.53±2.87	72.61±2.43	67.51±3.51
15	85.44±2.32	86.32±3.21	70.73±2.42
30	88.21±2.65	88.24±3.23	81.23±1.98
45	94.19±1.34	92.35±1.44	87.76±2.45
60	98.23±1.97	97.65±2.31	92.25±1.51

Table 8: Stastical data of ANOVA (dunnet's t test) and similarity factor analysis

Brand name	t	Similarity factor (f_2)	Difference factor (f_1)
MNB	1.08	33.88	2.26
MAB	21.4	-32.15	10.50

Table 9: Comparison of release profiles of hydrochlorthiazide in buffer

Time in min.	% cumulative drug release(mean ± SD, n=12)		
	ARB	MNB	MAB
5	39.35	58.21	30.36
15	48.84	67.81	35.65
30	56.92	73.44	42.39
45	61.21	83.35	51.72
60	75.23	91.25	74.21

Table 10: Stastical data of ANOVA (Dunnet's t test) and similarity factor analysis

Brand name	t	Similarity factor (f_2)	Difference factor (f_1)
ARB	75.38	-1.0223	19.38
MAB	28.76	-79.588	37.35

Table 11: Comparison of release profiles of hydrochlortzide in 0.1N HCl

Time in min.	% Cumulative drug release (mean ± SD, n=12)		
	ARB	MNB	MAB
5	66.34	10.21	16.45
15	73.45	16.32	29.33
20	79.88	19.36	38.92
30	83.23	37.82	51.66
45	88.91	48.96	68.21
60	93.56	59.72	75.51

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Table 12: Stastical data of ANOVA (Dunnet's t test) and similarity factor analysis

Brand name	t	Similarity factor (f_2)	Difference factor (f_1)
MNB	Not found	107.74	60.36
MAB	12.48	42.34	-92.38

Table 13: comparison of release profiles of amlodipine in 0.1 N HCl

Time in min.	% cumulative drug release(mean ± SD, n=12)		
	ARB	MNB	MAB
5	60.43	17.65	43.87
15	68.56	28.98	57.30
30	78.98	48.64	74.76
45	86.76	63.75	82.52
60	93.43	81.32	90.66

Table 14: Stastical data of ANOVA (Dunnet's t test) and similarity factor analysis

Brand name	t	Difference factor (f_1)
MNB	0.692	38.08
MAB	1.432	10.06

5. CONCLUSION

Dissolution testing is an essential in vitro technique for evaluating medicinal products. Amlodipine and Hydrochlorothiazide which are used to treat hypertension , has no credible dissolution conditions in its BP or USP monograph. In this work, we compared discriminating conditions like medium of 0.1 N HCl & 6.8 phosphate buffer medium, paddles, and a stirring speed of 75 rpm for dissolution testing of, Amlodipine and Hydrochlorothiazide **.** The method can be employed in formulation development research as well as biobatch selection for bioequivalent studies. The proposed method is particular, accurate, exact, linear, and robust, and it can be used to properly evaluate the batch-to-batch quality and stability of the medical product.

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