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QbD based method development forsimultaneous quantification for Amlodipine Besilate, Hydrochlorothiazide and Olmesartan medoxomil Film-coated tablet dissolutions in different dissolution media by RP-HPLC

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ABSTRACT

The scientific way to develop a simple and robust analytical HPLC method for the critical separationsis QbD approach. Quality-by-design (QbD) is a systematic approach to product or process development, which begins with predefined objectives, and uses science and risk management approaches to gainproduct and process understanding and ultimately process control. The concept of QbD canbe extended to analytical methods. A simple Analytical method was developed and used to identify and quantify simultaneously the three active pharmaceutical ingredients Amlodipine (AML), Hydrochlorothiazide (HCTZ) and Olmesartan medoxomil (OLM)in presence of major degradants, sample matrix and other extraneous peaks from different dissolution medias, namely pH1.2 Hydrochloric acid ,pH 4.5 Acetate buffer and pH 6.8 Phosphate buffer solutionsby reverse phase HPLC method. The identified CQA (Critical quality attributes) are resolution between acetate peak from HCTZ peak, resolution between HCTZ and Olmesartan (Metabolite of Olmesartan medoxomil) and the resolution between OLM and AML which will effects the quality of the product and Analytical method performance. The CPP (critical process parameters) were identified in initial phase of method development and design space developed for the robust method. The optimized methodology was achieved on C18 (typically 75mm length, 4.6mm ID and 3.5µm) column with optimized conditionsof Mobile phase 0.1% Orthophosphoric acid (pH-2.1): Acetonitrile: Methanol (67:28:5 v/v/v).35°C Column temperature, sampling rate 5pts/sec at 230nm. The method was validated for specificity, reproducibility, accuracy, linearity, robustness and solution stability and can be used for the assessment of quality ofdrug product in development and stability samples of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil film-coated tablets.

Keywords: QbD, HPLC, DOE, degradants, quantification

INTRODUCTION

Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil Film-coated tablets are available with two brand names Sevikar HCT and Tribenzor .Amlodipine besilate, chemically 3-ethyl-5methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3,5pyridinedicarboxylate, monobenzenesulphonate with empirical formula is $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ [2]. The structure is shown in Fig.1. Olmesartan medoxomil, chemically 2, 3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate with empirical formula is $C_{29}H_{30}N_6O_6$[1].The structure is shown in Fig.2. Hydrochlorothiazide, chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiazidiazine-7-sulfonamide 1, 1-dioxide. Its empirical formula is $C_7H_8ClN_3O_4S_2$[3].The structure is shown in Fig.3 The drug product is used for the treatment of hypertension, to decrease blood pressure. Lowering blood pressure reduces the risk of fatal and nonfatalcardiovascular problems, primarily strokes and myocardial infarctions [4]

Several analytical methods were reported so far for analysis of this drug product in pharmaceutical formulations include UV-Visible spectrophotometry [5],[11].Several LC methods for simultaneous quantification of Amlodipine,Olmesartan and Hydrochlorothiazide have previouslypublished [6],[9],[10].There are also methods reported for simultaneous determinations of Amlodipine and olmesartan medoxomil by spectrophotometer technique[8] and by HPLC [7].All the reported methods are lagging in the QbD approach for the method development and the present study focus on the QbD principles and DOE experimentation for method development.

The present work is focused on QbD approach [12] to analytical method development and for simple isocratic Reverse phase LC method, which can achieve critical separations with shorter development time.With the use of mathematics and statistical approaches, the DOE will proves theoretical critical control points in the analytical method.

Traditional Analytical development vs QbD
Traditional analytical method development:

- Limiting to little-robust and non-superior method.
- Variability's during continuous utilization of method.
- Method-transfer issues.
- Uni-variate (One factor at a time)

QbD based analytical method development:

- More robust, knowing the design space.
- Control strategy.
- Minimum variability, thorough understanding of the method parameters(Material, method parameters)
- Multivariate (cumulative effect)

Development of analytical method is the study that results by the experimental trails with different conditions for the separations. If the separations are affected with two or more number of parameters. The optimization of such methods has further complication and takes much amount of time for method development. The results of experiments are not known in advance until they have been made experimentally. Using the DOE software statistical experiments are conducted in Situations in which researchers can optimize the conditions of the experiment and can control the factors that are irrelevant to the research objectives. For example, Temperature parameter has no effect on the resolution of the components and one can optimize the other parameters to get the required objectives to finalize an HPLC method

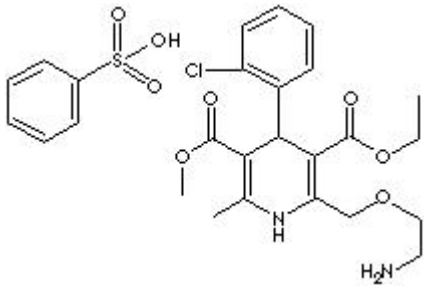

Fig.1 Amlodipine besilate

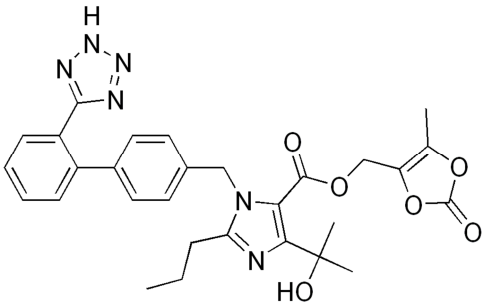

Fig.2Olmesartan medoxomil

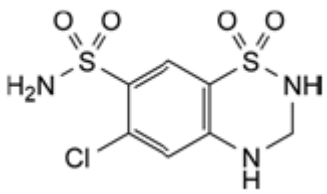

Fig.3 Hydrochlorothiazide

MATERIALS AND METHODS

1.1 High performance liquid chromatograph with UV detector –Waters-Empower soft ware or equivalent

1.2 Kinetex C18 75mm length,4.6mm internal diameter and 2.6µ particale size or equivalent(Used for DOE experiments)
1.3 X-Bridge C18 75mm length,4.6mm internal diameter and 3.5µparticle size or equivalent
1.4 Design-Expert 8.0.7.1 soft ware-Stat ease or equiavlent
1.5 Orthophosphoric acid 88%-Merck GR garde or equivalent
1.6 Acetonitrile-Fisher scintific HPLC grade or equivalent
1.7 Methanol-Merck HPLC grade or equivalent
1.8 Sodium acetate trihydrate-Merck GR garde or equivalent
1.9 Potassium dihydrogen phosphate-Merck GR grade or equvivalent
1.10 Sodium hydroxide-Merxk GR grade or equvivalent
1.11 Hydrochloric acid-37%-Merck
1.12 Demineralised water
1.13 Purified water- 0.45 Millipore Milli-Q water

EXPERIMENTAL

1.14 **Chromatography:** Waters e2695 model chromatograph equipped with 2489 UV-dual wavelength detector eqipped withKinetex C18 75mm length,4.6mm internal diameter and 2.6µ particale size was emloyed for the design experiments.Detection was done at 230nm(as shown in Fig.4).UV spectra of Amlodipine besilate,Hydrochlorothiazide and Olmesartan medoxomil was recorded uing 2998-PDA detector for the selection of wavelength.

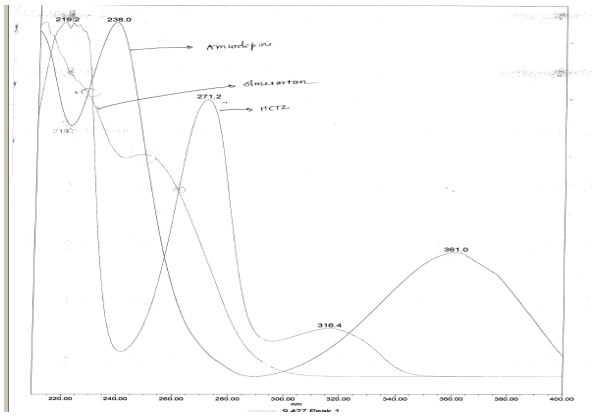

Fig.4 Overlaid spectra of Amlodipine,Hydrochlorothiazide and Olmesartan medoxomil

1.15 **Chemicals and reagents:**

Amlodipine,Hydrochlorothiazide and Olmesartan medoxomil were obtained fromAET Laboratories pvt.Ltd,Hyderabad,India.Chemicals and reagents are stated in the 'Materials and Methods'.

1.16 **Preparation of Standard solution:**

Preapred Solution containing 0.014mg/mL of Amlodipine besilate,0.028mg/mL of Hydrochlorothiazide and 0.044mg/mL of Olmesartan medoxomil respectively in Water:Acetonitrile (1:1).

1.17 **Preparation of Test solution:**

Dissolved one tablet of Amlodipine,Hydrochlorothiazide and Olmesartan medoxomil 10/25/40 Film coated tablet containing 10mg of Amlodipie as Amlodipine besilate,25mg of Hydrochlorothiazide and 40mg of Olmesartan medoxomil into 1000mL volumetric flask,added 900mL of pH-4.5 Acetate buffer sonicated for 15min ,shaked well.Filtered through 0.45µ PVDF Millipore filter ,by discarding 20mL of solution and filled into 2mL HPLC vial.The vials are injected into Chromatographic system.

2.0 METHOD DEVELOPMENT

2.1 **Objective**

The main objective of the chromatographic metthod was to separate Acetate peak and olmesaratan peak with Hydrochlorothiazide ,Amlodipine and Olmesartan medoxomil.The optimised conditions were obtained with *design experiments.*

After the DOE X-bridge column employed for the method instead of Kintex column due to more tailing of Amlodipine peak.

File Version 8.0.7.1 **Study Type** Response Surface
Design Type Central Composite
Design Model Quadratic

2.2 Optimisation of Chromatographic conditions

2.2.1 Design Summary with two factors(Flow and temperature)

Two chromatographic factors column oven temperature(°C) and Flow rate(mL/min) were choosen as CPP for the design experiments and three resolution factors(CQA) were studied and represented in Table-1 and counter plots are represented in Fig.5,6&7

Table-1 :Design of Experiments-DOE trails(Flow & Temperatur

Run	Temp (degree C)	Flow (mL/min)	Resolution-1 (R-1)	Resolution-2 (R-2)	Resolution-3 (R-3)
1	35.00	1.00	2.00	2.30	4.10
2	42.07	1.00	1.60	2.70	3.30
3	27.93	1.00	2.20	1.40	4.60
4	30.00	0.80	2.10	1.50	4.60
5	40.00	0.80	1.70	2.60	3.50
6	40.00	1.20	1.60	2.70	3.30
7	35.00	1.28	1.90	2.30	4.00
8	35.00	0.72	1.90	2.40	4.00

Where, R1-Resolution between Acetate peak with Hydrochlorothiazide**,** R2-Resolution between Hydrochlorothiazide with Olmesartan and R3-Resolution between Olmesaratn medoxomil and Amlodipine

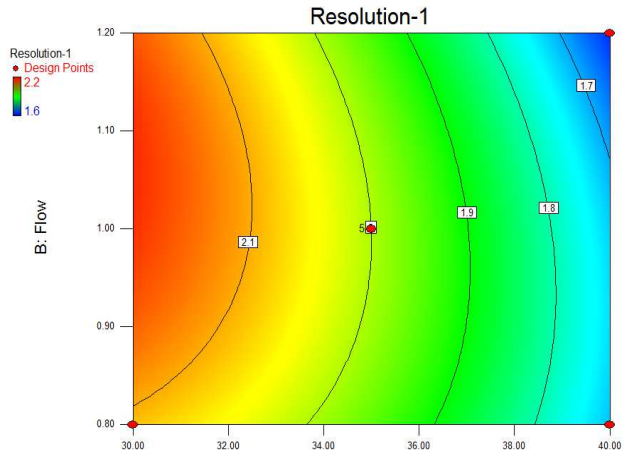

Fig-5:Counter plot for the respone-1

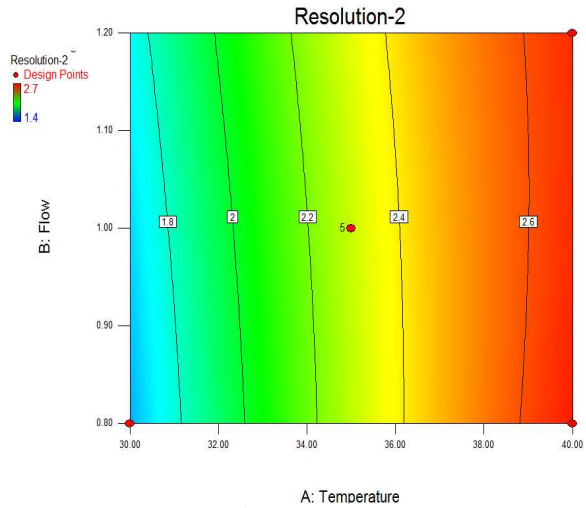

Fig-6:Counter plot for the respone-2

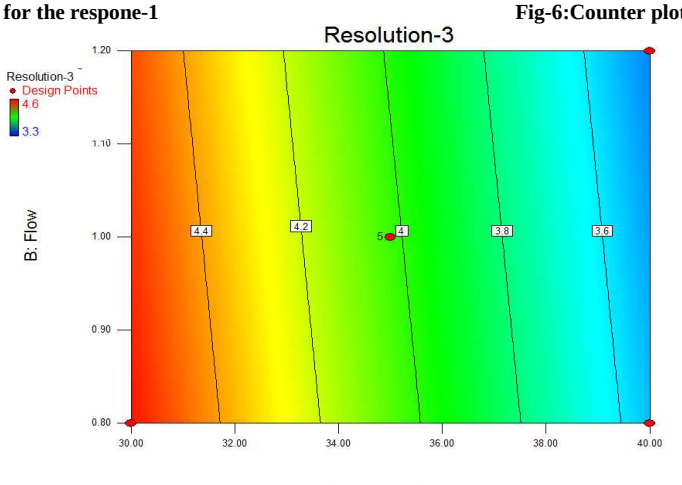

Fig-7: Counter plot for the respone-3

From the above design of experiments and contour plots it was observed that the Resolution 2 and 3 are not much significant for the changes in the Temperature and Flow, but the Resolution 1 i.e Resolution between Acetate peak and Hydrochlorothiazide ishighlyvarying and was optimised using DOE experiments.

2.2.2 **Design Summary with one factor(Methanol composition in Mobile phase)** Chromatographic factor Methanol composition was choosen for the design experiments and three resolution factors were studied and are shown in table-2.

Table-2: Design of Experiments-DOE trails (Methanol composition)

Run	Factor 1 A: Methanol %	Response 1 Resolution-1 Re1	Response 2 Resolution-2 Re2	Response 3 Resolution-3 Re3
1	4	1.90	2.30	4.00
2	3	1.80	1.90	3.20
3	5	1.90	2.70	4.60

From the above experiments,it was observed that the Resolution 2 and 3 are increased by increase in the Methanol composition and Resolution 1 is improved slightly and finalised using DOE experiments

2.2.3 **Design Summary with one factor(Buffer composition in Mobile phase)** Chromatographic factor Buffer composition was choosen for the design experiments and three resolution factors were studied and are shown in table-3. **Conclusions:**From the above experiments all the three responses improved much with increase in buffer concentartions and finalised using DOE experiments.

Table-3: Design of Experiments-DOE trails (Buffer composition)

Run	Factor1 A: Buffer %	Response 1 Resolution-1 Re1	Response 2 Resolution-2 Re2	Response 3 Resolution-3 Re3
1	64	1.9	2.7	4.6
2	65.5	2	3.5	5.6
3	67	2.3	4.7	6.8

2.2.4 **DOE Conclusions:**From the above design of experiments and contour plots it was observed that the Resolution 2 and 3 are much increased for the increase in the Methanol composition ,Resolution 1 is improved slightly and finalised using DOE experiments.The chromatogram(Fig.8) was obtained finalised DOE conditions.

2.2.5

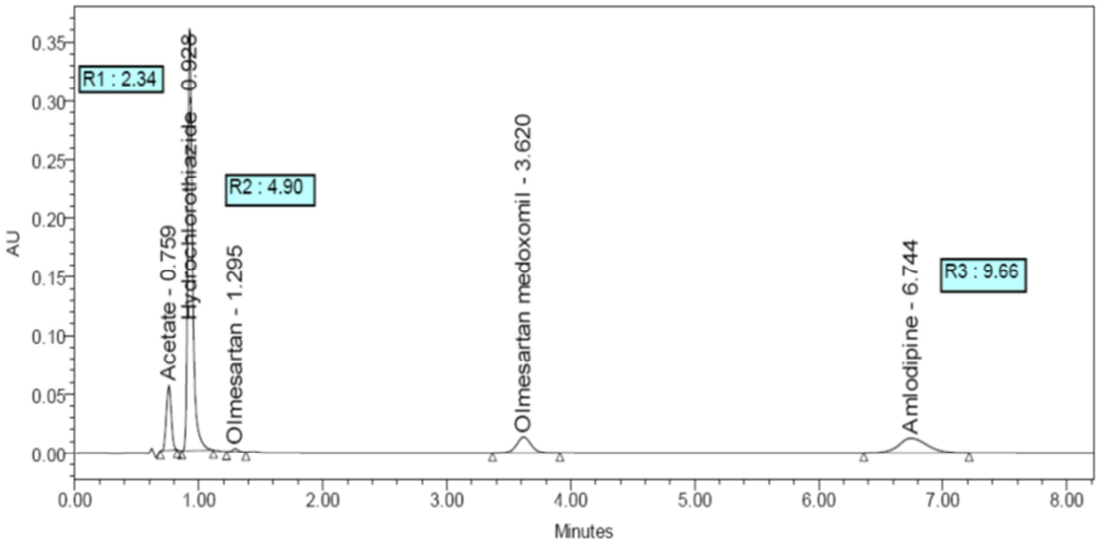

Fig.8: Typical chromatogram of Amlodipine, HCTZ and Olmesartan medoxomil tablet in pH 4.5 Acetate dissolution media in X-Bridge column

3.0 VALIDATION

3.1 The validation is performed as per ICH Guidelines [13]

3.2 **System suitability:** System suitability parameters are integral part of liquid chromatographic methods. It is used for chromatography that the equipment was suitable for its intended use.Summary validation results were shown in Table-4.

Table-4: Summary of Validation

Validation	Parameter	Acceptance criteria	Amlodipine	HCTZ	Olmesartan medoxomil
System suitability	Symmetry factor	Not more than 2.0	1.01	1.29	1.08
	Plate count	Not Less than 2000	5451	2412	4694
	%RSD #	Not more than 2.0%	0.57	0.69	0.55
	Resolution#	Not less than 2.0	8.95	NA	15.58
Specificity	Retention times#	NA	4.727	0.898	2.791
Precision	%RSD#	Not more than 2.0%	1.97	1.48	1.47
Accuracy#	20% Level	% Recovery should be within 2% of the specified range	18	21	20
	100% Level		98	102	101
	150% Level		147	149	150
Linearity	Correlation coefficient(r)	Not less than 0.99	1.000	0.998	1.000
	Regression coefficient(r^2)	Not less than 0.94	1.000	0.997	1.000

3.3 **Specificity:** Specificity is the ability of the method that in the presense of impurities,matrix components,degradants and diluent peaks should not interfere with the analytes under intrest.Specificity will provide an accurate result for the conyent of the analyte that was present in the sample.For the following method specificty was proved by analysing the individual componentts of the matrix for the interference with the analytes.

The above results shows that,there was no interference was observed at retention times of Active molecules and specificity was proved.

3.4 **Reproducibility:** Reproducibility is the ability of the method to get the consistent results for the six individual preparations.For the following method reproducibility was proved by adding known concentartions of analyte at 100% Level to the matrix on six individual preparations.% Relative standard deviations were evaluated.

The above reslts shows that,the proposed method was reproducible.

3.5 **Accuracy:** The accuracy of the analytical method was the closeness of the results obtained between the reference value added to the sample to the real value obtained from the analytical procedure. For the following method linearity was proved from 0.00217mg/mL ,0.01087, 0.01631mg/mL of Amlodipine,0.00548mg/mL ,0.02738, 0.04107mg/mL of Hydrochlorothiazide and 0.00906mg/mL ,0.04528, 0.06792 mg/mL of Olmesartan medoxomil.

The above reslts shows that,the proposed method was Accurate within specified range.

3.6 **Linearity:** The linearity of the Analytical method is its ability to obtain the results which will corrrelate with the Concentration of the analyte to its response choosen in the methodology.i.e the concentration or amount of the analyte is directly propotional to the responses. For the following method linearity was proved from 0.00217mg/mL to 0.01631mg/mL of Amlodipine,0.00548mg/mL to 0.04107mg/mL of Hydrochlorothiazide and 0.00906mg/mL to 0.06792 mg/mL of Olmesartan medoxomil.Correlation coefficient and regression coefficient were evaluated to prove the linearity.Linearity graphs for Amlodipine(Fig.9),Hydrochlorothiazide(Fig.10) and Olmesartan Medoxomil(Fig.11) were showing linear range for concentartion and Area response.

Linearity of Amlodipine

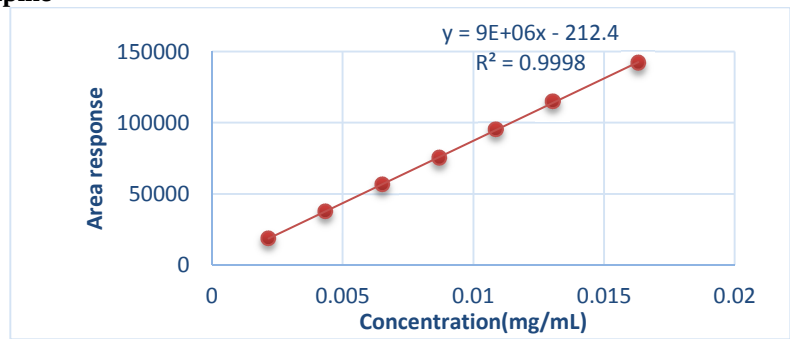

Fig.9:Linearity of Amlodipine for Concentration (mg/mL) vs Area response

Linearity of Hydrochlorothiazide

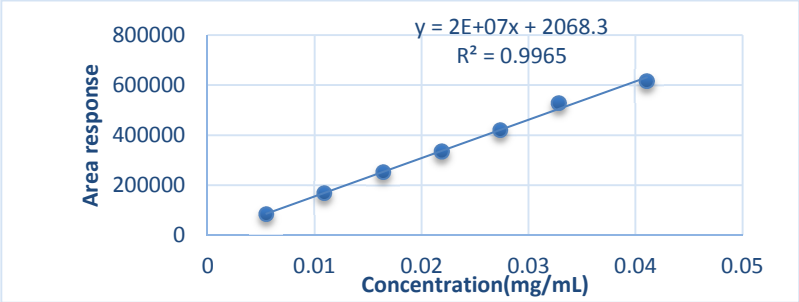

Fig.10:Linearity of Hydrochlorothiazide for Concentration (mg/mL) vs Area response

Linearity of Olmesartan medoxomil

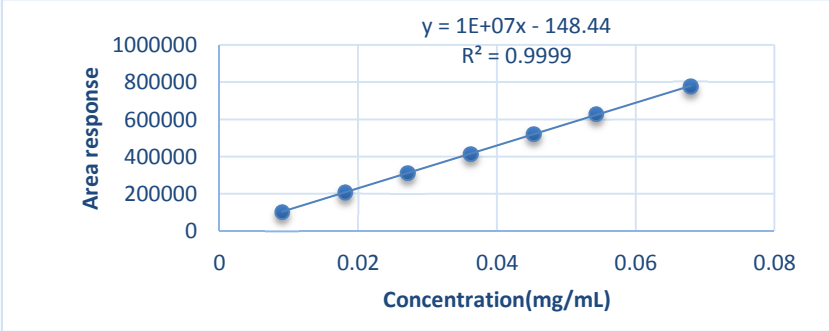

Fig.11:Linearity of Olmesartan medoxomil for Concentration (mg/mL) vs Area response

3.7 **Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The method is found robust with the variations in the analytical method and the solutions are stable at 10°C for 12hrs.

CONCLUSION

The LC method developed for quantitative assay of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil in Formulation products in different dissolution Medias is specific, precise, accurate and robust. The validation results of the method are found satisfactory. The method was stability indicating and useful for Analytical research development labs for multimedia dissolution(pH1.2 Hydrochloric acid media,pH 4.5 Acetate buffer media,pH 6.8 phosphate buffer media) analysis of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil Film coated tablets and their stability samples. The statistical tool (DOE) and QbD principles are more useful for the HPLC method development. This process helps in thorough understanding of the parameters and less amount of timefor the development cycle of the analytical method.

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