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HPTLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND FELODIPINE IN BULK DRUGS AND COMBINED DOSAGE FORMS.

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ABSTRACT

A combination of Metoprolol and felodipine tablet has achieved more pronounce antihypertensive. For this combination a simple, specific, accurate and precise HPTLC method has been developed for simultaneous determination of metoprolol succinate and felodipine in active pharmaceutical ingredient and tablet dosage form. Thin layer Chromatographic separation of the drugs was carried on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase using chloroform: toluene: methanol: glacial acetic acid 6: 3: 1: 0.04 (v/v/v/v) as mobile phase. Densitometric evaluation was performed at 238 nm. The two drugs were resolved with R_F values at 0.26 ± 0.02 and 0.53 ± 0.01 for Metoprolol succinate and Felodipine respectively. The validation parameters of method were performed as per ICH guidelines Q2A (R1). The linearity of method was seen in the range of 100-600 ng/spot and 10–60 ng/spot for Metoprolol succinate and Felodipine respectively. The % RSD for intraday precision was 1.15-1.76% of Metoprolol succinate, 1.03-1.23% of Felodipine and interday precision was 0.6-1.7 of Metoprolol succinate, 1.06-1.47% of felodipine. % recovery studies from tablets were in between 99.73 ± 0.70 % and 100.3 ± 0.06 % for Metoprolol succinate and Felodipine respectively. The developed method may be used for routine quality control analysis of Metoprolol succinate and Felodipine in active pharmaceutical ingredient and tablet dosage form.

KEYWORDS: ***Simultaneous Estimation, HPTLC, Metoprolol succinate, Felodipine.***

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INTRODUCTION

Metoprolol Succinate

Chemically Metoprolol Succinate (MT) is 1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanolhemisuccinate with Molecular formula $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$& molecular weight 684.81.

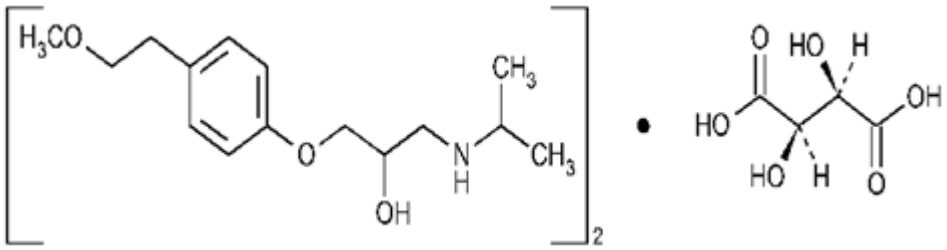

Figure 1
Structure of Metoprolol Succinate

Metoprolol Succinate is a selective β1-adrenergic blocking agent used in acute myocardial infarction (MI), heart failure, angina pectoris hypertension. It may also be used for prophylaxis for migraine headaches. At low doses, Metoprolol succinate shows little activity against β2-adrenergic receptors of the lungs and vascular smooth muscle. The drug is quite sensitive, even a small dose of the drug gives a sufficient blockade of the beta-adrenergic receptors. It is officially given in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP).

Felodipine

Felodipine chemically known as ethyl methyl-4(2, 3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-5methyl ester with molecular formula of Felodipine is $C_{18}H_{19}C_{l2}NO_4$ and molecular weight 384.254 g/mol. It is mostly used in the treatment of hypertension, heart failure and angina pectoris.

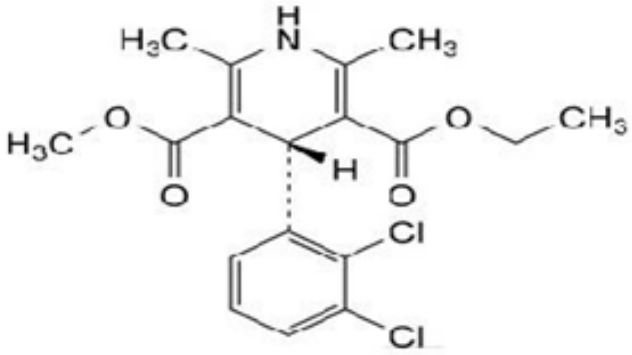

Figure 2
Structure of Felodipine

Felodipine is a Calcium antagonist. Felodipine is act as a long-acting 1,4-dihydropyridine calcium channel blocker (CCB). Its mechanism of action is on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive form, then felodipine binds to a number of calcium-binding proteins, exhibit competitive antagonism of the mineral corticoid receptor which inhibits the activity of cyclic nucleotide phosphodiesterase, and blocks calcium influx through voltage-gated T-type calcium channels. Felodipine is used in treatment of mild to moderate essential hypertension.[1-6] Only a few methods have been reported in the literature for the determination of Metoprolol and felodipine in both combination and with other drug combinations dosage form by using hplc, uv methods. No any HPTLC-densitometric methods have been reported for simultaneous determination of Metoprolol and felodipine in their pharmaceutical combinations until now.[7-23.]

MATERIALS AND METHODS

Material and reagents

The gift sample of Metoprolol succinate (Purity NLT 98%) was procured from Ipca Laboratories Limited Kandivli (West) Mumbai. Felodipine (Purity NLT 97.5%) gift sample was supplied by Nivedita Chemicals PVT Ltd. Andheri East, Mumbai. Logimax tablet containing Metoprolol succinate 50mg and Felodipine 5 mg was procured from local market.

Instrumentation and chromatographic conditions

TLC was performed on aluminium plates precoated with 0.2-mm thickened layers of silica gel $60F_{254}$. Before use, plates were pre washed with methanol then dried and activated by keeping in an oven at 115^0C for period of 10 minutes. Drug samples were applied on the plates, as 6-mm bands by means of a Camag Linomat 5 sample applicator syringe. Plates were developed with

chloroform: toluene: methanol: glacial acetic acid 6: 3: 1: 0.04 (v/v/v/) as mobile phase in a Camag twin trough glass chamber previously saturated with mobile phase vapour for 30 minute at 25 ± 2°C. The development distance was kept approximately 80 mm. The plates were scanned in absorbance mode at 238 nm by using Camag TLC Scanner 3 operated by winCATS software. The slit dimensions were set at 5 mm × 0.45 mm with deuterium lamp as radiation source of emitting a continuous UV spectrum light in the range 190–400 nm.

Experimental

Preparation of standard solutions and calibration curves

25mg of Metoprolol succinate and were accurately weighed and transferred separately in 25ml volumetric flasks, dissolved using methanol to give 1000µ g/ml. Both the solutions were mixed together in ratio of 10:1 for Metoprolol and Felodipine respectively to get working standard solution. Calibration was performed by applying mixture of working standard solution within range of 1.0 –6.0 µL using 100µL Hamilton syringe with the help of LinomatV auto sprayer on HPTLC plate. The final concentration was found to be 100-600 ng/spot for Metoprolol succinate and 10-60 ng/spot for felodipine. The plates were developed using mobile phase and detected at 238 nm by TLC Scanner 3. From the results, calibration curves for both drugs were recorded by plotting of concentration verses area under curve. The values of regression coefficient, slope and intercept were reported on basis of the standard curve regression analysis.

Assay of tablets

Assay of marketed formulation was performed using twenty tablets of LOGIMAX containing Metoprolol succinate 50 mg and Felodipine 5 mg. Each tablet was weighed and average weight of tablet was calculated. The tablets were crushed as fine powder by using mortar and pestle. Sample equivalent to the average weight of 50mg of Metoprolol succinate and 5mg of Felodipine was weighed and dissolved in 10 ml of methanol, and ultra sonicated for about 15 minutes. The solution was filtered. From the above solution 0.01 ml is taken and diluted up to 10 ml of methanol. 5µl from the finally obtained solution was applied for six times on a TLC plate followed by development and scanning.

RESULTS AND DISCUSSION

Method Validation- Linearity And Range

Linearity of the method was performed using aliquots of working standard solution of Metoprolol Succinate (100–600µl) and Felodipine (10– 60µl) which were applied on to the plate, to obtain concentration in the range 100 to 600 ng/spot and 10 to 60 ng/spot. Calibration curve were given by plotting concentration vs. peak area as shown in figure 3 and 4.Least square regression analysis was performed to generate the calibration equation and R^2 value which is given in table 1. Residual graphs were shown in fig 5 and 6.

Table 1
Linearity and range of Metoprolol Succinate and Felodipine

Linearity and range	Metoprolol Succinate	Felodipine
Range (ng/spot)	100-600	10-60
Regression coefficient (r^2)	0.9998	0.9997
Linearity equation	y = 149.75x + 8613.1	y = 1421.x + 89.68

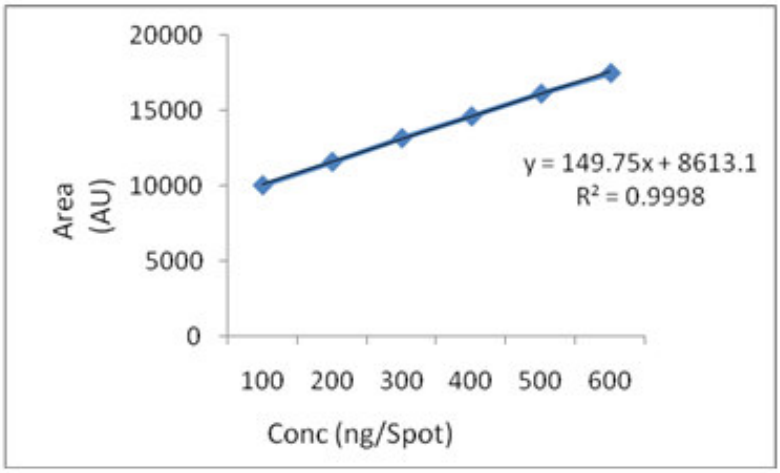

Figure 3
Linearity graph of Metoprolol Succinate

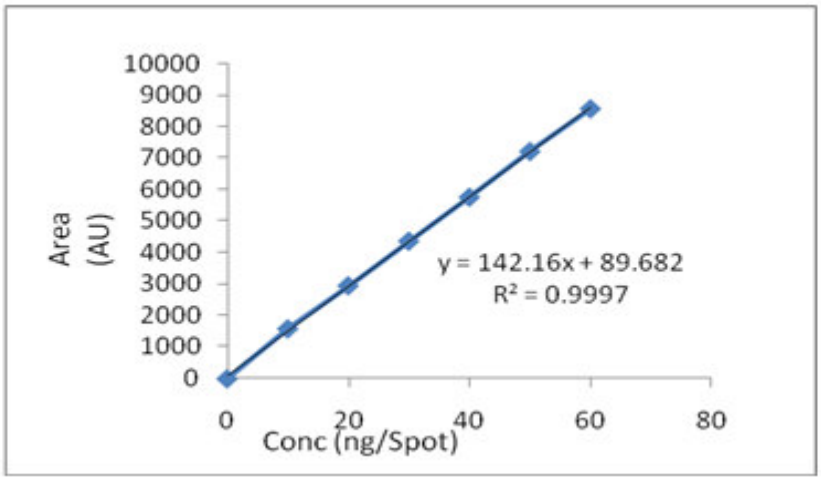

Figure 4
Linearity graph of Felodipine

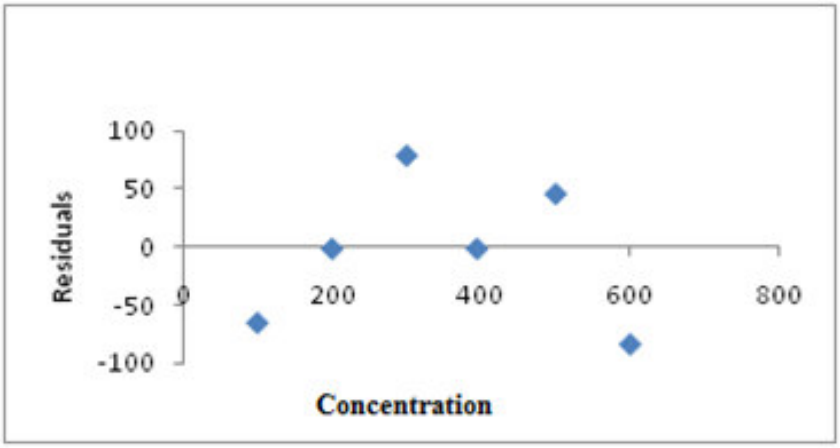

Figure 5
Residual graph of Metoprolol succinate.

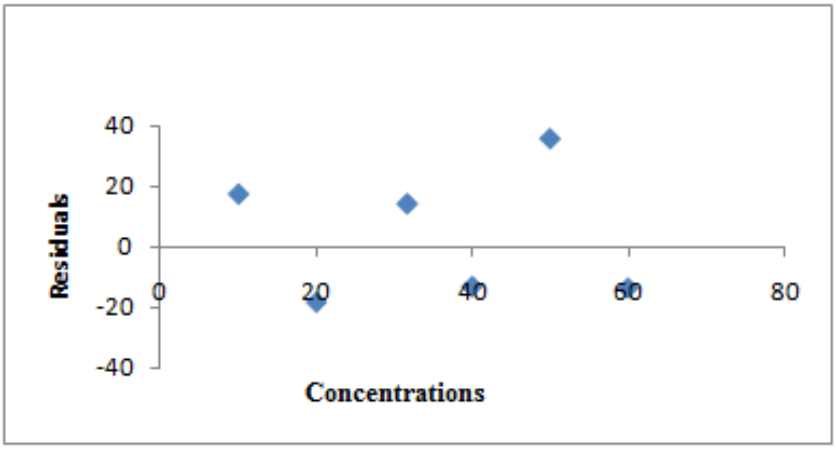

Figure 6
Residual graph of Felodipine.

Precision

The precision study of developed method was performed by repeatability studies. Repeatability studies were carried out by analysis of three different applied volumes viz. 200, 400, 600µl for Metoprolol Succinate and 20, 40, 60µl for Felodipine respectively, from the working standard solution on a TLC plate followed by development of the plate. The %RSD was assessed by analyzing standard drug solutions within the calibration range as 200,400,600 ng/spot of Metoprolol succinate and 20,40,60 ng/spot of felodipine, three times on the same day for intra-day precision and on three different days over a period of a week for Inter- day precision given in table 2 and 3.

Table 2
Intraday Precision details of Metoprolol succinate and Felodipine

	Metoprolol Succinate			Felodipine		
S.No.	Conc (ng/spot)	Area (mcV.sec) * avg of 6	% RSD	Conc (ng/spot)	Area (mcV.sec) *avg of 6	% RSD
1	200	11614.41	1.76	20	3075	1.03
2	400	15667.93	1.15	40	5727.23	1.23
3	600	16583.09	1.34	60	8449.9	1.04

Table 3
Interday Precision details of Metoprolol succinate and Felodipine

	Metoprolol Succinate			Felodipine		
S.No.	Conc (ng/spot)	Area (mcV.sec) * avg of 6	% RSD	Conc (ng/spot)	Area (mcV.sec) *avg of 6	% RSD
1	200	11653.02	1.7	20	3076.3	1.303
2	400	15254.8	1.40	40	5772.1	1.47
3	600	16696.1	0.6	60	8497.3	1.06

Accuracy
The % recovery studies of Metoprolol succinate and felodipine were carried out at 80%,100%,120% level. Accuracy results are given in table 4.

Table 4
Recovery studies of Metoprolol succinate and Felodipine tablet.

Lable claim LOGIMAX	% Level	Conc. (ng/spot)	API added (ng/spot)	Ammount found	% Recovery	Mean (%) Recovery
Metoprolol Succinate (50mg)	80	500	400	900.26	100.6	99.73
	100	500	500	1097.3	99.43	
	120	500	600	1198.32	99.29	
Felodipine (5mg)	80	50	40	90.6	99.9	100.03
	100	50	50	100.2	100.5	
	120	50	60	109.2	99.7	

Limit of Detection and Limit of Quantification
Limits of detection (LOD) and quantification (LOQ) is stand for the concentration of the analyte that would give signal-to-noise ratio of 1:3 for LOD and LOQ, respectively. The LOD and LOQ of Metoprolol succinate and felodipine are given in table 5.

Table 5
LOD and LOQ of Metoprolol Succinate and Felodipine

S.No.	Drug	LOD (ng/spot)	LOQ (ng/spot)
1	Metoprolol Succinate	3.95	11.97
2	Felodipine	0.026	0.081

Robustness of the Method
Robustness was evaluated by making small change in the chromatographic conditions such as time from mobile phase ratio, time from spotting to chromatography and development to scanning. Robustness results are given in Table 6.

Table 6
Results of robustness study

S.No.	Change process parameters	Name of drug	Conc. (ng/spot)	Mean area	% RSD
1	Time from development to scanning	Metoprolol Succinate	500	16473.3	0.82
	+ 10 min	Felodipine	50	7232.9	1.03
2	Mobile phase ratio	Metoprolol Succinate	500	16222.7	0.14
	(2:8) ±0.1 v/v	Felodipine	50	7052.5	0.98
3	Time from spotting to	Metoprolol	500	16434.2	0.85
	chromatography (+10 min)	Felodipine	50	7453.6	1.35

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The specificity result gives well resolution of both the drugs. HPTLC chromatogram is shown in Figure 7.

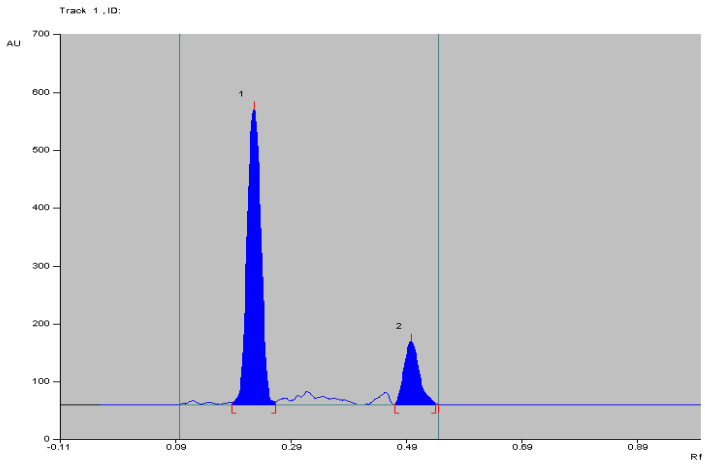

Figure 7

HPTLC chromatogram of Metoprolol Succinate and Felodipine API.

Assay

Assay was performed using LOGIMAX tablets containing Metoprolol succinate 50 mg and Felodipine 5 mg. The % label claim is found to be 99.98 % for Metoprolol succinate and 99.01 % for Felodipine (Fig. 8 and Table.7).

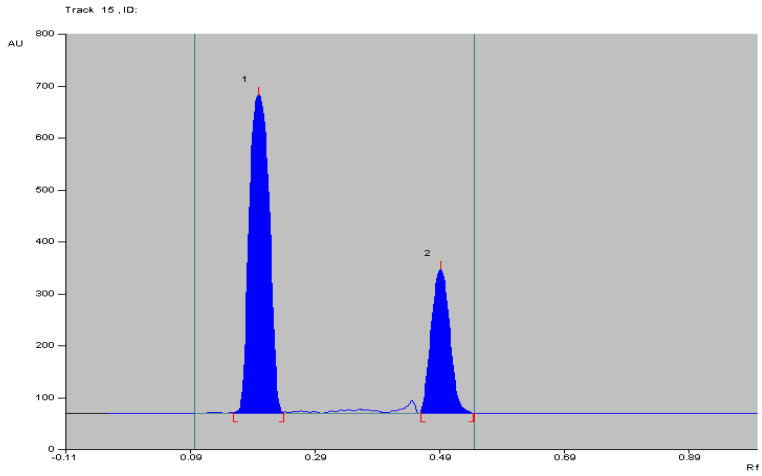

Figure 8

HPTLC chromatogram of Metoprolol succinate and Felodipine LOGIMAX tablet

Table 7

Assay of marketed formulation for Metoprolol succinate and Felodipine

Lable claim	Conc. of Drug (ng/spot)	% Assay	% mean
Metoprolol succinate (50mg)	500	98.6	99.98
	500	99.0	
	500	99.3	
Felodipine (5mg)	50	98.91	99.01
	50	98.63	
	50	99.50	

CONCLUSION

The validated HPTLC method was simple, precise, accurate, specific, reproducible and more economic than the reported methods without interference from the excipients. However, the present HPTLC method is fast with compared to economic sophisticated chromatographic techniques. The method gives excellent linearity with a limit of quantification of 11.97 and 0.081, limit of detection of 3.95 and 0.026 mg/ml for Metoprolol succinate and Felodipine respectively. Linear regression analysis was found to be R^2 = 0.9998 and

0.9997 for Metoprolol succinate and Felodipine respectively. %RSD from precision studies found to be not more than two and recovery results were within rage at 80, 100 and 120% level. Hence developed method seems to be suitable for routine analysis of pharmaceutical formulations in quality-control laboratories.

CONFLICT OF INTEREST

Conflict of interest declared none.

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