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Stability indicating LC estimation of ramipril and hydrochlorthiazide in its bulk and tablet formulation

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Abstract

A simple, rapid and sensitive HPLC method has been developed for the simultaneous determination of Ramipril and Hydrochlorothiazide in their dosage forms. Acetonitrile: Phosphate buffer (0.01 M) adjusted to pH 2.6 with O-phosphoric acid, was used as the mobile phase. A CHROMO Sil C-8 (4.6*250 mm) column was utilized as stationary phase. Detection was affected spectrophotometrically at 210 nm. The method was also applied for the determination of Ramipril in the presence of its degradation products. Linearity ranges for Ramipril and Hydrochlorothiazide were 10-50 and 10-150 mg/ml, respectively. The proposed method was further applied to the analysis of tablets containing the two drugs, the percentage recoveries S.D. (*n*_5) were 99.66% and, 99.63% for Ramipril and Hydrochlorothiazide respectively.

Keywords: Ramipril, Hydrochlorothiazide, High Performance Liquid Chromatography, Limit of Quantification, Limit of Detection.

Introduction

Ramipril,2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-30] octan ecarboxylic acid, is an angiotensin-convertingenzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduce the degradation of bradykinin. Hydrochlorothiazide,6-chloro-3,4-dihydro-2H-1,2,4-benzothia-zine-7-sulphonamide-1,1-dioxideis a thiazide diuretic. It increases sodium and chloride excretion by distal convolated tubule.

Literature survey reveals few analytical methods for the determination of Ramipril in pharmaceutical preparations and biological fluids, viz., radioimmunoassay, spectrophotometry , and HPLC. Ramipril is frequently co-formulated with hydrochlorothiazide in a medicinally recommended ratio of 1:5. Analysis of such mixture is challenging, because Ramipril (the minor component) is poorly absorbing light in the UV region (The value ofA1%:1 cm at 257 nm is _8), while Hydrochlorothiazide (the major component) is strongly absorbing light in the UV region (value of A1%:1 cm at 272nm is _644). The aim of this work is to develop a simple, rapid, sensitive and reliable HPLC assay procedure for the quality control of Ramipril and Hydrochlorothiazide in pharmaceutical preparations.[1-2]

Material and methods[3-8]

Reagents and Standards

Ramipril hcl and Hydrochlorothiazide standards were obtained from FDC and Lupin Laboratory, Mumbai, India. HPLC-grade methanol, Acetonitrile and formulation containing Ramipril and Hydrochlorothiazide (CARDACE–H tablets) were used. The labelled Ramipril and Hydrochlorothiazide content of each tablet was 5 mg and 12.5 mg, respectively.

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Selection of stationary phase[8]

On the basis of RP-HPLC mode and number of carbon present in the molecule CROMOSIL C-8 (4.6*250 mm) column was selected for further study.

Selection of mobile phase[9-11]

It was found that Acetonitrile and 0.01M Potassium dihydrogen phosphate buffer (P^H-2.6) gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase was determined to be 0.01M phosphate buffer solution:Acetonitrile (50:50 v/v, P^H 2.6). This mobile phase produced good resolution, reasonable retention times and acceptable peak symmetry for both the drugs.

Preparation of mobile phase

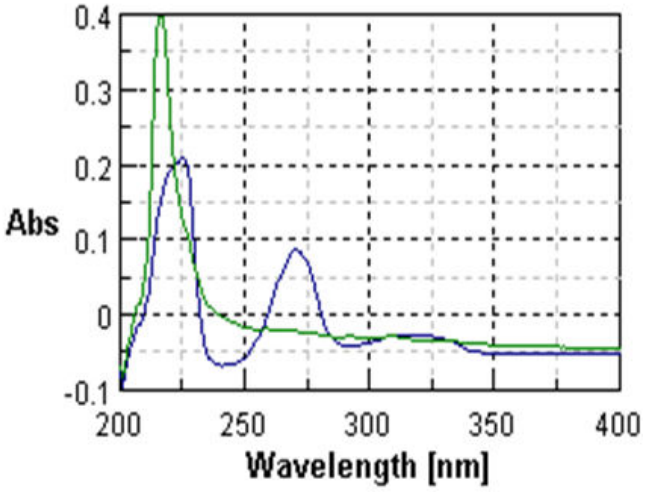
Phosphate buffer 0.01 M solution was prepared by dissolving accurately about 1.369 gms of potassium dihydrogen phosphate in a 1000 ml of glass double distilled water.. Mobile phase was prepared by mixing 250 ml of 0.01M potassium dihydrogen phosphate solution with 250 ml of Acetonitrile and its P^H is adjusted to 2.6 by ortho phosphoric acid. This mobile phase was ultrasonicated for 20 min, and then it was filtered through 0.45 µm Nylon, 47 mm membrane filter paper.

Preparation of standard stock solution

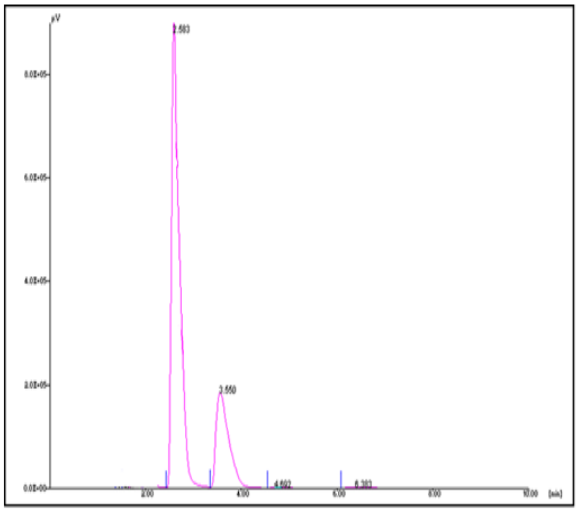
Accurately about 50 mg of each of reference standard of Hydrochlorthiazide and Ramipril 20mg was weighed and transferred to 50ml volumetric flask. Both drugs were dissolved in 50ml of mobile phase with shaking and then volume was made up to the mark with mobile phase to get 1000µg/ml & 400 µg/ml of standard stock solution of each drug. These stock solutions were filtered through 0.2µm Nylon 6, 13 mm membrane filter paper.5 ml of above stock solution was then pipette out in 50 ml volumetric flask and diluted up to the mark with methanol to get 100 µg/ml & 40 µg/ml Hydrochlorthiazide & Ramipril respectively

Selection of analytical wavelength

Each solution was scanned using double beam UV visible spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid. The wavelength selected was 210 nm (Graph 1).



Graph 1: Overlain spectra of Ramipril and Hydrochlorthiazide



Graph 2: Chromatogram of Ramipril and Hydrochlorthiazide

Chromatographic condition

Using the optimized mobile phase, the flow rate was set to 1.0 ml/min and UV detection was carried out at 210 nm. The mobile phase and samples were degassed by ultrasonic vibrations for 20 min and filtered through 0.45µm Nylon, 47 mm membrane filter paper. The peak areas were plotted against the corresponding concentrations to obtain the standard calibration curves.

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Standard calibration curves for Hydrochlorthiazide & Ramipril are shown in fig. The Lambert- Beer's law was obeyed in the concentration range of 75-150 µg/ml &10-50 for Hydrochlorthiazide and Ramipril respectively. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and also standard deviation (S.D.) for intercept value was less than 2 % (Graph 2).

Table 1: System suitability parameters

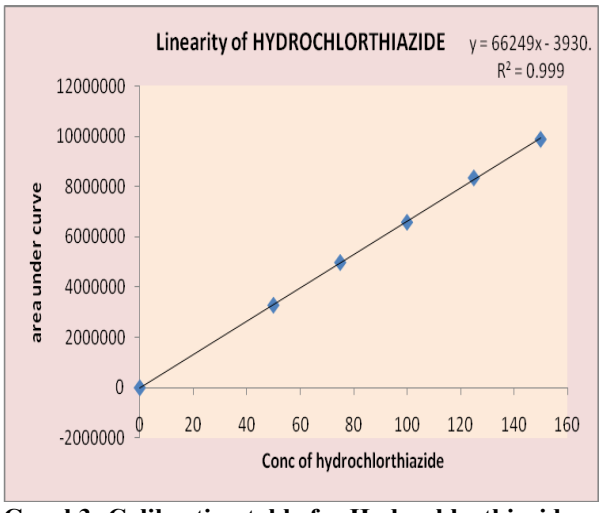
Parameter	Hdrochlorthiazide	Ramipril
Tailing factor	1.25	1.5
Resolution (Rs)	3.8	
Separation factor	1.857	
Capacity factor	2.07	4.15
Theoretical plates (N)	2541	1570
Retention time	2.583	3.550

Results and Conclusion

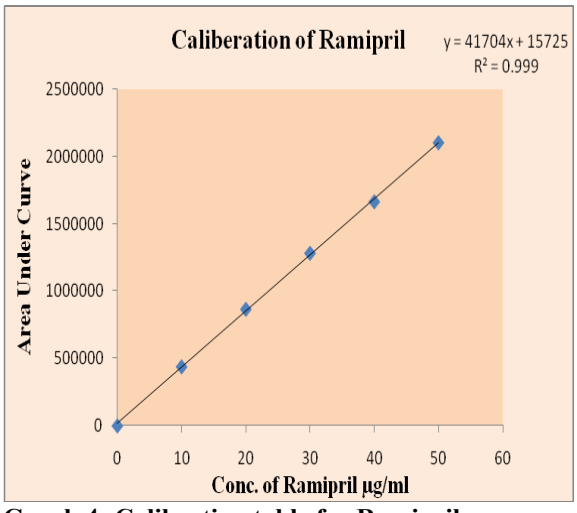
Evaluation of analytical method [12-16]

Linearity

Suitable dilutions using mobile phase were made from the standard stock solutions containing 1000 µg/ml of Hydrochlorthiazide and 400 µg/ml of Ramipril, to prepare range of standard solutions of five different concentrations of analyte for further experimental work (Graph 3, 4). Five replicates of each concentration were injected. The linearity of the relationship between peak area and concentration was determined by analyzing five working standards over the concentration range 50-150 µg/ml for Hydrochlorthiazide and 10-50 µg/ml for Ramipril. The results obtained are shown in table 2,3,4,5 respectively.



Graph3: Calibration table for Hydrochlorthiazide



Graph 4: Calibration table for Ramipril

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Table 2: Calibration table for Hydrochlorthiazide

Concentration Hydrochlorthiazide (mcg/ml)	Area under curve (AUC)
0	0
50	3286457
75	4983295
100	6586457
125	8354967
150	9889564

Table 4: Calibration table for Ramipril

Concentration Ramipril (mcg/ml)	Area under curve (AUC)
0	0
10	438557
20	865426
30	1281355
40	1663454
50	2101153

Table 3: Linearity of Hydrochlorthiazide

Standard Conc.→	50µg/ml	75µg/ml	100µg/ml	125µg/ml	150µg/ml
Replicates ↓	Peak area				
1	3256455	4926784	6563549	8209546	9838645
2	3279649	4952489	6558645	8216894	9847582
3	3249856	4918645	6576548	8225464	9865485
4	3291254	4932594	6569548	8245978	9856544
5	3284198	4968754	6585494	8256498	9874865
Mean	3261987	4932639	6566247	8217301	9850571
SD	15647.85	17665.44	9251.492	7966.814	13667.31
%RSD	0.479703	0.358134	0.140895	0.096952	0.138746

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Table 5: Linearity of Ramipril

Standard Conc. →	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
Replicates ↓	Peak area				
1	438957	865626	1281655	1663554	2105123
2	438148	865247	1282456	1665628	2106489
3	437956	865426	1281356	1664562	2107495
4	438236	865048	1283659	1662544	2109856
5	438555	865845	1281699	1661473	2109564
Mean	438353	865433	1281822	1664581	2106369
SD	531.2479	189.5969	568.7709	1037.135	1190.544
%RSD	0.121192	0.021908	0.044372	0.062306	0.056521

Precision

One set of three different concentrations of combined working standard solution of Ramipril and Hydrochlorthiazide were prepared. All the solutions were analyzed thrice, in order to record any intra-day variation in the result. The result obtained for intra-day variations are shown in the table no.6 and 7. For inter-day variation study, three different concentrations of the combined standards were analyzed for three days. The result obtained for inter-day variations are shown in the table no 8 and 9.

Table 6: Intra-day variability of Hydrochlorthiazide

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
75	4926784	4952489	4918645	4932639	17665.44	0.358134
100	6563549	6558645	6576548	6566247	9251.492	0.140895
125	8209546	8216894	8225464	8217301	7966.814	0.096952

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Table 7: Intra-day variability of Ramipril

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
20	865626	865247	865426	865433	189.59	0.022
30	1281655	1282456	1281356	1281822	568.77	0.045
40	1663554	1665628	1664562	1664581	1037.1	0.062

Table 8: Inter-day variability of Hydrochlorthiazide

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
75	4918645	4932594	4968754	4939998	25861.92	0.523521
100	6576548	6569548	6585494	6577197	7992.766	0.121522
125	8225464	8245978	8256498	8242647	15782.92	0.191479

Table 9: Inter-day variability of Ramipril

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
20	865426	865048	865845	865439.7	398.67	0.046
30	1281356	1283659	1281699	1282238	1242.5	0.097
40	1664562	1662544	1661473	1662860	1568.5	0.094

Accuracy

To check the accuracy of proposed method, level of recovery carried out at 80, 100 and 120 % of the concentration as per standard addition method. To perform recovery studies of the test concentration, a powder of pre analysed tablet sample containing 5 mg of Ramipril and 12.50 mg of Hydrochlorthiazide was weighed then transferred into 100 ml volumetric flask, add about 100 ml of mobile phase and sonicated for 20 minutes with intermediate shaking and volume make up to the mark take 8 ml solution in 10 ml volumetric and make up volume. 40 µg/ml and 100 µg/ml of Ramipril & Hydrochlorthiazide pure drugs were used as standard concentrations, finally % recovery was calculated and results and statistical validation are shown in table 10, 11.

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Table 10: Recovery studies

Tablet sample	Level of recovery (%)	Amount present (µg/ml)		Amt of std. added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
		RAM	HTZ	RAM	HTZ	RAM	HTZ	RAM	HTZ
CARDACE-H	80	40	100	32	80	71.87	178.49	99.81	99.16
	80	40	100	32	80	71.53	179.38	99.34	99.71
	80	40	100	32	80	71.67	178.59	99.54	99.21
	100	40	100	40	100	79.88	198.64	99.85	99.32
	100	40	100	40	100	79.66	199.21	99.57	99.32
	100	40	100	40	100	79.91	198.68	99.88	99.60
	120	40	100	48	120	87.90	219.87	99.88	99.34
	120	40	100	48	120	87.81	218.98	99.78	99.53
	120	40	100	48	120	87.88	219.57	99.84	99.80

Table 11: Statistical validation

Tablet Sample	Type of recovery %	(%) Mean		SD		SEM	
		RAM	HTZ	RAM	HTZ	RAM	HTZ
CARDACE -H	80	99.56	99.36	0.306	0.304	0.346	0.3061
	100	99.66	99.42	0.158	0.151	0.157	0.158
	120	99.81	99.75	0.208	0.208	0.208	0.214

Limit of detection (LOD)

LOD is calculated from the formula

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = Standard deviation of the response, S= slope of the calibration curve

Ramipril- 0.07 µg/ml

Hydrochlorthiazide – 1.25 µg/ml

Limit of quantification (LOQ)

LOQ is calculated from the formula

$$LOQ = \frac{10\ \sigma}{S}$$

σ = Standard deviation of the response, S= slope of the calibration curve

Ramipril _ 0.21µg/ml

Hydrochlorthiazide – 3.5 µg/ml

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Range
The range shown by ramipril and hydrochlorthiazide is given as 10 – 50 µg/ml, 10 – 150 µg/ml respectively.

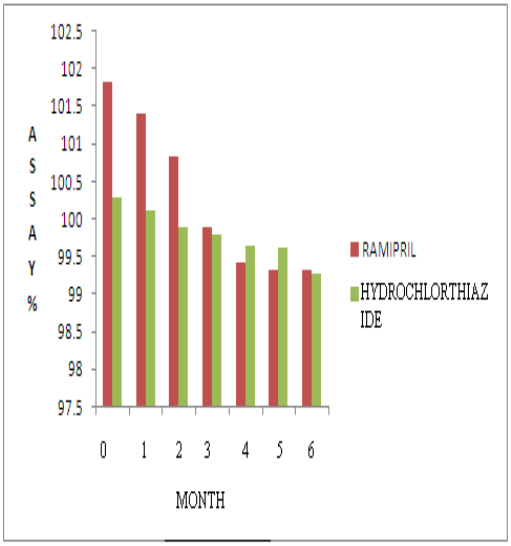
Specificity
A blend of commonly used excipients was treated as per developed procedure and the chromatogram showed no inferring peaks at retention time of the both drugs.

Stability study of Ramipril and Hydrochlorthiazide tablets as per ICH guidelines[17-20]
To carry out the stability study of CARDACE-H tablets, 80 tablets were procured from market. 4 strips each were stored in Programmable environmental test chamber calibrated previously and labelled as

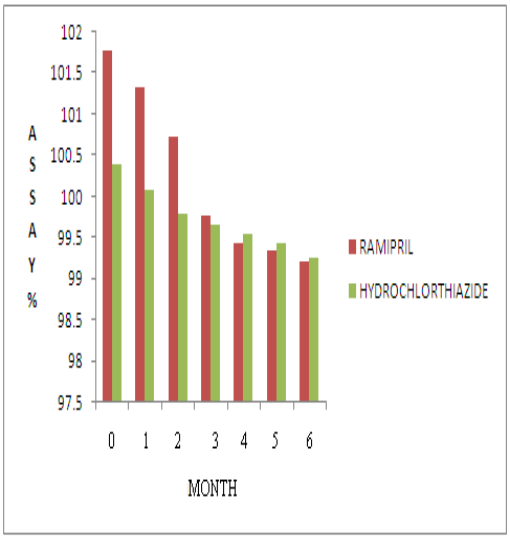
- 30ºC and 65% RH (serial no. ICH-2530)
- 40ºC and 75% RH (serial no. ICH-2531)

Initial testing (at 0th month) of tablet was done before keeping the strip for both intermediate and accelerated stability testing. Further testing was done at the end of each month for six months as per ICH guidelines.
The physical parameters such as hardness, friability and disintegration time were checked and the content of Ramipril and Hydrochlorthiazide was determined by carrying out assay by HPLC method at the end of each month for six months as per ICH guidelines.

Assay by HPLC of Cardace-H
Weigh powder equivalent to 5mg and 12.5 mg of Ramipril and Hydrochlorthiazide transferred to 100 ml volumetric flask. Sufficient amount of mobile phase was added to dissolve the content. Then it was sonicated, cool to room temperature and then volume was made up to the mark with mobile phase. Withdrawing 8 ml of this solution and diluted to 10 ml with mobile phase. Sample was filtered through 0.2 µ nylon membrane filter and injected into the HPLC column.(table 12,13),(Graph 5,6,7,8).
The stability study was carried out for a period of six months at 30^{0}C & 65% RH and 40^{0}C & 75% RH for CARDACE-H, which shows that both Ramipril and Hydrochlorthiazide were found to be stable and comply with IP and BP limits respectively upto 6th month. There is no effect of elevated temperature and humidity on physical stability of product but the assay result showed that the chemical stability of drug has negligible effect.



Graph 5: Assay of CARDACE-H stored at 30ºC and 65% RH by HPLC



Graph 6: Assay of CARDACE-H stored at 40ºC and 75% RH by HPLC

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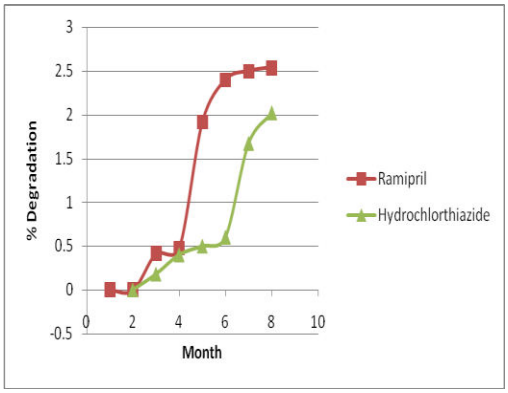
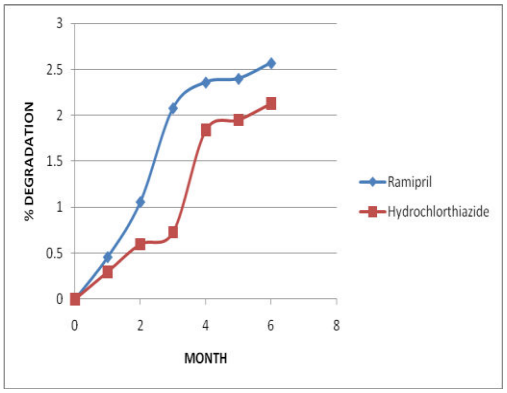


Graph 7: Loss of active ingredient from tablet stored at 30°C/65% RH



Graph 8: % Loss of active ingredient from tablet stored at 40°C/75% RH

Table 12: Assay of CARDACE-H stored at 30°C and 65% RH by HPLC

Month	Amount present (mg)		Amount found (mg)		Assay (%)		% Loss of active ingredient	
	RAM	HTZ	RAM	HTZ	RAM	HTZ	RAM	HTZ
0th month	5	12.5	5.09	12.53	101.82	100.28	-	-
1st month	5	12.5	5.07	12.51	101.40	100.18	0.42	0.18
2nd month	5	12.5	5.04	12.48	100.84	99.88	0.98	0.4
3rd month	5	12.5	4.95	12.47	99.90	99.78	1.92	0.5
4th month	5	12.5	4.92	12.45	99.42	99.65	2.4	0.6
5th month	5	12.5	4.91	12.32	99.32	99.61	2.5	1.67
6th month	5	12.5	4.91	12.28	99.28	99.20	2.54	2.02

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Table 13: Assay of CARDACE-H stored at 40°C and 75% RH by HPLC

Month	Amount present (mg)		Amount found (mg)		Assay (%)		% Loss of active ingredient	
	RAM	HTZ	RAM	HTZ	RAM	HTZ	RAM	HTZ
0th month	5	12.5	5.08	12.51	101.78	100.38	-	-
1st month	5	12.5	5.06	12.47	101.32	100.08	0.46	0.3
2nd month	5	12.5	5.03	12.45	100.72	99.78	1.06	0.6
3rd month	5	12.5	5.006	12.44	100.12	99.65	2.08	0.73
4th month	5	12.5	4.98	12.34	99.77	99.54	2.36	1.84
5th month	5	12.5	4.97	12.30	98.42	99.43	2.44	1.95
6th month	5	12.5	4.98	12.27	98.81	99.23	2.57	2.13

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