ESTIMATION OF PERINDOPRIL AND INDAPAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM AND ITS VALIDATION BY HPLC

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Abstract

A simple, accurate and a precise method was developed for the simultaneous estimation of Perindopril and Indapamide in tablet dosage form using HPLC and UPLC on agilent eclipse XDB column of dimensions (150x4.6mm, 3.5µ) using 0.1% tri ethyl amine and acetonitrile within the ratio of 60:40 v/v as pumped through a column with a flow of 1 ml/min. The retention time of perindopril was 3.49 min in HPLC and 1.75 min in UPLC, Indapamide was 4.08 min in HPLC and 3.17 min in UPLC. The total run time in HPLC method was 6 min and in UPLC method was 4 min. These methods provide good linearity (R^2=0.999) over a range of 4-60 µg/ml of perindopril and 1.25-18.75 µg/ml of indapamide in both HPLC and UPLC methods. The optimized method gives good recovery results in perindopril and indapamide. The method was validated in terms of specificity, linearity, accuracy, precision, robustness and degradation.

Key words: *Perindopril, Indapamide, Development, HPLC, UPLC.*

1. Introduction

Perindopril may be a long acting ACE inhibitor [1-2] want to treat high vital sign [3-4], coronary failure [5-6] are stable arteria coronaria disease [7-8] in sort of perindopril arginine (trade names include coversyl, coversum) or perindopril erbumine (aceon). Consistent with the Australian governments pharmaceutical benefits scheme website supported data provided to the Australian department of health and ageing by the manufacturer. Perindopril arginine and Perindopril erbumine are therapeutically equivalent and should be interchanged without differences in clinical effects. However, the dose prescribed to realize an equivalent effect differs. Thanks to different molecular weights [9] for the two forms. A prodrug perindopril is hydrolyzed to its active metabolite [10-11], perindoprilat, within the liver [12].

Indapamide may be a thiazide-like diuretic generally utilized in the treatment of hyper tension [13] also as decompensated coronary failure. Combination preparations with Perindopril (an ACE inhibitor anti hypertensive) also are available. The thiazide like diuretics [14] (Indapamide and Chlorthalidone) are simpler than the thiazide type diuretics (including hydrochlorothiazide) for reducing the danger of attack, stroke and coronary failure in persons with high vital sign and therefore the thiazide like and thiazide type diuretics have similar rates of adverse effects [15].

High performance liquid chromatography we can separate the delicate and complex natural mixtures, which chemical composition needs to be well established in biological fluids, environmental samples and drugs. Another important application of LC-MS include the analysis of food, pesticides and plant phenols. UPLC-MS/MS is a powerful technique used for the many applications which has very high sensitivity and sensitivity. Generally its application is oriented towards the general detection and potential identification of chemicals in the presence of other chemicals.

2. Materials and Method

Chemicals and Reagents:

Acetonitrile, Tri ethyl amine (TEA), water and methanol were purchased from Merck (India) Ltd, Worli, Mumbai, India. All APIs of perindopril and Indapamide as reference standards were purchased from Glenmark, Mumbai.

Instrumentation

HPLC Conditions:

Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower 2.0 was used.

UPLC conditions:

Waters Acquity UPLC with quaternary pump, PDA detector with empower software 2.0 was used.

Chromatographic conditions:

Chromatographic separation was administered in isocratic mode at temperature employing a agilent eclipse XBD column (150x4.6mm, 3.5 µ). A mixture of 0.1% tri ethyl amine: acetonitrile in 60:40 v/v was used as mobile phase with a flow of 1 ml/min. The injection volume was 10 µl and eluents were monitored at 215 nm using PDA detector. The run time was 6 min using HPLC and 4 min using UPLC.

Preparation of stock and dealing standards

Preparation of ordinary solution:

Accurately weighed 40 mg of Perindopril and 12.5 mg of Indapamide working standards into 100 ml volumetric flask, 70 ml of diluents was added to flask and sonicated it to 15 min to dissolve the components and make up to the mark with diluents.
Take 5 ml of the above solution into 50 ml volumetric flask and made up to the mark with diluents.

Preparation of sample solution:

5 tablets were weighed and calculate the typical weight of every tablet then the load like one tablet. Crush the 5 tablets into powder form, take the equivalent weight of 40 mg of Perindopril and 12.5 mg of Indapamide sample into 100 ml volumetric flask, add 70 ml of diluents and sonicated for 30 min to dissolve the components, then diluted up to the mark with diluents. Further dilute 5 ml of the above solution to 50 ml and it has been filtered through 0.45 µ nylon syringe filter.

Method Development

From the literature survey there are few analytical methods was established in combination of Perindopril and Indapamide. But there are no common method in HPLC and UPLC. The present study was proposed a unique method was applicable in both HPLC and UPLC. In development process they have many trials were performed different buffers like water, phosphoric acid, formic acid finally tri ethyl amine was selected as buffer. In development process tri ethyl amine ratio was gradually increased with decreasing of acetonitrile at final stage we get good resolution and exists the system suitable conditions.

Method validation

The analytical method using HPLC and UPLC was validated as per ICH Q_2 R1 guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) and degradation.

System sutiability:

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, tailing and a couple of RSD are calculated and located to be within the bounds.

Specificity:

Specificity is that the ability to access unequivocally the analyte within the presence of other components, which can be expected to be present within the standard and sample solution. It had been checked by examining the chromatograms of blank samples and samples spiked with Perindopril and Indapamide.

Accuracy:

Accuracy is that the closeness of the test results obtained by the tactic to truth value. It had been assessed by the recovery studies at three different concentration levels. In each level a minimum of three injections got and amount of the drug present, percentage recovery and related variance were calculated.

Precision:

Precision of an analytical method is that the degree of agreement among individual test results. It had been studied by analysis of multiple samplings of homogeneous sample. The precision of this method was assessed in terms of repeatability, intraday and inter-day variations. It had been checked by analyzing the samples at different time intervals of an equivalent day also as an different days.

Linearity and range:

Linearity of an analytical method is its ability to get results directly proportional to the concentration of the analyte within the sample within a particular range. The six series of ordinary solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the quality solution and therefore the regression equation was calculated. The smallest amount squares method was want to calculate the slope, intercept and coefficient of correlation.

LOD and LOQ:

LOD is that the lowest amount of analyte during a sample which will be detected while LOQ is that is that lowest amount of analyte during a sample which will be determined with acceptable precision and accuracy. LOD and LOQ were separately determined supported the calibration curves. The LOD and LOQ for Perindopril and Indapamide were determined by injecting progressively low concentrations of ordinary solutions using the developed RP-HPLC and UPLC method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines. Where s/n indicates signal to noise.

Stress degradation:

Stress degradation should be no interference between peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R_2). The degradation peaks should be separated from one another and therefore the resolution between the peaks should be a minimum of 1.0 and therefore the peak purity of the

principle peaks shall pass. Forced degradation studies were performed by differing types of stress conditions to get the degradation of about 20%.

Robustness:

The robustness of an analytical procedure may be a measure of its ability to stay unaffected by small but deliberate variations in method parameters and provides a sign of its reliability during normal usage. Robustness study was performed by injecting standard solution into HPLC and UPLC systems and altered chromatographic conditions like flow rate (±10%), organic content within the mobile phase (±10%). The separation factor, retention time and the peak symmetry were calculated by determining the affect of the modified parameters.

3. Results and Discussion

Method development and Optimization:

The most suitable isocratic condition to resolve Perindopril and Indapamide using agilent eclipse XDB column, after the chromatographic conditions were optimized for specificity, resolution and retention time was a mobile phase consisting of 0.1% tri ethyl amine acetonitrile within the ratio of 60:40. When a better percentage of mobile phase was used the resultant chromatogram had a rise either in a back background noise or peaks indicating the tailing effect. Thus supported the above mentioned parameters in HPLC Perindopril and Indapamide were eluted at a retention time of 3.49 min and 4.08 min shown in figure 1 and by using UPLC the Perindopril and Indapamide were eluted at a retention time of 1.75 min and 3.17 min shown in figure 2 respectively. Table 1 depicts the chromatographic parameters applied for the tactic using HPLC and UPLC.

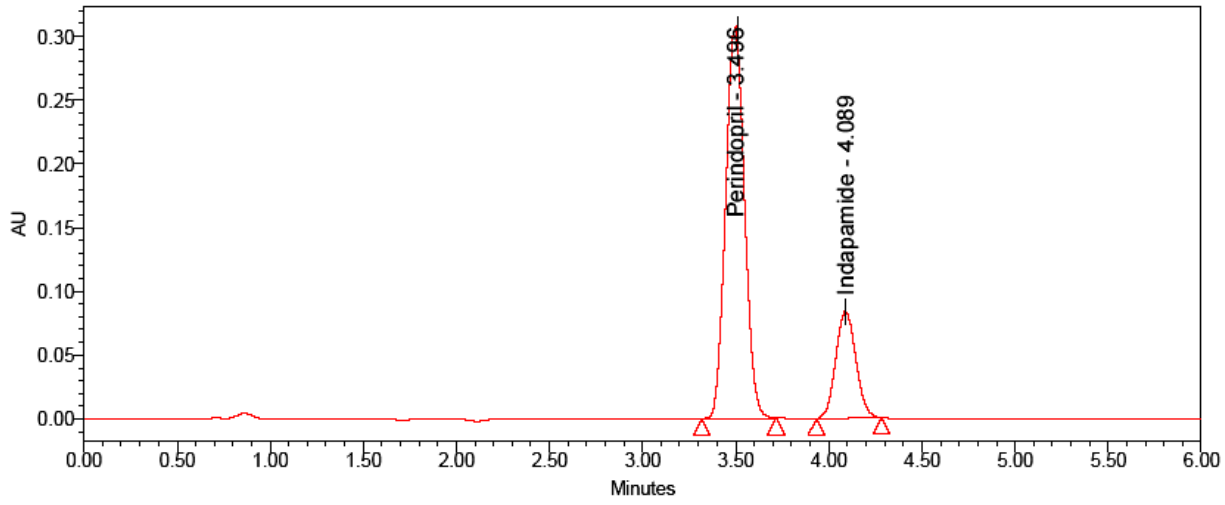

Fig. 1: Representative chromatogram of Perindopril and Indapamide standard using HPLC

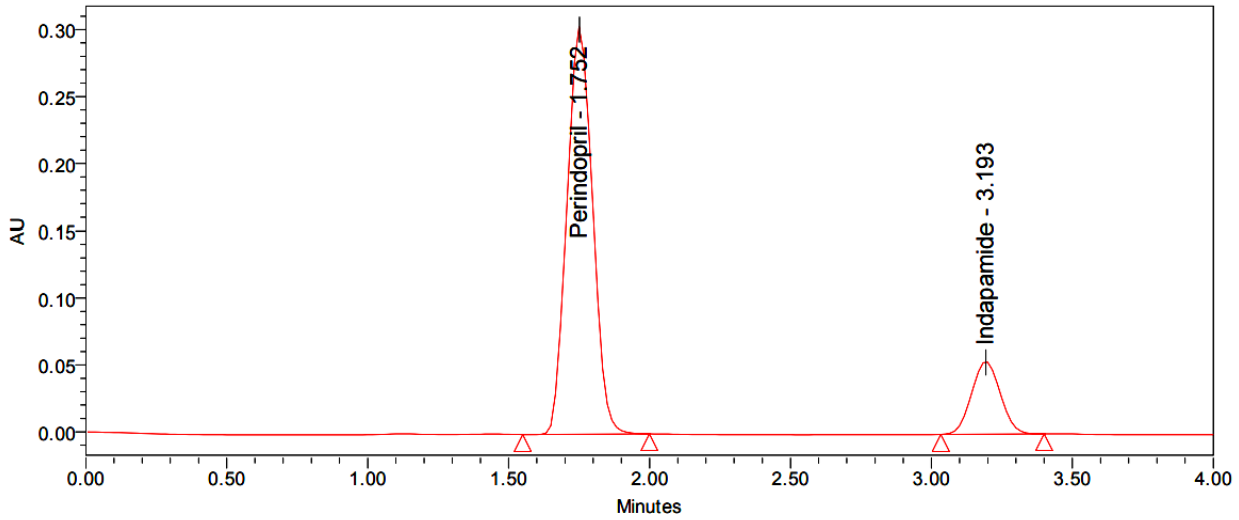

Fig. 2: Representative chromatogram of Perindopril and Indapamide standard using UPLC

Table 1: HPLC and UPLC conditions for Perindopril and Indapamide

Condition	HPLC conditions	UPLC conditions
Instrument	Waters alliance e-2695	Waters Acquity
Column	Agilent eclipse XDB (150x4.6mm, 3.5µ)	Agilent eclipse XDB (150x4.6mm, 3.5µ)
Mobile phase	0.1% Tri ethyl amine:acetonitrile (60:40 v/v)	0.1% Tri ethyl amine:acetonitrile (60:40 v/v)
Flow rate	1 ml/min	1 ml/min
Injection volume	10 µl	10 µl
Wavelength	215 nm	215 nm
Run time	6 min	4 min
Retention time	Perindopril- 3.496 min Indapamide- 4.089 min	Perindopril- 1.754 min Indapamide- 3.174 min

Method validation:

The HPLC and UPLC methods were validated consistent with the validation of analytical procedures provided within the ICH guidelines and draft guidance for the industry analytical procedures and method validation.

Specificity:

Specificity was used to test the facility of the assay method to eliminate the results of all interfering substances on Perindopril and Indapamide peak results, specificity by comparing the chromatograms to the blank (figure 3). The validated method showed that the drug contents eluted with no interfering peaks generated by the excipients within the market products.

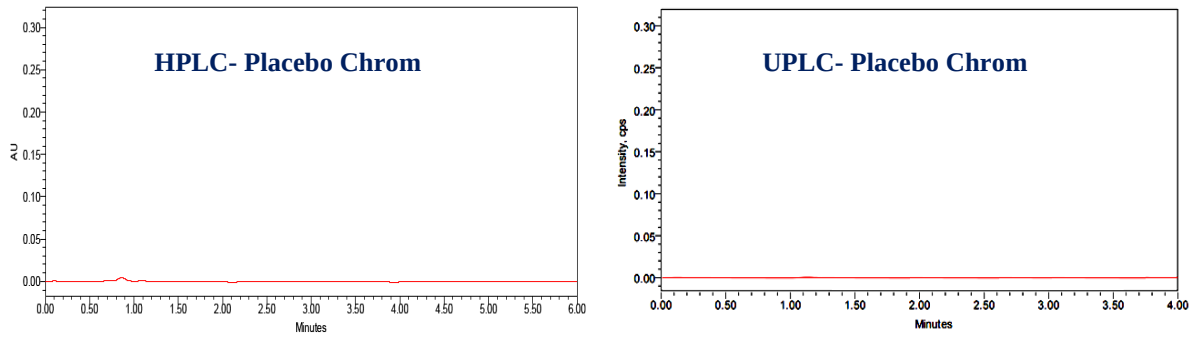

Fig. 3: HPLC and UPLC chromatogram of placebo

System suitability:

Standard solution of Perindopril 40 µg/ml and Indapamide 12.5 µg/ml was injected six times record six injections area to calculate % RSD was less than 2.0 and USP tailing is less than 2.0 and USP plate count is more than 2000 for six injections for both Perindopril and Indapamide in HPLC and UPLC. The values are tabulated in table 2.

Table 2: Sytem suitability results for Perindopril and Indapamide in HPLC and UPLC

Drug name	USP plate count		USP tailing		USP Resolution		% RSD	
	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
Perindopril	5874	8963	0.98	0.54	-	-	0.89	0.71
Indapamide	4985	10542	0.78	0.43	3.02	8.36	1.02	0.59

Linearity and range:

Linearity of the tactic was evaluated by preparing a typical solution containing 40 µg/ml of perindopril and 12.5 µg/ml of indapamide (100% of the targeted level of the assay concentration). Sequential dilutions were performed to the given solutions at 10%, 25%, 50%, 100%, 125% and 150% of the targeted concentrations. These were injected into both HPLC and UPLC systems and therefore the peak areas are wont to plot calibration curves against the concentrations. The coefficient of correlation values of those analytes were 0.999. The mean standard calibration curves are shown in figure 4.

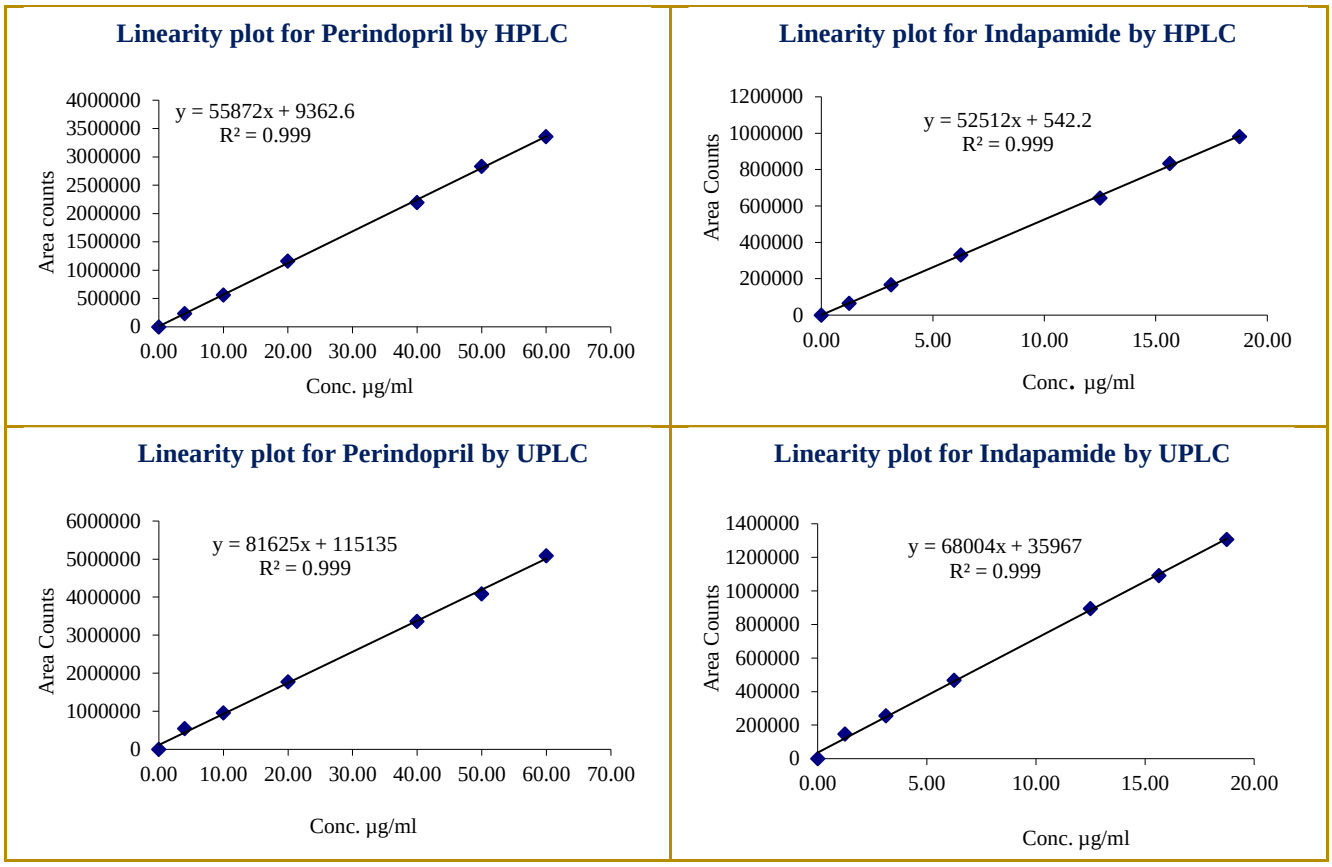

Fig. 4: Linearity plot of Perindopril and Indapamide by HPLC and UPLC

Table 3: HPLC & UPLC linearity data of Perindopril and Indapamide

Linearity	Perindopril			Indapamide		
	Conc. (µg/ml)	HPLC area	UPLC area	Conc. (µg/ml)	HPLC area	UPLC area
Linearity-1	4	234558	547808	1.25	65854	146581
Linearity-2	10	561383	958753	3.13	166815	255651
Linearity-3	20	1164355	1771560	6.25	331041	468128
Linearity-4	40	2193378	3366503	12.5	643875	895033
Linearity-5	50	2834604	4087600	15.63	833857	1090570
Linearity-6	60	3357714	5092881	18.75	982351	1306743
Slope		55872.03	81625.86		52514.19	68006.85
Intercept		9362.66	115135.33		603.86	36044.57
% RSD		0.9997	0.999		0.9998	0.9993

LOD and LOQ:

Limit of detection and quantification minimum concentration level at which the analyte are often reliably detected. Quantified by using the quality formulas. LOD values for Perindopril and Indapamide were 0.04 µg/ml, 0.0125 µg/ml and LOQ values for Perindopril and Indapamide were 0.4 µg/ml, 0.125 µg/ml respectively using HPLC and UPLC.

Table 4: LOD and LOQ results of Perindopril and Indapamide

Drug Name	LOD		LOQ	
	HPLC (S/N)	UPLC (S/N)	HPLC (S/N)	UPLC (S/N)
Perindopril	3	3	10	10
Indapamide	3	3	10	10

Accuracy:

Accuracy decided by recovery studies which were administered in three different concentration levels namely 50%, 100% and 150%. APIs with concentration 20 µg/ml, 40 µg/ml, 60 µg/ml of perindopril and 6.25 µg/ml, 12.5 µg/ml, 18.75 µg/ml of indapamide were prepared. As per the test method the test solution was injected to three preparations each spike level and therefore the assay was performed. The share recovery values were found to be within the range of 98-101%.

Table 5: Accuracy results for HPLC and UPLC

	HPLC		UPLC	
% of target concentration	Perindopril (% Recovery)	Indapamide (% Recovery)	Perindopril (% Recovery)	Indapamide % Recovery)
50%	99.5	100.2	99.7	99.9
100%	99.9	99.8	100.1	100.1
150%	99.9	99.5	98.8	99.5
Mean % accuracy	99.8	99.8	99.5	99.8

Precision:

Precision was established in two types namely method (intraday) precision and intermediate (inter-day) precision.

Method precision was investigated by the analysis of six separately prepared samples of an equivalent batch. From these six separate sample solutions was injected into both HPLC and UPLC and the peak area obtained wont to calculate mean assay and % RSD values. This method was found to be precise as % assay in between 98-102% and % RSD is less than 2.0%.

Intermediate precision was performed by three variations instrument, column and analyst variations. Intermediate precision was investigated by the analysis of six separately prepared samples of an equivalent batch. From these six separate sample solutions was injected into different HPLC and UPLC and peak area obtained wont to calculate mean assay and percentage RSD values.

Table 6: Precision results using HPLC and UPLC

		HPLC				UPLC			
Analyte	Amount present	Method precision		Intermediate precision		Method precision		Intermediate precision	
		% Assay	% RSD	% Assay	% RSD	% Assay	% RSD	% Assay	% RSD
Perindopril	40	98.52	1.18	98.41	1.02	100.02	0.89	99.78	1.54
Indapamide	12.5	100.63	0.95	99.93	0.76	100.41	1.35	99.67	1.06

Robustness:

Robustness of the method was found to be % assay should be between 98-102%. Slightly variations were wiped out the optimized chromatographic conditions like flow (±10%), organic content in the mobile phase (±10%).

Table 7: Robustness results using HPLC

Drug name	Flow plus (1.1 ml/min)	Flow minus (0.9 ml/min)	Org plus (44:56)	Org minus (36:64)
	% Assay			
Perindopril	98.5	98.7	99.2	98.6
Indapamide	100.9	101.2	98.6	100.8

Table 8: Robustness results using UPLC

Drug name	Flow plus (1.1 ml/min)	Flow minus (0.9 ml/min)	Org plus (44:56)	Org minus (36:64)
	% Assay			
Perindopril	99.56	99.65	99.65	98.89
Indapamide	100.05	99.31	99.54	99.36

Stability:

To assess the stableness of sample solution, they were analyzed initially to 24 hrs at different intervals of time. No significant degradation was observed during this era and thus the mean deviation from the mean and therefore the mean was not quite 5.0% suggesting that the solutions were stable for a minimum of 24 hrs which was sufficient for the both analytical procedures of HPLC and UPLC.

Table 9: HPLC results of stability

Stability	Perindopril		Indapamide	
	% of lable claim	% of deviation	% of lable claim	% of deviation
Initial	99.8	0.00	101.8	0.00
6 hrs	99.6	0.04	101.7	0.10
12 hrs	99.4	0.12	101.2	0.15
18 hrs	99.3	0.25	100.8	0.21
24 hrs	99.1	0.36	100.4	0.23

Table 10: UPLC results of stability

Stability	Perindopril		Indapamide	
	% of lable claim	% of deviation	% of lable claim	% of deviation
Initial	99.7	0.00	99.6	0.00
6 hrs	99.1	-0.6	99.1	-0.5
12 hrs	98.5	-1.2	98.8	-0.8
18 hrs	98.1	-1.6	98.2	-1.41
24 hrs	97.3	-2.41	97.8	-1.81

Forced degradation:

Forced degradation studies were performed to means the tactic is suitable for degraded products the studies provide information about the conditions during which the drug is unstable so as that measures are often taken during formulation to avoid potential instabilities.

Acid degradation:

Acid degradation of Perindopril and Indapamide were studied in various stress conditions finally maximum degradation was obtained in addition of 1 ml of 1N HCl in sample stock solution at 60°C for 30min after that cool to room temperature then neutralize with 1 ml of 1N NaOH.

Alkali degradation:

Alkali degradation of Perindopril and Indapamide were studied in various stress conditions finally maximum degradation was obtained in addition of 1 ml of 1N NaOH in sample stock solution heat the solution at 60°C for 30 min after that cool to room temperature then neutralize with 1 ml of 1N HCl.

Peroxide degradation:

Peroxide degradation of Perindopril and Indapamide were studied in various conditions maximum degradation was formed in 1 ml of 30% hydrogen peroxide in sample stock solution and heat the solution at 60°C for 30 min after that cool to room temperature then make up to the mark with diluents.

Reduction degradation:

Reduction degradation of Perindopril and Indapamide were studied in 10% sodium bi sulphate solution.

Thermal degradation:

In thermal degradation sample was exposed to 105°C for 6 hrs.

Photolytic degradation:

Sample was exposed in photo stability chamber minimum of 1.2 million lx h and 200 W h/m2 light. The most commonly accepted wave length of lights in the range of 300-800 nm to cause the photolytic degradation.

Hydrolysis degradation:

Hydrolysis degradation of Perindopril and Indapamide were studied in addition of 5 ml water in sample solution reflux for 30 min.

Table 11: Forced degradation results of Perindopril

Degradation condition	% Assay by HPLC	% deg by HPLC	% Assay by UPLC	% deg by UPLC
Control	100.26	0.26	100.18	0.18
Acid deg	84.43	15.83	83.97	16.21
Alkali deg	84.64	15.62	84.10	16.08
Peroxide deg	85.17	15.09	86.69	13.49
Photolytic	98.99	1.27	98.62	1.56
Reduction	83.92	16.34	82.2	17.98
Thermal	98.46	1.8	98.23	1.95
Hydrolysis	97.64	2.62	97.62	2.56

Table 12: Forced degradation results of Indapamide

Degradation condition	% Assay by HPLC	% deg by HPLC	% Assay by UPLC	% deg by UPLC
Control	100.37	0.37	100.45	0.45
Acid deg	84.69	15.68	84.73	15.72
Alkali deg	84.15	16.22	84.76	15.69
Peroxide deg	86.39	13.98	86.17	14.28
Photolytic	100.18	0.19	99.33	1.12
Reduction	84.69	15.68	84.47	15.98
Thermal	100.26	0.11	99.05	1.4
Hydrolysis	98.68	1.69	98.28	2.17

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5. Conclusion

The developed method for HPLC and UPLC exists for all variation parameters as per ICH guidelines. The unique chromatographic conditions are applied two chromatographic systems. But UPLC method was more sensitive than HPLC. They have good resolution between Perindopril and Indapamide and short run time. An isocratic method for the determination of Perindopril and Indapamide was developed and is precise and reliable. The regression curve equation is capable of reliably predicting the drug concentration within the range of 4-60 µg/ml of Perindopril and 1.25-18.75 µg/ml of Indapamide respectively, from the height area obtained. The method was successfully validated and allowed the reliable, sensitive, robust and specific detection of Perindopril and Indapamide during a common marketed preparation.

6. References

1. Tucker, Bryan M, Perazella, Mark A. What are the major adverse affect on the kidney of ACE inhibitor and ARBs nephrology secrets, Elsevier 2019;78-93.
2. Bezalel S, Mahlab Guri K, Asher I, Werner B, Sthoeger ZM. Angiotensin converting inhibitor induced angio edema. The American journal of medicine 2015;128(2):120-5.
3. Lackland DT, Weber MA, Weber. Global burden of cardiovascular disease and stroke, hypertension at the core. The Canadian journal of cardiology 2015;31(5):569-71.
4. DiNicolantonio JJ, Mehta V, O Keefe JH. Is salt a culprit or an innocent brystander in hypertension. A hypothesis challenging the ancient paradigm. The American journal of medicine 2017;130(8):893-899.
5. Abraham WT, Smith SA. Devices in the management of advanced chronic heart failure. Nature reviews, Cardiology 2013;10(2):98-110.
6. Page RL, O Bryant CL, Cheng D, Dow TJ, Ky B, Stein CM. Drugs that may cause or Exacerbate heart failure. A scientific statement from the American heart association. Circulation 2016;134(6):e32-69.
7. Rezende PC, Scudeler TL, da Costa LM, Hueb W. Conservative strategy for the treatment of stable coronary artery disease, World journal of clinical cases 2015;3(2):163-70.
8. Petersen JW, Pepine CJ. Microvascular coronary dysfunction and is chemic heart disease. Where are we in 2014. Trends in cardiovascular medicine 2015;25(2):98-103.
9. Neufeld R, Stalke D. Accurate molecular weight determination of small molecules via DOSY-NMR by using external calibration curves with normalized diffusion coefficients. Chem Sci 2015;6:3354-3364.

10. Haffen E, Paintaud G, Berard M, Masuyer C, Bechtel Y, Bechtel PR. On the assessment of drug metabolism by assays of codeine and its main metabolites, Therapeutic drug monitoring 2000;22(3):258-65.
11. Coller JK, Christrup LL, Somogyi AA. Role of active metabolites in the use of opiods, European journal of clinical pharmacology 2009;65(2):121-39.
12. Saxena, Romil, Theise, Neil, Canals of Hering. Recent insights and current knowledge, Seminars in liver disease 2004;24(1):43-48.
13. Shaukat Shah MD, Ibrahim Khatri MD, Edward D, Fries MD. Mechanism of anti hypertensive effect of thiazide diuretics. American heart journal St. Louis 95(5).
14. Rejnmark L, Vestergaard P, Pedersen AR, Heickendorff L, Andreasen F, Mosekilde L. Dose effect relations of loop and thiazide diuretics on calcium homeostasis, a randomized double blinded latin square multiple cross over study in postmenopausal osteopenic women. Eur J Clin Invest 2003;33(1):41-50.
15. Olde Engberink RH, Frenkel WJ, van den Bogaard B, Brewster LM, Vogt L, Vanden BJ. Effects of thiazide types and thiazide like diuretics on cardiovascular events and mortality. Systematic review and meta-analysis. Hypertension 2015;65(5):1033-40.