

Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR THE ESTIMATION OF PERINDOPRIL AND INDAPAMIDE

VARSHA BHOIR¹, VIRAJ HIBARE, MRINALINI C. DAMLE*

¹Department of Quality Assurance, AISSMS College of Pharmacy, Pune, India, ²Department of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Kennedy road, Near RTO, Pune 411001, Maharashtra, India.
Email: mcdamle@rediffmail.com

Received: 19 Aug 2013 Revised and Accepted: 22 Sep 2013

ABSTRACT

Objective: To develop a validated stability indicating HPTLC method for determination Perindopril and Indapamide.

Methods: The method was based on the separation of two drugs on plates precoated with silica gel 60 F₂₅₄ using Dichloromethane: Methanol: Glacial acetic acid in the ratio of 9.5:0.5:0.1 v/v/v as mobile phase followed by scanning in absorbance mode at 215 nm.

Results: The optimized chromatographic conditions gave compact spot for Perindopril and Indapamide at R_f value of 0.30 ± 0.02 and 0.60 ± 0.02 respectively. The calibration curve was found to be linear in range of 1000-5000 ng/band and 200-1000 ng/band for perindopril and indapamide respectively. The limit of detection and quantification were found to 164 ng/band and 491 ng/band for Perindopril and 41.41 ng/band and 125.49ng/band for Indapamide. The method has been successfully applied to tablets and was validated according to ICH Harmonized Tripartite guidelines.

Conclusion: A new sensitive, simple, rapid and precise high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous determination of Perindopril and Indapamide in pharmaceutical dosage form. The proposed method can be applicable for simultaneous determination of Perindopril and Indapamide in bulk and formulation.

Keywords: Perindopril arginine, Indapamide, HPTLC, Stability indicating method.

INTRODUCTION

Perindopril arginine; (2S,3aS,7aS)-1-[(S)-N-[(S)-1-carboxybutyl] alanyl] hexahydro-2-indoline carboxylic acid 1-ethyl ester, is one of the non-peptide Angiotensin II receptor antagonists, and is used for the treatment of patients with hypertension and symptomatic heart failure.[1] Indapamide; 3-(aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl) benzamide, is a diuretic of the class of Benzothiadiazines. The combined oral administration of perindopril with indapamide has been found to be more effective than either of the drugs alone in the treatment of hypertension. Structures of Perindopril arginine and Indapamide are shown in Figure 1. A literature survey revealed that Perindopril arginine is official in the British Pharmacopoeia[2], Indapamide is official in the British Pharmacopoeia[3]. However, the combination is not official in any pharmacopoeia. Upon detailed literature survey it was found that, individually these drugs have been analyzed by many methods[4-11], however very few methods viz. one spectrophotometric method, two HPLC methods and one stability indicating RP-HPLC method has been reported for this combination.[12-14] To the best of our knowledge, no stability indicating HPTLC (High Performance Thin Layer Chromatography) method has been reported for simultaneous estimation of both the drugs in tablet dosage form. The present work describes the simple, accurate, precise, sensitive stability indicating HPTLC method for the determination of Perindopril arginine and Indapamide in combination. The method was validated as per the ICH guidelines.[15]

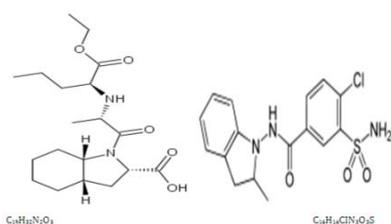


Fig. 1: Structure of Perindopril Arginine and Indapamide

MATERIALS AND METHODS

Chemicals and Reagents

Perindopril arginine and Indapamide was provided as a gift sample by Mylan Laboratories, Hyderabad. Methanol (AR grade), Dichloromethane (AR grade) and Glacial acetic acid (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai, India

Instruments and Chromatographic conditions

Chromatographic separation of drugs was performed on precoated silica gel 60 F₂₅₄, (10 cm × 10 cm with 250 μm layer thickness) purchased from E-Merck, Darmstadt, Germany. Samples were applied on the plate as a band with 4 mm width using Camag 100 μl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) and a densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS software (Version 1.4.3, Camag). Chamber saturation time was 15 min. Migration distance was 90 mm, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Marketed Formulation

A commercial pharmaceutical preparation, COVERSYL PLUS (Serdia Pharmaceuticals Pvt. Ltd., Mumbai, India) was assayed. Its label claim was as follows:

Each uncoated scored tablet contains:

PERINDOPRIL ARGININE B.P..... 4 mg
INDAPAMIDE U.S.P..... 1.25 mg

Selection of wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm and the overlain spectra were obtained. Both the drugs showed considerable absorbance at 215 nm. So, 215 nm was selected as the detection wavelength (Figure 2).

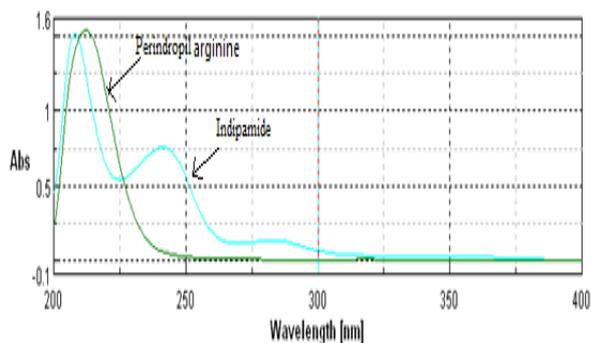


Fig. 2: Overlain spectra of Perindopril arginine and Indapamide.

Method Development

Method development for resolution of Perindopril arginine and Indapamide was started with the development of densitogram with solvents in different ratios and combinations of Toluene, Ethyl acetate, Methanol, Dichloromethane, Triethylamine, and Glacial acetic acid. Finally, Dichloromethane: Methanol: Glacial Acetic acid (9.5:0.5:0.1 v/v/v) was selected as a mobile phase with a good resolution at Rf 0.30 and 0.60 for Perindopril arginine and Indapamide respectively.

Preparation of Stock and Standard Solutions

Standard stock solutions of Perindopril arginine and Indapamide were prepared by separately dissolving 10 mg of drug in 10 ml of methanol to get concentrations of 1000 µg/ml. From the resultant solution of Perindopril, 1, 2, 3, 4 and 5 µl were applied on 5 X 10 cm pre-coated TLC plate as a band of length 4 mm, at a distance of 10 mm from both x-axis and y-axis. 1 ml of standard stock solution of Indapamide was diluted to 10 ml with methanol in 10 ml volumetric flask to get working standard solution 100 µg/ml.

From the resultant solution, 2, 4, 6, 8, and 10 µl were spotted on the plate. Plate was then developed in Camag 10 X 10 cm development chamber using selected mobile phase.

Stress degradation studies of bulk drug

Stress degradation studies were carried under condition of acid/base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared: the blank subjected to stress in the same manner as the drug solution, working standard solution of Indapamide subjected to stress condition, working standard solution of Perindopril subjected to stress degradation. Dry heat and photolytic degradation were carried out in solid state.

Alkaline hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml of 0.1N NaOH. The solution was diluted to 10 ml with methanol and kept for 90 min. 6 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril with 0.01N NaOH, and spotted immediately.

First the degradation was tried with 0.1N NaOH for half hour reduced to 0.01 N NaOH for 15mins. Degradation observed was too high. After addition of base in the standard solution of Perindopril, exothermic reaction was observed. So finally 0.01 NaOH was used for the degradation.

Acidic hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml of 0.1 N HCl. The solution was diluted to 10 ml with methanol and kept for 90 min. 2 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril with 0.1N HCL after 15min.

Neutral Hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml water. The solution was diluted to 10 ml with methanol and kept for 48 Hrs. 6 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril and was spotted immediately after exposure to neutral condition.

Oxidation hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml 3% solution of H₂O₂. The solution was diluted to 10 ml with methanol and was kept for 48 Hrs. 6µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of and Perindopril spotted immediately after exposure to oxidative condition.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample of Indapamide in oven (80° C) for a period of 24 hours. Samples were withdrawn after 24hrs, dissolved in methanol and diluted to get 10µg/ml as final concentration of 6µl was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Perindopril was exposed to 40°c for 15min and solution was prepared as above. First the degradation was tried for half hour but the degradation observed was high. The storage is strictly recommended in cold condition.

Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. Samples were weighed, dissolved and diluted to get 10 µg/ml. 6µl of this solution was spotted. 10 times higher concentration was also spotted to locate degradation product, if any. Same procedure was followed for Perindopril.

Preparation of Tablet solution for Assay

10 tablets were accurately weighed and powdered. From the powdered mixture, certain amount (equivalent to 4 mg of perindopril arginine and 1.25 mg of Indapamide) of the powder was accurately weighed and transferred to 10 ml volumetric flask. Methanol was added in the volumetric flask and the resultant mixture was sonicated for 10 min at room temperature to disperse the powder completely. The resultant mixture was further diluted to 10 ml with methanol and then filtered through Whatmann filter paper no 1, to get the clear solution. 1 ml aliquot of the filtered solution was then diluted to 10 ml with methanol in 10 ml volumetric flask to get the final concentration 100 µg/ml of Indapamide separately. 3 µl of 1000 µg/ml and 6µl of 100 µg/ml of this solution was spotted on TLC plate as a band of length 4 mm on two separate tracks for the assay of Perindopril arginine and Indapamide respectively.

Method Validation

Linearity and Range

Linearity was studied by analyzing five concentrations of the drug, and process was repeated for five times each. It was done over the concentration range of 1000-5000 ng/band and 200-1000 ng/band for perindopril arginine and Indapamide respectively.

Precision

Precision of the system and method was evaluated by analyzing independent sample preparations obtained from homogenous sample and % RSD value obtained was calculated to determine any variation.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample

solution at three different levels of 80, 100 and 120 %. Mean percentage recovery for both the drugs was then determined.

Limit of detection and limit of quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on TLC scanner 3 in the range of 200-400 nm using WinCats software (version 1.4.3).

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. Robustness of the method was determined by making slight deliberate changes like chamber saturation time and $\pm 2\%$ variation in mobile phase compositions.

RESULTS AND DISCUSSION

Optimization of mobile phase

TLC procedure was optimized with a view to develop a method for simultaneous estimation of Perindopril arginine and Indapamide. The drug reference standards were spotted on TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve the peaks of Perindopril arginine and Indapamide. Best suited mobile phase was found to be Dichloromethane: Methanol: Glacial Acetic Acid in the ratio of 9.5:0.5:0.1 v/v/v. Developed mobile phase resulted in resolution of Perindopril arginine and Indapamide at $R_f 0.30 \pm 0.02$ and 0.60 ± 0.02 respectively. Well-defined bands were obtained when the chamber was saturated for 15 min. with the mobile phase at room temperature before chromatographic development. (Figure 3)

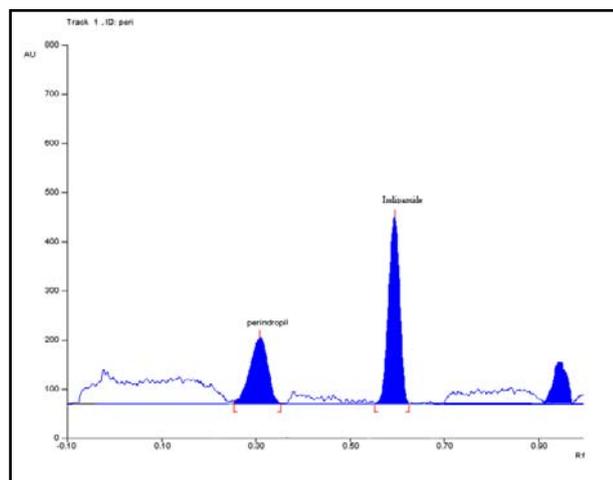
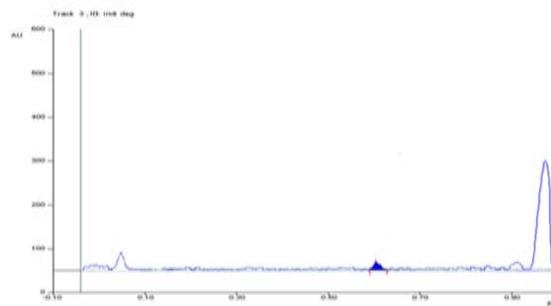


Fig. 3: Representative Densitogram of Perindopril arginine ($R_f 0.30 \pm 0.02$) and Indapamide ($R_f 0.60 \pm 0.02$)

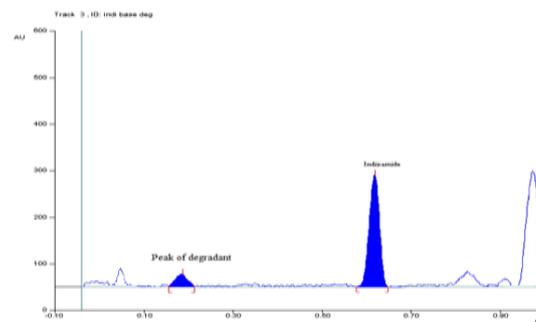
Stress degradation study

Hydrolysis at basic pH

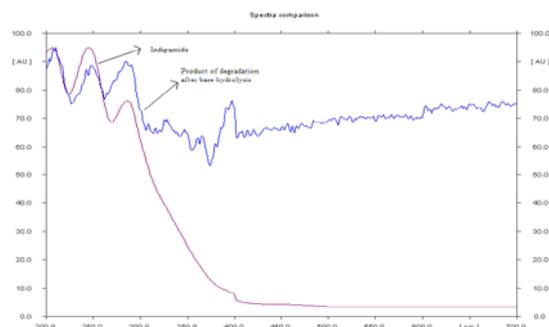
After alkaline degradation, 71.21% Indapamide was recovered with 1 peak of degradation product. (Figure 3 A, B and C) While no degradation peak was observed for Perindopril with 58.08% recovery.



A)



B)



C)

Fig. 3: A) Blank NaOH, B) Indapamide (600ng/band) treated with NaOH, C) Overlain spectra of Indapamide and product of degradation.

Hydrolysis at acidic pH

After acid hydrolysis, 70.71% Indapamide was recovered with 1 peak of degraded product while 77.36% Perindopril was recovered with no peak of degraded product. [Fig.4 A, B and C]



A)

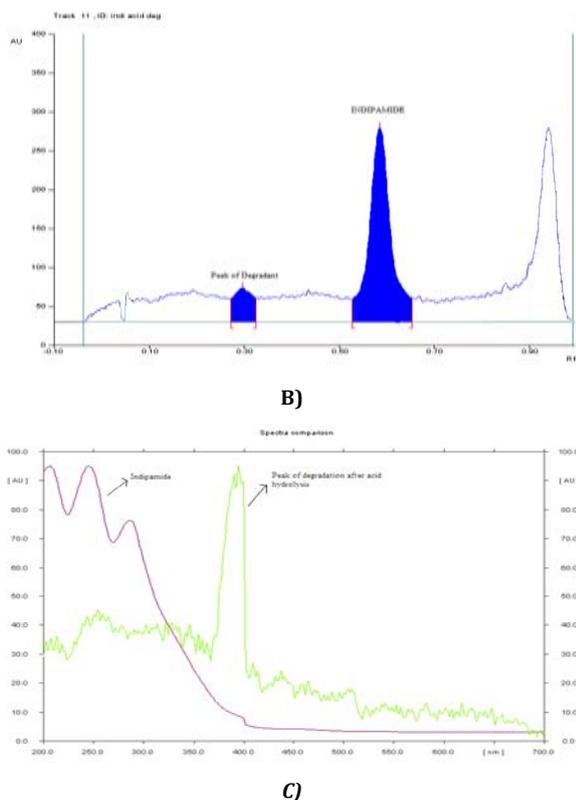


Fig. 4: A) Blank HCL, B) Indapamide (600ng/band) treated with HCL, C) Overlay spectra of Indapamide and product of degradation.

Under neutral hydrolysis

After neutral hydrolysis, 71.94% Indapamide was recovered and 65.66% Perindopril was recovered.

After oxidative condition

After oxidation, 74.34% Indapamide was recovered while 60.23% Perindopril was recovered with no peak of degraded product.

Table 1: Summary of stress degradation study of Indapamide

S. No.	Stress Degradation Condition	Peak Area	% Recovery	R _f of Degradation product
1	Base (0.1 N NaOH, kept for 90 mins)	4682	71.21	0.20
2	Acid (0.1 N HCl, kept for 90 mins)	4658	70.71	0.28
3	Water (kept for 48 hrs.)	4718	71.94	-
4	H ₂ O ₂ 3% (kept for 48 hrs)	4835	74.34	-
5	Heat dry (80°C, 24 hrs.)	5696	91.99	-
6.	Photo stability [UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs]	6050 6087	99.26 100.01	- -

After the photo degradation

After the photo degradation study for UV light and Florescence light, 100.01% Indapamide was recovered with no peak of degradation while 83.49% Perindopril with no peak of degradation.

Degradation under dry heat

After dry heat, 91.99% Indapamide was recovered with no peak of degraded product while 67.79% Perindopril was recovered with no peak of degraded product.

Stress degradation results are summarized in Table 1 and 2 & results of method validation are given in Table 3.

Table 2: Summary of stress degradation study of Perindopril

S. No.	Stress Degradation Condition	Peak Area	% Recovery	R _f of degradation Product
1	Base (0.01 N NaOH, immediately)	2963	58.08	-
2	Acid (0.1 N HCl, kept for 15mins)	3752	77.36	-
3	Water (immediately)	3267	65.66	-
4	H ₂ O ₂ 3% (immediately)	3072	60.23	-
5	Heat dry (40°C, 15 min)	3354	67.79	-
6	Photo stability UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs	4391 4001	93.01 83.49	- -

Method Validation

The linearity in the proposed HPTLC method for determination of Perindopril arginine and Indapamide was found in the concentration range of 1000-5000 ng/band and 200-1000 ng/band with r₂ value of 0.991 and 0.998 respectively (Figure 5).

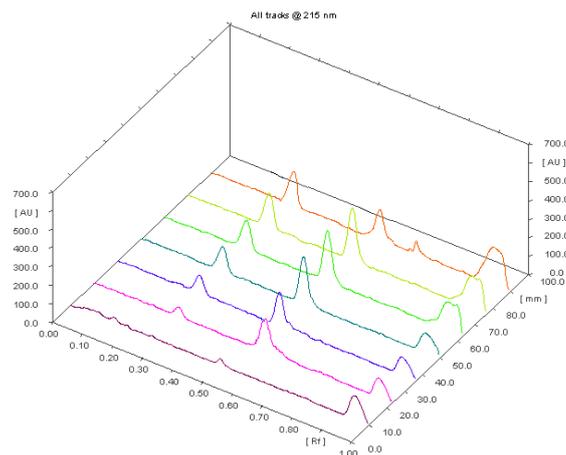


Fig. 5: Linearity for Perindopril Arginine (R_f 0.30 ± 0.02) and Indapamide (R_f 0.60 ± 0.02).

Marketed sample of tablet was analyzed and the percentage label claim was found to be 99.98 % and 99.94 % for Perindopril arginine and Indapamide respectively. HPTLC method was validated as per the ICH guidelines. The accuracy of method was determined at 80, 100 and 120 % level. The % recovery was found to be within the limits of 98 % to 102 % for both the drugs. Precision was calculated as interday and intraday variations. For System and Method precision, % RSD (Relative Standard Deviation) was found to be not more than 2 % for both drugs. For robustness studies, there were no significant changes in R_f and peak areas, which demonstrated that the developed HPTLC method is robust. Peak purity was found to be more than 0.995, which demonstrated that the method is specific. The results of validation are summarized in Table 3.

Table 3: Summary of validation parameters

S. No.	Validation Parameter	Results	
		Indapamide	Perindopril
1.	Linearity	$y = 8.128x + 1210$ ($R^2 = 0.998$)	$y = 1.363x + 588.7$ ($R^2 = 0.991$)
2.	Range	200-1000 ng/band	1000-5000 ng/band
3.	Precision	(%RSD)	(%RSD)
	A) System precision	1.83	1.75
	A) Method precision	1.13	1.56
4.	Accuracy	% Recovery	% Recovery
	80%	99.53	99.63
	100%	98.70	101.25
	120%	99.48	99.28
5.	LOD	41.41 ng/band	164 ng/band
6.	LOQ	125.49 ng/band	491 ng/band
7.	Specificity	Specific	Specific
8.	Robustness	Robust	Robust

The degradation conditions mentioned above were arrived at, after number of initial trials for optimization of extent of degradation. Overall study comprised of stability indicating method development for Indapamide and Perindopril arginine. This method can be used for stability testing of this drug in dosage forms.

CONCLUSION

The validated HPTLC method was found to be simple, accurate and rapid for the routine determination of Perindopril arginine and Indapamide in tablet formulation. The proposed method can therefore, be successfully used for simultaneous estimation of Perindopril arginine and Indapamide in combined dosage form.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENT

Authors are thankful to Mylan Laboratories, Ltd. Hyderabad for providing working standard of Perindopril arginine and working standard of Indapamide. Authors are also thankful to the Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

REFERENCES

1. Erk N. Comparison of spectrophotometric and LC method for the determination of Perindopril and Indapamide in pharmaceutical formulations. *J of Pharm and Biomedical Analysis* 2001;26:43-52.
2. British Pharmacopoeia. Vol 2. London, UK:British Pharmacopoeial Commission Office;2007:1609-11.
3. British Pharmacopoeia. Vol 2. London, UK:British Pharmacopoeial Commission Office;2007:1078-80.
4. Medenica M, Ivanovic D, Maskovic M, Jancic B, Malenovic A. Evaluation of impurity level of perindopril tert-butylamine in tablets. *J of Pharm and Biomedical Analysis* 2007;44:1087-1094.
5. Simoncic Z, Roskar R, Gartner A, Kogej K, Kmetec V. The use of Microcalorimetry and HPLC for the determination of degradation kinetics and thermodynamic parameters of Perindopril arginine in aqueous solutions. *Int J of Pharm* 2008;356:200-05.
6. Lakshmi KS, Sivasubramanian L, Pandey AK. A validated RP-HPLC method for simultaneous determination of losartan and perindopril in solid dosage form. *J Pharm Rev* 2010;131-3.
7. Saleh, Hanna M, Amin, Alaa S, E-Mamml, Magda. New colorimetric methods for the determination of indapamide and its formulations. *J Mikrochimica Acta* 2001;137(3-4):185-9.
8. Sahoo S, Panda PK, Mishra SK, Sahoo S. HPLC Method Development For Simultaneous Estimation Of Hydrochlorothiazide And Perindopril in Tablet Dosage form. *Asian J Pharm Clin Res* 2012, 5(2), 136-8.
9. Tarkase KN, Jadhav MB, Tajane SR, Dongare US. Development and Validation of UV-Spectrophotometric methods for estimation of Indapamide in bulk and tablet dosage form. *J Der Pharm Chemica* 2012, 4 (3), 1128-32.
10. Barot TG, Prajapati V, Patel PK, Shah N, Patel LD. A Validated RP-HPLC Method for Simultaneous Estimation of Indapamide impurity (Methyl Nitrosoindoline) API form. *Int J of Pharm Tech Res* 2009, 1(4), 1287-96.
11. Jinay P, parul P, Mandev P. Development and Validation of Spectrophotometric methods for simultaneous estimation of Amlodipine besylate and Indapamide in combined dosage form. *Int J Pharm Pharm Sci* 2012;4(3):257-61.
12. Bharadwaj V, Gulecha B, Madgulkar A, Damle MC. RP-HPLC method for simultaneous estimation of perindopril and indapamide in tablet formulation. *J Indian Drugs* 2007;44(7):504-08.
13. Jogha H, Khandelwal U, Gandhi T, Singh S. Development and validation of stability-indicating assay method for simultaneous determination of Perindopril and Indapamide in combined dosage form by reversed-phase high-performance liquid chromatography. *J of AOAC Int* 2010;93(1):108-15.
14. Juddy J, Blessen P, Sundarapandian M. Method development and validation for simultaneous estimation of Perindopril erbumine and Indapamide by RP-HPLC in pharmaceutical dosage forms. *Int J Pharm Pharm Sci* 2011;3(Suppl 4):288-93.
15. Validation of Analytical Procedures:Text and Methodology Q1A (R2). *J ICH Harmonized Tripartite Guideline* 2003.