IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ENALAPRIL MALEATE BY QBD APPROACH

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Abstract

A simple, specific, quick, accurate, and prudent converse steps high-performance liquid chromatography (HPLC) technique has been developed for the simultaneous evaluation of Enalapril maleate in bulk formulation. In terms of exactness, precision, linearity, breaking point of identification, and cut off of quantitation, the developed strategy was accepted. In the future, these drugs can be evaluated in connected measurement structures using the suggested method. The current work focuses on the cutting-edge methods that have been employed thus far and paves the way for future research where the work is still being done. The proposed method can be used for the estimation of these drugs in biological fluids**.** The design, development, standardizing, and quality control of pharmaceutical drugs all depend on rather precise and sensitive analytical procedures. Since the medication product involves human life, quality is crucial. Strong primary healthcare initiatives around the world must focus on the proper production and quality control of drugs. Quality is the complete sum of all elements which contribute directly or indirectly to the safety; efficacy and acceptability of the product.

Keywords: Enalapril Maleate, Oxfendazole, HPLC, Linearity, Validation.

Introduction

Enalapril male ate is an ACE inhibitor to treat hypertension, high blood pressure, diabetic kidney disease, and heart failure. It is typically administered by mouth or injection into a vein in tandem with a diuretic such as furosemide to manage heart failure. The manifestations usually start within an hour and extend for approximately a day when administered orally. Due to the apparent inexpensive therapy and administration availability, the oral route is extensively used to deliver therapeutic drugs to manage hypertension, leading to high patient adherence. At the early stages of the treatment of hypertension, it can be useful to choose monotherapy to observe the effect and the side effects of the drug. However, monotherapy can be insufficient to reach the target blood pressure in a majority of patients. A greater therapeutic benefit can be achieved with two or even more antihypertensive drugs. Therefore, fixed-dose combinations (FDCs) are frequently used in cardiovascular diseases such as hypertension. In order to

develop an FDC product including two drugs, certain conditions must be met. For instance, a synergistic effect can be observed using two drugs together, or a side effect related to a drug may be eliminated using the other drug concurrently. In the treatment of hypertension, there is a synergistic effect between calcium channel blockers (CCBs) and angiotensin-converting enzyme inhibitors (ACEIs)**.** To know about the composition and structure of matter, Analytical chemistry, a branch ofscience, is used, by acquiring, practicing and conveying information. It is not confined to definite compounds or reactions and it deals with the study of the natural and artificial materials. Geometrical features like molecular morphologies and species identity are constituted in the properties of analytical chemistry. The development of its various concepts and theories include safety and quality of food, pharmaceuticals and water, environmental monitoring, biomedical applications and also to support the legal processes (forensics) and diagnose diseases, etc., Analytical chemists play a vital role to support this. To identify and measure the chemical species in a sample, analytical chemists use different techniques. By comparison of the known substance to a similar substance(whose concentration is known), which is called a standard reference material, and almostevery technique will be carried out. In general, the drugs may be new or partially modified in structure of the existing ones with combinations releasing into the market annually. Frequently, from its introduction into the market to the inclusion of pharmacopoeias, it is being delayed, so there is a lack of analytical methods for these drugs, and for such drugs, this can lead to the development of newer analytical methods.

High Performance Liquid Chromatography (HPLC):

HPLC is used to figure out the amount of specific compound in a solution. It supports reliable quantitative range to allow the determination of substances in a single run. This method is considered to be rapid, accurate, precise and specific and offers the ease of automation. It is because methods using HPLC have more advantages over the conventional methods. For analytical measures and method authentication, U.S FDA has given some manufacturing directions. The ICH guidance also provides clear text on the method validation of drug analysis. The USP has precise strategies for method validation for compound assessment. USP describes eight steps for endorsement. They are [6-7], Linearity, Accuracy, Precision, Selectivity and Specificity, Limit of quantification, Limit of detection, Robustness and Ruggedness.

Enalapril Maleate

Enalapril is an ACE inhibitor. ACE stands for angiotensin converting enzyme. Enalapril is used to treat high blood pressure (hypertension) in adults and children who are at least 1 month old. Enalapril is also used to treat congestive heart failure (CHF). CHF is a disorder of the ventricles (the lower chambers of the heart) which decreases the heart's ability to pump blood to the body. The active pharmaceutical ingredient Enalapril is applied in pharmaceutical oral formulations as a salt (1:1) with maleate to ensure its physicochemical stability.

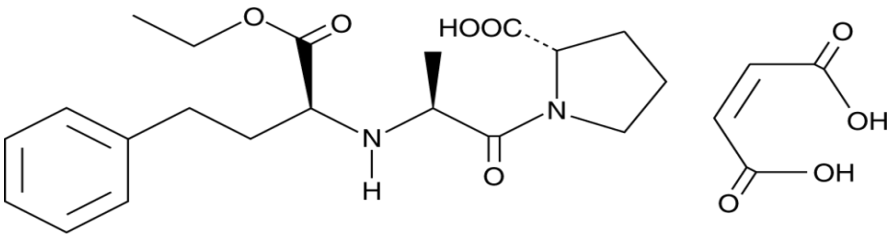


Fig No.1 Structure of Enalapril Maleate

Material and Method

Table No. 1: Active Pharmaceutical Drug

Sr. No.	Name	Description
1.	Enalapril Maleate	• White powder, • use as Hypertension
2.	Envas10	• 10 mg drug contain each tablet, • Manufactured by Cadila pharmaceutical LTD

Table No. 2: List of Chemicals use in Research work

Sr. No.	Name of Chemical	Molecular Formula	Properties	Manufacturer
1.	Acetonitrile	C_2H_3N	Solvent, BP 76-81.6°C	Merck Life Science
2.	Acetonitrile	CH_3OH	Flammable Solvent	Merck Life Science
3.	PhosphateBuffer	KH_2PO_4	White Crystalline Powder	S D Fine Chem. Ltd, Mumbai
4.	DistilledWater	H_2O	Universal Solvent, BP 100°C	In-house

Optimization

Screening design for suitable chromatographic condition:

Determination of suitable column and solvent system based on peak parameters.

- Water: Methanol: Acetonitrile
- Buffer: Methanol: Acetonitrile

These two types of mobile phases were selected for screening study on C18 columns at pH 4.0, 5.0 and 6.0. These mobile phases were screened by varying the organic phase composition from 50 to 70 % v/v and flow rate 1.0 mL/ min.

Results of various trials, having aqueous phase composition 50 % v/v are shownin following tables.

Table 3 Trials performed at mobile phase (50:25:25 v/v) with aqueous phasepH 4

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (50:25:25 v/v/v)	Less Theoretical Plates with greater peak asymmetry	Very Dissatisfactory
2	Buffer: Methanol: ACN (50:25:25 v/v/v)	Less peak asymmetry but less theoretical plates	Satisfied

Table 4 Trials performed on mobile phase (50:25:25 v/v) with aqueous phasepH 6

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (50:25:25 v/v/v)	Greater Peak asymmetrywith less retention time	Satisfactory
2	**Buffer: Methanol: ACN (50:25:25 v/v/v)**	**Good peak properties, less retention time withGreater Theoretical plates**	**Extremely Satisfactory**

Results of various trials, having aqueous phase composition 60 % v/v are shownin following tables.

Table 5 Trials performed at mobile phase (60:20:20 v/v/v) with aqueousphase pH 4

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (60:20:20 v/v/v)	More retention time	Not satisfactory
2	Buffer: Methanol: ACN (60:20:20 v/v/v)	Less peak asymmetrywith more theoreticalplates	Partly Satisfactory

Table 6 Trials performed at mobile phase (60:15:15 v/v) with aqueous phasepH 5

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (60:20:20 v/v/v)	More retention time	Not satisfactory
2	Buffer: Methanol: ACN (60:20:20 v/v/v)	Greater Peak Asymmetry	Not Satisfactory

Table 7 Trials performed at mobile phase (60:20:20 v/v) with aqueous phasepH 6

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (60:20:20 v/v/v)	Good peak properties	satisfactory
2	Buffer: Methanol: ACN (60:20:20 v/v/v)	Less Peak Height	Not satisfactory

Results of various trials, having aqueous phase composition 70 % v/v are shownin following tables.

Table 8 Trials performed at mobile phase (70:15:15 v/v/v) with aqueousphase pH 4

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (70:15:15 v/v/v)	Lower theoretical plates	Not satisfactory
2	Buffer: Methanol: ACN (70:15:15 v/v/v)	Lower theoretical plates	Not satisfactory

Table 9 Trials performed at mobile phase (70:15:15 v/v/v) with aqueousphase pH 5

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (70:15:15 v/v/v)	Less Peak Height but more retention time	Partly Satisfactory
2	Buffer: Methanol: ACN (70:15:15 v/v/v)	Less Peak height and good theoretical plates	Satisfactory

Table 10 Trials performed at mobile phase (70:15:15 v/v/v) with aqueous phasepH 6

Sr. no.	Composition	Observation	Remarks
1	Water: Methanol: ACN (70:15:15 v/v/v)	Less peak asymmetry with more theoretical plates with more theoretical Plates	Satisfactory
2	Buffer: Methanol: ACN (70:15:15 v/v/v)	Good Peak Properties but More retention time	Not satisfactory

Trials performed at mobile phase Water: Methanol: Acetonitrile (50:25:25v/v/v) with aqueous phase pH 6 are extremely Satisfactory.

Effect of independent variables on retention time (X):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 119.89, *p* value less than 0.005 and R^2 value of 0.9756. There is only a 0.01% chance that a "Model F-Value" this large couldoccur due to noise. Values of % C.V. and adjusted R^2 were 8.02 and 0.9675 respectively. The model for response X (Retention time) is as follows: The equation for response surface quadratic model is as follows

Retention Time = +7.67 +3.86 * A -0.41 * B

Fig.3 (b) shows a graphical representation of pH of buffer (B) and amount of Buffer (A), while flow rate (C) is maintained constant at its optimum of 1 mL min^{-1}. Changein pH of buffer showed slightly change in retention time (X), also decrease in amountof buffer showed decreases the retention time.

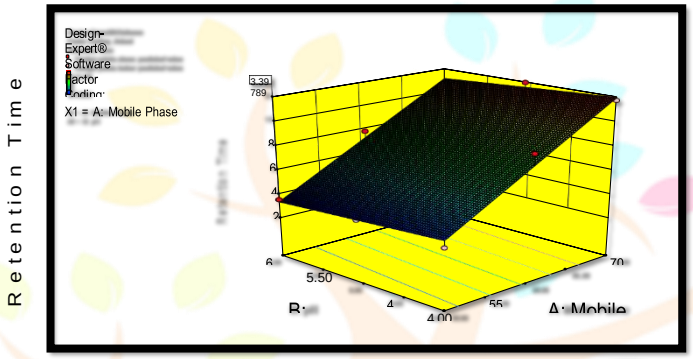


Fig. No. 2 Three-dimensional plot for retention time as a function of pH ofbuffer and amount of buffer.

Constant factor (flow rate- 1mL min^{-1})

Effect of independent variables on tailing factor (Y):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 35.33, *p* value less than 0.005 and R^2 value of 0.9217. There is only a 0.05% chance that a "Model F-Value" this large couldoccur due to noise. Values of % C.V. and adjusted R^2 were 9.46 and 0.8956 respectively. The model for response

Asymmetric Factor = +1.59 + 0.083 * A - 0.51* B

Fig.6.(b) shows a graphical representation of pH of buffer (B) and amount of Buffer (A), while flow rate (C) is maintained constant at its optimum of 1.0 mL min^{-1}. As increases in pH of buffer decrease the tailing factor, it is synergistic effect on response (Y) while increase in amount of Buffer showed no drastic change in the asymmetry.

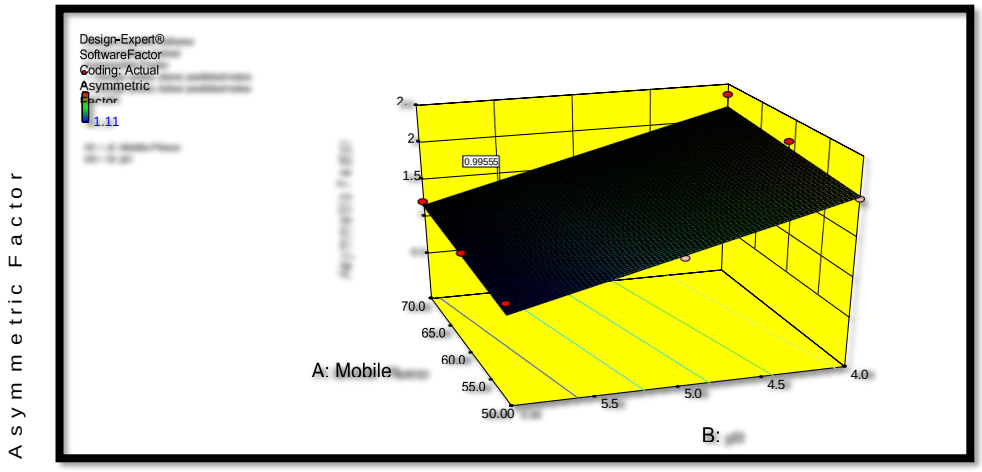


Fig. no. 3 Three-dimentional plot for tailing factor as a function of pH of bufferand % v/v of buffer. Constant factor (flow rate- 1mL min^{-1})

Effect of independent variables on theoretical plates (Z):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 19.34, *p* value less than 0.005 and R^2 value of 0.8657. There is only a 0.24% chance that a "Model F-Value" this large couldoccur due to noise. Values of % C.V. and adjusted R^2 were 21.75 and 0.8210 respectively. The model for response Z (theoretical plates) is as follows:

Theoretical Plates = +6032.67 -96.17*A +3330.83* B

Fig.9.(b) shows a graphical representation of amount of Acetonitrile (A) and pH of buffer (B), while flow rate (C) is maintained constant at its optimum value 1mL min^{-1}. As increases in pH of buffer showed positive effect on number of theoretical plates(Z), while increase in amount of buffer does not show significant change.

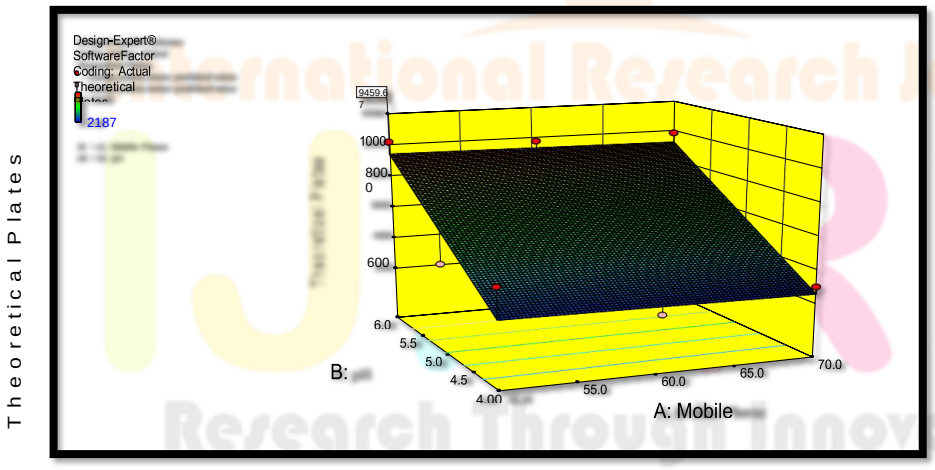


Fig. no. 4 Three-dimentional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1 mL min^{-1})

Result and Discussion

The HPLC Validation of Optimized result of Oxfendazole is at 6 pH, Mobile Phase of Acetonitrile: Buffer (60:30) at Maximum Wavelength 227 nm. The proposed HPLC method was validated in terms of system suitability,

specificity, precision, accuracy and robustness as per the International Conference on Harmonization (ICH) guidelines (7).

Linearity:

The linearity of peak area response for Enalapril Maleate was determined from 10 % to 50 % level of working concentration of Enalapril Maleate. The stock solutions of standard Enalapril Maleate was diluted to six different known concentrations. Linearity graph of concentration (as x-value) versus area (as y-value) were plotted and correlation coefficient, y-intercept and slope of the regression were calculated.

Table 11: Linearity Result of Enalapril Maleate

Sr.No.	Concentration (µg/ml)	Peak Area Enalapril Maleate
1	10	112620
2	20	225495
3	30	339243
4	40	460920
5	50	563611

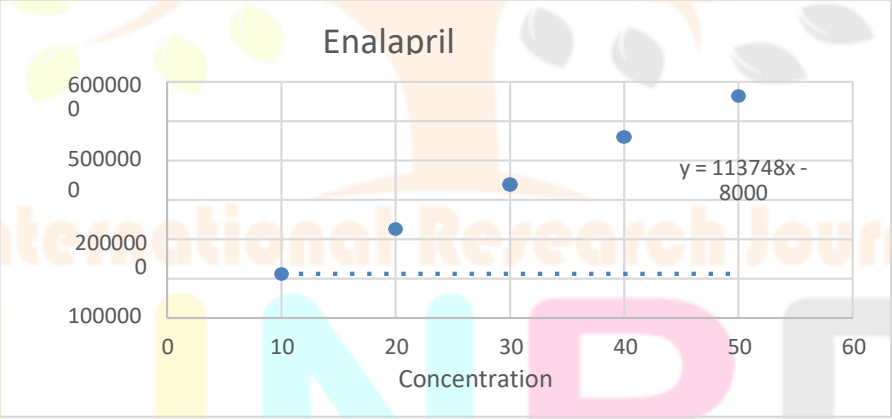


Figure 5: Calibration Curve of Enalapril Maleate

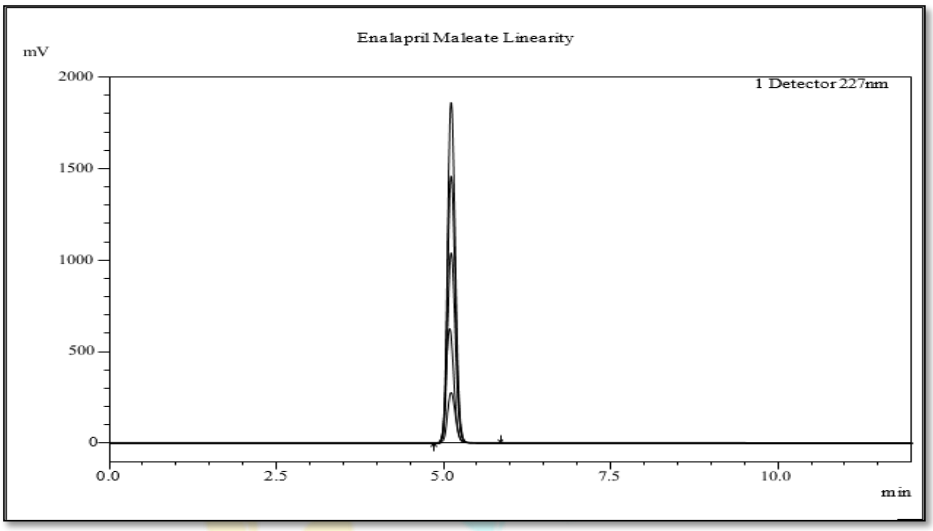


Figure 6 : Overlain of Enalapril Maleate

Table No. 12 Characteristic parameters of Enalapril Maleate for the proposedHPLC method.

Parameter	**Result**
	Enalapril Maleate
Calibration range (µg/ml)	10-50
Detection wavelength (nm)	227
Solvent (Methanol: Buffer)	65:35
Regression equation (y*)	y = 113648x + 8000
Slope (b)	113648
Intercept (a)	8000
Correlation coefficient(r2)	0.9995
Limit of Detection (µg/ml)	0.0061
Limit of Quantitation (µg/ml)	0.0262

System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40 µg/ml. The results which are given in Table No 13.Were within acceptable limits.

Table No 13. System suitability studies of Enalapril Maleate by HPLC method.

Sr. No.	Properties	Values
1.	Retention time	**5.1 ± 0.26**
2.	Area	**8023 ± 660**
3.	Asymmetry	**1.1 ± 0.36**

Specificity

Chromatogram of blank was taken as shown in Fig No.6.3-6.5. Chromatogram of Enalapril Maleate showed peak at a retention time of 5.103 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Enalapril Maleate was 5.103 ± 0.0098min. The wavelength 227 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Enalapril Maleate from the tablet formulation was Enalapril Maleate.

Table No 14: Specificity of Enalapril Maleate by HPLC method

Concentration	API Area	Tablet Area
40	461020	462227
40	468998	456625
40	465992	461666
40	460990	466498
40	460990	453866
40	462990	454998
Mean	466829	459313
SD	42209.96	49227.39
RSD	0.90	1.06

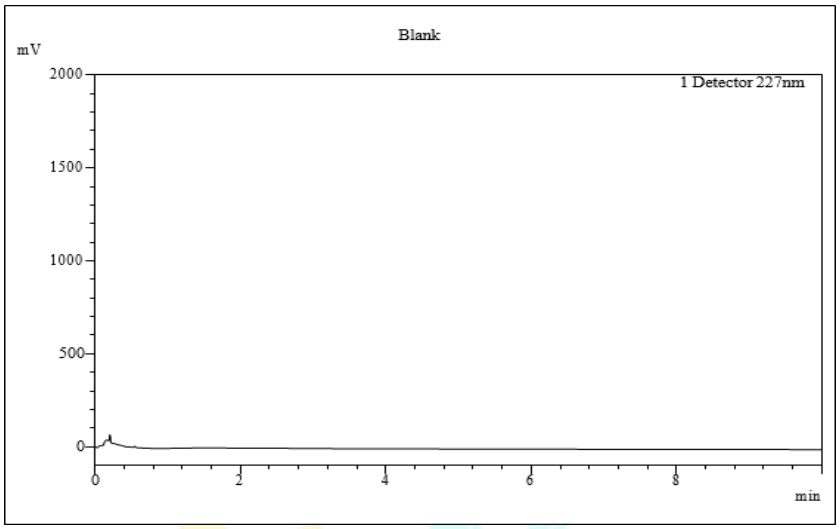


Fig. No. 7 typical chromatogram of Blank

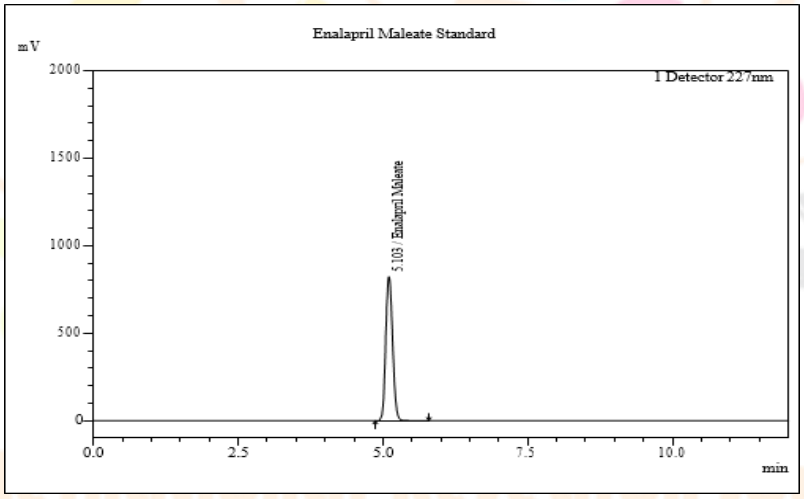
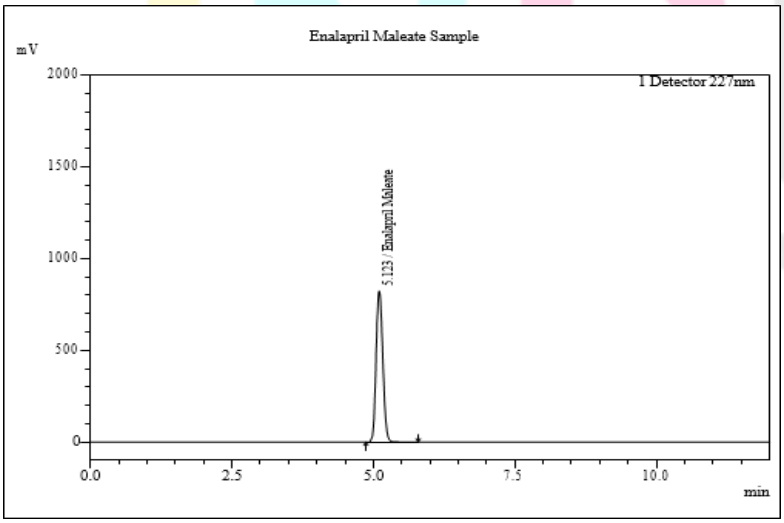


Fig. No. 8 typical chromatogram of Enalapril Maleate Standard [Concentration40ug/ml



Fi Fig. No. 9 typical chromatogram of Enalapril Maleate Sample [Concentration40ug/ml]

Sensitivity:

The sensitivity of measurement of Enalapril Maleate by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ were calculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level, while ten times the noise value gave the LOQ. LOD and LOQ were foundto be 0.006526 and 0.02648 respectively.

Precision:

Demonstration of precision was done under two categories. The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Enalapril Maleate and the % RSD of the replicate injections was calculated. In addition,to demonstrate the precision of method (Method Precision), six samples from the same batch of formulation were analysed individually and the assay content of each sample was estimated. The average for the six determinations was calculated along with the % RSD for the replicate determinations.

Both the system precision and method precision were subjected for inter-day and intra-day variations as reported in Table.

Table No. 15: Intraday Precision of Enalapril Maleate at 227

Concentration	Peak Area		
	0min	1 hr	2hr
40	461020	469020	461020
40	468990	465090	456991
40	465990	460990	465990
40	460990	460590	468994
40	460990	460090	459966
40	462990	459990	468996
Mean	466829	464295	463662
SD	42209.96	44659.32	50432.94
RSD	0.90	0.96	1.09

Table No.16: Interday Precision of Enalapril Maleate at 227

Concentration	Peak Area		
	1 day	2 day	3 day
40	461020	469593	468686
40	468990	466132	466954
40	465990	469018	465420
40	460990	469018	469358
40	460990	456293	466232
40	462990	469596	469846
Mean	466829	466942	462933
SD	42209.96	48133.00	49568.93
RSD	0.90	1.03	1.05

Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Enalapril Maleate (20 µg/ml) were spiked with 80, 100, and 120 % extra Enalapril Maleate standard and themixtures were analyzed by the proposed method.

Standard deviation of the % recovery and % RSD were calculated and reported in Table No. 6.6.

Table No. 17 Accuracy of Enalapril Maleate at 227 nm.

Sr. No.	Concentration	Peak Area	recovery%
1	80	460991	100.08
2	80	466990	100.42
3	80	460981	100.03
4	100	563414	99.96
5	100	563638	100.44
6	100	563632	100.03
6	120	666466	100.06
8	120	666486	100.33
9	120	665984	99.93

Robustness:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase composition (Methanol: Water and Methanol: buffer) and mobile phase flow rate by 1.0 ml/min (0.9 and 1.1 ml/min) had no significant effect on the retention time and chromatographic response of the 20 µg/ml solution, indicating that the method was robust. The results are shown in Table No. 6.8.

Table No. 18 Robustness of Enalapril Maleate at 227 nm

Concentration	Peak Area	
	Methanol: Buffer	Methanol: Water
40	461020	148699
40	468990	145348
40	465990	146248
40	460990	145348
40	460991	148652
40	462992	144236
Mean	466829	146605
SD	42209.96	19065.91
RSD	0.90	1.30

The contents of the thesis have been divided into eight chapters and appropriatereferences have been placed after the 9th chapter. Spectrophotometric method was developed for the estimation of Enalapril Maleate in Pharmaceutical Formulation by QbD approach.

- Designed of Experiment by Design expert software.
- Optimized and Developed method for Spectrophotometry.
- Spectrophotometric method was validated for Linearity, Accuracy, Interday&Intraday Precision, Specificity & Selectivity, Sensitivity and Robustness.
- Designed of Experiment by Design expert software.
- Optimized and Developed method for Chromatography.

- Chromatographic method was validated for Linearity, Accuracy, Interday&Intraday Precision, Specificity & Selectivity, Sensitivity and Robustness.

All the developed methods were successfully applied to determine the drugs inPharmaceutical preparation.

Conclusion

Designing techniques able to accurately and precisely analyse a large number of samples in a short amount of time is always important for regular analytical purposes. The Indian Pharmacopoeia officially recognizes Enalapril maleate. There aren't many analytical techniques for determining Enalapril maleate in the literature. These include UV-visible spectrophotometric methods, HPLC, and HPTLC techniques. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical. In the present investigation HPLC method (Using Quality by Design) for the quantitative estimation of Enalapril Maleate in bulk drug and per ICH guidelines pharmaceutical formulations has been developed.HPLC methods were validated as and results of linearity, precision, accuracy, Specificity,System suitability and robustness pass the limit. The HPLC method is more sensitive, accurate and precise compared to the previously reported method. There was no any interference of excipients in the recovery study. The molar extinction coefficient (L mol-1 cm-1) and %RSD values were low, indicating the sensitivity of the established approaches. The accuracy, precision, and robustness of the suggested high-performance liquid chromatographic approach have also been assessed, and it has shown to be practical and efficient for the quality control of Enalapril maleate. The developed method for Enalapril Maleate quality control was proven to be straightforward and economical. Furthermore, the reduced solvent usage results in an economical and ecologically sustainable spectroscopy process. As a result, the suggested methodology is quick, accurate, needs little effort in sample preparation, and is a good approach for Enalapril maleate.

References

1. Khopkar SM. Basic Concept of Analytical Chemistry. 2nd ed. New Delhi: New Age International Ltd. Publishers; 1998. p. 1, 178-179.
2. Christen GD. Analytical Chemistry. 5th ed. John Wiley and Sons; 2003. p. 35-42, 131-132.
3. Skoog DA, Holler FJ, Crouch SR. Principle of Instrumental Analysis. 5th ed. Thomson Brooks/Cole.; 2004. p. 1-2, 678-688.
4. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. Part-2. 4th ed. New Delhi: CBS Publishers and Distributors; 2002. p. 275-325.
5. Chatwal GR, Sharma AK. Instrumental Methods of Chemical Analysis. 5thed. Mumbai: Himalaya Publishing House; 2002. p. 2.149-2.184.
6. Instruction Manual Pharmaspec, UV 1601 Series, Operation Guide, ShimadzuSpectrophotometer. Japan: Shimadzu Corporation Koyoto; 2001. p. 5.16, 8.2.
7. Swarbrick J, Boylan JC. Encyclopedia of Pharmaceutical Technology. Vol-1. 3rd ed. New York: Marcel Dekker Inc.; 1997. p. 538-540.
8. Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis. 6th ed., Thomson Brooks/Cole.; 2007. p. 762-63,816-827.

9. Dong MW. Modern HPLC for practicing scientists. New Jersey: John Wiley & Sons, Inc., Publication, Hoboken; 2006.p. 1-9.

10. Sethi PD. High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations. 1st ed. New Delhi: CBS Publishers and Distributors; 2001.p. 3-11, 116-120.

11. Weston A, Brown PR. High Performance Liquid Chromatography, and CE – Principles and practice. 1st ed. California: Academic Press; 1997. p. 1-4, 8-11, 24-26, 29-32, 71.

12. Willard HH, Meritt LL, Dean JA, Settle F. Instrumental Method of Analysis, 7th ed. Delhi: CBS Publishers and Distributors; 2001.p.14.

13. Swadesh JK. HPLC Practical and Industrial Applications. 2nd ed. USA: CRC Press; 2000. p. 141-143.

14. ICH, (Q2A), Harmonized Tripartite Guideline, Text on Validation of Analytical Procedures. IFPMA. Geneva; 1994. p. 1-5.

15. ICH, (Q2B), Harmonized Tripartite Guideline, Validation of Analytical Procedures:Methodology, Text and Methodology, IFPMA. Geneva; 1996. p. 1-8.

16. The United state Pharmacopoeia, The National formulary, Asian ed. USA: published byUnited state pharmacopoeial convention; 2003. p. 960.

17. Nash RA, Wachter AH. Pharmaceutical process validation. 3rd ed. New York: MarcelDekker Inc.; 2003. p. 1-10.

18. Gennaro AR. Remington: The Science and Practice of Pharmacy. 20th ed. vol-1.Philadelphia: Lippincott Williams and Wilkin; 2000. Page no.587.

19. Anjeneyulu Y, Chandrasekhar K, Manikam V. A Textbook of Analytical Chemistry.Pharmamed Press BSP Books; 2008.p.1-2.

20. Somenath M. Sample Preparation Techniques in Analytical Chemistry. 1st ed. New York: Wiley Interscience; 2003. p.13-16.

21. Raynord P. Liquid Chromatography for the Analyst. New York: Marcel Dekker Inc; 1994.p.2.

22. Bahadur KV, Thapa C, Ghimire P, Chaudhari P, Yadhav J. Formulation and optimization of enalapril maleate-loaded floating microsphere using box–behnken design in vitro study. J Appl Pharm Sci. 2020;10:095-104.

23. Verma S, Nagpal K, Singh SK, Mishra DN. Unfolding type gastroretentive film of cinnarizine based on ethyl cellulose and hydroxypropylmethylcellulose. Int J Biol Macromol. 2014;64:347-52. doi: 10.1016/j.ijbiomac.2013.12.030, PMID 24370473.

24. Abbas Z, Marihal S. Gellan gum-based mucoadhesive microspheres of almotriptan for nasal administration: formulation optimization using factorial design, characterization, and in vitro evaluation. J Pharm Bioallied Sci. 2014;6(4):267-77. doi: 10.4103/0975-7406.142959, PMID 25400410.

25. Semalty M, Verma D, Semalty A. Development evaluation of microspheres of verapamil hydrochloride. Ind Drugs. 2011;48:34-8.

26. Alagusundaram M, Chetty MS, Umashankari K, Badarinath AV, Lavanya C, Ramkanth S. Microspheres as a novel drug delivery system: a review. Int J Chem Tech Res. 2009;1:526-34.

27. Swain S, Patro CN, Sruti J, Rao MEB. Design evaluation of sustained release solid dispersions of verapamil

hydrochloride. Int J Pharm Sci Nanotechnol. 2011;3:1252-62.

28. Wang W, Zhang J, Yang S, Zhang H, Yang H, Yue G. Experimental study on the angle of repose of pulverized coal. Particuology. 2010;8(5):482-5. doi: 10.1016/j.partic.2010.07.008.

29. Subham B, Gaurav C, Dilipkumar P, Gosh AK. Investigation on crosslinking density for development of novel interpenetrating polymer network (IPN) based formulation. J Sci Ind Res. 2010;69:777-84.

30. Hemraj Ramteke K, Balaji Jadhav V, Kulkarni NS, Kharat AR, Diwate SB. Preparation, evaluation and optimization of multi particulate system of mebendazole for colon-targeted drug delivery by using natural polysaccharides. Adv Pharm Bull. 2015;5(3):361-71. doi: 10.15171/apb.2015.050, PMID 26504758.

31. Bhardwaj P, Chaurasia D, Singh R, Swarup A. Development and characterization of novel site-specific hollow floating microspheres bearing 5-Fu for stomach targeting. Scientific World Journal. 2014;2014:705259. doi: 10.1155/2014/705259. PMID 25383377.

32. Farooq U, Khan S, Nawaz S, Ranjha NM, Haider MS, Khan MM. Enhanced gastric retention and drug release via development of novel floating microspheres based on Eudragit E100 and polycaprolactone: synthesis and in vitro evaluation. Des Monomers Polym. 2017;20(1):419-33. doi: 10.1080/15685551.2017.1326702, PMID 29491813.

33. Beringhs AO, Fonseca ABDS, De Campos AM, Sonaglio D. Association of PLGA microspheres to carrier pellets by fluid bed coating: a novel approach towards improving the flowability of microparticles. J Pharm (Cairo). 2018;2018:3874348. doi: 10.1155/2018/3874348. PMID 30057848.