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Method Development and Validation for Simultaneous Estimation of Nifedipine and Triamterene by Using RP-HPLC in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the Nifedipine and Triamterene in Tablet dosage form. Chromatogram was run through Inertsil-ODS C18, 250x 4.6 mm, 5µ. Mobile phase containing Methanol and Water in the ratio of 80:20 was pumped through column at a flow rate of 1ml/min. Optimized wavelength for Nifedipine and Triamterene was 238nm. Retention time of Nifedipine & Triamterene were found to be 2.955min and 3.538 min. %RSD of the Nifedipine and Triamterene were and found to be 0.27 and 0.21 respectively. %assay was obtained as 99.24% and 99.82% for Nifedipine and Triamterene respectively. LOD, LOQ values are obtained from regression equations of Nifedipine & Triamterene were 0.08ppm, 0.25ppm and 0.03ppm, 0.08ppm respectively. Regression equation of Nifedipine is y= 10831x - 34273, and y = 21030x + 31232 of Triamterene.

Keywords: Nifedipine, Triamterene, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Nifedipine is belongs to the class of organic compound known as dihydro pyridine carboxylic acids and derivative [1,2,3]. These are compounds containing a dihydro pyridine moiety bearing a carboxylic acid group. Nifedipine is chemically named as 3,5-di methyl 2,6-di methyl-4-(2-nitro phenyl)-1,4-di hydro pyridine-3,5-dicarboxylate[4,5].

Trimterene is a class of organic compound known as pteridines and derivatives. These are polycyclic aromatic compounds containing a pteridine moieties [6,7]. Which consist of a pyrimidine fused toa pyrazine ring to form pyrimido (4,5-b) pyrazine. Triamterene is chemically named as 6-phenylpteridine-2,4,7-triamine[8,9].

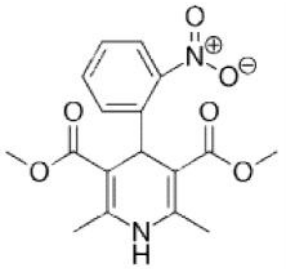

Figure 1: Structure of Nifedipine

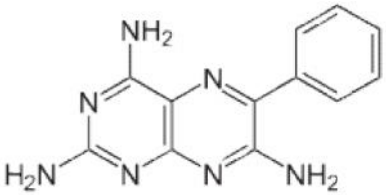

Figure 2: Structure of Triamterene

2. Materials and Methods

Materials:

Nifedipine and Triamterene , Combination Nifedipine and Triamterene tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc[10].

Instrument: HPLC instrument used was of WATERS HPLC 2695 system with Auto Injector and PDA Detector. Software used Empower 2. UV-VIS spectrophotometer Systrnonics Instruments and matched quartz was be used for measuring absorbance for Nifedipine & Triamterene solutions.

Methods:

Standard Preparation: Accurately Weighed and transferred 250mg of NIFEDIPINE and 10mg TRIAMTERENE of working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml[11].

Sample Preparation:

For analysis of commercial formulation, 20 tablets of Nifedipine 500mg and Triamterene 2.5mg were weighed the average weight was calculated and powdered. A quantity equivalent to 500mg of Nifedipine and 2.5mg of Triamterene was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins.The solution was allowed to stand at a room temperature for 20-30mins and filterd it through a whatmann filter paper.

Linearity:

Linearity solutions are prepared such that 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml,0.8ml from the Stock solutions Nifedipine and Triamterene are taken in to 7 different volumetric flasks and diluted to 10ml with diluents to get 20ppm, 30ppm, 40ppm, 50ppm, 60ppm, 70ppm,80ppm of Nifedipine and 2.5ppm, 5ppm, 10ppm, 15ppm, 20ppm, 25ppm,30ppm of Triamterene [12,13].

Accuracy:

Standard Preparation: Accurately Weighed and transferred 250mg of Nifedipine and 10mg Triamterene of working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml[14,15].

Preparation of 50% Spiked Solution:

75mg of drug was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents [16,17].

Preparation of 100% Spiked Solution:

150 mg of drug was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 100% Sample stock solution [18]. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents [19].

Preparation of 150% Spiked Solution:

215mg of drug was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 150% Sample stock solution [20,21]. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents [22,23].

Method Development

Many trials were done by changing columns and Mobile phases and were reported below.

Trial: 1

Column Used : Inertsil - C18, BDS 250 x 4.6 mm, 5µ.
Mobile phase : Methanol: Water (45:55)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

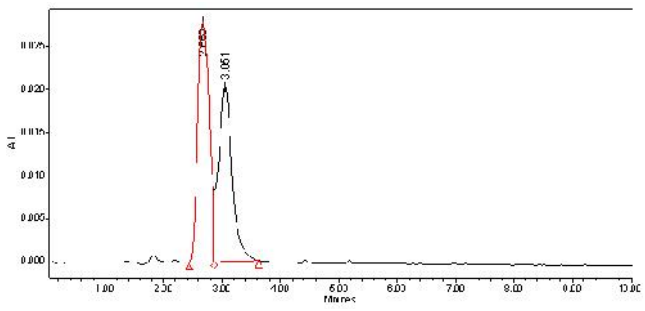

Figure 3: Trial chromatogram 1

Observation: peak shapes were not good.

Trial: 2

Column Used : Kromasil 150 x 4.6 mm, 5µ.
Mobile phase : Buffer (Kh2po4): Acetonitrile (40:60)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

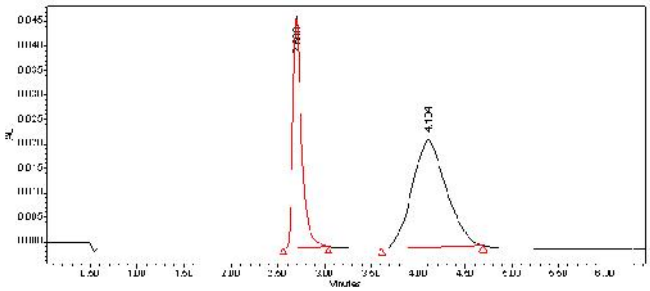

Figure 4: Trial chromatogram 2

Observation: The two peaks are separated completely but peak shapes are not good.

Trial: 3

Column Used : KROMOSIL 150 x 4.6 mm, 5µ.
Mobile phase : Buffer: Acetonitrile (85:15)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

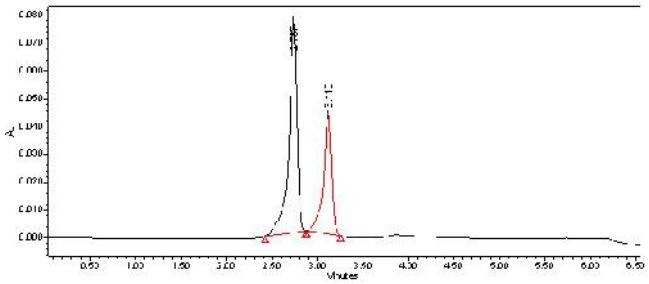

Figure 5: Trial chromatogram 3

Observation: Triamterene tailing was not passed so further trials are carried out.

Trial: 4

Column Used : BDS 150 x 4.6 mm, 5µ.
Mobile phase : Buffer: Acetonitrile (35:65)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

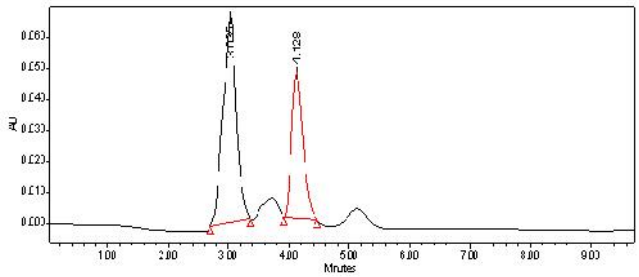

Figure 6: Trial chromatogram 4

Observation: Nifedipine, Triamterene peaks were sharp, but extra peak was observed, so further tail is carried out.

Trial: 5

Column Used : BDS 150 x 4.6 mm, 5µ.
Mobile phase : Buffer: Acetonitrile (40:60)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

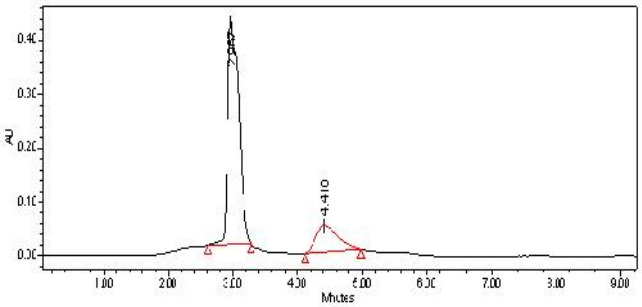

Figure 7: Trial chromatogram 5

Observation: peak shapes are not good.

Optimized Method:

Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Inertsil -ODS C18 (250 x 4.6 mm, 5 µ)
Mobile phase : Methanol: Water (80:20)
Flow rate : 1ml/min
Wavelength : 238nm
Temperature : Ambient
Injection Volume : 20µl

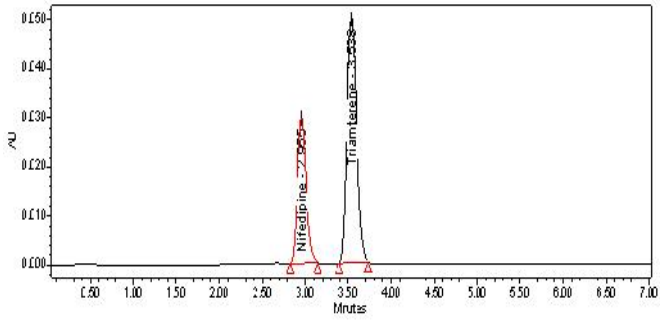

Figure 8: Optimized chromatogram of Nifedipine and Triamterene

Observation: peak shape and retention time is good.

3. Results and Discussion

Method Validation:

3.1 Precision:

Intraday precision (Repeatability):

Intraday Precision was performed and % RSD for Nifedipine and Triamterene were found to be 0.27% and 0.21% respectively.

Table 1: Inter day precision results for Nifedipine and Triamterene

Sr. No	Nifedipine	Triamterene
1	805783	1152293
2	801690	1146923
3	801496	1147283
4	806432	1152490
5	797564	1139272
6	801496	1147283
Mean	801593	1147591
Std. Dev.	3262.714	4815.615
%RSD	0.406614	0.419628

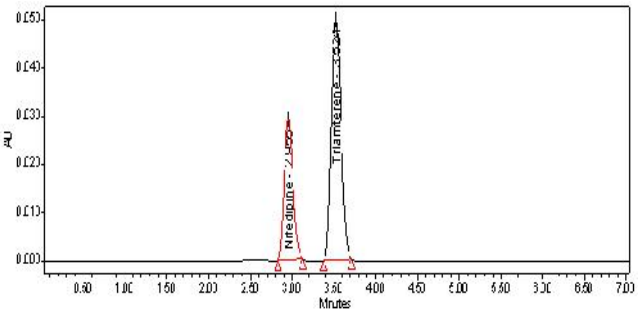

Figure 9: Repeatability Chromatogram of Nifedipine and Triamterene

Inter day precision:

Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Nifedipine and Triamterene were 0.40% and 0.42%.

Table 2: Inter day precision results for Nifedipine and Triamterene

Sr. No	Nifedipine	Triamterene
1	805783	1152293
2	801690	1146923
3	801496	1147283
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Mean	801593	1147591
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%RSD	0.406614	0.419628

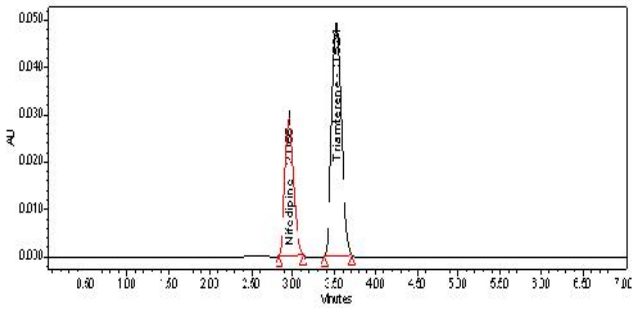

Figure 10: Inter Day precision Chromatogram of Nifedipine and Triamterene

3.2 Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 3.

Table 3: Table of Accuracy

Sample	Conc. (%) (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Nifedipine	50	49.79	100.81	0.93
	100	100.11	99.70	0.53
	150	150.03	100.21	0.28
Triamterene	20	20.01	100.22	0.59
	40	39.98	100.02	0.054
	60	59.88	99.98	0.08

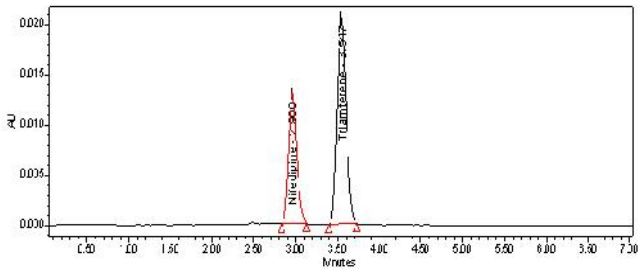

Figure 11: Accuracy 50% Chromatogram of Nifedipine and Triamterene

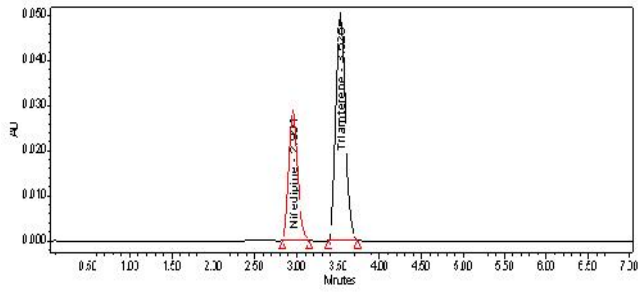

Figure 12: Accuracy 100% Chromatogram of Nifedipine and Triamterene

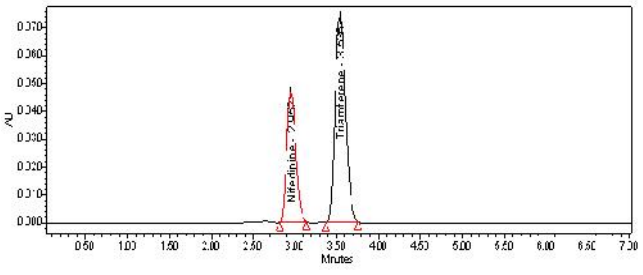

Figure 13: Accuracy 150% Chromatogram of Nifedipine and Triamterene

3.3 Linearity:

Six Linear concentrations of Nifedipine (20-80ppm) and Triamterene (2.5-30ppm) are prepared and injected. Regression equation of the Nifedipine and Triamterene are found to be, y = 20193x + 1902, and y = 31282x + 11218. And regression co-efficient was 0.999.

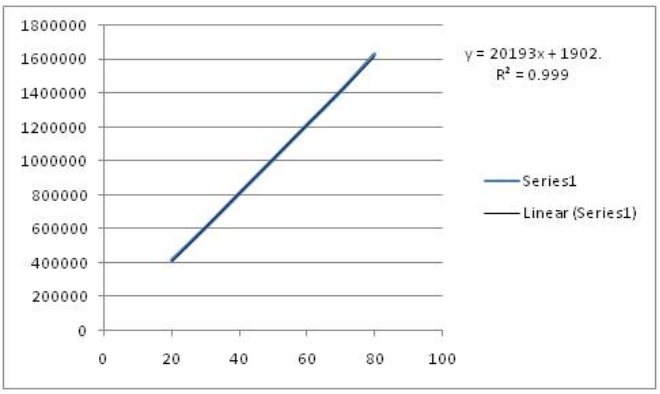

Figure 14: Calibration curve of Nifedipine

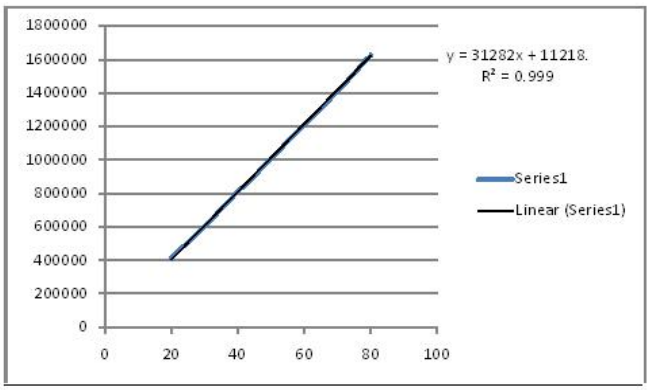

Figure 15: Calibration curve of Triamterene

Table 4: Calibration data of Nifedipine and Triamterene method

S.N	Conc. Nifedipine (µg/ml)	Response	Conc. Triamterene (µg/ml)	Response
1.	0	0	0	0
2.	20	412977	2.5	523467
3.	30	605369	5	829544
4.	40	807564	10	1139272
5.	50	1007428	15	1448018
6.	60	1210925	20	1728926
7.	70	1409560	25	2089505
8.	80	1627087	30	2407574

4. Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Nifedipine and Triamterene in Tablet dosage form. Retention time of Nifedipine and Triamterene were found to be 2.764min and 3.162 min. %RSD of the Nifedipine and Triamterene were and found to be 0.507 and 0.51 respectively. %assay was obtained as 99.24% and 99.82% for Nifedipine and Triamterene respectively. LOD, LOQ values are obtained from regression equations of Nifedipine and Triamterene were 0.07ppm, 0.20ppm and 0.03ppm, 0.10ppm respectively. Regression equation of Nifedipine is y = 10831x - 34273, and y=21030x + 31232 of Triamterene. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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