A
SYNOPSIS OF THE THESIS ENTITLED

DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS FOR ESTIMATION OF SOME ANTIHYPERTENSIVE AGENTS AND THEIR COMBINED DOSAGE FORMS

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RESEARCH GUIDE
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INTRODUCTION \[^{[1-3]}\]:

Hypertension is an abnormal increase in diastolic and/or systolic pressure. 1 billion people worldwide and is a leading cause of mortality. About 20% of people of world are hypertensive, and one-third of are not even aware they are hypertensive. Therefore, this disease is sometimes called the "silent killer." A condition present when blood flows through the blood vessels with a force greater than normal. Hypertension can strain the heart, damage blood vessels, and increase the risk of heart attack, stroke and kidney problems and cause death.

The newer Antihypertensive drugs and their combinations are approved by FDA. These newer drugs and their combinations are not official in any pharmacopeias and take more time to include in pharmacopoeia.

In order to overcome analytical difficulties for newer antihypertensive drugs, it is required to develop and validate newer analytical methods for simultaneous estimation drugs in pharmaceutical formulations.

Elevated blood pressure develops gradually over many years usually without a specific identifiable cause. However, possible medical causes, such as medications, kidney disease, adrenal problems or thyroid problems, must first be excluded. High blood pressure that develops over time without a specific cause is considered benign or essential hypertension. Blood pressure also tends to increase as a person ages. A primary risk factor for prehypertension is being overweight. Other risk factors include a sedentary lifestyle, eating high sodium foods, smoking and excessive alcohol intake. Blood pressure levels appear to be familial, but there is no clear genetic pattern.
2. AIM OF PRESENT WORK:
To develop and validate of analytical methods for simultaneous estimation of anti-hypertensive drugs in pharmaceutical dosage form.

- There are numbers of newer antihypertensive drugs and their formulations are approved by FDA which is either new molecule or partial modification of existing molecule.
- These newer drugs and their combinations are not official in any pharmacopeias and are take more time to include in pharmacopeias.
- Because of continuous and longer use of these drugs individually newer side effects, toxicity, resistances are observed.

So present investigation was undertaken with a view to develop and validate new analytical methods for simultaneous estimation of
1. Olmesartan medoxomil (20 mg) and Indapamide (1.5 mg) tablets
2. Olmesartan medoxomil (20 mg) and Chlorthalidone (12.5 mg) tablets
3. Metoprolol succinate (50 mg) and Chlorthalidone (12.5 mg) tablets
4. Nebivolol Hydrochloride (5 mg) and Chlorthalidone (12.5 mg) tablets
5. Rosuvastatin calcium (10 mg) and Hydrochloorthiazide (12.5 mg) tablets

OBJECTIVES
- To develop RP-HPLC methods for simultaneous estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorothiazide, Metoprolol succinate and Chlorthalidone in combination drug products.
- To develop UV or HPTLC methods (wherever UV method is not feasible) for simultaneous estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorothiazide, Metoprolol succinate and Chlorthalidone in combination drug products.
- All developed methods to be validated for specificity, linearity, accuracy, repeatability (precision), ruggedness, limit of detection and limit of quantification, robustness and system suitability.
- To perform statistical comparison of developed methods.
3. Experimental work

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Simultaneous estimation of Olmesartan medoxomil and Indapamide from bulk and commercial products using a validated reverse phase high Performance liquid chromatographic technique[^4].

A simple, rapid, accurate, sensitive, precise and economical reverse phase high performance liquid chromatographic (RP-HPLC) method is developed and validated (as per ICH guidelines) for simultaneous separation and quantification of two anti-hypertensive drugs Olmesartan Medoxomil and Indapamide. The separation of both the drugs was achieved on ACE C\textsubscript{18} AR column (250 x 4.6 mm, 5 \( \mu \)m) column using a mobile phase of Sodium Perchlorate and triethylamine buffer solution (at pH 3): Acetonitrile (60:40 v/v). The flow rate was 1 ml/min and detection was done at 280 nm. The retention time for Indapamide was 5.3 min and for Olmesartan Medoxomil was 6.8 min. Olmesartan medoxomil and Indapamide showed a linear response in the concentration range of 50-300 \( \mu \)g/ml and 3.75 - 22.5 \( \mu \)g/ml, respectively. The correlation co-efficient ('r' value) for Olmesartan Medoxomil and Indapamide was 0.9999 and 0.9998, respectively. The percentage recoveries obtained for Olmesartan medoxomil and Indapamide ranges from 99.3% to 100% and 99% to 100.7%, respectively. The results of analysis have been validated as per ICH guidelines. The extremely low flow rate, simple mobile phase composition makes this method cost effective, rapid and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Simultaneous estimation of Olmesartan medoxomil and Chlorthalidone from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique[^5-6].

A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromatographic method is developed and validated (as per ICH guidelines) for simultaneous separation and quantification of two anti-hypertensive drugs, Chlorthalidone and Olmesartan medoxomil. The separation of both the drugs was achieved on Inertsil ODS 3V C\textsubscript{18} column (250 x 4.6 mm id, 5 \( \mu \)m particle size) column using a mobile phase of sodium dihydrogen phosphate buffer solution (at pH 3): acetonitrile (48:52 v/v). The flow rate was 1 ml/min and detection was done at 270 nm. The retention time for Chlorthalidone was 3.65 min and Olmesartan medoxomil was 6.23 min. Chlorthalidone and Olmesartan medoxomil showed a linear response in the
concentration range of 6.25-37.50 μg/ml and 10-60 μg/ml, respectively. The correlation co-
efficients for Chlorthalidone and Olmesartan medoxomil were 0.9999 and 0.9998, respectively. 
The percentage recoveries obtained for Chlorthalidone and Olmesartan medoxomil ranges from 
99.5% to 100.1% and 99.1% to 100.4%, respectively.

Simultaneous estimation of Chlorthalidone and Nebivolol hydrochloride from bulk and 
commercial products using a validated reverse phase high performance liquid 
chromatographic technique [7-10].

A simple, rapid, accurate, precise and economical reverse phase high performance liquid 
chromatographic method is developed and validated (as per ICH guidelines) for simultaneous 
separation and quantification of two anti-hypertensive drugs, Chlorthalidone and Nebivolol 
hydrochloride. The separation of both the drugs was achieved on zorbax eclipse XDB C8 column 
(250 x 4.6 mm id, 5 μm particle size) column using a mobile phase of Potassium dihydrogen 
phosphate and triethylamine buffer solution (at pH 3): acetonitrile (65:35 v/v). The flow rate was 
1 ml/min and detection was done at 280 nm. Results: The retention time for chlorthalidone was 
4.36 min and Nebivolol hydrochloride was 9.68 min. Chlorthalidone and Nebivolol 
hydrochloride showed a linear response in the concentration range of 31.25-187.5 μg/ml and 
12.5 - 75 μg/ml respectively. The correlation co-efficients for Chlorthalidone and Nebivolol 
hydrochloride were 0.9998 and 0.9999 respectively. The percentage recoveries obtained for 
Chlorthalidone and Nebivolol hydrochloride ranges from 99.4% to 100.5% and 99.4% to 99.8% 
respectively. Validation results indicated that method shows satisfactory results.

Simultaneous estimation of Metoprolol succinate and Chlorthalidone in pharmaceutical 
solid dosage form by using a developed and validated reverse phase high performance liquid 
chromatographic technique [11-13].

The separation of both the drugs was achieved on Inertsil ODS 3 column (100 x 4.6 mm, 5 μm) 
column using a mobile phase of diammonium hydrogen phosphate buffer solution (at pH 5.5): 
Methanol (70:30 v/v). The flow rate was 1.0 ml/min and detection was done at 254 nm. The 
retention time for Metoprolol succinate was 6.91 min and Chlorthalidone was 9.94 min. 
Metoprolol succinate and Chlorthalidone showed a linear response in the concentration range of 
50- 300 μg/ml and 12.5 - 75 μg/ml respectively. The correlation co-efficients for Metoprolol 
succinate and chlorthalidone were 0.9999 Metoprolol succinate and Chlorthalidone and 0.9998,
respectively. The percentage recoveries obtained for ranges from 98.9% to 100.6% and 98.5% to 99.2% respectively. The results of analysis have been validated as per ICH guidelines. Validation results indicated that method shows satisfactory linearity, accuracy, precision and ruggedness.

**Simultaneous estimation of Rosuvastatin calcium and Hydrochlorothiazide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique** [14-15].

A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromatographic (RP-HPLC) method is developed and validated (as per ICH guidelines) for simultaneous separation and quantification of two cardiovascular drugs, viz., Rosuvastatin calcium and Hydrochlorothiazide. The separation of both the drugs was achieved on ACE C18 AR column (250 x 4.6 mm, 5 µm) column using a mobile phase of sodium perchlorate buffer solution (at pH 3.0): Acetonitrile (60:40 v/v). The flow rate was 1 ml/min and detection was done at 280 nm. Results: The retention time for Hydrochlorothiazide was 3.9 min and for Rosuvastatin calcium was 10.3 min. Rosuvastatin calcium and Hydrochlorothiazide showed a linear response in the concentration range of 5-30 µg/ml and 6.25 - 37.5 µg/ml respectively. The correlation co-efficients for Rosuvastatin calcium and Hydrochlorothiazide were 0.9998 and 0.9999 respectively. The percentage recoveries obtained for Rosuvastatin calcium and Hydrochlorothiazide ranges from 99.3% to 100.4% and 99.2% to 100.4% respectively.

**UV spectrophotometric method**

**Simultaneous estimation of Olmesartan medoxomil and Indapamide by standard addition UV Spectrophotometric method in their combined dosage forms** [16-17]

A simple, accurate, precise, reproducible and economical UV spectroscopic method for simultaneous estimation of Olmesartan medoxomil and Indapamide in marketed formulation is developed and validated as per ICH guidelines. Method employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths 256 nm and 240 nm which are the λmax values of Olmesartan medoxomil and Indapamide in methanol. A predetermined concentration of Indapamide (6 g/ml) was added to standard and sample solution to obtain concentration of Indapamide in range of UV estimation and to decrease effect of low dose. This predetermined concentration is deducted from final results to get exact concentration
of drug. The linearity of method was investigated in range of 5-25 \( \mu g/ml \) for Olmesartan medoxomil and 5-25 \( \mu g/ml \) for Indapamide respectively. The results of analysis have been validated for linearity, accuracy and precision, ruggedness, solution stability, LOD and LOQ of the proposed method. This method can be successfully employed for simultaneous estimation of all drugs in commercial products.

**Simultaneous UV spectrophotometric method for simultaneous estimation of Olmesartan medoxomil and Chlorthalidone in their combined dosage forms**\(^{[18-19]}\).

A simple, accurate, precise, reproducible and economical UV spectroscopic method for simultaneous estimation of Chlorthalidone and Olmesartan medoxomil in marketed formulation is developed. Method employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths 220 nm and 254 nm which are the \( \lambda_{max} \) values of Chlorthalidone and Olmesartan medoxomil in methanol. The linearity of method was investigated in range of 5-25 \( \mu g/ml \) for Chlorthalidone and 5-25 \( \mu g/ml \) for Olmesartan medoxomil respectively. The results of analyses have been validated for linearity, accuracy and precision, ruggedness, LOD and LOQ of the proposed method. This method can be successfully employed for simultaneous estimation of all drugs in commercial products.

**Simultaneous UV spectrophotometric method for simultaneous estimation of Chlorthalidone and Nebivolol hydrochloride in their combined dosage forms**\(^{[20]}\).

A simple, accurate, precise, reproducible and economical UV spectroscopic method for simultaneous estimation of Chlorthalidone and Nebivolol Hydrochloride in marketed formulation is developed. Method employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths 226 nm and 282 nm which are the \( \lambda_{max} \) values of Chlorthalidone and Nebivolol hydrochloride in methanol. The linearity of method was investigated in range of 5-25 \( \mu g/ml \) for Chlorthalidone and 5-25 \( \mu g/ml \) for Nebivolol hydrochloride respectively. This method can be successfully employed for simultaneous estimation of all drugs in commercial products.
Simultaneous UV spectrophotometric method for simultaneous estimation of Rosuvastatin calcium and Hydrochlorothiazide in their combined dosage forms\textsuperscript{[21-24]}.

A simple, accurate, precise, reproducible and economical UV spectroscopic method for simultaneous estimation of Rosuvastatin calcium and Hydrochlorothiazide in marketed formulation is developed and validated (as per ICH guidelines). Method employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths 243 nm and 270 nm which are the λmax values of Rosuvastatin calcium and Hydrochlorothiazide in methanol. The linearity of method was investigated in range of 5-15 μg/ml for Rosuvastatin calcium and 6.25-18.75 μg/ml for Hydrochlorothiazide respectively. The results of analyses have been validated statistically for linearity, accuracy and precision, ruggedness, LOD and LOQ of the proposed method. This method can be successfully employed for simultaneous estimation of all drugs in commercial products.

**HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD\textsuperscript{[25-27]}**

Simultaneous estimation of Metoprolol succinate and Chlorthalidone in pharmaceutical solid dosage form by using a developed and validated high performance thin layer chromatographic technique

This work represents development and validation (as per ICH guidelines) of novel, accurate, precise, rugged, robust and reproducible high performance thin-layer chromatographic technique for the simultaneous estimation of Metoprolol succinate and Chlorthalidone from marketed formulation. High performance thin-layer chromatography was performed using HPTLC aluminium plates precoated silica gel plate 60 F\textsubscript{254} as stationary phase. The solvent system consisted of toluene: ethyl acetate: methanol: triethylamine (6: 1: 1: 0.6 v/v/v/v) as the mobile phase for the separation of Metoprolol succinate and Chlorthalidone at 230 nm. The method was found to be linear in the range of 500-7000 ng spot\textsuperscript{-1} for Metoprolol succinate and 125-1750 ng spot\textsuperscript{-1} for Chlorthalidone. R\textsubscript{f} value for Metoprolol succinate and Chlorthalidone is 0.53 and 0.24 respectively. The relative standard deviation (% RSD) values of the precision study were <2% which indicated that the developed method was precise; recovery was found to be 99.9% - 100.1% for Metoprolol succinate and 99.9% - 100.3% Chlorthalidone respectively. The extremely simple mobile phase composition makes this method cost effective, rapid and nontedious.
4. SUMMARY

- RP-HPLC methods for simultaneous estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorothiazide, Metoprolol succinate and Chlorthalidone in combination drug products were developed.

- UV spectroscopic methods for simultaneous estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorothiazide, in combination drug products were developed.

- HPTLC method for simultaneous estimation of Metoprolol succinate and Chlorthalidone in combination drug products was developed.

- All developed methods to be validated for specificity, linearity, accuracy, repeatability (precision), ruggedness, limit of detection and limit of quantification, robustness and system suitability.

- Statistical comparison of methods is performed.
5. CONCLUSION

Validated simultaneous RPHPLC method have been developed and successfully applied for estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorthiazide, Metoprolol succinate and Chlorthalidone in their combination drug products on HPLC with PDA detector and isocratic elution. Optimization of buffer solution pH, ratio of mobile phase concentration and use of proper organic phase in mobile phase to improve peak shape was a critical part of method. The shorter retention time cuts down cost of experiment. Good resolution between all three drugs was best key part of developed method. Specificity and selectivity of method was an added as set to method for routine analysis of drug samples in Analytical development laboratories and Quality control laboratories.

Validated simultaneous UV spectrophotometric methods have been developed and successfully applied for simultaneous estimation of Olmesartan medoxomil and Indapamide, Olmesartan medoxomil and Chlorthalidone, Nebivolol HCl and Chlorthalidone, Rosuvastatin calcium and Hydrochlorthiazide in their combination drug products using UV Spectrophotometer. Low interference in UV method gives precise and reproducible results.

Validated HPTLC analytical method has been developed for simultaneous estimation of Metoprolol succinate and Chlorthalidone in their combination drug products. The developed method is simple, precise specific and selective for simultaneous drug estimation. Data analysis proves that method is reproducible and accurate. The advantages of method are low cost of reagents, rapid analysis by method and excellent peak shapes. Developed HPTLC methods meet system suitability criteria, peak integrity and proper peak resolution. A low detection and quantitation limit of method proves high sensitivity of method. An acceptable % CV values confirms that method is precision of method.
6. REFERENCES


7. PUBLICATIONS


9. Avani Sheth, C N Patel, Nehal Shah, Ragin shah. Simultaneous estimation of Olmesartan medoxomil and Indapamide by standard addition UV Spectrophotometric method in their combined dosage forms, Indian drugs, ISSN No:0019-462X.

LIST OF ACCEPTED ARTICLES