STRUCTURED ABSTRACT

Simultaneous Estimation of Some Antihypertensive Drugs Using Spectroscopic and Chromatographic Methods

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Key Words: Simultaneous Estimation, Analytical Method Validation, RP-HPLC, RP-HPLC/MS, Anti-hypertensive drugs.

Background: In Pharmaceutical market, many newer Antihypertensive combinations drugs are available to control hypertension as well as to keep society healthy and stress free. All presently available old drugs have frequent dosing schedule and unpleasant side effects. So there arises need to develop simple, accurate, precise, cost effective, robust, rugged, and perfect analytical methods for new combinations of drugs. So, Development & Validation of various analytical methods for newer Antihypertensive drug combinations becomes a notable contribution to society as well as for Pharmaceutical companies.

Henceforth this particular research is of genuine use to Pharmaceutical Society.

Aim: The objective of present work was, to develop new RP-HPLC, UV-Spectroscopic and HPTLC methods for combination drug products. Validation of newly developed analytical methods should be Simple, Accurate, Precise, Selective, Specific, Reproducible, Highly sensitive Solvent stability according to ICH Q2R1 Guidelines.

Materials and Methods: In proposed work, drug substances used are Amlodipine Besylate, Indapamide, Olmesartan medoxomil and
Hydrochlorothiazide. The major Instruments that utilized are UV-1700 double beam UV-Visible spectrophotometer (Japan), HPLC system (sample-cooled model LC–2010CHT, Shimadzu Corporation, Japan) with UV detector and Class VP software version 2.31, Electronic analytical balance (Sartorius CD 2150, Gottinge Germany), pH meter (pH Tutor Range 0-14, Model 313927, Eutec Instruments), HPTLC system (Camag Linomat 5), Infrared Spectrophotometer (Spectrum GX FT-IR) and Melting point apparatus (Model: VMP – D). Absorption correction, first derivative spectrophotometry, and RP-HPLC were developed for Indapamide and Amlodipine Besylate in their combined tablet formulation. The simultaneous equation, first derivative spectrophotometry, and stability indicating RP-HPLC were developed for the estimation of Indapamide and Olmesartan Medoxomil in their combined formulation. HPTLC method for Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorothiazide in combined tablet formulation was developed. Force Degradation study of Indapamide and Olmesartan Medoxomil determined the drug peak and also for degradation products. Also, an isocratic LC method was developed for the separation of OLM in base degradation products, and degradation product was isolated. The structure of the major degradation products was predicted by MASS data, and it was also supported by IR and NMR.

**Results and Discussion:** In Both UV method for Amlodipine Besylate and Indapamide methanol was used as a solvent. The first method was absorption correction method. In this method wavelength used for determination was 256 nm and 360 nm for Indapamide and Amlodipine Besylate respectively. Linearity was observed in the range of 3–18 μg/mL for Indapamide and 10–60 μg/mL for Amlodipine Besylate. Assay value of was found to be 99.32 % and 101.34 % of labeled claim for Indapamide and Amlodipine Besylate respectively. Second method was derivative spectrophotometric. In this method wavelength used for determination was 237.4 nm and 241 nm for Indapamide and Amlodipine Besylate respectively. Linearity was observed in the range of 1.5–9 μg/mL for Indapamide and 5–30 μg/mL for Amlodipine Besylate. Assay value was found to be 99.72 % and 100.28 % of labeled claim for Indapamide and Amlodipine
Besylate respectively. RP–HPLC method was developed using Kromasil C–18 column (5 μm, 250 mm x 4.6 mm i.d) and ACN : 0.02M Na$_2$HPO$_4$ (70:30 v/v) pH was adjusted 7 with ortho-Phosphoric acid as the mobile phase. In this method, the linearity range was found between 40–120 μg/mL for Indapamide and 125–375 μg/mL for Amlodipine Besylate. Assay value of was found to be 98.44 % and 99.33 % of labeled claim for Indapamide and Amlodipine Besylate respectively. Two UV methods and stability indicating RP–HPLC method were developed for Olmesartan Medoxomil and Indapamide. In Both UV method methanol was used as a solvent. First method was simultaneous equation method. In this method wavelength used for determination was 240 nm and 256 nm for Indapamide and Olmesartan Medoxomil respectively. Linearity was observed in the range of 1–30 μg/mL for Indapamide and 10–70 μg/mL for Olmesartan Medoxomil. Assay value was found to be 99.33 % and 100.4 % of labeled claim for Indapamide and Olmesartan Medoxomil respectively. Second method was derivative spectrophotometric. In this method wavelength used for determination was 225.4 nm and 256.6 nm for Olmesartan Medoxomil and Indapamide respectively. Linearity was observed in the concentration range of 1–30 μg/mL for Indapamide and 10–70 μg/mL for Olmesartan Medoxomil. Assay value was found to be 98.66 % and 100.75 % of labeled claim for Indapamide and Olmesartan Medoxomil respectively. RP–HPLC method was developed using Phenomenex C–18 (250 mm x 4.6 mm i.d.) 5 μm column and Acetonitrile : 0.02 M Na$_2$HPO$_4$ (45:55 v/v) pH was adjusted to 7 with orthophosphoric acid as the mobile phase. Linearity range was found between 11.25–26.5 μg/mL for Indapamide and 150–350 μg/mL for Olmesartan Medoxomil. The retention time of Olmesartan Medoxomil & Indapamide were found to be around 4.79 min & 7.59 min respectively. Assay value was found to be 100.20 % and 99.93 % of labeled claim for Indapamide and Olmesartan Medoxomil respectively.

In forced degradation study of Olmesartan Medoxomil and Indapamide, Maximum degradation of OLM observed in base hydrolysis and maximum degradation of IND observed in oxidation followed by base degradation. The number of degraded products observed in OLM was I to IX and in IND A to J.
Degradation products of OLM under alkali conditions were studied. The structure of the degradation products of OLM in base also predicted based on the fragmentation pattern.

High performance thin layer chromatographic separation for Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorothiazide was performed on aluminium plates pre-coated with 0.2 mm layers of silica gel 60 F 254 (20 cm × 10 cm, E. Merck, Germany) and of n-Hexane: Dichloroethane: Methanol: GAA (4.5:3.5:1:1 v/v) as optimized mobile phase. The detection wavelength was 268 nm. $R_f$ values of 0.53 for Olmesartan Medoxomil, 0.36 for Amlodipine Besylate, 0.24 for Hydrochlorothiazide respectively.

**Conclusions:** All the developed methods were new and followed the ICH guidelines, so methods were found to be suitable for routine quantitative analysis.
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Selected Drug combination for research
- Amlodipine Besylate & Indapamide
- Olmesartan Medoxomil & Indapamide
- Amlodipine Besylate, Olmesartan Medoxomil & Hydrochlorothiazide

Analytical method development and validation
- UV method
  - First Derivative
  - Absorption Correction
- RP-HPLC method
- HPTLC method
  - Simultaneous Equation
  - First Derivative
- RP-HPLC method
  - Method Development
  - Forced Degradation
  - Characterization of Base Degradation of Olmesartan Medoxomil