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Development and Validation of Simultaneous Equation Method for Simultaneous Estimation of Amlodipine Besylate and Indapamide in Combined Dosage Form

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ABSTRACT

The manuscript describes validated simultaneous equation method for the simultaneous estimation of Amlodipine Besylate and Indapamide in combined dosage form. Simultaneous equation method was based on the estimation of both the drugs at two wavelengths i.e. absorption maxima of both drugs. An absorption maxima of Amlodipine besylate was 240 nm and absorption maxima of Indapamide was 215 nm in methanol. The linearity was obtained over the concentration range of 10 – 30 µg/mL for amlodipine besylate and 2 – 10 µg/mL for Indapamide with mean recovery of 99.45 ± 0.84 % and 99.46 ± 0.33 % for Amlodipine Besylate and Indapamide, respectively. These methods were found to be simple, sensitive, accurate, precise and economical and applicable for the simultaneous determination of Amlodipine Besylate and Indapamide in combined dosage form.

Keywords: Amlodipine Besylate, Indapamide, simultaneous equation method, absorption maxima.

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INTRODUCTION

Amlodipine (AMLO) is a dihydropyridine calcium antagonist and chemically it is 3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy) methyl]-4-(2-chloro 6- methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulphonate (Figure 1); $C_{20}H_{25}ClN_2O_5 \cdot C_6H_5SO_3H$.¹ Indapamide (INDA) is Indapamide is a non-thiazide sulphonamide diuretic drug and chemically it is 4-chloro-N(2-methyl-1-indolinyl)-3-sulfamoyl benzamide hemihydrates (Figure 1); $C_{16}H_{16}ClN_3O_3S \cdot$ ²

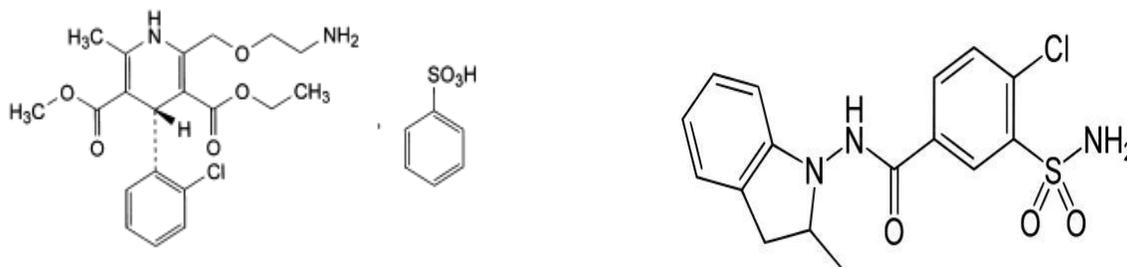


Figure 1: Structure of Amlodipine Besylate and Indapamide

Both the drugs are official in IP³, BP⁴ and USP⁵. Literature survey reveals that various methods like Spectrophotometric⁶⁻⁹, HPLC¹⁰⁻¹², Stability indicating method¹³⁻¹⁴ are reported for the estimation of AMLO. Literature survey also reveals various methods like Spectrophotometric¹⁵⁻¹⁶, HPLC¹⁷⁻¹⁸, LC-MS¹⁹ are reported for estimation of Indapamide. The present manuscript describes simple, sensitive, accurate, precise, reproducible and economical simultaneous equation method for the simultaneous estimation of AMLO and INDA in combined dosage form. The proposed method was validated according to ICH guideline. The method was found to be suitable for simultaneous and accurate determination of these drugs in tablet dosage forms without any interference from the excipients.

MATERIALS AND METHODS

Instrument & Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1800, Japan), attached to a computer software UV probe 3.2, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Analytical balance (Sartorius Balance), Corning volumetric flasks, pipettes of borosilicate glass were used in the study.

Reagents and Materials

Amlodipine Besylate (AMLO) and Indapamide (INDA) bulk powder was gifted by Reputed Pharmaceutical Industry, Gujarat (India), with 99.96% purity, The commercial fixed dose combination product containing 5 mg AMLO and 1.5 mg INDA was procured from the local

pharmacy, AR grade Methanol (S.D. Fine Chemical Ltd., Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England).

Preparation of Standard Stock Solutions

Accurately weighed AMLO (10 mg) and INDA (10 mg) was transferred to a separate 100 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard solutions having concentration AMLO (100 µg/mL) and INDA (100 µg/mL).

Determination of the Analytical Wavelengths

The standard solutions of AMLO (25 µg/mL) and INDA (8 µg/mL) were scanned separately in the UV range of 200-400 nm. Data were recorded at an interval of 1 nm. Overlain spectra show 240 nm as the λ_{\max} of AMLO and 215 nm as the λ_{\max} of INDA.

METHOD VALIDATION

Calibration curve

Calibration curves were plotted over a concentration range of 10 – 30 µg/mL for AMLO and 2 – 10 µg/mL for INDA. Accurately measured standard working solutions of AMLO (1.0, 1.5, 2.0, 2.5 and 3.0 mL) and INDA (0.2, 0.4, 0.6, 0.8 and 1.0 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with methanol, and the absorbance was measured at 215 nm and at 240 nm for both drug. The calibration curves were constructed by plotting absorbances Vs concentrations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of AMLO and INDA by the standard addition method. Known amounts of standard solutions of AMLO and INDA were added at 50, 100 and 150 % levels to prequantified sample solutions of AMLO and INDA (15 + 4.5 µg/mL respectively). The amounts of AMLO and INDA were estimated by using the equations.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of AMLO (20 µg/mL) and INDA (6 µg/mL) without changing the parameters for the simultaneous equation method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMLO (15, 20 and 25 µg/mL) and INDA (4, 6 and 8 µg/mL). The results were reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines²⁰.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where

σ = the standard deviation of the response ,

S = Slope of calibration curve.

ANALYSIS OF AMLO AND INDA IN COMBINED DOSAGE FORM

The absorbance of sample solution was measured at 215 nm and at 240 nm. These values were then equated in equation 1 and 2 mentioned in below section and the concentration of each drug was calculated.

RESULTS AND DISCUSSION

Simultaneous equation method depends on the property that, if a sample contains two absorbing drugs each of which absorbs at the λ_{max} of each other. In this method the absorbance was measured at two wavelengths i.e. λ_{max} of two components. For this measurement, the solutions of AMLO (25 $\mu\text{g/mL}$) and INDA (8 $\mu\text{g/mL}$) were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. From the overlain spectra of the two drugs (Figure 2) absorbances were measured at selected wavelength i.e. 240 nm λ_{max} of AMLO and 215 nm, λ_{max} of INDA. The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation; to obtain the concentration

$$C_X = (A_2 a_{Y1} - A_1 a_{Y2}) / (a_{X2} a_{Y1} - a_{X1} a_{Y2}) \quad (1)$$

$$C_Y = (A_1 a_{X2} - A_2 a_{X1}) / (a_{X2} a_{Y1} - a_{X1} a_{Y2}) \quad (2)$$

Where,

C_X and C_Y = The concentration of AMLO and INDA respectively.

A_1 = The absorbance of mixture at 215 nm

A_2 = The absorbance of mixture at 240 nm

a_{X1} = absorptivity of AMLO at 215 nm

a_{X2} = absorptivity of AMLO at 240 nm

a_{Y1} = absorptivity of INDA at 215 nm

a_{Y2} = absorptivity of INDA at 240 nm

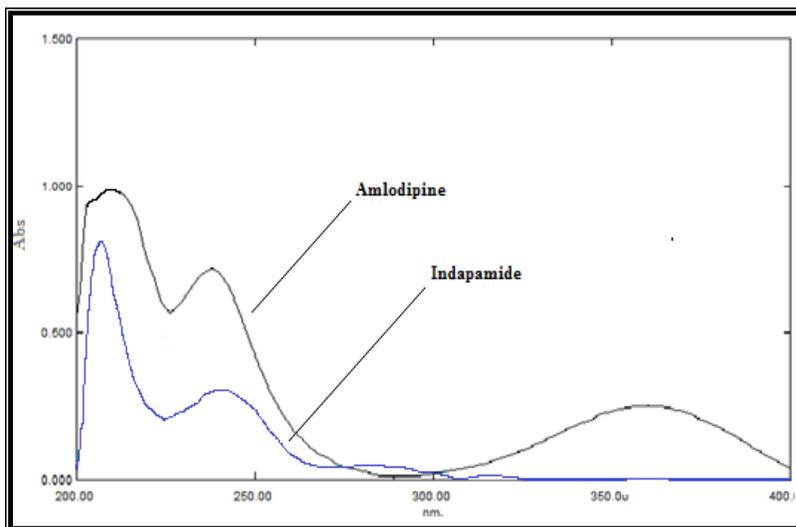


Figure 2: Overlain Absorption Spectra of Amlodipine Besylate and Indapamide from Standard Solution in Methanol

VALIDATION OF THE PROPOSED METHOD

Calibration curve

Linear correlation was obtained between absorbance versus concentrations of AMLO in the range of 10 – 30 $\mu\text{g/mL}$ and INDA in the ranges of 2 – 10 $\mu\text{g/mL}$. Regression parameters are mentioned in table 1 and the calibration curves of these two drugs at 215 nm and 240 nm were validated by the high value of correlation coefficients of regression (Table 1).

Table 1: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

| Parameters | AMLO | | INDA | |
|--|-----------------------|-----------------------|-----------------------|-----------------------|
| | 215 nm | 240 nm | 215 nm | 240 nm |
| Concentration range ($\mu\text{g/mL}$) | 10 – 30 | 10 – 30 | 2 – 10 | 2 – 10 |
| Sandell's sensitivity ($\mu\text{g/cm}^2$) | 2.24×10^{-2} | 2.76×10^{-2} | 9.60×10^{-3} | 1.75×10^{-2} |
| Slope | 0.048 | 0.043 | 0.103 | 0.054 |
| Intercept | 0.068 | 0.113 | 0.001 | 0.014 |
| Correlation coefficient (r) | 0.9990 | 0.9995 | 0.9980 | 0.9985 |
| LOD ($\mu\text{g/mL}$) | 0.51 | 0.15 | 0.12 | 0.13 |
| LOQ ($\mu\text{g/mL}$) | 1.56 | 0.73 | 0.35 | 0.40 |
| Repeatability (RSD, n=6), % | 0.55 | 0.31 | 0.60 | 0.55 |
| Precision (RSD), % | - | - | - | - |
| Interday (n = 6) | 0.59 – 1.24 | 0.39 – 0.86 | 0.60 – 0.99 | 0.87 – 1.38 |
| Intraday (n = 6) | 0.55 – 1.16 | 0.31 – 0.38 | 0.21 – 1.20 | 0.55 – 0.66 |

^a LOD = Limit of detection.

^b LOQ = Limit of quantification.

^c % RSD = Percent relative standard deviation.

Accuracy

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.45 ± 0.84 and 99.46 ± 0.33 % for AMLO and INDA, respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

Table 2: Recovery Data for the Proposed Method

| Drug | Level | Amount of sample taken ($\mu\text{g/mL}$) | Amount of standard spiked ($\mu\text{g/mL}$) | Mean % Recovery \pm SD* |
|------|-------|---|--|---------------------------|
| AMLO | 50 % | 15 | 7.5 | 100.34 ± 0.59 |
| | 100 % | 15 | 15 | 98.68 ± 0.65 |
| | 150 % | 15 | 22.5 | 99.34 ± 0.39 |
| INDA | 50 % | 4.5 | 2.25 | 99.50 ± 1.35 |
| | 100 % | 4.5 | 4.5 | 99.77 ± 1.37 |
| | 150 % | 4.5 | 6.75 | 99.12 ± 0.61 |

* Mean % Recovery \pm Standard Deviation of five observations

Method precision

The RSD values for AMLO and INDA were found to be 0.55 and 0.60 %, respectively at 215 nm and 0.31 and 0.55 % respectively at 240 nm (Table 1). Low value of relative standard deviation indicates that the proposed method is repeatable.

Intermediate precision

The low RSD values of interday (0.59 – 1.24 and 0.60 – 0.99 %) and intraday (0.55 – 1.16 and 0.21 – 1.20 %) variations for AMLO and INDA, respectively at 215 nm and interday (0.39 – 0.86 and 0.87 – 1.38 %) and intraday (0.31 – 0.38 and 0.55 - 0.66 %) variations for AMLO and INDA, respectively at 240 nm, reveal that the proposed method is precise (Table 1).

LOD and LOQ

LOD values for AMLO and INDA were found to be 0.51 and 0.12 $\mu\text{g/mL}$, respectively at 215 nm and 0.15 and 0.13 $\mu\text{g/mL}$ respectively at 240 nm and LOQ values for AMLO and INDA were found to be 1.56 and 0.35 $\mu\text{g/mL}$, respectively at 215 nm and 0.73 and 0.40 $\mu\text{g/mL}$ respectively at 240 nm. (Table 1). These data show that this method is sensitive for the determination of AMLO and INDA.

Assay of the Pharmaceutical Formulation

The proposed validated method was successfully applied to determine AMLO and INDA in their combined dosage form. The spectrum for AMLO and INDA in sample was recorded and is shown in Figure 3. The result obtained for AMLO and INDA was comparable with the

corresponding labeled amount (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine estimation of AMLO and INDA in pharmaceutical combined dosage form.

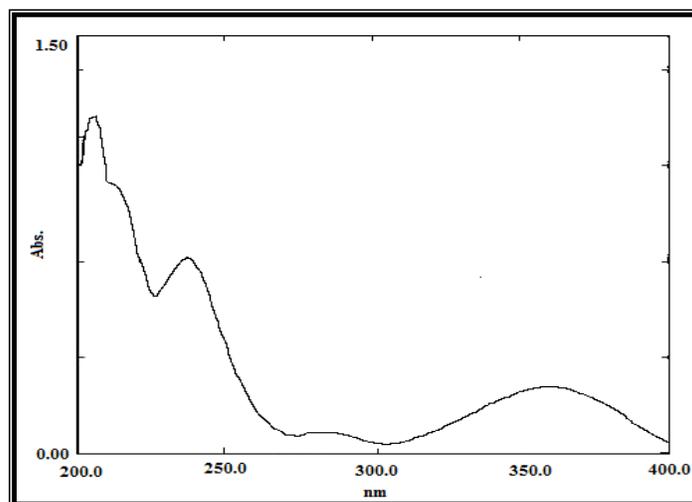


Figure 3: Absorption Spectra of the Sample Solution

Table 3: Assay Results for the Combined Dosage Form Using the Proposed Method

| Sample | Label Claim (mg/tab) | Amount Found \pm SD (n = 5) | % Label Claim \pm SD (n = 5) |
|--------|----------------------|----------------------------------|-----------------------------------|
| AMLO | 5 | 5.01 \pm 0.04 | 100.19 \pm 0.79 |
| INDA | 1.5 | 1.50 \pm 0.02 | 99.81 \pm 0.97 |

^aSD = Standard deviation.

^bn = Number of determinations.

CONCLUSION

In this proposed method, for AMLO the linearity is observed in the concentration range of 10 – 30 μ g/mL with co-efficient of correlation, (r) = 0.9990 and (r) = 0.9995, respectively at 215 nm and 240 nm. For INDA, the linearity is observed in the concentration range of 2 – 10 μ g/mL with co-efficient of correlation, (r) = 0.9980 and (r) = 0.9985, respectively at 215 nm and 240 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the AMLO and INDA in combined dosage form without any interference of the excipients.

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