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# High Performance Liquid Chromatographic Determination of Methyldopa in Bulk and Dosage forms

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**Abstract:** High performance chromatographic determination of methyldopa in bulk and tablet form was carried out on a $C_{18}$ column (250 x 4.6 mm with particle size of 5 micrometer) using a mixture of phosphate buffer pH 2.8 and acetonitrile (95: 5 v/v) as the mobile phase pumped at flow rate of 1 ml/min. Constructed calibration curve was linear in the concentration range (25-250 µg/ml) with correlation coefficient of 1.0000. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.91µg/ml and 2.77µg/ml, respectively. The accuracy and the precision of the developed method were very good (RSD < 2%). The validity of the proposed method was confirmed through the statistical comparison of the obtained data with those of the official USP method.

**Keywords**: Chromatography; HPLC; Methydopa; USP method.

## INTRODUCTION

Methyldopa **(Figure 1)**is an antihypertensive that is thought to have mainly a central action. It may also act as a false neurotransmitter, and have some inhibitory actions on plasma renin activity[1].Both the British Pharmacoepia (BP) [2] and the United States Pharamacoepia (USP) [3]employ spectrophotometric determination in the visible region for assay of methyldopain tablets after its reaction with ferrous iron in acidic medium.

Literature review revealed that chromatographic and spectrophotometric methodshave been developed for the determination of methyldopa in bulk and tablet dosage form[4-10]. In the present work we intend to adopt our earlier developedHPLC methodfor the determination of levodopa and carbidopa[11]tothe determination of methyldopa in bulk and tablet form.

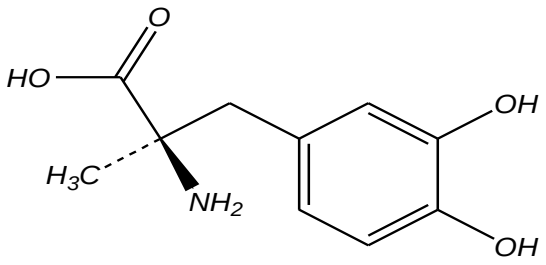


**Figure 1:** Chemical structure of methyldopa (2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid)

## MATERIALS AND METHODS

**Instruments and apparatus:** Experiments were performed on a Shimadzu Prominence HPLC system consisted of: degasser (Model DGU-20A3R), pump (Model LC-20AB), automatic injector (Model Sil-20A) and variable wavelength UV–VIS detector (ModelSPD-20AV). Chromatographic separations were carried out on an Intersustain® (5 µm particle size, 250 mm×4.6 mm I.D.) from GL Sciences Inc., Japan. Shimadzu UV-1800 240V spectrophotometer.

**Standard and sample:** Levodopa (99.3%), methyldopa (99.8%) and Carbidopa (100.2%) were kindly provided by National Medicines Quality Control Laboratory (NMQCL) - Sudan. G.M. Methyldopa® tablets (250 mg) were purchased from the local market.

### *Chemicals and reagents*

**Chemicals:** Sodium dihydrogen phosphate dehydrate, phosphoric acid (analytical grade) and acetonitrile (HPLC grade) were purchased from Scharlau, Spain.

**Diluent:** Phosphoric acid (0.1M) was used as the diluent throughout the experimental work.

**Phosphate buffer pH 2.8:** Eleven grams of sodium dihydrogen phosphate were dissolved in 900 ml of distilled water, the pH was adjusted to 2.8 using phosphoric acid and the volume was made to one litre with distilled water.

### *Preparation of standards and sample solutions*

**Standard stock solution:** An accurately weighed methyldopa standard (125 mg) was transferred into a 100 ml volumetric flask, 70 ml of the diluent was added and the solution was sonicated for 5 minutes. The volume was then completed to the mark with diluent (1.25 mg/ml).

**Sample stock solution:** Amount of powdered tablets equivalent to 125 mg methyldopa was accurately weighed and transferred into 100 ml volumetric flask, 70 ml of the diluent was added and solution was

sonicated for 15 minutes with occasional shaking. The volume was then completed to the mark with diluent and filteredusing 0.45 µ nylon syringe filter (1.25 mg/ml).

**Linearity standard solutions:** Five points calibration solution was prepared by transferring aliquot volumes (2-20 ml) from the stock standard solution into five 100 ml volumetric flasks; the volumes of the flasks were then made to mark with the diluent.

**Sample solution:** Five milliliter of the sample stock solution was diluted to 100 ml with the diluent, 20 µl of the resulting solution were injected into the HPLC system and peak area was recorded.

***Procedure***

**Determination of $\lambda_{max}$:** Two milliliters of the stock standard solution were diluted to 100 ml with the diluent. The solution was then scanned in a UV spectrophotometer over the range of 190-400 nm to determine the suitable detection wavelengthof the analyte.

**Method validation:** The proposed method was validated in accordance with ICH guidelines [(12)], linearity, precision; accuracy and robustness were investigated as method validation parameters. Quantification was performed with five point external calibration curves. Precision was determined as repeatability (three samples) and intermediate precision (three samples on a different day). Accuracy was determined by sample spiking and robustness was determined by intentionally varying the method parameters within small range.

**Linearity:** Triplicate 20 µl injections were made from the linearity standard solution. The calibration curve of the analyte was obtained by plotting its average peak area against its corresponding concentration. The regression analysis data (slope, intercept and correlation coefficient) were calculated, further the limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the regression analysis data according to the following formulae[11]:

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ = the standard deviation of the response, S = the slope of the calibration curve

**Accuracy:** To assess the accuracy of the method, recovery studies were carried out by spiking the sample with standard to 20%, 100%, and 200% of the nominal sample concentration (n= 3). The recovery% was then calculated using the following equation[(11)]:

$$\% \text{ Recovery} = (C_s - C_u)/C_A \times 100$$

Where

$C_s$=concentration of spiked samples.

$C_u$= concentration of unspiked samples.

$C_A$= concentration of analyte added to the test sample

**Precision:** The method's within the day precision was determined by analyzing three sample solutions containing different concentrations of the analytes in the range of (20 -200%) of the nominal sample concentration, prepared by proper dilution from Solution C. Each sample was injected three times; the mean,

standard deviation (SD) and the relative standard deviation (RSD) were calculated. To determine the between the days precision the procedure was repeated by a different analyst on a different day using a different instrument.

**Robustness:** The method robustness was investigated by varying the following method critical parameters: pH (± 0.1), organic solvent ratio (± 1%), flow rate (± 0.1 ml/min), column supplier (different brand) and detection wavelength (± 5 nm).

## RESULTS AND DISCUSSION

**Determination of λmax: The** wavelength away from the stray light in the ultraviolet region and giving reasonable response for the analytes (280 nm) was selected as the chromatographic detection wavelength **(Figure 2).**

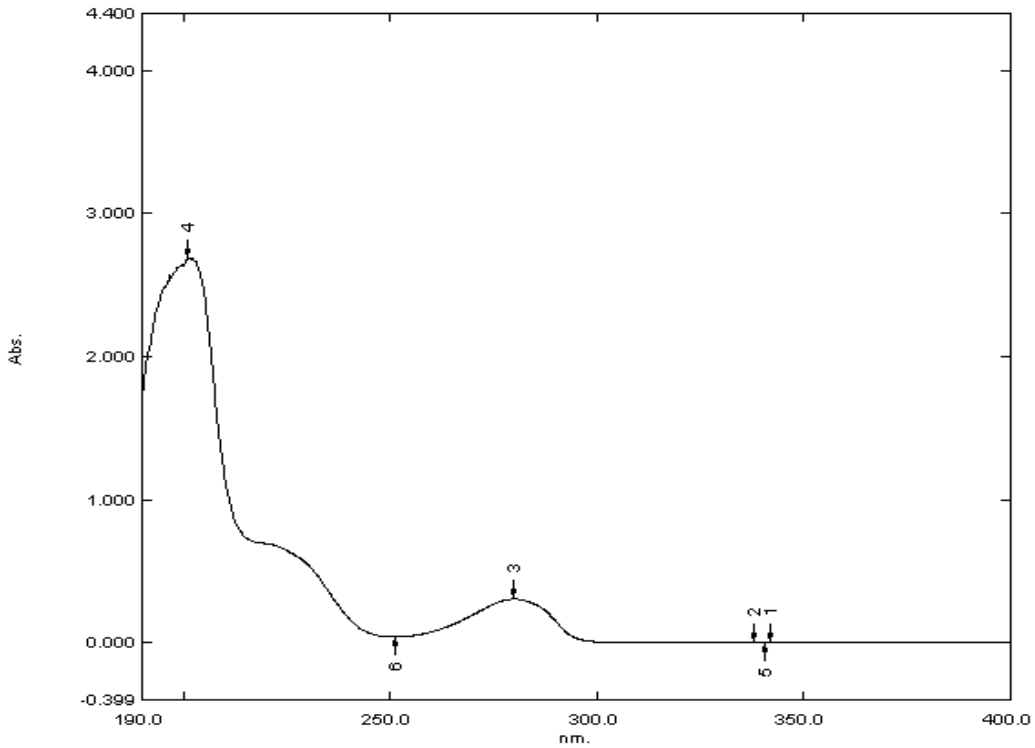


**Figure** 2: UV spectrum of methyldopa solution (25 µg/ml)

**Method development:** In this study, our earlier developed method[11] used for the analysis of levodopa and carbidopa in combined dosage form was adopted for the analysis of methydopa as well. This approach was encouraged by the fact that the three drugs share many structural features, moreover the log P (octanol/water) of methyldopa (-1.8) is intermediate between that of carbidopa and levodopa (-1.0 and -2.4) respectively, as such it was expected that it will elute in the middle between levodopa and carbidopa.

Accordingly the same system used in our earlier work was tried for the separationof the three drugs mixture. Optimum separation of the three analytes was very evident as shown in **Figure 3**, it is interesting to note that the three analytes had eluted from the system according to the increasing order of their log P values in total consistence with reversed phase elution principles.

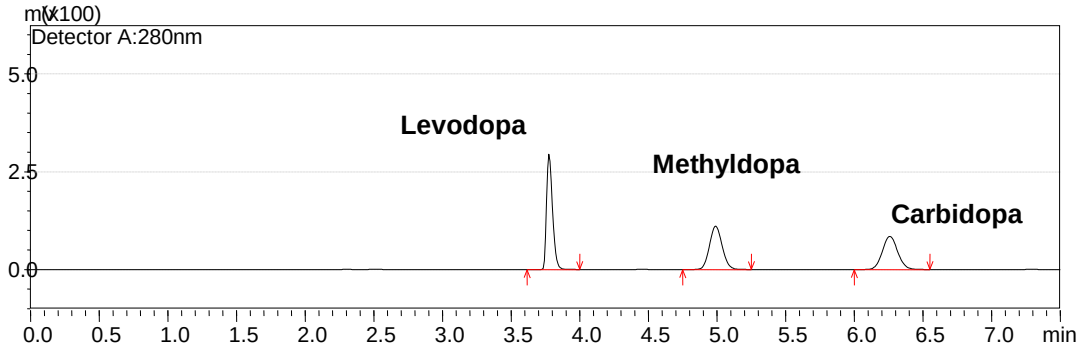


**Figure 3:** Typical chromatogram for drugs mixture using 5: 95 v/v ratio of acetonitrile and buffer solution

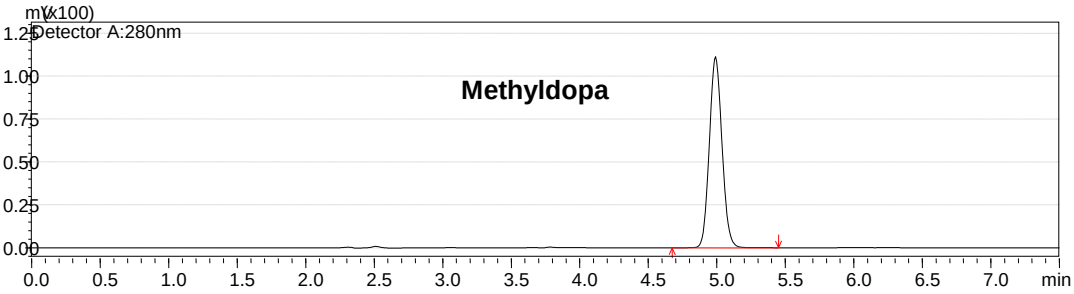


**Figure 4:** Typical chromatogram of methyldopa solution (25µg/ml)

## Method validation

**Linearity:** The constructed calibration curve was linear over the concentration range 25 – 250 µg/ml with a correlation coefficient 1.000, indicating the proportionality of the analyte responses with the concentration. Table 1 summarizes the regression analysis data and the analytes LOD and LOQ.

**Table 1:** Linearity data of the developed method

| Parameter | Value |
|---|---|
| Retention time | 4.98 |
| Concentration range (µg/ml) | 25-250 |
| Slope± SE (*$10^6$) | 13.65 ± 0.01 |
| Intercept± SE (*$10^3$) | 12.12 ± 1.86 |
| Correlation coefficient ($r^2$) | 1.0000 |
| Limit of detection (µg/ml) | 0.91 |
| Limit of quantitation (µg/ml) | 2.77 |
| $R^2$ | 1.00 |

**Accuracy:** The obtained percentage recoveries at three levels (20, 100 &200%) were 99.5% -100.3%; n=3. These high values indicate the accuracy of the developed method and freedom from interference.

**Precision:** The method repeatability precision (within the day) was proved by the low RSD% values; 0.20 - 0.43%. Further the intermediate precision (between the days) performed with a different analyst on a different day using a different instrument resulted in RSD% values of 0.56%

**Robustness:** Robustness was investigated by varying the following method critical parameters: pH (± 0.1), organic solvent ratio (± 1%), flow rate (± 0.1 ml/min), column supplier (different brand) and detection wavelength (± 5 nm). The results of this investigation showed the method's capability to withstand small but deliberate changes of its parameters without significantly affecting the system suitability outcomes **(Table 2).**

**Table 2:** Method robustness data

| **Parameter** **Factor** | Rt (min) | RSD% (n=5) | Theoretical plates # | Tailing factor | Resolution |
|---|---|---|---|---|---|
| Normal conditions | 5.30 | 0.03 | 10069.78 | 1.13 | 6.28 |
| Different column brand | 4.12 | 0.06 | 11884.53 | 1.15 | 4.61 |
| Flow rate 0.9 ml/min | 5.86 | 0.06 | 10901.84 | 1.12 | 6.43 |
| Flow rate 1.1 ml/min | 4.81 | 0.08 | 9348.30 | 1.13 | 6.01 |
| Wavelength 275 nm | 5.31 | 0.31 | 10168.47 | 1.13 | 6.32 |
| Wavelength 285 nm | 5.28 | 0.14 | 10033.25 | 1.13 | 6.09 |
| ACN 4% | 6.02 | 0.26 | 10259.30 | 1.11 | 9.44 |
| ACN 6% | 4.70 | 0.20 | 9639.69 | 1.14 | 2.67 |
| Buffer pH 2.7 | 4.63 | 0.06 | 8760.27 | 1.14 | 2.76 |
| Buffer pH 2.9 | 5.62 | 0.03 | 9405.45 | 1.13 | 7.88 |

**Assay:** The developed method was applied for the determination of content uniformity. Good assay results were obtained (97.14 ± 0.64; n=3).

The validity of the method was also assessed by comparing the statistical results obtained with those of the official USP liquid chromatography method. As the calculated t- values were less than tabulated ones (n =4, P=0.05), the result of the developed method can be considered as accurate and precise as the official liquid chromatographic method (**Table 3).**

**Table 3:** Comparison results between the developed method and USP official method

| Method | Mean ± SD; n=3 | t-value (t-tabulated) | F-value (F tabulated) |
|---|---|---|---|
| **Developed** | 97.14 ± 0.64 | 1.380 (2.78) | 1.03 (19) |
| **Official** | 97.87 ± 0.65 | | |

## CONCLUSION

The developed method was proved to be selective, accurate and precise for the analysis of methyldopa in bulk and tablets form. The simplicity and cost effectiveness of the method suggest its possible use for routine quality analysis of methyldopa.

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