# Spectrophotometric Determination of Amlodipine Besylate Using 2-Hydroxynaphthaldehyde as a Derivatizing Reagent

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A new spectrophotometric method has been developed for the determination of the amlodipine besylate by derivatization with 2-hydroxynaphthaldehyde. The molar absorptivity of amlodipine besylate after derivatization was calculated $1.05 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at $\lambda_{max}$ 416 nm. The derivative obeyed the Beer's law within the concentration range 4-20 µg/mL. The color reaction was highly stable and did not show any change in absorbance up to 12 h. The method was applied for the analysis of amlodipine besylate from tablets of various pharmaceutical companies and the amounts found were observed within 2.48, 4.95 and 9.97 mg/tablet with relative standard deviations (RSD) ranging 2.7-0.01 % (n = 3), respectively.

**Key Words: Amlodipine besylate, 2-Hydroxynaphthaldehyde, Spectrophotometry.**

## INTRODUCTION

Amlodipine besylate (APB) is a long acting calcium channel blocker drug[1-2]. It is used in the treatment of hypertension and angina (chest pain) alone or with other drugs. Different analytical methods have been reported for the analysis of amlodipine besylate, based on spectrophotometric[3-8], flourimetric[9,10], mass spectrometric[11-13], thin layer chromatographic[14], voltametric[15-16], high performance liquid chromatographic[17-24], GC[25], capillary electrophoretic[26] and enzyme linked immunosorbent[27] techniques. For spectrometric analysis, the determination is carried out using either the natural absorbance of amlodipine besylate at 360 nm or it is derivatized with a suitable reagents such as 1,10-phenanthroline, 2,2'-bipyridyl, ammonium heptamolybdate tetrahydrate, ρ-Chloranilic acid iron(III) and ferricyanide, eriochrome black-T, indigo carmine and methyl orange[3,4,7,8,10]. The derivatization either increases the molar absorptivity of the drug or produces bathochromic shift, therefore, in present work 2-hydroxynaphthaldehyde (HN) is examined for the first time as a derivatizing reagent for spectrophotometric determination of amlodipine besylate in pharmaceutical preparations.

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## EXPERIMENTAL

All the reagents and chemicals of pharmaceutical or analytical grades were used. The double distilled water used throughout the study was obtained from distillation plant all made of glass. Pure amlodipine besylate (APB) was obtained from Novartis Pharmaceuticals (Pvt.) Ltd. (Pakistan), 2-hydroxynaphthaldehyde, (HN) and acetic acid from E. Merck, (Germany), sodium acetate from Fluka, (Switzerland) and ethanol from BDH, (UK). Buffer solutions between pH 1-10 at unit interval were prepared from hydrochloric acid (1 M), potassium chloride (1 M), acetic acid (1 M), sodium acetate (1 M), sodium bicarbonate (1 M), sodium carbonate (saturated solution), ammonium chloride (1 M) and ammonia solution (1 M). The solution of HN (2 % w/v) was prepared by dissolving 2 g HN in 100 mL ethanol. The spectrophotometric studies were carried out with a double beam spectrophotometer UV-1601 (Shimadzu Corporation, Japan) with dual silica 1 cm cuvettes.

**Analytical procedure:** The ethanolic solution (0.2-1.0 mL) containing amlodipine besylate (20-100 µg) was transferred to 5 mL calibrated well stoppered volumetric flasks and were added 1.5 mL HN (2 % in ethanol w/v), followed by acetate buffer pH 5(0.5 mL). The contents were heated on water bath at 95 ºC for 15 min. The solutions were cooled at room temperature and the volumes were adjusted to mark with ethanol. The absorbance was measured at 416 nm against reagent blank which was prepared in a similar way only omitting the addition of amlodipine besylate.

**Reference UV method:** The solution (0.5-2.5 mL) containing amlodipine besylate (50-250 µg) was transferred to 05 mL calibrated well stoppered volumetric flasks and the volumes were adjusted to mark with ethanol. The absorbance was measured at 360 nm against ethanol and the molar absorptivity was observed $5.44 \times 10^3$ L mol$^{-1}$ cm$^{-1}$.

**Analysis of amlodipine besylate from pharmaceutical preparations:** Thirty two samples of different pharmaceutical companies were collected and were subjected for the analysis of APB. The sample (0.01 g) from each preparation adoptin (Tread Pharmaceuticals (Pvt.) Ltd. Lahore, Pakistan), amdipine (Nabiqasim Industries (Pvt.) Ltd.., Karachi, Pakistan), amdocal (Bex Pharma (Pvt.) Ltd. Lahore, Pakistan), amlobest (Pearl Pharmaceuticals (Pvt.) Ltd., Islamabad, Pakistan), aml (Batala Pharmaceutical (Pvt.) Ltd., Gujranwala, Pakistan), amlocard (Pharmatec (Pvt.) Ltd., Karachi, Pakistan), amlod (Atco Laboratories (Pvt.) Ltd., Karachi, Pakistan), amlotac (Bio-Mark Farma (Pvt.) Ltd., Lahore, Pakistan), amodip (Mass Pharma Lahore (Pvt.) Ltd., Lahore, Pakistan), ampress (Barret Hodgson (Pvt.) Ltd., Karachi, Pakistan), anam (Tagma Pharma (Pvt.) Ltd., Lahore, Pakistan), Ca-B (Saydon Pharmaceutical Ind.(Pvt.) Ltd., Peshawar, Pakistan), cabok (Platinium Pharmaceuticals (Pvt.) Ltd., Karachi, Pakistan), caloc (Bosch Pharmaceuticals (Pvt.) Ltd., Karachi, Pakistan), cardiovasc (Werrick Pharmaceuticals (Pvt.) Ltd., Karachi, Pakistan), endip (English Pharmaceuticals (Pvt.) Ltd., Lahore, Pakistan), hibiohit (Unipharma (Pvt.) Ltd., Lahore, Pakistan), hypocard (Caylex (Pvt.) Ltd., Pakistan), lodipin (Schazoo Zaka

(Pvt.) Ltd., Lahore, Pakistan), norvasac (Pfizer Laboratories (Pvt.) Ltd., Karachi, Pakistan), lodivasc (Dosaco Laboratories (Pvt.) Ltd., Lahore, Pakistan), onato (Sami Pharmaceuticals (Pvt.) Ltd., Karachi, Pakistan), prescard (Epla Laboratories (Pvt.) Ltd., Karachi, Pakistan), quvasc (Novartis Pharma (Pvt.) Ltd., Karachi, Pakistan), rasdipin (Rasco Pharma (Pvt.) Ltd., Lahore, Pakistan), ravapin (Rakaposhi Pharmaceuticals (Pvt.) Ltd., Peshawar, Pakistan), sicknor (Everest Pharmaceuticals (Pvt.) Ltd., Islamabad, Pakistan), sofvasc (Willson's Pharmaceuticals (Pvt.) Ltd., Islamabad, Pakistan), vasodil (Pharmedic Laboratories (Pvt.) Ltd., Lahore, Pakistan), vespin (Amson vaccines & Pharma (Pvt.) Ltd., Rawalpindi, Pakistan), wincoram (Polyfine Chempharma (Pvt.) Ltd., Peshawar, Pakistan), zodip (Zafa Pharmaceutical Laboratories (Pvt.) Ltd., Karachi, Pakistan), was dissolved separately in ethyl alcohol and the volumes were adjusted to 100 mL. The solution (0.6 mL) from each of the 100 mL solutions of all brands was further transferred to 5 mL calibrated well stoppered separately flask and the procedure was repeated as described in analytical procedure. The amount of amlodipine besylate from each of the sample was calculated using the external calibration curve.

**Recovery (%) of amlodipine besylate from samples by standard addition technique:** Amlodipine besylate standard (0.01 g) was dissolved in 100 mL double distilled ethyl alcohol. Two portions, each consisting 0.6 mL were taken in two different volumetric flasks (05 mL). One was added with 0.4 mL of sample solution containing 100 µg/mL of amlodipine besylate and the derivatization procedure was repeated for both solutions as described in analytical procedure. The % recoveries were calculated from the increase in the absorbance with added standard.

## RESULTS AND DISCUSSION

Amlodipine besylate (APB) or 2-(2-amino-ethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-ethyl ester 5-methyl ester benzenesulfonic acid reacts with 2-hydroxynaphthaldehyde (HN) to form an imine APB-HN derivative (Fig. 1) which absorbs at 416 nm maximally with bathochromic shift having molar absorptivity of $1.05 \times 10^4$ L mol$^{-1}$ cm$^{-1}$. HN was then examined as a derivatizing reagent for the spectrophotometric determination of amlodipine besylate. The amount of HN added, effects of pH, heating time, temperature and the stability of the (APB-HN) derivative were studied carefully.

### Optimization of parameters

**Analytical wavelength:** For the quantitative analysis, the wavelength of maximum absorbance plays an important role. It is compulsory to ensure that derivatizing reagent should not absorb near to the region where the analyte derivative absorbs. This may cause inaccuracy in absorption of the drug because the derivatizing reagent is added in excess to complete the reaction quantitatively. To avoid this setback, it is obligatory to select the wavelength where the analyte derivative shows maximum absorbance value and the derivatizing reagent indicates minimum absorbance. The absorbance value of 08 µg/mL of amlodipine besylate as 2-hydroxynaphthaldehyde derivative

was recorded at different wavelengths between 280-550 nm after heating for 15 min at 90 ºC using buffer pH 5. It was noted that the maximum absorbance occurs at 416 nm against reagent blank. Therefore, the wavelength of 416 nm was selected as optimum wavelength ($\lambda_{max}$).
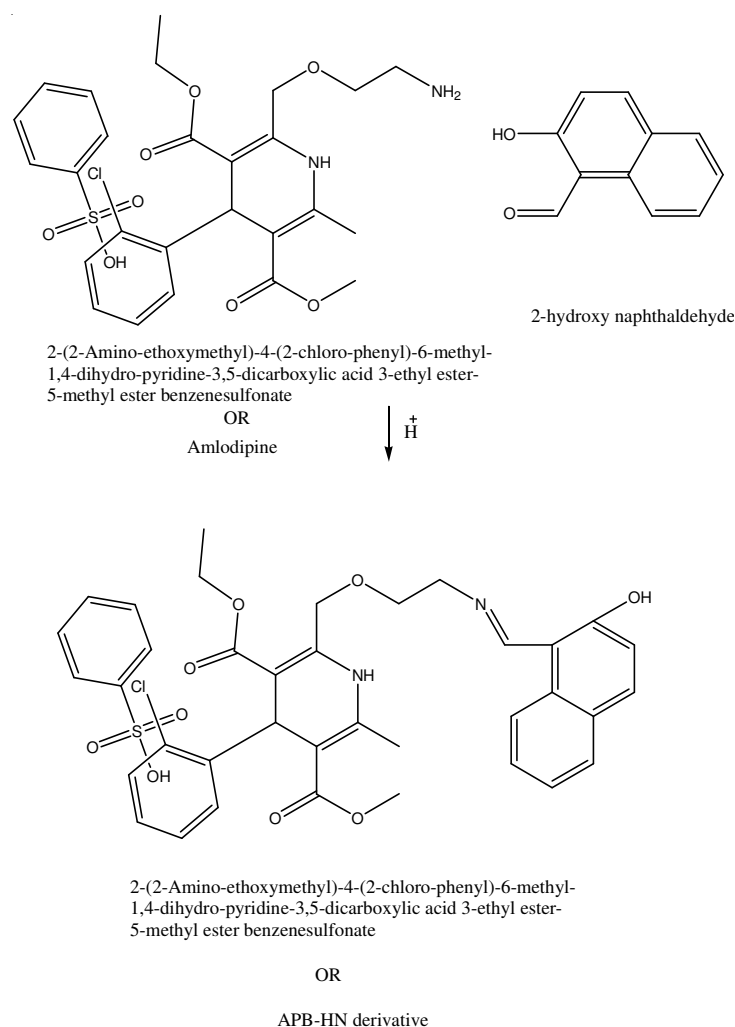


Fig. 1. Formation of amlodipine besylate derivative with 2-hydroxy naphthaldehyde

**Effect of reagent concentration:** The effects of adding various amounts of HN solution on absorbance of 08 μg/mL APB is given in Fig. 2. The volume of 2 % ethanolic HN was varied between 0.5-2.5 mL with an interval of 0.5 mL and the absorbance was measured at $\lambda_{max}$ 416 nm. Initially the absorbance was increased slowly and then the similar absorbance was observed with addition of 1.5 mL and above, it was therefore the addition of 2.5 mL (2 % w/v in ethanol) HN solution was selected.
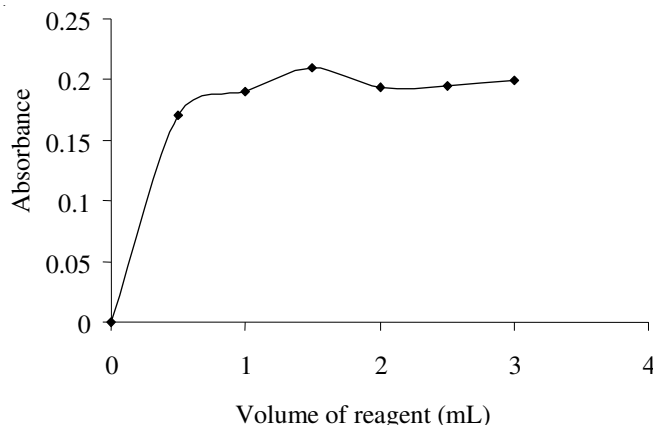
Fig. 2. Effect of volume of reagent HN 2 % ethanolic on absorbance of APB derivative

**Effect of order of mixing the reagents:** The order of adding reagents during derivatization process has significant role in precision of results and improvement of absorbance. In this study, it was observed that the addition of reagent to APB solution (1 mL) followed by buffer pH 5 (0.5 mL) resulted in a decrease in absorbance value. Taking the buffer pH 5 first and then adding the reagent HN, followed by APB solution also gave lower absorbance value. But it was observed that the maximum absorbance value achieved when 1.5 mL of reagent HN was added to the standard solution of APB followed by buffer (0.5 mL) pH 5. The contents were then heated on water bath and the volume was adjusted to the mark with ethanol.

**Optimization of heating time and temperature for the formation of derivative:** To obtain the maximum absorbance value for an analyte, the selection of optimum time and temperature for the formation of stable derivative are essential parameters. The effect of time and temperature on absorbance of 8 μg/mL of APB solution in the presence of 2 % HN solution was checked at 416 nm from 0-30 min with an interval of 5 min at 90 ℃. A similar absorbance was observed after heating for 15 min, so, heating for 15 min at 90 ℃ was considered as optimal heating time and temperature for derivatization.

**Effect of solvents:** The effect of various solvents such as methanol, 1-propanol, 1-butanol, amyl alcohol, isoamyl alcohol, acetonitrile, ethyl acetate, toluene, nitro-benzene and carbon tetrachloride on the absorbance of 12 μg/mL of APB was examined. Each of the solvent 0.6 and 1.2 mL was added after the addition of 2 % ethanolic solution of HN and 0.5 mL acetate buffer pH 5 followed by heating for 15 min. The ethanol proved to be the best choice.

**Effect of pH:** The effect of adding 0.5 mL of 1 M buffers of pH range 1-10 on the absorbance of 8 μg/mL APB solution at already maximized conditions was studied. The absorbance increased gradually from buffer pH 1 and it was maximum at pH 5 (Fig. 3). Addition of buffer above pH 8 produced precipitation. Therefore, the acetate buffer of pH 5 was selected as optimal.
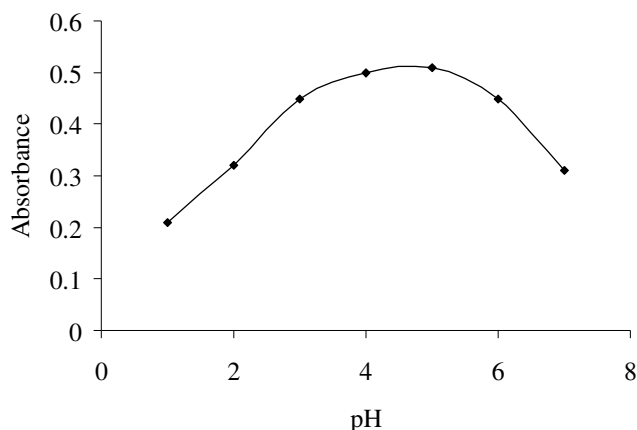
Fig. 3. Effect of pH on derivatization of amlodipine besylate

**Interference study:** The effect of possible presence of associated materials such as mannitol, sorbitol, lactose, sucrose, glucose, galactose and fructose was investigated at 10 times the concentration of APB and it was observed that none of these substances interfered by showing any change in absorbance more than 5 %.

**Stability of the derivative:** The stability of APB-HN derivative was examined in terms of absorbance at the concentration of 4 μg/mL of APB, but no change in absorbance of more than 4 % was observed within 48 h.

**Calibration plot:** The effect of variation in the concentration of APB on its absorbance as derivative APB-HN was studied.

A linear calibration curve was obtained which obeyed the Beer's law within the concentration range 4-20 μg/mL of APB with coefficient of determination $r^2$ 0.9995 (Fig. 4). The Sandell's sensitivity (0.004) was observed at 2 μg/mL of APB-HN. The validity of the calibration curve was obtained by the analysis of test solution of APB and the per cent relative error was found ±1-3.5 %. Thirty two samples containing APB available in the local market were analyzed to determine the amount of APB quantitatively (Table-1). The mean values and % (95% confidence limit) 2.5 mg, 5 mg and 10 mg APB tablets were 0.403 ± 0.2107, 2.197 ± 3.65 and 0.774 ± 0.338, respectively (Table-2).

TABLE-1
EFFECTS OF DIFFERENT POSSIBLE ADDITIVES ON
THE ABSORBANCE OF 20 μg/mL APB-HN DERIVATIVE

| Chemical added | Absorbance | Relative error (%) |
|---|---|---|
| - | 0.210 | 00 |
| Mannitol | 0.220 | 1 |
| Sorbitol | 0.200 | -1 |
| Glucose | 0.210 | 00 |
| Galactose | 0.198 | -1.2 |
| Fructose | 0.199 | -1.1 |
| Sucrose | 0.200 | -1 |

TABLE-2
ANALYSIS OF APB FROM PHARMACEUTICAL PREPARATIONS

| Name of drug | Amount labeled/tablet (mg) | Amount found/tablet (mg) relative standard deviations) (%) | Relative deviations from labeled values ± (%) | Recovery (%) by standard addition technique |
|---|---|---|---|---|
| Adoptin | 05 | 4.87 (1.54) | 2.6 | 98.6 |
| Amdipine | 05 | 4.91 (1.27) | 1.8 | 99.1 |
| Amdocal | 05 | 4.90 (1.94) | 2.0 | 97.9 |
| Amlobest | 05 | 4.93 (0.40) | 1.4 | 99.0 |
| Aml | 05 | 4.91 (0.31) | 1.8 | 95.6 |
| Amlocard | 10 | 9.91 (0.25) | 0.9 | 99.2 |
| Amlod | 05 | 4.96 (0.87) | 0.8 | 100.2 |
| Amlotac | 10 | 9.95 (0.38) | 0.5 | 100.0 |
| Amodip | 2.5 | 2.47 (0.01) | 0.8 | 98.2 |
| Ampress | 10 | 9.97 (0.20) | 0.3 | 96.5 |
| Anam | 10 | 9.97 (0.02) | 0.3 | 97.2 |
| Ca-B | 10 | 10.02 (0.69) | 0.2 | 99.4 |
| Cabok | 10 | 10.00 (0.26) | 00 | 97.5 |
| Caloc | 05 | 4.95 (0.84) | 1.0 | 98.8 |
| Cardiovasc | 2.5 | 4.95 (0.40) | 0.4 | 100.3 |
| Endip | 05 | 4.96 (0.64) | 0.8 | 98.5 |
| Hibiohit | 05 | 4.96 (0.92) | 0.8 | 99.9 |
| Hypocard | 10 | 9.98 (0.10) | 0.2 | 95.6 |
| Lodipin | 05 | 4.97 (0.30) | 0.6 | 98.2 |
| Norvasac | 05 | 4.87 (1.54) | 2.6 | 98.0 |
| Lodivasc | 2.5 | 2.47 (0.40) | 0.8 | 99.6 |
| Onato | 10 | 9.99 (0.10) | 0.1 | 99.9 |
| Prescard | 05 | 5.06 (2.30) | 1.2 | 98.8 |
| Quvasc | 2.5 | 2.46 (0.93) | 0.8 | 99.5 |
| Rasdipin | 05 | 5.04 (2.70) | 0.8 | 98.0 |
| Ravapin | 10 | 9.97 (0.20) | 0.3 | 96.0 |
| Sicknor | 10 | 10.0 (0.76) | 0.1 | 97.2 |
| Sofvasc | 2.5 | 2.51 (0.83) | 0.4 | 95.9 |
| Vasodil | 05 | 5.10 (2.00) | 2.0 | 98.6 |
| Vespin | 05 | 5.01 (0.99) | 0.02 | 100.5 |
| Wincoram | 10 | 9.96 (0.49) | 0.4 | 98.6 |
| Zodip | 05 | 4.96 (1.10) | 0.8 | 100.2 |

**Day to day reproducibility/repeatability:** For the determination of intra and interday reproducibility of the method, the ethanolic standard solution (0.2 mL) of 100 μg/mL of APB was taken in three different calibrated volumetric flasks (5 mL) and the procedure was followed for each solution as described in analytical procedure. The absorbance was measured against reagent blank at 416 nm. The above procedure was repeated for three days (n = 3). The mean absorbances of intraday and interday reproducibilities were observed as 0.096 and 0.094 with (RSD) values 0.36 % and 0.15 %, respectively.
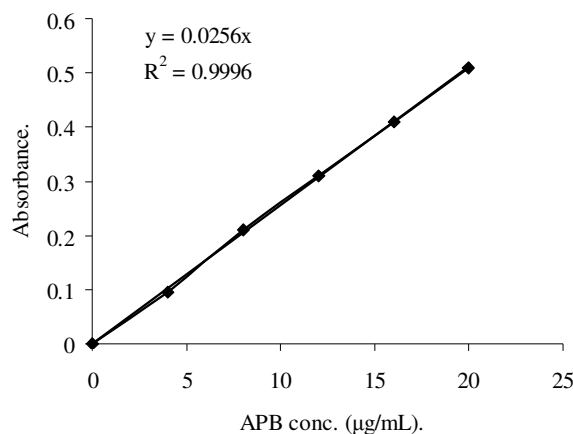
Fig. 4.   Calibration curve of amlodipine besylate 0.01% using 2-hydroxynaphthaldehyde as derivatizing reagent

TABLE-3
RESULTS OF OPTIMIZATION, PRECISION AND ACCURACY

| Parameters | Selected values |
|---|---|
| Wave length $\lambda_{max}$ of APB-HN derivative | 416 nm |
| Beer's law limits (µg/mL) of APB-HN derivative | 4-20 |
| Wave length $\lambda_{max}$ of APB | 360 nm |
| Beer's law limits (µg/mL) of APB | 10-50 |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) of APB-HN derivative | $1.05 \times 10^4$ |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) of APB | $5.4 \times 10^3$ |
| Sandells sensitivity of APB-HN (Conc. at 0.005 ?g/mL absorbance unit) | 0.5 |
| Regression equation (y)a | |
| Slope (b) | 0.0256 |
| Intercept (a) | 0 |
| Coefficient of determination ($r^2$) | 0.9996 |
| Relative Standard deviation (%) | 2.7-0.01 |
| Relative deviation (± %) | 2.6-0.02 |
| Mean value mg/tablet ± range of error at (95 % confidence limit) of five tablets (2.5 mg) of various pharmaceutical companies | 2.48 ± 0.0094 |
| Mean value mg/tablet ± range of error at (95 % confidence limit) of ten tablets (5 mg) of various pharmaceutical companies | 4.95 ± 0.0294 |
| Mean value mg/tablet ± range of error at (95 % confidence limit) of eleven tablets (10 mg) of various pharmaceutical companies | 9.97 ± 0.0200 |

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