SEPARATION AND ANALYSIS OF AMLODIPINE/BENAZEPRIL COMBINATION IN CAPSULES BY A NOVEL ION PAIR LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The objective of this study was to develop and validate a novel ion-pair liquid chromatography method, in order to separate and assay of amlodipine/benazepril combination in capsules. This method was a fast, practical and additional choice in quality control laboratories.

Methods: The chromatographic conditions comprised of a classical C18-type stationary phase (250 × 4.6 mm, 5 μ), with a mobile phase consisting of 45% of 10⁻³ M of cetrimide and 55% acetonitrile. The flow rate was 1 ml/min; the detection wavelength was at 242 nm, under ambient temperature.

Results: The method was validated for linearity with correlation coefficients very close to one, the accuracy with mean recovery values between 95.0-105.0%, precision with relative standard deviations of the calculated concentrations less than 5.0% and specificity in the presence of degradation products and excipients.

Conclusion: The results presented in this paper showed that the developed method was fast and applicable, for the separation and determination of amlodipine/benazepril combination in capsules.

Keywords: HPLC, Ion pair liquid chromatography, Amlodipine, Benazepril

INTRODUCTION

Amlodipine besilate (fig. 1) 3-Ethyl 5-methyl (4RS)-2-[2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate is a long-acting calcium channel blocker, used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina, it increases blood flow to the heart muscle.

It is also a second-generation 1,4-dihydropyridine derivative of the prototypical molecule nifedipine. Like most of the second-generation dihydropyridine derivatives, it has greater selectivity for the vascular smooth muscle than myocardial tissue, a longer half-life (34 h), and less negative inotropy than the prototypical nifedipine. Amlodipine is used in the treatment of chronic stable angina and in the management of mild-moderate hypertension. It is marketed as the benzene sulfonic acid salt.

Benazepril hydrochloride (fig. 1) [(3S)-3-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid hydrochloride is an angiotensin-converting enzyme inhibitor. It acts on the renin-angiotensin-aldosterone system, by inhibition of the conversion of the inactive angiotensin I to the highly potent vasoconstrictor angiotensin II. It also reduces the degradation of bradykinin. Benazepril is applied in pharmacotherapy as a first choice drug for the treatment of arterial hypertension, ischemic heart disease, hypertrophy of the left heart ventricle and postinfarction heart dysfunction [1].

The combination of amlodipine besylate and benazepril HCl is very useful in the treatment of hypertension, the brand name of this oral combination is Lotrel which is developed by Novartes.

Fig. 1: Chemical structures of amlodipine besilate/benazepril hydrochloride [2]
salts on the pumps, arising from the use of buffers. This technique of elution using a surfactant such as cetrimide diminishes the pressure applied on the column.

In addition, we must update the analytical methods in a regular manner in quality control laboratories, in order to choose the best one. Therefore, the objective of this work was to develop and validate a novel ion-pair HPLC method, for the assay of amlodipine/benazepril in capsules.

To our knowledge, this is the first ion-pair liquid chromatography that used cetrimide as a surfactant, to determine amlodipine/benazepril.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Working standards of amlodipine/benazepril were gifted by Ultra Medica pharmaceutical industries, Damascus-Syria. The capsules (brand name-Norkand and Lowcor plus), as received, were stored in the dark at ambient temperature and humidity. They were all analyzed within expiry dates. All the other used reagents were of HPLC grade: acetonitrile (PROLABO), cetrimide (TCI Chemicals), deionized water for HPLC and syringe filters 0.45 µm.

**Instrumentation**

The HPLC instruments used were an Agilent 1260 infinity, equipped with a UV detector and a Shimadzu LC 20 -AT with a diode array detector. The pH meter used was from Crison.

**Method development and optimization of chromatographic conditions**

**Selection of detection wavelength**

The utilized detection wavelength was at 242 nm.

**Column selection**

A NUCLEODUR-C18ec-Machrey-Nagel reversed phase column, 250 x 4.6 mm 5-Micron was utilized.

**Mobile phase preparation**

The mobile phase is consisting of: 45% of 10^{-3} M cetrimide and 55% acetonitrile. The pH was 10.0 without adding any buffer.

**Reference solutions preparation**

A precise quantity of the working standards was accurately weighed, then dissolved in a sufficient volume of distilled water, to obtain the starting standard solutions: 10 mg/50 ml water of benazepril, 5 mg/50 ml water of amlodipine. These starting standard solutions were used for the preparation of the linearity solutions.

**Tablet samples preparation**

Twenty capsules of Norkand 10 (amlodipine 5 mg/benazepril 10 mg), were emptied, then a quantity of the powder containing 2 capsules was transferred into a 100 ml volumetric flask containing deionized water; the content was dispersed under magnetic stirring during 20 min and sonicated for 10 min, until the two active pharmaceutical ingredients were well dissolved (C=5 mg amlodipine/100 ml) and (C=10 mg benazepril/100 ml).

The same precedent protocol was applied, in order to prepare Lowcor plus (amlodipine 5 mg/benazepril 20 mg).

Later, the tablets solution prepared as mentioned above (Norkand 10), was standing at room temperature and sunlight for 60 d. Then, it was analyzed for specificity test demonstration.

**Analytical method validation**

Method validation was performed under a variety of international regulations and quality standards, for the validation of pharmaceutical analytical methods [15-18].

**RESULTS AND DISCUSSION**

**HPLC analysis**

The chromatographic conditions comprised of a C18 reversed phase column, 250 x 4.6 mm 5-Micron, with a mobile phase consisting of: 45% of 10^{-3} M of cetrimide and 55% acetonitrile. The flow rate was 1 ml/min, the utilized detection wavelength was 242 nm, under ambient temperature.

A solution containing a mixture 1:1 of the reference solutions was injected under the previous chromatographic conditions, the retention times were amlodipine 3.12 min, benazepril 5.8 min (fig. 2).

![Fig. 2: Chromatogram of a standard solution containing a mixture of amlodipine/benazepril](image)

**Analytical method validation**

**Linearity**

The linearity of analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of an analyte in the sample. It may be demonstrated directly on the analyte, or on spiked samples using at least five concentrations over the whole working range [15-18].

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Five concentrations over the working range were prepared for each drug; this process was done three different times during three weeks.
(n=3). Fig. 3 showed the regression lines of amlodipine/benazepril with the correlation coefficients ($R^2$) given in Table 1. All the correlation coefficients were very close to one, so the developed method was linear for analyzing the two drugs.

![Fig. 3: Linearity lines of amlodipine/benazepril](image)

<table>
<thead>
<tr>
<th>Table 1: Correlation coefficients of amlodipine/benazepril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amlodipine</strong></td>
</tr>
<tr>
<td>Correlation coefficients ($R^2$)</td>
</tr>
<tr>
<td>Equation $y$</td>
</tr>
<tr>
<td>$R^2 = 0.997$</td>
</tr>
</tbody>
</table>

$n = 3$: five concentrations over the working range, were prepared for each drug; this process was done three different times during three weeks.

**Range**

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations), for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity [15-18].

The linearity was demonstrated in the interval (0.1-0.00625 mg/ml) for amlodipine, (0.2-0.0125 mg/ml) for benazepril.

**Accuracy**

The accuracy of an analytical procedure: expresses the closeness of agreement between the value which is accepted, either as a true conventional value or an accepted reference value and the value found. For the quantitative approaches, at least nine determinations across the specified range should be obtained; for example, three replicates at three concentration levels each. The percentage recovery or the difference between the mean and the accepted true value together with the confidence intervals are recommended[15-18].

Three concentration levels (0.1, 0.05, and 0.025 mg/ml) have been used to study the accuracy of amlodipine. The results indicated that the individual recovery ranged from 97.72 % to 102.98 %. The recovery of amlodipine by the proposed method was accepted, as the mean recovery value was 100.82 % between 95.0-105.0% with RSD value 2.73 not more than 5.0%.

Three concentration levels also (0.1, 0.05, and 0.025 mg/ml) have been used to study the accuracy of benazepril. The individual recovery ranged from 100.12% to 106.19%. The recovery of benazepril by the proposed method was accepted, as the mean recovery value 103.57 was between 95.0-105.0% with RSD value 3.00 not more than 5.0%.

<table>
<thead>
<tr>
<th>Table 2: Mean recoveries of three concentration levels of amlodipine/benazepril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amlodipine</strong></td>
</tr>
<tr>
<td>Mean concentration level$_1$ (0.1 mg/ml) %$_a$</td>
</tr>
<tr>
<td>97.72</td>
</tr>
<tr>
<td>Mean concentration level$_2$ (0.05 mg/ml) %$_a$</td>
</tr>
<tr>
<td>102.98</td>
</tr>
<tr>
<td>Mean concentration level$_3$ (0.025 mg/ml) %$_a$</td>
</tr>
<tr>
<td>101.75</td>
</tr>
<tr>
<td>Mean recovery % ($\pm$) SD</td>
</tr>
<tr>
<td>100.82±2.75</td>
</tr>
<tr>
<td><strong>RSD</strong></td>
</tr>
<tr>
<td>2.73</td>
</tr>
</tbody>
</table>

$\text{mean, } n = 3$

**Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Various precision levels are system or instrument precision, intermediate precision, repeatability, reproducibility.

Repeatability, this short-term variability includes, in addition to the system precision, the contributions from the sample preparation, such as weighing, aliquoting, dilution, extraction, homogenization, etc.

Intermediate precision includes the influence of additional random effects according to the intended use of the procedure, in the same laboratory and can be regarded as an [initial] estimate for the long-term variability. Relevant factors, such as operator, instrument, and days should be varied. Reproducibility, according to the ICH definition is obtained varying further factors between laboratories and is particularly important in the assessment of official compendial methods or if the method is applied at different sites [15-18].

Repeatability and intermediate precision results: The solutions 0.1 mg/ml of amlodipine, 0.1 mg/ml of benazepril have been prepared at
three different times, by three analysts during three weeks, each solution was injected three times \( (N = 9) \). Relative standard deviations of the calculated concentrations (RSD) were given in table 3.

The RSD of the nine determinations of solutions \( \left( 0.1 \text{ mg/ml} \right) \) of amlodipine/benazepril, were 1.8 \% and 1.27 \% for benazepril, (not more than 2.0 \%). These results indicated that the repeatability and intermediate precision of this method were correct for both amlodipine/benazepril.

### Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection: is the lowest amount of an analyte in a sample which can be detected but not necessarily quantified as an exact value. Limit of quantitation is the lowest concentration of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy \([15-18]\). The calculated LODs and LOQs for amlodipine/benazepril, by this new method, were mentioned in table 4.

#### Table 3: Relative standard deviation of the nine determinations of solutions of amlodipine/benazepril

<table>
<thead>
<tr>
<th>N</th>
<th>Amlodipine (0.1 mg/ml)</th>
<th>Benazepril (0.1 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0956</td>
<td>0.0616</td>
</tr>
<tr>
<td>2</td>
<td>0.0956</td>
<td>0.0611</td>
</tr>
<tr>
<td>3</td>
<td>0.0955</td>
<td>0.0612</td>
</tr>
<tr>
<td>4</td>
<td>0.0976</td>
<td>0.0597</td>
</tr>
<tr>
<td>5</td>
<td>0.0976</td>
<td>0.0592</td>
</tr>
<tr>
<td>6</td>
<td>0.0980</td>
<td>0.0604</td>
</tr>
<tr>
<td>7</td>
<td>0.0999</td>
<td>0.0599</td>
</tr>
<tr>
<td>8</td>
<td>0.0998</td>
<td>0.0606</td>
</tr>
<tr>
<td>9</td>
<td>0.0994</td>
<td>0.0597</td>
</tr>
<tr>
<td></td>
<td>0.0977±0.0017</td>
<td>0.0604±0.000771</td>
</tr>
<tr>
<td>RSD</td>
<td>1.82</td>
<td>1.27</td>
</tr>
</tbody>
</table>

\(^n = 9\)

#### Table 4: Limit of detection and quantification of the HPLC method

<table>
<thead>
<tr>
<th></th>
<th>Amlodipine (mg/ml)</th>
<th>Benazepril (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection</td>
<td>0.062</td>
<td>0.037</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>0.187</td>
<td>0.114</td>
</tr>
</tbody>
</table>

### Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components, which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. \([15-18]\).

The chromatogram of the tablets solution before degradation indicated no additional peaks other than those of amlodipine 2.9 min/benazepril 5.9 min (fig. 4).

In order to demonstrate the specificity of the method, tablets solution was exposed to sunlight for 60 d at room temperature. Then, it was recorded.

The chromatogram of the standing tablets solution of amlodipine/benazepril showed many additional peaks \( (3, 3.70, 4.34, 6.65 \text{ and } 9 \text{ min}) \), but they were well resolved from the two main peaks 2.6 for amlodipine and 5.5 for benazepril. (fig. 5). So, the developed method is able to assess amlodipine/benazepril unequivocally in the presence of degradation products and the excipients.

Fig. 4: Chromatograms of the standing tablets solution of amlodipine/benazepril before degradation
Finally, we applied our method to commercial assay capsules purchased from Syria. The data of capsules contents were reported in table 5. The specification of the USP Pharmacopeia recommended that capsules should contain not less than 90% and not more than 110% of the labeled amount of the active pharmaceutical ingredient for amlodipine and benazepril [19]. Amlodipine and benazepril of Syrian capsules contained the active pharmaceutical ingredient, within the range 90-110% of the stated concentration with RSD not more than 5%. Norkand 10 contained 95.39% amlodipine/105.76% benazepril, Lowcor plus 5/20 contained 104.60% amlodipine/92.59% benazepril, Norkand 20 contained 100.00% amlodipine/101.64% benazepril. These results reported herein demonstrated that the quality of capsules of amlodipine/benazepril sold in Syria was correct.

Abhi Kavantha et al. results of assaying amlodipine/benazepril tablets were 98.00±1.89 for amlodipine and 99.04±1.62 for benazepril [3]. Hemdan A et al. result of assaying amlodipine/benazepril capsules was 100.45±0.248 for benazepril and 99.86±0.485 for amlodipine [4]. Safwan Ashour et al. results of analyzing capsules purchased in Syria, by their proposed method were between 99.7±1.11 and 101.8±1.26 for benazepril and between 102.0±1.30 and 105.4±0.71 for amlodipine [5].

Our results of assaying capsules were in accordance with their of the president studies.

<table>
<thead>
<tr>
<th>Formulation name</th>
<th>Active ingredient and potency</th>
<th>Batch number</th>
<th>Manufacturer name</th>
<th>% of nominal concentration amlodipine /benazepril</th>
<th>SD amlodipine /benazepril</th>
<th>RSD amlodipine /benazepril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norkand 10</td>
<td>amlodipine 5 mg</td>
<td>F1952</td>
<td>Ultra Medica-Syria</td>
<td>95.39/105.76</td>
<td>2.06/0.33</td>
<td>2.16/0.31</td>
</tr>
<tr>
<td></td>
<td>mg/benazepril 10 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>amlodipine 5 mg/benazepril 20 mg</td>
<td>0026</td>
<td>National Company-Syria</td>
<td>104.60/92.59</td>
<td>0.049/0.058</td>
<td>0.047/0.063</td>
</tr>
<tr>
<td>Lowcor plus 5/20</td>
<td>mg/benazepril 20 mg</td>
<td>62211</td>
<td>Ultra Medica-Syria</td>
<td>100.00/101.64</td>
<td>0.90/0.032</td>
<td>0.90/0.031</td>
</tr>
<tr>
<td>Norkand 20</td>
<td>mg/benazepril 20 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION
A novel ion-pair HPLC method has been developed for the determination of amlodipine/benazepril in capsules. This method was utilized to separate a mixture of amlodipine/benazepril in capsules and to determine the quantity of the two active ingredients as declared on the label. This method could be an additional analytical technique particularly in the quality control of raw materials, active pharmaceutical ingredients and pharmaceutical formulations.

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AUTHORS CONTRIBUTIONS
The study was carried out in collaboration among all the authors. The idea was developed by Saleh Trefi and Yaser Bitar, the analysis was done by Saleh Trefi and Yaser Bitar, optimization was done by Yaser Bitar. The manuscript was written by Saleh Trefi.

CONFLICT OF INTERESTS
There is no conflict of interest between authors.

REFERENCES


