DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR THE ESTIMATION OF PERINDOPRIL AND INDAPAMIDE

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ABSTRACT

Objective: To develop a validated stability indicating HPTLC method for determination Perindopril and Indapamide.

Methods: The method was based on the separation of two drugs on plates precoated with silica gel 60 F 254 using Dichloromethane: Methanol: Glacial acetic acid in the ratio of 9.5:0.5:0.1 v/v/v as mobile phase followed by scanning in absorbance mode at 215 nm.

Results: The optimized chromatographic conditions gave compact spot for Perindopril and Indapamide at RF value of 0.30 ± 0.02 and 0.60 ± 0.02 respectively. The calibration curve was found to be linear in range of 1000-5000 ng/band and 200-1000 ng/band for perindopril and indapamide respectively. The limit of detection and quantification were found to be 16 ng/band and 491 ng/band for Perindopril and 41.41 ng/band and 125.49 ng/band for Indapamide. The method has been successfully applied to tablets and was validated according to ICH Harmonized Tripartite guidelines.

Conclusion: A new sensitive, simple, rapid and precise high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous determination of Perindopril and Indapamide in pharmaceutical dosage form. The proposed method can be applicable for simultaneous determination of Perindopril and Indapamide in bulk and formulation.

Keywords: Perindopril arginine, Indapamide, HPTLC, Stability indicating method.

INTRODUCTION

Perindopril arginine; [2S3aS7aS]-1-[(S)-N-[(S)-1-carboxybutil] alanyl] hexahydro-2-indole carboxylic acid 1-ethyl ester, is one of the non-peptide Angiotensin II receptor antagonists, and is used for the treatment of patients with hypertension and symptomatic heart failure.[1] Indapamide; 3-(aminosulfonyl)-4-chloro-(2,3-dihydro-2-methyl-1H-indol-1-yl) benzamide, is a diuretic of the class of Benzothiadiazines. The combined oral administration of perindopril with indapamide has been found to be more effective than either of the drugs alone in the treatment of hypertension. Structures of Perindopril arginine and Indapamide are shown in Figure 1. A literature survey revealed that Perindopril arginine is official in the British Pharmacopoeia[2]. Indapamide is official in the British Pharmacopoeia[3]. However, the combination is not official in any pharmacopeia. Upon detailed literature survey it was found that, individually these drugs have been analyzed by many methods[4-11], however very few methods viz. one spectrophotometric method, two HPLC methods and one stability indicating RP-HPLC method has been reported for this combination.[12-14] To the best of our knowledge, no stability indicating HPTLC (High Performance Thin Layer Chromatography) method has been reported for simultaneous estimation of both the drugs in tablet dosage form. The present work describes the simple, accurate, precise, sensitive stability indicating HPTLC method for the determination of Perindopril arginine and Indapamide in combination. The method was validated as per the ICH guidelines.[15]

MATERIALS AND METHODS

Chemicals and Reagents

Perindopril arginine and Indapamide was provided as a gift sample by Mylan Laboratories, Hyderabad. Methanol (AR grade), Dichloromethane (AR grade) and Glacial acetic acid [AR grade] were purchased from S. D. fine chemical Laboratories, Mumbai, India.

Chromatographic separation of drugs was performed on precoated silica gel 60 F 254 (10 cm × 10 cm with 250 µm layer thickness) purchased from E-Merck, Darmstadt, Germany. Samples were applied on the plate as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) and a densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS software (Version 1.4.3, Camag). Chamber saturation time was 15 min. Migration distance was 90 mm, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Marketed Formulaion

A commercial pharmaceutical preparation, COVERSYL PLUS (Serdia Pharmaceuticals Pvt. Ltd., Mumbai, India) was assayed. Its label claim was as follows:

Each uncoated scored tablet contains:

PERINDOPRIL ARGININE B.P.………………….4 mg  
INDAPAMIDE U.S.P.………………….1.25 mg

Selection of wavelength

After chromatographic development, bands were scanned over the range of 200-400 nm and the overlain spectra were obtained. Both the drugs showed considerable absorbance at 215 nm. So, 215 nm was selected as the detection wavelength (Figure 2).
Method Development

Method development for resolution of Perindopril arginine and Indapamide was started with the development of densitogram with solvents in different ratios and combinations of Toluene, Ethyl acetate, Methanol, Dichloromethane, Triethylamine, and Glacial acetic acid. Finally, Dichloromethane: Methanol: Glacial Acetic acid, Methanol, Dichloromethane, Triethylamine, and Glacial Indapamide was started with the development of densitogram with a good resolution at Rf 0.30 and 0.60 for Perindopril arginine and Indapamide respectively.

Preparation of Stock and Standard Solutions

Standard stock solutions of Perindopril arginine and Indapamide were prepared by separately dissolving 10 mg of drug in 10 ml of methanol to get concentrations of 1000 μg/ml. From the resultant solution of Perindopril, 1, 2, 3, 4, and 5 µl were applied on 5 X 10 cm pre-coated TLC plate as a band of length 4 mm, at a distance of 10 mm from both x-axis and y-axis. 1 ml of standard stock solution of Indapamide was diluted to 10 ml with methanol in 10 ml volumetric flask to get working standard solution 100 μg/ml.

From the resultant solution, 2, 4, 6, 8, and 10 µl were spotted on the plate. Plate was then developed in Camag 10 X 10 cm development chamber using selected mobile phase.

Stress degradation studies of bulk drug

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared: the blank subjected to stress in the same manner as the drug solution, working standard solution of Indapamide subjected to stress condition, working standard solution of Perindopril subjected to stress degradation. Dry heat and photolytic degradation were carried out in solid state.

Alkaline hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml of 0.1 N NaOH. The solution was diluted to 10 ml with methanol and kept for 90 min. 6 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril with 0.01 N NaOH, and spotted immediately.

First the degradation was tried with 0.1 N NaOH for half hour reduced to 0.01 N NaOH for 15 mins. Degradation observed was too high. After addition of base in the standard solution of Perindopril, exothermic reaction was observed. So finally 0.01 N NaOH was used for the degradation.

Acidic hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml of 0.1 N HCl. The solution was diluted to 10 ml with methanol and kept for 90 min. 2 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril with 0.1N HCl after 15 min.

Neutral Hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml water. The solution was diluted to 10 ml with methanol and kept for 48 Hrs. 6 µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of Perindopril and was spotted immediately after exposure to neutral condition.

Oxidation hydrolysis

2 ml of solution of Indapamide was mixed with 2 ml 3% solution of H2O2. The solution was diluted to 10 ml with methanol and was kept for 48 Hrs. 6µl of the solution was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Same procedure was repeated for working standard solution of and Perindopril spotted immediately after exposure to oxidative condition.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample of Indapamide in oven (80°C) for a period of 24 hours. Samples were withdrawn after 24hrs, dissolved in methanol and diluted to get 10µg/ml as final concentration of 6µl was spotted. 10 times higher concentration were also spotted to locate degradation product, if any. Perindopril was exposed to 40°C for 15 min and solution was prepared as above. First the degradation was tried for half hour but the degradation observed was high. The storage is strictly recommended in cold condition.

Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hr. Samples were weighed, dissolved and diluted to get 10 µg/ml. 6µl of this solution was spotted. 10 times higher concentration was also spotted to locate degradation product, if any. Same procedure was followed for Perindopril.

Preparation of Tablet solution for Assay

10 tablets were accurately weighed and powdered. From the powdered mixture, certain amount (equivalent to 4 mg of perindopril arginine and 1.25 mg of Indapamide) of the powder was accurately weighed and transferred to 10 ml volumetric flask. Methanol was added in the volumetric flask and the resultant mixture was sonicated for 10 min at room temperature to disperse the powder completely. The resultant mixture was further diluted to 10 ml with methanol and then filtered through Whatmann filter paper no 1, to get the clear solution. 1 ml aliquot of the filtered solution was then diluted to 10 ml with methanol in 10 ml volumetric flask to get the final concentration 100 μg/ml of Indapamide separately. 3 µl of 1000 μg/ml and 6µl of 100 μg/ml of this solution was spotted on TLC plate as a band of length 4 mm on two separate tracks for the assay of Perindopril arginine and Indapamide respectively.

Method Validation

Linearity and Range

Linearity was studied by analyzing five concentrations of the drug, and process was repeated for five times each. It was done over the concentration range of 1000-5000 ng/band and 200-1000 ng/band for perindopril arginine and Indapamide respectively.

Precision

Precision of the system and method was evaluated by analyzing independent sample preparations obtained from homogenous sample and % RSD value obtained was calculated to determine any variation.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample.
solution at three different levels of 80, 100 and 120 %. Mean percentage recovery for both the drugs was then determined.

**Limit of detection and limit of quantitation**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

**Specificity**

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on TLC scanner 3 in the range of 200-400 nm using WinCats software (version 1.4.3).

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was determined by making slight deliberate changes like chamber saturation time and ± 2% variation in mobile phase compositions.

**RESULTS AND DISCUSSION**

**Optimization of mobile phase**

TLC procedure was optimized with a view to develop a method for simultaneous estimation of Perindopril arginine and Indapamide. The drug reference standards were spotted on TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve the peaks of Perindopril arginine and Indapamide. Best suited mobile phase was found to be Dichloromethane: Methanol: Glacial Acetic Acid in the ratio of 9.5:0.5:0.1 v/v/v. Developed mobile phase resulted in resolution of Perindopril arginine and Indapamide at Rf 0.30 ± 0.02 and 0.60 ± 0.02 respectively. Well-defined bands were obtained when the chamber was saturated for 15 min. with the mobile phase at room temperature before chromatographic development. (Figure 3)

**Stress degradation study**

**Hydrolysis at basic pH**

After alkaline degradation, 71.21 % Indapamide was recovered with 1 peak of degradation product. (Figure 3 A, B and C) While no degradation peak was observed for Perindopril with 58.08% recovery.

**Hydrolysis at acidic pH**

After acid hydrolysis, 70.71% Indapamide was recovered with 1 peak of degraded product while 77.36% Perindopril was recovered with no peak of degraded product. [Fig 4 A, B and C]

![Fig. 3: A) Blank NaOH, B) Indapamide (600ng/band) treated with NaOH, C) Overlain spectra of Indapamide and product of degradation.](image-url)
Fig. 4: A) Blank HCL, B) Indapamide (600ng/band) treated with HCL, C) Overlain spectra of Indapamide and product of degradation.

Under neutral hydrolysis
After neutral hydrolysis, 71.94% Indapamide was recovered and 65.66% Perindopril was recovered.

After oxidative condition
After oxidation, 74.34% Indapamide was recovered while 60.23% Perindopril was recovered with no peak of degraded product.

Table 1: Summary of stress degradation study of Indapamide

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Stress Degradation Condition</th>
<th>Peak Area</th>
<th>% Recovery</th>
<th>Rf of Degradation Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Base (0.01 N NaOH, kept for 90 mins)</td>
<td>4682</td>
<td>71.21</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>Acid (0.1 N HCl, kept for 15 mins)</td>
<td>4658</td>
<td>70.71</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>Water (immediately)</td>
<td>4718</td>
<td>71.94</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>H2O2 3% (immediately)</td>
<td>4835</td>
<td>74.34</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Heat dry (40°C, 15 min)</td>
<td>5696</td>
<td>91.99</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Photo stability UV, 200 watt hrs/square meter Fluorescence, 1,2 million Lux. Hrs</td>
<td>6050</td>
<td>99.26</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6087</td>
<td>100.01</td>
<td>-</td>
</tr>
</tbody>
</table>

Method Validation
The linearity in the proposed HPTLC method for determination of Perindopril arginine and Indapamide was found in the concentration range of 1000-5000 ng/band and 200-1000 ng/band with r2 value of 0.991 and 0.998 respectively (Figure 5).

Fig. 5: Linearity for Perindopril Arginine (Rf 0.30 ± 0.02) and Indapamide (Rf 0.60 ± 0.02).

Marketed sample of tablet was analyzed and the percentage label claim was found to be 99.98% and 99.94% for Perindopril arginine and Indapamide respectively. HPTLC method was validated as per the ICH guidelines. The accuracy of method was determined at 80, 100 and 120 % level. The % recovery was found to be within the limits of 98 % to 102 % for both the drugs. Precision was calculated as interday and intraday variations. For System and Method precision, % RSD (Relative Standard Deviation) was found to be not more than 2 % for both drugs. For robustness studies, there were no significant changes in Rf and peak areas, which demonstrated that the developed HPTLC method is robust. Peak purity was found to be more than 0.995, which demonstrated that the method is specific. The results of validation are summarized in Table 3.
Table 3: Summary of validation parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Validation Parameter</th>
<th>Results</th>
<th>Indapamide</th>
<th>Perindopril</th>
</tr>
</thead>
</table>
| 1.    | Linearity            | $y = 8.128x + 1210$ (R² = 0.998) | $y = 1.363x + 588.7$ (R² = 0.991)
| 2.    | Range                | 200-1000 ng/band    | 1000-5000 ng/band |
| 3.    | Precision            |                     |            |             |
|       | A) System precision  | 1.83                | 1.75       |
|       | A) Method precision  | 1.13                | 1.56       |
| 4.    | Accuracy             |                     |            |             |
|       | % Recovery           | 99.53               | 99.63      |
|       | 80%                  | 98.70               | 101.25     |
|       | 100%                 | 99.48               | 99.28      |
| 5.    | LOD                  | 41.41 ng/band       | 164 ng/band |
| 6.    | LOQ                  | 125.49 ng/band      | 491 ng/band |
| 7.    | Specificity          | Specific            | Specific   |
| 8.    | Robustness           | Robust              | Robust     |

The degradation conditions mentioned above were arrived at, after number of initial trials for optimization of extent of degradation. Overall study comprised of stability indicating method development for Indapamide and Perindopril arginine. This method can be used for stability testing of this drug in dosage forms.

CONCLUSION

The validated HPTLC method was found to be simple, accurate and rapid for the routine determination of Perindopril arginine and Indapamide in tablet formulation. The proposed method can therefore, be successfully used for simultaneous estimation of Perindopril arginine and Indapamide in combined dosage form.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENT

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REFERENCES