Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

SIMULTANEOUS ESTIMATION OF PERINDOPRIL ERBUMINE AND INDAPAMIDE IN BULK DRUG AND TABLET DOSAGE FORM BY HPTLC

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Received: 26 November 2010; Revised: 31 December 2010; Accepted: 3 January 2011; Available online: 11 January 2011

ABSTRACT

Perindopril erbumine is one of the newly used non-peptide Angiotensin II receptor antagonist, and is used for the treatment of patients with hypertension and symptomatic heart failure. Indapamide is a diuretic of the class of Benzothiadiazines. The combined oral administration of perindopril erbumine with indapamide has been found to be more effective than either drug alone in the treatment of hypertension. A new sensitive, simple, rapid and precise high performance thin layer chromatographic (HPTLC) method has been developed for simultaneous determination of both the drugs in Pharmaceutical dosage form. The method was based on the separation of two drugs on plates precoated with silica gel 60 F_{254}. The mobile phase used was Dichloromethane : Methanol : Glacial acetic acid in the ratio of 9.5:0.5:0.1 v/v/v. Both the drugs showed considerable absorbance at 215 nm. Linearity was obtained in the concentration range of 1-5 µg/band and 100-500 ng/band for perindopril and indapamide respectively. The method has been successfully applied to tablets and was validated according to ICH Harmonized Tripartite guidelines.

Keywords: Perindopril erbumine, Indapamide, HPTLC.

INTRODUCTION

Perindopril erbumine; (2*S*,3a*S*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-carboxybutyl] alanyl] hexahydro-2-indoline 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-*N*-(2,3-dihidro-2-methyl-1*H*-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.[1] Structures of Perindopril erbumine and Indapamide are shown in Figure **1**.

A literature survey revealed that perindopril, an active pharmaceutical ingredient (API) is official in the *British Pharmacopoeia*[2], indapamide API is official in the *British Pharmacopoeia*[3] and *U.S. Pharmacopeia*[4]. Indapamide tablets are official in the *British Pharmacopoeia*[5] and *U.S. Pharmacopeia*[6]. 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[7-10], however very few methods viz. one spectrophotometric method, two HPLC methods and one stability indicating RP-HPLC method has been reported for this combination.[1, 11, 12]

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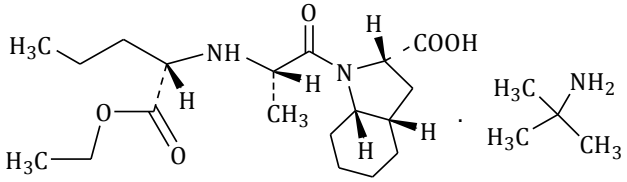
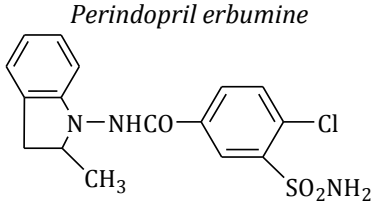
To the best of our knowledge, no HPTLC (High Performance Thin Layer Chromatography) method has been described for simultaneous estimation of both the drugs in tablets. The present work describes the simple, accurate, precise, sensitive HPTLC method for the determination of Perindopril erbumine and Indapamide in combination. The method was validated as per the ICH guidelines.[13]

Figure 1. Structure of Perindopril erbumine and Indapamide.



Perindopril erbumine



Indapamide

MATERIALS AND METHODS

Chemicals and Reagents

Perindopril erbumine was provided as a gift sample by Matrix Laboratories, Hyderabad. Indapamide was provided as a gift sample by Glenmark Pharmaceuticals, Nasik. Drugs were used as such, without any further purification. Methanol (AR grade), Dichloromethane (AR

grade) and Glacial acetic acid (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai, India.

Instruments and Chromatographic conditions

Chromatographic separation of drugs were performed on Aluminium plates precoated with 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 Formulation

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

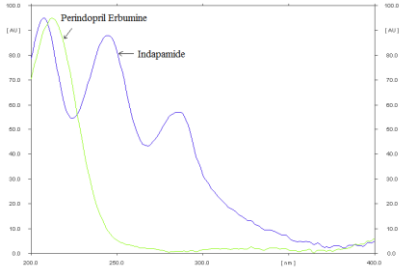
Each uncoated scored tablet contains:

PERINDOPRIL ERBUMINE B.P..... 4 mg
INDAPAMIDE U.S.P..... 1.25 mg

Selection of detection 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**).

Figure 2. Overlay spectra of Perindopril erbumine and Indapamide.



Method Development

Method development for resolution of Perindopril erbumine and Indapamide was started with the development of densitogram with neat solvents in different ratios and combinations of Toluene, Ethyl acetate, Methanol, Dichloromethane, Triethylamine, and Glacial acetic acid. Finally, Dichloromethane: Methanol: Glacial Acetic acid (9.5:0.5:0.1 v/v/v) was selected as a mobile phase with a good resolution at R_f 0.30 and 0.50 for Perindopril erbumine and Indapamide respectively.

Preparation of Stock and Standard Solutions

Standard stock solutions of Perindopril erbumine and Indapamide were prepared by separately dissolving 10 mg of drug in 10 ml of methanol to get concentrations of 1000µg/ml. 5 ml of standard stock solution of Perindopril erbumine was then diluted to 10 ml with methanol in 10 ml volumetric flask to get working standard solution 500µg/ml. From the resultant solution, 2, 4, 6, 8, and 10 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 100µg/ml. From the resultant solution, 1, 2, 3, 4, and 5 µl were spotted on the plate. Plate was then developed in Camag 10 X 10 cm development chamber using selected mobile phase.

Preparation of Tablet solution for Assay

10 tablets were accurately weighed and powdered. From the powdered mixture, certain amount (equivalent to 10 mg of perindopril erbumine and 4 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 125 mm Ø Whatmann filter to get the clear solution. 4 ml aliquot of the filtered solution was then diluted to 10 ml with methanol in 10 ml volumetric flask to get the final concentration 400µg/ml and 125µg/ml of Perindopril erbumine and Indapamide respectively. 10 µl and 2 µl of this solution were applied on 5 X 10 cm pre-coated TLC plate as a band of length 4 mm on two separate tracks for the assay of Perindopril erbumine and Indapamide respectively.

Method Validation

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 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 1-5 µg/band and 100-500 ng/band for perindopril erbumine and indapamide respectively.

Precision

Precision of the system was evaluated by analyzing six independent sample preparations obtained from homogenous sample and % RSD value obtained was calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day 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. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

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.

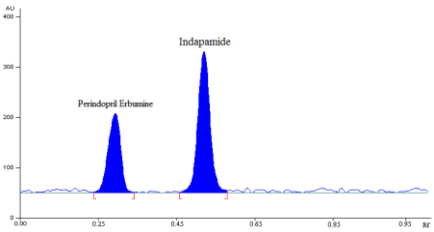
Robustness of the method was determined by making slight deliberate changes like chamber saturation time and ± 2% variation in mobile phase compositions.

RESULTS AND DISCUSSION

Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a method for simultaneous estimation of Perindopril erbumine 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 erbumine 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 erbumine and Indapamide at R_f 0.30 ± 0.02 and 0.50 ± 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. The representative densitogram is shown in Figure **3**.

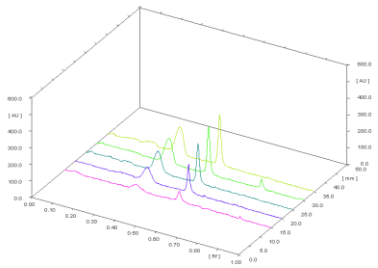
Figure 3. Representative Densitogram of Perindopril erbumine (Rf 0.30 ± 0.02) and Indapamide (Rf 0.50 ± 0.02)



Method Validation

The linearity in the proposed HPTLC method for determination of Perindopril erbumine and Indapamide was found in the concentration range of 1-5 µg/band and 100-500 ng/band with r^2 value of 0.995 and 0.991 respectively (Figure **4**).

Figure 4. Linearity for Perindopril Erbumine (R_f 0.30 ± 0.02) and Indapamide (R_f 0.50 ± 0.02).



Marketed sample of tablet was analyzed and the percentage label claim was found to be 99.98 % and 99.94 % for Perindopril erbumine 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 Intraday precision, % RSD (Relative Standard Deviation) was found to be not more than 1 % and for Interday precision it was found to be not more than 1.5 % 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. Validation parameters are summarized in Table **1**.

Table 1. Summary of validation parameters.

Validation Parameter	Perindopril Erbumine	Indapamide
Linearity Equation (r^2) Range	y = 1.024x + 149.2 r^2 = 0.995 1 – 5 µg/band	y = 6.938x + 3097 r^2 = 0.991 100 – 500 ng/band
Precision (% RSD) Intraday Interday	 NMT 1 % NMT 1.5 %	 NMT 1 % NMT 1.5 %
Accuracy (% recovery)	Within limits (100 ± 2 %)	Within limits (100 ± 2 %)
Limit of Detection	250 ng/band	25 ng/band
Limit of Quantitation	825 ng/band	83 ng/band
Specificity Peak purity	Specific Front and Tail > 0.995	Specific Front and Tail > 0.995

CONCLUSION

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

ACKNOWLEDGMENT

Authors are thankful to Matrix Laboratories, Hyderabad for providing working standard of Perindopril erbumine and Glenmark Pharmaceuticals, Nasik for providing working standard of Indapamide. Authors are also thankful to the Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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