Analytical Study of Certain Amide Containing Drugs

A Thesis

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"Analytical Chemistry"

By

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Preface

Pharmaceutical compounds form main category of our daily deals. This category is subjected to some degradation due to bad storage, transportation, humidity or usage. Degradation products produced may affect the pharmacological and toxicological aspects or bioavailability of the active constituents. Stability of these compounds has to be investigated to determine their sensitivity against different conditions. Furthermore, stability indicating procedures which can quantify the intact drug in presence of its degradation products without any interference of other co-formulated drugs or excipients have to be developed.

Recently, multicomponent formulations have gained a great importance due to patient’s appliance, synergistic effects, multiple action and fast onset of action. Thus, simultaneous estimation of co-formulated drugs is an important part in the field of pharmacy as it cancels effort and time of extraction, separation and gets accurate and precise results.

This work deals with the analysis of some pharmaceutical compounds with different pharmacological activities, namely, rebamipide, torsemide, perindopril arginin and amlodipine besylate either in single or binary pharmaceutical formulations. Simple spectrophotometric, ion selective electrode based potentiometric and chromatographic methods were applied in this study.

The aims of this work

- To investigate the stability of the drugs under study according to ICH guidelines against different stress conditions.
- To develop stability indicating analytical procedures which afford selective determination of those drugs in presence of their degradation products.
- To check the applicability of the developed methods for the accurate and precise analysis of the target drugs in raw materials, binary mixtures and in pharmaceutical formulations.
Abstract

This thesis demonstrates four parts which are; general introduction and three other parts describe literatures and different techniques for the quantitative analysis of the cited drugs in presence of their degradation products in raw material and in their pharmaceutical preparations and an appendix in addition to references and summary in Arabic.

**Part I: General introduction**

A general introduction about functional group amide, its importance, naming, properties, hydrolysis and analysis.

**Part II: Stability Indicating Methods for Determination of Perindopril Arginin and Amlodipine Besylate in Binary Mixture and in Presence of Their Alkaline Degradation Products**

This part includes:

**Introduction and literature review**

An introduction describes the pharmacological action of each of perindopril and amlodipine, their chemical structures, physical properties, stability and brief review of the published analytical procedures developed for their analysis.

**Section A: Spectrophotometric Methods for Determination of Perindopril Arginin and Amlodipine Besylate in Binary Mixture**

This section describes two spectrophotometric methods for simultaneous determination of perindopril and amlodipine in binary mixture. In both methods amlodipine (AML) can be determined directly at 360 nm but perindopril is determined
differently according to the theory of each method. The first method depends on the theory of absorptivity factor so, perindopril is determined at 222.2 nm by subtraction then multiplication by $1/F = 4$. The second one is the absorbance correction method at which the absorbance of AML at 360 nm is converted to its correspondence at 208 nm. The absorbance corresponding to perindopril (PER) is calculated by subtraction. Good linearity was obtained in the range of 10-70 µg mL$^{-1}$ and 4-28 µg mL$^{-1}$ for PER and AML, respectively, with mean recoveries of 100.11±0.60 for PER at 208 nm and 100.00±0.51, 100.12±0.60 and 99.57±0.84 for AML at 208, 222.2 and 360 nm, respectively. The suggested methods were applied successfully for the determination of both drugs in binary mixtures with different ratios and in their pharmaceutical formulations.

**Section B: Stability Indicating TLC-Densitometric Method for Determination of Perindopril Arginin and Amlodipine Besylate in Presence of Their Alkaline Degradation Products**

In this section, a satisfactory separation of PER, AML from their alkaline degradation products was achieved by using ethyl acetate – methanol – toluene – ammonia solution 33% (6.5: 2: 1: 0.5 by volume) as a developing system and UV detection at 215 nm for linearity of PER and 237 nm for linearity of AML. The plot of integrated peak area of each drug to its respective concentration was found to be linear in the range of 4-28 µg/band for PER and 2-8 µg/band for AML. The proposed method was used to determine both drugs in synthetic mixtures and in commercial tablets. Furthermore, standard addition technique was applied to assess the validity of the proposed method.
Section C: Stability Indicating HPLC Method for Determination of Perindopril Arginin and Amlodipine Besylate in Presence of Their Alkaline Degradation Products

A stability indicating HPLC method was introduced for simultaneous determination of PER and AML in presence of their alkaline degradation products. The mobile phase that allowed good separation was found to be phosphate buffer (pH 2.5, 0.01M) – acetonitrile – tetrahydrofuran (60: 40: 0.1% by volume) at flow rate 1 mL min^{-1} and UV detection at 215 nm. The suitability of the chromatographic conditions was ascertained by the determination of system suitability parameters. Validity and applicability of the method were verified by applying the suggested method for determination of PER and AML in laboratory prepared mixtures and pharmaceutical formulations.

Section D: Stability Indicating Ion Selective Electrode Method for Determination of Perindopril Arginin and Amlodipine Besylate in Presence of Their Alkaline Degradation Products

In this section, novel miniaturized polyvinyl chloride membrane sensors in all-solid state graphite supports were described for the determination of perindopril (sensors 1&2) and amlodipine (sensors 3&4). The sensors were based on the formation of an ion association complex between PER (sensors 1&2) or AML (sensors 3&4) and tetraphenylborate anionic exchanger as electroactive material and the use of 2-hydroxypropyl-β-cyclodextrin as neutral ionophore dispersed in a PVC matrix with either dioctyl phthalate (sensors 2&3) or dibutyl sebasate (sensors 1&4) as plastisizer. The performance characteristics of these sensors were evaluated according to IUPAC recommendations, revealing fast, stable and linear response for PER and AML in dosage form and plasma.
Section E: Stability Indicating Chemometric Methods for Determination of Perindopril Arginin and Amlodipine Besylate in Presence of Their Alkaline Degradation Products

Multivariate spectral analysis techniques were investigated and developed in this section for simultaneous determination of PER and AML in presence of their alkaline degradation products. The method was based on UV spectrophotometry, and the resulting heavily overlapping responses are processed by chemometrics. Nineteen mixtures were used for building the calibration models, while six mixtures were chosen to be used as an external validation set. PLS and CRACLS multivariate calibration models were constructed using the data obtained. The suggested methods were applied for determination of PER and AML in their dosage forms and the results were compared to those obtained by the manufacturer method using t and F tests to assess the validity of the method.


This part includes:

Introduction and literature review

This introduction comprises a brief idea about the structure, properties, stability and different methods for the analysis of rebamipide either in pharmaceutical formulations or in biological fluids.
Section A: First Derivative of Ratio Spectra Spectrophotometric Method for Determination of Rebamipide in presence of Its Hydrolytic Degradation Products

This section investigates the stability of rebamipide (REB) under different stress conditions as well as isolation and characterization of the degradation products. Then a first derivative of ratio spectra spectrophotometric method was developed for determination of intact rebamipide in presence of its degradation products at 249.4, 259 nm and at the total peak amplitude (DD_{249.4+259 nm}) using the spectrum of 20 µg mL\(^{-1}\) degradation products as a divisor. Linear correlation was obtained in the range of 2-14 µg mL\(^{-1}\) with good accuracy. The proposed method succeeded in the determination of REB in pharmaceutical formulations.

Section B: Dual Wavelength Spectrophotometric Method for Determination of Rebamipide in presence of Its Hydrolytic Degradation Products

An accurate and precise stability indicating method for determination of REB was described. In this method, 254 & 269 nm were selected so that, the absorbance difference between these wavelengths is zero for the degradation products thus, the absorbance difference of a mixture is corresponding to REB only. It shows good linearity in the range of 5-65 µg mL\(^{-1}\) with mean recovery of 99.84±1.06. Satisfactory results were obtained upon applying the suggested method in the pharmaceutical formulations which encourage the use of the proposed method in quality control laboratories.
Section C: TLC-Densitometric Method for Determination of Rebamipide in presence of Its Hydrolytic Degradation Products

In this section, TLC separation of REB from its degradation products was obtained by using methanol – chloroform – ammonia solution 33% (8.5:1.5: 0.5 by volume) as a developing system. The bands were measured quantitatively at 329 nm and the pure drug was determined over the concentration range of 0.5-4.5 µg/band. The suggested method could be considered as a stability indicating one as it could determine the drug in presence of its hydrolytic degradation products without pretreatment. The results obtained by the proposed method were statistically compared with the reported HPLC method and there was no significant difference between them.

Section D: HPLC Method for Determination of Rebamipide in presence of Its Hydrolytic Degradation Products

A simple HPLC method was applied for separation and quantitative determination of intact rebamipide in presence of its hydrolytic degradation products. The mobile phase of choice was found to be methanol – phosphate buffer pH 6 (1:1, v/v) at a flow rate 1mL min⁻¹ and UV detection at 254 nm. The chromatographic conditions were optimized to obtain maximum resolution and peak symmetry. Validity and applicability of the method were confirmed by the analysis of REB in its dosage form and the application of standard addition technique.

Part IV: Stability Indicating Methods for Determination of Torsemide

This part contains:
Introduction and literature review

It discusses the pharmacological action of the studied drug as well as its chemical structure and properties. In addition to concise review of the different methods reported in the literatures for the determination of the drug in pure form, pharmaceutical formulations and biological fluids.

Section A: Second Derivative Spectrophotometric Method for Determination of Torsemide

The stability characteristics of TOR were studied according to ICH guidelines and the degradation products were characterized and confirmed. A second derivative spectrophotometric method was introduced for the selective determination of torsemide (TOR) at 262.4 nm without any interference of its degradation products over the concentration range 5-30 µg mL\(^{-1}\). The results obtained by the application of the proposed method for the analysis of TOR in its commercial tablets encourage its use in pharmaceutical laboratories.

Section B: First Derivative of Ratio Spectra Spectrophotometric Method for Determination of Torsemide

In this section, \(^1\)DD method was applied to determine torsemide in presence of its degradation products, where TOR was determined by measuring the peak amplitudes at 232.4, 244.6 nm and at the total peak amplitude (\(^1\)DD \(232.4+244.6\) nm) using the spectrum of 30 µg mL\(^{-1}\) degradation products as a divisor. The linearity range of TOR was 4-28 µg mL\(^{-1}\). It was determined successfully in laboratory prepared mixtures using the proposed method. The validity was checked by the analysis of TOR in its pharmaceutical formulations and the standard addition technique assessed this validity.
Section C: TLC-Densitometric Method for Determination of Torsemide

This method depends on the separation and quantitative evaluation of thin layer chromatography of TOR in presence of its degradation products using acetone – chloroform – ethyl acetate (4: 4: 2 by volume) as a developing system. Chromatogram was scanned at 287 nm in the range of 0.5-5.0 µg/band with mean recovery of 99.86±0.70. Statistical studies were done showing no significant difference in comparison with the official method.

Section D: HPLC Method for Determination of Torsemide

This section utilized HPLC technique which allowed complete separation of TOR from its degradation products using a mobile phase consisted of phosphate buffer pH 4 – acetonitrile (3: 2, v/v) with UV detection at 287 nm and flow rate of 1mL min⁻¹. The proposed procedure was successfully applied for the determination of TOR in its pure powder form, laboratory prepared mixtures and dosage form.

Section E: Ion Selective Electrode Method for Determination of Torsemide

This section demonstrates a comparative study of the proposed four sensors for the stability indicating determination of torsemide in presence of its degradation products. The study revealed that sensor 1 is the least selective and sensitive electrode due to the absence of 2-HP-β-CD in its fabrication. Sensors 2-4 showed higher selectivity and sensitivity with less response time due to the presence of 2-HP-β-CD. On the other hand, performance characteristics of sensors 2-4 were compared from the point of the type of plasticizer. It was found that sensor 4 with o-NPOE has the most sensitive, selective, fast and precise response. The four sensors succeeded in the
determination of TOR in pure form, its pharmaceutical formulation and in presence of its degradation products to different extents.

The thesis contains: 61 Figure, 93 Table and 171 Reference. In addition to Appendix and Summary in Arabic
I.1. Introduction

Amide is a very important group in nature since it is the link by which amino acids join together to form peptides, which make up proteins in our bodies. This deceptively simple group has an unexpected feature, which is responsible for much of the stability of proteins\(^{(1)}\). Amides are organic compounds that contain the functional group consisting of an acyl group (R-C=O) linked to a nitrogen atom. The term refers both to a class of compounds and a functional group within those compounds. Also, it refers to deprotonated form of ammonia or amine, often represented as anions R\(_2\)N\(^{-}\).

I.2. Nomenclature

Amides have NH\(_2\), NHR, or NR\(_2\) group in place of the OH group of a carboxylic acid. Amides are named by replacing the term “oic acid”, “ic acid” or “ylic acid” in the parent’s acid name with amide. Cyclic amides are called lactams, they are necessarily secondary or tertiary amides. Their nomenclature is similar to that of lactones; they are named as 2-azacycloalkanone\(^{(2)}\).

I.3. Structure and bonding

The simplest amides are derivatives of ammonia where one hydrogen atom has been replaced by an acyl group. The ensemble is generally represented as RC(O)NH\(_2\). Amides are usually regarded as derivatives of carboxylic acids in which the hydroxyl group has been replaced by an amine or ammonia.

The lone pair of electrons on the nitrogen is delocalized onto the carbonyl, thus forming a partial double bond between N and the carbonyl carbon which is responsible for the restricted rotation about this C-N bond\(^{(1, 3)}\).

I.4. Properties

I.4.1. Basicity

Compared to amines, amides are very weak bases and their tendency to attract
hydrogen ions is so slight. This lake of basicity is explained by bond nature of amides at which the nitrogen lone pair is delocalized by resonance. This resonance has two effects which prevent the lone pair acting as a base. First, because the lone pair is no longer located on an atom as intense negative region, there is no attraction. Second, delocalization makes molecules more stable, so to reclaim lone pair and attract hydrogen ion, the delocalization has to be broken and that will cost energy. On the other hand, amides are much stronger bases than carboxylic acids, esters, aldehydes and ketones.

I.4.2. Solubility

Typically, amides are less water soluble than comparable amines and carboxylic acids since these compounds can both donate and accept hydrogen bonds. But small amides can participate in hydrogen bonding with water and other protic solvents, hence their water solubility is greater than that of corresponding hydrocarbons\cite{3}.

I.5. Amide synthesis

Amides are commonly formed via reactions of a carboxylic acid or its derivatives (acid chloride and acid anhydride) with an amine. Also, other methods with different substrates and catalyzing reagents are known.

In addition, the simple direct reaction between an alcohol and an amine was reported to be effective using special ruthenium-based catalyst in a so called dehydrogenative acylation\cite{4}.

I.6. Amide reactions

Owing to their resonance stabilization, amides are less reactive under physiological conditions, which is comforting, since proteins are composed of amino acids linked together by amide bonds. However, amides undergo many chemical reactions, usually through an attack on the carbonyl breaking the carbonyl double bond. Some of these reactions are: dehydration, Hofmann rearrangement, amide reduction and nitrosation.
I.7. Amide hydrolysis

Amides can be hydrolyzed in hot alkali as well as in strong acidic conditions. Acidic conditions yield the carboxylic acid and the ammonium ion while basic hydrolysis yields the carboxylate ion and ammonia. Acid hydrolysis of amides involves the formation of protonated carbonyl group which is electrophilic enough to be attacked by water. Then, protonation of the nitrogen atom to be good leaving group.

I.8. Stability assessment of amides

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy. Instability of pharmaceuticals can cause changes in the physical, chemical, pharmacological, and toxicological properties of the active pharmaceutical ingredients. Pharmacists should therefore take various factors into consideration, for example drug stability, possible degradation products, and potential interactions with excipients used in the formulation, to ensure successful therapy. To assess stability of drug product, methods are used which enable accurate and precise quantification of the drug, its degradation products, and interaction products, if any.

As mentioned before, amide can be hydrolyzed in acidic or basic conditions into degradation products which may be with no pharmacological activity. Thus stability indicating methods are required to afford the selective determination of a drug substance in presence of its decomposition and reaction products\(^5\).

Different techniques have differing degrees of sophistication, sensitivity, selectivity, cost and also time requirements. The selection of the best procedure for a given determination is an important task. The following considerations are main factors in the choice of an analytical technique for stability studies and quality control laboratories\(^6\):

1. The nature of material to be investigated: thermolabile, radioactive or photosensitive
2. Possible interference from components of the material other than those of interest and the possible need for sample clean-up to avoid matrix interference.

3. The concentration range which need to be investigated, limit of detection, level of accuracy and the degree of selectivity required for a quantitative analysis.

4. The time required to complete the analysis and the facilities available.

5. The type and purpose of analysis required: elemental or molecular, routine or occasional.

In this thesis, different selective analytical techniques were adopted for the determination of intact rebamipide, torsemide, perindopril and amlodipine either alone, in their binary mixture or in presence of their degradation products. These techniques include: spectrophotometry, chromatography and potentiometry.

The suggested methods are simple, accurate, selective and sensitive for determination of the cited drugs. Application of the proposed methods to the analysis of the aforementioned drugs in their pharmaceutical formulations shows that neither the excipients nor the degradation products interfere with the determinations.
II.1. Introduction

High blood pressure is associated with decreased life expectancy and increased risk of stroke, coronary heart disease and other end organ disease e.g. retinopathy or renal failure. Other risk factors for vascular disease that may be synergistic include: smoking, obesity, hyperlipidaemia, diabetes and left ventricular hypertrophy. In some patients with mild hypertension, changing life style may be sufficient but in other cases without additional risk factors treatment is required. Several groups of drugs by different mechanisms reduce blood pressure. The main groups are:

- β-adrenoceptor antagonists (β-blockers) e.g. propranolol and atenolol.
- Thiazide diuretics e.g. hydrochlorothiazide.
- Angiotensin converting enzyme inhibitors (ACE inhibitors) e.g. perindopril.
- Angiotensin antagonists e.g. losartan.
- Calcium channel blockers e.g. amlodipine and nifedipine.
- α-adrenoceptor antagonists e.g. prazosin.
- Centrally acting drugs e.g. clonidine and methyldopa.

β-blockers and thiazide diuretics are the first line drugs in the treatment of hypertension. While ACE inhibitors and calcium channel blockers reduce the risk of stroke, coronary heart disease and cardiovascular death. α-adrenoceptor antagonists have favorable effects on blood level\(^7\).

II.2. Perindopril

Perindopril (PER) is an angiotensin converting enzyme inhibitor (ACE inhibitor) used in the treatment of hypertension and heart failure. Angiotensin II is a powerful circulating vasoconstrictor and inhibition of its synthesis in hypertensive patients results in a fall in peripheral resistance and a lowering of blood pressure. Perindopril is also used to reduce the risk of cardiovascular events in patients with stable ischaemic heart disease. Angiotensin converting enzyme inhibitors do not impair
cardiovascular reflexes and are devoid of many of the adverse effects of the diuretics and β-blockers\(^{(7)}\).

Perindopril is available in two salts forms, perindopril arginin which is more stable than perindopril erbumine.

**II.2.1. Structure**

Perindopril (PER), chemically it is \((2S,3aS,7aS)-1-[(2S)-2-[[1(S)-1-(Ethoxycarbonyl)butyl]amino]-1-oxopropyl]octahydro-1H-indole-2-carboxylic acid\(^{(8)}\).  

![Chemical structure of Perindopril](image)

**II.2.2. Physical properties\(^{(9)}\)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>(C_{19}H_{32}N_{2}O_{5}). (C_{6}H_{14}N_{4}O_{2})</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>542.7</td>
</tr>
<tr>
<td>Description</td>
<td>A white or almost white, slightly hygroscopic, crystalline powder. It exhibits polymorphism.</td>
</tr>
<tr>
<td>Solubility</td>
<td>Freely soluble in water, alcohol and in chloroform; soluble or sparingly soluble in dichloromethane.</td>
</tr>
</tbody>
</table>

**II.2.3. Stability**

Perindopril acts as a prodrug of the diacid perindoprilate, which is the active form to increase its protein binding and absorption. After oral doses perindopril is rapidly absorbed with a bioavailability of about 65 to 75%. It is extensively metabolized mainly in the liver, to perindoprilate which is the active drug. The bioavailability of
perindopril is 25%\(^{(9)}\). Dewani et al\(^{(10)}\) studied the stability of perindopril erbumine under different stress conditions as acid, base, neutral, thermal hydrolysis and oxidation and it was found that perindopril is degraded under these different conditions with different ratios to give one degradation product. Also, it was found that alkaline degradation produced the highest yield of degradation product, indicating that alkaline hydrolysis is the least hard conditions with the highest probability. The high tendency of PER for hydrolysis into perindoprilate makes its analysis in presence of its alkaline degradation product (active metabolite) an analytical task of potential.

II.2.4. Methods of analysis of Perindopril

Perindopril erbumine is an official drug. Its analysis was described in British Pharmacopoeia as well as non pharmacopeial literatures either in pharmaceutical formulations or in biological fluids in binary mixtures with co-formulated drugs and in presence of its metabolites. But up to now, there is no reported literature for the analysis of perindopril arginin. The methods of analysis of perindopril erbumine include:

II.2.4.1. Pharmacopeial method

Perindopril erbumine was assayed in BP\(^{(11)}\) via non aqueous titration method by dissolving PER in anhydrous acetic acid and using 0.1M perchloric acid as a titrant. The end point was determined potentiometrically.

II.2.4.2. Spectroscopic methods

(a) Spectrophotometric methods

Erk\(^{(12)}\) described spectrophotometric methods based on first derivative with zero crossing technique and first derivative of the ratio spectra at 226.5 nm for simultaneous quantitative determination of PER and indapamide in binary mixtures.
The same binary mixture was resolved spectrophotometrically using the absorbance correction principle for perindopril at 210 nm\(^{(13)}\).

**(b) Colorimetric methods**

Colorimetric determination of PER through the reaction with either 2,3-dichloro-5,6-dicyano-p-benzo-quinone (DDQ), 7,7,8,8-tetracyano-quinodimethane (TCNQ), tetracyanoethylene (TCNE), chloranil or p-chloranilic acid was described for determination of PER in dosage form\(^{(14)}\). UV detection at 252 nm for the copper complex formed by the flow of PER through packed reactor of CuO for derivatization was also applied for the analysis of PER in dosage form\(^{(15)}\).

One more method used direct spectrophotometry and atomic absorption spectrometry for the analysis perindopril and ramipril through ternary complex formation with CuSO\(_4\)/eosin solution at 535 & 327.7 nm, respectively\(^{(16)}\).

**II.2.4.3. Chromatographic methods**

**(a) Gas chromatography**

Few gas chromatographic procedures were introduced for the determination of PER and its metabolite in biological fluid after derivatization with either heptafluorobutyric anhydride or NO-bis(trimethylsilyl) acetamide pyridine using helium as carrier gas and mass fragmentographic detection\(^{(17)}\) or helium carrier gas and electron capture detection\(^{(18)}\). In pharmaceutical formulation, it was determined by GC on glass column coated with methylsilicone with helium gas\(^{(19)}\).

**(b) High performance liquid chromatography**

Some HPLC methods were developed for determination of PER in pharmaceutical dosage forms in presence of co-formulated drugs or in human plasma for screening and bioequivalence studies. These methods are summarized in Table 1 as follows:
Table 1: Different HPLC methods described in literature for determination of Perindopril

<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>Detection</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt;</td>
<td>0.1% ammonia solution – methanol (20: 80, v/v)</td>
<td>MS</td>
<td>Estimation of PER &amp; its metabolite in human plasma&lt;sup&gt;(20)&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Gradient elution of 0.15% formic acid – water – acetonitrile – ammonium acetate (pH 10)</td>
<td>MS</td>
<td>Screening of basic drugs in equine urine&lt;sup&gt;(21)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyanopropyl C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Phosphate buffer (pH 6) – methanol (55: 45, v/v)</td>
<td>UV at 210 nm</td>
<td>Simultaneous determination of PER &amp; indapamide in binary mixture&lt;sup&gt;(22)&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Phosphate buffer pH (2.4) – acetonitrile (7: 3, v/v)</td>
<td>UV</td>
<td>Determination of PER and indapamide in dosage form&lt;sup&gt;(12)&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMC-pack C&lt;sub&gt;8&lt;/sub&gt;</td>
<td>Phosphate buffer – acetonitrile (37: 63, v/v) pH adjusted to 2.5</td>
<td>UV at 215 nm</td>
<td>Evaluation of impurities level of PER in tablets&lt;sup&gt;(23)&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Ammonium acetate – acetonitrile (30:70, v/v)</td>
<td>MS</td>
<td>Determination of PER in plasma&lt;sup&gt;(24)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Li Chrosorb C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Phosphate buffer (pH 2-6) – acetonitrile (3: 2, v/v) pH adjusted to 2.5</td>
<td>UV at 211 nm</td>
<td>Determination of PER in tablets&lt;sup&gt;(25)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>