Method development work on separation and simultaneous determination of specified and unspecified impurities of Amlodipine Besilate, Hydrochlorothiazide and Olmesartan medoxomil Film-Coated tablets

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Abstract: The three active moieties present in the drug product has specified impurities in their individual monographs of Pharma Europe (Ph. EUR.). Amlodipine Besilate has 8 impurities, Impurity-A, B, C, D, E, F, G and H in which 4 impurities are specified impurities, which are Impurity-A, D, E and F. Olmesartan medoxomil also have 4 impurities, Impurity-A, B, C and D in which Impurity-A and Impurity C are specified impurities. Hydrochlorothiazide is also having 3 impurities, Impurity-A, B and C and is specified as per Monograph. Hence, initially the target of the method is separate all impurities and active molecules, total number of peaks for the separation were 15 peaks. A robust method developed for the separation of all the peaks was done on High performance liquid chromatographic system(HPLC) with Photo-diode array detector(PDA) and using Polar RP-5, 250mmX 4.6mm I.D and 4µm particle size and with pH-3.0 phosphate buffer as Mobile phase-A and Acetonitrile as Mobile phase-B with a simple gradient of (Time in min/%B)-0/15,6/15,10/25,45/35,80/20,90/15 and 95/15. Column oven temperature is maintained at 45°C, Flow rate 0.8mL/min and chromatogram monitored at 230nm. The developed method is able to separate all the impurities with minimum resolution of 2.0 and good response of peaks was found. The method can be used for the method development of simultaneous determination of impurities of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil in drug products.

Key words: HPLC, PDA, Specified, Unspecified impurities

INTRODUCTION

Amlodipine Besilate: A white or almost white powder. Slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol, slightly soluble in 2-propanol. No polymorphism encountered for this molecule[1]. The structure and IUPAC name is shown in fig.1

Molecular formula: C26H31ClN2O8S
Molecular Weight: 567.05

(RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulfonate.

Fig. 1 Structure and Nomenclature of Amlodipine Besilate.

The MHRA granted Pharmaceutical Services Incorporated (PSI) NV Marketing Authorisations (licenses) for the medicinal products Amlodipine 5mg Tablets (PL 19156/0033) and Amlodipine 10mg Tablets (PL 19156/0034), on 21st May 2007. These are prescription only medicines (POM) for the treatment of high blood pressure (hypertension) or a certain type of chest pain called angina, a rare form of which is Prinzmetal’s or variant angina.

Amlodipine Tablets contain the active ingredient Amlodipine Besilate, which is a type of medicine known as a calcium-channel blocker. It relieves heart problems by widening blood vessels to allow more blood through. This helps reduce blood pressure and relieve the strain on the heart muscles. [1]

Organic Impurities: The pharmacopeial impurities were shown in fig. 2 (Ph. Eur 7.4) [2]
Fig.2: Pharmacopeial impurities of Amlodipine Besilate (ph.eur 7.4)

Chromatographic conditions: (Ph. Eur 7.4) The related substances of Amlodipine Besilate can be analyzed using C18, 250mm x 4.0mm, 5µm or its equivalent with 1.5mL/min, 20µL of injection volume and column temperature 30°. The retention time is the twice the retention time of Amlodipine. The eluent is a 2.3g/L Ammonium acetate buffer: Methanol (30:70) v/v. The eluent is the diluent for sample preparations. The eluent is monitored at 237nm. The system suitability is resolution between Impurity B and G should not less than 2.0 [2].

Olmesartan Medoxomil: It is white to off white powder and it is an angiotensin II receptor antagonist which has been used for the treatment of high blood pressure. It was developed by Sankyo in 1995, and is sold under the trade name Benicar and Olmecip (Cipla). An ester prodrug, it is completely and rapidly hydrolyzed to the active acid form, olmesartan [3]. It is practically insoluble in water and sparingly soluble in methanol [4]. The structure and IUPAC name [5] is shown in fig. 3.

Molecular formula: C_{29}H_{30}N_{6}O_{6}

Molecular Weight: 558.6

(5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-((4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl)-1H-imidazole-5-carboxylate

Fig.3 Structure and Nomenclature of Olmesartan medoxomil.

Organic Impurities: The pharmacopeia impurities were shown in fig. 4 (Ph. Eur 7.4) [5]

Specified impurities: A and C

Unspecified impurities: B and D
Impurity-A  
[Chemical structure image]

Impurity-B  
[Chemical structure image]

Impurity-C  
[Chemical structure image]

Impurity-D  
[Chemical structure image]

Fig.4: Pharmacopeial impurities of Olmesartan medoxomil (Ph.eur 7.4)

Chromatographic conditions: (Ph. Eur 7.4) The related substances of Olmesartan medoxomil can be analyzed using C8, 100mm x 4.6mm, 3.5µm or its equivalent with 1.0mL/min, 20µL of injection volume and column temperature 40°C. The eluent uses buffer 2.04g/L Pot. dihydrogen phosphate, pH 3.4 with OPA and mobile phase with gradient elution having M. P-A: Buffer: ACN (80:20); M. P-B: Buffer: ACN (20:80) and the gradient program time (min) /%B 0/25,10/25,3/100,45/100. Acetonitrile is used as diluent for sample preparations. The eluent is monitored at 250nm. The system suitability is resolution between Impurity B and Olmesartan medoxomil should not less than 3.5 [5]

Hydrochlorothiazide: It is white to off white powder and it is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones.[6]. The structure and Nomenclature of Hydrochlorothiazide is shown in fig.5

Molecular formula: C_{7}H_{8}ClN_{3}O_{4}S_{2}

Molecular Weight : 297.7

Fig.5 Structure and Nomenclature of Hydrochlorothiazide.
Organic Impurities: The pharmacopeial impurities were shown in fig.6 (Ph. Eur 7.4) [7]

Specified impurities: A, B and C

Un specified impurities: B and D

![Impurity-A](image1.png) ![Impurity-B](image2.png) ![Impurity-C](image3.png)

Fig.6: Pharmacopeial impurities of Olmesartan medoxomil (ph.eur 7.4)

Chromatographic conditions: (Ph. Eur 7.4) The related substances of Olmesartan medoxomil can be analyzed using C18, 100mm x 4.6mm, 3µm or its equivalent with 0.8 mL/min, 20µL of injection volume and column temperature 25°C. The eluent uses buffer pH 3.2 phosphate buffer and mobile phase with gradient elution having: M.P-A: Buffer: Methanol: THF (940:60:10); M.P-B: Buffer: Methanol: THF (500:500:50) and the gradient program time (min) /%B 0/0, 17/45, 30/45, 35/0, 50/0. (1:1) Methanol: Acetonitrile, 200mL of buffer mixture is used as diluent for sample preparations. The eluent is monitored at 224nm. The system suitability is resolution between Impurity A and Hydrochlorothiazide should not less than 2.5 [7]

Hypertension (Blood pressure) is the force exerted by blood against the walls of the blood cells, and the magnitude of this force is dependent on the cardiovascular output and the resistance of blood vessels. Hypertension is having blood pressure higher than 140mm Hg over 90mm Hg. In the US alone, about a third of all people over the age of 20 years have hypertension, as measured by high blood pressure and taking antihypertensive medications in 2011-2012. Control of hypertension has become a key national priority in the US[8].

Drugs are usually started as monotherapy (just one drug) and at a low dose initially. If there are any side-effects associated with drugs, they are usually minor.

A number of different classes of drug are available and all are suitable for lowering blood pressure:

- Diuretics (including thiazides, chlorthalidone and indapamide), which have been a cornerstone of treatment since 1977 E.g. Hydrochlorothiazide
- Beta-blockers E.g. Olmesartan and Amlodipine
- Calcium antagonists E.g. Amlodipine Besilate
- Angiotensin-converting enzyme (ACE) inhibitors
- Angiotensin receptor blockers.

The choice of drug depends on the individual and any other conditions they may have. While a single drug is usually tried in monotherapy first, a combination of at least two antihypertensive drugs is usually required [8].
Triple therapy of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil had a significant benefit when compared to its single and dual combinations of individual moieties in patients with moderate to hypertension [9].

The triple combination of three molecules is commercially available under the brand name, Tribenzor and it is provided as a tablet for oral administration, is a fixed combination of olmesartan medoxomil (ARB), Amlodipine (CCB), and hydrochlorothiazide (thiazide diuretic) [10]. The increase in patients with hypertension is going higher and the need for the combination of these drugs shows considerable benefits to its individual components and their dual combinations. For any drug that is to be medicated must have high quality. The quality of the drug products can be assessed by using multiple analytical techniques depends on the sensitivity and application of the methods. The test methods include Identification, Assay, Dissolution, water content, Content uniformity tests, Microbiology tests and related substances method.

As per my knowledge and in literature, there are no analytical methods for simultaneous determinations related substances of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil in a single method. There are several methods were reported for the related substances determinations of individual components or with combination of other moieties. There are several methods reported for related substances of Amlodipine includes chiral determination [11] for the determination of isomers, simultaneous impurity determination in Amlodipine Besilate and Benzepril HCl combination drug product [12] and Development and validation of stability indicating RP-UPLC method for simultaneous determination of related substances of s (-) Amlodipine and s (-) metoprolol succinate in fixed dose combination tablet dosage form [13].

**MATERIALS AND METHODS**

HPLC equipped with PDA detector 2998 series from Waters, Chemicals are obtained from Merck. HPLC grade water obtained in house using Milli-Q water system. HPLC grade Acetonitrile is obtained from Rankem. Amlodipine besilate and its impurity standards are obtained from Moehs, Olmesartan medoxomil and its impurity standards are obtained from Hetero laboratories. Hydrochlorothiazide and impurity standards are obtained from Unichem laboratories.

**RESULTS AND DISCUSSION**

The initial experiments for separation of a mixture of components done using pH 2.5 buffer and Accucore XL C18 column. Henceforth low pH is not right choice for the development. With this intention higher pH of 6.8 buffer selected for separation. Even though separations are not improved and also peak shapes are broad. Hence pH and selection of variety of buffers to be selected. In order to change the selectivity of components triflouric acetic buffer is used, with this Amlodipine and Olmesartan peak are merged. Here on pH optimization trials are taken. A pH value of 3.0 potassium dihydrogen phosphate buffer used. In effect of pH, peaks are well separated, but the baseline noise is high. To avoid the noise inertsil C8 column used, considerable decrease in baseline noise observed. Under those circumstances polar hydrochlorothiazide impurities are not well separated. Hence Phenomenex Polar RP-5 column with 250mm length X 4.6mm I. D and 5µ particle size is used for polar impurity resolution and required separations obtained as shown in fig.7. Table-1 gives the information about the sequential order of development trails. The selection of wavelength is done based on the wavelength maxima of the active moiety. But in this case selection of far more difficult because of having
different UV spectra of the three molecules. The wavelength is selected on overlapping the spectra of three moieties as shown in fig. 8. The selected wavelength 230nm gives optimum response to three activies Amlodipine, Hydrochlorothiazide, Olmesartan medoxomil and its impurities.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Column</th>
<th>Flow (mL/min)</th>
<th>CT(°C)</th>
<th>Buffer</th>
<th>pH</th>
<th>M.P.-A</th>
<th>M.P.-B</th>
<th>Gradient Time(min)/%B</th>
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<tbody>
<tr>
<td>Trail-1</td>
<td>Accucore XL C18 (2.6µm)</td>
<td>1.00</td>
<td>35</td>
<td>10mM KH2PO4</td>
<td>2.5</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 5 40 45 5 5 55 65</td>
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<tr>
<td>Observations: Amlodipine and Olmesartan medoxomil peaks are closely eluting.</td>
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<td>Trail-2</td>
<td>Accucore XL C18 (2.6µm)</td>
<td>1.00</td>
<td>45</td>
<td>10mM KH2PO4</td>
<td>6.8</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 5 8 20 30 5 5 20 70 70</td>
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<td>Observations: Amlodipine and Olmesartan medoxomil peaks are broad.</td>
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<td>Trail-3</td>
<td>Accucore XL C18 (2.6µm)</td>
<td>1.00</td>
<td>45</td>
<td>0.1% TEA</td>
<td>2.1</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 5 15 25 30 5 5 30 65 65</td>
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<tr>
<td>Observations: Amlodipine and Olmesartan medoxomil peaks were merged.</td>
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<tr>
<td>Trail-4</td>
<td>Accucore XL C18 (2.6µm)</td>
<td>1.00</td>
<td>45</td>
<td>10mM KH2PO4</td>
<td>3.0</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 5 15 25 30 5 5 30 65 65</td>
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<tr>
<td>Observations: Amlodipine and Olmesartan medoxomil peaks were well separated. Base line peaks are high unable to identify retentions of impurities.</td>
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<tr>
<td>Trail-5</td>
<td>C8 columns (5µm)</td>
<td>1.00</td>
<td>45</td>
<td>10mM KH2PO4</td>
<td>3.0</td>
<td>Buffer</td>
<td>ACN</td>
<td>Set of gradient trails with variations in compositions</td>
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<td>Observations: Base line noise reduced drastically. Separations were not much effective. Increase in initial composition of Mobile phase-B, the noise is considerably decreased but the initially eluted Hydrochlorothiazide impurities were merging.</td>
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<td>Trail-6</td>
<td>Phenyl columns (5µm)</td>
<td>0.80</td>
<td>40</td>
<td>10mM KH2PO4</td>
<td>3.5</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 15 75 80 10 10 90 90</td>
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<tr>
<td>Observations: Separations were improved but not much effective.</td>
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<tr>
<td>Trail-7</td>
<td>Polar RP-5 (5µm)</td>
<td>0.80</td>
<td>45</td>
<td>10mM KH2PO4</td>
<td>3.0</td>
<td>Buffer</td>
<td>ACN</td>
<td>0 6 10 45 80 85 15 1 5 25 35 80 80</td>
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<tr>
<td>Observations: All the impurities were well separated and Base line is reduced. Root-cause for the base line peaks were identified and were controlled.</td>
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Table-1: Set of development sequential events of development trials for the separations of Amlodipine, Hydrochlorothiazide, Olmesartan medoxomil and their specified impurities.

Fig.7: Chromatogram with separation of specified impurities of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil

Fig.8: Overlaid UV spectra of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil

CONCLUSION

Total 15 peaks were separated using chromatographic techniques, for the impurity profiling of Amlodipine besilate, Hydrochlorothiazide and Olmesartan medoxomil Film-Coated tablets. The developed method is able to separate all the impurities with minimum resolution of 2.0 and good response of peaks was obtained. The method can be used for the method development of simultaneous determination of impurities of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil in drug products.

References

[1] AMLODIPINE 5MG TABLETS PL 19156/0033, AMLODIPINE 10MG TABLETS PL 19156/0034, UKPAR-MHRA

[2] Ph.eur 7.4 Amlodipine Besilate


[5] Ph.eur 7.4 Olmesartan medoxomil


[7] Ph.eur 7.4 Hydrochlorothiazide.


[12] Simultaneous determination of Amlodipine Besylate and Benazepril HCl impurities in finished pharmaceutical products by UPLC