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Euro Polymer Science 2019: Validated Chromatographic methods for the simultaneous determination of co-formulated drugs in pharmaceutical formulation - Dina A.El-Cairo University

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Two specific, sensitive, and precise stability-indicating chromatographic methods have been developed, optimized and validated for determination of perindopril arginin (PER) and amlodipine besylate (AML) in their mixtures and in the presence of their degradation products. The first method was based on thin-layer chromatography (TLC) combined with densitometric determination of the separated bands. Adequate separation was achieved using silica gel 60 F254 TLC plates and ethyl acetate–methanol–toluene–ammonia solution, 33% (6.5:2:1:0.5 by volume), as a developing system. The second method was based on high-performance liquid chromatography, by which the proposed components were separated on a reversed-phase C18 analytical column using a mobile phase consisting of phosphate buffer (pH 2.5, 0.01 M)–acetonitrile–tetrahydrofuran (60:40:0.1% by volume) with ultraviolet detection at 218 nm. Different parameters affecting the suggested methods were optimized for maximum separation of the cited components. System suitability parameters of the two developed methods were also tested. The suggested methods were validated in compliance with the ICH guidelines and were successfully applied for the quantification of PER and AML in their commercial tablets. Both methods were also statistically compared to each other and to the reference methods with no significant differences in performance.

Perindopril (PER), (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)butyl] amino]-1-oxopropyl] octahydro-1H-indole-2-carboxylic acid, is an angiotensin-converting enzyme inhibitor that is used in the treatment of hypertension and heart failure. It is also used to reduce the risk of cardiovascular events in patients with stable ischaemic heart disease (1). PER is converted in the body into its active metabolite, perindoprilate. Amlodipine (AML), 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5 pyridinedicarboxylic acid 3-ethyl 5-methyl ester, is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina. Recently, perindopril arginin has been marketed in combination with amlodipine besylate in tablets for the treatment of essential hypertension.

This combination is advised for patients whose blood pressure is not adequately controlled by either drug alone, because it has been shown to be superior in lowering systolic and diastolic blood pressures when compared with either of the monotherapy regimens. The combination, thereby also has significantly fewer dose-dependent adverse experiences than high-dose calcium antagonist monotherapy.

PER is formulated in dosage form in two salt forms, perindopril erbumine and perindopril arginin. The advantage of arginin salt over erbumin is that it imparts a certain stability to perindopril and inhibits the cyclyzation process in open containers or at high temperatures.

Instruments:

The liquid chromatography system consisted of an isocratic pump, Model G1310A (Agilent Technologies, Santa Clara, CA), an ultraviolet (UV) variable wavelength detector (Model G1314A, Agilent 1100 Series), a Rheodyne injector, Model 7725 I (Sigma-Aldrich, Taufkirchen bei München, Germany) equipped with a 20 µL injector loop (Agilent Technologies). The stationary phase was a 250 × 4.6 mm i.d. Nucleosil C18 analytical column from Alltech Associates (Deerfield, IL). The mobile phase was a phosphate buffer solution (1.74 g of dipotassium hydrogen phosphate anhydrous was dissolved and diluted to a volume of 900 mL with deionized water, and adjusted to a pH of 2.5 with 85% phosphoric acid)–acetonitrile–tetrahydrofuran (60:40:0.1% by volume). The mobile phase was filtered through a 0.45 µm Millipore membrane filter and was degassed for 15 min in an ultrasonic bath before use. UV detection was conducted at 218 nm. The samples were filtered also through a 0.45 µm membrane filter, and were injected by the aid of a 20 µL Agilent analytical syringe.

Materials and reagents

Pure standard

Standard PER (99. 68%) and AML (100.14%) were supplied by Servier Egypt Industries (Cairo, Egypt). Their purities were assessed according to the manufacturer method and official (5) HPLC methods, respectively.

Pharmaceutical formulation

Coveram tablets are available in several different strength combinations, including 5/5, 10/10, 5/10 and 10/5 mg of PER and AML, respectively. They are manufactured by Servier (Ireland) Industries Limited for Les Laboratories Servier (France). Batch numbers 77597, 376112, 73935 and 59423 were purchased from the Egyptian markets.

Degraded sample

Both PER and AML were stressed for alkali degradation studies. Solutions were prepared by dissolving, separately, 25 mg of pure PER and AML powders in 25 mL of 0.1 M and 1 M NaOH, respectively, and the solutions were refluxed for 2 h in the case of PER and 1 h for AML. The degradation process was followed by TLC using 33% ethyl acetate–methanol–toluene–ammonia solution (6.5:2:1:0.5 by volume) as the developing system. Degradation products were

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precipitated at specific pH for each degradation product by controlling the pH using hydrochloric acid, washed three times each with 10 mL of distilled water and re-crystallized from methanol. The degradation products were elucidated by IR and MS.

Chemicals and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. These included hydrochloric acid, 33% ammonia solution, toluene, ethyl acetate, sodium hydroxide, methanol and chloroform (El-Nasr Pharmaceutical Chemicals Cairo, Egypt); tetrahydrofuran (THF) and acetonitrile; HiPerSolv, HPLC grade (Merck); o-phosphoric acid (85%) (BDH, Poole, England); de-ionized water, bi-distilled from an Aquatron Automatic Water Still A4000.

Standard solutions

Stock standard solutions of both perindopril arginin and amlodipine besylate (2 mg/mL) were prepared in methanol (for the TLC densitometric method) and 0.1 mg/mL in the mobile phase for the HPLC method.

Stock standard solutions of the alkaline degradation products derived from the complete degradation of standard solutions of both PER and AML (1 mg/mL) were prepared in methanol (for the TLC densitometric method) and 0.1 mg/mL in the mobile phase for the HPLC method.

All stock standard solutions were freshly prepared on the day of analysis and stored in the refrigerator to be used within 24 h.

Procedure

Chromatographic conditions

TLC was performed on 20 × 10 cm TLC aluminum sheets precoated with 0.25 mm silica gel 60 F254; the samples were applied as bands (bandwidth: 4 mm; spacing: 13.8 mm; 15 mm from the bottom edge of the plate). Linear ascending development was conducted in a chromatographic tank, previously saturated with 33% ethyl acetate–methanol–toluene–ammonia solution (6.5:2:1:0.5, by volume) for 1 h at room temperature. The developed plates were air dried and scanned at 218 nm for PER and 237 nm for AML on a Camag TLC scanner 3 operated in the absorbance mode, with a deuterium lamp as a source of radiation.

HPLC was conducted on an RP-C18 column (250 × 4.6 mm i.d, 5 µm p.s.). The mobile phase consisted of phosphate buffer (pH 2.5, 0.01 M)–acetonitrile–tetrahydrofuran (60:40:0.1% by volume). The mobile phase was filtered through a 0.45 µm Millipore membrane filter (Billerica, MA) and was degassed for 15 min in an ultrasonic bath before use. UV detection was conducted at 218 nm. The system was operated at ambient temperature. The flow rate was isocratic at 1 mL/min. The samples were also filtered through a 0.45 µm membrane filter, and were injected by the aid of a 20 µL Hamilton analytical syringe.

Conclusion

Perindopril arginin and amlodipine besylate are co-formulated in antihypertensive formulations, and because they are widely used drugs, it is important to find simple, rapid and inexpensive methods for their analysis, especially in quality control laboratories. The suggested chromatographic methods provide simple, accurate and reproducible stability-indicating methods for their quantitative analysis in the presence of their degradation products. The developed TLC method is sensitive and has the advantages of short time for analysis of different concentrations in one run, large sample capacity and use of a minimal volume of solvents. The HPLC method offers good resolution between the four proposed components within a suitable analysis time. It is highly specific, but more expensive. The proposed methods have advantages over other published methods for analyzing the binary mixture in the presence of their degradation products. Therefore, the applied methods may be useful for stability investigation of the active drugs and for checking the extent of degradation in pharmaceutical formulations.

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