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Simultaneous Determination of Amlodipine with H_1-Receptor Antagonists by Reversed Phase High Performance Liquid Chromatography and Application to Interaction Studies

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ABSTRACT

A rapid, fast and precise method has been developed and validated for the simultaneous determination of amlodipine with H_1-receptor antagonists (cetirizine, fexofenadine, and buclizine) from dosage forms. The chromatography was performed on a Purospher® Star, C_{18} (5 µm, 250 × 4.6 mm) column using acetonitrile: buffer (0.01 mM) (40:60, v/v, pH adjusted to 3.0), as a mobile phase. The mobile phase was pumped at a flow rate of 1.0 mL·min^{-1} and UV detection was performed at 240 nm. The method was validated for linearity, accuracy, precision and specificity. The method was applied to study the interaction between amlodipine and H_1-receptor antagonists. These interactions were carried out in simulated gastric juice (pH 1), simulated full stomach (pH 4), blood pH (pH 7.4) and simulating GI (pH 9). The interacting drugs were heated at 37˚C with intermittent shaking and the samples were withdrawn every thirty minutes for three hours and drug contents were analyzed by RP-HPLC techniques. In most cases the *in vitro* availability of amlodipine was decreased. It was observed that the change in *in vitro* availability was pH dependent.

Keywords: Amlodipine; Cetirizine; Fexofenadine; Buclizine; Interactions; Reversed Phase High Performance Liquid Chromatography

1. Introduction

Amlodipine (**Figure 1**) is a 1, 4-dihydropyridine-based calcium antagonist, chemically it is R, S-2 [(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-ethoxy carbonyl-5-methoxycarbonyl-6-ethyl-1,4-dihydro pyridine or 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxy cabonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine. It differs from other members of this group including the trial product nifedipine, by the presence at 2-position of a dihydropyridine ring of a side-chain which carries a basic amino group [1]. According to Burges and his colleagues [2] it is the presence of this side-chain with its basic amino group which is primarily responsible for setting this particular antagonist apart from other chemically similar antagonists.

A number of HPLC methods are reported for the determination of amlodipine in literature. Some of them quantitate amlodipine in pharmaceutical formulations [3,4], while the others in human serum [5-11] or in combination with other formulations [12-15].

H_1-receptor antagonists are the mainstay of treatment for several allergic disorders, particularly rhinitis, conjunctivitis, dermatitis, urticaria and asthma [16,17]. Antihypertensive drugs and H_1-receptor antagonists can be co-administered in a number of cases. Numerous HPLC methods were reported for the quantitation of cetirizine dihydrochloride or fexofenadine hydrochloride with pseudoephedrine in combined pharmaceutical dosage forms [18], a method for cetirizine dihydrochloride and related impurities is also reported [19]. Simultaneous quantification of cetirizine or levocetirizine with cefpirome was reported by Arayne *et al*. [20], and buclizine with pyridoxine and meclizine were reported by Arayne *et al*. [21].

The objective of our study was to develop a new method for the simultaneous determination of amlodipine with H_1-receptor antagonists (cetirizine, fexofenadine hydrochloride and buclizine hydrochloride) since these drugs are co-prescribed at high frequency. The proposed method

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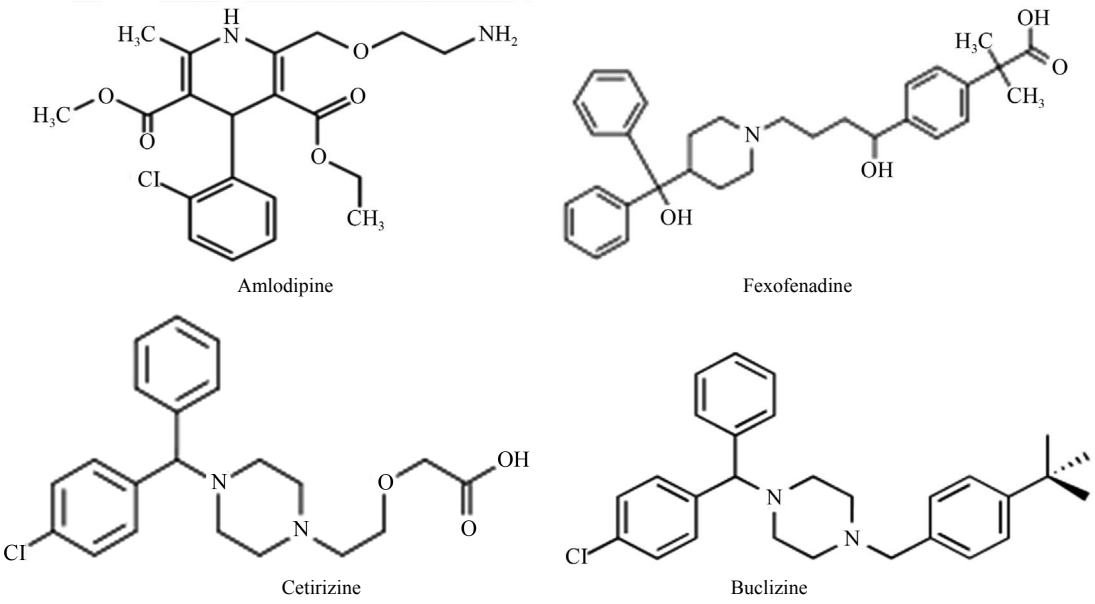

Figure 1. Chemical structures of amlodipine and H_1-receptor antagonists used in the present study.

was successfully applied for the determination of H_1-receptor antagonists in commercial tablets. The method was validated with respect to linearity, limit of detection and quantification, precision, accuracy, specificity and applied in interaction studies.

2. Experimental

2.1. Reagents

Reference standard of amlodipine (Sofvasc®, 5 mg) was obtained from Wilson's Pharmaceuticals. Cetirizine (Rigix®, 10 mg), fexofenadine (Fexet®, 60 mg) and buclizine (Longifene®, 25 mg) were obtained from Ali Gohar Pharmaceuticals, UCB Farchim SA, Switzerland, Getz Pharma Pakistan and Ali Gohar Pharmaceuticals respectively. Methanol and acetonitrile (HPLC grade) (TEDIA®, USA), hydrochloric acid (11 N; Merck Marker), glacial acetic acid, orthophosphoric acid (85%, from Merck Damstabt, Germany), potassium chloride, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate, sodium chloride, ammonium chloride, ammonia solution 26% were obtained from Sigma Aldrich (Germany).

2.2. Preparation of Buffers

Hydrochloric acid (0.1 N) was prepared by diluting 9 mL hydrochloric acid (36%, 11 N) in a liter volumetric flask and the volume was made up to the mark with deionized water. Buffer of pH 4 (chloride buffer) was prepared by dissolving 3.725 g of potassium chloride in one liter deionized water; pH was adjusted with 0.1 N hydrochloric acid. For the preparation of buffer of pH 7.4 (chloride buffer) 3.725 g of KCl was dissolved in 250 mL deionized water and pH was adjusted with 0.1 N HCl. Buffer of pH 9 (ammonia buffer) was prepared by dissolving 4.98 g of ammonium chloride in 100 mL of deionized water and adjusted to pH 9 with 10% ammonia.

2.3. Instrumentation and Chromatographic Conditions

A liquid chromatographic system equipped with Shimadzu model LC-10AT VP pump, a Shimadzu model SPD-10AT VP, variable wavelength UV-visible detector was used. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module to a Pentium 4 PC. The chosen conditions were: mobile phase acetonitrile: buffer (0.01 mM) (40:60, v/v), pH adjusted to 3.0, with flow rate of 1.0 $mL \cdot min^{-1}$. Column used was Purospher® Star, C_{18} (5 µm, 250 × 4.6 mm). The absorption maxima of amlodipine, cetirizine, fexofenadine and buclizine is 240 nm, 232 nm, 210 nm and 230 nm respectively, however, 240 nm was selected for the quantification as all these drugs gave good response at this wavelength.

2.4. Preparation of Stock and Working Standard Solutions

Stock solutions of amlodipine, and H_1-receptor antagonists (cetirizine, fexofenadine and buclizine) (100 $\mu g \cdot mL^{-1}$) were individually prepared freshly by dissolving appropriate amount of each reference standard in their respective mobile phase ratios to yield final drug concentrations. The stock solutions were diluted with mobile phase to yield working standard solutions (5 - 50 $\mu g \cdot mL^{-1}$) for preparation of calibration curve for each drug.

2.5. Analysis of Pharmaceutical Dosage Forms

Stock and working standard solutions of pharmaceutical dosage forms were also prepared by the same procedure as described above. For pharmaceutical dosage forms, twenty tablets each of amlodipine 5 mg (Sofvasc®) and interacting drugs, cetirizine (Rigix®, 10 mg), fexofenadine (Fexet®, 60 mg) and buclizine (Longifene®, 25 mg) were weighed and finely powdered in a mortar. An amount equivalent to drug content in each tablet was weighed, transferred to a 100 mL volumetric flask, dissolved by stirring for 10 minutes and the final volume made up with mobile phase. The primary stock solution was filtered through 0.45 µm Millipore filter paper and the filtrate was further diluted to prepare a secondary stock solution. Aliquots of the secondary stock solutions were diluted to their respective concentration and the samples were analyzed using proposed method.

2.6. Interaction Studies

Stock solutions (100 µg·mL^{-1}) of amlodipine, H_1-receptor antagonists (cetirizine, fexofenadine and buclizine) were prepared in simulated gastric juice, buffers of pH 4, 7.4 and 9 individually. These solutions were mixed in 1:1 ratio in flasks individually and kept on a water bath at 37˚C for three hours with stirring. The samples were withdrawn after 30 minutes time interval for 3 hours and drug contents were filtered through a millipore filter (0.45 µ) and analyzed by RP-HPLC. The % availability of each drug was then calculated with respect their standard samples.

3. Results and Discussions

3.1. Linearity, Limit of Detection and Quantification

A representative chromatogram of amlodipine with H_1-receptor antagonists (cetirizine, fexofenadine and buclizine) is shown in **Figure 2**, indicating complete separation of all these analytes. To evaluate the linearity of method, different dilutions in the range 0.5 - 25 µg·mL^{-1} were analyzed. The limit of detection (LOD) for this assay for amlodipine, cetirizine, fexofenadine and buclizine was 0.06, 0.12, 0.11 and 0.05 µg·mL^{-1} respectively. While limit of quantification (LOQ) was found to be 0.22, 0.41, 0.38 and 0.18 µg·mL^{-1} for amlodipine, cetirizine, fexofenadine and buclizine, respectively (**Table 1**).

3.2. Precision and Accuracy

To determine accuracy of the method, absolute recoveries at three different concentrations of amlodipine and H_1-receptors in placebo of respective dosage forms, were determined by assaying the samples and comparing peak areas of sample solution with respective standards. %RSD was calculated by standard method. These results showed that the method was precise (%RSD from 0.13% to 1.48%) and accurate (accuracy from 98.58% to 102.01%). Recovery tests were performed by adding known amounts of stock solutions to samples with known contents. The percentage of recovery was calculated by comparing the determined amount of these standards with the added amount (**Table 2**).

3.3. Specificity

No peak of excipients was found in chromatogram when these drugs were tested in presence of excipients, which proved that method can be applied successfully to dosage formulation. Furthermore, the mean % recovery values obtained also verified that this method could be applied in pharmaceutical dosage formulations.

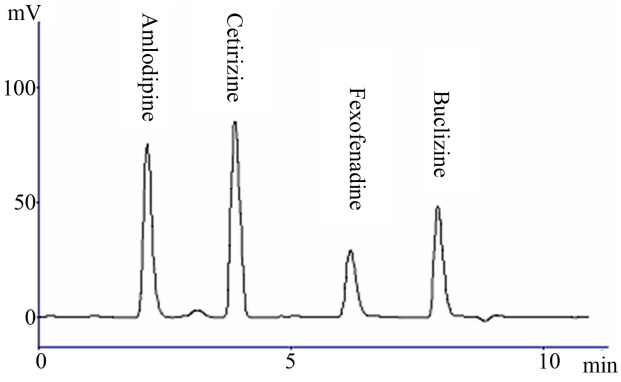

Figure 2. Chromatogram showing simultaneous determination of amlodipine with H_1-receptor antagonists.

Table 1. Linear regression functions and their statistical parameters of amlodipine and H_1-receptor antagonists.

Analyte	Concentration range (µg·mL^{-1})	Regression equation	r^2	LOD (µg·mL^{-1})	LOQ (µg·mL^{-1})
Amlodipine	0.5 - 25	y = 10171x + 2473.3	0.9984	0.06	0.22
Cetirizine	0.5 - 25	y = 6128.7x − 1950.0	0.9995	0.12	0.41
Fexofenadine	0.5 - 25	y = 4984.7x + 12743	0.9988	0.11	0.38
Buclizine	0.5 - 25	y = 14429x − 12309.0	0.9996	0.05	0.18

Table 2. Accuracy and precision of amlodipine and H_1-receptor antagonists.

Analyte	Spiked concentration ($\mu g \cdot mL^{-1}$)	Mean measured concentration ($\mu g \cdot mL^{-1}$)	Accuracy %	Precision %RSD
Amlodipine	8	8.00	100.00	0.13
	10	10.09	100.89	1.35
	12	12.24	102.01	1.06
Cetirizine	8	7.93	99.15	1.45
	10	9.86	98.61	0.74
	12	11.59	98.58	1.07
Fexofenadine	8	7.95	99.37	0.21
	10	10.80	100.08	1.81
	12	11.84	99.54	1.18
Buclizine	8	7.96	99.55	0.93
	10	10.09	100.90	1.48
	12	12.05	100.42	1.27

Table 3. *In vitro* interactions studies of amlodipine with H_1-receptor antagonists.

%Avail→			←At pH 1→				←At pH 4→					
↓Time(min)	Aml	Cet	Aml	Fexo	Aml	Buc	Aml	Cet	Aml	Fexo	Aml	Buc
30	70	50	81	79	100	102	55	65	49	53	101	103
60	70	68	84	85	101	103	69	66	76	78	100	100
90	75	71	88	76	100	48	77	75	99	89	102	101
120	79	71	87	84	100	51	79	72	115	101	101	102
150	79	77	81	87	100	67	80	79	120	124	100	101
180	80	74	83	87	100	73	83	70	128	130	100	100
%Avail→			←At pH 7.4→						←At pH 9→			
30	49	29	52	79	100	100	61	37	59	59	101	102
60	53	47	65	83	101	100	67	49	66	82	100	100
90	59	61	66	83	99	105	71	75	87	94	100	100
120	71	68	70	83	101	100	74	81	92	127	100	101
150	75	63	73	91	103	108	76	94	107	141	100	100
180	81	101	81	95	106	100	76	98	128	165	100	108

Aml: Amlodipine; Cet: Cetirizine; Fexo: Fexofenadine; Buc: Buclizine; %avail: %Availability.

3.4. Interaction Studies

The validated method as described above was used to monitor drug interactions between amlodipine and H_1-receptor antagonists. These interactions were carried out at physiological pHs, in simulated gastric juice (pH 1), simulated full stomach (pH 4), blood pH (pH 7.4) and simulating GI (pH 9). The results of these interactions are given in **Table 3**. The % availability of all drugs was 100% at the zero time point, but with time, the availability changed.

Amlodipine and cetirizine showed slightly reduced % availability, showing the formation of charge transfer complex of low molar absorptive values in buffers of pH 1, 4, 7.4 and 9. In amlodipine-fexofenadine interaction, a noticeable change in % availability was observed which might be due to formation of a complex between these two molecules, showing evidence of an interaction. On the contrary, there was no convincing change in availability % of amlodipine in presence of buclizine, indicative of absence of an interaction; which is due to lack of electron donating functionalities in buclizine.

4. Conclusion

We described the simultaneous determination of amlodipine with H_1-receptor antagonists in pharmaceutical dosage forms. The method was linear in the concentration range of (5 - 50 $\mu g \cdot mL^{-1}$).The method was applied to study the interactions. The interaction results showed that in most cases the availability of amlodipine was decreased

in the presence of H_1-receptor antagonists. This suggested that for coadministration of amlodipine with H_1-receptor antagonists, a proper interval should be given to avoid such interactions.

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