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Simultaneous determination of antihypertensive drugs in human plasma

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Abstract : A simple, touchy and inexpensive procedure got its start applying solid-phase extraction, combined with high performance liquid chromatographic procedure along with UV detection intended for determination of candesartan cilexetil and azilsartan medoxomil residues in human plasma. The evaluated parameters include the extracts by using silica gel the use of a aggregate of ethyl acetate/ 0.5 % formic acid (1:4, v/v) as eluent. The method was established the usage of plasma samples spiked with candesartan cilexetil and azilsartan medoxomil at different concentration levels (0.03 and 0.3 mg/L). Average recoveries (using each concentration six replicates) ranged 84-92%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.03-10.0 mg/L and limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg/mL and 0.03 mg/L respectively.
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Keywords : Solid-phase extraction; Candesartan; Azilsartan; Silica gel; HPLC LOQ; LOD.

INTRODUCTION

Antihypertensive are a class of drugs which might be used to treat hypertension (excessive blood pressure). Antihypertensive therapy seeks to prevent the complications of excessive blood strain, along with stroke and myocardial infarction. evidence suggests that reduction of the blood pressure via five mmHg can decrease the risk of stroke with the aid of 34%, of ischaemic heart ailment with the aid of 21%, and reduce the chance of dementia, coronary heart failure, and mortality from cardiovascular disease[1]. There are numerous training of antihypertensives, which lower blood stress by way of exclusive approach. a few of the maximum critical and maximum widely used capsules are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers[2].

Candesartan, an angiotensin-receptor blocker (ARB), is utilized alone or with other antihypertensive specialists to treat hypertension. Candesartan rivals angiotensin II for tying at the AT1 receptor subtype[3]. The chemical name of Candesartan cilexetil is((±)-1-hydroxyethyl-2-ethoxy- 1-[p-(o-1Htetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate cyclohe-xyl carbonate. Candesartan cilexetil is metabolised completely by esterases in the intestinal wall during absorption to the active candesartan moieity[4,5].

Azilsartan medoxomil is an angiotensin II receptor antagonist which has the chemical names (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1-{[2'-(5-oxo-4,5-dihydro-1,2,4oxadiazol-3 yl)biphenyl-4-yl]methyl}-1H-benzimidazole-7-carboxylate monopotassiuml salt and1H-Benzimidazole-7-carboxyliclacid,1-[[2'-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)[1,1'- biphenyl]-4-yl]methyl]-2-ethoxy-,(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester[6]. Azilsartan medoxomil is rapidly hydrolysed to the active moiety azilsartan by esterases in the gastrointestinal tract

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and/or during drug absorption. The enzyme carboxymethylenebutenolidase is a recently discovered hydrolysis mechanism for azilsartan medoxomil in the intestine and liver[7,8]. Azilsartan is an inverse agonist of the AT1 receptor, is a highly potent, selective and competitive antagonist of the angiotensin II type 1 receptor. Molecular basis of Azilsartan medoxomil reveals that it may be responsible for its clinical efficacy[9,10].

The target of this investigation is to build up an effective, basic, fast, validated and reliable technique for the routine biological sample analysis for the simultaneous determination of candesartan cilexetil and azilsartan medoxomil in human plasma.

EXPERIMENTAL

Standards, reagents and samples

The analytical standards of candesartan cilexetil (99.2%) and azilsartan medoxomil (99.5%) were obtained from Sigma Aldrich. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, ethyl acetate, formic acid and silica gel were supplied from Merck Limited and Blank human plasma was obtained from the local blood bank and stored at -18± 2°C prior to use.

Standard stock solutions

The candesartan cilexetil and azilsartan medoxomil standard stock solutions were individually prepared in acetonitrile at a concentration level 100 mg/L and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample preparation

Representative 5.0 g portions of human plasma fortified with 5 µL of working standard solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction procedure

The representative sample was homogenized using a high speed blender. Accurately weighed 5 g of plasma sample in different stoppered glass flasks and extracted with 100 mL of extraction solvent (ethyl acetate/ 0.5 % formic acid (1:4, v/v)) using an end over end shaker for 30 minutes. Filtered the contents and the filtrate was re-extracted with 50 ml of extracting solvent

Clean-up procedure

The pooled extract was reduced to small volume by evaporation and to this 2 g of alumina was added and filtered. The filtered extract was passed through a Silica gel cartridge and eluted with 50 ml of extraction solvent. The collected solvent extract was evaporated under vacuum to near dryness at 40°C using buchi rota vapour. Residues were reconstituted with 5 ml of acetonitrile, for quantification by HPLC-UV method.

Chromatographic separation parameters

The HPLC-UV system used, consisted Shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (X Bridge -C18) Column temperature was maintained at 30°C. The injected sample volume was 10µL. Mobile Phases A and B was Acetonitrile and HPLC water (pH-3 adjusted with Acetic Acid) (80:20 (v/v)). The flow-rate used was kept at 1.0 mL/min. A detector wavelength was 230 nm. The external standard method of Calibration was used for this analysis.

Method validation

Method validation[6] ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.03 and 0.3 mg/kg. Linearity was determined by different known concentrations (0.03, 0.1, 0.5, 1.0, 5.0 and 10.0 mg/L) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, mg/L) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Specificity was confirmed by injecting the plasma control. There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in (Figure 1 and 2). Furthermore, the retention times of candesartan cilexetil and azilsartan medoxomil were constant at 5.3 ± 0.2and 4.4 ± 0.2, min.

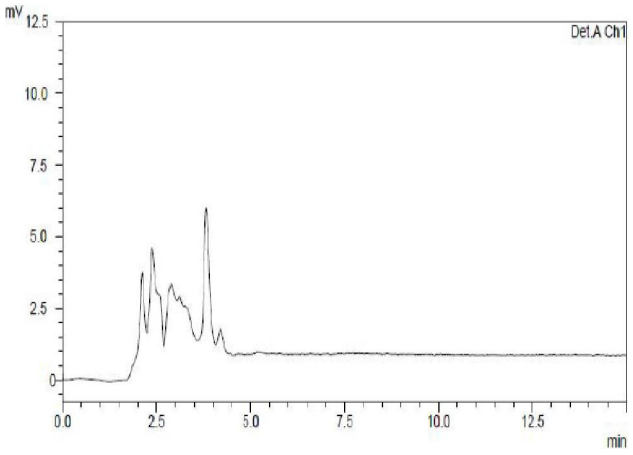

Figure 1 : Representative chromatogram at plasma control

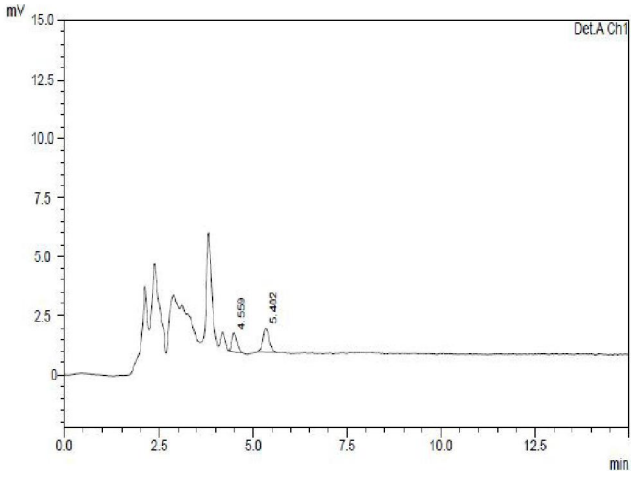

Figure 2 : Representative chromatogram at fortification level of 0.03 µg/mL

Linearity

Different known concentrations of standards (0.03, 0.1, 0.5, 1.0, 5.0 and 10.0 mg/L) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of standards were used to calculate linear regression equations. These were Y=8333.71X + 9.22 and Y=10107.54X + 66.10, with correlation coefficients of 0.9999 and 1.0000 for candesartan cilexetil and azilsartan medoxomil respectively. A calibration curve showed in (Figure 3).

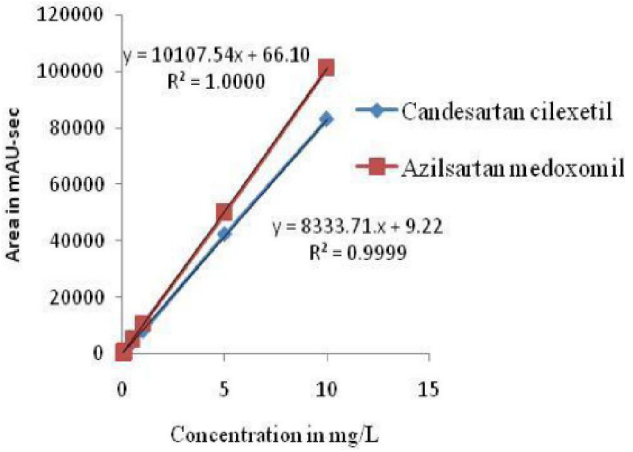

Figure 3 : Representative calibration curve of candesartan cilexetil and azilsartan medoxomil

Accuracy and precision

Recovery studies were carried out at 0.03 and 0.3 mg/L fortification levels for candesartan cilexetil and azilsartan medoxomil in plasma. The recovery data and relative standard deviation values obtained by this method are summarized in TABLE 1.

These numbers were calculated from four (6) replicate analyses of given sample (Candesartan cilexetil and azilsartan medoxomil) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

Detection and quantification limits

The limit of quantification was determined to be 0.03 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (84-92%, RSD<2%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.03 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

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TABLE 1 : Recoveries of the candesartan cilexetil and azilsartan medoxomil from fortified plasma control sample (n=6)

Fortification Concentration in mg/L	Replication	Recovery (%)	
		Candesartan cilexetil	Azilsartan medoxomil
0.03	R1	86.12	85.62
	R2	85.25	86.12
	R3	82.69	84.36
	R4	83.65	83.96
	R5	84.19	85.17
	R6	85.97	86.07
	Mean	84.65	85.22
	STDEV	1.37	0.90
	RSD	1.61	1.05
0.3	R1	90.23	91.78
	R2	90.58	90.86
	R3	91.56	92.04
	R4	92.03	91.47
	R5	91.24	91.78
	R6	90.78	91.89
	Mean	91.07	91.64
	STDEV	0.67	0.42
	RSD	0.73	0.46

TABLE 2 : Storage stability Details (n=6)

Fortification Concentration in mg/L	Storage Period in Days	Recovery in %	
		Candesartan cilexetil	Azilsartan medoxomil
0.1	0	93.21	93.91
		95.26	94.75
		96.32	95.19
		94.25	95.29
		94.00	94.19
		93.00	94.56
	Mean	94.34	94.65
	STDEV	1.26	0.54
	RSD	1.34	0.57
	30	92.36	92.26
		93.26	91.79
		93.59	91.45
		92.25	92.39
		91.28	92.15
		92.79	93.03
	Mean	92.59	92.18
	STDEV	0.82	0.54
	RSD	0.89	0.59

Storage stability

A storage stability[2] study was conducted at -20 ± 2°C with plasma samples spiked with 0.1 µg/mL of candesartan cilexetil and azilsartan medoxomil Samples were stored for a period of 30 days at this temperature. Analysed for the content of candesartan cilexetil and azilsartan medoxomil before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 2% for candesartan cilexetil and azilsartan medoxomil showing no significant loss of residues on storage. The results are presented in TABLE 2.

CONCLUSIONS

This paper describes a fast, simple sensitive analytical method based on SPE-HPLC-UV simultaneous determination of candesartan cilexetil and azilsartan medoxomil residues in human plasma. The SPE extraction procedure is very simple and inexpensive method for determination of candesartan cilexetil and azilsartan medoxomil residues in human plasma. The

mobile phase Acetonitrile and HPLC water (pH-3 adjusted with Acetic Acid showen good separation and resolution and the analysis time required for the chromatographic determination of the candesartan cilexetil and azilsartan medoxomil were very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of candesartan cilexetil and azilsartan medoxomil residues on a large number of biological samples.

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