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Research Article

CHROMATOGRAPHIC SEPARATION FOR CILAZAPRIL AND RELATED IMPURITIES DETERMINATION

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Abstract: A simple and rapid reverse-phase high-performance liquid chromatographic (HPLC) method for the simultaneousseparation and determination of Cilazapril and its process-related impurities in bulk drugs has been developed. Fourprocess-related impurities of Cilazapril have been separated on an Inerstsil ODS-3V (C18) column and detected at 254 nmusing a photo diode array (PDA). This HPLC method was successfully applied to the analysis of Cilazapril bulk drug. Therecoveries of Cilazapril and process-related impurities were in the range of 92.86 – 106.23%, and found to be specific,precise and reliable for the determination of un-reacted raw materials, intermediates in the reaction mixtures and bulk drugs.

Key**words:** Cilazapril, HPLC, process-related impurities

INTRODUCTION

Cilazapril is a novel drug which is beingevaluated for the treatment of essentialhepertension and congestive heart failure. Cilazaprilis rapidly hydrolysed by non-specific esterases tothe active acid metabolite cilazaprilat, which is apotent inhibitor of angiotension convertingenzyme (ACE). Cilazapril is a pyridazine angiotensinconvertingenzyme inhibitor (ACEinhibitor).Cilazapril works by causing blood vesselsto relax. This lowers blood pressure by decreasingproduction of a strong chemical in the body. It helpsthe heart work more effectively. It improves bloodflow and increases the supply of blood and oxygen to the heart. ACE inhibitors may be used for thetreatment of several heart related problems & helps to decrease the risk of heart attacks. IC represents a universal analyticaltechnique for the separation and quantitativedetermination of specific ion species. Complexmixtures of anions or cations can be separated tothe level of specific ions and then quantified in arelatively short time. The main applications of IC methods arein the determination of trace anions in ultra pure water in the pharmaceutical industry, electronics,power plants, pulp and paper production.[1] The IC method can detect and quantifysubstances that cause color, smell and slime in the production process, as well as salts and other corrosive substances. These disturbing substances include volatile organic acids (acetic, formic, lacticand butyric) and inorganic salts present as anions: chloride, fluoride, sulfate, nitrate.[2-9] A number of spectrophotometric methods were reported for the determination of Cilzapril inits binary mixtures.[10, 11] And also HPLC methods for the specific determinations of Cilazapril along withother angiotensin-converting enzyme inhibitors isreported by [12, 13] A Validated method for thedetermination of Cilzapril and its metabolites inpresence of other enzyme inhibitors are alsoreported [14.] Shalini Joshi reported a method forformulations by TLC, HPLC and RPTLC.[15]

EXPERIMENTAL

Reagents and chemicals

All reagents were of analytical grade unless stated otherwise.HPLC-grade water was provided by a Milli-Q® water purificationsystem, Millipore Corp. (USA), HPLC-grade acetonitrile andammonium acetate procured from Merck India Ltd. (India).

Apparatus

The HPLC system included two LC-10AT VP pumps, anSPD-M10A VP photo-diode array detector, a CTO-10AS VPoven and SCL-10A VP controller (all from Shimadzu, Japan).A reverse-phase Inertsil ODS-3V (GL Sciences Inc., Japan)column (25 cm × 4.6 mm i.d.; particle size, 5 µm) was used forseparation, and chromatograms were integrated using Class software.

Chromatographic conditions

The mobile phase was 1% ammonium acetate and acetonitrile(55:45 v/v); before delivering into the column it was filteredthrough a 0.45µm nylon filter and degassed. The analysis wascarried out under isocratic condition using a flow rate of 1.0 ml/min at 30°C. Chromatograms were recorded at 254 nmusing a PDA detector.

Analytical procedure

Solutions of (1000 µg/ml) Cilazapril and its process-relatedimpurities were prepared by dissolving known amounts ofcomponents in a mobile phase. These solutions were furtherdiluted to determine the accuracy, precision, linearity and limitof detection and limit of quantification.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Figure 1 shows the synthetic process for the synthesis ofCilazapril. It can be seen from Fig. 1 that there

are fourcompounds that include the starting material and intermediatesthat could be present as a potential impurity in Cilazapril. Thepresent study was aimed at developing a chromatographicsystem capable of eluting and separating Cilazapril and itssynthetic impurities.All of the impurities and Cilazapril were subjected to separationby reverse-phase HPLC using different columns and mobilephases. The separation and peak shapes were good on InertsilODS-3V (4.6 × 250 mm, 5 µm) column using 1% ammoniumacetate and acetonitrile (55:45 v/v). A typical chromatogram ofCilazapril spiked with 25 ppm of each impurity is shown in Fig. 2. It is evident from Fig. 2 that all of the compounds wereeluted and well separated with good peak shapes and resolutions.The UV wavelength at 254 nm was chosen for detection andquantification, since Cilazapril and its impurities have goodabsorption at that wavelength. The peaks were identified byinjecting and comparing the retention times of individualcompounds and studying the absorption spectra using a PDAdetector (Fig. 3). The developed method was validated withrespect to accuracy, precision, linearity and robustness.

Specificity

To demonstrate the specificity of the method, Cilazapril bulkdrug was spiked with known amounts of impurities, andchromatographed. All of the impurities were well separatedfrom Cilazapril; the chromatographic peak purity and thehomogeneity were evaluated with a PDA detector. The peakswith a flat-top indicated that Cilazapril has a homogeneous peakwith no impurities embedded in it. Also, the specificity waschecked by stressing pure Cilazapril under UV light at 254 nm, and under extreme conditions, such as 0.1 N NaOH, 0.3 N HCland 3% H_2O_2 at 60°C for 24 h. Under UV and acidic conditionsthere was no change in the purity, but in alkaline and oxidativeconditions the degradation products were formed. However,they are well separated from Cilazapril and the process impurities,indicating that the method is specific for the separation andestimation of Cilazapril and its process impurities. It can be seenfrom the HPLC chromatogram of Cilazapril bulk drug (Fig. 4)that the process-related impurities were well separated fromunknown impurities.

System suitability

The system suitability was evaluated by using 0.1% of allimpurities spiked to Cilazapril (100 µg/ml), and evaluated bymaking five replicate injections. The system suitabilityparameters (retention time (t_r), relative retention time (RRT)and tailing factors) were evaluated, and are recorded in Table 1.

Linearity

The linearity of the detector response to different concentrationsof impurities was studied by analyzing Cilazapril spiked witheach impurity at seven levels ranging over 0.1 – 2.0 µg/ml;similarly, the linearity of Cilazapril was studied by preparingstandard solutions at seven different levels, ranging over25 – 300 µg/ml. The data were subjected to statistical analysisusing linear-regression model. The correlation coefficients (R^2)for Cilazapril and its impurities were in the range of 0.995 – 0.999.

Accuracy and precision

The accuracy of the method for impurities was checked byspiking each impurity at three different concentration levels,ranging over 0.1 – 1.0 µg/ml (0.1, 0.4 and 1.0 µg/ml) to theCilazapril at a specified level (100 µg/ml). All estimations weremade in triplicate (n = 3); the recoveries for all four impuritieswere found to be 92.86 – 106.23%. The precision of the methodfor impurities was tested by injecting six individual preparationsof 100 µg/ml of Cilazapril spiked with 0.1 µg/ml of eachimpurity. The recoveries of each impurity were calculated, andthe RSD was found to be in the range of 1.07 – 3.27%.

The accuracy and precision for determining the assay ofCilazapril was checked at three different levels: *i.e*., 50, 100 and200 µg/ml each, in triplicate. Also, the RSD values were foundto be below 1.0%. The intermediate precision is the inter-dayvariation at the same concentration levels determined on successive days. The inter-day variations calculated for threeconcentration levels are expressed in terms of %RSD, at eachconcentration level, the %RSD, values were below 1.5%,indicating a good intermediate precision.

Robustness

In order to evaluate the robustness of the method, the influenceof a small and deliberate variation of the analytical parameterson Cilazapril and its impurities was studied. The robustness wasstudied by varying ±0.2 ml of the flow rate, ±2 ml of theacetonitrile composition in the mobile phase and ±2°C in thecolumn temperature to the actual method parameters. In all ofthe above variations, test samples were injected in triplicate.There was a slight change in the retention times of Cilazapril andits impurities upon changing the mobile-phase concentration,but all peaks were well separated without affecting the accuracyof the quantitative estimation of Cilazapril and the impurities.There was no significant change observed upon changing theflow rate and the temperature. The result indicates that themethod is suitable for the separation and estimation of Cilazapriland its synthetic impurities.

Limit of detection and quantification

The limit of detection (LOD) and quantification (LOQ)represent the concentrations of the analytes that would yield asignal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively.LOD and LOQ were determined by measuring the magnitude ofthe analytical background by injecting blank samples andcalculating the signal-to-noise ratio for each compound byinjecting a series of solutions until the S/Nratio is 3 for LODand 10 for LOQ. LOD and LOQ of all compounds lie in therange of 0.016 – 0.029 and 0.045 – 0.095 µg/ml, respectively.

Analysis of samples

First, accurately weigh 200 mg of aCilazapril bulk drugsample into a 100-ml volumetric flask, and dissolve in themobile phase and make up. This solution was used to estimateimpurities. The impurities in bulk drug samples were identifiedby comparing the retention times and the UV spectral curveswith that of standard impurities. The results are recorded inTable 2. Almost all impurities were found in

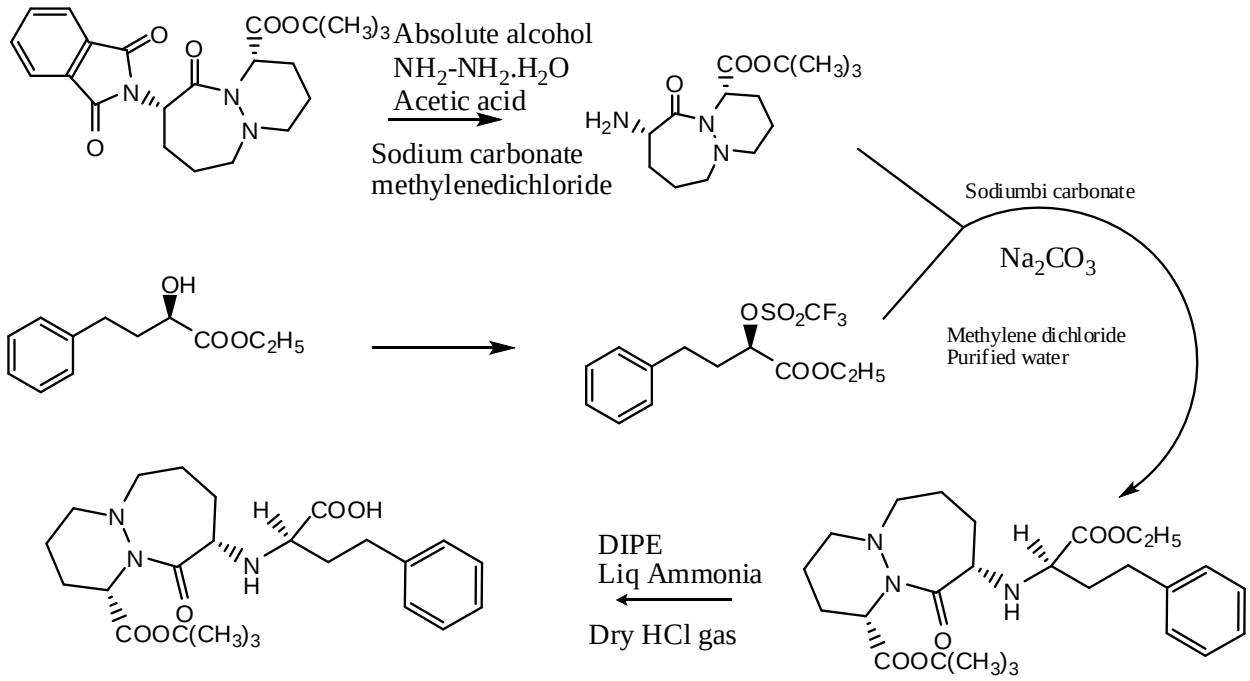
different quantitiesin all studied samples. Cilazapril -2 has the highest amount of impurity(0.14%), of which impurity D alone was 0.05%. The assay fordetermining Cilazapril when carried out by diluting the abovesolution to 200 ppm with the mobile phase ranged from 99.67 to99.80%.

Table1.System suitability data

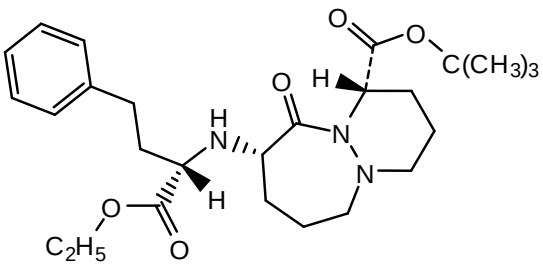
Compound	t_R/min	RRT	Tailing factor	λmax/nm (ε)
A	6.75	0.45	1.26	231 (20889)
B	8.948	0.59	1.20	254 (15341)
C	11.904	0.79	1.08	252 (26380)
D	2.997	0.20	1.13	241 (57506)
Cilazapril	15.091	1.0	1.03	246 (42966)

Table2.Results of analysis of bulk drugs

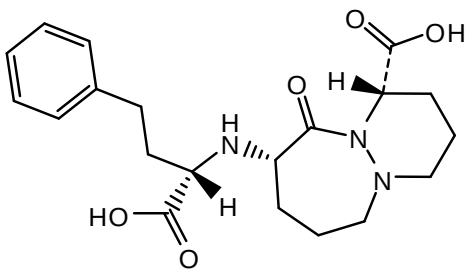
	Impurities, % (w/w) ± %RSD			
Sample	A	B	C	D
Cilazapril – 1	-	0.01 ± 1.03	-	0.03 ± 0.82
Cilazapril– 2	0.03 ± 3.53	0.04 ± 2.44	0.01 ± 2.87	0.05 ± 2.87
Cilazapril– 3	0.03 ± 1.10	0.02 ± 1.21	0.02 ± 0.23	0.03 ± 1.37



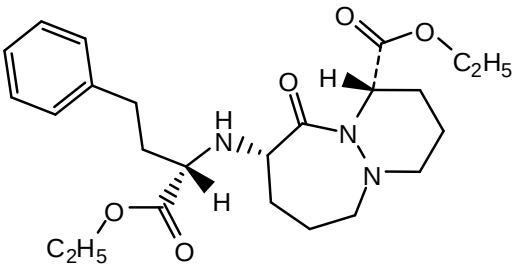
Cilazapril



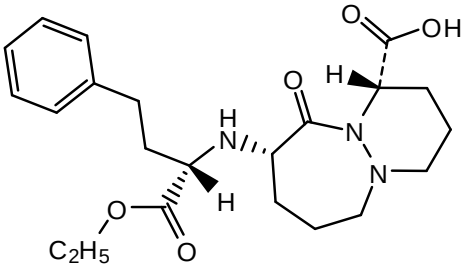
Impurity - A



Impurity - B



Impurity - C



Impurity - D

Fig. 1. Chemical synthesis of Cilazapril and its related Impurities

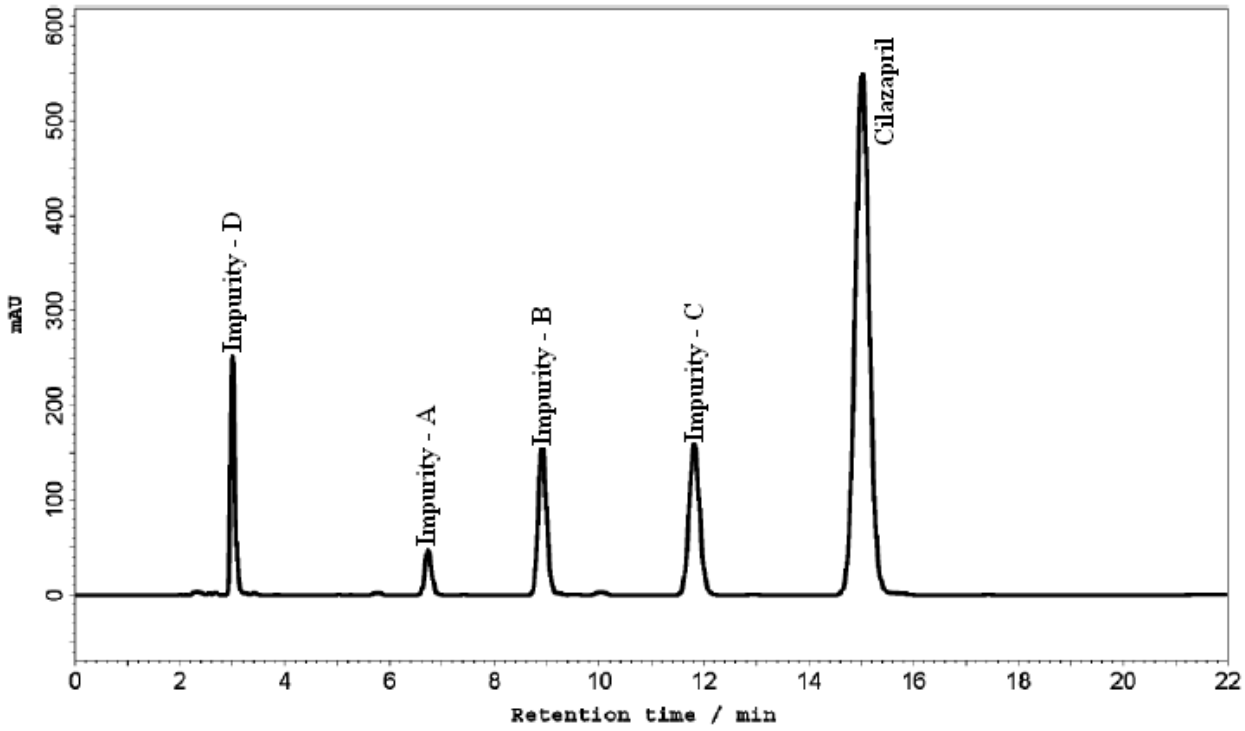


Fig.2.Typical chromatogram of Cilazaprilspiked with 25 ppm ofeach impurity (A, B, C and D)

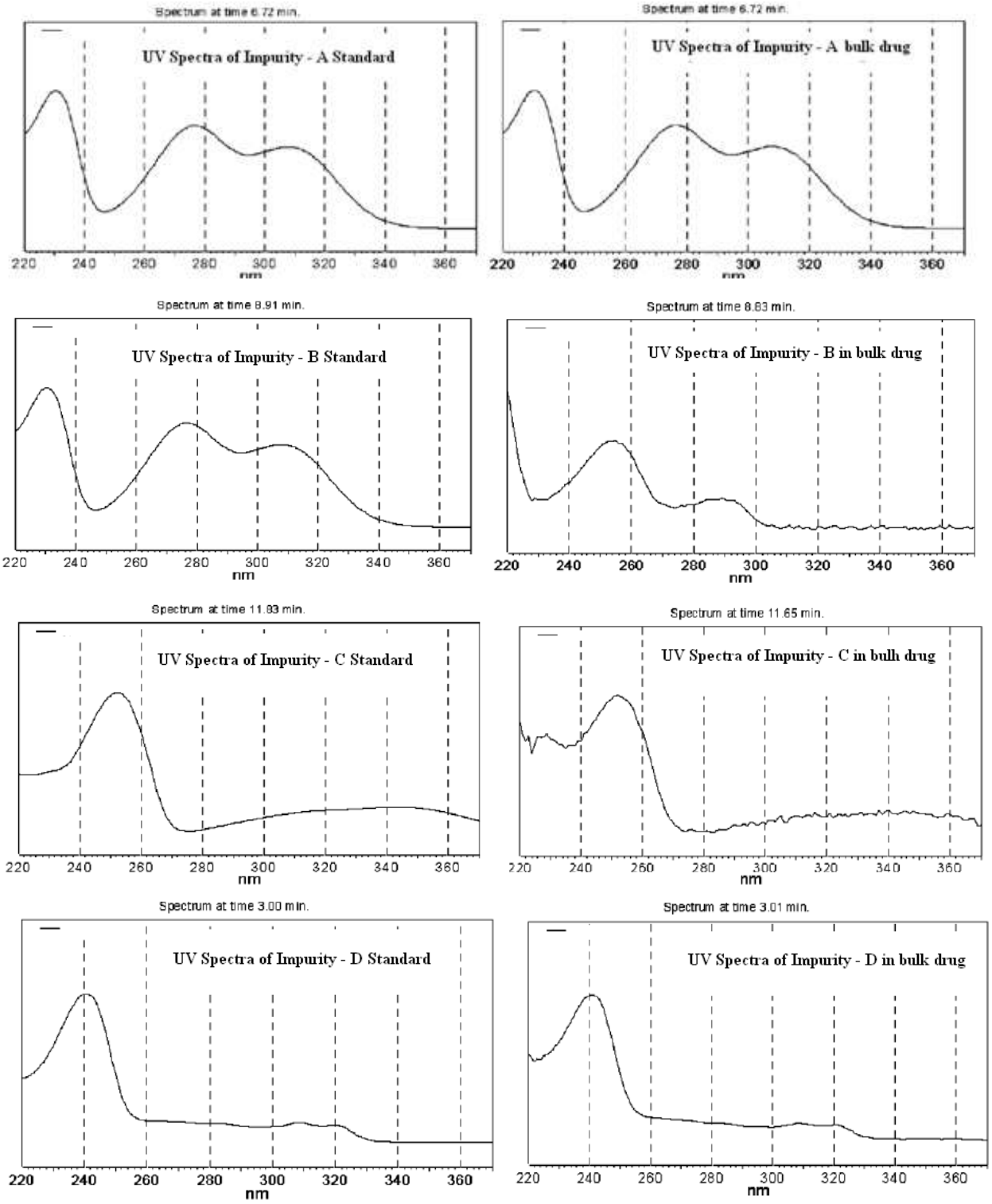


Fig.3.Comparative UV absorption spectra of impurities (A, B, C and D) in standard and bulk drugs.

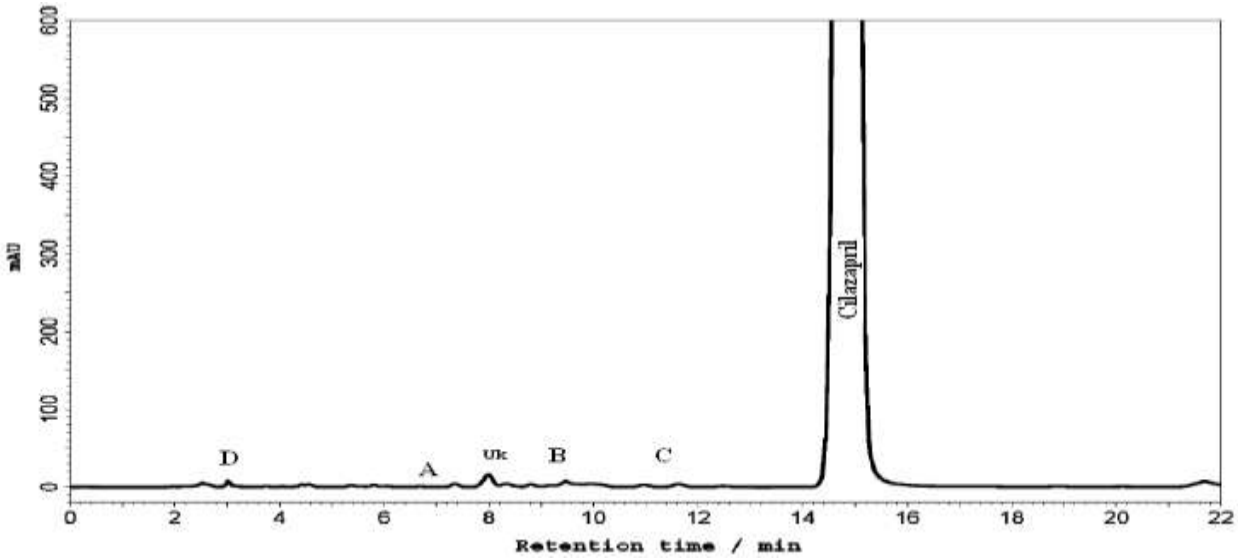


Fig.4.Typical chromatogram of a bulk drug (Uk, unknownimpurity).

CONCLUSIONS

A simple reverse phase-HPLC method was developed andvalidated for the simultaneous estimation of Cilazapril and itsprocess-related impurities in Cilazapril bulk drug. This method is selective, sensitive and precise for estimating Cilazapril and itsprocess-related impurities, which may be present in trace levelsin bulk drugs.

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