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ANALYTICAL DEVELOPMENT AND FORMULATION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE IN COMBINATION WITH SELECTIVE EXCIPIENTS

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ABSTRACT

The present study is carried out for the analytical development and formulation of ramipril and hydrochlorothiazide in combination with selective excipients. The objective of drug and excipient compatibility considerations and practical studies is to delineate, as quickly as possible, real and possible interactions between potential formulation excipients and the Active Pharmaceutical Ingredient (API). This is an important risk reduction excercise early in formulation of drugs. A specific and accurate reverse phase ultra-performance liquid chromatographic method was developed for the excipient compatibility studies of selective excipients in bulk drugs (Ramipril and Hydrochlorothiazide). The developed method consists of mobile phase, is a mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70. Mobile Phase A(Buffer and methanol in the ratio of 93:7, the buffer used is Sodium phosphate). Mobile Phase B (100 % Acetonite) with gradient programming, Hypersil BDS C18, The size of the column is 100 mm x 2.1 mm, 1.7 µm column as stationary phase with a flow rate of 0.1 mL/min and the PDA detector is employed. With the proposed method the compatibility of the excipients with bulk drugs was found to be acceptable under the guidelines of ICH-Q8 (R2) and the excipients with bulk drugs are then subjected forauthenticated formulations and are assayed for purity of formulated tablets with marketed product for comparable studies. The in vitro dissolution studies were also carried out. The drug content obtained from the prepared formulations is also within the limits and comparable with the marketed product, Cardace. The formulated tablets have shown promising results in the invitro dissolution studies.

Keywords:-Ramipril, Hydrochlorothiazide, ICH-Q8 (R2), Ultra Pressure Liquid Chromatography (UPLC), Impurities, Compatibility Studies.

INTRODUCTION

Ramipril, (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid and Hydrochlorothiazide, 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide[1],comes under the antihypertensive class of drugs that are used to treat hypertension.There are many classes of antihypertensive drugs, which lower blood pressure by different means; among them the most important and used are the thiazide diuretics and the Angiotensin Converting Enzyme (ACE) inhibitors [2-3].The type of medication to use initially for hypertension has been the subject of several large studies and resulting national guidelines. The fundamental goal of treatment should be the prevention of the important endpoints of hypertension, such as heart attack, stroke and heart failure.The most instable combination antihypertensivedrugsare Ramipril and Hydrochlorothiazide combination, although this formulation is very beneficial giving supraaddictiveproperty[4-7], it was facing much instabilitybecause of the decomposition of the Active Pharmaceutical Ingredient (API) by mode of oxidation. To solve the instabilities we need to modify the formulation by adding or replacing the excipients[8], in this study ascorbic acid,

butylatedhydroxytoulene, butylatedhydroxyanesole are used.

A detailed literature revealed that several analytical methods available forexcipient compatibility studiesinclude Related Substance (RS) method in Reversed Phase Liquid Chromatography when present with other drugs, UPLC Method[9-12]. The present work involves the related substance (RS) method in RP-UPLC for the analytical development and formulation of ramipril and hydrochlorothiazide with selective excipientsin combined dosage form and their in vitro dissolution studies.[13-14]

MATERIALS AND METHODS

Materials: Ramipril was donated byAurobindoPharma, Hyderabad.Hydrochlorothiazidewas generously gifted byHetero Drugs, Ltd; Hyderabad. Acetonitrile (HPLC grade) was purchasedfromQualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for HPLC and UPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

Instrumentation: An Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Bandlinesonerexsonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. The mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70 v/v. Mobile Phase A (Buffer and methanol in the ratio of 93:7, the buffer used is Sodium phosphate). Mobile Phase B (100 % Acetonitrile) with gradient programming was used as mobile phase at 0.1 mL/min. The column was maintained at ambient temperature.

Chromatographic Conditions: The chromatographic elution was carried out in gradient mode using a mobile phase consisting of mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70 v/v. Mobile Phase A (Buffer and methanol in the ratio of 93:7, the buffer used is Sodium phosphate)the maintained pH is 4.1 with Ortho Phosphoric Acid (OPA). Mobile Phase B (100 % Acetonite) the maintained pH is 2 with Ortho Phosphoric Acid (OPA). The analysis was performed at ambient temperature using a flow rate of 0.1 mL/min with a run time of 70 mins. The eluent was monitored using PDA detector. The mobile phase was filtered through 0.45 µm micron filter prior to use.

Preparation of Stock and Standard Solutions: The stock standard solution of ramipril and hydrochlorothiazide was prepared with methanol to a concentration of 100 µg ml.

Sample Preparation: The average tablet mass was calculated from the mass of tablets of Cardace (10 mg and 12 mg of ramipril and hydrochlorothiazide tablet, which was composed of ramipril, hydrochlorothiazide and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 100 ml brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 15 min to aid dissolution and then filtered through a Whatman no 42 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of ramipril and hydrochlorothiazide in the final solution was within the working range and then analyzed by Ultra Performance LiquidChromatography (UPLC). The results were given in the tables, 01 and 02.

Excipient Compatibility Studies: The typical drug-excipient interactions can be studied using both, binary (1:1; or customised) and prototype formulations powder mixes are prepared by triturating API with the individual excipients.In the present work thefirst approach is to conduct short term (3months) stability studies using generic prototype formulations under stressed conditions with binary systems as diagnostic back- up. Two batches of powder mixed in the ratio of 1:1 with bulk drugs (Ramipril and Hydrochlorothiazide combination) and selected excipients (Dicalcium Phosphate, Magnesium Stearate, Mannitol, Maize Starch, Ferric Oxide, Butylatedhydroxyanisole, Butylatedhydroxytoluene, Ascorbic Acid) are prepared and one batch is stored in stressed condition using incubator at a temperature of 60°C of Lab Hosp Co-Op Pvt Ltd and the other batch in ambient temperature conditions. The idea is to diagnose any observed incompatibility from the prototype formulation work then hopefully identifies the culprit excipient from the binary mix data.Hopefully a prototype formulation can be taken forward as a foundation for product development, analytically. The impurities obtained in the present study are evaluated by Ultra Performance Liquid System (UPLC).

Preparation of Combination Tablet Dosage Form: Wet granulation method has been employed to

prepare combination tablet dosage form of ramipril and hydrochlorothiazide in combination with selective excipients. Dicalcium phosphate is used as diluent along with mannitol as an osmotic diuretic agent, ascorbic acid, butylatedhydroxytoulene and butylatedhydroxyanisole as an anti-oxidant agents are tirtuarated in a mortar and sufficient 95% ethanol solution was used as a granulating agent; Magnesium stearate was used as lubricant, maize starch as a thickening agent and red ferric oxide as a colouring agent. Two formulations are prepared; Formulation 1 containing Butylatedhydroxyanisole as an anti-oxidant agent and in Formulation 2, Butylatedhydroxytoulene is employed as an anti-oxidant.

Application of Method forAssay Studies inTablet Dosage Forms: The developed and compatibleUPLC method was applied for determination of Ramipril and Hydrochlorothiazide from dosage forms, to determine the content of API (Ramipril and Hydrochlorothiazide)in tablets (label claim: 10 mg of Ramipril and 12mg of Hydrochlorothiazide), 20 tablets were taken and contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask and was dissolved in 25 mL of deionized water and volume was made up to the mark with deionized water. Theflask was sonicated for 15 min to affect complete dissolution. The solution was filtered through a 0.45 µm micro filter. Suitable aliquot of the filtered solution was transferred into a 100 mL volumetric flask and made up to the volume with mobile phase to yield the concentration of 20µg/mL. The experiments were performed under the chromatographic conditions described above. The eluent was monitored using PDAdetector and concentration in the sample was determined by comparing the area of sample with that of the standard.

Invitro Dissolution Studies:

Invitro dissolution studies of our two formulations and a marketed product were studied in USPXXIII tablet dissolution test apparatus –II (Lab Hosp, Corp.). Employing a paddle stirrer at a speed of 50 rpm using 900ml of 0.1N Hcl as dissolution medium at 37±0.50C, one tablet was used in each test. At predetermined time intervals 5ml of the samples were withdrawn by means of a syringe fitted with a pre filter. The volume withdrawn at each interval was replaced with the same quantity of fresh dissolution medium maintained at 37±0.5ºC. The samples were analyzed for drug release by measuring the absorbance at 210nm using High Pressure Liquid Chromatography (HPLC) after suitable dilutions. The mobile phase used in this technique is a liquid mixture of sodium phosphate buffer and methanol in the ratio of 82:18 and the column used in this technique is Licrosphere Recursive Partitioning (RP) select B (250 x 4.0 mm, 5 µm) and the column temperatre was 450ºC. The flow rate is 1.5 ml per min and the injection volume is 20 µl. The run time was 10 mins. All the studies were conducted in triplicate. The results are given in tables no: 03and 04.

RESULTS AND DISCUSSION

In this study analytical development and formulation of ramipril and hydrochlorothiazide in combination with selective excipients are performed, the first approach in this study is to conduct short term (3months) stability studies using generic prototype formulations under stressed conditions with binary systems as diagnostic back- up. Two batches of powder mixed in the ratio of 1:1 with bulk drugs (Ramipril and Hydrochlorothiazide combination) and selected excipients (Dicalcium Phosphate, Magnesium Stearate, Mannitol, Maize Starch, Ferric Oxide, Butylate dhydroxyanisole, Butylate dhydroxytoluene, Ascorbic Acid) are prepared and one batch is stored in stressed condition using incubator at a temperature of 60°C of Lab Hosp Co-Op Pvt Ltd and the other batch in ambient temperature conditions. To evaluate the presence of impurities obtained under such conditions the Ultra Pressure Liquid Chromatography (UPLC) was performed to the samples containing the combination of ramipril and hydrochlorothiazide with selective excipients. With the proposed method the compatibility of the excipients with bulk drugs was found to be acceptable under the guidelines of ICH-Q8 (R2).

Confirmation of Stability: The resultant chromatogram(Figure No: 01) shows the presences of impurities are under the limits of impurities which confirm the formulation is stable.

Impurity Content: The impurity content shows us the amount of respective impurities obtained in the studied formulations.

Confirmation of Purity: The Ultra Pressure Liquid Chromatography (UPLC) was performed to the prepared tabletformulations and marketed product Cardace and the resultant chromatogram(Figure No: 02, 03, and 04) shows that the formulated products are comparable with the marketed product, "Cardace". Mobile phase optimization was initiated using the chromatographic conditions as mentioned abovethe flow rate was increased from 0.1mL/min to

1.0 mL/min to estimate main drug content from the tablet formulations. The peak shape and separation was found to be good when a mobile phaseconsisting of mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70v/v. Mobile Phase A (Buffer and methanol in the ratio of 93:7, the buffer used is Sodium phosphate) the maintained pH is 4.1 with Ortho Phosphoric Acid (OPA). Mobile Phase B (100 % Acetonite) the maintained pH is 2 with Ortho Phosphoric Acid (OPA). The analysis was performed at ambient temperature using a flow rate of 1.0 mL/min with a run time of 15 mins. The retention times of Hydrochlorothiazide and Ramiprilwere found to be 2.181, 2.176, 2.137 mins and 8.461, 8.266, 7.996 mins; in Formulation 1, Formulation 2 and in Marketed product(Cardace) respectively, indicating good separation of both analytes from each other. The theoretical plate number for Hydrochlorothiazide and Ramiprilwere found to be 1901.8, 2190.6,2879.0and 7675.9,8254.9, 6949.5in Formulation 1, Formulation 2 and in Marketed product(Cardace) respectively, thus indicating good column efficiency. The specific chromatograms were recorded using PDA detector, shown in Figure no: 02, 03 and 04.

Percentage Purityby Assay Studies: The percentage purity of the active pharmaceutical ingredient is estimated in the formulations, (Formulation 1andFormulation 2) and the marketed product, Cardace are as above mentioned in the table no: 02.

Confirmation of Dissolution: The dissolution studies of Ramipril and Hydrochlorothiazide in our formulations (Formulation 1 and Formulation 2) are comparable with the marketed product, Cardace.

CONCLUSION

The present study is an attempt to perform analytical development and formulation of ramipril and hydrochlorothiazide in combination with selective excipients.The proposed RP-UPLC method for Related Substance is specific andaccurate for the compatibility studies of bulk drugs with excipients and quantification of Ramipril and Hydrochlorothiazidefrom its tablet dosage form. The method has been found to be better than previously reported methods, because of its long runtime, use of readily available mobile phase, detection and elution of analytes of minute particle sizeand low tR. All these factors make this method suitable for the compatibility studies of bulk drugs with excipientsthecompatabilitystudieswere studied and the presence of impurities within the limits was confirmedand quantification of Ramipril and Hydrochlorothiazide in tablet dosage forms was comparable with the marketed product, Cardace. The method can be successfully used for routine analysis of compatibility studies in bulk drugs and pharmaceutical dosage forms without interference. With the marketed formulation dissolution studies, the formulations of ramipril and hydrochlorothiazide with selective excipients showed promising results. Hence they may be considered as comparable formulations. So with these studies we may conclude that among the two formulations (Fromulation 1 and Formulation 2), the formulation 1 is apparently comparable with the marketed product, Cardace as their impurity is comparably less than Cardace, and it is apparently suitable for further pharmacodynamic and pharmacokinetic studies to evaluate clinical safety in suitable animal and human models.

Figure1: Specimen ChromatogramRelated Substances

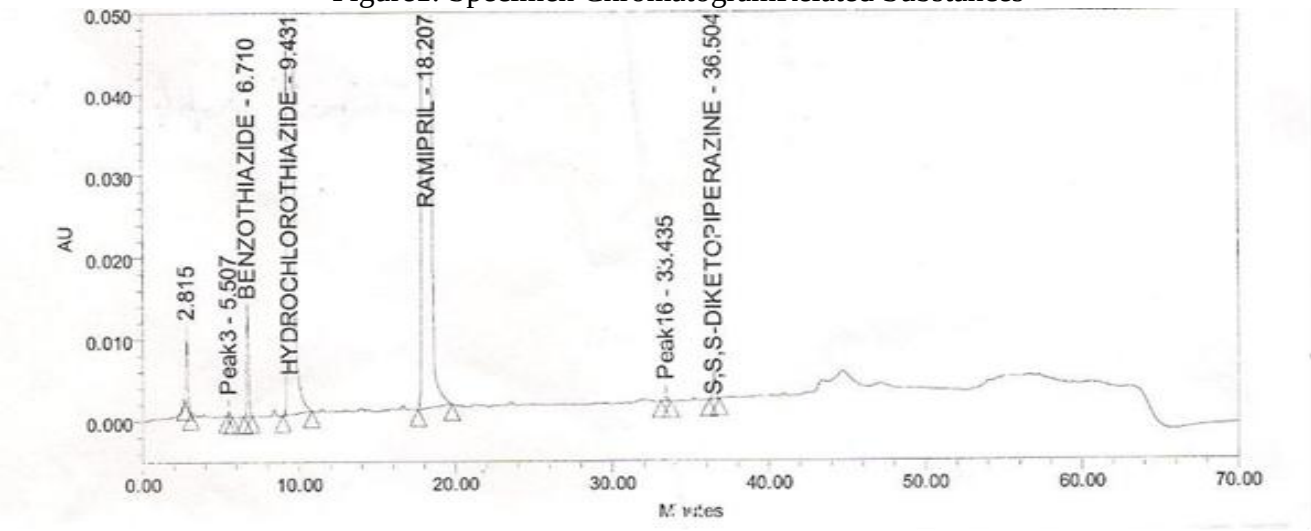


Figure No: 02: Specimen Chromatogram of Percentage Purity In Formulation 1.

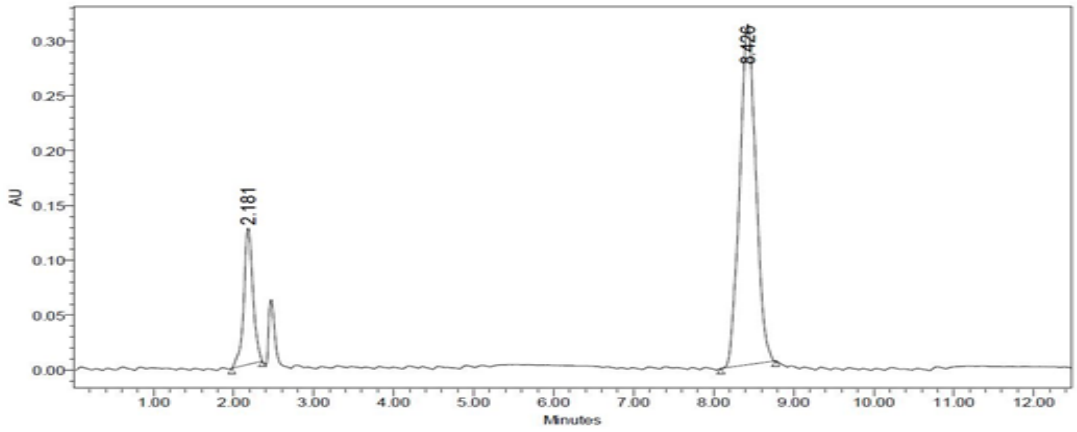


Figure No: 03: Specimen Chromatogram of Percentage Purity In Formulation 2.

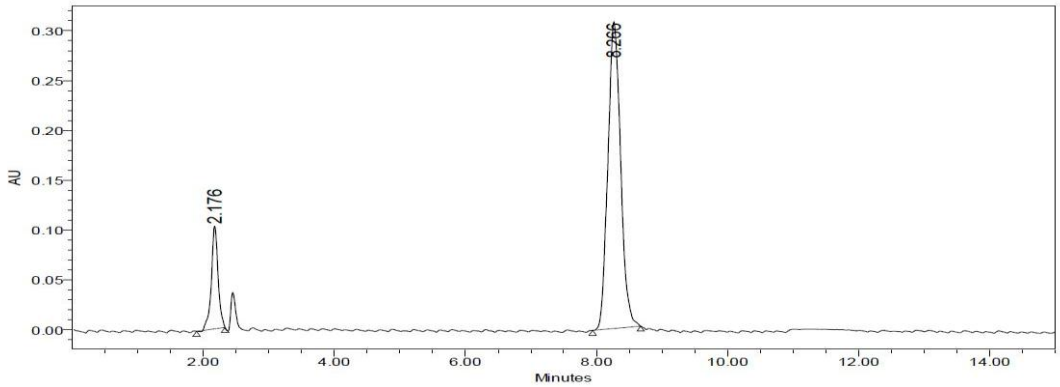


Figure No: 04: Specimen Chromatogram of Percentage PurityinCardace Formulation.

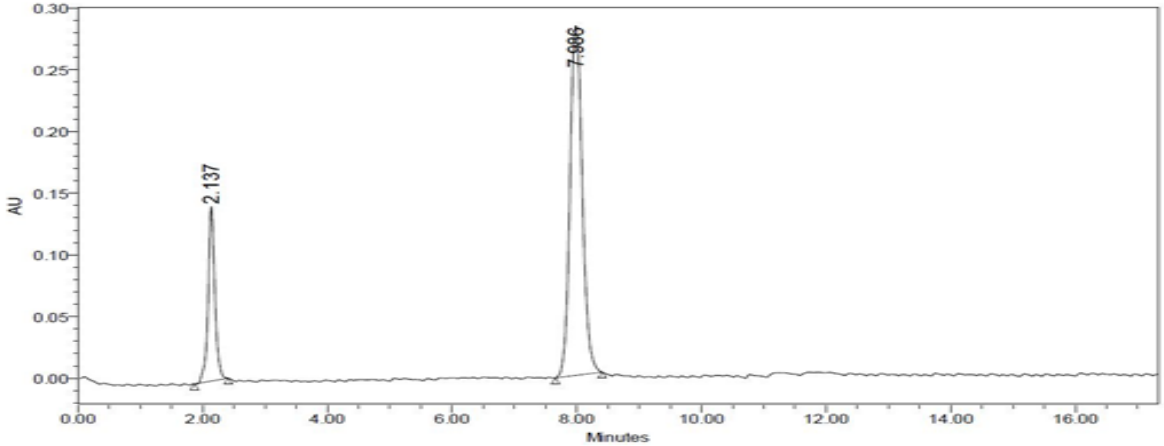
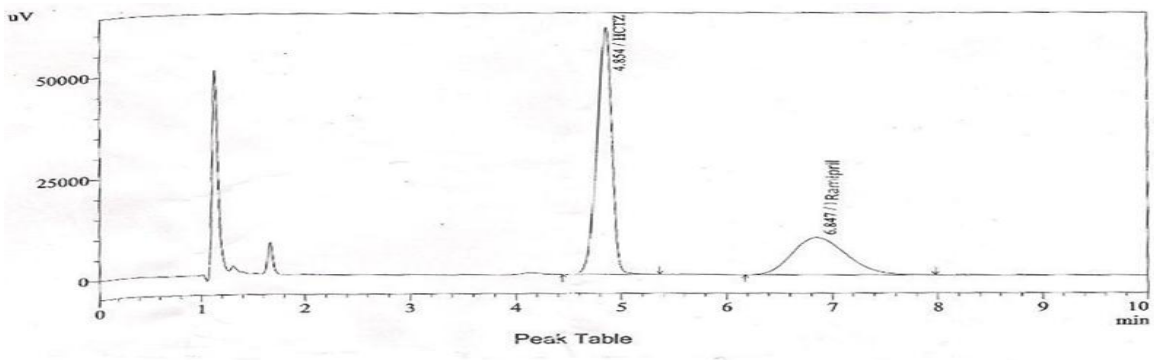
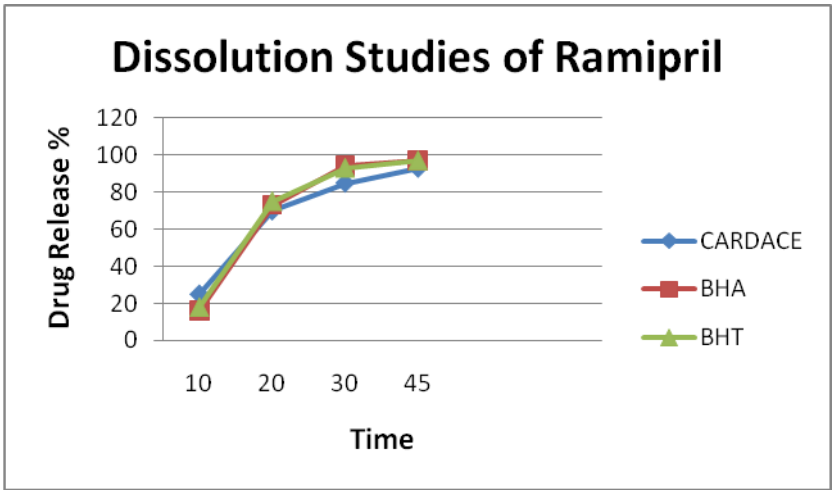
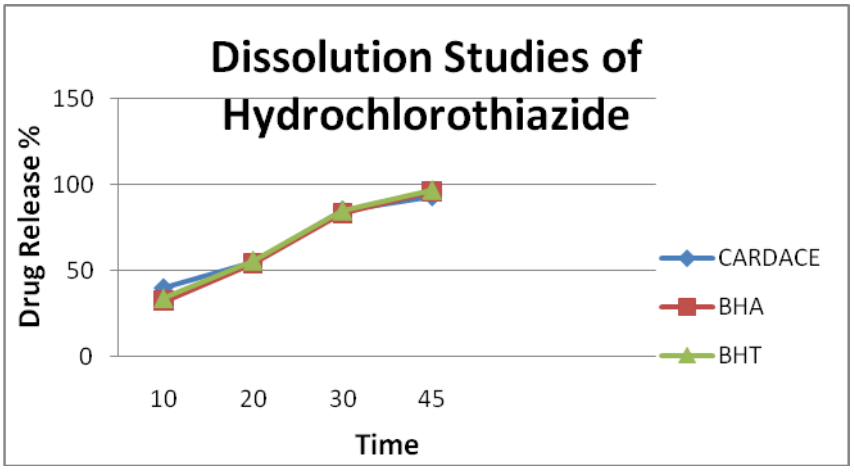


Figure No: 05: Specimen Chromatogram of Dissolution Studies..





Graph No: 01: Dissolution studies of Ramipril.



Graph No: 02: Dissolution studies of Hydrochlorothiazide.

Table No: 01. Impurity Content:

S.No	Impurity Name	Relative Retention Time	Formulation 1	Formulation 2	Cardace
1	Benzothiazide	0.71	0.2	0.31	0.42
2	Unknown	0.58	0.01	0.01	0.15
3	Lat Impurity	1.83	NOT DETECTED	NOT DETECTED	0.51
4	Diketopiparazine	2.00	0.06	0.05	0.12

Table No: 02.Percentage Purity by Assay Studies:

% Drug found	Label Claim per Tablet (mg)	Formulation 1	Formulation 2	Cardace
Ramipril	10	99.2%	99.3%	96.1%
Hydrochlorothiazide	12	98.6%	93.2%	96.3%

Table No: 03.Dissolution Studies of Ramipril

S.No	Time(mins)	Cardace	Formulation 1	Formulation 2
1	10	25	16	18
2	20	70	73	75
3	30	85	94	93
4	45	93	97	97

Table No: 04. Dissolution Studies ofHydrochlorothiazide

S.No	Time(mins)	Cardace	Formulation 1	Formulation 2
1	10	40	32	34
2	20	55	54	56
3	30	85	83	85
4	45	93	96	97

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