



ISSN Print 2231 – 3648  
Online 2231 – 3656

Available Online at: [www.ijpir.com](http://www.ijpir.com)

## International Journal of Pharmacy and Industrial Research

### Development and validation of RP-HPLC method for the simultaneous estimation of Chlordiazepoxide, Clidinium bromide, Dicyclomine hydrochloride and Rabeprazole sodium in bulk and pharmaceutical dosage forms

P. Chandrakiran\*, K. Praveen, S. Mabooni, S.A. Rahaman, A. Shanta Kumari

Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Mangalagiri, Guntur-522503, Andhra Pradesh, India

#### ABSTRACT

A simple, specific and accurate RP-HPLC method was developed for the simultaneous determination of Chlordiazepoxide, Clidiniumbromide, Dicyclomine hydrochloride and Rabeprazole sodium in tablet dosage forms. A waters C<sub>18</sub> column (50 mm × 4.6 mm, 5 μm) with mobile phase consisting of acetonitrile and phosphate buffer 30:70 (v/v) (pH 2.5 adjusted with triethylamine) were used. The flow rate was 1.0 ml/ min and effluents were monitored at 250 nm. The retention time of Chlordiazepoxide, Clidinium bromide, Dicyclomine hydrochloride and Rabeprazole sodium in tablet formulation were found to be 4.0 min, 7.3 min, 5.9 min, 6.8 min respectively. The method was validated according to the ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity, ruggedness and robustness. The method showed good reproducibility and recovery with % RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear. Hence it can be applied for routine analysis of Chlordiazepoxide, Clidinium Bromide, Dicyclomine Hydrochloride and Rabeprazole sodium in pharmaceutical combined dosage forms.

**Keywords:** RP-HPLC, Chlordiazepoxide, Clidiniumbromide, Rabeprazolesodium, ICH guidelines.

#### INTRODUCTION

Now a days combined dosage forms are mostly preferred when compared to single dosage forms. Chlordiazepoxide, Clidinium bromide, Dicyclomine hydrochloride, Rabeprazole sodium is available in combined pharmaceutical dosage form. The brand name is [1] Coliwin –R tab containing

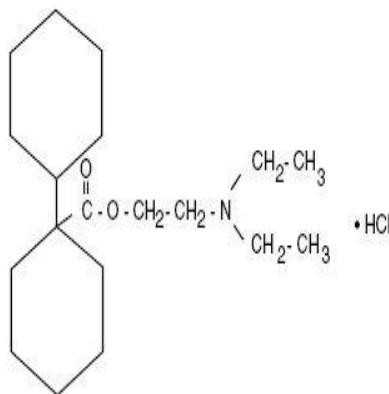
5mg of chlordiazepoxide, 2.5 mg of clidinium bromide, 10 mg of dicyclomine hydrochloride, 10 mg of rabeprazole sodium [1] used in the treatment of anxiety and Alcohol withdrawal. Literature survey reveals that various analytical methods have been reported for single dosage forms. The present paper aims to report a simple, accurate, precise, RP-HPLC method for estimation of

#### Author for Correspondence:

P.Chandrakiran  
Department of Pharmaceutical Analysis,  
Nirmala College of Pharmacy,  
Mangalagiri, Guntur-522503,  
Andhra Pradesh, India

chlordiazepoxide, clonidine bromide, combined dosage forms.  
 dicyclominehydrochloride, rabeprazole sodium in

### Drug profile of dicyclomine hydrochloride

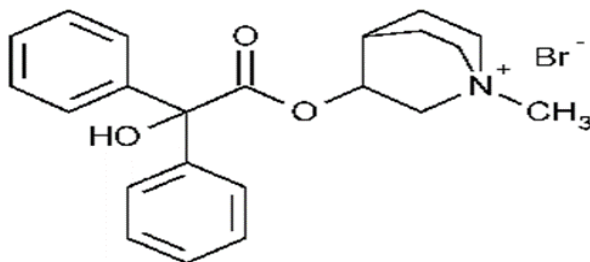


### Dicyclominehydrochloride [2, 9]

It is a 2-Diethylaminoethyl bicyclohexyl-1-carboxylate hydrochloride .CAS number is 77-19-0, and it acts as a antispasmodic agent and it is

white odourless crystalline powder .and it is soluble in water, alcohol, chloroform, and insoluble in ether.

### Drug profile of clidinium bromide

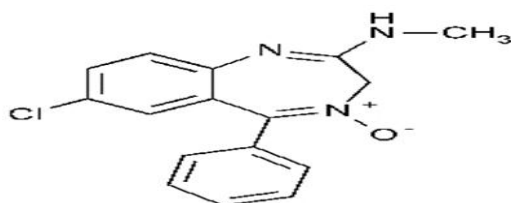


### Clidinium bromide [3, 4, 9]

It is a 3-[(2-hydroxy-2,2-diphenylacetyl)oxy]-1-methyl-1-azabicyclo[2.2.2]octan-1-ium bromide,CAS number is 3485-62-9,and it acts as a

Antimuscarinics and it is white crystalline substance and it is soluble in water and alcohol,and methanol.

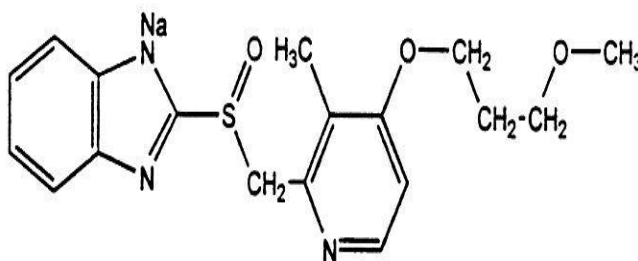
### Drug profile of chlordiazepoxide [5, 6, 9]



It is a 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-Oxide, CAS number is 58-25-3. It belongs to benzodiazepine category and it is a

white crystalline substance and it is soluble in water.

### Drug profile of rabeprazole sodium



It is a [7, 9] 2-[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl[sulfinyl]-benzimidazole sodium, CAS number is 117976-90-6, it acts as a proton pump inhibitor and it is white crystalline solid and it is soluble in water and methanol, freely soluble in ethanol, chloroform, and ethyl acetate and insoluble in ether and n-hexane

## MATERIALS AND METHODS

### Optimised chromatographic conditions [8]

Suitable High Performance Liquid Chromatography equipped with UV-visible detector was used. HPLC (waters, 2695 separation module) SOFTWARE (Empower, version 2.0)

Column : Waters C18, 50 mm x 4.6 mm, 5 $\mu$ m.

Wavelength : 250 nm

Injection Volume : 10 $\mu$ L

Column Temperature : Ambient

Flow rate : 1.0 mL/min

Retention time of Chlordiazepoxide (4.0mins), Dicyclomine HCL (5.9mins), Rabeprazole (6.8mins), clidiniumbromide (7.3mins).

### Chemicals and Reagents

Tri ethyl Amine (HPLC Grade), Octane-1-Shellphonic Acid (AR grade), Acetonitrile (HPLC grade), HPLC grade Water (Milli Q or equivalent) and Ortho Phosphoric Acid (HPLC Grade).

### Drug samples

Dicyclomine hydrochloride, Chlordiazepoxide procured from lifeline laboratories and Clidinium bromide, Rabeprazole sodium were procured from icon laboratories, Vijayawada, Krishna district.

### Preparation of diluent

HPLC grade water and HPLC grade methanol (20+80 v/v) was prepared.

### Preparation of standard solution

#### Chlordiazepoxide

Accurately 5mg of Chlordiazepoxide working standard was transferred into a 100 mL volumetric flask. 70 mL of diluent was added, sonicated to dissolve and diluted to final volume with diluent.

#### Dicyclomine HCl

Accurately 10 mg Dicyclomine HCl working standard was transferred into a 100 mL volumetric flask. 70 mL of diluent was added, sonicated to dissolve and diluted to final volume with diluent.

#### Rabeprazole Sodium

Accurately 10 mg Rabeprazole Sodium working standard was transferred into a 100 mL volumetric flask. 70 mL of diluent was added, sonicated to dissolve and diluted to volume with diluent.

#### Clidinium Bromide

Accurately 2.5 mg Clidinium Bromide of working standard was prepared and transferred into a 100 mL volumetric flask. 70 mL of diluent was

added, sonicated to dissolve and diluted to final volume with diluent. Further diluted each 5mL of Solution- A, B, C and D to 50 mL with the diluent.

### Preparation of sample solution

Weighed 10 capsules or tablets and crushed into powder and then one tablet weight equivalent of sample is transferred into a 250 mL volumetric flask. 200 mL of diluents was added, sonicated to dissolve and diluted to volume with diluent. Further diluted 5 mL to 100 mL with the diluent. Filtered through 0.45µ Nylon syringe filter.

### Procedure

10µL of standard preparation, sample preparation were injected five times into chromatographic column. Recorded the chromatograms and measured the peak responses for Chlordiazepoxide, DicyclomineHCl, Rabeprazole Sodium, Clidinium Bromide. The System suitability parameters were satisfied. From the peak responses, calculated the content of Chlordiazepoxide, DicyclomineHCl, Rabeprazole Sodium, Clidinium Bromide in the sample.

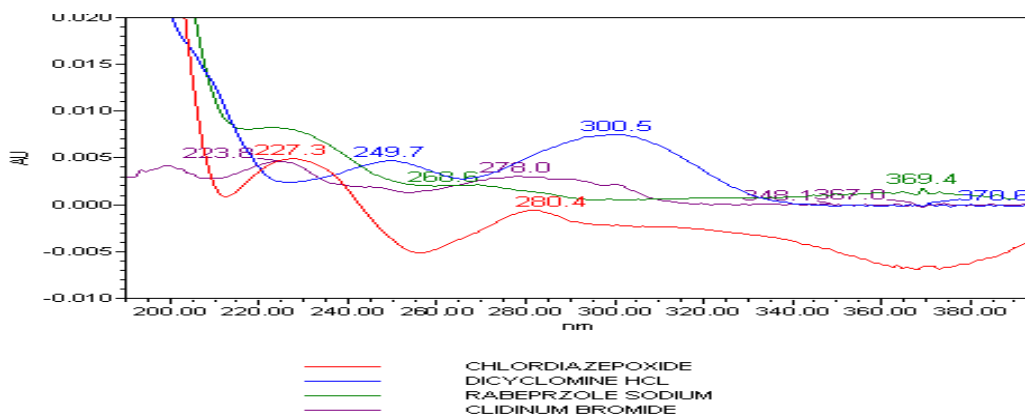
### Assay calculations

DRUG	LABELED AMOUNT(mg)	AMOUNT PRESENT(mg)	% ASSAY
Dicyclomine hydrochloride	10	10.5	99.8
Chlordiazepoxide	5	5.1	100.2
Clidinium bromide	2.5	2.6	100.4
Rabeprazolesodium	10	10.1	100.8

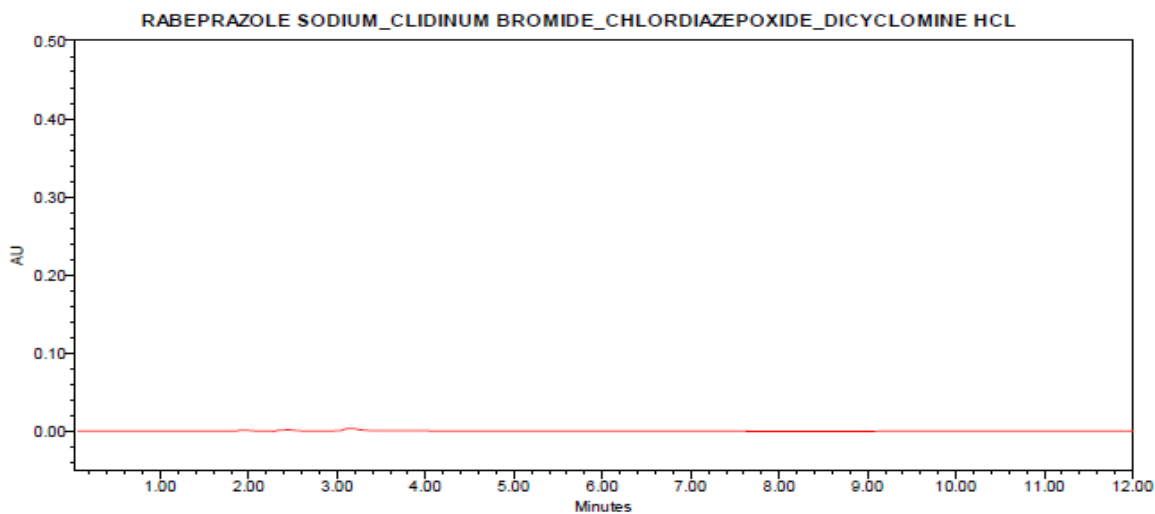
### Validation parameters

Parameter	Acceptance Criteria	Chlordiazepoxide	Clidinium Bromide	Dicyclomine Hydrochloride	Rabeprazole Sodium
Linearity	Correlation coefficient	$r^2 = 0.9985$	$r^2 = 0.9982$	$r^2 = 0.9985$	$r^2 = 0.999$
Range	$r^2 > 0.999$				
System Precision	RSD < 2%	%RSD = 0.43	%RSD = 0.317	%RSD = 0.119	%RSD = 0.148
Intermediate Precision	RSD < 2%	%RSD = 0.627	%RSD = 1.909	%RSD = 1.896	%RSD = 1.755
Method precision	RSD < 2%	%RSD = 0.130	%RSD = 0.868	%RSD = 0.336	%RSD = 0.879
Accuracy	Recovery 98- 102% (individual)	% recovery=100.3	%recovery=100.5	% recovery=104.5	% recovery=100.5
Solution Stability	> 12 hour	Stable up to 24 hour	Stable up to 24 hour %RSD = 0.741	Stable up to 24 hour %RSD = 0.253	Stable up to 24 hour %RSD = 0.187
Robustness	RSD NMT 2% in modified condition	Complies %RSD= 0.415	Complies %RSD= 0.268	Complies %RSD= 1.638	Complies %RSD= 1.070
	Flow minus	%RSD= 0.669	%RSD= 1.077	%RSD= 0.749	%RSD= 0.861
	Flow plus	%RSD=0.338	%RSD=0.168	%RSD=0.865	%RSD=0.171
	Organic plus	%RSD=0.272	%RSD=0.085	%RSD=0.698	%RSD=1.530
	Organic minus	%RSD= 0.35	%RSD= 0.46	%RSD= 0.4	%RSD= 0.35
	Wavelength plus	%RSD= 0.099	%RSD= 0.236	%RSD= 0.489	%RSD= 0.239
	Wavelength minus				

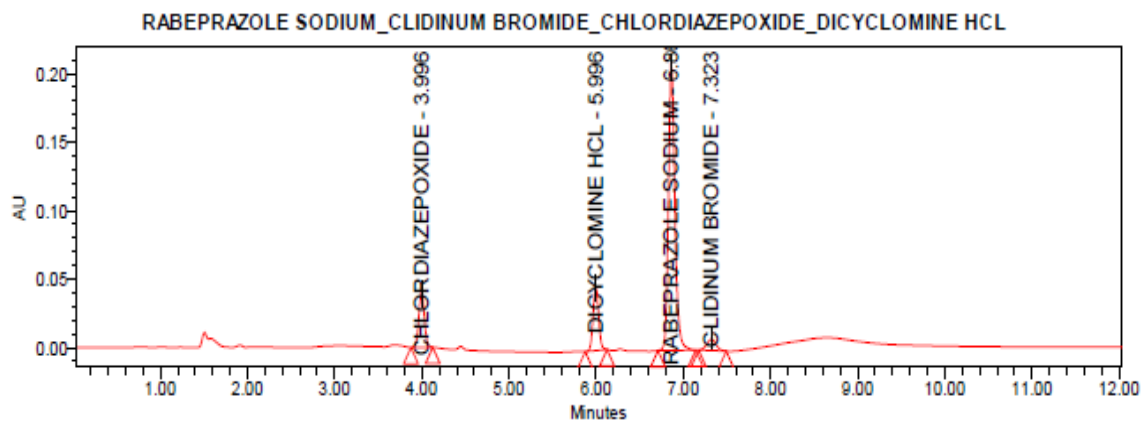
**Tables/Figures**



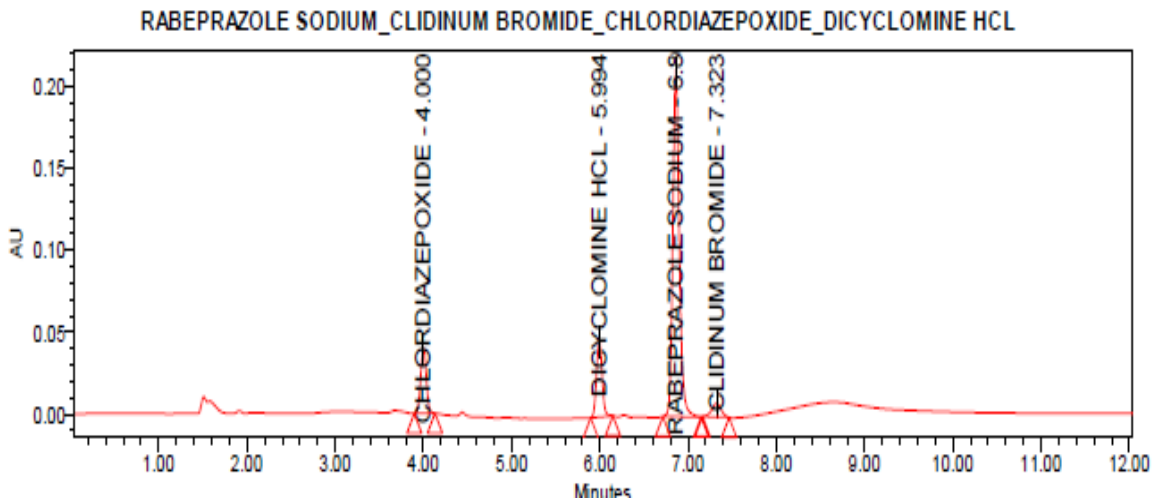
Overlay Spectrum of Chlordiazepoxide, Clidinium Bromide, Dicyclomine Hydrochloride, Rabepazole Sodium, (250nm)



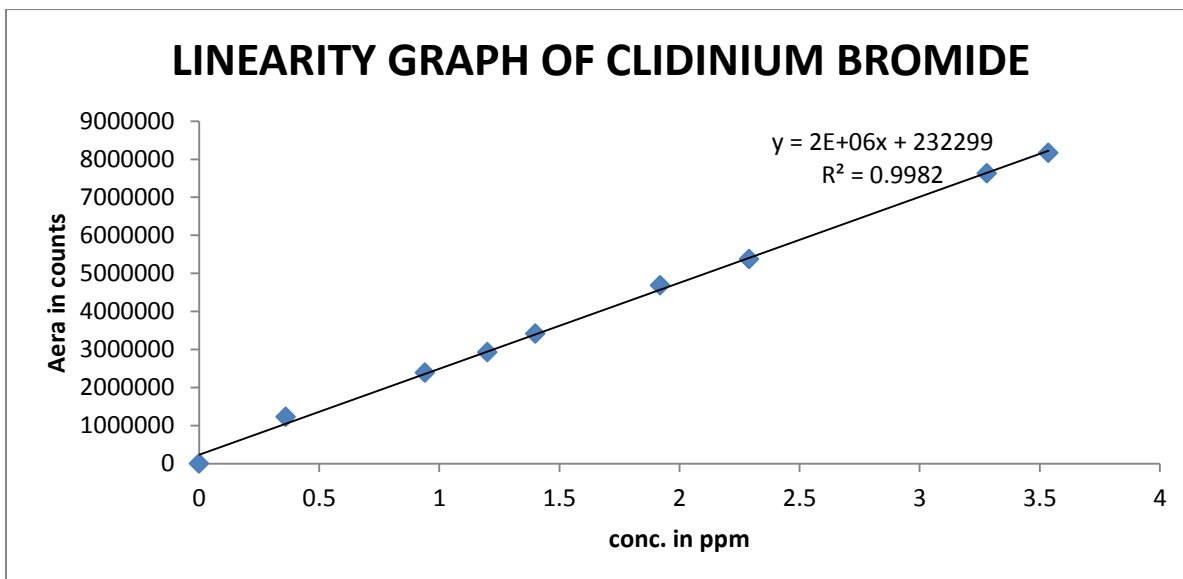
*A Representative chromatogram of blank*

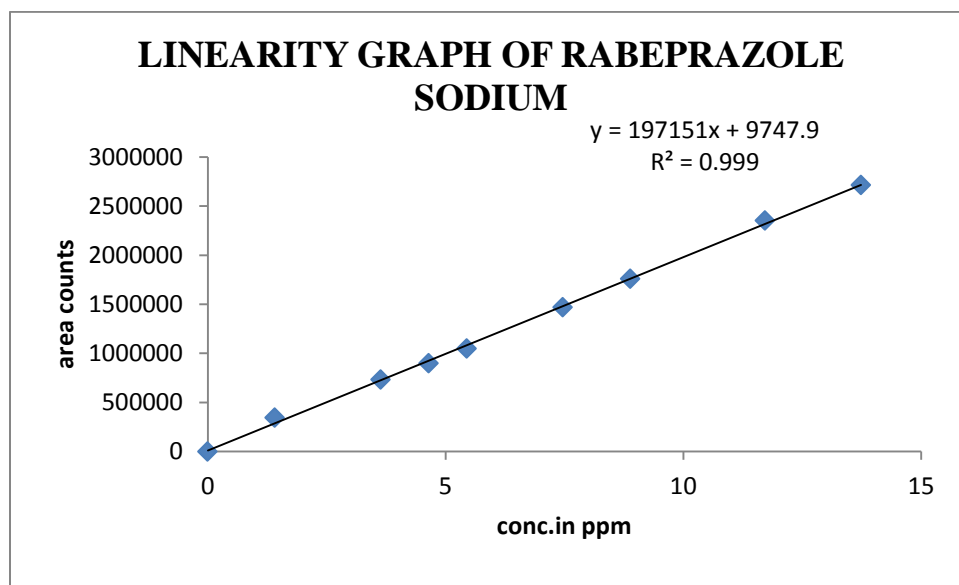
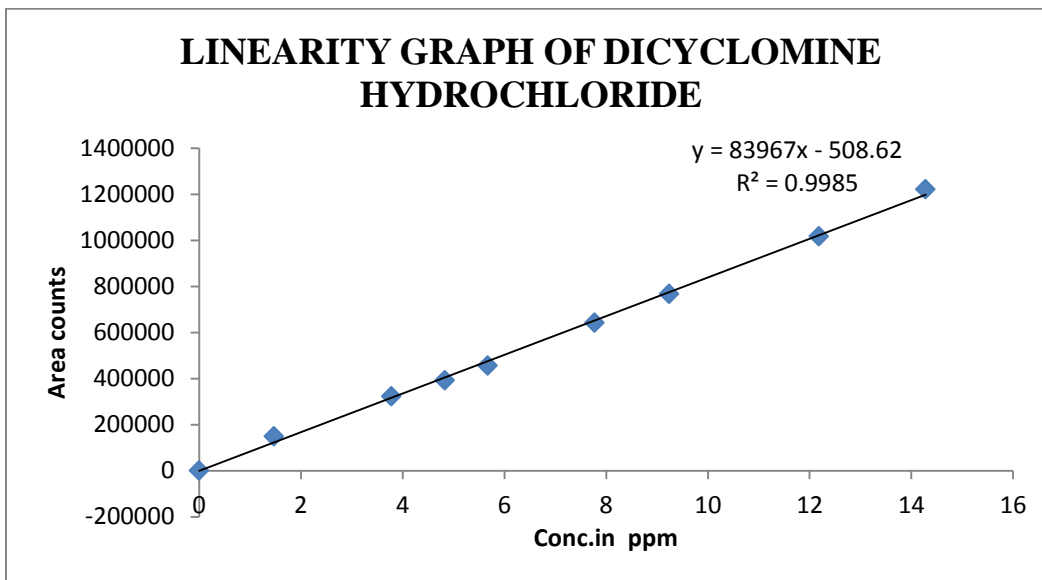


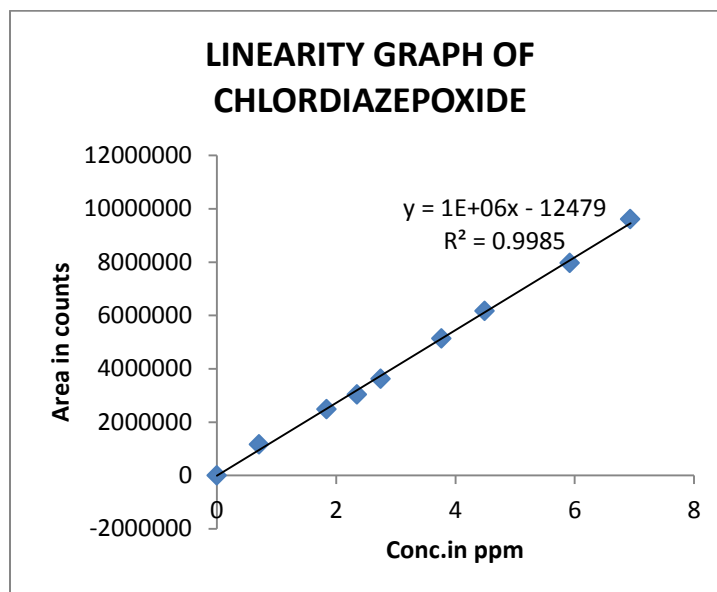
*A Representative chromatogram of Standard*



A Representative chromatogram of sample







## DISCUSSION

In the present investigation new analytical method has been developed for the estimation of potent drugs Chlordiazepoxide, Clidinium bromide, DicyclomineHCl and Rabeprazole sodium in combined dosage form. Since there is no analytical method developed for this combined dosage form, the developed method provides a wide choice for the routine determination of Chlordiazepoxide,

Clidinium bromide, DicyclomineHCl and Rabeprazole sodium in bulk and Pharmaceutical dosage forms according to ICH guidelines.

## Acknowledgements

I am very much indebted to ICON LABORATORIES, Vijyawada, my industrial guide Mr.Narendra kumar for providing an opportunity to undertake this dissertation.

## REFERENCES

- [1]. [www.Medindia.net](http://www.Medindia.net)
- [2]. <http://www.drugs.com/ingredient/Dicyclomine.html>
- [3]. "Clidinium bromide". Drugs.com. Retrieved 24, 2013.
- [4]. "Clidinium Bromide Monograph". Drugs.com. Retrieved 24, 2013.
- [5]. [.http://www.webmd.com/drugs/2/drug-148989/Chlordiazepoxide-oral/details](http://www.webmd.com/drugs/2/drug-148989/Chlordiazepoxide-oral/details)
- [6]. [.http://en.wikipedia.org/wiki/Chlordiazepoxide](http://en.wikipedia.org/wiki/Chlordiazepoxide)
- [7]. drugs.com International availability of Rabeprazole via drugs.com
- [8]. ICH Tripartite Guidelines, Q2R1 Validation of Analytical Procedures: Text and Methodology, ICH, Geneva, Switzerland, 2005.
- [9]. Tripathi K.D. Essentials of Medicinal Pharmacology, 6th ed., New Delhi, India, 2008
- [10]. AshutoshPathak, PallaviRai, and Sadhana J. Rajput\*Stability-Indicating HPLC Method for Simultaneous Determination of Clidinium Bromide and Chlordiazepoxide in Combined Dosage Forms, Journal of Chromatographic Science, 48, 2010.
- [11]. RK Venisetty\*1 and Kamarapu Sk2Rp-Hplc Method Development And Validation For Simultaneous Estimation Of Clidinium Bromide, Chlordiazepoxide And Dicyclomine Hydrochloride In Bulk And Combined Tablet Dosage Forms International Journal of Advanced Biomedical & Pharmaceutical Research *RK Venisetty and Kamarapu SK. Int J of Ad Biomed & Pharm Res.* 2(1), 2013, 35-40. e- ISSN 2321-4961



- [12]. Manish Hiranand Bachani\*, Dhaval Suresh Acharya, Krunal Vasantkumar Shah, Development and Validation Of Hplc Method For Simultaneous Estimation Of Dicyclomine Hydrochloride, Acetaminophen And Clidinium Bromide In Solid Dosage Form International Journal of Pharmacy and Pharmaceutical Sciences 5(2), 2013, ISSN- 0975-1491.
- [13]. ShreenidhiSurve\*, ArpitPatwari, Jiten Patel, IshwarsinhRathod, Mahesh Chhabria HPTLC and HPLC Method Development And Validation For Simultaneous Estimation Of Rabeprazole Sodium And Levosulpiride In Bulk And Its Pharmaceutical Dosage Form International Journal of Pharmacy and Pharmaceutical Sciences 5(3), 2013, ISSN- 0975-1491.
- [14]. Alaa S. Amin\*, Hassan A. Dessouki, Moustafa M. Moustafa, Mohammed S. Ghoname Spectrophotometric methods for sertraline hydrochloride and/or clidinium bromide determination in bulk and pharmaceutical preparations Chemical Papers 63(6), 2009, 716–722, DOI: 10.2478/s11696-009-0069-8
- [15]. T. Sireesha\*, K.Shantakumari, R.V.Durgasai and K.SudhaNaik Development And Validation Of New Analytical methods For The Simultaneous Estimation Of Paracetamol And Dicyclomine Hydrochloride In Bulk And Pharmaceutical Dosage Forms By Using Rp-Hplc And Uv Methods World Journal of Pharmaceutical Research SJIF Impact Factor 5.045 3(10), 1584-1602. Research Article ISSN 2277– 7105
- [16]. Baldha R1. G., Patel Vandana. B.2\* and Mayan Simultaneous Spectrophotometric Estimation of Rabeprazole Sodium and Domperidone in combined dosage forms International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN: 0974-4304, 2(2), 2010, 1563-1568.
- [17]. Aravind.Doki\* And Kamarapu.Sk Method Development And Validation Of Rp-Hydrochloride In Bulk And Combined Tablet Dosage Forms Hplc Method For Simultaneous Estimation Of Clidinium Bromide, Chlordiazepoxide And Dicyclomine Research Article Pharmaceutical Sciences *Ijpbs* 3(3), 2013, 152-161
- [18]. D. Umamaheswari\* , M. Kumar. Jayakar, Rajesh Chatakonda Method development and validation of Itopride Hydrochloride and Rabeprazole Sodium in pharmaceutical dosage form by Reversed Phase High Performance Liquid Chromatography *Journal of Chemical and Pharmaceutical Research J. Chem. Pharm. Res.*, 2(5), 2010, 399-417, ISSN No: 0975-7384 CODEN(USA): JCPRC5B.