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RESEARCH ARTICLE

Determination of some derivative amine with Bethanechol by Ion Chromatography

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Abstract

Amines and derivative amine Compound are detected by Ion Chromatography measuring Detected at baseline 0.15µs cm^{-1} of complex formed with the post column Methane sulphonic acid as eluent .Using this method , derivative amine will coelunt with the Bethanechol. Rapid, efficient, cost effective and reproducible isocratic reversed phase method has been developed and validated for the determination of Bethanechol in active an organic ions using mobile phase 30mM Methane sulphonic acid having pH 4.5 Mobile phase was pumped at a flow rate of 0.25 mL min^{-1} using isocratic elution through Ion pac ,Cs_{18} (250×2 mm, 5 µm) column. Conductivity detection was performed at 0.15µs cm^{-1} . Method was validated following the IC Isocratic. Calibration curve was linear in concentration range [Diethyl amine (A)=0.2-1.0 , Ethylene diamine (B)=0.1-0.5 , Trimethyl amine (C)=0.3-1.0), bethanechol chloride (D)=5-25] µg mL^{-1} (n=3) with correlation coefficient 0.996-0.999, and lower limits of detection and quantitation as 1.050 ,0.529 ,1.495 and ,26.400 ng mL^{-1} and 3.184 ,1.605 4.532 and 80.000 ng mL^{-1} in standard material respectively. Recovery was found to be in the range 97-100% and precision less than 1%. Developed method was successfully applied for analytic analysis of standard solution and also to study the interaction of Derivative Amine and Bethanechol temperature (25-30°C) .

INTRODUCTION

One of the most e important applications of Ion chromatography IC is the termination of amine and derivative amine compound in active pharmaceutical Drugs (APDs) Approximately 5% of Biochemical industries and antibiotic drugs on the market are developed in salt for certain physicochemical and Biopharmaceutical propertied of (APDs) can be improved by pairing basic or acidic drug[1] Amines and derivative amine are widely used in various industries ,such as pharmaceutical , power and Biochemical industries , in pharmaceutical , Amines and derivatives may be used in the production some agents and drugs[2] . Diethyl amine (A),Ethylene diamine (B), Trimethyl amine (C) ,and bethanechol chloride (D) 2-[(amino carbonyl) oxy] – N ,N ,N,trimethyl propane ammonium chloride is quaternary ammonium compound but (B) derivative amine compound is di- amine ,(A)
and (C)derivative amine compound is mono – amine that are structurally and pharmacologically related to acetylcholine and different chemical compound[3] .its administered either as injection or tablet for the treatment of urinary retention . some methods are treated derivative amines and Bethanechol such as Ion chromatography and gravimetric , titremetric to assay this compounds[4] . But the best method to determination bethanechol and

derivative amine by ion chromatography exchange because it is more specific method and Stability[5]. The proposed method specifies the use of a Dionex ion pac Cs18 separator column using a manually prepared methane sulfonic acid (MSA) eluent and suppressed conductivity detection in this application were studies re reducibility ,linearity, method detection limits potential interference during the determination at derivative amine and bethanecol. Chromatography techniques with conductivity Detection plays an important role in the derivative amine and Bethanechol by baseline 0.15 µs/cm and pressure 170 Bar at 25 -30 ^{o}C with Cs 18 Column also 690 Ion Chromatography system . Analytical method that have been used for determining derivative amine in metrics by IC system with conductivity Detector has advantages of µl /min flow rate and low eluent consumption ,which allows continuously run and available IC system with out additional[6] consumable replacement costs and saving time ,spent on equilibrating and recalibration typically need after each start – up [7]. The Ion chromatography method allow for the analysis of derivative amine and Bethanechol samples according to 690 IC system and high sensitivity method[8].

Goal:

To develop an efficient and comprehensive LC (IC) method for the analysis and determination of Derivative amine and Bethanchol in Biochemical ,pharmaceutical antibiotic and some drug reagents. This method must separate the Four main active components (Diethyl amine, Ethylene diamine, Trimethyl amine, Bethanechol) .

Experimental :-

A Ion Pac – Cs_{18} ; 5µm , 2×250 mm *(P/N* 060478*)* was chosen for this separation because its sulphonyl groups are structurally similar to the phenyl groups and aromatic structures contained in the compound at interest[9]. The separation at derivative amine and bethanechol sample can be completed within Run time 17min using a 30 mM methane sulphonic acid[MSA].

Equipment:-

A home-mad IC-UV system including :-
LKB Bump 2150 –HPLC ,Bromma -
- Dionex Ion Pac column - Cs18 ; 5µm , 2×250 mm *(P/N* 062878*)*
- Electric injection valve 100 µL loop inject in system before flow Conductive cell .
– - Conductive Detector
- Flow cell , 1.5 µL

Recommended Equipments

Ahome - made designed IC by metrohm 690 with two detector (conductivity and UV. Vis) and LKB Isocritic pump C2150.

Standards

100 mg/liter of Bethanechol Compound and 50mg/l for each derivative amines can be prepared in the laboratory or it is available commercially from chemical companies provided such these standard solutions used applying forIon Chromatography , Such these standard solution dissolved in the solution of pH values = 4 - 4.5 which can be used as standard solutions by applying chromatography.

Chloride ion for Bethanechol cation and proton for derivative amine[10] .

Deionized water is used by 18.2 MΩ . cm to prepare standard solutions.

Cation Exchange of Simultaneous separation , Bethanechol with derivative amines:

- **Analytes**
  Diethyl amine $C_4H_{11}N$, Ethylene di-amine $C_2H_8N_2$, Trimethyl amine C_3H_9N, Bethanechol $C_4H_8O_2N_2^+Cl^-$.

All peaks (n = 3) were well resolved and the precision was acceptable. System suitability of the method was studied through method development by calculating LOD, LOQ, Intercept , Slope, Retention Time standard error, standard

error estimate and repeatability favorable for the system are summarized in Table **1,2** and **3**.the values in table 4 referred to high accuracy and success method project[11] .

Discussion of method:

Separation column used in this method is Ion pac Cs_{18} for a time 17minutes and the linear gradient ranged between 3-14 minutes of Methane sulphonic acid while the total time of separation is 19 minutes. All Amines can be detect under Baseline Conductivity 0.15µs / cm for cations complex with MSA . This reagent can be remained under effect of H_2 or N_2 gas [12] .

Derivative amine with Bethanechol can be separated by applying cation simultaneous exchange technique [13] is dissolved by its solution in this experiment can be achieved by Chromatography separation benefiting on the electronic selectivity differences between two types amine [14] the singular and dual cation making stable selectivity to separated with methane sulphonic acid allowing with separation while Bethanechol are remained non-separated in the column and upon a time of displacing derivative , it is displaced with the most stable cation using MSA . Total time of both ions about 17 -19 minutes and that All amine can be detected by measuring Conductivity at 0.15µs cm^{-1} . Accounts of Intercept and Slope by using Five solutions in the concentration (n=3) against peak s height for each ion table 2 .

Lower limits of detection and quantitation:-

Lower limit of detection (LLOD) and quantitation (LLOQ) are the concentrations that give signal to noise ratio of 3:1 or 10:1 respectively which can be detected and verified by the relation of standard deviation of response (SD) to the slope of calibration curves (S) [15] :

LLOD=3.3 SD/S

LLOQ=10 SD/S

Linearity and range [16]

Linearity of method was tested in order to demonstrate a proportional relationship of response verses analyte concentration. It was studied at Five concentration level in the range (A=0.2 – 1.0) ,(B=0.1 -0.5) ,(C =0.3 – 1.5) ,(D= 5 -25)µg mL^{-1} (n=3). The regression equation was found linear by plotting peak area verses amine concentration, correlation coefficient obtained for the regression line was greater than0.996 - 0.999. Table 2 represents the regression data including, correlation coefficient, slope, intercept, standard error and standard error estimate .

Accuracy/recovery studies [17]

The accuracy of an analytical method is determined by how close the test results obtained by that method come to the true value. It can be determined by recovery studies, where a known amount of standard is spiked in the sample to be analyzed. The results of accuracy studies are shown in Table 3 and it is evident that the method is accurate within the desired range .

Ruggedness

Ruggedness of proposed method was evaluated for two days in two different labs with two different instruments i.e HEC Lab, Department of Chemistry, University of Bsrah and the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Bsrah. The instruments in both laboratories were Swiss model ICS 690. % RSD and % recovery obtained are tabulated in Table 3 and 4 which was found within acceptable limits and proved the ruggedness of method table1.

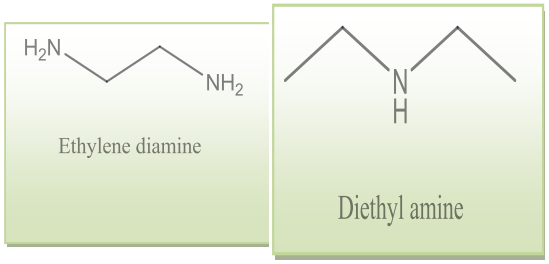
Precision [18]

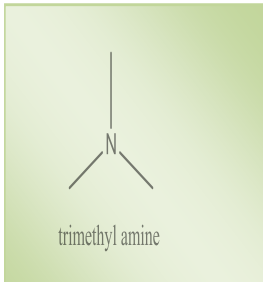
Precision of method, reported as %RSD, was estimated by measuring repeatability (intra-day assay) on seven replicate injection at concentration of (A =0.6,B =0.3 ,C= 0.9 D = 15) µg mL^{-1} in Table 3, and intermediate precision (inter-day variation) was studied for two days using Self that concentration solution (n=3). All the results given in Table 4 .

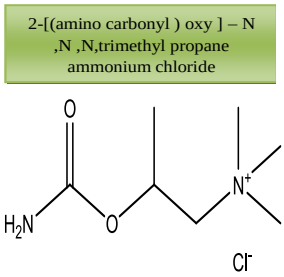
Conclusion :-

A simple, rapid, accurate, precise, low cost and least time consuming IC method for the quantitative analysis of The derivative amine as standard solution, has been developed and validated. The intra-run and inter-run variability and accuracy results were found in acceptable limits. Simplicity of the method[19] , shorter run time, economical nature and low limits of detection and quantitation makes the method superior to the other reported IC methods. The method could be applied analysis of studied organic materials. The method has been applied to study cations metal

interactions and the results reveal that the presence of metals ion has a more pronounced effect on availability of cations is the main factor responsible for decreased or increased availability of cation [20] . Therefore, a concomitant administration of derivative amine Compound with Bethanechol seem advisable, Moreover, a proper time interval should be maintained .







Parameters	*Conditions*
Description Column	**Ion Pac CS18, 2 mm CSRS® ULTRA II 2 mm Auto Suppression® r**
Iso Critic Mobil phase	**Methane**
Detection System	*Conductivity detection*
Back Ground Conductivity	*.15µs / Cm0*
Flow Rate	*0.25 mL / min*
Temperature	*30 °C*
pressure Background	**170 Bar**
Run Time	*17 Min*
Injection Volume	*100µL*

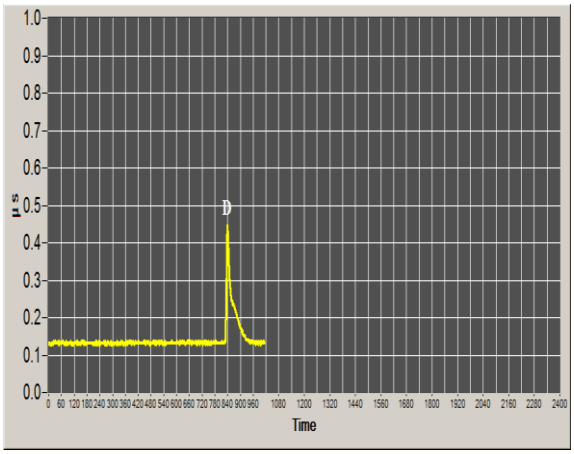


Fig 1 Bethanechol Grave

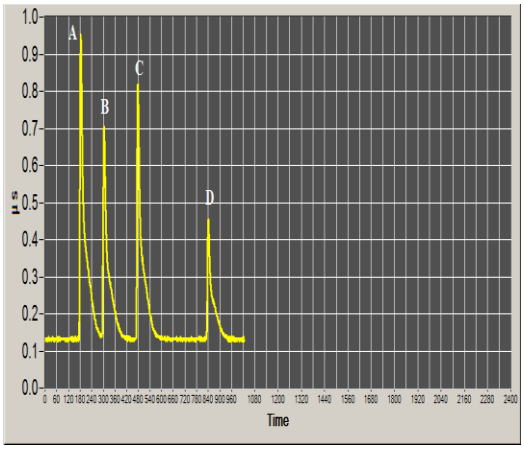


Fig 2 Derivative amine with Bethanechol Grave

Table 2: Regression statistics of the proposed method with LOD, LOQ, Intercept and Slope for Bethanechol and derivative Amines

Ions Compound	Retention Time(min)	R^2	Std Er[a]	Std Er Est[b]	Intercept	Slope	LOD ng mL^{-1}	LOQ ng mL^{-1}
A	4	0.999	1.149	1.0954	-0.571	127.143	1.050	3.184
B	6	999.0	1.149	1.0954	-0.762	159.714	0.529	1.605
C	9	0.997	1.9810	1.8886	-1.190	69.52	1.495	4.532
D	15	0.999	.7660	0.766	-0.381	2.057	26.400	80.000

[a]standard error, [b]standard error estimate

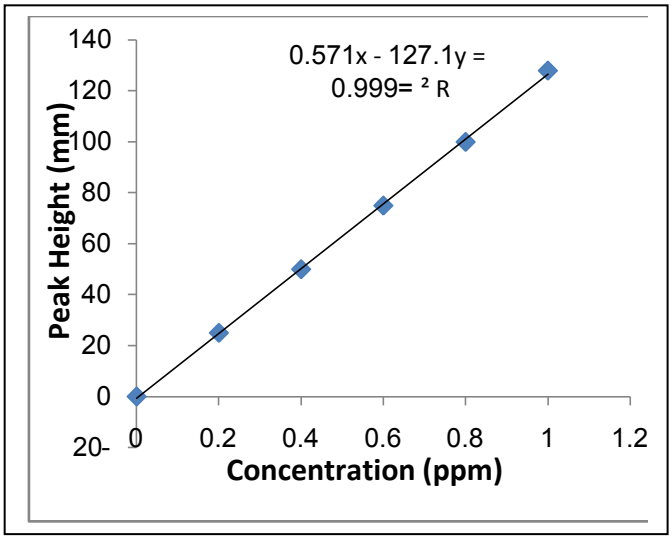


Fig 3 :linearity A Compound

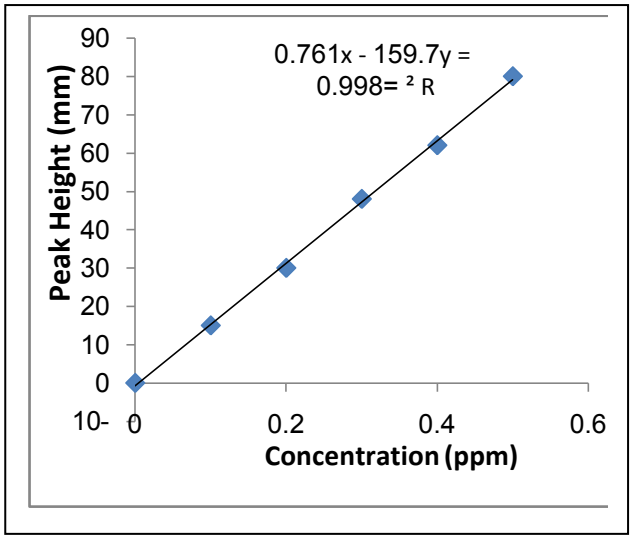


Fig 4 : linearity B Compound

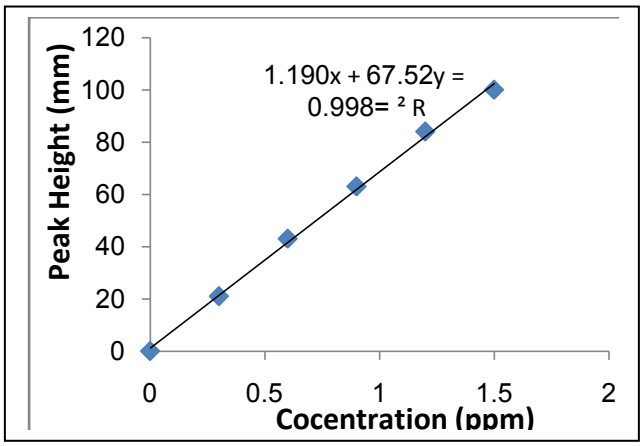


Fig 5 : linearity C Compound

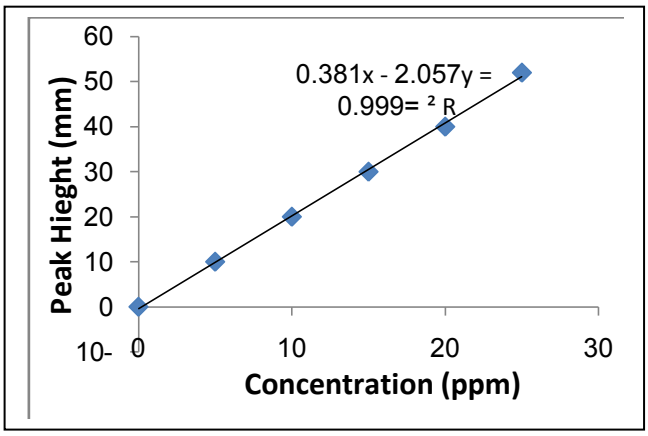


Fig 6 : linearity D Compound

Table 3: Method accuracy from recovery assays for the studied analyses.

Ions Compound	Recovered conc. ($\mu g\ mL^{-1}$)	% Recovery	%RSD
A	**0.6**	**100**	**0.84**
B	**0.29**	**96**	**0.77**
C	**0.89**	**98**	**0.75**
D	**15**	**100**	**1.12**

Table 4: Intra and inter-day precision and accuracy of analytes added **(A= 0.6, B = 0.3,C= 0.9, D =15)** $\mu g\ mL^{-1}$ (n=3) .

	Intra-day			**Inter-day**		
Ions Compound	**Found**	**%Rec**	**%RSD**	**Found**	**%Rec**	**%RSD**
A	**0.6**	**100**	**0.84**	**0.59**	**98**	**0.84**
B	**0.29**	**96**	**0.77**	**0.29**	**96**	**0.75**
C	**0.89**	**98**	**0.75**	**0.88**	**0.97**	**0.76**
D	**15**	**100**	**1.12**	**15**	**100**	**072**

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