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Novel Approach and Cloud Point Extraction Method for Determination of Acetazolamide Drug

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Abstract

Acetazolamide was hydrolyzed to primary aromatic amine by using (0.4) M NaOH under reflux. The product was evaluated by two a ways. The first way, Diazotization coupling reaction (approach) as simple, sensitive, rapid and selective Spectrophotometric method, using 8-hydroxyquinoline as Chromogenic reagent to give Azo dye (red) in basic medium. The second way involves applying Cloud point extraction, using Triton-x_{114} as surfactant. The Azo dye was diagnosed by FT-IR, ^{1}HNMR and UV-Visible technique. The analytical data for Approach and Cloud point extraction method, involve concentration rang (5-150), (0.5-6) µg.mL^{-1}, molar absorptivity (2.3×10^3), (1.3×10^4) L.mol^{-1}.cm^{-1}, Sandall's sensitivity (0.096) µg.cm^{-2} (0.017) µg.cm^{-2} and detection limits (0.952) µg.mL^{-1} and (0.043) µg.mL^{-1} respectively. In addition the measurement enrichment factor (100) and preconcentration factor (6.30), The proposed methods don't affect by the existence of excipients so the methods were applied *successfully in determining Acetazolamide in pharmaceutical preparations.*

Keywords: *Hydrolysis Acetazolamide, spectrophotometric determination, Cloud point extraction, Diazotization coupling reaction, 8-Hydroxyquinoline.*

Introduction

Acetazolamide a carbonic anhydrase inhibitor, which is used primarily to reduce intraocular pressure by decreasing aqueous humor formation, therapeutically for treatment of glaucoma, epilepsy and as a diuretic and has been used clinically since 1954 [1-4]. Acetazolamide in either medicinal forms or biological fluids were estimated in several techniques and methods have been declared in the literature, including HPLC for the quantification of acetazolamide in human and rat plasma [5-7], LC/MS and GC/MS [8-10], LC-UV [11-13], and spectrophotometry for determination acetazolamide and other sulfonamide drugs [14-19]. Applications of cloud point extraction techniques for estimation of some elements and drugs [20,21]. The present paper involves a novel determination of acetazolamide in pure and pharmaceutical formulations by Diazotization coupling reaction (approach) and Cloud point extraction spectrophotometric method, depending on basic hydrolysis of acetazolamide to primary aromatic amine, and coupling the product with 8-Hydroxyquinoline as a chromogenic reagent to give Azo-dye in alkaline medium.

Experimental

Instruments

The scanning of all spectrums and measurements of the absorbance at selected wavelengths achieved by T80 UV-Visible Spectrometer PG Instrumental Ltd, UK, with quartz cell matched 1 cm, Infrared spectra were registered using FT-IR, Shimadzu, Japan, ^{1}HNMR spectrum was registered using NMR Burker DPX 400 spectrophotometer operating at 300 MHz. the chemical shift δ is quoted in ppm relative to DMSO-d^6, while the pH was adjusted using 340i pH-meter WTW, Germany, and Heating-Cooling Water Bath – Haak Fe, Sartorius

Chemicals and reagents

Highest purity of Acetazolamide ($C_4H_6N_4O_3S_2$) was gained from state company for drug Industries and Medical Appliance-(SDI) Samarra-Iraq. 8-hydroxyquinoline (C_9H_7NO),Sodium nitrite ($NaNO_2$),Absolute ethanol (C_2H_5OH),Sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3) were gained from the BDH Company with Purity 99.00%. Hydrochloric acid (HCl) was gained from a BDH Company with

concentration 37.00%. Triton X-114 from Arcos organics, new jersey, USA with purity 100%. Pharmaceutical preparations that were used in this study were Cidamex and Diamox 250mg/acetazolamide from CID Egypt and France respectively.

Hydrolysis of Acetazolamide

0.1 g of Acetazolamide was hydrolyzed in alkaline medium by using 0.4 M NaOH under reflux for 2 hours. During the reaction, the secondary amide is converted to the primary amine [22]. The synthetic path of the product is shown in Scheme I. The hydrolysis product was cooled and diluted to suitable volume using distilled water to obtain a stock solution (2000 $\mu g \cdot mL^{-1}$). More dilute solution was prepared daily by neutralize of stock solution with dilute hydrochloric acid and dilute to final suitable volume using distilled water. The tablet sample (Cidamex and Diamox 250mg/acetazolamide) were prepared in the same manner.

2 mol. from the hydrolysis product was converted into dizonium salt ion by using 0.3 mL HCl 1 M and 0.2 mL $NaNO_2$ 1%. The dizonium salt ion was coupled with 1 mol. 8-hydroxyquinoline in alkaline medium to give azo-dye. The synthetic path of the azo-dye is shown in Scheme II. The azo-dye was precipitate, purified and diagnosed by FT-IR, ^{1}HNMR and UV-Visible technique as shown in Fig.2, 3 and 4.

General procedure

General procedure of Approach method

The calibration curve of acetazolamide was constructed by using a series of (10 mL) volumetric flask. Increasing volumes (0.1-3 mL) from Acetazolamide solution after hydrolysis (500 $\mu g .ml^{-1}$), mixed well with (0.3, 0.2 mL) from Hydrochloric acid (1 M) and (1%) Sodium Nitrate respectively, and stand for (5 min) to complete formation of dizonium salt. Then added (2.5 mL) 8-Hydroxyquinoline (0.03M) and (0.7 mL) Sodium Hydroxide (0.4 M). The volume was made up to mark with distilled water. The absorbance for all solutions was measured at (502 nm) at (25ºC) against solution blank.

General procedure of Cloud point extraction method

A series of (10 mL) volumetric flask, increasing volumes (0.05-0.6 mL) from a solution of Acetazolamide after hydrolysis (100 $\mu g .ml^{-1}$) mixed well with (0.1, 0.2 mL) from Hydrochloric acid (1M) and Sodium Nitrate (1%) respectively, and stand for (5 min) to complete formation dizonium salt. Then added (0.5 mL) 8-Hydroxyquinoline (0.005M) at pH=7.2 by using Sodium carbonate (0.2 M). The volume was made up to the mark with distilled water. The solutions were transferred into a centrifuge tube with added (0.5 mL) Triton X-114. The mixture was transferred into hot water bath about (10 min) at (55℃) to form a cloud solution. The mixture separated into two phases by centrifuge (10 min) at (3500 ramp), The aqueous phase decanted and the surfactant-rich phase diluted with (0.3 mL) absolute ethanol. The absorbance of final solutions was measured at (502 nm) against blank solution was prepared in the same away.

Result and Discussion

Identification of the prepared drug and Azo Dye

Identification of the Hydrolysis product and Azo Dye. The hydrolysis product was prepared as explained in paragraph 2.3. The qualities experimental were involved tests for amine group by using Nitrous acid test and Azo-dye test. [23-25] The hydrolysis product (5-amino-1,3,4-thiadiazole-2-sulfonamide) give yellow solution result from a reaction between a primary aromatic amine with concentrated HCl and $NaNO_2$ to formation diazonium salt ion, while the acetazolamide before hydrolysis not reaction. The two compounds were tested also by azo-dye test. The hydrolysis product after formation diazonium salt ion was coupled with 8-Hydroxyquinoline in alkaline medium to give azo-dye, while the Acetazolamide before hydrolysis not reaction. This azo-dye was prepared by taking a stoichiometric amounts was precipitate, purified and diagnosed by FT-IR, ^{1}HNMR and UV-Visible technique.

IR spectrum of azo-dye showed in Fig.1. one peak at 3417 cm-1 assigned for stretching (O-H) of phenolic group, peak at 1426 cm-1 assigned of stretching (N=N), and other peak 1575,1145 and 879 cm-1 assigned for stretching (C=N) thiadiazole, (S-C) and (C-S-C) groups respectively. This mean the formation of the aromatic primary amine group by hydrolysis of Acetazolamide and converted the amine in to diazonium salt in the presence of HNO_2 and coupling the last with 8-Hydroxyquinoline in alkaline medium. The ^{1}H-NMR spectrum (DMSO-d6, 400 MHz) of azo- dye complex showed chemical shifts at δ 1.66 (1H, $-SO_2NH_2$), Singlet broad band at δ 3.5 refer to water in the solvent, DMSO, where its band δ 2.5. δ 5.5 (1H, aromatic -C-OH), δ 7.1(2H,C-H_5

quinoline),δ 7.4 and 7.5 (3H,2H, C-$H_{22,4}$ quinoline), δ 8.3 and 8.85 (2H,1H, C-$H_{2,21}$ quinoline) [26-28] as shown in Fig.1. The qualitative and quantitative study of drug after hydrolysis, also done by UV-Visible technique. The Azo-dye complex (color product) scanned at (700-400 nm), and give a maximum absorption λmax at (502 nm) versus blank solution. while the scan of the blank solution versus water doesn't give any absorption at λmax for colored product as shown in Fig.3. This property was adopted in the estimation of trace amounts from Acetazolamide in pure and pharmaceutical preparations.

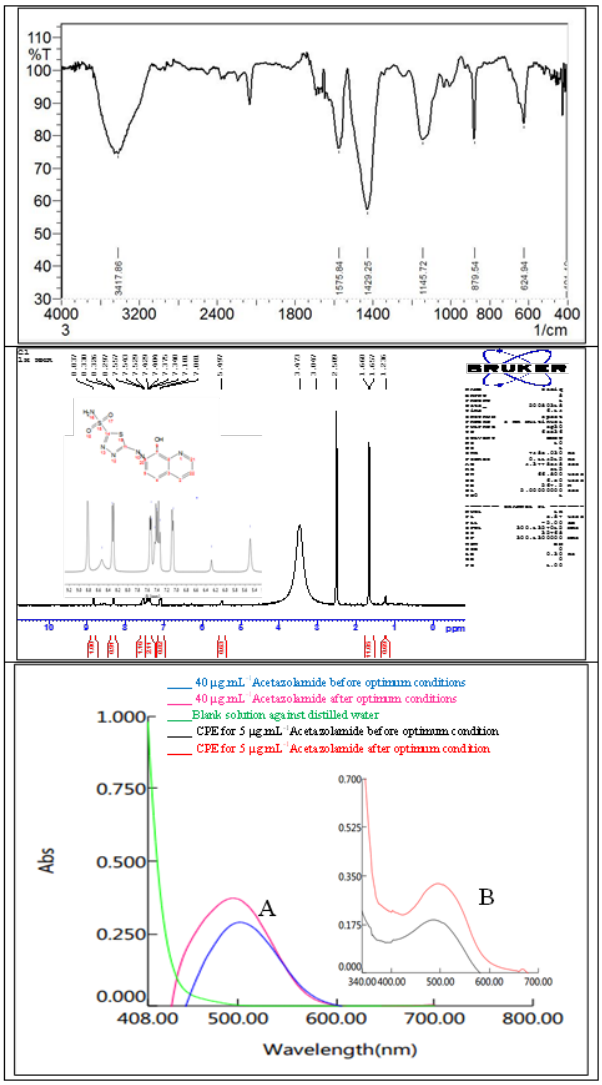

Figure 1. Identification of Azo Dye by FT-IR, ^{1}H-NMR and UV-Vis A: Azo-dye in Batch method, B: Azo- dye in Cloud point extraction method

Optimization of Experimental Conditions

Selection Optimal Experimental Conditions of Approach method

The effect of various experimental conditions as concentration, volumes from 8-Hydroxyquinoline reagent, volumes of HCl (1M), volumes of (1%) Sodium Nitrate, different types of bases, various volumes of Sodium Hydroxide (0.4 M) and temperatures of formation of (40 µg.ml^{-1}) azo dye were studied. The starting point involves using (0.5mL) from (1M) Hydrochloric acid with (0.5 mL) from (1%) Sodium nitrate, stand for (5 min), then (0.5mL) from (0.05M) 8-Hydroxyquinoline, and (0.5mL) from (0.4 M) Sodium hydroxide were added in (10 mL) volumetric flask. The absorbance of the solutions was measured at (λmax=502nm) against blank solutions after (5 min) since the beginning of the coupling reaction. The effect of various experimental conditions shown in Fig.2.

Selection Optimal Experimental Conditions of CPE method

The effect various concentrations, volumes of 8-Hydroxyquinoline reagent, volumes of HCl (1M), optimum pH value, volume of Triton X-114 5%, also effect of temperature and incubation time on formation of surfactant of (5 µg.ml^{-1}) azo dye were studied. The starting point involves (0.3 mL) of Hydrochloric acid (1M) fellow (0.2 mL) of Sodium nitrate (1%), stand for (5 min), then (0.3 mL) from 8-Hydroxyquinoline (0.005M), at (pH = 7) Sodium carbonate with (0.3 mL) Triton X-114 5% were added in (10 mL) volumetric flask. The mixture was heated (10 min) at 55 ℃ in water bath. The mixture was separated by centrifuge Ramp=3500 at (10 min). The surfactant rich phase was diluted with (0.3 mL) absolute ethanol and the absorbance of the solution was measured at (λmax=502nm) against the blank solution The effect of various experimental conditions shown in Fig.2.

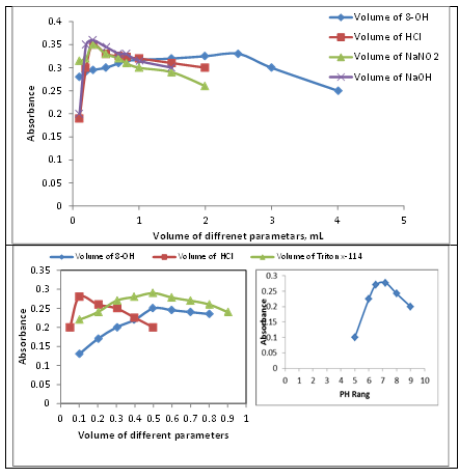

Figure 2 The effect of various experimental conditions on formation of Azo dye

Calibration curve

After fixing all the optimum conditions for the reaction of acetazolamide after hydrolysis with 8-Hydroxyquinoline of approach and cloud point extraction method the calibration curves were constructed as shown in Fig.3. The all analytical values were calculated with accuracy and precision result are summarized in Table.1. The results shown each method has a good accuracy and precision.

Table 1 Summary of analytical value of calibration curves with accuracy and precision resulting of approach and CPE method with

N	Parameter	Value	
		Approach method	**CPE method**
1	Regression equation	Y= 0.0103x+0.0099	Y= 0.0593x+0.0024
2	Slope	0.0103	0.0593
3	Intercept (a)	0.0099	0.0024
4	Correlation coefficient	0.9981	0.9992
5	Linear Range (µg.mL^{-1})	5-150	0.5-6
6	Molar absorptivity (Ɛ) (L.mol^{-1}.cm^{-1})	2.3×10^{3}	1.3 ×10^{4}
7	Sandall's sensitivity (S) (µg.cm^{-2})	0.096	0.017
8	Limit of Detection LOD (µg.mL^{-1})	0.952	0.043
9	Limit of Quantitation LOQ (µg.mL^{-1})	3.174	0.140
10	Preconcentration factor	---	100
11	Enrichment factor	---	6.3

accuracy and precision

Acetazolamide	Concentration µg mL^{-1}				
	Taken	**Found**	**Error*%**	**Recovery*%**	**RSD*% n= 5**
	Approach method				
	40	39.929	-0.175	99.824	0.362
	50	49.603	-0.793	99.206	0.103
	60	59.450	-0.916	99.080	0.963
	Cloud point extraction method				
	1.5	1.47	-2.00	98.00	0.212
	4	3.98	-0.50	99.50	0.163
	5	5.05	1.00	100.10	0.071

Stoichiometry of Reaction and mechanism

The methods of Mole ratio and Continuous variation, Jobs method were used to detect the stoichiometry of azo-dye formation from reaction 8-Hydroxyquinoline reagent with acetazolamide drug. The results obtained in Fig.4 shown that 2:1 acetazolamide to 8-hydroxyquinoline was formed at 502 nm. And The average conditional stability constant of the colored products in water at optimum conditions was 6 × 10^{12} L^2 mol^{-2} . The azo dye has high stability because the acetazolamide have electron with drawing group (sulfonamide) makes) $^{\oplus}N{\equiv}N$) group more positive charge and 8-Hydroxyqunolie have electron donating group makes ring very active so easy formation of high stability azo-coupling reaction between acetazolamide and 8-hydroxyquinoline[26]. The proposed mechanism of reaction illustrated in Scheme.II.

Pharmaceutical applications

The proposed methods approach and cloud point extraction were applied successfully for determination three concentrations form Acetazolamide in (Cidamex and Diamox 250mg) tablets as shown in the Table.3, after studying the effect of additives by adding separately excess amounts about (10:1) from additives to (40 µg.mL^{-1}) acetazolamide solutions under optimum reaction conditions followed in the calibration curves. As shown in a Table. 2.

Table 2. Determination of (40 µg.mL^{-1}) from Acetazolamide in the presence of additives

Excipient	Acetazolamide (40 µg.mL-^{1})		
	Conce found µg.mL^{-1}	Erorr%	Recovery*%
Pvp	39.83	-0.425	99.75
Lactose	40.05	0.125	100.13
Starch	39.69	-0.775	99.225
Mg stearate	40.07	0.175	100.175

*Average of five determinations

Table 3. Application of proposed methods on pharmaceutical preparation for Acetazolamide

	Concentration µg mL^{-1}				
	Taken	found	Error*%	Recovery*%	RSD*% n=5
(Cidamex) 250 mg			Batch method		
	40	39.74	-0.650	99.35	1.069
	50	49.41	-1.172	98.83	1.350
	60	60.06	0.111	100.11	0.970
			Cloud point extraction method		
	1.5	1.477	-1.53	98.46	0.743
	4	3.996	-0.10	99.90	0.793
	5	4.988	-0.24	99.76	1.107
(Diamox) 250 mg			Batch method		
	40	39.29	- 1.777	98.22	0.339
	50	49.83	- 0.330	99.67	1.120
	60	59.88	- 0.186	99.81	0.821
			Cloud point extraction method		
	1.5	1.495	-0.333	99.67	0.606
	4	3.962	-0.950	99.05	1.012
	5	4.972	-0.560	99.44	0.430

Evaluate the results of the proposed methods

The standard method for estimation Acetazolamide in the British pharmacopoeia was applied for determination of acetazolamide in pure drug and Pharmaceutical Preparations. The results of standard method comparison with proposed methods approach and cloud point extraction (F and t test value). The results summarized in the Table.4. Shown no significant differences between the two methods.

Table 4. Application of F, and t test for comparison between proposed and standard methods

	Pharmaceutical preparation	Proposed method		Standard method	
		Rec.*%		Rec.*%	
Batch method	Pure Acetazolamide	99.37	0.0009	99.89	0.1296
	Cidamex	99.43	0.0081	98.90	0.3969
	Diamox	99.23	0.0100	99.80	0.0729
	F-Value (experimental) = 0.1000, Critical F-Value (19.000) t-Value (experimental) = -0.9944 , Critical t-Value (2.776)				
CPE method	Pure Acetazolamde	99.20	0.0120	99.89	0.1296
	Cidamex	99.37	0.0025	98.90	0.3969
	Diamox	99.39	0.0049	99.80	0.0729
F-Value (experimental) = 0.0979, Critical F-Value (19.000)) t-Value (experimental) = -1.0990, Critical t-Value (2.776))					

Conclusions

A simple, rapid, sensitive and new selective Spectrophotometric methods have been developed, not affected by excipients, successfully applied for determination of trace amounts of acetazolamide drug in pure and pharmaceutical formulations based on basic hydrolysis of acetazolamide and coupling the hydrolysis product with 8-hdroxyquinoline reagent depending on the diazonium coupling reaction.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

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