Determination of Glimepiride, Amlodipine and Nitrofurantoin by Reversed phase - High Performance Liquid Chromatography (RP- HPLC Technique)

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ABSTRACT
The drugs were studied in this research are Glimepiride(GLM) is insulin independent and glucose reduction level in blood of human body ,Amlodipine (AML)is lowering blood pressure and Nitrofurantoin(NTF) is used to treat urinary tract infection. In spite of many analytical techniques were used to determine these drugs. Modified HPLC was used in present work ,this analytical technique is rapid, accurate, sensitive and applied for determination of drug in its formulations. Analytical parameters for determination of drugs GLM, AML and NTF by RP-HPLC at UV detector were as following ; mobile phas acetonitrile:water:ethanolsodiumphalate(60:20:20),cetonitrile:water:triethyamine(45:45:10), acetonitrile:buffer solution(30:70),flow rate (1.5,1.5,1.0) ml/minute, wave length (230,245,254) nm, retention time (8.169,6.771,4.413) minutes ,stationary phase C_{18} column, linear range 10-50, 2-10,5-25 µg/ml, limit of detection (2.8×10^{-5}),(2.9×10^{-3}),(9.3×10^{-3}) µg/ml, standard deviation (0.054,1.8439,0.0625) and the percentage of recovery (100.06%,100.9%,100.8%) respectively.

تقدير بعض الادوية بواسطة الطور العكوس تقنية كروموتوغرافيا السائل عالي الاداء

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الخلاصة
استخدمت الادوية غليميبيرايد ، وأملوديبين ونايتروفيورنتين في هذه الدراسة. لا يعتمد غليميبيرايد على الانسولين وانه يقلل كمية السكر في الدم البشري . يقلل الأميلودبين ضغط الدم و يستخدم نايتروفيرونيتن لعلاج ألتهاب المجاري اليولية . بالرغم من وجود عدد من التقنيات التحليليه المستخدمة لقياس الادوية لكن استخدم في هذا البحث تقنية كروموتوغرافيا السائل عالي الاداء المطور والتي كانت سريعة ودقيقة وحساسة لتقدير المواد الفعالة في الادوية . المتغيرات التحليليه المستخدمة لتقدير الادويه غليميبيرايد، وأملوديبين ونايتروفيورنتين بواسطة الطور العكوس تقنية كروموتوغرافيا السائل عالي الاداء بأستخدام كاشف الاشعة فوق البنفسجيه كانت على النحو الاتي: الطور المتحرك اسيتونترايل:ماء:فثالات الصوديوم الاثيليبه(60:20:20) والاسيتونترايل:ماء:ثلاثي الاثيل الاميني(45:45:10) والاسيتونترايل: محلول منظم(30:70) وسرعة الجريان(1.5,1.5,1.0)مل/دقيقه والطول الموجي(230,245,254) نانوميتر. وزمن الاحتجاز(8.169,6.771,4.413)دقيقه والطور الثابت عمودC_{18} والمدى الخطي(10-50, 2-10,5-25) مايكروغرام/مل وحد الكشف(2.8×10^{-5}) , (2.9×10^{-3}), (9.3×10^{-3}) (مايكروغرام/مل) والانحراف القياسي (0.054, 1.0849, 0.625) والنسبة المئوية للاسترجاعية (100.06%,100.9%,100.8%) لأدوية غليميبيرايد ، وأملوديبين ونايتروفيورنتين على التوالي .

1. INTRODUCTION

HPLC is separation technique that can be used for determination of organic molecules and ions. HPLC is based on mechanisms of adsorption partition and ion exchange. The efficiency of the column used in HPLC depends upon the height and the number of the plates[1 - 3]. Mobile phase, flow rate and detector play an important role in separation and detection of analytic in matrix of sample [4 - 6].The direct determination of drugs by HPLC depend upon the physical properties of drugs[7 - 9] while the indirect

determination of drugs depend upon the chemical properties of drug with metal to form metallic - drug [10] , or oxidative coupling of drug with other reagent in presence of acid or alkaline media [11] . There are three properties such as peak height, peak area and internal standard used for determination of drug by HPLC [12]. Although normal phase (NR - HPLC) and reversed phase HPLC (RP - HPLC) used in determination of drug, the present work was used RP - HPLC for determination of GIM, AML and NTF. In spite of many detectors used in HPLC techniques such as mass spectrometry [13], the recent work was used uv/visible detector due to its availability, cheap and reliable for use. There are many analytical methods used for quantitative determination of GLM, AML and NTF in their formulations such as tablets, syrup ,injection and capsules[14] .all the principle of previous methods depend upon the physical and chemical properties of the drugs.

2. MATERIALS AND METHODS

Materials

Acetonitrile for HPLC use, dimethyl foramide were bought from BDH company. Drugs were taken as gift from SDI company. Apparatus of HPLC of type shimadzu LC-20 AD HPLC Kyoto-japan supplied with column c_{18} (25 cm *4.6mm)25µm. pH meter type c830 multi-parameter analyzer

Methods

20 µl of GLM and AML were injected in HPLC technique using UV detector while 100 µl of NTF was injected and many experiments were run to get the best separation of drug using different analytical conditions for each drugs

Preparation solution

Preparation of 50µg/ml GLM

It is prepared by dissolve 0.05 gm of GLM in mobile phase contains acetonitrile , water , and ethanol sodium phalate (60 : 20 : 20) and complete the volume to 100 ml volumetric flask with mobile phase , then 10 ml of this solution diluted to 100 ml with mobile phase to get 50µg / ml GLM

Stationary phase: The same column was used for NTF drug

Mobile phase: Acetonitrile: water :ethanol sodium Phalate(60:20:20)

Preparation of 20 µg / ml AML

It is prepared by dissolving 0.025 gm from pure AML powder in mobile phase solution of acetonitrile , water and triethyl amine at ratio(45 : 45 : 10) and complete the volume to 50 ml in volumetric flask with mobile phase . 1 ml is diluted to 25 ml with mobile phase in volumetric flask to get 20 µg / ml of the drug .

Stationary phase:Column C18 (250mm × 4.6mm × 5 - 10µm)

Mobile phase: Acetonitrile: water: Triethylamine(45:45:10)**.**

Preparation of 1000 µg / ml NTF

It is prepared by dissolve 1.0 gm. of pure NTF powder in 1000 ml volumetric flask on which 50 ml of dimethyl formide was added and the volume was completed by solution to the mark . From this solution 1ml is diluted to 10 ml in volumetric flask by (Acetonitrile: Buffer) to obtain a solution of 100 µg / ml.

Buffer solution

It is prepared by dissolving 6.8 gm K_2HPO_4 in 500 ml distilled water , 30 ml of 1M NaOH is added and the pH adjusted to 7.0 and complete the volume with distilled water to one liter .

Mobile phase

The mobile phase was Acetonitrile : Buffer (30 : 70)

Stationary phase Column C_{18} (250mm × 4.6mm× 5 - 10µm)

3. RESUITS

Calibration Curves for the drugs

Solution of different concentrations (10-50) µg/ml were prepared from stock solution , 20 µl of GLM was injected by HPLC and response was measured at 230 nm . The calibration curve showing in figure 1

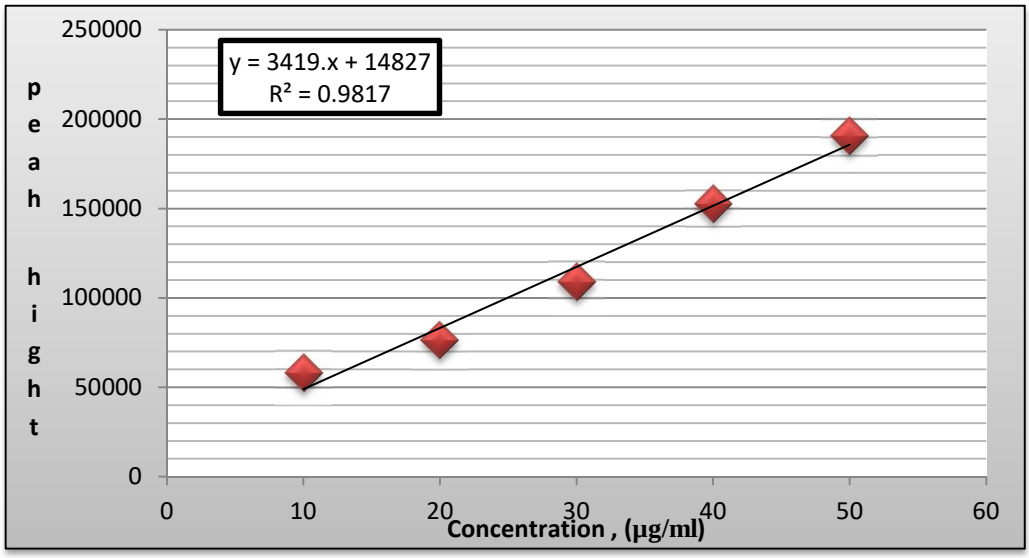

Figure 1:Calibration curve of GLM

Solution of different concentrations (2-10) µg/ml were prepared from stock solution , 20 µl of GLM was injected by HPLC and response was measured at 245 nm . The calibration curve showing in figure 2

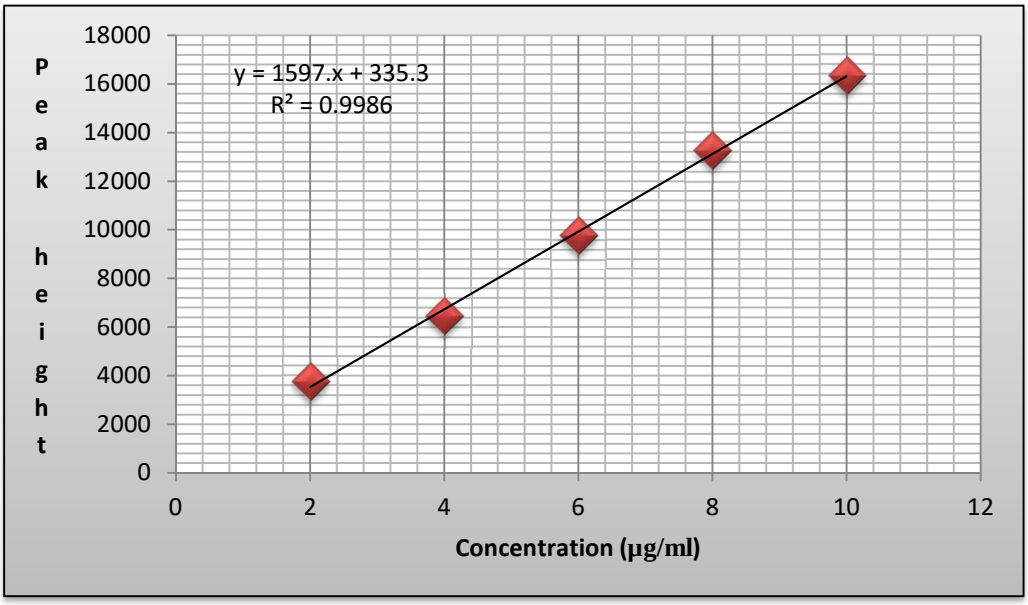

Figure 2:Calibration curve of AML

Serious of solution (5 - 25) µg / ml were prepared from the stock solution 100 µl of the NTF was injected in HPLC and the response was measured at 254 nm . The calibration Curve is showing in Figure -3-

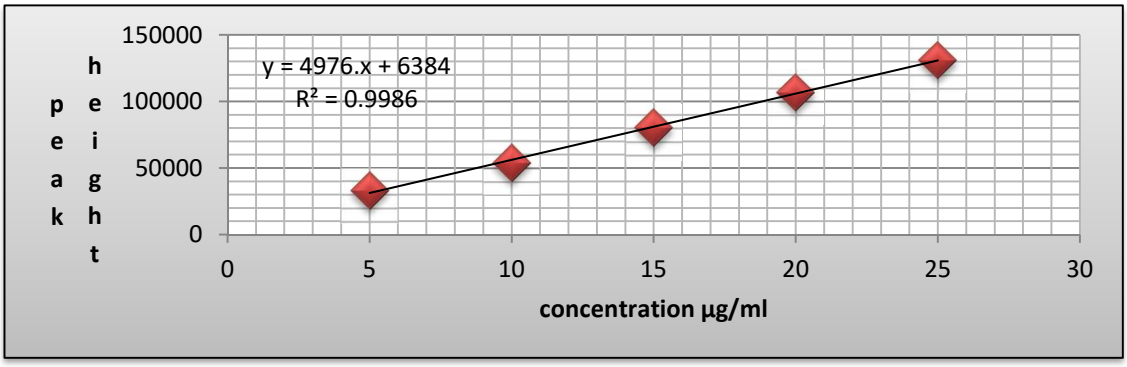

Figure 3 Calibration of NTF

Accuracy and precision

The accuracy and precision of the methods for determination of drugs were calculated in Tables1,2,3

Table. 1. determination of GLM(%R.S.D=0.054)

No	Conc. Of drug (ppm)	Peak height (mv)	Peak area(mv)	Conc.ofdruge(found)	Recovery%
1	30	107981	2640948	27.2460	**90.8200**
2	30	107984	2640951	27.2469	**90.8230**
3	30	107988	2640949	27.2480	**90.827**
4	30	107999	2640953	27.2512	**90.8373**
5	30	108987	2640941	27.5402	**91.8007**

Table. 2. determination of AML(%R. S.D=1.8439)

No	Conc. Of drug ($\mu g.ml^{-1}$)	Peak height(mv)	Peak area (mv)	Calculated concentration	Recovery.%
1	6	9788	184647	5.9190	**98.6500**
2	6	9784	184653	5.9165	**98.6090**
3	6	9801	184649	5.9272	**98.7863**
4	6	9906	184675	5.9929	**99.8800**
5	6	9911	184681	5.9961	**99.9343**

Table . 3 . determination of NTF(%R.S.D=0.0625)

No.	Conc. Of drug ($\mu g.ml^{-1}$)	Peak height (mv)	Peak area (mv)	Calculated concentration	Recovery.%

1	15	80600	20830362	14.91	**99.43**
2	15	81225	20925401	15.04	**100.27**
3	15	81280	20783212	15.05	**100.34**
4	15	80996	20809621	14.99	**99.96**
5	15	80527	20800123	14.90	**99.30**

Evaluation of the Results

The results were evaluated by using t-test and F-Value for comparison between proposed method for determination of these drugs and standard methods used in British pharmacopeia B.P 2005 , t - test for these experiment is 0.285 less than tabular value 2.776 at reliable level 95 % .F.value for experiments is also 1.142 less than tabular value 6.390 at reliable level 95% .

Studying the Interference Effect in Recent Analytical Technique

100 µg / ml of Glucose, Maltose Fructose and Starch solution were added to 10 µg / ml of the drug. The % recovery of each drug was calculated using HPLC technique. The results were illustrated in Table

Table - 4 - Effect of Carbohydrate on Determination of Drugs

Carbohydrate Solution	%Recovery of 10 µg / ml before addition 100 µg/ml			%Recovery of 10 µg / ml after addition 100 µg/ml		
	GLM	AML	NTF	GLM	AML	NTF
Glucose	100.8	100.3	99.17	100.2	100	99.0
Maltose	100.0	99.6	101.17	100	100	101
Fructose	99.9	100.8	99.63	100	100.0	99.7
Starch	100.2	100.1	100.67	100	100.1	100.7

Determination of drugs in its formulations Tablets

20 g of Tablets were weighted and Crushed then an accurately weighted 10 mg of each drug is extracted with 3 × 25 ml methanol and filtration in 100 ml measuring flask, the residue is washed with methanol and filtration to complete the volume in volumetric flask to 100 ml, dilute to get 10 mg / ml of drug. The dilution should be with mobile phase, the recovery was calculated and found to be 95 .1% , 100.4 % , 100.8 for GLM , AMD , NTF respectively .

Capsules

20 capsule are weighted and them an accurately weighted quantity equivalent to 100 mg in 100 ml volumetric flask , 25 ml of dimethylforamide are added and shake for is minutes 1 ml of solution diluted to 100 ml with mobile phase . The recovery was calculated and found to be 99% , 100% , 100.8 %for GLM , AML and NFT respectively .

Suspensions

Pipette 5 ml of suspension equivalent to 25 mg of drug in 50 ml volumetric flask, 10 ml of solution was added and the volume was complete to mark with dimethylforamide, pipette 1 ml of the solution and diluted with mobile phase to get to 10 mg / ml of the drug. The recovery was calculated for and found to be 100% , 99.8 % and 100.4% for GLM , AML , NTF respectively . The ideal analytical parameters for determination of GLM,AML and NTF were listed in Table(5)

Table(5) Analytical parameters for determination of drugs

Analytical parameters	GLM	AML	NTF
Mobile phase	Acetonitrile: water : Ethanolsodiumphalate (60 :20 : 20)	Acetonitrile: water : triethyle amine (45 : 45 : 10)	Acetonitrile; Buffer solution (30:70)
Stationary phase	C_{18} COLUMN 250 mm	C18 COLUMN 250mm	C_{18} COLUMN 250mm
Linear range µg /ml	10 - 50	2 - 10	5 - 25
Recovery	100.06%	100.9 %	100.8%
Correlation coffient	0.9817	0.9986	0.9986
λ_{max} nm	230	245	254
Detection limit µg /ml	2.8×10^{-5}	2.9×10^{-3}	9.3×10^{-3}
Standard deviation	0.054	1.8439	0.0625
Flow rate ml / min	1.5	1.5	1.0
pH	3.5	3.0	5.4
Pharmaceutical formulation	Tablet and Capsules	Tablet, Capsules and suspension	Tablet ,Capsules and suspension
Type of HPLC	RP – HPLC	RP – HPLC	Rp – HPLC
Regression line	Y = 3419 X + 14827	Y = 1597 X + 335.3	Y = 4976 X + 6384
Retention time (min)	8.169	6.771	4.413
Number of theoretical plates	1512.4	3328.8	932.12
High Equivalent theoretical plates	0.1653	0,0751	0.2682

4. DISCUSSION

The results in this research indicate that analytical method used in the work is accurate (high recovery) and precise (low standard deviation) . The detection limit in this study was also calculated by taken as average of five readings, it was observed that detection limit of GLM<AML<NTF , i . e the sensitivity of drug using RP- HPLC is $GLM > AML > NTF$.

The t-test and F-test for recent method is less than tabular valve at same reliable level at confidence 95% indicating that no significant differences between standard method and proposed method. The best separation of drug gives good band, low HETP and high

recovery. This fact agrees with result obtained in research . The results in Table (4) indicate that no significance differences between original recovery before addition and after addition of carbohydrate solution, this indicates that the method is not suffering from interfaces effect , therefore this method is highly recommended for determination of drug by HPLC due to sensitively and decrease the retention time of drug leading to less using quantity of mobile phase , which is good in view of economic situation .

The results in Table(5) illustrate that the present analytical methods are suitable for determination of drugs in theirs formulations

5. CONCLUSION

Reversed phase high performance liquid chromatography technique is rapid, sensitive ,accurate, precise and reliable for determination of GLM,AML and NFT drugs.

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