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Use of Acidic Hydrolysis and Diazo Coupling Reaction for Spectrophotometric Determination of Furosemide in Urine and Pharmaceutical Formulation

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Abstract

Simple and rapid spectrophotometric determination of furosemide (FUR) has been investigated .The method is based on acid hydrolysis of FUR to free primary aromatic amine and diazotization followed by coupling with 3, 5 di methyl phenol (3, 5-DMPH) at basic medium. The absorbance was measured at 434 nm, the method was optimized for best condition, and beers' law is obeyed over the range of 0.4-50 $\mu g.mL^{-1}$ with molar absorptivity and sandal's sensitivity 1.3899 $x10^4$ L moL^{-1} $.cm^{-1}$ and 0.0238$x10^4$ $\mu g.cm^{-2}$ respectively. Analysis of solution containing nineteen different concentrations of FUR gave a correlation coefficient of (0.9999) and limit of detection, limit of quantitation were 0.127, 0.464$\mu g.mL^{-1}$ respectively. The reaction stoichiometry was evaluated by Job's and mole ratio method was found to be 1:1(diazotized FUR: 3, 5-DMPH) .The method was applied in synthetic urine and pharmaceutical formulation. The recovery of FUR in spiked urine was satisfactory resulting in the values of (99±3.32) %, the results of the suggested method was compared with available official literature method.

Keywords: Furosemide, 3,5-Dimethyl Phenol Spectrophotometry, Diazotization.

1. Introduction

Furosemide or Frusemide (4-chloro-N-furfuyl -5-sulfamayl- anthranilic acid is formally a sulfonamide, an antibacterial agent. However, the intense and fast dieresis produced by this drug, has extended its application as a powerful acidic diuretic for diverse treatment in human and veterinary medicine. Furosemide is often classified as a loop diuretic due to its predominate action in the nephron [1].

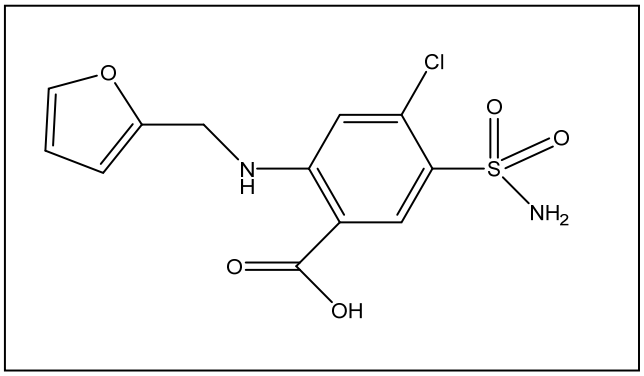
Fur acts inhibiting of sodium on Henle, s 100g and inhibiting the co-transportation of sodium, potassium and chloride, and causes excretion of calcium, magnesium and bicarbonate ion. Intense and fast dieresis may also mask the ingestion of other doping agents by reducing their concentration in urine [2].

Fur have a large number of analytical technique to determinate it in pharmaceutical and biological samples a number of spectrophotometric method have also been reported for furosemide [3-8].

Also electrochemical detection and capillary electrophoresis have been used to quantify fur [9-11].

HPLC is generally the method of choice for diuretics quantitation, due to the required time and cost of the analysis [12-13].

The aim of this study was to develop and validate a new spectrophotometric method for quantitative determination after acidic hydrolysis with strong acid media to yield salumine that can be azotization and coupled with 3.5 dimethyl phenol to form a new orange complex have a maximum absorption at 434nm.



Scheme (1): Chemical structure of furosemide

2. Experimental

Apparatus

1-All spectrophotometric measurements were made on Shimadzu U.V- visible double beam spectrophotometer equipped with 1 cm quartz cells (Kyoto, japan) the uv-vis spectra of standard and solution were recorded in 1cm quartz cell at wave length of 434nm.

2-Sartorius BL210 electronic balance was used for weighing the samples.

3-pH measurements were taken with Jan way pH-meter 3310.

Material and reagents:

All chemicals used were of analytical grade or chemically pure, distilled water was used for all dilutions of reagents and samples Expect 3, 5- DMPH which was dissolved in methanol. Standard Grade of furosemide was kindly provided by State Company for Drug Industries and Medical Appliances (S.D.I), Samara-Iraq.

Standard hydrolyzed furosemide (100 µg.mL^{-1})

Standard FUR was prepared by dissolving 0.25 g of pure drug in methanol and diluting to the mark in 250ml volumetric flask with distilled water to prepare (1000 µg.mL^{-1}). Transfer 150mL of prepared solution with 25mL HCl (M≈ 11.8) to volumetric flask of 250mL and diluting to the mark with distilled water to prepare (600µg.mL^{-1}) of FUR. The obtained solution was reflexed 3hr at 70C° until it would be clear and yield a light yellowish solution pointed to complete the acidic hydrolysis, leave it to cool in ice bath then transfer 16.6mL to 100mL volumetric flask then diluted to the mark with distilled water to prepare (100µg.mL^{-1}) of hydrolyzed FUR .

Reagent solutions

1-Sodium nitrate solution (1%) was prepared by dissolving 1g of $NaNO_2$ (Merck) in distilled water and dilute to the mark in 100mL volumetric flask.

2-Sodium hydroxide solution (1.5M) was prepared by dissolving 1.5g of NaOH (Merck) in distilled water and dilute to the mark in 25mL volumetric flask.

3- Buffer solution (pH11) was prepared by mixing 100mL of 0.025 M borax with 41mL of 0.1 M HCl

4-Sodium carbonate solution (1.5M) was prepared by dissolving 3.9g of salt in distilled water and dilute to the mark in 25mL volumetric flask.

Pharmaceutical and synthetic urine solutions

A_ synthetic urine sample was spiked in order to achieve concentration close to (4.0X10^{-4}) M. A complete description of the components of the synthetic urine is given in Table (1)

B_ Solutions for tablets were weighted out grinded and mixed well. A portion of the result powder (containing 0.25 g FUR) was used for the preparation of the solution as described before.

Calibration Curve procedure.

Calibration curve was prepared by taking appropriate aliquots (0.1–4) mL of working standard solution of hydrolyzed FUR in 5mL volumetric flask to give (0.4_50) µg.mL^{-1}. Samples for each test were prepared by adding 0.5 mL of freshly 1% $NaNO_2$, then 0.5 mL of 3,5–DMPH added to the resulting solutions followed addition of 0.7 mL buffer solution. The solutions were making up to the mark with distilled water and mixed well, the absorbance of the light orange dye was measured at 434 nm against the reagent blank.

Standard addition procedure

A– In synthetic urine

The method was used for assay FUR present in synthetic urines samples. To a series of seven (5mL) calibrated flasks , transfer 0.5 ml of synthetic urine sample containing 4.25 mL of 600.µg.mL^{-1} hydrolyzed FUR followed by addition (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6

mL)of standard FUR solution of 100 µg.ml^{-1}**.** The result mixtures were then treated **as** described in calibration curve procedure and measured the absorbance at 434nm.

B- In pharmaceutical samples

To a series of seven 5mL volumetric flask, transfer 0.5mL of 100µg.ml^{-1} tablet solution followed by addition (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mL)of standard FUR of 100 µg.ml^{-1} .The result mixture were then treated as described in calibration procedure and measure the absorbance at 434 nm.

3. Result and discussion

Absorption spectra

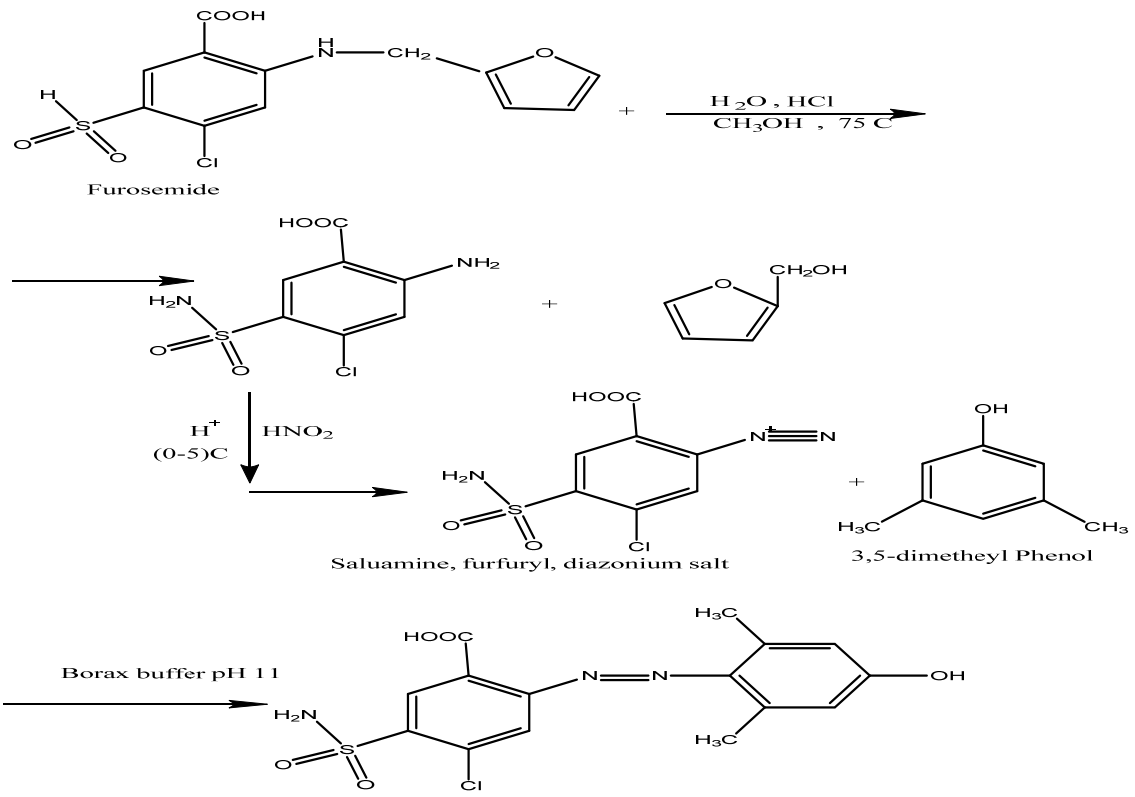
The primary test of the present method involved diazotization of hydrolyzed FUR (The pH of FUR 5) with sodium nitrate then reacted with 3, 5-DMP in basic medium to form colored azo dyes product. The absorbance and ⅄ max of azo dye was measured against the reagent blank. Figure (1) shows the maximum absorption obtained at wavelength 434nm.

Optimization of reaction conditions

The optimum conditions for color development were established by varying the parameters one at time keeping the others fixed and observing the effect produced on the absorbance of the colored product. Preliminary investigation showed that the light orange product resulted upon treating FUR solution with 3, 5-DMPH after diazotization in alkaline medium.

Study of the variables affecting the color development of reaction product, namely : the amount of reagent for the range (0.1 – 2)mL , the amount of $NaNO_2$ for the range (0.1 – 2) mL, the pH of the coupling reaction by using different volumes (0.1- 2) mL of borax buffer, the effect of diazotization reaction time was studied at different times (0-30 min) , and the order of mixing reagent solutions, were performed on 10 µg.mL^{1} of FUR solution. 0.5 ml of 3, 5-DMPH solution was selected as an optimum amount Figure (2), while 0.5mL was the optimum volume of $NaNO_2$ Figure (3), of buffer solutions different volumes are tested to control the pH of coupling reaction medium. The study showed using 0.7 mL of borax gave the best absorbance of the colored product Figure (4) while the study of reaction time showed the reaction spontaneously occur at zero time .The optimum order which gave the highest absorbance was ; FUR+ reagent + buffer.

The suggested mechanism for the hydrolysis and diazotization with coupling reaction is shown in scheme (2):



Scheme (2): The hydrolysis and diazotization with coupling reaction of FUR with 3, 5-DMPH

Final absorption spectra

The absorption of the spectrum colored produced under optimum reaction condition, shows, maximum absorption at 434 nm against reagent blank Figure (5)

Calibration curve and analytical data

Employing the optimum conditions, the measured absorbance at 434 nm versus different standard concentrations of FUR were plotted to construct calibration curve. The linearity of the obtained plot was in the concentration range (0.4 – 50) $\mu g.mL^{-1}$ as shown in Figure (6).

Evaluating the liner regression

Evaluating the liner regression of the suggested method is done by plotting the standardized concentration residuals vs the predicated concentration of the tested standard solutions. The residuals for FUR in all points appear to be randomly distributed around zero as show in Figure (7)

Stoichiometry of the product

Jobs and mole ratio methods have been used to determine the stoichiometry of the colored product. FUR and 3, 5-DMPH solution of $1.38x10^{-3}$M were prepared and mixed in various molar ratio in 5mL volumetric flask. According to the suggested procedure, the absorbance was measured at434nm. The graph of the results obtained as in Figure (8) & (9) gave maximum mole ratio X_{max}= 0.5, X= 1 in Jobs and mole ratio method respectively and showed that1:1 FUR: 3, 5-DMPH.

Precision and accuracy

The precision and accuracy of the proposed method was tested by analyzing five replicate of FUR in three different levels (within Beers' law range). The result list in Table (3) indicates an acceptable accuracy and precision to suggested work.

Interferences study

The presence of different quantity (2, 6 and 12ml) of 1000µg.mL^{-1} for Excipients can be tolerates in the analysis 5µg.mL^{-1} FUR since it would cause relative error percent less than 5% as it shown in Table (4).

Application in pharmaceutical forms

In order to demonstrate the applicability of the proposed method in determination of FUR, the method was applied to different manufacturing source containing FUR:

1- Furosemide (Tablet 40 mg) Ajanta (India)
2- Lazine (Furosemide injection 20mg) (Syria)

To apply the suggested method on pharmaceutical formulation, an accurately weighed 1.212g from tablet (40mg) and 12.5mL^{-1} (20mg) from ampule which contains 0.25g of pure furosemide to prepare the solutions as described before. Five replicate of three concentration levels (2, 6 and 12µg.mL^{-1}) were taken and calculated the recovery and relative standard deviation. The result indicated the suitability of suggested work for routine analysis of FUR asset shown in Table (5).

Standard additions method

The proposed method was applied to the determination of FUR in synthetic urine as described before. 4.25mL of 600µg.mL^{-1} hydrolyzed FUR was transferred to the prepared urine sample and completed the volume to the mark in 25mL volumetric flask with distilled water. Seven solutions were prepared in seven-5 mL volumetric flasks, by the addition of 0.5mL of synthetic urine solution and different volumes (0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mL) of 100 µg.mL^{-1} standard FUR drug solution and the same procedure of calibration curve were to give a good recovery in drug sample which indicate no interferences from the matrix affect the determination of FUR. Figure (10) and table (11) show the standard addition plots and Table (6) shows the recovery.

Comparison with the reported methods

Table (7), shows the between the proposed method and other literature spectrophotometric methods throughout some measured analytical parameter.

Table (1): The Components of the synthetic urine [22].

Compound	Weight (g)
NaCl	0.05
Na_2SO_4	0.05
KH_2PO_4	0.05
Kcl	0.05
$CaCl_2$	0.05
$NH_4(SO_4)_2$	0.05
Urea	0.62

Table (2): Summary of optical characteristics, validation parameters and statistical data for the proposed method.

Parameter	Value
λ_{max} (nm)	434
Color	Light orange
Linear range ($\mu g.mL^{-1}$)	0.4-50
Regression equation	y =0.042x+0.0112
Slop	0.042
Intercept	0.0112
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.3889×10^4
Correlation coefficient	0.9999
Detection Limit ($\mu g.mL^{-1}$)	0.127
Quantitative limit($\mu g.mL^{-1}$)	0.464
Shandell's sensitivity ($\mu g.cm^{-2}$)	0.0238×10^{4}

Table (3): Evaluation of the accuracy and precision of the propose method

FUR Conc. ($\mu g.mL^{-1}$)		Relative Error %	R.S.D.* %
Taken	Found*		
2	2.021	1.050	0.043
6	5.891	-1.816	0.016
12	11.931	-0.575	0.021

*Average of five replicate

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Table (4): The relative error value for 10µg.mL^{-1}of FUR in The presence of different excipients

Excipients	Taken 1000	Relative error (%)
Acacia	2	0.2783
	6	0.3671
	12	0.4559
Glucose	2	0.1214
	6	0.3801
	12	0.7345
Starch	2	0.3889
	6	0.6100
	12	0.9842
Vanillin	2	0.1851
	6	0.4141
	12	0.8148
Maltose	2	0.1960
	6	0.3944
	12	0.5784
Sucrose	2	0.0971
	6	0.1784
	12	0.3012

Table (5): Recovery data obtained by application the present method in drug formulations

Pharmaceutical preparation	Labeled amount mg	Conc.taken µg/mL	Conc.found* (µg)	Recovery%	S.D
Movineer, Tablet ,actavis England	40	6.000	5.902	98.366	0.130
		12.000	12.000	100.000	0.044
Movineer,Tab,ajanta/India	40	6.000	5.734	95.566	0.033
		12.000	11.947	99.558	0.175
Lazine Ampule Syria	20	1.000	0.968	96.800	0.049
		2.000	1.993	99.650	0.086

Table (6): Recovery data obtained by standard additions method for FUR in rigin tablet and synthetic urine

Pharmaceutical preparation	Conc.Taken µg/ml	Conc.found µg/ml	Recovery
Sample FUR +0.5ml tablet	10	10.01	100.3
Sample FUR with synthetic urine	12	11.82	98.5

*Average of three replicate

Table (7): Analytical parameter for the determination of Fur by the proposed method comparing to other methods

Technique used	D.L	Liner range	Rec.%	Ref.
Electrochemical	($3.8x10^{-7}$) M	($1.0x10^{-2}$-$5.0x10^{-7}$)M	-	14
Electrochemical	($7.0x10^{-8}$)----	($1.0x10^{-7}$– $7.0x10^{-6}$) ----	-	15
HPLC	-	(20-200)µg.mL^{-1}	99.9	16
HPLC	(5.2)ng.mL^{-1}	(5.20-25.00) ng.mL^{-1}	-	17
Fluorescence	(0.007) µg.mL^{-1}	(0.03– 140.00) µg.mL^{-1}	97.8-100.8	18
Spectrophotometric	-	($8X10^{-3}$-$1.20x10^{2}$) -----	99.14	19
Spectrophotometric	(0.50)------	(20-160)-------	-	20
Spectrophotometric	(0.23) ----	(5-30)------	99.69-100.83	21
Present method	(0.12)-----	(0.4-50) ------	99	-

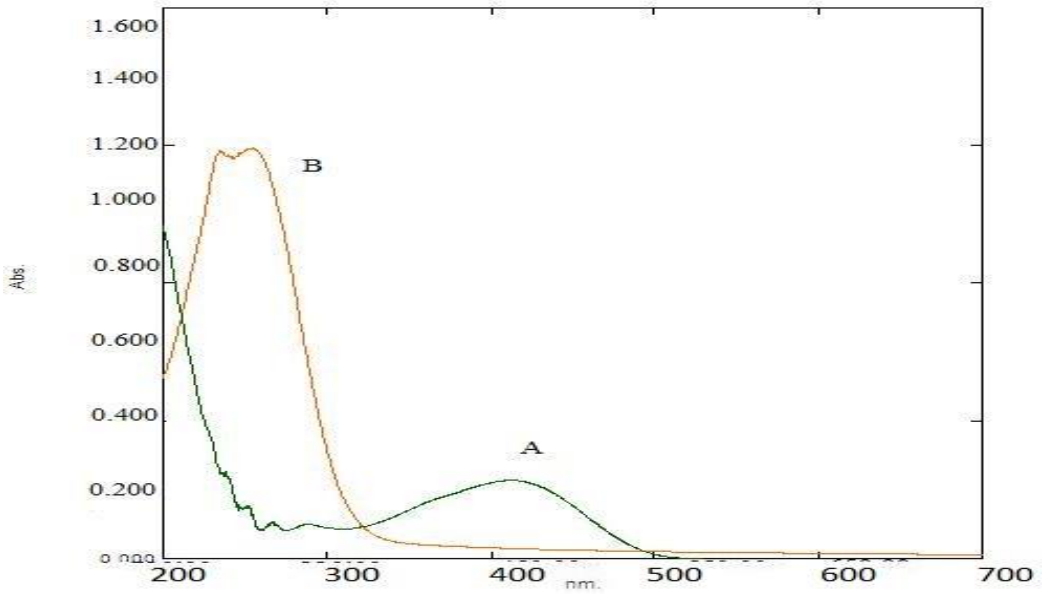

Figure (1): Absorption spectra of (A) 10µg.mL^{-1} FUR against reagent blank, (B) blank solution

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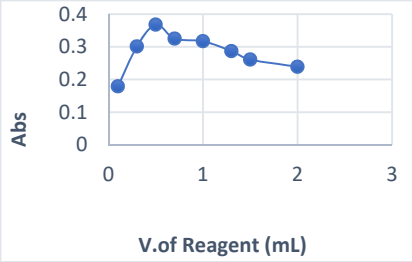

Figure (2): Effect of the volume of 3, 5-dimethyl phenol on colour

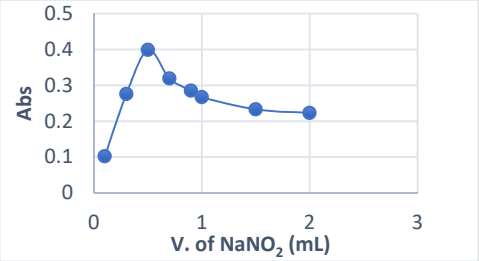

Figure (3): Effect of the sodium nitrate volume on colour development

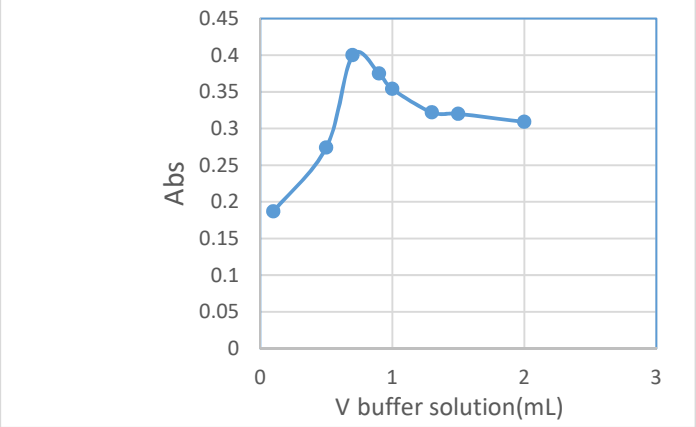

Figure (4): Effect of the volume of Buffer solution on color development

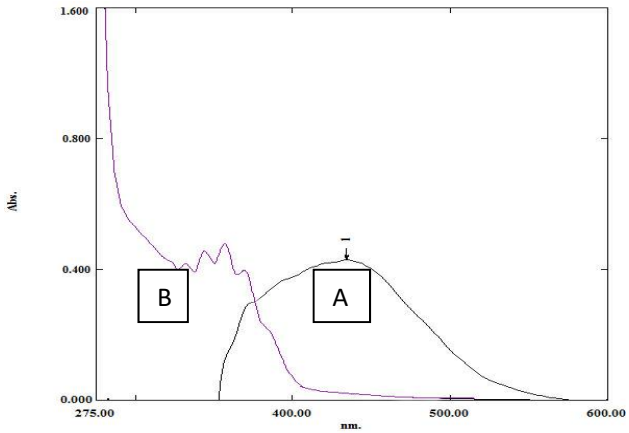

Figure (5): Absorption spectra of, (A) 10µg.mL^{-1}of colored compound under optimum condition, (B) lank solution against solvent

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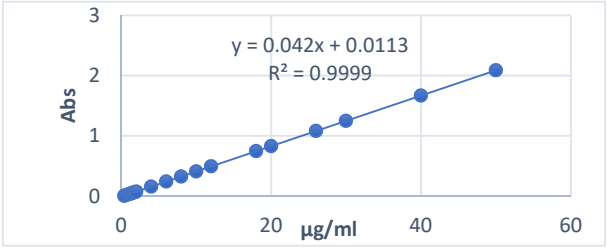

Figure (6): Calibration curve for the determination of FUR under optimum conditions

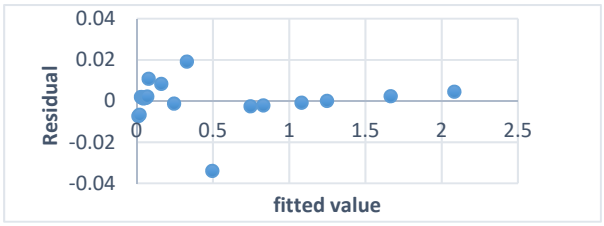

Figure (7): The residual error of liner regression model

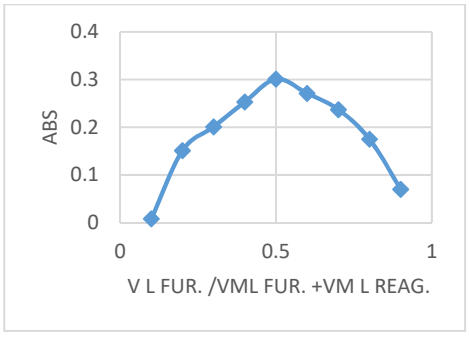

Figure (8): Job's curve of 1.38×10^{-3}M FUR and 3, 5-DMPH

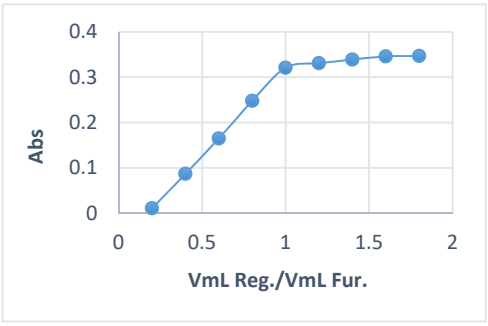

Figure (9): Mole ratio of 1.38×10-3M for each FUR and 3, 5-DMPH

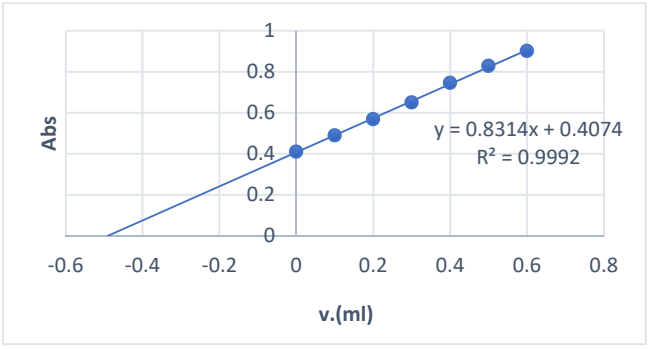

Figure (10): Plot of standard additions method for determination of Fur in tablet

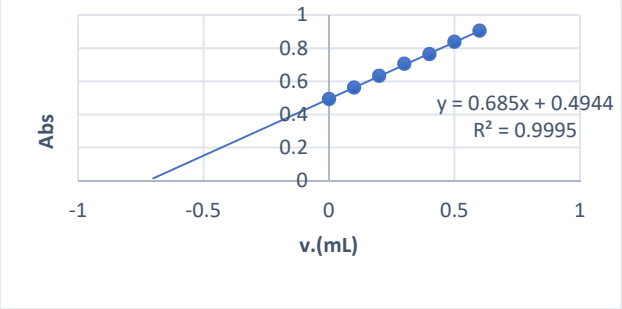

Figure (11): Plot of standard additions method for determination of Fur. in urine

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