**New Spectrophotometric procedure for the assay of Sulfadiazine drug based on Diazotization Coupling Reaction**

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**ABSTRACT**

A sensitive, simple and rapid Spectrophotometric procedure for the assay of trace quantities of Sulfadiazine (SDZ) drug as bluk and in diluted solution is characterized. The procedure is depended on the Diazotization reaction which is produced by coupling Sulfadiazine with (4-amino-2-Hydroxy acetophenon) (AHA) to produce an intense colored complex with highly absorption at 410 nm. beer’s law was Applied on the range of concentration between( 0.5 – 15) ppm ,the molar absorptivity and the sensitivity of Sandell were 2.8484 x104 l.mol-1.cm-1, 0.008 μg.cm-2 . the limit detection (LOD) was 0.443 µg/ml-1 . LOQ was 0.249µg/ml-1. The procedure does not resort to the extraction by solvents, The perfect circumstances for all colour increasing are characterized and the suggested procedure has been very good application for the assay of quantities of Sulfadiazine in pure drug and in its pharmaceutical Preparations by using fixed time procedure. The additives and general excipients materials did not affect in the studied procedure.

Keys words: - **New Spectrophotometric procedure; Diazotization reaction; Sulfadiazine drug.**

**Introduction:-**

Sulfa derivatives drugs are widely used in medicine because of their inhibitory effect on the growth in many bacteria(1) .that was considered to be the major cause of death before the discovery of sulfa drugs and other antibiotics(2). it acts by inhibiting the production of folic acid inside the bacterial cell. Sulfadiazine is used for the treatment of urinary tract infections All sulfa derivatives involved in being contain one benzene ring with amino group and sulfate group. Among the most important of these derivatives was Sulfamethoxazole (SDZ)(3).The drug has the structure formula C10H10N4O2S and the chemical structure was drawen in figure(1), the mass molar of drug was 250.3 g / mol(4).



**Figure(1) The chemical structure of Sulfadiazine (SDZ)**

Scientifically the name of (SDZ) by system of (IUPAC) was 4-amino-N-pyrimidin-2-yl benzene sulfonamide.It was white, pinkish-white or yellowish-white, crystals or crystalline powder, water insoluble , very slightly soluble in alcohol ,slightly soluble in acetone,. It was dissolved in solutions of dilute mineral acids and in alkali hydroxides, the melting point 255 Co with decomposition. also it was effected with exposure to air or light. The (SDZ) Pharmaceutical Preparations of were floumizin cream, tablets(5). It also prepares a combination drug with silver metal (6,7). ​​many researchers in the analytical chemistry field have made a lot of methods for determination for the drugs a like in its bulk form or pharmaceutical Preparations and in biological fluids. The main procedures for assay of (SDZ) were making direct or indirect Spectrophotometric procedure. Which based on schiff base, the oxidation-reduction, charge- transfer and diazo-coupling reactions (10)? and then subsequent measurement of absorption for Colored complex compounds that result from these reactions(8 -21). the another analytical procedures are used for assay (SDZ) drug like amperometric titration(22) ion-selective electrode (ISE) potentiometric(23), potentiometric titration(24,25), differential pulse polarographic(26), differential scanning calorimetric(27), thin layer chromatographic (TLC)(28),reverse phase high performance liquid chromatographic (RP-HPLC)(29-30),capillary zone electrophoresis(CZE)(31), sequential injection(SI) chemiluminescence (CL)(32), flame atomic absorption Spectrophotometric(AAS)(33) Fourier transform Raman spectroscopy(34), spectrofluorimetric(35) and nuclear magnetic resonance (NMR)(36) In the studied procedure characterized a newly sensitive, simple and rapid kinetic Spectrophotometric procedure for the assay of the trace quantities of Sulfadiazine (SDZ) basing on the azo-coupling reaction between Sulfadiazine and(4-amino-2-Hydroxy acetophenon )(AHA) to produce an intense colored complex and The perfect circumstances for this reaction were studied. the procedure was used for assay of the (SDZ) content on some of the different types of its pharmaceutical Preparations that containing different doses and forms from the drug with and the pure substance with high precision and accuracy.

**Experimental**

**Apparatus**

* all absorbance and spectral measurements were performed on applied double - beam UV-Visible 160 digital recording spectrometer.
* sencetive balance , ice-water bath pH meter , Jenway 3020 .

 **Material and reagents**

The Chemicals which were used in the procedure with highly degree of purity and did not need to purification, their solutions were prepared by the following :-

1. Sulfadiazine:- (SDZ) pure substance was processed from the state company for drug industries and medical appliances (SDI) Samara –Iraq. The(100) ppm standard concentration solution of( SDZ) was prepared by dissolution (0.01 g) of (SDZ) in 5 ml of ethanol and the volume is completed to 100 ml with deionized water in a volumetric flask.The solution was transferred to a dark flask and it is stable for at least one week the solution was stayed stable for more of one month after keeping away from the light.the working solution prepared from this solution.
2. Sodium nitrite NaNO2(0.05) M, It was supplied by (BDH Chemicals Ltd, Laboratory reagent) company by Dissolution (0.345)gm of substance (pure) in(100)ml deionized water.
3. Hydrochloride acid (1M):- the solution was prepared from (GCC)at percentge (%98) company, that was using for preparation (1M) solution
4. Sudium hydroxide (1M):- It was supplied from the Merck company ,the solution was prepared by dissolving (4)gm of substance in(100)ml deionized water.
5. 4-amino-2-Hydroxy acetophenon (SDA) (0.01) M:- it was supplied from (BDH Chemicals Ltd, Laboratory reagent) company by dissolution (0.075)gm of substance (pure) in (50 ml) abselute ethanol from(BDH Chemicals Ltd,%99.9).

**Preliminary investigation**

a series of volumetric flasks of (25ml), Equal amounts of standard solutions of, Sulfadiazine (SDZ),the range of concentrations were between (0.5 – 15)ppm, respectively the final volumes were adding separately, after that adding of (1ml) Hydrochloride acid (1M) and (1ml) of Sodium nitrite (0.01) M, then we leave the solutions for ten minutes to complete the azo-coupling reaction, after that It has been added (1) ml of Sudium hydroxide (0.5M) for The drug solution ,then followed by addition of (3ml) from the Reagent (AHA) and complete the volumes by deionized water.they were reminded for ten minutes at the room tempeture the measuring OF absorbance was at (410 nm), against reagent blank solution and a calibration curve was built.

**Procedure for Assay of Sulfadiazine (SDZ) in Pharmaceutical Preparations (5).**

 a number of Sulfadiazine (SDZ) pharmaceutical preparations which was containing (SDZ) as active ingredient which were taking and it involved the following:-

**Tablets (100 µg/ml)**:- Two tablets (1.0 g sulfadiazine /tablet) of the drug are weighed and crushed to powder. A 0.021g of this powder which is equivalent to 0.01 g of SDZ is weighed and dissolved in a portion of distilled water containing 2 drops of 1 M of NaOH solution. The resulting solution is then mixed well, filtered to get clear solution and diluted to 100 ml with deionized water in a volumetric flask. Each ml from the solution contains100 µg SDZ.

**Floumizin cream**(Ag.SDZ) (100 µg Ag.SDZ/ml). To 1.0 g of cream (containing 0.01g of Ag.SDZ), added 50 ml ether, shake well and the mixture is transferred to a separating funnel. The Ag. SDZ is then extracted with 25 ml of deionized water (three times). The aqueous layer is collected, filtered and diluted to 100 ml with deionized water in a volumetric flask .

**RESULTS AND DISCUSSION;-**

the ideal conditions were studied for reaction:-

the all conditions which are influencing on the absorbance of the compound formed so as to Increase of it.

**1- Reagent volume effect:**-

The Reagent volume was affected on the absorbance we .It was made from (0.5 – 6)ml of the reagent (4-amino-2-Hydroxy acetophenon ) (AHA) (0.01M) with Presence(1ml) Hydrochloride acid (1M) and (1ml) of Sodium nitrite (0.01) M and (0.5) ml of Sudium hydroxide (0.5M) . (3 mL) is the better volume for the reagent, which was giving the highest absorption that was applied in the next experiments.

**2 - Sodium nitrite** **volume effect:**-

 the volume of sodium nitrite which affected on the absorption intensity were studied. .it was made from (0.5 – 5)ml of NaNO3 at concentration (0.05M). with (3ml) from the reagent and (1ml ) of HCl solution and (0.5) ml of Sudium hydroxide (0.5M). It was show that (1 mL) is the better volume which was giving the maximum absorption that was applied in the next experiments.

**3- Acid effect:-**

the existence of acid was leading to give the highly intensity of the produced complex, acids such as H2SO4 ,HCl, HNO3 and CH3COOH are studied at (1M) as concentration, they were show that all these acids made the absorbance of coloring product , so; HCl was the favorite acid that shows the highly absorption which selected in the following experiments. The volume ( 1 ml) of the acid gave highly sensitivity which applied in the following experiments.

**4-The time effect for completing the azo-coupling reaction:-**

 It was found (10) minutes that the best time for completing the azo-coupling reaction which applied in the following experiments.

5- **Temperature effect:**-

 The product which was resulting of the procedure was examined at different temperatures. They show that the values of absorbance studied between the range of temperature (0-70ºC), the absorbance value decrease at higher temperatures, that resulting from the dissociation of the product by heat. The colored product was stable at range of temperature between (5 - 30ºC) that was producing the highly absorbance. The temperature room was applied in this method.

**6- Reaction Time effect :**-

The intensity of colour show its highly after the drug (SDZ) had been directly reaction with (AHA) in the being of sodium nitrite and HCl as acidic solution remained stable after ten minutes. ten minutes time was applied as ideal time in the studied procedure. The colour was giving stable at more than 24 hour.

**7- Absorption Spectra**:-

The scanning spectral was built to give the wavelength that showing highly absorption for producing compound after establishing the perfect circumstances a gainst the solution of blank which was containing the sodium nitrite, the acid and reagent.

Figure (2) gives the spectra of colour compound and the blank, the maximum absorption at 410 nm where (A) spectrum represents colour product from the reaction (B) the blank spectum .



A

(A) spectrum compound product

(B) blank spectum

B

Figure(2) gives the spectra of yellow product at (4 ppm) of (SDZ) (A) and, the blank(B)at (0.01M) of reagent ,sodium nitrite(0.05M) , and (1M) HCl acid.

**8- Calibration curve:-**

Appling the perfect circumstances was showing by the procedure, a calibration curve linear for Sulfadiazine (SDZ) is showed in (Figure 3),from this curve the Beer’s law is applied between the range of concentration of (0.5 – 15) ppm, the correlation coefficient was 0.9993, the slope of curve was 0.1138 ,and an intercept of 0.0545..The highly molar absorptivity of the yellow product was founding 2.8484 x104 L.mol-1.cm-1.The sensitivity Sandal was 0.008 (μg.cm –2). the limit detection (LOD) was 0.443 µg/ml-1 and the LOQ was 0.249 µg/ml-1.

Figure(3) shows the Calibration curve of (SDZ)

**9- Precision and Accuracy:-**

The precision and accuracy for this procedure were gave by calculating the sulfadiazine (SDZ) at different three concentrations. The obtaining results showed in Table (1),the procedure was satisfactory. and have highly precision and accuracy

Table (1) Precision and Accuracy of the studied procedure.

|  |  |  |  |
| --- | --- | --- | --- |
| Conc. Of (SDZ) ppm | % Error  | % Recovery  | % R.S.D  |
| 1.00 | - 4.3O | 95.70 | 0.562 |
| 8.00 | + 3.20 | 103.20 | 0.432 |
| 14.00 | + 0.24 | 100.24 | 0.177 |

**10 – Stoicheiometry of reaction(37.38) :-**

The stoicheiometry was studied for the reaction of sulfadiazine (SDZ) and (AHA) was obtained by using mole ratio method and Job’s method, the results showed that 1:1 (SDZ) to (AHA) complex was formed at 410 nm . The product was soluble in ethanol; The stability constant of the coloring product was calculated by using the absorbance that measured for the solution containing, Equal quantity of sulfadiazine (SDZ ) and the reagent with ideal quantity (1ml) of (2.5×10-4 M).and other (AHA) solution with five times the concentration of the main concentration. The stability constant average of the coloring product in ethanol under the made experimental circumstances was 1.72 ×106l1.mol-1.

The the colour product was formed between(SDZ)and (AHA) may probably occur as shown in scheme on the following equations(39.40) Fig (4):



Figure(4) scheme of the azo - coupling reaction

**11- Interference**s:-

The study of excipients were, talc, lactose, Acacia ,starch**,** , Sucrose, magnesium stearate Glucose, benzoic acid, aspartate, and polyvinylpirrolidone (PVP). , and There is no effect on the measurements, in this procedure, solution was formed by (SDZ) and each one of the excipients was obtained separately in concentrations ten-times more than of (SDZ) were examined by the like procedure in the Calibration curve,(2 ml) of (100)ppm (SDZ) and (2ml) for each type of excipients was applied for this study and making the dilution to the mark of volumetric flask (25ml). the interference level was supposed to make acceptable when the error was not more than ± 2% according to the expected No interferences were showed in the procedure of assay of (SDZ) in the being of the excipients studied(Average of three Investigations).

Table(2) Investigation of (4ppm) sulfadiazine(SDZ)in the being of excipients.

|  |  |  |
| --- | --- | --- |
| **Interference** | **% Error** | **% Recovery** |
| Talc | - 4.360 | 95.640 |
| lactose | - 3.130 | 96.870 |
| starch | + 2.850 | 102.850 |
| Acacia | - 4.440 | 95.560 |
| Sucrose | - 4.200 | 95.800 |
| Glucose | -.3.250 | 96.750 |
| magnesium stearate | + 3.500 | 96.500 |
| benzoic acid | + 2.480 | 102.480 |
| Aspartate | - 3.350 | 96.650 |
| PVP | - 4.450 | 95.550 |

**13- Application of the procedure**

the procedure was applied for the assay of pharmaceutical preparations of the drug, was studied. The results from the procedure of assay for available formulations of sulfadiazine SDZ) drugs are showed in following Table (3) .

Table (3) : sulfadiazine(SDZ)in pure and dosage forms .

|  |  |
| --- | --- |
|  Pharmaceutical preparations containing (SDZ) | Average recovery % |
| Proposed method | Standard method(8) |
| Pure (SDZ) | 95.634 | 95.150 |
| Tablets (100 µg/ml) (SDZ) | 96.770 | 95,350 |
| Floumizin cream (Ag.SDZ) (100 µg Ag.SDZ/ml) | 97.120 | 95.410 |

 the average of three determinations was giving .the standard procedure for drug assay were obtained from British Pharmacopoeia (2009). The reproducible results were showing and the procedure of assay for formulations was cross examined by the Standard procedure.

**CONCLUSION**:- A sensitive, rapid, precise, and simple Spectrophotometric procedure has been built for the assay of trace quantities of Sulfadiazine (SDZ) in aqueous solution by using the azo coupling reaction with 4-amino-2-Hydroxy acetophenon (AHA)and Hydrochloride acid in the being of sodium nitrate. The studied procedure does not need the solvent extraction or temperature control step; the procedure was applied, successfully for the assay of trace quantities commercial (SDZ)drug.

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