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Abstract

A new trend describes the development and validation of a simple, sensitive and selective kinetic spectrophotometric methods for the determination of sulfadiazine in pharmaceutical formulations has been conducted. In this paper, sulfadiazine was dervitized as a new organic compound 4(4-sulphophenylazo) pyrogallol, 4-SPAP, by coupling pyrogallol with diazotized sulfadiazine in medium of controlled pH. 4-SPAP was characterized by techniques of FT-IR, H-NMR, GC-mass, TG and DSC thermal analysis methods. Solvatochromic behavior in solvents of various polarities was also investigated.

The determination of sulfadiazine was accomplished by initial rate and fixed time methods. These methods were based on the reaction of the compound containing sulfadiazine, 4-SPAP, with Ca(II) to form colored product with a maximum absorbance at 520nm. The two methods were adopted for constructing the calibration curves and examined for their suitability for the quantitation of sulfadiazine in pharmaceuticals. The limit of detection (LOD)and limit of quantification(LOQ)were found to be, by initial rate method, 0.35 and1.05 $\mu g.mL^{-1}$and that by fixed time method were found to be 0.69 and2.07$\mu g.mL^{-1}$, respectively. The percent relative standard deviations (%RSD) for the results ranged from 1.04% to 1.76% and 0.85% to 1.42% for the initial rate and fixed time methods of the proposed kinetic spectrophotometric method, respectively. The existence of common excipients in the pharmaceutical formulation did not produce any significant interference. Statistical comparison was reported as indicated from the F- and t-test data of the proposed methods with that of reference method showing excellent agreement and indicating no significant difference in their accuracy and precision.

Keywords: sulfadiazine; kinetic methods of analysis; pharmaceutical analysis; solvatochromic.

1. Introduction

A considerable deal of attention has been simulated for the preparation and studies of organic reagents in recent years. Numerous of these organic reagents have drawn much attention as they are sensitive, chromogenic reagents in addition to being interesting complex-forming agents. Azo compounds with –N=N– moiety, are among the most deep explored classes of organic reagents both from the theoretical and practical view point. The existence of an azo linkage in aromatic compounds makes them highly important in dyestuff industry, dosimetry and pharmacy[1-3]. The medicine of sulpha drugs is the core of a variety of drugs which functions to treat allergy, coughing and also used for other uses. It should be taken into consideration that sulfa drugs and sulfites, do not sulfonamide and do not have same effect of sulfonamides[4,5].

Fig. 1

One of the most important compounds of sulfa antibiotics is sulfadiazine which has been listed by the world health organization (WHO) as essential medicines of sulfonamides[6]. It works by stopping the growth of bacteria and other organisms. Sulfadiazine is used to treat many different types of infections, such as urinary tract infections, ear infections, meningitis, malaria, toxoplasmosis, and others [7]. Several analytical methods are available to quantify amount of sulfadiazine and other drugs in pharmaceutical dosage forms. Among these methods, kinetic method has acquired specific attention in chemical and pharmaceutical analysis due to their selectivity and elimination of additive interferences, which affect direct spectrophotometric methods. Furthermore, such methods show high selectivity since they involve the measurement of the absorbance as a function of reaction time[8,9].

Such kinetic methods, reported in recent publications[10-22], are employed for the determination of important molecules used in various fields.

To our knowledge at the date of writing, there is no reference in the analytical literature reviews demonstrating the kinetic spectrophotometric methods for the determination of the sulfadiazine. For that reason, our aim is to develop a novel kinetic spectrophotometric method for sulfadiazine drug determination. In the present method, sulfadiazine was precipitated through coupling reaction with pyrogallol in medium of controlled pH, then the amount of sulfadiazine is determined by measuring the absorbance of solution prepared from the precipitate at 520nm by both initial and fixed time methods.

2. Experimental

2.1. Apparatus

UV-Visible spectra were measured with matched 1 cm quartz cells by T80 uv-visible spectrophotometer, PG instruments Ltd., UK. Infrared spectra were recorded using prestige-21 FT-IR, Shimadzu, Japan. Melting points were determined using SMP30 stuart melting point apparatus, UK and are uncorrected while the pH was monitored using 340i pH-meter WTW, Germany. Thermal analysis conducted by thermogravimetry and differential scanning calorimeter were run using Stapt-1000 Linseis. Thermal analysis of the synthesized compound and its starting materials were performed under a continuously purged nitrogen atmosphere (flow rate 50mL/min). The instrument was calibrated for temperature and enthalpy using indium. The samples were crimped in non-hermetic aluminum pans and scanned from 30 to 500 ºC at a heating rate of 10 ºC/min by platinum crucible. The ^{1}H-NMR spectrum was recorded in DMSO using NMR Bruker DPX 400 spectrophotometer operating at 300MHz. TMS was used as internal standard and

chemical shifts δ recorded in ppm. GC-Mass analysis was accomplished using GCMS-QP2010, Shimadzu, Japan.

2.2. Chemicals and reagents

All chemicals used were of analytical reagent grade. All solutions were prepared with deionized water. The solvents were used namely cyclohexane, chloroform, benzene, ethanol, methanol were purchased from BDH(London, England); diethyl ether, ethyl acetate, acetic acid were purchased from RDH(Buchs, Switzerland); propanol, 1,4-dioxane, toluene, n-butanol were purchased from Merck(Darmstadt, Germany); DMSO, and THF were purchased from GCC(Slidell, USA). Solvents were used as received. Sulfadiazine, sodium nitrite, pyrogallol and calcium chloride were supplied from Fluka (Buchs, Switzerland).

2.3. Synthesis of 4(4-sulphophenylazo)pyrogallol

A 5.44g(0.02mmol) of sulfadiazine was diazotized by adding 5mL of concentrated HCl and 5mL of $NaNO_2$ which prepared by dissolving amount equivalent to 0.02mmol. The diazotization reaction was accomplished with good stirring at (0-5)ºC for 30min. The prepared diazotized solution was then added drop-wise to beaker containing 2.52g(0.02mmol) of pyrogallol. The coupling reaction was maintained at (0-5)ºC, and pH of the mixture was raised by adding cooled solution of conc. sodium hydroxide and controlled within range of 5.0-6.0. The resulting solution formed a colored precipitate. The mixture was diluted with appropriate amount of deionized water and was allowed to stand in ice bath for 1 hr. with constant stirring, left overnight then filtered and allowed to dry. The product was purified with absolute ethanol. Yield of the product was 43%.

The synthetic route of the product is shown in the following figure:

Fig. 2

2.4 Preparation of solutions for analytical application of 4-SPAP: sulfadiazine determination

4-SPAP stock solution (0.01M)

This was prepared by dissolving 0.1930g of 4-SPAP in absolute ethanol in 50 mL volumetric flask. Working standard solutions were freshly prepared by diluting the stock solution with absolute ethanol to obtain the appropriate concentration.

Calcium(II) solution (0.03M)

Prepared by dissolving 0.1651g of $CaCl_2$ in deionized water and the volume was completed to 50 mL in a volumetric flask with the same solvent.

Sodium hydroxide solution (1M)

It was prepared by appropriate dilution of the standard concentrated solution (5M) with deionized water in volumetric flask.

Pharmaceutical preparations

Cream. Ten plastic tube containers of cream as sulfadiazine- containing form (each contains 1% silver sulfadiazine) were weighed. A weighed amount of the cream equivalent to 1g of sulfadiazine was dissolved in hot water, cooled and made up to 50 mL with deionized water. The resulting solution was filtered and treated as described under recommended procedure.

2.5. Recommended procedure for the determination of sulfadiazine

2.5.1 Initial–rate method

In the first procedure, the initial rates of the reactions were determined by measuring the slopes of the initials tangents to the absorbance-time curves. An increasing volume of 4-SPAP working standard solution (0.01M) were transferred into a series of 10 mL standard flask to cover the range of the calibration graph(2×10^{-5}-3×10^{-4}) at 25ºC. To each flask, 1.4mL of 0.03M Ca(II) was added in the same sequence and diluted up to the mark with ethanol then the contents of the flask were well mixed. An increase in absorbance with increase in time was recorded at 520nm compared to a reagent blank prepared in the same way but containing no 4-SPAP. At selected concentration of 4-SPAP, the initial rates of the reactions were achieved by determining the slopes of the initial tangents to the absorbance-time curves. A plot of calibration graph of log initial rate of the reaction versus the log of the molar concentration of sulfadiazine-containing product.

2.5.2 Fixed time, absorbance difference (ΔA) method

In this procedure of analysis, two series of 10 mL calibrated flask, increasing volume of stock solution (0.01M) of 4-SPAP was transferred to cover the range of the calibration graph (2×10^{-5}-2×10^{-4}) then 0.5mL of 1M sodium hydroxide solution was added and followed by 1.4 mL of 0.03M Ca(II). The contents were well shaken and the solution was diluted to the mark with ethanol. The reaction mixture was allowed to stand for 5 min at 25ºC, and the absorbance of 4-SPAP was measured at 520nm versus a reference reagent.

2.5.3 Determination of sulfadiazine from dosage form

An accurate weight of plastic tube container equivalent 1% of the silver sulfadiazine was dissolved in deionized water and the volume was made up to 50mL. The residue of sample was filtered by using membrane filter. The stock solution was diluted according to need and analyzed by the recommended procedures.

3. Results and discussion

3.1 Physical and chemical properties of 4-SPAP

The purity of the synthesized azo compound was examined by melting point and TLC on silica gel plates using 2:3 methanol:toluene as solvent. The reagent is deep orange powder, slightly soluble in water. It is soluble in ethanol, methanol, acetone, dioxane and easily soluble in DMF and DMSO. Its solution with pH less than 5 is distinguished by yellow color, while its alkaline solution is of brown color.

4-SPAP melts at 167 ºC. FT-IR spectrum of 4-SPAP, (Fig. 3), showed strong bands at (3421.08 and 3379.28cm^{-1}) which belong to the stretching vibration of OH group, medium band at 1525cm^{-1} attribute to stretching of N=N group, strong band at 1334cm^{-1} to stretching SO_2(asym.)[23].

The arylazo dye synthesized in the current study may be found in two tautomeric forms (Fig. 4). The infrared spectrum of the dye revealed the absence of a carbonyl band, but an intense hydroxyl band in the region 3379-3421 cm^{-1}, suggesting that this compound exist predominantly in the 3-hydroxytautomeric form in the solid state.

Fig. 3

Fig. 4

(Fig. 5) shows the ^{1}H-NMR spectrum of reagent with broad band at δ= 12.3ppm belonging to the more acidic OH group of pyrogallol moiety. Band at δ=11.9 ppm(br, 1H, –SO_2NH–). 4-SPAP showed broad band at δ= 9.5 refer to protons of 2OH involved in strong intramolecular hydrogen bonding. Further bands; δ=8.4ppm (s, 3H,

pyridine-H), group of peaks at δ=7.82 and 8.0 ppm belonging to 4H of benzene ring of sulfadiazine moiety and those absorbed at 6.5 to 7.0 belonging to protons of pyrogallol and aromatic –CH–N–. Singlet broad band at δ = 3.5ppm refer to water in the solvent, DMSO, where its band at δ =2.5ppm[24-26].

Fig. 5

3.2. Thermal analysis of 4-SPAP, Thermogravimetry(TG) and differential scanning calorimetry(DSC)

The data are extracted from thermal analysis that is related to the variation in the characteristics of material regarding the effect of temperature especially by combining the two (TG-DSC) techniques.

Figures 6, 7 and 8 show combined TG-DSC analysis of pyrogallol, sulfadiazine and the reagent respectively. The TG and DSC of the raw materials and of the product of their synthesis were markedly different. Unlike inorganic materials, organic compounds show degradation at low temperatures; therefore, thermal data obtained from thermograms, listed in table 1, reveal that the initial temperature begins with low thermal stability.

Thermal stability of the compound of interest (the synthesized one) demonstrated from figure 8 which can be used in applied field when it is used without any changes in its structure [27]. TG curve for the synthesized compound presented in figure 8 shows three stages of decomposition. The first stage is attributed to water loss from the surface and pores of the powder sample. Large mass changes due to high percentage loss stems in the second stage from about 200-400ºC which referred to maximum amount of degradation of the organic reagent. End of decomposition appeared in the third stage which may be attributed to residue of the compound.

The DSC curve, shows two peaks; endothermic and exothermic. Broad endothermic peak attributed dehydration/water loss with melting process did not appear significantly. Exothermic peak is attributed to decomposition of the prepared reagent.

Fig. 6

Fig. 7

Fig.8

Table (1)

Table (2)

3.3 GC-Mass study

The GC method is an effective method for separating and detectig compounds that can volatilize and are thermally constant. The difference between the normal GC and GC-MASS method is that of the detecting carrier gas, where MS is used as a detector instead of FID or any other detector that generates the molecules and evaporates into a gas where they are ionised into charged particles. The MS is uesed to identify constitunt elements of some material or part. It is also used to illustrate chemical structures of molecules, that is, by depending on the work method of MS to expel chemical compounds for generating charged particles and measure the mass ratio to their charge. As a result of thousands of compounds stored in the memory of system, they are matched with compounds that are analyzed by a certain identical percentage or by identifying the unknown compound. The reason to prepare the new reagent is that it is not available in memory of the system. The compound sulfadiazine is chemically stable when it is transformed into a volatile compound through the highest peak with retention time 26.611min. As for the pyrogallol, it is thought to decompose

into different parts through the other peaks as shown in Fig. 9, or it is not found in the library of the device.

Fig. 9

3.4. Effect of pH on the reagent

The orange solution of 4-SPAP dissolved in ethanol was shown peak in 438nm assaign to N=N absorption, Fig. 10, which emphesize that the dye was formed where this band was not found in all reacting molecules.

Fig. 10

Based on the acidity of the medium, the solution of 4-SPAP involves three variable acid-base forms: LH_4, LH_3^-, LH_2^{2-}, Fig.10. The full protonated neutral species, LH_4, yellow colour 424nm exist in acidic medium of pH until 1.0 is reached. The orange neutral form predominated when pH rangeed between 4.0-6.0 and exhibited maximum absorption at 438nm. In media of pH >7.0, the spectrum of 4-SPAP shows absorption band at 470nm with isobestic point at 365nm, Fig.11. This behavior explains the presence of an equilibrium between ionic and neutral forms of 4-SPAP [28, 29].

Fig. 11

Dissociation constant of 4-SPAP

The dissociation constant of 4-SPAP was evaluated by a spectrophotometric method[30, 31] through the absorbance–pH plot at specific wavelengths which exhibits S-shaped curve. The values of pKa were 5.10 and 9.7

Fig. 12

Fig. 13

3.5. Solvent effect

The absorption spectra of the azo compound under study were scanned in different solvents of varying polarities such as acetic acid, ethanol, methanol, n-propanol, 2-propanol and butanol which belong to protic solvents and ethyl acetate, acetonitrile, dioxane, diethyl ether, DMSO, DMF and THF as aprotic solvents.

The behavior of electronic spectra in different solvents is complex. In other words different phenomena are present in various media. Idealized theories[32,33] considering the solvent dielectric constant to act as a quantitative measure of solvent polarity. This view is insufficient as the researches lack to dealing with certain solute-solvent interactions like hydrogen-bonding and electron pair donor /electron pair accepter(EPD/EPA) interactions, which has a great effect in those interactions. According to the already described definition of solvent polarity and from fig. 13 which shows variation of λmax as a function of solvent dielectric constant, it is clear that sole physical quantity can't represent such solvent dielectric constant. Thus, the linear solvation energy relationship(LSER) as multiparameter scale of solvent polarity as proposed by Kamlet and Taft is considered an effective and aspirational approach for solvation effects, which entails significant interactions of a solute with its environment and can make an estimation for the compounds under study to form hydrogen bonds. Kamlet-Taft solvatochromic equation is of the following form[34].

$$\nu = \nu o + s\pi^* + b\beta + a\alpha \qquad \text{..... (1)}$$

where π^* is an index of the solvent dipolarity/polarizability, β is a measure of the solvent hydrogen-bonding acceptor (HBA) basicity, α is a measure of the solvent hydrogen-bonding donor (HBD) acidity and νo is the regression value of the solute

property in cyclohexane as the reference solvent. The regression coefficients s, b and a in Eq. 1 measure the relative susceptibilities of the absorption frequencies to the indicated solvent parameters.

The solvent parameters [35] are presented in table 3. The correlations of the spectroscopic data were accomplished by means of multiple linear regression analysis. It was observed that absorption frequencies for azo compound in different solvents, show agreeable correlation with π*, β and α parameters. The results of the multiple regressions are given in Table 3, and coefficients vo, s, b and a have confidence intervals at a level of significance of 95%. The degree of success of eq 1 is shown in figure 14 by means of a plot of calculated absorption wavenumbers (νcalc) as a function of the corresponding theoretical values. The negative sign of b coefficient for the synthesized azo compound in protic solvents denote a bathochromic shifts with increasing solvent hydrogen bond acceptor basicities. This indicates stabilization of the electronic excited state relative to the ground state. The positive sign of s and a coefficients point out a hypsochromic shifts with increasing solvent dipolarity/polarizability and solvent hydrogen bond donor acidities. This proposes stabilization of the ground state relative to the electronic excited state.

For aprotic case-solvent, coefficient s of negative sign indicates a bathochromic shifts with increasing solvent dipolarity/polarizability. Also, this proposes stabilization of the electronic excited state relative to the ground state. The positive sign of b and a coefficients refer to a hypsochromic shifts with rising solvent hydrogen bond acceptor basicities and solvent hydrogen bond donor acidities. This suggests stabilization of the ground state relative to the electronic excited state.

Table 3

Table 4

Fig. 14

Fig. 15

3.6. Preliminary investigations between 4-SPAP and Ca(II)

Throughout the preliminary investigation on the reaction between 4-SPAP and Ca(II) in an alkaline medium, a soluble colored chelate complex was formed. Calcium ion was selected because it's the only metal ion that reacts with 4-SPAP and gives distinct color change from more than 30 elements of periodic table at different conditions of concentration, pH and temperature. The colored product is quantified spectrophotometrically at 520nm. The absorbance of the colored product measured versus reagent blank increases with time and then the stability of complex remained for at least 60 min. This was used as a footing for a suitable kinetic method for the determination of sulfadiazine drug in pharmaceutical preparations using at least of 20 fold excess amount of Ca(II) in the existence of sodium hydroxide solution.

Therefore, the reaction was remarked as pseudo-first order reactions.

The initial rate and fixed time methods [36, 37] were tested and the most proper analytical merit was chosen regarding the applicability, sensitivity, the values of the intercept and correlation coefficient (r).

3.7. Optimization of the experimental conditions

Optimum reaction conditions affecting the reaction of 4-SPAP with calcium(II) was studied. In subsequent experiments, 1mL of 2×10^{-4}M of 4-SASP was taken in 10mL final volumes and the absorbance was measured at room temperature(25°C) for a series of solutions by varying one and fixing the other parameters at λmax versus reagent blank. All the results recorded in tables and figures are the average of five measurements.

3.7.1. Selection of reaction medium

In medium of acidify nature, the product showed pale color and low absorbance was coherent. Such solutions emphasize weakness of the complex formation; therefore, they were avoided.

To find a suitable medium for the reaction and to investigate the sensitivity of 4-SPAP -Ca(II) complex, different aqueous bases and buffer solutions were used, such as sodium hydroxide, ammonium hydroxide, sodium carbonate, phosphate, glycine and borate buffers. No significant influence of the buffer type on the absorbance was observed, and the good results were obtained when sodium hydroxide was used.

In order to determine the optimum concentration of sodium hydroxide, various volumes of 1M sodium hydroxide solution (0.1-2.0) mL were used. The results proved that 0.5mLof sodium hydroxide (1M) solution was the optimum for the determination of 4-SPAP. Larger volumes decreased the absorbance of the colored species. The above optimum volume was used in all subsequent experiments.

3.7.2. Effect of volume of calcium(II)

The volumes (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.5)mL of metal ion for the maximum color of the product to attain at 520nm were studied when the reagent concentration is 0.03 M. The absorbance increased with the increase in the volume of Ca(II) and becomes the greatest at 1.4mL. Thus, 1.4mL was selected as optimum value for the determination process.

3.7.3. Effect of temperature

The influence of temperature was examined in the range of (0-50)ºC. It was noticed that the color intensity increased with the increase in temperature up to 30 ºC. After that it was gradually decreased. Therefore, 25 ºC was selected for the determination process.

3.8. Quantitation Method(Evaluation of the kinetic methods)

Initial rate method

Under the above specified optimum conditions, the absorbance time curves for the reaction of 4-SPAP with Ca(II) were constructed, fig. 15. The initial rates of the reaction were determined from the slopes tangents of the absorption-time curves which measured for 19min at intervals of 2min starting from 1min at room temperature.

The initial rates of 4-SPAP reaction with Ca(II) would follow a pseudo-first order with respect to 4-SASP concentration, and were found to obey the following equation:

$$V = \Delta A/\Delta t = K' C^n \quad \ldots\ldots\ldots\ldots (2)$$

where V is the reaction rate, A is the absorbance, t is the measuring time, K′ is the pseudo-first order rate constant, C is the molar concentration of 4-SPAP, and n is the order of the reaction. The logarithmic form of the above equation as follows:

$$Log\ V = log\ \Delta A/\Delta t = log\ K' + n\ log\ C \quad \ldots\ldots\ldots\ldots (3)$$

Regression analysis using the method of least square was done to assess the slope, intercept and correlation coefficient. The analytical figures of merit and results of regression analysis are given in table 5. Specific rate constant of the kinetic reaction of 4-SPAP with Ca(II) (k, at 25ºC) was determined and found to be 11.46min^{-1} or 0.69s^{-1}. The value of n in the regression equation was 1.0693(≈1), confirming that the reaction of 4-SPAP with Ca(II) was in first order with respect to the 4-SPAP concentration. The limit of detection (LOD)and limit of quantification(LOQ)were found to be 0.35 and1.05 µg.mL^{-1}, respectively. These low values confirmed the good sensitivity of the initial rate method and consequently its capability to determine low amounts of 4-SPAP.

Fig. 16

Table 5

Fig. 17

Fixed time

In this method, the absorbance of varying concentrations retaining 4-SASP was inspected at a pre-selected fixed time of 1, 3, 5, 7 and 9min. Plots of absorbance against the concentrations of 4-SPAP were conducted at the fixed periods of time for the reaction, table 6. The regression equations, correlation coefficients, and the LOD and LOQ are given in table 6. The lowest limits of detection and quantification were obtained with fixed times of 1, 3, 5, 7 and 9min. However, the fixed time of 5 min exhibited broader concentration range for quantification. According to the ICH guidelines for validation of analytical procedures[38], the limit of detection is not necessary to be part of the validation. Therefore, depending on wider concentration range and minimal time of analysis, the fixed time of 5 min is recommended for the determination of 4-SPAP.

Table 6

3.9. Stoichiometry and the probable structure

The stoichiometry of the reaction of (4-SPAP–Ca(II)) was examined by mole ratio method[39]. Mole ratio procedure was achieved at 1×10^{-3}M solution of each of the reagent and metal ion to find the stoichiometry of complex. In this way a sequence of

solutions were ready in which the concentration of the reagent is kept constant and that of the metal ion is diverse and the optimum condition was set at maximum wavelength for the product, the absorbance of the solutions were measured versus blank and plotted versus the ratio of the moles. The stoichiometric ratio between 4-SPAP and Ca(II) at 520nm was found to be 1:1.

Based on the obtained molar reactivity, and depending on the nature of 4-SPAP,

the suggested structure of the colored product is shown in fig.18.

Fig. 18

3.10. Validation of the proposed methods

Accuracy and precision. The accuracy and precision for determination of current kinetic spectrophotometric method were inspected at three concentration levels of 4-SPAP[40] by examining five replicate samples of each concentration by both the initial rate and fixed time methods. The percent relative standard deviations (%RSD) for the results ranged from 1.04% to 1.76% and 0.85% to 1.42% for the initial rate and fixed time methods of the proposed kinetic spectrophotometric method, respectively (Table 7) confirming the high reproducibility of the results and the precision of the method. This good value of precision was advisable for quality control analysis of sulfadiazine in its pharmaceutical forms.

Table 7

3. 11. Specificity and interference

The specificity of the proposed kinetic spectrophotometric methods(initial rate and fixed time method) for determination of the studied drug in the presence of frequently

encountered excipient like; sucrose, glucose, lactose, sodium salicylate, sodium benzoate, starch and benzoic acid was examined, table 8.

Table 8

The interference was considered acceptable for error lower than ± 2%. It was found that there was no interferences from these excipients and additives, so the proposed method can be considered selective one.

3.12. Application of the proposed methods

The initial rate and fixed time methods of the proposed kinetic spectrophotometric method have been performed on commercial pharmaceutical formulations containing sulfadiazine.

The method involves weighing appropriate amount of the commercial formulation and dissolving it in conc. HCl. The solution formed was diazotized by addition of sodium nitrite at 10 ºC. The diazotized product was coupled with pyrogallol under controlled condition of pH (less than 7.0) and temperature(5 ºC). The resulting precipitate was filtered, washed and purified. The precipitate contains sulfadiazine became ready for analysis within 24 hrs.

The concentration of sulfadiazine was computed from its corresponding regression equations. The results obtained from the investigations are summarized in table 9, which indicate that there were no interferences from excipients and the proposed methods have good selectivity. In these states the proposed methods involved (initial rate or fixed time) which was statistically compared with official method[41] as shown in table 10. The determination of t-test and F-test indicated no significant differences between both the current and the reported methods at 95% confidence

level. That refers to good precision and accuracy in the analysis of sulfadiazine in pharmaceutical dosage forms.

Table 9

4. Conclusion

Sulfadiazine, for first time, was successfully determined by incorporating it as an organic solid compound, 4-SPAP, through coupling of pyrogallol with diazotized sulfadiazine in medium of controlled pH within 5.0-6.0. The procedure of synthesis which yields 4-SPAP did not prevent the ease of its purification, characterization and solvatochromic behavior with solvents of different polarities which were studied by applying Kamelt-Taft equation. The determination process was accomplished using initial rate and fixed time kinetic spectrophotometric methods. The results showed that these two methods were simple, accurate and precise with low limit of detection. References methods for sulfadiazine determination require extraction procedures due to difficulty of solubility of sulfadizine in aqueous media. The presented method does not need such tedious treatments. Furthermore, the suggested method of analysis requires simple apparatus for its performance available in any analytical laboratory. In accordance to all these features, the proposed method can be regarded valuable and appropriate for quality control and routine determination of the studied drug in pharmaceutical dosage forms. Further investigation is required to test other forms of pharmaceuticals containing sulfadiazine such as tablet and gauze.

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References

1- E. R. Bandalaa, L. Gonzeza, F. de la Hozb, M. A. Pelaezc, D. D. Dionysiouc, P. S.M. Dunlopd, J. Anthony Byrned, J. Luis Sancheza, Application of azo dyes as dosimetric indicators for enhanced photocatalytic solar disinfection (ENPHOSODIS), Journal of Photochemistry and Photobiology A: Chemistry 218 (2011) 185–191.

2- E. Weglarz-Tomczak, Ł. gorecki, Azo dyes – biological activity and synthetic trategy, Chemik Science-Technique-Market, 66, 12, 1298-1307(2012).

3- A. Shaikh and J. S. Meshram, Design, synthesis and pharmacological assay of novel azo derivatives of dihydropyrimidinones, Cogent Chemistry, 1(2015) 1-9.

4- H. Otten, "Domagk and tbe development of the sulphonamldes" Journal of Antimicrobial Chemotherapy 17(1986) 689-696.

5- Alka Garg and William Smith, "Sulfur allergies can be misleading " Journal of pharmacy practice and research, 43(2013) 332-339.

6- 19th WHO Model List of Essential Medicines(April 2015).

7- Jeffrey Wallace and Douglas S. Paauw, "Appropriate Prescribing and Important Drug Interactions in Older Adults" Med Clin N Am , 99(2015) 295–310.

8- Safwan Ashour, Mouhammed Khateeb, " Kinetic spectrophotometric determination of pravastatin in drug formulations via derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)" Arabian Journal of Chemistry 4(2011) 299–305.

9- D. Perez-Bendito, A. Gomez-Hens and M. Silva, " Advances in drug analysis by kinetic methods" Journal of pharmaceutical and biomedical analysis, 14(1996) 917-930.

10- Ashraf M. Mahmoud, Hanaa M. Abdel-Wadood, Niveen A. Mohamed, "Kinetic spectrophotometric method for determination of amlodipine besylate in its pharmaceutical tablets" Journal of Pharmaceutical Analysis, 2(2012) 334–341.

11- Ashraf M. Mahmoud, Hanaa M. Abdel-Wadood, Niveen A. Mohamed, " Kinetic Spectrophotometric Method for the Estimation of Ofloxacin in Pharmaceutical Formulations" J. Mex. Chem. Soc., 56(2012) 355-361.

12- Safwan Ashour, "New Kinetic Spectrophotometric Method for Determination of Atorvastatin in Pure and Pharmaceutical Dosage Forms", Pharm Anal Acta 4(2013) 232-437.

13- Raafia Najam, Gh Mohd Shah and S Muzaffar Ali Andrabi," Kinetic spectrophotometric determination of an important pharmaceutical compound, pregabalin" Journal of Analytical Science and Technology, 4(2013) 22-30.

14- Mohammed G. Abdel Wahed, Ragaa El Sheikh, Ayman A. Gouda and Sayed Abou Taleb," Kinetic spectrophotometric determination of some fluoroquinolone antibiotics in bulk and pharmaceutical preparations" Bull. Chem. Soc. Ethiop., 27(2013) 329-346.

15- Elrasheed A. Gadkariem, Magdi A. Mohamed and Maha A.A. Jabbir, " Assay of Tranexamic Acid via Coupling with Ascorbic Acid Using Kinetic Methods, Elixir online journal, 54(2013) 12331-12334.

16- Akram M. EL-Didamony, M. Z.Saad, N. O. Saleem, "kinetic spectrophotometric method for the determination of morphine, nalbuphine and nal trexone drugs in bulk and pharmaceutical formulations" J. Chil. Chem. Soc., 58(2013) 1907-1913.

17- Safwan Ashour and Mouhammed Khateeb, "New Kinetic Spectrophotometric Method for Determination of Fexofenadine Hydrochloride in Pharmaceutical Formulations, International Journal of Spectroscopy , Volume 2014, 8 pages(2014).

18- Sayed Mohamed Sayed Derayea, " Validated selective spectrophotometric methods for the kinetic determination of desloratidine in tablets and in the presence of its parent drug, Pak. J. Pharm. Sci., 27(2014) 1781-1788.

19- Safwan Fraihat, " kinetic spectrophotometric methods for the determination of gentamicine in pharmaceutical forms" International Journal of Pharmacy and Pharmaceutical Sciences, Int J Pharm Pharm Sci, 7(2015) 302-305.

20- Mohauman Mohammad Al-Rufaie, "Modern kinetic spectrophotometric procedure for estimation of furosemide drug as bulk form and in pharmaceuticals preparations, Curr. Issues Pharm. Med. Sci., 29(2016) 184-189.

21- Akram M. El-Didamony, Mona O. Abo-Elsoad, " Kinetic spectrophotometric method for the determination of some fourth generation fluoroquinolones in bulk and in pharmaceutical formulations, Journal of Saudi Chemical Society 21(2017) 558-566.

22- César Soto, Cristian Poza, David Contreras, Jorge Yáñez, Fallon Nacaratte, and M. Inés Toral," A New Kinetic Spectrophotometric Method for the Quantitation of Amorolfine" Journal of Analytical Methods in Chemistry Volume 2017, 8 pages(2017).

23- D. L. Pavia, G. M. Lampon and G. S. Kriz, "Introduction to spectroscopy", 3rd ed, Thomson Learning, Inc., USA, pp. 24-29(2001).

24- Moustafa, M.A.,EL-Mossalamy, E.H.; and Amine, A.S., Monatshefte fur Chim., 126(1995)901-908.

25- J. Guo, L. Li, Y. Ti, J. Zhu, Synthesis and properties of a novel pH sensitive poly(N-vinyl-pyrrolidone-co sulfadiazine) hydrogel, eXPRESS Polymer Letters, 1 (2007) 166–172.

26- J. Turczan and T. Medwick, Identification of sulfonamides by NMR spectroscopy, Journal of pharmaceutical sciences, 61(1972) 434-443.

27- G. Simeon , A. Dragenov and D. Rustschev , J. Termal Anal., 36 (1990) 721.

28- L.E.Vidal Salgado,C.Vargas-Hernandez .American Journal of Analytical Chemistry, 5(2014) 1290-1301.

29- N. A. Naser, K. H. Kahdim and D. N. Taha, Synthesis and characterization of an organic reagent 4-(6-bromo-2-benzothiazolylazo)pyrogallol and its analytical application, J. Oleo Sci. 61 (2012) 387-392.

30- A. A. El-Ansary, A. A. Ali, spectrophotometric determination of ionization constants of some 8-hydroxyquionline azo dyes, Chimie, 30(1984) 145-150.

31- M. S. Abu-Bakr, A. S. El-Shahawy and S. M. Ahmed, spectrophotometric study of acid-base equilibrium of 4- (2-benzothiazolylazo)resorcinol and 4-(2-benzothiazolylazo)salicylic acid in water-organic solvent media, J. Solu. Chem. 22, (1993) 663-675.

32- Onsager, L. J. Am. Chem. Soc. 58(1936) 1486–1493.

33- Kirkwood, J. G. J. Chem. Phys. 2(1934) 351–361.

34- Kamlet, M. J.; Taft, R. W. Acta Chemica Scandinavica. 39(1985) 611–628.

35- Reichardt, C. Solvents and Solvent Effects in Organic Chemistry. 3rd ed, VCH, 2003; p.433.

36- K.B. Yatsimirskii, "Kinetic Methods of Analysis", Pergamon Press, Oxford, UK, 1966, 43.

37- H. A. Laitimen; W. E. Harris, "Chemical Analysis", 2nd ed., Mc-Graw-Hill, New York, USA, 1975.

38- International Conference on Harmonization, ICH Harmonised Tripartite Guidline-Text on Validation of Analytical Procedures, Federal Registar 60, 1995, p. 11260.

39- P. O. Okolo and E. E. Ukpebor, Stoichiometry of quinol/ammonium-nitrogen complex using spectrophotometry, Jour. Chem. Soc. Pak., 26, No. 3, 207-211(2004).

40- G.W. Ewing, Instrumental Methods of Chemical Analysis, 5th ed., Lippincott-Raven, Philadelphia, 1995, pp. 484–486.

41- "British Pharmacopoeia on CD-ROM", Version 11, 5th Ed., Vol. I&II, Copyright by System Simulation Ltd, The Stationery Office Ltd., London, 2007.

Table 1. Effect of temperature on mass changes of analyzed materials.

Materials	Step 1			Step 2			Step 3			Loss on Ignition%
	Tonset ºC	Toffset ºC	Mass changes%	Tonset ºC	Toffset ºC	Mass changes/%	Tonset ºC	Toffset ºC	Mass changes%	
Pyrogallol	30	305.631	-69.8284	305.631	494.503	-28.4374	-	-	-	-98.2658
Sulfadiazine	25	315.128	-28.6071	423.244	494.573	-24.0122	494.573	500	-3.98835	-56.6.765
Reagent	40	157.482	-2.6528	433.581	494.762	-29.7011	494.762	500	-7.4284	-39.7823

Table 2. Effect of temperature on DSC of analyzed materials.

Materials	1 Effect endo (due to water)			2 Effect endo			3 Effect exo		
	Tonset ºC	Peak max ºC	Toffset ºC	Tonset ºC	Peak max ºC	Toffset ºC	Tonset ºC	Peak max ºC	Toffset ºC
Pyrogallol	128.6	-82.402	143.5	215	-20	265	360	74	430
Sulfadiazine	256.3	-37.167	266.4	-	-	-	275	-3.5	349
Reagent	60.80	94.07	130.59	-	-	-	188.62	190.41	194.69

(Tonset – the initial temperature of thermal degradation for each stage, Toffset - the final temperature at which the degradation process for each stage ends, mass loss (W%) and residue at the end of decomposition processes)

Table 3. Absorption maxima of azo compound in various solvents and selective Kamlet-Taft solvent parameters.(ε: dielectric constant)

A: protic solvents						
Solvent	**λmax**	**ν, cm^{-1}**	**π***	**β**	**α**	**ε**
acetic acid	456	21929.82	0.64	0.45	1.12	6.2
Methanol	428	23364.49	0.6	0.63	0.93	32.6
Ethanol	430	23255.81	0.54	0.75	0.83	24.6
Propanol	434	23041.47	0.52	0.9	0.84	20.33
2-propanol	438	22831.05	0.48	0.84	0.76	19.92
Butanol	438	22831.05	0.47	0.84	0.84	18
B: aprotic solvents						
Dioxane	434	23041.47	0.62	0.48	0	2.3
Ether	420	23809.52	0.27	0.47	0	4.3
ethyl acetate	432	23148.15	0.45	0.45	0	6
DMF	426	23474.18	0.88	0.69	0	36.7
DMSO	436	22935.78	1	0.76	0	47
THF	432	23148.15	0.55	0.55	0	7.6
Acetonitrile	402	24875.62	0.66	0.4	0.19	37.5

Table 4. Regression fits to solvatochromic parameters of Eq 1.

Azo compound in	**νo**	**S**	**B**	**a**	**R**	**F**
protic solvent	20399.17	5015.253	-2337.14	2275.391	0.9145	7.131585
aprotic solvent	22770.57	-1474.66	11081.79	2431.977	0.9332	13.95984

R- Correlation coefficient; F- Fisher's test

Table 5. Analytical information for the initial rate method of the proposed kinetic spectrophotometric method for determination of 4-SPAP.

Linear range(M)	Leastsquareequation(Log $V=\log K' + n \log C$)[a]		Correlation coefficient(r)	LOD(M)	LOQ(M)
	Intercept (log K)	Slope(n)			
2×10^{-5}- 2×10^{-4}	1.6164	0.10693	0.9812	0.09×10^{-5} (0.35)[b]	0.27×10^{-5}(1.05)

[a] V is the reaction rate, K′ is the conditional rate constant, n is the order of reaction, and C is the molar concentration of 4-SPAP.

[b] Figures in parenthesis are the linear range in $\mu g.mL^{-1}$.

Table 6. Analytical parameters for the fixed-time method of the proposed kinetic spectrophotometric method for the determination of 4-SPAP.

Reaction time(min)	Linear range(M)	Correlation coefficient(r)	Slope (b)	Intercept(a)	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
1	1×10^{-5}- 1×10^{-4}	0.9856	0.0044	0.0021	1.06	3.18
3	1×10^{-5}- 2×10^{-4}	0.9745	0.0067	-0.0001	0.81	2.43
5	1×10^{-5}- 2×10^{-4}	0.9901	0.0147	-0.0022	0.69	2.07
7	1×10^{-5}- 2×10^{-4}	0.9882	0.0202	-0.0025	0.59	1.77
9	1×10^{-5}- 2×10^{-4}	0.9868	0.0243	-0.0037	0.40	1.20

Table 7. Evaluation of the accuracy and precision of the initial rate and fixed time methods of the proposed kinetic spectrophotometric method for determination of 4-SPAP.

Concentration,, M	Initial rate method			Fixed time method		
Present	Found, M	E%	Rec.%	Found, M	E%	Rec.%
3.500×10^{-5}	3.609×10^{-5}	+3.11	103.11	3.602×10^{-5}	+2.91	102.91
5.500×10^{-5}	5.387×10^{-5}	-2.05	97.95	5.392×10^{-5}	-1.96	98.04
1.000×10^{-4}	9.865×10^{-5}	-1.35	98.65	9.897×10^{-5}	-1.03	98.97

Table 8. Effect of excipient (10 folds) on the recovery of the colored product.

Excipient 2.0×10^{-4}M	Sulfadiazine-containing product, found, M	E%	Rec.%
Sucrose	-5.0×10^{-5}	-0.25	99.75
Glucose	-2.2×10^{-5}	-1.1	98.9
Lactose	-8.0×10^{-5}	-0.4	99.6
sodium salicylate	-5.5×10^{-5}	-0.275	99.73
sodium benzoate	-9×10^{-5}	-0.45	99.55
Starch	-2.2×10^{-5}	-1.1	98.9
benzoic acid	1.0×10^{-5}	-0.05	99.95

Table 9. Determination of sulfadiazine in its pharmaceutical dosage forms using the proposed method(initial rate and fixed time methods)and comparison with standard BP method using t- and F- statistical tests

Pharmaceutical preparations	Proposed method				Standard method	
	Initial rate method		Fixed time method			
	Rec.%	$(Xi-\bar{X1})^2$	Rec.%	$(Xi-\bar{X1})^2$	Rec.%	$(Xi-\bar{X2})^2$
No-Burn Philadelphia Pharmaceuticals, Jordan.	100.22	0.0009	100.19	0.0016	99.85	0.0576
Silverdin, DEVA HOLDING, Turkey.	100.33	0.0064	100.28	0.0025	100.25	0.0256
Hamazine HAYAT drug productions Co., Iraq.	100.20	0.0025	100.22	0.0001	100.17	0.0064
	ΣXi= 300.75	$\Sigma(Xi-\bar{X1})^2=$ *0.0098*	ΣXi= 300.69	$\Sigma(Xi-\bar{X1})^2=$ *0.00*42	ΣXi= 300.27	$\Sigma(Xi-\bar{X2})^2=$ *0.0896*
	X1= 100.25		$\bar{X1}$= 100.23		$\bar{X2}$= 100.09	
	t (theor.)[a] =0.586 (2.78) F *(theor.)*=0.109 (19.03)		*t (theor.)*=0.528 (2.78) F *(theor.)*=0.043 (19.03)			

[a]Figures in parenthesis are the calculated values of t and F at 95%confidencelimit, respectively. The tabulated t- and F-values

Figure Captions

Fig. 1: General chemical structure of sulfonamides.

Fig. 2: Synthetic route of 4-SPAP.

Fig. 3: FT-IR spectrum of 4-SPAP.

Fig. 4: Tautomeric forms of 4-SASP.

Fig. 5: ^{1}H-NMR spectrum of 4-SPAP.

Fig. 6: TG and DSC curves for pyrogallol thermal decomposition(heating rate10 ^{o}C/min)

Fig. 7: TG and DSC curves for sulfadiazine thermal decomposition(heating rate10 ^{o}C/min)

Fig. 8: TG and DSC curves for 4-SPAP thermal decomposition(heating rate10 ^{o}C/min)

Fig. 9: GC-Mass spectrum of 4-SPAP.

Fig. 10: Absorption spectrum of 4-SPAP in ethanol.

Fig. 11: Acid–base forms of 4-SPAP at pH range 1.0-11.

Fig. 12: Effect of pH on 4-SPAP; pH=1.98, 2.69, 3.7, 4.3, 5.3, 6.8, 7.0, 8.22, 9.8, 10.0, 11.25

Fig. 13: Effect of pH on 4-SPAP, selected spectra from figure 11 show isobestic point at 365nm.

Fig. 14: Variation of λmax of azo compound as a function of solvent dielectric constant. A. protic solvents; B. aprotic solvents.

Fig. 15: The plot of λmax observed against λmax calculated from Eq. 1

for azo compound in different solvents A. protic solvents; B. aprotic solvents.

Fig. 16: Absorbance-time curves for the reaction of varying concentrations of 4-SPAP with Ca(II).

Fig. 17: Calibration plots reliance of the log.rate of the reaction vs. log of molar concentration of 4-SPAP for initial rate method.

Fig. 18: Suggested structure of the 4-SPAP–Ca(II) colored product.

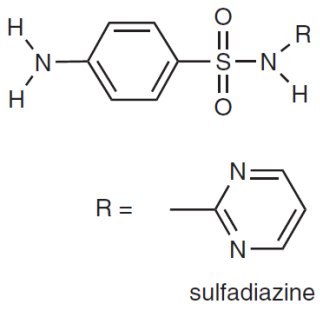


Figure 1

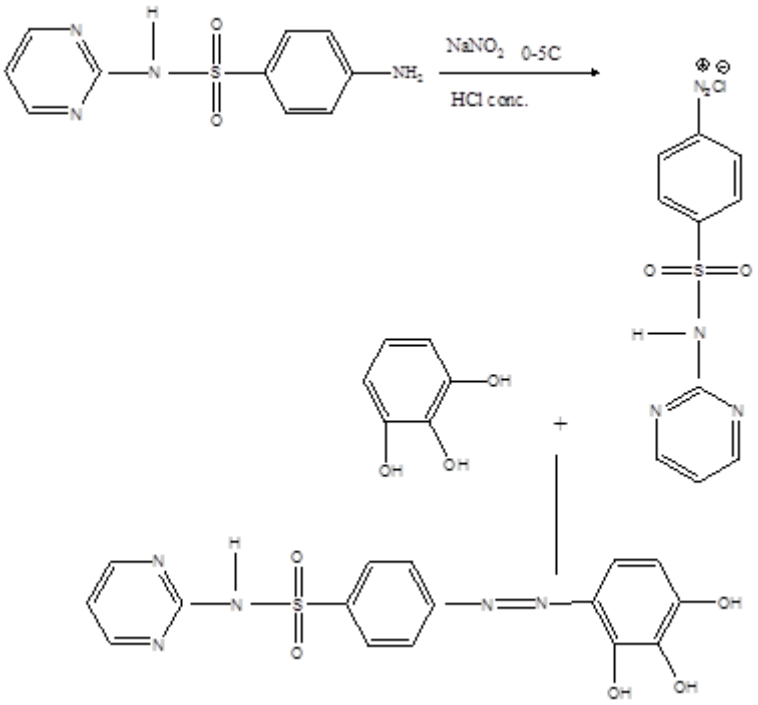


Figure 2

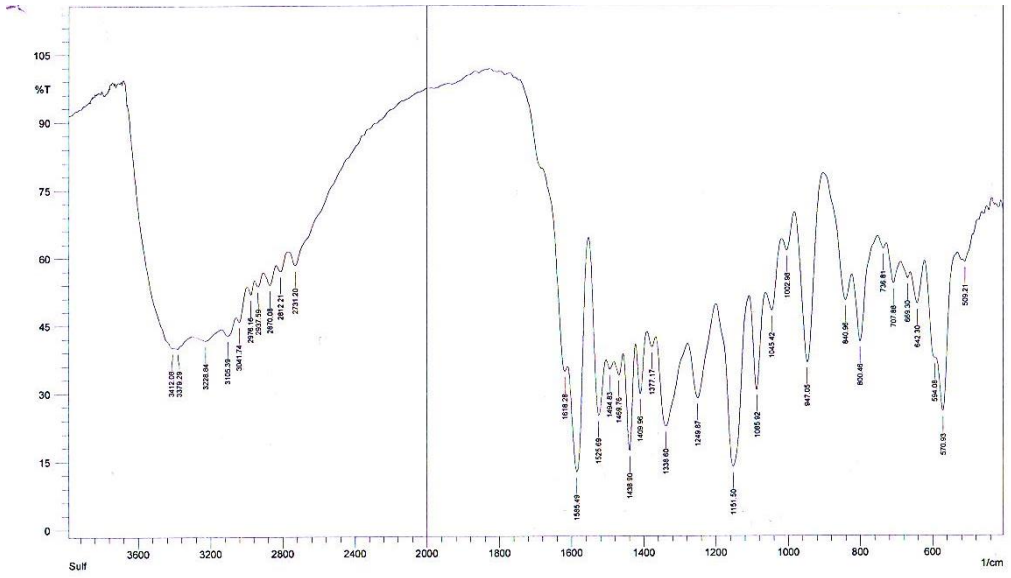


Figure 3

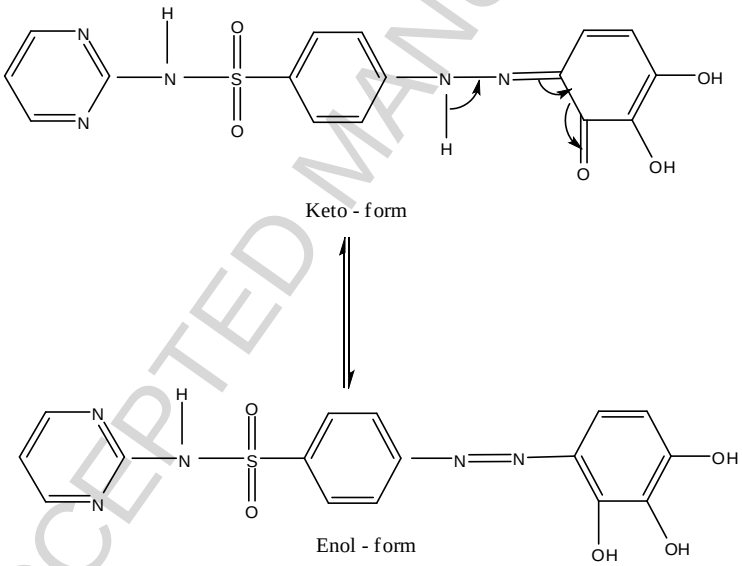


Figure 4

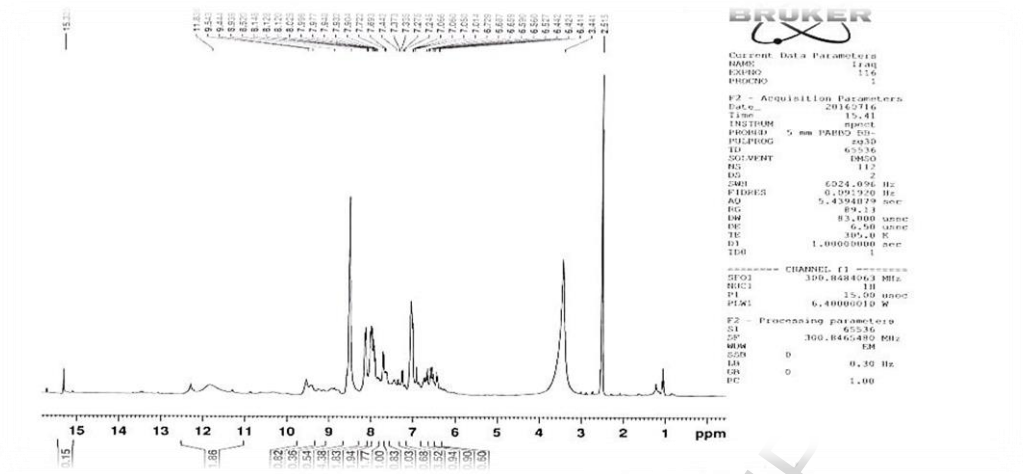
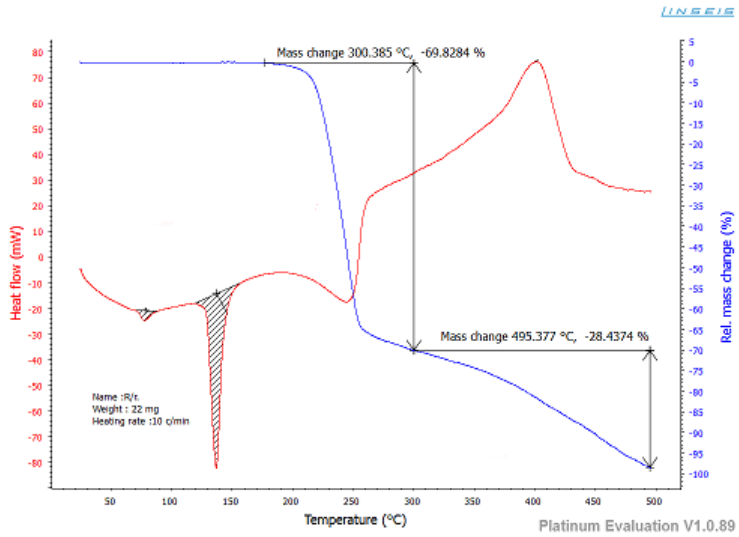


Figure 5



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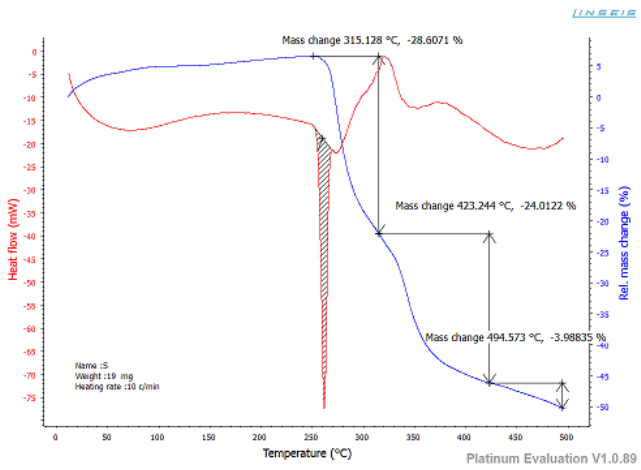


Figure 7

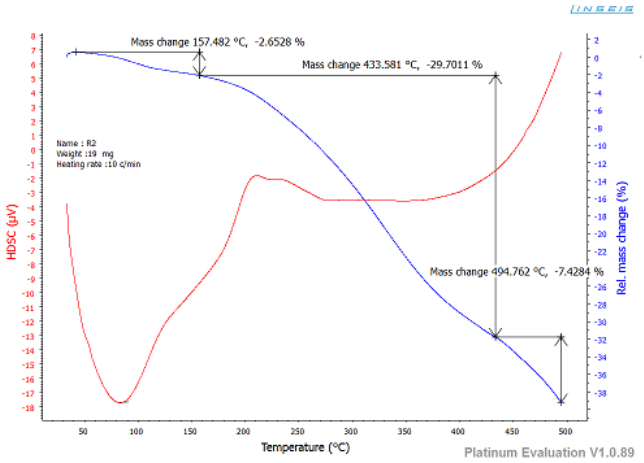
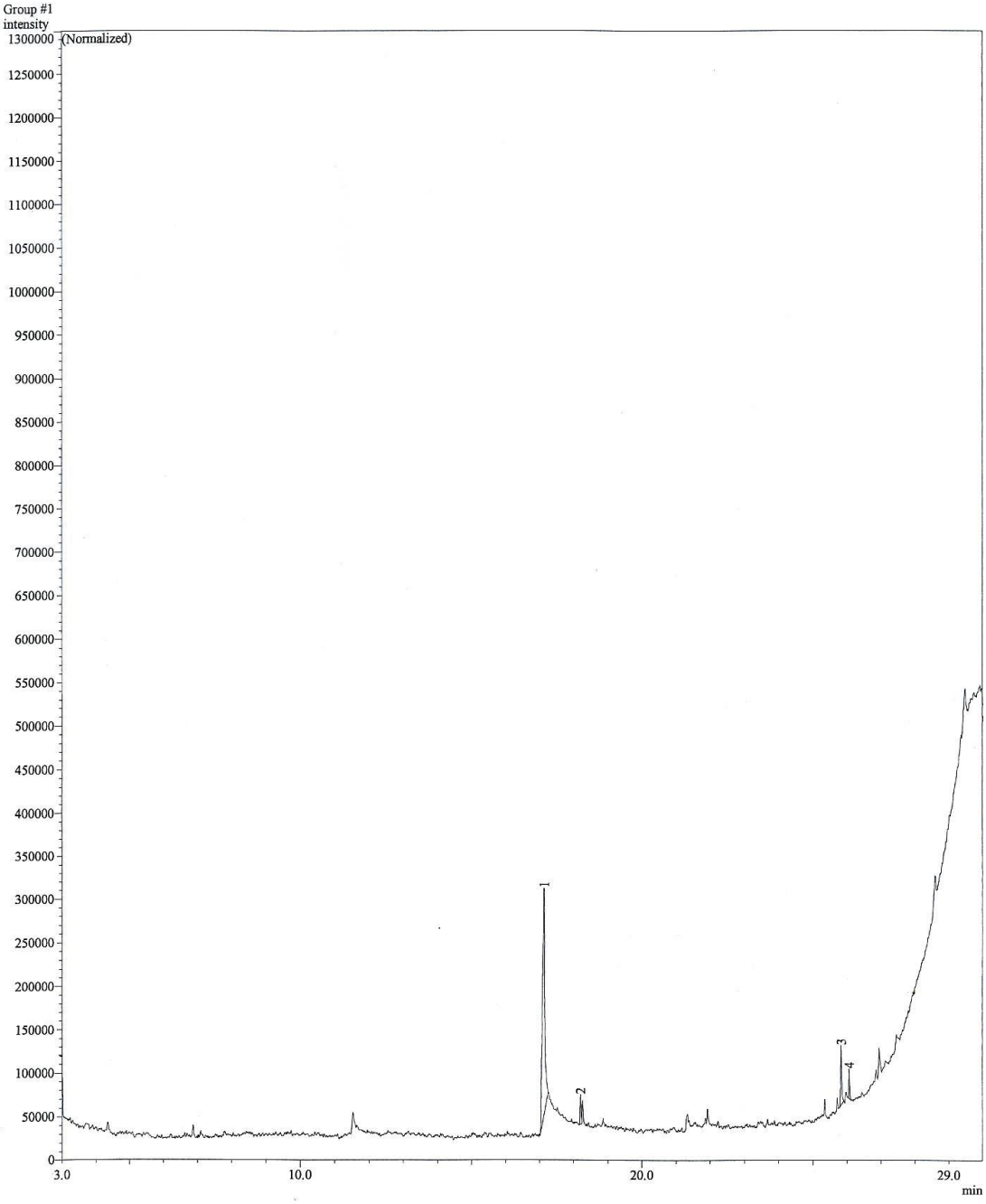


Figure 8



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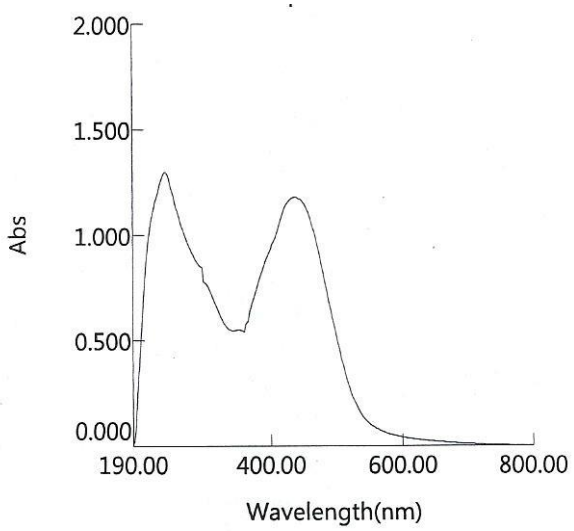

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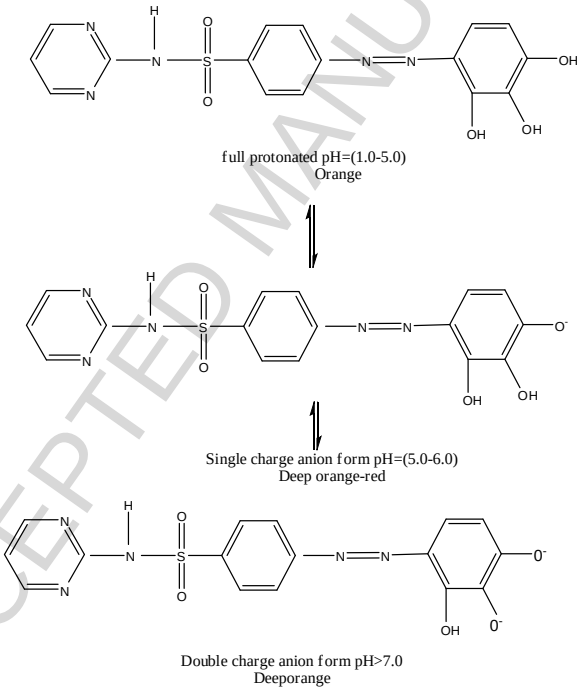
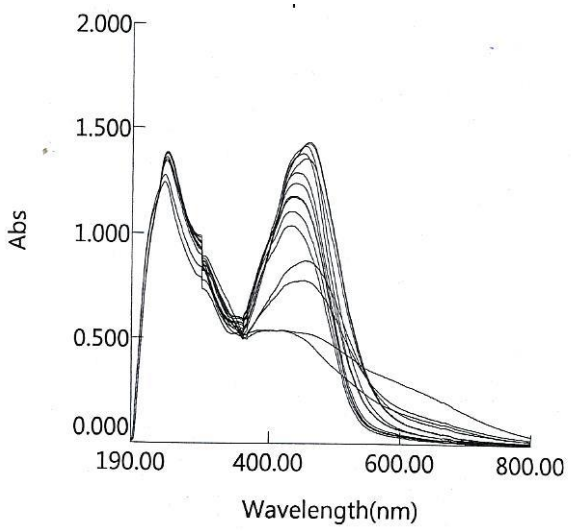


Figure 11



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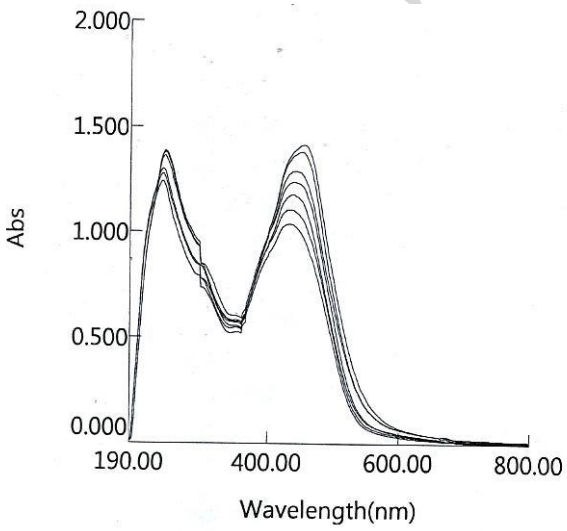

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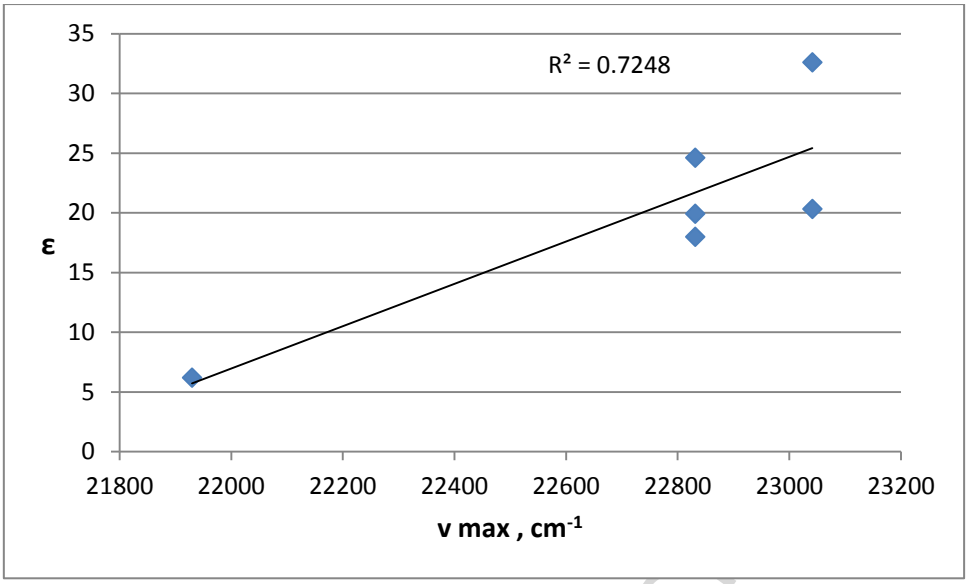

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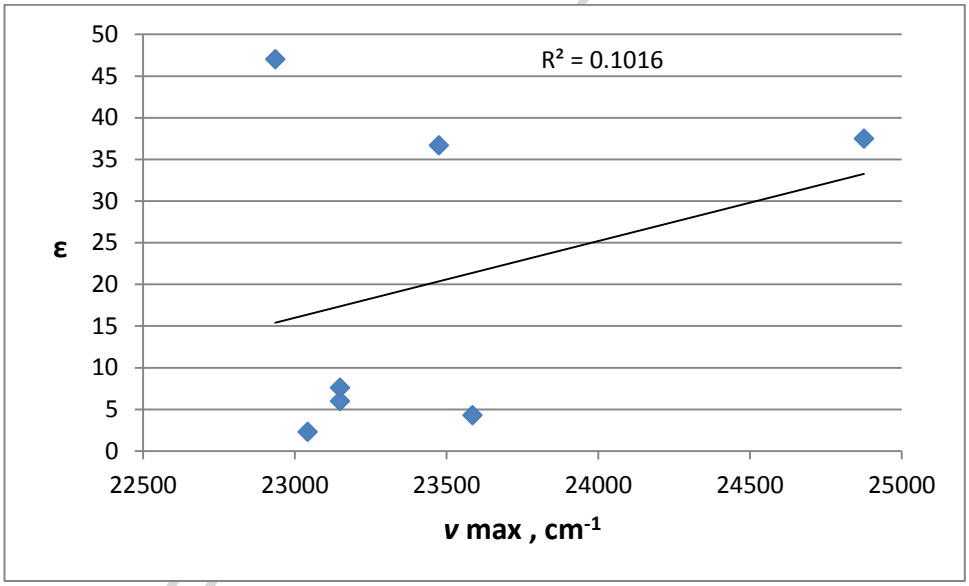
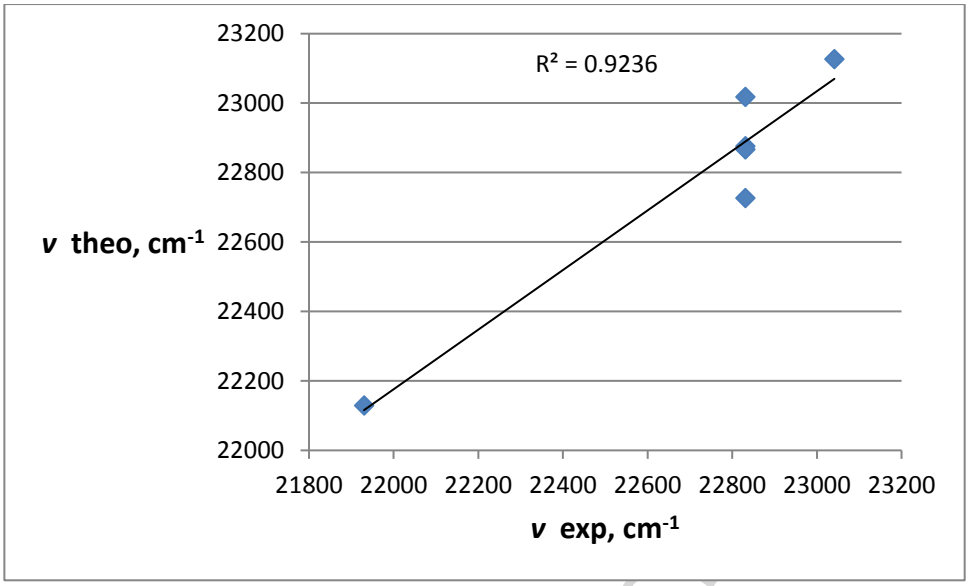


Figure 14

A.



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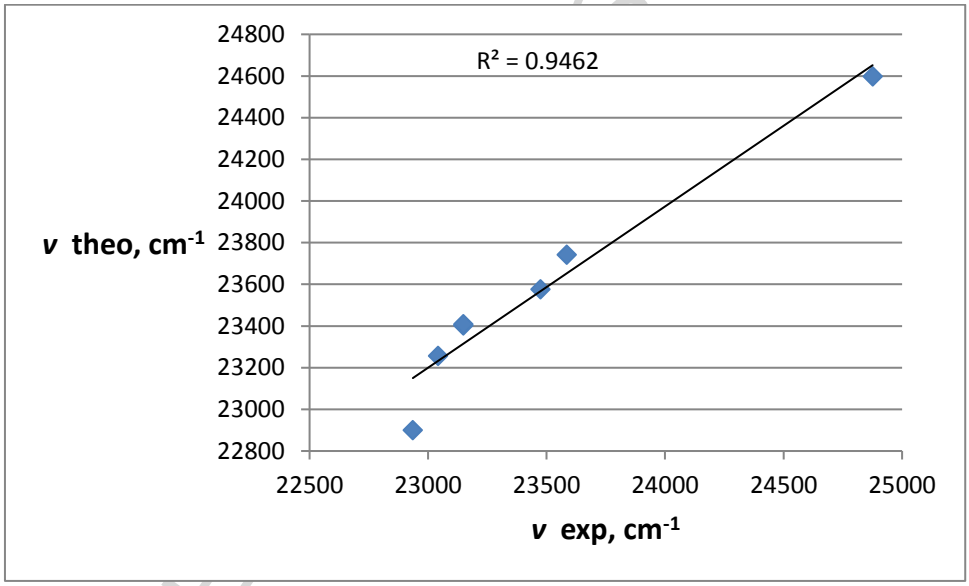


Figure 15

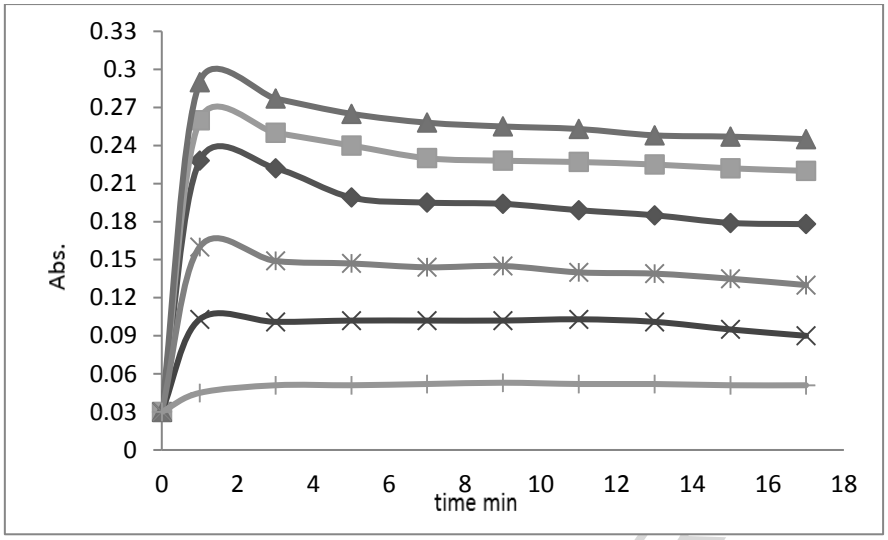


Figure 16

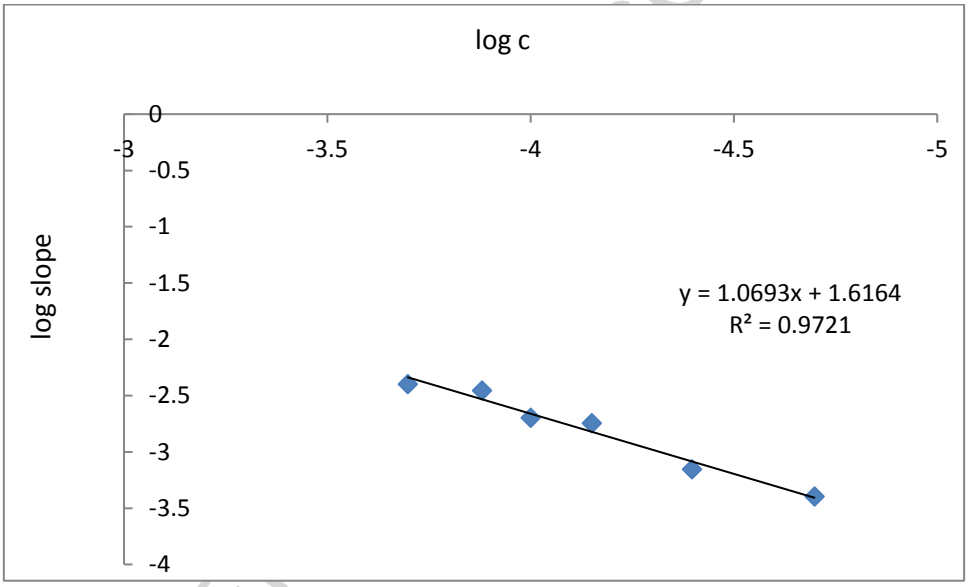


Figure 17

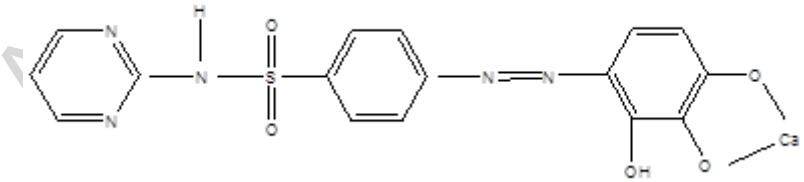
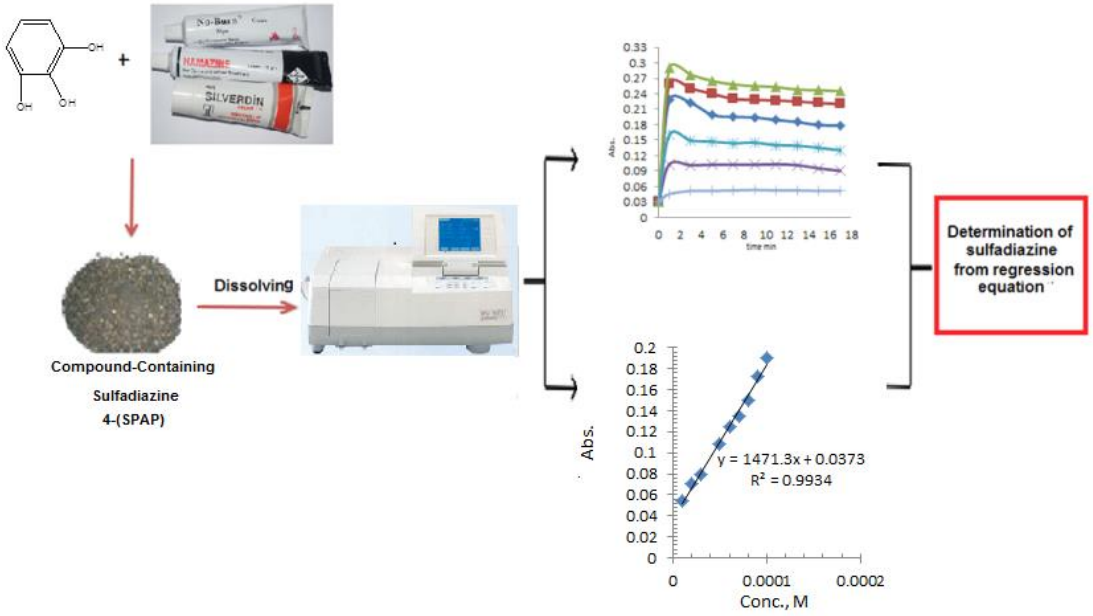


Figure 18

Graphic abstract



Highlights

- Synthesis of new organic reagent, (4(4-sulphophenylazo)pyrogallol.
- Kinetic methods of analysis (initial rate and fixed time) were used for the quantitation of sulfadiazine after easy dissolution of the synthesized compound.
- Methods of analysis were characterized with good features such as simplicity, short time of analysis, good precision and accuracy, high selectivity and low limit of detection.