Research Article

Pregabalin and Tranexamic Acid Evaluation by Two Simple and Sensitive Spectrophotometric Methods

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This paper demonstrates colorimetric visible spectrophotometric quantification methods for amino acid, namely, tranexamic acid and pregabalin. Both drugs contain the amino group, and when they are reacted with 2,4-dinitrophenol and 2,4,6-trinitrophenol, they give rise to yellow colored complexes showing absorption maximum at 418 nm and 425 nm, respectively, based on the Lewis acid base reaction. Detailed optimization process and stoichiometric studies were conducted along with investigation of thermodynamic features, that is, association constant and standard free energy changes. The method was linear over the concentration range of 0.02–200 μg mL⁻¹ with correlation coefficient of more than 0.9990 in all of the cases. Limit of detection was in range from 0.0041 to 0.0094 μg mL⁻¹ and limit of quantification was in the range from 0.0137 to 0.0302 μg mL⁻¹. Excellent recovery in Placebo spiked samples indicated that there is no interference from common excipients. The analytical methods under proposal were successfully applied to determine tranexamic acid and pregabalin in commercial products. t-test and F ratio were evaluated without noticeable difference between the proposed and reference methods.

1. Introduction

Pregabalin (PG) chemically is 3-amino methyl hexanoic acid, with the chemical formula C₉H₁₇NO₂. Its structural and pharmacological features correspond to the mammalian neurotransmitter gamma-aminobutyric acid (GABA), and it is primarily used as anticonvulsant drug. However, its effects are much broader being analgesic, antiepileptic, antiadipetic, and anti-inflammatory drug. Further more, it has been recommended for gastrointestinal damage, alcoholism, and insomnia [1]. Exact mechanism is not known; however, it has been an established fact that it binds to calcium channel in the central nervous system which decreases calcium influx at nerve endings and therefore reduces the liberation of several associated neurotransmitters which subsequently results in the above-mentioned activities [2]. Tranexamic acid (TXA) is chemically designed as trans-4-(aminomethyl) cyclohexanecarboxylic acid, with chemical formula C₈H₁₄NO₂. It is the closed cyclic analogous structure of lysine. TXA has been very potent antifibrinolytic agent, vastly used in haemorrhagic diseases. Its significant use is to treat ovarian tumors and it is recommended to manage pregnancy and reduce blood lose in surgery. It is considered as the best substitute to surgery in cases of menorrhagia [3, 4]. Chemical structure of TXA and PG is shown in Figure 1.

PG is not yet part of any pharmacopoeial monograph. However, analytical method reported for PG includes spectrophotometry [5–9], spectrofluorimetry [10], and high pressure liquid chromatography with UV detection [11], with mass detection [12], and with fluorescence detection [13]. TXA quantification has been reported through colorimetric spectrophotometry [14–18], HPLC [19], and spectrofluorometry [20]. Although chromatographic method offers high level of selectivity and specificity, the associated expenses are alarming and cannot be shouldered by every pharmaceutical...
industry. Moreover, the molecules of TXA and PG lack UV absorbing chromospheres, so in case of HPLC high load on column will deteriorate and reduce its life which turned out to be huge burden on low and middle economy-based industries. Amino acids like TXA and PG are highly polar and cannot be easily volatilized, so gas chromatographic method would not be easy or straightforward. Spectrophotometers are not the common instruments in all the labs. All these suggest that the proposed analytical methods are highly important from quality control point of view. The colorimetric reagents used in this study are trinitrophenol (TNP) and dinitrophenol (DNP). These reagents have wide range utility as quality colorimetric analyzing agents in pharmaceutical industry [21–27].

A glance over the literature revealed few reports regarding charge transfer complexes for PG and TXA. A structural analogous of PG and TXA gabapentin has been determined by colorimetry-visible spectrophotometry by reacting with iodine, chloranil, chloranilic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetracyanomethylene, methyl orange, hydroxy benzaldehyde, picric acid and with ninhydrin [28–30]. Colorimetric spectrophotometric methods for PG include reaction with 1,2-naphthoquinone-4-sulphonate sodium and 2,4-dinitrofluorobenzene [5], ascorbic acid and salicylic aldehyde [6], 7,7,8,8-tetracyanoquinodimethane, 2,3-dichloro-5,4-benzoquinone, tetracyanoethylene and 2,3,5,6-tetrachloro-6-dicyano-1,4-benzoquinone, 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone, tetracyanoethylene and 2,3,5,6-tetrachloro-1,4-benzoquinone [7], quinalizarin and alizarin [8], and vanillin, acetyl acetone, and formaldehyde [9]. Colorimetric quantification reagent for TX includes ninhydrine [14, 15], ferric chloride [15], 1,2-naphthoquinone-4-sulphonate sodium [16], ascorbic acid [17], and Azodye [18].

There is no report on TXA and PG determination which utilizes TNP or DNP reagents. Most of the reported spectrophotometric methods are insensitive or need complicated extractions and heating/cooling procedures or use reagents which do not produce linear response. Moreover, most of the methods are based on the absorbance in near UV region and thus specificity is questioned. The method under proposal is highly selective and extremely simple, so it can be usefully adopted for routine analysis in quality control laboratories. The proposed methods are accompanied by the reaction of amine group of amino acid with hydroxyl group, activated by neighboring nitro groups of both TNP and DNP. As a result, yellow color complexes are formed on simple addition of the two reagents and no extra process is required. Thermodynamics features in respect of association constant and standard free energy changes have been evaluated for both reactions. Both of methods have been proved to be very useful from routine quality control prospective and can be considered as superior to most of the published methods with respect to pace, ease, low cost, and sensitivity.

2. Experimental

2.1. Instrument. A UV-Visible Shimadzu Spectrophotometer 1601 with 1 cm path length quartz cells controlled by Shimadzu UV Probe 3.9 version software was used. MS Excel sheet was used to evaluate F, t tests, and other statistical parameters.

2.2. Materials and Reagents. Analytical grade reagents were used. Pregabalin and tranexamic acid pure drugs were a kind gift from a local pharmaceutical agency having 99% plus purity. Tranex capsules 250 and 500 mg and Syngab capsules 200 mg, 50 mg, and 100 mg (Atco Laboratories Ltd., Karachi, Pakistan) were procured from the market. TNP and DNP were acquired from Merck Sigma Aldrich, Germany. Microcrystalline cellulose (Avicel pH 101, maize starch, magnesium stearate, PVP, Aerosil R-200, crospovidone-X, HPMC (606), and PEG (6000)), titanium dioxide, and isopropyl alcohol were a gift from a local pharmaceutical agency. The entire chemicals were used according to their safety precautionary measures.

2.3. General Procedure

2.3.1. Analytical Method Development

Reagents and Standard Stock Solutions Preparation. A 2.27 gl⁻¹ TNP and 1.84 gl⁻¹ 2,4-DNP solutions were prepared individually in dichloromethane corresponding to 10 mMol of the concerned reagent.

160 µgL⁻¹ of PG and 155 µgL⁻¹ of TXA were prepared individually by taking 16 and 15.5 mg weights, respectively, in 100 mL calibrated volumetric flasks, dissolved in 10 mL of water, and diluted to the mark level with the same solvent.

Stoichiometric Study. To study stoichiometry of the reaction, Job’s method of continuous variation was followed [31]. Equimolar solution (1 mMol) of each of the coloring reagents and drugs were prepared. In a series of 10 mL calibrated amber volumetric flasks, solutions of PG and TXA were prepared individually, comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, ... 9:1) each with both of the reagents (TNP and DNP) separately. After 10 minutes of reaction the absorbance of the complex was measured at the wavelength of maximum absorption against the reagent blanks treated similarly.

DNP Method. 3 mL of drug PG or TXA stock solution was taken individually in 10 mL amber volumetric flasks to
which 4 mL of the coloring reagent DNP was added. Sample was diluted with acetonitrile up to volume. Absorbance was measured after 10 minutes of reaction at 418 nm using appropriate blank treated similarly.

**TNP Method.** 3 mL of drug PG or TXA stock solution was taken individually in 10 mL amber volumetric flasks to which 4 mL of the coloring reagent TNP was added. Acetonitrile was added up to the mark level. Absorbance was measured after 10 minutes of reaction at 425 nm using appropriate blank treated similarly.

### 2.3.2. Procedures for Pharmaceuticals Formulation

Homogeneous samples were obtained for each drug molecule. Bulk homogeneous powder equivalent to 10 mg of pregabalin or tranexamic acid was dissolved in methanol by sonication for 15 min and shaking thoroughly for about 30–40 min. The samples were cooled down and diluted up to the mark level with methanol, mixed well, and filtered using a Whatman number 42 filter paper to give 100 μg mL⁻¹ of PG and TXA each, individually. Further dilution and reactions were continued as described above under general procedures.

### 2.3.3. Excipients Interference Evaluation

10 mg of each PG and 10 mg of TXA were spiked individually with common excipients like magnesium stearate, HPMC (hydroxypropyl methylcellulose), glucose, pyrrolidone, lactose, talc powder, and starch. It was further processed as described under general procedures. Samples were subjected to multiple analyses and percent recovery was calculated.

### 2.3.4. Reaction Mechanism

This reaction is based on the proton transfer reaction from Lewis acid such as TNP and DNP to Lewis base such as TXA and PG, which produces intensely yellow color ion-pair complex as depicted by Saito and Matsunaga [26]. Mechanistic view has been expressed in Figure 2.

### 2.3.5. Thermodynamic Studies

Standard free energy changes (ΔG°) and association constant (Kc) were determined for both of the methods to evaluate thermodynamic aspects. The reaction of TNP and DNP each with PG and TXA was investigated for the association constants by the application of Benesi-Hildebrand equation [32]:

\[
\frac{C_a}{A} = \frac{1}{\varepsilon} + \frac{1}{K_c \cdot \varepsilon} \cdot \frac{1}{C_b},
\]

where \( C_a \) and \( C_b \) are the concentrations of the drug (PG or TXA) and coloring reagent (DNP or TNP) respectively, \( A \) is the absorbance of the complex, \( \varepsilon \) is the molar absorptivity of the complex, and \( K_c \) is the association constant of the complex.

Utilizing the above equation, \( A \) was plotted against \( C_a \) in both cases and straight lines were obtained. Standard free energy changes were calculated by the following equation:

\[
\Delta G^° = -2.356RT \log K_c,
\]

where \( \Delta G^° \) is free energy change associated with complex (kJ mol⁻¹), \( R \) is the thermodynamic gas constant (1.987 cal mol⁻¹ deg⁻¹), \( T \) is the Kelvin temperature (273+°C), and \( K_c \) is the equilibrium constant.

### 2.4. Assay Validation

ICH guideline was followed for the validation purpose and various experiments were performed [33].

#### 2.4.1. Linearity

Linearity is the ability of method to show absorbance corresponding to the analytes concentration. Least square procedure was adopted to develop the regression equations which showed linear relation of the concentration of complex with absorbance, complying Lambert Beer’s law. Under these experimental set-ups, absorbance at the given
wavelength was found to vary directly with the concentrations of both the donor and the acceptor molecules.

2.4.2. Precision and Accuracy. Accuracy of the analytical method is that the parameter confirms that the test results obtained with the method are close to the true or accepted values, while precision is the reproducibility of test result when the same homogenous sample is subjected to multiple testing. Solutions containing three different concentrations of pregabalin and tranexamic acid were arranged and investigated in triplicate for accuracy and precision.

2.4.3. Specificity. Specificity is the quantification of the analytes in presence of component mixtures, excipients, and additives. The effect of common excipients and additives was evaluated by designing spiking experiments. Both TXA and PG were evaluated with common excipients at different concentration levels individually.

2.4.4. Limit of Detection (LOD) and Limit of Quantification (LOQ). Both reactions were evaluated for LOD and LOQ values. The empirical formulas $3\sigma/S$ and $10\sigma/S$ were used to establish LOD and LOQ for the method, where $S$ is the slope and $\sigma$ is the standard deviation of the response statistically inferred from calibration curve. A signal to noise ratio of 3:1 was defined for LOD and of 10:1 was defined for LOQ, respectively [33].

3. Results and Discussion

The reaction of coloring reagent TNP or DNP as Lewis acids with amino acid (PG and TXA) as Lewis base afforded intense yellow charge transfer complex. The intensity in color is attributed to the formation of phenolate ion [26] shown in reaction scheme in Figure 2.

3.1. Method Optimization. In order to achieve optimum experimental condition, various factors were evaluated including time, temperature, solvent of choice, and concentration of drugs and reagent. Those experimental factors which were affecting the absorbing abilities of the resulting complex were optimized to enhance selectivity and sensitivity. The reaction and stability of the complex were evaluated with respect to time in intervals at room temperature. Maximum absorbance was obtained in 5- to 10-minute time period and prolonged period of time did not affect the absorbance of the complex. However, a negative impact was noted when reaction mixture was heated especially beyond 60°C. Similarly, effect of coloring reagent was evaluated by adding it to the fixed concentration of drug substance. To 100 $\mu$g mL$^{-1}$ of drug substance, reagents TNP and DNP were added in the range from 1 to 9 mL. The amount of coloring reagent linearly increased the absorbance up to equimolar stage beyond which the absorbance remains constant at maximum absorbance, which corresponds to 4.0 mL of solution of each DNP and TNP. Different solvents systems including chloroform, acetone, 2-propanol, acetonitrile, and dichloromethane were evaluated for optimum results.

Water was used to prepare stock solution of PG and TXA because of the free solubility of the drugs and working range solutions were prepared in acetonitrile. It has been known that methanol interferes in charge transfer complex formation so acetonitrile was used in working range solutions in subsequent analysis [25]. Dichloromethane was found to be suitable for preparation of DNP and TNP solutions. The solutions were scanned in visible range (800 to 350 nm) by spectrophotometer using the corresponding blank. It was found that, for TNP complex, $\lambda_{max}$ was 425 nm, while for DNP it was 418 nm which justifies intensity in color of complex derived from TNP as compared to that of DNP as in Figure 3. In view of these results, all working solutions to reaction were prepared in acetonitrile in sequence of drug-reagent solvent, maintained at 25 ± 2°C. Absorbance is measured at 418 and 425 nm for DNP and TNP complexes, respectively, after 10 minutes of mixing.

3.2. Stoichiometry. Job’s continuous variations method suggested a 1:1 molar ratio for both reagents with both drugs. However, experimental design considered the drug molecules to be limiting reactants, so the chromogenic reagent was taken in slight excess in subsequent analysis in order to make the reaction drug concentration dependent and to counter any possible interference.

3.3. Thermodynamic Study of the Complex. Complexes were studied in detail by applying Bensi-hilbrand theory. Association constant ($K_a$) was in the range of $1.51\times10^3$ for DNP, while it was in the range of $2.74\times2.86\times10^3$ for TNP and standard free energy changes were $-2.336$ to $-2.358$ for complex derived from DNP and $-2.498$ to $-2.508$ for TNP-based complex. Results are portrayed in Table 1.

| Drug molecule | DNP | TXA | PG | TNP |
|---------------|-----|-----|----|-----|
| Molar absorptivity (Ma) | 1326.85 | 1337.35 | 1404.77 | 1418.36 |
| $K_a$ | $1.51 \times 10^3$ | $1.62 \times 10^3$ | $2.74 \times 10^3$ | $2.86 \times 10^3$ |
| $\Delta G$ | $-2.336$ | $-2.358$ | $-2.498$ | $-2.508$ |

Figure 3: Spectrum of complexes and blanks.
Standard free energy changes value indicates spontaneity of the reaction. High wavelength, high association constant, and low standard free energy changes with complex involving TNP as compared to DNP justify the resonance phenomenon in the phenolate ion derived from TNP compared to DNP [26].

3.4. Validation Studies. After development method validation studies were conducted in line with the above-mentioned protocol. Using linear regression equation, methods showed linear response in concentration range from 0.02 to 200 \( \mu \text{g/mL} \) with correlation coefficient of more than 0.9990 in both cases. In all the cases studied, Beer’s law was obeyed with small intercept values (−0.0028 to 0.0021). Slopes were found to be in the range of 0.01018 to 0.0146. Small intercept and insignificant variation of slope are attributes of the excellent response of the methods. Low LOQ values of (0.095–0.109) \( \mu \text{g/mL} \) explained sensitivity of the method as in Table 2. Obtained results in Tables 3 and 4 are very close to 100% label claim, indicating excellent accuracy, while very good precision is obvious from the % relative standard deviation which is less than 2. During spiking experiments no interference was found which indicated that none of those additives possess enough basicity to cause interference in analysis of TXA and PG. In addition, good % recoveries in common excipients spiked samples that ranged from 98.3 to 101.4% further confirmed the specificity of the projected methods.

3.5. Application of the Method in Pharmaceuticals. General procedures described above were followed to determine content of capsule dosage pharmulations for both of the drugs. The projected colorimetric methods based on charge-transfer complexes were applied to determine PG and TXA, along with the reference methods 9 and 15 for PG and TXA, respectively, to evaluate the test results. \( t \)-test and \( F \)-test were carried out with MS Excel, and the proposed methods were found to be comparable to the referenced methods and no considerable variation was found between them which indicated similar precision and accuracy. Results are presented in Table 5.

4. Conclusions

To estimate the quantity of tranexamic acid and pregabalin in commercial products, two simple and sensitive colorimetric methods were developed and validated. Picric acid and 2,4-dinitrophenol, the two coloring reagents, have been utilized to develop two simple, much more common but sensitive and selective visible range spectrophotometric methods for routine analysis of tranexamic and pregabalin in bulk raw material and finished or semifinished dosage form. The
suggested methods are superior to the already established spectrophotometric methods in terms of simplicity; t and F tests guaranteed high accuracy and precision. Hence, the proposed methods can be eagerly adopted by pharmaceutical quality control laboratories for routine quantitative analysis.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

References

[1] L. A. Houghton, C. Fell, P. J. Whorwell, I. Jones, D. P. Sudworth, and J. D. Gale, “Effect of a second-generation α₉δ ligand (pregabalin) on visceral sensation in hypersensitive patients with irritable bowel syndrome,” Gut, vol. 56, no. 9, pp. 1218–1225, 2007.

[2] S. I. Johannessen and T. Tomson, “Pharmacokinetic variability of newer antiepileptic drugs: when is monitoring needed?” Clinical Pharmacokinetics, vol. 45, no. 11, pp. 1061–1075, 2006.

[3] A. R. Gennaro, Remington. The Science and Practice of Pharmacy, Mack, Easton, Pa, USA, 1995.

[4] L. Good, E. Peterson, and B. Lisander, “Tranexamic acid decreases external blood loss but not hidden blood loss in total knee replacement,” British Journal of Anaesthesia, vol. 90, no. 5, pp. 596–599, 2003.

[5] M. I. Walash, F. F. Belal, N. M. El-Enany, and M. H. El-Maghrabe, “Utility of certain nuclophilic aromatic substitution reactions for the assay of pregabalin in capsules,” Chemistry Central Journal, vol. 5, no. 1, article 36, 2011.

[6] F. S. Hammad and O. M. Abdallah, “Optimized and validated spectrophotometric methods for the determination of pregabalin in pharmaceutical formulation using ascorbic acid and salicylaldehyde,” Journal of American Science, vol. 8, no. 12, pp. 118–124, 2012.

[7] H. Salem, “Analytical study for the charge-transfer complexes of Pregabalin,” E-Journal of Chemistry, vol. 6, no. 2, pp. 332–340, 2009.

[8] A. A. Gouda and Z. A. Malah, “Development and validation of sensitive spectrophotometric method for determination of two antiepileptics in pharmaceutical formulations,” Spectrochimica Acta—Part A: Molecular and Biomolecular Spectroscopy, vol. 105, pp. 488–496, 2013.

[9] H. M. Saleh, M. M. El-Henawee, G. H. Ragab, and O. F. Mohamed, “Spectrophotometric and spectrofluorimetric determination of pregabalin via condensation reactions in pure form and in capsules,” International Journal of Pharmaceutical, Chemical and Biological Sciences, vol. 4, no. 3, pp. 738–747, 2014.

[10] M. I. Walash, F. Belal, N. El-Enany, and M. H. El-Maghrabe, “Simple and sensitive spectrofluorimetric method for the determination of pregabalin in capsules through derivatization with fluorescamine,” Luminescence, vol. 26, no. 5, pp. 342–348, 2011.

[11] R. S. Gujral, S. K. M. Haque, and S. Kumar, “A novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine samples,” African Journal of Pharmacy and Pharmacology, vol. 3, no. 6, pp. 327–334, 2009.

[12] V. V. Vaidya, S. M. Yetal, S. M. N. Roy, N. A. Gomes, and S. S. Joshi, “LC-MS-MS determination of pregabalin in human plasma,” Chromatographia, vol. 66, no. 11-12, pp. 925–928, 2007.

[13] M. Dousa, P. Gibala, and K. Lemr, “Liquid chromatographic separation of pregabalin and its possible impurities with fluorescence detection after postcolumn derivatization with o-phthalaldehyde,” Journal of Pharmaceutical and Biomedical Analysis, vol. 53, no. 3, pp. 717–722, 2010.

[14] T. M. Ansari, A. Raza, and A.-U. Rehman, “Spectrophotometric determination of tranexamic acid in pharmaceutical bulk and dosage forms,” Analytical Sciences, vol. 21, no. 9, pp. 1133–1135, 2005.

[15] M. S. Arayne, N. Sultana, F. A. Siddiqui, A. Z. Mirza, and M. H. Zuberi, “Spectrophotometric techniques to determine tranexamic acid: kinetic studies using ninhydrin and direct measuring using ferric chloride,” Journal of Molecular Structure, vol. 891, no. 1–3, pp. 475–480, 2008.

[16] S. Agarwal and R. S. R. Murthy, “A novel and sensitive colorimetric method for estimation of tranexamic acid in bulk and in pharmaceutical dosage form,” International Journal of Research in Pharmaceutical and Biomedical Sciences, vol. 3, no. 2, pp. 947–950, 2012.

[17] E. A. Gadkariem, M. A. Mohamed, and M. A. A. Jabhir, “Assay of Tranexam acid via coupling with Ascorbic acid using kinetic methods,” Elixir Pharmacy, vol. 54, pp. 12331–12334, 2013.

[18] M. Y. Dhamra, “Spectrophotometric determination of tranexam acid by azo-dye formation-application to pharmaceutical preparations,” Journal of Education & Science, vol. 24, no. 3, pp. 21–33, 2011.

Table 5: F and t tests of the method.

| Taken (mg) | PG found (mg) | TXA found (mg) | PG found (mg) | TXA found (mg) | PG [9] found (mg) | TXA [15] found (mg) |
|-----------|--------------|----------------|--------------|----------------|------------------|-------------------|
| 100.1     | 100.2        | 100.6          | 101.6        | 100.7          | 100.3            | 101.1             |
| 101.1     | 99.8         | 100.3          | 98.6         | 99.4           | 100.8            | 99.8              |
| 100.2     | 100.1        | 99.8           | 100.9        | 100.3          | 99.7             | 101.2             |
| 100.4     | 100.2        | 99.6           | 99.1         | 98.7           | 99.3             | 100.7             |
| 100.3     | 100.6        | 100.6          | 98.4         | 97.9           | 99.5             | 100.4             |
| 99.6      | 100.5        | 101.4          | 97.5         | 98.1           | 100.5            | 100.3             |
| MEAN      | 100.23       | 100.38         | 99.35        | 99.13          | 100.01           | 100.58            |
| SD        | 0.288        | 0.646          | 1.576        | 1.184          | 0.601            | 0.527             |
| RSD       | 0.287        | 0.644          | 1.586        | 1.195          | 0.601            | 0.524             |
| t-Test    | 0.44         | 0.569          | 0.355        | 0.222          |                  |                   |
| F-Test    | 0.131        | 0.665          | 0.054        | 0.110          |                  |                   |
[19] J. F. Huertas-Pérez, M. Heger, H. Dekker, H. Krabbe, J. Lankelma, and F. Ariese, “Simple, rapid, and sensitive liquid chromatography-fluorescence method for the quantification of tranexamic acid in blood,” Journal of Chromatography A, vol. 1157, no. 1-2, pp. 142–150, 2007.

[20] G. Iskender and S. Atmaca, “Spectrofluorometric determination of tranexamic acid in pharmaceutical dosage forms,” Pharmazie, vol. 43, no. 4, p. 290, 1988.

[21] M. H. Abdel-Hay, S. M. Sabry, M. H. Barary, and T. S. Belal, “Spectrophotometric determination of bisacodyl and pirebidil,” Analytical Letters, vol. 37, no. 2, pp. 247–262, 2004.

[22] M. Y. El-Mammli, “Spectrophotometric determination of fluclavoxacillin in pharmaceutical preparations using some nitrophenols as a complexing agent,” Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 59, no. 4, pp. 771–776, 2003.

[23] E. Regulska, M. Tarasiewicz, and H. Puzanowska-Tarasiewicz, “Extractive-spectrophotometric determination of some phenothiazines with dipicyramine and picric acid,” Journal of Pharmaceutical and Biomedical Analysis, vol. 27, no. 1-2, pp. 335–340, 2002.

[24] I. Hussain, M. I. Tariq, and H. L. Siddiqui, “Structure elucidation of chromogen resulting from Jaffé’s reaction,” Journal of the Chemical Society of Pakistan, vol. 31, no. 6, pp. 937–948, 2009.

[25] N. Sultana, M. S. Arayne, and S. N. Ali, “Charge transfer complexes of gamma aminobutyric acid-analogue, a neurotransmitter: synthesis and spectrophotometric determination,” Journal of Bioanalysis & Biomedicine, vol. 528, no. 2, pp. 12–21, 2013.

[26] G. Saito and Y. Matsunaga, “Charge-transfer and proton-transfer in the formation of molecular complexes. I. The complex isomerization of some anilinium picrates by melting,” Bulletin of the Chemical Society of Japan, vol. 44, pp. 3328–3335, 1971.

[27] M. S. Refat, L. A. El-Zayat, and O. Z. Yeşilel, “Spectroscopic characterization of charge-transfer complexes of morpholine with chloranilic and picric acids in organic media: crystal structure of bis(morpholinium 2,4,6-trinitrocyclohexanolate),” Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 75, no. 2, pp. 745–752, 2010.

[28] M. S. Saleh, A. K. Youssef, E. Y. Hashem, and D. A. Abdel-Kader, “A novel Spectrophotometric method for determination of gabapentin in pharmaceutical formulations using 2,5-dihydroxybenzaldehyde,” Computational Chemistry, vol. 2, pp. 22–30, 2014.

[29] F. A. Siddiqui, M. S. Arayne, N. Sultana et al., “Spectrophotometric determination of gabapentin in pharmaceutical formulations using ninhydrin and π-acceptors,” European Journal of Medicinal Chemistry, vol. 45, no. 7, pp. 2761–2767, 2010.

[30] F. A. Siddiqui, N. Sher, N. Shafi, H. Shamshad, and A. Zubair, “Kinetic and thermodynamic spectrophotometric technique to estimate gabapentin in pharmaceutical formulations using ninhydrin,” Journal of Analytical Science and Technology, vol. 4, p. 17, 2013.

[31] Ph. Job, “Recherches sur la formation de complexes minéraux en solution, et sur le stabilité (formation and stability of inorganic complexes in solution),” Annali di Chimica, vol. 9, pp. 113–203, 1928.

[32] H. A. Benesi and J. Hildebrand, “A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons,” Journal of the American Chemical Society, vol. 71, no. 8, pp. 2703–2707, 1949.