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Research Article

Sensitive Spectrophotometric Determinations of Paracetamol and Protriptyline HCl Using 3-Chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one

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A new spectrophotometric method is developed for the determination of Paracetamol (PCT) and protriptyline HCl (PTP) in pure forms and in pharmaceutical formulations. The experiment involves the use of 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one as a novel chromogenic reagent for the determination of PCT and PTP. The method is based on the formation of charge transfer complex between the drugs and chromogenic reagent. Beer's law is obeyed in the concentration ranges 10.00–60.00 μg mL^{-1} for PCT at 545 nm and 40.00–160.00 μg mL^{-1} for PTP at 468 nm. The molar absorptivity, Sandell, sensitivity, and limit of detection and quantification are also calculated. The method has been successfully applied for the determination of both PCT and PTP in pharmaceutical samples with acceptable results.

1. Introduction

Paracetamol (PCT), *N*-(4-hydroxyphenyl)ethanamide is one of the most commonly used home medicine to reduce pain and fever [1] (Figure 1). These actions are known, respectively, as analgesic and antipyretic [2]. PCT is also used to treat headache, muscle ache, arthritis, backache, toothache, cold, and fever. An overdose of PCT may cause some toxic effects such as fulminating hepatic necrosis, which causes about 450 deaths in the USA every year [3]. Thus, the development of simple methods for the determination of PCT is obviously significant in pharmaceuticals.

Protriptyline HCl (PTP) is one of the tricyclic antidepressant [4], chemically known as *N*-methyl-5*H*-dibenzo[a,d]-cycloheptene-5-propanamine hydrochloride (Figure 2). It works by blocking the transporters responsible for reuptake of neurotransmitters like norepinephrine and serotonin [5]. This medication is primarily used to treat the anxiety, depression, bipolar disorder, and obsessive-compulsive disorder [6]. They are also known as effective analgesic used for the treatment of chronic pain especially neuropathic or neuralgic pain [7].

A number of methods are available in the literature for the determination of PCT, such as high-performance liquid chromatography [8–10], Chemiluminescence [11], Fluorescence [12], pulse perturbation technique [13], Spectrofluorimetric [14], and several others. A very few methods are reported in the literature for the determination of PTP in pharmaceutical formulations which include high performance liquid chromatography [15–17], Liquid chromatography [18], and flow injection technique [19].

Above mentioned techniques are not widely used in the basic clinical laboratories, because they require more expensive equipments and complicated sample preparation. All the reported methods require expensive reagents and are time consuming. Literature search has revealed that only a few visible spectrophotometric methods [20–23] are available for the determination of PCT and PTP in bulk drug and pharmaceutical formulations. UV-VIS spectrophotometry is a simple analytical method for quantitative analysis which provides practical and significant economic advantages over other methods. Therefore, they are of frequent choice for pharmaceutical analyses. Thus, there is a lot of scope for development of simple and suitable analytical spectrophotometric

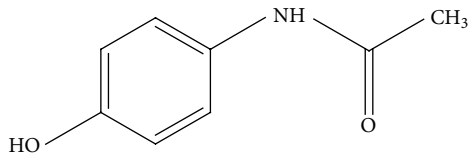


Figure 1: Paracetamol.

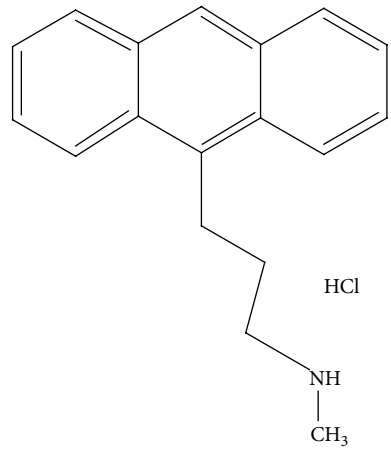


Figure 2: Protriptyline HCL.

method for the determination of drugs in pure and pharmaceutical formulations.

In the present work we report 3-chloro-7-hydroxy-4-methylcoumarin as a novel reagent for spectrophotometric determination of PCT and PTP in pure and pharmaceutical formulations (Tablets). The main advantage of the developed method is being simple and sensitive and involving rapid reaction of PCT and PTP with 3-chloro-7-hydroxy-4-methylcoumarin.

2. Materials and Methods

2.1. Apparatus. A UV-Visible spectrophotometer (SHIMADZU, UV 2550) with 1 cm quartz cells was used for the absorbance measurements.

2.2. Reagents. All solutions were prepared with double distilled water. Chemicals used were of analytical reagent grade. Solution of 3-chloro-7-hydroxy-4-methylcoumarin (4.7483×10^{-4}) was prepared by dissolving 500 mg in 100 mL ethanol and NaOH (2 M) was prepared by dissolving 8 g in 100 mL of water. An accurately weighed amount of pure PCT and PTP was dissolved in ethanol and diluted stepwise to get working concentration.

2.3. Analytical Method. Aliquots of PCT (10.00–60 μg mL^{-1}), and PTP (40.00–160 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks. To each flask, 0.5 mL of reagent and 0.5 mL of NaOH were added and heated at 40°C for 10 minutes and cooled to room temperature. Afterwards reaction mixture was diluted to 10 mL by using ethanol. Obtained violet colored adduct of PCT and brown colored adduct of PTP were measured at 545 and 468 nm, respectively, against the reagent blank.

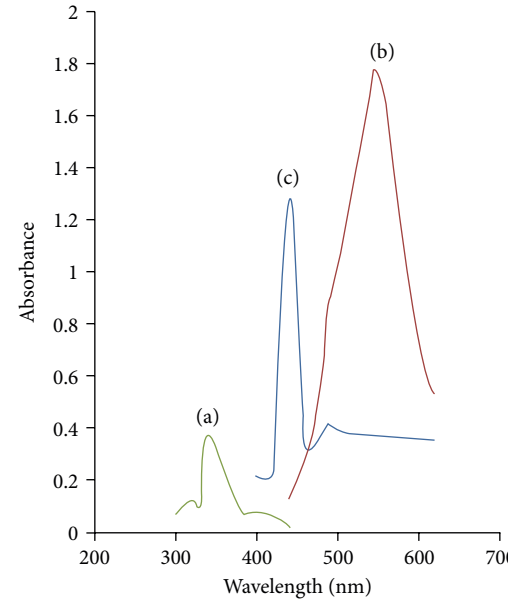


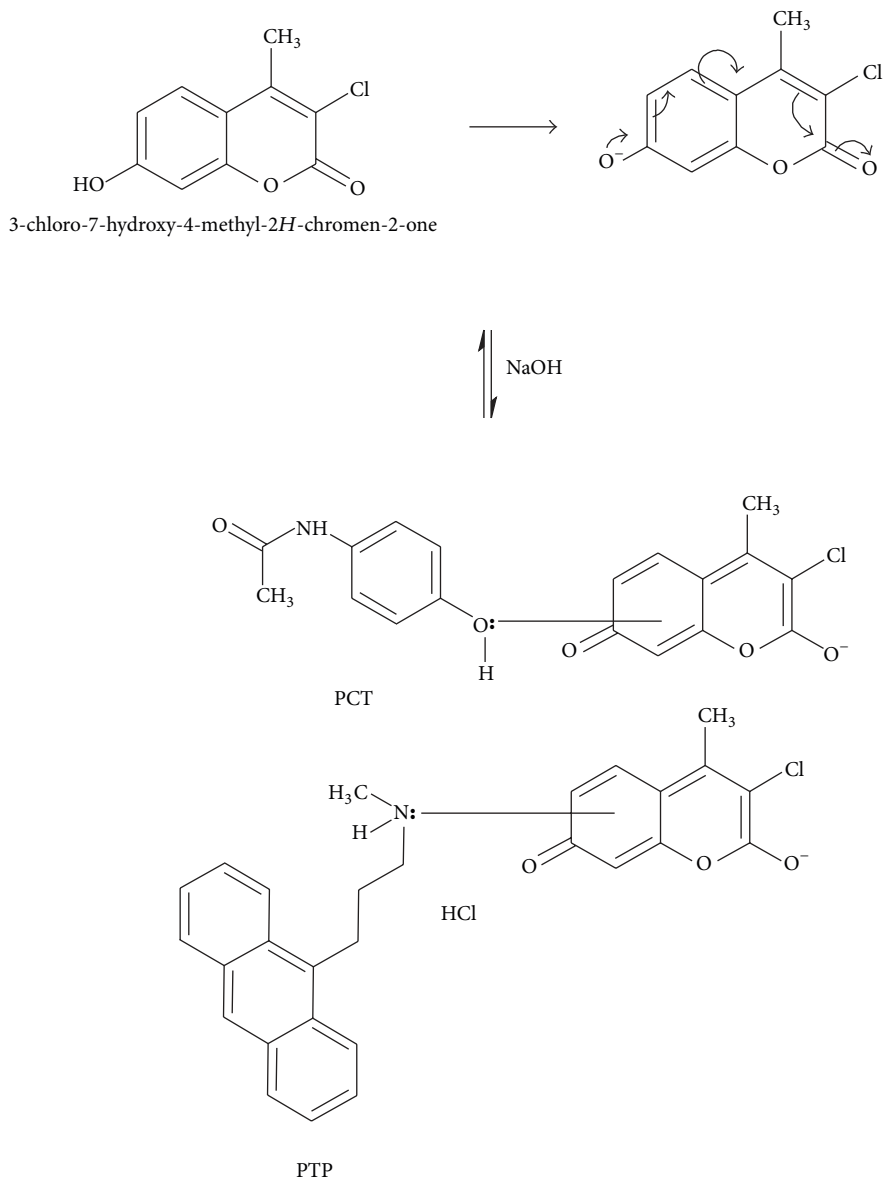
Figure 3: Absorption maximum for (a) blank, (b) PCT, and (c) PTP.

2.4. Preparation of Pharmaceutical Formulation. Commercial tablets of PCT (Paracetamol 500 mg) and PTP (Vivactil 10 mg) were analyzed by the proposed method. Both the tablets were crushed separately in a mortar and dissolved in ethanol; the solution was filtered through Whatman filter paper no. 41 and diluted quantitatively with ethanol to obtain a suitable concentration for the analysis. A convenient aliquot was then subjected to the analysis by using proposed method.

3. Results and Discussion

In order to establish the experimental conditions, the effect of reagent concentrations and temperature and the evaluation of the time required to complete the reaction with respect to maximum sensitivity have been studied. 0.5 mL of reagent and 0.5 mL of NaOH were found to be optimum for maximum absorbance for both the drugs. It was also found that the absorbance reaches its maximum after heating for 10 minutes at 40°C. Characterization of the reaction was carried out by the evaluation of reaction stoichiometry. The reaction proceeds with the formation of highly stable violet colored adduct for PCT and brown colored adduct for PTP (Scheme 1). The absorption spectrum of the colored products is shown in Figure 3.

3.1. Stoichiometry of the Reaction Product. Job's method of continuous variation is an effective approach to study the stoichiometry of the chemical reaction [24]. In this method, the total molar concentration of the two species (i.e., drug and reagent) is held constant, but their mole fractions are varied. As shown in Figure 4, the molar ratio which gave maximum



Scheme 1: Reaction of PCT and PTP with 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one.

absorbance is found to be 1 : 1 (drug : reagent). In view of this result a reaction mechanism is proposed considering the transfer of free electron of the (oxygen atom present in one molecule of PCT and nitrogen atom present in one molecule of PTP) drugs to the charge-deficient center of reagent molecule.

3.2. Method Validation

3.2.1. Spectral Characteristics and Sensitivity. The absorption spectra of the violet colored product with λ_{max} of 545 nm and brown colored product with λ_{max} of 468 nm were used in the studies of PCT and PTP, respectively. The reagent blank practically showed negligible wavelength (Figure 3). Under the experimental conditions, absorbance is linearly proportional to concentration over the range of 10.00–60.00 μg mL^{-1} for PCT (Figure 5) and 40.00–160.00 μg mL^{-1} for PTP (Figure 6). Slope, intercept, and correlation coefficient are obtained by the method of least squares which is described by the equation: $Y = a + bX$ (where Y = absorbance, a = intercept, b = slope and X = concentration in μg mL^{-1}). Regression analysis results are summarized in Table 1.

The limit of detection (LOD) and the limit of quantification (LOQ) are calculated as per the ICH guidelines [25] from the expression LOD = 3.3σ/S and LOD = 10σ/S (σ = standard deviation of the residuals, S = slope of calibration curve). The calculated values are given in Table 2.

3.2.2. Accuracy and Precision. In order to determine the accuracy and precision, recovery studies are carried out by standard addition technique at three different concentrations (10, 20, and 30 μg mL^{-1} for PCT and 40, 60, and 80 μg mL^{-1}

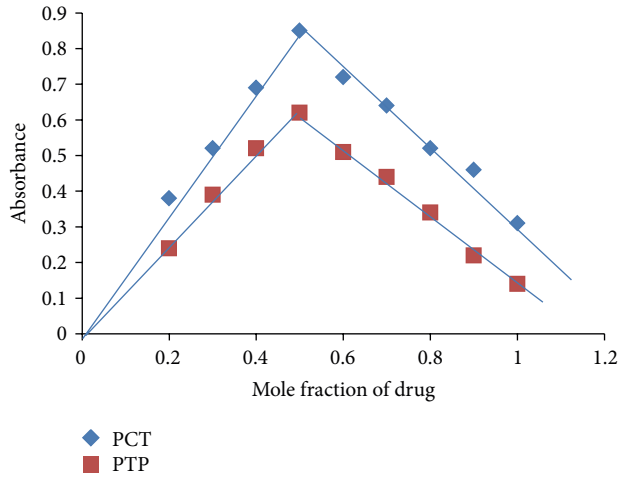


Figure 4: Application of Job's method to the reaction between reagent and the studied drugs.

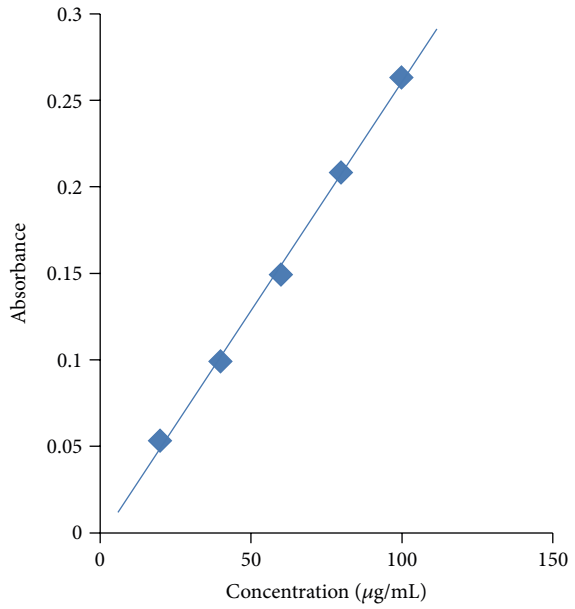


Figure 5: Calibration curve for PCT.

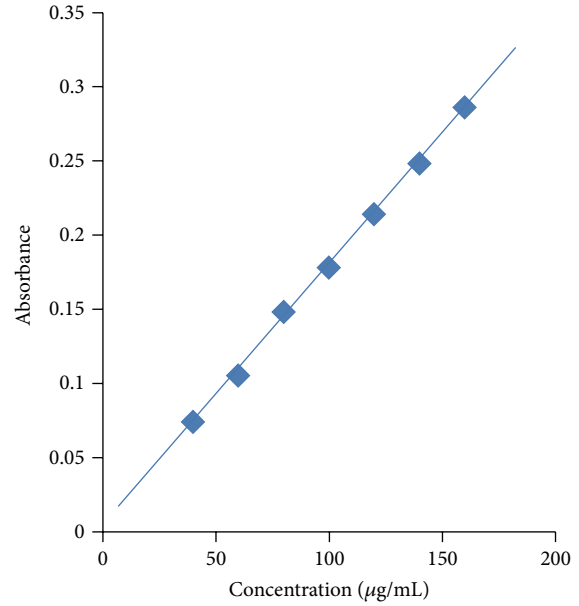


Figure 6: Calibration curve for PTP.

Table 1: Spectral and statistical data for the determination of drugs.

Parameters	Drugs	
	PCT	PTP
λ_{max} (nm)	545	468
Beer's law limits (μg/mL)	10.00–60.00	40.00–160.00
Molar absorptivity ($L\,mol^{-1}\,cm^{-1}$)	0.12×10^4	0.55×10^4
Sandell's sensitivity ($\mu g\,cm^{-2}$)	0.11×10^{-2}	0.54×10^{-2}
Limit of detection* ($\mu g\,mL^{-1}$)	0.0578	0.1527
Limit of quantification* ($\mu g\,mL^{-1}$)	0.1753	0.5840
Regression equation**	$Y = a + bX$	$Y = a + bX$
Slope (b)	0.0065	0.0017
Intercept (a)	0.0280	0.0025
Correlation coefficient (r)	0.9963	0.9994

*Limit of detection calculated according to ICH guidelines.
**Y is the absorbance and X concentration in $\mu g\,mL^{-1}$.

for PTP). Accuracy is calculated as the percentage recoveries of the drugs in their pure form. Precision of the methods is assessed as RSD % at different levels. Obtained results are found to be less than 3% and are acceptable. Results are given in Table 2.

3.2.3. Interference Study. The specificity of the method is indicted by the interference study. It is found that presence of some common excipients (starch, lactose, citric acid, and sodium carbonate) will not cause any analytical problem, thus proposed method can be successfully applied for the determination of PCT and PTP in various pharmaceutical formulations.

4. Analytical Application

The developed methodology is very adequate for the determination of PCT and PTP in pure form and in pharmaceutical formulation (Paracetamol 500 mg and Vivactil 10 mg). Moreover, the proposed procedure is very economical when compared to other methods. The performance of proposed method is assessed by calculation of Student's t-test at 95% confidence limits for four degrees of freedom and also by the recovery studies. The results given in Table 3 reveal that the calculated t values for both the drugs are less than theoretical values and are equally accurate.

Table 2: Evaluation of accuracy and precision.

Amount taken (μg mL^{-1})	Amount found* (μg mL^{-1})	RE (%)	SD (μg mL^{-1})	RSD (%)
PCT				
10.00	9.88	1.20	0.15	1.50
20.00	19.54	2.30	0.26	1.33
30.00	29.91	0.46	0.27	0.46
PTP				
40.00	39.37	1.57	0.74	1.87
60.00	59.60	0.66	0.61	1.02
80.00	79.83	0.21	1.30	1.74

*Mean value of five determinations.
RE: relative error; SD: standard deviation; RSD: relative standard deviation.

Table 3: Result of assay of formulation by the proposed method.

Drug	Trade name	Labeled amount (mg)	Found ± SD using
Paracetamol	Paracetamol	500	506.76 ± 0.46 $t = 0.53$
Protriptyline HCl	Vivactil	10	10.05 ± 0.73 $t = 0.15$

*Mean value of five determinations.
Tabulated t value at 95% confidence level is 2.7.

5. Conclusions

The proposed spectrophotometric method is simple, sensitive, and easily applicable for the determination of PCT and PTP. Developed method require the minimum chemicals, is easy to perform, and does not contain any intermissive experimental variables, which affect the reliability of the results. These characteristics make the proposed method very suitable for the analysis of PCT and PTP in quality control laboratories.

Acknowledgments

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References

[1] T. Németh, P. Jankovics, J. Németh-Palotás, and H. Koszegi-Szalai, "Determination of paracetamol and its main impurity 4-aminophenol in analgesic preparations by micellar electrokinetic chromatography," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 47, no. 4-5, pp. 746–749, 2008.

[2] A. Afkhami, N. Sarlak, and A. R. Zarei, "Spectrophotometric determination of salicylamide and paracetamol in biological samples and pharmaceutical formulations by a differential kinetic method," *Acta Chimica Slovenica*, vol. 53, no. 3, pp. 357–362, 2006.

[3] Á. N. Mhaoláin, B. D. Kelly, E. G. Breen, and P. Casey, "Legal limits for paracetamol sales," *The Lancet*, vol. 369, no. 9570, p. 1346, 2007.

[4] American Society of Health-System Pharmacists, AHFS Drug Information, 2002.

[5] F. Sériès and Y. Cormier, "Effects of protriptyline on diurnal and nocturnal oxygenation in patients with chronic obstructive pulmonary disease," *Annals of Internal Medicine*, vol. 113, no. 7, pp. 507–511, 1990.

[6] Ultram, Protriptyline, Ortho-McNeil Pharmaceutical, 2007.

[7] J. Kirchheiner, K. Nickchen, M. Bauer et al., "Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response," *Molecular Psychiatry*, vol. 9, no. 5, pp. 442–473, 2004.

[8] M. Kartal, "LC method for the analysis of paracetamol, caffeine and codeine phosphate in pharmaceutical preparations," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 26, no. 5-6, pp. 857–864, 2001.

[9] S. Grujić, T. Vasiljević, and M. Laušević, "Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography-ion trap-tandem mass spectrometry," *Journal of Chromatography A*, vol. 1216, no. 25, pp. 4989–5000, 2009.

[10] M. E. El-Kommos and K. M. Emara, "Determination of phenyltoloxamine salicylamide, caffeine, paracetamol, codeine and phenacetin by HPLC," *Talanta*, vol. 36, no. 6, pp. 678–679, 1989.

[11] W. Ruengsitagoon, S. Liawruangrath, and A. Townshend, "Flow injection chemiluminescence determination of paracetamol," *Talanta*, vol. 69, no. 4, pp. 976–983, 2006.

[12] J. C. L. Alves and R. J. Poppi, "Simultaneous determination of acetylsalicylic acid, paracetamol and caffeine using solid-phase molecular fluorescence and parallel factor analysis," *Analytica Chimica Acta*, vol. 642, no. 1-2, pp. 212–216, 2009.

[13] N. Pejić, L. Kolar-Anić, S. Anić, and D. Stanisavljev, "Determination of paracetamol in pure and pharmaceutical dosage forms by pulse perturbation technique," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 2, pp. 610–615, 2006.

[14] K. W. Street Jr. and G. H. Schenk, "Spectrofluorometric determination of acetylsalicylic acid, salicylamide, and salicylic acid as an impurity in pharmaceutical preparations," *Journal of Pharmaceutical Sciences*, vol. 70, no. 6, pp. 641–646, 1981.

[15] U. Huber, *Analysis of Tricyclic Antidepressants By HPLC*, Agilent Technologies, 1998.

[16] P. Koteel, R. E. Mullins, and R. H. Gadsden, "Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum," *Clinical Chemistry*, vol. 28, no. 3, pp. 462–466, 1982.

[17] S. J. Bannister, V. D. W. S. van der Wal, J. W. Dolan, and L. R. Snyder, "Liquid-chromatographic analysis for common tricyclic antidepressant drugs and their metabolites in serum or plasma with the technicon "FAST-LC" system," *Clinical Chemistry*, vol. 27, no. 6, pp. 849–855, 1981.

[18] F. A. Beierle and R. W. Hubbard, "Liquid chromatographic separation of antidepressant drugs: I. Tricyclics," *Therapeutic Drug Monitoring*, vol. 5, no. 3, pp. 279–292, 1983.

[19] M. E. Georgiou, C. A. Georgiou, and M. A. Koupparis, "Rapid automated spectrophotometric competitive complexation studies of drugs with cyclodextrins using the flow injection gradient technique: tricyclic antidepressant drugs with α-cyclodextrin," *Analyst*, vol. 124, no. 3, pp. 391–396, 1999.

[20] A. Ruiz Medina, M. L. Fernández de Córdova, and A. Molina Díaz, "A very simple resolution of the mixture paracetamol and salicylamide by flow injection-solid phase spectrophotometry," *Analytica Chimica Acta*, vol. 394, no. 2-3, pp. 149–158, 1999.

[21] G. V. S. R. Kumar, V. R. Devi, K. V. D. Lakshmi, and L. R. Bs Murty, "Detection and spectrophotometric determination of paracetamol using NBS," *Analytical Chemistry*, vol. 12, no. 2, pp. 62–65, 2013.

[22] L. Nejem, S. Antakli, and H. Bagdashe, "Spectrophotometric determination of paracetamol and orfinadrin citrate in tablets," *Asian Journal of Chemistry*, vol. 2, no. 25, pp. 1079–1082, 2013.

[23] K. Divya and B. Narayana, "New visible spectrophotometric methods for the determination of protriptyline HCl in bulk and pharmaceutical formulations," *Journal of Chemical and Pharmaceutical Research*, vol. 4, no. 9, pp. 4352–4358, 2012.

[24] G. D. Christian and J. E. O'Rilly, "Jobs method," in *Instrumental Analysis*, Prentice Hall, Upper Saddle River, NJ, USA, 2nd edition, 1980.

[25] International Conference on Harmonization (ICH) Guidelines, http://www.ich.org/.