Journal of Photochemistry & Photobiology A: Chemistry 368 (2019) 248–253

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

# Development of a fluorimetric method for assessing paracetamol in pharmaceuticals tablets

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ARTICLE INFO

*Key words:*
Paracetamol
Fluorimetric method
Oxidation
Fluorescence quenching

ABSTRACT

A simple, sensitive, quick, cost effective, and reliable fluorimetric method for the determination of paracetamol has been developed. The method was based on oxidation of paracetamol by potassium chlorate in the presence of sulphuric acid followed by monitoring of the fluorescence quenching of quinine sulphate by chloride ions at the excitation wavelength of 360 nm and emission wavelength of 446 nm. The fluorescence emission was corrected with a correction factor using absorbance for every sample. All absorbances and emissions were monitored by exiting samples at $\lambda$ = 360 nm with optimized conditions. There was a linear relationship between the quantum yield ratio and the paracetamol concentration, with a correlation coefficient of 0.996. The detection limit and quantification level were 56.0 ng $mL^{-1}$ and 189.0 ng $mL^{-1}$, respectively. The precision and accuracy of the method were satisfactory and the standard deviation of recovery was not more than 2%. The amount of paracetamol estimated by the developed fluorimetric method was compared with the standard method. The percentage of weights recovery of standard paracetamol from the fluorimetric method were found to be 98 to 102% suggesting the high reliability of the developed method.

## 1. Introduction

Acetaminophen (N-acetyl-4-aminophenol) is the main active ingredient in pharmaceutical formulations of paracetamol. Acetaminophen appears as a white, odorless and crystalline powder possessing a bitter taste which is commonly used as an analgesic and antipyretic drug [1]. It is extensively used in the treatment of moderate pains such as headaches and minor body aches. Paracetamol might also be used to manage more severe pain such as post-surgical pain. Although paracetamol is used to treat inflammatory pain, it exhibits only weak anti-inflammatory activity [2]. It is available as an over the counter drug in worldwide. Eventhough, the paracetamol is a very safe analgesic at therapeutic doses, overdosing may lead to hepatic necrosis or renal failure [3]. Therefore, rapid determination of paracetamol content in pharmaceutical formulations and in biological fluids (overdose monitoring) is very important [4]. Paracetamol consists of a benzene ring core-substituted by a hydroxyl group and an amide group in the para position resulting a conjugated system as shown in Fig. 1.

The therapeutic importance of this type of drugs has promoted the various analytical methods for its quantitative determination of active ingredient. These methods include micellar liquid chromatography [5], high performance liquid chromatography (HPLC) [6–22], capillary electrophoresis [23–26], radioimmunoassay [26], and electrochemical analysis which have been recently reviewed [27]. Most of these methods were developed based on body fluids such as whole blood [6], red blood cells [7], plasma [8–10], serum [11], and urine [12]. The most recent methods have been applied for paracetamol determination in pharmaceuticals is chromatographic techniques [19,7–22]. However, these procedures lack sensitivity [19] besides being tedious and requiring selective and expensive detectors which may not available in the laboratories [21]. HPLC and Gas Chromatography (GC) methods have good accuracy and precision but they are time consuming. Further, it requires derivatization of paracetamol and the use of costly, highly specialized instruments [2–4]. However, Fluorimetric and spectrophotometric methods are considered to be more convenient alternative technique because of their simplicity and high sensitivity. Few fluorimetric and spectrophotometric methods have been reported [28–34] for the determination of these types of drugs in their pharmaceutical dosage forms. Unfortunately, these methods need laborious time and require derivatization of drugs. Hence, the main aim of this research is to develop a new simple, reliable, quick, sensitive, cost effective fluorimetric method to overcome the difficulties in analyzing paracetamol in pharmaceuticals dosage forms.

Paracetamol is not fluorescent by itself. Potassium chlorate was

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https://doi.org/10.1016/j.jphotochem.2018.09.039
Received 2 August 2018; Received in revised form 21 September 2018; Accepted 23 September 2018
Available online 29 September 2018

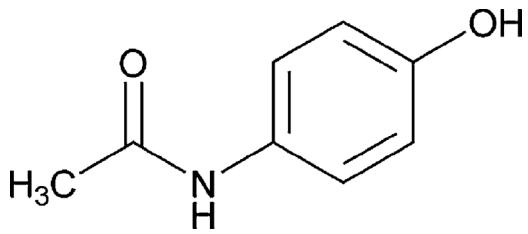


**Fig. 1.** Structure of paracetamol (Acetaminophen).

used as an oxidizing agent. During the oxidation reaction, potassium chlorate produces chloride ions which can quench the fluorescence emission of quinine sulphate. This study focuses a fluorimetric method measuring fluorescence quenching of quinine sulphate by chloride ions yielded by the oxidation reaction of paracetamol by potassium chlorate in acidic medium at $\lambda_{excitation}$ 360 nm and $\lambda_{emission}$ 445 nm.

## 2. Experimental

### 2.1. Instrumentation

FL 2700 fluorimeter (Hitachi- Japan) with 1-cm quartz cells were used for all measurements. The slit width of excitation and emission monochromators were set to be 5 nm. The calibration and linearity of the instrument were frequently monitored with standard quinine sulphate (10 ng $mL^{-1}$). Wavelength calibration was performed by measuring $\lambda_{excitation}$ at 360 nm and $\lambda_{emission}$ at 445 nm. No variation in wavelength was observed. Absorption spectra were measured with an UV spectrophotometer, UV2900 (Hitachi-Japan). HPLC measurements were carried out with HPLC Jasco 802 SC (Hitachi, Japan). Data were recorded using Clarity light software. The purity determination was performed on a stainless steel column which is 150 mm long with an internal diameter of 4.6 mm, filled with octadecylsilane chemically bonded to porous silica particles of 5 mm diameter ($C_{18}$, 150 mm × 4.6 mm, Shimadzu, Japan). The oven temperature was set at 50 °C. Pump was in isocratic mode and the solvent system was Methanol: Water (20:80). The flow rate was kept at 1 ml/min, and the elution was monitored at 257 nm by UV visible detector.

### 2.2. Materials, reagents and solutions

All the chemicals used were of analytical grade. Standard paracetamol, quinine sulphate, methanol, sulphuric acid and potassium chlorate were from Sigma Aldrich Co. Ltd. 2 L of 1 mol $L^{-1}$, L of 0.10 mol $L^{-1}$. A concentration of 1.0 mol $L^{-1}$ sulphuric acid was prepared by dilution of concentrated sulphuric acid with double distilled water. Potassium chlorate was prepared by dissolution of Potassium chlorate in 1.0 mol $L^{-1}$ sulphuric acid and the concentrations ranged from 0.10 to 0.50 mol $L^{-1}$. Methanol: water (20:80) mixture (mobile phase) was prepared by adding 300 ml methanol and 1200 ml of double-distilled water into a 2000 ml beaker (and sonicating for ten minutes. Quinine sulphate solution was prepared by dissolving 79.1 mg of quinine sulphate in 1.0 mol $L^{-1}$ sulphuric acid and made up to 100.0 ml with same acid. The concentration of the final solution was $1.0 \times 10^{-4}$ mol $L^{-1}$. 20.0 ml of this solution was diluted until the concentration was $1.0 \times 10^{-6}$ mol $L^{-1}$ with 1.0 mol $L^{-1}$ sulphuric acid.

### 2.3. Preparation of samples for analysis

#### 2.3.1. Preparation of samples for HPLC study

From the standard (authentic) paracetamol powder sample, 21.0 mg was weighed and transferred into a 50.0 ml volumetric flask. Then it was topped up with the mobile phase, and sonicated for ten minutes to dissolve the drug molecules. A series of standards were prepared using this solution, with the concentration range of 8.4 μg $mL^{-1}$ to 42.0 μg $mL^{-1}$

From the commercially available paracetamol, ten tablets were weighted, and finely powdered. 20 mg of paracetamol powder was weighed from each sample and then transferred into a 50 ml volumetric flask. Mobile phase was added to make up the volume and sonicated for 5 minutes for the drug molecule to dissolve. Thereafter, each solution was filtered into clean beakers. From each filtrate, 5.00 ml of the solution was taken into separate 50.0 ml volumetric flasks, and then mobile phase was added to make up the volume [18].

#### 2.3.2. Preparation of samples for fluorescence study

From the standard (authentic) paracetamol powder sample, 100.0 mg of paracetamol was weighed, transferred into a 100.0 ml round bottom flask, and diluted with 50 ml of 0.10 mol $L^{-1}$ potassium chlorate solution. The resulting solution was heated for 20 minutes at 90 °C. Then it was transferred into a 100 ml volumetric flask, and topped up with 1.0 mol $L^{-1}$ sulphuric acid. Finally it was filtered and allowed to reach the room temperature. Using standard paracetamol stock solution $1.0 \times 10^{-6}$ mol $L^{-1}$ quinine sulphate solution and 1.0 mol $L^{-1}$ sulphuric acidsulphuric acid solution, series of standard solutions were prepared.

Five brands were selected from the commercially available paracetamol, for the analysis. The indicated weights of all the brands were 500 mg, but the actual weights had small variation. Therefore, ten tablets each of the commercially available paracetamol were accurately weighed and crushed to a powder. Amount close to 100.0 mg was weighed, and dissolved in 50.0 ml of 0.10 mol $L^{-1}$ potassium chlorate in round bottomed flask. It was heated to 90 °C for 20 minutes, transferred to a 100.0 ml volumetric flask and made to the volume with 1.0 mol $L^{-1}$ sulphuric. Then, it was filtered into a 150 ml beaker. This was the stock solution for fluorimetric analysis. Same method was followed for all five commercially available samples of paracetamol

## 3. Results and discussion

Paracetamol was oxidized in acidic medium by potassium chlorate and the oxidation reaction yielded chloride ions quantitatively. In acidic medium, the resulting chlorides ions quenched the fluorescence emissions of quinine sulphate. Fig. 2 illustrates the reduction of fluorescence emission signals when the emissions were recorded with $\lambda_{excitation}$ 360 nm and $\lambda_{emission}$ 446 nm.

It is interesting to note that potassium chlorate is a strong oxidizing agent which has high reduction potential value in acidic medium. Even though it has a high reduction potential, kinetically it does not react spontaneously with paracetamol molecules. Therefore, the reaction medium has to heat to activate the reaction with paracetamol.

### 3.1. Optimization of reaction conditions

A series of experiments were carried out to identify the optimum experimental conditions to develop the fluorimetric method. Experimental conditions such as Concentration of potassium chlorate,

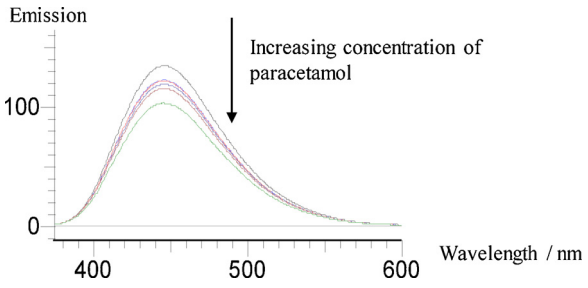


**Fig. 2.** Fluorescence quenching spectra for standard paracetamol solutions.

solvent used to dissolve regents, type of the acid and its concentration, heating temperature and heating time and concentration of quinine sulphate can influence the emission intensity. Hence, these variables were optimized by altering one variable at a time while keeping the rest constant.

### 3.1.1. Concentration of potassium chlorate

The effect of potassium chlorate was investigated by conducting a series of reactions using different concentrations while keeping other variables constant. The concentration range was $1.0 \times 10^{-2}$ mol $L^{-1}$ to 0.50 mol $L^{-1}$. When Potassium chlorate concentration is increased, the relative fluorescence intensity was decreased. This may be due to the fact that at very high concentrations, molecular collisions have quenched the fluorescence signal. Very low strength of Potassium chlorate was not sufficient to oxidize paracetamol quantitatively. Since the reaction stoichiometry was not established yet, the favorable concentration must be low and it must be in excess than the analyte. Further investigations were carried out using Potassium chlorate concentration of 0.10 mol $L^{-1}$ in acidic medium.

### 3.1.2. Solvents used

Since paracetamol is to a large molecule, it was difficult to dissolve in water. Therefore, suitability for the dissolution was checked with methanol water mixture, acetone water mixture, and acidic water mixture. Methanol and acetone has an ability to dissolve paracetamol tablet but not completely in water. A series of experiments were carried out with acetone and methanol water mixture by preparing paracetamol samples and blank samples to investigate the effect of solvents. However, it was found that Relative Fluorescent Intensity (RFI) was decreased when methanol concentration was increased in water. This may be due to methanol being oxidized with potassium chlorate in acidic medium. With heated acidic water, RFI did not change and small amounts of paracetamol can be completely dissolved in it. Therefore, further investigations were conducted with acidic double distilled water.

### 3.1.3. Acid type and its concentration

In order to determine the most suitable acid for the reaction, different acids (sulphuric and nitric) were tested. Hydrochloric acid was not tested because chloride ions ($Cl^-$) ions can quench the fluorescence of quinine sulphate. Nitric was not used for acidifying purpose because it may act as strong oxidizable acid when the reaction medium temperature is elevated. According to the literature [25], quinine sulphate exhibit fluorescence in protonated form as well as de-protonated form. However, in order to quench the photoactivity the protonated form is preferable. It can be protonated with sulphuric acid solution as described in previous work [25]. Since the oxidation reaction must be performed in acidic medium for the fluorescence measurements, 1.0 mol $L^{-1}$ sulphuric acid solution was used for further investigations.

### 3.1.4. Temperature and heating time

The effect of heating temperature and heating time on oxidation of paracetamol with potassium chlorate in 1.0 mol $L^{-1}$ sulphuric acid medium was studied by carrying out the reaction at room temperature, at temperatures ranged was 30 °C–100 °C. The results revealed that optimum temperature was 90 °C (Fig. 3).

The effect of heating time was studied between 5 minutes–60 minutes. Reaction is complete after 20 minutes (Fig. 4). Longer heating time and refluxing did not have a negative impact on the reaction. For precision consideration, 20 minutes was selected under reflux condition for further experiments.

### 3.1.5. Concentration of quinine sulphate

A series of experiment were carried out to determine the best concentration of quinine sulphate. The concentration range of quinine sulphate was $1.0 \times 10^{-5}$ mol $L^{-1}$ to $1.0 \times 10^{-8}$ mol $L^{-1}$ in 1 mol $L^{-1}$ sulphuric acid. When, concentration of quinine sulphate is increased, it was observed that the relative sensitivity gradually decreased, but when the concentration is decreased higher noise was observed. Therefore, it was identified that $1.0 \times 10^{-6}$ mol $L^{-1}$ as the optimum concentration for this work and used for further investigations.

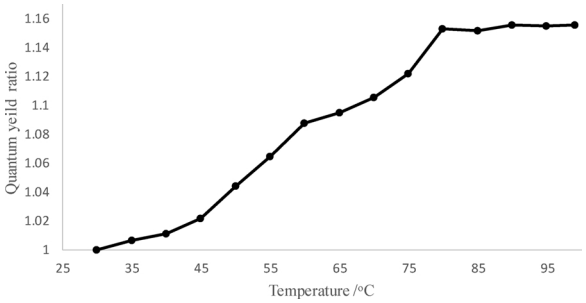


**Fig. 3.** Plot of variation of quantum yield ratio with reaction temperature.

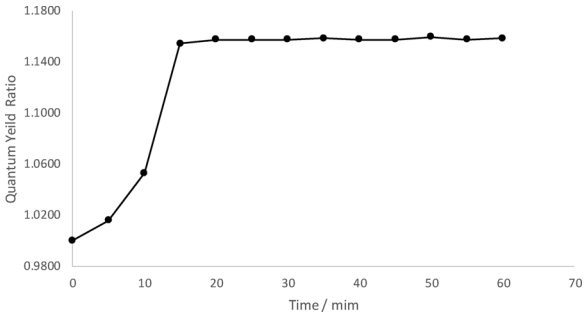


**Fig. 4.** Plot of quantum yield ratio with reaction time of standard paracetamol.

## 3.2. Validation of proposed method

### 3.2.1. Linearity, detection, quantification and Upper limits

Under the described optimum reaction conditions, the calibration curve for paracetamol oxidation was constructed by analyzing a series of standard solutions of paracetamol. In all cases, a linear relationship was found between measured values of quantum yield ratio and concentration of paracetamol. The regression equation for the results was derived using the least square method. The slope and intercept of the calibration curve was 0.0010 and 0.9809 respectively. The plots (n = 5) were linear with good correlation coefficients (0.9961 - 0.9946), in the general concentration range of 100–2500 ng $mL^{-1}$. The limits of detection (LOD) and limits of quantification (LOQ) were determined using the formula LOD or LOQ = $k(SD_a)/b$, where k = 3 for LOD and 10 for LOQ, $SD_a$ is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values were 56 ng $mL^{-1}$ and 189 ng $mL^{-1}$. Upper limits were detected under the optimum reaction conditions. It was observed that the upper limits of detection is 2274 ng $mL^{-1}$ with linear and good correlation. Therefore the best range of determination of paracetamol is 189–2274 ng $mL^{-1}$ under the optimum reaction conditions.

### 3.2.2. Accuracy and precision

Precision of the method was determined by assessing the reproducibility with preparation of blind samples. Also replicate analysis was done for ten separate samples of standard solutions at the concentration level of 1500 ng $mL^{-1}$. The relative standard deviations (RSD) were not more than 2%. This level of precision was suitable for quality control laboratories for the analysis of pharmaceutical dosages of paracetamol

Also, the accuracy and precision of the proposed method was determined by comparing the fluorimetric results with results obtained from the official method which was HPLC method for standard

**Table 1**
Recovery values of standard paracetamol by the proposed method.

| Sample No. | Weight of paracetamol/g | Average recovery /g (n = 3) | % Recovery | % Error |
|---|---|---|---|---|
| 1 | 0.0691 | 0.0690 | 99.9 | −0.1 |
| 2 | 0.0566 | 0.0580 | 102 | 2 |
| 3 | 0.1002 | 0.0986 | 98 | −2 |
| 4 | 0.1344 | 0.1320 | 98 | −2 |
| 5 | 0.1501 | 0.1495 | 99.6 | −0.4 |
| | | mean | 99.7 | |
| | | Standard deviation | 2 | |

paracetamol samples. From the calculation of absolute error and relative error, the accuracy of proposed method was high under the specified conditions. For the calculation, HPLC data were compared with fluorimetric data. The standard deviation of the percentage recovery was found to be more than 2%, (Table 1) and hence it has high precision.

Accuracy was determined by calculating relative error. It ranged from -2% to 2% (Table 1) and sample number 1, 3, 4 and 5 are under estimate and sample number 2 is over estimate. The low error indicates a higher accuracy of proposed method [28,29] (Fig. 5).

### 3.2.3. Interferences studies

Before proceeding with the analysis of commercially available paracetamol in pharmaceutical preparations, interference liabilities were examined to investigate the impact on the performance of the proposed method.

Chloride ions contained in many regent may interfere with the experimental results. Also, substances that are liable to be oxidized by potassium chlorate may interfere with the final results. By carrying out blank experiments along with the actual samples, the interferences can be minimized. This was checked by carrying out blank experiments at high concentrations of potassium chlorate, ranging from 0.10 mol $L^{-1}$ to 2.0 mol $L^{-1}$, and concentration of Potassium chlorate around 1.0 mol $L^{-1}$ or above, a negative impact was observed. Other common excipients such as titanium dioxide, Citric acid, stearic acid and potassium sorbate were spiked with pure paracetamol samples to examine the interference liabilities of the performance of proposed method. Samples were prepared by mixing known amounts of the excipients. It was found that no interference was observed from the tested excipients with the proposed method. The percentage of recovery values were 100.0–102 (Table 2) in the presence of common excipients. Although, the proposed method is based on an oxidation reaction, it is not selective. Obtained results of recoveries show that the suitability of the method for the analysis of paracetamol, since there was no interference from common excipients.

### 3.2.4. Robustness and ruggedness

Robustness was evaluated by influencing small variation in the

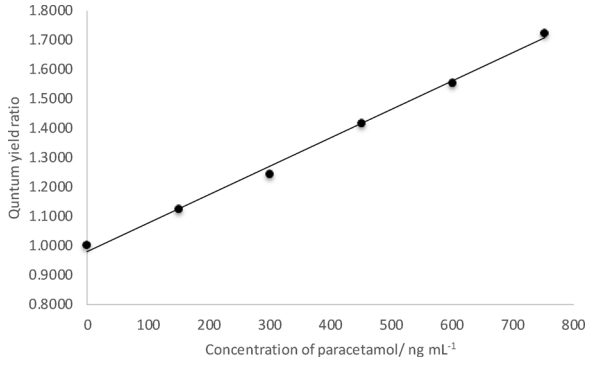


**Fig. 5.** Plot of quantum yield ratio vs. concentration of standard paracetamol.

**Table 2**
Analysis of paracetamol in the presence of common excipients.

| Common Excipients | Recovery (% = S.D.)[a] |
|---|---|
| Titanium dioxide (50)[b] | 100.0 ± 0.8 |
| Citric acid (50)[b] | 102 ± 1 |
| Stearic acid (50)[b] | 101 ± 1 |
| Potassium sorbate (50)[b] | 100.4 ± 0.6 |

[a] Values are mean of three determinations.
[b] Figures in parenthesis are the amounts of excipients added in mg per 100 mg of paracetamol.

**Table 3**
Influence of the small variation of the reaction parameters on the analysis of pure paracetamol by proposed method.

| Optimum Reaction Parameters | Reaction Parameters varied | Recovery (% ± S.D.)[a] |
|---|---|---|
| | No variation [b] | 99.3 ± 0.8 |
| **Potassium chlorate concentration** | | |
| 0.10 mol $L^{-1}$ | 0.08 mol $L^{-1}$ | 99.1 ± 0.6 |
| | 0.15 mol $L^{-1}$ | 100.0 ± 0.4 |
| **Sulphuric acid concentration** | | |
| 1.0 mol $L^{-1}$ | 0.5 mol $L^{-1}$ | 99.9 ± 0.8 |
| | 1.5 mol $L^{-1}$ | 100.1 ± 0.8 |
| **Quinine sulphate concentration** | | |
| $1.0 \times 10^{-6}$ mol $L^{-1}$ | $0.5 \times 10^{-6}$ mol $L^{-1}$ | 98.9 ± 0.3 |
| | $1.5 \times 10^{-6}$ mol $L^{-1}$ | 99.1 ± 0.5 |
| **Reaction time** | | |
| 20 minute | 15 minute | 99.4 ± 0.4 |
| | 25 minute | 99.2 ± 0.7 |
| **Reaction temperature** | | |
| 90 °C | 85 °C | 99.6 ± 0.4 |
| | 95 °C | 100.2 ± 0.9 |

[a] values are mean of three determinations ± S.D.
[b] the conditions were: Potassium chlorate concentration was 0.1 mol $L^{-1}$, sulphuric acid concentration was 1.0 mol $L^{-1}$ and quinine sulphate concentration was $1.0 \times 10^{-6}$ mol $L^{-1}$, reaction time was 20 min. and reaction temperature was 90 °C.

experimental parameters and observing analytical performance of the proposed method. The Table 3 indicates the reaction parameters that has been changed by small variations. During these experiments, one parameter was changed while the other parameters were kept constant at the optimum reaction conditions as shown in Table 3, and the recovery percentages were calculated at each time. It was observed that none of these variables significantly influence the performance of the proposed method. The recovery values were 98.9 - 100.2. These results indicate that the reliability of the proposed fluorimetric method throughout the routine analysis was acceptable. Ruggedness was tested by applying the proposed method to analysis of authentic paracetamol samples using blind sample preparation at two different laboratories, by two different persons and on different days. The results obtained from the two labs were reproducible and RSD value did not exceed 2%.

### 3.3. Assay of commercially available pharmaceuticals

The proposed method gave satisfactory results. Therefore, the different pharmaceuticals were subjected to analysis for their content of active ingredient by proposed method and official method which is already published [35]. Those results were compared with the results obtained from published method (standard method) by statistical analysis with respect to the accuracy (*t*-test) and precision (*F*-test). The recovery percentages found to be from 97.9 to 102 (Table 4) and no significant difference were observed between the calculated and theoretical values of both the official and proposed method at 95% confidence level. This shows almost similar accuracy and precision in the analysis of commercially available paracetamol by the proposed

**Table 4**
Comparison of weight recovered for commercially available paracetamol.

| Product | Weight recovered by proposed method ± SD[a] / mg | Weight recovery by official method ± SD[a] / mg | % Recovery ± SD[a] | *F* value[b] | *t* value[b] |
|---|---|---|---|---|---|
| Sample 1 | 486 ± 1 | 497 ± 1 | 97.9 ± 0.3 | 6.24 | −9.08 |
| Sample 2 | 473 ± 3 | 484 ± 2 | 98 ± 1 | 4.08 | −4.65 |
| Sample 3 | 402 ± 2 | 405 ± 1 | 100 ± 2 | 1.15 | −1.29 |
| Sample 4 | 438 ± 3 | 447 ± 1 | 97.9 ± 0.9 | 3.92 | −5.64 |
| Sample 5 | 420 ± 2 | 414 ± 1 | 102 ± 2 | 9.90 | −15.98 |

[a] Values of three determinations ± S.D.
[b] Theoretical values for *t* and *F* at 95% confidence limit (n = 3) were 2.78 and 19 respectively.

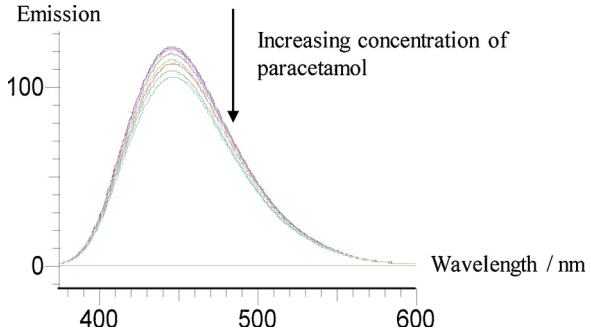


**Fig. 6.** Fluorescence quenching spectra of commercially available paracetamol.

method (Fig. 6).

## 4. Conclusion

The present study describes a very simple, rapid, cost effective, reliable and sensitive fluorimetric method for analysis of paracetamol content in pharmaceuticals. The method was based on oxidation of paracetamol (acetaminophen) by potassium chlorate in the presence of sulphuric acid, and subsequent monitoring of fluorescence quenching of quinine sulphate by chloride ions formed from the oxidation reaction, at 446 nm.

This method is reliable for the accurate determination of paracetamol in the absence of chloride ions and other reducing agents present in reagents. In an economical point of view, all the analytical regents are inexpensive and available in any analytical laboratory. Therefore, this method can be recommended for routine analysis in quality control laboratories.

## Acknowledgements

The authors thank Sri Lanka Pharmaceutical Cooperation, University of Colombo and Institute of chemistry Ceylon for facilitating to access all required instruments and commercial samples.

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