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Original Article

New and Highly Sensitive Spectrophotometric Method for the Determination of Paracetamol in Preformulation and Dosage Forms

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Abstract
A simple and highly sensitive spectrophotometric method for the determination of paracetamol has been developed. The employed methodology involves reaction of paracetamol with iron(III) and subsequent reaction with ferricyanide in acid medium, which yield a Prussian blue product with maximum absorption at 730 nm. Beer’s law was obeyed for the paracetamol concentration range from 0.20 - 3.00 μg ml⁻¹. The relative standard deviation (n = 8) was vary between 0.26 and 2.30 % for 1.0 μg ml⁻¹ paracetamol. The commonly encountered excipients and additives that accompany pharmaceutical preparations did not interfere with the determination. The method was applied for the determination of paracetamol in pharmaceutical preparations. This method is cheap, rapid, easy, accurate and precise which can be used for the routine estimation of paracetamol in pharmaceutical preparations.

Keywords: Paracetamol; Prussian Blue; spectrophotometry; pharmaceutical preparations.

1. Introduction
Paracetamol (PC) as a drug is known for effective analgesic and antipyretic properties. It is non-steroidal, anti-inflammatory and is extensively used for pain management [1-4]. Paracetamol is readily absorbed after administration and widely gets distributed throughout most of the body fluids. Its metabolic pathway comprises conjugation to from glucuronide and sulphate derivatives. About 90% of the therapeutic dose gets excreted along with the urine in 24 h as conjugated derivatives; 1 to 4 % of the excreted material being unchanged drug [5]. Paracetamol is prescribed for variety of patients including children, pregnant women and the elderly including those with osteoarthritis, simple headaches and non-inflammatory musculoskeletal condition [6]. The molecular structure of paracetamol reveals of lone pair of electrons of oxygen and nitrogen and aromatic ring which make it a potential electron donor [7]. This property is explored and forms the basis of spectrophotometric method. Development of new methods which are precise and sensitive for determining drug concentrations in pharmaceutical formulations is important. Based on the aforementioned observations the main objective was to carry out the development of more efficient analytical techniques, destined to quality control of one of the medicaments more widely used.

Contemporary analytical methodologies adopted to determine paracetamol in different matrices include electroanalytical techniques [8, 9], chromatographic methods [10, 11] and automated methods such as flow injection analysis [12, 13]. However, these methods lack specificity, sensitivity, simplicity and less time for analysis. Other methods to determine paracetamol both in pharmaceutical mixtures and biological fluids include continuous flow spectrophotometry, capillary electrophoresis [14], fluorimetry [15], gas chromatography [16], second derivative UV absorption’s spectroscopy [17, 18], atomic absorption spectroscopy [19], Raman spectra [20], chemiluminescence [21] and nuclear magnetic resonance mass spectroscopy [22]. Both the BP [23] and USP [24] recommend an HPLC method for the determination of paracetamol in pharmaceutical formulations; Chromatographic methods are preferred for identification of impurities in preformulations or metabolites in biological matrices rather than routine quantitative analysis. Further, they need specialized equipments and maintenance of which is costly which requires high sophistication and very expensive cost of equipment. Electroanalytical techniques have been used for the determination of a wide range of pharmaceutical preparations with advantages and in most cases, there is no
need for derivatization and these methods are less sensitive to matrix effects compared to other analytical techniques [25]. Direct UV–visible spectrometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, often demanding low-cost equipments. Up to now, several UV–visible spectrophotometric methods have been reported in the literature for the determination of PC. The majority published spectrophotometric methods are based on indophenol dye, and Schiff’s base formation, nitrosation and subsequent chelation, oxidation, oxidative coupling with some special reagent, UV absorption, pH-induced spectral changes [26–41]. Some of those are time-consuming, need heating for acceleration of the detection reaction, sometime auxiliary ligand, concentrated H2SO4 is necessary. All of the previously published methods are not sensitive enough to be used for paracetamol analysis [26–41]. Therefore an attempt was made to meet the ever-increasing demand for the stringent quality control in the pharmaceutical industries by developing simple, sensitive and selective spectrophotometric procedure for the determination of paracetamol in both preformulations and dosage forms. In the proposed procedure reduction of iron(iii) to iron(ii) takes place which reacts with ferricyanide and forms a Prussian blue product in acidic medium with maximum absorption at 730 nm. This method has distinct advantages of sensitivity and stability and does not require heating or distillation and is reliable due to reproducibility. Besides, the method offers clear advantages over most of the chromogenic reagents currently used for the purpose and the procedure has positive features over the existing methods. A comparison of the analytical features of the developed method with those of previously published methods for direct spectrophotometric determination of paracetamol are given in Table 1

2. Experimental
2.1. Apparatus
UV-Vis spectrophotometer UVIDEC-610 type with 1.0-cm matched cell (Jasco, Tokyo, Japan) was employed for measuring the absorbance.

2.2. Reagents
All chemicals and solvents used were of analytical grade. Double distilled water was used throughout. A stock solution of Paracetamol (SG Pharmaceutical, India), An accurate mass 5 mg of weighted paracetamol was transferred into 100 ml volumetric flask and dissolved in water to obtain a standard solution of 50 µg ml⁻¹. Aqueous solution of 0.1% (w/v) ferric chloride (BDH, India) containing few drops of 5M hydrochloric acid and 0.065% (w/v) potassium ferricyanide (BDH, India) was prepared in double distilled water; acetic acid (2M) was prepared in double distilled water.

2.3. Procedure
Aliquots of standard solutions of paracetamol (50 µg ml⁻¹) were transferred into 25-ml calibrated flask. Ferric chloride, potassium ferricyanide and acetic acid, each 1.0 ml were added to each flask, the contents were mixed well and kept aside for 15 min at room temperature. It was diluted to the mark with 1N hydrochloric acid. The absorbance of Prussian Blue color was measured at 730 nm against the corresponding reagent blank and calibration graphs were constructed.

2.4. Pharmaceutical preparations
Twenty tablets of analgesic and antipyretic drug were finely powdered in a small dish. Fifty mg of the powdered drug was dissolved in 20 ml of double distilled water and filtered through Whatman no 42 filter paper. The filtrate was made up to 100 ml with water in a volumetric flask. The filtrate was diluted with water to get 1.0 µg ml⁻¹ concentration. An aliquot of this solution was treated as described above.

3. Results and discussion
The determination of paracetamol involves the reaction of drug with ferric salt in the presence of potassium ferricyanide under acidic condition to produce a Prussian blue colored product with maximum absorption at 730 nm. The reaction involves the reduction of iron(iii) by paracetamol to iron(ii), which subsequently reacts with ferricyanide to give a Prussian blue (PB) colored product in acidic medium.

The factors affecting the color development such as reproducibility, sensitivity and adherence to Beer’s law were investigated.
3.3. Calibration ranges.

The linear calibration range for standard PC solutions from 0.2 to 3.0 \( \mu \text{g} \text{ml}^{-1} \). The regression curve obtained can be expressed by the following equation: \( \lambda_{730} \text{nm} = 0.1981C + 0.0345 \) where \( C \) is the concentration of PC in the final solution. The apparent molar absorptivity of PC was calculated from the slope of the calibration graph to be \( \epsilon = 3.60 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1} \). The limit of detection (LD) achieved for PC estimated by 3sB/m (where sB is the standard deviation of 8 measurements of the blank and m is the slope of the calibration line), was 0.05 \( \mu \text{g} \text{ml}^{-1} \) for the analyte with linear regression coefficient of 0.9996. The relative standard deviation for a 1.0 \( \mu \text{g} \text{ml}^{-1} \) PC (n = 8) was found to be vary between 0.26 and 2.30 %.

3.4. Color stability

Under the optimum reaction condition described the formation of the product in the proposed method was studied at different temperatures. The result indicated that the absorbance values remained constant in the temperature range of 10-90\(^{\circ}\)C. Beyond 90\(^{\circ}\)C the absorbance values decreased indicating the dissociation of the product formed on prolonged heating. The colored product was stable upto 24 h at room temperature.

3.5. Interference

Various additives and excipients that usually accompany paracetamol in pharmaceutical preparations were studied. Commonly encountered additives and excipients such as lactose, glucose, fructose, maltose, starch, dextrose, gum acacia, talc, and other additives used in combination with paracetamol - like substance including caffeine, ibuprofen,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Greenish blue</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>730</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>~24</td>
</tr>
<tr>
<td>Beer's law (( \mu \text{g} \text{ml}^{-1} )) range</td>
<td>0.2 - 3.0</td>
</tr>
<tr>
<td>Molar absorptivity ( \epsilon ) (L mol(^{-1}) cm(^{-1}))</td>
<td>( 3.60 \times 10^4 )</td>
</tr>
<tr>
<td>Sandell’s sensitivity (( \mu \text{g} \text{cm}^{-2} ))</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Regression equation:

\[ \text{Slope}(a) = 0.198 \pm 0.00365 \]
\[ \text{Intercept}(b) = 0.0346 \pm 0.04747 \]
\[ \text{Correlation coefficient} = 0.9996 \]

\[ \text{Limit Of Detection (LOD) \( \mu \text{g} \text{ml}^{-1} \)} = 0.05 \]

*\( y = ax + b \) where \( x \) is the concentration of paracetamol in \( \mu \text{g} \text{ml}^{-1} \).

NR: not reported.
diclofenac sodium, and nimuselide did not interfere, while ascorbic acid was the only substance that interfered seriously. The results are presented in Table 3.

3.6. Analysis of pharmaceutical formulations

The applicability of the method to assay commercial pharmaceutical preparations was examined. Tablets containing Paracetamol sold in the market or by the druggist were analyzed by the proposed method. Comparison of the results statistically (by means of the Student’s t-test and the variance ratio F-test) with those obtained by the official method [43]. The obtained results were compared with those of the standard addition method, and presented in Table 4 indicated that there is no significant difference in the proposed method of analysis as compared with the existing methods.

Table 4: Determination of paracetamol in commercial samples by the proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Paracetamol (mg per tablet)*</th>
<th>Recovery (% mean ± R.S.D.)a</th>
<th>Proposed Method</th>
<th>Official Method [43]</th>
<th>F-Value**</th>
<th>t-Found ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpol</td>
<td>500</td>
<td>100.97 ± 0.67</td>
<td>100.10 ± 1.20</td>
<td>3.87</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>325</td>
<td>102.00 ± 2.30</td>
<td>100.20 ± 0.62</td>
<td>2.65</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>Sumo</td>
<td>500</td>
<td>102.10 ± 2.30</td>
<td>99.90 ± 1.30</td>
<td>1.96</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Imol</td>
<td>325</td>
<td>99.30 ± 0.68</td>
<td>100.60 ± 0.52</td>
<td>2.67</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>Molifen</td>
<td>500</td>
<td>101.00 ± 1.00</td>
<td>99.90 ± 0.69</td>
<td>2.81</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>Dolopar</td>
<td>500</td>
<td>99.90 ± 0.30</td>
<td>101.20 ± 0.26</td>
<td>4.46</td>
<td>1.76</td>
<td></td>
</tr>
</tbody>
</table>

*aAverage of five determinations, assayed as a percentage of label claim.
R.S.D., Relative Standard Deviation (n = 8).
*Label claimed as per the manufacturer
**Tabulated F-value at 95% confidence level is 6.39
***Tabulated t-value at 95% confidence level is 2.78

4. Conclusion

The procedure described for analysis of paracetamol meets most of the requirements including simplicity, rapidity, sensitivity, selectivity and cost of analysis. It is evident from the results that the recommended procedure is well suited for the routine assay and evaluation of drugs, in preformulation and dosage forms, to assure high standard of quality control. With increasing consumer awareness, the pharmaceutical industry and the drug control authority have long been interested in the development of simple, accurate and sensitive methods for the assay and evaluation of drugs in bulk and in dosage form, to maintain high standard in quality control. The use of common reagents and simplicity of the method is a step forward to assure high standard in quality control. Such simple methods based on spectrophotometry have become an accepted analytical tool for the assay and evaluation of drugs. In brief, the spectrophotometric method proposed in this study can be applied for direct determination of paracetamol in drug control laboratories as a rapid, reliable, sensitive and cost effective method for getting accurate results.

References

41. J.M. Murfin, Analyst 97 (1972) 663.

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