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# طريقة طيفية جديدة لتعيين الفيبانتيل بشكليه النقي والجرعة المركبة

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## الملخص:

تم تطوير طريقة قياس طيفي جديدة، بسيطة، دقيقة وسريعة لضبط جودة فيبانتيل في شكليه النقي وفي جرعاته المركبة. تعتمد الطريقة على استغلال مستقبلات-π مثل 6,3-ثنائي كلور-5,2-ثنائي هيدروكسي-بيتا-بنزوكوينون (CAA) أو 3,2-ثنائي كلور-6,5-ثنائي سيانو-بيتا-بنزوكوينون (DDQ) لتعيين فبانتيل كمانح إلكترونات ليكون معقد ملون له امتصاص عند 524 وعند 579 نانوميتر، على التوالي. تم التدليل على تطبيقية الطريقة بتعيين كمية الدواء المراد المعني في الأقراص التجارية ومن ثم تقييم النتائج إحصائيا وفقا لضوابط ICH. هذه الطريقة الطيفية القياسية الجديدة الواردة في هذه الورقة تتميز بالسرعة والتفرد في التعيين الكمي لفبانتيل في طوره النقي وفي شكل جرعاته المركبة.

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ORIGINAL ARTICLE

# A novel spectrophotometric approach for the determination of febantel in pure and dosage forms

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**KEYWORDS**

Febantel;
Spectrophotometry;
2,5-Dichloro-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione;
4,5-Dichloro-3,6-dioxo-1,4-cyclohexadiene-1,2-dicarbonitrile;
Charge transfer complex

**Abstract** A new, simple, accurate and rapid spectrophotometric method was developed for the quality control of febantel (FBT), in pure and dosage forms. The method was based on the utility of π-acceptors such as 3,6-dichloro-2,5-dihydroxy-*p*-benzoquinone (CAA) or 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) for the determination of FBT which act as an e-donor to form a highly colored complex with absorption bands at 524 and 579 nm, respectively. The applicability of the method was demonstrated by the determination of the studied drug in commercial tablets and the results were statistically evaluated as per the ICH guidelines. The new spectrophotometric method described in this paper is fast, convenient and has the novelty of carrying out the quantitative determination of FBT in pure and dosage forms.



## 1. Introduction

Febantel (FBT) chemically, (*RS*)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one (Fig. 1) is widely used in veterinary medicine (Rose, 1999) which is rapidly absorbed and metabolized in animals (Booth and McDonald, 1988) and thus it is used both in monogastric and ruminant animals. Because of its wide range of antiparasitic activity it is qualified as a broad spectrum anthelmintic which is used for the treatment of gastrointestinal parasitism in cattle, sheep and swine (Wollweber et al., 1978). Additionally it has larvicidal and ovicidal properties (Taylor, 1999). Moreover, it has high degree of efficiency, good margin of safety and versatility of administration (Pontes et al., 2013). Febantel is a pro-drug, which is known to convert into an active compound soon after administration (World Health Organization, 2009). The recommended therapeutic doses for cattle, sheep and swine are 10, 5 and 5 mg/kg respectively (Wollweber et al., 1978). Over dosage of the drug may lead to the unfavorable effect on human health when people consume products containing veterinary drug residues. To prevent the abuse of veterinary drugs including febantel, the Department of Health announced the revised "Tolerances for Residues of Veterinary Drugs" in January 2001 (Department of Health, 2001), and the maximum residue limits for veterinary drugs were set. Therefore, it is an important issue to establish a standard analytical method for monitoring drug in pure and

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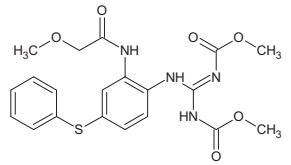


**Figure 1** Structure of FBT.

dosage forms. Literature survey reveals very few analytical methods for the analysis of FBT. Reported methods include determination of FBT in combination with the other anthelmintic drugs like flubendazole, oxfendazole and oxfendazole sulfone etc. These include High performance liquid chromatography (HPLC) (Dusi et al., 2005; Wanda and Anna, 2010; Delatour et al., 1983; Landuyt et al., 1993; Chou et al., 2004) with ultraviolet (UV) (Kawasaki et al., 1999) or gas chromatography/mass spectrometry (GC/MS) (Wilson et al., 1991). So far there is no visible spectrophotometric method developed for the analysis of FBT in pure and dosage forms.

The aim of the present work is to develop a fast, accurate and simple spectrophotometric method for the determination of FBT. The present work describes two new spectrophotometric procedures for the quality control of FBT in pure and dosage forms through charge transfer complex.

A charge transfer complex is a stable molecular system formed between an electron donating molecule (having sufficiently low ionization potential) and an electron accepting molecule (having high electron affinity). The principal feature of this type of complex formation is the appearance of new and intense absorption bands in ultraviolet or visible region of the spectrum. Absorption bands of this type are known as charge transfer bands, since they involve electronic transitions from orbital of the donor to the vacant orbital of the acceptor. In the present work we used two such charge transfer reagents (CAA and DDQ) for the quantitative determination of drug in pure and dosage forms.

## 2. Materials and methods

### *2.1. Apparatus*

A UV–Visible spectrophotometer (SHIMADZU, UV 2550 Japan) with 1 cm quartz cells was used for the absorbance measurements. The infrared spectra of the complexes were recorded using KBr discs on a SHIMADZU FT-IR spectrometer.

### *2.2. Reagents and chemicals*

All reagents and chemicals used were of analytical grade and the solvents were spectroscopic grade. FBT pure drug was obtained as gift sample from CAD Pharma Inc., Bangalore, India. Pharmaceutical formulations of FBT were obtained commercially, in the form of tablets namely Drontal® plus (113.4 and 680.4 mg). The 0.5% of chromogen reagents CAA (CAS No. 87-88-7, Spectrochem Private Ltd., Mumbai, India) and DDQ (CAS No. 84-58-2, Spectrochem Private Ltd., Mumbai, India) were prepared in methanol.

#### *2.2.1. Stock solution of FBT*

A mass containing about 0.1 g of pure FBT was accurately weighed and diluted to 100 mL with methanol (1000 μg $mL^{-1}$). The stock solution was diluted appropriately to get working concentration.

### *2.3. Procedures*

#### *2.3.1. Assay procedure by using CAA*

Aliquots of FBT containing 5.00–35.00 μg $mL^{-1}$ were transformed into a series of 10 mL volumetric flasks followed by the addition of 1 mL CAA. The volumes were completed to the mark with methanol. The solution was left to stand for few minutes at room temperature and absorbance was measured at 524 nm (Fig. 2) for FBT–CAA complex against a blank. (Blank solution prepared in the same manner without drug).

#### *2.3.2. Assay procedure by using DDQ*

For DDQ aliquots of FBT containing 20.00–120.00 μg $mL^{-1}$ were transformed into a series of 10 mL volumetric flasks followed by the addition of 1 mL DDQ. The volumes were completed to the mark with methanol. The solution was left to stand for few minutes at room temperature and absorbance was measured at 579 nm (Fig. 3) for a FBT–DDQ complex against a blank. (Blank solution prepared in the same manner without drug).

#### *2.3.3. Assay of pharmaceutical formulation*

Ten commercial tablets of FBT from each dosage form (Drontal® plus 113.4 mg and Drontal® plus 680.4 mg) were crushed into a fine powder using a Pestle and Mortar. An amount of tablet powder equivalent to 0.1 g of FBT was accurately weighed and transferred into a 100 mL volumetric flask. About 50 mL of methanol was added and the mixture was shaken for about 20 min. The volume of the mixture was adjusted to 100 mL with methanol and filtered through Whatman filter paper No. 41. Obtained filtrate was diluted quantitatively with methanol to obtain a suitable concentration for the analysis. A

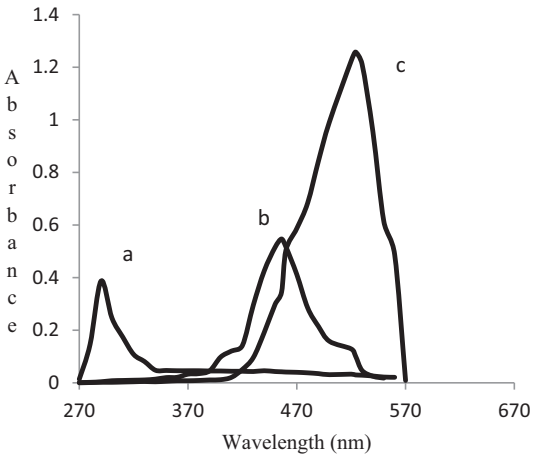



**Figure 2** Absorption spectra of (a) FBT in methanol, (b) CAA in methanol, (c) FBT–CAA complex in methanol.

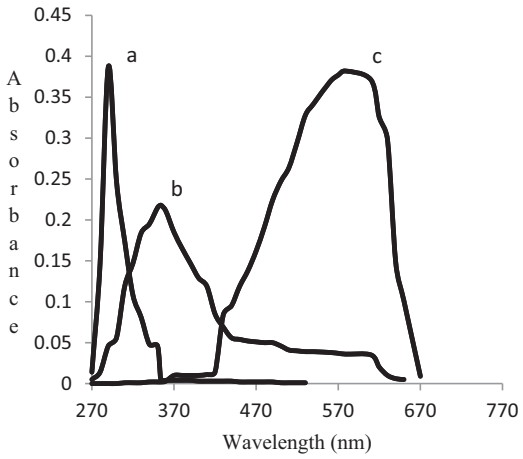



**Figure 3** Absorption spectra of (a) FBT in methanol, (b) DDQ in methanol, (c) FBT–DDQ complex in methanol.

convenient aliquot was then subjected to the analysis using proposed methods.

*2.3.4. Assay procedure for method validation*

The developed method was validated statistically as per the ICH guidelines (ICH- Q2 (R1), 2005). The amount of FBT present in the sample was calculated using a calibration curve. In order to study the accuracy of the proposed methods, three concentrations of pure FBT within the linearity range are analyzed and each determination being repeated five times. The precision (intra-day and inter-day) of the methods was determined separately from the response obtained by five replicates of a fixed amount of drug. Sandall's sensitivity was calculated as the minimum concentration of drug required to produce an absorbance of 0.001 nm. The limit of detection (LOD) and the limit of quantification (LOQ) are obtained from the expression

$$\text{LOD} = 3.3\ \sigma/\text{S} \text{ and } \text{LOD} = 10\sigma/\text{S}$$

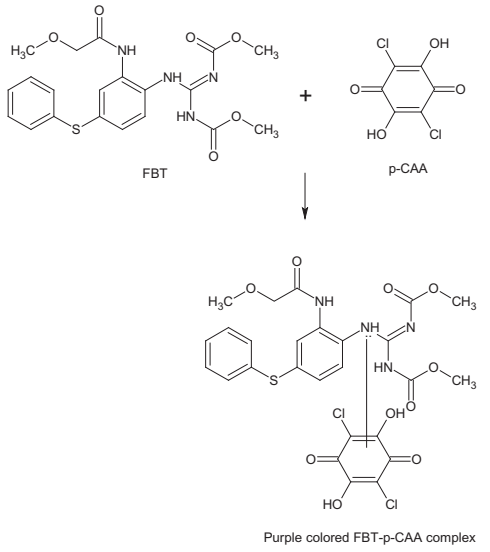
$\sigma$ = standard deviation of the blank
$S$ = slope of calibration curve.

## 3. Synthesis of charge transfer complexes

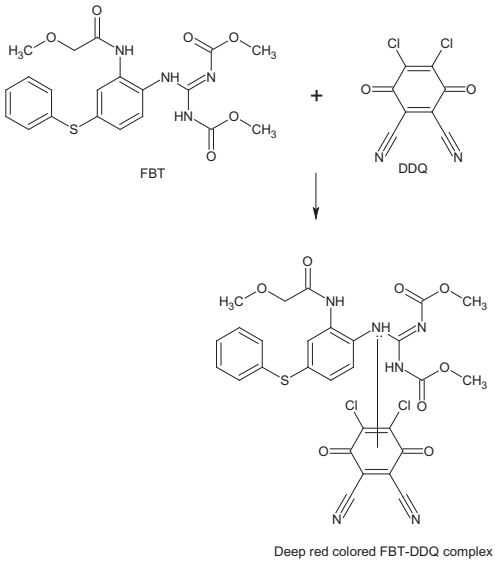
The solid charge transfer complexes of FBT with CAA and DDQ were prepared by mixing 0.01 M of the drug and CAA or DDQ in 20 mL methanol with continuous stirring for about one hour at room temperature. The colored complexes developed and the solution was allowed to evaporate slowly at room temperature. Colored solid complexes were formed, filtered, and dried under vacuum over anhydrous calcium chloride.

## 4. Results and discussion

FBT exhibits weak UV-absorption in the range 250–300 nm ($\lambda_{max}$ is 290 in methanol). Therefore, it is necessary to develop a sensitive spectrophotometric determination of FBT in pure form, dosage form and if possible, in biological fluids. In the present work suitable chromogen is used which reacts with FBT to obtain a light absorbing charge transfer complex derivative. Molecular charge-transfer complexes are of particular




**Scheme 1** Reaction of FBT with CAA.




**Scheme 2** Reaction of FBT with DDQ.

interest in pharmaceutical science which can be applied as useful means in the qualitative and quantitative analyses of different pharmaceutical compounds.

Charge transfer reagents like CAA and DDQ has been used for the spectrophotometric determination of drugs containing electron donors such as nitrogen and oxygen (Kenneth et al., 2011; Lories et al., 1999). The interaction of FBT (which containing nitrogen as n donors) with π acceptors like CAA and DDQ gave rise to a bathochromic shift and can be attributed to the formation of new molecular complexes (Schemes 1 and 2). Newly formed purple colored FBT–CAA complex and deep reddish brown color FBT–DDQ complex can be measured at 524 and 579 nm, respectively. The formation of highly intense absorption bands is an evidence of the formation of new charger-transfer complexes.

**Table 1** Spectral and statistical data for the determination of FBT.

| Parameters | | Reagents used |
|---|---|---|
| | P-CAA | DDQ |
| $\lambda_{max}$ (nm) | 524 | 579 |
| Beer's law limits (μg/ml) | 5.00–35.00 | 20.00–120.00 |
| Molar Absorptivity (L $mol^{-1}$ $cm^{-1}$) | $2.992 \times 1\ 0^4$ | $0.9059 \times 10^4$ |
| Sandell's sensitivity (μg $cm^{-2}$) | $0.10 \times 10^{-2}$ | $0.34 \times 10^{-2}$ |
| Limit of detection* (μg $mL^{-1}$) | 0.9428 | 0.1571 |
| Limit of quantification* (μg $mL^{-1}$) | 2.8571 | 0.4761 |
| Regression equation** | $Y = a + b\ X$ | $Y = a + b\ X$ |
| Slope ($b$) | 0.0070 | 0.0021 |
| Intercept ($a$) | 0.0077 | 0.0224 |
| Correlation coefficient ($r$) | 0.9989 | 0.9964 |

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

### *4.1. Analytical data*

#### *4.1.1. Validity of Beer's law*

It is also important to know the concentration limits of drug at which these reactions are quantitative. Under optimized condition it is found that, Beer's law is valid over the concentration ranges from 5.00–35.00 to 20.00–180.00 μg $mL^{-1}$ of drug using CAA and DDQ reagents, respectively. The calibration graphs in both the methods are described by the equation: $Y = a + b\ X$ (Where $Y$ = absorbance, $a$ = intercept, $b$ = slope and $X$ = concentration in μg $mL^{-1}$) is obtained by the method of least squares. Slope, intercept, correlation coefficient, Sandell's sensitivities, and molar absorptivity (ε) values are given in Table 1. The small values of Sandell's sensitivity indicate the high sensitivity of the proposed method and low values of limits of detection (LOD) and quantification (LOQ) indicate the possibility of applying CAA and DDQ reagents in routine analysis of the drugs under investigation.

#### *4.1.2. Robustness*

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the proposed method was examined by evaluating the small variations in reagent concentration and reaction time. It was found that none of these variables had a significant effect on the determination of the investigated drug.

#### *4.1.3. Accuracy and precision*

Accuracy of the proposed methods is measured by calculating the percentage relative error (% RE) and precision of the

**Table 2A** Evaluation of accuracy and precision by using *p*-CAA.

| | Amount taken (μg $mL^{-1}$) | Amount found* (μg $mL^{-1}$) | RE (%) | SD (μg $mL^{-1}$) | RSD (%) |
|---|---|---|---|---|---|
| Intraday | 10.00 | 9.86 | 1.40 | 0.28 | 2.83 |
| | 15.00 | 14.84 | 1.06 | 0.37 | 2.49 |
| | 20.00 | 19.72 | 1.40 | 0.39 | 1.97 |
| Interday | 10.00 | 9.95 | 0.50 | 0.03 | 0.30 |
| | 15.00 | 14.85 | 1.00 | 0.04 | 0.26 |
| | 20.00 | 19.41 | 2.95 | 0.03 | 0.15 |

* Mean value of five determinations.

**Table 2B** Evaluation of accuracy and precision by using DDQ.

| | Amount taken (μg $mL^{-1}$) | Amount found* (μg $mL^{-1}$) | RE (%) | SD (μg $mL^{-1}$) | RSD (%) |
|---|---|---|---|---|---|
| Intraday | 20.00 | 19.84 | 0.80 | 0.17 | 0.85 |
| | 40.00 | 39.57 | 1.07 | 0.38 | 0.96 |
| | 60.00 | 59.56 | 0.73 | 0.22 | 0.36 |
| Interlay | 20.00 | 19.98 | 0.02 | 0.1 | 0.5 |
| | 40.00 | 39.92 | 0.15 | 0.2 | 0.41 |
| | 60.00 | 59.88 | 0.11 | 0.24 | 0.40 |

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

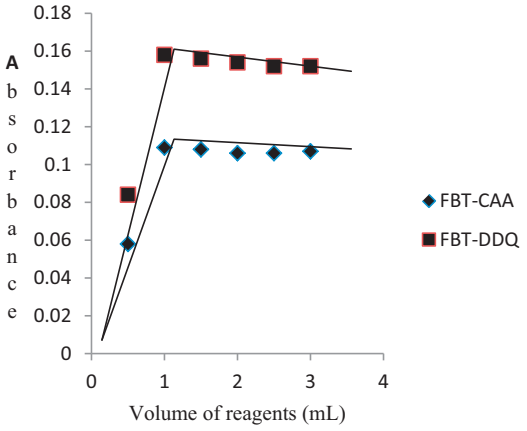



**Figure 4** Effect of reagent volume on the formation of FBT–CAA and FBT–DDQ complexes.

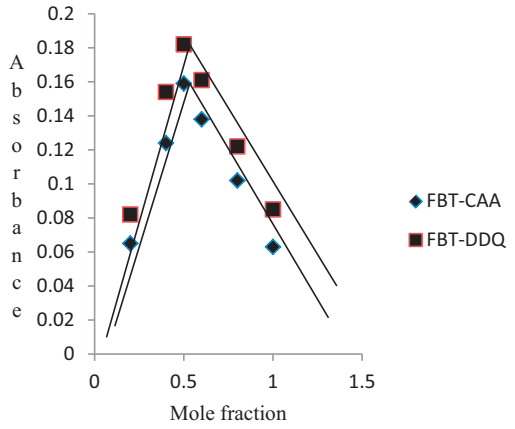



**Figure 5** Job's continuous-variation plots of FBT–CAA and FBT–DDQ complexes.

methods is assessed as percentage relative standard deviation (% RSD) at different concentration levels. The precision of the proposed methods is calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of the studied drug are analyzed in five replicates during the same day (intra-day precision) and for seven consecutive days (inter-day precision). Obtained analytical results shows a low SD and RSD value (less than 3%) which indicates the good accuracy and precision of the methods. The results of this study are summarized in Tables 2A and 2B.

### *4.2. Optimization of the reaction conditions*

To optimize the volume of the reagents, various volumes of 0.5% CAA or DDQ solutions are added to a constant concentration of drug. It is found that the highest absorbance at the relevant maxima was obtained upon using 1 mL of chromogenic reagents and higher concentration of reagents does not affect the color intensity (Fig. 4). Complete color development was attained at room temperature between the drug reagents and remained stable for further 6 h with CAA or 5 h with DDQ. Different solvents like ethanol, acetone, methanol and water were tried for the reaction where the use of methanol yielded maximum color intensity. From the optimization studies it is found that reaction can be carried out at room temperature by using 0.5% of 1 mL reagents in methanol medium.

### *4.3. Stoichiometry of the charge transfer complexes*

Job's method of continuous variation (Job, 1964) is generally an applicable and widely used technique in order to determine the suitable ratio between drug and reagents (CAA and DDQ). A series of solutions are prepared in which the sum of concentration of reagents and drug are same while their concentration varies. Fig. 5 shows that the interaction between this drug and reagents occurs in equimolar basis, i.e. the two straight lines are intersected at 1:1 [Drug]:[Reagents]. This means that, 1:1 complexes were formed between the drug and CAA or DDQ reagents. The charge transfer complexes formed between CAA and DDQ with FBT drug takes place through the transfer of electron from a donor (drug) to the π-acceptor reagent.

### *4.4. Application of the developed method to pharmaceutical formulations*

The proposed method has been applied for the determination of FBT in tablets (Drontal plus, 113.4 and 680.4 mg). The observed *t*-test values as compared to the corresponding tabulated values at 95% confidence level indicated that the calculated *t*-values are less than the tabulated ones. The obtained results with excellent % RE suggest that the method can be successfully applicable for the analysis of FBT in pure and pharmaceutical analysis. Obtained values are given in Table 3.

### *4.5. IR spectral studies*

IR spectrum of the molecular complex of CAA and DDQ with FBT indicates that the band of *v* (NH) of the free donor molecule which exhibited at 3271 $cm^{-1}$ is shifted to lower wave

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

| Brand name | Labeled amount (mg) | Found* ± SD using CAA | Found ± SD using DDQ |
|---|---|---|---|
| Drontal® plus | 113.4 | 113.46 ± 0.02<br>$t = 0.38$ | 113.5 ± 0.06<br>$t = 0.69$ |
| Drontal® plus | 680.4 | 680.09 ± 0.06<br>$t = 0.41$ | 680.16 ± 0.09<br>$t = 0.52$ |

Tabulated t value at 95% confidence level is 2.77.
* Mean of five determinations.

**Table 4** UV–Vis and IR data for the new charge transfer complexes.

| Comp. | $\lambda_{max}$(UV–vis) (nm) | IR ($cm^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | $\nu$(C═O) | $\nu$(C≡N) | $\nu$(C─Cl) | $\nu$(NH) | $\nu$($R_2C$═N─R) |
| FBT | 290 | 1737 | – | – | 3271 | 1653 |
| CAA | 456 | 1665 | – | 578 | – | – |
| DDQ | 354 | 1678 | 2260 | 746 | – | – |
| FBT–CAA | 524 | 1687 | – | 574 | 3159 | 1637 |
| FBT–DDQ | 579 | 1693 | 2254 | 742 | 3182 | - |

number values of 3159 and 3182 $cm^{-1}$ in FBT–CAA and FBT–DDQ complexes, respectively. This is mainly due to the accepted symmetry and electronic structural changes upon complexation. The IR spectral data of the molecular complex of CAA and DDQ with FBT are given in Table 4.

## 5. Conclusions

The proposed charge transfer complexation method is rapid and simple. The developed method is a novel spectrophotometric method for the analysis of FBT in a short time period with high accuracy and precision. The suggested method has many advantages of being simple, accurate, sensitive and suitable for routine analysis in control laboratories. This method utilizes a single step reaction at room temperature by using a simple chromogenic reagent. The recommended procedures are validated and are well-suited for the analysis of drug and pharmaceuticals.

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