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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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1. Introduction

Febantel (FBT) chemically, (*RS*)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-*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 quali“ed 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 ef“ciency, 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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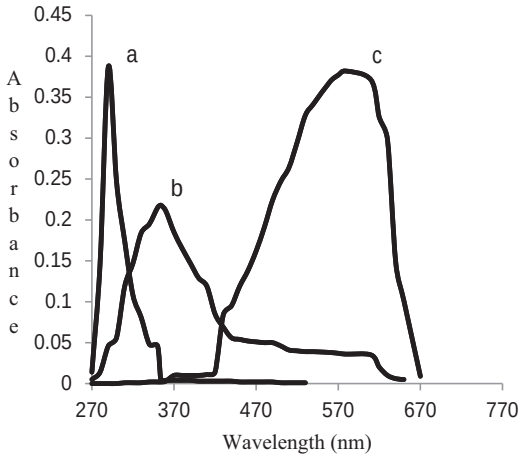


Figure 3 Absorption spectra of (a) FBT in methanol, (b) DDQ in methanol, (c) FBT…DDQ complex in methanol.

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 “ve times. The precision (intra-day and inter-day) of the methods was determined separately from the response obtained by “ve replicates of a “xed 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 quanti“cation (LOQ) are obtained from the expression

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

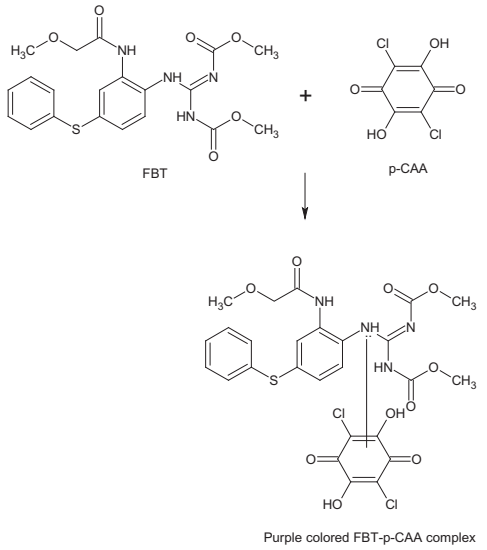
σ = standard deviation of the blank
S = slope of calibration curve.

3. Synthesis of charge transfer complexes

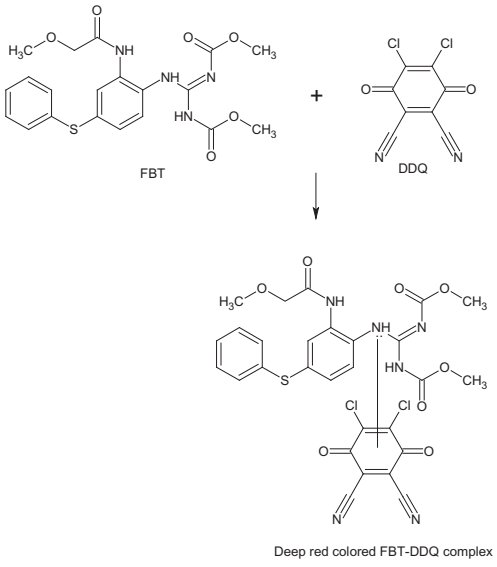
The solid charge transfer complexes of FBT with CAA and DDQ were prepared by mixing 0.01 M of the drug and CAA or DDQ in 20 mL methanol with continuous stirring for about one hour at room temperature. The colored complexes developed and the solution was allowed to evaporate slowly at room temperature.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

4. Results and discussion

FBT exhibits weak UV-absorption in the range 250…300 nm (λ_{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 ”uids. 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 interest in pharmaceutical science which can be applied as useful means in the qualitative and quantitative analyses of different pharmaceutical compounds.



Scheme 1 Reaction of FBT with CAA.



Scheme 2 Reaction of FBT with DDQ.

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
λ_{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· 1 0^4	0.9059· 10^4
Sandell•s sensitivity (µg cm^{-2})	0.10· 10^{-2}	0.34· 10^{-2}
Limit of detection* (µg mL^{-1})	0.9428	0.1571
Limit of quanti"cation* (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 coe cient (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 coef"cient, 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 quanti"cation (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 signi"cant 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 "ve 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 "ve determinations.

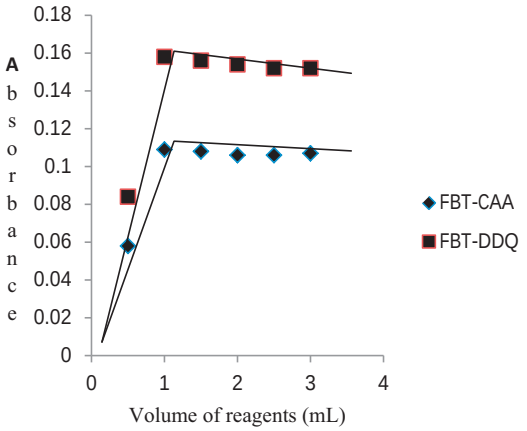


Figure 4 Effect of reagent volume on the formation of FBT…CAA and FBT…DDQ complexes.

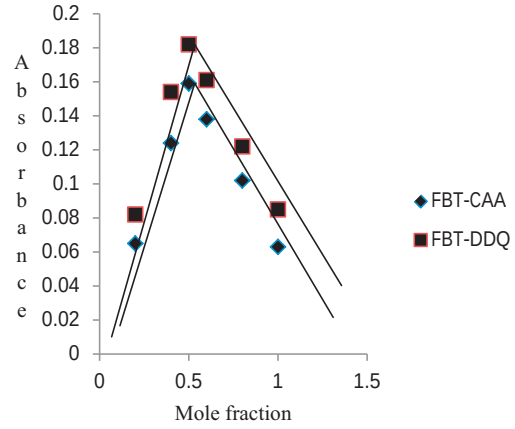


Figure 5 Job•s continuous-variation plots of FBT…CAA and FBT…DDQ complexes.

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 "ve 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% con"dence 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 ν (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 t = 0.38	113.5 ± 0.06 t = 0.69
Drontal plus	680.4	680.09 ± 0.06 t = 0.41	680.16 ± 0.09 t = 0.52

Tabulated t value at 95% con"dence level is 2.77.
* Mean of "ve determinations.

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

Comp.	λ_{max}(UV…vis) (nm)	IR (cm^{-1})				
		ν(C═O)	ν(C≡N)	ν(CACl)	ν(NH)	ν(R_2C═NAR)
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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