

# Sensitive Extractional Colorimetric Analysis of Fexofenadine Hydrochloride and Irbesartan Bases Through Acid-Dye Complexation Using Naphthol Blue Black in Pure Form and Pharmaceuticals

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**Abstract:** A simple, accurate and sensitive method has been presented for the determination of fexofenadine hydrochloride (FEX) and irbesartan (IRB) in bulk and pharmaceutical preparations. The method is based on the reaction of the above cited drugs with naphthol blue black (NBB) dye in solutions containing Britton buffer to form ion-pair complexes extractable with chloroform and subsequently measured spectrophotometrically at 625 nm. All the reaction conditions for the proposed methods have been studied. The reactions were extremely rapid at room temperature and the absorbance values remained unchanged for at least 24 hrs. Beer's law was obeyed in the concentration ranges 2.7–53.8 and 10–244  $\mu\text{g mL}^{-1}$  with detection limit of 0.013 and 0.75  $\mu\text{g mL}^{-1}$  for FEX and IRB, respectively. The proposed methods were applied successfully for the determination of FEX and IRB in pharmaceutical formulations. Interferences of the other ingredients and excipients were not observed. The results obtained were compared statistically with those obtained by the official method and showed no significant differences regarding accuracy and precision.

**Keywords:** Extractive Colorimetry, Fexofenadine Hydrochloride, Irbesartan, Naphthol Blue Black, Pharmaceuticals

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## 1. Introduction

Fexofenadine (Figure 1),  $\alpha, \alpha$ -dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl] butyl]-benzene acetic acid [1] is the active carboxylic acid analogue of the antihistamine terfenadine. Fexofenadine is a second generation antihistamine drug useful to available treatments of allergic diseases with a wide margin of safety [2]. The drug is official in BP [3] and USP [4], which describe HPLC methods for the assay of fexofenadine hydrochloride. Several analytical methods for the determination of fexofenadine hydrochloride in pharmaceutical formulations have been reported including high performance liquid chromatography [5–8], spectrophotometry [8–12], conductometry [13], extractive spectrophotometry with bromothymol blue, bromocresol green, bromocresol purple and bromophenol

blue [13, 14], spectrofluorometry [15], potentiometry [16] and capillary electrophoresis [17, 18]. Fexofenadine hydrochloride has been determined in combination with other drugs using high performance liquid chromatography [19–23], high performance thin layer chromatography [24] and spectrophotometry [25–27] in combined dosage forms.

Irbesartan (Figure 1), an anti-hypertensive is chemically designated as 2-butyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one. It is used for the treatment of hypertension [3]. The drug is official in BP [3] and USP [4], which describe HPLC methods for the assay of irbesartan. Few methods have been described for the determination of irbesartan in pharmaceutical dosage forms by HPLC [28–32], extractive

spectrophotometry with Picric acid, bromocresol green, bromothymol blue, cobalt thiocyanate and molybdenum (V) thiocyanate [33], spectrophotometry [34–36] and HPTLC [37]. Several HPLC [38–45], spectrophotometry [44–50] and capillary electrophoresis methods [51, 52] are reported in the literature for the determination of irbesartan in combined pharmaceutical formulations.

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs. Therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [13, 14, 33, 53-55]. So far, there

has been no ion-pair extractive spectrophotometry method reported for an estimation of fexofenadine hydrochloride and irbesartan with naphthol blue black. Fexofenadine and irbesartan have basic cationic nitrogen reacts with anionic dye at a suitable pH, to form highly colored chloroform extractable ion pair complex. Therefore, this paper suggests simple and sensitive colorimetric procedures for the determination of fexofenadine and irbesartan in tablets and capsules. The methods are based on the ability of the cited drugs to form ion-pair complex with naphthol blue black, as acidic dye. Optimum conditions were established and the methods were validated for linearity.

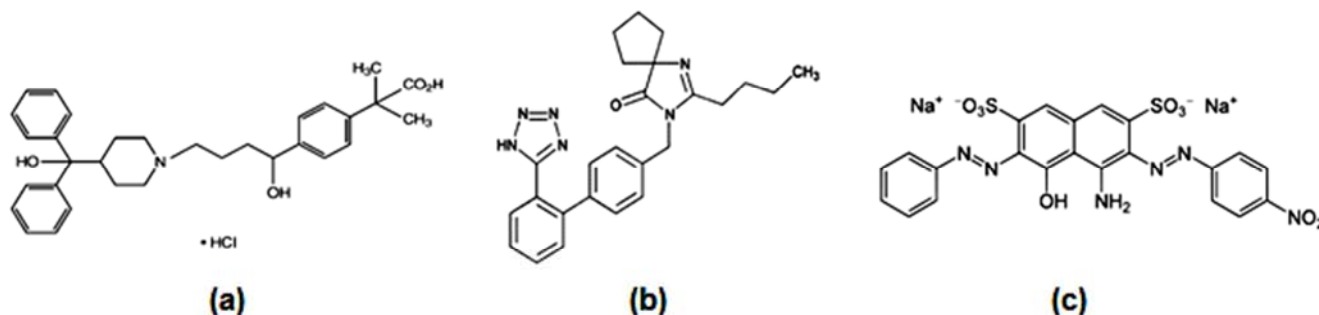


Figure 1. Chemical structure of fexofenadine hydrochloride (a), irbesartan (b) and naphthol blue black (c).

## 2. Materials and Methods

### 2.1. Instrumentation

A Jasco V-530 UV-VIS spectrophotometer (Japan) provided with 1 cm matched quartz cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 500–700 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. A pH meter model Suntex-SP 701 (Taiwan) equipped with combined glass pH electrode was used. The desired temperature was maintained at 20°C.

### 2.2. Reagents and Drugs

Pharmaceutical grade fexofenadine hydrochloride (FEX) was obtained from Chem Pharma, India, and used after its purity was determined which found to be 100.05% according to BP [3]. Irbesartan (IRB) was obtained from Cipex Specialities Pvt Ltd, India. Its purity was found to be 99.67% according to BP [3]. Tablets and capsules containing fexofenadine hydrochloride and irbesartan were purchased from Syrian market. All chemicals and solvents used were of analytical reagent grade of E. Merck (Germany). Absolute methanol and bi-distilled water were used. Naphthol blue black (NBB) (Fig. 1) was prepared daily as  $1 \times 10^{-3}$  M in bi-distilled water. Britton buffer solutions (pH range from 1.3 to 4.0) were prepared by mixing specific volumes of acetic acid 0.2 M, phosphoric acid 0.2 M and boric acid 0.2 M.

### 2.3. Standard Stock Solutions

$1 \times 10^{-3}$  M solution of pure FEX was prepared in bi-distilled water.  $1 \times 10^{-3}$  M solution of pure IRB was prepared by

dissolving the appropriate weight of IRB in 5 mL of glacial acetic acid in 100 mL volumetric flask, the volume was then diluted to the mark with bi-distilled water. The solutions were stored in dark bottles and kept in the refrigerator for not more than 10 days. Other concentrations of working solutions were then prepared by suitable dilution of the stock solution with bi-distilled water.

### 2.4. Recommended Procedures

#### 2.4.1. FEX Method

Into a series of 50 mL separating funnels, 2 mL of buffer of pH 2.4 and 5 mL of  $1 \times 10^{-3}$  M NBB solution were added. Varying aliquots (50–1000  $\mu$ L) of a standard FEX ( $1 \times 10^{-3}$  M) solution were accurately transferred to each funnel. The funnels were shaken vigorously with 10 mL chloroform for 2 min, and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a beaker, dried over anhydrous sodium sulfate and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with chloroform. The absorbance of the blue colored ion-pair complex was measured at 625 nm against the reagent blank.

#### 2.4.2. IRB Method

Into a series of 50 mL separating funnels, 5 mL of buffer solution of pH 1.5 and 4 mL of  $1 \times 10^{-3}$  M NBB solution were added. An appropriate volume of  $1 \times 10^{-3}$  M standard IRB solution (0.24–5.73 mL) was added to each funnel and mixed well. The funnels were shaken vigorously with 10 mL chloroform for 2 min, and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 50 mL beaker, dried over anhydrous

sodium sulfate, and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with chloroform and mixed. The absorbance of the solution was measured at 625 nm against the reagent blank.

#### 2.4.3. Procedure for Dosage Forms

Twenty tablets or the contents of 20 capsules containing FEX or IRB were weighed and finely powdered. In the case of FEX, an amount of the powder equivalent to 100 mg of FEX was weighed into a 100 mL volumetric flask, 30 mL methanol was then added and sonicated for about 5 min. The volume was diluted to the mark with methanol, mixed well and filtered. The combined filtrate was evaporated to the dryness. The remaining portion of the solution was dissolved with bi-distilled water in a 100 mL volumetric flask, and the resulting solution was used for analysis by the recommended procedure in the concentration range mentioned above.

In the case of IRB, an amount of the powder equivalent to 25 mg of IRB was weighed into a 25 mL volumetric flask, 5 mL of glacial acetic acid was then added and mixed for about 15 min. The volume was diluted to the mark with bi-distilled water, mixed well and filtered. The general procedure was then followed in the concentration range mentioned above.

#### 2.4.4. Procedure for Stoichiometric Ratio

The reaction stoichiometry between the studied drugs and NBB has been determined spectrophotometrically by applying molar ratio and continuous variation methods. In the former method, equimolar solutions of the studied drugs and NBB ( $1 \times 10^{-3}$  M) were used. Different aliquots of NBB were added to fixed aliquots of drug solution –total volume 10 mL– and the absorbance was measured at 625 nm against the reagent blanks treated similarly. While in the latter method, a series of drug–NBB solutions was kept at 2.0 mL (0:2, 0.2:1.8, 0.4:1.6, ..., 2:0) where  $C_{\text{FEX}} + C_{\text{NBB}} = 2 \times 10^{-4}$  M and  $C_{\text{IRB}} + C_{\text{NBB}} = 6 \times 10^{-4}$  M. The reagent was mixed in various proportions and then diluted to volume in a 10 mL calibrated flask with chloroform. The absorbance of the resulting solutions was measured at 625 nm against the reagent blanks treated similarly.

### 3. Results and Discussion

#### 3.1. Absorption Spectra

FEX and IRB form ion-pair complexes in acidic buffer with NBB dye and these complexes are quantitatively extracted into chloroform. Absorption spectra of the blue FEX–NBB and IRB–NBB ion-pair complexes extracted into chloroform with its  $\lambda_{\text{max}}$  at 625 nm, respectively, are shown in Figure 2. The colorless reagent blank under similar conditions showed negligible absorption.

Containing cationic nitrogen, the cited drugs react with NBB to form ion-pair complexes between the basic nitrogen of FEX and IRB in Britton buffer and NBB. Each drug–NBB complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction. The complex is quantitatively extracted into chloroform.

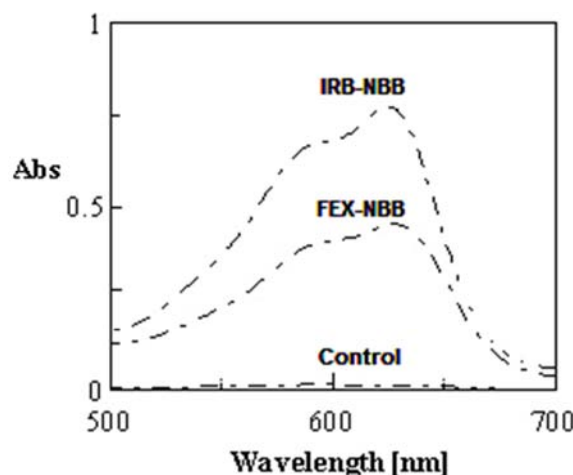


Figure 2. Absorption spectra of: FEX–NBB complex ( $20 \mu\text{g mL}^{-1}$  of FEX) and IRB–NBB complex ( $90 \mu\text{g mL}^{-1}$ ) against their respective blank vs. chloroform.

#### 3.2. Optimization of Variables

Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments. Britton buffer was found to be suitable for NBB method. Chloroform was preferred to other solvents (carbon tetrachloride, dichloromethane, and ether) for both methods for its selective and quantitative extraction. Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 625 nm for FEX–NBB and IRB–NBB.

The effect of pH and volume of buffer was studied by extracting the colored complex species at different pH value and volume of buffer. Maximal absorbance was observed at the pH 2.4 and 1.5 using 2 and 4 mL of Britton buffer for FEX and IRB, respectively, (Figures 3 and 4).

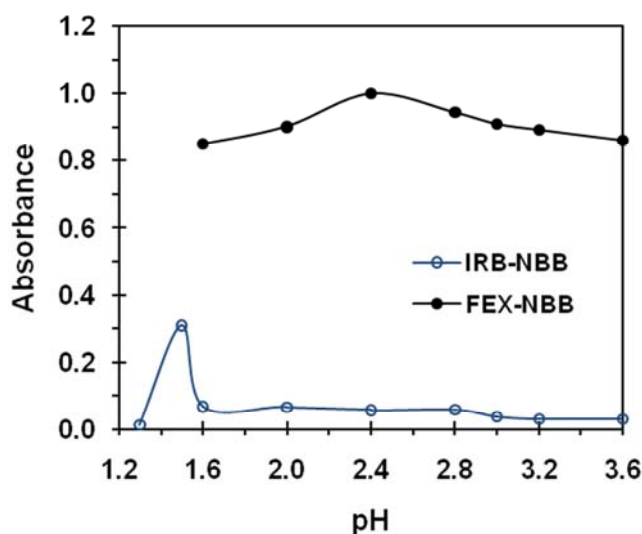


Figure 3. Effect of pH and volume of buffer on the absorbance at  $\lambda_{\text{max}}$  of FEX ( $42 \mu\text{g mL}^{-1}$ )–NBB and IRB ( $31 \mu\text{g mL}^{-1}$ )–NBB complexes.

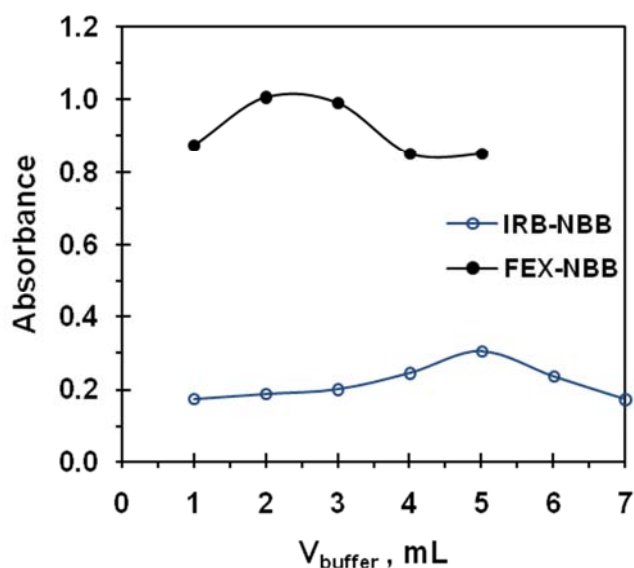


Figure 4. Effect of volume buffer on the absorbance of at  $\lambda_{\max}$  of FEX ( $42 \mu\text{g mL}^{-1}$ )-NBB at pH 2.4 and IRB ( $37 \mu\text{g mL}^{-1}$ )-NBB at pH 1.5.

A volume of 5 and 4 mL of  $1 \times 10^{-3}$  M NBB was found to be optimal for complete complexation between FEX and NBB, and IRB and dye, respectively, since the absorbance at maximum wavelength was found to be maxima at the mentioned volumes. The effect of the reagent's concentration on the absorbance of the colored complex species is shown in Figure 5.

### 3.3. Stoichiometric Relationship

The stoichiometric ratio and conditional stability constant

of the FEX-NBB or IRB-NBB complex formed were determined by applying Job's method of continuous variation and molar ratio method [56]. In all cases of Job's method (Figure 6a), the plots reached maximum value at a mole fraction of 0.5, indicating that ion pair complex with drug to dye ratio 1:1 are formed. Also, the plots of the mole ratio between drug and reagent *versus* the absorbance values were prepared (Figure 6b), and the results revealed that the formation of ion-pair complex between drug and reagent followed a 1:1 reaction stoichiometry.

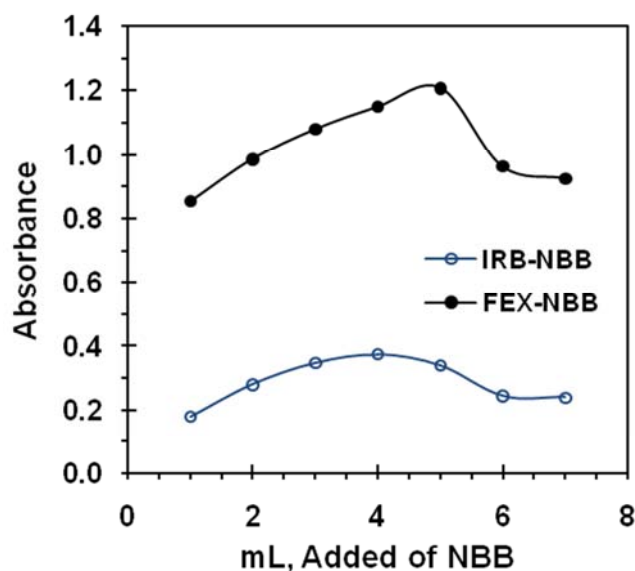


Figure 5. Effect of reagent volume on the formation of the colored ion-pair complexes FEX ( $50 \mu\text{g mL}^{-1}$ )-NBB and IRB ( $44 \mu\text{g mL}^{-1}$ )-NBB.

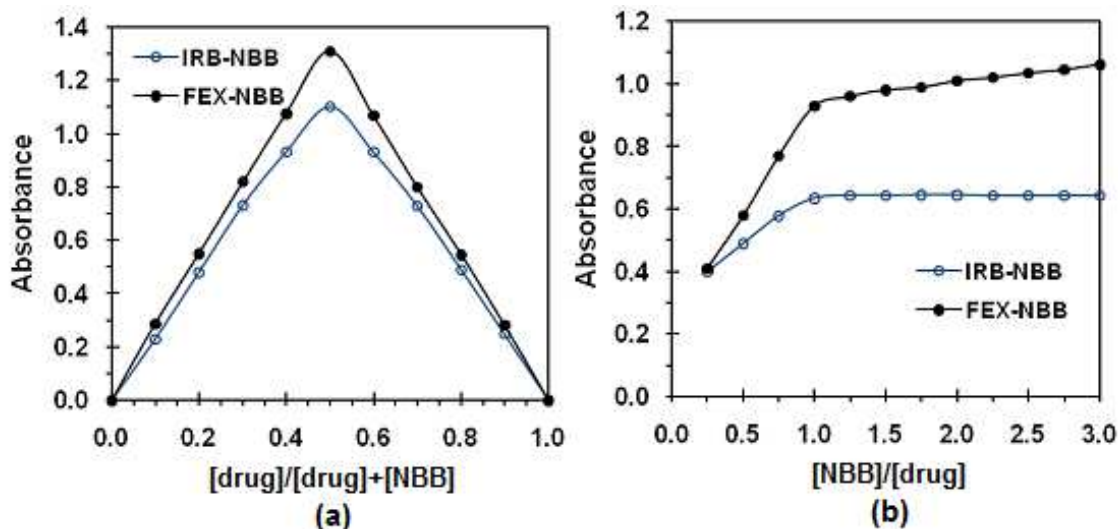


Figure 6. (a) Job's method of continuous variation of drug-NBB complexes,  $C_{\text{FEX}} + C_{\text{NBB}} = 2 \times 10^{-4}$  M and  $C_{\text{IRB}} + C_{\text{NBB}} = 6 \times 10^{-4}$  M, (b) mole-ratio method of drug-NBB complexes ( $C_{\text{FEX}} = 5 \times 10^{-5}$  M,  $C_{\text{IRB}} = 3 \times 10^{-4}$  M).

### 3.4. Conditional Stability Constant ( $K_f$ ) of Ion-pair

The conditional stability constant ( $K_f$ ) of the ion-association complex formed by FEX or IRB with NBB, was calculated from the continuous variation data using the equation (1).

$$K_f = \frac{A / A_m}{[1 - (A / A_m)]^{n+2} \cdot C_M(n)^n} \quad (1)$$

where  $A$  is the maximum observed absorbance and  $A_m$  is the

absorbance value when all the amount of drug is associated.  $C_M$  is the mole concentration of drug at the maximum absorbance and  $n$  is the combination ratio of the ion-pair considered [57]. The log  $K_f$  values obtained for the FEX-NBB and IRB-NBB ion-pair, are 6.52 and 7.23, respectively.

### 3.5. Validation of the Method

A linear relationship was found between the absorbance at  $\lambda_{\max}$  and the concentration of FEX and IRB in the range of 2.7–53.8 and 10–244  $\mu\text{g mL}^{-1}$ , respectively. Regression analysis of the Beer's law plots at  $\lambda_{\max}$  reveals a good correlation (Table 1). The graphs show negligible intercept and are described by the regression equation,  $A = mC + b$  (where  $A$  is absorbance of 1 cm layer,  $m$  is the slope,  $b$  is the intercept and  $C$  is the concentration of the measured solution in  $\mu\text{g mL}^{-1}$ ) obtained by the least-squares method [58].

**Table 1.** Statistical data of the regression equations for the determination of FEX and IRB with the proposed method.

Parameter	FEX	IRB
$\lambda_{\max}$ (nm)	625	625
Beer's law range ( $\mu\text{g mL}^{-1}$ )	2.7–53.8	10–244
Ringbom optimum range ( $\mu\text{g mL}^{-1}$ )	8.0–40.0	16.0–120
LOD ( $\mu\text{g mL}^{-1}$ )	0.013	0.750
LOQ ( $\mu\text{g mL}^{-1}$ )	0.24	1.36
$\epsilon$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	$1.40 \times 10^4$	$0.36 \times 10^4$
Stability (hrs) up to 30 °C	72	24
Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ )	0.073	0.238
Regression equation*: Slope ( $m$ )	0.0220	0.0086
Intercept ( $b$ )	0.0751	–0.0022
Correlation coefficient ( $r$ )	0.9998	0.9998
Range of error%	$\pm 1.37$	$\pm 0.82$

\*  $A = mC + b$ , where  $A$  is absorbance and  $C$  is the concentration ( $\mu\text{g mL}^{-1}$ ).

The minimum level at which the investigated compound can be reliably detected (limit of detection, LOD) and

quantified (limit of quantitation, LOQ) was determined experimentally for the proposed methods. The LOD was expressed as the concentration of drug that generated a response to three times of the signal to-noise (S/N) ratio, and the LOQ was 10 times of the S/N ratio. The LOD of FEX and IRB attained as defined by IUPAC [59],  $\text{LOD}_{(k=3)} = k \times S_a / b$  (where  $b$  is the slope of the calibration curve and  $S_a$  is the standard deviation of the intercept), was found to be 0.013 and 0.750  $\mu\text{g mL}^{-1}$  for FEX and IRB, respectively. The LOQ was also attained according to the IUPAC definition,  $\text{LOQ}_{(k=10)} = k \times S_a / b$ , and was found to be 0.24 and 1.36  $\mu\text{g mL}^{-1}$ , respectively. Sandell's index represents the number of micrograms or nanograms of the determinant per milliliter of a solution having an absorbance of 0.002 for the cell path length of 1 cm and is a suitable parameter for expressing and comparing the sensitivity of developed spectrophotometric method. For more accurate analysis, Ringbom optimum concentration range was calculated [60]. Table 1 shows the analytical parameters for the determination of FEX and IRB using the proposed method.

The accuracy and precision of the proposed methods was established by measuring the content of FEX or IRB in pure form at four different concentration levels. The intra-day precision of the proposed method was performed by carrying out six independent analyses at each concentration during the same day. In the same manner, the inter-day precision was also evaluated by measuring the cited drugs content at each concentration level on 5 consecutive days by the proposed method (Table 2). The RSD% values of intra-day and inter-day studies showed that the precision was good (Table 2). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error ( $E_r\%$ ) between the measured concentrations and taken concentrations for FEX and IRB (Bias%). The results obtained are compiled in Table 2 and show that the accuracy was good.

**Table 2.** Analysis of FEX and IRB with NBB in bulk powder.

Method	Drug taken ( $\mu\text{g mL}^{-1}$ )	Intra-day (n=6)			Inter-day (n=6)		
		Found $\pm$ SD ( $\mu\text{g mL}^{-1}$ )	RSD%	$E_r\%$	Found $\pm$ SD ( $\mu\text{g mL}^{-1}$ )	RSD%	$E_r\%$
FEX–NBB	5.00	5.08 $\pm$ 0.06	1.18	1.60	5.08 $\pm$ 0.07	1.38	1.60
	10.00	9.97 $\pm$ 0.08	0.80	–0.30	9.98 $\pm$ 0.06	0.60	–0.20
	20.00	20.25 $\pm$ 0.12	0.59	1.25	20.20 $\pm$ 0.10	0.49	1.00
	40.00	40.12 $\pm$ 0.21	0.52	0.30	40.07 $\pm$ 0.19	0.47	0.18
	10.00	9.98 $\pm$ 0.14	1.40	–0.20	9.97 $\pm$ 0.10	1.00	–0.30
IRB–NBB	50.0	50.72 $\pm$ 0.56	1.10	1.44	50.69 $\pm$ 0.47	0.93	1.38
	100.0	100.08 $\pm$ 1.03	1.03	0.08	100.02 $\pm$ 0.90	0.90	0.02
	200.0	200.05 $\pm$ 1.32	0.66	0.02	200.03 $\pm$ 1.28	0.64	0.01

### 3.6. Application to Analysis of Pharmaceutical Formulations

The proposed techniques were applied to the tablets and capsules. The ingredients in the tablets and capsules did not interfere in the experiments. The applicability of the proposed methods for the assay of FEX in formulations was examined by analyzing various formulations and the results

are tabulated in Table 3 were compared to the official non-aqueous titration method for FEX and IRB [3] by means of  $t$ - and  $F$ -values at 95% confidence level. In all cases, the average results obtained by the proposed methods and official method were statistically identical, as the difference between the average values had no significance at 95% confidence level. The low values of RSD show the results are reproducible. The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of FEX



and IRB in pure form and in formulations. The commonly used additives such as starch, lactose, glucose, titanium

dioxide, and magnesium stearate do not interfere with the assay procedures.

**Table 3.** Determination of FEX and IRB in different pharmaceutical formulations by the proposed and official methods.

Formulation	Label claim	% Recovery <sup>a</sup> ±SD	
		Proposed method	Official method [3]
Fexodine <sup>b</sup>	60 mg FEX/cap	100.80±0.58	99.71±0.52
		$t=1.72$	$t=1.25$
		$F=1.24$	
Fenadin-120 <sup>c</sup>	120 mg FEX/tab	100.50±0.85	99.69±0.55
		$t=1.31$	$t=1.24$
		$F=2.39$	
Fenadin-180 <sup>c</sup>	180 mg FEX/tab	102.40±0.81	100.52±0.64
		$t=2.48$	$t=1.82$
		$F=1.60$	
Rovil-75 <sup>c</sup>	75 mg IRB/tab	101.12±0.86	100.39±0.91
		$t=1.94$	$t=2.06$
		$F=1.12$	
Rovil-150 <sup>c</sup>	150 mg IRB/tab	100.35±0.94	101.03±0.1.13
		$t=1.76$	$t=1.49$
		$F=1.45$	

<sup>a</sup> Five independent analyses. At 95% confidence level t-value is 2.776 and F-value is 6.26. <sup>b</sup> Supplied by KIMI, Syria and <sup>c</sup> supplied by BPI, Syria.

## 4. Conclusion

The developed spectrophotometric method describes the use of extractive ion-pair complexation reaction with acid dye for the determination of FEX and IRB in pure form and pharmaceutical formulations. The proposed method is accurate, precise and use simple and lower reagent consumption. Therefore, this approach could be considered for the analysis of FEX and IRB in the quality control laboratories. Method is sufficiently sensitive to permit determination even down to 0.013 and 0.75 µg mL<sup>-1</sup> of FEX and IRB, respectively. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulation excipients in the estimation. The commonly used additives such as starch, lactose and magnesium stearate do not interfere with the assay procedures.

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