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Simultaneous resolution of febuxostat and diclofenac potassium in pure form and in their multi-ingredient formula

Shimaa A. Mahmoud[1]*, Amira M. El-Kosasy[2] and Fatma A. Fouad[1]

[1] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
[2]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University.
*Correspondence: shimaa.ahmed22@azhar.edu.eg

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Abstract: Easy, precise, and accurate three spectrophotometric procedures for successive resolution of febuxostat and diclofenac potassium in their multi-ingredient formula without prior separation were established and validated. Second derivative (^{2}D), dual-wavelength (DW), and ratio subtraction (RS) are the names of these procedures. They showed good linearity in the ranges of 2.0-20.0 µg/mL for febuxostat and 5.0–50.0 µg/mL for diclofenac potassium with strong determination coefficients (r^2). For ^{2}D (r^2 = 0.9995 - 0.9998), DW (r^2 = 0.9997 -0.9998) and RS (r^2 = 0.9995 - 0.9999) for febuxostat and diclofenac potassium; respectively. By using the proposed procedures, the limits of detection and the limits of quantitation for febuxostat and diclofenac potassium were 0.135–0.580 µg/mL and 0.408–1.756 µg/mL, respectively. They were also successfully used to test two drugs in their multi-ingredient formula, with the findings being checked, statistically analyzed, and found to be consistent with those obtained by a previously published method.

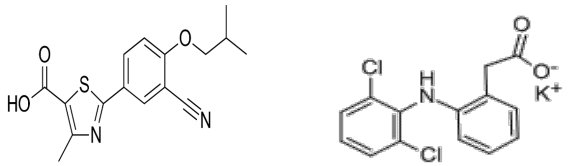
Keywords: Febuxostat; Diclofenac; Dual-wavelength; Second derivative; Ratio subtraction.

1. Introduction

Febuxostat (FEB) is 2 (3-cyano 4-[2-methyl propoxy] phenyl) 4 methyl-1, 3-thiazole 5-carboxylic acid[1] (Figure 1a). It works as a xanthine oxidase inhibitor and can be used to treat hyperuricemia and chronic gout[2]. It is a nonpurine selective xanthine oxidase inhibitor. FEB was found to be more effective than allopurinol at lowering serum uric acid levels[3]. Some drug forms containing an NSAID such as diclofenac potassium (DIC) are co-formulated to reduce inflammation and control pain in gout attacks. DIC is the salt of potassium 2-(2, 6-dichloranilino) phenyl acetic acid[1] (Figure 1b). Few analytical techniques for simultaneous study of FEB and DIC were found in the literature review. These methods include: spectrophotometry [4-7], HPTLC [7, 8] and HPLC methods [9, 10].

1.1. Theoretical background of the suggested procedures.

1.1.1. Derivative spectrophotometry is a benefit analytical method for extracting qualitative and quantitative data from overlapping curves, as well as removing the effects of standard shifts and standard tilts induced by finding other compounds in a sample. The properties recorded can permit evaluation of one or a few analytes without first separating or purifying them[11]. Derivative spectrophotometry has become very useful in recent years as an additional method for resolving various analytical problems. It's used in a variety of fields, including pharmaceutical, forensic, clinical, and biochemical analysis, as well as inorganic and organic analysis[12]. Furthermore, the second derivative procedure can be used to evaluate drug substances in the presence of impurities by choosing a wavelength with no influence from the impurity (zero crossing) and a suitable value for the drug to be measured. For the assay of drugs in mixtures and multi-ingredient formulas, it has become a well-established technique[13, 14]



(a) **(b)**

Figure 1: Chemical structures of (a) Febuxostat and (b) Diclofenac potassium (Sweetman SC[1]).

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1.1.2. Dual wavelength: In the presence of an interfering object, dual wavelength spectroscopy is an effective technique for analysing a component. Dual analytical wavelengths were chosen to eliminate interference by making the absorbance difference zero for one drug when analysing the other [15-17].

1.1.3. Theory of ratio subtraction technique [18-21] The method is dependent on this; in case you have a mixture of two drugs (A) and (B) with overlapping spectra and (B spectrum) is extended than (A), you can determine (A) by dividing the mixture's spectrum by a certain concentration of (B) as a divisor (B'). (A / B' + constant) would be the new curve as a result of the division. We can get the original curve by subtracting this constant, then multiplying the new curve obtained after subtraction by (B') (The divisor). This may be summarized within taking after equations[18-21]:

$$(A+B) / B' = (A / B') + (B / B') = (A / B') + \text{Constant} \quad (1)$$

$$(A / B') + \text{Constant} - \text{Constant} = (A / B') \quad (2)$$

$$(A / B') \times (B') = (A) \quad (3)$$

The constant can be calculated from the curve (A+B) / B' by the straight line which is parallel to the wavelength axis within the region where (B) is extended.

2. Methods

2.1. Instrumental

All absorbance estimations were performed with a Shimadzu UV Vis 1601 PC spectrophotometer (Tokyo, Japan) associated to an IBM compatible device and an HP laser jet printer, and a quartz cell with a 1 cm path length. UV- Probe personal spectroscopy software version 2.21 (Shimadzu) is the included software. The speed of scanning is 2800 nm/min and the band spectral is 2 nm.

2.2. Chemicals & reagents

- Pure febuxostat: B. No. OP-FAB/06/16/001 was kindly provided by Mash Premiere with purity 100.61% according to supplier.
- Pure diclofenac potassium: B. No. DK/ 1808 /0080B was kindly provided by The Arab Company for Gelatin & Pharmaceutical products with purity 99.85% according to supplier.
- Methanol (Sigma – Aldrich, USA) analytical grade.
-Pharmaceutical multi-ingredient formula
- Xanfeb DSR® tablets, it is labelled to contain 40 mg FEB and 100 mg DIC manufactured by Indoco Remedies, India) were purchased from pharmacies.

2.3. Standard solutions

FEB & DIC stock standard solutions were made with methanol at a concentration of 1 mg/mL.The stock solutions were diluted with methanol to prepare the working solutions of both drugs at a concentration of (100µg/mL).

2.4. Procedures

2.4.1. Spectral characteristics

Utilizing methanol as a blank, ^{0}D of FEB (2.0-20.0 µg /mL) and DIC (5.0-50.0 µg /mL) were recorded over the extend of 200 – 400 nm and put away within the computer. The stored data were subjected to different procedures to obtain second derivative spectra (^{2}D), dual wavelength (DW) and ratio subtraction spectrophotometry (RS).

^{2}D spectra of the drugs were recorded and the peak amplitude at 353 nm for FEB and 246 nm for DIC (Figures 2, 3) was calculated using scaling factor 300 and = 16000 against methanol as a blank.

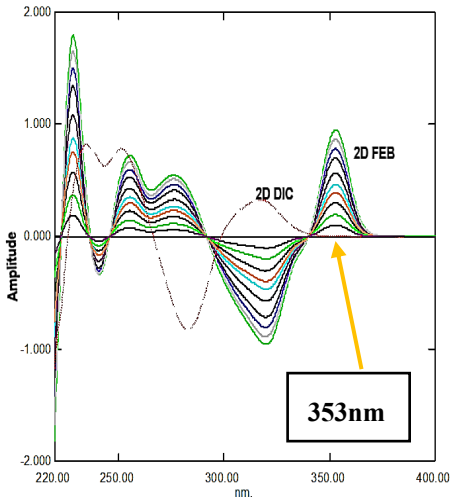


Figure 2: Second derivative of different concentrations of FEB spectra (2-20 µg/ mL) at 353 nm and second derivative of DIC (20 µg /mL).

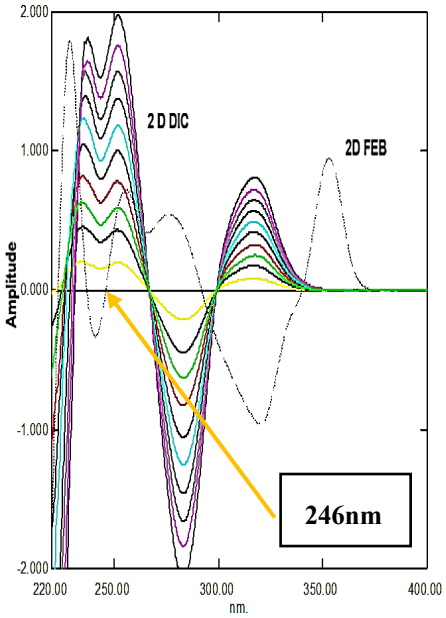


Figure 3: Second derivative of different concentrations of DIC spectra (5-50 µg/mL) at 246nm and second derivative of FEB (20 µg /mL).

DW method was chosen for a mixture of two drugs because it allowed for a zero-absorbance difference for one drug and a high absorbance difference for the

other. The spectra of the prepared standard solutions are scanned from 200 to 400 nm. FEB & DIC solution absorbance differences were estimated at 299.8 and 265 nm or 279.8 and 249.2 nm for FEB & DIC, respectively (Figure 4).

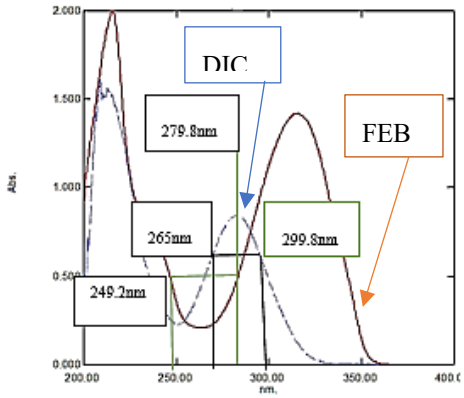
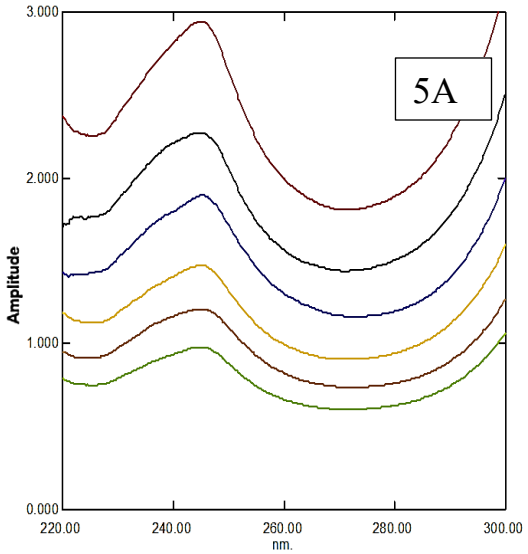
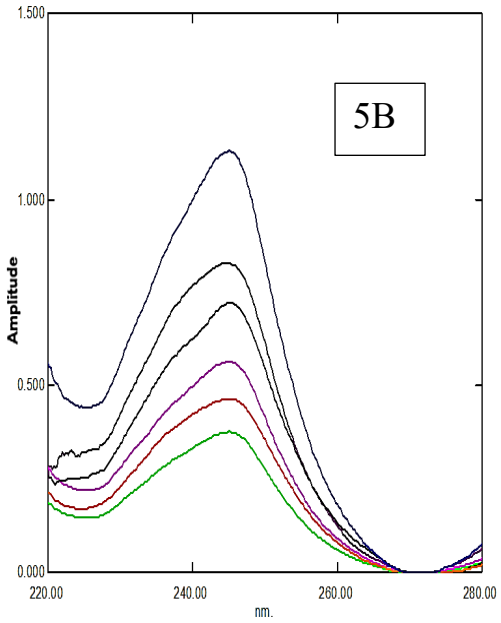
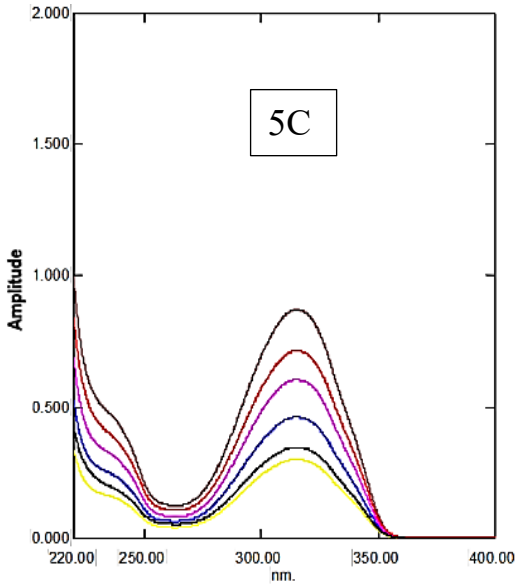


Figure 4: Zero order spectra of (20 µg /mL) of FEB & DIC in methanol.

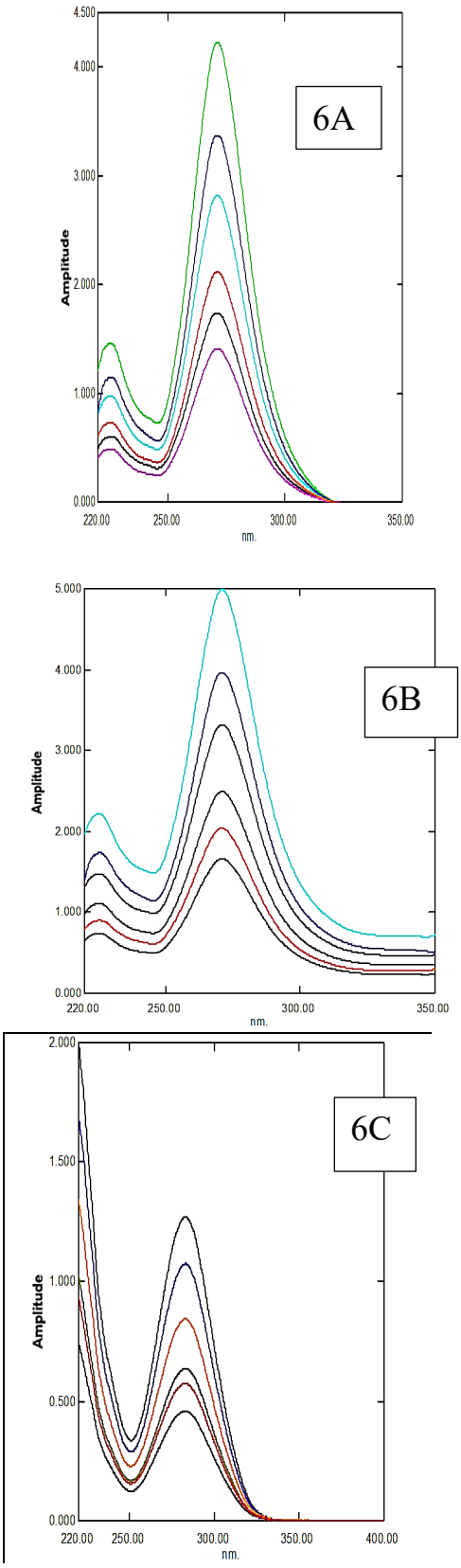
RS procedure, to obtain the ratio spectra, divide the stored FEB spectra by the spectrum of DIC (20 µg /mL) (Figure 5A). The constant was subtracted from every ratio spectrum (the value of amplitude at 271 nm in the ratio spectra), then the obtained spectra were multiplied by the divisor spectrum (Figure5B). At 316 nm, the amplitude values were calculated (Figure 5C). To determine DIC, divide the stored DIC spectra by the spectrum of FEB (20 µg /mL) to obtain the ratio spectra (Figure 6A). The constant was subtracted from every ratio spectrum (amplitude value at 321 nm in the ratio spectra), then the spectra obtained were multiplied by the divisor spectrum (Figure 6B). At 283 nm the amplitude values were measured. (Figure 6C).







Figures 5: Ratio spectra of laboratory prepared mixture of FEB and DIC **(A):** After using (20 µg/mL) of DIC as divisor. **(B):** After subtraction of the constant at 271 nm. **(C):** After multiplying by the divisor again.



Figures 6: Ratio spectra of laboratory prepared mixture of FEB and DIC **(A):** After using (20 µg/mL) of FEB as divisor. **(B):** After subtraction of the constant at 321 nm. **(C):** After multiplying by the divisor again.

2.4.2. Laboratory prepared mixtures

Mixtures of different ratios for FEB & DIC were developed by taking aliquots from the corresponding working solutions (100 µg /mL), mixing well, and volume was completed with methanol to be analysed by the proposed methods.

^{2}D procedures, FEB or DIC concentrations determined from the corresponding regression equation by measuring peak amplitude at 353 nm or at 246 nm for FEB or DIC; respectively.

DW The absorbance difference calculated between at 299.8 and 265 nm or at 279.8 and 249.2 nm for FEB or DIC; respectively and the concentrations of the drug were calculated from the corresponding regression equation.

RS procedures for calculation of FEB the absorption spectra of two drugs were divided by that of DIC (20µg/mL), to get the ratio spectra. The constant (amplitude value at 271 nm in the ratio spectra) was subtracted from each ratio spectrum, followed by multiplication of the obtained spectra by the divisor spectrum and the amplitude values were measured at 316 nm (Figures 5A, B and C)**.** While for determination of DIC, the absorption spectra of mixture were divided by that of FEB (20µg/mL), to get the ratio spectra. The constant (amplitude value at 321 nm in the ratio spectra) was subtracted from each ratio spectrum, followed by multiplication of the gotten spectra by the divisor spectrum and measuring the amplitude values at 283 nm (Figures 6A, B and C)**.**

2.4.3. Pharmaceutical multi-ingredient formula

Ten tablets of Xanfeb DSR® were adequately weighed and pulverized. In a 50-mL volumetric flask, a quantity of powder equivalent to 80 mg FEB or 200 mg DIC was transferred. A volume of 25 mL methanol was applied to the flask, which was sonicated for 30 minutes before being completed to volume with methanol followed by filtration. One milliliter of this solution was diluted in 100 mL of methanol to yield a final solution containing 16 µg /mL FEB or 40 µg /mL DIC. The nominal contents of the tablets were calculated utilizing the corresponding regression equations or the previously plotted calibration graphs.

3. Results

3.1. Methods Validation

The developed spectrophotometric methods were validated using the guidelines of ICH [22].

3.1.1. Linearity

Aliquots of standard FEB and DIC solution (100.0 µg /mL), equivalent to (2.0-20.0 µg) and (5.0-50.0 µg) for FEB and DIC, respectively, were correctly transferred into a series of 10-mL volumetric flasks and methanol was used to complete

the flasks to the desired volume. The prepared solutions' UV absorption spectra were screened from 200 to 400 nm using methanol as a blank.

Second Derivative procedures**:** The amplitude values of second derivative of FEB measured at 353 nm versus the final drug concentrations in µg/mL were plotted to get the calibration graph and the regression equation was derived. While DIC measured amplitude values of second derivative of DIC at 246 nm versus the final drug concentrations in µg/mL were plotted to get the calibration graph and the regression equation was derived. The parameters of the regression equations were presented in Table 1.

Dual wavelength procedures: The absorbance difference between 299.8 and 265 nm measured for determination of FEB. While absorbance difference measured between 279.8 and 249.2 nm was selected for determination of DIC. Calibration curve was designed by plotting the absorbance difference versus drug concentrations in µg/mL to get the calibration graph and the regression equation was derived. The parameters of the regression equations were presented in Table 1. Ratio subtraction procedures**:** The calibration graph was generated by plotting the calculated amplitude values of FEB at 316 nm against the final drug concentrations in µg/ mL, and the regression equation was obtained. Also, the calibration graph was generated by plotting the calculated amplitude values of DIC at 283nm against the final drug concentrations in µg/ mL, and the regression equation was obtained. Table 1.

3.1.2. Limits of detection and quantitation

According to ICH guidelines (LOD) and (LOQ) were calculated from the following equations:

$LOD = 3.3\ \sigma / S$ and $LOQ = 10\ \sigma / S$

Where σ: the residual standard deviation of a regression lines while S: the slope of the calibration curve.

LOD and LOQ values were calculated, and the obtained results showed the sensitivity of the proposed methods as shown in Table 1.

3.1.3. Accuracy

The accuracy of the methods was checked by using the proposed procedures to evaluate five different concentrations (4, 8, 12, 16 and 20 µg/mL) for FEB and (10, 15, 20, 25, 30, 35 µg/mL) for DIC, each was evaluated in triplicate, in their pure form, on the same day (intra-day) and three days later (inter-day), and the accuracy was calculated as percent recovery % R. The % recoveries for the proposed spectrophotometric methods were in the range of (99.56 to 100.52%) for both drugs as shown in Table1.

3.1.4. Precision

The precision of the methods was estimated by using the suggested procedures to assess three different concentrations (4, 8 and 16) µg/mL (10, 20 and 30) µg/mL for FEB and DIC; respectively. Precision was measured as percent relative standard deviation percent RSD for each in triplicate, in their pure state on the same day (intra-day) and three days later (inter-day) as shown in Table 1.

3.1.5. Robustness

The robustness of the posited procedures was examined by repeating each procedure with small alterations in the wavelength at which results are obtained (0.2 nm). No marked changes were observed in the results, confirming robustness of the proposed methods.

Table 1: The regression parameters and validation results for determination of FEB and DIC by the proposed methods

Parameter	^{2}D		DW		RS	
	FEB	DIC	FEB	DIC	FEB	DIC
Wave length (nm)	353	246	(299.8 - 265)	(279.8 - 249.2)	316	283
Linearity range µg/ mL	2.0-20.0	5.0-50.0	2.0-20.0	5.0-50.0	2.0-20.0	5.0-50.0
Slope	0.0476	0.0322	0.048	0.0296	0.0751	0.0415
Intercept	0.0052	0.0262	0.0074	0.0126	0.0031	0.0205
Determination coefficient (r2)	0.9995	0.9998	0.9997	0.9998	0.9995	0.9999
Accuracy[a] (%R)	99.78	100.05	100.17	100.52	100.05	99.56
Inter-day precision[b] RSD%	0.989	1.734	1.052	0.601	0.521	0.207
Intra-day precision[b] RSD%	0.998	0.170	1.585	0.731	1.115	0.512
LOD µg/ mL	0.253	0.580	0.412	0.395	0.137	0.135
LOQ µg/ mL	0.767	1.756	1.250	1.197	0.414	0.408

[a] mean of 5 determinations, [b] mean of 3 determination

3.1.6. Selectivity

The specificity of the proposed methods was confirmed by testing them on laboratory-prepared mixtures of FEB and DIC at various concentrations within the range of linearity. Good recoveries were obtained as shown in Table 2.

Table 2: Determination of FEB and DIC in laboratory prepared mixtures by the proposed methods

Lab prepared mixture µg/ mL		^{2}D		DW		RS	
		% R					
FEB	**DIC**	**FEB**	**DIC**	**FEB**	**DIC**	**FEB**	**DIC**
4	10	100.4	99.23	101.78	98.54	101.95	101.05
5	12.5	98.54	98.84	101.78	99.89	97.52	99.86
6	15	102.08	100.44	101.77	100.75	98.57	100.55
8	20	99.31	101.19	99.41	99.39	101.84	100.77
10	25	101.98	98.66	98.49	101.08	98.76	99.43
12	30	101.27	101.84	100.53	99.47	100.88	99.51
10	10	98.17	101.03	99.69	101.64	99.79	101.12
5	15	102.02	99.41	97.96	98.87	101.72	98.8
15	5	100.93	99.42	101.34	98.24	99.67	102.06
10	20	98.14	99.73	99.87	98.69	101.83	98.76
Mean % ± SD		100.28 ± 1.618	99.98 ± 1.082	100.26 ± 1.405	99.35 ±1.161	100.25 ±1.533	100.19 ±1.090

3.1.7. Application in multi-ingredient formula

For the determination of FEB and DIC in their tablets, the proposed procedures were used. The results were satisfactory and in line with the label argument, suggesting that there was no disruption from excipients or additives. In addition, the standard addition technique was used to verify the specificity of the procedures mentioned. It was carried out by adding known quantities of FEB or DIC in their pure forms to already analyze multi-ingredient formula and the percent recovery %R of the pure added concentrations was calculated Table 3. The obtained results were statistically compared with the results obtained using the reported procedure[10] which involved RP-HPLC using mixture of KH_2PO_4 (0.02 M) and sodium hydroxide: acetonitrile: methanol (35:9:56, v/ v/v) as mobile phase. Detection was carried out at 290 nm is determined in concentration range of 12.5-75 µg/ mL and 5-30 µg/ mL for DIC & FEB; respectively. Using the t-test and F-value[23] at a 95% confidence level, no major variations were observed, suggesting that the proposed methods for the study of two drugs in their multi-ingredient formula were accurate and precise as shown in Table 4.

Table 3: Determination of FEB and DIC in pharmaceutical dosage forms by the proposed methods and results obtained by standard addition technique.

Xanfeb DSR®			^{2}D			DW			RS	
	Claimed taken (µg/ml)	Pure added (µg/mL)	Mean% ± SD	Pure found (µg/mL)	R% of pure added	Mean% ± SD	Pure found (µg/mL)	R% of pure added	Mean% ± SD	Pure found (µg/mL)	R% of pure added
FEB	4	4	99.78±1.380	3.913	97.83	99.81±1.344	3.918	97.95	99.85±1.330	3.916	97.90
	4	8		7.961	99.51		7.966	99.58		7.974	99.68
	4	12		12.038	100.32		12.044	100.37		12.035	100.29
	Mean% ± SD			99.22 ± 1.27			99.30± 1.234			99.29 ± 1.242	
DIC	10	10	100.21±1.086	10.095	100.95	100.20±1.208	10.091	100.91	100.26± 0.988	10.087	100.87
	10	20		19.614	98.07		19.626	98.13		19.628	98.14
	10	30		29.505	98.35		29.505	98.35		29.496	98.32
	Mean% ± SD			99.12 ± 1.588			99.13±1.545			99.11 ± 1.527	

Table 4: Statistical comparison for the results obtained by the proposed methods and the reported methods [10] for the analysis of FEB and DIC.

	^{2}D method			DW method			RS method			Reported method [10]		
	Taken (μg mL^{-1})	Found* (μg/mL)	% Recovery	Taken (μg/mL)	Found* (μg/mL)	% Recovery	Taken (μg/mL)	Found* (μg/mL)	% Recovery	Taken (μg/mL)	Found * (μg/mL)	% Recovery
FEB	4	3.930	98.25	4	4.035	100.88	4	3.935	98.38	5	5.06	101.19
	8	7.977	99.71	8	7.916	98.95	8	7.967	99.59	10	10.136	101.36
	12	12.047	100.39	12	11.894	99.12	12	12.049	100.41	15	14.822	98.81
	16	16.282	101.76	16	16.104	100.65	16	16.291	101.82	20	19.812	99.06
	20	19.758	98.79	20	19.82	99.1	20	19.808	99.04	25	25.273	101.09
Mean %± S.D.	99.78±1.380			99.81±1.344			99.85±1.330			100.30±1.255		
t-test	0.626(2.306)			0.598(2.306)			0.555(2.306)			-		
F-test	1.210(6.388)			1.148(6.388)			1.124(6.388)			-		
DIC	10	10.192	101.92	10	10.032	100.32	10	10.186	101.86	20	19.99	99.95
	20	19.882	99.41	20	20.27	101.35	20	19.854	99.27	30	29.817	99.39
	25	25.168	100.67	25	24.973	99.89	25	25.113	100.45	40	40.268	100.67
	30	29.853	99.51	30	29.838	99.46	30	29.973	99.91	50	50.105	100.21
	40	39.816	99.54	40	40.104	100.26	40	39.924	99.81	60	59.898	99.83
Mean %± S.D.	100.21±1.086			100.20±1.208			100.26±0.988			100.01 ± 0.473		
t-test	0.378(2.306)			0.371(2.306)			0.510 (2.306)			-		
F-test	5.262(6.388)			4.722(6.388)			4.354 (6.388)			-		

*Average of three separate determinations. The values between parentheses are the tabulated t and F values at $P = 0.05$[23].

4. Discussion

The literature review for the determination of cited drugs includes spectrophotometry [4-7], Habib et al.[4] developed spectrophotometric methods for determination FEB in concentration range 2–10 μg/mL and DIC 2.5-25 μg/mL, while linear range of Modi et al.[5] were 3-15 μg/mL and 6-30 μg/mL for FEB and DIC; respectively, and Derasari et al.[6] determined FEB and DIC in concentration range 2-9 μg/mL and 5-22.5 μg/mL for FEB and DIC; respectively, On the other hand, El-Yazbi et al.[7] developed spectrophotometric methods for determination of FEB and DIC in linear range 2–14 and 4–18 μg/mL for FEB and DIC; respectively, which give priority of the proposed methods to be determined FEB and DIC in wider range as our linear range 2–20 and 5–50 μg/mL for FEB and DIC; respectively, comparison between the proposed methods and other spectrophotometric reported methods was presented in table 5. The literature review showed also HPTLC and HPLC methods [7-10] for the determination of cited drugs, El-Yazbi et al. developed two HPTLC methods and one HPLC method for determination of FEB and DIC [7-9]. In the first one [7] used chloroform–methanol as mobile phase, in [8] used petroleum ether-chloroform-ethyl acetate-formic acid as mobile phase. While in [9] used methanol-formic acid as mobile phase. Vaibhav et al. [10] used KH_2PO_4 and sodium hydroxide: acetonitrile: methanol as mobile phase. Our methods were found to be simpler, less cost, non-destructive and more eco-friendly than others reported methods.

Zero order spectra of two drugs show severed over lapping (Figure 4). Therefor ^{2}D, DW and RS methods were selected for resolving over lapping of two drugs without previous separation. The survey of literature review indicated the application of ^{1}D-spectrophotometry for the simultaneous determination of two cited drugs [6]. Hence ^{2}D determination of FEB and DIC. It was noteworthy to mention that although the peak of DIC at 292 nm is very sensitive however it gave non-linear result. In RS method different concentrations of FEB (4, 8, 12, 14 and 20 μg/mL) and DIC (5, 10, 15 and 20 μg/mL) were tried to choose the best divisor. The best one was (20 μg/mL), as it gave best results in accordance with selectivity and sensitivity.

Table 5: Comparison for the results obtained by the proposed methods and the reported methods for the analysis of FEB and DIC.

Parameter	Proposed methods		Reported method[4]		Reported method[5]		Reported method[6]		Reported method[7]	
Linearity range µg/ mL	**FEB**	**DIC**	**FEB**	**DIC**	**FEB**	**DIC**	**FEB**	**DIC**	**FEB**	**DIC**
	2–20	5–50	2-10	2.5-25	3-15	6-30	2-9	5-22.5	2–14	4–18
Inter-day precision RSD%(range)	0.989-1.303	0.601-1.734	0.96	1.35	0.55-0.95	0.71-1.38	0.30-1.19	0.39-1.08	0.47-2.03	0.83-1.95
Intra-day precision RSD%(range)	0.744-1.585	0.170-0.731	0.66	0.45	1.04-1.42	0.88-1.69	0.22-1.07	0.22-1.16	0.34-1.90	1.08-2.11
LOD µg/ mL (range)	0.137-0.412	0.135-0.580	-	-	0.02-0.16	0.23-0.80	0.054-0.145	0.145-0.84	0.56-0.58	1.16-1.19
LOQ µg/ mL (range)	0.141-1.250	0.408-1.756	-	-	0.07-0.49	0.70-2.43	0.165-0.439	0.439-2.56	1.87-1.92	3.85-3.96

5. Conclusion

The proposed study describes three different spectrophotometric methods for the simultaneous estimation of FEB and DIC in bulk or in their combination; second derivative, dual wavelength and ratio subtraction methods. The proposed methods are easy, fast, and inexpensive from an economic point of view**.** They can be taken into account another choice tools for the regular analysis of the two drugs with minimum sample preparation since it takes less time and does not necessitate multiple elaborate treatments or time-consuming extraction procedures.

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Ethics statement: NA.

Author contribution: Authors AE and FF designed the study and wrote the protocol. SM performed the experimental work and statistical analysis. Authors AE and FF supervise the analyses of the study. Author SM wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript.

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