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Smart Spectrophotometric Methods Based on Feasible Mathematical Processing and Classical Chemometry for the Simultaneous Assay of Alcaftadine and Ketorolac in Their Recently Approved Pharmaceutical Formulation.

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Abstract

Alcaftadine (ALC) and Ketorolac tromethamine (KET) binary combination is a new co-formulated pharmaceutical product that is used to treat allergic conjunctivitis. The present work utilizes three simple, precise, accurate, and rapid spectrophotometric and chemometric methods to determine the recently released pharmaceutical formulation. The methods include the first derivative of absorbance spectrum (Method A), dual-wavelength (Method B), and absorbance subtraction methods (Method C). ICH guidelines have been used to validate the developed methods. Linear regression lines were found over the concentration range of 1-9 and 1-12 µg mL^{-1}, 1-8 and 2-10 µg mL^{-1}, and 2-10 and 2-10 µg mL^{-1} of ALC and KET for methods A, B, and C respectively. All the methods showed correlation coefficients higher than 0.999, in addition to low LOQ and LOD which indicate high sensitivity of the proposed methods.

Keywords: Alcaftadine, Ketorolac tromethamine, spectrophotometric, chemometric, pharmaceutical formulation.

1. Introduction

Allergic conjunctivitis is a highly common eye disease, as it affects up to 40% of human beings. Allergic conjunctivitis has symptoms of increasing eye redness, swelling, and itching [1].

Alcarex KT ® is a recently approved eye drops in India, used to treat allergic conjunctivitis [2]. Composed of ALC 0.25% and KET 0.4%.

ALC is 6,11-dihydro-11-(1-methyl-4-piperidiny-lidene)-5H-imidazo [2, 1-b] [3] benzazepine-3-carboxaldehyde (Fig. 1). ALC is an antihistaminic and mast cell stabilizer drug used for the prevention of allergic conjunctivitis-related itching. It also exhibits modulatory activity on the mobilization of immune cells and the stabilizing effects of mast cells. It works by blocking the release of histamine from mast-cell [3, 4]. Alcaftadine is not extensively degraded by microsomal cytochromes, but it is quickly transformed to the carboxylic acid metabolite by one or more cytosolic enzymes [5]. ALC is stable under photolytic, thermal and neutral hydrolysis conditions. Under acidic hydrolysis, it is transformed to ALC DP 1 (fig. 1). Under alkaline hydrolysis, it is transformed to ALC DP 2 and 3 (fig. 1). Under oxidative conditions, it is transformed to ALC DP 4 and 5 (fig. 1) [6].

KET is 2-Amino-2- (hydroxymethyl) propane1, 3-diol (1RS) -5- benzoyl-2, 3-dihydro-1Hpyrrolizine-1-carboxylate (Fig. 2). KET is a non-steroidal anti-inflammatory drug (NSAID). It is a derivative of heterocyclic acetic acid and used as an analgesic and to treat eye inflammation with an effect similar to that of the family of opioids [7]. The metabolic products of Ketorolac Tromethamine are the parent drug's hydroxylated and conjugated forms. The liver is responsible for the majority of the metabolism of Ketorolac Tromethamine [8]. Under acidic hydrolysis, Ketorolac Tromethamine is transformed to KET DP 1, 2 and 3 (fig. 2). Under alkaline and neutral hydrolysis, it is transformed to KET DP 3 (fig. 2). Under oxidative conditions, it is transformed to KET DP 5, 6 and 7 (fig. 2). Under alkaline and neutral photolytic conditions, it is transformed to KET DP 1, 6 and 9 (fig. 2). Under acidic photolytic conditions, it is transformed to KET

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DP 4, 6, 8 and 9 (fig. 2). Under thermal condition, it is transformed to KET DP 3 (fig. 2) [9].

Different methods have been reported for the assay of ALC and KET, including Spectrophotometric [4, 7, 10-15], LC-MS [6], HPLC [7, 16-20], HPTLC [21-24], fluorescence [25, 26] and electrochemical methods [27, 28].

Derivative spectrophotometry (DS) is a sophisticated modern method. It is based on derivative spectra originating from zero-order spectra as a parent. The removal of overlapping signals and the absence of background induced by other components present in a sample can be achieved by derivatizing the zero-order spectrum [29]. The above characteristics will make it possible to quantify one or a few compounds without having to separate or purify them first. Nowadays, DS is a very useful method for resolving a wide range of methodological issues. DS has applications in pharmaceutical, biological, and biochemical analysis, as well as inorganic and organic analysis [29].

ALC and KET cannot be assayed in a combined dosage form by direct spectrophotometric methods because of the overlap of their absorbance spectrum (Fig. 3). Therefore, the proposed work successfully developed three different spectrophotometric and chemometric methods for determining the stated drugs in their synthetic dosage formulation.

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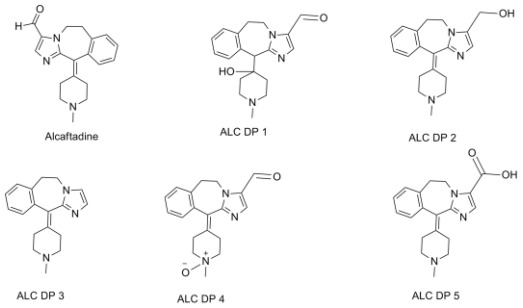

Fig.1: Chemical structures of Alcaftadine and its degredation products.

2. Experimental

2.1. Instrumentation

A T80 double beam UV–VIS spectrophotometer (pg instruments, Leicestershire, UK) connected to UVWin software was used for the spectrophotometric measurements. The measurements were taken in one-centimeter quartz cells.

Aquatron water still a4000d, double-distilled (Cole-Parmer, Staffordshire, UK).

2.2. Materials and reagents

ALC and KET were kindly supplied by Orchidia pharmaceuticals (Al-Obour, Cairo, Egypt) and Kahira pharmaceuticals (Shoubra City, Cairo, Egypt) for ALC and KET, respectively. Hydrochloric acid was supplied by Acros (Geel, Belgium). Ethanol was supplied by Fischer Scientific (Loughborough, U.K). Methanol and Acetonitrile were supplied by Merck (Darmstadt, Germany). Sodium hydroxide was supplied by El Nasr pharmaceutical chemical co. (Cairo, Egypt). Orchinohist ® eye drops labeled to contain 0.25% of ALC (B.N. 10-1219137), obtained from Orchidia pharmaceuticals (Al-Obour, Cairo, Egypt).

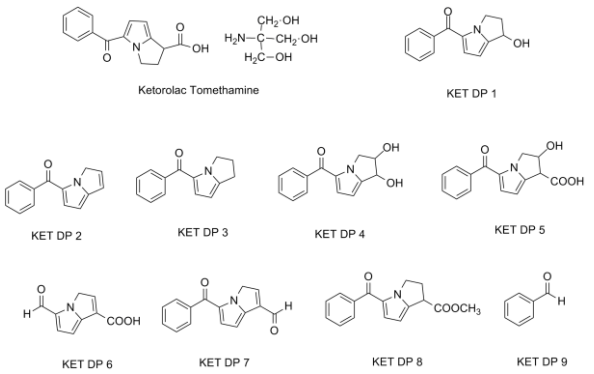

Fig.2: Chemical structures of Ketorolac tromethamine and its degredation products.

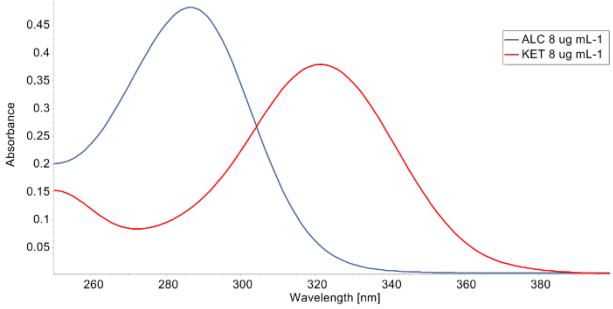

Fig. 3: Zero order UV spectrum (Both ALC and KET are 8 µg mL^{-1}).

2.3. Preparation of standard solutions

Stock standard solutions (200 µg mL^{-1}) of ALC and KET were prepared by dissolving 50 mg of each drug in 250- mL double distilled water then kept at 4°C. These stock solutions were further diluted with 0.01M NaOH (prepared by dissolving 0.4 g of NaOH in 1000 mL double distilled water) to obtain working standard solutions. Accurately calibrated aliquots of the standard solutions of ALC and KET were moved into separate sets for preparing various mixtures within the linearity range. Each set is made up of 10-mL volumetric flasks which have been diluted with 0.01M NaOH. All these solutions were stable for at least 20 days.

2.4. Preparation of a synthetic and separated ALC and KET pharmaceutical formulation

Unfortunately, Alcarex KT ® eye drops are not available in the local market of Egypt; Orchinohist ® eye drops labeled to contain 0.25% of ALC is available. So, to prepare synthetic Alcarex KT ® eye drops, 12 mg of KET were added to Orchinohist ® eye

drops to give a final concentration of 0.25% and 0.4% for ALC and KET, respectively. For separated ALC and KET determination, (Orchinohist ®) eye drops and (Acular LS ®) eye drops were determined

2.5. Optimizing of diluent solvent

Water, methanol, ethanol, acetonitrile, 0.01M NaOH, and 0.01M HCl were tested for dilution of stock solution.

2.6. Procedures

2.6.1. The first derivative of the absorbance spectrum method (Method A)

In this method, the first derivative of the original spectrum was done, then assaying each drug at a wavelength where the other drug was zero crossing as shown in (Fig. 4). So, any increase in absorbance is due to the selected drug only [30]. Procedure: A specific volumes were pipetted into a 10-mL volumetric flask in the range of 10-90 µg mL^{-1} and 10-120 µg mL^{-1} for ALC and KET, respectively, and diluted with 0.01M NaOH to get 1-9 µg mL^{-1} and 1-12 µg mL^{-1} for ALC and KET, respectively. The absorbance was scanned from 250 nm to 400 nm. After that first-order derivative was done using UVWin computer software. The absorbance was measured at 272 nm and 351 nm for ALC and KET, respectively.

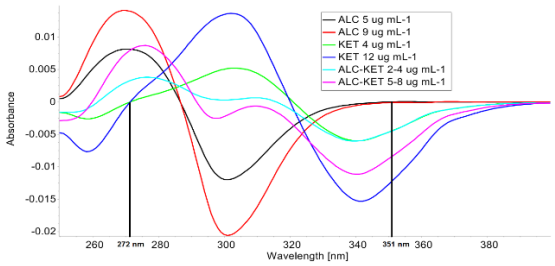

Fig. 4: First derivative method.

2.6.2. Dual-wavelength method (Method B)

In this method, two wavelengths for ALC were selected, at these wavelengths, KET absorbance was equal, so the absorbance difference was directly proportional to ALC concentration [8]. The concentration of KET in the mixture could be calculated directly as shown in (Fig. 5).

Procedure: A specific volumes were pipetted into a 10-mL volumetric flask in the range of 10-80 µg mL^{-1} and 20-100 µg mL^{-1} for ALC and KET, respectively, and diluted with 0.01M NaOH to get 1-8 µg mL^{-1} and 2-10 µg mL^{-1} for ALC and KET, respectively. The absorbance was measured at 289 nm and 351 nm for ALC and 342 nm for KET.

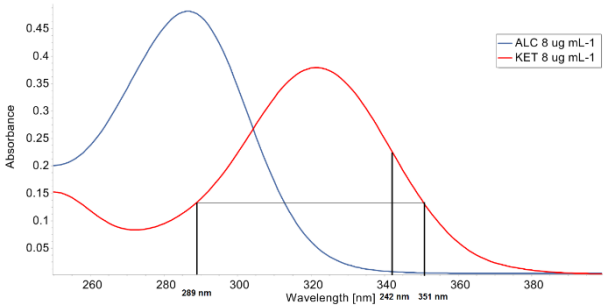

Fig. 5: dual wavelength method (Both ALC and KET are 8 µg mL^{-1}).

2.6.3. Absorbance subtraction method (Method C)

At the iso-absorption point, ALC and KET act as a one-component as they show equal absorptivity values. Consequently, by measuring the absorbance value at the selected iso-absorption point (λ_{iso}), the total concentration of both ALC and KET could be calculated using absorbance at (λ_{iso}), where the concentration of KET in the mixture could be calculated directly [31] as shown in (Fig. 6).

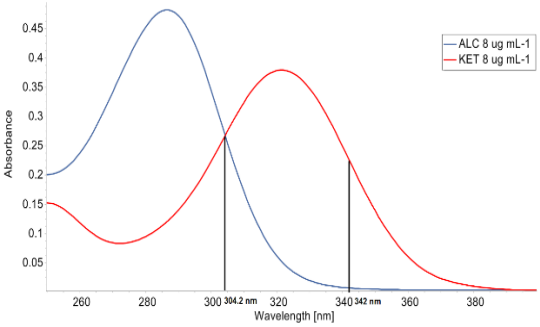

Fig. 6: Absorbance subtraction method (Both ALC and KET are 8 µg mL^{-1}).

Table 1: The regression and validation parameters for the proposed chemometric methods

Parameter	Method A		Method B		Method C	
	ALC	KET	ALC	KET	ALC	KET
Linear range (µg mL^{-1})	1.0– 9.0	1.0 – 12.0	1.0 – 8.0	2.0 – 10.0	2.0 – 20.0	2.0 – 10.0
Slope	0.00152	-0.00102	0.05554	0.02577	0.02851	0.02577
SD of slope (S_b)	0.000119	0.000013	0.00038	0.00038	0.00027	0.00038
Intercept	0.000269	-0.00020	0.01271	0.00971	0.01803	0.00971
SD of intercept (S_a)	0.000087	0.000095	0.00196	0.00229	0.00306	0.00229
Correlation Coefficient	0.9996	0.9996	0.9999	0.9996	0.9995	0.9996
SD of residuals ($S_{y,x}$)	1.1×10^{-7}	8.9×10^{-8}	3.8×10^{-5}	3.5×10^{-5}	2.9×10^{-4}	3.5×10^{-5}
LOD (µg mL^{-1})	0.19	0.31	0.12	0.30	0.35	0.30
LOQ (µg mL^{-1})	0.57	0.94	0.35	0.89	1.07	0.89

Table 2: Accuracy of the proposed chemometric methods using standard addition method

Amount taken (µg mL^{-1})		Amount added (µg mL^{-1})		Amount found (µg mL^{-1})		% Recovery ± SD	
ALC	**KET**	**ALC**	**KET**	**ALC**	**KET**	**ALC**	**KET**
Method A							
1.25	2.00	0.00	0.00	1.24	2.00	99.20 ± 2.35	100.00 ± 2.20
1.25	2.00	0.75	2.00	1.99	4.02	99.50 ± 1.80	100.38 ± 1.35
1.25	2.00	3.75	6.00	5.01	8.06	100.20 ± 1.18	100.31 ± 0.67
1.25	2.00	5.75	9.00	6.95	11.03	99.23 ± 0.49	100.29 ± 0.49
Method B							
1.25	2.00	0.00	0.00	1.25	1.99	100.00 ± 0.79	99.55 ± 3.63
1.25	2.00	0.75	1.00	2.02	3.02	100.80 ± 0.49	100.80 ± 0.53
1.25	2.00	2.75	3.00	4.06	4.98	101.45 ± 0.61	99.66 ± 1.17
1.25	2.00	4.75	5.00	5.89	6.97	98.20 ± 0.15	99.53 ± 0.50
Method C							
2.50	4.00	0.00	0.00	2.54	3.98	101.63 ± 2.91	99.37 ± 1.54
2.50	4.00	0.50	1.00	3.08	4.95	101.58 ± 0.55	98.93 ± 0.41
2.50	4.00	2.50	4.00	4.97	7.93	99.39 ± 0.80	99.15 ± 0.66
2.50	4.00	5.50	6.00	7.95	9.97	99.37 ± 0.92	99.65 ± 0.49

Procedure: A specific volumes were pipetted into a 10-mL volumetric flask in the range of 20-200 µg mL^{-1} and 20-100 µg mL^{-1} for ALC and KET, respectively, and diluted with 0.01M NaOH to get 2-20 µg mL^{-1} and 2-10 µg mL^{-1} for ALC and KET, respectively. The absorbance was measured at 342 nm for KET alone and 304.2 nm for the total.

2.6.4. Procedure for the analysis of pharmaceutical formulation

0.5 mLs of five synthetic pharmaceutical formulations were diluted in a 10-mL calibrated flask using 0.01M NaOH. Then 0.4 mL of resulted solution was further diluted in a 10-mL calibrated flask using 0.01M NaOH.

3. Results and discussion

3.1. Selection of diluting solvent

0.01M NaOH was selected as it gave the highest absorbance and fulfill all spectrophotometric and chemometric criteria (Fig. 7) [12].

Table 3: Evaluation of the intra-day and inter-day precision for the proposed methods.

Method	Conc. Level (µg mL^{-1})		%Recovery ± SD		RSD	
	ALC	**KET**	**ALC**	**KET**	**ALC**	**KET**
			Intra-day	**precision**		
Method A	2.0	4.0	99.70 ± 1.63.	101.60 ± 1.05	1.63	1.03
	5.0	8.0	100.88 ± 0.56	100.71 ± 0.53	0.56	0.53
	7.0	11.0	99.27 ± 0.41	100.21 ± 0.48	0.41	0.48
Method B	2.0	3.0	101.07 ± 0.37	101.27 ± 0.72	0.37	0.71
	4.0	5.0	101.00 ± 0.38	100.79 ± 0.44	0.38	0.44
	5.0	7.0	98.13 ± 0.16	99.86 ± 0.32	0.16	0.32
Method C	3.0	5.0	101.56 ± 0.18	99.02 ± 0.32	0.18	0.32
	5.0	8.0	99.53 ± 0.21	99.35 ± 0.42	0.21	0.42
	8.0	10.0	99.54 ± 0.78	100.03 ± 0.95	0.78	0.95
			Inter-day	**precision**		
Method A	2.0	4.0	99.30 ±1.36	100.08 ± 1.47	1.37	1.47
	5.0	8.0	100.36±0.73	100.37± 0.56	0.73	0.56
	7.0	11.0	99.14 ± 0.44	100.80±0.89	0.44	0.88
Method B	2.0	3.0	100.91± 0.42	100.95± 0.81	0.42	0.80
	4.0	5.0	101.28± 0.53	99.93± 0.1.32	0.52	1.32
	5.0	7.0	98.38± 0.42	99.24±1.04	0.43	1.05
Method C	3.0	5.0	101.75± 0.35	99.17± 0.97	0.34	0.98
	5.0	8.0	100.65± 0.59	99.44± 0.64	0.59	0.64
	8.0	10.0	99.83± 0.60	100.09± 0.80	0.60	0.80

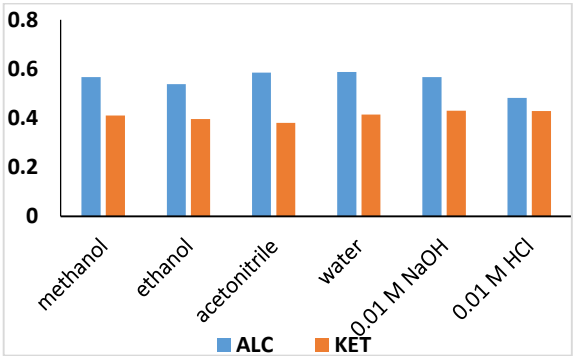

Fig. 7: Effect of the diluting solvent on the proposed chemometric methods intensity (Both ALC and KET are 10 µg mL^{-1}).

3.2. Methods validation

ICH guidelines [32] were followed to validate the suggested methods.

Linearity and range

By use of the defined experimental parameters, the analytical response was plotted against ALC, and KET concentrations formed linear relationships. Following calculating several analytical values, elevated results of the correlation coefficients (r) and minor results of the intercept (Sa), the standard deviations of the residuals (Sy/x), and the slope (Sb) were obtained (Tables 1). These data determine the linearity of the analytical response and the outcomes agreement with Beer's law.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined according to ICH [32] recommendations using the following equations:

LOD = 3.3 σ / slope

LOQ = 10 σ / slope

Where σ is the standard deviation of intercept, the resulted values were listed in (Table 1).

Accuracy and precision

Accuracy was done using the standard addition method at three concentration levels. The resulted values were listed in (Table 2). Intra- and inter-day precision for the suggested methods was calculated at three concentration levels for each drug. The resulted values were listed in (Table 3). The obtained standard deviations (SD) were all less than 2% which indicates good accuracies and precisions of the proposed methods.

Robustness

To investigate the suggested methods' robustness, small variations in NaOH concentration were done. The methods could afford a successfully small change in NaOH concentration. The resulted values were listed in (Table 4) which indicates the proposed methods are robust.

3.3. Solution stability

Stability was investigated for ALC and KET standard and sample solutions for 1, 2, and 3 hours. There was no significant difference in the results, which indicated the stability of solutions during analytical procedures. The resulted values were listed in (Table 5).

Table 4: Robustness for the proposed chemometric methods.

Method	NaOH concentration	Concentration (µg mL^{-1})		% Recovery ± RSD	
		ALC	KET	ALC	KET
Method A	0.009	5.0	8.0	98.53 ± 0.81	99.53 ± 0.18
	0.011	5.0	8.0	100.84 ± 0.57	101.70 ± 0.78
Method B	0.009	4.0	5.0	100.57 ± 0.88	98.22 ± 0.83
	0.011	4.0	5.0	101.25 ± 0.47	100.59 ± 0.54
Method C	0.009	5.0	8.0	99.74 ± 0.37	99.74 ± 0.37
	0.011	5.0	8.0	101.33 ± 0.63	99.54 ± 0.66

Table 5: Solution stability for ALC 5 µg mL^{-1} and KET 8 µg mL^{-1}.

Time (Hour)	ALC to zero time	KET to zero time
1	99.87%	99.92%
2	99.75%	99.81%
3	99.69%	99.7%

Table 6 A: Application of the proposed chemometric methods to pharmaceutical formulation.

Parameters	Method (A)		Method (B)		Method (C)	
	ALC	KET	ALC	KET	ALC	KET
% Recovery [a]	99.62	99.76	99.99	99.12	99.80	99.26
SD	0.55	0.52	0.56	0.56	0.53	0.42
t-value [b]			1.06	1.88	0.51	1.69
F-value [b]			0.98	0.86	1.10	1.58

[a] Mean of 5 determinations
[b] Tabulated value at 95% confidence limit; t=2.306 and F=6.338.

Table 6 B: Application of the proposed chemometric methods to separate pharmaceutical formulation [4, 33].

Parameters	Method (A)		Method (B)		Method (C)	
	ALC	KET	ALC	KET	ALC	KET
% Recovery [a]	99.62	99.76	99.99	99.12	99.80	99.26
SD	0.55	0.52	0.56	0.56	0.53	0.42
t-value [b]			1.06	1.88	0.51	1.69
F-value [b]			0.98	0.86	1.10	1.58

[a] Mean of 5 determinations
[b] Tabulated value at 95% confidence limit; t=2.306 and F=6.338.

3.4. Pharmaceutical application

These three methods were successfully applied to determine the cited drugs in their pharmaceutical formulation. There is no reported method for the recently approved pharmaceutical formulation, so the second and third methods were statistically compared with the first method by using student's *t*- and F-tests. There was no significant difference between the proposed methods' results, owing to calculated values are lower than the theoretical values at 95% confidence limit. The resulted values were listed in (Table 6 A). The recoveries achieved by the suggested technique for separated ALC (Orchinohist ®) and KET (Acular LS ®) determination in their pharmaceutical dosage forms were found to be in good agreement with those obtained by analyzing the same dosage forms using the previously published spectrophotometric methods (Table 6 B) [4, 33].

4. Conclusion

Different spectrophotometric and chemometric methods were presented showing various simplicity degrees in their application and differences in LOQ and LOD values resulted from each method. All investigated spectrophotometric and chemometric methods: first derivative, dual-wavelength and absorbance subtraction display good analytical parameters concerning accuracy and precision. The proposed methods were successfully employed for the assay of recent pharmaceutical formulation. The proposed methods were found to be time-saving, cheap, and easy. The proposed methods could be regarded as reference methods for assaying the new combination in their pharmaceutical formulation.

Conflicts of interest

The authors have no known competing personal relationships or financial interests that could have seemed to influence the reported work in this paper.
The authors declare no interest conflicts.

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Author statement

Ahmed S. Ahmed: Conceptualization, methodology, data curation, software, and preparation of original draft writing. **Khalid M. Badr El-din:** Conceptualization and data curation. **Ahmed Khorshed:** investigation, Visualization, and supervision. **Mohamed Oraby***: Methodology, validation, reviewing, and writing. **Sayed M. Derayea**: Methodology, validation, reviewing, writing, and editing.

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