International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 4, 2018

Original Article

EXTRACTIVE-SPECTROPHOTOMETRIC DETERMINATION OF SOME ANTIMUSCARINIC ANTAGONIST IN TABLET FORMULATIONS USING ERIOCHROME CYANINE R

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Received: 16 Dec 2017 Revised and Accepted: 24 Feb 2018

ABSTRACT

Objective: To develop and validate simple, rapid and sensitive spectrophotometric method for the assay of four antimuscarinic antagonists, namely oxybutynin (OXB), solifenacin (SOL), tolterodine (TOL) and fesoterodine (FES) in bulk and pharmaceutical formulations.

Methods: The proposed method is based on the reaction of the selected drugs with eriochrome cyanine R (ECR) in buffered aqueous solution at pH 1.0. The formed ion-pair complexes were extracted with dichloromethane and measured quantitatively with maximum absorption at 464 nm. All variables that affect on color intensity such as pH, buffer volume and concentration of ECR and extractive solvents were studied and optimized.

Results: The calibration graphs were linear over the concentration range of 4–24, 4–32, 4–32 and 2–22 µg/ml for OXB, SOL, TOL and FES, respectively. The stoichiometry of the reaction was found to be 1:1 in all cases. Molar absorptivity values were found to be 2.043×10^4, 1.856×10^4, 1.798×10^4 and 2.856×10^4 l/mol/cm for OXB, SOL, TOL and FES, respectively. Excipients which used as an additive in commercial formulations did not interfere in the analysis.

Conclusion: The developed method was successfully applied to determine OXB, SOL, TOL and FES in pharmaceutical preparations. The developed method can be used for quality control and routine analysis where time, cost effectiveness and high specificity of analytical technique are of great importance.

Keywords: Spectrophotometry, Ion-pair complexes, Oxybutynin chloride, Solifenacin, Tolterodine, Fesoterodine, Eriochrome cyanine R

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i4.24300

INTRODUCTION

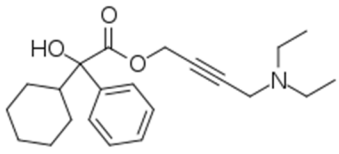
Oxybutynin, solifenacin, tolterodine and fesoterodine as an antimuscarinic/anticholinergic muscle relaxant, used to reduce urinary frequency, urinary urgency and urinary incontinence in people with an overactive bladder. They works by relaxing the involuntary detrusor muscle in the wall of the bladder by blocking muscarinic/cholinergic receptors present on the surface of the muscle cells and thus prevents acetylcholine from acting on these receptors [1–4].

Oxybutynin (OXB), (*α*-cyclohexyl-*α*-hydroxybenzenacetic acid-4-(diethylamino)-2-butynyl esters) (fig. 1a) an anticholinergic agents is a tertiary amine that mainly acts as a direct smooth muscle relaxant and displays weak antimuscarinic activity [5, 6]. A survey of the literature revealed that few methods have been reported for the determination of OXB such as UV and visible spectrophotometry [7–9], voltammetric [10], HPLC methods [11–14], gas chromatography in serum [15], Polymeric matrix membrane sensors were used for stability-indicating potentiometric determination of OXB and differential pulse polarography [16].

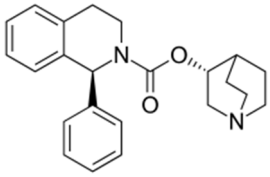
Solifenacin (SOL) [17–20], chemically, is butanedioic acid (3R)-1-azabicyclo [2.2.2] octan-3-yl(1S)-1-phenyl-1,2,3,4-tetrahydro-isoquinoline-2-carboxylate (fig. 1b), having an empirical formula of $C_{27}H_{32}N_2O_6$ and molecular weight of 480.5528 g/mol. Literature survey reveals chromatographic methods for the analysis of solifenacin and have been applied for the quantification of SOL in bulk, biological fluids and commercial formulations [21–27]. Though the chromatographic methods are precise the instrumentations are expensive and required critical experimental conditions. Hence these techniques are not applied for routine analysis of SOL. The spectrophotometric methods are still popular for the routine analysis in quality control laboratories because they are precise, simple, fairly accurate and cost-effective. Very few literatures are cited on spectrophotometric methods [28, 29].

Tolterodine (TOL) has chemical name of ®-N, N-diisopropyl-3-(2-hydroxy-5-methyl phenyl)-3-phenyl-propanamine L-hydrogen tartrate (fig. 1c). The methods reported in the literature for its determination either in biological matrix or in pharmaceutical formulations are spectrophotometric [30–33], LC [34–36], HPLC [37–41], potentiometric [42] and electrochemical methods [43, 44].

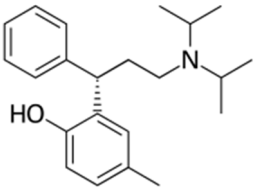
Fesoterodine (FES) [45, 46] chemically, is designated as isobutyric acid 2-(®-3-diisopropylamino-1-phenylpropyl)-4-hydroxymethyl) phenyl ester hydrogen fumarate (fig. 1d). A survey of chemical literature as shown that few articles are available for the determination of fesoterodine fumarate using LC and LC-MS [47–49], HPLC [50–52], UV [53–55] and electrophoresis [56]. An attempt has been made to develop a simple and rapid visible spectrophotometric method for the assay of selected drugs in tablet dosage forms.



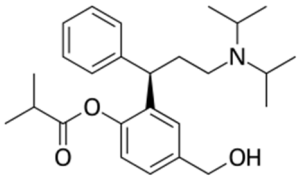
a- OXB [5]



b- SOL [17]



c- TOL [38]



d- FES [45]

Fig. 1: Chemical structure of the studied drugs

MATERIALS AND METHODS

Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK), with wavelength range 190 nm ~ 1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells.

Reagents and solutions

All the chemicals were of analytical or pharmaceutical grade and used without further purification. Double distilled de-ionized water was used to prepare all solutions. A stock solution of pure selected drugs was prepared by dissolving 20 mg of the selected drugs in a 100 ml calibrated flask. Working solutions of lower concentrations were freshly prepared by appropriate dilution with water. A 2×10^{-3}M of eriochrome cyanine R (ECR) (Sodium 4-[(1-hydroxy-naphthalen-2-yl-hydrazinylidene]-7-nitro-3-oxo-Y-naphthalene-1-sulfonate) stock solution were prepared by dissolving 107.28 mg of dye (99% purity) in distilled water and diluting to 100 ml in a measuring flask with distilled water. Series of buffer solutions of KCl-HCl (pH=1.0–2.2), potassium hydrogen phthalate-HCl (pH=2.2–4.0) and NaOAc-HCl (pH=3.2–6.8) were prepared by standard methods.

All pharmaceutical preparations were obtained from local market. Tolterodine tablets (2 mg TOL/tablet) manufactured by Sabaa International Company for Pharmaceuticals and Chemical Industries, Egypt, Uripan (5 mg OXB/tablet) manufactured by ADWIA Co. S.A.E. 10th of Ramadan City, Egypt, Sofenacin (5 mg SOL/tablet) manufactured by Marcyrl Pharmaceutical Industries, El Obour City, Egypt and fesoterodine fumarate is Toviaz 4 mg/tab (Pfizer Company, Egypt).

General recommended procedures

Into a series of separating funnels, accurately measured aliquots of 4–24 µg/ml for OXB, 4–32 µg/ml for SOL, 4–32 µg/ml for TOL and 2–22 µg/ml for FES were pitted out. Then, 2.5 ml of 2×10^{-3}M of ECR, 1.0 ml of buffer solution of pH=1.0 were added and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 2.0 min and then, the combined dichloromethane extracts were dried over anhydrous sodium sulphate. The absorbance of colored ion-pair complexes was measured within 20 min of extraction at 464 nm against a reagent blank prepared in the same manner except for addition of drugs. A calibration graph was constructed for each drug and the concentration of unknown samples can be deduced by using of calibration graph.

Procedure for tablets

At least ten tablets of each commercial pharmaceutical formulation of the drugs were weight into a small dish, powdered and mixed well. An accurate weight of the powder equivalent to 10 mg of each drugs was dissolved in distilled water, filtered into a 100 ml calibrated flask and diluted to the mark with water. Further dilution was made to obtain working range concentration and analyzed as the above procedure for the analysis.

RESULTS AND DISCUSSION

Absorption spectra

Ion-pair extraction spectrophotometry has received considerable attention for quantitative estimation of many pharmaceutical compounds [57–60]. This technique depends on the reaction of a drug that has basic cationic nitrogen and an anionic dye, where a highly colored ion-pair complex is formed. OXB, SOL, TOL and FES reacted with an anionic dye (ECR) in acidic buffer to form ion-pair complexes, which are soluble in dichloromethane. These complexes have an absorption maximum at 464 nm against a reagent blank and hence (fig. 2) and this wavelength was used for all subsequent measurements.

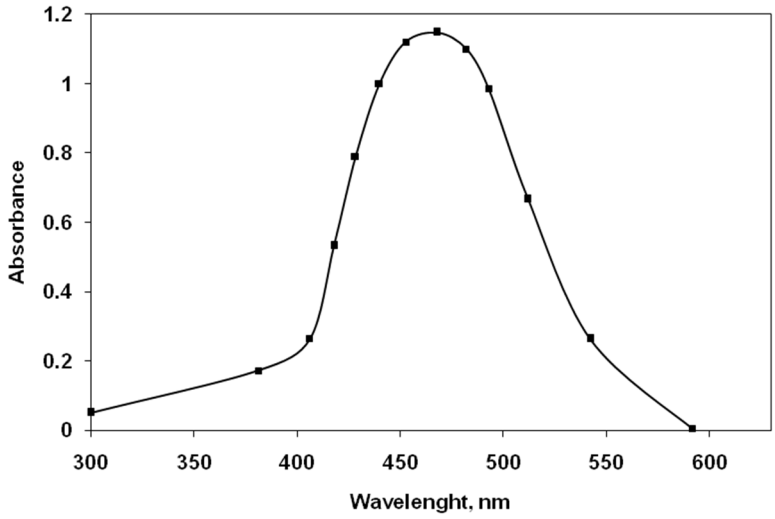


Fig. 2: Absorption spectra of 24 µg/ml OXB with ECR dye complex extracted in dichloromethane

Optimization of the reaction condition

The optimization of the methods were carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complexes were found by studying with preliminary experiments such as pH of the buffer, type of organic solvent, dye concentration and shaking time for the extraction of ion-pair complexes.

Effect of pH

It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers, such as KCl-HCl (pH 1.0–2.2), NaOAc–HCl (pH 1.9–4.92) and NaOAc–AcOH (pH 3.4–5.6). It was noticed that the maximum color intensity and constant absorbances were observed in KCl-HCl of pH=1.0 for all the drugs. The volume of buffer solution added was studied and adding 1.0 ml buffer solution of pH=1.0 attained complete color development. For the highest color intensity and maximum absorbance, the buffer solution should be added after mixing the drug–dye solution at neutral pH.

Choice of organic solvent

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained the highest absorbance with dichloromethane. It was also observed that only one extraction was adequate to achieve a quantitative recovery of the complexes and the shortest time to reach the equilibrium between both phases. Shaking time of 0.5–5 min provided constant absorbance and hence, 1.0 min was selected as the optimum shaking time.

Effect of dye concentration

The effect of ECR concentration on the intensity of the color developed at the selected wavelength and constant drugs concentration was tested using different volumes of ECR (0.5–5 ml). It was observed that 2.5 ml of 2×10^{-3}M ECR were necessary for the maximum color development of the ion-pair complexes. After this volume, the absorbance remains constant by increasing the volume of the reagent.

Sequence of addition and phase ratio

Although it is not a fundamental factor, the most favorable sequence is drug-reagent–buffer–dichloromethane) for the highest absorbance and stability. The complexes with these sequences remain stable at least for 12 h. The ratio of aqueous to organic phase was ineffective and the ratio 1:1 was chosen for extraction of the colored species.

Stability of the ion-pair complexes

The stability of the ion-pair complexes formed between the studied drugs and ECR was evaluated. Although the ion-pairs were obtained instantaneously, constant absorbance readings were obtained after not less than 5.0 min of standing at room temperature (25±2 °C). Ion-pairs were stable for at least 12 h without any change in color intensity or in $\lambda_{max.}$

Composition of ion–pair complexes

In order to establish a molar ratio between OXB and ECR dyestuff used, Job's method of continuous variation was applied. In this method, solutions of drug and dyestuff with identical molar concentrations were mixed in varying volume ratio in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug. This measurement showed that 1:1 complex was formed through the electrostatic attraction between positive protonated OXB^+ and negative ECR^-. The possible reaction mechanism is proposed and given in fig. 3.

Validation of proposed method

The following validation parameters were tested according to the guidelines set by the international conference on harmonization (ICH) [61]: linearity, accuracy, precision, limit of detection (LOD) and the limit of quantitation (LOQ).

Linearity and range

The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, slope, intercept and correlation coefficient determined for each drug are given in table 1. A linear relationship was found between the absorbance and the concentration of each drug in the range of 4–24 µg/ml for OXB, 4–32 µg/ml for SOL, 4–32 µg/ml for TOL and 2–22 µg/ml for FES (fig. 4). Regression analysis of Beer's law plotted at λ_{max} reveals a good correlation (r^2 = 0.9976–0.9989). The graphs showed a negligible intercept, which was calculated by the least-squares method's regression equation, A = a+bC (where A is the absorbance of 1.0 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in µg/ml.

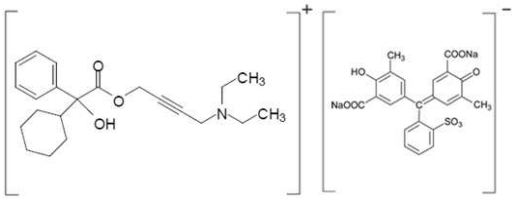


Fig. 3: Structure of OXB–ECR ion pair complex

Table 1: Analytical parameters and optical characteristics of the proposed method

Parameters	Drugs			
	OXB	SOL	TOL	FES
Beer's law limit, µg/ml	4–24	4–32	4–32	2–22
Molar absorptivity, l/mol/cm	2.04×10^4	1.85×10^4	1.79×10^4	2.85×10^4
Sandell's sensitivity, ng/cm^2	19.27	25.88	26.44	18.47
Correlation coefficient ®	0.9976	0.9994	0.9989	0.9983
Linear regression equation*				
$S_{y/x}$	0.0263	0.0125	0.0143	0.0110
Intercept (a)	0.1499	0.2378	0.2436	0.1556
Slope (b)	0.0462	0.0315	0.0301	0.0457
SD of slope (S_b)	1.57×10^{-3}	5.01×10^{-4}	6.05×10^{-4}	1.32×10^{-3}
SD of intercept (S_a)	0.0540	0.0237	0.0265	0.0227
LOD, µg/ml	0.0357	0.0523	0.0548	0.0361
LOQ, µg/ml	0.1082	0.1587	0.1661	0.1094

OXB: oxybutynin, SOL: solifenacin, TOL: tolterodine, FES: fesoterodine, *A = a+bc, where A is the absorbance and C is the concentration of drug in µg/ml.

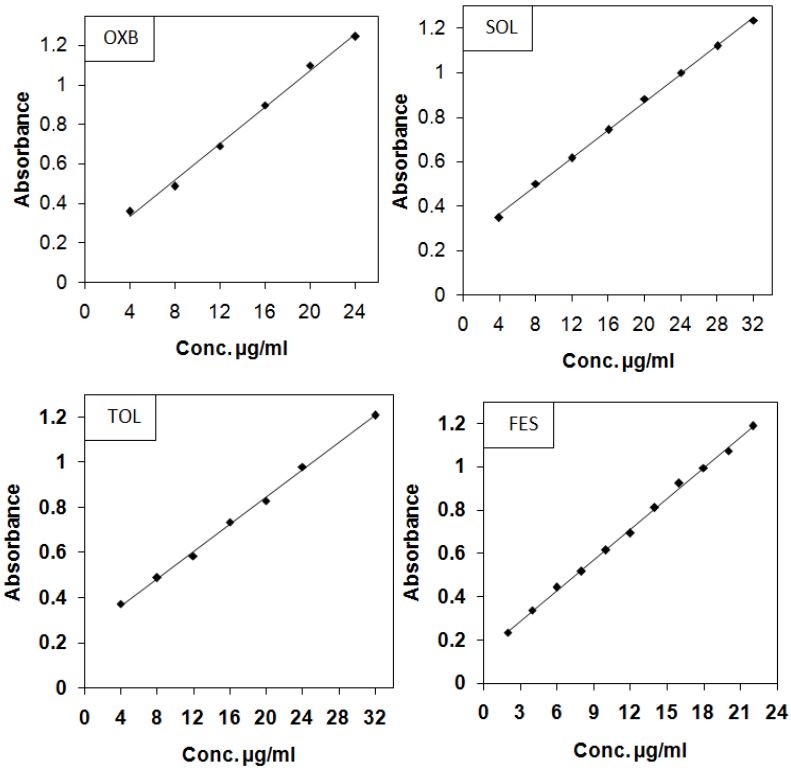


Fig. 4: Calibration curve of ion-pair complexes of OXB, SOL, TOL and FES with ECR against a blank

Detection and quantification limits

The detection limit (LOD) for the proposed method was calculated using the following equation [61].

LOD = 3.3 s/k

Where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the drug and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were found to be 0.0357 µg/ml for OXB, 0.0523 µg/ml for SOL, 0.0548 µg/ml for TOL and 0.0361 µg/ml for FES.

The limits of quantification, LOQ, defined as:

LOQ = 10 s/k

According to this equation, the limits of quantification were found to be 0.1082 µg/ml for OXB, 0.1587 µg/ml for SOL, 0.1661 µg/ml for TOL and 0.1094 µg/ml for FES. A comparison of the performances between the proposed method and those of reported methods for the studied drugs is summarized in table 2. It can be seen that the detection limit of the present work is lower than that found in the reported methods.

Accuracy and precision

In order to determine the accuracy and precision of the recommended procedure five replicate determinations at three different concentrations of the studied drugs were carried out. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (table 3) and indicate that the proposed method is highly accurate and reproducible.

Effect of interferences

In order to evaluate the selectivity of the proposed method for the analysis of pharmaceutical formulations, the effects of the presence of excipients and additives, which can occur in real samples, were investigated. It was found that the presence of the common excipients of tablets such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate did not interfere with the determination of the studied drugs at the levels normally found in dosage forms.

Table 2: Comparison of linear range and detection limits for the studied drugs with reported methods

Drug	Methods	Linear range, µg/ml	Detection limit, µg/ml	Ref
OXB	Squarewave cathodic adsorptive stripping voltammetry	1-18	0.100	10
	Differential pulse cathodic adsorptive stripping voltammetry	1-17.6	0.2300	10
	Ion-association complex with Tropaeoline OOO	1.0-7.5	0.0621	9
	Ion-association complex with Alizarin Red-S.	2.0-15	0.1112	9
	Extractive-spectrophotometric with eriochrome cyanine R	4-24	0.0357	Proposed method
SOL	HPLC method	2-100	0.0700	22
	RP-HPLC	20-70	0.100	24
	Extractive-spectrophotometric with eriochrome cyanine R	4-32	0.0523	Proposed method
TOL	Zero order derivative	30-180	0.6600	31
	RP-HPLC	100-300	0.1600	38
	Extractive-spectrophotometric with eriochrome cyanine R	4-32	0.0548	Proposed method
FES	Capillary electrophoresis	2-100	0.5700	56
	Second-order derivative UV spectrophotometric	2-24	0.3800	55
	Extractive-spectrophotometric with eriochrome cyanine R	2-22	0.0227	Proposed method

Table 3: Evaluation of intra-day accuracy and precision of the proposed method

Drugs	Intra-day accuracy and precision				
	Taken µg/ml	Found[a] µg/ml	Recovery, %	RSD, %	RE[b], %
OXB	4	3.9999	99.998	2.107	-0.002
	12	11.9995	99.996	1.527	-0.004
	20	19.9992	99.996	1.156	-0.004
SOL	8	7.9995	99.994	1.581	-0.006
	20	19.998	99.994	1.703	-0.006
	28	27.998	99.996	2.095	-0.004
TOL	8	7.9995	99.994	1.760	-0.006
	20	19.9988	99.994	2.059	-0.006
	32	31.9980	99.994	1.786	-0.006
FES	4	3.9998	99.996	1.126	-0.004
	10	9.9994	99.994	1.393	-0.006
	16	15.9993	99.996	1.911	-0.004

OXB: oxybutynin, SOL: solifenacin, TOL: tolterodine, FES: fesoterodine, [a]Mean value of five determinations, [b]RE: Relative error.

Tablets analysis

The proposed method was successfully applied to the determination of OXB, SOL, TOL and FES in their commercially tablets. Various formulations were analyzed to examine the applicability of the developed method and the results were tabulated in table 4. The results were reproducible with low RSD values. The average percent recoveries obtained were quantitative (95.78–100.73), indicating the good accuracy of the method.

The results of analysis of the commercial tablets and the recovery study of drugs suggested that there is no interference from any excipients (such as starch, talc, gelatin, magnesium stearate, sulfate, glucose, acetate and phosphate), which are present in tablets.

Table 4: Assay results of OXB, SOL, TOL and FES in pharmaceutical formulations by the proposed method

Drug formulations	Drug taken µg/ml	Drug found[a] µg/ml	Recovery, %	RSD, %	RE[b], %
Uripan tablets[c], 5 mg/tab	4	3.899	97.494	2.965	-2.50
	12	12.051	100.430	1.442	0.43
	20	19.772	98.860	2.542	-1.14
sofenacin tablets[d], 5 mg/tab	8	7.976	99.706	3.084	-0.294
	20	19.878	99.392	2.329	-0.608
	28	28.206	100.738	2.283	0.738
Tolterodine tablets[e], 2 mg/tab	8	7.754	96.936	3.649	-3.064
	20	19.736	98.680	2.934	-1.32
	32	31.107	97.210	3.655	-2.79
Toviaz[f], 4 mg/tab	4	3.908	97.700	2.626	-2.3
	10	9.578	95.784	3.724	-4.216
	16	15.796	98.726	2.039	-1.274

[a]Mean value of five determinations, [b]RE: Relative error, [c]Produced of Adwia pharmaceuticals Company, Egypt, [d]Produced of Marcyrl Pharmaceutical Industries, Egypt, [e]Produced of Sabaa international company for Pharmaceutical and chemical industry, Egypt, [f]Produced of Pfizer Company, Egypt.

CONCLUSION

The proposed method characterized by using simple reagents which can be afforded by any ordinary analytical laboratory. The method is sufficiently sensitive to permit determination even down to 2.0 µg/ml. The developed method is highly reliable owing to the stability of the dye and ion-pair complexes, which are finally measured. The proposed method is simple, precise, accurate and convenient. Therefore, it can be useful for routine analyses and quality control assay of the examined drugs in raw material and in tablets without fear of interference caused by the excipients expected to be present in tablets. This is for the first time that spectrophotometric method is being reported for the assay of FES in pure form and also in its pharmaceutical formulation.

AUTHORS CONTRIBUTIONS

AKRAM M. EL-DIDAMONY: Developed original idea and design of work. The experimental work was carried out by Nora O. Saleem, AKRAM M. EL-DIDAMONY, MONUIR Z. SAAD: Data interpretation and manuscript writing. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no competing interests.

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