Simultaneous square wave voltammetric determination of two anti-inflammatory drugs using a bare carbon paste electrode

Rehab O. El-Attar[a], Ahlam M. Fathi[b], Ibrahim H.I. Habib[a]

[a]Applied Organic Chemistry Department, Microanalysis Laboratory, National Research Centre, Dokki, Giza, [b]Physical Chemistry Department, National Research Centre, Cairo

Correspondence to Rehab Attar, PhD, Microanalysis Laboratory, Applied Organic Chemistry Department, National Research Centre (NRC), El Bohouth Street, Dokki, 12622 Giza, Egypt. Tel: +20 128 740 1708; Fax: 02 33371931; e-mail: rhbattar@yahoo.com

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Background
Diclofenac (DIC) is a nonsteroidal anti-inflammatory drug (NSAID) that is more potent than indomethacin. There is an extended-release form of this drug available for long-term treatment.
Diflunisal (DIF) is a NSAID with less anticoagulant activity than aspirin. It is more potent than aspirin and is not converted to salicylic acid *in vivo*.
Objectives
A simple, direct, and sensitive method for the simultaneous determination of two types of NSAIDs, namely, diclofenac 'DIC' and diflunisal 'DIF' by square wave adsorptive anodic stripping voltammetry and applied at the same time in synthetic and pharmaceutical samples.
Materials and methods
In this method, both cited ingredients were oxidized by a bare carbon paste electrode (CPE) forming two well-separated peaks at the potential of 0.65 and 0.85 V, respectively, in 0.04 M universal buffer solution at pH 5 with 100 mV/s scan rate by square wave adsorptive anodic stripping voltammetry technique with an Autolab system of potentiostat/galvanostat.
Results and conclusion
The prepared sensor showed good linear regression curves over the concentration ranges of 1–6 and 5–25 μg/ml for DIC and DIF, respectively, while the detection limit 'LOD' and quantification limit 'LOQ' and were 0.204 and 0.68 μg/ml for DIC and 1.366 and 4.553 μg/ml for DIF, in turn. The cited sensor was applied with accurate and precise results for determining DIC and DIF at the same time in synthetic and pharmaceutical samples and gave close results in comparison with the official method, specifically by UV-high performance liquid chromatography.

Keywords:
diclofenac, diflunisal, square wave voltammetry, synthetic and pharmaceutical samples

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Introduction

Diclofenac sodium (DIC) is a phenylacetic acid derivative and known as 2-(2,6-Dichloranilino) phenylacetic acid monosodium salt, while diflunisal (DIF) is a salicylate derivative and known as 2',4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid [1] as shown in Fig. 1, both are classed as nonsteroidal anti-inflammatory drugs (NSAID) and can be co-formulated in suppository dosage form known as Rheumafen Forte suppositories and used in painful and inflammatory conditions including osteoarthritis, rheumatoid arthritis, renal and biliary colic, acute gouty attack, and dysmenorrhea [2,3]. The combination of diclofenac sodium with other NSAIDs or salicylates (e.g., diflunisal) is not recommended due to the increased gastrointestinal toxicity risk [4].

There are many analytical methods for determining DIC individually in the pharmaceutical and biological samples, as exemplified by spectrophotometry [5], spectrofluorimetric [6] or capillary zone electrophoresis [7], while DIF was determined based on LC-MS [8], LC–Densitometry and high performance liquid chromatography (HPLC) [9] and HPLC-UV method [10] but there is a recent trend to focus on the most preferred methods that depend principally on the simultaneous analysis, binary or ternary, as they are characterized by saving time and cost for cases that require rapid and accurate analysis in the pharmaceutical industries. From this point, DIC was determined simultaneously with other ingredient such as paracetamol and chlorzoxazone by spectrophotometry [11], or aceclofenac by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS) [12], while DIF was determined with naproxen by spectrophotometry [13], spectrofluorimetric [14] or HPLC [15]. Only few reports showed the simultaneous determination of both DIC and DIF using chemometric methods

[16] and HPLC [17], however they do not lack drawbacks since they need well-qualified and experienced analysts, as well as the instruments, which are expensive and a lot of non-eco-friendly organic solvents consuming. Alternatively, the electrochemical techniques, specifically voltammetry, have been used successfully for rapid, sensitive and accurate determination of electro-active analytes in biological fluids and require little sample preparation and less time consumption. Several voltammetry methods have been applied to quantify both drugs alone [18–28] or concurred with other drugs in the same sample [29].Therefore, the present study aims to develop a simple, accurate, reliable, and specific voltammetric method for the simultaneous analysis of both DIC and DIF in synthetic and real pharmaceutical samples.

Materials and methods

All reagents were of the analytical grade and bi-distilled water was used throughout the experiments. Diflunisal ($C_{13}H_8F_2O$, 250.198 g/mol) and diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$, 318.13 $gmol^{-1}$) were purchased from Sigma. The multi-walled carbon nanotubes (MWCNTs), the graphite powder (synthetic 1–2 μm) and paraffin oil, all were purchased from Sigma-Aldrich. BR stock buffer solution with concentration 4×10^{-2} $molL^{-1}$ was prepared by dissolving 2.47 g boric acid in a mixture of 2.7 ml H_3PO_4 and 2.3 ml glacial CH_3COOH and diluted to 1 l with deionized water. The desired pH value was adjusted with sodium hydroxide solution [30].

The two standard stock solutions (250 μg/ml) of DIC and DIF were prepared daily by dissolving appropriate quantity of them about 25 mg individually; in small amount of 0.05 mol/l NaOH and then completed to 100 ml with distilled water and then stored in a refrigerator at 4–6°C in order to avoid possible degradation.

A Rheumafen Forte suppository (GlaxoWellcome Egypt S.A.E., El-Salam City, Cairo, Egypt, BN 082345A) containing diflunisal (200 mg) and diclofenac sodium (100 mg) was purchased from the local Egyptian market.

Apparatus and measurements

The apparatus used was Square wave voltammetry '(SWV)' technique with an Autolab system of potentiostat/galvanostat (PGSTAT 302 NU tract, the Netherlands) equipped with three electrodes, Ag/AgCl- 3 M KCl as a reference electrode, a platinum wire as a counter electrode and working electrode prepared as described below. The digital pH-meter Metrohm was used to carry out the pH measurements.

Sensor preparation

For preparation of bare carbon paste electrode (CPE), graphite powder (200 mg) was mixed with paraffin oil (100 μl) in agate mortar, the resulting mixture was then packed into insulin plastic syringe (1 ml), (which had 2.5 mm internal diameter and 70 mm high), its surface area was estimated at ~0.049 cm^2 and the external electric contact was obtained by inserting a copper wire into it. The electrode was smoothed on a witted Whitman filter paper. Similar CPE was prepared but with 10% MWCNTs.

Procedure

Universal buffer solution at pH 5.0 was prepared and about 10 ml of it were taken and introduced into the voltammetric cell, then, several cyclic sweeps from 0.4 to 1.2 V were applied at a scan rate 100 mV/s to obtain minimum background current using bare CPE. Different aliquots of standard solutions of DIC, and DIF, over the concentration range of 1–6 and 5–25 μg/ml, respectively, were transferred to the voltammetric vessel and preconcentration was implemented at 0.4 V for 30 s with stirring at 2000 rpm. The equilibrium time was carried out for 10 s, then the voltammogram was recorded by square wave scanning over an oxidation potential range from 0.4 to 1.2 V with scan rate of 100 mV/s, pulse amplitude of 30 mV and frequency of 25 Hz. Standard addition method was used to measure the mean of triplicate content in the sample at room temperature.

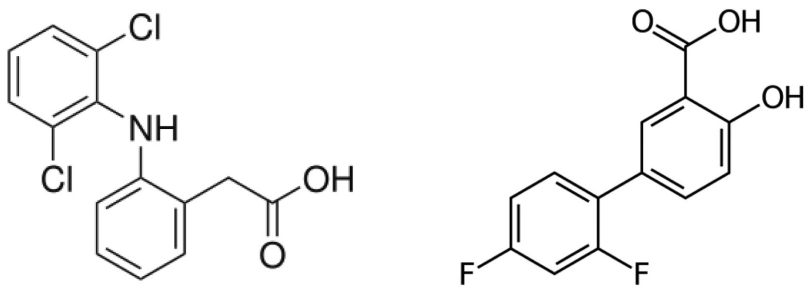
A Rheumafen Forte suppository was transferred to NaOH solution (0.05 M) about 5 ml and the solution was shaked and warmed for 15 min, then cooled to solidify the paraffin wax, after that transferred into a volumetric flask and completed to 100 ml distilled water. Three different aliquots of solution were transferred into voltammetric vessel and the same procedure as mentioned above was followed.

Result and discussion

Electrochemical behavior of DIF and DIC on bare electrode

Square wave voltammetry was carried out on the bare and the modified carbon paste electrodes with MWCNTs (10%) by scanning over 0.4 to +1.0 V potential rang at pH 7 in universal buffer (0.04 M)

Figure 1



Diclofenac **Diflunisal**

Chemical structure of diclofenac sodium and diflunisal.

solution with 100 mV/s scan rate. From the results, two distinct anodic oxidation peaks by both electrodes corresponding to DIC and DIF were appeared at potentials around +0.65 and +0.85 V, respectively. However, there is no noticeable change in the peak heights in the presence of MWCNTs as shown in Fig. 2. The same results were obtained when different concentrations of MWCNTs (5%-7.5%-10%-12.5%-15%) were changed. So, the bare electrode was selected for the next studies in the simultaneous determination of DIC and DIF.

Effect of pH

One of the most important parameters that affects peak current and potential is pH range. The study was carried out using universal buffer solutions in pH range from 2 to 9 at 100 mV s^{-1} scan rate and using SWV. In the anodic direction from 0.4 to 1.2 V, the two oxidation peaks were shifted simultaneously towards less-positive potential values by increasing the alkalinity of pH up to 9, and the corresponding peak currents were also increased, but reached maximum values at pH 3 for DIC, and at pH 5 for DIF, after which there were gradual decrease in the peak height due to the reduced participation of the proton numbers in the oxidation reaction of the cited molecules, as shown in Fig. 3. So, pH 5 was selected in the next experiments.

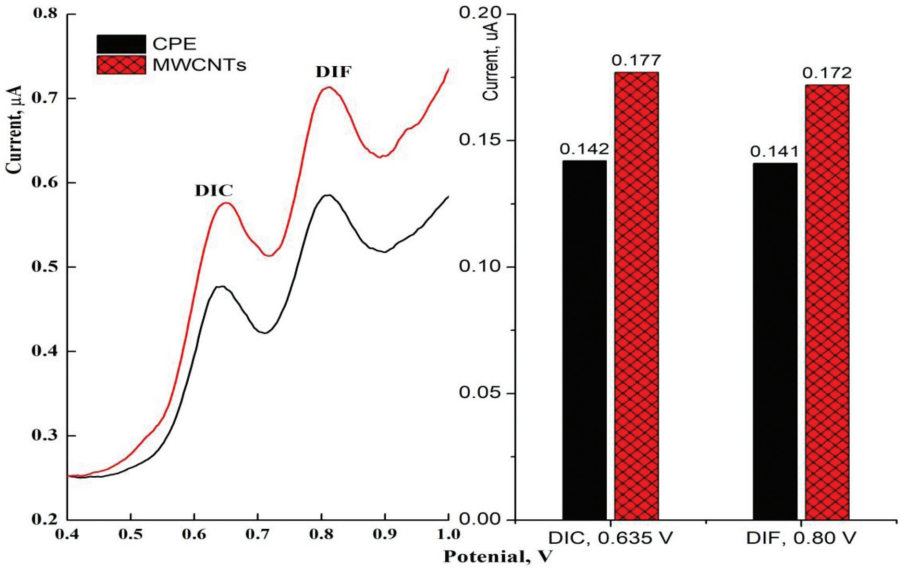
From the plot, a linear relationship with $E_p$vs pH was expressed with regression equations:

Ep_a (V)=−0.054 pH + 0.944, (R=0.995), for DIC,

Ep_a (V)=−0.057 pH + 1.236, (R= 0.992), for DIF

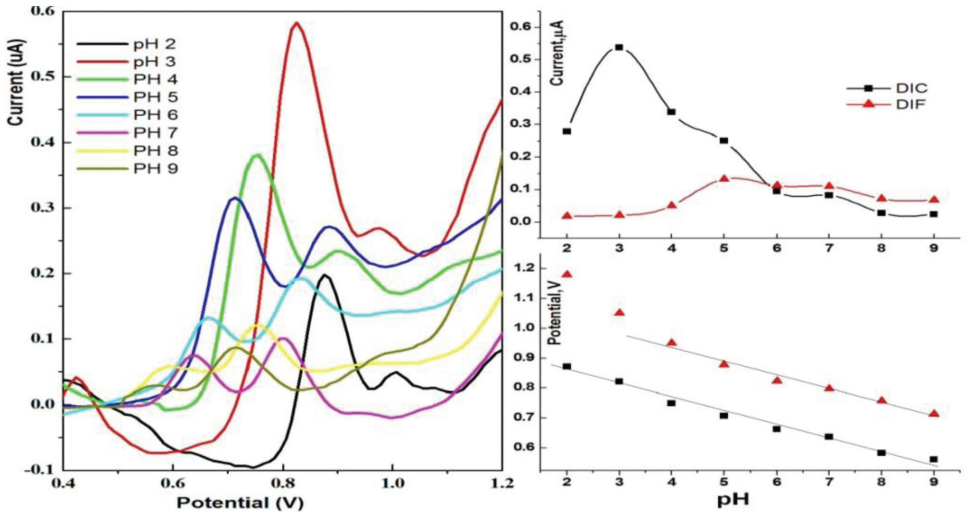
Slope values can be an indicative of involvement of equal number of both protons and electrons during the

Figure 2



Square wave electrochemical behavior of 100 µg/ml DIC and DIF on bare and MWCNTs electrodes.

Figure 3



Effect of pH on the oxidation of 100 µg/ml of DIC and DIF using the bare carbon paste.

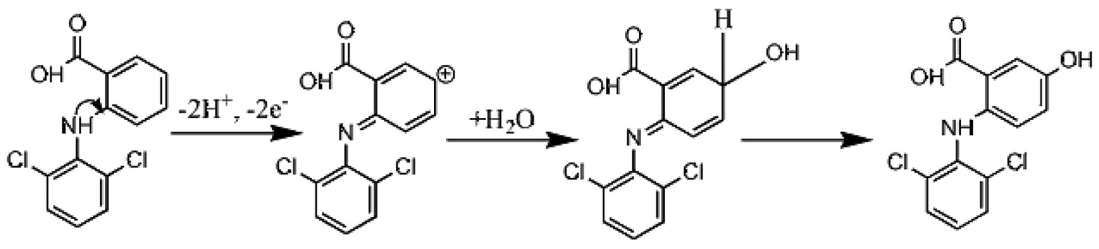
oxidation for DIC and DIF. This result explains the peak height of DIC was higher relative to DIF by half although both were in equal concentration (ug/ml).In the cathodic direction, no peaks were observed in the same electrolyte media, suggesting that the electrochemical process is irreversible. Accordingly, the electrochemical mechanism of DIC may be proposed as given in Fig. 4 which in accordance with others [31]. While DIF as indicated by its structure resembling to salicylic derivative [32,33], the mechanism can be proposed as given in Fig. 5.

Analytical performance evaluation

The analytical performance should be tested for the developed method as demonstrated in Fig. 6 where the anodic current was increased as a function of the standards added from 1 to 6 µg/ml for DIC and from 5 to 25 µg/ml for DIF, above these concentration ranges the current deviations were shown. Calibration curves show good linearity as indicated by high correlation coefficient of 0.99, and the standard deviations of the intercept (S_a) and slope (S_b) were found to be less than 1.0% relative to the slope. The LOD and LOQ were determined in accordance with the ICH guidelines, utilizing the formulas LOD = 3.3 SD/S and LOQ = 10 SD/S. From the calculations above LOD and LOQ values were found to be 0.204 and 0.680 µg/ml and 1.37 and 4.55 µg/ml for DIC and DIF as given in Table 1.

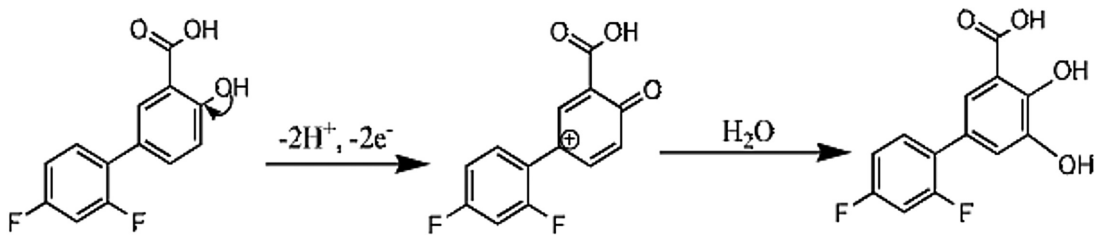
To show that the overlapped peaks did not affect the method accuracy, some analysis parameters were studied such as the percentage relative error (bias, %) and the percentage relative standard deviation (RSD %) values. The difference value between the average of the result x and the real value x should not be greater than the standard deviation SD at a mostly confidence level of 95% as elucidated by the following confidence CI formula where *t* is the *t*-test value tabulated from the two tailed *t*-test table at the degree of freedom n. As depicted in Table 2, the left side value of bias 0.7 is less than the right side value of confidence limit of 2.43 for determination of DIC, and similarly, the bias of 1.1 is less than 2.84 for

Figure 4



Proposed mechanism of electrochemical oxidation of DIC.

Figure 5



Proposed mechanism of electrochemical oxidation of DIF.

determination of DIF indicating that the source of error is mainly random [34].

$$\bar{x} - x_t = \pm \frac{\text{ts}}{\sqrt{n}}$$

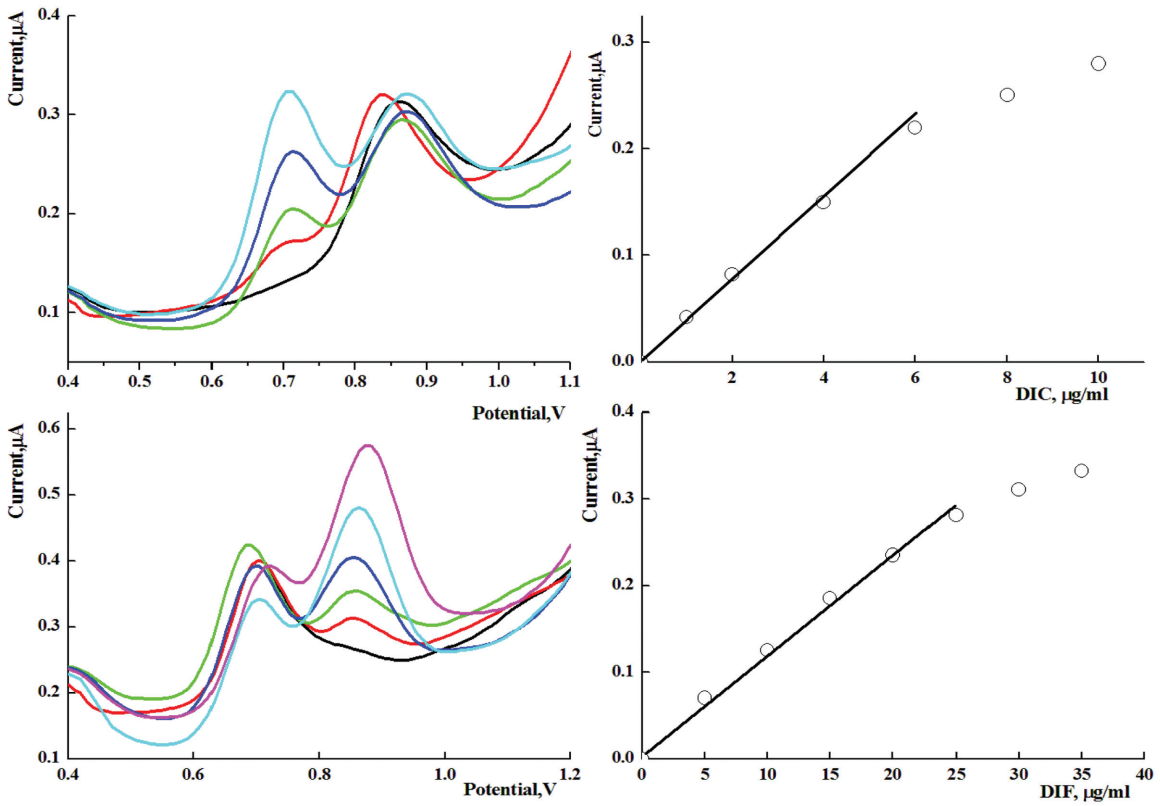
Determination of diclofenac and diflunisal in real samples

The developed method was applied successfully to determine DIC and DIF in real samples with an average recovery of 100.7 and 103.1%, respectively. The sensor reproducibility was investigated via an intraday study. No significant changes were observed in the peak current with the relative standard deviation (RSD) value of 4.16% and 4.74% for DIC and DIF, respectively. It confirms that the proposed method show high stability and reproducibility, which is necessary in analytical applications. Furthermore, the reference method by RP-HPLC [17] was applied for the estimation of DIC and DIF in their combined formulation; Rheumafen suppositories and synthetic samples as in Table 3. From the results that we

Table 1 The regression parameters and validation results for diclofenac and diflunisal determination by the proposed method

Parameter	DIC	DIF
Intercept±S_a	0.0088±0.0024	0.0196±0.0051
Slope±S_b	0.0035±6.25E-5	0.0011±3.06E-5
Linearity range, µg/ml	1–6	5–25
R	0.994	0.993
LOD, µg/ml	0.204	1.366
LOQ µg/ml	0.680	4.553

Figure 6



Calibration curve for the determination of DIC and DIF by square wave voltammetry on the bare carbon paste electrode in pH 5.0 buffer solution.

Table 2 Determination of diclofenac and diflunisal in synthetic samples by square wave voltammetry technique in pH 5.0 buffer solution

Taken, μg/ml		Found, μg/ml		Recovery, %	
DIC	DIF	DIC	DIF	DIC	DIF
0	100	-	101.5	-	101.5
10	100	9.4	102.8	94	102.8
20	100	20.7	103.4	103.7	103.4
40	100	40.0	103.2	100.0	103.2
60	100	59.9	103.8	99.8	103.8
50	0	54.8	-	109.5	-
50	50	51.3	47.4	102.7	94.7
50	100	51.1	99.1	102.1	99.1
50	150	49.4	155.5	98.7	103.6
50	200	48.5	202.4	97.0	101.2
50	250	49.6	245.7	99.3	98.3
Average, %				100.7	101.1
RSD, %				4.19	4.89
Bias				0.7	1.1
Confidence interval (CI) at α=0.05 and n=10				2.430	2.835

Table 3 Determination of diclofenac and diflunisal in (Rheumafen suppositories) by the proposed method

Taken, μg/ml		Found, Mean±SD, % Proposed Method		Reference HPLC [14]	
DIC	DIF	DIC	DIF	DIC	DIF
2.5	5	97.382	95.398	102.690	94.737
3.75	7.5	100.289	102.158	101.123	99.060
5	10	99.592	103.759	98.722	103.634
Accuracy, bias,%	-	0.912	–0.439	–0.845	0.856
Precision, RSD	-	1.532	4.418	1.981	4.488
CI at α=0.05	-	0.949	2.739	1.228	2.782
t-test*	-	1.21305	0.3569	-	-
F-test*	-	0.5769	0.048	-	-

*t Critical and F Critical one-tail at n=9, 95% confident are 1.86 and 3.44, respectively.

observed the proposed method is applicable successfully for the assay of the real samples by comparison with the reference method and show selectivity, high sensitivity and accuracy.

Conclusion

The present work introduced a simple, sensitive and selective method for simultaneous square wave voltammetric determination of dicolfenac and diflunisal in real and synthetic samples using bare 'CPE'. An attempt was made to improve the efficiency of CPE by incorporating multiwalled carbon nanotubes, and a slight improvement was found in the peak current compared with the CPE, and therefore this nanomaterial was excluded. The cited CPE showed good linearity by both drugs with concentration ranges from 1 to 6 for DIC and 5 to 25 μg/ml for DIF, while the quantification limit LOQ and detection limit LOD were 0.68 and 0.204 μg/ml for DIC and 4.553 and 1.366 μg/ml for DIF. The method was applied in real and synthetic samples and found acceptable average recoveries in comparison with the official method.

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Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

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