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An LC–MS/MS quantification method development and validation for the dabrafenib in biological matrices

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ABSTRACT

An accurate, specific, and robust liquid chromatography–tandem mass spectrometry technique was developed and validated for the quantification of dabrafenib in plasma samples. Internal standard and drug components were subjected to extraction by utilizing liquid–liquid extraction method by utilizing ethyl acetate. high performance liquid chromatography of reverse phase was operated with Phenomenex (50×4.60 mm, 5.0 µm) C_{18} analytical column, isocratic system of mobile solvent comprised acetonitrile and formic acid (0.1%) (85:15, %V/V). Mass triple quadrupole detection system was utilized for the analysis. Electrospray ionization in the positive ionization approach method operated in multiple reactions monitoring with ionic transition of m/z 520.10–176.98, m/z 465.09–244.10 for dabrafenib, sorafenib, respectively. Rectilinear plot was processed in concentration levels of 74–2,956 ng.ml^{-1} and the method validation was executed as per the United States Food and Drug Administration strategies for bio analytical methods. The recovery findings obtained were more than 92.5% and the accurateness was fall in between–1.53% and 2.94% of relative error and % relative standard deviation findings were <4.65%. The high sensitiveness, better accuracy, and precision with good recovery findings for the plasma samples of a developed method prove its applicability for pharmacokinetics and bioequivalence studies.

INTRODUCTION

Dabrafenib chemically designated as N- {3- [5- (2-amino pyrimidin-4-yl) -2-tert- butyl-1, 3-thiazol -4-yl]-2-fluorophenyl} -2, 6-difluoro benzene-1-sulfonamide. Its chemical formula and molecular mass are $C_{23}H_{20}F_3N_5O_2S_2$ and 519.56 g/mol, respectively (Fig. 1). It (Tafinlar is a trade name) is concomitant with a mutated type version of a *BRAF* gene and useful in the management of cancer (Gibney *et al*., 2013; Huang *et al*., 2013; Lawrence *et al*., 2014). This drug is related to the B-RAF enzyme, which will exhibit important activity in cellular growth regulation, and inhibits to produce anticancer action. During the phase I and II trials, this drug has shown safety management profiles in all the patients associated with mutated metastatic melanoma B-RAF (V600) (Maverakis *et al*., 2015; Suttle *et al*., 2015).

First, this drug is utilized singly for the management of metastatic melanoma or unresectable with B-RAF (V600E) mutations as an inhibitor of kinases. This was identified by US-FDA confirmed tests (Ascierto *et al*., 2012; FDA, 2013,2018). Utilization of combined formulation was created a demonstrational strong response rate. Development in disease-related symptoms or complete existence has not at confirmed for this drug when combined with trametinib (Dhillon *et al*., 2007; Hauschild *et al*., 2017; Long *et al*., 2017).

Literature review on dabrafenib reveals that only two analytical approaches on LC-tandem mass spectroscopy (Merienne *et al*., 2017; Svante *et al*., 2017) were described for the assessment of dabrafenib in sample plasma. The reported works were in combination with the other components. Therefore, current work was aimed to develop an accurate, specific, and robust liquid chromatography–tandem mass spectrometry (LC–MS/MS) technique for the assessment of dabrafenib in human's plasma, as a single drug.

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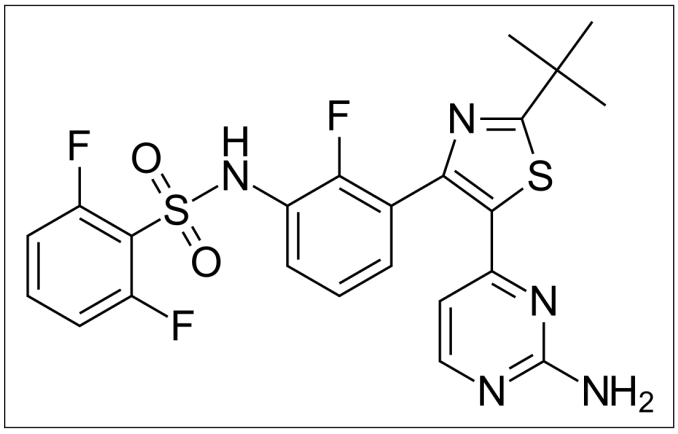

Figure 1. Dabrafenib chemical structure.

MATERIALS AND METHODS

Chemical reagents

The dabrafenib (purity: 99.68%) standard and sorafenib (purity: 99.87%) utilized as internal standard (IS) were obtained from the MSN Labs, Hyderabad, India. Acetonitrile (ACN) and methyl alcohol of LC purity were acquired from Merck, Mumbai, India. Deionization of water has been processed from Milli-Q waters system (Millipore, USA).

LC–MS/MS system and its settings

The LC–MS/MS system comprising an Agilent 1200 liquid chromatographic instrument with a dual pumps-SL and an Agilents/6164 triple quadrupole mass spectroscopic system with electro spraying ionizations source (CA). Chromatographic data was processed by a Mass Hunters version B.010.004 software. Through Phenominex (50 × 4.6mm id, 5 µm), C_{18} analytical column, an isocratic solvent system comprised ACN and HCOOH (0.1%), (85:15, %*v/v*) was passed. The assessment of analytes was executed in a triple quadrupoles mass system retaining electro spraying ionizations method, functioning in multiple reactions monitoring, and transitions were m/z 520.10–176.98, m/z 465.09–244.10 for dabrafenib, sorafenib, respectively, in the positive ionizations mode (Raviraj *et al*., 2016; Vijaykumar and Raviraj, 2019). The MS/MS settings were fixed as: nebulize gas pressures, 45.0 psi, source temperature, 500ºC; capillaries voltage, 6.00 kV, and dryer gas (N_2) flow, 10 l/minute. The injection volume and auto-sampler temperatures were set to 10 µl and 5.0ºC, respectively. The flowing rates of 0.80 ml.minute^{-1} and collisional energies of 20 eV were utilized in the chromatographic elution.

Protocol quality control samples

1.0 mg/ml individual dabrafenib stock solution and IS were executed in ACN (diluent) separately. The stock solution of dabrafenib was then subjected for serial dilution with diluent to attain the operational standards. An IS operational solution at 250 ng.ml^{-1} executed by dilution of IS primary solutions with diluent. Processed standard samples were retained at −20ºC before the utilization.

Calibration standards of dabrafenib (74, 180, 450, 850, 1,300, 1,800, 2,350, and 2,956 ng/ml,) were processed by spiking method applied to working standards to blank plasmas. Quality controls at lower, medium, and higher concentration (207, 1,478, and 2,217 ng.ml^{-1}), were executed separately in a similar mode.

Protocol for samples preparation

A 200 µl aliquots of plasmas samples were located in 10 ml plastic tubes, follows by adding 100 µl of IS operational solution was added in all the sample solutions excluding the blank sample. The resulting mixture was extracted with 5.0 ml of ethyl acetate after the sonication for 20 minutes. Next, the sample was subjected for centrifugation for 15.0 minutes at 5.0ºC and 5,000 rpm. The supernant organic solvent phase was relocated into fresh glass tube and nitrogen steam was applied to evaporate the same. Resultant dried residue was reconstructed with 100 µl of movable solvent and 10µl aliquots were infused to and LC–MS/MS equipment for examination.

Analytical method validation

Developed analytical technique was validated as per the regulatory guidelines of US-FDA for different validation constraints to meet the respective guidelines (EMA, 2011; FDA, 2001).

RESULT AND DISCUSSIONS

Mass spectrometric instrument

In the development stage, fresh dabrafenib solution was injected for the optimization of the product and parent ion. Precursor ion at 520.10 m/z value was detected in the positive ionizations approach. Upon fragmentation of the precursor ion, fragments of m/z 176.98 and 94.04 were noticed. The daughter ion of dabrafenib at 176.98 m/z was identified with a maximum intensity value. Sorafenib is having similar physicochemical properties with the dabrafenib to select as an IS for this bioanalytical method development and for good recovery during the sample preparation and validation process. Multiple reactions monitoring (MRM) scan was executed for the identification of the product and parent ions for both drugs and transitions finalized as m/z 520.10 –176.98 for dabrafenib and m/z 465.09–244.10 for sorafenib. (Bhamare *et al*., 2019; Kulkarni *et al*., 2016a, 2016b; Patel *et al*., 2017)

Specificity

Plasma blank and spiked plasma at lower limit of quantifiacation (LLOQ) level (74 ng/ml) of dabrafenib and IS were ran in the LC-MS/MS system and the outcomes were represented in Figure. 2. No intervention peaks were noticed for dabrafenib and sorafenib from sample plasmas. The drug and the IS were isolated from the system within 4 minutes and the retaining times of dabrafenib and sorafenib were 2.40 and 3.0 minutes, correspondingly (ICH, 2005; Vikingsson *et al*., 2017).

Sensitivity and linearity

The LLOQQC of drug component was fixed at 74 ng.ml^{-1}, because at this concentration level the signal/noises findings were >10.0, and the accurateness and precision findings were <2.82 %relative standard deviation (RSD). Rectilinear plots were processed for every batch between the concentration levels of 74.0–2,596.0 ng.ml^{-1} for dabrafenib in plasmas (Table 1). The equation of regression plot was calculated to form the average values of six replica calibration standards and was found as:

$y = 0.00034\ x + 0.00018$ for dabrafenib, where "x" represents the plasmas concentration (Nirav *et al*., 2017; Jaivik *et al*., 2017) and "y" indicates the peaks ratio, i.e., analytes/IS.

Recovery, accuracy, and precision

Inter-day, and intra-day accurateness, and precision findings were given in Table 2 and Figure. 3. Within a day precision finding existed in between 2.17% and 3.94% (RSD) for dabrafenib, whereas the accurateness findings were within -1.53%–2.94% of relative error. Likewise, for between the days experimental, precision was changed between 1.54% and 3.58% (RSD) for dabrafenib, whereas the accurateness was between −1.37% and 4.65% of relative error (Patel *et al*., 2011).

The dabrafenib average recovery findings were in the range of 95.35%–101.37% at 3-QC concentrations (Table 3). The executed LLE for the sampling process proved that dabrafenib and sorafenib (97.49%) were recovered with higher percentage values from plasma (Titier *et al*., 1997). These resulted findings were

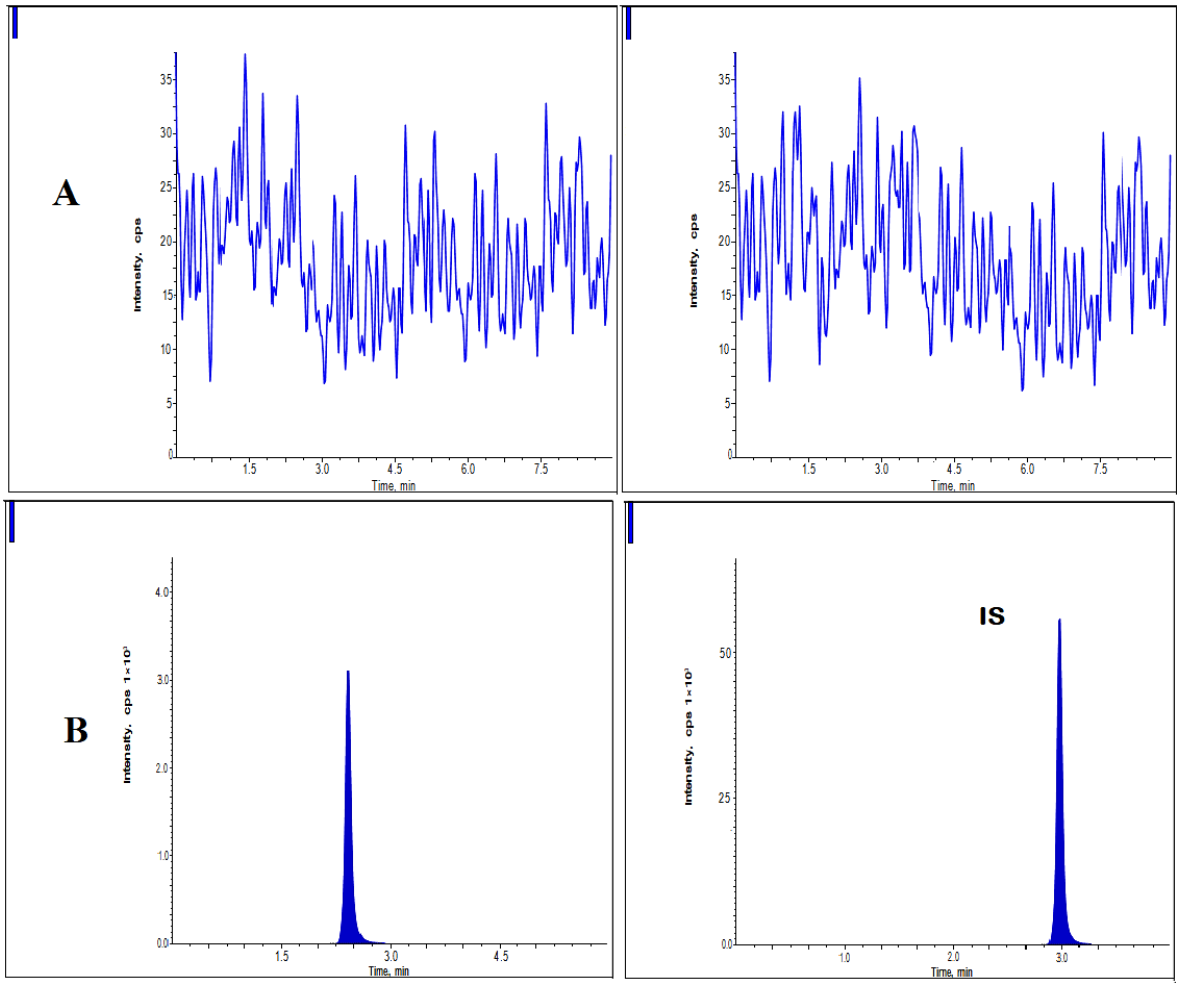

Figure 2. Dabrafenib processed chromatograms at A) Blank and B) LLOQ samples.

Table 1. Linearity standard levels for dabrafenib.

LS-ID	Concentration (ng.ml^{-1})	Mean[a] (ng.ml^{-1})	% RSD	% RE
LS-1	74	73.12	0.88	2.15
LS-2	180	175.87	4.13	3.25
LS-3	450	433.57	16.43	1.95
LS-4	850	865.98	−15.98	3.87
LS-5	1,300	1,329.04	−29.04	2.69
LS-6	1,800	1,863.96	−63.96	4.32
LS-7	2,350	2,275.13	74.87	2.24
LS-8	2,956	3,043.28	−87.28	3.16

[a]Six replicates.
RE, Relative error.

Table 2. Intra, and inter-day accurateness and precision of dabrafenib in plasma.

Spiked conc. (ng/ml)	Intraday (n = 6)			Interday (n = 6×3)		
	Measured conc. (mean ± SD; ng/ml)	Precision (RSD %)	Accuracy (RE %)	Measured conc. (mean ± SD; ng/ml)	Precision (RSD %)	Accuracy (RE %)
74	71.82 ± 1.56	2.17	2.94	70.56 ± 2.53	3.58	4.65
207	235.01 ± 9.25	3.94	−1.53	209.84 ± 3.24	1.54	−1.37
1,478	1,448 ± 32.24	2.23	2.02	1,441.35 ± 30.52	2.12	2.48
2,217	2,189 ± 62.84	2.87	1.26	2,178.32 ± 59.65	2.74	1.75

RE, Relative error; RSD, Relative standard deviation.

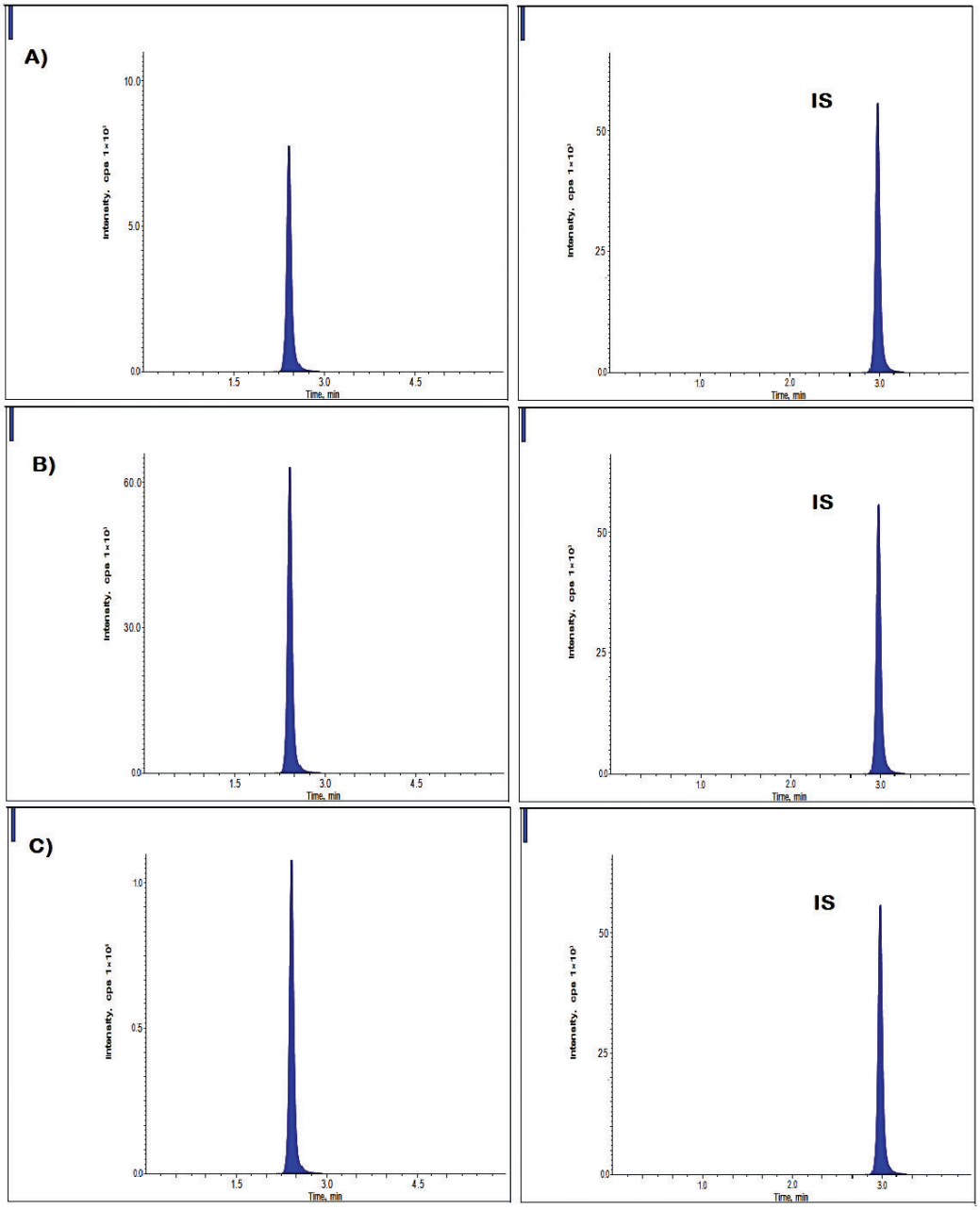

Figure 3. Representative dabrafenib chromatograms at A) LQC B) MQC, and C) HQC levels.

Table 3. Recovery rates of dabrafenib and sorafenib.

Concentration level	*x*	*y*	% Recoveries	% Mean recoveries	% RSD
LQC	8,752	8,345	95.35	98.62	2.52
MQC	62,495	61,957	99.14		
HQC	93,743	95,027	101.37		
Sorafenib	56,846	55,419	97.49		

x, average recovery of un-extracted sample; *y*, average recovery of extract sample.

Table 4. Matrix effect for dabrafenib at LQC and HQC levels.

	LQC			HQC		
S. No	Peak area without matrix	Peak area with of matrix	Matrix factor	Peak area without matrix	Peak area with of matrix	Matrix factor
1	8,366	8,477	101.32	94,135	90,671	96.32
2	8,263	8,104	98.07	93,061	91,377	98.19
3	8,613	8,332	96.74	92,721	95,280	102.76
4	9,373	9,592	102.34	87,223	88,654	101.64
5	8,317	7,952	95.62	86,494	82,705	95.62
6	8,656	8,467	97.81	95,879	93,501	97.52
Mean			98.65			98.675
± SD			2.63			2.89
% RSD			2.67			2.93

RSD: Relative standard deviation; SD, Standard deviation.

Table 5. The stabilities of dabrafenib in humans plasma under dissimilar storage environments ($n = 3$).

Storage condition	LQC-207 ng.ml^{-1}		MQC-1478 ng.ml^{-1}		HQC-2217 ng.ml^{-1}	
	Accuracy (average %)	Precision (RSD %)	Accuracy (average %)	Precision (RSD %)	Accuracy (Average %)	Precision (RSD %)
Room temp., 8 hours	93.89	2.95	101.85	1.79	96.31	2.65
30 days at −20.0°C	96.24	3.27	98.37	4.01	98.12	3.85
Three freeze-thawed cycles	103.27	1.52	95.65	4.21	102.47	2.85
Extracts, 24.0 hours at 4.0°C	98.56	1.87	94.98	2.34	103.41	4.08

RSD, Relative standard deviation.

proven the accuracy in terms of recovery and precision in terms of %RSD.

Matrix effect

The findings of matrix effect values were represented in Table 4. The respective peaks area ratio of the drug/IS solubilized in the plasma blank extract to those solubilized with movable solvent existed in between 95.62% and 102.34% for dabrafenib at lower quality control (LQC) level and 95.62%–102.76% at high quality control (HQC) level. These results proposed that interfering matrix component effect of the analytes were insignificant when exposed to the developed LC–MS/MS circumstances (Jaivik *et al*., 2017; Titier *et al*., 1997).

Stability tests

The dabrafenib stability of was established subsequently subjecting control sample solutions to dissimilar storage environments (FDA, 2001; Nirav *et al*., 2017). The subjected environments comprise longer time stabilities after the storing at -20.0°C for 30.0 days, shorter time stabilities at the room conditions for a period of 8.0 hours, processed samples (extracts) stabilities after 24.0 hours at 4.0°C, and three completely freeze-thawed cycle (frozen at −20.0°C for 12.0 hour). The finding for stabilities for control sample solutions in the plasmas was represented in a Table 5. The assessed accurateness values for dabrafenib drug were existed in between 94.98% and 103.27%, which were acceptable as per the regulatory guidelines.

CONCLUSION

A specific, accurate, and new validated LC–MS/MS technique was produced for the quantitation of US-FDA approved dabrafenib anticancer drug in plasmas of humans. Within a day precision finding existed in between 2.17% and 3.94% (RSD) for dabrafenib, whereas the accurateness findings were within −1.53%–2.94% of relative error. Likewise, for between the days experimental, precision was changed in between 1.54% and 3.58%

(RSD) for dabrafenib, whereas the accurateness was in between −1.37% and 4.65% of relative error. The findings of matrix effect values were existed in between 95.62% and 102.34% for dabrafenib at LQC level and 95.62%–102.76% at HQC level. The LLOQQC of drug component was fixed at 74 $ng.ml^{-1}$, because at this concentration level the Signal/Noise findings were >10.0. Equation of regression plot was $y = 0.00034\ x + 0.00018$ for dabrafenib. Finally, the produced method was within the limits of bioanalytical method validation guidelines and can be successfully applicable to quantitate the dabrafenib in variable bio-samples.

The intra-day and inter-day accuracies were within −1.37% to 4.65% of relative error and the RSD of precision were less than 3.94%. The drug was sufficiently stable under different analytical conditions. liquid liquid extraction method was optimized for dabrafenib extracting from plasma with mean percent recoveries of 98.62% by utilizing the sorafenib as an IS. The sensitivity, good validation criteria, and high percent recoveries from plasma of the proposed method rendered it applicable for the bioequivalence and pharmacokinetic studies.

CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

FUNDING

There is no funding to report.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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