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Review Article

Active Enatiomeric Form of Pregabalin Based on Sole and Coupled Chromatography Methods

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ABSTRACT

Objective: Pregabalin (PGB) is an antiepileptic drug, named (S)-3-(aminomethyl)-5-methyl hexanoic acid. Since the discovery, PGB has remained the main representative drug from the antiepileptic category, due to its unique mode of action which concerns the treatment of painful diabetic neuropathy postherpetic neuralgia by lagging down the impulses in the brain which causes seizures.

Chemically, PGB shows amphoteric (zwitterionic) properties and the presence of a chiral center reflects the comparatively high biological activity of one of its isomers. Analytically, the ring closure and ring-opening of PGB depend on the solubility and the method or instrument adopted. The analytical techniques in pharmaceuticals are used for analysis and drug monitoring remains important in perceiving factors like bioavailability, bioequivalence, and therapeutic monitoring.Due to all mentioned features, it becomes necessary to compile the various analytical methods that have been reported in the literature for its analysis. The present critical review assesses the compilation of relevant articles that have already been published in recent ten years, describing analytical methods like UV, HPLC (single and combined dosage form), HPTLC, LC-MS/MS, and GC/MS method for the same.

Conclusion: The present comprehensive review disclosed a summary of pharmacokinetics-dynamics, pharmaceutical preparations, and analytical methods of bulk drug and biological matrices since the time of discovery.

Keywords: Pregabalin, RP-HPLC, LC-MS/MS, HPTLC, GC-MS, Pharmacokinetic, Pharmacodynamics, Synthesis.

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ABBREVATIONS

PGB: Pregabalin; NT: Neurotransmitter; GBP: Gabapentin; VGP: Vigabatrin; TOP: Topiramate; BLF: Baclofen; VGSCs: Voltage-Gated Sodium Channels; VGPCs: Voltage-gated Potassium Channels; VGCCs: Voltage-Gated Calcium Channels; DDQ: 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone; TCNQ: 7,7,8,8-Tetracyanoquinodimethane; ALA: Alpha-Lipoicacid; λmax: Lamda Max; RF: Retention Factor; RT: Retention Time; FR: Flow Rate; UV-VIS: UV-Visible Spectrometry; HPLC: High-Performance Liquid Chromatography; RP-HPLC: Reverse-Phase High-Performance Liquid Chromatography; HPTLC: High Performance Thin Later Chromatography; LC-MS/MS: Liquid Chromatography-Mass Spectrometry/Mass Spectrometry; GC/GC-MS: Gas Chromatography/Gas Chromatography-Mass Spectrometry; DCC: Dicyclohexylcarbodiimide; NHS: N-Hydroxysuccinimide; EDC:1-ethyl-3-(3-Dimethylaminopropyl) Carbodiimide; ECF: Ethyl Chloroformate; MCF: MethylChloroformate; IBCF: Isobytyl-Chloroformate; OPA: Orthophosphoric Acid; IUPAC: International Union of Pure and Applied chemistry; CNS: Central Nervous System; ATP: Adenosine Triphosphate; Ca^{2+}: Calcium2^{+}; MIP: Indian Pharmacopoeia; Cm: Centimeter; mm: Millimeter; nm: Nanometer; µl: Micro liter; µg: Microgram; NaOH: Sodium hydroxide; ACN: Acetonitrile; DW: Distilled water; LOD: Limit of Detection; LOQ: Limit of Quantification; ®: Registered Trademark.

INTRODUCTION

FNeuropathic pain is the result of hyper-excitability of neurons in damaged areas of nerves which are similar to that of convulsion [1]. Drugs like Gabapentin (GBP) and its analog Pregabalin (PGB) were designed against pains associated with trigeminal neuralgia and diabetic neuropathy [2]. Both analogs GBP and PGB are essential in treatment because of their minimum drug interactions and side effect [3]. Most of the drugs of the epilepsy section work on varied mechanisms and so considered to be broad-spectrum drugs [4]. The basic mechanism of antiepileptic drug based on the most important inhibitory neurotransmitter (NT) of the Central Nervous System (CNS), γ-aminobutyric acid (GABA) enhancement. Modulation of voltage-gated ion channel and attenuation of glutamate-mediated NT in the brain is considered as other mechanisms for drugs like PGB[5,6]. The GABA is considered to exert major and rapid inhibitory action from all classes of NT in the brain. It is believed that GABA is ionotropic [7] and metabotropic [8] in its sub-forms. The GABA-A and GABA-C are considered ionotropic while GABAB is metabotropic [9,10]. GABA-A is the preferred target for anticonvulsant drugs like PGB and GBP [11,12]. Moreover, GABA-B receptors with a subunit of GABA-B RS are also abundant at the spine and dendrites, where they are influencing synaptic and dendritic functions, result in modulating calcium (Ca^{2+}) signals[13]. Considering another mechanism of modulation of voltage-gated ion channel (Ca^{2+}, $Na^{+}$and K^{+}), the calcium channel plays a vital role in regulating Ca^{2+} signaling united with the action of PGB. Such channels are also known as voltage-gated calcium channels (VGCCs) [14]. For the basic anticonvulsant drugs like phenytoin, the preferred target is voltage-gated sodium channels

(VGSCs) at the presynaptic nerve terminal of excitatory glutamate receptors[15]. These drugs cause highly neuronal firing when binding to an inactivation gate nearby resulting inactivation of VGSCs. The intimately associated voltage-gated K^+ channels with the membrane repolarization process are again considered to be important for drugs like Levetiracetam[16,17]. The drug PGB is structurally related to both the inhibitory NT GABA and GBP. Chemically PGB is 3-(aminomethyl)-5-methyl hexanoic acid or 4-amino-3-(2-methyl propyl)butanoic acid with molecular formula C8H18O2N, melting point 186-188 ºC and the molecular weight 159.23 g Mol-1 [18,19]. The PGB is available in the enantiomeric form (R) and (S) (Fig. 1) where the (S)-enantiomer is approximately 10 times more active than the (R)-enantiomer[20]. The compound has one stereogenic center and in preclinical studies, there was no indication of racemization of PGB S-enantiomer to the R-enantiomer [21]. The approved product LYRICA®(USP 6,197,819 B1-2001) capsules contain 25, 50, 75, 100, 150, 200, 225 or 300 mg of PGB. The LYRICA® with active ingredients contains S-(+)-4-amino-3-(2-methyl propyl) butanoic acid [22]. PGB is a potent ligand of alpha-2-delta subunit of voltage-gated calcium channel in the central nervous system which shows analgesic, anticonvulsant and anxiolytic activity [23]. It is a structural analog, but functionally dissimilar, of naturally occurring transmitter GABA (γ-aminobutyric acid). It is generally used for epilepsy, neuropathic pain, and anxiety condition. It is soluble in an aqueous solution and partially soluble in nonpolar solvents like DMSO, ethanol, DMF. It's a crystalline substance that occurs in a single morphic form and non-hygroscopic. It is thermally stable and not solvated [24]. Aside from the chemical, pharmacodynamic, and pharmacokinetic parts, which are older but accurate, the manuscript is being designed using the most recent spectroscopical data available from 2010 to 2021, as well as the LYRICA patent (2005) (Fig. 1).

LITERATURE REVIEW

Synthesis

PGB the best drug for the treatment of epilepsyis available in the enantiomeric form. As discussed, the S-(+) enantiomer is more potent than the R-(-) enantiomer and hence the synthesis of more active enantiomer or separation of enantiomeric form remains in focus during the designing of PGB. The schemes describe a few crucial industrial and patented methods to synthesize the PGB.

As per Fig. 2, the patented method of synthesis of PGB includes chiral oxazolidinone alkylation (asymmetric) chemistry. The chloride derivative (1.2) of (1.1) was used for acylation of the anion of chiral (1.3) to get (1.4) which was then alkylated with benzyl bro¬moacetate to produce (1.5) with greater than 95% enantio¬meric excess. The chiral auxiliary was treated with lithium hydroxide/hydrogen peroxide followed by sodium bisulfite to produce acid (1.6). The resulting acid was reduced to alcohol (1.7) using boranedimethyl sulfide. The obtained (1.7) was converted to the tosylate derivative using tosyl chloride in pyridine to produce (1.8) and then to azide (1.9) derivative by using sodium azidein dimethyl sulfoxide. Finally, the hydrogenation of azide in the presence of palladium on charcoal, catalyst gives PGB [25] (Fig. 2).

The amino acid (2.1) in Fig. 3 can be converted to get (2.2) by treating sodium nitrite and sodium bromide in dilute acid. The retention of configuration can be seen in the proceeded reaction. In another step, the bromo acid was esterified using t-butyl acetate to produce (2.3). The compound (2.4) can be produced by displacement of bromide with diethylsodiomalonate, which was then hydrolyzed to the acid (2.5). Further, the chiral lactone (2.6) can be produced by reduction with boranedimethyl¬sulfide. The ring openings of the lactone with trimethylsilyl iodide produce (2.7) which gives (2.8) in treatment with sodium azide. At last, treatment of azide *via* hydrolysis of acid and hydrogenation produces PGB[26] (Fig. 3).

Thesynthesis of PGB can be carried out by Stobbe condensation (Fig. 4) by utilizing (3.1) and (3.2) using di-n-propylamine/acetic acid as a catalyst to produce (3.3). Then, the hydro-cyanation product (3.4) can be obtained to get the racemic compound (3.5). Further, by hydrolysis and decarboxyl¬ation the final product of PGB can be obtained[27] (Fig. 4).

The synthesis of enantio-selective PGB can be carried out by the introduction of a nucleophile in β-position of a ά, β-unsaturated carbonyl using Iminium catalyst (4.3). The proclivity of catalyst (4.3) with (4.1) and (4.2) was used in the three-step synthesis of PGB[28] (Fig. 5).

It describes the enantio-selective synthesis of PGB with a single chlorogenic center using enantio-selective hydrogenation of (5.1). The reaction carried out at 55oC in methanol using (5.2) asapre-catalyst to get (5.3) a high enantio-selective compound. Finally, the reaction scaled up with hydrogenation of nitrile to amino-group over a heterogeneous nickel, catalyst to obtain PGB [29] (Fig. 6).

Mechanism of action

PGB considerably resembles the (GBP) and accepted for the treatment of neuropathic pain since 2004. They are considered derivatives of inhibitory GABA. The evolution of PGB was coming into existence to suppress the anti-spastic effects of GBP. The mechanism of action of both the drugs used to demonstrate the efficacy in the treatment of both seizure disorders and pain syndromes. From the various mechanisms of actions of PGB, the action of voltage-gated sodium channels (VGSCs), voltage-gated potassium channels (VGPCs), and voltage-gated calcium channels (VGCCs), the action of VGCCs are considered as the primary mechanism. In the recent literature, it was found that neither PGB nor GBP affects sustained repetitive firing in the spinal cord or cortical neurons following acute exposure. The inadequacy of action at batrachotoxin binding sites in rat suggests that blocking VGSCs rarely contribute to the action of PGB [30,31].Considering the action of VGPCs, in a study, it was concluded that enlarging the exposure of PGB produces a late-onset allosteric enhancement of VGPCs in dorsal root ganglion neurons in rats. The action may be due to the activation of a specific protein named Kinase-A[32] (Fig. 7).

PGB shows high-affinity binding to the α2δ subunit of the P/Q type of voltage-gated channel. The voltage-gated calcium channel is closed to resting membrane potential, the depolarization by action potential causes the channel to open which leads to the entry of Ca^{2+}into the cell axonal membrane depolarizes when the action potential travels down to the neuron. When a voltage-gated calcium channel opens which causes an intrinsic current, the NT releases it from the synaptic vesicle and the multiplication of neurotransmission. In the presence of Ca^{2+}exocytosis of NT and membrane fusion occurs [33,34].

PGB targets the voltage-gated calcium channel which consists of four subunits (Fig. 7a). The α1 subunit is a transmembrane array from a pore through which Ca^{2+} enters into the cell. The α2δ subunit contains δ protein linked by a disulfide bond to α2 protein, which has a higher affinity for the PGB binding site. The β subunit is intracellular and it modifies the functioning of the α2δ subunit, while the γ subunit is a glycoprotein that Inline in the cell membrane as shown in Fig. 7b [35-38].

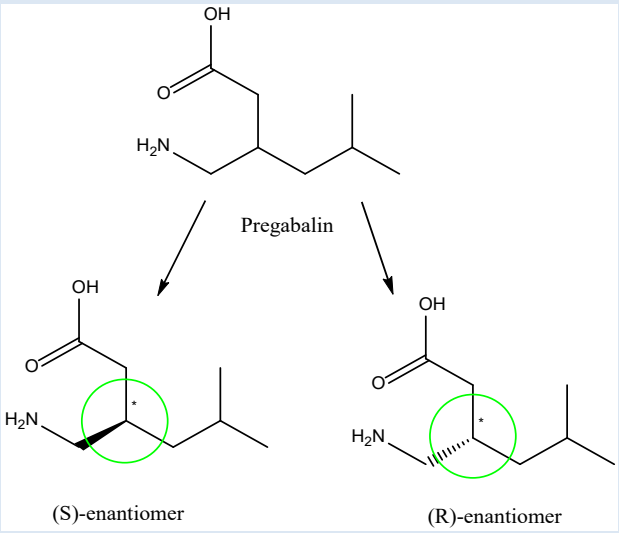


Figure 1: Structure of PGB with enantiomers.

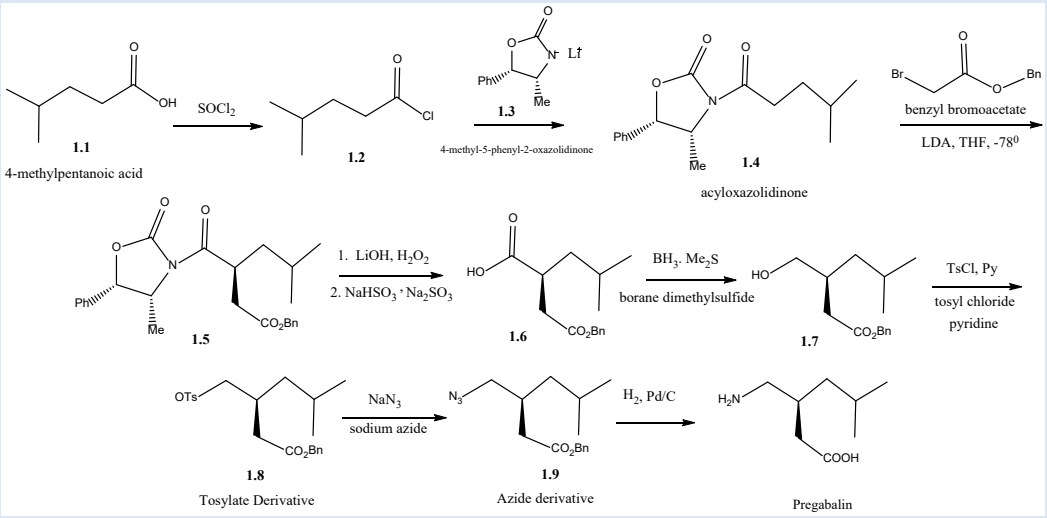


Figure 2: Synthesis of PGB (LYRICA)®.

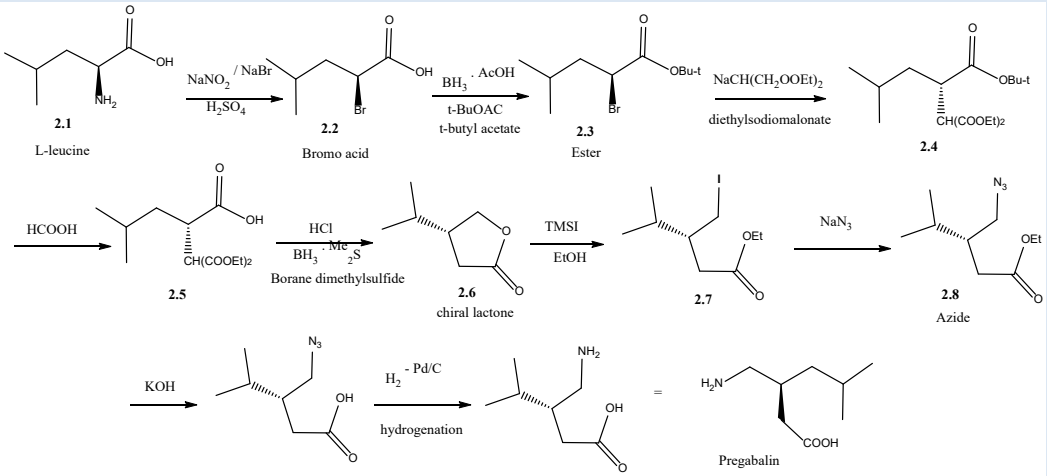


Figure 3: Synthesis of PGB.

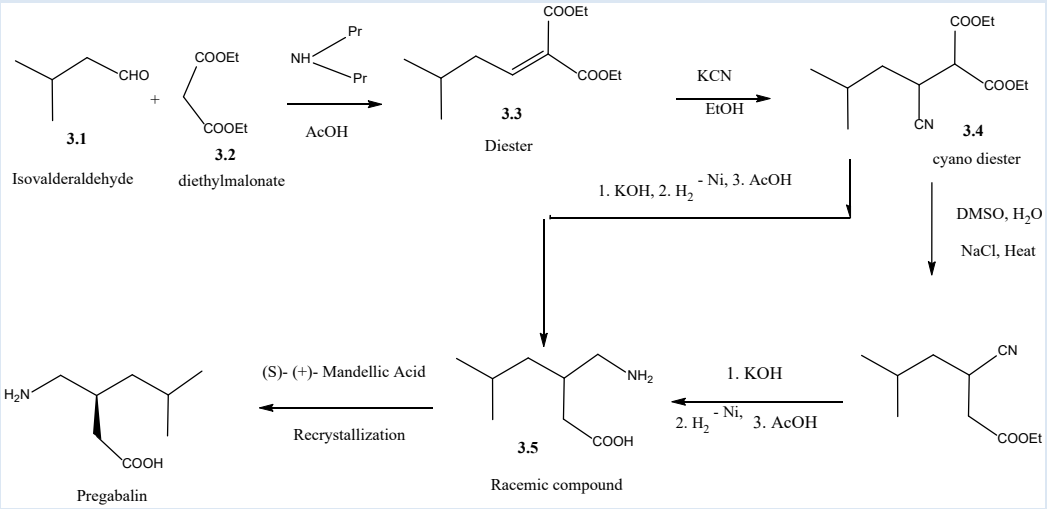


Figure 4: Synthesis of PGB by Stobbe condensation.

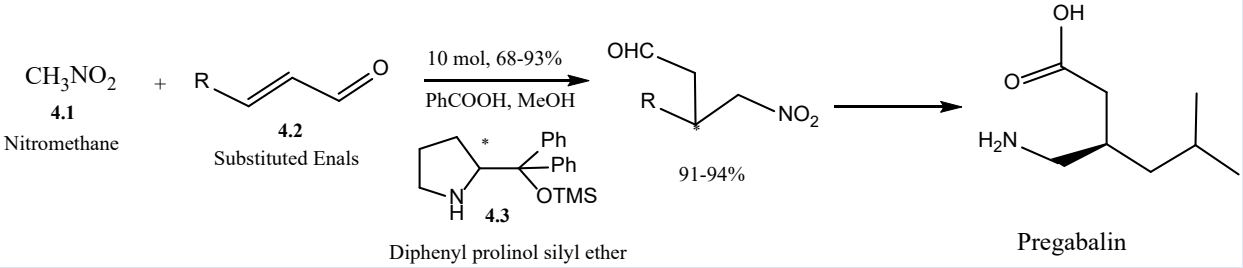
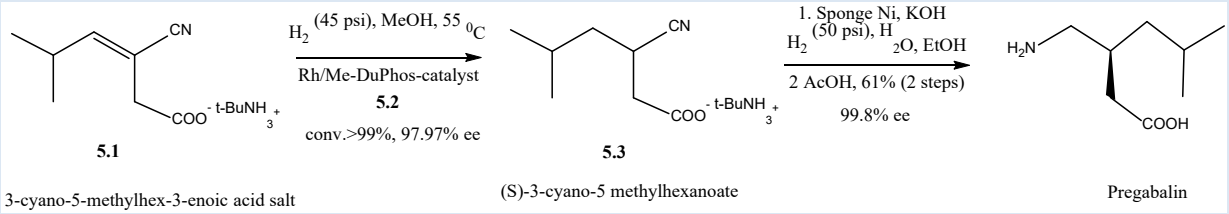


Figure 5: Synthesis of PGB with Iminium catalyst.



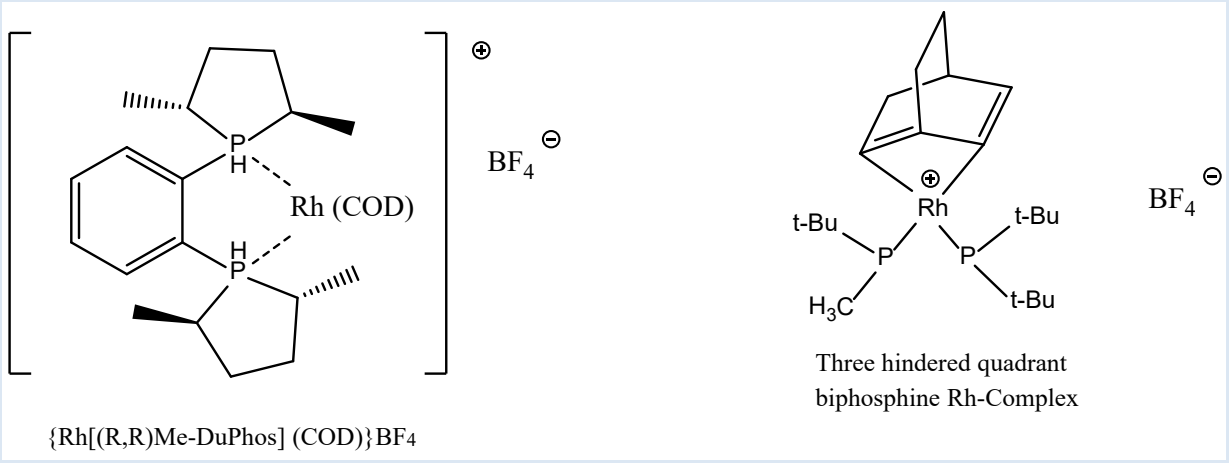


Figure 6: Catalytic synthesis of PGB.

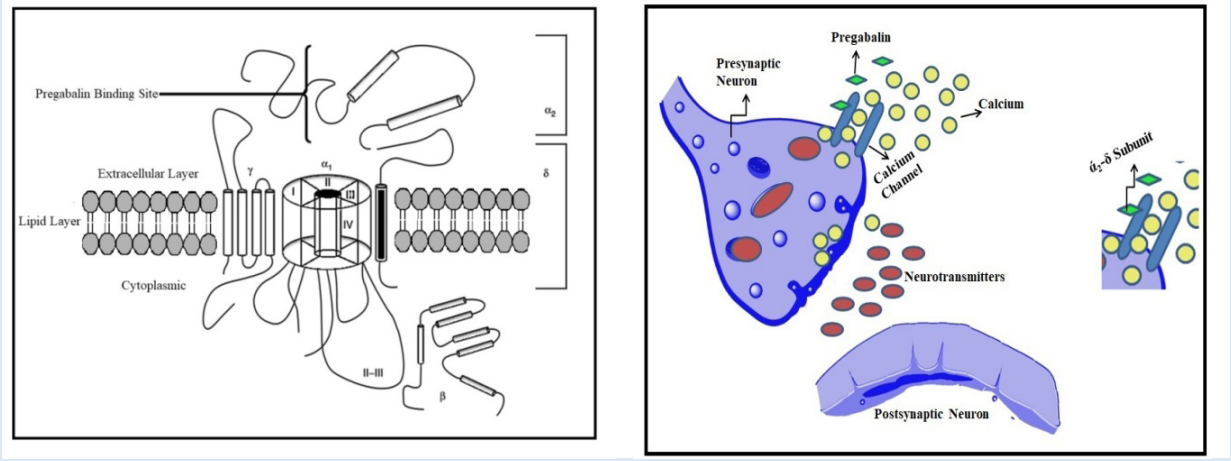


Figure 7: Binding site and mechanism of action of PGB, a) Represents thebinding Site of PGB at VGCCsdifferent units; b) Represents the mechanism of action of PGB at pre and post synaptic neurons.

PGB pharmacokinetics

PGB is quickly absorbed and shows linear pharmacokinetics after oral administration. Its oral bioavailability is ≥ 90% peak plasma concentration arises 1 hr after oral administration and constant concentration achieve within 24-48 hrs. 20-25% peak plasma level decreases by the food intake and increase time to peak level by 3 hrs [39-41].This study includes single-dose and multi-dose tolerance studies. PGB has a comparatively short half-life which has a volume of distribution of 0.5 L kg-1 which does not bind to plasma protein. Pharmacokinetics investigation of clinical studies shows that pharmacokinetics PGB was not significantly influenced by sex or race [42,43].

Adsorption

PGB is quickly and widely absorbed after oral administration in the fasted state which shows the maximum plasma concentration after 1 hr in single or multiple doses and steady-state has been obtained within 24-48 hours after repeated dosing [44]. These fast absorption properties reflect the observed onset of efficacy as soon as a weak one in clinical trials performed in patients with partial epilepsy.

Distribution, metabolism, and elimination

PGB is a substrate of the system L carrier is capable of the transport of large amino acids across the brain and the gut. Coherent with this PGB can speedily cross the blood-brain barrier conducted in mice during the preclinical studies which are an obvious advantage for a drug that increases the CNS activity [45,46]. The biotransformation studies of PGB state that it gets absorbed almost completely (98%) and only approx. 2% of the dose can be recovered from the urine (as metabolites). This is considered as a minimum or negligible metabolism where PGB excreted unchanged in the urine. The principal metabolite obtained was N-methylpregabalin [47,48] (Fig. 8).

Due to the negligible amount of metabolite, it does not inflict hepatic metabolism or impaired liver functions and does not cross or restrict enzymes like the cytochrome P450 system. PGB could not bind to the plasma protein and also doesn't inhibit drug metabolism *in vitro* that's why PGB is improbable to cause or subject to pharmacokinetic drug-drug interaction and the anticipation that has been proved in clinical pharmacokinetic studies [49-51].

Physicochemical properties

PGB (trade name-LYRICA®) exists in white to off-white crystal structure and single morphic form. The compound was found to be non-hygroscopic, non-solvated, stable, and water-soluble. In water/aqueous medium solubility was >30 mg ml-1 at RT. The pH of the drug ranges from 1 to 13 [52].The boiling point of PGB was found to be 274 ± 230 Cat 760 mm Hg [53] and thesolubility was observed in the water freely and also in basic and acidic solutions [54]. The vapor pressure was observed as 2.02 × 10-9 mm Hg at 25°C. The LogP value was observed as log Kow=-1.78 with optical Specific rotation as +10.52 degD-1 (c=1.00 in water) and Dissociation Constants-pKa1=4.2 and Pka2 of 10.6 [55-57]. The log of the partition coefficient (n-octanol/0.05 M phosphate buffer) at pH 7.4 was -1.35. Henry's Law constant=6.89 × 10-11 atm-cu m mol-1 at 25°C[58].

Analytical accounts on PGB

Although drug analysis is an important method need to regulate in all the stages during development, from designing to the post-marketing phases, but here in the article we focus mainly on the phases of analytical development and pharmacokinetics investigation [59-62]. Therefore, an abundance of data related to drugs may be obtained through analytical techniques, including data such as physical and chemical stability of the drug, bioavailability, and bioequivalence of the drug, design of the dosage form, quantification, and identification of impurities in the marketed product, and the quantification of the drug content [63-65]. In addition, therapeutic drug monitoring was also performed using analytical techniques through the determination and investigation of the pharmacokinetic parameters of all the methods available, HPLC-based methods [single (Table 1) and combination with PGB (Tables 2 and 3)] have mostly used techniques for pharmaceutical analysis [66-81]. As PGB was easily soluble in water and at room temperature, ring closure does not occur; HPLC [82-94] is the most preferred method for its analysis. Several other methods like HPTLC [95-98], Ultraviolet-Visible [80,81] have been used for the determination of PGB in bulk and pharmaceutical formulation (Table 1).

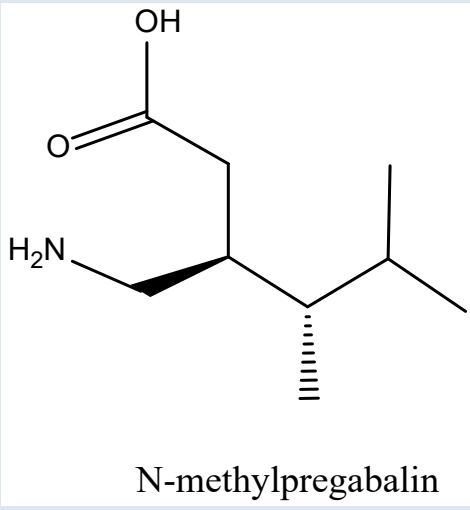


Figure 8: Active metabolite of PGB.

Table 1: Spectrophotometric method for analysis of PGB.

Sr. No	Matrix	Method	Detection (λmax) nm	Solvent	Linearity	LOD and LOQ	Ref
1	Bulk and Pharmaceutical Dosage form	UV spectrophotometric	210 nm	Double DW	6-14 µg/ml	2.457 mg/ml 7.448 mg/ml	[71]
2	Pharmaceutical preparations	UV spectrophotometric	DDQ	494	methanol	2.0-30.0	[72]
			TCNQ	841	Water	1.5-10	
			Ninhydrin reagent	573	DW	40-180.0	
3	Bulk drug and Capsule	Spectrophotometric	460 nm	DW	0.5–7.0	0.019 and 0.0647	[73]
		Spectrofluorimetric	558 nm	Chloroform	40–400 ngmL−1	0.049 and 0.165	
4	Bulk, Capsule and in Human Urine Samples	spectrophotometric	353 nm	DW	0.5–3.5	2.46 × 10−1 8.154 × 10−2	[74]
5	pure form and in capsules	spectrophotometric method	402.6 nm	Phosphate buffer pH 7.4	50-1000 µg mL-1	60 and 200 µg mL-1	[75]
6	Pure drug and pharmaceutical formulation	UV spectrophotometric	223 nm	Methanol	2.5-12.5	0.31-0.87	[76]
7	Pure form and capsule form	Spectrophotometric	333 nm	DW	20-160	0.545-1.652	[77]
		Spectrofluorimetric	470 nm	DW	0.2-3	1.95 × 10-3-5.9*10-3	
8	Capsule dosage form	spectrophotometric	365 nm	Water	2-18	-	[78]
9	Pure form and capsule	spectrophotometric	385 nm	NaOH solution	2-10	0.24 and 0.74	[79]

Note: Compound: PGB alone in all reported methods.

These reported methods describe the evaluation of PGB in the various dosage forms in a single constituent and in combination with GBP, MCA, Paracetamol (PCM), Methylcobalamin, Vigabatrin, Sildenafil, Amitriptyline spectacles different analytical method carries out for estimation of PGB. With the combination of four second-generation antiepileptic drugs, including PGB, GBP, VGB, and Topiramate, the analysts were elicited from blood plasma by the help of extensive solid-phase extraction derivatized with 4-chloro-7-nitrobenzofurazan and detection of HPLC with fluorescence detection. The scheme is confirmed acceptable for all four analysts and relevant for daily use [97-104]. Currently, the use of LC-MS/MS [105-107] has been gaining interest due to the high levels of analytical sensibility achieved and the lower limit of detection that this tool is able to provide for drug quantification and identification (Tables 2 and 3).

Spectrophotometric methods

To date, numerous spectroscopic methods have been accounted for the determination of PGB sole and in combination. The present review highlights the analytical methods used for the quantification and identification of PGB in pharmaceuticals and biological samples, particularly focusing on the HPLC-based methods. Even though the clinical use of PGB has been approved for a long, but the active participation of enantiomers LYRICA®22(2005), synthesis, and separation of enantiomers remains keen interesting points for the study of PGB. Therefore, the present review describes only those analytical methods that were published in the scientific literature for PGB since its discovery. The analytical studies of PGB were done in bulk and pharmaceutical formulation which involves several calculations of absorbance ranges starting from 210 nm [71]. In research, the addition of the chromophoric group for UV spectroscopy was made through the reaction of benzoylation to give a sensitive derivative of PGB [76]. From the study of Armagan and co-workers, it was found that PGB acts as anelectron donor with the DDQ and TCNQ as π acceptors which give extremely colored compounds at 494 and 841 nm. Here TCNQ was found to be more preferable based on higher molar absorptivity and lower detection limit [72]. The Spectro-fluorometric method of PGB with sensitive fluorogenic and chromogenic indicator 7-chloro-4-nitrobenzofurazon was used for routine quality control analysis without the intrusion of excipients [73]. In another study, by collecting a biological sample (Human Urine) by RS Gujral and co-workers, the PGB was blended with potassium iodate and potassium iodide to provide excellent accuracy and precision. It may assist in determining the influence of this drug on a human being meanwhile the treatment [74].

Chromatographic overview

In the widely used methods of spectroscopy like RP-HPLC for Isocratic elusion, hypersil C18 column (250 mm × 4.6 mm) was used. The combined mobile phase system like methanol: acetonitrile: potassium hydrogen orthophosphate (3:1:16v/v/v) [82] and potassium dihydrogen orthophosphate buffer (balanced for pH 6 by utilizing NaOH solvent): acetonitrile: methanol (75:10:15v/v/v) [94] were used for very easy, cost-efficient, quick, and effective method development. The main benefit of this method involves short retention time, without depletion with another reagent, the stability of solvent, no requirement for the earlier separation and purification. The less chromatographic time creates this method appropriate for the processing of numerous samples indefinite periods of time. In another estimation of PGB in human plasma, it was concluded that the method based on the derivatization of PGB with FDED in alkaline solution provides satisfactory results. The colored product can be found by a UV detector at less concentration [86].

HPTLC method

Another sensitive and reliable spectroscopy after HPLC is the HPTLC method. According to Patil and co-workers the concurrent determination of PGB and Aceclofenac stability-indicating method, chromatographic departure was carried out on aluminum plate smear with silica gel 60 F254, and the mobile phase was selected as toluene: methanol: formic acid (7:3:0.2v/v/v). In this method, all parameters were met with acceptable standards [95,96]. In another method, PGB and amitriptyline hydrochloride estimation was done with densitometry in pharmaceutical preparations. The silica gel 60F254 was used as a static phase and for the mobile phase toluene, methanol, and formic acid (7:2.5:0.5v/v/v) are used. This scheme concludes that the established method has many benefits like less cost consuming, relatively fast, stable, distinct, and easily reproducible [97,98] (Table 4).

LC-MS/MS method

Currently, the LC-MS and LC-MS/MS is the adaptable analytical tool that blends liquid chromatography resolute strength with mass spectrometry detection specificity. Sample compounds are isolated by liquid chromatography and then added to the mass spectrometer. The mass spectrometer generates the charge ions which then tracked [99-102]. The estimation of PGB alone and in combination is shown in Tables 5 and 6 which includes different parameters like stationary phase, mobile phase, detector, internal standard, etc. The research of N. Kostic and co-workers include the determination ofPGB by novel LC-MS method in the dried matrix sport (DMS) [99-108] (Table 5).

The appealing method of sample accumulation in micro quantity was utilized in the form of dried blood spot (DBS) and dried plasma spot (DPS) followed by the pre-column derivatization method [109-115]. From the analysis, it was concluded that the DPS is certainly can become an appropriate component for all parameters using a plasma matrix. Nevertheless, the potential depreciation of plasma by DBS depends on overcoming the hematocrit issue[116-119] (Table 6).

GC and GC/MS method

Yet another interesting method for detection of the PGB is GC and GC/MS method. Theuniqueness in chemical structure (presence of acidic and basic functional group), provides amphoteric physicochemical properties to PGB results in selective solubility. PGB being amphoteric shows free solubility in water, but sparingly in methanol and ethanol [120]. The presence of a chiral center (Fig. 1) suggests chiral derivatization of its amine moiety, which is yet another challenge because of zwitterionicproperties of PGB which prevents convectional GC-MS methods. The zwitterionic structure (Fig. 9) of PGB shows the negative charge on active site (carboxylic acid), results poor chromatography inGC-MS (Fig. 9).

Moreover, the dehydration of PGB, due to high temperature in GC-MS, may result in formation of Prega-L or 4-isobutyl-2-pyrrolidinone or lactam (Fig. 9) which is considered as a highly toxic substance. Determination of PGB *via* GC and GC-MS methods are listed in Table 7 . Generally the ethyl chloroformate (ECF) was used in several GC-MS methods as derivatizing reagent, but the use of newer agents like (S)-TPC [120], IBCF, DCC and NHS also provides us very satisfactory results during estimation of PGB in different matrices like urine, blood and human plasma (Table 7).

The method of protection of carboxylic acid groupconverting to methyl ester [120], Salt induces precipitation, micro-extraction techniques (solid-phase micro-extraction and dispersive liquid–liquid micro-extraction) are listed in Table 7.

Table 2: Limit of detection and limit of quantification.

Sr. No.	Drug	Matrix	Method	Detection (nm)	Linearity	LOD and LOQ	Ref
1	PGB+ MCA+ ALA	Multi component dosage form	First order derivative spectroscopic method	436.24	100-140	5.0915 and 15.4290 µg/ml	[80]
				307.03	1-1.4	0.01893 and	
				0.05737	Tenofovir	Tenofovir	
				383	130-170	5.4640 and 16.5576	
2	PGB + PCM	Bulk and tablet formulation.	UV spectroscopic method	210	2-14	0.0215 and 0.0651	[81]
				246		0.0540 and 0.1638	Tenofovir

Note: Solvent: water for all the reported methods.

Table 3: HPLC methods for analysis of PGB.

Sr. No	Matrix	Method	Stationary Phase	Mobile Phase	Detection (nm)	FR ml/min	RT	Ref
1	Bulk, and human urine sample	RP-HPLC	ODS hypersil column (250 mm × 4.6 mm)	methanol acetonitrile - 0.02 M di - potassium hydrogen orthophosphate (K2HPO4) (pH - 7.00)	210	1.0	4.63	[82]
2	Pharmaceutical tablet dosage form	RP-HPLC	Phenomenex C18 column (150 × 4.6 mm Id, ODS 2, 5 µm)	50:50 % (v/v) of MeOHand 10 mM Ammonium Acetate	210	0.7	3.39 ± 0.10	[83]
3	In bulk/ formulation form	RP-HPLC	Kromasil, C18, 100 × 4.6 mm, 5 µm column	phosphate buffer pH 6.9 and ACN (90:10)	210	1.0	4.7	[84]
4	In bulk and human urine samples	RP-HPLC	C18 5 µm ODS hypersil column (250 mm × 4.6 mm)	MeOH:CAN 0.02 M di - potassium hydrogen orthophosphate (K2HPO4) (pH - 7.00)	210	1.0	3.9	[85]
5	Human plasma	HPLC	TRACER EXCEL ODS-A stainless steel column, (5 μm, 150 × 4.6 mm	Methanol and Na2HPO4 (65:35)	360	1.0	-	[86]
6	In bulk drug, and human serum has	RP-HPLC	KROMASIL 100-5 C-18 column (250×4.6 i.d. mm)	buffer pH 7 and ACN (96:4, v/v)	210	1.0	4.6	[87]
7	Bulk drugs and in capsule dosage forms.	HPLC	Inertsil ODS -3V, C18 (250 × 4.6 mm Id, 5 µm) column	80: 10: 10 (v/v/v) of Disodium Hydrogen Phosphate Buffer: ACN: MeOH.	210	1.0	4.7	[88]
8	Pharmaceutical and bulk formulation	RP-HPLC	C18 5 µm BDS hypersil column (250 mm × 4.6 mm)	phosphate buffer solution (pH 6.9) and acetonitrile (94:6)	210	1.0	9.0	[89]

Note: Compound: PGB is used as drug sample in all reported methods

Table 4: HPTLC methods for analysis of PGB in combination.

Drug	Matrix	Method	Stationary phase	Mobile phase	Detection (nm)	Rf	Ref
Aceclofenac +PGB	In bulk and in formulation	stability-indicating HPTLC	Silica Gel 60 F254 HPTLC Plate	Toluene: MeOH: Formic acid (7: 3: 0.2 v/v/v)	210	0.68 ± 0.03 (ACF)and 0.27 ± 0.03(PGB)	[95]
PGB + Amitriptyline HCl	Pharmaceutical dosage form	HPTLC Densitometry	Silica gel 60 F254 HPTLC method	Toluene: Methanol: Formic acid (7: 2.5: 0.5 v/v/v)	205	0.27 ± 0.03(PGB) 0.68 ± 0.03(AMTR)	[96]
GBP+ PGB	Pharmaceutical dosage forms.	HPTLC method	Silica Gel G60 F254	Ethyl Acetate: MeOH: Ammonia (6.0: 4.0: 0.1 v/v)	210	0.993 (GBP) 0.992 (PBG)	[97]
Milnacipran HCl+ Duloxetine HCl+ PGB	Bulk drug and pharmaceutical formulation	Staility indicating HPTLC method	Silica gel 60 F254	ACN-water-ammonia (6:0.6:1.6, v/v/v)	220	0.9996	[98]
				dichloromethane-MeOH(8:1, v/v)	230	0.9995	
				ethyl acetate-MeOH: ammonia (6:3:0.1, v/v/v)	210	0.9999	

Table 5: LC-MS/MS methods for analysis of PGB.

Sr No	Stationary phase	Mobile phase	Detection\ Detector	IS	FR	Ref
1	YMC-Pack Octyl column (50 × 4.0 mm, 3 µm particle size)	ACN: 0.15% formic acid (85:15, v/v).	A TSQ Quantum 104 Access MAX triple quadrupole	-	550 µL/min	[99]
2	Kromasil 100 C18 (3.5 µm, 3, 30 mM) column	ACN-0.5% formic acid (80:20)	triple quadrupole mass spectrometer	Tramadol	1 mL/min	[100]
3	Hypurity, 5 mm C-18 (50 ´ 4.6 mm i.d.)	buffer-MeOH 20:80 (v/v)	Biosystems MDS Sciex (API 2000)	Imipramine	0.9 mL/min	[101]
4	Waters Symmetry® C18, 100 mm × 4.6 mm, 3.5 m	formic acid and ACN (30:70, v/v)	API 4000 triple quadrupole instrument	Rosuvastatin	1.0 mL/min	[102]
5	Shiseido Capcell Pak MG C18 column	ammonium acetate and ACN (15:85, v/v)	API 2000	Losartan	0.2 mL/min	[103]
6	ThermoHypurity C18 5 lm analytical column	ACN a2 mM ammonium acetate 80:20 (v/v)	API 2000 instrument	_	1.0 mL/min	[104]

Note: Method applied: LC-MS/MS and Matrix: Human Plasma for all reported methods

Table 6: LC-MS/MS methods for analysis of PGB in combination.

Sr No	Drug	Matrix	Stationary Phase	Mobile phase	Detection/detector	FR mL/min	Ref
1	PGB Sildenafil and Its Active Metabolite	Rat plasma	Chromolith Speed Rod RP-18e, 50 9 4.6 mm Column	10:90 (v/v) MeOH:water	API 3000 Triple Quadrupole	3	[105]
2	PGB and GBP	In urine	PhenomenexKinetex, 2.1 mm 50 mm 2.6 µm	1:1:1 MeOH:ACN:water (v/v)	AB SCIEX API 4000	0.4	[106]
3	BLF, GBP and PGB	human post-mortem blood	Poroshell 120 EC-C18 HPLC column	Mobiphase A: deionized water:formic acid (0.1%). Mob phase B: ACN: Formic acid (0.1%).	Agilent 6460 Triple Quadrupole mass spectrometer	-	[107]

Note: Method applied: LC-MS/MS for all reported methods

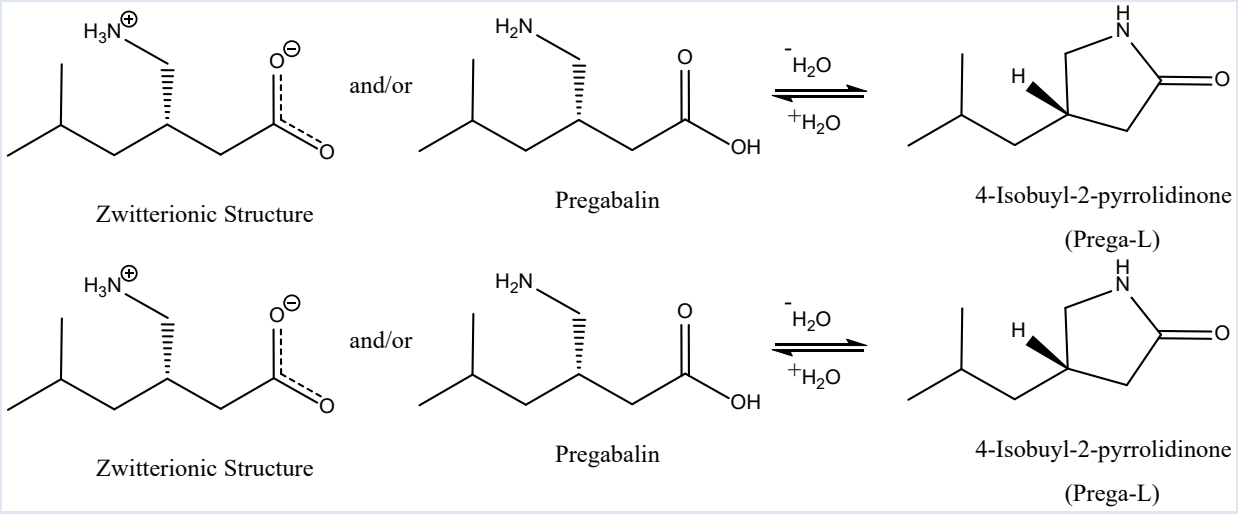


Figure 9: Zwitterionic ion and ring closure.

Table 7: GC/GC-MS methods for analysis of PGB.

SrNo	Matrix	Method	Model number	Internal Std	Column	Derivatizing reagent	Injector Temp °C	Ref
1	Bulk Drug	GC-MS	Agilent – 7693/ GC-MS (7890B/5977A)	-	30 m, 250 µm i.d., 25 µm film thickness	(S) trifluoroacetyl-prolyl chloride or (S)-TPC	280	[120]
2	Tissues and Blood	GC-MS	Agilent 6890	Ibuprofen	HP-5-MS; 0.25 mm × 60 m × 0.25 um capillary, 60 m × 250 µm	-	-	[121]
3	Urine Sample	GC-MS	GC Ultra with Triplus autoSampler +TSQ Quantum XLS Mass spectrometer	-	Elite-5 MS 60 m × 0.25 µm film thickness	ECF+MCF+ IBCF	270	[122]
4	Human Plasma	GC	Shimadzu -2014, Japan	-	Rtx-5 cross bond 30 m × 0.25 µm	ECF	170	[123]
5	Bulk drug and Tablet	GC	Shimadzu-2014, Japan	-	Rtx-5 cross bond 30 m × 0.25 µm	ECF	170	[124]
6	Human Plasma	GC-MS	Agilent- 7890 B	GBP	30 m × 0.25 µm	EDC+ NHS+ DCC	280	[125]

Note: Drug: PGB is used for all reported methods.

DISCUSSION AND CONCLUSION

PGB is a potent ligand of alpha-2-delta subunit of voltage-gated calcium channel in the central nervous system which shows analgesic, anti-convulsant and anxiolytic activity. It is a structural analog of GABA (γ-aminobutyric acid) but functionally dissimilar to naturally occurring transmitter. Although the drug received approval for since long, but, due to highly active enantiomeric form generation, only a few analogs are able to reach till stages of clinical development. The unique mechanism of action renders PGB substantially important for medical and clinical use. Furthermore, PGB exhibits suitable pharmacokinetic properties and high stability with a creation of negligible amount (2%) of metabolites. Considering zwitterionic properties and solubility in water, the HPLC-based methods are widely preferred for estimation of PGB as the ring closure does not occur at room temperature. Several other coupled methods like UV, LC-MS/MS, or mass spectroscopy, are the major analytical techniques available in the literature for the determination of PGB in bulk and biological samples. The manuscript is being designed on considering the recent data available (for 10 years). The pharmacokinetic, mechanism of action and synthetical part may be older than selected years but the compiled spectroscopical methods are from 2010-2021 with the consideration of the patent of LYRICA (2005). The advantages of the methods based on all spectrometers may be attributed to accuracy and sensitivity with high specificity which can be determined by these methods. Critically applied methods like LC-MS/MS and HPTLC are also mentioned enantiomeric separation of PGB. The presence of a chiral group and ring closure due to high temperature suggests the use of selected chiral derivatizing agents in GC and GC-MS, which are compiled in the present review. The present review elaborates various analytical approaches exercised for the appraisal of PGB and the current scenario to apply the state of the art of analytical development. Further methods are reported for its pharmacokinetics as well as bioequivalence studies.

DECLARATION

Ethics approval and consent to participate: Not Applicable

Consent for publication: Not Applicable

Availability of data and material: The data, if required, will be available upon request

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SB, KA and US contributed equally for preceding this research. Concept and guidance was from AS and final manuscript was prepared and checked by AN and PS. We declare that all authors have read and approved the manuscript before submission.

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