New spectrophotometric method for the determination of gabapentin in bulk and dosage forms using p-dimethylaminobenzaldehyde

Olajire A. Adegoke, Olayemi M. Adegbolagun, Elizabeth O. Aiyenale and Olusegun E. Thomas

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

ABSTRACT
A new simple, accurate and economic spectrophotometric method based on azo dye derivatization for the determination of gabapentin (GBP) was developed. Critical factors were optimized. The method was validated and assay of dosage forms was done. Spot tests and TLC confirmed the formation of azo adduct. A 0.3 M NaNO2 solution using 2 M HCl was used for diazotization. The optimal temperature and time were 30°C and 10 min. Azo adducts were determined at 430 nm. Methanol was found to be the best solvent. Gabapentin coupled at a ratio of 1:1 with DMAB. The assays of GBP were linear over the range 1–6 µg/mL ($r = 0.9973$) and LOD of 0.8322 µg/mL. The methods were accurate (error $< 2\%$) and precise (RSD $< 0.5\%$). The methods were successfully applied to the assay of GBP in dosage forms and compared favorably with reference method ($p > .05$). The successful diazotization of gabapentin and the azo adduct formation with DMA8 is reported for the first time.

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1. Introduction
Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures. Epilepsy is not a uniform condition but comprises many different seizure types as well as epilepsy syndromes based on heterogeneous etiologies [1]. Studies have revealed increases in postsynaptic glutamate receptors and decreases in gamma-aminobutyric acid (GABA) receptors in microgyric cortex which could promote epileptogenesis [2]. Drug therapy is the mainstay of epilepsy treatment. In very severe treatment-resistant cases, some patients are subjected to brain surgery (in the Netherlands approximately 50/year). For other patients implantation of a nervus vagus, stimulator is a treatment option. It has also been discovered that patients can be treated with a ketogenic diet [3]. Carbamazepine, ethosuximide, phenobarbital, phenytoin and valproate are the most frequently used conventional anti-epileptics. The therapeutic failure in 20–25% of patients has stimulated intensive research into novel anti-epileptic drugs and so far nine of them have been developed and licensed mainly as adds-on treatment in patients poorly responding to conventional therapy. These nine new products are felbamate, gabapentin (GBP), lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, vigabatrin and zonisamide [4].

GBP is recommended for use in treating difficult complex partial seizures, which are difficult to control, with or without secondary generalization in adults and partial seizures in children between 3 and 12 years of age.

GBP is structurally related to GABA, but it is not known how it functions to control seizures. The side effects that were reported in trials include sedation, dizziness, fatigue, unsteadiness, nausea and terror. In children, within the age 3–12, other effects were viral infection, somnolence and hostility [5]. GBP is the most frequently prescribed adjunctive anti-epileptic drug (beating out lamotrigine, topiramate, levetiracetam, tiagabine and zonisamide, in that order). GBP chemically is 1-(aminomethyl) cyclohexaneacetic acid with a molecular formula of C9H17NO2 and a molecular weight of 171.24. The drug does not bind to plasma proteins. Pharmacokinetics of GBP is not affected by foods and other drugs. GBP can be actively transported across the brain–blood barrier and the gut via the L-system amino acid transporter, which recognizes L-isoleucine, L-leucine, L-phenylalanine and L-valine [6,7].

GBP has no significant UV, visible or fluorescence absorption due to the lack of significant number of chromophores. In this regard, direct analysis has been fraught with major disadvantages. Due to this

CONTACT Olajire A. Adegoke ao.adegoke@mail.ui.edu.ng, jireade@yahoo.com

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deficiency, several methods have been previously reported for the determination of GBP in dosage forms and these methods include: spectrophotometric [8–15], spectrofluorimetric [16,17], capillary electrophoresis [18], potentiometric [19], HPLC [20–22] and HPTLC with derivatization using ninhydrin [23]. Majority of the spectrophotometric and spectrofluorimetric methods, as well as some previously reported HPLC techniques, involve prior derivatization with a variety of reagents. Some of these reactions suffer some setbacks in terms of speed of analysis, concentration levels adopted and use of very expensive instrumentation and reagents. The only UV azo derivatization procedure reported in the literature was by Dalvi et al. [13] using β-naphthol as the coupling component. This also suffers the demerit of using high concentration levels of 10–50 μg/mL. In recent years, we have demonstrated the accurate and simple determination of diazotizable drug molecules using p-dimethylaminobenzaldehyde (DMAB) as the coupling component [24–27]. The present study seeks to extend the advantages of speed, simplicity, accuracy and excellent calibration range to the determination of GBP in bulk and dosage form and using readily available laboratory reagents.

2. Materials and method

2.1. Chemicals and reagents

Methanol, ethanol, ethyl acetate, sulphuric acid, hydrochloric acid, sodium nitrite (All are analytical reagent grade obtained from BDH-Poole, England), pharmaceutical grade lactose, magnesium stearate, talc, starch and gelatin were used in the assessment of interference liabilities. The diazo coupling component p-DMAB was obtained from BDH-Poole, England while GBP chemical reference substance was obtained from Sigma-Aldrich (USA). Bi-distilled water was used to make sample solutions.

2.2. Equipment

Analytical balances (Mettler AE 160 and Mettler PC 400), thermostated water bath (Buchi Switzerland), melting point apparatus, oven Spectrophotometer (Lambda 25 UV/UV Spectrometer; Perkin Elmer Inc., Singapore) were utilized in the study.

2.3. Recrystallization of DMAB

Para-dimethylaminobenzaldehyde, DMAB (2 g) was dissolved in 60 ml of ethanol in a beaker over a water bath. The solution was filtered while hot, concentrated and the crystals were obtained by drop wise addition of ice-cold water. The beaker was left overnight undisturbed. The crystals were collected by filtering and washed with excess ice-cold water, and then oven dried (50°C). The procedure was repeated two times. The melting point was then determined (72–75°C).

2.4. Preparation of stock solutions

2.4.1. Preparation of DMAB

A 0.3%w/v solution of DMAB was prepared by dissolving 0.075 g of the crystals in a beaker with 0.0625 M sulphuric acid and then transferred into a 25 ml volumetric flask and made up to volume with 0.0625 M sulphuric acid.

2.4.2. Preparation of diazotized drugs for DMAB adduct

A 0.0344 g of GBP reference substance was dissolved in a mixture of 2 ml of water and 0.12 ml of 2 M HCl in a 100 ml beaker and 5 ml of 0.3 M sodium nitrite solution was added to the entire mixture and stirred for 20 min in an ice bath. The diazonium was then transferred into a 10 ml volumetric flask and made up to volume with cold distilled water.

2.5. Evidence of coupling reaction

2.5.1. Spot test

A 0.5 ml aliquot of the DMAB stock was measured into a test tube, and 0.5 ml of the GBP stock solution was added and vortex-mixed for 10 s. The yellow adduct formed was kept at room temperature (30°C) for 5 and 20 min. The procedure was repeated, and samples were incubated at 70°C for 5 and 20 min to observe any further colour changes or any visible evidences of reactions or decomposition. Each determination was repeated twice.

2.5.2. Thin-layer chromatography

Thin-layer chromatography (TLC) assessment was carried out using ethyl acetate:methanol (9:1), ethyl acetate:methanol (8:2) and ethyl acetate:methanol (5:5) for normal phase as well as methanol:water (4:6; for reversed phase) as the mobile phases. Pre-coated TLC plates (GF254 0.2 mm, Merck, Germany) were spotted with freshly prepared stock solutions of GBP, DMAB and the adduct formed between GBP and DMAB, and then developed in chromatographic tanks containing the respective mobile phases. Spots were visualized under UV light at 254 nm.

2.6. Effect of diluting solvents

The effects of the diluting solvents: methanol, ethanol, propan-1-ol, ethyl acetate and water were investigated to select the optimal solvent for measurement at optimal temperature, time, reagent and acid concentrations.
2.7. Selection of analytical wavelength (λ_{max})

The immediate golden yellow-coloured complex formed for the DMAB adduct was allowed to stay at room temperature for 10 min. The solution was made up to 5 ml with methanol. The UV–VIS spectrum of the adduct formed was scanned from 190 to 900 nm with methanol as solvent blank. Also, 0.5 ml aliquots of DMAB and GBP solutions in methanol were scanned separately.

2.8. Optimization studies

2.8.1. Optimization of sodium nitrite concentration for diazotization

The effect of varying the concentrations of sodium nitrite solution required for diazotization was assessed using concentration levels of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 M. Under optimal conditions, 5 ml of each concentration was used to diazotize GBP solution and 0.5 ml from each of the diazonium was added to 0.5 ml DMAB solution in a test tube, vortex-mixed and incubated at the optimum temperature and time. The absorbance reading was taken after addition of 4 ml of methanol. The optimal concentration was taken as the concentration corresponding to the maximal absorbance of the adduct. All procedures were carried out in duplicate.

2.8.2. Optimization of hydrochloric acid concentration

The effect of varying the concentration of hydrochloric acid used for the diazotization procedure was evaluated with acid concentrations of 0.5, 1.0, 2.0, 2.5 and 3.0 M. Under optimal conditions, diazotization and coupling reactions were carried as usual and the absorbance of the azo adduct formed was determined at the λ_{max} of 430 nm. All procedures were carried out in duplicate.

2.8.3. Optimization of volume of hydrochloric acid

The effect of varying the volume of hydrochloric acid used in the diazotization step was measured using 0.12, 0.25, 0.5, 1.0 and 2.0 ml aliquots of 2 M HCl. Under optimal conditions, respective volumes of hydrochloric acid were used to effect diazotization of GBP and 0.5 ml from each of the diazonium was added to 0.5 ml DMAB solution in a test tube, vortex-mixed and incubated at optimum temperature and time. All procedures were carried out in duplicate.

2.8.4. Optimization of coupling temperature

Optimization of temperature and time was done using the method of steepest ascent [28]. Aliquots of the diazotized drug solution (0.5 ml) were added to 0.5 ml DMAB solution in test tubes and the reaction mixtures were mixed by shaking followed by incubation at 30°C, 50°C, 60°C and 70°C for 5 and 20 min. Each determination was done in duplicate. The absorbance reading of the azo adduct was taken at 430 nm after making them up to 5 ml with methanol. The optimal temperature was taken as the temperature corresponding to the maximal absorbance of the azo adduct. Each determination was done in duplicate.

2.8.5. Optimization of coupling time

Aliquots of the diazotized drug solution (0.5 ml) were added to 0.5 ml DMAB solution in test tubes and the reaction mixtures were mixed by shaking followed by incubation at 30°C. The reaction was terminated inserting the test tube in an iced bath and thereafter making up to 5 ml with methanol at 0, 2, 5, 10, 15, 20 and 30 min. Each determination was done in duplicate. The absorbance readings of the azo adduct were taken at 430 nm at the λ_{max} and the optimal reaction time was taken as the time corresponding to the maximal absorbance of drug. Each determination was done in duplicate.

2.8.6. Effect of concentration of acid used in the preparation of DMAB solution

The effect of varying the concentration of sulphuric acid used in preparing the DMAB solution was determined by preparing the solution of DMAB in 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1 or 2 M H_{2}SO_{4}. A 0.5 ml aliquot of the drug solution was added to 0.5 ml DMAB solution at each concentration of acid in a test tube, vortex-mixed and incubated at optimum temperature and time. The absorbance reading was taken after addition of 4 ml of methanol. All procedures were carried out in duplicate.

2.8.7. Effect of DMAB concentration

The effect of varying the concentration of DMAB was assessed with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0 and 3.0%w/v in 0.0625 M H_{2}SO_{4}. Under optimal conditions, after mixing 0.5 ml of each concentration with 0.5 ml of diazotized drug solution, the reaction mixture was made up to 5 ml with methanol. The absorbance of the azo adduct formed was read at 430 nm. All procedures were carried out in duplicate.

2.9. Stoichiometric ratio determination

Job’s method of continuous variation [29] was used to determine the optimal stoichiometric ratio at which the diazotized GBP will combine with DMAB. Equimolar solutions (0.02011 M) of the reagent and the drug stock solution were prepared using the procedure described above. Into nine different test tubes, 0, 0.2, 0.25, 0.33, 0.5, 0.67, 0.75, 0.8 and 1.0 ml of the reagent solution was added, respectively. Each tube was then made up to 1.0 ml with diazotized drug stock solution. The reaction was then maintained at 30°C for 10 min. At the end of this time interval, the reaction was stopped by cooling in ice and the reaction volume made up to 5 ml...
with methanol. The absorbance readings were taken at 430 nm using methanol as blank. All procedures were carried out in duplicate.

2.10. Validation studies

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and reproducibility using the current ICH guidelines [30].

Calibration curves were prepared under the established conditions, by plotting absorbance as a function of the corresponding concentrations on each of three consecutive days. A linear relationship was observed between absorbance at 430 nm and concentrations of GBP in the range 1–6 μg/ml.

The accuracy of this new analytical method was assessed using recovery studies at concentration levels of 2, 3.4 and 5 μg/ml representing the low, mid and high concentration regions of the calibration range.

The intra-day precision for GBP in pure form was evaluated by assaying the three different concentrations of GBP within the calibration curve range in four replicates on the same day (intra-day) and on three consecutive days (inter-day) in the same manner as mentioned under the general procedure.

The LOQ and LOD were calculated according to the equations 1 and 2

\[ \text{LOD} = 3.3 \frac{\sigma}{S} \]
\[ \text{LOQ} = 10 \frac{\sigma}{S} \]

where \( \sigma \) is the standard deviation of the blank signals and \( S \) is the slope of the calibration graph.

2.11. Interference liabilities

The selectivity of the method was investigated by observing any interference encountered from the commonly utilized pharmaceutical excipients and additives such as starch, lactose, magnesium stearate, talc, gelatin and their mixtures. Recovery studies of diazotized GBP (3.4 μg/ml) from the matrices containing these excipients were carried out. Four replicates were determined in each instance.

2.12. Stability of the azo adduct under diffuse light

Standard test solutions containing 3.4 μg/ml of GBP were prepared in four sample vials. Two of the vials were wrapped with aluminium foil, while the other two were left unwrapped. Both sets were kept on the laboratory bench. The absorbance reading of the solutions at 430 nm was taken at 30 min interval for a period of 3 h and thereafter after 24 and 72 h.

2.13. Infra-red analysis of azo adduct

The IR spectrum of the azo adducts formed between GBP and DMAB was recorded using KBr disk after isolating the formed azo adduct from methanol.

2.14. Dosage form analysis

Three readily accessible brands of GBP capsules (300 mg strength) were utilized for this part of the study. Weight uniformity of each formulation was carried out. The quantity of the powder equivalent to 172 mg of GBP was dissolved in 10 ml of bi-distilled water and allowed to disperse effectively. The solution was filtered. 2 ml of the filtrate was transferred into 100 ml beaker and diazotized as previously outlined in Section 2.8. An aliquot of the diazotized drug was carried through the coupling process with the DMAB. For comparison, a reference method using non-aqueous titrimetric analysis was carried out using 0.01 M perchloric acid. Results obtained for the new method and the reference procedure were compared statistically at \( p = 0.05 \).

3. Results and discussion

GBP, when diazotized, was found to avidly react with DMAB. The diazotized drug was colourless. The DMAB reagent was also colourless. The colour of the azo adduct produced intensified when longer time of reaction and increasing temperature were allowed for the reaction. It was also discovered that the colour of the adduct was stable for more than 72 h when kept under ordinary laboratory environmental storage. Evidence for the formation of new chemical species by diazotization of GBP and coupling with DMAB was established by TLC analysis. The \( R_f \) values showed the adduct was more non-polar than the coupling reagents as shown in Table 1 for mobile phase 1 (ethyl acetate:methanol; 9:1). In all the three mobile phase systems adopted, the adduct was obtained as a single spot justifying that only one product was formed. In all instances, the \( R_f \) values were distinct from that of the diazotized GBP and coupling reagent.

3.1. Selection of analytical wavelength

The UV–VIS absorption spectra of the GBP, diazotized GBP, DMAB and the azo adduct formed between them are shown in Table 1. Spot tests and TLC analysis of reaction mixture.

| Time (minutes) | 30°C | 70°C | \( R_f \) value (mobile phase) 1a |
|---------------|------|------|---------------------------------|
| 0             | Yellow       | Yellow       | DMAB, 0.63; Adduct, 0.69       |
| 5             | Golden yellow | Golden yellow | DMAB, 0.63; Adduct, 0.69       |
| 20            | Golden yellow | Golden yellow | DMAB, 0.63; Adduct, 0.69       |

aMobile phase mixture consists of ethyl acetate: methanol (9:1).
are presented in Figure 1. The spectra show the formation of a new chromogen distinct from the starting materials. Bathochromic shift was observed. DMAB exhibited two prominent maxima at 330 and 350 nm while the drug GBP has an insignificant absorption centred on 225 nm. On diazotization, the drug absorbed at 235 and 360 nm. This is anticipated since conversion of free amino group to the diazonium ion produces an increase in electronic conjugation of a molecule and hence the observed bathochromic shift between the undiazotized and diazotized GBP. On coupling the diazotized GBP with DMAB, a completely different absorption spectrum was obtained as presented in Figure 1. The azo adduct exhibited three significant maxima at 235, 335 and 430 nm with a shoulder at 350 nm. This result shows a pronounced bathochromic shift with respect to the drug and the reagent. This thus provides an avenue for the quantitative determination of the drug using a relatively simple methodology of azo dye derivatization. The wavelength maximum at 430 nm for the azo adduct also gave optimal detector response. The increase in the wavelength of absorption of the azo adduct compared to the diazotized drug can be attributed to the elongation of conjugation due to coupling of the diazotized primary aliphatic amino group with DMAB.

3.2. Optimization studies

The effect of the concentration of sodium nitrite (NaNO₂) was studied by measuring the absorbance of the coloured product at 430 nm. The result obtained is presented in Figure 2. It was found that 0.3 M solution (5.0 ml) of sodium nitrite was sufficient to accomplish the diazotization of GBP. From Figure 2, it is evident that the absorption of the azo adduct increased gradually from 0.05 M and thereafter plateau at 0.3 M. Beyond this concentration, the absorbance did not significantly increase producing only a 0.005 absorbance units increase at 0.5 M NaNO₂. The need for a comprehensive evaluation of the amount of sodium nitrite required for the diazotization is borne out of the fact that GBP possesses an aliphatic primary amine. The diazotization of aliphatic amines is often difficult to accomplish due to the lack of π electrons for effecting delocalization of the developed charge as obtainable for their aromatic counterparts. The successful diazotization of GBP points to one of the great peculiarities of this new method for the assay of GBP.

In all diazotization procedures involving amino groups, the amount of mineral acid utilized for the reaction influences the nature and stability of the diazonium ions produced. The influence of the volume of the optimal 2 M hydrochloric acid concentration on the diazotization reaction was therefore studied using 0.1, 0.25, 0.5, 1.0 and 2.0 ml. Maximum and constant absorption intensities were achieved following the addition of 0.1 ml of 2 M HCl (Figure 3), after which the absorbance of the reaction product began to decrease. The reason for favourable low volume of acidity is explained based on the fact that high-acid concentration may lead to permanent polarization of the amino carboxylic acid in GBP. In addition, this can lead to the precipitation and denaturation of the amino acid since HCl is a stronger acid than the carboxylic acid moiety within the GBP molecule. This adduced reason further accounts for the requirement for optimization of HCl concentration used in the diazotization process.

The time allowed for diazotization was also found to produce a critical result. The absorbance of the new azo adduct was found to peak at 20 min using the optimal concentrations and volumes of acid and NaNO₂.
Figure 3. Effect of HCl concentration on diazotization (absorbance of azo adduct measured at 430 nm with methanol as solvent).

The absorbance of the azo adduct thereafter drastically declined to a plateau from 25 to 30 min reaction time. Hence, subsequent diazotization procedure was accomplished at 20 min reaction time.

The method of steepest ascent was utilized in establishing the optimal temperature and time required for the coupling reaction between diazotized GBP and the coupling component, DMAB. The effect of temperature allowed for the coupling reaction to take place was studied at 30°C, 50°C, 60°C and 70°C at two-time levels of 5 and 20 min. The results are presented in Figure 4. Critical evaluation of the results produced at 5 min reaction time shows that there is a gradual decrease in absorbance values beyond 30°C temperature. This is most likely due to thermal decomposition of the azo adduct produced. This behaviour implies that the reaction between DMAB and GBP is thermodynamically favoured hence confirming the avidity with which the reaction took place during the spot test procedure at ordinary laboratory environmental temperature. The inability of the reaction product to withstand a rise in temperature indicates the peculiar nature of this new adduct formed between the reacting species. In our previous experience with cephalosporins [27], some of the compounds coupled with DMAB at elevated temperatures. Thus, the ability of DMAB to produce coloured adducts with diazotized drugs and for such adducts to withstand high temperature must be dependent on the structure of the drug molecules. GBP being an alicyclic molecule will obviously find it difficult to withstand high temperature just as it fails to withstand high-acid concentrations.

The time required at the optimum temperature of 30°C was thereafter investigated using time levels of 0, 2, 5, 10, 15, 20, 25 and 30 min and monitoring the absorbance of the resultant product at 430 nm. The results for this assessment are presented in Figure 5.

The absorbance gradually increased from 0 min and attained a peak at 10 min. Thereafter a slight drop in the absorbance to a near plateau was found up to 30 min. It should, however, be noted that the absorbance drop between 10 and 15 min is just 5.49% and the absorbance of the azo adduct was found stable for up to 48 h.

3.3. Effect of acid concentration for DMAB preparation

The effect of varying acid concentrations for the preparation of DMAB on the absorbance of the azo adduct formed was studied and the results are presented in Figure 6. At an acid concentration below 0.0625 M, p-DMAB particles did not dissolve. It was observed that absorbance of azo adduct reduced with an increase
in the concentration of the acid used in the preparation of \( p \)-DMAB. If the reaction medium is too acidic, the amine on DMAB molecule becomes protonated and non-nucleophilic, inhibiting the activating influence of the dimethylamino group and thus leading to inability to form azo adducts. This may explain why lower acid concentration was just sufficient for the formation of the azo adducts. The second peak observed at 0.5 M may be due to the presence of some other products from the reaction, probably the protonated DMAB.

### 3.4. Effect of DMAB concentration

The optimal concentration for DMAB was found to be 0.3%w/v. Increase in absorbance values was observed with increasing concentrations of DMAB, indicating that the reaction was not complete at concentrations less than 0.3%. The result is presented in Figure 7. The solutions of DMAB between 0.5% and 3% were supersaturated and the reaction mixture had precipitates which scattered light and resulted in high absorbance values. The result obtained here is in consonance with previous application of DMAB as a coupling component for the spectrophotometric determination of diazotizable drugs [24–27].

### 3.5. Effect of diluting solvent

The diluting solvents methanol, ethanol, propan-1-ol, water and ethyl acetate were investigated in order to be able to select the optimal solvent for measurement at optimal conditions following the azo dye formation. Ethyl acetate formed a two-layer mixture with the azo adduct formed and it did not sufficiently extract the new azo adduct hence the possibility of carrying out extraction was ruled out. This is, however, advantageous as extraction of reaction mixture may not be specific and may be fraught with reduced accuracy. The utilization of alcoholic solution (particularly methanol) repressed the deactivating influence of aldehyde group while the activating potential of the dimethylamino group was optimal. This led to ready substitution of the incoming electrophile and hence formation of a yellow-coloured solution. This has been observed in our previous research experiences with DMAB as a coupling component.

### 3.6. Stoichiometric ratio determination for DMAB-GBP adduct

The result for the stoichiometric ratio determination is presented in Figure 8. The absorbance rose gradually from 0.2 mole of DMAB and peaked at 50% DMAB ratio. This, therefore, implies that the mole ratio 1:1 was found to give the highest absorbance value for the DMAB-GBP adduct and this ratio was, therefore, selected as the stoichiometric ratio for subsequent determinations. This confirms the presence and availability of only one primary aliphatic amino group for diazotization and coupling on GBP structure. It also affirms that multiple products are not produced as observed for the TLC analysis of the reaction mixture.
3.7. IR spectroscopic study

The IR spectra of the adduct formed between GBP and DMAB also confirmed the formation of a new compound. The IR spectra of GBP showed the expected doublet of the primary NH2 group at 2857 and 2925 cm$^{-1}$, C-Nstr at 1165 cm$^{-1}$ and the carbonyl stretch frequency of COOH group at 1542 cm$^{-1}$. The formation of the azo adduct was evidenced by the disappearance of the twin peak of the primary amino group indicating that a tertiary amino group is now present. The appearance of a medium to strong absorption band at 1600 cm$^{-1}$ is evidently due to a stretching vibration of the N9N bond while a new C-Nstr was observed at 2086 cm$^{-1}$. The presence of a broad band at 3423 cm$^{-1}$ in the new azo adduct confirms that the carboxylic acid group is now present in the azo adduct bringing about the O-Hstr.

3.8. Validation studies

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well-defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of regression line calculated according to an established Beer–Lambert’s mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. By using the above-mentioned procedure, a linear regression equation was obtained. The regression plots showed that there was a linear dependence of adduct intensity on the concentration of the drug over the concentration range selected. The absorbances of the azo adduct and GBP concentration was found to vary linearly within the concentration range of 1–6 μg/ml (correlation coefficient, $r = 0.9986$).

Further validation parameters are presented in Table 2. The limits of detection and quantitation were found to be 0.83 and 2.52 μg/ml, respectively. These limits compare favourably with previously reported procedures for the determination of GBP in pure and dosage forms.

The accuracy and precision were carried out over a three-day period. The results are presented in Table 3. The mean recoveries of GBP from the spiked pure samples ranged between 99 and 107 μg/ml for the intra-day determination. While for the inter-day assessments of these parameters, the values obtained were 100 and 104.5 μg/ml. The intra-day and inter-day assessments of precision gave relative standard deviations less than 0.5% in all instances. This low imprecision value attests to the suitability of this new method for the assay of GBP.

3.9. Interference studies of commonly used excipients

The results for the interference liabilities of the new method to the presence of commonly utilized excipients are presented in Table 4. In the presence of all the excipients, except gelatin, good recoveries of GBP were obtained. The result obtained with gelatin is anticipated since it contains a terminal amino group that is also diazotizable. The advantage nowadays is that gelatin is hardly utilized in drug formulation hence the interference may not be greatly important. The mixture also presented evidence of interference due to the presence of gelatin.

3.10. Analytical signal stability

The result for the analytical signal stability is presented in Figure 9. The absorbance was fairly constant, resonating within ±7.1% over the three hour period. The analytical signal stability was further carried out at 24 and 72 h and the same degree of stability was obtained. This result attests to the suitability of the DMAB system for the spectrophotometric determination of GBP.

Table 2. Analytical and validation parameters for the assay of GBP.

| Parameter                                      | Results                        |
|-----------------------------------------------|--------------------------------|
| Beer’s law limit (μg ml$^{-1}$)               | 1–6                            |
| Limit of detection (μg ml$^{-1}$)             | 0.8322$^a$                     |
| Limit of quantification (μg ml$^{-1}$)        | 2.5218$^a$                     |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)   | 5.996 $\times$ 10$^3$         |
| Sandell’s sensitivity (μg cm$^{-2}$)          | 28.56                          |
| Calibration curve parameters                  |                                |
| Intercept, (a)                                | 0.0206                         |
| Intercept ± 95% C.I.                          | 0.0206 ± 0.002114              |
| Slope (b)                                      | 0.0312                         |
| Slope ± 95% C.I.                              | 0.0312 ± 0.007423              |
| Correlation coefficient, ($r^2$)              | 0.9973                         |
| Coefficient of determination, (r)             | 0.9986                         |
| Standard deviation of (a ($S_a$))             | $6.6 \times 10^{-3}$          |
| Standard deviation of (b ($S_b$))             | $1.9 \times 10^{-3}$          |

$^a$Using ICH guidelines.

Table 3. Intra-day and inter-day assessment of accuracy and precision.

| Concentration (μg/ml) | Day 1$^a$ Mean recovery (%) | Day 2$^b$ Mean recovery (%) | Day 3$^a$ Mean recovery (%) | Inter-day statistics$^b$ |
|-----------------------|-----------------------------|-----------------------------|-----------------------------|-------------------------|
|                       | RSD (%)                     | RSD (%)                     | RSD (%)                     | Mean recovery (%)       | RSD (%) |
| 2                     | 103.2 ± 0.01                | 0.28                        | 107.2 ± 0.01                | 0.25                    | 103.2 ± 0.01       | 0.28 |
| 3.4                   | 103.1 ± 0.00                | 0.15                        | 105.5 ± 0.01                | 0.15                    | 105.8 ± 0.01       | 0.14 |
| 5                     | 106.6 ± 0.01                | 0.10                        | 102.2 ± 0.00                | 0                       | 99.0 ± 0.01       | 0.12 |
|                       | 100.6 ± 0.00                | 0.10                        | 102.2 ± 0.00                | 0                       | 100.6 ± 0.00       | 0.09 |

$^a$n = 4.

$^b$n = 12, sample preparation and assay carried out using optimized conditions of the experiment.
Table 4. Interference liabilities with common excipients.

| Drug Conc. (μg/ml) | Starch | Lactose | Talc | Magnesium Stearate | gelatin | Mixture of all excipient |
|--------------------|--------|---------|------|--------------------|--------|--------------------------|
| 3.4                | 98.0 ± 0.01 | 101.0 ± 0.01 | 104.3 ± 0.01 | 98.0 ± 0.01 | cloudy | Interference |

Recovery (%)= mean ± SD, n = 4.

Table 5. Comparative assay of GBP dosage form.

| Drug formulation | New DMAB method | Reference method | Statistics (p-Values) |
|------------------|-----------------|-----------------|-----------------------|
|                  | %Recovery ± SDb | %Recovery ± SDb | F-test | t-Test |
| TEVA             | 103.93 ± 1.28  | 102.0 ± 2.83    | 1.15 | 0.41 |
| Biopentin        | 103.59 ± 1.06  | 102.0 ± 2.83    | 0.18 | 0.43 |
| Akobal-G         | 101.59 ± 2.16  | 98.83 ± 0.41    | 0.11 | 0.11 |

*aMean value, n = 6. %Content of GBP stated by USP Pharmacopoeia ranges from 90% to 110%.
*b% recovery calculated as a function of amount of sample utilized.
*cStatistical analyses done between the results obtained from the proposed method and the reference method.

3.11. Comparative dosage form analysis

The new DMAB method was compared with a reference non-aqueous titration method reported by Basavaiah and Abdulrahman [19]. This reference method involved the use of 0.01 M perchloric acid, 0.2% crystal violet solution as an indicator and 2 mg/ml GBP stock solutions. The results obtained are presented in Table 5. Statistical significance test was done between the two sets of results obtained using F-ratio and Student t-test. There were no significant differences between the results obtained from the DMAB method and the reference non-aqueous titration for the three brands of analysed.

Some clearly evident advantages of this new DMAB method over previously reported methods are obvious. This is the first report of the successful diazotization of GBP and coupling to DMAB. The azo adduct produced was stable for up to 72 h. The method was found to be simple and uses readily available reagents. It also does not have any recourse to extraction which can compromise accuracy. In addition to the foregoing, the LOD and LOQ obtained were low signifying the ability of the method to determine minute concentrations of GBP in sample matrices. Comparing this new approach to previously reported UV–VIS methods also reveals some major advantages. The UV determination was carried out a longer wavelength in the visible region thereby guaranteeing some measure of selectivity and lack of interference often encountered in the UV regions. The method does not involve any solvent extraction as opposed to most ion pair complexation reactions that have been previously reported for the assay of GBP. In this new DMAB reaction, no buffers were utilized as opposed to majority of the HPLC techniques and the derivatization technique with 1,2-naphthoquinone sulphate.

3.12. Mechanism of coupling reaction

The mechanism of the formation of the new product between DMAB and GBP is presented in Scheme 1. The diazonium ion generated coupled with DMAB at a site next to the dimethylamino functional group since the dimethylamino group as an activating substituent is ortho-para directing. The COOH is deactivating and therefore meta directing. The net effect is that the ortho position to the dimethylamino group determines the substitution pattern hence substitution takes place next to the dimethylamino group of DMAB. The use of a polar but non-aqueous medium as methanol represses the deactivating influence of COOH and thereby optimizing the activating effect of the dimethylamino group. The 3D optimization of the azo adduct formed is presented in Figure 10. Examination of both structures revealed that all the bonds are staggered without undue crowding around a particular centre. The stereochemistry is also of the $E$ type around the diazo linkage thus ensuring that no obvious disturbance occurs between cross bonds between the residual alicyclic and aromatic skeletons. Although there seem to a discontinuation of conjugation in the structure of the azo adduct, the 3D optimization revealed a...
near tricyclic structural arrangement which will eventually lead to stability. The staggered nature will obviously facilitate the stability of the molecule. This might account for the profound stability of the azo adduct for even up to 72 h.

The resultant effect of the new structure proposed for the azo adduct, therefore, adds to the advantages generated from the adoption of the DMAB method for the spectrophotometric determination of GBP and makes it more suitable than previously reported methods.

4. Conclusions
A new spectrophotometric method has been successfully developed for the determination of GBP in bulk and dosage forms using the formation of an azo adduct as a derivatization technique. Optimal conditions for the diazotization in terms of the sodium nitrite concentration, acid concentration and volume, temperature and time were investigated and utilized for the assay of GBP. The new procedure was carried out with excellent calibration data, good validation parameters in terms of low inaccuracy and imprecision. The new method also has added advantages of simplicity, speed and accuracy. It could find application as a ready methodology for the quality control of gabapentin.

Disclosure statement
No potential conflict of interest was reported by the authors.

ORCID
Olajire A. Adegoke http://orcid.org/0000-0002-8623-3635
Olayemi M. Adegboyegan http://orcid.org/0000-0002-1396-850X
Elizabeth O. Aiyenale http://orcid.org/0000-0002-8688-5414
Olusegun E. Thomas http://orcid.org/0000-0001-8519-2125

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