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"From safe source to safe sink" development of colorimetric assay for gabapentin in bulk drug and capsules using naturally derived genipin

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ABSTRACT

A novel colorimetric assay for gabapentin in bulk drug and capsules has been developed via a safety-and-sustainability concerning concept. The method relied on the reaction of primary amino group of drug with non-toxic and eco-friendly genipin in totally aqueous medium to form the blue product which was subsequently measured by visible spectrophotometry at 590 nm. Under the optimized conditions, Beer's law was obeyed in the concentration range of 0.15–0.50 mM (r^2=0.9998). It was accurate, precise and insensitive to the interferences from all related compounds specified in the United States Pharmacopeia as well as commonly used excipients. Furthermore, it gave the assay results in agreement with the pharmacopeial chromatographic method. Owing to the environmental concern and responsibility, a fast and facile method was also proposed for the treatment of waste generated from the assay based on the decoloration by using gypsum as a cheap and commonly available adsorbent. After the treatment, more than 95% of the initial blue product was removed from the waste solution and the treated waste was proven to be safe for aquatic organisms, as studied in brine shrimp and guppy fishes. Therefore, this work not only reports for the first time the application of naturally derived genipin to drug analysis, but also presents a new and contemporary paradigm that illustrates the fully benign-by-design development of the analytical methodologies in the era of Green Chemistry, starting from the safe source of reagents toward the safe sink when waste is released into the environment.

1. Introduction

Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) is a neurotransmitter γ-aminobutyric acid (GABA) analogue which is used for the treatment of partial seizures and neuropathic pain [1,2]. At present, a number of assays have been developed for the quality control of this drug in bulk and pharmaceutical formulations such as high performance liquid chromatography (HPLC) [3–5], capillary electrophoresis [6,7], chemiluminometry [8], potentiometry [9], voltammetry [10], spectrofluorimetry [11,12] and spectrophotometry [13–19]. Among them, colorimetric assays are usually considered as simple, effective and rapid means and require minimal and common, inexpensive instrumentation. From literatures, gabapentin which has very low UV absorption could be assayed on the visible spectrophotometry after it was derivatized via different reactions. Abdellatef and khalil [13] reported the methods based on the reactions of gabapentin with vanillin in McIlvain buffer and with *p*-benzoquinone in ethanol. Ninhydrin reagent in *N*-*N*-dimethylformamide [13,16] and in methanol medium [17] have been used to derivatize gabapentin for the spectrophotometric assays. The method proposed by Al-Zehouri et al. [14] relied on the condensation of gabapentin with acetylacetone and formaldehyde via Hantzsch reaction. The assay based on charge transfer reactions of gabapentin with various π-acceptors e.g. 2,3,5,6-tetrachloro-1,4-benzoquinone, chloranilic acid, tetracyanoethylene in acetonitrile were developed by Salem et al. [17]. Recently, the formation of ion-pair complexes as a result of a proton transfer from picric acid or 2,4-dinitrophenol to the primary amino group of gabapentin was used as a basis for the colorimetric assay of this drug [19]. Although these methods have shown several advantages, some chemical reagents and/or organic solvents with known or suspicious toxicity were employed in the assays. For instance, besides the organic solvents which potentially cause harmful effects on health and environment, a common derivatizing reagent, ninhydrin, is known to have the toxicity including somnolence,

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regional or general arteolar or venous dilation, dyspnea, skin irritation [20,21] and potentially promotes tumor development on mouse skin [22]. By this reason, the development and use of not only facile and efficient but also safe colorimetric assays for this drug is still on the demand.

In accordance with the principles of green analytical chemistry, the elimination or substitution of toxic reagents in the analysis with less harmful alternatives is a viable means to develop safe, green and sustainable assays [23]. Nowadays, a number of compounds originating from natural sources such as plants have been proven to be attractive options that can be used in the role of assay reagents as purified substances or the extracts with no or little purification or modification [24]. For example, curcumin in *Curcuma longa* L. was used as a chromogenic reagent for the analysis of boron in sea water samples [25]. Chlorophyll from pea plant was used in the fluorescence assay of Hg(II) [26].

Genipin is an iridoid aglycone of geniposide extracted from fruits of gardenia such as *Gardenia jasmonoides* Ellis [27], which has long been used in oriental traditional medicines for the treatment of various disorders such as inflammation, hepatic disease and abdominal pain due to dysentery [28]. Genipin itself is colorless, but it reacts with primary amino groups to form the blue pigments which have the maximum absorption at about 590 nm [29]. Since the colored adducts are safe, edible and heat stable, they are used in East Asia as food colorants and fabric dyes [30]. For its biodegradability and low cytotoxicity, genipin has recently been investigated as a natural reagent used to crosslink the polymers e.g. gelatin and chitosan in tissue engineering and drug delivery [31–33]. It was found that gelatin crosslinked with genipin was much less cytotoxic about 5,000 to 10,000 times than that crosslinked with glutaraldehyde [34]. For the analytical applications, genipin has been proposed as a reagent in a forensic science to develop clearer, more visible and stable fingerprints than classical ninhydrin which is a harmful reagent. In addition, the reaction of genipin with primary amino groups has been used to develop the colorimeric methods for the quantitation of amino acids [35], free amino groups in water soluble chitin derivatives and other amino sugars such as D-mannosamine and D-galactosamine [36]. However, to date, no report regarding the application of this natural compound to the assay of drugs containing primary amines has been found.

In this work, a novel assay for gabapentin in bulk drug and capsules has been proposed based on the colorimetric reaction with genipin. Unlike most other previous works which ended up once the satisfactorily accurate and reproducible methods were obtained; in this study a simple and facile treatment of waste generated from the assay was further proposed and the ecological effects of the treated waste was evaluated to confirm its safety on aquatic organisms. Therefore, this is the first report that not only presents the application of naturally derived genipin to drug analysis, but also fully illustrates the benign-by-design development of the analytical methodologies starting from the safe source of reagents toward the safe sink when the wastes from the assay are released into the environment.

2. Experimental

2.1. Materials and apparatus

The reference standard gabapentin and its related compounds including 2-aza-spiro[4.5]decan-3-one or gabapentin lactam (Compound A), (1-cyano-cyclohexyl)-acetic acid (Compound B), (1-(3-oxo-2-aza-spiro[4.5]dec-2-ylmethyl)-cyclohexyl)-acetic acid (Compound D) and carboxymethyl-cyclohexanecarboxylic acid (Compound E) were purchased from the United States Pharmacopeia (USP) (Rockville, MD). Genipin (as crystal-like powders, reagent grade) was supplied by Challenge Bioproducts Co. (Yun-Lin, Taiwan). All other chemicals were of analytical grade from Merck (Damstadt, Germany). Distilled water was used to prepare all solutions. Raw material of gabapentin was a gift from Thailand Government Pharmaceutical Organization and the commercial dosage forms (300 mg gabapentin per capsule) were obtained from local drug stores in Thailand.

The absorbance measurement was performed by using an Agilent 8453 model UV–visible spectrophotometer (Agilent, USA) using a semi-micro quartz cuvette (1 cm path length). For the HPLC standard assays, the system consisting of an Agilent 1100 Serie and a diode array detector (Agilent, USA) was used. The chromatographic conditions were followed using the methods described in USP 33 [37].

2.2. Preparation of standard solution of gabapentin

The reference standard gabapentin of about 0.085 g was accurately weighed, transferred into a 20 mL volumetric flask and dissolved with water to prepare 25 mM standard gabapentin solution. Then, 0.4 mL of the resultant solution was diluted with water in a 20 mL volumetric flask to give a final concentration of 0.5 mM which was used for the preparation of standard curve.

2.3. Preparation of sample solutions from gabapentin bulk drug and capsules

To assay the raw material, bulk drug was prepared as a working solution containing 0.5 mM gabapentin by following the same procedure that was used to prepare the standard solution. For the capsules, the contents of twenty capsules were weighed and powder equivalent to 0.085 g gabapentin was accurately weighed and transferred into a 20 mL volumetric flask. About 15 ml of water was added and the content was sonicated for 5 min to obtain the complete dissolution. The mixture was then added up to 20 ml with water and filtered through 0.45 μm membrane filter, discarding the first portion of the filtrate. Further dilutions were made to give a nominal concentration of 0.5 mM gabapentin which was ready for the analysis.

2.4. Preparation of genipin cocktail solution

In order to minimize pipetting steps and increase the assay precision and accuracy, genipin solution was freshly prepared as a cocktail with buffer solution. This was done by adding 0.009 g of genipin and 10 mL of 100 mM potassium phosphate buffer, pH 7.0 into a 20 mL volumetric flask. Distilled water was used to dissolve the content and made up to the volume, resulting in a stock cocktail composed of 2 mM genipin in 50 mM potassium phosphate buffer, pH 7.0.

2.5. Assay procedure

Different aliquots (180, 240, 360, 480, 600 μL) of 0.5 mM standard gabapentin solution or 420 μL of 0.5 mM sample solution were accurately transferred into a series of 1.5 mL microcentrifuge tubes by the use of micropipettes. Water in different volumes was precisely added into each tube to bring the total volume to 600 μL. This resulted in a set of standard genipin solutions with the concentration of 0.15, 0.20, 0.30, 0.40, 0.50 mM and the sample solution with the concentration of 0.35 mM. Subsequently, 600 μL of genipin cocktail was accurately pipetted to each tube. All tightly closed tubes were placed in a water bath set at 80 °C for 60 min, then cooled by plunging into an ice bath for 5 min. After leaving the reaction at room temperature for

5 min, the absorbance of the solutions was read at 590 nm. A reagent blank composed of 600 μL of water and 600 μL of genipin cocktail and run by the same assay procedure was also prepared and its absorbance was used to subtract the absorbance values obtained from the reactions which contained gabapentin. A standard curve was constructed by plotting the blank-corrected absorbance values versus gabapentin concentration. The concentration of the unknown sample was computed from the regression equation derived from the Beer's law data.

2.6. Procedure for the treatment of laboratory waste

The treatment of whole waste generated from the assay was carried out in a 50 mL conical tube by adding 0.5 g of gypsum ($CaSO_4.2\,H_2O$) into 25 mL of waste solution. The tube was vortexed for 2 min at the ambient temperature and the mixture was then filtered through a Whatman filter paper No.1. Since the pH of the solution decreased after the adsorption step, the filtrate obtained was neutralized with sodium hydroxide solution to pH 7 and the mixture was finally filtered to obtain a clear, colorless and neutral filtrate which was considered as the treated waste.

2.7. Acute toxicity assessment of treated laboratory waste

The waste after the treatment was evaluated for the acute toxicity to the two aquatic orgamisms namely brine shrimp (*Artemia salina*) and male, adult guppies (*Poecilia reticulate*). Prior to the test, brine shrimp cysts were hatched for 36 h in 35 ppt salinity artificial seawater which were well aerated at room temperature (27 °C). The treated waste was diluted with artificial seawater where the final salinity of all test solutions was adjusted to 35 ppt. (for test on brine shrimp) or distilled water (for test on guppies) to different concentrations i.e. 0 (control), 6.25, 12.5, 25, 50 and 100% by volume of wastewater. At test initiation, ten test organisms were randomly added to each replicate test chamber ($n=2$). The tests on brine shrimp were conducted under static conditions in 6 well polystyrene plates. In each well, 5 mL of waste solutions with different concentrations were added. The test on guppies was carried out in 1 L beakers containing 500 mL of waste solutions. The organisms were not fed during the test. After the exposure period of 24 h, the test organisms were observed and the number of live organisms per replicate was recorded. The test results were acceptable if control survival equaled to or exceeded 90%. The data analysis consisted of transforming the observed percentage mortalities with a probit transformation and transforming the wastewater concentrations to $\log_{10}$. From the graph, the mean lethal concentration which caused 50% mortality of the test organisms (LC50) was determined at the probit value of 5 and the acute toxic unit (TUa) defined as 100/LC50 was calculated. The criterion of toxicity was based on the Aquatic Toxicity Testing Guideline for Waste Effluents [38]: TUa value <3 (non-toxic), 3–10 (slightly toxic), 10–50 (toxic), 50–100 (very toxic) and >100 (extremely toxic).

3. Results and discussion

3.1. "From safe source to safe sink" concept and the assay reaction

While most works related to the assay development usually end up once the methods have been established and validated, we proposed here a full route via the so-called "from safe source to safe sink" concept for the design of the efficient, benign-to-operator and environmentally friendly analytical methodologies. In this study, the concept was applied to the development of a novel assay of gabapentin in the pharmaceutical samples. As shown in Fig. 1, the development process started from the selection of naturally derived genipin as a colorimetric reagent owing to its ability to react with the primary amino group in gabapentin and form blue color (Fig. 2) where the intensity could be measured with ease by visible spectrophotometry at the maximum wavelength of 590 nm, the typical absorption characteristic of the blue products obtained from the reaction of genipin with various amino acids [35]. Notably, genipin itself is less harmful and more eco-friendly than some commonly used amino group-derivatizing agents such as ninhydrin and the colored products formed between genipin and amino acids or proteins are considered to be safe [30]. Since the reaction of genipin with primary amines could take place in water, after it was chosen, the safer assay was designed by conducting the entire procedures in water medium, rendering the method free from the use of any organic solvents. Furthermore, to minimize the reagent consumption and waste generation, the volume of the reaction was deliberately set at as low as 1.2 mL per reaction which was adequate for the

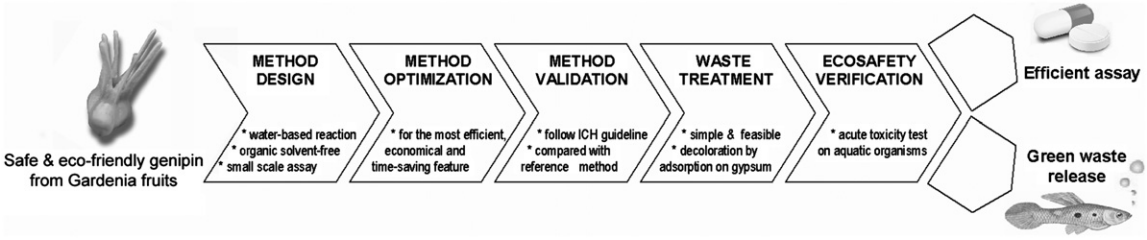

Fig. 1. Schematic representation of the development of the assay for gabapentin based on the "from safe source to safe sink" concept.

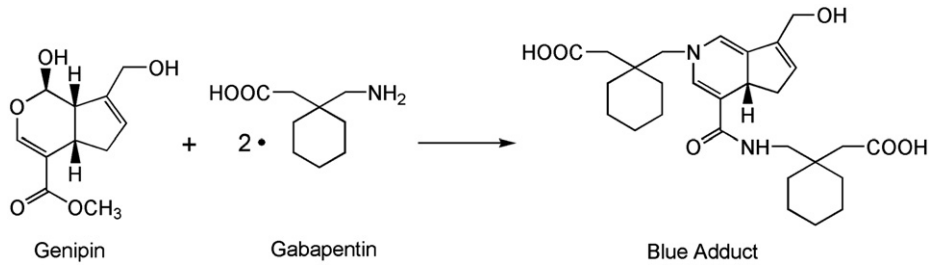

Fig. 2. Proposed scheme for the formation of blue colored adduct from the reaction of gabapentin with genipin.

absorbance measurement by using typical semi-micro cuvette. After the design, the experimental parameters were optimized to obtain the most efficient, economical and time-saving assay, followed by the validation of the analytical performance and the comparison with the standard method. In contrast to the accustomed manner where no special attention is paid to the polluting potential or the intrinsic toxicity of the assay waste, the "from safe source to safe sink" concept has called for the awareness and responsibility to the fate of waste coming out after the assay. Therefore, a simple, fast and feasible method for the treatment of the whole waste containing the genipin and gabapentin adduct was further investigated and proposed. Since adsorption has been found to be superior to other decontamination techniques in terms of the initial cost, simplicity of design, ease of operation and it does not result in the formation of harmful substances like many other cases, here commonly available and inexpensive material namely gypsum was used as an adsorbent for the removal of colored adducts from waste solution. Moreover, in the last step the treated waste was evaluated for the acute toxicity to the aquatic organisms i.e. brine shrimp and guppies prior to the final implementation of the method. By this way, it was assured that the developed assay was acceptably safe, beginning from the reagent use, all through the operating steps and after the assay where the waste was released into the environment.

3.2. Optimization of the method

The experimental parameters that could affect the formation of blue products between gabapentin and genipin were optimized independently. These studies aimed to obtain the most efficient, economical and time-saving assay.

3.2.1. Optimal pH

The pH influence on the formation of the blue product was studied by setting up the reactions in various buffers i.e. potassium hydrogen phthalate-HCl (pH 4), potassium hydrogen phthalate-NaOH (pH 5), potassium hydrogen phosphate–NaOH (pH 6, 6.5, 7) and Tris–HCl (pH 8, 9). It was found that the reaction conducted at pH 7 produced the most intense blue color as measured by the absorbance at 590 nm (Fig. 3). Hence, it was chosen as the optimal pH for the assay.

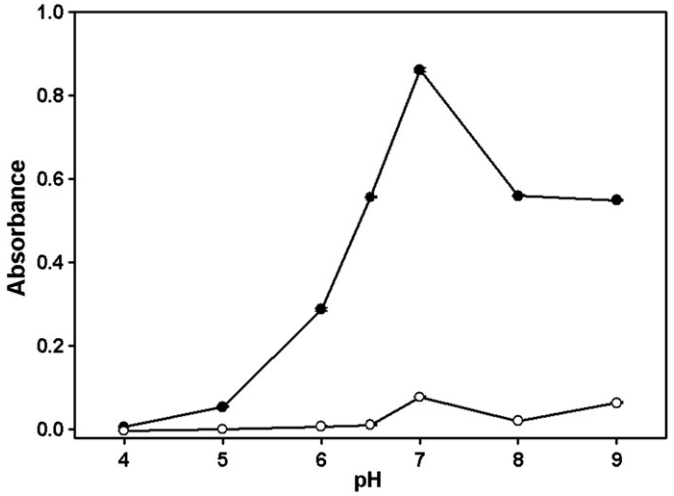

Fig. 3. Effect of pH on the reaction of genipin with 0.3 mM gabapentin (●) and without gabapentin or blank (○). In this experiment, 600 μL of 2 mM genipin in the buffers with different pH was used and the reaction was heated at 80 °C for 1 h.

3.2.2. Optimal concentration of genipin

The optimal concentration of genipin was examined by using the varied concentrations of genipin (1, 2, 4, 6 and 8 mM) for the construction of standard curves over the gabapentin concentration range of 0.20–0.50 mM. As shown in Fig. 4, the use of 1 mM genipin solution gave a line with the lowest slope, indicating a poor sensitivity of the method. In contrast, when the concentration of genipin was raised to 2 mM, the steepest slope with the highest absorbance values was obtained. The use of genipin solutions at the higher concentration than 2 mM did not further increase the slope or the sensitivity. Moreover, the plotted lines became lower due to the higher absorbance background which was used for the subtraction as a reagent blank (data not shown). Thus, 2 mM genipin was the most effective and economical.

3.2.3. Optimal heating temperature

By varying the heating temperature (60, 70, 80 and 90 °C) and fixing the heating time at 60 min, it was found that the slope of standard curves increased with the increasing temperature (Fig. 5). Since heating at 80 °C gave a line which was not significantly

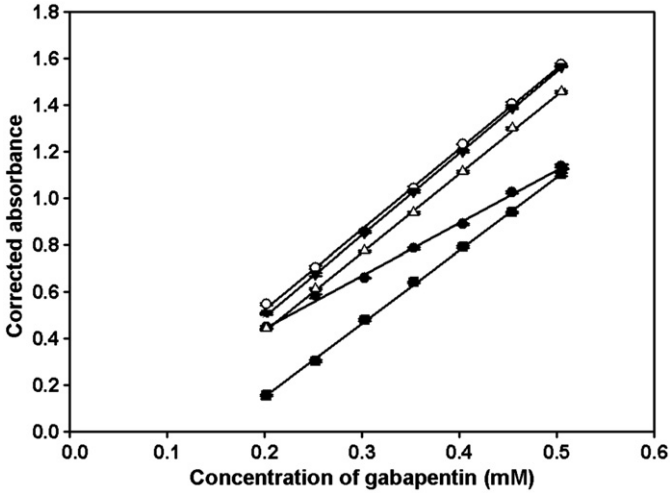

Fig. 4. Effect of concentration of genipin solution: 1 mM (●), 2 mM (○), 4 mM (▼) and 6 mM (△) and 8 mM (■). In this experiment, genipin solutions were prepared in 50 mM potassium phosphate buffer, pH 7.0 and the reaction was heated at 80 °C for 1 h.

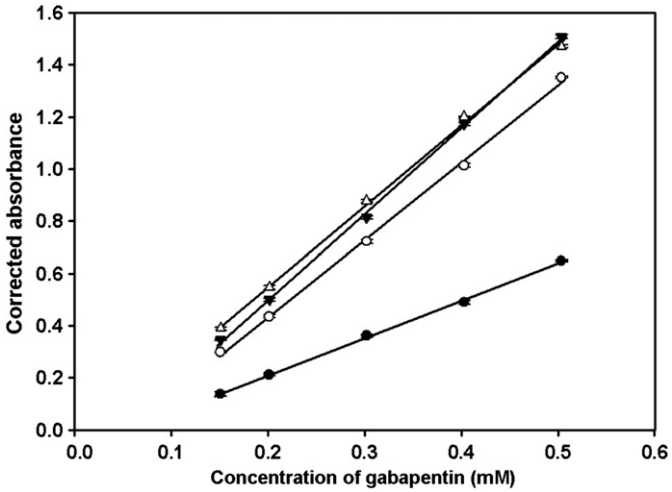

Fig. 5. Effect of heating temperature: 60 °C (●), 70 °C (○), 80 °C (▼) and 90 °C (△). In this experiment, 600 μL of 2 mM genipin in 50 mM potassium phosphate buffer, pH 7.0 was used and the reaction was heated at different temperature for 1 h.

different from that obtained from heating at 90 °C, it was chosen for the standard protocol.

3.2.4. Optimal heating time

After 80 °C was selected as the optimal heating temperature, the optimized heating time was subsequently studied at this temperature. As shown in Fig.6, the color intensity of the solution gradually increased with time until it reached a constant value after 60 min. Therefore, heating was allowed for 60 min to obtain a complete, energy-minimizing and time-saving reaction.

3.2.5. Stability of blue color

Since the color intensity of the solution must be measured after the steady blue color was obtained, the stability is inevitably an important issue to be concerned with. From the experiment, the color was satisfactorily stable with only slight decrease of the absorbance (less than 1%) within 1 h when the solution was placed under ambient light (Fig. 7). Furthermore, the color stability significantly improved if the solution was protected from light as evidenced by more than 99% remaining absorbance values over the 6-hr period.

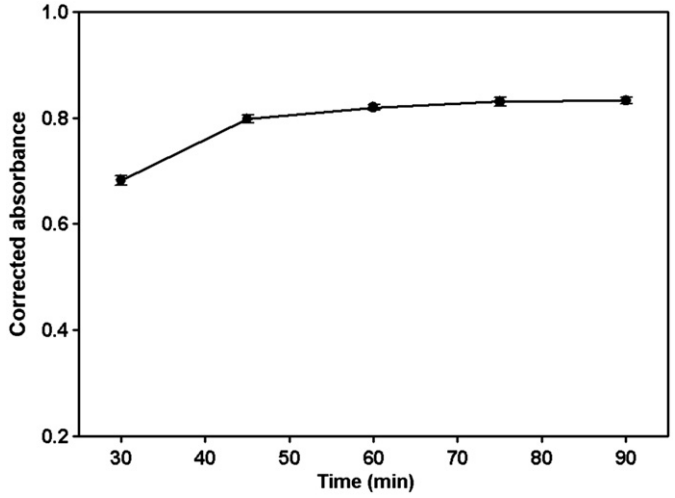

Fig. 6. Effect of heating time. In this experiment, 600 μL of 0.25 mM gabapentin and 600 μL of 2 mM genipin in 50 mM potassium phosphate buffer, pH 7.0 was used and the heating temperature was set at 80 °C.

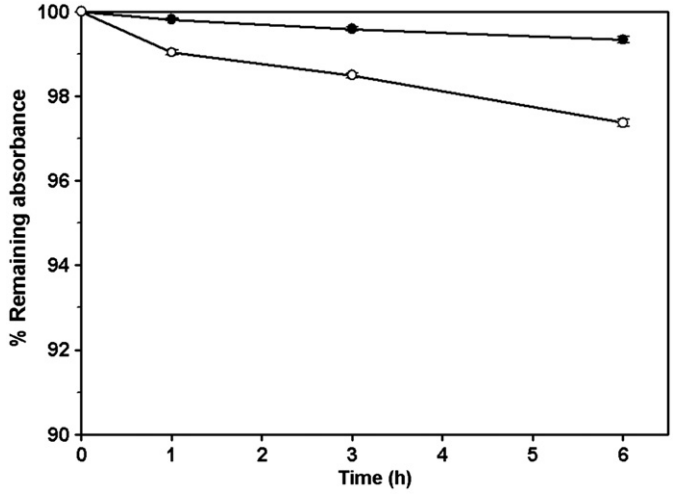

Fig. 7. Color stability of the blue product kept under light (○) and dark (●) condition. In this experiment, the blue colored solution was prepared by heating 600 μL of 0.35 mM gabapentin and 600 μL of 2 mM genipin in 50 mM potassium phosphate buffer, pH 7.0 at 80 °C for 1 h.

3.3. Method validation and comparison to the standard method

After the optimal protocols were established, the method validation was carried out to verify its overall performance. It was found that an excellent linear response of absorbance in relation to the concentration of gabapentin was observed over the range of 0.15–0.50 mM with the regression coefficient of 0.9998. The linear equation relating A (absorbance) to C (concentration, mM) was $A=3.410C+0.143$. LOD ($3.3\sigma/m$) and LOQ ($10\sigma/m$) as determined from the linear regression were 0.004 and 0.014 mM, respectively.

The accuracy of the proposed method was determined by spiking three different levels of standard gabapentin (0.15, 0.20, 0.25 mM) into the pre-analyzed drug solutions (0.15 mM) prepared from the capsules and determining the added concentration by the proposed method. It was found that the percentage recoveries were in the range of 100.37–100.59% (Table 1). Therefore, the method was found to be satisfactory accurate as the percentage recovery values were in the range of 98–102%. In term of the precision which was studied in bulk drug and capsules, the % relative standard deviation (RSD) was 0.37–0.63% for the repeatability (intra-day precision) and 0.04–0.22% for the intermediate precision (inter-day precision) (Table 2). Since both % RSD values were less than 2%, the method was acceptably precise.

To investigate the specificity of the proposed method, the interferences from the gabapentin related compounds specified in the USP including Compound A, B, D and E as well as the excipients commonly used in capsule formulations i.e. lactose, microcrystalline cellulose and colloidal silicon dioxide were studied. From the experiments, all substances tested did not react with genipin to form the blue colored product (data not shown) because of the absence of primary amino group in the structures. In addition, when they coexisted with the gabapentin at the concentration levels which were limited by the USP monograph (for the related compounds) or commonly used in capsules (for the excipients), the % drug recovery values were in the range of 99.75–100.40% (Table 3). This finding indicated the specificity of the method and the absence of interference from the related compounds and excipients.

The performance of the proposed method was further tested by applying it to the analysis of gabapentin in bulk and capsule samples in the comparison with the USP chromatographic method. The mean values of % content or % labeled amount found

Table 1
Accuracy of the proposed method.

Spiked concentration of gabapentin standard (mM)	Average recovery*(%)	RSD (%)
0.15	100.37	0.44
0.20	100.59	0.33
0.25	100.49	0.60

* Number of measurement (n) in each concentration level was equal to 3.

Table 2
Precision of the proposed method.

Precision	Bulk drug		Capsules	
	Content found (%)	RSD (%)	Labeled amount (%)	RSD (%)
Intra-day*	100.31	0.37	99.76	0.63
Inter-day**	100.28	0.04	99.75	0.22

* Number of measurement (n) was equal to 6.
** Number of measurement (n) was equal to 3.

Table 3
Recovery of gabapentin in the presence of various interferences.

Impurities or excipients	Added concentration (%)*	Recovery of gabapentin** (%)	RSD (%)
Related compound A	0.5	100.31	0.13
Related compound B	0.5	100.40	0.58
Related compound D	0.5	100.40	0.59
Related compound E	0.5	100.26	0.37
Lactose	20	100.11	0.38
Microcrystalline cellulose	20	100.09	0.39
Colloidal silicon dioxide	1	99.75	0.06

* The % values mean the percent of weight of impurities or excipients added by weight of gabapentin in the samples.
** Number of measurements (n) was equal to 3.

Table 4
Results of the analysis of gabapentin by the proposed method and USP HPLC method.

Sample	Proposed method	USP method
Bulk drug		
Content found (%)	100.31	100.35
RSD (%)	0.37	0.20
Capsules		
Labeled amount (%)	99.76	99.90
RSD (%)	0.63	0.45

Number of measurement (n) was equal to 6.

with RSD are shown in Table 4. In all cases, the samples complied with the official content requirement (98.0–102.0% for bulk drug and 90.0–110.0% for capsules). In addition, the statistical analysis confirmed that there was no significant difference between the proposed method and the pharmacopeial reference method at 95% confidence level.

3.4. Removal of blue adducts from waste solution

Due to the eco-concern and the responsibility on the disposal of colored effluents into the environment, the colored species from either industries or laboratories should be removed from wastewater before they are discharged. In this study, batch adsorption by using gypsum as an adsorbent was employed for the decontamination of the blue adducts in the assay waste because of the simplicity of the operation as well as the low cost and common availability of materials and equipments. It was found that using the adsorbent for the wastewater at the ratio of 1:50 (w/v) in cooperation with vigorous shaking could remove > 95% of the blue colored product from the solution rapidly within 2 min. Thus, the proposed treatment was one of the effective and practical ways to decontaminate and decolorize the waste prior to the release into the environment.

3.5. Acute toxicity of laboratory waste

The results of the acute toxicity evaluation on non-vertebrate brine shrimp showed that the untreated waste caused 0% mortality of the tested organisms at 50% v/v dilution, the maximum concentration that could be prepared in the experiment since the untreated waste at the higher concentration than this level was likely to precipitate in 35 ppt saline solution. Nevertheless, 100% of the organisms safely survived in the treated waste even at the undiluted (100%) concentration. In the vertebrate guppy fishes, LC50 was found to be 17% v/v for the waste solution before the treatment (Fig. 8). This gave the TUa value of 5.8 and thus the waste prior to the treatment was classified as "slightly toxic" substance. However, after the decolorization, LC50 of waste solution was noticeably raised up to 41% and the TUa value was lowered to 2.4 which satisfactorily met the criteria of "non-toxic" effluent. From this result, it clearly demonstrated the performance of the proposed waste treatment method by using gypsum adsorbent to both decolorize and detoxify the assay waste and to produce the resultant cleaner waste which was proven safe for the aquatic life in the environment.

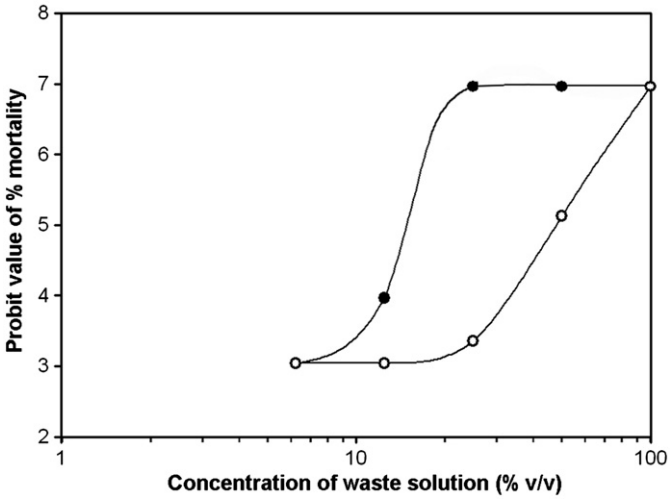

Fig. 8. The percentage mortality of guppy fishes at different concentrations of waste solutions before (●) and after (○) the gypsum adsorption treatment.

4. Conclusion.

In the present study, a novel assay for gabapentin in pharmaceutical samples has been successfully developed based on the colorimetric reaction with naturally derived genipin. The philosophy of the method design relied on the so-called "from safe source to safe sink" concept in which all the reagents used as well as the procedures performed were delicately chosen and optimized to obtain the efficient, safe and environmentally friendly assay. Furthermore, a practical procedure for the treatment of waste after the analysis was also proposed and the treated waste was finally guaranteed for its eco-safety prior to the release into the environment. Therefore, this work not only presents a new route for the assay of gabapentin which is applicable to the pharmaceutical industries, but also calls for the environmental awareness and responsibility from all the analytical chemistry communities to carefully develop the analytical methodologies through this fully green concept for our world's sustainability.

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