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Short communication

Development and application of a validated HPLC method for the analysis of dissolution samples of gabapentin drug products☆

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Abstract

A simple isocratic reversed-phase HPLC method was developed and validated for the analysis of dissolution samples of gabapentin tablets and capsules. Separation of gabapentin from its major degradation impurity, 3,3-pentamethylene-4-butyrolactam was achieved on a Phenomenex Luna Cyano column using a methanol–acetonitrile–20 mM KH_2PO_4 (pH 2.2) (5:5:90, v/v/v) mobile phase. The compounds were eluted isocratically at a flow rate of 1.25 mL/min. Both compounds were analyzed with UV detection at 210 nm. The method was validated according to USP Category I requirements for gabapentin. The validation characteristics included accuracy, precision, linearity, range, specificity and limit of quantitation. Robustness testing was also conducted to evaluate the effect of minor changes to the chromatographic system and to establish appropriate system suitability parameters. Validation acceptance criteria were met in all cases. This method was used successfully for the quality assessment of five gabapentin drug products.
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Keywords: Gabapentin; HPLC; Impurity; Lactam; Drug products; Dissolution

1. Introduction

Gabapentin [1-(aminomethyl)cyclohexaneacetic acid; structure I] is a γ-aminobutyric acid (GABA) analog used for treatment of partial seizures in adults and children [1]. It has also been shown to be effective for neuropathic pain [2]. Gabapentin increases GABA levels in the brain clinically [3]. However, its mechanism of action is still not clear. It had been suggested that gabapentin may bind to an undefined receptor or binding site in the brain [4]. More recently it has been proposed that gabapentin inhibits calcium influx by inhibiting calcium channels in presynaptic terminals [5]. Gabapentin is rapidly absorbed following oral dosing. The T_{max} is approximately 2–3 h and the plasma half-life is between 5 and 7 h [1]. Gabapentin, whose protein binding is <3%, is eliminated by renal excretion without significant metabolism [1].

Gabapentin is a white to off-white crystalline solid with a pK_{a1} of 3.7 and a pK_{a2} of 10.7. It is freely soluble in water and in both basic and acidic aqueous solutions [6]. It degrades via intramolecular cyclization to form a γ-lactam: 3,3-pentamethylene-4-butyrolactam [lactam, structure II].

I II

Various analytical methods for therapeutic monitoring have been reported in the literature for the quantitative determination of gabapentin in human plasma or serum [7–15]. Methods for the analysis of gabapentin in pharmaceutical formulations are quite limited and typically involve a derivatization step [16–19]. The

☆ This scientific contribution is intended to support regulatory policy development. The views presented in this article have not been adopted as regulatory policies by the Food and Drug Administration at this time.

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authors had earlier reported a novel method for the determination of gabapentin and its major degradation impurity in tablets and capsules which did not require derivatization [20].

To the best of our knowledge there is only one reference for the analysis of gabapentin dissolution samples. No details for this HPLC method are given except for detector wavelength [21]. Hence, an attempt has been made to develop a simple, efficient and selective method for the analysis of the dissolution samples of gabapentin tablets and capsules. The method requires no extraction or derivatization steps. HPLC instrumentation with UV detection, which is readily available in most analytical and pharmaceutical laboratories, was used. A total analysis run time of less than 10 min was achieved. The method was used successfully to evaluate the dissolution profiles of five marketed gabapentin drug products.

2. Experimental

2.1. Materials

Gabapentin and 3,3-pentamethylene-4-butyrolactam (lactam) certified reference standards were purchased from the United States Pharmacopeia (Rockville, MD, USA). Gabapentin drug substance was purchased from Interchem Corporation (Paramus, NJ, USA). Nylon syringe filters were purchased from Millipore Corp. (Bedford, MA, USA). HPLC grade monobasic potassium phosphate (KH_2PO_4), ACS grade phosphoric acid and ACS grade hydrochloric acid (HCl) were purchased from Fisher Scientific (Fairlawn, NJ, USA). HPLC grade acetonitrile and methanol were purchased from Burdick and Jackson (Muskegon, MI, USA). HPLC ready deionized 18 MΩ water was obtained, in-house, from a Milli-Q Gradient A-10 water purification system, Millipore Corp., (Bedford, MA).

2.2. Dissolution

A calibrated dissolution apparatus (USP II) was used with paddles at 50 rpm and bath temperature maintained at 37 ± 1 °C. Nine hundred millilitre freshly prepared and degassed 0.06N HCl solution was used as the dissolution medium.

Six tablets/capsules were evaluated for each drug product tested. Dissolution samples were collected at 5, 10, 20 and 30 min for the capsule drug products and at 10, 20, 30 and 45 min for the tablet drug products [22]. At each time point, a 5 mL sample was removed from each vessel using an auto-sampler and filtered through a nylon filter (0.45 μm, 25 mm) into labeled glass tubes and analyzed by HPLC.

The amount of gabapentin in the test samples was calculated, as percentage dissolved, from the measured peak area for the test samples and compared it with the peak area for the standard gabapentin solution using the following equation:

$$\text{Dissolved}\,(\%) = \frac{900}{\text{Drug Load}} \times \frac{\text{Peak Area (sample)}}{\text{Peak Area (standard)}} \times \text{Concentration (standard)} \times 100$$

where drug load is 600 or 800 for the tablets and 300 for the capsules.

2.3. Instrumentation and chromatographic conditions

The HPLC system consisted of a Hewlett Packard 1050 series (Agilent Technologies, Wilmington, DE, USA) equipped with a quaternary pump, online degasser, column heater, autosampler and diode array-detector (DAD). Data collection and analysis were performed using ChemStation software (Agilent Technologies). Separation was achieved on a Phenomenex Luna cyano column 250 mm × 4.6 mm, 5 μm fitted with a 4.0 mm × 3.0 mm, Phenomenex cyano security guard cartridge (Phenomenex, Torrance, CA, USA). The elution was isocratic at 1.25 mL/min with a mobile phase of methanol–acetonitrile–20mM KH_2PO_4 (pH 2.2) (5:5:90, v/v/v). The column temperature was maintained at 26 °C. The injection volume was 40 μL with UV detection at 210 nm.

2.4. Preparation of standard solutions

2.4.1. Preparation of gabapentin calibration standards

Gabapentin stock solution I of 5 mg/mL was prepared in water using the USP gabapentin reference standard. Calibration standard solutions at eight levels were prepared daily by diluting the stock solution I to concentrations of 0.05, 0.10, 0.15, 0.20, 0.25, 0.35, 0.50 and 0.65 mg/mL.

2.4.2. Preparation of gabapentin quality control standards

Gabapentin stock solution II of 5 mg/mL was prepared in water using the gabapentin reference standard. Quality control (QC) standard solutions were prepared by diluting the stock solution II for the final QC concentrations of 0.06, 0.16, 0.24, 0.44 and 0.64 mg/mL. Gabapentin stock solution III of 5 mg/mL was prepared in water using the gabapentin drug substance.

2.4.3. Preparation of lactam standard

Lactam stock solution I of 1 mg/mL was prepared in water using the USP lactam reference standard.

2.5. Method validation

The method was validated according to the United States Pharmacopeia Category I requirements. The following validation characteristics were addressed: linearity, range, accuracy, precision, specificity, limit of quantitation and robustness.

2.5.1. System suitability standard

System suitability standard solution which contained 0.4 mg/mL gabapentin and 4 μg/mL lactam was prepared by diluting and mixing the gabapentin and lactam stock solutions with mobile phase. System suitability was determined from six replicate injections of the system suitability standard before sample analysis. The acceptance criteria were less than 2% relative standard deviation (R.S.D.) for peak area, greater than 6000 column plates, USP tailing factor less than 2.0 and resolution

between gabapentin and the lactam of at least 10. Resolution was calculated using the following equation:

$$R = 1.18\left[\frac{t_2 - t_1}{W_2 + W_1}\right]$$

where t_2 and t_1 are the retention times of lactam and gabapentin, respectively, and W_2 and W_1 are the peak widths at half height. The results were used to monitor critical operational parameters of the chromatographic system to confirm that the resolution and precision were adequate immediately prior to analysis.

2.5.2. Linearity and range

Standard calibration curves were prepared with eight calibrators over a concentration range of 0.05–0.65 mg/mL (0.05, 0.10, 0.15, 0.20, 0.25, 0.35, 0.50 and 0.65 mg/mL) for gabapentin. The data of peak area versus drug concentration were treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day linearity. The range was the interval between the highest and lowest concentration of analyte where acceptable linearity, accuracy and precision were obtained.

2.5.3. Accuracy and precision

Accuracy and precision of the method were determined for the drug substance by analyzing QC standard samples at five concentrations of gabapentin (0.06, 0.16, 0.24, 0.44 and 0.64 mg/mL). The method precision was established by injecting three standard QC samples at each concentration level for the intra-day precision and on 3 days for the intermediate precision. Precision was expressed by the %R.S.D. of the analyte peaks. Accuracy was established by evaluating the amount determined from the quality control standards and comparing to the respective nominal value expressed as percent recovery.

Accuracy of the method was also tested on all drug products at three concentrations with three respective samples. The method of standard additions was utilized. For the tablet drug products, 20 tablets were ground into a fine powder using a glass mortar and pestle. For capsule drug products, 20 capsules were weighed and the contents emptied into a glass mortar. The empty capsule shells were weighed to determine the average fill weight in each capsule. The fill material was gently ground using a glass pestle for 1 min to break any aggregated or cemented material. From the powdered drug product, a portion equivalent to about 30% of the nominal (100%) dissolution of gabapentin in the drug product was accurately weighed and transferred to a 100 mL volumetric flask (for 600 mg and 800 mg strengths) and 250 mL (for 300 mg strength). Approximately 75 mL of dissolution medium was added to the flask. Drug product was then spiked with gabapentin stock solution III up to the target concentration. The target concentrations were 40, 80 and 120% gabapentin with respect to the nominal (100%) dissolution. In addition samples were analyzed containing 30% of the nominal amount of gabapentin in the drug product without spiking. All samples were sonicated for 15 min followed by 15 min on a mechanical shaker at 100 rpm. The flasks were adjusted to volume and mixed well. The resulting solution was filtered using a 0.45 μm nylon filter into labeled glass tubes. Samples were diluted 60:40 with 6:1:1 250 mM KH_2PO_4:methanol:acetonitrile dilution mix and injected into the HPLC for analysis. Percent recovery was calculated by comparing the known spiked amount of gabapentin to the amount detected in the spiked sample after subtracting the amount detected in the unspiked (30% product) samples.

2.5.4. Limit of quantitation

The limit of quantitation for gabapentin is the lowest concentration where acceptable accuracy and precision were obtained. In addition an estimate of the limit of quantitation for gabapentin was calculated from ten times the noise value.

2.5.5. Robustness

The robustness of the method was evaluated by analyzing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±10%), auto-sampler injector volume (±50%) and column compartment temperature (±4 °C).

2.5.6. Specificity

Specificity of the method was determined by analyzing samples containing a mixture of the drug product excipients and samples containing gabapentin's main degradation product, the lactam. All chromatograms were examined to determine if gabapentin and the lactam co-eluted with each other or with any excipient peak.

3. Results and discussion

3.1. Selection and optimization of analytical method

Gabapentin is a weak ultraviolet (UV) absorber requiring spectral analysis at short UV wavelengths. At 210 nm gabapentin absorbance is approximately one order of magnitude less than the lactam. Thus, traditional dissolution analysis by direct UV was not used since low levels of the lactam may interfere with gabapentin quantitation.

When direct UV detection is not feasible for dissolution analysis due to potential interferences, HPLC is often used. Interferences from impurities are separated from the main product and analysis can still be achieved with UV detection. For HPLC analysis the lactam is strongly retained on reversed-phase columns since it is relatively non-polar. Gabapentin is a small, highly polar molecule which can exist as a cation, anion or zwitterion due to its acid pK_a of 3.7 and base pK_a of 10.7. Thus, it is poorly retained on most reversed-phase HPLC columns. Therefore, it is difficult to elute both compounds efficiently using a simple isocratic system. The optimization goal was to develop a simple and efficient chromatographic method for two molecules with very different chemical selectivities. The authors have previously reported a HPLC method for the analysis of gabapentin potency samples at 210 nm using a cyano column and 8:92 acetonitrile:pH 6.2 phosphate buffer (20 mM) [20]. This method was found to be unsuitable for the present analysis due to the large difference in pH between the test samples which were prepared in 0.06N HCl and the mobile phase which contains pH 6.2 buffer. Hence, the mobile phase was modified to include a pH

Table 1
Parameters and linearity data of gabapentin calibration curves

Standard curve	Analytical range (mg/mL)	Calibrators	Slope	*y*-Intercept	R^2 value
Validation set 1	0.05–0.65	8	581.44	−0.9606	0.9999
Validation set 2	0.05–0.65	8	583.08	−0.8702	0.9999
Validation set 3	0.05–0.65	8	582.22	3.2756	0.9999

Table 2
Accuracy: drug substance ($n = 3$)

Sample	0.06 mg/mL	0.16 mg/mL	0.24 mg/mL	0.44 mg/mL	0.64 mg/mL
Validation set 1	101.30	97.87	98.38	99.27	99.62
Validation set 2	100.25	97.67	99.27	99.85	99.47
Validation set 3	97.92	98.65	100.84	99.86	100.84

2.2 phosphate buffer (20 mM). The resulting system provided good resolution but resulted in significant tailing for both the gabapentin and the lactam peaks.

To improve peak symmetry, the concentration of the organic portion of the mobile phase, i.e., of acetonitrile, was varied between 4 and 12%, individually, and in combination with tetrahydrofuran or methanol. Best results were obtained when 5% acetonitrile and 5% methanol was used, although the peak of the void volume was found to be chromatographically noisy in all cases resulting in minor interference to the early eluting gabapentin peak. Diluting the dissolution samples 60:40 with a dilution mix (consisting of methanol, acetonitrile and monobasic potassium phosphate (250 mM) in the ratio 1:1:6) to bring the pH of the sample to 2.2 removed this chromatographic noise. Both, a Phenomenex Luna Cyano and a Brownlee Spheri-5 Cyano column were tested and both generated an efficient run time of less than 10 min. The Phenomenex column provided superior peak symmetry and was selected for the study. The USP tailing factor improved from 1.96 to 1.62 on the Phenomenex column. Since the dissolution samples (in 0.06N HCl) were diluted with the dilution mix, all samples and standards prepared from the stock solutions were diluted using a solution containing 60 parts of 0.06N HCl and 40 parts of the dilution mix.

3.2. Method validation

The following method validation characteristics were addressed for gabapentin: accuracy, precision, specificity, limit of quantitation, limit of detection, linearity, range and robustness. The method was validated for accuracy, precision, specificity and limit of quantitation using sets of three quality control (QC) standards. Standard calibrators were used to establish linearity and range. Robustness was established using the system suitability standard. The validation characteristics met the acceptance criteria for USP Category I.

3.2.1. System suitability

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested met the acceptance criteria on all days. Adequate resolution of >10 between the gabapentin and the lactam peaks ensured the specificity of the method.

The system suitability assessment for the analytical HPLC method established instrument performance parameters such as peak area %R.S.D., column efficiency (N) and USP tailing factor (T_f) for the gabapentin peak. The mean ($n = 18$) peak area %R.S.D. was 1.2% and the mean T_f and N were 1.66 and 6933 plates/m with CVs of 1.0 and 1.8%, respectively. All critical parameters tested met the acceptance criteria on all days.

3.2.2. Linearity and range

Linearity of the method was confirmed by preparing gabapentin standard curves for the analytical range of 0.05–0.65 mg/mL. Excellent correlation between analyte peak area and concentration of the drug was observed with $r^2 \geq 0.999$ for all standard curves (Table 1). Precision and accuracy were established for drug substance from 0.06 to 0.64 mg/mL. Therefore the range for the method is 0.06–0.64 mg/mL. This range represents 30–320, 15–160 and 12–118% dissolution, respectively, for the 300, 600 and 800 mg gabapentin drug products.

Table 3
Precision: drug substance (%R.S.D., $n = 3$)

Sample	0.06 mg/mL	0.16 mg/mL	0.24 mg/mL	0.44 mg/mL	0.64 mg/mL
Validation set 1	3.43	0.87	2.43	1.15	0.67
Validation set 2	2.18	1.12	0.60	0.18	0.35
Validation set 3	1.99	2.38	1.32	0.66	0.68
Intermediate	2.71	1.45	1.78	0.73	0.82

Table 4
Accuracy: drug product (%recovery, $n = 3$)

Product	30% drug product spiked to		
	40%	80%	120%
A: Capsule	99.5	98.8	97.5
B: Capsule	99.1	99.2	99.0
C: Tablet	99.9	98.7	98.3
D: Tablet	97.1	98.5	101.6
E: Tablet	97.3	99.7	98.0

3.2.3. Accuracy and precision

Accuracy and precision were established across the analytical range for gabapentin and the lactam. The accuracy and intra-day and inter-day precision were calculated from the QC samples for gabapentin and the lactam. Results for the intra-day accuracy of gabapentin are summarized in Table 2. Results for the intra-day and inter-day precision are summarized in Table 3. The accuracy results for gabapentin in all drug products showed good recovery and are summarized in Table 4. Results for the accuracy of gabapentin tested in drug products at three concentration levels by the technique of standard addition ranged from 97.5 to 99.5% for capsule drug product A and 97.1 to 101.6% for tablet drug product D. The recovery was $100 \pm 3\%$ for all samples.

3.2.4. Limit of quantitation

The limit of quantitation for gabapentin based on the lowest concentration where acceptable accuracy and precision were obtained is 0.06 mg/mL. An estimate of the limit of quantitation based on 10× S/N is 0.050 mg/mL, which is the same as the lower level of the calibration curve. An estimate of the limit of detection based on 3× S/N is 0.015 mg/mL for gabapentin.

3.2.5. Robustness

To ensure the insensitivity of the HPLC method to minor changes in the experimental conditions it is important to demonstrate robustness of the method. None of the alterations caused a significant change in resolution between gabapentin and lactam,

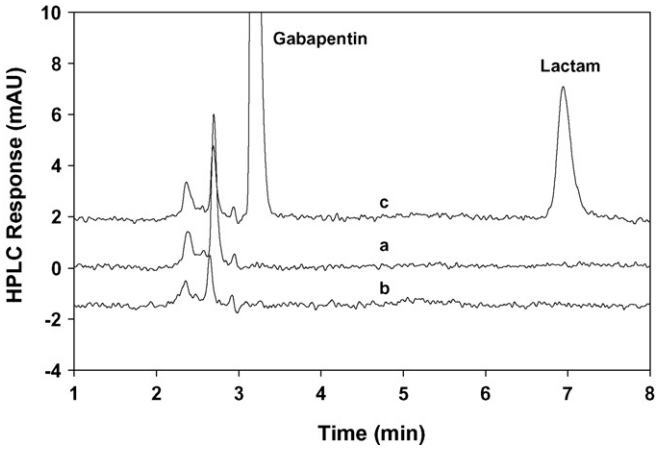

Fig. 1. Chromatography of (a) excipient mix; (b) 60:40 mix of 0.06N HCl and dilution mix and (c) the system suitability standard.

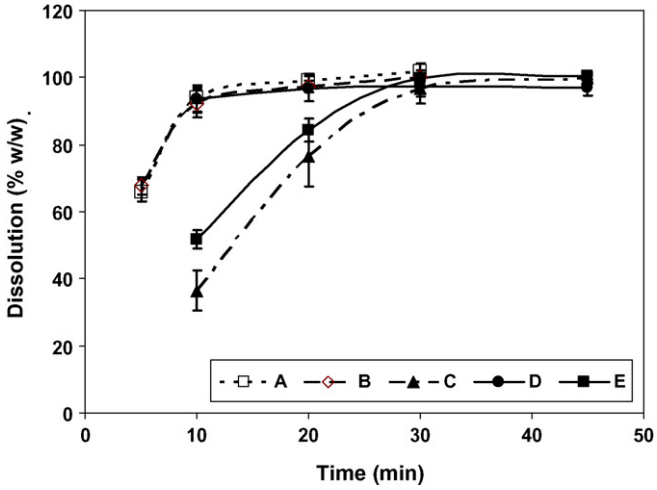

Fig. 2. Dissolution profile of gabapentin capsule products A and B and tablet products C–E.

peak area R.S.D., USP tailing factor, peak width or theoretical plates.

3.2.6. Specificity

The excipient mixture showed the absence of any peaks beyond the void volume (Fig. 1). The 60:40 mix of 0.06N HCl and dilution mix also had no peaks beyond the void volume. In addition, resolution between gabapentin and the lactam was always greater than 15. Due to the absence of any co-eluting peaks we determined this method to be specific for gabapentin.

3.3. Analysis of the marketed products

The validated method was used in the analysis of five gabapentin drug products. This included drug products from four different manufacturers, as two different dosage forms (capsules and tablets), and three different dose strengths (300, 600 and 800 mg). Dissolution profiles of each product are presented in Fig. 2. The two capsule products (products A and B) showed similar dissolution profile with >90% dissolution within 10 min and >99% dissolution in 30 min. However, significant differences were observed within the dissolution profiles of the three tablet products tested (products C–E). Tablet product D displayed significantly more rapid dissolution than tablet products C and E and its profile was similar to the capsule products. Products C and E showed <90% dissolution within 20 min, while product D was >90% dissolved within 10 min. However, all three tablet products tested showed >97% dissolution in 45 min.

4. Conclusion

A simple and efficient reverse-phase HPLC method was developed and found to be accurate, precise and linear across the analytical range. The method was specific for the determination and quantification of gabapentin in dissolution samples. The method may be used to assess the quality of commercially available gabapentin drug products.

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