Chemical Stability of 4-Aminopyridine Capsules

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ABSTRACT

Background: 4-Aminopyridine (4-AP) is a known potassium channel blocker that has been used to treat multiple sclerosis and spinal cord injury. 4-AP capsules must be prepared extemporaneously.

Objective: To determine the chemical stability of 4-AP capsules containing 10 mg of active ingredient.

Methods: Ten-milligram capsules were prepared from 4-AP obtained from 2 suppliers, with either lactose or microcrystalline cellulose as diluent. The hard gelatin capsules were stored in amber snap-top prescription vials at room temperature (20°C to 25°C) with protection from light. Two capsules were collected from each group on days 0, 14, 28, 62, 96, 125, 180, and 365 and stored in a rubber-stoppered glass test tube containing desiccant material at –70°C. On the day of analysis, solutions were prepared from the contents of the capsules, which had been accurately weighed and appropriately diluted; the solutions were assayed, in duplicate, by means of a stability-indicating high-pressure liquid chromatography assay.

Results: Ten-milligram capsules of 4-AP, prepared from material obtained from each supplier and diluted with either lactose or microcrystalline cellulose, retained at least 94% of the initial content for 365 days when stored in plastic prescription vials at room temperature with protection from light.

Conclusions: Extemporaneously prepared 10-mg capsules of 4-AP were considered stable for 365 days when stored in plastic prescription vials at room temperature with protection from light.

Key words: 4-aminopyridine, capsules, lactose, microcrystalline cellulose, stability, high-pressure liquid chromatography

RÉSUMÉ

Historique : La 4-aminopyridine (4-AP) est un inhibiteur des canaux potassiques connu, qui a été utilisé dans le traitement de la sclérose en plaques et des lésions médullaires. Les capsules de 4-AP doivent être préparées extemporanément.

Objectif : On a mené une étude pour déterminer la stabilité chimique des capsules de 4-AP renfermant 10 mg de principe actif.

Méthodes : On a préparé des capsules de 10 mg avec de la 4-AP provenant de deux fournisseurs, et du lactose ou de la cellulose microcristalline comme excipient. Les capsules de gélatine dure ont été conservées à la température de la pièce (entre 20 et 25 ºC) et protégées de la lumière dans des flacons d'ordonnance de couleur ambre à bouchon pression. On a recueilli deux capsules de chaque groupe aux jours 0, 14, 28, 62, 96, 125, 180 et 365, qu'on a entreposées à -70 ºC dans des éprouvettes de verre munies d'un bouchon de caoutchouc et contenant un dessicatif. Le jour de l'analyse, on a préparé des solutions à partir du contenu des capsules, qu'on a pesé avec précision et dilué de façon appropriée; on les a ensuite soumises à un double dosage au moyen d'une épreuve de stabilité par chromatographie liquide à haute pression.

Résultats : Les capsules de 10 mg de 4-AP, préparées à partir du produit de chaque fournisseur dilué avec du lactose ou de la cellulose microcristalline, ont conservé au moins 94 % de leur contenu initial pendant 365 jours, lorsqu'elles étaient conservées dans des flacons d'ordonnance de plastique à la température de la pièce et à l'abri de la lumière.

Conclusions : Les capsules de 10 mg de 4-AP préparées extemporanément et conservées dans des flacons d'ordonnance de plastique à la température de la pièce et à l'abri de la lumière ont été jugées stables pendant une période de 365 jours.

Mots clés : 4-aminopyridine, capsules, lactose, cellulose microcristalline, stabilité, chromatographie liquide à haute pression

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INTRODUCTION

Neural and muscular dysfunctions, such as multiple sclerosis and spinal cord injury, have been treated successfully with 4-aminopyridine (4-AP).[1] Various strengths of immediate-release formulations of 4-AP are prepared extemporaneously in pharmacies throughout Canada.

In a stability study of 5-mg capsules of 4-AP with lactose as the diluent, Trissel and others[2] found that the active ingredient was stable for 6 months when stored at either 4°C or 23°C and for 1 month at 37°C. Potter and others[3] used 10-mg capsules of 4-AP prepared with lactose diluent to treat patients with spinal cord injury. They stated that the product was stable for only 20 days because of possible polymerization between the drug and the gelatin. Where stability information about a specific product is unavailable, a beyond-use date of 6 months or 25% of the supplier's expiry date, whichever is shorter, may be assigned, according to USP guidelines.[4] At the author's institution, 10-mg capsules of 4-AP have been assigned an arbitrary 3-month expiry date for storage at room temperature with protection from light.

A review of the literature revealed only one chemical stability study of 4-AP,[2] for 5-mg capsules stored at room temperature. A study was therefore undertaken to determine (by means of a high-pressure liquid chromatography [HPLC] system) the chemical stability of 4-AP from 2 sources, prepared as 10-mg capsules, with either lactose or microcrystalline cellulose powder used as the diluent and storage at room temperature with protection from light.

METHODS

Preparation of Capsules

4-AP capsules were prepared from 2 sources of active ingredient (Regis Chemical Company, Morton Grove, Illinois, lot 3194; Wiler Fine Chemicals Ltd, London, Ontario, lot 68150) and either lactose USP (Wiler, lot 10338) or microcrystalline cellulose NF (FMC Corporation, Philadelphia, Pennsylvania, lot 1851) as the diluent. The active ingredient was accurately weighed out and then geometrically triturated with one of the diluents. A capsule-filling machine was used to fill #3 hard gelatin capsules with each powder, at 10 mg of 4-AP per capsule. The capsules were then placed in amber polypropylene prescription vials and stored at room temperature (20°C to 25°C) with protection from light.

Collection of Samples

Immediately after packaging, 2 capsules were collected from each of the 4 groups, placed in a glass test tube containing desiccant material, and stoppered. These samples were stored at –70°C for analysis at a later date. Subsequently, on days 14, 28, 62, 96, 125, 180, and 365, additional samples were collected and frozen in a similar manner.

Chemical Stability Study

HPLC System

The mobile phase for the 4-AP assay was a combination of 15 parts acetonitrile (EM Science, Gibbstown, New Jersey, lot 36065) and 85 parts of a phosphate buffer. The phosphate buffer was prepared by dissolving 25 mmol/L of monobasic phosphate (monohydrate) (BDH, Toronto, Ontario, lot 115184/38578) and 1 mg/mL of 1-heptanesulphonic acid sodium (Aldrich Chemical Company, Milwaukee, Wisconsin, lot HR 12521ER) in HPLC-grade water. The pH of the final solution was adjusted to 3.0 ± 0.1 with concentrated orthophosphoric acid (BDH, lot 91892); the solution was then filtered through a 0.45-µm nylon filter and degassed.

The mobile phase was pumped through a 4.6 mm ± 250 mm, 5-µm, C_{18} column (Luna ODS [octadecylsilane] 18[2], lot 256161; Phenomenex, Torrance, California) at a rate of 1.0 mL/min by means of an isocratic delivery pump (model LC-10AS, Shimadzu Corporation, Kyoto, Japan). A photodiode array detector (model SPD-M6A, Shimadzu Corporation) was set at 263 nm to monitor the peaks of the 4-AP and the internal standard. An autoinjector (model SIL-10AXL, Shimadzu Corporation) was used to inject 10-µL samples. Caffeine citrate (Sel-Win Chemicals Ltd, Vancouver, British Columbia, 10 mg/mL, lot 8960) was used as the internal standard.

HPLC Assay Validation

Forcibly degraded samples of 4-AP were used to prove the specificity of the HPLC method. Three separate solutions of 4-AP, containing approximately 10 mg/mL each, were prepared. One solution was adjusted to a pH of approximately 1.5 with concentrated hydrochloric acid (BDH, lot 109521/11031) and heated on a hot plate (Thermix stirring hotplate, model 210T, Fisher Scientific Ltd, Nepean, Ontario) for 121 h. A second solution was adjusted to a pH of about 11 with 5N sodium hydroxide (BDH, lot 9112019) and heated for 121 h on a hot plate. One millilitre of 30% hydrogen peroxide (BDH, lot

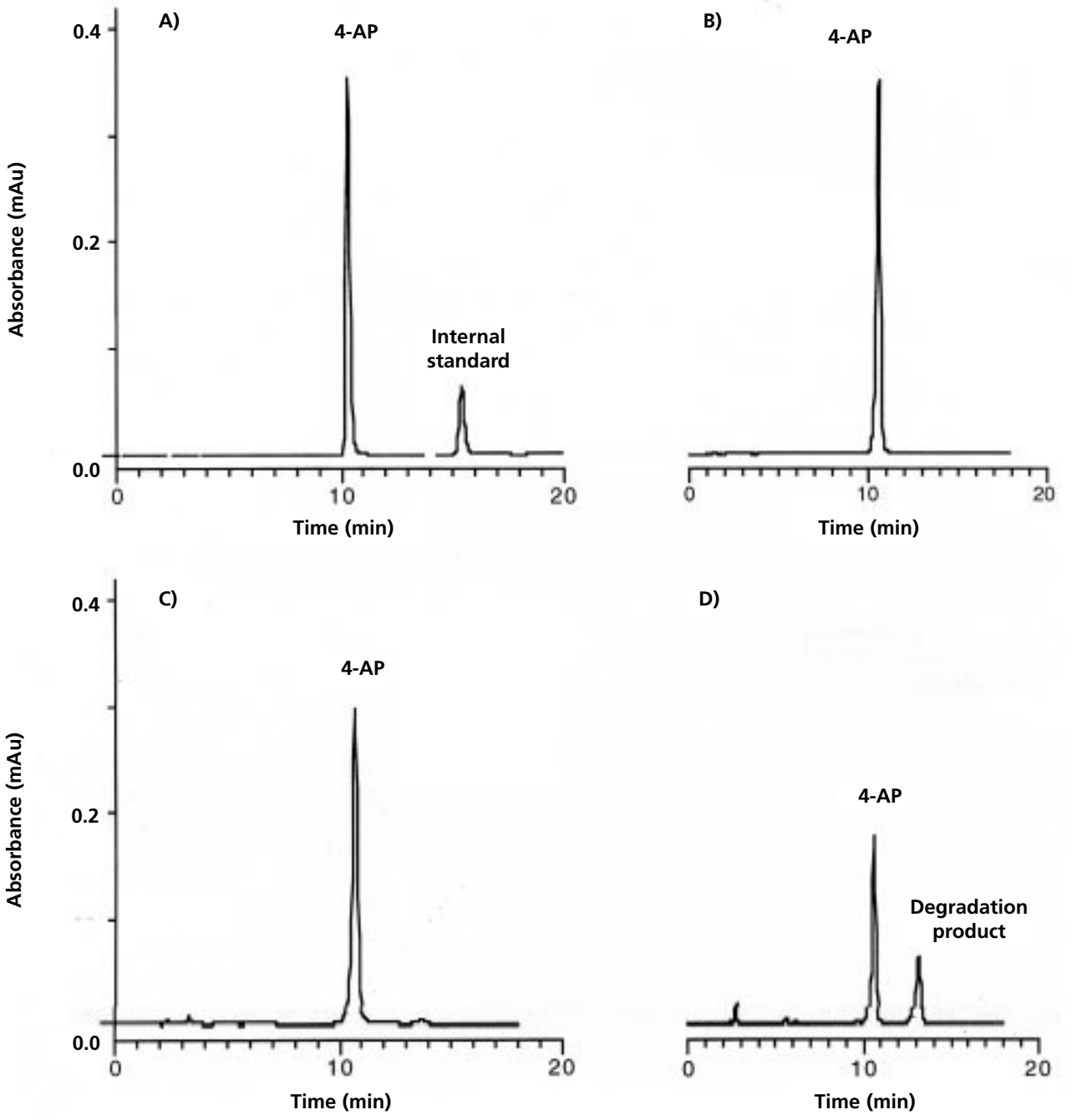

Figure 1. Sample chromatograms of 4-aminopyridine and degradation products. A: Sample of 4-aminopyridine (4-AP) prepared with lactose and internal standard, after storage for 365 days at 22°C with protection from light. B: Acid-degraded sample after heating for 121 h. C: Alkaline-degraded sample after heating for 121 h. D: Oxidized sample after 121 h at room temperature.

113439-30995) was added to the final solution, which was stirred at 22°C for 121 h. All solutions were analyzed at time 0 and then at 3, 23, 48, and 121 h for interfering peaks.

Multiwavelength (254 and 263 nm) and ultraviolet (UV) spectral analysis (200-350 nm) were used to determine the purity of all peaks. The UV spectra of the 4-AP peak from the degradation samples were compared with the UV spectra of the 4-AP reference material, and correlation coefficients were determined.

Stability Study

On the day of analysis, solutions were prepared by accurately weighing the contents of each capsule and transferring the material into 10-mL volumetric flasks. To each flask, 5 mL of a 15% solution of acetonitrile in water was added, and the flask was then sealed and sonicated for 5 minutes. Solutions were then brought to volume, mixed, and filtered through a 0.45-µm nylon filter disk (Acrodisc 4, Gelman Sciences, Ann Arbour, Michigan, lot 8915). The samples were then further diluted with mobile phase after the addition of an internal standard and were analyzed by the stability-indicating HPLC method described above.

Statistical Analysis

Least-squares regression analysis was used to assess the linearity of all standard curves. Intraday variation was assessed by comparing the average areas of 5 replicate injections at 3 separate time points and was reported as the coefficient of variance. The coefficients of variance of the slopes, linear coefficients, and average areas from 5 separate days were used to compare the interday variation of the method. Values from 5 recovery samples (prepared from solutions containing known weights of 4-AP) were used to measure the accuracy of the method. The sensitivity of the assay was defined as the concentration that generated a detectable peak while still retaining a linear relationship.

RESULTS

HPLC Assay Validation

Heating of the acidic solution resulted in no degradation of the parent compound after 121 h (Figure 1B). Alkaline conditions produced an approximately 25% decrease in 4-AP concentration after 121 h of heating (Figure 1C). Oxidation with hydrogen peroxide resulted in nearly 50% destruction of the parent compound after 121 h (Figure 1D). None of the detected degradation peaks interfered with the parent compound. The purity of all parent peaks was confirmed by multiwavelength and UV spectral analysis. When the spectra of the parent compound were compared with those of the reference material, correlation was good ($r > 0.990$).

The intraday coefficient of variance was 1.51% when area ratios were compared. When slopes, linear

Table 1. Stability of 10-mg Capsules of 4-Aminopuridine (Source: Wiler Fine Chemicals) Stored at Room Temperature with Protection from Light

Diluent	Initial Conc'n* (mg/capsule)	Mean % of Initial Concentration Remaining†						
		Day 14	Day 28	Day 62	Day 96	Day 125	Day 180	Day 365
Lactose	10.0 ± 0.01	100.7 ± 5.2	100.3 ± 1.0	108.0 ± 5.4	101.1 ± 5.7	101.5 ± 1.9	102.3 ± 0.5	100.0 ± 0.6
Microcystalline cellulose	10.4 ± 0.07	103.0 ± 1.7	98.6 ± 1.1	101.6 ± 5.9	98.2 ± 2.0	102.3 ± 1.2	94.7 ± 0.6	99.9 ± 1.0

*Mean (± standard deviation) of 4 determinations, calculated from standard curve.
†Mean (± standard deviation) of 4 determinations, based on 100% at time zero.

Table 2. Stability of 10-mg Capsules of 4-Aminopuridine (Source: Regis Chemical Company) Stored at Room Temperature with Protection from Light

Diluent	Initial Conc'n* (mg/capsule)	Mean % of Initial Concentration Remaining†						
		Day 14	Day 28	Day 62	Day 96	Day 125	Day 180	Day 365
Lactose	10.5 ± 0.55	98.9 ± 0.6	99.5 ± 1.4	98.4 ± 3.0	100.7 ± 1.9	102.0 ± 0.9	98.9 ± 0.8	96.9 ± 0.8
Microcystalline cellulose	9.8 ± 0.09	103.6 ± 5.8	105.1 ± 1.2	109.4 ± 1.5	98.6 ± 2.0	111.8 ± 1.7	102.2 ± 5.6	99.5 ± 2.1

*Mean (± standard deviation) of 4 determinations, calculated from standard curve.
†Mean (± standard deviation) of 4 determinations, based on 100% at time zero.

coefficients, and average areas from 5 separate days were compared, the coefficients of variance were 1.79%, 0.026%, and 1.59%, respectively. The average recovery (± standard deviation) from samples prepared from known concentrations was 101.1% ± 1.14%. The sensitivity of the assay was determined to be 10.4 ng of 4-AP.

Stability Study

The results of the chemical stability study are summarized in Tables 1 and 2. Capsules extemporaneously prepared with either source of 4-AP powder and diluted with either lactose or microcrystalline cellulose were stable for 365 days when stored at room temperature with protection from light. All samples retained at least 94% of the original strength over the 365-day study period.

DISCUSSION

Trissel and others[2] studied the stability of 4-AP prepared as 5-mg capsules diluted with lactose and stored at 4°C, 23°C, and 37°C. The capsules were stable for 6 months at both 4°C and 23°C and for 1 month at 37°C. Potter and others[3] reported, in a paper describing the use of 4-AP in 3 cases, that capsules of 4-AP prepared with lactose were stable for only 20 days at room temperature. The study reported here has demonstrated a period of stability of 365 days for 10-mg capsules stored at room temperature with protection from light. There was no difference in stability between capsules prepared with lactose and those prepared with microcrystalline cellulose as the diluent.

Degradation products were adequately separated from the parent compound and the internal standard under all chromatographic conditions. 4-AP was stable under acidic conditions, showed some signs of destruction under alkaline conditions, and was most affected by harsh oxidative conditions. The purity of all of the parent peaks of 4-AP was confirmed by multiwavelength and UV spectral analysis.

In conclusion, 10-mg capsules of 4-AP compounded with either lactose or microcrystalline cellulose as the diluent were chemically stable for 365 days when stored at room temperature with protection from light. The author's institution has now adopted a 365-day beyond-use date, which allows for larger production batches and cost savings to the hospital through economy of scale.

References

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