Stability of 4-Aminopyridine and 3,4-Diaminopyridine Oral Capsules

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Abstract

The objective of this study was to evaluate the chemical stability of 4-aminopyridine 5-mg capsules and 3,4-diaminopyridine 5-mg capsules under a variety of storage conditions. Each of the two drug preparations was extemporaneously prepared in hard gelatin capsules; lactose and micronized silica gel were used as excipients. Samples were stored under three conditions: refrigeration at 4°C and protected from light for 6 months, protected from light at room temperature that ranged from 22°C to 24°C for 6 months, and at a temperature of 37°C and protected from light for 1 month.

Once each month, visual inspection of the capsules and their powder contents was performed to identify observable changes (color, texture, etc), and the weight of the capsule content was measured individually. Chemical stability was assessed initially and at monthly intervals by means of a stability-indicating high-pressure liquid chromatography (HPLC) analytical technique based on the determination of drug content.

No visible changes were observed in any of the samples under any of the storage conditions. The hard gelatin capsules remained clear and colorless, and the content of the capsules remained an off-white powder when viewed under normal fluorescent room light. Capsule content weight did not change during the study. Both 4-aminopyridine and 3,4-diaminopyridine exhibited excellent chemical stability under all study conditions. Little or no loss of drug content occurred in either product under refrigeration, at room temperature, and even at the elevated temperature of 37°C.

The oral 5-mg capsules of 4-aminopyridine and 3,4-diaminopyridine did not undergo decomposition or other adverse changes within 6 months at refrigerated or room temperature or within 1 month of storage at 37°C.

Introduction

The drugs 4-aminopyridine (Figure 1) and 3,4-diaminopyridine (Figure 2) are not available commercially. Nevertheless, they are used as orphan drugs and as extemporaneously prepared dosage forms for the treatment of neural or muscular dysfunction, such as that produced by multiple sclerosis or spinal cord injury.[1] Although 4-aminopyridine and 3,4-diaminopyridine have been used clinically for some time, published stability information on those drugs is lacking. In the absence of specific published information, a beyond-use date of 6 months from the date of preparation has been applied to capsules that are filled with drugs in dry-powder form.[2] Even so, specific information is needed to establish the stability of those drugs in oral dosage forms to substantiate beyond-use dating.

The purpose of this study was to evaluate the chemical stability of 4-aminopyridine 5-mg capsules and 3,4-diaminopyridine 5-mg capsules stored for up to 6 months at 23°C and 4°C and for up to 1 month at 37°C (an elevated temperature that might be encountered during transportation).

Figure 1. Chemical Structure of 4-Aminopyridine.

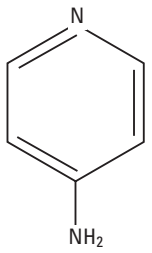
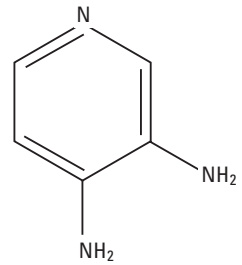


Figure 2. Chemical Structure of 3,4-Diaminopyridine



Materials and Methods

Materials

Extemporaneously compounded hard gelatin capsules containing 4-aminopyridine 5 mg (Lot # IPS-4-121300, Innovative Pharmacy Solutions, Edmond, Oklahoma) and 3,4-diaminopyridine 5 mg (Lot # IPS-34-111200, Innovative Pharmacy Solutions) were obtained from a compounding pharmacy. The 4-aminopyridine reference standard (Lot #73315, Professional Compounding Centers of America [PCCA], Houston, Texas) and 3,4-diaminopyridine reference standard (Lot #76620, PCCA) were obtained from a commercial source. The reference standards were used without further purification. The mobile phase components were all of a grade suitable for HPLC analysis. The water used was also HPLC grade.

Table 1. Capsule Formulations of 4-Aminopyridine and 3,4-Diaminopyridine Evaluated for Stability.

Component	4-Aminopyridine	3,4-Diaminopyridine
4-Aminopyridine	5 mg	—
3,4-Diaminopyridine	—	5 mg
Silica gel micronized	1 mg	1 mg
Lactose hydrous, NF	248 mg	240 mg
Total theoretical weight	254 mg	246 mg

Table 2. High-Pressure Liquid Chromatography Analytical Method Used To Evaluate the Stability of 4-Aminopyridine and 3,4-Diaminopyridine Oral Capsules.

	4-Aminopyridine[a] 3,4-Diaminopyridine[b]
Mobile phase	1650 mL sodium phosphate monobasic 50 mM and 1-octanesulfonic acid buffer 4 mM 350 mL acetonitrile
Column	Prodigy C_{18}[c] (250 x 4.6 mm, 5 µm) Model # 00G-4097-E0
Flow rate	0.8 mL/min
Detection	
4-aminopyridine	266 nm, 0.5 AUFS
3,4-diaminopyridine	290 nm, 0.5 AUFS
Sample	
Injection volume	10 µL
Retention times	
4-aminopyridine	6.3 min
3,4-diaminopyridine	6.5 min

[a] Diluted 20-fold for analysis. Precision: 25.0 ± 0.2 µg/mL, mean ± SD, n = 10; percent relative standard deviation, 0.8%; standard curve range, 5 to 40 µg/mL; correlation coefficient, ≥ 0.9993; intraday and interday coefficients of variation, 1.2% and 0.8%, respectively.
[b] Diluted 20-fold for analysis. Precision: 24.8 ± 0.1 µg/mL, mean ± SD, n = 10; percent relative standard deviation, 0.4%; standard curve range, 10 to 40 µg/mL; correlation coefficient, ≥ 0.9998; intraday and interday coefficients of variation, 1.4% and 1.2%, respectively.
[c] Phenomenex, Torrance, California.

Table 3. Stability of 4-Aminopyridine 5-mg[a] Capsules.

Time (months)	Average Drug Content (mg) per Capsule[b] 4°C	23°C	37°C
0	4.96 ± 0.11	4.96 ± 0.11	4.96 ± 0.11
1	4.86 ± 0.20	4.79 ± 0.15	4.76 ± 0.19
2	4.81 ± 0.15	4.87 ± 0.11	—[c]
3	4.89 ± 0.16	4.88 ± 0.19	—[c]
6	4.93 ± 0.13	4.83 ± 0.14	—[c]

[a] Nominal drug content. Actual initial amount of 4-aminopyridine per capsule was 4.96 ± 0.11 mg (mean ± SD of triplicate determinations of 10 capsules).
[b] Mean ± SD for triplicate determinations of 5 capsules at each evaluation point.
[c] Not tested at this interval.

Table 4. Stability of 3,4-Diaminopyridine 5-mg[a] Capsules.

Time (months)	Average Drug Content (mg) per Capsule[b] 4°C	23°C	37°C
0	5.15 ± 0.16	5.15 ± 0.16	5.15 ± 0.16
1	5.14 ± 0.12	4.94 ± 0.09	4.95 ± 0.06
2	5.00 ± 0.12	4.99 ± 0.17	—[c]
3	5.12 ± 0.19	5.08 ± 0.25	—[c]
6	4.98 ± 0.25	4.89 ± 0.14	—[c]

[a] Nominal drug content. Actual initial amount of 3,4-diaminopyridine per capsule was 5.15 ± 0.16 mg (mean ± SD of triplicate determinations of 10 capsules).
[b] Mean ± SD for triplicate determinations of 5 capsules at each evaluation point.
[c] Not tested at this interval.

Methods

Preparation and Sampling

The 4-aminopyridine and 3,4-diaminopyridine 5-mg capsules were compounded according to the formulas in Table 1. One hundred capsules of each preparation were supplied for evaluation. The capsules were subdivided and packaged in 16-dram standard amber polypropylene plastic prescription vials (L-16, Owens-Brockway, Berlin, Ohio) with child-resistant closures (SL-35, Owens-Brockway). Ten capsules of each preparation were evaluated initially. Forty capsules of each preparation were stored at 4°C, 40 capsules of each were stored at 23°C, and 10 capsules of each were stored at 37°C. The humidity of the storage room, which was measured periodically during the study, ranged from 62% to 67%. Five capsules from each storage condition were evaluated after 1 month at all storage conditions and after 2, 3, and 6 months of storage at 4°C and 23°C.

Evaluation and HPLC Analysis

The physical stability of the preparations was assessed by visual examination of the finished capsules and also by evaluating the capsule contents emptied from the capsules. Visual examinations were performed in normal diffuse fluorescent room light with the unaided eye. In addition, the individual capsule contents were weighed at each time point.

Sample capsules of 4-aminopyridine and 3,4-diaminopyridine were prepared for analysis by transferring the contents of each capsule individually into a glass test tube. Ten milliliters of HPLC-grade water was added to each tube, and the tubes were vortex-mixed for 30 seconds. The solution was delivered through a 0.45-µm filter and was diluted 20-fold with HPLC water for analysis.

The 4-aminopyridine and 3,4-diaminopyridine concentrations were determined by means of a stability-indicating HPLC assay method adapted from the method of Capacio et al.[3] The details of the analytical method used in this study are cited in Table 2. A high-performance liquid chromatograph (LC Module 1 Plus, Waters Corporation, Milford, Massachusetts) was used to analyze the drugs. It consisted of a multisolvent delivery pump, an autosampler, and an ultraviolet light detector in one unit. Separation was performed by means of a C_{18} column (Prodigy, 5 µm, 250 x 4.6 mm, Phenomenex, Torrance, California). A second high-performance liquid chromatograph (Alliance 2960, Waters) with a photodiode array detector (Model 996, Waters Corporation) was used to confirm drug peak purity. The systems were controlled and integrated by personal computers by means of chromatography management software (Millennium^{32} Chromatography Manager, Waters Corporation). Triplicate HPLC determinations on each of the samples

were performed at each test interval to determine drug concentration.

The analytical method for each of the drugs was demonstrated to be stability indicating by accelerated degradation. The sample solutions were mixed with each of the following: 0.1 N sodium hydroxide, 0.1 N hydrochloric acid, or 3% hydrogen peroxide. Each of those three mixtures was subjected to heating, and loss of the intact drugs was observed with each of these treatments. No ultraviolet-absorbing degradation product peaks that interfered with the peaks of the intact drugs evaluated by the analytical method described were produced by any of the decomposition techniques. Stability of the drug preparations was defined as not less than 90% of the initial drug content remaining in the capsules.

Results and Discussion

Under normal fluorescent room light, both of the preparations appeared as off-white powder inside colorless, hard gelatin capsules. No visible changes were observed in the capsules or in the powder that was removed from the capsules. Visual observation indicated that the powder remained unchanged throughout the study.

The mean individual capsule content weight initially for 4-aminopyridine capsules was 254.5 ± 4.8 mg (n = 10); for 3,4-diaminopyridine capsules, the individual content weight initially was 242.8 ± 4.8 mg (n = 10). Individual capsule content weights in the evaluated samples were similar throughout the study. There was no evidence of weight change during storage.

The results of the HPLC analysis for each of the test drugs are shown in tables 3 and 4. Both 4-aminopyridine and 3,4-diaminopyridine were found to be very stable under the conditions of this study. Losses did not exceed 5% in any of the samples and were generally much less after storage for 6 months at both 4°C and 23°C and after storage for 1 month at 37°C. Photodiode array purity analysis of the drug peaks from the capsule samples confirmed both the purity of the drug peaks and their having remained identical to the standards throughout the study.

The stability of 4-aminopyridine in the capsule preparation evaluated in this study is much greater than that indicated previously in public commentary.[4] The drug did not exhibit its anticipated relatively rapid and extensive decomposition. 3,4-Diaminopyridine appears to be similarly stable. The stability of both of those oral capsule preparations was quite good. Stability was maintained throughout a reasonable expiration period of 6 months from the date of preparation at a normal storage temperature and even at an elevated temperature such as that encountered during short-term transportation.

Conclusion

Both 4-aminopyridine and 3,4-diaminopyridine as encapsulated powder preparations were stable for at least 6 months at room temperature or under refrigeration and for at least 1 month at an elevated temperature (37°C).

References

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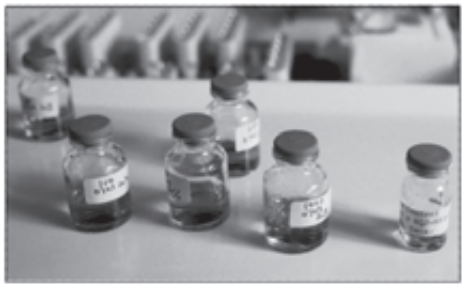

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