Published in final edited form as:

Am J Health Syst Pharm. 2015 December 15; 72(24): 2182–2188. doi:10.2146/ajhp150023.

Stability of Furosemide and Chlorothiazide^a

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Abstract

PURPOSE—Furosemide is a loop diuretic and chlorothiazide is a thiazide diuretic which are commonly used in pediatric patients for varying reasons. Furosemide and chlorothiazide can be used concomitantly to maximize diuresis. It has been a practice at some institutions to combine furosemide and chlorothiazide in the same syringe even though stability data is currently lacking for this combination. The purpose of this study was to determine whether furosemide and chlorothiazide are stable either alone or when mixed together.

METHODS—Chlorothiazide and furosemide were diluted in 5% dextrose USP to a final concentration of 10 mg/mL of chlorothiazide and 1 mg/mL of furosemide and combined. Samples of chlorothiazide in dextrose, furosemide in dextrose and dextrose alone were also prepared for control purposes. Compounds were detected using an Agilent 6460 Triple Quad LC/MS/MS equipped with an ESI source.

RESULTS—Chlorothiazide typically eluted from the chromatogram at 2.6 min and furosemide at 4.8 min. Mixtures and samples were diluted 10,000 fold prior to LC/MS/MS analysis to give 1,000 ng/mL chlorothiazide and 100 ng/mL furosemide so that both would be in the linear range of the assay. Each compound was degraded by exposure to strong UV light in a time-dependent manner. Both chlorothiazide and furosemide retained over 90% of the original concentration when stored separately or together for up to 96 h.

^aFunding source: This work, in part, was supported by Nemours Biomedical Research and a NIH grant, P20GM103464.

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This work, in part, was presented as an abstract at the Cardiology 2014 17th Annual Update on Pediatric and Congenital Cardiovascular Disease, abstract #737, Orlando, FL, USA February 20, 2014.

CONCLUSIONS—Chlorothiazide (10 mg/mL) and furosemide (1 mg/mL) are stable for up to 96 hours at room temperature when protected from light, either alone or together in dextrose 5%.

Keywords

chlorothiazide; furosemide; stability

Background

Furosemide is a loop-diuretic that interferes with the chloride-binding co-transport system inhibiting the re-absorption of sodium and chloride in the ascending loop of Henley and distal renal tubule causing increased excretion of sodium and water. Chlorothiazide is a thiazide type diuretic that inhibits sodium re-absorption in the distal tubule, also leading to increased excretion of sodium and water. The combination of furosemide and a thiazide diuretic (i.e. chlorothiazide or hydrochlorothiazide) has been shown to increase the natriuresis compared with that induced by furosemide alone in animal models ¹. Furthermore, the combination of loop diuretic and a thiazide diuretic has been shown to increase the natriuresis compared with that induced by furosemide alone in addition to increasing the amount of weight loss in adult heart failure and fluid overloaded patients ²⁻⁵.

There are also data in adults suggesting that continuous infusion of loop diuretics produces a greater diuresis as compared with intermittent dosing of loop diuretics ^{6,7}. Combinations of loop diuretics are also used in children to reduce tolerance or resistance to individual compounds.⁸ Since the goal of using both furosemide and chlorothiazide is to cause fluid removal, combining the two drugs in the same syringe for continuous infusion could be an advantage by limiting the total amount of fluid delivered to the patient during therapy. Thus, it has become a practice at some institutions to combine furosemide and chlorothiazide in the same syringe even though stability data are currently lacking for this combination. The purpose of this study was to determine whether injectable formulations of furosemide and chlorothiazide are stable either alone or when mixed together.

Methods

Sample preparation

Chlorothiazide^b (sodium salt, 500 mg vial APP Pharmaceuticals, Schaumburg, IL) was reconstituted with 18 ml of Bacteriostatic Water for Injection, USP (APP Pharmaceuticals, Schaumburg, IL), resulting in a final concentration of 28 mg/mL. Next, 3.57 mL of the 28 mg/mL solution was added to 1 mL of furosemide injection^c (20mg/2mL Hospira Inc, Lake Forest, IL) and 5% dextrose USP (Baxter, Deerfield, IL) was added to a final volume of 10 mL. All compounds were used prior to expiration. The resulting final concentrations were 1 mg/mL of furosemide and 10 mg/mL of chlorothiazide. Samples of furosemide 1 mg/mL and chlorothiazide 10 mg/mL were also prepared separately for analysis. One mL of the 10 mg/mL of furosemide was mixed with 9 mL of 5% dextrose, and the 10 mg/mL chlorothiazide

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was prepared as described for the mixture except for substituting the 1 mL of furosemide with 1 mL of dextrose. Three separate preparations of each formulation were prepared. The mixtures were visually examined for color change against a white background and for haze, turbidity, gas bubbles, and precipitation against a black background. These evaluations were done immediately and after the samples were stored at 25°C in the dark for up to 96 hours to simulate storage under normal clinical use. All samples were stored in 15 ml polypropylene tubes (352097, BD Biosciences, Bedford, MA).

Fresh samples of chlorothiazide and furosemide (in triplicate) were prepared each day using the same pharmaceutical grade compounds each day prior to liquid chromatography tandem mass spectroscopy (LC/MS/MS) analysis to account for any variation in MS signals. Experimental values were adjusted based upon the fresh samples being 100% of the signal. Daily variations in signals were less than 5% during the study period.

Data are expressed as mean with standard deviation. Stability was defined as not less than 90% and not more than 110% of furosemide and chlorothiazide remaining.

Reference standards of furosemide^d and chlorothiazide^e were obtained from USP (Rockville, MD) and dissolved in equimolar NaOH (sodium hydroxide) solution to a final concentration of 1 and 10 mg/mL, respectively. The freshly prepared USP standards were compared directly with freshly prepared pharmaceutical products.

To force degradation, pharmaceutical grade samples were exposed to UV light (254 nm) using a UVG-11 lamp (UVP, Upland, CA) for up to 16 h. Samples were aliquoted into quartz cuvetts that were placed approximately 2 cm away from the light source. At timed intervals, aliquots were removed and diluted for LC/MS/MS analysis. Assays were performed in triplicate and with three independent experiments.

LC/MS/MS analysis

Prior to the stability study, LC/MS/MS methods were developed to separate, detect and measure furosemide, chlorothiazide and degradation products. Chlorothiazide, furosemide and degradation products were separated by chromatography on a Poroshell 120 EC-C18 column (2.7 μ m, 3.0 X 50 mm) at 50°C and eluted with a gradient from 0.1% formic acid (solution A) to 100% methanol in 0.1% formic acid (solution B) using an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) system. The gradient was set up as follows: start (0 min) 100% A, 1 min 100% A, 3 min linear gradient to 100% B, 3.5 min hold 100% B, 3.6 min linear gradient to 100% A, 6 min hold 100% A. Solvent flow rate was 0.4 mL/min and total run time per sample was 7 min, including column equilibration. Compounds were detected using an Agilent 6460 Triple Quad LC/MS/MS equipped with an ESI source. MS conditions were: gas temperature 350°C and flow rate 10 L/min; sheath gas temperature 400°C, flow rate 12 L/min; nebulizer pressure 45 psi; capillary 3500 V and detector in negative ion mode. Chlorothiazide primary ion was 294⁻ and fragment ion 214.1⁻ with fragmentor set at 150 V and collision energy 12 electron volts (eV). Furosemide

dlot # L0H311
elot # I0L188

primary ion was 329⁻ and fragment ion 285.1⁻ with fragmentor set at 110 V and collision energy 12 eV. A major product of UV degradation of chlorothiazide was detected with a primary ion 259.9⁻ and fragment ion 180⁻ with fragmentor set at 135 V and collision energy 25 eV. A major product of UV degradation of furosemide was detected with a primary ion 311.1⁻ and fragment ion 202.2⁻ with fragmentor set at 120 V and collision energy 20 eV. Samples were diluted 10,000 fold prior to LC/MS/MS unless otherwise noted. 10 µl each sample was added to 990 µl water, mixed and then 10 µl of the mixture was added to an additional 990 µl of water and mixed prior to analysis by LC/MS/MS.

Results

During preparation of the samples and on storage, visual examination did not detect any color changes or evidence of haze, turbidity, gas bubbles, or precipitation, indicating that both drugs remained in solution during the course of the experiment.

Chlorothiazide typically eluted from the chromatogram at 2.6 min and furosemide at 4.8 min (Fig 1A). An initial dilution range was prepared to determine the linear range of the LC/MS/MS assay. The assay for chlorothiazide was linear from 8 to 1,250 ng/mL (Fig 2A) and furosemide was linear from 8 to 1,000 ng/mL (Fig 2B). Above 1,250 ng/mL, the signal intensity of chlorothiazide samples very high and no longer linear so these points were not used to generate the calibration curve (open circles, Fig 2A). Thus 10,000 fold dilution of samples prior to LC/MS/MS analysis (to give 1,000 ng/mL chlorothiazide and 100 ng/mL furosemide) ensured that both compounds were measured in the linear range of the assay. The LC/MS/MS system used in this study gave consistent results that did not vary by more than 1% for repeat injections of the same sample. The commercial sources of the two drugs were found to have identical chromatographic and molecular properties to the USP standards. When freshly prepared, the area under the curve for peaks of the pharmaceutical formulations of chlorothiazide and furosemide were within 95% of values obtained using the USP standards.

To force loss of compound and validate the assay method, both compounds were exposed to UV light. MS1 scans of exposed chlorothiazide samples revealed appearance of a new compound of mass/charge ratio (m/e) 259.9. A 180⁻ fragment was generated in the collision cell of the MS as described in the methods section to quantify appearance of this UV degradation product. The product eluted at 1.4 min and was separated from chlorothiazide (Fig 1B). Similarly, the major degradation product generated by UV exposure of furosemide was detected as a peak m/e 311.1⁻ and fragment ion 202.2⁻ that eluted at 4.5 min just before elution of furosemide (Fig 1C). The peak corresponding to chlorothiazide decayed in a linear fashion over 4 h and this decay continued such that only 21% of the parent compound remained after 16 h of UV exposure (Fig 3A). The mass of the degradation product is consistent with loss of chloride from the parent compound. The degradation product increased linearly over the first 4 h of UV exposure, indication that this product is stable relative to the parent compound. Furosemide was less stable to UV exposure, with approximately 25% of the original compound remaining after 3 h exposure to UV light (Fig 3B). A product with a reduced m/e of 311.1 appeared as the original compound was lost, but this was also unstable during UV exposure. After 16 h of UV exposure, neither the parent

compound nor the fragment of m/e 311.1 could be detected. These results clearly show that the assay measuring area under the curves that correspond to chlorothiazide and furosemide indicates levels of each compound and that this assay can be used to indicate sample stability.

Using the developed LC/MS/MS method, the commercial sources of chlorothiazide and furosemide when stored separately or together at 25°C and protected from light, retained at least 90% of the active compounds for the 96 h of this study (Table 1). Levels of chlorothiazide were significantly lower in both formulations 48 and 96 h after preparation, but did not change significantly between 72 and 96 h, indicating that mixing the two drugs does not affect stability and that there is an initial small reduction in levels of chlorothiazide. The degradation products produced by forced degradation were not detected in the 96 h samples.

Discussion

Both chlorothiazide and furosemide can be degraded by exposure to strong UV light at 254 nm. Loss of parent compound was time dependent and corresponded to appearance of degradation products. The commercial drugs contained excipients, including mannitol and dextrose. MS/MS signals from the commercial drugs were identical to those of the pure USP standards, indicating that the excipients did not affect the measurement of drug. For chlorothiazide, UV dependent loss of the parent compound was directly proportional to appearance of a degradation product with a m/e of 259.9, consistent with photodehalogenation. Similar (but slower) degradation has been reported after exposure to UV at 313 nm in methanol solutions⁹. Furosemide was more sensitive to UV exposure with more than 50% of the compound degraded after 3 h of treatment. Amongst a range of degradation products, a major fragment with m/e 311.1 appeared at the first time point measured, peaking after 2 h of UV exposure. A recent study detected at least 12 novel UV absorbing peaks appearing during the degradation of furosemide, although their molecular characteristics were not determined¹⁰. While our studies show that chlorothiazide and furosemide are stable for up to 96 h at 25°C and protected from light as formulated in our hospital, these same drugs may not be stable using different formulations. For example a related compound, hydrochlorothiazide, is stable in acid or in suspension, but degrades above pH 6.5¹¹. The USP standard of chlorothiazide is the free acid and when dissolved in equimolar sodium hydroxide it slowly decomposed on storage. Furthermore, after long-term storage at room temperature, there may be microbiological concerns if drugs are not prepared using aseptic techniques. The use of bacteriostatic water provides antimicrobial properties to the formulation during reconstitution. The preservatives used to reconstitute the vial are then diluted out into the larger combination product such that patients see a negligible amount of preservative. We have not used this combination product in the most vulnerable patient population, i.e. premature infant < 34 weeks gestational age.

Conclusion

We conclude that both chlorothiazide and furosemide are stable when stored at 25°C and protected from light either alone or in a 1:1 combination of 10 mg/mL of chlorothiazide and 1 mg/mL furosemide, each in dextrose 5% USP for up to 96 h.

Acknowledgments

Funding source: This work, in part, was supported by Nemours Biomedical Research and a NIH grant, P20GM103464.

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Figure 1. Chromatographic separation of chlorothiazide and furosemide

Panel A shows a typical chromatogram of chlorothiazide and furosemide detected by MS/MS. Original samples of a mixture of the two compounds were diluted 1:10,000 and 5 μ l of the sample applied to the LC column. The peaks of the two compounds are shown at 2.8 and 4.8 min, respectively. Panel B shows a typical chromatogram of chlorothiazide and a fragment eluting at 1.4 min after exposure of the parent compound to UV light. Panel C shows a typical chromatogram of furosemide and a fragment eluting at 4.5 min after

exposure of the parent compound to UV light. In each chromatogram the y-axis is ion counts for each separate compound normalized to the highest peak (set at 100).

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A

chlorothiazide - 10 Levels, 7 Levels Used, 10 Points, 7 Points Used, 0 QCs

B

furosemide - 10 Levels, 10 Levels Used, 30 Points, 30 Points Used, 0 QCs

Figure 2. Chlorothiazide and furosemide calibration curves

Serial two-fold dilutions of chlorothiazide were prepared to generate 10 levels of concentration and 5 μ l of each sample was applied to the LC column and quantified by MS/MS (A). The three higher data points for chlorothiazide were beyond the linear phase and consequently were not used in the calibration curve. Serial two-fold dilutions of furosemide were prepared to generate 10 levels and 5 μ l of each sample was applied to the LC column and quantified by MS/MS (B). Three separate dilutions were prepared to give 30 separate data points to show consistency of the instrument.

Figure 3. Time dependent UV degradation of chlorothiazide and furosemide

Chlorothiazide and furosemide were exposed to UV light and at timed intervals samples were analyzed by LC/MS/MS to quantify remaining parent compounds and new fragments. Area under the curve corresponding to chlorothiazide is shown after UV exposure for up to 960 min (squares, panel A). Loss of parent compound was accompanied by appearance of a fragment (triangles, panel A). Area under the curve corresponding to furosemide is shown after UV exposure for up to 180 min (squares, panel B). This was accompanied by

appearance of a fragment (triangles, panel B). The UV degradation product was only diluted by 100 to enable detection of this fragment at early time points.

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Table 1

Stability of furosemide and chlorothiazide

Formulation ^a	Percent remaining (mean +/- standard deviation)				
	0 h	24 h	48 h	72 h	96 h
Chlorothiazide alone	100.0+/-1.2	98.6+/-1.3	94.5+/-0.2 ^b	91.2+/-3.2	91.4+/-2.0 ^b
Furosemide alone	100.0+/-1.2	107.2+/-11.1	107.0+/-2.3 ^b	96.4+/-3.3	96.7+/-2.3
Chlorothiazide mixed	100.0+/-0.7	93.8+/-0.7 ^b	92.2+/-0.8 ^b	92.8+/-0.8 ^b	96.2+/-1.7 ^b
Furosemide mixed	100.0+/-8.8	101.3+/-2.1	110.2+/-1.3	95.7+/-1.5	96.7+/-3.1

^aFurosemide (1 mg/mL) and Chlorothiazide (10 mg/mL) were prepared separately (marked as alone) or as a 1:1 mixture (marked as mixed). Data were normalized to the starting drug concentrations as 100%

^bp < 0.5 compared to time t = 0 h..