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Analysis of diltiazem in Lipoderm® transdermal gel using reversed-phase high-performance liquid chromatography applied to homogenization and stability studies

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Abstract

A simple and novel method for the extraction and quantification of diltiazem hydrochloride was developed and applied to homogenization and stability studies. The method used solid phase extraction coupled with reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Validation showed inter-day recoveries ranging from 84.00 to 96.52% with relative standard deviations ranging from 12.01 to 15.94%. Intra-day recoveries ranged from 67.95 to 106.1% with relative standard deviations less than 5%. The method showed excellent linearity from 50 to 250 mg/ml in undiluted gel ($R^2 = 0.996$). The homogenization study showed good homogenization using both 50 and 100 depression techniques. Diltiazem was stable at a concentration of 246 mg/ml for 30 days and at a concentration of 99.6 mg/ml for 60 days no matter the storage conditions explored in this study.

Keywords: Diltiazem; Reverse-phase liquid chromatography; Transdermal gel; Drug stability; Compounded drug analysis

1. Introduction

Diltiazem is a calcium channel antagonist used in the treatment of angina, hypertension, and arrhythmias in humans as well as hypertrophic cardiomyopathy in cats [1,2]. The structure of diltiazem is presented in Fig. 1. Oral preparations undergo significant first pass metabolism that decrease the terminal half life. There is also evidence of significant pharmacokinetic differences between felines and humans. These include significant differences in bioavailability, clearance, and volume of distribution [3]. Current treatment regimens include multiple day dosing or the use of sustained release preparations in order to maintain therapeutic plasma concentrations [4]. Such intensive and chronic dosing regimens in veterinary medicine leads to lack of owner compliance as well as increased stress in the feline patient. Therefore, transdermal drug delivery has been developed as an alternative dosing method [5]. Currently, there are no published reports on the efficacy of a diltiazem transdermal gel in any species.

Transdermal gel formulations are compounded at individual pharmacies which can result in day to day variations within the concentration of the parent drug. There is no information published regarding the stability or homogeneity of any drug within Lipoderm® transdermal gels. Analytical methods have been developed to extract diltiazem and its metabolites from human, rabbit, canine, and feline plasma [6–11]. However, there are no published methods for the detection of diltiazem in a gel formulation. Given the differences in interspecies pharmacokinetics and the novel delivery system, a reliable and accurate method for determining dilti-

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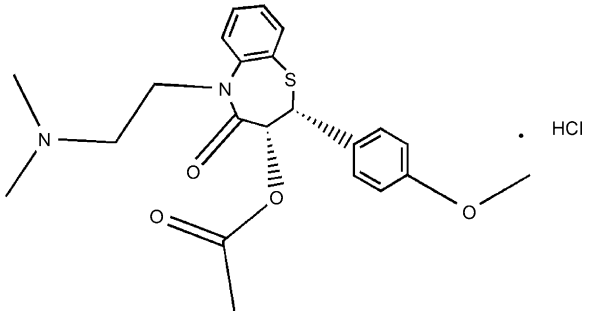

Fig. 1. Structure of diltiazem hydrochloride.

azem concentrations in Lipoderm® transdermal gel is mandatory for appropriate therapeutic dose regimens and pharmacokinetic studies. This paper describes a novel method for the extraction of diltiazem from Lipoderm® transdermal gel and shows its application to pharmaceutical formulation quality control through stability and homogenization studies.

2. Experimental

2.1. Chemicals

Diltiazem hydrochloride, USP (Lot: SN0303, purity of 98.5–101.5%) was purchased from Spectrum Chemical Company (Gardena, CA, USA). Ethoxydiglycol (Lot: C102336) and Lipoderm® (Lot: C102361) were purchased from Professional Compounding Centers of America (Sugarland, TX, USA). high-performance liquid chromatography (HPLC) grade methanol, acetonitrile, *O*-phosphoric acid 85%, and reagent grade ammonium hydroxide were purchased from Fisher (Fair Lawn, NJ, USA). HPLC grade sodium phosphate monobasic monohydrate was purchased from Baker (Phillipsburg, NJ, USA) and reagent grade hydrochloric acid 37% was purchased from Mallinckrodt (Paris, KY, USA). All water was purified with an ultra high purity water filtration system (Dracor Water Systems, Durham, NC, USA).

2.2. Apparatus

The HPLC system was a Waters 2695 Alliance Separation Module equipped with a Waters 2487 Dual λ Absorbance Detector (Milford, MA, USA). All data were collected on a Dell Optiplex GX60 computer (Round Rock, TX, USA) utilizing Empower Applications Version 1.0.0.1 software. An Atlantis C18 (particle size 5 μm, 4.6 mm i.d. × 150 mm length) column and guard column were used for the separations and purchased from Waters (Milford, MA, USA). The 24-port vacuum manifold was obtained from VWR Scientific Products (S. Plainfield, NJ, USA). Waters Oasis HLB 3 ml extraction cartridges (sorbent weight 60 mg) were obtained from Waters (Milford, MA, USA). All components were weighed on an Ohaus Top loading Metric Balance (Pine Brook, NJ, USA).

2.3. HPLC conditions

The mobile phase composition was acetonitrile–sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M) (33:67 v/v). The guard and analytical column temperature was set at $30.0 \pm 0.5\,^{\circ}$C. The detector wavelength was 237.0 nm. The flow rate and injection volume were 1.0 ml/min and 25 μl, respectively. Auto sampler temperature was set at $25.0 \pm 0.5\,^{\circ}$C. Diltiazem concentrations were determined by comparing the peak areas of diltiazem from the samples to an external standard calibration curve.

The sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M) was prepared daily by dissolving 2.76 g of sodium phosphate monobasic monohydrate crystals into 1 l of water. The pH was adjusted to 2.5 using *O*-phosphoric acid and was filtered through a 0.45 μm filtration system and degassed via inline degasser before use.

2.4. Standard solutions

The diltiazem stock solution (1000 μg/ml) was prepared by dissolving 0.0100 g of diltiazem into 10.0 ml of acetonitrile and stored at $-4\,^{\circ}$C for 14 days in opaque vials. Two working solutions of diltiazem were prepared daily by diluting the stock solution with sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M) with end concentrations of 5 and 1 μg/ml. Standards were prepared by spiking sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M) with the working solutions of diltiazem to have final diltiazem concentrations of 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005 μg/ml. Concentrations spanned the expected range of concentrations for the diluted sample. Since diltiazem is sensitive to light, all stock and standard solutions as well as samples were stored in opaque bottles to protect from light degradation.

Stock transdermal gel preparations were prepared by a registered compounding pharmacist using standard compounding pharmacy protocols provided only to registered compounding pharmacists. Concentrations were based on the target therapeutic concentration of 100 mg/ml based on an unpublished pilot study. Specifically, 5 g of diltiazem hydrochloride 50 mg/ml gel, 5 g of diltiazem hydrochloride 100 mg/ml gel and 5 g of diltiazem hydrochloride 250 mg/ml gel were formulated. After being weighed out (1250, 500, and 250 mg for end concentrations of 250, 100, and 50 mg/ml, respectively), the diltiazem hydrochloride was then transferred to the barrel of a sterile 6 cc polypropylene luer lock syringe attached to the luer lock syringe adapter. The actual amount of diltiazem was calculated by reweighing the weigh paper and subtracting residual amounts from the original amount weighed. The plunger was carefully replaced, pushing all powder towards the hub of the syringe. Another sterile 6 cc luer lock syringe containing 1.5 ml of ethoxydiglycol reagent was locked on to the other port of the luer lock syringe adapter.

Gentle pressure was applied to introduce the ethoxydiglycol reagent into the syringe containing the diltiazem hydrochloride. Diltiazem hydrochloride and ethoxydiglycol were then swirled until dissolution was visibly achieved. The empty syringe (formerly containing ethoxydiglycol) was removed and replaced by a 6 cc syringe containing Lipoderm® gel. Lipoderm® was introduced into the dissolved diltiazem hydrochloride in a quantity sufficient to make a total volume of 5 cc. The remainder of the Lipoderm® (if any) was ejected from the syringe and the syringe reattached to the syringe adapter. Using firm pressure, the contents were transferred from syringe to syringe via the adapter until a total of 50 depressions had been made on the syringes. Aliquots from each batch were then transferred to sterile 1 cc tuberculin syringes and capped with sterile tip caps. Syringes were stored protected from light in brown opaque zip lock plastic bags.

2.5. Sample preparation

Approximately 0.02 g of diltiazem transdermal gel (of known and unknown concentration) was weighed out and diluted into 500 ml of water using a 500 ml volumetric flask. Waters Oasis HLB 3 ml cartridges were placed into the vacuum manifold, conditioned with 1 ml methanol that was allowed to flow by gravity, and followed by 1 ml of sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M). Two hundred-fifty microliters of diluted sample was added and allowed to flow through by gravity. The cartridge was washed with 1 ml of a methanol–sodium phosphate monobasic monohydrate buffer (pH 2.5, 0.02 M) (30:70 v/v). Once the wash had flowed through the disk, the cartridges were dried under high vacuum (15 in Hg) for 30 s. Elution was with 1 ml methanol and again dried under high vacuum for 30 s. Elution solvents were evaporated to dryness under 15 psi reagent grade nitrogen gas at 60 °C. The residue was dissolved in 1 ml sodium phosphate monobasic monohydrate buffer and gently vortex-mixed for 30 s.

2.6. Method validation

Recovery samples and blanks were run with every batch of samples as part of quality control. Intra-day precision and accuracy was determined by extraction of five replicates from three different transdermal gel concentrations. Inter-day precision and accuracy was determined by extraction of one replicate of three different transdermal gel concentrations on five different days.

2.7. Homogenization study

Transdermal gel homogenization was quantified by the extraction of diltiazem from batches made with a technique using either 50 or 100 depression of the syringes through the syringe adapter as described above. Two different transdermal gel concentrations for each technique listed were aliquoted into five samples. Those samples were further subaliquoted into five replicates. Each replicate was extracted and diltiazem was quantified.

2.8. Stability study

Batches of diltiazem Lipoderm® transdermal gel were prepared at target concentrations of 250 and 100 mg/ml. Each batch was divided into three different storage condition environments: room temperature (25 °C), refrigeration (4 °C), and freezer (−25 °C) and stored in brown opaque zip lock plastic bags. For each day of analysis, a sample from each condition was brought to room temperature before being analyzed. Analysis was performed on days 1, 3, 7, 14, 30, 45, and 60.

2.9. Statistical analysis

Both studies were statistically analyzed by analysis of variance using SAS (Version 8.01, Cary, North Carolina, USA) and assumed a randomized complete block design with subsampling. Subsamples were averaged to create the sample statistic. A p-value less than 0.05 was considered significant.

3. Results and discussion

3.1. Chromatography

Fig. 2 shows a representative chromatogram for a sample containing 50 mg/ml of diltiazem hydrochloride extracted from the Lipoderm® gel. The corresponding blank samples did not contain any peaks eluting at or near the same retention time as diltiazem hydrochloride. Diltiazem hydrochloride typically eluted at 7.9 min under the conditions described. Any variation of elution time was monitored with external standards in every daily run. Standards were run interspersed throughout the sample set.

3.2. Linearity

The calibration line ($y = 66732x$) was obtained by injecting the standard solutions described above. The slope of the external standard plot, covering a diltiazem concentration of 0.005–5.0 μg/ml was 66732 ± 6442.506 (slope ± S.D., $n = 10$) with the y-intercept assigned a value of zero. The external standard curve was linear in the range of concentrations that were detected in the diluted samples with a correlation coefficient of 0.9994 ± 0.0007 ($R^2 \pm$ S.D., $n = 10$). External standard curves were run with every batch on every day of the validation.

Linearity was seen in the presence of the transdermal gel through the concentration range of 50–250 mg/ml. The slope was 572 ± 117.5 (slope ± S.D., $n = 4$) and had a correlation coefficient of 0.996 ± 0.03 ($R^2 \pm$ S.D., $n = 4$) with the y-intercept assigned a value of zero.

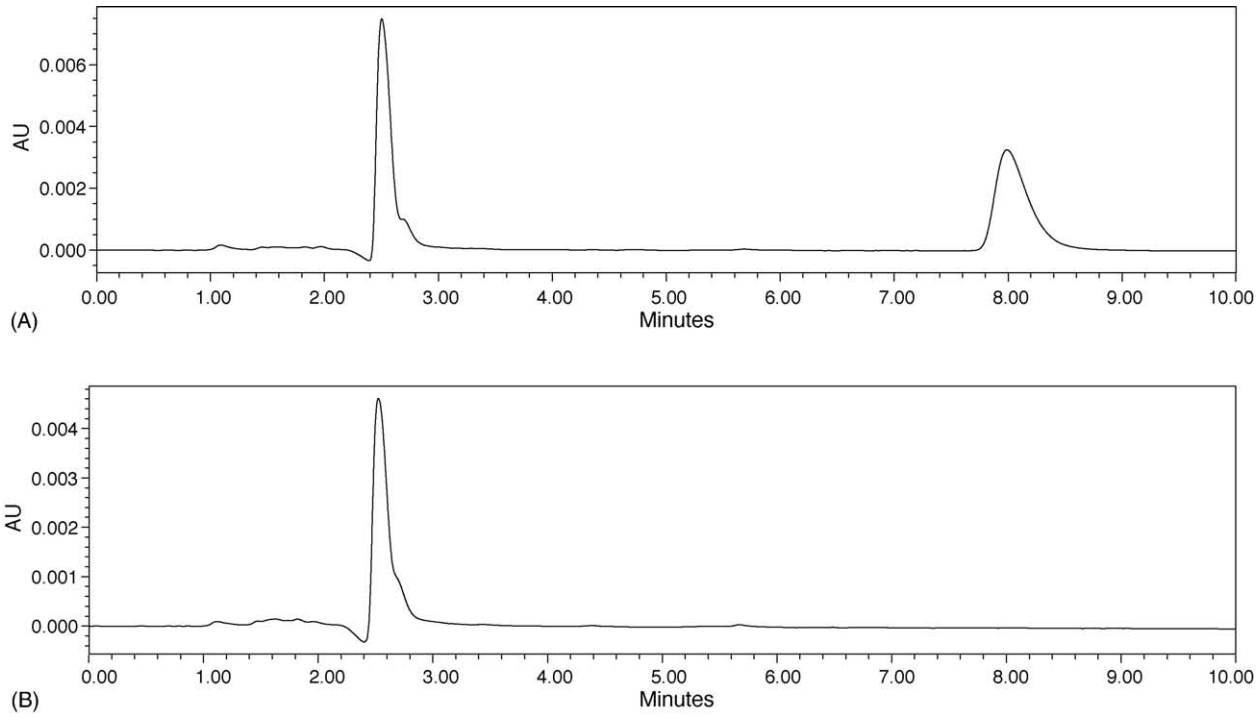

Fig. 2. Representative chromatogram of Lipoderm® transdermal gel spiked with diltiazem at a concentration of 50 mg/ml (A) and not spiked with diltiazem (B).

The limit of detection (LOD) was defined as being a peak-signal-to-noise ratio of at least 3:1. The LOD was 0.005 μg/ml diltiazem hydrochloride standard which corresponded to 5 mg/ml of undiluted transdermal gel. The lowest concentration of gel used therapeutically (100 mg/ml) falls well above the LOD. Fifty milligrams per mililiter was the lowest concentration evaluated in this study. The lower limits of this system were not investigated in this study since therapeutic concentrations center around 100 mg/ml.

3.3. Precision and accuracy

The results of the inter-day and intra-day precision and accuracy are listed in Table 1 for the diltiazem transdermal gel. Mean recoveries for the inter-day diltiazem transdermal gel ranged from 84.00 to 96.52% with a relative standard deviation (R.S.D.) ranging from 12.01 to 15.94%. It should be noted that only 4 days were run for validation of the 250 mg/ml. Since all gels were made fresh daily for the validation study, it was impossible to accurately prepare the

Table 1
Inter and intra-day precision and accuracy of diltiazem in Lipoderm® transdermal gel

Target concentration (mg/ml)	Day	Spiked concentration (mg/ml)	Calculated intra-day concentration (mean ± S.D., $n = 5$) (mg/ml)	Intra-day R.S.D. (%)	Intra-day percent recovery (mean ± S.D., $n = 5$)	Average inter-day recovery (mean ± S.D., $n = 5$) (%)	Inter-day R.S.D. (%)
250	1	238	237.9 ± 3.16	1.33	99.96 ± 1.0	84.00 ± 13.0	15.94
	2	248	167.3 ± 4.3	2.58	67.45 ± 2.0		
	3	221	191.0 ± 1.8	0.95	86.44 ± 1.0		
	4	219	179.9 ± 4.1	2.28	82.13 ± 2.0		
100	1	91	70.8 ± 2.0	2.88	77.81 ± 2.0	90.65 ± 13.0	14.20
	2	93	72.5 ± 1.8	2.52	77.94 ± 2.0		
	3	82	82.4 ± 1.9	2.30	100.45 ± 2.0		
	4	105	95.4 ± 2.6	2.69	90.9 ± 2.0		
	5	99	106.1 ± 2.7	2.61	106.16 ± 2.0		
50	1	42	40.8 ± 1.3	3.21	97.13 ± 3.0	96.52 ± 12.0	12.01
	2	51.5	50.1 ± 1.3	2.52	97.36 ± 2.0		
	3	48	49.2 ± 1.7	3.54	102.46 ± 4.0		
	4	50	39.1 ± 1.0	2.64	78.15 ± 2.0		
	5	52	50.0 ± 2.2	4.38	99.67 ± 4.0		

exact same concentrations each day. Thus, each spiked concentration is listed for each day along with the target concentration. For example in Table 1, individual day concentrations for the target concentration of 250 mg/ml ranged from 219 to 248 mg/ml. Intra-day recoveries ranged from 67.45 to 99.96%, 77.81 to 106.16%, and 78.15 to 102.46% for the target concentrations of 250, 100, and 50 mg/ml, respectively. The mean intra-day R.S.D. range for all target concentrations were less than 5%.

The high inter-day R.S.D. can be explained by the inherent variability in the gel preparation method. The standard operating procedure used for this study is the same as the one used in compounding pharmacies and was chosen to make the studies as realistic as possible. This included the use of syringes to measure volume. Measuring in this way decreased the ability to accurately calculate the true concentration of the gel matrix. This inaccuracy occurred with the preparation of each new batch. Since the true concentration is incorporated into each calculation of recovery and the recoveries are compared for each inter-day R.S.D., inaccuracies due to mixing and measurement of volumes using syringes are amplified. The inaccuracy in the gel preparation thus magnified itself for each new batch of gel made and ultimately resulted in an increase in inter-day R.S.D. The extremely good intra-day variation (less than 4.38%) shows that method itself is valid and robust.

3.4. Homogenization study

Table 2 shows the results of the homogenization study. There is no evidence of any statistical difference between sample aliquot concentrations within each technique (p-value 0.9998). The relative standard deviations were 1.79 and 2.58% for target concentration of 250 mg/ml for the 50 depression and 100 depression techniques, respectively.

The relative standard deviations of the target concentration of 100 mg/ml were 11.07 and 0.96% for the 50 and 100 depression techniques, respectively. There was a significant difference between the relative standard deviations (p-value 0.002) for the 50 depression and 100 depression techniques at the 100 mg/ml target concentration. However, since there was no statistical difference between the sample aliquot concentrations within each batch, it was concluded that there was adequate homogenization using the 50 depression technique.

3.5. Stability study

The actual concentrations of the bulk transdermal gel were 246 and 99.6 mg/ml for the target concentrations of 250 and 100 mg/ml, respectively. Fig. 3 shows the concentration of diltiazem hydrochloride in the Lipoderm® transdermal gel at various conditions. There was no evidence of any effect due to temperature of storage (p-value 0.569). Relative standard deviations of the assay allow for a 15% variation before any significance could be noted. For the 246 mg/ml concentration there is a significant change from the original concentration at day 30 (p-value 0.001). This is also seen for days 45 (p-value 0.001) and 60 (p-value 0.001). However, taking the assay into account the only true significance is on day 60 when the concentration was lower than 209.1 mg/ml. There is no evidence of significant change in concentration for the 99.6 mg/ml concentrations at day 60 (p-value 0.0884). Over the course of the study, several physical characteristics of the gel subjectively changed over time. Starting at day 14, the gels stored at room temperature had a tendency to separate into a lipid and an organic layer. There was no subjective difference between the amount of separation between days 14, 30, 45, and 60. Gels stored in the refrigerator developed by day 60 a crystalline appearance and did not dissolve as easily as in previous days. Gels stored in the freezer underwent a separation similar to that seen in the room temperature group upon thawing. There was no difference between the concentrations of the transdermal gels with respect to the physical characteristic changes noted. These physical changes could effect the disposition of diltiazem within the matrix itself. For example, diltiazem may partition out of the organic phase and into the polar phase after prolonged storage. This could significantly alter the dose applied to each patient, the dose absorbed to

Table 2
Transdermal homogenization study variability of aliquots taken throughout a transdermal gel using a 50 or 100 depression homogenization technique

Actual concentration (mg/ml)		Aliquot concentration (mean ± S.D., $n = 5$) (mg/ml)		Aliquot R.S.D. (%)		Batch concentration (mean ± S.D., $n = 5$) (mg/ml)		Batch R.S.D. (%)	
50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]
254	252.8	241.8 ± 8.3	224.1 ± 8.7	3.44	3.88	243.0 ± 4.3	230.0 ± 5.9	1.79	2.58
		236.9 ± 2.1	224.0 ± 3.5	0.89	1.56				
		246.2 ± 2.1	230.8 ± 4.1	0.85	1.79				
		248.0 ± 3.8	237.3 ± 0.5	1.54	0.22				
		241.9 ± 2.8	233.9 ± 2.9	1.18	1.24				
103.5	109	99.5 ± 2.6	98.7 ± 4.1	2.61	4.18	112.9 ± 12.5	99.7 ± 1.0	11.07	0.96
		115.6 ± 1.8	99.1 ± 2.2	1.58	2.18				
		104.2 ± 2.3	101.2 ± 2.8	2.2	2.79				
		113.0 ± 2.7	99.5 ± 3.5	2.43	3.48				
		131.9 ± 2.2	99.8 ± 2.7	1.68	2.76				

[a] Indicate number of depressions used in the technique.

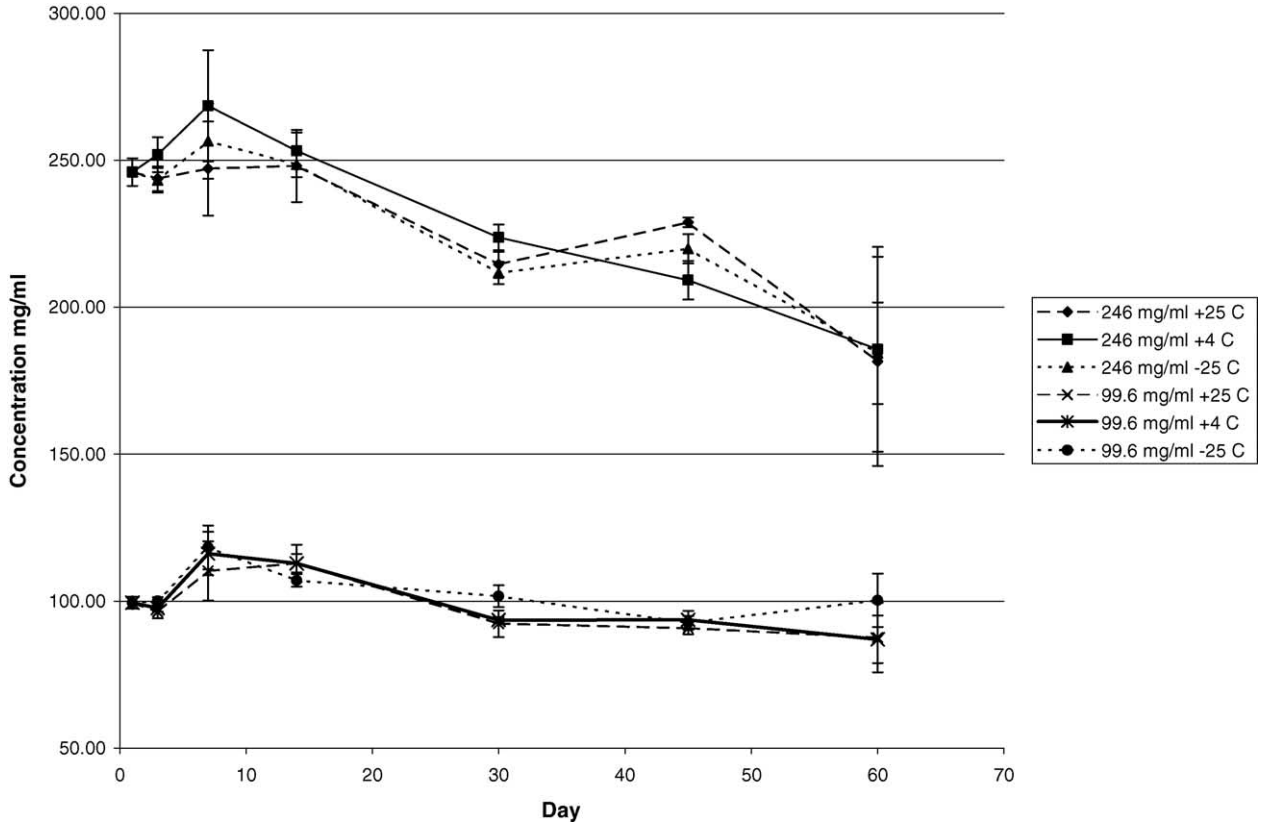

Fig. 3. Stability of diltiazem in Lipoderm® transdermal gel at 246 and 99.6 mg/ml under different storage conditions.

each patient, as well as the actual amount assayed. To correct for this change, each sample that underwent a physical change was re-emulsified before subsequent analysis.

4. Conclusion

This paper described a method for the quantification of diltiazem hydrochloride in a Lipoderm® transdermal gel. The technique showed excellent reproducibility within each day. The variability seen between days is most likely associated with the inability to accurately measure the true concentration of each batch of transdermal gel. This inaccuracy could possibly account for therapeutic failures or toxicity related to this low therapeutic index drug and should be further investigated. This method showed adequate sensitivity, specificity, accuracy, and precision and was applied to pharmacuetic studies investigating the homogenization of compounded formulations and the stability of diltiazem with the Lipoderm® formulation at various storage conditions. There was no difference between the 50 depression and 100 depression homogenization techniques. Diltiazem was stable at a concentration of 99.6 mg/ml for 60 days and at 246 mg/ml for 30 days no matter the storage conditions described in this study. This technique can be further applied by doctors, veterinarians, and pharmacists to investigate therapeutic failures by accurately determining dose, stability, and homogenization of individually compounded products. This will ultimately provide for better patient care.

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