

Long-Term Stability Study of L-Adrenaline Injections: Kinetics of Sulfonation and Racemization Pathways of Drug Degradation

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ABSTRACT: Injectable formulations of L-adrenaline are commonly used in emergency medicine. Despite numerous studies, the comparative contribution and kinetics of the L-adrenaline inactivation pathways during storage have not been conclusively evaluated. We examined the kinetics of L-adrenaline degradation in a prospective study and determined the extent of drug inactivation by different pathways during and beyond the stipulated product shelf-life in 42 batches of adrenaline ampules stored under controlled conditions. The content of L-adrenaline and degradation products was determined with a chiral high-performance liquid chromatography (HPLC) assay, and the degradation products were identified by mass spectrometric detection as D-adrenaline and L- and D-adrenaline sulfonate. The kinetics of the content change with storage was analyzed simultaneously for L-adrenaline and the degradation products using kinetic modeling. The lower acceptable level of adrenaline content in the formulation stated by US Pharmacopoeia (90% as a sum of L- and D-isomers) was attained after 2.0 years of storage, at which time the content of the therapeutically active L-isomer amounted to as low as 85%. The modeling revealed significant differences in the degradation kinetics in the formulations produced before and after 1997, whose cause remained unidentified in this study. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 93:969–980, 2004

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INTRODUCTION

Adrenaline is a catecholamine compound that is commonly applied by intravenous injection in emergency medicine due to its effects on the cardiovascular system. In accordance with the United States Pharmacopeia (USP), the injections contain an aqueous solution of L-adrenaline (as a bitartrate salt) that is several-fold more potent than its optical isomer.¹

Adrenaline in solution is subject to degradation; therefore, numerous studies addressed the effect of formulation variables on the drug inactivation kinetics, and attempts have been made to improve the formulation stability.^{2–9} The results of these studies indicate that L-adrenaline in solution is inactivated by racemization and oxidation or to reaction with auxiliary formulation components (e.g., sodium metabisulfite) employed as an antioxidant (Fig. 1). The products of these reactions (including D-adrenaline and adrenaline sulfonate) possess little or no pharmacological activity compared with the parent compound.^{6,10} The reversibility of reactions involved in L-adrenaline degradation should be taken into account for

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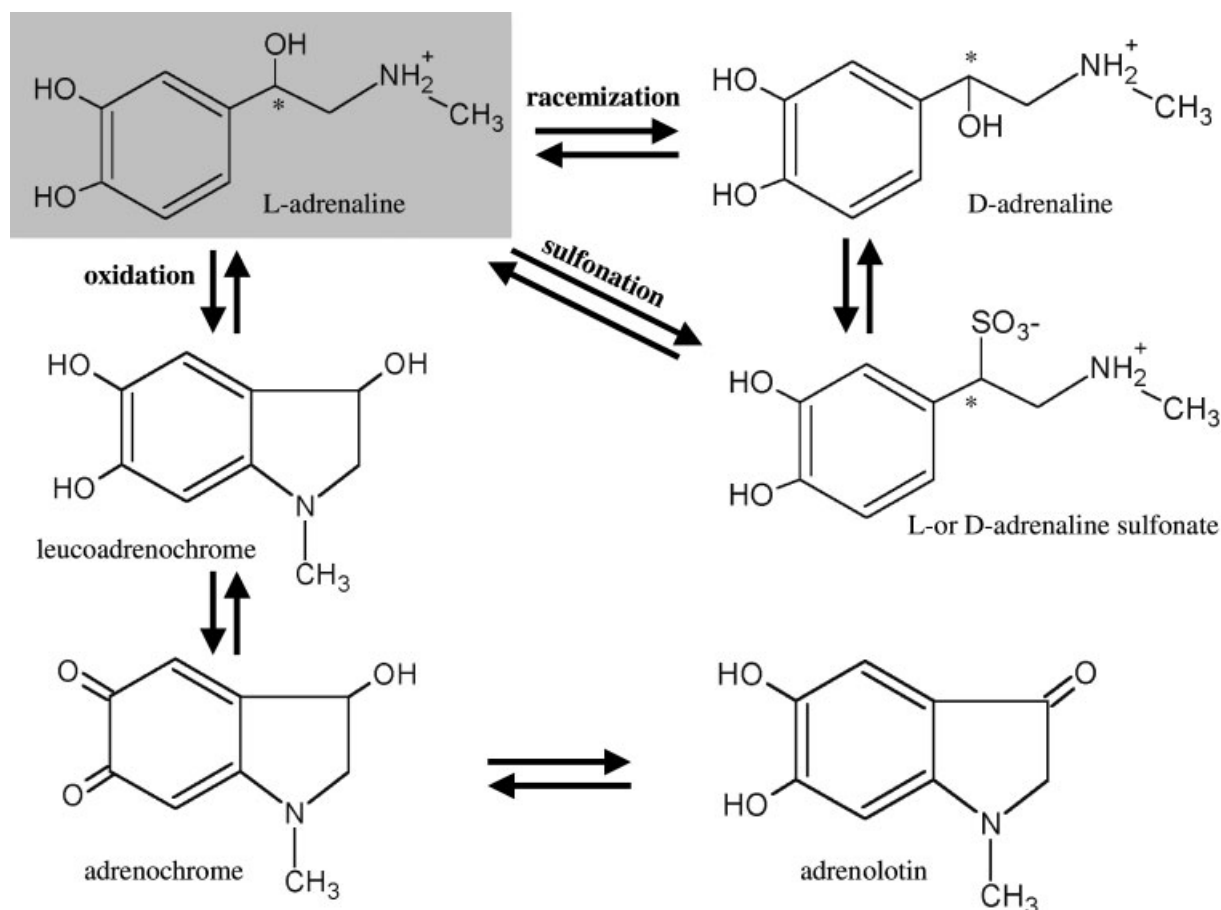


Figure 1. Degradation reactions of L-adrenaline.

long-term stability studies. For instance, D-adrenaline, which is formed from L-adrenaline by the racemization process, degrades by racemization (to produce L-adrenaline isomer), bisulfite addition, and oxidation reactions.

Despite the obvious significance of L-adrenaline optical isomerization in the overall drug inactivation process, the USP assay for adrenaline injections does not provide quantification of the optical isomers in the formulation.¹¹ Moreover, most of the adrenaline injections stability data available in the literature were obtained using comparatively nonspecific colorimetric, fluorimetric, or bioassay techniques that do not allow for accurate determination of the pharmacologically active drug isomer. The conclusive evaluation of the comparative contribution and kinetics of the drug degradation pathways requires well-characterized analytical methods that provide enantiomeric separation and reliable quantification. Examples of such methods based on chiral liquid chromatography have recently been published.^{8,12,13}

The objective of this research was to examine in a prospective study the kinetics of the different pathways of L-adrenaline degradation in commercially available preparations acquired by Israel Defense Forces (IDF), and determine the extent of the drug inactivation therein during and beyond the stipulated product shelf-life. The analysis was conducted using a chiral high-performance liquid chromatographic (HPLC) method with ultraviolet-visible (UV-vis) detection, and the degradation products were identified by mass spectrometry (MS-MS).

EXPERIMENTAL

Samples of Adrenaline Injections

Adrenaline injections (1 mg of adrenaline base per milliliter) were produced by manufacturer A (Teva Ltd, Israel; 28 batches, manufactured 1985–1998) and manufacturer B (Biogal Ltd., Hungary; 14 batches, manufactured 1998–2002)

using a similar preparation process, except for a higher minimal initial content of adrenaline starting from 1997. The minimal initial drug amount in the formulation was changed from 90 to 103% of the declared content, corresponding to 0.90 and 1.03 mg/mL adrenaline base, respectively, because of concerns regarding the limited stability of the preparations. The additional components of the formulation were 1 mg/mL of sodium metabisulfite, 8 mg/mL of sodium chloride, and water for injection.

All the studied samples were received shortly (1–2 months) after the production of the corresponding batch. Following receipt, all batches were stored under controlled conditions recommended by the manufacturers. On the date of analysis, the storage period was 5.8–17.3 years for the batches produced by manufacturer A and 0.2–5.4 years for the batches produced by manufacturer B.

Chemicals and Reagents

L-Adrenaline bitartrate and potassium chloride were from Sigma (St. Louis, MO). Glacial acetic acid and HPLC-grade acetonitrile was from J.T. Baker (Deventer, Holland). Water was purified with a tandem RiOs (reverse osmosis)/Milli-Q Gradient A-10 system (Millipore, Molsheim, France). All other chemicals used in this study were of analytical or HPLC grade.

Chiral HPLC Assay

The chiral assay was a modification of the method provided by Showa Denko K.K., Tokyo, Japan. The mobile phase used for the chiral HPLC assay was 0.2 M potassium chloride in water: 0.2 M potassium chloride and 0.4% (v/v) acetic acid in water: acetonitrile (96:1:3, v/v). The mobile phase was filtered under vacuum through 0.45- μ m nylon filters (Millipore, Bedford, MA).

The chromatographic system consisted of an HPLC model HP 1100 (Hewlett Packard, Palo Alto, CA) interfaced to an HP ChemStation, and a chiral Shodex ODS 5 μ m column, 150 $\times$ 4.6 mm (Showa Denko K.K., Tokyo, Japan). The volume of injection was 50 μ L, the column temperature was 10°C, the flow rate was 0.7 mL/min, and the run time was 20 min. The mobile phase was deaerated by on-line degasser, and detection was performed by UV-vis photodiode-array detector at 280 nm. A stock solution of L-adrenaline bitartrate, obtained by dissolving 21.84 mg of the compound in 10.0 mL of water, was further diluted in the mobile

phase to prepare calibration standards in the 10–120% nominal drug concentration range.

Samples for analysis were prepared by diluting 1.0 mL of adrenaline injection solution with the stipulated content 1.8 mg/mL of adrenaline bitartrate (equivalent to 1 mg/mL of adrenaline base) with mobile phase to 20 mL.

HPLC-MS-MS Assay

A non-chiral HPLC method applying volatile mobile phase was developed for identification of the degradation products in adrenaline injections. The mobile phase was ammonium acetate buffer (5 mM, pH 7.0)/acetonitrile/formic acid (89.03:10:0.07, v/v/v) pumped at a flow rate 1.0 mL/min. The HPLC-MS-MS system consisted of Thermo Separation Products HPLC system (Egelsbach, Germany), Millipore Solvent delivery system (Millipore Corp., Milford, MA), MS-MS detector (Micromass Ltd., UK), UV detector (HPLC detector 432, Kontron Instruments, Switzerland), and 5 μ m Luna Phenyl-Hexyl HPLC column (250 $\times$ 4.6 mm; Phenomenex, Inc., Torrance, CA).

Samples of aged adrenaline batches were prepared for analysis by diluting 1.0 mL of the adrenaline injection solution with mobile phase to 20 mL, and 20 μ L of the obtained solution was injected into HPLC-MS-MS system.

After the HPLC column, the eluent was split to obtain the flow rate of $\sim$ 0.25 mL/min and was modified by continuous injection of 0.65% formic acid in acetonitrile at a rate of 25 μ L/min. The UV detector was set to 280 nm. HPLC-MS-MS was performed using electrospray ionization (EI) in the positive ion mode, with nitrogen as the nebulizer and drying gas. The mass range was 50–500 amu, and the dwell time was 0.1 s. The molecular ion masses of the degradation product and adrenaline were identified, and daughter scans of $m/z = 248$ (degradation product) and $m/z = 184$ (adrenaline) were measured with the collision energies of 11 and 18 eV.

pH Measurements

The pH values of the studied samples were determined using Mettler Titroprocessor (model 796, Herisau, Switzerland) with a combined glass electrode.

Determination of Aluminum Concentrations

Aluminum concentrations in the samples of adrenaline batches were determined by furnace

atomic absorption analysis using Analyst 300 apparatus (Perkin Elmer, Norwalk, CT) following dilution 1:100 with 0.2% nitric acid. The analysis was performed versus standard solutions at 309.3 nm applying the recommended pretreatment and atomization conditions.¹⁴

Analysis of Degradation Kinetics of Adrenaline

Exponential Regression

Data analysis by exponential regression and calculation of 95% confidence intervals was performed according to the method described by Zar.¹⁵

Modeling

The goal of modeling was identification of the most parsimonious model that could appropriately describe the experimental outcomes. The structure of the proposed models was based on available data concerning adrenaline degradation pathways in injections (see Discussion),⁶ and models with different kinetic order of underlying chemical reactions have been studied.

Analysis of the content versus storage period data was performed with ADAPT II Pharmacokinetic/Pharmacodynamic Systems Analysis Software (Biomedical Simulations Resource, Los Angeles, CA) applying the generalized least-

square function.¹⁶ The variance was described by the linear model: $\text{Var } R = (a + b \cdot R)^2$, where a and b are the variance parameters. Goodness of fit for the individual model was assessed from the graphs of the predicted and observed data, the coefficients of variation of the resulting parameters, and the values of the Akaike and Schwartz criteria.¹⁷ The modeling was performed separately for the batches produced before and after 1997 (see Results), and for each period, the four sets of data (content of L-adrenaline, D-adrenaline, L-adrenaline sulfonate, and D-adrenaline sulfonate in the injections) were fitted simultaneously.

RESULTS

The Chiral HPLC Method and Its Validation

The chiral assay applied in this study enabled quantification of the optical isomers of adrenaline and its degradant in injectable preparations. The chromatograms of aged formulations obtained by this method (see Fig. 2) show four peaks corresponding to L-adrenaline ($t = 15.0$ min), D-adrenaline ($t = 16.3$ min), and two degradation products ($t = 3.4$ and 3.6 min, respectively).

The number of theoretical plates obtained for the L-adrenaline, D-adrenaline, and L- and D-degradation products were 6966, 4695, 3510, and

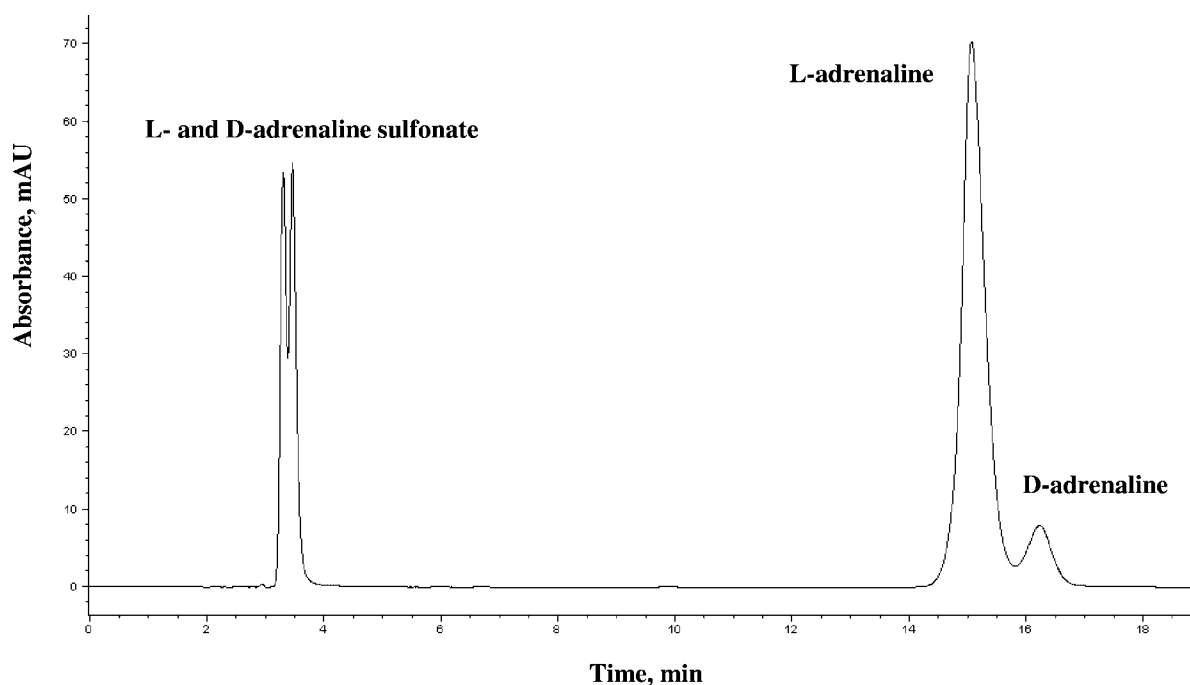


Figure 2. Chromatogram of aged adrenaline injection.

2988, respectively. The corresponding peak symmetry factors were 0.743, 1.01, 0.911, and 0.687, respectively.

The assay exhibited high linearity, precision, and accuracy. Linearity of the chiral assay was determined in the concentration range 9.1–109.2 μg L-adrenaline bitartrate per milliliter, corresponding to 10–120% of the nominal concentration of the diluted samples. The detector response expressed as the peak area was linear with concentration. The correlation coefficient (r^2), intercept, and slope of the calibration curve were 0.9999, $-4.12 \text{ mAU}\cdot\text{s}$, and $32.5 \text{ mAU}\cdot\text{s}\cdot(\mu\text{g}/\text{mL})^{-1}$, respectively. The detection and quantitation limits were 0.12; and 0.40% of the nominal drug concentration, respectively.

Accuracy was assessed by testing solutions of L-adrenaline bitartrate prepared in duplicate at 100% of the nominal concentration. The solutions were processed according to the sample preparation method prior to their analysis. The recovery was $100.6 \pm 0.1\%$.

Selectivity was assessed by chromatography of standard, aged, and stressed samples of adrenaline injections. Aged samples revealed only four peaks corresponding to the optical isomers of adrenaline and its degradation product. These peaks were resolved to the baseline from additional compounds appearing in samples subjected to forced degradation, and the selectivity of the separation was confirmed by assessment of corresponding peak purity factors. The shapes of the UV-vis absorption spectra for the isomers of adrenaline and degradation product were similar, indicating possible similarity in the chemical structure.

Identification of the Degradation Product

The degradation products were identified by HPLC-MS-MS, applying a nonchiral separation method. The degradation product eluted as a single peak ($t = 2.9 \text{ min}$), and its mass spectrum (Fig. 3, panel A) revealed molecular ions $m/z = 248, 265$, and 270 , which are attributed to adrenaline sulfonate ($\text{M} + \text{H}^+$, $\text{M} + \text{NH}_4^+$, and $\text{M} + \text{Na}^+$, respectively) that exist in the formulations as two optical isomers. Adrenaline eluted as a single peak ($t = 4.8 \text{ min}$) with its mass spectrum showing $m/z = 184$ and 166 , which are attributed to the molecular ions $\text{M}' + \text{H}^+$, and $\text{M}' + \text{H}^+ - \text{H}_2\text{O}$, respectively (data not shown). The daughter spectra of the degradation product at m/z 248 were obtained with different collision energies

(see Fig. 3, panels B and C), and showed major peak at m/z of 166, which was attributed to $\text{M} + \text{H}^+ - \text{H}_2\text{SO}_4$.

Degradation Kinetics of Adrenaline

The chiral method was applied for estimation of the long-term stability of adrenaline injections. The amounts of the isomers of adrenaline and the degradation product were determined using L-adrenaline as a reference assuming that the relative absorption of L- and D-adrenaline sulfonate at 280 nm is identical to that of L-adrenaline. This assumption was substantiated by nearly identical light absorption spectra of new and aged batches with close initial drug contents (data not shown) and by mass balance calculations: the total observed substance in the formulations (sum of L- and D-adrenaline and L- and D-adrenaline sulfonate expressed as their stoichiometric equivalents to the drug) remained constant with the storage period and was ~ 100 and 105% in batches produced before and after 1997, respectively (see Fig. 4). The results of the study show that the content of L-adrenaline in the formulation rapidly decreased with storage (Fig. 4). The content of D-adrenaline increased during the first 8–10 years of storage and decreased afterwards. The content of L-adrenaline sulfonate was similar to that of D-adrenaline sulfonate in all batches and increased with storage.

The measured pH values of the studied samples are presented in Figure 5. The pH values of all the studied samples were in the 3.25–3.70 range and remained unchanged with storage.

Aluminum concentrations in the tested batches increased in a linear fashion from 0 to $\sim 35 \mu\text{g}/\text{L}$ after 17-years of storage period (see Fig. 6). The aluminum level increased at a similar rate in the batches obtained from Manufacturers A and B, without significant differences in the slopes and intercepts of the respective linear functions.

Exponential Regression of Adrenaline Content versus Time Data

The change in the contents of L-adrenaline and L- and D-adrenaline together in the injections was appropriately described by a simple exponential regression (see Fig. 7 and Table 1). The difference in the initial drug content for the batches produced before and after 1997 was reflected by differences in the intercept of the respective

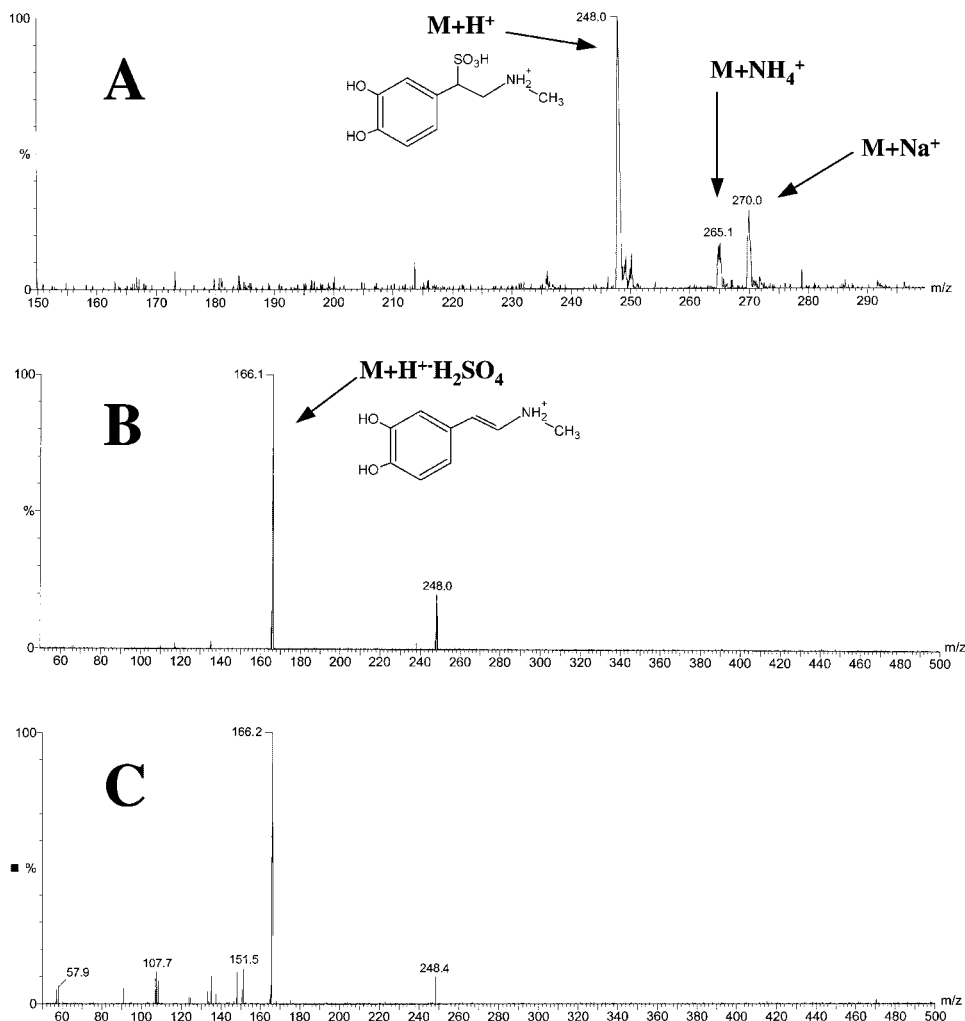


Figure 3. ESI mass spectra of adrenaline degradation product (panel A) and its daughter scans at $m/z=248$, with collision energies of 11 and 18 eV (panels B and C, respectively).

exponential decay equations (e.g., the intercept of 109.4 ± 4.3 versus 99.5 ± 5.2 for L+D-adrenaline; see Figure 7, panel B and Table 1). For each time period, narrow confidence intervals of the drug degradation temporal profiles were obtained, indicating low interbatch variability. Exponential regression resulted in lower coefficients of variation for the initial contents compared to those of the degradation rate constants (3.9–5.9 versus 19–22%, respectively; see Table 1).

Modeling

The structure of the model that was selected to describe the degradation of adrenaline is presented in Figure 8 (panel A), and the underlying

differential equations are as follows:

$$dX_1/dt = k_1X_2 + k_5X_4 + k_6X_3 - (k_1 + k_2 + k_3)X_1 \quad (1)$$

$$dX_2/dt = k_1X_1 + k_5X_3 + k_6X_4 - (k_1 + k_2 + k_3)X_2 \quad (2)$$

$$dX_3/dt = k_2X_1 + k_3X_2 + k_4X_4 - (k_4 + k_5 + k_6)X_3 \quad (3)$$

$$dX_4/dt = k_2X_1 + k_3X_1 + k_4X_3 - (k_4 + k_5 + k_6)X_4 \quad (4)$$

where X_1 , X_2 , X_3 , and X_4 are content of L-adrenaline, D-adrenaline, L-adrenaline sulfonate, and D-adrenaline sulfonate in the injections,

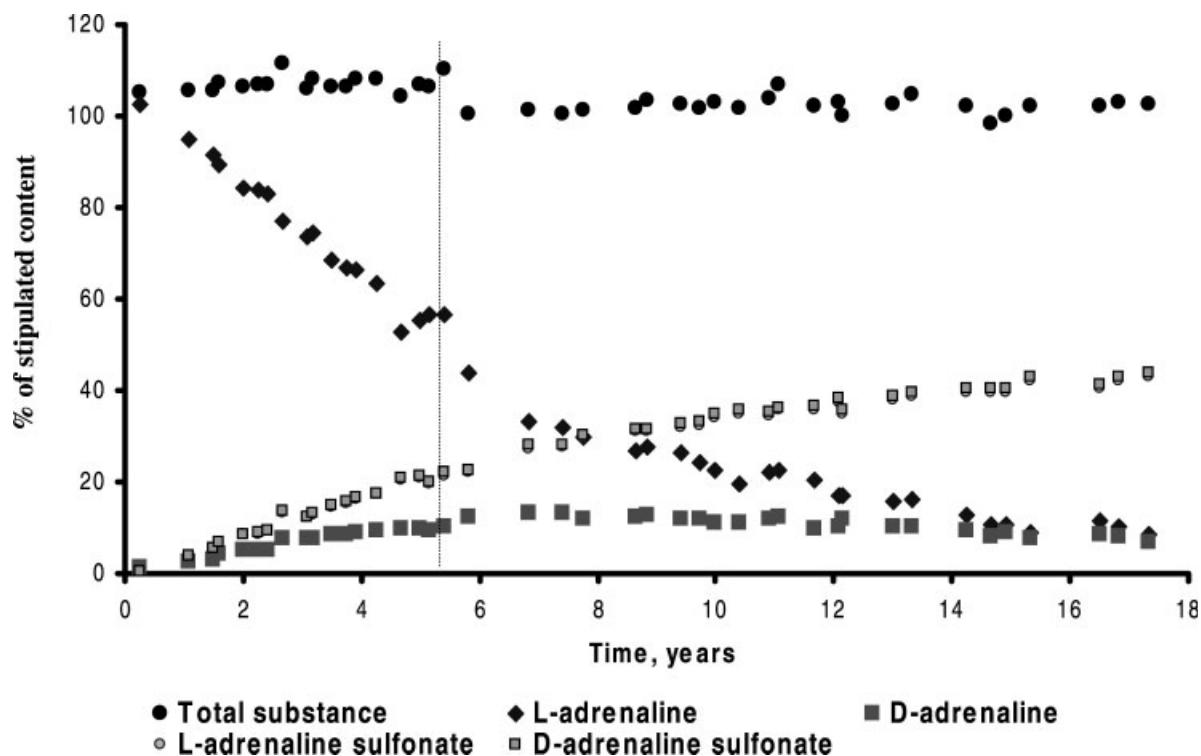


Figure 4. The content of L- and D-adrenaline and L- and D-adrenaline sulfonate (expressed as their stoichiometric equivalents to adrenaline) in injections as a function of storage period. Total substance was calculated as the sum of L- and D-adrenaline and L- and D-adrenaline sulfonate contents in each batch. The dashed line indicates the time point of the change in the minimal initial drug amount (see Experimental).

respectively (% of the stipulated content). The rate constants are k_1 for adrenaline racemization, k_2 for adrenaline sulfonation without change of conformation, k_3 for adrenaline sulfonation with change of conformation, k_4 for adrenaline sulfonate racemization, k_5 for conversion of adrenaline sulfonate to adrenaline with change of con-

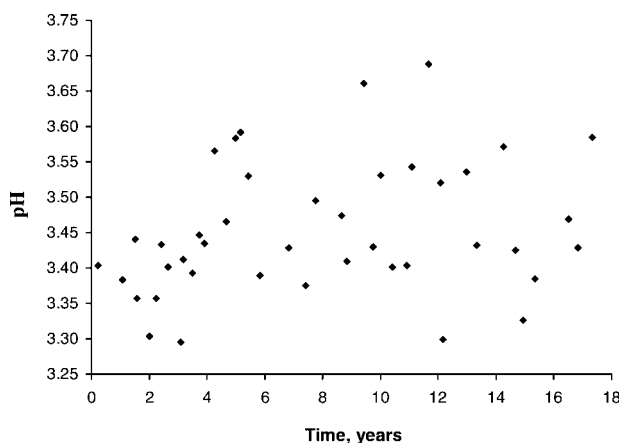


Figure 5. The pH values of the injections as a function of storage period.

formation, and k_6 for conversion of adrenaline sulfonate to adrenaline without change of conformation (see Fig. 8). All the degradation and racemization reactions were assumed to occur according to first-order kinetic processes. The

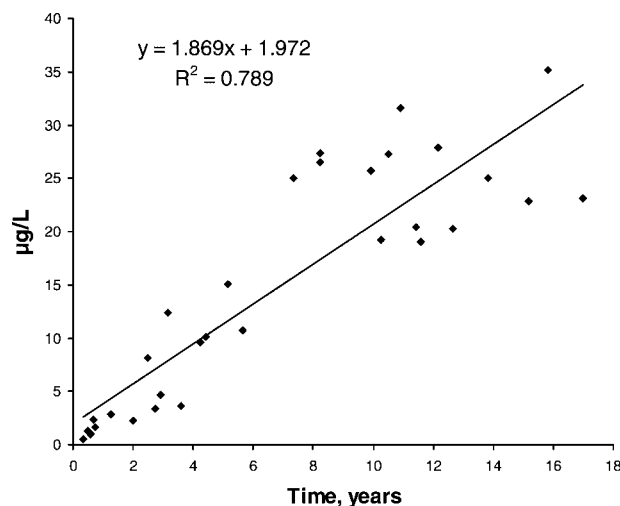


Figure 6. The concentration of aluminum in injections as a function of storage period.

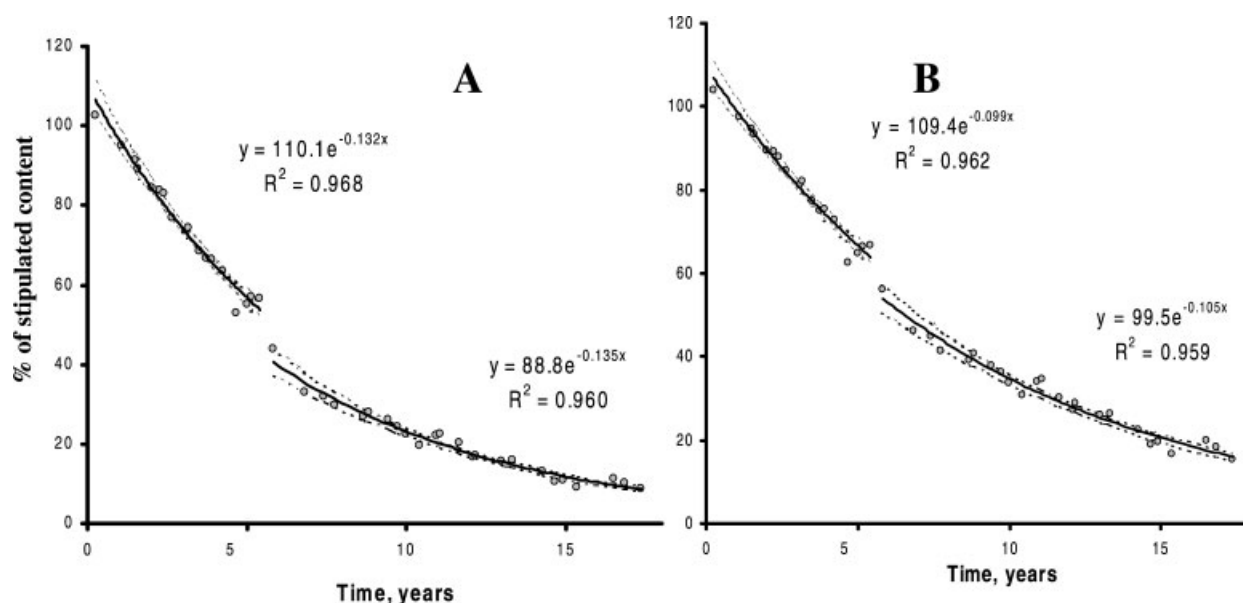


Figure 7. The content of L-adrenaline (panel A) and sum of L- and D-adrenaline (panel B) in injections as a function of storage time. The circles are the observed data, the thick lines are the mean estimated content according to the exponential regression, and the dotted lines are the 95% confidence intervals.

constants were assumed to be equal for L- and D-isomers.

Attempts to model the results as a single data set were unsuccessful and led to a significant discrepancy with the actual findings. Therefore, the data sets of the batches produced before and after 1997 were modeled separately. According to the results of modeling, the rates of the reactions with k_4 , k_5 , and k_6 rate constants (see Fig. 8, panel A) were negligible compared with the other reaction rates. This result allowed application of a reduced model (see Fig. 8, panel B) that was capable of adequately describing the content change with storage period for all analyzed substances (see Fig. 9). The estimated kinetic parameters for the two sets of adrenaline samples are presented in Table 2. The values of the Akaike and

Schwartz criteria for the batches produced before 1997 were 445 and 455, respectively, and for the batches produced after 1997 were 314 and 323, respectively. Low values of the Akaike and Schwartz criteria indicate close fits of the experimental data by the applied model.¹⁷

For short storage times, the kinetic parameters provided by the exponential regression analysis agreed well with the more inclusive kinetic models developed in this study, but revealed considerable discrepancies at the longer storage periods. For instance, the initial amount of L-adrenaline calculated from the exponential decay model in the batches produced after 1997 ($110.1 \pm 4.3\%$, see Table 1) was in accordance with the corresponding values estimated from the reduced model ($107.4 \pm 0.6\%$, see Table 2). In contrast, the values

Table 1. Kinetic Parameters of Exponential Regression: Initial Content and the Degradation Rate Constant (k) of L-Adrenaline and the Sum of L- and D-Adrenaline in Injections Produced Before and After 1997

Isomer	Required Initial Drug Content Parameter	90–115% (Manufacturing Dates: 1985–1997)			103–115% (Manufacturing Dates: 1997–2002)		
		Estimate	SD	%CV	Estimate	SD	%CV
L-Adrenaline	Initial content, %	88.8	5.3	5.9	110.1	4.3	3.9
	k , year ⁻¹	0.135	0.029	21	0.132	0.026	19
L- and D-Adrenaline	Initial content, %	99.5	5.2	5.2	109.4	4.3	3.9
	k , year ⁻¹	0.105	0.023	22	0.099	0.021	21

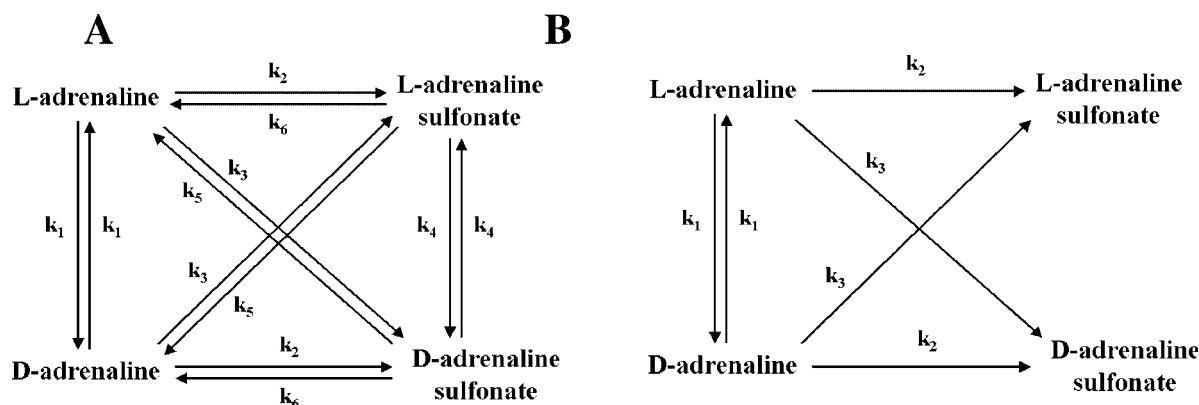


Figure 8. The full (panel A) and reduced (panel B) models of adrenaline degradation pathways in injections. The reactions are assumed to have first-order kinetics (see eqs. 1–4).

provided by the exponential and reduced models for the batches produced before 1997 were as different as 88.8 ± 5.3 versus $102.3 \pm 0.5\%$, respectively.

DISCUSSION

A chiral stability-indicating HPLC method was applied to study the kinetics of L-adrenaline degradation under controlled storage conditions and to determine the extent of drug degradation by the different pathways during and beyond the stipulated product shelf-life. Compared with the previous studies that did not detect optical isomers of adrenaline and utilized complicated sample preparation techniques,^{2–9,12} application of the chiral method provided a simple and reliable procedure for selective determination of L-adrenaline and the degradation products in the tested samples.

Rapid oxidation of L-adrenaline in aqueous solutions to strongly colored, pharmacologically inactive adrenochrome and adrenolotin⁶ necessitated use of auxiliary compounds in the injectable formulations. Several studies addressed

the potential drug-stabilizing effects of sodium metabisulfite, EDTA, ascorbic acid, boric acid, acetylcysteine, and other substances,^{7,9} as well as optimized conditions of the preparation and packaging of the adrenaline solutions. Combinations of several approaches (i.e., use of sodium metabisulfite as an antioxidant, removal of oxygen from the ampules by packing under nitrogen, and keeping the pH in the 3.0–3.8 range^{2,5,6,8}) effectively prevented the oxidative drug inactivation. Use of metabisulfite, although leading to formation of pharmacologically inactive adrenaline sulfonate, remains a common method for stabilization of adrenaline solutions because the rate of bisulfite addition is normally low compared with the rate of the drug oxidation.¹⁸

Based on mass balance considerations, we conclude that in the metabisulfite-stabilized commercial preparations examined in this study, the only pathways of L-adrenaline degradation were racemization and sulfonation (see Fig. 1) and that there was no significant degradation to additional products that were not detected by the chiral HPLC assay method used herein. Insignificance of additional degradation pathways (e.g., oxidation)

Table 2. Kinetic Parameters of Racemization and Sulfonation of Adrenaline Estimated using the Reduced Model (see Fig. 8, panel B) in Injections Produced Before and After 1997

Required Initial Drug Content Parameter	90–115% (Manufacturing Dates: 1985–1997)			103–115% (Manufacturing Dates: 1997–2002)		
	Estimate	SD	%CV	Estimate	SD	%CV
k_1 (year ⁻¹)	0.0575	0.0017	3.00	0.0334	0.0008	2.36
k_2 (year ⁻¹)	0.0527	0.0005	0.918	0.0458	0.0008	1.68
k_3 (year ⁻¹)	0.0544	0.0005	0.915	0.0469	0.0008	1.67
Initial content of L-adrenaline (%)	102.3	0.5	0.442	107.4	0.6	0.605

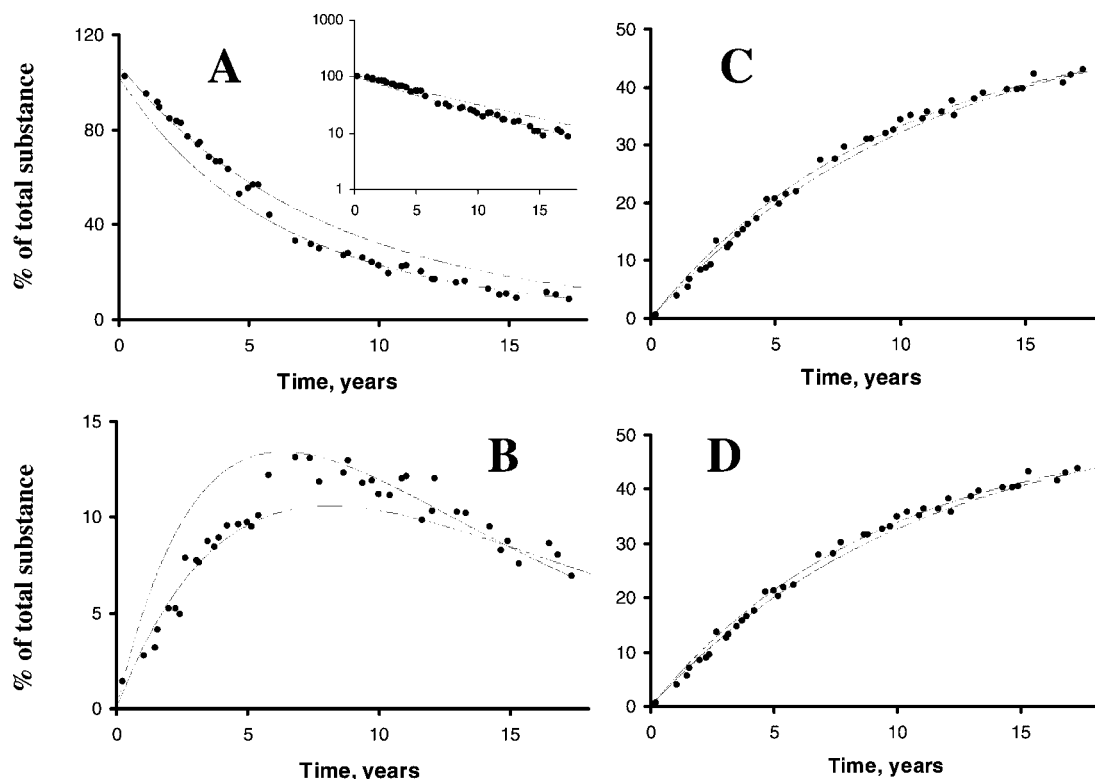


Figure 9. The content of L-adrenaline (panel A), D-adrenaline (panel B), L-adrenaline sulfonate (panel C), and D-adrenaline sulfonate (panel D) in injections as a function of storage period. The circles are the observed data, and the lines are the best fits according to the reduced model described in Figure 8. The modeling was performed separately for two data sets corresponding to batches produced before and after 1997 (see text for details). The insert is the semilogarithmic presentation of panel A.

was supported by absence of additional peaks on the chromatograms on one hand and the purity of the observed substances confirmed by MS examination on the other hand. The drug exhibited rapid degradation by reactions with first-order kinetics in adrenaline when stored under recommended conditions. In the batches produced after the application of the new requirement for the minimal drug content, the lowest acceptable limit (90% as a sum of L- and D-isomers) was attained after 2.0 years of storage, whereas the L-adrenaline content in the formulation at that time point was notably lower and equal only to 85%. The 5.6% racemization of adrenaline during a 2-year storage period is consistent with the results of previous studies showing 10% racemization into the D-isomer after ~ 4 years of storage at pH 2.4⁸ or after 3 years at pH 3.0–3.5.¹⁹ These outcomes indicate that during the storage of adrenaline injections, L-adrenaline may be converted to a significant extent into the much less pharmacologically active D-isomer, considerably

affecting the actual shelf-life of the pharmaceutical product in comparison with the officially stated limits. Use of chiral analytical assay methods, therefore, appears to be necessary for the more realistic estimation of adrenaline injections shelf-life based on selective determination of the pharmacologically active compound that can not be accomplished by the nonchiral assay currently provided by the USP.¹¹

Analysis of L-adrenaline stability data by exponential regression, assuming first-order overall degradation process, appears to be of limited use. At extended storage periods when D-adrenaline is present in significant concentrations, the rate of the reverse optical isomerization (conversion of D-adrenaline to L-adrenaline) becomes substantial, resulting in a distortion from the assumed first order. Consequently, the initial drug amount estimated from the exponential approximation for the longer storage periods was $88.8 \pm 5.3\%$ (see Table 1) that is significantly lower than the required initial drug content (103–115%). This

outcome prompts an erroneous conclusion about the poor initial quality of the formulations that in fact had been shown to have an acceptable drug amount upon receipt (data not shown).

In contrast to the results of the exponential regression, the kinetic modeling approach provided a more accurate estimation of the L-adrenaline stability profile and fitted well the experimental data (see Fig. 9 and Table 1). Kinetic analysis according to the proposed model showed that adrenaline was initially present in the formulations only as the L-isomer, and its estimated initial amount correlated well with the initial drug amount calculated from the total observed substance (Fig. 4), which provides another indirect confirmation of the model adequacy.

The applied model provided appropriate description of the content change with storage period for all analyzed substances. However, the comprehension of the model and the obtained kinetic parameters (the rate constants) may be hampered due to the complex mechanism and reversible nature of the underlying chemical reactions that are involved in the degradation of L-adrenaline in injections. Racemization of adrenaline was proposed to occur through formation of an intermediate adrenaline carbocation compound.⁶ Alternatively, a mechanism involving a quinonoid-type intermediate was proposed by Venter.²⁰ On the other hand, adrenaline sulfonate may be produced by reaction between adrenaline (SN2 reaction) or the carbocation compound (SN1 reaction) and the metabisulfite-related ions (metabisulfite, bisulfite, and sulfite) that are in equilibrium in solution. It has been also determined that at pH values lower than 5 the kinetics of sulfonation is first order in adrenaline, and zero order in metabisulfite-related ions.⁶

Because the concentrations of the intermediate adrenaline carbocation compound can not be readily measured, the modeling approach was applied to determine the integral rate constants of the degradation reactions rather than the rate constants of the intermediate steps involved in each pathway. However, the outcomes of this study are consistent with the mechanisms previously proposed for adrenaline degradation. Because the concentrations of L- and D-adrenaline sulfonate in each of the studied samples were similar (see Fig. 4), we conclude that SN1 reaction involving the intermediate carbocation compound and resulting in formation of the racemic product was the predominant mechanism of adrenaline sulfonate formation, whereas the contribution of

the SN2 reaction was minor. The overall kinetic rate of the racemization reaction was first order in adrenaline, indicating that formation of the carbocation compound may be the rate-limiting step of the racemization process. The rates of conversion of adrenaline sulfonate to adrenaline (reactions with k_5 and k_6 rate constants, see Fig. 8) appear to be insignificant due to the high stability of the aliphatic C–S bond forming in the sulfonate addition that makes the reaction practically irreversible.

Interestingly, the results of the modeling indicate that the adrenaline degradation behavior was different in the batches produced before and after 1997 (see Fig. 9). For each interval alone, the model successfully described the kinetic profiles of the observed compounds as evidenced by the respective curves fitting well the experimental data, the low values of the Akaike and Schwartz criteria, and the low coefficients of variation of the estimated kinetic parameters (Table 2). The racemization rate constant (k_1) of the newer batches decreased 1.72-fold, whereas the rate constants of adrenaline sulfonation with or without the change in conformation (k_3 and k_2) decreased 1.15- and 1.16-fold, respectively, compared with the longer stored drug lots. The observed differences in the degradation kinetics most probably resulted from concomitant changes in the drug manufacturing process and/or transfer of the adrenaline ampoules production from Manufacturer A to Manufacturer B.

The change in the initial content of L-adrenaline in the formulations ca. 1997 is not expected to have affected the rate constants of racemization and sulfonation *per se*, because the kinetics of these processes are first order in adrenaline, as demonstrated by the outcomes of the exponential regression and kinetic modeling presented here (see Figs. 7 and 9) and the results of previous studies.⁶ The observed differences in the rate constants might however be potentially associated with a change in the relative amounts of other formulation components or impurities contributing to adrenaline deactivation. For example, Milano et al.¹⁸ demonstrated significantly increased rates of adrenaline racemization in solution due to trace levels of aluminum ions in the order of 2 mg/L, whereas no increase in the reaction rate was observed in the presence of other multivalent metal cations. However, the results of aluminum assay in the batches examined in this study showed a trend of gradual increase in aluminum concentrations with storage time that was similar for both manufacturers, and the maximal

aluminum concentrations in the tested batches were ~50-fold lower than 2 mg/L (see Fig. 6). This result indicates that the differences in the degradation kinetics were not related to the presence of aluminum and probably originated from additional matrix components not assessed in this study. In addition, the effect of possible differences in the raw materials or packaging procedure cannot be excluded, despite the claimed consistency of the manufacturing protocol between the manufacturers.

In conclusion, the results of this investigation indicate that during the storage of metabisulfite-stabilized adrenaline injections, the drug (the pharmacologically active L-adrenaline isomer) rapidly degrades due to racemization and sulfonation processes. At the time point when the lower acceptable drug content stated by the USP is approached, the content of the pharmacologically active substance may be lower by ~5% due to the racemization process. Periodic testing of adrenaline batches by an extensively characterized and reliable chiral stability-indicating method is necessary to ensure the quality of injections during the stipulated storage period.

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