

Enantiomeric degradation of epinephrine in autoinjector products



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Clinical Implications

Medications must retain 90% active drug to be considered effective. Epinephrine autoinjectors typically have short expiration dating; however, expired adult doses appear to reliably retain activity for at least 18 months, whereas pediatric doses may degrade more quickly after expiration.

A previous study using reverse-phase high-performance liquid chromatography (HPLC) demonstrated that approximately 80% of 46 studied epinephrine autoinjectors (EAI) had retained 90% or more of total epinephrine concentrations up to 30 months beyond their labeled expiration dates.¹ Epinephrine is a chiral molecule where only the L(−) configuration demonstrates biological activity and the D(+) configuration is not considered biologically active. Inactive epinephrine is present as an impurity, formed as a byproduct of active epinephrine degradation.

Separating chiral compounds is important as each enantiomer poses different pharmacokinetic and pharmacological activities, as active epinephrine is 10 times more potent than inactive epinephrine. Manufactured EAI are enantiopure products that contain only L(−) epinephrine, used to counter symptoms of anaphylaxis. Upon storage, racemic conversion may reduce pharmacological and, potentially, clinical responses. Therefore, the quantitative enantiomeric composition of the active ingredient should be considered in determining expiry dates. Several analytical methods have been used to separate epinephrine enantiomers and determine the enantiomeric purity of epinephrine-containing drug products.²⁻⁴ Epinephrine's degradation includes more compounds than just these 2 enantiomers.⁵

This study determined the concentration of active and inactive epinephrine in expired EAI using the chiral separation technique. An HPLC equipped with a diode array detector was employed using a chiral stationary phase column (ORpak CDBS-453, 4.6 × 150 mm; Shodex, New York, NY). The chiral separation technique offers good selectivity and low limits of quantification. Each sample was examined over a 20-minute run through the HPLC chiral column, allowing further exploration of the peaks formed. The peaks for L(−) and D(+) epinephrine occur around 7.75 minutes and 8.23 minutes, respectively. The chiral HPLC chromatogram peak areas for each enantiomer were used to calculate the corresponding concentrations. Pure standards of L(−) epinephrine (99%; ThermoFisher Scientific; Waltham, MA) and D(+) epinephrine (Santa Cruz Biotechnology, Inc.; Dallas, TX) were used to generate calibration curves that correlate the peak areas to concentrations. The EAI were collected from friends, relatives, colleagues, and selected pharmacies and clinics.

A total of 155 autoinjectors were collected and analyzed between 2018 and 2021. Adult and pediatric autoinjectors (0.3 mg and 0.15 mg, respectively) from multiple manufacturers were analyzed, including AdrenaClick 0.3 mg (n = 3), AdrenaClick 0.3 mg generic (n = 19), AdrenaClick generic 0.15 mg (n = 4), Auvi-Q 0.3 mg (n = 5), EAI generic (Teva) 0.3 mg (n = 2), EAI generic (Teva) 0.15 mg (n = 5), EpiPen (n = 53), EpiPen generic (n = 38), EpiPen Jr (n = 19), and EpiPen Jr generic (n = 7). The labeled expiration dates for the assayed EAI range from March 2008 to September 2021. The EAI content was transferred into preweighed HPLC vials by direct injection that simulates the clinical delivery from the autoinjector. The injection was completed by maintaining pressure for 10 seconds on the vials and analyzed immediately to avoid further degradation and racemic conversion. The amount of medication transferred from each EAI was determined by weighing the vial before and after transfer. All 155 EAI ejected contents, indicating a working firing mechanism in each EAI beyond the expiration date listed. The investigators were not blinded to the expiration dates on the studied EAI.

The remaining active epinephrine was analyzed and is depicted in Figure 1. Approximately two-thirds (65%, n = 101) of the expired EAI retained more than 90% active epinephrine. The overall inverse correlation between the active epinephrine and the number of months past the expiration date, 0.66, was similar to the strength of association found in a previous study (r = 0.63).⁶ However, when the adult and pediatric doses were examined individually, the inverse correlations were 0.64 and 0.93, respectively. The 3 EAI with less than 90% active epinephrine in the first 18 months were all pediatric doses. In the first 36 months after expiration, 93% of adult doses retained activity, versus only 66% of pediatric doses.

The median duration between the expiration and assay dates was 23 months. As shown in Table 1, all EAI (n = 12) that had recently expired (0-2.9 months) contained more than 90% active epinephrine.

Recently expired (0-23.9 months) EAI (n = 79) had less than 5% inactive epinephrine (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). These results are similar to the work by Peterson et al,⁷ which reported that 2.2% to 2.3% of inactive epinephrine was detected in epinephrine-containing pharmaceutical products, which had expired within 12 months. It is important to note that the 2 enantiomers should not equal 100%. Companies must adhere to certain parameters when manufacturing medications, and the variation allowed is increased (90%-115%, per United States Pharmacopeia) when small doses are used. Because of the variation allowed and the measurable and unmeasured products developed in degradation, the percentages are unlikely to total 100%.

This study has many demonstrated strengths, including that it is the first (to our knowledge) to report on the chiral separation of epinephrine from EAI with respect to retaining at least 90% active epinephrine. It also suggests that pediatric doses may be more susceptible to degradation than adult doses. Although there are strengths, the limitations must be acknowledged. This is an *in vitro* study, and no anaphylactic reactions were blunted. The EAI were collected from multiple community resources, and

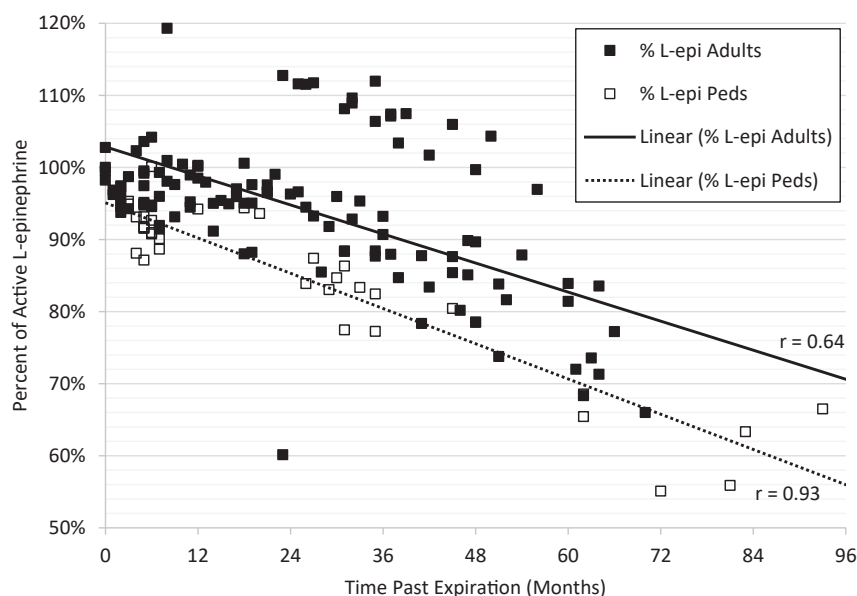


FIGURE 1. L(–)epinephrine in expired autoinjectors.

TABLE I. Epinephrine autoinjectors by months from expiration dating and percentage of the active form remaining

Time of device testing (months past the expiration date)	No. of EAI	Percent L(–) epinephrine remaining			
		100% or above	99.9%-95.0%	94.9%-90.0%	Less than 90%
0-2.9	12	2	7	3	—
3.0-5.9	19	2	7	8	2
6.0-8.9	17	5	3	8	1
9.0-11.9	6	1	3	2	—
12.0-14.9	7	2	3	2	—
15.0-17.9	4	—	3	1	—
18.9-20.9	8	1	3	2	2
21.0-23.9	6	1	4	—	1
24.0-29.9	12	3	2	3	4
30.0-35.9	18	5	2	1	10
36.0-41.9	10	4	—	2	4
42.0-47.9	9	2	—	—	7
48.0-59.9	10	1	2	—	7
60.0-71.9	12	—	1	—	11
>72.0	5	—	—	—	5
Total	155	29	40	32	54

EAI, Epinephrine autoinjector.

although the storage conditions of the EAI cannot be confirmed, this likely increases the external validity of the results. The donated EAI likely reflect how users and the community treat the EAI. Although the data presented here are encouraging, it is important to note that there is wide variation in the remaining active epinephrine, even among the same manufacturer at similar data points. For example, 2 EAI from 1 manufacturer with the same expiration date demonstrated a 2-fold difference, though this may be attributed to storage conditions. A minimum sample was not calculated; however, the reliance on donations produced an uncertain pipeline to acquiring the expired EAI.

In summary, approximately 65% of the expired EAI retained at least 90% of the active epinephrine over a median duration of

almost 2 years. Expired doses for adults appear to reliably retain activity for at least 18 months, whereas pediatric doses may be more susceptible to degradation after expiration. This may raise further questions regarding the expiration dates of these expensive but potentially life-saving products.

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TABLE E1. Inactive epinephrine formed in EAls by months from expiration dating

Time of device testing (months past the expiration date)	No. of EAls	Percent D(+) epinephrine remaining			
		0%-2.49%	2.50%-4.99%	5.0%-7.49%	More than 7.5%
0-2.9	12	7	5	—	—
3.0-5.9	19	15	4	—	—
6.0-8.9	17	14	3	—	—
9.0-11.9	6	3	3	—	—
12.0-14.9	7	4	3	—	—
15.0-17.9	4	2	2	—	—
18.0-20.9	8	2	6	—	—
21.0-23.9	6	1	5	—	—
24.0-29.9	12	3	7	2	—
30.0-35.9	18	4	10	4	—
36.0-41.9	10	—	1	6	3
42.0-47.8	9	—	1	2	6
48.0-59.9	10	—	1	—	9
60.0-71.9	12	1	—	2	9
>72.0	5	—	—	3	2
Total	155	56	51	19	29

EAI, Epinephrine autoinjector.