

Impact of Temperature Exposure on Stability of Drugs in a Real-World Out-of-Hospital Setting

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Study objective: The aim of this study is to determine the content of 5 important emergency medical services (EMS) drugs after being stored at the recommended refrigerated temperature, room temperature, or in an emergency physician transport vehicle operating under real-world working conditions.

Methods: Adrenaline hydrochloride, cisatracurium besylate, lorazepam, methylethergonovine maleate, and succinylcholine chloride were stored for 1 year under the 3 conditions. For each storage condition, samples of the drugs were taken after 1, 2, 3, and 4 weeks and after 2, 4, 6, 8, 10, and 12 months. For adrenaline hydrochloride, however, the samples were taken after 1, 2, 4, 6, 8, 10, and 12 months. The samples were analyzed with a validated high-performance liquid chromatography assay. A drug was considered stable if its content was above 90%.

Results: Adrenaline hydrochloride and methylethergonovine maleate remained stable for 1 year at room temperature and in the emergency physician transport vehicle. At room temperature and in the emergency physician transport vehicle, lorazepam became unstable within 4 weeks. Succinylcholine chloride was stable for 2 months at room temperature and for 1 month in the emergency physician transport vehicle. Cisatracurium besylate became unstable within 4 months at room temperature. However, it remained stable for 4 months in the emergency physician transport vehicle.

Conclusion: When stored at room temperature or in the emergency physician transport vehicle, lorazepam became unstable within weeks, whereas succinylcholine chloride and cisatracurium besylate became unstable within months. Adrenaline hydrochloride and methylethergonovine maleate remained stable for several months, even under room temperature and emergency physician transport vehicle conditions. Thus, real-world EMS working conditions pose challenges for maintaining optimal efficacy of these important EMS drugs. [Ann Emerg Med. 2013;62:380-387.]

Please see page 381 for the Editor's Capsule Summary of this article.

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INTRODUCTION

Background

As with hospital-based medications, drugs used in out-of-hospital emergency medical services (EMS) should be principally subject to the same recommended storage restrictions. These are specified in the summary of product characteristics.¹ The out-of-hospital emergency environment, however, often does not allow strict adherence to recommended storage conditions. The main stress factors for stored drugs include sunlight, vibration, and extreme temperature. Exposure to such factors in the out-of-hospital EMS setting may lead to decreased stability of stored drugs and unreliable or deleterious clinical effects.²⁻⁸ Of all the environmental factors that contribute to drug degradation, temperature is the most critical.⁹ In response to concerns about temperature extremes

and on-ambulance storage of medications, the United States Pharmacopeia Convention published a new general information chapter in 2004 entitled "Emergency Medical Services and Ambulances—Storage of Preparations."¹⁰

For most drugs, manufacturers must guarantee a potency of 90% to 110%.¹¹ Drug expiration date and storage conditions are based on the results of stability tests performed under a range of temperatures for a specified period. The manufacturer guarantees the stability and activity of a drug if it is used by its expiration date and if it is stored as recommended.¹² Degradation of drugs is mostly caused by chemical reactions (eg, oxidation because of oxygen exposure, hydrolysis because of water exposure) and physical reactions (eg, alteration of particle size, disintegration of a suspension/emulsion, adsorption of water), both of which are temperature dependent.^{9,13} Therefore,

Editor's Capsule Summary*What is already known on this topic*

The stability of medications depends on storage conditions. Conditions in emergency medical services (EMS) vehicles vary.

What question this study addressed

What is the stability of 5 medications recommended for refrigeration when stored in a refrigerator, at room temperature, and within an EMS vehicle?

What this study adds to our knowledge

During 1 year, adrenaline hydrochloride, cisatracurium besylate, lorazepam, methylethergonovine maleate, and succinylcholine chloride all remained stable when refrigerated. In the EMS vehicle, the estimated time to 10% drug degradation was longest for methylethergonovine (87.3 months) and shortest for succinylcholine (1.2 months) and lorazepam (1.0 month). The difference between room temperature and EMS vehicle was not uniform.

How is this relevant to clinical practice

The storage conditions for medications should be considered when determining their real-world shelf life, especially in EMS settings.

when drugs labeled for refrigeration are not stored at the appropriate temperatures, they are prone to degradation.

Studies on the effect of environmental temperatures on drugs requiring refrigeration have reported a wide range of results (Table 1). High-temperature studies of drug stability and potency are typically conducted in controlled environments, in which temperature can be experimentally varied. Few data are available, however, on the effect of temperature fluctuations and exposure time outside recommended storage conditions. Only 1 study has determined the biological consequence of temperature-induced degradation of a drug (ie, adrenaline).¹⁴ Such studies are complex because degradation also depends on other factors, such as drug concentration and solvent pH,¹⁴⁻¹⁹ yet manufacturers are not required to perform stability tests under actual storage conditions, such as in EMS settings.¹⁰

Importance

Drugs requiring refrigeration are more at risk for degradation when stored inappropriately. To date to our knowledge, no study has investigated how exposure to temperature variations for long periods affects a broad range of out-of-hospital drugs specifically requiring refrigeration. In the chapter "Emergency Medical Services and Ambulances—Storage of Preparations," the

Table 1. Drugs recommended for refrigeration: stability at high temperatures.

Drug Name	Stability Information
Adrenaline hydrochloride	3 mo at 70°C (158°F): no degradation ⁷ 1 y at 40°C (104°F): no degradation ⁶ Exposure to cyclical cooling and heating (−6°C and 54°C) (21.2°F and 129°F) every 12 h: unstable after 14 days ⁷ 12 wk at 70°C (158°F): 1 mg/mL of drug: no degradation ¹⁴ 0.1 mg/mL of drug: 64% degradation, with a significant loss (30%) of cardiovascular potency ^{14*} Exposure to cyclical heating (65°C (149°F) for 8 h/day for 4–12 wk or 672 h of heat exposure): 31% degradation ¹⁵ Exposure to constant heat [65°C (149°F) for 7 days or 169 h of heat exposure]: 100% degradation ¹⁵
Cisatracurium besylate	21 days at RT: stable ²⁶ 45 days at 23°C (73.4°C): 5%–7% degradation ¹⁹
Lorazepam	4 mo at RT: 16.6% degradation ²⁷ 5 mo at 15°C–30°C (59°F–86°F): 10% degradation ^{22†}
Methylethergonovine maleate	1 y at 30°C (86°F) (stored in the dark): 18% degradation ²⁸ 2–4 wk at 40°C–50°C (104°F–122°F): stable ²⁹
Succinylcholine chloride	Exposure to cyclical cooling and heating (−6°C and 54°C) (21.2°F and 129°F) every 12 h: unstable after 14 days ⁷ 4 wk at 25°C (77°F): 10% degradation ¹⁶ 5 mo at 20°C–26°C (68°F–78.8°F): 10% degradation ¹⁷ 1 mo at 35°C (95°F): 10% degradation ¹⁷ 1 day at 70°C (158°F): 10% degradation ¹⁷ 1 mo at RT: 20 mg/mL of drug: 1.2% degradation ¹⁸ 50 mg/mL of drug: 2.1% degradation ¹⁸ 1 mo at 37°C (98.6°F): 20 mg/mL of drug: 5.4% degradation ¹⁸ 50 mg/mL of drug: 8.1% degradation ¹⁸ 90 days in ambulance (13°C–32°C) (55.4°F–89.6°F): 10% degradation ²³

RT, room temperature.

*Determined through blood pressure and pulse rate responses of conscious rats.

†Lorazepam was more unstable when stored at 25°C to 30°C (77°F to 86°F).

United States Pharmacopeia recommends that "environmentally sensitive" medications should not be stored in ambulances unless they are stored in a temperature-controlled storage cabinet, or unless each medication package is attached to an individual time-temperature indicator.¹⁰ Because ambulances transport to the emergency scene many lifesaving drugs under severe time constraints, it is not practical to store these drugs elsewhere other than in the ambulances themselves. Refrigerated compartments could be used to facilitate safe storage. For these

Table 2. List of drugs studied.

Drug Name	Brand Name (Manufacturer)	Concentration	Expiration Date	Batch Number
Adrenaline hydrochloride	Adrenaline (Sterop, Brussels, Belgium)	0.8 mg/mL	Oct 2011	080251
Cisatracurium besylate	Nimbex (GlaxoSmithKline, Wavre, Belgium)	20 mg/10 mL	Jul 2011	9046
Lorazepam	Temesta (Pfizer, Brussels, Belgium)	4 mg/mL	Oct 2010	09K13A
Methylergonovine maleate	Methergin (Novartis Pharma, Vilvoorde, Brussels)	0.2 mg/mL	Jan 2013	S0104
Succinylcholine chloride	Lysthenon (Nycomed, Konstanz, Germany)	100 mg/2 mL	May 2011	10504696

reasons, it is critical to determine when drugs, especially “environmentally sensitive” drugs, exposed to typical ambulance temperature fluctuations are no longer safe for human use.

Goals of This Investigation

The aim of this study was to evaluate the stability of drugs requiring refrigeration under actual out-of-hospital EMS conditions.

MATERIALS AND METHODS

Study Design and Setting

This was a longitudinal study to evaluate the stability of drugs requiring refrigeration and that are used in an out-of-hospital EMS system operating in a temperate climate zone. The studied drugs were adrenaline hydrochloride, cisatracurium besylate, lorazepam, methylergonovine maleate, and succinylcholine chloride. These drugs are used in our emergency drug kit (Department of Emergency Medicine, UZ Leuven, Leuven, Belgium) and are not stored according to the manufacturer’s recommendations. Although it is not frequently used, methylergonovine maleate was included in the study because it is used for controlling out-of-hospital postpartum hemorrhage. The drugs were considered to be stable at contents above 90%.^{9,11,13}

Drug content was determined quantitatively by validated high-performance liquid chromatography, which is considered to be the criterion standard in the pharmaceutical industry for assessing drug stability and for quantitative testing operations.

This study was conducted at the emergency department (ED) of Leuven University Hospital, a 2,000-bed tertiary care hospital in Leuven, Belgium. The EMS in Belgium is based on a 2-tiered Franco-German model, in which a paramedic composes the first tier, and for life-threatening situations, a physician and a nurse compose the second tier. The latter is called the Mobile Urgency Group. The EMS system in Belgium is considered to be an extension of the local hospital. There is 1 Mobile Urgency Group for every 140,000 inhabitants. In our hospital, the Mobile Urgency Group receives a median of 4.68 calls per 24 hours. The median response time is 16 minutes, and the median trail is 45 minutes. On average, the Mobile Urgency Group drives a distance of 13 km to the call destination. Our hospital has 2 emergency physician transport vehicles, which are located in a garage at the EMS station. The garage is not heated in the winter. In the summer, its air conditioning is set to a target temperature of 15°C (59°F). Which emergency physician

transport vehicle responds first to an EMS request alternates every month. For practical reasons, all drugs (labeled both for room temperature and for refrigeration) are stored together in a portable emergency EMS backpack.

The emergency physician transport vehicle has no refrigerator for drugs that require refrigeration. Backup portable emergency EMS backpacks are stored in the ED at room temperature. Emergency physician transport vehicle emergency backpacks are replaced with backup packs when a drug is depleted, when used out-of-hospital for EMS, or when a drug has reached its expiration date. The previous year, the turnover of EMS drugs labeled for refrigeration was 109 ampoules per year for adrenaline tartrate (0.8 mg/mL), 140 ampoules per year for adrenaline hydrochloride (10 mg/10 mL), 60 ampoules per year for cisatracurium besylate (20 mg/10 mL), 1 ampoule per year for methylergonovine maleate (0.2 mg/mL), 48 ampoules per year for lorazepam (4 mg/mL), and 76 ampoules per year for succinylcholine chloride (50 mg/mL).

During the 1-year study period from January 14, 2010, to January 14, 2011, the abovementioned drugs were stored in 3 places: in a refrigerator at 2°C (36°F) to 8°C (46°F), in the ED at a room temperature of 20°C (68°F) to 25°C (77°F) (room temperature), or in the emergency physician transport vehicle. Guided by the literature search summarized in Table 1, we extracted 2 samples of each drug stored at each condition after 1, 2, 3, and 4 weeks and after 2, 4, 6, 8, 10, and 12 months. For adrenaline hydrochloride, samples were taken only after 1, 2, 4, 6, 8, 10, and 12 months because published stability test results and the summary of product characteristics indicate that adrenaline hydrochloride is stable even when exposed to high temperatures.^{6,14,15} Thus, adrenaline hydrochloride was not expected to degrade below the 90% threshold within 1 month. Refrigerated samples served as control samples. All ampoules of drugs were unopened and contained a fresh batch of each respective drug (adrenaline hydrochloride, cisatracurium besylate, lorazepam, methylergonovine maleate, and succinylcholine chloride).

All ampoules of each drug had identical lot numbers and, with the exception of lorazepam, had expiration dates beyond the study completion date. A comprehensive list of investigated drugs is shown in Table 2. All ampoules (94 total) were divided into their convenience storage group and placed into sealed plastic bags. The ampoules were labeled 1 and 2 with a refrigerator, room temperature, or emergency physician transport vehicle subscript and the storage period (1 week, 2

weeks, etc.). Room temperature and emergency physician transport vehicle ampoules were placed into the regular drug box in the portable emergency EMS backpack and stored, respectively, either in the ED or on the back floor of the emergency physician transport vehicle, where they were protected from ultraviolet exposure caused by direct sunlight. These ampoules were not used on any patients because the backpacks were locked and labeled “not for human use.” Emergency physician transport vehicle ampoules were transferred monthly into the emergency physician transport vehicle assigned to respond first on EMS request. Refrigerator ampoules were stored in a refrigerator located in the laboratory performing the pharmacologic analysis. Ampoules were removed at the designated intervals. Room temperature and emergency physician transport vehicle ampoules were transported within 15 minutes to the laboratory. Thereafter, refrigerator, room temperature, and emergency physician transport vehicle ampoules were stored in a freezer at -80°C (-112°F) until analysis. Pilot data showed that samples of the included drugs were stable at -80°C (-112°F). Detailed experimental conditions of the assay can be found in Appendix E1 (available online at <http://www.annemergmed.com>).

Methods of Measurement

Maximum, minimum, and average outdoor temperatures (emergency physician transport vehicle ambient temperature) in Leuven during the study period were obtained from the National Belgian Meteorological Service and recorded. The meteorologic measurements were performed at the Korbeek-lo station, which is 10 km from the EMS station.

At the end of the study period and for the 3 conditions (refrigerator, room temperature, and emergency physician transport vehicle), the content of each drug was determined by high-performance liquid chromatography at each designated storage time. The content was reported as a percentage (%) of the label claim, plus or minus a standard deviation (SD) of 6 measurements.

The estimated time to reach 10% degradation of each drug was determined by linear regression analysis for samples subjected to room temperature and emergency physician transport vehicle conditions. All the graphs were constructed with Excel 2003 software (version 11.8012.6568) SP2 (Microsoft, Redmond, WA).

Outcome Measures

The primary outcome measure was stability of drugs labeled for refrigeration but stored in the emergency physician transport vehicle. The secondary outcome measure was estimated time the drugs degraded by 10%.

Primary Data Analysis

Differences between the regression lines of refrigerator, room temperature, and emergency physician transport vehicle conditions for each drug were determined by comparing their

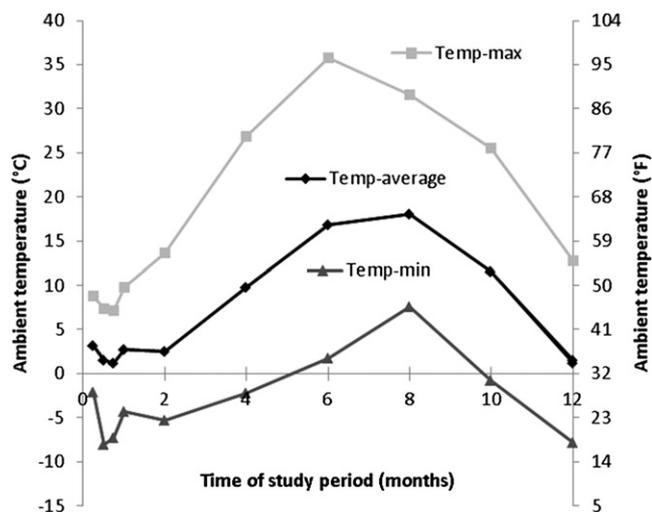


Figure 1. Time plot for ambient temperatures during the 12-month study period ($^{\circ}\text{C}$). *

*Ambient temperature data obtained from the National Belgian Meteorological Service; sampled location temperatures within 10 km of stored drugs.

slopes (GraphPad Prism version 5 software; GraphPad, La Jolla, CA). F tests were performed, and *P* values of .05 or less were deemed to be statistically significant.

To assess whether starting the study in the summer (June 21 until September 21) versus the winter (December 21 until March 21) period would have shown different degradation rates, nonparametric tests (SPSS, version 20.0; SPSS, Inc., Chicago, IL) were used to compare medians of temperatures (logged every week) and slopes of regression lines between 2, 4, and 6 months and 6, 8, and 10 months, characterizing degradation rate in winter versus summer, respectively. *P* values of less than .05 were considered as statistically significant.

RESULTS

Ambient temperatures (average, minimum, and maximum temperatures) recorded during the 12-month study period are shown in Figure 1. Ambient temperatures (January 14, 2010, to January 14, 2011) ranged from -8°C (17.6°F) to 35.8°C (96.4°F), with a mean of 10.3°C (50.5°F) (SD 7.3°C or 45.1°F).

The percentage content of each of the 5 drugs stored at refrigerator, room temperature, and emergency physician transport vehicle is shown in Figure 2. All of the control samples, which were stored in a refrigerator at 2°C (35.6°F) to 8°C (46.4°F), remained stable for the entire duration of the study because their content remained above the 90% lower limit.

Our results have shown that adrenaline hydrochloride and methylergonovine maleate remained stable for at least 1 year at room temperature and in the emergency physician transport vehicle. Although lorazepam became unstable within 4 weeks at room temperature, it remained stable up to 4 weeks in the

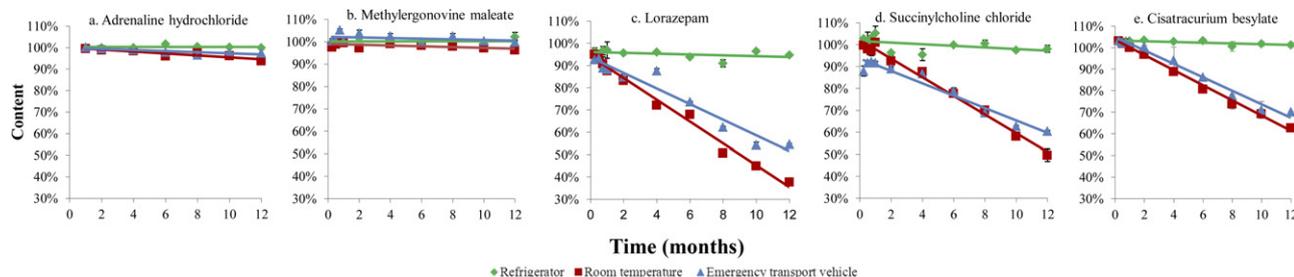


Figure 2. Content of the individual drugs by storage condition and length of time stored.

Table 3. Differences between slopes of regression lines.*

	F/RT	F/EPTV	RT/EPTV
Adrenaline hydrochloride	Significant <i>P</i> =.003	Significant <i>P</i> =.02	None <i>P</i> =.14
Cisatracurium besylate	Significant <i>P</i> <.001	Significant <i>P</i> <.001	Significant <i>P</i> =.03
Lorazepam	Significant <i>P</i> <.001	Significant <i>P</i> <.001	Significant <i>P</i> =.0012
Methylergonovine maleate	None <i>P</i> =.16	None <i>P</i> =.39	None <i>P</i> =.85
Succinylcholine chloride	Significant <i>P</i> <.001	Significant <i>P</i> <.001	Significant <i>P</i> <.001

F, Refrigerator; RT, room temperature; F/RT, F versus RT; F/EPTV, F versus EPTV; EPTV, emergency transport vehicle; RT/EPTV, RT versus EPTV.

**P*<.05 was considered significant.

emergency physician transport vehicle. Succinylcholine chloride was stable for 2 months at room temperature but only 1 month in the emergency physician transport vehicle. Cisatracurium besylate became unstable within 4 months at room temperature, even though it remained stable for up to 4 months in the emergency physician transport vehicle.

Regression analysis revealed that the time estimated to 10% degradation was 22.2 months at room temperature and 38.2 months in the emergency physician transport vehicle for adrenaline hydrochloride, 3.8 months at room temperature and 4.7 months in the emergency physician transport vehicle for cisatracurium besylate, 0.8 months at room temperature and 1.0 month in the emergency physician transport vehicle for lorazepam, 52.9 months at room temperature and 87.3 months in the emergency physician transport vehicle for methylergonovine maleate, and 2.8 months at room temperature and 1.2 months in the emergency physician transport vehicle for succinylcholine chloride. Table 3 shows the significant differences between the slopes of the regression lines for each drug in relation to its storage condition.

LIMITATIONS

This study has several limitations. First, for each drug, we used only 2 ampoules per storage condition. This small sample size, however, was similar to those of previous studies.

Second, our study began in the winter. One would expect the emergency physician transport vehicle group of drugs to degrade faster if the study had begun in the summer. However,

degradation rates were similar in winter versus summer for all drugs (adrenaline hydrochloride, *P*=1.00; cisatracurium besylate, *P*=0.44; lorazepam, *P*=1.00; methylergonovine maleate, *P*=0.44; succinylcholine chloride, *P*=0.44) despite significantly higher temperatures in summer versus winter months (*P*<.001). Therefore, we can state that starting in the winter or summer does not have any effect on the degradation rate. In our opinion, exposure to environmental temperature as such, not according the manufacturer’s recommendation, is the most important factor resulting in degradation.

Third, our study findings may have limited generalizability. The many factors that contribute to drug degradation and the many unique EMS configurations could make it very difficult to generalize our findings to other EMS systems. Performing a study in an actual EMS environment is associated with unpredictable variation. To address this issue and facilitate comparison with other studies, we reported as many details as possible about our EMS system (climate zone, call volume, trail, location of vehicles, and absence of temperature-controlled compartments).

Of all the environmental factors that contribute to drug degradation, temperature is the most critical. Hence, we conducted our study under 3 temperature conditions: in the refrigerator at 2°C (35.6°F) to 8°C (46.4°F) (control), at room temperature (20°C to 25°C) (68°F to 77°F), and in emergency physician transport vehicles (ambient temperature, which varied as a function of climate zone and season). This study was performed in Belgium, which has a temperate climate like that of other countries in Northern Europe. For convenience in assigning shelf-life stability, experts have divided the world into 4 climate zones. In Europe, most of the population lives in climatic zone I (temperate) or II (Mediterranean). In Japan, 99% of the population lives in temperate or Mediterranean climates, whereas in the United States 94% of the population lives in these climates. However, because of actual exposure in an EMS setting, variations cannot be denied. We could not simulate other variations in the ambient temperature of the emergency physician transport vehicle caused by, for example, hotter summer temperatures or colder or warmer winter temperatures because the study was conducted in a single year, 2010. Thus, our findings represent just a portion of our out-of-hospital EMS. EMS providers could alter the temperature in the emergency physician transport vehicle, which could have

influenced the results of our study. Despite these concerns, we believe that our room temperature and emergency physician transport vehicle results are useful, clearly supporting the notion that medications stored under EMS conditions degrade faster at different rates than those stored according to manufacturers' specifications.

Sunlight or vibration can also influence drug degradation. In our study, room temperature and emergency physician transport vehicle ampoules were stored in the regular drug box of a portable emergency EMS backpack, where they were protected from ultraviolet exposure. All the studied drugs are light sensitive. Although light could influence drug degradation, it was difficult to take into account different actual EMS scenarios involving light exposure (eg, opening the backpack during a intervention on a sunny day). Rather than addressing this issue, we believe that it is more important to anticipate this problem by encouraging manufacturers to use dark glass for light-sensitive drugs, especially those used in out-of-hospital EMS. Alternatively, hospital pharmacists can repackage light-sensitive drugs used in EMS.

Vibration is another factor that could contribute to drug degradation. Emergency physician transport vehicle ampoules were subject to vibrations during the study period, whereas other ampoules (room temperature) were not. Determining the factors related to the degradation process was, however, beyond the scope of this study. Further research on the effect of vibrations could be valuable.

A fourth limitation of this study involves how the emergency physician transport vehicle drugs were stored. In our hospital, EMS backpacks in emergency physician transport vehicles are exchanged for "new" backpacks at regular intervals. Before the exchange, the new backpacks containing drugs are stored in the ED at room temperature. Thus, drugs in the new backpacks were exposed to both room temperature and emergency physician transport vehicle ambient temperatures for variable times, depending on the turnover of backpacks, which was difficult to simulate.

DISCUSSION

To our knowledge, this study is the first year-long study to investigate the effects of actual temperature variations on a broad range of out-of-hospital drugs that manufacturers recommend refrigerating. Our findings confirmed that exposure to environments outside the manufacturer's storage recommendations can result in the degradation of out-of-hospital drugs. The degree of degradation, however, varied across the drugs studied. Lorazepam stored at room temperature and in emergency physician transport vehicles became unstable within weeks, whereas succinylcholine chloride stored in emergency physician transport vehicles became unstable within 1 month but at room temperature became unstable within 2 months. On the other hand, cisatracurium besylate stored at room temperature became unstable within 4 months but when stored in emergency physician transport vehicles remained stable for 4 months. Although these drugs have a narrow therapeutic

range, they are typically administered according to patient response. However, in the literature, no adverse events involving ineffectiveness and toxicity have been reported.

Cisatracurium, a nondepolarizing neuromuscular blocking agent with an intermediate duration of action, undergoes Hofmann elimination, a process dependent on pH and temperature, to form laudanosine.²⁰ Limited data are available about the potential toxic systemic effects of laudanosine; however, it may cause excitement and seizure activity.²¹

Lorazepam is the first-line treatment out-of-hospital and in-hospital for the initial treatment of status epilepticus. Lorazepam can undergo rearrangement to form a less polar carboxaldehyde derivative; this rearrangement is accelerated at higher temperatures.²² No data are available about the activity and toxicity of this degradation product.

Succinylcholine chloride, which is a short-acting depolarizing neuromuscular blocking agent used to assist intubation, has a narrow dosing range of 0.5 to 1 mg/kg. Its chemical stability and pharmacologic action are limited by hydrolysis. Stepwise degradation first results in the formation of succinyl monocholine, which undergoes much slower hydrolysis to yield choline and succinic acid.²³ The rate of hydrolysis is increased by temperature, light, and acidity. If succinic acid is formed, which decreases the pH, the degradation reaction accelerates. The degradation products seem to be inactive, but there are limited data available on their toxicity.

In a recent study, Merlin et al²³ analyzed the degradation of succinylcholine chloride before and after exposure to ambulance cabin temperatures for 7 months. Using mass spectrometry, they found that its concentration decreased, whereas concentrations of its degradation products increased during the study period. The study reported the estimated 10% degradation of succinylcholine chloride at precise intervals, but the intervals the study used were different from ours: 3.0 and 2.4 months for indoor and outdoor vehicles, respectively, versus 2.8 months and 1.2 months for room temperature and emergency physician transport vehicles, respectively. The authors found no evidence that ambient temperatures contributed to the degradation of succinylcholine. In addition, the trends of degradation indoors and outdoors were only marginally significant. By contrast, our results showed a significant, extremely robust difference in trends of degradation for succinylcholine chloride stored in a refrigerator, at room temperature, and in emergency physician transport vehicles. However, our study cannot be compared satisfactorily with theirs. We used samples stored in a refrigerator as controls for the duration of the study period. We also included a room temperature group, wherein backpacks containing drugs were stored in the ED. Moreover, our emergency physician transport vehicles were operated outdoors but were housed in a partially temperature-conditioned garage. Although both studies began collecting data during the winter and the mean temperature and the minimum temperature in both studies

were similar, the maximum temperature was higher in our study.

The present study highlights the importance of taking into account the correct drug storage recommendations because our results demonstrated a significant difference in degradation trends for drugs stored in the refrigerator versus at room temperature and drugs stored in the refrigerator versus in emergency physician transport vehicles. This was the case for adrenaline hydrochloride, cisatracurium besylate, lorazepam, and succinylcholine chloride. However, differences in degradation trends did not always necessarily lead to instability, which was defined as a drug content of less than 90%. Accordingly, the content of adrenaline hydrochloride was greater than 90% when stored at room temperature or in emergency physician transport vehicles for 1 year.

Because adrenaline hydrochloride, cisatracurium besylate, lorazepam, methylethylergonovine maleate, and succinylcholine chloride are stored in emergency physician transport vehicles throughout the world, the procedure for removing unused ampoules needs to be standardized according to evidence-based results, such as those of our study. Significant protocol inconsistencies exist within regions: Some replace the ampoules daily or monthly, whereas others adhere to expiration dates. Although the clinical efficacy of these drugs when unstable is yet to be established, data on how long it takes for these drugs to degrade by 10% can serve as important guidelines for appropriate use in patients.^{10,24,25} At present, Belgium does not have guidelines on which specific drugs are to be used in EMS. Therefore, other environmentally sensitive medications not studied in this report are currently used in out-of-hospital EMS. Similar studies should be performed for other environmentally sensitive drugs.

In conclusion, lorazepam stored at room temperature and in operational ambulances became unstable within weeks and succinylcholine chloride became unstable within months. Indeed, lorazepam degraded by 10% when stored 0.8 months in light-resistant environments at room temperature and when stored 1 month in an operational ambulance. For succinylcholine chloride, the 10% degradation times were 2.8 months when stored in light-resistant environments at room temperature and 1.2 months when stored in an operational ambulance; and for cisatracurium besylate, the degradation times were 4.7 months in an operational ambulance and 3.8 months when stored in light-resistant environments at room temperature. These drugs should be removed from EMS backpacks at the end of these time frames to maintain optimal potency and avoid adverse effects resulting from drug degradation. Adrenaline hydrochloride and methylethylergonovine maleate were more stable because they did not degrade below 90% when stored at room temperature or in ambulances for 1 year.

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APPENDIX: E1

Experimental conditions.

Chromatography Instrumentation and Conditions

The high-performance liquid chromatography (HPLC) apparatus consisted of an Ultimate 3000 pump (Dionex, Germering, Germany) and an ASI-100 automated sample injector (Dionex) combined with a deuterium lamp cathodeon detector (Thermo Fisher Scientific, Waltham, MA). The temperature of the column, immersed in a waterbath, was maintained using a heating circulator (Julabo, Seelbach, Germany). Chromeleon software (Dionex Softron) on a personal computer connected to the detector was used to record the signals. Five different stationary phases and experimental conditions were chosen according to the European Pharmacopoeia (Ph. Eur.) and United States Pharmacopoeia. The details are shown in the Table below.

Reagents and Samples

Details of all samples used in this study are listed in Table 2 of the article. Reference adrenaline (98.7%) and succinylcholine chloride dihydrate (99.5%) were obtained from Sigma-Aldrich (Steinheim, Luxembourg). Lorazepam (100.0%) was obtained from SA Fagron NV (Waregem, Belgium). Methylegonovine maleate (100.0%) was from Tocris Bioscience (Bristol, UK). Atracurium besylate (98.5%) Chemical Reference Substance was obtained from European Directorate for the Quality of Medicines

(EDQM; Strasbourg, France). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from VWR International (Leuven, Belgium). Sodium dihydrogen phosphate dihydrate (NaH_2PO_4) and potassium dihydrogen phosphate anhydrous (KH_2PO_4) were obtained from Merck (Darmstadt, Germany). Dipotassium hydrogen phosphate anhydrous (K_2HPO_4) was obtained from Chem-Lab NV (Zedelgem, Belgium). Ammonium carbamate, 1-pentanesulphonic acid sodium salt monohydrate, and 1-octanesulphonic acid sodium salt monohydrate were from Acros Organics (Geel, Belgium). A Milli-Q gradient water purification system (Millipore, Bedford, MA) was used to purify deionized water.

Sample Preparation

Adrenaline hydrochloride (0.8 mg/mL) and methylegonovine maleate (0.2 mg/mL) ampoule solutions were injected directly without any further treatment. A 0.5 mL sample of lorazepam was diluted to 10.0 mL with mobile phase (0.2 mg/mL). For succinylcholine chloride, 0.8 mL of the sample was diluted to 5.0 mL with mobile phase (8 mg/mL). For cisatracurium besylate 0.2%, 1.0 mL was diluted to 10.0 mL (0.2 mg/mL) with mobile phase. All the reference standards were weighed to a certain amount and dissolved in mobile phase to achieve the concentration mentioned in the prepared ampoule sample solutions. All the prepared solutions were filtered with a 0.2- μm regenerated cellulose filter before injection into the HPLC system.

Table. Experimental conditions for high-performance liquid chromatography analysis of 5 drugs used in out-of-hospital EMS.

Drug name	Column	T (°C)	WL (nm)	Inj. vol. (μL)	Flow rate (mL/min)	Mobile phase	Reference
Adrenaline hydrochloride	Zorbax Eclipse XDB Phenyl, 5 μm , 250 \times 4.6 mm (Agilent Technologies)	50	210	20	2.0	ACN-mixture (5:95, v/v) Mixture containing 5 g/L NaH_2PO_4 and 2.6 g/L sodium octanesulphonate in H_2O (pH 2.8)	Adapted from Ph. Eur. 7.0 Adrenaline tartrate (01/2008:0254)
Cisatracurium besylate	Hypersil Keystone-C ₁₈ , 5 μm , 250 \times 4.6 mm (Thermo Scientific)	RT	280	20	1.0	MeOH-ACN-10.2 g/L KH_2PO_4 (20:20:60, v/v/v)	Adapted from Ph. Eur. 7.0 Atracurium besylate (01/2008:1970)
Lorazepam	Zorbax Extend-C ₁₈ , 5 μm , 250 \times 4.6 mm (Agilent Technologies)	RT	235	20	1.0	ACN-3.5 g/L K_2HPO_4 (pH 10.5) (5:95, v/v)	Adapted from Ph. Eur. 7.0 Lorazepam (01/2008:1121 corrected 6.0)
Methylegonovine maleate	Hypersil BDS C ₁₈ , 5 μm , 100 \times 4.6 mm (Thermo Scientific)	RT	310	20	2.0	2 g/L Ammonium carbamate-ACN- H_2O (65: 17.5:17.5, v/v/v)	Adapted from Ph. Eur. 7.0 Methylegometrine maleate (07/2009:1788)
Succinylcholine chloride	Platinum EPS C ₁₈ , 3 μm , 4.6 \times 150 mm (Mandel Scientific, Canada)	RT	214	20	1.0	ACN-mixture (20:80, v/v) Mixture containing 4.9 g/L sodium 1-pentanesulfonate and 1 % (v/v) 1 N H_2SO_4 in H_2O	Adapted from USP Succinylcholine chloride for injection

Abbreviations: EMS, emergency medical service; T, column temperature; WL, wavelength; Inj. vol., injection volume; Ph. Eur., European Pharmacopoeia; RT, room temperature; USP, United States Pharmacopoeia; USA, United States of America; XDB, extra densely bonded; BDS, base deactivated silica; EPS, extended polar selectivity.