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Original Article

Accelerated stability testing of a transdermal patch composed of eserine and pralidoxime chloride for prophylaxis against (±)-anatoxin A poisoning

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ABSTRACT

The current study evaluated the stability potential of a transdermal patch composed of eserine and pralidoxime chloride for prophylaxis against (±)-anatoxin A poisoning. The drug combinations were fabricated in an adhesive matrix system supported by a backing membrane and attached to a temporary release liner. Stability testing of the optimized formulation was established for 6 months under accelerated study conditions as per International Conference on Harmonisation guidelines. Results obtained after 6 months showed that the optimized patch formulation was stable with respect to drugs content, pH, diffusion, visual inspection, and other analytical parameters.

1. Introduction

The notion of stability in pharmaceutics is understood as the ability of a pharmaceutical product to maintain its properties within the specified limits during its declared shelf life [1]. In order to define the shelf life for a drug, investigations/tests must be performed, according to a prescheduled program, resulting in information about various aspects of stability (chemical, physical, and microbiological) [2]. Essential goals of stability test performance are as follows: (1) selection of adequate formulation and primary packaging material; and (2) determination of shelf life and storage conditions for the drug.

(±)-Anatoxin A, a guanidinemethyl phosphate ester isolated from the freshwater cyanobacterium (blue-green algae), produces its effects primarily by binding selectively and

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stereospecifically to nicotinic cholinergic receptors of the nervous system in rat brain membranes, where it acts as a potent agonist but it is not hydrolyzed by cholinesterase. Eserine and 2-PAM are one of a number of medical drugs against certain psychochemical warfare agents. *In vivo* pretreatment with eserine and high concentrations of pralidoxime chloride (2-PAM) are the only effective antagonists against a lethal dose of (±)-anatoxin A poisoning [3]. Again, the protective effects of a nonselective muscarinic antagonist (atropine), a cholinesterase-reactivating oxime (2-PAM), and two reversible acetyl cholinesterase inhibitors (physostigmine and pyridostigmine) against fonofos and phosphamidon induced lethality in 24 h *Artemia* is well documented. The concentrations of eserine and 2-PAM were selected based on the clinically available plasma concentrations in human, without considerable dermal irritation, skin sensitization and acute dermal toxicity [4]. An eserine transdermal delivery system is an attractive option given the potent nature of the drug and bypass of the hepatic first-pass elimination effect. A controlled-release device of eserine is desirable because it can avoid the need for multiple doses, improve drug stability, and release eserine in a controlled fashion. For this reason, eserine has been suggested as an alternative prophylactic against Such Lethal poisoning because it is an unquaternized carbamate that penetrates the central nervous system [5]. However, eserine has a short plasma half-life and a narrow therapeutic index, which limited its clinical use in humans and needs the use of a sustained release formulation [6]. For this reason, we developed a sustained-release, combinational, prophylactic transdermal drug-in-adhesive matrix type therapeutic system containing eserine and 2-PAM for the delivery to the skin.

In the present study, we evaluated the stability potential of a transdermal patch formulation composed of eserine and 2-PAM developed at our laboratory for prophylaxis against (±)-anatoxin A poisoning at different time intervals according to the International Conference on Harmonisation (ICH) guidelines.

2. Materials and methods

2.1. Reagents

Eserine and 2-PAM were provided by Sigma–Aldrich (St. Louis, MO, USA). Pressure-sensitive adhesives were obtained from Henkel Corporation (Bridgewater, NJ, USA). Backing polyester film laminate and release liner were kindly donated as a gift sample by 3M (St. Paul, MN, USA). All reagents and solvents used were of analytical grade and used as received.

2.2. Preparation of drugs-in-adhesive matrix type transdermal patches

Drugs-in-adhesive matrix type transdermal patches containing eserine and 2-PAM were prepared with the blends of two pressure-sensitive adhesives in different volume ratios and different concentrations of permeation enhancer until a homogeneous mixture was obtained. The mixture was degassed, cast on drug-impermeable backing membrane, dried, and finally attached with a protective release liner.

2.3. Accelerated stability testing study

The accelerated stability testing study of the best optimized transdermal patch was performed for 6 months, according to the ICH guidelines [7] under the following conditions: 40 ± 2°C temperature and 75 ± 5% relative humidity (RH) to confirm the stability potential of the drugs present in the best optimized formulation. The parameters determined during the stability study are listed in Table 1. Points of analysis 1 and 2 gave the eserine and 2-PAM content in best optimized transdermal patch formulation, respectively. Surface pH, diffusion analysis, visual inspection, attenuated total reflectance (ATR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) analysis of the samples were carried out at Points 3–8, respectively. The samples were withdrawn periodically (0, 2 weeks, 1 month, 2 months, 3 months and 6 months) and evaluated for different points of analysis. After 6 months, all points of analysis were carried out to assess the physicochemical stability of the optimized drugs-in-adhesive transdermal patch under accelerated study conditions. The adopted study plan design including the points of analysis under the accelerated stability study conditions is shown in Table 2.

2.4. Drug content uniformity

Samples consisting of optimized drugs-in-adhesive matrix type transdermal patches were stored according to the study plan design to determine the effect of changes in drug content on the stability of the formulations. The patches composed of eserine and 2-PAM were transferred to a 100-mL volumetric flask and extracted with ethanol. The flask was shaken for ~2 hours by mechanical means and then centrifuged at 6000*g* for 10 minutes and the supernatant was collected. The supernatant was then transferred to a 10-mL volumetric flask and diluted with mobile phase to complete the volume. All samples were filtered through a 0.45-μm filter before injection on the high-performance liquid chromatography system. The simultaneous chromatographic determination and estimation of eserine and 2-PAM present in drugs-in-adhesive matrix type transdermal patches were performed using the method developed and validated in our laboratory, as per the ICH guidelines, using an isocratic mode of elution and reverse-phase liquid chromatography under the following chromatographic conditions [8]: (1) composition of mobile phase: acetonitrile: 10 mM potassium dihydrogen phosphate and 10 mM heptane-1-sulfonic acid sodium salt

Table 1 – Points of analysis.

Points	Analysis
1	Eserine content in transdermal patch
2	2-PAM content in transdermal patch
3	Surface pH
4	Diffusion study
5	Visual inspection
6	Attenuated total reflectance
7	Differential scanning calorimetry
8	X-ray diffraction

2-PAM = pralidoxime chloride.

Table 2 – Accelerated stability testing study plan design including the points of analysis.

ICH conditions	Time period (mo)					
	0	0.5	1.0	2.0	3.0	6.0
(40 ± 2°C)/75 ± 5% RH	1–8	1–5	1–5	1–5	1–5	1–8

ICH = International Conference on Harmonisation; RH = relative humidity.

monohydrate in water (adjusted to pH 3.0 with orthophosphoric acid) at a ratio of 30:70, v/v, respectively; (2) mobile phase flow rate: 1.0 mL/min; (3) injection volume: 20 μL; (4) detection wavelength: 238 nm with a UV detector; and (5) column: C_{18} column (CHOMASIL, particle size 5 μm, 250 mm × 4.6 mm internal diameter).

2.5. *Surface pH*

The patches were kept in contact with casting solvent for 30 minutes. The surface pH of the patch was measured by means of a potentiometer [9].

2.6. *Diffusion study*

The *ex vivo* skin permeation through depilated rat abdominal skin of the optimized transdermal patch kept under accelerated stress conditions was conducted using a modified Franz diffusion cell apparatus at different periods of time.

2.7. *Visual inspection*

All the prepared patches were visually inspected for shape, clarity, smoothness, homogeneity, stickiness, uniformity and flexibility.

2.8. *ATR analysis*

ATR analysis (Bruker, Bremen, Germany) of an optimized transdermal patch before initiation and after completion of the stability study was carried out to assess the integrity and compatibility of the drugs with the pressure-sensitive adhesive component in the drugs-in-adhesives matrix type transdermal patch. The sample was placed in the sample holder and spectral scanning was undertaken in the wave number region between 4000 cm^{-1} and 500 cm^{-1} at a resolution of 4 cm^{-1} and scan speed of 2 mm/s.

2.9. *DSC analysis*

DSC analysis was performed on the optimized transdermal patch before and after the stability study. Initially, the moisture was removed by heating the samples and then each sample was accurately weighed into a platinum crucible 40-μL aluminum pan under hermetically sealed conditions, where α-alumina powder was used as a reference. Thermograms were recorded from 30°C to 500°C at the heating rate of 10°C/min under a constant flow of an inert nitrogen gas atmosphere at a flow rate of 20 mL/min. These analyses were done on a Perkin–Elmer® Instruments Pyris-Diamond TG/DTA (Osaka, Japan).

2.10. *XRD studies*

X-ray diffractograms were recorded for optimized transdermal patches before and after the stability study using an X-ray diffractometer (X-pert Pro; PANalytical, Netherlands) equipped with Ni-filtered CuKα radiation (θ = 1.5418 Å), at 30 kV and 15 mA. The samples were mounted on a sample holder and XRD scans were recorded up to 2 θ plane in the angle range of 1°–60° at a scan speed of 1°/min to estimate the crystallinity of the samples.

3. Results and discussion

The best optimized drugs-in-adhesive matrix type prophylactic transdermal patches composed of eserine and 2-PAM were subjected to accelerated stability testing as per ICH guidelines. The patches were stored at a temperature of 40 ± 2°C and 75 ± 5% RH for 180 days (6 months). The accelerated stability testing study data of the best-optimized formulation is shown in Table 3. The optimized formulation was found to be stable

Table 3 – Points of analysis performed in optimized transdermal patch formulation during accelerated stability testing conditions.

Points of analysis	Time period (mo)					
	0	0.5	1.0	2.0	3.0	6.0
1[a]	87.05 ± 2.53%	89.31 ± 2.15%	83.61 ± 1.63%	90.14 ± 1.71%	88.80 ± 3.40%	84.84 ± 2.71%
2[a]	91.40 ± 2.09%	89.77 ± 2.79%	90.98 ± 4.86%	93.17 ± 2.49%	88.08 ± 3.58%	90.31 ± 4.40%
3[b]	6.1	6.5	6.1	6.2	6.6	6.3
4[b]	89.65% in 72 h for eserine 94.35% in 72 h for 2-PAM	88.62% in 72 h for eserine 95.02% in 72 h for 2-PAM	90.51% in 72 h for eserine 96.58% in 72 h for 2-PAM	88.31% in 72 h for eserine 93.05% in 72 h for 2-PAM	87.44% in 72 h for eserine 91.12% in 72 h for 2-PAM	86.13% in 72 h for eserine 96.71% in 72 h for 2-PAM
5	Complies	Complies	Complies	Complies	Complies	Complies
6	Complies	—	—	—	—	Complies
7	Complies	—	—	—	—	Complies
8	Complies	—	—	—	—	Complies

— = not determined; 2-PAM = pralidoxime chloride.
[a] Indicates mean values of three observations, along with their standard deviation values.
[b] Single value.

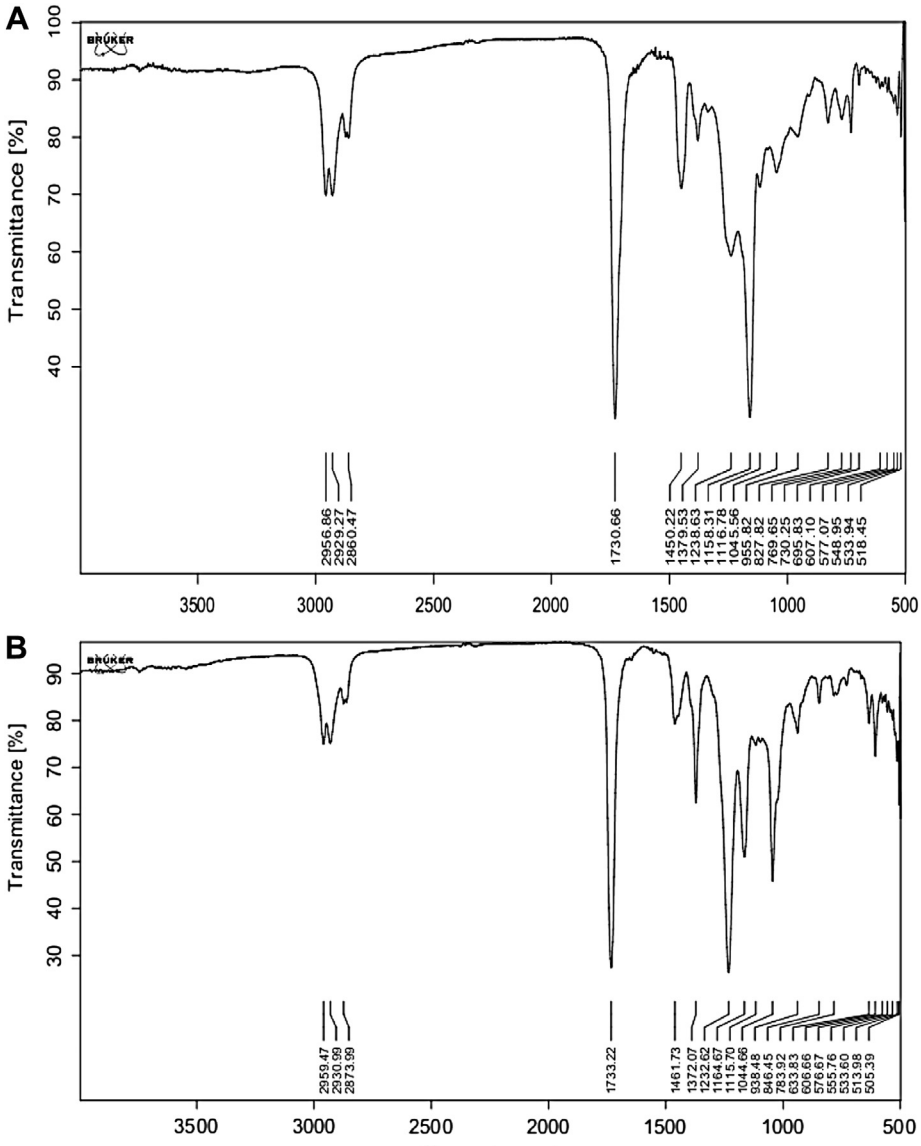

Fig. 1 – Attenuated total reflectance spectra of optimized transdermal patch (A) at 0 months and (B) at 6 months under accelerated stability testing conditions.

with respect to its drug content uniformity, surface pH, cumulative percentage release, and by visual inspection.

Furthermore, in order to assess the integrity, compatibility and stability of the final optimized formulations, the samples were analyzed by ATR, DSC and XRD. For this purpose, 0 and 6 months ATR, DSC and XRD were performed in order to rule out any alteration in drugs and excipient compatibility studies, changes in glass transition profile, and to access the appearance of crystallinity during the stability period. After 6 months, ATR, DSC and XRD revealed that there were no physicochemical changes under such conditions, as supported by the figures generated from the instruments, showing the integrity of the product.

3.1. *Drug content uniformity of the patches*

Results obtained after 0–6 months storage are presented in Table 3. The drug content of eserine and 2-PAM in optimized transdermal patches ranged from 83.61 ± 1.63% to 90.14 ± 1.71% and 88.08 ± 3.58% to 93.17 ± 2.49%, respectively. There were no significant changes in drug content of eserine and 2-PAM in optimized transdermal patches during the study. Observed variations were due to the drug-loading process. Drug contents were found to be uniform with relatively low standard deviation values. The results of content uniformity studies clearly indicate that the drug was uniformly distributed throughout the pressure-sensitive adhesives (PSAs) matrix. Temperature and RH did not show any significant effect on drug content uniformity of the transdermal patches. None of the main and other degradation products of eserine and 2-PAM were obtained in the optimized transdermal patches during high-performance liquid chromatography analysis. The drug content in the transdermal patches was stable under the accelerated stress conditions during the study. Minute variations in drug content were observed, but within allowed limits.

3.2. *Surface pH*

The surface pH of optimized transdermal patch solution was found to be in the range of 6.1–6.6, which indicated the absence of skin irritancy and stability of the product. No significant changes in pH value were observed in 6 months. Thus, the pH of the formulation was stable throughout the study.

3.3. *Diffusion study*

The *ex-vivo* skin permeation profiles of eserine and 2-PAM were investigated by modified Franz diffusion cell apparatus at different periods of time. The cumulative amounts of released drugs remained stable at 40°C/75% RH for 6 months, with a small amount of variation in complete drug release profile at 72 hours. The observed variation may have been due to the variation in theoretical drug loading during the fabrication process.

3.4. *Visual inspection*

There was no alteration in the visual character of the optimized transdermal patch during the period of analysis, especially in terms of shape, clarity, smoothness, homogeneity, stickiness, uniformity and flexibility. No red tint coloration over the patch formulation was observed under accelerated stress conditions for >6 months. The red tint coloration appeared due to the air, heat, light, traces of metals, and moisture sensitivity of eserine [10]. This might be due to the rigidity of the adhesive matrix system that compactly entrapped the drug moiety into its matrix, by preventing the presence of free eserine over the surface of the matrix. It was also evident with DSC and XRD analysis of the optimized patch that the drugs were homogeneously dispersed over the adhesive matrix system. No corresponding DSC and XRD peaks of pristine drugs were found, indicating the absence of free drugs.

3.5. *ATR analysis*

ATR analysis of optimized transdermal patches was performed before and after the stability study (Fig. 1A and B). The ATR profiles of optimized transdermal patches remained stable during storage under accelerated stress conditions. We

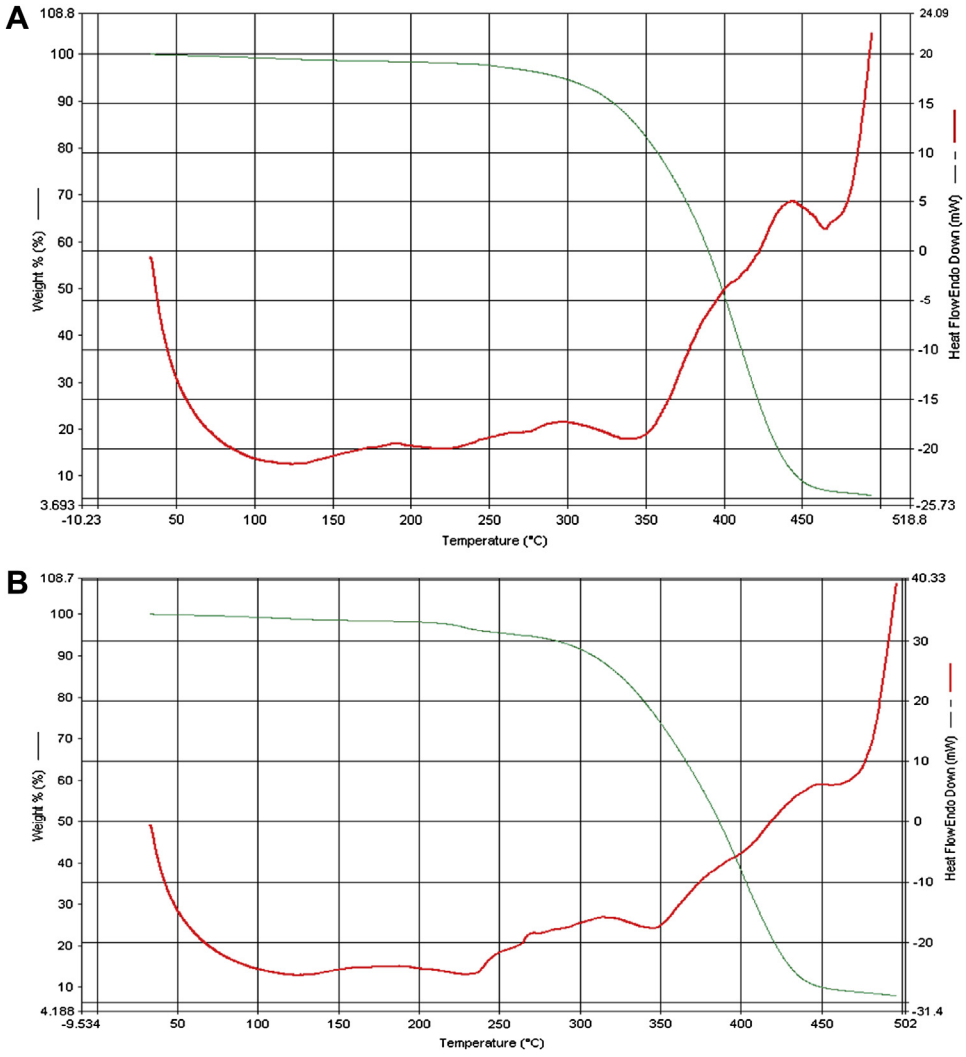

Fig. 2 – Differential scanning calorimetry thermogram of optimized transdermal patch (A) at 0 months and (B) at 6 months under accelerated stability testing study conditions.

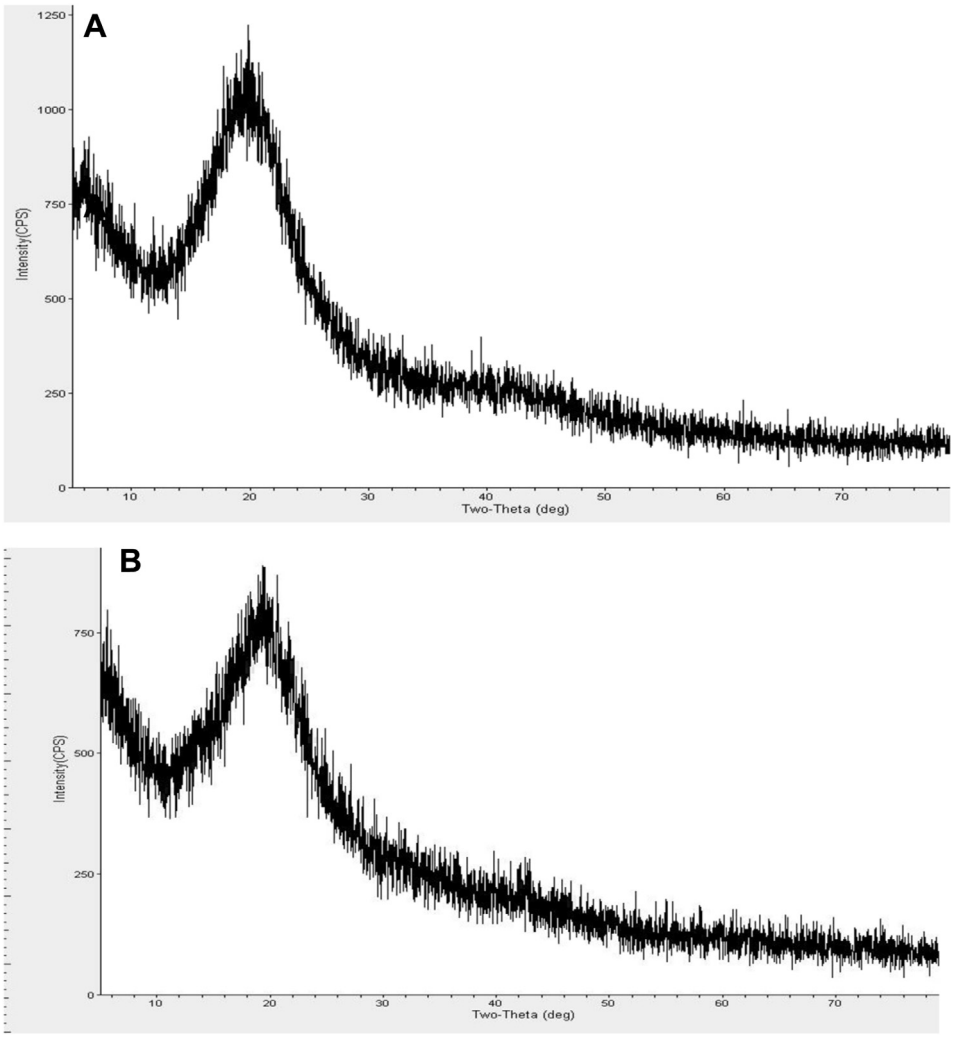

Fig. 3 – X-ray diffractograms of optimized transdermal patch (A) at 0 months and (B) at 6 months under accelerated stability testing study conditions.

observed a small shift in the characteristic peaks during ATR analysis. The observed variation was probably due to the drug-loading process and complete loss of organic solvents during storage.

3.6. *DSC and XRD analysis*

DSC (Fig. 2A and B) and XRD (Fig. 3A and B) profiles of optimized transdermal patches before and after stability testing did not show any significant changes, indicating the stability and non-crystallinity of the samples during storage.

4. Conclusion

The study showed successful results, and the optimized transdermal patches composed of eserine and 2-PAM were stable for 6 months at 40°C/75% RH. No significant changes in test parameters were noticed. To the best of our knowledge, this has not been shown previously for this type of product. The patches used in this study gave a complete drug-release profile for a period of 72 hours. The optimized prophylactic transdermal patch used in the study constitutes an interesting formulation for prophylaxis against (±)-anatoxin A poisoning.

Conflicts of interest

The authors declare that they have no conflicts of interest to declare in connection with the contents of this manuscript.

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