Development and Evaluation of Combined Drug Formulation for Autoject-injector, for Emergency Application in Organophosphate Poisoning

Neeti Jain[#*], Pravin Kumar[#], Deo Kumar[#], Yogendra Mavai[†], and R. Vijayaraghavan[#]

[#] *Defence Research Development Establishment, Gwalior–474 002*
[†] *ASBASJSM College of Pharmacy, Bela, Ropar, Punjab*
[*]*E-mail: neetijain1@rediffmail.com*

ABSTRACT

Atropine sulphate and pralidoxime chloride are considered as essential antidotes in the treatment of nerve agent poisoning. Now in India these antidotes are available in the form of self injectable autoinjectors. This study is designed with aim to replace two individual autoinjectors with single one. Stability of the components plays a vital role in the development of any dosage form, in this study we investigated the stability of the antidotes in combination (atropine sulphate+2 PAMCl) in single drug cartridges. In the present work shelf life of pralidoxime chloride (300 mg/ml) and atropine sulphate (1 mg/ml) solution in combination was evaluated by accelerated studies. The derived model is based on the rate equation and Arrhenius equation was used for extrapolation. Further, antidotal efficacy of atropine sulphate *in vitro*, using rat's isolated ileum and pralidoxime chloride by survival studies *in vivo* against dichlorvas in mice were evaluated, for further confirmation of analytical findings. The constituted formulation was found to be stable for 24 months.

Keywords: Atropine sulphate, pralidoxime chloride, autoinjectors, nerve agents, cholinesterase, oximes, bioassay

1. INTRODUCTION

Most of the organophosphorous (OP) compounds are cholinesterase (ChE) inhibitors and extremely toxic. Some of the OP compounds are regarded as nerve agents and are listed in the schedules of the chemical weapons convention. The deleterious actions of nerve agents and related OP pesticides are due to their ability to potently inhibit acetylcholinesterase (AChE) irreversibly, that leads to accumulation of acetylcholine in synaptic cleft and further to cholinergic crisis[1,2]. There is still a possibility of deliberate use of OP compounds on humans with the primary intention of inflicting a casualty or reducing the combat efficiency during war and by terrorists[3-6]. OP or nerve agent intoxication can be readily identified by its characteristic signs and symptoms such as constriction of pupil (miosis), hypersecretion, tremors and convulsions[7,8]. The most critical effects of nerve agent exposure are paralysis of respiratory muscles and inhibition of respiratory centre that leads to death[9]. Death is immediate on high dose exposure of toxicants, the only solution is immediate administration of atropine sulphate followed by pralidoxime chloride (2 [(hydroxyimino) methyl]-1-methylpyridinium chloride)[10]. Atropine sulphate is a competitive inhibitor of muscarinic receptor and pralidoxime chloride (PAM *Cl*) acts as an acetylcholine esterase reactivator. In the field presence of medical personnel for immediate drug administration during the exposure of nerve agents is not possible, for such emergencies self injectable autoinjectors containing drugs are in vogue, and are designed to permit a rapid and convenient means of intramuscular administration of the drugs[10-14]. This establishment has designed and developed the reusable autoject injectors in which the cartridges can be replaced after the expiry of shelf life of the drug (Fig. 1)[13]. It

has generally been considered that pralidoxime chloride is less stable in solution compared to atropine sulphate. The antidotal effectiveness of PAM *Cl* in autoinjector was reported against sarin, dichlorvas (DDVP) and diisopyropylphosphorofluridate (DFP), and found to be stable up to three years[13-15]. Three sets of two autoinjectors are provided to army persons for administration of required amount of PAM *Cl* (~1800 mg) along with atropine sulphate (~ 6 mg)[16]. In an emergency it is possible that soldiers may get confused which one should be used first. With the view of replacing six autoinjectors by three we have combined atropine sulphate (1 mg/ml) and PAM *Cl*

Figure 1. Traditional indigenous designed autoject-injector containing PAM *Cl* and atropine sulphate drugs for nerve agent poisoning.

(300 mg/ml) in a single drug cartridge for autoinjector. This combination has come out as a new formulation thus we have calculated the shelf life by accelerated stability testing as well as evaluated the biological activity of both the antidotes in separate models. The current regulations for stability tests are drafted by the International Commission for Harmonisation (ICH)[17,18]. Accelerated testing at high temperatures allows a significant reduction in testing time. The stress testing includes the effect of temperature in 10 °C increments as an accelerated temperature mode for the shelf life prediction of drug using Arrhenius theory.

The recommended storage condition for autoinjector is preferably 25 °C or less. The climatic conditions are highly variable in several parts of the world. The possible temperature and humidity fluctuations outside the labeled storage conditions affect the drug. The objective of present work is to make a reliable stability prediction for pralidoxime chloride and atropine sulphate in combination with self-injectable autoinjector drug cartridges for OP or nerve agent toxicity.

2. EXPERIMENT

2.1 Materials and Methods

Pralidoxime chloride and atropine sulphate were of Indian Pharmacopoeia (IP) grade, purchased from trade. Acetonitrile (HPLC grade) was purchased from J.T. Baker chemicals (India). DDVP was purchased from the trade. All other chemicals were of pharmaceutical or analytical grade and obtained from Qualigens (India) or Ranbaxy Fine Chemicals (India). For autoinjector cartridge materials viz., borosilicate glass cartridge (Borosilicate, India), bromobutyl septums (Bharat Rubber, India), and stainless steel needles (Iscon Engineering, India) were used.

2.2 Cartridges Preparation

Glass cartridge, convoluted needle and the septums were sterilized in a steam sterilizer. The needle was introduced in the glass cartridge and the back septum was placed and aligned. Pralidoxime chloride (300 mg/ml) along with atropine sulphate (1 mg/ml) was prepared in methyl paraben (1%w/v) and filtered through a 0.22 -μm membrane filters (Millipore Corp., Mass, USA). The final pH of drug solution was 4.0-4.5 and 2.2 ml of drug solution was filled in the glass cartridges, covered with the front septum under nitrogen purging and sealed with an aluminium cap using the hand operated crimping device. A set of 10 autoinjector containing the cartridges was used for the study and each were kept in a photostability chamber (Thermolabs Scientific, India) with 65 % (± 5 %) relative humidity as follows:

Group I : stored at 30 °C
Group II: stored at 40 °C.
Group II: stored at 50 °C
Group IV: stored at 60 °C

2.3 Assay of Pralidoxime Chloride

Aliquots of samples for chemical assay were withdrawn aseptically at the required intervals using a Hamilton syringe through out the study. The solution was subjected to assay procedure as per Indian Pharmacopoeia (IP). In brief, an aliquot was diluted with distilled water to a concentration of 100 μg/ml, and then re-diluted with 1N *NaOH* to obtain a final concentration of 10 μg/ml. Samples were assayed by UV analysis at 332 nm using a spectronic 1201 spectrophotometer (Spectronic Corporation, USA) within 10 minutes after the addition of *NaOH*. Standard solutions were prepared by dissolving reference standard in distilled water for making samples of various concentrations (50 mg/ml – 400 mg/ml). Atropine sulphate has not given any absorbance when followed by the same procedure that indicates no interference of atropine in the estimation of PAM *Cl*.

2.3.1 In-vivo Protection Studies Against DDVP

Randomly bred Swiss female mice (30-35 g) from the Institute's animal facility were used for the study. The animals were housed in polypropylene cages under controlled experimental conditions with free access to food (standard pellet diet, Ashirwad Ltd, India) and water until 2 h before and after the experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).This study has the approval of the Institutional Animal Ethical Committee.

This study is designed to assess the bioactivity of PAM Cl stored at various temperatures in combination with atropine sulphate in autoinjector drug cartridges. This study was carried out according to the method reported by Kumar[14], *et al*. Protective index (PI) was determined by the given formula:

In brief, for this study each group consisted of ten mice. Mice were treated with PAM *Cl* (30 mg/kg) and atropine sulphate (10 mg/kg) after DDVP (80 mg/kg, 160 mg/kg and 320 mg/kg, s.c) administration and observed up to 24 hrs for any mortality. For this study freshly prepared solution of atropine sulphate was used and for PAM *Cl*, required volume of the sample was taken from the prepared cartridges aseptically and diluted in water for injection (WFI). Both atropine sulphate and PAM *Cl* were injected immediately after injecting various doses of DDVP and PI was calculated according to previous reports[14]. The PI of stored samples was compared to PI of freshly prepared sample and per cent activity of PAM *Cl* was calculated.

2.4 Assay of Atropine Sulphate

The concentration of atropine sulphate was assayed according to the method reported by Vijayaraghavan[13], *et al*. with slight modifications. Waters (600 controller) HPLC system consisted of rheodyne manual injector with 10 μl sample loop with Waters Xterra MS C_{18} column was used for drug assay. Mobile phase consisted of 50 mM phosphate buffer (KH_2PO_4, pH 3.2 with Phosphoric acid) and acetonitrile (85+15). Sample was run at 22 °C column temperature and absorbance was recorded at 211 nm by 2487 UV detector. In the same mobile phase PAM Cl has not given any peak.

2.4.1 In-vitro Bioassay of Atropine Sulphate

Atropine sulphate was assayed by its muscarinic receptor blocking action. The percent blocking effect of acetylcholine (ACh) was determined on rat's isolated ileal muscle *in vitro*

in isolated organ bath. Isolated segment of rat's distal ileum, 1.5 cm to 2 cm in length, was vertically suspended in oxygenated Krebs' solution in 30 ml organ bath. The temperature of the bath was maintained at 36 °C. Before the administration of any drug, the tissues were stabilised at a resting tension of 0.5-1 g and equilibrated for 30 min washing out every 10 min.

Acetylcholine was dissolved in distilled water and added to the organ tube of bath. In all cases, after the maximal contractile effect had been obtained (1 min), the preparation was washed by an overflow perfusion of bath with 30–60 ml of Kreb's ringer solution, and the chamber was continuously superfused between drug administrations. Concentrations are expressed as final drug concentrations (e.g. ACh 1, 2, and 4 µg/ml) actually in contact with ileum tissue, and cover the full range from no effect to maximal contractile response. For selection of atropine sulphate (e.g. 1, 2, 4 ng/ml) dose in a separate set of experiments, it was added 10 min prior to ACh (1, 2 and 4 µg/ml) in three different doses. Atropine sulphate in dose of 2 ng/ml was found to block the effect of ACh in all three doses and this dose is selected for further studies (Table 2).

3. CALCULATION OF SHELF LIFE

For the first order reaction the rate constant at various temperature was noted from the slope of the graph by the formula:

Slope $= -K/2.303$

The recorded rate constant at various temperatures are given in Table 1. From the slope of the graph and rate constant the degradation rate was calculated.

The Arrhenius plot was drawn for the logarithm of velocity constant against the reciprocal of the absolute temperature.

$$K = Ae^{-E_a/RT}$$

where E_a is the activation energy, A is the Arrhenius constant, R is the molar gas constant and T is the absolute temperature.

The K value at desired temperature of 25 °C was obtained by extrapolating the Arrhenius plot. The obtained K value was then placed in the first order equation and $t_{90\%}$ was calculated from equation:

$$\log C = \log C_0 - Kt/2.303$$

where C is the potency at time t, and C_0 is potency at time t_0

$$t_{90\%} = \frac{\log(100/90) \times 2.303}{K}$$

4. STATISTICAL ANALYSIS

All the variables were analyzed by one-way ANOVA with student Newman-Keuls multiple comparison procedure. A probability of <0.05 is taken as statistically significant. Sigma Stat (Jandel Sci. USA) was used for statistical calculations.

5. RESULTS AND DISCUSSION

The shelflife of the drug or any formulation is the time on which it loses 10 per cent of its potency or biological activity or sterility when stored according to manufacturer's instruction. The use of kinetic and predictive studies for establishing credible expiration dates for pharmaceutical products are now accepted worldwide.

Our initial studies showed that PAM *Cl* and atropine sulphate drug cartridges are stable for two years at room temperature in autoinjector.

Both atropine sulphate and PAM *Cl* are currently used to protect human from nerve agent poisoning and are the recommended drugs for immediate application after nerve agent exposure[19]. In case of sarin exposure atropine sulphate

Table 1. Percent protective index of various temperature stored sample of PAM Cl + freshly prepared ATR against DDVP *in vivo*

Storage temperature (°C)	Per cent PI of PAM					
storage duration (months)	0.5M	1M	2M	4M	8M	16M
30	100.2±0.21	99.8±0.61	98.6±0.29	96.9±0.61	95.3±0.37	88.4±0.62
40	100.4±0.61	96.3±0.37	93.5±0.42	89.3±0.28	85.4±0.26	-
50	99.8±0.73	93.6±0.39	87.6±0.58	-	-	-
60	98.5±.61	91.6±0.35	84.3±0.54	-	-	-

LD_{50} of DDVP= 11.9 (7.78-18.7) mg/kg. PI of freshly prepared PAM solution + ATR= 26.9

Table 2. Selection of atropine sulphate concentration for isolated rat ileum against acetylcholine for bioassay

Sample	Con. ATR (ng/ml)	Ach (µg/ml)	% Blocking
ATR+PAM	16	1,2,4	100
ATR+PAM	8	1,2,4	100
ATR+PAM	4	1,2,4	100
ATR+PAM	2	1,2,4	100
ATR+PAM	1	1,2,4	53

antagonizes the attachment of acetylcholine at muscarinic receptor and PAM *Cl* reactivates the acethycholinesterase inhibited by nerve agent exposure[13]. In emergencies when there is a need to quickly obtain as adequate blood concentration of the above drugs and intravenous route of administration is not an option, a multicomponent containing autoinjector gives many advantages. In this study we have mixed both the drugs in single drug cartridges and develop the new formulation. In the development of pharmaceutical dosage form, one of the persistent challenges is the assurance of acceptable stability. The stability refers to the storage time allowed before any

degradation product of the drug reaches to a sufficient level to cause a risk to the patient. The content of PAM *Cl* and atropine sulphate in the cartridge was calculated as a percentage of the initial concentration after accelerated stability study. The biological activity of both the drugs was estimated at various time intervals on different models to assure the chemical analysis.

In the present experiment no difference was observed in the degradation curve from 30–60 °C indicating that the reaction follows the same order of kinetics. The temperature changes during the accelerated aging can alter the pH and leads to non-Arrhenius behavior of drug degradation[20]. In the study the pH of drug solution was noted through out the study and that was in the range of 3 to 4, indicating that the degradation reaction follows Arrhenius behavior.

Figure 2 shows log concentration of PAM *Cl* against different time point's at all four temperatures. This straight line confirms the first order reaction for the degradation of PAM *Cl*. It is well known that the drug degradation reaction usually follows first order kinetics in liquid preparations[21]. It was observed that higher the storage temperature, the larger the degradation constant values obtained.
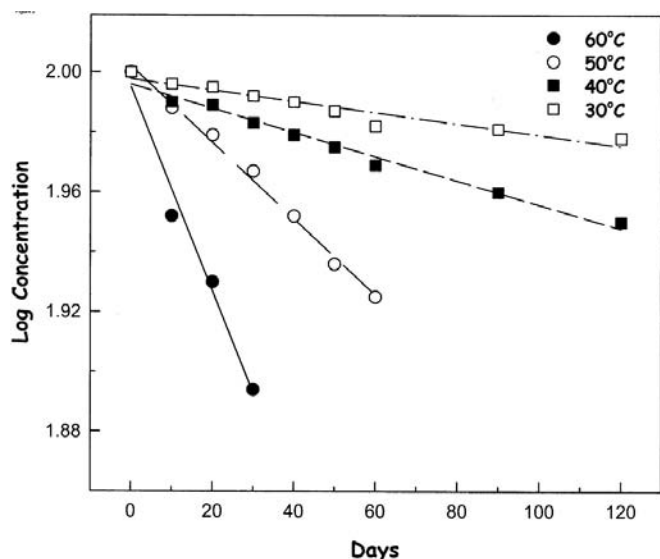

Figure 2. Effect of temperature on log concentration of PAM Cl (300 mg/ml) stored in combination with atropine sulphate (1 mg/ml).

Extrapolation presupposes that the same rate determining reaction is valid at both the accelerated and the extrapolated conditions. However, increasing deviation from the experimental conditions increases the risk for a change in the reaction pattern. The real time stability data of the sample were also calculated by alternatively keeping the samples at the room temperature.

Figure 3 curve shows degradation reaction constant (log K) against the inverse of storage temperature (1/T) for 300 mg/ml of PAM Cl solution in combination of 1 mg/ ml of atropine sulphate. With the help of this curve we have extrapolated the value of log K for PAM Cl at 25 °C.

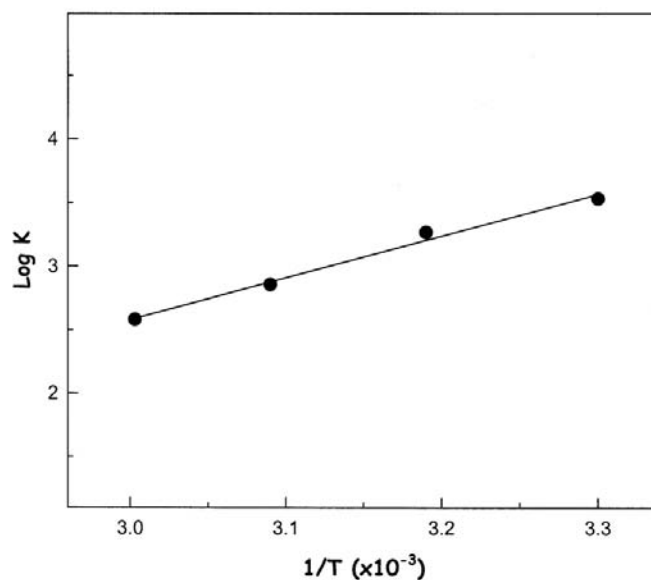Table 1 indicates the percent protective index (PI) of stored samples (30-60 °C) of PAM *Cl* against DDVP *in-vivo*

Figure 3. Log K *vs* 1/T for PAM Cl.

system. Results show that PAM *Cl* and atropine sulphate samples stored at room 30 °C are showing similar PI compared to a freshly prepared solution even after eight months. Thus the PAM *Cl* in combination with atropine sulphate is stable for more than one year at 30 °C. In previous studies it is reported that the administration of PAM along with atropine sulphate results in better protection compared to individual protection from each drug[13,14]. In this protection studies also we have used freshly prepared atropine solution along with diluted PAM *Cl* samples, even though atropine was incorporated with PAM *Cl*, After ten times dilution the concentration of atropine gets very low and not able to produce any effect as recommended dose is quite high (10 mg/kg). To overcome this problem we have used freshly prepared solution of atropine sulphte for protection studies.

Results indicate that samples of PAM *Cl* stored at 40 °C was having a PI of more than 94 per cent compared to freshly prepared PAM *Cl* solution even after one month storage and start reduction in PI after that. These biological findings match with our analytical findings in which also concentration of PAM get less than 90 per cent only after 45 days storage at 40 °C.

Figure 4 represents the log concentration of atropine sulphate against different time point's at all four temperatures. The straight line obtained confirms the first order reaction for the degradation. Figure 5 shows degradation reaction constant (log K) against the inverse of storage temperature (1/T) for 1 mg/ml of atropine sulphate solution stored in a combination of 300 mg/ml of PAM *Cl*. With the help of this curve we have extrapolated the value of log K for atropine sulphate at 25 °C.

Table 2 represents the effect of various concentrations of atropine against acetylcholine (ACh) on isolated rat's ileum *in vitro*. Data shows that at a minimum dose (2 ng/ml) atropine sulphate is able to block the effect of 4 µg/ml of ACh. On the basis of data represented we have selected 2 ng/ml for further studies.

Table 3 shows the per cent blocking effect of atropine sulphate samples stored at various temperatures against ACh
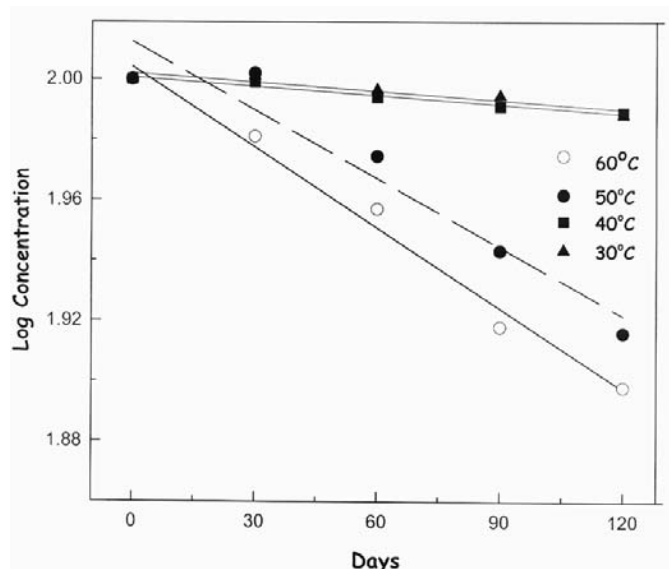
Figure 4. Effect of temperature on log concentration of atropine sulphate (1 mg/ml) stored in combination with PAM Cl (300 mg/ml).
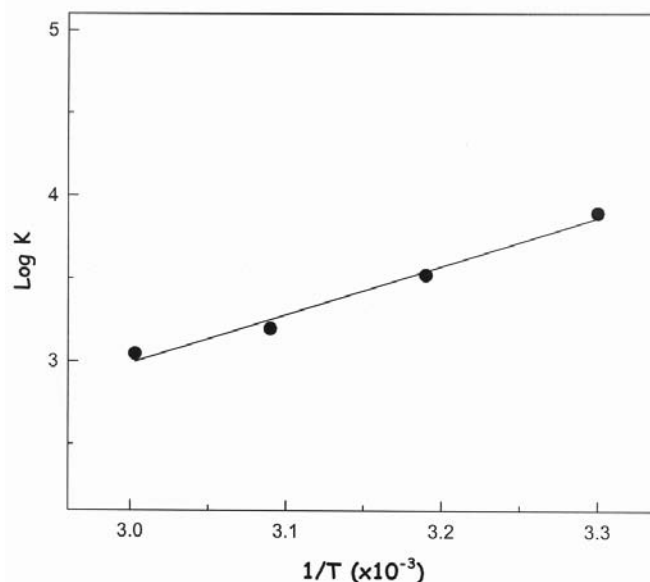

Figure 5. Log K *vs* 1/T for atropine sulphate.

Table 3. Per cent blocking effect of atropine (2 ng/ml) stored at various temperature at various days against various concentration of Ach (4 µg/ml)

Storage temperature (°C)	Per cent blocking effect of ATR					
storage duration (months)	0.5M	1M	2M	4M	8M	16M
30	99.8±0.53	99.4±0.36	99.2±0.19	99.4±0.72	99.7±0.41	98.9±0.33
40	99.3±0.48	99.4±0.31	98.9±0.51	98.3±0.29	97.3±0.36	95.3±0.18
50	98.9±0.36	99.1±0.27	98.1±0.35	96.3±0.26	94.1±0.16	92.4±0.49
60	98.7±0.29	97.7±0.32	96.5±0.34	94.1±0.19	90.2±0.23	85.1±0.31

on rat's isolated ileum. Biological studies showed that atropine sulphate is stable for more than 2 yrs even in addition to PAM *Cl*. Sample contain PAM *Cl* (300 mg/ml) along with atropine i.e. 300 time higher concentration, for observing the effect of PAM *Cl* on isolated rat's ileum we have used 10 times higher dose of PAM (20 ng/ml) compared to atropine against ACh and results showed that PAM *Cl* is not having any effect on ACh induced ileum constriction. Thus, this rat's isolated ileum model very well worked as an ideal model for atropine bioassay in drug solution. Atropine was found to be stable analytically as well as biologically by both the test methods for more than 1 year at 30 °C. Kumar[14], *et al.* reported that PAM *Cl* is biologically active for more than 36 months against DDVP and DFP, whereas in combination with atropine sulphate the stability get reduced for PAM *Cl*, but no effect was observed in atropine sulphate shelf life.

On the extrapolation stability of PAM Cl (300 mg/ml) in combination of atropine sulphate at 25 °C is more than 2 yr (810 days). The remaining content of the drug solution after storage at 30 °C for 1 year was found to be about 94.32 per cent for PAM *Cl*. Addition 10 per cent overages (330 mg/ml) will double the shelf life of PAM *Cl*, theoretical data reveal that PAM *Cl* is stable for more than 4 yrs with addition of 10 per cent overages, atropine sulphate is stable for a longer

time compared to PAM *Cl*. On the basis of stressed condition storage and real time data it can be predicted that the remaining concentration after the long–term study with duration almost equal to half of the extrapolated shelf life is much higher than 90 per cent.

6. CONCLUSION

Preliminary studies indicate that PAM *Cl* (300 mg/ml) in combination with atropine sulphate (1 mg/ml) is stable in clear glass cartridges for more than two years at room temperature in autoinjectors, with the addition of 10 per cent overages shelf life can be further enhanced.

REFERENCES
1. Shih, T.M.; Koviak,T.A. & Capacio, B.R. Anticonvulsants for poisoning by the organophosphorus compound soman: Pharmacological mechanisms. *Neurosci. Biobehaviour Rev.*, 1991, **15**(3), 349–62.
2. Taysse, L.; Calvet, J.H.; Buee, J.; Christin, D.; Delamanche, S. & Breton, P. Comparative efficacy of diazepam and avizafone against sarin induced neuropathology and respiratory failure in guinea pigs: Influence of atropine dose. *Toxicology,* 2003, **188**(2-3), 197–09.

3. Namba, T. Cholinestrase inhibition by organophosphate compound and its clinical effects. *Bulletin WHO*, 1971, **44**, 289-307.

4. Zoch; E. DieWirkung organischer Phosphors¨ aureester bzw. Phosphors¨ aureester auf versch. Enzyme. Arzneim. *Forsch*, 1971, **21**, 181-87. (French)

5. Namba, T.; Nolte, C.T.; Jackrel, J. & Grob, D. Poisoning due to organophosphate insecticides : Acute and chronic manifestations. *Amer. J. Med.,* 1971, **50**(4), 475-91.

6. Petroianu, G.A.; Hasan, M.Y.; Arafat, K.; Nurulain, S.M. & Schmitt, A. Weak inhibitors protect cholinesterases from stronger inhibitors (Dichlorvos). *Int. J. Toxicol.*, 2005, **24**(2), 79-86.

7. Holmstedt,B. Pharmacology of organophosphorus cholinesterase inhibitors. *Pharmacology Review,* 1959, **11**(3), 567-688.

8. Lallement, G.; Clarencon, D.; Brochier, G.; Baudichon, D.; Galonnier, M.; Blanchet, M.; Galonnier, M.; Blanchet, G. & Mestries, J.C. Efficacy of atropine/ Pralidoxime/ Diazepam or atropine/HI-6/prodiazepam in prmate's intoxication by soman. *Pharmacol. Biochem. Behaviour,* 2000, **56**(2), 325-31.

9. Worek, F.; Kirchner, T. & Szinicz, L. Effect of atropine and bispyridine oxime on respiratory and curculatory function in guinea pigs poisoned with sarin. *Toxicology,* 1995, **95**(1-3), 123-33.

10. Koelle, G.B. Clinical and experimental toxicology of organophosphates and carbamates, edited by Ballantyne, B.; Marrs, T.C. Butterworth-Heineman, Oxford, 1992. pp. 33–37.

11. Schroeder, A.C.; Digiovanni, J.H.; Bredow, J.V. & Heiffer, M.H. Praldoxime chloride stability indicating assay and analysis of solution samples stored at room temperature for ten years. *J. Pharma. Sci.,* 1989, **78**(2), 132–36.

12. Schier, J.G.; Ravvikumar, P.R.; Nelson, L.S.; Hellan, M.B.; Howland, M.A. & Hoffman, R.S. Preparing for chemical terrorism: stability of injectable atropine sulfate. *Acad Emerg. Med.,* 2004, **11**(4), 329–34.

13. Vijayaraghavan, R.; Jain, N.; Gautam, A.; Sharma, M.; Singh, S.; Kumar, D.; Singh, R.; Kumar, P.; Bhasker, A.S.B.; Gupta, A.K. & Jain, S. Evaluation of antidotal efficacy of atropine sulphate and pralidoxime chloride given by autoinjectors against nerve agents (Sarin) *Toxicity. J. Med. CBR Def.,* 2007, **5**, 1-12.

14. Kumar, P.; Vijayaraghavan, R.; Kumar, D.; Jain, N.; Swarnkar, H.M.; Waghmare, C.K.; Bhatacharya, B.K.; Sharma, M. & Jain, S. Shelf life studies of parlidoxime chloride solution in autoinjector cartridge stored at room temperature. *Curr. Tren. Biotech. Pharm.,* 2008, **2**, 251–59.

15. Sharma, R.; Gupta, P.K.; Mazumdar, A.; Dubey, D.K.; Ganesan, K. & Vijayaraghavan, R.A. Quantiatative NMR protocol for simultaneous analysis of atropine and obidoxime in parentral injection devices. *J. Pharm. Biomed. Anal.,* 2009, **49**(4), 1092-096.

16. Holstege, C.P.; Krik, M. & Sidell, F.R. Chemical warfare : Nerve agent poisoning. *Crit. Care Clin.,* 1997, **13**(4), 923-42.

17. Ivana, I.; Ljilana, Z. & Mira, Z. A stability indicating assay method for cefuroxime axetil and its application to analysis of tablets exposed to accelerated stability test conditions. *Journal of Chromatography A,* 2006, **A1119**(1-2), 209-15.

18. Gilalegre, M.E.; Bernabeu, J.A. & Camacho, M.A. Torres-Suarez AI. Statistical evaluation for stability studies under stress storage conditions. *Il Farmaco*, 2001, **56**(11), 877-83.

19. Connors, K.A.; Amidon, G.L. & Stella, V.J. Chemical stability of pharmaceuticals. John Wiely and Sons. USA. 1986.

20. Newton, D.W. & Miller, K.W. Estimating shelf life of drugs in solution. *J. Hosp. Pharm.*, 1987, **44** (7), 1633-640.

21. Carstensen, J.T. Solution kinetics. *In* Drug stability, principals and Practice, edited by J.T. Carstensen, C.T. Rhodes. Eds. 3ʳᵈ. Marcel Dekker*,* New York, 2000. pp. 19-56.

Contributors

Dr Neeti Jain obtained her masters in pharmacology from Pune University and PhD from Hamdard University, New Delhi. Currently working at Defence Research Development Establishment (DRDE), Gwalior. She extensively worked for pharmacological and toxicological profiling of chemical warfare agents and evaluation of efficacy and safety of newly synthesized antidotes against CW agents. She has expertise in animal model development for pharmacological screening of drugs and development of novel drug. She has more than 10 national and international research articles in her credit. She has supervised 4 MS and M. Pharmacy students.

Dr Pravin Kumar obtained his MSc (Medical) in Pharmacology from Smt NHLM Medical College, Ahmedabad (Gujarat University) and PhD (Pharmacology) from Jiwaji University, Gwalior. Currently working as Scientist 'F' at DRDE, Gwalior. His area of research include: Pharmacological and toxicological studies of chemical warfare (CW) agents, evaluation of efficacy and safety of newly synthesised antidotes against CW agents. He has expertise in inhalation toxicology including quantitative assessment of potential of peripheral sensory irritant compounds (tear gas) following inhalation exposure. He has published more than 55 research papers in national and international journals of repute.

Mr Deo Kumar obtained his MSc (Biochemistry) from University of Poona, Pune. Currently working as Technical Officer `C' at DRDE, Gwalior. He has expertise in recording and analysis of cardio-respiratory parameters using multi-channel polygraph in rodents. He is actively involved in the pharmacological and toxicological evaluation compounds defence interest. He has published more than 20 research papers in highly reputed journals with high impact factors.

Mr Yogendra Mavai obtained Masters in pharmacy in pharmacology from ASBASJSM College of Pharmacy, Bela, Ropar (Punjab). Presently he is working as Lecturer at Sri Ram Collage of Pharmacy, Banmore, Morena and engaged in research for screening of new drugs in various animal models.

Dr R. Vijayaraghavan obtained his MSc and PhD in Medical Pharmacology from JIPMER, Pondicherry and Jiwaji University, Gwalior, respectively. He is superannuated as Director and Outstanding Scientist from DRDE, Gwalior. Presently working as Director, Research at Saveetha University, Chennai. He was awarded with *DRDO Agni Award of Excellence in Self Reliance* in 2004 and *DRDO Titanium Trophy* in 2007. He has developed several products, viz., personal decontamination kit, reusable autoinjectors for nerve gas poisoning, first aid kit for CBW agents, and insect repellent spray and cream. He has about 200 research publications in reputed journals and about 60 patents, copyrights and designs.