

Intravenous application of HI-6 salts (dichloride and dimethansulphonate) in pigs: comparison with pharmacokinetics profile after intramuscular administration

Jana ŽDÁROVÁ KARASOVÁ^{1,2}, Filip ZEMEK², Martin KUNEŠ¹, Jaroslav KVĚTINA³, Jaroslav CHLÁDEK⁴, Daniel JUN^{1,2}, Jan BUREŠ³, Ilja TACHECÍ³, Kamil KUČA^{1,2}

¹ Biomedical Research Center, University Hospital, Hradec Králové, Czech Republic

² Departments of Public Health, Toxicology and Center of Advanced Studies, Faculty of Military Health Sciences, Hradec Králové, Czech Republic

³ 2nd Department of Internal Medicine – Gastroenterology, Charles University Faculty of Medicine & University Hospital, Hradec Králové, Czech Republic

⁴ Department of Pharmacology, Faculty of Medicine Charles University, Hradec Králové, Czech Republic

Correspondence to: Dr. Jana Zdarova Karasova, PhD.
Department of Public Health, Faculty of Military Health Sciences
Trebesska 1575, 500 01 Hradec Králové, Czech Republic
TEL: +420-973-253-116; FAX: +420-495-518-094; E-MAIL: karasova@pmfhk.cz

Submitted: 2013-06-21 *Accepted:* 2013-08-30 *Published online:* 2013-11-10

Key words: **HI-6 salts; intravenous administration; pharmacokinetic; nerve agents; oximes; pigs**

Neuroendocrinol Lett 2013;34(Suppl.2):74–78 PMID: 24362096 NEL341013A11 ©2013 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVES: Oxime HI-6 is an acetylcholinesterase reactivator therapeutically efficient against nerve agents. Because of their physico-chemical properties, oximes are typically applied intramuscularly (i.m.). This route of administration has also some disadvantages, and alternative strategies ought to be examined. We evaluated the pharmacokinetic profiles of two HI-6 salts after their intravenous (i.v.) administration, and compare the results with the known pharmacokinetics after i.m. administration.

METHODS: Pigs were administered with HI-6 salts (i.v.), either HI-6 dichloride (10.71 mg/kg) or molar equivalent HI-6 dimethansulphonate (13.59 mg/kg). Doses of the HI-6 salts corresponded with a standard HI-6 dichloride dose in one autoinjector (500 mg) and were recalculated for one kilogram of body weight.

RESULTS: The main pharmacokinetic parameters are comparable after i.v. and i.m. HI-6 administration. The compared pharmacokinetic parameters were half-life, terminal rate constant, mean residence time of the molecule in the body, clearance, and the apparent volume in the terminal phase. The bioavailability after i.m. administration was comparable with that of i.v.; these results suggest that the oxime is well released from the muscle depot. Significant differences were found in parameters C_{max} and T_{max} which are important in cases of emergency when rapidity and bioavailability are paramount for the success of treatment.

CONCLUSIONS: I.v. administration should solve the problem of rapid clearance. Infusion or bolus administration may be considered as a logical subsequent step in oxime treatment strategy. The main advantage is in maintenance of an effective therapeutic plasma concentration, a more easily achievable effective therapeutic concentration, and fewer local adverse reactions.

Abbreviations:

HI-6	- Oxime (Acetylcholinesterase reactivator)
DMS	- dimethansulphonate
AChE	- acetylcholinesterase
i.v.	- intravenous
i.m.	- intramuscular
C _{max}	- maximum of drug concentration in blood
T _{max}	- time when C _{max} occurred
TLC	- thin layer chromatography
NMR	- nuclear mass resonance
HPLC	- high performance liquid chromatography
UV/VIS	- ultraviolet/visible
AUC	- area under the curve

INTRODUCTION

Oxime HI-6 is a promising acetylcholinesterase (AChE; EC 3.1.1.7) reactivator. The high effectiveness is due to its wide spectrum of therapeutic activity against different structures of nerve agents. Oxime HI-6 is considered to be more effective than the commonly-used oximes (pralidoxime and obidoxime); relatively good reactivation potency was found in the treatment of VX, Russian VX, sarin, cyclosarin and also soman intoxication (Karasova *et al.* 2010; Lundy *et al.* 2011). The other advantage is its relatively low toxicity compared with the traditional oximes (Clement *et al.* 1995).

Oximes are typically applied intramuscularly (i.m.) mainly because of their physicochemical properties. HI-6 dichloride is supplied to military personnel in wet/dry autoinjectors as the first self-aid (Lundy *et al.* 2005). The most important factor that critically influences oxime therapeutic effectiveness is its rapid bioavailability in plasma and tissue (Jokanovic 2009); thus for example, application via the gastrointestinal tract is not considered as adequate.

According to previously published *in vivo* data, oxime HI-6 is relatively rapidly released from the muscle depot, and an effective plasma concentration is achieved in a few minutes after i.m. application (Karasova *et al.* 2013a). The maximal concentration is achieved in the time interval 20–30 min after administration. Subsequently, there is typically rapid elimination via the kidney. However, this route of application has also some disadvantages. The dose of oxime is limited by its solubility, and the application of a higher volume into the muscle is painful. The other problem relates to the solubility of oxime HI-6 in the autoinjector chamber. The time interval for autoinjector application is relatively short: the oxime HI-6 has to be dissolved in the injection vehicle in a very short time frame. Undissolved crystals may block the injection needle and lead to the reduction of total dose (Thiermann *et al.* 1998; Lundy *et al.* 2005). In the case of severely poisoned patients repeated autoinjector application has been recommended. However, intramuscular administration is invasive, and its repetition can be painful (Lamson *et al.* 2011). Thus new strategies should be examined.

The main aim of our work was to describe the pharmacokinetic profiles of two HI-6 salts, the dichloride

and dimethansulphonate (DMS), after i.v. administration, and to compare these results with the known pharmacokinetics profiles for i.m. administration. The applied doses were derived from the standard autoinjector dose (i.m.), and recalculated to correspond with the weight of the experimental animals.

MATERIAL AND METHODSChemicals

Salts of oxime HI-6 were synthesized in our laboratory by the process previously described. The structural parameters and purity of HI-6 oxime were confirmed using TLC, NMR and HPLC analysis. (Jun *et al.* 2008, 2010; Kuca *et al.* 2008). Purity was 99%. Other chemicals (analytical reagent grade) were purchased from standard commercial sources (Merck, Darmstadt, Germany and Sigma-Aldrich, Steinheim, Germany). Double-distilled and deionized water was used for mobile phase preparation.

Instrumentation

All analyses were performed on 1260 Infinity series Agilent liquid chromatograph (Palo Alto, CA, USA), composed of degasser, quaternary pump, light-tight autosampler unit set, thermostated column compartment and UV/VIS detector. The maximum HI-6 absorption is 310 nm. Agilent ChemStation software (Palo Alto, CA, USA) was used for analysis of results.

Separation conditions for HI-6 salts in plasma and brain samples

Analytical column LiChrospher® 60, 250×4.6 (5 µm) was used for analysis with installed guard column (4×4 RP-select B; Merck, Darmstadt, Germany). The mobile phase composition was 80:20 (v/v) purified water/acetonitrile, with aqueous component 3 mmol/l octane sulphonic acid and 1 mmol/l tetramethylammonium chloride, pH=1.8. The flow rate of the mobile phase was 1.4 ml/min. All chromatograms were obtained at conditioned temperature (30 °C) (Karasova *et al.* 2012).

Animal treatment

The use of animals in this study was under the supervision of the Ethics Committee (Faculty of Military Health Sciences in Hradec Kralove, University of Defence in Brno, Czech Republic). The presented study was performed on juvenile female Landrace pigs, *Sus scrofa domestica* (VEMAS Inc., Zamberk, Czech Republic). Animals were housed indoors at the vivarium (temperature 18±2 °C, humidity 55±5%), and under standard 12h light/dark cycles. The animals received standard laboratory diet A1 (VEMAS Inc., Zamberk, Czech Republic) and were allowed tap water *ad libitum*.

The animals were divided into two groups (n=3) and labeled by ear tags. The average body weight was approximately 22±2 kg. Experiments were conducted after 14 days of acclimatization. All animals were

premedicated (i.m.) with ketamine 30 mg/kg (Narkamon, Spofa, Czech Republic) in combination with azaperone 2 mg/kg (Stresnil, Janssen Pharmaceutica, Belgium) and atropine 0.05 mg/kg (Atropin Biotika A.U.V., Slovak Republic). Subsequently, the animals were placed in the dorsal recumbent position on an operating table, intubated with an ET 6.0-6.5 and anaesthetized by isoflurane inhalation (at concentration 2–0.5%). Venous access was established by inserting a 16 gauge i.v. catheter (Cavafix Cetro, B-Braun, Germany) into vena jugularis externa. Catheter outlet was via subcutaneous tunnel behind the ear, and fixed to the skin tissue (Lundy *et al.* 2005; Karasova *et al.* 2013a).

After anesthesia stabilization, the oxime HI-6 dichloride (i.v.; 10.71 mg/kg, prepared in situ using 0.9% saline) or the oxime DMS salt (i.v.; 13.59 mg/kg, prepared in situ using 0.9% saline) was administered. Doses applied i.v. correspond with doses applied i.m. Blood samples (500 µl) were serially removed at regular time intervals: 1, 3, 5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, 180 and 240 min after i.v. administration, and were drawn into heparinized tubes. Plasma was prepared by centrifugation (1600 g, 10 min, 4°C, Universal 320R, Hettich, Germany) and frozen at –80°C prior to analysis (one week).

Sample preparation for HPLC analysis

200 µl plasma samples were mixed with 50 µl trichloroacetic acid in order to precipitate proteins. The samples were spun at 21 000 g at 4°C for 15 minutes (M 240R, Hettich, Germany), and the supernatant used for HPLC analysis.

Calibration

A calibration curve was established using plasma samples spiked with oxime HI-6 (1.25; 2.50; 5.00; 10.00; 20.00; and 40.00 µg/mL samples, in triplicate). The retention time of oxime HI-6 was ~6.8 min. The amounts of oxime HI-6 in each sample were converted to concentration by interpolation of the calibration curve using the data analysis and statistical software program Prism4 (Graph Pad Software, USA).

Pharmacokinetics modeling

Pharmacokinetics modeling was performed using Kinetica software, version 4.0 (InnaPhase Corporation, Thermo Fisher Scientific Inc. Waltham, MA, USA). Population parameter values were estimated using the measured plasma concentrations of the animals and then the animal's pharmacokinetic parameters were obtained according to the maximum a posteriori Bayesian fitting method. Moreover, a standard non-compartmental approach was used to determine C_{max} and T_{max} from the experimentally assayed concentrations. The area under the plasma concentration-time curve (AUC) total or in the time interval was calculated by the linear trapezoidal method.

RESULTS

The *absorption curves* of both HI-6 salts after i.v. administration were measured and compared to examine the possibility that the DMS salt might have better pharmacokinetic profile than the dichloride salt. In addition, the achieved i.v. results were compared with the known pharmacokinetics profile after i.m. administration of the same doses of both HI-6 salts.

Figure 2 shows the plasma HI-6 concentrations measured following i.v. injection of 10.71 mg/kg of HI-6 dichloride compared with values obtained after similar administration of the molar equivalent of HI-6 DMS (13.59 mg/kg). The pharmacokinetic constants calculated for i.v. administration are detailed in Table 1. In the same table the statistically important constants of the i.v. and i.m. pharmacokinetic profiles are also compared.

Many of the main pharmacokinetic parameters are comparable between the i.v. and i.m. routes of administration. No significant differences between HI-6 salts were found. The bioavailability after i.m. administration was comparable with that of i.v. Significant differences were found in parameters C_{max} and T_{max} .

DISCUSSION

The therapeutic effectiveness of all oximes is based on their bioavailability and fast absorption after administration (Jokanovic *et al.* 2009). Although they are quickly released from the muscle depot into the vascular system and plasma (Karasova *et al.* 2013a), the subsequent transport to the point of action is limited by biological barrier (Karasova *et al.* 2013b). The main therapeutic target is AChE in the central and peripheral nervous system and neuromuscular junctions. Their lower biological barrier to permeation is the main reason why oximes are applied i.m. Other non-invasive routes of administration are still considered as non-effective (Voicu *et al.* 2010a,b).

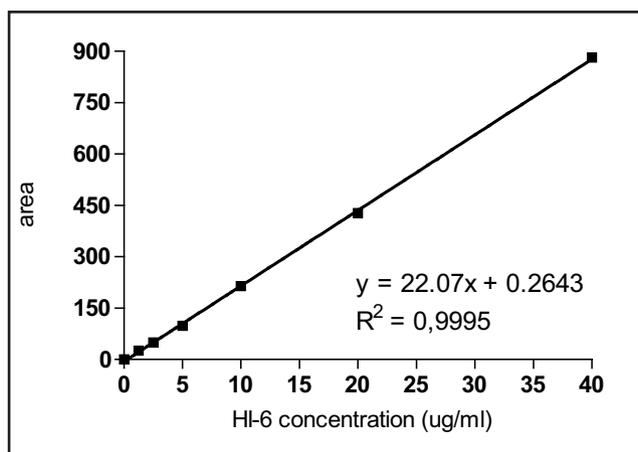
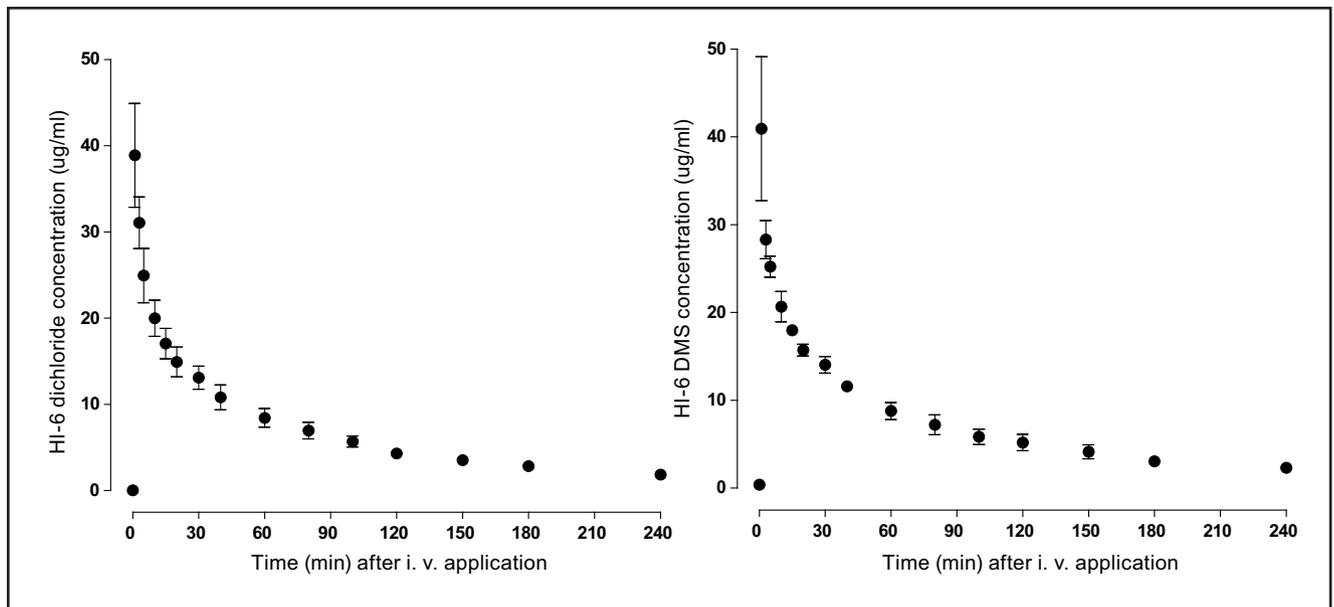


Fig. 1. Calibration curve of HI-6 for chromatographic analysis

Tab. 1. Pharmacokinetic parameters of HI-6 obtained after a single i.v. and i.m. injection of HI-6 dichloride (10.71 mg/kg) and HI-6 DMS (13.59 mg/kg).

Parameter	HI-6 2 Cl (i.v.)	HI-6 DMS (i.v.)	HI-6 2 Cl (i.m.)	HI-6 DMS (i.m.)
C_{max} ($\mu\text{g/ml}$)	38.9 \pm 6.02	52.59 \pm 19.63	19.0 \pm 0.93	23.8 \pm 3.08
T_{max} (min)	–	–	18.3 \pm 7.27	13.3 \pm 3.33
AUC_{total} (min. $\mu\text{g/ml}$)	2171 \pm 233	2396 \pm 180	1969 \pm 254	2259 \pm 329
$AUC_{0-240\text{min}}$ (min. $\mu\text{g/ml}$)	1931 \pm 182	2069 \pm 139	1747 \pm 235	1965 \pm 232
λ_z (1/min)	0.0079 \pm 0.0008	0.0069 \pm 0.0005	0.0093 \pm 0.0012	0.0086 \pm 0.0014
Half-life (min)	89.53 \pm 8.68	101.33 \pm 8.02	76.8 \pm 10.3	84.6 \pm 12.0
MRT (min)	107 \pm 7.9	119 \pm 13.7	108 \pm 10.3	109 \pm 10.5
Clearance (ml/min/kg)	4.53 \pm 0.63	3.96 \pm 0.33	–	–
V_z (l/kg)	0.57 \pm 0.042	0.58 \pm 0.061	–	–
Clearance/F (ml/min/kg)	–	–	4.31 \pm 0.59	3.81 \pm 0.63
V_z/F (l/kg)	–	–	0.48 \pm 0.084	0.044 \pm 0.008

All values are means \pm S.E.M. (n=3). Symbols: C_{max} = maximum concentration of HI-6 in the plasma, T_{max} = time to reach C_{max} , AUC_{total} = area under the concentration-time curve of plasma HI-6 from zero up to infinity, $AUC_{0-240\text{min}}$ = area under the concentration-time curve of plasma HI-6 from zero up to 240 min, λ_z = terminal rate constant, MRT = mean residence time of molecule in the body, CL/F = apparent total plasma clearance (i.m.), V_z/F = apparent volume of distribution during terminal phase (i.m.). Pharmacokinetic parameters after i.m. application were previously published in Toxicology Letters (Karasova et al. 2013a).

**Fig. 2.** Absorption curves of HI-6 salts (dichloride and DMS) following i.v. administration in equimolar doses – concentration of HI-6 salts ($\mu\text{g/ml}$) versus time (min) after administration. Each point in the time curves represents the mean with SEM (n = 3).

The ionization of oximes at physiological pH also plays a crucial role in their distribution process. The oxime ionized state explains the pharmacodynamic effect (reactivation potency) at the AChE active site (Worek *et al.* 2011). On the other hand, the passive transport through the biological barriers is strictly limited by this structural state (Mercey *et al.* 2012).

The other factor that influences oxime efficacy is the oxime elimination process. Oximes are rapidly

eliminated from plasma via the kidneys. This fast elimination would be explained by their low barrier permeation, such that much of the oximes stay in plasma (Karasova *et al.* 2013a). A second important factor is their low plasma protein binding. The fast elimination that leads to reduction of plasma oxime levels should be considered as a major reason for failure of therapy.

From the pharmacokinetics point of view, the main focus of investigation should be on simplifying

the application routine, reducing adverse effects, and improving oxime bioavailability (Kušic *et al.* 1991; Clement *et al.* 1995). In recent years, intravenous formulation for HI-6 administration has been discussed. Intravenous administration may be preferred over an i.m. application in some case of emergency (Pawar *et al.* 2006).

If we compare important pharmacokinetic parameters after i.v. and i.m. administration, many of them are comparable. Also, no significant differences between the HI-6 salts were found. Comparable pharmacokinetic parameters were half-life, terminal rate constant, mean residence time of the molecule in the body, clearance, and apparent volume in the terminal phase. The bioavailability after i.m. administration was comparable to that of i.v.; these results suggest that oxime is well released from muscle depot. As we expected, significant differences were found in classical parameters such as C_{max} and T_{max} . Both these parameters are important in the case of emergency when rapidity and bioavailability is critical for the success of treatment (Yanagisawa *et al.* 2006).

Intravenous application should also solve the problem of rapid clearance. Infusion application may be considered as a logical subsequent step in oxime treatment strategy. The main reason is the maintenance of an effective therapeutic plasma concentration, more easily achievable effective therapeutic concentration, and fewer local adverse reactions. Especially for in-hospital use, it is preferable that the oxime is delivered independently from other treatments in order to allow the physician to titrate the dose of oxime and atropine individually, according to the casualty's condition.

ACKNOWLEDGEMENTS

The study was supported by the research grant IGA NT/14270 (Ministry of Health, Czech Republic) and Ministry of Defence; A long-term organization development plan 1011.

REFERENCES

- Clement JC, Bailey DG, Madill HD, Tran LT, Spence JD (1995). The acetylcholinesterase reactivator HI-6 in man: Pharmacokinetics and tolerability in combination with atropine. *Biopharm Drug Dist.* **16**: 415–425.
- Jokanovic M (2009). Medical treatment of acute poisoning with organophosphorus and carbamate pesticides. *Toxicol Lett.* **190**: 107–115.
- Jun D, Stodulka P, Kuca K, Koleckar V, Dolezal B, Simon P, et al (2008). TLC Analysis of Intermediates Arising During the Preparation of Oxime HI-6 Dimethanesulfonate. *J Chrom Sci.* **46**: 316–319.
- Jun D, Stodulka P, Kuca K, Dolezal B (2010). High-performance liquid chromatography analysis of by-products and intermediates arising during the synthesis of the acetylcholinesterase reactivator HI-6. *J Chrom Sci.* **48**: 694–696.
- Karasova JZ, Kassa J, Pohanka M, Musilek K, Kuca K. (2010). Potency of HI-6 to reactivate cyclosarin, soman and tabun inhibited acetylcholinesterase – In vivo study. *Lett Drug Des Discov.* **7**: 516–520.
- Karasova JZ, Hnidkova D, Pohanka M, Musilek K, Chilcott RP, Kuca K (2012). Pharmacokinetics of acetylcholinesterase reactivator K203 and consequent evaluation of low molecular weight antioxidant/markers of oxidative stress. *J Appl Biomed.* **10**: 71–78.
- Karasova JZ, Pavlik M, Chladek J, Jun D, Kuca K (2013a). Hyaluronidase: Its effects on HI-6 dichloride and dimethanesulphonate pharmacokinetic profile in pigs. *Toxicol Lett.* **220**: 167–171.
- Karasova JZ, Zemek F, Kassa J, Kuca K (2013b). Entry of oxime K027 into the different parts of rat brain: comparison with obidoxime and oxime HI-6. *J Appl Biomed.* **11**; *In press.* DOI 10.2478/v10136-012-0037-4.
- Kuca K, Stodulka P, Hrabanova M, Hanusova P, Jun D, Dolezal B (2008). Preparation of oxime HI-6 (dichloride and dimethanesulphonate) – antidote against nerve agents. *Defence Sci J.* **58**: 399–404.
- Kušic R, Jovanović D, Radlejevic S, Joksovic D, Todorovic V, Boskovic B, et al (1991). HI-6 in man: efficacy of the oxime in poisoning by organophosphate insecticides. *Hum Exp Toxicol.* **10**: 113–118.
- Lamson MJ, Sitku-Green D, Wannarka GL, Mesa M, Andrews P, Pellock J (2011). Pharmacokinetics of diazepam administered intramuscularly by autoinjector versus rectal gel in healthy subject A Phase I, randomized, open-label, single-dose, crossover, single-centre study. *Clin Drug Invest.* **31**: 585–597.
- Lundy PM, Hill I, Lecavalier P, Hamilton MG, Vair C, Davidson C, et al (2005). The pharmacokinetics and pharmacodynamics of two HI-6 salts in swine and efficacy in the treatment of GF and soman poisoning. *Toxicology.* **208**: 399–409.
- Lundy PM, Hamilton MG, Sawyer T, Mikler J (2011). Comparative protective effects of HI-6 and MMB-4 against organophosphorus nerve agent poisoning. *Toxicology.* **285**: 90–96.
- Mercey G, Verdelet T, Renou J, Kliachyna M, Baati R, Nachon F, et al (2012). Reactivators of acetylcholinesterase inhibited by organophosphorus nerve agents. *Accounts Chem Res.* **45**: 756–766.
- Pawar KS, Bhoite RR, Pillay CP, Chavan SC, Malshikare DS, Garad SG (2006). Continuous pralidoxime infusion versus repeated bolus injection to treat organophosphorus pesticide poisoning: a randomised controlled trial. *Lancet.* **368**: 2136–2141.
- Thiermann H, Schreiner R, Eyer P (1998). Dissolution kinetics of unstable drugs in two-compartment autoinjectors: Analysis of the individual shaking behaviour and influence of various shaking parameters on the dissolution rate of HI-6 in an automated system. *Int J Pharm.* **170**: 23–32.
- Voicu VA, Thiermann H, Radulescu FS, Mircioiu C, Miron DS (2010a). The toxicokinetics and toxicodynamics of organophosphonates versus the pharmacokinetics and pharmacodynamics of oxime antidotes: Biological consequences. *Bas Clin Pharm Toxicol.* **106**: 73–85.
- Voicu V, Sora I, Sarbu C, David V, Medvedovici A (2010b). Hydrophobicity / hydrophilicity descriptors obtained from extrapolated chromatographic retention data as modeling tools for biological distribution: Application to some oxime-type acetylcholinesterase reactivators. *J Pharm Biomed Anal.* **52**: 508–516.
- Worek F, Aurbek N, Wille T, Eyer P, Thiermann H (2011). Kinetic analysis of interactions of paraoxon and oximes with human, Rhesus monkey, swine, rabbit, rat and guinea pig acetylcholinesterase. *Toxicol Lett.* **200**: 19–23.
- Yanagisawa N, Morita H, Nakajima T (2006). Sarin experiences in Japan: acute toxicity and long-term effects. *J Neurol Sci.* **249**: 76–85.