Open Access Scientific Reports

Research Article Open Access

Keywords: Guinea Pig; Galanthamine; Liquid chromatography; Mass spectrometry; Chemical warfare agents; Nerve agents

Introduction

Organophosphorus compounds, since their early introduction in agriculture as a pesticide and later as Weapons of Mass Destruction (WMD) in the form of Chemical Warfare Agents (CWA), are a global concern for their toxicity in humans [1-3]. Organophosphorus G-Series (Sarin (GB), Cyclosarin (GF), Soman (GD), Tabun (GA)), and V-Series (VE, VG, VM, VX) compounds are one classi cation of CWA by convention, which act to severely disrupt neurological regulation within biological systems through their irreversible inhibition of the enzyme Acetylcholinesterase (AChE) [4-6]. e degree of toxicity (e.g. chronic to acute) or severity of the disruption (e.g. mild71((e.g.)-1719reverel

Citation:

Citation: Steiner WE, Pikalov IA, Williams PT, English WA, Hilmas CJ (2012) Development of a Novel LC/MS/MS Extraction Assay for Galanthamine in Guinea Pig Plasma and its Application to Nerve Agent Countermeasures. 1: 149. doi:10.4172/scientificreports.149

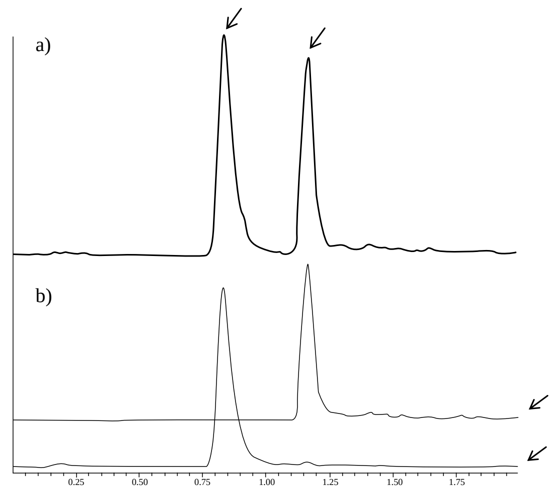

Figure 2: Positive ion mode chromatograms with a) being the total precursor ion current (TIC) chromatogram of a mixture of GAL and DPH and b) the extracted precursor ion current (EIC) chromatograms for GAL and DPH with retention times being found to be 0.83 and 1.17 min, respectively.

acceptors), xd (interaction with dioxane - proton donors) and xn (interaction with nitromethane - dipole-dipole interactions) that could be ne tuned for chromatographic separation integrity and extraction recovery selectivity. Consideration was also given in the selection process to organic solvents with lower boiling points in an e ort to increase the rate at which the upper organic layer could be evaporated in the sample preparation process. ereby, minimizing the rate-limiting time step needed for sample preparation. Ultimately, trichloromethane with a P' value of 4.1 (e.g. 0.1 for non-polar hexane and 9.0 for polar water) and a boiling point of around 61°C was used. Optimal extraction recoveries as determined by comparing the peak area for plasma samples for three replicate inter-batch assays that were spiked before and a er extraction were obtained for both GAL (0.025 (8.69 nM), 0.25 (86.9 nM), and 1.00 µg/mL (348 nM)) and DPH (0.25 µg/mL (97.9 nM)) in 10 min with a trichloromethane liquid-liquid extraction.

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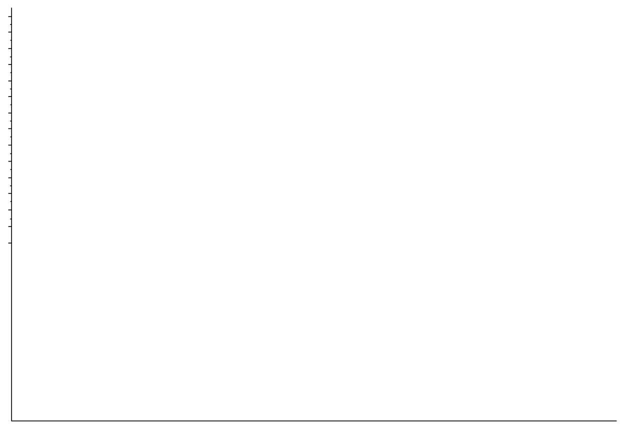
Mass spectrometry development

Chromatographic mobile phase composition with a continuous isocratic ratio of (65:35 v/v) mobile phase A (0.05% formic acid, 0.2% glacial acetic acid, 99.75% HPLC grade water) to mobile phase B (0.1% formic acid, 9.9% HPLC grade water, 90% methanol) was found to be optimal for both chromatography and mass spectrometry ionization e ciency a er many trials when combined with a Waters Symmetry Shield 3.5 µm packed RP18 guard (3.9 mm x 20 mm) and analytical (4.6 mm x 75 mm) column assembly. From a chromatography perspective the combination of select mobile phase solvent constituents (e.g. methanol organic modi er and formic acid to displace ion pairs) and stationary phase media type (e.g. shielded silanol non-polar RP18 packing) provided an environment in which equilibrium chemistry could take place between the two phases to provide improved Gaussian peak shapes with consistent baseline resolution, as seen in Figure 2, for GAL and DPH in a relatively short amount of time (i.e. 0.83 and 1.17 min, respectively). From a mass spectrometry perspective the use of key mobile phase solvent constituents (e.g. formic and acetic acid) helped to greatly improve the sensitivity of the analysis by increasing the positive ionization e ciency in the source region of the mass spectrometer.

Mass spectrometric positive ion Turbo IonSpray mode quanti er precursor to product ion (M+H)+ transitions of 288-to-213 m/z and 256-to-167 m/z were found a er a few trials to be the most sensitive and selective for GAL and DPH respectively, in this study. Here, the precursor ions (e.g. 288 and 256, GAL and DPH) were selected in Quadrupole 1 (Q1), fragmented by collisionally induced dissociation (CID) in Quadrupole 2 (Q2), and then product ions (e.g. 213 and 167, GAL and DPH) were selected in Quadrupole 3 (Q3). e quanti er precursor to product ion (M+H)+ transition of 288-to-213 m/z for GAL is shown in Figure 3 as a) full scan Q1 showing the GAL precursor 288 m/z ion and b) full scan Q3 showing the GAL product 213 m/z ion. In Figure 4, the quanti er precursor to product ion (M+H)+ transition of 256-to-167 m/z for DPH is shown as a) full scan Q1 showing the DPH precursor 256 m/z ion and b) full scan Q3 showing the DPH product 167 m/z ion. e same type of mass spectrometry ion selection was also done for the quali er precursor to product ion (M+H)+ transitions of 288-to-231 m/z and 256-to-152 m/z for GAL and DPH, respectively. is ensured that if a case did happen to arise in which the quanti er



ion transitions for GAL and DPH were not present the quali er ion transitions could be used for the quantitation of GAL. e modes of fragmentation of the 288 m/z GAL and 256 m/z DPH precursor ions producing product ions at an m/z of 197, 209, 213, 225, 231, 270 for GAL and 152, 167, 183, 230, for DPH have been established in literature [31,32,34,35].

Conclusions

A new high throughput sample preparation extraction LC/MS/MS analysis assay has been developed with commercially available materials to enable future researchers the ability to reproducibly and sensitively quantitate galanthamine in guinea pig plasma. e sample preparation employing a 10 min trichloromethane liquid-liquid extraction gave consistent extraction recoveries of GAL and the internal standard DPH from 160 µL plasma sample volumes. e LC/MS/MS analysis method operated in the positive ion MRM Turbo Ionspray mode yielding highly selective quanti er precursor to product ion (M+H)+ transitions for GAL (288-to-213 m/z) and DPH (256-to-167 m/z). Sample run times where on the order of 1.50 min per sample. Most importantly, it is envisioned that this new extraction assay with low sample volume requirements, rapid GAL extraction recovery, and rapid sample runtimes would be bene cial to not only a wide range of researchers researching pharmacokinetics, bioavailability, or bioequivalence studies but of more speci c interest to researchers exploring applications of organophosphorus nerve agent poisoning countermeasures in guinea pigs and humans.

Acknowledgements

The authors would like to acknowledge: Dr. James Bruce's Laboratory from the University of Washington Genome Sciences, Seattle, WA; The University of Washington's Proteomics Resource (UWPR95794); Dr. Jeff Jones's Laboratory from Washington State University, Pullman, WA for open use of a variety of liquid chromatography and mass spectrometry instrumentation; and the United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, MD, for their continued scienti fc correspondence and support.

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