ADNI Alzheimer's Disease Neuroimaging Initiative

Plasma 4,4'- dichlorodiphenyldichloroethylene (DDE) quantification

Douglas I. Walker[1,2], Thomas S. Wingo[3], Jason R. Richardson[4], Dean P. Jones[2], Kurt D. Pennell[1], Allan I. Levey[3]

1. Department of Civil and Environmental Engineering, Tufts University, Medford MA
2. Clinical Biomarkers Laboratory, Emory University School of Medicine, Atlanta GA
3. Emory Alzheimer's Disease Research Center, Emory University School of Medicine, Atlanta GA
4. Department of Environmental and Occupational Medicine, Rutgers Robert Wood Johnson Medical School, Piscataway NJ

Contents

Summary

A previous study examining body burden of the insecticide metabolite 4,4'-dichlorodiphenyldichloroethylene (4,4'-DDE) in 86 Alzheimer's disease (AD) patients and 79 healthy controls indicated a 3.8 fold increase in DDE levels within the diseased population and increased risk for AD (Richardson, Roy et al. 2014). The goal of this study was to utilize gas chromatography with tandem mass spectrometry and stable isotope dilution quantification to measure plasma DDE levels within a nationwide population consisting of a well characterized AD and control population. The resulting data provides whole plasma levels of 4,4'-DDE within each individual, expressed as µg/L.

4,4'-DDE analysis

Sample extraction and instrumental analysis for 4,4'-DDE quantification was adapted from previously established methods developed for the trace quantification of organohalogens (e.g. Richardson, Shalat et al. (2009), Hatcher-Martin, Gearing et al. (2012), Bradner, Suragh et al. (2013)). Prior to analysis of samples, method fitness of purpose was determined for each analyte using the procedures described in the method verification and quality control section. Method optimization was utilized to increase recovery, decrease interferences and minimize the method detection limit (MDL).

Method verification and quality control

Method recovery efficiencies were determined by spiking pooled plasma with a 1 µg/L standard and carrying the spiked matrix through the entire extraction procedure. Recovery efficiency of the spiked matrix was determined by comparing the analyte peak area from the blank matrix spiked prior to the extraction procedure to extracted matrix spiked post extraction and lipid cleanup. Method recoveries were observed to range from 75-90%. Blank matrix and method blanks were included within each batch (10 samples + 1 pooled reference sample + method blank). All reagents used were minimum ACS grade. Analytical methods including both sample preparation and instrumental analysis indicated method fitness of purpose by meeting the required internal standard corrected or absolute recovery of greater than 70%, and coefficient of variation of less than 30% for no less than 7 samples at 3 different concentrations within the range of expected levels and method detection limits, determined using the EPA method defined in 40 CFR Part 136 Appendix B.

Sample preparation

4,4'-DDE was extracted from the plasma using the procedures described by Brock, Burse et al. (1996), Sandau, Sjodin et al. (2003) and Rivera-Rodriguez, Rodriguez-Estrella et al. (2007). Briefly, 415 µL of plasma was placed in an amber glass vial, to which a 20 µL aliquot of 2-propanol containing 50 µg/L ^{13}C 4,4'-DDE, ^{13}C 2,4'-DDE, ^{13}C 4,4'-DDD, and ^{13}C 2,4'-DDD (Cambridge Isotope Laboratories, Tewksbury MA) was added. Plasma proteins were denatured by adding 500 µL of formic acid, vortex mixing for 30 sec and placing in a sonicating bath for 20 min. The extract was transferred to a 1g solid phase extraction (SPE) cartridge consisting of a C-18 absorbent (UCT, Bristol PA) pre-conditioned with 6 mL dichloromethane (DCM), 6 mL methanol (M-OH) and 6 mL of a wash solution (0.1 M HCl and 5% M-OH). Polar interferences were removed by rinsing with an additional 6 mL of wash solution. The cartridge was dried for 30 minutes via vacuum followed by centrifuging at 3000 rpm for 10 min. Analytes were eluted using a 12 mL DCM rinse and the liquid sample in each tube reduced to dryness by heating at 35ºC under a gentle stream of high purity N2 gas using a 30 position multivap heating block. The extract was reconstituted in 100 µL Optima grade hexane containing 1 ng octachloronapthalene (OCN, Cambridge Isotope Laboratories, Tewksbury MA), vortexed gently and transferred to an amber glass autosampler vial containing a low volume glass insert, sealed immediately with a Teflon-lined screw cap and analyzed by GC-MS/MS.

Gas chromatography-mass spectrometry methods

Plasma extracts were analyzed using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 7000A triple quadrupole mass spectrometer and an Agilent 7683 autosampler injector. A 4 µL aliquot of extract was injected into an inlet operated a 250ºC in pulsed split-less mode. The analytes were separated on an Agilent DB-5MS capillary column (30m length × 0.25mm inner diameter × 0.25µm film thickness) using high purity helium (99.999% purity) as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program consisted of an initial temperature of 100ºC for 1 min, increased to 180ºC at 25ºC/min; followed by a temperature ramp to 250ºC at 6ºC/min, and finally increased to 280ºC at 20ºC/min and held for 5 min, resulting in a total run time of 25.4 min. Analyte retention times were determined using individual standards and confirmed by matching analyte spectra obtained in full scan mode to the 2008 National Institute of Standards and Technology mass spectral library, with 4,4'-^{13}C-DDE and 4,4'-DDE eluting at 14.4 min.

Mass spectra were obtained using an electron ionization source, operated at a source temperature of 250°C, electron impact energy of -70 eV and transfer line temperature maintained at 275°C. High purity He (99.999% purity) and N_2 (99.999% purity) at a flow rate of 2.25 and 1.00 mL/min were used as the quench and collision gas, respectively. Front and back quadrupole temperatures were maintained at 150°C. Quantification and confirmation of analyte identity were completed by operating the mass spectrometer in multiple-reaction-monitoring (MRM) mode. The use of MRM, in which collision-induced disassociation of selected precursor ions yields additional fragmentation of analyte ions provides for increased sensitivity, decreased matrix interferences and reduced analyte false positives (Martinez Vidal, Moreno Frias et al. 2000, Medina, Pitarch et al. 2009, Dorman and Reiner 2012) in comparison to a micro electron capture detector (µECD) or single quadrupole mass spectrometer operating in selective ion monitoring (SIM) mode.

Collision cell energy, detector gain, quantification and qualification ions were optimized during method development to select analyte ions that provided the greatest abundance while minimizing interferences. Parent ions were first selected by analyzing spiked extracts with both quadrupoles operating in full scan mode. Possible candidates, selected based on abundance and uniqueness to the analyte, were then monitored at various collision energies with the front quadrupole in SIM mode and the back quadrupole operated in full scan mode. Optimum collision energy, detector gain, quantification and qualification ions were then selected for each analyte based on maximizing the signal-to-noise ratio (S/N). The quantification and qualification transfers are given in table 1.

Table 1: MS/MS quantification parameters summary

Analyte	Type	Precursor Ion	Product Ion	Collision Energy
^{13}C 4,4'-DDE	Quant	257.4	187.1	25
^{13}C 4,4'-DDE	Qual	329.3	259.2	25
4,4'-DDE	Quant	245.3	175.3	25
4,4'-DDE	Qual	317.2	248.0	25

Calibration and data processing

Final ^{13}C 4,4'-DDE and 4,4'-DDE concentrations in the extracted samples were determined by comparison of the peak area of each analyte quantification ion normalized by the area of product ion 332.8 m/z for OCN using an independent 6-point calibration curve. Only peaks within the analyte retention time +/- 0.2 minutes and exhibiting a qualifier ratio within 35% of the standard ratio were considered for quantification. Calibration standards contained both the stable isotopic form and unlabeled form of 4,4'-DDE and were spiked with OCN at the same volumetric ratio as the extracts. Stock solutions were prepared individually at 1000 µg/L. Calibration standards were then prepared in hexane from the working solution over a range of 0.1 to 50 µg/L. The method detection limit (MDL) was determined using the EPA method (USEPA [2003]) based upon repeated analysis (n=7) of the lowest spiking level, and was calculated to be 0.04 and 0.07 µg/L for ^{13}C 4,4'-DDE and 4,4'-DDE, respectively. Following acquisition of the data, peaks were extracted and integrated using the MassHunter Quantitative Analysis Software, Version B.03.01/Build 3.1.170.0. Analyte recovery was determined using the calculated concentration of ^{13}C 4,4'-DDE and expected concentration in the extract. The resulting whole plasma concentrations were calculated based on the initial volume of plasma extracted (415 µL).

ADNI Alzheimer's Disease Neuroimaging Initiative

Version 1.0

This document is the first version uploaded to the ADNI database containing data on 4,4'-DDE levels within an ADNI subpopulation.

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
ADRC_Emory_DDE - Analysis Version 1.0	November 3, 2014

About the Authors

This document was prepared by Douglas Walker (Tufts University/Emory University), Thomas Wingo (Emory University), Jason Richardson (Rutgers University), Dean Jones (Emory University), Kurt Pennell (Tufts University) and Allan Levey (Emory University). For more information please contact Douglas Walker at 404-727-5984 or by email at douglas.walker@emory.edu

Notice: This document is presented by the author(s) as a service to ADNI data users. However, users should be aware that no formal review process has vetted this document and that ADNI cannot guarantee the accuracy or utility of this document.

References

Bradner, J. M., T. A. Suragh, W. W. Wilson, C. R. Lazo, K. A. Stout, H. M. Kim, . . . W. M. Caudle (2013). "Exposure to the polybrominated diphenyl ether mixture DE-71 damages the nigrostriatal dopamine system: role of dopamine handling in neurotoxicity." Exp Neurol 241: 138-147.

Brock, J. W., V. W. Burse, D. L. Ashley, A. R. Najam, V. E. Green, M. P. Korver, . . . L. L. Needham (1996). "An improved analysis for chlorinated pesticides and polychlorinated biphenyls (PCBs) in human and bovine sera using solid-phase extraction." J Anal Toxicol 20(7): 528-536.

Dorman, F. L. and E. J. Reiner (2012). Emerging and Persistent Environmental Compound Analysis, Elsevier.

Hatcher-Martin, J. M., M. Gearing, K. Steenland, A. I. Levey, G. W. Miller and K. D. Pennell (2012). "Association between polychlorinated biphenyls and Parkinson's disease neuropathology." Neurotoxicology 33(5): 1298-1304.

Martinez Vidal, J. L., M. Moreno Frias, A. Garrido Frenich, F. Olea-Serrano and N. Olea (2000). "Trace determination of alpha- and beta-endosulfan and three metabolites in human serum by gas chromatography electron capture detection and gas chromatography tandem mass spectrometry." Rapid Commun Mass Spectrom 14(11): 939-946.

Medina, C. M., E. Pitarch, T. Portoles, F. J. Lopez and F. Hernandez (2009). "GC-MS/MS multi-residue method for the determination of organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in human breast tissues." J Sep Sci 32(12): 2090-2102.

Richardson, J. R., A. Roy, S. L. Shalat, R. T. von Stein, M. M. Hossain, B. Buckley, . . . D. C. German (2014). "Elevated serum pesticide levels and risk for Alzheimer disease." JAMA Neurol 71(3): 284-290.

Richardson, J. R., S. L. Shalat, B. Buckley, B. Winnik, P. O'Silleaghain, R. Diaz-Arrastia, . . . D. C. German (2009). "Elevated Serum Pesticide Levels and Risk of Parkinson Disease." Archives of Neurology 66(7): 870-875.

Rivera-Rodriguez, L. B., R. Rodriguez-Estrella, J. J. Ellington and J. J. Evans (2007). "Quantification of low levels of organochlorine pesticides using small volumes (<or=100 microl) of plasma of wild birds through gas chromatography negative chemical ionization mass spectrometry." Environ Pollut 148(2): 654-662.

Sandau, C. D., A. Sjodin, M. D. Davis, J. R. Barr, V. L. Maggio, A. L. Waterman, . . . D. G. Patterson, Jr. (2003). "Comprehensive solid-phase extraction method for persistent organic pollutants. Validation and application to the analysis of persistent chlorinated pesticides." Anal Chem 75(1): 71-77.