DETECTION OF SELEGILINE AND SELEGILINE METABOLITES IN AN ELISA ASSAY FOR AMPHETAMINE

B.A. Mayer, T. Tobin†, H.H. Tai‡, J.D. Harkins†, J. Carbajal, C. Scherer, T. Hawkins, C. Bratton, and D.E. Schroedter

Neogen Corporation, Lexington, Kentucky 40505, †Maxwell H. Gluck Equine Center, University of Kentucky, Lexington, Kentucky 40506, ‡College of Pharmacy, University of Kentucky, Lexington, Kentucky 40506

Selegiline acts as a monoamine oxidase inhibitor and is used to treat Parkinson's disease and dementia in humans. Selegiline is readily available and is a Class 2 drug in the RCI Uniform Classification Guidelines for Foreign Substances (2001). The drug is similar in structure to amphetamine ((±)-□-methylbenzeneethanamine), has well documented CNS stimulatory effects and can alter the performance of sport animals. Amphetamine is classified as a Class 1 drug in the RCI Uniform Classification Guidelines for Foreign Substances (2001). While immunoassays are currently available for the detection of amphetamine, we are reporting on a new assay, which offers greater sensitivity for amphetamine and a host of other illegal phenethylamines. In particular, this improved assay is highly sensitive for the selegiline metabolite, desmethylselegiline.

Antisera were produced in rabbits by injecting an amphetamine:protein immunogen. The same drug hapten was conjugated to horseradish peroxidase to form a drug:enzyme conjugate. Antisera coating onto microwells was optimized with the enzyme conjugate using a TMB substrate system. Standard curves were generated by competing the conjugate with known levels of amphetamine in buffer. The I-50 (measure of assay sensitivity) was optimized to 1 ng/mL.

Background studies using cleared equine and canine track samples revealed slight interferences with the assay. This interference was alleviated by diluting equine urine samples 1:5 or 1:9 with assay buffer and by diluting equine serum and plasma samples 1:1 with assay buffer. Canine urine samples were diluted 1:9 with assay buffer.

Cross-reactivates for several illegal drugs, therapeutic drugs, masking agents, vitamins, and drug vehicles were determined. The assay was more sensitive for the major urinary metabolite of selegiline, desmethylselegiline, than amphetamine. The parent compound was also detected in the assay, although with less cross-reactive than the metabolite. Specific cross-reactivity was also seen with several other drugs of interest on the RCI list (i.e., methamphetamine, ephedrine, fenfluramine, and mephentermine). Several other compounds had less cross-reactivity, but may also be detected with this assay. These drugs include: methcathinone, cathinone, pseudoephedrine, isoxsuprine, clenbuterol, ractopamine, and metaraminol.

Amphetamine and desmethylselegiline standard curves were generated in four common sample matrices (equine plasma, equine serum, equine urine and canine urine). The sensitivity of the assay in these matrices were:

Matrix	Amphetamine I-50 ng/mL	Desmethylselegiline I-50 ng/mL
Equine Urine (1:9)	27	2.6
Equine Plasma (1:1)	3.3	0.35
Equine Serum (1:1)	4.2	0.51
Canine Urine (1:9)	31	3.0

Equine urine samples were collected after selegiline administration. Equivalents of selegiline and selegiline metabolites were readily detectable in the urine even after dilution.

Immunoassay screening methods have not been previously described for selegiline. The Neogen amphetamine assay detects selegiline, and is very sensitive to the urinary metabolite, desmethylselegiline.