LC/MS/MS Determination of Urinary Concentrations of Insecticides and Herbicides in Professional Applicators

Frederic L. Ciner[1,2], Roy W. Plunkett, Jr.[1], Michael F. Martin[1], Shelley A. Harris[2,3] and Timothy R. Croley[1,2]

1. Commonwealth of Virginia, Department of General Services, Division of Consolidated Laboratory Services, Richmond, VA 23219

2. Center for Environmental Studies, Virginia Commonwealth University, Richmond, VA 23284

3. Department of Preventive Medicine and Community Health, Virginia Commonwealth University, Richmond, VA 23284

Introduction

The objective of this work was the development of a robust, high-throughput, and sensitive method for the determination of specific pesticides in urine of professional turf applicators following occupational exposures. The compounds of interest in this study are: the herbicides dicamba, mecoprop (MCPP), 4-chloro-2-methylphenoxyacetic acid (MCPA), and 2,4-D; the insecticides imidacloprid and bifenthrin, and their urinary metabolites 6-chloronicotinic acid (6-CNA) and 2-methyl-3-phenylbenzoic acid (MPA), respectively. Quantitative analysis was performed using solid-phase extraction (SPE) followed by negative ion electrospray ionization HPLC/MS/MS from approximately 120 professional applicators. In 2003, twenty (20) applicators provided 19 consecutive 24-hour urine samples. In 2004, approximately 100 applicators will provide a total of six, 24 hour urine samples over three different time periods (spring, summer and fall). Measured concentrations of these analytes are to be used in conjunction with pesticide application information to predict the total absorbed dose following multiple exposures in a large occupational field study. This information, along with information collected from each subject by questionnaire, will be used to conduct an external validation of previously developed epidemiologic prediction models. Data obtained in this biomonitoring study will also be used to develop recommendations to reduce occupational exposure and for health risk assessments.

Experimental

Test subjects collected composite, 24 hour urine samples in three one liter containers and the samples were kept on ice. In the field, specific gravity, volume and urine mass were measured. An aliquot was taken and frozen at -20ºC for subsequent HPLC/MS/MS analysis. Samples were thawed at room temperature prior to solid-phase extraction. SPE was performed using C-18 cartridges (Varian, Harbor City, CA). The cartridges were conditioned with MeOH and H_2O, 1ml of urine was diluted with 2 ml of 0.1% formic acid and loaded onto the cartridge. The analytes were eluted with 1ml of MeOH. Separation was performed using an Agilent 1100 HPLC (Wilmington, DE) with the mobile phase consisting of 0.1% formic acid:0.1% formic acid in ACN. Gradient separation was used (total run time = 16 minutes) on a 150 x 2 mm x 4 µm

Phenomenex Synergi RP-18 column (Torrance, CA). A Bruker Esquire 3000plus quadrupole ion trap mass spectrometer (Billerica, MA) was utilized for MS/MS determination of analytes. A time-segmented acquisition was utilized in the analyses (Figure 1). The segments consisted of:

1. Diversion (t = 0 - 3 min) to waste
2. Optimized MS/MS analysis for imidacloprid and 6-CNA
3. Optimized MS/MS analysis for dicamba, 2,4-D, MCPP, MCPA and MPA

Results

The extraction efficiencies were between 75-85% for all analytes in this study. Calibration curves were generated using spiked urine to account for matrix effects. All curves yielded an $r^2 \geq 0.995$. Analytes were detected in the low picogram on column range. The method was tested for robustness through blind studies and all analytes were quantitated within 1 standard deviation of the true value.

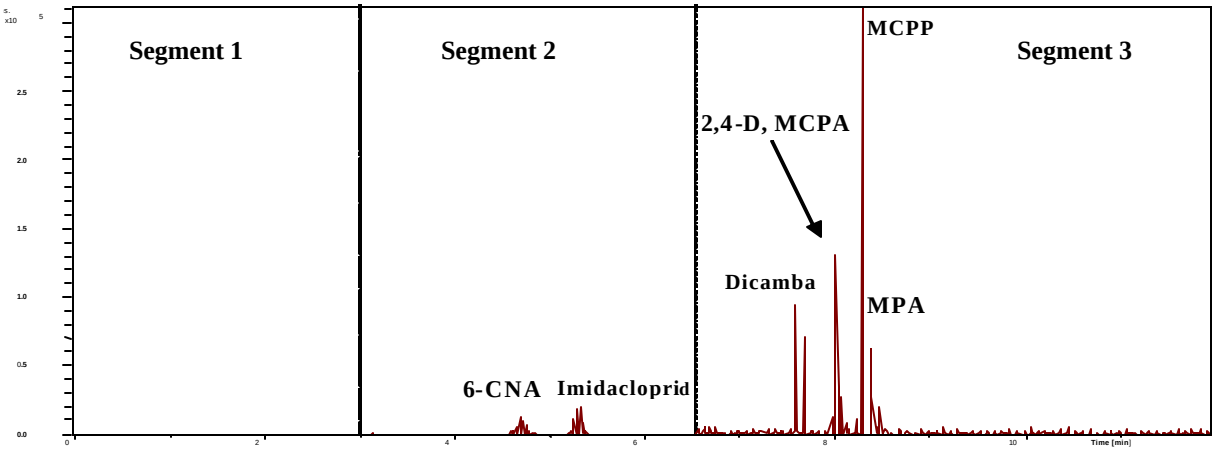

Figure 1: LC/MS/MS Chromatogram showing the time-segmented acquisition windows

Conclusions

We have developed a sensitive and robust method for the biological monitoring of select herbicides and pesticides in professional turf applicators. The resulting data, from this analytical protocol, will assist in validating the epidemiological model for occupational exposure among this group. The validated method provides ample sensitivity for this biomonitoring study; in addition, the method has the potential for automation allowing for high-throughput analysis. This would, in turn, allow data to be transferred more readily from the laboratory to the field for both epidemiologic modeling and occupational risk assessment.

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