Development of a targeted LC/MS approach to screen for 182 pesticides in urine samples

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Abstract

Pesticides are used all over the world and are found in many environments such as farmlands, water plants, hospitality and agriculture facilities. Exposure to high levels of pesticides can be harmful to agricultural workers and their families. Our goal was to develop a urinary screening method to determine whether individuals might be exposed to various pesticides. We used mixtures of pesticide standards to develop a liquid chromatography tandem mass spectrometry method. A semi-quantitative curve was created by diluting the standard pesticide solutions. Urine samples were prepared by the addition of acetonitrile to precipitate proteins. Standards and samples were analyzed using a Waters Xevo tandem mass spectrometry system. We monitored 182 pesticides using this method. We screened approximately 120 urines from adults and children. We found 45 pesticides present in the urine samples with the lower limits of detection ranging from 0.1 to 10 ng/mL. Using this method, we can screen for pesticide exposure in urine samples from adults and children. This screening technique will inform which pesticides should be studied using specific and sensitive assays for more quantitative measurements of exposure.

Introduction

- Results from the Agricultural Health Study, an ongoing study of pesticide exposures in rural families, show that farmers who used agricultural insecticides experienced an increase in headaches, fatigue, insomnia, dizziness, hand tremors, and other neurological symptoms.
- Evidence suggests that children are particularly susceptible to adverse effects from exposure to pesticides, including neurodevelopmental effects.
- Research on pesticides is imperative to better understand their cause and effect over time in humans.
- Effective screening methodologies are necessary to determine whether individuals have been exposed to pesticides.

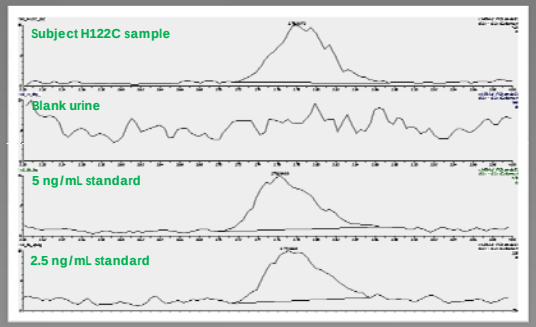
Methods

- **Samples.** Urine samples were collected from farm workers, non-farm workers, and their children in Washington State from 2001 to 2004. The study was approved by the University of Washington Institutional Review Board.
- **Standard preparation.** Pesticide standards were diluted in blank urine resulting in final concentrations ranging from 0.1 to 100 ng/mL.
- **Urine sample preparation.** For each urine sample, 800 uL of ice-cold acetonitrile was added to 200 uL of urine. Samples were vortexed and centrifuged at 15,000 rpm for 10 min at 4˚C. 900 ul of supernatant was transferred and evaporated under nitrogen gas. Samples were reconstituted with 25 uL of methanol and 25 uL of 0.4% acetic acid, vortexed and centrifuged. 40 uL of supernatant was transferred to LC vials for analysis.
- **LCMS procedure.** We injected 10 uL of each prepared sample onto a Waters ultra high-pressure liquid chromatography system coupled to a Waters Xevo TQD UPLC-MS/MS. Chromatograhic separation was achieved using a Agilent 2.1x100 mm C18 column with a 1.8 micron particle size. The mobile phases consisted of 0.2% acetic acid in water and methanol. We used a gradient to separate the pesticides with a flow rate was 0.5 mL/min. The total run time was 25 minutes per sample.

Table 1. List of pesticides found In urine samples.

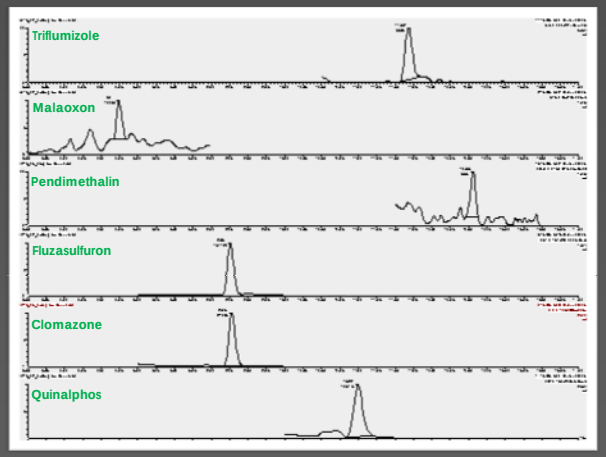
Compound	Parent ion (m/z)	Daughter ion (m/z)	Retention time (min)	Lowest Detectable Concentration (ng/ml)
Azaconazole	300	159	8.8	0.4
Azinphos-methyl	318	160	9.1	2.5
Bifenazate	301.1	170	10.2	2.5
Carbendazim	192.1	132.1	3.8	0.25
Carfentrazone-ethyl	412	346	10.9	10
Clomazone	240	125	9.2	0.5
Cyproconazole	292.2	70.2	10.6	2.5
Dimethoate	230.01	198.97	5	0.5
Epoxiconazole	330	121.04	10.6	0.5
Ethoprophos	243.2	131	10.5	5
Fenpyroximat	422.2	366.1	13	0.25
Fluzasulfuron	408.1	181.9	9.2	0.25
Flumetsulam	326.1	129	5.1	0.1
Forchlorfenuron	248.1	93	8.6	0.5
Furalaxyl	302.14	95.01	9.6	0.5
Hydramethylnon	495.2	323.15	10.7	5
Imidacloprid	256.1	209.1	4.7	2.5
Indoxacarb	528	150	11.9	0.5
Isofenphos-methyl	354.09	252.97	11	2.5
Lenacil	235.2	153.1	8.5	5
Linuron	249.1	160.1	9.3	0.5
Malaoxon	315	98.9	7.7	0.1
Mecarbam	330	227.1	10.4	5
Mepanipyrim	224.1	106	10.2	1
Mesosulfuron-methyl	504	82.9	8.6	0.5
Methabenzthiazuron	222	165	8.4	0.5
Methacrifos	241.1	209.1	8.9	2.5
Methidathion	303	145	8.8	2.5
Methoprotryne	272.2	198.2	9	0.5
Metolachlor	284.1	252.1	10.6	1
Metsulfuron methyl	382	167	7.6	0.5
Molinate	188	126	9.9	1
Myclobutanil	289.1	70.2	10	0.5
Paclobutrazol	294.1	70.2	9.9	0.5
Pendimethalin	282.2	212.2	12.5	25
Procymidone	284.1	256.1	10	5
Propyzamide	256.1	190	9.8	1
Quinalphos	299	162.9	10.9	0.25
Rotenone	395	192.1	10.7	10
Spinosad A	732.6	142	10.6	5
Spinosad D	746.52	142	10.8	5
Spirotetramat	374.2	330.21	10.5	1
Thiofanox	241.1	184.08	8.1	1
Tralkoxidym	330.2	284.3	12.6	5
Triflumizole	346	277.9	11.7	0.25

Figure 2. Detection of carbendazim. We monitored the mass transition of the precursor (parent) to daughter ion for carbendazim. In the urine of subject H122C, a peak was observed at 3.78 min which is consistent with the retention time of the carbendazim standards (bottom panels).



Results

Figure 1. Representative chromatograms for various pesticides that are detectable by MS/MS. Each pesticide has a characteristic mass transition and retention time permitting identification of the pesticide in urine samples.



- A total 182 pesticides could be extracted and detected using this method (Figure 1)
- Samples from approximately 120 adults and children were screened
- We found a variety of pesticides in various urine samples (Table 1 and Figure 2)
- This method provides a rapid screen of potential pesticide exposure in a run time of 25 min.

Conclusions

- We developed a rapid semi-quantitative screening method to determine the presence of 182 pesticides in urine
- Using the results from this screening, samples from exposed individuals can be analyzed with more quantitative methods
- By identifying exposed individuals, further work can be done to study pesticide toxicity and strategies for personal protection

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