Determination of Parathion Metabolite, *p*-Nitrophenol in Urine of Parathion Factory Workers

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Parathion is an organophosphate pesticide being legally applied for the purpose of agriculture and is being manufactured in Korea. A gas chromatography/mass spectrometric method was developed for the determination of parathion urinary metabolite, *p*-nitrophenol. *p*-Nitrophenol was extracted from weak acidic urine, and then measured by gas chromatography-mass spectrometry (selected ion monitoring). The recovery of *p*-nitrophenol in the overall procedure was 88.2%. The detection limit of the assay was 1.0 μg/L based upon assayed urine of 2.0 mL. The method was applied to the determination of *p*-nitrophenol in urine of workers of a parathion industry. Spot urines of workers of a parathion industry were sampled at the end of shift and *p*-nitrophenol was analyzed using above developed method. *p*-Nitrophenol could be detected in all of the urine samples at concentrations varying from 3.0 to 681 μg/L.

Key Words : Parathion, Urinary *p*-nitrophenol, GC/MS

Introduction

Parathion is extremely toxic from acute (short-term) inhalation, oral, and dermal exposures.[1-5] Acute exposure of humans to parathion may result in nausea, vomiting, abdominal cramps, diarrhea, excessive salivation, headache, weakness, difficult breathing, blurring or dimness of vision, convulsions, central nervous system depression, paralysis, coma, and respiratory failure. The central nervous system, blood, respiratory system, eyes and skin are the organs most affected by acute exposure of humans to parathion.[1] Chronic (long-term) inhalation and oral exposure of humans and animals to parathion have been observed to result in depressed red blood cell cholinesterase activity, nausea, and headache.[1] Depressed plasma and red blood cell cholinesterase activity have also been observed in humans chronically exposed to parathion by ingestion.[1]

Parathion is metabolized to *p*-nitrophenol in the urine, and *p*-nitrophenol in the urine is quantified as an indicator of parathion exposure. There is a need to develop a sensitive quantification method of *p*-nitrophenol in order to provide insight into the extend of the exposure of parathion to worker or farmer.

To date, many methods for determination of *p*-nitrophenol have been described containing spectrophotometric methods,[6,7] high performance thin layer liquid chromatographic technique (HPTLC),[8] high performance capillary zone electrophoresis,[9] high performance liquid chromatographic technique (HPLC)[10-14] and gas chromatographic technique (GC).[15,16] A research was published for the determination of *p*-nitrophenol in the urine by SPME and GC-MS.[16] Detection limit of the method was 18 μg/L in urine, and the method can not been used to determine *p*-nitrophenol in concentrations of low μg/L in urine. Mass spectrometry has the potential to become a standard analytical tool for detecting *p*-nitrophenol.

This paper describes analysis without derivatization of trace *p*-nitrophenol in urine.

Experimental

Chemicals and reagents. *p*-Nitrophenol and pyren-d10 were purchased from Sigma (St. Louis, MO, USA). Analytical grade of potassium bishydrogen phosphate, sodium sulfate, hydrochloric acid and sodium chloride (Sigma, St. Louis, MO, USA) were used as reagents and methylene chloride, methanol and ethyl acetate (E. Merck, Darmstadt, Germany) were used as solvents.

Sample preparation. The urine samples of workers of a parathion industry were collected in polypropylene bottles at the end of shift and stored at −20 °C until sample analysis preparation was carried out. After mixing, an aliquot of 2 mL urine was transferred to a 15 mL glass tube with a cap and was spiked with 20 μL of the internal standard solution (pyren-d10, 1.0 mg/L in methanol). The sample was then extracted twice with 5 mL of methylene chloride and centrifuged at 1500 g for 10 minutes. The organic phases were combined and concentrated in a gentle stream of nitrogen to a final volume of 200 μL. Two micrometers of this solution were analyzed by gas chromatography/mass spectrometry/electron impact ionization (GC/MS/EI) in selected ion monitoring (SIM). Blank urine was collected from a normal and healthy man.

Gas chromatography-mass spectrometry. All mass spectra were obtained with a Agilent 6890/5973 N instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230 C). Full-scan mass spectra (m/z 40-400) were recorded for analyte identification. The operating parameters

Table 1. GC-MS condition of *p*-Nitrophenol

Parameter	Conditions	
Column	HP-5MS (Cross-linked 5% phenylmethylsilicon), 30 m × 0.2 mmI.D. × 0.25 μm F.T	
Carrier	He at 1.0 mL/min	
Oven Temp	130 °C (1 min) → 250 °C (5 min)	
Injector type	split mode (1:10)	
Injector Temp	260 °C	
Transfer line	270 °C	
Selected Ion	Start time (min)	Selected Ions, m/z
Group	2.5	65, 109, 139, 188

were as Table 1.

Calibration and quantification. Calibration curve for *p*-nitrophenol was established by extraction after adding 5, 10, 20, 50, 100, 250, 700 and 1400 ng of standard and 40 ng of internal standard in urine 2.0 mL. The ratio of the peak area of standard to that of internal standard was used in the quantification of the compound.

Results and Discussion

Chromatography. For the GC separation of *p*-nitrophenol, the nonpolar stationary phase was used. The peaks of *p*-nitrophenol and internal standard are symmetrical and resolutions of the analytes from the background compounds in urine were above 2.0 (Figure 1). The relative retention time of *p*-nitrophenol to internal standard (pyren-d10) was 0.701. There were no extraneous peaks observed in a chromatogram of blank urine in near of the retention time of 3.56 min.

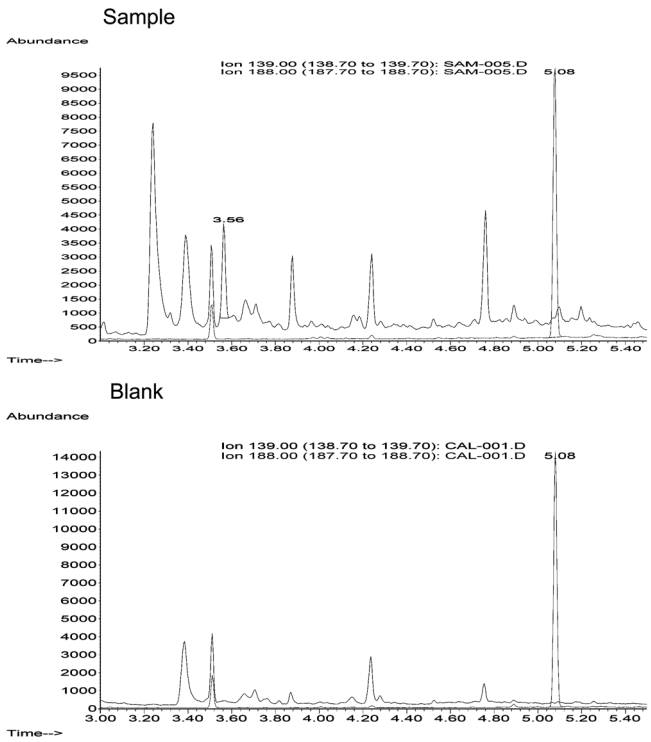

Figure 1. Chromatogram of *p*-nitrophenol from real sample and blank urine (3.56 min: *p*-nitrophenol, 5.08 min: ISTD).

Mass spectrometry. The mass spectrum of *p*-nitrophenol (Figure 2) was obtained. *p*-Nitrophenol illustrates molecular and base ion at 139 amu, and characteristic ions at 81, 93, 109 and 123 amu. The ions at 123 and 109 amu were from the losses of [O] and [NO] from the molecular ion.

139 amu was selected for the quantitation, and 65 and 109 amu were selected for the qualification for *p*-nitrophenol.

Extraction and clean up. In spite of the condition improvement of other alternative extraction techniques, liquid-liquid extraction (LLE) is still the most efficient techniques for the routinely performed analysis of *p*-nitrophenol in urine.

p-Nitrophenol has phenol group, which present in ionic form at pH above 9.0. Optimum pH can be obtained in slightly acidic condition and methylene chloride was selected as an extraction solvent.

Test samples at 5.0 and 20.0 μg/L in urine were prepared and the recovery was calculated by percentage of the analytes recovered. The recoveries of the test compounds were about 85.6-90.8% (CV = 7.4%) for *p*-nitrophenol (n = 5).

Linearity and detection limit. Examination of typical standard curve of peak area ratios of *p*-nitrophenol to internal standard using a least-squares fit demonstrated a linear relationship with correlation coefficients being greater than 0.997. Linear equation of *p*-nitrophenol was y = 0.0102x–0.01 (r^2 = 0.997) in the concentration range of 1.0-700 μg/L. The limit of quantitation (LOQ) was 1.0 μg/L based upon an assayed sample of 2 mL. LOQ was defined by 10 times of coefficients of variation for replicate determinations (n = 7) of sample.

Precision and accuracy. The range and standard deviation values for the precision and accuracy are given in Table 2. For five independent determinations at 10 and 50 μg/L, the coefficient of variation was less than 10%. This low relative error is based on simple extraction. The method is considered to give enough precision and accuracy for the detection of *p*-nitrophenol in urine.

Sample analysis. The method was applied to the determination of *p*-nitrophenol in urine of workers of a parathion industry. Spot urines of workers of a parathion industry were sampled at the end of shift.

We analyzed *p*-nitrophenol in 12 urine samples. *p*-Nitro-

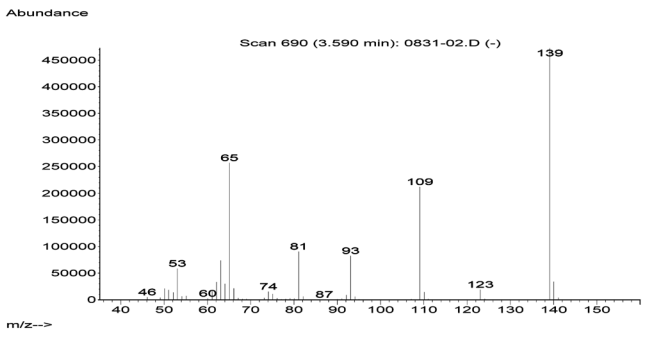

Figure 2. Mass spectrum of *p*-nitrophenol.

Table 2. Precision and accuracy by extraction of *p*-nitrophenol in urine

Spiked Conc (μg/L)	Measured Conc. (μg/L)	Mean ± SD (RSD%)
10	9, 8, 9, 10, 10	9 ± 0.8 (9.1)
50	48, 47, 52, 50, 48	49 ± 2 (4.1)

SD = standard deviation; RSD = relative standard deviation

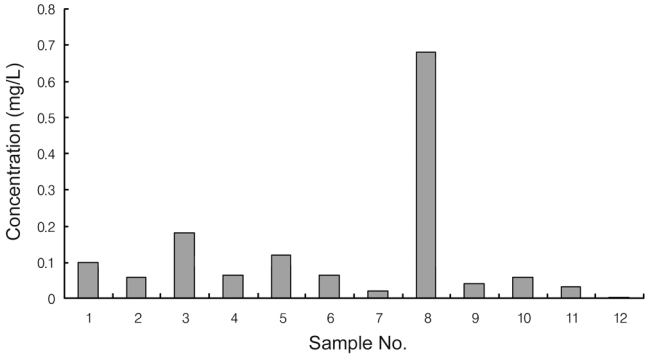

Figure 3. The concentrations of *p*-nitrophenol in urines from 12 workers of parathion factory.

phenol could be detected in all of the urine samples at concentrations varying from 3.0 to 681 μg/L (Figure 3). Especially, we identified a worker (No 8 of Figure 3), who was exposed with high concentration of parathion, and should be identified the areas of risk where he might be exposed.

The urinary monitoring takes the cost in perspective, and is a successful and safe method of managing worker's health. With these tests, the high-risk cases and negligence can be picked up. It can be also help us to identify areas of risk where workers at a certain point may be exposed, which can, *via* the worker's urine test, be identified.

Conclusion

p-Nitrophenol offers good chromatographic property and sensitive response for the EI-MS (SIM) without derivatization. The extraction of the compound from urine with methylene chloride also gave high recovery with small variation. Quantitation of *p*-nitrophenol is excellent, with linear calibration curve over a range of 1.0-700 μg/L and detection limit of 1.0 μg/L. The present method developed for the determination of *p*-nitrophenol in urine as an indicator for the exposure of parathion, and may be applicable to monitor parathion-, methyl parathion- and ethyl parathion exposure of factory worker or field worker.

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