II.7.7 Diazine and triazine herbicides

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Introduction

Diazine and triazine herbicides are being widely used in the world. These herbicides inhibit the electron-transport system in the higher plants and thus suppress the photosynthesis, resulting in the herbicidal action. These compounds are also important as pollutants for crops, soil and groundwater [1, 2]. The attention is usually directed toward chronic toxicities of the herbicides [3]. Although the acute toxicities of the compounds are usually considered low, there are reports dealing with acute poisoning by them; they should be taken into consideration as poisoning-causative substances. As acute poisoning symptoms provoked by diazine and triazine herbicides, nausea, vomiting, skin- and mucosa-stimulating actions, contact dermatitis, circulation insufficiency, shock state, dyspnea, metabolic acidosis and renal insufficiency can be mentioned; as a subacute poisoning symptom, polyneuropathy due to triazines is known [4].

For analysis of diazine and triazine herbicides, methods by GC, GC/MS and immunoassays were reported. However, they were GC analysis of atrazine in bovine tissues [5], ELISA analysis of atrazine and its metabolites in human urine [6] and other methods dealing with surface-water and cow milk [7–9]. There are almost no reports on GC or GC/MS analysis of diazine and triazine herbicides in human body fluids except those reported by the authors' group [10, 11]. There is a review on analysis of herbicides in biomedical specimens from a broader point of view [12]. In this chapter, detailed procedures for GC analysis of diazine and triazine herbicides in human body fluids are described.

Reagents and their preparation

i. Reagents

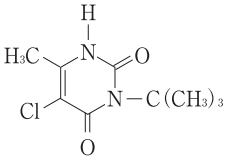
Figure 7.1 and *Table 7.1* show structures of diazine and triazine herbicides, respectively. The authentic standards of all herbicides can be purchased from either Wako Pure Chemical Industries, Ltd., Osaka, Japan or Kanto Chemicals, Tokyo, Japan. Other common chemicals used were of the highest purity commercially available.

ii. Preparation

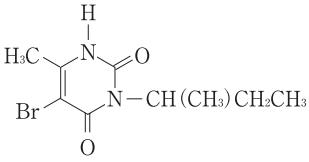
A 1-mg aliquot of each compound is dissolved in 1 mL methanol (1 mg/mL)[a] as a stock solution. The solution is diluted to a desired concentration with methanol just before use.

Figure 7.1

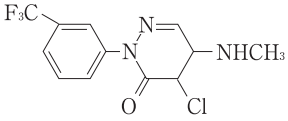
terbacil



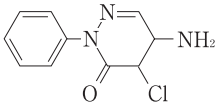
bromacil



norflurazon



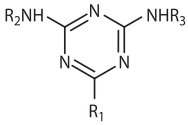
pyrazon

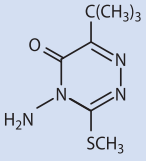


Structures of diazine herbicides.

Table 7.1

Structures of triazine herbicides



	R_1	R_2	R_3
ametryn	SCH_3	CH_2CH_3	$CH(CH_3)_2$
atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$
cyanazine	Cl	CH_2CH_3	$CCN(CH_3)_2$
prometon	OCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$
prometryn	SCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$
propazine	Cl	$CH(CH_3)_2$	$CH(CH_3)_2$
simazine	Cl	CH_2CH_3	CH_2CH_3
metribuzin			

GC conditions

Columns: DB-1 and DB-17 fused silica capillary columns (both 30 m × 0.32 mm i.d., film thickness 0.25 μm, J & W Scientific, Folsom, CA, USA) used for diazine herbicides, and the DB-1 column used for triazine herbicides.

GC conditions for diazine herbicides; instrument[b]: a GC-4CM gas chromatograph (Shimadzu Corp., Kyoto, Japan); detector: FID; column (oven) temperature: 100 °C (1 min) → 10 °C/min → 280 °C; injection temperature: 230 °C; detector temperature: 280 °C; carrier gas: He; its flow rate: about 3 mL/min; injection mode: splitless (1 min).

GC conditions for triazine herbicides; instrument[b]: an HP5890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA); detector: FID or nitrogen-phosphorus detector (NPD)[c]; column (oven) temperature: 120 °C → 2.5 °C/min → 160 °C; other conditions the same as above.

Procedures for diazine herbicides

i. Liquid-liquid extraction

i. A 1-mL volume of whole blood, containing diazine herbicides, (both target and IS compounds) is mixed with 1 mL distilled water and 2 mL diethyl ether, capped and shaken for 1 min.
ii. After centrifugation at 3,000 rpm for 5 min, the ether layer is transferred to a vial. To the above aqueous layer, 2 mL diethyl ether is again added, shaken and centrifuged in the same way to obtain the second ether layer; this procedure is repeated once more to obtain the third ether layer. The three ether layers are combined and evaporated to dryness under a stream of nitrogen in the vial.
iii. The residue is dissolved in 100 μL methanol, and a 1-μL aliquot of it is injected into GC.

ii. Solid-phase extraction with Bond Elut C_{18}

i. A Bond Elut C_{18} cartridge (Varian, Harbor City, CA, USA) is activated by passing 10 mL methanol and 20 mL distilled water; this procedure is repeated twice[d] to remove impurities being contained in the cartridge.
ii. A 1-mL volume of plasma or urine, containing diazine herbicides (both target and IS compound), is mixed with 4 mL distilled water; in the case of whole blood, the 1-mL specimen is well mixed with 9 mL distilled water to hemolyze it completely.
iii. The above sample solution is poured into the activated cartridge, followed by washing with 20 mL distilled water and elution with 3 mL of chloroform/methanol (9:1).
iv. After a small amount of the upper aqueous layer is removed with a Pasteur pipette, the lower organic phase is evaporated to dryness under a stream of nitrogen. The residue is dissolved in 100 μL methanol, and a 1-μL aliquot of it is injected into GC.
v. For determination of terbacil or bromacil, norflurazon (final, 5 μg/mL) is used as IS; for that of norflurazon or pyrazon, bromacil (final, 5 μg/mL) used as IS. Various amounts (0.16, 0.31, 0.63, 1.25, 2.5, 5.0 and 10 μg) of a target compound together with 5 μg of IS are spiked into 1-mL volume blank body fluid specimens and subjected to the above solid-phase extraction to construct a calibration curve. The peak area ratio of a target compound to IS obtained from a test specimen is applied to the calibration curve to calculate its concentration.

Procedure for triazine herbicides

i. A Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) is washed with 10 mL methanol and 20 mL distilled water. This washing procedure is repeated not less than twice for activation[d].
ii. One of the triazine herbicides is chosen as IS (5 μg for FID and 0.5 μg for NPD) and spiked into 1-mL of a test serum or urine specimen, followed by dilution with 4 mL distilled water.
iii. The above mixture is poured into the activated cartridge, followed by washing with 20 mL distilled water and elution with 3 mL of chloroform/methanol (9:1) or 3 mL chloroform only.
iv. After a small amount of the upper aqueous layer is removed with a Pasteur pipette, the eluate is evaporated to dryness under a stream of nitrogen; the resulting residue is dissolved in 100 μL methanol and 1 μL of it is injected into GC. The quantitation procedure is essentially the same as described in the above v step of the solid-phase extraction for diazine herbicides.

Assessment of the methods

i. Diazine herbicides

Figure 7.2 shows gas chromatograms for diazine herbicides with various combinations of an extraction method and a GC column used. The left panels show the chromatograms for the authentic standard directly injected into GC (50 ng each on-column); the right panels those for the extracts of whole blood, into which 5 μg/mL each of diazine herbicides was spiked. It is clear that solid-phase extraction with a Bond Elut C_{18} cartridge gives cleaner chromatograms than the liquid-liquid extraction with diethyl ether. This was also true for human serum and urine specimens.

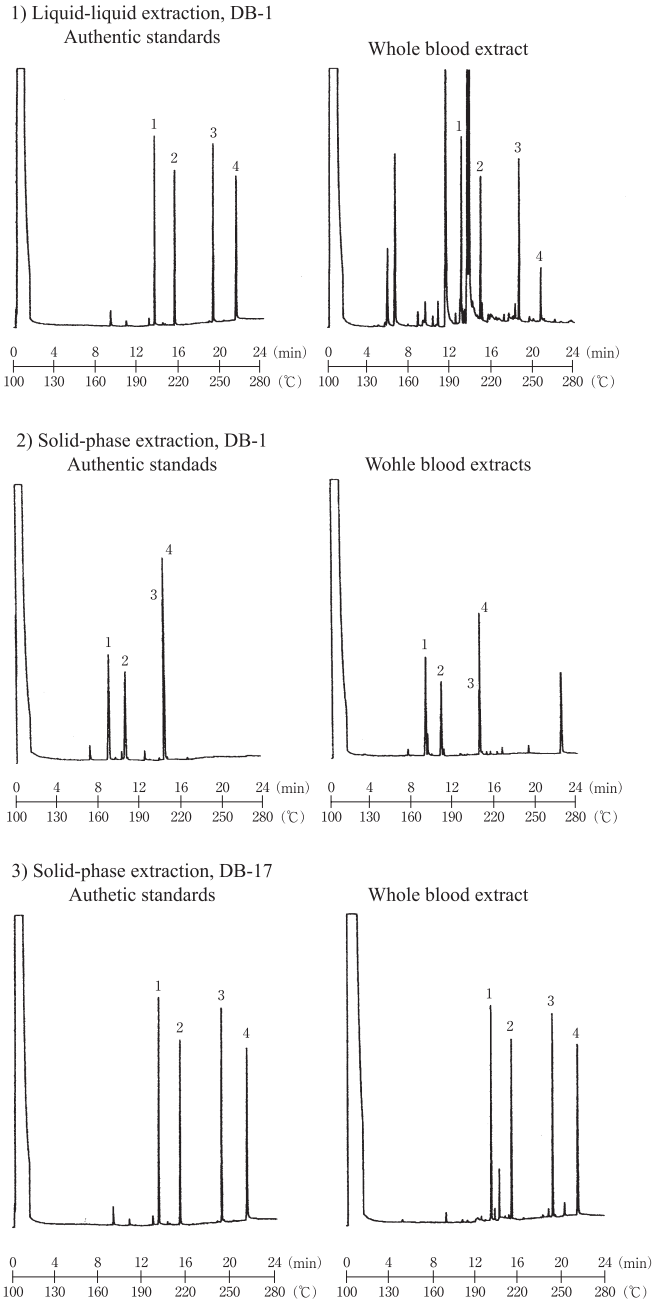
Good linearity was observed in the range of 16 ng–10 μg/mL for diazines. Their detection limits were 1.2–1.4 ng on-column for whole blood and plasma and 1.1–1.2 ng on-column for urine.

ii. Triazine herbicides

Figure 7.3 shows gas chromatograms for triazine herbicides obtained by solid-phase extraction with Sep-Pak C_{18} cartridges with different elution solvents and different detectors. The left panels show the chromatograms for the authentic triazine herbicides directly injected into GC (50 ng each on-column for the FID and 5 ng each on-column for the NPD); the right panels those for the extracts of serum specimens, into which 5 μg/mL each of triazine herbicides was spiked. There was almost no difference between elutions with chloroform only and chloroform/methanol (9:1); but the time required for evaporation of the eluates was much shorter for the chloroform only than for the chloroform/methanol mixture. The recovery rates for the FID detection were not less than 65 and 97 % for the serum and urine specimens, respectively. The detection limits by the FID detection were 2 and 14 ng on-column for propazine and simazine, respectively.

In the chromatograms with the NPD detector, slight tailing was observed for all peaks, because this phenomenon becomes more obvious at the ten times lower concentration (5 ng

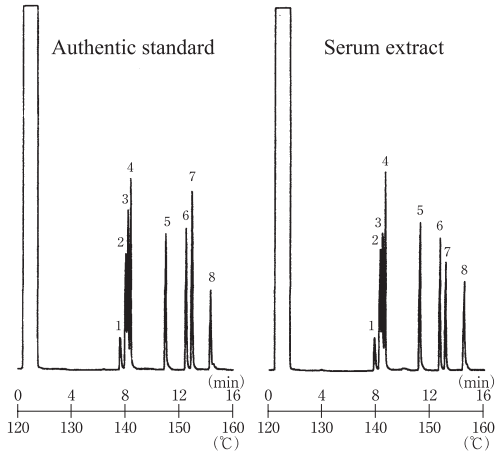
Figure 7.2



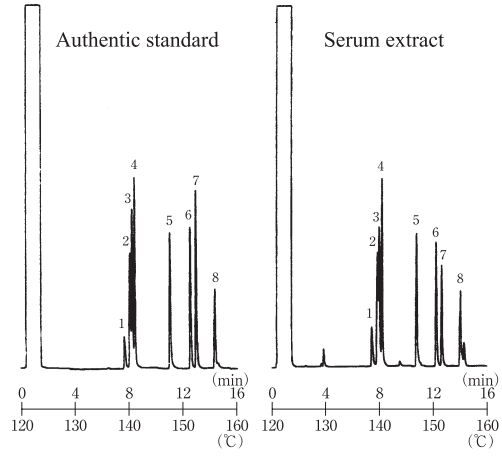
GC-FID chromatograms for diazine herbicides using different extraction methods and GC capillary columns. 1: terbacil; 2: bromacil; 3: norflurazon; 4: pyrazon. For the authentic standards, the amount for injection was 50 ng on-column each; into the blank blood specimens, 5 µg/mL each of the compounds was spiked.

Figure 7.3

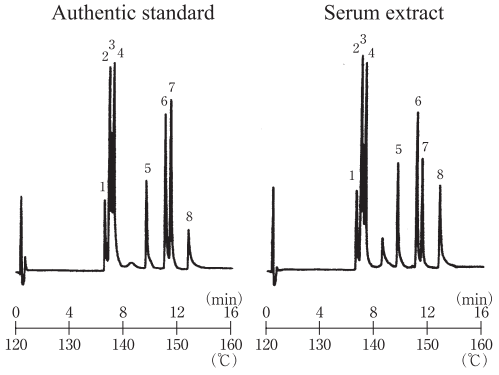
1) Solid-phase extraction, $CHCl_3$ elution, FID



2) Solid-phase extraction, $CHCl_3$/MeOH (9:1) elution, FID



3) Solid-phase extraction. $CHCl_3$ elution, NPD



on-column for the authentic standards and 0.5 μg/mL in the serum specimen). The recovery rates were not less than 60 and 78 % for serum and urine, respectively; it was more than 100 % for cyanazine. The sensitivity with NPD was about ten times higher that with FID; the detection limits of triazines with NPD were 0.2–0.6 ng on-column.

Poisoning cases and toxicities

Case 1 [13]: a 38-year-old male ingested 500 mL of a herbicide product containing 100 g atrazine, 25 g aminotriazole (amitrole), 25 g ethylene glycol and 0.15 g formaldehyde. The plasma atrazine concentration was 2.0 μg/mL 1 h after the ingestion. Although the treatment of metabolic acidosis, hemodialysis and administration of ethanol against the ethylene glycol poisoning were carried out, he provoked coma, circulation insufficiency, metabolic acidosis, bleeding from the digestive tract, necrosis of hepatic cells and DIC, and died 3 days later.

Case 2 [3]: an adult male ingested 1,000 g of a 50 % atrazine powder product; when he was vomiting and being excited, he was found by his family member. At an early stage, atropine was administered to him, because organophosphorus herbicide poisoning was suspected. Fortunately he recovered without any severe poisoning symptom except only a slight one due to atropine.

The LD_{50} values for diazine herbicides are said to be about 5 g/kg in humans; those for triazine herbicides except cyanazine 1–5 g/kg [14].

Notes

a) The herbicides dissolved in methanol at 1 mg/mL is stable for at least 2–3 weeks at 4 °C.
b) Any type of gas chromatograms for capillary columns can be used, regardless of its manufacturer.
c) It is the same as a flame thermionic detector (FTD) and is specific for compounds including nitrogen or phosphorus in their structures; they are being sold by many manufacturers.
d) In the original method reported by Suzuki et al. [15], they used chloroform/methanol (9:1), methanol and distilled water,10 mL each, for activation. When this procedure is applied to a recent product of C_{18} cartridges, the elevation of baselines and impurity peaks due to the cartridge matrix are frequently observed. It is recommendable to simply use methanol and distilled water for washing and activation to obtain good results.

◀ **Gas chromatograms for the authentic triazine herbicides and solid-phase extracts of serum specimens, into which triazine herbicides had been spiked. 1: simazine; 2: atrazine; 3: prometon; 4: propazine; 5: metribuzin; 6: ametryn; 7: prometryn; 8: cyanazine. For the authentic standards, 50 ng each of the compounds was injected on-column, and 5 μg each was spiked into 1 mL serum to detect peaks with an FID in the panels 1) and 2). With an NPD, the amounts were reduced to 5 ng each on-column and 0.5 μg each spiked into 1 mL serum, respectively, in the panel 3). The GC column used was a DB-1 medium-bore capillary (30 m × 0.32 mm i. d., film thickness 0.25 μm).**

References

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