II.4.3 Acetaminophen (paracetamol)

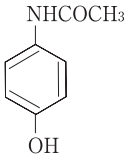
by Einosuke Tanaka

Introduction

Acetaminophen (paracetamol, APAP) *(Figure 3.1)* has been being used as an excellent analgesic-antipyretic for a long time, and is included as an ingredient in many over-the-counter drugs of analgesics and cold drugs. However, when APAP is ingested in large amounts, it was reported to cause liver disorders [1].

For analysis of APAP, HPLC [2–18], LC/MS [19], LC/MS/MS [20], GC [21], GC/MS [22, 23] and capillary electrophoresis [24, 25] are being used. Among the methods, HPLC is most popular for its analysis. In this chapter, HPLC methods for analysis of APAP and its metabolites are presented.

Figure 3.1



Structure of acetaminophen.

HPLC analysis of APAP and its metabolites in serum [18]

Reagents and their preparation

- APAP (Sigma, St. Louis, MO, USA) is dissolved in methanol to prepare 1 mg/mL solution.
- Theophylline (internal standard, IS, Sigma) is dissolved in 6 % perchloric acid aqueous solution to prepare 10 mg/mL solution.
- APAP and its metabolites[a] (APAP-glucuronide and APAP-*N*-sulfate) are dissolved in methanol to prepare 1–200 μg/mL solutions for calibration curves.

HPLC conditions

Column: a reversed phase column[b] (C_{18}, 150 × 4.6 mm i. d., particle diameter 5 μm, Supelco, Bellefonte, PA, USA).

Mobile phase: 0.05 mM sodium sulfate solution (pH 2.2)[c]/acetonitrile (93:7, v/v).

Detection wavelength: 254 nm; flow rate: 1.5 mL/min; column (oven) temperature: 30 °C.

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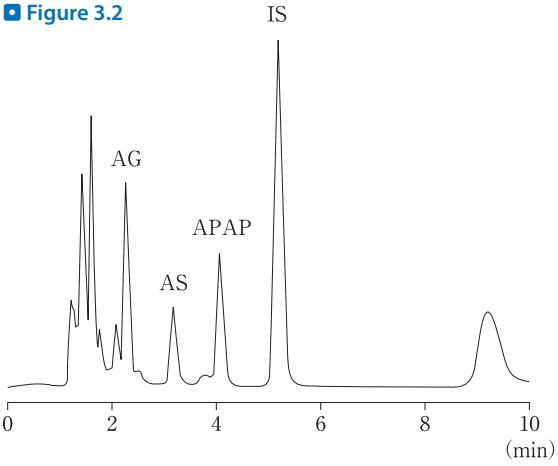
Procedure

i. A 10-μL[d] aliquot of serum and 20 μL IS solution[e] are placed in a centrifuge tube.
ii. The tube is vortex-mixed for 5 s.
iii. It is centrifuged at 1,700 *g* and 4 °C for 5 min.
iv. The supernatant fraction is transferred to a clean glass test tube.
v. A 10-μL aliquot of it is injected into HPLC.
vi. The various concentrations of the standard solutions are processed according to the above procedure.

Assessment of the method

Figure 3.2 shows an HPLC chromatogram for an extract of rat serum, to which APAP and its metabolites had been added. In this method, APAP and its metabolites can be simultaneously measured with a small amount of a specimen. Linearity could be obtained in the range of 1.56–200 μg/mL for APAP and its sulfate conjugate, and in the range of 3.5–500 μg/mL for APAP-glucuronide. The detection limit of all compounds was about 0.05 μg/mL, and recovery rates were 98–103 %.

Figure 3.2



HPLC chromatogram for acetaminophen (APAP) and its metabolites in an extract of rat serum [18]. APAP: acetaminophen (3.1 μg/mL, retention time 4 min); AG: APAP- glucuronide (7.8 μg/mL, 2.3 min); AS: APAP-*N*-sulfate (3.1 μg/mL, 3.1 min); IS: internal standard (theophylline) (20 μg/mL, 5.1 min).

HPLC analysis of APAP and its metabolites in urine [4]

Reagents and their preparation

- APAP (Eastmann, Rochester, NY, USA) and APAP metabolites[a] (APAP-glucuronide, catechol 3-hydroxyaminophen, APAP-*N*-sulfate, 3-cysteinyl APAP, 3-methoxy APAP and APAP-3-mercapturic acid) are dissolved in methanol.
- The concentrations of APAP and its metabolites to be prepared for calibration curves are 0.2–500 μg/mL.

HPLC conditions

Column: a reversed phase column[f], μBondapak C_{18} (300 × 4.6 mm i.d., particle diameter 10 μm, Waters, Milford, MA, USA).

Mobile phase: methanol/0.1 M potassium dihydrogenphosphate containing 0.75 % acetic acid (7:93, v/v).

Detection wavelength: 248 nm or an electrochemical detector[g] (+ 0.60 V).

Flow rate: 1.5 mL/min; column (oven) temperature: room temperature.

Procedure[h]

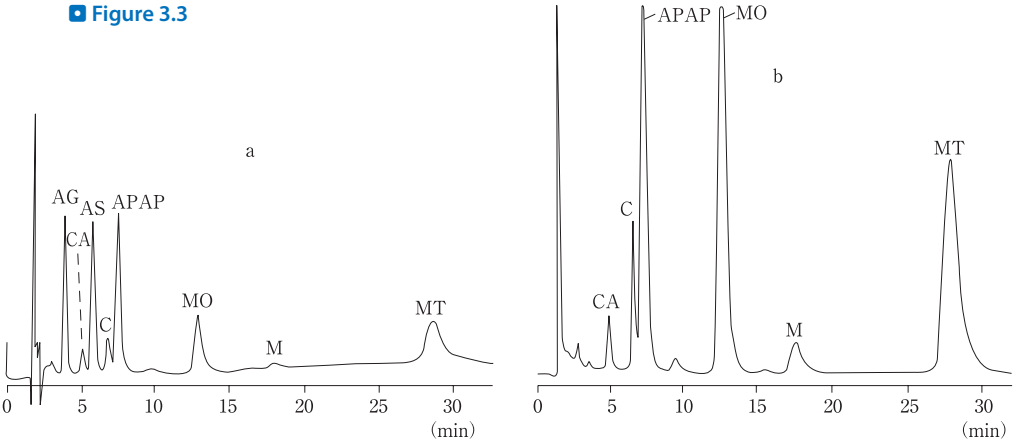
i. A 1-mL volume of urine and 4 mL of 2 M acetate buffer solution (pH 5.0) are placed in a centrifuge tube with a stopper in duplicate.
ii. A 50-μL aliquot of β-glucuronidase-sulfatase (Sigma) is added to one of the tubes, and 50 μL of 2 M acetate buffer (PH 5.0) to the other tube (control).
iii. Both tubes are incubated at 37 °C overnight with shaking.
iv. After the incubation, the tubes are cooled with ice to stop the enzymatic reaction.
v. After centrifugation, the supernatant solution is subjected to the procedure described in the above section for HPLC analysis in serum of this chapter; a fixed volume of the resulting specimen is injected into HPLC.
vi. For constructing calibration curves, various concentrations of standard solutions are processed in the same way.

Assessment of the method

Figure 3.3 shows HPLC chromatograms for extract of urine, to which APAP and its metabolites had been added. The electrochemical detector showed much higher sensitivity than the UV detector (about 5 times for APAP and 5–10 times for some metabolites).

About 95 % of APAP is excreted into urine in its glucuronide-conjugate form [26]; therefore, the conjugate can be converted to free APAP with β-glucuronidase-sulfatase to be measured without any authentic standard of APAP-glucuronide.

Figure 3.3



HPLC chromatograms for acetaminophen (APAP) and its metabolites extracted from human urine [4]. APAP: acetaminophen (4.5 µg/mL); AG: APAP-glucuronide (5.4 µg/mL); CA: catechol 3-hydroxyaminophen (3.1 µg/mL); AS: APAP-*N*-sulfate (4.7 µg/mL); C: 3-cysteinyl APAP (1.7 µg/mL); MO: 3-methoxy-APAP (2.2 µg/mL); M: APAP-3-mercapturic acid (1.5 µg/mL); MT: 3-methylthio-APAP (5 µg/mL); a: UV detector (248 nm); b: electrochemical detector (+ 0.60 V).

Toxic and fatal concentrations

See [27, 28]

For therapeutic use, a daily dose of more than 1.2 g of APAP should not be administered for more than 10 days. Its oral toxic doses in adults are 5–10 g; that in infants is 150 mg/kg. The oral fatal dose is 25 g or more. Blood therapeutic concentrations: 2.5–25 µg/mL; its toxic concentrations: 150–300 µg/mL; its fatal concentration: not less than 160 µg/mL (average 250 µg/mL).

Poisoning cases

Case 1 [29]: a 28-year-old black male was hospitalized for treatments of abdominal pain and hematemesis; the pain existed in the area of the upper abdomen and radiated towards the back. He had ingested 12–14 capsules (6–7 g) of APAP "Extra Strength" during 24 h. He was a chronic alcoholic and narcotic abuser, but he denied the use of illicit drugs at the time. The biochemical tests for liver and kidney functions showed abnormal data. At 36 h after the admission, it was disclosed that he had ingested a large amount of APAP; the blood APAP concentration was 60 µg/mL. On day 17 after the admission, the liver biopsy showed the findings of liver dysfunction (fibrosis and regenerated nodules), but the symptoms were gradually improved. He was discharged on day 20 after admission.

Case 2 [29]: a 28-year-old black male was admitted to a hospital, because of headache and fever. His general conditions had been good until 5 days before, when headache and fever were aggravated. He said that he had ingested 2–4 tablets every 4–6 h; it was considered that the total amount ingested had been 5–6 g (10–12 tablets) during 24 h. He denied his massive in-

gestion or suicide attempt. At 36 h after admission, extensive and abnormal pain of his trunk associated with icterus, dark urine, nausea and vomiting appeared. The excretion amounts of urine had decreased gradually before admission; for about 24 h before admission, he had not been able to urinate by himself. He had drunk a lot of beer in his daily life and had habitually ingested glutethimide, methaqualone and drug syrup obtainable without prescription; but he denied his drug abuse. The biochemical tests for liver and kidney functions showed abnormal data. The blood APAP concentration 17 h after admission was 237 μg/mL; it was decreased to 137 μg/mL 24 h later. At 48 h after admission, flapping tremor appeared. Peritoneal dialysis was performed, but he died on the next day.

Case 3 [29]: a 40-year-old male was admitted to a hospital because of the pain radiating towards the back; he had a past history of alcoholism and chronic pancreatitis. Just before admission, he had ingested 25–35 tablets of "Extra Strength" together with another kind of drug of APAP. During about 3 weeks before admission, he had drunk 12–18 cans of beer daily; but for 2 days just before admission, he did not drink. He had noticed his dark urine; for 3 days just before admission, nausea and vomiting appeared. The biochemical tests for liver and kidney functions showed slight abnormal data. Blood APAP concentration 72 h after admission was 14.5 μg/mL. Liver dysfunction was observed, but his conditions were gradually improved. He was discharged 14 days after admission.

Notes

a) The APAP metabolites (APAP-glucuronide, catechol 3-hydroxyaminophen, APAP-*N*-sulfate, 3-cysteinyl APAP, 3-methoxy APAP and APAP-3-mercapturic acid) are not commercially available; they should be synthesized [18].
b) In many reports for HPLC analysis, reversed phase chemical-bonded octadecyl (C_{18}) columns are being used.
c) The pH of the solution is adjusted to 2.2 with phosphoric acid. When only APAP is analyzed, the mobile phase at pH 7.0 or 9.0 can be used (see the analytical application data of Waters and other literature).
d) This method was established for small amounts of specimens of rat. By increasing the specimen volume, higher sensitivity can be obtained.
e) Since theophylline is contained in tea and coffee, other ISs, such as 2-acetaminophenol and 4-fluorophenol can be used.
f) Recently, columns with 10–15 cm length and 2.5–5 μm particle size are being well used.
g) The electrochemical detector gives much higher sensitivity than the UV detector.
h) For solid-phase extraction of APAP, the following procedure can be used:
    i. A 1-mL volume of methanol and 1 mL distilled water are passed through an Oasis™ HLB 30 mg/1 mL column (Waters) to activate it.
    ii. A 1 mL volume of serum is poured into the column.
    iii. A 1 mL volume of 5 % methanol in water is passed through the column to wash it.
    iv. APAP is eluted with 1 mL methanol.
    v. The eluate is evaporated to dryness under a stream of nitrogen with warming at 40 °C.
    vi. The residue is dissolved in 100 μL of the mobile phase.

vii. A fixed volume of the solution is injected into HPLC.
viii. Various concentrations of the authentic solution of APAP are processed according to the above procedure to construct a calibration curve.

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