II.4.4 Acetylsalicylic acid

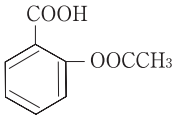
by Einosuke Tanaka

Introduction

Acetylsalicylic acid (ASA, aspirin) (*Figure 4.1*) has been being used as an analgesic-antipyretic for a long time; it is contained in many of over-the-counter drugs. Although ASA is relatively safe, various poisoning symptoms, such as lowered consciousness levels, hypotension, pulmonary edema and convulsion, were reported upon ingestion of a large amount of this drug [1].

For analysis of ASA, methods by HPLC [2–19], GC [20–23], GC/MS [24] and capillary electrophoresis [25–27] were reported; among these methods, HPLC is most popular. In this chapter, the methods for ASA analysis by HPLC [9, 16] and GC [23] are presented.

Figure 4.1



Structure of acetylsalicylic acid (ASA).

HPLC analysis of ASA and its metabolites in plasma [16]

Reagents and their preparation

- ASA, salicylic acid, gentisic acid and salicyluric acid can be purchased from Sigma (St. Louis, MO, USA).
- ASA is dissolved in acetonitrile to prepare 1 mg/mL solution.
- 2-Methylbenzoic acid (MBA) (internal standard, IS; Bayer, Leverkusen, Germany and other manufacturers) is dissolved in purified water to prepare 100 μg/mL solution.
- For constructing each calibration curve, methanolic solutions of ASA and its metabolites at various concentrations in the range of 0.2–100 μg/mL are prepared.

HPLC conditions

Column: a reversed phase column[a, b] (Novapak, 150 × 3.9 mm i.d., particle diameter 4 μm, Waters, Eschborn, Germany).

Mobile phase: purified water/85 % phosphoric acid/acetonitrile (740 mL:900 μL:180 mL, v/v) (pH about 2.5).

Detection wavelength: 237 nm; flow rate: 1 mL/min; column (oven) temperature: 30 °C.

Procedure

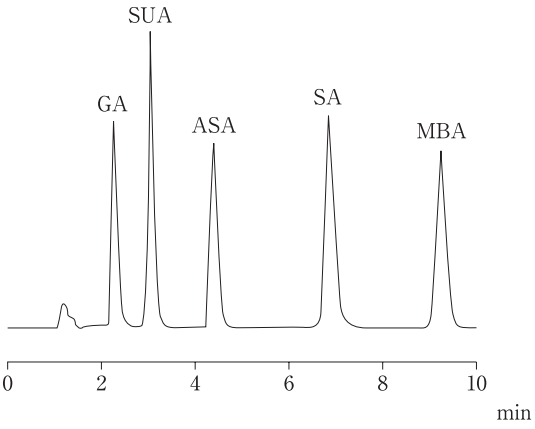
i. A 200-µL volume of plasma[c] and 200 µL of MBA[d] solution are placed in a 1.5-mL volume microtube, and mixed well for 1–2 s.
ii. The pH of the mixture is adjusted to about 2.7.
iii. A 400-µL volume of acetonitrile is added to the above mixture.
iv. It is vortex-mixed well at 4 °C for 15 min and centrifugal at 10,500 g for 1 min.
v. The supernatant fraction is transferred to another 1.5-mL volume microtube, followed by addition of 100–120 mg NaCl[e].
vi. The microtube is vortex-mixed and left at 4 °C for 10 min.
vii. It is centrifuged at 10,500 g for 1 min.
viii. A 10-µL volume of the supernatant fraction (acetonitrile layer) is injected into HPLC.
ix. For solutions of various concentrations of ASA and its metabolites, 200-µL aliquot each was processed according to the above procedure for construction of calibration curves.

Assessment of the method

Figure 4.2 shows an HPLC chromatogram for ASA and its metabolites extracted from human plasma. By this method, ASA, salicylic acid, gentisic acid and salicyluric acid, which is formed by glycine conjugation of salicylic acid, can be measured.

ASA and salicylic acid can be quantitated down to 100 ng/mL; the recoveries of ASA and its metabolites were 107–122 %.

Figure 4.2



HPLC chromatogram for the authentic acetylsalicylic acid and its metabolites [16]. GA: gentisic acid; SUA: salicyluric acid; ASA: acetylsalicylic acid; SA: salicylic acid; MBA: 2-methylbenzoic acid (IS). Each compound was dissolved in 0.01 M hydrochloric acid solution to prepare 50 µg/mL solution.

HPLC analysis of ASA and its metabolites in plasma, tissues and urine [9]

Reagents and its preparation

ASA (Sigma) is dissolved in methanol; for calibration curves, solutions of ASA and its metabolites at 0.2–10 μg/mL are prepared.

HPLC conditions

Column: a reversed phase column[a] (LiChrosorb RP-18, 150 × 4 mm i.d., particle diameter 5 μm).

Mobile phase[f]: methanol/purified water (60:40, v/v) (pH 3).

Detection wavelength: 280 nm; flow rate: 1.5 mL/min; column (oven) temperature: 45 °C.

Procedures[g]

i. Plasma

i. A 200-μL volume of plasma[c], 50 μL phosphoric acid and 600 μL ethyl acetate are placed in a small centrifuge tube.
ii. The tube is voltex-mixed for 30 s and centrifuged at 600 g for 10 min.
iii. A 400-μL volume of the organic phase is transferred to a small glass vial, and evaporated to dryness under a stream of air in an ice bath.
iv. The residue is dissolved in 200 μL of the mobile phase and injected into HPLC.
v. The solutions of ASA and its metabolites at various concentrations are processed according to the above procedure.

ii. Organ tissues

i. A 500-mg aliquot of an organ tissue is minced in 2 mL of purified water and homogenized with cooling with ice.
ii. It is centrifuged at 40,000 rpm for 30 s.
iii. The supernatant fraction is decanted to a test tube, and a 200 μL of it is subjected to the procedure of the above i. plasma.

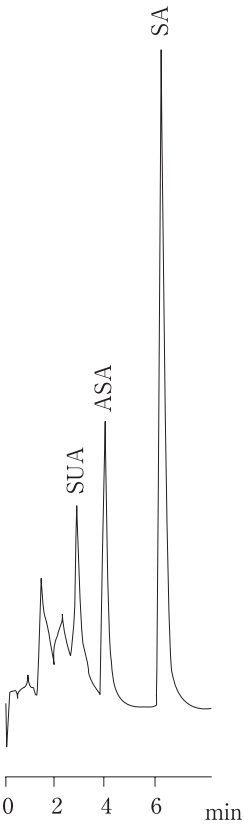
iii. Urine

i. Urine is diluted 10-fold with purified water.
ii. The diluted specimen is subjected to the procedure of the above i. plasma.

Assessment of the method

Figure 4.3 shows an HPLC chromatogram for ASA and its metabolites extracted from plasma of a rabbit, to which ASA had been administered intravenously 15 min before sampling

Figure 4.3



HPLC chromatogram for ASA and its metabolites extracted from plasma of a rabbit, to which 50 mg/kg ASA had been administered intravenously 15 min before sampling of its blood [9]. SUA: salicyluric acid; ASA: acetylsalicylic acid; SA: salicylic acid.

of its blood. By this method, ASA and its two metabolites in human plasma, tissues and urine can be analyzed. Quantitation limit of ASA and salicylic acid was about 500 ng/mL; the recoveries were 89–101 %.

GC analysis of ASA and its metabolite in serum [23]

Reagents and their preparation

- p-Hydroxybenzoic acid ethyl ester (IS, Sigma) is dissolved in purified water to prepare 15 μg/mL solution.
- ASA is dissolved in methanol. For its calibration curve, ASA solutions at 10–250 μg/mL are prepared.

GC conditions

Column[h]: a packed glass column, 2 % OV-225 Gas Chrom W (80–100 mesh, 1.2 m × 4 mm i. d., obtainable from many manufacturers).

Temperatures: column 110 °C, injection port 250 °C, detector 300 °C; detector: FID; carrier gas (flow rate): nitrogen (60 mL/min); detector gas (flow rate): air (100 mL/min) and hydrogen (30 mL/min).

Procedure

i. A 100-μL volume of serum[c, i], 2 mL of 1 M hydrochloric acid solution and 1 mL *p*-hydroxybenzoic acid ethyl ester (IS) solution are placed in a 10-mL volume glass centrifuge tube with a ground-in stopper.
ii. A 5-mL volume of ethyl ether is added to the above mixture, shaken and centrifuged; this procedure is repeated once.
iii. The combined organic phase (upper layer) is transferred to a 10–20 mL volume test tube.
iv. The phase is condensed to a small amount (about 1 mL) under a stream of nitrogen with warming at 42–44 °C. The condensed extract is transferred to a 4-mL volume glass vial with a silicone cap and evaporated to dryness under a stream of nitrogen.
v. The residue is mixed with 10 μL acetonitrile and 5 μL *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce, Rockford, IL, USA) and heated at 60 °C for 10 min for silylation.
vi. A 2–3 μL aliquot of it is injected into GC.
vii. Solutions of ASA or salicylic acid at various concentrations are treated according to the above procedure for constructing calibration curves.

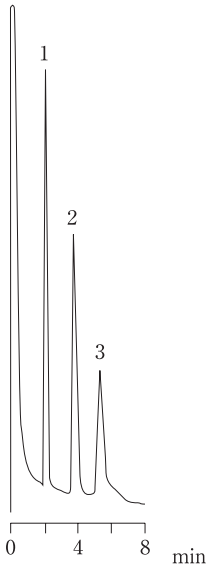
Assessment of the method

Figure 4.4 shows a gas chromatogram for ASA and its metabolite salicylic acid extracted from human serum. Quantitative analysis of both compounds can be made in the range of 25–250 μg/mL.

Toxic and fatal concentrations [28, 29]

When 150–300 mg/kg of ASA is ingested orally, various poisoning symptoms, such as nausea, vomiting and tinnitus, appear; when the dose exceeds 300 mg/kg, the symptoms become serious. Dangerous oral doses of ASA for adults and infants are about 20 and 1.5 g, respectively. Therapeutic blood ASA concentrations: 20–100 μg/mL; toxic concentrations: 150–300 μg/mL; fatal concentration: not lower than 500 μg/mL.

Figure 4.4



Gas chromatogram for the spiked ASA and salicylic acid extracted from human serum [23]. 1: salicylic acid; 2: *p*-hydroxybenzoic acid ethyl ester (IS); 3: acetylsalicylic acid (ASA). A 15-μg each of the compounds was added to 1 mL serum.

Poisoning cases

Case 1 [30]: a 25-year-old white female had been healthy physically; but she had been diagnosed to be the borderline-type personality disorder. She had attempted suicide several times. She had been habitually taking tranylcypromine (a monoamine oxidase inhibitor). At about 7:00 p. m., she ingested all Ecotrin tablets (enteric coating) in a bottle. This means that she ingested about 30 g of ASA, because the bottle contained 90–100 tablets each containing 325 mg ASA. She vomited the tablets and their residue repeatedly. At 11:00 p. m., she was brought to an emergency hospital in the comatose state for admission. The blood tests showed respiratory alkalosis and metabolic acidosis. As a result of treatments and observation, she was transferred to the psychiatric department of the hospital 4 days after. The blood specimens were sampled at some intervals after the ingestion. The blood concentrations of salicylic acid at 6, 12 and 17 h after ingestion were 30, 200 and 300 μg/mL, respectively. The salicylic acid concentrations increased thereafter; the peak concentration was attained at 24 h after slowly ingestion.

Case 2 [31]: a 64-year-old female received laminectomy, because of chronic articular rheumatism. After the operation, she was administered the long-lasting enteric coating tablets of ASA; she took two tablets (800 mg ASA each) of Solprin twice (in total 3,200 mg ASA) daily. During the admission, the Solprin tablets were changed to Ecotrin tablets each containing 325 mg ASA; she took 3 tablets of Ecotrin 4 times (in total 3,900 mg) daily. After recovery, she returned to her sanatorium, where she took overdoses of ASA; she took 3 tablets (325 mg each ASA) of Ecotrin at 7:00 a. m., 2 tablets (800 mg each ASA) of Solprin at 8:00 a. m., 3 tablets of Ecotrin at 11:00 a. m., 3 tablets of Ecotrin at 4:00 p. m. and 3 tablets of Ecotrin plus 2 tablets

of Solprin at p.m. 9:00. Therefore, she ingested 7.1 g ASA daily (97 mg/kg/day, body weight 73.2 kg) for 10 days. From about 24 h before the second admission to the hospital, slight fever and somnolence appeared. The last ingestion of ASA tablets was made at 9:00 p. m. on the previous day of admission. In the morning of the day for admission, she fell into the comatose state. The blood ASA concentration at 17 h after the last ingestion was 924 μg/mL; the concentration decreased to 748 μg/mL on day 2 of admission, but she died on day 3.

Notes

a) In many reports on HPLC analysis of ASA, reversed phase chemical-bonded octadecyl silica gel columns are being used.
b) To prevent the peak of salicylic acid from tailing, 400 μL di-*n*-butylamine is mixed with 200 mL of mobile phase, and passed through the column at a flow rate of 0.3 mL/min before injection of a sample solution.
c) ASA is easily converted into salicylic acid by the action of esterase in blood. To prevent ASA from its postmortem conversion, 4 mg sodium fluoride and 50 I. U. heparin should be added to 1.5 mL blood just after sampling. Blood specimens are preferably stored at not higher than −70 °C. It is also recommended that the final extract solution prepared is analyzed as soon as possible, and all procedure for extraction is made under cooling with ice.
d) MBA is dissolved in 0.2 M hydrochloric acid solution/0.2 M phosphoric acid solution (50:50, v/v) to prepare 5 μg/mL solution.
e) NaCl is added to prevent the test solution from its evaporation. For rapid analysis, the steps vi.–viii. can be skipped.
f) The pH of the mobile phase is adjusted to 3 by using 5 mM NaOH and 5 mM phosphoric acid solutions.
g) In this method, no IS is used.
h) The column should be heated at 225 °C with nitrogen flow overnight for its aging. The silylation of the packing material with hexamethyldisilazane (HMDS) is useful to obtain sharp peaks.
i) Plasma, serum and whole blood can be used as specimens.

References

1) Japan Poison Center (ed) (2000) Poisoning Accidents and their Countermeasures with Special Reference to Actual Cases, revised edn. Jiho Inc., Tokyo, pp 127–131 (in Japanese)
2) Blair D, Rumack BH, Peterson RG (1978) Analysis for salicylic acid in serum by high-performance liquid chromatography. Clin Chem 24:1543–1544
3) Cham BE, Johns D, Bochner F et al. (1979) Simultaneous liquid-chromatographic quantitation of salicylic acid, salicyluric acid, and gentisic acid in plasma. Clin Chem 25:1420–1425
4) Cham BE, Ross-Lee L, Bochner F et al. (1980) Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay. Ther Drug Monit 2:365–372
5) Harrison LI, Funk ML, Ober RE (1980) High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine. J Pharm Sci 69:1268–1271
6) Wahlin-Boll E, Brantmark B, Hanson A et al. (1981) High-pressure liquid chromatographic determination of acetylsalicylic acid, salicylic acid, diflunisal, indomethacin, indoprofen and indobufen. Eur J Clin Pharmacol 20:375–378

7) Rumble RH, Roberts MS, Wanwimolruk S (1981) Determination of aspirin and its major metabolites in plasma by high-performance liquid chromatography without solvent extraction. J Chromatogr 225:252–260
8) Buskin JN, Upton RA, Williams RL (1982) Improved liquid-chromatography of aspirin, salicylate, and salicyluric acid in plasma, with a modification for determining aspirin metabolites in urine. Clin Chem 28:1200–1203
9) Reidl U (1983) Determination of acetylsalicylic acid and metabolites in biological fluids by high-performance liquid chromatography. J Chromatogr 272:325–331
10) Bakar SK, Niazi S (1983) High-performance liquid chromatographic determination of aspirin and its metabolites in plasma and urine. J Pharm Sci 72:1020–1023
11) O,Kruk RJ, Adams MA, Philp RB (1984) Rapid and sensitive determination of acetylsalicylic acid and its metabolites using reversed-phase high-performance liquid chromatography. J Chromatogr 310:343–352
12) Ogunbona FA (1986) Simultaneous liquid chromatographic determination of aspirin and the metabolites in human urine. J Chromatogr 377:471–474
13) Siebert DM, Bochner F (1987) Determination of plasma aspirin and salicylic acid concentrations after low aspirin doses by high-performance liquid chromatography with post-column hydrolysis and fluorescence detection. J Chromatogr 420:425–431
14) Gaspari F, Locatelli M (1987) Determination of aspirin and salicylic acid in uremic patients plasma using reversed-phase high-performance liquid chromatography. Ther Drug Monit 9:243–247
15) Legaz ME, Acitores E, Valverde F (1992) Determination of salicylic acid by HPLC in plasma and saliva from children with juvenile chronic arthritis. Tokai J Exp Clin Med 17:229–237
16) Kees F, Jehnich D, Grobecker H (1996) Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by high-performance liquid chromatography. J Chromatogr B 677:172–177
17) Krivosikova Z, Spustova V, Dzurik R (1996) A highly sensitive HPLC method for the simultaneous determination of acetylsalicylic, salicylic and salicyluric acids in biologic fluids: pharmacokinetic, metabolic and monitoring implications. Method Find Exp Clin Pharmacol 18:527–532
18) McMahon GP, Kelly MT (1998) Determination of aspirin and salicylic acid in human plasma by column-switching liquid chromatography using on-line solid-phase extraction. Anal Chem 70:409–414
19) Pirola R, Bareggi SR, De Benedittis G (1998) Determination of acetylsalicylic acid and salicylic acid in skin and plasma by high-performance liquid chromatography. J Chromatogr B 705:309–315
20) Walter LJ, Biggs DF, Coutts RT (1974) Simultaneous GLC estimation of salicylic acid and aspirin in plasma. J Pharm Sci 63:1754–1758
21) Rance MJ, Jordan BJ, Nichols JD (1975) A simultaneous determination of acetylsalicylic acid, salicylic acid and salicylamide in plasma by gas liquid chromatography. J Pharm Pharmacol 27:425–429
22) Tam YK, Au DS, Abbott FS (1979) Improved gas-liquid chromatographic-flame ionization detection assay of acetylsalicylic and salicylic acids. J Chromatogr 174:239–244
23) Belanger PM, Lalande M, Dore F et al. (1983) Rapid gas chromatographic determination of serum salicylates after silylation. J Pharm Sci 72:1092–1093
24) Tsikas D, Tewes KS, Gutzki FM et al. (1998) Gas chromatographic-tandem mass spectrometric determination of acetylsalicylic acid in human plasma after oral administration of low-dose aspirin and guaimesal. J Chromatogr B 709:79–88
25) Goto Y, Makino K, Kataoka Y et al. (1998) Determination of salicylic acid in human serum with capillary zone electrophoresis. J Chromatogr B 706:329–335
26) Hansen SH, Jensen ME, Bjornsdottir I (1998) Assay of acetylsalicylic acid and three of its metabolites in human plasma and urine using non-aqueous capillary electrophoresis with reversed electroosmotic flow. J Pharm Biomed Anal 17:1155–1160
27) Zaugg S, Zhang X, Sweedler J et al. (2001) Determination of salicylate, gentisic acid and salicyluric acid in human urine by capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr B 752:17–31
28) Naito H (2002) Poisoning of Industrial Products, Gases, Pesticides, Drugs, and Natural Toxin. Cases, Pathogenesis and its Treatment, 2nd edn. Nankodo, Tokyo, pp 354–355 (in Japanese)
29) Stead AH, Moffat AC (1983) A collection of therapeutic, toxic and fatal blood drug concentrations in man. Hum Toxicol 2:437–464
30) Kwong TC, Laczin J, Baum J (1983) Self-poisoning with enteric-coated aspirin. Am J Clin Pathol 80:888–890
31) Shkrum MJ, Gay RM, Hudson P (1989) Fatal iatrogenic salicylate intoxication in a long-term user of enteric-coated aspirin. Arch Pathol Lab Med 113:89–90