II.4.5 Antiepileptics

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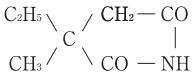
Introduction

As antiepileptics, phenytoin, mephenytoin, nirvanol and ethotoin are being used for treatments of grand mal and complex partial seizures. A iminostilbene derivative carbamazepine, phenobarbital (one of the barbiturates) and some benzodiazepines are also being well used as antiepileptics (*Figure 5.1*). Poisoning cases due to accidental and suicidal ingestion of these antiepileptics were reported [1].

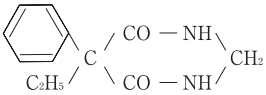
For analysis of antiepileptics, methods using HPLC [2–15], LC/MS [16], GC [17, 18] and GC/MS [19,20] were reported. Among them, HPLC methods are being used most commonly. In this chapter, two HPLC methods, dealing with analysis of phenytoin and other antiepileptics together with their metabolites in serum [11] and plasma [15], are presented.

Figure 5.1

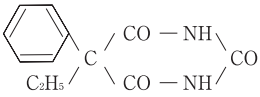
1 ethosuximide



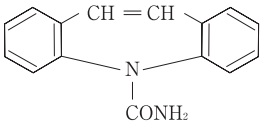
2 primidone



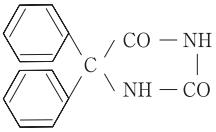
3 phenobarbital



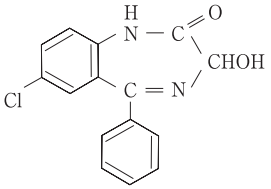
4 carbamazepine



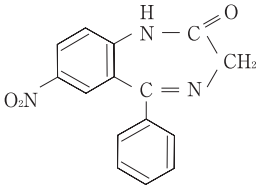
5 phenytoin



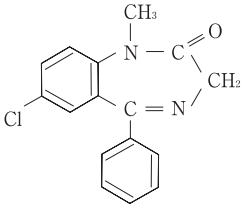
6 oxazepam



7 nitrazepam



8 diazepam



Structures of antiepileptics and their analogs.

HPLC analysis of antiepileptics in serum [11]

Reagents and their preparation

- Carbamazepine, ethosuximide, phenytoin, primidone, phenobarbital, diazepam, oxazepam, nitrazepam and 5-(4-methylphenyl)-5-phenylhydantoin (internal standard, IS) can be all purchased from Sigma (St. Louis, MO, USA).
- Each of above drugs is dissolved in methanol to prepare 1 mg/mL solution.
- By diluting the 1 mg/mL solution with methanol, solutions at various concentrations in the range of 0.05–5 μg/mL are prepared for drugs to be quantitated, because they are used for constructing each calibration curve.

HPLC conditions

Column: a reversed phase column[a] (TSK gel super-ODS; 100 × 4.6 mm i. d., particle diameter 2 μm, Tohso, Tokyo, Japan).

Mobile phase: acetonitrile/8 mM phosphoric acid aqueous solution (3:7, v/v); its flow rate: 0.6 mL/min; detection wavelength: 215 nm; column (oven) temperature: room temperature.

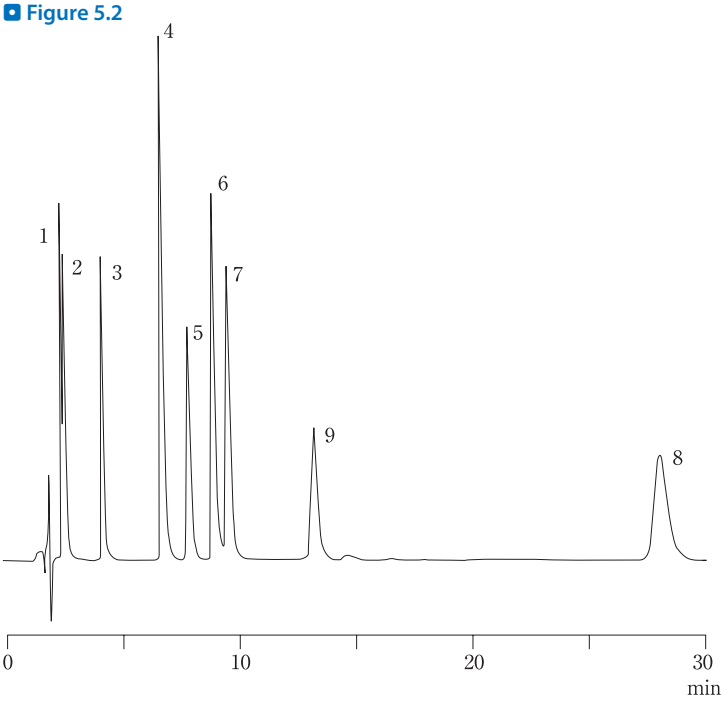
Procedure

i. A 0.5-mL volume of serum[b], 100 μL of 0.2 M hydrochloric acid solution, 50 μL of IS solution and 3 mL dichloromethane are placed in a glass centrifuge tube with a ground-in stopper.
ii. After shaking for 1 min, the tube is centrifuged.
iii. The resulting organic phase (lower layer) is transferred to a glass vial with a conical bottom.
iv. The organic extract is evaporated to dryness under a stream of nitrogen.
v. The residue is dissolved in 100 μL mobile phase.
vi. A fixed amount of the above solution is injected into HPLC.
vii. For constructing calibration curves, various concentrations of standard solution are mixed with 50 μL IS solution each and processed according to the above procedure.

Assessment of the method

Figure 5.2 shows an HPLC chromatogram for the eight antiepileptics listed in *Figure 5.1*, which had been spiked into human serum. The linearity of each compound could be obtained in the range of 0.05–1 μg/mL in serum; recovery rates were 96–104 %. The detection limits were: 0.05 μg/mL for carbamazepine; 0.1 μg/mL for primidone, phenobarbital, phenytoin[c], oxazepam, ethosuximide, and nitrazepam; and 0.5 μg/mL for diazepam.

Figure 5.2



HPLC chromatogram for 8 antiepileptics spiked into human serum [11]. 1: ethosuximide (10 µg/mL, retention time 2.6 min); 2: primidone (1 µg/mL, 2.7 min); 3: phenobarbital (1 µg/mL, 4.3 min); 4: carbamazepine (1 µg/mL, 6.9 min); 5: phenytoin (1 µg/mL, 8.0 min); 6: oxazepam (1 µg/mL, 9.0 min); 7: nitrazepam (1 µg/mL, 9.6 min); 8: diazepam (1 µg/mL, 28.1 min); 9: 5-(4-methylphenyl)-5-phenylhydantoin (IS, 1 µg/mL).

HPLC analysis of antiepileptics and some metabolites in plasma [15]

Reagents and their preparation

- Ethosuximide, primidone, phenobarbital, carbamazepine, phenytoin and carbamazepine-epoxide can be purchased from Sigma. Lamotrigine, carbamazepine-diol and 9-hydroxy-methyl-10-carbamyl acridan were reported to be obtainable from Chiba-Geigy (Basel, Switzerland)[d]. These compounds are dissolved in methanol to prepare 1 mg/mL solution.
- By diluting the methanolic 1 mg/mL solution, various concentrations of some drugs in the range of 0.5–200 µg/mL are prepared for constructing each calibration curve.

HPLC conditions

Column: a reversed phase column[a], Supelcosil LC-18 (150 × 4.6 mm i.d., particle diameter 5 µm, Supelco, Bellefonte, PA, USA).

Mobile phase: 0.01 M potassium dihydrogenphosphate solution/methanol/acetonitrile (65:18:17, v/v) (pH 7.5); detection wavelength: 220 nm; flow rate: 1 mL/min: column (oven) temperature: room temperature.

Procedure[e]

i. A 100-μL aliquot of plasma[b] and 20 μL IS solution are mixed for several seconds in a 2-mL volume microtube.
ii. A 1-mL volume of diethyl ether is added to the above mixture.
iii. After shaking for 5 min, the tube is centrifuged at 1,000 g for 10 min.
iv. The organic phase (upper layer) is transferred to another clean microtube.
v. The phase is evaporated to dryness under a stream of nitrogen.
vi. The residue is dissolved in 100 μL of the mobile phase.
vii. A 20-μL aliquot of the solution is injected into HPLC.
viii. For constructing calibration curves, various concentrations of standard solutions are mixed with 20 μL IS solution each and processed according to the above procedure.

Assessment of the method

In this method, 6 kinds of antiepileptics (ethosuximide, primidone, lamotrigine, phenobarbital, phenytoin and carbamazepine) and two carbamazepine metabolites (carbamazepine-diol and carbamazepine- epoxide) can be detected from human plasma (*Figure 5.3)*. The detection limits of each drug was about 0.2 μg/mL; recovery rates were 71–104 %.

Toxic and fatal concentrations in blood [21]

Phenytoin: therapeutic, 7 μg/mL (10–20 μg/mL); toxic, 48 μg/mL (30–60 μg/mL); fatal, not lower than 70 μg/mL (average 94 μg/mL).

Carbamazepine: therapeutic, 6.4 μg/mL (3.5–9.4 μg/mL): toxic, 10.1 μg/mL (3.2–20.6 μg/mL); fatal, 19.6 μg/mL.

Ethosuximide: therapeutic, 44 μg/mL (13–71 μg/mL); toxic, 100–200 μg/mL; fatal, 250 μg/mL.

Primidone: therapeutic, 10.5 μg/mL (6–17.8 μg/mL): toxic, 20–50 μg/mL; fatal, 65 μg/mL.

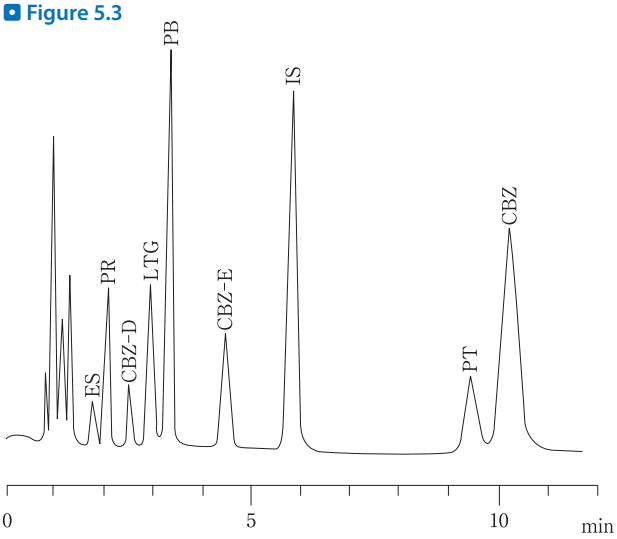
Diazepam: therapeutic, 0.05–2 μg/mL; toxic, 2–5 μg/mL; fatal, not lower than 5 μg/mL.

Phenobarbital: therapeutic, 12.3 μg/mL (4–26.2 μg/mL); toxic, 16.7 μg/mL (3.7–90 μg/mL); fatal, 45 μg/mL (4.3–120 μg/mL).

Oxazepam: therapeutic, 0.5–2 μg/mL; toxic, not lower than 2 μg/mL; fatal, not clear.

Nitrazepam: therapeutic, 0.044 μg/mL (0.026–0.066 μg/mL); toxic, 0.2 μg/mL (a single oral intake); fatal, 5.2–9 μg/mL.

Figure 5.3



HPLC chromatogram for 6 antiepileptics and two metabolites spiked into human plasma [15]. ES: ethosuximide (25 mg/mL, retention time 1.80 min); PR: primidone (2.5 mg/mL, 2.10 min); CBZ-D: carbamazepine-diol (1 mg/mL, 2.55 min); LTG: lamotrigine (1 mg/mL, 2.95 min); PB: phenobarbital (5 mg/mL, 3.33 min); CBZ-E: carbamazepine-epoxide (1 mg/mL, 4.48 min); IS: 9-hydroxymethyl-10-carbamyl acridan (IS, 5.92 min); PT: phenytoin (2.5 mg/mL, 9.45 min); CBZ: carbamazepine (2.5 mg/mL, 10.2 min).

Poisoning cases

Case 1 [22]: a 85-year-old female suffered from fever, muscle stiffness and itching exanthemas at home just before admission to a hospital. As her past history, she had experienced hysterectomy, ischemic heart disease and diabetes mellitus (type II). About one month before admission, she had been diagnosed as complex partial epileptic seizure and had daily taken 300 mg phenytoin (once at night), 500 mg metformin (twice a day), 500 mg tolbutamide (twice a day), 10 mg nifedipine (twice a day) and 1 mg risperidone (once a day). She had no history of smoking, and had quitted drinking alcohol since the appearance of the epileptic attack. Upon medical examination for admission to a hospital, she complained of her bad general conditions; her physical conditions were: body temperature 38 °C, heart beat 90/min and blood pressure 120/70 mmHg. There were erythematous exanthemas and the characteristic stiffness of the neck; but neither photophobia nor other pathological neurological findings were observed. Antibiotic, corticosteroid and antihistaminic drugs were administered intravenously. All drugs for oral intake except phenytoin were discontinued on day 3 after admission. The administration of phenytoin was also stopped on day 7.

Benzodiazepine antiepileptics were used to control the attack; the functions of the kidney, liver and heart were aggravated. She died 12 days after admission. The concentration of blood phenytoin at admission was 13.6 μg/mL.

Case 2 [23]: a 61-year-old female had been suffering from chronic headache and frequent vertigo. As a result of clinical tests, a meningioma had been discovered in the left fronto-peri-

etal lobe of her brain. It had been removed by surgery without any complication. After discharge from the hospital, she took 100 mg phenytoin every 8 h to prevent her from epileptic attack. Four days after her discharge, fever and nausea appeared; on the next day (on day 5 after discharge), itching erythematous exanthemas appeared and extended to the face, chest and extremities. Seven days after the appearance of the exanthemas, icterus, epigestric and right upper abdominal pains, bile-containing urine and polyuria appeared. Her family doctor stopped the administration of phenytoin and prescribed an antihistaminic drug. She had a past history of allergy against lorazepam, but not history of hepatitis, blood transfusion or alcohol intake. Upon visiting a university hospital, her physical conditions were: fever 38 °C, heart beat 90/min and blood pressure 120/80 mmHg. The erythematous exanthemas extended over almost whole skin of the body; in the neck and back, the exanthemas became desquamative. Clinical tests showed normal data except for the liver function. The blood phenytoin concentration was 0.95 μg/mL (normal value 10–20 μg/mL). The findings of liver biopsy showed extensive damages of hepatocytes associated with disarrangement of the lobules and with inflammatory cell infiltration into the portal vein and the parenchyma.

Case 3 [24]: a 18-year-old male (body weight 60 kg) had attempted suicide by ingesting phenytoin capsules (amount ingested not clear); he had no history of epilepsy. Twelve hours after ingestion, he had been brought to a hospital in the semicomatose state. Just after admission, the blood phenytoin concentration was 45 μg/mL, but his vital signs were stable. The gastro-lavage was performed; but no capsules or their debris could not be found in the lavage fluids. The sounds of the intestinal peristalsis decreased slightly. Thereafter, the blood phenytoin level increased up to 114 μg/mL on the 5th day; in the second week, the level was 105 μg/mL. The patient was still in the state of delirium, which was not improved. Grand mal, which seemed to be secondary to the toxic encephalopathy, appeared twice. By the end of the 2nd week, the sound of intestinal peristalsis became audible; the blood phenytoin concentration decreased to 75 μg/mL. After 3 weeks of admission, the phenytoin could not be detected from his blood.

Notes

a) In many reports, reversed phase octadecyl (C_{18}) chemical-bonded silica gel columns are being used.
b) Blood, serum and plasma specimens are stable at –20 °C for at least 4 weeks; they are stable at 25 °C for 24 h.
c) Phenytoin has optical isomers. Their determination method is described in the reference [6].
d) Lamotrigine and carbamazepine-diol are not obtainable in Japan; the analytical data of these compounds has been shown only as useful informations.
e) For extraction of antiepileptics by solid-phase extraction, the following procedure can be recommended.
    i. A 5-mL volume of 20 % methanol aqueous solution and 5 mL distilled water are passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) to activate it.
    ii. A 1-mL volume of a serum specimen is poured into the cartridge.
    iii. The cartridge is washed with 5 mL distilled water.
    iv. The target compounds are eluted with 5 mL methanol.

v. The residue is evaporated to dryness under a steam of nitrogen with warming at 40 °C.
vi. The residue is dissolved in 100 μL of the mobile phase.
vii. A fixed amount of the solution is injected into HPLC.
viii. For construction of calibration curves, various concentrations of the standard solutions are processed according to the above procedure.

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