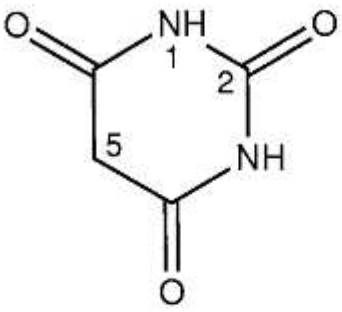
Barbiturates

Barbiturates are 5,5'-disubstituted derivatives of barbituric acid. In addition, the nitrogen atom at position 1 may be methylated as in methylphenobarbital, while substitution of sulfur for oxygen at position 2 gives thiobarbiturates such as thiopental.

The structure of barbituric acid is shown below:



Some commonly occurring barbiturates are listed in Table 18. Other barbiturates that may be encountered include cyclobarbital, cyclopentobarbital, heptabarbital, hexobarbital, methohexital and vinbarbital. Note that barbituric acid itself is no longer used as a drug.

Barbiturates are potent hypnotics and sedatives, but in many countries only phenobarbital and (intravenous) thiopental find wide application nowadays. Barbiturates may also be used for euthanasia in veterinary medicine, and barbital sodium is used as a laboratory chemical, especially in buffer solutions.

In acute poisoning it may be important to ascertain whether barbital or phenobarbital (so-called long-acting barbiturates), or a short- or medium-acting compound has been taken. This is because alkaline diuresis (see section 2.2.3) can enhance the excretion of barbital and phenobarbital, but not of other barbiturates.

Table 18. Some barbiturate hypnotics

Compound	Chemical name	Relative molecular mass
Amobarbital	5-Ethyl-5-isopentylbarbituric acid	226
Barbital	5,5-Diethylbarbituric acid	184
Pentobarbital	5-Ethyl-5(1-methylbutyl)barbituric acid	226
Phenobarbital	5-Ethyl-5-phenylbarbituric acid	232
Secbutabarbital	5-*n*-Butyl-5-ethylbarbituric acid	212
Secobarbital	5-Allyl-5-(1-methylbutyl)barbituric acid	238
Thiopental	5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric acid	242

There is no reliable simple test for these compounds and a qualitative analysis is best performed by thin-layer chromatography of a solvent extract of urine, stomach contents or scene residues (see section 5.2.3). This should also permit identification of the type of barbiturate present, if not the actual compound ingested.

The method given below will permit measurement of total barbiturate in a solvent extract of the specimen, and relies on the characteristic spectral shift shown by barbiturates on going from pH 11 to pH 2. However, ideally a double-beam spectrophotometer is required (see section 4.5). Accurate measurement of individual barbiturates normally requires gas-liquid or high-performance liquid chromatography.

Quantitative assay

Applicable to whole blood, plasma or serum (5 ml).

Reagents

1. Borate buffer, pH 8.4. Mix 22.4 g of disodium tetraborate with 76 ml of aqueous hydrochloric acid (1 mol/l) and dilute to 2 litres with purified water.
2. Aqueous hydrochloric acid (2 mol/l).
3. Concentrated sulfuric acid (relative density 1.83).
4. Concentrated ammonium hydroxide (relative density 0.88).
5. Sodium sulfate/charcoal mixture. Add 100 mg of activated charcoal to 100 g of anhydrous sodium sulfate, mix thoroughly and heat in an evaporating basin at 100 °C for 8 hours. Allow to cool and store in a tightly stoppered bottle.

Standards

Solutions containing barbital at concentrations of 5, 10, 25 and 50 mg/l in blank human plasma, prepared by dilution from an aqueous solution of barbital sodium (1.12 g/l, equivalent to diethylbarbituric acid at a concentration of 1.00 g/l).

Method

1. Add 5 ml of sample, 2 ml of hydrochloric acid and 60 ml of diethyl ether (with care) to a 250-ml separating funnel.
2. Lubricate the stopper of the funnel with purified water, insert and shake gently for 2 minutes.
3. Allow to stand for 5 minutes, and then discard the lower, aqueous phase through the tap of the separating funnel.

4. Add the diethyl ether extract to 10 ml of borate buffer in a second separating funnel and mix for 1 minute.
5. Allow to stand for 5 minutes and again discard the lower, aqueous phase through the funnel tap.
6. Wash round the funnel with 5 ml of purified water, allow to stand for 5 minutes and again discard the lower, aqueous phase through the funnel tap.
7. Add about 4 g of sodium sulfate/charcoal mixture to the ether extract in the funnel, shake to disperse, and filter the extract through phase-separating filter-paper into a 150-ml conical flask.
8. Add a further 20 ml of diethyl ether to the separating funnel, shake and add to the extract in the flask through the filter funnel.
9. Evaporate the extract to dryness on a water-bath at 40 °C under a stream of compressed air or nitrogen.
10. Add 5.0 ml of purified water to the dry extract in the flask, swirl gently and allow to stand for 5 minutes.
11. Filter the reconstituted extract through phase-separating filter-paper into a 12.5-cm test-tube.
12. Check the spectrophotometer zero at 240 nm using purified water in both sample and reference positions (1 × 1 × 4-cm fused silica cells, see section 4.5.2).
13. Add 4 ml of filtrate from the test-tube to a clean, dry cell, add 50 µl of concentrated ammonium hydroxide and mix using a plastic paddle. Check that the pH is about 10 (universal indicator paper).
14. Quickly measure the absorbance at 240 nm against a purified water blank (see section 4.5.2). If necessary, accurately dilute a portion of the extract with purified water to bring the reading on to the scale, and record the magnitude of the dilution. If a scanning spectrophotometer is available, scan in the region 200–450 nm.
15. Repeat the reading or scan after 5 minutes.
16. Add 0.1 ml of concentrated sulfuric acid to the cell, mix using the plastic paddle, and check that the pH is about 2 (universal indicator paper).
17. Repeat the reading (240 nm) or scan (200–450 nm).

Results

A number of compounds can interfere. Glutethimide is hydrolysed rapidly at alkaline pH values, so that the absorbance at 240 nm will markedly decrease after 5 minutes at pH 11 (step 15 above) if this compound is present. The presence of other compounds, such as methaqualone or phenazone (e.g., dichloralphenazone), can be revealed by scanning in the region 200–450 nm. Addition of 0.1 ml of aqueous sodium hydroxide (2 mol/l) to the ammoniacal extract (step 14 above)

produces a further characteristic spectral change (Fig. 10) which can be useful in qualitative work.

To perform a quantitative measurement, measure the difference between absorbance at pH 10 and at pH 2, construct a calibration graph by analysis of the standard barbiturate solutions, and calculate the barbiturate concentration in the sample.

Alternatively, use the following formula:

((absorbance at pH10) − (absorbance at pH2)) × dilution factor (if any) × 25 = barbiturate (mg/l)

Sample volumes of less than 5 ml may be used, but there will be a corresponding loss of sensitivity unless "micro"-volume fused silica spectrophotometer cells are available.

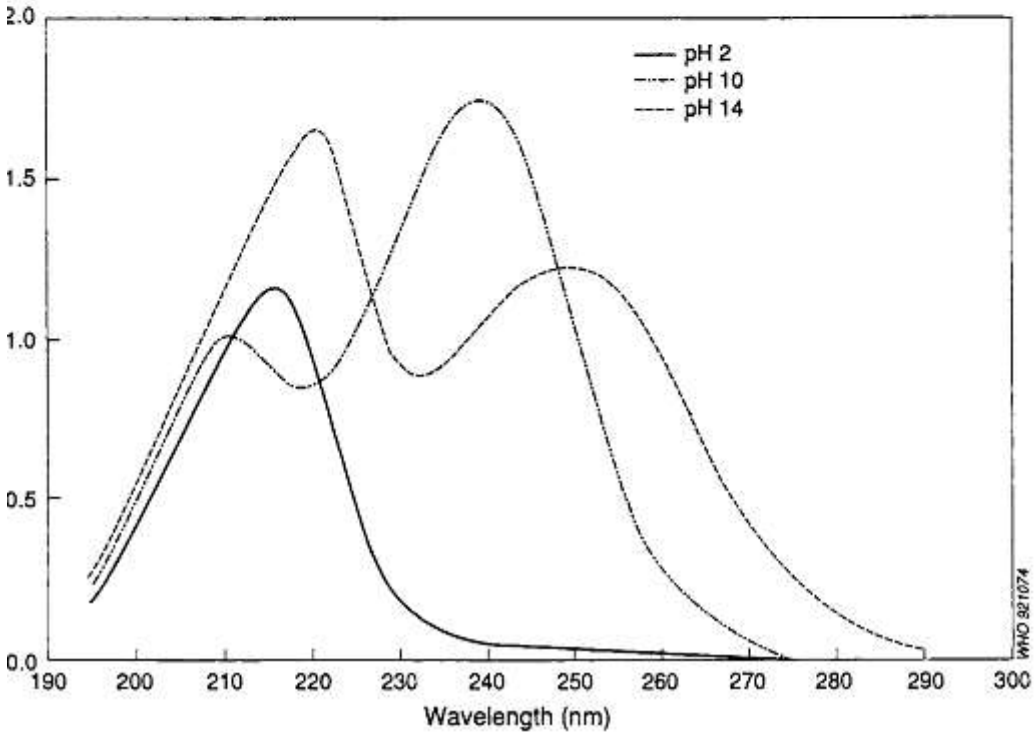
Sensitivity

Barbiturate, 2 mg/l.

Clinical interpretation

Barbiturates are very toxic in overdose and may cause peripheral vasodilation, hypotension, shock, hypoventilation, hypothermia, coma,

g. 10. Ultraviolet spectra of aqueous barbital sodium (50 mg/l) at different pH values



convulsions and acute renal failure. Death normally follows respiratory or cardiorespiratory arrest or respiratory complications.

Plasma barbiturate concentrations greater than 10 mg/l (50 mg/l barbital and phenobarbital) may be associated with serious toxicity. Repeated oral doses of activated charcoal and/or alkaline diuresis may be valuable in severe poisoning with barbital and phenobarbital. Charcoal haemoperfusion has been used to treat severe poisoning with short- and medium-acting barbiturates (see section 2.2.3).

2.2.3 *Active elimination therapy*

There are four main methods of enhancing elimination of the poison from the systemic circulation: repeated oral activated charcoal; forced diuresis with alteration of urine pH; peritoneal dialysis and haemodialysis; and haemoperfusion.

The systemic clearance of compounds such as barbiturates, carbamazepine, quinine and theophylline (and possibly also salicylic acid and its derivatives) can be enhanced by giving oral activated charcoal at intervals of 4–6 hours until clinical recovery is apparent. To reduce transit time and thus reabsorption of the poison, the charcoal is often given together with a laxative. This procedure has the advantage of being totally noninvasive but is less effective if the patient has a paralytic ileus resulting from the ingestion of, for example, phenobarbital. Care must also be taken to avoid pulmonary aspiration in patients without a gag reflex or in those with a depressed level of consciousness.

The aim of forced diuresis is to enhance urinary excretion of the poison by increasing urine volume per unit of time. It is achieved by means of intravenous administration of a compatible fluid. Nowadays, forced diuresis is almost always combined with manipulation of urine pH. Renal elimination of weak acids such as chlorophenoxy herbicides and salicylates can be increased by the intravenous administration of sodium bicarbonate. This can also protect against systemic toxicity by favouring partition into aqueous compartments such as blood. Indeed, alkalinization alone can be as effective as traditional forced alkaline diuresis, and has the advantage that the risk of complications resulting from fluid overload, such as cerebral or pulmonary oedema and electrolyte disturbance, is minimized. However, the pK of the poison must be such that renal elimination can be enhanced by alterations in urinary pH within the physiological range. It is also important to monitor urine pH carefully to ensure that the desired change has been achieved. Acidification of urine was thought to enhance the clearance of weak bases such as amfetamine, procyclidine and quinine, but this is no longer generally accepted.

Dialysis and haemoperfusion remove the poison directly from the circulation. In haemodialysis, blood is passed over a membrane which is in contact with the aqueous compartment in an artificial kidney, while in peritoneal dialysis an appropriate fluid is infused into the peritoneal cavity and then drained some 2–4 hours later. In haemoperfusion, blood is pumped through a cartridge of adsorbent material (coated activated charcoal or Amberlite XAD-4 resin). Haemodialysis is preferred for water-soluble substances such as ethanol, and haemoperfusion for lipophilic poisons such as short-acting barbiturates, which have a high affinity for coated charcoal or Amberlite XAD-4 resin. The decision to use dialysis or haemoperfusion should be based on the clinical condition of the patient, the properties of the poison ingested and its concentration in plasma. Haemodialysis and haemoperfusion are only effective when the volume of distribution of the poison is small, i.e., relative volume of distribution less than 5 l/kg.