Ultraviolet Spectrophotometric Measurement of Chlordiazepoxide in Plasma

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A simple ultraviolet spectrophotometric method is described for measurement of chlordiazepoxide in blood. The drug is extracted into chloroform at pH 7.4 and, after a NaOH wash, extracted back into HCl. The drug is identified by its characteristic ultraviolet spectra at acidic and basic pH's, and quantified from the absorbance value measured at the major acid peak of 247 nm. Although certain weakly basic drugs theoretically could interfere if present concurrently with chlordiazepoxide, we have not seen this during three years of experience. This procedure is easily combined with established methods for barbiturates and glutethimide. Specimens from more than 60 cases of documented chlordiazepoxide ingestion have had concentrations ranging from 0.2 to 6.6 mg/100 ml by this procedure.

Chlordiazepoxide ("Librium"; Hoffmann-LaRoche, Nutley, N.J. 07110), a benzodiazepine derivative, is widely used for the treatment of anxiety. Its structure is shown in Figure 1. Chlordiazepoxide is frequently involved in cases of adult drug overdose, both by itself and in combination with other drugs. In our experience, its involvement has been exceeded only by that of barbiturates and alcohol. Chlordiazepoxide reaches appreciable concentrations (greater than 1.0 mg/100 ml) in blood in clinically significant overdose (1). For these reasons, the identification and quantification of chlordiazepoxide in blood can be considered a relevant part of an emergency toxicology program in the clinical laboratory.

Published methods designed for clinical use are based on fluorometry (2), colorimetry (3), and gas chromatography (4). With the exception of the last, these methods are directed specifically toward chlordiazepoxide, and are not suited to rapid screening. None of the above are optimal for 24-h emergency use by the clinical laboratory.

The ultraviolet absorption of chlordiazepoxide, both as the free base and the salt, is well established. It is insufficiently appreciated that chlordiazepoxide, a very weak base, is efficiently extracted at neutral pH concurrently with weak acids (barbiturates) and neutral drugs (glutethimide1). We have clinically applied the proposed spectrophotometric method for more than three years, and have measured chlordiazepoxide blood concentrations in more than 60 cases of confirmed overdose. This paper describes our procedure.

Materials and Methods

Apparatus

Double-beam recording spectrophotometer. We used the Model 402, Perkin-Elmer Corp., Norwalk, Conn. 06852.

Reagents

All reagents are AR grade.

Chloroform.
NaOH. 0.45 mol/liter.
Phosphate buffer. 0.5 mol/liter, pH 7.4.
HCl. 0.5 mol/liter.
NaOH. 5 mol/liter.

Standard. Stock standard consists of 1 mg of chlordiazepoxide per milliliter as the hydrochloride (Hoffmann-LaRoche) in methanol. This is stored in a freezer, and not exposed unduly to light. Appropriate aliquots of stock standards are added to plasma to form desired working standards.

Procedure

Three milliliters of plasma, 2.0 ml of phosphate buffer, and 30 ml of chloroform are placed in 50-

Fig. 1. Chemical structure of chlordiazepoxide

1 Glutethimide is actually a very weak acid.
ml round-bottomed stoppered centrifuge tubes, shaken for about 5 min, and centrifuged (1500 × g, 5 min). Twenty-five milliliters of the chloroform is collected, after filtration through Whatman No. 1 filter paper, into a second centrifuge tube containing 5 ml of NaOH (0.45 mol/liter). The contents are shaken for about 5 min and centrifuged. The NaOH (upper) layer is completely removed and either discarded or saved for barbiturate analysis. Five milliliters of HCl (0.5 mol/liter) is added to the remaining chloroform and the tubes are again shaken and centrifuged. The aqueous layer is removed and divided into two 2-ml aliquots, A and B. One-half milliliter of HCl (0.5 mol/liter) is added to A, and 0.5 ml of NaOH (5 mol/liter) to B. A and B are each scanned separately, but on the same recording, against an identically processed aqueous blank. They are identified from the characteristic configuration of the ultraviolet absorption spectra at the two pH's. Absorbance at the major acid peak (245–250 nm) is used for quantification. Calculations are based upon absorbance values obtained from identically processed plasma standards. We have found a constant derived from previously run standards to be satisfactory for emergency use.

Results

Standard curve. A standard curve was obtained by analysis of plasma containing various amounts of added chlordiazepoxide. Absorbance values at 247 nm were linear with concentration to an absorbance of at least 0.8, which is equivalent to a plasma concentration of 3.3 mg/100 ml. Figure 2 illustrates the absorption spectra of chlordiazepoxide added to HCl or NaOH.

Sensitivity. Plasma concentrations as low as 0.2 mg/100 ml could be reliably identified and quantified.

Precision. The coefficient of variation for 10 different sera, each containing 1.7 mg of added chlordiazepoxide per 100 ml, was 3%.

Recovery. The actual physical recovery of chlordiazepoxide from plasma was determined by spectrophotometry by comparison with nonextracted primary aqueous standards. After correction for aliquoting, recovery averaged 84%. Drug concentrations of actual patient samples were calculated from factors derived from similarly processed plasma standards, which corrects for the incomplete recovery.

Specificity. Diazepam was not extracted from chloroform by HCl (0.5 mol/liter). Only 7% of oxazepam was extracted from chloroform under these conditions. This drug, rarely used, would only interfere if present concurrently with chlordiazepoxide, at an extremely high concentration in blood. Other weak bases with sufficiently strong ultraviolet absorbance, and which could be recovered under the conditions of assay, do interfere: methapyrilene, chlorpheniramine, and tripelemamine. If present alone, they were not confused with chlordiazepoxide, because their own spectra were different. Chlorpromazine, imipramine, and amitriptyline, added to plasma at concentrations of 1.7 mg/100 ml, were not recovered and did not interfere. We have encountered no interferences in clinical specimens. Twenty drug-free plasmas had an average absorbance of less than 0.02 at 247 nm when taken through this procedure for chlordiazepoxide analysis.

More than 200 specimens submitted for emergency toxicology have been analysed for chlordiazepoxide, and more than 60 patients had concentrations of 0.2 mg/100 ml to 6.6 mg/100 ml. Figure 3 shows a representative spectrum obtained.

Fig. 2. Spectra of chlordiazepoxide hydrochloride (0.8 mg/100 ml)

a, spectrum of salt (in 0.5 molar HCl); b, spectrum of free base (in 0.5 molar NaOH)

Fig. 3. Spectra obtained from analysis of plasma of patient who had ingested chlordiazepoxide

a, spectrum of salt (in HCl); b, spectrum of free base (in NaOH)
from the blood of a patient after a documented chlordiazepoxide overdose.

Almost one-third of these cases had concentrations greater than 1 mg/100 ml, and one sixth were greater than 2 mg/100 ml. Almost all of the patients had some evidence of central nervous system depression, but genuine coma that could be attributed to chlordiazepoxide alone was rare. Even patients with concentrations exceeding 6 mg/100 ml were not in coma. Patients who were asleep, but arousable, and had ingested only chlordiazepoxide had an average concentration of more than 4 mg/100 ml; levels greater than 2 mg/100 ml were associated with drowsiness. In mixed ingestions the clinical picture was often more serious than could be attributed to either drug alone.

Discussion

The extraction procedure does not differ appreciably from those described for barbiturates and glutethimide by Goldbaum (5, 6), or more generally applied to the assay of weak acid and neutral drugs. General extraction procedures recommend a separate alkaline extraction for basic drugs. Chlordiazepoxide exists predominitely as the free base at neutral pH and is efficiently extracted at pH 7.4 concurrently with acid and neutral drugs. It is sufficiently basic to be extracted from chloroform by HCl (0.5 mol/liter).

The procedure described here is not specific in the strict sense. Obviously, any basic drug that can be extracted at neutral pH and recovered into HCl (0.5 mol/liter) will potentially interfere. In practice, they interfere only if they reach detectable blood concentrations and are ingested simultaneously with chlordiazepoxide. Diazepam and oxazepam are not extracted from chloroform by HCl (0.5 mol/liter), and so will not interfere. The phenothiazines and tricyclic antidepressants (amitriptyline and imipramine) are stronger bases (pKa > 9) and are not recovered by this procedure. Evidence of overdosage with these drugs is usually detected by examination of the urine. Many of the antihistamines absorb strongly in the ultraviolet spectra and are recovered by this procedure. Those tested have sufficiently characteristic spectra that they would not be confused with chlordiazepoxide. Although they could interfere if ingested concomitantly with chlordiazepoxide, we have not seen this. The narcotics and amphetamines do not reach measurable blood concentrations and must be sought in urine. The drugs which in our experience have been most frequently ingested with chlordiazepoxide have been barbiturates, glutethimide, and alcohol—none of which interferes with this procedure.

Generally the spectrum of chlordiazepoxide in the salt form is sufficient for identification and quantification. The characteristic change that occurs with alkalinization lends confirmation. Although it has been recommended that plasma be used for extraction of basic drugs, we have successfully identified chlordiazepoxide in sera from clinical cases. When an aliquot of heparinized blood to which chlordiazepoxide had been added was made to clot, the drug concentration in the serum was identical to that in plasma.

Chlordiazepoxide is widely prescribed, and is implicated often enough in drug overdose to justify its emergency measurement by the clinical laboratory. Although coma and death from chlordiazepoxide ingestion alone is unusual, less severe alterations in consciousness are not uncommon. Combined with other drugs, it can alter the prognosis and clinical picture.

Many laboratories perform emergency spectrophotometric measurements of barbiturates and glutethimide by the methods of Goldbaum (5, 6) or closely related modifications (7, 8). With the same extraction, an additional 20 min of effort is sufficient to identify and quantify chlordiazepoxide. Extensive clinical experience with this procedure in our laboratory indicates that it is well suited for emergency toxicology.

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References