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Note

Determination of alcuronium dichloride in plasma by high-performance liquid chromatography without solvent extraction

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A number of analytical procedures have been used for the determination of the neuromuscular blocking agent alcuronium dichloride (diallylnortoxiferine, Alloferin) and include bioassay [1], assay of radioactively labelled drug [2] and a spectrofluorimetric technique involving extraction with rose-bengal [3].

A high-performance liquid chromatographic (HPLC) method has recently been reported [4] for the assay of plasma samples of alcuronium dichloride. The method uses ion-pair extraction of the picrate salt to isolate the drug followed by chromatographic separation and ultraviolet detection, *d*-tubocurarine chloride being used as an internal standard. A major disadvantage of this method is that it involves a time-consuming extraction step of 5 h and an evaporation process. Each plasma determination also requires 1 ml of plasma which necessitates taking quite a large total volume of blood from patients if one wishes to do detailed pharmacokinetic studies.

A new selective, rapid and sensitive method for the determination of alcuronium dichloride in 250 μl of plasma is the subject of this report.

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EXPERIMENTAL

Standards and reagents

Acetonitrile and methanol were HPLC grade (Waters Assoc., Milford, MA, U.S.A.) and potassium dihydrogen orthophosphate was AR grade (Ajax Chemicals, Sydney, Australia). Alcuronium dichloride was a gift from Roche Products (Sydney, Australia) and a stock solution of 0.2 mg in 1 ml was prepared in distilled water and stored at 4°C until required.

Instrumentation

Reversed-phase HPLC was performed using a Waters Model M45 solvent delivery system, a Rheodyne Model 7125 40-μl injector and a Brownlee RP-8 column (25 cm × 4.6 mm I.D., 10 μm particle size). A Rheodyne column inlet filter (Model 7302) was placed between the injection valve and column. An Omniscribe recorder (Houston Instruments) was used in conjunction with a LC-4A amperometric detector (BioAnalytical Systems) and an LC-17 oxidative flow-cell. The LC-17 flow-cell is composed of a TL-5 cube with a glassy carbon working electrode and a silver/silver chloride reference electrode. Injections were made with a 100-μl SGE syringe.

Assay procedure

To 250 μl of plasma were added 500 μl of acetonitrile. This solution was vortexed for 1 min, then centrifuged at approximately 2000 *g* for 5 min. An aliquot (40 μl) of the clear supernatant was injected onto the column. The mobile phase consisted of acetonitrile—0.001 *M* potassium dihydrogen orthophosphate—methanol (10:22:68). The chromatogram was run at ambient temperature with a flow-rate of 1.5 ml/min. The electrochemical detector was operated with an applied voltage of 1.0 V and the recorder at a setting of 10 mV and a chart speed of 2.5 mm/min. The procedure was standardised by analysing drug-free plasma samples spiked with known quantities of alcuronium dichloride solution. Peak heights were used to establish calibration curves.

RESULTS AND DISCUSSION

This assay procedure produces a good, almost noise-free baseline after the initial plasma peaks except for one endogenous plasma peak with a retention time of 16 min (Fig. 1B), the alcuronium peak having a retention time of 12 min. (Fig. 1A).

It has been reported [5] that direct injection of plasma samples onto a reversed-phase column leads to an increase in back pressure of the system due to proteinaceous materials being deposited onto the head of the column. To avoid this problem we used a column inlet filter between the injector and column to protect the column inlet frit from being blocked. These inline filters are inexpensive, easily replaced and there seems to be no loss of resolution. In our preliminary work we initially diluted plasma with an equal volume of acetonitrile but found that the filter needed to be changed regularly. Now that we dilute the plasma with a two-fold volume of acetonitrile we have had no problem with increasing back pressure and the operating pressure

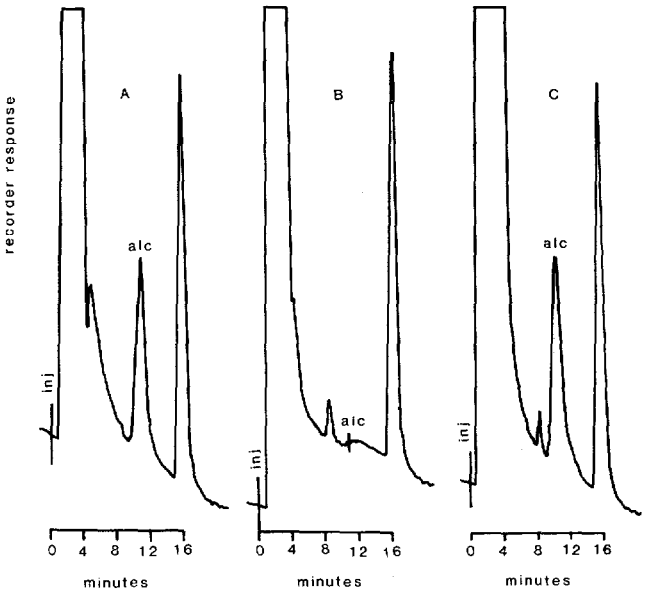

Fig. 1. Chromatograms of (A) plasma spiked with alcuronium dichloride (alc) at a concentration of 1 μg/ml; (B) patient plasma collected at zero time, and (C) patient plasma collected at 10 min after drug administration, concentration approximately 1.1 μg/ml.

remains at approximately 7 MPa. The filters are changed occasionally when the operating pressure rises above 10 MPa.

The analytical method reported here is selective and linear calibration curves were derived over the range 0.1—6.0 μg/ml of alcuronium dichloride giving a correlation coefficient greater than 0.995. There is no extraction or evaporation step and an internal standard is not needed. The sensitivity for this method is at least equal to the previously described procedure (0.1 μg/ml) and with appropriate modification even lower concentrations of alcuronium could be measured. After deproteinating the plasma samples with acetonitrile the supernatant was found to contain 100% of the added alcuronium dichloride. The method is rapid and a single plasma sample could be assayed within 20 min of receipt. Although we routinely use 250 μl of plasma as little as 100 μl of plasma could be used if diluted with 200 μl of acetonitrile. This would be particularly important if one wanted to study the pharmacokinetics of alcuronium in patients where it is difficult to take large quantities of blood.

A 7-days analytical study was undertaken to determine the coefficient of variation of the procedure using a pooled-plasma specimen containing

TABLE I

DRUGS TESTED FOR INTERFERENCE IN PLASMA

Caffeine	Codeine	Diazepam
Fentanyl	Indomethacin	Methadone
Metoprolol	Morphine	Nitrazepam
Oxycodone	Paracetamol	Pethidine
Phenoperidine	Pholcodine	Thiopentone

alcuronium dichloride at 1.0 μg/ml and was found to be 4.7%. The intra-day coefficients of variation were 2.3%, 3.0% and 4.2% at 2.0 μg/ml, 1.0 μg/ml and 0.6 μg/ml, respectively ($n=8$).

Under the above analytical conditions no interference has been observed by a number of drugs used routinely during anaesthesia. Drugs that have been tested for interference are listed in Table I.

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