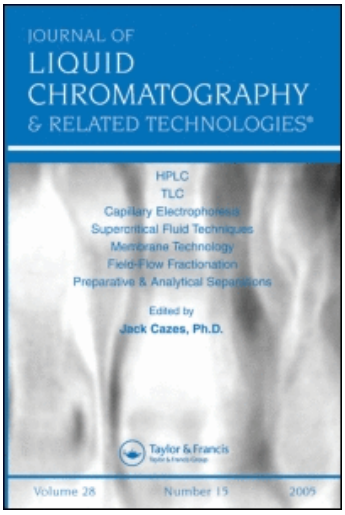

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HPLC DETERMINATION OF METHYLPHENIDATE IN HUMAN PLASMA

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ABSTRACT

A simple, rapid and sensitive method for measuring methylphenidate in human plasma by HPLC has been developed. After the addition of the internal standard, ethylphenidate, the two compounds are extracted under basic conditions. The residue obtained is resuspended in acetonitrile and analysed on an ODS reversed phase column with detection by UV absorbance at 192 nm. The limit of sensitivity is 5 ng/ml and the procedure is linear over the 5-50 ng/ml concentration range.

INTRODUCTION

Methylphenidate (MPH, α-Phenyl-2-piperidineacetic acid methyl ester) is a sympathomimetic agent used for the treatment of mild psychiatric disorders and hyperactivity in children (1). Numerous gas-liquid chromatographic assays with either nitrogen-phosphorus or mass spectrometric detection are available, but all require an acetylation step due to the instability of the compound in the injection port (7). On the other hand, high performance liquid chromatography (HPLC) has been used for the quantitation of the

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enantiomers of MPH in water (8,9) as well as for its total content in plasma (10). Unfortunately, this latter assay, with a lower limit of reliable determination of 20 ng/ml, is not sufficiently sensitive to characterize the pharmacokinetics of MPH after a 15 mg oral dose. In fact, a sensitivity of 5 ng/ml has to be achieved for the estimation of the elimination phase (11).

This report describes a sensitive high performance liquid chromatographic procedure for measuring methylphenidate in human plasma after single low doses as well as for monitoring of therapeutic levels.

MATERIALS AND METHODS

Equipment

The HPLC equipment consisted of: an Altex Model 100 pump; a Waters U6K injector; a Schöeffel Spectroflow UV detector Model 770 with the wavelength set at 192 nm; a Perkin-Elmer Model 56 chart recorder and a 4.6 mm I.D. x 15 cm ODS reversed phase, 5 µm particule size Ultrasphere column.

Materials

HPLC grade acetonitrile (J.T. Baker Chemical Co., Phillipsburg, N.J.) and triethylamine (Eastman Co., Rochester, NY) were used. Glass distilled hexane and ethyl acetate were obtained from Caledon Laboratories Ltd. (Georgetown, Ontario). Methylphenidate was USP standard. Ethylphenidate was synthesised by transesterification of methylphenidate in acidic ethanol for 72 hours under reflux. Upon analysis of the basic extract of the reaction mixture a single peak was obtained with the HPLC conditions described below. No evidence of residual methylphenidate was observed. The mass spectrum of MPH shows a base peak at m/z 180 corresponding to the derivatized piperidine moiety and diagnostic peaks at 150 and 125. Ethylphenidate also shows a base peak at m/z 180 but has diagnostic peaks at 164 and 125. Both mass spectra were obtained on a Hewlett-Packard Model 5985 GC-MS and are identical to the ones reported (11).

Chromatographic Conditions

The mobile phase is a mixture of 35% (V:V) acetonitrile in 0.07% triethylamine adjusted to pH 3.4 with concentrated H_3PO_4. It is run at room temperature at a flow rate of 1.5 ml/min. The detector wavelength is set at 192 nm with a sensitivity of 0.01 AUFS. Injection volume is 75 µl.

Procedure

Whole blood is drawn into a Vacutainer® tube (Beckton-Dickinson) containing EDTA and is centrifuged (1000xg) for 15 minutes to obtain the plasma which was immediately frozen at -15° until analysis.

To 1 ml of plasma is added 40 µl of the stock solution of ethylphenidate (1 µg/ml), 1.0 ml of a 0.2 M carbonate buffer pH 9.1 and 5 ml of a Hexane:Ethyl Acetate mixture (75:25). After 10 minutes of mixing and 5 minutes of centrifugation (1000xg), 4.5 ml of the organic phase is transferred to another tube and evaporated to dryness in a dry bath at 55°C under a stream of nitrogen. The residue obtained is reconstituted in 100 µl of acetonitrile for injection into the chromatograph.

Calibration

A stock solution containing exactly 1 µg/ml of methylphenidate, as free base, is prepared in methanol. Different volumes of this stock solution are added to human plasma to give final concentration of 0, 5, 10, 20, 30, 40 and 50 ng/ml. These spiked plasma samples are then subjected to the above extraction procedure. From the chromatograms obtained, a standard curve is constructed by plotting the methylphenidate:ethylphenidate peak height ratio against the MPH concentration. For the determination of the percentage of recovery, a standard is prepared by diluting a known amount of the two stock solutions in mobile phase. After analysis of these standards, the recovery is determined by comparing the peak heights obtained from experimental specimens with the ones obtained from the recovery standards.

RESULTS

Figure 1 shows typical chromatograms of: (a) a drug-free plasma and (b) a plasma spiked with 15 ng/ml of MPH. The retention times of methylphenidate and ethylphenidate are 3.2 and 4.6 minutes, respectively. Although there is a peak at 9.0 minutes, it does not interfere with the drug or the internal standard and the analysis time remains relatively short i.e. 12 minutes. Different standard curves run on 5 separate days gave a mean slope value of 0.026, a mean intercept value of 0.01, a coefficient of determination (r^2) higher than 0.995 and a inter-day slope variation of 6% (Table 1) with a 13% c.v. at the lowest concentration. The back-calculated concentrations from these curves showed a precision and accuracy over the range studied with deviations of less than 5% from the nominal values. The percentage of recovery is 74.3 ± 2.2% and 72.3 ± 1.6% for MPH, with similar values (75%) for internal standard at 5 and 20 ng/ml. The limit of detec-

Table 1. Inter-Day Variation (n=5)

Conc (ng/ml)	Mean PHR*	C.V (%)
5	0.14	13.4
10	0.27	6.6
20	0.54	6.9
40	1.03	10.9
50	1.33	4.4
RF†	0.026	5.7

* PHR = peak height ratio

† RF = mean response factor

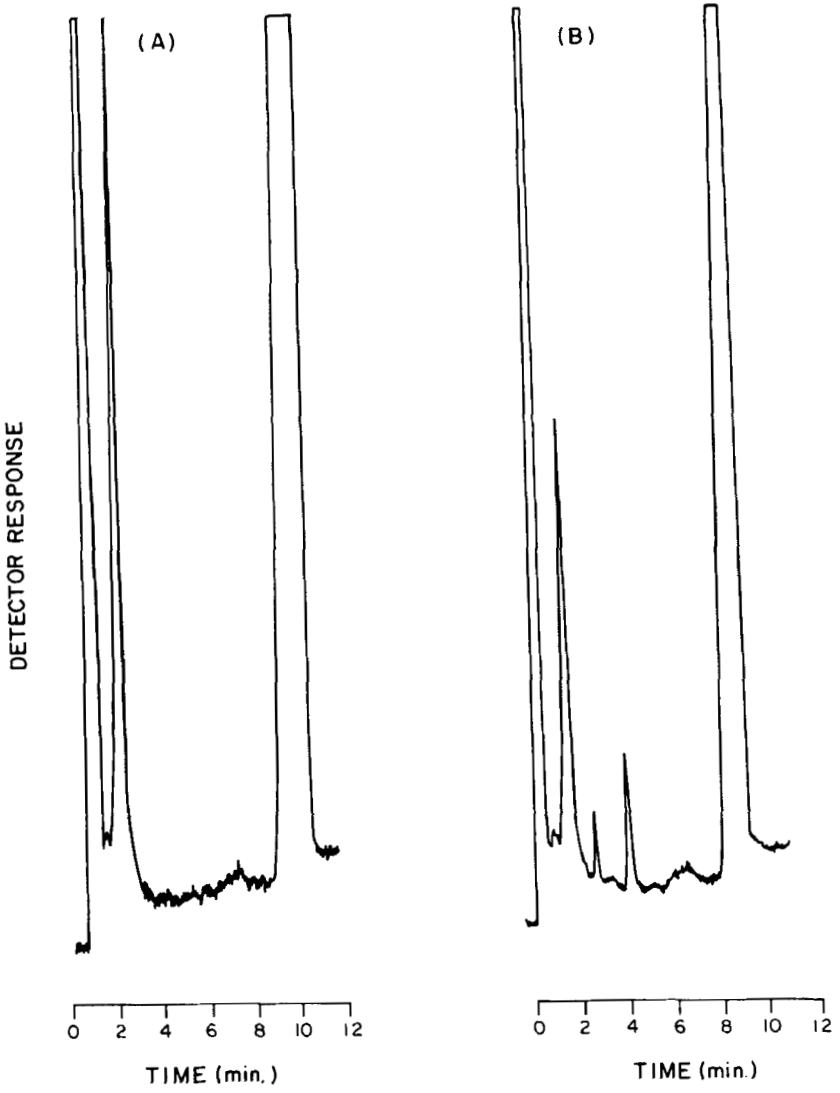

Figure 1. HPLC chromatograms obtained upon analysis of a) drug-free plasma containing no internal standard and b) drug-free plasma spiked with 15 ng/ml of MPH and internal standard.

tion, assuming a signal to noise ratio of 2, is set at 2.5 ng/ml but the lowest reliable determination from day to day is 5 ng/ml. The intra-day variation, measured by the analysis of five replicates, is 4 and 2% for concentrations at 5 and 20 ng/ml respectively (Table 2).

Other basic drugs that could be extracted in these conditions such as cimetidine, diazepam, flurazepam, procainamide, acetaminophen, caffeine, theophylline, quinidine, diltiazem, verapamil, nifedipine and doxepin do not interfere with the analysis. A 10 mg tablet dose of MPH was administered to a healthy male volunteer (88.5 kg; 0.11 mg/kg). Blood samples were taken over 7 hrs after dosing in evacuated tubes with EDTA as anticoagulant. This tested the procedure in the extreme range as shown in Table 3.

DISCUSSION

This method is simpler and faster than those currently available because it does not require a derivatisation step. Moreover, having a limit of sensitivity of 5 ng/ml, it is more

Table 2. Intra-Day Accuracy and Precision (n=5)

Nominal Conc. (ng/ml)	Back Calculated Conc. (ng/ml)	C.V (%)	MRE* (%)
5	5.09	4.4	+ 1.8
20	20.09	1.9	+ 0.5

* MRE = mean residual error

Table 3. Plasma Concentration of MPH in a Volunteer After a 10 mg Single Oral Dose (n=2)

Time (h)	Concentration MPH ng/ml
0.5	3
1	5.1
2	4.6
3	2.8
4	2.3
5	N.D.*

* Below limit of detection

sensitive than other HPLC methods (8-10). Therefore, this simple method is adequate for the therapeutic monitoring of MPH in hyperactive children as well as for single dose pharmacokinetic studies. The example given of the application of the method gave values expected from a low single dose (12).

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