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Identification and Quantification of Trihexyphenidyl and Its Hydroxylated Metabolite by Gas Chromatography with Nitrogen-Phosphorus Detection

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Abstract

A sensitive and specific assay for the simultaneous quantification of trihexyphenidyl and its hydroxylated metabolite in plasma and urine is described. The method is based on the extraction of the drugs with an organic solvent and separation on a 3% OV-17 Chromosorb Q column in a gas chromatograph equipped with a nitrogen-phosphorus detector. The procedure employs SKF 525 A as the internal standard and requires no derivatization. The detection limit was found to be 2 ng/mL for trihexyphenidyl and 1 ng/mL for its metabolite. The precision of the assay procedure for both compounds is about 4 to 7%.

Introduction

Trihexyphenidyl (THP), also known as benzhexol, is a synthetic anticholinergic drug used both for the treatment of Parkinson's syndrome and for the control of the extrapyramidal side effects of neuroleptics. In adults, therapeutic doses are about 1 to 15 mg daily (1). Several side effects of THP have been noted in the literature, including bradycardia (2), disturbances of recent memory (3,5), and myasthenia gravis (6). Moreover, in psychotic patients the drug has demonstrated hallucinogenic and euphoric properties, leading to its frequent abuse alone or in combination with opiates and other narcotics (7,8).

Despite the extensive use of THP, its plasma determination and pharmacokinetic profile remain somewhat unstudied. This seems to be due to the absence of a simple and sensitive method for assaying the substance. In 1985, THP was quantified in serum by a radioreceptor assay, which is unsuitable for routine analyses (9). Some GC methods have been reported, but they are only suitable for the determination of THP in single tablets (10,11) or provide no information on THP metabolites (12). Therefore, we have developed a new, direct GC method that has permitted the simultaneous monitoring of THP and its hydroxylated metabolite (OH-THP) in human plasma and urine.

Experimental

Materials. Trihexyphenidyl chlorhydrate (THP) and RP 23 843 (hydroxycyclohexyl-1-phenyl-1-piperidino-3-propanol, he hydroxylated metabolite of trihexyphenidyl, OH-THP) were obtained from Rhône-Poulenc Santé. SKF 525 A chlorhydrate was purchased from Smith Kline and French Laboratories. Solvents and chemicals were HPLC and analytical grade, respectively. Stock solutions (100 μg/mL, free base) of drugs and SKF 525 A (internal standard) were prepared in methanol and stored at 4°C. The THP and OH-THP standard concentrations were prepared separately by dilution of the primary stock solution in plasma. Each drug was at concentrations of 5, 10, 20, 50, 100, and 200 ng/mL. Phosphate buffer was prepared with a saturated solution of KH_2PO_4, 40% in deionized water and adjusted to pH 9.2 with 2M NaOH.

Chromatographic conditions. Gas chromatography was performed on a 6-ft × 2-mm i.d. glass column with 3% OV-17 on 100/120 mesh Chromosorb Q (Alltech). The GC system consisted of a Perkin-Elmer (8500) chromatograph with a nitrogen-phosphorus detector and a Perkin-Elmer data collector (Sigma 15). The operating conditions were as follows: column, injector port, and detector temperatures were 248, 300, and 300°C, respectively. N_2 carrier gas pressure was 35 psi. Quantification was done for THP and OH-THP by plotting peak area ratios (drug/IS) against the concentration of standards to produce standard curves and by comparing the results for the case samples with the curves.

Procedure. Plasma or urine (2 mL), 1 mL of phosphate buffer (40%, pH 9.2), 20 μL of SKF 525 A (200 ng/20 μL), and 5 mL cyclohexane were pipetted into a 20-mL Pyrex centrifuge tube. After vortexing and centrifugation, the solvent phase was evaporated to dryness at 45°C in a Speed Vac Concentrator (Savant Instruments, Inc.). The residue was dissolved in 20 μL of methanol and 1 μL was injected into the column.

Results and Discussion

The procedure as described has been found to be both sensitive and specific for the analysis of THP and OH-THP in plasma and urine. Retention times were 4.14, 4.86, and 10.04 min for THP, SKF 525 A, and OH-THP, respectively. Figure 1 is a typical chromatogram of an extracted plasma obtained from a medical examiner's case. As seen, no interferences from plasma components were observed. Urine samples exhibited similarly clean extracts.

Results were linear over the range of 5–200 ng/mL for THP and OH-THP. The mathematical expressions and the correlation

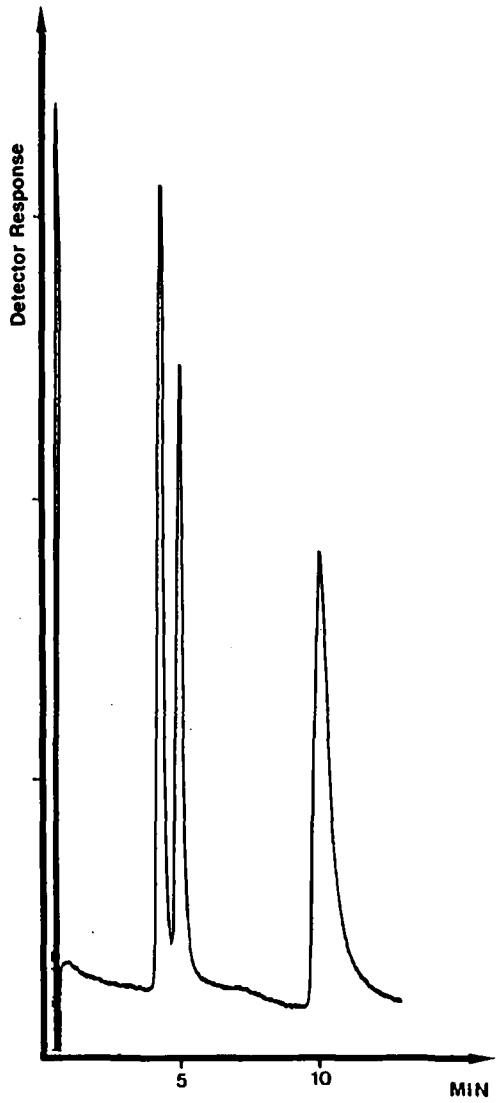

Figure 1. Gas chromatogram of an extract of test plasma sample obtained from a subject under trihexyphenidyl treatment. 4.14 min, trihexyphenidyl (52 ng/mL); 4.86 min, SKF 525 A; 10.04 min, hydroxytrihexyphenidyl (37 ng/mL).

Table I. Mean Values (%) of Reproducibility in Plasma

	Trihexyphenidyl (ng/mL)		Hydroxytrihexyphenidyl (ng/mL)	
Mean values	20	200	20	200
Within-run precision (N=9)	4.7	3.8	5.6	4.3
Day-to-day precision (over 3 weeks)	6.1	6.3	7.2	6.9

Table II. Comparison of Organic Solvents for Extraction of THP and OH-THP from Plasma at pH 9.2*

Solvent	THP recovery (%)	OH-THP recovery (%)
Chloroform	69.3	81.4
Chloroform/isopropanol/ *n*-heptane (50:17:33)	59.4	36.4
1,2-Dichloroethane	79.7	84.7
Dichloromethane	80.1	85.6
n-Hexane	35.8	21.4
Cyclohexane	83.3	86.3
Ethyl acetate	83.7	32.1
Ethyl ether	87.6	32.1
n-Heptane/isoamyl alcohol (98.5:1.5)	80.3	56.4

* Samples were spiked with 50 ng/mL of THP and OH-THP. Extraction recovery was determined by comparing the representative peak areas of extracted plasma with the peak areas of methanolic standards at the same concentration.

Table III. Retention Times for Tested Drugs

Drug	Retention time (min)
Meperidine	1.14
Methadone	3.20
Propoxyphene	3.46
Methaqualone	4.70
Codeine	8.89
Dextromoramide	24.24

coefficients of THP and OH-THP are respectively $y = 1.09x - 0.07$ (N = 4, $r = 0.994$) and $y = 0.98x + 0.04$ (N = 4, $r = 0.993$). The results indicated a good linear proportionality between the NPD response and the concentration of both compounds in plasma. Day-to-day precision was studied by adding THP and OH-THP to blank plasma at therapeutic and toxic concentrations. Analyses were performed every day over 3 weeks. The precision was found to be 4–7% (Table I). The within-run studies are also summarized in Table I.

During the initial stages of this work, various common extraction solvents were investigated for recovering THP and OH-THP from plasma. Extraction recovery was determined for THP and OH-THP by comparing the representative peak areas of extracted plasma with the peak areas of methanolic standards at the same concentration (external standard quantification). The results are presented in Table II.

Cyclohexane was chosen as the extraction solvent on the basis of its ability to minimize emulsion formation during the extraction and to produce a suitable recovery. When chloroform was used, alone or in mixture with other solvents, an extraction artifact that eluted closely with THP was produced. This artifact was not identified. Since 1894 it has been known that chloroform contains phosgene gas, which can react with ethanol to form ethyl chloroformate (13). The latter substance, when reacted with normeperidine, will form normeperidine ethyl carbamate (14). As for normeperidine, chloroform use should be avoided in the extraction of THP. Several extraction buffers were tested (pH 7.4, 8.2, 9.2, 10.0), and pH 9.2 was found to be the most suitable.

The limit of detection was determined by spiking plasma with decreasing concentrations of both molecules until a response equivalent to 3 times the background noise was observed. The lower limits of detection were found to be 2 ng/mL and 1 ng/mL in plasma for THP and OH-THP, respectively. These detection limits are adequate for forensic and clinical analyses.

Some narcotics were tested on the OV-17 column, since they are often associated with THP. All the analgesics examined were eluted at retention times different from those of THP, OH-THP, and SKF 525 A (Table III), except methaqualone, which today is rarely prescribed. Chlorpromazine, sometimes associated with THP in pharmaceutical tablets, is not extracted by the present method and thus will not interfere with the assay.

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