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CAPILLARY GAS CHROMATOGRAPHY OF TRIHEXYPHENIDYL, PROCYCLIDINE AND CYCRIMINE IN BIOLOGICAL FLUIDS

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SUMMARY

A sensitive (50–100 pg/ml) method is described for the analysis of the anticholinergic drugs cycrimine, procyclidine and trihexyphenidyl by capillary gas chromatography with flame thermionic detection Since these anticholinergic drugs are frequently administered in combination with antipsychotic medication for the treatment of mental illness, the potential interference by antipsychotic drugs in this assay was examined No interference was observed from a series of antipsychotic drugs in the quantitation of cycrimine, procyclidine or trihexyphenidyl The use of this technique to study trihexyphenidyl pharmacokinetics in man is described

INTRODUCTION

Trihexyphenidyl (benzhexol) and its analogues procyclidine and cycrimine (Fig 1) are synthetic anticholinergic drugs used in the treatment of Parkinson's disease and neuroleptic-induced parkinsonism [1]. The pharmacokinetics of trihexyphenidyl in man has been described [2] using a cholinergic radioreceptor binding assay [3] However, the interpretation of these data is difficult since this method is not compound-specific; it quantitates cholinergic receptor binding activity without regard for chemical structure Several procedures have been developed for the instrumental analysis of trihexyphenidyl including gravimetric [4], volumetric [5], spectrophotometric [6–8], polarographic [9,10], potentiometric [11–13] and gas chromatographic (GC) [14–16] methods. GC techniques have also been described for the analysis of procyclidine [17,18] Unfortunately, the majority of these methods have been de-

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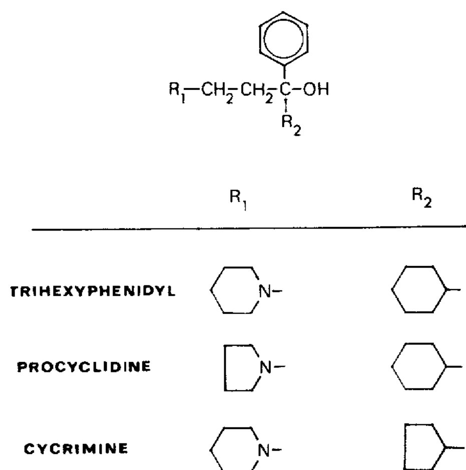

Fig 1 Chemical structures of trihexyphenidyl, procyclidine and cycrimine

veloped for drug quantitation in pharmaceutical formulations or in vitro preparations Accordingly, with the exception of a GC–mass spectrometric method [16], current instrumental procedures do not possess the sensitivity required for pharmacokinetic studies of these drugs in man

In this paper we describe a method for the quantitation of trihexyphenidyl, procyclidine and cycrimine in biological fluids by capillary GC with flame thermionic (alkali flame) detection. This procedure has a lower limit of sensitivity of 50 pg for trihexyphenidyl and 100 pg for procyclidine and cycrimine per ml of sample for a 2.0 ml sample volume. The application of this assay to the single-dose pharmacokinetics of trihexyphenidyl is described

EXPERIMENTAL

Materials

Reagent-grade chemicals and HPLC-grade solvents were used throughout Procyclidine·HCl, trihexyphenidyl·HCl and cycrimine·HCl were the kind gifts of Burroughs Wellcome (Kirkland, Canada), Cyanamid Canada (Baie d'Urfe, Canada) and Eli Lilly (Indianapolis, IN, U.S A.), respectively. Other drugs used in this study were of pharmaceutical grade from various manufacturers. All glassware was siliconized with 0.2% Aquasil (Pierce, Rockford, IL, U S A) in water, followed by two distilled water rinses and oven drying (100°C, 1 h)

Apparatus

Analyses were performed using a capillary gas chromatograph (Shimadzu GC-9A) equipped with a splitless injector (Shimadzu SPL-G9) and flame thermionic detector (Shimadzu FTD-9) (Shimadzu, Columbia, MD, U S.A.)

Sample aliquots were injected on the fused-silica capillary column [25 m$\times$0 25 mm I D , 0 1 μm chemically bonded OV-17 stationary phase (Quadrex, New Haven, CT, U S.A)] with a 0.5-min splitless period. For 0 6 min following sample injection the column temperature remained steady at 150°C, after which it increased at 30°C/min to 230°C, then at 5°C/min to 245°C and finally at 30°C/min to 280°C. The detector and injector were maintained at 280°C. Carrier gas (helium) pressure was 2 3 kg/cm^2; other gas flow-rates were· hydrogen 3 5 ml/min, air 110 ml/min and auxiliary (helium) 35 ml/min The recommended air flow-rate for this detector is 150–200 ml/min, however, sensitivity was optimal in this application with a reduction of air flow to 110 ml/min Chromatogram peak heights and retention times were determined by an electronic integrator (Model CR-3A, Shimadzu, Rockford, MD, U S A).

Sample preparation

A 2 0-ml aliquot of sample (plasma, serum or urine) was added to a glass test tube containing buffer (200 μl of 1.0 *M* glycine, pH 11 0), internal standard solution (100 μl of 150 ng/ml cycrimine for trihexyphenidyl or procyclidine analysis, 100 μl of 150 ng/ml procyclidine for cycrimine analysis) and extraction solvent [2.0 ml of ethyl acetate–hexane (1 3)] The tube was capped, shaken gently (15 min) and centrifuged (10 min, 250 *g*) The drug-containing organic phase (upper) was transferred to a second glass test tube containing 1 5 ml of 1.0 *M* hydrochloric acid, and the tube was capped, shaken vigorously and incubated (room temperature) for a minimum of 12 h. Following this back-extraction, the organic phase (upper) was discarded, the aqueous phase alkalinized (1 0 ml of 10 *M* sodium hydroxide), 1 5 ml extraction solvent were added, and the tube was capped, shaken and centrifuged as before. The drug-containing organic phase was removed and evaporated in a 1.5-ml polyethylene centrifuge tube (BelArt, Pequannock, NJ, U.S.A.) under a stream of nitrogen The residue was redissolved in extraction solvent (40 μl) and a 2 0-μl aliquot injected on to the column

RESULTS AND DISCUSSION

Sample chromatograms for drug-free plasma and plasma containing 3.0 ng/ml each of cycrimine, procyclidine and trihexyphenidyl are shown in Fig 2 No endogenous compounds were observed to significantly interfere with the analysis of these drugs in human plasma. By this method the minimum detectable quantities of cycrimine, procyclidine and trihexyphenidyl are 100, 100 and 50 pg/ml of plasma respectively.

This extraction procedure is sufficiently complex to require an internal standard for the accurate quantitation of these drugs in a biological fluid. An appropriate internal standard should have similar structural, extraction and chromatographic properties to the compounds of interest, but must not be

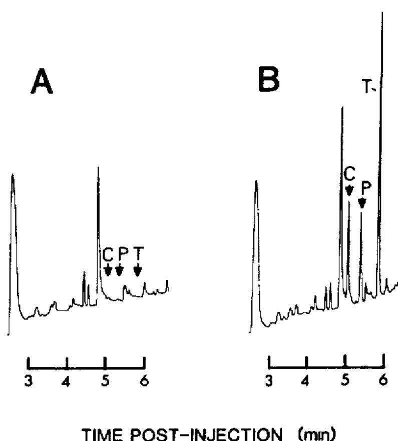

Fig 2 Sample chromatograms of (A) plasma containing no cycrimine (C), procyclidine (P) or trihexyphenidyl (T) and (B) plasma containing 2 ng/ml cycrimine (C), procyclidine (P) and trihexyphenidyl (T)

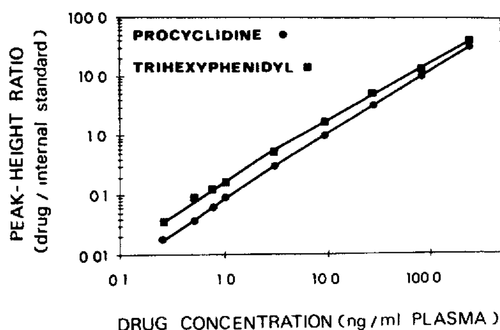

Fig 3 Typical standard curves for plasma samples containing known concentrations of procyclidine and trihexyphenidyl Cycrimine was the internal standard employed in this case

present in samples received for analysis. Since combinations of these anticholinergic drugs are not commonly prescribed, one compound may be selected as the internal standard for the quantitation of the other two In our case, cycrimine represents an ideal internal standard for trihexyphenidyl or procyclidine as it is not available as a prescription drug in Canada However, we have also investigated the analysis of cycrimine-containing plasma samples with procyclidine as the internal standard.

Standard curves for plasma samples spiked with known concentrations of trihexyphenidyl and procyclidine are shown in Fig. 3. These calibration curves, obtained by plotting the ratio of drug to internal standard peak heights against known drug concentrations, were not linear throughout their entire range (0.25–243 ng/ml). As a result, plasma drug concentrations were determined by in-

terpolation within linear sections (as determined by least-squares linear regression) of approximately a 40-fold concentration range of the standard curve In this way, accurate predictions of known concentration samples were obtained while maintaining predicted concentrations of the blank samples insignificantly different from zero Standard curves for the analysis of plasma samples containing known concentrations of cycrimine (with procyclidine as the internal standard) were similar and were treated in the same manner Within-day reproducibility was assessed by repeatedly analysing a plasma sample containing the appropriate drug at a concentration of 3 0 ng/ml eight times during the day Between-day reproducibility was determined through the analysis of plasma samples containing a range of trihexyphenidyl, procyclidine and cycrimine concentrations over six separate occasions These results are listed in Table I

This assay was developed to examine the pharmacokinetics of trihexyphen-

TABLE I

REPRODUCIBILITY DATA FOR THE ANALYSIS OF PLASMA SAMPLES CONTAINING EITHER PROCYCLIDINE AND TRIHEXYPHENIDYL OR CYCRIMINE

Cycrimine was used as the internal standard for trihexyphenidyl and procyclidine analysis, procyclidine was the internal standard for cycrimine determinations See text for details

Compound	Actual concentration (ng/ml)	Concentration determined (mean ± S D) (ng/ml)	Coefficient of variation (%)
Between-day reproducibility over a six-day period			
Procyclidine	0 25	0 26 ± 0 04	13 6
	1 0	0 93 ± 0 08	8 2
	3 0	2 97 ± 0 17	5 9
	9 0	8 96 ± 0 33	3 6
	27 0	28 30 ± 1 52	5 4
Trihexyphenidyl	0 25	0 25 ± 0 04	17 0
	1 0	0 98 ± 0 05	5 5
	3 0	2 90 ± 0 19	6 4
	9 0	9 17 ± 0 35	3 8
	27 0	28 52 ± 1 38	4 8
Cycrimine	0 25	0 23 ± 0 05	20 4
	1 0	1 01 ± 0 07	7 2
	3 0	3 02 ± 0 17	5 6
	9 0	9 08 ± 0 36	4 0
	27 0	27 98 ± 1 42	5 1
Within-day reproducibility for eight determinations			
Procyclidine	3 0	2 99 ± 0 22	7 2
Trihexyphenidyl	3 0	3 01 ± 0 16	5 4
Cycrimine	3 0	2 96 ± 0 21	7 0

idyl and procyclidine, drugs used primarily for the treatment of antipsychotic drug-induced Parkinson's symptoms Accordingly, the potential for interference from drugs commonly prescribed for the treatment of schizophrenia was examined. These agents and their retention times in this system are listed in Table II Clearly, the compounds of interest are completely resolved from the drugs surveyed The possible interference from antipsychotic drug metabolites was not investigated in this study The chromatographic behaviour of benztropine, another commonly prescribed anticholinergic drug, was also determined Although the retention time of this drug (6.74 min) lends itself to quantitation by this method, the poor efficiency of the current extraction procedure ($\approx 5\%$) precludes its analysis in biological fluids at pharmacologically relevant concentrations

The specificity of a chromatographic assay procedure is a combination of column resolving power, detector specificity and the selectivity of the sample preparation Even with the excellent resolving power of capillary columns (typically 2000–5000 theoretical plates per meter) and the selectivity of flame thermionic detectors for compounds containing nitrogen and phosphorus, the

TABLE II

RETENTION TIMES FOR SEVERAL ANTIPSYCHOTIC AND ANTICHOLINERGIC DRUGS

Compound	Retention time[a] (min)
Triflupromazine	5 03
Cycrimine	5 11
Procyclidine	5 45
Trihexyphenidyl	5 94
Perphenazine	6 17
Promazine	6 31
Benztropine	6 74
Chlorprothixene	7 33
Chlorpromazine	7 35
Methotrimeprazine	7 52
Molindone	7 93
Loxapine	8 01
Pipotiazine	9 22
Haloperidol	10 91
Prochlorperazine	10 93
Fluspirilene	N D
Mesoridazine	N D
Pericyazine	N D
Pimozide	N D
Thioridazine	N D
Thiothixene	N D

[a]N D , drug was not detected or had a retention time in excess of 12 min

myriad of such compounds in a biological matrix places emphasis on selective extraction procedures Dean et al [18] have described a cyclohexane extraction of biological fluids for the GC analysis of procyclidine in plasma and urine. This extraction procedure is sufficiently selective to allow quantitation of procyclidine in human plasma with a lower limit of sensitivity of 20 ng/ml For the GC analysis of biperiden, an anticholinergic drug structurally related to trihexyphenidyl, Le Bris and Brode [19] combined a hexane extraction of plasma with an acidic back-extraction to further reduce interfering substances and permit quantitation of the drug to 250 pg/ml. Few interfering substances are extracted from plasma by either hexane or cyclohexane, however, their poor extraction efficiency for this group of drugs (60% for trihexyphenidyl with equal aqueous and organic phase volumes) necessitates increased solvent volumes, multiple extractions or both to maintain reasonable drug recoveries Unfortunately, similar extraction efficiencies were observed for trihexyphenidyl with several solvents (ethyl acetate, methylene chloride, chloroform, *n*-butanol, diethyl ether). However, a binary mixture of ethyl acetate and hexane (from 1 3 to 3 1) extracted trihexyphenidyl, procyclidine and cycrimine with 98% efficiency An ethyl acetate/hexane ratio of 1 3 was selected for this procedure since fewer detectable endogenous compounds were extracted from plasma with this composition than with mixtures containing greater proportions of ethyl acetate.

The combination of ethyl acetate and hexane not only increases extraction efficiency over hexane alone, but also alleviates the difficulty of phase separation of the emulsion frequently formed with hexane- and protein-containing fluids However, in contrast to hexane, back-extraction of these anticholinergic drugs from ethyl acetate–hexane into an acidic aqueous phase is very poor

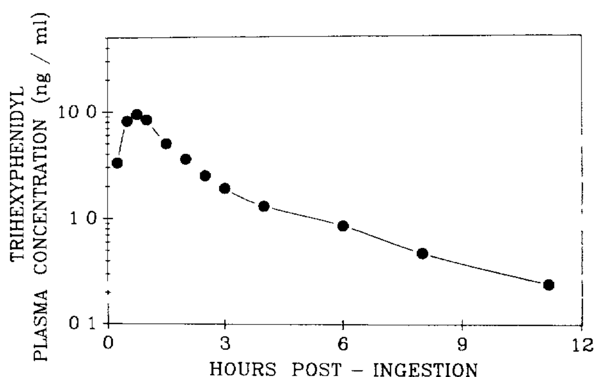

Fig 4 Plasma trihexyphenidyl concentration–time curve from an individual taking a single 2-mg tablet of trihexyphenidyl hydrochloride

(5–10% efficiency) regardless of the acid concentration To circumvent this problem, the drug-containing organic phase is incubated with acid for at least 12 h, the resultant acid-catalyzed hydrolysis of ethyl acetate improves the back-extraction efficiency such that the majority (90–95%) of drug is recovered in the aqueous phase

The application of this method to the study of trihexyphenidyl pharmacokinetics was investigated by monitoring plasma levels of the drug in human volunteers following a single 2-mg oral dose A typical plasma drug concentration–time curve is shown in Fig. 4 Clearly, the drug is rapidly absorbed from the gut and cleared from the plasma These results underscore the requirement for a highly sensitive assay procedure for the study of anticholinergic drug pharmacokinetics under clinically relevant dosage regimes

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