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Flame-Ionization and Electron-Capture GLC Determination of 1-(2,6-Dimethylphenoxy)-2-aminopropane in Plasma

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Abstract □ A sensitive flame-ionization GLC method is described for the determination of the new anticonvulsant, 1-(2,6-dimethylphenoxy)-2-aminopropane, in plasma. The method is compared with a simultaneously developed electron-capture technique, which was found to give no advantage applicable to the analysis. Relative standard deviations of replicate samples extracted for analysis by the flame-ionization method are less than 7%. The lower limit of detection of this method is 7 ng/ml.

Keyphrases □ 1-(2,6-Dimethylphenoxy)-2-aminopropane—flame-ionization and electron-capture GLC analysis in human plasma □ GLC, flame ionization and electron capture—analysis, 1-(2,6-dimethylphenoxy)-2-aminopropane in human plasma

The new anticonvulsant drug, 1-(2,6-dimethylphenoxy)-2-aminopropane (I), was tested clinically and shown to be effective in the treatment of psychomotor seizures (1). Two urinary metabolites were characterized (2), and a method for the quantitation of I in urine was published (3). To determine the half-life of this drug and to establish an easily measurable parameter as a basis for dosage adjustment, a method for quantitation of I in plasma was developed[1].

EXPERIMENTAL

Reagents—1-(2,6-Dimethylphenoxy)-2-aminopropane[2] (I) and the internal standard, 1-(2,3-dimethylphenoxy)-2-aminopropane[2] (II), were supplied as the hydrochloride salts and were used without further purification. All extracting solvents were reagent grade for flame work and specially purified[3] for electron-capture work. Water for standards and buffers used in the electron-capture procedure was made by extracting 0.1 *N* HCl (distilled water) four times with 0.1 volume of chloroform, adjusting the pH to 12 with sodium hydroxide pellets, and repeating the extractions. The resulting aqueous phase was redistilled in an all-glass system and stored in a ground-glass stoppered bottle. Trifluoroacetic anhydride[4] and pentafluoropropionic anhydride[5] were used for derivatization.

Apparatus—A gas chromatograph[6] equipped with flame-ionization and scandium tritide (4) electron-capture detectors was used. The columns were 91.5-cm × 2-mm (i.d.) glass U-tubes packed with 2.8% OV-210–3.2% OV-1 on 80–100-mesh Chromosorb W-HP for flame-ionization analysis and 3% OV-17 on 100–120-mesh Gas Chrom Q for electron-capture analysis. Instrument temperatures were 270, 250, and 120° at the detector, injector, and column oven, respectively. The electron-capture detector was operated in the dc mode at a potential of 90 v. The flow of nitrogen through the OV-210–OV-1 column was set at 25.5 ml/min, giving retention times of 4.58 and 6.00 min for the trifluoroacetamide derivatives of I and II, respectively. Nitrogen was also used as carrier gas with the electron-capture detector at a flow rate of 12.0 ml/min, with a detector purge of 35.0 ml/min giving retention times of 3.00 and 4.37 min for the pentafluoropropionamide derivatives of I and II, respectively.

Special centrifuge tubes[7], tapered to a 1.0–1.5-mm diameter capillary at the bottom, were used to obtain almost total recovery of the final chloroform extract.

Procedure—In a 16 × 150-ml culture tube (Teflon-lined screw cap), 2.00 ml of plasma, 0.50 ml of the internal standard (800 ng II/ml of distilled water), 0.5 ml of 2.0 *N* NaOH, and 5 ml of ethyl acetate are combined and shaken for 10 min. After centrifugation, the ethyl acetate is transferred to a tapered centrifuge tube, 1.0 ml of 0.1 *N* HCl is added, and the mixture is shaken for 5 min. The ethyl acetate is aspirated completely after centrifugation, 0.5 ml of 2.0 *N* NaOH and 0.1 ml of chloroform are added to the aqueous phase, and the tube is shaken by hand for 1 min. The tube is centrifuged and the lower phase is transferred to a conical vial[8] with a microsyringe. Trifluoroacetic anhydride (50 μl) is added, and the vial is capped and allowed to stand at room temperature for 30 min. The chloroform and excess reagent are evaporated at room temperature under a stream of nitrogen, and the residue is dissolved in 25 μl of redistilled carbon disulfide. Approximately 1 μl is injected into the chromatograph. Quantitation is by peak height ratio (I/II).

For analysis by electron-capture detection, 1 ml of plasma is extracted and the internal standard concentration is lowered to 80 ng II/ml. All extractions prior to derivatization are performed as already described. Pentafluoropropionic anhydride is substituted for trifluoroacetic anhydride as the derivatizing reagent. After the 30-min reaction time, the chloroform and excess reagent are evaporated at room temperature under a stream of nitrogen and the residue is taken up in 100 μl of chloroform. The chloroform is transferred to a tapered centrifuge tube containing 0.5 ml of 2.0 *N* NaOH and the final extraction is repeated as already described. The organic phase is transferred and evaporated under nitrogen at

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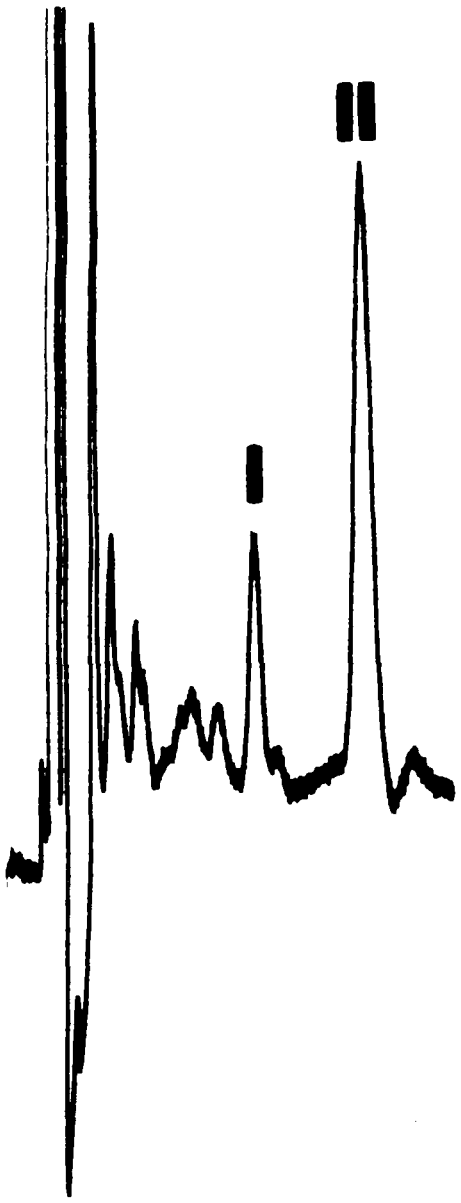

Figure 1—*Electron-capture chromatogram of extract of standard plasma (1 ml) with I and II added; I and II denote the pentafluoropropionamide derivatives of the drug (7.2 ng/ml) and internal standard (40.7 ng/ml), respectively.*

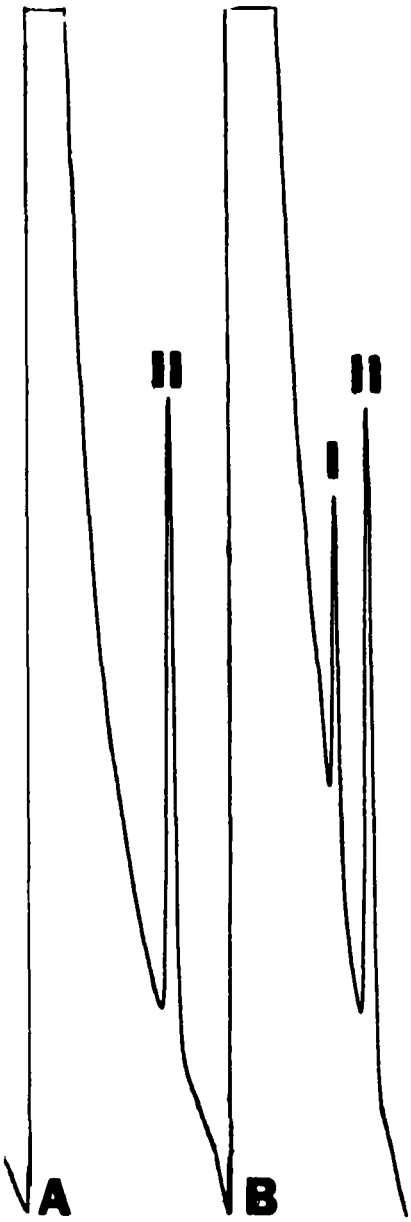

Figure 2—*Flame-ionization chromatograms of an extract of standard plasma (A) and an extract of plasma drawn from a patient taking 400 mg I/day in multiple doses (B) with the internal standard added to both; I and II denote the trifluoroacetamide derivatives of the drug and internal standard (202 ng/ml), respectively. The measured concentration of the drug in the patient sample was 99.3 ng/ml.*

room temperature, and the residue is dissolved in 25 μl of toluene. About 1 μl is injected into the chromatograph.

RESULTS AND DISCUSSION

Two primary factors prompted the consideration of both flame-ionization and electron-capture GLC during method development. Electron-capture GLC was indicated by the required lower limit of detection of 0–10 ng/ml. However, a scan of plasma samples from test patients on high doses of I (1200 mg/day) showed that the upper limit had to be at least 2000 ng/ml. Therefore, a wide linear dynamic range, characteristic of the flame-ionization detector, was also required.

The chromatogram of a plasma sample extracted according to the electron-capture procedure is shown in Fig. 1. Although the lower limit of detection of this technique was almost an order of magnitude lower than that of the flame-ionization technique, the linear range of the detector was such that multiple calibration curves were needed to cover the required concentration range. By using carbon disulfide as the final solvent, a lower limit of 7 ng I/ml was obtained with flame-ionization detection. Since this was well below the lowest recorded plasma concentration (16.8 ng/ml) of any patient taking the drug, this technique was chosen for routine analyses.

Figure 2 shows chromatograms of standard plasma and a patient's plasma extracted for flame-ionization detection. The standard plasma used was taken from a pool made up of samples from patients taking one or more of the common anticonvulsants, including ethosuximide, phenobarbital, primidone, diphenylhydantoin, and carbamazepine. No interference resulted from the presence of these drugs.

The stability of the trifluoroacetamide derivatives of I and II and the reproducibility of the flame-ionization method were checked simultaneously by chromatographing five replicate samples (102 ng/ml) six times over 90 hr. Table I shows the peak height ratios of the samples at various times after evaporation of the derivatizing reagent. The relative standard deviations of the ratios for each sample (RSD_{stab}) show that there is no apparent

Table I—Stability of Trifluoroacetamide Derivatives and Reproducibility of Flame-Ionization Procedure

Injection Time	Peak Height Ratio[a] Sample Number 1[c]	2[c]	3[c]	4[c]	5[c]	σ_{repr}	$\bar{X}$	RSD_{repr}, %
0.5 hr[b]	0.544	0.481	0.511	0.536	0.499	0.026	0.514	5
3.0 hr[b]	0.540	0.485	0.498	0.541	0.506	0.026	0.514	5
17.0 hr[b]	0.571	0.502	0.495	0.554	0.516	0.034	0.528	7
43.0 hr[b]	0.560	0.510	0.479	0.542	0.519	0.031	0.522	6
69.0 hr[b]	0.544	0.507	0.512	0.564	0.546	0.025	0.535	5
90.0 hr[b]	—	0.486	0.510	0.549	0.497	0.028	0.511	6
σ_{stab}	0.014	0.013	0.013	0.011	0.018			
$\bar{X}$	0.552	0.495	0.501	0.548	0.514			
RSD_{stab}, %	2.6	2.7	2.6	2.0	4			

[a] I-trifluoroacetamide/II-trifluoroacetamide. [b] Time after formation of trifluoroacetamide derivatives. [c] Replicate extracts of standard plasma with I and II added at 102 and 202 ng/ml, respectively.

degradation of the derivatives after 90 hr at room temperature in carbon disulfide. Sample 1 went to dryness between 69 and 90 hr, and the 90-hr result was rejected by application of the Q-test at a 99.5% confidence level. The relative standard deviations of the ratios of the five replicates at each time (RSD_{repr}) show satisfactory reproducibility of the technique. Standard calibration curves typically had linear correlation coefficients of 0.999, and the slopes of three separate curves run over a 2-month period had a relative standard deviation of 5%.

The particular sequence of extractions before the derivatization step served to eliminate any drying of a solution containing I in the free base form. Results indicated a large decrease in recovery and reproducibility if the amine was taken to dryness, even at room temperature. With this procedure, recovery was 70.3 ± 2.7% at a concentration of 65 ng I/ml and 73 ± 5% at 650 ng I/ml. Glassware was not silanized, but recovery may be increased by this technique.

The range of plasma concentrations found in patients taking normal amounts of I (200–400 mg/day in multiple doses) varied from 20 to 650 ng/ml. In single-dose studies, peak plasma levels were reached in 1–3 hr and the subsequent half-life varied from 4 to 7.5 hr. Chronic administration of I resulted in an increase in the low plasma levels over what would be expected from an equivalent dose given for 1 day; however, the peak plasma levels did not increase. For example, one patient was given 100 mg of I at 8:00 am, 2:00 pm, and 10:00 pm for 8 days. Blood was drawn at 1 and 4 hr after the initial morning dose. The plasma concentration at 1 hr increased from 45.9 to 193 ng/ml in 8 days, while the concentration at 4 hr remained at 243 ± 48 ng/ml over the entire period. The drug appears to be stable in refrigerated plasma for at least 1 month. Samples analyzed at the beginning and end of this period gave identical results within the limits of precision of the technique.

The metabolites of I (2) do not interfere with quantitation of the parent drug since the retention times of their trifluoroacetamide derivatives are much longer than those of the drug and internal standard under the chromatographic conditions employed. Preliminary investigation has indicated that the hydroxymethyl metabolite [1-(2-hydroxymethyl-6-methylphenoxy)-2-aminopropane] may be present in plasma in the low nanogram range; however, conclusive proof has not yet been obtained.

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