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Note

Gas chromatographic method for the determination of flumecinol in biological fluids

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Flumecinol (Zixoryn®*; RGH-3332; 3-trifluoromethyl-α-ethyl-benzhydrol) synthesized by Tóth et al. [1] is a new enzyme inducer that induces — on the basis of animal and human experiments — the endoplasmic reticulum-bound mixed-function oxidase system in the liver [2—4].

In order to study the role of flumecinol in humans and dogs it was necessary to elaborate a specific and sensitive method for the quantitative determination of flumecinol in biological samples. Our method is based on a simple extraction and gas—liquid chromatography (GLC) of flumecinol-containing samples.

EXPERIMENTAL

Materials

Flumecinol and internal standard (3-trifluoromethyl-benzhydrol) were the products of G. Richter Ltd. (Budapest, Hungary). Chloroform, potassium hydroxide and sodium citrate were the products of Reanal (Budapest, Hungary) and were of analytical grade. Diethyl ether puriss. (Ferak, Berlin, G.F.R.) and chloroform were carefully purified by distillation before use.

The specific activity of [1-^{14}C]flumecinol was 10.92 × 10^7 Bq/mmol (2.95 mCi/mmol).

Preparation of calibration curves

To 2 ml of human plasma or 1 ml of dog plasma, 600 ng of 3-trifluoromethyl-benzhydrol (internal standard) and flumecinol (5—300 ng for human

*Manufacturer: Chemical Works of Gedeon Richter Ltd., Budapest, Hungary.

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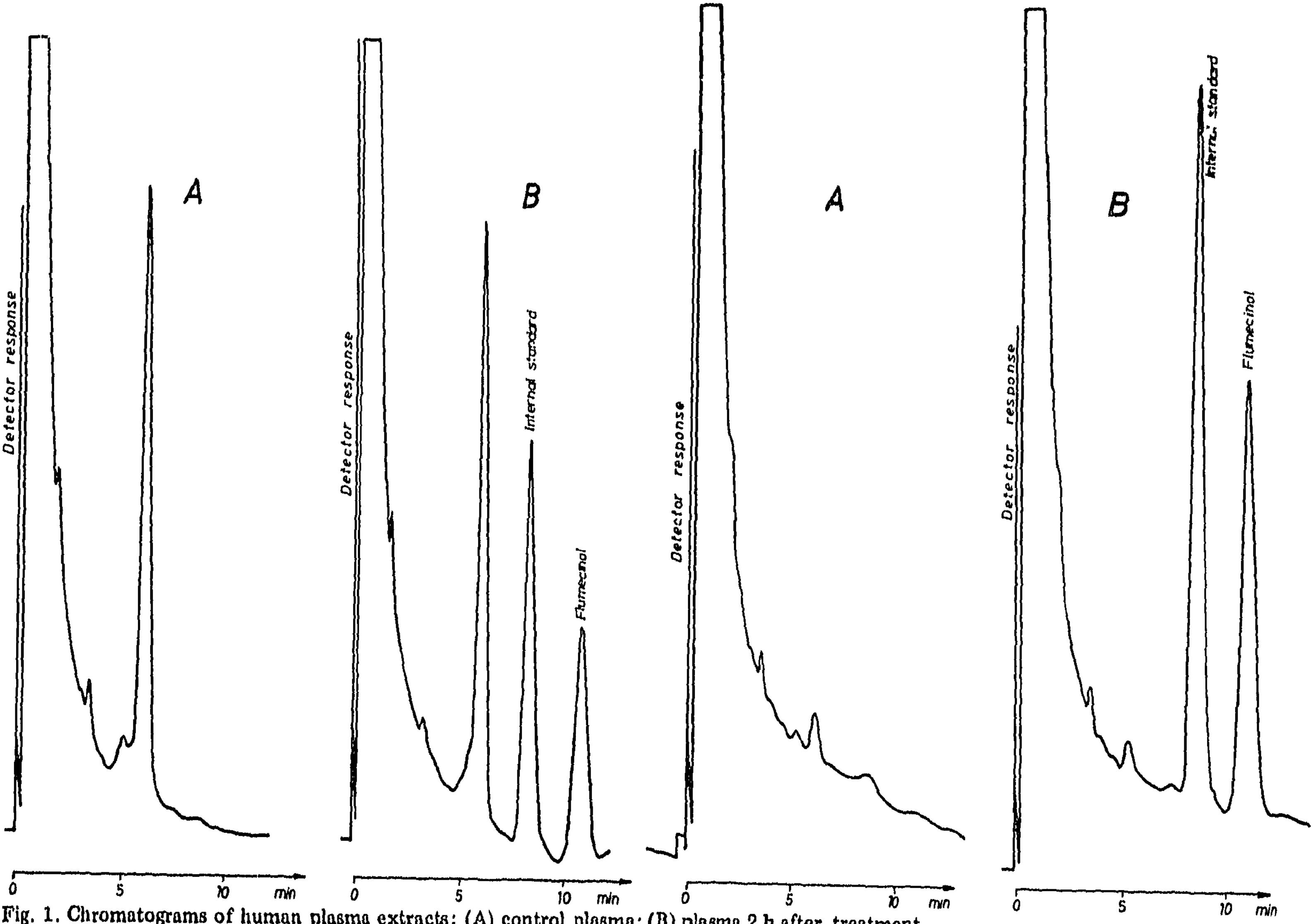

Fig. 1. Chromatograms of human plasma extracts: (A) control plasma; (B) plasma 2 h after treatment.

Fig. 2. Chromatograms of urine extracts: (A) control urine; (B) urine containing flumecinol and internal standard.

samples, and 100—800 ng for dog samples) were added in chloroform. After the addition of 0.6 ml of 2 *N* KOH the samples were extracted with 4 ml of diethyl ether by shaking for 15 min and centrifugation at 3000 *g* for 15 min. The diethyl ether was transferred to test-tubes and evaporated at room temperature. The dry residue was dissolved in 30 μl of chloroform and 1 μl was injected into the gas chromatograph.

For quantification the ratio of peak area of drug to that of the internal standard was used. The recovery of flumecinol was detected by using [^{14}C] flumecinol.

Gas—liquid chromatography

A Hewlett-Packard Model 5736A gas chromatograph with flame-ionization detector was used. The electronic parameters were: range 1, attenuation 2.

Nitrogen of high purity was used as carrier gas. The column packing was a phase containing 10% OV-1 on 80—100 mesh Chromosorb G AW DMCS (Applied Science Labs., State College, PA, U.S.A.) packed in a 0.9 m × 4 mm I.D. glass tube. The column was conditioned at 240°C for 24 h under a gas flow of 30 ml/min. The column was operated at 185°C with a nitrogen flow-rate of 20 ml/min. The temperature settings were injector port 300°C, detector 300°C.

Determination of radioactivity

Radioactivity of liquid samples was counted in a Packard Tri-Carb liquid scintillation spectrometer Model 3310. The aqueous samples were counted in Insta-Gel^{R} (Packard, Downers Grove, IL, U.S.A.) and the diethyl ether samples in a toluene-based liquid scintillation solution (5 g of PPO, 0.1 g of dimethyl POPOP, 100 ml of toluene; Reanal). For the determination of the absolute activity the external standard—channel ratio method was used.

In vivo experiments

Flumecinol was administered in a single oral dose for the pharmacokinetic examination of the drug. The human dose was 100 mg and that for dog 40 mg/kg.

Sodium citrate (3.8%, w/v) was added to the human or dog blood samples in a ratio of 1:9, to prevent coagulation. To 2 ml of human plasma, 1 ml of dog plasma, or 1 ml human urine, 600 ng of 3-trifluoromethyl-benzhydrol (internal standard) were added in 60 μl of chloroform and the samples were processed as above.

RESULTS AND DISCUSSION

Flumecinol is a compound specifically developed for inducing the mixed-function oxidases in liver and thus increasing the metabolic elimination of endogenous and exogenous compounds. As flumecinol influences the pharmacokinetics of many drugs and possibly even the elimination of the drug itself, it seemed rather important to have a specific and sensitive method for the quantitative determination of flumecinol in biological fluids.

Earlier experiments [5] have shown that the application of electron-capture detection for the sensitive detection of the compound failed, most probably

Fig. 3. Calibration curves for flumecinol in human (×) and dog (○) plasma.

Fig. 4. Human (×) and dog (○) plasma flumecinol concentration—time curves after oral administration.

due to steric effects. Therefore, we turned our attention to the less-sophisticated flame-ionization detection.

As flumecinol does not contain any ionizable group it was not possible to purify samples prior to GLC analysis by the acid—base washing technique generally used. We have found that samples extracted under basic conditions (as described in Methods) with diethyl ether gave samples pure enough for the GLC analysis (Fig. 1). The retention times of flumecinol and the internal standard (3-trifluoromethyl-benzhydrol) were 12 and 9 min, respectively.

The method can also be applied to dog plasma and to urine (Fig. 2).

The calibration curves obtained with human and dog plasma show a good linearity between 5 and 800 ng/ml flumecinol. The standard deviation (S.D.) for the calibration curve of human and dog samples was found to be 7.13% and 5.76%, respectively (Fig. 3). Recovery of flumecinol from plasma samples was checked by using radiolabelled drug and was found to be 75.02 ± 1.847% (S.D.).

In Fig. 4 the plasma concentration—time curves are shown for human and dog experiments, demonstrating that the method is sensitive enough for monitoring plasma concentrations after the administration of therapeutic doses.

CONCLUSIONS

A method for determining flumecinol (Zixoryn®) concentrations in biological fluids (human plasma and urine, and dog plasma) at minimum levels of 5 ng/ml has been elaborated. The simple and rapid method is appropriate for routine analysis and pharmacokinetic studies.

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