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Determination of the Antiallergic Agent KB-2413 in Plasma by Means of Capillary Gas Chromatography with a Nitrogen-Sensitive Detector[1)]

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A gas chromatographic method for the quantitative determination of the new antiallergic agent, 1-(2-ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate (KB-2413), in plasma has been developed. This method, which was based on solvent extraction and flexible fused-silica capillary gas chromatography with a nitrogen-sensitive detector and a moving needle solvent cut sample injector, is simple, sensitive and selective.

The detection limit was about 1 ng (as free base)/ml and the calibration curve was linear over the range of 2 to 100 ng/ml. Moreover, the intra- and inter-assay coefficients of variation for the determination of KB-2413 were 3.1—4.0% and 1.5—6.7%, respectively. This assay was applied to the determination of the plasma concentration of KB-2413 base after oral administration to dogs.

Keywords——antiallergic agent; 1-(2-ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate; KB-2413; determination; capillary gas chromatography; nitrogen detector; plasma

1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate (KB-2413) is a new benzimidazole derivative having strong antiallergic activities and lower toxicities in animals as compared with other known antiallergic drugs.[2)]

For pharmacokinetic studies on this compound, it was necessary to establish a simple and sensitive method for the determination of its concentration in the body fluid. A gas chromatographic method for the quantitative determination of KB-2413 base in plasma was developed, and is described here.

Experimental

Materials——KB-2413 was synthesized and supplied by Fuji Chemical Industry, Co., Ltd. (Toyama, Japan). A KB-2413 related compound, 1-(2-ethoxyethyl)-2-(4-methyl-1-piperazinyl)benzimidazole 3/2 fumarate (KB-1688), used as an internal standard was synthesized in our Pharmaceuticals Research Center.[3)]

Benzene for pesticide analysis (used as an extraction solvent) and the other reagents of special grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Gas Chromatography (GLC)——A Shimadzu GC-7A gas chromatograph equipped with a nitrogen-sensitive detector (model FTD-8, Shimadzu, Kyoto, Japan) and a moving needle solvent cut sample injector (Shimadzu) was used for all analyses.

The column used was a flexible fused-silica capillary column coated with OV-1701 (25 m × 0.2 mm i.d., Shimadzu). The injection port and column oven were maintained at 300 and 250 °C, respectively. Helium was used as the carrier gas and make-up gas at flow rates of 1.4 and 40 ml/min, respectively. The detector output was set at 10—

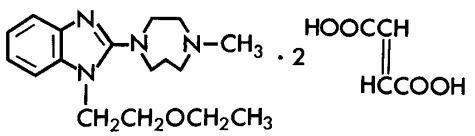

Fig. 1. Chemical Structure of KB-2413

plasma, 1 ml
↓
←—add 1 ml of 0.2 N NaOH
←—extract with 6 ml of benzene
↓
organic phase, 5 ml
↓
←—back-extract with 2 ml of 1 N HCl
↓
aqueous phase
↓
←—add 0.5 ml of 5 N NaOH
←—extract with 6 ml of benzene
↓
organic phase, 5 ml
↓
←—add 20 ng of internal standard
←—evaporate under nitrogen stream
↓
residue
↓
←—redissolve in 50—100 μl of methanol–25—28% ammonia water (50 : 1)
↓
gas chromatography

Chart 1. Schematic Flow Diagram for the Determination of KB-2413 Base in Plasma

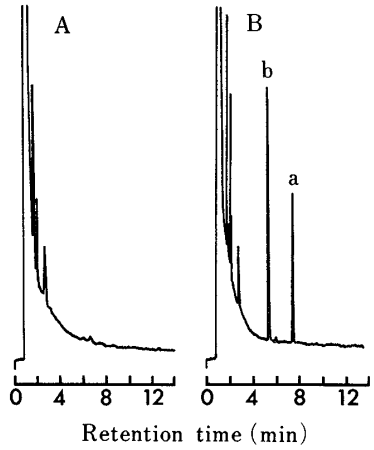

Fig. 2. Chromatograms of (A) Blank Dog Plasma and (B) Dog Plasma Containing 25 ng of KB-2413 Base and 20 ng of KB-1688

Peak a, KB-2413 base; peak b, KB-1688 base.

20%, and the flow rates of air and hydrogen were adjusted to 110 and 2.8 ml/min, respectively.

A split injector (model CLH-702, Shimadzu) was also used at a split ratio of 40 : 1.

Extraction Procedure——A flow diagram of the procedure is shown in Chart 1.

A 1 ml sample of plasma was mixed in a 10-ml glass-stoppered centrifuge tube with 1 ml of 0.2 N sodium hydroxide and 6 ml of benzene. The mixture was vigorously shaken for 10 min and centrifuged for 10 min at 3000 rpm. Five ml of the organic layer was transferred into another centrifuge tube containing 2 ml of 1 N hydrochloric acid, and the mixture was shaken and centrifuged under the same conditions as described above. The organic layer was carefully discarded with an aspirator, and the remaining aqueous phase was alkalinized again by adding 0.5 ml of 5 N sodium hydroxide.

The mixture was re-extracted with 6 ml of benzene by shaking and centrifuging in the same manner as described above.

Then 5 ml of the organic layer was transferred into another centrifuge tube and 20 ng of KB-1688 dissolved in ethanol (1 μg/ml) was added as an internal standard. After being stirred for 2—3 s, the mixture was evaporated to dryness under a stream of nitrogen at about 40 °C. The residue was redissolved in 50—100 μl of methanol–25—28% ammonia water (50 : 1, v/v) and a 1 μl aliquot was injected onto the column. Ammonia water was added to prevent the adsorption of the drug on the glass vessels.

Calibration Curve——Various amounts (2 to 100 ng) of KB-2413 base were added to 1 ml aliquots of blank dog plasma, and extraction was performed as shown in Chart 1. The calibration curve was obtained by plotting the peak height ratio of KB-2413 base to KB-1688 base against the amount of KB-2413 base added.

Application to Animal Experiment——Male beagle dogs (9—11 kg b.w.) fasted overnight were used. One ml of an aqueous solution of KB-2413 was filled into a No. 1 gelatin hard capsule and immediately administered orally at a dose of 5 mg/kg with about 50 ml of water to dogs. Blood samples were taken before administration and at 0.5, 1, 1.5, 2, 3, 4 and 6 h after administration.

Results

Selectivity

Chromatograms obtained from blank dog plasma and dog plasma containing 25 ng of KB-2413 base and 20 ng of KB-1688 are shown in Fig. 2.

No interfering peak was observed in the plasma extracts and the separation of the two compounds added was complete. This method allowed us to detect KB-2413 base at a

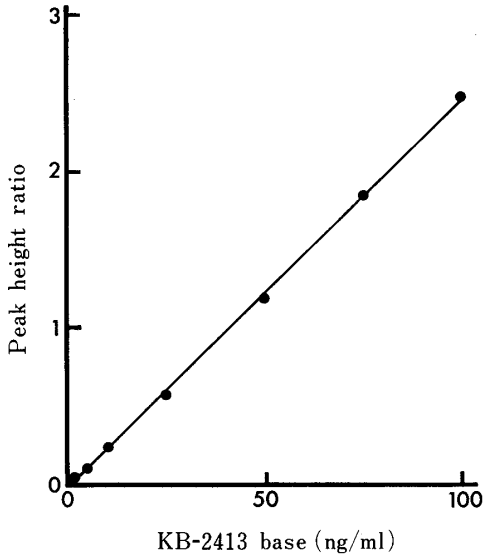

Fig. 3. Calibration Curve for the Determination of KB-2413 Base in Plasma

Various amounts in the range of 2—100 ng of KB-2413 base and 20 ng of KB-1688 were added to each sample.

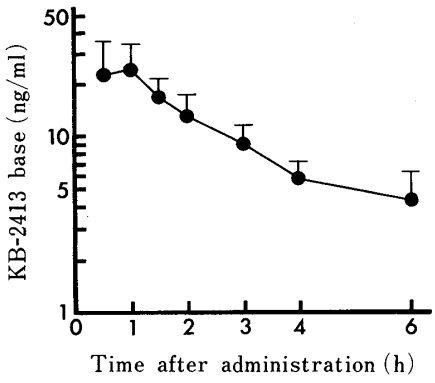

Fig. 4. Plasma Level of KB-2413 Base Following Oral Administration of KB-2413 at a Dose of 5 mg/kg to Dogs

Each point represents the mean of 5 animals and the vertical bars represent the standard deviation of the mean.

Table I. Intra-assay Analytical Precision for the Determination of KB-2413 Base in Plasma

KB-2413 base (ng/ml)		Coefficient of variation (%)	n
Added	Found		
5	4.8	4.0	5
25	24.6	3.2	5
50	50.2	3.1	5
75	74.9	3.7	5
100	101.3	3.4	5

Table II. Inter-assay Analytical Precision for the Determination of KB-2413 Base in Plasma

KB-2413 base (ng/ml)		Coefficient of variation (%)	n
Added	Found		
5	5.0	6.7	4
10	9.8	2.4	4
25	24.0	3.1	4
50	50.6	1.5	4

concentration as low as 1 ng/ml. Moreover, the back-extraction method was useful not only in improving the selectivity but also in minimizing the fouling of the column and the detector, so that a large number of samples could be measured under comparable conditions.

Calibration Curve

The calibration curve for KB-2413 base obtained over the range of 2 to 100 ng/ml is shown in Fig. 3. Good linearity was obtained with the correlation coefficient of 0.9997 ($n=7$).

Accuracy and Reproducibility

The accuracy and reproducibility of the method were evaluated at concentrations of 5—100 ng of KB-2413 base/ml. The intra- and inter-assay data are summarized in Tables I and II, respectively.

In the intra-assay study, the overall recoveries of KB-2413 base from plasma samples averaged 99.2 ± 2.07 (S.D.)% and the coefficient of variation was 3.1 to 4.0% in the concentration range of 5—100 ng/ml. On the other hand, in the inter-assay study, the overall recoveries of KB-2413 base from plasma samples averaged 98.9 ± 4.20 (S.D.)% and the coefficient of variation was 1.5 to 6.7% in the concentration range of 5—50 ng/ml.

Application to Animal Experiments

The applicability of this method was tested by analyzing plasma samples obtained after oral administration of KB-2413 at a dose of 5 mg/kg to dogs. The plasma level reached the maximum (24.1 ± 10.37 ng/ml) at 1 h after dosing and then decreased with a half-life of about 2 h, as shown in Fig. 4.

Discussion

A preliminary study showed that KB-2413 had a strong affinity for the stationary phases and supports of the column. Thus, it was found that the quantitative determination of KB-2413 could not be achieved on conventional packed columns. The recent introduction of inert fused-silica capillary columns has facilitated reliable and reproducible analysis of a variety of drugs.[4] The use of a fused-silica capillary column coated with OV-1701 permitted the selective determination of KB-2413 base in plasma, substantially without interferences from adsorption. However, because a capillary column generally has low capacity, only a small portion of the sample can be applied to the column. For example, the range of quantitative determination tends to be considerably limited if a conventional split injection system is used.

In the present study, a moving needle solvent cut sample injector proved to be very useful for the straight-forward and reproducible determination of KB-2413 base. However, when the carrier gas flow to the splitter was completely stopped, slight broadening or tailing of chromatographic peaks tended to occur. This problem seemed to be due to the spreading of the sample into a small space in the injector system before being introduced into the capillary column. Nevertheless, the sensitivity of KB-2413 base determination with this injector under the optimum conditions (assumed to be approximately 5 : 1 split ratio) was about 8 times higher than that with a conventional split injector system.

Furthermore, a hydrogen flame ionization detector, ^{63}Ni electron capture detector and nitrogen-sensitive detector were examined for suitability to detect minute amounts of KB-2413 base. In view of the chemical structure of KB-2413, which has a benzimidazole nucleus with a homopiperazine ring, a highly sensitive and selective determination was expected to be established by using a nitrogen-sensitive detector. It was indeed found that the sensitivity of KB-2413 base determination with a nitrogen-sensitive detector was much higher than with the other two detectors.

Because of its simplicity, sensitivity and selectivity, the present method is well suited for the routine analysis of a large number of plasma samples. The results of the determination of plasma concentrations after oral administration of KB-2413 at a dose of 5 mg/kg in beagle dogs confirmed the usefulness of this method for application to animal experiments.

References and Notes

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