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GAS CHROMATOGRAPHIC DETERMINATION OF OXAZEPAM FROM TABLETS AND MICROCAPSULES IN URINE

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ABSTRACT

A gas-chromatographic method for the determination of oxazepam and its glucronid in tablets and microcapsules through its hydrolysis product, 2-amino-5-chlorobenzophenone, is described. The drug is extracted into methylene chloride by acidic hydrolysis. Lorazepam is used as an internal standard. The analysis is performed on 80-120 mesh celite coated with 5% of OV-17, packed into a glass column in a gas chromatograph with electron capture detector.

The elimination rates of oxazepam tablets and microcapsules are investigated through urine samples. The calculated elimination rate constants decreased in the following order, T_1, T_2, T_3, M_2, and M_3.

INTRODUCTION

Oxazepam is a pharmacologically active 1,4-benzodiazepine derivative. It has therapeutical application

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and is an important metabolite of diazepam and medazepam. Its elimination is faster than the other benzodiazepine derivatives. Oxazepam reaches to a maximum blood level in two hours and its mean plasma half-life is 3.9 hours (1).

All benzodiazepines appear to undergo biotransformation in the liver prior to excretion and they are excreted by kidneys (2).

Measurements of oxazepam based on colorimetry (3), fluorometry (3), thin layer chromatography (4), gas chromatography (1,5,6) and high performance liquid chromatography (2,7) have been reported.

In this paper, a rapid, simple and sensitive gas chromatographic method used by Vessman et al (6) is modified and applied to the oxazepam tablets in Turkish Drug Market and to the microcapsules prepared with differing core:wall ratios. Oxazepam and its glucuronide is determined by urine analysis.

EXPERIMENTAL

Materials- Oxazepam (M-020), Wyeth; Lorazepam, Wyeth; ethyl cellulose, Fluka; Celite (80-120mesh), Chrompack; OV-17 (methyl phenyl silicon), Chrompack; bromothymol blue, Merck; methylene chloride, Merck; chloroform, Merck; n-heptane, Merck; sulfuric acid,Merck; sodium hydroxide, Merck; potassium hydrogen phosphate, Merck; disodium hydrogen phosphate, Merck; Oxazepam tablets in Turkish Drug Market (Serepax,T_1; Adumbran,T_2; Tranil,T_3); ethyl cellulose coated microcapsules (1:1 and 1:2 core:wall ratio); distilled water.

Preparation of Oxazepam Microcapsules- Microcapsules are prepared by phase separation-coacervation method, (8). Phase separation is obtained by adding 10% disodium hydrogen phosphate to the dispersion of oxazepam in methyl ethyl ketone.

Microcapsules with core:wall ratios of 1:1 and 1:2 are prepared. The different sizes of microcapsules present in each batch are separated by sieving on a mechanical shaker using a range of standard sieves (710-125 μm). Microcapsules corresponding to about 10 mg oxazepam shown in Table 1 are used in the in vivo elimination experiments.

Oxazepam Tablets- Tablets containing oxazepam as an active principal, present in Turkish Drug Market are used in the in vivo experiments. These tablets are shown in Table 2.

Plan of the in vivo Experiments- The oxazepam tablets in Turkish Drug Market and the oxazepam microcapsules prepared (Tables 1,2) are taken by six healty subjects. The specifications of the subjects are shown in Table 3.

All the preparations are given as single dose to the same subject group. The volunteers emptied their bladders before the experiment and this urine is used as reference.

The preparations are taken by the subjects in the morning after a light breakfast with 100 ml water. All the urine is measured at the following intervals, (0-2), (2-4), (4-6), (6-8), (8-12), (12-24) and (24-48) hours. The subjects did not take any other drug during the test period. A new preparation is given to every subject

TABLE 1

Microcapsules Used In in vivo Tests

Preparations	Core:Wall Ratio	Particle Size (μm)	Code
Microcapsule	1:1	500/355	M_2
	1:2	500/355	M_3

TABLE 2

Tablets Used In in vivo Tests

Preparations	Properties	Content mg Oxazepam	Code
Tablets	uncoated	10	T_1
	uncoated	10	T_2
	film coated	10	T_3

after five days has passed from the time they have taken the last formulation.

Standards of the Apparatus- Packard Model 409 gas chromatograph equipped with a Model 714 ^{63}Ni electron capture detector is used. The instrument is fitted with a 2 m glass column (0.3 i.d.) packed with 5% OV-17 on celite (80-120mesh). The chromatographic condi-

TABLE 3

Specifications of the Subjects

No of Subjects	Sex	Age	Weight	Height
1	F'	34	48	1.62
2	F	38	47	1.52
3	F	32	65	1.60
4	M"	34	80	1.69
5	M	38	70	1.72
6	M	18	60	1.73

'= female
"= masculine

tions are the following: injection port and detector temperature 250°C, column temperature 235°C and column activation temperature 235°C. The nitrogen gas flow is 30 ml min^{-1}.

Calibration Curve- The calibration curve is constructed from four standard samples containing 0,50,100,200 µl of oxazepam standard solution (oxazepam dissolved in chloroform to a final concentration of 500 ng ml^{-1}) together with 100 µl of the internal standard solution (Lorazepam dissolved in chloroform to a final concentration of 750 ng ml^{-1}) and 2 ml of water. These samples are treated according to the following procedure:

- 4 ml phosphate buffer (pH 7.4), 8 ml methylene chloride are added. After extraction for 15 minutes by

a laboratory shaker (140 strokes min^{-1}) and centrifugation, the organic layer is filtered into another test tube,

- 2 ml n-heptane is added to the methylene chloride phase and the mixture is extracted for ten minutes with 2 ml 12N sulphuric acid. The organic layer is discarded and the tube heated for 6 minutes in boiling water bath.

- Then the tubes are cooled, 1 drop of bromothymol-blue solution is added and the content alkalized with 5N sodium hydroxide. 0.2 ml of n-heptane is added and after extraction for 10 minutes and centrifugation, the aqueous phase is removed and discarded.

- 3-5 µl of the n-heptane layer is injected into the gas-chromatograph.

The area under the curve is determined and the relative calibration is constructed. The relative calibration curve is preferred to absolute calibration because with the former method, the recovery factors of the respective compounds measured are assumed to remain constant. This prevents to draw new calibration curve for every day of the experiment.

Extraction Procedure=Acidic Hydrolysis of Glucronide in Urine- Oxazepam and its glucronide in urine is determined through its hydrolysis product, 2-amino-5-chlorobenzophenone. The urine samples are treated according to the following procedure:

- To a 2 ml volume of urine, 1.5 ml 12N sulphuric acid and 100 µl internal standard is added.

- The mixture is heated on a water bath for 10 minutes.

- After neutralization, the benzophenones are extracted into n-heptane and analyzed as described under the construction of the calibration curve.

RESULTS AND DISCUSSION

The bioavailability tests from urine samples are preferred to other biological fluids because it is easy to determine the amount excreted; easy to collect samples and to find volunteers to participate in the experiments.

All the benzodiazepines are subject to biotransformation in the liver prior to excretion (2). Since they are excreated by kidneys, the amount excreted by uremic patients is slower than the normal subjects (9). In our experiments, the subjects are chosen from healthy people and the recovery of dose is well within the limits (%70-90) given in literature (10,11) (Table 4).

Retantion Times- The hydrolysis of lorazepam (internal standard) was completed under the conditions applied to oxazepam. Lorazepam, which has the similar structural features as oxazepam, gave accurate and reproducible results as an internal standard.

The separation of the two benzophenones from each otherare easily achieved on OV-17 columns. The retantion times of oxazepam and lorazepam are 6.08 and 1.42 minutes, respectively. Gas chromatograms achieved by urine extraction are shown in Figure 1.

Data Obtained From Urine Analysis- The amuont of oxazepam (mg) eliminated and the elimination rates (mg s^{-1})

TABLE 4

Recovery of Dose of Oxazepam Eliminated by Urine

Preparation	% Recovery	S_D (n=6)
T_1	87.09 ±	0.3956
T_2	87.32 ±	0.1817
T_3	83.51 ±	0.4579
M_2	80.53 ±	0.1926
M_3	65.26 ±	0.1310

S_D= Standard Deviation

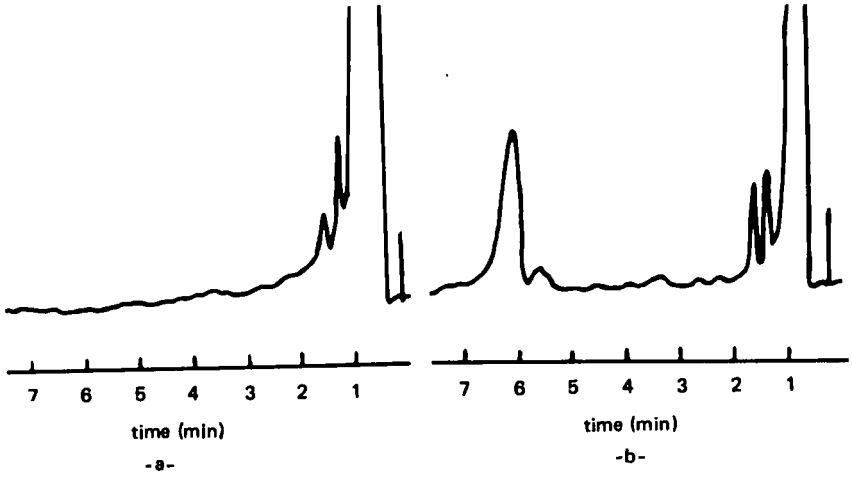

FIGURE 1

Gas Chromatograms Obtained by Urine Extracts

a- only internal standard, Lorazepam

b- internal standard and Oxazepam.

are determined. The elimination rate constants of oxazepam are determined by plotting the natural logarithms of the elimination rate against time. The slopes of the lines are the elimination rate constantsfor eaoh subject. The mean elimination rate constants and elimination half-lifes (hr) of oxazepam preparations are shown in Table 5. The elimination rates and elimination rate constants give variation coefficients up to 75%.

The elimination rate constants of the tablets are approximatly twice the microcapsules; that is the elimination rates of the microcapsules are slower than the tablets investigated, (Table 5). The elimination half-lifes of the microcapsule formulations are longer than the tablets, (Table 5).

The elimination rate constants of the oxazepam tablets (T_1- T_3) are 0.1281, 0.1131 and 0.0904 mg s^{-1}, respectively. The elimination rate constants of the micro-

TABLE 5

Mean Elimination Rate Constants (k_d) and Elimination Half-Life (hr) of Oxazepam Preparations

Preparations	$k_d \times 10^2$	t %50
T_1	12.81	5.41
T_2	11.31	6.13
T_3	9.04	7.67
M_2	6.16	11.25
M_3	5.33	13.04

TABLE 6

Difference Between the Means of Oxazepam Eliminated By Urine

PREP. / t (hr)	$\bar{T}_1$	$\bar{T}_2$	$\bar{T}_3$	$\bar{M}_2$	$\bar{M}_3$	1-2 P	1-3 P
1	1.9870 (0.5420)	2.1383 (0.2653)	1.4686 (0.3193)	0.6922 (0.0694)	-	P > 0.800 INS	P > 0.200 INS
3	1.4453 (0.3185)	2.1145 (0.6550)	2.3192 (0.6630)	0.7645 (0.1322)	-	P > 0.200 INS	P > 0.200 INS
5	2.5558 (0.5063)	2.5830 (0.1687)	2.4880 (0.4897)	2.0290 (0.1695)	1.1903 (0.1219)	P > 0.800 INS	P > 0.800 INS
7	2.3113 (0.4720)	1.1183 (0.2434)	1.6097 (0.4022)	1.8438 (0.2113)	1.7777 (0.2159)	P < 0.050	P > 0.200 INS
10	1.2285 (0.0225)	1.4.60 (0.1255)	1.0660 (0.3119)	1.4937 (0.2100)	1.6635 (0.2264)	P > 0.100 INS	P > 0.500 INS
18	-	-	-	1.1130 (0.1857)	1.2918 (0.1605)	-	-
36	-	-	-	0.2853 (0.0643)	1.1525 (0.1405)	-	-

TABLE 6 (continued)

1-4 P	1-5 P	2-3 P	2-4 P	2-5 P	3-4 P	3-5 P	4-5 P
P < 0.050	-	P > 0.100 INS	P < 0.001	-	P < 0.050	-	-
P < 0.050	-	P > 0.800 INS	P < 0.001	-	P < 0.050	-	-
P < 0.050	P < 0.050	P > 0.800 INS	P < 0.050	P < 0.001	P > 0.200 INS	P < 0.050	P < 0.010
P < 0.050	P < 0.050	P > 0.200 INS	P < 0.050	P < 0.050	P > 0.200 INS	P > 0.100 INS	P > 0.080 INS
P < 0.050	P < 0.050	P > 0.200 INS	P < 0.050	P < 0.050	P > 0.200 INS	P < 0.100	P > 0.500 INS
-	-	-	-	-	-	-	P > 0.200 INS
-	-	-	-	-	-	-	P < 0.001

P= probability between the means INS= insignificant
( ) = data in brackets are standard difference between the means

capsules changed according to the core:wall ratios of the microcapsules. The elimination rate of M_2 is 0.0616 mg s^{-1} and is slower than the tablets. The elimination rate of M_3 is the slowest of all the preparations investigated and as a result has the longest elimination half life. The values are 0.0533 mg s^{-1} and 13.04 hr., respectively. The elimination from M_3 is slower than M_2 and tablets (T_1- T_3), but the recovery of M_3 from urine is 65.26 %. The elimination continues till the 48 th hour but there is no oxazepam in urine in the first three hours. This long lag time seen at the beginning of the elimination might be due to the thicker wall material, less porosity on the microcapsule surface and less space for the uncapsulated oxazepam, in other words 'free oxazepam', (12). This long lag time and the low recovery of oxazepam made M_3 an unsuitable formulation.

It is found that microcapsules having 1:1 core:wall ratio and 355 um particle size ,M_2, have slowed the release rate characteristics and prolonged the elimination of oxazepam. When the formulation is taken, elimination started without any lag time and lasted till the 48 th hour. The in vivo recovery of dose is calculated as 80.53 %.

The release of the active material from the microcapsule without causing a delay could be from the 'free oxazepam' that sticks to the walls of the microcapsules during washing and drying process. The continuing release, could be through the pores left after the coacervating agent, disodium hydrogen phosphate, dissolved from the microcapsule wall by the aqueous dissolution medium.

The statistical difference of the amount of oxazepam eliminated by urine is also investigated. The difference between the amount of oxazepam eliminated from tablets are found insignificant whereas the difference between the tablets and microcapsules are significant, Table 6.

The results of the elimination experiments show that the formulated microcapsules prolonged the release of oxazepam when compared to the tablets. These in vivo results are in accordance with the in vitro dissolution characteristics (13). The in vitro release rate of oxazepam decreased as in the in vivo elimination rate in the following order, T_1, T_2, T_3, M_2, and M_3.

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