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DISSOLUTION RATE AND BIOAVAILABILITY OF SPIRONOLACTONE TABLETS: EFFECT OF VARIOUS TECHNOLOGICAL FACTORS

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ABSTRACT

The effects of the magnesium stearate, talc and gelatin contents of spironolactone tablets on dissolution rate and bioavailability of the drug have been evaluated. The dissolution test results for eight formulations corresponding to a 2x2x2 factorial design showed dissolution rates to be chiefly dependent on gelatin content. Testing the bioavailability of the eight formulations using a balanced incomplete block design revealed no evidence of bioinequivalence, but in view of the possibility that the absence of significant differences had been due to the large number of formulations (and consequent lack of sensitivity on the part of experimental design) three representative formulations were re-tested using a 3x3 Latin square design. The results confirmed those of the balanced incomplete block study. Use of the balanced incomplete block design is advocated for bioavailability studies in which a large number of formulations are to be compared.

INTRODUCTION

Spironolactone is usually included among the active principles considered to be prone to bioinequivalence problems when administered in solid oral dosage forms (1), and in recent years considerable attention has been paid to this aspect of the drug (2; 3; 4; 5). However, relatively little has been published regarding the influence of technological factors on its bioavailability in tablets, the only variables whose effects have been studied with some detail being particle size (6; 7) and the presence of surfactants (8; 9; 10). This article reports a study of the effects of three technological factors (gelatin, talc and magnesium stearate content) on the dissolution rate and bioavailability of spironolactone tablets.

EXPERIMENTAL

Formulations: Micronized spironolactone was supplied by Claudio Barcia S.A. (lot 78/91). Eight formulations containing 25 mg of spironolactone per tablet were prepared by conventional wet granulation method from a basic mixture composed of 12.8% spironolactone, 52.32% wheat starch and 34.88% lactose. The proportions of gelatin, talc and magnesium stearate were chosen to fit a 2x2x2 factorial design (Table I). Gelatin was dispersed in water and added to the mixture during granulation. Tablets were formed using a rotary press with flat 6 mm punches.

Analytical methods: Spironolactone was determined in dissolution test samples using the spectrophotometric method of Raptis et al. (4). Canrenone was determined in urine samples by spectrofluorometric method of Gochman et al. (11). Urine samples were stored at -30ºC before their analysis.

Dissolution test: Dissolution tests were carried out at 37ºC in the USP XXI Apparatus I using a 3 liters vessel, a speed of 150 r.p.m. and destilled water as the dissolution medium. Dissolution curves were characterized by the mean residence times (MRT_{dis}) (12).

TABLE 1

Gelatin, talc and magnesium stearate content of the eight formulations studied.

Formulation	% Gelatin	% Talc	% Magnesium stearate
A	2	2.5	1
B	2	2.5	2
C	2	5.0	1
D	2	5.0	2
E	4	2.5	1
F	4	2.5	2
G	4	5.0	1
H	4	5.0	2

Protocol: The eight formulations were administered inmediatly before a standard breakfast to 14 volunteers of either sex, none of whom had any history of renal insufficiency. Administration conformed to a random balanced incomplete block design (13). In a complementary study, three of the formulations were administered to 15 volunteers in accordance with a 3x3 Latin square design with five replicates per square. Urine was collected: 1; 2; 3; 4; 5; 6; 8; 10; 12; 24 and 48 hours after administration of the formulation.
Pharmacokinetic Analysis: The urinary excretion curves of canrenone, the main metabolite of spironolactone, were characterized by the three statistical moments proposed by Yamaoka et al. (14) for plasma level curves and adapted by Vila-Jato et al. (15) for urinary excretion curves. These moments are the total quantity of canrenone excreted after 48 hours, in µg (E^{48}); the mean residence time, in h (MRT) and the variance in residence time, in h^2 (VRT)
Statistical Analysis: The values of E^{48}, MRT and VRT obtained in the bioequivalence studies were subjected to two-way multivariant analysis of variance (MANOVA), the independent variables being volunteer and formulation. In the balanced incomplete block study the null hypothesis was tested using the Wilks' test (16), and

TABLE 2

Technological characteristics of the eight formulations studied. Mean value ± standard deviation

Formulation	Mean weight mg	Friability $\%.10^4$	Hardness Kg	Disintegration time (min.)
A	212.0±6.65	332.8	6.02±0.48	3.35±0.68
B	213.4±6.88	964.0	4.87±0.59	4.83±0.49
C	224.2±8.96	44.1	7.05±0.18	5.08±0.13
D	215.8±6.00	1033.1	4.50±0.44	4.08±0.20
E	224.3±7.54	0.0	0.92±0.38	15.17±4.35
F	223.2±4.78	1153.5	2.58±0.20	16.17±4.10
G	230.4±5.21	390.4	3.42±0.38	13.08±2.84
H	224.9±5.12	1613.2	2.67±0.26	17.08±2.25

in the Latin square by the greatest eigenvalue test (17). The MRT_{dis} results were subjected to analysis of variance in order to detect significant differences between the dissolution rates of the various formulations and to identify the technological factors responsible.

RESULTS AND DISCUSSION

Table 2 lists the technological characteristics of the eight formulations studied. Figure 1 shows the mean dissolution curves to differ considerably, and the MRT_{dis} of each formulation is listed in Table 3

The results of the analysis of variance (Table 4) show that the main factor responsible for the significant differences among the MRT_{dis} values was the quantity of gelatin and that this effect was modulated by the quantity of talc. The magnesium stearate content of the formulations had no significant effect.

Figures 2 and 3 show the mean distributive urinary excretion curves obtained for the eight formulations tested

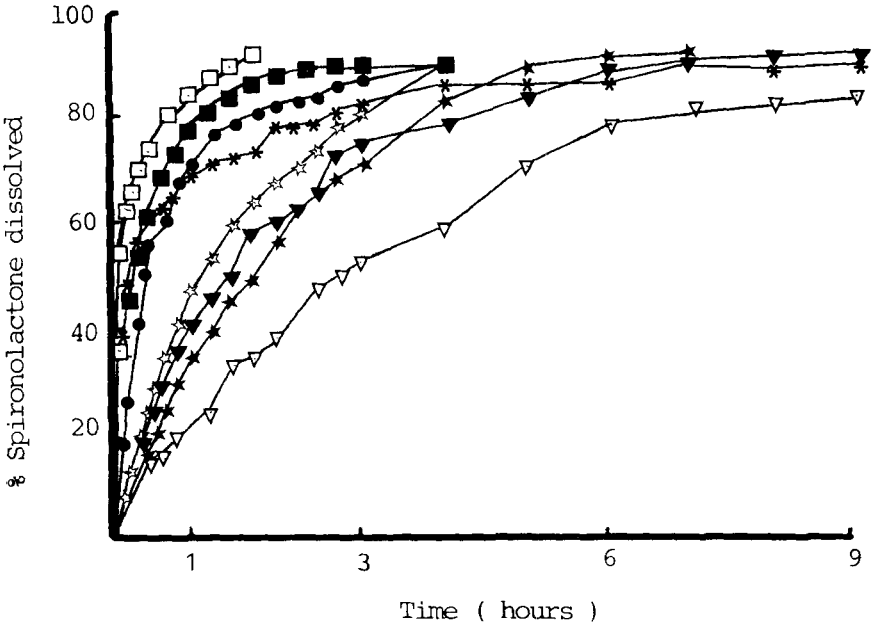

FIGURE 1-. Mean dissolution curves of the eight spironolactone tablets studied. A (*), B (●), C (□), D (■), E (☆), F (★), G (▼) and H (▽)

TABLE 3

MRT values (mean of 6 determinations) corresponding to the dissolution curves of the eight formulations studied.

Formulation	$MRT_{dis.}$ (min.)
A	50.48
B	39.85
C	15.92
D	28.57
E	60.50
F	107.46
G	111.52
H	169.48

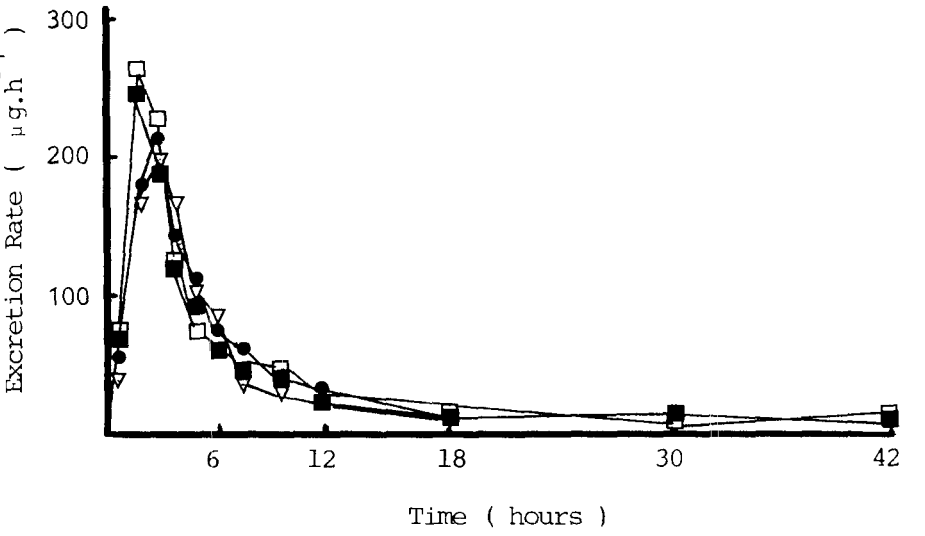

FIGURE 2-. Spironolactone mean urinary excretion curves of A (▽), B (●), C (□) and D (■) formulations

TABLE 4

ANOVA of the MRT_{dis} values of the dissolution curves corresponding to the eight formulations studied.

Source of Variation	D.F	S.S	M.S.	F	α
Formulations	7	111658.7504	15951.2500	10.70	0.01
Gelatin (G)	1	81257.2605	81257.2605	54.52	0.01
Talc (T)	1	2044.5436	2044.5436	1.37	N.S.
Magnesium stearate (MS)	1	5890.7930	5890.7930	3.95	N.S.
G x T	1	14818.1866	14818.1866	9.94	0.01
G x MS	1	5797.6646	5797.6646	3.89	N.S.
T x MS	1	1822.7442	1822.7442	1.22	N.S.
G x T x MS	1	27.5578	27.5578	0.02	N.S.
Error	40	59612.1497	1490.3037		
Total	47	171270.9002			

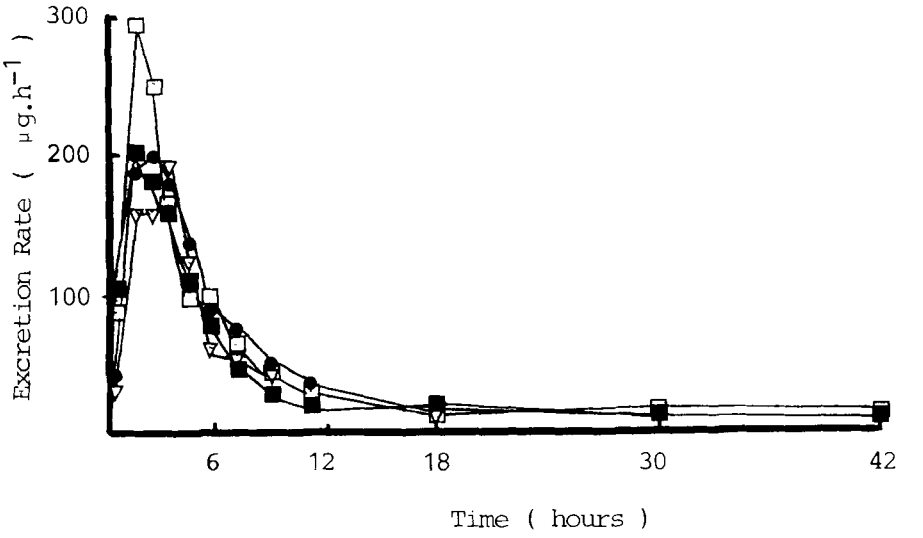

FIGURE 3-. Spironolactone mean urinary excretion curves of E (▽), F (●), G (□) and H (■) formulations

in the balanced incomplete block study, and Table 5 the values of E^{48}, MRT and VRT for each individual and formulation. The multivariant analysis of variance (Table 6) and Wilks´test fail to justify rejection of the null hypothesis that the formulations were bioequivalent (χ^2 = 58.91 with 75 d.f.). In view of the possibility of this result´s being due to lack of sensitivity on the part of the experimental design, formulations C, E and G (those with the lowest, the highest and approximately the mean values of E^{48}) were subjected to further testing in a 3x3 Latin square study with five replicates per square. Figure 4 shows the mean urinary excretion curves obtained in this complementary study, and the values of E^{48}, MRT and VRT are listed in Table 7. Again, no significant differences were detected among the three formulations tested with respect to these variables (Table 8).

The results reported above show that formulations whose in vitro behaviours are appreciably different and which

TABLE 5

E^{48} (μg), MRT (h.) and VRT (h^2) values for each volunteer and formulation corresponding to the Balanced Incomplete Block study

Volunteer	Formulation	E^{48}	MRT	VRT	Formulation	E^{48}	MRT	VRT	Formulation	E^{48}	MRT	VRT	Formulation	E^{48}	MRT	VRT
1	A	1073.5	10.70	100.12	B	1082.4	8.59	99.90	C	1190.9	9.45	104.49	D	1287.4	11.12	114.85
2	E	2233.5	12.16	111.17	F	1934.0	9.91	66.70	G	2442.5	12.12	125.59	H	2058.6	10.34	95.83
3	A	1678.9	9.75	86.03	B	1637.7	9.11	92.71	G	1582.7	8.65	91.96	H	1545.2	7.14	78.92
4	C	765.0	9.41	98.24	D	1407.2	9.04	97.46	E	1192.6	10.38	125.36	F	1187.4	9.05	107.13
5	A	1340.1	8.25	103.27	C	1321.8	9.73	109.99	F	1288.7	9.74	120.31	H	1431.6	10.33	109.48
6	B	1620.3	8.47	76.53	D	1782.0	9.23	106.86	E	1431.3	11.32	103.55	G	1793.8	9.22	105.02
7	A	1075.6	8.12	82.84	D	1043.3	10.00	122.39	F	1110.5	8.42	102.74	G	1174.7	9.17	116.20
8	B	645.6	8.52	105.49	C	1591.7	4.67	44.48	E	1073.2	8.66	104.19	H	615.7	8.57	112.84
9	A	1537.5	7.38	72.99	B	1482.2	9.15	80.82	E	1642.4	8.60	65.08	F	1918.3	9.47	84.15
10	C	1452.5	8.63	79.02	D	2156.1	7.90	70.33	G	1781.5	6.95	72.83	H	1700.9	7.83	87.48
11	A	775.5	8.33	90.09	C	1315.2	6.64	75.39	E	915.7	6.69	82.66	G	1175.3	7.25	87.16
12	B	996.3	8.06	87.88	D	1094.6	7.94	92.40	F	1025.6	9.43	105.79	H	1033.9	9.01	103.54
13	A	1297.0	7.68	73.43	D	1041.0	7.18	77.80	E	1110.4	5.54	42.98	H	1267.0	6.41	54.07
14	B	2242.6	9.59	93.05	C	2390.9	9.09	84.80	F	2388.9	9.40	79.96	G	2110.4	8.19	85.32

TABLE 6

Results of the multivariant analysis of variance in the Balanced Incomplete Block Design.
χ^2 = 58.91 with 75 d.f. 90.53 is the Critical Value for α = 0.01

Source of Variation	Matrix	Sum of squares and products		
Formulations	H	9470027.9160		
		11367.4007	70.9508	
		- 25719.9895	508.7214	9842.2841
Volunteers	S	271415.9567		
		- 49.5974	3.6315	
		8454.3322	23.8796	2613.4230
Error	E	1518323.0253		
		- 1777.3413	41.0974	
		- 27261.2979	399.0652	13349.2387
Total	T	11259766.8980		
		9540.4620	115.6798	
		- 44526	931.6663	25804.9458

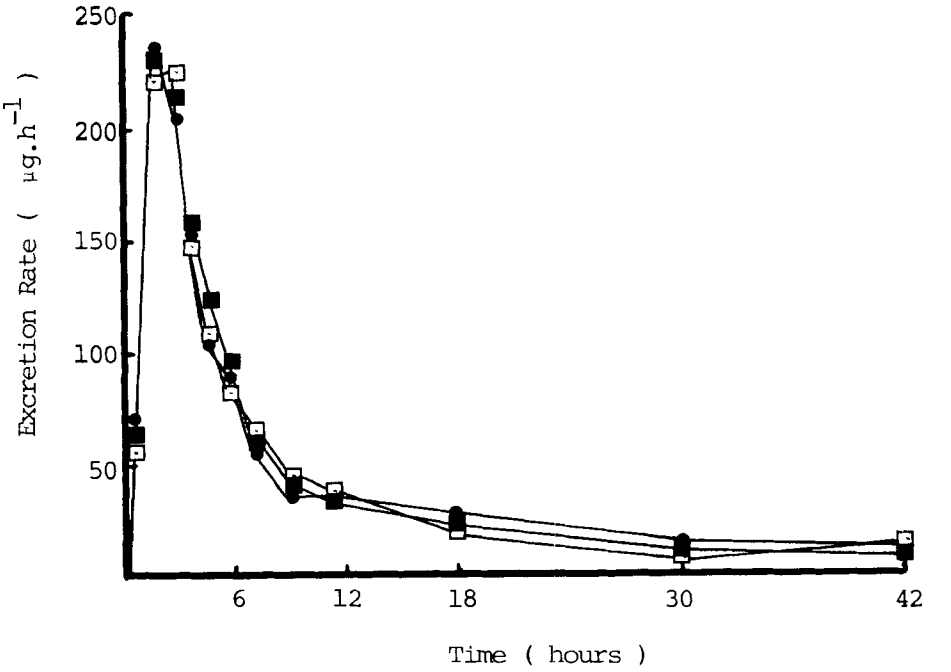

FIGURE 4-. Spironolactone mean urinary excretion curves of C (■), E (●) and G (□) formulations.

TABLE 7

E^{48} (µg), MRT (h.) and VRT (h^2) values for each volunteer and formulation corresponding to the Latin square design study.

Volunteer	Formulation	E^{48}	MRT	VRT	Formulation	E^{48}	MRT	VRT	Formulation	E^{48}	MRT	VRT
1	C	1291.3	11.38	130.09	E	1234.8	10.38	110.05	G	1187.4	10.08	102.43
2	C	2443.9	8.66	88.88	E	2314.7	9.79	93.77	G	2774.4	10.50	106.14
3	C	2327.4	9.36	94.38	E	2268.0	8.25	81.80	G	2676.0	10.32	118.03
4	C	2026.1	8.05	88.73	E	2155.1	10.05	97.80	G	1514.9	9.93	133.23
5	C	1827.3	6.58	60.08	E	1747.7	7.84	76.88	G	1570.6	7.73	87.79
6	E	1619.7	8.67	87.23	G	1642.9	9.94	91.41	C	1798.4	9.19	86.99
7	E	1248.0	9.06	107.84	G	941.2	9.20	113.38	C	1233.9	11.00	125.08
8	E	1269.1	10.68	110.41	G	1211.4	9.73	79.44	C	1460.3	9.38	90.52
9	E	1801.6	10.46	116.96	G	1570.9	9.25	101.57	C	1779.2	11.95	100.20
10	E	1109.7	8.07	84.59	G	853.3	7.38	80.49	C	924.8	8.21	99.39
11	G	1204.4	7.38	82.37	C	1297.8	10.54	109.58	E	1235.4	9.17	102.14
12	G	1123.3	8.33	91.57	C	1244.4	9.19	107.83	E	1359.6	9.95	108.51
13	G	1111.5	6.82	77.23	C	1110.2	6.49	60.77	E	683.1	9.57	120.40
14	G	2270.5	8.59	64.02	C	2066.1	7.55	71.48	E	1934.0	6.52	60.60
15	G	1767.9	10.68	112.68	C	1117.4	8.29	100.23	E	1257.4	11.37	130.95

TABLE 8

Results of the multivariant analysis of variance (MANOVA) of the Latin square study. The greatest eigenvalue of $H.E^{-1}$ is C_S= 0.034 and $C_S/1 + C_S$ = 0.033; the parameters of its distribution are s=2, m= 0 and n = 12. Null hypothesis is not rejected at α = 0.05 level

Source of Variation	D.F.	Sum of squares and products		
Formulations	H	18615.8093		
		- 26.9587	0.7076	
		- 1096.5609	11.0991	199.8775
Volunteers	S	8834290.3120		
		775.5698	47.8162	
		- 65750.1098	543.6550	8785.5775
Error	E	1010435.2707		
		1169.8521	35.1148	
		- 15286.1938	362.2836	6588.6757
Total	T	9863341.3920		
		1918.4632	83.6386	
		- 82132.8625	917.0377	15574.1507

are distinguishable in dissolution studies are nevertheless bioequivalent. The factors identified as responsible for the differences in dissolution rate therefore have no effect on bioavailability, at least within the concentration ranges studied. These findings should be interpreted in the light of those of an earlier study (18) in which the two factors chiefly responsible for controlling the biopharmaceutical behaviour of spironolactone were identified as its poor solubility and the long elimination half-life of canrenone. The failure to detect bioinequivalences in the present study cannot be attributed to canrenone's high distribution MRT making the statistical moments insensitive towards changes in absorption rate, because analysis of variance of the quantities of canrenone excreted after three hours shows that this parameter, which is more sensitive than the others to absorption rate (18), likewise fails to exhibit signifi-

cant differences (F = 0.02 with 2; 28 d.f.). It must be therefore be concluded that in the present case the absence of significant differences between the canrenone urinary excretion profiles of the various formulations studied is due to the low solubility of spironolactone, which results in there being little difference between the formulations as regards the rate at which the active principle is made available. This hypothesis is supported by the fact that when large increases in the dissolution rate of spironolactone tablets have been achieved by reduction of particle size (6; 7) or inclusion in solid dispersions or complexes (19; 20) the bioavailability of the drug has also improved significantly. As a consequence of the above conclusion, it may be expected that the technological factors involved in the production of spironolactone tablets with conventional formulations will give rise to no bioinequivalence.

Finally, it is worth pointing out that in spite of the least significant differences in the balnaced incomplete block design being much larger than in the Latin square design (314.06 µg as against 142.60 µg), the former was nevertheless sensitive enough for the purposes of the study. This means that balanced incomplete block designs may usefully be employed in evaluating the effects of technological factors on bioavailability or in other bioequivalence studies in which large numbers of formulations must be studied simultaneously.

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