10. Zilpaterol hydrochloride

First Draft prepared by
Joe Boison, Saskatoon, Canada,
Fernando Ramos, Coimbra, Portugal,
Pascal Sanders, Fougères, France,
Al Chicoine, Saskatoon, Canada
and
Stefan Scheid, Berlin, Germany

Identity

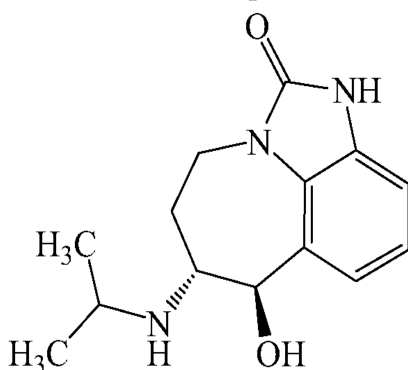
International Non-proprietary name (INN): Zilpaterol hydrochloride

Synonyms: RU 42173, zilpaterol HCl, Zilmax®, Zilmax® Pre-mix.

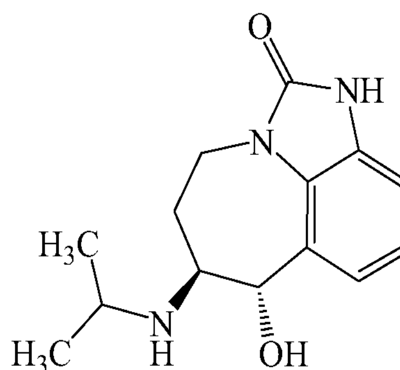
IUPAC Names: (±)-Trans-4,5,6,7-Tetrahydro-7-hydroxy-6-(isopropylamino)-imidazo[4,5,1-jk]-[1]benzazepin -2(1H)- one, monohydrochloride
Trans(±)-4,5,6,7-Tetrahydro-7-hydroxy-6-[(1-methyl-ethyl)amino]-imidazo[4,5,1-jk]-[1]benzazepin-2(1H)-one, monohydrochloride

Chemical Abstract Service Number: Zilpaterol hydrochloride: 119520-06-8.

Structural Formula (zilpaterol free base):



(6R,7R)



(6S,7S)

Molecular Formula: $C_{14}H_{19}N_3O_2$ • HCl (zilpaterol hydrochloride)

Molecular Weight: 297.56 g for zilpaterol hydrochloride, and
261.113 g for zilpaterol free base.

Other information on identity and properties

Pure active ingredient: Zilpaterol has two chiral carbons and consequently four optical enantiomers. These enantiomers are: "(6R,7R)", "(6R,7S)", "(6S,7R)" and "(6S,7S)".
RU 42173 corresponds to racemic trans zilpaterol hydrochloride, a mixture of the (6R,7R) and (6S,7S) enantiomers.

Appearance: White to practically white powder

Melting point: 219.5–220.5°C

Solubility: Zilpaterol hydrochloride is very soluble in water and in other aqueous media (about 50% of product dissolved) at different pH values (1–10). It is only slightly soluble in methanol (about 3%) and practically insoluble in most organic solvents (<0.1%) such as

ethanol, acetone, ethyl-acetate, isopropyl ether, hexane, toluene, chloroform, dichloromethane or n-octanol.

Residues in food and their evaluation

Conditions of use

Zilmax®, the commercial formulation of zilpaterol hydrochloride, is composed of 4.8% w/w zilpaterol hydrochloride as active ingredient, 8% polyoxyl 35 castor oil, 4.3% povidone 30 (40% aqueous solution) and 82.9% corn cob grit. Zilmax® is used to increase rate of bodyweight gain, improve feed efficiency, and increase carcass leanness in cattle fed in confinement for a period of 20-40 consecutive days at the end of the feeding period before slaughter.

Dosage

Zilpaterol hydrochloride should be mixed into the feed at a level of 7.5 mg/kg on a 90% dry matter basis. This represents a dose of approximately 0.15 mg/kg bw or 60 to 90 mg zilpaterol hydrochloride per animal per day.

Registered uses

Zilmax® has been registered for use in Columbia, Costa Rica, the Dominican Republic, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and South Africa. Marketing authorization has been obtained for Zilmax® in Brazil, Canada, Kazakhstan and the United States of America. Import licences have recently been granted for the use of Zilmax® in Lebanon and Pakistan, while registration procedures are currently ongoing in Australia, Belarus, Indonesia and Taiwan. A procedure for registering "import MRLs" is currently active in Japan. The product was registered initially in South Korea as a feed additive, but this licence was withdrawn and the product was recently registered as a medicated pre-mix product. Zilpaterol hydrochloride is not permitted for use in lactating dairy cattle.

Pharmacokinetics and metabolism

Test material used in radiolabel pharmacokinetic and metabolism studies

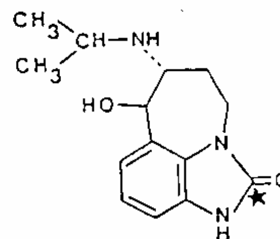
Pharmacokinetic and metabolism studies were conducted with [^{14}C]zilpaterol hydrochloride (trans (±)-6(1-methyletylamino)-7-hydroxy-4,5,6,7-tetrahydro-[2-^{14}C]azepino[1.2.3-cd]benzimidazole-2(1H)-one, hydrochloride) where the label is in the carbonyl position of the molecule (Figure 10.1). The position of this ^{14}C label has been shown to be in a stable, non-metabolized, part of the molecule (Veltz, 1999; Tremblay *et al.*, 1988).

Specific activity: 6.06-6.27 MBq/mg.

Purity: > 99.0% (by TLC and HPLC methods)

The good radio-purity, together with radiolabelling in a stable, non-metabolized position, allowed use of this product to assess pharmacokinetics and metabolism in laboratory animal species and in cattle.

Figure 10.1. The structure of [^{14}C]zilpaterol free base showing the position of the label



Pharmacokinetics and metabolism in laboratory animals

Rats

A study (non-GLP-compliant) was conducted with [^{14}C]zilpaterol hydrochloride in 12 male fasted Sprague-Dawley rats (mean weight 202 g) to assess absolute oral bio-availability, the feeding status/effect of repeated oral administrations and then the distribution into the body (Tremblay *et al.*, 1990a). The rats were allocated to two groups of 6 animals each. The rats were fasted 21 h and administered 1 mg/kg bw zilpaterol hydrochloride by either an oral or intravenous route, and fasted 6 h after dosing; the oral bio-availability was determined to be 99.3% based on urinary excretion. Administration into the food at a dose of 0.055 mg/kg bw produced lower systemic exposure (both area under the curve (AUC) and maximum concentration (plasma) (C_{max})) than after oral gavage, with a slightly higher exposure in females than in males. C_{max} was approximately proportional to the dose.

Another study (non-GLP compliant) was reported in which [^{14}C]zilpaterol hydrochloride was administered in a single oral dose of 1 mg/kg by gastric intubation to 10 male and 10 female Sprague-Dawley rats, mean weight 203 g (Tremblay *et al.*, 1989). The 10 rats were divided into two groups of 5 each. The first group was anaesthetized and killed 0.5 h after drug administration and the second group 24 h after drug administration. The total radioactivity in the different tissues and plasma collected was determined by liquid scintillation counting (LSC). The ratio of tissue radioactivity concentration to that of plasma ($R_{t/p}$) was calculated for each tissue collected (Table 10.1). The $R_{t/p}$ results of the study conducted to determine the tissue distribution of zilpaterol as a function of time in the rat after a single oral dose administration showed that the radioactivity concentration of the drug depletes between 0.5 h and 24 h for all tissue matrices and organs of the males or females tested. Kidneys and liver involved in the metabolism and elimination of zilpaterol hydrochloride and its metabolite displayed the highest $R_{t/p}$. At 24 h, the residual radioactivity was low and there was no retention in the organ samples, with no marked difference between male and female rats.

The $R_{t/p}$'s measured for plasma, liver, kidneys, skeletal muscle and lung tissues are given in Table 10.2 for the male rats used in the above study and sacrificed at 0.5 and 24 h after the oral dose. These results show that the concentration of residues likely to be found in muscle are lower than would be found in kidney, liver and lung tissue.

Table 10.1. Tissue distribution of zilpaterol at 0.5 and 24 h following a single administration of 1 mg/kg [^{14}C]zilpaterol hydrochloride by gastric intubation to male and female Sprague-Dawley rats (Tremblay *et al.*, 1989)

	$R_{t/p}$ >>>1	$R_{t/p}$ <<<<1
0.5 h male rats	Vascular system (heart, spleen, bone marrow) Respiratory system (diaphragm, lung) Endocrine system (thyroid, adrenals, pituitary) Digestive system (pancreas, duodenum, stomach) Liver – 5.96; kidney – 34.4	CNS (cortex, cerebellum, medulla) Eyes Fat (subcutaneous, perirenal) Testis and thymus
0.5 h female rats	Reproductive system (vagina, oviducts, uterus, & ovaries) Skeletal muscle, adrenals, liver – 7.24; kidneys – 37.4	Subcutaneous fat
24 h male rats	Respiratory system (lung, diaphragm) Vascular system (blood, erythrocytes) Relational system (skin, skeletal muscle) Adrenals – 7.0; stomach – 13.3; kidney – 16.6; urinary bladder – 24.2; liver – 75	CNS (cortex, cerebellum, medulla) Endocrine system (thyroid, pituitary) Vascular system (heart, bone marrow) Thymus, pancreas, eyes, perirenal fat
24 h female rats	Ovaries, liver – 71; kidneys – 11.4	

NOTES: $R_{t/p}$ = ratio of tissue radioactivity concentration to that of plasma.

A GLP compliant study was undertaken in which 70 male (mean bodyweight 272 g) and 70 female (mean bodyweight 213 g) Sprague-Dawley rats (about 8 weeks old) were allocated to two groups of 15 animals/sex/group, which received a dietary admixture, and two groups of 20 animals/sex/group dosed by gavage (Sauvez, 1995). Unlabelled zilpaterol doses used were 0.05 or 1.10 mg/kg/day (gavage and dietary admixture) for 13 days. All the animals were fasted for gavage purposes. Blood samples were collected Days 2–3 and Days 13–14, and harvested plasmas were analysed for unchanged zilpaterol using a validated radioimmunoassay method with a LOQ of 0.025 ng/ml. After a 2-week repeated administration by oral route (dietary or gavage) at a dose of 0.05 mg/kg or 1.0 mg/kg bw in male and female rats, the mean plasma $AUC_{(24h\ period)}$/dose were generally 2–4 times higher in females than in males; the mean plasma $AUC_{(24h\ period)}$ and C_{max} were 1.5–4 and 10–13 times higher, respectively, by gavage than by dietary admixture (Table 10.3).

Table 10.2. Ratio ($R_{t/p}$) of concentrations of [^{14}C]zilpaterol in male Sprague-Dawley rats killed 0.5 and 24 h after a single oral dose (Tremblay *et al.*, 1989)

Tissue	0.5 h withdrawal		24 h withdrawal	
	n	Mean ±S.D.	n	Mean ±S.D.
Plasma	5	1	5	
Liver	5	5.96 ±0.24	5	75 ±14
Kidneys	5	34.4 ±3.7	5	16.6 ±3.7
Skeletal muscle	5	1.24 ±0.08	5	2.46 ±0.44
Lung	5	1.65 ±0.27	5	1.43 ±0.19

Table 10.3. Mean pharmacokinetic parameters for zilpaterol in Sprague-Dawley rat plasma after dosing by dietary admixture or gavage (Sauvez, 1995)

Route of administration	Days	Dose (mg/kg/day)	Sex	T_{max} (h)	C_{max} (ng/ml)	$AUC_{(0–24\ h)}$ or $AUC_{(19–19\ h)}$ (ng.h/ml)
Dietary admixture	2–3	0.055	M	23	0.24	3.64
			F	23	1.90	29.5
		1.10	M	3	3.65	56.1
			F	23	8.71	159
	13–14	0.055	M	3	0.18	2.92
			F	7	0.68	10.0
		1.10	M	7	3.78	47.2
			F	7	9.46	157
Gavage	2–3	0.055	M	0.75	2.50	5.44
			F	0.25	5.99	13.4
		1.10	M	0.50	44.5	139
			F	0.50	11.5	356
	13–14	0.055	M	0.75	2.35	6.35
			F	0.25	8.29	18.4
		1.10	M	0.75	46.0	188
			F	0.75	95.5	397

NOTES: T_{max} = time at which C_{max} occurs; C_{max} = maximum concentration (plasma); AUC = area under the curve.

In a non-GLP-compliant study of the *in vitro* binding of zilpaterol to rat serum proteins, 30 fasted male Sprague-Dawley rats with a mean bodyweight of 200 g were administered [^{14}C]zilpaterol hydrochloride at a concentration of 0.005, 0.05, 0.5, 10 and 100 µg/ml (Tremblay, Biechler and Cousty, 1990b). Blood samples were collected from all the rats. After dialysis to equilibrium point, the percentage binding was calculated from the concentrations measured by counting radioactivity using a liquid scintillation counter. The results presented in Table 10.4 indicate that a mean percentage binding of zilpaterol to rat serum protein was 14% (13.7%) at 37°C.

Table 10.4. *In vitro* determination of percentage binding of zilpaterol to Sprague-Dawley rat serum proteins (Tremblay, Biechler and Cousty, 1990b)

Concentration of [^{14}C]zilpaterol hydrochloride (µg/ml)	% Mean binding ±S.D. to Sprague-Dawley rat serum protein
0.005	15.8 ±1.1
0.05	10.9 ±1.7
0.5	12.0 ±1.0
10	14.3 ±0.7
100	15.4 ±0.3
Overall mean % binding	13.7 ±2.1

Two studies (non GLP-compliant) were conducted to determine the routes of excretion, characterize the residues in the excreta and compare the metabolites observed in cattle and rat excreta. The 3 male and 3 female Wistar rats (Tulliez, 2000a) and 3 male and 3 female Sprague-Dawley rats (Tulliez, 2000b) weighing 200–250 g were administered a single oral dose of [^{14}C]zilpaterol hydrochloride synthetic diet by gavage at 0.2 mg/kg bw. In the first study, two Wistar rats (one male and one female) were sacrificed at 12 h and 48 h post-dose and the remaining two Wistar rats were sacrificed after 8 days. Urine and faeces were collected daily, and liver and remaining carcass tissue were collected at 12 h, 48 h and 8 days post-dose and analysed by LSC, combustion, HPLC and mass spectrometry. In the second study, the Sprague-Dawley rats were assigned to three groups of 2 (1 male and 1 female) and urine and faeces were collected for 8 days. Rats were killed 8 days post-dose and liver samples were collected.

The metabolic profile in the Wistar rat urine (Table 10.5) contains predominantly unchanged zilpaterol at 60% of the administered dose the first day, and at about 47% on Day 2. There were five other metabolites observed in the urine, of which the de-isopropyl-derivative is the most abundant at about 20%. This metabolite is undetectable in the faeces, while hydroxy-zilpaterol is the most abundant metabolite in faeces at about 58%, together with small percentages of acetylated de-isopropyl-zilpaterol and the glucuronate conjugate of hydroxy-zilpaterol (Table 10.5).

The metabolic profiles in the Sprague-Dawley rat urine and faeces were similar to those observed in the Wistar rat (Table 10.6).

Table 10.5. Distribution of metabolites in urine and faeces in Wistar rats treated with [^{14}C]zilpaterol hydrochloride (Tulliez, 2000a)

Metabolite Number	Identification	Faeces (%)		Urine (%)	
		Day 1	Day 2	Day 1	Day 2
A	Acetylated de-isopropyl- zilpaterol	7.1	8.6	4.4	4.2
B	Unidentified	3.1	1.3	2.2	0.7
C	Glucoronate conjugate of hydroxyl-zilpaterol	3.3	5.3	9.3	22.3
D	Unidentified	ND	ND	0.5	ND
E_1	De-isopropyl-zilpaterol	Trace	Trace	19.5	14.2
E_2	Hydroxy-zilpaterol	60.3	55.8	—	—
F	Zilpaterol	15.8	3.9	60.3	46.7

NOTES: ND = not detectable.

Table 10.6. Percentage distribution of metabolites in urine and faeces after a single dose of [14C]zilpaterol administered to Sprague-Dawley rats (Tulliez, 2000b)

Metabolite Number	Identification	Faeces (%) Days 1–3 M+F	Urine (%) Day 1		Urine (%) Day 2	
			M	F	M	F
A	Acetylated de-isopropyl-zilpaterol	3.7	11.1	3.1	14.3	3.7
B	Unidentified	2.7	3.7	0.6	9.1	0.4
C	Glucoronate conjugate of hydroxy-zilpaterol	1.5	0.5	7.1	5.3	6.0
D	Unidentified	ND	0.4	0.1	0.4	ND
E_1	De-isopropyl-zilpaterol	4.1	15.2	8.4	10.9	7.3
E_2	Hydroxy-zilpaterol	68.8	5.9	3.3	16.6	6.4
F	Zilpaterol	7.1	50.0	73.2	24.9	70.9

NOTES: ND = not detectable.

The data collected to monitor the cumulative excretion of radioactivity indicated that more than 60% of the dose at 48 h and about 92% of the dose 8 days after dosing could be accounted for (data not shown). While no sex-related differences were observed for the excretion of total radioactivity, there was nevertheless an apparent more rapid urinary excretion for male rats compared with female rats (30% versus 20%). Radioactivity concentrations in the liver and carcass amounted to less than 1% of the administered dose. The results of the comparative metabolic profile study for zilpaterol in the two rat species is shown in Table 10.7.

When [14C]zilpaterol hydrochloride is administered to rats by oral gavage, the compound is excreted almost equally in the urine (49–51%) and faeces (42–44%). Less than 0.1% of the radioactive concentration was retained in the liver 8 days post-dose. The structure of the major metabolite in faeces was confirmed by GC-MS to be the hydroxy-zilpaterol. It is concluded that the metabolic profile for zilpaterol in the urine of the Sprague Dawley rat is quantitatively and qualitatively similar to that obtained for the Wistar rats.

Table 10.7. Metabolic balance in Wistar and Sprague Dawley rats administered [14C]zilpaterol hydrochloride (Tulliez, 2000a, b)

Rat #	Sex	Urine	Faeces	Liver	Carcass	Total
Metabolic Balance in Wistar rats (% administered dose)						
1	M	54.3	39.9	0.07	0.46	94.7
2	M	47.8	44.0	0.04	0.63	92.5
3	M	51.1	40.0	0.06	0.63	91.8
4	F	51.1	44.8	0.06	0.45	96.4
5	F	49.0	44.3	0.09	0.90	94.3
6	F	40.0	40.8	0.05	1.00	81.9
Mean ±SD		48.9 ±4.9	42.3 ±2.3	0.06 ±0.02	0.68 ±0.23	91.9 ±5.2
Metabolic Balance in Sprague-Dawley rats (% administered dose)						
7	M	63.3	34.6	0.05		98.0
8	M	52.4	46.7	0.06		99.0
9	M	51.1	41.3	0.06		93.0
10	F	42.4	47.4	0.04		89.9
11	F	45.4	48.0	0.05		93.4
12	F	50.3	44.0	0.06		94.3
Mean ±SD		50.8 ±7.2	43.7 ±5.1	0.05 ±0.01		94.6 ±3.4

In a non-GLP-compliant study, 10 adult Wistar rats (5 males and 5 females) each received a single 0.2 mg/kg of [^{14}C]zilpaterol hydrochloride by oesophageal catheter (Zalko, 1993). Two rats (1 male and 1 female) were killed at 12 and 48 h. The other six were killed after 8 days. The radioactivity present in the liver, kidneys, perirenal fat, muscle and the rest of the carcass was measured after grinding the tissue. Urine and faeces from two animals in the last group were collected each day and their radioactivity contents were measured (Table 10.8).

On average, nearly 91% of the administered radioactivity was eliminated in the urine (49%) or faeces (42%) during the 8 days, with 63% of the radioactivity excreted during the first 24 h. At kill time on the 8th day, only 0.72 ±0.19% of the administered radioactivity was detectable in the carcass. The liver at this same time-point contained 0.06 ±0.02% of the administered radioactivity. In liver, the concentrations of zilpaterol HCl equivalents decreased quickly from 36 µg/kg after 12 h to 2 µg/kg on the 8th day. The same trend was seen in the kidney, with much lower concentrations (7 µg/kg to ND). There was no significant radioactivity determined in the fat or muscle (Table 10.8).

Table 10.8. Radioactivity in rat tissues, expressed in µg/kg, after a single oral administration of [^{14}C]zilpaterol hydrochloride at a dose of 0.2 mg/kg (Zalko, 1993)

Post-dose	Rat # & sex	Liver	Kidney	Muscle	Fat
12 h	1 M	33	7	ND	ND
	2 F	38	5	2	1
48 h	3 M	12	3	ND	ND
	4 F	20	4	ND	2
8 days	5 M	2	ND	ND	ND
	6 F	3	ND	ND	ND

NOTES: ND = not detectable

The main metabolites were deisopropyl-zilpaterol, hydroxy-zilpaterol and unchanged zilpaterol, accounting for 2, 9 and 81% respectively of the total radioactivity detected in urine. In faeces, these were 7, 40 and 44% respectively, totalling about 91% of the total areas under the radioactive HPLC peak. This high percentage supports the view that conjugation metabolic pathways are absent (or occur to only a very slight extent), which is in contrast to the behaviour of other β-agonists, which have always shown that glucorono-conjugation or sulfo-conjugation are the dominant mechanisms. This difference, the study authors presume, may be due to the chemical structure, which is not strictly of the phenyl-ethanolamine type. The hydroxy-metabolite of zilpaterol is the dominant extractable metabolite in the liver.

Dogs

An open dose randomized cross-over study using 4 fasted male beagle dogs (mean weight of 10 kg) in a non-GLP-compliant study was undertaken to measure the absolute bio-availability of [^{14}C]zilpaterol hydrochloride in the dog after a single dose administration of 1 mg/kg bw intravenously or orally (Tremblay *et al.*, 1990c; Tremblay, Biechler and Cousty, 1990d). The dogs were fasted for 24 h before and 8 h after drug administration. Urine samples were collected over a 48 h period and analysed for zilpaterol by LSC. The overall mean binding to dog serum proteins at 37°C was calculated to be 15% (Table 10.9). After intravenous administration, the radioactivity concentration was 22.8 ±2.1% of the dose, and 23.9 ±2.4% after oral administration. The absolute bio-availability of zilpaterol in the beagle dog was calculated as 100%. This represents a renal clearance of 23% of total clearance.

Table 10.9. Mean binding constants for zilpaterol to beagle dog serum proteins (Tremblay, Biechler and Cousty, 1990b)

Concentration of [^{14}C]zilpaterol hydrochloride (µg/ml)	% Mean binding ±S.D. to beagle dog serum protein
0.005	22.7 ±4.5
0.05	13.2 ±0.9
0.5	14.2 ±2.0
10	13.0 ±1.1
100	12.2 ±0.7
Overall Mean% Binding	**15.1 ±4.3**

Humans

A study (non-GLP-compliant) was conducted with 9 healthy male fasted volunteers aged between 28 and 55 years weighing between 56 and 76 kg, using a single-blind protocol versus a placebo to measure the clinical tolerance of humans to zilpaterol (Sutton and Budhram, 1987; Tremblay and Mouren , 1988). Zilpaterol was administered as a solution at single doses of 0.25, 0.50, 1.0 and 2.0 mg to the healthy volunteers and blood was collected from each volunteer at 15 minutes following drug administration, then 1, 2, 3, 4, 5, 6, 8 and 24 h after dosing and zilpaterol concentrations in plasma were analysed by radioimmunoassay (LOQ = 0.1 ng/ml). Time (T_{max}) to reach the maximal concentration (C_{max}) was observed 1 hour after dosing whatever the dose, and there was a linear relationship between both the C_{max} or AUC, and the dose. The plasma concentrations were proportional to the dose administered and the $t_{½}$ was independent of the administered dose. In this study, it was observed that the 1.0 mg dose was badly tolerated by volunteers and as result, none of the volunteers was given a dose greater than 2.0 mg.

Pharmacokinetics and metabolism in food producing animals

Pigs

In a GLP-compliant study (Sauvez, 1993), two healthy 8-month-old pigs (1 male and 1 female), each weighing about 20 kg, were administered a single oral dose at 1 mg/kg bw of [^{14}C]zilpaterol hydrochloride by gavage to assess the absorption of the drug into swine. Seven millilitres of blood were taken from each animal 1, 2, 4, 7, 24 and 48 h after dosing, into heparinized tubes. The data in Table 10.10 show that the highest radioactivity concentration C_{max} of 414 ±212 µg-eq/kg was achieved at T_{max} of 1 h for both male and female pigs following the single dose administration. There was still low but detectable radioactivity concentration of 5±2 µg-eq/kg of zilpaterol 48 h after the single dose administration. The $AUC_{(0-48\ h)}$ was 3720 ±244 µg-eq.h/kg.

Table 10.10. Total radioactive concentration of [^{14}C]zilpaterol hydrochloride in plasma after administration of a single dose by gavage to pigs (Sauvez, 1993)

Sampling time (h)	Swine	Radioactivity concentration (ng-eq/g)	Mean radioactivity concentration (ng-eq/g)
1	M	500	414 ±122
	F	328	
2	M	364	319 ±64
	F	274	
4	M	278	253 ±35
	F	229	
7	M	145	170 ±35
	F	195	
24	M	15	21 ±9
	F	27	
48	M	4	5 ±2
	F	6	
T_{max}		1 h	
Mean Concentration at 1 h (ng-eq/g)		414 ±122	
Mean concentration 24 h after dosage (ng-eq/g)		5 ±2	
$AUC_{(0-48h)}$ (ng-eq.h/g)	M	3547	3720 ±244
	F	3893	

In a GLP-compliant study (Chevolleau, 2004), two 8-month-old Camborough 15 × DRX strain pigs (1 castrated male, 122 kg, and 1 female, 118 kg) were administered 0.3 mg/kg bw [^{14}C]zilpaterol hydrochloride in a single oral dose poured onto a 300 g pellet, and killed 24 h after administration. Urine and faeces were collected during the 24 h period following the labelled drug administration. At slaughter, liver, kidneys and samples of muscle (*longissimus dorsi*), perirenal (PR) and subcutaneous (SC) adipose tissue, were excised, trimmed of any extraneous tissue, weighed and homogenized for analysis. Four samples were analysed for each matrix by LSC, combustion, HPLC and radio-HPLC. The results of these analyses are shown in Table 10.11.

Table 10.11. Zilpaterol residues in excreta and tissues of Camborough strain swine administered 0.3 mg/kg bw [^{14}C]zilpaterol hydrochloride (Chevolleau, 2004)

	Concentration of zilpaterol excreted in excreta and tissues (µg/kg)		
	Male	Female	Average
Urine	12 062	13 655	12 859
Faeces	1 399	5 320	3 360
Liver	58	42	50
Kidney	73	29	51
Muscle	20	13	17
Fat (perirenal)	2	1	1
Fat (subcutaneous)	2	1	1.5

After oral administration to swine, more than 85% of the administered dose is eliminated in urine and about 3% in faeces of female pigs (Table 10.12). In liver, about 80% of the administered radioactivity was extractable, although approximately 90% is extractable from other tissues (Table 10.12).

In urine and faeces, unchanged zilpaterol accounts for about 90% of the total residue, the remainder being deisopropyl-zilpaterol and hydroxy-zilpaterol (Table 10.13). In all tissues, zilpaterol represents about 92% of the extractable radioactivity, followed by deisopropyl-zilpaterol (about 5%) and hydroxy-zilpaterol (3%).

Table 10.12. Measurement of extractable radioactivity from Camborough strain swine administered 0.3 mg/kg bw [^{14}C]zilpaterol hydrochloride (Chevolleau, 2004)

	Male		Female	
	% dose adminis-tered	Extract-able radio-activity (%)	Extract-able radio-activity (%)	% dose adminis-tered
Urine	88.5			85.8
Faeces	2.5	97.1	99.6	3.3
Liver	0.2	80.2	84.9	0.2
Kidney	0.1	91.0	95.6	0.00
Muscle	—	84.9	92.0	—

Table 10.13. Metabolic profiles (as percentage of extractable radioactivity for Camborough swine administered 0.3 mg/kg bw [^{14}C]zilpaterol hydrochloride (Chevolleau, 2004)

	% Zilpaterol extractable		% Deisopropyl-zilpaterol extractable		% Hydroxy-zilpaterol extractable	
	Male	Female	Male	Female	Male	Female
Urine	89	91	5	4	2	1
Faeces	92	94	4	4	4	2
Liver	89	90	6	7	5	4
Kidneys	94	93	6	7	-	-
Muscle	94	93	6	8	-	-
Range	92 ±3	92 ±2	5 ±1	4 ±2	4 ±2	2 ±2

Cattle

A GLP-compliant study was conducted with four Salers steers and four Charolais × Salers heifers averaging 295 kg bw and allotted to four groups of two animals, each group comprising one steer and one heifer (Tulliez, 1992). The first group was kept on feed supplemented with unlabelled zilpaterol and was used as control. Animals in the three other groups were given a single dose of 0.2 mg/kg bw of [^{14}C]zilpaterol hydrochloride by gavage of the pellet and were killed at 12 h, 48 h and 8 days, respectively. Plasma was collected from each animal during the first 10 h and then at the 14th, 21st and 24th hours, and then every day until they were killed. Urine and faeces were collected daily from the individual animals for the 8-day surviving animals. At kill point, liver, kidneys and samples of muscle (*longissimus dorsi*), perirenal and visceral fat and of the four stomachs were excised and frozen until analysis.

Table 10.14. Excretion of [^{14}C]zilpaterol in steers (Salers) and heifers (Charolais × Salers) during the eight days following a single administration of [^{14}C]zilpaterol by gavage (Tulliez, 1992)

	Radioactivity excreted as % of administered dose	
	Steer	**Heifer**
Urine	88.2	84.3
Faeces	8.7	8.6
Total	96.9	92.9

There was a rapid increase in radioactivity in plasma, which reached a maximal value 12 h and 10 h following drug administration in the male and female, respectively. The corresponding highest concentrations in plasma were 16.8 ng/ml and 22.4 ng/ml zilpaterol equivalents. Depletion of radioactivity in plasma occurred on a biphasic basis. The $T_{1/2}$ for the first phase was observed at 11.9 and 13.2 h for the male and female, respectively. The second phase corresponded to a very slow decrease of radioactivity but could not be accurately described because the signal was not significantly different from the background. Over 90% of the dose (97% in steers and 93% in heifers) was excreted over the 8 days (Table 10.14). In males, 88% of the excreted material was in the urine and 8.7% was in the faeces, while in females 84% was in the urine and 8.6% was in the faeces.

At 12 h post-dose, the radioactive concentrations were observed in the following order: liver=kidney >reticulum >omasum >abomasum >rumen >muscle >fat. Radioactivity was not detectable in any tissues except liver at 192 h post-dose (Table 10.15).

Table 10.15. [^{14}C]zilpaterol hydrochloride equivalents (µg/kg of fresh sample) in tissues and stomachs of steers and heifers at different withdrawal periods following a single administration of [^{14}C]zilpaterol hydrochloride by gavage (Tulliez, 1992)

		[^{14}C]zilpaterol hydrochloride equivalents (µg/kg) post-dosing		
		12 h	**48 h**	**192 h**
Liver	M/F	112/116	42/39	15/11
	Average	(114)	(41)	(13)
Kidney	M/F	110/118	25/23	NS/NS*
	Average	(114)	(24)	NS
Perirenal fat	M/F	2/2	1/NS	NS/NS
	Average	(2)	NS	NS
Visceral fat	M/F	7/3	4/2	NS/NS
	Average	(5)	(3)	NS
Muscle	M/F	17/15	4/3	NS/NS
	Average	(16)	(4)	NS
Rumen	M/F	61/43	20/20	NS/NS
	Average	(52)	(20)	NS
Reticulum	M/F	83/147	14/16	NS/NS
	Average	(115)	(15)	NS
Omasum	M/F	82/79	60/34	NS/NS
	Average	(81)	(47)	NS
Abomasum	M/F	50/63	12/14	NS/NS
	Average	(57)	(14)	NS

NOTES: NS = not significant. The result in brackets represents the average of the readings from the 2 animals.

Analysis of the urine showed that unchanged zilpaterol represented more than 60% of the radioactivity, with the remainder distributed among four metabolites. In tissues, unchanged zilpaterol was the main residue, and one major metabolite, which represents about 20% of the extractable residue in tissues and about 13% of the radioactive residue in urine, was identified by GC-mass spectral analysis as deisopropyl zilpaterol (Table 10.16).

Table 10.16. Metabolites identified in the urine and tissues of cattle following a single administration of [14C]zilpaterol by gavage (Tulliez, 1992)

Metabolite peak identification (1)	Name	Urine	Liver	Kidney	Muscle
		Percentage distribution (%)			
F	Zilpaterol	65.7	62.3	69.5	71.9
E_1	Deisopropyl-zilpaterol	13.2	20.6	17.9	21.2
E_2	Hydroxy-zilpaterol	ND (2)	ND	ND	ND
A	Acetylated deisopropyl-zilpaterol	7.0	ND	ND	ND
B	Unidentified	11.3	ND	ND	ND
C	Hydroxy-Zilpaterol-Glucuronide	Trace (3)	ND	ND	ND
D	Unidentified	3.5	3.3	5.7	ND

NOTES: (1) Metabolite peak identifications are based on those assigned in the rat studies (Tulliez, 2000a, b). (2) ND = not detected. (3) Trace: metabolite detected as trace in urine from steers, but not detected in urine from heifers.

In a GLP-compliant pilot steady state study, four groups of two animals each (one Charolais steer and one Charolais heifer, 200–220 kg bw) were used in 4 consecutive trials (multi-dose administration) (Tulliez, 2000c). The animals were administered daily an oral dose of [14C]zilpaterol at 0.15 mg/kg bw for 10, 12, 15 and 21 days, and killed 20–24 h after the last dose administration. Another group of 2 non-medicated animals served as controls. Radio analysis of the extractable radioactivity from liver, muscle, kidneys showed that, other than parent drug, the only other major metabolite was deisopropyl zilpaterol (10–15%). Blood samples were collected daily before the daily dosing, and at kill time, liver, kidneys and muscle (*longissimus dorsi*) and fat (perirenal) were collected. Total radioactivity in the tissues was determined by LSC, and zilpaterol-related metabolites were isolated, purified by HPLC and identified by mass spectrometry (ESI-MS, GC-MS, and thermospray mass spectrometry – TSP-MS). Radioactivity levels reached a steady state concentration of 20 µg/kg in plasma after 4–6 days of dosing.

No significant radioactivity could be detected in fat samples. Total residue concentrations in the liver, kidney and muscle of male and female cattle after 10, 12, 15 and 21 days of dosing, respectively, are shown in Table 10.17. The concentrations of the different components in the extractable radioactivity in liver, muscle and kidney are presented in Table 10.18.

Table 10.17. Total residues determined in liver, kidney and muscle of cattle killed 20–24 h after the last dose of [14C]zilpaterol (Tulliez, 2000c).

Days of treatment	Residue concentrations in tissues (µg/kg of [14C]zilpaterol hydrochloride equivalents)		
	Liver (M / F)	Kidney (M / F)	Muscle (M / F)
10	249 / 292	129 / 154	23 / 18
12	361 / 417	183 / 240	28 / 32
15	329 / 456	228 / 251	34 / 31
21	383 / 344	234 / 166	21 / 22

NOTES: M = males; F = females.

Table 10.18. Distribution of extractable [14C]zilpaterol-related metabolites in tissues of cattle killed 20–24 h after the last dose of [14C]zilpaterol (Tulliez, 2000c)

Treatment days	Concentrations of zilpaterol and deisopropyl-zilpaterol in [14C]zilpaterol hydrochloride equivalents (µg/kg)					
	Liver		Kidney		Muscle	
	Zilpaterol	Deisopropyl-zilpaterol	Zilpaterol	Deisopropyl-zilpaterol	Zilpaterol	Deisopropyl-zilpaterol
10	68	16	62	13	73	13
12	76	8	87	5	85	10
15	67	12	79	6	86	15
21	69	13	72	7	94	13

Comparative metabolism

Zilpaterol is readily absorbed and the parent compound and metabolites are readily eliminated, primarily in the urine (80% in cattle, 85% in swine and 50% in rats), with the remainder in the faeces. Unchanged parent compound is the main compound excreted in the urine of the three species and is the main residue found in cattle tissues (liver, kidney and muscle). In cattle, swine and rats, the main non-parent metabolite excreted in urine is deisopropyl-zilpaterol. This metabolite and unchanged zilpaterol are the only metabolites (at >10% of the radioactivity) found in edible tissues from cattle. Furthermore, rats of both strains (Wistar and Sprague-Dawley) produce all the metabolites that are found in cattle edible tissues, in relatively similar proportions, and these represent approximately 90% of the entire extracted radioactivity.

In conclusion, the results of the studies demonstrate that the metabolic profiles of zilpaterol in Sprague-Dawley and Wistar rats, swine and cattle (Table 10.19) are qualitatively similar.

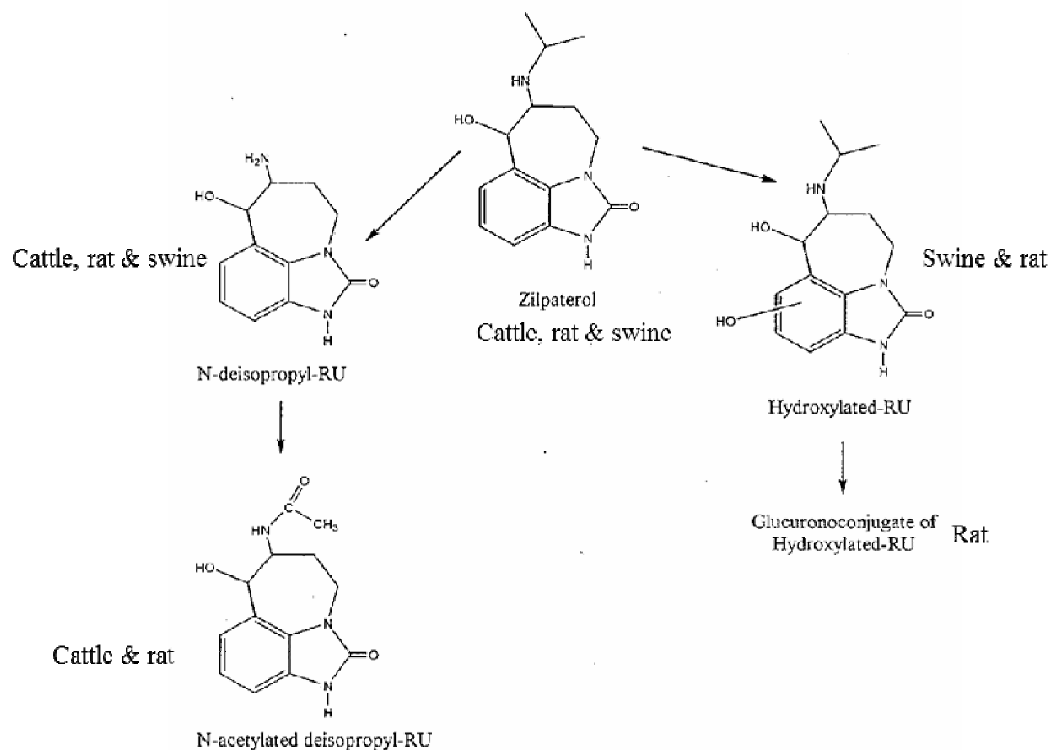
Table 10.19. Comparison of the percentage distribution of metabolites in urine of cattle and rats after radiolabel dosing with [^{14}C]zilpaterol hydrochloride

Metab olite	Identification	Males			Females		
		Wistar rat	Sprague-Dawley rat	Steer	Wistar rat	Sprague-Dawley rat	Heifer
A	Acetylated deisopropyl-zilpaterol	5.2	11.1	5.8	3.5	3.1	3.3
B	Unidentified	3.5	3.7	13.5	1.0	0.6	6.8
C	Glucoronate conjugate of hydroxy-zilpaterol	10.0	0.5	TR	8.5	7.1	ND
D	Unidentified	0.4	0.4	3.6	15.3	0.1	3.1
E_1	De-isopropyl-zilpaterol	23.7	15.2	14.7	15.3	8.4	7.9
E_2	Hydroxy-zilpaterol		5.9	TR		3.3	ND
F	Zilpaterol	51.5	50.0	62.3	69.1	73.2	78.7

NOTES: TR = Trace; ND = Not determined.

On the basis of the experimental observations, the metabolic pathway shown in Figure 10.2 has been proposed.

Figure 10.2. Proposed metabolic scheme for zilpaterol hydrochloride



Tissue residue depletion studies

Radiolabelled residue depletion studies

Cattle

A GLP-compliant study was conducted in which 17 healthy Hereford cattle (9 steers, 6 heifers) weighing between 200 and 230 kg were allocated into six groups (Tulliez, 1999). Group I (1 male and 1 female) was a non-medicated group designed to provide control samples. Each of the remaining Groups (II–VI) comprised 3 animals (2 males and 1 female, or the opposite). During the experimental period, each animal received the radiolabelled [^{14}C]zilpaterol and unlabelled zilpaterol at 0.15 mg/kg bw/day for 12 days. The Group II animals were killed 12 h after the last dose on the 12th day, Group IV 24 h, Group V 48 h and Group VI 96 h after the last dose. Group III animals were fed for 15 days and killed 12 h after the last dose. Liver samples were collected as follows: LL – left lobe; RL – right lobe; CL – caudate lobe; SL – square lobe. Adipose tissue was either PR – perirenal; or SC – subcutaneous. A validated liquid chromatographic/fluorescence method was used for the analysis of zilpaterol and zilpaterol metabolites in edible tissues and fat of cattle. The tissue samples were analysed for total radioactivity (Table 10.20) and percentage of extractable radioactivity (Table 10.21), as well as for unchanged zilpaterol and deisopropyl-zilpaterol metabolite by HPLC with radiometric detection. Extractable parent zilpaterol in tissues was measured using HPLC with fluorescence detection (Table 10.22).

A mass balance for unchanged zilpaterol and its metabolites in tissues was calculated from the recovery of the radioactivity after different extraction steps. Labelled zilpaterol and labelled metabolites were extracted from liver, kidney and muscle using an ammonia-acetonitrile-methanol mixture and then purified by solid phase extraction. Liver was again the tissue containing the highest total residue concentrations, expressed as zilpaterol HCl-equivalents, with concentrations of 291 ±56, 205 ±14, 157 ±23, and 113 ±17 µg/kg at 12, 24, 48 and 96 h, respectively, after the last dose for the animals administered 12 daily doses of the drug (Table 10.20). The next highest total residue concentrations were observed in kidney, with concentrations of 184 ±31, 100 ±5, 37 ±25 and 9 ±4 µg/kg at 12, 24, 48 and 96 h, respectively, after administration of the final dose. The total residue concentration in muscle was already very low 12 h after the last dose, at 22 µg/kg, and depleted quickly to non-detectable concentrations 96 h after the last dose.

Table 10.20. Total residues in tissues of cattle fed 0.15 mg /kg bw/day of [^{14}C]zilpaterol hydrochloride for 12 days (Tulliez, 1999)

Withdrawal time	Liver (µg/kg ±SD)	Kidney (µg/kg ±SD)	Muscle (µg/kg ±SD)	Fat (µg/kg)
12 h (1)	291 ±55.9	184 ±30.7	22 ±3.2	10.5
24 h	205 ±13.8	100 ±4.9	12 ±2.7	ND
48 h	157 ±22.5	37 ±24.9	ND	ND
96 h	113 ±17.0	9 ±3.5	ND	ND

NOTES: (1) Data from the 12- and 15-day feeding period were combined. (2) ND = not detected.

Table 10.21. Percentage extractability (mean ±SD) of [^{14}C]zilpaterol-related residues from cattle tissues (Tulliez, 1999)

Withdrawal time	Extractable zilpaterol-related residues in cattle tissues (%)		
	Liver	Muscle	Kidney
12 h	49 ±6	102 ±9	68 ±21
24 h	40 ±1	99 ±6	85 ±3
48 h	33 ±7	98	62 ±31
96 h	24 ±2		38 ±4

Residues in tissues were similar in animals administered zilpaterol when slaughtered at zero withdrawal time (12 h after the last dose) whether the drug was administered for 12 or 15 days. The residue levels reached a steady state by 12 days after dosing. Analysis of the total [^{14}C]zilpaterol-related residues showed that percentage of extractability decreased from about 50% in liver at 12 h to 24% at 96 h (Figure 10.3). In kidney, percentage of extractability also decreased with time (Figure 10.4). Essentially all of the residues in muscle were extractable at the 12 and 24 h withdrawal periods (Table 10.21).

The radioactivity extracted from tissues was analysed using radio-HPLC. Radioactivity extracted from liver and kidney is mainly associated with unchanged zilpaterol and deisopropyl-zilpaterol. Very minor metabolites are also present. No difference is observed between sexes, and the distribution between zilpaterol and deisopropyl zilpaterol does not vary significantly with the withdrawal time. In muscle, the same pattern is generally observed, although in some samples, deisopropyl-zilpaterol is not detectable. The results are shown in Table 10.22.

Parent zilpaterol together with smaller amounts of deisopropyl-zilpaterol were the predominant compounds found in the extractable residues from tissues. Parent zilpaterol was approximately 4–8 times more abundant than the deisopropyl-zilpaterol.

Table 10.22. Measurement of [^{14}C]zilpaterol and [^{14}C]deisopropyl-zilpaterol residues in cattle tissues, mean ±SD expressed as zilpaterol HCl equivalents in µg/kg (Tulliez, 1999)

Withdrawal time	Residues of [^{14}C]zilpaterol and [^{14}C]deisopropyl-zilpaterol (µg/kg)					
	Liver		Kidney		Muscle	
	zilpaterol	deisopropyl-zilpaterol	zilpaterol	deisopropyl-zilpaterol	zilpaterol	deisopropyl-zilpaterol
12 (1)	104.7 ±33.3	11.2 ±1.7	127.1 ±22.3	14.9 ±1.9	13.3 ±1.8	1.6 ±0.1
12 (2)	84.4 ±19.8	15.7±2.3	92.6 ±28.5	16.3 ±3.4	12.7 ±3.8	3.7 ±0.4
24 (1)	48.4 ±5.3	6.5 ±1.4	57.9 ±5.0	7.8 ±1.7	4.8 ±2.0	ND (3)
48 (1)	22.9 ±13.3	2.5 ±0.3	18.9 ±22.8	1.4 ±0.8	ND	ND
96 (1)	7.5 ±3.4	1.1 (0.2) (4)	0.3 (0.3) (4)	0.14	ND	ND

NOTES: (1) Group was fed medicated feed for 12 days. (2) Group was fed medicated feed for 15 days. (3) ND = Not detectable. (4) Only one value available for the 96-h samples, so no mean and SD were calculated.

Parent zilpaterol was also measured by a validated HPLC/FL method. At 12 h, it represented 28 ±7% of the total radioactivity residue (TRR) and 57 ±11% of extracted radioactive residue (ERR) in liver. The MR:TRR and MR:ERR ratios decreased with time to reach, respectively, 1.2 ±0.1 and 5.2 ±0.1% at 96 h. For kidney, a similar trend was observed (Table 10.23). Zilpaterol residues in liver show a biphasic curve of depletion for total radioactive residue related to a slow decrease of non-extractable radioactive residue. It should also be noted that there was a difference in the sensitivities of the radiometric versus the fluorescence detection method used for the quantification of zilpaterol hydrochloride.

Table 10.23. Distribution of zilpaterol-related residues in kidney, muscle and liver over the four-day (96 h) tissue withdrawal period (Tulliez, 1999)

Withdrawal time	TRR Eq µg/kg	ERR Eq µg/kg	LC-R Zilpaterol HCl (MR*) µg/kg	LC-F Zilpaterol HCl (MR) µg/kg	MR*:TRR %	MR:TRR %	MR*:ERR %	MR:ERR %
Liver								
12 h	291 ±56	143 ±29	95 ±27	81 ±22	66 ±13	28 ±7	76 ±12	57 ±11
24 h	205 ±14	82 ±4	48 ±5	40 ±1	59 ±4	20 ±1	59 ±4	49 ±1
48 h	157 ±23	52 ±19	23 ±13	15 ±12	42 ±9	9 ±6	42 ±9	25 ±12
96 h	113 ±17	27 ±3	7.5 ±3.4	1.4 ±0.2	27 ±11	1.2 ±0.1	27 ±11	5.2 ±0.1
Kidney								
12 h	184 ±31	68 ±21	110 ±30	106 ±25	60 ±12	58 ±8	100 ±48	96 ±44
24 h	100 ±5	85 ±3	58 ±5	58 ±5	58 ±4	57 ±3	68 ±4	67 ±4
48 h	37 ±25	62 ±31	19 ±23	21 ±23	40 ±25	46 ±23	70 ±34	83 ±37
96 h	9 ±4	38 ±4	0.3 ±0.3		3.2 ±1.5		8.2 ±3.1	
Muscle								
12 h	22 ±2.4	22 ±3.8	13 ±3	15 ±2	60 ±10	69 ±5	59 ±8	68 ±8
24 h	12 ±2.6	12 ±2.0	5 ±2	8 ±2	39 ±8	63 ±4	39 ±10	64 ±8
48 h	6	2	2	5	38	75	39	76
96 h	ND	ND	ND	ND	–	–	–	–

NOTES: TRR = Total radioactive residue (as Zilpaterol HCL equivalents). ERR = Extracted radioactive residue (as Zilpaterol HCL equivalents). MR* = Parent zilpaterol (Marker residue) determined by radio-HPLC. MR = Parent zilpaterol (Marker residue) measured by HPLC-fluorescence. ND = not detected.

Bio-availability of zilpaterol bound residue

A GLP-compliant study was conducted to determine the bio-availability of non-extractable residues from cattle liver (Girkin, 1999). The bio-availability of non-extractable residues remaining in the liver from cattle administered labelled zilpaterol was determined using Sprague-Dawley rats (16 male, 16 female) ranging in age from 6 to 10 weeks and weighing 200–239 g. Liver was obtained from cattle killed at 12, 24, 48 and 96 h after 12 repeated daily doses and following 12 h withdrawal after the last of 15 repeated daily doses. Pooled liver samples from each dosage×withdrawal time were extracted, lyophilized, finely powdered and pelleted. Groups of 4 rats (2 males and 2 females) were surgically altered. After a 24-hour recovery, the rats were fed lyophilized pelleted control liver. In addition, 2 groups of surgically altered rats fed with either rat diet or control rat liver were administered an intra-gastric dose of labelled zilpaterol in aqueous solution at a nominal dose of 1 mg/kg. Following extraction of cattle liver from the animals killed at 12, 24, 48, and 96 h after the last of 12 repeated daily doses of labelled zilpaterol, the proportions of unextracted radioactivity were 36.8, 53.6, 65.2 and 70.7%, respectively.

After extraction of cattle liver from animals killed 12 h after the last of the 15 repeated daily doses of labelled zilpaterol, the proportion of unextracted radioactivity was 48.3%. Following administration of labelled zilpaterol by gastric cannulae to bile-duct cannulated rats, zilpaterol was well absorbed (mean >88% of the administered dose) by rats fed with rat diet or control pelleted liver. The results show that the non-extractable residues from livers of cattle at all sacrifice points were only poorly absorbed by the rats, with a mean maximum of 3.3% of the dose being absorbed and therefore bio-available (Table 10.24).

Table 10.24. Recovery of [^{14}C]zilpaterol radioactivity. Concentration expressed as % of administered dose following intragastric administration to Sprague-Dawley rats (Girkin, 1999)

	% Radioactivity (n = 4)				
	Group III	**Group IV**	**Group V**	**Group VI**	**Group VII**
Days of administration (days)	12	15	12	12	12
Withdrawal period (hours)	12	12	24	48	96
		Absorbed			
Urine	2.4	2.2	2.0	0.8	1.1
Bile	0.0	0.2	0.0	0.0	0.1
Carcass & Tissues	3.3	2.5	2.0	0.8	1.2
		Non-absorbed			
Faeces	88.0	97.2	101.9	96.1	99.3
GIT contents	2.4	0.5	0.1	0.0	0.1
Cage washes	0.0	0.2	0.0	0.0	0.0
Total non-absorbed	90.4	97.8	101.9	96.1	99.4
Total Recovery	93.3	100.3	104.0	96.8	100.6

Figure 10.3. Depletion of zilpaterol residues in cattle liver.

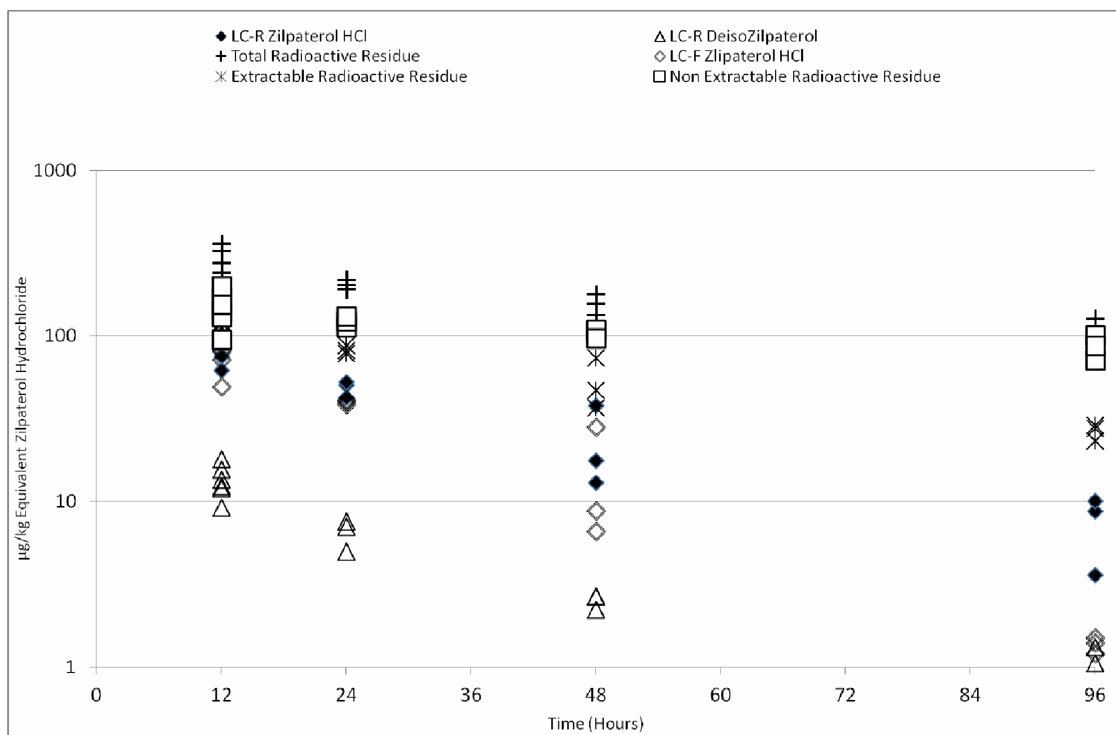
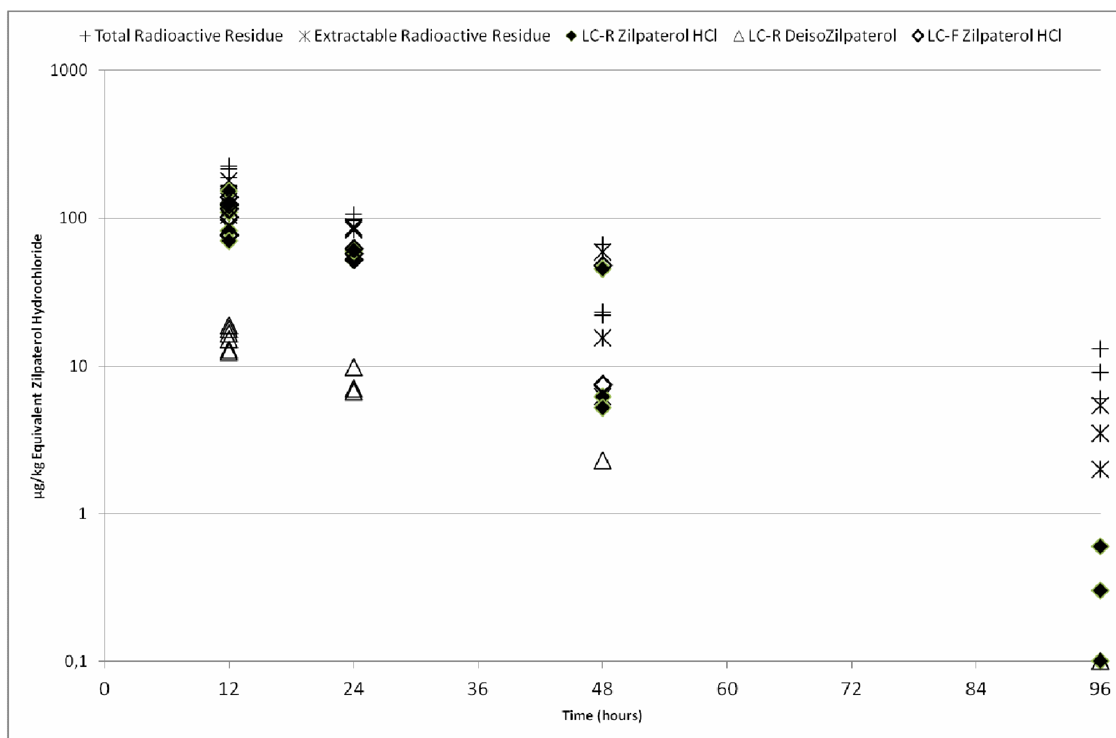


Figure 10.4. Depletion of zilpaterol residues in cattle kidney



Residue depletion studies with unlabelled drug

Cattle

In the first of three pivotal GLP-compliant tissue residue depletion studies conducted to measure the concentration of zilpaterol in the liver, muscle and kidney tissues of cattle (Hughes, McDonald and Bomkamp, 1999), 18 crossbred beef cattle (9 steers weighing 455 to 595 kg and 9 heifers weighing 480 kg to 573 kg at the initiation of treatment) were randomly assigned to four groups (2 of each sex per group). The cattle were treated for 12 consecutive days with the commercial pre-mix medicated feed at the recommended dosage of 0.15 mg/kg bw per day or 7.5 mg/kg in feed. After receiving the final dose via medicated feed, one group of animals was killed at each of 12, 24, 48 or 96 h post-dose. Two animals were non-medicated control animals. These animals were considered representative of standard feedlot cattle.

Samples of liver, muscle and kidney from the four-day withdrawal study were assayed by the validated HPLC/FL method (Table 10.25). Recoveries of marker residue were 91.8 ±3.72%, 86.1 ±13.9% and 98.4 ±4.57%, respectively, for the liver, muscle and kidney. The LOQs for the method were 3 µg/kg, 1 µg/kg and 1 µg/kg, respectively, for liver, muscle and kidney, while LODs were 1 µg/kg, 0.1 µg/kg and 0.5 µg/kg, respectively, for the liver, muscle and kidney. The mean concentrations of zilpaterol in liver depleted from 28.3 µg/kg 12 h after the last 12th-day dose to 11.4 µg/kg 24 h after the last dose and to 4.5 µg/kg 48 h after the last dose. At 12, 24 and 48 h after the last dose, the concentrations of residues in kidney were 51, 13 and 6 µg/kg, respectively. It was noted that in this particular study the residue concentrations in kidney were slightly higher than the residue concentrations in liver.

Table 10.25. Mean zilpaterol concentrations in cattle liver, muscle and kidney tissues in the four-day (96-h) withdrawal period pivotal study (Hughes, McDonald and Bomkamp, 1999)

Withdrawal Period	Mean zilpaterol hydrochloride equivalents (µg/kg) (n = 4)		
	Liver	Muscle	Kidney
Group II (12 h)	28.3 ±9.1	5.0 ±1.9	50.8 ±33.1
Group III (24 h)	11.4 ±2.8	2.1 ±0.5	1.3 ±1.54
Group IV (48 h)	4.5 ±4.0	<LOQ [1]	5.7 ±5.2
Group V (96)	<LOD [2]	<LOD [3]	<LOD [4]
LOD (µg/kg)	1	0.1	0.5
LOQ (µg/kg)	3	1	1

NOTES: (1) LOQ = 1 µg/kg; (2) LOD = 1 µg/kg; (3) LOD = 0.1 µg/kg; (4) LOD = 0.5 µg/kg.

In the remaining two pivotal GLP-compliant studies, a total of 25 steers and 25 heifers, including 48 treated and 2 controls, forming 9 groups, were used in each of the studies (Crouch, 2011a, b). The group assignments, treatment and withdrawal periods are shown in Table 10.26.

Table 10.26. Experimental design used in the two 10-days withdrawal period pivotal studies for zilpaterol hydrochloride (Zilmax) residue depletion study in cattle (Crouch, 2011a, b)

Group	Withdrawal time (days)	Zilmax dose (mg/head/day)	Dosing period (consecutive days)	Steers	Heifers
I	0.5	90	20	3	3
II	1	90	20	3	3
III	2	90	20	3	3
IV	3	90	20	3	3
V	4	90	20	3	3
VI	6	90	20	3	3
VII	8	90	20	3	3
VIII	10	90	20	3	3
Control	NA	NA	NA	1	1

NOTES: NA = not applicable

For the purpose of these two studies, cattle were administered Zilmax® either via component feeding (Crouch, 2011a) or via a pelleted type C top dress supplement (Crouch, 2011b) at the recommended dosage regimen of 90 mg zilpaterol hydrochloride per head, and for 20 consecutive days. The males were castrated and no female was pregnant. The bodyweights ranged from 433 kg to 574 kg for heifers, and from 480 kg to 584 kg for steers. Samples (muscle and liver) were assayed by the validated HPLC/FL method. The LOD for the method was 0.90 µg/kg with an LOQ of 2.0 µg/kg for liver, while the LOD and LOQ were 0.53 µg/kg and 2.0 µg/kg, respectively, for muscle tissue. The concentrations of residues in liver were significantly lower than the residue levels observed in the earlier pivotal study (Hughes, McDonald and Bomkamp, 1999). Residues in muscle tissue were too low to permit a depletion curve plot (Table 10.27).

Table 10.27. Mean zilpaterol free base residue concentrations in liver and muscle at withdrawal times of 12, 24, 48, 72, 96, 144, 192 and 240 h in cattle

Slaughter time	Top dress supplement (Crouch, 2011b)		Component feeding (Crouch, http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/Dietary_Exposure_AssessmentMethodologies_for_Residues_of_Veterinary_Drugs.pdfa)	
	Liver (µg/kg)	**Muscle (µg/kg)**	**Liver (µg/kg)**	**Muscle (µg/kg)**
12 h	12.9 ±5.3	3.0 ±0.7 (1)	13.9 ±7.3	3.8 ±0.5 (2)
24 h	All values but one (3.6) <LOQ (4)	All values <LOQ	5.7 ±2.4	All values <LOQ
48 h	All values <LOQ	All values <LOQ	3.8 ±1.0 (3)	Al values <LOQ
72 h	All values but one (2.9) <LOQ	All values <LOD	2.3 ±0.4 (3)	All values <LOD
96 h	All values <LOD (5)	All values <LOD	All values <LOQ	All values <LOD
144 h	All values <LOD	All values <LOD	All values but one (2.01) <LOQ	All values <LOD
192 h	All values <LOQ	All values <LOD	All values <LOQ	All values <LOD
240 h	All values <LOD	All values <LOD	All values <LOD	All values <LOD

NOTES: (1) 4 out of 6 values >LOQ. (2) 2 out of 6 values >LOQ. (3) 3 out of 6 values >LOQ. (4) LOQ = 2 µg/kg. (5) LOD = 0.527 µg/kg.

Methods of analysis for residues in tissues

Residues of unlabelled zilpaterol in the three pivotal residue depletion studies (Hughes, McDonald and Bomkamp, 1999; Crouch, 2011a, b) were measured using a validated HPLC method with fluorescence detection, HPLC/FL (Nandihalli, Hughes and Bomkamp, 1999). The HPLC/FL method involves the extraction of unchanged zilpaterol from liver, kidney and muscle homogenate with a basic mixture of acetonitrile and methanol. The extract is filtered and the filtrate partitioned with iso-octane to remove non-polar co-extractives. Following removal of the organic fraction, ammonium acetate is added to the concentrated aqueous extract and the mixture is centrifuged. The aqueous supernatant is cleaned up on a conditioned SPE cartridge from which the retained zilpaterol is eluted with ammonium acetate. The eluate is further cleaned up on a C8 RP Select B cartridge. Zilpaterol is eluted from the cartridge with a mixed solution of ammonium acetate/methanol/water. After evaporating to dryness, the residue is taken up into an ammonium acetate solution containing sodium azide. The chemically stable fluorescent zilpaterol derivative is separated from other co-extractives and non-fluorescent compounds by HPLC using a Puropher RP-18 column and detected with fluorescence detection at 640 nm (excitation at 285 nm).

In two of these pivotal studies, significant matrix interference effects were observed from the analysis of liver tissue, necessitating modification to the validated method in order to minimize the matrix effect (Crouch, 2011a, b). The LOQ was set at 2 µg/kg for both muscle

and liver, although the LODs were 0.53 µg/kg and 0.90 µg/kg in muscle and liver, respectively. The average recoveries from fortified Quality Control samples were 71.4% and 71.8% at levels of 6 µg/kg and 24 µg/kg, respectively, in incurred liver, and 76.2% and 72.9% at 5 µg/kg and 20 µg/kg , respectively, in incurred muscle (Crouch, 2011b). They were 69.2% and 68.5% at levels of 6 µg/kg and 24 µg/kg, respectively, in liver, and 78.5% and 72.7% at 5 µg/kg and 20 µg/kg, respectively, in muscle (Crouch, 2011a).

All the concentrations were reported as zilpaterol hydrochloride equivalents by the sponsor. In view of the fact that it is zilpaterol free base and not zilpaterol hydrochloride that is being analysed with these methods, the operational parameters of the method have been re-calculated to reflect this, as shown in Table 10.28.

Table 10.28. Validation parameters of the routine HPLC/FL method for the quantification of zilpaterol in cattle tissues

Validation criterion	Liver	Kidney	Muscle
Precision (%) RSD	8.76	6.95	6.96
Accuracy (% Recovery) (mean ±SD)	93.5 ±8.2	92.1 ±6.4	84.3 ±5.9
LOQ (µg/kg) – Zilpaterol hydrochloride – Zilpaterol free base	3.0 2.6	1 0.9	1 0.9
LOD (µg/kg) – Zilpaterol hydrochloride – Zilpaterol free base	1.0 0.9	0.5 0.4	0.1 0.09
Linearity 0.5 to 200 µg/kg as Zilpaterol HCl tissue equivalents	R^2 >0.98	R^2 >0.98	R^2 >0.98
Selectivity [(1)]	No interference	No interference	No interference
Residue Stability 3 Freeze/thaw cycles [(2)] Low, High (% Recovery)	88.4 ±1.2 to 95.5 ±4.6	79.9 ±5.5 to 96.2 ±14.7	84.8 ±10.2 to 99.2 ±2.1
Stability of extract 24 hour or 1 month [(3)] (% Recovery)	72.7 ±0.4 to 84.3 ±10.1	85.5 ±7.1 to 110.8 ±4.3	81.2 ±3.9 to 85.9 ±3.7

NOTES: (1) No interference with other feed additive products or therapeutic β-agonist compounds. (2) Low = samples fortified with 3 µg/kg, and high = samples fortified with 12 µg/kg. (3) Range of values for samples stored for 24 hours in HPLC autosampler or stored in the freezer for one month. Samples fortified at 3 and 12 µg/kg. For the purpose of this table, the molecular weights of 247.113 g for zilpaterol free base and 283.56 g for zilpaterol hydrochloride were retained.

LC-MS/MS Method

In another GLP-compliant study, an LC-MS/MS method developed by the Sponsor was validated for the determination and confirmation of zilpaterol residues in bovine liver and muscle tissue (Wrzesinski, 2012). Briefly, after homogenization with dry ice, about 1 g of tissue is fortified with the internal standard, and 0.5, 1.0 and 2.0 times the concentrations of 10 and 12 µg/kg in muscle and liver, respectively, and extracted with 5 ml methanol. Following centrifugation, 500 µl of supernatant are applied to an Oasis® MCX 96-well plate (10 mM) in metanol. After sequential washing, plates are eluted with 2 × 200 µl 0.1% ammonium acetate in methanol and evaporated to dryness. Reconstitution is performed with 150 µl 90:10 ammonium acetate 10 mM containing 0.1% formic acid:methanol. The final extract is analysed by LC-MS/MS. The extraction recovery was reported as 78% and 82% for bovine liver and muscle tissues, respectively. The LOD and LOQ are similar to those achieved with the HPLC/FL method. A summary of the performance characteristics of the LC-MS/MS method developed by the Sponsor is provided in Table 10.29.

Unlike other β-agonists such as clenbuterol and ractopamine, there are very few analytical methods published for zilpaterol. The first methods published used gas chromatography-mass spectrometry (GC-MS) to measure residues of zilpaterol in feeds (Bocca *et al.*, 2003a) and tissues (Bocca *et al.*, 2003b). Sensitive liquid chromatography-mass spectrometric (LC-MS) methods were later reported for zilpaterol in urine, faeces and tissues (Stachel, Radeck and Gowik, 2003; Blanca *et al.*, 2005; Van Hoof *et al.*, 2005). In one of these published validated methods (Stachel, Radeck and Gowik, 2003), zilpaterol residues in urine, retina and

plasma and muscle tissue are extracted after hydrolysis with protease, and zilpaterol residues in liver and kidney tissues are extracted after hydrolysis with glucoronidase/arylsulfatase mix, cleaned up using solid-phase extraction and analysed by LC-MS/MS. The method was used to quantify incurred zilpaterol residues in muscle, kidney, retina and liver samples collected from 2 cattle administered zilpaterol HCl at 0.15 mg/kg bw once daily for 14 days, and killed 1 and 10 days post-dosing (i.e. 1 animal on each sampling date). The concentrations of zilpaterol measured in muscle, kidney and liver samples collected were 0.01 µg/kg, 0.03 µg/kg and 0.03 µg/kg at 10 days, respectively. This LC-MS/MS method demonstrated detection limits 10–100 times better than those of the HPLC/FL and the LC-MS/MS methods used in the pivotal residue depletion studies provided to the Committee (Hughes, McDonald and Bomkamp, 1999; Crouch, 2011a, b).

More recently, rapid analytical methods have been developed using polyclonal and monoclonal antibody-based immunoassay and immune-biosensor analyses that are applicable for the analysis of residues of zilpaterol in swine, cattle and sheep (Shelver and Smith, 2011).

Table 10.29. Validation parameters of the LC-MS/MS method for zilpaterol free base quantification in cattle liver and muscle tissue (Wrzesinski, 2012)

	Liver samples		**Muscle samples**	
	Fortified (1)	**Incurred (2)**	**Fortified (1)**	**Incurred (2)**
Intra-day accuracy (% bias)	-4 – +3		-4 – +3%	
Intra-day precision (% CV)	2 – 6%	2 -6%	1 – 5%	1 – 5%
Inter-day accuracy	-2 – +2%		-1 – +1%	
Inter-day precision	3 – 4%	4%	3 – 4%	3 – 4%
LOQ / LOD µg/kg	3.0 / 0.06		2.5 / 0.02	
Curve range	2 – 30 ng/g equivalents		2 – 24 ng/g equivalents	
Linearity (r^2)	0.9987 – 0.9989		0.9978 – 0.9985	
Specificity/selectivity (3)	No interference observed		No interference observed	
Matrix effect (4)	-17 – +37%		6 – 58%	
Ruggedness testing (5)	Acceptable		Acceptable	
Extraction recovery	76 – 79%		81 – 83%	
Stability: – Freeze-thaw (6)	4 cycles		4 cycles	
– Room temperature (6)	24 hours		24 hours	
– Extract (7)	246.5 hours		246.5 hours	
– Stock solution (8)	81 days			
Confirmatory analysis:				
– Incurred samples (9)	<10%		<10% with 2 exceptions (11)	
– Fortified samples (9)	<10% with 2 exceptions (10)		<10% with 2 exceptions (11)	

NOTES: (1) Fortified levels were ½ ×, 1× and 2× concentrations of 10 µg/kg in muscle and 12 µg/kg liver.
(2) Incurred samples in the range of 0.5–2× concentrations of 10 µg/kg in muscle and 12 µg/kg liver.
(3) Determined using control muscle and liver from 6 sources from different regions in the country. Additionally, the absence of interference from 20 other drugs registered for use in cattle was demonstrated.
(4) Comparison of chromatographic analyte peak area of matrix fortified post-extraction (1/2× and 2× tolerance limits) and solvent standard at same concentration in six matrix lots from different regions. Lowest to highest values are presented.
(5) Mobile phase composition, alternate analytical column, SPE plate, and alternate LC-MS/MS platform.
(6) Conducted using incurred samples.
(7) Conducted at room temperature and normal light conditions with incurred and fortified extracts.
(8) Refrigerated stability.
(9) Relative abundance ratios of confirmatory transitions were required to be within 10% of the comparison standard.
(10) Two fortified QC1 samples ratios are outside 10% of standards.
(11) Two incurred and four fortified samples ratios are outside 10% of standards.

Stability of residues in frozen tissues

The stability of incurred zilpaterol residues was evaluated in liver, kidney and muscle during storage at temperature of ≤10°C for up to 12 months (Hughes and Halverson, 2000). Tissues from control animals fortified with 6 or 12 µg/kg zilpaterol hydrochloride were selected for storage and re-analysis at 0, 1, 3, 6 and 12 months of storage. Tissues from three treated animals that had two different levels of incurred zilpaterol residues at the first analysis were also selected and stored for periodic re-analysis. The zilpaterol HCl content of the tissues was measured by the HPLC/FL method, and the recovery was at least 73% (Nandihalli, Hughes and Bomkamp, 1999; method also described in Hughes, McDonald and Bomkamp (1999). The results show that the stability of residues of zilpaterol in liver, kidney and muscle tissues was not adversely influenced by storage for up to 12 months at or below -10°C. Table 10.30 shows the results of tissues from treated-animals with two different levels of incurred residues.

Table 10.30. Storage stability of zilpaterol in frozen tissue samples from treated cattle that were stored at or below -10°C for up to 12 months (Hughes and Halverson, 2000)

Tissue Matrix	Animal No.	Zilpaterol HCl equivalents found (µg/kg)				
		0 Month	1 Month	3 Months	6 Months	12 Months
Liver	2911	6.9	7.2	8.0	6.8	6.5
	2910	27.1	26.4	26.1	26.5	30.6
Muscle	2910	4.2	4.1	4.2	3.5	4.4
	2913	8.0	7.6	8.2	7.5	8.6
Kidney	2911	8.3	8.0	7.3	7.4	7.3
	2910	49.2	47.3	45.8	47.8	52.9

The methods available for the analysis of zilpaterol in biological matrices are summarized in Table 10.31.

Table 10.31. Summary of analytical methods for zilpaterol residues

Method	Species and tissues	LOD / LOQ (µg/kg)	Reported validation	Reference
LC-MS/MS	cattle urine and pig liver	CCα (urine) = 0.11 [(1)] CCα (liver) = 0.13 CCβ (urine) = 0.26 +[(2)] CCβ (liver) = 0.52	Validated according to the Commission Decision 2002/657/EC	Blanca *et al.*, 2005
GC-MS/MS	retinal extracts (cattle)	CCα = 65.7 CCβ = 73.9	Validated according to SANCO/1805/2000	Bocca, *et al.*, 2003b
GC-MS	commercial feeds for cattle	LOD = 7.5 LOQ = 25.0	Internal validation	Bocca *et al.*, 2003a
HPLC/FL	cattle liver and muscle	LOD(liver) = 0.53 LOQ (liver) = 2 LOD (muscle) = 0.90 LOQ (muscle) = 2	Internal validation	Crouch, 2011a, b
HPLC/FL	cattle liver, muscle, kidney	LOD (liver) = 1.0 LOD (muscle) = 0.1 LOD (kidney) = 0.5 LOQ (liver) = 3.0 LOQ (muscle) = 1.0 LOQ(kidney) = 1.0	Validated method	Hughes, McDonald and Bomkamp, 1999

Method	Species and tissues	LOD / LOQ (µg/kg)	Reported validation	Reference
HPLC/FL	cattle liver, kidney and muscle	LOD (liver) = 1 LOD (kidney) = 0.5 LOD (muscle) = 0.1 LOQ (liver) = 3 LOQ (kidney) = 1 LOQ (muscle) = 1	Internal validation	Nandihalli, Hughes and Bomkamp, 1999
ELISA	horse urine	LOD/LOQ ≤0.8	External validation (3)	Shelver and Smith, 2011
LC-MS/MS	pig liver, kidney, muscle cattle liver, kidney, muscle	LOD/LOQ (liver) ≤0.15 LOD/LOQ (kidney) ≤0.28 LOD/LOQ (muscle) ≤0.020 LOD/LOQ (liver) ≤ 003 LOD/LOQ (kidney) ≤0.03 LOD/LOQ (muscle) ≤0.01	Internal validation	Stachel, Radeck and Gowik, 2003
HPLC/FL	cattle liver, kidney, muscle	LOD (liver) = 1 LOD (kidney) = 1 LOD (muscle) = 0.1 LOQ (liver) = 3 LOQ (kidney) = 5 LOQ (muscle) = 1	Internal validation	Tulliez, 1999
LC-MS LC-MS/MS	calf urine, faeces	CCβ ≤1 (urine) CCβ ≤1 (faeces)	Internal validation	Van Hoof *et al.*, 2005
LC-MS/MS	cattle liver, muscle	LOD (liver) = 0.06 LOD (muscle) = 0.02 LOQ (liver) = 3.0 LOQ (muscle) = 2.5	Internal validation	Wrzesinski, 2012

NOTES: (1) CCα = Decision limit. (2) CCβ = Detection capability; (3) External validation was conducted using chemical standards.

Appraisal

Zilpaterol hydrochloride, (±)-trans-4,5,6,7-tetrahydro-7-hydroxy-6-(isopropylamino)imidazo [4,5,1-jk]-[1]benzazepin-2(1H)-one hydrochloride, is a β2-adrenergic repartition agent used in cattle for increased rate of bodyweight gain, improved feed efficiency, and increased carcass leanness in cattle fed in confinement for slaughter for a period of 20–40 consecutive days before withdrawal from the feed. Zilpaterol hydrochloride has not been previously reviewed by the Committee.

Zilpaterol hydrochloride should be mixed into the feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level in the feed is designed to treat the animals with approximately 0.15 mg zilpaterol hydrochloride/kg bw or 60–90 mg zilpaterol hydrochloride per animal per day. The product Zilmax® is not permitted for use in lactating dairy cattle. Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.

Studies conducted in rats, swine and cattle demonstrated that the metabolism was qualitatively and quantitatively comparable in these three species following oral administration of zilpaterol hydrochloride, with two major metabolites, deisopropyl-zilpaterol and hydroxy-zilpaterol, together with the parent zilpaterol free base being observed. The drug is readily absorbed and the parent compound and metabolites are readily eliminated, primarily in the urine (80% in cattle, 85% in swine and 50% in rats) with the remainder in the faeces. Unchanged parent compound is the main compound excreted in the urine of these three species. Zilpaterol residue concentrations were approximately 4 to 8 times higher than those of the only significant metabolite, deisopropyl-zilpaterol, in tissues and urine. In rat faeces, the major metabolite is the hydroxyl-zilpaterol. A metabolism study conducted in cattle with [^{14}C]zilpaterol shows that radioactive residues are detectable in liver 8 days following a single oral dose of 0.2 mg/kg bw.

Radiolabelled residue depletion studies conducted in cattle after treatment at the

recommended dose of 0.15 mg/kg bw/day demonstrated that a steady state is achieved in 12 days of treatment. However, the data available from these studies was from a limited number of animals and only provided a depletion curve of total residue for a period limited to 96 h post-dose. No residues were detected in fat after 12 hours and no residues were detected in muscle after 24 hours. Residues were detected in liver and kidney at 96 h post-dose. Extractable residues account for 24–58% of total residues in liver, whereas residues in muscle are approximately 100% extractable. Extractable residues in kidney vary between 28 and 90% between animals. The extractable fraction of the total residue in liver and kidney decreased with time, so that the ratios between zilpaterol and extractable residue in liver and kidney decrease with time, as do the ratios between zilpaterol and total residue in liver and kidney.

A bio-availability study conducted with cannulated rats using liver containing incurred residues of [^{14}C]zilpaterol, obtained from treated animals, demonstrated that less than 3% of the bound residues in liver were absorbed and considered to be biologically available. The bio-availability of non-extractable residues in kidney and muscle was not determined.

The residue data provided are insufficient to identify a marker residue correlated with the total residue depletion curve observed in liver and kidney after a period of 4 days. The LOQs of the analytical methods used in the depletion studies submitted by the Sponsor were insufficient to provide the data required to calculate an estimated daily intake and the percentile concentrations associated with the depletion curves used for the recommendation of MRLs. MRLs based on the method LOQ of 2 µg/kg, if used to recommend MRLs for muscle and fat, would result in an EDI or TMDI that exceeds the upper bound of the ADI. The validated methods provided by the Sponsor were considered to have an inadequate LOQ for effective residue control of zilpaterol.

Maximum Residue Limits

In recommending MRLs for zilpaterol, the Committee considered the following factors:

- An ADI of 0–0.04 µg/kg bw for zilpaterol was established by the Committee, corresponding to an upper bound of acceptable intake of 2.4 µg/day for a 60 kg person.
- Zilpaterol HCl is registered to be mixed into feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level is designed to treat animals with approximately 0.15 mg/kg bw or 60–90 mg zilpaterol HCl per animal per day.
- Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.
- Zilpaterol HCl is not permitted for use in lactating dairy cattle.
- Zilpaterol has two major metabolites: deisopropyl zilpaterol (tissues and urine) and hydroxy-zilpaterol (faeces).
- The Committee agreed that parent zilpaterol was an appropriate marker residue in muscle. Only limited data were available for tissues other than muscle, and the Committee was unable to determine a suitable marker residue in other edible tissues. Liver and kidney contained the highest concentration of zilpaterol at all sampling times, followed by muscle. The data provided are not sufficient to determine the total residue half-life in the liver after 96 hours. There are no measurable residues in adipose fat.
- The ratios of the concentration of zilpaterol to the concentration of the total residues for liver and for kidney over the 96-hour withdrawal period after the last drug administration could not be determined with any confidence due to the very limited data available and lack of sensitivity of the methods used.
- The ratio of zilpaterol to total radioactive residues in muscle is approximately 50%.

- The analytical methods used in the depletion studies do not allow the characterization of the pharmacokinetics at times when, even at the LOQ, the concentrations are not compatible with dietary exposures below the ADI, particularly in liver.

A marker residue could not be established in any edible tissue other than muscle, and the Committee concluded that an appropriate marker residue for other tissues should be identified. In the absence of an appropriate marker residue for liver and kidney, a marker residue to total residue ratio could not be established for these tissues.

The Committee used the highest concentrations of total residues to estimate dietary exposure, because no median residue levels could be determined and no marker residue in liver and kidney was defined. These highest concentrations of extractable radioactivity, expressed as zilpaterol equivalent, were 1.0, 28.6 and 5.4 µg/kg at 96 hours for muscle, liver and kidney, respectively. These calculations indicated that the dietary exposure was higher than the ADI for the withdrawal times for which data were provided. It was also noted that the ADI is based on an acute end-point and is applicable to both acute and chronic exposure.

The Committee concluded that it was not possible to recommend MRLs for zilpaterol. The following data are needed to establish MRLs:

- results from studies investigating marker residue in liver and kidney;
- results from studies determining marker residue to total residue ratio in liver and kidney; and
- results from depletion studies to enable the derivation of MRLs compatible with the ADI.

All such studies should use sufficiently sensitive, validated analytical methods capable of measuring zilpaterol and its major metabolites in edible tissues of cattle.

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