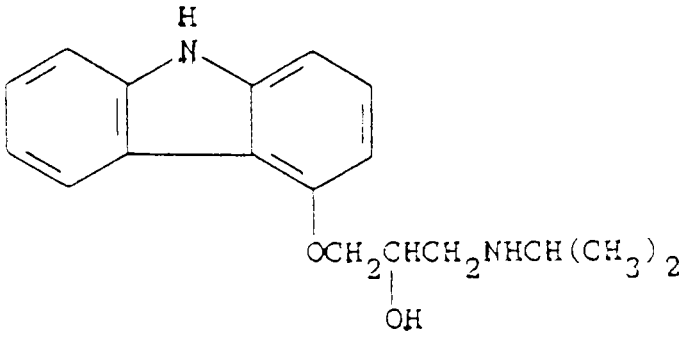
CARAZOLOL

IDENTITY

Chemical: 4-(2-hydroxy-3-isopropyl-amino-propoxy)-carbazole,
1-(carbazol-4-yl-oxy)-2-hydroxy-3-isopropylamino-propane,
1-(4-carbazolyloxy)-3-(isopropylamino)-2-propanol

Synonyms: Carazolol

Structural formula:



Molecular formula: $C_{18}H_{22}N_2O_2$

Molecular weight: 298.34

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Carazolol (98.0 to 102% by titrimetric assay with respect to dry substance)

Appearance: Pale yellow crystalline powder.

Melting point: 135^{o} - 138^{o}

Solubility:
Water.....practically insoluble
Chloroform.....slightly soluble
Ethanol (96%).....soluble
Acetone.....soluble
Methanol.....very soluble

UV maxima: 286 ± 1 nm

Storage: Should be stored in tightly closed containers free from light.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Carazolol is a ß-blocking agent used as a tranquiliser to prevent stress in animals and humans. It is most widely used in pigs to ameliorate stress when they are transported thus preventing losses due to death and deterioration of the meat quality. It is also used in female pigs to relieve the stress of parturition and in male pigs to placate and prevent frenzy during mating. A less widespread use is in the prevention of reproductive stresses in dairy cows. The drug may alleviate stress during calving and may also have a positive effect on conception rate.

The dose for pigs is 10 μg per kg BW administered as an intramuscular (IM) injection behind the ear. The dose used in the cattle in the research studies cited was either 5 mg intravenously (IV) or 10 mg IM to cows weighing approximately 500 kg.

The full effects of the drug are obtained about 20-30 minutes after intramuscular injection and within 5 minutes after intravenous administration. The effects are maintained for 8-12 hours in the pig and for about 4 hours in cattle.

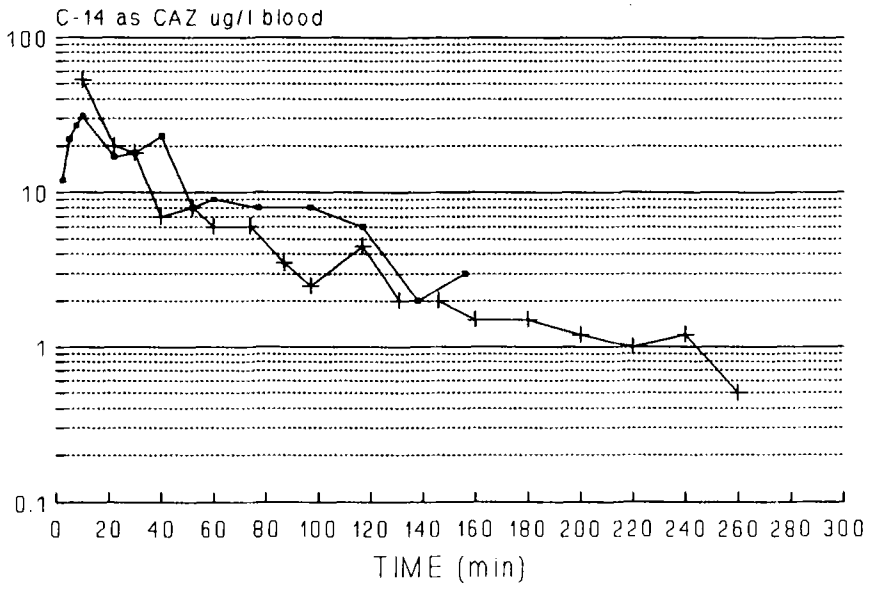
METABOLISM

Pharmacokinetics

Pigs

Two castrate male pigs were cannulated in the left jugular vein. 24 hours later they were each injected intramusculary behind the ear (left or right ear not specified) with 10 μg per kg BW ^{14}C-Carazolol (SA 24.37 μCi per mg). Blood samples were collected at intervals for 30 hours and the total radioactivity in the blood was determined as concentration equivalents of Carazolol. The sensitivity of the assay was 0.05 μg per kg. The activity of samples collected after 5 hours was below the sensitivity of the method (Rudloff, 1982). The results are shown in figure 1. In both animals Carazolol is absorbed rapidly with peak concentrations in the blood seen at 10 minutes. Thereafter the concentration falls rapidly and is not detectable 5 hours after administration.

Figure 1. Carazolol Kinetics in the Blood of Pigs.



Three further castrate male pigs weighing 57, 58 and 63 kg, were administered a dose of 10 μg per kg BW ^{14}C-Carazolol and urine and faeces collected for either 4 hours (1 pig) or 6 hours (2 pigs). Tissues were collected at slaughter and the radioactivity in the tissues was measured.

The total activity excreted in the urine and faeces and expressed as a percentage of original dose was 47.5% at 4 hours after administration and 56.3% and 58.0% in the two pigs killed 6 hours after administration. About 25% original dose was excreted in the urine within 4.5 hours of administration (Rudloff, 1982).

The concentrations (μg/kg) in the urine and bile collected at the time of slaughter are:

	Withdrawal time (hours)		
	4	**6**	**6**
Urine	185	159	201
Bile	143	242	374

The concentrations in the tissues are given later in table VI. After absorption, cumulation of the substance takes place particulary in the liver, lungs, kidney and spleen. Relatively lower concentrations are found in the fat and muscle. Nevertheless all the values are higher than in blood. A further interesting observation was that activity was present in the stomach contents and also in faeces when the faeces were sampled just 10 minutes after administration. This indicates that Carazolol appears to be able to cross the epithelia at high blood concentrations.

Cattle

A series of experiments were conducted in Germany (Steinhart, 1987) to investigate the pharmokinetics in the blood and milk of dairy cattle following either IV or IM administration of Carazolol using the commercially available preparation.

In experiment 1, 5 mg Carazolol were administered IV to three cows and IM to two cows. The injections were made at 12 noon and samples of serum and milk were collected for 7 hours and 20 hours respectively. The concentrations of Carazolol were determined by the HPLC method (Rudolph and Steinhart, 1987, Rudolph, 1988) and the results are shown in table I. The lower limit of detection of the method is 0.3 µg/L. No measurable amount of residues were found in serum at 3 hours WT or in milk at 20 hours WT. There was considerable variation in the profiles of the cows and may be the result of problems incurred during the injection of the drug. If one assumes that the results for cows numbered 1 and 5 are erroneous because of injection problems then the half lives of Carazolol in serum are in the range 3.7 to 8.3 minutes (see table I).

Table I. Kinetics of Carazolol in serum and milk of dairy cattle.

WT (h:min)	cow 1 530 kg (IV)	cow 2 520 kg (IV)	cow 3 540 kg (IV)	cow 4 470 kg (IM)	cow 5 430 kg (IM)
Serum:					
:05	20.1	8.3	13.4	2.5	ND
:10	7.5	5.4	5.6	2.7	0.8
:15	49.4	2.8	1.8	2.0	1.5
:30	12.4	0.9	0.3	0.7	1.0
1:00	1.6	0.7	ND	ND	0.4
2:15	0.7	ND	ND	ND	ND
3:00	ND		ND		
4:00	ND				
7:00	ND				
Milk:					
4:00	1.3	3.3	3.2	1.8	2.8
18:00	ND	0.4	1.4	ND	ND
20:00	ND	ND	ND	ND	ND
Half life in serum (min):					
	?	3.8	7.1	8.3	?

In another experiment reported by Steinhart, (1987), (No. 5) and Rudolph (1988) to investigate blood kinetics of Carazolol, three cows were injected IV with 5 mg Carazolol and serum sampled were prepared at various intervals after administration of the drug. The concentration of Carazolol was determined by the HPLC method of Rudolph and

Steinhart (1987). 5 days later the same cows were given 10 mg Carazolol as an IM injection and the concentration of Carazolol again determined in serum samples. The results are shown in table II. As in experiment 1 one of the animals, No. 8, produced strange results which again might be caused by incorrect injection into a vein.

Table II. Kinetics of Carazolol in Serum of Dairy Cows. (Results expressed as µg Carazolol per L)

WT (min)	cow 13 561 kg (IV)	cow 6 505 kg (IV)	cow 8 570 kg (IV)	cow 13 562 kg (IM)	cow 6 504 kg (IM)	cow 8 572 kg (IM)
5	10.4	14.2	0.9	4.1	ND	14.8
10	5.3	8.6	4.5	5.1	6.5	20.3
20	4.8	6.7	7.2	5.6	5.8	21.4
30	2.8	2.9	26.1	7.2	7.6	18.2
40	2.3	4.9	15.0	8.2	10.4	17.2
60	3.2	3.8	19.8	5.3	7.7	6.9
Half life (min)						
	17.6	18.5	?	111.7		22.4

ND is not detectable (<0.05 µg/L)

Three further experiments (numbers 2, 3 and 4) were carried out to investigate the excretion of Carazolol into milk of dairy cows administered IV or IM doses ranging between 2.5 mg and 10 mg.

One cow weighing 530 kg was administered increasing doses of Carazolol IV on three separate occasions (see table III). The concentration of Carazolol in milk collected 2.5 hours and 17 or 18 hours after injection are shown in table III. The residues of Carazolol in the milk were dose dependent at 2.5 hours WT and no residues were detectable (<0.05 µg/L) at 17 or 18 hours WT.

Table III. Concentrations of Carazolol (µg/L) in milk after IV injection.

Dose (mg)	2.5 hours WT	17 or 18 hours WT
5.0	2.40	ND
3.5	1.14	ND
2.5	0.58	ND

ND is not detected (<0.05 µg/L)

Seven cows were injected IV with 2.5 mg Carazolol and milk collected at 2.5 hours and 18 hours after injection. The concentration of Carazolol in the milk (by HPLC method) was 1.67 ± 0.52 (mean ± SD) µg/L, n = 7 at 2.5 hours WT. In six cows no residues were detected at 18 hours WT , a seventh cow had residues of 0.26 µg/L.

Five cows were administered 5.0 mg Carazolol IV and two days later administered 10 mg Carazolol IM Milk samples were collected at 2.5 hours and 18 hours after each injection. No residues were detectable by the HPLC method in the samples collected 18 hours after either injection. The residues (mean ± SD) at 2.5 hours were 1.96 ± 0.56 μg/L after IV injection and 3.72 ± 1.15 μg/L after IM injection.

Metabolism in Food and Laboratory Animals.

General

Lipophilic beta-receptor blockers undergo extensive metabolism (Riess et al, 1975) which usually includes; Glucuronidation of the hydroxyl group of the side chain; hydroxylation and subsequent glucuronidation; oxidative desamination; cleavage of the ether link to form a phenol; n-dealkylation.

There is little information available on the metabolism of Carazolol in animals. Limited information on three metabolites in urine is provided for the pig and human but there is no information on metabolism in cattle or in laboratory species other than the dog.

There are two sets of residue data in the pig. The first gives the total residues using radiometric methods but without giving an indication of concentrations of either parent drug or individual metabolites. The second set of data uses an HPLC method which measures parent drug (in urine three other metabolites were measured) but gives no indication of the total residues in the edible tissues. The metabolism studies reported do not provide evidence for a possible marker substance.

Pig

Rudolph (1988) developed an HPLC method to measure the concentrations of three metabolites; Carazolol-monoglucuronide, Carazolol-lactate and Carazolol-acetate. The lower limits of detection (μg/L) for the metabolites were Carazolol-gluc, 0.48 as Carazolol; Carazolol-La, 5.5; Carazolol-Ac, 3.5. He used the method to investigate the metabolism of Carazolol in the urine of pigs after IM administration of 10 μg/kg Carazolol to sows and collecting urine for 4.5 hours after injection. The results are shown in table IV.

Table IV. Residues of Carazolol and metabolites in pig urine.

Sow	Caraz.		Caraz.-Gluc		Caraz.-La		Caraz.-Ac	Total
No.	µg/L	%dose	µg/L	%dose	µg/L	%dose	µg/L	% dose
I	15.4	0.77	9.4	0.47	20.4	1.02	trace	2.3
II	19.4	0.88	10.9	0.50	16.0	0.73	trace	2.1
III	9.8	0.56	16.5	0.94	24.6	1.41	trace	2.9
IV	18.4	0.92	5.6	0.28	24.3	1.21	trace	2.4
V	11.3	0.53	24.5	1.14	123.8	5.76	trace	7.4

(The residues are all expressed as equivalents of Carazolol)

The residues found in the Rudolph study can be compared with the total residue values from a radiometric study (Rudloff, 1982). In the radiometric study 25% of the original dose is excreted in the urine in 4.5 hours, and from the values in the above table one has to conclude that the majority of residues in pig urine are not identified.

Bartsch et al. (1979), carried out a radiometric study and used the same dose and withdrawal time (2 hours) as Rudolph and Steinhart (1987). The residues in tissues reported in the two studies are compared in table V. The results for HPLC method have to treated with caution because only one pig was used. Whereas there is evidence that the majority of residues in muscle tissue are parent drug, only 15% and 21% of the residues are Carazolol in liver and kidney respectively. The other residues are unidentified but Rudolph and Steinhart (1987) claim that treatment of the tissues with glucuronidase failed to liberate Carazolol and concluded that the Carazolol-glucuronide was not present in the three tissues.

Table V. Residues (µg/kg) in pigs by radiometric or HPLC methods.

	^{14}C-Caraz. (5)	^{14}C-Caraz. (6)	Caraz.-HPLC (5)	HPLC/^{14}C-Caraz. (1)
Liver	28.4	33.7 ± 11.7	5.1	0.15
Kidney	17.5	20.9 ± 6.0	4.4	0.21
Muscle	2.1	2.5 ± 0.8	2.9	1.14

^{14}C-Carazolol measured by Bartsch et al. (1979), using six pigs. One pig gave very low values (see table VI) and if omitted, the values are indicated in the second column of figures.

Dog

The metabolism of ^{14}C-Carazolol was investigated in 24-hour urine study from dogs (Hindermayr et al, 1977). The drug was administered by IV injection and 31% of the original radioactivity was recovered in the urine over a 24 hour withdrawal period. The metabolites identified were those formed by:

Oxidative desamination ----> side chain carboxylic acid

Hydroxylation -------> phenol group

Glucuronidation ------> 2-propyl-glucuronide

Human

Two humans weighing, 72 kg and 88 kg were each given an oral dose of 5 mg Carazolol and their urine collected for 4.5 hours. The residues of Carazolol and three metabolites were measured by an HPLC method (Rudolph and Steinhart, 1987).

Human	Caraz. % dose	Caraz.-Gluc % dose	Caraz.-La % dose	Caraz.-Ac % dose	Total % dose
A	0.34	6.62	6.18	0.55	13.7
B	0.18	8.82	9.7	1.11	19.8

Less than 0.5% of the drug is excreted as parent drug and a significant quantity is excreted as the glucuronide and lactate. No total residues were measured and no other metabolites identified.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Pig

^{14}C-Carazolol (SA. 29.3 μCi/mg - purity >96%) in 0.9% saline was administered intamusculary behind one ear at a dose of 10 μg/kg BW to 10 castrate male pigs weighing 40 - 50 kg and the total residues, as equivalents of Carazolol, were determined in the tissues of pigs slaughtered 2, 8 and 16 hours after dosing (Bartsch et al, 1979). The results are shown in table VI.

Table VI. Total Residues (μg Carazolol equiv. per kg or L) of ^{14}C-Carazolol in pigs.

WT **Tissue**	2 hours n = 6	2 hours n = 5	8 hours n = 2	16 hours n = 2
Muscle	1.77 ± 0.78	2.00 ± 0.57	0.35, 0.44	0.14, 0.02
Liver	28.4 ± 16.8	33.7 ± 11.7	2.8, 6.8	16.2, 6.2
Kidney	17.5 ± 9.9	20.9 ± 6.2	2.3, 5.0	4.7, 2.7
Fat	3.7 ± 3.6	4.3 ± 3.6	12.5, 14.8	0.03, 0
Inj. site	8.8 ± 12.0	10.2 ± 12.8	0.6, 11.6	2.4, 18.1
Brain	1.4 ± 1.3	1.62 ± 1.16	0, 0.09	0.6, 0.2
Lungs	41.1 ± 20.8	49.3 ± 5.8	5.5, 11.9	8.1, 5.1
Contents small intestine	227 ± 60	272 ± 110	20.8, 43.2	75, 75
Bile	290 ± 185	344 ± 148	229, 355	188, 209
Serum	4.9 ± 4.0	5.9 ± 3.6	0.24, 2.6	0.56, 0.34

The radioactivity in 1 ml serum or 0.2 ml urine was determined following the addition of hydroxylamine hydrochloride and toluene scintillation fluid. The radioactivity in faeces and tissues was determined by oxidation and measuring the activity of the CO_2 produced. The detection limit was 0.05 μg/kg.

The concentrations of residues in one of the six pigs in the group slaughtered two hours after dosing were very much lower and different from the values for the other five pigs. This may have been due to incomplete administration of the dose. The results for this pig are not included in the second column of values.

The highest concentration of residues in tissues were in the liver, kidney and lungs with much lower levels in the muscle, fat and brain. The values were highest at 2 hours after dosing except in the fat where the maximum value was at the 8 hour time point. Residues were present in all the tissues at 16 hours after dosing. There is no information on the identity of the residues but taking into account the extensive metabolism of beta-receptor blockers one might assume that much of the residues are metabolites and not parent drug.

^{14}C-Carazolol (SA. 24.4 μCi/mg - purity >96%) in 0.9% saline was administered intamusculary behind one ear at a dose of 10 μg/kg BW to 3 castrate male pigs and the total residues, as equivalents of Carazolol, were determined in the tissues of pigs slaughtered 4 and 6 hours after dosing (Rudloff, 1982). The results are shown in table VII.

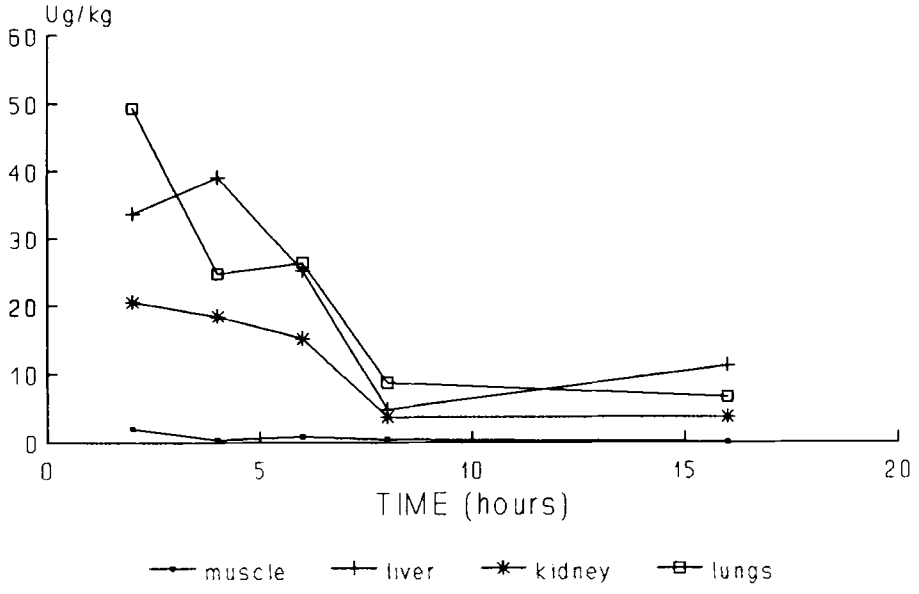
Table VII. Total Residues (μg Carazolol equiv. per kg or L) of ^{14}C-Carazolol in pigs.

Tissue	**WT**	4 hours (63 kg)	6 hours (58 kg)	6 hours (57 kg)
Muscle (loin & chop)		0.18 - 0.36	0.23 - 0.48	0.47 - 2.12
Liver		39.1	25.1	25.3
Kidney		18.4	15.1	15.5
Fat		1.3	1.0	0.2
Injection site		9.1	5.1	3.0
Brain		3.4	1.1	2.3
Lungs		24.7	23.5	29.0
Contents of stomach		18.5	8.8	58.1

The radioactivity in faeces and tissues was determined by oxidation and measuring the activity of the CO_2 produced. The detection limit was 0.05 μg/kg.

The dose of Carazolol used in the two studies is the same and therefore the results from the two radiometric are combined to give more time points. The depletion curves for the edible tissues are shown in figure 2.

Figure 2. Depletion of Carazolol in Edible Tissues of the Pig.



Other Residue Depletion Studies

Pigs

A castrate male pig weighing 80 kg was injected IM with Carazolol at a dose of 10 μg/kg BW. Samples were collected from six sites in the liver, three in the kidney and in two sites in the filet and the residues of Carazolol were determined by the HPLC method Rudolph and Steinhart, (1987). The values in μg/kg were: Muscle, 2.7, 3.0; Liver, 5.1 ± 1.4; Kidney, 4.4 ± 1.0. Thus, there is some evidence of uneven distribution of the residues of parent drug within an organ.

Rudolph (1988) reported that the concentration of incurred residues of Carazolol in pig livers and kidneys was unchanged after storage for four months at -18^{O}.

Eight pigs (90 - 100 kg) were injected IM with Carazolol at a dose of 10 μg/kg BW and slaughtered at 1.75 hours after injection. The Carazolol content in the kidneys was measured and the mean ± SD uncorrected for recovery was 17 ± 4 μg/kg. Correcting for recovery gave a mean value of 19 μg/kg. (Engelsma and Simons, 1985).

Cattle

Twelve cows were collected in a slaughterhouse and injected either IM with 10 mg Carazolol or in an abdominal vein with 5 mg Carazolol. The cows were slaughtered at various time intervals after injection and samples of liver, kidney, muscle and fat collected

for analysis of Carazolol by the HPLC method of Rudolph & Steinhart (1986). The weights of the cows, their treatment and the results for residues of Carazolol are given in table VIII.

Table VIII. Residues of Carazolol in cattle by HPLC method. (μg/kg)

WT	Weight		Muscle		Liver		Kidney		Fat	
(hours)	IM	IV	IM	IV	IM	IV	IM	IV	IM	IV
2.75	255	215	8.8	5.5	31.5	19.0	44.6	86.9	<3.5	7.5
4.5	203	198	7.7	7.8	35.3	15.9	63.9	38.1	<3.5	3.6
7.75	241	243	4.2	5.9	10.0	10.2	22.9	18.9	<3.5	<3.5
14.0	227	256	3.8	4.1	12.4	13.1	20.2	16.1	<3.5	<3.5
18	221	272	1.7	1.4	3.5	5.8	15.3	8.5	<3.5	<3.5
23.5	232	250	<1.3	<1.3	7.5	5.5	6.8	7.9	<3.5	<3.5

The residues after IM and IV do not essentially differ at the two doses used. The sponsors combined the results and calculated the mean elimination half lives of Carazolol. The values were; muscle, 8.9 hours; liver, 7.8 hours and kidney, 4.9 hours.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

There are several methods used by various countries for detecting and determining residues of carazolol in porcine and bovine tissues (usually kidney) and fluids. Carazolol is extracted from tissues or fluids, purified by chromatography using mini-columns, TLC or HPLC and measured by UV or fluorescence. The lower limits of detection are in the low μg/kg or μg/L range.

HPLC

Much of the data on residues of Carazolol submitted by the sponsors was obtained using the HPLC method developed by Rudolph (1988) and Rudolph and Steinhart (1987). This method is suitable for routine monitoring of animals and fresh meat. An outline of the main stages of the method is shown in the flow diagram:

Sample (usually liver or kidney) 50g

Extract with acetonitrile-methanol-ammonia

Centrifuge, decant and dry

Dissolve in NaOH

Extract with diethyl-ether

Kieselgel chromatography, eluting with methanol-ammonia

Dry

Dissolve in NaOH

Extract with diethyl-ether

Dry ether phase

Dissolve in 0.5 ml methanol

HPLC (20 μl) by fluorescence with excitation at 245nm and emission at 345nm; Column, Spherisorb ODS II; Mobile phase, acetonitrile-1.25% aqueous ammonia-dioxane, (90:10:2)

The recoveries for pig liver tissues spiked with 1 to 15.5 μg/kg was 74 - 88% with a correlation coefficient (r = 0.9999) for linearity of mass versus response. Similar values for recovery were observed with muscle and kidney. The lower limit of detection is 0.48 μg/kg for tissues.

An HPLC method (Van Ginkel et al, 1989) for detecting tranquilisers and Carazolol was used by the Netherlands in a surveillance programme. Positive results could be confirmed by an additional two-dimensional TLC method although often was not necessary. Pig kidney was homogenised and made alkaline with NaOH before extraction with diethyl ether. The ether extract was applied to a Baker 10 SPE cartridge column and after washing with organic solvents was eluted with acetonitrile-water-ammonia into NaOH. The Carazolol is extracted into hexane and an aliquot applied to a reverse phase SAS-Hypersil column. The mobile phase was acetonitrile-water-ammonium acetate and the eluate monitored at 235nm in the UV region. The limit of detection was $<1\,\mu$g/kg.

The fraction suspected of containing the Carazolol was spotted on a TLC plate and run in two dimensions using 1) acetone-dichloromethane-ammonia (40:60:1, v/v/v) and 2) acetone-25% ammonia (100:0.1, v/v). The Carazolol spots were detected after spraying with vanillin-phosphoric acid and heating at 120^{O} for 5 minutes.

Arneth (1990) has developed another HPLC method which detects 5 tranquilizers and Carazolol in pig kidneys or urine. 5 g fresh tissue is homogenised in acetonitrile and centrifuged. The clear supernatant is diluted with aqueous ammonia an an aliquot placed on an OCTYL solid phase mini-column. The analytes are eluted from the column in ammoniated-methanol. After drying and redissolving in the mobile phase (for HPLC) an aliquot was analysed on HPLC. The column was a LiChrospher 60 RP-select B, the mobile phase was 0.05M phosphate buffer/tetrahydrofurane/acetonitrile (60:5:35, v/v/v). A fluorescent detector was used with emission measured at 345nm and

excitation at 245nm. Recoveries for Carazolol (mean ± SD) from spiked samples were urine (spike = 15 µg/L) 78 ± 8.0%, kidney (spike = 10 µg/kg) 77 ± 14%.

TLC

A TLC method using fluorescent detection was developed (Ellens et al., 1982) for detecting Carazolol in pig kidneys. The lower limit of detection is 1 µg/kg. 40 g pig kidney were cut up and homogenised in alkaline diethyl-ether. The supernatant was dried, taken up in acetone and put on a 1 g silica mini-column. The eluate with acetone-methanol was dried and back extracted between acid-ether-alkali-ether. The dried extract was dissolved in methanol and an aliquot run on TLC plates (silica gel). The plates were developed in chloroform:methanol:25% ammonia (150:15:0.5, v/v/v) and the spots viewed under short wave UV light. The spots could be quantitated using a fluorodensitometer with excitation at 313nm and emission at 354nm. Contents of 1 µg/kg give a clearly positive result.

The positives were confirmed by a second TLC analysis. The extract was dansylated and the derivatives run on high performance TLC plates in two dimensions. The mobile phases were 1) chloroform:acetone (9:1, v/v); 2) ethyl acetate:n-hexane:dichloro-methane (150:20:40, v/v/v). The plate is viewed under short-wave UV light. A sensitivity of 1 µg/kg is claimed.

The method was evaluated using six pigs injected IM with Carazolol at a dose of 5 - 50 µg/kg BW. The pigs were transported to the slaughterhouse and samples of muscle, liver and kidneys collected for analysis. No time interval is indicated but the pigs were killed within a few hours of the administration of Carazolol. All the kidneys were positive in both TLC systems, five of the six livers were positive and all the muscles (loins) were negative.

Mini-column Chromatography

Engelsma and Simons (1985) developed a simple method for routine screening of Carazolol in pig kidneys. 20 g kidney was homogenised, mixed with NaOH and heated for 1 hour. After cooling Carazolol was extracted with diethyl-ether and an aliquot of the extract run through two mini-columns in series. The first column was a C-18 bonded reversed phase cartridge and the second column a Sep-Pak silica cartridge. The Carazolol was eluted from the silica cartridge with methanol/HCl. The fluorescence spectra (excitation 245nm) was recorded from 300-400nm and the amount of Carazolol determined by iteration with a standard curve. Blank values for untreated pigs were 0.1 - 0.3 µg/kg and recoveries from kidneys using 5, 10 and 15 µg/kg spikes were 87 - 88% with CVs 3.1 - 3.7%. The lower limit of detection was at least 1 µg/kg.

A mean value of 19 µg/kg (see above) was recorded for Carazolol in the kidneys of eight pigs slaughtered 1.75 hours after dosing. This value is higher than other results using TLC or HPLC and is similar to the value found in the radiometric studies. This method may determine several Carazolol metabolites either because they are unresolved in the mini-columns or some of the metabolites may be hydrolysed to Carazolol in the heating with alkali stage.

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