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List of Abbreviations

MAA	:	Marketing Authorisation Application
SmPC	:	Summary of Product Characteristic
MHRA:		Medicines and Healthcare products Regulatory Agency
UK	:	United Kingdom
CVS	:	Cardiovascular System
CNS	:	Central Nervous System
GIT	:	Gastrointestinal tract
GLP	:	Good Laboratory Practice
CSD	:	Cortical Spreading Depression
cAMP	:	Cyclic Adenosine Monophosphate
5-HT	:	5-hydroxytryptamine
RVM	:	Rostroventromedial
NO	:	Nitric Oxide
IL	:	Intraluminal
EL	:	Extraluminal
µg	:	microgram
kg	:	kilogram
mmHg	:	millimetre Hg
µm	:	micrometre
min	:	minute
L	:	Litre
ng	:	nanogram
hr	:	Hour
ip	:	intraperitoneal
g	:	gram
ED_{50}	:	median effective dose
icv	:	intracerebroventricular
iv	:	intravenous
ip	:	intraperitoneal
°C	:	degree Celsius
sc	:	subcutaneous
V	:	volt

ml	:	millilitre
AUC	:	Area under Curve
V_d	:	Volume of distribution
V_{dss}	:	Volume of distribution at steady state
F	:	oral bioavailability
SE	:	Standard error
CSF	:	Cerebro Spinal Fluid
CBT	:	Core Body Temperature
IBI	:	Inter breath interval
ALD_{50}	:	Approximate Median Lethal Dose
LD_{50}	:	Median Lethal Dose
ECG	:	Electrocardiogram
µM	:	micromolar
NPY	:	neuropeptide Y
HSP	:	heat-shock protein
DNA	:	Deoxyrebonucleic acid
RNA	:	ribonucleic acid
IPA	:	internal pudendal artery
SAP	:	systemic arterial pressure
ICP	:	intracorporal pressure
IPAF	:	internal pudendal artererial flow
PRL	:	prolactin
NA	:	noradrenaline
MHPG	:	3-methoxy-4-hydroxyphenylethylene glycol
NK	:	Natural killer
ODC	:	ornithine decarboxylase
ADI	:	Acceptable Daily Intake
WHO	:	World Health Organization
MHB	:	Methyl parahydroxybenzoate
MTDI	:	Maximum Tolerated Daily Intake

2.4 Non-Clinical Overview

In this marketing authorisation application (MAA), approval is sought by Syri Limited t/a Thame Laboratories for a marketing authorisation for Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution in accordance with Article 10(1) of Directive 2001/83/EC, as amended.

Clonidine is indicated for the following conditions (SmPC 2014):

- The prophylactic management of migraine or recurrent vascular headache.
- The management of vasomotor conditions commonly associated with the menopause and characterised by flushing.

Medicines and Healthcare products Regulatory Agency (MHRA) granted a marketing authorization to Boehringer Ingelheim Limited, for Clonidine (Dixarit Tablets 25 micrograms) in 1986 and has therefore been authorised for more than 30 years in the UK. At present, Clonidine is available within the European Union in the form of tablets, solution for injection, capsules, transdermal patches and eye drops.

Clonidine (ATC code: N02C X02) is chemically 2-(2,6-Dichloroanilino)-2-imidazoline. Its molecular formula is $C_9H_9Cl_2N_3$ and molecular weight is 230.1 (Sweetman SC 2009).

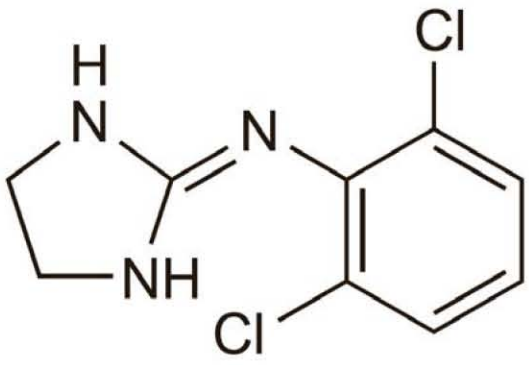

Figure 1: Structural formula of Clonidine (Sweetman SC 2009).

2.4.1 Overview of the Nonclinical Testing Strategy

As stated previously, the proposed application is in accordance with Article 10(1) of Directive 2001/83/EC as amended, a generic application of reference product, Dixarit Tablets 25 micrograms marketed by Boehringer Ingelheim Limited. Since this is a generic application and Clonidine has been used within clinical practice for more than 30 years, no non-clinical studies, toxicological or animal pharmacological studies have been performed by the applicant and none are required. All the information provided in this section has been sourced from the public domain such as published literature and articles.

In preparation of this non-clinical overview the applicant has reviewed the published data from the following sources using the stated search criteria:

Pubmed: Search terms used include Clonidine, Clonidine pharmacodynamics (in vitro, in vivo, receptor binding), Clonidine safety pharmacology, Clonidine toxicity, Clonidine pharmacokinetics (absorption, distribution, metabolism and excretion).
Google Scholar: Search terms used include Clonidine, epidemiology and prevalence of schizophrenia, Clonidine pharmacodynamics (in vitro, in vivo, receptor binding), Clonidine toxicity (acute, chronic, genotoxicity, carcinogenicity, reproductive, developmental in rodents/non-rodents) Clonidine pharmacokinetics (absorption, distribution, metabolism, excretion), safety pharmacology (effect on CVS, CNS, respiration, GIT, kidney).
Science Direct: search terms used include Clonidine, Clonidine pharmacodynamics, Clonidine toxicity, Clonidine pharmacokinetics.
JSTAGE: Search terms used include Clonidine, Clonidine pharmacodynamics (in vitro, in vivo, receptor binding studies in rodents and non-rodents), Clonidine toxicity (acute, chronic, genotoxicity, carcinogenicity, reproductive, developmental in rodents/non-rodents).
EBSCO: Search term used was Clonidine.
Books: Goodman and Gilman 2008, Martindale 36th Edition, 2009. Search term used was Clonidine.

The reference product Dixarit Tablets 25 micrograms is a well-established pharmaceutical product (over 30 years of clinical use) with a favourable risk/benefit profile, which is considered to provide sufficient reassurance of the favourable risk/benefit profile of the new product proposed for marketing. For this reason discussion is restricted to the brief description

of the pharmacological and toxicological properties of Clonidine drug substance. The non-clinical overview will address some in-vivo and in-vitro studies that have been undertaken elsewhere which support this application, and will also address the impurity profile and suitability and safety of excipients in the formulation.

Any comment on the GLP status for cited studies is not possible. However, it is assumed that these were conducted to the standards prevailing at the time.

2.4.2 Pharmacology

Mechanism of Action

Clonidine is an antihypertensive agent which acts centrally by stimulating alpha$_2$-adrenergic receptors and producing a reduction in sympathetic tone, resulting in a fall in diastolic and systolic blood pressure and a reduction in heart rate. Treatment with Clonidine diminishes the responsiveness of peripheral vessels to constrictor and dilator stimuli, thereby preventing the vascular changes associated with migraine. The same direct action on peripheral vessels moderates the vascular changes associated with menopausal flushing (Jarrott B et al 1987, Houston MC 1981, Onesti G et al 1971 and Toxnet 2012).

Both α_2- and β-adrenergic mechanisms control the propagation of cortical spreading depression (CSD) in rat probably via the release of glutamate. This effect might depend on the regulation of presynaptic cAMP concentration, which is known to determine glutamate release from the terminals. This theory is further corroborated by the finding that CSD is also blocked by 5-HT1α receptors that also regulate cAMP level and glutamate release. The prophylactic effect of b-adrenoceptor antagonists in human migraine could therefore be based on decreased neuronal excitability and/or inhibition of CSD (Richter F et al 2005).

The role of α_2-adrenoreceptor agonists in pain is based on enhancement of descending inhibition in the rostroventromedial (RVM) nuclear complex, inhibition of nitric oxide (NO) release, and blockade of glutamate release. In addition, some α_2 agonists such as clonidine potentiate muscarinic M1 and M4 receptors (Ramadan NM 2004, Kang YJ et al 2003).

Pharmacodynamics Studies

In vitro studies

With the aim of evaluating whether Clonidine and methysergide produce vasoconstriction, which might be contributing to the mechanism of action of migraine, an isolated tissue perfusion experiment was designed. Rabbit auricular artery was isolated and was suspended in a 3 ml organ bath and perfused with Krebs solution at 37°C by means of a constant output pump. Drugs were administered either as bolus injections directly into the fluid perfusing the artery immediately proximal to the cannula, or were added to the perfusion reservoir. Field

stimulation of the intramural sympathetic nerves was effected by means of two platinum electrodes arranged opposite each other, one on each side of the artery. Pulses of 1 msec duration and supramaximal voltage were given for 5 sec at 1--25 Hz. both clonidine and methysergide caused vasoconstriction. When added to the perfusion fluid the threshold concentration in each case was between 2 and 20 ng/ml and the rise in perfusion pressure was well maintained for long periods of time. The result of this experiment were indicative that the both of this drugs cause vasoconstriction and that might be one of the mechanism by which they act as migraine prophylactic (Fozard JR 1976).

In a study, the vasoconstrictor action of clonidine on arteries from rabbit ear, rat tail and helicle strips from the tail artery of rat was evaluated. Arteries were cannulated in order to evaluate the pressor-depressor response of the clonidine. In this set, the drug was applied both intraluminal and extraluminal into the cannulated and mounted artery. In cannulated arteries clonidine increase the perfusion pressure independent of method of administration and this effect was maintained up to maximum of 30min. Similarly, noradrenaline increase the perfusion pressure in cannulated arteries. However, this effect was abolished by phentolamine (1µg/ml). In rat tail artery strip, clonidine was less potent in producing a contractile response than noradrenaline and which was only 25% of that produced by noradrenaline. Clonidine shows dose dependent relaxation of smooth muscle when add at cumulative dose in strips contracted with noradrenaline. Hence, from the results of the study, it was concluded that clonidine producing vasoconstriction by a direct activation of adrenergic alpha receptors, rather than indirectly by the release of noradrenalin from sympathetic nerves (Hodge RL et al 1972).

In a study, the effect of clonidine on postjunctional α-adrenoceptors in rabbit aorta was evaluated. The thoracic aorta removed from the rabbit and cut spirally into two strips and suspended in Krebs-Henseleit solution. In isolated aortic strips, clonidine shows partial agonist activity on postjunctional α-adrenoceptors and cause smooth muscle contraction. This contractile response was competitively antagonized by phentolamine. Also, clonidine competitively antagonized the contractile effect of noradrenaline (Medgett IC et al 1978).

In another study, the effect of clonidine on isolated heart is evaluated. Heart from the guinea pigs weighing 300-450 g were isolated and mounted for recording the response. Clonidine (2.5-40 µg) was injected into the aortic cannula. After administration of 10 µg, clonidine exerted a positive inotropic effect and without significant change in heart rate. However, in

the presence of burimamide, positive inotropic effect of clonidine is nearly abolished and show significant negative chronotropic effect. Additionally, clonidine showed dose dependent increase in the amplitude of the inotropic effect (Csongrady A et al 1974).

In vivo studies

The spectrum of activity of clonidine at different dose and in different animals are summarised in table below (Stahle H 2000):

Table 1: Effects of clonidine in animal experiments

Effect	Approximate threshold dose (µg/kg)	Experimental animal
Sedation	10	Dogs, cats
Secretory inhibition	50	Rats
Contraction of nictitating membrane	10	Dogs, cats
Blood sugar increase	10	Rats
Analgesia (several tests)	20-400	Mice
Adrenolysis	100	Rabbits, cats
Initial increase in blood pressure	1	Dogs, cats, rabbits
Decrease in blood pressure	1	Dogs, cats, rabbits
Local anaesthesia	0.1%	Guinea-pigs
Bradycardia	1	Dogs, cats, rabbits

The vascular theory which attributes migraine to spasm of a cerebral artery causing local hypoxia and transient focal symptoms followed by neurogenically mediated extra- and/or intracranial vasodilation causing headache is one of the two important theories for the cerebral mechanism of migraine (Lauritzen M 1987). Effect clonidine on the cerebroarterial constriction was evaluated in cats. Mongrel cats were anaesthetized with slow intravenous injection of (80 mg/kg) chloralose. Experimental set was prepared in order to measure the cerebrospinal fluid pressure, for measuring the blood pressure and for measuring the pial artery diameter.

a) Cerebrospinal Fluid Pressure:

In a group of 6 cats Basal cerebrospinal fluid pressure (10.3 ± 2.01 mm Hg), i.v. injection of 30 µg/kg clonidine increased the cerebrospinal fluid pressure from 7.0 ± 0.89 to 9.2 ± 1.31 mm Hg, however, this effect was not significant and subsided within 15 min. In

another group of 4 cats 3 µg/kg clonidine was injected i.v. The basal cerebrospinal fluid pressure (2.9 ± 0.24 mm Hg) was not affected by the drug. Pretreatment with yohimbine abolish the inhibitory effect of clonidine. However, clonidine still increased arterial blood pressure indicating that no or incomplete inhibition of the postjunctional vascular α-adrenoceptors was caused by the infusion of yohimbine.

b) Pial artery diameter:

In a total of 6 untreated cats the mean resting diameter was 138 ± 14.6 µm. After bilateral electrical stimulation of the efferent cervical sympathetic nerves with DC pulses of 20 Hz, tended to increase when measured 15, 30, 45 and 60 min thereafter (143 ± 17.0, 155 ± 16.5,175 ± 8.9 and 168 ± 21.6 µm).

In another group of 9 cats 30 µg/kg clonidine i.v. reduced the vessel diameter by a maximum of 9 ± 2.0%. The effect reached a maximum immediately after the injection and subsided completely within 10-15 min.

The inhibitory effect of clonidine in neurosympathetic transmission might explain the efficacy of the drug in the treatment of migraine (Reichl R et al 1980).

Cortical spreading depression (CSD) play important role in the migraine and the development of the penumbra zone after stroke. Therefore in a study was conducted to evaluate the effect of clonidine on CSD. Clonidine applied topically to an area of the exposed cortex of anesthetized adult rats and the migration of CSD-related DC potential deflections across the treated area was observed. Clonidine (0.56 mmol/L dissolved in 165 mmol/L NaCl) was applied in 7 rats. In three of the rats CSD migration was inhibited, in the other three rats CSD initiation was blocked. In a washout period of 45 to 120 mins, CSD was restored in 3 of the 6 rats. The results of the study suggest that the interference of clonidine with CSD may contribute to their beneficial therapeutic effect (Richter F et al 2005).

The actions of clonidine which is mediated through the Central Nervous System have been examined in Greyhound dogs. Dogs (20 to 30 kg) were anaesthetized and prepared for the experiment. In the experimental, intravascular pressures were measured by Statham pressure transducers connected to polythene catheters and heart rate was recorded from the electrocardiograph with a G rass cardiotachometer. Clonidine was administered through vertebral artery at a dose of 2µg/min for 10-20min. The observation made during study are summarised below (Katic F et al 1972):

a. The clonidine infusion decreased the arterial pressure with mean reduction of 16.6mmHg. Within next hour, pressure gradually returned to normal. Similarly, heart rate was also increase by mean 11.3 beats/min and returned to normal with blood pressure.
b. In same study, the effect of clonidine on central effect of angiotensin was evaluated. Angiotensin was infused into the vertebral artery at the dose of 32 ng/min for five min, before and after an intravertebral infusion of clonidine. Angiotensin caused an increase in the heart rate and arterial pressure and in the presence of clonidine these effects was abolished or reduced.

From the above data it was concluded that there are central adrenergic neurones which inhibit cardiovascular autonomic reflexes and that the central autonomic effects of clonidine are due to stimulation of inhibitory adrenoceptors.

In order to investigate the mechanism of antihypertensive effect of Clonidine in cats, cats were anaesthetized with 80 mg/kg of chloralose intraperitoneal injection. Blood pressure was measured from a femoral artery with a Statham pressure transducer and heart rate was measured from electrocardiograph leads using a cardiotachometer. Clonidine (5-40 µg/kg) injected intravenously into the anaesthetised cat increases the blood pressure followed decrease within 90-150min. These two phases (initial increase followed by decrease in blood pressure) was dose dependent. Similarly, after administration of clonidine heart rate increase immediately followed by decrease in same manner as blood pressure. Phentolamme diminished the contraction of the nictitating membranes produced by clonidine at high doses (10-40µg/kg). Therefore, it was concluded that the effect of clonidine on heart was not because of the direct action on the beta adrenoceptors but due to the reduction of the central sympathetic tone to the heart (Rand MJ et al 1968).

A study was conducted to evaluate the effect of clonidine on the arterial pressure and heart rate (HR) after administration through different route in hypertensive rat (coarctation of abdominal aorta artery (CoA) model). Wistar rats (240–270 g) were used to the 7 days of the CoA or a sham operation (SO). The MAP rats were significantly higher after CoA as compared to SO. Rats were treated with clonidine via intravenous (i.v.), intracerebroventricular (i.c.v.) and intrathecal (i.t.) anesthetized with pentobarbital (40 mg/kg i.p.). After i.v. administration (3-30µg/kg), clonidine increases the blood pressure in the rats SO and in the CoA animals followed by a decrease in arterial pressure. Similarly, after i.c.v. via (10µg) and i.t. (3µg) administration of clonidine produce a greater decrease in MAP in the hypertensive rats than in the controls SO animals. The results of the study concluded that,

these hypertensive animals would be sensitive to the antihypertensive action of clonidine administered by different ways, suggesting a great sensitivity of the post-synaptic α_2-adrenoceptor of central nervous system (Gorzalczany SB et al 2003).

In a study, intravenous administration of clonidine caused an immediate and significant decrease of intraocular pressure of rabbit eyes. The pupillary dilation response of clonidine was inhibited by i.v. administration of phenoxybenzamine suggests that hypotensive response to be dependent on a n intact adrenergic innervation of the ocular tissues (Allen RC et al 1976). Similarly, in rat's clonidine (10µg/kg) reverse the hypertensive effect of chronic intravenous infusion of a low dose (4.0ng/min) of angiotensin II via the sympathetic nervous system (Gorbea-Oppliger VJ et al 1994).

Antinociceptive activity of clonidine was evaluated in acetylcholine induced abdominal constriction test in the mouse, hot-plate test in the mouse, tail-immersion test in the mouse and rat, paw pressure test in the rat, the formalin test in the rat, electrical tail stimulation in the rat and dental pulp stimulation in the conscious dog. Clonidine was dissolved in saline and injected either subcutaneously (sc), in dose volumes of 0.2ml/20g and 0.4ml/100g in the mouse and rat, respectively, or intracerebroventricularly (icv), in dose volumes of 5ul/mouse and 10ul/rat. Dogs received sc injection in a dose volume of 0.1ml/kg. The results of the study concluded that, clonidine has potent antinociceptive properties against several types of noxious stimuli tested (Skingle M et al 1982).

Safety Pharmacology

In a study, dogs were treated with clonidine at dose of 25 µg/kg or 12.5 µg/kg followed by placebo via the catheter, once daily for 14 consecutive days. Sedation was observed in dogs receiving high dose clonidine while no other changes in general appearance or neurologic behaviour was observed during the whole study period. The body weight did not change significantly and no gross lesions were observed in the spinal cord and the brain at autopsy (Gordh TE et al 1984).

In another study, dogs were treated with clonidine orally (500 µg/day) for seven days. On eighth day, dogs were given a final oral dose and then anaesthetized and blood pressure and heart rate were measured. The blood pressure and heart rate were not significantly different as compared to normal dogs (Katic F et al 1972).

Clonidine was shown to produce a dose-dependent decrease in locomotor activity of mice (0.5-25.0 µg/kg) and rats (0.05-1.0 mg/kg) in spite of marked sympathomimetic effects (Maj J et al 1972; Johnston AL et al 1988 and Tilson HA et al 1977).

Clonidine (0.03 or 0.1 mg/kg) prevent the gastric lesions induced by oxotremorine plus neostigmine, probably through a α_2-agonist mechanism. Similar effect was observed in gastric damage induced by dimaprit in rats at dose 0.3 or 1.0 mg/kg given intravenously (Del Soldato P 1986).

The safety profile of clonidine was observed in six different in vivo models (Delaunois A et al 2014):

1). Male Sprague Dawley rats (weight: 340–430 g) were anesthetized with sodium pentobarbital and received a single i.p. injection of clonidine (0.01, 0.03, 0.1, and 0.3 µmol/kg). Clonidine induced hypotension in pentobarbital-anesthetized rats.
2). Male SHR rats (weight: 300–350 g) were given an i.v. bolus of clonidine (0.03, 0.1 or 0.3 µmol/kg). Clonidine induced hypotension in conscious spontaneous hypertensive rats.
3). Telemetered conscious normotensive Male Sprague Dawley rats (weight: 300–350 g) received a single i.p. dose of clonidine (0.1 or 0.3 µmol/kg). Clonidine reduced heart rate and body temperature in telemetered rats.
4). Four male naive Beagle dogs (8–19 months old) received ascending doses of clonidine (0.01 and 0.03 µmol/kg) by intravenous slow infusion (10 min) in the cephalic vein, with a minimum of 3 days between each administration. Clonidine induced hypotension in telemetered dogs.
5). Anesthetized pithed Male Sprague Dawley rats (weight: 210–300 g), without sympathetic nerve stimulation were given four cumulative intravenous injections of clonidine (0.001, 0.01, 0.1, 1 µmol/kg). Rats with sympathetic nerve stimulation were given four increasing doses of clonidine (0.01, 0.03, 0.1, and 0.3 µmol/kg). In anesthetized pithed rats, clonidine showed dose-dependent hypertension.
6). Male NMRI mice (weight: around 25 g) were administered with a single i.p. dose of clonidine at several doses, ranging from 0.003 to 100 µmol/kg. In a mouse Irwin test, sedative effects of clonidine started from threefold its ED_{50} in the mouse formalin test.

Sprague–Dawley rat pups at p7 of both sexes (n = 20/treatment group) were injected with IP clonidine (40, 200 and 400 µg/kg). At all doses studied, clonidine treated animals, had significantly lower RR (respiratory rate) at 10 and 90 min after drug administration, compared

to saline treated animals. Clonidine treated animals had significantly prolonged IBI (inter-breath interval) compared to saline treated animals at similar time points. CBT (core body temperature) did not increase in clonidine treated rat pup. Oxygen consumption (V_{O2}) and carbon dioxide production (V_{CO2}), after clonidine treatment was significantly lower compared to saline treated animals. In the newborn rat, clonidine causes minimal respiratory depression when compared to morphine and stabilizes CBT (Kesavan K et al 2014).

In male Dunkin Hartley guinea pigs (450±50 g), intravenous clonidine injection was given with cumulatively increasing doses (1.0–3.0–10.0 mg/kg), with an interval of 30 min, and the ECG was recorded 5, 10, 20, 30 minutes after each dose. Clonidine induced a lengthening of the ECG parameter of RR, without any corresponding increase of QT (Testai L et al 2007).

Since clonidine is there in clinical practice for more than 30 years, cases of clonidine toxicity are well documented by various clinicians. The primary action of clonidine is to reduce blood pressure. Therefore there are sincere chances of getting hypotension. There are several cases reported of hypotension due to clonidine treatment in humans. Other effects due to clonidine overdose are hyperthermia, coma, and bradycardia. It can also cause miosis. Majority of these effects are also documented in animal studies (Toxnet 2012).

2.4.3 Pharmacokinetics

In order to study the pharmacokinetics of clonidine, Groups of 8 male Sprague-Dawley rats (150-250 g) received 10, 50, or 250 µg/kg of clonidine as a rapid bolus injection through the tail vein. One animal in each group was then sacrificed 1, 5, 15, 30, 60, 120, 180, and 240 min after injection, trunk blood was collected, and the various organs and tissues were rapidly dissected and processed for pharmacokinetic measurements (Conway EL et al 1982).
The pharmacokinetic parameters estimated in the experiment are described below:

Table 2: The pharmacokinetic parameters of clonidine:

Parameter	Dose		
	10 µg/kg	**50 µg/kg**	**250 µg/kg**
Half life (min)	1.6	1.4	3.9
Total body clearance (ml/min)	-	11.83	6.48
Renal Clearance (ml/min)	-	3.78	2.07
Volume of Distribution (ml)	-	828	846
Area Under Curve (ng/ml/min)	-	845	7714

Equations used for the calculations of pharmacokinetic parameters $Cp = Ae^{-\alpha t}$ for 10 µg/kg dose; $Cp = Ae^{-\alpha t} + Be^{-\beta t}$ for other doses

In another study in order to evaluate the pharmacokinetics of clonidine, rat and cat both were used. Male Sprague-Dawley rats, weighing 175-200 g and cats of both sexes, weighing 2.5-3.0 kg, were used throughout the study. Three doses of clonidine 500, 250 and 125 µg/kg were evaluated intravenously. Provisions were made to collect blood for pharmacokinetic evaluation. By applying appropriate models for pharmacokinetic, all the pharmacokinetic parameters were evaluated at all three doses that are described below (Paalzow LK et al 1979).

Table 3: Pharmacokinetic Parameters of Clonidine Given Intravenously to Rats.

Dose clonidine (µg/kg)	AUC (min-ng/ml)	AUC dose normalized (min-ng/ml)	Vd_{area} (ml/kg)	V_c (ml/kg)	Vd_{ss} (ml/kg)	Clearance (ml/min/kg)
125	1817.55	1817.55	4826.24	945.25	4349.29	68.77
250	5707.98	2853.99	4888.21	1160.52	4551.64	43.79
500	15610.35	3902.59	2829.51	1355.90	1658.43	32.03

Absorption

After subcutaneous administration of ^{14}C-clonidine at a dose of 0.25, 0.5 and 1 mg/kg in male rats, the maximum concentrations in plasma reached (Cmax) at 2 hr in all dose groups. Similarly in female rats, after s.c. administration of 1mg/kg ^{14}C-clonidine show similar value of Cmax. The absorption parameters in male and female rats are summarised below:

Table 4: Absorption parameters of ^{14}C-clonidine

Sex	Dose (mg/kg)	Parameters		
		Tmax (hr)	Cmax (ng·hr/ml)	AUC (µg·hr/ml)
Male	0.25	2	41.4	0.554
	0.5	2	90.4	1.22
	1	2	168.9	2.52
Female	1	2	171.7	2.81

The above data clearly indicates that the Cmax and AUC are almost dose-proportional (Yamahata T et al 1996a).

After administration of ^{14}C-clonidine at a dose of 1 mg/kg for 21 days in male rats, the Cmax was found to be 205.0 ng/ml at 2 hr and AUC was 4.58 µg • hr/ml. As compared to single dose, similar Tmax was observed while Cmax and AUC were 1.2 and 1.8 times higher in multi dose (Yamahata T et al 1996b).

In the male dogs, Cmax of 980.7 ng/ml was observed after 4hr of administration of ^{14}C-clonidine at a dose of 1 mg/kg and AUC was 40.6µg•hr/ml. During initial 1st hr, the concentration of clonidine was higher in blood as compared to that in plasma and decrease after 2 hr, which suggest that transfer of clonidine to the blood cells was low. In the male dogs, the Cmax was 6 times higher than observed in the male rats (Yamahata T et al 1996a).

Similarly, in the male monkey, Cmax of 703.0 ng/ml was observed at 8hr of ^{14}C-clonidine administration at a dose of 1 mg/kg and AUC was 40.6µg•hr/ml. During initial 2 hr, the concentration of clonidine was higher in blood as compared to that in plasma and decrease after 2 hr, which suggest that transfer of clonidine to the blood cells was low as decrease in dogs (Yamahata T et al 1996a).

In rats, pharmacokinetics of clonidine was evaluated using three different blood collection techniques. The oral bioavailability of clonidine (1 mg/kg oral/0.1mg/kg i.v.) using three different blood collection methods is as described below (Hui YH et al 2007).

Table 5: Oral bioavailability of clonidine

Blood collection method	F (%)
Tail	97±17
Cannula	>100
Orbital	>100

Distribution

Clonidine is well distributed in various tissues. The penetration of clonidine into tissues is rapid. The distribution half-life of clonidine in rat after intravenous injection of three different doses (125, 250 and 500µg/kg) was found to be less than 2 min. In cats also the distribution of clonidine (50µg/kg) was found to be rabid with distribution phase half life of less than 1 min. From the doses tested and the distribution rate constant; the distribution of the clonidine was found to be dose dependant (Paalzow LK et al 1979).

The *in-vitro* binding of ^{14}C-clonidine in the rat, dog and monkey plasma proteins are summarised below (Yamahata T et al 1996c):

Table 6: Binding of ^{14}C-clonidine to male rat, dog and monkey plasma proteins

Concentration (ng/ml)	Ratio of binding (%)		
	Rat plasma	Dog plasma	Monkey plasma
20	39.2 ± 0.8	46.1 ± 1.5	33.2 ± 0.6
60	38.9 ± 0.6	46.3 ± 0.4	32.2 ± 0.9
108	34.6 ± 0.2	45.2 ± 1.5	27.6 ± 0.9

In the same study, in-vivo binding of ^{14}C-clonidine to male rats, dogs and monkey are summarised below. The binding ratios increased gradually with the elapse of time (Yamahata T et al 1996c).

Table 7: Binding of ^{14}C-clonidine to male rat, dog and monkey plasma proteins

Time	Ratio of binding (%)		
	Rat plasma	Dog plasma	Monkey plasma
30 min	33.0 ± 1.8	-	-
1 hr	-	53.8±16.6	44.1
2 hr	37.3±1.7	-	-
4 hr	-	68.1± 1.3	56.4
8 hr	43.7±3.1	-	72.3
24 hr	73.5±7.6	93.7 ± 0.6	90.9

After subcutaneous administration of ^{14}C-clonidine to male rats at a dose of 1 mg/kg, high level of radioactivity was observed at the administration site, urine in bladder, preputial gland, gastric contents, intestinal contents, exorbital lacrimal gland, intraorbital lacrimal gland, nasal cavity and bulbourethral gland, followed by the levels in the mandibular gland, kidney and liver after 2 hrs of administration. The concentration observed in the mandibular gland, kidney, liver and stomach was 11 to 16 times higher than that in plasma. The radioactivity in spleen, hypophysis, pancreas, adrenal gland, bone marrow, thyroid gland, lung and brain were higher than that in blood. The total radioactivity in the body was decrease after 8 and 24hr of administration (Yamahata T et al 1996a).

After administration of ^{14}C-clonidine at a dose of 1 mg/kg for 21 days in male rats, maximum concentrations were observed at 2 hr in all tissues except the eyeball and fat and concentration was higher than that in the plasma. In the kidney, mandibular gland and liver higher concentration was observed which is 12 to 15 t imes higher than that in the plasma (Yamahata T et al 1996b).

Similarly in dogs, after subcutaneous administration of ^{14}C-clonidine to male dogs at a dose of 1 mg/kg, compared to plasma high radioactivity was observed in the aorta, moderate higher in the thyroid gland, liver and lung, slightly higher in the kidney, similar in the adrenal gland and less in the others (Yamahata T et al 1996a).

After subcutaneous administration of ^{14}C-clonidine to lactating rats, a maximum of clonidine observed in the milk was 399.2 ng/ml at 30 min of administration. After 24 hr, it decreased to 5% and to undetectable level at 72 hr of administration. The concentration in the milk was 5.5

times higher than that in the plasma at 30 min, and then comparable with that in the plasma after 8 hr (Yamahata T et al 1996c).

Both the steady-state plasma and CSF concentrations of clonidine were proportional to the dose; the ratio of CSF to plasma concentration was approximately 0.5 (Yaksh TL et al 1994).

Metabolism

Clonidine has been reported to undergo extensive metabolism in several species, including man, rat and dog. About 50% of a dose is metabolised in the liver. Only two metabolites, p-hydroxy-clonidine and 2,6-dichlorophenylguanidine, a product of imidazoline ring cleavage. Clonidine was incubated with the liver microsomal enzymes and 2-(2,6-dichlorophenylamino)-imidazole, was the major metabolite produced in microsomal incubations. In the perfused liver, on the other hand, 2, 6-dichlorophenylguanidine was the principle metabolite while 4-oxo-clonidine was also formed in significant amounts, only trace quantities of p-hydroxyclonidine were detected in both incubation and perfusion (Baillie TA et al 1978).

After single and multiple subcutaneous administration of ^{14}C-clonidine to male rats at a dose of 1 mg/kg, the metabolic pathway of clonidine is presented below:

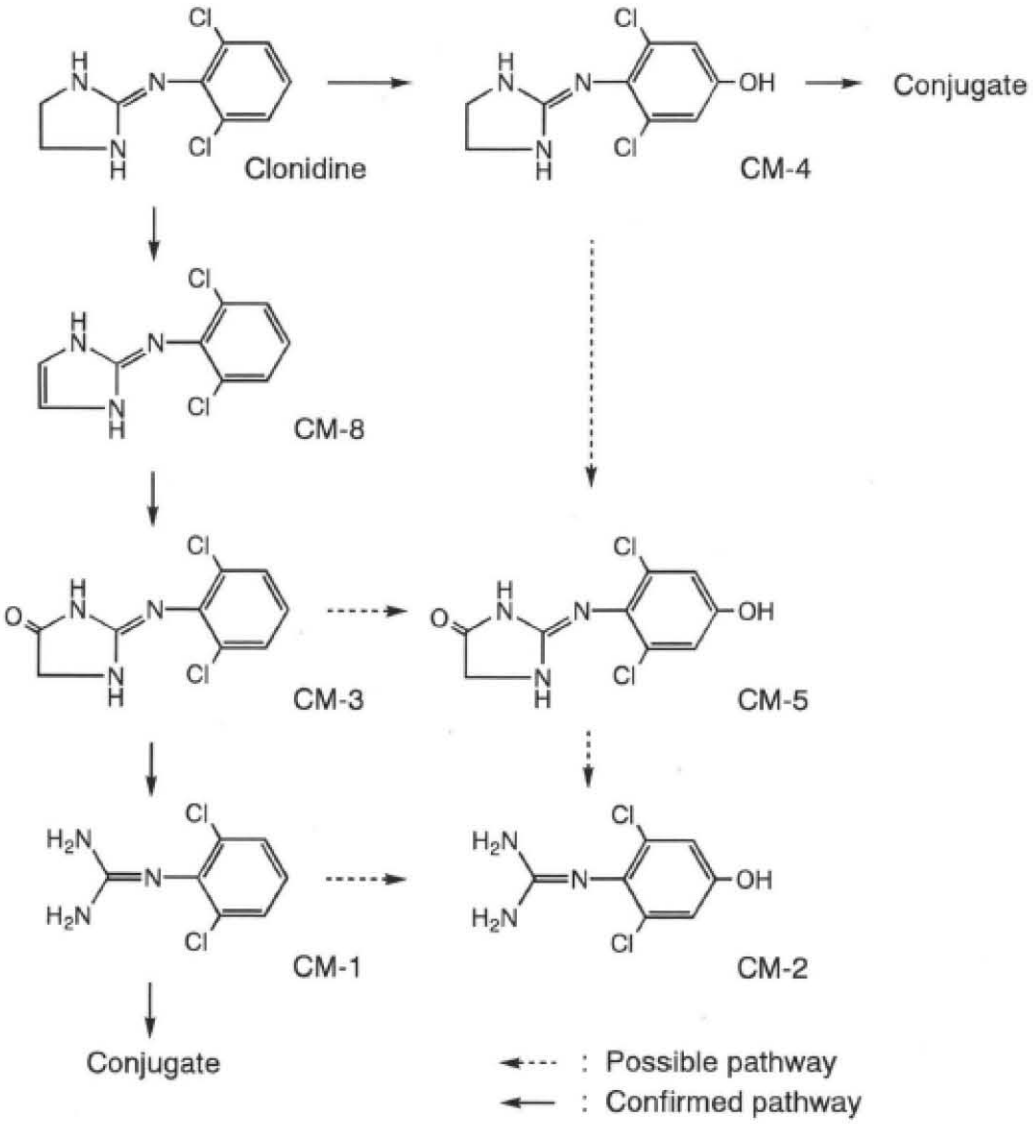

Figure 2: Speculated metabolic pathway of clonidine

Unchanged clonidine was mainly found in the plasma after 30 min of administration. At the same time clonidine metabolite, CM-3 was also detected. After that the % of unchanged clonidine decrease and CM-3 metabolite % increase with the time. CM-1 was detected after 2 hr and 8 hr of administration and CM-4 was detected at 2 and 8 hr. In brain, unchanged clonidine was found after 2 hr of administration along with the metabolite CM-3. After 8hr, percentage of unchanged clonidine decreased and CM-3 increased. In liver, CM-4, CM-8, CM-3, CM-1 and CM-2 were detected along with the unchanged clonidine. No difference was observed in the metabolic patter after single and multiple doses. In the kidney, CM-3, CM-8, CM-1 and CM-4 were also detected along with the unchanged clonidine. However, CM-5 was detected after 21 days repeated administration only (Yamahata T et al 1996d).

Excretion

In the rat about 45% of the given dose is excreted as unchanged drug in urine. From the doses tested and the elimination rate constant found in the study the elimination of the clonidine was found to be dose dependant. The value of elimination rate constant reduced with the reduction of the dose. The vasoconstrictor and antidiuretic property of clonidine could be responsible for the reduction in the rate of elimination. Total body clearance of clonidine is mainly dependant on the urinary excretion at lower doses. At high doses as described earlier tissue distribution is affected and that also adds up in the process of clearing clonidine from the body (Paalzow LK et al 1979).

Direct estimates of the renal clearance of clonidine were obtained after administration of both 50 and 250 µg/kg. After the 50µg/kg dose, the renal clearance was found to be 4.9 ml/min in contrast to 3.0 ml/min after the 250µg/kg dose. Thus there is approximately a 40% decrease in renal clearance at the higher dose. The cumulative urinary excretion in the group of rats given 50µg/kg clonidine (weight = 236 ± 9 g, n = 4) was 3.7 ± 0.17µg over 4 hr or 32% of the dose. In the group given 250µg/kg (weight = 229 ± 15 g, n = 3), it was 18.6 ± 0.73µg over 4 hr again representing 32% of the dose (Conway EL et al 1982).

After subcutaneous administration of ^{14}C-clonidine to male and female rats at a dose of 1 mg/kg, the radioactivity was completely excreted in the urine and feces by 24 and 48 hr after administration, respectively. In the male rats, within 168 hr of administration, 69.5 and 28.4% of the dose were excreted in the urine and feces, respectively while in the female rats; the ratio of excretion in the urine was slightly higher than that in the male rats. The total radioactivity excreted in the male and female rats were 97.8% and 95.2% of the dose respectively. However, less than 0.2 and about 1 % of the dose remained in the administration sites and carcasses, respectively, in rats. The cumulative excretions of radioactivity in urine and feces after subcutaneous administration of ^{14}C-clonidine to rats are summarised below (Yamahata T et al 1996a):

Table 8: Cumulative excretions of radioactivity in urine and feces

Time (hr)	Excretion of radioactivity (% of dose)					
	Male			Female		
	Urine	Feces	Total	Urine	Feces	Total
0-4	31.5 ± 1.2	-	-	40.6 ± 3.0	-	-
8	45.9 ± 4.9	-	-	57.1 ± 3.8	-	-
24	66.2 ± 5.0	23.9 ± 1.9	90.1 ± 3.2	76.3 ± 3.9	9.9 ± 1.9	86.2 ± 2.7
48	68.5 ± 4.0	27.3 ± 3.8	95.8 ± 1.6	78.8 ± 3.4	14.6 ± 3.1	93.3 ± 0.4
72	68.9 ± 3.8	27.8 ± 4.0	96.7 ± 1.6	79.3 ± 3.5	15.1 ± 3.1	94.4 ± 0.5
96	69.1 ± 3.8	28.0 ± 4.1	97.2 ± 1.5	79.5 ± 3.5	15.3 ± 3.1	94.8 ± 0.5
120	69.3 ± 3.8	28.2 ± 4.2	97.5 ± 1.6	79.7 ± 3.5	15.3 ± 3.1	94.9 ± 0.5
144	69.4 ± 3.8	28.3 ± 4.2	97.6 ± 1.6	79.8 ± 3.6	15.3 ± 3.1	95.1 ± 0.5
168	69.5 ± 3.8	28.4 ± 4.3	97.8 ± 1.7	79.9 ± 3.6	15.3 ± 3.1	95.2 ± 0.5
Administration site (168hr)			0.2 ± 0.1			0.1 ± 0.1
Carcass (168hr)			1.1 ± 0.6			0.8 ± 0.1

Similarly, in the male dogs and monkeys receiving same dose as rats, about 80.0% and 73.1% of the dose was excreted in the urine and feces within 24 hr of administration, but after that the radioactivity was excreted slowly. Within 480 hr of drug administration, 87.0 and 10.2% in dogs and 86.1% and 8.6% of the dose in monkey were excreted in the urine and feces, respectively, and the total excretion was 97.2% and 94.7% in dogs and monkey respectively (Yamahata T et al 1996a).

In bile-duct cannulated male rats, after subcutaneous administration of 14C-clonidine at a dose of 1 mg/kg, 33.2, 61.9 and 0.2% of the dose were excreted in the bile, urine and feces, respectively, within 48 hr. No radioactivity was detected in the gastro-intestinal contents after 48 hr of administration. However, 0.2 and 1.1% of the dose remained in the administration site and carcass, respectively (Yamahata T et al 1996a).

After administration of ^{14}C-clonidine at a dose of 1 mg/kg for 21 days in male rats, the total excretion of radioactivity in the urine and feces during each 24 hr after daily administration was almost constant after the 2nd dosing, accounting for 93 to 98% of the cumulative dose. Within 168 hr of last administration, 71.6 and 26.4% of the dose was excreted in the urine and feces, respectively, and the total excretion was 98.1% of dose (Yamahata T et al 1996b).

2.4.4 Toxicology

Single Dose Toxicity/Acute Toxicity

In an acute toxicity test, mice (either sex; weight: 20-25 grams), were orally administered single dose of clonidine. The approximate lethal dose (ALD_{50}) values were determined by finding mortality within 25 hours of the drug administration. The clonidine have ALD_{50}>1000mg/kg. In another study, cats of either sex (weight: 2.5 to 4 kg), clonidine produced hypotension and bradycardia at dose of 10mg/kg (Malhotra V et al 2011).

The oral LD50 of clonidine in rats was 465 mg/kg, and in mice 206 mg/kg. The LD50 in 24 hours when given intravenously to mice is 17.6 mg/kg; the LD50 during a 14-day observation period following a single oral dose is over 30 mg/kg in dogs (Product monograph, 2012).

Male Sprague-Dawley rats (weight: 300 to 400 g) were anaesthetized and intravenous clonidine injections were given into a lateral tail vein at dose of 1 - 30µg/kg. Clonidine caused a dose-dependent mydriasis and bradycardia (Hsu WH et al 1984).

Sprague-Dawley Rat pups aged 3, 7 or 21 postnatal (P) days were given intrathecal injections of clonidine in escalating doses of 0.1, 0.3, 1, 3, 10, and 30mg/kg. Even at doses much greater than required for analgesia, clonidine did not produce signs of spinal cord toxicity (Walker SM et al 2012).

In a study on mice, clonidine was found to induce hypothermia and sedation in mice, which were then prevented by verapamil, nifedipine and cinnarizine (Czarnecka E et al 1994).

Repeat Dose Toxicity/Chronic Toxicity

Rats were administered with clonidine HCl at a dose of 0.8 mg/kg for 15 days. Clonidine, both acutely (on day 1) and chronically (on day 15), caused hypoactivity and loss of interest and investment in the environment. Acute and chronic imipramine pretreatments reversed these effects of clonidine (Enginar N et al 1990).

Adult male Beagle dogs were prepared with chronic lumbar epidural catheters and epidurally administered constant infusions of either saline (N = 10), or 80 µg/hr (N= 6), 200 µg/hr (N = 6), or 320 µg/hr (N = 12) clonidine hydrochloride at a rate of 4 ml/24 hr for 28 days. Saline

infusion showed no effect upon any behavioral measure. Clonidine produced a dose-dependent increase in thermal skin-twitch response latency (antinociception), lowering of respiration rate, heart rate, and blood pressure, and increased sedation. These effects were maximum at around Day 1 to 3, when a progressive adaptation was observed over the course of the study, except the respiration, which remained depressed. Clonidine produced no negative effects on body weight, body temperature, motor function, bowel or bladder function, or clinical pathology values. The dogs were deeply anesthetized and terminated after 28 days of continuous infusion. No clinically significant differences in protein or glucose concentration were displayed in cisternal cerebrospinal fluid taken at termination. Including control, dogs in all groups had a chronic inflammatory response in the epidural space, as indicated by fibrosis, foreign body giant cells, and lymphocytes, but no spinal cord pathology. Conclusively, in absence of any change in CSF composition, significant spinal cord pathology, or signs of tissue or organ toxicity, the epidurally administered clonidine appears to be safe at infusion rates up to 320 µg/hr and at infusate concentrations up to 2 mg/ml (Yaksh TL et al 1994).

After administration of clonidine (0.1 mg/kg) orally to rats for 2 months, clonidine produces an up-regulation of α-adrenergic, cholinergic (muscarinic) and serotonergic (5-HT,) receptors in brain regions mainly involved in cardiovascular regulation (Gulati A et al 1991).

Subacute (12-13 weeks) and chronic (26-78 weeks) toxicity studies have ruled out any increased morbidity or mortality due to a cumulative effect or possible organ damage. No abnormality has been recorded in blood, urine or internal organs after subacute dosages. In rats, there is a clear dose-related lag in weight gain, and sedation with a brief hyperactive phase immediately following the administration of the drug. Dogs show a dose-related restriction of growth; female dogs in subacute i.v. toxicity studies were anovulatory with high daily doses (0.5 mg/kg). Glycosuria has been found in rabbits receiving 1 mg/kg daily for 30 days. No significant drug induced pathological or histological change in the circulatory and parenchymatous organs of the rat or in the endocrine organs of mice and rabbits has been observed (Product monograph, 2012).

Male albino Swiss mice (weight: 25–30 g) were given intraperitoneal injection of clonidine 2 mg/kg/day, chronically for 10 days. Clonidine was found to induce physical dependence in mice, which can be prevented by lithium. The withdrawal signs of clonidine are very similar to opioid withdrawal signs (Dehpour AR et al 2002). In rats, withdrawal of chronic clonidine

in low dosage (30 µg/kg/day i.c.v., 7 days) elicited short-lasting blood pressure elevations (upswings) and withdrawal of a higher dose (300 µg/kg/day s.c., 7 days) induced tachycardia and upswings (Jonkman FA et al 1985).

Four adult male baboons (*Papio anubis*) weighing 25.5–29.5 kg were surgically prepared with a chronically indwelling silastic catheter for intravenous self-administration of clonidine. Baboons, who were abusing cocaine, started abusing clonidine at doses of 0.0001–0.056 mg/kg per injection after at least 15 days (Weerts EM et al 1999).

Chronic administration of drugs acting on monoamines like clonidine, disturb the spontaneous activity and behavioral state dependency of the monoaminergic cells, influences the neurotransmitter turnover and change the sensitivity of both pre- and post-synaptic receptors (Mirmiran M et al 1986).

Carcinogenicity

Using a gene in vivo-derived expression profile classifier, clonidine is categorized as non-carcinogen (Doktorova TY et al 2012). In a 132-week (fixed concentration) dietary administration study in rats, clonidine hydrochloride administered at 32 to 46 t imes the maximum recommended daily human dose was unassociated with evidence of carcinogenic potential (Product monograph, 2012).

Genotoxicity and Mutagenicity

In mutagenicity studies on different test systems, such as Ames *Salmonella Typhimurium*, *E. Coli* and UDS Rat Hepatocytes, the mutagenicity results for clonidine were found to be negative (CCRIS 1998).

In rats, clonidine-treatment (50 µg/kg, s.c., once daily) suppressed neuropeptide Y (NPY) gene expression in the adrenal gland, probably at the level of transcription, by activation of the α_2-adrenoceptor (Higuchi H et al 1991). In rats, clonidine produced some unusual transcriptional regulatory mechanisms, which induced heat-shock protein (HSP) expression in the aorta (Moen RJ et al 1998). Clonidine was injected into the rat fetal brain (5 µg in 5 µl of saline) or subcutaneously to the rat pups (1, 10 µg in 50 µl of saline). After 3 days, clonidine increased the level of apoptotic enzyme caspase-3 mRNA expression and enhanced the DNA fragmentation in the brainstem of the 21-day-old fetuses and 8-day-old rats, suggesting that clonidine facilitates cell death in the developing brainstem (Dygalo NN et al 2004).

In an in vitro assessment model of drug toxicity from chronic exposure, in rat hepatocyte cultures, clonidine was cytotoxic at 170 µ M but did not induce changes at lower concentrations during 24 h exposure. In co-culture of rat or human hepatocytes with rat liver epithelial cells, clonidine gave no significant cytotoxic effect after 7 days. Clonidine 100 µM did not affect cell morphology of rat hepatocytes in co-culture, even after 12 days of treatment (Ratanasavanh D et al 1988).

Reproductive and Developmental Toxicity

In studies on male rats, clonidine (0.25 mg/kg, 6 min pretest) induced a profound deficit in intromissive and ejaculatory behavior in mating tests and decreased the incidence of seminal emission and the number of penile responses (erections, cups and flips) in ex copula penile reflex tests. After 0.005, 0.025 or 0.25 mg/kg clonidine, erectile dysfunction was observed at a similar degree, whereas 0.0005 mg/kg was without effect (Clark JT et al 1990).

In Dogs (weight: 8.5 to 10 kg), a low dose (0.2 - 0.4 µg/kg) of clonidine suppress the intracorporal pressure (ICP)-increase markedly via direct injection into the internal pudendal artery (IPA), which supplies the penile blood flow. Clonidine intravenous injection at a dose of 1.6 - 3.2 µg/kg also profoundly reduced the ICP-increase, but only negligibly lowered the systemic arterial pressure (SAP). In both routes, when the ICP-increase was reduced, the IPA blood flow (IPAF) decreased coincidentally. These results suggest clonidine could suppress penile erection by acting locally in the penile structure, possibly resulting from a penile vasoconstriction involving α_2 adrenoceptor (Lin SN et al 1988).

In lactating female Wistar rats, clonidine 10 µg/kg decreased prolactin (PRL) release. In male Wistar rats treated with clonidine 10 µg/kg, a significant decrease of plasma prolactin was observed (Navarra P et al 1991).

In fetal lambs, clonidine was infused into lateral ventricle for up to 24 hours at 128 to 135 days' gestation. The incidence and episode duration of fetal breathing was significantly reduced for the duration of the clonidine infusion period. Electrocortical activity cycling became irregular and rapid, and the incidence of high-voltage electrocortical activity (corresponding to quiet sleep) was reduced. Fetal heart rate slowed but arterial pressure was unchanged. On the contrary, after the infusion was over, both the breathing incidence and episode duration significantly increased compared to control, with continuous high-amplitude

breathing for several hours. Conclusively, long-term use of clonidine during pregnancy could slow lung development by reducing fetal breathing activity (Bamford OS et al 1990).

In eight chronically prepared normotensive near term ewes, clonidine 300 µ g was intravenously injected. Clonidine produced significant toxicity - intraamniotic pressure increased, uterine blood flow decreased, maternal and fetal serum glucose increased, and maternal and fetal PO_2 decreased. Maternal and fetal blood pressure and serum cortisol were unaffected, while heart rate reduced. At serum clonidine concentrations <1.0 ng/ml, no adverse maternal or fetal effects were noted. Direct fetal infusion of clonidine did not reduced fetal arterial PO_2 levels, although heart rates reduced and serum glucose levels increased. These effects of clonidine are because of its actions on α_2-adrenergic receptors. Conclusively, clonidine may adversely affect the fetus by direct actions and by changing the maternal physiology (Eisenach JC et al 1989).

In a series of experiments, male rat pups were subcutaneously injected with clonidine 100µg/kg twice daily from postnatal day 8 - 21. After the last clonidine injection, on day 21 postnatally, free noradrenaline (NA) metabolite MHPG (3-methoxy-4-hydroxyphenylethylene glycol) concentrations were decreased in all brain regions, suggesting a clonidine-induced decrease in NA neuronal activity during development (Mirmiran M et al 1988).

Pregnant Wistar rats were given clonidine (20 or 100 µg/kg) in the drinking water over gestation days 1 to 21. Maternal weight gain, litter size or litter weight were unchanged as were physical and behavioral development measures. At maturity, male but not female rats weighed less than the controls. Prenatal exposure to clonidine did not alter the beneficial effects of enriched rearing on maze solving. Clonidine-exposed (100 µg/kg) female rats that were raised in enrichment had a significantly increased mortality rate (Ryan CL et al 1990).

Fertility of male or female rats was unaffected by clonidine hydrochloride doses as high as 150 µg/kg or about 3 t imes the maximum recommended daily human dose (MRDHD). Fertility of female rats did, however, appear to be affected (in another experiment) at dose levels of 500 to 2000 µg/kg or I 0 to 40 times the MRDHD (Product monograph, 2012).

Reproduction studies performed in rabbits of doses up to approximately 3 times the maximum recommended daily human dose (MRDHD) of clonidine hydrochloride have revealed no evidence of teratogenic or embryotoxic potential in rabbits. In rats, however, doses as low as

1/3 the MRDHD were associated with increased resorptions in a study in which dams were treated continuously from 2 months prior to mating. Increased resorptions were not associated with treatment at the same or at higher dose levels (up to 3 times the MRDHD) when dams were treated on days 6-15 of gestation. Increased resorptions were observed at much higher levels (40 times the MRDHD) in rats and mice treated on days 1-14 of gestation (lowest dose employed in that study was 500 µg/kg) (Product monograph, 2012).

Neurotoxicity

In female beagle dogs, clonidine 12.5 or 25 µg/kg was administrated via intrathecal or extradural catheters once daily for 14 consecutive days. Clonidine does not show any significant neurotoxic effects (Gordh TE et al 1984). Rats were injected daily with clonidine 1.63 or 16.3 µg for 14 consecutive days via intrathecal catheters. Clonidine gave rise to no detectable neurotoxic changes in these doses (Gordh T Jr et al 1986). Pregnant CD rat dams were given daily subcutaneous injections of clonidine on days 8 through 20 of gestation. Maternal body weight was decreased dose dependently. At postnatal day 1, brain ornithine decarboxylase (ODC) activity was unchanged in clonidine -exposed pups of both sexes (Ali SF et al 1988).

Other Toxicity Studies

Clonidine causes severe hypoxemia after bolus intravenous administration and can cause mild hypoxemia after bolus intraspinal administration in sheep (Eisenach JC et al 1987 and Eisenach JC 1988). A review of the literature in a wide array of animal models showed that in the tissues around the spinal cord, clonidine produce vasoconstriction, but the clinical consequences of this response in humans is not well defined (Rowlingson JC 1993).

[redacted]

[redacted]

Excipients:

Table 10: List of Excipients

Name of ingredient	Function	Reference to Standard	Content in 5ml of product*
Clonidine hydrochloride	Active ingredient	Ph. Eur.	0.050mg
Methyl parahydroxybenzoate	[redacted]	Ph. Eur.	[redacted]
Sodium dihydrogen phosphate monohydrate	[redacted]	B.P.	[redacted]
Disodium hydrogen phosphate anhydrous	[redacted]	Ph. Eur.	[redacted]
Sucralose	[redacted]	Ph. Eur.	[redacted]
Purified water	Vehicle	Ph. Eur.	Qs to 5.0ml

*Each 5ml of Clonidine hydrochloride Sugar Free Oral Solution contains [redacted] Sodium dihydrogen phosphate monohydrate [redacted] Disodium hydrogen phosphate anhydrous equivalent to 1.8280mg of total sodium.

All of the excipients used in the formulation satisfy the relevant European Pharmacopoeial monograph requirements. These excipients have been widely used in pharmaceutical products and their safety profile is well established. The rationale for using the excipients is discussed in detail in Module 3.2.P.2, Pharmaceutical Development. The safety of the excipients and the content relative to the Acceptable Daily Intake (ADI) as specified by the European Union and WHO is discussed below.

Methyl parahydroxybenzoate

The WHO has recommended an acceptable daily intake (ADI) limit for Methyl parahydroxybenzoate as 10mg/kg body weight (WHO JECFA, Methyl parahydroxybenzoate, 1973).

Based on the maximum daily dose of the proposed Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution, the maximum possible daily exposure to Methyl parahydroxybenzoate (MHB) in target population has been demonstrated in the table below:

Table 11: Calculation of the quantity of Methyl parahydroxybenzoate ingested following administration of Clonidine Oral Solution

Target population	Average Body Weight for age group (Kg, Lower limit)	Maximum Daily Dose (MDD) of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution for age group (mg)	Volume of Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution at the MDD (ml)	Content of MHB in 5ml of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution (mg)	Daily MHB exposure (mg) at the MDD of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution (mg)	Maximum acceptable daily intake of Methyl parahydroxybenzoate (ADI = 10mg/kg bw) (mg)	Is the daily Methyl parahydroxybenzoate exposure at the MDD exceeding ADI proposed by WHO? (Yes/No)
Adults (18 years and above)	70	0.15	15	[redacted]	[redacted]	700	No

Based on the proposed posology and the calculations in the table immediately above on the safety of Methyl parahydroxybenzoate, the levels are well below the nominal threshold level and consequently an important safety margin of error or tolerance is built in to the formulation. Therefore, the safety concerns are within the safety-risk benefit profile typically applied by the industry and the currently accepted regulatory directives.

Phosphorus (From Sodium dihydrogen phosphate monohydrate and Disodium hydrogen phosphate anhydrous)

Sodium dihydrogen phosphate monohydrate is widely used in pharmaceutical formulation and not usually associated with adverse effects. Oral consumption of phosphates act as mild saline laxatives only when administered orally (2–4g of monobasic sodium phosphate in an aqueous solution is used as a laxative) (Rowe RC et al, Sodium phosphate monobasic, 2009).

The quantity of sodium dihydrogen phosphate monohydrate, which could be ingested daily at the maximum daily dose by the target population, [redacted] Hence sodium dihydrogen phosphate monohydrate level used for target population is below the thresholds level of laxative effective, even at the highest doses as per SmPC and therefore the product can be considered safe to consume for the population.

Disodium phosphate anhydrous occurs as a white powder. It is used in a wide variety of pharmaceutical formulations as a buffering agent and as a sequestering agent. It is very soluble in water, more so in hot or boiling water; practically insoluble in ethanol (95%). The anhydrous material is soluble 1 in 8 parts of water. It is incompatible with alkaloids, antipyrine, chloral hydrate, lead acetate, pyrogallol, resorcinol and calcium gluconate, and ciprofloxacin (Rowe RC et al, Sodium phosphate dibasic, 2009).

In proposed formulation, [redacted] The quantity of disodium hydrogen phosphate anhydrous, which could be ingested daily at the maximum daily dose by the target population, [redacted] Hence, disodium hydrogen phosphate anhydrous level used for target population is below the thresholds level of laxative effective, even at the highest doses as per SmPC and therefore the product can be considered safe to consume for the population.

The WHO has recommended a Group MTDI for phosphorus from all sources, as 70mg/kg body weight, expressed as P (WHO JECFA, Sodium dihydrogen phosphate, 1982).

Based on the maximum daily dose of the proposed Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution, the maximum possible daily exposure to Phosphorus (From Sodium dihydrogen phosphate monohydrate and Disodium hydrogen phosphate anhydrous) in target population has been demonstrated in the table below:

Table 12: Calculation of the quantity of Phosphorus (From Sodium dihydrogen phosphate monohydrate and Disodium hydrogen phosphate anhydrous) ingested following administration of Clonidine Oral Solution.

Target population	Body Weight (Kg)	Maximum Daily Dose (MDD) of the Clonidine hydrochloride 50micrograms/5 ml Sugar Free Oral Solution for age group (mg)	Volume of Clonidine hydrochloride 50micrograms/5 ml Sugar Free Oral Solution at the MDD (ml)	Daily exposure at the MDD (mg)		Daily P exposure in at the MDD (mg)		Total Daily P exposure at the MDD of the Clonidine hydrochloride 50micrograms/5 ml Sugar Free Oral Solution (mg)	Maximum acceptable daily intake of Phosphorus (ADI = 70mg/kg bw) (mg)	Is the daily Phosphorus exposure at the MDD exceeding ADI proposed by WHO? (Yes/No)
				Sodium dihydrogen phosphate monohydrate	Disodium hydrogen phosphate anhydrous	Sodium dihydrogen phosphate monohydrate	Disodium hydrogen phosphate anhydrous			
Adults (18 years and above)	70	0.15	15	[redacted]					4900	No

Phosphorus (From Sodium dihydrogen phosphate monohydrate and Disodium hydrogen phosphate anhydrous)

Based on the proposed posology and the calculations in the table immediately above on the safety of Phosphorus (From Sodium dihydrogen phosphate monohydrate and Disodium hydrogen phosphate anhydrous), the levels are well below the nominal threshold level and consequently an important safety margin of error or tolerance is built in to the formulation. Therefore, the safety concerns are within the safety-risk benefit profile typically applied by the industry and the currently accepted regulatory directives.

Sucralose

Sucralose is used as a sweetening agent in beverages, foods, and pharmaceutical applications. It has a sweetening power approximately 300–1000 times that of sucrose and has no aftertaste. It has no nutritional value, is noncariogenic, and produces no glycemic response. Sucralose is used in concentration of 0.03 to 0.24%. [redacted]

Sucralose is generally regarded as a nontoxic and non-irritant material and is approved, in a number of countries, for use in food products. Following oral consumption, sucralose is mainly unabsorbed and is excreted in the feces (Rowe RC et al, Sucralose, 2009).

The WHO has recommended an acceptable daily intake (ADI) limit for Sucralose as 15mg/kg body weight (WHO JECFA, Sucralose, 1990).

Based on the maximum daily dose of the proposed Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution, the maximum possible daily exposure to Sucralose in target population has been demonstrated in the table below:

Table 13: Calculation of the quantity of Sucralose ingested following administration of Clonidine Oral Solution.

Target population	Average Body Weight for age group (Kg, Lower limit)	Maximum Daily Dose (MDD) of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution for age group (mg)	Volume of Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution at the MDD (ml)	Content of Sucralose in 5ml of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution (mg)	Daily Sucralose exposure (mg) at the MDD of the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution (mg)	Maximum acceptable daily intake of Sucralose (ADI = 15mg/kg bw) (mg)	Is the daily Sucralose exposure at the MDD exceeding ADI proposed by WHO? (Yes/No)
Adults (18 years and above)	70	0.15	15	[redacted]		1050	No

Based on the proposed posology and the calculations in the table immediately above on the safety of Sucralose, the levels are well below the nominal threshold level and consequently an important safety margin of error or tolerance is built in to the formulation. Therefore, the safety concerns are within the safety-risk benefit profile typically applied by the industry and the currently accepted regulatory directives.

2.4.5 Integrated Overview and Conclusions

In reference of this application, clonidine is indicated for the following two conditions:

- The prophylactic management of migraine or recurrent vascular headache.
- The management of vasomotor conditions commonly associated with the menopause and characterised by flushing.

Clonidine is centrally acting analgesic agent. Clonidine was initially developed as an centrally acting antihypertensive agent, acting through the central mechanisms which regulate blood pressure in the body. One of the well documented actions of clonidine is cerebral vasodilation. This effect of clonidine has led its use in the management of migraine. Clonidine acts to reduce the CSD in the migraine, which is supported by the reports of the various studies. There are several studies in animals to support the proposed indication. In-vitro and in -vivo studies to support its vasodilatory action and its usefulness in migraine are described in the relevant sections of this document.

Safety of clonidine is well documented. Acute studies in animals like rat, mice, dog, guinea pig as well as spontaneously hypertensive rats; Repeat dose toxicity studies in rat, mice, dogs, baboons; Carcinogenicity in rat; Genotoxicity in various organisms; in-vitro cytotoxicity in rat hepatocytes.; Reproductive toxicity in rats lambs and ewes. Apart from these studies the specific physiological system were also documented like immunotoxicity in rat and rabbit; neurotoxicity in dogs; ocular toxicity in rats. The excipients used in the Clonidine hydrochloride 50micrograms/5ml Sugar Free Oral Solution are strictly controlled to the monograph requirements given in the current Ph. Eur. edition and in-house specifications. The excipients presented in the formulation are widely used in pharmaceutical products and are well-established.

Clonidine is well absorbed and distributed orally. Its distribution in various tissues is rapid. It is extensively metabolised in liver. It is excreted in urine. Clonidine follows linear pharmacokinetics up to certain dose in animals. After which the urinary excretion gets affected and the clearance of clonidine gets affected in such a way that its pharmacokinetics no longer remains linear.

Non-clinical data revealed no special hazard for humans based on conventional studies of safety pharmacology, repeated-dose toxicity, genotoxicity, carcinogenic potential and toxicity to reproduction and development.

Overall preclinical pharmacological, pharmacokinetic and toxicological data indicate that Clonidine may be used effectively and safely in the proposed indications. The product proposed will be administered in an equivalent manner to the currently marketed UK reference product, Dixarit Tablets 25 micrograms. The dose recommendations and indications are the same as that of the reference product and therefore based upon the data provided no non-clinical issues are to be expected that might be deleterious to the grant of a product licence.

2.4.6 Literature References

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