Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Meaning of Abbreviation
AUC	Area Under the Curve
AUC_{0-t}	Area under the concentration time curve up to last measurable concentration
ATC	Anatomical Therapeutic Chemical
CEP	Certificate of Suitability
C_{max}	Maximum concentration observed
CSM	Committee on Safety of Medicines
EDQM	European Directorate for the Quality of Medicines
EMA	European Medicines Agency
FDA	Food & Drug Administration (USA)
GLP	Good Laboratory Practice
IBS	Irritable Bowel Syndrome
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Abbreviation	Meaning of Abbreviation
LD_{50}	Dose which causes death of 50% of tested population
MSDS	Material Safety Datasheet
NDA	New Drug Application
NHP	Non-human primate
OECD	Organisation for Economic Co-operation and Development
Ph.Eur	European Pharmacopoeia
QOS	Quality Overall Summary
q.s.	Quantum satis
SmPC	Summary of Product Characteristics
T_{max}	Time at which the maximum concentration (C_{max}) is observed
$t_{1/2}$	Time for the concentration levels to fall to 50% of their value.

Introduction

As outlined in Module 2.2, Rosemont Pharmaceuticals Limited have developed a new liquid formulation and proposes to apply for UK National Marketing Authorisations for two strengths of oral solutions:

Spironolactone 50mg/5ml Oral Solution

Spironolactone 25mg/5ml Oral Solution

The active ingredient, spironolactone, is an established active substance. Spironolactone products have been available on the UK market and widely used in clinical practice for many years since 1957 (*Kagawa 1957*). The therapeutic use and safety profile of spironolactone is well established within the United Kingdom and European Union.

The new Spironolactone Oral Solutions are proposed as hybrid applications under Regulation 52 (previously Article 10(3) of 2001/83/EC), referring to the reference product Aldactone 100 mg Film-Coated Tablets, which have been authorised in the UK and European Community for more than 10 years. The UK licences for Aldactone 25 mg, 50 mg and 100 mg Film-Coated Tablets are currently held by Pfizer Limited (PL00057/0927 - 0929).

Aldactone Film-Coated Tablets, as approved in UK, are indicated for treatment of: Congestive cardiac failure; Hepatic cirrhosis with ascites and oedema; Malignant ascites; Nephrotic syndrome; and Diagnosis and treatment of primary aldosteronism.

The Aldactone Tablets Summaries of Product Characteristics (SmPCs) include posology for adults, plus both elderly and paediatric populations, subject to cautionary statements.

These Spironolactone Oral Solution products have been developed as new formulations equivalent to an existing marketed product, Aldactone Film-Coated Tablets, with equivalent indications and posology proposed.

As described in Module 2.5, a bioequivalence study has been conducted to compare Spironolactone 50mg/5ml Oral Solution (dose of 10 mL i.e. 100 mg) to the reference medicinal product Aldactone 100 mg Film-Coated Tablets (PL00057/0927, Pfizer Healthcare). The results of the biostudy demonstrated equivalent extent of absorption (AUC_{0-t}), but differences in C_{max} between the Test (oral solution) and Reference (originator tablet) products resulting from T_{max} differences.

Therefore, while the drug substance is well known, the new applications cannot be filed as simple generic applications under Article 10.1, since the pharmaceutical form differs from that of the reference product and due to the differences in C_{max}. UK National applications for these products are therefore proposed as hybrid applications under Article 10(3) of 2001/83/EC.

The applications will rely in part on the established safety and efficacy for the existing registered product, supplemented by the pharmacokinetic data from the bioequivalence study, but with no new clinical efficacy, clinical safety, or non-clinical studies undertaken by the applicant.

Overview of the Nonclinical Testing Strategy

No new non-clinical studies have been conducted as none are considered necessary, with the established safety profile of spironolactone products over many years use.

This Non-Clinical Overview has been compiled to provide a concise review of the scientific literature relating to the pharmacology, pharmacokinetics and toxicological data of spironolactone. The up to date non-clinical scientific literature cited in this Non-Clinical Overview provides all the required evidence to support the licensing of these products and their proposed use. A literature search was undertaken using the Google scholar and PubMed databases on 5th March 2021. The search terms used included: spironolactone toxicity, carcinogencity, chronic, acute, preclinical, nonclinical, regulatory toxicology submissions. The searches were limited to publications in English.

The Non-Clinical Overview has been written by an appropriately qualified person, providing an appropriate review of the relevant non-clinical pharmacology, pharmacokinetics and toxicology.

2.4.1 Pharmacology

Pharmacotherapeutic group: Diuretic; potassium-sparing agents; Aldosterone antagonists

ATC code: C03DA01

2.4.1.1 Mechanism of Action

Spironolactone, as a competitive aldosterone antagonist, increases sodium excretion whilst reducing potassium loss at the distal renal tubule. It has a gradual and prolonged action (*SPC Aldactone Tablets*).

2.4.1.2 Primary Pharmacodynamics

Spironolactone is a competitive inhibitor of the binding of aldosterone to its receptor. Its most important site of action is the distal portion of the renal tubules, where it combines with soluble cytoplasmic aldosterone receptors to form complexes which are inactive and which do not bind to nuclear acceptor sites, thus preventing a chain of biochemical events leading to the synthesis of physiologically active proteins. Thus, it promotes a diuresis and acts as an antihypertensive agent. Administration of spironolactone is associated with a reversal of the electrolytic changes attributed to aldosterone and with a dose-dependent increase in plasma-renin activity in rats (*Dollery, 1998*).

A separate but less important effect is direct inhibition of adrenal synthesis of aldosterone (*Dollery, 1998*).

Spironolactone has been shown to inhibit the formation of the aldosterone complex in the nuclei of kidney epithelial cells of adrenalectomised rats. This occurs at concentration ratios that inhibit the action of aldosterone in the rat in vivo. In vitro studies with kidney tissue slices from rats demonstrated displacement of aldosterone from its specific intracellular receptors by spironolactone (*Ochs, 1978*).

2.4.1.3 Secondary Pharmacodynamics

Given the long-established safe use of spironolactone, no new information on secondary pharmacodynamics has been identified.

2.4.1.4 Safety Pharmacology

Given the long-established safe use of spironolactone, no new information on safety pharmacology has been identified.

2.4.2 Pharmacokinetics

2.4.2.1 Absorption and Bioavailability

Studies of the absolute oral bioavailability of spironolactone in animals using a solution of spironolactone in ethanol and water indicated rapid and nearly complete gastrointestinal absorption of the drug (*Karim, 1978*).

Spironolactone is well absorbed orally and is principally metabolised to active metabolites: sulfur containing metabolites (80%) and partly canrenone (20%). Although the plasma half-life of spironolactone itself is short (1.3 hours) the half-lives of the active metabolites are longer (ranging from 2.8 to 11.2 hours). (*SPC Aldactone Tablets*)

2.4.2.2 Distribution

Both spironolactone and canrenone are over 90% bound to plasma proteins. (*SPCs for Genethics Spironolactone 25mg Tablets and Accord Spironolactone 25mg film-coated Tablets*)

2.4.2.3 Metabolism

Following the administration of 100 mg of spironolactone daily for 15 days in non-fasted healthy volunteers, time to peak plasma concentration (t_{max}), peak plasma concentration (C_{max}), and elimination half-life ($t_{1/2}$) for spironolactone is 2.6 hr., 80 ng/ml, and approximately 1.4 hr., respectively. For the 7-alpha-(thiomethyl) spironolactone and canrenone metabolites, t_{max} was 3.2 hr. and 4.3 hr., C_{max} was 391 ng/ml and 181 ng/ml, and $t_{1/2}$ was 13.8 hr. and 16.5 hr., respectively. (*SPC Aldactone Tablets*)

2.4.2.4 Excretion

Elimination of metabolites occurs primarily in the urine and secondarily through biliary excretion in the faeces. The renal action of a single dose of spironolactone reaches its peak after 7 hours, and activity persists for at least 24 hours. (*SPCs for Aldactone Tablets, Genethics Spironolactone 25mg Tablets and Accord Spironolactone 25mg film-coated Tablets*)

2.4.2.5 Pharmacokinetic Drug Interactions

The proposed Summary of Product Characteristics is aligned with that of the reference product, Aldactone 25mg Tablets, and therefore known drug interactions will be included in SmPC Section 4.5.

2.4.3 Toxicology

It appears that most spironolactone toxicology studies pre-date the adoption of GLP in 1976. In addition, no associated toxicokinetic data was available.

2.4.3.1 Single Dose Toxicity

The acute toxicity of spironolactone is low in rats, mice and rabbits, so that there is a high potential therapeutic ratio.

The oral LD50 for rat, mouse, and rabbit was greater than 1000 mg/kg suggesting that spironolactone was well tolerated by these 3 species (*EMA, 2012*).

2.4.3.2 Repeat-Dose Toxicity

In a 26-week rat study dosing up to 700 mg/kg/day, there were no treatment-related findings except for a dose-related increase in liver weight. This increase in liver weight was considered to be an adaptive change, probably due to metabolism of spironolactone rather than a toxic change (*Product monograph, Pfizer, 2015*).

In a 13-week NHP study and a 26-week dog study dosed up to 250 mg/kg/day, there were no treatment-related findings. Unfortunately, no reasons were given why two non-rodent species studies were undertaken or were not given a higher dose levels of spironolactone (*Product monograph, Pfizer, 2015*). It is considered probable that both species could not tolerate higher dose levels.

A single 13-week study in dogs dosed up to 20 mg/kg/day was considered by the EMA to be GLP compliant. The finding in this study was an increase in the liver weight in males only at the highest dose (20 mg/kg/day). In addition, there was an increase in progesterone, decrease in testosterone and prostate atrophy at all dose levels including the therapeutic dose (2 mg/kg/day) (*EMA, 2012*). Most dogs used in toxicology studies are less than 1 year old and are sexually immature. Therefore, it is likely that the dogs used in this 13-week study were immature and the prostate had not fully developed. Thus, it is probable that there was delayed prostate maturity rather than prostate atrophy.

During chronic testing, histological changes were noted in rat liver, thyroid gland and male genitalia. There were also changes in monkey testes and male mammary glands (*Dollery 1998*).

In a 78-week study in rats, a number of malignant tumours occurred, mainly affecting skin and connective tissue, liver, thyroid and kidney. However, by comparison with a control group, it was not clear whether the incidence of tumours was greater than would be expected in any ageing rat population. Thus, whether spironolactone predisposes to tumour formation remains an unresolved question.

Evidence of carcinogenicity in rats resulted in licences for spironolactone being amended to exclude its use in essential hypertension and idiopathic oedema (*CSM 1988; Drug and Theraputics Bulletin, 1988*).

2.4.3.3 Reproductive Toxicity

There are a number of reproductive toxicology studies with spironolactone, in rat, mouse and rabbits.

In female rats and mice spironolactone induced infertility by inhibiting ovulation. In male rats there was a decreased sperm count. These studies did not establish a no-effect level (*EMA, 2012*)

In rats dosed at 200 mg/kg/day between gestation days 13 and 21 late embryogenesis and foetal development) resulted in femininization of male pups. Dose levels of 100 and 50

mg/kg/day resulted in reproductive tract changes including delayed prostate development. It is considered that these changes are due to hormonal disruption. There were similar changes in mice and rabbits (*FDA, NDA 12-151/S, 2009* & *Pfizer MSDS, 2014*).

In addition, canrenoate is a major active metabolite of spironolactone which a rat carcinogen can be found in human milk (*FDA, NDA 12-151/S, 2009*).

Overall, spironolactone is classified as a human male teratogen which femininizes the foetuses due its antiandrogenic action. The FDA recommends that women of child bearing potential should only be treated with spironolactone in conjunction with suitable contraception (*FDA, NDA 12-151/S, 2009*).

2.4.3.4 Genotoxicity

Spironolactone was negative in the Ames test (bacterial) and in the mammalian cell test without activation. The major metabolite of spironolactone, canrenoate was also negative in the Ames test, but it was positive in the mammalian cell test with activation. In an in vivo mammalian study canrenoate was not mutagenic up to 270 mg/kg (*FDA, NDA 12-151/S, 2009* & *Pfizer MSDS, 2014*).

2.4.3.5 Carcinogenicity

There were two carcinogenicity studies with spironolactone in the rat. The first was an 18-month study dosed via the diet at 50,150 and 500 mg/kg/day. There were statistically significant increases in benign adenomas of the thyroid and testes. In addition, male rats showed a dose-related increase of hepatocellular hypertrophy and hyperplastic nodules in the liver. The second carcinogenicity study was 24-months using the same strain of rat and the dietary route of administration dosed at 10, 30 and 100 mg/kg/day. There was a statistically significant dose-related increase in benign adenoma of the thyroid and a dose-related increase in liver weight.

There were no carcinogenicity studies with spironolactone in the mouse. This would be expected under current OECD guidelines.

Canrenoate:

Canrenoate is a pharmacologically active major metabolite of spironolactone. Mutagenic studies required metabolic activation before showing a positive (mutagenic) effect in bacteria and yeast. In a 26-week rat study dosed up to 360 mg/kg/day, the high dose induced hypertrophy of the thyroid and adrenal and a few mammary tumours. In a 52-week rat study dosed up to 270 mg/kg/day which induced granulocytic leukaemia in peripheral blood and bone marrow of both sexes. In addition, in the females there were several mammary tumours. In a 24-month rat carcinogenicity study dosed up to 270 mg/kg/day resulted in treatment-related increases in myelocytic leukaemia, hepatic, thyroid, testicular and mammary tumours (*Product monograph, Pfizer, 2015* & *FDA, NDA 12-151/S, 2009*).

Spironolactone has been shown to produce tumours in rats when administered at high doses over a long period of time. The significance of these findings with respect to clinical use is not certain. However, the long term use of spironolactone in young patients requires careful consideration of the benefits and the potential hazard involved. Spironolactone or its metabolites may cross the placental barrier. With spironolactone, feminisation has been observed in male rat foetuses. The use of Aldactone in pregnant women requires that the anticipated benefit be weighed against the possible hazards to the mother and foetus. (*SPC Aldactone Tablets*)

2.4.3.6 Local Tolerance

There was no effect of spironolactone on the immune system.

OECD guideline studies with spironolactone showed a mild degree of eye and skin irritation. Skin sensitisation studies showed conflicting results. Overall, it is considered that spironolactone has shown a potential for skin sensitisation (*EMA, 2012*).

2.4.3.7 Toxicity Studies in Combination with Other Drugs

Given the long-established safe use of spironolactone, no new information has been identified.

The proposed Summary of Product Characteristics is aligned with that of the reference product, Aldactone 25mg Tablets, and therefore known drug interactions will be included in SmPC Section 4.5.

2.4.3.8 Safety assessment of impurities and degradation

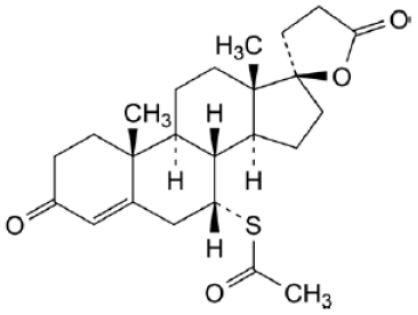
Impurity profile of the active substance

Spironolactone active substance is manufactured by [redacted] and is the subject of an EDQM Certificate of Suitability (CEP), held [redacted] The Certificate of Suitability has been granted by EDQM, confirming compliance with the requirements of Ph.Eur. monograph 0688 for spironolactone. The details have been assessed by the EDQM and a copy of the current certificate is provided in Module 3.2.R.

The active substance spironolactone is a white or yellowish-white powder, with molecular formula $C_{24}H_{32}O_4S$, molecular mass 416.6, and chemical name:
S-[(2′*R*)-3,5′-Dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′furan]-7α-yl] ethanethioate

Spironolactone is practically insoluble in water, but soluble in alcohol. The structural formula is as follow:

Figure 1: Spironolactone structural formula



The quality of the drug substance is controlled in accordance with the respective monograph of the European Pharmacopoeia (Monograph 0688) and the impurity profile testing for related substances is in accordance with the monograph, with additional tests as required by the CEP for residual solvents.

The control of impurities in the active substance is carried out by the following specification, as further detailed in QOS and Module 3.2.S.4:

Table 1 Impurity specification for active substance: Spironolactone (Manufacturer: [redacted]

IMPURITY	Chemical name	LIMIT (%)
Related substances (according to Ph. Eur. monograph):		
Impurity A	S-[(2′*R*)-3,5′-dioxo-5′*H*-spiro[androst-4-ene-17,2′furan]-7α-yl] ethanethioate (Δ20-spironolactone)	[redacted]
Impurity C	(2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′furan]- 3,5′-dione (aldone)	[redacted]
Impurity D	S-[(2′*R*)-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′furan]-7α-yl] ethane(dithioperoxate) (disulfanyl-spironolactone)	[redacted]
Impurity E	S-[(2′*R*)-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′furan]-7β-yl] ethanethioate (7β-spironolactone)	[redacted]
Impurity F	(2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4,6-diene-17,2′furan]- 3,5′-dione (canrenone)	[redacted]
Impurity I	S-[17α-(ethoxymethyl)-17β-hydroxy-3-oxoandrost-4-en-7α-yl] ethanethioate	[redacted]
Any other unspecified impurity	-	[redacted]
Total impurities	-	[redacted]
Residual Solvents (according to CEP requirements):		
[redacted]	-	[redacted]
[redacted]	-	[redacted]

NMT = Not More Than

These impurities have not been found as degradants during forced degradation or stability studies.

The impurities are controlled according to the limits established in Ph. Eur. Monograph for the drug substance and further controlled in the Finished Product Specification, as detailed below.

It is noted that the levels of residual solvents applied by the EDQM to methanol and acetone on the CEP for the active substance conform to the concentration limits permitted in pharmaceutical products without further justification (according to ICH guideline Q3C):

[redacted]

Suitability and safety of excipients in the formulation

Apart from the active ingredient, the proposed oral solution contains only two excipients, as shown in the following table.

Both excipients are the subject of and comply with current Ph.Eur. monographs and are widely used in pharmaceutical products.

Table 2 Excipients used in Spironolactone Oral Solutions

Ingredient	Specification	Function	Amount per 5 ml*
Peppermint Oil	Ph.Eur	Flavour	[redacted]
Medium Chain Triglycerides	Ph.Eur	Solvent	[redacted]

* same quantities apply to both product strengths

[redacted] and [redacted] are both MCT based liquid medicinal products. Both products have no reported safety or tolerability issues or complaints. An oil based (medium chain triglycerides) [redacted] was also approved in [redacted]

Spironolactone has an unpleasant taste, so peppermint oil is included to mask the drug taste and odour, to aid patient compliance. Peppermint oil is widely used in pharmaceuticals, traditional herbal remedies, confectionery and foods, as both a flavouring agent and as an active itself (e.g. for symptomatic relief of gastrointestinal problems such as IBS).

There are no known safety issues with these two excipients at the chosen levels.

Impurity profile of the drug product

Identified and unspecified degradation products are controlled in the finished product specification, as follows:

Table 3 Impurity specification for Spironolactone Oral Solutions

IMPURITY	Release Limit	Shelf-life Limit (%)
Related substances (according to Finished Product Specification):		
Specific identified degradation product: [redacted]	[redacted]	[redacted]
Any unspecified degradation product	[redacted]	[redacted]
Total degradation product	[redacted]	[redacted]

NMT = Not More Than

[redacted]

the patient will naturally become exposed to the molecule, and in fact, gains therapeutically from canrenone.

Individual unspecified organic degradation products are controlled in the finished product [redacted]

Total Degradation Products are controlled in the finished product specification at levels of: [redacted]

Risk assessments on Spironolactone 50mg/5ml Oral Solution described in Module 3.2.P.5.5 state that:

- [redacted]
- [redacted]

Integrated overview and conclusions

Spironolactone is a well-established substance which is currently available in a number of other licensed products in the United Kingdom.

Spironolactone has been shown to inhibit the binding of aldosterone to its receptor. A separate, but less important effect, is direct inhibition of adrenal synthesis of aldosterone.

No toxicological issues are considered to be raised by the use of spironolactone in this oral solution formulation. The excipients in the formulation would not be anticipated to influence the pharmacology or toxicology of the drug.

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Summary of Product Characteristics 06/2018, Accord Spironolactone 25mg film-coated Tablets (Accord Healthcare Limited PL20075/0456)– *available at MHRA website*