EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

PROTEIN BINDING OF CHLORTHALIDONE AND ITS INTERACTION WITH FOOD

*Gayathri V., Jeeshna G., Nandhini S. and Sangavi M. S.

Karur Tamilnadu India.

*Corresponding Author: Gayathri V.

Karur Tamilnadu India.

ABSTRACT

Chlorthalidone is a thiazide-like diuretic that leads to fluid retention and was approved for the treatment of hypertension either individually or in combination with other Hypertension drugs. Chlorthalidone is poorly soluble in water at room temperature and soluble in Ethanol, Methanol and Sodium hydroxide. The objective of this study is to estimate the protein binding of chlorthalidone individually and in presence of grape juice. UV spectral studies authenticate the spectra obtained were matched with standard pure drug UV spectra show the maximum absorbance peak at 260 nm. The concentration of Chlorthalidone having affinity towards Protein across the Semipermeable membrane is determined. Interactions between food and drugs can unintentionally reduce or increase the effect of the drug, resulting in alteration of desired therapeutic activity. Food drug interaction is conducted for this drug using Grape juice as a food content and the concentration of the bound and unbound drug is determined using UV spectral absorbance of the drug. Hence based on the observation performed at invitro, protein binding studies of chlorthalidone individually and in combination with grape juice, it was observed that the concentration of unbound drug was found to increase in presence of grape juice at various time intervals as compared to the drug individually administered. Hence it can be concluded that there was a significant interaction between chlorthalidone and grape juice at invitro level which has to further conformed by pursuing the invivo study.

KEYWORDS: chlorthalidone, interaction, protein binding.

INTRODUCTION

Chlorthalidone is used to control and treat high blood pressure. It falls under the category of medications known as thiazide-like diuretics (water pills), which helps to stop the body from taking in too much salt, which can lead to fluid retention. Chlorthalidone has few advantages as a therapy for hypertension, oedema, and calcium nephrolithiasis. **(Connor C. Kerndt et al, 2022)**

Since 1960, the FDA has approved Chlorthalidone for the treatment of hypertension. A first-line medication for hypertension is Chlorthalidone. Both alone and in combination with other antihypertensive medications, such as beta-blockers or clonidine, this medicine is used to treat hypertension. Further to that, oedema is treated with it.**(Connor C. Kerndt et al, 2022)**

Oedema in pregnancy could be caused on by disease pathogenesis as well as the physiologic and mechanical implications of pregnancy. When oedema is caused by pathologic factors during pregnancy, chlorthalidone is as recommended as just it is in the absence of pregnancy. Dependent oedema in pregnancy is caused by limitation of venous return by the swollen uterus, and it is effectively treated by elevating the lower extremities and using a support hose. (www.accessdata.fda.gov)

Despite having similar structural properties, chlorthalidone and hydrochlorothiazide have very different pharmacokinetics. For example, despite having a half-life of about 40 to 60 hours and a large volume of distribution, chlorthalidone gradually leaves the plasma compartment through tubular secretion. • The administration of furosemide, the most used diuretic in the loop diuretic class, might be confounded by its wildly unpredictable bioavailability, which ranges from 12% to 112%. • In terms of lowering blood pressure over the course of the night, 25 mg of chlorthalidone is significantly more powerful than 50 mg of hydrochlorothiazide. **(Sica et al, 2011)**

It's important to note that the ALLHAT (Antihypertensive and Lipid-Lowering Therapy to Prevent Heart Attack) trial evaluated chlorthalidone with other first-line antihypertensives including calcium channel blockers and angiotensin-converting-enzyme inhibitors (ACE-I). Chlorthalidone had a lower correlation with stroke than ACE-I and a lower association with heart failure than calcium channel blockers, according to ALLHAT, therefore thiazide-like diuretics should be thought of as the first-line treatment treating hypertensive patients. According to the results of this research, chlorthalidone reduces the blood pressure,

specifically the systolic pressure, earlier and more significantly than lisinopril and amlodipine.**(Connor C. Kerndt et al, 2022)**

Although chlorthalidone is a structurally related sulfanilamide derivative to conventional thiazides, its pharmacokinetics do differ significantly. Chlorthalidone has a half-life of about 50 hours, which is extremely lengthy, whereas data on the half-life of HCTZ has been contradictory, with one study suggesting a lowest limit as brief as 2.4 hours while other studies show a half-life between 6.5 and 9 hours.**(Connor C. Kerndt et al, 2022)**

Although being often recommended, hydrochlorothiazide (HCTZ) has little or no proof that it lowers morbidity and mortality when compared to a placebo or a control. Chlorthalidone and indapamide have, however, been shown to be more effective than placebo in lowering morbidity and mortality. Despite this, neither the JNC-8 nor the Canadian Hypertension Education Programme recommendations have given CTD or IDP the edge over HCTZ. Nonetheless, suggestions from the 2011 NICE HTN guidelines have this regrettable gap (Box 1) [6]. Arguments in favour of these suggestions can be made based on the striking pharmacological discrepancies between the structures of CTD and IDP and those of HCTZ, which are both devoid of the traditional thiazide benzothiadiazine dioxide rings and hence differ from each other pharmacologically [4]. Thus, the term "thiazide-like diuretics" is misplaced. **(DiNicolantonio et al, 2015)**

A biphasic elimination pattern with a rapid phase and a slow secretory phase is typical of the excretion, which accounts for around 50% of the injected dose that is excreted by the kidney unmetabolized. Moreover, it has a mean time to peak blood concentration of six hours following a single dose. By comparing blood concentrations following administration of various dose strengths, it can be seen that there are some limits in dose-response.**(Connor C. Kerndt et al, 2022)**

MECHANISM OF ACTION
A long-acting oral diuretic with antihypertensive properties is chlorthalidone. The distal convoluted tubule of the nephron appears to be the site of the action. Chlorthalidone's diuretic effects lead to reductions in cardiac output, plasma volume, total exchangeable sodium, glomerular filtration rate, and renal plasma flow. Sodium and water depletion appear to be the basis for chlorthalidone and related medications' antihypertensive impact, despite the fact that their exact mode of action is not fully understood. Similar to the thiazide diuretics, chlorthalidone lowers serum potassium levels in a dose-dependent manner, raises blood sugar and uric acid levels, and has the potential to lower sodium and chloride levels.(www.accessdata.fda.gov)

ADMINISTRATION
Chlorthalidone is available only as an oral medication.
Strength: 25 mg, 50 mg
Hypertension: starting from 12.5 to 25 mg daily, maximum dose: 100 mg daily
Heart failure: starting from 12.5 mg or 25 mg daily, maximum dose: 100 mg daily
Generalized edema: starting from 50 g or 100 mg daily, maximum dose: 200 mg daily
Calcium nephrolithiasis: 25 mg/daily

When choosing the dose, the patient's age must also be taken into account. Chlorthalidone should be used at lower doses to the elderly (those over 65), commencing at 6.25 mg to 12.5 mg per day and titrating gradually as previously suggested. **(Connor C. Kerndt et al, 2022)**

Chlorthalidone is only available as an oral medication. Chlorthalidone comes in 25 mg and 50 mg pills, which can be divided for proper dosing. The dosage regimens change depending on the clinical indication. The recommended starting dose for the treatment of heart failure is 12.5 mg or 25 mg once daily, with titrations up to 100 mg once daily as needed. Dosing for generalized edema starts at 50 to 100 mg per day and can be titrated up to 200 mg per day. Chlorthalidone, which is typically provided at 25 mg/daily, can be used to treat calcium nephrolithiasis as previously mentioned. When choosing the dose, the patient's age has to be considered. Those over 65 who fall under the geriatric population should receive lower doses of chlorthalidone, starting at 6.25 to 12.5 mg and gradually increasing up to a daily maximum of 25 mg. Chlorthalidone is a Beers criterion drug and should only be taken under proper control. **(Connor C. Kerndt et al, 2022)**

ADVERSE EFFECT
Most organ systems are affected negatively by chlorthalidone, however to varying degrees and in different ways. Moreover, electrolyte derangement is a frequently reported side effect of this medicine because it promotes diuresis and changes the physiology of the nephron. Chlorthalidone typically causes hypokalemia, but it can also lead to hyponatremia or hypochloremia. For individuals receiving chlorthalidone regularly throughout hypertension treatment, monitoring serum electrolytes is critical due to these recognised derangements. **(Connor C. Kerndt et al, 2022)**

Cardiovascular System: Orthostatic hypotension. Central Nervous System: dizziness, vertigo, headache, paraesthesia, xanthopsia, weakness, restlessness and insomnia. **(Khoy et al, 2001)**

GI: Anorexia, gastric irritation, nausea, vomiting, bloating, diarrhoea, constipation, jaundice, pancreatitis, GU: impotence, hematologic, leukopenia, thrombocytopenia, agranulocytosis, aplastic anaemia; Dermatologic: purpura, photo-sensitivity dermatitis, rash, urticaria, necrotizing angiitis, cutaneous vasculitis,

exfoliative dermatitis, erythema multiforme, metabolic: hyperglycemia, Which is not the effect of Diuretic but by an effect of hypokalemia, hyperuricemia, hyponatremia. **(Khoy et al, 2001)**

PROTEIN BINDING (DRUG DISTRIBUTION)

Protein-drug binding refers to the quantity of a medication that binds to proteins. There are two types of drug binding to proteins: irreversible and reversible. Weak chemical bonds are typically involved in reversible. Covalent bonding causes irreversible to develop. Moreover, the drug's tissue toxicity or carcinogenicity can promote irreversible binding. The pharmacokinetics and pharmacodynamics of a medicine are significantly influenced by the plasma protein binding of the drug. The amount of medicine that is bound to plasma protein determines how much of the drug is free-active. Drug efficiency may be increased or decreased by protein binding. Drugs may be more effectively distributed throughout the body if they bind to proteins. It might also alter the time that medicine takes to work. Drug-protein complexes that are themselves biologically active are formed when a protein binds to a drug, altering the therapeutic effect. **(Jennifer L. Davis, 2018)**

There are two drug types in the blood sample.
• Bound
• Unbound.

The medication that is protein-bound is neither metabolised nor eliminated. It is pharmacologically inactive because of the drug's pharmacokinetic and pharmacodynamic inertness. the unbound drug undergoes metabolism in the liver and the other tissues. If a medicine is less bound, it will be able to disperse or traverse cell membranes more effectively. Unbound fraction provides information about pharmacologic effects. The unbound portion can be broken down and/or eliminated. Except in vast paracellular spaces like capillaries, the protein-bound medication cannot pass membranes. This limits the medication, which is strongly bound to plasma protein, to vascular compartments. There is no action with the bound fraction. Despite this, it balances with the plasma's unbound portion. When elimination reduces the concentration of the unbound fraction, it dissociates. Plasma protein binding is comparable to the temporary storage of the medication as a result. **(Lavisha Jindal, Gurmeet Singh, 2021)**

One medication has the ability to attach to several protein binding sites. Additionally, many drugs may bind to a single site. Drugs binding to the same location develop displacement interactions. The drug that binds with a high affinity will displace the drug that binds with a low affinity. The amount of free-form will double in concentration if the drug bound is displaced. This, however, is only temporary because the substance that was displaced will diffuse into tissues and also get metabolised or eliminated. The two separate highly bonded medications won't compete with one another, and it's possible that their binding sites won't even overlap. **(Lavisha Jindal, Gurmeet Singh, 2021)**

Protein binding is a quick-acting equilibrium mechanism that is reversible. Due to its compliance with the law of mass of action, it is also regarded as an absorption process. For a simple 1:1 protein-drug complex, the interaction between a protein and a drug molecule can be illustrated as follows:

$$P+D = PD$$

P represents the concentration of free binding sites for the unbound protein. D is the concentration of the medication that is not bound. The concentration of a protein-drug combination is known as PD. The phrase becomes: when the law of mass action is applied.

$$K= [PD]/ [P][D]$$

K is the association constant.

$$[PD]=K[P][D] \quad (1)$$

If total protein concentration is given by [Pt], then,

$$[Pt]=[P]+[PD] \quad (2)$$

Total protein concentration is the sum of unbound protein and protein present in the complex. Or,

$$[P]=[Pt]-[PD] \quad (3)$$

Substituting the value of [P] in equation (1), then
[PD]=K[D]([Pt]-[PD])
[PD]=K[D][Pt]-K[D][PD]
[PD]+K[D][PD]=K[D][Pt]
[PD](1+K[D])=K[D][Pt]
[PD] /[Pt]=K[D] /1+ K[D]

Where [PD]/[Pt] represents an average number of drug molecules bound per mole of protein [Pt].
Substituting [PD]/[Pt] by r,

$$r =K[D] /1+ K[D]$$

If there are n number of independent binding sites, then:
r=n K[D] /1+ K[D] (4)
r(1+K[D])=nK[d] r+rK[D]=nK[D]
r= nK[D]–rK[D]
r=[D] (nK–rK)
r/[D]=nK-rK (5)
This is known as a **Scatchard plot.**
If the quantity and type of protein in the experimental system are unknown, equation (5) is not employed to analyse the results. A different equation is then applied,

$$[Db] /[D]=-K[Db]+ nK[Pt]$$

Where Db gives the concentration of the bound drug. The ratio of [Db]/[D] is plotted against [Db]. K is obtained from the slope and nK[Pt] is determined from the intercept. **(Lavisha Jindal, Gurmeet Singh, 2021)**

SIGNIFICANCE OF PROTEIN BINDING

Protein binding may improve or decrease a drug's effectiveness. Agents with limited protein binding generally penetrate tissue easier than those with high protein binding, although they are eliminated much more quickly. Differences seem to be of minimal therapeutic significance for medicines that are less than 80–85 percent protein bound. Yet, the tissue penetration and half-life of highly and minimally protein-bound agents may differ significantly from one another. Many different plasma proteins, including albumin, are targets for dr binding. The clinician should be concerned that the agent may be bound in vivo to one of these "minority" plasma proteins if the percentage of protein-bound medication is higher in human blood than in a straightforward albumin solution. (**Richard T. Scheife, 1989**)

Stress, surgery, liver or kidney failure, and pregnancy are just a few of the many events that might affect the concentration of a number of plasma proteins. Free drug concentrations are a better indicator of clinical benefit in these situations than total concentrations are. When comparing competing treatments, formulary committees must understand the therapeutic importance of qualitative and quantitative variations in protein binding.(**Richard T. Scheife, 1989**)

Drug protein binding can affect a drug's pharmacodynamics (interaction with receptors and enzymes) as well as pharmacokinetics (absorption, distribution, and clearance). The ability of medications to attach to plasma proteins, and more especially to human serum albumin, is important among all the proteins they may potentially do so. Hence, estimating plasma protein binding is crucial for characterising a novel chemical during drug development. (**Anagha A. Damre, Krishna R. Iyer, 2012**)

Adsorption

The concentration of free drugs is reduced by the absorbed drug's binding to plasma proteins. First-order kinetics is employed in the conventional dose form. Hence, increased protein binding may disrupt the equilibrium of absorption. As a result, the sink state and concentration gradient are established, and they now serve as the driving force for further absorption. (**Lavisha Jindal, Gurmeet Singh, 2021**)

Systemic solubility of drugs

By attaching to lipoprotein, which serves as a barrier hydrophobic substance, neutral endogenous macromolecules, a water-insoluble medication such as steroids, and heparin are circulated and dispersed in tissue. Drug metabolism is reduced after binding to the protein, and the biological half-life of the medication is thus extended. Only the portion that is unbound is metabolised. (**Lavisha Jindal, Gurmeet Singh, 2021**)

Distribution

With several medicines, concentration-dependent protein binding has been seen. It has been demonstrated that several antibiotics, such as example beta-lactams and macrolides, exhibit saturable concentration-dependent PB within the concentration range that may come from a typical therapeutic dose. Higher percentages of an unbound drug are present in plasma as a result of the binding sites on proteins being more saturated when a drug's plasma concentration rises. Yet, at high drug concentrations, nonlinear protein binding can also take place in the other direction with reduced unbound fractions. (**Markus et al 2011**)

Plasma protein displacement interaction

Drug A is persistently administered to steady-state. Afterwards, drug B is introduced, which raises the free fraction of drug A. Will the effect of A be greatly boosted given that its effects are caused by its free concentration? the quantity of medication A that is displaced will be available to interact with its receptor as well as be disseminated to the rest of the body. Redistribution will reduce the amount of the rise in the free concentration of A, with the increase being largest for medicines with the smallest starting distribution volumes. This will also lessen the degree to which the action of A increases, as drug distribution from plasma to its receptor is sometimes not instantaneous but is typically characterized by a first-order process with comparable rate to general drug distribution. However, any subsequent increase in free A will likewise be available for drug eradication. Free concentrations of A will revert to the pre-B level for medications with low clearance, where intrinsic clearance of the free drug is the only factor affecting mean steady-state free drug concentration. Hence, any improvement in A's pharmacological action will be momentary and unsustainable. (**P. E. ROLAN, 1994**)

Elimination

Only the medicine that is unbound can be eliminated. Drugs cannot enter the glomerulus filtration or metabolizing organ due to protein binding. Glomerular filtration is mainly eliminated by Glomerular filtration process.
• Have an effect on how drugs are distributed to body tissues.
• Reduces the amount of free medication that can be used to reach cell action sites.
• Lessens or eliminates the drug's pharmacological action since it can no longer bind to the receptor site.
• The drug-protein complex has biological activity on its own.
• Displacement of the bound drug due to co - administration of a special medication that also binds to plasma proteins may result in substantial toxicity because the free medication interacts with the receptor to elicit a pharmacological response.
• Extends the duration of action of a drug.

• Delays a drug's elimination and increases its accumulation. **(Lavisha Jindal, Gurmeet Singh, 2021)**

FOOD DRUG INTERACTION
Unintentionally reducing or increasing a drug's effect because of its interactions between food and drug can result either therapeutic failure or increased toxicity. This could have an adverse effect on patient care, increase morbidity, and prolong the duration of hospitalisation or treatments. There is a need for strategies to recognize and stop the emergence of food drug interactions, even though the extent of the issue is unknown. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

MECHANISM
Pharmacokinetic and pharmacodynamic interactions are two categories that describe interactions between food and drugs. By far the most frequent interactions are pharmacokinetic ones, in which a food changes how a medicine is absorbed, distributed, metabolised, or eliminated. Only a few pharmacodynamic interactions exist where food or food derivatives have an impact on the receptor-level activity of a medication. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

1.1 CHARACTERISTICS OF THE DRUG
A drug's physical and chemical properties have a significant impact on how it might interact with food. Various drugs from the same drug class or different dosage forms of the same medication can have entirely distinct chemical properties and thus completely different food-drug interactions. The physicochemical characteristics of the drug alone typically cannot accurately predict food-drug interactions, hence interaction investigations of drug pharmacokinetics and effects with or without concurrent food intake are necessary. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

1.2 CHARACTERISTICS OF THE MEAL
The amount and nature of a meal, as well as the precise time of drug intake in relation to a meal, may all affect the development of food-drug interactions. For instance, a high fat content often increases the bioavailability of lipophilic medications, either because the drugs are more soluble in the body (such as albendazole and isotretinoin) or due the stimulation of bile production (e.g. griseofulvin and halofantrine). The bioavailability of some medications, such as digoxin and lovastatin, may also be decreased by a high fibre content due to the medicines' binding to the fibre. The definitions of fasting are varied, nevertheless, and these circumstances are sometimes not thoroughly examined. Unless otherwise specified, the word "fasting" in this review refers to abstaining from eating for at least an hour before and at least two hours after taking a medicine. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

1.3 PHARMACOKINETIC EFFECT PARAMETERS
The majority of medications have a correlation between their bioavailable and their effect, making alterations in bioavailability a crucial effect parameter of food-drug interactions. Absorption and first-pass metabolism are required for bioavailability. The most significant drug-food interactions occur when changes in a drug's absorption either from chemical interactions between the medication and food (such as chelation) or from physiological responses to food intake (changes in gastric acidity, bile secretion or gastrointestinal motility). Food-drug interactions that solely affect the rate of drug absorption are frequent but rarely have therapeutic significance. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

1.4 CLINICAL EFFECT PARAMETERS
It is not always easy to understand how pharmacokinetic parameters relate to pharmacological effects, and typically, food-induced changes in a drug's bioavailability can only be interpreted as a sign of a food-drug interaction. Only when the influence of food consumption on the pharmacological effect of the drug is quantified can the clinical importance of a specific food-drug interaction be assessed. Depending on the drug type (antibacterial, antihypertensive, lipid-lowering, or anticoagulant, for example), the relevant effect characteristics will vary, and for many pharmaceuticals, the pharmacological effect is not easily quantified. **(Lars E. Schmidt and Kim Dalhoff, 2002)**

MATERIALS AND EQUIPMENTS
APPARATUS
Open-ended cylinder, beaker, pipette, glass rod, standard flask.

MATERIALS

	MATERIALS	SOURCE
1.	Chlorthalidone	Gift sample
2.	Ethanol	Changshu fine chemical co., ltd.
3.	Egg albumin flakes	Labogens., ltd.
4.	Conc. HCL	NICE chemicals.
5.	Egg	

EQUIPMENTS

S.NO	EQUIPMENT	SOURCE
1.	UV Visible spectrophotometer	JASCO V-530 UV 1600.
2.	Melting point apparatus	MP-DS TID-2000

EXPERIMENTAL SECTION

1. Optimization of standard curve
i) 10mg of chlorthalidone is dissolved in 10ml of ethanol and made upto 100ml with distilled water

ii) 20, 40, 60, 80 and 100ug/ml are prepared by diluting 2, 4, 6, 8 & 10ml of stock solution respectively with water.

iii) 1ml of each sample is added to 1 ml of ethanol, shaken well and is allowed to stand for 3 minutes.

iv) Absorbance at 260 nm is noted and a graph of concentration [X-axis] Vs absorbance [Y axis] is plotted.

2. Determination of melting point
The melting point of a drug can be determined by introducing a tiny amount into a small capillary tube, attaching this to the stem of a thermometer centred in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete.

3. Preparation of 2.8 x 104 M solution of egg albumin
0.315g of egg albumin flakes is dissolved in 25ml of distilled water. It is shaken well [till flakes are completely dissolved] and is kept aside.

4. Preparation of egg membrane
Soak whole egg in 0.5N HCL solution. The outer calcareous shell get dissolved and then cut off the part of eggshell membrane and remove the inner contents. Wash the obtained membrane thoroughly in distilled water and store in the refrigerator.

5. Studies on Protein Binding
i) 25ml of 2.57×10 M of Chlorthalidone is prepared using a buffer of pH 7.

ii) A open ended cylinder is taken and a semipermeable membrane is tied on to the neck of the boiling tube.

iii) The egg albumin solution is taken in the semipermeable membrane.

iv) The tube is then immersed into the buffer solution containing the drug Chlorthalidone taken in a beaker.

v) Immediately at zero time 4ml of the solution is pipetted out from the beaker and is replaced with 4ml of ethanol.

vi) Similar readings are taken at 25, 40 and 60 minutes intervals and percentage of drug bound in the form of a protein complex can be determined.

6. Studies on Protein Binding drug interaction
i) 50ml of 2.57×10 M of Chlorthalidone is prepared using a buffer of pH 7.

ii) A boiling tube open on both sides is taken and a semipermeable membrane is tied on to the neck of the boiling tube.

iii) The egg albumin solution is taken in the semipermeable membrane.

iv) 50ml of edible grape juice is taken in a beaker containing the buffer solution.

iv) The tube is then immersed into the buffer solution containing the drug Chlorthalidone taken in a beaker.

v) Immediately at zero time 4ml of the solution is pipetted out from the beaker and is replaced with 4ml of ethanol.

vi) Similar readings are taken at 25, 40 and 60 minutes intervals and percentage of drug interact with grape juice can be determined. **(C.Vijaya Ragavan)**

RESULT AND DISCUSSION

1). SOLUBILITY
Solubility test for chlorthalidone was carried out in different solvents such as water, ethanol, methanol, sodium hydroxide and the results are given in below.

Table 1: Solubility of Chlorthalidone in different solvents.

SOLVENTS	SOLUBILITY
Water	Insoluble
Ethanol	Completely soluble
Methanol	Completely soluble
NaOH	Soluble

2). PREPARATION OF STANDARD GRAPH OF CHLORTHALIDONE
In the preparation of standard graph, by using methanol and sodium hydroxide the linearity was not obtained.
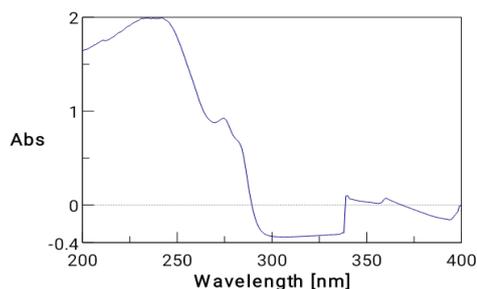
Figure 1: UV Spectrum of Methanol.
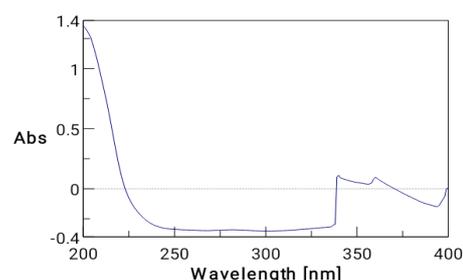
Figure 2: UV spectrum of NaOH.

By using ethanol as a solvent, the linearity was obtained.
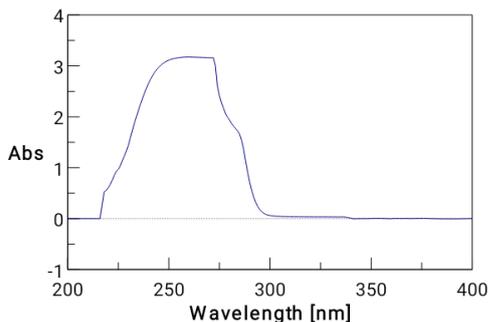
Figure 3: UV Spectrum of Ethanol.

In the preparation of standard graph, by using ethanol the linearity was obtained between 0.6 – 0.9 mcg/ml concentration of Chlorthalidone and the regression value was found to be r^2 = 0.998273. Thus Chlorthalidone obeys Beer lambert's law at the concentration between 0.6 – 0.9 mcg/ml. The results are shown in fig.1 Beer lambert's law at the concentration between 0.6 – 0.9 mcg/ml. The results are shown in fig.4

From the peak, the obtained λ max was found to be 260nm.
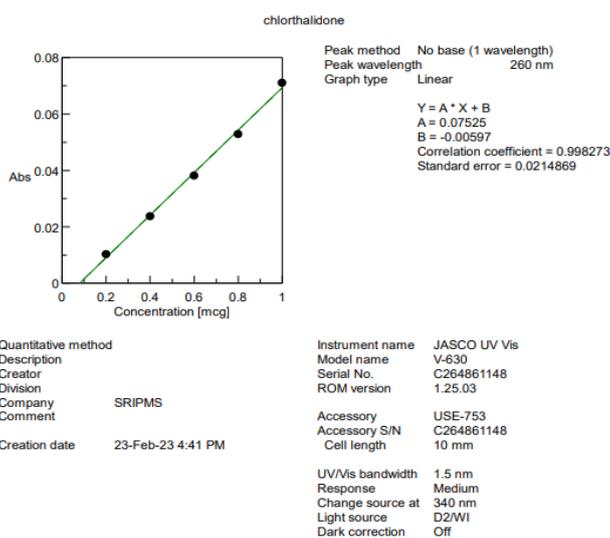
Figure 4: Standard graph of Chlorthalidone.

Table 2: Concentration & Absorbance of Chlorthalidone.

No.	Sample	Comment	Conc. [mcg]	Abs	260.0 nm
*Blank					
1			0.2	0.0103	
2			0.4	0.0237	
3			0.6	0.0381	
4			0.8	0.0528	
5			1	0.071	

3). MELTING POINT

The melting point of chlorthalidone was determined by using the melting point apparatus. The obtained melting point was found to be 239℃.

4). PROTEIN BINDING STUDIES OF CHLORTHALIDONE

The unbound/free drug present in the sample solution was estimated by withdrawing 4ml of the sample solution at various intervals like immediately after the experiment is started, 25, 40, 60minutes and replaced with equal volume of ethanol respectively. The absorbance of each sample withdrawn was measured using UV spectrophotometry at 260nm. The concentration of free drug present in the solution is calculated with the help of standard graph.

The absorbance was plotted and was intrapolated to get the concentration in mcg/ml.

From the concentration (mcg/ml), the concentration in moles is calculated.

Thus, the calculation was done and the percentage of chlorthalidone bound to protein was found.

Table 3: Concentration of Chlorthalidone after Protein Binding.

Time Min.	Absorbance Nm	Concentration Mcg/ml	Concentration Moles
0	0.6030	8.0926	23.888
25	0.4626	6.2268	18.380
40	0.4539	6.1112	18.039
60	0.4053	5.4653	16.132

5). PROTEIN BINDING STUDIES OF CHLORTHALIDONE WITH GRAPES

The unbound/free drug present in the sample solution was estimated by withdrawing 4ml of the sample solution at various intervals like immediately after the experiment is started 25, 40, 60minutes and replaced with equal volume of ethanol respectively. The absorbance of each sample withdrawn was measured using UV spectrophotometry at 260nm. The concentration of free drug present in the solution is calculated with the help of standard graph.

The absorbance was plotted and was intrapolated to get the concentration in mcg/ml.

From the concentration (mcg/ml), the concentration in moles is calculated.

Thus, the calculation was done and the percentage of Chlorthalidone bound to protein was found.

Table 3: Concentration of Chlorthalidone after Drug-Food Interaction.

Time Min	Absorbance Nm	Concentration mcg/ml	Concentration Moles
0	0.5841	7.8414	23.146
25	0.5742	7.7099	22.758
40	0.5392	7.2447	21.385
60	0.5212	7.0055	20.679

The concentration of unbound drug at **25 min** was found to be **6.2268 mcg/ml.**

The concentration of unbound drug along with grape juice at **25 min** was found to be **7.7099 mcg/ml.**

The concentration of unbound drug at **40 min** was found to be **6.1112 mcg/ml.**

The concentration of unbound drug along with grape juice at **40 min** was found to be **7.2447 mcg/ml.**

The concentration of unbound drug at **60 min** was found to be **5.5653 mcg/ml**

The concentration of unbound drug along with grape juice at **60 min** was found to be **7.0055mcg/ml.**

The concentration of the unbound drug after **25 minutes** was found to be **1.2996** fold decreases.

The difference in concentration of the unbound drug after **40 minutes** was found to be **1.3242** fold decreases.

The difference in concentration of the unbound drug after **60 minutes** was found to be **1.4807** fold decreases

The difference in concentration of the unbound drug along with grape juice after **25 minutes** was found to be **1.017** fold decreases.

The difference in concentration of the unbound drug along with grape juice after **40 minutes** was found to be **1.0823** fold decreases.

The difference in concentration of the unbound drug along with grape juice after **60 minutes** was found to be **1.1193** fold decreases.

The concentration of unbound drug was low compared to that of unbound drug along with grape juice. The presence of unbound drug in the concentrations increases with food drug interaction.

SUMMARY AND CONCLUSION

Chlorthalidone (Water Pill) is a thiazide -like diuretics class of drug used in the management and treatment of Hypertension by Antagonizing sodium chloride symporter in the distal convoluted tubule of the nephron.

At therapeutic concentrations in plasma, many drugs exist mainly in bound form. The fraction of drug that is free in aqueous solution can be as low as 1%, the remainder being associated with plasma protein. It is a unbound drug that is pharmacologically active.

The physical characteristic (Solubility, Melting point) of pure drug is checked as per Indian pharmacopoeia guidelines. There is no marked change in characteristic of the drug.

UV spectral studies authenticate the spectra obtained were matched with standard pure drug UV spectra show the maximum absorbance peak at 260 nm.

The concentration of Chlorthalidone having affinity towards Protein across the Semipermeable membrane is determined.

Interactions between food and drugs can unintentionally reduce or increase the effect of the drug, resulting in therapeutic failure or increased toxicity.

Food drug interaction is conducted for this drug using Grape juice as a food content and the concentration of the bound and unbound drug is determined using UV spectral absorbance of the drug.

The concentration of unbound drug after 25min, 40 min and 60 min was found to be 6.2268 mcg/ml, 6.1112 mcg/ml and 5.5653 mcg/ml respectively. The concentration of unbound drug was found to be decrease at 40 min and 60 min by 1.32 and 1.48 fold respectively as compared to the concentration at 25 min which infers that the drug has more affinity towards protein as the contact time of drug and protein is increased.

The concentration of unbound drug after 25min, 40 min and 60 min in the presence of grape juice was found to be 7.7099 mcg/ml, 7.2447 mcg/ml and 7.0055 mcg/ml respectively. The concentration of unbound drug was found to increase by 1.01 fold at 25, 40 and 60 min in presence of grape juice which infers that there is a significant interaction between drug-food interaction.

Hence based on the above observation performed at invitro level there is a significant interaction of chlorthalidone and grape juice as the concentration of drug increases in presence of grape juice. Hence this has to be further pursued at invivo level to find out whether there is significant interaction between drug and grape juice when co-administered.

BIBLIOGRAPHY

1. David S. Kountz, Andrew Goldman, John Mikhail, Mayer Ezer, Chlorthalidone: The Forgotten Diuretic, January 2021, Research share.
2. Jennifer L. Davis, in Equine Internal Medicine (Fourth Edition), 2018.
3. Richard T. Scheife, protein binding: what does it mean? DICP, The Annals of Pharmacotherapy, 1989 July-August; Volume 23, S27.
4. Lars E. Schmidt and Kim Dalhoff, Department of Clinical Pharmacology, Rigshospitalet, Copenhagen, Denmark, Food-Drug Interactions, February 2002; Pg. No: 1482-1486.
5. www.accessdata.fda.gov
6. Indian Pharmacopoeia, 2010; 2: 1076-1077.
7. Zvetanka D. Zhikova, Studies on drug- Human serum Albumin binding: The current state of the matter, 2015.
8. Brigitte Buscher' Bio analysis for plasma protien binding studies in drug delivery & drug development, 2017.
9. Wolfgang. A Ritschel, Binding of Drugs to biological material, American pharmacist Association, 2016, Seventh Edition.
10. Mirko Koziolek, Department of Biopharmaceutics and Pharmaceutical Technology, Centre of Drug Absorption and Transport, Germany, 15 June 2019; 134: 31-59.
11. Sandra J.Taler, Should Chlorthalidone be the diuretic of choice for Antihypertensive therapy, 2008; 293-297.
12. Giulia Dell'omo, Chlorthalidone improves Endothelial Medical vascular response in Hypertension, 2005; 10(4): 271.
13. C. Vijaya Ragavan, A Practical Handbook of Physical Pharmaceutics, Pg. No: 74-79.
14. Lavisha Jindal, Gurmeet Singh, Role and application of protein binding in drug distribution process, Pharmaspire, June 2022; 13: 35-41.
15. L J Francis, J B Houston, D Hallifax, Impact of Plasma Protein Binding in Drug Clearance Prediction: A Data Base Analysis of Published Studies and Implications for In Vitro-In Vivo Extrapolation, Epub, December 2020.
16. Anna Vangone and Alexandre M J J Bonvin, Contacts-based prediction of binding affinity in protein–protein complexes, April 2019.
17. Andreas Svennebring, The impact of plasma protein binding on toxic plasma drug concentration Article, January 2016.
18. Karolina Wanat, Biological barriers, and the influence of protein binding on the passage of drugs across them, March 2020.
19. Rang and Dales Pharmacology, Sixth edition, Pg-no: 102.
20. Camelia Harapu Diaconu 1, Magdalena Cuciureanu, L Vlase, Rodica Cuciureanu, Food-drug interactions: grapefruit juice, 2011 Jan-Mar.
21. Md. Mostafizur Rahman, Srinivas Mukund Vadrev, Arturo Magana-mora et al, A novel Graph mining approach to predict and evaluate food drug Interaction, 2022.
22. Partha Pratim Mahata, Abhimunayu Thakur et al, A Review on pharmacist's role in Mitigation of Food-Drug Interaction, 2015.
23. Judy K. Anderson, Potential Food-Drug Interactions in long turn care, Journal of Gerontological Nursing, 2012; 38: 4.
24. McCabe, Beverly, Prevention of Food-Drug interactions with special emphasis on older adult, Current opinion in Clinical Nutrition and Metabolic care, 2004; 7(1): 21-26.
25. Connor C. Kerndt; Jayesh B. Patel, Chlorthalidone statpearls, November 1, 2022.
26. Anagha A. Damre, Krishna R. Iyer, The Significance and Determination of Plasma Protein Binding Part III. General Principles of ADME. Drug Transporters and Other Mechanisms of Transport, 15 October 2012.
27. P.E.Rolan, Plasma protein binding displacement interactions- Why are they still regarded as clinically important?
28. Markus A. Zeitlinger, Hartmut Derendorf, Johan W. Mouton, Otto Cars, William A. Craig, David Andes, and Ursula Theuretzbacher, Protein Binding: Do We Ever Learn? July 2011; 3067–3074.
29. Lakshmi Manasa S. Chekka, Arlene B. Chekka et al, Race-Specific Comparisons of Antihypertensive and Metabolic Effects of Hydrochlorothiazide and Chlorthalidone, The American Journal of Medicine, July 2021; 134(7): 918-925.e2
30. Domenic A Sica 1, Barry Carter, William Cushman, Lee Hamm, Thiazide and loop diuretics, Epub 2011 Jul 27.

31. Mohammad Khoyi University of Nevada-Reno, Reno, USA, David Westfall University of Nevada-Reno, Reno, USA, Chlorthalidone, 2007 Elsev.