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Effects of Chronic Kidney Disease and Ochratoxin A and their interaction in some Biochemical Parameters of Humans in Karbala Province

A Thesis Submitted to the Council of the College of Applied Medical Sciences University of Kerbala in Partial Fulfillment of the Requirements for the Degree of Master in Clinical Laboratories

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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We, the examining committee, certify that we have read the thesis entitled "Effects of Chronic Kidney Disease and Ochratoxin A and their interaction in some Biochemical Parameters of Humans in Karbala Province" and have examined the student (**Othaim Razzaq Falayh**) in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories** with **Excellent** degree.

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10 / 9 / 2023

Dedication

Thanks, and praise to God Almighty first for the blessing of patience and the ability to accomplish the work, for God is praise for these blessings.

My angel in life, in the sense of love and compassion, who taught me the meaning of life and their presence is the reason for my success. Teach me to trust God and that I can reach them despite the difficulty of the road.

My parents

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Summary

Numerous studies have been conducted on food contamination with Ochratoxin A (OTA) in various nations throughout the world. More than 5 billion people in developing nations are found to be at risk of chronic OTA exposure according to studies. OTA is nephrotoxic with a range of adverse health effects, It also contributes to the development of Balkan endemic nephropathy. The aim of the study was detection the correlation between Ochratoxin A and Chronic kidney disease (CKD) effect in some level of a biochemical parameters, and their effect on thyroid hormones Triiodothyronine (T3), Tetraiodothyronine (T4), and Thyriod stimulating hormone (TSH).

This study was carried out in laboratories of the College of Applied Medical Sciences / University of Kerbala, private laboratories, and the Ministry of Science and Technology, /Baghdad, Iraq. During the period from 1/12/2022 to 1/4/2023, blood samples were collected from 100 patients with CKD and from 100 healthy control.

The results showed a significant difference in the levels of ochratoxin A in the blood plasma, as its levels in CKD patients reached 99%, and its levels in the blood plasma of healthy controls reached 32%. Where the percentage of females was more than males in the presence of OTA in the blood plasma of patients, and the percentages were as follows: females 56% and males 43%.

The results also showed that the concentration of OTA in the blood plasma of patients was significantly higher than those of healthy control, with male and females patients' concentrations reaching 23.475 and 23.943 ng/ml, respectively, while healthy control concentration was 2.075 and 2.171 ng/ml, respectively.

The results showed that levels of urea in healthy males and females were both within the normal range, whereas the presence of ochratoxin A was associated with an increase in urea levels in male patients to 140.34 mg/dL and in female patients to 121.53 mg/dL.

The a verage creatinine levels in male and female patients with chronic kidney disease increased to 3.655, 3.680 mg/dL, respectively, which is substantially higher than those in healthy control.

This study proved that ochratoxin A and CKD affected the normal levels of the studied hormones if it led to TSH levels decreasing in patients with CKD with a highly significant difference *P* value = 0.000192 when compared to levels in healthy individuals; where that levels

of TSH in blood serum of patients Male(PM) and patients Female (PF)with OTA and PF, without OTA decreased to 0.227, 0.247, and 0.241 µIU/mL respectively With high significantly different levels of TSH in the blood serum of healthy Male(HM) and healthy Female **(**HF) with OTA, HM and HF without OTA were 6.07, 3.757, 1.852, 1.692 µIU/mL respectively.

On the other hand, ochratoxin and chronic kidney disease caused an increase in the level of the T4 hormone ranged between 244.24- 322.627 nmoL/L, while the level of the hormone in healthy control ranged between 107.28_118.76 nmoL/L.

The result showed that the levels of T3 hormone increased to 19.461, 18.896, and 23.013 nmoL/L in the blood serum of PM, with OTA, PF, with OTA, and PF, without OTA, respectively with significantly different levels in the blood serum of HM, with OTA, HF, with OTA, HM, without OTA, and HF, without OTA which they were 1.988, 2.018, 2.108, and 1.946 nmoL/L respectively.

Examining physiological blood parameters revealed that the result of WBC was the presence of no significant differences compared to the healthy control in *P* value 0.062, the result of Hemoglobin (HGB) was the presence of significant differences in *P* value 0.00018 between each of them compared to the healthy control. Additionally, there was a very significant difference between the percentages of RBC in people with CKD and healthy patients *P* value 0.000107. The results of the physiological blood parameters represented by the MCV, and Platelets (PLT) did not show any substantial differences between CKD patients compared to healthy control

Concluded

This study showed that the level of OTA concentration in CKD is much higher than the permissible levels in the blood, and the presence of the same toxin in the blood of healthy people indicates the presence of food contamination. This study proved that ochratoxin A causes an imbalance in the levels of the studied hormones (TSH, T3, and T4), and this imbalance reflects negatively on metabolism and therefore may be associated with many diseases that affect humans.

Keywords: Ochratoxin A (OTA), Chronic kidney disease (CKD), Triiodothyronine (T3), Tetraiodothyronine (T4),Thyriod stimulating hormone (TSH), White Blood Cells(WBC), Red Blood Cells(RBC), Mean Corpuscular Volume(MCV).

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List of Abbreviations

NO.	Abbreviations	Item
1.	AKD	Acute Kidney Disease
2.	AF	Aflatoxins
3.	b.w	Body weight
4.	BUN	Blood urea nitrogen
5.	CBC	complement picture account
6.	CKD	Chronic Kidney Disease
7.	D.M	Diabetes mellitus
8.	DNA	Deoxyribonucleic acid
9.	FAO	Food and Agriculture Organization
10.	FDA	Food and Drug Administration
11.	FSH	Follicle-stimulating hormone
12.	GFR	Glomerular filtration rate
13.	GIT	Gastrointestinal tract
14.	GLD	Glutamate dehydrogenase
15.	GSH	Glutathione
16.	HCG	Human chorionic gonadotropin

17.	HPLC	high-performance liquid chromatography
18.	HPT	hypothalamic-pituitary-thyroid
19.	I	Iodine
20.	JECFA	Joint FAO/WHO Expert Committee on Food Additives
21.	LH	Luteinizing hormone
22.	MDRD	Modification of diet in renal disease
23.	MIT.T1	Mon-iodotyrosine
24.	NAD	Nicotinamide adenine dinucleotide
25.	NADH	Reduced Nicotinamide adenine dinucleotide
26.	NPN	Non-protein nitrogenous
27.	OTA	Ochratoxin A
28.	PRC	Professional Regulation Commission
29.	Proteinase K	Proteinase Keratin
30.	T3	Triiodothyronine
31.	T4	Tetraiodothyronine
32.	TBG	Thyroxine binding globulin
33.	TBP	Thyroxine binding proteins
34.	TBPA	Thyroxine binding prealbumin

35.	TC	Trichothecenes
36.	TLC	Thin-layer chromatography
37.	TRH	Thyrotropin-releasing hormone
38.	tRNA	Transfer Ribonucleic acid
39.	TSH	Thyriod stimulating hormone
40.	UV	Ultraviolet
41.	WHO	World health organization
42.	ZEN	Zearalenone

Chapter One

Introduction

1.1. Introduction:

Fungi is one of the many sources of food contamination, mycotoxin which are classified as the secondary metabolites produced by certain fungi, putting people at risk for a variety of health problems, including mycoses (Navale *et al.*, 2021).

Mycotoxins are produced when molds develop on certain plants and some food products. Mycotoxins can be consumed directly, or they can be consumed through the flesh and derivatives of animals that ate infected feed. The World Health Organization in 2018. Specific environmental elements, such as high temperatures and humidity, help fungi manufacture these toxins (Alina Marc, 2022).

Mycotoxicosis, a word used to describe the disease brought on by mycotoxins, is a condition in which an animal or human who consumes these mycotoxins develops an acute or chronic illness(Sharma and Bhandari, 2020).

When a significant dose is exposed over a short period, acute toxicity occurs, and in rare situations, it can cause a fatal condition (Barac, 2019a). Chronic toxicity over an extended period can damage cells, the nervous system, The immune system, and even cause cancer in people(Shahba, *et al.*, 2021).

Mycotoxins have been related to cancer and liver damage in both humans and animals. Both human and animal cell genomes are highly susceptible to mycotoxins' penetration, where they dramatically alter the nucleotide sequence and cause catastrophic, irreversible defects in the genome (Adam *et al.*, 2017).

Since mycotoxins can cause gangrene, respiratory problems, convulsions, liver cancer, changes to protein metabolism, and weakened immunity, they have an impact on human health. Premature death and increased medical costs would affect the economy (Freire and da Rocha, 2017a).

The fungi *Penicillium, Aspergillus, Claviceps,* and *Fusarium* produce mycotoxins like Ochratoxins, Aflatoxins, T-2/HT-2 Toxins, Deoxynivalenol (DON, Vomitoxin), Ergot Alkaloids, Zearalenone, Cyclopiazonic Acid, Mycophenolic Acid, and Fumonisins. The contaminated food is due to these mycotoxins (Alina Marc, 2022).

OTA is teratogenic, nephrotoxic, immunotoxin, neurotoxic, and hepatotoxic, among other effects, according to scientific studies. Previous studies have linked OTA's toxicological effects, including teratogenicity, neurotoxicity, and carcinogenicity, with a range of adverse health effects,It also contributes to the development of Balkan endemic nephropathy (Barac, 2019b).

Several food crops are contaminated by ochratoxin A, a mycotoxin produced by Aspergillus and Penicillium (Gurikar *et al.,* 2023). The health danger posed by OTA to both living things and people is significant. The three types of ochratoxins that frequently contaminate grains are ochratoxin A (OTA), ochratoxin B (OTB), and ochratoxin C (OTC) (Samuel *et al.,* 2021).

Mycotoxins have been demonstrated to significantly lower life expectancy, according to the Food and Agriculture Organization (FAO) of the United Nations, making it a serious concern to human health to consume food contaminated with these compounds (Hajok *et al.*, 2019a).

Consuming contaminated foods made from plants may be the direct cause of human exposure to ochratoxin A. Ochratoxin A has been found in a variety of foods, including wine, beer, grape juice, dried fruits, spices, legumes, cereals and cereal goods, coffee beans, dried fruits, and dried vegetables (Hajok *et al.*, 2019b). Consuming meat from farm animals that were fed mycotoxin-contaminated fodder could serve as an indirect form of exposure (Belkacem-Hanfi *et al.,* 2014).

A progressive condition known as CKD affects the kidney's structure and function and is brought on by several different circumstances. An estimated glomerular filtration rate (eGFR) of less than 60 mL/min per 1 73 m2 or signs of kidney damage, such as albuminuria, hematuria, or abnormalities found through laboratory testing or imaging and present for at least three months, are typical indicators of chronic kidney disease. Chronic renal illness is anticipated to increase to the fifth most prevalent cause of death worldwide, one of the highest forecasted increases of any major cause of mortality (Kalantar-Zadeh *et al.*, 2021).

The causes of CKD are as the following: Type 2 diabetes mellitus 30% to 50%, high blood pressure 27.2%, Type 1 diabetes mellitus 3.9%, Chronic tubulointerstitial nephropathy 3.6%, Inherited or cystic diseases 3.1%, Primary glomerulonephritis 8.2%, Vasculitis or secondary glomerulonephritis 2.1%, Neoplasma cell dyscrasias and neoplasms 2.1%, and Other Diseases (Webster *et al.*, 2017a). Clinically, disorders of the thyroid gland such as hypothyroidism and Euthyroid Sick Syndrome often occur in CKD patients, especially in CKD stage 5 (Zoccali *et al.*, 2006).

Thyroid hormones are crucial for organismal development and homeostasis, the function of the thyroid gland is one of the most important in the human body as it regulates the majority of the body's physiological actions. The thyroid produces hormones T3 and T4 that have many actions including metabolism, development, protein synthesis, and the regulation of many other important hormones. Any dysfunction in the thyroid can affect the production of thyroid hormones T3 and T4 which can be linked to various pathologies throughout the body(Nilsson and Fagman, 2017a).

1.2. Aim of the study

Investigation the relationship between Ochratoxin A and chronic kidney disease and their interection on thyroid hormones T3, T4, and TSH and physiological blood parameters.

The general steps of this study are:

1-Collection of blood from patients with CKD and healthy control.

2- Determine of physiological parameters CBC and some parameters of the CKD Urea, Creatinine, and GFR.

3- - Investigation of OTA in blood serum by using thin-layer chromatography (TLC).

4- Investigation of OTA in blood serum by using Thin-layer chromatography (TLC) and High-performance liquid chromatography(HPLC).

5- Determine the quantitaty of the following hormones: TSH, T3, and T4.

Chapter Two

Literatures Review

2.1. The kidney

Anatomy and structure: Kidneys same as beans in shape (Radi, 2019). A mature kidney weighs around 150 g and is about the size of a human fist (Webster *et al.*, 2017b), They play a crucial role in the urinary system (Mahajan *et al.*, 2021).

kidneys are situated on the back of the abdomen, one on either side of the spine, behind the peritoneum, and beneath the diaphragm (Tambur *et al.*, 2021), As in the figure 2-1.

Up to 2 million nephrons, which filter blood, can be found in each human kidney (Takasato *et al.*, 2015), Nephron is the structure and mechanism through which Kidney Functional Units (Fattah et *al.*, 2019), which have a renal corpuscle and renal tubule in each (Tambur *et al.*, 2021).

The expanded apex of a nephron is found within each renal corpuscle. also called the glomerular capsule or the bowman's capsule, its network of capillaries, termed the glomerulus, and calculating the rate of filtration, called the glomerular filtration rate, is commonly used to evaluate renal function (Staruschenko *et al.*, 2023).

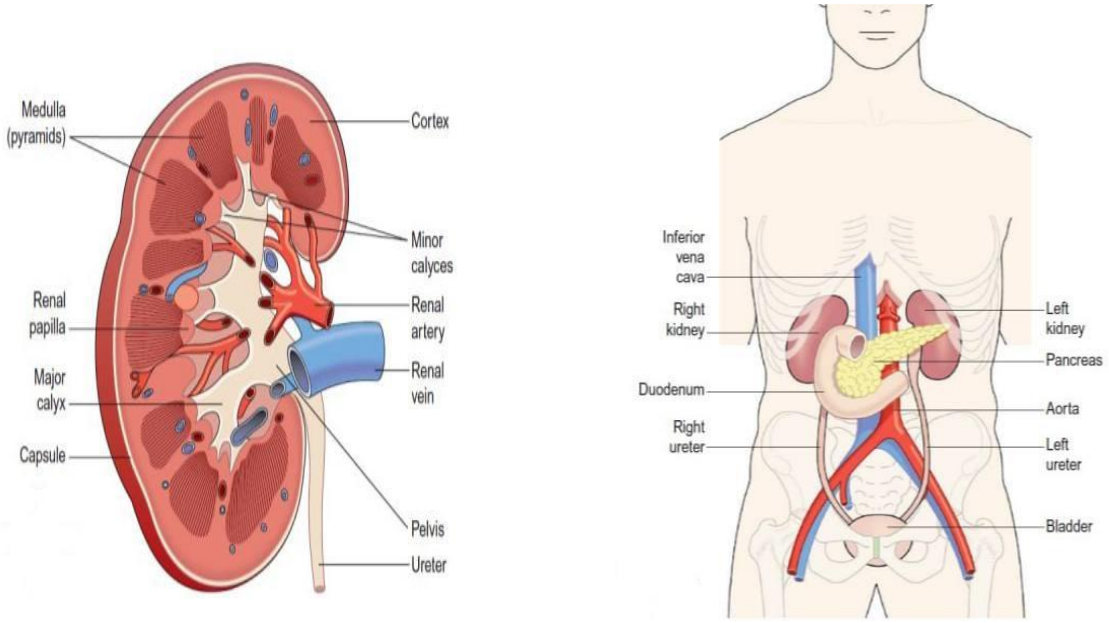

Figure 2-1: kidney anatomy and urinary system (Waugh, 2014).

The main functions of the kidneys are:

1. Urine production and the regulation of fluid, electrolyte, and pH levels.
2. Insulin, glucagon, and parathyroid hormone are just a few of the polypeptide hormones that they break down (Burggren and Bautista, 2019).
3. Elimination of toxic by-products of metabolism, including urea, creatinine, and uric acid.
4. Erythropoietin, a hormone that promotes the development of new red blood cells, and 1,25 dihydroxy vitamin D are synthesized and secreted.
5. The synthesis and release of renin, a key enzyme in regulating blood pressure (Inker and Titan, 2021).

2.1.1 Renal function test

Accurate diagnosis, risk assessment, and implementing treatment that improves clinical outcomes all benefit greatly from the use of biochemical indicators.

2.1.1.1. Urea and creatinine

Human nitrogen metabolism produces the waste products of blood urea nitrogen (BUN) and creatinine (Cr). Since they are quite small, the nephrons have no trouble filtering them out. Tubular reabsorption of BUN is typically between 30% and 40%, while Cr reabsorption is poor (Ok *et al.*, 2021).

Urea is a by-product of protein metabolism and is referred to as blood urea nitrogen (BUN) when it is detected in the blood. The breakdown products of nitrogen (BUN) are classified as non-protein nitrogen (NPN) waste products. Deamination of amino acids from protein catabolism results in the formation of ammonia. Liver enzymes catalyze the transformation of ammonia into urea. Therefore, the concentration of urea is determined by the amount of protein consumed, the efficiency with which the body catabolizes protein, and the rate at which urea is excreted by the kidneys. Eighty percent to ninety percent of NPNs in the human body are accounted for by urea. Because urea must be excreted by the kidneys, it can be used as a biomarker of kidney health. High-protein diets and impaired kidney function both contribute to elevated BUN (Prigent, 2008).

2.1.1.2. Glomerular filtration rate (GFR)

The filtration capacity of the glomerulus can be shown by analyzing creatinine concentrations in plasma and urine samples. The glomerulus filters creatinine freely since it is created endogenously within the body. Due to its aforementioned qualities, creatinine serves as a reliable endogenous marker for creatinine clearance. Reduced glomerular filtration rate (GFR), as seen in renal disease, leads to impaired creatinine clearance by the kidneys. A rise in plasma creatinine concentration is the result of a decreased GFR. Renal function cannot be determined by measuring plasma levels alone. It is possible that plasma creatinine levels will not change until severe renal impairment has occurred. Furthermore, a normal reference range for plasma creatinine is not indicative of a healthy renal system (Prigent, 2008).

The glomerular filtration rate (GFR) is the gold standard for gauging kidney function in both healthy individuals and those with chronic renal disease (Inker and Titan, 2021).

2.1.2. Renal failure

Acute nephritic syndrome, acute kidney disease (AKD), chronic kidney disease, nephropathy related to systemic disease, and other diverse disorders have all been recognized as causes of renal disease. Variations in AKD and CKD incidence, prevalence, and outcomes by race may reflect regional, ethnic, and ancestral influences, and may be the result of differences in biological, socioeconomic, behavioral, healthcare access, and quality (Levey *et al.*, 2020).

Acute glomerulonephritis: Renal failure due to Acute glomerulonephritis is common and assumed to be the result of autoimmune injury to the kidney. Patients with glomerulonephritis have elevated levels of blood urea nitrogen (BUN) and serum creatinine and reduced glomerular filtration rate (Vinen *et al.*, 2003).

Acute kidney injury: describes a rapid decline in kidney function over a week, as measured by increased serum creatinine levels (a marker of renal excretory function) and decreased urinary output (oliguria) (a quantitative indication of urine production) (Kellum *et al.*, 2021).

Chronic kidney disease: renal impairment is defined as persistently abnormal signs of kidney injury, a deterioration in renal function (glomerular filtration rate (GFR) 60 mL/min per 1.73m^2), or both for at least 3 months (Yuan *et al.*, 2022),(Wilkinson *et al.*, 2021).

Large-scale epidemiological studies suggest that between 5 and 10 percent of the population has CKD, and this number is expected to rise (Ellam *et al.*, 2009).

Chronic Kidney Disease: nephron loss, inflammation, myofibroblast activation, and extracellular matrix deposition are the hallmarks of chronic renal dysfunction syndrome. Nephron loss, which includes tubules, glomeruli, and endothelium, is driven by lipotoxicity and oxidative stress (Yuan *et al.*, 2022).

2.1.3. Pathophysiology of CKD

Chronic and progressive nephropathies cause increasing renal fibrosis and loss of normal kidney architecture, in contrast to acute kidney injury. The glomeruli, tubules, interstitials, and arteries of the kidney are all impacted. Histologically, it manifests as fibrosis of the tubulointerstitial, interstitial, and vascular spaces, as well as glomerulosclerosis (Webster *et al.*, 2017b).

2.1.4. Etiology of CKD

The following Table 2-1 lists the most common major conditions that lead to chronic kidney disease and, ultimately, end-stage renal disease.

Table 2-1: Major Causes of Severe Chronic Kidney Disease (Hanna Abboud, 2010).

NO.	Cause	Percent of causes
1	Diabetes mellitus	44.9 %
A	Diabetes mellitus type 1	3.9 %
B	Diabetes mellitus type 2	41.0 %
2	Hypertension	27.2 %
3	Glomerulonephritis	8.2 %
4	Chronic interstitial nephritis or obstruction	3.6 %
5	Hereditary or cystic disease	3.1 %
6	Secondary glomerulonephritis or vasculitis	2.1 %
7	Neoplasms or plasma cell dyscrasias	2.1 %
8	Miscellaneous conditions	4.6 %
9	Uncertain or unrecorded cause	5.2 %

Using the modified diet for renal disease formula, can calculate the glomerular filtration rate (GFR) in patients with end-stage renal disease. Diabetic kidney disease (DKD) causes over half of all new cases of end-stage renal disease (Niewczas *et al.*, 2019) as shown in the following Table 2-2:

Table 2-2: Stages of Chronic Kidney Disease and Prevalence in Adults(Hanna Abboud, 2010).

stage	Description	Estimated GFR Ml/min/1.73m²	Prevalence %	No. of Patients Million
I.	Damage to the kidneys despite an increased or normal GFR	>90	1.78	3.6
II.	Damage to the kidneys despite an increased or normal GFR	60-89	3.24	6.5
III.	Damage to the kidneys despite an increased or normal GFR	30-59	7.69	15.5
IV.	Damage to the kidneys despite an increased or normal GFR	15-29	0.35	0.7
V.	Damage to the kidneys despite an increased or normal GFR	<15	0.25	0.5

2.2. Thyroid

2.2.1. Thyroid gland anatomy

The thyroid gland is the first endocrine gland and the largest gland to form in humans. It begins in the fourth week of the series and begins to function accurately at the end of the third month (Benvenga *et al.*, 2018a), secretes thyroid hormone to regulate metabolic rate and is situated in the front of the neck just below the thyroid cartilage (Benvenga *et al.*, 2018b).

It consists of two lobes, which rest on the right and left sides of the tracheal wall, respectively. As seen in the following diagram (2-2), they are joined by an isthmus, a narrow band of thyroid tissue that spans the trachea's front surface (Waugh, 2014).

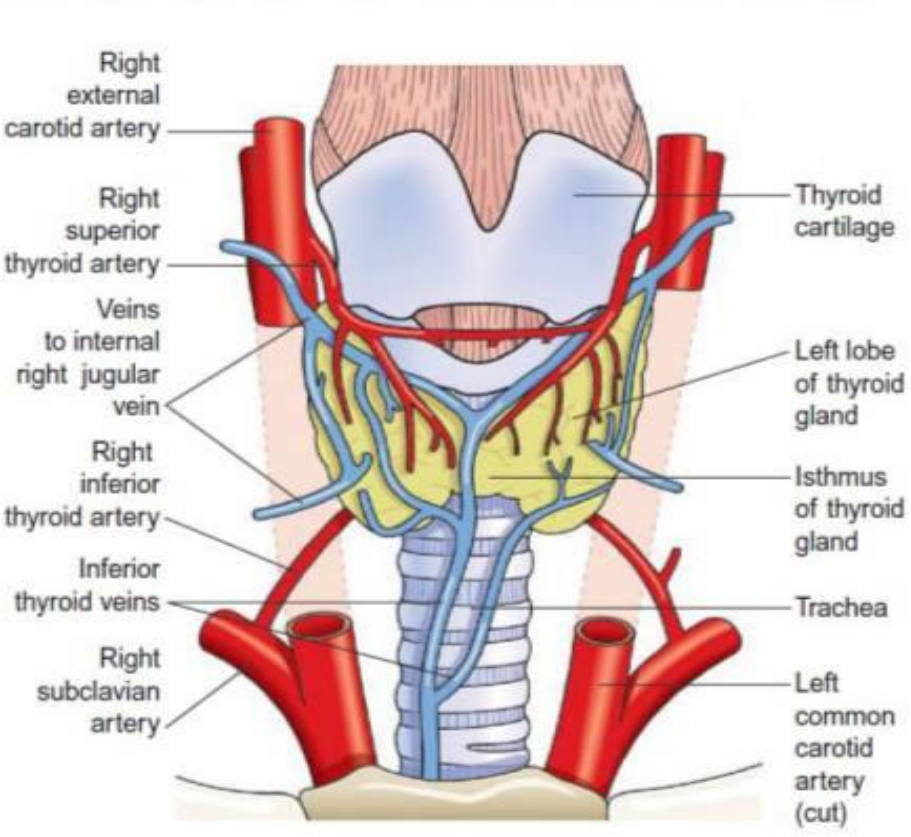

Figure 2-2: Thyroid gland anatomy (Waugh, 2014).

Thyroid hormone plays an important role in the development and function of every organ system in the body. The presence of triiodothyronine (T3) occupied receptors in the fetal brain demonstrated by 10 weeks of gestation provides evidence that thyroid hormone has a critical role in brain development(LaFranchi, 2021).

It is important to note that the thyroid gland uses iodine obtained from the diet to produce its hormones and the weight of the thyroid gland varies from person to person based on factors such as body size and dietary iodine levels; however, the average weight of the thyroid gland is between 25 and 30 grams (Messina and Redmond, 2006).

Thyroid hormones are important regulators of metabolism, energy use, and the proper functioning of vital organs like the heart and brain (Stathatos, 2019).

Hormonogenesis in the thyrocyte can be subdivided into three main steps: iodide uptake; iodide oxidation and organification; and secretion of thyroid hormones. These steps are summarized, all living beings are capable of taking up iodine and incorporating it into proteins (Benvenga *et al.*, 2018a). In humans and most vertebrates, the thyroid gland has evolved to save and store iodine. The thyroid produces iodinated molecules, iodothyronine, and iodothyronines, the latter including thyroid hormones (T4 and T3) (Nilsson and Fagman, 2017b).

The only biological function attributed to iodine concerned its incorporation into thyroid hormones (THs), synthesized by the thyroid gland, namely T4 (3,5,3′,5′-tetraiodo-L-thyronine) and T3 (3,5,3′-triiodo-L-thyronine), are characterized by the presence of four and three iodine atoms within the molecule, respectively, and play a prominent role in human body development and homeostasis (Sorrenti *et al.*, 2021).

Iodine is ingested with several foods including dairy products, grains, and meat. Upon ingestion, organic iodine is reduced to inorganic iodide (I-), the chemical form needed for the biosynthesis of thyroid hormones(Benvenga *et al.*, 2018a). The thyroid and kidney are the most iodine-hungry organs. Indeed, the thyroid actively takes up iodine from the bloodstream, where its concentration is approximately 30 times lower than in the thyroid (Benvenga *et al.*, 2018c).

2.2.2. Thyrotrophin stimulates hormone (TSH)

Human Thyroid Stimulating Hormone (TSH) or thyrotropin is a glycoprotein with a molecular weight of approximately 28,000 daltons, synthesized by the basophilic cells (thyrotropes) of the anterior pituitary (Pierce, 1971).

TSH is composed of two non-covalently linked subunits designated alpha and beta. Although the alpha subunit of TSH is common to the luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), the beta subunits of these glycoproteins are hormone specific and confer biological as well as immunological specificity. Both alpha and beta subunits are required for biological activity(Nguyen, *et al.*, 2023).

Thyrotrophin stimulates hormone stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by interacting with a specific receptor on the thyroid cell surface (Carvalho and Dupuy, 2017).

In a classic negative feedback loop, increasing T3 and T4 levels inhibit TSH production. The hypothalamus may have both a stimulatory and an inhibitory effect on pituitary TSH synthesis, as somatostatin and dopamine have been shown to have an inhibitory effect on TSH release. Hypothyroidism and hyperthyroidism originate from abnormalities in the hypothalamic-pituitary-thyroid axis, which controls the synthesis of T4 and T3. Low levels of T3 and T4 with a markedly increased TSH characterize primary hypothyroidism (Burger and Patel, 1972).

Thyroxine and 3,3′,5-triiodothyronine are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development and metabolic and neural activity. The synthesis and secretion of TSH are stimulated by thyrotropin-releasing hormone (TRH), the hypothalamic tripeptide, in response to low levels of circulating thyroid hormones (Nillni, 2010).

Elevated levels of T3 and T4 suppress the production of TSH via a classic negative feedback mechanism (Waugh, 2014).

Failure at any level of regulation of the hypothalamic-pituitary-thyroid axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T4 and/or T3. In cases of primary hypothyroidism, T3 and T4 levels are low and TSH levels are significantly elevated (Mammen, 2023).

2.2.3. Thyroxine (T4)

Thyroxine (T4) is an iodine-containing hormone that has a molecular weight of approximately 777 daltons and is secreted by the thyroid gland, T4 and its associate thyroid hormone T3 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal metabolic and neural activity(Glass and Holloway, 1990).

Although T3 has greater biological potency(Sawin *et al.*, 1977), T4 is normally present in human serum in approximately 50-fold excess of circulating T3 and accounts for more than 90% of the circulating protein-bound iodine. T4 is 99.9% bound to serum thyroxine-binding proteins (TBP). The hormone is transported bound primarily to thyroxine-binding globulin (TBG) and secondarily by thyroxine-binding prealbumin (TBPA) and albumin (Pardridge and Landaw, 1984).

Less than 0.05% of the total circulating T4 is unbound and therefore biologically active(Oliveira *et al.*, 2001). Clinically, T4 measurements have long been recognized as an aid in the assessment and diagnosis of thyroid status. Elevated T4 values are characteristically seen in patients with overt hyperthyroidism, while T4 levels are generally depressed in patients with overt hypothyroidism, Normal T4 levels accompanied by high T3 values are seen in patients with T3-thyrotoxicosis(Wang *et al.*, 1998).

T4 levels are altered by physiological or pathological changes in TBP capacity. Thyroxine binding globulin (TBG) capacity has a pronounced effect on the concentration of thyroid hormones. Consequently, T4 levels may be elevated with increased concentrations of TBG, Conversely, when TBG levels are decreased, such as in nephrotic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness, or congenital decrease of TBG, T4 may be reduced. Drugs that compete for protein binding sites, such as phenylbutazone, diphenylhydantoin, or salicylates, can result in a depressed T4 measurement. (Larsen, 1972).

Thyroid hormones have effects on a wide variety of other tissues and systems. When the thyroid gland is underactive or overactive, T3 and T4 can have significant physiological effects on the heart, skeletal muscles, skin, digestive system, and reproductive system, especially in children (VanPutte *et al.*, 2021).

2.2.4. (T3) 3,3′,5-triiodothyronine

3,5,3' Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons1 and a half-life in serum of 1.5 days, T3 circulates in the blood as an equilibrium mixture of free and protein-bound hormone (Moroz *et al.*, 1983).

T3 is bound to thyroxine-binding globulin (TBG), prealbumin, and albumin. The actual distribution of T3 among these binding proteins is controversial as estimates range from 38-80% for TBG, 9-27% for prealbumin, and 11-35% for albumin, The binding of these proteins is such that only 0.2-0.4% of the total T3 is present in solution as unbound or free T3 (Sinha *et al.*, 2019), this free fraction represents the physiologically active thyroid hormone (Ali and Majeed, 2022).

It has become apparent in recent years that T3 plays an important role in the maintenance of the euthyroid state. Serum T3 measurements can be a valuable component of a thyroid screening panel in diagnosing certain disorders of thyroid function as well as conditions caused by iodine deficiency. Clinically, measurements of serum T3 concentration are especially valuable in diagnosing hyperthyroidism and in following the course of therapy for this disorder (Marsden And Mckerron, 1975).

Dietary iodine deficiency results in inadequate production of thyroid hormones despite the presence of normal thyroid tissue. In these cases, the serum T4 concentration is often low while the Thyroid Stimulating Hormone (TSH) concentration is elevated. Elevated TSH associated with low T4 is normally indicative of hypothyroidism. However, in iodine deficiency, these results together with normal or slightly elevated serum T3 are indicative of euthyroid status in most individuals(Ermans, 1986).

T3 levels are also affected by conditions that affect TBG concentration (Sinha *et al.*, 2019). In patients with severe or chronic illnesses such as CKD, many abnormalities of thyroid hormone balance occur. T4 production and the extent of serum thyroid hormone binding may be independently abnormal, resulting in a low, normal, or high free T4 estimate. Serum T3 concentrations are often low; TSH levels may be normal or slightly elevated. Total T3 measurements may be valuable when hyperthyroidism is suspected and the free T4 estimate is normal (Kaplan *et al.*, 1982).

In previous studies thyroid disorders may affect renal function, The pathophysiology of thyroid disorders in CKD is multifactorial. The causal relationship between thyroid disorders and CKD seems to be bidirectional(Dousdampanis *et al.*, 2014).

In a previous study CKD affects the pituitary-thyroid axis and the peripheral metabolism of thyroid hormones, a decrease in renal function also accounts for an ineffective clearance of abnormal serum constituents, inflammatory cytokines, iodide excretion, and an increase in nitrogen conservation. suggest that nephrologists must consider the dangers of thyroid disease and its appropriate treatment in conjunction with treating CKD (Mohamedali *et al.*, 2014).

2.3. Mycotoxins

Mycotoxins are secondary metabolites produced by fungi that infect crops before harvest, increasing the likelihood that mycotoxins may develop during the drying, shipping, and storage of the harvested product (Koletsi *et al.*, 2021; Ranaldi *et al.*, 2009). Pose a considerable risk to human and animal health and are therefore among the most important mycotoxins in agriculture (Nolan *et al.*, 2021).

Mycotoxins in food and their derivatives are not just an issue in third-world countries, though. According to World health organization (WHO) statistics, the prevalence of mold-derived mycotoxins in consumable food is not decreasing. A lot of work is put into identifying mycotoxins in food and then getting rid of them because of the obvious rise in the number of diseases caused by mycotoxins (Freire and da Rocha, 2017b).

The prevalence of mycotoxin contamination is higher in nations with inadequate harvesting, production technology, or storage conditions, but it affects the entire world. Molds and mycotoxins are another potential health risk in damp houses. Mycotoxin generation requires the growth of mycotoxigenic mold, although the presence of mold does not guarantee the existence of mycotoxins. Mycotoxins can only be secreted under very specific circumstances. Mycotoxin synthesis is sensitive to changes in temperature, water activity, pH, oxygen, and substrate composition (Janik *et al.*, 2020a).

Direct or indirect contamination of the food chain can allow mycotoxins to reach humans and animals. Indirect contamination occurs in foods and animal feed when any ingredient has been contaminated by a toxigenic fungus before processing, and the mycotoxins remain after the fungus has been removed. On the other hand, mycotoxins can be directly introduced into a product, food, or feed when a toxigenic fungus infects it. Toxigenic fungus can grow and flourish in most food and feed products at any point in the supply chain, including harvesting, processing, transportation, and storage (Edite Bezerra da Rocha *et al.*, 2014).

Mycotoxins are primarily ingested by people who consume contaminated plant products, while they can also be present in foods made from animals, such as milk, cheese, meat, and other animal products (Edite Bezerra da Rocha *et al.*, 2014).

Molds grow at temperatures between 20 and 37 °C, and the optimum temperature for toxin production is 25.5 °C. Even at lower temperatures (below 10 °C), mycotoxin secretion is possible; however, the production period is lengthened and the toxin concentration is lowered. Water activity is another important element in mycotoxin generation; the ideal range for this parameter is between 0.83 and above 0.9 aw (Janik *et al.*, 2020a). Hydrolysis of OTA by microorganisms in the gastrointestinal tract produces Ochratoxin A, a far less harmful byproduct (Buszewska-Forajta, 2020).

OTA is nephrotoxic and causes gradual renal failure by destroying the renal tubular epithelium (Raghubeer *et al.*, 2015; Moretti *et al.*, 2017).

Mycotoxins have been linked to illness and death in both mammals and birds, thus controls are warranted in this case. Time of exposure, the health of the organism, the amount of toxin, and how sensitive it is all play a role in determining how dangerous it is. Toxins in the polluted fodder accumulate in the body and eventually lead to chronic poisoning if the animals eat it (Buszewska-Forajta, 2020).

The low molecular weight products of filamentous fungi are the source of all mycotoxins. Since the need for grain storage arose with the development of settled agricultural communities, mycotoxins have existed for at least two millennia. Following the abrupt demise of 100,000 young turkeys in England that had consumed peanut meal tainted with *Aspergillus flavus*, the word "mycotoxin" was first used in 1962 (Freire and da Rocha, 2017b).

2.3.1. Ochratoxin A (OTA)

Aspergillus and *Fusarium* species produce metabolites referred to as ochratoxins, which are defined chemically as derivatives of 3,4-dihydromethylisocoumarin that are amide-bound to the amino group of L-B-phenylalanine. The secondary metabolite ochratoxin A (OTA) was first identified in the fungus *Aspergillus ochraceous* in 1965 (Wang et al., 2016). It has been suggested that it contributes to the development of Balkan endemic nephropathy (BEN), which is known to be linked to malignancies of the urinary system (Knasmüller *et al.*, 2004).

Natural OTA is a powerful nephrotoxic mycotoxin with additional negative effects including hepatotoxicity, teratogenicity, and immunosuppression. The kidney and liver are particularly vulnerable to OTA's carcinogenic effects (Wang *et al.*, 2016).

Mycotoxin OTA is known to cause harm to developing fetuses since it can penetrate the placental barrier and reach the kidneys of an unborn baby. Male and female reproductive health suffers greatly from OTA exposure(Niaz, 2020).

Coffee, beans, oats, wheat, maize, wine, dried fruit, spices, eggs, meat, and so on all make up its food commodity. Consequences for health effects on the kidneys, liver, genes, embryos, and immune system Neurotoxicity (Janik *et al.*, 2020b).

Since 1990, numerous reviews have explored *Penicillium's* toxic secondary metabolites and mycotoxins from other fungal species. Several species of *Penicillium*, which now produce ochratoxin, used to be present. Ochratoxin A, the most common mycotoxin in human blood, has been linked to a nephropathic condition that is especially prevalent in Eastern Europe (D'Mello, 1997).

Mycotoxins pose a major threat to human health because of their tiny molecular weights, which do not stimulate the immune system, and their resistance to high temperatures, which prevents them from being destroyed by the temperature needed to cook food (Janik *et al.*, 2020a).

The number of mycotoxins in food, the duration of exposure, the individual's weight, the presence of other mycotoxins (synergistic effect), and environmental factors (primarily how commodities are stored) all play a role in determining whether a mycotoxin exposure causes an acute or chronic infection in the human body (Janik *et al.*, 2020a).

OTA is a well-known mycotoxin that is widely distributed around the world and has harmful effects on both human and animal health (Hussein and Brasel, 2001). Therefore, it is essential to research OTA's hazardous effects and take precautions against their harm. One of the target organs most susceptible to OTA-induced damage is the kidney(Lee *et al.*, 2018).

2.3.2. Ochratoxin A's chemical composition

Chemically described as a 3,4-dihydro-methylisocoumarin derivate, Ochratoxins are derivatives of an isocoumarin moiety linked to phenylalanine by an amide bond as in Figure (2-3). The most important ochratoxins are ochratoxin A (OTA), ochratoxin B (OTB), and ochratoxin C (OTC); its negative effects are thought to result from its apparent competition with natural phenylalanine in the protein synthesis process involving phenylalanine-tRNA synthetase. The immune system is repressed, immune organ size is reduced, antibody responses are muted, immune cell activity is changed, and cytokine synthesis is altered, among other effects of OTA that have been noted (Wang *et al.*, 2016).

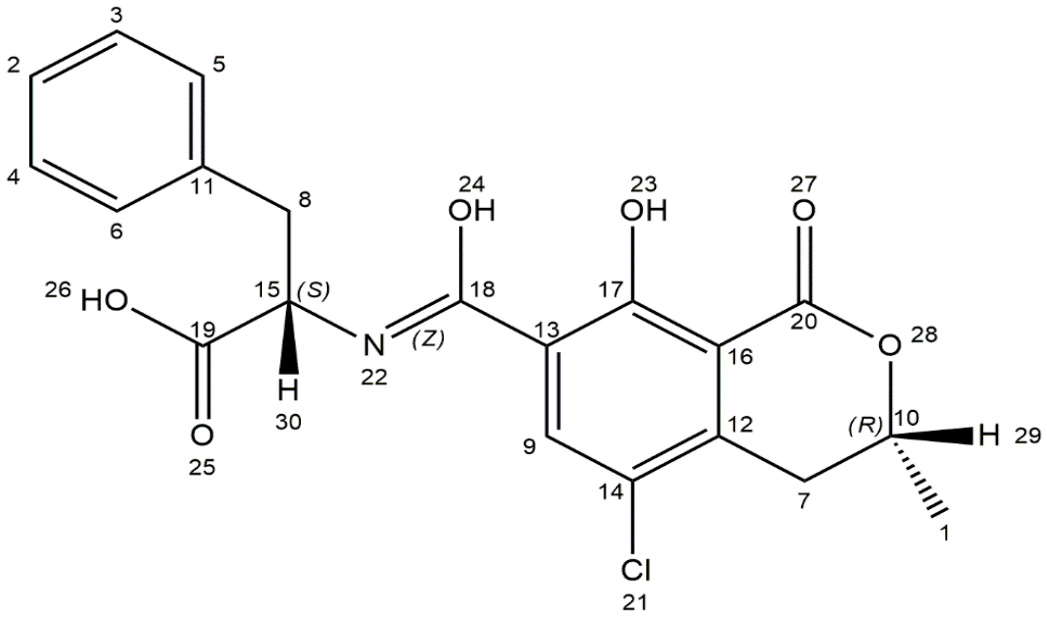

Figure 2-3: Chemical structure of ochratoxin A (Janik *et al.*, 2020a).

The most important ochratoxins are ochratoxin A (OTA), ochratoxin B (OTB), and ochratoxin C (OTC) as in Figure 2-4.

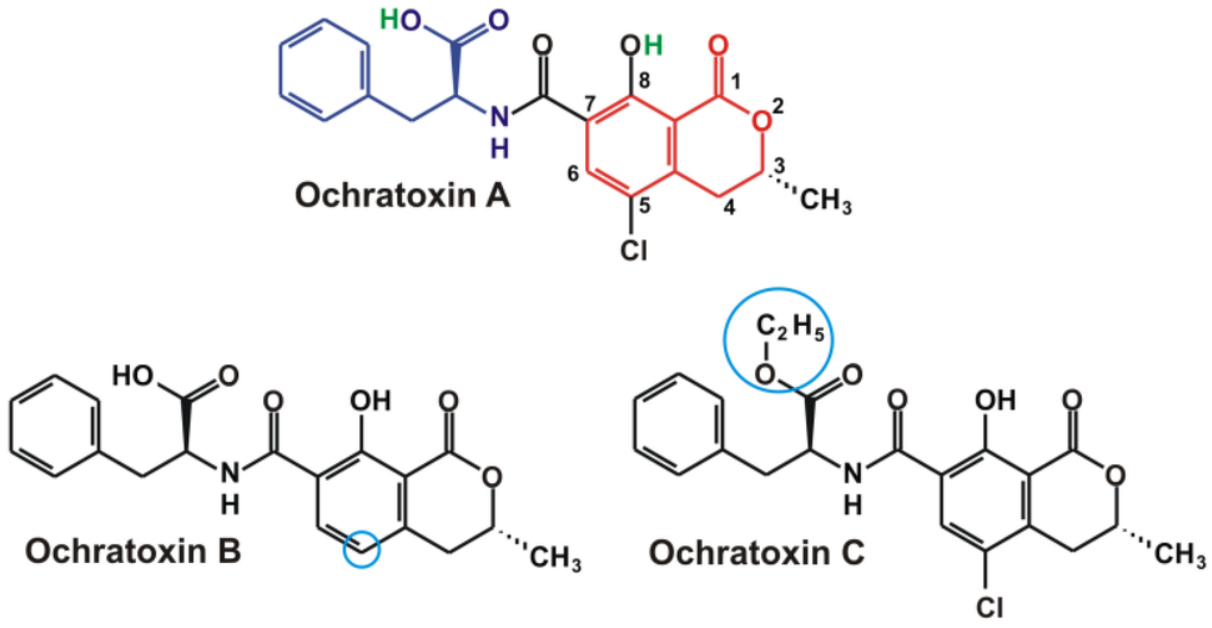

Figure 2-4: Chemical structure of ochratoxin A (OTA), ochratoxin B (OTB), and ochratoxin C (OTC) (Kőszegi and Poór, 2016).

2.3.3. Physical characteristics of OTA

Ochratoxin A is a white, crystalline chemical that dissolves easily in polar organic solvents, water, and weak aqueous bicarbonate solutions (Coronel *et al.*, 2010).

2.3.4. Distribution and accumulation in tissues

The kidneys reabsorb OTA and excrete it in the urine; the enterohepatic system recycles it, and the feces include its metabolites. Dangerous metabolites can be detoxicated by microorganisms in the digestive tract. In addition, cytochrome P450 enzymes in many tissues can break down ochratoxin A and B (Kőszegi and Poór, 2016).

High levels of intestinal OTA absorption have been seen in several animal species. Following oral administration, the rates of absorption in the systemic circulation varied from 40% in chickens to 56% in rabbits to 66% in pigs gastrointestinal tract (Galtier, 1977).

The largest concentration in the blood occurs after oral administration of OTA, according to studies on the absorption of OTA, and the toxin can be immediately detected in the peripheral circulation (Vartiainen *et al.*, 2020). The time it takes for a single oral dose of OTA to reach its peak blood concentration varies greatly between species; it has been measured to be 0.33ng/ml hours in chickens and 1 hour in rabbits (Galtier *et al.*, 1981).

Numerous studies have revealed that the stomach serves as the majority of an animal's gastrointestinal tract's absorption site, is that OTA is more lipid-soluble in acidic environments because it is uncharged (Roth *et al.*, 1988).

Following single administrations of OTA to rats, examination of intestinal contents and serum revealed additional peaks in OTA concentration profiles over time, supporting the theory that OTA is eliminated through the biliary system. These findings show that OTA is carried via the enterohepatic system throughout the body (Kumagai and Aibara, 1982).

2.3.5. Metabolism of OTA

Assessment and management of mycotoxin contamination in food and feed require knowledge of mycotoxin biotransformation and metabolism in humans, animals, plants, and microbes (Wu *et al.*, 2011).

Therefore, elucidating the Ochratoxin phases of Animal and human metabolisms is particularly helpful in assessing the quality of food. The ingestion of plants that have metabolized the toxin ochratoxin A can lead to digestive issues in animals and humans (Mally *et al.*, 2004).

The majority of ochratoxin A's metabolites are not very harmful to the intestines and can be broken down by either stage I or stage II enzymes. Ochratoxin A is metabolized by bacterial enzymes to form Ochratoxin Alpha (Madhyastha *et al.*, 1990).

Glucuronide and glutathione are two more Ochratoxin A metabolites found in animal and human tissues, blood, and urine. Commonplace techniques for detection have been used, such as enzyme-linked immunosorbent assays, thin-layer chromatography, and high-performance liquid chromatography. Simultaneously, straightforward and on-site detection approaches have been evolving, mostly driven by the discovery of novel functional nanomaterials and particular DNA aptamers (Wang *et al.*, 2016).

2.3.6. Excretion of Ochratoxin A

In most animals, OTA is slowly being eradicated. The two main ways that OTA is eliminated from the blood are via glomerular filtration into the urine or through the bile duct into the feces(Magan, 2004).

There may be significant species-specific variations in the quantity and rate of OTA clearance through the bile duct and kidney (Magan, 2004) because enterohepatic circulation and serum protein binding influence have an impact on the amount of OTA eliminated from the bloodstream. OTA is reabsorbed from the gut by enterohepatic circulation after being discharged into the bile. Biliary excretion of OTA is the main excretory pathway for OTA administered orally in laying hens. In two experiments, the OTA clearing mechanism was examined. Human organic anion transporter 4 (hOAT4) carries out OTA transport in the proximal tubule's apical side. and the proximal tubule's basolateral side by the hOAT1 and 3 transporters(Armorini, 2015).

In the researcher's (Schaaf *et al.*, 2002)study, he explained that Balkan endemic nephropathy (BEN), a disease characterized by progressive renal fibrosis in human patients, has been associated with exposure to ochratoxin A (OTA). This mycotoxin is a frequent contaminant of human and animal food products and is toxic to all animal species tested. OTA predominantly affects the kidney and is known to accumulate in the proximal tubule (PT). The induction of oxidative stress is implicated in the toxicity of this mycotoxin.

2.3.7. Toxic Effects of Ochratoxin A

After being consumed either directly or indirectly, the OTA toxin is absorbed by the digestive system and binds with the blood protein (Taghizadeh *et al.,* 2018). The OTA toxin attaches to albumin, one of the two major blood proteins, along with globulin. Albumin is a protein produced in the liver and is also referred to as serum albumin or albuminuria. In addition, there is blood plasma, which makes up about 50% of human plasma. That regulates the osmotic pressure in the blood compartment (Nicholson *et al.,* 2000).

Any exposure to mycotoxins is damaging to both humans and animals, but ochratoxin toxins are especially dangerous because of their wide range of adverse effects on the body, including toxicity, renal toxicity, neurotoxicity, immunotoxicity, reproductive toxicity, and carcinogenicity. Numerous negative consequences of the OTA toxin on humans have been reported. As a chain of connected occurrences, toxicity is A toxicant that enters the body, undergoes metabolic and nutritional changes, and then interacts with various cell components, such as protein and DNA, to have a variety of toxicological consequences (Khairnar, 2022).

2.3.8. Nephrotoxicity of OTA

After renal cells experience oxidative stress, OTA appears to have a strong nephrotoxic effect. The kidney is the most common site of OTA poisoning, and the middle and terminal sections of the proximal convoluted tubules are the most affected (Jung *et al.,* 2001).

Nephrotoxicity is described as a dramatic reduction in kidney function caused by a substance's or a drug's harmful effects (Ma, 2019). Because of the high binding of this toxin to albumin, the first demonstration mechanism of OTA nephrotoxicity indicated that tubular secretion is the primary mode of OTA excretion. Its clearance via glomerular filtration is minimal, and greater levels in proximal tubular cells might be due to the reabsorption of tubular toxins. In the latter, OTA raised the concentration of reactive oxygen species (ROS), which increased the production of 8-oxo guanine (Tao *et al.,*2018).

Cells and tissues become more vulnerable to oxidative stress as a result of decreased glutathione synthesis, oxidized glutathione recycling, and oxidoreductase activity brought on by inhibition of the erythroid oxidative stress response pathway 2-like 2 and its Keap-1 inhibitor(Limonciel *et al.,*2014).

In rabbit kidneys, low dosages of OTA, either separately or together, induced apoptosis and lipid peroxidation, which appears to be a major factor in nephrotoxicity characterized by nuclear fragmentation and cytoplasmic flow(Kumar *et al.,*2014).

Birds with OTA showed substantial and persistent changes in the ultrastructure of the proximal convoluted tubules, including severe mitochondrial and reticulum endoplasmic lesions. In the kidney, OTA causes microscopic, inflammatory, necrotic, and degenerative problems (Patial *et al.,* 2013).

Chapter Three

Materials and Methods

3.1. The materials of the study

3.1.1. The Instruments

The devices that are summarized in Table 3-1 were used according to the manufacturer and the origin.

Table 3-1: Laboratory Instruments for the study

No	Instrument	Manufacturer	Country
1	Autoclave	Gallemkamp	England
2	C4000, Architect	Abbott	Japan
3	Candle Jar	China Mheco	China
4	Centrifuge	Hettich/Ebazo	Germany
5	Chemistry Analyzer	Abbott C4000	USA
6	Deep Freeze	Royal	England
7	Electric Shaker	Bioneer	England
8	Hematology Auto Analyzer CBC	Abbott Ruby	USA
9	Hormone Analyzer	Abbott I1000	USA
10	HPLC	Sykam	Germany
11	Uv Source	Cleaver	England
12	Water Distiller	Dahin	Korea

3.1.2. The Equipment's

The Equipment that is summarized in Table 3-2 was used according to the manufacturer and the origin.

Table 3-2: Laboratory Equipment used in the study

NO	Equipment	Manufacturer	country
1	Test tube	Mhco	China
2	Acetonitrile	Supelco	USA
3	Beaker	Colac	England
4	Distilled Water for HPLC	Scharlau	Spain
5	EDTA tube	Plastilab	China
6	Eppendrof (1.5ml tube)	Vitrex, Meae	China
7	Ethanol 70%	Scharlau	Spain
8	Gel tube	Plastic	China
9	Graduated cylinder	Colac	England
10	Latex Examination Gloves powdered	RZ	Malaysia

11	Medical cotton	Medicare Hygiene Limited	Lebanon
12	Micropipette (10_100ul)	HUMAN Humapette	Germany
13	Micropipette (100_1000uL)	HUMAN Humapette	Germany
14	Phosphate buffer pH7.4(PBS)	BDH	England
15	Screwed glass test tub	China Mheco	China
16	Stand	Mhco	China
17	Syringes (10) ml	Provi	China
18	Test tube rack	Mhco	China
19	Thin layer chromatography plate (TLC)	Chmlab	Spanish
20	Tip Micropipette (10_100ul)	AFCO-DISPO	Jordan
21	Tip Micropipette (100_1000uL)	AFCO-DISPO	Jordan
22	Tube Plain Without Additives	AFCO-DISPO	Jordan
23	Volumetric Flask	Volac	England

3.1.3. Kits and Chemicals

Chemicals and tests were used according to the manufacturer and origin, as shown in the following Table 3-3.

Table 3-3 Kits and chemicals

NO	Reagents (Kits and Chemicals)	Manufacturer	country
1	Chloroform (stabilized with amylene) extra pure Minimum assay (GC)99.0%	THOMAS BAKER	India
2	Creatinine	Architect, Abbott	Japan
3	Methanol	Scharlau	Spain
4	Proteinase K	Intron Biotechnology	Korea, Geneaid
5	Standard OchratoxinA HPLC	Sigma	USA
6	Standard OchratoxinA in TLC	High media	India
7	T3 Kit	Abbott	Ireland
8	T4 Kit	Abbott	Ireland
9	TSH Kit	Abbott	Ireland
10	Urea	Architect, Abbott	Japan

3.2. Methods

3.2.1. Study design

Case-control research was conducted, between December 2022 and April 2023. CKD patient samples were collected from Department's Dialysis, and Internal Medicine - Nephrology Consultation in the Imam AL-Hussain Medical City, and the Imam Al-Hassan Al-mujtaba Teaching Hospital in Kerbala province. Self-reported data (questionnaire) was used to collect sociodemographic information about the patients, such as their ages, gender, weight, height, family medical histories, and the presence or absence of chronic disorders such as diabetes mellitus and hypertension. CKD patients were compared with healthy controls, as shown in diagram 3-1.

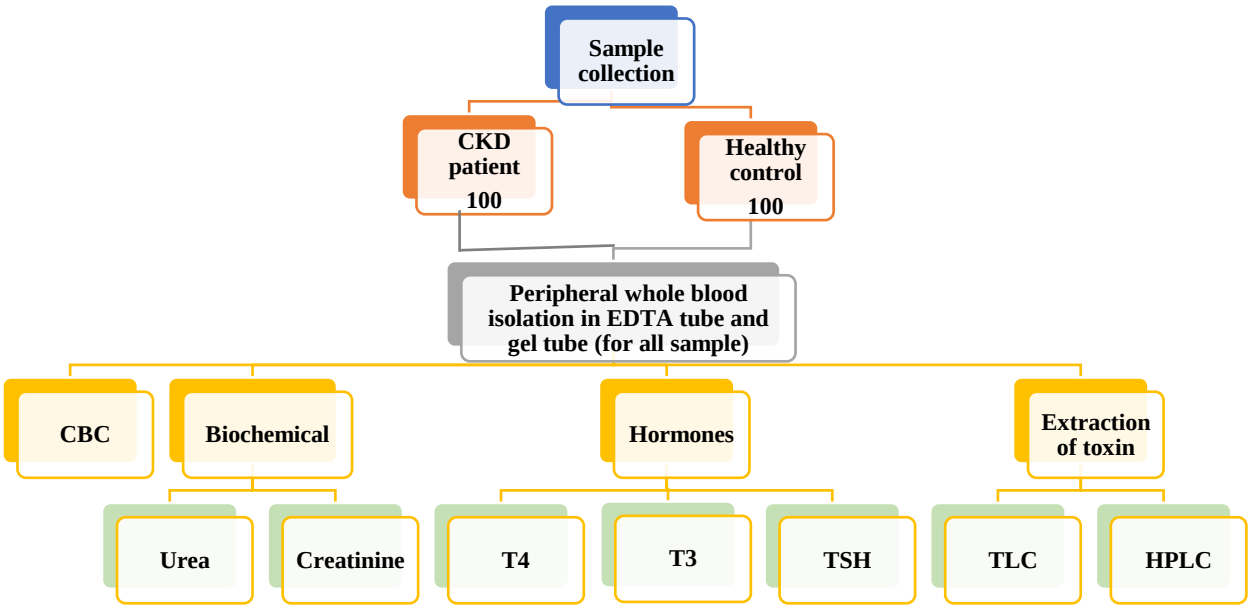

Figure 3-1: Study design

3.2.2. Study groups

One hundred participants with nephropathy and one hundred healthy controls participated in the study. The study was divided into groups, as shown in Table (3-4)

Table 3-4 The symbols of the Study groups

NO.	Groups	Description
1.	PF with OTA	Patient female with OTA
2.	PF without OTA	Patient female without OTA
3.	PM with OTA	Patient male with OTA
4.	PM without OTA	patient male without OTA
5.	HF with OTA	Healthy female with OTA
6.	HF without OTA	Healthy female without OTA
7.	HM with OTA	Healthy male with OTA
8.	HM without OTA	Healthy male without OTA

3.2.3. Patient Criteria

The study comprised 100 patients with CKD. A questionnaire was used to evaluate significant socioeconomic factors, general health status, family history of chronic diseases, gender, and age.

3.2.4. Exclusion Criteria

Dialysis patients and those who reported having a congenital kidney disease were excluded from the study.

3.2.5. Control Criteria

One hundred healthy volunteers were selected to serve as the study's control group. The healthy control had no history of kidney problems, and blood samples were taken from them. The participants' ages tended to be the same as those of CKD patients. The self-report (questionnaire) was also used to acquire participants' demographic details.

3.2.6. statement of ethics

The study's protocol received the seal of approval number 226 on the date2022\12\7 from the Karbala-based College of Applied Medical Sciences Ethical Committee. The consent to collect samples was given by the patients or their legal guardians.

3.2.7. Collection of the data

Important patient data, including names, ages, genders, weights, etc, are shown in the appendix (Questionnaire form). Questionnaire as well as shown in page appendix (XXVI).

3.2.8. Blood Collection and Storage of blood Samples

10 ml of blood venous was drawn from the vein of each of the CKD patients and the healthy controls using a sterile syringe; 8 ml were placed in two gel tubes, and 2 ml were placed in an EDTA tube before being transported in a container to the lab's main facility. The serum was separated by centrifuging the samples for 15 minutes at 4000 rpm after they had settled for 15 minutes.

Each serum was split in half using a micropipette and then placed in a sterile container (an Eppendorf 1.5 ml tube) at - 20 °C until analyses could be performed. Previously collected serum samples were then utilized to evaluate:

1. Test of Renal Function (Serum urea, Serum creatinine).

2. Investigation of OTA in blood serum by TLC.

3. Measurement (Qualitative and Quantitative) of OTA in blood serum by HPLC.

4. Thyroid Function Test T3, T4, and TSH.

5. CBC is used to analyze the blood profile.

3.2.8.1. Investigation of Ochratoxin A by TLC (Qualitative) in blood serum

This research followed the stages outlined by AL-Musoui (2015). All glasses were autoclaved for 15 minutes at 120°C before use, and ethanol alcohol 70% was used to wipe all surfaces in preparation for each day's work.

3.2.8.1.1. Proteinase K solution

Following the protocols provided by the Korean Junaid Company, a proteinase K solution was made. Proteinase K powder 28900 M.wt was dissolved in 1.1 ml pure sterile distilled water.

3.2.8.1.2. Standard OTA preparation for TLC

A stock solution of OTA standard 200µg/ml was prepared by dissolving 1mg of OTA in 5ml chloroform.

3.2.8.1.3. Ochratoxin A extraction from serum samples using TLC

500µL of Serum was taken in a test tube and added to 50µL from proteinase K solution and left to react for 10 minutes, then centrifugation for 15 minutes at 4000 xg, and from the sample the supernatant was taken, and the precipitate was neglected.

after that chloroform 1ml was added to the supernatant (twice its volume) and shaken in a vortex, where it formed two layers (chloroform layer and serum layer), The chloroform layer was separated by separating the funnel and put in a sterile other glass tube and let to evaporate (Mousa, 2013). as well as shown in page appendix(XXV).

3.2.8.1.4. Detection of OTA by Thin Layer Chromatography

A thin layer of chromatography plates was used coated with silica gel, a dimension of (20×10 cm) was used after activated in an electric oven at 120°C for 1 hour before use. A light straight line was made at a distance of 1.5 cm from the bottom and top base of the plate, the bottom line was used for loading samples and the top line was used for numbering. The mobile phase is used to separate OTA a mobile phase of chloroform, methanol, and water 90: 8: 2.

Ochratoxin A stock standard 15µl was added as a spot on the TLC plate by a capillary tube, then 15µl from each extracted sample was added to the plate with a distance of 2 cm between samples. After that, the spots were left to dry at room temperature. Next, the plate was put in the separation jar which contained a mobile phase. The thin layer plate was left in the jar until the mobile phase reached 2cm from the upper edge of the plate.

The TLC plate was removed from the jar and left to dry at room temperature. Then, the plate was examined under UV light 360nm, and compare the color and compare the color and relative flow (RF) of extracted samples with the standard toxin (Sotanaphun, 2009).

3.2.8.2. Qualitative and Quantitative Analysis of Serum Ochratoxin A by HPLC

HPLC model Sykamn (Germany) was used to analyze the add detection of Ochratoxin. The mobile phase was isocratic acetonitrile :D.W:formic acid 50:47:3 at a flow rate of 1.0mL/min, the column was C18-ODS (25 cm* 4.6mm), and the detector Florescent (Ex=365 nm, Em =445 nm) (Skarkova *et al.*, 2013). Figure 3-2 also shows how the device works. as well as shown in page appendix(XXIV).

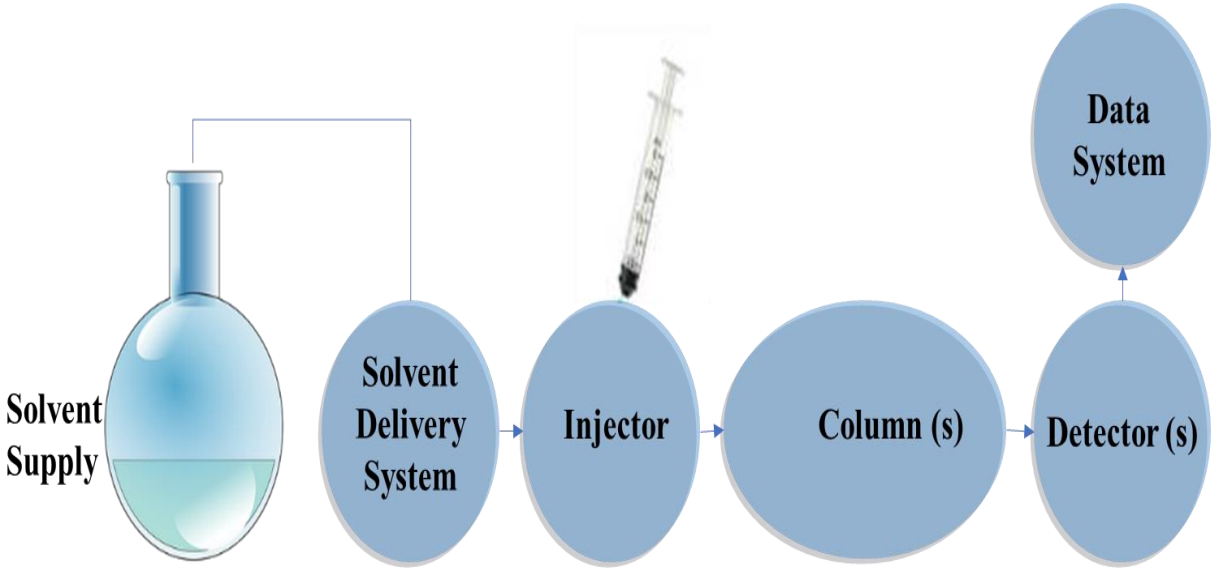

Figure 3-2: HPLC system and essential parts

3.2.8.2.1. Standard OTA preparation for HPLC

In a 10 ml conical flask, 1 mg of OTA standard has been dissolved in acetonitrile. The injected concentration was prepared to be 100 ppm using the dilution law.

3.2.8.2.2. HPLC-based Ochratoxin A extraction from serum samples

Serum 250 µL was extracted with 15% trichloroacetic acid 0.5 ml and dichloromethane 1 ml by vigorous overtaxing for 30s in a 2 ml safe-lock polypropylene conical-bottom centrifuge tube. The mixture was allowed to stand for 24 h at room temperature and then centrifuged at 14.000 x g for 10 minutes (Herzallah, 2009).

The lower dichloromethane phase was carefully drawn by a Pasteur pipette and transferred to a 1.5 ml safe lock polypropylene conical bottom centrifuge tube. The acidic phase and the compact precipitate layer formed between the two phases were re-extracted with dichloromethane (0.5 ml) for 30 s on a vortex mixer and then centrifuged for 5 min at 14.000 x g. The pooled dichloromethane extract was evaporated to dryness at 40°C under a gentle nitrogen flow. The remaining residue was dissolved in methanol 80 ml and transferred to a 1 ml HPLC vial (milićević, 2009). This is applied according to the equation.

Calculation:

$$\text{Con. of sample (ng/ml)} = \frac{\text{conc. of standard} \times \text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Dilution factor (D.F)}}{\text{Volum sample}}$$

3.2.8.3. Method for Quantitation of Kidney Functions

Kidney function was evaluated for both CKD and healthy control. The specific tests for each variable are summarized below:

3.2.8.3.1. Method for Quantitation Serum Urea level

Japanese company Abbott offered detailed instructions for conducting the analysis. The correlation studies were carried out utilizing NCCLS EP9-A, a protocol created by CLSI. serum samples were used to compare the aero set system's urea nitrogen assay to a commercially available urease technology.

Principles of the procedure

The Urea Nitrogen assay is a modification of an enzymatic procedure first described by Talke and Schubert (1965). The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited time. Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample.

3.2.8.3.2. Method for Quantitation Serum Creatinine Level

Abbott, a Japanese firm, provided specific guidelines for doing the analysis. The elimination of creatinine from the blood is carried out by glomerular filtration. The level of creatinine in the blood increases when renal function is impaired. The assessment of renal dialysis patients, the calculation of glomerular filtration rate (GFR), and the diagnosis and follow-up of acute and chronic renal illness all depend on serum creatinine levels. Creatinine in the serum can be measured and used as a reference quantity when determining the albumin/creatinine ratio or determining if a 24-hour sample was complete (Thomas, 1998).

Principles of the procedure

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.

3.2.8.3.3. Measurement of Estimated Glomerular Filtration Rate

Calculating eGFR by using CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation (Rosner and Bolton, 2006a):

GFR = (141 – Age) * (Weight) ÷ (72 *Scr) = [if male]
GFR = (141 – Age) * (Weight) ÷ (72 *Scr) * 0.85 = [if femal]

3.2.8.4. Measurement of physiological blood parameters

Auto analyzer (Abbott ruby) hematological (CBC) device analysis was performed in the private laboratory (Aldeqa lab) in Karbala province Iraq.

3.2.8.5. Method for Quantitation Hormones of the thyroid gland

Thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) levels were analyzed in the private laboratory (Aldea lab) in Kerala province Iraq using an American hormone analyzer (Abbott i1000).

3.2.8.5.1. Method for Measurement of TSH Levels in the Blood

The American Abbott i1000 hormone analyzer was used under protocols provided by the Irish corporation Abbott.

Biological principles of the procedure

The TSH assay is a two-step immunoassay that detects Thyroid Stimulating Hormone (TSH) in human serum and plasma:

1. Sample, anti-β TSH antibody-coated paramagnetic microparticles, and TSH Assay Diluent are combined. TSH present in the sample binds to the anti-TSH antibody-coated microparticles.

2. After washing, anti-α TSH acridinium-labeled conjugate is added to create a reaction mixture.

3. Following another wash cycle, Pre-Trigger, and Trigger Solutions are added to the reaction mixture.

4. The resulting chemiluminescent reaction is measured as relative light units. There is a direct relationship between the amount of TSH in the sample and the relative light units detected by the Architect system optics.

3.2.8.5.2. Method for Measurement of T4 levels in the blood

The American Abbott i1000 hormone analyzer was used to analyze as per Abbott's specifications in Ireland.

Biological principles of the procedure

The Architect Total T4 assay is a two-step immunoassay to determine the presence of thyroxine (Total T4) in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and anti-T4 coated paramagnetic microparticles are combined. Bound T4 is removed from the binding sites on thyroxine-binding globulin, prealbumin, and albumin. The T4 present in the sample binds to the anti-T4 coated microparticles.
2. After washing, T3 acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger, and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units. There is an inverse relationship between the amount of Total T4 in the sample and the relative light units detected by the Architect system optics

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3.2.8.5.3. Method for Measurement of T3 levels in the blood

The American Abbott i1000 hormone analyzer was used under protocols provided by the Irish corporation Abbott**.**

Biological principles of the procedure

The ARCHITECT Total T3 assay is a two-step immunoassay to determine the presence of Total T3 in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and anti-T3 coated paramagnetic microparticles are combined. The T3 present in the sample binds to the anti-T3-coated microparticles.
2. After washing, T3 acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger, and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units. There is an inverse relationship between the amount of Total T3 in the sample and the relative light units detected by the Architect system optics.

3.2.9. Statistical analysis

The experiments were conducted according to the complete design and results analysis by using one-way ANOVA and mean comparison according to T-test and X^2, as well as Duncan Test. The SPSS Program Version 24 was used to obtain the statistical values.

Chapter Four

Results and Discussion

4.1. Investigation of OTA in blood serum of chronic kidney disease by using TLC

The results showed that blood serum collected from patients had the OTA in 99 (99%) samples where one patient was without OTA. On another hand the number of blood serums collected from the health control, the OTA exists in 32 (32%) samples, while 68 (68%) samples are without OTA. When compared to the healthy the number of samples taken from patients shows a highly significant difference in the results, Table 4-1. as well as shown in page appendix(XXIII).

Table 4-1: Distribution of patients with and without OTA compared to the number of healthy with and without OTA according to the TLC test, Based on Cases

Cases	With OTA	Without OTA	Total	*P value*
Patients	99 (99%)	1 (1%)	100	0.0000**
Healthy	32 (32%)	68 (68%)	100	0.00032**
Total	131	69	200	0.0000**
P value	0.00001**	0.0000**	NS	

**** Means significant differences (*p<0.01*)** ***** Means high significant differences (*p<0.001*)**

NS: No significant differences

The results also illustrated the number of blood samples collected from female and male patients with OTA was 56 (56%) and 43 (43%) respectively with no significant differences between them while the number of samples that collected from healthy females and males with OTA were 22 and 46 respectively, Table 4-2.

Table 4-2: Distribution of the patients (females and males) with and without OTA compared to healthy control with and without OTA according to the TLC test, Based on Gender

Gender	Patients		Healthy		
	With OTA	Without OTA	With OTA	Without OTA	*P value*
Female	56 (56%)	1 (1%)	22 (22%)	22 (22%)	0.00012 ***
Male	43 (43%)	0	10 (10%)	46 (46%)	0.00001***
Total	99	1	32	68	0.00001***
P value	0.19137		0.03389*		

*** Means significant differences (*p<0.05*)** ***** means high significant differences (*p<0.001*)**

The result agrees with Hassan and Ali (2022) who found that 90% of blood samples collected from patients infected with chronic kidney disease of uncertain etiology with OTA have a significant difference of blood samples with OTA 30% that are collected from healthy controls. Also, it was found the number of blood samples collected from female and male patients affected with OTA was 48.8% and 51.1% respectively.

The result agrees with Hassan and Ali (2022) who found that female and male patients affected with the OTA were 44 (48.8%) and 46 (51.1%) respectively with no significant difference between them, while Sami Abdel Reda, (2017) showed that women with chronic kidney diseases are more sensitive to ochratoxin A than men, as the percentage of women with toxin A was 60 %, also disagreed with AL-Musoui(2015), he demonstrated that females had a lower OTA infection rate 70% than males 87.5%.

The result disagrees with Hadi Al-Mhanaa (2020) where the study is conducted on 94 patients (renal failure) 54 male and 40 female and 15 healthy people 10 male and 5 female as the control group. where it was qualitative diagnosis by using TLC, 29 cases have an OTA in serum and 65 cases without ochratoxin.

4.2. Assessment of OTA concentration in blood serum of patients with chronic kidney disease compared with the control group by using HPLC.

The findings revealed that the levels of OTA in the blood serum of female and male patients were 23.943 and 23.475 ng/ml, respectively, whereas the levels of female and male healthy controls were 2.171 and 2.075 ng/ml, respectively, with significant differences between healthy and patients, Table 4-3, as well as shown in page appendix (I _ XXII).

Table 4-3: Duncan's test for measuring the concentration of OTA in patients and healthy blood serum using HPLC

Groups	Mean(ng/ml)	SD	Duncan Test	*P* value
Patient Females	23.943	2.413	a	0.0015***
Patient Males	23.475	2.592	a	
Healthy Females	2.171	0.121	b	
Healthy Males	2.075	0.124	b	

***** means high significant differences (*p<0.001*)**

The research agrees with Al-Mhanaa (2020) the study's findings showed a statistically significant rise in the concentrations of OTA in both the control group and all patients with chronic kidney diseases, with *P* values of 0.001measur of concentrations (1.298 ng/ml and 0.543 ng/ml), respectively.

The above results are also similar to the research conducted by Özçelik *et al.,* (2001b) where were his results, The healthy group's average OTA concentration was 0.40 ng/ml. The group of patients receiving hemodialysis had the highest mean concentration, measuring 2.11 ng/ml, all patient groups had greater average toxin concentrations than the control group. but the concentration of the ochratoxin A varies from our study because patients with chronic kidney diseases had undergone dialysis, compared to the condition of patients without dialysis.

The current study agrees with Hassan and Ali's (2022b) investigation which found the concentration range of OTA in the blood serum of female and male patients with CKD was 7.015 ng/ml and 7.071 ng/ml respectively

Breitholtz-Emanuelsson *et al.,* (1994b) illustrated that the healthy group's mean and median ochratoxin A values were 0.53 and 0.44 ng/ml serum, respectively. The group of patients receiving dialysis had the highest mean levels, which was 1.4 ng/ml serum. The dialysis group had a greater frequency of samples with >0.44 ng ochratoxin A/ml serum.

The result showed no significant differences in the gender Table 4-3, disagreeing with Breitholtz-Emanuelsson *et al.,* (1994b) the concentrations of OTA in the male and female kidney failure patients were significantly higher at (1.136 ng/ml) and (1.231 ng/ml), respectively, compared to the control group, which had concentrations of (OTA) of (0.573 ng/ml) and (0.48) ng/ml, respectively. However, the results of the sex differed, with the results being found to be significantly higher.

Among 40 analyzed serum samples, 27.5% were found to contain OTA with levels going from 0.1 to 11.98 ng/ml with a mean concentration of 0.73±2.35 ng/ml (Ghali *et al.*, 2008).

In France, two patients with chronic renal failure showed incredibly high blood levels of OTA 205 and 367 ng/ml, respectively Fuchs and Peraica (2005) found that in Egypt, patients with nephropathic syndromes had the highest OTA level of 10.15 ng/ml compared to 0.91 ng/ml in healthy controls(Wafa *et al.*, 1998).

As shown that OTA concentrations in blood were higher in patients with chronic renal insufficiency treated with dialysis than in healthy individuals, OTA accumulation brought on by renal failure cannot be eliminated by dialysis. This is supported by the finding that there was no difference in OTA concentration in those with chronic renal insufficiency assessed before and after dialysis (Malir *et al.*, 2001).

4.3. Measurement of OTA-positive group of CKD patients with biochemical parameters of the kidney:

4.3.1. Urea

The results of Table (4-4) show that there are significant differences in the level of urea in the blood serum of patients, as its levels increased at the mean of 140.34 mg/dL, 121.53 mg/dL, and 74.00 mg/dL for PM and PF, with OTA, and PF, without OTA, respectively, compared to the healthy group, where the levels of urea in the blood serum showed at the normal level were 25.20 mg/dL, 24.63 mg/dL, 28.47 mg/dL, and 24.00 mg/dL for HM and HF with OTA, HM and HF, without OTA respectively.

Table 4-4: Comparison between urea

Groups	Mean (mg/dL)	SD	Duncan Test	*P* value
PM, with OTA	140.34	86.98	a	0.00029***
PF, with OTA	121.53	64.89	a	
PF, without OTA	74.00	0.00	ab	
HM, with OTA	25.20	3.46	b	
HF, with OTA	24.63	4.89	b	
HM, without OTA	28.47	5.09	b	
HF, without OTA	24.00	6.40	b	

***** Means high significant differences (*p<0.001*)**

The results obtained by Heussner *et al.*, (2006) confirm the potential for interactive (synergistic) effects of Citrinin and OTA and possibly other mycotoxins in cells of renal origin.

The study by Nourbakhsh and Tajbakhsh (2021) the study explains the causes of damage to cells through the main possible mechanisms of neurotoxicity are oxidative DNA, protein and lipid damage, and apoptosis of cells.

The increase in urea and creatinine is often associated with renal insufficiency Rosner and Bolton (2006a). The resulting agreement with Hassan and Ali (2022 b) who found that increased levels of urea in patients with nephropathy were 115 mg/dl in male patients while the level was 99.1 ng/dl compared to the levels in healthy which were 21.6 mg/dl.

Many studies found a significant positive relationship between urea and creatinine in progress chronic kidney disease as a result of the loss of glomerular filtration rate (Alaini *et al.*, 2017).

Also, increased levels of urea in blood serum are considered greater, whereas protein levels are significantly lower than in the healthy group indicating that the kidney is impaired, damaged, and toxic (Mir and Dwivedi, 2010).

The result agrees with Sharma *et al.*, (2011) that urea and creatinine concentrations change oppositely with the glomerular filtration rate, which is a particularly sensitive sign of kidney failure.

The result agrees with Lippold *et al.*, (1992) that urea levels of the blood are significantly higher than in the control group, although protein levels are significantly lower, indicating that kidney function is compromised.

OTA is nephrotoxic and causes gradual renal failure by destroying the renal tubular epithelium (Raghubeer *et al.*, 2015;Moretti *et al.*,2017).

4.3.2. Creatinine

The results of Table 4-5 show that there are highly significant differences in the level of creatinine in the blood serum of patients, as its levels increased at the mean of 3.655 mg/dL, 3.680 mg/dL, and 1.620 mg/dL for PM and PF with OTA, and PF without OTA, respectively, compared to the healthy group, where the levels of creatinine in the blood serum showed at the normal level were 0.700 mg/dL, 0.500 mg/dL, 0.739 mg/dL, and 0.536 mg/dL for HM and HF with OTA, HM and HF without OTA respectively.

Table 4-5: Comparison between creatinine

Groups	Mean (mg/dL)	SD	Duncan Test	*P* value
PM, with OTA	3.655	2.64	a	0.00018***
PF, with OTA	3.680	2.44	a	
PF, without OTA	1.620	0.00	b	
HM, with OTA	0.700	0.141	b	
HF, with OTA	0.500	0.083	b	
HM, without OTA	0.739	0.161	b	
HF, without OTA	0.536	0.113	b	

***** means high significant differences (*p<0.001*)**

The results of this study agreed with Hassan and Ali (2022b) who found that the level of creatinine in female patients with OTA was 3.73 mg/dl compared with the level in the blood serum of the female control was 0.571mg/dl. Also, Kareem (2021) reported that the level of creatinine in the blood serum of patients with chronic kidney diseases was 3.92 mg/dl compared with healthy levels of 0.66 mg/dl.

The increase in the concentration of serum creatinine indicates the progression of kidney disease. Also, increased creatinine in blood serum is considered a marker of renal damage (Bożentowicz-Wikarek *et al.*, 2016).

The result agrees with Stoev *et al.*, (2012) that blood creatinine levels were significantly increased and indicated severe kidney injury, According to experiments, the kidneys have a higher concentration of OTA.

A prominent marker of kidney damage and toxicity is an elevated blood creatinine level (Mir and Dwivedi, 2010).

The first stage in regulating the glomerular filtration rate (GFR) is the measurement of creatinine, which is frequently used to assess renal function(Rosner and Bolton, 2006b).

4.3.3. Glomerular filtration rate (GFR)

The result showed that GFR decreased in PM and PF with OTA, and PF without OTA groups to 25.69, 21.48, and 36.00 ml/min/1.7m² respectively with high significantly different as compared with HM and HF with OTA, HM, and HF without OTA which was 111.60, 114.64, 113.84 and 118.63 ml/min/1.7m², respectively, Table 4-6.

Table (4-6): Comparison between GFR

Groups	Mean (ml/min/1.73m2)	SD	Duncan Test	*P* value
PM, with OTA	25.69	14.24	b	0.000017***
PF, with OTA	21.48	14.52	b	
PF, without OTA	36.00	0.00	b	
HM, with OTA	111.60	8.60	a	
HF, with OTA	114.64	11.40	a	
HM, without OTA	113.84	13.85	a	
HF, without OTA	118.63	11.44	a	

***** Means high significant differences (*p<0.001*)**

The result agrees with Kareem (2021) found that OTA decreased GFR in the serum of patients with chronic kidney disease and serum contamination with OTA.

Studies by Dai *et al.*, (2002); Bertelli *et al.*, (2005), permit us to speculate that OTA's harmful effects are partly related to how it affects enzymatic activities and partially to how it directly affects the formation of ROS. The glomerular filtration rate is inversely proportional to chronic kidney disease(Pan *et al.*, 2019b).

The OTA nephrotoxicity mechanism involves oxidative stress, according to research on rats, kidney function is negatively impacted by OTA exposure, which also causes significant alterations; as the researcher explained that the reduction in GFR, which is connected to the generation of oxygen free radicals, and the increase in creatinine shown in rats treated with OTA are related; which proved that oxidative stress mediates the kidney toxic effects of OTA on a functional and histological level; a marker of membrane lipid damage, in the kidneys of rats, and that Glutathione peroxidase activity was primarily decreased (Damiano *et al.*, 2020).

In reality, oxidative stress can encourage the production of several vasoactive mediators that can directly impact renal functions by producing renal vasoconstriction or lowering the glomerular capillary ultrafiltration coefficient and, thus, lowering GFR (Craven *et al.*, 1992).

The mechanisms of OTA-induced nephrotoxicity include inhibition of protein synthesis, DNA damage, cell cycle arrest, and cell apoptosis (Gong *et al.*, 2019). OTA is recognized as a strong nephrotoxin of its accumulation in proximal tubule epithelial cells and initiating cellular damage through oxidative stress, DNA damage, apoptosis, and inflammatory response (Özcan *et al.*,2015).

OTA-induced nephrotoxicity has been addressed by a variety of treatments, however, the mechanism of action of this mycotoxin is still unclear and complex. However, in recent years, numerous in vivo and in vitro investigations have acknowledged that one of the mechanisms behind the toxicity of OTA is the production of oxidative stress caused by its exposure(Abdel-Wahhab *et al.*, 2017; Costa *et al.*, 2016).

It is generally recognized that oxidative stress results from an imbalance between the oxidant and antioxidant systems, which may be brought on by higher levels of free radical production and lower antioxidant activity. Numerous studies indicate that OTA exposure causes an excess of free radicals to be produced both in vitro and in vivo, causing lipids in membranes, one type of cell component, harm (Ciarcia *et al.*, 2016; Costa *et al.*, 2016b).

4.4. Evaluation of concentration of levels of the hormone in the blood serum of patients with chronic kidney disease compared with the control group

The following are the results of the effects of Ochratoxin A and chronic kidney disease on hormones.

4.4.1. Comparison between serum levels of TSH hormone

The result showed that mean levels of TSH in blood serum of PM and PF with OTA and PF without OTA decreased to 0.227 µIU/mL,0.247 µIU/mL, and 0.241 µIU/mL, respectively. With significantly different levels of TSH in the blood serum of HM and HF with OTA, HM, and HF without OTA were 6.072 µIU/mL, 3.757 µIU/mL, 1.852 µIU/mL, 1.692 µIU/mL respectively, Table 4-7.

Table 4-7: Comparison between serum levels of TSH hormone

Groups	Mean µIU/mL	SD	Duncan Test	*P* value
PM, with OTA	0.227	0.046	c	0.000192***
PF, with OTA	0.247	0.045	c	
PF, without OTA	0.241	0.000	c	
HM, with OTA	6.072	7.003	a	
HF, with OTA	3.757	4.757	b	
HM, without OTA	1.852	0.666	b	
HF, without OTA	1.692	0.795	b	

***** Means high significant differences (*p<0.001*)**

4.4.2 Comparison between serum levels of T_4 hormone

Table 4-8 showed that the levels of T4 hormone increased to 244.24 nmoL/L, 252.84 nmoL/L, and 322.627 nmoL/L in the blood serum of PM and PF with OTA, and PF without OTA, respectively with significantly different levels in the blood serum of HM and HF with OTA, HM and HF without OTA which they were 118.76 nmoL/L, 113.09 nmoL/L, 112.69 nmoL/L, and 107.28 nmoL/L respectively.

Table 4-8: Comparison between serum levels of T4 hormone

Groups	Mean (nmoL/L)	SD	Duncan Test	*P* value
PM, with OTA	244.24	82.4	a	0.000146***
PF, with OTA	252.84	87.5	a	
PF, without OTA	322.627	0.00	a	
HM, with OTA	118.76	9.68	b	
HF, with OTA	113.09	19.5	b	
HM, without OTA	112.69	15.9	b	
HF, without OTA	107.28	14.3	b	

***** means high significant differences (*p<0.001***

4.4.3. Comparison between serum levels of the T_3 hormone

Table 4-9 showed that the levels of T3 hormone increased to 19.461 nmoL/L, 18.896 nmoL/L, and 23.013 nmoL/L in the blood serum of PM and PF, with OTA, and PF without OTA, respectively with significant different levels in the blood serum of HM and HF, with OTA, HM and HF without OTA which they were 1.988 nmoL/L, 2.018 nmoL/L, 2.108 nmoL/L, and 1.946 nmoL/L respectively.

Table 4-9: Comparison between serum levels of T3 hormone

Groups	Mean (nmoL/L)	SD	Duncan Test	*P* value
PM, with OTA	19.461	4.31	b	0.000258***
PF, with OTA	18.896	5.09	b	
PF, without OTA	23.013	0.00	a	
HM, with OTA	1.988	0.25	c	
HF, with OTA	2.018	0.219	c	
HM, without OTA	2.108	0.210	c	
HF, without OTA	1.946	0.63	c	

***** Means high significant differences (*p<0.001*)**

From the results of Table 4-7, we note a significant decrease for all samples of chronic kidney patients compared to healthy samples in the level of TSH hormone in the blood, and there were statistically significant differences between the level of the hormone in the blood. From this result, we conclude that ochratoxin A affects the level of the hormone in the blood synergistically with CKD.

According to the results of tables 4-8 and 4-9, the level of the hormones T4, and T3 in the blood increased significantly for all samples of patients with CKD when compared to healthy samples. From this result, we conclude that ochratoxin A affects the level of the hormone in the blood synergistically with CKD. Numerous studies have demonstrated the alteration in T4, and T3 hormone levels that take place in patients with CKD.

The current study disagrees with Pan *et al.,* (2019) where 905 non-dialysis participants, based on the estimated glomerular filtration rate (eGFR), The lowest level of T3 was reported in CKD5. It increased with increasing eGFR, where no significant differences were found between groups in T4, and TSH ($p > 0.05$).

Wang *et al.,* (2020) findings demonstrated that subclinical hypothyroidism was significantly associated with a higher risk of CKD independent of some conventional risk factors among the community population and age might.

The current study disagrees with Elaroussi *et al.,* (2006) showed a highly significant decrease in the level of T3 and T4 hormones with the presence of OTA in the broiler diets. and an inverse relationship was shown between the dietary OTA level and the serum T4 concentration.

Showed an increasing trend for the population of low T3 according to the increase in CKD stage with normal level of TSH ($eGFR \geq 90$, 8.2%; $60 \leq eGfr < 90$, 10.9%; $30 \leq eGFR < 60$, 20.8%; $15 \leq eGFR < 30$, 60.6%; $eGFR < 15$, 78.6%). Similar to previous studies, a large proportion of CKD 4–5 patients also had ESS (62.1 and 69.1%). Meanwhile, low T3 syndrome now is used as a marker of severe disease(Pan *et al.,* 2019a).

The current study disagrees with Hassan *et al.,* (2010)showed mycotoxins caused a significant decrease ($P<0.05$) in T3, T4, and T3 / T4 ratio compared to controls in laboratory animals.

In a previous study, the investigation was conducted to evaluate the adverse effects of Ochratoxin A (OTA) administered orally for 30 days caused significant alterations in the serum levels of various body hormones such as thyroid hormones (triiodothyronine and thyroxin) of adult male Wistar rats(Kumar *et al.*, 2008; Kumar *et al.*, 2011).

The current study agrees with Elsayed *et al.,* (2019) showed that serum T4 levels significantly increased ($p<0.05$) in the OTA group as compared to the control group (in animals).

4.5. Measurement of OTA-positive group of CKD patients with blood physiological parameters:

4.5.1. White blood cells (WBC)

The number of WBCs was 10.45, 9.32, and 10.29 *10^3/UL in the PM and PF with the OTA, and PF without OTA, respectively. Without significantly different healthy control than HM and HF with OTA, HM, and HF without OTA the mean number of WBC in these was 7.00,7.41, 7.04, 8.25* 10^3/UL respectively Table 4-10.

Table 4-10: Comparison between WBC

Groups	Mean 10^3/UL	SD	Duncan Test	*P* value
PM, with OTA	10.45	4.24	NS	0.062
PF, with OTA	9.32	3.31	NS	
PF, without OTA	10.29	0.00	NS	
HM, with OTA	7.00	1.32	NS	
HF, with OTA	7.41	2.25	NS	
HM, without OTA	7.04	1.48	NS	
HF, without OTA	8.25	2.82	NS	

NS: No significant differences

Low-grade inflammation is common in patients with CKD and end-stage renal disease (ESRD) and has already been recognized as playing a unique role in the pathophysiology and taking into account future cardiovascular diseases and all-cause mortality risk (Akchurin and Kaskel, 2015).

In a previous study, WBC count was found to be a better predictor of CKD risk in African Americans relative to whites (Bash *et al.*, 2009).

There was no difference in the levels of albumin, CRP, ESR, and WBC in women with renal insufficiency, but there was a significant difference between levels of serum albumin and ESR in male patients(Yildirim *et al.*,2013a).

In a study of 97 patients with renal failure, it was found that the median white blood cell count was 8.4, while the white blood cell count was 7.6 for 47 healthy control, there was no difference in the levels of WBC with renal failure (Yildirim *et al.*,2013b)

The previous study found low WBC count to be independently associated with CKD progression in patients aged over 60 years with pre-dialysis CKD stages G2–G5. Accumulating evidence indicates a high WBC count is associated with a worse prognosis among patients with CKD as a high WBC count is generally recognized to reflect an activated inflammatory state (Arai *et al.*, 2018).

These results agree with Hassan and Ali (2022b) who found that the mean of WBC was 9.078 $*10^3$/UL in CKD with OTA.

In CKD patients, several interventions, such as general lifestyle modifications, dietary supplementation, and pharmacological agents, have already been proposed to target inflammation(Machowska *et al.*, 2016).

Mycotoxins are dangerous chemical substances of low molecular weight that the immune system can not identify, in contrast to bacterial toxins, which are proteins that the body's immune system can recognize. As a result, there is no reaction when the body comes into contact with mycotoxins (Buszewska-Forajta, 2020).

4.5.2. Red Blood Cells (RBC)

The results of RBC shown in Table 4-11 appearance number of RBC in the blood of PM and PF with OTA, and PF without OTA were 4.31, 3.75, and 3.99 $*10^6$ cell/ul, respectively with significantly different healthy control HM and HF with OTA, and HM and HF without OTA were 5.27, 4.73, 4.84, and 4.81$*10^6$ cell/ul, respectively.

Table 4-11: Comparison between RBC

Groups	Mean (10^6 cell/ul)	SD	Duncan Test	*P* value
PM, with OTA	4.31	0.92	b	0.000107***
PF, with OTA	3.75	0.72	b	
PF, without OTA	3.99	0.00	b	
HM, with OTA	5.27	0.81	a	
HF, with OTA	4.73	0.46	a	
HM, without OTA	4.84	0.56	a	
HF, without OTA	4.81	0.44	a	

***** Means high significant differences (*p<0.001***

Red blood cells can be damaged from both internal and external sources. The dominant factor of oxidative stress within the RBC is Hb. Oxygen derivative free radicals are generated as a result of autooxidation of Hb associated with the inner surface of the membrane, mainly with cytoskeleton proteins(Rifkind and Nagababu, 2013), Oxidative damage to the erythrocyte plasma membrane leads to impaired oxygen supply and leads to accelerated aging of red blood cells (Mohanty *et al.*,2014).

Advanced chronic kidney disease (CKD) is almost invariably associated with anemia that is primarily caused by depressed production of erythropoietin, oxidative stress, and inflammation (Vaziri and Zhou, 2008)

The decrease in the number of red blood cells belongs to the body's unable to absorb iron and vitamins from food which leads to the depletion of iron stores in the body (AL-Jumiley, 2014)

They discovered that nephropathy plays a significant role in the development of anemia (Ragen, 1960). The elimination of circulation brought on by OTA-induced extravasation of the blood could explain the drop in RBC(Jordan *et al.*,1977).

4.5.3. Hemoglobin level (HGB)

The result shown in Table 4-12 was the presence of significant differences in *P* value 0.000018 between each of them PM and PF with OTA, and PF without OTA, were 11.16, 9.85, and 11.03 g/dl, respectively compared to healthy control of them HM and HF with OTA, HM and HF without OTA were 14.22, 12.26, 13.65, and 11.92 g/dl, respectively.

Table 4-12: Comparison between HGB

Groups	**Mean (**g/dl)	**SD**	**Duncan Test**	***P* value**
PM, with OTA	11.16	2.08	d	0.00018***
PF, with OTA	9.85	1.64	d	
PF, without OTA	11.03	0.00	d	
HM, with OTA	14.22	1.64	a	
HF, with OTA	12.26	1.75	ab	
HM, without OTA	13.65	1.41	ab	
HF, without OTA	11.92	0.94	b	

***** Means high significance differences (*p<0.001*)**

The results of this study agree with Hassan and Ali (2022), who found a decrease in the level of HGB to 11.44 g/dl and 11.8 g/dl in the blood serum of females.

This outcome supports the Miale (1972)finding that OTA reduced hemoglobin levels. Further research revealed that OTA significantly reduced hemoglobin levels(Huff *et al.*, 1979a).

discovered that iron losses are related to the anemia of nephropathy. Patients with nephropathy who frequently lose iron have a functional iron shortage due to poor dietary iron absorption from the gastrointestinal tract(Babitt and Lin, 2012).

In a previous study in animals treated with OTA total erythrocyte counts, leukocyte count, PCV, and Hb were decreased (Ahmad *et al.*, 2012).

The reason for the low level of hemoglobin may be due to the association of ochratoxin A with the membrane proteins of the red blood cells, which affects the energy supply of the cells represented by ATP, as well as the lack of transport of sugars into the cells, and this process leads to inflammation in the short life of the cells and thus their death, which is reflected in the decrease The amount of hemoglobin in the blood(Cloherty *et al.*,2001)

The result of the study approached with Al-Khalid(2010) who found the level of hemoglobin (HB) in the blood serum of rats treated with Geotrichum toxin caused a decreased level of HB to 9.2 gm/100ml. Also, the similarity to al-robbery (2007) study showed that HB decreased in the blood serum of rats to 9.3gm/100ml.

The decrease in HB level belongs to the mycotoxin induce an increase of oxidate possession in cells the result of this possession produces free radical that invade red blood and lysis leading to a decrease in HB level (Gautier *et al.*, 2001).

This present study agrees with Solak *et al.*, (2013), the HGB values show a significant difference in CKD where the *p*-value was <0.001.

4.5.4. Hematocrit

The result shown in Table (4-13) was the presence of significant differences in *P* value 0.000104 between each of PM and PF with OTA, and PF without OTA, were 35.17, 30.66, and 34.85 %, respectively compared to healthy control of them HM and HF with OTA, HM and HF without OTA were 42.82, 38.91, 40.99, and 36.85%, respectively.

Table 4-13: Comparison between HCT for study groups

Groups	Mean %	SD	Duncan Test	*P* value
PM, with OTA	35.17	6.05	d	0.000104***
PF, with OTA	30.66	5.09	d	
PF, without OTA	34.85	0.00	d	
HM, with OTA	42.82	5.83	a	
HF, with OTA	38.91	4.47	ab	
HM, without OTA	40.99	4.24	ab	
HF, without OTA	36.85	2.86	b	

***** Means high significant differences (*p<0.001*)**

The decrease belongs to the toxin OTA possibly attributable to a defensive reaction against toxicity through stimulation of erythropoiesis or may be associated with decreased erythrocyte hemoglobin and HTC (Al-Jumyily,2014)

The present study results agree with Paul *et al., (*1979) who found that OTA impact time hemoglobin level was significantly reduced compared to the level in blood serum of healthy on the other hand HCT decreased by up to 30% in blood serum of patients with kidney chronic disease.

The reduction of HCT belongs to removal from circulation as a result of OTA-induced blood extraversion (Jordon *et al.*,1977). Hassan and Ali (2022) found that OTA caused decreased hematocrit in the blood serum of male patients with nephropathy to 32.68% compared with 52% in the blood serum of male healthy.

4.5.5. Mean Corpuscular Volume (MCV)

The Duncan test did not show any significant differences in Mean Corpuscular Volume between patients and healthy control where the *P* value > 0.3451 as shown in Table (4-14).

Table (4-14): Comparison between MCV

Groups	Mean/ fl	SD	Duncan Test	*P* value
PM, with OTA	82.82	7.14	NS	0.3451
PF, with OTA	83.30	6.48	NS	
PF, without OTA	87.24	0.00	NS	
HM, with OTA	81.68	5.74	NS	
HF, with OTA	82.28	7.61	NS	
HM, without OTA	84.43	4.35	NS	
HF, without OTA	85.75	3.87	NS	

NS: No significant different

In the current study, the mobilized cell volume was without a significant *P* value of 0.3451. agree with Huff *et al.*, (1979b) that the total volume of erythrocyte count cells in circulating erythrocytes did not change significantly with graded levels of dietary OTA.

In this present study same as Solak *et al.*, (2013), the MCV values did not show a significant difference in CKD where the *p*-value was 0.341.

4.5.6. Platelets (PLT)

As shown in Table 4-15, there were no significant differences in platelets and the value of *P* value 0.31260.

Table 4-15: Comparison between PLT

Groups	Mean $\times 10^3$ /UL	SD	Duncan Test	*P* value
PM, with OTA	266.36	87.77	NS	0.31260
PF, with OTA	247.22	117.31	NS	
PM, without OTA	0.000	0.000	NS	
PF, without OTA	299.5	0.00	NS	
HM, with OTA	245.8	31.09	NS	
HF, with OTA	252.72	74.13	NS	
HM, without OTA	220.06	52.98	NS	
HF, without OTA	250.95	71.33	NS	

NS: No significant differences

This finding conflicts with other studies that claim anemia is a common nephropathy consequence that can worsen the likelihood of bleeding by causing platelet dysfunction(Miale, 1972). Additionally, other trials revealed that OTA decreased PLT levels and had an impact on clotting time(‘Miale, 1972).

4.6. Evaluate the relationship between the parameters:

4.6.1. The correlation coefficient (r) between OTA and other parameters

Shows the results of the statistical analysis of Table 4-16, which includes finding a correlation r between OTA and the rest of the factors in this study, where there was a direct correlation between OTA and the factors Urea, Creatinine, WBC, PLT, T3, T4 if their correlation coefficient values 0.474, 0.363, 0.320,0.188, 0.519, 0.431 were respectively.

While the correlation relationship was inverse between OTA and each of the following factors's CKD, GFR, RBC, HGB, HCT, MCV, and TSH, and their correlation coefficient values were - 0.554, - 0.521, - 0.209, - 0.362, - 0.338, - 0.150, and - 0.212, respectively. It should be noted that the relationship between OTA and the following factors Gender was non-significant *P* value >0.05.

Table 4-16: The correlation coefficient (r) between OTA and other parameters

Parameters	The correlation r	*P* value
CKD	- 0.554*	0.000
Gender	0.044	0.534
Urea	0.474***	0.000
Creatinine	0.363***	0.000
GFR	- 0.521***	0.000
WBC	0.320***	0.000
RBC	- 0.209***	0.003
HGB	- 0.362***	0.000
HCT	- 0.338***	0.000
MCV	- 0.150*	0.034
PLT	0.188***	0.008
TSH	- 0.212***	0.003
T3	0.519***	0.000
T4	0.431***	0.000

*** Means significant differences (*p<0.05*)** ***** Means high significant differences (*p<0.001*)**

4.6.2. The correlation coefficient (r) between CKD and other parameters:

Demonstrates the findings of the statistical analysis of Table 4-18, which includes determining a correlation r between CKD and the other factors in this study. If the correlation coefficient values of the factors GFR, RBC, HGB, HCT, and TSH were 0.959, 0.452, 0.585, 0.553, and 0.452, respectively, then there was a direct correlation between CKD and those factors.

The correlation between CKD and each of the following variables was inverse, with their correlation coefficient values being OTA, Urea, Creatinine, WBC, T3, and T4. It should be mentioned that there was no statistically significant correlation between CKD and the following parameters Gender, MCV *P* value >0.05.

Table 4-17: The correlation coefficient (r) between CKD and other parameters

Parameters	The correlation (r)	*P* value
OTA	- 0.554*	0.000
Gender	- 0.130	0.067
Urea	- 0.696***	0.000
Creatinine	- 0.644***	0.000
GFR	0.959***	0.000
WBC	- 0.369***	0.000
RBC	0.452***	0.000
HGB	0.585***	0.000
HCT	0.553***	0.000
MCV	0.069	0.332
PLT	- 0.112	0.114
TSH	0.452***	0.000
T3	- 0.931***	0.000
T4	- 0.749***	0.000

*** Means significant differences (*p<0.05*)** ***** means high significant differences (*p<0.001*)**

Conclusions

and

Recommendations

Conclusions

1. This study showed that the levels of OTA concentration in CKD are much higher than the permissible levels in the blood, and the presence of the same toxin in the blood of healthy people indicates the presence of food contamination.
2. This study proved that ochratoxin A causes an imbalance in the levels of the studied hormones (TSH, T3, and T4), and this imbalance reflects negatively on metabolism and therefore may be associated with many diseases that affect humans.
3. The study showed that there was a significant relationship between ochratoxin A and chronic renal failure, and the nature of the relationship was synergistic between them.
4. The effect of ochratoxin A poison on most of the studied physiological and biochemical parameters led to a deviation from their normal levels.
5. This study is the first locally and globally according to the available sources, especially the effects of ochratoxin A poison on hormone levels (TSH, T3, and T4).

6. The influence of Ochratoxin A on reducing GFR and raising urea and creatinine levels was demonstrated in the current study.

Recommendations:

1. Considering Ochratoxin A as a risk factor for chronic kidney disease in Iraq.
2. Examination of the presence of ochratoxin A in the blood of patients with renal failure, and if the results are positive, the treatment of the poison with antitoxins is part of the renal failure treatment plan.
3. The safety of foods, especially those imported from countries in very tiny quantities and within legal limits, must be carefully considered because they are the principal source of human exposure to this and other microbial toxins.
4. A study of the effect of ochratoxin A on the human immune system.
5. Investigating how ochratoxin A affects several hormones, particularly testosterone, estrogen, and others. Moreover, further studies should be done to determine the effects of OTA on the reproductive health of patients and its correlation with TRH and the Hypothalamic-Pituitary-Thyroid-Adrenal (HPA) axis.
6. Investigations about the gender difference with OTA, CKD, and thyroid disorders.
7. Detection of the effects of OTA one erythropoietin (EPO).

References

References:

- Abdel-wahhab, m.a. et al. (2017) ‘chitosan nanoparticles plus quercetin suppress the oxidative stress, modulate dna fragmentation and gene expression in the kidney of rats fed ochratoxin a-contaminated diet’, food and chemical toxicology, 99, pp. 209–221. Available at: https://doi.org/10.1016/j.fct.2016.12.002.
- Adam, m.a.a. et al. (2017) ‘effects of different mycotoxins on humans, cell genome and their involvement in cancer’, oncology reports, 37(3), pp. 1321–1336. Available at: https://doi.org/10.3892/or.2017.5424.
- Ahmad, muhammad fakhar-ud-din, et al. Effects of ochratoxin a feeding in white leghorn cockerels on hematological and serum biochemical parameters and its amelioration with silymarin and vitamin e. Pakistan veterinary journal, 2012, 32.4.
- Akchurin, o.m. and kaskel, f. (2015) ‘update on inflammation in chronic kidney disease’, blood purification, 39(1–3), pp. 84–92. Available at: https://doi.org/10.1159/000368940.
- Alaini, a., malhotra, d., rondon-berrios, h., argyropoulos, c. P., khitan, z. J., raj, d. S., ... & tzamaloukas, a. H. (2017). Establishing the presence or absence of chronic kidney disease: uses and limitations of formulas estimating the glomerular filtration rate. World journal of methodology, 7(3), 73.
- Ali, n. And majeed, a. (2022) ‘thyroid hormone concentration and receptor’, egyptian academic journal of biological sciences, b. Zoology, 14(1), pp. 221–230. Available at: https://doi.org/10.21608/eajbsz.2022.233650.
- Alina marc, r. (2022) ‘implications of mycotoxins in food safety’, in mycotoxins and food safety -recent advances. Intechopen. Available at: https://doi.org/10.5772/intechopen.102495.
- Al-jumiley, s.a.a. (2014) mycotoxin. Book house, karbala, iraq, pp422.
- Al-mhanaa, h.a.h., & a.-o.a.b. (2020) ‘the evaluation of ochratoxin a in patients suffer from renal failure.’, indian journal of forensic medicine & toxicology, 14(3).
- Al-musoui, h.r., (2015) study the relationship between some fungi and its toxins with kidney failure unknown case. College of Science /Al-qadisiya university.

References

- Arai, y., kanda, e., iimori, s., naito, s., noda, y., sasaki, s., ... & uchida, s. (2018). Low white blood cell count is independently associated with chronic kidney disease progression in the elderly: the ckd-route study. Clinical and experimental nephrology, 22, 291-298.
- Armorini, s., al-qudah, k. M., altafini, a., zaghini, a., & roncada, p. (2015). Biliary ochratoxin a as a biomarker of ochratoxin exposure in laying hens: an experimental study after administration of contaminated diets. Research in veterinary science, 100, 265-270.
- Aycicek, h., aksoy, a. And saygi, s. (2005) 'determination of aflatoxin levels in some dairy and food products which consumed in ankara, turkey', food control, 16(3), pp. 263–266. Available at: https://doi.org/10.1016/j.foodcont.2004.03.004.
- Babitt, j.l. and lin, h.y. (2012) 'mechanisms of anemia in ckd', journal of the american society of nephrology, 23(10), pp. 1631–1634. Available at: https://doi.org/10.1681/asn.2011111078.
- Barac, a. (2019a) 'mycotoxins and human disease', in clinically relevant mycoses. Cham: springer international publishing, pp. 213–225. Available at: https://doi.org/10.1007/978-3-319-92300-0_14.
- Barac, a. (2019b) 'mycotoxins and human disease', in clinically relevant mycoses. Cham: springer international publishing, pp. 213–225. Available at: https://doi.org/10.1007/978-3-319-92300-0_14.
- Bash, l. D., erlinger, t. P., coresh, j., marsh-manzi, j., folsom, a. R., & astor, b. C. (2009). Inflammation, hemostasis, and the risk of kidney function decline in the atherosclerosis risk in communities (aric) study. American journal of kidney diseases, 53(4), 596-605.
- Belkacem-hanfi, n., fhoula, i., semmar, n., guesmi, a., perraud-gaime, i., ouzari, h. I., ... & roussos, s. (2014). Lactic acid bacteria against post-harvest moulds and ochratoxin a isolated from stored wheat. Biological control, 76, 52-59.
- Benvenga, s., tuccari, g., ieni, a., & vita, r. (2018). Thyroid gland: anatomy and physiology. Encyclopedia of endocrine diseases, 4, 382-390.
- Bertelli, a. A., migliori, m., filippi, c., gagliano, n., donetti, e., panichi, v., ... & giovannini, l. (2005). Effect of ethanol and red wine on ochratoxin a-induced experimental acute nephrotoxicity. Journal of agricultural and food chemistry, 53(17), 6924-6929.

References

- Bożentowicz-wikarek, m., owczarek, a., kocełak, p., olszanecka-glinianowicz, m., więcek, a., & chudek, j. (2016). C-terminal to intact fibroblast growth factor 23 ratio in relation to estimated glomerular filtration rate in elderly population. Kidney and blood pressure research, 41(5), 519-526.
- Breitholtz-emanuelsson, a., minervini, f., hult, k., & visconti, a. (1994). Ochratoxin a in human serum samples collected in southern italy from healthy individuals and individuals suffering from different kidney disorders. Natural toxins, 2(6), 366-370.
- Burger, h.g. and patel, y.c. (1972) 'the value of serum thyrotrophin measurement in the diagnosis and management of hypothyroidism.', the medical journal of australia, 2(6), pp. 293–297. Available at: https://doi.org/10.5694/j.1326-5377.1972.tb47302.x.
- Burggren, w., & bautista, n. (2019). Invited review: development of acid-base regulation in vertebrates. Comparative biochemistry and physiology part a: molecular & integrative physiology, 236, 110518.
- Buszewska-forajta, m. (2020) 'mycotoxins, invisible danger of feedstuff with toxic effect on animals', toxicon. Elsevier ltd, pp. 34–53. Available at: https://doi.org/10.1016/j.toxicon.2020.04.101.
- Carvalho, d.p. and dupuy, c. (2017) 'thyroid hormone biosynthesis and release', molecular and cellular endocrinology, 458, pp. 6–15. Available at: https://doi.org/10.1016/j.mce.2017.01.038.
- Chu, f.s. (1996) 'recent studies on immunoassays for mycotoxins', in, pp. 294–313. Available at: https://doi.org/10.1021/bk-1996-0621.ch022.
- Ciarcia, r., damiano, s., squillacioti, c., mirabella, n., pagnini, u., florio, a., ... & florio, s. (2016). Recombinant mitochondrial manganese containing superoxide dismutase protects against ochratoxin a-induced nephrotoxicity. Journal of cellular biochemistry, 117(6), 1352-1358.
- Cloherty, e.k., levine, k.b. and carruthers, a. (2001) 'the red blood cell glucose transporter presents multiple, nucleotide-sensitive sugar exit sites', biochemistry, 40(51), pp. 15549–15561. Available at: https://doi.org/10.1021/bi015586w.
- Costa, j.g. et al. (2016a) 'ochratoxin a-induced cytotoxicity, genotoxicity and reactive oxygen species in kidney cells: an integrative approach of complementary endpoints', food

and chemical toxicology, 87, pp. 65–76. Available at: https://doi.org/10.1016/j.fct.2015.11.018.

- Costa, j.g. et al. (2016b) ‘ochratoxin a-induced cytotoxicity, genotoxicity and reactive oxygen species in kidney cells: an integrative approach of complementary endpoints’, food and chemical toxicology, 87, pp. 65–76. Available at: https://doi.org/10.1016/j.fct.2015.11.018.
- Craven, p.a., melhem, m.f. and derubertis, f.r. (1992) ‘thromboxane in the pathogenesis of glomerular injury in diabetes’, kidney international, 42(4), pp. 937–946. Available at: https://doi.org/10.1038/ki.1992.370.
- Dai, j., park, g., wright, m. W., adams, m., akman, s. A., & manderville, r. A. (2002). Detection and characterization of a glutathione conjugate of ochratoxin a. Chemical research in toxicology, 15(12), 1581-1588.
- Damiano, s., andretta, e., longobardi, c., prisco, f., paciello, o., squillacioti, c., ... & ciarcia, r. (2020). Effects of curcumin on the renal toxicity induced by ochratoxin a in rats. Antioxidants, 9(4), 332.
- D′ Mello, J. P. F., Porter, J. K., Macdonald, A. M. C., & Placinta, C. M. (1997). Fusarium mycotoxins. Handbook of plant and fungal toxicants, 287-301.
- Dousdampanis, p., trigka, k., vagenakis, g. A., & fourtounas, c. (2014). The thyroid and the kidney: a complex interplay in health and disease. The international journal of artificial organs, 37(1), 1-12.
- Edite bezerra da rocha, m. Et al. (2014) ‘mycotoxins and their effects on human and animal health’, food control, pp. 159–165. Available at: https://doi.org/10.1016/j.foodcont.2013.08.021.
- Elaroussi, m. A., mohamed, f. R., el barkouky, e. M., atta, a. M., abdou, a. M., & hatab, m. H. (2006). Experimental ochratoxicosis in broiler chickens. Avian pathology, 35(4), 263-269.
- Ellam, t., el-kossi, m., prasanth, k. C., el-nahas, m., & khwaja, a. (2009). Conservatively managed patients with stage 5 chronic kidney disease—outcomes from a single center experience. Qjm: an international journal of medicine, 102(8), 547-554.

References

- Elsayed, m. A. E., mohamed, n. E. S., hatab, m. H., & elaroussi, m. A. (2019). Oxidative stress of in-ovo ochratoxin a administered during chick embryonic development. Brazilian journal of poultry science, 21.
- Ermans am. (1986) ‘disorders of iodine deficiency.’, Philadelphia: jb lippincott co, 705(721).
- Fattah, h., layton, a. And vallon, v. (2019) ‘how do kidneys adapt to a deficit or loss in nephron number?’, physiology (bethesda, md.). Nlm (medline), pp. 189–197. Available at: https://doi.org/10.1152/physiol.00052.2018.
- Folin, o. (1904) ‘beitrag zur chemie des kreatinins und kreatins im harne.’, hoppe-seyler´s zeitschrift für physiologische chemie, 41(3), pp. 223–242. Available at: https://doi.org/10.1515/bchm2.1904.41.3.223.
- Freire, f.d.c.o. and da rocha, m.e.b. (2017a) ‘impact of mycotoxins on human health’, in fungal metabolites. Cham: springer international publishing, pp. 239–261. Available at: https://doi.org/10.1007/978-3-319-25001-4_21.
- Freire, f.d.c.o. and da rocha, m.e.b. (2017b) ‘impact of mycotoxins on human health’, in fungal metabolites. Springer international publishing, pp. 239–261. Available at: https://doi.org/10.1007/978-3-319-25001-4_21.
- Fuchs, r. And peraica, m. (2005) ‘ochratoxin a in human kidney diseases’, food additives &contaminants,22(sup1),pp.53–57.Availableat: https://doi.org/10.1080/02652030500309368.
- Galtier, p. (1977). Contribution of pharmacokinetic studies to mycotoxicology—ochratoxin a. Veterinary science communications, 1, 349-358.
- Galtier, p., alvinerie, m., & charpenteau, j. L. (1981). The pharmacokinetic profiles of ochratoxin a in pigs, rabbits and chickens. Food and cosmetics toxicology, 19, 735-738.
- Zhang, g., lu, y., yang, l., dong, y., wen, j., xu, j., & zhang, q. (2020). Methylene blue post-treatment improves hypoxia-ischemic recovery in a neonatal rat model. Neurochemistry international, 139, 104782.
- Ghali, r. Et al. (2008) ‘hplc determination of ochratoxin a in a low volume of human blood serum’, analytical letters, 41(5), pp. 757–766. Available at: https://doi.org/10.1080/00032710801934528.

References

- Gautier, J. C., Holzhaeuser, D., Markovic, J., Gremaud, E., Schilter, B., & Turesky, R. J. (2001). Oxidative damage and stress response from ochratoxin A exposure in rats. Free Radical Biology and Medicine, 30(10), 1089-1098.
- Glass, c.k. and holloway, j.m. (1990) 'regulation of gene expression by the thyroid hormone receptor', biochimica et biophysica acta (bba) - reviews on cancer, 1032(2–3), pp. 157–176. Available at: https://doi.org/10.1016/0304-419x(90)90002-i.
- Gong, l., zhu, h., li, t., ming, g., duan, x., wang, j., & jiang, y. (2019). Molecular signatures of cytotoxic effects in human embryonic kidney 293 cells treated with single and mixture of ochratoxin a and citrinin. Food and chemical toxicology, 123, 374-384.
- Gurikar, c. Et al. (2023) 'impact of mycotoxins and their metabolites associated with food grains', grain & oil science and technology, 6(1), pp. 1–9. Available at: https://doi.org/10.1016/j.gaost.2022.10.001.
- Hadi al-mhanaa, h.a., & a.-o.a.b. (2020) 'the evaluation of ochratoxin a in patients suffer from renal failure.', Indian journal of forensic medicine & toxicology, 14(3).
- Hajok, i. Et al. (2019a) 'a risk assessment of dietary exposure to ochratoxin a for the polish population', food chemistry, 284, pp. 264–269. Available at: https://doi.org/10.1016/j.foodchem.2019.01.101.
- Hajok, i. Et al. (2019b) 'a risk assessment of dietary exposure to ochratoxin a for the polish population', food chemistry, 284, pp. 264–269. Available at: https://doi.org/10.1016/j.foodchem.2019.01.101.
- Hanna abboud, m.d., and w.l.h.m.d. (2010) 'stage iv chronic kidney disease', new england journal of medicine, 362(1).
- Hassan, a. A., rashid, m. A., & koratum, k. M. (2010). Effect of aflatoxin b1, zearalenone and ochratoxin a on some hormones related to fertility in male rats. Life sci. J, 7(3), 64-72.
- Hassan and ali. (2022) 'investigation of ochratoxin in blood of chronic kidney disease of uncertain etiology.', turkish journal of physiotherapy and rehabilitation, 32(3).
- Heussner, a. H., dietrich, d. R., & o'brien, e. (2006). In vitro investigation of individual and combined cytotoxic effects of ochratoxin a and other selected mycotoxins on renal cells. Toxicology in vitro, 20(3), 332-341.

References

- Huff, w. E., chang, c. F., warren, m. F., & hamilton, p. B. (1979). Ochratoxin a-induced iron deficiency anemia. Applied and environmental microbiology, 37(3), 601-604.
- Hussein, h.s. and brasel, j.m. (2001) toxicity, metabolism, and impact of mycotoxins on humans and animals, toxicology. Available at: www.elsevier.com/locate/toxicol.
- Inker, l.a. and titan, s. (2021) 'measurement and estimation of gfr for use in clinical practice: core curriculum 2021', american journal of kidney diseases. W.b. saunders, pp. 736–749. Available at: https://doi.org/10.1053/j.ajkd.2021.04.016.
- Jackson, i.m. (1982) 'thyrotropin-releasing hormone', new england journal of medicine, 306(3).
- Janik, e., niemcewicz, m., ceremuga, m., stela, m., saluk-bijak, j., siadkowski, a., & bijak, m. (2020). Molecular aspects of mycotoxins—a serious problem for human health. International journal of molecular sciences, 21(21), 8187.
- Jordan, w. H., carlton, w. W., & sansing, g. A. (1977). Citrinin mycotoxicosis in the mouse. Food and cosmetics toxicology, 15(1), 29-34.
- Jung, k. Y., takeda, m., kim, d. K., tojo, a., narikawa, s., yoo, b. S., ... & endou, h. (2001). Characterization of ochratoxin a transport by human organic anion transporters. Life sciences, 69(18), 2123-2135.
- Kalantar-zadeh, k., jafar, t. H., nitsch, d., neuen, b. L., & perkovic, v. (2021). Chronic kidney disease. The lancet, 398(10302), 786-802.
- Kaplan, m. M., larsen, p. R., crantz, f. R., dzau, v. J., rossing, t. H., & haddow, j. E. (1982). Prevalence of abnormal thyroid function test results in patients with acute medical illnesses. The american journal of medicine, 72(1), 9-16.
- Kareem (2021) the features of aflatoxinb1 effects on chronic kidney diseases. College of Medicine /University of Kerbala .
- Department of chemistry and biochemistry
- Kellum, j. A., romagnani, p., ashuntantang, g., ronco, c., zarbock, a., & anders, h. J. (2021). Acute kidney injury. Nature reviews disease primers, 7(1), 52.
- Khairnar, b. A., dabhane, h. A., dashpute, r. S., girase, m. S., nalawade, p. M., & gaikwad, v. B. (2022). Study of biogenic fabrication of zinc oxide nanoparticles and their applications: a review. Inorganic chemistry communications, 110155.

References

- Knasmuller, s., cavin, c., chakraborty, a., darroudi, f., majer, b. J., huber, w. W., & ehrlich, v. A. (2004). Structurally related mycotoxins ochratoxin a, ochratoxin b, and citrinin differ in their genotoxic activities and in their mode of action in human-derived liver (hepg2) cells: implications for risk assessment. Nutrition and cancer, 50(2), 190-197.
- Koletsi, p., schrama, j. W., graat, e. A., wiegertjes, g. F., lyons, p., & pietsch, c. (2021). The occurrence of mycotoxins in raw materials and fish feeds in europe and the potential effects of deoxynivalenol (don) on the health and growth of farmed fish species—a review. Toxins, 13(6), 403.
- Kőszegi, t., & poór, m. (2016). Ochratoxin a: molecular interactions, mechanisms of toxicity and prevention at the molecular level. Toxins, 8(4), 111.
- Kumagai, s., & aibara, k. (1982). Intestinal absorption and secretion of ochratoxin a in the rat. Toxicology and applied pharmacology, 64(1), 94-102.
- Kumar, m., dwivedi, p., sharma, a. K., sankar, m., patil, r. D., & singh, n. D. (2014). Apoptosis and lipid peroxidation in ochratoxin a-and citrinin-induced nephrotoxicity in rabbits. Toxicology and industrial health, 30(1), 90-98.
- Kumar, s. N., jain, a. K., singh, k. P., shrivastava, n., & telang, a. G. (2008). Sub-acute toxic effect of ochratoxin a and endosulfan alone and their combination on hormonal disorder in adult male rats. Toxicology letters, 180(1), s188.
- Kumar, s. N., telang, a. G., singh, k. P., jain, a. K., afroz, m., & patil, r. D. (2011). Experimentally induced toxicity of ochratoxin a and endosulfan in male wistar rats: a hormonal disorder. J. Anim. Vet. Adv, 10, 1750-1755.
- Lafranchi, s. H. (2021). Thyroid function in preterm/low birth weight infants: impact on diagnosis and management of thyroid dysfunction. Frontiers in endocrinology, 12, 666207.
- Larsen, p. R. (1972). Triiodothyronine: review of recent studies of its physiology and pathophysiology in man. Metabolism, 21(11), 1073-1092.
- Lee, hyun jung, et al. Renal toxicity through ahr, pxr, and nrf2 signaling pathway activation of ochratoxin a-induced oxidative stress in kidney cells. Food and chemical toxicology, 2018, 122: 59-68.

References

- levey, a. S., titan, s. M., powe, n. R., coresh, j., & inker, l. A. (2020). Kidney disease, race, and gfr estimation. Clinical journal of the american society of nephrology: cjasn, 15(8), 1203.
- Lippold, c. C., stothers, s. C., frohlich, a. A., boila, r. J., & marquardt, r. R. (1992). Effects of periodic feeding of diets containing ochratoxin a on the performance and clinical chemistry of pigs from 15 to 50 kg body weight. Canadian journal of animal science, 72(1), 135-146.
- Liu, h., jiang, y., luo, y., & jiang, w. (2006). A simple and rapid determination of atp, adp and amp concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. Food technology & biotechnology, 44(4).
- Ma, y., liu, h., wu, j., yuan, l., wang, y., du, x., ... & zhang, h. (2019). The adverse health effects of bisphenol a and related toxicity mechanisms. Environmental research, 176, 108575.
- Machowska, a., carrero, j. J., lindholm, b., & stenvinkel, p. (2016). Therapeutics targeting persistent inflammation in chronic kidney disease. Translational research, 167(1), 204-213.
- Madhyastha, s.m. Et al. (1990) 'effects of different cereal and oilseed substrates on the growth and production of toxins by aspergillus alutaceus and penicillium verrucosum', journal of agricultural and food chemistry, 38(7), pp. 1506–1510. Available at: https://doi.org/10.1021/jf00097a017.
- Magan, V., & Sanchis, N. (2004). Environmental conditions affecting mycotoxins. Mycotoxins in food: Detection and control, 174-189.
- Mahajan, t. Et al. (2021) 'histological and histochemical studies on the development of nephron and juxta glomerular apparatus in goat foetii (capra hircus)', ~ 572 ~ journal of entomology and zoology studies, 9(2), pp. 572–577. Available at: http://www.entomoljournal.com.
- Malir, f., severa, j., roubal, t., kacerovsky, j., fixa, p., moucka, p., ... & cerna, m. (2001). The dialysis of ochratoxin a (ota). Mycotoxin research, 17, 129-131.

References

- Mally, a., zepnik, h., wanek, p., eder, e., dingley, k., ihmels, h., ... & dekant, w. (2004). Ochratoxin a: lack of formation of covalent dna adducts. Chemical research in toxicology, 17(2), 234-242.
- Mammen, j.s.r. (2023) 'thyroid and aging', endocrinology and metabolism clinics of north america, 52(2), pp. 229–243. Available at: https://doi.org/10.1016/j.ecl.2022.10.008.
- Marsden, p. And mckerron, (1975) 'serum triiodothyronine concentration in the diagnosis of hyperthyroidism', clinical endocrinology, 4(2), pp. 183–189. Available at: https://doi.org/10.1111/j.1365-2265.1975.tb01525.x.
- Messina, m., & redmond, g. (2006). Effects of soy protein and soybean isoflavones on thyroid function in healthy adults and hypothyroid patients: a review of the relevant literature. Thyroid, 16(3), 249-258.
- 'Miale, j. B. (1972). The erythrocyte-quantitative and qualitative aspects. Laboratory medicine, hematology. Fourth edition. St. Louis, cv mosby co, 631-71.
- Milićević, d. R., jurić, v. B., stefanović, s. M., vesković-moračanin, s. M., & janković, s. I. (2009). Analysis of ochratoxin a in pig tissues using high pressure liquid chromatography (hplc) and liquid chromatography tandem mass spectrometry (lc/ms/ms) as confirmative methods. Zbornik matice srpske za prirodne nauke, (117), 51-61.
- Mir, m. S., & dwivedi, p. (2010). Ochratoxin a-induced serum biochemical alterations in new zealand white rabbits (oryctolagus cuniculus). Turkish journal of veterinary & animal sciences, 34(6), 525-531.
- Mohamedali, m., reddy maddika, s., vyas, a., iyer, v., & cheriyath, p. (2014). Thyroid disorders and chronic kidney disease. International journal of nephrology, 2014.
- Mohanty, j. G., nagababu, e., & rifkind, j. M. (2014). Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Frontiers in physiology, 5, 84.
- Moretti, a., logrieco, a. F., & susca, a. (2017). Mycotoxins: an underhand food problem. Mycotoxigenic fungi: methods and protocols, 3-12.
- Morley, j. E. (1981). Neuroendocrine control of thyrotropin secretion. Endocrine reviews, 2(4), 396-436.

References

- Moroz, l. A., meltzer, s. J., & bastomsky, c. H. (1983). Thyroid disease with monoclonal (immunoglobulin g λ) antibody to triiodothyronine and thyroxine. The journal of clinical endocrinology & metabolism, 56(5), 1009-1015.
- Mousa, w., ghazali, f. M., jinap, s., ghazali, h. M., & radu, s. (2013). Modeling growth rate and assessing aflatoxins production by aspergillus flavus as a function of water activity and temperature on polished and brown rice. Journal of food science, 78(1), m56-m63.
- Staruschenko, A., Ma, R., Palygin, O., & Dryer, S. E. (2023). Ion channels and channelopathies in glomeruli. Physiological Reviews, 103(1), 787-854
- Navale, v., vamkudoth, k. R., ajmera, s., & dhuri, v. (2021). Aspergillus derived mycotoxins in food and the environment: prevalence, detection, and toxicity. Toxicology reports, 8, 1008-1030.
- Brenner, b. M. (1985). Nephron adaptation to renal injury or ablation. American journal of physiology-renal physiology, 249(3), f324-f337.
- Nguyen, thi mong diep, klett, d., & combarnous, y. (2022). Undissociable chemically cross-linked and single-chain gonadotropins. Theriogenology.
- Niaz, k., shah, s. Z. A., khan, f., & bule, m. (2020). Ochratoxin a–induced genotoxic and epigenetic mechanisms lead to alzheimer disease: its modulation with strategies. Environmental science and pollution research, 27(36), 44673-44700.
- Nicholson, j. P., wolmarans, m. R., & park, g. R. (2000). The role of albumin in critical illness. British journal of anaesthesia, 85(4), 599-610.
- Niewczas, m. A., pavkov, m. E., skupien, j., smiles, a., md dom, z. I., wilson, j. M., ... & krolewski, a. S. (2019). A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. Nature medicine, 25(5), 805-813.
- Nillni, e. A. (2010). Regulation of the hypothalamic thyrotropin releasing hormone (trh) neuron by neuronal and peripheral inputs. Frontiers in neuroendocrinology, 31(2), 134-156.
- Nilsson, m. And fagman, h. (2017a) ‘development of the thyroid gland’, development, 144(12), pp. 2123–2140. Available at: https://doi.org/10.1242/dev.145615.

References

- Nilsson, m. And fagman, h. (2017b) ‘development of the thyroid gland’, development (cambridge). Company of biologists ltd, pp. 2123–2140. Available at: https://doi.org/10.1242/dev.145615.
- Nolan, p., auer, s., spehar, a., oplatowska-stachowiak, m., & campbell, k. (2021). Evaluation of mass sensitive micro-array biosensors for their feasibility in multiplex detection of low molecular weight toxins using mycotoxins as model compounds. Talanta, 222, 121521.
- Fakhri, y., omar, s. S., mehri, f., hoseinvandtabar, s., & mahmudiono, t. (2022). Global systematic review and meta-analysis on prevalence and concentration of aflatoxins in peanuts oil and probabilistic risk assessment. Reviews on environmental health, (0).
- Ok, f., erdogan, o., durmus, e., carkci, s., & canik, a. (2021). Predictive values of blood urea nitrogen/creatinine ratio and other routine blood parameters on disease severity and survival of covid-19 patients. Journal of medical virology, 93(2), 786-793.
- Oliveira, j. H., persani, l., beck-peccoz, p., & abucham, j. (2001). Investigating the paradox of hypothyroidism and increased serum thyrotropin (tsh) levels in sheehan’s syndrome: characterization of tsh carbohydrate content and bioactivity. The journal of clinical endocrinology & metabolism, 86(4), 1694-1699.
- European food safety authority (efsa). (2006). Opinion of the scientific panel on contaminants in the food chain [contam] related to ochratoxin a in food. Efsa journal, 4(6), 365.
- Özcan, z., gül, g., & yaman, i. (2015). Ochratoxin a activates opposing c-met/pi3k/akt and mapk/erk 1-2 pathways in human proximal tubule hk-2 cells. Archives of toxicology, 89, 1313-1327.
- Özçelik, n., koşar, a., & soysal, d. (2001). Ochratoxin a in human serum samples collected in isparta-turkey from healthy individuals and individuals suffering from different urinary disorders. Toxicology letters, 121(1), 9-13.
- Pan, b., du, x., zhang, h., hua, x., wan, x., & cao, c. (2019). Relationships of chronic kidney disease and thyroid dysfunction in non-dialysis patients: a pilot study. Kidney and blood pressure research, 44(2), 170-178.

References

- Pardridge, w.m. and landaw, e.m. (1984) ‘tracer kinetic model of blood-brain barrier transport of plasma protein-bound ligands. Empiric testing of the free hormone hypothesis.’, journal of clinical investigation, 74(3), pp. 745–752. Available at: https://doi.org/10.1172/jci111490.
- Patial, v., asrani, r. K., patil, r. D., ledoux, d. R., & rottinghaus, g. E. (2013). Pathology of ochratoxin a–induced nephrotoxicity in japanese quail and its protection by sea buckthorn (hippophae rhamnoides l.). Avian diseases, 57(4), 767-779.
- Pierce, j. G. (1971). Eli lilly lecture: the subunits of pituitary thyrotropin—their relationship to other glycoprotein hormones. Endocrinology, 89(6), 1331-1344.
- Prigent, a. (2008, january). Monitoring renal function and limitations of renal function tests. In seminars in nuclear medicine (vol. 38, no. 1, pp. 32-46). Wb saunders.
- Radi, z. A. (2019). Kidney pathophysiology, toxicology, and drug-induced injury in drug development. International journal of toxicology, 38(3), 215-227.
- Ragen, patrick a., albert b, hagedorn & owen, c. A. (1960). Radioisotopic study of anemia in chronic renal disease. Ama archives of internal medicine, 105(4), 518-523.
- Ranaldi, g., caprini, v., sambuy, y., perozzi, g., & murgia, c. (2009). Intracellular zinc stores protect the intestinal epithelium from ochratoxin a toxicity. Toxicology in vitro, 23(8), 1516-1521.
- Rifkind, j. M., & nagababu, e. (2013). Hemoglobin redox reactions and red blood cell aging. Antioxidants & redox signaling, 18(17), 2274-2283.
- Rosner, m.h. and bolton, w.k. (2006a) ‘renal function testing’, american journal of kidney diseases, 47(1), pp. 174–183. Available at: https://doi.org/10.1053/j.ajkd.2005.08.038.
- Roth, a., chakor, k., ekuécreepy, e., kane, a., roschenthaler, r., & dirheimer, g. (1988). Evidence for an enterohepatic circulation of ochratoxin a in mice. Toxicology, 48(3), 293-308.
- Samuel, m. S., jeyaram, k., datta, s., chandrasekar, n., balaji, r., & selvarajan, e. (2021). Detection, contamination, toxicity, and prevention methods of ochratoxins: an update review. Journal of agricultural and food chemistry, 69(46), 13974-13989.

References

- Sowers, j. R., carlson, h. E., brautbar, n., & hershman, j. M. (1977). Effect of dexamethasone on prolactin and tsh responses to trh and metoclopramide in man. The journal of clinical endocrinology & metabolism, 44(2), 237-241.
- Schaaf, g. J., nijmeijer, s. M., maas, r. F. M., roestenberg, p., de groene, e. M., & fink-gremmels, j. (2002). The role of oxidative stress in the ochratoxin a-mediated toxicity in proximal tubular cells. Biochimica et biophysica acta (bba)-molecular basis of disease, 1588(2), 149-158.
- Shahba, s., mehrzad, j., & malvandi, a. M. (2021). Neuroimmune disruptions from naturally occurring levels of mycotoxins. Environmental science and pollution research, 28(25), 32156-32176.
- Sharma, a., hirulkar, n. B., wadel, p., & das, p. (2011). Influence of hyperglycemia on renal function parameters in patients with diabetes mellitus. Ijpba, 2(suppl 2), 734-9.
- Sharma, neeta, and avantina s. Bhandari, eds. Bio-management of postharvest diseases and mycotoxigenic fungi. Crc press, 2020.
- Sinha, m. H., mehtab, t., asha, u. H., sikder, m. M., akter, k., & mahbub, m. R. (2019). Effect of chandraprabha batika on thyroid hormone profile in male sprague-dawley rats. Biol med (aligarh), 11, 457.
- Skarkova, j., ostry, v., malir, f., & roubal, t. (2013). Determination of ochratoxin a in food by high performance liquid chromatography. Analytical letters, 46(10), 1495-1504.
- Solak, y., yilmaz, m. I., saglam, m., demirbas, s., verim, s., unal, h. U., ... & kanbay, m. (2013). Mean corpuscular volume is associated with endothelial dysfunction and predicts composite cardiovascular events in patients with chronic kidney disease. Nephrology, 18(11), 728-735.
- Sorrenti, s., baldini, e., pironi, d., lauro, a., d'orazi, v., tartaglia, f., ... & ulisse, s. (2021). Iodine: its role in thyroid hormone biosynthesis and beyond. Nutrients, 13(12), 4469.
- Sotanaphun, u., phattanawasin, p., & sriphong, l. (2009). Application of scion image software to the simultaneous determination of curcuminoids in turmeric (curcuma longa). Phytochemical analysis, 20(1), 19-23.
- Stoev, s. D., gundasheva, d., zarkov, i., mircheva, t., zapryanova, d., denev, s., ... & schneider, y. J. (2012). Experimental mycotoxic nephropathy in pigs provoked by a mouldy

References

diet containing ochratoxin a and fumonisin b1. Experimental and toxicologic pathology, 64(7-8), 733-741.

- Taghizadeh, s. F., rezaee, r., davarynejad, g., asili, j., nemati, s. H., goumenou, m., ... & karimi, g. (2018). Risk assessment of exposure to aflatoxin b1 and ochratoxin a through consumption of different pistachio (pistacia vera l.) Cultivars collected from four geographical regions of iran. Environmental toxicology and pharmacology, 61, 61-66.
- Takasato, minoru, er, p. X., chiu, h. S., maier, b., baillie, g. J., ferguson, c., ... & little, m. H. (2015). Kidney organoids from human ips cells contain multiple lineages and model human nephrogenesis. Nature, 526(7574), 564-568.
- Talke, h. S. G. E., & schubert, g. E. (1965). Enzymatic urea determination in the blood and serum in the warburg optical test. Klinische wochenschrift, 43, 174-175.
- Tambur, a. R., kosmoliaptsis, v., claas, f. H., mannon, r. B., nickerson, p., & naesens, m. (2021). Significance of hla-dq in kidney transplantation: time to reevaluate human leukocyte antigen–matching priorities to improve transplant outcomes? An expert review and recommendations. Kidney international, 100(5), 1012-1022.
- Tao, y., xie, s., xu, f., liu, a., wang, y., chen, d., ... & yuan, z. (2018). Ochratoxin a: toxicity, oxidative stress and metabolism. Food and chemical toxicology, 112, 320-331.
- Thomas, l., & boege, f. (1998). Kidney and urinary tract. Thomas l, ed. Clinical laboratory diagnostics. 1st ed. Frankfurt/main: th-books-verl.-ges, 362-400.
- Vanputte, cinnamon l., jennifer l. Regan, and andrew f. Russo. Seeley's essentials of anatomy & physiology. Mcgraw-hill, 2021.
- Vartiainen, s., yiannikouris, a., apajalahti, j., & moran, c. A. (2020). Comprehensive evaluation of the efficiency of yeast cell wall extract to adsorb ochratoxin a and mitigate accumulation of the toxin in broiler chickens. Toxins, 12(1), 37.
- Vaziri, n. D., & zhou, x. J. (2009). Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease. Nephrology dialysis transplantation, 24(4), 1082-1088.
- Vinen, c.s., & o.d.b.g. (2003) ‘acute glomerulonephritis’, postgraduate medical journal, 79(930).

References

- Wafa, e. W., yahya, r. S., sobh, m. A., eraky, i., el-baz, m., el-gayar, h. A., ... & creppy, e. E. (1998). Human ochratoxicosis and nephropathy in egypt: a preliminary study. Human & experimental toxicology, 17(2), 124-129.
- Wang, s. T., pizzolato, s., & demshar, h. P. (1998). Diagnostic effectiveness of tsh screening and of t4 with secondary tsh screening for newborn congenital hypothyroidism. Clinica chimica acta, 274(2), 151-158.
- Wang, x., zhao, x., & huang, x. (2020). Association of subclinical thyroid dysfunction with chronic kidney disease: a systematic review and meta-analysis. Endocrine research, 45(1), 41-49.
- Wang, y., wang, l., liu, f., wang, q., selvaraj, j. N., xing, f., ... & liu, y. (2016). Ochratoxin a producing fungi, biosynthetic pathway and regulatory mechanisms. Toxins, 8(3), 83.
- Waugh, anne, and allison grant. Ross & wilson anatomy and physiology in health and illness e-book. Elsevier health sciences, 2014.
- Morton, r., webster, a., masson, p., & nagler, e. (2017). Chronic kidney disease.
- Webster, a.c. et al. (2017b) ‘chronic kidney disease’, the lancet. Lancet publishing group, pp. 1238–1252. Available at: https://doi.org/10.1016/s0140-6736(16)32064-5.
- Wilkinson, t. J., miksza, j., yates, t., lightfoot, c. J., baker, l. A., watson, e. L., ... & smith, a. C. (2021). Association of sarcopenia with mortality and end-stage renal disease in those with chronic kidney disease: a uk biobank study. Journal of cachexia, sarcopenia and muscle, 12(3), 586-598.
- Yildirim, i., hur, e., & kokturk, f. (2013). Inflammatory markers: c-reactive protein, erythrocyte sedimentation rate, and leukocyte count in vitamin d deficient patients with and without chronic kidney disease. International journal of endocrinology, 2013.
- Yildirim, ibrahim, ender hur, and furuzan kokturk. "inflammatory markers: c-reactive protein, erythrocyte sedimentation rate, and leukocyte count in vitamin d deficient patients with and without chronic kidney disease." international journal of endocrinology 2013 (2013).
- Yuan, qian, ben tang, and chun zhang. "signaling pathways of chronic kidney diseases, implications for therapeutics." signal transduction and targeted therapy 7.1 (2022): 182.

References

- Zoccali, c., mallamaci, f., tripepi, g., cutrupi, s., & pizzini, p. (2006). Low triiodothyronine and survival in end-stage renal disease. Kidney international, 70(3), 523-528.

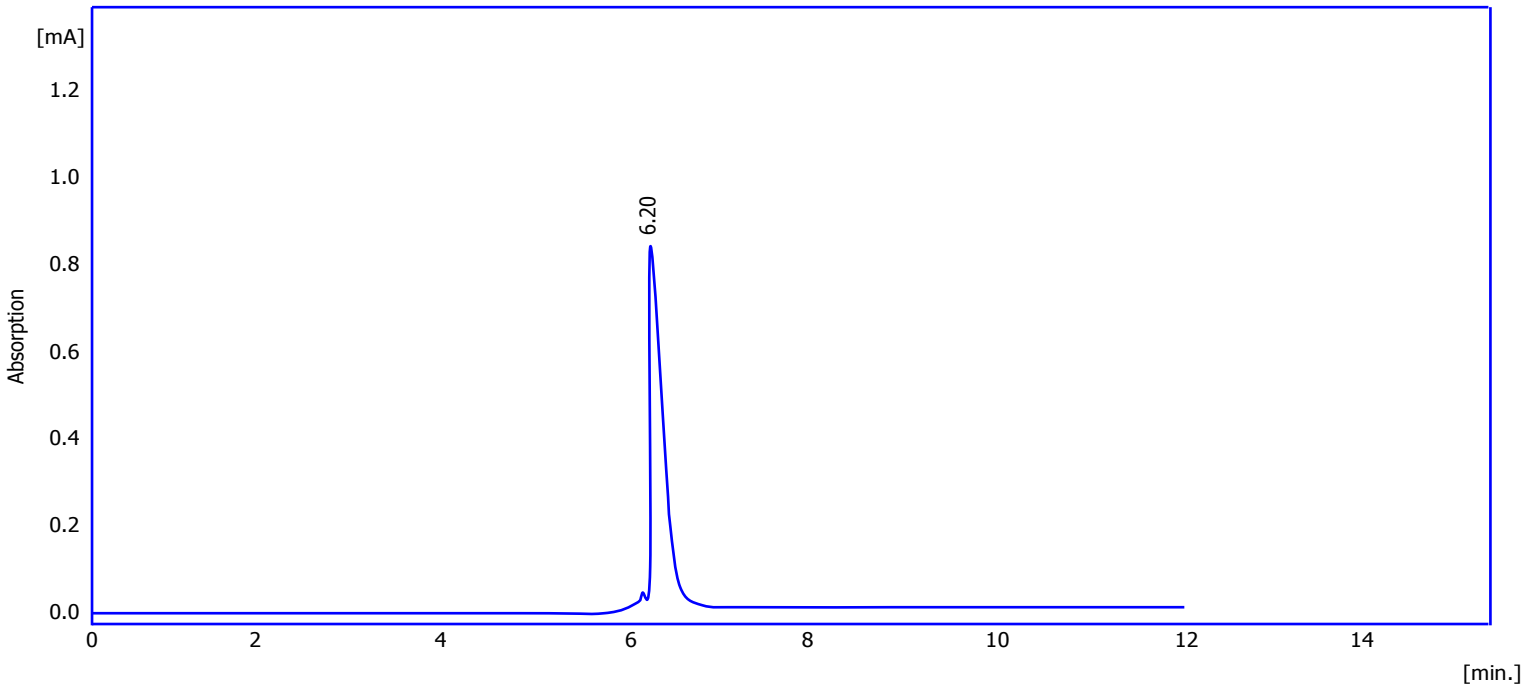
Appendix

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: Ocratoxine A (0.5 ppb)	Amount	0
Sample	: Ocratoxine A (0.5 ppb)	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ Ocratoxine A (0.5 ppb)

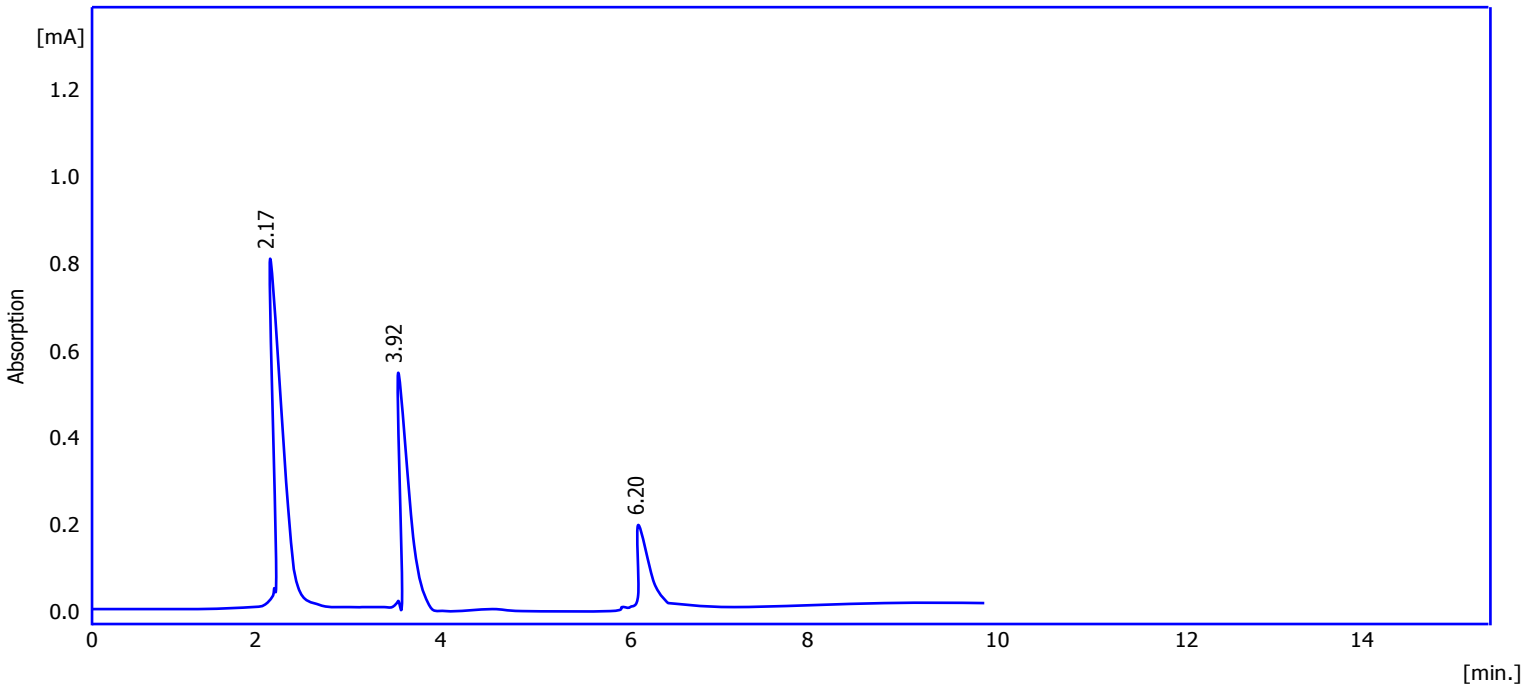
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	6.20	856.08	800.28	100.00	100.00	0.25	
	Total	856.08	800.28	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 1	Amount	0
Sample	: control 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 1)

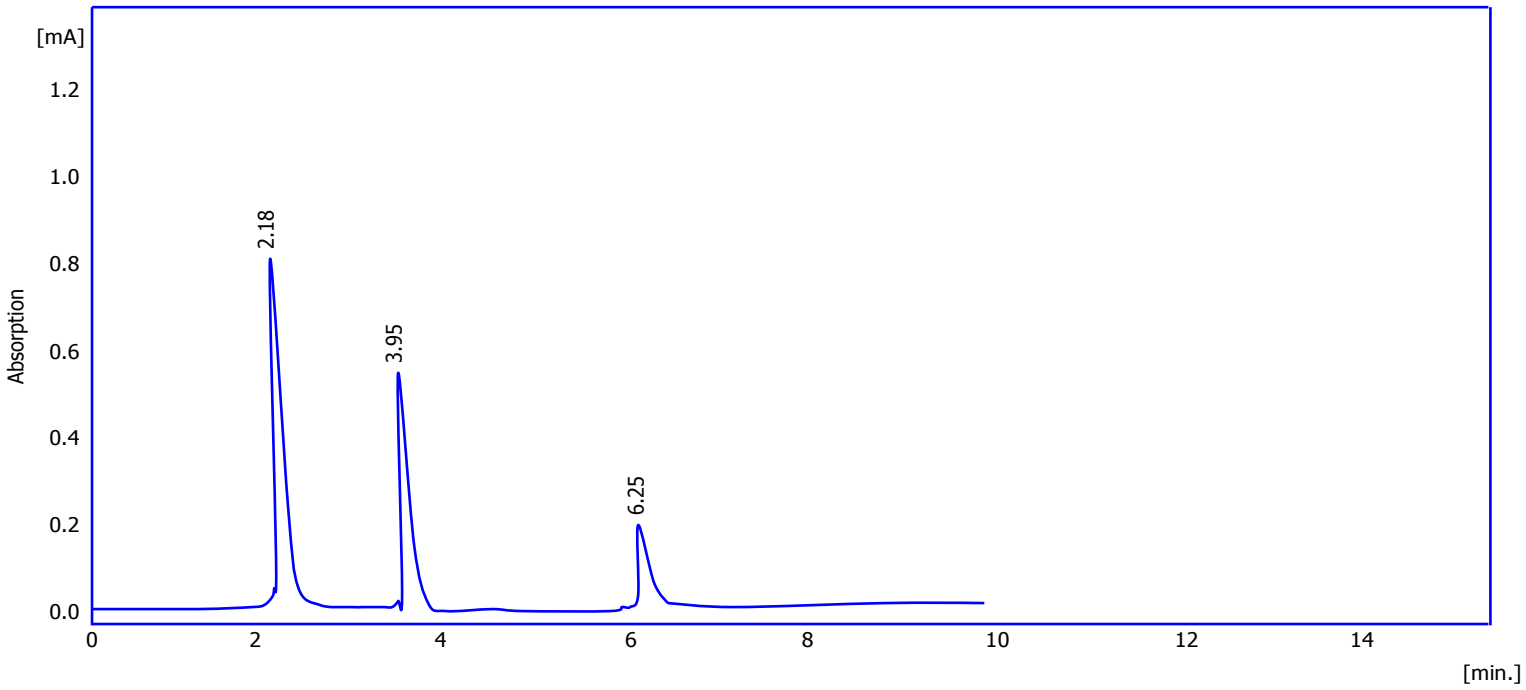
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.17	1265.98	794.25	50.00	50.00	0.30	
2	3.92	2855.26	599.85	35.00	35.00	0.20	
3	6.20	985.65	194.32	15.00	15.00	0.10	
	Total	5106.98	1588.98	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 2	Amount	0
Sample	: control 2	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 2)

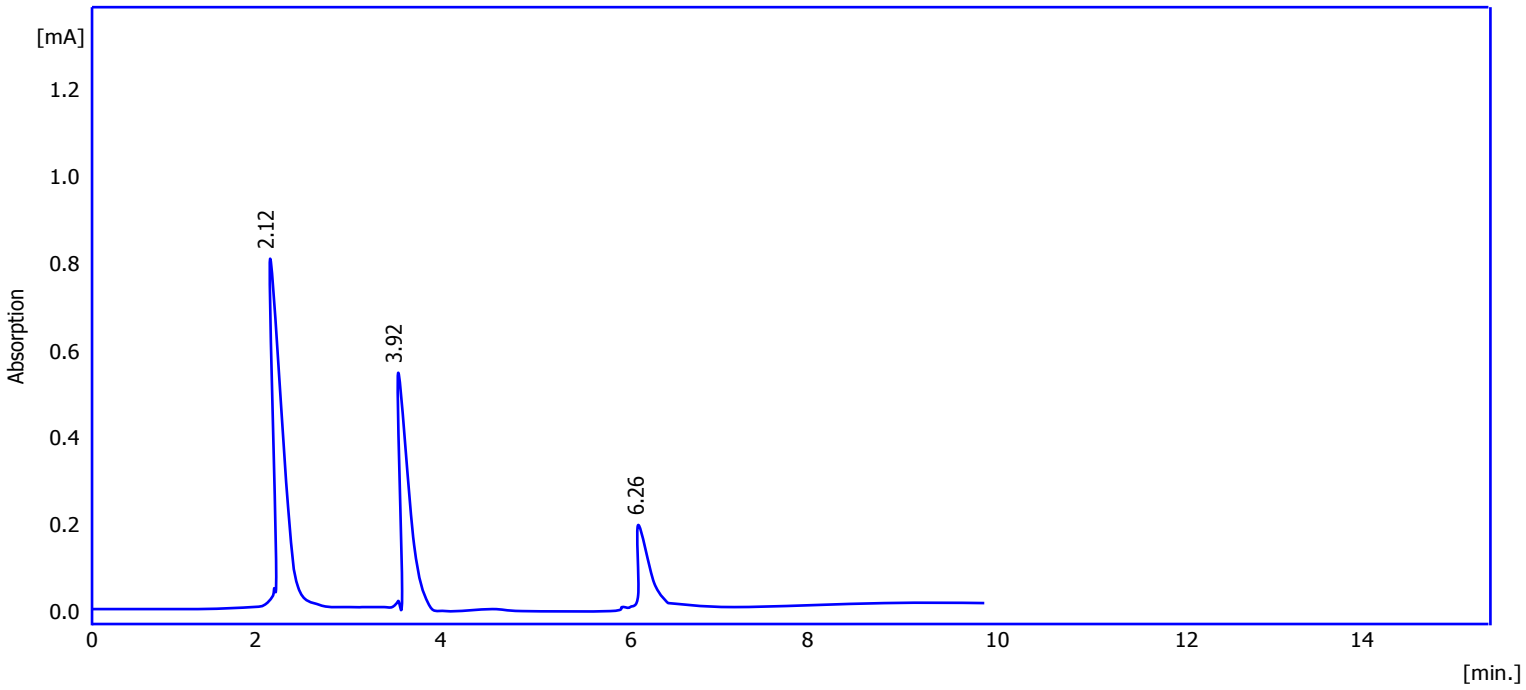
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.18	1221.08	793.25	50.00	50.00	0.30	
2	3.95	2653.01	596.14	35.00	35.00	0.20	
3	6.25	962.11	192.49	15.00	15.00	0.10	
	Total	4863.25	1570.55	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 3	Amount	0
Sample	: control 3	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 3)

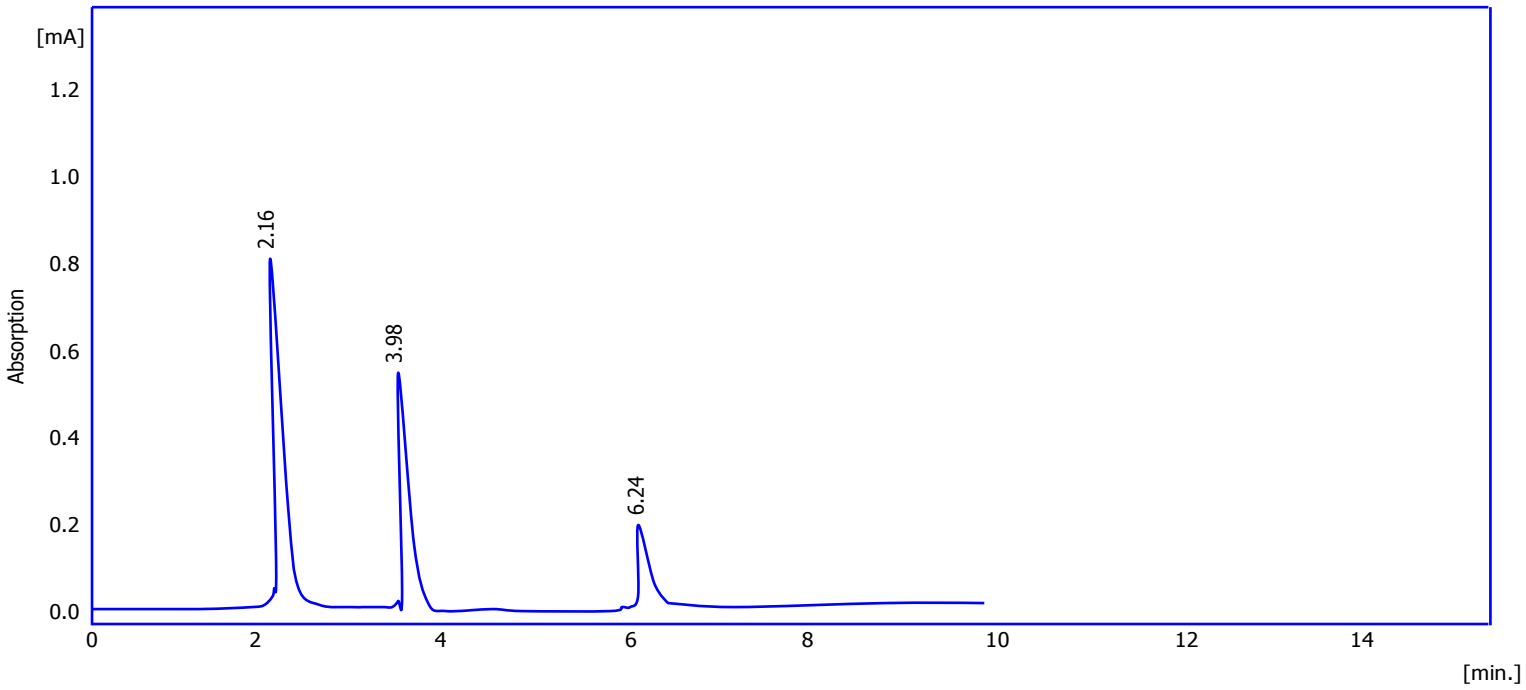
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.12	1185.64	792.44	50.00	50.00	0.30	
2	3.92	2788.94	595.98	35.00	35.00	0.20	
3	6.26	980.10	196.98	15.00	15.00	0.10	
	Total	4954.89	1578.55	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 4	Amount	0
Sample	: control 4	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 4)

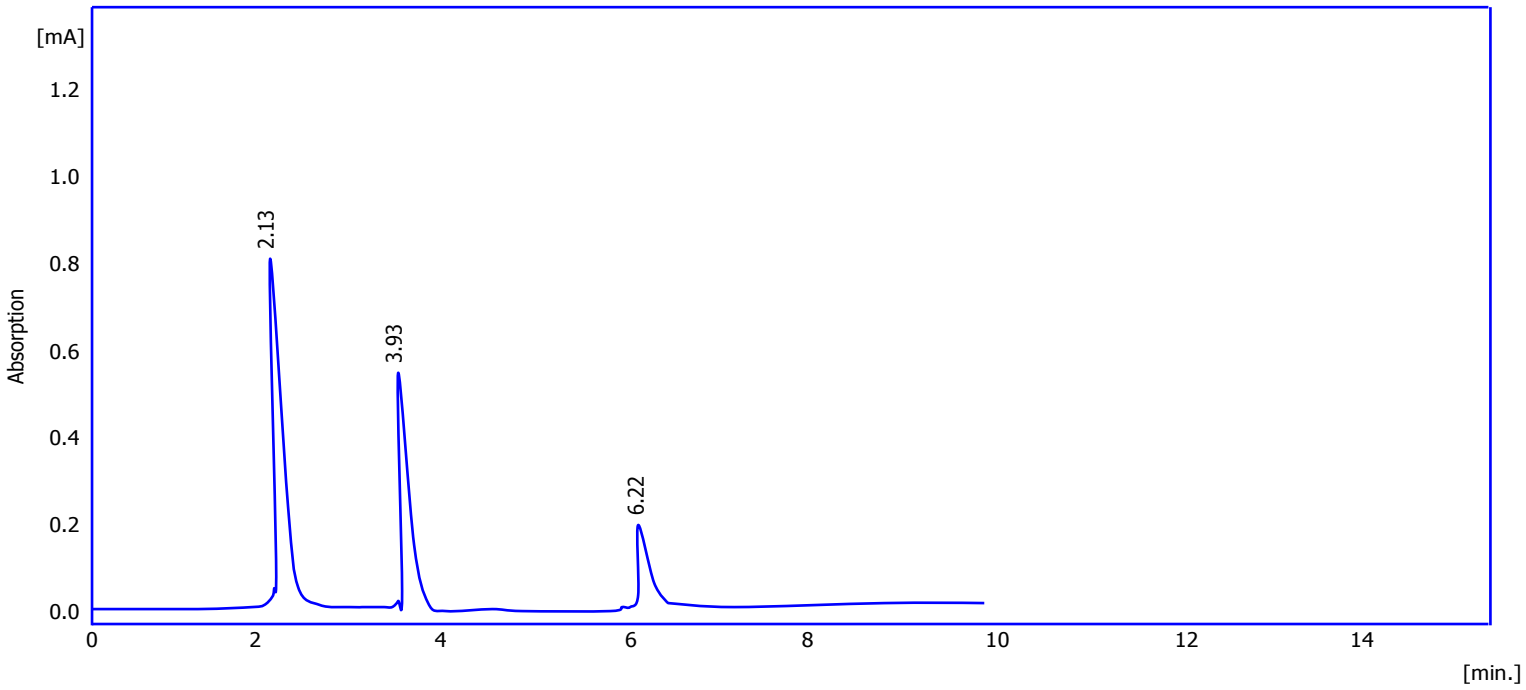
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.16	1192.14	793.25	50.00	50.00	0.30	
2	3.98	2778.55	594.15	35.00	35.00	0.20	
3	6.24	885.24	195.29	15.00	15.00	0.10	
	Total	4855.19	1570.88	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 5	Amount	0
Sample	: control 5	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 5)

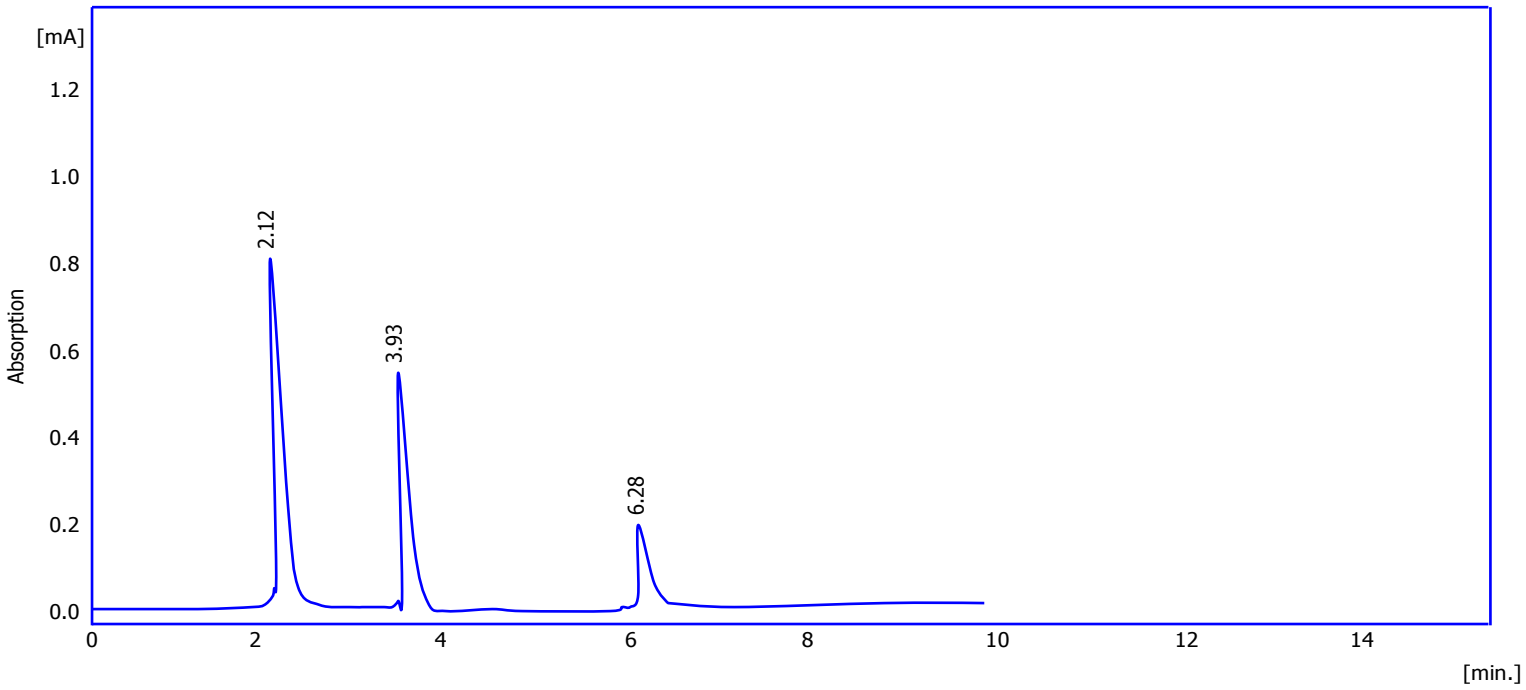
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.13	1174.25	794.15	50.00	50.00	0.30	
2	3.93	2791.24	593.05	35.00	35.00	0.20	
3	6.22	913.26	194.15	15.00	15.00	0.10	
	Total	4878.98	1565.29	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 6	Amount	0
Sample	: control 6	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 6)

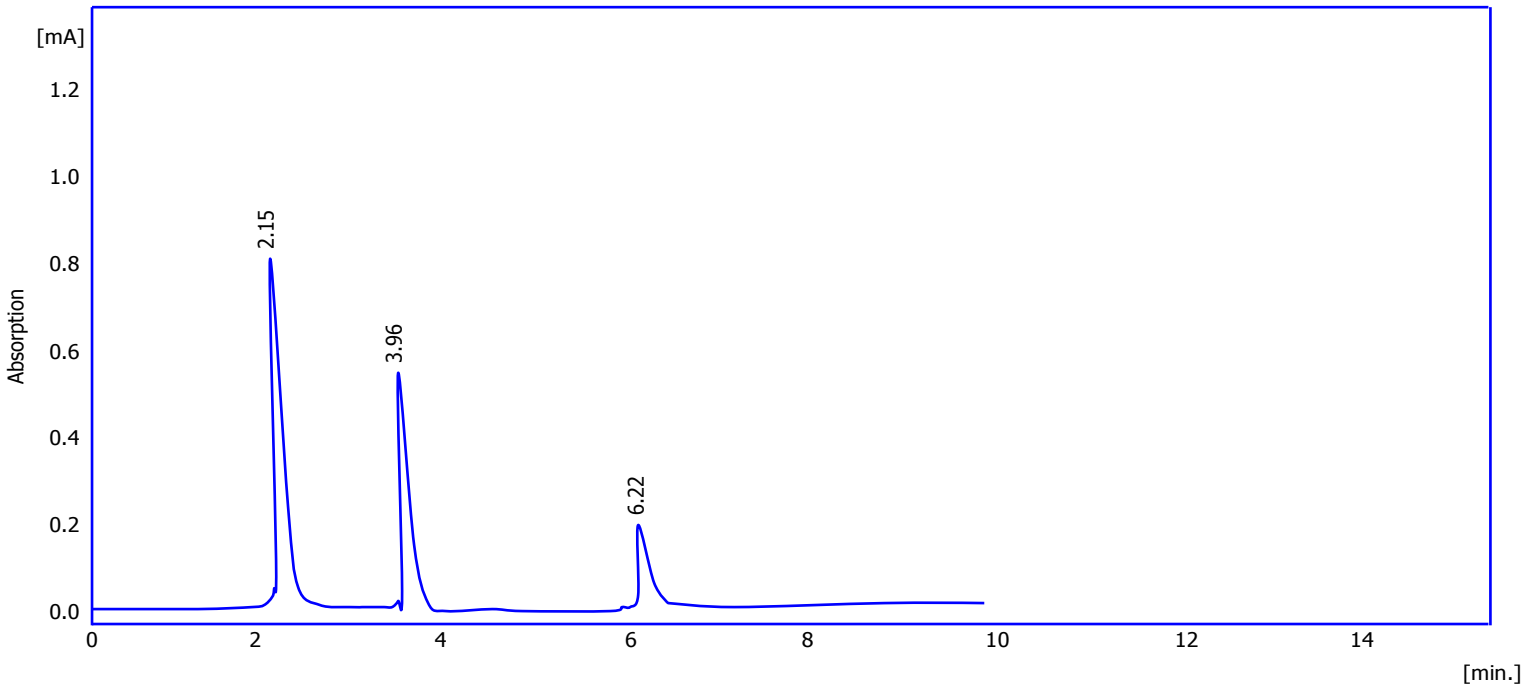
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.12	1180.55	796.23	50.00	50.00	0.30	
2	3.93	2784.15	595.08	35.00	35.00	0.20	
3	6.28	919.85	196.99	15.00	15.00	0.10	
	Total	4884.55	1573.98	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 7	Amount	0
Sample	: control 7	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 7)

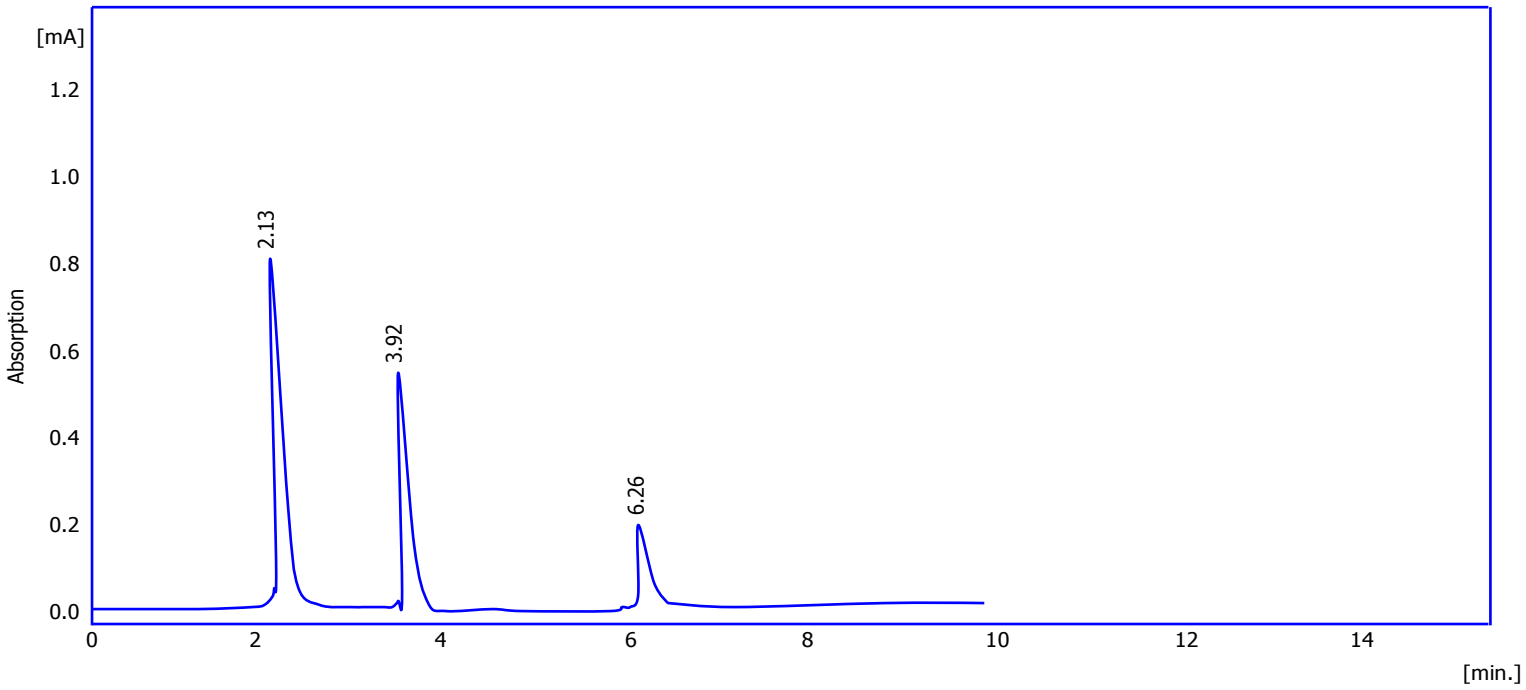
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.15	1195.49	791.25	50.00	50.00	0.30	
2	3.96	2786.22	593.25	35.00	35.00	0.20	
3	6.22	841.25	191.59	15.00	15.00	0.10	
	Total	4822.65	1562.58	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 8	Amount	0
Sample	: control 8	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 8)

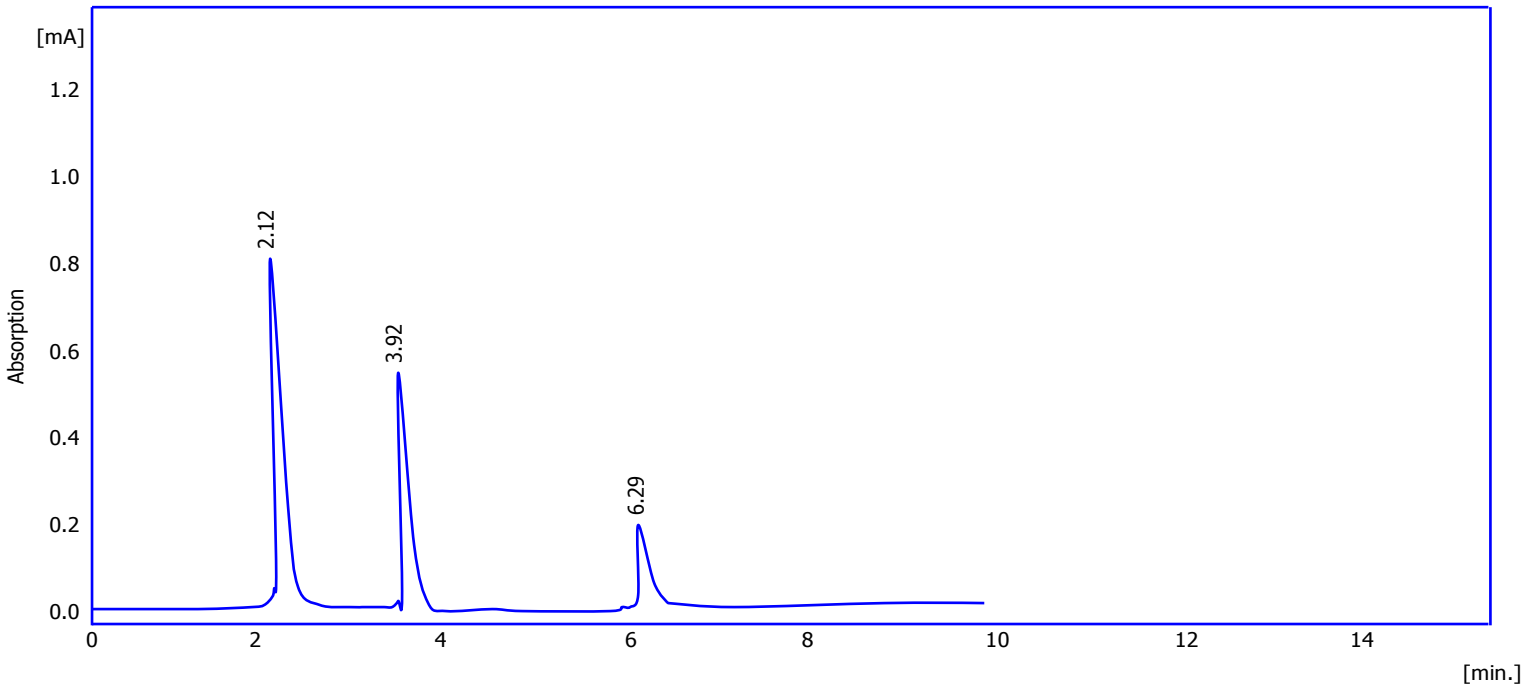
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.13	1204.56	792.54	50.00	50.00	0.30	
2	3.92	2811.65	592.05	35.00	35.00	0.20	
3	6.26	836.65	193.65	15.00	15.00	0.10	
	Total	4852.68	1574	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 9	Amount	0
Sample	: control 9	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 9)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.12	1200.15	793.65	50.00	50.00	0.30	
2	3.92	2820.66	595.98	35.00	35.00	0.20	
3	6.29	864.28	194.58	15.00	15.00	0.10	
	Total	4885.09	1583.65	100.00	100.00		

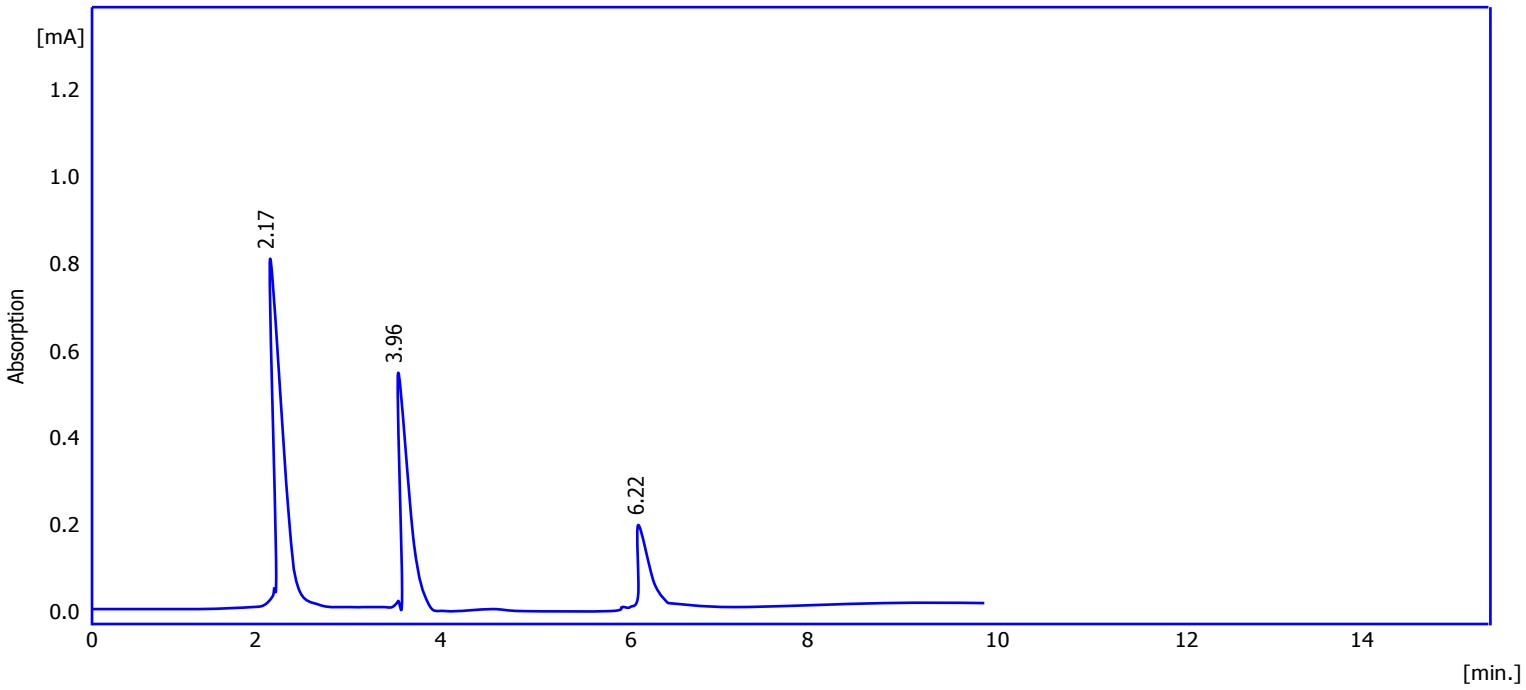
X

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 10	Amount	0
Sample	: control 10	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 10)

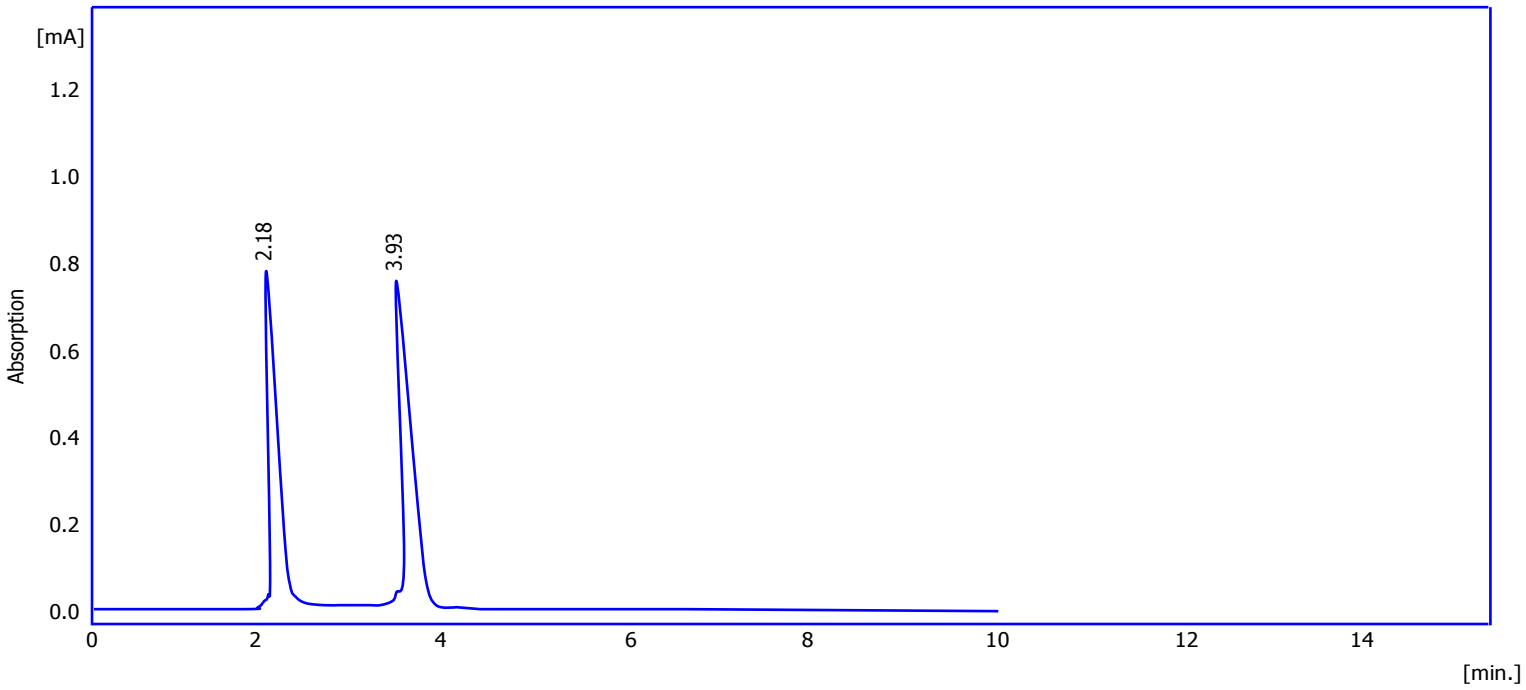
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.17	1215.65	795.25	50.00	50.00	0.30	
2	3.96	2885.64	594.14	35.00	35.00	0.20	
3	6.22	905.23	190.25	15.00	15.00	0.10	
	Total	5006.25	1588.99	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: control 11	Amount	0
Sample	: control 11	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 11)

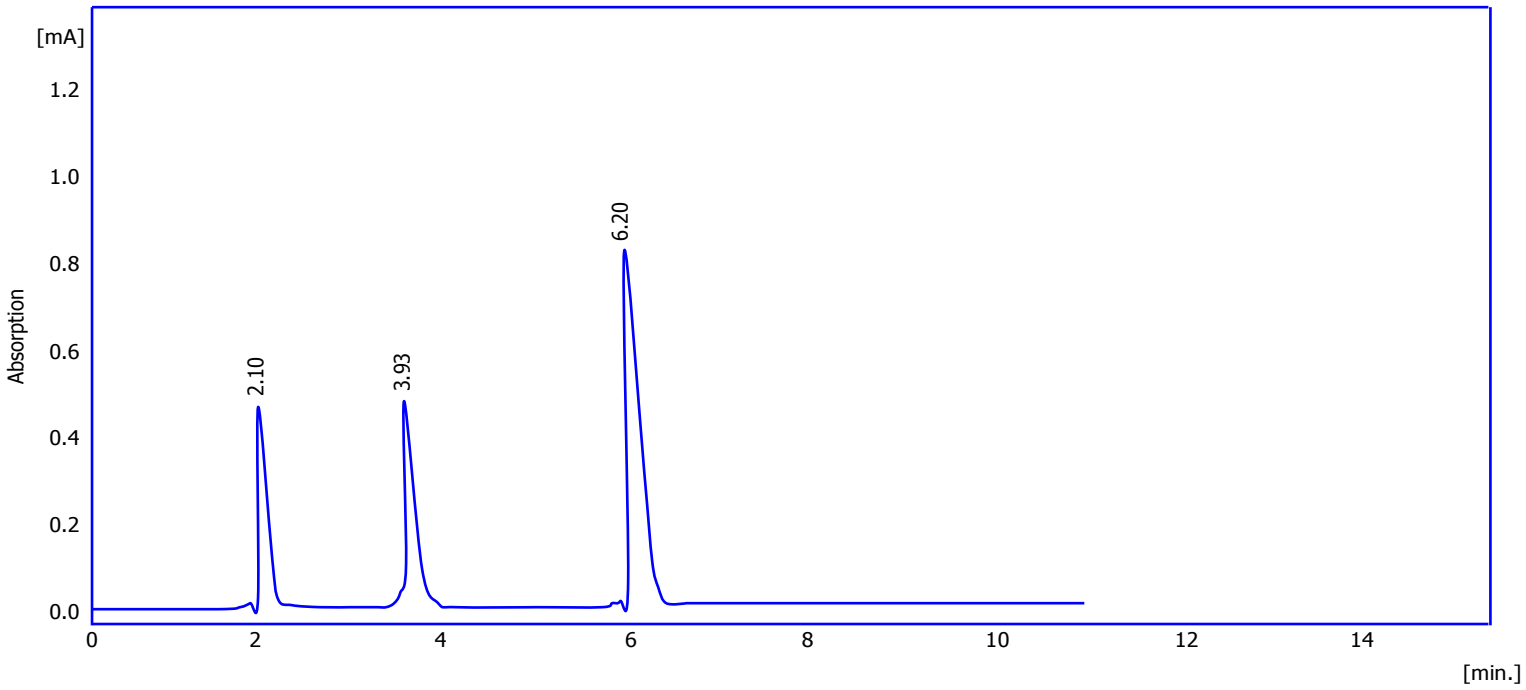
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.18	1220.55	796.59	50.00	50.00	0.25	
2	3.93	2712.05	790.11	50.00	50.00	0.25	
	Total	3962.60	1586.70	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 1	Amount	0
Sample	: sample 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 1

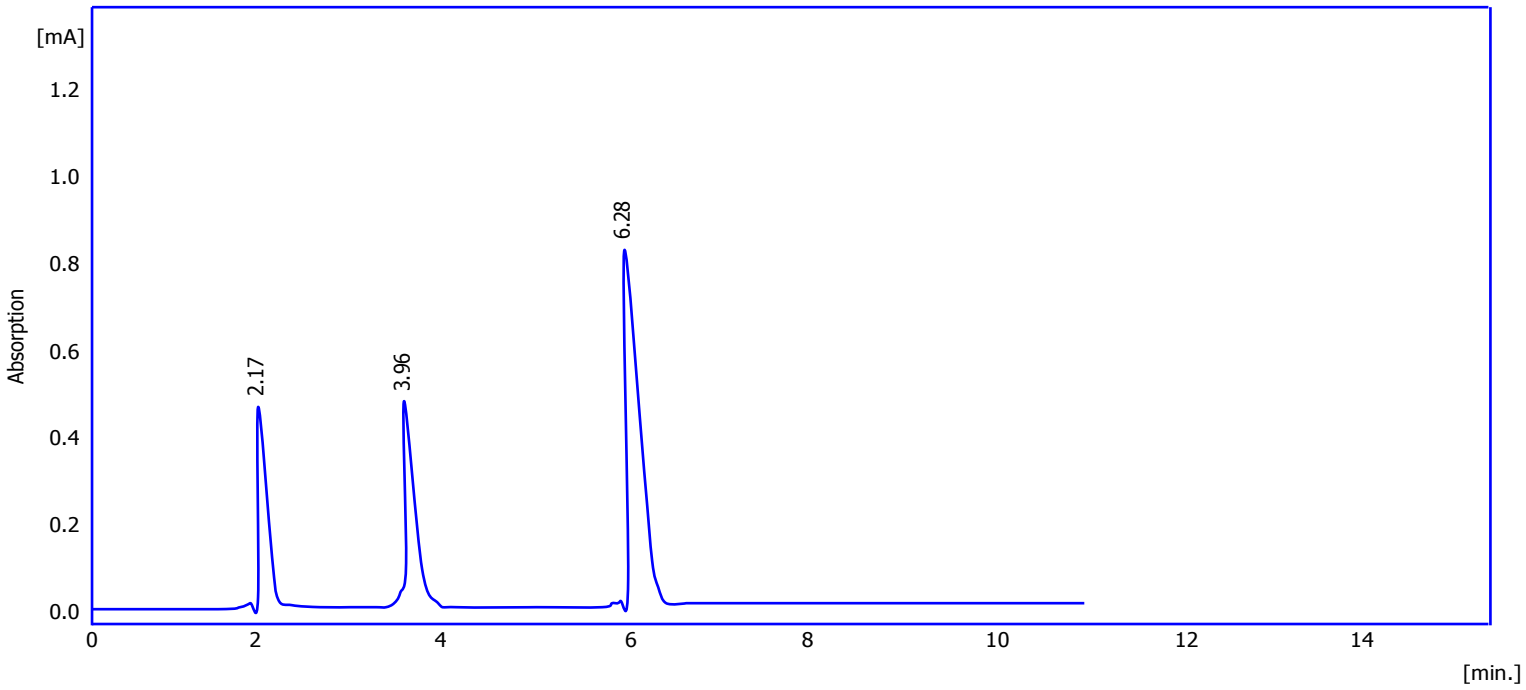
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.10	1256.59	462.58	25.00	25.00	0.15	
2	3.93	2100.49	465.99	25.00	25.00	0.15	
3	6.20	9586.28	800.12	50.00	50.00	0.30	
	Total	12943.59	1729.64	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 2	Amount	0
Sample	: sample 2	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 2)

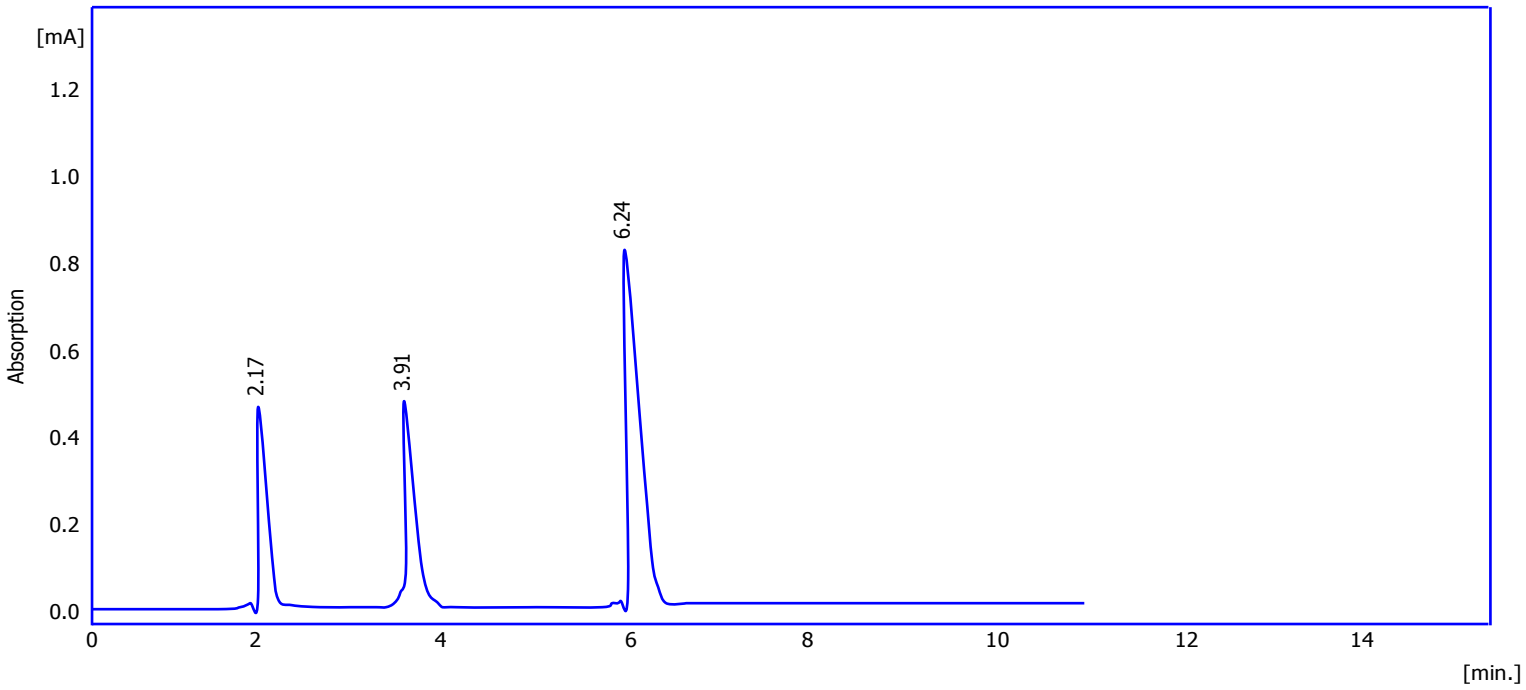
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.17	1262.15	461.49	25.00	25.00	0.15	
2	3.96	2110.59	464.11	25.00	25.00	0.15	
3	6.28	8952.14	802.58	50.00	50.00	0.30	
	Total	12322.88	1730.15	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 3	Amount	0
Sample	: sample 3	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 3)

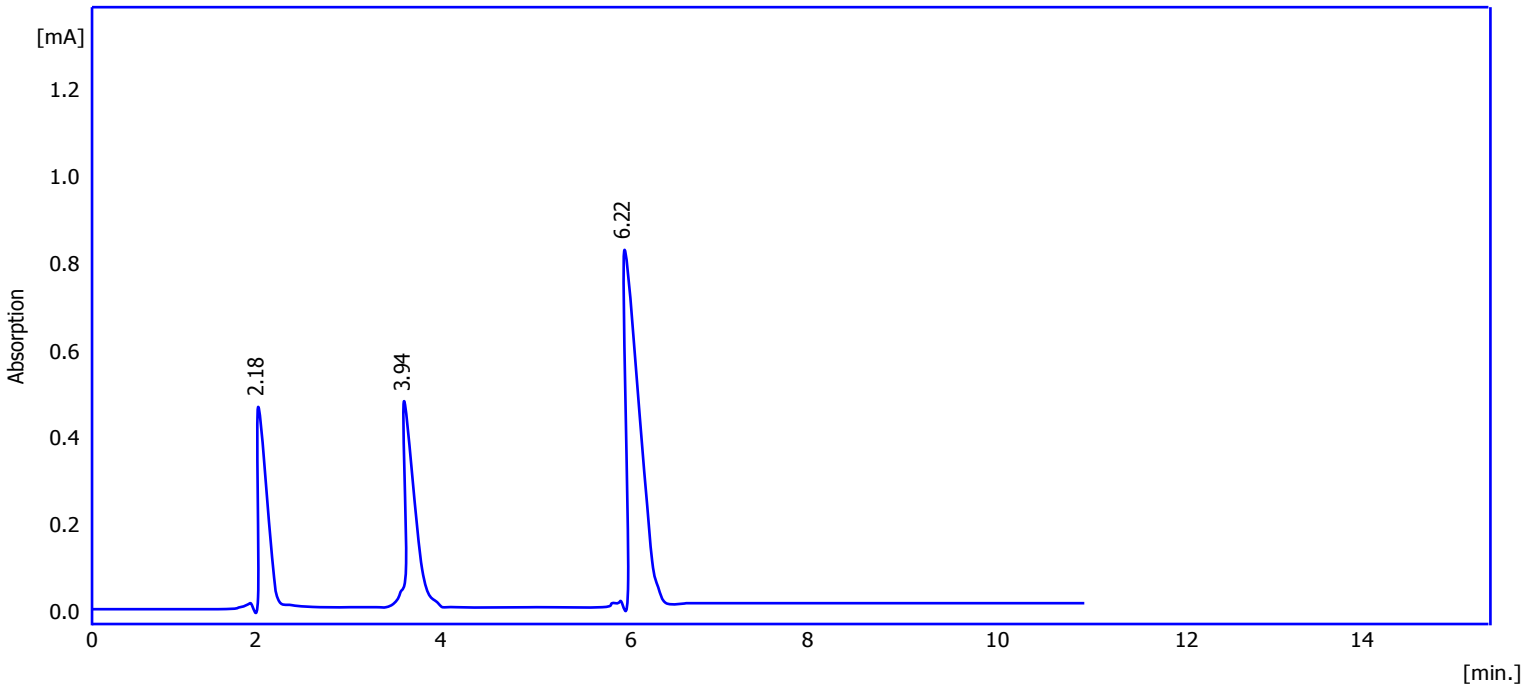
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.17	1301.25	463.56	25.00	25.00	0.15	
2	3.91	2189.28	462.58	25.00	25.00	0.15	
3	6.24	10556.28	801.44	50.00	50.00	0.30	
	Total	14046.59	1736.58	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 4	Amount	0
Sample	: sample 4	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 4)

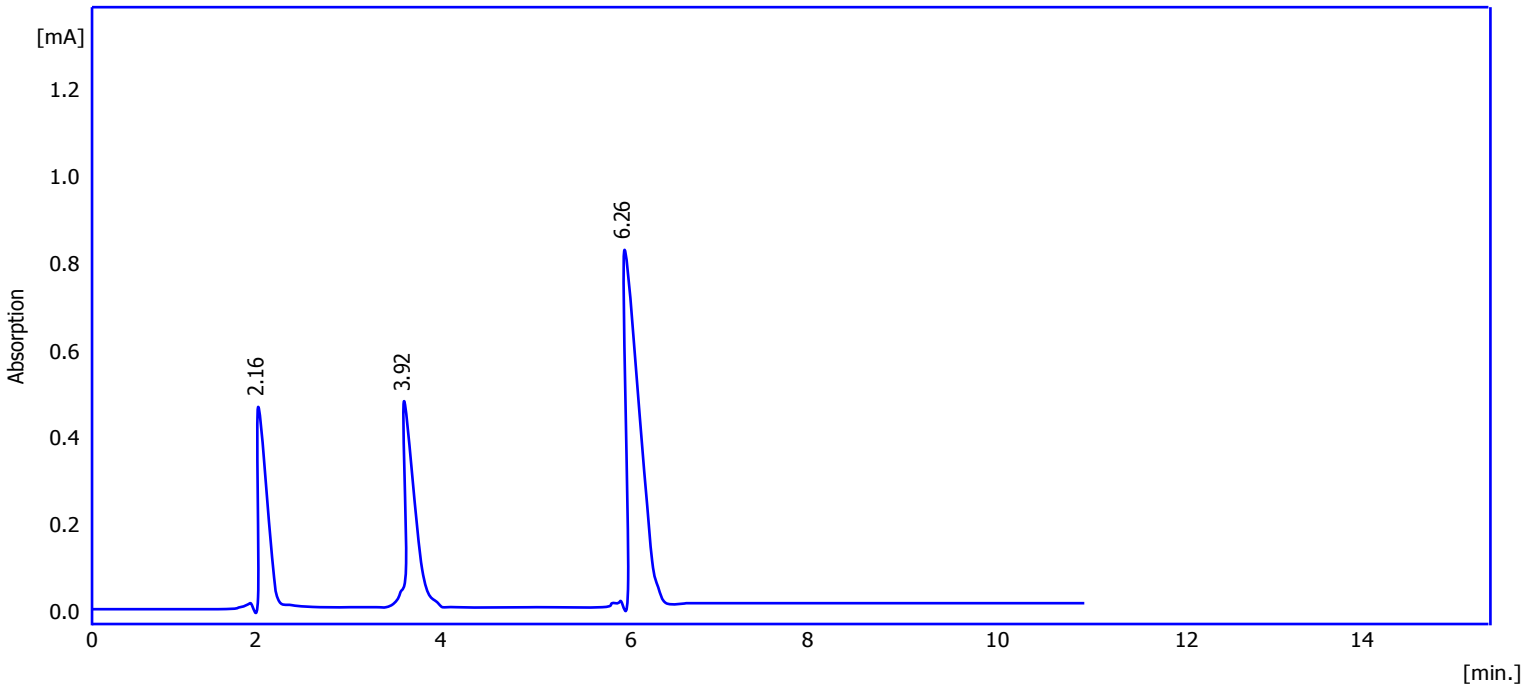
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.18	1251.49	460.10	25.00	25.00	0.15	
2	3.94	2104.11	462.88	25.00	25.00	0.15	
3	6.22	10265.08	801.65	50.00	50.00	0.30	
	Total	13620.14	1732.89	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 5	Amount	0
Sample	: sample 5	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 5)

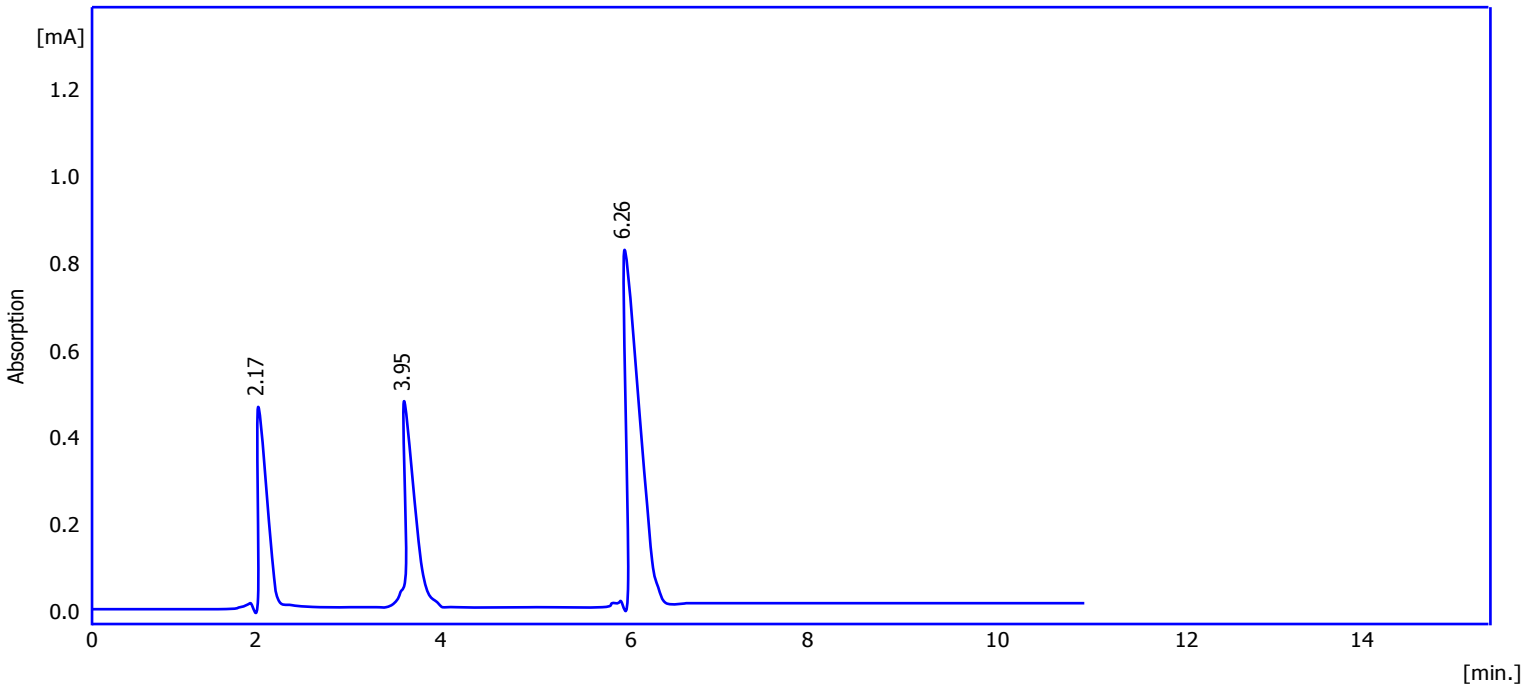
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.16	1264.18	461.25	25.00	25.00	0.15	
2	3.92	2360.22	463.65	25.00	25.00	0.15	
3	6.26	11552.65	800.88	50.00	50.00	0.30	
	Total	15177.48	1730.68	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 6	Amount	0
Sample	: sample 6	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 6)

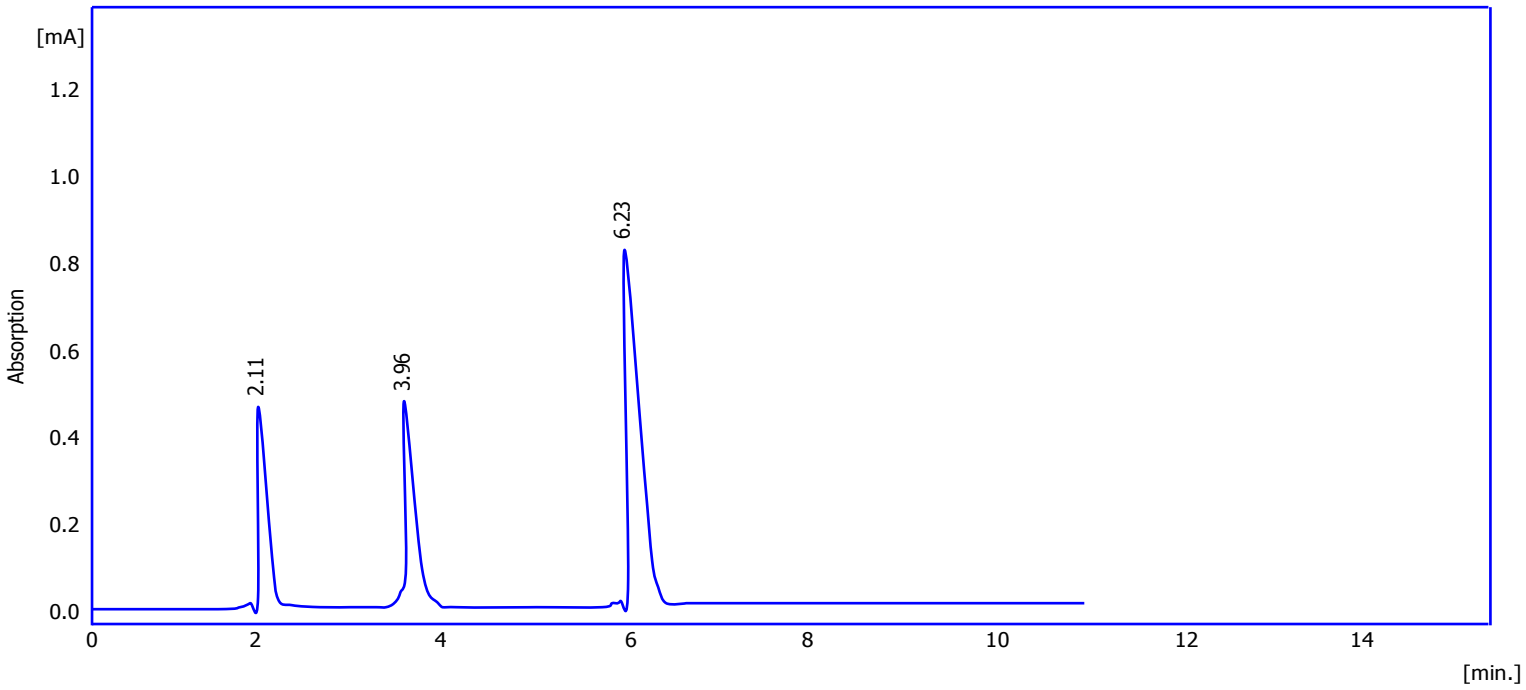
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.17	1295.46	462.65	25.00	25.00	0.15	
2	3.95	2117.99	468.97	25.00	25.00	0.15	
3	6.26	9620.47	800.51	50.00	50.00	0.30	
	Total	13033.25	1727.98	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 7	Amount	0
Sample	: sample 7	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 7)

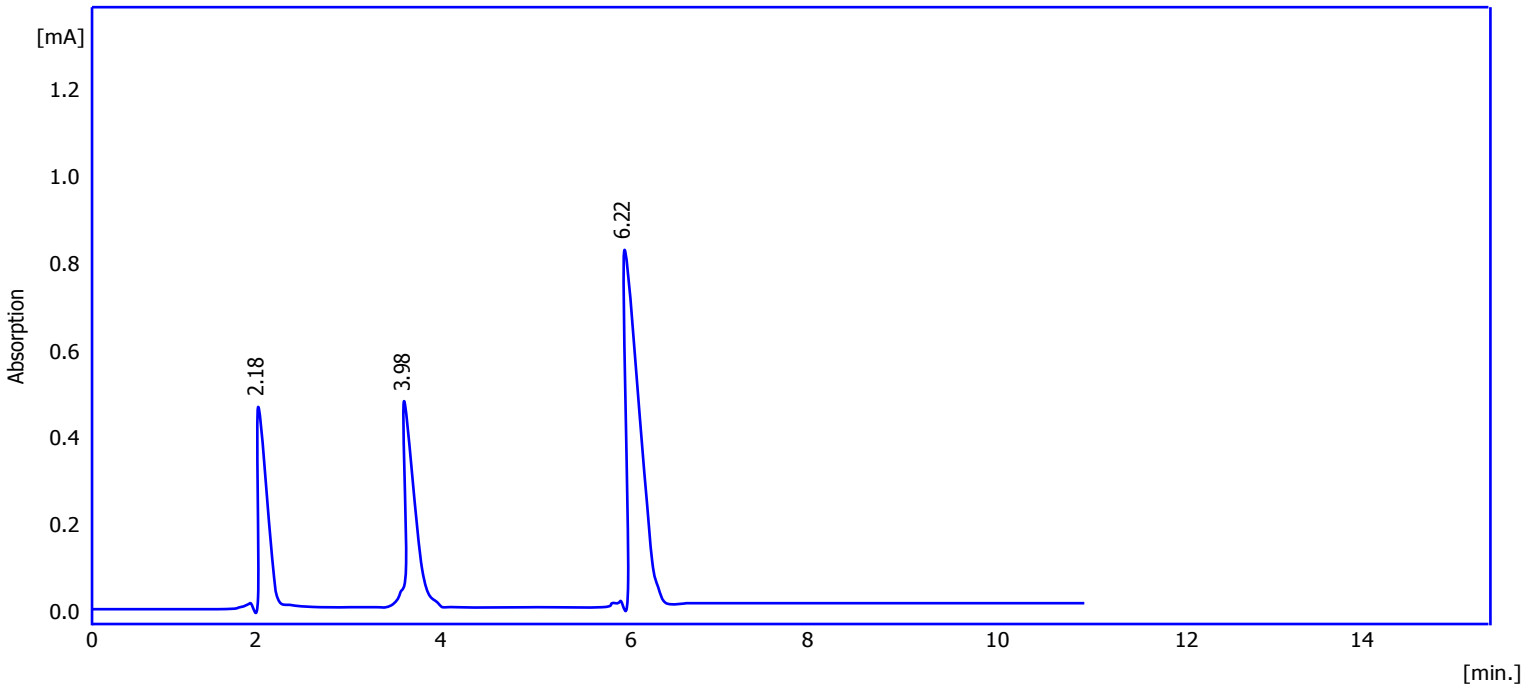
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.11	1198.54	460.22	25.00	25.00	0.15	
2	3.96	2166.59	462.65	25.00	25.00	0.15	
3	6.23	10856.22	801.58	50.00	50.00	0.30	
	Total	14221.59	1724.98	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 8	Amount	0
Sample	: sample 8	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 8)

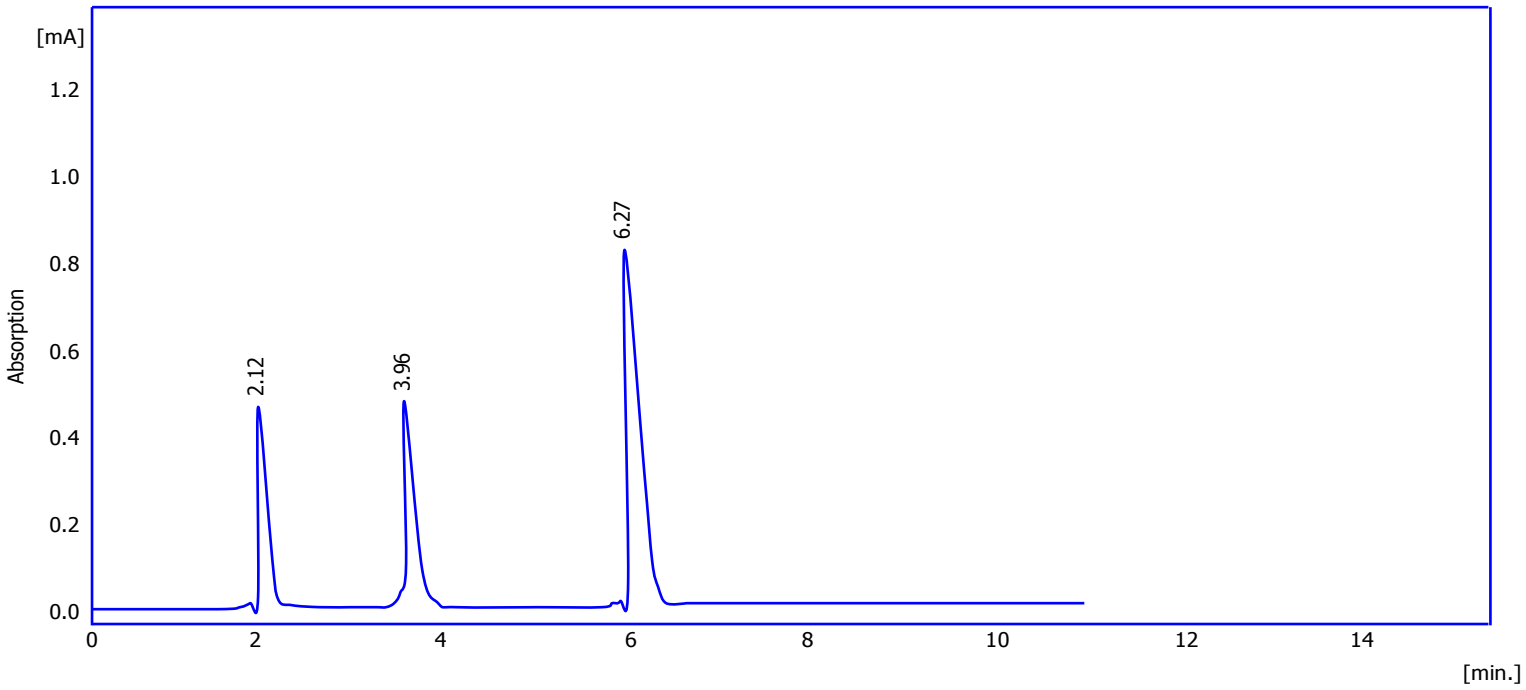
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.18	1274.19	460.11	25.00	25.00	0.15	
2	3.98	2120.66	462.65	25.00	25.00	0.15	
3	6.22	11582.64	805.98	50.00	50.00	0.30	
	Total	14977.58	1731.59	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 9	Amount	0
Sample	: sample 9	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 9)

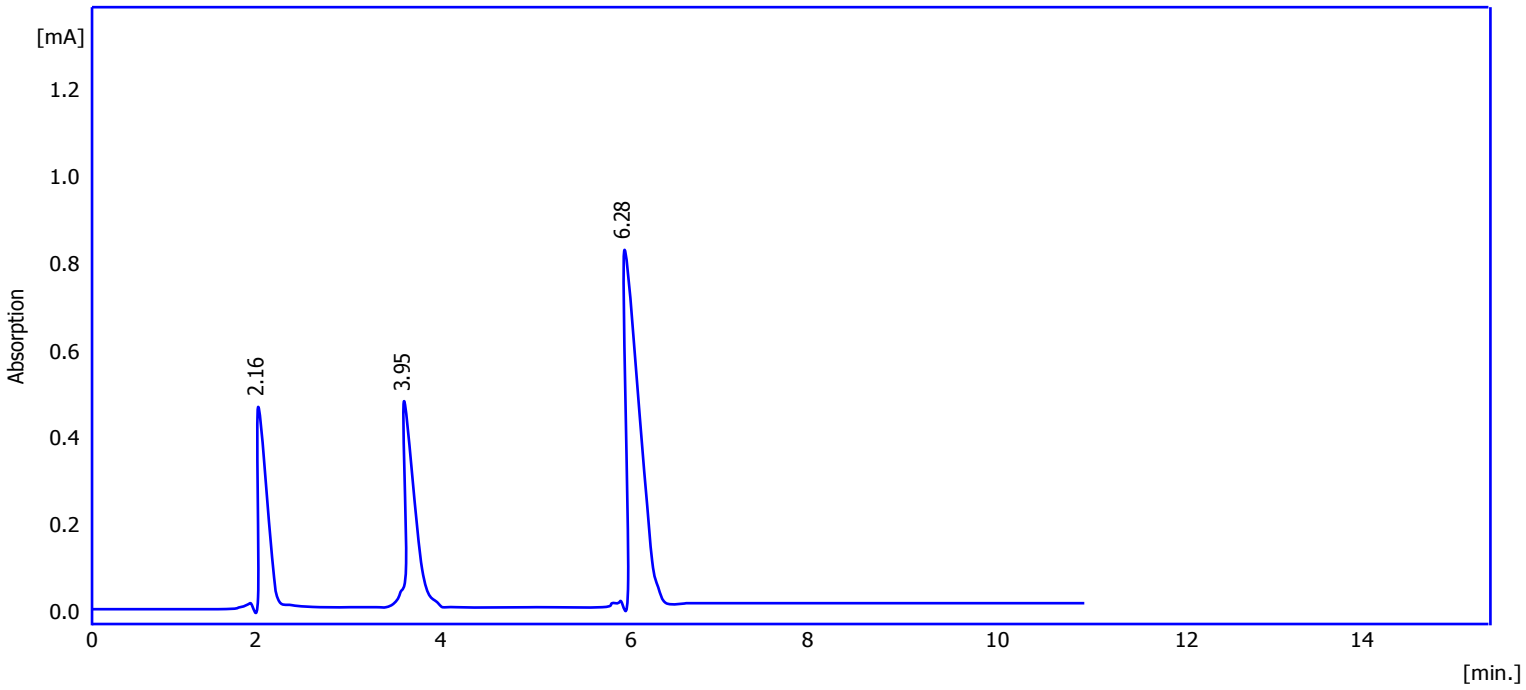
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.12	1260.23	463.56	25.00	25.00	0.15	
2	3.96	2104.98	461.25	25.00	25.00	0.15	
3	6.27	8562.49	802.49	50.00	50.00	0.30	
	Total	11862.14	1724.98	100.00	100.00		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 10	Amount	0
Sample	: sample 10	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 10)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.16	1252.16	462.65	25.00	25.00	0.15	
2	3.95	2120.44	462.00	25.00	25.00	0.15	
3	6.28	9950.24	800.49	50.00	50.00	0.30	
	Total	13298.58	1734.08	100.00	100.00		

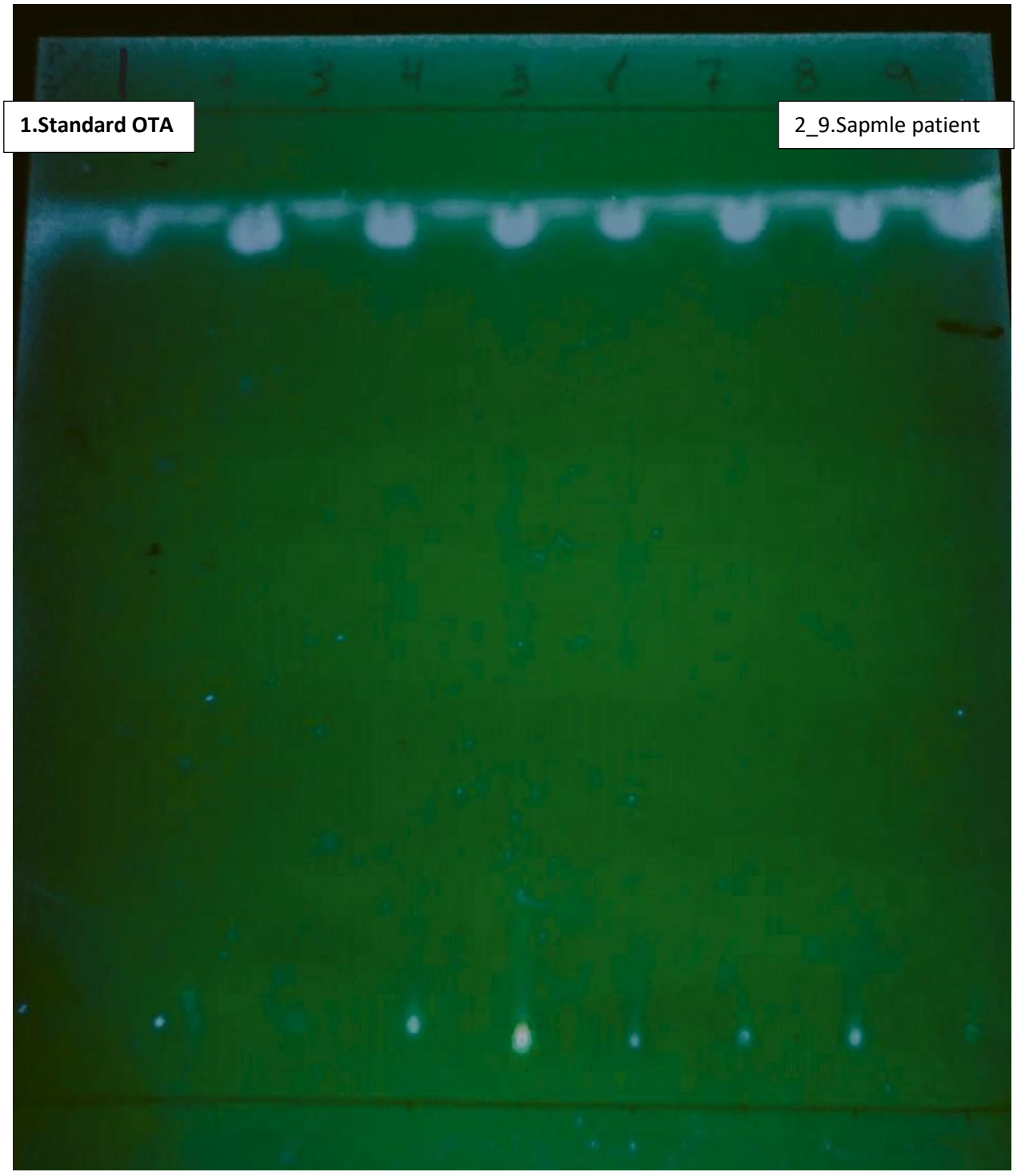
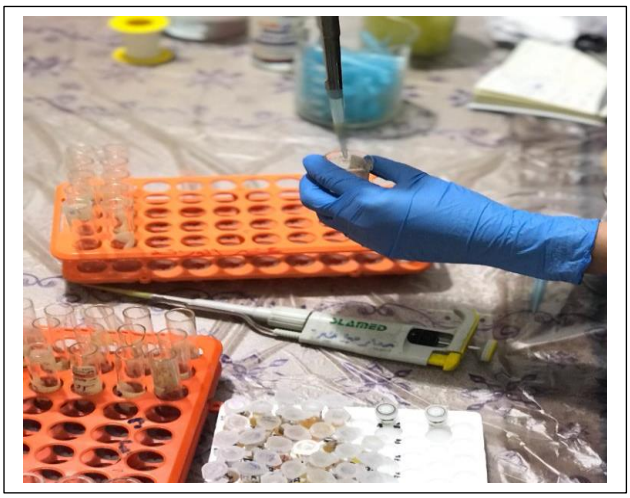
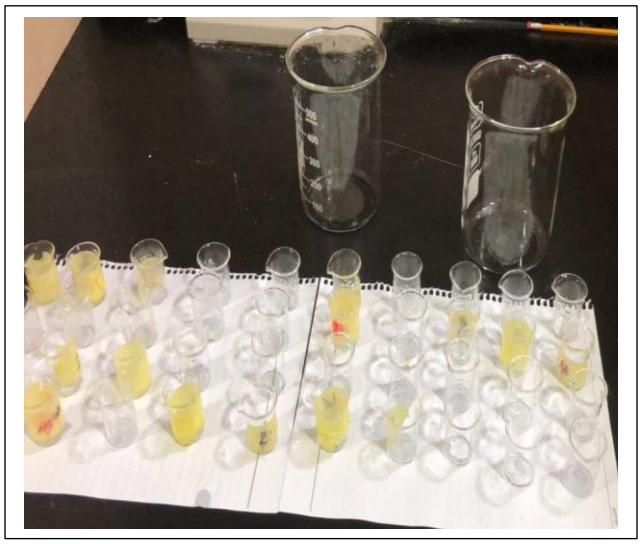


Figure 1: The result of examining samples of patients with chronic kidney disease and detection of Ochratoxin A by TLc test.

Figure 2: Injector of HPLC

Figure 3: *High Performance Liquid Chromatography*





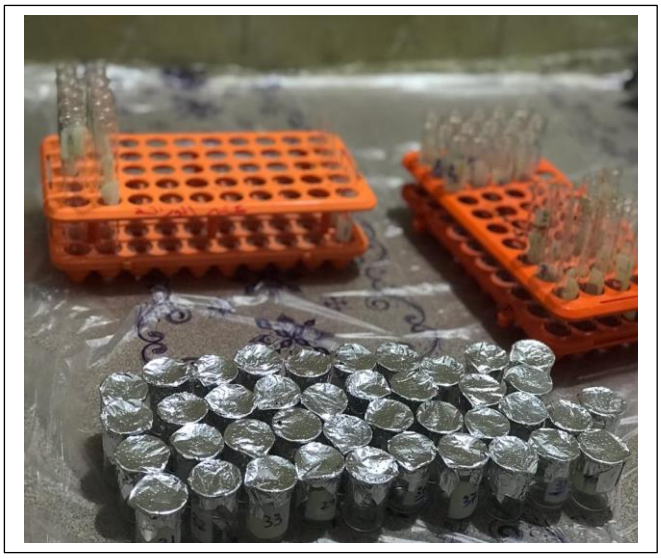


Figure 4: Ochratoxin A extraction from serum samples using TLC

QUESTIONNAIRE FORM

Date: / /
Serial number:

Name:	**Age:**	**Gender:**
Phone:	**Address:**	

Symptoms

Hypouresis ☐ Vomiting ☐ Nausea ☐ Fatigue ☐ Weakness ☐

Anemia ☐ Peripheral edema ☐ Difficulty sleeping ☐

Other ☐

Family History

Family history of CKD: Yes ☐ No ☐

Case status

Dialysis: Yes ☐ No ☐

Other:

Medical History

Diabetes: ☐ Other disease: ☐

Hypertension: ☐

Other

Wight: Length:

الخلاصة

تم إجراء العديد من الدراسات حول تلوث الأغذية الأوكراتوكسين أ في دول مختلفة في جميع أنحاء العالم. وجد أن أكثر من 5 مليارات شخص في الدول النامية معرضون لخطر التعرض المزمن لسم الأوكراتوكسين أ وفقًا للدراسات. بالإضافة إلى ذلك، فإن الأوكراتوكسين أ ومستقلباته توجد في الأنسجة الحيوانية وفي غذاء الإنسان (مثل لحوم البقر والأغنام). الأوكراتوكسين أ هو مادة سامة كلوية وله اثار ضارة مختلفة على الصحة، كما أنها تساهم في تقدم اعتلال الكلية المتوطن في البلقان. وكان الهدف من الدراسة هو الكشف عن الارتباط بين الأوكراتوكسين أ وتأثير مع مرض الكلى المزمن في بعض المعايير البيوكيميائية المستوى وتأثيرها على هرمونات الغدة الدرقيةT3 ، T4، TSH.

أجريت هذه الدراسة في مختبرات كلية العلوم الطبية التطبيقية/ جامعة كربلاء والمختبرات الخاصة ووزارة العلوم والتكنولوجيا/ بغداد/ العراق. خلال الفترة من 2022/12/1 إلى 2023/4/1 تم جمع 100عينة دم من مرضى الكلى المزمن و100 من الأصحاء.

أظهرت النتائج اختلافًا معنويًا في مستويات الأوكراتوكسين أ في بلازما الدم، حيث بلغت مستوياتها في مرضى الفشل الكلوي المزمن 99٪ ومستوياتها في بلازما الدم من الاصحاء 32٪. حيث كانت نسبة الاناث أكثر من الذكور بوجود الأوكراتوكسين أ في بلازما الدم للمرضى، وكانت النسب كالاتي الإناث 56٪ والذكور 43٪.

كما أظهرت النتائج أن تركيز الأوكراتوكسين أ في بلازما الدم لدى المرضى كان أعلى بشكل واضح من الأصحاء، حيث بلغت تركيزه في المرضى الذكور والإناث 23.475 و23.943 نانو غرام/ مل، على التوالي، بينما كانت تركيز الأصحاء في الذكور والإناث (2.075 و2.171) نانو غرام / مل على التوالي.

أظهرت النتائج أن مستويات اليوريا لدى الذكور والإناث الأصحاء كانت ضمن المعدل الطبيعي، بينما ارتبط وجود الأوكراتوكسين أ بزيادة مستويات اليوريا لدى المرضى الذكور إلى 140.34 مجم / ديسيلتر وفي المرضى الإناث إلى 121.53 مجم / ديسيلتر.

ارتفع متوسط مستويات الكرياتين في المرضى الذكور والإناث المصابين بأمراض الكلى المزمنة إلى 3.655، 3.680 ملجم / ديسيلتر، على التوالي، وهو أعلى بكثير من الاصحاء.

الخلاصة

أثبتت هذه الدراسة أن الأوكراتوكسين أ ومرض الفشل الكلوي المزمن أثر على المستويات الطبيعية لهرمونات الغدة الدرقية إذا أدى ذلك إلى انخفاض مستويات هرمون TSH في المرضى مع اختلاف كبير في قيمة0.000192 value *P* عند مقارنتها بمستويات الأصحاء؛ حيث انخفض مستوى TSH في مصل الدم حيث كان المرضى الذكور والاناث مع الأوكراتوكسين أ والمرضى الاناث بدون الأوكراتوكسين أ هي (0.227, 0.247 و 0.241) µIU / mL على التوالي مع مستويات مختلفة بشكل كبير من TSH في مصل الدم حيث كان الاصحاء الذكور والاناث مع الأوكراتوكسين أ والاصحاء الذكور والاناث بدون الأوكراتوكسين أ كانت (6.072 ، 3.757 ، 1.852، 1.692)µIU / mL على التوالي.

من جانب اخر، تسبب الأوكراتوكسين أ مع مرض الفشل الكلوي المزمن في زيادة مستوى هرمون T4 وتراوح nmoL / L (244.24 – 322.627)بينما تراوح مستوى الهرمون في الاصحاء بين (107.28_118.76) nmoL / L .

بينت النتائج أن مستويات هرمون T3 ارتفعت إلى (19.461، 18.896، 23.013) nmoL / L في مصل الدم حيث كان المرضى الذكور والاناث مع الأوكراتوكسين أ والمرضى الاناث بدون الأوكراتوكسين أ على التوالي مع مستويات مختلفة في مصل الدم لكل من كان الاصحاء الذكور والاناث مع الأوكراتوكسين أ والاصحاء الذكور والاناث بدون الأوكراتوكسين أ كانت والتي كانت (1.988، 2.018، 2.108 ،1.946) nmoL /L على التوالي.

أظـــهر فحص معايير الـدم الفسيولوجية أن نتيجة WBC كانت بدون فرق معنوي مقارنة بالأصحاء حيث كـــانت قيمـــة (0.062) *P* value، وكانت نتيجة HGB ايضاً بفروق معنوية عالية وكانت قيمة (0.00018) *P* value مقارنة مع الاصحاء. بالإضافة إلى ذلك، تبين فرق معنوي بين نسب خلايا الدم الحمراء لدى الأشخاص المصابين بأمراض الكلى المزمنة والأصحاء وكانت قيمة (0.000107)*P* value . بينما لم تظهر أي فروق ذات دلالة إحصائية للمعاير (PLT،MCV) بين مرضى CKDمقارنة بالأصحاء.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

تأثير مرض الفشل الكلوي المزمن وسم الأوكراتوكسين A وتفاعلهما في بعض المقاييس البيوكيميائية للإنسان في محافظة كربلاء

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

عُثيم رزاق فليح حسن الاسـدي

بكالوريوس علوم الحياة/ جامعة كربلاء/2014

بأشراف

ا.د. سامي عبد الرضا الجميلي

1445 هـ

2023 م