CHAPTER I:

INTRODUCTION

Body fluid analysis has played a crucial role in reconstructing events during crime scene investigation. It is often presumed that crimes that involve violence and mental disturbances such as murder or sexual assault provide good sources of body fluids such as blood, saliva, semen, vaginal secretions, urine and tears. Tears are secreted in response to any emotional or stressful situations. In the absence of the commonly noted body fluids such as blood or saliva, tears can play an important role that can lead to personal identification by examining the biochemistry and molecular aspects to obtain a full DNA profile.

Tears are a clear liquid secreted by the glands known as tear ducts or lacrimal glands/tear gland found in the eyes of all land mammals. Their functions include lubricating the eyes (basal tears), removing irritants (reflex tears), and aiding the immune system. Tears also occur as a part of the body's natural pain response. Lachrymal fluid impregnates the cornea, conjunctiva and the nasolachrymal ducts. It ensures the moisture of the cornea, allows blinking and enhances tear elimination. Lachrymal fluid is organised into a structural film comprising three different layers and it protects the conjunctiva and the cornea against physical and chemical agents. It contains many exogenous and endogenous compounds. Lachrymal fluid is constantly secreted by basal glands, but their secretions can be affected by physical or emotional stimulation.

The process of lacrimation is classified into three types based on differences in their composition:

1. **Basal tears** are secretions that are present in the superficial layers of the eye that helps in maintaining the lacrimal film for both visual and corneal surface and maintains the homeostatic condition. Basal tears also help in improving the refraction by maintaining a constant layer of tear secretion to obtain a smooth surface. It aids in better focus of the visual images on the retina

2. **Reflex tears** are lacrimal secretions produced in response to external stimuli which may be by physical or chemical factors. Upon minor stimulation, it increases basal tear secretions and when intense, it causes excess tear secretions causing a person to weep. Reflex tears are formed due to various external stimuli and are of different types. Most of the reflex tears are caused due to irritation, it causes damage to the ocular surface by external irritants such as dust, pollen, sand, chilli powder, structural damage or disturbance to the eye due to infections, allergies or inflammation. Since it causes immediate discomfort and leads to pain, it stimulates the reflex hypersecretion of the lacrimal glands
3. **Psycho emotional tears** or **psychic tears** can be caused due to many states of mind. Those that causes sensorial pain, emotional pain and a variety of other emotional states such as anger, depression, fear, anxiety, panic, restlessness, frustration, grief, humiliation, nervousness, nostalgia, sentiments, rejection, embarrassment, oppression, compassion, joy, solitude, sadness, admiration, condolence, pity, tenderness, affection, victory from a sports, music closely associated with the weeper etc.

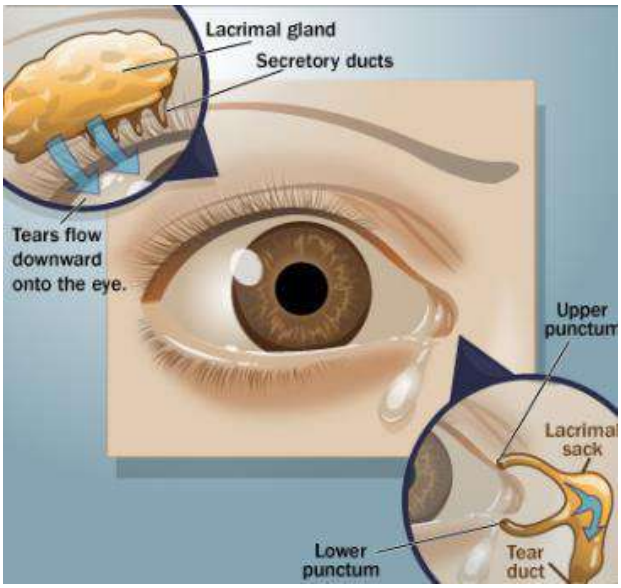


Fig: The eye and lacrimal glands

Composition of tears:

1. Water
2. Electrolytes: Sodium, potassium, chloride, bicarbonate, magnesium and calcium
3. Lipid (outer layer): Triglyceride, fatty acids, phospholipids, lysozymes, lactoferrin, lipocalin and cholesterol esters
4. Aqueous (middle layer): Inorganic salts, enzymes, metabolites and proteins
5. Mucin (inner layer): Glycoproteins

General significance of tears:

After blinking, a film of tear fluid coats the surface of the eye at a certain thickness, and is maintained for a while. This is called tear stability. Tears not only keep the eye moist but also have an important role in maintaining the healthy functioning of the eye. These includes:

- Preventing dryness
- Supplying oxygen and nutrients to eye
- Preventing infection
- Healing damage to the surface of the eye
- Creating a smooth surface on the eye

Forensic significance of tears:

Tears can be considered as powerful forensic evidence in the absence of other body fluids. Although tears are secreted in very less volume, they may be potentially used to establish the identity. Tears may be found in a dry state deposited on substrates such as tissue papers, handkerchief, pillows, bedding or any other surface that will retain the small volume of tears. The possibility of finding tears as evidence on various substrates such as a tissue or cloth have been less explored. It could offer immense clues both on qualitative and quantitative analysis for personal identification. DNA profiling from tears is done in same way as profiling from other biological fluids. Tears on the other hand can be useful in determining the drug constituents which may be psychotropic or therapeutic drugs.

What is the Schirmer's Test or Tear Test?

The eye maintains a stable level of moisture and eliminates foreign particles by producing tears. When your eyes are too dry or too wet, doctor may perform the Schirmer's Test. The Schirmer's Test is primarily used to diagnose dry eye syndrome. This is a condition that occurs when the tear glands are unable to produce enough tears to keep eyes moist. As a result, the eyes can't get rid of dust and other irritants. The doctor collects tears using Schirmer test strips (generally a filter paper).

How drugs are secreted into tears?

Systemically administered drugs, particularly those administered orally, are subjected to first-pass metabolism by the liver, where the concentration of the drug is reduced prior to entering the bloodstream. Following transport in the bloodstream, a drug seeking to reach target ocular tissues encounters the blood-retinal barrier. Composed of retinal capillary endothelial cells and retinal pigment epithelium cells, the blood-retinal barrier restricts the flow of drugs from the blood to the posterior segment. The outer layer of the barrier, which consists of the RPE, restricts intercellular permeation due to its tight junctions.[2] While drugs can easily enter the choroid due to its high vasculature, the tight junctions of the RPE restrict drug from further transport into the retina. Less than 2 percent of the systemically administered drug reaches the target ocular tissue following the first-pass metabolism and permeation of the blood-retinal barrier.

About the Instrument:

Introduction:

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Principle:

The principle of HPLC is adsorption. When a mixture of compounds are introduced into HPLC column, they travel according to their relative affinities towards their stationary phase. The component which has more affinity towards the stationary phase travel slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

Instrumentation:

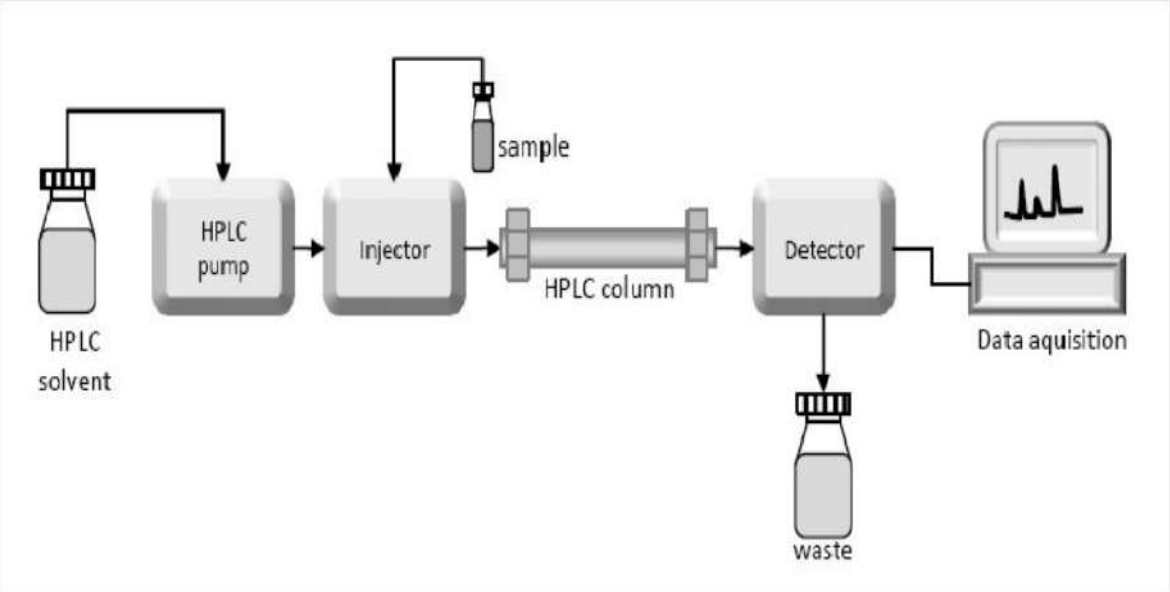


Fig: Instrumentation of HPLC

Working:

Mixture of analytes are introduced as a band to the column, which contains a non-polar reversed phase stationary phase. There is a mobile phase. As the mixed analytes band is applied to the column, the mobile phase pushes the analytes down the column. As they move down the column, they come into contact with the stationary phase. Analytes that have a higher affinity for the stationary phase will be retained more strongly and elute later in the run. Thus, you can separate the analytes based upon how strongly they interact with the stationary phase. The stationary phase consists of hydrophobic, non-polar and most often a C18. The mobile phase consists of a polar, hydrophilic, aqueous component, usually water and acetonitrile or methanol. Analytes will be separated based upon their relative affinity for these two phases. Hydrophobic compounds, such as benzopyrene, will have a strong affinity for the hydrophobic stationary phase, and will be strongly bound. Hydrophilic compounds such as ethyl sulphate will have little affinity for the stationary phase and will stay primarily in the mobile phase and be rapidly carried through the column.

UV detectors:

An Ultraviolet detector is a type of non-destructive chromatography detector which measures the amount of ultraviolet or visible light absorbed by components of the mixture being eluted off the chromatography column. They are often used as detectors for high-performance liquid chromatography. The standard ultraviolet (UV) detector for high performance liquid chromatography (HPLC) measures the absorbance of monochromatic light of fixed wavelength in the UV or visible wavelength range (typically between 190 nm [UV] and 400 nm [blue light]) against a reference beam and relates the magnitude of the absorbance to the concentration of analyte in the eluent passing through a flow cell contained within the instrument.

Fig: HPLC with UV detectors.

This project work entitles to determine the presence of drugs in tears. This work involves collecting samples from patients and volunteers, preparing the sample for analysis and using sophisticated instruments for qualitative and quantitative analysis of the tear samples. By this we can know if drug constituents are distributed into lachrymal fluid, if so, then doctors can collect tear samples from patients as well as drug abusers to determine the drug present in tear samples.

CHAPTER II:

LITERATURE REVIEW

Dave C Crandall, Irving H Leopold, 1979. They conducted a research on the topic **"The influence of systemic drugs on tear constituents."** Antibiotic penetration into tears, drugs stimulating or retarding lacrimation, and effects on tear electrolytes, lysozyme, and immunoglobins are reviewed from the literature. Important applications to clinical practise such as contact lens wear, general anesthesia, eye infections, and epiphora or dry eye symptoms are discussed.

Wolfgang Thormann, Sabine Lienhard, Paul Wernly, 1993. Their research entitled **"Strategies for the monitoring of drugs in body fluids by micellar electrokinetic capillary chromatography."** Electrokinetic capillary techniques can exploit numerous separation principles, making them flexible and easily applicable to a variety of separation problems. In recent publications, this emerging technology has been shown to be well suited for monitoring drugs and metabolites in body fluids, including tears, saliva and urine. Most attention has been focused on micellar electrokinetic capillary chromatography (MECC) because it permits the separation and determination of drugs with discrimination being largely based on differences in hydrophobicity.

Vincent Baeyens, Robert Gurny, 1997. This paper titled **"Chemical and physical parameters of tears relevant for the design of ocular drug delivery formulations"** provides a summary of the most important chemical and physical parameters of tears that can help the formulator in the development of new ocular formulations and in the conception of innovative ophthalmic delivery approaches. The aim of this review is also to give a description of the main analytical techmiques available in ophthalmology that can be used for pharmacokinetic studies of active compounds. The importance of tear sampling is also discussed.

Sandra Zaugg, Wolfgang Thormann, 2000. This paper titled **"Enantioselective determination of drugs in body fluids by capillary electrophoresis."** The chiral capillary electrophoresis (CE) emerged as a promising, effective and economic approach for the

enantioselective determination of drugs in body fluids, hair and microsomal preparations. This review discusses the principles and important aspects of CE-based chiral bioassays, provides a survey of the assays developed and presents an overview of the key achievements encountered. Applications discussed encompass the pharmacokinetics of drug enantiomers, the elucidation of the stereoselectivity of drug metabolism and bioanalysis of drug enantiomers of toxicological and forensic interest.

Bita Esmaeli, MD; M. Amir Ahmadi, MD; Edgardo Rivera, MD; et al 2002. Their research paper entitled "Docetaxel secretion in tear." The secretion of docetaxel in tears may be a mechanism for canalicular inflammation and tear drainage obstruction, which are known to occur as an adverse effect of the drug. ear fluid was collected from 4 patients receiving docetaxel weekly and 2 patients receiving docetaxel every 3 weeks as a single agent for the treatment of metastatic breast cancer. Tear samples were collected once prior to and again within 30 minutes following the end of the 1-hour docetaxel infusion. The tear and plasma samples were analyzed for drug content using high-performance liquid chromatography and tandem mass spectrometry. Docetaxel was found in the tear samples collected from all 6 patients.

Sekiyama E, Matsuyama Y, Higo D, Nirasawa T, Ikegawa M, et al.,2008. Their research paper entitled **"Applying Magnetic Bead Separation / MALDI-TOF Mass Spectrometry to Human Tear Fluid Proteome Analysis."** The proteins and peptides in tears play an important role in preserving the integrity and stability of the ocular surface. Proteomic analysis of tear films will enable us to detect early biological markers of eye diseases, however, it is often hampered by the small amount of tear volume and the low protein concentration. They concluded that magnetic beadbased separation combined with MALDI-TOF-MS (ClinProt MALDI-TOF) appears to be ideally suited for the first-line screening of peptides and proteins in tears.

Byrro R M, de Oliveira F G, da Silva Cunha A, Cesar I C, Chellini P R, Pianetti G A, 2012. Their study entitled **"Determination of ofloxacin in tear by HPLC-ESI-MS/MS."** In which they studied that A liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) method for quantitation of ofloxacin in rabbits' tears was

developed and validated. The tear was collected with tear strips, extracted by a liquid extraction procedure. The validated method was successfully applied to determine the ofloxacin concentration in tears of rabbits treated with a mucoadhesive chitosan films and a conventional eye drop formulation.

Guo H, Lee C, Shah Mihir, Jagna S R, Edman M C, Klinngam W, Hamm- Alvarez S F, Mackay J A, 2018. Their study entitled **"A novel elastin-like polypeptide drug carrier for cyclosporine A improves tear flow in a mouse model of Sjögren's syndrome"** As a potent macrolide immunosuppressant, cyclosporine A (CsA) is used to treat multiple autoimmune diseases, including non-autoimmune and autoimmune-mediated dry eye disease, rheumatoid arthritis and psoriasis. cyclosporine A increased tear production relative to CA192 alone. Moreover, CA192 delivery reduced indications of CsA nephrotoxicity relative to free CsA. CA192 represents a viable new approach to deliver this effective but nephrotoxic agent in a modality that preserves therapeutic efficacy but suppresses drug toxicity.

Matthias Boerger, Sebastian Funke, Andreas Leha, Anna- Katrin wusstermann, Fabian Maass, Mathias Bahr, Franz Grus, Paul Lingor, 2019. They conducted a research entitled **"Proteomic analysis of tear fluid reveals disease-specific patterns in patients with Parkinson's disease."** The diagnosis of Parkinson's disease (PD) is still challenging and biomarkers could contribute to an improved diagnostic accuracy. Tear fluid (TF) is an easily accessible body fluid reflecting pathophysiological changes in systemic and ocular diseases and is already used as a biomarker source for several ophthalmological disorders. Here, we analysed the TF of patients with Parkinson's Disease and controls (CTR) to describe disease-related changes in TF and identify putative biomarkers for the diagnosis of Parkinson's disease.

R Aparna, R Shanti Iyer, 2019. Their study entitled **"Tears and Eyewear in Forensic Investigations."** This paper provides a systematic review of the possibility of using tears and eyewear for the purpose of forensic investigation and to statistically support the inferences with prescription databases which may be initiated across different populations. It is speculated that the last seen image referred to as an 'Optogram' of an individual may be

captured in the retina since our eye functions like a camera. Although this claim is considerably unexplored, it is quite possible that the last seen image of a criminal, objects or a place may be noted that can positively help in linking individuals at the scene of crime or identify the primary crime location.

Terra Beek MSN, FNP-C, 2020. They did a research on the topic **"Examination of the role of platelet-rich plasma in meniscal tears."** The role of platelet-rich plasma in meniscal tears in humans has yet to be fully examined in the literature. Meniscal tears remain a prevalent and common orthopedic injury. Numerous methods of treatment for this condition are available, ranging from conservative methods to surgical options. Platelet-rich plasma is a biologic agent that is minimally invasive, can have regenerative properties, and may aid healing for patients. This article offers a current review of the literature examining platelet-rich plasma in meniscal tears and recovery.

CHAPTER III:

AIM AND OBJECTIVE

AIM:

The aim of this research is to determine the presence of drugs (psychotropic and therapeutic drugs) in tears using instruments such as high performance liquid chromatography with UV detectors.

OBJECTIVE:

The objectives of this study are:

- To investigate the tear concentrations for drug over time
- To determine the categories of drugs that are secreted in tears
- To investigate the presence of psychotropic drugs in tears

CHAPTER IV:

MATERIALS AND METHODOLOGY

Materials Required:

Schirmer test strips, gloves, micro capillary tubes, 1 ml, 10 ml Pipettes, cuvette, test tubes, calorimeter, spectrophotometer, micropipette and tips.

METHODOLOGY:

Sample Collection:

Schirmer's test uses paper strips is used to collect tears from the patients. This strip is generally used to perform dry eye test. In this experiment we will collect sample tears using Schirmer test strips. For this study, tear samples from 30 patients and 10 volunteers are collected (by their consent) who use medicinal drugs on daily basis. The procedure for collecting tears are given below.

Procedure:

- Remove two strips from the sterile packet and label them ‘R’ (right) and ‘L’ (left)
- Bend each strip, at the notch, to a 90 degree angle
- Ask the patient to look up and, with an index finger, gently pull down the lower eyelid.
- Hook the bent end of the strip over the centre of the lower eyelid and allow it to ‘sit’ inside
- Repeat the procedure for the other eye.
- Ask the patient **not to squeeze**, but just to keep the eyes gently closed.
- After five minutes, ask the patient to open both eyes and look upwards.
- Carefully remove both strips.
- Place the strips in a sterile paper bag for laboratory examination.

Sample Preparation:

The collected tear sample is then processed for protein extraction because the drug binds along with the protein. So, the first step is to estimate the concentration of protein in the given sample using Lowry's protein assay.

Reagents Required:

1. BSA stock solution (1mg/ml)
2. Analytical reagents
3. 2x Lowry concentrate
4. 0.2 N Folin reagent
5. Folin - Ciocalteau reagent solution

Reagent Preparation:

1. Analytical reagent:

   Dissolve 20 gm sodium carbonate in 260 ml water, 0.4 gm cupric sulphate (5x hydrated) in 20 ml water, and 0.2 gm sodium potassium tartarate in 20 ml water. Mix all three solutions to prepare the copper reagent. Prepare 100 ml of a 1% solution (1 gm/100 ml) of sodium dodecyl sulphate (SDS). Prepare a 1 M solution of NaOH (4 gm/100 ml).
2. 2x Lowry concentrate:

   Mix 3 parts copper reagent with 1 part SDS and 1 part NaOH. Solution is stable for 2-3 weeks. Warm the solution to 37 degrees C if a white precipitate forms, and discard if there is a black precipitate. Better, keep the three stock solutions, and mix just before use.
3. 0.2 N Folin's reagent:

   Mix10 ml 2 N Folin reagent with 90 ml water.
4. Folin - Ciocalteau reagent solution

   (1N) dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water).

Procedure:

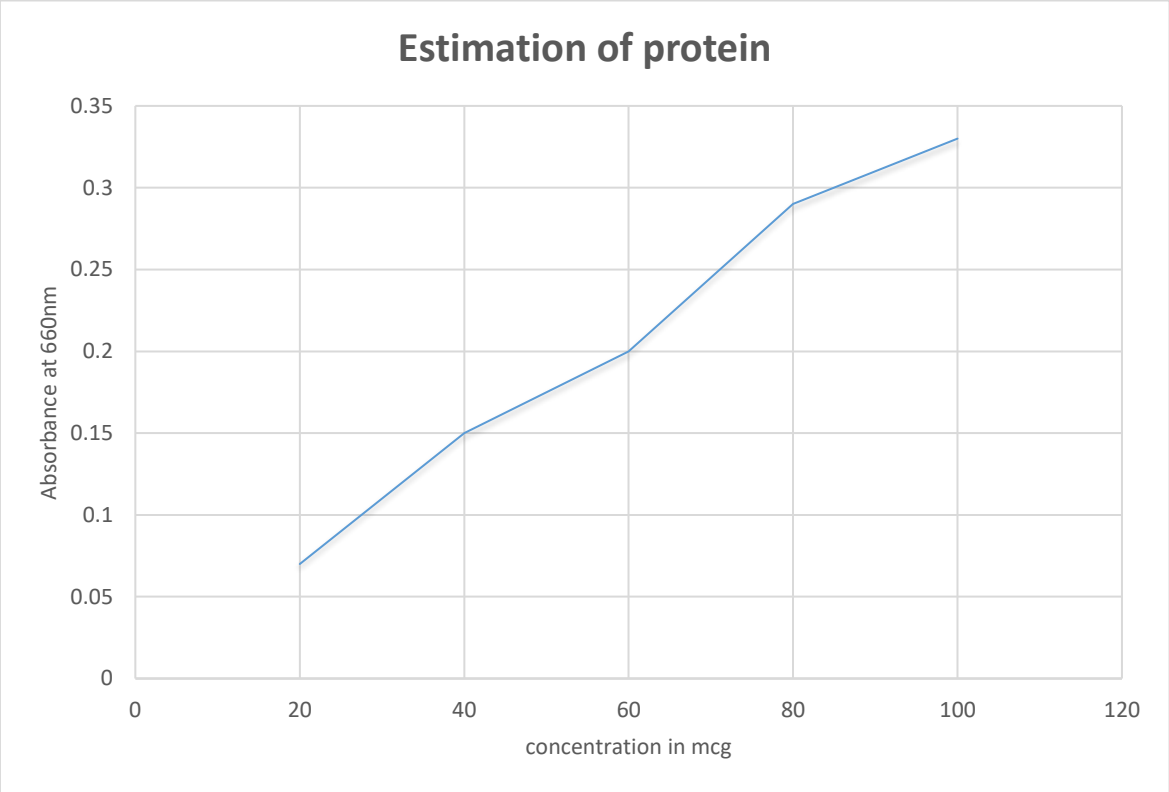
1. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ ml.
2. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well.
3. This solution is incubated at room temperature for 10 mins.
4. Then add 0.2 ml of reagent Folin- Ciocalteau solution (reagent solutions) to each tube and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm.
5. Plot the absorbance against protein concentration to get a standard calibration curve.
6. Check the absorbance of unknown sample and determine the concentration of the unknown sample using the standard curve plotted above.

Observation Table:

BSA (ml)	Water (ml)	Sample conc. (mg/ml)	Folin's reagent (ml)	Alkaline $CuSO_4$	O.D 660 nm
0.0	1.0	Blank	0.5	4	0.0
0.2	0.8	20	0.5	4	0.07
0.4	0.6	40	0.5	4	0.15
0.6	0.4	60	0.5	4	0.20
0.8	0.2	80	0.5	4	0.29
1.0	0.0	100	0.5	4	0.33
1.0	0.0	To estimate	0.5	4	0.24

Graph:

A graph is plotted by taking the values of absorbance on y- axis and concentration of proteins on x- axis.



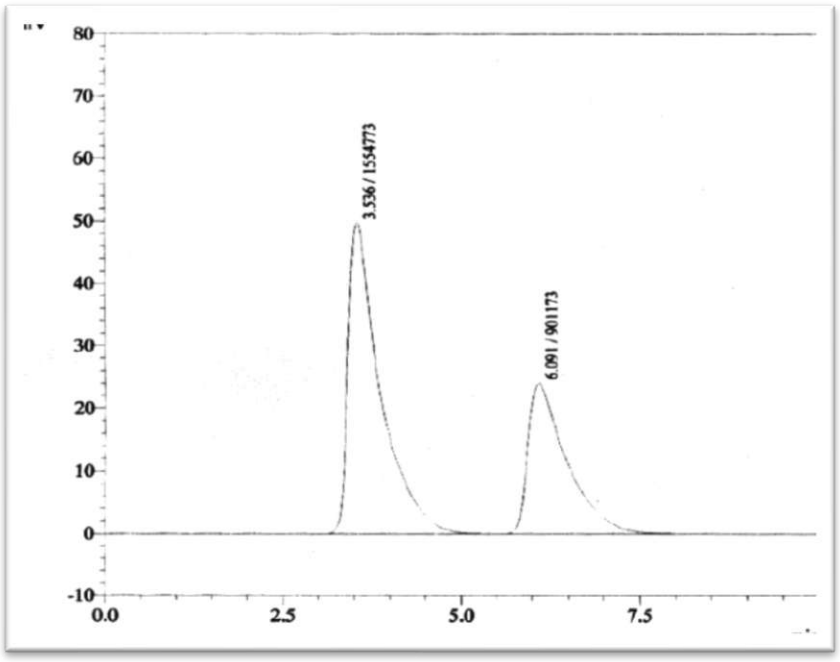
Result:

The protein is extracted from the tear sample. This tear sample contains 14.2% of lysozyme, 9.5% of human serum albumin, 27.7% of secretory IgA, and 30.9% of mucin. The concentration of protein present is 72 mcg/ml.

INSTRUMENTS:

The extracted protein from the tear sample is then injected into HPLC-UV for determining any drug constituents in the sample.

The mobile phase selected was acetonitrile and water as maximum detection and sensitivity was observed in mobile phase ratio 60 : 40 v/v/ (Acetonitrile and Water). All the solvent were filtered through 0.45 µ Millipore filler before used and degassed. Standard sample solutions were also filtered through 0.25 µ membrane and degassed. 20 µL of standard solution was injected to Rhenodyne injector several time to optimize the condition. A steady baseline was recorded at the flow rate 1 mL/ min at the retention time 3.536 minute and UV detection at 210 nm. A typical chromatogram obtained is given below.



This is the standard chromatogram obtained. From this graph it is evident that the chromatographic value of 3.536 belongs to Acetaminophen (paracetamol).

CHAPTER V:

RESULT AND CONCLUSION

RESULT:

Tears belong to alternative biological materials, which are not widely used for determination of drugs. It is noted that a very small category of drugs are secreted into tears which includes anti-histamine drugs and neoplastic agents (cancer drugs). From the project it is clear that the concentration drugs that are excreted into tears is in a very less volume. In a total of 40 samples, 12 samples had the presence of acetaminophen or commonly called as paracetamol which is used as pain killer and to treat fever and rest of the sample did not show any presence of drugs.

CONCLUSION:

The current trends in investigation are under tremendous progress and refinement in the laboratory-based methods that have been utilized for personal identification and in crime reconstruction. Tears are not widely used as an evidence. One of the main reasons for this is the requirement of expensive, highly-sensitive and highly-selective methodologies. In spite of these problems, analysis of unconventional bio samples (e.g., tears) may be advantageous and provide information that would not be accessible in materials routinely analysed.

Although they are secreted in trace quantities, they can be of great help and this is only possible when its biochemical characteristics are well understood to be utilized effectively for laboratory analysis. It is also possible to perform ABO typing and DNA profiling from tears deposited on various substrates

Human tear samples have been employed for pharmacokinetic study of some anti-microbial drugs and antibiotic drugs, which are administered for treatment of allergic conjunctivitis, ocular infections or skin infections and respiratory system diseases. The chief purpose of determinations of these drugs was to evaluate the potential applicability of tears as an alternative material for evidence or therapeutic drug monitoring.

CHAPTER VI:

REFERENCES

1. Van Haeringen N.J. 1981. Clinical biochemistry of tears. Survey Ophthalmol. 26(2):84-96
2. Sitaramamma T., Shivaji S. & Rao G.N. 1998. Effect of storage on protein concentration of tear samples. Curr. Eye Res. 17(10):1027-1035
3. Markoulli M., Papas E., Petznick A. & Holden B. 2011. Validation of the Flush Method as an alternative to basal or reflex tear collection. Curr. Eye Res. 36(3):198-207
4. Ananthi S., Chitra T., Bini R., Prajna N.V., Lalitha P. & Dharmalingam K. 2008. Comparative analysis of the tear protein profile in mycotic keratitis patients. Molecular Vision 14:500-507.
5. M.S. Norn Tear secretion in normal eyes. Estimated by a new method: the lacrimal streak dilution test Acta Ophthalmol., 1965 (43) (1965), pp. 567-573
6. R. Avisar Tear secretion in patients with nonspecific eye complaints Israel J. Med. Sci., 14 (1978), pp. 339-341
7. K. Virkler, I.K. Lednev Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene Forensic Sci. Int., 188 (July (1-3)) (2009), pp. 1-17
8. G.A. De Souza, L.M. Godoy, M. Mann Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors Genome Biol., 7 (2006), p. R72
9. G. Strong, The Spectacles Of Murder, The Trials And Tribulations Of A Forensic Optometrist (2006)
10. W.H. Frey, D. Desota-Johnson, C. Hoffman, J.T. McCall Effect of stimulus on the chemical composition of human tear Am. J. Ophthalmol., 92 (4) (1981), pp. 559-567
11. R. Brunish The protein components of human tears Arch. Ophthalmol., 57 (1957), p. 554
12. S. Marshall, A. Rodrigues, H.J. Hird Tears: The Forgotten Evidence Type (2014)

13. R.A. Wickenheiser, R.M. Jobin Comparison of DNA recovered from a contact lens using PCR DNA typing J. Can. Soc. Forensic Sci., 32 (1999), pp. 67-73
14. Worakul N, Robinson JR. Ocular pharmacokinetics/pharmacodynamics. Eur J Pharm Biopharm 1997;44:1627:71.
15. Gaudana R, Ananthula HK, Parenky A, et al. Ocular drug delivery. AAPS J 2010;12:3:348-60
16. Kim YC, Chiang B, Wu X, et al. Ocular delivery of macromolecules. J Control Release 2014;28:190:172-81.
17. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:5:487-97.
18. Hornof M, Toropainen E, Urtti A. Cell culture models of the ocular barriers. Eur J Pharm Biopharm 2005;60:2:207-25.
19. Cuhna-Vaz JG. The blood-ocular barriers: Past, present, and future. Doc Ophthalmol 1997;93:1-2:149-57.
20. Hosseini K, Matsushima D, Johnson J, Widera, Nyam K, Kim L, Xu Y, Yao Y, Cormier M. Pharmacokinetic study of dexamethasone disodium phosphate using intravitreal, subconjunctival, and intravenous delivery routes in rabbits. J Ocul Pharmacol Ther 2008;24:3:301-8.
21. Comprehensive ophthalmology by A.K Khurana
22. Kanski's clinical ophthalmology: A systematic approach
23. https://www.reviewofophthalmology.com/article/the-hows-and-whys-of-pharmacokinetics
24. https://blog.domypapers.com/thesis-topics-ophthalmology/
25. https://jamanetwork.com/journals/jamaophthalmology/article-abstract/633652
26. http://ijpsr.com/bft-article/ocular-drug-delivery-system-an-overview/?view=fulltext
27. https://www.ncbi.nlm.nih.gov/pubmed/380030