

A Review on: Assessment of the Application of Qualitative and Quantitative Assays for the Hormone-Releasing Disorder Known as Hypogonadism in Male Gonads.

Mr. Komal Thakre^{*1}, Manaswi Lokhande², Mr. Anil Dhawade³

¹ Assistant Professor, Department of Pharmaceutical Science, Shri Sadaguru Datta Institute of Pharmacy, Kuhi, Nagpur

² Bachelor in Pharmacy, Shri Sadaguru Datta Institute of Pharmacy, Kuhi, Nagpur.

³ Associate Professor, Shri Sadaguru Datta Institute of Pharmacy, Kuhi, Nagpur.

Submitted: 15-15-2023

Accepted: 25-12-2023

ABSTRACT: Hypogonadism is a clinical disease caused by hypothalamic-testicular axis disorders that prevent the testis from producing testosterone and spermatozoa. Hypogonadism is an endocrine condition characterized by insufficient serum testosterone synthesis by the testicular Leydig cells. Aphrodisiacs are substances (food or chemicals) that stimulate sexual desire. Aphrodisiac drugs are used to improve the sexual functioning of humans. Methods for estimation of hypogonadism includes TLC, HPLC, UV, Molecular docking. Software mentioned for molecular docking are Uniprot, PubChem, PyRx, Autodock/ ML tool, PyMOL.

KEYWORDS: Hypogonadism, Aphrodisiac, Kallmann's syndrome, Analytical methods, Molecular docking.

I. INTRODUCTION

Hypogonadism is a clinical syndrome resulting from the testis's inability to produce testosterone and spermatozoa due to hypothalamic-testicular axis pathologies. The disruption of the HPG axis normalizes the functioning of Leydig cells in the testis, leading to a condition. The process of spermatogenesis is hindered by this, causing disturbances in normal reproductive physiology. Hypogonadism is organic when a disorder causes permanent dysfunction, and functional when reversible conditions temporarily suppress gonadotropin and/or testosterone production.^[1,2]

Male hypogonadism, whether acquired or congenital, can be caused by abnormalities in the hypothalamic-pituitary-testicular axis. It is critical to distinguish between primary hypogonadism (originating in the testicles) and secondary hypogonadism (originating in the hypothalamus or pituitary gland). Hypogonadism symptoms include diminished spontaneous reactions, decreased

nocturnal penile tumescence, decreased libido, and decreased testicular volume. This exercise examines the diagnosis and treatment of male hypogonadism, as well as which people are most likely to benefit from screening. This activity emphasizes the importance of the interprofessional team in enhancing care for male hypogonadism patients.^[3,4]

An aphrodisiac is a substance (food or chemical) that promotes sexual desire. Aphrodite is the Greek goddess of love, sexuality, and beauty. Aphrodisiac medicines are used to improve humans' poor sexual functions. These agents have been employed for a long time, and there is ample evidence of their use by ancient Greek and Arab physicians such as Hippocrates (460 B.C.), Dioscorides (70 A.D.), Raazi (926 A.D.), and Ibn-e-Sina (1038 A.D.) etc. Erectile dysfunction is described as the inability to achieve and maintain a sufficient penile erection for acceptable sexual performance.^[5,6]

Aphrodisiac refers to any chemical that increases sexual pleasure. Anxiety, depression, stress, fear of sex, neurological disorders, stroke, cerebral trauma, Alzheimer, Parkinson's disease, and chronic disorders-diabetes, hypertension, vascular insufficiency, Atherosclerosis, penile disease-phimosis, peyronies, lifestyle-chronic alcohol abuse, cigarette smoking, aging, decrease in hormone level with age are all causes of sexual dysfunction. Cardiovascular, hepatic, renal, pulmonary, and cancer disorders are examples of systemic diseases.^[7]

1.1. Causes of Male Hypogonadism.

• Primary Hypogonadism

- Anorchidism is a condition that occurs during birth.
- Cryptorchidism

- Orchitis due to the mumps
- Genetic and developmental issues:
- Sertoli cell only syndrome, Klinefelter syndrome, androgen receptor and enzyme deficiencies
- Chemotherapy and radiation therapy
- Testicular injury
- Anti-Leydig cell illnesses (autoimmune syndromes)

• **Secondary Hypogonadism**

- Kallmann's syndrome and Prader-Willi syndrome are two genetic diseases.
- Pituitary tumors, granulomas, and abscesses are all examples of pituitary disorders.
- Hyperprolactinemia
- Trauma to the skull
- Radiation therapy
- Several drugs

• **Mixed Hypogonadism**

(both primary and secondary)

- Alcoholism
- Ageing
- HIV infection is a chronic infection.
- Corticosteroid therapy
- Hemochromatosis
- Systemic illness (liver failure, uremia, sickle cell disease) *Mixed hypogonadism is frequently classified as secondary hypogonadism.^[5]

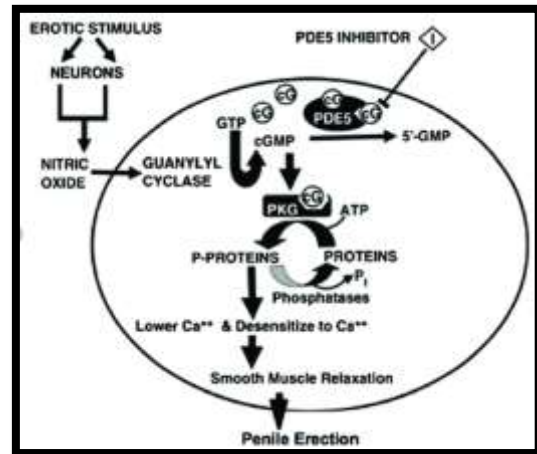


Fig1 :Pathophysiology Of Aphrodisiac^[2]

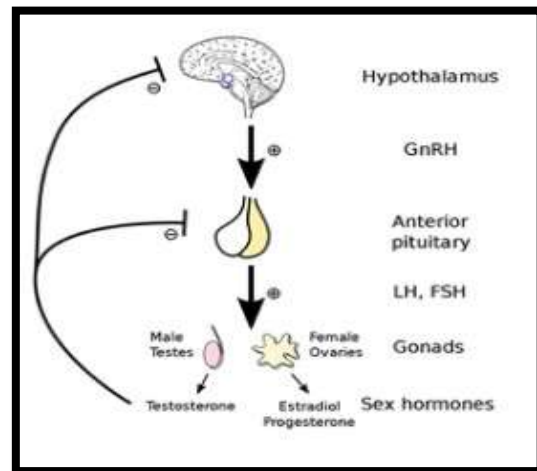


Fig2: Pathophysiology Of Hypogonadism^[2]

1.2 Pathophysiology

Aphrodisiacs are products that stimulate libido or sexual drive. They do this by stimulating the release of nitric oxide from the hypothalamus, which in turn dilates blood vessels of the corpus carvenosum and activates the enzyme guanylatecycase (GC), which converts guanosine triphosphate (GTP) into Cyclicguanosine monophosphate (cGMP). This increases the flux of bood into penile tissue and results in a penis erection. The erection lasts only briefly because cGMP is hydrolyzed into inactive GMP by the Phosphodiesterase type-5 enzyme (PDE-5), which is located in the penile tissues. Aphrodisiacs can inhibit PDE-5's hydrolyzing action, allowing active cGMP to accumulate 'Undisturbed' and prolong the erection through increased blood flow

1.3 Etiology

There are both acquired and congenital caussesof hypogonadism. In prepubescent males, ambiguous, genitalia, micropenis, and bilateral cryptorchidism are all indicators of a testosterone deficit.

Young adults undergo kyrotype testing to rule out diseases like Turner syndrome and Klinefelter syndrome, which can cause a testosterone shortage.^[3]

1.4 Epidemiology

Oftentimes, hypogonadism goes under-reported. Studies have shown that about 50% of men in their 80s and 40% of men over 45 are hypogonadal. It has been discovered that every ten years, testosterone levels drop by 100ng/Dl. The relationship between racial and ethnic groupings with hypogonadism doesn't seem to exist.^[3]

The etiology of hypogonadism in men has not received enough attention. Only Klinefelter

syndrome, which has a probable population prevalence of > 5:10,000 men (potentially as high as 10–25:10,000), is one of the known causes of endogenous androgen insufficiency. Although the prevalence of mild traumatic injury (5–10:10,000 men) may also be a prevalent cause of androgen shortage, large-scale, long-term investigations are needed to substantiate this perhaps excessively high prevalence estimate.

Rare (prevalence < 10,000 men) are the classic causes of male androgen deficiency: endogenous Cushing syndrome, hyperprolactinemia, pituitary macroadenoma, and iron overload syndrome.

The most prevalent iatrogenic causes of male androgen insufficiency are lymphoma and leukemia, radiation and chemotherapy for testicular cancer, androgen deprivation therapy for prostate cancer, as well as radiation therapy for primary brain tumors and head and neck cancers prevalence.^[3, 10]

2. Materials And Method

2.1. TLC

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. To do Thin-layer chromatography, a sheet of glass, plastic, or aluminum foil is coated thinly with an adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper). This adsorbent layer is the stationary phase. After the sample is applied to the plate, a solvent or combination of solvents (known as the mobile phase) is drawn up the plate by capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

2.1.1. Chemicals :

All reagents used were of analytical grade. Precoated Silica gel 60 F254 HPTLC plates, Standard β -sitosteroland Lupeol .

2.1.2. Instrumentation:

Chromatographic sample applicator fitted with a Hamilton Syringe and a Camag TLC Scanner IV.

The data gathering program Wincats 1.4.7 was utilized. Chromatographic sample applicator with a Hamilton Syringe and a Camag TLC Scanner IV. The data collection program Wincats 1.4.7 was used. CAMAG Linomat V was used for separation^[8].

1.3. Enhanced Condition Of The Chromatography:

- Stationary phase: Precoated aluminum sheets (8 × 8 cm) with silica gel 60 F254
- Phase of mobility: Lithuene: Acetate ethyl: Formic acid (V/V/V = 15: 3: 0.1)
- Saturation time of the chamber: 10 minutes
- Front of solvent: 70 mm
- Slit size: 0.4 x 0.3 mm
- Width of band: 6 mm
- Derivatization: 10 minutes at 1100C and anisaldehyde-sulphuric acid
- Wavelength of scanning: 520 nm.^[8]

2.1.4. Procedure Of TLC

TLC Plate Preparation

- Fill a 100 ml beaker with 10 to 15 ml of solvent, then set it aside on a level surface to remain undisturbed. Put some parafilm, petri dish, or aluminum foil over the beaker.
- Cut a piece of TLC plate in reverse phase so that it fits snugly inside the beaker.
- To avoid uneven solvent flows up the TLC plate, which could impact the results, make sure the plate bottom is absolutely horizontal.
- Using a pencil, draw a line parallel to the plate's width such that it is above the solvent surface.
- Mark the samples you wish to run below the line.
- Take a capillary tube out of the capillary bottle with tweezers.
- Submerge it in the intended solution, allowing capillary action to draw the solution into the tube.
- Place the capillary tube on the pencil line, then gently push it on the plate until a tiny liquid dot emerges.
- Proceed with the remaining fixes. The dots need to be far enough apart to avoid blending when the solvent is running.
- Prior to developing the TLC plates, let the spots to dry.
- Gently drop the plate into the beaker and release it just above the solvent surface. . Grasp the top of the plate with tweezers and hold it upright vertically.
- If everything is done correctly, the plate should drop in and the adsorbed solvent should form a straight horizontal solvent line.
- Hold off until the solvent line reaches just 1 centimeter above the plate's top.
- Carefully remove with tweezers, then pencil-mark the solvent line before it disappears.^[8,9]

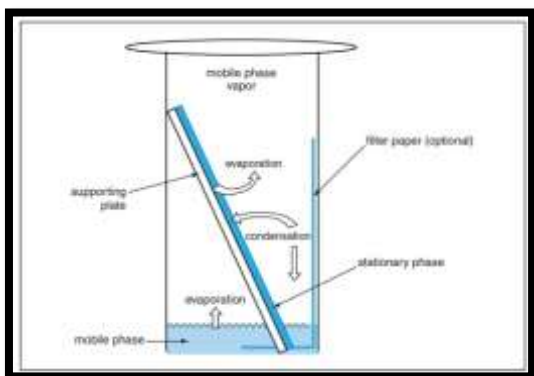


Fig3: Schematic representation of TLC ^[9]

2.2 HPLC

HPLC is the preferred technique for determining a new chemical entity's peak purity, assessing new formulations, monitoring reaction changes throughout synthetic processes or scale-ups, and performing quality control and assurance on the finished medicinal products. Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. ^[11, 12]

2.2.1. Principle of HPL

The stationary phase of an HPLC column is made of porous material, and the mobile phase, or liquid phase, is pumped through the column at a greater pressure once the sample solution has been injected into it. The adsorption of solute on stationary phase based on its affinity towards stationary phase is the separation principle that is adhered to.

The HPLC technology has the following characteristics.

- mobile phase
- Regulated mobile phase flow rate. ^[Excellent clarity]
- Glass column, stainless steel, small diameter
- Quick analysis
- Slightly elevated pressure ^[11]

2.2.2. Basic Operating Procedure of HPLC

- Examine each material's SDS.
- In a fume hood, prepare the analyte solution.
- Prior to operating the instrument, confirm that the solvent reservoirs are filled.
- Verify that the appropriate mobile phase is being used.

- Before using, thoroughly wash all lines and columns if you are altering the mobile phase.
- Before starting the study, make sure that any air bubbles have been removed from the pump lines and inspect the system for leaks.
- Switch on the HPLC and let the mobile phase solution reach equilibrium.
- Verify that the pressure is substantially below the HPLC system's maximum pressure and that it is acting normally.
- Never allow the solvent mobile phase bottles to run out of solvent; always check their levels.
- Press the button labeled "injection" (or the equivalent on your device). Put the background music into the device Inject your sample into the device after the background has steadied.
- To make sure the HPLC column is thoroughly cleaned, it should be rinsed for at least thirty minutes following each run. Depending on the instrument and the sample being examined, there are differences in the process for cleaning the column and injecting.
- *Shut down the HPLC.
- Make sure all waste is disposed of in the proper hazardous waste containers. ^[11,12]

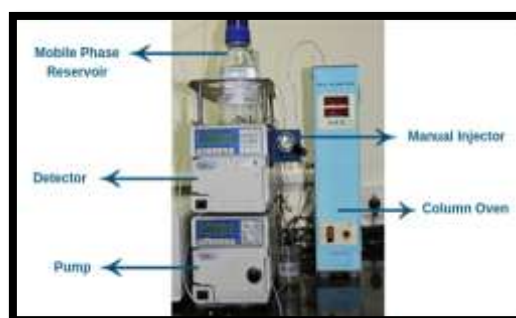


Fig4: HPLC instrument ^[17, 18]

2.3. UV Spectroscopy

Ultraviolet-visible spectroscopy, often known as UV-Vis or UV/Vis spectrophotometry, is the study of absorption or reflection spectroscopy in the ultra-visible spectrum. This indicates that it makes use of light in the visible and near-infrared (NIR) and near-UV spectrums. The apparent hue of the compounds is directly impacted by their absorption or reflectance in the visible spectrum. Molecules change electrically in this area of the electromagnetic spectrum. Since absorption monitors transitions from the ground state to the excited state, while fluorescence spectroscopy deals with transitions from the excited state to the ground

state, this approach is a complement to fluorescence spectroscopy.^[13]

Procedure

- You must ensure that your sample satisfies the required minimum sample size.
- Prior to accessing the program or the instrument, confirm that it is turned on and/or that a "Experiment in progress" card is present.
- In order to make sure there are no samples or the integrated sphere within the UV-Vis chamber, open the chamber if no other experiment is underway.
- After shutting the cover, activate the device.
- Using the desktop shortcut, launch the application.
- It will open several windows. All but the window and the window should be deleted. (Note: We lack the fixtures and samples required for the calibration option.
- Permit the instrument to finish initializing.
- The technician can now install it if they choose to use the inbuilt sphere.
- Configure the scan method. Select Transmission or Absorbance as specified.
- Decide which wavelengths to start and stop at.
- For bandwidth and data interval, use the default settings.
- All of the lamp types are xenon.
- Enter how many samples there are in a single run.
- Ensure that a file name seed is included.
- Adjust the display parameters as directed.
- Decide which background correction to use. (Normally, 100% T).
- Insert blanks into the measurement and reference beam lines.
- Start the background. A line with two arrows pointing downward is the toolbar icon.
- Take the blank out of the measurement beam once the background is complete. Keep the blank in its current location in the reference beam.
- Insert the sample into the beam of measurement.
- To conduct the measurement, close the chamber and select the running man icon.
- A CSV file with the data can be saved. Nonetheless, the analysis software is accessible both on the MUE 170 lab PCs and through download.
- Ensure that the results are saved to the workgroup computer's shared folder.

- Please don't forget to turn off the instrument and log out of the software after you're done.
- Never use external media on the instrument computer; instead, get your data from the workgroup computer.^[13,14]

2.4. Molecular Docking

Molecular docking is an important method in structural molecular biology and computer-assisted drug design. The goal of ligand—protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Docking may be used to perform virtual screening on vast libraries of compounds, rate the results, and provide structural theories about how the ligands inhibit the target, which is extremely useful in lead optimization. The docking input structures are just as crucial as the docking process, and interpreting the results of stochastic search methods can be difficult at times.^[15]

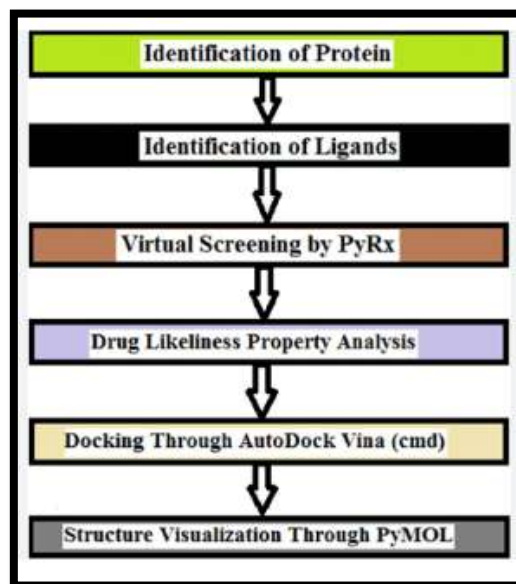


Fig:4Steps Involved In Molecular Docking^[16]

2.4.1. Identification of Protein by Uniprot

A database was used to find the Protein Islet amyloid polypeptide, which is a diabetes-causing protein. Uniprot provided the structure of the protein molecule Islet Amyloid polypeptide (IAPP). The protein structure was retrieved in ".pdb" format from Uniprot (RCSB PDB).

2.4.2. Identification of Compounds by PubChem

For the docking study, the natural chemicals Alpha-Amyrin acetate, Myrcene, and

Vasicine were employed. For the docking investigation, all of these chemicals were chosen from the PubChem online database. Compound 3D structures were retrieved in.sdf format from the PubChem web database. The downloaded structures of the provided compounds were then translated from.sdf to.pdb format using the 'online SMILES Translator and structure file generator' and downloaded in.pdb format. These.pdb file compounds were used to execute PyRx and Auto dock tools/software in the following process.

2.4.3. Virtual Screening by using PyRx

PyRx is the important software used in molecular docking for virtual screening of the compounds. Software PyRx was used to screen the compounds and its binding affinity with the given protein target. Compounds were selected based on their minimum binding affinity for further process of drug likeness property analysis. In PyRx procedure the protein molecule was loaded first and it was converted from .pdb format to pdbqt format. In next stage natural compounds were imported in .sdf format and converted from .sdf format to .pdbqtformat, then docking virtual screening process was run through Vina wizard and result were analyzed according to its binding affinity between protein molecule and natural compounds.

2.4.4. Drug Likelihood Property Analysis

For drug likelihood property analysis, the online web server Swiss ADME was employed. This screening chemical was assessed for its drug property using the Lipinski rule of five. Screened compounds were copied from PubChem using their CID number followed by the Canonical SMILES that were provided. The copied Canonical SMILES were pasted into the online web server Swiss ADME dialog box and the process was launched. It exhibits all of the drug-like qualities. Lipinski's rule of five was used to screen the compounds. The Lipinski rule of five indicates that: -

- 1) Molecular mass less than 500 Dalton
- 2) Donors of less than 5-Hydrogen bonds
- 3) A maximum of ten hydrogen bond acceptors
- 4) A maximum of 5 -Partition co-efficient LogP
- 5) There can be no more than one rule.

2.4.5. Docking ThroughAutoDock Vina (cmd)/MLT tool

The protein target was loaded into Auto Dock Vina's graphical panels in.pdb format. By clicking on 'edit,' the protein target was prepared by eliminating its water molecules, adding hydrogen

polar atoms, and adding Kollman charges. The protein molecule was then transformed into.pdbqt format, and the protein was eventually saved in.pdbqt format by going on grid. Compound was imported, transformed, and stored in.pdbqt format. The compound and protein in.pdbqt format were then reloaded on graphical windows, and a grid box was selected and saved as by grid notepad, followed by a config txt on notpad for the last command, Using the command prompts shown below, the findings were processed and the output file was automatically stored in.pdbqt format.

2.4.6. Structure Visualization ThroughPyMOL

PyMOL tool was used for structure visualization which was a freely available tool. The protein in .pdbqt format and output in .pdbqt format were loaded on PyMOL graphical screen. The protein and compound binding structure was visualized and analyzed.^[16]

II. CONCLUSION

Defects in the gonadal response to the manufacture of sex hormones, or gonadotropins, are known as hypogonadism. It can be difficult to diagnose and evaluate people clinically, especially before puberty. It is easier to diagnose hypogonadism when it is detected using analytical methods. After the above review, we can conclude that the techniques covered in this article—TLC, HPLC, UV spectroscopy, and molecular docking—are more exact and accurate.

REFERENCES

- [1]. YleniaDuca , Antonio Aversa , Rosita Angela Condorelli 1, Aldo Eugenio Calogero 1 andSandro La Vignera 1ce, Substance Abuse and Male Hypogonadism, JCMReceived: 1 May 2019; Accepted: 18 May 2019; Published: 22 May 2019, Page no.1-26, doi:10.3390/jcm8050732.
- [2]. ShubhadeepRoychoudhury, SaptaparnaChakraborty, Arun Paul Choudhury, Anandan Das, Niraj Kumar Jha, PetrSlama, Monika Nath, Peter Massanyi, JanneRuokolainen and Kavindra Kumar KesarEnvironmental Factors-Induced Oxidative Stress: Hormonal and Molecular Pathway Disruptions in Hypogonadism and Erectile Dysfunction,MDPI, Received: 2 April 2021 Accepted: 20 May 2021 Published: 24 May 2021 ,Page no.1-39,

- Antioxidants 2021, 10, 837. doi.org/10.3390/antiox10060837.
- [3]. OmeedSizar, Janice Schwartz, Hypogonadism-StatPearls-NCBI Bookshelf,2023Jan, last update: June27,2022 , Page no. 1-5.
- [4]. Christina Carnegie,MB,BS,FFPM, Diagnosis Of Hypogonadism : Cinical Assessments And Abratry Tests , MEDREVIEWS, 2004, Page no. 1-8.
- [5]. P. Dandona,¹ M. T. Rosenberg², A practical guide to male hypogonadism in the primary care setting,THE INTERNATIONAL JOURNAL OF CINICAL PRACTICE, ^a 2010 Blackwell Publishing Ltd Int J ClinPract, May 2010, 64, 6, 682–696, Page No.682-696 , 682 doi: 10.1111/j.1742-1241.2010.02355.
- [6]. Tajuddin, Shamshad Ahmad, Abdul Latif and Iqbal Ahmad Qasmi Aphrodisiac activity of 50% ethanolic extracts of MyristicafragransHoutt. (nutmeg) and Syzygiumaromaticum (L) Merr. & Perry. (clove) in male mice: a comparative study, BioMed Central, Published: 20 October 2003, page no.
- [7]. Ramandeep Singh , Ashraf Ali , Gaurav Gupta , AlokSemwal , G. Jeyabalan¹, Some medicinal plants with aphrodisiac potential: A current status, ESEVIER, Received 28 May 2013 Received in revised form 3 June 2013 Accepted 18 June 2013 Available online 20 September 2013, Page no. , doi: 10.1016/S2221-6189(13)60124-9.
- [8]. Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, At-Changa, Dist-Anand, 388421, Gujarat, India, Quantification of Two Marker Compounds from unsaponifiable matter of Hygrophilaspinoso Seeds Using Validated TLC-Densitometric Method NIRAJ VYAS*#, MANAN RAVAL*,ReserchGate, | Oct – Dec 2015 | Vol 7 | Issue 4|, Page no.39-45 .
- [9]. Li Cai , Thin Layer Chromatography ,ReserchGate, 18 October 2018, page no.1-19 , DOI: 10.1002/9780470089941.et0603s08.
- [10]. ArchanaBele, AnubhaKhale , An overview on thin layer chromatography,ReserchGate, Received on 28 July, 2010; received in revised form 16 November, 2010; accepted 18 January, 2010, Page no. 256-267.
- [11]. Bradley D. Anawalt, MD, ArthiThirumalai, MBBS, Epidemiology of Male Hypogonadism, HHS Public Access, 2022 March ; Page no. 1-39, doi:10.1016/j.ecl.2021.11.016.
- [12]. Santosh Kumar Bhardwaja,b , K. Dwivedia and D. D. Agarwala, A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry, Received 04 November 2015; accepted 20 November 2015,Page no.76-81.
- [13]. PriyaSadapha, KavitaDhamak, Review Article on High-Performance Liquid Chromatography (HPLC) Method Development and Validation, International Journal of Pharmaceutical Sciences Review and Research, Published on: 15-06-2022, Page no.: 23-29, DOI: 10.47583/ijpsrr.2022.v74i02.003.
- [14]. GahanduleMangal and Banerjee S. K, Development of UV spectrophotometric methods and validation for estimation of furosemide in bulk and tablet dosage form by absorbance maxima and Area Under the Curve method, International Journal of Advances in Pharmaceutic, Volume 5 Issue 6 [2016],Page no.160-170, DOI: 10.7439/ijap.
- [15]. Diya Patel¹ , Diya Panchal¹ , Kunj Patel¹ , Prof. Mitali Dalwadi² , Dr.Umesh Upadhyay³, “A Review on UV Visible Spectroscopy, International Journal of Creative Research Thoughts, 10 October 2022 , Volume 10, Page no.1-13.
- [16]. Morris, G.M., Lim-Wilby, M. (2008). Molecular Docking. In: Kukol, A. (eds) Molecular Modeling of Proteins. Methods Molecular Biology, vol 443. Humana Press.doi.org/10.1007/978-1-59745-177-2_19.
- [17]. Tushar Kumar, NoopurKhare, NehaShukla , Abhimanyu Kumar Jha, Molecular Docking Studies of Possible Treatment of Diabetes using Vasicine against Islet Amyloid Polypeptide, ReserchGate, June 2021, vol.9, Page no.1-11.
- [18]. Mahima Sharma, DharmendraArya, KiranBhagour, R.S. Gupta, Natural aphrodisiac and fertility enhancement measures in males: A revie, ELSEVIER,

- 15 February 2017, Page no, doi.org/10.1016/j.cmrp.2017.02.007.
- [19]. Q. Xia, J.M. Jiang, X. Gong, et al., Experimental study of tongxia purgative method in ameliorating lung injury in acute necrotizing pancreatitis, *World J. Gastroenterol.* 6 (2000) 115–118.
- [20]. B. Zhang, W.X. Li, et al., The Influences of pre and post processed pieplant and compatibility of pieplant and glauber's salt on gastrointestinal motility of rat, *Guid. J. Tradit. Chin. Med. Pharm.* 14 (10) (2008) 72–74.
- [21]. Ang, HH, Chan, KL, Gan EK, Yuen KH. Enhancement of sexual motivation in sexually naive male mice by *Eurycomalongifolia*. *Int J Pharmacol* 1997; 35: 144-146.
- [22]. Ramandeep Singh, Sarabjeet Singh, G. Jeyabalan, Ashraf Ali. An overview on traditional medicinal plants as aphrodisiac agent. *J Pharm Phytochem* 2012; 1(4): 43-56.
- [23]. Ramandeep Singh, Ashraf Ali, G. Jeyabalan, AlokSemwal. Current status of Indian medicinal plants with aphrodisiac potential. *J Acute Dis* 2013; 2(1): 13-21.
- [24]. Singhal S., Singhal N., Agarwal S., *Pharmaceutical Analysis II, Thin layer chromatography*, PragatiPrakashan, First edition, 2009, 98-111.
- [25]. Kasture A.V., Mahadik K.R, Wadodkar S.G, More H.N., *A textbook of pharmaceutical analysis, Instrumental methods*, NiraliPrakashan, 9th edition, 2005, vol II, 18-30.
- [26]. Quach, H. T, Steeper, R L, Griffin, G. W, Separation of plant pigments by thin layer chromatography. *Journal of chemical education*.2004, 81, 385-7.
- [27]. Barratt CLR, Björndahl L, De Jonge CJ, et al. The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance-challenges and future research opportunities. *Hum Reprod Update*. 2017;23:660–680. [PubMed: 28981651].
- [28]. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an Endocrine Society clinical practice guideline. *J ClinEndocrinolMetab.* 2018;103:1715–1744. [PubMed: 29562364].
- [29]. Mulhall JP, Trost LW, Brannigan RE, et al. Evaluation and management of testosterone deficiency: AUA guideline. *J Urol.* 2018;200:423–432. [PubMed: 29601923].
- [30]. P.K. Singh, M. Pande, L.K. Singh, R.B. Tripathi, steps to be considered during method development and validation for analysis of residual solvents by gas chromatography, *Int. Res J Pharm. App Sci.*, 3(5) (2013) 74-80.
- [31]. B. Prathap, G.H.S. Rao, G. Devdass, A. Dey, N. Harikrishnan, Review on Stability Indicating HPLC Method Development, *International Journal of Innovative Pharmaceutical Research*.3(3) (2012) 229- 237.
- [32]. Jungwirth, A.; Giwercman, A.; Tournaye, H.; Diemer, T.; Kopa, Z.; Dohle, G.; Krausz, C. European Association of Urology guidelines on male infertility: The 2012 update. *Eur. Urol.* 2012, 62, 324–332. [CrossRef] [PubMed]
- [33]. Tüttelmann, F.; Nieschlag, E. Classification of andrological disorders. In *Andrology, Male Reproductive Health and Dysfunction*, 3rd ed.; Nieschlag, E., Behre, H.M., Nieschlag, S., Eds.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 87–92.
- [34]. Salonia, A.; Rastrelli, G.; Hackett, G.; Seminara, S.B.; Huhtaniemi, I.T.; Rey, R.A.; Hellstorm, W.J.G.; Palmert, M.R.; Corona, G.; Dohle, G.R.; et al. Paediatric and adult-onset hypogonadism. *Nat. Rev. Dis. Primers* 2019, 5, 38. [CrossRef] [PubMed]
- [35]. Sizar, O.; Schwartz, J. *Hypogonadism; Stat Pearls Publishing: Treasure Island, FL, USA, 2019.*
- [36]. T. Bhagyasree, N. Injeti, A. Azhakesan, U.M.V. Rao, A review on analytical method development and validation, *International Journal of Pharmaceutical Research & Analysis*, Vol 4 (8) (2014) 444-448.
- [37]. A. Shrivastava, V.B. Gupta, HPLC: Isocratic or Gradient Elution and Assessment of Linearity in Analytical Methods, *J AdvScient Res*, 3(2) (212) 12-20
- [38]. Lefievre L, De Lamirande E, Gagnon C. The cyclic GMP-specific phosphodiesterase inhibitor, sildenafil,



- stimulates human sperm motility and capacitation but not acrosome reaction. *J Androl* 2000;21:929-37.
- [39]. Tomova M, Gjulemetova R, Zarkova S, Peeva S, Pangarova T, Simova M. Steroidal saponins from *Tribulusterrestris* L. with a stimulating action on the sexual functions. Varna, Bulgaria: International Conference of Chemistry and Biotechnology of Biologically Active Natural Products; 1981.
- [40]. Burger M, Sikka SC, Bivalacqua TJ, Lamb DJ, Hellstrom WJ. The effect of sildenafil on human sperm motion and function from normal and infertile men. *Int J Impot Res* 2000;12:229-34.
- [41]. Matsumoto AM. Effects of chronic testosterone administration in normal men: Safety and efficacy of high dosage testosterone and parallel dosedependent suppression of luteinizing hormone, follicle-stimulating hormone, and sperm production. *J ClinEndocrinolMetab* 1990;70:282-7.