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Review Article

Different Analytical Methodology for The Analysis of Methotrexate

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ABSTRACT

There has been an increase in the number of diseases observed in this situation before this drug hits the market, it must undergo approval and testing methods are crucial to ensure its safe and reliability. This process includes using various analytical techniques to collect information about the drug. An increase in infections has been observed in this current situation, but certain procedures must be followed before this drug is released to the market. The endorsement and explanatory tactics are key methods that help ensure its quality and reliability. This process includes using various investigative methods to collect information about the drug. This study uses various analytical techniques like UV-visible Spectrophotometric and chromatography methods such as high-performance liquid chromatography, hyphenation techniques such as LC-MS for measuring specific anti-cancer medications. Different methods like UV-spectroscopy, HPLC MS are used to analyze methotrexate for testing chemotherapeutic drugs. This review focuses on the examination of various analytical methods.

INTRODUCTION

An uncontrolled cell growth disorder is abnormal cell growth that interferes with cell growth, resulting in a solid cell mass known as growth factor or liquid disease (for- example, bone marrow, blood-related diseases)[1]. Disease affects people. Cancer can affect people of all ages, even in the womb, causing weakness and disfigurement throughout the body. Radiation therapy and chemotherapy are the main clinical treatments used to control early stages of tumor cells. Nature has many useful resources, mainly plants, for the discovery and development of medicines against serious diseases. The medicinal plant is an effective treatment for tumor cells. Medicines derived from medicinal plants have less toxicity and side effects. Cancer can be prevented by the use of a number of chemical agents whose toxicity prevents their use . [2]. methotrexate MTX 4aminon10-methylpteroylglutamic corrosive an antifolate drug created as the first designated anti - cancer

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specialist in 1940[3] it is directed at a high portion to treat aa select tumor types, such as severe human leukemia Bosom sickness Head and neck cancers, osteogenic sarcoma, prostate, and bladder disorders[[4,5] it is likewise directed at a low portion as a solution for various immune system and provocative infections like rheumatoid joint pain psoriasis sarcoidosis and lupus erythematosus systemic.[6] MTX utilized in tall measurements in cancer treatment can moreover cause exceptionally genuine, life-threatening side impacts exceedingly subordinate on the treatment length, age and condition of the persistent. made by a few companies[7], hence quality control is basic withi High mobile phase flow rates through big porous particles (30–60 mM) are the foundation of the TFC. Larger molecules and matrix components are kept out of the stationary phase's pores by the small molecules, which results in wasteAfter that, the trapped molecules are back-flushed with an organic solvent to remove them from the TFC column and add them to the analytical LCeMS/MS Devices. Protein precipitation was accomplished using methanol, which contains formic acid. Cyclone-P (50 mm 0.5 mm) and Hypersil Gold C8 (2.1 mm 50 mm, 5 mm) columns were used for HPLC separation. The mobile phases A and B were utilized to be water and methanol, with each containing 10 mM ammonium formate, at a flow rate of 0.7 mL/min. Acetone with a ratio of 2-propanol to acetonitrile.n the pharmaceutical industry to guarantee a high-quality, secure and viable item. Methotrexate is in a course of drugs called antimetabolites. Methotrexate treats cancer by reduce the development of cancer cells. Methotrexate treats psoriasis by reduce the development of skin cells to halt scales from create. Methotrexate may treat rheumatoid joint pain by diminishing the movement of the resistant framework. Methotrexate (MTX) has well established uses in the treatment of psoriasis, rheumatological disorders, different cancers, and medically assisted pregnancy termination. Nausea, fatigue, fever, elevated risk of infection, decreased white blood cell counts, and deterioration of the oral mucosa are typical adverse effects. Liver illness, lung disease, lymphoma, and excruciating skin rashes are possible additional side effects Long-term patients should have their side effects monitored on a regular basis . Using it while nursing is unsafe. Lower dosages can be required for people with kidney issues. It works by preventing the body from utilizing folic acid. The purpose of this study was to present an overview of the analytical techniques used to identify MTX and its metabolites in pharmaceutical, living and eco -friendly samples In two single-dose (10 mg and 2.5 mg) bioequivalence studies of the test 2 mg/ml methotrexate oral solution using licenced tablets, 24 male subjects in good health were asked to rate the taste of the oral solution as either bitter, sour, salty, sweet, or not apparent. They were also allowed to make additional comments. Long-term ICH stability experiments at ambient circumstances (25°C/60%RH) and in-use shelf life studies were carried out concurrently with clinical investigations. Medical supervision using methotrexate When discussing ectopic pregnancy, the phrase "medical management" refers to the usage of a medication known as methotrexate. Methotrexate is a potent medication that functions by momentarily disrupting the body's ability to digest folate, an important nutrient required to support rapidly dividing cells during pregnancy. The medication prevents the pregnancy from progressing further, and the Fallopian tube remains intact as the medicine is progressively reabsorbed by the body. Treatment with methotrexate works best in the early stages of pregnancy.

CHEMICAL STRUCTURE :

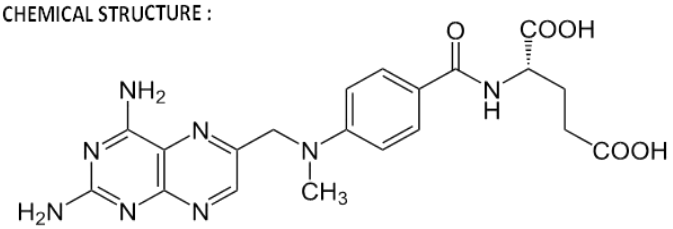

FIG1:CHEMICAL STRUCTURE OF METHOTRIXATE

CHEMICAL PROPERTIES

Molecular formula	C20H22N805
Molecular weight	454.44 g/mol
Molecular mass	454.46
Freezing point	365 to 399 (Decay)
Water Dispersible	Less than 1 mg/ml at.66°
pKa -value	4.8 and 5.5
log p	1.85

MTX structure is exceptionally comparable to that of folic acid (FA) .MTX comprises of a petridine -diamine center and a p-amino benzyl parcel, connected to a glutamic acid portion containing two profoundly ionizable carboxylic acid bunches .its solvency is PH -dependent .as impartial or fundamental arrangement are required for its dissolvability . The presence of an asymmetric carbon in the structure creates S and R stereoisomers. S- MTX is the active form, with R isomer added as an important.

Pharmacological Action :-

Reversibly inhibiting the dihydrofolate reductase enzyme, MTX causes a reduction in decreased folate levels, which is important for the manufacture of purines, thymidylate, and methionine. Consequently, in malignant cells, the reduction of nucleotides inhibits DNA synthesis. Furthermore, dihydrofolatemetabolites and MTX's polyglutamate forms impede the activity of folate-dependent enzymes, such as thymidylate synthase[10,11] .Rephrase Depending on the type of tumor, the patient's age, body size, and the protocol followed, MTX is administered via a variety of routes, including oral, intravenous, subcutaneous, intrathecal, and intramuscular ones, with wide variations in dose (from 7.5 mg to 12 g/m2)[12] .At dosages of up to 30 mg/m2,[13]the oral absorption is almost total. Because of the decreased absorption in dosages, the medication is supplied intravenously in high-dose regimens. Red blood cells (RBCs) have the ability to absorb MTX and subsequently undergo poly glutamation Follyl polyglutamate synthase in the cells converts MTX into MTXPGs .Monitoring intracellular MTXPGs is crucial for evaluating the pharmacokinetic and pharmacodynamic profile of MTX in the treatment of RA, psoriasis, ankylosing spondylitis, and cancer since the amount of these compounds is linked to the effectiveness and toxicity of MTX . The kidneys are primarily responsible for the elimination of MTX, and the clearance varies greatly according on factors such as age, race, gender, regimen, and renal function diffusions. The hepatic, gastrointestinal, mucosal, and hematologic side effects of MTX therapy are the most significant ones. Furthermore, excessive dosages of MTX may cause kidney damage as a result of the parent drug and its metabolites crystallizing in the nephrons, and systemic toxicity results from the delay in renal clearance .Dihydrofolate reductase inhibition The main way that methotrexate works is by blocking the enzyme dihydrofolate reductase (DHFR), which stops dihydrofolate

(DHF) from being converted to tetrahydrofolate (THF). De novo purine synthesis requires THF. Stopping the production of DNA When administered intradermally, methotrexate can stop DNA production in both psoriatic and normal skin for 12 to 16 hours. Cutting down on inflammation Inflammation can be decreased by methotrexate by: promoting the release of adenosine from fibroblasts Neutrophil adhesion reduction preventing neutrophils from producing leukotriene B4 preventing the synthesis of IL1 locally lowering IL-6 and IL-8 levels inhibiting immunity mediated by cells Taking care of ectopic pregnancy If there has been no rupture of the fallopian tube, methotrexate is used to treat ectopic pregnancies. The management of molar pregnancies Molar pregnancies are treated with methotrexate along with dilatation and curettage.

Sample Treatment :

The online technique offers high sensitivity, minimal contamination, automation, and comprehensive analysis . A pre-column housed in a six-port high-pressure switching valve is applied in the on-line SPE-LC. Prior to the injection and elution of analytes in the analytical column using valve switches, the sample is pre-concentrated on a pre-column . Molecularly imprinted polymers (MIPs) are artificial recognition materials with highly specific recognition properties. Liu et al. used MIPs in conjunction with SPE as a selective separation method to determine the presence of MTX in human serum using HPLC. They used trimethoprim as a pseudo template for the preconcentration, purification, and measurement of MTX in clinical samples in order to boost the procedure's sensitivity. imprinting that is covalent, noncovalent, and hybridization of the two techniques are the three imprinting techniques for applying MIPs in SPE to create a complex template-functional monomer . MIP allows for the application of both online and offline SPE; however, the off-line form is typically utilized for the extraction of target compounds from biological materials. MIPs can provide high selectivity, stability, and an easy-to-make, low-cost preparation . Ion exchange SPE is a useful tool for extracting charged chemicals from solutions. Anionic and cationic materials have been separated using liquid chromatography-strong anion exchanger (LC-SAX), liquid chromatography-strong cation exchanger (LC-SCX), or liquid chromatography-weak cation exchanger (LC-WCX) bonded silica cartridges, respectively. Strong cations are extracted using the LC-SCX material, which is made of silica with aliphatic sulfonic acid groups bound to the surface and employed without regard to recovery or elution. A weak cationic species that contains an aliphatic carboxylic acid group bound to the silica surface is recovered using LC-WCX . For MTX isolation, the strong cation exchange (Bondesil SPE) SPE technique was used . The aforementioned separation was performed using methanol as the eluent with formic acid and 5% ammonia. Ion exchange SPE is based on the electrostatic interaction between the positively charged groups of the molecule attached to the silica surface and the charged carboxylate functional groups of MTX. LLE works by combining an aqueous solution with an immiscible solvent .Target analytes were extracted following phase separation by carefully separating the analytes and contaminants between the phases. Large amounts of extremely pure organic solvents are required, evaporation is used, and solvent removal is one of the disadvantages of LLE methods Microwave aided extraction (MAE) has also been employed as an MTX preparation method for some environmental matrices The extraction process was finished by heating the mixture to 110 C for 10 minutes and then maintaining it there for an additional 30 minutes using 25 mL of

MeOHeH2O (pH ¼ 2) at a 1:1 ratio. The extraction solvent and target are heated via microwave radiation, which is the foundation of MAE. MAE achieves rapid preparation, little solvent extraction.

Different Analytical Methodology

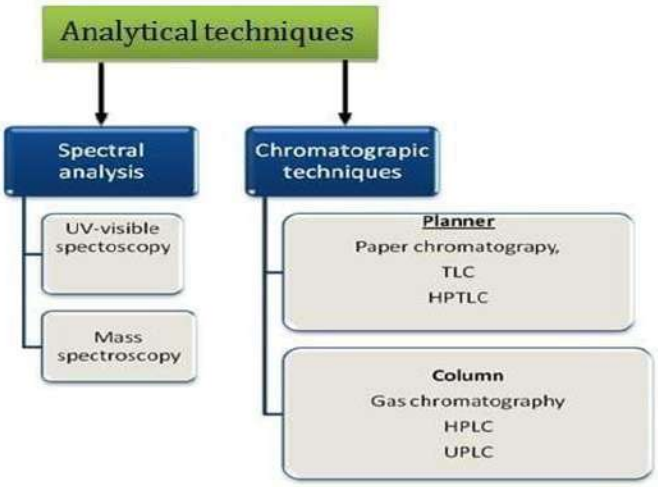

Fig 2: Analytical Method

A) HPLC:

The most often used methods for analyzing MTX and its metabolites are mass spectrometer detection, fluorescence, and UV -visible detection. Fluorimetry, evaporative light scattering detectors (ELSD), and electrochemical detection (ECD) may also be used with LC. In 14 out of 24 evaluations, the most commonly reported wavelength for HPLCUV-visible detection is between 302 and 400 nm, while 5 out of 24 quantitation were carried out at a wavelength of 300 nm. One measurement was made using an electrochemical detector while three investigations were conducted using HPLC-fluorescent detection . Chromatographic techniques have employed a wide range of stationary and mobile phases; nonetheless, C18 columns were utilized for the majority of HPLC experiments. Certain researchers used LC techniques with either pre-column or post-column derivatization . Reagents are added to the samples before to HPLC injection in precolumn derivatization, Reagents are introduced to the elution between the column and detector in a post-column technique As a quick and automated process, post-column derivatization happens without adding chemicals to the column. As a result, unreacted peaks and reagent are absent from the chromatograms. In contrast, post-column derivatization results in the creation of the product prior to detection; nevertheless, in pre-column derivatization, sample stability throughout storage and analysis is crucial. The hydrodynamic radius is used in SEC to separate biomolecules. This method uses an aqueous buffer as the mobile phase, allowing molecules to be depending on the biomolecules' molecular size and ability to diffuse in the stationary phase's spherical porous particles ince MTX polyglutamates (MTXPGs) aggregate in erythrocyte cells, an HPLC fitted with fluorescence detection can measure all MTXPGs in erythrocyte cells at a cheap cost and in a biocompatible manner before post-column derivatization. There was a 25e400 nmol/L linearity range SPE was used by Michaila et al. to distinguish between MTX and indomethacin (IND) in human urine. straightforward and accurate HPLC approach for figuring out MTX, 7-OH-MTX, and DAMPA in human plasma was presented by Uchiyama et al. Proteins were extracted from plasma using an isocratic elution

method on a Cap cell Pak C18 UG120 (150 mm ~ 4.6 mm, 5 ìm) column with phosphate buffer (50 mmol/mL at pH 5.3) and acetonitrile (ACN) (9:1, v/v) as the mobile phase at a flow rate of 0.5 mL/min. A combination of 5% trichloroacetic acid and 5% aqueous ACN solution was employed to extract proteins from plasma. By subjecting a PTFE knitted capillary coil (3 m ~ 0.5 mm) positioned between the column and the detector to UV light (245 nm), fluorescent photolytic degradation products were generated. At 368 and 425 nm, respectively, were the excitation and emission wavelengths. MTX, caffeine, diclofenac, glimepiride, and ibuprofen were found in Jordanian wastewater using an HPLC/UV/fluorescence technique. An RP-C8 (250 mm ~ 4.6 mm, 5 mm) column was used for chromatographic separation, and the mobile phase consisted of ACN and H2O containing 0.1% trifluoroacetic acid at a 1:1 ratio and a flow rate of 1 mL/min. To identify caffeine, nifidipen (internal standard), diclofenac, glimepiride, and ibuprofen, UV detection was performed at 225 nm. The fluorescence method was utilized for MTX detection. at lem ¼ 463 nm and lex ¼ 367 nm. 0.9 and 3.0 mg/L for MTX, 0.6 and 1.9 mg/L for caffeine, 1.7 and 5.8 mg/L for diclofenac, 1.4 and 4.5 mg/L for glimepiride, and 2.6 and 3.8 mg/L for ibuprofen were determined to be the limit of detections (LODs) and limit of quantifications (LOQs) . An HPLC/UV technique with a 6-minute elution time was described by Hu et al. for the simultaneous measurement of warfarin, guanabenz, MTX, desipramine, carbamazepine, and propranolol. In the macromolecular conjugates, Ciekot et al. used an HPLC system with a UV visible detector for the measurement of total MTX and an SEC for the detection of free MTX . At a wavelength of 372 nm, the total MTX was quantified, with a linear range of 1.204e40.13 mM, a LOD of 0.3150, and a LOQ of 1.050 mM. For SEC, the following values were obtained: 2.006e200.6 mM, 0.2761, and 0.9203 mM for the linear range, LOD, and LOQ, respectively. In a different investigation, MTX and FA were simultaneously measured using ion chromatography fitted with an electrochemical detector in the urine and human plasma of RA patients. Quaternary amine functionalized multi-wall carbon nanotubes (q-MWNTs) were added to the glassy carbon electrode (GCE) in order to increase the sensitivity of the detection. There are numerous uses for HPLC in both clinical and laboratory science. Because it is a reliable method of obtaining and guaranteeing product purity, it is frequently employed in pharmaceutical development. Even while HPLC is capable of producing incredibly pure and high-quality products, it is not always the main technique employed in the manufacturing of bulk medicinal materials. The European Pharmacopoeia states that just 15.5% of syntheses employ HPLC. Nevertheless, according to US pharmacopoeia, it is involved in 44% of syntheses. Given that large-scale HPLC can be an expensive technology, this could potentially be the result of disparities in time and financial restrictions. Unfortunately, HPLC's increased specificity, accuracy, and precision come at the expense of increased cost.

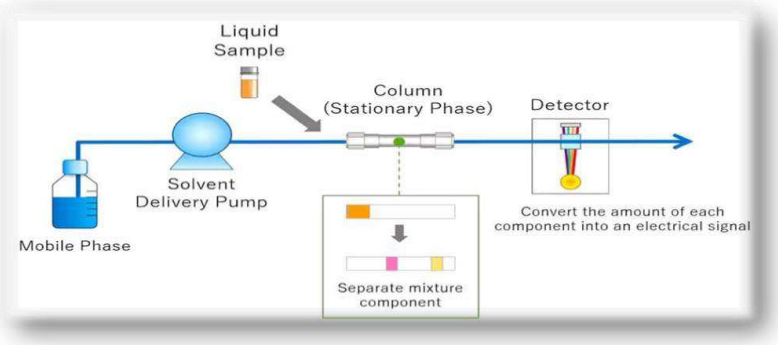

FIG 3: High Performance liquid Chromatography

LOQ values were found to be MTX, 7-OH-MTX, and DAMPA had respective concentrations of 3, 0.38, and 3.4 ng/mL. Begas et al. reported utilising HPLC to monitor MTX in patients with osteosarcoma. Phenyl cartridges were used to increase the method's selectivity for SPE of MTX in human urine serum sample. In a different study, two different types of nanogels containing MTX were created using the ionic gelation process in order to look at the neuro-pharmacokinetic effects of the drug. This study used HPLC to measure the concentrations of MTX in liver, brain, and plasma homogenates . Because of the ionic interaction that results in the production of ionic gelation, two aqueous solutions including a polyanion sodium tripolyphosphate and a chitosan polymer were utilized in the ionic gelation method to transfer the substances from liquid to gel medium. statistical calculations of MTX loaded on biodegradable microparticles using HPLC and UV spectrophotometric techniques revealed that both approaches produce findings that are comparable and can be used to monitor the quality of MTX in drug delivery systems . Raichur and Devi used HPLC-UV to determine the amount of MTX in rat plasma. 4 and 5 ng/mL, respectively, were the LOD and . The process was also expanded to determine how much MTX should be included in tablet formulation. Roy and associates conducted comparable measurements using tablet and bulk dose forms. The concentration range of 8–60 mg/mL showed linearity, and the retention time was 3.28 min . The suggested technique for figuring out MTX in polymeric nanoparticles worked effectively.

B) Uv-Spectroscopy :

Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) that is released and absorbed when a sample's molecules, atoms, or ions transition between different energy levels .UV-VIS Spectroscopy: Using light in the visible, ultraviolet, and nearinfrared regions, ultraviolet (UV) spectroscopy is a physical method of optical spectroscopy. According to the Beer-Lambert equation, a solution's absorbance is directly correlated with the route length and the concentration of the absorbing species in the solution. Thus, the concentration of the absorber in a solution can be found using UV/VIS spectroscopy for a particular route length. The rate at which the absorbance varies with concentration must be understood Spectroscopy is essentially concerned with the relationship between light and matter The excitation of electrons from their ground state into a higher energy state happens when UV radiation is absorbed. In order to excite their electrons to higher anti-bonding molecular orbitals, molecules containing π-electrons or non-bonding electrons . Transitions can be classified into four types: π–

π*, n–π*, σ–σ*, and n–σ*. They can be arranged in the following order: A > n-σ* > σ–π* > n–π. The wavelength range of 800– 200 nm corresponds to the 1.5–6.2 EV UV–visible area of energy in the electromagnetic spectrum. The fundamental idea underlying absorbance spectroscopy is the Beer-Lambert Law. In this case, absorptivity (a), concentration (c), route length (b), and absorbance (A). A = a bc C is equal to A/a b. To gather UV-visible spectra, three different kinds of absorbance equipment are used: Allin-one spectrometer. spectrometer with two beams. Concurrent spectroscopy. A straightforward, quick, economical, and exractive UV spectrophotometric technique was created to measure the amount of gemcitabine hydrochloride (GMCT) in pharmaceutical and bulk drug formulations. The drug's reaction with gold nanoparticles (AuNP) to generate a dark blue solution with an absorption maximum at 688 nm modifies the original color of AuNP, according to UV spectrophotometric studies that served as the basis for the Uv spectrophotometric is most important parameters is adsorption of compound . it obeys the beers lamberts law. Uv spectrophotometer instrument most important requirement for cuvette . A cuvette is a tiny, clear rectangular container that is available in a range of sizes, materials, and quality levels. For visiblerange measurements between 310 and 2500 nm, glass cuvettes are used. Quartz cuvettes, on the other hand, offer precise results from 200 to 2500 nm in the visible and ultraviolet spectra. The manufacturing tolerance decreases with increasing measurement efficiency and consistency. In order to assess a sample's absorption, transmittance, fluorescence emission, fluorescence polarization, or fluorescence longevity, a light beam is sent through the cuvette. Liquid samples are used in conventional UV-visible or fluorescence spectroscopy. The researchers use a spectrophotometer to test the experimental sample after pouring it into a cuvette.

Mainly two types of cuvette.

a. glass cuvette
b. glass cuvette

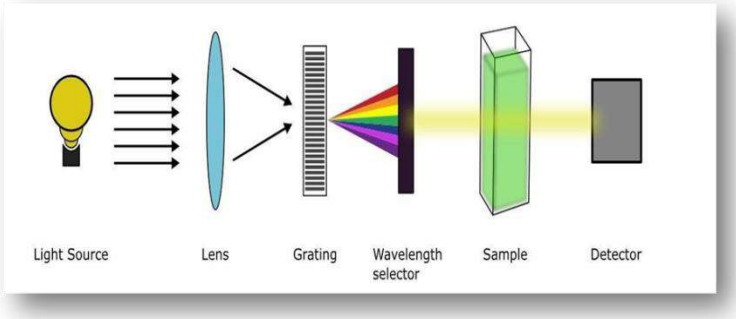

Fig 4 : Uv -Spectrophotometer

Because of the heteroaromatic pterin chromophore in the MTX structure, which allows the molecule to strongly absorb UV light, the amount of MTX can be measured using inexpensive UV light spectrophotometry. The pterin chromophore exhibits its highest molar extinction at neutral pH, while absorbance decreases with acidic pH . Furthermore, the first or higher order derivatives can be found using UVevisible spectrophotometry of absorbance against wavelength in order to identify overlapping spectra, particularly in pharmaceutical analysis . The investigation was carried out by the researchers using the area under the curve (AUC) approach or zero-order derivatization. The AUC approach is used,

however zero-order derivatization is completed in a single wavelength.

C)Mass Spectrometery :

Roland et al. prefere using triple quadrupole mass spectrometry (MALDI-QqQ-MS/MS) and ultrafast matrix-assisted laser desorption (MALDI) to determine MTX and MTXPGs in erythrocyte lysates. Operate aminopterin as the inside standard, the process comprised SPE of MTX and MTX-MTXPG ingestion from dephytinization erythrocyte lysates. There were 0.3 and 10 nmol/L as the LLOQ and LOD,apart .

The method was used to measure the levels of MTX and MTX-MTXPG metabolites in RA patients who had received oral MTX at modest doses.. The scientists stated that by removing LC and using MALDI instead, analysis times were shortened. As a result, this approach can be used for high-throughput measurements of a large number of samples . It was shown that MTXPG5 has the maximum glutamylation. No MTXPG6 or MTXPG7 was found in the erythrocyte pellets from RA patients. One analytical method for determining the mass-to-charge ratio of ions is mass spectrometry (MS). A mass spectrum, or a plot of intensity as a function of the mass-tocharge ratio, is used to display the results. Mass spectrometry is applicable to both complicated mixtures and pristine samples in a wide range of fields. Plotting the ion signal as a function of the mass-to-charge ratio is known as a mass spectrum The massof particles and molecules, the elemental or isotopic signature of a sample, and chemical identity or structure of molecules and other chemical compounds can all be ascertained using these spectra. A sample, which might be solid, liquid, or gaseous, is ionized in a standard MS technique, for instance, by subjecting it to an electron beam. This could result in some of the molecules in the sample being positively charged without breaking up or breaking up into positively charged pieces. Then, by accelerating them and exposing them to an electric or magnetic field, for instance, these ions (fragments) are sorted based on their mass-to-charge ratio; ions with the same mass-to-charge ratio will deflect to the same degree. A device that can identify charged particles, such an electron multiplier, picks up the ions. The findings are shown as spectra of the detected ions' signal intensity as a function of the mass-tocharge ratio. Three parts make up a mass spectrometer: a detector, a mass analyzer, and an ion source. A fraction of the material is transformed into ions by the ionizer. Depending on the sample's phase (solid, liquid, or gas) and the effectiveness of different ionization methods for the unidentified species, there are many different ionization approaches. After being extracted from the sample by an extraction system, the ions are sent into the detector via the mass analyzer. The mass analyzer can sort the ions according to their mass-to-charge ratio because of the variations in the masses of the fragments. The detector offers information for determining the abundances of each ion present by measuring the value of an indicator quantity. A multichannel plate is one example of a detector that provides spatial information as well.

D)Capillary Electrophoresis (Ce) :

Different migration rates are produced by CE, which is used to separate charged analytes and is based on differences in ion electrophoretic mobilities . In cases where the medication concentration is more than mg/L or g/L, direct injection of biological fluids to CE is feasible The reason CE equipped with a UV detector is not sensitive enough to test pharmaceuticals in mg scale is because CE in order to improve the sensitivity of CE, more samples are injected into the capillary column and the detector's sensitivity is changed .CE has been suggested as a further method to separate MTX in various matrices. It uses a small sample size, inexpensive capillaries,

high speed, high resolution, and high efficiency. Triplestacking CE was used to evaluate MTX and its metabolites in CSF because of the critical role that CSF plays during treatment and relapse . In this article, samples were injected hydrodynamically because the sample ions had a low charge. Although UV visible detection is a popular detector in CE, enantiomeric impurity identification in biomedical materials is not a good use for it, Consequently, chiral MTX was separated using electrokinetic chromatography (EKC), a kind of CE with luminescence detection . amperometric biosensors , voltammetric techniques , potentiometric membrane electrodes TLC ,HPLC and CE. Capillary electrophoresis can be performed with comparatively simple equipment. depicts a basic design of a capillary electrophoresis apparatus. A capillary, electrodes, a high voltage power supply, a detector, a sample vial, source and destination vials, and a data output and handling device are the major parts of the system. An aqueous buffer solution or other electrolyte is poured into the capillary, source vial, and destination vial. The capillary inlet is inserted into a vial of the sample to introduce it. The capillary is returned to the source vial once the sample has been inserted using capillary action, pressure, siphoning, or electrokinetics. An electric field generated between the source and the analytes starts the migration of the analytes.

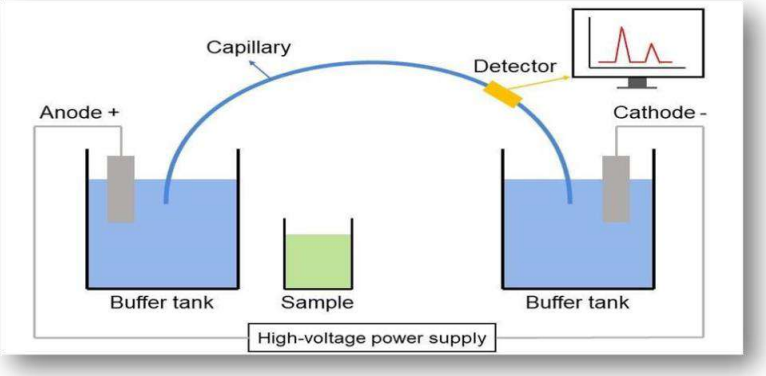

Fig:5 Capillary Electrophoresis

CE uses the speed at which charged ions travel in a capillary tube in response to an applied electric field to separate them. Because the capillary walls are negatively charged, the buffer solution's positive ions are drawn to them. This enables molecules to move uniformly through the center of the tube by covering the capillary walls with an electrical double layer. The buffer drags the molecules with it as it passes from the anode to the cathode when an electric current is applied. Benefits CE offers various benefits, such as: High resolution: Compared to high performance liquid chromatography (HPLC), CE yields narrower peaks and superior resolution. High speed: Compared to slab gel DNA separations, CE is quicker. Low sample use: CE needs very little in the way of buffers and samples. Drawbacks Additionally, CE has several drawbacks.

E) Electrochemical Method :

Electrical stimulation is used in a class of analytical procedures called electrochemical methods to determine a chemical's concentration or reactivity. Electrochemical methods are a quick, easy, and affordable option for quantifying MTX among analytical techniques that don't require laborious sample preparation Three steps and two electron/two proton transmissions characterize the MTX reduction reaction in neutral and acidic conditions. MTX undergoes a three-step eduction reaction in neutral and acidic

liquids that involves two electron/two proton transmissions [5, 8-dihydro-MTX is created in the first stage and tautomerizes to become 7, 8-dihydro-MTX. The next step involves separating the C(9)eN(10) bond of 7, 8-dihydro-MTX, and in the end, petridine is changed into its 5, 6, 7, 8-tetrahydro derivative. As a consequence of a very long tautomerization step, MTX is reduced in alkaline solutions in a single step with two-electron/two-proton transmissions to produce the reactant for further Static mercury drop electrodes (SMDE) or hanging mercury drop electrodes (HMDE) are used for the electrochemical analysis of MTX with cyclic voltage monitoring (CV)differential pulse. voltammetry (DPV), or adsorptive stripping differential pulse voltammetry (AdSDPV) have been employed . Several researchers have recently employed modified electrodes containing nanoparticles to lower the overpotential and speed up the response rate of numerous electroactive substrates Promising opportunities for sensing systems have been opened up by carbon nanotubes, gold, and silver nanoparticles A glassy carbon electrode(GCE) was modified using multiwalled carbon nanotubes functionalized with quaternary amine(q-MWCNTs) and a nanostructured patent was created by modifying the GCE with MWCNTs embedded within a dihexadecyl hydrogen phosphate film. Recent research have reported on the modification of GCE for MTX quantification using nafion composite film and DNA functionalized single-walled carbon nanotube (DNA/SWCNT). In certain studies, detection limits at the nanomolar (nM) level were attained. IN this method mostly used different types of electrode . Several detection tools can identify capillary electrophoresis separation. UV or UV-Vis absorbance is the main method of detection used by most commercial devices. A portion of the capillary itself serves as the detecting cell in these systems. Separated analytes can be detected via on-tube detection without sacrificing resolution. For greater flexibility, capillaries used in capillary electrophoresis are typically coated with a polymer, most commonly Teflon or polyimide. However, the capillary's UV detection section needs to be optically transparent. For capillaries coated with polyimide, a section of the coating is usually scraped or burned off to reveal a naked window that is a few millimeters long .

N = μ V 2 D m {\displaystyle N= {\frac {\mu V}{2D_{m}}}} is the formula for the number of theoretical plates, or separation efficiency, in capillary electrophoresis. Here, N {\displaystyle N} represents the number of theoretical plates, μ {\displaystyle \mu } represents the apparent mobility in the separation medium, and D m {displaystyle D_{m} is the analyte's dispersion coefficient. Although practical constraints limit the electric field strength to several hundred volts per centimeter, this equation shows that the effectiveness of separation is proportional to the electric field strength and is only limited by dissemination. Applying extremely high potentials (>20–30 kV) could cause capillary breakdown or arcing. Additionally, resistive heating (Joule heating) results from the application of high electric fields.

F)Liquid Chromatography-Mass Spectroscopy (Lc-Ms) :

Because of its inexpensive cost, LCeMS has shown to be one of the best methods for analyzing anticancer medications. The interface between the mass spectrometer and the HPLC eluent was made up of many ionization sources. The two most popular ionization sources are air pressure chemical ionization (APCI) and electrospray ionization (ESI); nevertheless, ESI is the ion source that is most frequently employed in the methods under discussion To investigate MTX, multiple reaction mode (MRM) and SRM modes have been employed. For assays that are

quantitative, We utilize SRM mode. The researchers employed DMSO as a solvent for the MTX medicinal product to avoid esterified contaminants . The LCeESIeMS/MS approach was used to simultaneously quantify eight cytotoxic medications, including MTX, cytarabine (CYT), gemcitabine (GEM), etoposide phosphate, cyclophosphamide (CP), if osfamide (IFO), irinotecan, doxorubicin, epirubicin, and vincristine .The 10 pharmaceuticals were analyzed simultaneously on a variety of surfaces, including coated paperboard, latex gloves, glass, polypropylene, polystyrol, stainless steel, and computer mice. The wipe sampling technique was combined with LCeMS/MS in SRM mode. The LOQs were 0.1 ng/cm for each of the cytotoxic medications. Using LC-MS, the following tasks were completed: measuring MTX and MTXPGs in whole-blood quantifying MTX and MTXGlun (n ¼ 2e5) in Caco-2 cells and analyzing MTXPG2 to MTXPG7 in RA patients receiving low-dose oral methotrexate therapy [The further LC-MS investigations include measuring MTX in human plasma , TDM of plasma MTX levels , estimate of MTX in human plasma and quantification of MTX in serum of rheumatoid arthritis patients .Without the need for additional sample preparation, Boer et al. devised a UeHPLCESIMS/MS stable isotope dilution approach to identify MTX in plasma. Simple sample preparation, such as dilution and protein precipitation, was combined with a quick (3-min) run time in this analysis. The approach provided here has a high dynamic range (3e250 mM) and an enhanced limit of quantification (LOQ; 5 nM) when compared to the standard Abbott TDx fluorescence polarization immunoassay (FPIA) for MTX TDM. A tiny but considerable negative constant error was found, despite the method's outstanding agreement with the FPIA method. As a result, it might be used for standard clinical TDM Ultra-performance liquid chromatography, or U-HPLC, is a growing chromatographic separation method where the stationary phase's particle size is less than 2.5 mm Guichard et al. created a general UHPLC-MS approach with the use of chromatographic modeling software for the analysis of 24 antitumor medicines, including MTX, utilized in hospital pharmacy units. The technique was effectively used to wipe materials from work areas and analyze pharmaceutical compositions. Boer and colleagues applied stable-isotope-labelled internal standards for the first time to the LC-MS/MS analysis of MTXPG1-5 in RBC. A study was published that examined the pharmacokinetics of 5-FU in mouse plasma, brain, and urine when combined with low-dose MTX using LC-MS. Bluett et al. measured the amounts of MTX and 7OHMTX in human urine using LC-MS adjusted in SRM mode. Urine samples were collected up to 105 and 98 hours following drug administration, respectively, to measure MTX and 7-OH-MTX in order to increase specificity and reduce cross-reaction .Schofield et al. originally reported using turbulent flow liquid chromatography (TFCeLC) in conjunction with electrospray positive ionization tandem mass spectrometry (TFCeLCeMS/MS) to quickly determine MTX, 7-OH MTX, and 4-DAMPA in serum samples .

High mobile phase flow rates through big porous particles (30–60 mM) are the foundation of the TFC. Larger molecules and matrix components are kept out of the stationary phase's pores by the small molecules, which results in waste. The trapped molecules are then removed from the TFC column and added to the analytical LCeMS/MS system by back-flushing with an organic solvent. Protein precipitation was accomplished using methanol, which contains formic acid. Cyclone-P (50 mm 0.5 mm) and Hypersil Gold C8 (2.1 mm 50 mm, 5 mm) columns were used for HPLC separation. The

mobile phases A and B were utilized to be water and methanol, with each containing 10 mM ammonium formate, at a flow rate of 0.7 mL/min. Acetone with a ratio of 2propanol to acetonitrile. Because LC can separate delicate and complicated natural mixtures whose chemical composition needs to be clearly established (such as biological fluids, environmental materials, and pharmaceuticals), the coupling of MS with LC systems is appealing. Additionally, LC-MS is used in the study of volatile explosive residues.[29] Since over 85% of natural chemical compounds are polar and thermally labile and cannot be processed by GC-MS, LC-MS has emerged as one of the most popular chemical analysis methods in use today.[5] For instance, HPLC-MS is thought to be the most advanced analytical method for pharmaceutical and proteomics labs.

G) Nanomaterial Optical Probes :

suitability of nanomaterials for sensing applications has been demonstrated. With higher sensitivity and reduced detection limits of several orders of magnitude, the clever use of such nano-objects produced observably better results. To find MTX on fluorescent probes made of nanomaterials, some research was done. Compared to traditional fluorescence techniques, nanomaterial fluorescent sensors MTX can be determined using a straightforward, user-friendly, and pre-treatment process-free method that does not require complex derivatization to transform it into luminous chemicals. These probes function according to the inner filter effect (IFE), electron transfer, or fluorescence resonance energy transfer (FRET) principles. Bovine serum albumin stabilized gold nanoclusters were first used by Chen et al. as fluorescent probes, and they produced good results for MTX detection in actual samples. The sensitive detection of MTX in the range of 0.0016e24 mg/mL was made possible by the reduction in fluorescence intensity of the gold nanoclusters brought on by MTX. MTX was shown to have a de.9 ng/mL .N, S-codoped fluorescent carbon nanodots (NSCDs) were created by Wang et al. using glucose and ammonium persulfate. In contrast to pure CDs, ethylene diamine showcased exceptional chemical stability, consistent shape, outstanding water solubility, vibrant blue emission, and a relatively high fluorescence quantum yield . A wide linear span of 50%, covering a range of 6%, is available. An absence of concentration, paired with a detection limit close to zero. The fluorescence displayed by NSCDs experienced a notable reduction due to the phenomenon of FRET occurring between NSCDs and MTX, enabling the precise identification of 33 nM of MTX sensitivity. The technique effectively identified MTX in human serum during the experiment mentioned in reference . Zhao and colleagues. Citric acid and L-cysteine were employed in their research. Recently, there have been advancements in the development of carbon quantum dots that are doped with nitrogen and sulfur. The dots reveal a strong response.

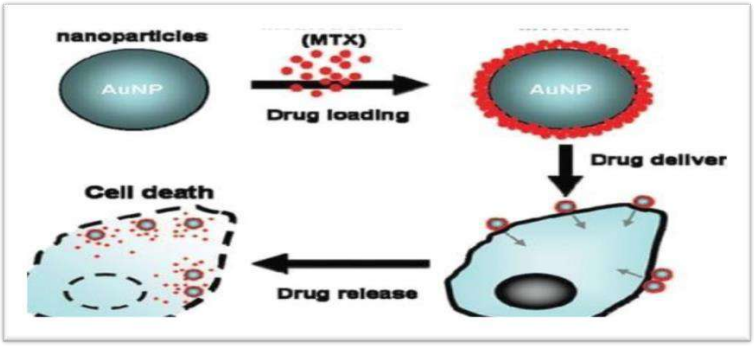

Fig:6 Nanamaterial Optical Probe

CONCLUSION:

The analysis of MTX and its metabolites from 2008 to 2019 was examined in this review. A review of the literature found that MTX and its metabolites have been identified in pharmaceutical, biological, and environmental samples using a variety of analytical techniques, including capillary electrophoresis, UVevisible spectrophotometry, HPLC using various detector systems, and electrochemical methods. A comparison of several methods of analysis . The UV spectrophotometric approach contributes significantly to method development and validation and yields findings that are adequately fast. Based on the results, it can be said that purified or distilled water is the most commonly used solvent because it is readily available, most drugs dissolve easily in it, it is practical for the majority of method development processes, and it yields better results when the results are validated .Over the past few decades, the number of cancer humans (including patients and medical personnel) made the development of reliable analytical methods for assessing these substances crucial. The initial analytical techniques, mostly utilizing LC-UV, made it possible to establish the groundwork for the application of cytotoxic medications in the treatment of human cancers by comprehending how the drugs interact with the body, creating pharmaceutical formulations, and assessing the toxicity.

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