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PHARMACEUTICAL ANALYTICAL APPROACHES FOR INOSINE PRANOBEX: A COMPREHENSIVE REVIEW

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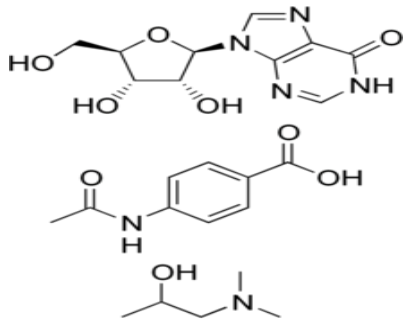
ABSTRACT

Inosine Pranobex (IP), commonly known as inosine acedoben dimepranol, isoprinosine and methisoprinol, has been proven to positively impact the host's immune system, by enhancing T-cell lymphocyte proliferation and activity of natural killer cells, increasing levels of pro-inflammatory cytokines and thereby restoring deficient responses in immunosuppressed patients. At the same time, it has been shown that it can affect viral RNA levels and hence inhibit growth of several viruses. Due to its immunomodulatory and antiviral properties, and its safety profile, it has been widely used since 1971 against viral infections and diseases, among which subacute sclerosis panencephalitis, herpes simplex virus, human papilloma virus, human immunodeficiency virus, influenza and acute respiratory infections, cytome - galovirus and Epstein–Barr virus infections. This article examines published analytical methods that are reported so far for the determination of inosine pranobex in Pharmaceutical formulations and biological samples. They include various techniques like high performance liquid chromatography (HPLC), Ultra performance liquid chromatography (UPLC), Liquid chromatography-mass chromatography (LC-MS), Hyphenated HPLC and tandem MS techniques and Thin layer chromatography (TLC).

KEYWORDS: Inosine pranobex, Antiviral; Herpes; HPV; Immunomodulation; Infection; Influenza, Analytical methods.

1. INTRODUCTION

Inosine pranobex (also known as **Inosine acedoben dimepranol** (**methisoprinol**, inosiplex or Isoprinosine) is an antiviral drug that is a combination of inosine and dimepranol acedoben (a salt of acetamido benzoic acid and dimethyl amino isopropanol) in a ratio of 1 to 3. It is used primarily in European countries, especially as a treatment for acute viral infections, such as the common cold.[31]



STURUCTURE OF INOSINE PRANOBEX

1.1 HISTORY

The first studies conducted with the drug happened as early as the 1970s. It was licensed in 1971[1] with the first robust preliminary review of its efficacy having been published in 1986.[2] Since the beginning, the drug was praised for its wide array of use cases, it was note early on that it has a clinically noticeable effect on the immune function. In the 1990s, the possibility of the drug being used for HIV infection has also been investigated thoroughly, with the results typically highlighting improved immune function.[3] Nonetheless, following the development of more effective HIV drugs, this use case has been largely discontinued.

Throughout the 21st century, Inosine pranobex has been used mainly in Central and Eastern Europe, in contrast to the United state, where the medication is not as widely available. In Eastern Europe, namely poland, the medication is available over-the-counter under the brand name of Groprinosin® thanks to its safety and low risk of overdose.

In 2020, inosine pranobex was found to be a cheap and effective treatment for SARS-CoV-2 in cases not requiring hospitalization with fatality rate effectively halved as a result of its use.[1]

Tabel 1: History Of Inosine Pranobex.[30][31]

YEAR	EVENT
1970	Development of Inosine Pranobex begins by Wellcome Research Labs/AB Astra
1971	First clinical trials conducted to evaluate immunomodulatory and antiviral effects.
1974	Marketed in Europe under names like Isoprinosine, Delimmun, etc.
1976	US Patent granted (e.g., US3937741A)-Inosine acedoben dimepranol composition
1980s	Widely used for viral infection such s herpes, simplex, subacute sclerosing penencephalitis (SSPE). Influenza, etc.
1990s	Enters developing markets (Asia, Latin, America, Eastern Europe). Generics begin to appear.
2000	Original patents expire in most countries, increasing global generic production.
2005-2015	Studies continue on its immunomodulatory properties, especially in HIV, HPV and respiratory viruses.
2020	Investigated during COVID-19 Pandemic for its immune – enhancing potential (no official approval)
2024-2025	Still marketed in countries like India, Poland, Romania and some Latin American countries.
Present	Not FDA Approved in the USA, but widely used internationally as a generic drug.

1.2 PATENT[32-33]

Inosine Pranobex was first developed in **the United States**. It was initially synthesized by **Lederle Laboratories**, which was later acquired by **Pfizer**. The drug was developed in the 1960s and patented in the 1970s for its potential as an antiviral and immune-modulating agent.

The development of inosine Pranobex was part of research aimed at finding new treatments for viral infections, and it has been used in various countries for conditions such as herpes simplex virus infections, warts, and other viral illnesses. Its immunomodulatory properties have also been a focus, as it is believed to enhance the body's natural immune response.

Though it was first developed in the U.S., it has been marketed and used worldwide, including in Europe, Asia, and Latin America, under various brand names.

Table 2: Patent Element Details.

PATENT ELEMENT	DETAILS
DRUG NAME	Inosine Pranobex
CHEMICAL NAME	Inosine acedoben dimepranol /Methisoprinol
CAS NUMBER	36703-88-5
THERAPEUTIC USE	Antiviral, Immunomodulatory
FIRST PATENT HOLDER	Newport Pharmaceutical /AB Astra (Historical origin)
ORIGINAL PATENT NUMBER	US3937741A(example)
ORIGINAL PATENT DATE	Granted: February 10, 1976
PATENT EXPIRATION	Expired in most countries (typically 1996-2000)
CURRENT PATENT STATUS	Expired – original product now generic in most countries
GENERIC AVAILABILITY	Widely available as generic drug
FORMULATION PATENTS	Some countries may have patents on new formulations (e.g., sustained release drug)
COMBINATION PATENTS	May exist if combined with other drugs (check country- specific database)
INDIAN PATENT STATUS	No active primary patent; generic manufacturing permitted
OTHER NOTES	Not FDA- approved in USA; approved in Europe, Asia, and Latin America.

2. MECHANISM OF ACTION[34]

One of the primary mechanisms of Inosine Pranobex is its ability to modulate the immune system. The compound has been shown to enhance the proliferation of T-lymphocytes, a type of white blood cell that plays a crucial role in the immune response. By promoting T-cell proliferation, Inosine Pranobex boosts the body's ability to mount an effective immune response against viral infections. Specifically, it has been found to increase the production of interleukin-2 (IL-2), a cytokine that is important for the growth, proliferation, and differentiation of T-cells. This upregulation of (IL-2) subsequently enhances the activity of natural killer (NK) cells, which are vital for the immediate response to virally infected cells.

In addition to its immunomodulatory effects, Inosine Pranobex exhibits direct antiviral activity. One proposed mechanism is the inhibition of viral RNA synthesis, which restricts virus replication. This inhibition is thought to occur through the interference with the viral RNA-dependent RNA polymerase, an enzyme essential

for the replication of viral genetic material. By impeding this enzyme, Inosine Pranobex reduces the ability of the virus to reproduce and spread within the host. Furthermore, it has been observed that Inosine Pranobex can induce the production of interferon gamma, a cytokine that possesses antiviral properties and enhances the antigen-presenting capabilities of macrophages.

Moreover, Inosine Pranobex is believed to restore the function of immune cells that have been compromised by chronic viral infections. For instance, chronic infections can lead to T-cell exhaustion, a state where T-cells lose their effectiveness over time. Inosine Pranobex can help rejuvenate these exhausted T-cells, thereby restoring their ability to fight infections. This property is particularly valuable in the management of persistent viral infections where the immune system's prolonged engagement with the virus leads to diminished immunity.

Another important aspect of Inosine Pranobex mechanism is its antioxidant properties. Viral infections often result in oxidative stress, which can damage cellular structures and impair immune function. Inosine Pranobex has been shown to reduce oxidative stress by scavenging free radicals and enhancing the activity of antioxidant enzymes. This reduction in oxidative stress not only helps in protecting cells from damage but also supports the overall immune response.

In clinical settings, Inosine Pranobex has demonstrated efficacy in the treatment of various viral infections, further validating its dual role as an immunomodulator and antiviral agent. It is important to note that while the drug is generally well-tolerated, it may have side effects such as gastrointestinal disturbances, headache, and dizziness. Therefore, its use should be guided by a healthcare professional, especially in patients with pre-existing health conditions.

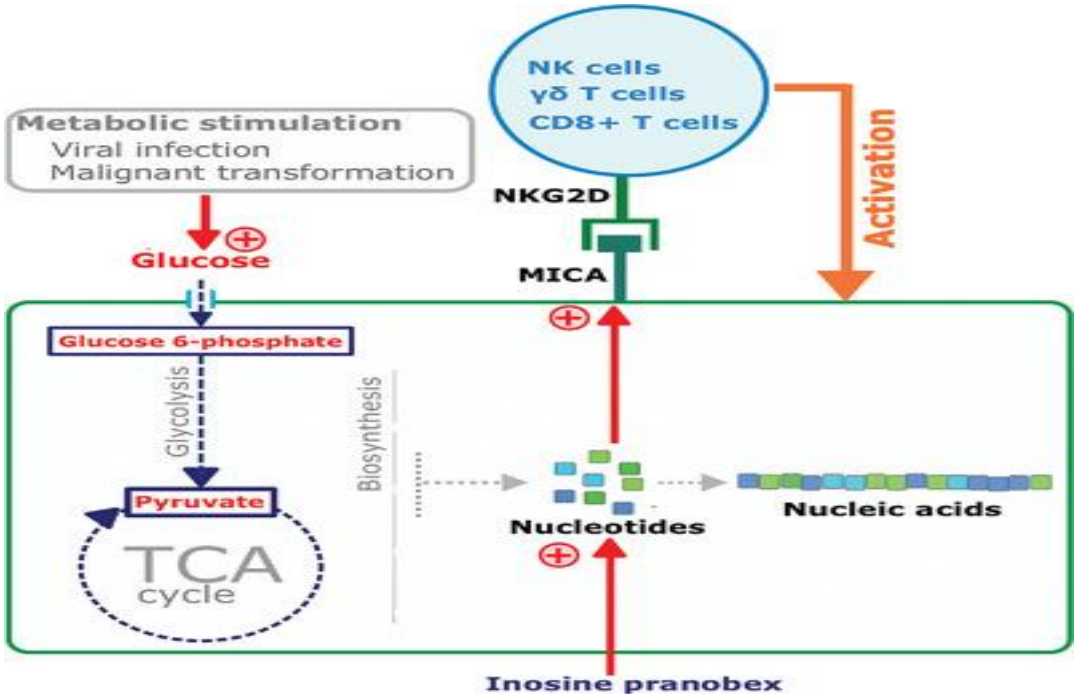


Figure 1: Mechanism action of Inosine Pranobex.

Table 3: "Mechanism of Action of Inosine Pranobex: An Overview"

MECHANISM ASPECT	DETAILS/EXPLANATION
Drug type	Synthetic **immunomodulator** and **antiviral agent**
Main components	Combination of Inosine, Acedoben and Dimepranol
Immunostimulatory Action	Enhances T-Lymphocyte proliferation and differentiation
Cytokine stimulation	Increase production of interleukin -2 (IL-2) and interferon – gamma (IFY – gamma)
Macrophage activation	Promotes activation of macrophages and natural killer (NK) cells
Antiviral mechanism	Inhibits viral RNA synthesis: interferes with viral replication in host cells.
Purine derivative role	Inosine supports nucleic acid metabolism and energy production
Cell – mediated immunity	Boost activity of cytotoxic T-cells and helper T- cells (CD4+)
Duration of effect	Immune response persists longer than plasma half life (50-90 minutes)
Overall impact	Enhances the body's defense mechanisms against viral infection and immune suppression

Table 4: Clinical Significance.

USED FOR	REASON
Chronic viral infections	Enhances immune clearance of viruses (e.g., herpes, HPV, influenza)
Immunocompromised conditions	Boosts cell-mediated immunity (e.g., HIV Supportive therapy)
Warts and subacute sclerosing panencephalitis (SSPE)	Due to **antiviral** and **immune modulation action**

2.1 PHRMACODYNAMIC PROPERTIES[35-36]

Inosine Pranobex is a synthetic purine derivative with immunomodulatory and antiviral properties, which result from an apparent *in vivo* enhancement of host immune responses due to the drug.

In clinical studies Inosine Pranobex has been shown to normalise a deficient or dysfunctional cell-mediated immunity by evoking a Th1 type response which initiates T lymphocyte maturation and differentiation and potentiation of induced lymphoproliferative responses, in mitogen or antigen-activated cells. Similarly, the drug has been shown to modulate T-lymphocyte and natural killer cell cytotoxicity, T8 suppressor and T4 helper cell functions and also to increase the number of IgG and complement surface markers.

Inosine Pranobex increases cytokine IL-1 production and enhances IL-2 production, upregulating the expression of the IL-2 receptor *in vitro*. It significantly increases endogenous IFN -γ secretion and decreases the IL-4 production *in vivo*. It has also been shown to potentiate neutrophil, monocyte and macrophage chemotaxis and phagocytosis.

In vivo, inosine acedoben dimeparanol enhances potentiation of depressed lymphocytic mRNA protein synthesis and translational ability while inhibiting viral RNA synthesis achieved by yet-to-be-clarified degrees of
(1) Incorporation of inosine-mediated orotic acid into polyribosomes;
(2) Inhibition of polyadenylic acid attachment to viral messenger RNA and
(3) Molecular reorganisation of lymphocyte intramembrane plasma particles (IMP) that results in a nearly threefold increase in density.

Inosine pranobex inhibits cGMP phosphodiesterase only at high concentrations *in vitro* and at levels not involved in the *in vivo* immunopharmacological effects.

Table 5: Pharmacodynamic Studies on Inosine Pranobex.

STUDY/MODEL	VIRUS/ INFECTION	PHARMACODYNAMIC MECHANISM
In vitro+ IFN α (A549 cells)	Adenovirus HAdv-2&5 RNA viruses: CA-16, EV17, HPIV-16	IP significantly reduced viral titers; synergy with interferon – α showed stronger inhibition than either alone. Combined IP+IFN α reduced viral titers by demonstrating synergism.
Phase 3RCT in symptomatic COVID-19 patients	Mild – moderate SARS-CoV-2 infection	Early clinical and cure response; enhanced NK cell cytotoxicity and induction of NKG2D ligand on infected cells.
Ex vivo lymphocyte cultures	Human lumphocytes+ mitogenic stimulation	Ip upregulated Th1 cytokines (IL-2, IFN-gamma, TNF- α) and suppressed Th2 cytokine IL-10 in dose dependent fashion.
Phase 4 RCT (double-blind, placebo-controlled	Acute respiratory virus infections (ILI)	Accelerated symptom resolution in <50 year, non-obese subgroup; indicated immune-mediated response rather than direct virucidal effect.

2.2 PHARMACOKINETIC PROPERTIES[37-38]

Absorption: When administered orally in man, Inosine pranobex is rapidly and completely absorbed (≥ 90 %) from the gastrointestinal tract and appears in the blood. Similarly, 94-100% of IV values of DIP [N,N-dimethylamino-2-propanol] and PacBA [p-acetamidobenzoic acid] components are recovered in urine after oral administration in Rhesus monkeys.

Distribution: Radiolabelled material was found in the following tissues in order of decreasing specific activity when drug was administered to monkeys: kidneys, lung, liver, heart, spleen, testes, pancreas, brain and skeletal muscle.

Metabolism: In human subjects following a 1 g oral dose of Inosine pranobex, the following plasma levels were found for DIP and PAcBA, respectively: 3.7 µ g/ml (2 hours) and 9.4 µ g/ml (1 hour). In human dose tolerance studies, peak post-dose elevation of uric acid levels as a measurement of drug-derived inosine are not linear and can vary + 10 % between 1-3 hours.

Excretion: The 24-hour urinary excretion of PAcBA and its major metabolite under steady-state conditions at 4 g per day amounted to approximately 85 % of the administered dose. 95 % of the DIP-derived radioactivity in urine was recovered as unchanged DIP and DIP N-oxide. The elimination half-life is 3.5 hours for DIP and 50 minutes for PAcBA. The major metabolites in

humans are the N-oxide for DIP and the o-acyl glucuronide for PAcBA. Because the inosine moiety is degraded by the purine degradation pathway to uric acid, radiolabelled experiments in humans are inappropriate. In animals up to about 70 % of the administered inosine can be recovered as urinary uric acid following oral tablet administration and the remainder as the normal metabolites, xanthine and hypoxanthine.

Bioavailability/AUC: Urinary recoveries under steady state conditions of the PAcBA moiety and its metabolite were found to be > 90 % of the expected value from solution. The recovery of the DIP moiety and its metabolite was >76 %. The plasma AUC was >88 % for DIP and > 77 % for PABA.

Table 6: Pharmacokinetic studies on Inosine Pranobex.

PK PARAMETER	DETAILS
ABSORPTION	Rapid oral absorption; peak plasma concentration reached within 1-2 hours post-dose.
BIOAVAILABILITY	Moderate; not significantly affected by food intake
DISTRIBUTION	Widely distributed; crosses cell membranes; no significant accumulation observed.
PLASMA PROTEIN BINDING	Low (<50%); contributes to efficient tissue distribution
METABOLISM	Rapidly metabolized; inosine component follows purine metabolism (uric acid formation)
ELIMINATION	Primarily renal; metabolites excreted via urine within 24 hours
HALF LIFE	Short; approximately 50-90 minutes
EXCRETION	>95 % excreted in urine as metabolites (hypoxanthine, uric acid etc.)

2.3 SAFETY

The most commonly found effects are nausea and vomiting, hypotension, drowsiness and skin irritation may also occur. Metabolism of the inosine component of the drug can lead to an increase in uric acid levels in both blood and urine. The occurrence of transient reversible hyperuricaemia occurs in about 10 % of patients taking Inosine pranobex.[5] Due to the potential risk of hyperuricosuria and the development of urate nephrolithiasis, increased fluid intake and exclusion of acidic foods is recommended during isoprinosine therapy. Its administration is not recommended in combination with immunosuppressing medicine.

Tolerance studies in healthy individuals and patients have consistently shown that Inosine pranobex has no serious side effects and is remarkably well tolerated by the organism. Continuous administration of the drug for up to 7 years, at doses ranging from 1 to 8 g per day, has only occasionally caused transient nausea. This nausea was associated with a large number of tablets ingested. In addition, transient increases in serum and urinary uric acid levels have been reported. This increase in serum uric acid concentration is more common in male patients than in females.[6]

Neither long-term damage not death from overdose have been reported in relation to inosine pranobex, doses upward of 1 g/kg of body weight were found to be toxic in rodents.[4] The drug is metabolized very quickly; therefore, any side effects should subside quickly with no long-term effects.

2.4 INDICATIONS

Preventative use

Inosine pranobex can also be helpful in managing and decreasing the incidence of common viral infections, such as the common cold or influenza.[7] As such, it is commonly prescribed preventatively, albeit at a lower dose. Several studies have investigated the benefits of inosine pranobex therapy in frequently ill children[8][19] and returned positive results in both clinical and immunological outcomes.

Herpesvirus infections

Inosine Pranobex is indicated as a safe antiviral for herpesviruses, such as herpes simplex virus types 1 and 2, cytomegalovirus (CMV), and Epstein- Barr virus (EBV).[10] The drug also proved helpful in managing complicated cases of lengthy reactivations of herpesviruses such as EBV, and subsequent post-viral fatigue.

Human Papilloma Virus (HPV) infections

Inosine Pranobex may be prescribed for the treatment of HPV infections both benign and oncogenic,[11] as a very safe and effective alternative therapy Usually it is administered in combination with other treatment methods, such as CO_2 laser and Podophyllotoxin.

Influenza and Rhinovirus infections

The evidence in treating rhinovirus infections is mixed. While no statistically significant effect was observed in rhinovirus 44 or 32 infection,[12] its administration in rhinovirus 21 infection led to statistically improved health outcomes in patients, shortened infectivity and decreased viral shedding.[15] In Influenza and Influenza-(RSV, adenovirus and parainfluenza virus) infections, inosine pranobex did lower the symptom severity and duration.[13][14]

COVID-19

When the global coronavirus pandemic hit in 2020, inosine pranobex was one of the first medication used experimentally to treat the SARAS-CoV-2 induced virosis, mainly due to its remarkably wide area of use

and general antiviral properties. Several clinical trials were conducted returning largely positive results.

Its use was pioneered in the Czech Republic, where it was first noted that use greatly decreases mortality among elderly.[16][17] In 2022, a large Phase 3 trial concluded that administration of inosine pranobex should start as early as possible with greatly improved outcomes in mild to moderate COVID-19 patients.[18]

Type B and C Viral Hepatitis

In type B hepatitis, inosine pranobex was found ineffective during the acute phase of the infection, though in 28-days lower bilirubin and transaminase levels were detected. Greater number of patients became antigen-negative within a 90-days' time-frame indicating a faster recovery rate.[19]

Type C hepatitis was not studied as extensively, hence not so much data is available. It has been shown that inosine pranobex therapy in combination with ribavirin normalizes aminotransferase levels in patients unresponsive to interferon treatment.[20]

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)

In 2021, the US ME/CFS Clinician Coalition recommended the use of inosine pranobex for "immune dysfunction" symptoms, specifically "frequent viral infections, herpes simplex outbreaks, low natural killer cell activity, sore throat, tender nodes, low grade fevers".[21]

Subacute Sclerosing Panencephalitis (SSPE)

Although the effect is unclear, several case reports have suggested that inosine pranobex may provide beneficial therapeutic effects in managing the illness. Several long-term studies suggested that the drug both increased survival and decreased neurological deficiencies.[22] It is not a cure for the illness though, as currently no cure exists.

Human Immunodeficiency Virus (HIV) and AIDS

Inosine pranobex has been proven to delay AIDS progression in HIV positive patients. In a large Phase I study of 831 HIV-positive patients, it was found to be very safe with no serious side effects reported.[23]

2.5 DOSAGE

For acute infection, the typical dose is 50 mg/day/kg of body weight. For prevention of chronic issues lower doses are typically recommended, usually under 2 g/day. The maximum dose permitted is around 4 g/day. The toxicity of the drug in humans is unknown, but doses upward of 1 g/kg of body weight were toxic in rodents.[4]

2.6 REGULATORY CLASSIFICATION AND APPROVED STATUS

Inosine Pranobex is not approved by the U.S, FDA but is approved in several other countries (e.g.., some parts of Europe, Latin America, and Asia) for its immunomodulatory and antiviral properties.

It is commonly marketed under brand name such as **Isoprinosine®, Delmunyl®, Isojol®** and **Imunovir®.**

Regulatory authorities such as EMA, CDSCO (India) and TGA (Australia) may have specific requirements for may have specific requirements for drug's quality documentation and analytical validation for product registration.[41]

2.7 MELTING POINT[27]

If Inosine Pranobex has a melting point range of **140℃-145℃**

2.8 SOLUBILITY PROFILE[26]

Inosine Pranobex, a synthetic immunomodulatory agent, exhibits high solubility in distilled water, this property is largely attributed to the hydrophilic nature of its constituents- inosine Pranobex when tested in distilled water at room temperature (25 ± 2 ℃), inosine pranobex dissolves completely, indicating it is **freely soluble in water.**

3. SOLUBILITY

K. kafedijiski et al. developed the solubility study of Inosine Pranobex is described as freely soluble in water but the biopharmaceutics classification system (BCS) requires data on the solubility at 37℃ and in different media with different pH values. For the reason, the following solubility tests were undertaken: to different samples of IAD (approximately 10 mg, 50.0 mg and 690.0 mg) in a glass – stoppered graduated cylinder (10.0 ml) were added increasing volumes of solvent at 20℃ and 30℃. After each addition of the solvent to the indicated total volume, the mixture was shaken vigorously for 10 min and was visually checked for any undissolved parts in the sample.[26] Solubility conditions of inosine pranobex was show on (Table 7).

Table 7: Solubility Of Inosine Pranobex In Different Conditions.[26]

SOLVENT- MEDIA TEMPERATURE	APPROXIMATE SOLUBILITY OF IAD (mg/mL)	APPROXIMATE SOLUBILITY OF IAD (%w/v)
Purified water,20℃	230.0	23.0
Purified water, 37℃	345.0	34.5
HCl, 0.1mol/l PH=1.2, 20℃	<1.0	<0.1
HCl, 0.1mol/l pH=1.2, 37℃	<1.0	<0.1

Buffer, pH=4.5, 20℃	153.3	15.3
Buffer, PH=4.5, 37℃	345.0	34.5
Buffer, pH=6.8,2 30℃	230.0	23.0
Buffer, PH=6.8, 37℃	345.0	34.5

4. ANALYTICAL METHODS

4.1 TLC[24]

Taghreed A. Mohamed et al. developed the thin layer chromatography (TLC) with densitometry has been established for the identification and the quantification of inosine pranobex in drug substance and drug products. Inosine pranobex is a combination of inosine, acetamidobenzoic acid, and dimethylaminoisopropanol. UV densitometry was performed in absorbance mode at 260 nm. The separation was carried out on aluminum sheet of silica gel 60 f 254 [chloroform - methanol- - toluene -10 % ammonia solution (6:5:1: 0.1 % v/v)] as mobile phase. Linearity range was found to be 1-12, 2-12, 2-20 and 2-16 µg/ml for inosine pranobex, inosine, acetamidobenzoic acid, and dimethylaminoisopropanol with the mean percentage recoveries 99.74 ± 1.73 %, 99.88 ± 1.75 %, 99.56 ± 1.08 %, and 99.36 ± 0.71 % respectively, (Correlation coefficient r2 = 0.9998 for inosine pranobex, r2 = 0.09999 for inosine, r2 = 0.9998 for acetamidobenzoic acid and r2= 0.9998 for dimethylaminoisopropanol). The detection and quantification limits for inosine pranobex and other components are also reported. The presented method was validated according to ICH guidelines. Statistical comparison of the results was performed using Student's t-test and F-ratio at 95 % confidence level, and there was no significant difference between the reference and proposed method with regard to accuracy and precision. It could be said that the validated TLC- densitometry method is suitable for the routine analysis of inosine pranobex in quantity control laboratories.

4.2 HPLC (SHARC 1 COLUMN)[29]

Chromatography of these two compounds can be difficult due to their high polarity. But both compounds can be well retained and separated using anhydrous (water-free) conditions using HPLC on SHARC 1 column, which uses hydrogen-bonding as a separation mechanism. The method uses a gradient of acetonitrile (ACN) and methanol (MeOH) mobile phase with volatile buffer containing Formic Acid 0.1 % and Ammonium formate 0.01 %, making the method MS-compatible. Both compounds can also be UV detected at 270 nm.

Table 8: Chromatographic Conditons of Hplc Sharc Column 1.

COLUMN	Sharc 1
MOBILE PHASE	ACN/MeOH
BUFFER	Formic acid 0.1 %, Ammonium formate 0.01 %
FLOW RATE	1.0 ml/min
DETECTION	UV 270 nm
ANALYZING COMPOUNDS	Inosine, Acedoben

4.3 HYPHENATED HPLC AND TANDEM MS TECHNIQUES[28]

Mo chen et al. developed the Inosine pranobex is a compound formulation composed of inosine and p-acetaminobenzoic acid (PABA) salt of N,N-dimethylamino-2-propanol (DIP). This study was to investigate the clinical plasma pharmacokinetic properties of DIP and PABA after single and multiple oral doses of inosine pranobex tablets in healthy volunteers. The established LC/MS/MS method for plasma DIP determination had a linear range of 0.02-10 mg/mL, and the HPLC method for plasma PABA determination had a linear range of 0.05- 40 mg/mL. Linear pharmacokinetic characteristics were found with single oral doses of 0.5, 1.0 and 2.0 g. No obvious accumulation effects were observed for DIP and PABA.

4.4 UPLC and LC-MS/MS[25]

K. Santhakumari et al. developed the simultaneously evaluate Inosine pranobex and levamisole in pharmaceutical formulations using UPLC, a reliable and simple method was developed. The chromatographic separation used in this method a Phenomenox C18 column (50mmx2.1mm, 3.5) 0.1 % Trifluoroacetic Acid (TFA) and Methanol were employed in a mobile phase with a flow rate of 0.5 mL/min and room temperature. At 223 nm, a UV observation was made. With these settings, we were able to successfully use UPLC to achieve good linearity throughout a range of 12.5-75 g/ml of inosine pranobex and 2.5-15 g/mL of levamisole. The results of other UPLC validation parameters, such as system precision, method precision, accuracy, robustness, and degradation studies, were present within the permitted limit while employing the aforementioned assay method, in accordance with ICH criteria.

Table 9: Reported Analytical Methods Of Inosine Pranobex.

1. TLC

STUDY	MOBILE PHASE	COLUMN	DETECTOR	WAVELENGTH
Validated analytical method development Inosine Pranobex in Drug Products by Thin Layer Chromatography[24]	Chloroform - methanol- - toluene -10 % ammonia solution (6:5:1: 0.1 % v/v)	Aluminum sheet of silica gel 60	UV	254 nm

2. UPLC/LC-MS

STUDY	MOBILE PHASE	COLUMN	DETECTOR	WAVELENGTH	FLOW RATE
Validated Method Development of Levamisole and Inosine pranobex by using UPLC &Characterization of Degradants by LC-MS/MS[25]	Trifluoroacetic Acid (0.1 %) and Methanol	C_{18} column	UV	233 nm	0.5 ml/min

3. HPLC/ LC-MS

STUDY	MOBILE PHASE	COLUMN	DETECTOR
Development, Validation, and Application of the LC-MS/MS Method for determination of 4-Acetamidobenzoic acid in Pharmacokinetic pilot studies in pigs.[42]	Mobile phase A (0.2 %) formic acid in water Mobile phase B (0.2 %) formic acid in acetonitrile 9:1 v/v	C_{18}	LC-MS
An HPLC method for determination of inosine and hypoxanthine in human plasma from healthy volunteers and patients presenting with potential acute cardiac ischemia[28]	Acetonitrile – 0.5% F.A and 0.5% acetate solution (75:35 v/v)	C_{18}	LC-MS

4. HPLC

STUDY	MOBILE PHASE	COLUMN	DETECTOR	WAVELENGTH	FLOW RATE	RT
Analytical Method Development Validation and Degradation of Inosine Pranobex by Using RP-HPLC Method.[27]	Methanol (with 0.1 % Water) and Ortho-Phosphate Buffer (45:55 %)	C_{18} Column	UV	259 nm	0.8 ml/ min	5.7mins
HPLC analytical research method development and validation of Inosine pranobex by using the QbD Approach[43]	Methanol to (0.1 %) OPA buffer (60:40 % v/v)	C_{18} Column	UV	259 nm	1.00 ml/ min	6.1mins

6. CHALLENGES OF INOSINE PRANOBEX IN ANALYTICAL METHOD[39-40]

Inosine Pranobex (IP), a combination of inosine and p-acetamidobenzoate of 4-amino-phenyl-acetamide, is used as an immunomodulatory agent. When developing analytical methods to quantify and characterize inosine Pranobex in pharmaceutical formulations, several challenges can arise due to the chemical properties and complexity of the compound. Here are some common analytical challenges:

6.1. Chemical Instability and Degradation

- **Hydrolysis:** Inosine pranobex is prone to hydrolytic degradation, particularly under acidic or basic conditions, which can complicate the development of stable analytical methods.
- **Oxidation and Photodegradation:** Inosine, a component of inosine pranobex, is sensitive to oxidation and UV light, leading to degradation products. This instability can complicate the

extraction, quantification, and identification of the drug.

- **Solution Stability:** When dissolved in solvents, inosine pranobex may undergo chemical changes over time, requiring careful optimization of solution conditions, including pH, temperature, and solvent choice.

6.2. Chromatographic Challenges

- The separation of inosine pranobex components using chromatographic methods like HPLC or thin-layer chromatography (TLC) can be difficult because of the similar chemical properties of inosine and para-acetamidobenzene.
- The mobile phase composition, column type, and temperature need to be optimized to ensure proper separation.

6.3. Separation and identification of compounds

- **Complexity of Mixture:** Inosine pranobex is a combination of inosine and two other chemical entities (p-acetamidobenzoate and 4-amino-phenyl-acetamide). Separating and quantifying these components can be challenging, especially if there are impurities or degradation products.
- **Co-elution of Related Compounds:** In chromatographic methods like HPLC, the active ingredients and degradation products may have similar chemical structures, leading to co-elution, which can interfere with accurate quantification.

6.4. Lack of Suitable Chromatographic Methods

- **Hydrophilicity of Inosine:** Inosine is hydrophilic and may not readily interact with the stationary phases used in reversed-phase chromatography. This may require the use of specialized columns or mobile phase modifiers.
- **Solvent Selection:** The polar nature of inosine Pranobex can make solvent selection tricky in chromatography. The solvent system needs to be carefully optimized to ensure efficient separation without degradation of the active ingredients.
- **Peak Resolution:** Achieving high-resolution separation of the individual components of inosine pranobex from its degradation products can be difficult, especially when using techniques like HPLC.

6.5. Sensitivity and Detection Limitations

- **UV Detection:** Inosine pranobex may not absorb well in the UV range, making UV-based detection methods (like UV-VIS spectrophotometry or UV-HPLC) less sensitive.
- **Low Signal Response:** The low concentration of active ingredients in pharmaceutical preparations can lead to low signal responses in various detection methods. Enhancing sensitivity may require specialized detection techniques such as mass spectrometry (MS) or fluorescence detection, but these are often more complex and costly.

6.6. Standardization and Reference Materials

- **Lack of Stable Reference Standards:** Obtaining stable, well-characterized reference standards for inosine pranobex and its degradation products can be challenging, especially since the compound may degrade over time. The lack of reliable reference materials can complicate method validation and calibration.
- **Homogeneity of Formulation:** The combination of different active components in a single formulation may lead to heterogeneity in tablet or suspension samples. Achieving uniform extraction and accurate sample preparation can be difficult without optimized protocols.

6.7. Validation and Regulatory Requirements

- **Method Validation:** Analytical methods for inosine pranobex need to meet stringent validation criteria (e.g., accuracy, precision, specificity, linearity, robustness) for regulatory approval. The complexity of the compound and potential for degradation can make method validation more time-consuming and challenging.
- **Regulatory Scrutiny:** Given its use in clinical settings, any analytical method must comply with regulatory guidelines (such as those from the FDA or EMA), which require comprehensive method validation and robust testing. This includes ensuring the method can detect both the active Pharmaceutical ingredients (APIs) and degradation products under varying conditions.

6.8. Quantitative Analysis in Biological Samples

- **Matrix Interference:** Biological samples (such as blood, plasma, or urine) can contain complex matrices that may interfere with the detection of inosine pranobex. This is especially challenging for bioanalytical methods like liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- **Extraction and Sample Preparation:** Efficient extraction and cleanup methods must be developed to isolate inosine pranobex and its metabolites from complex biological matrices without introducing bias or loss of analyte.
- **Sensitivity and Selectivity in Biological Matrices:** Achieving high sensitivity and selectivity when analyzing inosine pranobex in biological matrices is crucial for accurate pharmacokinetic studies, but matrix effects can make these analyses more difficult.

6.9. Interference from Excipients

- **Excipients in Formulation:** Pharmaceutical formulations of inosine pranobex often contain excipients such as fillers, binders, or preservatives, which can interfere with the analysis by contributing to background noise or overlapping signals in spectroscopic methods. Careful separation and optimization are needed to minimize these effects.

6.10. Analytical Techniques to Address Challenges

To overcome some of these challenges, several advanced analytical techniques can be employed.

- **High-Performance Liquid Chromatography (HPLC):** Can be used for the separation of inosine pranobex components, with different detectors like UV, fluorescence, or MS enhancing sensitivity.
- **Mass Spectrometry (MS):** Coupling HPLC with MS (LC-MS) allows for detailed analysis of molecular structure and degradation products.
- **Thin-Layer Chromatography (TLC):** Simple and cost-effective for preliminary qualitative analysis.
- **Capillary Electrophoresis (CE):** Useful for separating and quantifying polar compounds like inosine pranobex.

  Addressing these challenges requires methodical optimization of extraction procedures, chromatographic conditions, and detection strategies, ensuring the accuracy and reliability of the analytical method for inosine pranobex.

7. CONCLUSION

Inosine pranobex (IP), a compound with antiviral and immunomodulatory effects, has demonstrated its potential in treating various viral infections and conditions related to immune dysfunction. Pharmacologically, IP is recognized for its ability to stimulate the immune system, enhance the production of interferon, and boost cellular immunity, making it valuable in treating diseases like herpes simplex virus infections and other viral conditions. Moreover, its antiviral properties extend to improving host defence mechanisms.

In terms of analytical methods, various techniques such as chromatography, spectrophotometry, and high-performance liquid chromatography (HPLC) and LC-MS have been employed for the quantification and analysis of inosine pranobex. These methods are crucial for ensuring the purity, dosage, and overall quality of the drug in Pharmaceutical formulations. The review highlights the importance of robust analytical procedures in maintaining consistency and efficacy in therapeutic use.

In conclusion, inosine Pranobex holds promise as a therapeutic agent with significant antiviral and immunomodulatory effects. Continued research and refinement of both its Pharmacological applications and the analytical methods used to assess its quality are essential for optimizing its clinical use and ensuring patient safety and efficacy.

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REFERENCES

1. Beran J, Spajdel M, Slíva J, “Inosine Pranobex Deserves Attention as a Potential Immunomodulator to Achieve Early Alteration of the COVID-19 Disease Course Viruses”, Drugs, 2021; **13**(11): 2246.
2. Campoli-Richards DM, Sorkin EM, Heel RC, “Inosine Pranobex A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy", Drugs, 1986; **32**(5): 383–424.
3. De Simone C, Famularo G, Tzantzoglou S, Moretti S, Jirillo E, "Inosine Pranobex in the treatment of HIV infection: a review", International Journal of Immunopharmacology, 1991; **13**(Suppl 1): 19–27.
4. Inosine Pranobex. go.drugbank.com. Retrieved 2024-05-02.
5. You Y, Wang L, Li Y, Wang Q, Cao S, Tu Y, "Multicenter randomized study of inosine pranobex versus acyclovir in the treatment of recurrent herpes labialis and recurrent herpes genitalis in Chinese patients", J Dermatol, 2015; **42**(6): 596–601.
6. Campoli-Richards DM, Sorkin EM, Heel RC, "Inosine pranobex. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy", Drugs, 1986; **32**(5): 383–424.
7. Waldman RH, Ganguly R, "Therapeutic efficacy of inosiplex (Isoprinosine) in rhinovirus infection", Ann N Y Acad of Sciences, 1977; **284**(1): 153–160.
8. Gołębiowska-Wawrzyniak M, Markiewicz K, Kozar A, Derentowicz P, Siwińska-Gołębiowska H, “The study on Therapeutic Efficacy of Inosine Pranobex in Children. Polish Journal of Food and Nutrition Sciences”, J Pharm Biomed Anal, 2004; **54**(2s): 33–36.
9. Melekhina E, Muzyka A, Lysenkova M, Gorelov A, “A comparative analysis of therapeutic regimens in children with monthly respiratory infections and reactivation of infection caused by human herpesvirus type 6", J Pharm Biomed Anal, 2018; **13**(5): 74–82.".
10. Hashimoto K, Hosoya M, “Advances in Antiviral Therapy for Subacute Scelerosing panencephalitis”, Drugs, 2021; Molecules. **26**(2): 427.
11. Simunkova M.” Inosine Pranobex www.tribune.cz (in Czech). Retrieved 2024-05-02.
12. Pachuta DM, Togo Y, Hornick RB, Schwartz AR, Tominaga S, “Evaluation of Isoprinosine in experimental human rhinovirus infection”, Antimicrob Agents and Chemothera, 1974; **5**(4): 403–408.
13. Beran J, Šalapová E, Špajdel M, “Inosine Pranobex is safe and effective for the treatment of subjects with confirmed acute respiratory viral infections: analysis and subgroup analysis from a phase 4, randomised, Placbo-controlled, double study”, BMC Infect Dis, 2016; **16**(1): 648.

14. Sliva J, Pantzartzi CN, otava M, "Inosine pranobex: A key Player in the Game Against a Wide Range of Viral infections and Non- infectious Diseases", Adv Ther, 2019; 36(8): 1878–1905.
15. Waldman RH, Ganguly R, "Therapeutic efficacy of inosiplex (Isoprinosine) in rhinovirus infection", Ann N Y Acad Sci, 1977; **284**(1): 153–160.
16. Beran J, Špajdel M, Slíva J, "Inosine Pranobex Deserves Attention as a Potential Imuunomodulator to Achieve Early Alteration of the COVID-19 Disease Course", Viruses, 2021; **13**(11): 2246.
17. Beran J, Spajdel M, Katzerova V, Holousova A, Malys J, Finger Rouskova J, "Inosine Pranobex Significantly Decreased the Case-Fatality Rate among PCR Positive Elderly with SARS-CoV-2 at Three Nursing Homes in the Czech Republic", Pathogens, 2020; **9**(12): 1055.
18. Jayanthi CR, Swain AK, Ganga RT, Halnor D, Avhad A, Khan MS, "Efficacy and Safety of Inosine Pranobex in COVID-19 Patients: A Multicenter phases 3 Randomized Double -Blind Placebo-Controlled Trail", Adv Ther, 2022; **5**(12): 2200159.
19. Indries M, "Clinical and histological correlations in chronic viral hepatitis C.", BMC Infect Dis, 2013; **13**(S1): 52.
20. Pardo M, Carreno V, "Lack of efficacy of inosine pranobex in the treatment of chronic hepatitis C", J Hepatol, 1994; **21**(2): 278.
21. Campling F, Sharpe M, "Some myths about CFS/ME". Chronic fatigue syndrome (CFS/ME)", Oxford University Press Oxford, 2008; 57–60.
22. Huttenlocher PR, Mattson RH, "Isoprinosine in subacute sclerosing panencephalitis", Neurology, 1979; **29**(6): 763–771.
23. Glasky AJ, Gordon JF, "Isoprinosine (inosine pranobex BAN, INPX) in the treatment of AIDS and other acquired immunodeficiencies of clinical importance", Cancer Detection and Prevention. Supplement, 1987; **1**: 597–609.
24. Taghreed A. Mohamed "Validated analytical method development of inosine pranobex in drug products by thin layer chromatography", Sci J Anal Chem, 2014; 2:2(6): 59-66.
25. K, Santhakumari, S. Mohan, K. Prasada Rao, "Validated Method Development of Levamisole and Inosine Pranobex by Using UPLC and Characterisation of Degradants by LC-MS/MS", YMER Digital, 2022; 21(07): 734-44.
26. K. Kafedjiiski, V.Belcheva, M.Radeva-llieva, E.Grigorov "Development of New Formulation of Inosine Acedoben Dimepranol powder for Oral solution in Sachets", Acta Medica Bulgaria, 2024; 51(3).
27. Charushila p. Ahire, "Analytical Method Development Validation and Degradation of Inosine Pranobex by Using RP-HPLC Method", Int J Pharm Res, 2024; 9(04): 1350-1395.
28. Mo chen, Yuan Zhang Xian- Ting Que, Ya Ding, Ling Yang, Ai-Dong Wen, Tai-Jun Hang "Pharmacokinetic Study of Inosiplex tablets in healthy Chinese volunteers by hyphenated HPLC and tandem MS techniques", J Pharm Anal, 2013; 3(6): 387-393.
29. HPLC Separation of Acedoben and Inosine on SHARC 1 Column https: // sielc.com
30. Kovachev SM, Vasilev N, Kovachev D, "Inosine pranobex Immunotheraphy in cervical HPV-positive Patients; An Update", Med Mal Infect, 1990; 20(3): 158-163.
31. Wikipedia Contributors. Inosine Pranobex. https// en.wikipedia.org/wiki/Inosine Pranobex
32. Gedeon Richter Plc. Tablet Formulation containing Inosine Pranobex and Zinc Gluconate. WIPO. https: // patents. google.com/patent /WO2020162773A1
33. European patent office. EP3651803A1 and EP3920892B1 https://www.epo.org
34. Deray G.et al. "Clinical Use and Mechanism of Action of Isoprinosine", Med Mal Infect, 1990; 20(3): 158-163.
35. Stankiewicz A, Gralla E, Wnuk A, "Pharmacokinetics of Inosine Pranobex in Healthy Volunteers Drugs", Exp Clin Res, 1981; 7(07): 499-507.
36. Wasilewska-Bak D, Mikolajczak PL, "Pharmacokinetics and Metabolism of Inosine in Humans", Acta Pol Pharm, 1993; 50(05): 387-391.
37. Sabadashka NV, Pankiv LA, Kurchevskyi IH, "Use of inosine pranobex in viral infections: mechanism and efficacy", Wiad Lek, 2020; 73(2): 274-278.
38. Jin X et al," Immunomodulatory effects of inosine pranobex in vitro and in vivo", Int Immunopharmacol, 2008; 07-18.
39. European Pharmacopeia online. Inosine pranobex monograph. https://www.edqm.eu.
40. Bonato PS, Silva AR, Lanchote VL, "Determination of Purine Metabolites by HPLC in Biological Fluids: A Review, J Chromatogr B, 2002; 781(1-2): 267-296.
41. Pederson C, Sandstrom E, Petersen CS, Norkrans G, Gerstoft J, Karlsson A, Chirstensen KC, Hakansson C, Pehrson PO, Nielsen JO, "The efficacy of inosinr pranobrx in preventing the acquired immunodeficiency syndrome in patient with human Immunodeficiency virus infection", N Engl J Med, 1990; 322(25): 1757-63.
42. Brys M, Salat K, Cegielka A, Skiba A, Giermansinski P, Niewiadomy A, "Development and validation of LC-MS/MS Method for determination of 4- Acetamidobenzoic acid in pig, plasma", Molecules, 2021; 26(15): 4437.
43. Sharma R J , Shelar M, Andhale G, Kadam J, Singh S " HPLC analytical research method development and validation of Inosine pranobex by using the QbD Approach", IJPQA, 2024; 15(4): 2146-2150.