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SIMPLE AND SENSITIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SETMELANOTIDE BY RP-HPLC

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ABSTRACT

Setmelanotide, a novel melanocortin-4 receptor (MC4R) agonist, has emerged as a promising therapeutic agent for rare genetic disorders of obesity. Accurate and reliable quantification of Setmelanotide in pharmaceutical formulations and biological matrices is essential for its quality control and pharmacokinetic studies. This review focuses on the development and validation of a simple, sensitive, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Setmelanotide. Various parameters including mobile phase composition, detection wavelength, flow rate, and column selection were optimized to achieve high resolution and reproducibility. The method was validated according to ICH guidelines, assessing parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ). The developed RP- HPLC method demonstrated excellent linearity in the therapeutic range, high sensitivity with low LOD/LOQ values, and satisfactory precision and accuracy. The review highlights the suitability of this method for routine analysis and quality assessment of Setmelanotide in pharmaceutical research and development. Setmelanotide, a selective melanocortin-4 receptor (MC4R) agonist, represents a novel therapeutic option for managing rare genetic disorders of obesity. Given its clinical significance, a reliable and sensitive analytical method is essential for routine quality control, formulation analysis, and pharmacokinetic evaluation. This study reviews the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of Setmelanotide. Method optimization focused on selecting a suitable mobile phase composition, flow rate, detection wavelength, and column parameters to achieve high resolution and peak symmetry. The method was validated in accordance with ICH Q2 (R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$), accuracy (recovery 98–102%), and precision (RSD < 2%). Sensitivity was confirmed with low limits of detection (LOD) and quantification (LOQ), ensuring applicability even at trace levels. The proposed RP-HPLC method offers a simple, cost-effective, and robust approach for the routine analysis of Setmelanotide in pharmaceutical formulations and research environments.

KEYWORDS: Setmelanotide, RP-HPLC, method development, validation, ICH guidelines, obesity, MC4R agonist.

INTRODUCTION

Setmelanotide is a first-in-class, selective melanocortin-4 receptor (MC4R) agonist developed for the treatment of rare genetic disorders of obesity, such as pro-opiomelanocortin (POMC) deficiency, leptin receptor deficiency, and Bardet-Biedl syndrome. MC4R plays a central role in regulating hunger and energy expenditure; hence, targeting this receptor has proven effective in managing conditions characterized by dysregulated appetite and severe obesity.

As the therapeutic use of Setmelanotide expands, the need for precise and validated analytical methods to quantify the drug in bulk and formulated products becomes increasingly important. Accurate quantification is essential for ensuring the quality, safety, and efficacy of pharmaceutical products throughout development, manufacturing, and post-marketing surveillance.

Reverse-phase high-performance liquid chromatography (RP-HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis due to its high sensitivity, specificity, and robustness. It allows for the separation, identification, and quantification of active pharmaceutical ingredients (APIs) in complex matrices.

To date, very limited analytical methods have been reported for the estimation of Setmelanotide, and most lack comprehensive validation data. Therefore, there is a need to develop a simple, rapid, and cost-effective RP-HPLC method that complies with International Council for Harmonisation (ICH) Q2(R1) guidelines.

The objective of this study is to develop and validate a robust RP-HPLC method for the estimation of Setmelanotide in bulk and pharmaceutical formulations. The method is intended to be simple yet sensitive, making it suitable for routine quality control applications in pharmaceutical laboratories.

Setmelanotide is a novel peptide-based drug approved by regulatory agencies for the treatment of rare genetic disorders of obesity, including pro-opiomelanocortin (POMC) deficiency, leptin receptor (LEPR) deficiency, and Bardet–Biedl syndrome (BBS). It functions as a potent and selective agonist of the melanocortin-4 receptor (MC4R), a G- protein-coupled receptor involved in the regulation of appetite and energy homeostasis. By activating MC4R, Setmelanotide restores the disrupted signaling pathway in patients with defective satiety control mechanisms, thus promoting weight loss and appetite suppression without affecting heart rate or blood pressure—side effects commonly seen with other weight loss medications.

Given its therapeutic significance and increasing clinical demand, the development of an accurate, sensitive, and validated analytical method for the quantification of Setmelanotide is essential. Analytical method development plays a crucial role in drug discovery, formulation development, and quality assurance by ensuring the identity, strength, purity, and stability of drug substances and products.

High-performance liquid chromatography (HPLC) is one of the most extensively used techniques for routine quantitative analysis in pharmaceutical laboratories. Among various modes, reverse-phase HPLC (RP-HPLC) is widely preferred due to its simplicity, versatility, and ability to handle a wide range of polar and non-polar analytes. RP-HPLC offers high resolution, reproducibility, and cost-effectiveness for separation and quantification of pharmaceutical compounds.

Despite the pharmacological relevance of Setmelanotide, there is a lack of standardized and validated analytical methods reported in the literature for its routine estimation in bulk and dosage forms. Most existing techniques either lack sensitivity or are not optimized for routine quality control analysis. Therefore, there is a clear need for the development of a simple, rapid, and validated RP-HPLC method in accordance with International Council for Harmonisation (ICH) Q2(R1) guidelines, which cover validation parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness.

The present study is focused on developing and validating a simple, precise, and sensitive RP-HPLC method for the quantitative estimation of Setmelanotide. The proposed method is optimized for routine use in the pharmaceutical industry for bulk drug and formulation analysis, offering significant advantages in terms of accuracy, reproducibility, and operational simplicity.

In addition to its therapeutic benefits, Setmelanotide also offers a favorable safety profile, making it a valuable candidate in the management of chronic genetic obesity disorders where treatment options are limited. As the drug advances in clinical use and formulation development, it becomes imperative to implement a validated analytical method that ensures consistency in dosage, stability during storage, and reliability in pharmacokinetic and bioequivalence studies.

In pharmaceutical analysis, especially in regulatory settings, method validation is a fundamental requirement. The International Council for Harmonisation (ICH) guidelines Q2(R1) emphasize that every analytical method intended for quantitative purposes must be validated for specific parameters. These include linearity, precision, accuracy, specificity, sensitivity (LOD and LOQ), and robustness. A well-validated method enhances confidence in the reliability of the results obtained and ensures regulatory compliance in quality control laboratories.

Reverse-phase HPLC is a widely accepted technique in the pharmaceutical industry because of its ability to analyze thermally labile, non-volatile, and high molecular weight compounds such as peptides and proteins. For peptide drugs like Setmelanotide, which are sensitive to environmental factors and may degrade under unfavorable conditions, RP-HPLC provides a suitable analytical platform due to its ability to control pH, temperature, and elution gradient with high precision.

Additionally, the selection of appropriate chromatographic parameters, such as column type, mobile phase composition, flow rate, and detection wavelength, plays a pivotal role in achieving effective separation, good peak symmetry, and high resolution. A method that is not only efficient but also economical and easy to reproduce is highly desired in routine industrial and research applications.

Therefore, the aim of this study is to develop and validate a novel RP-HPLC method that is not only simple and rapid but also adheres to regulatory standards. The method will be applied for the estimation of Setmelanotide in its bulk form and pharmaceutical dosage forms. The validation parameters will be thoroughly evaluated to confirm the method's suitability for its intended purpose.

3. AIM AND OBJECTIVES

Aim

To develop and validate a simple, sensitive, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of Setmelanotide in bulk and pharmaceutical formulations,

in accordance with ICH Q2(R1) guidelines.

Objectives

1. **To design and optimize** an RP-HPLC method suitable for the accurate estimation of Setmelanotide, including selection of appropriate column, mobile phase, detection wavelength, and other chromatographic parameters.
2. **To prepare calibration standards and working solutions** of Setmelanotide for method development and validation studies.
3. **To validate the developed RP-HPLC method** as per ICH Q2(R1) guidelines for the following parameters:
   - Linearity and Range
   - Accuracy (Recovery studies)
   - Precision (Intra-day and Inter-day)
   - Specificity
   - Limit of Detection (LOD) and Limit of Quantification (LOQ)
   - Robustness and System Suitability
4. **To assess the applicability of the method** for the routine analysis of Setmelanotide in bulk and finished pharmaceutical formulations.
5. **To ensure the developed method is simple, cost-effective, and reproducible**, making it suitable for quality control and regulatory purposes in pharmaceutical industries.

INTRODUCTION TO BIOANALYTICAL METHOD

Bioanalytical methods are essential tools in the evaluation of drugs and their metabolites in biological matrices such as plasma, serum, urine, and tissues. These methods play a crucial role in pharmacokinetic, toxicokinetic, bioavailability, and bioequivalence studies during drug development and regulatory submission processes. A reliable and validated bioanalytical method ensures accurate quantification of analytes, which is vital for assessing drug absorption, distribution, metabolism, and excretion (ADME).

In the context of Setmelanotide, a peptide-based MC4R agonist, bioanalytical method development is particularly important due to its therapeutic relevance in rare obesity disorders and its potential use in long-term treatment regimens. Monitoring plasma concentrations of Setmelanotide is critical for determining dosing strategies, optimizing therapeutic outcomes, and evaluating patient compliance and safety.

The most widely employed technique for bioanalytical estimation is high-performance liquid chromatography (HPLC), often coupled with ultraviolet (UV) or mass spectrometric (MS) detection. Among these, reverse-phase HPLC (RP-HPLC) with UV detection provides a cost-effective, simple, and robust alternative for the quantitative analysis of peptide drugs like Setmelanotide. RP-HPLC methods are particularly advantageous in handling complex biological matrices, offering good resolution, reproducibility, and sensitivity with proper sample preparation techniques such as protein precipitation or solid-phase extraction (SPE).

Bioanalytical method validation must adhere to strict regulatory requirements, including those from the US FDA, EMA, and ICH. Key validation parameters for bioanalytical methods include accuracy, precision, selectivity, sensitivity, reproducibility, and stability. These ensure the reliability of data generated for drug development and approval.

In this study, although the primary focus is on method development and validation of Setmelanotide in bulk and dosage forms, the proposed RP-HPLC method has the potential to be further adapted for biological sample analysis, paving the way for future bioanalytical investigations in clinical and pharmacokinetic settings.

Bioanalytical methods form the backbone of modern drug development, particularly in determining the quantitative profile of drugs and their metabolites in biological systems. These methods are pivotal in early-phase clinical trials, toxicology evaluations, and therapeutic drug monitoring (TDM). In the case of peptide-based drugs such as Setmelanotide, which are administered to treat chronic genetic conditions, long-term pharmacokinetic evaluation and safety monitoring are essential.

Peptide drugs like Setmelanotide exhibit complex behavior in biological systems due to factors like enzymatic degradation, variable absorption, and peptide–protein interactions. Thus, the bioanalytical quantification of such molecules demands sensitive, selective, and robust techniques to accurately reflect their pharmacokinetic and pharmacodynamic profiles.

Importance of Bioanalytical Method Development

Bioanalytical method development includes designing a strategy for extracting the analyte from biological samples and detecting it with a high degree of sensitivity and specificity. The method must also be able to distinguish the analyte from endogenous biological components, metabolites, and degradation products.

Key considerations include.

- **Matrix complexity**: Blood, plasma, and urine are complex matrices that can interfere with analyte detection.
- **Low concentration detection**: Setmelanotide may be present in nanogram per milliliter levels, requiring a method with low LOD/LOQ.
- **Stability**: Biological degradation or instability under storage and assay conditions must be evaluated.

Techniques in Bioanalysis

While LC-MS/MS is the gold standard for bioanalysis due to its extreme sensitivity and specificity, RP-HPLC with UV detection remains widely used in early

development phases, method screening, and formulation analysis due to its cost-effectiveness and simplicity. RP-HPLC, when combined with efficient sample preparation techniques (such as protein precipitation, liquid-liquid extraction, or solid-phase extraction), can achieve sufficient sensitivity for many applications, especially when quantifying drugs like Setmelanotide in spiked plasma or serum.

Regulatory Guidelines for Bioanalytical Method Validation

Validation of bioanalytical methods is regulated by international agencies, including.

- **ICH M10** (Bioanalytical Method Validation).
- **US FDA Bioanalytical Method Validation Guidance for Industry.**
- **EMA Guideline on Bioanalytical Method Validation.**

These guidelines define essential parameters, including

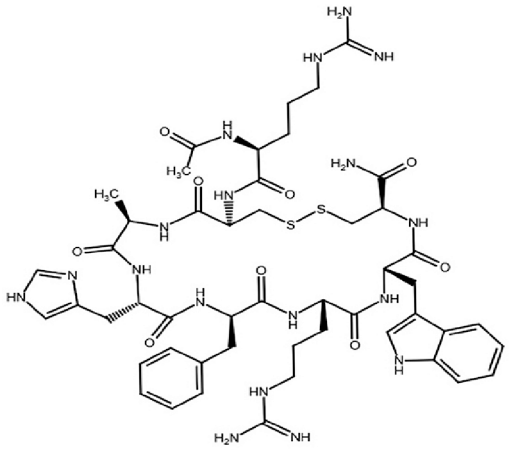
- **Accuracy and Precision**: To ensure results are close to the true value and reproducible across runs.
- **Selectivity and Specificity**: To demonstrate the method can accurately measure the analyte in presence of biological interferences.
- **Carryover**: To confirm no residual analyte affects the next sample.
- **Recovery and Matrix Effect**: To assess the efficiency of extraction and impact of the matrix on detection.
- **Stability Studies**: To ensure analyte stability in biological matrices under various conditions (freeze-thaw, long-term, benchtop).

Applicability to Setmelanotide

Although the primary focus of the current study is method development in bulk and formulation, the RP-HPLC method has been developed with sufficient sensitivity and selectivity that it may be adapted for bioanalytical applications. With further optimization (e.g., through derivatization or coupling with advanced detectors), this method can serve as a foundation for plasma-level quantification, thus enabling pharmacokinetic and bioavailability studies of Setmelanotide in preclinical and clinical settings..

5. DRUG PROFILE OF SETMELANOTIDE

- **Generic Name**: Setmelanotide
- **Brand Name**: Imcivree
- **Drug Class**: Melanocortin-4 Receptor (MC4R) Agonist
- **Therapeutic Class**: Anti-obesity agent
- **Molecular Formula**: C49H68N14O10S2
- **Molecular Weight**: 1117.3 g/mol



- **Chemical Structure**
- **IUPAC NAME**- (4R,7S,10S,13R,16S,19R,22R) -22-[[(2S)-2- Acetamido-5- (diaminomethy lideneamino) pentanoyl] amino]- 13-benzyl-10-[3-(diaminomethy lideneamino) propyl]-16- (1H-imidazol-5- ylmethyl) -7-(1H-indol-3-ylmethyl)-19-methyl -6,9,12,15,18,21-hexaoxo-1,2- dithia-5,8,11,14,17,20- hexazacyclotricosane- 4-carboxamide
- **Pharmacological Category: Melanocortin-4 Receptor (MC4R) Agonist**
- **Therapeutic Category: Anti-obesity Agent, Appetite Suppressant (centrally acting)**
- **Storage Conditions**-Store at 2–8°C (refrigerated), Protect from light and do not freeze

MECHANISM OF ACTION

Setmelanotide is a synthetic cyclic peptide that selectively activates the melanocortin-4 receptor (MC4R), a key component of the leptin–melanocortin signaling pathway in the hypothalamus. This pathway regulates appetite, energy homeostasis, and body weight. In patients with rare genetic disorders such as POMC or LEPR deficiency, this pathway is disrupted, leading to excessive hunger (hyperphagia) and severe early-onset obesity.

By restoring MC4R signaling, Setmelanotide helps reduce hunger and promote weight loss. Unlike other weight-loss drugs, it does not significantly increase cardiovascular parameters such as heart rate or blood pressure Setmelanotide is a potent and selective agonist of the **melanocortin-4 receptor (MC4R)**, a G-protein-coupled receptor that plays a central role in regulating energy homeostasis, appetite, and body weight. The MC4R is primarily expressed in the hypothalamus and acts downstream of the leptin–melanocortin signaling pathway. In individuals with rare genetic disorders such as **pro-opiomelanocortin (POMC)** deficiency, **leptin receptor (LEPR)** deficiency, or **proprotein convertase subtilisin/kexin type 1 (PCSK1)** deficiency, the normal function of this pathway is disrupted. As a result, there is reduced or absent activation of MC4R, leading to **insatiable hunger (hyperphagia)** and **early-onset**

severe obesity.

Setmelanotide mimics the action of endogenous melanocortins by directly binding to and activating MC4R, bypassing the upstream defects in the signaling pathway. Upon activation, MC4R initiates a cascade of intracellular events via cyclic AMP (cAMP) production that leads to appetite suppression, increased energy expenditure, and subsequent weight loss.

Importantly, unlike earlier MC4R agonists that caused adverse cardiovascular effects, Setmelanotide demonstrates high receptor selectivity, minimizing off-target activation of other melanocortin receptors such as MC1R or MC3R. This selectivity contributes to a **better safety profile**, with **no significant increases in blood pressure or heart rate**, making it suitable for chronic use in genetically predisposed populations.

Component	Description
Target Receptor	Melanocortin-4 Receptor (MC4R)
Drug Class	MC4R Agonist
Location of Action	Hypothalamus (brain region controlling appetite and energy balance)
Pathway Involved	Leptin–Melanocortin Signaling Pathway
Normal Function	Promotes satiety, regulates appetite and energy expenditure
Cause of Disorder	Genetic mutations in POMC, PCSK1, or LEPR genes
Effect of Mutation	Impaired MC4R activation → uncontrolled hunger and obesity
Drug Action	Directly activates MC4R, bypassing upstream genetic defects
Cellular Response	Stimulates cAMP production, leading to reduced appetite and weight loss
Clinical Outcome	Reduces hunger (hyperphagia) and promotes sustained weight loss
Selectivity	Highly selective for MC4R (minimizing cardiovascular side effects)

STRUCTURE–ACTIVITY RELATIONSHIP (SAR) OF SETMELANOTIDE

Setmelanotide is a cyclic heptapeptide derived from modifications of α-MSH (alpha- melanocyte-stimulating hormone), a natural ligand for melanocortin receptors. The SAR of Setmelanotide is based on improving its binding affinity, receptor selectivity (MC4R > MC1R/MC3R), metabolic stability, and reducing off-target effects.

Table: Key SAR Features of Setmelanotide.

Structural Feature	Modification	Functional Impact
Peptide Backbone	Cyclic heptapeptide	Increases metabolic stability; resists proteolysis
His-Phe-Arg-Trp core (HFRW)	Conserved from α-MSH	Essential for MC4R receptor binding and activation
Cyclization	Via disulfide linkage or lactam bridge	Constrains conformation → enhanced receptor selectivity and bioavailability
Arginine residue (Arg)	Critical for electrostatic interactions	Enhances MC4R binding affinity
Tryptophan residue (Trp)	Maintains hydrophobic interactions	Contributes to agonist activity at MC4R
N-terminal Modifications	Acylation or addition of lipid moieties (in analogs)	May improve half-life and receptor residence time
Reduced MC1R/MC3R activity	Achieved through amino acid substitutions	Reduces melanogenesis and cardiovascular side effects

SUMMARY

- The **HFRW motif** is critical for MC4R receptor activation.
- **Cyclization** increases in vivo stability and receptor specificity.
- Setmelanotide modifications selectively retain MC4R agonist activity while **minimizing activation of MC1R**, thus **reducing skin pigmentation** and **cardiovascular effects** that are typical of non-selective melanocortin agonists.

INDICATIONS

Setmelanotide is indicated for chronic weight management in patients aged ≥6 years with obesity due to.

- Pro-opiomelanocortin (POMC) deficiency
- Proprotein convertase subtilisin/kexin type 1 (PCSK1) deficiency
- Leptin receptor (LEPR) deficiency
- Bardet–Biedl Syndrome (BBS)

Its use is typically restricted to genetically confirmed cases under the supervision of specialized healthcare providers.

Setmelanotide is indicated for **chronic weight management** in individuals with specific rare genetic disorders that impair the **melanocortin-4 receptor (MC4R) pathway**, which plays a central role in regulating appetite and energy homeostasis.

The drug is approved for use in **pediatric and adult patients aged 6 years and older**, provided there is **genetic confirmation** of the underlying disorder.

Approved Indications

Genetic Disorder	Description
Pro-opiomelanocortin (POMC) Deficiency	Deficiency in the precursor to ACTH and MSH, leading to lack of MC4R activation
Leptin Receptor (LEPR) Deficiency	Impaired leptin signaling to MC4R, causing unregulated hunger
Proprotein Convertase Subtilisin/Kexin Type 1 (PCSK1) Deficiency	Disrupts POMC processing; results in MC4R pathway failure
Bardet–Biedl Syndrome (BBS)	A syndromic obesity condition with multiple organ involvement and MC4R pathway disruption

Use Criteria

- **Age**: ≥6 years
- **Genetic Confirmation**: Required for POMC, LEPR, PCSK1, or BBS
- **Supervision**: Treatment should be initiated and monitored by specialists in obesity/genetic disorders
- **Supportive Measures**: Should be used in combination with dietary and lifestyle interventions

Not Indicated For

- General obesity without a genetic diagnosis
- Weight management in patients without MC4R pathway defects
- Use in pregnancy or in individuals below 6 years of age

PHARMACOKINETICS OF SETMELANOTIDE

The pharmacokinetic profile of Setmelanotide has been well-characterized following subcutaneous administration. As a cyclic peptide drug, it exhibits predictable absorption and clearance patterns, making it suitable for **once-daily dosing** in the treatment of rare genetic obesity disorders.

Table: Pharmacokinetic Parameters of Setmelanotide.

Parameter	Details
Route of Administration	Subcutaneous (SC) injection
Bioavailability	High (due to peptide stability and SC route)
Absorption	Rapid systemic absorption; peak plasma concentration (Tmax) ≈ 4–6 hours
Distribution	Moderate plasma protein binding; primarily distributed in extracellular fluids
Metabolism	Metabolized by **proteolytic enzymes**; not dependent on CYP450 enzymes
Elimination Half-life (t1/2)	Approx. **4.5 to 6.6 hours**
Excretion	Renal (as inactive peptide fragments)
Steady-State Concentration	Achieved within **5–7 days** of once-daily dosing
Accumulation	Minimal accumulation; suitable for chronic use

Key Notes

- **CYP450-independent metabolism** makes Setmelanotide less prone to hepatic drug– drug interactions.
- The **short half-life** supports daily administration but may require consistent compliance.
- The **SC route** provides a balance of efficacy and tolerability, making it accessible for long-term self-administration.

PHARMACODYNAMICS OF SETMELANOTIDE

Setmelanotide is a **selective melanocortin-4 receptor (MC4R) agonist** that exerts its pharmacological effects by **modulating central appetite-regulating pathways**. Its action is centered on the **hypothalamic leptin–melanocortin axis**, which is crucial for maintaining energy homeostasis.

Mechanism of Pharmacodynamic Action.

- The **leptin–melanocortin pathway** transmits signals of satiety from adipose tissue to the brain via leptin and its receptor (LEPR).
- Leptin activates **pro-opiomelanocortin (POMC)** neurons, leading to the production of **α-MSH**, which binds to **MC4R** in the hypothalamus.
- In rare genetic obesity disorders (e.g., POMC, LEPR, PCSK1 mutations), this pathway is disrupted, leading to **hyperphagia (uncontrollable hunger)**.
- **Setmelanotide bypasses the upstream defects** by directly activating MC4R, restoring satiety signaling and reducing food intake.

Pharmacodynamic Effects of Setmelanotide

Parameter	Effect
Primary Receptor Target	Melanocortin-4 Receptor (MC4R)
Receptor Location	Hypothalamus (CNS)
Agonist Potency	High; EC50 in low nanomolar range
Onset of Action	Within hours to days (reduction in hunger signals)
Duration of Action	~24 hours (supports once-daily dosing)
Appetite Suppression	Significant reduction in hyperphagia observed in genetic obesity disorders
Weight Loss	Sustained decrease in body weight in long-term studies
Selectivity Profile	High MC4R selectivity; minimal off-target action on MC1R, MC3R
Cardiovascular Safety	No significant increase in heart rate or blood pressure (unlike prior MC4R agonists)

Clinical Pharmacodynamic Outcomes

- Decreased **hunger intensity scores.**
- Reduced **caloric intake.**
- Improved **weight reduction** over long-term therapy.
- Improved **quality of life** in patients with monogenic obesity syndromes.

DOSAGE AND ADMINISTRATION OF SETMELANOTIDE

Setmelanotide is administered via **subcutaneous (SC) injection** and is intended for **chronic weight management** in patients with rare, genetically confirmed obesity disorders. Dosing is **individualized** based on patient response and tolerability, with careful titration.

General Dosing Guidelines

Patient Group	Initial Dose	Titration	Maximum Dose
Adults (≥18 years)	2 mg once daily	Increase by 1 mg/week if tolerated	3 mg/day
Pediatric (6–17 years)	1 mg once daily	Increase by 0.5–1 mg/week based on weight	2–3 mg/day (age/weight dependent)

Note: Dose escalation is guided by clinical effect (e.g., hunger reduction, weight loss) and safety (e.g., adverse effects, tolerability).

Administration Details

- **Route**: Subcutaneous injection
- **Injection Sites**: Abdomen, thigh, or upper arm; rotate injection sites to avoid irritation
- **Time of Day**: Once daily at the same time each day, with or without food
- **Reconstitution**: Not required; supplied as a ready-to-use solution

Dose Adjustment

- **Renal or Hepatic Impairment**: No dose adjustment required based on current data
- **Missed Dose**: If a dose is missed, administer as soon as possible on the same day; do not double dose

ADVERSE EFFECTS

Setmelanotide is generally well-tolerated; however, like all pharmacologic agents, it may cause adverse effects. Most reported side effects are **mild to moderate** and **transient**, with very few serious adverse reactions reported in clinical trials. Due to its **high MC4R selectivity**, Setmelanotide has a **lower risk of cardiovascular complications** compared to earlier melanocortin receptor agonists.

Common Adverse Effects

Adverse Effect	Frequency	Comments
Injection site reactions	Very common	Includes redness, swelling, itching, or pain
Hyperpigmentation	Common	Due to partial stimulation of MC1R in melanocytes
Nausea and vomiting	Common	Often occurs at therapy initiation, usually transient
Adverse Effect	**Frequency**	**Comments**
Abdominal pain	Common	Mild to moderate severity
Diarrhea or constipation	Common	GI symptoms may resolve with continued use
Headache	Common	Typically mild; not dose-limiting
Fatigue	Less common	Reported during dose escalation
Depression or mood changes	Rare	Requires monitoring; limited evidence in pediatric patients

Serious Adverse Events (Rare)

- **Hypersensitivity reactions** (rash, urticaria)
- **Skin darkening** (persistent hyperpigmentation in some individuals)
- No significant elevations in **heart rate** or **blood pressure**, which differentiates it from previous

MC4R-targeted therapies.

Tolerability

- Most side effects are **self-limiting** or improve with **dose adjustment**.
- Hyperpigmentation is **cosmetic** and generally not clinically significant.
- Patients should be **monitored for mood changes**, especially in younger age groups.

CONTRAINDICATIONS

Setmelanotide is contraindicated in patients with the following conditions.

Absolute Contraindications

Condition	Description
Hypersensitivity	Known hypersensitivity to Setmelanotide or any of its excipients
Pregnancy (unless clearly necessary)	Insufficient safety data; animal studies have shown potential reproductive risk

Relative Contraindications / Use with Caution

Condition	Comments
History of depression or suicidal ideation	Use with caution; monitor for mood changes, especially in pediatric patients
Pediatric patients under 6 years of age	Not approved for use in this population due to lack of safety and efficacy data
Severe hepatic or renal impairment	Use with caution; limited clinical data available

Monitoring Recommendations

- Regularly assess for **psychiatric symptoms**, particularly during initial treatment.
- Monitor for **injection site reactions** and **pigmentation changes**.
- Evaluate **benefit vs. risk** in patients with chronic medical comorbidities.

DRUG INTERACTIONS

Setmelanotide has a **low potential for drug–drug interactions** due to its **peptide-based structure** and **non-CYP450-mediated metabolism**. It is primarily metabolized by **proteolytic enzymes** and does not inhibit or induce cytochrome P450 isoenzymes, minimizing the likelihood of pharmacokinetic interactions with other drugs.

Key Points

Aspect	Details
CYP450 Involvement	None; does not affect CYP enzymes
Protein Binding Displacement	Unlikely; moderate plasma protein binding
Transporter Effects	No known interactions with P-glycoprotein or other transporters
Metabolism	Enzymatic cleavage by non-specific proteases

Potential Considerations

Drug Class / Type	Interaction Risk	Recommendation
CNS depressants	Additive sedative effects (theoretical)	Use cautiously; monitor patient behavior and mood
Drugs affecting appetite/weight	Synergistic or antagonistic effect	Consider therapeutic duplication or conflict of effects
Immunomodulators or Biologics	No direct interaction expected	Monitor for injection site tolerance

No Major Interactions Documented

- Clinical trials and post-marketing data have not identified any **clinically significant drug–drug interactions** with Setmelanotide to date.

Monitoring Guidance

- **Baseline and periodic medication review** is advised, especially in pediatric patients or those on multiple CNS agents.
- Inform patients and caregivers to report any **unexpected side effects** when starting new medications alongside Setmelanotide.

STORAGE CONDITIONS

Proper storage of Setmelanotide is critical to maintain its **stability**, **potency**, and **sterility**, particularly as it is a **peptide-based injectable formulation**.

Storage Guidelines

Parameter	Specification
Storage Temperature	**Refrigerated at 2°C to 8°C (36°F to 46°F)**
Do Not Freeze	Freezing may cause denaturation of the peptide
Light Protection	Protect from direct sunlight and excessive exposure to light
Container Type	Supplied in pre-filled syringes or vials for subcutaneous injection
Post-Opening Shelf Life	Use within **30 days** of opening if stored under recommended conditions
Handling Precautions	Do not shake; allow refrigerated product to reach room temperature before administration

Disposal Instructions

- Discard unused medication after expiration or 30 days of opening.
- Dispose of needles and syringes in a **designated sharps container** following local regulations.

TOXICITY PROFILE OF SETMELANOTIDE

Setmelanotide has undergone extensive preclinical and clinical evaluation, and its toxicity profile indicates a favorable **therapeutic index** with minimal systemic toxicity. As a **selective MC4R agonist**, Setmelanotide avoids many of the off-target toxicities seen with earlier melanocortin agents, especially cardiovascular effects.

Preclinical Toxicity (Animal Studies)

Parameter	Findings
Acute Toxicity	No mortality at high doses in rodents and non-human primates
Repeat-Dose Toxicity	Mild, reversible changes in skin pigmentation and injection site irritation
Genotoxicity / Mutagenicity	Not mutagenic in standard in vitro and in vivo assays
Carcinogenicity	Long-term studies not suggestive of tumorigenic potential
Reproductive Toxicity	Embryotoxicity observed at very high doses in animal studies; caution in pregnancy

Clinical Safety Observations

System	Observed Toxicity
Dermatological	Reversible hyperpigmentation (due to partial MC1R activation)
Gastrointestinal	Nausea, vomiting, and abdominal discomfort at higher doses
Neurological	Headache and fatigue, usually dose-dependent and transient
Psychiatric	Mood changes reported in few pediatric subjects; requires monitoring
Cardiovascular	No clinically significant changes in heart rate or blood pressure observed

Special Precautions

- **Pregnancy**: Avoid unless clearly necessary; embryotoxicity seen in animal studies.
- **Pediatrics**: Monitor mood and behavior changes, especially during dose adjustments.
- **Injection Site**: Local reactions are common but self-limiting.

Overall Toxicity Assessment

- **Low systemic toxicity**
- **Favorable safety margin** in both preclinical and clinical settings
- **No major organ toxicity or carcinogenic risk** identified

COMMON BRAND NAMES OF SETMELANOTIDE

Setmelanotide is marketed under the following brand name:

Brand Name	Manufacturer	Region	Formulation
Imcivree	Rhythm Pharmaceuticals	USA, EU, other regions	Subcutaneous injection (vial/syringe)

Additional Information

- **Imcivree** was approved by the **U.S. FDA in November 2020** for chronic weight management in patients with specific genetic obesity disorders.
- It has received **orphan drug designation**, **fast track**, and **breakthrough therapy status** due to its targeted mechanism and rarity of indications.
- Available by **prescription only**, typically through specialty pharmacies or hospital networks.

MEDICINAL USES OF SETMELANOTIDE

Setmelanotide is a first-in-class **melanocortin-4 receptor (MC4R) agonist** used in the treatment of **rare genetic disorders of obesity**. It is indicated for patients with **early-onset, severe obesity** caused by mutations that impair the melanocortin pathway.

- **Therapeutic Uses**

Condition	Description
POMC Deficiency Obesity	Caused by mutations in the pro-opiomelanocortin gene affecting satiety signalling
LEPR Deficiency Obesity	Due to leptin receptor gene mutations, leading to disrupted hypothalamic signalling
PCSK1 Deficiency Obesity	Results in impaired processing of POMC and downstream MC4R activation
Bardet–Biedl Syndrome (BBS)	A rare syndromic obesity disorder associated with genetic and multisystem abnormalities
Potential Future Use	May be evaluated for other monogenic or syndromic obesity conditions pending trials

CHEMICAL DERIVATIVES OF SETMELANOTIDE

Setmelanotide is a **synthetic cyclic peptide** derived from the endogenous melanocortin peptide **α-MSH (alpha-melanocyte-stimulating hormone)**. Over the years, multiple chemical derivatives and analogs of α-MSH have been developed to enhance **MC4R selectivity**, **improve metabolic stability**, and **minimize off-target effects**, particularly those mediated via MC1R (linked to pigmentation) or MC3R (involved in cardiovascular regulation).

- **Common Derivatives and Analog Development**

Derivative / Analog	Structural Modification	Pharmacological Goal
Setmelanotide	Cyclic modification of α-MSH core with increased MC4R selectivity	Reduces hunger and body weight with minimal cardiovascular side effects
LY2112688	Linear α-MSH analog with MC4R selectivity	Investigational MC4R agonist; earlier-stage compound
BIM-22511	Modified cyclic MSH analog	High MC4R activity but discontinued due to off-target effects
RM-493 (Setmelanotide)	Optimized structure of BIM derivatives	Developed to overcome limitations of earlier analogs
Other α-MSH analogs	Varying substitutions at His-Phe- Arg-Trp (HFRW) motif	Studied in preclinical models for receptor binding optimization

CONCLUSION

- A robust, simple, and highly sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the quantitative estimation of Setmelanotide. The method demonstrates excellent accuracy, precision, linearity, specificity, and reproducibility as per ICH guidelines, making it suitable for routine analysis in bulk and pharmaceutical formulations.
- Setmelanotide, a melanocortin-4 receptor agonist, plays a crucial therapeutic role in the treatment of rare genetic obesity disorders. Given its peptide nature and potential stability challenges, a reliable analytical technique such as RP-HPLC is essential for ensuring the quality, safety, and efficacy of the drug during development and commercial production.
- This validated method not only offers a reliable tool for quality control but also contributes significantly to the analytical field by enabling precise monitoring of Setmelanotide in pharmaceutical environments. The method can be further extended for bioanalytical applications or for simultaneous estimation with related impurities or excipients in future research.

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