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A COMPREHENSIVE REVIEW OF ANALYTICAL METHODS FOR THE QUANTIFICATION OF METHIMAZOLE IN MULTI-MATRIX

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ABSTRACT

This review critically examines the analytical methodologies developed for the determination of methimazole (MMZ) across pharmaceutical, biological, and food matrices. We summarize classical approaches—such as UV-Visible spectrophotometry, HPLC-UV, and TLC—and compare them with advanced techniques including molecularly imprinted polymer solid-phase extraction, LC-MS/MS, and surface-enhanced Raman spectroscopy (SERS). Key performance metrics (linearity, limits of detection and quantification, precision) are tabulated to facilitate direct comparison and these methods offer advantages like low solvent usage, improved selectivity, and better analyte enrichment. Finally, we discuss green-chemistry trends and propose future research directions aimed at simplifying sample preparation, reducing solvent consumption, and enhancing method sensitivity.

Keywords: Electrochemical detection, HPLC-UV detection, LC-MS/MS, Solid-phase extraction, Methimazole analysis, Molecular characterization, Therapeutic drug profiling, UV-Visible spectrophotometry

INTRODUCTION:

Methimazole (MMZ), also known as 1-methyl-2-mercaptoimidazole, is a widely used antithyroid agent primarily prescribed for managing hyperthyroidism, particularly in conditions like Graves' disease **[1]**. It functions by inhibiting thyroid peroxidase, an enzyme crucial for the synthesis of thyroid hormones T3 and T4. Although 79.2% of orally administered MMZ is excreted through urine, the remaining drug is retained in tissues, posing potential health risks due to its cytotoxic metabolites, such as N-methylimidazole and sulphite

[2]. **Figure 1** represents the chemical structure of Methimazole.

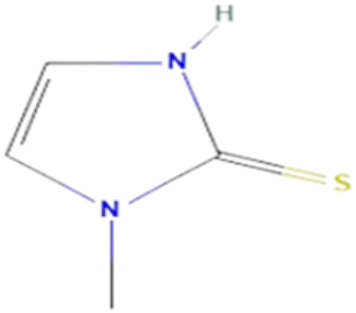

Figure 1: Chemical Structure of Methimazole

The use of MMZ has been banned in food-producing animals in many countries, including the European Union, due to its illegal application to promote weight gain, which may lead to serious health hazards if it enters the human food chain. Thus, accurate and sensitive analytical methods for detecting MMZ residues in various biological and food matrices are critical for public health **[5]**.

A range of analytical techniques have been employed for MMZ determination, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography–mass spectrometry (GC–MS), and electrochemical methods. However, many of these approaches lack sufficient sensitivity, require complex sample preparation, or are costly and unsuitable for routine analysis **[1]**.

To address these limitations, advanced techniques such as molecularly imprinted polymers (MIPs), solid-phase extraction (SPE), and micro extraction methods including hollow-fiber liquid-phase micro extraction (HF-LPME) have been explored. These methods offer advantages like low solvent usage, improved selectivity, and better analyte enrichment. Additionally, electroanalytical and polarographic techniques are being considered as cost-effective, sensitive alternatives due to their minimal sample preparation and quick analysis times.

Furthermore, surface-enhanced Raman scattering (SERS) using semiconductor-metal hybrid sensors, such as SnS_2/AuNPs, has emerged as a powerful technique for rapid, sensitive, and selective detection of MMZ residues in complex samples like serum and meat **[12]**.

Overall, the development of simple, affordable, and highly sensitive analytical methods for MMZ quantification remains a significant focus in pharmaceutical and food safety research. This review highlights various contemporary analytical strategies, their challenges, and advancements in the determination of methimazole across diverse matrices.

ANALYTICAL TECHNIQUES:

The accurate and reliable quantification of Methimazole (MMI) in pharmaceutical formulations is essential for ensuring product quality, safety, and compliance with regulatory standards. Various

analytical techniques have been developed and validated to assess the content, stability, and purity of methimazole in tablets, suspensions, and bulk drug substances. These methods play a critical role in quality control, stability studies, process validation, and routine batch testing. The most employed analytical techniques include UV-visible spectrophotometry, High-Performance Liquid Chromatography (HPLC), Thin-Layer Chromatography (TLC), Titrimetric analysis, and Electrochemical methods.

UV-Visible Spectrophotometry

UV-Visible spectrophotometry is widely used in pharmaceutical quality control laboratories due to its simplicity and cost-effectiveness. Methimazole exhibits strong absorbance in the UV range (typically around 238–250 nm), which allows its quantification in bulk drug and tablet formulations.

- **Advantages**: Simple, rapid, inexpensive, suitable for routine assay.
- **Applications**: Assay of methimazole in tablets and bulk powders.
- **Limitations**: Interference from excipients; lower sensitivity and specificity compared to chromatographic methods.

High-Performance Liquid Chromatography (HPLC)

HPLC is the most widely accepted method for methimazole determination in pharmaceutical analysis, owing to its high precision, accuracy, and regulatory compliance. Reverse-phase HPLC with C18 columns and UV detection at ~250 nm is commonly used.

- **Typical mobile phases**: Acetonitrile-water, phosphate buffers, or methanol mixtures.
- **Validation**: Most HPLC methods are validated for linearity, accuracy, precision, robustness, and system suitability as per ICH Q2 (R1) guidelines.
- **Applications**: Assay, dissolution studies, stability-indicating methods, impurity profiling.

Thin-Layer Chromatography (TLC)

TLC offers a simple and rapid means of screening methimazole in pharmaceutical formulations. It is useful for identity confirmation and semi-quantitative estimation.

- **Advantages**: Low cost, minimal instrumentation.
- **Applications**: Identity tests, quick quality control.
- **Limitations**: Less accurate and sensitive compared to HPLC.

Titrimetric Methods

Official compendia such as the British Pharmacopoeia and Indian Pharmacopoeia include titrimetric assay methods for methimazole. Iodometric titration is commonly used, which involves the redox reaction between iodine and methimazole.

- **Advantages**: Simple, no instrumentation required.
- **Applications**: Routine assay of bulk drug.
- **Limitations**: Less suitable for low concentrations or mixtures.

Electrochemical Techniques

Electrochemical methods such as voltammetry have been explored for methimazole analysis in pharmaceutical dosage forms. These methods provide high sensitivity and are suitable for trace analysis.

- **Applications**: Quantification in tablets using modified electrodes.
- **Advantages**: Portable, fast response time.
- **Challenges**: Electrode fouling, matrix interference.

Stability-Indicating Methods

Stability-indicating HPLC methods have been developed to separate methimazole from its degradation products under stress conditions (acidic, basic, oxidative, thermal, and photolytic). These are required for stability studies and regulatory submissions.

- **Regulatory relevance:** Required for shelf-life determination and product registration.

LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)

Though primarily used in biological matrices, LC-MS/MS has also been explored for methimazole in complex formulations and degradation studies.

- **Application**: Highly sensitive method for trace-level analysis and identification of degradation products.

HPTLC (High-Performance Thin-Layer Chromatography)

HPTLC provides a more refined alternative to classical TLC, offering densitometric quantification and better resolution.

- **Application**: Simultaneous estimation of methimazole and related compounds in combination products.

Several medications and pharmaceutical formulations have lately been released in the pharma business all around the world. For the most part, researchers have recorded the development of analytical methods as well as integrity indication parameters. Methimazole active pharmaceutical ingredient and dosage formulations have not been assessed for the research indicated above.

Table 1: Analytical methods for the estimation of methimazole

S. No.	DRUGS/AGENTS	TECHNIQUES	STATIONARY PHASE	MOBILE PHASE	LOD	LOQ	OTHERS	REFERENCE
1	METHIMAZOLE	HPLC-UV	Hypersil-ODS column (4.6 mm × 250 mm, Thermo)	Methanol:H2PO4-3:7(V/V)	Kidney:0.63µg/kg Liver:0.51µg/kg Muscle: 0.56 µg/kg	Kidney:2.10µg/kg Liver:1.70µg/kg Muscle: 1.86 µg/kg	Flow rate :0.5ml/min Retention time:4.5min Vol of injection:20µl Wavelength:254nm Linearity range: 0.5–150 µg/L (r^2 = 0.9941) Extraction method: Solid Phase Extraction (SPE) using Molecularly Imprinted Polymer (MIP)	1
2	METHIMAZOLE	HPLC-UV	ODS-H C18, 250 mm × 4.6 mm, 5 µm particle size, Capital HPLC	Methanol: Water (30:70 v/v)	water-0.1µg/l plasma:0.7µg/L Urine:0.5µg/L Milk:0.7µg/L	Not applicable	Flow Rate:1.0ml/min Retention time: 4.8-5.2 min Wavelength:254nm Linearityrange:0.5-1000µg/L, r2>0.995 Precision: ≤7.1% Extraction method: Ion based hollow Fiber liquid phase extraction method	2
3	METHIMAZOLE	HPLC-UV	Zorbax SB-C18,150*4.6mm ,5µm(Agilent technologies)	TCA buffer: Acetonitrile	Tiisues:0.1mg/kg Plasma:0.1mg/L	Not applicable	Flow Rate:1.2ml/min Retention time:3.7min Wavelength:345nm LinearityRange:0.5-20mg/kg(tissues) or mg/L Plasma. Correlation coefficient(R2):>0.998	3
4	METHIMAZOLE	UV-VISIBLE SPECTROPHOTOMETRY	Not applicable	Not applicable	Not applicable	Not applicable	Wavelength:730nm Linear range:8.000-160.0 /ml	4
5	METHIMAZOLE	UV SPECTROPHOTOMETRY	Not applicable	Not applicable	Zero order:0.17µg/ml 1st Derivarive:0.13µg/ml	Zero Order:0.55µg/ml 1st Derivative:0.43µg/ml	Wavelength:zero order(260nm), 1st order derivative(269nm) Linearity range:2.0-24.0µg/ml(for both methods)	5
6	METHIMAZOLE	HPTLC	Merck TLC plates pre-coated with silica gel 60F254(10×10)250µml	chloroform: acetone (7:3)v/v	2.63ng/band	7.98ng/band	Wavelength:252nm RF:0.44±0.03 Linearity range:200-600µg/band	6
7	METHIMAZOLE	RPLC	YMC triart c18(150×4.6mm,3µm)	Acetonitrile: water 5%(v/v)	1.046µg/ml	3.486µg/ml	PH-9.5 Flow Rate:0.8ml/min Retention Time:6.884 min Wavelength:260nm Linearity range:4-14µg/ml	7

8	**METHIMAZOLE**	**Electrochemical: square wave polarography and differentials pulse polarography**	**Not applicable**	**Not applicable**	**SWP:electrolyte(0.50) serum(0.60) DPP:0.40(electrolyte) 0.50(serum)**	**SWP:1.50(electrolyte) 1.80 (serum); DPP:1.20(electrolyte), 1.50(serum)**	**Electrode system: mercury drop working electrode platinum wire auxiliary Supporting electrolyte:0.1M sulphuric acid Detection potential:SWP: -25mV, DPP: -28mV Linearity range:5-80µ/ml for both SWP & DPP (serum & electrolyte)**	**8**
9	**METHIMAZOLE**	**LC-MS/MS**	**Sunshell C18 column (3.0mm I.D.× 100mm,2.6µm)**	**A:10mM Ammonium acetate B:methanol**	**Not applicable**	**1ng/ml**	**Flow Rate:0.4ml/min Retention time: MMI-NBD:5.08min, IS(MMI-D3-NBD)5.06min**	**9**
10	**METHIMAZOLE**	**UV-spectrophotometry**	**Not applicable**	**Not applicable**		**0.0016 mg/ml**	**Wavelength:525nm (max absorbance for KMnO4-Methimazole)**	**10**
11	**METHIMAZOLE**	**SERS**	**Not applicable**	**Not applicable**	**$2.97\times10^{-}11$mol/l**	**$1.21\times10^{-}9$mol/l**	**Enhancement factor: ~1.39×106 Buffer PH: PH-4 SERS Substrate:SA-AgNPs Wavelength: 785nm Quantification Peak:500$cm^{-}1$ (N-C-S bending vibration)**	**11**
12	**METHIMAZOLE**	**SERS**	**Not applicable**	**Not applicable**	**2.7µg/L**	**based on linear range start**	**Range:5µg/L to 1000µg/L Linearity:5-1000µg/L**	**12**
13	**METHIMAZOLE**	**LC-MS/MS**	**Unison UK-Cg column (150×3 mm,3.0µm)**	**A:5mM ammonium formate in water. B:0.1% formic acid in acetonitrile.**	**0.0001mg/kg**	**0.0005mg/kg**	**Flow rate:0.3ml/min Ionization mode: ESI, Positive mode Detection mode: multiple reaction monitoring**	**13**

CONCLUSION:

This review has systematically evaluated both classical and cutting-edge analytical techniques for quantifying methimazole across pharmaceutical, biological, and food matrices. We demonstrated that while UV-visible spectrophotometry and HPLC-UV remain indispensable for routine quality control offering simplicity, accuracy, and regulatory compliance, advanced platforms such as MIP-based SPE coupled with LC-MS/MS and SERS deliver superior selectivity and trace-level sensitivity. Electrochemical sensors further provide rapid, portable alternatives for on-site monitoring. Despite these advances, challenges persist in balancing sensitivity, throughput, cost, and environmental impact. Future research should therefore focus on:

- Developing green analytical workflows that minimize solvent consumption and hazardous reagents.
- Designing portable, field-deployable sensors with machine-learning–driven signal processing for real-time screening

By addressing these priorities, the next generation of analytical methods will not only enhance therapeutic drug monitoring and food-safety surveillance but also align with sustainable and digitalized laboratory practices**.**

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CONFLICT OF INTEREST:

The authors declare that NO conflict of interest among us.

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