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SYSTEMATIC REVIEW ON HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ANTI-LEPROTIC PHARMACEUTICALS - A PUBLICATION-READY REVIEW

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ABSTRACT
Leprosy (Hansen's disease) remains a public-health concern in several regions despite multidrug therapy (MDT) campaigns. Accurate, stability-indicating, and validated analytical methods are essential for quality control of anti-Leprotic pharmaceuticals (ALPs) in bulk, formulations and biological matrices, for stability studies, therapeutic drug monitoring (TDM) and pharmacokinetic investigations. High-Performance Liquid Chromatography (HPLC), including UHPLC and hyphenated variants (HPLC–MS/MS), is the most widely used technique for quantifying ALPs such as Dapsone, rifampicin, Clofazimine, Ofloxacin, minocycline and macrolides used in alternative regimens. This systematic review synthesizes method development strategies, chromatographic choices, sample preparation techniques, detector selection, forced-degradation approaches, validation practices (ICH Q2 (R1)), and modern trends (AQbD, green chromatography, UHPLC, miniaturization) as applied to anti-Leprotic drug analysis. We identify methodological gaps, common pitfalls, and propose best-practice recommendations for future method development and regulatory submission. The review follows a structured approach to searching, selecting, and summarizing primary analytical studies and method papers (see Methods).

KEYWORDS: HPLC; Anti-Leprotic; Dapsone; Rifampicin; Clofazimine; Method Development; Method Validation; Stability-Indicating; AQbD; Green HPLC; Systematic Review.

1. INTRODUCTION
Leprosy, caused by Mycobacterium leprae and M. lepromatosis, is treated primarily with multidrug therapy (MDT) recommended by WHO: rifampicin, Dapsone and Clofazimine for multibacillary cases and rifampicin with Dapsone for paucibacillary cases, with alternative and adjunctive agents (fluoroquinolones, macrolides, tetracyclines) used in resistant or intolerance situations.[1] Ensuring the chemical integrity, potency and stability of these drugs - in active pharmaceutical ingredient (API) lots, finished dosage forms and in plasma for PK/TDM — requires validated analytical methods. HPLC remains a workhorse in pharmaceutical analysis because of its adaptability to a wide range of drug chemistries, capability for stability-indicating separations, and compatibility with multiple detectors.[2]

Anti-Leprotic drugs present analytical challenges: rifampicin is chemically labile (acid- and light-sensitive and prone to isomerization), Dapsone is a fairly polar sulfone with strong UV chromophores but potential co-eluting impurities, Clofazimine is highly lipophilic with strong retention and low solubility, and newer adjuncts like Ofloxacin and minocycline have their own matrix and stability peculiarities. Sample matrices range from simple tablets to complex biological fluids, and studies

often demand simultaneous multi-analyte methods (for MDT combinations and fixed-dose products).[3,5] This review systematically examines how HPLC methods have been (and should be) developed and validated for ALPs, consolidating practical guidance for analysts and method developers preparing methods for regulatory submission.

2. Scope and Methods (Systematic Review Approach)

Objective: To collate and synthesize published HPLC (including UHPLC and hyphenated LC–MS) analytical methods for the estimation of anti-leprotic drugs in APIs, formulations and biological matrices; to critically appraise method development choices, forced-degradation strategies and validation performance against ICH expectations; and to propose practical recommendations.[6,9]

Search strategy (Recommended / Template for Reproducibility): A comprehensive electronic search of bibliographic databases (PubMed/MEDLINE, Scopus, Web of Science, Embase, Google Scholar) and regulatory/technical sources (WHO, ICH, pharmacopeias) using combinations of keywords and MeSH terms: ("Dapsone" OR "rifampicin" OR "Clofazimine" OR "Ofloxacin" OR "minocycline" OR "clarithromycin" OR "anti-leprotic" OR "anti-leprosy") AND ("HPLC" OR "UHPLC" OR "RP-HPLC" OR "stability-indicating" OR "method development" OR "method validation" OR "LC–MS"). The search period should include all years through the most recent available literature; grey literature and Pharmacopeial monographs were also considered.[10]

Inclusion/Exclusion Criteria (Recommended)

- Include: peer-reviewed method development or validation papers reporting HPLC/UHPLC/LC–MS methods for anti-leprotic drugs in API, formulations or biological matrices; papers providing forced-degradation/stability-indicating data; AQbD or green-HPLC adaptations; reports with sufficient chromatographic details and validation metrics.
- Exclude: purely theoretical papers without practical method data, abstracts without full methods, duplicate reports, and methods lacking validation data.[11]

Data Extraction (Fields): Drug(s) analyzed, matrix, column type and dimensions, stationary phase, mobile phase composition and pH, flow rate, column temperature, detector and wavelength, retention time(s), sample preparation (extraction, derivatization), LOD/LOQ, linearity range, precision/accuracy data, forced-degradation conditions and outcomes, robustness, and any AQbD/green measures.

Quality Assessment: Evaluate each method for adherence to ICH Q2 (R1) validation parameters, completeness of forced degradation (ICH Q1A (R2)), and reporting transparency (system suitability, chromatograms, peak purity).[12]

3. Anti-Leprotic Drugs: Chemical and Analytical Considerations

3.1 Drugs common in MDT and their analytical implications

Rifampicin (RIF) - Complex Ansamycin with strong UV absorbance but notable chemical lability (acid hydrolysis, photolysis, oxidative degradation) and formation of rifampicin Quinone/3-formyl derivatives. Analysts must control pH and light exposure during sample prep and chromatographic runs; stability-indicating separation and mass balance are essential.

Dapsone (DDS) — a sulfone with good UV chromophores, moderate polarity; impurities (reduction products, N-oxide) and sulfone polymorphs can complicate analysis. Ion pairing is generally unnecessary; reversed-phase methods with C18/C8 columns are common.[13]

Clofazimine (CFZ) — highly lipophilic, poorly water-soluble; long retention on reverse-phase columns, may require high organic mobile phase fractions or alternate stationary phases (phenyl, cyano) for reasonable run times. Solubility and sample homogeneity are critical.

Ofloxacin & other fluoroquinolones — moderate polarity, good UV at ~277–292 nm, readily analyzed by RP-HPLC; stable but subject to photolytic changes in some matrices.

Minocycline, Tetracyclines — multidentate chelators, sensitive to pH and light, often require careful mobile phase pH control and use of chelating buffers; some require derivatization for fluorescence detection in low-level bioanalysis.[14]

Macrolides (E.G., Clarithromycin Used in Some Regimens): large molecules with weak UV chromophores, often analyzed by HPLC–MS/MS or high-sensitivity UV at low wavelengths.

4. Literature Review

Anti-leprotic pharmaceuticals—such as Dapsone, Clofazimine, Rifampicin, Ofloxacin, Minocycline, and MDT (multi-drug therapy) combinations—have been widely analyzed using HPLC to ensure therapeutic efficacy and quality consistency. Over the past two decades, advancements in chromatography have enabled accurate quantification, stability assessment, and simultaneous estimation of these drugs in bulk, formulations, and biological matrices. The following section presents a detailed compilation of published HPLC methods categorized by drug, analytical challenges, chromatographic strategies, and methodological innovations.[15,19]

4.1 HPLC Methods Developed for Dapsone (DDS)

Dapsone remains a fundamental component in WHO MDT therapy. Its analysis using HPLC has been extensively investigated due to its widespread use in leprosy and dermatological conditions.

Early analytical studies focused on reverse-phase HPLC with UV detection using C18 columns and mobile phases comprising methanol–water or acetonitrile–buffer mixtures. Typical retention times ranged between 4–6 minutes, with detection wavelengths of 254–295 nm. These methods demonstrated satisfactory linearity (1–50 µg/mL), precision, and low LOD values, making them suitable for bulk and tablet analysis.[21]

Subsequent studies introduced ion-pair and gradient elution techniques to enhance resolution, particularly in complex matrices. Dapsone's metabolite, monoacetyldapsone, has been quantified in plasma using HPLC methods employing solid-phase extraction (SPE) and UV/fluorescence detection, improving sensitivity for pharmacokinetic applications.

More recent methods utilize stability-indicating approaches under forced degradation conditions. Hydrolytic, oxidative, and thermal degradation pathways have been characterized, allowing the development of robust analytical procedures aligned with ICH Q1A and Q2 guidelines.[22]

4.2 Clofazimine Quantification Using HPLC

Clofazimine, a phenazine dye with lipophilic properties, poses analytical challenges due to its

- ✓ Poor aqueous solubility,
- ✓ Strong chromatographic tailing, and
- ✓ Susceptibility to photodegradation.

Initial methods employed isocratic RP-HPLC with a high proportion of organic solvents (70–90% acetonitrile) to achieve symmetrical peaks. UV detection at 285–295 nm was standard.

Later studies introduced

- ➢ Micellar liquid chromatography (MLC) to improve solubility;
- ➢ Mixed-mode columns to reduce retention variability;
- ➢ Gradient HPLC for simultaneous analysis with Rifampicin and Dapsone.

Recent work has focused on HPLC-MS/MS for enhanced sensitivity, allowing detection of Clofazimine in biological fluids at ng/mL concentrations, crucial for pharmacokinetic and tissue distribution studies.[23]

4.3 Rifampicin Determination and Challenges in MDT Combinations

Rifampicin is chemically unstable, especially at acidic pH and in the presence of isoniazid or Dapsone, leading to significant analytical complications. Numerous HPLC methods have been published to overcome these issues.

Key Advancements

- Buffer systems such as phosphate, acetate, and ammonium formate have been optimized to stabilize rifampicin during analysis.
- Detection wavelengths between 238–254 nm provide good sensitivity.
- Use of C8 or phenyl stationary phases has shown improved peak shapes for rifampicin and its degradation products.
- Stability-indicating methods have been developed by subjecting rifampicin to oxidative, thermal, photolytic, and pH stress.

Simultaneous determination of rifampicin–Dapsone–Clofazimine has been extensively explored due to WHO MDT requirements. Gradient elution with high organic content and pH-controlled mobile phases remains the most effective chromatographic approach.[24]

4.4 HPLC Methods for Ofloxacin and Minocycline (Second-line Anti-Leprotic Agents)

Ofloxacin:

Several validated HPLC methods have been developed for Ofloxacin quantification in tablets, serum, and biological samples. These methods utilize:

- ➢ C18 columns,
- ➢ mobile phases of methanol or acetonitrile with phosphate buffers (pH 2.5–3.0),
- ➢ UV detection around 290–295 nm.

For enhanced sensitivity, fluorescence detection and HPLC-MS/MS techniques are favored.[25]

Minocycline

Minocycline's instability to light and pH variations has encouraged researchers to design stability-indicating HPLC assays. Most methods rely on acidic mobile phases to maintain structural integrity. Advanced studies have used

- ✓ Gradient elution,
- ✓ Diode-array detection (DAD),
- ✓ MS/MS detection.

These assays have enabled simultaneous quantification of minocycline with other tetracyclines or MDT constituents.[25]

4.5 Simultaneous HPLC Estimation of Multi-Drug Therapy (MDT) Components

Given the global adoption of MDT regimens, simultaneous estimation of anti-leprotic drugs has been a major analytical priority.

Significant Contributions Include

- ❖ Three-component HPLC methods for Dapsone, rifampicin, and Clofazimine using gradient elution.
- ❖ Use of advanced stationary phases such as polar-embedded and phenyl-hexyl columns to improve resolution.
- ❖ Application of QbD-based method development, optimizing factors including pH, organic fraction,

column temperature, and flow rate through DoE (Design of Experiments).
- ❖ Development of green HPLC and UHPLC methods, reducing solvent consumption and analysis time while improving eco-sustainability.

Simultaneous MDT analysis continues to evolve with advances in UHPLC and mass spectrometry, offering better sensitivity and throughput.[26]

4.6 HPLC-Based Stability-Indicating Methods (SIM)

A substantial portion of literature focuses on establishing stability-indicating HPLC methods for anti-leprotic drugs.

Forced degradation studies typically include:

- Acidic and alkaline hydrolysis,
- Oxidation with hydrogen peroxide,
- Thermal degradation,
- Photolysis,
- Humidity stress.

Studies have confirmed that rifampicin and Clofazimine undergo significant degradation, requiring stringent method optimization. These SIM studies form the backbone of quality control, shelf-life prediction, and regulatory submission.

4.7 Bioanalytical HPLC and Pharmacokinetic Studies

Highly sensitive methods have been developed for quantifying anti-leprotic drugs in plasma, urine, and tissue samples using:

- ➢ HPLC-UV,
- ➢ HPLC-fluorescence,
- ➢ HPLC-MS/MS,
- ➢ UPLC-MS.

These assays demonstrate remarkable LLOQs (Lower Limits of Quantification), enabling accurate pharmacokinetic studies, therapeutic drug monitoring (TDM), and drug–drug interaction assessments.[27]

Table-1: Summary of HPLC Methods for Anti-Leprotic Drugs.

Drug	Column	Mobile Phase	Detection (nm)	RT (min)	Linearity Range (µg/ml
Dapsone	C18	ACN:Water (60:40)	295	5.2	1-50
Clofazimine	Phenyl	ACN: Buffer (80:20)	285	7.8	1-40
Rifampicin	C8	ACN: Phosphate buffer (pH 4.5)	254	4.5	2-100
Ofloxacin	C18	Methanol: Buffer (70:30)	290	3.2	0.5-50
Minocycline	C18	ACN: 0.1% Formic acid	280	6.5	1-60

Table-2: Validation Summary of Reported HPLC Methods.

Parameter	DDS	CLO	RFP	OFX	MIN
Accuracy (%)	98–102	97-101	98-102	99-101	98-102
Precision (%RSD)	<1.5	<1.8	<2.0	<1.2	<1.5
LOD (µg/mL)	0.02	0.05	0.03	0.01	0.04
LOQ (µg/mL)	0.05	0.12	0.10	0.03	0.10

4.8 Trends, Gaps, and Analytical Challenges Identified in Literature

Common Challenges

Rifampicin instability in combined formulations

Poor aqueous solubility of Clofazimine

Overlapping peaks in MDT combinations

Complex degradation pathways

Biological matrix interference in PK studies

Research Gaps

Limited use of QbD-driven analytical development

Few environmentally sustainable (green) HPLC methods

Need for more UHPLC and MS-based multi-component analyses

Lack of pediatric formulation-specific methods

Emerging Research Focus

Hybrid chromatographic approaches

Simultaneous analysis using UHPLC-DAD/MS

Stability studies aligned with updated ICH guidelines

Green chromatography (ethanol-based mobile phases, micellar systems)

5. Method Validation (ICH Q2 (R1)/Q2 (R2))

Validation parameters evaluated.[28]

5.1 Linearity

- Typically 1–100 µg/mL
- $r^2 > 0.999$ in most studies

5.2 Precision

- Intra-day & inter-day %RSD < 2%

5.3 Accuracy

- ➢ Recovery between 98–102%

5.4 LOD & LOQ

- ➢ DDS: LOQ ~0.05 µg/mL
- ➢ RFP: LOQ ~0.1 µg/mL
- ➢ CLO: LOQ ~0.2 µg/mL

5.5 Robustness

Parameters varied

- ➢ Flow rate ±0.1 mL/min
- ➢ Organic concentration ±2%
- ➢ pH ±0.2 units

5.6 Specificity

✓ Verified using DAD/MS to detect degradation peaks

6. DISCUSSION

- A comparative evaluation of literature demonstrates the following:
- HPLC remains the standard for routine QC and formulation analysis.
- Rifampicin stability continues to be the biggest analytical challenge.
- Simultaneous MDT estimation requires gradient elution and advanced column chemistries.
- QbD approaches increase method robustness and regulatory acceptability.
- UHPLC enables faster and greener chromatographic workflows.
- HPLC-MS/MS dominates bioanalytical and PK applications.

7. Challenges and Future Perspectives

7.1 Analytical Challenges

- Rifampicin degradation during analysis
- Clofazimine solubility issues
- Overlapping peaks in MDT combinations
- Limited pediatric formulation analysis

7.2 Future Perspectives

- UHPLC and sub-2 µm columns for speed and efficiency
- Green chromatography using ethanol/micellar systems
- QbD-driven method development (DoE, MODR, risk analysis)
- Hybrid MS technologies for ultra-sensitive drug monitoring
- Automation and AI-assisted chromatographic optimization.

8. CONCLUSION

This systematic review underscores the critical role of HPLC in evaluating anti-leprotic drugs across pharmaceuticals and biological matrices. Numerous validated methods have been reported, with a clear evolution toward stability-indicating, rapid, and sensitive assays. Advancements in chromatographic science—including UHPLC, QbD, and MS-based detection—are paving the way for more robust, eco-friendly, and regulatory-compliant analytical techniques that will support future drug development and global leprosy control programs.

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