Utilization of ^{1}H NMR Spectroscopy for the Analysis and Quality Verification of Berberine in Herbal Dietary Supplements

A Technical White Paper on Quantitative Proton NMR Methodology, Orthogonal Verification, and Quality Control Applications

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Abstract

Berberine, a quaternary protoberberine isoquinolinium alkaloid derived principally from species of Berberis, Coptis, Phellodendron, and Hydrastis, has been established over the past decade as one of the highest-volume single-alkaloid dietary supplement ingredients sold in North American and European markets, driven by consumer interest in its reported effects on glucose and lipid metabolism through AMP-activated protein kinase (AMPK) signaling. Independent testing programs conducted between 2018 and 2025 have repeatedly documented substantial noncompliance between labeled and actual berberine content in commercial formulations, with failure rates for potency claims ranging from roughly 40% to over 60% depending on the survey and the product category examined, and with particularly severe deficiencies noted among gummy and low-cost formulations. This white paper examines the analytical chemistry underlying the use of one-dimensional proton nuclear magnetic resonance (^{1}H NMR) spectroscopy as a quantitative and structurally unambiguous method for berberine determination in raw materials, extracts, and finished dosage forms. The isoquinolinium ring system of berberine gives rise to a set of well-resolved aromatic and methylenedioxy proton resonances between δ 6.0 and δ 9.9 that are minimally overlapped by excipient, matrix, or co-formulated ingredient signals, permitting direct integration-based quantification without chromatographic separation. The present work reviews the structural basis for these assignments, the methodological considerations that govern accurate quantitative ^{1}H NMR (qHNMR) work with an unstable, quaternary, positively charged alkaloid — including relaxation delay selection, solvent and salt-form effects, and internal- versus external-calibration strategies such as ERETIC/PULCON — and synthesizes the published literature comparing high-field (500 MHz) and benchtop (60 MHz) ^{1}H NMR results against orthogonal ultra-high-performance liquid chromatography (UHPLC) and HPLC-UV determinations across more than sixty commercial formulations. Chemometric extensions of low-field ^{1}H NMR for simultaneous adulterant screening are also considered. The evidence assembled here supports the conclusion that qHNMR, whether performed at high field or on compact benchtop instrumentation, constitutes a validated, rapid, and orthogonal analytical platform well suited to the quality control, label-claim verification, and food-fraud investigation needs of the berberine supplement sector, while several practical limitations — including the alkaloid's degradability once in solution and the persistent ambiguity in industry practice between "berberine" and "berberine hydrochloride" label declarations — are identified as continuing sources of measurement and compliance uncertainty.

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1. Introduction and Market Context

Berberine is classified chemically as a protoberberine alkaloid, a subgroup of the broader isoquinoline alkaloid family characterized by a planar, fully aromatic isoquinolinium ring system bearing a permanent positive charge on the ring nitrogen. It is most commonly isolated, or present as the dominant alkaloid, in the roots, rhizomes, and bark of Berberis species (barberry, Oregon grape), Coptis chinensis (goldthread, huanglian), Phellodendron amurense and Phellodendron chinense (cortex phellodendri, huangbai), Hydrastis canadensis (goldenseal), and Tinospora species [1]. Its long history of traditional use in Ayurvedic and East Asian medicine, principally for gastrointestinal and antimicrobial indications, has more recently been supplemented by a substantial body of preclinical and clinical work describing its activation of AMP-activated protein kinase (AMPK) and consequent effects on hepatic lipogenesis, glucose uptake, and gut microbial composition [2,3,4,5,6,7].

This pharmacological profile, amplified considerably by social-media-driven consumer interest characterizing berberine as a nutraceutical alternative to pharmaceutical glucagon-like peptide-1 (GLP-1) receptor agonists, produced a marked expansion of the North American berberine supplement category during 2023 through 2025. That expansion has been accompanied by a body of independent testing evidence indicating that a substantial fraction of marketed berberine products fail to deliver their labeled potency. Industry-conducted HPLC-UV surveys have reported that as many as eighteen of thirty-three tested brands contained less than 40% of labeled potency, with several products found to contain 1% or less of the declared berberine content, and gummy formulations disproportionately represented among the worst-performing products [8,9,10,11]. Independent academic surveys employing HPLC-UV and UHPLC-MS/MS methodology have reported comparable findings, with Funk and coworkers documenting substantial variability in the quantity of berberine measured across commercial preparations sold in the United States [12], and Marcheluzzo and coworkers reporting analogous variability among berberine- and monacolin-containing lipid-control supplements marketed in Italy [13]. A further complication, distinct from simple underdosing, arises from inconsistent industry practice regarding whether a declared milligram quantity refers to berberine free base, berberine hydrochloride, or berberine hydrochloride dihydrate; because the hydrochloride dihydrate salt form is used at approximately 85–90% berberine potency by mass, a product declaring 500 mg of "berberine" in hydrochloride form should properly contain on the order of 550–575 mg of berberine hydrochloride dihydrate to meet its label claim, and ambiguity on this point between brand owners and their contract manufacturers has itself been identified as a source of label noncompliance independent of any deliberate underfilling [14].

Against this backdrop, the present white paper undertakes a technical review of ^{1}H NMR spectroscopy as an analytical tool for berberine quantification and quality verification, situating the technique relative to the chromatographic methods that dominate current industry testing practice. NMR spectroscopy offers particular advantages for this analyte class: because the isoquinolinium chromophore of berberine produces well-resolved, non-overlapping proton resonances that are directly diagnostic of its planar aromatic structure, quantification can proceed by direct signal integration without prior chromatographic separation, without a matrix-matched calibration curve constructed from an authentic reference standard of identical provenance, and with concurrent structural confirmation of analyte identity in the same spectrum used for quantification. These properties have led several independent laboratories, most notably groups at the University of Toulouse and the University of Nantes, to develop and validate both high-field and compact benchtop ^{1}H NMR methodologies specifically for berberine-containing dietary supplements and botanical extracts [15,16,17]. The sections that follow examine the structural basis for ^{1}H NMR assignment of berberine, the methodological requirements for rigorous quantitative work with this quaternary and

chemically unstable alkaloid, the comparative literature evaluating NMR against orthogonal chromatographic techniques, and the practical and regulatory considerations relevant to a commercial testing laboratory considering ^{1}H NMR as a berberine quality-control platform.

2. Chemistry and Structural Characterization of Berberine

Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium, $C_{20}H_{18}NO_4^+$) possesses a fully conjugated, planar isoquinolinium ring system in which rings A and D are aromatic and bear, respectively, a methylenedioxy bridge (ring A) and two methoxy substituents (ring D), while the central rings B and C complete the tetracyclic dibenzo[a,g]quinolizinium framework. The permanent positive charge resides on the quinolizinium nitrogen, and berberine is accordingly isolated and marketed almost exclusively as a salt, most commonly the chloride or sulfate, with the hydrochloride dihydrate being the dominant commercial form used in dietary supplement manufacture [14]. The intense yellow color long associated with barberry, goldenseal, and related botanicals is a direct consequence of this extended, charge-delocalized chromophore, which absorbs strongly in the near-UV/visible region and has historically supported the use of UV detection (typically at or near 345 nm, with some methods employing 265 or 226 nm) in HPLC-based quantification [14,18].

Berberine belongs to a structurally related family of protoberberine and aporphine-type alkaloids that frequently co-occur in the same botanical matrices and must be distinguished analytically from berberine itself. These congeners include palmatine and jatrorrhizine (which lack the methylenedioxy bridge and instead carry additional methoxy or hydroxyl substituents on ring A), coptisine (which, unlike berberine, bears two methylenedioxy bridges), columbamine, epiberberine, and the reduced, non-aromatic tetrahydroprotoberberine canadine, along with the structurally related furanoquinoline alkaloid hydrastine and its hydrolysis product hydrastinine, which co-occur with berberine specifically in Hydrastis canadensis (goldenseal) [15,19]. Because HPLC-UV methods rely principally on retention time and UV absorbance for identification, co-eluting or spectrally similar protoberberine congeners can complicate peak assignment in complex botanical extracts unless chromatographic resolution is carefully validated; ^{1}H NMR spectroscopy, by contrast, resolves these congeners on the basis of distinct proton chemical shift patterns arising directly from their differing substitution patterns, a point elaborated further in Section 3.

Berberine is not indefinitely stable once dissolved. The Q-QUIPU quantitative NMR study of goldenseal root extract by Le and coworkers specifically identified berberine as an "unstable natural product" within the extract matrix, noting that its concentration in solution was observed to decline measurably within the first hour following sample preparation, in contrast to the comparatively stable co-occurring alkaloids hydrastine and canadine [15,19]. This degradability, generally attributed to oxidative and photochemical pathways that can convert berberine to oxidized derivatives such as oxyberberine and to dihydroberberine-type reduction products under certain conditions, has direct methodological consequences: it disfavors quantitative approaches that require extended acquisition times (such as two-dimensional quantitative NMR experiments applied to overlapping or low-intensity signals) in favor of rapid one-dimensional ^{1}H acquisition, and it necessitates standardized, minimized, and documented sample-handling intervals between dissolution and spectral acquisition in any validated qHNMR protocol intended for regulatory or litigation-support use.

3. ^{1}H NMR Spectral Assignment of Berberine

The diagnostic and quantitatively useful signals in the ^{1}H NMR spectrum of berberine arise from the aromatic and olefinic protons distributed across its four-ring isoquinolinium framework, together with two well-separated singlets from the N^+-adjacent methylene protons and the aromatic methoxy substituents. The single most diagnostic and most frequently exploited resonance for quantitative work is the H-13 proton, a signal that is frequently reported together with the overlapping H-8 resonance as a composite low-field singlet, appearing in the far-downfield aromatic region, generally in the range of δ 8.6 to δ 9.9 depending on solvent, concentration, and salt form; this resonance is well separated from essentially all excipient, matrix, and co-formulated ingredient signals encountered in a typical dietary supplement matrix, which is the principal reason it has been adopted as the integration target of choice in the majority of published quantitative methods [18,17]. Hasada and coworkers, working with commercial reagent-grade protoberberine alkaloid standards, quantified purity directly from the ratio of this H-13 singlet intensity (at approximately δ 8.7 in their solvent system) to that of an internal hexamethyldisilane reference whose own concentration had been established with metrological (SI-traceable) rigor using certified potassium hydrogen phthalate, and found that NMR-determined purities of several commercial berberine, palmatine, and coptisine reagents were systematically lower than the values claimed by the supplying manufacturers, a discrepancy carrying direct consequences for HPLC methods calibrated against those same reagents [17].

Beyond H-13, the berberine ^{1}H NMR spectrum presents a second aromatic doublet pair (assigned to H-11 and H-12, the vicinal protons on ring D, appearing as mutually coupled doublets in the δ 6.8–8.2 region depending on solvent) and two further isolated aromatic singlets (H-1 and H-4 on ring A, flanking the methylenedioxy bridge, typically observed between δ 6.9 and δ 7.9). The methylenedioxy bridge itself gives rise to a highly diagnostic two-proton singlet near δ 6.0–6.2 (–O–CH_2–O–), a chemical shift region essentially unoccupied by any common excipient (starches, celluloses, gelatin, glycerin, stearates) or by co-formulated ingredients typically paired with berberine in combination products (such as milk thistle, chromium picolinate carriers, or alpha-lipoic acid). Two aromatic methoxy singlets (ring D, δ 4.0–4.4) and the aliphatic dihydroisoquinolinium methylene protons (H-5 and H-6, appearing respectively as a triplet near δ 3.2 and a triplet near δ 4.9–5.2, reflecting the deshielding influence of the adjacent quaternary nitrogen) complete the principal proton envelope. Collectively, this pattern of two isolated aromatic singlets, a mutually coupled aromatic doublet pair, a methylenedioxy singlet, two methoxy singlets, and two aliphatic methylene multiplets constitutes a spectral fingerprint that is both diagnostic of the intact isoquinolinium structure (and therefore capable of distinguishing berberine from degraded, hydrolyzed, or congeneric alkaloid species) and, through the H-13/H-8 composite singlet in particular, well suited to rapid, high-precision integration-based quantification [18,17,1].

Solvent selection materially affects both the observed chemical shifts and the practical utility of the method. Berberine chloride and berberine sulfate are appreciably water soluble, permitting direct analysis in D_2O for aqueous extracts or reconstituted formulations, whereas hydroalcoholic or DMSO-d_6 solvent systems are more frequently employed for botanical extracts containing lipophilic co-constituents or for reference-standard purity work of the kind described by Hasada and coworkers [17]. Because the H-13/H-8 resonance is reported across the literature at values ranging from approximately δ 8.6 in some hydroalcoholic systems to δ 9.7–9.9 in DMSO-d_6-based methods used for related benzyl-substituted protoberberine derivatives, a validated quantitative method must fix and document solvent, pD/pH, temperature, and reference compound for any chemical-shift-based signal assignment to be reliably reproduced across laboratories; the safer and more broadly applicable practice, consistent with recommendations from the quantitative NMR literature generally, is to confirm signal identity for a given solvent system directly against an authentic,

independently characterized berberine reference standard (ideally supported by two-dimensional correlation experiments such as COSY and HSQC in the initial method-development phase) rather than relying on literature chemical shift values transferred across differing solvent conditions [20].

4. Quantitative ^{1}H NMR Methodology (qHNMR): General Principles

Quantitative ^{1}H NMR occupies a distinctive position among analytical techniques applicable to natural product and dietary supplement analysis because the NMR signal intensity is, to first approximation, directly proportional to the molar concentration of the nuclei giving rise to it, independent of the chemical environment or electronic structure of the analyte, provided that instrumental and experimental conditions (principally full T_1 relaxation between scans and the absence of nuclear Overhauser or other intensity-distorting effects) are properly controlled. This universality has been the subject of extensive methodological review by Pauli and coworkers, whose original 2005 review and subsequent 2012 update, together comprising several hundred literature citations, established the experimental parameters — adequate relaxation delay (typically five times the longest relevant T_1), sufficient spectral width and digital resolution, appropriate window function selection, and rigorous baseline and phase correction — required for qHNMR results to achieve pharmaceutical-grade accuracy and precision [21,22]. Because qHNMR quantification depends on proton count and signal integration rather than on a compound-specific detector response factor, it does not require that the calibration standard be chemically identical to the analyte, a property of particular value when authentic, well-characterized reference material for minor or unusual natural product constituents is difficult to obtain; this same property, however, places a correspondingly greater burden on rigorous validation of the calibration compound's own purity and on the metrological traceability of its assigned concentration, a point that has led directly to the incorporation of qHNMR into the United States Pharmacopeia as an accepted purity-determination method and to its endorsement by the Journal of Medicinal Chemistry as an acceptable primary method for reference standard purity assignment [23,24].

Two principal calibration strategies are employed in published berberine qHNMR work. The internal standard approach, used by Hasada and coworkers [17] and by Li and coworkers in their cortex phellodendri method [18], introduces a known quantity of a non-interfering reference compound (hexamethyldisilane in the former case, anthracene in the latter) directly into the NMR sample, and calculates analyte concentration from the ratio of analyte-to-reference signal integrals corrected for differing proton counts. The external, or electronic, referencing approach — most widely implemented through the ERETIC (Electronic REference To access In vivo Concentrations) method originally described by Akoka, Barantin, and Trierweiler and subsequently generalized as PULCON (PULse length-based CONcentration determination) — instead generates or references a calibrated synthetic signal that does not physically share the NMR tube with the sample, avoiding both the risk of spectral overlap between an added internal standard and the analyte of interest and the practical inconvenience of adding a reference compound to every sample [25,26]. Both strategies have been applied successfully to berberine-relevant qHNMR work, and the choice between them in a given laboratory setting is generally governed by instrument capability (ERETIC/PULCON requires appropriate spectrometer calibration and software support) and by the risk, discussed further below, that an added internal standard's own resonances might overlap the H-1, H-4, or methylenedioxy region of a complex botanical matrix.

A methodological consideration of particular relevance to berberine, distinct from qHNMR practice for more chemically robust natural products, concerns the interaction between the alkaloid's documented in-solution instability (Section 2) and the relaxation delay requirements of rigorous quantitative acquisition.

Because full T_1 relaxation of the relevant aromatic protons can require acquisition times on the order of several minutes to tens of minutes for a properly relaxed, high-precision one-dimensional spectrum, and because berberine concentration in solution has been observed to decline measurably within the first hour after sample preparation, a validated berberine qHNMR protocol must either demonstrate, through dedicated stability experiments, that the chosen acquisition window does not introduce a measurable degradation-related bias, or must adopt a calibration-curve-based approach under conditions of deliberately incomplete relaxation, as implemented by Danoun and coworkers for their benchtop method (Section 6), in which a calibration curve constructed under matched incomplete-relaxation conditions is used in place of a fully relaxed absolute integration, trading some theoretical rigor for acquisition speed matched to the analyte's practical stability window [16]. Le and coworkers reached a directly analogous conclusion when they found that the more time-intensive two-dimensional Q QUIPU HSQC method, despite its demonstrated value for the overlapping and low-intensity hydrastine and canadine signals in their goldenseal extract, was "not competitive" for berberine quantification specifically because of the alkaloid's degradation kinetics, and that rapid one-dimensional ¹H NMR remained "the direct method of choice" for this particular analyte notwithstanding the general analytical advantages of two-dimensional approaches for resolving overlapped resonances [15,19].

5. Published Berberine-Specific ¹H NMR Methods: A Literature Survey

The application of ¹H NMR specifically to berberine quantification in botanical and supplement matrices has been documented across several independent research groups over roughly the past two decades, with methodology evolving from purity assessment of pharmaceutical reagents toward routine, high-throughput quality control of commercial finished products. Hasada and coworkers, working at a Japanese pharmaceutical research institution, applied internal-standard ¹H NMR to establish that commercial reagent-grade protoberberine alkaloid standards frequently carried assigned purities higher than those independently verified by NMR, with the consequence that HPLC methods calibrated against those reagents systematically overestimated alkaloid content when subsequently applied to Coptidis Rhizoma crude drug material; the same ¹H NMR method was shown to be directly applicable to alkaloid content determination in the crude drug itself, without requiring chromatographic separation from the coptisine, palmatine, and other protoberberine congeners naturally co-occurring in that matrix [17].

Li and coworkers subsequently developed and validated a rapid ¹H NMR method for simultaneous protoberberine alkaloid determination (berberine and palmatine) in cortex phellodendri and in complex, multi-ingredient traditional Chinese medicine prescriptions, using anthracene as internal standard and reporting recoveries of berberine and palmatine from Phellodendron amurense material in the range of 95–106%, with limits of detection and quantitation of 1.0 and 1.8 µg/mL respectively, and a total analysis time of approximately five minutes without any pre-purification or chromatographic separation step [18]. This work established, in a peer-reviewed and independently reproducible setting, that quantitative ¹H NMR could match conventional chromatographic performance benchmarks (accuracy, recovery, and detection limit) for berberine while eliminating the sample preparation and separation time that chromatographic methods require.

Le and coworkers, applying the more recently developed quantitative two-dimensional Q QUIPU (Quick QUantItative Perfected and pUre shifted) NMR technique in combination with conventional one-dimensional ¹H NMR to goldenseal (Hydrastis canadensis) root extract, demonstrated the complementary use of the two approaches: berberine, whose H-6/H-7 signals are non-overlapped in the one-dimensional spectrum but whose in-solution instability disfavors extended two-dimensional acquisition, was quantified

by rapid one-dimensional ¹H NMR, while the overlapped β-hydrastine signals and the low-intensity canadine signals — neither of which could be reliably integrated in the one-dimensional spectrum — were instead quantified using the two-dimensional Q QUIPU HSQC approach. The combined methodology achieved agreement within approximately 10% relative difference compared to coupled UHPLC-MS/UV reference measurements across the three alkaloids studied, directly demonstrating both the accuracy of the NMR-based approach and the value of matching NMR experiment selection to the physicochemical behavior of the specific analyte under study [15,19].

The most directly relevant recent work for the finished-dietary-supplement use case addressed by this white paper is that of Danoun, Balayssac, Gilard, Martino, and Malet-Martino, who examined eighteen commercial berberine food supplements purchased from retail websites during 2020, applying three distinct quantitative ¹H NMR methodologies at a 60 MHz benchtop (compact) spectrometer and cross-validating the results against a 500 MHz high-field reference measurement [16]. Berberine was quantified in approximately fifteen minutes on the benchtop instrument, a practical throughput advantage directly relevant to routine quality-control testing volumes, and the benchtop-derived quantities agreed closely with the 500 MHz reference values, establishing that compact, comparatively low-cost NMR instrumentation — feasible for deployment directly at a manufacturing site or within a regulatory testing laboratory without the capital and infrastructure demands of a superconducting high-field magnet — is capable of pharmaceutically meaningful quantitative accuracy for this analyte. Applying their validated method to the eighteen commercial products against their respective label claims, the authors found that only 56% of the formulations examined contained the labeled quantity of berberine, and that the daily berberine dose actually delivered by the surveyed products varied extremely widely, corroborating, through an entirely independent NMR-based analytical route, the potency deficiencies previously reported using HPLC-based methods by Funk and coworkers and by industry testing programs [16,12,8,9].

Considered collectively, this body of published work establishes several converging conclusions of direct relevance to a testing laboratory: (i) the H-13/H-8 composite singlet, and secondarily the methylenedioxy and H-1/H-4 aromatic singlets, provide non-overlapped, chemically diagnostic integration targets suitable for quantification across a range of solvent systems and matrix complexities; (ii) both internal-standard and electronic/external-reference calibration strategies have been successfully validated for this analyte; (iii) berberine's documented degradation kinetics in solution favor rapid one-dimensional acquisition, whether at high field or benchtop field strength, over extended two-dimensional or multi-scan protocols; and (iv) independently, and using distinct instrumentation, sample sets, and calibration strategies, multiple laboratories applying ¹H NMR to commercial berberine products have reached label-compliance findings that are quantitatively consistent with those obtained by orthogonal HPLC-UV and UHPLC-MS/MS methods, providing a meaningful degree of cross-method corroboration for the underlying finding of widespread commercial noncompliance.

6. Comparison with Orthogonal Chromatographic Methods

Reversed-phase HPLC with UV detection, typically employing a C18 stationary phase and detection near 345 nm (with some methods using 226 or 265 nm), remains the dominant analytical platform used by industry testing programs and by contract testing laboratories for berberine potency determination, reflecting the compound's strong native UV chromophore, its favorable retention behavior on standard reversed-phase columns, and the wide commercial availability of certified berberine chloride reference standards from suppliers including Sigma-Aldrich, USP, and PhytoLab. UHPLC-MS/MS methods, as applied by Funk and coworkers, extend this platform with mass-selective detection capable of resolving

berberine from co-eluting matrix components and structurally related congeners on the basis of mass-to-charge ratio in addition to retention time, at correspondingly greater instrumental cost and method complexity [12].

The principal analytical advantage that ^{1}H NMR offers relative to these chromatographic platforms is the combination, within a single acquisition, of structural confirmation and quantification: because the full complement of diagnostic berberine resonances (methylenedioxy singlet, aromatic singlets and doublets, methoxy singlets, aliphatic methylenes) is observed simultaneously in every spectrum, an atypical or unexpected spectral pattern immediately signals either analyte degradation, the presence of a structurally related but distinct protoberberine congener, or an interfering co-formulated ingredient, without requiring a separate confirmatory injection or a second chromatographic method. This property is of particular value in a food-fraud or litigation-support context, where the analytical question at issue is frequently not merely how much berberine is present but whether the material is genuinely berberine, or has instead been diluted, substituted, or misrepresented — a question to which a full ^{1}H NMR spectrum speaks more directly than a single UV or mass chromatogram peak. HPLC-based methods, by contrast, offer superior chromatographic resolving power for complex mixtures containing many minor congeners at trace concentration, and superior raw sensitivity at the low nanogram-to-picogram level achievable with mass-spectrometric detection, an advantage relevant to trace-level adulterant screening (for example, of pharmaceutical drugs illegally added to herbal products) that exceeds the practical detection limits of routine ^{1}H NMR [12,27].

A further, non-trivial practical consideration favoring NMR-based methods in the berberine context specifically is the salt-form ambiguity discussed in Section 1: because HPLC-UV quantification against a certified reference standard yields a result expressed in the units and salt form of that reference standard (typically berberine chloride or berberine hydrochloride), any downstream reporting or label-compliance calculation still requires an explicit and consistently applied conversion between "berberine," "berberine HCl," and "berberine HCl dihydrate," and confusion on precisely this point has been documented as a contributing source of nominal label noncompliance independent of genuine underdosing [14]. Quantitative ^{1}H NMR, because it measures molar proton content directly and is agnostic to counterion identity (the chloride, sulfate, or other anion contributes no observable ^{1}H NMR signal of its own in the diagnostic region), in principle permits berberine cation content to be reported on a chemically unambiguous molar or free-base-equivalent basis, provided the analyzing laboratory documents this convention clearly and consistently in its reporting — a discipline that is not uniformly observed across the industry HPLC testing landscape.

Finally, several groups have extended low-field, benchtop ^{1}H NMR beyond single-analyte quantification into chemometric, multivariate screening applications directly relevant to the food-fraud detection context emphasized throughout this white paper series: Wu and coworkers applied chemometric analysis of low-field ^{1}H NMR spectra to detect pharmaceutical adulteration of slimming dietary supplements, and Pujol and coworkers applied a comparable benchtop NMR–chemometric workflow to detect hidden drug ingredients in honey-based supplement products, in both cases exploiting the same practical advantages of speed, minimal sample preparation, and structural specificity that underlie the berberine-specific work reviewed above, coupled with multivariate pattern-recognition methods (principal component analysis and partial least squares discriminant analysis) to flag spectral deviations from an authentic reference population without requiring a priori knowledge of the specific adulterant present [28,29]. This chemometric extension is directly transferable to the berberine supplement category, where multi-ingredient combination products (frequently co-formulated with milk thistle, alpha-lipoic acid, chromium, or other metabolic-health ingredients) present exactly the kind of complex, variable matrix for which a targeted single-analyte HPLC

method may be blind to unanticipated adulterants or substituted botanicals, but for which a full-spectrum ^{1}H NMR fingerprint, interrogated chemometrically, retains discriminating power.

7. Regulatory and Quality-Control Context

Berberine-containing products are regulated in the United States principally as dietary supplements under the Dietary Supplement Health and Education Act of 1994 (DSHEA), a framework now marking its thirtieth year of operation and one that places primary responsibility for identity, purity, strength, and composition verification on the manufacturer and distributor rather than requiring premarket analytical approval by the U.S. Food and Drug Administration [30]. This regulatory structure, combined with the documented economic incentive toward underfilling of a raw material (berberine hydrochloride) whose bulk commodity price has risen alongside consumer demand, situates berberine squarely within the broader analytical category of economically motivated adulteration (EMA), a term applied within the food and supplement fraud literature to the fraudulent, intentional substitution, dilution, or misrepresentation of a product or ingredient for economic gain [31].

Within this regulatory and economic context, analytical methods capable of rapid, structurally specific, and cost-effective verification carry particular value for manufacturers seeking to demonstrate label-claim compliance, for contract testing laboratories serving that manufacturer base, and for enforcement or litigation-support investigations initiated in response to independent testing findings of the kind summarized in Section 1. The compact benchtop ^{1}H NMR methodology validated by Danoun and coworkers is explicitly framed by its authors as intended for practical deployment at manufacturers and in laboratories of food regulatory bodies, reflecting a deliberate design objective of matching analytical rigor to the throughput, cost, and infrastructure constraints of routine industrial and regulatory quality-control settings, as distinct from the research-laboratory context in which high-field NMR methodology has traditionally been developed and validated [16].

It is worth noting explicitly, in the interest of a calibrated technical assessment, that ^{1}H NMR spectroscopy — whether performed at high field or on benchtop instrumentation — has not to date achieved the same breadth of regulatory codification specifically for berberine that HPLC-UV enjoys through decades of pharmacopeial precedent and the wide commercial availability of validated, single-analyte HPLC methods; the United States Pharmacopeia's General Chapter <761> establishes qNMR generally as an accepted quantitative technique and the Journal of Medicinal Chemistry has endorsed qHNMR as an acceptable purity assay, but a monograph-level, berberine-specific compendial NMR method comparable in regulatory standing to an established USP or AOAC HPLC monograph has not been identified in the literature reviewed for this white paper [23,24]. A laboratory adopting ^{1}H NMR for berberine quality control should accordingly expect to conduct and document its own method validation (linearity, accuracy, precision, specificity, and robustness, following ICH Q2-type validation principles) against the specific instrument, solvent system, and product matrix in use, rather than relying on direct adoption of a compendial monograph, and should retain HPLC-UV or UHPLC-MS/MS as a documented orthogonal cross-check where regulatory, contractual, or litigation-support requirements call for confirmation by an independently validated, compendially recognized method.

8. Practical Considerations and Limitations

Several practical limitations of the ^{1}H NMR approach to berberine analysis warrant explicit acknowledgment, consistent with a balanced technical assessment rather than an unqualified endorsement

of the technique. First, the degradation kinetics discussed in Sections 2 and 4 impose a genuine constraint on method design: any laboratory implementing qHNMR for berberine must establish, through its own stability studies, the maximum interval between sample dissolution and spectral acquisition that can be tolerated without introducing a measurable low bias, and must standardize sample-handling procedures (dissolution solvent, temperature, and time-to-acquisition) accordingly; this is a nontrivial validation burden not shared by chromatographic methods employing rapid, automated sample injection immediately following extraction.

Second, raw sensitivity remains a comparative limitation. Published limits of detection and quantitation for HPLC-based protoberberine methods (for example, 1.0 and 1.8 µg/mL respectively in the method of Li and coworkers) are achievable with routine reversed-phase UV detection, whereas one-dimensional ^{1}H NMR, even at high field, is intrinsically a comparatively insensitive technique relative to UV or mass-spectrometric detection, and the benchtop 60 MHz methodology of Danoun and coworkers was validated down to a minimum practical quantity of approximately 10 mg of berberine per capsule or tablet using a 15 mg test sample — entirely adequate for verifying compliance with typical 300–1500 mg per-dose label claims, but not competitive with HPLC-MS/MS for trace-level contaminant or adulterant screening in the low microgram-per-milliliter range or below [18,16].

Third, gummy and other complex-matrix dosage forms, disproportionately represented among the worst-performing products in recent industry testing surveys, present a genuine analytical challenge to any method: gelatin, pectin, sugar alcohols, and flavoring/coloring agents used in gummy formulations introduce a dense, broad, and variable aliphatic and carbohydrate proton background that, while generally not overlapping the diagnostic aromatic and methylenedioxy region exploited for berberine quantification, can complicate baseline definition and integration in a one-dimensional spectrum acquired without additional matrix cleanup; a rigorous method-validation program for this dosage form should specifically characterize matrix-related baseline effects and, where necessary, incorporate a simple liquid-liquid or solid-phase extraction cleanup step prior to NMR acquisition [10,11].

Fourth, and consistent with the broader qHNMR literature reviewed in Section 4, the accuracy of any qHNMR determination is only as good as the purity assignment and metrological traceability of the internal or external calibration reference employed; the finding by Hasada and coworkers that several commercially available reagent-grade berberine, palmatine, and coptisine standards carried NMR-verified purities below their manufacturer-assigned values underscores that a testing laboratory cannot simply accept a purchased reference standard's certificate of analysis at face value, but should independently verify reference-standard purity — ideally by qHNMR against a metrologically traceable primary reference such as certified potassium hydrogen phthalate — before that standard is used to calibrate a routine method [17].

9. Conclusions and Recommendations

The published literature reviewed in this white paper supports several converging technical conclusions. Berberine's isoquinolinium ring system produces a set of well-resolved, structurally diagnostic ^{1}H NMR resonances — most usefully the H-13/H-8 composite aromatic singlet and the methylenedioxy singlet — that are minimally overlapped by common excipients or co-formulated ingredients and that permit direct, integration-based quantification without chromatographic separation. Both internal-standard and electronic external-referencing (ERETIC/PULCON) calibration strategies have been independently validated for qHNMR generally, and both high-field (500 MHz) and compact benchtop (60 MHz) instrumentation have been shown, in directly comparative published work, to yield quantitatively consistent berberine results,

indicating that ^{1}H NMR-based berberine quality control need not require capital-intensive high-field infrastructure to achieve pharmaceutically meaningful accuracy. Applied to commercial finished products, ^{1}H NMR-based determinations have independently corroborated, via an analytical route entirely distinct from HPLC-UV or UHPLC-MS/MS, the now well-documented finding that a substantial fraction of marketed berberine supplements — on the order of 40–60% across the several independent surveys reviewed here — fail to deliver their labeled potency.

For a commercial analytical laboratory evaluating ^{1}H NMR as a berberine testing platform, the evidence reviewed here supports several specific recommendations. Method validation should explicitly characterize and document the acceptable interval between sample dissolution and spectral acquisition, informed by dedicated stability experiments against the specific solvent and sample-preparation protocol in use, given berberine's documented in-solution degradation behavior. Reference standard purity should be independently verified by qHNMR rather than accepted from a supplier certificate of analysis alone. Results should be reported on an explicitly defined chemical basis (berberine cation, berberine hydrochloride, or berberine hydrochloride dihydrate, as appropriate to the specific label claim under evaluation) to avoid perpetuating the salt-form ambiguity identified in this white paper as an independent source of apparent, as distinct from genuine, label noncompliance. Where gummy or other complex-matrix dosage forms are analyzed, matrix-related baseline effects should be specifically characterized and a sample cleanup step incorporated if warranted. Finally, and consistent with general good analytical chemistry practice for consequential compliance or litigation-support determinations, ^{1}H NMR results should be corroborated where feasible against an orthogonal, compendially recognized chromatographic method, both because HPLC-UV and UHPLC-MS/MS retain superior raw sensitivity for trace-level work and because cross-method agreement provides the strongest available evidentiary basis for a compliance finding subject to potential challenge.

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Summary

This white paper has reviewed the chemical, spectroscopic, and methodological basis for the use of one-dimensional ¹H NMR spectroscopy in the quantitative analysis of berberine, a quaternary protoberberine isoquinolinium alkaloid that has become one of the most heavily marketed single-ingredient dietary supplements in the current North American and European nutraceutical landscape. The isoquinolinium ring system of berberine gives rise to a well-resolved and structurally diagnostic proton spectrum, most notably the low-field H-13/H-8 composite aromatic singlet and the methylenedioxy singlet, neither of which is substantially overlapped by common excipients or by co-formulated ingredients, permitting rapid, direct-integration quantification without chromatographic separation. Published work spanning purity assessment of pharmaceutical reagents, quantification within complex botanical extracts such as goldenseal and cortex phellodendri, and, most directly, quality evaluation of eighteen commercial berberine food supplements using both high-field and compact benchtop instrumentation, has established that qHNMR achieves accuracy consistent with orthogonal UHPLC-MS/MS and HPLC-UV determinations while offering the practical advantages of speed, minimal sample preparation, and simultaneous structural confirmation of analyte identity. This body of NMR-based evidence independently corroborates the troubling but now well-replicated finding, drawn from multiple industry and academic testing programs employing entirely distinct analytical platforms, that a substantial proportion of commercial berberine products fail to deliver their labeled potency. Persistent practical limitations — berberine's in-solution degradation kinetics, the comparatively modest raw sensitivity of ¹H NMR relative to mass-spectrometric detection, and the ongoing ambiguity in industry labeling practice between berberine free base and its hydrochloride salt forms — are identified as continuing priorities for method validation and for industry-wide standardization, but do not diminish the conclusion that qHNMR constitutes a scientifically sound, practically deployable, and increasingly well-precedented addition to the analytical toolkit available for berberine quality control and food-fraud investigation.