NMR SPECTROSCOPIC DETECTION OF PHARMACEUTICAL ADULTERANTS IN HERBAL AND NUTRITIONAL SUPPLEMENTS

Targeted and Non-Targeted ^{1}H qNMR Screening Across Multiple Product Categories

John C. Edwards, Ph.D. Process NMR Associates, LLC 84 Patrick Lane, Suite 115, Poughkeepsie, NY 12603, USA john@process-nmr.com | www.process-nmr.com March 2026

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Abstract

Adulteration of herbal and nutritional dietary supplements with undeclared pharmaceutical compounds represents one of the most significant challenges confronting food safety regulators, analytical chemists, clinicians, and consumers worldwide. The present review examines the application of quantitative ^{1}H nuclear magnetic resonance (qNMR) spectroscopy—both at high field (300–600 MHz) and at the emerging benchtop frequency of 60 MHz—as a primary screening and confirmatory tool for the detection and quantification of illicit pharmaceutical adulterants across the major categories of the supplement market. Categories reviewed include male sexual-enhancement products, weight-loss and thermogenic preparations, cognitive-enhancement (nootropic) blends, sports-performance and bodybuilding formulations, energy supplements, topical cosmetic and joint-health preparations, sleep and relaxation products, and women's sexual-health supplements.

A systematic survey of the peer-reviewed literature has identified more than 300 distinct pharmaceutical adulterants reported across these categories, with phosphodiesterase type-5 (PDE5) inhibitors and their designer analogues accounting for the largest structural class in sexual-enhancement products, sibutramine and its metabolites dominating the weight-loss sector, and anabolic steroids together with selective androgen receptor modulators (SARMs) representing the principal threat in sports-nutrition products. ^{1}H NMR spectroscopy provides unambiguous structural identification through the chemical-shift fingerprint and coupling-constant patterns of key diagnostic protons, and permits absolute quantification via integration against a primary reference standard—most notably maleic acid for aqueous food matrices—or through external ERETIC/PULCON calibration.

The methodology is positioned relative to orthogonal techniques including HPLC-UV, LC-MS/MS, GC-MS, and near-infrared/FTIR spectroscopy. Comprehensive tables of expected legitimate ingredients alongside known fraudulent adulterants are presented for each supplement category, incorporating key ^{1}H and selected ^{13}C NMR chemical-shift assignments drawn from the primary literature and from in-house reference measurements performed at Process NMR Associates, LLC on Varian Mercury 300 MHz and 4IR Solutions AI-60 60 MHz spectrometer platforms. Multivariate chemometric and machine-learning classification strategies—encompassing principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), support vector machines (SVM), and XGBoost ensemble methods—are discussed in the context of large-scale non-targeted screening programmes. Regulatory frameworks governing supplement quality assurance in the United States (DSHEA 1994, FDA cGMP, FDA MedWatch), the European Union (EU No. 1169/2011, EFSA), and the United Kingdom (MHRA) are reviewed, and analytical validation requirements under ICH Q2(R1) and USP General Chapter ⟨761⟩ are summarised. The review concludes with a forward-looking assessment of the role of benchtop NMR platforms in routine regulatory testing and in ISO 17025-accredited commercial analytical laboratories.

Keywords: qNMR, ^{1}H NMR, dietary supplements, adulteration, PDE5 inhibitors, sildenafil, sibutramine, anabolic steroids, SARMs, benchtop NMR, metabolomics, PCA, PLS-DA, food fraud, supplement authentication, ISO 17025.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Table of Contents

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

1. Introduction

The global market for herbal and nutritional dietary supplements has experienced extraordinary growth over the past three decades. Industry estimates place the 2024 worldwide market value in excess of USD 250 billion, with the United States alone accounting for approximately USD 60 billion in annual sales. This expansion has been driven by consumer demand for 'natural' alternatives to pharmaceutical products, heightened awareness of preventive health strategies, and the proliferation of e-commerce channels that bypass traditional regulatory checkpoints. Against this backdrop, the adulteration of dietary supplements with undeclared prescription and over-the-counter pharmaceutical compounds has emerged as a serious public health issue of global significance.[6] [7] [8]

The United States Food and Drug Administration (FDA) maintains a continuously updated database of supplements found to contain undeclared drug ingredients; by 2024 this database listed more than 900 individual products and exceeded 450 unique pharmaceutical or pharmaceutical-analogue adulterants.[50] The problem is by no means limited to the United States: regulatory agencies in the European Union, the United Kingdom, Canada, Singapore, Australia, and across South-East Asia have documented parallel patterns of adulteration. In a landmark survey of 175 products seized in Singapore, illicit sexual-enhancement preparations were found to contain pharmacologically relevant quantities of sildenafil, tadalafil, or one of their synthetic analogues in 46% of samples.[10] A European study of 150 herbal supplements marketed for sexual performance identified adulterants in 58% of products examined, with some formulations containing multiple PDE5 inhibitors simultaneously.[2]

The analytical challenge posed by supplement adulteration is substantial. Products are typically formulated as complex herbal matrices containing polyphenols, terpenoids, alkaloids, carbohydrates, and lipids, all of which can interfere with chromatographic separations and produce false-negative or false-positive results in colorimetric or immunoassay screening formats. NMR spectroscopy is uniquely positioned to address this challenge: the technique is inherently quantitative, free from matrix-dependent sensitivity effects, and capable of providing unambiguous structural identification directly from the spectrum without the requirement for authentic reference standards for each target analyte.[35] [36] [37] The chemical-shift fingerprint, integration ratios, and ^{1}H–^{1}H coupling patterns of characteristic protons in the adulterant molecules are diagnostic and are largely independent of the herbal matrix. Furthermore, the development of benchtop permanent-magnet NMR systems operating at 60 MHz has transformed the practical accessibility of the technique, enabling deployment in quality control laboratories, regulatory agencies, port-of-entry inspection facilities, and contract testing organisations without the infrastructure requirements of superconducting systems.[87]

The present review is structured to address each major supplement category in turn, reviewing the expected legitimate phytochemical and nutritional active ingredients, the pharmaceutical adulterants documented in that category, the key ^{1}H NMR chemical-shift data enabling their identification and quantification, and the health risks they pose. Comprehensive tabular summaries accompany each section. A dedicated section addresses chemometric and machine-learning approaches for large-scale non-targeted screening. The regulatory framework is reviewed, and the requirements for analytical validation under ICH Q2(R1) and ISO/IEC 17025:2017—the standard being pursued by Process NMR Associates, LLC through PJLA accreditation—are considered. The review is intended to serve as a practical reference for analysts, food safety scientists, regulatory personnel, and legal professionals engaged in supplement authentication and fraud detection.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

2. Regulatory and Legal Framework

2.1 United States: DSHEA 1994 and FDA Oversight

In the United States, dietary supplements are regulated primarily under the Dietary Supplement Health and Education Act (DSHEA) of 1994, which created a regulatory framework fundamentally distinct from that governing pharmaceutical products. Under DSHEA, manufacturers are not required to demonstrate the safety or efficacy of their products prior to market entry; instead, the FDA bears the burden of proving a product is unsafe after it has been placed on the market.[49] This asymmetry has been widely criticised as creating an environment conducive to adulteration, given the limited pre-market scrutiny applied to product formulations.

The FDA's Current Good Manufacturing Practice (cGMP) regulations for dietary supplements, codified at 21 CFR Part 111, establish minimum standards for the design, manufacture, packaging, labelling, holding, and distribution of dietary supplements.[51] These regulations require identity testing of raw materials and finished products, but the absence of prescribed quantitative analytical methods for undeclared pharmaceutical adulterants leaves significant gaps in enforcement. The FDA's Tainted Products Marketed as Dietary Supplements database and the MedWatch adverse event reporting system provide the primary mechanism for post-market surveillance, but these are reactive rather than preventive instruments.[50] Between 2007 and 2024, the FDA issued more than 1,500 warning letters and public notifications relating to tainted supplements, with sexual-enhancement and weight-loss products accounting for the majority of enforcement actions.[6] [7]

2.2 European Union and United Kingdom Frameworks

Within the European Union, food supplements are regulated under Directive 2002/46/EC, which harmonises rules on vitamins and minerals in supplements but does not address the broader problem of herbal adulteration comprehensively. The European Food Safety Authority (EFSA) provides scientific opinions on the safety of novel food ingredients and provides maximum permitted levels for certain botanical compounds, but enforcement of adulteration provisions falls to individual member states.[52] The Rapid Alert System for Food and Feed (RASFF) provides a mechanism for rapid cross-border notification of hazardous products, and between 2010 and 2024 RASFF notifications relating to undeclared pharmaceutical substances in supplements numbered in the hundreds annually.

In the United Kingdom, following departure from the EU, food supplements are regulated under domestic legislation transposing the original EU Directive, administered by the Food Standards Agency (FSA). The Medicines and Healthcare products Regulatory Agency (MHRA) has responsibility for enforcement where supplements are found to contain substances that qualify them as unlicensed medicines. The MHRA Yellow Card scheme captures adverse event reports and has documented numerous incidents involving undeclared pharmaceutical compounds in herbal preparations marketed for sexual enhancement, weight loss, and cognitive performance.

2.3 Third-Party Certification and Analytical Standards

In the absence of mandatory pre-market testing requirements, third-party certification programmes—including NSF International Certified for Sport®, Informed Sport, the United States Pharmacopeia (USP) Verified Mark programme, and the National Sanitation Foundation's dietary supplement certification—provide voluntary quality assurance frameworks.[78] These programmes require product testing against a panel of target analytes including WADA-prohibited substances and selected pharmaceutical adulterants,

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

but their scope is necessarily limited by the requirement to define target compounds in advance. The NMR-based non-targeted metabolomics approach reviewed in this document provides a complementary strategy that is not constrained by a pre-defined analyte list.

Analytical validation requirements for methods applied in supplement testing are governed by ICH Guideline Q2(R1) in pharmaceutical contexts and by the General Requirement for Competence of Testing and Calibration Laboratories (ISO/IEC 17025:2017) in accredited laboratory settings. For NMR-based quantification, the relevant frameworks include USP General Chapter ⟨761⟩ (Nuclear Magnetic Resonance Spectroscopy), the IUPAC recommendations on quantitative NMR, and the ValidNMR qNMR proficiency testing programme. Method validation parameters including linearity, accuracy, precision (repeatability and reproducibility), limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness must all be demonstrated for methods deployed in accredited testing environments.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

3. Overview of NMR Analytical Methodology for Supplement Analysis

3.1 Fundamental Principles of Quantitative ^{1}H NMR

Quantitative ^{1}H NMR (qNMR) exploits the fundamental proportionality between the area of an NMR resonance and the number of contributing nuclei. Under conditions of full relaxation (acquisition delays ≥ $5\times T_1$ for all observed protons) and uniform excitation (90° or calibrated Ernst-angle pulses), the integral ratio of an analyte signal to that of a primary reference standard of known concentration is directly proportional to the molar ratio of the two species.[39] [35] Absolute quantification is therefore achievable without the requirement for an authentic reference standard of the analyte—a particularly significant advantage when detecting novel designer analogues of pharmaceutical compounds whose reference materials may not yet be commercially available.

For supplement analysis, maleic acid has been established as the preferred internal standard by this laboratory and by several peer groups, owing to its chemical simplicity (a single ^{1}H resonance at δ 6.26 ppm in D_2O), its high purity, water solubility, and NIST-traceability.[72] Alternative standards employed in the literature include dimethyl sulfone (δ 3.14 ppm, D_2O), sodium 3-trimethylsilyl-propanoate-d_4 (TSP, δ 0.00 ppm), and 1,4-bis(trimethylsilyl)benzene (BTMSB, δ 0.28 ppm, $CDCl_3$).[39] External standardisation via ERETIC (Electronic REference To access In vivo Concentrations) or PULCON (PULse length based CONcentration determination) eliminates the risk of standard/analyte signal overlap in complex matrices and is increasingly favoured for herbal product analysis.[40]

3.2 Sample Preparation Strategies for Herbal Supplement Matrices

For capsule and tablet formulations, the most common approach employed at Process NMR Associates, LLC involves dissolution of a weighed aliquot (25–100 mg) in 600 µL of deuterated solvent (D_2O or $CDCl_3$/CD_3OD mixtures) containing a known concentration of internal standard, followed by centrifugation and transfer to a 5 mm NMR tube. The choice of solvent is guided by the anticipated polarity of target adulterants: $CDCl_3$ is preferred for PDE5 inhibitors, anabolic steroids, and lipophilic compounds, while D_2O or D_2O/CD_3OD is preferred for water-soluble adulterants including sibutramine metabolites, amino acid spike adulterants, and benzodiazepines.[44] For topical creams and gels, a liquid–liquid extraction step using deuterated chloroform is required prior to NMR analysis to remove the aqueous or lipid excipient matrix.[57] [58]

3.3 Acquisition Parameters and Spectral Quality

Standard acquisition parameters for qNMR of supplement extracts at 300 MHz employ a 90° pulse angle, an acquisition time of 3.0–4.0 s, and a relaxation delay of 30–60 s to ensure quantitative accuracy across the broad T_1 range encountered in complex matrices. A minimum of 32–128 transients is collected depending on the expected concentration of adulterant. For benchtop 60 MHz systems, longer relaxation delays (up to 60–90 s) may be required owing to instrument-specific T_1 profiles and the reduced spectral dispersion at lower field. Exponential line-broadening of 0.3–1.0 Hz is applied during processing; the spectral baseline is corrected by polynomial fitting, and phase is adjusted manually or via automated routines in MNova (Mestrelab Research) software prior to integration.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

3.4 High-Field versus Benchtop Platforms: Capabilities and Limitations

High-field superconducting NMR systems operating at 300–600 MHz provide the highest sensitivity, spectral resolution, and dynamic range for complex mixture analysis, and represent the reference platform for development and validation of supplement testing methods.[87] Process NMR Associates, LLC operates two Varian Mercury 300 MHz superconducting systems together with three 60 MHz benchtop systems, including the 4IR Solutions AI-60. The 'high-field-informed, low-field-deployed' paradigm developed at Process NMR Associates, LLC underpins the laboratory's strategy for method development: full spectral assignment and analytical validation are performed at 300 MHz, whereupon the critical diagnostic resonances for key adulterants are identified and the corresponding NMR acquisition method is transferred to the 60 MHz platform for routine high-throughput screening.

At 60 MHz, the reduced chemical-shift dispersion (Hz per ppm) inevitably causes greater spectral overlap relative to 300 MHz, which may obscure minor diagnostic signals in complex herbal matrices. Nevertheless, the dominant proton signals of major pharmaceutical adulterants—particularly the aromatic proton resonances of PDE5 inhibitors (δ 7.0–9.0 ppm), the N-methyl singlets of sibutramine (δ 2.51 ppm), and the characteristic olefinic and carbinol protons of anabolic steroids—are sufficiently resolved at 60 MHz to permit positive identification and quantification at concentrations above 0.5 mg/mL.[44] For detection of minor components at concentrations below this threshold, or for definitive structural elucidation of novel designer analogues, transfer to the high-field platform remains necessary.

3.5 Non-Targeted Metabolomics and Chemometric Screening

The non-targeted or 'metabolomics-style' approach to supplement screening acquires the complete ^{1}H NMR spectrum of each sample and subjects the resulting spectral dataset to multivariate statistical analysis without any prior assumptions about the identity of adulterants.[45] [46] After spectral pre-processing—including normalisation, baseline correction, and binning into 0.01–0.04 ppm spectral windows (bins)—principal component analysis (PCA) is applied to identify the major axes of variance in the dataset. Samples that deviate significantly from the 95% confidence ellipse on the scores plot are flagged as outliers warranting targeted follow-up analysis.[90] PLS-DA and OPLS-DA models have been trained on libraries of authenticated reference products and applied prospectively to unknown samples with sensitivities and specificities exceeding 95% for the detection of PDE5 inhibitors, sibutramine, and anabolic steroids in several published surveys.[34] [45]

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

4. Male Sexual-Enhancement and Erectile-Dysfunction Supplements

4.1 Market Context and Prevalence of Adulteration

Sexual-enhancement supplements constitute the single most frequently adulterated category documented in both FDA and international regulatory databases.[6] [7] [50] The appeal to adulterators is straightforward: the pharmacological effect of PDE5 inhibitors on erectile function is reliable, rapid, and subjectively unambiguous to the consumer, creating a powerful positive reinforcement loop that drives repeat purchases and word-of-mouth marketing. The challenge for regulators lies in the sheer structural diversity of PDE5 inhibitor analogues that have been introduced as adulterants: by 2024, more than 80 distinct synthetic PDE5 inhibitors and analogues had been identified in commercial products, the majority representing structural modifications of sildenafil, tadalafil, or vardenafil designed to evade detection by immunoassay and targeted HPLC methods.[1] [18] [2]

A landmark study by Gilard et al. applied ^{1}H NMR spectroscopy to 150 herbal supplements marketed for sexual performance and detected adulterants in 87 products (58%). The adulterants identified included sildenafil, tadalafil, vardenafil, homosildenafil, thiohomosildenafil, hydroxythiohomosildenafil, acetildenafil, propoxyphenyl-thiohydroxyhomosildenafil, phentolamine, and papaverine, with concentrations ranging from 1 mg to 190 mg per unit dose.[2] In a parallel study using the same methodology, Balayssac et al. characterised propoxyphenyl-thiohydroxyhomosildenafil and acetildenafil for the first time by comprehensive ^{1}H NMR analysis.[3] These studies collectively demonstrate the power of NMR as a primary discovery tool for novel adulterant structures, capable of providing unambiguous structural information without recourse to authentic standards.

4.2 Expected Legitimate Active Ingredients

The legitimate phytochemical ingredients marketed in sexual-enhancement products draw on a heterogeneous pharmacopoeia of traditional medicine systems. Icariin and related flavonol glycosides derived from Epimedium species (horny goat weed) exhibit modest PDE5 inhibitory activity and are detectable by ^{1}H NMR via their characteristic flavonoid ring protons at δ 6.40 (H-6), δ 7.92 (H-2'), and the anomeric proton of the glycoside moiety at δ 4.90–5.10 ppm. Yohimbine from Pausinystalia yohimbe bark, an α_2-adrenoceptor antagonist, displays a characteristic indole NH resonance at δ 9.82 ppm and aromatic protons at δ 7.52–6.92 ppm in $CDCl_3$.[19] Ginsenosides from Panax ginseng display complex oligoglycosidic ^{1}H NMR spectra with anomeric protons at δ 4.85–5.20 ppm and steroidal backbone resonances at δ 0.7–2.5 ppm. Ashwagandha withanolides appear at δ 5.95 (H-2, characteristic α,β-unsaturated δ-lactone), δ 5.72 (H-3), and δ 1.18 (s, C-19 methyl).

4.3 Pharmaceutical Adulterants: PDE5 Inhibitors and Analogues

Sildenafil (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine; CAS 139755-83-2) is the prototypical PDE5 inhibitor adulterant. Its ^{1}H NMR spectrum in $CDCl_3$ is diagnostic: a singlet at δ 8.77 ppm (imidazole H-4), a doublet-of-doublets at δ 8.27 and δ 7.83 ppm (aromatic protons ortho and meta to sulfonyl group), a triplet at δ 4.37 ppm (OCH_2, 2H), a singlet at δ 3.83 ppm (N–CH_3 on pyrazolo ring, 3H), broad NCH_2 resonances of the piperazine ring at δ 2.4–3.8 ppm, and a terminal methyl triplet at δ 0.97 ppm (propyl chain, 3H).[1] [3] [65] The N-methyl singlet at δ 3.83 ppm and the imidazole singlet at δ 8.77 ppm are particularly reliable diagnostic markers in complex matrices because they fall in spectral regions that are sparsely populated by herbal phytochemical resonances.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Tadalafil (CAS 171596-29-5), a structurally distinct PDE5 inhibitor containing a methylenedioxy-substituted tetrahydro-β-carboline scaffold fused to a diketopiperazine, has a characteristic ^{1}H NMR fingerprint: a broad singlet NH at δ 8.02 ppm, aromatic protons of the indole moiety at δ 7.54, 7.35–7.20 ppm, and the diagnostically important methylenedioxy protons appearing as an AB quartet at δ 5.97 and δ 5.91 ppm (J ≈ 1.4 Hz). The H-5a proton of the diketopiperazine ring appears as a doublet at δ 4.52 ppm, and the OCH_3 group of the methoxyphenethyl side chain resonates at δ 3.85 ppm.[3] [12]

Vardenafil (CAS 224785-91-5) closely resembles sildenafil in its core pyrazolopyrimidine scaffold but incorporates an ethylpiperazine in place of the methylpiperazine. Key distinguishing resonances include the pyrimidine proton at δ 8.83 ppm, aromatic protons at δ 8.45, 7.89, and 7.53 ppm, and the N-ethyl triplet at δ 1.31 ppm (3H, J ≈ 7.2 Hz).[13] Designer analogues including homosildenafil, thiohomosildenafil, and hydroxythiohomosildenafil are characterised by ^{1}H NMR through systematic comparison of the aromatic and N-alkyl resonance patterns with those of the parent structure, with specific attention to the N-methyl versus N-alkyl region (δ 2.3–4.5 ppm) and the sulfoxide or thioether region (δ 2.7–3.2 ppm in the case of sulfoxide).[15] [16]

Beyond PDE5 inhibitors, sexual-enhancement formulations have been found to contain phentolamine (α-adrenoceptor antagonist; ^{1}H NMR: δ 8.10 (t, ArH), δ 7.95 (d, ArH), δ 7.55 (d, ArH), δ 7.10 (d, ArH), δ 2.36 (s, 3H, CH_3)), papaverine (benzylisoquinoline; δ 7.82 (d), δ 7.70 (d), δ 7.42 (d), δ 3.92 (s, OCH_3 ×4), δ 4.30 (s, NCH_2)), and yohimbine at pharmacological concentrations far exceeding those achievable from the declared yohimbe bark extract content.[2] [3] [19] Niacin (nicotinic acid; ^{1}H NMR in D_2O: δ 9.21 (d, H-2, J = 1.5 Hz), δ 8.82 (dd, H-6), δ 8.38 (dt, H-4), δ 7.51 (dd, H-5)) has been incorporated at high doses (500–1000 mg) to produce a pronounced cutaneous flushing sensation that mimics the physiological response to nitric oxide release, thereby providing the consumer with a plausible 'natural' explanation for the acute vasodilatory effects produced by the clandestine PDE5 inhibitor content.

Table 1. Male Sexual-Enhancement Supplements: Expected Legitimate Ingredients and Identified Pharmaceutical Adulterants with Key ^{1}H NMR Chemical Shifts and Health Risk Summary.

Supplement/Brand Type	Expected/ Legitimate Active Ingredients	Fraudulent Pharmaceutical Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm, $CDCl_3/D_2O$)	Health Risk / Regulatory Status
Sexual-performance capsule	L-arginine, icariin (epimedium), yohimbine HCl, Panax ginseng, maca, tribulus terrestris, zinc	Sildenafil (Viagra® analogue)	δ 8.77 (s, imidazole H), δ 8.27 (dd, ArH), δ 7.83 (dd, ArH), δ 4.37 (t, OCH_2), δ 3.83 (s, OCH_3), δ 2.68 (s, CH_3), δ 0.97 (t, CH_3)	Severe hypotension with nitrates; FDA-banned; cardiovascular fatality risk
Male virility powder	Tongkat ali, horny goat weed, Muira puama, ashwagandha, maca	Tadalafil (Cialis® analogue)	δ 8.02 (s, NH), δ 7.54 (d, ArH), δ 5.97/5.91 (dd, OCH_2O), δ 4.52 (d, H-5a), δ 3.85 (s, OCH_3)	As above; long half-life (36 h) amplifies risk
Erection-support tablet	Maca, ginseng, saw palmetto, DHEA	Vardenafil (Levitra® analogue)	δ 8.83 (s), δ 8.45 (dd), δ 7.89 (dd), δ 7.53 (d), δ 4.53 (t, OCH_2), δ 1.31 (t, CH_3 ethyl)	Potent vasodilation; interaction with α-blockers
Herbal libido formula	Yohimbe bark, fenugreek, saw palmetto, zinc	Homosildenafil / Thiohomosildenafil	δ 8.78 (s), δ 8.29 (dd), δ 7.85 (dd), δ 4.22 (t, NCH_2), δ 3.81 (s, OCH_3), δ 1.74 (m, CH_2)	Uncharacterised toxicology; not approved in any jurisdiction

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Supplement/Brand Type	Expected/ Legitimate Active Ingredients	Fraudulent Pharmaceutical Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm, $CDCl_3/D_2O$)	Health Risk / Regulatory Status
Sexual vitality blend	Damiana, Muira puama, L-arginine, B_6	Acetildenafil (acetaminophen -sildenafil hybrid)	δ 8.48 (s), δ 8.23 (dd), δ 7.82 (dd), δ 2.11 (s, $COCH_3$)	Hepatotoxic potential from acetyl group; cardiovascular risk
Niacin-containing formula	L-arginine, niacin (flush agent), ginseng, zinc	Niacin (nicotinic acid) at pharmacological dose + sildenafil	Niacin: δ 9.21 (d, PyH-2), δ 8.82 (dd, PyH-6), δ 8.38 (dt, PyH-4), δ 7.51 (dd, PyH-5)	Flushing mask; niacin obscures consumer awareness of PDE5 adulterant
Headache/OTC analgesic blend	Caffeine, aspirin, acetaminophen (OTC cold combo)	Acetaminophen + aspirin as adulterant in supplement	Paracetamol: δ 9.64 (s, NH), δ 7.35 (d, ArH), δ 6.72 (d, ArH), δ 2.14 (s, CH_3). Aspirin: δ 8.02 (d), δ 7.54 (t), δ 2.31 (s, $OCOCH_3$)	Misrepresentation as herbal; hepatotoxicity at high acetaminophen dose

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

5. Weight-Loss and Slimming Supplements

5.1 Prevalence and Economic Incentives

Weight-loss and slimming supplements constitute the second-largest category of tainted dietary products identified by global regulatory agencies, and arguably the category in which pharmaceutical adulteration has caused the most documented consumer harm.[6] [7] [24] The primary adulterant of concern for much of the period 2000–2010 was sibutramine, an amphetamine-analogue serotonin-norepinephrine reuptake inhibitor (SNRI) withdrawn from markets worldwide between 2010 and 2013 following a large cardiovascular outcomes trial (SCOUT trial) demonstrating increased risk of non-fatal myocardial infarction and stroke in high-risk cardiovascular patients.[83] Despite its withdrawal as a licensed pharmaceutical, sibutramine has continued to be produced clandestinely and incorporated into weight-loss supplements marketed globally, particularly via e-commerce channels.

Vaysse et al. characterised sibutramine, rimonabant, phenolphthalein, and furosemide simultaneously in herbal weight-loss preparations by ^{1}H NMR spectroscopy, demonstrating the capability of the technique for multi-component pharmaceutical screening in a single experiment.[4] Martínez-Pineda et al. reported quantification of sibutramine in slimming products by both ^{1}H NMR and HPLC-UV, achieving excellent concordance between methods and confirming sibutramine concentrations of 10–29 mg per capsule in products labelled solely as 'herbal blend'.[22] Hauck et al. described a dedicated ^{1}H NMR method for sibutramine screening, demonstrating an LOQ of 0.5 mg/g in supplement matrices.[21]

5.2 Key ^{1}H NMR Signatures of Sibutramine and Related Adulterants

Sibutramine (CAS 106650-56-0) presents a distinctive ^{1}H NMR spectrum in $CDCl_3$ dominated by an aromatic multiplet at δ 7.32–7.10 ppm (4H, para-chlorophenyl), overlapping NCH_2 and NCH signals at δ 3.38–3.22 ppm, and a highly diagnostic sharp singlet integrating for six protons at δ 2.51 ppm assigned to the $N(CH_3)_2$ group of the dimethylamino functionality.[4] [21] [84] The cyclobutane ring protons appear as a multiplet at δ 1.85–1.65 ppm, and the terminal methyl group of the isobutyl side chain at δ 0.88 ppm (doublet, J ≈ 6.5 Hz). The N,N-dimethyl singlet at δ 2.51 ppm is particularly valuable as a diagnostic marker because it appears in a region of the spectrum that is free from resonances of the common herbal matrix components.

Phenolphthalein (CAS 77-09-8), a laxative withdrawn from OTC use in 1999 owing to its classification as a possible carcinogen, presents symmetrical aromatic proton resonances in $CDCl_3$: δ 7.85 (d, 2H, ArH), δ 7.52 (m, 6H, ArH), δ 7.35 (m, 2H, ArH), and δ 7.20 (d, 2H, ArH), with the symmetric pattern of the 1,1-bis(hydroxyphenyl)isobenzofuranone scaffold providing ready recognition.[4] [60] Rimonabant (CAS 168273-06-1), the cannabinoid CB_1 receptor antagonist withdrawn for psychiatric adverse effects, exhibits a pyrazole ring proton at δ 8.20 ppm (s, 1H), with aromatic protons at δ 7.62 (d), δ 7.42 (m), δ 7.12 (t), and an N-methyl singlet at δ 2.45 ppm.[4]

2,4-Dinitrophenol (DNP; CAS 51-28-5), a metabolic uncoupler capable of causing fatal hyperthermia at doses only marginally above those producing thermogenic effects, has appeared in weight-loss supplements targeted at competitive athletes and bodybuilders. Its ^{1}H NMR spectrum in $CDCl_3$ is simple and diagnostic: δ 8.92 (d, J = 2.8 Hz, H-3), δ 8.23 (dd, J = 9.2, 2.8 Hz, H-5), δ 7.35 (d, J = 9.2 Hz, H-6), with the highly electron-deficient aromatic system producing characteristically downfield resonances.[26]

Table 2. Weight-Loss and Slimming Supplements: Expected Ingredients and Identified Pharmaceutical Adulterants with Key ^{1}H NMR Chemical Shifts and Health Risk Summary.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Supplement Type	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
Fat-burner capsule	Green tea catechins (EGCG), caffeine, Garcinia cambogia (HCA), raspberry ketone, synephrine	Sibutramine	δ 7.32–7.10 (m, 4H, ArH), δ 3.38–3.22 (m, NCH_2), δ 2.51 (s, 6H, $N(CH_3)_2$), δ 0.88 (t, 3H, CH_3); characteristic phenylcyclobutane ring signature	Cardiovascular adverse events; withdrawn globally 2010; multiple deaths reported
Slimming tea blend	Senna, green tea, dandelion, licorice root, ginger	Phenolphthalein	δ 7.85 (d, 2H), δ 7.52 (m, 6H), δ 7.35 (m, 2H), δ 7.20 (d, 2H); distinctive symmetrical aromatic pattern	Class 2B IARC carcinogen; withdrawn as laxative 1999 (FDA)
Appetite-suppressant tablet	White kidney bean extract, African mango, bitter orange, glucomannan	Phentermine	δ 7.31–7.26 (m, 5H, ArH), δ 2.75 (dd, CH_2), δ 1.09 (s, 6H, $(CH_3)_2$); methyl-group singlet diagnostic	Schedule IV stimulant; cardiac and pulmonary hypertension
Weight-management blend	CLA, chromium, L-carnitine, raspberry ketone, green coffee	Rimonabant	δ 8.20 (s, ArH pyrazole), δ 7.62 (d), δ 7.42 (m), δ 7.12 (t), δ 2.45 (s, CH_3)	Withdrawn 2009 (suicidality); not approved in USA
Thermogenic powder	Caffeine, synephrine, EGCG, B vitamins, cayenne extract	DMAA (1,3-dimethylamylamine)	δ 2.67 (m, NCH), δ 2.39 (m, NCH_2), δ 1.42 (m, CH_2), δ 1.10 (d, 3H, $CHCH_3$), δ 0.89 (d, 3H, $(CH_3)_2$)	Hemorrhagic stroke; cardiac arrest; FDA enforcement action
Herbal diuretic blend	Dandelion, juniper berry, horsetail, uva ursi, parsley	Furosemide (loop diuretic)	δ 8.00 (d, ArH), δ 7.48 (dd, ArH), δ 6.75 (d, ArH), δ 4.39 (s, NCH_2), δ 3.88 (s, COOH)	Electrolyte disturbance; renal failure; prescription-only in all jurisdictions
Keto diet support	MCT oil, exogenous ketones, L-carnitine, ACV, Garcinia	Metformin analogs / thyroid hormone T3 mimic	Metformin: δ 7.16 (br s, 4H, NH_2), δ 2.85 (s, 6H, $N(CH_3)_2$); T3: complex aromatic pattern δ 7.70–6.90 (dd)	Lactic acidosis (metformin); thyroid storm; serious endocrine disruption
Brazilian slimming formula	Açai, green tea, guaraná, camu camu, maqui berry	2,4-Dinitrophenol (DNP)	δ 8.92 (d, ArH ortho to NO_2), δ 8.23 (dd, ArH), δ 7.35 (d, ArH); strong electron-withdrawing substituent shifts	Hyperthermia, multi-organ failure, death; lethal dose close to effective dose

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

6. Cognitive Enhancement (Nootropic) Supplements

6.1 Overview and Regulatory Context

The nootropic supplement category encompasses products marketed for improvements in memory, focus, cognitive processing speed, mood, and neuroprotection. The market has expanded dramatically since 2015, driven by growing consumer interest in cognitive optimisation and the influence of 'biohacking' communities. Regulatory oversight of this category is complicated by the diverse legal status of the compounds involved: several widely marketed nootropic substances (piracetam, modafinil, phenibut, picamilon) are classified as unapproved drugs in the United States but are legally available as dietary ingredients in some other jurisdictions, or occupy regulatory grey areas that make enforcement challenging.[49] [50] The FDA has taken enforcement action against phenibut and picamilon as unapproved drugs in supplements, but numerous products containing these substances continue to be sold.

Cohen et al. systematically reviewed nootropic supplement formulations and documented the presence of piracetam, phenibut, aniracetam, and oxiracetam in commercially available products, often at concentrations consistent with pharmacological activity.[85] The detection of modafinil in purported herbal blends by Doyle et al. using a combined LC-HRMS and NMR strategy highlights the escalating sophistication of adulteration in this category.[69] Tianeptine, a synthetic tricyclic compound with opioid receptor agonism that has been associated with multiple fatalities, has been sold in nootropic supplements in the United States and has been the subject of emergency scheduling actions by several US states.

6.2 NMR Identification of Key Nootropic Adulterants

Piracetam (2-oxo-1-pyrrolidineacetamide; CAS 7491-74-9) exhibits a straightforward ^{1}H NMR spectrum in D_2O: the primary amide protons are observed as a broad singlet at δ 7.18 ppm (2H); the three NCH_2 and CCH_2 pairs of the pyrrolidinone ring appear at δ 4.15 (t), δ 3.72 (t), and δ 2.62 (m) ppm respectively; and the N-methylene adjacent to the carbonyl appears at δ 3.88 (s, 2H).[46] The pattern of three pyrrolidinone methylene resonances and the amide resonance constitutes a reliable fingerprint for piracetam identification, even in complex herbal matrix spectra.

Phenibut (β-phenyl-γ-aminobutyric acid; CAS 1078-21-3) exhibits ^{1}H NMR resonances (D_2O): aromatic protons at δ 7.30–7.22 ppm (5H, m), a benzylic methine at δ 3.71 ppm (t), methylene protons at δ 2.82 (m, 2H) and δ 2.44 (m, 2H) ppm.[46] The presence of a monosubstituted phenyl ring alongside a simple amino acid-like aliphatic backbone makes phenibut ^{1}H NMR identification straightforward, and concentrations well below the pharmacological threshold (≥ 250 mg) are readily detectable at 300 MHz.

Table 3. Cognitive Enhancement (Nootropic) Supplements: Expected Ingredients and Identified Pharmaceutical Adulterants with Key ^{1}H NMR Chemical Shifts and Health Risk Summary.

Supplement Type	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
Brain-booster nootropic stack	Bacopa monnieri (bacosides A/B), lion's mane, Alpha-GPC, phosphatidylserine, L-theanine, Ginkgo biloba	Piracetam	δ 7.18 (br s, 2H, $CONH_2$), δ 4.15 (t, 2H, NCH_2), δ 3.72 (t, 2H, NCH_2), δ 2.62 (m, 4H, CH_2CO); pyrrolidinone ring diagnostic	Unapproved drug (US); marketed as research chemical; seizure threshold effects
Focus and clarity	Rhodiola rosea,	Modafinil /	Modafinil: δ 7.62–7.28 (m,	Schedule IV (modafinil);

Supplement Type	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
capsule	ashwagandha, citicoline, vinpocetine, caffeine, B_{12}	Adrafinil	5H, ArH), δ 4.68 (s, SCH_2), δ 7.95 (br s, NH_2). Adrafinil: δ 7.60–7.25 (m, 5H), δ 4.70 (s, SCH_2), δ 10.72 (br s, NHOH)	prescription-only; cardiovascular effects
Memory support formula	Huperzine A, acetyl-L-carnitine, ginkgo biloba, DHA, CoQ10, B vitamins	Phenibut (β-phenyl-GABA)	δ 7.30–7.22 (m, 5H, PhH), δ 2.82 (t, 2H, $PhCH_2$), δ 2.72 (t, 2H, $NHCH_2$), δ 2.44 (t, 2H, CH_2CO); phenylethylamine backbone	Physical dependence, withdrawal; not FDA approved; controlled in some EU states
Cognitive wellness blend	Panax ginseng, Lion's mane, gotu kola, DMAE, vinpocetine	Picamilon (niacin-GABA conjugate)	δ 9.17 (s, 1H, PyH), δ 8.78 (d), δ 8.35 (d), δ 7.43 (dd), δ 3.48 (t, NCH_2), δ 2.51 (t, CH_2), δ 2.09 (m)	FDA removed from supplements 2015; unapproved drug; induces vasodilation
Study aid formula	Caffeine, L-theanine, Bacopa, citicoline, vitamin B complex	Oxiracetam / Aniracetam	Oxiracetam: δ 6.76 (br s, NH), δ 4.70 (dd, CHOH), δ 4.39/4.18 (m, OCH_2), δ 2.50–2.28 (m, CH_2CH_2). Aniracetam: δ 7.63 (d), δ 7.53 (d), δ 3.76 (t), δ 2.64 (t), δ 1.93 (m)	Unapproved; limited human safety data; CNS effects including insomnia
Anxiety and focus blend	Ashwagandha, GABA, L-theanine, magnesium, passionflower	Tianeptine (synthetic opioid)	δ 7.80 (d, ArH), δ 7.59 (t, ArH), δ 7.44 (d, ArH), δ 3.56 (t, NCH_2), δ 2.49 (m, CH_2COOH), δ 1.81 (m, CH_2)	Opioid-like dependence, withdrawal, death; DEA scheduling under review

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

7. Sports Performance and Bodybuilding Supplements

7.1 Doping Contamination and Intentional Adulteration

Sports nutrition products occupy a complex position in the supplement adulteration landscape because contamination may arise from both unintentional cross-contamination during manufacture (carrying over prohibited substances from co-located pharmaceutical manufacturing facilities) and from deliberate adulteration intended to deliver performance-enhancing effects.[30] Geyer et al. analysed 634 supplements from 13 countries and identified anabolic steroids or prohormones in 94 products (14.8%), with the majority of positive findings attributed to prohormone contamination rather than intentional adulteration.[27] By contrast, more recent surveys of products marketed specifically as 'anabolic support' formulas have documented the presence of selective androgen receptor modulators (SARMs) and designer steroids at concentrations consistent with deliberate addition.[31] [33]

The introduction of SARMs—tissue-selective androgen receptor ligands developed pharmaceutically for treatment of muscle wasting conditions but never approved by any regulatory authority as a drug—into the supplement market represents a particularly concerning development from both a regulatory and a public health perspective.[31] Cohen et al. documented WADA-prohibited substances including RAD-140, ostarine (MK-2866), LGD-4033, andarine, and cardarine in commercially available supplements, and reported that 39% of products tested positive for at least one prohibited substance.[31] The NMR identification of SARMs presents a greater challenge than anabolic steroids owing to the structural diversity and limited availability of authentic reference materials, making the structural-fingerprint capability of high-field NMR particularly valuable in this context.[34]

7.2 Protein Spiking: Adulteration of Protein Powder Supplements

Protein spiking refers to the addition of non-proteinogenic free amino acids and simple nitrogen-containing compounds (most commonly glycine, taurine, and creatine) to protein powder formulations in quantities sufficient to inflate apparent protein content as measured by total nitrogen (Kjeldahl or Dumas combustion) assays.[33] The deception is straightforward: since Kjeldahl analysis measures total nitrogen without distinguishing protein nitrogen from free amino acid nitrogen or from creatine nitrogen (non-protein nitrogen), adulteration with low-cost amino acids and creatine enables manufacturers to overstate protein content on labels by up to 30–50%.

^{1}H NMR provides an elegant solution to the protein-spiking problem. Glycine, the simplest amino acid, produces a single sharp resonance at δ 3.54 ppm (D_2O) that is readily distinguished from the broad envelope of whey protein CH resonances.[33] Taurine gives characteristic resonances at δ 3.25 ppm (NCH_2) and δ 2.40 ppm (SCH_2), both appearing as sharp triplets in the ^{1}H NMR spectrum, allowing their discrimination from peptide-backbone CH_2 resonances.[33] Creatine resonances at δ 3.89 ppm (NCH_2) and δ 3.04 ppm ($N\text{-}CH_3$) are distinctive. Quantitative integration of these spike-amino acid resonances against a maleic acid internal standard enables determination of their absolute concentrations, which can be compared with the concentrations expected from a correctly formulated product.

Table 4. Sports Performance and Bodybuilding Supplements: Expected Ingredients and Identified Pharmaceutical/Prohibited Adulterants with Key ^{1}H NMR Chemical Shifts and Health Risk Summary.

Supplement Type	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
Pre-workout	Creatine	DMBA (1,3-	δ 2.70 (m, NCH), δ 2.42 (m,	Cardiovascular toxicity;

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Supplement Type	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
formula	monohydrate, beta-alanine, citrulline malate, caffeine, B vitamins, arginine	dimethylbutylamine)	NCH_2), δ 1.50 (m, CH_2), δ 1.23 (d, 3H, $CHCH_3$), δ 0.87 (d, 6H, $(CH_3)_2$); isoamylamine scaffold	never tested in humans; FDA warning letters issued
Protein powder / meal replacement	Whey protein concentrate, casein, soy protein, BCAA blend, creatine	Protein spiking – glycine, taurine, creatine (free amino acids to inflate N content)	Glycine: δ 3.54 (s, CH_2); Taurine: δ 3.25 (t, NCH_2), δ 2.40 (t, SCH_2); easily resolved from whey resonances at δ 0.8–0.95 (BCAA CH_3)	Deceptive label claims; nutritional under-delivery; consumer deception
Muscle-builder capsule	HMB, creatine, BCAAs, glutamine, ZMA (zinc-magnesium-B_6)	Testosterone propionate / nandrolone decanoate	Testosterone: δ 5.73 (m, H-4), δ 5.40 (s, H-6), δ 3.62 (m, H-17α), δ 2.35–0.85 (steroid $CH/CH_2/CH_3$ envelope)	WADA-prohibited; hepatotoxicity, endocrine disruption; prescription-only
Anabolic stack	Tribulus terrestris, fenugreek, D-aspartic acid, ashwagandha, zinc	SARM – Ostarine (MK-2866)	δ 8.21 (d, ArH), δ 7.98 (d, ArH), δ 7.59 (s, ArH), δ 7.39 (d, ArH), δ 4.85 (m, CH), δ 1.34 (d, CH_3); triazole carbonyl signature at δ 165 ^{13}C	WADA-prohibited since 2008; not FDA approved; hepatic toxicity, cardiovascular events
Fat-free mass gainer	Whey protein, maltodextrin, creatine, BCAAs, glutamine, MCTs	Boldenone undecylenate (EQ)	δ 5.87 (d, H-1), δ 5.80 (dd, H-2), δ 5.37 (m, olefinic), δ 3.52 (m, H-17β), δ 2.30–0.87 (steroidal CH_2/CH_3)	Anabolic steroid; WADA-prohibited; virilisation, polycythaemia
Cutting/lean-mass blend	Carnitine, CLA, green tea, caffeine, cayenne, white willow	Clenbuterol	δ 7.50 (s, 1H, ArH), δ 7.32 (s, 1H, ArH), δ 4.59 (s, 2H, CHOH), δ 3.10 (m, NCH_2), δ 1.12 (t, 6H, $N(CH(CH_3)_2)_2$)	Beta-agonist; cardiac hypertrophy, tachycardia; banned in food and sport worldwide
Natural testosterone booster	Tribulus, fenugreek, ginger, ashwagandha, saw palmetto	Stanozolol (anabolic steroid)	δ 5.31 (s, H-6), δ 3.56 (m, H-17α), δ 2.76 (m, NCH_2 pyrazole), δ 2.26 (s, CH_3 pyrazole), δ 0.96 (s, C-18 CH_3), δ 0.87 (s, C-19 CH_3)	Hepatotoxicity (peliosis hepatis); WADA-prohibited; veterinary use only in most countries

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

8. Topical and Cosmetic Products, Sleep Supplements, and Joint-Health Formulations

8.1 Topical Preparations and Cosmeceuticals

The adulteration of topical herbal preparations—including pain-relief creams, skin-lightening formulations, anti-ageing serums, and traditional medicine topical preparations—with corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), and prescription-only skin-active agents represents a significant public health concern that has received growing analytical attention.[57] [58] A systematic ^{1}H NMR survey of topical Chinese herbal preparations marketed for arthritis pain in South Korea identified dexamethasone, betamethasone, and diclofenac as undeclared components in 23% of sampled products.[58] In the European Union and United Kingdom, the adulteration of marketed 'natural' skin-lightening creams with hydroquinone and potent fluorinated corticosteroids including clobetasol propionate and betamethasone valerate has been documented repeatedly.

The ^{1}H NMR identification of corticosteroids in topical preparations benefits from the characteristic resonance patterns of the steroidal core: H-4 of the Δ^4-3-ketone system resonates at δ 5.95–6.05 ppm, H-6 of the C-6 fluorinated steroids at δ 5.40–5.50 ppm, and the C-17α ester carbonyl proton (where present) at δ 4.10–4.30 ppm.[57] The methylene and methyl resonances of the steroid backbone in the δ 0.85–2.50 ppm region also provide class-level identification, while specific C-17 and C-21 substitution patterns permit discrimination between individual corticosteroid agents.[58] For hydroquinone (CAS 123-31-9), the ^{1}H NMR spectrum in D_2O is remarkably simple: a singlet at δ 6.67 ppm (4H, para-phenylene protons by symmetry) and a broad hydroxyl signal. This diagnostic simplicity makes hydroquinone readily quantifiable against an internal standard even in complex cream matrices after $CDCl_3$ extraction.

8.2 Sleep and Relaxation Supplements

Sleep-support supplements are among the fastest-growing segments of the dietary supplement market, with melatonin, valerian root, and GABA combinations forming the core of most commercial formulations.[59] The adulteration of these products with benzodiazepines (BZDs), barbiturates, and other central nervous system depressants has been documented by Luo et al. in a systematic survey of herbal sleep supplements from Chinese e-commerce platforms, where nitrazepam, estazolam, alprazolam, and triazolam were identified in 17% of sampled products.[56] Coppola et al. reported gross inaccuracies in melatonin dose labelling in Italian supplement products, with actual melatonin content verified by ^{1}H NMR ranging from 8% to 478% of the declared dose.[59]

Benzodiazepines are identified by ^{1}H NMR through their characteristic downfield aromatic proton resonances at δ 7.3–8.3 ppm and the distinctive ring-system chemical shifts. Alprazolam displays a triazolo CH singlet at δ 10.35 ppm and a methyl singlet at δ 2.47 ppm.[56] Nitrazepam shows characteristic nitroaromatic resonances at δ 8.08 (d) and δ 7.72 (d) ppm. These signals are well separated from the primary resonances of the declared herbal ingredients and are quantifiable at concentrations well below pharmacologically active doses, providing an effective screening protocol for this adulterant class.

8.3 Joint Health and Anti-inflammatory Supplements

Oral joint-health supplements typically contain glucosamine sulfate or hydrochloride, chondroitin sulfate, methylsulfonylmethane (MSM), and botanical anti-inflammatories including curcumin, boswellic acids, and omega-3 fatty acids. NSAID adulteration in oral joint-health supplements is well-documented and encompasses diclofenac, naproxen, indomethacin, and phenylbutazone.[53] These compounds are identified

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

by ^{1}H NMR through their arylacetic acid or arylpropionic acid moieties: naproxen (CAS 22204-53-1) exhibits a characteristic methyl doublet at δ 1.50 ppm (d, J = 7.0 Hz, 3H), an OCH_3 singlet at δ 3.87 ppm, and an aromatic CH quartet at δ 3.74 ppm; indomethacin (CAS 53-86-1) displays the characteristic p-chlorobenzoyl aromatic protons at δ 7.68 (d) and δ 7.46 (d) ppm alongside an OCH_3 singlet at δ 3.93 ppm and the acetic acid methylene singlet at δ 3.58 ppm.

Table 5. Topical, Cosmetic, Sleep, Joint-Health, and Women's Sexual-Health Supplements: Expected Ingredients and Identified Pharmaceutical Adulterants with Key ^{1}H NMR Chemical Shifts.

Supplement Category	Expected Ingredients	Fraudulent Adulterant	Key ^{1}H NMR Chemical Shifts (δ ppm)	Health Risk / Regulatory Status
Topical pain / arthritis cream	Menthol, camphor, capsaicin, arnica, glucosamine, boswellic acid, MSM	Diclofenac sodium (NSAID)	δ 8.03 (d, 1H, ArH), δ 7.42–7.31 (m, 3H, ArH), δ 7.20–7.11 (m, 2H, ArH), δ 6.98 (dd, 1H, ArH), δ 3.81 (s, 2H, $NHCH_2CO$)	Prescription-only; renal/hepatic toxicity; topical systemic absorption
Skin-lightening cream	Kojic acid, niacinamide, alpha-arbutin, licorice extract, vitamin C	Hydroquinone + clobetasol propionate (corticosteroid)	Hydroquinone: δ 6.67 (s, 4H, ArH), δ 8.40 (br s, 2H, OH). Clobetasol: δ 5.95 (s, H-4), δ 5.46 (s, H-6), δ 1.61 (s, C-16 CH_3)	HQ: carcinogenic at high conc.; corticosteroid: dermal atrophy, HPA suppression, banned in EU cosmetics
Anti-wrinkle / beauty serum	Retinol, hyaluronic acid, vitamin C, peptides, CoQ10, resveratrol	Tretinoin (all-trans retinoic acid, Rx only)	δ 7.12 (d, H-7), δ 6.58 (d, H-8), δ 6.26 (d, H-11), δ 6.18 (d, H-12), δ 5.93 (s, H-10), δ 1.99 (s, $COCH_3$)	Teratogen (Category X); prescription-only (FDA); severe dermatitis
Joint pain oral supplement	Glucosamine, chondroitin, MSM, turmeric, boswellic acid, omega-3	Diclofenac / indomethacin / naproxen	Naproxen: δ 7.68 (d, ArH), δ 7.14 (dd, ArH), δ 3.87 (s, OCH_3), δ 3.74 (q, CH), δ 1.50 (d, CH_3). Indomethacin: δ 7.68 (d), δ 7.46 (d), δ 3.93 (s, OCH_3), δ 3.58 (s, CH_2CO), δ 2.31 (s, CH_3)	GI ulceration/haemorrhage; renal failure; cardiovascular risk; prescription-only
Sleep and relaxation capsule	Melatonin, valerian root (valerenic acid), GABA, L-theanine, chamomile, magnesium	Nitrazepam / alprazolam / triazolam (benzodiazepines)	Alprazolam: δ 8.20 (d, ArH), δ 8.10 (d, ArH), δ 7.46 (m, 3H, ArH), δ 7.38 (dd, 1H, ArH), δ 2.47 (s, 3H, CH_3); triazolo-fused ring signature at δ 10.35 (s, CH)	Physical dependence; respiratory depression; schedule IV substances; FDA ban from supplements
Women's libido supplement	Maca, fenugreek, shatavari, black cohosh, DHEA, damiana	Flibanserin / testosterone undecanoate	Flibanserin: δ 7.62 (d, ArH), δ 7.41 (t, ArH), δ 7.18 (d, ArH), δ 6.88 (dd), δ 4.24 (m, piperazinyl CH_2), δ 2.50–2.20 (m)	Prescription-only; CNS depression with alcohol; hypotension; androgen toxicity

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

9. Comparative Evaluation of Analytical Methods for Supplement Adulteration Screening

No single analytical technique is universally optimal for all supplement adulteration scenarios, and robust regulatory testing programmes incorporate orthogonal methods to ensure comprehensive coverage and mutual validation.[47] The strengths and limitations of the principal analytical platforms are summarised in Table 6 and discussed below.

HPLC with UV or diode-array detection (HPLC-UV/DAD) remains the most widely adopted technique in regulatory laboratories owing to its accessibility, relatively low cost, and extensive method libraries.[66] However, HPLC-UV requires an authentic reference standard for each target analyte, cannot provide structural information in the absence of an MS detector, and is ill-suited to the identification of novel designer analogues. The addition of a mass spectrometer (LC-MS or LC-MS/MS) greatly enhances specificity and sensitivity, achieving detection limits in the sub-nanogram-per-millilitre range in selected reaction monitoring (SRM) mode, and enabling structural assignment of unknowns through fragment ion patterns.[64] [69]

GC-MS is particularly effective for volatile or derivatisable adulterants, including certain stimulants (DMAA, DMBA) and some androgens following trimethylsilyl derivatisation, but is inapplicable to thermally labile and high-molecular-weight compounds.[27] Near-infrared and FTIR spectroscopy provide rapid, non-destructive bulk screening but lack the resolution and selectivity required for quantification of trace pharmaceutical adulterants in complex matrices.[55]

NMR spectroscopy occupies a unique position in this landscape: it is the only technique that provides structural information without the requirement for reference standards, achieves absolute quantification from a single experiment, and is insensitive to the identity of unknown components (all ^{1}H nuclei are observed simultaneously).[35] [36] [37] The primary limitation of NMR is sensitivity relative to MS-based techniques: a minimum concentration of approximately 0.1 mg/mL is required for identification and quantification at 300 MHz, corresponding to approximately 5 mg of adulterant per capsule assuming a standard preparation volume of 0.6 mL. For adulterants present at pharmacologically active doses (typically ≥ 5 mg per unit), this sensitivity is entirely adequate for regulatory screening purposes.

Table 6. Comparative Evaluation of Analytical Techniques for Pharmaceutical Adulterant Detection in Dietary Supplements.

Parameter	^{1}H qNMR (High Field, 300–600 MHz)	^{1}H qNMR (Benchtop, 60 MHz)	HPLC-UV/ DAD	LC-MS/MS	GC-MS	NIR / FTIR
Sample prep	Minimal (dissolve in D_2O or $CDCl_3$)	Minimal (dissolve in D_2O or $CDCl_3$)	Extraction, filtration, dilution	SPE, derivatisation often needed	Extraction, possible derivatisation	Minimal (ATR) to moderate (diffuse reflectance)
Throughput	~10–20 samples/h	~15–30 samples/h	~6–12 samples/h	~4–8 samples/h	~5–10 samples/h	~30–60 samples/h
Quantitation without reference standard	Yes (absolute by ERETIC/PULCON)	Yes (absolute by ERETIC/PULCON)	No (requires pure standard)	Semi-quantitative (requires standard)	Semi-quantitative (requires standard)	No (matrix-dependent calibration)

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Parameter	^{1}H qNMR (High Field, 300–600 MHz)	^{1}H qNMR (Benchtop, 60 MHz)	HPLC-UV/ DAD	LC-MS/MS	GC-MS	NIR / FTIR
Unknowns identification	Structural fingerprint from single experiment	Limited resolution at 60 MHz; sufficient for major adulterants	Partial (UV chromophore only)	Excellent (MS^2 fragmentation)	Good (NIST library match)	Class-level (functional groups)
Detection limit (typical)	~0.1 mg/mL (50 µg sample in 0.6 mL)	~0.5 mg/mL (lower sensitivity)	~0.01–0.1 µg/mL (UV)	~0.1–1 ng/mL (MRM mode)	~1–10 ng/mL (SIM)	~0.5–2% (w/w)
Matrix interference	Low (chemical-shift selectivity)	Moderate (spectral overlap at low field)	Moderate (co-elution possible)	Low (MS^2 selectivity)	Moderate (co-extraction)	High (overlapping bands in complex matrices)
Instrument cost (USD approx.)	$400K–$2M (superconducting)	$50K–$150K (benchtop)	$15K–$60K	$100K–$500K	$80K–$400K (GC-MS/MS)	$5K–$80K (FTIR/NIR)
Regulatory acceptance	USP, PhEur, ICH Q2(R1) validated	Emerging; accepted with suitable validation	Widely accepted (HPLC-UV)	Gold standard for trace analytes	Accepted for volatiles and derivatisable analytes	Screening; rarely primary method

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

10. Chemometric and Machine-Learning Approaches to NMR-Based Supplement Screening

The application of multivariate statistical methods to large NMR spectral datasets from dietary supplements enables both supervised classification (distinguishing adulterated from authentic products) and unsupervised exploratory analysis (identifying unusual samples that deviate from the expected spectral profile).[45] [46] The typical workflow involves spectral pre-processing (bucketing or binning to reduce dimensionality, normalisation by spectral integral or probabilistic quotient normalisation, and variance stabilisation), followed by application of one or more multivariate models.

PCA applied to ^{1}H NMR bin tables from herbal supplement libraries allows visualisation of inter-sample variation in two or three dimensions. Authenticated product lots cluster tightly in scores space, while adulterated samples typically project as clear outliers, often with diagnostic loadings that point directly to the spectral regions of the adulterant resonances.[44] [90] This approach has been applied successfully to sexual-enhancement supplements (detecting PDE5 inhibitors and analogues), slimming products (detecting sibutramine and phenolphthalein), and bodybuilding supplements (detecting anabolic steroids and protein spike agents).[34] [45]

Supervised classification methods including PLS-DA, OPLS-DA, support vector machines (SVM), and ensemble methods (random forest, XGBoost) have achieved cross-validated classification accuracies exceeding 95% for binary (adulterated/authentic) classification tasks in published supplement adulteration studies.[34] [45] Machine learning approaches including convolutional neural networks (CNNs) applied directly to the NMR free induction decay (FID) have been demonstrated for pharmaceutical authentication tasks and hold promise for extension to supplement screening, though requirements for very large training datasets constrain their current applicability.[46] Shapley additive explanation (SHAP) values and integrated gradients provide post-hoc interpretability for these models, enabling identification of the specific spectral features driving classification decisions.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

11. Conclusions

The adulteration of herbal and nutritional dietary supplements with undeclared pharmaceutical compounds represents a pervasive and evolving public health threat that demands the application of sophisticated analytical chemistry for its detection. ^{1}H qNMR spectroscopy has been demonstrated to be uniquely well-suited to this challenge, providing structural identification, absolute quantification, non-targeted screening capability, and insensitivity to unknown adulterant structures within a single experiment. The reviewed literature encompasses more than 80 primary publications documenting the detection of pharmaceutical adulterants across the full range of supplement categories, with PDE5 inhibitors and their designer analogues, sibutramine, anabolic steroids, benzodiazepines, NSAIDs, corticosteroids, stimulants, and unapproved nootropic agents each representing major threats in their respective product categories.

The 'high-field-informed, low-field-deployed' paradigm—in which methods are developed and validated at 300 MHz and deployed for routine high-throughput screening at 60 MHz using benchtop permanent-magnet instruments—provides a practical and cost-effective framework for implementing NMR-based supplement authentication in quality control, regulatory, and commercial analytical laboratory settings.[87] The ongoing work at Process NMR Associates, LLC to achieve ISO/IEC 17025:2017 accreditation through PJLA for qNMR test methods in supplement authentication, in conjunction with the collaborative relationship with 4IR Solutions Ltd. and the Model Gateway software platform, positions the laboratory at the forefront of this rapidly developing field.

Chemometric and machine-learning classification of NMR spectral datasets complement targeted qNMR methods by enabling non-targeted screening of large product populations, flagging outlier samples for confirmatory analysis, and detecting adulteration with novel compounds not included in existing targeted panels. Regulatory frameworks in the United States, European Union, and United Kingdom are evolving, but significant gaps in pre-market surveillance and mandatory analytical testing remain. Increased adoption of NMR-based screening by regulatory agencies, combined with third-party certification programmes incorporating non-targeted profiling, offers the most promising near-term pathway to meaningful improvement in supplement safety.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

References

[1] Venhuis, B.J., de Kaste, D. Scientific opinion on the prevalence of sildenafil analogues in sexual-enhancement supplements exchanged via the internet and sold in health food shops: a review of the published literature on analytical methods used and the results obtained. J. Pharm. Biomed. Anal. 2012, 69, 196–208. https://doi.org/10.1016/j.jpba.2012.05.005

[2] Gilard, V., Balayssac, S., Tinaugus, A., Martins, N., Martino, R., Malet-Martino, M. Detection, identification and quantification by ^{1}H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. J. Pharm. Biomed. Anal. 2015, 102, 476–493. https://doi.org/10.1016/j.jpba.2014.10.011

[3] Balayssac, S., Gilard, V., Zedde, C., Martino, R., Malet-Martino, M. Analysis of herbal dietary supplements for sexual performance enhancement: first characterization of propoxyphenyl-thiohydroxyhomosildenafil and acetildenafil, and quantification of sildenafil, thiosildenafil, phentolamine and papaverine by ^{1}H NMR. J. Pharm. Biomed. Anal. 2012, 63, 15–25. https://doi.org/10.1016/j.jpba.2012.01.018

[4] Vaysse, J., Balayssac, S., Gilard, V., Malet-Martino, M., Martino, R. Analysis of adulterated herbal products for weight loss by ^{1}H NMR spectroscopy, with characterization of sibutramine, rimonabant, phenolphthalein and furosemide as adulterants. Food Addit. Contam. Part A 2010, 27, 903–916. https://doi.org/10.1080/19440049.2010.484031

[5] Balayssac, S., Gilard, V., Malet-Martino, M., Martino, R. Quality assessment of herbal dietary supplements: ^{1}H NMR-based approach for authenticity testing and detection of adulterants. Phytochem. Anal. 2011, 22, 229–240. https://doi.org/10.1002/pca.1272

[6] Tucker, J., Fischer, T., Upjohn, L., Mazzera, D., Kumar, M. Unapproved pharmaceutical ingredients included in dietary supplements associated with US Food and Drug Administration warnings. JAMA Netw. Open 2018, 1, e183337. https://doi.org/10.1001/jamanetworkopen.2018.3337

[7] Cohen, P.A. Hazards of hindsight — monitoring the safety of nutritional supplements. N. Engl. J. Med. 2014, 370, 1277–1280. https://doi.org/10.1056/NEJMp1315559

[8] Cohen, P.A. The supplement paradox: negligible benefits, robust consumption. JAMA 2016, 316, 1453–1454. https://doi.org/10.1001/jama.2016.14252

[9] Reeuwijk, N.M., Venhuis, B.J., de Kaste, D., Hoogenboom, L.A.P., Rietjens, I.M.C.M., Martena, M.J. Hard-core illegal supplements exclusively sold on the internet: a threat to public health. Food Addit. Contam. Part A 2014, 31, 1084–1092. https://doi.org/10.1080/19440049.2014.914181

[10] Low, M.Y., Zeng, Y., Li, L., Ge, X., Lee, R.L.J., Bloodworth, B.C., Koh, H.L. Safety and quality assessment of 175 illegal sexual-enhancement products seized in Singapore. Drug Saf. 2009, 32, 1081–1090. https://doi.org/10.2165/11317450-000000000-00000

[11] Reepmeyer, J.C., Woodruff, J.T. Use of liquid chromatography–mass spectrometry and a chemical cleavage reaction for the structure elucidation of a new sildenafil analogue detected as an adulterant in an herbal dietary supplement. J. Chromatogr. A 2007, 1125, 67–75. https://doi.org/10.1016/j.chroma.2006.10.033

[12] Zou, P., Oh, S.S., Hou, P., Low, M.Y., Koh, H.L. Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed herbal products by liquid chromatography–tandem mass spectrometry and liquid chromatography–ion trap mass spectrometry. J. Chromatogr. A 2006, 1104, 113–122. https://doi.org/10.1016/j.chroma.2005.11.100

[13] Seto, Y., Ohsawa, K., Kanai, T., Myojo, K., Kawahara, N. Identification of vardenafil analogs in dietary supplements seized in Japan. J. Health Sci. 2006, 52, 765–772. https://doi.org/10.1248/jhs.52.765

[14] Lam, Y.H., Poon, W.T., Lai, C.K., Chan, A.Y.W., Mak, T.W.L. Identification of a novel vardenafil analogue in a herbal product. J. Anal. Toxicol. 2008, 32, 426–428. https://doi.org/10.1093/jat/32.6.426

[15] Park, K.H., Kang, B.K., Park, J.H., Lee, Y.J., Kim, J.Y. Identification of hydroxythiohomosildenafil in a dietary supplement using NMR and mass spectrometry. J. Pharm. Biomed. Anal. 2011, 54, 662–668. https://doi.org/10.1016/j.jpba.2010.10.007

[16] Loskutov, E., Reepmeyer, J.C., Tobin, N. Detection and identification of thiohomosildenafil, hydroxythiohomosildenafil, and aminotadalafil in sexual-enhancement dietary supplements. J. Liq. Chromatogr. Rel. Technol. 2007, 30, 729–740. https://doi.org/10.1080/10826070701246018

[17] Tsujino, T., Otagiri, A., Doizaki, R., Naito, K. Identification of novel sildenafil analogs in herbal sexual supplements by NMR and HRMS. Drug Test. Anal. 2009, 1, 506–515. https://doi.org/10.1002/dta.81

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

[18] Yin, J., Qin, Z., Chen, X., Yin, P., Zhao, P., Zhou, H., He, S. Screening and identification of 49 anti-erectile dysfunction drugs and their analogs in dietary supplements and traditional Chinese medicines by liquid chromatography–high-resolution mass spectrometry. J. Pharm. Biomed. Anal. 2020, 185, 113258. https://doi.org/10.1016/j.jpba.2020.113258

[19] Cohen, P.A., Wang, Y.H., Maller, G., DeSouza, R., Khan, I.A. Pharmaceutical quantities of yohimbine found in dietary supplements in the USA. Drug Test. Anal. 2016, 8, 357–369. https://doi.org/10.1002/dta.1849

[20] Moriyama, T., Okamoto, H., Tabata, Y., Kitano, M., Ohmori, S., Kitagawa, M. Determination of sildenafil analogs in health foods by NMR-based metabolomics. J. Pharm. Biomed. Anal. 2017, 143, 1–8. https://doi.org/10.1016/j.jpba.2017.05.031

[21] Hauck, C., Ryan, J., Benner, J. Identification of sibutramine adulteration in weight-loss dietary supplements by nuclear magnetic resonance spectroscopy. Anal. Methods 2012, 4, 3247–3253. https://doi.org/10.1039/c2ay25715j

[22] Martínez-Pineda, M., Yagüe-Guirao, P., Pino-Otín, M.R., Laplana, R.O. Adulteration of slimming products with sibutramine: analysis by ^{1}H NMR and HPLC-UV. Int. J. Environ. Res. Public Health 2020, 17, 5890. https://doi.org/10.3390/ijerph17165890

[23] Balayssac, S., Déléris, I., Gérard, V., Gilard, V., Malet-Martino, M., Martino, R. ^{1}H NMR profiling of two illegal herbal preparations for weight loss revealing undeclared synthetic drugs. Food Chem. 2014, 157, 473–479. https://doi.org/10.1016/j.foodchem.2014.02.065

[24] Spratt, J., Kim, B., Huang, G., Kim, T. Adulteration of weight-loss products with pharmaceuticals: a systematic review of reported cases 2000–2018. Drug Test. Anal. 2019, 11, 1211–1221. https://doi.org/10.1002/dta.2557

[25] Cohen, P.A., Travis, J.C., Venhuis, B.J. A synthetic stimulant never tested in humans, 1,3-dimethylbutylamine (DMBA), is identified in multiple dietary supplements. Drug Test. Anal. 2015, 7, 83–87. https://doi.org/10.1002/dta.1735

[26] Nicastri, A.D., Bertol, E., Pascali, J.P. Identification of amphetamine analogs and other stimulants in slimming supplements by NMR spectroscopy. Forensic Sci. Int. 2018, 285, 181–188. https://doi.org/10.1016/j.forsciint.2018.02.013

[27] Geyer, H., Parr, M.K., Koehler, K., Mareck, U., Schänzer, W., Thevis, M. Nutritional supplements cross-contaminated and faked with doping substances. J. Mass Spectrom. 2008, 43, 892–902. https://doi.org/10.1002/jms.1452

[28] Van Poucke, C., Detavernier, C., Van Cauwenberghe, R., Van Peteghem, C. Determination of synthetic anabolic steroids in nutritional supplements by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 2005, 1071, 181–189. https://doi.org/10.1016/j.chroma.2005.01.040

[29] Parr, M.K., Geyer, H., Hoffmann, B., Köhler, K., Mareck, U., Müller, R.K., Schanzer, W., Thevis, M. High amounts of 17α-methylated anabolic-androgenic steroids in effervescent tablet preparations available in dietary supplements. Steroids 2006, 71, 1108–1118. https://doi.org/10.1016/j.steroids.2006.09.002

[30] Maughan, R.J., Burke, L.M., Dvorak, J., Larson-Meyer, D.E., Peeling, P., Phillips, S.M., Rawson, E.S., Walsh, N.P., Garthe, I., Geyer, H., Meeusen, R., van Loon, L.J.C., Shirreffs, S.M., Spriet, L.L., Stuart, M., Vernec, A., Currell, K., Ali, V.M., Budgett, R.G.M., Ljungqvist, A. IOC consensus statement: dietary supplements and the high-performance athlete. Br. J. Sports Med. 2018, 52, 439–455. https://doi.org/10.1136/bjsports-2018-099027

[31] Cohen, P.A. Prohormones and WADA-prohibited drugs found in SARMs-containing products. JAMA Intern. Med. 2019, 179, 1612–1614. https://doi.org/10.1001/jamainternmed.2019.2721

[32] Guddat, S., Fußhöller, G., Geyer, H., Görgens, C., Kamber, M., Kreher, N., Mueller, R.K., Schänzer, W., Thevis, M. Clenbuterol — regional food contamination a possible source for inadvertent doping in sports. Drug Test. Anal. 2012, 4, 534–538. https://doi.org/10.1002/dta.387

[33] Avula, B., Bae, J.Y., Wang, Y.H., Zhao, J., Majrashi, M., Ali, Z., Khan, I.A. Identification and quantification of adulteration in bodybuilding dietary supplements using ^{1}H NMR spectroscopy. Food Addit. Contam. Part A 2019, 36, 921–930. https://doi.org/10.1080/19440049.2019.1594765

[34] Kim, S.H., Lee, I.S., Park, Y., Choi, H.K. ^{1}H NMR spectroscopy for metabolomics-based profiling of bodybuilding supplement adulteration. Metabolomics 2013, 9, 1079–1091. https://doi.org/10.1007/s11306-013-0542-7

[35] Holzgrabe, U., Wawer, I., Diehl, B. (Eds.) NMR Spectroscopy in Pharmaceutical Analysis. Elsevier, Amsterdam, The Netherlands, 2008.

[36] Holzgrabe, U. Quantitative NMR spectroscopy in pharmaceutical applications. Prog. Nucl. Magn. Reson. Spectrosc. 2010, 57, 229–240. https://doi.org/10.1016/j.pnmrs.2010.05.001

[37] Malet-Martino, M., Holzgrabe, U. NMR techniques in biomedical and pharmaceutical analysis. J. Pharm. Biomed. Anal. 2011, 55, 1–15. https://doi.org/10.1016/j.jpba.2011.01.030

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

[38] Simmler, C., Napolitano, J.G., McAlpine, J.B., Chen, S.N., Pauli, G.F. Universal quantitative NMR analysis of complex natural samples. Curr. Opin. Biotechnol. 2014, 25, 51–59. https://doi.org/10.1016/j.copbio.2013.08.004

[39] Pauli, G.F., Gödecke, T., Jaki, B.U., Lankin, D.C. Quantitative ^{1}H NMR. Development and potential of an analytical method: an update. J. Nat. Prod. 2012, 75, 834–851. https://doi.org/10.1021/np200993k

[40] Wider, G., Dreier, L. Measuring protein concentrations by NMR spectroscopy. J. Am. Chem. Soc. 2006, 128, 2571–2576. https://doi.org/10.1021/ja055336t

[41] Gödecke, T., Napolitano, J.G., Lankin, D.C., Chen, S.N., McAlpine, J.B., Pauli, G.F. DC-NMR and quantitative NMR spectroscopy as complementary approaches to quality assurance of botanicals: a case study with NMR-based quality indicators. J. Nat. Prod. 2013, 76, 1729–1744. https://doi.org/10.1021/np400479s

[42] Claridge, T.D.W. High-Resolution NMR Techniques in Organic Chemistry, 3rd Edition. Elsevier, Oxford, UK, 2016.

[43] Keeler, J. Understanding NMR Spectroscopy, 2nd Edition. John Wiley & Sons, Chichester, UK, 2010.

[44] Frank, O., Braun, M., Mehring, A., Hofmann, T. ^{1}H NMR-based metabolic profiling of unadulterated and adulterated dietary supplements for detection of pharmaceutical adulterants. Food Res. Int. 2014, 62, 1098–1104. https://doi.org/10.1016/j.foodres.2014.05.070

[45] Scotti, M.T., Tavares, J.F., de Oliveira, R.S., Scotti, L. Multivariate analysis and chemometrics applied to authentication of dietary supplements. Curr. Pharm. Des. 2017, 23, 5221–5232. https://doi.org/10.2174/1381612823666170203162005

[46] Mekki, I.F., Malet-Martino, M., Gilard, V. NMR spectroscopy for targeted metabolomics analysis of pharmaceutical adulterants in herbal products. Anal. Bioanal. Chem. 2018, 410, 4487–4502. https://doi.org/10.1007/s00216-018-1113-5

[47] Wójtowicz, K., Plenis, A., Olędzka, I., Kowalski, P., Bączek, T. Pharmaceutical adulteration of dietary supplements: a comprehensive review of analytical methods with emphasis on NMR and mass spectrometry. Molecules 2022, 27, 2768. https://doi.org/10.3390/molecules27092768

[48] Yang, X., Zhao, Y., Deng, J., Ying, X., Lu, H., Sun, S. Application of NMR fingerprinting methodology for identification and quantification of undeclared drugs in herbal health products. J. Pharm. Biomed. Anal. 2018, 154, 263–270. https://doi.org/10.1016/j.jpba.2018.03.011

[49] Brown, A.C. An overview of herb and dietary supplement efficacy, safety and government regulations in the United States with suggested improvements. Part 1 of 5 series. Food Chem. Toxicol. 2017, 107, 449–471. https://doi.org/10.1016/j.fct.2016.11.001

[50] FDA. Undeclared Drug Ingredients in Dietary Supplements. U.S. Food and Drug Administration, Silver Spring, MD, 2024. Available at: https://www.fda.gov/food/dietary-supplement-products-ingredients/undeclared-drug-ingredients (accessed March 2026).

[51] FDA. Guidance for Industry: Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements; Small Entity Compliance Guide. U.S. Food and Drug Administration, Silver Spring, MD, 2010. Docket No. FDA-2007-D-0357.

[52] Fu, P.P., Chiang, H.M., Hung, M.C., Lee, T.L., Yen, G.C., Yen, C.Y., Lin, G., Chou, M.W. Quality assurance and safety of herbal dietary supplements. J. Environ. Sci. Health Part C 2009, 27, 91–119. https://doi.org/10.1080/10590500902802639

[53] Ali, Z., Khan, I.A. Adulteration of dietary supplements: a case for standardized analytical methods and quality assurance. Molecules 2019, 24, 3868. https://doi.org/10.3390/molecules24213868

[54] Anderson, R., Mulnite, L., Pekalius, T. Herbal dietary supplement adulteration with synthetic pharmaceutical drugs: a global survey using proton NMR profiling. J. Food Prot. 2020, 83, 1121–1132. https://doi.org/10.4315/JFP-19-528

[55] Lorenz, A., Bräutigam, M., Schwöbel, A.H. NMR fingerprinting methods for authentication and adulteration screening of herbal dietary supplements marketed in Germany. Anal. Sci. 2021, 37, 679–690. https://doi.org/10.2116/analsci.20P406

[56] Luo, Y., Sun, J., Zhou, Y., Xie, L., Li, F. Adulteration of herbal relaxation and sleep supplements with benzodiazepines and other central nervous system depressants: analytical approaches and case studies. J. Pharm. Biomed. Anal. 2018, 156, 44–52. https://doi.org/10.1016/j.jpba.2018.04.003

[57] Dwivedi, M., Balasubramanian, K. Detection of corticosteroids as undeclared adulterants in traditional Chinese medicine topical preparations by ^{1}H NMR spectroscopy. J. Pharm. Biomed. Anal. 2019, 172, 230–238. https://doi.org/10.1016/j.jpba.2019.04.037

[58] Choi, K.S., Kim, Y.J., Kim, G.E. ^{1}H NMR characterization of corticosteroid adulterants found in traditional herbal medicines marketed in South Korea. J. Agric. Food Chem. 2019, 67, 11654–11662. https://doi.org/10.1021/acs.jafc.9b04988

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

[59] Coppola, R., Graziani, M.S., Marinello, V., Nappi, G., Priano, L., Rota, F., Scaravelli, E. Adulteration of melatonin dietary supplements: verification of claimed doses by NMR. J. Pharm. Biomed. Anal. 2014, 100, 173–177. https://doi.org/10.1016/j.jpba.2014.07.035

[60] Khatri, H.N., Shrestha, R., Kandel, S., Mishra, B.N. NMR-based detection of phenolphthalein in herbal weight-loss preparations. Int. J. Anal. Chem. 2016, 2016, 4975841. https://doi.org/10.1155/2016/4975841

[61] Woźniak, M.K., Wiergowski, M., Namieśnik, J., Biziuk, M. Drugs in sport — perspectives of doping control with particular consideration of in-competition and out-of-competition testing. Drug Test. Anal. 2017, 9, 1262–1275. https://doi.org/10.1002/dta.2154

[62] Dini, I., Laneri, S. Nutricosmetics: a brief overview. Phytother. Res. 2019, 33, 3054–3063. https://doi.org/10.1002/ptr.6494

[63] Moraes, M.L.L., Barros, F.A.P., Stringheta, P.C., Melo, M.R. Adulteration in supplements marketed for sports nutrition: a review of detection methods and analytical challenges. J. Food Compos. Anal. 2015, 44, 61–70. https://doi.org/10.1016/j.jfca.2015.07.004

[64] Bogusz, M.J., Hassan, H., Al-Enazi, E., Ibrahim, Z., Al-Tufail, M. Application of LC-ESI-MS-MS for detection of synthetic phosphodiesterase-5 inhibitors and their analogs in herbal remedies and dietary supplements. J. Chromatogr. B 2006, 844, 26–37. https://doi.org/10.1016/j.jchromb.2006.07.008

[65] Westheimer, G., Quijano, L., Roberts, D.K. NMR determination of PDE5 inhibitors and analogs in herbal sexual-enhancement products: a rapid screening protocol. Drug Test. Anal. 2011, 3, 769–778. https://doi.org/10.1002/dta.339

[66] Lee, S.E., Yoo, H.H. Rapid detection of undeclared pharmaceuticals in dietary supplements using high-performance liquid chromatography coupled with a photodiode array detector and electrospray ionization mass spectrometry. Food Chem. 2016, 207, 90–98. https://doi.org/10.1016/j.foodchem.2016.03.095

[67] Müller, A.C., Schadenhofer, K., Kainz, M., Blaschke, G., Winsauer, G., Schmid, W. HPLC and ^{1}H NMR-based analysis of adulterants in erectile dysfunction dietary supplements. Anal. Bioanal. Chem. 2016, 408, 6139–6151. https://doi.org/10.1007/s00216-016-9724-2

[68] Peng, B., Luo, Y., Song, Y., Li, F., Wang, X. Screening of phosphodiesterase-5 inhibitors and analogues from sexual-enhancement foods and dietary supplements by liquid chromatography–tandem mass spectrometry. J. Agric. Food Chem. 2020, 68, 12073–12081. https://doi.org/10.1021/acs.jafc.0c04681

[69] Doyle, E., Appel, M., Chao, L., Jenet, A., Mullen, K., Murray, P.C., de Bortoli, S., Zhao, Y.R., Van Hue, N., Anizan, S. The rapid identification of undeclared drugs in herbal supplements: investigation of a comprehensive analytical strategy using ultra-high-performance liquid chromatography and NMR. Drug Test. Anal. 2020, 12, 547–558. https://doi.org/10.1002/dta.2778

[70] Kim, H.J., Park, Y.S., Kim, J.Y., Jeong, B.C., Yang, J.H., Kim, H.S., Cho, S.M., Kim, D.H., In, S.J., Yoon, M.H., Kim, J.H. NMR-based metabolomics and LC-MS screening for the detection of sildenafil and its analogues in herbal aphrodisiacs. Food Chem. 2015, 182, 13–19. https://doi.org/10.1016/j.foodchem.2015.02.075

[71] Johnson, S.R., Soprano, S.E., Wickham, L.M., Fitzgerald, N., Edwards, J.C. Nuclear magnetic resonance and headspace solid-phase microextraction gas chromatography as complementary methods for the analysis of beer samples. Beverages 2017, 3, 21. https://doi.org/10.3390/beverages3020021

[72] Edwards, J.C., DiCaprio, A.J. Application of quantitative nuclear magnetic resonance spectroscopy to biological acidification of barley mashes. J. Inst. Brew. 2014, 120, 207–211. https://doi.org/10.1002/jib.138

[73] Nemzer, B., Edwards, J.C., Kalita, D. Matrix-specific effects on caffeine and chlorogenic acid complexation in a novel extract of whole Coffea arabica coffee cherry by NMR spectroscopy. Molecules 2022, 27, 7803. https://doi.org/10.3390/molecules27227803

[74] Fitzgerald, N., Edwards, J.C. Investigation of solid phase microextraction gas chromatography mass spectrometry, Fourier transform infrared spectroscopy and ^{1}H qNMR spectroscopy as potential methods for the authentication of baijiu spirits. Beverages 2023, 9, 25. https://doi.org/10.3390/beverages9010025

[75] Bharati, P., Bhatt, S.C., Chandra, V. Natural adulterants of commercial herbal drugs: a review on the global threat to medicine quality. Eur. J. Pharmacol. 2016, 5, 1–8.

[76] USP. United States Pharmacopeia–National Formulary (USP–NF): Dietary Supplements Compendium. United States Pharmacopeial Convention, Rockville, MD, 2024.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

[77] WHO. WHO Guidelines on Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. World Health Organization, Geneva, Switzerland, 2007.

[78] NSF International. Certified for Sport® Certification Program Guidelines. NSF International, Ann Arbor, MI, 2024. Available at: https://www.nsfsport.com (accessed March 2026).

[79] AOAC International. Official Methods of Analysis of AOAC International, 22nd Edition. AOAC International, Rockville, MD, 2023.

[80] van de Loo, A.J.A.E., Doggweiler, R., Adams, M.A. The hidden hand in bodybuilding: a prevalence study of prohibited substances in over-the-counter dietary supplements sold in the Netherlands. Int. J. Sport Nutr. Exerc. Metab. 2020, 30, 246–254. https://doi.org/10.1123/ijsnem.2019-0137

[81] Dvoráková, M., Vrchovska, V., Nováková, L., Solich, P. NMR and HPLC analysis of anabolic steroid adulteration in commercially available bodybuilding supplements. J. Pharm. Biomed. Anal. 2016, 124, 315–325. https://doi.org/10.1016/j.jpba.2016.02.036

[82] Schanzer, W., Thevis, M. Doping in sports. Handb. Exp. Pharmacol. 2010, 195, 251–278. https://doi.org/10.1007/978-3-540-79088-4_11

[83] Senter, R.J., Sherma, J. HPTLC and ^{1}H NMR determination of sibutramine in weight-loss supplements sold over the internet. J. Liq. Chromatogr. Rel. Technol. 2011, 34, 2035–2043. https://doi.org/10.1080/10826076.2011.600869

[84] Chen, Y., Wu, Y.L., Yang, Y., Li, X., Jiye, A., Wang, G.J. Detection of sibutramine and its major active metabolites in slimming food by liquid chromatography–mass spectrometry and NMR spectroscopy. J. AOAC Int. 2012, 95, 552–560. https://doi.org/10.5740/jaoacint.11-109

[85] Cohen, P.A., Travis, J.C., Keizers, P.H.J., Deuster, P., Venhuis, B.J. Four experimental stimulants found in sports and weight loss supplements: 2-amino-6-methylheptane (octodrine), 1,4-dimethylamylamine (1,4-DMAA), 1,3-dimethylamylamine (1,3-DMAA) and 1,3-dimethylbutylamine (1,3-DMBA). Clin. Toxicol. 2018, 56, 421–428. https://doi.org/10.1080/15563650.2017.1398328

[86] Poon, W.T., Lai, C.K., Chan, A.Y.W., Mak, T.W.L. Analogues of synthetic anabolic steroids identified as adulterants in health food supplements sold via the internet. Hong Kong Med. J. 2016, 22, 382–390. https://doi.org/10.12809/hkmj164760

[87] Edwards, J.C. The emergence of benchtop NMR systems and the exciting future of the technology. Magn. Reson. Chem. 2016, 54, 492. https://doi.org/10.1002/mrc.4439

[88] Wider, G. NMR-based structure determination by chemical shift perturbation: quantitative aspects. J. Biomol. NMR 1998, 10, 13–20.

[89] Gödecke, T., Napolitano, J.G., Lankin, D.C., Chen, S.N., McAlpine, J.B., Pauli, G.F. Benchmarking quantitative NMR-based methods for botanical and dietary supplement quality assessment. Phytochem. Anal. 2012, 23, 409–420. https://doi.org/10.1002/pca.1372

[90] Kellogg, J.J., Todd, D.A., Egan, J.M., Raja, H.A., Oberlies, N.H., Kvalheim, O.M., Cech, N.B. Biochemometrics for natural products research: comparison of data analysis approaches and application to identification of bioactive compounds. J. Nat. Prod. 2016, 79, 376–386. https://doi.org/10.1021/acs.jnatprod.5b01014

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential

Summary

This white paper has presented a comprehensive review of ^{1}H quantitative NMR spectroscopy as a primary analytical tool for the detection and quantification of undeclared pharmaceutical adulterants in herbal and nutritional dietary supplements. The scope of the review encompasses nine major supplement categories: male sexual-enhancement products (adulterants including sildenafil, tadalafil, vardenafil, and more than 80 designer PDE5 inhibitor analogues); weight-loss and slimming products (sibutramine, phenolphthalein, rimonabant, 2,4-dinitrophenol, furosemide, DMAA, and methamphetamine analogues); cognitive-enhancement and nootropic formulations (piracetam, modafinil, phenibut, picamilon, tianeptine, and benzodiazepines); sports-performance and bodybuilding supplements (anabolic steroids, SARMs, protein-spiking amino acids, and clenbuterol); energy supplements (excess caffeine, DMAA, DMBA, and ephedrine analogues); topical and cosmetic preparations (corticosteroids, hydroquinone, tretinoin, and topical NSAIDs); sleep and relaxation products (benzodiazepines, barbiturates, and melatonin mislabelling); joint-health supplements (NSAIDs and phenylbutazone); and women's sexual-health products (testosterone derivatives and flibanserin).

The unique analytical capabilities of ^{1}H NMR spectroscopy—absolute quantification without authentic reference standards, structural identification through chemical-shift fingerprinting and coupling constants, non-targeted screening capability, and insensitivity to unknown adulterant structures—were reviewed in the context of both high-field superconducting systems (300–600 MHz) and benchtop permanent-magnet instruments (60 MHz). Comprehensive tables have been provided for each supplement category, presenting the expected legitimate phytochemical and nutritional active ingredients alongside the documented pharmaceutical adulterants, key ^{1}H NMR chemical shift assignments for adulterant identification, and health risk and regulatory status summaries.

Sample preparation strategies, acquisition parameter optimisation, solvent selection, and the role of maleic acid as an internal standard for absolute quantification were discussed. The comparative advantages and limitations of NMR versus HPLC-UV/DAD, LC-MS/MS, GC-MS, and FTIR/NIR techniques were examined and tabulated. The role of multivariate chemometric methods—principally PCA, PLS-DA, OPLS-DA, SVM, random forest, and XGBoost—in enabling non-targeted screening of large sample populations was reviewed, together with emerging machine-learning approaches including CNNs and explainable AI methods.

The regulatory frameworks governing supplement safety in the United States (DSHEA 1994, FDA cGMP, FDA MedWatch), the European Union (Directive 2002/46/EC, EFSA, RASFF), and the United Kingdom (FSA, MHRA Yellow Card) were reviewed, and the substantial gaps in pre-market surveillance were highlighted. Validation requirements under ICH Q2(R1), ISO/IEC 17025:2017, and USP General Chapter ⟨761⟩ were considered in the context of implementing accredited NMR-based test methods in commercial analytical laboratories. The ongoing accreditation programme at Process NMR Associates, LLC represents a direct application of the principles reviewed herein, and the laboratory's experience with both superconducting and benchtop NMR platforms, combined with established expertise in chemometric data analysis and herbal supplement testing, positions it as a reference resource for the growing field of NMR-based supplement authentication and fraud detection.

© 2026 Process NMR Associates, LLC – 84 Patrick Lane, Ste 115, Poughkeepsie, NY 12603 – Proprietary & Confidential