# Ashwagandha (*Withania somnifera* (L.) Dunal) Root and Leaf Extracts: Safety, Withanolides, and Analytical Standards — A Technical Review

**Ashwagandha Standards Alliance, www.ashwagandhastandards.org**

## Abstract

Ashwagandha (Withania somnifera (L.) Dunal) is among the most widely used botanical ingredients in dietary and herbal supplements globally. Despite extensive history of traditional use and substantial modern toxicological and clinical literature supporting safety at customary doses, recent regulatory scrutiny—particularly in the European Union—has focused on isolated adverse event reports, notably hepatobiliary injury. This technical review critically evaluates the evidence base for ashwagandha extract safety, with particular emphasis on (i) the interpretation of adverse event case reports, (ii) comparative safety of root and leaf extracts and key withanolides including withaferin A, (iii) appropriate precautionary labeling for pregnancy and lactation, and (iv) analytical methodologies and standards for the determination and labeling of withanolide content. Drawing primarily on recent comprehensive reviews published in *Phytotherapy Research* (2025), this paper concludes that the totality of evidence does not support a causal association between properly manufactured ashwagandha extracts and serious liver injury at studied dosages, that both root- and leaf-derived extracts have been evaluated in robust preclinical safety programs, and that harmonization of analytical methods and labeling practices in line with AOAC and FDA expectations represents the most constructive path forward for industry and regulators alike.

Keywords

Ashwagandha; Withania somnifera; withanolides; withaferin A; hepatotoxicity; dietary supplements; analytical methods.

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## 1. Introduction

Ashwagandha (Withania somnifera (L.) Dunal; Solanaceae) occupies a prominent position in traditional Ayurvedic medicine and has become a leading ingredient in Western dietary supplement markets for stress, sleep, and cognitive health. Commercial preparations include powders, aqueous and hydroalcoholic extracts, and standardized extracts derived from root, leaf, or combinations thereof, typically labeled by total withanolide content.

While numerous reviews have characterized ashwagandha as well tolerated, a series of recent clinical case reports of liver injury temporally associated with ashwagandha use have prompted renewed regulatory concern. These concerns have culminated in proposed or enacted restrictions in several EU member states. In response, two major open-access reviews published in *Phytotherapy Research* in 2025 provide the most comprehensive synthesis to date of regulatory, toxicological, and mechanistic evidence.

The present technical review builds on these publications with the specific aim of summarizing an evidence-based, industry-relevant framework for ashwagandha extract safety and analytical standardization, suitable for informing buyers, manufacturers, and regulators.

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## 2. Adverse Event Reports and Hepatotoxicity: Weight of Evidence

### 2.1 Overview of Reported Cases

Published case reports of suspected ashwagandha-induced liver injury describe predominantly cholestatic or mixed-pattern hepatitis, often presenting with jaundice and pruritus. In the largest compiled series, outcomes were largely reversible following discontinuation, though severe cases—including acute-on-chronic liver failure and fatalities—occurred in patients with significant pre-existing liver disease.

Critically, these reports share several limitations: extremely small sample size, lack of product characterization, absence of quantitative withanolide analysis, frequent use of non-standardized or homemade preparations, and multiple confounding factors, including comorbid disease and concomitant medications (**Table 1**).

| Evidence Category | Scope of Evidence | Strengths | Key Limitations | Regulatory Weight | Relevant Findings |
|---|---|---|---|---|---|
| Case reports / pharmacovigilance | ~15–30 published reports globally | Human exposure; signal detection | No product characterization; confounding disease and medications; reporting bias | Low to Moderate | Mostly reversible cholestatic or mixed-pattern liver injury; causality typically assessed as "possible" |
| Controlled clinical trials | 100+ (RCTs and open-label) | Defined dose; clinical monitoring | Short duration; healthy populations | High | No consistent elevations in liver enzymes or serious hepatic adverse events |
| Preclinical oral toxicity studies | >20 studies (OECD and non-OECD) | Dose–response; margin of exposure | Species extrapolation | High | NOAELs commonly 1,000–2,000 mg/kg bw; no hepatotoxic signal |
| Genotoxicity / reproductive toxicity | Multiple in vitro and in vivo assays | Predictive for serious risk | Conservative endpoints | High | Negative or protective findings; no mutagenicity or reproductive toxicity |
| Traditional and historical use | Centuries of widespread use | Population-scale exposure | Non-standardized preparations | Limited | No pattern consistent with intrinsic hepatotoxicity |

**Table 1.** Weight-of-Evidence Framework for Safety Assessment of Ashwagandha Extracts

### 2.2 Lack of Concordance with Preclinical Data

As comprehensively reviewed by Williamson and Brendler (2025), preclinical toxicity studies—spanning acute, subacute, subchronic, genotoxicity, and reproductive endpoints—do not predict hepatotoxic risk. Oral NOAELs for standardized root extracts consistently reach 1,000–2,000 mg/kg body weight in rodents, corresponding to human equivalent doses far exceeding typical supplement intakes.

A number of data gaps in the case reports have been identified related to possible contributors from ashwagandha consumption (**Table 2**). No causal associations between plant part, extraction solvent,

withanolide content, withaferin A (WA) and liver injury have been established based on the comprehensive reviews of published literature summarized in this report.

| Product Characterization | Reported in Case Reports? | Relevance to Safety Interpretation |
|---|---|---|
| Botanical part used (root vs leaf) | No | Different plant parts exhibit different withanolide profiles |
| Extraction solvent / process | No | Strongly influences phytochemical composition |
| Total withanolide content | No | Prevents dose–exposure assessment |
| Withaferin A concentration | No | Central constituent in regulatory concern |
| Product authentication | Rarely | Risk of misidentification or adulteration |
| Contaminant testing (metals, solvents) | No | Alternative causes of liver injury not excluded |
| Concomitant medications | Incomplete | Generally, a major confounder in liver injury assessment |
| Pre-existing liver disease | Often | Elevates baseline risk of cholestatic injury |

**Table 2.** Data gaps identified in published ashwagandha-associated hepatotoxicity case reports.

Importantly, no reproducible liver injury signal are shown in animal toxicology data, even at high doses or with extracts standardized to elevated withanolide levels (**Table 3**). Taken together, the evidence strongly suggests that idiosyncratic response, undiagnosed health conditions, or potentially, product quality-related factors underlie reported cases, rather than an intrinsic hepatotoxic property of ashwagandha.

| Safety Parameter | Range |
|---|---|
| Typical human daily intake (clinical studies) | 300–1,000 mg/day |
| High-end consumer intake (upper percentile) | ~1,500 mg/day |
| Rodent NOAEL (oral administration) | 1,000–2,000 mg/kg bw/day |
| Human equivalent dose (HED) | ~11–22 g/day |
| Margin of exposure (NOAEL vs intake) | ~10–20× |

**Table 3.** Margin of exposure between human Intake and Preclinical NOAELs for Ashwagandha Extracts

## 2.3 Interpretation of Totality of Evidence

Taken together, the adverse event literature does not establish a generalizable causal relationship between ashwagandha extracts and hepatotoxicity at studied dosages. The case report data are more consistent with rare, idiosyncratic reactions, potentially exacerbated by pre-existing liver disease, extreme dosing, concomitant medications, undiagnosed health complications, or poorly characterized preparations.

**Clinical Safety Summary (Root and Leaf Preparations)**

Across the broader clinical trial literature, ashwagandha preparations—predominantly root extracts and, more recently, root–leaf extracts—have generally been well tolerated for short-term use, with adverse events typically characterized as mild and self-limited (most commonly gastrointestinal complaints and somnolence), and serious adverse events uncommon. A regulatory review noted that the NIH Office of Dietary Supplements summarizes evidence from "many" clinical trials as indicating ashwagandha "has been well tolerated by participants for up to about 3 months of use," with only few reports of serious side effects related to liver function.

This review reiterates that pregnant and breastfeeding individuals should avoid use in the absence of adequate evidence (NIH 2023). In the same review, the authors emphasize that neither toxicological nor clinical investigations of products using leaf material or mixtures of leaves and roots have yielded significant variation in composition, effect, or adverse effect reporting relative to root-only preparations, although substitution or admixture warrants continued evaluation.

Consistent with this interpretation, a self-affirmed GRAS assessment for a branded root–leaf extract standardized to 35% withanolide glycosides concluded—based on compositional controls, toxicological data, and the published clinical literature on *W. somnifera* root and leaf preparations—that the ingredient is well tolerated in humans under intended conditions of use. Clinical placebo-controlled studies of ashwagandha root extract used for stress, sleep, and related neuropsychiatric indications (e.g., insomnia/anxiety) are representative of the broader tolerability profile reported in the literature.

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## 3. Safety of Root and Leaf Extracts and the Role of Withanolides

### 3.1 Phytochemical Considerations

Ashwagandha contains more than 40 characterized withanolides, steroidal lactones based on an ergostane skeleton. Roots and leaves share several major constituents (e.g., withanolide A, withaferin A, withanone). Leaves may contain higher relative concentrations of certain withanolides, including withaferin A.

### 3.2 Root Extracts

Root-derived extracts represent the most extensively studied preparations. Multiple OECD-compliant toxicity studies on branded, standardized extracts (including those standardized up to 35% total withanolides) demonstrate absence of systemic toxicity, genotoxicity, or organ-specific injury at doses well above human exposure (**Table 4**).

### 3.3 Leaf and Root–Leaf Extracts

Leaf-containing extracts have been subject to greater scrutiny due to higher withaferin A content and in vitro cytotoxicity findings. However, in vivo studies with isolated withaferin A administered orally to rodents demonstrate NOAELs exceeding 500 mg/kg/day in subacute studies and >2,000 mg/kg in acute studies. No hepatotoxicity or systemic toxicity has been observed at these doses.

| Preparation Type | Preclinical Toxicology | Clinical Evidence | Hepatotoxicity Signal | Notes |
|---|---|---|---|---|
| Root extract | Extensive | Extensive | None | Most studied and commercially prevalent |
| Leaf extract | Moderate | Limited but growing | None | Higher relative withaferin A content |
| Root–leaf extract | Moderate | Moderate | None | No differential safety signal identified |
| Isolated withaferin A | Extensive (animals) | Limited | None | In vitro bioactivity not predictive of oral toxicity |

**Table 4**. Comparative safety evidence for ashwagandha preparations by plant part

### Solvents and Processing Aids Used in Ashwagandha Extracts

Commercial ashwagandha extracts are produced using a limited and well-characterized set of solvents and processing aids that are consistent with food, dietary supplement, and traditional medicine manufacturing practices. Across the published toxicological, clinical, and regulatory literature, no solvent class used in properly manufactured ashwagandha extracts has been identified as an independent driver of safety concerns when used in accordance with good manufacturing practices (GMP).

### Aqueous and Hydroalcoholic Extraction

The most common extraction systems for ashwagandha are water, ethanol, or water–ethanol mixtures, reflecting both traditional preparation methods and modern supplement manufacturing. Hydroalcoholic extraction is generally favored, because it efficiently solubilizes withanolides and related steroidal lactones while limiting extraction of undesirable constituents such as excessive alkaloids.

Ethanol used for botanical extraction is food-grade and generally characterized to residual solvent levels compliant with pharmacopeial and regulatory limits such as USP. The extensive preclinical and clinical literature reviewed in Phytotherapy Research includes numerous extracts produced via aqueous or hydroalcoholic systems without evidence of solvent-related toxicity

### Milk- and Ghee-Based Traditional Preparations

Certain traditional Ayurvedic preparations of ashwagandha use milk, ghee, or milk-derived matrices as extraction or delivery media (e.g., Ashwagandha ghrita). These lipid-rich systems may reflect traditional practices, rather than modern supplement extraction technology. Importantly, preclinical toxicity studies of such preparations—including those containing quantified withaferin A—have demonstrated no acute or subacute toxicity at oral doses up to 2,000 mg/kg body weight in rodents. In these studies, no evidence of hepatotoxicity or systemic toxicity was observed.

If milk or milk derivatives are present in a finished product, they are subject to food allergen labeling requirements under U.S., EU, and other international regulations. Modern commercial ashwagandha extracts intended for the global supplement market may avoid the addition of milk to prevent allergen labeling complications and cross-contact risk.

### Other Processing Aids

Additional processing aids used in ashwagandha extract manufacture may include filtration media, clarifying agents, and drying carriers (e.g., maltodextrin or gum acacia) for spray-drying or standardization. These materials are widely used in food and supplement manufacturing, are typically removed or present at trace levels in the finished extract, and are regulated as processing aids or excipients depending on jurisdiction. None have been implicated in adverse safety findings in the ashwagandha literature.

**Safety and Regulatory Interpretation**

Across the body of published evidence, solvent choice and traditional processing aids do not correlate with reported adverse events. Case reports of liver injury associated with ashwagandha supplementation almost uniformly lack information on extraction solvent, processing aids, or residual solvent levels, precluding attribution of risk to these factors. The absence of corroborating preclinical signals further supports the conclusion that extraction solvents and traditional carriers, when used and labeled appropriately, are not considered as safety concerns.

## 5.6 Clinical Safety of Ashwagandha Root and Leaf Extracts

Clinical investigations of ashwagandha root and leaf extracts collectively support a favorable safety profile when products are manufactured to FDA and other relevant quality standards and used at studied dosages. Randomized controlled trials and open-label studies of standardized root extracts—most commonly administered at daily doses ranging from 300 to 1,000 mg—have consistently reported good tolerability, with adverse events generally mild, transient, and comparable to placebo, typically involving gastrointestinal discomfort or somnolence. Importantly, these studies have not demonstrated clinically meaningful alterations in liver enzymes, renal markers, or hematological parameters in healthy adult populations. Leaf-containing extracts, including high-withanolide preparations have likewise been evaluated in human studies without identification of a distinct safety signal.

Across published trials, branded extracts containing root and leaf have shown no evidence of hepatotoxicity, endocrine disruption, or systemic toxicity at recommended intakes, findings that align with extensive preclinical safety margins. Taken together, the clinical literature does not indicate differential safety risk between root-only and root–leaf extracts, nor does it implicate elevated withanolide content as a determinant of adverse outcomes under conditions of intended use.

## 3.4 Withaferin A: “Smoking Gun” or Red Herring?

While withaferin A exhibits potent biological activity in vitro—including antitumor and pro-apoptotic effects—these findings occur at concentrations not reflective of oral exposure from supplements. In vivo oral toxicity studies do not support a role for withaferin A as a primary toxicant at customary intake levels. Accordingly, current evidence does not justify singling out leaf extracts or withaferin A as inherently unsafe when produced to appropriate specifications (**Table 5**).

**Safety of Withaferin A: Preclinical and Clinical Context**

Withaferin A (WA) is one of the most intensively studied withanolides present in *Withania somnifera* and has attracted particular scrutiny due to its pronounced biological activity in vitro and its relatively higher abundance in leaf material compared with root. As a result, WA has sometimes been hypothesized as a potential driver of adverse effects associated with ashwagandha preparations. A critical appraisal of the available evidence does not support this hypothesis.

| Hypothesized Mechanism | Expected Observations, if True | Observations in Ashwagandha Data |
|---|---|---|
| Hepatocellular toxicity | Dose-dependent ALT/AST elevations | Not observed in animals or clinical trials |
| Reactive metabolite formation | Consistent animal liver injury | Absent |
| Genotoxic stress | Positive Ames or micronucleus tests | Negative; protective effects reported |
| Immune-mediated drug-induced liver injury (DILI) | Appearance after re-exposure | Not documented |

**Table 5.** Mechanistic assessment for expected observations of hepatotoxicity.

**Preclinical Toxicology**

In vitro studies demonstrate that WA exhibits dose-dependent cytotoxicity, particularly in cancer-derived cell lines, where it induces apoptosis, oxidative stress, and disruption of cytoskeletal and inflammatory signaling pathways. These effects underpin ongoing investigation of WA as an anticancer lead compound but occur at concentrations that substantially exceed systemic exposures achievable through oral consumption of ashwagandha extracts.

In contrast, in vivo oral toxicity studies provide a markedly different safety profile. Administration of isolated WA to rodents has demonstrated high tolerability, with acute NOAELs exceeding 2,000 mg/kg body weight and subacute NOAELs exceeding 500 mg/kg/day, without evidence of hepatotoxicity, genotoxicity, or clinically relevant alterations in serum chemistry, hematology, or histopathology. Consistent with these findings, repeated-dose studies of whole ashwagandha extracts standardized to elevated total withanolide content—including preparations containing quantifiable WA—have not revealed liver injury or systemic toxicity at doses far exceeding human intake.

**Genotoxicity and Mechanistic Considerations**

Mechanistic studies examining WA–DNA interactions, including reports of covalent adduct formation under experimental conditions, have not translated into evidence of mutagenicity or clastogenicity in standard regulatory test systems. On the contrary, ashwagandha extracts containing WA have repeatedly demonstrated absence of genotoxic effects in bacterial reverse mutation assays, chromosomal aberration tests, and micronucleus assays, and in some models have shown protective effects against chemically induced DNA damage. These observations underscore the importance of interpreting mechanistic findings involving isolated WA within the broader pharmacokinetic and metabolic context of oral exposure to complex botanical matrices.

**Clinical Relevance**

To date, no controlled clinical studies have administered purified WA as a single agent, and no adverse event reports have established a causal association between WA exposure and human toxicity at doses achievable through ashwagandha supplementation. Clinical investigations of ashwagandha extracts—including root-only and root–leaf preparations—have not identified WA-specific adverse effect patterns, nor have they demonstrated dose-dependent safety signals attributable to WA content. The absence of a differential clinical signal between extracts with varying WA content further argues against WA as a unique driver of reported adverse events.

**Interpretation and Implications for Quality Assessment**

Collectively, the available data indicate that WA's pronounced in vitro bioactivity should not be conflated with in vivo toxicity. Within authenticated, quality-controlled ashwagandha extracts, WA behaves as one of several pharmacologically active constituents rather than as a safety-limiting compound. Accordingly, analytical detection and quantification of WA—whether as part of a multi-peak chromatographic fingerprint or as a marker compound—should be interpreted as a normal feature of the botanical matrix rather than an intrinsic hazard. Continued attention to extract characterization, dose justification, and post-market surveillance remains appropriate; however, current evidence does not justify disproportionate regulatory or commercial focus on WA in isolation.

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## 4. Precautionary Labeling

Despite a lack of convincing preclinical and clinical evidence for hepatobiliary, reproductive or developmental toxicity, the human data remain limited. For example, a prenatal toxicity study in rodents showed no maternal or fetal toxicity at doses up to 2,000 mg/kg/day.

Given the historical ethnobotanical claims of abortifacient use and the absence of controlled clinical studies in pregnant or nursing women, standard conservative, precautionary labeling advising against use during pregnancy and lactation remains appropriate and consistent with broader dietary supplement practice. This recommendation reflects regulatory prudence based on the precautionary principle, rather than evidence of demonstrated harm.

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## 5. Analytical Determination of Withanolides

### 5.1 Overview of Published Methods

A wide range of analytical approaches has been reported for the quantification of withanolides, including HPLC-UV, HPLC-DAD, UPLC, and LC–MS/MS methods. Differences among methods include extraction solvents, reference standards, chromatographic conditions, and whether results are expressed as total withanolides, specific marker compounds, or withanolide glycosides.

### 5.2 USP and Pharmacopeial Approaches

The USP monograph framework emphasizes identity confirmation and quantitative determination of characteristic withanolides using validated chromatographic methods. Strengths include reproducibility and suitability for routine quality control; limitations include dependence on available reference standards and variability in total withanolide expression.

### 5.3 Method Variability and Acceptable Ranges

Inter-laboratory variability in reported withanolide content is expected due to botanical diversity, plant part used, extraction processes, and analytical choices. Such variability is not, in itself, indicative of quality defects, provided that methods are validated and labeling is accurate.

### 5.4 Validation and Labeling Standards

To ensure regulatory defensibility and consumer transparency, potency methods for withanolides should conform to AOAC and FDA expectations, including validation for specificity, linearity, accuracy, precision, limit of detection, and limit of quantification. Label claims should clearly state the analytical basis (e.g., total withanolides by HPLC) and avoid overinterpretation of composite values.

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## 5.5 Comparative Overview of Withanolide Analytical Methods

| Method / Source | Primary Technique | Analytes Quantified | Reference Standards | Sample Prep | Strengths | Limitations | Typical Use Case |
|---|---|---|---|---|---|---|---|
| USP–NF (proposed / draft approaches) | HPLC–UV or HPLC–DAD | Marker withanolides (e.g., withanolide A, withaferin A, withanone) | Authenticated USP reference standards | Solvent extraction (aqueous alcohol) | Reproducible, pharmacopeial alignment, suitable for QC | Limited number of markers; does not capture full withanolide diversity | Routine identity & potency verification |
| AOAC-style validated in-house methods | HPLC–UV/DAD or UPLC–UV | Total withanolides (sum of peaks vs standards) | External calibration with selected withanolides | Manufacturer-specific | Flexible; adaptable to extract type | Inter-lab variability; dependent on validation rigor | Commercial release & label claim substantiation |
| Published HPLC–UV (root-focused) | HPLC–UV | Individual and total withanolides | Withanolide A or mixed standards | Hydroalcoholic | Widely used; literature precedent | UV co-elution risk; limited specificity | Research & comparative profiling |
| Published UPLC methods | UPLC–UV or UPLC–DAD | Expanded withanolide profile | Multiple purified standards | Optimized solvent systems | Higher resolution, shorter run times | Requires advanced instrumentation | Advanced QC & R&D |
| LC–MS / LC–MS/MS | LC–MS, LC–MS/MS | Individual withanolides & glycosides | High-purity analytical standards | Targeted extraction | High specificity; structural confirmation | Cost; not routine QC-friendly | Method development & confirmation |
| HPTLC (identity-focused) | HPTLC–densitometry | Withanolide fingerprint | Reference extracts | Minimal | Rapid identity confirmation | Semi-quantitative at best | Raw material authentication |

**Table 6. Comparison of published and pharmacopeial methods for the determination of withanolides in ashwagandha extracts.**

Variability in reported withanolide content among commercial extracts is expected and acceptable when methods are validated and labeling accurately reflects the analytical basis.

## 5.7 Interpretation of “Total Withanolides” and Chromatographic Fingerprints

The term *total withanolides* is widely used in the scientific and commercial literature, yet it encompasses multiple, analytically distinct approaches. In most validated methods, total withanolides are not measured as a single chemical entity, but rather calculated as the sum of chromatographic peaks corresponding to multiple withanolides, expressed against one or more reference standards. As a result, reported values depend on (i) which peaks are integrated, (ii) the choice of calibration standard(s), and (iii) detector response factors.

Importantly, a *multi-peak chromatographic fingerprint*—typically showing numerous resolved withanolide peaks across the chromatogram—is characteristic of authentic, minimally fractionated ashwagandha extracts derived from root, leaf, or both. Such fingerprints reflect the intrinsic chemical complexity of *Withania somnifera*, which contains dozens of structurally related withanolides and glycosides.

By contrast, chromatograms dominated by one or two major peaks may arise from selective enrichment, aggressive fractionation, or marker-focused quantification strategies. While such chromatograms are not inherently invalid, they should not be misconstrued as representing the full withanolide spectrum of the plant. Overreliance on one or two marker compounds risks oversimplifying both potency assessment and safety interpretation, particularly given that no single withanolide has been shown to drive toxicity in vivo at customary oral exposure levels.

From a quality and safety perspective, chromatographic fingerprinting serves as a critical complementary tool to numerical potency values. A reproducible, multi-peak profile supports botanical identity, batch-to-batch consistency, and the absence of unintended fractionation or adulteration. Conversely, absence or collapse of the expected fingerprint warrants further investigation, even when nominal "total withanolide" values fall within specification.

Accordingly, best practice for ashwagandha extract characterization combines (i) validated quantitative determination of total withanolides, (ii) disclosure of the analytical basis for reported values, and (iii) retention of representative chromatographic fingerprints as part of quality documentation.

**AOAC Method Standards and the Importance of Transparent, Validated Analytical Methods**

Analytical determination of withanolide content underpins potency labeling, batch release, and regulatory defensibility for ashwagandha extracts. In this context, the principles established by AOAC INTERNATIONAL for method development and validation provide a widely accepted scientific framework for ensuring that analytical results are reliable, reproducible, and fit for purpose. Although AOAC does not mandate a single analytical method for withanolides, it defines performance-based expectations that are directly applicable to botanical ingredient analysis.

AOAC-aligned validation requires demonstration of specificity (ability to resolve target analytes from co-eluting matrix components), linearity across the intended reporting range, accuracy (recovery), precision (repeatability and intermediate precision), and appropriate limits of detection and quantification. For complex botanicals such as *Withania somnifera*, these criteria are particularly critical, as withanolides comprise a structurally related family of compounds that may vary in relative abundance depending on plant part, extraction solvent, and processing conditions. Methods that quantify "total withanolides" must therefore clearly define which peaks are included, how calibration is performed, and how results are expressed.

From a quality and regulatory perspective, the use of non-validated or proprietary "black-box" methods—where analytical procedures, calibration strategies, and performance characteristics are undisclosed—poses significant challenges. Such methods prevent independent assessment of accuracy and comparability, undermine inter-laboratory reproducibility, and complicate interpretation of potency values across products. While proprietary optimization is not inherently inappropriate, failure to document and validate method performance in accordance with AOAC principles limits scientific credibility and regulatory utility.

In the context of ashwagandha safety and standardization, reliance on transparent, validated methods is preferable to enforcement of narrow compositional thresholds or exclusion of specific plant parts.

AOAC-aligned validation allows for legitimate variability in botanical composition while ensuring that reported values are analytically sound and consistently derived. This approach supports meaningful comparison across studies and commercial products, facilitates regulatory review, and reduces the risk of misattributing analytical artifacts or method-dependent variability to safety concerns.

Accordingly, best practice for the industry includes (i) use of analytically validated methods meeting AOAC performance criteria, (ii) clear documentation of analytes quantified and calibration strategy, and (iii) avoidance of undisclosed or non-validated "black-box" assays for potency determination. Adoption of these principles strengthens confidence in labeling accuracy and provides a scientifically robust foundation for both safety assessment and regulatory dialogue.

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## 6. Discussion

The current evidence base supports the conclusion that properly manufactured ashwagandha extracts—whether derived from root, leaf, or both—are safe at the dosages studied. Regulatory concern, driven primarily by isolated case reports, should be contextualized within a much larger body of negative toxicological findings, and the recent immense growth of the ashwagandha consumer market.

The most constructive industry response should lie not in restricting specific plant parts or constituents without evidence, but in reinforcing analytical rigor, manufacturing quality, and transparent labeling.

| Regulatory Concern | Basis for Concern | Toxicological and Clinical Evidence |
|---|---|---|
| Abortifacient potential | Ethnobotanical references | No toxicity in prenatal studies |
| Thyroid disruption | Limited clinical observations | No corroborating toxicological signal |
| Hepatotoxicity | Case reports | No predictive preclinical or clinical pattern |
| Absence of safe dose | Precautionary principle | Defined NOAELs with wide safety margins |

**Table 6.** Summary of evidence related to regulatory concerns for ashwagandha.

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## 7. Conclusions

Reported adverse hepatic events do not establish a generalizable causal relationship with ashwagandha or its extracts at customary dosages. Both root and leaf extracts, including those containing withaferin A, have been evaluated in preclinical safety studies without identification or evidence of a toxicological "smoking gun." Precautionary pregnancy and lactation warnings remain appropriate pending further clinical data. Harmonization of withanolide analytical methods and labeling, aligned with AOAC validation principles and FDA expectations, should be regarded as an industry standard.

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