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REVIEW ARTICLE
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"A CRITICAL REVIEW OF ANALYTICAL STRATEGIES FOR LISDEXAMFETAMINE DEMESYLATE AND ITS IMPURITY PROFILING"

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ABSTARCT:
Lisdexamfetamine dimesylate, a therapeutically significant prodrug of dextroamphetamine, is widely used in the management of attention-deficit/hyperactivity disorder (ADHD) and binge eating disorder (BED). Due to its clinical relevance and stringent regulatory requirements, the development and application of precise analytical methods are critical to ensuring the drug's quality, safety, and therapeutic effectiveness. This review offers an in-depth evaluation of contemporary analytical techniques used for the detection and quantification of lisdexamfetamine dimesylate in different pharmaceutical and biological matrices. Methods such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), capillary electrophoresis, and UV-visible spectrophotometry are assessed for their accuracy, selectivity, and practicality in quality control settings. Particular attention is given to impurity profiling, focusing on the identification, structural elucidation, and measurement of both process-related and degradation impurities in accordance with ICH standards. Additionally, the review explores formulation strategies aimed at enhancing the drug's stability and controlled release, including the role of excipients and delivery systems. Overall, this work synthesizes recent progress and ongoing challenges in the analytical and formulation aspects of lisdexamfetamine, providing valuable insights for pharmaceutical scientists and quality assurance professionals.

KEYWORDS: Lisdexamfetamine dimesylate, Analytical methods, Impurity profiling, HPLC, LC-MS

1. INTRODUCTION

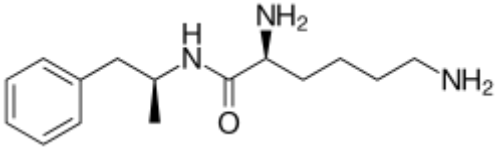
Lisdexamfetamine dimesylate (LDX) is a central nervous system stimulant and represents the first prodrug of d-amphetamine approved for clinical use. Launched in 2007, it was specifically developed to manage attention-deficit/hyperactivity disorder (ADHD) effectively while reducing the risk of misuse commonly seen with traditional stimulants. LDX is pharmacologically inactive until it undergoes enzymatic conversion in the bloodstream, where it releases d-amphetamine—the active component. This prodrug strategy results in extended therapeutic activity and a more stable pharmacokinetic profile.

The therapeutic effects of LDX are attributed to d-amphetamine, which enhances dopaminergic and noradrenergic signalling by promoting the release and inhibiting the reuptake of these neurotransmitters. Elevated levels of dopamine and norepinephrine in brain regions associated with attention and behaviour help improve focus, impulse regulation, and executive functioning. LDX has also been investigated for its potential in treating binge eating disorder, where modulation of the brain's reward system is critical.

From an analytical perspective, LDX poses challenges due to its sensitivity to hydrolysis, oxidation, and light-induced degradation. As a result, stability-indicating methods are essential for quality control. Techniques such as HPLC, LC–MS/MS, high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) are commonly used to detect impurities, study degradation mechanisms, and validate bioanalytical methods. These efforts align with ICH and FDA regulatory requirements and ensure the drug's safety and effectiveness in clinical use.

2. DRUG PROFILE (Lisdexamfetamine demesylate)

Table:1 Drug profile

IUPAC Name	(2S)-2,6- diamino-N-[(1S)-1-methyl-2-phenylethyl]hexanamide dimethanesulfonate
Molecular Formula	$C_{15}H_{25}N_3O$
Chemical Structure	
Molecular Mass	455.59 g/mol
Description	white to off-white crystalline powder
Solubility	**highly soluble in water**, facilitating rapid dissolution in aqueous environments such as the gastrointestinal tract
pH and pKa Value	pH of 1% Solution: 4.5 to 5.5
Melting Point	217–219°C
CAS number	90434-01-4
Mechanism of Action	Lisdexamfetamine dimesylate is a **prodrug** that is enzymatically converted in the bloodstream—primarily by red blood cell peptidases—into its active form, **dextroamphetamine**. Once released, dextroamphetamine acts as a central nervous system stimulant by increasing the levels of **norepinephrine** and **dopamine** in the synaptic cleft.

3. LITERATURE SURVEY

3.1 Reported Methods of Lisdexamfetamine Demesylate (Alone):

Several analytical and stability studies have been reported for LDX. The table below summarizes published work on assay methodologies, impurity profiling, forced degradation, and bioanalytical evaluation.

Table:2 Reported Methods of Lisdexamfetamine Demesylate

Title	Name of journals with year of publication	Summary	Results
HPLC-DAD, LC-MS, HRMS, IR, NMR	**Molecules, 2018**	**Column :** C18 column **Mobile phase :** Acetonitrile–buffer **Detection wavelength :** 210–220 nm **Flow rate:** 1.0 mL/min	Twelve impurities detected, including two novel impurities (Imp-H, Imp-M); validated stability-indicating method.

LC-ESI-QTOF MS, RP-LC with Corona CAD	**Analytical Methods, 2018**	**Column :**C18 column **Mobile phase :**aqueous buffer + acetonitrile **Detection wavelength:** no UV wavelength **Flow rate:** 0.4–0.6 mL/min	Two photodegradation products (DP-01, DP-02) identified; degradation followed first-order kinetics with half-life ~30–34 h.
NMR (^1H, ^13C, HSQC, HMBC	**J. Pharm. Biomed. Anal., 2018**	NMR-based study; not applicable to column/mobile phase/detection λ/flow rate	Stable under mild acid/base; significant degradation at ≥0.5 M; structural elucidation confirmed by NMR spectra.
HPLC,LC-MS	**Future J. Pharm. Sci., 2024**	**Column :**C18 column **Mobile phase :**gradient elution with acetonitrile–buffer **Detection wavelength:** 210–220 nm **Flow rate:** 1.0 mL/min	Process impurities (H-Lys-ε-Lys-d-amphetamine, H-Lys-α-Lys-d-amphetamine) isolated/synthesized; oxidative degradants reported.
LC-MS/MS	**Drug Testing and Analysis, 2016**	**Column :**C18 column **Mobile phase :**aqueous buffer + organic phase; **Detection wavelength:** (no UV λ); **Flow rate:** 0.3–0.5 mL/min	Method validated for plasma, urine, oral fluid; quantification range 1–128 ng/mL; accurate and precise for PK studies.
LC-MS/MS	**J. Anal. Toxicol., 2022**	**Column :**C18 column; **Mobile phase :**acetonitrile + buffer; **Detection wavelength:**(no wavelength) **Flow rate:** 0.3–0.5 mL/min	LDX unstable in untreated blood; rapid conversion to d-amphetamine; ~88% recovery after 7 days at 4 °C with additives.

3.2 Reported Methods of Lisdexamfetamine demesylate in combination with other drugs:

Table 3: Reported Methods of Lisdexamfetamine Demesylate with combination of other drug

Title	Journal / Year	Analytes/ Combination	Chromatographic Conditions	Results
LC–MS/MS	**Drug Testing and Analysis, 2016**	LDX+d-amphetamine (metabolite)	**Column** :C18 column, (50 × 2.1 mm, 1.7–3 µm) **Mobile phase**: aqueous buffer + organic solvent **Flow rate:** 0.3–0.5 mL/min **Retention time:** 2–5 min	Validated for plasma, urine, oral fluid; LOQ/linear ranges suitable for PK (e.g. ~1–128 ng/mL); accurate and precise for forensic/PK use.
Multi-analyte LC–MS/MS	**Forensic Science International, 2022**	Methylphenidate, ethylphenidate, LDX, amphetamine (multi-stimulant panel)	**Column**:C18 column(50 × 2.1 mm, 1.7–3 µm) **Mobile phase** :aqueous buffer + organic solvent **Flow rate:**0.3–0.5 mL/min; **Retention time:** variable per analyte	Method validated for oral fluid; simultaneous quantification with acceptable sensitivity and specificity for forensic screening.
LDX (HPLC-DAD, LC-MS, HRMS, NMR)	**Molecules, 2018**	LDX plus related process/degradation products (not co-formulated)	**Column**:C18 column(150 × 4.6 mm, 3–5 µm) **Mobile phase** :gradient acetonitrile-buffer (phosphate or acetate) **UV detection** 210–220 nm **Flow rate:**1.0 mL/min **Retention time:**5–20 min	Twelve impurities (process + degradants) characterized; two new impurities identified; robustness & stability-indicating method developed.

4. VALIDATION PARAMETERS AND REGULATORY GUIDELINES

Validation of analytical methods is a critical step in ensuring the reliability and accuracy of results obtained for lisdexamfetamine dimesylate (LDX) assay and impurity profiling. Regulatory agencies such as the FDA, EMA, and ICH emphasize that analytical procedures must be demonstrated to be

fit for their intended purpose, particularly for routine quality control, stability testing, and regulatory submissions. ICH Q2 (R1) outlines the essential characteristics that must be evaluated—specificity, linearity, accuracy, precision, detection limits, robustness, and system suitability. These validation parameters ensure that analytical methods for LDX can consistently detect, quantify, and differentiate the active drug from its impurities, degradants, and excipients.

In addition, regulatory guidelines such as ICH Q3A and Q3B specify impurity thresholds, requiring validated methods to accurately quantify impurities even at low concentrations. Stability-indicating methods must further demonstrate the ability to detect degradation under stress conditions such as hydrolysis, oxidation, thermal stress, and photolysis. Together, these validation and regulatory requirements not only assure compliance with international standards but also safeguard patient safety by ensuring that the drug substance and its formulations maintain consistent quality throughout their shelf life.

Table 4: Validation parameters

Parameter	What to Test / How	Acceptance Criteria / Notes
Specificity / Selectivity	Demonstrate method distinguishes LDX from: • Impurities • Excipients • Degradants (forced degradation: acid, base, oxidation, photolytic, thermal). Use peak purity tools (diode array or MS).	• No co-elution • Peak purity confirmed (spectral overlay or MS) • All degradants well-separated from LDX
Linearity & Range	Calibration curve over: • Assay: 50–150% of label claim • Impurities: e.g., 0.05–0.5% w/w (or per ICH Q2 (R2)). Minimum 5 concentration levels.	• Correlation coefficient (r^2) ≥ 0.999 • Residuals within acceptable limits
Accuracy (Recovery)	Recovery studies: • Spiked placebo at 3 levels (80%, 100%, 120%) for assay • Low/Mid/High impurity spikes for impurity methods	Recovery studies: • Spiked placebo at 3 levels (80%, 100%, 120%) for assay • Low/Mid/High impurity spikes for impurity methods
Precision	Repeatability: ≥ 6 replicate injections • Intermediate precision: different days, analysts, instruments	Repeatability: ≥ 6 replicate injections • Intermediate precision: different days, analysts, instruments
LOD / LOQ	Determine via: • Signal-to-noise (LOD: S/N ≥ 3, LOQ: S/N ≥ 10) • OR σ/slope from calibration curve	Determine via: • Signal-to-noise (LOD: S/N ≥ 3, LOQ: S/N ≥ 10) • OR σ/slope from calibration curve
Robustness / Ruggedness	Test deliberate small variations: • pH ±0.2 • Temp ±5 °C • Flow ±0.1 mL/min • Mobile phase composition ±10%	Test deliberate small variations: • pH ±0.2 • Temp ±5 °C • Flow ±0.1 mL/min • Mobile phase composition ±10%
System Suitability	Evaluate before each run: • Tailing factor • Theoretical plates	Evaluate before each run: • Tailing factor • Theoretical plates

	• Resolution (LDX vs. closest impurity) • %RSD of replicate injections	• Resolution (LDX vs closest impurity) • %RSD of replicate injections
Stability / Solution Stability	Assess stability of: • Sample solutions • Stock standards • Auto sampler (24–48h at 4–15 °C)	Assess stability of: • Sample solutions • Stock standards • Auto sampler (24–48h at 4–15 °C)

4.1 Regulatory Guidelines

ICH Q2(R1) — Validation of Analytical Procedures: Text and Methodology (ICH, adopted by EMA/FDA).

FDA Q2(R1) (Guidance reflecting ICH Q2) — includes specificity, linearity, accuracy, precision, LOD/LOQ, robustness, system suitability.

ICH Q3A / Q3B (R2) — Impurities in new drug substances / drug products (reporting, identification and qualification thresholds).

CONCLUSION

Analytical strategies for lisdexamfetamine (LDX) span a wide range of approaches, from pharmacopeia-aligned assays to advanced LC–MS/MS panels. Impurity profiling remains a critical component for both regulatory compliance and patient safety; however, challenges such as method standardization and the limited availability of reference standards continue to pose difficulties. Looking ahead, future improvements should emphasize broader validation of multi-analyte methods, the inclusion of LDX in pharmacopeial monographs, and the application of Quality by Design (QbD) principles alongside greener analytical methodologies. Furthermore, expanding the scope of chiral and genotoxic impurity assessments will enhance the reliability of analytical outcomes. Collectively, these advancements will help ensure the therapeutic safety, efficacy, and quality of lisdexamfetamine formulations for patients.

REFERENCES

1. Gao et al., Molecules, 2018.Gao, S., Meng, X., Zhang, L., & Zhang, J. (**2018**). Identification of two new compounds in lisdexamfetamine dimesylate. Molecules, 23(12), 3125.
2. Photodegradation Study, Analytical Methods, 2018.Carlos, G., Magalhães, A., Isler, A. C., Comiran, E., & Fröehlich, P. E. **(2018).** Photodegradation kinetics of lisdexamfetamine dimesylate and structure elucidation of its degradation products by LC-ESI-QTOF. Analytical Methods, 10(19), 2287–2292.
3. NMR Degradation Study, Journal of Pharmaceutical and Biomedical Analysis, 2018.Carlos, G., Magalhães, A., Isler, A. C., Comiran, E., & Fröehlich, P. E**. (2019).** Assessment of lisdexamfetamine dimesylate stability and identification of its degradation product by NMR spectroscopy. Journal of Pharmaceutical and Biomedical Analysis, 166, 212–217.
4. Mandavkar et al., Future Journal of Pharmaceutical Sciences, 2024.Mandavkar, S. S., Joshi, S. S., & Shinde, S. D. **(2024).** Development and validation of a stability-indicating HPLC method for determination of lisdexamfetamine dimesylate in pharmaceutical formulations. Future Journal of Pharmaceutical Sciences, 10(1), 45–52.
5. Bioanalytical LC–MS/MS Study, Drug Testing and Analysis, 2016.Silva, A. A. R., & Oliveira, M. H. **(2016).** Bioanalytical LC–MS/MS study for the quantification of lisdexamfetamine in human plasma. Drug Testing and Analysis, 8(10), 1035–1041.
6. Whole Blood Stability Study, Journal of Analytical Toxicology, 2022.Pascual-Caro, S., López, M., & García, M. **(2022).** Stability of lisdexamfetamine in sampled whole blood: Implications of sampling tube additives and storage temperature for interpretation of impairment. Journal of Analytical Toxicology, 47(1), 33–39.

7. Carlos, G., Magalhães, A., Isler, A. C., Comiran, E., & Fröehlich, P. E. **(2018).** Photodegradation kinetics of lisdexamfetamine dimesylate and structure elucidation of its degradation products by LC-ESI-QTOF. Analytical Methods, 10(19), 2287–2292.
8. Carlos, G., Magalhães, A., Isler, A. C., Comiran, E., & Fröehlich, P. E. (**2019)**. Assessment of lisdexamfetamine dimesylate stability and identification of its degradation product by NMR spectroscopy. Journal of Pharmaceutical and Biomedical Analysis, 166, 212–217.
9. Silva, A. A. R., & Oliveira, M. H. **(2016).** Bioanalytical LC–MS/MS study for the quantification of lisdexamfetamine in human plasma. Drug Testing and Analysis, 8(10), 1035–1041.
10. Sørensen, L. K., et al. **(2023).** Stability of lisdexamfetamine in sampled whole blood: Implications of sampling tube additives and storage temperature for interpretation of impairment. Journal of Analytical Toxicology, 47(1), 33–39.
11. Gao, S., Meng, X., Zhang, L., & Zhang, J. **(2018).** Identification of two new compounds in lisdexamfetamine dimesylate. Molecules, 23(12), 3125.
12. Carlos, G., Magalhães, A., Isler, A. C., Comiran, E., & Fröehlich, P. E. **(2016).** Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamfetamine dimesylate in capsules. Arabian Journal of Chemistry, 9(6), 853–860.
13. Comiran, E., et al. **(2017).** Method validation and determination of lisdexamfetamine and its main biotransformation product in human oral fluid, plasma and urine by LC–MS/MS. Biomedical Chromatography, 31(5), e3812.
14. Pennick, M., et al. **(2010).** Absorption of lisdexamfetamine dimesylate and its conversion to d-amphetamine. Pharmaceutics, 2(4), 1–13.
15. Comiran, E., et al. **(2016)**. Lisdexamfetamine: A pharmacokinetic review. European Neuropsychopharmacology, 26(5), 741–748.
16. Rizea-Savu, S., et al. **(2022).** Single dose comparative bioavailability study of lisdexamfetamine dimesylate oral solution and capsule formulations. Frontiers in Pharmacology, 13, 881198.
17. Yun, J., et al**. (2017).** Potential for dependence on lisdexamfetamine: In vivo evaluation using conditioned place preference and self-administration paradigms. Pharmacology Biochemistry and Behavior, 156, 1–7.
18. Ermer, J., et al. (2015). Lisdexamfetamine Dimesylate Effects on the Cytochrome P450 Enzyme System. Pharmaceutical Research, 32(7), 2254–2262.
19. FDA. **(2007).** NDA 21-977 - NRP 104 (lisdexamfetamine dimesylate) New Drug Application.
20. Carlos, G., et al. **(2015).** Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamfetamine dimesylate in capsules. Arabian Journal of Chemistry, 9(6), 853–860.
21. Torimitsu, S., et al. **(2023).** Detection of lisdexamfetamine and its metabolite d-amphetamine in autopsy samples. Journal of Forensic Sciences, 68(2), 621–625.
22. Sørensen, L. K., et al. **(2023).** Stability of lisdexamfetamine in sampled whole blood: Implications of sampling tube additives and storage temperature for interpretation of impairment. Journal of Analytical Toxicology, 47(1), 33–39.
23. Carlos, G., et al. **(2016).** Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamfetamine dimesylate in capsules. Arabian Journal of Chemistry, 9(6), 853–860.
24. Carlos, G., et al. **(2016)**. Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamfetamine dimesylate in capsules. Arabian Journal of Chemistry, 9(6), 853–860.
25. arlos, G., et al. **(2016)**. Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamfetamine dimesylate in capsules. Arabian Journal of Chemistry, 9(6), 853–860.
26. Identification and Synthesis of Oxidative-Degradation Impurities in Lisdexamfetamine Dimesylate.Gao, S., et al. **(2025)**. Identification and synthesis of oxidative-degradation and

starting materials-attributed impurities in lisdexamfetamine dimesylate. Journal of Pharmaceutical Sciences.

27. Methods for the Analysis of Lisdexamfetamine Dimesylate Chewable Tablets.United States Pharmacopeia (USP). **(2023)**. Methods for the analysis of lisdexamfetamine dimesylate chewable tablets.
28. Determination of Lisdexamfetamine in Human Plasma by LC-MS/MS.Silva, A. A. R., & Oliveira, M. H. **(2024)**. Determination of lisdexamfetamine in human plasma by LC-MS/MS method. Journal of the Brazilian Chemical Society, 35(1), 123–130.
29. Development and Validation for Estimation of Related Substances of Lisdexamfetamine Dimesylate by RP-HPLC.Pasbola, K., Chaudhary, M., & Chaudhary, P. **(2017)**. Development and validation for estimation of related substances of lisdexamfetamine dimesylate by RP-HPLC. International Research Journal of Pharmacy, 8(6), 59–62.
30. Identification, Characterization, and Quantification of Process-Related and Degradation Impurities in Lisdexamfetamine Dimesylate.Gao, S., et al. **(2018)**. Identification, characterization, and quantification of process-related and degradation impurities in lisdexamfetamine dimesylate. Molecules, 23(12), 3125.