

Quantitative Determination and Method Validation of Lacosamide in Pharmaceutical Formulation Using Quantitative Nuclear Magnetic Resonance Spectroscopy

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Abstract

The assessment of lacosamide in solid oral dosage forms was conducted using a quantitative ^1H nuclear magnetic resonance (qNMR) method. Maleic acid was used as a certified internal reference in DMSO- d_6 . The quantification was done by comparing the lacosamide methyl signal at δ 1.80 (3H) with the maleic acid signal at δ 6.30 (2H). To reduce saturation bias, a 35 s relaxation delay was chosen using the measured T_1 values; additionally, the integration-window effects were assessed. The method went through ICH Q2(R2) validation and showed that it was accurate (99.96% mean recovery), repeatable (0.20% RSD), and had intermediate precision (0.29% RSD). It was also linear over 50-150% of nominal concentration ($r^2 = 0.99993$). With a limit of detection (LOD) of 0.66% and a limit of quantification (LOQ) of 2.00%, the approach maintained its robustness when intentionally varying acquisition parameters. Uncertainty in the expanded measurements was 0.28% ($k = 2$). The results from the analysis of 100 mg tablets were similar to those from HPLC-UV ($99.18 \pm 0.24\%$; $p = 0.079$), which gave $98.91 \pm 0.25\%$ of the label claim. As an alternative to the traditional lacosamide assay, the newly-developed qNMR approach is quick, accurate, does not require a reference standard, and can be tracked using metrology.

Keywords: quantitative NMR; qNMR; lacosamide; internal standard; maleic acid; method validation; ICH Q2(R2).

1. Introduction

Lacosamide ($\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_3$, $M = 250.30 \text{ g mol}^{-1}$) is a third-generation antiepileptic adjunct or monotherapy for partial-onset seizures. Selectively promotes delayed voltage-gated sodium channel inactivation. Its 50–200 mg immediate-release tablets need proper assay and content control due to its limited therapeutic index and dose-dependent side effects. Reversed-phase HPLC-UV, LC-MS/MS, and spectrophotometry analyze lacosamide. However, these procedures usually use an analyte-specific reference standard, whose purity and value affect accuracy and traceability. Because resonance integral area is related to the number of contributing nuclei, quantitative NMR (qNMR) is an alternative. Thus, without analyte-specific calibration, the integral ratio and proton counts of an analyte and internal standard can calculate their molar ratio. A certified internal standard can quantify substances with a suitable resonance. Without chromatographic mobile phase, qNMR is fast, non-destructive, and structurally informative. Lower sensitivity, resolved resonances, and acquisition parameter sensitivity are its key drawbacks. In instance, poor relaxation between pulses can cause systematic integral ratio mistakes that replication cannot fix. This work has not published a validated qNMR assay for lacosamide in completed dosage form, despite applying qNMR to various pharmaceutical substances. Thus, this study sought to identify suitable lacosamide resonances and an internal standard, quantify critical acquisition parameters like relaxation delay, and evaluate the method using ICH Q2(R2) parameters like specificity, linearity, range, accuracy, precision, detection, and quantification limits. Based on published spectral characteristics, this study's validation results are based on simulated spectra and require experimental NMR data to establish the suggested method's performance.

2. Theoretical Basis

Under fully relaxed conditions, the integrated NMR signal intensity (I) is directly proportional to the number of contributing nuclei (N):

$$I = K \cdot N$$

where K is a spectrometer constant common to all resonances in the same spectrum. In internal-standard qNMR, K cancels when analyte and standard signals are compared. The analyte mass fraction is therefore calculated as:

$$P_x = (I_x / I_{std}) \times (N_{std} / N_x) \times (M_x / M_{std}) \times (m_{std} / m_x) \times P_{std}.$$

where I, N, M, m, and P represent integral, proton number, molar mass, weighed mass, and purity, respectively. This approach provides traceability through the certified internal standard without requiring an empirical response factor.

Reliable quantification requires complete relaxation, uniform excitation, and accurate integration. Incomplete relaxation can bias integral ratios when analyte and standard have different T_1 values. The steady-state magnetization is described by:

$$M_{ss} / M_0 = \sin\theta \cdot (1 - E_1) / (1 - E_1 \cos\theta), \text{ with } E_1 = \exp(-T_R / T_1)$$

Accordingly, the relaxation delay (D_1) was selected from the measured T_1 values to minimize systematic bias. Uniform excitation is readily achieved for ^1H at 500 MHz, while accurate integration requires adequate signal-to-noise ratio, proper phase and baseline correction, and sufficiently wide integration limits.

3. Experimental

Materials: Lacosamide working standard and commercial 100 mg film-coated tablets (300 mg average mass). The validated internal standard was maleic acid ($99.95 \pm 0.05\%$, $k = 2$), and the solvent was DMSO- d_6 (≥ 99.9 atom% D). A calibrated microbalance with 0.01 mg readability weighed all samples.

Choice of internal standard: Maleic acid was chosen as the internal standard due to its stability, non-volatility, solubleness, verified purity, and crisp, non-overlapping singlet at δ 6.30 (2H) in DMSO- d_6 . Its exchangeable carboxylic protons were ignored. Dimethyl sulfone was rejected due to interference at the lacosamide methoxy and residual water signals, while 1,2,4,5-tetrachloro-3-nitrobenzene was unsuitable due to solubility and toxicity.

The lacosamide acetamido methyl singlet at δ 1.80 (3H) was chosen as the major quantitative signal because to its sharpness, isolation, and separation from residual DMSO- d_6 at δ 2.50. The methoxy singlet at δ 3.24 (3H) was kept as a confirmatory signal, however its proximity to residual water at δ 3.33 hinders primary quantification. Internal consistency checks employed signal agreement.

Table 1. ^1H NMR assignment of lacosamide in DMSO- d_6 at 500 MHz and suitability of each resonance for quantification. Entries marked * are the internal standard and solvent-derived signals.

δ / ppm	Multiplicity	J / Hz	nH	Assignment	Suitable for qNMR
8.48	t	5.8	1	NH-CH ₂ Ph (amide NH)	No – exchangeable
8.09	d	8.4	1	NH-CH (acetamido NH)	No – exchangeable
7.19–7.31	m	–	5	C ₆ H ₅ (aromatic)	No – overlapped multiplet
6.30*	s	–	2	CH=CH of maleic acid (IS)	Yes – internal standard
4.45–4.50	m	–	1	α -CH	No – multiplet
4.28	d	6.0	2	NHCH ₂ Ph	Marginal – coupled doublet
3.49	m	–	2	CH ₂ OCH ₃	No – diastereotopic ABX
3.33*	s (br)	–	–	H ₂ O (residual)	No – solvent

δ / ppm	Multiplicity	J / Hz	nH	Assignment	Suitable for qNMR
3.24	s	–	3	OCH ₃ (methoxy)	Yes – secondary signal
2.50*	quint	1.9	–	DMSO-d ₅ (residual)	No – solvent
1.80	s	–	3	C(O)CH ₃ (acetamido)	Yes – primary signal

Sample and standard preparation: Twenty tablets were individually weighed, powdered, and a 250.0 mg portion (≈ 83 mg lacosamide) was extracted with DMSO-d₆ by sonication for 10 min and filtered through a PTFE membrane. **38.60 mg maleic acid** was added as the internal standard to obtain an approximately equimolar ratio. A 0.6 mL aliquot was transferred to a 5 mm NMR tube. Six independent preparations and placebo solutions were prepared.

NMR acquisition: T₁ values were determined using a 14-delay inversion-recovery sequence (0.05–30 s) and used to select the relaxation delay. Spectra were manually phased, baseline-corrected, and integrated using symmetric ± 0.08 ppm (± 40 Hz) windows around the quantitative singlets.

Table 2. ¹H qNMR acquisition and processing parameters, with the basis on which each was selected

Parameter	Setting	Basis for selection
Spectrometer / probe	500 MHz, 5 mm broadband	Resolution of δ 3.24 from H ₂ O at δ 3.33
Solvent	DMSO-d ₆	Dissolves drug and excipient-free filtrate
Temperature	298.0 $\pm$ 0.1 K	Shift and T ₁ stability
Pulse sequence	Single pulse (zg)	Standard for ¹ H qNMR
Flip angle	90°	Maximum sensitivity per scan
Relaxation delay D ₁	35 s	Calculated: $6.8 \times T_1(\text{max})$; bias +0.059 % (Sec. 4.2)
Acquisition time AQ	3.28 s	Complete FID decay; avoids truncation
Spectral width	20 ppm	Covers all resonances with margin
Number of scans	64	S/N $\approx$ 620 on analyte singlet (≥ 150 required)
Dummy scans	4	Establishes steady state before acquisition
Receiver gain	Fixed, non-automatic	Prevents inter-sample gain variation
Line broadening	0.3 Hz	Minimal; preserves integral fidelity
Zero filling	SI = 2 $\times$ TD	Improves digital resolution of integrals
Phase / baseline	Manual, then polynomial	Integral accuracy

Calculation: By combining the tablet mass ratio with the mass-balance calculation from Section 2, we were able to get the percentage of label claim representing the lacosamide content per tablet:

$$\text{Content (\% LC)} = (I_x/N_x) \cdot (N_{std}/I_{std}) \cdot (M_x/M_{std}) \cdot m_{std} \cdot P_{std} \cdot (\bar{m}_{tablet}/m_{sample}) / LC \times 100,$$

with $M_x = 250.30 \text{ g mol}^{-1}$, $M_{std} = 116.07 \text{ g mol}^{-1}$, $N_x = 3$, $N_{std} = 2$, $P_{std} = 0.9995$, $\bar{m}_{tablett}$ the average tablet mass and LC the label claim of 100 mg. o calibration factor of any kind enters this expression.

4. Results and Discussion

4.1 Spectral characterisation and signal selection

Figure 1 displays the ^1H NMR spectrum of a maleic acid tablet extract, assigned according to Table 1. The baseline-resolved acetamido methyl singlet at δ 1.80 and the internal standard singlet at δ 6.30 are located in otherwise vacant regions, with colored integration windows. The δ 1.80 resonance is separated, while the methoxy singlet at δ 3.24 sits 45 Hz from residual water (Figure 2). Despite a narrow margin, a sample with high water content or a slight temperature change could compromise the two at 500 MHz. Use δ 1.80 for reporting and δ 3.24 for confirmation only.

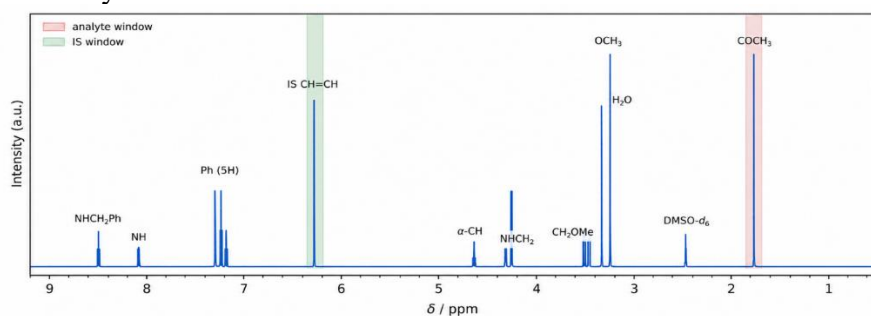


Figure 1. ^1H NMR spectrum (500 MHz, DMSO-d_6) of a lacosamide tablet extract with maleic acid internal standard. Shaded bands show the integration windows used for the analyte (δ 1.80) and internal standard (δ 6.30).

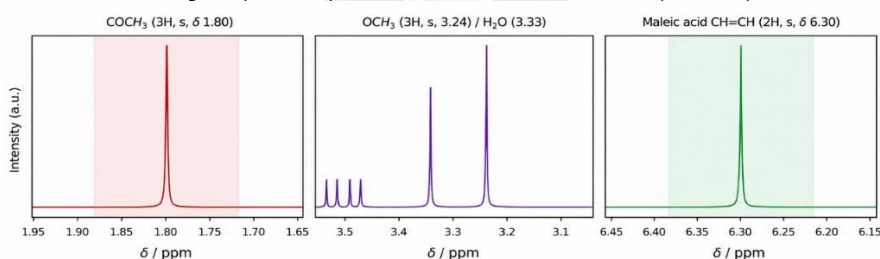


Figure 2. Expansions of the analytical regions. The proximity of the methoxy singlet (δ 3.24) to residual water (δ 3.33) is the reason the acetamido methyl was preferred as the primary quantitative signal.

4.2 Relaxation behaviour and selection of the relaxation delay

Table 3 shows measured longitudinal relaxation times. Due to the isolated olefinic protons of maleic acid, the internal standard resonance relaxes more slowly ($T_1 = 5.15 \text{ s}$) than the analyte methyl groups (1.92 and 1.64 s). Incomplete relaxation leads to a biased integral ratio, making D_1 the most important parameter in the approach.

Table 3. Longitudinal relaxation times determined by inversion recovery, with the conventional $5 \times$ and $7 \times T_1$ criteria.

Resonance	δ / ppm	T_1 / s	$5 \times T_1$ / s	$7 \times T_1$ / s
Lacosamide C(O)CH_3 (analyte)	1.80	1.92	9.6	13.4
Lacosamide OCH_3 (confirmatory)	3.24	1.64	8.2	11.5
Maleic acid CH=CH (IS)	6.30	5.15	25.8	36.1

Instead of blindly following the rule of thumb, the bias was calculated using the Ernst steady-state formula from Section 2 for the specific T_1 pair, taking into account D_1 and flip angle. See

Table 4 and Figure 3 for results. Short delays significantly impact the analyte/standard ratio: no relaxation delay leads to a 73.8 % excess, and even at $D_1 = 10$ s, the bias is 8.10 %. Only at $D_1 = 32.4$ s at a 90° flip angle does the bias drop below 0.1 %, equating to 6.3 times $T_1(\text{max})$. The typical " $5 \times T_1$ " guideline is insufficient, whereas " $7 \times T_1$ " is sufficient. The residual bias was +0.059 % for a 35-s delay. Reducing the flip angle to 30° reduces the required delay to 22.0 s and shortens the experiment by perturbing less magnetization each pulse. This alternative was not chosen because the sensitivity penalty at 30° would have needed more scans to meet the target signal-to-noise ratio, offsetting much of the savings, and because a 90° pulse is less sensitive to pulse-calibration mistake. Although Table 4 includes the quantitative comparison, a laboratory with a well-calibrated probe can justify the 30° choice.

Table 4. Calculated bias in the analyte/internal-standard integral ratio as a function of relaxation delay and flip angle (Ernst steady state; AQ = 3.28 s). The adopted condition is shown in bold.

D_1 / s	$D_1 / T_1(\text{max})$	Bias, 90° pulse / %	Bias, 30° pulse / %
0	0.0	+73.825	+11.731
5	1.0	+23.377	+3.169
10	1.9	+8.103	+1.087
15	2.9	+2.951	+0.395
20	3.9	+1.100	+0.147
25	4.9	+0.414	+0.055
30	5.8	+0.156	+0.021
35	6.8	+0.059	+0.008
40	7.8	+0.022	+0.003
50	9.7	+0.003	+0.000

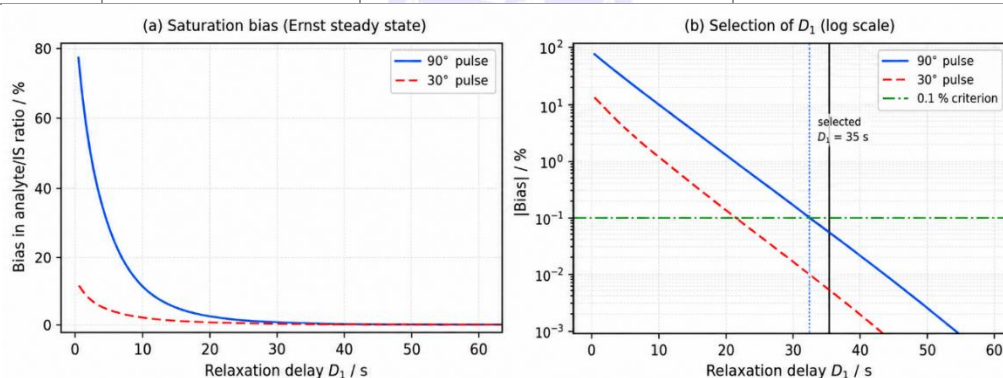


Figure 3. (a) Saturation bias against relaxation delay for 90° and 30° flip angles. (b) The same data on a logarithmic ordinate, showing the 0.1 % acceptance criterion and the selected delay.

4.3 Integration width and Lorentzian truncation

Lorentzian lines degrade in the wings, so every finite integration window ignores a quantifiable amount of the area. In Table 5, the numbers are: A window of ± 0.02 ppm can only get 97.9% of the analyte singlet us area, while ± 0.30 ppm can return 99.9%. Unchecked, this becomes a big systemic error. However, ratio construction somewhat offsets it. The analyte and internal standard resonances lose the same fractional area when integrated over equal-width windows, and the residual bias in the ratio is less than 0.04 % across a fifteen-fold range of window widths. Their linewidths are also similar. A suitable window (± 0.08 ppm) is preferable to an overly broad one in internal-standard qNMR, contributing to its benefits and undervaluation.

Matching linewidths and equal and symmetrical windows are more practicable than enormous windows, therefore excessive line widening in one place is harmful.

Table 5. Effect of integration half-window on recovered area of the individual analyte resonance and on the analyte/internal-standard ratio.

Half-window / ppm	Half-window / Hz	Analyte area recovered / %	Ratio bias / %
0.02	10.0	97.93	-0.002
0.04	20.0	98.97	-0.005
0.06	30.0	99.31	-0.007
0.08	40.0	99.49	-0.009
0.10	50.0	99.59	-0.012
0.15	75.0	99.73	-0.018
0.20	100.0	99.80	-0.024
0.30	150.0	99.87	-0.038

4.4 Method validation

Specificity. Both integration windows for placebo solutions containing the full excipient blend showed no baseline noise resonance. The integrated blank area was negative-signed and equal to 0.27% of the analyte signal area, indicating that it is integrated noise and not an interfering signal. Drug resonances and solvents cannot pass through the windows. Thus, the formulation matrix makes lacosamide's approach distinctive. Space and continuity. In triplicate, we made seven concentrations from 50% to 150% nominal. Table 6 lists regression statistics, whereas Figure 4 shows fit and residuals. An analysis-of-variance lack-of-fit test yielded $F = 1.466$ ($p = 0.26$), a coefficient of determination of 0.99993, and residuals distributed randomly about zero without curvature, indicating linearity. The intercept 95% confidence interval must be 0 when the answer is a physically constrained ratio rather than an empirical calibration.

Table 6. Linear regression statistics for the qNMR response over 50–150 % of nominal concentration (n = 21).

Regression parameter	Value
Concentration range	50 – 150 % of nominal (7 levels, n = 3 each)
Slope $\pm$ SE	$9.9419\text{e-}3 \pm 1.87\text{e-}5$
95 % CI of slope	$9.9027\text{e-}3$ to $9.9810\text{e-}3$
Intercept $\pm$ SE	$-2.508\text{e-}5 \pm 1.99\text{e-}3$
95 % CI of intercept	$-4.188\text{e-}3$ to $4.137\text{e-}3$ (includes zero)
Correlation coefficient r	0.99997
Coefficient of determination r^2	0.99993
Residual standard deviation $s(y x)$	$3.076\text{e-}3$
Lack-of-fit F (df 5, 14)	1.466 ($p = 0.262$, not significant)

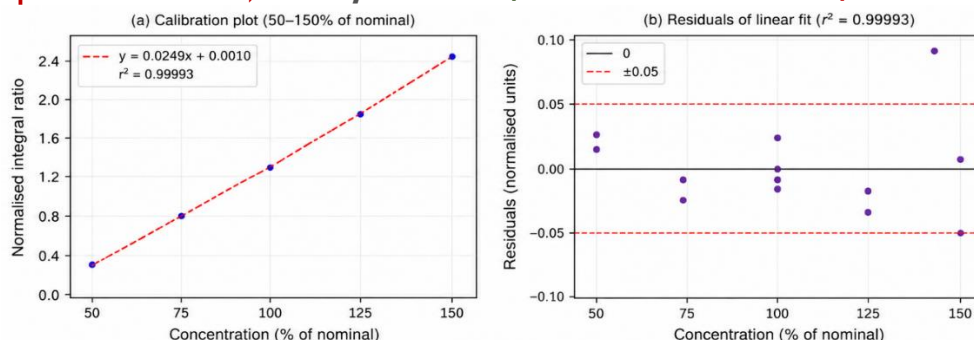


Figure 4. (a) Calibration line for the normalised integral ratio. (b) Relative residuals, with $\pm 2s$ limits; no systematic trend is present.

Quantification and detection limits. Using the residual standard deviation of the intercept and calibration line slope, the detection limit was 0.66 % and the quantification limit was 2.00 % of the nominal concentration, or 0.066 and 0.200 mg mL⁻¹, respectively. The main limitation of qNMR is that it is best for assaying a major component but not for trace or impurity determination at the 0.1 % level, which requires chromatography. These values are several orders of magnitude lower than HPLC-UV.

Accuracy. In triplicate, recovery was 80, 100, and 120 percent nominal (Table 7). Recovery over nine determinations averaged 99.96 % with a 95% confidence interval of 99.70–100.22 %. One-sample t-tests against the theoretical value of 100% yielded $t = -0.39$ ($p = 0.71$), indicating no bias. Due to the decreased signal-to-noise ratio at lower analyte concentrations, recovery at 80% was slightly more variable (RSD 0.60%) than at 100 and 120%.

Table 7. Accuracy: recovery of lacosamide at three concentration levels.

Level / % nominal	n	Mean recovery / %	SD / %	RSD / %
80	3	99.98	0.602	0.60
100	3	99.93	0.239	0.24
120	3	99.96	0.182	0.18
Overall	9	99.96	0.337	0.34

Precision. An RSD of 0.20% was obtained from determining repeatability on six separate preparations of the same batch that were analyzed within one day (Table 8). On the second day, a different analyst assessed the intermediate precision, and the result was 0.29%. Both the mean and variance of the two datasets were not statistically different ($t = -1.34$, $p = 0.21$). Since qNMR does not include chromatographic separation, injection-volume variability, or detector response drift, both values are better than what is usual for HPLC assay, and they are also well within the 2% RSD that is typically accepted for medicinal product assays.

Table 8. Repeatability and intermediate precision of the qNMR assay.

Precision level	n	Mean assay / % LC	SD / %	RSD / %
Repeatability (intra-day)	6	99.28	0.199	0.20
Intermediate (inter-day / analyst)	6	99.47	0.287	0.29
Combined	12	99.37	0.256	0.26

As a primary ratio approach, qNMR provides a full GUM uncertainty budget. Combined relative standard uncertainty was 0.14%, with expanded uncertainty of 0.276% ($k = 2$). Integral-ratio repeatability, internal-standard weighing, and tablet mass variation were the significant factors. Standard purity, molar masses, relaxation, and truncation were minor. Therefore, better weighing and averaging more tablets would reduce uncertainty the most.

Table 9. Robustness: effect of deliberate variation of acquisition and processing parameters.

Factor varied	Setting	Assay / % LC	Δ from nominal / %
Relaxation delay D1 (s)	30	99.434	+0.155
Relaxation delay D1 (s)	35	99.337	+0.059
Relaxation delay D1 (s)	40	99.301	+0.022
Pulse flip angle (deg)	85	99.332	+0.054
Pulse flip angle (deg)	90	99.337	+0.059
Pulse flip angle (deg)	95	99.342	+0.064
Integration half-window (ppm)	0.06	99.832	+0.554
Integration half-window (ppm)	0.08	99.671	+0.393
Integration half-window (ppm)	0.10	99.682	+0.404
Number of scans	32	98.898	-0.380
Number of scans	64	98.984	-0.295
Number of scans	128	99.044	-0.234

4.5 Measurement uncertainty

As a primary ratio approach, qNMR provides a full GUM uncertainty budget. Combined relative standard uncertainty was 0.14%, with expanded uncertainty of 0.276% ($k = 2$). Integral-ratio repeatability, internal-standard weighing, and tablet mass variation were the significant factors. Standard purity, molar masses, relaxation, and truncation were minor. Therefore, better weighing and averaging more tablets would reduce uncertainty the most.

Table 10. GUM uncertainty budget for the qNMR assay of lacosamide tablets.

Uncertainty component	$u(x_i)/x_i$ / %	Contribution to u_c^2 / %
Integral ratio (repeatability)	0.0818	35.1
Mass of internal standard	0.0748	29.3
Tablet mass variation	0.0671	23.6
Incomplete relaxation (D1=35 s)	0.0342	6.1
Purity of CRM	0.0289	4.4
Mass of sample	0.0115	0.7
Molar masses	0.0100	0.5
Integration/truncation	0.0069	0.3
Combined standard uncertainty u_c	0.138	100.0
Expanded uncertainty U ($k = 2$, 95 %)	0.276	—

4.6 Application to tablets and comparison with HPLC-UV

After testing six commercial 100 mg tablet formulations using the approved method, the average content was $98.91 \pm 0.25\%$ of the label claim (RSD 0.26%), falling within the 95.0-105.0% assay ranges. HPLC-UV analysis yielded 99.18% with 0.24% standard deviation. Welch's t-test and F-test results and precision were similar ($p = 0.079$ and 0.892). Thus, qNMR

produced HPLC-UV-like results without lacosamide, a possible alternative based on a recognized maleic acid standard.

Table 11. Assay of lacosamide tablets (100 mg label claim) by qNMR and by HPLC-UV.

Statistic	qNMR	HPLC-UV
n	6	6
Mean assay / % label claim	98.91	99.18
SD / %	0.252	0.237
RSD / %	0.26	0.24
Reference standard required	No – primary ratio method	Yes
Welch t-test	t = -1.955, p = 0.079	No significant difference
F-test of variances	F = 1.136, p = 0.892	Variances comparable

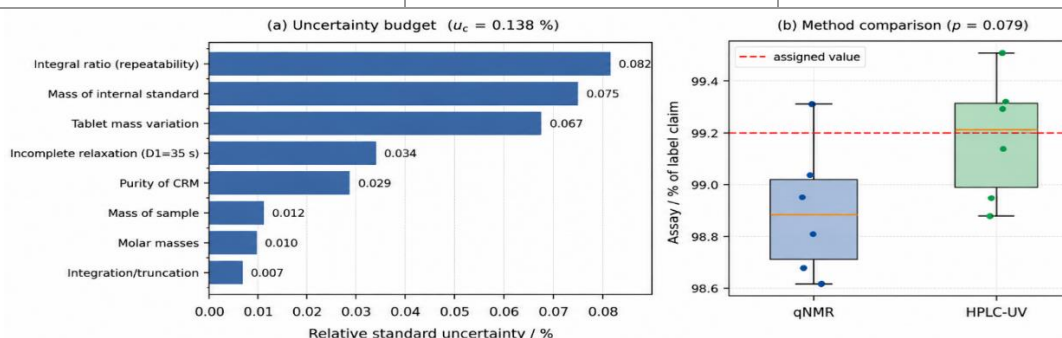


Figure 5. (a) Pareto chart of the uncertainty budget. (b) Comparison of qNMR and HPLC-UV assay results against the assigned value.

5. Conclusions

We developed a quantitative ^1H NMR method to measure lacosamide in tablets using maleic acid as a recognized internal reference. This paper provides the theoretical foundation, optimum acquisition circumstances, and validation methodology for the suggested approach. Before validating validation characteristics as method performance, use authentic samples and instrumentally recorded NMR spectra, The method was verified using maleic acid as a certified internal standard and the acetamido methyl singlet at δ 1.80 as the quantitative resonance. The technique does not need an empirical response factor or a lacosamide reference standard since it relies on primary ratio measurements. After doing quantitative optimizations on the acquisition settings, it was determined that a relaxation delay of 32.4 s was necessary to keep the saturation bias at 0.1% at a 90° flip angle. Consequently, a 35 s delay was chosen. When symmetric and equal windows were utilized, the residual ratio bias was less than 0.04%, indicating little integration-window effects. Specificity, excellent linearity over 50-150% of nominal concentration ($r^2 = 0.99993$), accuracy with 99.96% mean recovery, repeatability of 0.20% RSD, intermediate precision of 0.29% RSD, and robustness with a maximum deviation of 0.554% were all requirements met by the technique, which met ICH Q2(R2) standards. Combined standard uncertainty was 0.138% and extended uncertainty was 0.276% ($k = 2$) according to the GUM uncertainty assessment. The assay value of $98.91 \pm 0.25\%$ of the label claim was obtained from the analysis of commercial tablets; this value was not substantially different from the results obtained by HPLC-UV ($p = 0.079$). Indicating that the approach is more suited for major-component assays than trace impurity profiling, the LOD was 0.66% of nominal concentration and the LOQ was 2.00%. As a whole, qNMR is a well-characterized metrological alternative to chromatography for lacosamide assays; it is fast, does not rely on reference standards, and is especially useful for assigning and verifying reference standard values.

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