

Bio-based vs. conventional methanol for analytical HPLC workflows: A comparative performance study

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Abstract

The pharmaceutical industry is increasingly seeking to reduce the environmental impact of analytical laboratories while maintaining data quality and regulatory compliance. Methanol is one of the most widely used mobile phase solvents in HPLC methods for pharmaceutical research, development, and quality control (QC), making it an important opportunity to reduce the environmental footprint of routine analytical workflows.

This application note describes the properties, performance, and regulatory suitability of LiChrosolv® BioRenewable methanol, a bio-based alternative to conventional HPLC-grade methanol. Manufactured from renewable feedstocks, LiChrosolv® BioRenewable methanol delivers approximately 21.4% lower CO₂-equivalent emissions than conventional fossil-based methanol while meeting the purity specifications required for pharmaceutical HPLC and LC-MS applications.

LiChrosolv® BioRenewable methanol conforms to Ph. Eur. and ACS Reagent specifications and meets ICH Q3C requirements for methanol. Its chromatographic performance is comparable to that of conventional HPLC-grade methanol, supporting implementation in existing pharmaceutical HPLC and LC-MS methods with minimal disruption to established analytical workflows.

Key finding

LiChrosolv® BioRenewable methanol demonstrated chromatographic performance comparable to conventional fossil-based HPLC-grade methanol while reducing CO₂-equivalent emissions by approximately 21.4%. Equivalent performance was demonstrated across three USP pharmacopoeial API methods, supporting implementation in existing HPLC workflows with minimal changes.

Introduction

Methanol is one of the most widely used solvents in pharmaceutical analytical laboratories, serving as a primary or co-solvent in reversed-phase HPLC mobile phases for quality control, stability testing, dissolution testing, pharmacopoeial assays, impurity profiling, and analytical method development. Consequently, HPLC-grade methanol is consumed in large volumes across pharmaceutical research, development, and manufacturing laboratories.

Conventional methanol is produced from synthesis gas derived primarily from natural gas through steam reforming and related processes, a production route associated with substantial greenhouse gas emissions. As pharmaceutical companies increasingly seek to reduce Scope 3 greenhouse gas emissions across their supply chains, the environmental

Bio-Based HPLC Solvent Portfolio Launch (April 2026)

In April 2026, we launched the first bio-based HPLC solvent portfolio developed specifically for analytical laboratory applications, including LiChrosolv® BioRenewable methanol in HPLC and LC-MS grades. Manufactured from renewable feedstocks using a proprietary, patent-pending production process, these solvents deliver on average 21.4% lower CO₂-equivalent emissions than conventionally produced fossil-based methanol¹. The products are designed to meet the purity and performance requirements of analytical HPLC and LC-MS applications. Claims regarding reduced carbon impact are calculated using a cradle-to-gate carbon footprint analysis, evaluating raw material extraction and production processes for LiChrosolv® BioRenewable methanol compared to traditional petroleum-based manufacturing pathways. Calculated reductions do not include downstream distribution, customer storage, or waste incineration/disposal processes.

¹ The estimated product emissions footprint (in CO₂eq/L) provided here to customers of Merck KGaA, Darmstadt, Germany represents the cradle-to-gate GHG Scope 1, 2 and 3 emissions attributed to the specified product. Product footprint estimates are produced using internal tools with supplier data and third-party data, aligned with GHG Protocol and/or TFS PCF standards. GHG emissions results are subject to change due to updates and improvements in data quality and calculation methodology.

impact of laboratory reagents and solvents has become an important consideration. Sustainability initiatives such as the Science Based Targets initiative (SBTi), together with frameworks including ISO 14067 for product carbon footprint quantification and ISO 20400 for sustainable procurement, support organizations in integrating environmental considerations into sourcing decisions.

Scope of Study

This study compares the analytical performance of mobile phases prepared with conventional fossil-based methanol and bio-based methanol. Using three USP pharmacopoeial API methods, chromatographic

performance was evaluated by comparing key system suitability parameters, including retention time, resolution, peak symmetry, and assay results. The study demonstrates that LiChrosolv® BioRenewable methanol provides chromatographic performance comparable to conventional HPLC-grade methanol, supporting its use as a drop-in alternative for these analytical methods.

Drug substances evaluated

The following pharmaceutical substances were evaluated on both conventional and bio-based methanol as per United States Pharmacopoeia (USP) methods.

Table 1. Pharmaceutical drug substances evaluated using conventional and bio-based methanol according to applicable United States Pharmacopoeia (USP) methods

S.No	Product name	Chemical formula	IUPAC name/Therapeutic class
1	Lamivudine	C ₈ H ₁₁ N ₃ O ₃ S	4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one Antiviral (HIV/HBV)
2	Diclofenac sodium	C ₁₄ H ₁₀ Cl ₂ NNaO ₂	2-[2-(2,6-dichloroanilino)phenyl]acetic acid (sodium salt) NSAID/Anti-inflammatory
3	Clozapine	C ₁₈ H ₁₉ ClN ₄	8-chloro-11-(4-methylpiperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine Antipsychotic

Sustainability & Carbon Footprint Reduction

Carbon footprint comparison

Based on a life cycle assessment (LCA) using ecoinvent life cycle inventory data and supplier-specific emissions

data, the carbon footprint of LiChrosolv® BioRenewable methanol was compared with that of conventional fossil-based HPLC methanol. The results are summarized below.

Table 2. Carbon footprint comparison of LiChrosolv® BioRenewable methanol products with conventional fossil-based HPLC methanol

Product Name	Product No.	Bio-based CO ₂ e impact	Fossil-based CO ₂ e impact	CO ₂ e vs. Fossil	Grade
Methanol, BioRenewable, gradient grade for LC	1.06188	2.35 kg CO ₂ e/L	3.01 kg CO ₂ e/L	-21.9% CO ₂ e	HPLC Gradient Grade
Methanol, BioRenewable, hypergrade for LC-MS	1.06176	2.72 kg CO ₂ e/L	3.44 kg CO ₂ e/L	-20.9% CO ₂ e	LC-MS Hypergrade

Case Study on Potential CO₂-Equivalent Reductions

Based on the average 21.4% lower product carbon footprint of LiChrosolv® BioRenewable methanol compared with conventional fossil-based methanol, a pharmaceutical QC laboratory consuming approximately 5,000 L of HPLC-grade methanol annually could reduce the product-related CO₂-equivalent emissions associated with methanol procurement by an estimated 580–700 kg CO₂e per year. For a multi-site CDMO or pharmaceutical manufacturer consuming more than 50,000 L annually, the corresponding estimated reduction could exceed 5,800–7,000 kg CO₂e per year.

Note: These examples are provided for illustrative purposes only and are based on the reported carbon footprint. Total actual reductions will depend on methanol consumption, supply chain configuration, transportation, waste management practices, and the applicable life cycle assessment methodology.

Product portfolio & key specifications

LiChrosolv® BioRenewable methanol is available in two

analytical grades designed to cover the full range of pharmaceutical analytical applications.

Table 3. Comparison of key specifications, analytical applications, regulatory compliance, and sustainability attributes of conventional HPLC-grade methanol, LiChrosolv® BioRenewable methanol gradient grade, and LiChrosolv® BioRenewable methanol hypergrade LC-MS

Specification	Conventional HPLC grade	BioRenewable Gradient Grade (1.06188)	BioRenewable Hypergrade LC-MS (1.06176)
CAS number	67-56-1	67-56-1	67-56-1
Purity (GC)	≥99.9%	≥99.9%	≥99.97%
Water content	≤0.03%	≤0.03%	≤0.01%
Evaporation residue	≤2.0 mg/L	≤2.0 mg/L	≤1.0 mg/L
Filtration	0.2 µm	0.2 µm	0.2 µm
LC-MS suitability test	Varies	Not specified	Reserpine APCI/ESI ±
Applications	HPLC-UV, DAD, Pharmacopoeial QC	HPLC-UV, HPLC-DAD, Pharmacopoeial QC	HPLC-UV, LC-MS, LC-MS/MS, HRMS
Regulatory compliance	Ph. Eur., ACS	Ph. Eur., ACS	Ph. Eur., ACS
ICH Q3C classification	Class 2	Class 2	Class 2
Feedstock origin	Fossil fuel (natural gas)	Renewable biomass	Renewable biomass
CO ₂ e reduction	Baseline	21.9% lower	20.9% lower

Comparative Study on Pharmaceutical Products: Lamivudine, Diclofenac and Clozapine

Lamivudine, diclofenac, and clozapine were selected as representative pharmaceutical active pharmaceutical ingredients (APIs) to evaluate the chromatographic performance of LiChrosolv® BioRenewable methanol relative to conventional fossil-based HPLC-grade methanol. Comparative results for each method are presented below.

Lamivudine HPLC Analysis (USP Pharmacopoeia)

All analyses were performed under identical instrument conditions; the only variable was the methanol source in the mobile phase. Parameters assessed include

repeatability, system suitability, calibration linearity, LOD/LOQ, and recovery.

Comparative chromatographic performance

Table 4 compares the mean retention time, peak area, and USP tailing factor obtained from six replicate injections using conventional and bio-based methanol. The close agreement between the mean values indicates that substituting conventional methanol with LiChrosolv® BioRenewable methanol did not materially affect chromatographic performance under the conditions evaluated.

Table 4. Comparison of mean chromatographic parameters obtained from six replicate injections of the lamivudine API standard using mobile phases prepared with conventional fossil-based methanol and LiChrosolv® BioRenewable methanol

Parameter	Conventional methanol	Bio-Based methanol	Outcome
Mean retention time (min)	13.547	13.548	Comparable performance
Mean peak area	7,926,494	7,915,267	Comparable performance
USP tailing factor	0.971	0.970	Comparable performance (limit ≤2.0)

System suitability test (SST)

System suitability testing was performed using six replicate injections of the USP Lamivudine Resolution Mixture B RS solution under identical chromatographic conditions, with the methanol source in the mobile phase

as the only experimental variable. The chromatographic responses of the lamivudine diastereomer and lamivudine peaks were evaluated by comparing peak area precision (%RSD) and chromatographic resolution to assess compliance with the USP system suitability requirements.

Table 5. Comparison of system suitability parameters for the USP Lamivudine Resolution Mixture B RS solution using mobile phases prepared with conventional fossil-based methanol and LiChrosolv® BioRenewable methanol

Injection no.	Lamivudine diastereomer area		Lamivudine area	
	Conventional methanol	Bio-based methanol	Conventional methanol	Bio-based methanol
1	451,380	418,492	6,709,999	6,731,272
2	451,910	417,299	6,712,981	6,738,109
3	440,856	413,109	6,707,622	6,735,832
4	450,426	417,871	6,707,752	6,740,673
5	444,606	419,942	6,668,923	6,733,416
6	442,490	418,495	6,620,153	6,730,311
Mean	446,945	417,535	6,687,905	6,734,936
Standard deviation (SD)	4,875	2,340	37,014	4,023
RSD (%)	1.1	0.6	0.6	0.1
Resolution conventional methanol	1.87			
Resolution bio-based methanol	1.88			

As shown in **Table 5**, both conventional and bio-based methanol met the applicable USP system suitability requirements for chromatographic resolution and injection precision. LiChrosolv® BioRenewable methanol exhibited lower %RSD values for both the lamivudine diastereomer peak area (0.6% vs. 1.1% for conventional methanol) and the lamivudine peak area (0.1% vs. 0.6%),

indicating lower injection-to-injection variability under the conditions evaluated. All %RSD values were well within the USP acceptance criterion ($\leq 2.0\%$), while comparable chromatographic resolution (1.87 and 1.88) confirmed that replacing conventional methanol with LiChrosolv® BioRenewable methanol did not adversely affect system suitability or chromatographic performance.

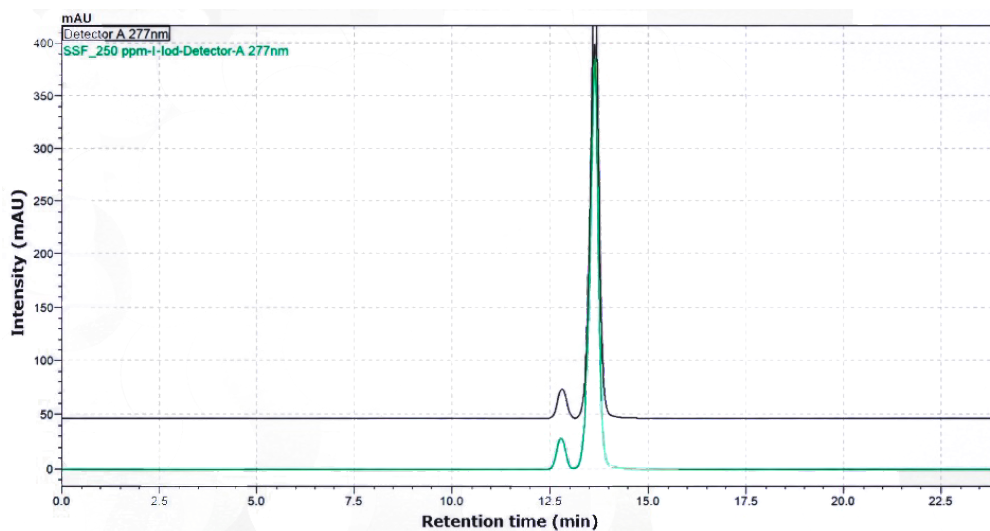


Figure 1. Overlay chromatograms of the USP Lamivudine Resolution Mixture B RS system suitability solution obtained using mobile phases prepared with conventional fossil-based methanol (black) and LiChrosolv® BioRenewable methanol (green).

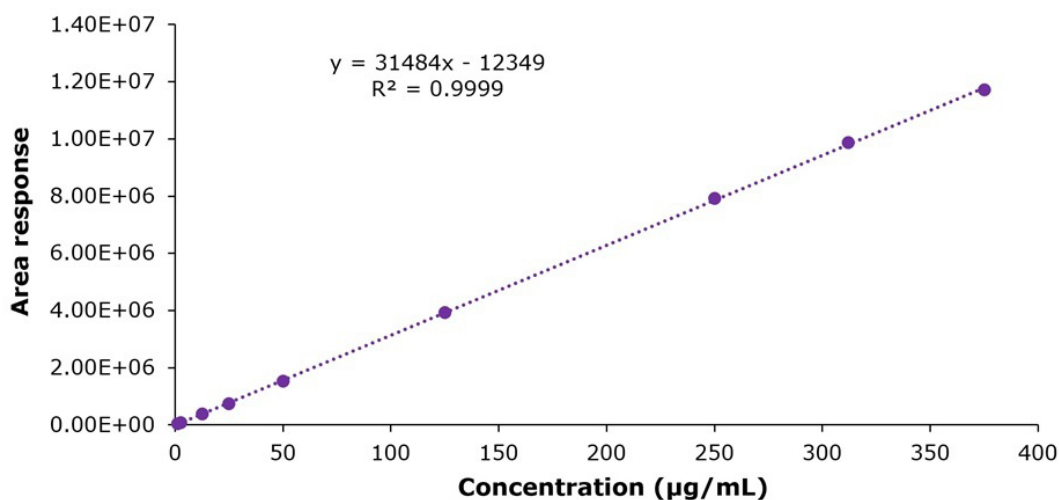
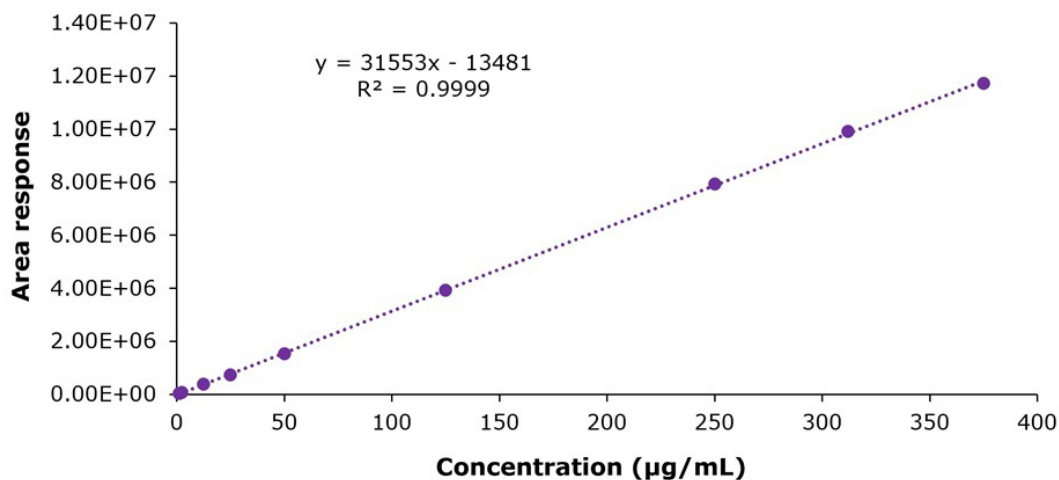
Linearity, LOD and LOQ (1.3–375 µg/mL)

As shown in **Table 6**, both conventional and bio-based methanol demonstrated excellent calibration linearity, as indicated by identical coefficients of determination ($R^2 = 0.9999$) across the evaluated concentration

range. LiChrosolv® BioRenewable methanol exhibited lower experimentally determined LOD and LOQ values than conventional methanol, indicating comparable or improved analytical sensitivity under the conditions evaluated.

Table 6. Comparison of calibration linearity, limit of detection (LOD), and limit of quantitation (LOQ) for lamivudine

Parameter	Conventional methanol	Bio-Based methanol	Outcome
Calibration range (µg/mL)	1.3–375	1.3–375	Equivalent
LOD (µg/mL)	5.64	5.00	Lower experimentally determined LOD (Bio)
LOQ (µg/mL)	17.10	15.16	Lower experimentally determined LOQ (Bio)
Correlation coefficient (R ²)	0.9999	0.9999	Equivalent

**Figure 2.** Calibration curve for lamivudine over the concentration range of 1.3–375 µg/mL using a mobile phase prepared with LiChrosolv® BioRenewable methanol.**Figure 3.** Calibration curve for lamivudine over the concentration range of 1.3–375 µg/mL using a mobile phase prepared with conventional methanol.

Recovery study

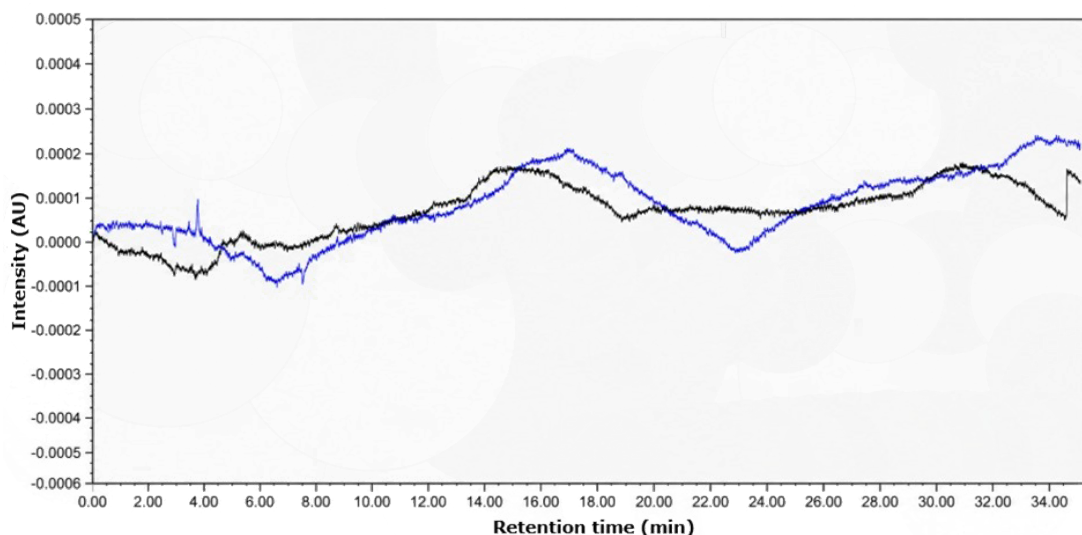
Recovery values were comparable for conventional and bio-based methanol across all evaluated spike levels, as summarized in **Table 8**. Both solvent systems met the applicable recovery acceptance criteria, with recovery values ranging from 98–102% at the mid- and high-spike

levels, demonstrating that the use of LiChrosolv® BioRenewable methanol maintained method accuracy. The elevated recovery observed at the LOQ spike level under both solvent conditions is consistent with the expected analytical variability near the limit of quantitation and does not indicate a solvent-dependent effect.

Table 7. Comparison of calibration linearity, limit of detection (LOD), and limit of quantitation (LOQ) for lamivudine

Parameter	Recovery (%)		Outcome
	Conventional methanol	Bio-Based methanol	
LOQ	113.85	116.15	Within expected variability at the LOQ
10%	100.28	101.32	Meets recovery acceptance criteria
50%	98.42	98.82	Meets recovery acceptance criteria
125%	99.22	99.67	Meets recovery acceptance criteria

Diclofenac Sodium HPLC Analysis (USP Pharmacopoeia)

**Figure 4.** Overlay chromatograms obtained for conventional methanol (black) and LiChrosolv® BioRenewable methanol (blue) used as the mobile phase in the USP HPLC analysis of diclofenac sodium.

Repeatability of the diclofenac sodium reference standard

Six replicate injections of the Diclofenac Sodium reference standard prepared with conventional methanol

were analyzed to assess method repeatability. The corresponding mean chromatographic values obtained using LiChrosolv® BioRenewable methanol are presented for comparison.

Table 8. Repeatability of the Diclofenac sodium reference standard (n = 6) with comparison of mean peak area obtained using LiChrosolv® BioRenewable methanol

Injection no.	Conventional methanol		Bio-based methanol	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	12.305	12,034,298	12.250	11,870,939
2	12.307	12,021,219	12.255	11,920,825
3	12.296	12,004,270	12.264	11,941,703
4	12.287	11,958,146	12.259	11,922,337
5	12.264	12,001,063	12.258	11,942,338
6	12.247	11,928,364	12.258	11,936,295
Mean	12.284	11,991,227	12.257	11,922,406
SD	0.024	40,169	0.005	26885
RSD (%)	0.2	0.3	0.04	0.2

The conventional methanol system demonstrated excellent repeatability, with % RSD values of 0.2% for retention time, 0.3% for peak area, and 0.5% for USP tailing factor, all of which are well within typical USP acceptance criteria. The mean retention time (12.284 vs. 12.257 min), peak area (11,991,227 vs. 11,922,406), and USP tailing factor (1.013 vs. 1.008) obtained with LiChrosolv® BioRenewable methanol

were comparable to those obtained with conventional methanol. The USP tailing factor remained well below the acceptance limit (≤ 2.0) under both solvent conditions, demonstrating comparable peak symmetry. These results indicate that LiChrosolv® BioRenewable methanol provides chromatographic performance comparable to conventional methanol for the USP analysis of Diclofenac Sodium.

System suitability test (SST)

The system suitability test (SST) for the USP method was performed using a three-component mixture comprising diethyl phthalate, Diclofenac related

substance A, and Diclofenac sodium. Six replicate injections were analysed for both conventional methanol and LiChrosolv® BioRenewable methanol to evaluate peak area repeatability and chromatographic resolution.

Table 9. Repeatability of the Diclofenac sodium reference standard (n = 6) with comparison of mean peak area obtained using LiChrosolv® BioRenewable methanol

Injection no.	Conventional methanol			LiChrosolv® bio-based methanol		
	Diethyl phthalate	Diclofenac related substance A	Diclofenac sodium	Diethyl phthalate	Diclofenac related substance A	Diclofenac sodium
1	83,990	94,725	696,508	83,721	94,706	695,120
2	83,717	94,649	694,932	83,717	94,792	694,755
3	83,845	94,371	697,610	82,579	95,135	694,083
4	83,621	95,034	695,327	82,349	93,992	695,621
5	84,163	93,439	696,617	82,794	94,492	698,376
6	82,425	93,827	694,241	84,108	95,606	699,355
Mean	83,627	94,341	695,873	83,211	94,787	696,218
RSD (%)	0.7	0.6	0.2	0.9	0.6	0.3
Resolution	-	5.8	11.63	-	5.65	11.37

All six replicate injections demonstrated excellent repeatability, with %RSD values below 1.0% for diethyl phthalate, diclofenac related substance A, and diclofenac sodium under both solvent conditions, meeting the USP acceptance criterion ($\leq 2.0\%$). Comparable mean peak areas were obtained for all three components using conventional methanol and LiChrosolv® BioRenewable methanol. Chromatographic resolution also remained comparable (5.80 vs. 5.65 for diethyl phthalate-

diclofenac related substance A and 11.63 vs. 11.37 for diclofenac related substance A-diclofenac sodium), demonstrating equivalent system suitability performance with the bio-based solvent. The overlay chromatograms (**Figure 5**) further demonstrate comparable retention behavior, peak shapes, and chromatographic resolution, confirming equivalent system suitability performance with LiChrosolv® BioRenewable methanol.

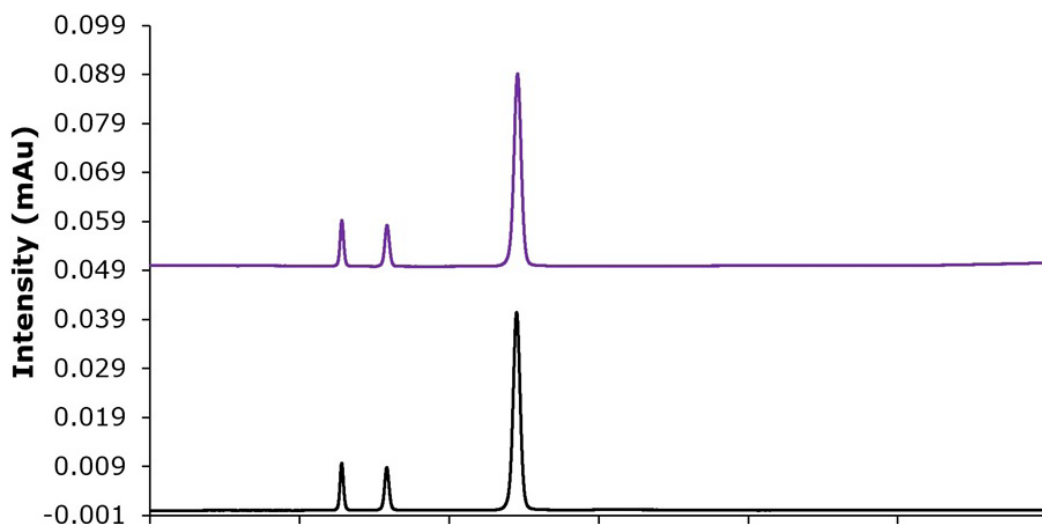


Figure 5. Overlay chromatograms of the system suitability solution containing diethyl phthalate, diclofenac related substance A, and diclofenac sodium obtained using LiChrosolv® BioRenewable methanol (violet) and conventional methanol (black) under USP chromatographic conditions.

Note: The chromatogram obtained using LiChrosolv® BioRenewable methanol is vertically offset for clarity.

Calibration linearity, LOD & LOQ

Calibration linearity was evaluated using a mixed standard solution containing diclofenac sodium, diclofenac related substance A, and diethyl phthalate over the concentration range of 0.15–2.25 µg/mL. Calibration curves were obtained for each analyte using

conventional methanol and LiChrosolv® BioRenewable methanol to assess linearity. The correlation coefficient (R^2) was determined for each component, while the limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for diclofenac sodium. The results are summarized in **Table 10**, and the corresponding calibration curves are presented in **Figures 6 and 7**.

Table 10. Comparison of calibration linearity, LOD, and LOQ for diclofenac using conventional methanol and LiChrosolv® BioRenewable methanol

Parameter	Conventional methanol	Bio-Based methanol	Outcome
Calibration range (µg/mL)	0.15–2.25	0.15–2.25	Equivalent
Diclofenac RS A LOD (µg/mL)	0.14	0.17	Equivalent
Diclofenac RS A LOQ (µg/mL)	0.44	–0.5	N Equivalent
Diethyl phthalate LOD (µg/mL)	0.25	0.13	Lower experimentally determined LOD
Diethyl phthalate LOQ (µg/mL)	0.76	0.4	Lower experimentally determined LOQ
Diclofenac LOD (µg/mL)	0.23	0.25	Equivalent
Diclofenac LOQ (µg/mL)	0.71	0.77	Equivalent
Correlation coefficient (diclofenac sodium)	0.9944	0.9935	Equivalent
Correlation coefficient (diclofenac RS A)	0.9970	0.9983	Equivalent
Correlation coefficient (diethyl Phthalate)	0.9936	0.9972	Equivalent

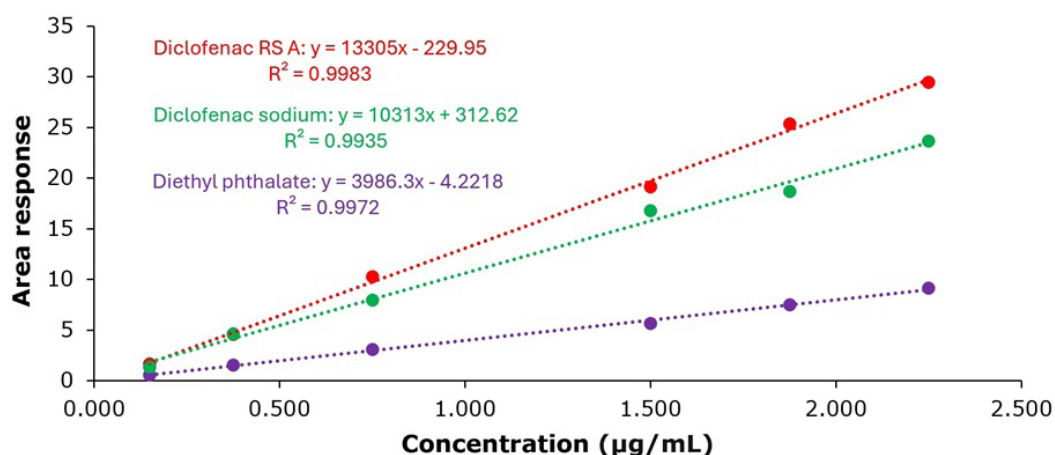


Figure 6. Calibration curves for diclofenac sodium, diclofenac related substance A, and diethyl phthalate using LiChrosolv® BioRenewable methanol.

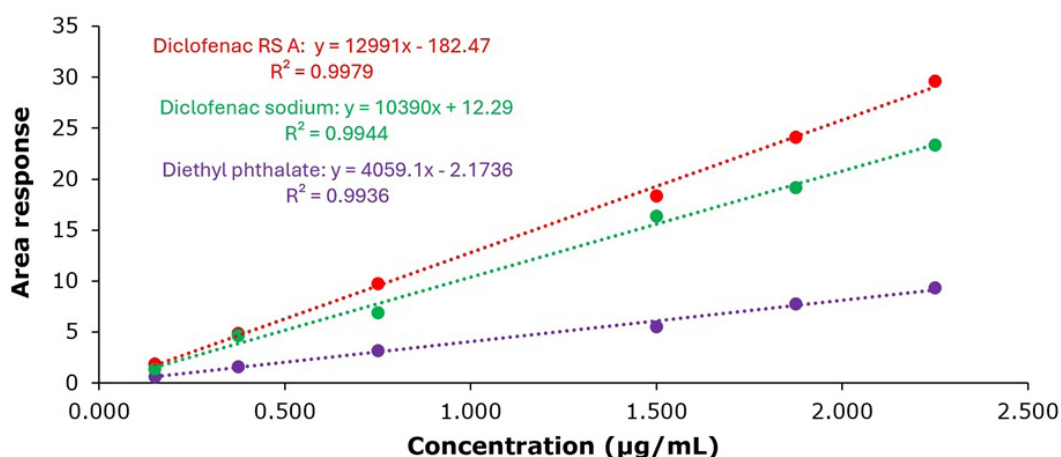


Figure 7. Calibration curves for diclofenac sodium, diclofenac related substance A, and diethyl phthalate using conventional methanol.

Calibration curves for diclofenac sodium, diclofenac related substance A, and diethyl phthalate demonstrated excellent linearity over the concentration range of 0.15–2.25 µg/mL using both conventional methanol

and LiChrosolv® BioRenewable methanol. Correlation coefficients ranged from 0.9935 to 0.9983, indicating a strong linear relationship between analyte concentration and detector response.

Recovery Study

Method accuracy was evaluated through recovery studies performed according to the USP monograph using conventional methanol and LiChrosolv® BioRenewable

methanol. Recovery was evaluated at the LOQ and multiple spike levels, and the resulting chromatographic performance was compared between the two solvent systems. The results are summarized in **Table 11**.

Table 11. Comparison of diclofenac sodium recovery using conventional methanol and LiChrosolv® BioRenewable methanol according to the USP monograph

Recovery (%)			
Spike level	Conventional methanol	Bio-Based methanol	Outcome
LOQ	100	100.0	Equivalent
25%	100	100.0	Equivalent
50%	96	101.33	Equivalent
125%	103	107.45	Equivalent

Recovery values were comparable between conventional methanol and LiChrosolv® BioRenewable methanol across all evaluated spike levels. Recoveries ranged from 96–103% for conventional methanol and 100.0–107.45% for the bio-based solvent, demonstrating

acceptable method accuracy. These results indicate that substituting conventional methanol with LiChrosolv® BioRenewable methanol does not adversely affect the recovery of diclofenac sodium under the USP method.

Clozapine HPLC Analysis (USP Pharmacopoeia)

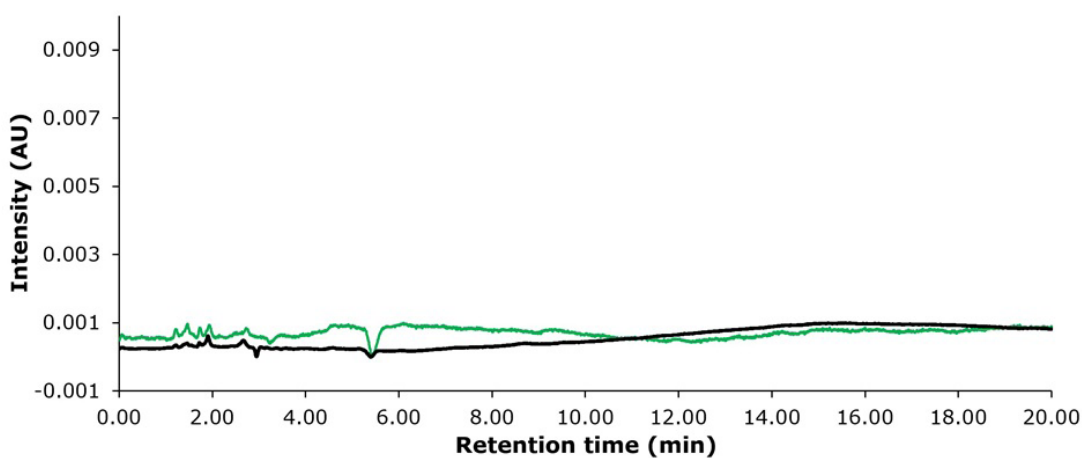


Figure 8. Overlay chromatograms obtained for conventional methanol (black) and LiChrosolv® BioRenewable methanol (green) used as the mobile phase in the USP HPLC analysis of clozapine.

Repeatability and system suitability

Repeatability and system suitability were evaluated using six replicate injections of the clozapine reference standard

prepared with conventional methanol and LiChrosolv® BioRenewable methanol. The resulting chromatographic performance parameters are summarized in **Table 12**.

Table 12. Repeatability and system suitability parameters for six replicate injections of the clozapine reference standard using conventional methanol and LiChrosolv® BioRenewable methanol

Parameter	Conventional methanol	Bio-renewable methanol
Mean peak area	3,011,719	3,023,219
Area % RSD	0.26%	0.16%
Mean retention time (min)	6.067	6.087
RT % RSD	0.08%	0.02%
USP tailing factor	1.1	1.1
Plate count/meter (USP reference standard)	15,689	17,464

Both solvent systems met the USP acceptance criterion for % RSD, demonstrating excellent repeatability. The bio-based solvent exhibited a slightly lower peak area %RSD (0.16%) than conventional methanol (0.26%), indicating comparable injection precision. Mean retention times were similar (6.067 and 6.087 min), with low retention time %RSD values observed for both solvent systems. In addition, both solvents produced an identical USP tailing factor of 1.1, indicating comparable peak symmetry. Collectively, these results demonstrate that LiChrosolv® BioRenewable methanol provides chromatographic

performance comparable to conventional methanol for the USP analysis of clozapine.

USP system suitability: Impurity resolution profile

The system suitability solution was analyzed using conventional methanol and LiChrosolv® BioRenewable methanol to evaluate the chromatographic resolution between clozapine and its degradation impurities according to the USP monograph. The chromatograms are shown in **Figure X**, and the corresponding resolution data are summarized in **Tables 13** and **14**.

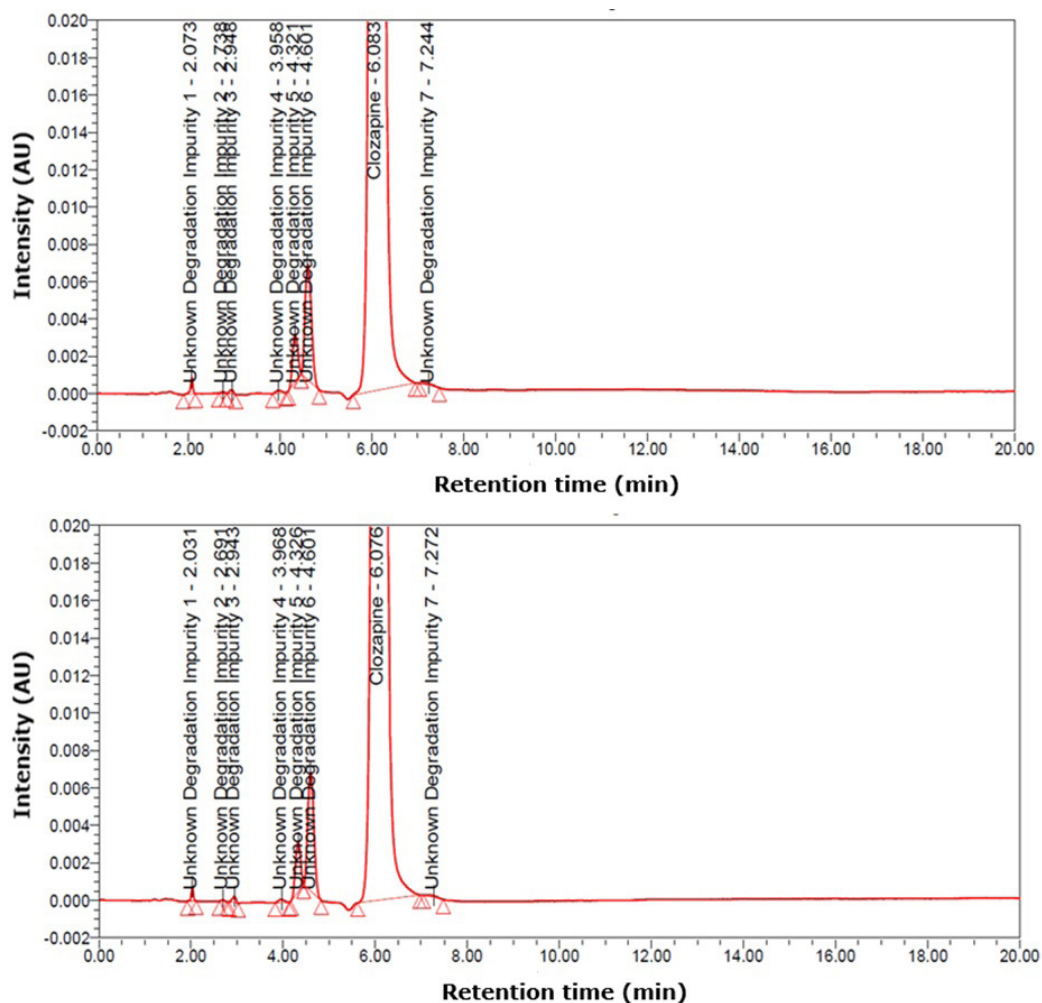


Figure 9. Chromatograms of the clozapine system suitability solution obtained using LiChrosolv® BioRenewable methanol (top) and conventional methanol (bottom).

Table 13. Retention time and resolution of clozapine and degradation impurities using conventional methanol

Sr. No.	Name	RT (min)	Resolution	USP criteria for resolution
1	Unknown degradation impurity 1	2.031	-	-
2	Unknown degradation impurity 2	2.691	6.05	-
3	Unknown degradation impurity 3	2.943	1.63	-
4	Unknown degradation impurity 4	3.968	5.16	-
5	Unknown degradation impurity 5	4.326	1.58	-
6	Unknown degradation impurity 6	4.601	1.14	-
7	Clozapine	6.076	4.80	NLT 1.5 between clozapine and any other peak
8	Unknown degradation impurity 7	7.272	3.01	

Note: Resolution values represent the separation between adjacent peaks. The USP acceptance criterion applies to the resolution between the clozapine peak and its closest adjacent peaks (Peak 7 and Peak 8).

Table 14. Retention time and resolution of clozapine and degradation impurities using LiChrosolv® BioRenewable methanol

Sr. No.	Name	RT (min)	Resolution	USP criteria for resolution
1	Unknown degradation impurity 1	2.073	-	-
2	Unknown degradation impurity 2	2.738	6.09	-
3	Unknown degradation impurity 3	2.948	1.42	-
4	Unknown degradation impurity 4	3.958	4.85	-
5	Unknown degradation impurity 5	4.321	1.51	-
6	Unknown degradation impurity 6	4.601	1.12	-
7	Clozapine	6.083	4.54	NLT 1.5 between clozapine and any other peak
8	Unknown degradation impurity 7	7.244	2.90	

Note: Resolution values represent the separation between adjacent peaks. The USP acceptance criterion applies to the resolution between the clozapine peak and its its closest adjacent peaks (Peak 7 and Peak 8).

Comparable chromatographic resolution was obtained using conventional methanol and LiChrosolv® BioRenewable methanol for the clozapine system suitability solution. Retention times of clozapine and the degradation impurities were similar under both solvent conditions, and the chromatographic profiles showed consistent separation of all detected peaks (**Figure 9**). The resolution between clozapine and adjacent peaks exceeded the USP acceptance criterion of NLT 1.5 for both solvent systems, demonstrating that substitution with LiChrosolv® BioRenewable methanol does not adversely affect impurity separation under the evaluated chromatographic conditions.

Calibration linearity, LOD, and LOQ

Calibration linearity and method sensitivity were evaluated using conventional methanol and LiChrosolv®

BioRenewable methanol over the concentration range specified in the USP monograph. The correlation coefficient (R^2), limit of detection (LOD), and limit of quantitation (LOQ) were determined to compare the analytical performance of the two solvent systems. The results are summarized in **Table 15**.

Excellent calibration linearity was obtained with both conventional methanol and LiChrosolv® BioRenewable methanol, with correlation coefficients of 0.9999 and 1.000, respectively. The calibration range was identical for both solvent systems (0.5–125 µg/mL). Lower LOD and LOQ values were obtained with the bio-based solvent, indicating comparable linearity with slightly improved method sensitivity under the evaluated conditions.

Table 15. Comparison of calibration performance for clozapine using conventional methanol and LiChrosolv® BioRenewable methanol

Parameter	Conventional methanol	Bio-Based methanol	Outcome
Calibration range (µg/mL)	0.5–125	0.5–125	Equivalent
LOD (µg/mL)	1.26	0.95	Equivalent
LOQ (µg/mL)	3.82	2.88	Equivalent
Correlation coefficient	0.9999	0.9999	Equivalent

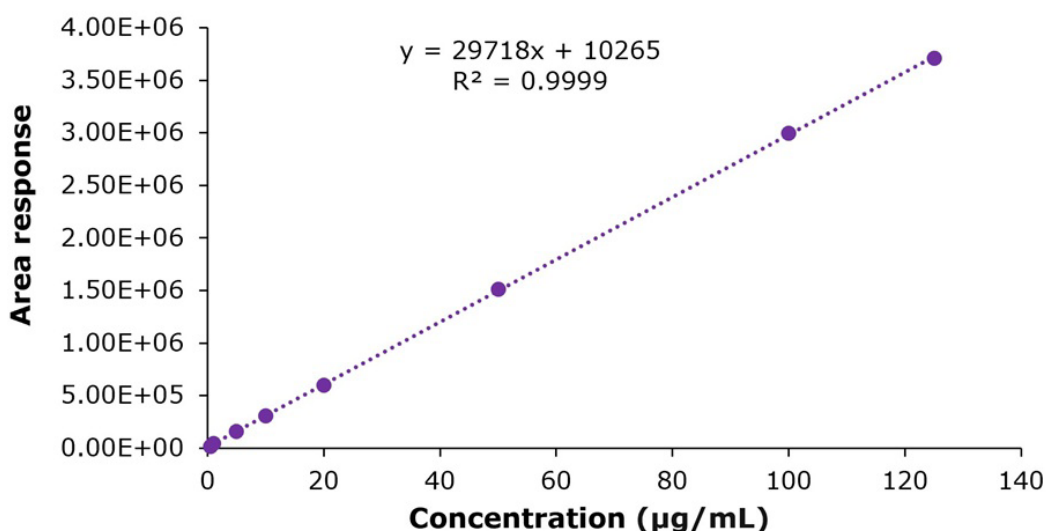


Figure 10. Calibration curves for clozapine over the concentration range of 0.5–125 µg/mL using conventional methanol.

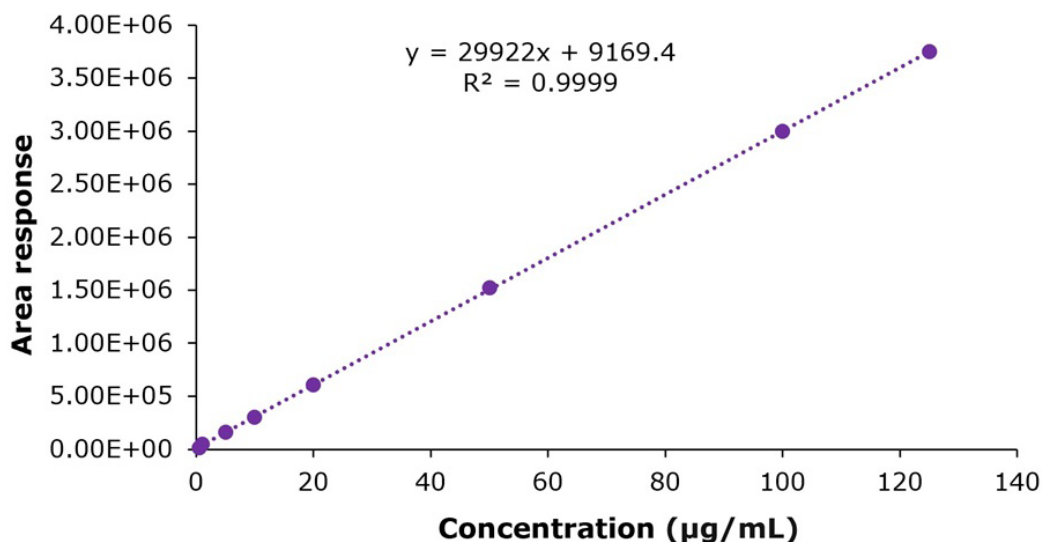


Figure 11. Calibration curves for clozapine over the concentration range of 0.5–125 µg/mL using LiChrosolv® BioRenewable methanol.

Recovery study

Table 16. Recovery of clozapine at different spike levels using conventional methanol and LiChrosolv® BioRenewable methanol

Spike level	Recovery (%)		Outcome
	Conventional methanol	Bio-Based methanol	
LOQ	100.0	100.0	Meets acceptance criteria
10%	102.0	99.0	Meets acceptance criteria
50%	99.2	98.6	Meets acceptance criteria
125%	101.7	101.5	Meets acceptance criteria

Recovery values were comparable between conventional methanol and LiChrosolv® BioRenewable methanol across all evaluated spike levels. All recovery values were within the commonly accepted validation limits for assay accuracy, demonstrating comparable method accuracy for both solvent systems. These results indicate that substituting conventional methanol with LiChrosolv® BioRenewable methanol does not adversely affect clozapine recovery under the USP method.

Comparison of USP method performance for three APIs using conventional methanol and LiChrosolv® BioRenewable methanol

The chromatographic performance of conventional methanol and LiChrosolv® BioRenewable methanol was compared across the USP methods for diclofenac, lamivudine, and clozapine. Key analytical performance parameters, including repeatability, system suitability, calibration performance, method sensitivity, and recovery, are summarized in **Table 17**.

Table 17. Repeatability of the Diclofenac sodium reference standard (n = 6) with comparison of mean peak area obtained using LiChrosolv® BioRenewable methanol

Performance parameter	Diclofenac		Lamivudine		Clozapine	
	Conventional methanol	Bio-based methanol	Conventional methanol	Bio-based methanol	Conventional methanol	Bio-based methanol
Retention time stability	12.284	12.257	13.547	13.548	6.067	6.087
Area RSD %	0.3	0.2	0.6	0.1	0.26	0.16
USP tailing factor	1.013	1.008	0.971	0.970	1.1	1.1
SST RSD-API peak (%)	0.2	0.3	0.6	0.1	0.2	0.4
SST RSD-Impurity/ref peak (%)	0.6–0.7	0.6–0.9	7.3	0.6	NA	NA

Performance parameter	Diclofenac		Lamivudine		Clozapine	
	Conventional methanol	Bio-based methanol	Conventional methanol	Bio-based methanol	Conventional methanol	Bio-based methanol
Calibration range (ug/mL)	0.15–2.25	0.15–2.25	1.3–375	1.3–375	0.5–125	0.5–125
LOD µg/mL	0.23	0.25	5.64	5.00	1.26	0.95
LOQ µg/mL	0.71	0.77	17.10	15.16	3.82	2.88
Recovery (mid spike) (%)	100–103	100–107	98–100	98–102	98–100	98–102

Conclusion

This in-house comparative study evaluated LiChrosolv® BioRenewable methanol using USP HPLC methods for diclofenac sodium, lamivudine, and clozapine. Across all three methods, the bio-based solvent demonstrated chromatographic performance comparable to conventional methanol, meeting the evaluated system suitability and method performance requirements while maintaining method accuracy and sensitivity.

- Comparable retention time and peak area reproducibility were observed under both solvent conditions for diclofenac sodium, lamivudine, and clozapine.
- All evaluated system suitability parameters met the applicable USP acceptance criteria. Lower %RSD values were observed for the lamivudine system suitability test using LiChrosolv® BioRenewable methanol.
- USP tailing factors were maintained within the specified acceptance limits for all three APIs under both solvent conditions.
- Comparable or lower LOD and LOQ values were obtained with LiChrosolv® BioRenewable methanol, indicating that method sensitivity was maintained.
- Comparable recovery values were obtained across all evaluated spike levels, demonstrating that method accuracy was maintained with LiChrosolv® BioRenewable methanol.

- LiChrosolv® BioRenewable methanol meets Ph. Eur. and ACS Reagent standards, satisfies ICH Q3C Class 2 and USP <467> requirements, and requires no method revalidation for like-for-like substitution.

- With approximately 21.4% lower CO₂ equivalent emissions than fossil-derived methanol, the use of LiChrosolv® BioRenewable methanol may contribute to reduced Scope 3 solvent-related emissions while maintaining comparable analytical performance under the evaluated USP methods.

The data presented in this study demonstrate that LiChrosolv® BioRenewable methanol provides chromatographic performance comparable to conventional methanol across the evaluated USP HPLC methods. Together with its reduced environmental footprint, these findings support its use as an alternative solvent for routine HPLC analytical workflows in pharmaceutical quality control.

Product Portfolio and Specifications

BioRenewable available grades

LiChrosolv® BioRenewable methanol is available in two analytical grades designed to cover the full range of pharmaceutical analytical applications.

Parameter	Gradient grade for LC (1.06188)	Hypergrade for LC-MS (1.06176)
CAS number	67-56-1	67-56-1
Molecular formula	CH ₃ OH	CH ₃ OH
Molecular weight	32.04 g/mol	32.04 g/mol
Regulatory compliance	Ph. Eur., ACS Reagent	Ph. Eur., ACS Reagent
Primary application	HPLC-UV, HPLC-DAD, Pharmacopeial QC	HPLC-UV, LC-MS, LC-MS/MS, HRMS
Purity (GC)	≥99.9%	≥99.97%
Water content	≤0.03%	≤0.01%
Evaporation residue	≤2.0 mg/L	≤1.0 mg/L
LC-MS suitability test	Not specified	Evaluated using (reserpine APCI/ESI ±)
Filtration	0.2 µm filter	0.2 µm filter
Feedstock origin	Renewable biomass	Renewable biomass
CO ₂ e reduction vs. fossil	21.9%	20.9%
Launch date	April 2026	April 2026

Regulatory compliance and pharmacopeial alignment

- **European pharmacopoeia (Ph. Eur.):** Conforms to the Ph. Eur. monograph for methanol (01/2012:1534). Both variants carry the "Reag. Ph. Eur." designation on the certificate of analysis (CoA).
- **ACS reagent grade:** Meets ACS Reagent specifications for methanol established by the American Chemical Society Committee on Analytical Reagents.
- **ICH Q3C residual solvents:** Methanol is classified as a Class 2 residual solvent under ICH Q3C (PDE: 30 mg/day; concentration limit: 3,000 ppm). The use of bio-based methanol does not alter these limits.
- **USP <467> residual solvents:** Methanol is classified as a Class 2 solvent under USP <467>. As the bio-based and fossil-derived solvents are chemically identical, the applicable USP <467> limits remain unchanged.
- **FDA 21 CFR / ICH Q7 (GMP):** The defined impurity profile and purity ($\geq 99.97\%$ GC) of the hypergrade are consistent with requirements for solvents used in GMP analytical testing environments.
- **Method revalidation:** Because bio-based methanol is chemically identical to fossil-derived methanol

(CAS 67-56-1), comparable chromatographic performance is expected when used as a like-for-like replacement. The need for method revalidation or change-control activities should be determined in accordance with site-specific quality systems and applicable regulatory requirements.

Regulatory note for pharmaceutical QC laboratories

Bio-based methanol is chemically identical to conventional methanol (CAS 67-56-1). The transition from fossil-derived to bio-based methanol represents a change in the manufacturing source of the raw material rather than a change in the chemical substance itself. Based on its chemical equivalence and the comparable analytical performance demonstrated in this study, such a substitution would generally not be expected to require changes to the validated analytical method. However, the need for method revalidation, regulatory notification, or internal change-control documentation should be determined in accordance with applicable regulatory requirements and the laboratory's quality management system (QMS). Laboratories are also advised to retain certificates of analysis (CoAs) for each solvent batch as part of their raw material documentation.

Related Products

Description	Cat. No.
Solvents	
Methanol, BioRenewable, gradient grade for liquid chromatography, LiChrosolv®, reag. Ph. Eur.	1.06188
Methanol, BioRenewable, $\geq 99.9\%$ (GC), suitable for LC-MS, LiChrosolv®	1.06176



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