3.2.S.4.3. VALIDATION OF ANALYTICAL PROCEDURES

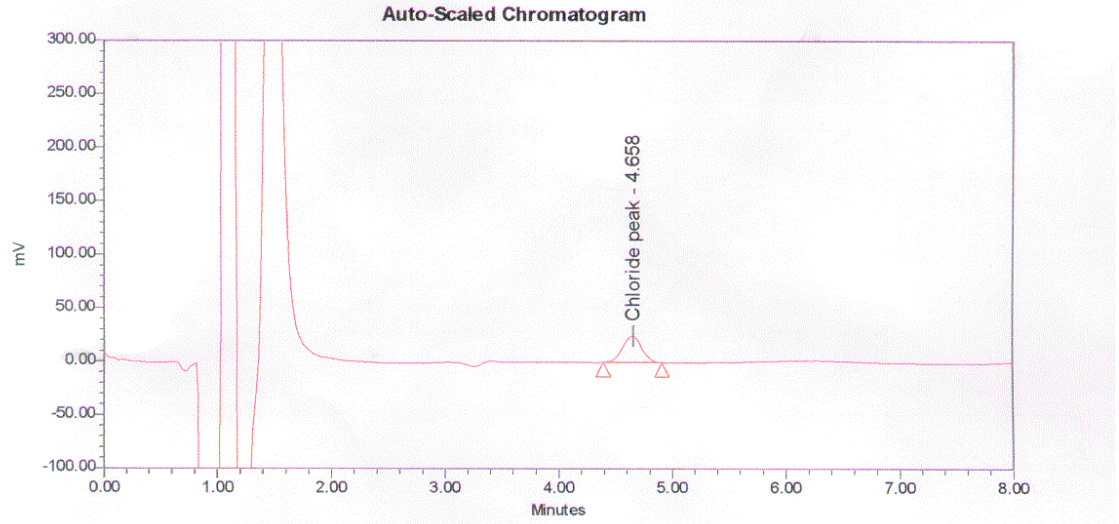
Validation of analytical procedures performed for gabapentin drug substance in accordance with the current edition of the European Pharmacopoeia (Ph Eur) gabapentin monograph or the current edition of the United States Pharmacopeia (USP) are not provided. Validation for the in-house Ionic Chloride, Residual Solvents by Gas Chromatography (GC), and Particle Size analytical gabapentin drug substance described Refer Table 3.2.S.4.3-1 in Section 3.2.S.4.2 Analytical Procedures are provided on the following pages.

3.2.S.4.3.1. Validation of Ionic Chloride Test

An Ion Chromatography (Nonsuppressed Conductivity) assay was validated to quantify trace levels of chloride in gabapentin drug substance. The specificity, linearity, system and method precision, accuracy, limit of quantitation (LOQ) range, limit of detection (LOD), and

Sample Information

SampleName	1.02 ppm Std (run 1)	Sample Type	Unknown
Vial	1	Date Acquired	4/17/2000 4:48:38 PM
Injection	1	Acq Method Set	Cl_MS_pda
Injection Volume	100.00 ul	Processing Method	Cl_pro
Channel	SATIN	Date Processed	4/18/2000 7:20:06 AM
Run Time	8.0 Minutes		



Peak Results

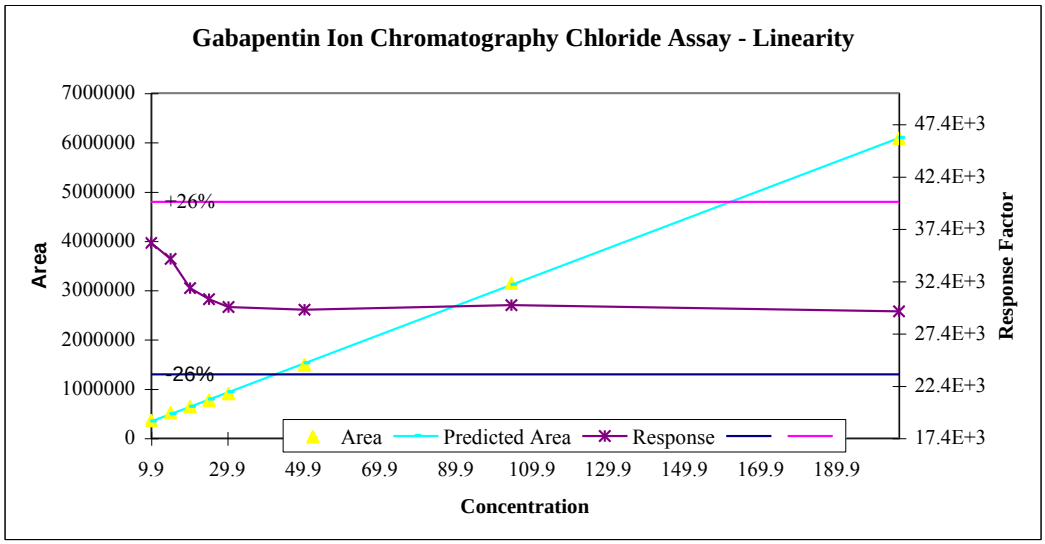
	Name	RT	Area	Tangent	USP Tailing
1	Chloride peak	4.658	288892	3.595052e+003	9.794051e-001

Linearity

Eight different samples of gabapentin were spiked with chloride to achieve final concentrations of 9.9, 15.0, 20.1, 25.1, 30.2, 50.2, 104.5, and 206.3 ppm. Samples were prepared and run using the assay conditions. The concentration (abscissa) was plotted versus the peak response (ordinate). A linear regression fit of the data gave a correlation coefficient of 0.99992, y-intercept of 48,692.4, and slope of 29,275.7 (see Figure 3.2.S.4.3-2).

A response factor plot (response factor versus the concentration) was also incorporated into the graph. The response factors were within ±26% over the entire concentration range.

Figure 3.2.S.4.3-2. Linear Regression and Response Factor Plots for Gabapentin Chloride Assay



Target Concentration	Target Area	Target Response	
20.1	637276	31705.27363	
Concentration	**Area**	**Predicted Area**	**Response**
9.9	356571	338521.9923	36017.27273
15	517366.3	487828.1476	34491.08667
20.1	637276	637134.3029	31705.27363
25.1	769427.3	783512.8866	30654.4741
30.2	902833.7	932819.0419	29895.15563
50.2	1487503.7	1518333.376	29631.54781
104.5	3141633.3	3108004.795	30063.47656
206.3	6081816	6088272.757	29480.44595

Regression Statistics	
Multiple R	0.99991861
R Square	0.999837226
Standard Error	27138.06893
Observations	8

	Coefficients	*Standard Error*
Intercept	48692.39673	13014.67774
Slope	29275.71673	152.4963481

System Precision

Six replicate injections were made of the spiked gabapentin drug substance solution at 9.9 ppm and at 102 ppm chloride content. The relative standard deviations (RSDs) are

shown in Table 3.2.S.4.3-1 and were below the acceptance criterion (not more than [NMT] 7%).

Table 3.2.S.4.3-1. System Precision

—	Chloride Content: 9.9 ppm	Chloride Content: 102 ppm
Run 1	301677	2654599
Run 2	312504	2634524
Run 3	340562	2647752
Run 4	355994	2665643
Run 5	335994	2647979
Run 6	301988	2655581
Mean (Area)	324763.7	2651013
Standard Deviation	22583.2	10387.4
RSD%	6.95%	0.39%

RSD = relative standard deviation.

Method Precision

Six separately weighed samples were run according to assay conditions versus a series of Chloride standards. A linear regression calibration curve was constructed and the amount of Chloride was calculated. The following equation was used for the lab samples.

$$\text{ppm Chloride} = \left(X_{smp}\right)\left(\frac{1}{C_{smp}}\right)\left(10^{6}\right)$$

X_{smp} = Concentration of Chloride in the gabapentin sample (from linear regression).
C_{smp} = Concentration of the gabapentin sample in mg/L (ppm).

The relative standard deviation (NMT 10%) for each sample indicates that the method is suitable for the determination of chloride in gabapentin drug substance. The results are summarized in Table 3.2.S.4.3-2.

Table 3.2.S.4.3-2. Method Precision

—	Spiked Sample	Sample Recovery
Assay 1	9.04 ppm	87.97%
Assay 2	9.47 ppm	93.67%
Assay 3	9.51 ppm	94.20%
Assay 4	11.03 ppm	114.33%
Assay 5	9.15 ppm	89.43%
Assay 6	9.45 ppm	93.40%
Assay Mean	9.61 ppm	—
Standard Deviation	0.72	—
RSD %	7.52%	—

— = not applicable; RSD = relative standard deviation

Accuracy

Accuracy was determined through spiked recovery studies. Four solutions of the gabapentin drug substance, 1 unspiked and 3 spiked with 10.2, 20.4, and 30.6 ppm of Chloride, were prepared and run according to assay conditions. A linear regression calibration curve was constructed using Chloride standards (1.02, 2.04, and 10.2 ppm) and the recovery was calculated. The percent recovery was within 74% to 126% of the theoretical value that was spiked to the samples as noted in Table 3.2.S.4.3-3.

Table 3.2.S.4.3-3. Accuracy for a Potential 20 ppm Chloride Specification

Actual (ppm)	Found (ppm)	Recovery (%)
10.2	9.49	91.6
20.4	19.59	95.3
30.6	29.89	97.2

In a separate experiment, accuracy was determined to support a potential 100 ppm chloride specification. Four solutions of the gabapentin drug substance, 1 unspiked and 3 spiked with 50.6, 101.9, and 151.5 ppm of Chloride, were prepared and run according to assay conditions. Two linear regression calibration curves were constructed using Chloride standards (1.02, 2.04, and 10.2 ppm; 5.1, 10.2, and 15.3 ppm) and the recovery was calculated using both calibration curves. The percent recovery was within 74% to 126% of the theoretical value that was spiked to the samples as noted in Table 3.2.S.4.3-4.

Table 3.2.S.4.3-4. Accuracy for a Potential 100 ppm Chloride Specification

	1.02, 2.04, 10.2 ppm Standards		5.1, 10.2, 15.3 ppm Standards	
Actual (ppm)	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
50.6	49.13	96.7	50.48	94.9
101.9	91.87	90.0	92.46	88.3
151.5	137.64	90.7	137.37	89.1

Percent recovery was calculated for Chloride according to the following equation:

$$\%\ \text{Recovery} = \frac{\text{found (ppm) spiked sample - found (ppm) unspiked sample}}{\text{actual (ppm) added to spiked sample}} \text{ x } 100\%$$

Limit of Quantitation

A sample of gabapentin drug substance spiked with the lowest level that was demonstrated to be within the linear range (9.9 ppm) was injected 6 times. The LOQ (in ppm) is then calculated using the following formula:

$$QL = \frac{10 \times SD \times \frac{C_i}{R}}{C_s} \times 10^6$$

Ci = Concentration of the Chloride (in ppm).
Cs = Concentration of the sample (in ppm).
R = Mean area response of the 6 injections.
SD = Standard Deviation.

The LOQ was determined to be 7 ppm. The actual specified value is 10 ppm.

Limit of Detection

LOD was calculated with the same samples as the LOQ except with the following formula:

$$DL = \frac{6 \times SD \times \frac{C_i}{R}}{C_s} \times 10^6$$

The LOD was determined to be 4 ppm. The actual specified value is 5 ppm.

Range

Based on the linearity and accuracy data, the range for the assay was 10 ppm to 200 ppm when incorporating the sample concentration (100 mg/mL).

Solution Stability

Chloride standard solutions (1.02, 2.04, and 10.2 ppm) and a sample solution spiked with 20 ppm Chloride were prepared and analyzed on the same day. The standard and spiked sample solutions from the initial day were analyzed (in duplicate) several times again, on various days, always against a fresh preparation of the Chloride standards. The values obtained each day were calculated for their chloride concentration and were then compared to the initial values for a percent difference calculation. Initial values used for the standards were their theoretical concentrations. The results are summarized in Table 3.2.S.4.3-5.

Table 3.2.S.4.3-5. Solution Stability

Time	1.02 ppm Standard	2.04 ppm Standard	10.2 ppm Standard	Gabapentin DS Spiked at 20 ppm[a]
Day 0	1.02	2.04	10.2	19.45
Day 1	1.1432 Δ%=12.08%	ND	ND	ND
Day 2	1.0903 Δ%=6.89%	1.9751 Δ%=3.18%	10.2470 Δ%=0.46%	19.80 Δ%=1.80%
Day 3	1.2364 Δ%=21.22%	ND	ND	ND
Day 4	ND	ND	ND	ND
Day 5	ND	ND	ND	ND
Day 6	1.3059 Δ%=28.03%[b]	2.0103 Δ%=1.46%	10.2617 Δ%=0.60%	20.88 Δ%=7.35%
Day 7	ND	2.1042 Δ%=3.15%	10.1069 Δ%=0.91%	21.37 Δ%=9.87%
Day 15	ND	2.1603 Δ%=5.90%	10.3126 Δ%=1.10%	21.92 Δ%=12.70%
Day 30	ND	2.1331 Δ%=4.56%	10.3213 Δ%=1.19%	24.79 Δ%=27.46%[c]

DS = drug substance; ND = not detected.
a. Drug substance lot number: 785712.
b. Stability failed on Day 6 for 1.02 ppm Standard.
c. Stability failed on Day 15 for Sample (Lot Number 785712) spiked with 20 ppm Chloride.

The sample solution was stable at room temperature for 15 days (360 hours), the 1.02 ppm Chloride standard was stable for 3 days (72 hours), and the 2.04 and 10.2 ppm Chloride standards were both stable for at least 30 days since the assay values did not change by more than 26% from the initial value.

Conclusion

A chloride ion chromatography assay for gabapentin drug substance has been validated. The method demonstrated acceptable specificity, linearity, system and method precision, accuracy, range, and analytical solution stability. The LOD and LOQ were determined to be below one-half a potential 20 ppm limit specification for chloride.

3.2.S.4.3.2. Validation of Residual Solvents Assay by Gas Chromatography (GC)

A new method for the determination of residual methanol, ethanol, isopropyl alcohol, (IPA), and toluene in gabapentin drug substance was developed and validated. Validation results demonstrated that the method is specific, linear, accurate, precise, reproducible, and robust. Based on the solution stability study, the Standard Stock Residual Solvent (Solution A) is stable for up to 7 days and the Sample Solution is stable for up to 2 days when stored at ambient conditions.

Background and Objectives

The validation parameters tested were: specificity, linearity, range, accuracy/method precision (repeatability), reproducibility, LOQ/reporting limit, and robustness (varying chromatographic parameters and solution stability). It should be noted that although the protocol requires analyzing each solvent at a LOQ/reporting limit of approximately 0.02% w/w, validation was inadvertently performed based on 20% of each solvent's specification limit. This did not have any impact on the LOQ/reporting limit for methanol, toluene and IPA since 20% of their specification limit is 0.02% w/w; however, since ethanol's specification limit is 0.5%, it resulted in a higher LOQ/reporting limit of 0.1% w/w. The validated range obtained for ethanol (0.1% w/w to 0.75% w/w) covers the specification limit of 0.5% w/w; therefore, it was concluded that there should be no impact during routine analysis.

Experimental

Standard and Sample Information

Reference standards used for the studies are provided in Table 3.2.S.4.3-6.

Table 3.2.S.4.3-6. Reference Standards used in Residual Solvents Assay by GC Method Validation

Reference Standard	Manufacturer(s)	Lot Numbers	Expiration Dates
Methanol	Sigma-Aldrich	01356BB	Jun 2007
	Aldrich	01658JC	Mar 2006
Ethanol	Aldrich	031664A1	Feb 2006
	Aldrich	BC01241LB	Apr 2008
IPA	Fluka	1200039	Sep 2009
	Aldrich	06439MC	Nov 2006
Toluene	Aldrich	PA02740KA	Jun 2007
	Aldrich	U13403	Nov 2006
THF	B & J	C0536	Jan 2006
	Aldrich	U13449	Nov 2006

GC = gas chromatography; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Gabapentin drug substance used for the studies is provided in Table 3.2.S.4.3-7.

Table 3.2.S.4.3-7. Gabapentin Drug Substance used in Residual Solvents Assay by GC Method Validation

Lot Numbers	Expiration Dates
KG-2-170	NA[a]
KG-2-71	NA[a]
KG-3-44	NA[a]

GC = gas chromatography; NA = not available.
a. Drug substance used was a laboratory batch, and no expiration date was assigned.

Equipment

The following instrumentation was used during the validation:

- GC System
- Autosampler
- Data Acquisition System

Analytical Procedures

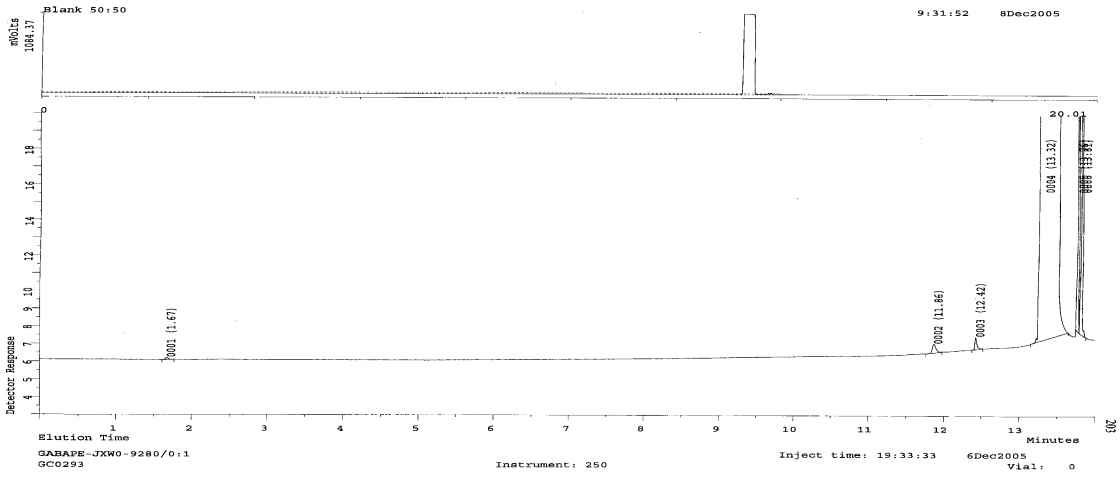
The proposed analytical method is described in Section 3.2.S.4.2 and was validated. The peak area response of residual methanol, ethanol, IPA, and toluene in gabapentin drug substance is measured by GC using flame ionization detection. The amount of residual solvent is quantitated using an internal standard method. Typical chromatograms obtained using this method are shown in the following section.

Results and Discussion

Specificity

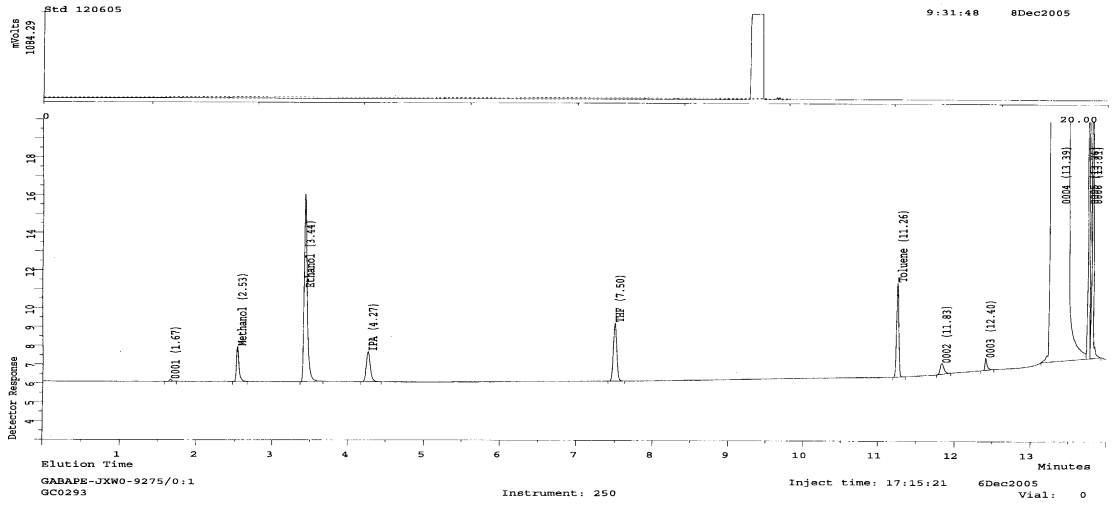
Specificity was demonstrated by injecting a Dimethylacetamide (DMAC)/Water blank (Solution C), a Reference Solution (Solution F), an Internal Standard Solution (Solution E), an unspiked sample, and a sample spiked with methanol, IPA, and toluene at approximately 0.1% w/w and ethanol at approximately 0.5% w/w. The chromatograms are shown in Figure 3.2.S.4.3-3 to Figure 3.2.S.4.3-7. The methanol, ethanol, IPA, and toluene peaks are adequately resolved from each other and other peaks present in the chromatograms. Furthermore, there were no peaks present in the blank, which co-eluted with the target solvents. Therefore, the acceptance criteria for specificity were met.

Figure 3.2.S.4.3-3. Representative Chromatogram of a DMAC/Water Blank (Solution C)



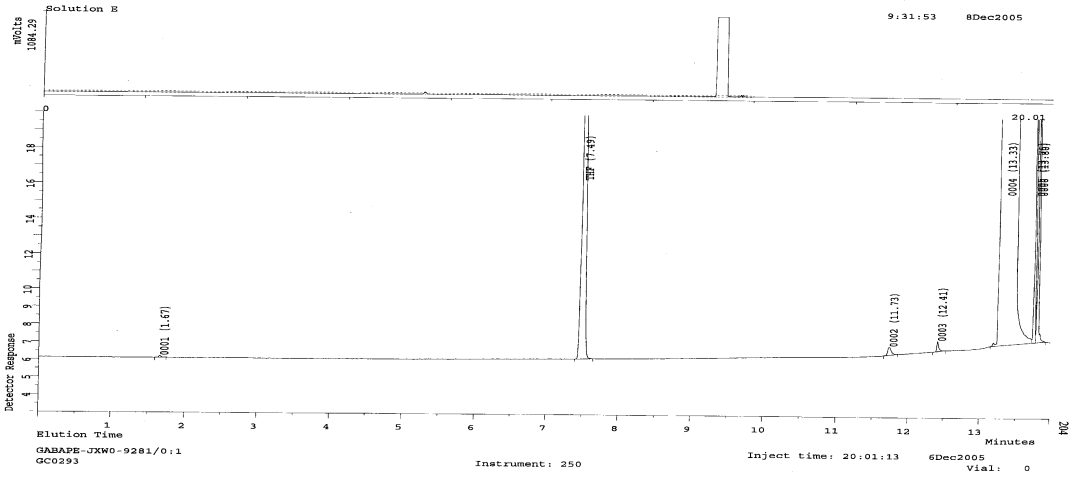
Note: Peak Identification: If present, methanol would elute at ~2.5 minutes, ethanol at ~3.4 minutes, IPA at ~4.3 minutes, THF at ~7.5 minutes and toluene at ~11.3 minutes. The peak eluting at 1.67 minutes is a system peak.
DMAC = Dimethylacetamide; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-4. Representative Chromatogram of Reference Solution (Solution F)



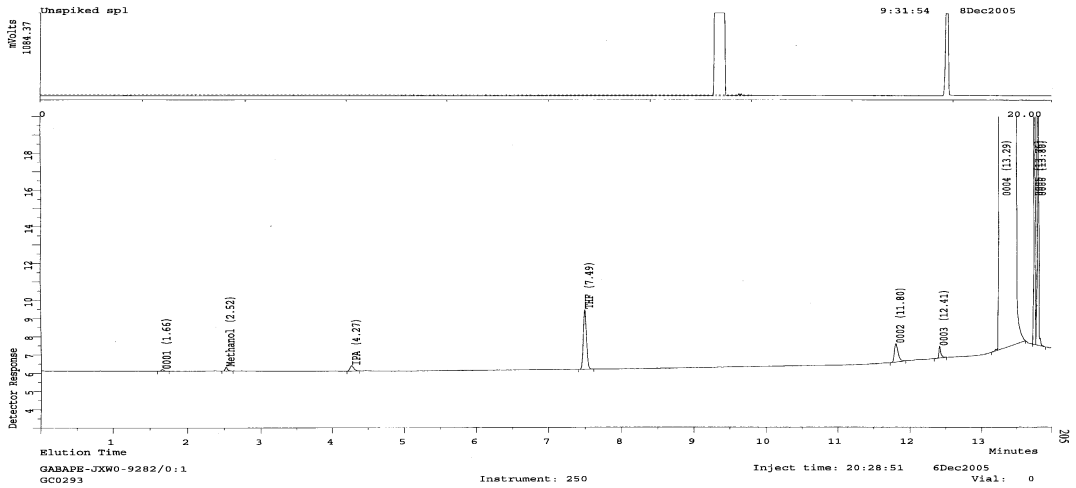
Note: Peak Identification: Methanol (2.53 minutes); ethanol (3.44 minutes); IPA (4.27 minutes); THF (7.50 minutes); and toluene (11.26 minutes). The peak eluting at 1.67 minutes is a system peak. Peaks eluting after 11.5 minutes are due to the solvent.
DMAC = Dimethylacetamide; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-5. Representative Chromatogram of Internal Standard Solution (Solution E)



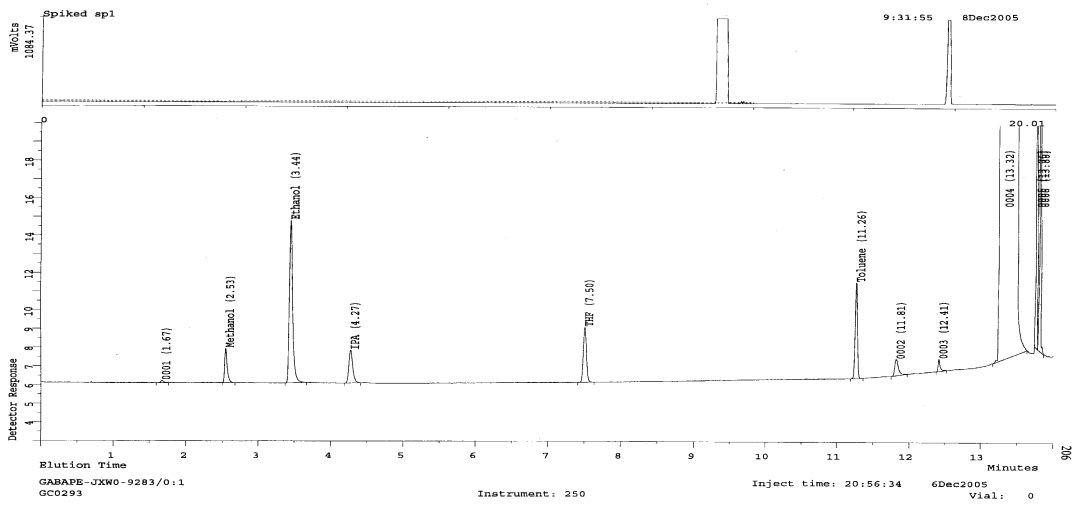
Note: Peak Identification: THF (7.49 minutes). The peak eluting at 1.67 minutes is a system peak. Peaks eluting after 11.5 minutes are due to the solvent.
THF = tetrahydrofuran.

Figure 3.2.S.4.3-6. Representative Chromatogram of an Unspiked Sample



Note: Peak Identification: Methanol (2.52 minutes); IPA (4.27 minutes); THF (7.49 minutes). The peak eluting at 1.66 minutes is a system peak. Peaks eluting after 11.5 minutes are due to the solvent.
IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-7. Representative Chromatogram of a Spiked Sample



Note: Peak Identification: Methanol (2.53 minutes); ethanol (3.44 minutes); IPA (4.27 minutes); THF (7.50 minutes); toluene (11.26 minutes). The peak eluting at 1.67 minutes is a system peak. Peaks eluting after 11.5 minutes are due to the solvent.
IPA = isopropyl alcohol; THF = tetrahydrofuran.

Linearity

Linearity of the peak area ratio (peak area of residual solvent/peak area of tetrahydrofuran [THF]) for methanol, IPA, and toluene was evaluated over the range of concentrations from the LOQ (0.02% w/w) to about 160% of the specification limit with respect to the concentration of gabapentin at 100 mg/10 mL solution. Linearity of the peak area ratio for ethanol was evaluated over the range of concentrations from the LOQ (0.1% w/w) to about 160% of the specification limit with respect to the concentration of gabapentin at 100 mg/10 mL solution. It should be noted that although the protocol requires analyzing each solvent at a LOQ/reporting limit of approximately 0.02% w/w, validation was inadvertently performed based on 20% of each solvent's specification limit. This did not have any impact on the LOQ/reporting limit for methanol, toluene and IPA since 20% of their specification limit is 0.02% w/w; however, since ethanol's specification limit is 0.5%, it resulted in a higher LOQ/reporting limit of 0.1% w/w (refer to Deviation Section below). The data and linear regression results are given in Table 3.2.S.4.3-8 to Table 3.2.S.4.3-11. Figure 3.2.S.4.3-8 to Figure 3.2.S.4.3-11 show the linear regression plots of the observed (dots) and calculated (line) values. The plots are linear over the concentration range tested and a correlation coefficient (r) ≥0.990 was obtained for each solvent. In addition, the y-intercept bias was within ±10.0% of the response of the standard for each solvent, thus the acceptance criterion for linearity was met (the protocol inadvertently states that the y-intercept bias must be ≤10.0% of the response for the standard.).

Table 3.2.S.4.3-8. Linearity of Methanol (GC System No. 468)

Approximate % of Specification	Concentration (µg/mL)	Peak Area Ratio[a]
20 (LOQ)	1.9621	0.113
39	3.9242	0.202
78	7.8485	0.410
98	9.8106	0.476
118	11.773	0.562
157	15.697	0.715

GC= gas chromatography; LOQ = limit of quantitation; r = correlation coefficient; THF = tetrahydrofuran.
a. Peak Area Ratio = Methanol Peak Area/THF Peak Area; Slope = 0.04420; y-Intercept = 0.03717; y-Intercept Bias = 7.8%; r = 0.998; Residual Sum of Squares = 0.001.

Table 3.2.S.4.3-9. Linearity of Ethanol (GC System No. 468)

Approximate % of Specification	Concentration (µg/mL)	Peak Area Ratio[a]
20 (LOQ)	9.85610	0.603
39	19.7122	1.16
79	39.4244	2.56
99	49.2805	2.99
118	59.1366	3.53
158	78.8488	4.60

GC = gas chromatography; LOQ = limit of quantitation; r = correlation coefficient; THF = tetrahydrofuran.
a. Peak Area Ratio = Ethanol Peak Area/THF Peak Area; Slope = 0.05836; y-Intercept = 0.08130; y-Intercept Bias = 2.7%; r = 0.998; Residual Sum of Squares = 0.048.

Table 3.2.S.4.3-10. Linearity of IPA (GC System No. 468)

Approximate % of Specification	Concentration (µg/mL)	Peak Area Ratio[a]
19 (LOQ)	1.9234	0.127
38	3.8467	0.240
77	7.6934	0.518
96	9.6168	0.615
115	11.540	0.730
154	15.387	0.947

GC = gas chromatography; IPA = isopropyl alcohol; LOQ = limit of quantitation; r = correlation coefficient; THF = tetrahydrofuran.

a. Peak Area Ratio = IPA Peak Area/THF Peak Area; Slope = 0.06150; y-Intercept = 0.01691; y-Intercept Bias = 2.7%; r = 0.999; Residual Sum of Squares = 0.001.

Table 3.2.S.4.3-11. Linearity of Toluene (GC System No. 468)

Approximate % of Specification	Concentration (µg/mL)	Peak Area Ratio[a]
20 (LOQ)	1.7990	0.214
40	3.5980	0.448
81	7.1959	0.881
101	8.9949	1.11
121	10.794	1.35
162	14.392	1.84

GC = gas chromatography; LOQ = limit of quantification; r = correlation coefficient; THF = tetrahydrofuran.

a. Peak Area Ratio = Toluene Peak Area/THF Peak Area; Slope = 0.12814; y-Intercept = -0.02513; y-Intercept Bias = -2.3%; r = 1.000; Residual Sum of Squares = 0.001.

Figure 3.2.S.4.3-8. Linearity of Detector Peak Response of Methanol Versus Concentration

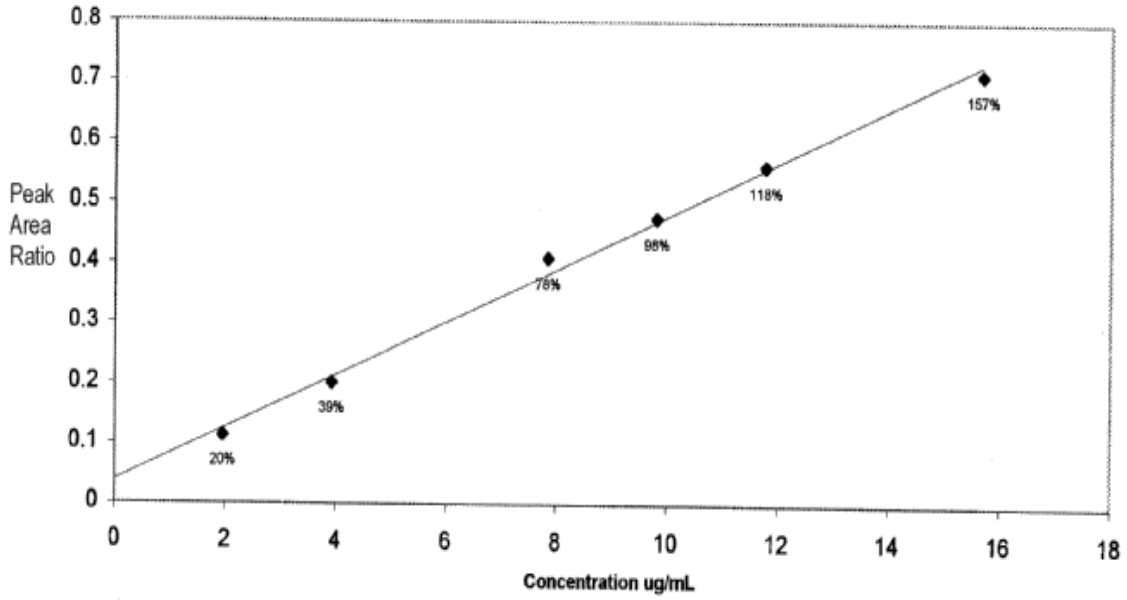


Figure 3.2.S.4.3-9. Linearity of Detector Peak Response of Ethanol Versus Concentration

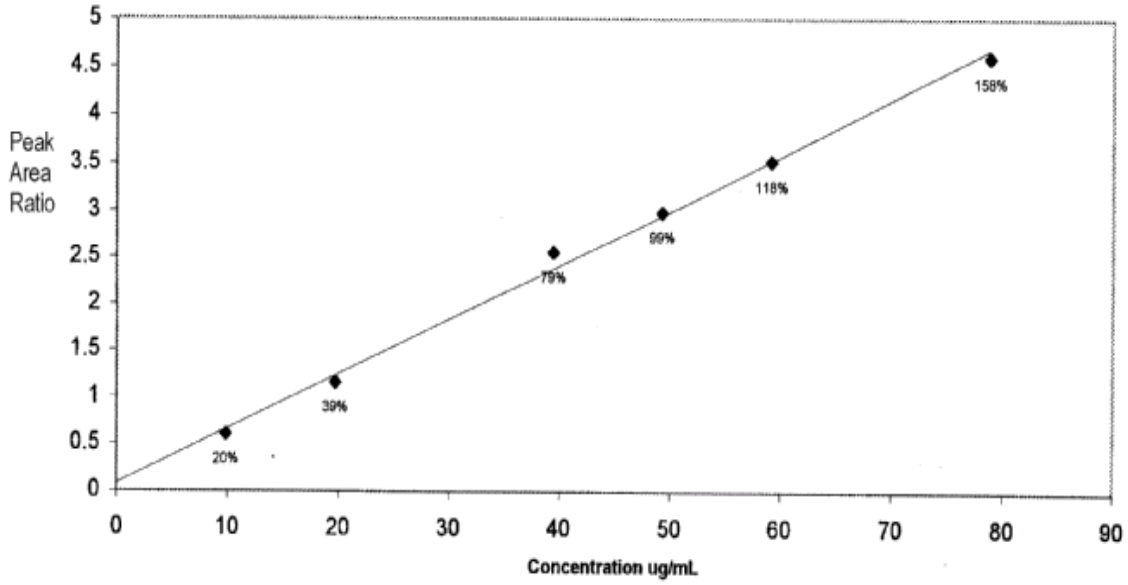


Figure 3.2.S.4.3-10. Linearity of Detector Peak Response of Isopropanol Versus Concentration

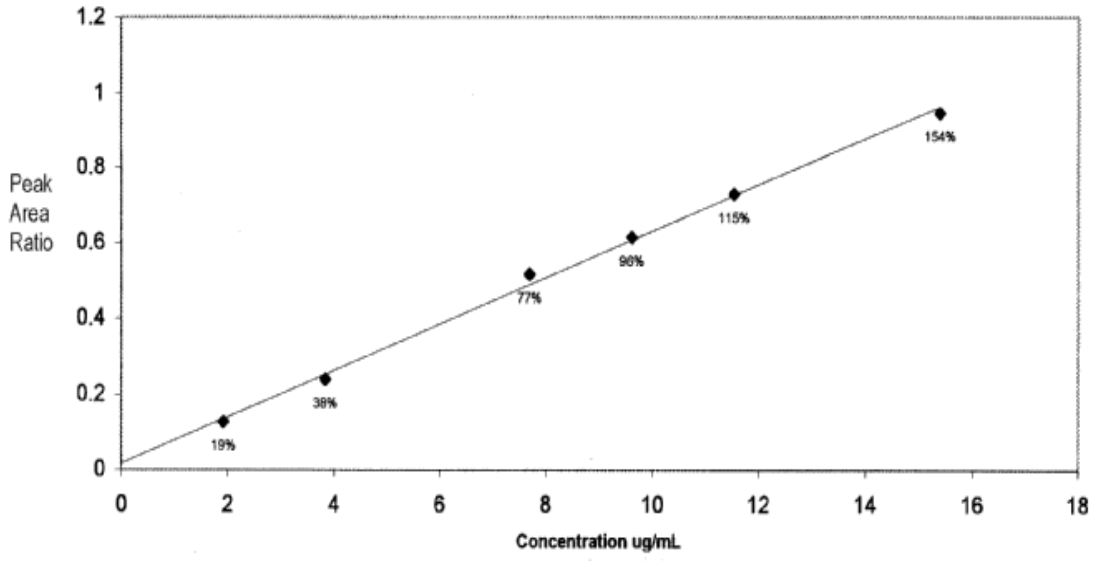
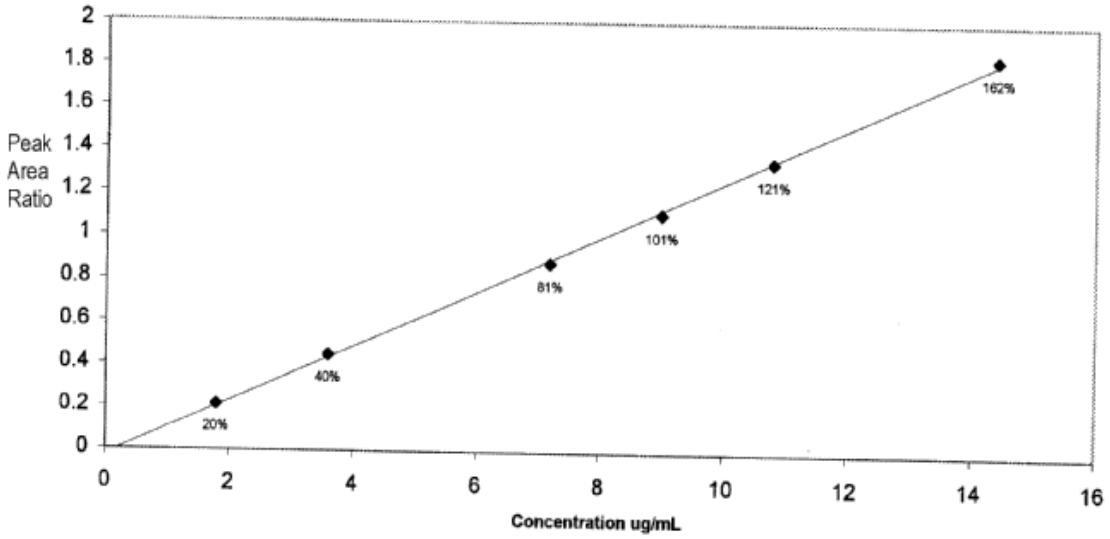


Figure 3.2.S.4.3-11. Linearity of Detector Peak Response of Toluene Versus Concentration



Range

Based on the results of the linearity, accuracy, and precision studies, the range of the method was demonstrated to be approximately 20% (LOQ/reporting limit) to 150% of the specification limits of ethanol (0.5% w/w); methanol and IPA (0.1% w/w); and toluene (0.089% w/w). This range is equivalent to approximately 0.02% to 0.15% w/w for methanol, IPA, and toluene and approximately 0.1% to 0.75% w/w for ethanol. It should be noted that although the protocol requires analyzing each solvent at a LOQ/reporting limit of approximately 0.02% w/w, validation was inadvertently performed based on 20% of each solvent's specification limit. This did not have any impact on the LOQ/reporting limit for methanol, toluene and IPA since 20% of their specification limit is 0.02% w/w; however, since ethanol's specification limit is 0.5%, it resulted in a higher LOQ/reporting limit of 0.1% w/w (refer to Deviations Section below).

Accuracy/Method Precision (Repeatability)

Precision

Method precision and accuracy were demonstrated by spiking a gabapentin drug substance sample with known amounts of methanol, ethanol, IPA, and toluene as follows: 3 separate preparations at about the LOQ level, 3 preparations at about 100%, and 3 preparations at about 150% of the specification limit. Validation was inadvertently performed using 20% of the specification limit as the LOQ/reporting limit instead of 0.02% w/w, as outlined in the protocol. This did not have any impact on the LOQ/reporting limit for methanol, toluene, and IPA since 20% of their specification limit is 0.02% w/w. However, since ethanol's specification limit is 0.5%, this resulted in a higher LOQ/reporting limit of 0.1% w/w (refer to Deviations Section below).

In order to determine the amount of methanol, ethanol, IPA, and toluene present in the sample, 3 unspiked samples were prepared as a control. The average amount of solvent present in the control samples was added to the amount spiked to determine the final amount of solvent that should be recovered from each sample preparation (target concentration). The sample preparations were analyzed according to the method and the results are shown in Table 3.2.S.4.3-12 to Table 3.2.S.4.3-15. Each individual and mean recovery value from each set of preparations met the acceptance criteria of being within ±20.0% (relative basis) for solvent levels at the LOQ; and within ±10.0% (relative basis) for solvent levels greater than the LOQ.

Table 3.2.S.4.3-12. Recovery Results for Methanol in Spiked Gabapentin Drug Substance - Analyst 1

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.004	—
Control-2	—	—	0.004	—
Control-3	—	—	0.004	—
Mean	—	—	0.004	—
(LOQ)-1	0.019	0.023	0.024	4.3
(LOQ)-2	0.020	0.024	0.023	-4.2
(LOQ)-3	0.020	0.024	0.024	0.0
Mean	—	—	—	0.0
100-1	0.098	0.102	0.102	0.0
100-2	0.098	0.102	0.104	2.0
100-3	0.097	0.101	0.101	0.0
Mean	—	—	—	0.7
150-1	0.146	0.150	0.154	2.7
150-2	0.146	0.150	0.141	-6.0
150-3	0.146	0.150	0.159	6.0
Mean	—	—	—	0.9

— = not applicable; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-13. Recovery Results for Ethanol in Spiked Gabapentin Drug Substance - Analyst 1

Approximate % of SpecificationLimit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.000	—
Control-2	—	—	0.000	—
Control-3	—	—	0.000	—
Mean	—	—	0.000	—
(LOQ)-1	0.099	0.099	0.101	2.0
(LOQ)-2	0.099	0.099	0.097	-2.0
(LOQ)-3	0.099	0.099	0.104	5.1
Mean	—	—	—	1.7
100-1	0.495	0.495	0.499	0.8
100-2	0.497	0.497	0.502	1.0
100-3	0.492	0.492	0.496	0.8
Mean				0.9
150-1	0.741	0.741	0.769	3.8
150-2	0.742	0.742	0.704	-5.1
150-3	0.738	0.738	0.806	9.2
Mean	—	—	—	2.6

— = not applicable; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-14. Recovery Results for IPA in Spiked Gabapentin Drug Substance - Analyst 1

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.012	—
Control-2	—	—	0.014	—
Control-3	—	—	0.012	—
Mean	—	—	0.013	—
(LOQ)-1	0.019	0.032	0.033	3.1
(LOQ)-2	0.020	0.033	0.033	0.0
(LOQ)-3	0.020	0.033	0.034	3.0
Mean	—	—	—	2.0
100-1	0.097	0.110	0.112	1.8
100-2	0.098	0.111	0.113	1.8
100-3	0.097	0.110	0.110	0.0
Mean	—	—	—	1.2
150-1	0.146	0.159	0.166	4.4
150-2	0.146	0.159	0.155	-2.5
150-3	0.145	0.158	0.170	7.6
Mean	—	—	—	3.2

— = not applicable; IPA = isopropyl alcohol; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-15. Recovery Results for Toluene in Spiked Gabapentin Drug Substance - Analyst 1

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.000	—
Control-2	—	—	0.000	—
Control-3	—	—	0.000	—
Mean	—	—	0.000	—
(LOQ)-1	0.019	0.019	0.017	-10.5
(LOQ)-2	0.019	0.019	0.018	-5.3
(LOQ)-3	0.019	0.019	0.018	-5.3
Mean	—	—	—	-7.0
100-1	0.093	0.093	0.093	0.0
100-2	0.094	0.094	0.094	0.0
100-3	0.093	0.093	0.093	0.0
Mean	—	—	—	0.0
150-1	0.139	0.139	0.144	3.6
150-2	0.140	0.140	0.147	5.0
150-3	0.139	0.139	0.137	-1.4
Mean	—	—	—	2.4

— = not applicable; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Reproducibility

To demonstrate reproducibility, a second analyst from a different laboratory performed a method precision/accuracy study. The sample preparations were analyzed according to the proposed method and the results are shown in Table 3.2.S.4.3-16 to Table 3.2.S.4.3-19. Each individual and mean recovery value from each set of preparations met the acceptance criteria of being within ±20.0% (relative basis) for solvent levels at the LOQ; and within ±10.0% (relative basis) for solvent levels greater than the LOQ. It should be noted that validation was inadvertently performed using 20% of the specification limit as the LOQ/reporting limit instead of 0.02% w/w, as outlined in the protocol. This did not have any impact on the LOQ/reporting limit for methanol, toluene, and IPA since 20% of their specification limit is 0.02% w/w. However, since ethanol's specification limit is 0.5%, it resulted in a higher LOQ/reporting limit of 0.1% w/w (refer to Deviations Section below).

Table 3.2.S.4.3-16. Reproducibility/Recovery Results for Methanol from Spiked Gabapentin Drug Substance - Analyst 2

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.012	—
Control-2	—	—	0.011	—
Control-3	—	—	0.011	—
Mean	—	—	0.011	—
(LOQ)-1	0.019	0.030	0.030	0.0
(LOQ)-2	0.019	0.030	0.032	6.7
(LOQ)-3	0.019	0.030	0.031	3.3
Mean	—	—	—	3.3
100-1	0.096	0.107	0.111	3.7
100-2	0.096	0.107	0.106	-0.9
100-3	0.095	0.106	0.108	1.9
Mean	—	—	—	1.6
150-1	0.143	0.154	0.151	-1.9
150-2	0.143	0.154	0.155	0.6
150-3	0.143	0.154	0.151	-1.9
Mean	—	—	—	-1.1

— = not applicable; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-17. Reproducibility/Recovery Results for Ethanol in Spiked Gabapentin Drug Substance - Analyst 2

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	ND	—
Control-2	—	—	ND	—
Control-3	—	—	ND	—
Mean	—	—	ND	—
(LOQ)-1	0.103	0.103	0.109	5.8
(LOQ)-2	0.103	0.103	0.109	5.8
(LOQ)-3	0.103	0.103	0.106	2.9
Mean	—	—	—	4.8
100-1	0.517	0.517	0.548	6.0
100-2	0.516	0.516	0.538	4.3
100-3	0.514	0.514	0.541	5.3
Mean	—	—	—	5.2
150-1	0.773	0.773	0.792	2.5
150-2	0.772	0.772	0.800	3.6
150-3	0.771	0.771	0.795	3.1
Mean	—	—	—	3.1

— = not applicable; LOQ = limit of quantitation; ND = none detected.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-18. Reproducibility/Recovery Results for IPA in Spiked Gabapentin Drug Substance - Analyst 2

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	0.025	—
Control-2	—	—	0.025	—
Control-3	—	—	0.025	—
Mean	—	—	0.025	—
(LOQ)-1	0.019	0.044	0.044	0.0
(LOQ)-2	0.019	0.044	0.047	6.8
(LOQ)-3	0.019	0.044	0.043	-2.3
Mean	—	—	—	1.5
100-1	0.094	0.119	0.126	5.9
100-2	0.094	0.119	0.123	3.4
100-3	0.094	0.118	0.126	6.8
Mean	—	—	—	5.4
150-1	0.141	0.166	0.173	4.2
150-2	0.141	0.166	0.177	6.6
150-3	0.140	0.165	0.172	4.2
Mean	—	—	—	5.0

— = not applicable; IPA = isopropyl alcohol; LOQ = limit of quantitation.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-19. Reproducibility/Recovery Results for Toluene in Spiked Gabapentin Drug Substance - Analyst 2

Approximate % of Specification Limit	Amount Spiked (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Control-1	—	—	ND	—
Control-2	—	—	ND	—
Control-3	—	—	ND	—
Mean	—	—	ND	—
(LOQ)-1	0.018	0.018	0.019	5.6
(LOQ)-2	0.018	0.018	0.020	11.1
(LOQ)-3	0.018	0.018	0.019	5.6
Mean	—	—	—	7.4
100-1	0.091	0.091	0.098	7.7
100-2	0.091	0.091	0.097	6.6
100-3	0.091	0.091	0.098	7.7
Mean	—	—	—	7.3
150-1	0.136	0.136	0.148	8.8
150-2	0.136	0.136	0.149	9.6
150-3	0.136	0.136	0.147	8.1
Mean	—	—	—	8.8

— = not applicable; LOQ = limit of quantitation; ND = none detected.
a. Target concentration = Average amount found in control + Amount spiked in sample.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Limit of Quantitation/Reporting Limit

To verify the LOQ in the presence of the sample matrix, 6 consecutive injections of one of the accuracy samples spiked at approximately the reporting limits (0.02% w/w for methanol, IPA and toluene, and approximately 0.10% w/w for ethanol) were performed and the precision of the replicate injections was determined for each solvent. Validation was inadvertently performed using 20% of the specification limit as the LOQ/reporting limit instead of 0.02% w/w, as outlined in the protocol. This did not have any impact on the LOQ/reporting limit for methanol, toluene and IPA since 20% of their specification limit is 0.02% w/w. However, since ethanol's specification limit is 0.5%, this resulted in a higher LOQ/reporting limit of 0.1% w/w (refer to Deviations Section below).

As presented in Table 3.2.S.4.3-20, the RSD of the peak area ratios for methanol, ethanol, IPA, and toluene from the 6 injections of the LOQ solution meet the acceptance criteria of ≤10.0%. In addition, as presented in Table 3.2.S.4.3-21, the signal to noise (S/N) ratios for methanol, ethanol, IPA, and toluene are above 10:1, thus meeting the acceptance criteria. Therefore, the concentrations of the solutions prepared at the reporting limits for methanol, ethanol, IPA, and toluene in gabapentin drug substance will be considered as the LOQ. No further attempts were made to verify the actual LOQ concentration that would give a S/N of approximately 10:1 since the reporting limits are well below the specification limits for each solvent, as shown in Table 3.2.S.4.3-21.

Table 3.2.S.4.3-20. System Precision for LOQ Concentration of Methanol, Ethanol, IPA, and Toluene (GC System No. 468)

Injection Number	Peak Area Ratio			
	Methanol	Ethanol	IPA	Toluene
1	0.119	0.650	0.220	0.213
2	0.109	0.657	0.220	0.216
3	0.106	0.623	0.216	0.221
4	0.116	0.686	0.225	0.212
5	0.115	0.627	0.220	0.218
6	0.117	0.630	0.219	0.217
Mean	0.114	0.645	0.220	0.216
%RSD	4.6	3.8	1.4	1.5

GC = gas chromatography; IPA = isopropyl alcohol; LOQ = limit of quantitation; RSD = relative standard deviation.

Table 3.2.S.4.3-21. Signal-to-Noise Ratios for Limit of Quantitation/Reporting Limit for Methanol, Ethanol, IPA, and Toluene

Solvent	µg/mL	% of Specification Limit	% w/w	Mean Signal-to-Noise Ratio
Methanol	1.9556	20	0.02	33:1
Ethanol	9.90383	20	0.10	140:1
IPA	1.9521	20	0.02	42:1
Toluene	1.8653	20	0.02	68:1

IPA = isopropyl alcohol.

Robustness

Varying Chromatographic Parameters

Small changes were made to critical GC parameters (initial oven temperature and first temperature ramp) to determine if slight variations in operating conditions would adversely affect the results. One injection each of unspiked and spiked gabapentin active pharmaceutical ingredient (API) samples at 0.1% w/w (methanol, IPA, and toluene) and at 0.5% w/w (ethanol) (Note: The protocol inadvertently gives the spiking concentration units for methanol, ethanol, IPA, and toluene as mg/mL instead of as % w/w) was made at the procedural condition and at each of the modified conditions. The initial oven temperature was modified by ±5°C (Note: The protocol inadvertently states to modify the initial oven temperature by ±5°C/minute) and the first temperature ramp by ±1°C/minute. System suitability was determined for each modified parameter (refer to Table 3.2.S.4.3-22). The acceptance criteria for system suitability were met under all conditions. It should be noted that the initial recovery results for ethanol at the first temperature ramp condition of 9°C/minute (-10.6%) did not meet the acceptance criteria of being within ±10.0%, relative basis, of the target concentration. An investigation was initiated; however, no assignable cause was determined (refer to Deviations Section below). Therefore, 3 spiked samples were analyzed at this modified condition, and the average of the 3 results are reported in Table 3.2.S.4.3-23 to Table 3.2.S.4.3-26. As shown in Table 3.2.S.4.3-23 to Table 3.2.S.4.3-26, for each set of varied conditions, the recoveries of methanol, ethanol, IPA, and toluene from a spiked sample at the analytical concentration agreed within ±10.0% (relative) of the adjusted target concentration. Furthermore, the chromatographic profiles under the modified conditions were similar to that obtained using the proposed method condition. Chromatograms under procedural and modified conditions are shown in Figure 3.2.S.4.3-12 to Figure 3.2.S.4.3-15.

Table 3.2.S.4.3-22. System Suitability Results for Gabapentin Drug Substance Under Robustness Conditions

Conditions	Reference	% RSD (≤10.0)				Resolution Between Ethanol and IPA Peaks (≥3)
		Methanol	Ethanol	IPA	Toluene	
Initial Oven Temperature (Procedural)[a]	DB 1922AX15, 16	4.0	4.3	3.2	1.2	10
Initial Oven Temperature 45°C	DB 1922AX46, 47	3.4	2.8	1.8	1.1	9
Initial Oven Temperature 35°C	DB 1922AX44, 45	2.7	3.1	2.5	1.3	11
First Temperature Ramp (Procedural)[a]	DB 1922AX112, 113	2.0	2.4	2.3	1.0	10
First Temperature Ramp 11°C/minute	DB 1922AX114, 115	2.9	3.2	3.0	0.6	10
First Temperature Ramp 9°C/minute[b]	DB 1922AX116, 117	3.4	3.8	3.5	1.3	10
First Temperature Ramp 9°C/minute[c]	DB 1935AX55, 56	2.8	3.1	1.9	1.0	9

IPA = isopropyl alcohol; RSD = relative standard deviation.

a. Procedural Conditions: Oven Temperature (Initial): 40°C; First Temperature Ramp: 40°C-100°C at 10°C/minute.
b. Initial results of 9°C/minute analysis.
c. Repeat analysis at 9°C/minute.

Table 3.2.S.4.3-23. Recovery of Methanol in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions

Conditions	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Procedural Conditions[c]	0.099	0.009	0.108	0.101	-6.5
Initial Temperature 45°C	0.099	0.010	0.109	0.105	-3.7
Initial Temperature 35°C	0.099	0.009	0.108	0.108	0.0
Procedural Conditions	0.097	0.009	0.106	0.102	-3.8
First Temperature Ramp 11°C/minute	0.097	0.009	0.106	0.104	-1.9
First Temperature Ramp 9°C/minute[d]	0.097	0.008	0.105	0.098	-6.7
First Temperature Ramp 9°C/minute[e]	0.098	0.008	0.106	0.103	-2.8

a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.
c. Procedural Conditions: Oven Temperature (Initial): 40°C; First Temperature Ramp: 40°C-100°C at 10°C/minute.
d Initial results of 9°C/minute analysis.
e. Average of 3 results from repeated 9°C/minute analysis.

Table 3.2.S.4.3-24. Recovery of Ethanol in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions

Conditions	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Procedural Conditions[c]	0.489	0.000	0.489	0.471	-3.7
Initial Temperature 45°C	0.489	0.000	0.489	0.489	0.0
Initial Temperature 35°C	0.489	0.000	0.489	0.501	2.5
Procedural Conditions	0.482	0.000	0.482	0.462	-4.1
First Temperature Ramp 11°C/minute	0.482	0.000	0.482	0.466	-3.3
First Temperature Ramp 9°C/minute[d]	0.482	0.000	0.482	0.431	-10.6[e]
First Temperature Ramp 9°C/minute[f]	0.491	0.000	0.491	0.494	0.6

a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.
c. Procedural Conditions: Oven Temperature (Initial): 40°C; First Temperature Ramp: 40°C-100°C at 10°C/minute.
d. Initial results of 9°C/minute analysis.
e. Out of acceptance criteria.
f. Average of 3 results from repeated 9°C/minute analysis.

Table 3.2.S.4.3-25. Recovery of IPA in Spiked Gabapentin API Sample Under Robustness Conditions

Conditions	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Procedural Conditions[c]	0.098	0.017	0.115	0.112	-2.6
Initial Temperature 45°C	0.098	0.017	0.115	0.114	-0.9
Initial Temperature 35°C	0.098	0.017	0.115	0.118	2.6
Procedural Conditions	0.095	0.016	0.111	0.108	-2.7
First Temperature Ramp 11°C/minute	0.095	0.015	0.110	0.108	-1.8
First Temperature Ramp 9°C/minute[d]	0.095	0.015	0.110	0.101	-8.2
First Temperature Ramp 9°C/minute[e]	0.099	0.014	0.113	0.113	0.0

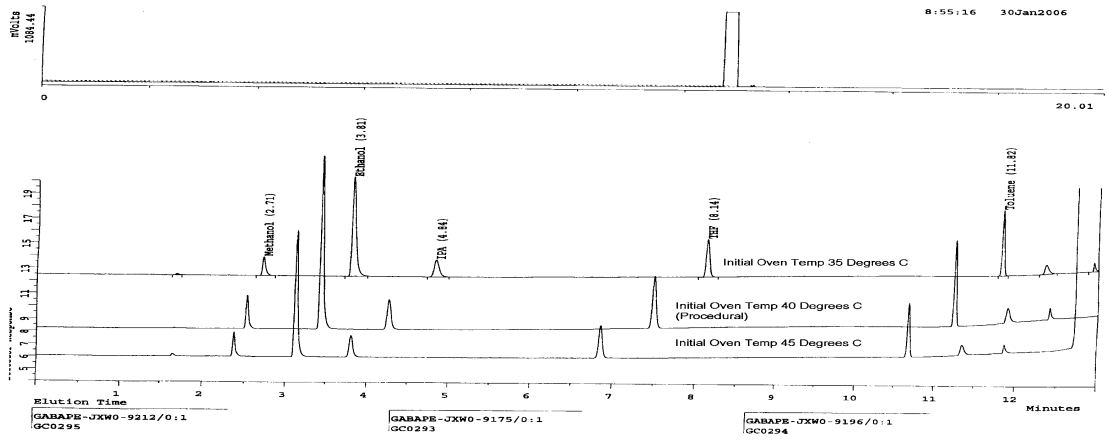
API = active pharmaceutical ingredient; IPA = isopropyl alcohol.
a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.
c. Procedural Conditions: Oven Temperature (Initial): 40°C; First Temperature Ramp: 40°C-100°C at 10°C/minute.
d. Initial results of 9°C/minute analysis.
e. Average of 3 results from repeated 9°C/minute analysis.

Table 3.2.S.4.3-26. Recovery of Toluene in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions

Conditions	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Procedural Conditions[c]	0.092	0.000	0.092	0.093	1.1
Initial Temperature 45°C	0.092	0.000	0.092	0.092	0.0
Initial Temperature 35°C	0.092	0.000	0.092	0.090	-2.2
Procedural Conditions	0.092	0.000	0.092	0.095	3.3
First Temperature Ramp 11°C/minute	0.092	0.000	0.092	0.094	2.2
First Temperature Ramp 9°C/minute[d]	0.092	0.000	0.092	0.096	4.3
First Temperature Ramp 9°C/minute[e]	0.093	0.000	0.093	0.092	-1.1

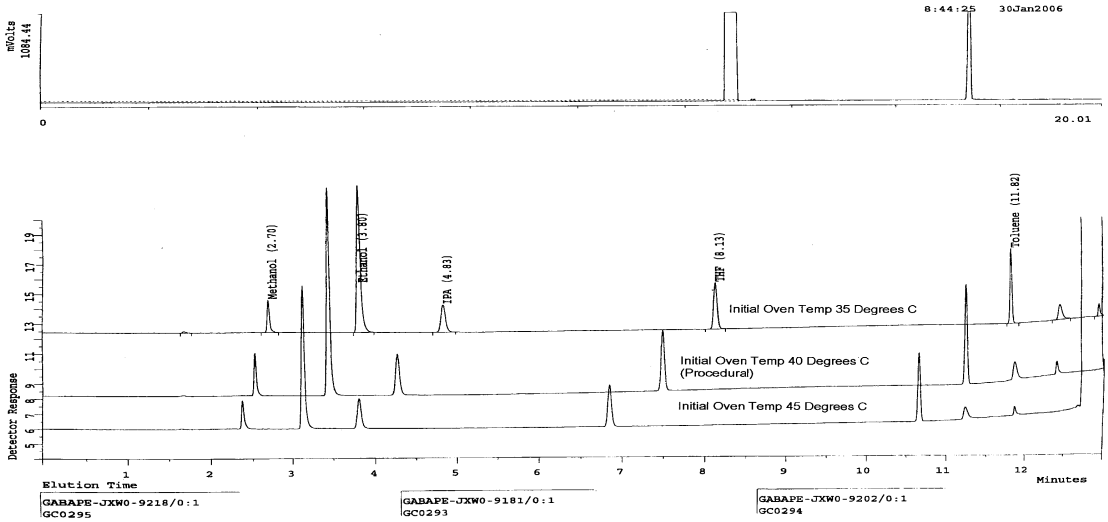
a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.
c. Procedural Conditions: Oven Temperature (Initial): 40°C; First Temperature Ramp: 40°C-100°C at 10°C/minute.
d. Initial results of 9°C/minute analysis.
e. Average of 3 results from repeated 9°C/minute analysis.

Figure 3.2.S.4.3-12. Overlaid Chromatograms of a Gabapentin Drug Substance Standard Solution under Modified Initial Oven Temperature. (Bottom) 45°C, (Middle) 40°C, (Top) 35°C



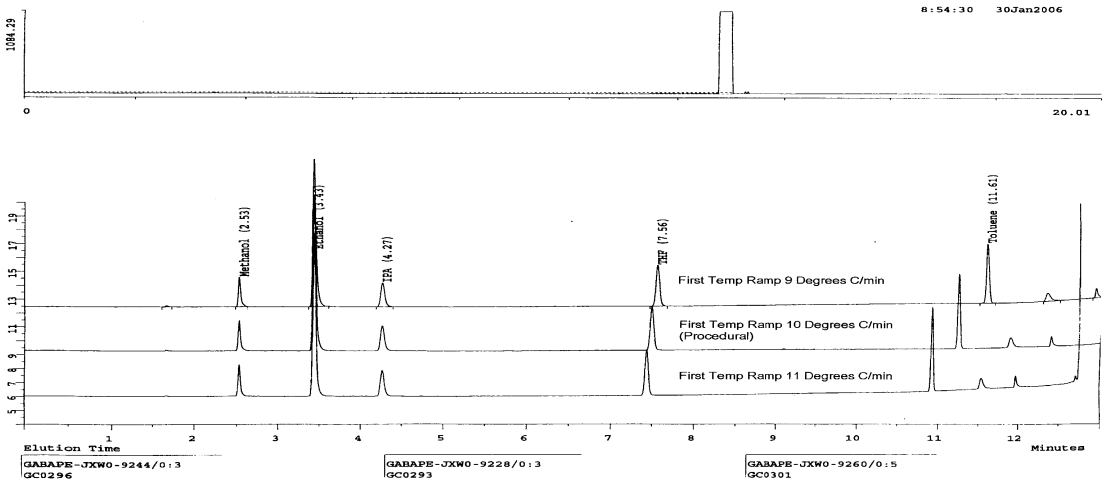
Note: Peak Identification: (Bottom to Top): Methanol (2.38, 2.53, 2.71 minutes); Ethanol (3.13, 3.43, 3.81 minutes); IPA (3.81, 4.27, 4.84 minutes); THF (6.86, 7.50, 8.14 minutes); Toluene (10.68, 11.26, 11.82 minutes).
ID = identification; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-13. Overlaid Chromatograms of a Gabapentin Drug Substance Spiked Sample Solution under Modified Initial Oven Temperature. (Bottom) 45°C, (Middle) 40°C, (Top) 35°C



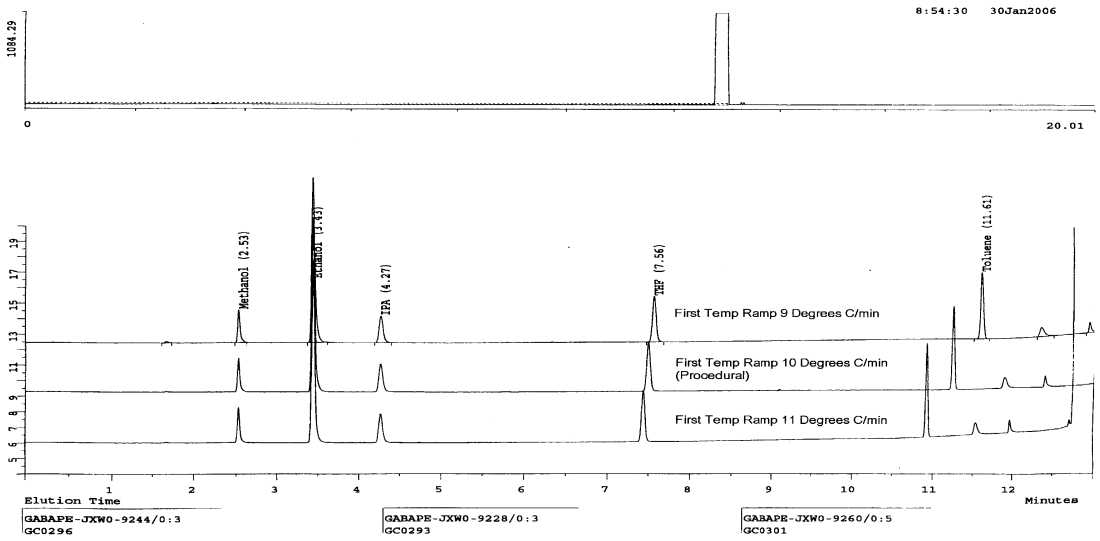
Note: Peak Identification: (Bottom to Top): Methanol (2.38, 2.53, 2.70 minutes); Ethanol (3.13, 3.44, 3.80 minutes); IPA (3.81, 4.27, 4.83 minutes); THF (6.85, 7.50, 8.13 minutes); Toluene (10.68, 11.26, 11.82 minutes).
ID = identification; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-14. Overlaid Chromatograms of a Gabapentin Drug Substance Standard Solution Under Modified First Temperature Ramp. (Bottom) 11°C/minute, (Middle) 10°C/minute, (Top) 9°C/minute



Note: Peak Identification: (Bottom to Top): Methanol (2.53, 2.53, 2.53 minutes); Ethanol (3.44, 3.44, 3.43 minutes); IPA (4.27, 4.27, 4.27 minutes); THF (7.44, 7.50, 7.56 minutes); Toluene (10.93, 11.26, 11.61 minutes).
ID = identification; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Figure 3.2.S.4.3-15. Overlaid Chromatograms of a Gabapentin Drug Substance Spiked Sample Solution Under Modified First Temperature Ramp. (Bottom) 11°C/minute, (Middle) 10°C/minute, (Top) 9°C/minute



Note: Peak Identification: (Bottom to Top): Methanol (2.53, 2.53, 2.53 minutes); Ethanol (3.44, 3.43, 3.44 minutes); IPA (4.27, 4.26, 4.27 minutes); THF (7.44, 7.49, 7.56 minutes); Toluene (10.93, 11.25, 11.61 minutes)
ID = identification; IPA = isopropyl alcohol; THF = tetrahydrofuran.

Solution Stability

Data was generated to assess the stability of methanol, ethanol, IPA, and toluene in the Standard Stock Residual Solvent (Solution A) and in the Spiked Sample Solution when stored at ambient conditions. As per the protocol, the sample solution was spiked with methanol, IPA, and toluene at approximately 0.1% w/w (Note: The protocol inadvertently gives the spiking concentration units for methanol, ethanol, IPA, and toluene as mg/mL instead of as % w/w) and with ethanol at approximately 0.5% w/w. The standard and sample solutions were evaluated against a freshly prepared standard solution on each day of analysis. The standard and sample solutions are assumed to be stable if the methanol, ethanol, IPA, and toluene concentrations are within ±10.0% of initial concentration, on a relative basis.

As presented in Table 3.2.S.4.3-27, the methanol, ethanol, IPA, and toluene concentrations in the Standard Stock Residual Solvent (Solution A) are within ±10.0% (relative basis) of initial concentration for up to 7 days. Therefore, the Standard Stock Residual Solvent is stable for up to 7 days when stored at ambient conditions.

The results for the Spiked Sample Solution are presented in Table 3.2.S.4.3-28. The percent difference for ethanol was 11.9% on Day 3 and did not meet the acceptance criteria of being within ±10.0% (relative basis) of initial concentration. Therefore, the Spiked Sample Solution is stable for up to 2 days when stored at ambient conditions.

Table 3.2.S.4.3-27. Solution Stability for Methanol, Ethanol, IPA, and Toluene in the Standard Stock Residual Solvent (Solution A) Stored at Ambient Conditions

Time Point	Methanol		Ethanol		IPA		Toluene	
	mg/mL	% Difference[a]	mg/mL	% Difference[a]	mg/mL	% Difference[a]	mg/mL	% Difference[a]
Initial (Day 0)	3.24	—	16.11	—	3.18	—	3.08	—
Day 1	3.20	-1.2	15.75	-2.2	3.13	-1.6	3.02	-1.9
Day 2	3.24	0.0	15.59	-3.2	3.08	-3.1	3.12	1.3
Day 3	3.29	1.5	16.27	1.0	3.21	0.9	3.06	-0.6
Day 7	3.10	-4.3	15.36	-4.7	3.07	-3.5	3.18	3.2

— = not applicable; IPA = isopropyl alcohol
a. % Difference (Relative) = [(Day X – Day 0)/Day 0] × 100.

Table 3.2.S.4.3-28. Solution Stability for Methanol, Ethanol, IPA, and Toluene in the Spiked Sample Solution Stored at Ambient Conditions

Time Point	Methanol		Ethanol		IPA		Toluene	
	mg/mL	% Difference[a]	mg/mL	% Difference[a]	mg/mL	% Difference[a]	mg/mL	% Difference[a]
Initial (Day 0)	0.102	—	0.462	—	0.108	—	0.095	—
Day 1	0.109	6.9	0.491	6.3	0.114	5.6	0.091	-4.2
Day 2	0.107	4.9	0.505	9.3	0.118	9.3	0.090	-5.3
Day 3	0.109	6.9	0.517	11.9[b]	0.118	9.3	0.088	-7.4

— = not applicable; IPA = isopropyl alcohol
a. %Difference (Relative) = [(Day X – Day 0)/Day 0] × 100.
b. Out of acceptance criteria (see above text).

System Suitability Testing

System suitability was established prior to each analysis by making 6 consecutive injections of the Reference Solution (Solution F). The required system parameters, such as precision (% RSD of the peak area ratio of each individual analyte) and resolution between the ethanol and IPA peaks were determined (Table 3.2.S.4.3-29). All system suitability requirements were met.

Table 3.2.S.4.3-29. Daily System Suitability Results for Gabapentin Drug Substance

Date of Experiment	Reference	% RSD of Peak Area Ratio (≤10.0)				Resolution Between Ethanol and IPA Peaks (≥3)
		Methanol/ THF	Ethanol/ THF	IPA/THF	Toluene/ THF	
30 Nov 2005	DB 1922AX15-16	4.0	4.3	3.2	1.2	10
5 Dec 2005	DB 1922AX112-113	2.0	2.4	2.3	1.0	10
6 Dec 2005	DB 1922AX179-180	1.6	1.6	2.2	1.0	10
7 Dec 2005	DB 1922AX181-182	3.3	4.1	2.7	1.7	10
8 Dec 2005	DB 1922BX7-8	4.0	4.7	3.4	2.3	11
12 Dec 2005	DB 1922BX59-60	5.9	6.1	4.9	2.8	9
19 Dec 2005	DB 1922BX112-113	5.8	5.4	3.9	2.4	10
20 Dec 2005	DB 1922BX114-115	1.0	0.6	1.5	0.4	9
25 Nov 2005 (Little Island)	DB 1922AX250	6.9	0.7	0.4	0.3	8
28 Nov 2005 (Little Island)	DB 1922AX250	1.1	0.6	0.9	0.4	7

IPA = isopropyl alcohol; RSD = relative standard deviation; THF = tetrahydrofuran.

Deviations

Upon review of the data from both sites (Morris Plains [MOPS] and Little Island), it was observed that instead of performing testing of each solvent at approximately the LOQ/reporting limit of 0.02% w/w (as stated in the protocol), testing was inadvertently performed based on 20% of the specification limit of each solvent. This did not have any impact on the LOQ/reporting limit for methanol, toluene, and IPA since 20% of their specification limit is 0.02% w/w. However, ethanol's specification limit is 0.5%, which resulted in a higher LOQ/reporting limit of 0.1% w/w. The validated range obtained for ethanol (0.1% w/w to 0.75% w/w) covers the specification limit of 0.5% w/w; therefore, it was concluded that there should be no impact during routine analysis.

During the Robustness study (varying of chromatographic parameters), it was observed that at the first temperature ramp modification of 9°C/minute, the recovery of ethanol from the spiked sample was -10.6%, which did not meet the acceptance criteria of being within ±10.0%, relative basis, of the target concentration (Table 3.2.S.4.3-31). An investigation was initiated which consisted of interviewing the analyst and also reviewing the data and instrument setup. Since the sample solution was out of stability, no re-injections were performed and no assignable cause could be determined. Therefore, 3 additional spiked samples were analyzed at the modified ramp condition of 9°C/minute for methanol, ethanol, IPA, and toluene. The initial results and the repeated results for each solvent are reported in Table 3.2.S.4.3-30 to Table 3.2.S.4.3-33. All individual results met the acceptance criteria of being within ±10.0% of the target concentration, on a relative basis. The average results obtained from the repeated study are also reported in the Robustness study for the first temperature ramp modified condition of 9°C/minute, (refer to Section Robustness, Table 3.2.S.4.3-23 to Table 3.2.S.4.3-26).

Table 3.2.S.4.3-30. Initial and Repeat Results for Recovery of Methanol in Spiked Gabapentin Drug Substance Under Robustness Conditions 9°C/minute

Sample	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] (% w/w)	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Initial results	0.097	0.008	0.105	0.098	-6.7
Repeat Sample 1	0.098	0.008	0.106	0.102	-3.8
Repeat Sample 2	0.098	0.008	0.106	0.102	-3.8
Repeat Sample 3	0.098	0.008	0.106	0.105	-0.9
Average of 3 Repeats	0.098	0.008	0.106	0.103	-2.8

a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-31. Initial and Repeat Results for Recovery of Ethanol in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions 9°C/minute

Sample	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] % w/w	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Initial results	0.482	0.000	0.482	0.431	-10.6[c]
Repeat Sample 1	0.492	0.000	0.492	0.489	-0.6
Repeat Sample 2	0.491	0.000	0.491	0.488	-0.6
Repeat Sample 3	0.490	0.000	0.490	0.506	3.3
Average of 3 Repeats	0.491	0.000	0.491	0.494	0.6

a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.
c. Out of acceptance criteria. See above text regarding out of specification result for recovery of ethanol.

Table 3.2.S.4.3-32. Initial and Repeat Results for Recovery of IPA in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions 9°C/minute

Sample	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] % w/w	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Initial results	0.095	0.015	0.110	0.101	-8.2
Repeat Sample 1	0.099	0.014	0.113	0.111	-1.8
Repeat Sample 2	0.099	0.014	0.113	0.113	0.0
Repeat Sample 3	0.099	0.014	0.113	0.116	2.7
Average of 3 Repeats	0.099	0.014	0.113	0.113	0.0

IPA = isopropyl alcohol
a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Table 3.2.S.4.3-33. Initial and Repeat Results for Recovery of Toluene in Spiked Gabapentin Drug Substance Sample Under Robustness Conditions 9°C/minute

Sample	Amount Spiked (% w/w)	Amount Found in Control (% w/w)	Target Concentration[a] % w/w	Amount Recovered (% w/w)	% Difference from Target Concentration[b]
Initial results	0.092	0.015	0.092	0.096	4.3
Repeat Sample 1	0.094	0.014	0.094	0.092	-2.1
Repeat Sample 2	0.093	0.014	0.093	0.093	0.0
Repeat Sample 3	0.093	0.014	0.093	0.091	-2.2
Average of 3 Repeats	0.093	0.014	0.093	0.092	-1.1

a. Target concentration = Amount found in Control + Amount spiked in samples.
b. % Difference (Relative) = [(Amount Recovered – Target Concentration)/Target Concentration] × 100.

Supplemental Validation for Acetone Quantitation

A supplemental method validation study was conducted to demonstrate that the analytical method is suitable to quantitate acetone. The supplemental method validation demonstrated that the test procedure is linear, accurate/repeatable, and able to detect acetone over the full concentration range of 50 ppm to 750 ppm in gabapentin API.

Linearity

Linearity was performed by preparing 6 solutions of acetone to cover the range of the method of 0.5 µg/mL to 100 µg/mL (approximately 0.005% w/w to 1.0% w/w relative to gabapentin in the sample preparation). The linearity data is shown in Figure 3.2.S.4.3-16 and the data is reported in Table 3.2.S.4.3-34.

Figure 3.2.S.4.3-16. Linearity Plot for Acetone

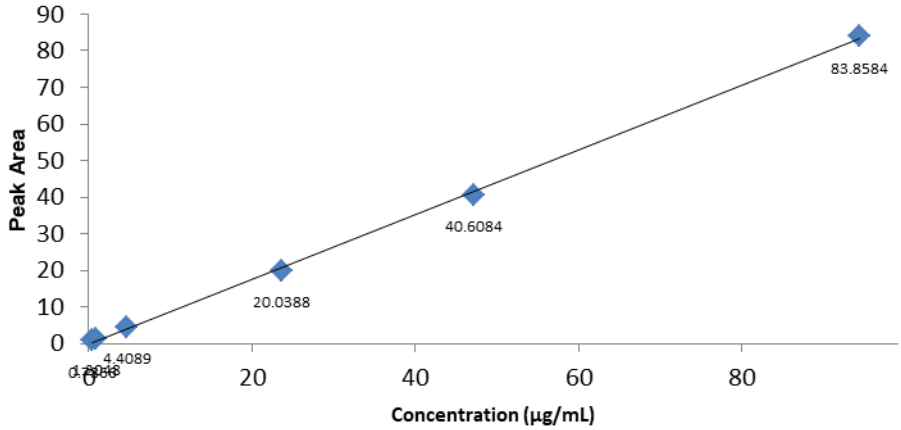


Table 3.2.S.4.3-34. Linearity Information for Acetone

Approximate % Analytical Concentration	Concentration (µg/mL)	Concentration (% w/w)	Peak Area
1%	0.472227	0.005	0.7866
2%	0.944454	0.01	1.2048
10%	4.72227	0.05	4.4089
50%	23.61135	0.24	20.0388
100%	47.2227	0.47	40.6084
200%	94.4454	0.94	83.8584

Range

Based on the results of the linearity, accuracy and precision studies, the range of the method was demonstrated to be from approximately 0.05% w/w to approximately 0.75% w/w relative to the analytical concentration of gabapentin. The range is equivalent to approximately 1% to 150% of the acetone specification limit (100 ppm). Acceptance criteria for linearity, precision and accuracy are met; therefore, the range has been determined to be acceptable.

Accuracy

Accuracy and method precision (repeatability) were demonstrated by analyzing the recoveries of acetone in the presence of other residual solvents (methanol, isopropanol, and toluene). Gabapentin API sample preparations, each spiked with the other residual solvents at 100% of their product (material) specifications, were spiked with known amounts of acetone as follows: Five preparations at the 0.005% (w/w), 3 preparations at the 0.01% (w/w), and 3 preparations at the 0.75% (w/w), relative to gabapentin were analyzed for accuracy and method precision (repeatability).

In addition, 3 unspiked samples were prepared as controls. The average amount of acetone present in the control samples was determined and was then added to the amounts spiked to determine the amount of acetone that should be recovered from each sample preparation.

The recovery results for acetone in the Gabapentin API are presented in Table 3.2.S.4.3-35.

Table 3.2.S.4.3-35. Accuracy of Acetone in Gabapentin API

Sample ID	Acetone Spiked (mg/mL)	Acetone Found (%)	Recovery (%)	Average (%)	% RSD
Unspiked Sample 1	-	0.0057	-	0.0053	-
Unspiked Sample 2	-	0.0050	-		-
Unspiked Sample 3	-	0.0053	-		-
QL Spiked Sample 1	0.0047	0.0104	104.4	96.2	8.0
QL Spiked Sample 2	0.0047	0.0103	103.3		
QL Spiked Sample 3	0.0047	0.0086	86.1		
QL Spiked Sample 4	0.0047	0.0093	92.5		
QL Spiked Sample 5	0.0047	0.0095	94.7		
0.01% Spiked Sample 1	0.0093	0.0143	97.9	95.5	4.4
0.01% Spiked Sample 2	0.0093	0.0133	90.6		
0.01% Spiked Sample 3	0.0093	0.0144	98.0		
0.75% Spiked Sample 1	0.7770	0.8238	105.3	107.1	2.0
0.75% Spiked Sample 2	0.7770	0.8560	109.4		
0.75% Spiked Sample 3	0.7770	0.8330	106.5		

- = not applicable; API = active pharmaceutical ingredient; ID = identification; QL = quantitation limit; RSD = relative standard deviation.

Precision (Repeatability)

Method Precision (Repeatability) was demonstrated as part of Accuracy (see Table 3.2.S.4.3-35). The % RSD for recovery of acetone at each level met the precision requirement of ≤15.0% for spiking levels <0.2% and ≤10.0% for spiking levels 0.2% to 1.0%. The precision of the method met the acceptance criteria.

Quantitation Limit

The quantitation limit (QL) for acetone was established at 50 ppm, equivalent to 0.005% (w/w). The QL was confirmed by analysis of 5 sample preparations spiked at the QL level during the accuracy study.

System Suitability Testing

System suitability was established prior to each analysis by making 6 consecutive injections of the Reference Solution. The % RSD of the peak area ratio relative to internal standard was determined for each residual solvent. The system suitability results are shown in Table 3.2.S.4.3-36.

Table 3.2.S.4.3-36. System Suitability Parameters

Date of Experiment	Acetone% RSD (NMT 10.0%)	Methanol % RSD (NMT 10.0%)	Isopropanol % RSD (NMT 10.0%)	Toluene % RSD (NMT 10.0%)	Average Resolution (n=6) (NLT 1.5)
30-Jan-2019	1.1	1.8	5.0	1.5	2.4
04-Feb-2019	0.5	0.7	0.7	1.0	2.5
08-Feb-2019	0.8	0.9	5.0	7.7	2.4

NLT = not less than; NMT = not more than; RSD = relative standard deviation.

Conclusions

The validation demonstrated that the proposed method used for the determination of residual methanol, ethanol, IPA, acetone, and toluene is specific, linear, accurate, precise, robust, and reproducible. Therefore, the method is considered suitable for its intended use. Based on the solution stability study, the Standard Stock Residual Solvent (Solution A) is stable for up to 7 days and the sample solution is stable for up to 2 days when stored at ambient conditions.

3.2.S.4.3.3. Validation of Particle Size Method

The in-house test method to determine particle size of gabapentin drug substance is a physical test utilizing an air-jet sieve procedure. The proposed analytical method is described in Section 3.2.S.4.2. As a physical test method, the key validation parameters are precision, including repeatability and intermediate precision, as well as robustness.

Accuracy is established by the physical dimensions of the sieve, and therefore was not evaluated during validation. Specificity is not applicable because the identity of gabapentin is established using other methods. Linearity, range, detection limit, and LOQ do not apply as this is not a quantitative test, but rather a ratio of masses that establish the particle size distribution. Therefore, validation of this method involved the evaluation of precision and robustness and results are summarized below.

Precision and Robustness

Precision and robustness were evaluated in a combined design of experiments. For intermediate precision, 3 analysts executed the method using 2 air-jet sieve instruments with 6 separate No. 100 sieves over 3 days. For robustness, the most significant variable of the test method, the applied vacuum, was evaluated over the acceptable limits of 9 to 11 inches of water vacuum. The experimental design is provided in Table 3.2.S.4.3-37. Results from the evaluation are provided in Table 3.2.S.4.3-38.

Table 3.2.S.4.3-37. Experimental Design – Particle Size Method Validation

Run No.	Analyst	Day	Number. of Samples	Instrument	Vacuum Setting (inches of water)
1	1	1	6	1	9
2	1	1	6	1	11
3	1	1	6	2	9
4	1	1	6	2	11
5	2	2	6	1	9
6	2	2	6	1	11
7	2	2	6	2	9
8	2	2	6	2	11
9	3	3	3	1	9
10	3	3	3	1	11

Table 3.2.S.4.3-38. Results – Particle Size Method Validation

	Result (% retained)					
	Analyst 1/Day 1		Analyst 2/Day2		Analyst 3/Day 3	
Instrument No.	9 inches water	11 inches water	9 inches water	11 inches water	9 inches water	11 inches water
Instrument 1	43.36	42.17	44.33	42.48	43.98	44.08
	43.15	40.67	43.41	43.15	43.61	43.14
	44.14	41.42	44.38	42.97	44.63	42.81
	44.05	43.83	44.37	43.01		
	43.71	41.70	43.73	42.61		
	43.94	41.58	43.48	43.45		
Instrument 2	41.75	42.68	43.04	42.47	Not tested	Not tested
	42.71	42.03	43.65	41.87		
	43.75	40.91	44.42	41.96		
	42.67	41.33	43.11	42.18		
	43.46	42.84	42.84	42.64		
	43.32	42.26	43.93	42.65		

Precision: Repeatability

Repeatability of the test method was established from the variation of the repeated preparations of each analyst. The repeatability of the method was determined to be 0.39% absolute (Percent Retained). Compared to the specification for percent remaining on the 100 mesh sieve of NMT 50%, this uncertainty is low and therefore acceptable.

Precision: Intermediate Precision

Intermediate precision was determined from the instrument-to-instrument and analyst/day to analyst/day variance. The instrument-to-instrument variance was 0.13% (Percent Retained) and the analyst/day to analyst/day was 0.14% (Percent Retained). Including the variance of preparation to preparation variability of 0.39% (Percent Retained) yields a total intermediate precision of 0.82% (Percent Retained). Compared to the specification for Percent Remaining on the 100 mesh sieve of NMT 50%, this uncertainty is low and therefore acceptable.

Robustness: Applied Vacuum

The robustness of the method to changes in the applied vacuum was assessed by the slope of the Percent Retained response as a function of the applied vacuum. The slope of the response was -0.595 Percent Retained per inch of water vacuum. This indicates that the expected impact of a 1 inch water variance from the nominal 10 inch set point would be approximately ±0.6% (absolute percent retained). Compared to the specification for Percent Remaining on the 100 mesh sieve of NMT 50%, this uncertainty is low and therefore the method is considered adequately robust.

Conclusion

The validation demonstrated that the proposed method used for testing the particle size of gabapentin drug substance was precise (including repeatability and intermediate precision) and robust for percent powder retained on a 100 mesh sieve across a vacuum range of 9 to 11 inches of water.