

Supplementary File

# Development and Validation of Analytical Method using Gas Chromatography with Triple Quadrupole Mass Spectrometry for the Detection of Alkyl Halides as Potential Genotoxic Impurities in Posaconazole

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## 1-(2,4-difluorophenyl) ethan-1-one (PGI-1) Standard Qualification Data

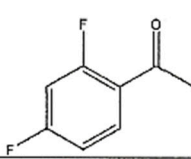
CERTIFICATE OF ANALYSIS		
Name of Product: 1-(2,4-difluorophenyl)ethan-1-one		
Molecular weight	: 156.13	Molecular Formula : C <sub>8</sub> H <sub>6</sub> F <sub>2</sub> O
Cat. No.	: NQ	CAS NO : 364-83-0
		
Chemical Name: 1-(2,4-difluorophenyl) ethan-1-one		
Sr. No.	Test	Result
1	Description	Pale yellow liquid
3	Chromatographic Purity by HPLC	99.93%
4	MASS by LCMS	Confirms to structure
5	<sup>1</sup> H NMR	Confirm to structure
6	<sup>13</sup> C NMR	Confirm to structure
7	FT-IR	Confirm to structure
8	Loss on drying (LOD) by TGA (%W/W)	0.23%
9	Potency (%W/W)	99.7%
Conclusion: The product complies as per above specifications.		
<ul style="list-style-type: none"> <li>• Long term Storage Conditions: Store at 2 to 8° C</li> <li>• Transportation Storage Condition : Room temperature</li> <li>• Short Term storage condition : Ambient Temperature</li> </ul>		

Figure S1: 1-(2,4-difluorophenyl) ethan-1-one (PGI-1) standard certificate of analysis.

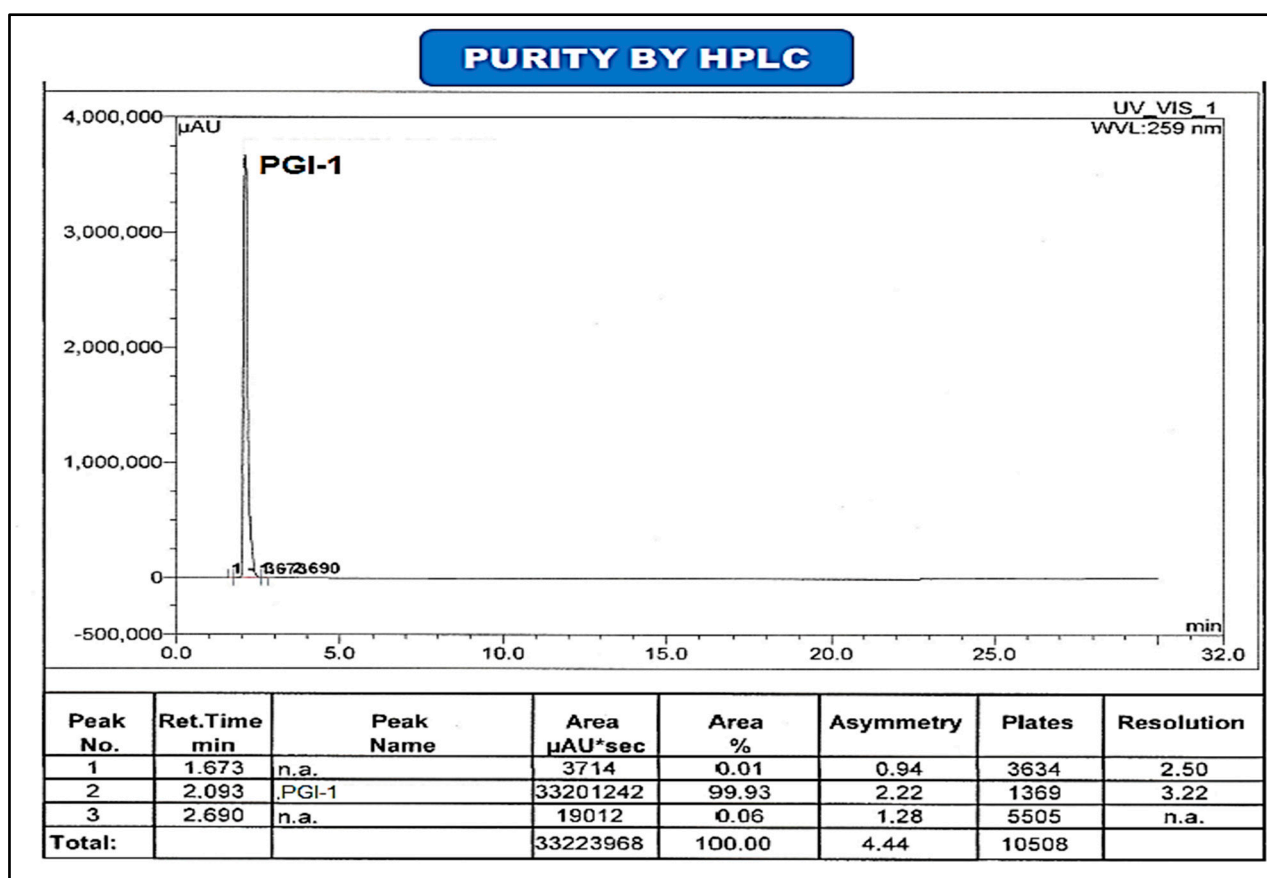


Figure S2: PGI-1 purity by HPLC chromatogram.

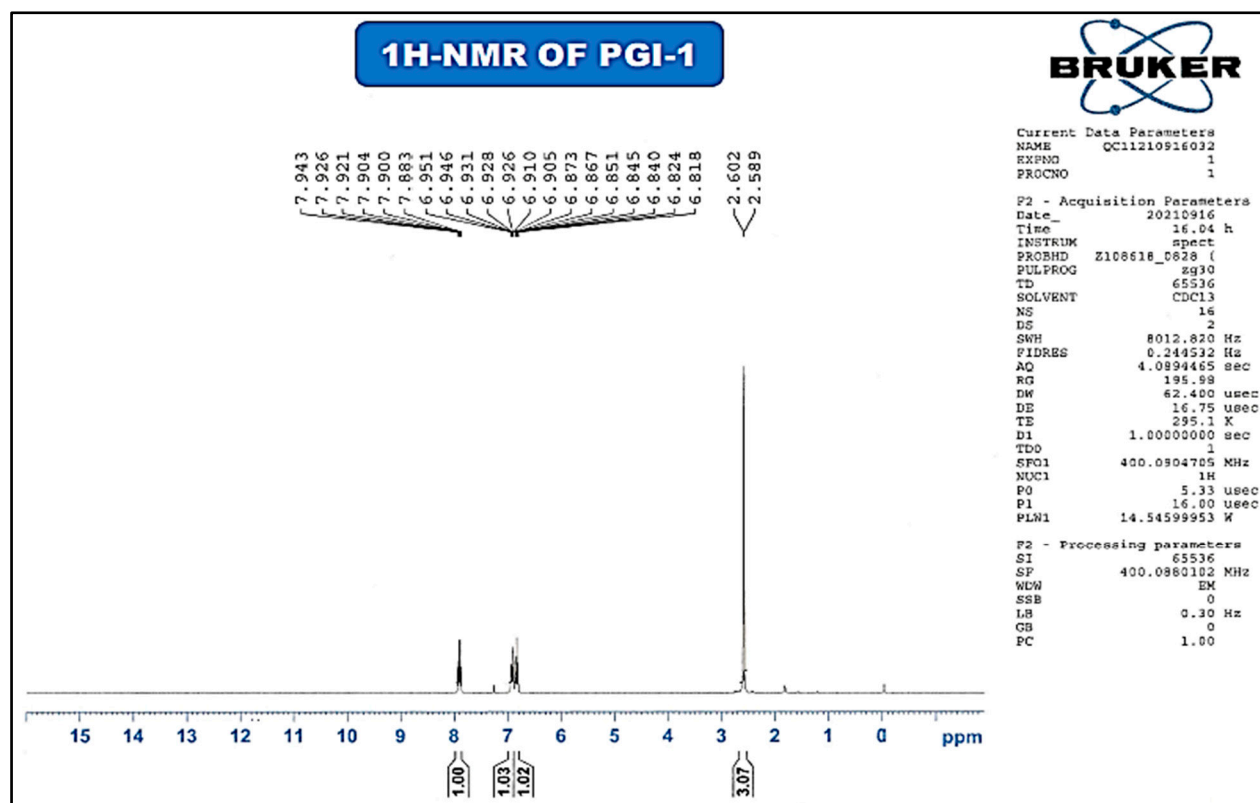


Figure S3: PGI-1 standard <sup>1</sup>H-NMR spectrum data.

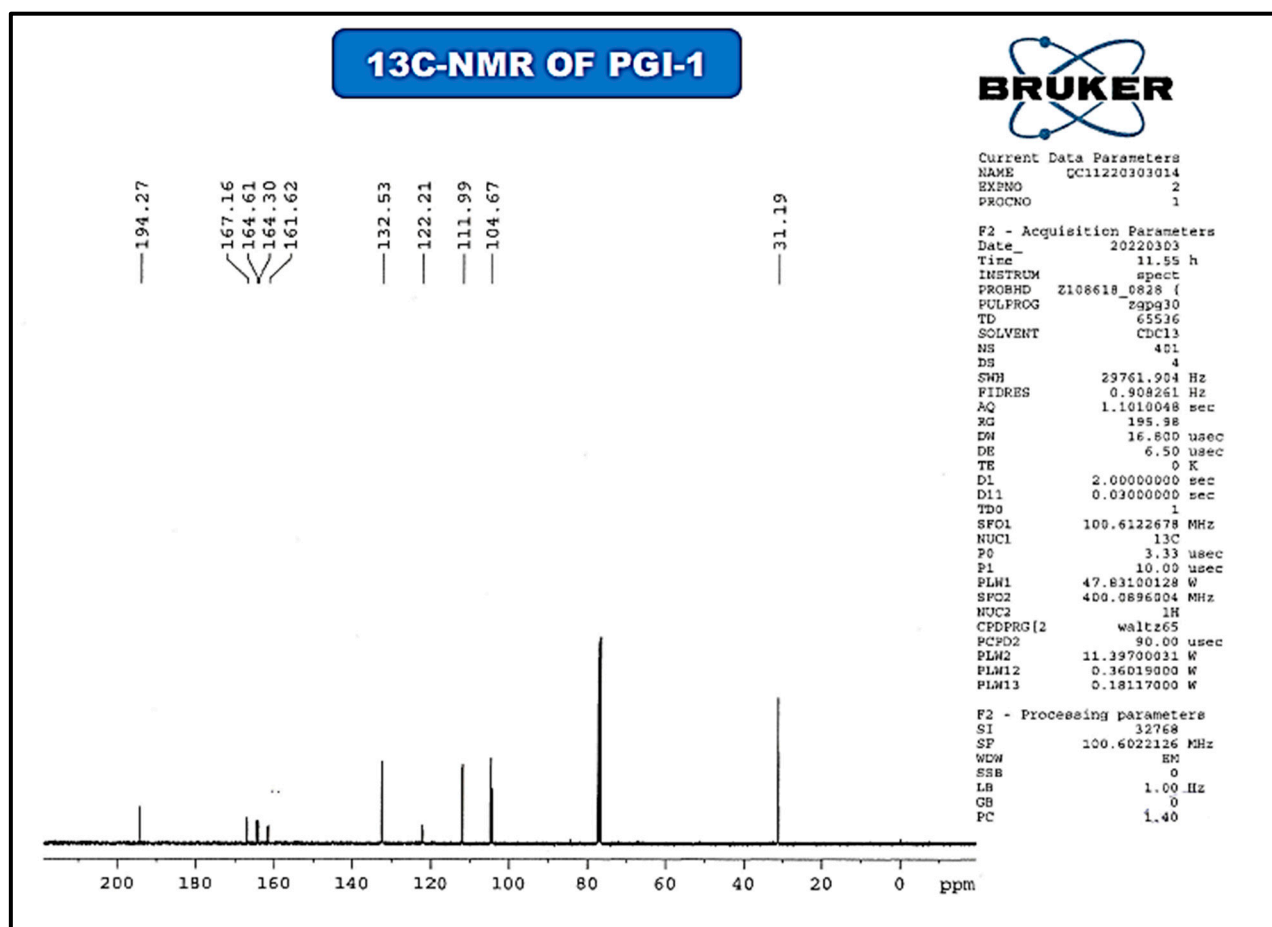


Figure S4: PGI-1 standard  $^{13}\text{C}$ -NMR spectrum data.

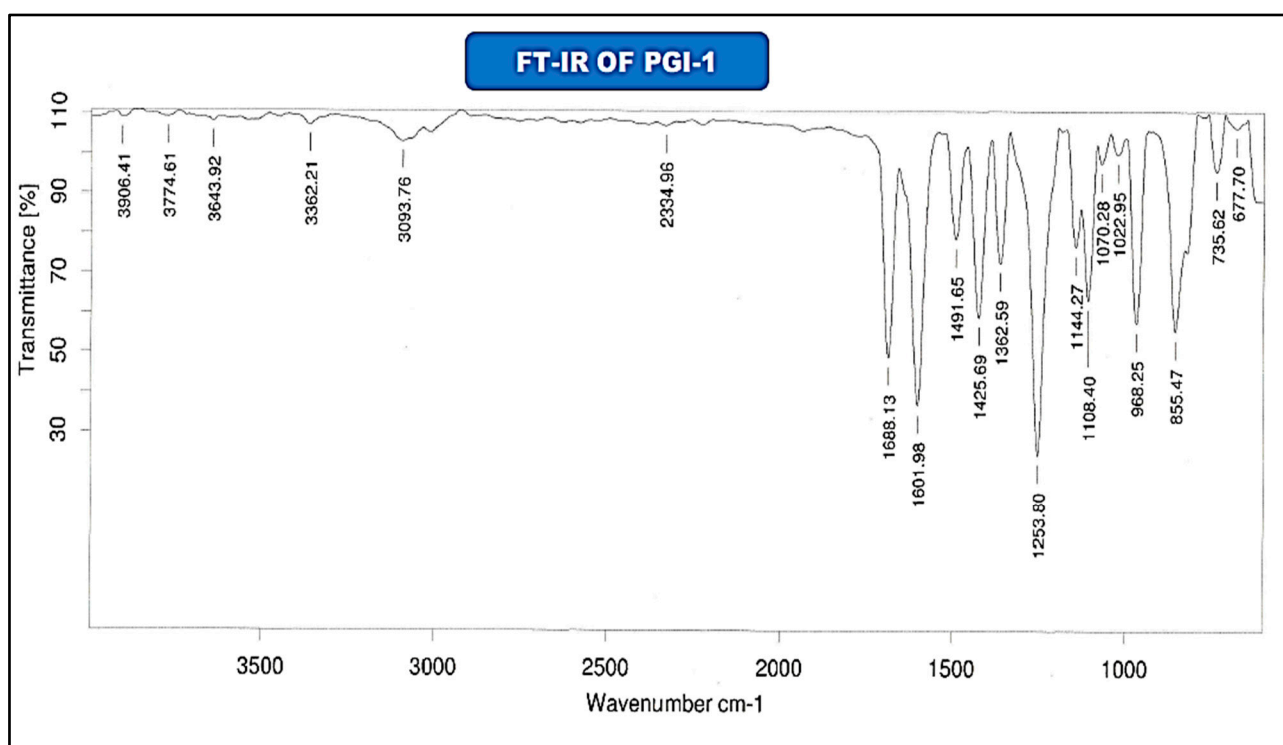


Figure S5: PGI-1 standard FT-IR spectrum.

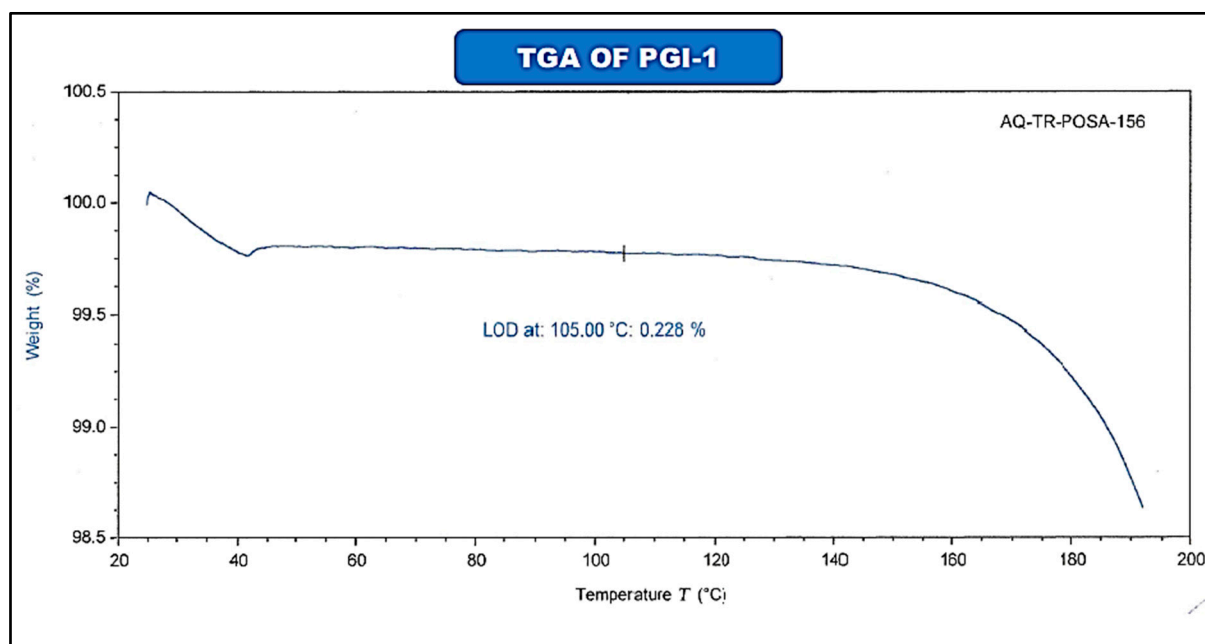


Figure S6: PGI-1 standard TGA graph.

### (Z)-1-(1-bromo prop-1-en-2-yl)- 2,4-difluorobenzene (PGI-2) Standard Qualification

#### CERTIFICATE OF ANALYSIS

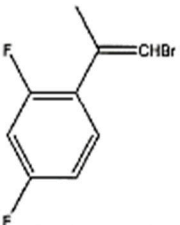
Name of Product: Z)-1-(1-bromoprop-1-en-2-yl)-2,4-difluorobenzene		
Molecular weight : 233.06		Molecular Formula : C <sub>9</sub> H <sub>7</sub> BrF <sub>2</sub>
		
ChemicalName: 1-(1-bromoprop-1-en-2-yl)-2,4-difluorobenzene		
Sr. No.	Test	Result
1	Description	Colourless Liquid
3	Chromatographic Purity by HPLC	99.12%
4	MASS by LCMS	No Ionization
5	<sup>1</sup> H NMR	Confirms to structure
6	<sup>13</sup> C NMR	Confirms to structure
7	FT-IR	Confirms to structure
8	Loss on drying (LOD) by TGA (%W/W)	0.26%
9	Potency (%W/W)	98.86%
Conclusion: The product complies as per above specifications.		
<ul style="list-style-type: none"> <li>• Long term Storage Conditions: Store at 2 to 8° C</li> <li>• Transportation Storage Condition : Room temperature</li> <li>• Short Term storage condition : Ambient Temperature</li> </ul>		

Figure S7: (Z)-1-(1-bromo prop-1-en-2-yl)- 2,4-difluorobenzene (PGI-2) standard certificate of analysis.

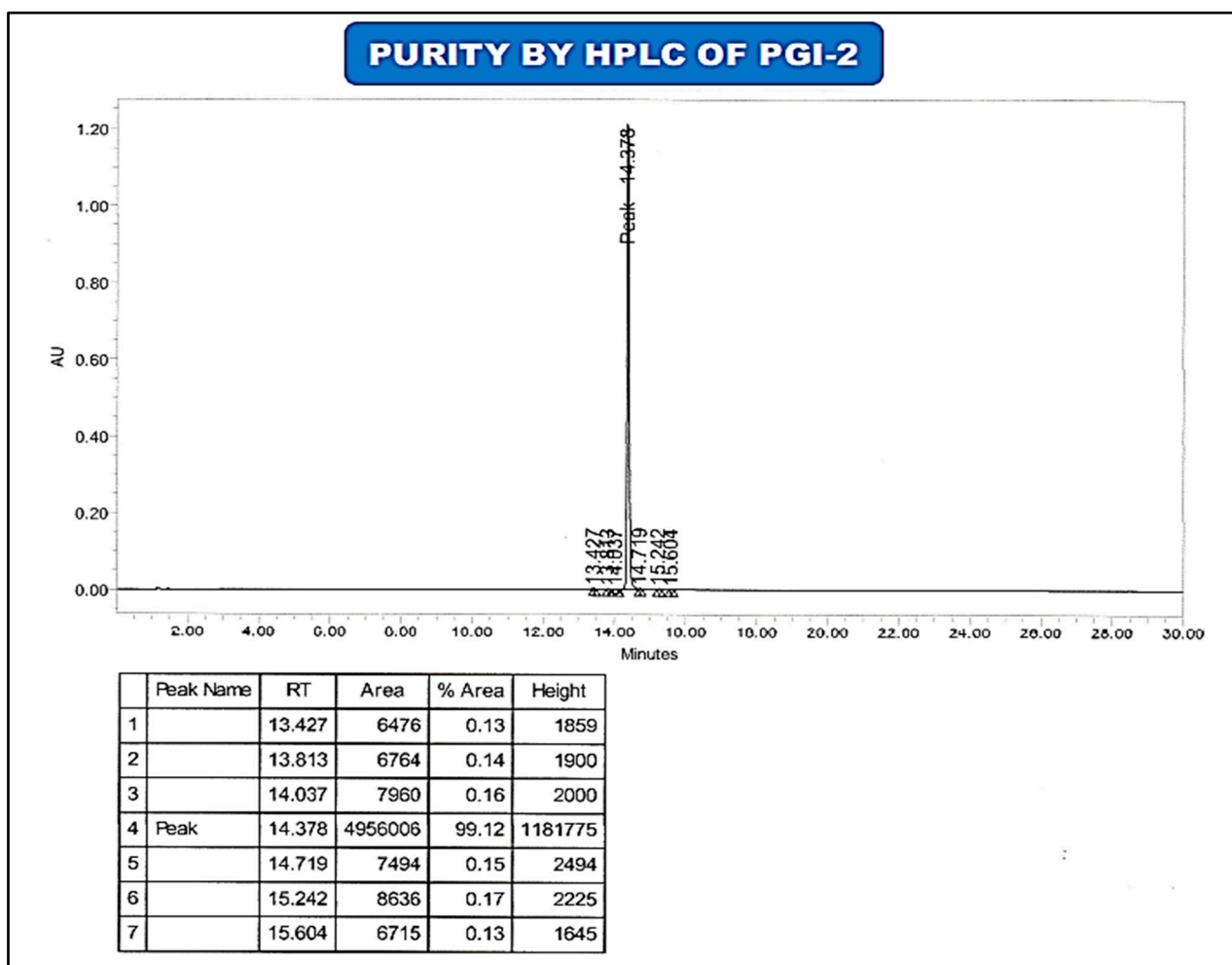


Figure S8: PGI-2 standard purity by HPLC chromatogram.

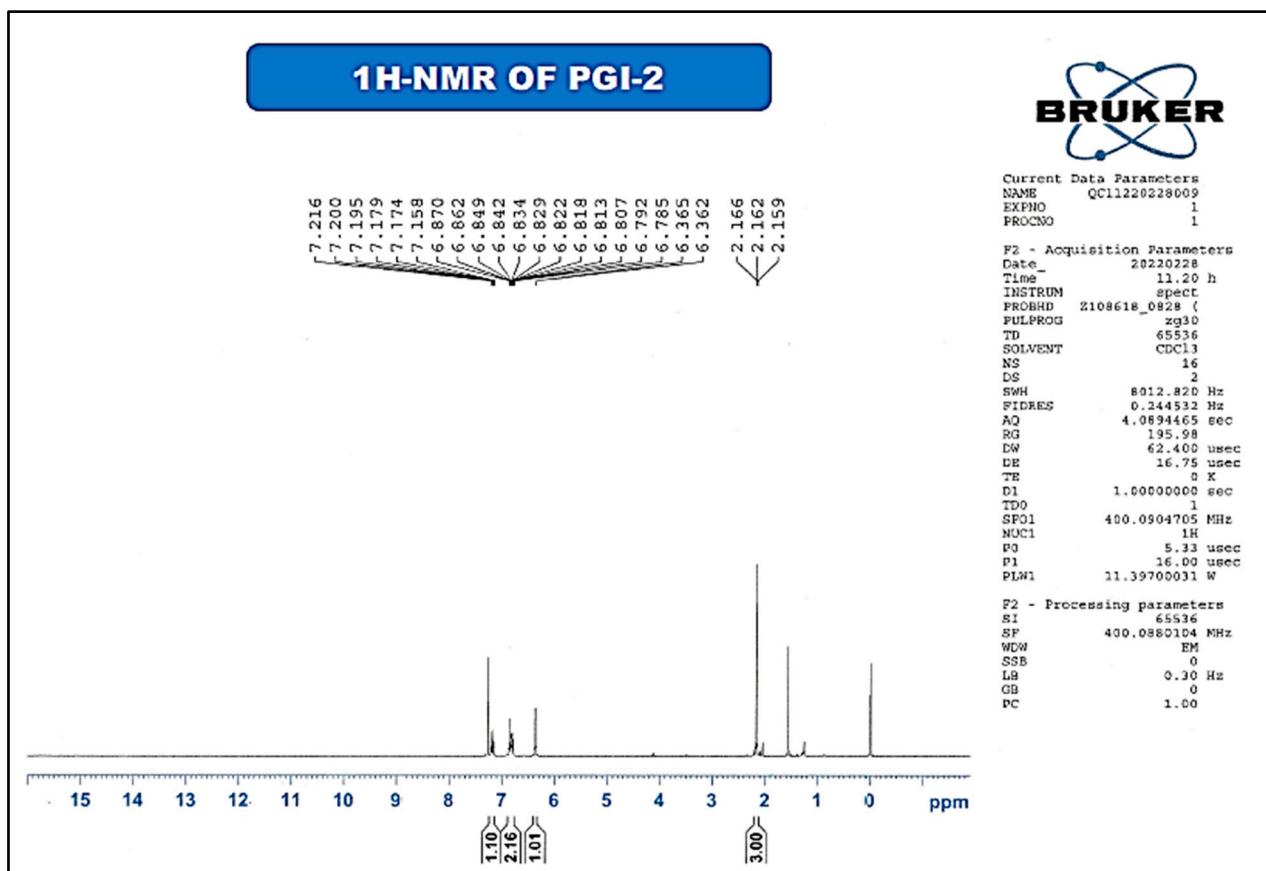


Figure S9: PGI-2 standard <sup>1</sup>H-NMR spectrum graph.

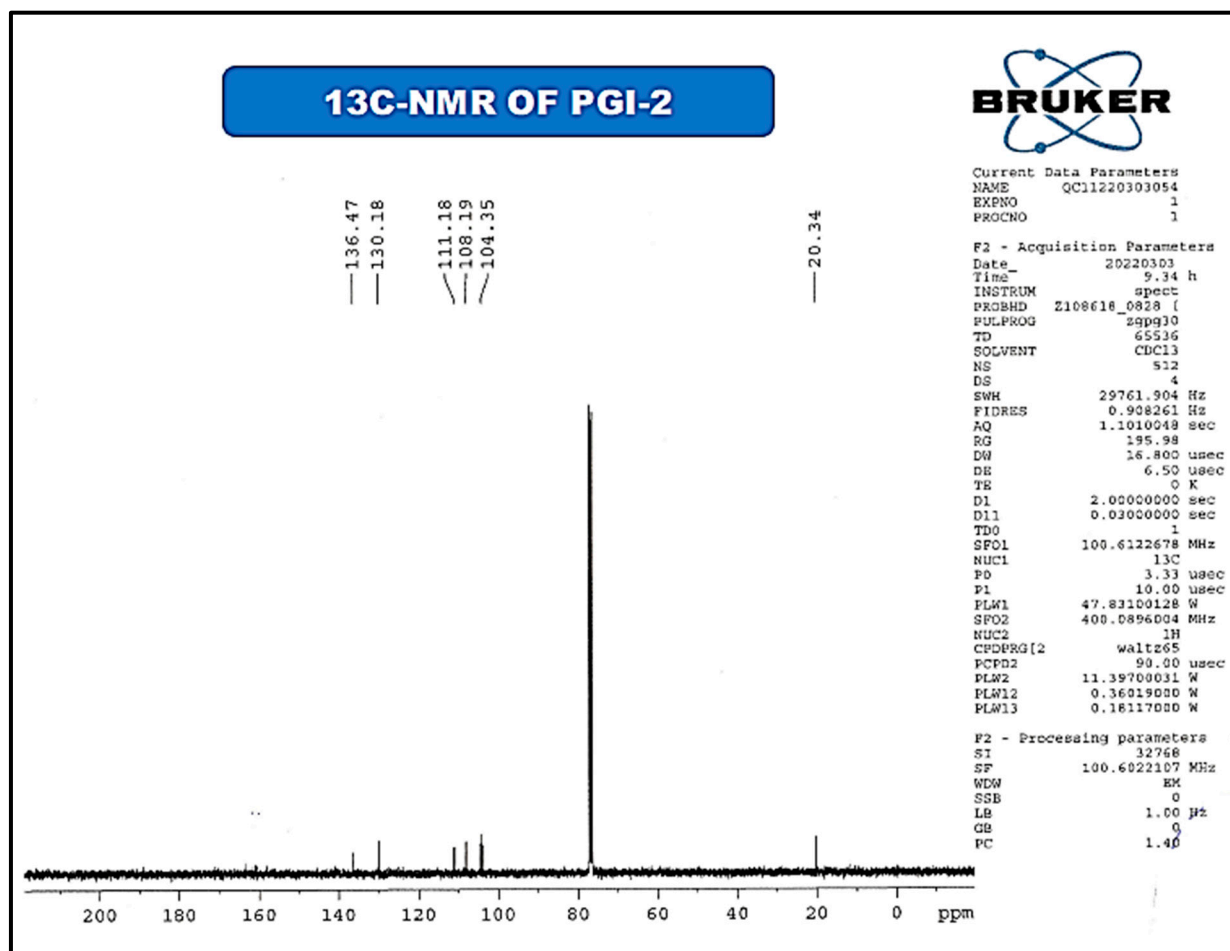


Figure S10: PGI-2 standard <sup>13</sup>C-NMR spectrum graph.

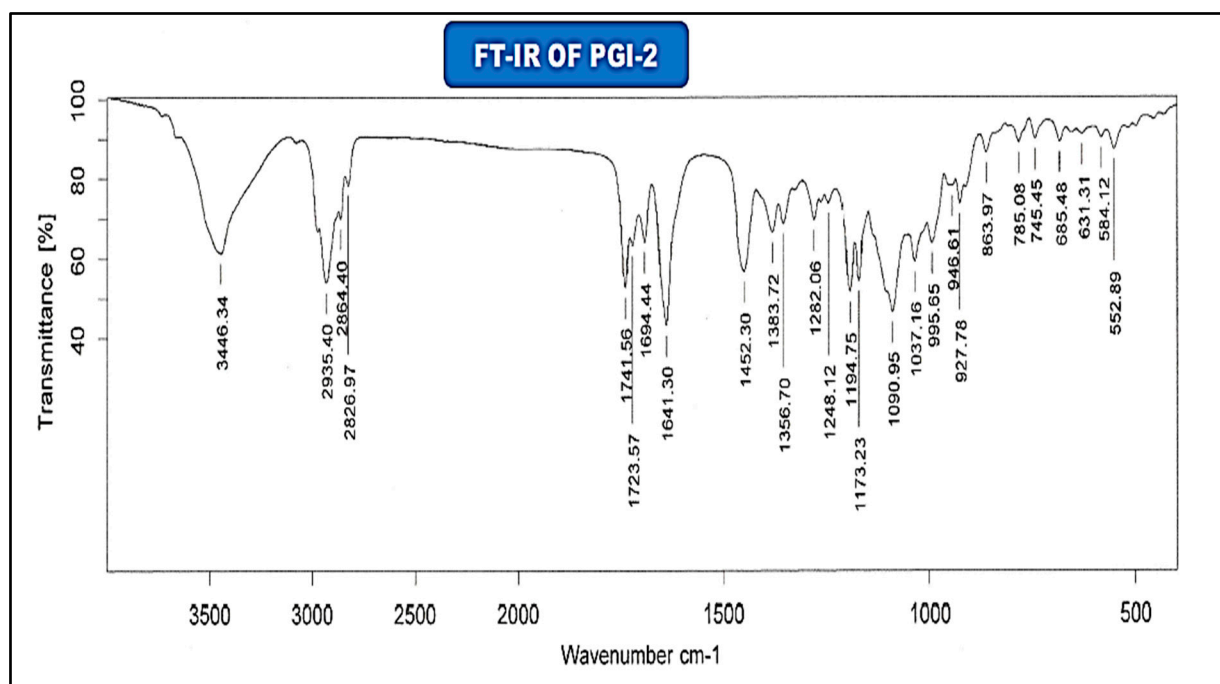


Figure S11: PGI-2 standard FT-IR spectrum.



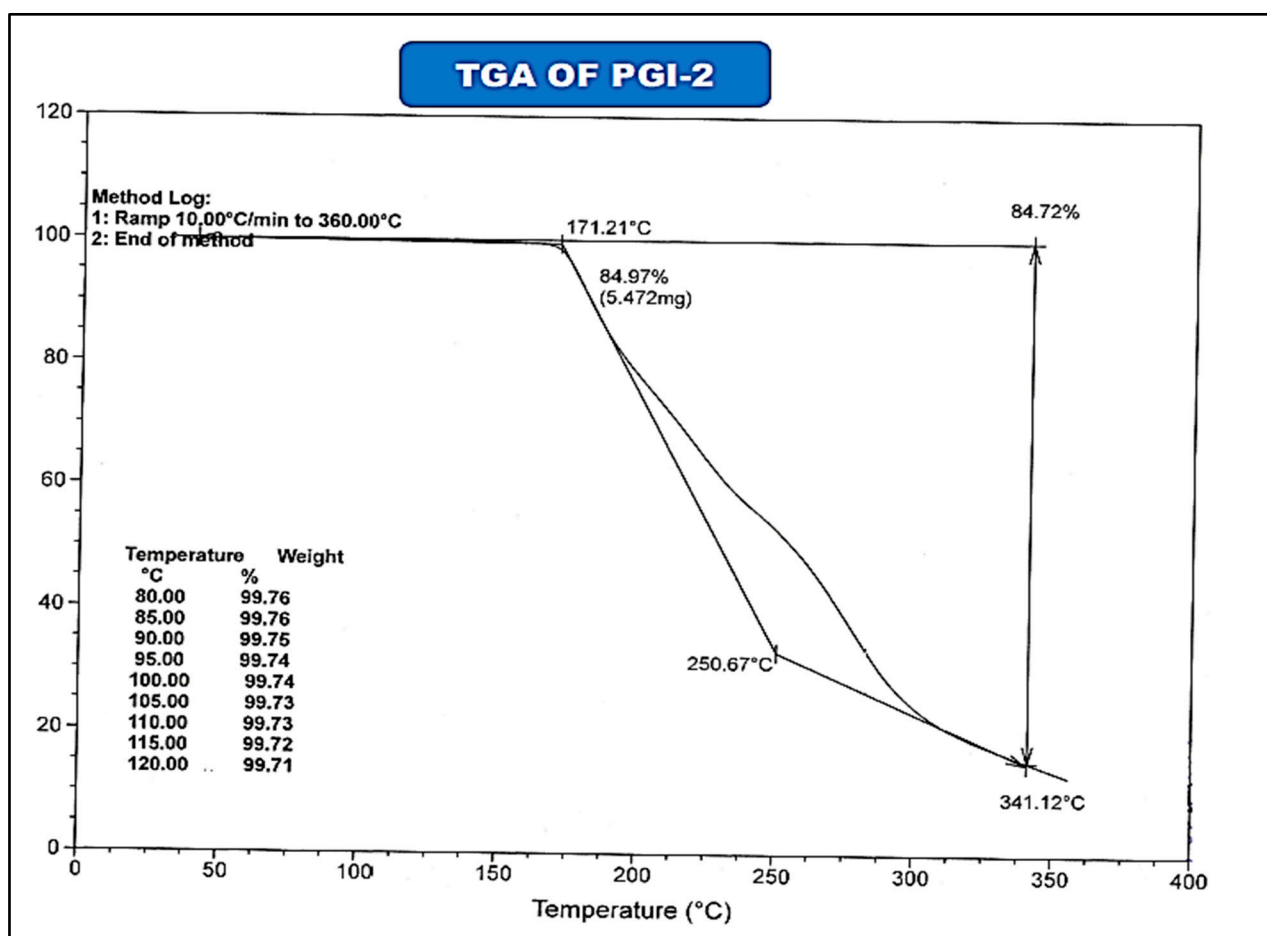
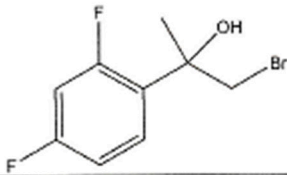


Figure S12: PGI-2 standard TGA graph.



# 1-bromo-2-(2,4- Difluoro phenyl) propan-2-ol (PGI-3) Standard Qualification Data

## CERTIFICATE OF ANALYSIS

Name of Product: 1-bromo-2-(2,4-Difluorophenyl)propan-2-ol		
Molecular weight	: 251.07	Molecular Formula : C <sub>9</sub> H <sub>9</sub> BrF <sub>2</sub> O
		
ChemicalName: 1-bromo-2-(2,4-difluorophenyl)propan-2-ol		
Sr. No.	Test	Result
1	Description	Colourless Liquid
3	Chromatographic Purity by HPLC	97.76%
4	MASS by LCMS	No Ionization
5	<sup>1</sup> H NMR	Confirms to structure
6	<sup>13</sup> C NMR	Confirms to structure
7	FT-IR	Confirms to structure
8	Loss on drying (LOD) by TGA (%W/W)	0.15%
9	Potency (%W/W)	97.61%
Conclusion: The product complies as per above specifications.		
<ul style="list-style-type: none"> <li>• Long term Storage Conditions: Store at 2 to 8° C</li> <li>• Transportation Storage Condition : Room temperature</li> <li>• Short Term storage condition : Ambient Temperature</li> </ul>		

**Figure S13:** 1-bromo-2-(2,4- Difluoro phenyl) propan-2-ol (PGI-3) standard certificate of analysis.

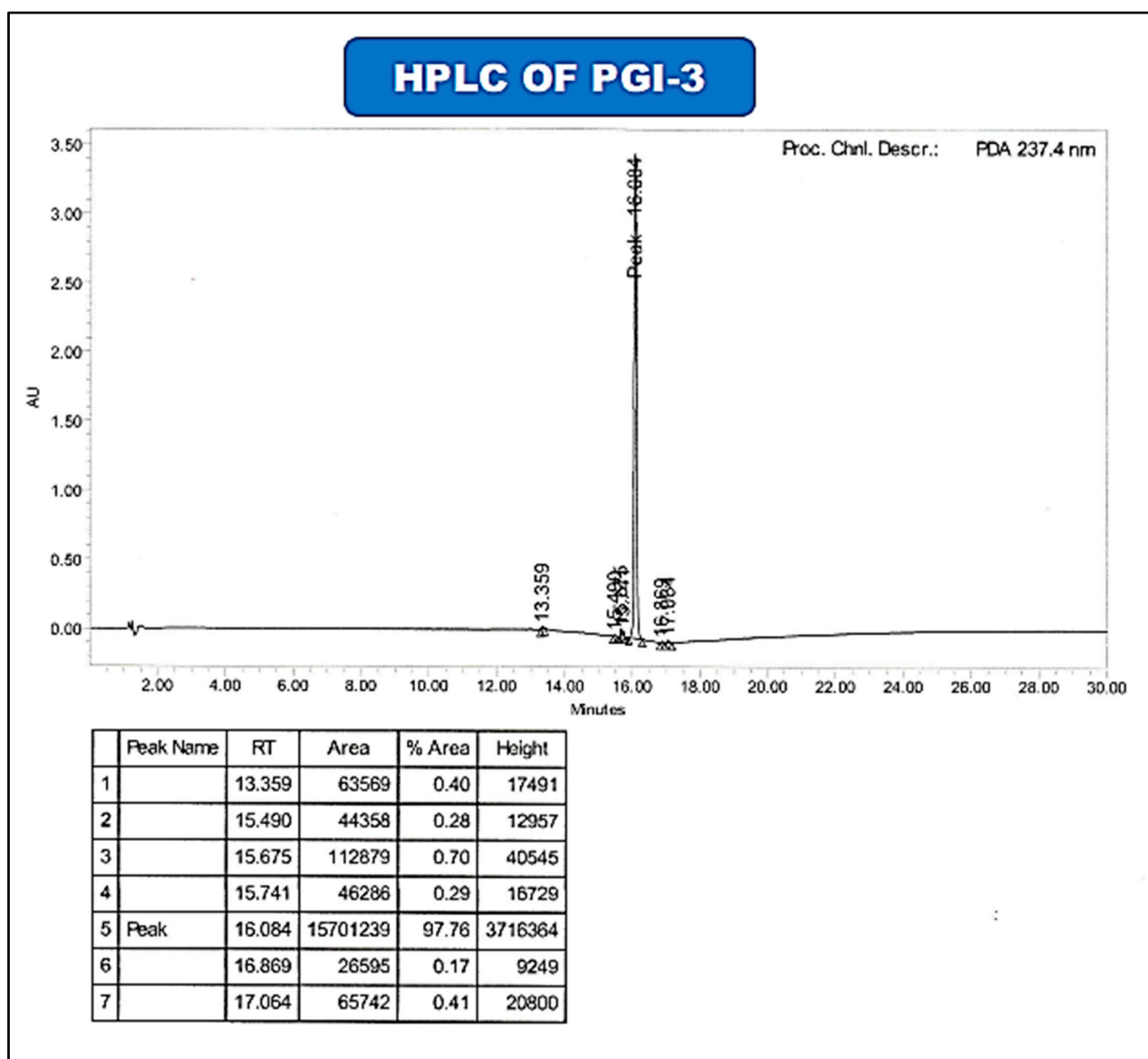


Figure S14: PGI-3 standard Purity by HPLC chromatogram.

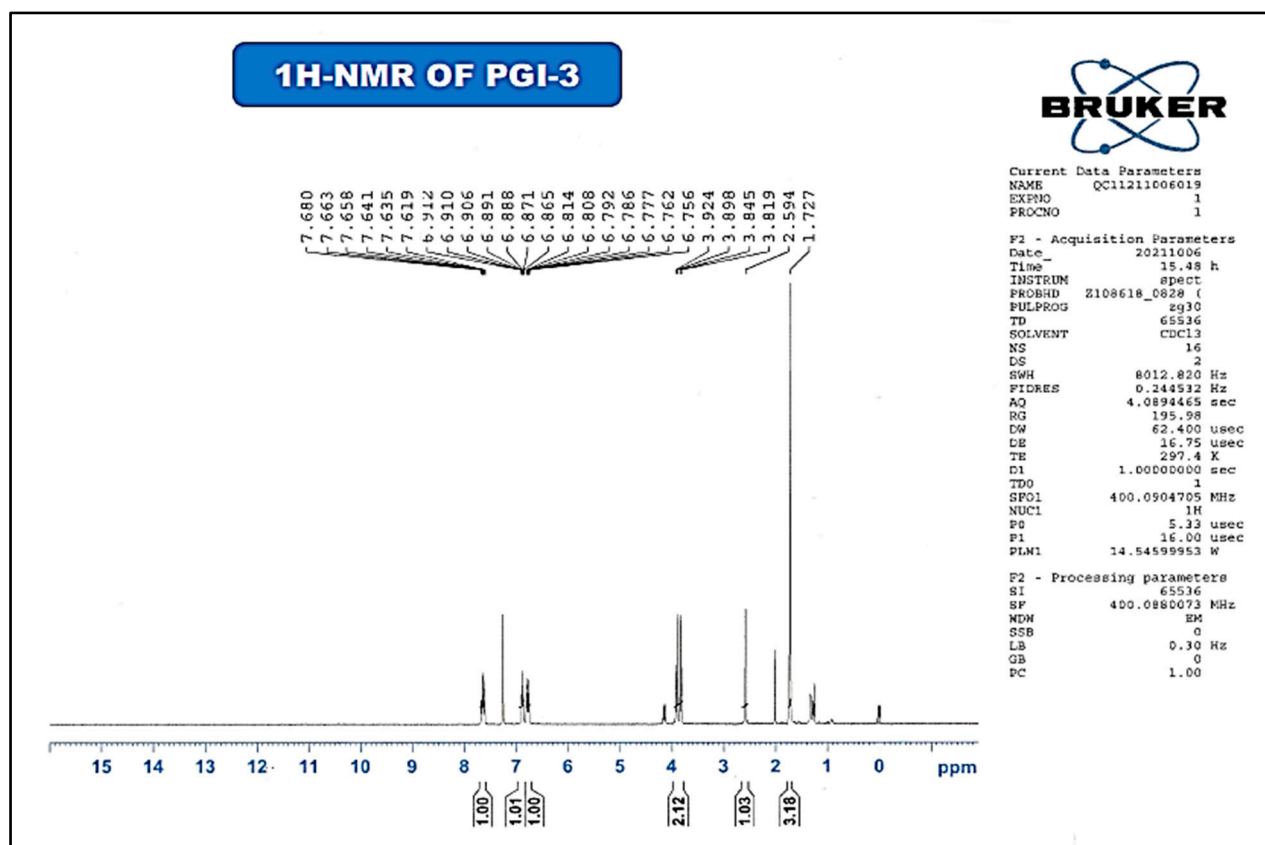


Figure S15: PGI-3 standard <sup>1</sup>H-NMR spectrum.

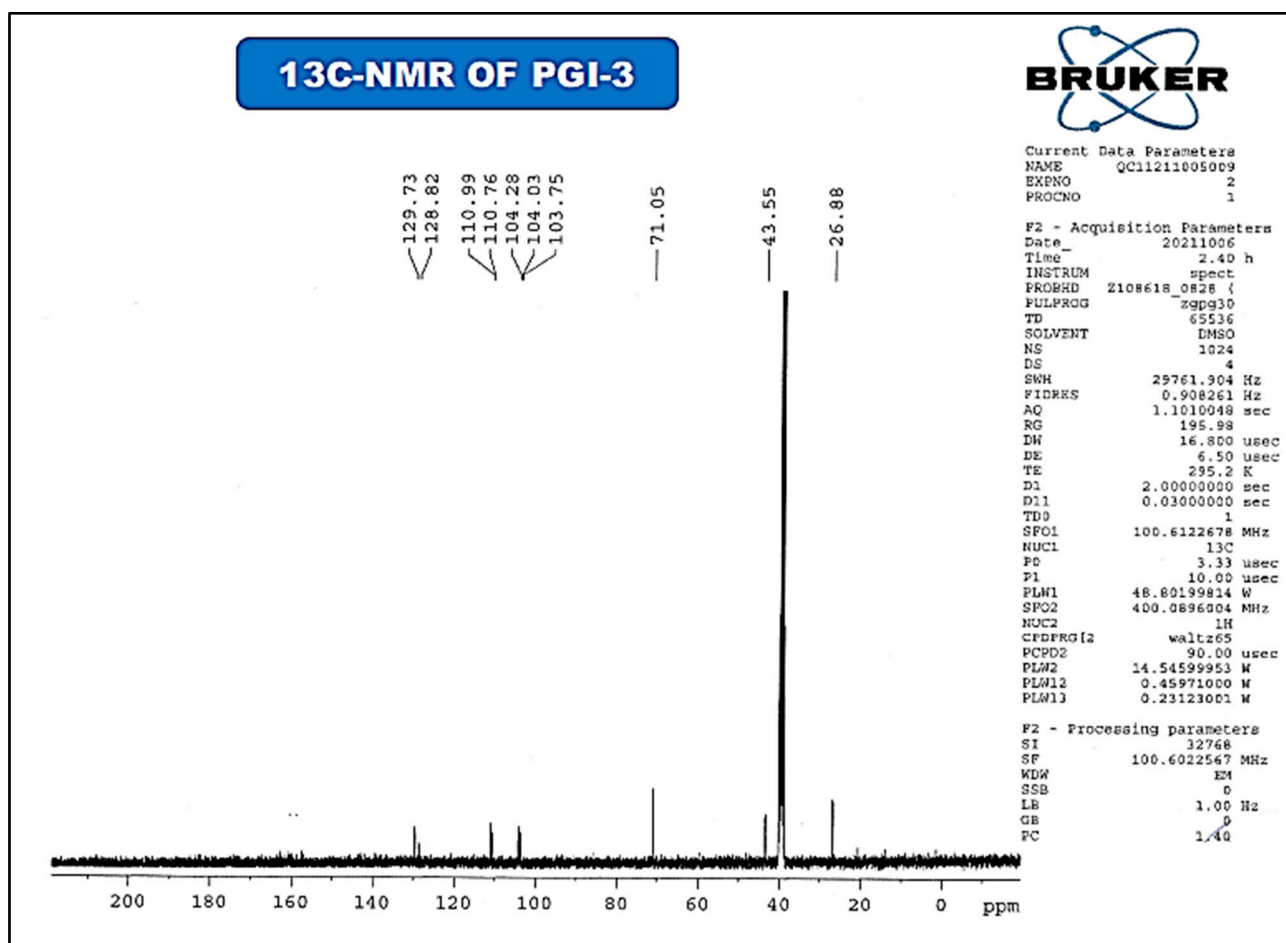


Figure S16: PGI-3 standard <sup>13</sup>C-NMR spectrum.

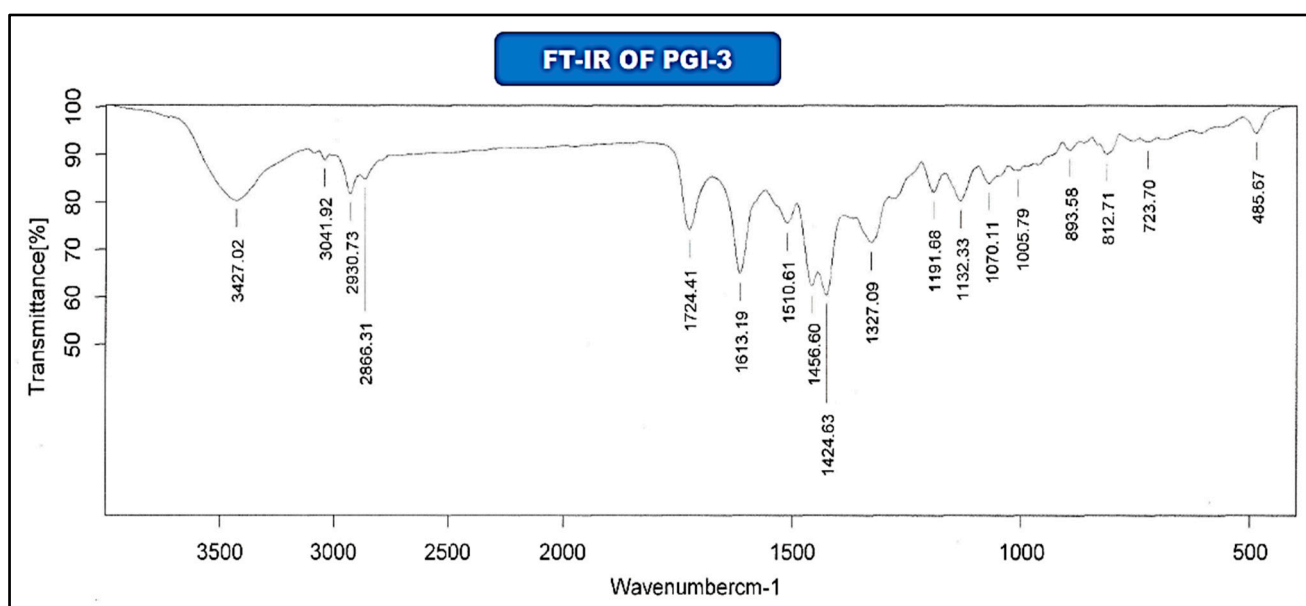


Figure S17: PGI-3 standard FT-IR spectrum.

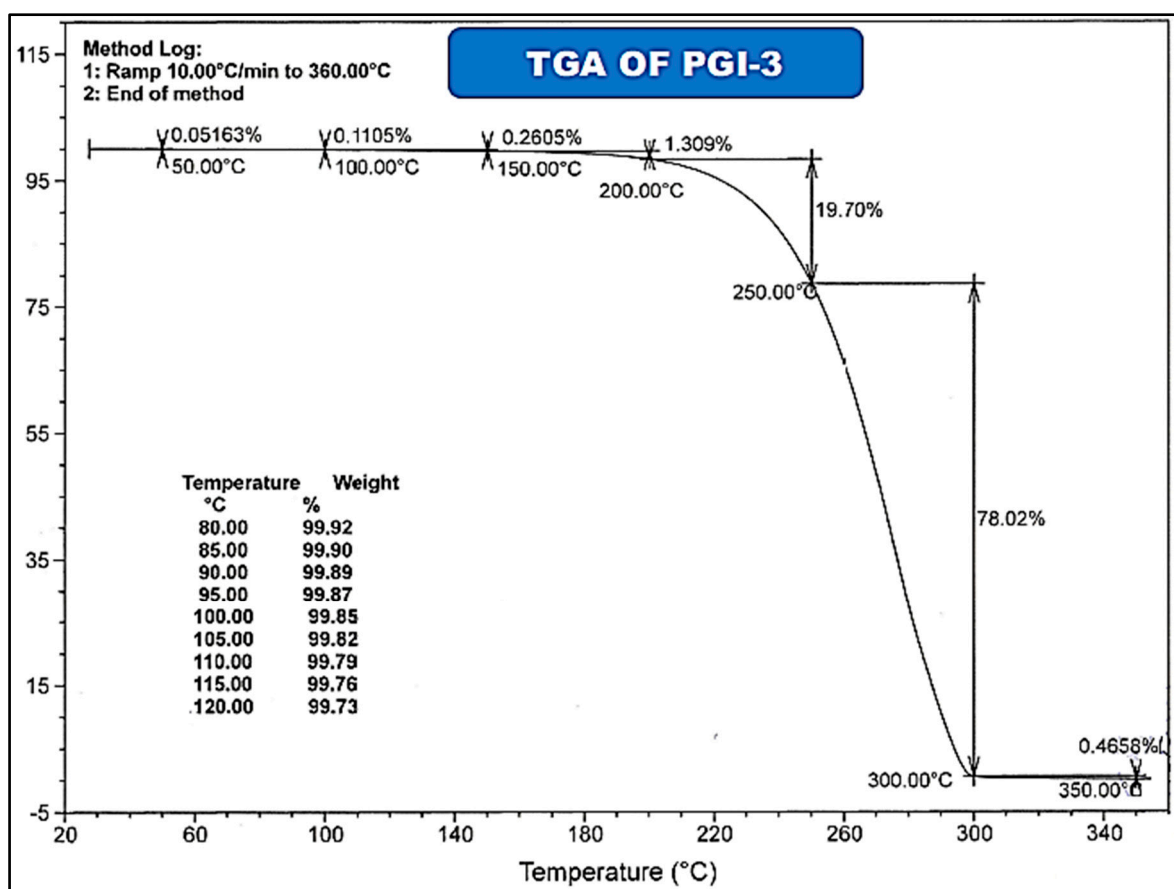


Figure S18: PGI-3 standard TGA graph.

## Method Validation Chromatograms

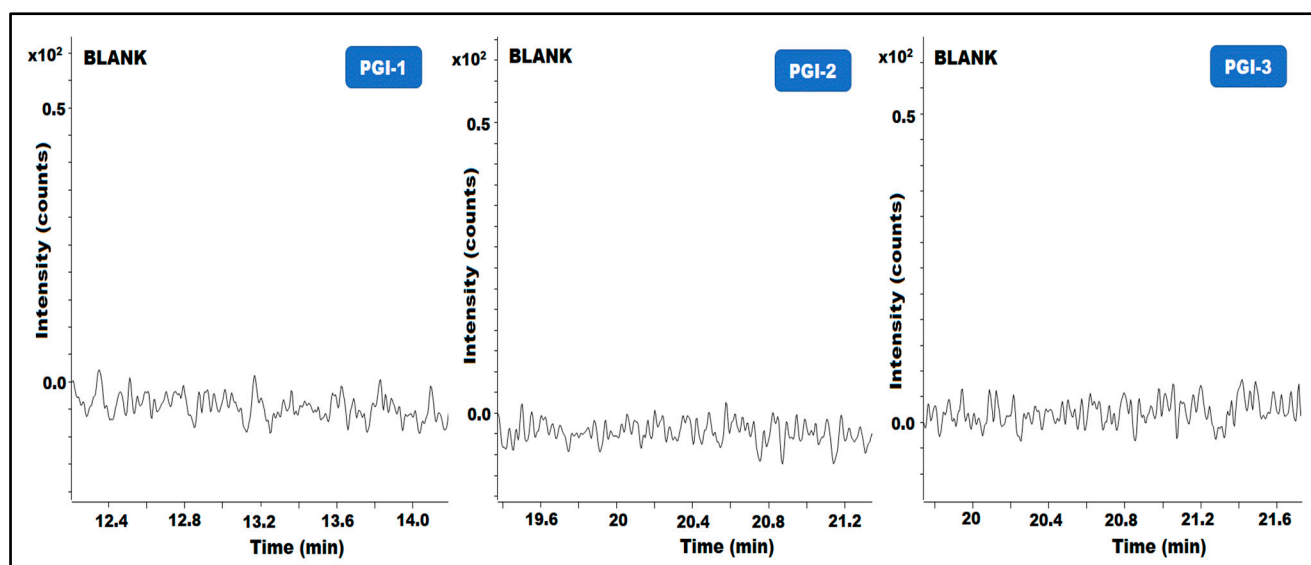


Figure S19: Blank solution chromatogram.

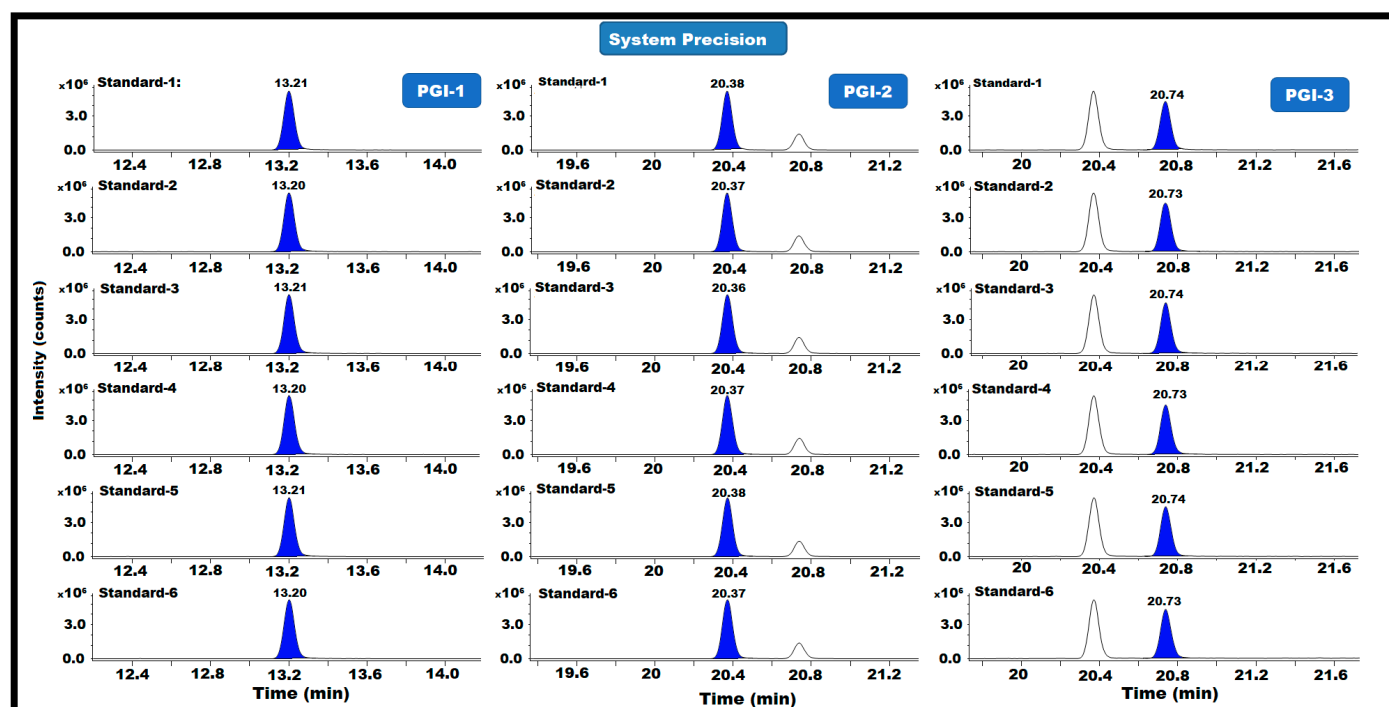


Figure S20: Standard solution chromatogram.

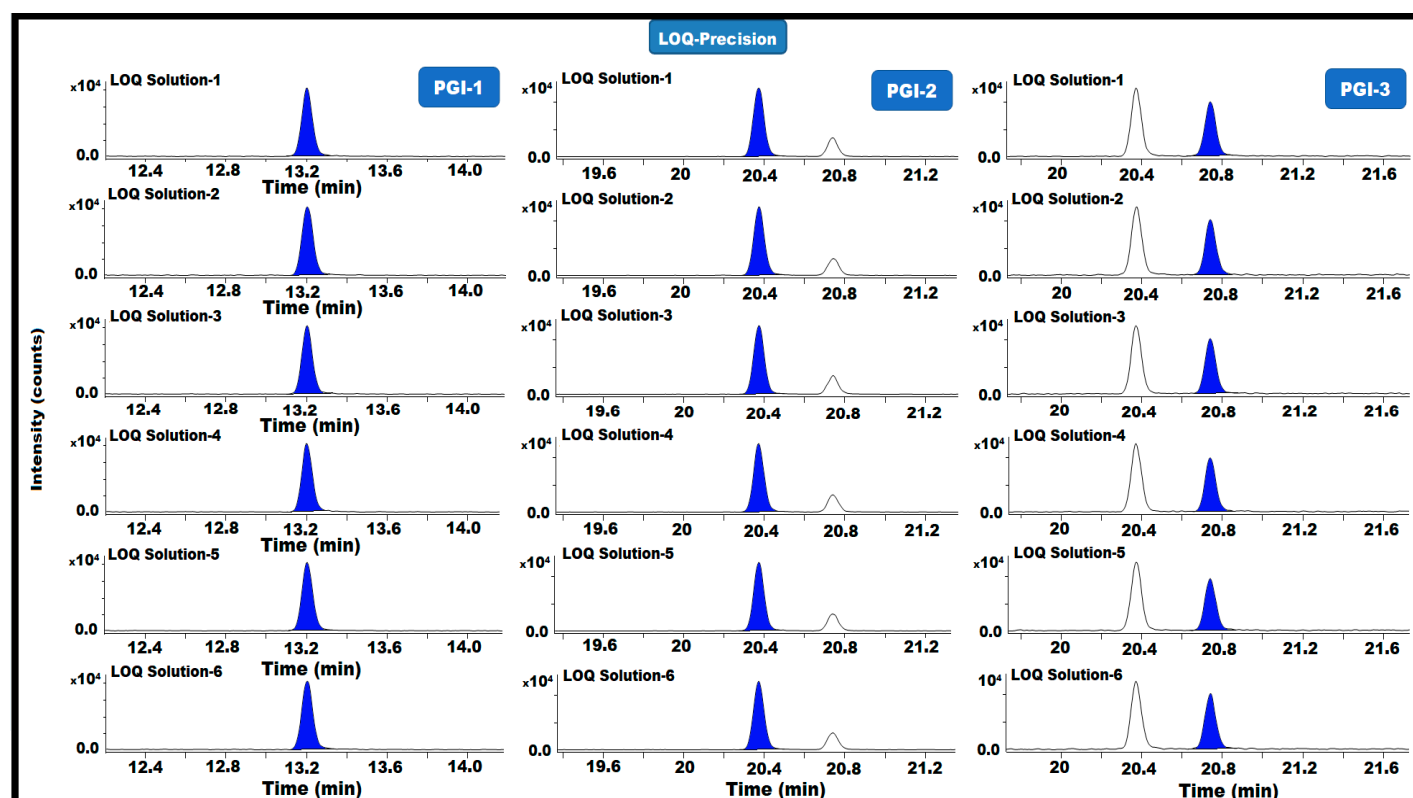


Figure S21: LOQ Precision chromatogram.

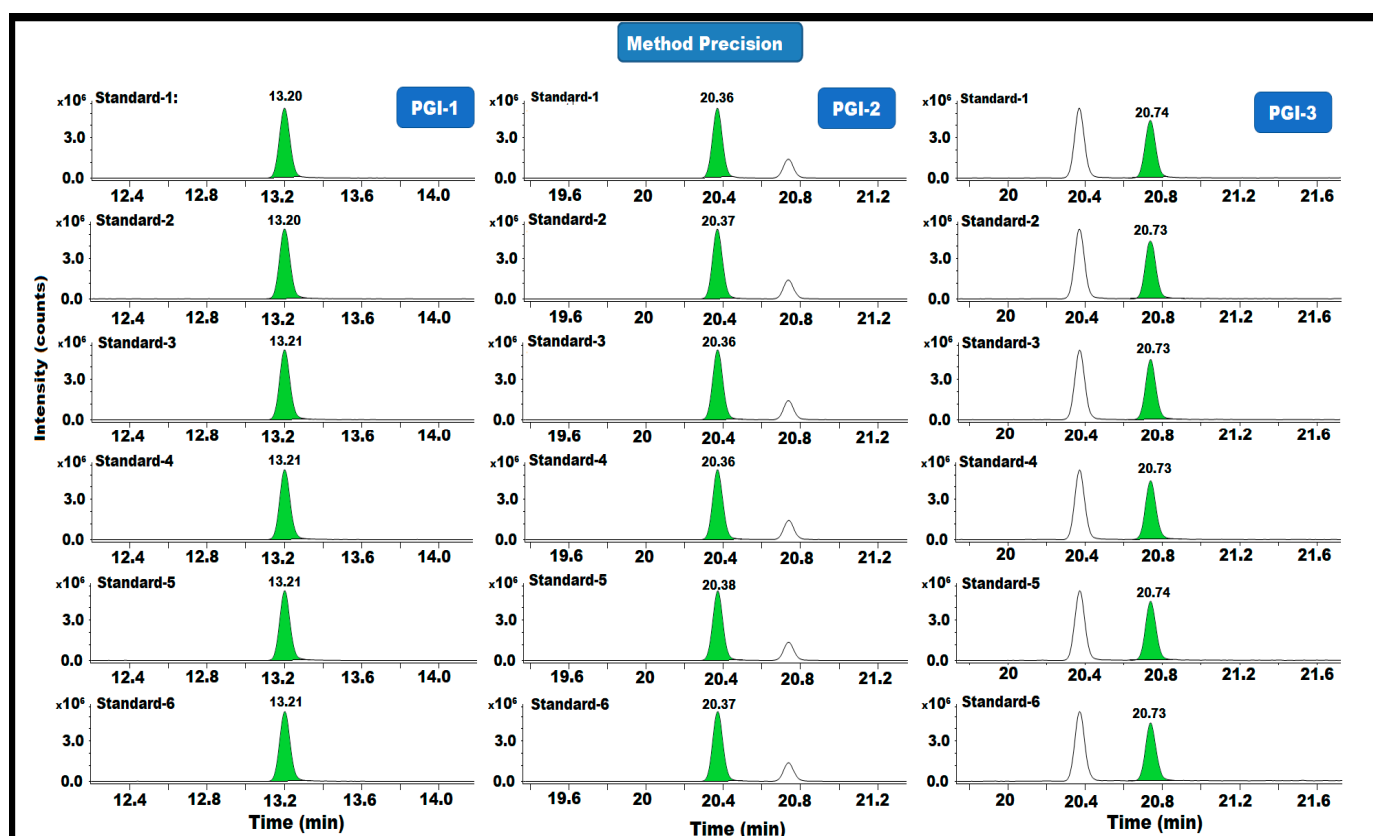


Figure S22: Method precision chromatogram.

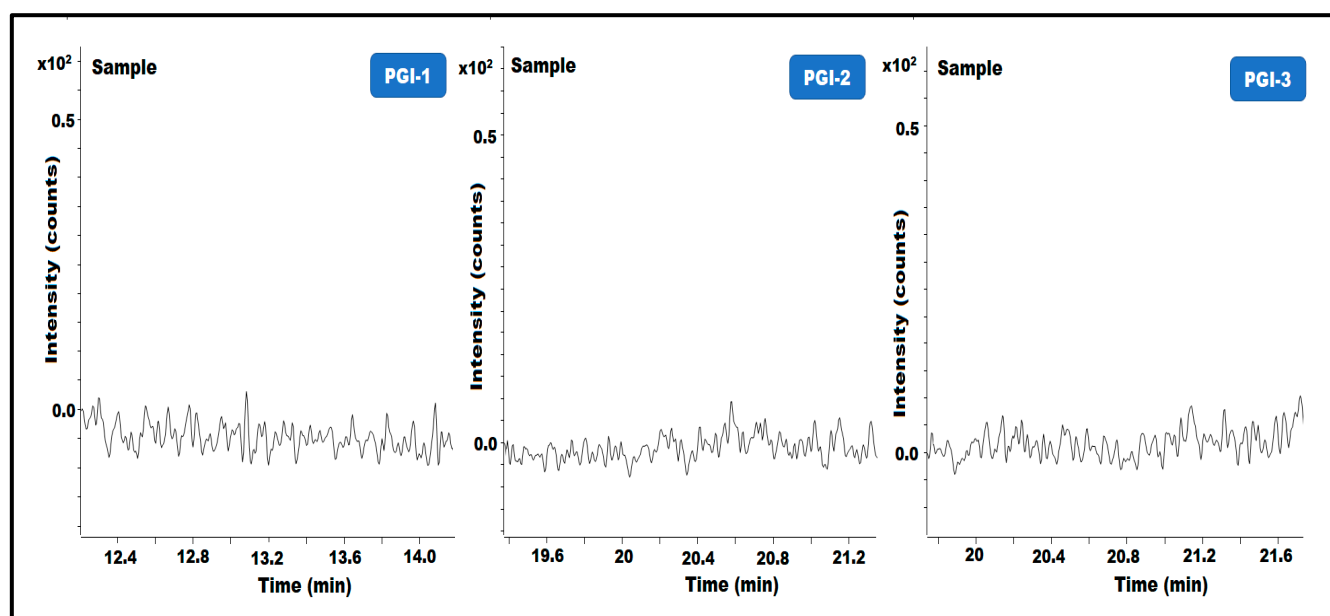


Figure S23: Sample solution chromatogram.



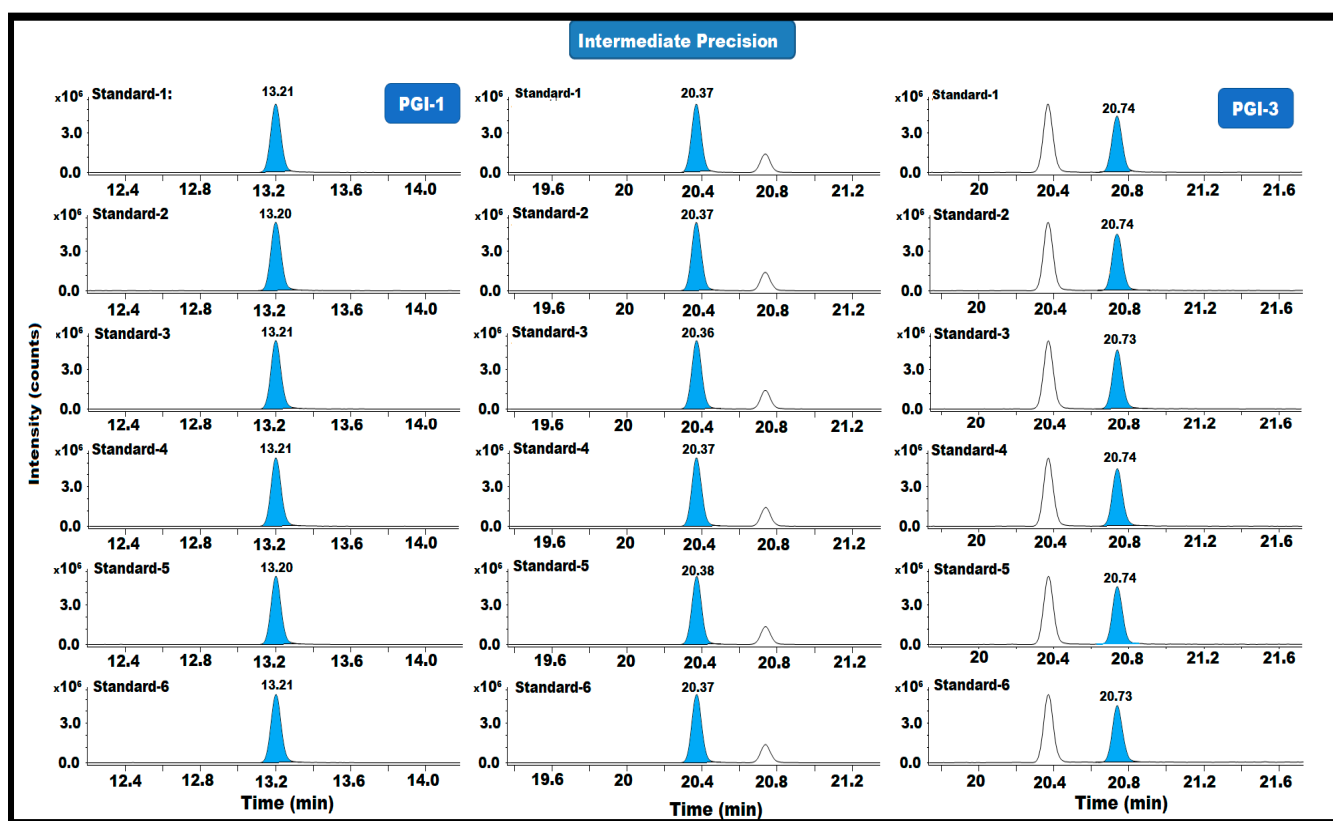


Figure S24: Intermediate precision chromatogram.

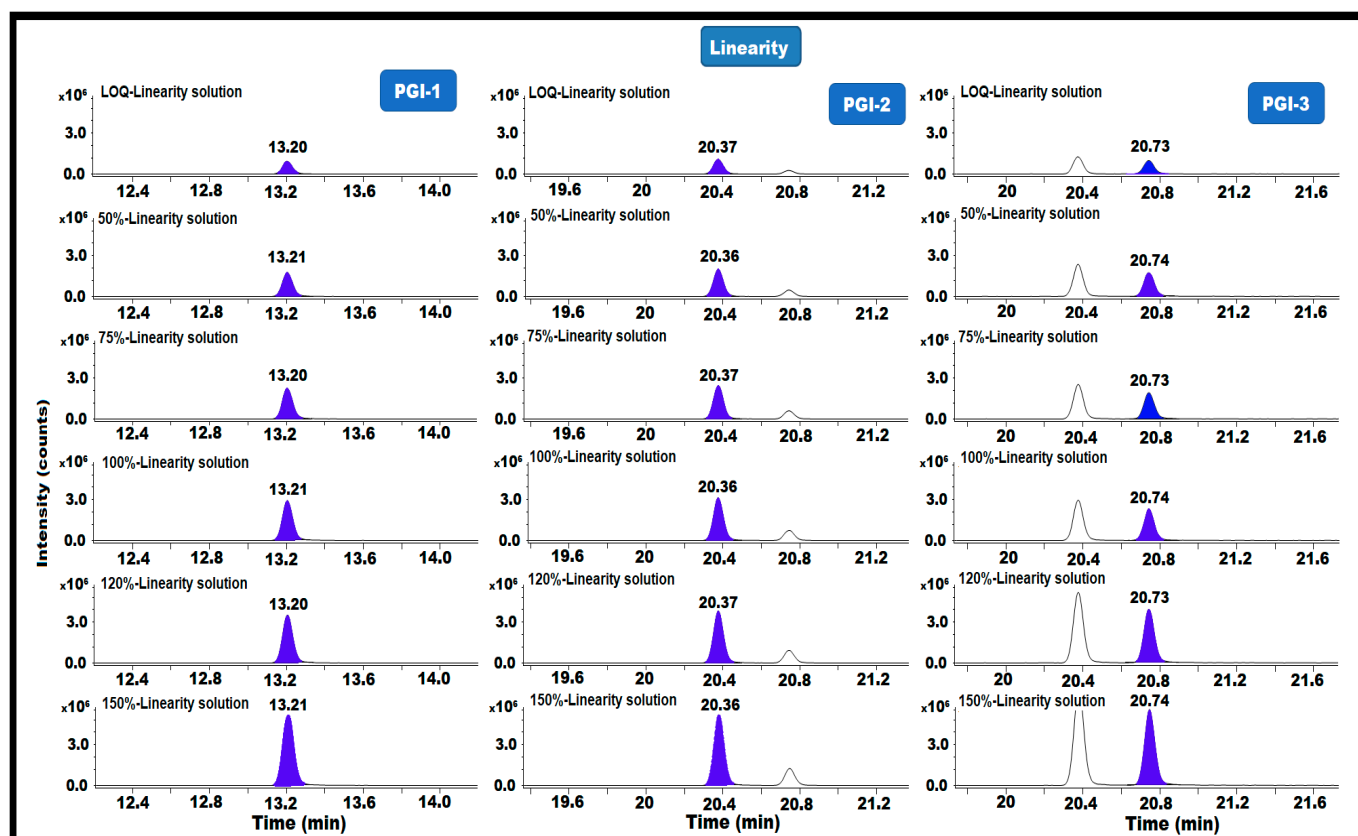
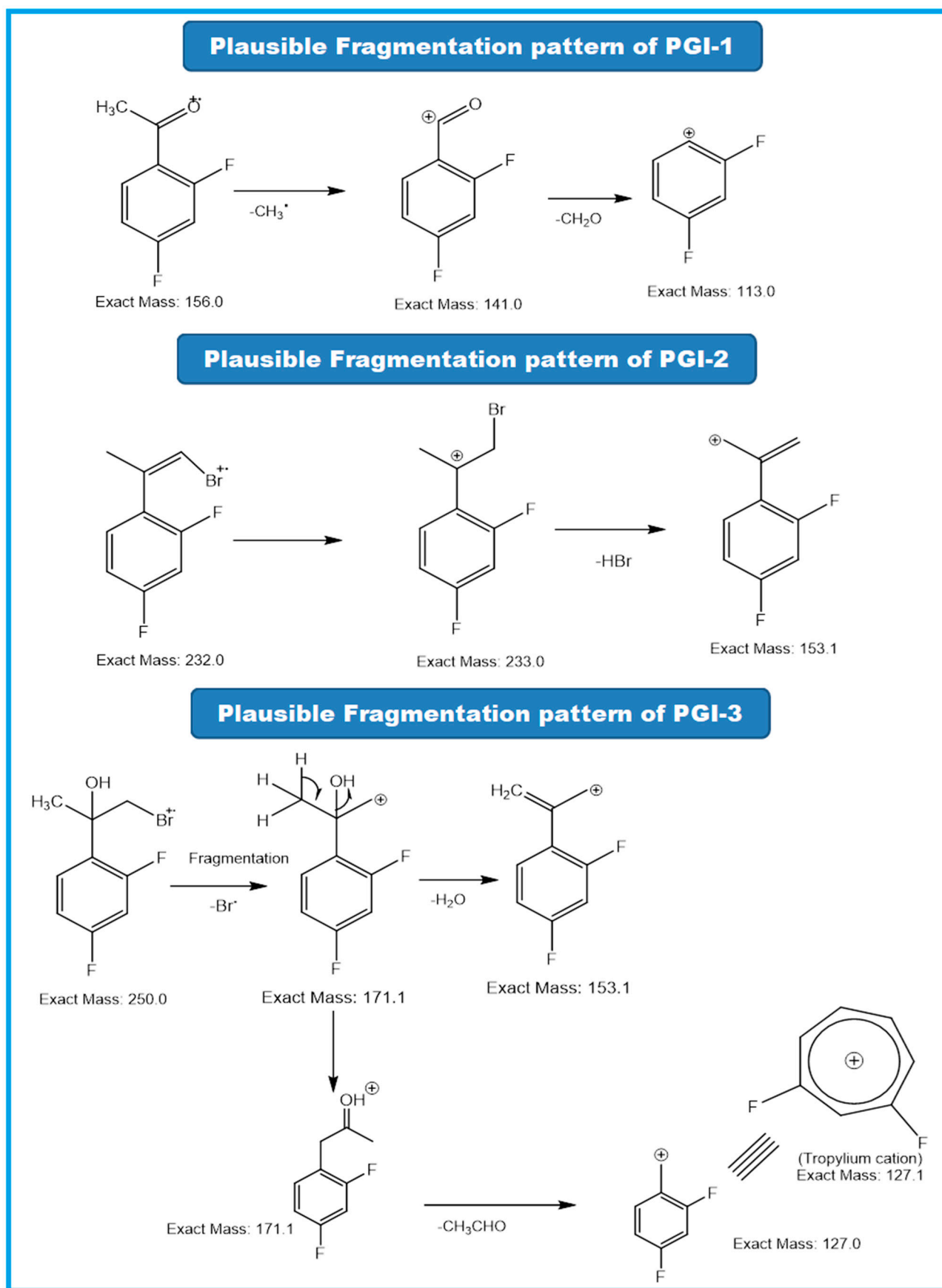


Figure S25: Linearity chromatogram.

Method Development Experiments by Using HPLC-PDA and LC-MRM-MS/MS	
Analytical Technique	Results
<p><b>HPLC-PDA :</b></p> <p>Liquid chromatography conditions:            Column: Symmetry C18 250mm x 4.6mm, 5 micron            Mobile phase A : 1 ml Formic acid in 1000ml water            Mobile phase B : Acetonitrile            Auto-sampler temperature 25 °C            Column temperature 30 °C            Flow rate: 1.0 mL/min            Injection volume: 100 µL            Gradient program (time/mobile phase B): 0/10, 20/80, 22/10, 30/10            Run time: 30 min            Note : Other conditions including different salt buffers, at different PH, column and organic solents used during the method development.</p>	<p>PGI-1,PGI-2 and PGI-2 peaks were detected at more than 100ppm concentration standard solution.</p> <p>However, the limit of targetted impurities was 0.1 ppm. Hence this technique found to be not suitable for the determination of these three Alkyl halide impurities (PGI-1,PGI-2 and PGI-3) in posocanozole</p>
<p><b>LC-MRM-MS:</b></p> <p>Column: Zorbax SB C18 100mm x 4.6mm, 3.5 micron            Mobile phase A : 1 ml Formic acid in 1000ml water            Mobile phase B : 1 ml Formic acid in 1000ml Methanol            Auto-sampler temperature 25 °C            Column temperature 30 °C            Flow rate: 0.6 mL/min            Injection volume: 50 µL            Gradient program (time/mobile phase B): 0/10, 10/90, 15/90, 17/10, 22/10            Run time: 22 min            Mass spectrometry conditions:            Source and Ionization mode: ESI- Positive            Detection mode: MRM            MS temperature: 400°C            Note : Other conditions including different salt buffers, at different PH, column and organic solents used during the method development.</p>	<p>The response of PGI-1,PGI-2 and PGI-2 peaks was very poor and observed that the response for injection to injection dropped down drastically.</p> <p>However, the limit of targetted impurities was 0.1 ppm, and LOD and LOQ should be established lower than this. Hence, this technique found to be not suitable for the determination of these three alkyl halide impurities (PGI-1,PGI-2 and PGI-3) in posocanozole</p>

**Figure S26:** Method development experiments result.



**Figure S27:** Plausible Fragmentation pattern of PGI-1, PGI-2 and PGI-3 impurities.