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Development and Characterization of a High-Purity Terpinen-4-ol Certified Reference Material by Mass Balance and qNMR

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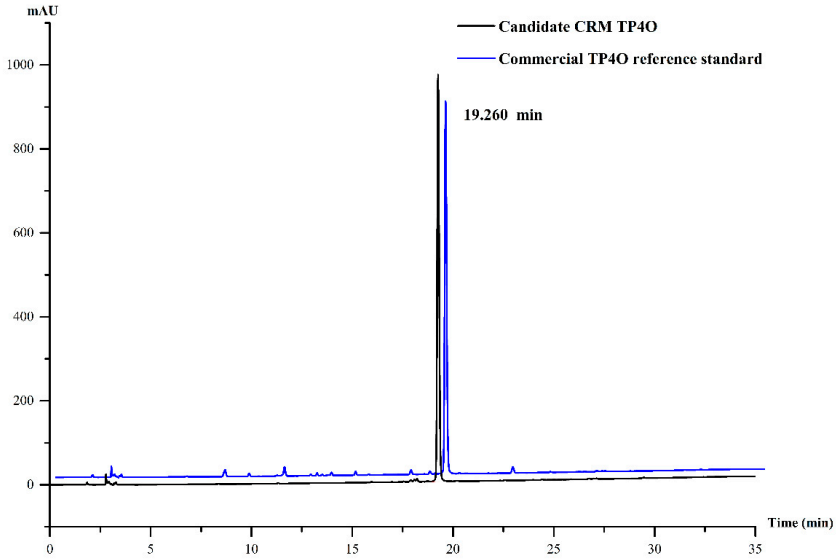


Figure S1. HPLC–PDA chromatograms of the candidate CRM TP4O (black line) and the commercial TP4O reference standard (blue line). Chromatographic separation was performed on a Chromolith® RP-18 column (150 mm x 4.6 mm). The column temperature was maintained at 35 °C, and the injection volume was 10 µL. Gradient elution was carried out using acetonitrile-water as the mobile phase at a flow rate of 0.8 mL min^{-1}. The gradient program was as follows: 0-30 min, 10-90% B; 30-40 min, 90% B; 40-42 min, 90-10% B; and 42-45 min, 10% B. Detection was performed at 205 nm. Both samples were analyzed at a concentration of 1000 mg L^{-1} under identical chromatographic conditions. The inset shows an expanded view of the main peak region, highlighting the identical retention time (19.26 min) and peak profile of TP4O in both materials.

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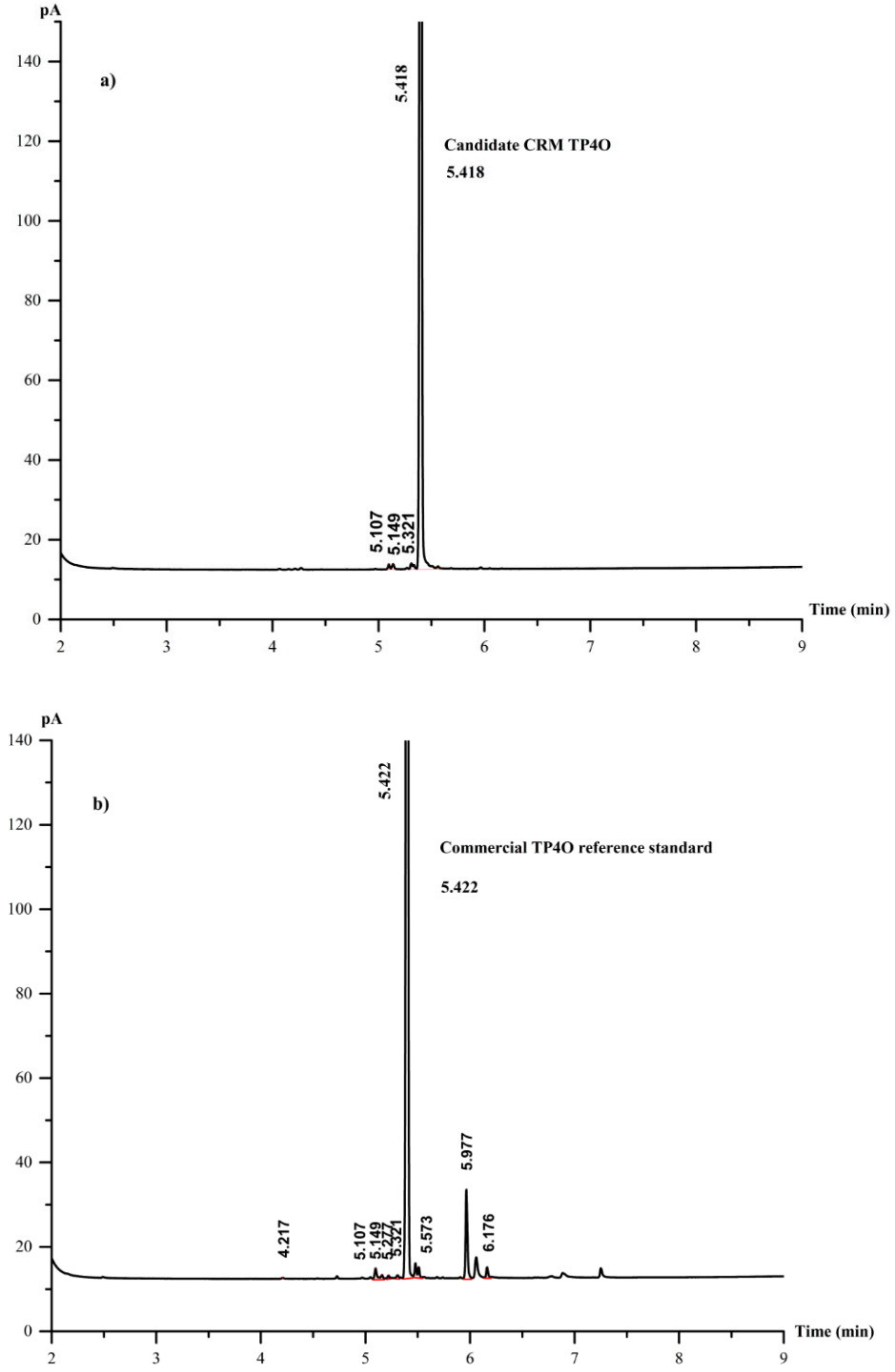


Figure S2. (a) GC-FID chromatogram of a candidate CRM TP4O, (b) GC-FID chromatogram of the commercial TP4O reference standard, showing comparable retention behavior with retention times of 5.418 and 5.422 min, respectively, under identical chromatographic conditions. All chromatograms were obtained using an HP-5MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). The oven temperature program was set at 80 °C for 1 min, followed by a ramp to 250 °C at 20 °C min^{-1}. Samples were injected in split mode (split ratio 1:38.8). The inlet and detector temperatures were maintained at 230 °C.

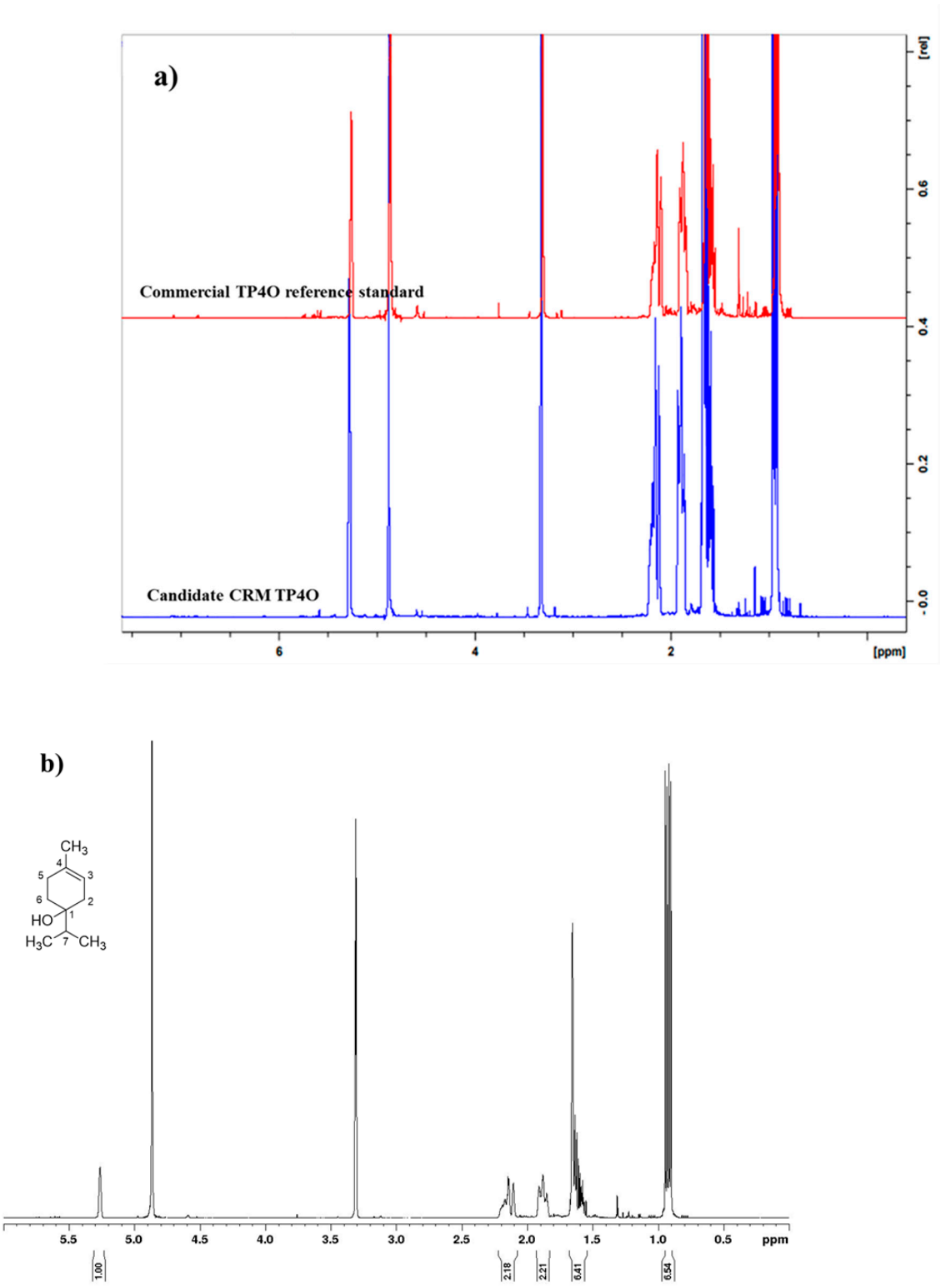


Figure S3. (a) ^{1}H NMR spectra (500 MHz) of the commercial TP4O reference standard (red line) and the candidate CRM TP4O (blue line) in MeOD. (b) ^{1}H NMR spectrum (500 MHz) of the candidate CRM TP4O in MeOD.

As shown in Figure S3b, the ^{1}H NMR chemical shifts of TP4O are detailed as follows: ^{1}H NMR (MeOD) δ 5.29-5.30 (m, 1H, olefinic H), 2.20-1.55 (m, aliphatic CH and CH_2), 1.55 (s, CH_3), and 0.94-0.92 (d, 6H, CH_3 of the isopropyl group).

Table S1. Trend analysis for packing order.

Slope (b_1)	SD of slope ($s(b_1)$)	$t_{b1} = \frac{\lvert b_1 \rvert}{s(b_1)}$	t_{crit}	Result	
0.00000007	0.00001735	0.00391	2.30600	$t_{b1} < t_{crit}$	No significant trend observed

Table S2. ANOVA analysis of homogeneity test results.

Parameters	Values
Mean square between bottle (s_1^2)	3.056
Mean square within bottle (s_2^2)	9.532
F (s_1^2/s_2^2)	0.321
$F_{0.05}$ (9,20)	2.393
Conclusion	$F < F_{0.05}$(9,20)

Table S3. Short-term stability study results of TP4O CRM stored at 45 °C.

Parameters	Results
slope (b_1)	-0.015
standard deviation of the slope s(b_1)	0.003
$t_{b1} = \frac{\lvert b_1 \rvert}{s(b_1)}$	4.942
$t_{0.95,n-2}$	12.706
Conclusion	$/b_1/ < t_{0.95,n-2} * s(b_1)$, No trend observed

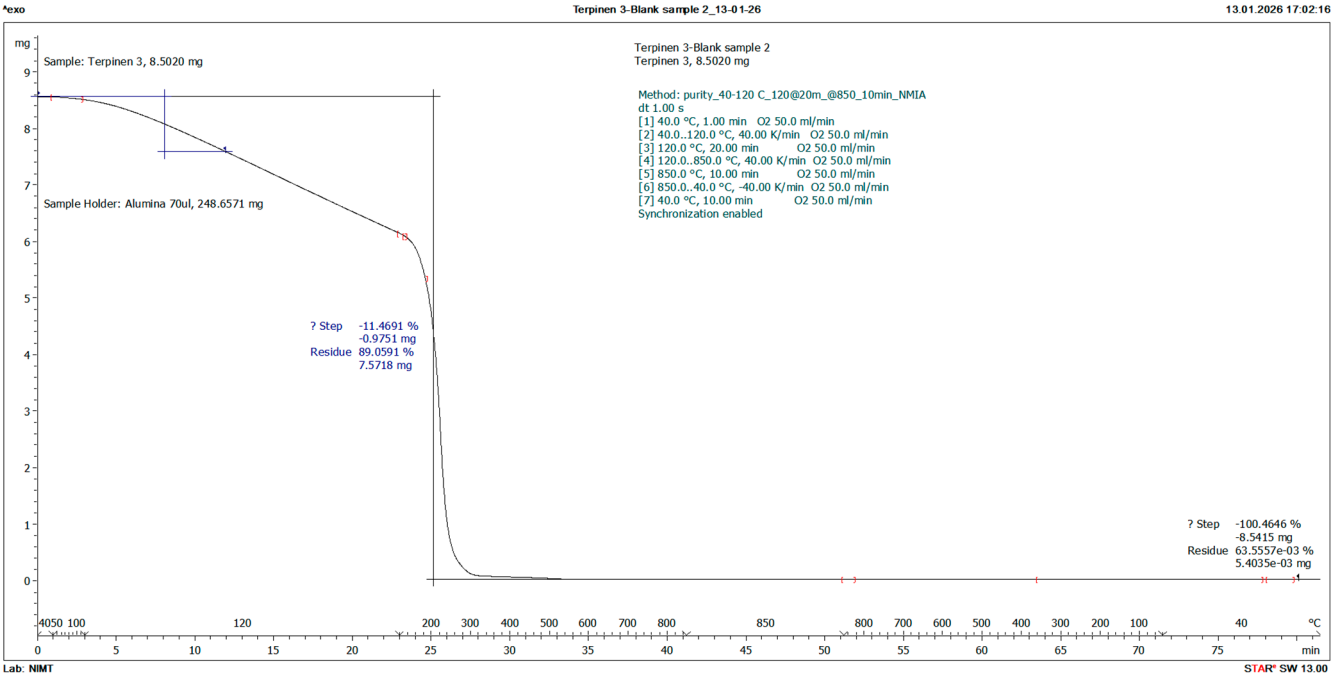


Figure S4. Thermogravimetric (TGA) thermogram of TP4O.

Table S4. Comparison of purity from the MB approach and qNMR technique.

	Technique	Mean purity (%)	Combined standard uncertainty (%)
1	MB	98.41%	0.09%
2	qNMR	99.13%	0.94%
Average purity value (%)		98.77%	0.95%
Statistical test		***t*-test: $p > 0.05$**	**The values are statistically equivalent**

Table S5. Combined uncertainty of purity of TP4O using qNMR.

Uncertainty source	Value	Standard uncertainty	Relative uncertainty (%)
I_x/I_{std}	0.80	0.007	0.8149
N_x	1.00	0.00	0.0000
N_{std}	9.00	0.00	0.0000
M_x	154.25	0.0019	0.0012
M_{std}	224.36	0.0052	0.0023
m_{std}/m_x	0.22	0.00075	0.3473
P_{std}	0.923	0.0030	0.3250
P_{qNMR} (%)	**99.13**	**0.94**	

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