

Research article

Simultaneous analytical determination of methyl salicylate and thymol in selected Malaysian traditional medicines

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Supplementary

Table S1. SST requirements [7].

Parameter	Acceptance Criteria
System precision	% RSD of standard retention time $\leq 1\%$ % RSD of standard peak area $\leq 1\%$ % RSD of standard peak height $\leq 1\%$
Theoretical plate count	≥ 2000
Tailing factor	≤ 2
Resolution	≥ 2

Table S2. Regression analysis for methyl salicylate and thymol.

Parameter	Methyl Salicylate	Thymol
Coefficient of determination, R^2	0.9994	0.9994
Slope, b	14.1120	6.2734
Slope standard deviation, S_b	0.0898	0.0376
Intercept, a	-6.8308	-9.1750
Intercept standard deviation, S_a	8.7282	3.5878
Residual standard deviation, S_{res}	16.7477	7.8599

Table S3. Confirmation test of linearity (methyl salicylate).

Sources of variation	Sum of squares	Degree of freedom	Mean squares	Fisher ratio	F critical value
Regression	6919152.0210	1	6919152.0210	28008.93	4.75
Lack-of-fit	1523.3658	4	380.8415	1.54	3.26
Pure error	2964.4054	12	247.0338		

Table S4. Confirmation test of linearity (thymol).

Sources of variation	Sum of squares	Degree of freedom	Mean squares	Fisher ratio	F critical value
Regression	1723871.456	1	172381.456	21635.76	4.75
Lack-of-fit	32.3198	4	8.08	0.1	3.26
Pure error	956.1237	12	79.677		

Table S5. Confirmation of y-intercept.

Parameter	Methyl Salicylate	Thymol
t_{cal}	0.783	2.557
t_{crit}	2.776	2.776

Table S6. Confirmation specificity.

Parameter	Methyl salicylate (liniment)	Methyl salicylate (ointment)	Thymol (liniment)	Thymol (ointment)
t_1	0.3342	1.2341	2.5593	3.6226
t_2	0.8951	0.1235	0.2473	0.0987
t_{crit}	9.9248	9.9248	9.9248	9.9248

Table S7. Test for matrix effect (methyl salicylate).

Parameter	Liniment		Ointment	
	Solvent (Methanol)	Sample matrix (Liniment)	Solvent (Methanol)	Sample matrix (Ointment)
Slope, b	0.0026	0.0027	0.0026	0.0025
Standard deviation, S	0.0026	0.0099	0.0026	0.0026
Residual variance, S^2_{res}	0.0000067	0.000098	0.0000067	0.0000069
F_{cal}	0.0682		0.9721	
F_{crit}	5.0503		5.0503	
t_{cal}	0.0008		0.0112	
t_{crit}	2.306		2.3060	

Table S8. Test for matrix effect (thymol).

Parameter	Liniment		Ointment	
	Solvent (Methanol)	Sample matrix (Liniment)	Solvent (Methanol)	Sample matrix (Ointment)
Slope, b	0.00074	0.00076	0.00074	0.00077
Standard deviation, S	0.00057	0.0012	0.00057	0.00064
Residual variance, S^2_{res}	0.00000032	0.0000014	0.00000032	0.00000042
F_{cal}	0.2248		0.7803	
F_{crit}	5.0503		5.0503	
t_{cal}	0.0009		0.003	
t_{crit}	2.306		2.306	

Table S9. Repeatability data for methyl salicylate.

Parameter	Liniment			Ointment		
	24.74	79.81	150.05	24.74	79.81	150.05
	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	24.59	79.92	152.69	24.49	82.52	154.81
Standard deviation	0.40	0.98	0.30	0.07	0.33	0.28
RSD (%)	1.65	1.23	0.20	0.30	0.40	0.18
95% CI	1.45	3.52	1.07	0.27	1.19	1.00

Table S10. Repeatability data for thymol.

Parameter	Liniment			Ointment		
	14.97	74.87	149.75	14.97	74.87	149.75
	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	15.47	75.60	154.53	15.09	72.25	154.16
Standard deviation	0.25	0.35	0.18	0.09	0.15	0.88
RSD (%)	1.63	0.46	0.12	0.62	0.20	0.57
95% CI	0.90	1.25	0.65	0.34	0.52	3.15

Table S11. Intermediate precision data for methyl salicylate.

Parameter	Liniment			Ointment		
	24.80	80.01	150.41	24.80	80.01	149.85
	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	26.98	81.48	147.54	25.91	81.42	146.73
Standard deviation	0.07	0.20	1.34	0.05	0.14	0.49
RSD (%)	0.27	0.24	0.91	0.20	0.18	0.34
95% CI	0.26	0.71	4.80	0.18	0.51	1.77

Table S12. Intermediate precision data for thymol.

Parameter	Liniment			Ointment		
	15.03 $\mu\text{g/mL}$	75.15 $\mu\text{g/mL}$	150.30 $\mu\text{g/mL}$	15.03 $\mu\text{g/mL}$	75.15 $\mu\text{g/mL}$	149.90 $\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	16.48	75.13	146.53	17.53	75.18	149.54
Standard deviation	0.03	0.07	1.24	0.05	0.05	0.28
RSD (%)	0.20	0.10	0.84	0.29	0.06	0.19
95% CI	0.12	0.26	4.43	0.18	0.17	1.00

Table S13. Recovery results for methyl salicylate.

Parameter	Liniment			Ointment		
	24.74 $\mu\text{g/mL}$	79.81 $\mu\text{g/mL}$	150.05 $\mu\text{g/mL}$	24.74 $\mu\text{g/mL}$	79.81 $\mu\text{g/mL}$	150.05 $\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	99.39	100.13	101.76	98.98	103.39	103.17
Standard deviation	1.64	1.23	0.20	0.30	0.42	0.19
RSD (%)	1.65	1.23	0.20	0.30	0.40	0.18
95% CI	5.86	4.42	0.71	1.08	1.49	0.67

Table S14. Recovery results for thymol.

Parameter	Liniment			Ointment		
	14.97 $\mu\text{g/mL}$	74.87 $\mu\text{g/mL}$	149.75 $\mu\text{g/mL}$	14.97 $\mu\text{g/mL}$	74.87 $\mu\text{g/mL}$	149.75 $\mu\text{g/mL}$
Mean ($\mu\text{g/mL}$)	104.18	100.98	103.19	100.81	96.50	102.95
Standard deviation	0.35	0.47	0.12	0.63	0.19	0.59
RSD (%)	0.34	0.46	0.12	0.62	0.20	0.57
95% CI	1.26	1.68	0.43	2.25	0.69	2.10



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