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Research article

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

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Abstract: Various topical formulations comprise of methyl salicylate and thymol due to their analgesic and anti-inflammatory properties. The huge demand has led traditional medicines being susceptible to adulteration with synthetic drugs or their analogues to enhance their efficacy and to minimise the cost of obtaining the limited natural substance. The objectives of this study are to analyse a suitable analytical method for simultaneous determination of methyl salicylate and thymol in selected Malaysian traditional medicines using High Performance Liquid Chromatography (HPLC) and to screen the selected Malaysian traditional medicines for possible methyl salicylate and thymol adulteration using the analytical method. Most literature search showed the determination of methyl salicylate and thymol as an individual compound or in combination with other compounds instead of both being detected simultaneously using a single method. Methyl salicylate and thymol were separated at about 3.8 and 6.2 min, respectively at a flow rate of 1 mL/min on C8 column with methanol and water (65: 35) as the mobile phase, column temperature of 35 °C and detector wavelength of 230 nm within 9 minutes of run time. Method validation was conducted and this method was sensitive, linear, specific, precise and accurate. The validated method was applied for screening of methyl salicylate and thymol in 10 samples of liniment and ointment. Half of the samples were detected with methyl salicylate and none with thymol. Majority of the positive samples were unregistered traditional medicines. As quantitation of both compounds is achievable with this method, it will be beneficial in the regulatory and industrial settings. This will ensure the safety and quality of traditional medicines, thereby safeguarding consumer's health. Hence, the method can be adopted for routine quality control (QC) analysis of methyl salicylate and thymol in traditional medicines.

Keywords: methyl salicylate; thymol; HPLC; simultaneous; traditional medicines

1. Introduction

Musculoskeletal injuries and rheumatic disorders have seen patients turning to over-the-counter (OTC) medicines for pain relief. This is mainly due to the inability of common treatment modalities such as opioids and surgical intervention to offer long term benefit to the patients. These OTC medicines consist of either single or multiple compounds that provide pain relief owing to their local analgesic action and anti-inflammatory property [1]. Various topical formulations comprise of methyl salicylate and thymol due to the aforementioned properties [2].

Methyl salicylate is a naturally occurring compound in wintergreen (*Gaultheria procumbens*) and sweet birch (*Betula lenta*). Following topical application, methyl salicylate will be metabolised to salicylic acid and produces its effects by the resulting vasodilation, enhancing the blood flow and increasing the temperature of the tissues [1]. Thymol can be found in abundance in any plants of the genus *Thymus* such as common thyme (*Thymus vulgaris*). Besides exhibiting analgesic and anti-inflammatory activities, thymol also demonstrates antimicrobial and wound healing properties [3].

Globally, the usage of traditional medicines has seen remarkable growth due to their alleged health benefits. Locally, the demand for traditional medicines is also on the rise as the annual sales of traditional medicines from 2000 to 2005 experienced almost a 5-fold increase to reach RM 4.5 billion. Majority of Malaysians were also found to be utilising traditional medicine throughout their lives [4]. The huge demand has led traditional medicines being susceptible to adulteration and contamination with synthetic drugs or their analogues to enhance their efficacy and to minimise the cost of obtaining the limited natural substance. Various reports found traditional medicines to be adulterated with anti-obesity agents, phosphodiesterase-5 inhibitors and non-steroidal anti-inflammatory drugs, consequently producing numerous adverse events upon chronic use [5].

Most literature search showed the determination of methyl salicylate and thymol as an individual compound or in combination with other compounds in traditional medicines instead of both being detected simultaneously using a single method. Hence, this method can be adopted for routine QC analysis of both compounds in traditional medicine. Therefore, in this study, a suitable analytical method for simultaneous determination of methyl salicylate and thymol using HPLC were analysed and later used for screening of possible methyl salicylate and thymol adulteration in selected Malaysian traditional medicines.

2. Materials and method

2.1. Reagent

The reference standards of methyl salicylate (purity: 97%) and thymol (purity: 98%) were obtained from Toronto Research Chemicals (TRC) Inc., Canada. HPLC grade methanol was supplied by Fisher Chemical. Ultrapure water was utilised throughout the study.

2.2. Instrumental conditions

This study were performed using an Agilent 1260 Infinity Quaternary Pump. For samples and standards, the injection volume was 5 µL. The mobile phase composition was 65% methanol: 35% water in isocratic mode. The separation was conducted using a Zorbax Eclipse Plus C8, 4.6 mm × 150 mm, 5 µm column at a flow rate of 1 mL/min. The column oven temperature was set at 35 °C and the detector wavelength setting was 230 nm. The run time of the analysis was 9 minutes.

2.3. Standard preparation

100 mg of weighed methyl salicylate and thymol reference standards were each transferred into a 25 mL volumetric flask. The flask content was dissolved and made up to volume by methanol. Then, 1 mL of the resulting solution from both volumetric flasks were pipetted into a 10 mL volumetric flask and made up to volume using methanol. The resulting concentration of both standard solutions was 0.4 mg/mL. Both standard solutions were kept in an amber vial and stored in a chiller at 4 °C.

2.4. Sample selection and preparation

Ten different brands of traditional medicines were purchased from traditional medicine stores in Malaysia. The chosen dosage forms were liniment and ointment. The samples were selected based on market availability and the absence of methyl salicylate and thymol as active ingredients on their labels. The samples must also be locally manufactured in Malaysia. Then, their product registration status was cross-checked with QUEST3+ Product Search, which can be accessed via the National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health Malaysia official website. From the ten selected samples, five samples were liniment and the rest were ointment. Regarding their registration status, five were registered traditional medicines while the remainder were unregistered.

For sample preparation, about 1.0 g of sample was weighed and transferred into 50 mL volumetric flask. 30 mL of methanol was then added and the mixture was heated on sonicator bath at 55 °C until fully liquified. The solution was cooled to room temperature and made up to 50 mL with methanol. 1 mL of the above sample solution was diluted to 50 mL with methanol. Then, 5 mL of the resulting solution was further diluted to 25 mL with methanol before filtered through a 0.45 μ m syringe filter.

2.5. Method validation

This method was validated in accordance to ICH Harmonised Tripartite Guideline. The performance characteristics that were validated include linearity, range, detection limit, quantitation limit, specificity, accuracy and precision. In addition, system suitability was also evaluated [6].

2.5.1. System suitability

Standard solutions from Section 2.3 were diluted together with methanol to produce methyl salicylate and thymol concentration of 0.1 mg/mL. The resulting mixture of methyl salicylate and

thymol standard solution was further filtered through a 0.45 µm syringe filter and six replicates were injected into the HPLC system for system suitability determination. Table S1 shows the system suitability test (SST) requirements of the analytical procedure.

2.5.2. Limit of detection and limit of quantitation

Both limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of residual. To achieve this, at least six levels of mixed standard solutions from Section 2.3. with regular distribution between each level were prepared. The mixed standard solutions were then analysed in three different batches under reproducibility conditions (standard solutions are freshly prepared and analysed on different days). Parameters for the calibration curve were then calculated, followed by the determination of LOD in Eq 1 and LOQ in Eq 2, as depicted below:

$$LOD = \frac{(3 \times Sres)}{b} \tag{1}$$

$$LOQ = \frac{(10 \times Sres)}{h} \tag{2}$$

where S_{res} is the residual standard deviation and b is the slope of the regression line.

2.5.3. Linearity and range

Linearity and range were established by preparing a series of calibration solution of methyl salicylate and thymol. Six concentration levels from mixed standard solutions (Section 2.3.) were prepared. These calibration solutions were then analysed in three different batches under reproducibility conditions. The concentration of the calibration solutions together with their response values were later subjected to regression analysis. To confirm the linearity of the analytical method, both the regression and linearity models need to be accepted. If F_{reg} was higher than the critical value $F_{(0.95,1,n(p-1))}$, the hypothesis was that the variation of y was explained by a regression model. $F_{(0.95,1,n(p-1))}$ was the value of Fisher distribution for 1 and n(p-1) degrees of freedom at 95% confidence level. If the first hypothesis was valid, the second hypothesis will be tested which concerns the validity of the linearity model. The linearity model was acceptable if F_{lof} was lower or equal to the critical value $F_{(0.95,n-2,n(p-1))}$. Following validity of both models, a further Student's t-test was carried out to determine whether a passed through the origin. If t_{cal} was lower or equal to the critical t value for n-2 degree of freedom at 95% confidence level (t_{crit}), it can be established that a passed through the origin. Generally, the coefficient of determination should be more or equal to 0.997 for the regression line [7]. In terms of the working range, it was determined based on the established linear range and a minimum of five concentration levels was needed to demonstrate linearity as stipulated by ICH guidelines [8].

2.5.4. Specificity

Specificity analysis was performed by the standard addition method. Four different samples (two each for liniments and ointments) were spiked with one selected concentration within the working range. The specificity was confirmed by adjusting a straight line between spiked

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concentration (v) and recovered (r) concentrations. The relationship between the spiked concentration (v) and recovered (r) concentration was expressed in the form of regression line (r = a + bv). If the specificity was valid, the slope and intercept were statistically not different from 1 and 0, respectively, which indicated that the overlap line (r = a + bv) was the equivalent of line y = x. Following least-square regression analysis, two hypothesis testing was carried out to confirm specificity. Firstly, t-test (t_1) was utilised to test the hypothesis that the slope was significantly equal to 1 while the second t-test (t_2) was utilised to test the hypothesis that the intercept was significantly equal to 0. Thus, if both conditions were accepted, it indicated that the overlap line was the equivalent to y = x, confirming the specificity of the method.

The specificity of the analytical method was also investigated by injecting blank solution (methanol), mixed standard solution, blank sample solution and spiked sample solution to demonstrate the absence of any interferences from other excipients and sample components. The excipients and other sample components should not interfere with the elution of methyl salicylate and thymol [7,9].

Also, a test for general matrix effect was also performed to study any significant difference between the calibration solution in solvent and calibration solution in the sample matrix. A set of calibration solution in methanol and another set each in both sample types (liniment and ointment) were prepared as established in the linearity studies. Least squares regression, F-test and t-test parameters were computed. An F-test to obtain F_{cal} and F_{crit} at the critical value of $F_{(0.95, n-l, n-l)}$ was conducted to examine the differences between all matrices' residual variances, S^2_{res} . Then, two hypothesis testing were carried out to compare the slope of the calibration lines using Student's t-test. Thus, if t_{cal} was less or equal to t_{crit} , it indicated that both of the slopes (methanol-liniment matrix or methanol-ointment matrix) were not different and the calibration for routine analysis can be performed in the solvent. Conversely, if the t_{cal} was more than t_{crit} , the calibration for routine analysis should be conducted in sample matrix solution.

2.5.5. Precision

Repeatability testing was conducted by spiking three batches of sample blanks in duplicates at three different concentrations covering the working range. Calibration solution based on the established linear range was also prepared. Then, the mean assay values for each batch were applied for determining mean value of the respective concentration levels. Repeatability was expressed in percentage of relative standard deviation (RSD). For repeatability, the percentage RSD of assay results should be $\leq 2\%$ [7]. In terms of intermediate precision, it was performed by a different analyst on different days utilising a different HPLC instrument with different standards and samples preparation. Similar methods as repeatability were applied for this testing. The acceptance criteria for intermediate precision requires the percentage RSD of both analysts' assay results to be $\leq 2\%$ [7,9].

2.5.6. Accuracy

The accuracy of the analytical method was determined by the standard addition method. Similar to precision, three batches of sample blanks in duplicates were spiked at three different concentrations covering the working range. This was in line with ICH recommendation by which a minimum of nine determinations over a minimum of three concentration levels covering the working

range was required for accuracy determination [8]. The percent recovery was calculated from the mean assay value of each batch. The mean recovery should be within the recovery limits depending on the concentration level. At the concentration of $10 \mu g/mL$, the acceptable recovery limit should be within 80 to 115% while at the concentration of $100 \mu g/mL$, the limit should be within the range of 85 to 110% [10].

2.6. Screening of methyl salicylate and thymol

Samples were screened for methyl salicylate and thymol as per the method. The screening was conducted by analysing the samples concurrently with a mixed standard solution of methyl salicylate and thymol prepared at LOD level. Following HPLC analysis, the samples were screened for the presence of methyl salicylate and thymol peaks by comparing their respective chromatograms to that of the LOD standard solution. If methyl salicylate and/or thymol peaks are present, the particular peak area will be compared to its corresponding peak area from the LOD standard solution. A sample is detected with methyl salicylate and/or thymol with the condition that the sample's peak area higher than that of the LOD standard solution.

3. Results and discussion

3.1. HPLC analysis of methyl salicylate and thymol standard

A mixture of methyl salicylate and thymol standard solution were injected into the HPLC system according to the aforementioned chromatographic conditions. Chromatogram of standard solutions exhibited peaks corresponding to methyl salicylate and thymol, which eluted at 3.861 minutes and 6.211 minutes, respectively as illustrated in Figure 1. Figure 1 clearly showed that both compounds were adequately resolved and free from any interferences. In terms of elution order for both compounds, it was found to be comparable with a previous study conducted by Bachute & Shanbhag (2016).

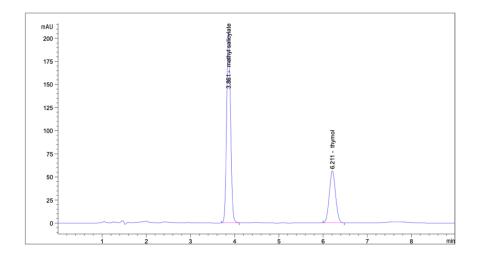


Figure 1. HPLC chromatogram of a mixture of methyl salicylate and thymol standard solution at $t_r = 3.861$ minutes for methyl salicylate and $t_r = 6.211$ minutes for thymol.

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3.2. Method validation

3.2.1. System suitability

The summarised results of SST are shown in Table 1. The method satisfied all the requirements [7].

Parameter	Acceptance Criteria	Methyl Salicylate	Thymol
System precision	% RSD of standard retention time ≤ 1%	0.048	0.036
	% RSD of standard peak area $\leq 1\%$	0.429	0.589
	% RSD of standard peak height ≤ 1%	0.576	0.590
Theoretical plate count	≥ 2000	8943	9194
Tailing factor	≤ 2	1.08	1.06
Resolution	≥ 2	_	11.13

Table 1. SST results.

3.2.2. Limit of detection and limit of quantitation

The LOD and LOQ for both compounds are given in Table 2. Additionally, the LOD and LOQ values for methyl salicylate from this study were comparable with findings by Shabir and Bradshaw (2011). However, LOQ for thymol were found to be higher than that of a previous study conducted by Hajimehdipoor et al. (2010), which was 8.6 µg/mL. Although the LOQ was higher, it was compensated by the comparable LOD value. Thus, the obtained detection limit and quantitation limit were found to be appropriate for routine analysis of methyl salicylate and thymol in traditional medicines.

Parameter	Methyl Salicylate	Thymol	
LOD (μg/mL)	3.56	3.76	
$LOQ (\mu g/mL)$	11.87	12.53	

Table 2. LOD and LOQ results.

3.2.3. Linearity and range

For linearity studies, six concentrations of calibration solutions for methyl salicylate (25.10, 40.33, 79.77, 100.38, 124.58, 149.68 μ g/mL) and thymol (14.97, 25.21, 75.25, 100.07, 125.28, 150.10 μ g/mL) were analysed and the calibration curves were constructed using regression analysis. The summary of regression analysis for methyl salicylate and thymol are shown in Table S2. The coefficient of determination (R²) of both calibration curves were more than 0.997, which indicated acceptable fit of the data to the regression line. In this study, six concentration levels of the working range for both compounds were assessed from data established from linearity analysis.

Both the regression and linearity models need to be accepted to confirm the linearity of the analytical method. As listed in Table S3 and Table S4, both compounds gave F_{reg} values higher than the critical value $F_{(0.95,1,n(p-1))}$. This indicated that the variation of peak area was explained by the regression model. In terms of the linearity model, both compound's F_{lof} was lower than the critical

value $F_{(0.95,n-2,n(p-1))}$. This signified the acceptance of the linearity model. Subsequently, confirmation of y-intercept was performed by calculating the t value for both compounds. As t_{cal} for both compounds was lower than t_{crit} , it indicated that the y-intercept passed through the origin. Table S5 shows the results for confirmation of y-intercept.

3.2.4. Specificity

Table S6 lists the results for specificity confirmation for methyl salicylate and thymol. From the results, all calculated values of t_1 and t_2 were lower than t_{crit} . This indicated that the slope and intercept were statistically not different from 1 and 0, respectively, which signified the overlap line was equivalent to y = x, confirming the analytical method's specificity.

Specificity were also demonstrated by the absence of any interferences from other excipients and sample components in blank solution (methanol), blank sample solution and spiked sample solution. All of these findings were in line with requirements set by Shabir (2005). Figure 2, 3, 4, 5 and 6 illustrates chromatogram of blank solution (methanol), blank sample solution (liniment), blank sample solution (ointment), spiked sample solution (liniment) and spiked sample solution (ointment), respectively.

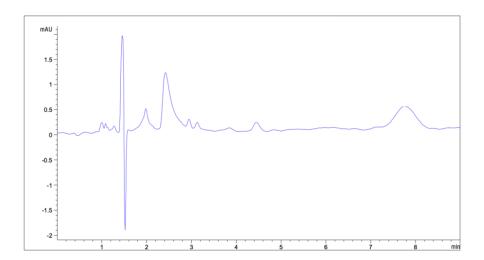


Figure 2. HPLC chromatogram of blank solution (methanol).

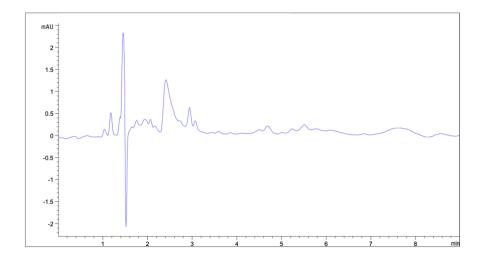


Figure 3. HPLC chromatogram of blank solution (liniment).

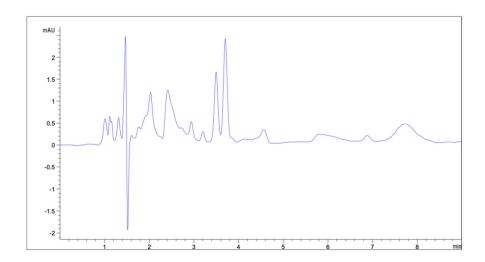


Figure 4. HPLC chromatogram of blank solution (ointment).

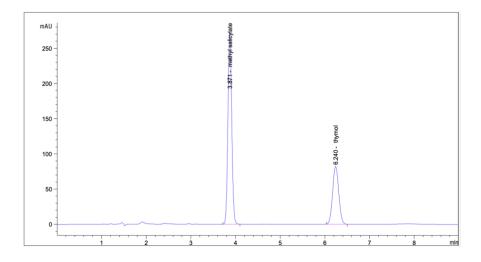


Figure 5. HPLC chromatogram of spiked sample solution (liniment).

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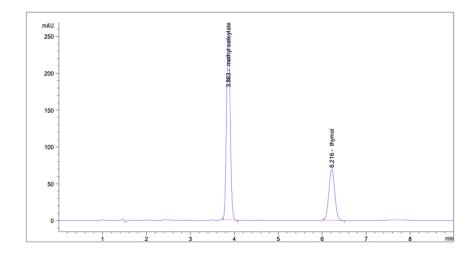


Figure 6. HPLC chromatogram of spiked sample solution (ointment).

In terms of test for matrix effect, the parameters for least squares regression, F-test and t-test were computed as listed in Table S7 and Table S8. For both compounds in both matrices, all residual variances were not different as illustrated in the lower value of F_{cal} when compared to the F_{crit} . Likewise, the calculated t value for all sets was less than the critical t value. This indicated that both slopes which referred to the methanol-liniment matrix or methanol-ointment matrix were not different. Hence, calibration can be conducted in solvent as there was no matrix effect observed for methyl salicylate and thymol.

3.2.5. Precision

For repeatability studies, all the percentage RSD assay values for methyl salicylate and thymol at three concentration levels were found to be lower than 1.65% and met the requirements set by Shabir (2005). Table S9 and Table S10 describes the repeatability data for both compounds in their respective matrices. The results indicated high repeatability of the method.

Regarding intermediate precision, the results for the first analyst was obtained from repeatability testing while the results for the second analyst are shown in Table S11 and S12. The RSD values for both analysts were calculated and found to be less than 1.65% for each concentration level. These results exhibited excellent precision for the analytical method as the assay results obtained by two analysts using two different instruments on different days should have a statistical RSD $\leq 2\%$ [7,9].

3.2.6. Accuracy

From the results obtained, mean recovery values of methyl salicylate and thymol at three concentration levels for both sample matrices were within a range of 96.5 to 104.18%, which fulfilled the requirements set by AOAC International (2013). These results indicated that the accuracy of the analytical method was satisfactory at the working range and the establishment of a close agreement within the spiked and recovered values. The recovery results are summarised in Table S13 and S14.

3.3. Screening of methyl salicylate and thymol

The summarised results of sample screening are shown in Table 3. Based on the HPLC analysis of all the samples, five were detected with methyl salicylate and none with thymol. As there is no available regulatory limit for both compounds, peak area at LOD concentration level was utilised as the benchmark for screening analysis. Methyl salicylate peak was present in sample LU1, LU2, LU3, OR3, OR2 and OU1. Although methyl salicylate peak was present in sample OR2, its peak area was lower than LOD level thus deeming it a negative sample.

Additionally, out of the five adulterated samples, unregistered traditional medicines made up the majority with four positive samples. Although these findings were expected, it was alarming to detect an adulterated registered sample (OR3). This might be due to the manufacturer's lack of transparency in regards to the active ingredients, whereby it was not declared in the label or during product registration. Methyl salicylate was not declared as the manufacturer will be required to provide substantial QC evidence concerning methyl salicylate in that particular product. It will be costly as QC testing requires personnel training and also relevant analytical equipment. Most manufacturers are small and medium-sized enterprises which have limited funding and facilities for QC. Furthermore, regulatory requirements for traditional medicines are more likely to be less stringent compared to that of pharmaceutical products. This may create a loophole that can be manipulated by manufacturers. Although such incidence is remote, it can be overcome by regular post-market surveillance of traditional medicines and facility inspection to ensure compliance towards Good Manufacturing Practice (GMP) principles. In terms of unregistered traditional medicines, the accompanying hazards can be minimised by Pharmacy Enforcement Division's active role in monitoring the sales of traditional medicines. This may include thorough investigation and laboratory analysis before any confiscation of operation and prosecution in a court of law [11]. Consumers can also play a part by ensuring the registration status of any traditional medicine prior consumption. Any exaggerated claims on the label should be received with caution as it might be adulterated.

It is noteworthy to mention only methyl salicylate was detected. The preference towards methyl salicylate in contrast to thymol was also evident as it was listed as one of the main adulterants for traditional medicines throughout the world [12]. Although thymol was not the adulterant in this study, this could be due to its presence in other dosage forms such as creams as evident in the cancellation of *Warisan Salju Langkawi Krim Susu Kambing Gamat Plus*'s product registration [13].

No.	Sample	Dosage	Registration	Peak Area of Methyl	Peak Area of Methyl
	Code	Form	Status	Salicylate (Sample)	Salicylate (LOD solution)
1.	LR1	Liniment	Registered	Not detected	64.8268
2.	LR2	Liniment	Registered	Not detected	64.8268
3.	LU1	Liniment	Unregistered	375.7730	64.8268
4.	LU2	Liniment	Unregistered	285.5094	64.8268
5.	LU3	Liniment	Unregistered	336.2017	62.4743
6.	OR1	Ointment	Registered	Not detected	64.8268
7.	OR2	Ointment	Registered	20.8065 (< LOD)	64.8268
8.	OR3	Ointment	Registered	363.0748	62.4743
9.	OU1	Ointment	Unregistered	263.1386	64.8268
10	OU2	Ointment	Unregistered	Not detected	62 4743

Table 3. Screening results of traditional medicines.

4. Conclusion

In this present study, an HPLC method for simultaneous determination of methyl salicylate and thymol in Malaysian traditional medicines was analysed and validated. Currently, there are no available HPLC methods for simultaneous determination of both compounds in traditional medicines. Previously reported methods mainly focused on the determination of methyl salicylate and thymol as an individual compound or in conjunction with other compounds. The run time of this method is the shortest in comparison to other literatures. In turn, the rapid analysis will help to save costs involving electricity, reagents and labour. The sensitivity of the analytical method was reflected by the acceptable LOD and LOQ values for both compounds. The linearity of the method was confirmed by the acceptance of regression and linearity models. Strong linear association between analyte concentration and detector response ($R^2 > 0.997$) was also demonstrated in a wide working range for both compounds. Specificity was also confirmed by t-test of the obtained slope and intercept as well as the absence of any interferences from excipients and sample components. Low percentage RSD values for repeatability and intermediate precision indicated the high precision of the analytical method. High degree of trueness was also exhibited as the recovery values for both compounds were within the range of 96.5 to 104.18%. From this study, it can be concluded that this sensitive, linear, specific, precise and accurate HPLC method can be utilised for routine QC analysis of methyl salicylate and thymol in traditional medicines.

By applying this method, selected Malaysian traditional medicines were screened for potential methyl salicylate and thymol adulteration. It should be noted that methyl salicylate was the only adulterant detected in all the samples. This indicated the manufacturers' preference towards methyl salicylate in contrast to thymol. Moreover, the findings revealed that unregistered traditional medicines were the main target for adulteration. This is not surprising as they do not undergo crucial QC and safety testing, which makes them susceptible to adulteration by unscrupulous manufacturers to gain lucrative profits [11].

Although this study only screened for potential adulteration, essentially this method can further quantitate both compounds in traditional medicines, specifically in liniment and ointment. This will be beneficial and practical once the limits are defined by regulatory bodies for both compounds. In another context, this method will also benefit traditional medicine manufacturers, who plans to conduct QC testing for their methyl salicylate and thymol-based products. The application of this

method in regulatory and industrial settings will ensure the safety and quality of traditional medicines, thereby safeguarding consumer's health.

In future investigations, it might be possible to include menthol and camphor in the analysis. Currently, only a gas chromatography method developed by Bachute and Shanbhag (2016) can simultaneously determine methyl salicylate, thymol, menthol and camphor in an ointment. These compounds resemble closely in terms of their physical and chemical properties as well as common active ingredients in many topical formulations. Unlike methyl salicylate and thymol, Drug Registration Guidance Document (DRGD) set a limit of 10% and 11% permitted content for menthol and camphor, respectively, when applied as active ingredients for external preparation [14,15]. The need for simultaneous determination of all these compounds is further highlighted by the product registration cancellation of *Minyak Seri Pala* as a result of undeclared menthol and thymol [13]. Another compound that warrant for future inclusion is carvacrol. Due to the isomeric nature of both carvacrol and thymol, complete separation can be difficult leading to inaccurate analysis [16]. To date, although carvacrol has not been reported as an adulterant, its addition is imperative as it can further enhance the QC of traditional medicines. Further studies on other sample matrices such as gels, lotions and creams will also need to be undertaken to ensure a comprehensive screening approach.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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