Method Development for Analysis of Essential Oils Authenticity using Gas Chromatography-Mass Spectrometry (GC-MS)

Novi Nur Aidha[1], Retno Yunilawati[1] and Irma Rumondang[1]
[1]*Badan Penelitian dan Pengembangan Industri, Kementerian Perindustrian*

Keywords: Essential Oil, Authenticity, GC-MS, Chemical Component.

Abstract: Essential oils widely used as fragrances and flavours in the food and cosmetics industry and also for the medical and pharmaceutical fields for various effects. The demand increasing of essential oil caused the cases of adulteration that affect the authenticity of essential oil. The authenticity is important in ensuring the quality of essential oil. This study was aimed to analyse the authenticity of essential oil use Gas Chromatography-Mass Spectrometry (GC-MS) by determining its chemical component. The experiment included repeatability, accuracy, and limit of detection. GC performed on HP-5MS capillary column operated in 60^0C-240^0C temperature programs. This method successfully applied to all types of essential oil with limit detection of clove oil was 0.02 ppm, citronella oil was 0.033 ppm, patchouli oil was 0.005 ppm, and lemongrass was 0.016 ppm. All types of essential oil also have good repeatability and accuracy with these methods. This study will facilitate the scientific community by enhancing the efficient method for essential oil.

1 INTRODUCTION

Essential oils are widely used as fragrances and flavor in the food and cosmetics industry, and in the medical and pharmaceutical fields for various effects (Mohamed et al., 2018; Wany et al., 2013). The purity of essential oils is very important in their use in various fields. The demand increasing of essential oil caused the cases of adulteration that affect the authenticity of essential oil. The adulteration occurs because of the prices for natural extracts higher than those of synthetic materials. Adulteration also is intended to gain volume or weight to get a higher profit. Adulteration essential oils in various ways are by mixing it using cheaper essential (Do et al., 2015), add compound isolate or synthesis- dilution with inert material (Ng et al., 2015; Ke et al., 2015; Schipilliti et al., 2010) or add with other oil include nutmeg oil contaminant with castor oil (Yunilawati et al., 2013), lemongrass oil identified kerosene or coconut oil as adulterants (Do et al., 2015) and sandalwood oil diluted with cedarwood oil (Howes et al., 2004). The adulteration can degrade the quality and can lead to safety issues, health hazards, or noncompliance with the natural grade.

The authenticity is important in ensuring the quality of essential oil. Authenticity can be defined as free from adulteration in the sense of absence of foreign matter, but it also suggests free from impurities. Control methods and standardization of essential oils are required to check compliance with the standards of quality. Many analytical techniques to analysis the authenticity of essential oil including isotope-ratio mass spectrometry (IRMS) (Schipilliti et al., 2010), nuclear magnetic resonance spectroscopy (NMR) (Cerceau et al., 2016), high-performance thin-layer chromatography (HPTLC) (Cerceau et al., 2016), high- performance liquid chromatography (HPLC) (Gaonkar et al., 2016) and gas chromatography (GC) (Esfahanizadeh et al., 2018; Abualhasan et al., 2017; Beale et al., 2017; Athar et al., 2013; Heuskin et al., 2009; Howes et al., 2004; Shellie et al., 2002). GC is the analytical technique for identification with controlled conditions and can be directly coupled to a mass spectrometer (MS) if information other than fingerprint is needed. Each type of essential oil has GC-MS qualitative fingerprint (Hu et al., 2006), which compared with the literature. Therefore, GC-MS has become a part of the routine testing for essential oil and commonly used for detecting adulteration of essential oil. Analysis using GC is very profitable and efficient because easy, faster separation, need short time, low cost, has sensitivity and good detection limit for volatile compound

Nur Aidha, N., Yunilawati, R. and Rumondang, I.
Method Development for Analysis of Essential Oils Authenticity using Gas Chromatography-Mass Spectrometry (GC-MS).
DOI: 10.5220/0009956000410046
In *Proceedings of the 2nd International Conference of Essential Oils (ICEO 2019)*, pages 41-46
ISBN: 978-989-758-456-5

(Vargas Jentzsch, 2019; Al-Rubaye et al., 2017; Chauhan, 2014).

This study was aimed to analyse the authenticity of essential oil use Gas Chromatography-Mass Spectrometry (GC-MS) by determining its chemical component. The essential oil used are patchouli, citronella, clove, massoia, and lemongrass oil, and the experiment included repeatability, accuracy, and limit of detection. The first experiment is validating the methods to ensure that it has reproducible and reliable results, and the results can be used to assess the quality, reliability, and consistency of the results of the analysis.

2 MATERIALS AND METHODS

2.1 Materials

Essential oils were used in this experiment are patchouli, citronella, clove, massoia, and lemongrass were obtained from Indonesia. The standard of essential oil was used are patchouli, citronella, and clove from France. The solvent was used is methanol (Merck).

2.2 Equipment

Gas chromatography with a mass spectrometer detector (GCMS) Agilent 6890 series with capillary column HP-5MS, 30 m x 0.25 mm id x 0.25 µm film thickness. Helium gas was used as the carrier gas at a constant pressure of 65 kPa. The essential oil was injected with a volume of 1 µL in a split ratio of 1:25 and a solvent delay of 2 minutes. The increasing oven temperature was programmed from 60-240°C with a step of 3°C per minute until reaching 240°C.

2.3 Methods

2.3.1 Repeatability

1 µl of the essential oil (patchouli, citronella, clove, massoia, and lemongrass) was diluted in 1 ml methanol, then injected of 1 µl into GC-MS. Repeatability is done by injecting essential oils 7 in times.

2.3.2 Accuracy

1 µl of the essential oil was diluted in 1 ml methanol (patchouli, citronella, clove oil from Indonesia as a sample, and patchouli, citronella, clove oil from France as a standard), then injected of 1 µl into GC-MS. The chromatogram data were compared between the essential oil from Indonesia and the standard.

2.3.3 Limit of Detection

The detection limit is done by injecting essential oils with various concentrations. Variable concentrations of clove, citronella, lemongrass and patchouli oil were made are 0.1 ppm; 0.05 ppm; 0.033 ppm; 0.025 ppm; 0.02 ppm and 0.016 ppm by diluted 1 µl of the essential oil into methanol (10 ml; 20 ml; 30 ml; 40 ml; 50 ml and 60 ml). Especially for patchouli oil, various concentration was also made to 0.005 ppm. The limit of detection was determined based on the lowest concentration that can be detected by the instrument. That concentration was observing the height of the major component in essential oil.

3 RESULT AND DISCUSSION

3.1 Methods Developments for Analysis of Essential Oil using GC-MS

The development methods for the analysis of the essential oil using GC-MS can be used to identify chemical compounds in essential oils, regardless of the type of essential oil, and also to analyze the authenticity of essential oils. The essential oils were used are patchouli, citronella, clove, lemongrass, and massoia oil because these are the major of essential oil produced in Indonesia (Ministry of Trade Republic of Indonesia, 2011). In this study, the method was created and optimized internally in the previous experiment. The analyze using GC Agilent 6890 with HP-5MS column and the performance of condition programs of GC-MS have been optimized and verified as have done by Cardoso et al. (2018) and Athar et al. (2013). The method validating included repeatability, accuracy, and limit of detection of the essential oil similar with the previous method were reported by Cardoso et al. (2018); Esfahanizadeh et al. (2018); Abualhasan et al. (2017); Chauhan (2014); Athar et al. (2013).

3.2 Repeatability

The repeatability experiments were established in order to evaluate the methods' trueness and precision, respectively. The repeatability was determined by the analytical procedure under normal conditions using seven (7) repetition on the same day (intraday precision). The precision of the development method

using GC-MS has established by comparing each chromatogram of the essential oil, and it was considered the peak profile of this compound. Figure 1. showed that all of the essential oil has good repeatability. Compared with an earlier study which is reported repeatability using GC-MS with triplicate (Esfahanizadeh et al., 2018; Athar et al. 2013) and six replicate (Cardoso et al., 2018).

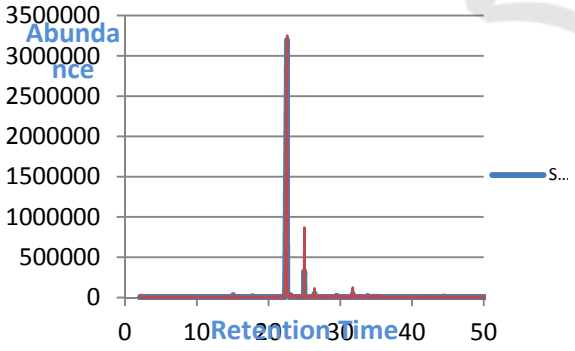
(a) (b) (c) (d) (e)

Figure 1: Repeatability of clove oil (a); patchouli oil (b); citronella oil (c); lemongrass (d) and massoia oil (e).

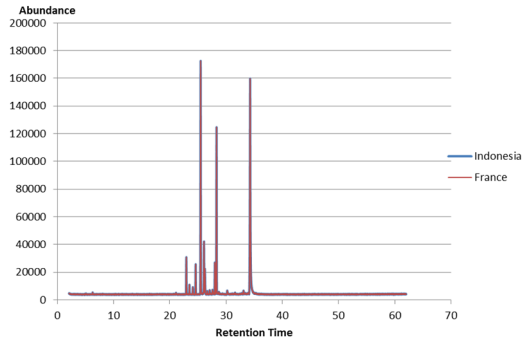
3.2 Accuracy

Accuracy was determined by comparing the chromatogram between essential oil from Indonesia with essential oil from France as standard (clove, patchouli, and citronella oil). Figure 2 showed that the essentials oil has good accuracy. The methods showed the spectra of Indonesian essential oil matched with the standard. The spectra have similar major components in each essential oil, although there is a difference in the high area of the spectra between Indonesian essential oils and standard. The variation of components and its concentration of essential oil depend on the type of regions. The main constituent in clove oil is eugenol; in patchouli oil is patchouli alcohol; and in citronella oil are citronellal, citronellal, and geraniol.

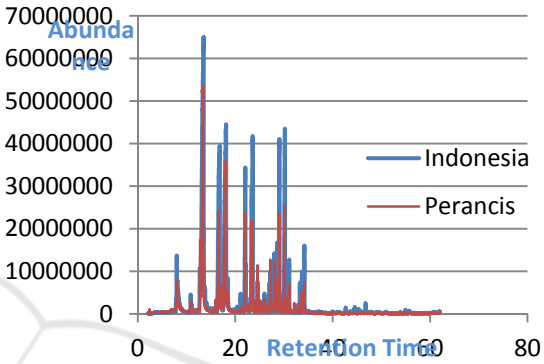
The accuracy of this method is comparable with previous research (Esfahanizadeh et al., 2018), the accuracy of eugenol from clove oil compared with standard eugenol (Sigma) (Athar et al., 2013), patchouli from China (Hu et al., 2006) and citronella oil compared with essential oil standard Sigma (Wany et al., 2014). The methods of those research validated the accuracy of one type of essential oil, but in this research can be used to validate the accuracy of all types of essential oil. If one of the peaks of the active ingredient was absent or there was absence an active component or impurities from other essential oils or foreign matter, it can be ensured that the essential oils are not authentic.



(a)



(b)



(c)

Figure 2: Accuracy of clove oil (a); patchouli oil (b); citronella oil (c).

3.3 Limit of Detection (LOD)

The limit of detection was showed the lowest concentration of essential oil could be detected using GC-MS. This method can be used for 4 (four) types of essential oil (clove, patchouli, citronella, and lemongrass oil) with the different LOD showed in Table 1. The value of LOD depended on the type of GC and detector, sensitivity on separations, and the experimental conditions (program) used. Athar et al. (2013) reported the LOD of eugenol from clove oil was 3µl/L used the same column and carrier gas but different in experimental conditions. Jumepaeng et al. (2014) obtained LOD of lemongrass values was in the range from 0.3 to 0.6 µg/mL using GC FID. The limit detection of citronella close with clove oil. Patchouli oil has the lowest limit detection than other essential oil.

Table 1: Limit detection of essential oil.

Essential oil	Limit of detection (ppm)
Clove oil	0.02
Patchouli	0.005
Citronella oil	0.033
Lemongrass oil	0.016

4 CONCLUSIONS

The validation method can be applied to all types of essential oil for analysis of authenticity. The repeatability and accuracy for the essential oil are good with limit detection of clove, citronella, patchouli, and lemongrass oil, which were 0,02 ppm; 0.033 ppm; 0.005 ppm and 0.016 ppm. This study will facilitate the scientific community by enhancing the efficient method for essential oil. Through this study can maintain Indonesia's reputation in the trade sector of essential oil, especially clove, patchouli, citronella, lemongrass, and massoia oils, and to facilitate identification of their purity and quality.

ACKNOWLEDGEMENTS

This research was supported by Center for Chemical and Packaging (CCP). We also thank to Technical Assistant EU-Indonesia Trade Support Programme (TSP) for the training to support the research.

REFERENCES

Abualhasan, M, N., Zaid, A, N., Jaradat, N., Mousa, A., 2017. GC Method Validation for the Analysis of Menthol in Suppository Pharmaceutical Dosage Form, *International Journal of Analytical Chemistry*.

Al-Rubaye, A, F., Hameed, I, H., Kadhim, M, J., 2017. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants, *International Journal of Toxicological and Pharmacological Research*, 9(1).

Athar, M, T., Tamboli, E, T., Ansari, S, H., Ahmad, S., 2013. Quantification of Eugenol in Hydro-Distilled Clove Oil (Eugenia Caryophyllus) and Its Marketed Products by Validated GC-MS Method, *Journal of Herbs, Spices And Medicinal Plants*, 19(4), 365–376.

Beale, D, J., Morrison, P, D., Karpe, A, V., Dunn, M, S., 2017. Chemometric Analysis of Lavender Essential Oils Using Targeted and Untargeted GC-MS Acquired Data for the Rapid Identification and Characterization of Oil Quality, *Molecules*, 22(8).

Cardoso, I, C., Nakamura, M, J., Correia, V, G., Pereira, H, M., Behrens, M, D., Tappin, M, R., 2018. Development, Optimization and Validation of a GC Method by Polarity Phase Constants and Statistical Design of Experiments for the Determination of Monoterpenes. *J. Braz. chem. Soc.*, 29(11), 2254–2265.

Cerceau, C, I., Barbosa, L, C, A., Filomeno, C, A., Alvarenga, E, S., Demuner, A, J., Fidencio, P, H., 2016. An Optimized and Validated 1H NMR Method for the Quantification of α-pinene in Essentials Oils, *Talanta. Elsevier*, 150, 97–103.

Chauhan, A., 2014. GC-MS Technique and Its Analytical Applications in Science and Technology, *Journal of Analytical & Bioanalytical Techniques*, 5(6).

Do, T, K, T., Minaglou, F, H., Antoniotti, S., Fernandez, X., 2015. Authenticity of Essential Oil, *Trends in Analytical Chemistry*, 66, 146–157.

Esfahanizadeh, M., Ayatollahi, S, A., Goodarzi, A., Bayat, M., Ata, A., Kobarfard, F., 2018. Development and Validation of a GC/MS Method for Simultaneous Determination of 7 Monoterpens in Two Commercial Pharmaceutical Dosage Forms, *Iranian Journal of Pharmaceutical Research*, 17(2), 24–32.

Gaonkar, R., Yallappa, S., Dhananjaya, B, L., Hegde, G., 2016. Development and Validation of Reverse Phase High Performance Liquid Chromatography for Citral Analysis From Essential Oils, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1036–1037. 50–56.

Heuskin, S., Godin, B., Leroy, P., Capella, Q., Wathelet, J, P., Verheggen, F., Haubruge, E., Lognay, G., 2009. Fast Gas Chromatography Characterisation of Purified Semiochemicals from Essential Oils of Matricaria chamomilla L. (Asteraceae) and Nepeta cataria L. (Lamiaceae)', *Journal of Chromatography A*, 1216(14), 2768–2775.

Howes, M-J, R., Simmonds, M, S., Kite, G, C., 2004. Evaluation of the Quality of Sandalwood Essential Oils by Gas Chromatography–Mass Spectrometry, *Journal of Chromatography A*. Elsevier, 1028(2), 307–312.

Hu, L, F., Li, S, P., Cao, H., Liu, J, J., Gao, J, L., Yang, F, Q., Wang, Y, T., 2006. GC-MS Fingerprint of Pogostemon Cablin in China, *Journal of Pharmaceutical and Biomedical Analysis*, 42(2), 200–206.

Jumepaeng, T., Jantayota, R., Hemmood, S., Komolwanich, S., Wuthisarn, S., Luthria, D., Chanthai, S., 2014. Determination and Comparison of Volatile Aroma Compounds in Fresh and Dried Leaves Samples of Citronella Grass, Lemongrass, and Citronella Incense Products by Micro-Hydrodistillation, *Acta Chromatographica*, 26(1), 177–190.

Ke, B., Dg, Y., Cl, W., Bl, S., Re, C., 2015. Detecting Essential Oil Adulteration' *Journal of Environmental Analytical*, 2(2), 2–4.

Ministry of Trade Republik Indonesia, 2011. *Indonesian Essential Oils : The Scents of Natural Life*, *Indonesian Essential Oil : The Scents of Natural Life*. Indonesia: Trade Policy Analysis and Development Agency Ministry of Trade, Republic of Indonesia.

Mohamed, M. ., Ibrahim, M, E., Wahba, H, E., 2018.

Flavoring Compounds of Essential Oil Isolated from Agriculture Waste of Coriander (Coriandrum sativum) Plant, *Journal of Materials and Environmental Sciences*, 9(1), 77–82.

Ng, B, T., Fang, F, E., Bekhit, A, E-D., Wong, J, H., 2015. *Methods for the Characterization, Authentication, and Adulteration of Essential Oils*, *Essential Oils in Food Preservation, Flavor and Safety*. Edited by V. R. Preedy. Elsevier Science & Technology.

Schipilliti, L., Tranchida, P, Q., Sciarrone, D., Russo, M., Dugo, P., Dugo, G., Mondello, L., 2010. Genuineness Assessment of Mandarin Essential Oils Employing Gas Chromatography-Combustion-Isotope Ratio MS (GC-C-IRMS)', *Journal of Separation Science*, 33(4–5), 617–625.

Shellie, R., Mondello, L., Marriott, P., Dugo, G., 2002. Characterisation of Lavender Essential Oils by Using Gas Chromatography–Mass Spectrometry with Correlation of Linear Retention Indices And Comparison with Comprehensive Two-Dimensional Gas Chromatography, *Journal of Chromatography A*, 970(1–2), 225–234.

Vargas Jentzsch, P., 2019. Detection of Essential Oils Adulteration: A Quick Overview and Current Challenges, *American Journal of Biomedical Science & Research*, 4(1), 10–11.

Wany, A., Jha, S., Nigam, V, K., Pandey, D, M., 2013. Chemical Analysis and Therapeutic Uses of Citronella Oil from Cymnopogon Winterianus: A Short Review, *International Journal of Advanced Research*, 1(6), 504–521. Available at: http://www.journalijar.com/uploads/2013-09 06_030154_471.pdf.

Wany, A., Kumar, A., Nallapeta, S., Jha, S., Nigam, V, K., Pandey, D, M., 2014. Extraction and Characterization of Essential Oil Components Based on Geraniol and Citronellol from Java citronella (Cymbopogon winterianus Jowitt), *Plant Growth Regulation*, 73(2), 133–145.

Yunilawati, R., Riyanto, A., Aidha, N, N., 2013. Pemurnian Minyak Pala Terkontaminasi untuk mendapatkan Minyak Pala Dengan Mutu sesuai SNI 06-2388-2006, *in Prosiding Pertemuan dan Presentasi Ilmiah Standardisasi 2013,* 173–182.