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Spraying of swine buildings with lemon grass (*Cymbopogon citratus*) essential oil does not produce blood absorption in swine

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Abstract

Aim of the study*: Cymbopogon citrates* (Lemon grass) essential oils have been used in swine buildings to reduce the offensive odor emanating from swine buildings. The present study was designed to investigate plasma residues of citral which is a major constituent of the essential oils of *Cymbopogon citratus.*

Methods: An HPLC method was established, validated and used for the determination of citral in swine plasma harvested from blood samples taken at the 14th, 21st and 28th day after spraying swine buildings with the 3% Lemon grass essential oil. Thereafter, analysis of the sample was conducted using HP ODS Hypersil column (200 4.6 mm, 5 µm) with a mobile phase consisting of methanol and 0.5% acetic acid; and a flow rate of 1mL/ min. The method was validated for parameters such as accuracy, precision, linearity and detection limits Results and discussion: Plasma spiked with standard citral(95%) revealed two chromatograms with retention times of about 10.7 and 12.2 minutes. The calibration curves for the citral isomers were found to be linear in the tested concentration ranges and mean recoveries wre101% and 99.17%. This method was used to determine the residues of citral in swine plasma pretreated with methanol. Surprisingly, there was no any detectable level of citral in swine plasma within the 28 days of exposure. Conclusion: spraying of swine buildings with essential oils of lemon grass oil does not cause plasma residues of citral.

Keywords : Citral, *C. citratus,* Plasma, Spray, Swine

Introduction

Pungent smelling of swine buildings has been attributed to incomplete degradation of carbohydrate, protein, and lipids in swine faeces [1]. Biofiltration and chemical methods were used to reduce the offensive odor emanating from these buildings. As a result of various disadvantages from the aforementioned methods, biological additives have been used to control the odor in swine buildings. Some of these additives include essential oils [2], microbial additives [3-4], and soybean oil [5-7]. Although these additives are less effective for odor reduction as compared with the biofiltration and chemical method, its low cost and relatively non-toxic nature to the animals makes them preferable [8].

Cymbopogon citratus (Lemongrass)is commonly used for its analgesic, anti-inflammatory, antispasmodic, anti-pyretic, sedative and diuretic effects [9]. Studies on its essential oils showed that it has antibacterial [10], antifungal [11-12] and anti-malarial activities [13].Furthermore, Volatile oil from the leaves of *C. citratus* is widely used by cosmetics and other chemical industries [14].

High contents of citral, a monoterpene aldehyde, contribute to the strong, pleasant, lemon-like aroma of essential oils of lemongrass [14]. Citral of lemongrass is a combination of two isomeric aldehydes, namely geranial (α-citral) and neral (β-citral) [15-6]. Other unusual active components are limonene, citronellal, β-myrcene and geraniol [17]. Despite the plant is used in swine farms of South Korea for its antimicrobial activity and to enhance odor of swine buildings, there has been no report with regard to the determination of active citral in swine plasma. In this study, an HPLC method was established, validated and used to assess the residues of citralin swine plasma.

Materials and methods

Experimental design

Swine with an average weight of 10 kg were obtained from the Goon-wi pig farm (Korea). They were kept in the animal house for 7 days before initiation of the experiment and were given with feed

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and water, *ad libitum.* Lemongrass essential oil (Polysorbate 80 and Polysorbate 20) was supplied from Biomist, Co. Ltd. Korea. A total of 30 L mixture of 3% lemongrass essential oils in water was sprayed in the animal house by an automatic sprayer for 2 minutes every 30 minutes for a total of 28 days. The research was approved by the institute of animal uses and care committee of Kyungpook National University, Republic of Korea.

Sample preparation

Blood samples were collected in heparin zed tube from ten swine at a time, before spraying and at the 14th, 21stand 28th day after spraying. Samples were then immediately stored at -70°C until analysis.

Chromatographic condition

The concentration of citral in plasma was assayed using a high-performance liquid chromatography (HPLC), with a Hewlett Packard 1100 system with an HPLC pump, HP ODS Hypersil column (200 4.6 mm, 5 µm), HP 1046A fluorescence detector, an auto injector and an on-line degasser. Standard citral was obtained from Sigma chemical and the purity was higher than 95%. Deionized water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). The mobile phase contains HPLC-graded methanol (Burdichand Jackson, Korea) and 0.5% acetic acid (Junsei chemical, Japan)(60%: 40% v/v) with pH adjusted to 3 and a flow rate of 1 mL/min. The column temperature and UV detection wave length were set at 30ºC and 254 nm, respectively.

Sample treatment

Plasma samples were deproteinated with equal volume of methanol and vortexed for 5min. After centrifugation at 14,000rpm for 5min, the supernatant was transferred to an eppendorf tube and concentrated using Speed Vac. The concentrated residue was re-dissolved in the mobile phase and 20 µL was injected into the HPLC system.

Validation of the methods

Calibration curve and linearity

Aliquots of 160, 80, 40, 20 and 10 µg/mL citral standard solution were diluted in methanol to obtain calibration curves at the concentration of 16, 8, 4, 2 and 1 µg/mL. The solutions at various test concentrations were made three times and triplicate injections were made in to the system. 95% citral prepared at five concentration levels was used to determine the linearity of the established method.

Accuracy and recovery

Recovery was used to determine accuracy in such a way that predetermined amount of citral was added to plasma. The recoveries of citral through the established extraction method were conducted by evaluating the mean peak areas obtained from samples spiked before and after extraction.

Limit of detection and Precision

Inter- day and intra-day precisions were assessed with low (1 µg/mL), medium (4 µg/mL) and high (16 µg/mL) quality control samples. Analysis of standard citral triplicates in a single day was used to determine he accuracy and intra-day precision. Whereas, replicate analysis of standard samples was conducted in three separate days determine the accuracy and inter-day precision.
Detection limit was defined as the minimum level at which the citral could be reliably detected.

Determination of citral in swine plasma

The established and validated method was used to determine citral concentration in swine plasma immediately before spraying and at 14th, 21st and 28th day after spraying.

Result and discussion

Validation of analytical methods is necessary to establish a method appropriate for its anticipated purpose. Based on various guidelines [18-9], specificity, precision, linearity, accuracy, quantitation limits, detection limits and robustness are among the most commonly evaluated parameters. Selection among these parameters for investigation is the decision of the analyst and it depends on the ultimate objective of the study [20].
Volatile oil of *C. citratus* consists of aldehydes, ketones, hydrocarbons and esters. Studies on essential oils of *C. citratus* showed the presence of high amount of citral (3, 7-dimethyl-2, 6-octadienal) which is composed of citral A (geranial, trans isomer) and citral B (neral, cis isomer) [15, 21]. Chromatographic analysis of standard citral (20 µg/mL) showed the presence two chromatographs (A and B) with a retention times of about 10.7 and 12.2 minutes (Figure 1).

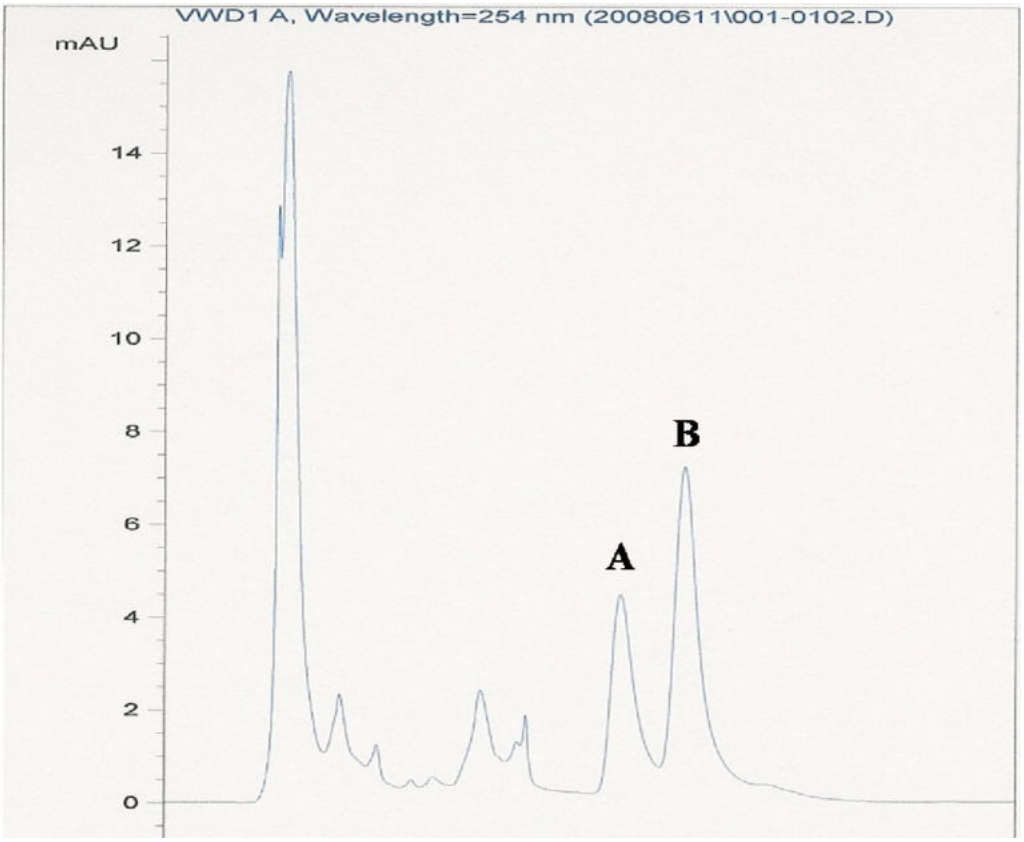

Figure 1. HPLC Chromatogram of 95 % citral (20 µg/mL)

The linearity of HPLC method established for this study was investigated at five concentrations ranging from 16- 1 µg/mL. The calibration curves of the two citral isomers (A and B) were plotted on concentration versus peak-area and the linearity was in the range of 16-1 µg/mL with 0.991 and 0.998 correlation coefficients (r) for citral A and B, respectively (Table 1). The representative linear equation for citral A and B was y=18.22x-6.591; r=0.991and y=9.635x-4.6; r=0.998, respectively; and the detection limit was 0.1 µg/mL.

Table 1. Test ranges, correlation coefficients (r^2) and standard curves of the citral in swine plasma

Citral	Standard curve	r^2	Concentration range(µg/mL)
Citral A (geranial)	y=18.22x-6.591	0.999	1.0-16.0
Citral B (neral)	y=9.635x-4.6	0.998	1.0-16.0
y: peak area ratio (analyte /internal standard); x: concentration of compound in swine plasma (µg/mL)			

Data on the intra and inter-day precision over three days of citral isomers are summarized in table 2 and 3, respectively. The mean recoveries of citral isomers (A and B) (Table 4) obtained from plasma spiked with different concentration ranges of the standard citral were 101% and 99.17%.

Table 2. Intra-day precision and accuracy citral in swine plasma (n=3)

Citral	Spiked concentration (µg/mL)	Measured concentration (µg/mL)[a]	Accuracy (%)
Citral A(geranial)	16	14.58±0.54	91.125
	4	4.01±0.04	100.25
	1	1.03±0.01	103
Citral B (neral)	16	17.02±0.27	106.375
	4	4.96±0.06	124
	1	1.34±0.04	134

[a] Mean ± SD; [b] accuracy = mean of measured concentration/nominal concentration

Table 3. Inter-day precision and accuracy of citral in swine plasma (n=3)

Citral	Spiked concentration (µg/mL)	Measured concentration (µg/mL)[a]	Accuracy(%)
Citral A (geranial)	16	14.83±1.09	92.6875
	4	3.55±0.23	88.75
	1	1.13±0.14	113
Citral B (neral)	16	13.81±1.31	86.3125
	4	3.43±0.29	85.75
	1	1.26±0.02	126

[a] Mean ± SD; [b] accuracy = mean of measured concentration/nominal concentration.

Table 4. Recoveries of citral in swine plasma (n=3)

Citral	Spiked (µg/mL)	Recovery (%)[a]	RSD (%)[b]
Citral A(geranial)	16	84.5	7.3
	4	91.5	6.5
	1	127	12.4
Citral B (neral)	16	79.5	9.5
	4	90	8.5
	1	128	1.6

[a] Recovery = 1 - [spiked concentration - measured concentration/spiked concentration] 100
[b] RSD, relative standard deviation.

Finally, chromatographic analysis of swine plasma harvested from blood sample taken at the 14th, 21st and 28th day after application of essential oils of *C. citrates* spray in swine buildings showed no detectable amount of citral in the plasma (Figure. 3).

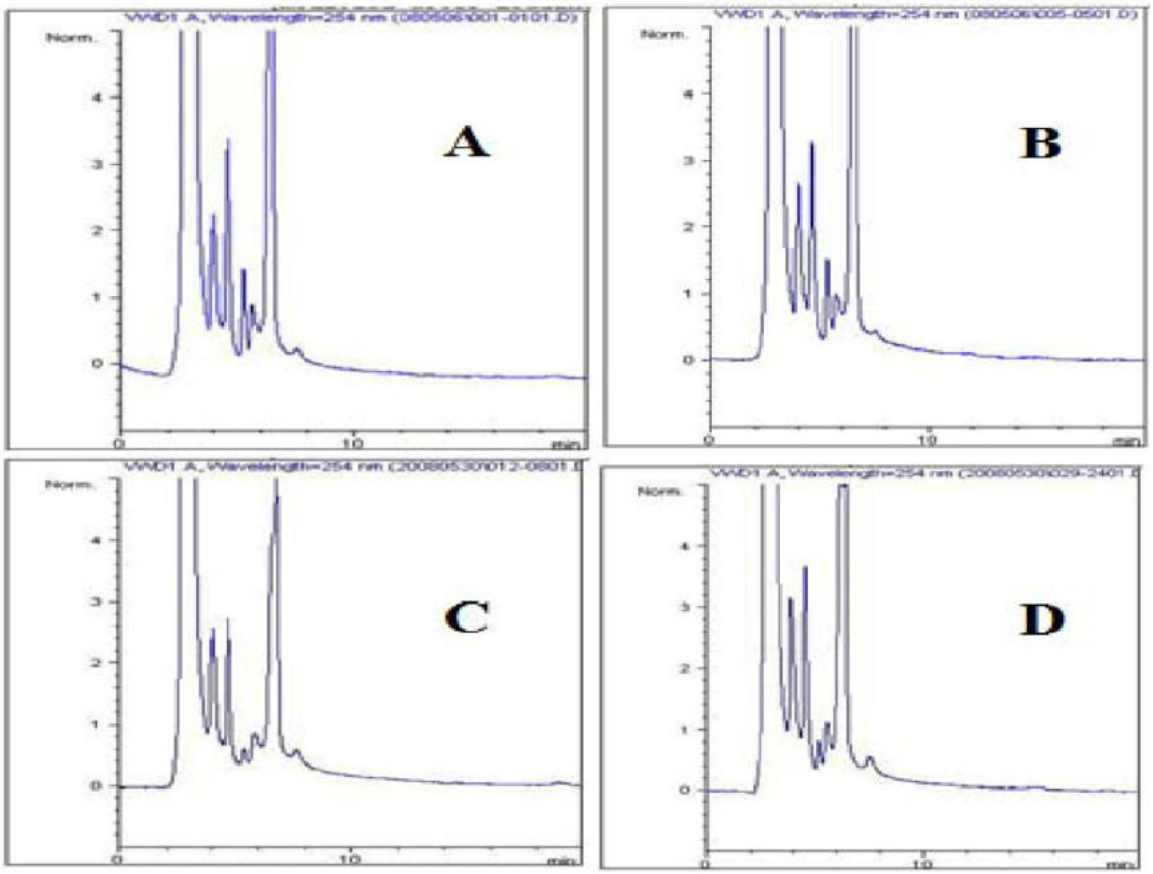

Figure 2. HPLC chromatograms of plasma sample prior to spraying (A); at day 14 (B), 21 (C) and 28 (D) after spraying of *C. citratus*

Conclusion

The method established in this study is the first HPLC-UV assay with simultaneous detection of citral in swine plasma. The analytical method set in this study can be considered as simple, fast, economic and reliable in routine analyses of complex matrices. In addition, there was no detectable level of citral in plasma of swine after being exposed to 3% concentration of essential oils of lemon grass for 28 days. The findings of this study warrants further investigation on plasma residues of citral at higher concentration of exposure and detailed pharmacokinetics parameters of citral.

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Competing interest

There is no conflict of interest.

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