



HAL
open science

A fast and reliable method to quantify neral and geranial (citral) in essential oils using ^1H NMR spectroscopy

Mathieu Paoli, Thomas Maroselli, Joseph Casanova, Ange Bighelli

► **To cite this version:**

Mathieu Paoli, Thomas Maroselli, Joseph Casanova, Ange Bighelli. A fast and reliable method to quantify neral and geranial (citral) in essential oils using ^1H NMR spectroscopy. *Flavour and Fragrance Journal*, 2023, 38 (6), pp.476-482. <10.1002/ffj.3760>. <hal-05034500>

HAL Id: hal-05034500

<https://hal.science/hal-05034500v1>

Submitted on 15 Apr 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

A fast and reliable method to quantify neral and geranial (citral) in essential oils using ^1H NMR spectroscopy

Mathieu Paoli | Thomas Maroselli | Joseph Casanova | Ange Bighelli

Université de Corse, UMR CNRS 6134 Sciences Pour l'Environnement, Equipe Chimie et Biomasse, Ajaccio, France

Correspondence

Mathieu Paoli, Université de Corse, UMR CNRS 6134 Sciences Pour l'Environnement, Equipe Chimie et Biomasse. Routes des Sanguinaires, 20000 Ajaccio, France.
Email: paoli_m@univ-corse.fr

Abstract

Citral (3,7-dimethyl-2,6-octadienal), a mixture of geranial and neral (two stereoisomeric monoterpene aldehydes), is considered an important raw material in the fields of fragrance, pharmaceutical, food and cosmetic industries. It is a well-known biologically active compound present in various essential oils. A fast and reliable experimental method was developed and validated in order to quantify citral in essential oils using ^1H NMR spectroscopy (experiment time at the minute level). The quantitative procedure, using anisole as internal standard, showed a perfect linearity of measurements ($R^2 = 0.9999$), a very good accuracy (relative errors comprised between -1.9% and 0.3%) and an excellent precision (reproducibility $17.8\text{ mg} \pm 1.0\%$). The procedure was also checked with three mixtures of weighted monoterpenes and the contents of citral, measured by GC and ^1H NMR, were absolutely comparable. Then, the method was successfully applied to measure the contents of citral in 10 commercially available essential oils. Among the investigated samples, citral varied from 2.9% (*Zingiber officinalis*) to 90.6% (*Bakhouisia citriodora*).

KEYWORDS

^1H NMR, citral, essential oils, quantitative determination

1 | INTRODUCTION

Citral (3,7-dimethyl-2,6-octadienal) is a mixture of two stereoisomeric monoterpene aldehydes, that is, geranial (E configuration of the double bond) and neral (Z configuration of the double bond) (Figure 1). Citral appears as a clear yellow coloured liquid with a lemon-like odour, less dense than water and insoluble in water. It is present in various essential oils at variable content and, in general, both stereoisomers are simultaneously present.

Citral is considered an important raw material; it is a basic product in the fragrance, pharmaceutical, food and cosmetic industries. For instance, it is used as an ingredient in aromatherapy or as scenting of soaps and toiletries.^{1,2} It is generally obtained

from lemongrass (*Cymbopogon flexuosus*) by fractional distillation (purity 95%), adduct formation and regeneration or chromatography column.³ In addition, it could be synthesized, for instance, using the Claisen–Cope tandem rearrangement,⁴ and could be selectively hydrogenated to nerol/geraniol, citronellal and citronellol which are also important perfumery products.⁵ Citral is a starting material for the synthesis, at industrial scale, of various commercially important compounds such as menthol, ionones and vitamin A.⁶

The biological activity of citral, as well as citral-rich essential oils, has been extensively investigated and covers various fields. Indeed, citral antimicrobial resistance was observed in various *Acinetobacter baumannii* strains with two strains confirmed as

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Flavour and Fragrance Journal* published by John Wiley & Sons Ltd.

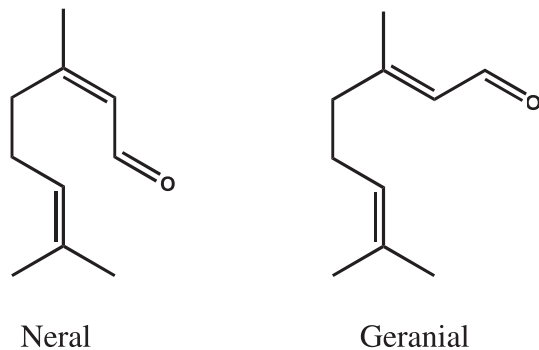


FIGURE 1 Chemical structures of neral and geranial (citral).

multi-drug resistant.⁷ Citral also exhibited excellent antifungal effects against common grain pathogens (*Aspergillus flavus* and *Aspergillus ochraceus*) in vitro and in situ⁸ and it has been demonstrated to have an antiproliferative effect in certain tumour cell lines.⁹ Furthermore, a group of mice treated with an “essential oil citral” showed a significant decrease in TNF- α levels in tests demonstrating anti-inflammatory activity.¹⁰ Vasodilation induced by citral was also investigated in pre-contracted vascular smooth muscle using thoracic aorta from Wistar Kyoto (WKY) rats¹¹ and the activity of citral on cytotoxicity, apoptosis and anti-proliferative effects using human lymphoma Ramos cells has been evaluated.¹² Citral has been reported to show cytotoxic effects on various cancer cell lines. In vitro studies revealed the significant cytotoxic activity of citral on the proliferation of pancreatic cell tumour lines and human B-lymphoma.⁹ Citral-rich essential oil of *Cymbopogon citratus* showed high ability to scavenge DPPH radicals. The essential oil of *C. citratus* was effective on prostate cell lines and glioblastoma cell lines.¹³ Other results suggest that citral could be a potential new agent, which can eliminate drug-resistant breast cancer cells in a spheroid model via inducing apoptosis.¹⁴ Otherwise, citral completely inhibited UVB-induced skin carcinogenesis by reducing levels of oxidative stress and pro-inflammatory cytokines, increasing apoptotic rate in the skin.¹⁵ Lastly, citral significantly inhibits hepatocellular carcinogenesis by restoring the antioxidants and phase II xenobiotic enzyme levels; thereby, it strongly proves as an antiproliferative agent against rat HCC.¹⁶

Due to the importance of citral in the fragrance, pharmaceutical, food and cosmetic industries and its availability in specific essential oils, the quantitative determination of this raw product is crucial and has been investigated by using various techniques. Obviously, due to the volatility of citral, GC (with or without using response factors) is the common technique developed in most analytical and quality control laboratories. Other techniques include reverse-phase high-performance liquid chromatography (HPLC),¹⁷ high-performance thin-layer chromatography (HPTLC)¹⁸ or stable-isotope gas chromatographic analysis.¹⁹ Concerning GC-MS, a specific procedure, developed by IFRA concerned the identification and quantification of citral (as well as 56 other allergens), achieved through the selected ion monitoring mode via the relative

abundance of characteristic fragments ions.²⁰ This procedure has a calibration range between 0.002% (20 $\mu\text{g/g}$) and 0.25% (2.5 mg/g) and it is not suited to the quantitation of citral in citral-rich EOs. Indeed, the concentration of citral in EOs varies drastically and may reach 90%, with more or less similar contents of neral and geranial. Preparation of the essential oil sample needs successive dilutions and it has been observed that sample dilutions greater than 250X may lead to problems of reproducibility. Otherwise, the GC signals of neral and/or geranial may overlap with those of other components and then quantitative determination remains erroneous. For instance, according to Adams (GC column = DB5), the signal with RI = 1235 may belong to neral, chrysanthenyl acetate, isobornyl formate or (E)-ocimene.²¹ The signal with RI = 1264, may belong to geranial or (E)-2-propenylphenol.²¹ According to Mass Finder (GC column = DB1), the signal with RI = 1215 may belong to neral, pulegone, cis-pulegol, thymol methyl ether or thymoquinone, and the signal with RI = 1244 may belong to geranial or trans-carvone epoxide.²²

Concerning NMR, quantitative determination of individual components using ¹H NMR spectroscopy has been preferably carried out on plant extracts and has been reviewed.^{23–27} Regarding the quantitative determination of components in essential oils, the first attempts date back to 1980 and concerned the quantitation of carvone in caraway and dill oils (60MHz spectrometer).²⁸ Since that time, a few studies have been reported concerning ¹H NMR quantitative determination of some components of essential oils: sesquiterpene hydrocarbons and benzyl benzoates (*Brickellia veronicaefolia*)²⁹; trans-anethole (fennel)³⁰; α -pinene (*Eucalyptus* and *Corymbia* species, *Schinus terebinthifolius*, turpentine)³¹; α -bisabolol (*Eremanthus erythropappus*)³²; menthofuran (*Mentha aquatica*)³³; geraniol, citronellol and citronellal (*Cymbopogon* species)³⁴; thymol and trans-anethole (*Foeniculum vulgare* and *Zataria multiflora*)³⁵; and thermolabile chemical markers (*Curcuma caesia*).³⁶ A comparative study of quantification of methyleugenol in foods concluded that GC-MS is suited for regulatory purposes but is time-consuming and NMR can be used for a faster screening analysis.³⁷

In this context, the aim of the present work was to develop a fast and reliable method to quantify citral, a product of commercial importance, in various essential oils by using ¹H NMR spectroscopy.

2 | EXPERIMENTAL

2.1 | Essential oil samples and pure compounds

Essential oil samples of *Bakhouisia citriodora* (origin: Australia), *Cymbopogon flexuosus* (origin: India), *Litsea citrata* (origin: China), *Melissa officinalis* (origin: Greece), *Leptospermum petersonii* (origin: South Africa), *Eucalyptus staigeriana* (origin: Brazil) and *Lippia citriodora* (origin: Morocco) were purchased from Aroma-Zone (Cabrière d'Avignon, France); sample of *Zingiber officinalis* (origin: Sri Lanka) from Paradesa (St Martin de la Brasque, France); and samples of *Thymus vulgaris* subsp. *canariensis* (origin: Spain) and *Cymbopogon citratus*

(origin: France) from *Kalyptoscorse* (Aghione, France). Pure compounds (citral, nonane and anisole) were provided by Sigma-Aldrich.

2.2 | Analytical gas chromatography (GC)

Analyses were carried out with a Clarus 500 Perkin Elmer apparatus equipped with two flame ionization detectors (FID), two fused-silica capillary columns (50 m, 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60°C to 220°C at 2°C/min and then held isothermal at 220°C for 20 min. The injector temperature was 250°C. The detector temperature was 250°C. The carrier gas was hydrogen (1.0 mL/min). The split was 1/60 and the injected volume was 0.5 μL . The neral and geranial contents were expressed as a percentage obtained using response factors (RF = 1.30 for the two compounds) according to Bicchi et al.³⁸ Nonane was used as internal reference.

2.3 | Nuclear magnetic resonance (NMR) spectroscopy

All NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 400.132 MHz for ^1H equipped with a 5-mm probe in deuterated chloroform (CDCl_3) with all chemical shifts referred to as internal tetramethylsilane (TMS). ^1H NMR spectra for quantitation of citral were recorded with the following parameters: flip angle 30°, acquisition time, 2.56 s for 32 K data table with a spectral width of 6410 Hz (16 ppm), relaxation delay $D_1 = 1.0$ s (total recycling time = 3.56 s) and spectra recorded with 16 scans.

The T_1 (longitudinal relaxation delay) values of the ^1H nuclei were determined by the inversion recovery method, using the standard sequence: 180°- τ -90°- D_1 , with a relaxation delay D_1 of 5.0 s for ^1H . Each delay of inversion (τ) was, thus, taken into account for the computation of the corresponding T_1 using the function $I_p = I_0 + p \cdot e^{-\tau/T_1}$. The limits of detection (LOD) and quantitation (LOQ) were determined experimentally using signal-to-noise ratio (S:N). According to Cerceau et al.,³² the concentrations for S:N = 10 and for S:N = 150 were set as LOD and LOQ respectively. Appropriate mass of citral (2.5–30.3 mg) was introduced in the NMR tube.

3 | RESULTS AND DISCUSSION

Quantitative determination of a component in a mixture can be determined by NMR spectroscopy by comparison of the area(s) of one or various signal(s) of the compound to be quantified, with those of an internal standard (internal calibration). Such a procedure requires a homogenous answer of selected nuclei of the compound and the internal reference, which depends mainly on the longitudinal relaxation time (T_1) of nuclei.

In our laboratories, quantitation of compounds such as ursolic and oleanolic acids in a dichloromethane extract from *Ilex aquifolium*³⁹ or 5-hydroxymethyl-2(5H)-furanone, the main compound of the *Helleborus lividus* subsp. *corsicus* dichloromethane leaf extract,⁴⁰ has been achieved using ^1H NMR, with a time of occupancy of the spectrometer at the minute level.

In order to quantify citral in essential oils by ^1H NMR, we selected the signals of the aldehydic protons of neral (doublet, 9.88 ppm) and geranial (doublet, 9.98 ppm) which are perfectly resolved and do not overlap with those of most components present in essential oils. Experiments have been carried out in deuterated chloroform (CDCl_3), and anisole was chosen as internal reference using the signal of its methoxy group (singlet, 3.80 ppm). Indeed, T_1 value of the methoxy protons of anisole, measured by the inversion recovery method in CDCl_3 ($T_1 = 0.7$ s), was similar to those of the aldehydic protons of neral and geranial ($T_1 = 0.6$ s for both protons) in the same solvent.

Then, to quantify citral in commercial essential oils, we selected a pulse sequence characterized by a flip angle of 30° and a total recycling time of 3.56 s (see Experimental). According to Becker et al.,⁴¹ we determined and plotted the percentage of recovered signal S:N (%) as a function of the pulse angle for the selected protons of neral, geranial and anisole. The difference in the steady-state magnetization ($\Delta M_z = 0.02\%$) is extremely small.

This quantitative procedure was validated by several experiments carried out on pure citral (accuracy, response linearity and precision, determination of limits of detection and quantification) and mixtures of various terpenes generally present in essential oils.

Firstly, we checked the accuracy of the procedure by comparing the amounts of pure citral, diluted in CDCl_3 , at different concentrations and measured by ^1H NMR with the weighted ones (in the range 2.5–30.3 mg), taking into account the relative areas of the methoxy protons of anisole and those of each aldehydic proton of neral and geranial (Table 1, contents calculated using equation 1). The relative errors, comprised between -1.9% and 0.3%, demonstrated a very good accuracy of measurements.

$$m_C = \frac{3 \times A_C \times M_C \times m_A \times P_A}{A_A \times M_A} \quad (1)$$

m_C : calculated mass (mg) of citral; A_C and M_C : area of the selected aldehydic signals and molecular weight (152.24 $\text{g} \cdot \text{mol}^{-1}$) of citral; m_A , M_A and A_A : amount (mg), molecular weight (108.14 $\text{g} \cdot \text{mol}^{-1}$) and area of the signal of the methoxy protons of anisole (A_A fixed at 1.0000 in all experiments); P_A : purity of anisole (99%). All the experiments have been recorded in 0.5 mL of CDCl_3 . Factor 3 is due to the integration of the three protons of the methoxy group of anisole.

Response linearity has been evaluated by plotting the calculated mass of citral versus the weighted one (calibration line). A perfect linearity of the measurements was observed (linear determination factor $R^2 = 0.9999$) (Figure 2).

Furthermore, five spectra of the same sample containing citral (18.0 mg) and anisole (13.4 mg) have been recorded with the same

Experience n°	Citral weighted mass ^a (mg)	Citral area (neral/geranial)	Citral (neral/geranial) calculated mass	RE (%)
1	2.5	0.0447 (0.0220/0.0227)	2.5 (1.2/1.3)	0.0
2	5.0	0.0894 (0.0443/0.0451)	5.0 (2.5/2.5)	0.0
3	10.3	0.1807 (0.0893/0.0914)	10.1 (5.0/5.1)	-1.9
4	13.9	0.2466 (0.1213/0.1253)	13.8 (6.8/7.0)	-0.7
5	18.0	0.3170 (0.1570/0.1600)	17.8 (8.8/9.0)	-1.1
6	23.8	0.4234 (0.2078/0.2156)	23.7 (11.6/12.1)	-0.4
7	30.3	0.5426 (0.2681/0.2745)	30.4 (15.0/15.4)	0.3

Note: Selected signals for neral δ = 9.88 ppm and geranial δ = 9.98 ppm; calculated mass according to Equation 1; A_A fixed at 1.0000 in all experiments; m_A : mass of anisole = 13.4 mg; RE: relative errors (%);

^aCitral weighted mass taking into account its purity (95%).

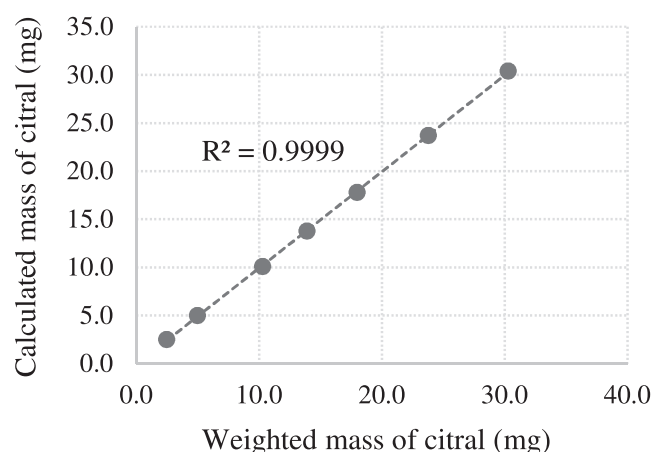


FIGURE 2 Response linearity of quantitation of citral using ¹H NMR.

experimental conditions to check the precision of the procedure (Table 2). The measured mass of citral, calculated using equation 1, was almost identical (17.7–17.9 mg). The relative errors between the calculated mass and the weighted one ranged from -1.7% to -0.6% (Table 2). The reproducibility (tube-to-tube variability) of 17.8 mg \pm 1.0% (17.8 mg \pm 0.18 mg), calculated with a confidence interval of 99% (Student's *t*-test), indicated a very good precision of measurements.

Finally, the limit of detection (LOD) and the limit of quantification (LOQ) (see Experimental) have been measured at 0.02 mg and 0.48 mg respectively.

We checked our experimental quantitative procedure on three mixtures of terpenes generally present in essential oils, that is, M1 = mixture of six acyclic compounds: citral (6.9 mg), neral (17.9 mg), geranial (9.7 mg) and neryl acetate/geranyl acetate (15.1 mg); M2 = mixture of six aldehydes: citral (5.1 mg), citronellal (18.3 mg), vanillin (15.2 mg), myrtenal (10.7 mg) and cinnamaldehyde (4.9 mg); and M3 = mixture of six monoterpenes with various functional groups: citral (18.8 mg), camphor (12.5 mg), linalyl acetate (9.1 mg),

α -pinene (6.2 mg) and terpinen-4-ol (7.4 mg) (Table 3). In each mixture, the content of citral, measured by ¹H NMR using the validated quantitative procedure, was compared with the weighted one, and with that measured by GC with response factors and an internal reference,³⁸ which is the common technique to quantify volatile compounds (Table 3). The results reported in Table 3 showed that the reliable determinations of the mass of citral in the mixtures M1–M3 are available from the ¹H NMR spectra. Relative errors between calculated masses by ¹H NMR and the weighted ones, comprised between -1.4% and 0.0%, certified the efficiency of the quantitative procedure. Otherwise, the masses of citral calculated by ¹H NMR are closer to the weighted ones than those calculated by GC using response factors. Therefore, the ¹H NMR procedure could be applied to quantify citral (neral and geranial) in commercial essential oil samples.

The validated quantitative method has been used to evaluate the content of citral (neral and geranial) in 10 commercial essential oils: *Bakhousia citriodora*, *Cymbopogon flexuosus*, *Litsea citrata*, *Cymbopogon citratus*, *Melissa officinalis*, *Thymus vulgaris* subsp. *canariensis* (Figure 3), *Leptospermum petersonii*, *Eucalyptus staigeriana*, *Lippia citriodora* and *Zingiber officinalis* (Table 4). Among the investigated oil samples, citral ranged from 2.9% to 90.6% of the whole composition (content calculated using ¹H NMR), although neral and geranial were the major components of most of the essential oils. *Bakhousia citriodora* essential oil was found to have the highest content of citral, that is, 90.6% (neral: 36.6% and geranial: 54.0%), while *Zingiber officinalis* essential oil exhibited the lowest content (citral 2.9%: neral 1.0% and geranial 1.9%). Similar quantitation has been carried out with the classical GC method using response factors.

4 | CONCLUSION

A fast and reliable experimental procedure, using ¹H NMR spectroscopy, was validated (accuracy, precision and linearity of measurements) in order to quantify citral (neral and geranial), a

TABLE 2 Precision of citral measurements using ^1H NMR.

	Test 1	Test 2	Test 3	Test 4	Test 5
Citral weighted mass ^a (mg)	18.0	18.0	18.0	18.0	18.0
Citral area (neral/geranial)	0.3170 (0.1570/0.1600)	0.3159 (0.1559/0.1600)	0.3172 (0.1559/0.1613)	0.3173 (0.1562/0.1611)	0.3193 (0.1566/0.1627)
Citral calculated mass (mg) (neral/geranial)	17.8 (8.8/9.0)	17.7 (8.7/9.0)	17.8 (8.8/9.0)	17.8 (8.8/9.0)	17.9 (8.8/9.1)
RE (%)	-1.1	-1.7	-1.1	-1.1	-0.6

Note: The signal area of the methoxy protons of anisole is fixed at 1.0000 in all experiments; citral area: area of the selected signals of citral (neral and geranial); mass of anisole = 13.4 mg; calculated mass according to Equation (1); RE: relative error.

^aCitral weighted mass taking into account its purity (95%).

TABLE 3 Quantitation of citral in mixtures M1, M2 and M3 using ^1H NMR.

	Citral weighted mass ^a (mg)	Citral % in mixture	^1H NMR			GC	
			Citral area (neral/geranial)	Citral (neral/geranial) calculated mass (mg)	RE % (citral)	Citral (neral/geranial) content %	Citral (neral/geranial) content %
M1	6.9	13.9	0.1488 (0.0725/0.0763)	6.8 (3.3/3.5)	-1.4	13.8 (6.7/7.1)	13.8 (6.7/7.1)
M2	5.1	9.4	0.1103 (0.0527/0.0576)	5.1 (2.4/2.7)	0.0	9.4 (4.4/5.0)	8.5 (4.2/4.3)
M3	18.8	34.8	0.3943 (0.1933/0.2010)	18.8 (9.2/9.6)	0.0	34.8 (17.0/17.8)	33.6 (16.5/17.1)

Note: Mixture M1 (neral, geranial, nerol, geraniol, neryl acetate and geranyl acetate), m1 = 49.6 mg; Mixture M2 (neral, geranial, citronellal, vanillin, myrtenal and cinnamaldehyde), m2 = 54.2 mg; Mixture M3 (neral, geranial, α -terpineol, linalyl acetate, camphor and α -pinene), m3 = 54.0 mg. The signal area of the methoxy protons of anisole is fixed at 1.0000 in all experiments; citral area: area of the selected signals of citral (neral and geranial); mass of anisole: 11.0 mg for mixtures M1 and M2 and 11.4 mg for mixture M3; calculated mass according to Equation (1); RE: relative error.

^aCitral weighted mass taking into account its purity (95%).

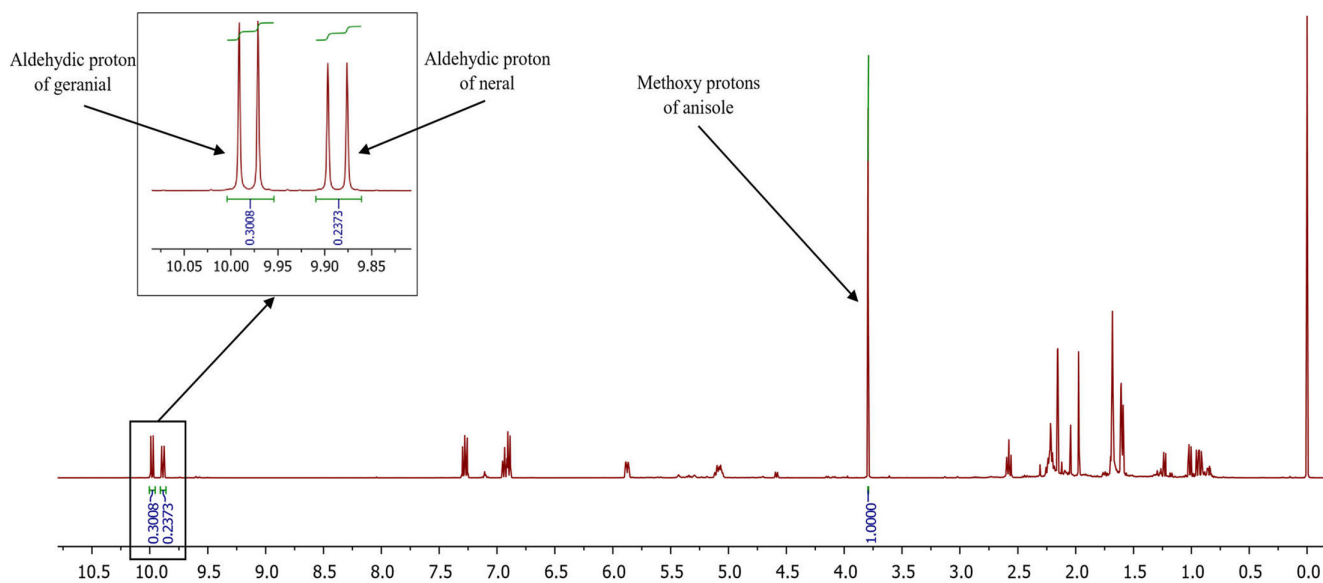


FIGURE 3 ^1H NMR spectrum of *Thymus vulgaris* subsp. *canariensis* commercial essential oil used for the quantitation of citral (neral and geranial).

well-known biologically active compound used in the field of pharmaceutical, food and cosmetic industries. This quantitative method was successfully applied to measure the content of citral in various commercially available essential oil samples. Therefore,

^1H NMR spectroscopy appeared as a powerful tool for quantitation of valuable components of essential oils with a short time of occupancy of the spectrometer that allows the use of this technique for quality control for instance.

TABLE 4 Quantitation of citral in commercial essential oils using ¹H NMR – comparison with GC.

Essential oil samples	¹ H NMR		GC	
	Essential oil weighted mass (mg)	Citral area (neral/geranial)	Citral calculated mass (mg) (neral/geranial)	Citral content % (neral/geranial)
<i>Bakhouisia citriodora</i>	58.3	0.9569 (0.3859/0.5710)	52.8 (21.3/31.5)	90.6 (36.6/54.1)
<i>Cymbopogon flexuosus</i>	54.3	0.7243 (0.3162/0.4081)	39.4 (17.4/22.5)	74.2 (32.2/42.0)
<i>Litsea citrata</i>	52.7	0.6559 (0.2744/0.3815)	37.3 (15.6/21.7)	71.7 (30.0/41.7)
<i>Cymbopogon citratus</i>	51.4	0.8926 (0.3711/0.5215)	35.7 (14.8/20.9)	68.4 (28.3/40.1)
<i>Melissa officinalis</i>	57.6	0.5984 (0.2516/0.3468)	33.0 (13.88/19.1)	57.0 (23.9/33.1)
<i>Thymus vulgaris</i> subsp. <i>canariensis</i>	54.3	0.5381 (0.2373/0.3008)	30.6 (13.5/17.1)	57.6 (25.3/32.3)
<i>Leptospermum petersonii</i>	52.1	0.4692 (0.2029/0.2663)	25.9 (11.2/14.7)	49.4 (21.3/28.1)
<i>Eucalyptus staigeriana</i>	52.2	0.2196 (0.0942/0.1254)	12.1 (5.2/6.9)	23.4 (10.0/13.4)
<i>Lippia citriodora</i>	52.5	0.1678 (0.0734/0.0944)	9.3 (4.1/5.2)	18.1 (8.0/10.1)
<i>Zingiber officinalis</i>	50.9	0.0307 (0.0105/0.0202)	1.5 (0.5/1.0)	3.0 (1.1/1.9)

Note: Signal area of the methoxy protons of anisole is fixed at 1.0000 in all experiments; mass of anisole (m_A): 13.2 mg for *Leptospermum petersonii*, *Lippia citriodora*, *Melissa officinalis*, *Eucalyptus staigeriana*, *Cymbopogon flexuosus* and *Bakhouisia citriodora* essential oil samples; 13.6 mg for *Litsea citrata* and *Thymus vulgaris* subsp. *canariensis* essential oil samples; 9.6 mg for *Cymbopogon citratus* essential oil sample; 11.4 mg for *Zingiber officinalis* essential oil sample; citral calculated mass (mg) according to Equation 1.

ACKNOWLEDGMENTS

The authors are grateful to the Collectivité de Corse for the research grant.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Breitmaier E. *Flavors, Fragrances, Pharmaca, Pheromones*. Wiley-VCH Tübingen; 2006:11-13.
- Idrees M, Hakkim L, Naikoo G, Ul HI. Recent advances in extraction, characterization, and potential use of citral. *Natural Bio-Active Compounds*. Springer Berlin; 2019:225-236.
- Savira A, Syahrani A, Rudyanto M. A simple method for isolation of citral using column chromatography. *Proceedings of the BROMO Conference Symposium on Natural Product and Biodiversity*. Science and Technology Publications; 2018:1-7. doi:10.5220/000984150002406
- Nowicki J. Claisen, cope and related rearrangements in the synthesis of flavour and fragrance compounds. *Molecules*. 2000;5:1033-1050. doi:10.3390/50801033
- Chen YZ, Liaw BJ, Chiang SJ. Selective hydrogenation of citral over amorphous NiB and CoB nano-catalysts. *Appl Catal A*. 2005;284:97-104. doi:10.1016/j.apcata.2005.01.023
- Pihlasalo J, Klika KD, Muezin DY, Nieminen V. Conformational equilibria of citral. *J Mol Struct THEOCHEM*. 2007;814:33-41. doi:10.1016/j.theochem.2007.02.031
- Adukwu EC, Bowles M, Edward-Jones V, Bone H. Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus*) and pure citral. *Appl Microbiol Biotechnol*. 2016;100:9619-9627. doi:10.1007/s00253-016-7807-y
- Tang X, Shao YL, Tang YJ, Zhou WW. Antifungal activity of essential oil compounds (geraniol and citral) and inhibitory mechanisms on grain pathogens (*aspergillus flavus* and *aspergillus ochraceus*). *Molecules*. 2018;23:2108. doi:10.3390/molecules23092108
- Di Mola A, Massa A, de Feo V, Basile A. Effect of citral and citral related compounds on viability of pancreatic and human B-lymphoma cell lines. *Medicinal Chem Res*. 2017;26:631-639. doi:10.1007/s00044-017-1779-z
- Braga Martins H, Das Neves Selis N, Leal Silva e Souza C, et al. Anti-inflammatory activity of the essential oil citral in experimental infection with *Staphylococcus aureus* in a model air pouch. *Evidence-Based Complementary and Alternative Medicine*. 2017;2017:2505610. doi:10.1155/2017/2505610
- Pereira S, Marques A, Sudo RT, Kaplan MA, Zapata-Sudo G. Vasodilator activity of the essential oil from aerial parts of *Pectis brevipedunculata* and its main constituent citral in rat aorta. *Molecules*. 2013;18:3072-3085. doi:10.3390/molecules18033072
- Dangkong D, Limpanasithikul W. Effect of citral on the cytotoxicity of doxorubicin in human B-lymphoma cells. *Pharm Biol*. 2014;53:262-268. doi:10.3109/13880209.2014.914233
- Bayala B, Bassole IHN, Maqdasly S, Baron S, Simpore J, Lobaccaro JMA. *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule. *Biochimie*. 2018;153:162-170. doi:10.1016/j.biochi.2018.02.013
- Nigjeh SE, Yeap SK, Nordin N, Kamalideghan B, Ky H, Rosli R. Citral induced apoptosis in MDA-MB-231 spheroid cells.

- BMC Complement Altern Med.* 2018;18:56. doi:10.1186/s12906-018-2115-y
15. Kremer JL, Melo GP, Marinello PC, et al. Citral prevents UVB-induced skin carcinogenesis in hairless mice. *J Photochem Photobiol B.* 2019;198:111565. doi:10.1016/j.jphotobiol.2019.111565
 16. Krishnan P, Sundaram J, Salam S, et al. Citral inhibits N-nitrosodiethylamine-induced hepatocellular carcinoma via modulation of antioxidants and xenobiotic-metabolizing enzymes. *Environ Toxicol.* 2020;35:971-981. doi:10.1002/tox.22933
 17. Gaonkar R, Yallappa S, Dhananjaya BL, Hedge G. Development and validation of reverse phase high performance liquid chromatography for citral analysis from essential oils. *J Chromatogr B.* 2016;1036-1037:50-56. doi:10.1016/j.jchromb.2016.10.001
 18. Faiyazuddin M, Ali J, Ahmad S, Ahmad N, Akhtar J, Baboota S. Chromatographic analysis of trans and cis-citral in lemongrass oil and in a topical phytonanocosmeceutical formulation, and validation of the method. *J Planar Chromatogr - Mod TLC.* 2010;23:233-236. doi:10.1556/jpc.23.2010.3.14
 19. Nhu-Trang TT, Casabianca H, Grenier-Loustalot MF. Authenticity control of essential oils containing citronellal and citral by chiral and stable-isotope gas-chromatographic analysis. *Anal Bioanal Chem.* 2006;386:2141-2152. doi:10.1007/s00216-006-0842-2
 20. The International Fragrance Association (IFRA), Analytical Working Group. Analytical method to quantify 57 suspected Allergens (and Isomers) in ready to inject fragrance materials by gas chromatography and mass spectrometry. Brussels. 2016 [https://ifrafragrance.org/docs/default-source/guidelines/23754_gd_2017_04_11_ifra_analytical_method_to_quantify_57_suspected_allergens_\(and_isomers\)_in_ready_to_inject_fragrance_materials_by_gc-ms-\(3\).pdf?sfvrsn=ad55ac1_6](https://ifrafragrance.org/docs/default-source/guidelines/23754_gd_2017_04_11_ifra_analytical_method_to_quantify_57_suspected_allergens_(and_isomers)_in_ready_to_inject_fragrance_materials_by_gc-ms-(3).pdf?sfvrsn=ad55ac1_6)
 21. Adams RP. *Identification of Essential Oil Components by Gas Chromatography-Mass Spectrometry.* 4th ed. Allured Publishing Corporation; 2007.
 22. Terpenoids Library Website. https://massfinder.com/wiki/Terpenoids_Library_List.
 23. Holzgrabe U, Deubner R, Schollmayer C, Waibel B. Quantitative NMR spectroscopy. Applications in drug analysis. *J Pharm Biomed Anal.* 2005;38:806-812. doi:10.1016/j.jpba.2005.01.050
 24. Pauli GF, Jaki BU, Lankin DC. Quantitative ¹H NMR: development and potential of a method for natural products analysis. *J Nat Prod.* 2005;68:133-149. doi:10.1021/np0497301
 25. Beyer T, Schollmayer C, Holzgrabe U. The role of solvents in the signal separation for quantitative ¹H NMR spectroscopy. *J Pharm Biomed Anal.* 2010;52:51-58. doi:10.1016/j.jpba.2009.12.007
 26. Bharti SK, Roy R. Quantitative ¹H NMR spectroscopy. *TrAC Trends Anal Chem.* 2012;35:5-26. doi:10.1016/j.trac.2012.02.007
 27. Wang Y, Wang X, Hu G, et al. Chemical ingredients characterization basing on ¹H NMR and SHS-GC/MS in twelve cultivars of *Coffea arabica* roasted beans. *Food Res Int.* 2021;147:110544. doi:10.1016/j.foodres.2021.110544
 28. Mossa JS, El-Obeid HA, Hassan MMA. PMR assay of essential oils. IV. Assay of carvone in caraway and dill oils. *Spectrosc Lett.* 1980;13:49-57. doi:10.1080/00387018008065070
 29. Rivero-Cruz B, Rivero-Cruz I, Rodriguez JM, Cerda-Garcia-Rojas CM, Mata R. Qualitative and quantitative analysis of the active components of the essential oil from *Brickellia veronicaefolia* by nuclear magnetic resonance spectroscopy. *J Nat Prod.* 2006;69:1172-1176. doi:10.1021/np060180b
 30. Abouzid S. Use of nuclear magnetic resonance spectroscopy in analysis of fennel essential oil. *Nat Prod Sci.* 2016;22:30-34. doi:10.20307/nps.2016.22.1.30
 31. Cerceau CI, Barbosa LCA, Filomeno CA, Alvarenga ES, Demuner AJ, Fidencio PH. An optimized and validated ¹H NMR method for the quantification of α -pinene in essential oils. *Talanta.* 2016;150:97-103. doi:10.1016/j.talanta.2015.10.087
 32. Cerceau CI, Barbosa LCA, Alvarenga ES, Ferreira AG, Thomasi SS. A validated ¹H NMR method for quantitative analysis of α -bisabolol in essential oils of *Eremanthus erythropappus*. *Talanta.* 2016;161:71-79. <https://doi.org/10.1016/j.talanta.2016.08.032>
 33. Manolache FA, Todasca C, Ionescu V, Iorga E, Berldie A, Matei M. Authentication of wild *mentha aquatica*, from Danube delta, using ¹H-NMR spectroscopy. *U.P.B. Sci Bull, Series B: Chemistry and Materials Science.* 2018;80:127-134.
 34. Cerceau CI, Barbosa LCA, Alvarenga ES, Maltha CRA, Ismail FMD. ¹H-NMR and GC for detection of adulteration in commercial essential oils of *Cymbopogon* ssp. *Phytochem Anal.* 2020;31:88-97. doi:10.1002/pca.2869
 35. Reyhani Y, Iranshahi M, Taghizadeh SF, Saberi S, Farhadi F. An evaluation of qH NMR: a complementary approach to GC-FID for quantification of thymol and trans-anethole in essential oils and supplements. *J Pharm Biomed Anal.* 2022;220:114992. doi:10.1016/j.jpba.2022.114992
 36. Mahanta BP, Sut D, Kemprai P, Paw M, Lal M, Haldar S. A ¹H-NMR spectroscopic method for the analysis of thermolabile chemical markers from the essential oil of black turmeric (*Curcuma caesia*) rhizome: application in post-harvest analysis. *Phytochem Anal.* 2019;31:28-36. doi:10.1002/pca.2863
 37. Grosch S, Monakhova YB, Kuballa T, Ruge W, Kimmich R, Lachenmeier DW. Comparison of GC/MS and NMR for quantification of methyleugenol in food. *Eur Food Res Technol.* 2013;236:267-275. doi:10.1007/s00217-012-1879-4
 38. Bicchi C, Liberto E, Matteodo M, et al. Quantitative analysis of essential oils: a complex task. *Flavour Fragr J.* 2008;23:382-391. doi:10.1002/FFJ.1905
 39. Palu D, Bighelli A, Casanova J, Paoli M. Identification and quantitation of ursolic and oleanolic acids in *Ilex aquifolium* L. leaf extracts using ¹³C and ¹H-NMR spectroscopy. *Molecules.* 2019;24:4413. doi:10.3390/molecules24234413
 40. Maroselli T, Paoli M, Bighelli A. A validated ¹H NMR method for the quantitation of 5-hydroxymethyl-2(5H)-furanone, a valuable chemical intermediate, in a dichloromethane extract of *Helleborus lividus* subsp. *corsicus* leaves from Corsica. *J Anal Methods Chem.* 2022;2022:9580338. doi:10.1155/2022/9580338
 41. Becker ED, Ferretti JA, Gambhir PN. Selection of optimum parameters for pulse Fourier transform nuclear magnetic resonance. *Anal Chem.* 1979;51:1413-1420. doi:10.1021/ac50045a016

How to cite this article: Paoli M, Maroselli T, Casanova J, Bighelli A. A fast and reliable method to quantify neral and geranial (citral) in essential oils using ¹H NMR spectroscopy. *Flavour Fragr J.* 2023;00:1-7. doi:10.1002/ffj.3760