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*Analytical Methods Section*

# Infrared spectrophotometric determination of citral corrected for limonene interference in lemon and orange essential oils

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An infrared spectrophotometric method is described to correct for interference by limonene in the determination of citral in orange and lemon essential oils by subtracting the limonene spectrum from the essential oil spectrum. The first derivative trough-to-peak distance between 1684 $cm^{-1}$ and 1677 $cm^{-1}$ was most reliable in determining citral, parameters of the zero order absorption spectrum (net absorbance and peak area) performed poorly in comparison.

## INTRODUCTION

The literature on essential oils is growing (Lawrence, 1984), the area of their identification being of particular interest (Motto, 1987). Citral from natural sources is a mixture of two geometric isomers (Fig. 1), geranial (citral *a* or *cis*-citral; CAS No. 141-27-5) and neral (citral *b* or *trans*-citral; CAS No. 106-26-3). Citral is used in the synthesis of vitamin A, ionone and methylionone, as a fragrance and flavoring, and for characterizing oils extracted from citrus fruit wastes (Oderinde, 1988). It forms part of the oxygenated fraction of essential oils, which is primarily responsible for flavouring properties (Melendreras *et al.*, 1985). This fact is suggested by the correlation between the composition of ethanol–water extracts of lemon essential oils and flavouring effect (Licandro *et al.*, 1990). Identification of this fraction is achieved simply by gas chromatography with oxygenate flame ionization detection (GC/O-FID), which has a high selectivity for oxygenated compounds (Lakszner & Szepesy,1988)

Quantitative differences in essential oils are due to many factors. The quality of winter oil is greater than that of summer oil. Mature fruit contains less essential oils than unripe fruit, but the ester index and the quantity of aldehydes and ketones, which indicate quality, increase with maturation (Sepulveda *et al.*, 1989). Climatological differences (Boelens & Jimenez, 1989) and extraction technology (Cotroneo *et al.*, 1988) also influence essential oil composition.

Chromatography is the most widely applied technique in the characterization of citrus oils. Thin layer chromatography has been used (Miramond & Giulianetti, 1986; Postaire *et al.*, 1988), especially to identify flavouring agents. The purity, quality and adulteration of citrus oils has been evaluated by high-resolution capillary gas chromatography (HRCGC) (Dugo & Cotroneo, 1988) employing either retention index differences of compounds found in oils from two columns of different polarities (Lancas & Cavicchioli, 1990) or, more commonly, detectors such as mass spectrometers (Inoma *et al.*, 1989). High-performance liquid chromatography is useful, particularly if microbore columns are employed (Benincasa *et al.*, 1990), or if used on-line with HRCGC (Yamauchi & Saito, 1990). There are also colorimetric methods to determine total aldehydes (Kloeti *et al.*, 1985). In all cases the data obtained can be correlated to sensory evaluation (Schieberle & Grosch, 1987).

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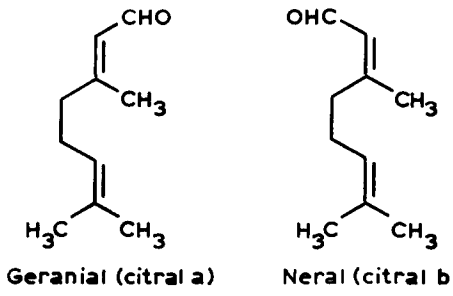


**Fig. 1.** Molecular structures for geranial and neral.

It is useful to be able to quantify citral in essential oils since it is an indicator of quality and imparts flavour and aroma. The method based on infrared spectrophotometry presented here quantifies citral in essential oils obtained from oranges and lemons, and offers a means of correcting for both the interference from limonene in this determination and the potential for graphical errors in a currently used infrared technique (Berner *et al.*, 1978).

## EXPERIMENTAL

### Apparatus

A Perkin-Elmer model 681 IR spectrophotometer was used with an appropriate communications interface. Data were processed on a Perkin–Elmer model 3600 data station using Perkin-Elmer software (PE680 and SNGLE; similar programs are supplied by other spectrophotometer manufacturers). Samples were held in a 0·15 mm path length NaCl cell.

### Reagents

Limonene (+) (purity ≥ 98%) was obtained from Fluka AG, Buchs SG (Ref. 62120); citral from Sigma (Ref. No. C-1645), the purity of which was tested by GC-FID (Fig. 2); lemon essential oils from Guinama, Escuder and Analema; and orange essential oils from Escuder and Guinama. In reporting the results these oils are labelled 1, 2, 3, 4 and 5, respectively.

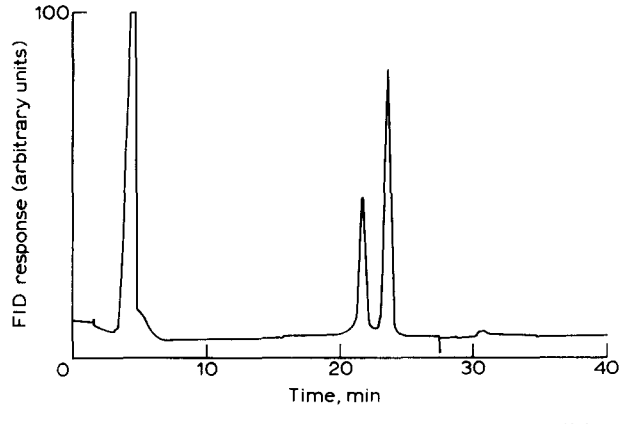


**Fig. 2.** Gas chromatogram of citral in ethanol. Conditions: A PE Sigma 300 gas chromatograph with a 2m × 3 mm i.d. glass column packed with 10% Carbowax 20 M on Chromosorb W AW-DMCS (80–100 mesh) was used. The carrier gas was helium N-48 (10 ml $min^{-1}$). FID detection ($H_2$ flow: 20 ml $min^{-1}$ and air flow: 300 ml $min^{-1}$). Injector and detector temperature were 190°C and column temperature gradient was 80–180°C at 3°C $min^{-1}$. *Trans*-citral is first to elute, followed by *cis*-citral.

**Table 1. Parameters of calibration lines constructed from 6 points in the range of 0·25–3% (w/v) citral in limonene**

| | Height | Area | D1 | D2 |
|---|---|---|---|---|
| Slope | 0·4136 | 5·3350 | 0·6396 | 0·1210 |
| Intercept | 0·1211 | 0·7804 | 0·5111 | 0·1071 |
| Error index (%) | 4·63 | 4·24 | 4·87 | 5·61 |
| Correlation coefficient | 0·9967 | 0·9972 | 0·9964 | 0·9952 |

### IR spectrophotometry

The spectrophotometer settings were:

- Scan: from 2000 to 1500 $cm^{-1}$ with data acquisition every 1 $cm^{-1}$
- Scan rate: 340 $cm^{-1}$ $min^{-1}$
- Slit: wide (W)
- Multiplier: 4
- Time: 88 s

### Procedure

*Direct IR method*

Standard solutions of citral in limonene of concentrations 0·25%, 0·5%, 1%, 1·5%, 2% and 3% (w/v) were prepared. Calibration lines were calculated with the SNGLE program using four features in the IR spectra: height of the 1680 $cm^{-1}$ peak due to —CHO above the baseline from 1696 $cm^{-1}$ to 1663 $cm^{-1}$; integrated peak area enclosed by this baseline; first derivative trough-to-peak distance between 1683 $cm^{-1}$ and 1673 $cm^{-1}$; and second derivative trough-to-peak distance between 1684 $cm^{-1}$ and 1678 $cm^{-1}$. Calibration line parameters are shown in Table 1 and typical absorbance and first derivative spectra in Figs 3 and 4, respectively.

*Correction for interference by limonene*

In the zero order spectra of the standard solutions the absorption band at 1779 $cm^{-1}$ due to limonene was subtracted by means of the SDIFF command in the PE680 program. Calibration lines were then calculated with SNGLE using: absorbance at 1680 $cm^{-1}$ above the baseline from 1699 $cm^{-1}$ to 1658 $cm^{-1}$; integrated peak area enclosed by this baseline; first derivative trough-to-peak distance between 1684 $cm^{-1}$ and 1677 $cm^{-1}$; and

**Table 2. Parameters of calibration lines constructed from 6 points in the range of 0·25–3% (w/v) citral in limonene after subtraction of limonene spectrum.**

| | Height | Area | D1 | D2 |
|---|---|---|---|---|
| Slope | 0·4591 | 5·9239 | 0·7545 | 0·1437 |
| Intercept | 0·0016 | −0·1641 | 0·1029 | 0·0269 |
| Error index (%) | 4·77 | 7·73 | 1·78 | 1·92 |
| Correlation coefficient | 0·9965 | 0·9909 | 0·9995 | 0·9994 |

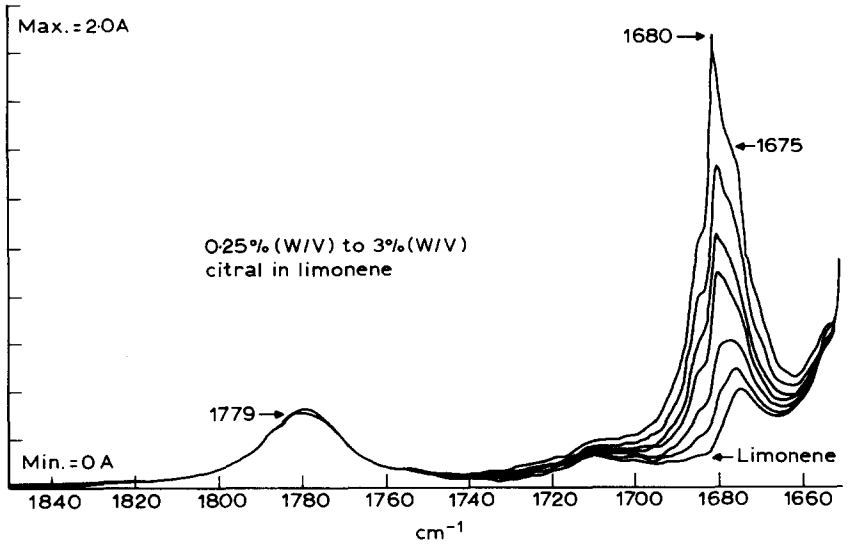


**Fig. 3.** Absorbance spectra of limonene and of 0·25–3% (w/v) citral in limonene.

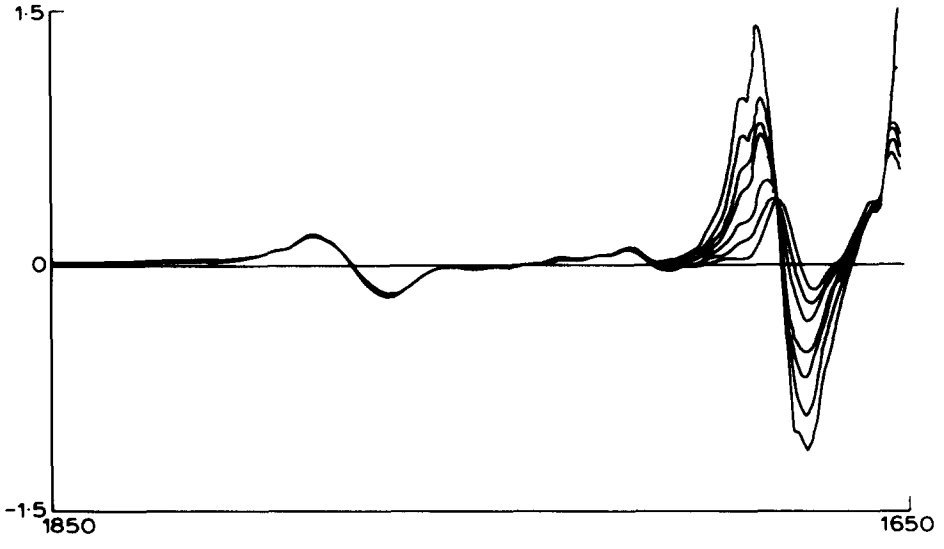


**Fig. 4.** First derivative spectra of limonene and of 0·25–3% (w/v) citral in limonene.

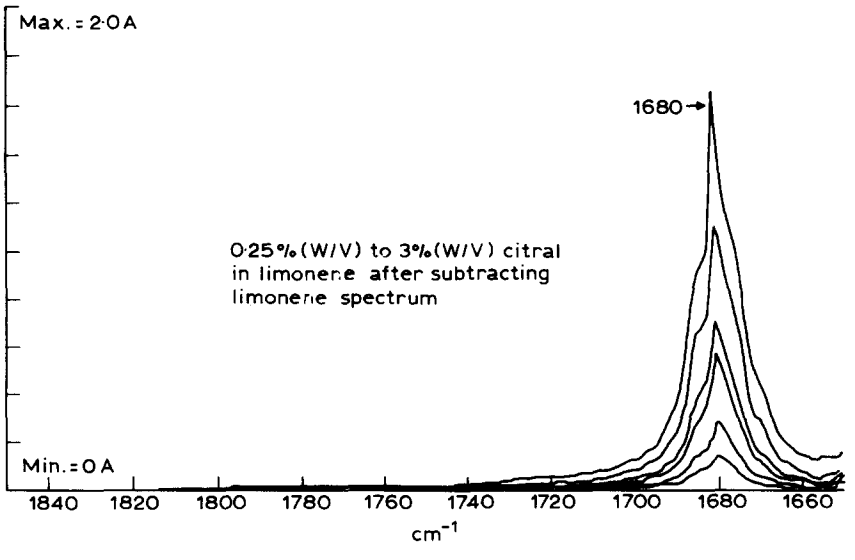


**Fig. 5.** Absorbance spectra of limonene and of 0·25–3% (w/v) citral in limonene after subtracting limonene spectrum.

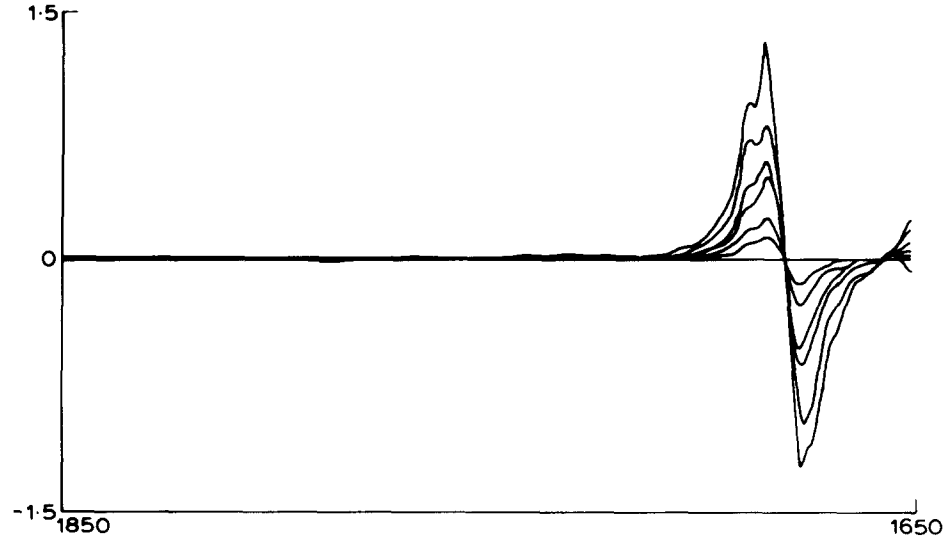


**Fig. 6.** First derivative spectra of limonene and of 0·25–3% (w/v) citral in limonene after subtracting limonene spectrum.

second derivative trough-to-peak distance between 1684 $cm^{-1}$ and 1679 $cm^{-1}$. Table 2 shows the parameters characterizing the calibration lines. Typical absorbance and first derivative spectra after subtracting the limonene spectrum are shown in Figs 5 and 6, respectively.

The citral content of the commercial essential oils was determined using the AOAC final action hydroxylamine method for the determination of total aldehydes in lemon oil (AOAC, 1984). Citral was also quantified separately for each oil using the four calibration curves obtained for the two spectrophotometric methods, diluting the essential oils as required to fit within the calibration range. Differences between the AOAC and spectrophotometric methods were assessed using the paired two-tailed *t*-test for each parameter.

## DISCUSSION

All the direct IR measurements of the citral content of the essential oils (Table 1) have large error indices and relative poor correlation coefficients. The *t*-test shows that they give values differing significantly from the citral content given by the AOAC method (Table 3). That the error is due to interference by limonene is suggested by the significant deviations from the AOAC method given by the direct method for six replicate determinations in limonene standards (Table 4).

Table 5 shows that the determination of citral by the first and second derivative trough-to-peak measurements in the corrected IR spectra was not significantly different to quantification by the AOAC method. The greater slope of the calibration line (Table 2) suggests that smaller changes in citral concentration may be determined by the first derivative trough-to-peak measurement.

## CONCLUSIONS

First derivative IR spectrophotometry, after subtracting the limonene spectrum from the sample spectrum, has been used to quantify citral in orange and lemon

**Table 3. Comparison of direct IR method with AOAC reference method using paired two-tailed *t*-test.**

| Sample | No. | AOAC method | Direct IR method | | | | Discrepancies | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Height | Area | D1 | D2 | Height | Area | D1 | D2 |
| Lemon oil | 1 | 1·10 | 0·98 | 0·99 | 1·27 | 1·25 | −0·12 | −0·11 | +0·17 | +0·15 |
| | 2 | 1·83 | 1·48 | 1·43 | 2·08 | 2·09 | −0·35 | −0·40 | +0·25 | +0·26 |
| | 3[a] | 1·99 | 1·68 | 1·60 | 1·90 | 2·03 | −0·31 | −0·39 | 0·00 | +0·04 |
| Orange oil | 4 | 1·52 | 1·32 | 1·26 | 1·82 | 1·82 | −0·20 | −0·26 | +0·30 | +0·30 |
| | 5 | 1·21 | 1·23 | 1·21 | 1·69 | 1·67 | +0·02 | 0·00 | +0·48 | +0·46 |
| Average | | | | | | | −0·192 | −0·232 | +0·240 | +0·242 |
| Standard deviation | | | | | | | ±0·149 | ±0·175 | ±0·176 | ±0·158 |
| Experimental *t* | | | | | | | 2·881 | 2·964 | 3·050 | 3·425 |

Theoretical *t* ($\alpha$ = 0·05, 4 degrees of freedom) = 2·776

[a] diluted ten-fold

**Table 4. Values given by AOAC and direct IR methods for six replicate determinations in limonene standard. Results are compared by paired two-tailed *t*-test.**

| Sample | No. | AOAC method | Direct IR method | | | |
|---|---|---|---|---|---|---|
| | | | Height | Area | D1 | D2 |
| Limonene | 1 | 0·00 | 0·11 | 0·04 | 0·04 | 0·23 |
| | 2 | 0·00 | 0·10 | 0·02 | 0·06 | 0·28 |
| | 3 | 0·00 | 0·13 | 0·05 | 0·05 | 0·25 |
| | 4 | 0·00 | 0·14 | 0·07 | 0·08 | 0·29 |
| | 5 | 0·00 | 0·11 | 0·03 | 0·09 | 0·33 |
| | 6 | 0·00 | 0·11 | 0·02 | 0·11 | 0·34 |
| Average | | | +0·117 | +0·038 | +0·072 | +0·287 |
| Standard deviation | | | +0·015 | +0·019 | +0·026 | +0·043 |
| Experimental *t* | | | 19·106 | 4·838 | 6·783 | 16·349 |
| Theoretical *t* ($\alpha$ = 0·05, 5 degrees of freedom) = 2·571 | | | | | | |

**Table 5. Comparison of corrected IR method with AOAC reference method using paired two-tailed *t*-test.**

| Sample | No. | AOAC method | Limonene spectrum subtracted | | | | Discrepancies | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Height | Area | D1 | D2 | Height | Area | D1 | D2 |
| Lemon oil | 1 | 1·10 | 1·00 | 0·93 | 1·20 | 1·13 | −0·10 | −0·17 | +0·10 | +0·03 |
| | 2 | 1·83 | 1·69 | 1·69 | 1·74 | 1·81 | −0·14 | −0·14 | −0·09 | −0·02 |
| | 3[a] | 1·99 | 1·69 | 1·64 | 1·82 | 1·95 | −0·30 | −0·35 | −0·17 | −0·04 |
| Orange oil | 4 | 1·52 | 1·41 | 1·42 | 1·43 | 1·48 | −0·11 | −0·10 | −0·09 | −0·04 |
| | 5 | 1·21 | 1·10 | 1·02 | 1·33 | 1·26 | −0·11 | −0·19 | +0·12 | +0·05 |
| Average | | | | | | | −0·152 | −0·190 | −0·026 | −0·004 |
| Standard deviation | | | | | | | ±0·084 | ±0·096 | ±0·129 | ±0·042 |
| Experimental *t* | | | | | | | 4·046 | 4·426 | 0·451 | 0·213 |
| Theoretical *t* ($\alpha$ = 0·05, 4 degrees of freedom) = 2·776 | | | | | | | | | | |

[a] diluted ten-fold

essential oils, whose quantification by conventional IR spectrophotometric methods is normally subject to interference by limonene.

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