Supporting Information

Is Low-field NMR a Complementary Tool to GC-MS in Quality Control of Essential Oils? A Case Study: Patchouli Essential Oil

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Dedicated to Professor Dr. Robert Verpoorte in recognition of his outstanding contribution to natural products research.
**Materials and Methods**

**Quantitation – calculation of relative response factor**

For the calculation of the relative response factor (RR$_F$), freshly distilled high-quality standard PEO, to which a known amount of internal standard had been added, was analysed in triplicate by GC-MS. Integration was done in ChemPattern. For the calculation, the following formula was used:

$$RR_F = \frac{A_x}{A_i} \times \frac{W_i}{W_x}$$ \[1\]

$A_x$ = area of all patchouli related peaks (minus internal standard)

$A_i$ = area of internal standard (E,E-farnesol)

$W_i$ = weight of internal standard in mg

$W_x$ = oil weight in mg

With the calculated response factor, the weight of all patchouli related peaks in different oils was calculated:

$$W_x = \frac{A_x}{A_i} \times \frac{1}{RR_F} \times W_i$$ \[2\]

The percentage PEO in the analysed oil was then finally calculated as:

$$\% \text{ PEO} = \frac{W_x}{W_{oil}} \times 100\%$$

In which $W_{oil}$ is the weight of the analysed oil

**Cosine similarity analysis**

Conducted similarity analysis was based on cosine similarity. Cosine similarity transforms obtained data into a vector and then compares the angle between two vectors, e.g., two TICs (chemmind.com/wiki).

Cosine similarity is defined as:

$$\cos(d_1, d_2) = \frac{d_1 \cdot d_2}{||d_1|| \cdot ||d_2||}$$ \[3\]

Whereby $d$ is the vectorised sample data

An example with random numbers to illustrate cosine similarity:

Sample 1 as vectorised data = (5, 0, 3, 0, 2, [...], $n_x$)

Sample 2 as vectorised data = (3, 0, 2, 0, 1, [...], $n_y$)
1) \( d_1 \times d_2 = 5 \times 3 + 0 \times 0 + 3 \times 2 + 0 \times 0 + 2 \times 1 + n_x \times n_y = 25 \)

2) \( ||d_1|| = \sqrt{5 \times 5 + 0 \times 0 + 3 \times 3 + 0 \times 0 + 2 \times 2 + n_x \times n_x} = 6.48 \)

3) \( ||d_2|| = \sqrt{3 \times 3 + 0 \times 0 + 2 \times 2 + 0 \times 0 + 1 \times 1 + n_y \times n_y} = 4.12 \)

4) \[
\cos(d_1, d_2) = \frac{d_1 \times d_2}{||d_1|| \times ||d_2||} = \frac{25}{6.48 \times 4.12} = 0.96
\]

**Calculated cosine value** | **Meaning for cosine analysis**
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X = 1 | Two vectors (TICs) are identical
1 > X > 0 | Two vectors are similar
X = 0 | Two vectors are in 90° (orthogonal) to each other

**Mahalanobis distance measure [1]**

Similarity analyses for NMR were based on Mahalanobis distance. The Mahalanobis distance measure performs the following actions:

- It transforms variables into uncorrelated variables
- It equals their variance to 1
- It calculates then Euclidean distance

\[
D^2 = (x - m)^T \times C^{-1} \times (x - m)
\]

\( D^2 \) = Mahalanobis distance

\( x \) = vector of data

\( m \) = vector of mean values of independent variables of selected (representative) samples

\( C^{-1} \) = inverse covariance matrix of independent variables of selected (representative) samples

**Reference**

**Fig. 1aS** 60 MHz $^1$H-NMR of PEO (neat) used for deliberate adulteration, 1 scan, S/N ratio $\approx 500$.

**Fig. 1bS** 60 MHz $^1$H-NMR of the same PEO (neat) as in **Fig. 1aS**, 32 scans, S/N ratio $> 3000$. 
**Fig. 1cS** 60 MHz $^1$H-NMR of PEO (neat) used for deliberate adulteration, 32 scans; recorded 2.5 months later and in a different NMR tube as the spectrum in **Fig. 1bS**.

**Fig. 1dS** 60 MHz $^1$H-NMR of PEO (neat) used for deliberate adulteration, zoom-in, 32 scans.
Fig. 1e 600 MHz $^1$H-NMR of standard PEO (in CDCl$_3$) used for deliberate adulteration.

Fig. 1f 600 MHz $^1$H-NMR of standard PEO (in CDCl$_3$) used for deliberate adulteration, zoom-in showing signals of pogostone (6) at 5.92 ppm and norpatchoulenol (8) at 5.71 and 5.49 ppm.
Fig. 2aS 60 MHz $^1$H-NMR of a commercial orange viscous PEO (neat + TMS), 25°C.

Fig. 2bS 400 MHz $^1$H-NMR of the same PEO (neat) as in Fig. 2aS, 25°C.
**Fig. 2c** 400 MHz $^1$H-NMR of a commercial non-viscous yellow PEO in CDCl$_3$, 25°C.

**Fig. 2d** 400 MHz $^1$H-NMR of the same PEO (neat) as in **Fig. S2ab**, 75°C.
Fig. 2eS 60 MHz $^1$H-NMR of a viscous orange PEO after dilution with 100 µL CDCl$_3$, 25°C.

Fig. 2fS 60 MHz $^1$H-NMR of a viscous orange PEO, 25°C after dilution with 200 µL CDCl$_3$. 
Fig. 2gS 60 MHz $^1$H-NMR of a viscous orange PEO, 25°C after dilution with 400 µL CDCl$_3$.

Fig. 2hS 60 MHz $^1$H-NMR of a viscous orange PEO, 25°C after dilution with 800 µL CDCl$_3$. 
Fig. 3S 60 MHz $^1$H NMR (neat) of a commercial viscous PEO, showing severe adulteration in spite of the significant line broadening due to the high viscosity. This PEO contained, according to GC-MS, 5% benzyl alcohol, 22% $\alpha$-gurjunene, 3% thujopsene, 7% alloaromadendrene, 2% $\gamma$-gurjunene, 3% cedrol, and 20% benzyl benzoate, i.e., 1 part PEO and 2 parts adulterants.
**Fig. 4a** 600 MHz $^1$H NMR of patchoulol in CDCl$_3$.

**Fig. 4b** Overlaid 60 MHz $^1$H-NMR spectra of standard PEO (bottom, in purple) and standard PEO adulterated with 20% benzyl alcohol (in green) showing shifts of various methyl groups of patchoulol. Both samples measured neat.
**Fig. 5S** 600 MHz $^1$H NMR spectrum of 1% pogostone in CDCl$_3$. Top: full spectrum and molecular structure including the unusual peak at 16.8 ppm from the hydroxyl. Bottom: zoom-in of the different NMR signals.
**Fig. 6S** A 600 MHz $^1$H NMR spectrum from 3.2-3.95 ppm of PEO used for spiking in CDCl$_3$. B 600 MHz $^1$H NMR spectrum from 3.2-3.95 ppm of Clearwood in CDCl$_3$. Signals at 3.28 and 3.50 ppm are due to patchoulol ethyl ether (not present in PEO). The signal at 3.74 ppm is a Clearwood-specific compound also, i.e., not present in PEO. All three could serve as adulteration markers.
Fig. 7aS Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 2 from the bottom in Fig. 2 (there in dark blue, here in green).

Fig. 7bS Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 3 from the bottom in Fig. 2.
**Fig. 7c** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 4 from the bottom in **Fig. 2**.

**Fig. 7d** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 5 from the bottom in **Fig. 2** (15% patchoulool).
**Fig. 7e** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 5 from the top in Fig. 2 (stem oil, rich in pogostone).

**Fig. 7f** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 4 from the top in Fig. 2.
**Fig. 7g** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 3 from the top in **Fig. 2**.

**Fig. 7h** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and spectrum 2 from the top in **Fig. 2**.
**Fig. 7iS** Overlaid 60 MHz $^1$H-NMR spectra of PEO used for deliberate adulteration (bottom, in purple) and the top spectrum in Fig. 2 (52% patchoulol).

**Fig. 8S** Overlaid 60 MHz $^1$H-NMR spectra of six buchu oils. Top two oils (red and orange): *Agathosma betulina*, diosphenol chemotype; middle two oils (olive green and blue green): *Agathosma betulina*, isomenthone chemotype; bottom two oils (light and dark blue): *Agathosma crenulata*. The bottom oil also occurs in Fig. 5. All oils contain limonene (peaks at 5.35 and 4.67 ppm). Only the top two oils contain (pseudo)diosphenol (peak at 6 ppm). Other main constituents are (iso)menthones (methyls at 0.8-1 ppm) and (iso)pulegone (methyls at 1.7-2 ppm). The top two
NMRs are very similar though not identical and clearly distinguishable from the other four oils. This is also true for the middle two oils. The two bottom oils differ more from one another due to one oil consisting of 55% (iso)menthones and 25% pulegone, while the bottom oil has 17.5% (iso)menthones and almost 50% (iso)pulegone. For more info on buchu oils, see:

Posthumus MA, van Beek TA, Collins NF, Graven EH. Chemical composition of the essential oils of *Agathosma betulina*, *A. crenulata* and an *A. betulina* x *crenulata* hybrid (Buchu). J Ess Oil Res 1996; 8: 223-228

Collins NF, Graven EH, van Beek TA, Lelyveld GP. Chemotaxonomy of commercial buchu species (*Agathosma betulina* and *A. crenulata*). J Ess Oil Res 1996; 8: 229-235

**Fig. 9S** Similarity analysis (NMR) based on Mahalanobis distance results. Segmented integration focusing on patchoulol and vetiver oil. Group 1 are genuine PEOs, representative samples, **dark red** circles. Group 2 are genuine PEOs, test samples, **orange** circles. Group 3 are commercial PEOs (6 genuine and 4 adulterated), **green** squares. Group 4 are deliberately adulterated samples, **black** asterisks. Group 5 are other EOs, **blue** pentagons. The larger the negative value, the more a sample deviates from the common pattern (green asterisk) based on the average of the representative samples.
Fig. 10S GC-MS fingerprint with assignment of major identified peaks. Common pattern of PEO as used in ChemPattern based on a combination of 73 different PEOs including genuine oils and genuine commercial PEOs. All constituents elute between 10-25 min.
Fig. 11S Biplot of principal component analysis (PCA) with ChemPattern of GC data. Eighty-nine independent variables were taken into account. Four constituents were assigned with a weight: patchoulol = 15, α-bulnesene = 20, α-guaiene = 30, seychellene = 40. Choice was based on high values of Eigenvalues and their explanatory power when no weights were assigned. Six groups are indicated: (1) representative genuine PEOs (dark red circles); (2) genuine PEOs (for testing of model, orange circles); (3) deliberately adulterated PEO (black stars); (4a) genuine commercial samples (light green squares); (4b) adulterated commercial samples (dark green squares); (5) other EOs (blue pentagons). As expected, groups (1), (2), and (4a) overlap to some extent. The outlier (bottom left quadrant) in group (2) is the stem PEO with high pogostone content. The outlier (top right quadrant) in group 3 is the 20% adulteration with Clearwood, it cannot be distinguished by PCA from a genuine oil. The four largest blue arrows correspond to alpha-guaiene (most left in upper right quadrant), alpha-bulnesene (middle in upper right quadrant), seychellene (most right in upper right quadrant), and patchoulol (bottom right quadrant).
Fig. 12S Similarity analysis (cosine) with ChemPattern based on GC-MS data. Six groups are indicated: (1) representative genuine PEOs (dark red circles); (2) genuine PEOs (for testing of model, orange circles); (3) deliberately adulterated PEO (black stars); (4a) genuine commercial samples (light green squares); (4b) adulterated commercial samples (dark green squares); (5) other EOs (blue pentagons). Upper: all samples. Lower: zoom-in on commercial samples and deliberately adulterated PEO with assignments.
Fig. 13S 60 MHz COSY spectrum of standard PEO (neat) used for deliberate adulteration.