SI-traceable purity assignment of volatile material ethylbenzene by quantitative nuclear magnetic resonance spectroscopy.

Elissa Ony, Ben Sinclair, Hannah Mault, and Carmen Dyck
Chem 2100 Dr. Kingsley Donkor

Objective

- To test quantitative nuclear magnetic resonance spectroscopy (qNMR) as a method to determine the SI-traceable purity of ethylbenzene, a volatile organic compound (VOC) by comparison with the traditionally accepted mass balance method.
- To find a method capable of accurately and precisely determining the purity of volatile substances despite their tendency to vaporize under normal temperature and pressure.
- qNMR was chosen because it can directly measure purity.

Background and Significance

Volatile organic compound (VOC): "Volatile organic compounds, or VOCs are organic chemical compounds whose composition makes it possible for them to evaporate under normal indoor atmospheric conditions of temperature and pressure" (3). Normal indoor atmospheric pressure not to be confused with STP (3).

NMR: Non-destructive method of identifying molecular structure and purity of an analyte (4).

1. Analyte is subjected to a magnetic field.
2. Nuclei that exhibit nuclear slip will "flip" or change spin states.
3. This flip generates a change in energy, which can be detected by the instrument.
4. Signals can then be used to calculate the purity of a compound.

- VOCs are all around: they're in lots of products, and some are even in the air we breathe, albeit normally in low concentrations, and many of them are classified as carcinogens (3).
  - It's important for manufacturers and consumers to know how much of a VOC is in a product, and whether it's enough to pose a risk.
  - To analyze how much of a substance is in a sample, standards are needed for comparison. If the components in standards aren't pure, the standard won't be the proper concentration, and therefore the analysis of the sample won't be accurate, so it's important to know how pure the material (ex. ethylbenzene) used in a standard is.
- This method, qNMR, has significant potential for determining the purity of other organic compounds and opens up a whole new world of analyzing the purity of other volatile compounds that are otherwise extremely difficult to analyze.

Future Work

- Provides accurate quantification of volatile organic materials by reducing measurement error caused by evaporation.
- Can be used in the development of pharmaceuticals to test drug potency (1).
- The versatility of qNMR makes it an ideal method for studying complex biological systems and processes (metabolomics) (1).
- Provides advantages over traditional chromatography techniques in the development and analysis of food and beverage products. (1).

References

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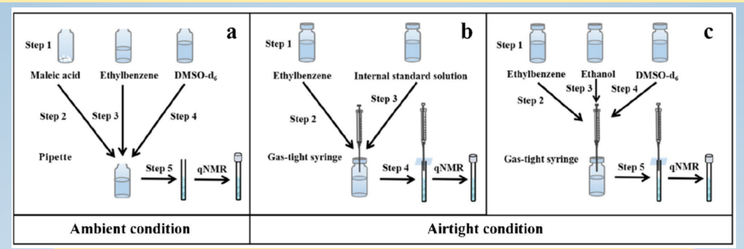

Figure 1. A graphic demonstrating the various experimental methods including internal standards, environmental conditions, and solvent.

Methodology

Purity assessment of ethylbenzene by mass balance method:

- Impurities in ethylbenzene were qualitatively determined by gas chromatography/mass spectrometry.
- Impurities in ethylbenzene determined by high performance liquid chromatography (HPLC) with diode array detection (DAD).
  - Determined the content of five major impurities and four minor ones.
  - Methylcyclohexane was determined by qNMR since it could not be detected by DAD.
  - For impurity verification ethylbenzene was used as a blank, and 1 uL impurity standard was mixed with 1 mL ethylbenzene under air tight conditions.
  - External standards were used to quantify impurities.
  - Impurity standard solutions were prepared in methanol and the corresponding peak area was given by liquid chromatography.
- Water content was determined by the Karl Fischer coulometric method.
- Inorganic impurities were determined by inductively coupled plasma–mass spectrometry.

Purity assessment of ethylbenzene by qNMR:

The experimental parameters:

1. 30° pulse
2. 64 k data points
3. 296 K probe temperature
4. 32 second relaxation delay
5. 16 scans
6. internal standard method (maleic acid and ethanol)

Maleic acid internal standard and ambient conditions:

In a 4 mL vial 5 mg of maleic acid, 10 mg of ethylbenzene, and 0.6 mL dimethyl sulfone, a polar solvent, were mixed for 20 seconds. Once visually confirmed to be completely dissolved, the solution was determined by NMR.

Maleic acid internal standard and airtight conditions:

The internal standard, maleic acid, was prepared by dissolving 35.999 mg of maleic acid in 4260.951 mg of DMSO. Using a gas tight syringe to maintain airtight conditions 10 mL of ethylbenzene were transferred into a 2 mL vial and weighed. Five-hundred uL of the internal standard was added. The solution was mixed and determined by qNMR.

Ethanol internal standard and airtight conditions:

Ethanol was chosen as an internal standard because it had a close boiling point to ethylbenzene. Using a gas tight syringe 30 uL of ethylbenzene, 15 uL of ethanol, and 500uL of DMSO were transferred to a 2 mL vial. The solution was mixed, transferred to an NMR tube covered in parafilm, covered with an NMR cap, and determined by qNMR.

Discussion

The reference value for ethylbenzene study material was obtained by the mass balance method as 99.89%with an error of 0.13%. This method has high precision and serves as a reference value for the results obtained with the quantitative NMR method. The first qNMR run was done in ambient conditions, the NMR tube was left uncovered, with an internal standard of maleic acid. This run gave a purity of 965.9 ± 62.1 mg/g, an imprecise and inaccurate result which suggested systematic error. To improve this, the second run was done with the same internal standard in airtight conditions. This result of 996.9 ± 3.4mg/g had acceptable precision but still poor accuracy. The systematic error was significantly improved by the third run in which the internal standard was switched to ethanol, which had a closer boiling point to the study material. The result of this run was a purity of 998.6 ± 3.8 mg/g which maintains acceptable precision while being very accurate. While the precision of the qNMR method was acceptable and showed potential for determining the purity of other VOCs, it was still three times less than the reference method.

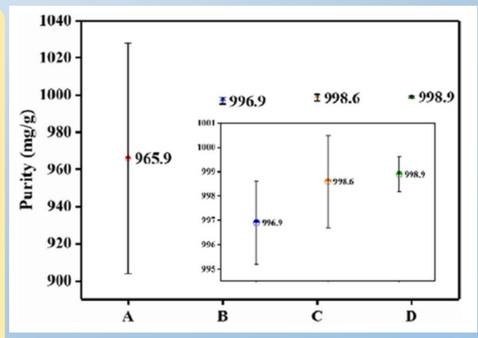

Figure 2. Quantitative results for the purity assessment of ethylbenzene. A) maleic acid internal standard and ambient conditions. B) maleic acid internal standard and airtight conditions. C) ethanol internal standard and airtight conditions. D) mass balance method.

Results

Purity assessment of ethylbenzene by mass balance method:

- Five major impurities were identified by their mass spectra: benzene, methylcyclohexane, isobutylbenzene, sec-butylbenzene and acetophenone.
- Four other impurities were not identified.
- Mean water content determined: n=6

Table 1. Results of mass balance purity assessment of ethylbenzene.

Impurity and method	Structurally Related Impurities by HPLC	Water content by Karl Fischer Coulometry	Inorganic by Inductively coupled plasma-mass spectrometry.
Mass percent of ethylbenzene study material	0.0633% major 0.0121% methylcyclohexane 0.018% minor	0.0110% ± 0.0008%	0.0020% ± 0.0010%

Total purity was calculated using the following equation:

$$\text{Purity} = P_0(1 - X_{RS} - X_W - X_{NV})$$

Where P0 is the purity of ethylbenzene only considering uncharacterized organic impurities, XRS is the percentage of structurally related organic impurities, XW is the water content, and XNV is the percentage of inorganic impurities.

Purity = 99.98(1 - 0.0754 - 0.0110 - 0.0020)

Finally, the purity was found to be **99.89% with an uncertainty of 0.13%. (k=2)**

Purity assessment of ethylbenzene by quantitative qNMR

- The equation used to calculate the mass fraction of the sample ethylbenzene:

$$P_{sam} = \frac{I_{sam} n_{std} M_{sam} m_{std}}{I_{std} n_{sam} M_{std} m_{sam}} P_{std}$$

- I was the peak area of the quantitative peak, n was the number of hydrogen (for quantification), M was the molecular weight, and m was the weight.
- The uncertainty associated with the purity was dependent on the uncertainties of NMR signal measurement, molar masses and masses of standard and sample and the purity of the standard.
- RSD was calculated using the following equation:

$$\frac{u(P_{sam})}{P_{sam}} = \sqrt{\left[\frac{u(I_{sam}/I_{std})}{I_{sam}/I_{std}}\right]^2 + \left[\frac{u(M_{sam})}{M_{sam}}\right]^2 + \left[\frac{u(M_{std})}{M_{std}}\right]^2 + \left[\frac{u(m_{std})}{m_{std}}\right]^2 + \left[\frac{u(m_{sam})}{m_{sam}}\right]^2 + \left[\frac{u(P_{std})}{P_{std}}\right]^2}$$

- Quantitative NMR was ran under three different conditions outlined in the methodology section.
- Each run's results were compared to the reference value obtained using the mass balance method.
- The first run with maleic acid did not agree with the reference value and also had low reproducibility.
- The second run was done under airtight conditions which gave a value of 996.9 ± 3.4 mg/g (k = 2). This value showed improved accuracy and reproducibility.
- The internal standard was then switched to ethanol, which has a boiling point much more similar to ethylbenzene. This produced a much more accurate and reproducible result of 998.6±3.8 mg/g, k=2 as compared to the mass balance method: 998.9±1.3 mg/g, k=2

Table 2. Summary of results from the four different methods.

Reference Value (Mass Balance Method)	qNMR using maleic acid in ambient conditions	qNMR using maleic acid in airtight conditions	qNMR using ethanol in airtight conditions
998.9±1.3 mg/g	965.9±62.1 mg/g	996.9 ± 3.4 mg/g	998.6±3.8 mg/g

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