Intelligent use of Relative Response Factors in Gas Chromatography-Flame Ionisation Detection

by Karen Rome and Allyson McIntyre, AstraZeneca, Macclesfield, SK10 2NA, UK

Quantitative analysis is required in pharmaceutical R&D to measure the quality of materials used in active pharmaceutical ingredient (API) manufacture, and to monitor the composition of reactions over time. Reagents and matrix typically contain organic carbon, are often volatile and cover a wide range of polarities; therefore Gas Chromatography-Flame Ionisation Detection (GC-FID) is often the appropriate technique.

Quantification by GC-FID is typically performed using external standards; this approach is selective and is accurate for low level analyses; however, it requires labourious sample preparation and has long cycle times. Quantification by proton Nuclear Magnetic Resonance (1H NMR) is typically performed using internal standards; this approach has simple sample preparation and short cycle times; however, it is not always selective and is not accurate for low level analysis. The use of pre-determined relative response factors (RRFs) is used in our laboratory for assay and quantification of solvent impurities by 1H NMR. This article discusses the concept and proof of principle of using pre-determined RRFs for quantification by GC-FID and its applications within our laboratory are presented.

Concept

The absolute responses of analytes in GC change from day to day and instrument to instrument. It is widely accepted that responses are corrected with an internal calibration standard as the resulting RRF, in general, remains constant and is not affected by changes in time of analysis or instrument. The use of a pre-determined RRF between two analytes can be then used to quantify an unknown concentration of one analyte in the presence of a known concentration of the other.

To determine the RRF between two analytes (A and B), the two analytes are analysed simultaneously within the same solution. Firstly, the peak area and concentration of the analyte are used to calculate the response for each analyte, as in Equation 1.

\[
\text{Response Factor} = \frac{\text{Peak area}}{\text{Concentration}}
\]

The response factors calculated for each analyte are then used to establish the RRF between the two analytes as in Equation 2.

\[
\text{Relative Response Factor (RRF)} = \frac{\text{Response Factor A}}{\text{Response Factor B}}
\]

The RRF can be used to calculate the unknown concentration of analyte A in the presence of a known concentration of analyte B, as in Equation 3.

\[
\text{Concentration A} = \frac{\text{Peak area A} \times \text{Concentration B}}{\text{Peak area B} \times \text{RRF}}
\]

Proof of Principle

Determination of RRF

Response factors for 31 common process solvents were calculated using Equation 1 with an established method (details in Table 1), an example chromatogram is given in Figure 1. The solvents were analysed simultaneously and the analysis was completed five times at three concentrations (0.015, 0.15 and 1.5 mg/mL). The response factors were calculated for each solvent in each analysis; the average response factors were calculated and reported; example details are in Table 2. RRFs could then be calculated for chosen solvent pairs using Equation 2.

Validation of RRF

A factorial experimental design study4 varying; solution matrix, solvent concentration and instrument was performed, this measured accuracy,
The RRF approach was repeated for multiple batches across several projects and when compared with the external standard approach, the two methods gave the same answer for each batch within experimental error.

Transfer of RRFs across chromatographic methods

Eight process solvents were selected and analysed on two more established methods, each method uses a different column (method 1 uses a DB1 30 m, 0.53 mm i.d., 3 µm film thickness and method 2 uses a RXI5 Sil MS 20 m, 0.18 mm i.d., 0.36 µm film thickness). Multiple solutions were prepared, varying the solution matrix and concentration. The pre-determined RRF’s for the solvent pairs were then used in Equation 3 to calculate the concentration of one solvent. These results were compared to the theoretical ‘true’ value, the two results gave the same answer within experimental error demonstrating that the RRF’s are independent of chromatographic method. A caveat to this is having good chromatography in the method.

Reactivity Considerations

During validation it was noticed the ethyl acetate peak area was reduced in some of the mixed solutions, experimental studies showed ethyl acetate was reacting with methanol in the presence of the basic solvents. To continue the validation we designed the solution mixtures so they didn’t contain all three types of solvent at any one time. As with all quantification methods considerations need to be made for reactivity of all analytes present in the sample, see below.

Method of Analysis

Preparation of calibration solution

1) Choose a suitable diluent solvent, where preferable properties include; good solubility for components of the sample, semi-volatile, inert, high purity and readily available at low cost. Typically our department uses dimethylformamide (DMF), dimethylsulfide (DMSO) or 1,3-Dimethy-2-imidazolidinone (DMI).

2) Add required concentration of calibration solvent and LOQ solvent (optional) and dilute to volume in diluent solvent; this can be stored and used for future analyses.

Sample analysis

Add sample to an appropriate size flask and dilute to volume with the calibration solution as required. Analyse the sample solution using the stipulated chromatographic conditions, use your response factor table to determine RRF and use the correct equation for the application to calculate the concentration of solvent in the sample. An example is given below, however, further application examples are given in the application section.

Example

A sample containing methanol (A) was analysed with dilute (B) used as the calibration solvent to determine the concentration of methanol present in a sample, see below.

Applications

Residual Solvent Content in Solid Samples – using an internal standards GC approach for the analysis of residual solvents, a reduction in sample preparation, analysis time and quantity of solvents is apparent.

Method

Accurately weigh samples and dilute to volume with the pre-prepared calibration solution as required. Analyse the sample solution using the stipulated chromatographic conditions, use your response factor table and Equation 4 to calculate the residual content of solvents in the solid sample.

Project example: 2-propanol was used as a process solvent, typically our department uses 1-propanol, methyl-tetrahydrofuran or decane.

### Table 1: Solvent method used for Proof of Principle

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Response factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.131</td>
</tr>
<tr>
<td>Isopropanol (IPA)</td>
<td>0.234</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.212</td>
</tr>
<tr>
<td>Methyl tert-butyl ether (MTBE)</td>
<td>0.303</td>
</tr>
<tr>
<td>Tetrahydrofuran (THF)</td>
<td>0.270</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.039</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.196</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.490</td>
</tr>
<tr>
<td>Decane</td>
<td>0.462</td>
</tr>
</tbody>
</table>
calibration solvent and chloroform was used as a LOQ solvent, six process solvents were quantified in a single chromatogram, details in Figure 2.

Benefits: Analysis using the internal standards GC approach gave a reduction in sample preparation from 3 hours to less than 30 minutes, a reduction in analysis from 3 hours to less than an hour and a 50% reduction in the quantity of solvents used. Overall, the internal standards GC method combines improved selectivity with a high level of accuracy to produce results in the same time frame as the internal standard NMR method.

Synthetic Organic Process Optimisation – use of internal standards approach in process optimisation allowed non-analysts the ability to analyse process samples in a simple and timely manner.

The quality and yield of a synthetic chemistry process is dependant on solvent composition. Solvent removal and solvent switching between stages of a reaction are used to reduce impurity formation, reduce process times and improve yield, therefore, timely information at this stage can help to improve understanding and determine critical end points.

Method: Take a solution of reaction mixture, filter and dilute to volume with pre-prepared calibration solution. Equation 3 was adapted to include the dilution factor applied to the reaction mixture, as in Equation 5. Analyse the sample solution using the stipulated chromatographic conditions, use your response factor table and Equation 5 to calculate the solvent content in the reaction mixture.

Equation 5

\[
\text{Conc} = \frac{\text{Peak area A}}{\text{Peak area B}} \times \frac{1}{\text{RF}} \times \text{Conc} \times \frac{1}{\text{dilution factor}}
\]

Project examples: i) Decane was used as an internal standard to quantify residual THF in an aqueous layer of reaction mixture, this allowed the synthetic chemist to optimise wash regimes with ethyl acetate to remove THF from the reaction mixture which improved the yield and quality of the process, chromatogram in Figure 3.

ii) The RRF between acetonitrile (ACN) and methyl tert-butyl ether (MTBE) was used to determine residual MTBE content in a single chromatogram, this allowed the process to move to the next stage quickly which reduced product degradation, chromatogram in Figure 4.

Benefits: The fast turnaround of results combined with simple sample preparation allowed the synthetic chemist to make decisions on distillation end points, washing regimes and phase separations to avoid detrimental effects to the reaction mixture.

In-Process Studies Support – the internal standards GC approach allows the replacement of an expensive specifically made standard with a cheap stable standard.
At AstraZeneca the in-process studies group monitor and improve reactions. The group are interested in quantifying starting materials, reagents, products and by-products during a reaction in order to understand the reaction scheme and optimise the reaction conditions. These compounds are typically difficult to make, reactive and expensive, as a consequence the external standards approach required calibration solutions to be specifically prepared before each analysis; this used expensive material and was time intensive. The internal standards GC approach was used to calculate the RRF for a common solvent, allowing the replacement of the calibrant to a common solvent.

Method: To determine a new RRF; prepare three solutions containing a known concentration of the analyte of interest and a known concentration of a selected calibration solvent. Analyse each solution five times, calculate the response using Equation 1 and then calculate the RRF using Equation 2. Calculate the relative standard deviation and measure against internal procedures, record the average results as the RRF in the method table. For sample analysis follow the method described for the process optimisation example using the new calculated RRF in Equation 5.

Project example: Tetrahydrofuran (THF) was used as a internal calibration standard to quantify formation and subsequent reaction of the reactive aldehyde compound, this allowed the synthetic chemist to build a kinetic model of the reaction and determine critical quality attributes in the synthetic process, chromatogram in Figure 5.

Benefits: A specifically made, reactive and expensive standard was replaced for a readily available, cheap and stable standard. Simple sample preparation and fast turnaround time of results encourages the use of process monitoring; the data was used to build a kinetic model to predict product and impurity formation under different conditions.

Preparative Chromatography - use of internal standards GC approach allows simple, fast analysis.

In preparative chromatography a solvent recycling programme is necessary to keep costs affordable. Control of the solvent composition in the solvent recycle programme by the chromatography suite is essential for successful delivery of preparative chromatography because the solvent composition affects the resolution of analytes.

Method: Analyse chromatography sample neat or dilute as appropriate for scale. Equation 3 is modified to calculate the solvent composition of a chromatography sample, as in Equation 6. Use your response factor table and Equation 6 to calculate solvent composition of chromatography sample.

Equation 6
Project example: The RRF between ACN and iso-propanol (IPA) was used to determine residual IPA content of preparative chromatography eluent in a single chromatogram, this allowed the analysts to optimise solvent recycling regimes reducing the quantity of waste solvent, chromatogram in Figure 6.

Benefit: Chromatography samples only contain volatile solvents, the combination of a short isocratic method and no sample preparation leads to turnaround of results in less than 4 minutes.

Assay of Materials in Solution – use of internal standards GC approach allows for simple, fast and accurate analysis.

Assay of materials used in the process is necessary to determine the correct charge for a process. Typically this is performed by 1H NMR, however, assay of a starting material stored in solution by 1H NMR is not always selective and often inaccurate due to the large solute concentration present.

Method: For sample analysis follow the method described for the in-process studies support example, calculate the RRF of the analyte for assay analysis against a suitable calibration solvent, then use the new calculated RRF in Equation 5.

Project example: THF was used as a calibration solvent to assay a starting material stored in toluene, chromatogram in Figure 7.

Benefit: Analysis using the internal standards GC approach increased the accuracy of the assay result and gave a reduction in analysis from 3 hours to less than an hour. This approach is an accurate and efficient way to assay multiple batches of materials in solution.

Conclusion
This approach has shown a range of internal standards can be used as calibration standards for quantification by GC. The internal standards approach for quantitative analysis by GC-FID has improved support in our department in various applications. The approach described allows simple and fast analysis that provides the following benefits:

- Reduced analysis time, the calibration standard is run simultaneously with the sample
- Simplified sample preparation; the calibration solution can be pre-prepared
- Reduced costs; less solvent is required per analysis
- Reduced costs, expensive standards can be replaced with cheap readily available standards
- Increased process understanding;
feedback shows in process analysis is routinely performed by synthetic organic chemists
- Increased colleague engagement;
simplified sample preparation and data interpretation has increased use of GC by non-analysts

References
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4. ICH Q2 R1 Validation of Analytical Procedures: Text and Methodology www.ich.org