

Solubility Determination in HFAs determined by coaxial NMR

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Introduction

Pressurized metered-dose aerosol inhalers (pMDIs) are the most common and convenient systems for delivering therapeutically active drug substances to the lungs. pMDIs can be formulated either as solution-based formulations, in which the drug is either dissolved in a propellant or a combination of the propellant and a co-solvent, or as suspension-based formulations, in which the drug particles are dispersed in a volatile propellant system. The solubility of a given drug in a propellant system is of critical importance when determining the most suitable type of MDI formulation to be developed. Therefore, quantitative determination of low levels of drug solubility in a given propellant is an important first step in the rational design of pMDI formulations. In addition, monitoring drug *kinetic* and *thermodynamic* solubility and stability in a given propellant can be a good initial indication of the physical / chemical stability of the formulation and suggest occurrence of crystal form conversion (solvation etc) or propellant / drug incompatibility.

Historically, determination of API solubility in suitable propellants has proved laborious, since the high volatility of the propellant means the measurement of API solubility must be undertaken using systems based on pressurized filtration, evaporation and transfer to an analytical medium, such as a carrier phase for HPLC measurements.

With the advent of commercial pressure NMR tubes, it is now possible to study HFA propellant *in situ*. These tubes can withstand pressures of up to 7 Bar, under which conditions HFA propellants can be maintained as liquids at room temperature. Furthermore, the fluorination of the propellant makes them ideal solvents for NMR studies, since their chemical shifts overlap only very rarely with those of the solute. In addition, because NMR is blind to solid particles, this method enables samples to be studied without the need of filtration. Samples can be prepared directly in the NMR tubes and using a minimal amount of powder. As such, low levels of sample can be studied “as-is” after a suitable equilibration time.

The quantification of the amount of solute in solution can be performed in two ways, either by an external electronic reference method, ERETIC, as proposed originally by Ph. Rogueda, and made public by Martin et al. (1), or with an internal reference, contained in a co-axial tube. The co-axial method achieves a much more robust signal, and superior accuracy to the ERETIC method.

Experimental

NMR has long been utilised as a technique for the quantification of APIs in the solution state, with the most precise methods involving the incorporation of an internal standard into the system. Direct incorporation into the analytical solution can provide excellent quantitative results, however the system is inherently altered and the acceptability of results gained becomes questionable. Further methods of internal standardisation are available and well documented; a) co-axial NMR systems where an internal standard solution is incorporated into the system, physically separated from the analytical solution by insertion into a glass capillary contained within the NMR tube or b) introduction of an electronic internal standard signal by utilisation of the NMR spectrometers hardware (ERETIC).

The inclusion of a physical internal standard is widely recognised as the most appropriate way of maximising both the accuracy and precision of quantitative measurements by solution state NMR, and also provides the opportunity to introduce a deuterium signal into the system allowing standard operation of the NMR spectrometer with respect to locking and shimming of the sample when analysing samples in non-deuterated solvent systems, such as the hydrofluoroalkanes (HFAs) widely used in pMDI formulations. This standard operation also provides the opportunity to implement an automated acquisition for the routine analysis of samples.

A model system was chosen for method development and associated validation activities. Budesonide was selected as the API, with 2H,3H-decafluoropentane (2H,3H-DFP) HFA propellant as the solvent system. ^1H , ^{13}C and 2-dimensional NMR spectra were obtained for budesonide and 2H,3H-DFP in deuterated solvents to gain a full understanding of the signals received and carry out spectral assignment.

Co-axial systems were investigated using a chloroform internal standard as a solution in deuterated acetonitrile (CD_3CN) in the stem of a co-axial NMR system (Wilmad Labglass, NJ, USA). This allowed standard locking of the spectrometer and gradient shimming to be performed on the deuterium signal of the CD_3CN and standardisation to the chloroform proton. Saturated solution of budesonide was generated at 25°C by equilibration in solution for > 24 hours located in the sample capacity of the co-axial set up. Experimental parameters were studied with this system, including scan number, relaxation times and solvent suppression methods to maximise signal : noise ratios, but at the same time keeping acquisition times to a minimum. Solvent suppression conditions were developed for incorporation into the method to suppress the large signal received from the 2H,3H-DFP, making the dynamic range of these signals more comparable to the low level resonances received from the budesonide.

The final system as operated was calibrated versus a standard series of known budesonide concentrations in 2H,3H-DFP in the range of 25 to 65 $\mu\text{g}/\text{mL}$, giving a linear response and a calibration factor that could be applied to calculations of saturated solutions of budesonide in 2H,3H-DFP for the system, accounting for volume differences of analyte and reference standard, distance from receiver coils in the probe etc. Saturated solutions of budesonide in 2H,3H-DFP at 25°C were analysed as a suspension with residual solids present to gain a quantitative result for the model system – the solid component giving no analytical signal and hence no contribution to the result.

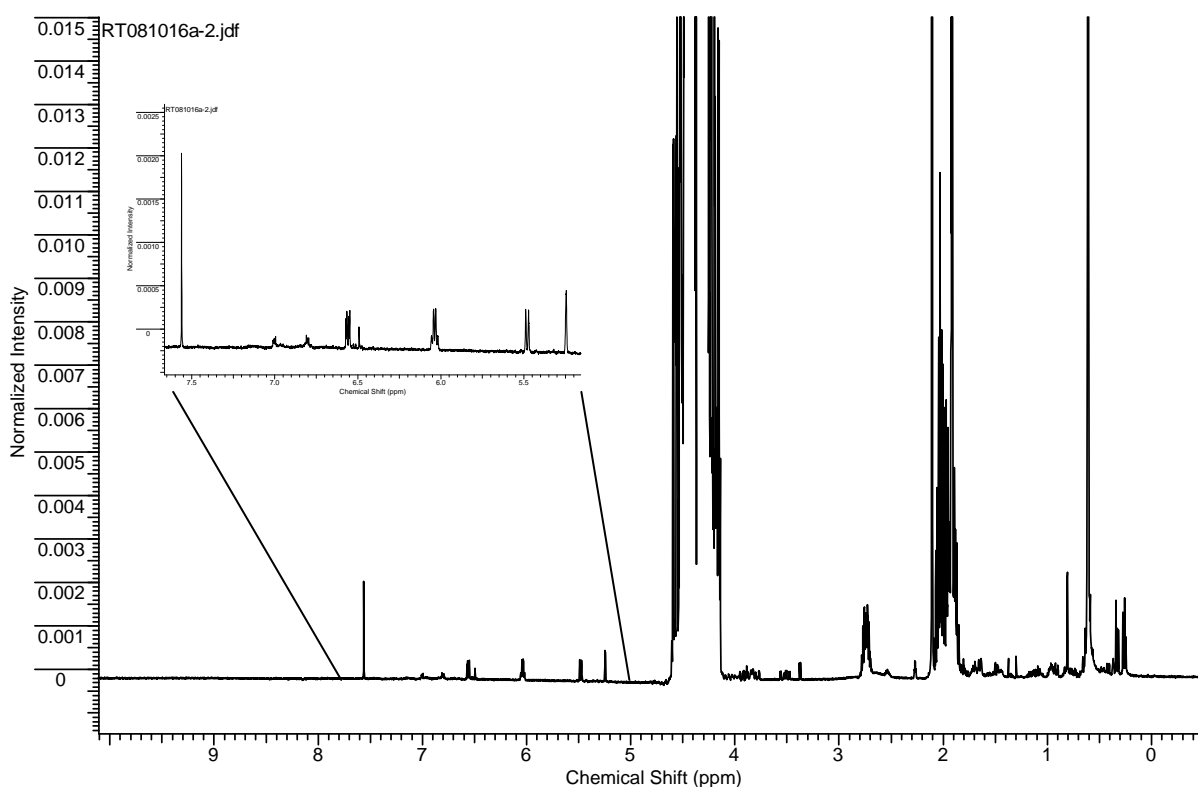
All analysis was carried out on a Jeol ECA 600 MHz FT spectrometer using a 5 mm autotune probe.

Validation of the method was performed using HPLC methods. Saturated solutions of budesonide in 2H,3H-DFP were created in the same manner as used for NMR experimentation. A filtration step was performed to remove any residual solids and suitable dilutions carried out to ensure complete dissolution of any budesonide in the system. The resultant solution was analysed vs. standard reference materials to obtain a quantitative result.

Results and Discussion

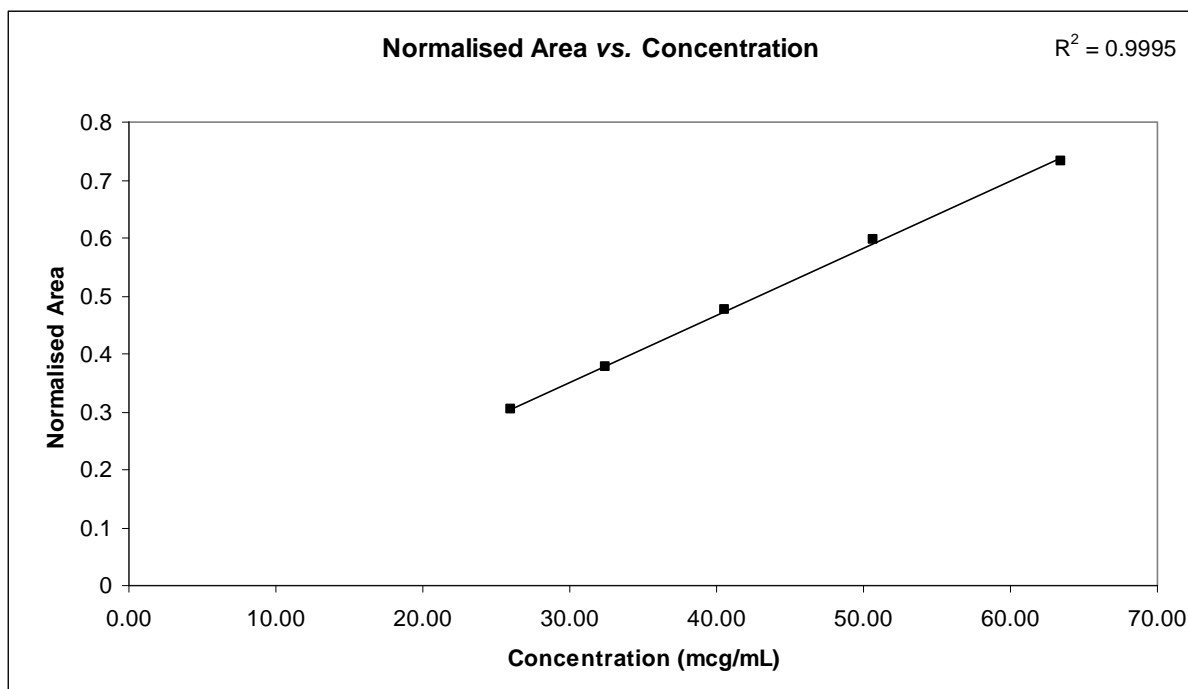
The spectrum obtained for the saturated suspension of budesonide in 2H,3H-DFP is shown in Figure 1, with three low field signals (5 to 7 ppm) attributable to the olefinic protons in the C1, C2 and C4 positions, and the internal standard (CHCl_3) at 7.56 ppm. These resonances were suitably separated from the large signals received from the 2H,3H-DFP to allow quantitation to be achieved without interference, and the magnitude of the solvent signals dramatically reduced by solvent suppression (separate experimentation had established solvent suppression magnitudes suitable so as not to affect the resonances used for quantification).

Figure 1 – Saturated suspension of budesonide in 2H,3H-DFP



The solubility of the budesonide in 2H,3H-DFP at 25°C was determined to be 87 $\mu\text{g/mL}$, with an LOQ for this system being ca. 20 $\mu\text{g/mL}$ (though longer acquisition times and re-calibration should yield significantly lower LOQ/LOD). Linearity curve for the standard series shown as Figure 2.

Figure 2 – Linearity series for the calibration of the system – budesonide solutions in the range 25 to 65 µg/mL.



Results obtained by HPLC analysis of the saturated solutions confirmed that the NMR method was highly accurate, with a result of 87 µg/mL being obtained by this methodology.

Conclusions

The study undertaken has provided a highly sensitive, accurate and precise co-axial NMR method of quantification for a model system of budesonide in 2H,3H-DFP. A small amount of further work should see the methodology become capable of determining the solubility of the model system at elevated pressures using the proprietary J Young co-axial system. The method will also be transferable to other APIs and propellants.