Assay of Tadalafil in Formulated Tablets via Benchtop qNMR

nanalysis

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Introduction

Nuclear Magnetic Resonance (NMR) is an extremely powerful characterization technique that is frequently used for pharmaceutical research to identify new compounds, assess purity and characterize and optimize chemical reactions, either online (with a flow cell), off-line or at-line(for NMR tube reactions or aliquot analysis). Although less common, it has also been established as a useful technique in pharma for routine quantitative analysis.[1,2,3,4] Benchtop NMR has emerged as an accessible technology to extend the use of NMR Spectroscopy to start-ups and SME pharmaceutical companies, as well as provide an easy-to-use, compact and automatable alternative to allow qNMR to be incorporated directly in quality control labs and used by technicians.

In this context, we describe the use of benchtop NMR for structure confirmation of an active drug ingredient; evaluation of degradation products and/or impurities, towards assessing the efficacy and performance of this technique for assay and content uniformity testing.

NMR has many advantages over commonly used methods, such as titration, infrared (IR) spectroscopy, Raman spectroscopy, and chromatography. It is chemically specific, non-targeted, non-destructive, inherently qualitative without calibration and requires very simple sample preparation. It does not require the preparation of dilution series, and does not consume large amounts of solvent, nor does it require an authentic standard substance[5] for analysis.

In this application note, we evaluate the purity of tadalafil bought as a reference standard (**Figure 1a**) using ^{1}H NMR and perform the assay test using two commercially available tadalafil-containing. These drugs are sold under the brand name Cialis and generic name Apo-Tadalafil. These drugs are used to treat benign prostatic hyperplasia,[6] erectile dysfunction,[7] and treat pulmonary arterial hypertension[8].

Dimethyl terephthalate (**Figure 1b**) was used as the internal calibrant in this quantitative NMR analysis. It was chosen because at least one of its ^{1}H resonances do not overlap with those resonances of the target compound, and its T_1 values are shorter than other possible qNMR internal standards. The example presented here is specific to pharma, but the qNMR method is general and can be validated for routine analysis in any field where purity determination is critical to the process.
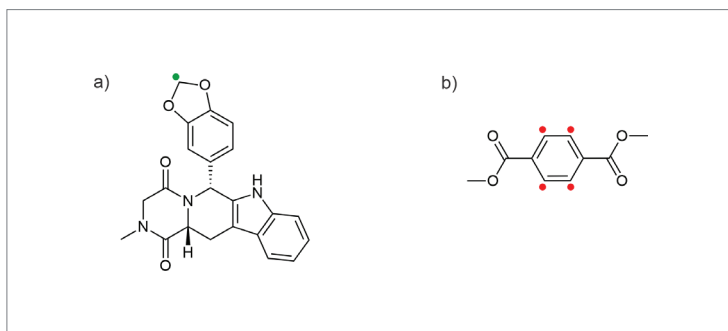

Figure 1: a) Structure of tadalafil, and b) structure of dimethyl terephthalate (DMT). The green and red circles represent the protons under evaluation in this study.

Experimental procedure: Tadalafil reference standard purity determination

The reference standard compound (tadalafil, 11.78 mg) Lot#: LRAB8867 and a certified internal calibrant (dimethyl terephthalate (DMT), 2.73 mg) Lot#: BCBT9974, were accurately weighed into a vial, and 600 µL of DMSO-d_6 was added. The solution was mixed using a vortex mixer until the sample and the internal standard were fully dissolved. The resultant clear solution was transferred to a 5 mm NMR tube, and the ^{1}H NMR spectrum was obtained after the T_1 values were determined. The T_1 value for the DMT signal at 8.08 ppm is 2.82 s, and the value for the tadalafil signal at 5.92 ppm is 0.53 s. All compounds were purchased from Sigma-Aldrich and used without further purification.

The spectra were obtained at 32 °C using a Nanalysis 60PRO benchtop NMR spectrometer at a 60 MHz proton frequency (1.418 tesla). The experiments were performed with the following acquisition parameters: number of complex points, 8192; spectra width, 24 ppm; number of scans, 32; scan delay, 20 s; spectral center, 8.03 ppm; acquisition time, 5.57 s; 90° pulse duration, 15.1 µs. Each spectrum was processed applying zero filling, number of points with zero filling equal 65536, phase and baseline correction were performed, Lorentz-to-Gauss window multiplication was applied to the FID (exponential line broadening (lb) and Gaussian line broadening (gb) were set to -0.1 and 0.3 Hz, respectively).[9] The spectrum acquisition was performed in quintuplicate.

Results and discussion: Tadalafil reference standard compound purity determination

The purity of the analyte P_X was calculated using the following **Equation (1)**:

$$P_x = \frac{A_x}{A_{DMT}} * \frac{N_{DMT}}{N_x} * \frac{M_x}{M_{DMT}} * \frac{m_x}{m_{DMT}} * P_{DMT} \qquad (1)$$

Where A_x is the integral value of the signal at 5.92 ppm which belongs to tadalafil; A_{DMT} is the integral value of the signal at 8.08 ppm that belongs to DMT; N_{DMT} and N_x correspond to the number of spins of DMT and tadalafil, respectively; M_x and M_{DMT} are the molecular weight of tadalafil and DMT, respectively; m_x is the weighted mass of tadalafil; and m_{DMT} and P_{DMT} are the weighted mass and the reported purity of DMT[10], respectively.

Table 1: Determination of tadalafil (X) reference compound purity using dimethyl terephthalate (DMT) as an internal calibrant.

	A_x	A_{DMT}	N_{DMT}	N_x	M_x (g.mol^{-1})	M_{DMT} (g.mol^{-1})	m_x (mg)	m_{DMT} (mg)	P_{DMT}[#] %	P_x %
1	5228386.44	4884533.93	4	2	389.4	194.186	2.73	11.78	99.95	99.4
2	5224447.26	4858522.55	4	2	389.4	194.186	2.73	11.78	99.95	99.9
3	5231152.01	4866928.42	4	2	389.4	194.186	2.73	11.78	99.95	99.9
4	5245944.95	4870028.1	4	2	389.4	194.186	2.73	11.78	99.95	100.1
5	5215474.05	4883227.2	4	2	389.4	194.186	2.73	11.78	99.95	99.2

[#]Value reported by the commercial supplier[10]

The pre-set integration region applied to each signal in **Figure 2** was: 8.2791 to 7.9320 ppm for DMT and 6.0283 to 5.7700 ppm for compound X. Replacing the variable values of **Equation 1** by the values reported in **Table 1**, a P_X purity value of 99.7 ± 0.4 is achieved. The calculated value agrees with the value range reported by the supplier company[11] (99.7 ± 0.1).
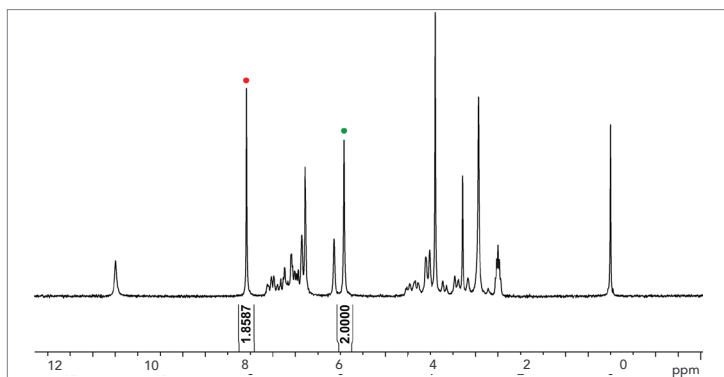
Figure 2. ^1H NMR spectrum of a mixture of tadalafil and DMT in DMSO-d_6 acquired using a Nanalysis benchtop 60PRO. The green and red circles represent the protons under evaluation of tadalafil and DMT, respectively.

Experimental procedure: Cialis and Apo-Tadalafil assay test

The assay tests were performed by weighing and powdering five tablets of each drug. The drug boxes indicate that each tablet contains 20 mg of tadalafil. A portion, representing approximately 20 mg of the active compound, of each well-mixed powder (210.91 mg for Cialis and 239.07 mg for Apo-Tadalafil) and the calibrant compound, DMT (2.25 mg for Cialis and 3.6 mg for Apo-Tadalafil), were weighed on an analytical balance (Mettler-Toledo MS105DU), followed by the addition of DMSO-d_6 (950 μL). The two vials containing the mixtures were vortexed for 5 minutes, followed by sonication for an extra 20 minutes. After centrifugation, the supernatants were filtered and added to a 5 mm standard NMR tube.[5] The acquisition and processing parameters used in each ^1H NMR spectrum were the same as described in the procedure for purity determination.

Results and discussion: Cialis and Apo-Tadalafil Assay test

To determine the mean mass of tadalafil in each tablet Equation 2 was used:

$$m_x = \frac{A_x}{A_{DMT}} * \frac{N_{DMT}}{N_x} * \frac{M_x}{M_{DMT}} * \frac{m_{DMT}}{m_{powder}} * P_{DMT} * T \qquad (2)$$

Where m_{powder} is the weighted mass of tablet powder sample taken for the assay test, and T is the average tablet weight. The meaning of any other parameters is identical as described in **Equation 1**.

Table 2: Determination of tadalafil average mass in Cialis tablets using dimethyl terephthalate (DMT) as a calibration compound.

	A_x	A_{DMT}	N_{DMT}	N_x	M_x (g.mol^{-1})	M_{DMT} (g.mol^{-1})	m_{DMT} (mg)	m_{powder} (mg)	P_{DMT} #	T (g)	m_x (mg)
1	278193.95	217851.59	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.8
2	277063.35	212686.38	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	20.2
3	273939.14	218779.56	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.4
4	277771.38	216568.53	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.9
5	276389.04	213979.22	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	20.0

#Value reported by the commercial supplier[10]

Table 3: Determination of tadalafil average mass in Apo-Tadalafil tablets using dimethyl terephthalate (DMT) as a calibration compound.

	A_x	A_{DMT}	N_{DMT}	N_x	M_x (g.mol^{-1})	M_{DMT} (g.mol^{-1})	m_{DMT} (mg)	m_{powder} (mg)	P_{DMT} #	T (g)	m_x (mg)
1	261260.33	314656.41	4	2	389.4	194.18	3.6	239.07	0.9995	0.41069	20.6
2	245126.49	301275.86	4	2	389.4	194.18	3.6	239.07	0.9995	0.41069	20.2
3	248098.42	304813.33	4	2	389.4	194.18	3.6	239.07	0.9995	0.41069	20.2
4	247394.95	303285.38	4	2	389.4	194.18	3.6	239.07	0.9995	0.41069	20.2
5	246545.00	300803.85	4	2	389.4	194.18	3.6	239.07	0.9995	0.41069	20.3

#Value reported by the commercial supplier[10]

The relative area of the chosen signals was determined by applying a pre-set integration region from 8.1964 to 7.9930 ppm for the DMT signal and from 5.9943 to 5.8340 ppm for the tadalafil signal (**Figure 2**). Replacing the values reported in **Tables 2** and **3** for its respective variables in **Equation 2**, an average mass of 19.8 ± 0.3 mg per tablet for Cialis and 20.3 ± 0.2 mg for each Apo-Tadalafil tablet was obtained. Both values are in the range accepted by the USP monograph for tadalafil, reported as being 20 ± 2 mg, as well as with the high field data, which was determined as being 20.0 mg and 20.9 mg, respectively for Cialis and Apo-Tadalafil.
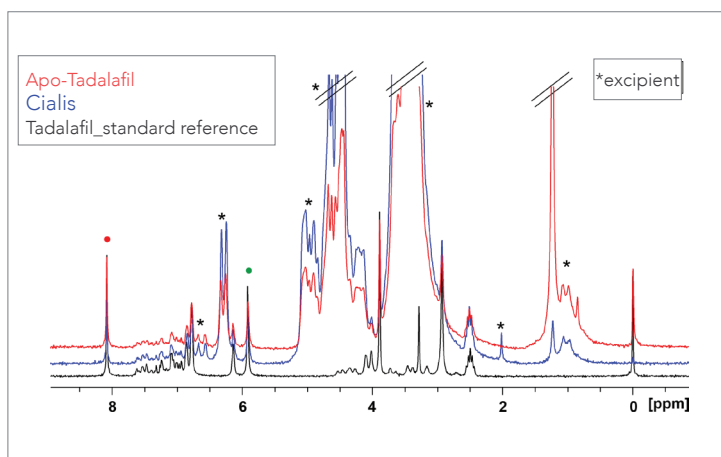

Figure 3. ^1H NMR spectrum of the tadalafil reference standard in DMSO-d_6 (black); ^1H NMR spectrum of the extraction of Cialis tablets with DMT in DMSO-d_6 (blue); ^1H NMR spectrum of the extraction of Apo-Tadalafil tablets with DMT in DMSO-d_6 (red). All spectra were acquired in a Nanalysis 60PRO.

In addition to the quantitative information obtained in the assay tests, a fingerprint of the excipients can be observed and used for quality control when using NMR analysis (**Figure 3**). For the studied case, the following excipients were reported by the suppliers: croscarmellose sodium, hydroxypropylcellulose, hydroxypropylmethylcellulose, iron oxide, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium lautyl sulfate, talc, titanium dioxide and triacetin for Cialis and croscarmellose sodium, hydroxypropylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium lautyl sulfate, titanium dioxide, ferric oxide red, ferric oxide yellow, hypromellose, poloxamer, and polyethylene glycol for Apo-Tadalafil.

Conclusion

Benchtop NMR was successfully used to evaluate the purity of tadalafil and perform the assay test of two different tablets containing tadalafil as the active ingredient. Like other methods, a sample preparation step is required before the data acquisition and evaluation. However, with NMR, the data can be acquired in minutes. In addition, the data evaluation is straightforward since the peak area in NMR is directly proportional to the number of protons present in the molecule. The internal calibrant, which has a known concentration and structure, has the role of providing one or more signals in areas that can be used for the quantification of the target analyte.

This study indicates how powerful benchtop NMR can be in quality control analysis and how it can be used as a complementary method in many steps of drug evaluation. Once the method is developed and validated, the results can be obtained quickly, with a low volume of waste solvent, no requirement for a specialist to run the sample or to evaluate the data, without mentioning the wealth of information obtained.

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