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^{1}H, ^{13}C, ^{15}N NMR analysis of sildenafil base and citrate (Viagra) in solution, solid state and pharmaceutical dosage forms

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Abstract

Sildenafil citrate (SC) (Viagra) and sildenafil base in pure form are easily and unequivocally characterized by multinuclear NMR spectroscopy. Analysis of chemical shifts indicates that: (i) N6–H forms intramolecular hydrogen bonds, (ii) N25 is protonated in the salt and (iii) intermolecular OH···N hydrogen bonds involving N2 and N4 are present in the solid sildenafil citrate. ^{13}C CPMAS NMR method has been proposed for the identification and quantitation of Viagra in its pharmaceutical formulations.

Keywords: Sildenafil citrate; Viagra; Solid-state NMR; Pharmaceutical formulations

1. Introduction

Sildenafil, 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate (Fig. 1), is an inhibitor of phosphodiesterase type 5 (PDE5), which is responsible for the degradation of cyclic guanosine monophosphate (cGMP). Sildenafil is claimed to having utility in a variety of therapeutic areas including the treatment of various cardiovascular disorders such as angina, hypertension, heart failure and atherosclerosis [1,2].

Sildenafil citrate (SC) (Viagra) has been approved for the treatment of erectile disorder. It has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of nitric oxide by inhibiting PDE5. When sexual stimulation causes local release of NO, inhibition of PDE5 by sildenafil causes increased levels of cGMP, resulting in smooth muscle relaxation and inflow of blood into the corpus cavernosum. Sildenafil at recommended doses has no effect in the absence of sexual stimulation [2,3].

The detailed study concerning solid-state structure of this compound is very important for understanding enzyme (PDE5)–inhibitor (sildenafil) interaction. It is also of interest to determine sildenafil's protonation sites since they may be responsible for its binding to the phosphodiesterase acidic amino acids.

HPLC [4] and HPLC–MS [5] procedures were developed for the determination of sildenafil in biological fluids. Reverse-phase high performance liquid chromatographic (RP-HPLC) method has been used [6] for the quantitation of sildenafil citrate in pure form and its pharmaceutical formulations. However, there is no official (pharmacopoeial) method for the assay of sildenafil citrate formulations. Existing techniques used to identify genuine and counterfeit products include HPLC [7,8] and NIR microscopy [9]. NMR spectroscopy, especially solid-state technique, could be an additional method for investigation of active compounds in drug formulation. The aim of this study was to collect ^{1}H, ^{13}C, ^{15}N NMR data required for identification and structural characterization of sildenafil base and citrate. Solid-state ^{13}C NMR is proposed as fast method for authentication of commercial products, tablet dosage forms with Viagra.

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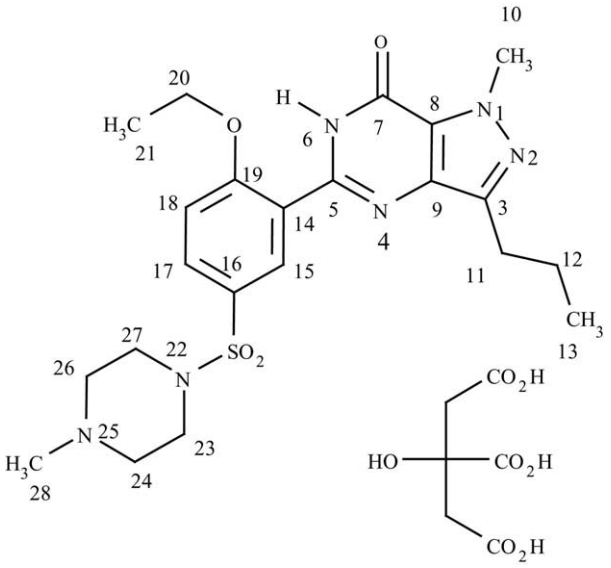

Fig. 1. Structure of sildenafil citrate with atom numbering.

2. Experimental

Sidenafil base and citrate were provided by Pharmaceutical Research Institute, Warsaw. The samples were stored in dry conditions at 280 K. Commercial Viagra tablets (Pfizer S.A.) contain sildenafil citrate, microcrystalline cellulose, calcium dibasic phosphate, crosscarmellose and magnesium stearate.

^{1}H, ^{13}C, ^{15}N NMR spectra for solution were recorded on a Bruker DRX500 spectrometer operating at 500.13 MHz for ^{1}H, 125.75 MHz for ^{13}C and 50.68 MHz for ^{15}N. All one- and two-dimensional experiments (HMQC, HSQC and HMBC) were run using the programs from the Bruker software library. Two-dimensional ^{1}H–^{15}N NMR measurements were carried out using the phase-sensitive gradient-selected inverse technique. The experiments were optimised for $^{2}J/^{3}J$ coupling constants of ca. 6 Hz (D6 = 0.08 s) and performed with four scans of 128 echo and four scans of 128 anti-echo accumulations; two-dimensional experimental data were zero-filled to 512 points along the nitrogen direction. The chemical shifts were referenced against internal TMS (^{1}H, ^{13}C) and external neat CH_3NO_2 (^{15}N).

Cross-polarisation (CP) magic angle spinning (MAS) solid-state ^{13}C and ^{15}N NMR spectra were recorded on a Bruker AVANCE-400 instrument at 100.13 and 40.55 MHz, respectively. Powder samples were spun at 7–10 kHz in 4 mm ZrO_2 rotor. A contact time of 5 ms, a repetition time of 8 s and a spectral width of 20 kHz were used for accumulation of 200–800 scans for standard ^{13}C MAS experiment. ^{13}C chemical shifts were calibrated indirectly (external reference) through the glycine CO signal recorded at 176.0 ppm relative to TMS. For ^{15}N MAS spectra a contact time of 7 ms, repetition time of 8 ms and spectral width of 32 kHz were used and ca. 8000 scans were accumulated. The chemical shifts were calibrated indirectly through the glycine NH signal at −350 ppm relative to CH_3NO_2.

The 25, 50 and 100 mg tablets of citrate were products of Pfizer Ltd.; the tablets were weighed and powdered. The weighed amount of solid was packed into NMR rotor.

3. Results and discussion

3.1. NMR in solution

^{1}H, ^{13}C, ^{15}N NMR spectra were recorded for base and salt in DMSO-d_6 in order to establish the protonation site. The inspection of ^{1}H chemical shifts (Table 1) showed that the differences between base and salt are significant only for the piperazine fragment; the most probable place of protonation is therefore the N25 nitrogen. ^{13}C and ^{15}N NMR spectra were assigned with the aid of 2D ^{1}H{^{13}C} and ^{1}H{^{15}N} correlation spectra, and the respective chemical shifts (in DMSO-d_6) are collected in Tables 2 and 3.

The ^{1}H–^{15}N correlation spectrum showed that N25 peak at −344.5 ppm was bonded to the ^{1}H at δ2.15 (CH_3), the N22 peak at −280.5 ppm was bonded to the piperazine protons at δ2.37 (CH_2), whereas the N2 peak to the CH_3 signal at δ4.16 and CH_2 at δ2.78. The ^{1}H peak at 12.2 ppm allowed easy assignment of the ^{15}N6 resonance at −211.1 ppm. The ^{1}H and ^{15}N chemical shifts for the citrate salt were assigned in similar way and are given in Table 3. The ^{15}N chemical shifts of N1, N2, N4 and N6 are the same for base and salt: differences of ca. 1 ppm are observed for piperazine nitrogens N22 and N15. The signal of N4 was of low intensity in the spectra recorded in the inverse detection mode, and its assignment was additionally confirmed by the direct detection of ^{15}N resonances.

The effect of protonation of the piperazine nitrogen atoms was determined by analysis of the ^{1}H and ^{13}C NMR spectra of buspirone analogues, bearing the same structural fragment [10]. The chemical shifts values of the carbon neighbours of N^+ in protonated compounds showed a diamagnetic effect

Table 1
^{1}H chemical shifts for sildenafil base and citrate in DMSO-d_6

Hydrogen atom	Base	Salt (citrate)
10 (N1–CH_3)	4.16	4.17
11 (CH_2)	2.78	2.78
12 (CH_2)	1.74	1.75
13 (CH_3)	0.94	0.94
15 (CH)	7.85	7.87
17 (CH)	7.82	7.84
18 (CH)	7.37	7.38
20 (OCH_2)	4.22	4.22
21 (CH_3)	1.34	1.34
23, 27 (CH_2)	2.91	2.98 (0.07)
24, 26 (CH_2)	2.37	2.56 (0.19)
28 (N25–CH_3)	2.15	2.28 (0.13)
N6–H	12.19	12.20
Citrate (CH_2)	–	2.67

The values $\Delta = \delta_{salt} - \delta_{base} > 0.05$ ppm are given in parentheses.

Table 2
^{13}C chemical shifts (δ, ppm) for sildenafil base and citrate (Viagra) in DMSO-d_6 and solid state

Carbon atom	Base		Salt			Δ' (ppm)
	δ (ppm), DMSO-d_6	δ (ppm), solid-state	δ (ppm), DMSO-d_6	δ (ppm), solid-state	δ (ppm), tablet[a]	
C3	137.93	137.8	137.89	135.4 (2.5)	135.5	2.5
C5 (NCN)	153.75	153.0 (0.8)	153.92	152.2 (1.7)	151.9	0.7
C7 (C=O)	148.20	144.7 (3.5)	148.24	146.7 (1.5)	146.8	−2.0
C8	124.52	123.6 (0.9)	124.51	124.1	124.3	
C9	145.02	144.7 (1.9)	145.07	146.7(−1.7)	146.8	−2.0
C10 (NCH_3)	38.15	38.3	37.88	37.1	37.2	1.1
C11 (CH_2)	27.66	30.2 (−2.5)	27.12	26.7 (0.4)	26.8	3.5
C12 (CH_2)	21.81	21.3 (0.6)	21.66	26.7 (−5.1)	26.8	−5.4
C13 (CH_3)	14.10	15.2 (−1.1)	13.81	15.8 (−2.0)	15.8	
C14	121.18	118.0 (3.2)	123.84	117.6 (6.3)	117.7	
C15 (C−H)	131.20	131.3	130.11	130.0	130.0	1.3
C16	128.80	125.0 (3.8)	126.21	125.8	125.6	−0.7
C17 (C–H)	131.70	131.3	131.63	131.1	131.0	
C18 (C–H)	113.08	113.8	113.38	112.2 (1.1)	112.3	1.6
C19	160.01	159.6	160.12	159.4	159.5	
C20 (OCH_2)	64.93	66.9 (−2.0)	64.92	65.9 (−1.0)	[b]	1.0
C21 (CH_3)	14.44	15.2	14.24	15.1 (−0.9)	15.3	
C23 (CH_2)	45.87	46.0	45.15	46.8 (−1.6)	46.9	−0.8
C24 (CH_2)	53.68	53.6	53.09	52.4 (0.7)	52.3	1.2
C26 (CH_2)	53.68	53.6	53.09	52.4 (0.7)	52.3	
C27 (CH_2)	45.87	46.0	45.15	46.8 (−1.6)	46.9	
C28 (CH_3)	45.64	45.2	44.57	43.6 (1.0)	43.7	1.6
Citrate: (CH_2)			43.10	43.6 (−0.5)	43.7	
C			72.22	75.6 (−3.4)	[b]	
2 × C=O			171.47	170.6 (0.9)	170.7	
C=O			175.32	177.9 (−2.6)	178.0	

The differences $\Delta = \delta_{liquid} - \delta_{solid} > 0.5$ ppm are given in parentheses; $\Delta' = \delta_{solid\ salt} - \delta_{solid\ base}$.
[a] Powdered 100-mg tablet with sildenafil citrate.
[b] Overlapped with signals of excipient.

(1.5–1.9 ppm for C_α and 2.4–3.7 ppm for C_β). In the case of sildenafil, the diamagnetic effect was observed (Table 2) for C28 (1.0 ppm) and for piperazine carbons C24, 26 (0.9 ppm) and C23, 27 (0.7 ppm), confirming that N25 is protonated in the citrate.

3.2. Multinuclear NMR in the solid-state

Although majority of the pharmaceutical products exist in solid form, solid-state NMR is rarely applied for their characterization [11,12]. It seemed interesting to gain some insight into the structure and intermolecular interactions of sildenafil in the solid phase; therefore, the ^{13}C CP MAS NMR spectra of base and salt were recorded. No rotational signals (spinning sidebands) are observed in the range 0–190 ppm when the rotation speed is 8–10 kHz. The resonances of carbons linked to nitrogen (C5 and also C7–C9) are slightly broader as a result of unaveraged residual coupling to quadrupolar ^{14}N. The ^{13}C NMR spectra of pure base and salt (citrate) are shown in Fig. 2. The spectra were assigned on the basis of liquid-state chemical shifts, dipolar dephasing and variable contact time experiments. The ^{13}C chemical shifts for solids are included in Table 2. The most interesting are the differences in chemical shifts $\Delta = \delta_{liquid} - \delta_{solid}$ since these values are indicative of rigid and conformationally flexible fragments of the molecule (the latter are expected to undergo larger changes). Compared to the averaged value in the liquid state, the signals of ethyl and of *n*-propyl carbons are shifted remarkably (+1.0 to −5.1 ppm).

Table 3
^{15}N NMR chemical shifts (δ, ppm) for sildenafil base and salt (citrate) in DMSO-d_6 and solid state; for pure compounds: $\Delta = \delta_{liquid,\ salt} - \delta_{solid,\ salt}$, $\Delta' = \delta_{solution,\ salt} - \delta_{solution,\ base} > 0.5$ ppm

Nitrogen atom	Base, DMSO-d_6	Salt, DMSO-d_6	Δ'	Salt, solid-state	Δ	Salt tablet[a]
N1	−198.8	−198.5		−203.2	4.7	−203.0
N2	−56.0	−57.2	1.2	−79.8	21.6	−79.3
N4	−148.0	−148.3		−159.6	11.3	−158.0
N6	−211.1	−211.3		−219.6	8.3	−219.2
N22	−280.4	−282.7	2.3	−284.3	1.6	−283.7
N25	−344.5	−343.6	−0.9	−341.3	−2.3	−240.6

[a] Powdered 100-mg tablet.

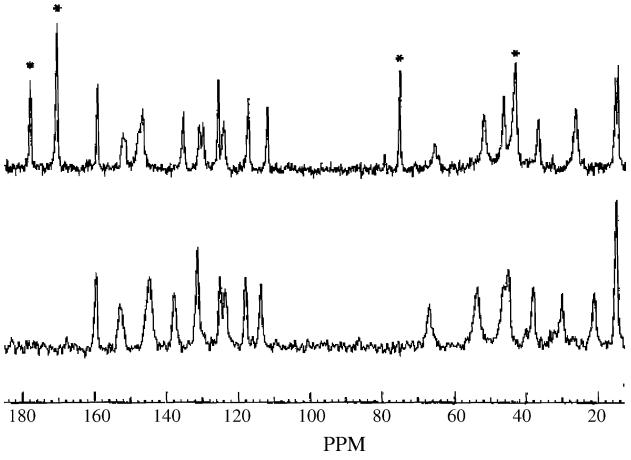

Fig. 2. ^{13}C CPMAS spectra of sildenafil base and citrate (the signals of citrate anion are marked with asterisks).

Molecular modelling shows that there is not enough space for free rotation of the alkyl substituents. The rotation of the ethyl group about the C19–O bond is hindered by the presence of heterocyclic system, the hindrance should be less if the planes of the aromatic and heterocyclic rings were perpendicular. However, the conformation with coplanar rings may be preferred since it is stabilised by the intramolecular hydrogen bond N6–H···O–Et, completing the six-membered cycle. The high-frequency position of N6–H signal at δ12.2 ppm in the ^{1}H spectra of base and citrate (Table 1) suggests that such an intramolecular bond does exist. The ethoxy group cannot be coplanar with the aromatic ring and directed to the C14–C5 bond; more probably, it is located above or below the plane of aromatic ring. Locked conformations of OMe or OEt groups usually result in chemical shifts changes of neighbouring *ortho* carbons. If the methyl of the ethoxy group points to C18–H, an increase of C18 shielding and a decrease of C14 shielding should be expected, such distinct changes are not observed (Table 2). The shielding of C14 is increased of 6.3 ppm for the citrate salt and of 3.2 ppm for the base, compared to the situation in solution.

The intramolecular rotation within the *n*-propyl chain, especially the rotation about C11–C12 bond, results in steric interaction of the terminal methyl group with either N4 or N2. Significant values of Δ (see Table 2) are due to the locking of this fragment into a particular conformation in the solid. The largest deshielding is observed for C12 ($\Delta = -5.4$ ppm) in the solid citrate salt.

One could expect that the N6–H and C7=O groups will form intermolecular hydrogen bonds in the crystal. The low frequency shift of C7=O in the spectra of the solid base and the citrate salt ($\Delta = 3.5$ and 1.5 ppm, respectively) suggests that the carbonyl group in not involved in C=O···HN bonds (steric crowding probably prevents free access to the HN–C=O fragment).

Further information on the interactions in the solids can be obtained from the analysis of ^{15}N MAS NMR chemical shifts.

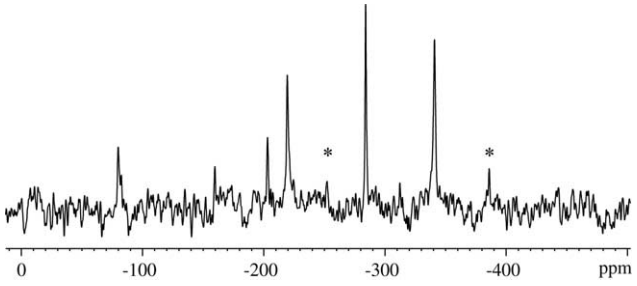

Fig. 3. ^{15}N CPMAS spectrum of sildenafil citrate (spinning side bands are marked with asterisks).

The ^{15}N spectrum of pure solid sildenafil citrate is shown in Fig. 3. Large differences, Δ, in chemical shifts between the solid and solution states of 11 and 21 ppm appear for N1 and N4, respectively. The nitrogens –N= are very sensitive to intermolecular interactions and the effects of about 10–20 ppm were observed (for example, for pyrazoles [13]) upon formation of hydrogen bonds. It is probable that hydroxyl groups of the citrate anion act as hydrogen bond donors and the OH···N1 and OH···N4 bonds exist in the crystals.

Crystal packing and intermolecular interactions are difficult to recognise in detail without crystallographic X-ray diffraction data. According to the Cambridge Structural Database, there is no structure of crystalline sildenafil, although the constants of citrate monoclinic lattice are given in [14].

The signal intensity in the cross-polarisation spectra is a function of the contact time t [15]: $I(t) = A(1 - T_{CP}/T_{1\rho}^{H})^{-1}[\exp(-t/T_{1\rho}^{H}) - \exp(-t/T_{CP})]$, where A is the intensity amplitude; the progress of cross-polarisation is characterised by the time constant T_{CP}. The decrease in intensities for longer contact times is due to the effects of ^{1}H spin–spin relaxation in the rotating frame, $T_{1\rho}^{H}$. The selected results of the variable-contact cross-polarisation experiments for sildenafil base are collected in Table 4; in our experiments the $T_{1\rho}^{H}$ relaxation time was long and the CP curves ended with plateaux. Therefore, the fitting of the experimental results was done with the reduced equation (neglecting the terms with $(T_{1\rho}^{H})^{-1}$): $I(t) = A[1 - \exp(-t/T_{CP})]$. The T_{CP} time depends on the magnitude of the ^{1}H–^{13}C dipolar coupling, it decreases with the number of hydrogen atoms bound to the carbon under study and increases with increasing mobility of the respective structural fragment. The carbons of *n*-propyl chain are characterised by very short T_{CP} times of 0.05 ms, for aromatic CH carbons the values of T_{CP} are: 0.08–0.13 ms. For quaternary carbons cross-polarisation is less effective and the T_{CP} times are longer: 0.62–0.68 ms. Maximum intensity of the *n*-propyl chain carbon signals is achieved with contact time of 1–2 ms. However, more suitable for quantification is the signal of quaternary carbon C19 (δ159.5 ppm), it is narrow, not overlapped by other resonances and reaches maximum intensity after $t = 4$ ms. Therefore, optimal contact time for measuring ^{13}C CP MAS spectra of sildenafil base is ca. 4–5 ms.

Table 4
Selected results of the variable-contact cross-polarisation experiments for solid sildenafil base and sildenafil citrate in 100-mg tablet

Carbon atom	Sildenafil base		Sildenafil citrate[a]	
	T_{CH} (ms)	$T_{1\rho}$ (ms)	T_{CH} (ms)	$T_{1\rho}$ (ms)
C5	0.68			
C7=O	0.62			
C9	0.61			
C11 (CH_2)	0.072	711		
C12 (CH_2)	0.049	160		
C13 (CH_3)	0.034		0.24	6.50
C14			1.36	6.45
C16			0.92	7.46
C17 (C–H)	0.13			
C18 (C–H)	0.08			
C19			1.23	6.69
C23 (CH_2)	0.049	155		
C24 (CH_2)	0.052	300		
C26 (CH_2)	0.050	300		
C28 (CH_3)	0.033			
C=O at 170 ppm (citrate)			0.59	6.42
C1 at 104 ppm (carbohydrate excipients)			0.18	4.45

[a] In 100-mg tablets.

3.3. Solid-state NMR of pharmaceutical dosage forms

The ^{13}C CP MAS spectra of the tablets containing different amounts of sildenafil citrate were recorded under identical experimental conditions. It is worth mentioning that chemical shifts of sildenafil citrate in pure form and in pharmaceutical dosage forms are the same (Table 2). Large peaks in the 50–110 ppm range are due to excipients (Fig. 4a). The CH resonances of carbohydrates may be suppressed using dipolar dephasing (delayed decoupling) pulse sequence with a delay of 40–70 μs inserted before the data acquisition. During this short period directly protonated carbons dephase rapidly and their signals are reduced whereas nonprotonated carbons (and methyl carbons of rotating groups) can be selectively observed (Fig. 4b). Sildenafil citrate is easily detected in the pharmaceutical dosage forms since only two of its carbon resonances (OCH_2 and quaternary carbon of the citrate anion) fall into carbohydrate-type region.

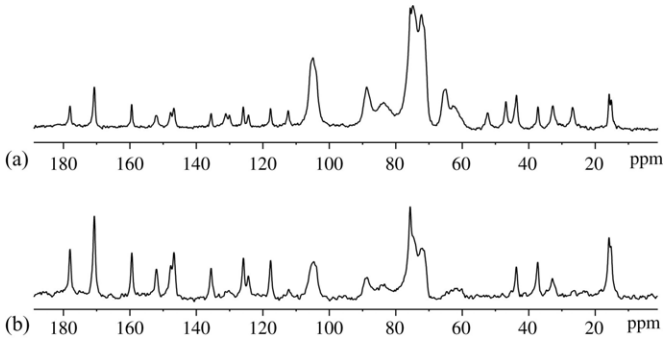

Fig. 4. ^{13}C CPMAS spectra of sildenafil citrate (Viagra) of the commercial tablet: (a) standard spectrum and (b) spectrum recorded with dipolar dephase pulse sequence (delay of 40 μs).

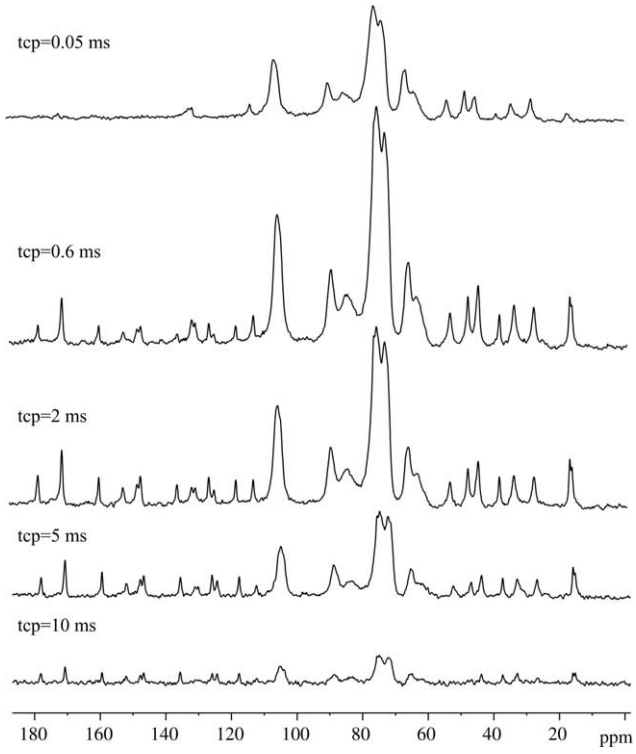

Fig. 5. ^{13}C CPMAS spectra of sildenafil citrate from commercial 100-mg tablet recorded with variable contact times.

Solid-state NMR measurement conditions, such as setting the magic angle, Hartmann–Hahn match, decoupling power and pulses, which are important for quantitative analysis [16], were thoroughly established and checked. Optimal recycle time and contact time were determined in separate experiments, repeated for particular dosage form. Variable contact time measurements performed for a 100-mg tablet are illustrated in Fig. 5. Maximum sensitivity for the CP experiment was determined by plotting signal intensities of selected resonances versus t_{CP} (Fig. 6). The values of T_{CP} are longer than those for pure sildenafil base; however, the $T_{1\rho}^{H}$ relaxation times are significantly shorter, of only 4–7 ms (Table 4). Different values $T_{1\rho}^{H}$ for pure sildenafil citrate and for the tablet suggest that the salt is molecularly dispersed in the excipients.

Somewhat unexpected, the signals in the spectra of 50- and 25-mg tablets (not shown) were of the same intensity as those from 100-mg tablet. It was evident that commercial tablets of Viagra containing 25, 50 and 100 mg of sildenafil citrate in the dosage form differ in the tablet's mass and size, but not in the concentration of the active substance. Therefore, a calibration plot was established using the mixtures of sildenafil citrate with hydroxymethyl cellulose (main component of excipients). The signals of C=O carbons of citrate (δ178.5 and 170.7 ppm) and the signals of C19, C16 and C14 (δ159.5, 125.8 and 117.6 ppm, respectively) are distinct in each spectrum (not shown) and can be used for quantitative analysis. Their intensities are linear functions of the amount

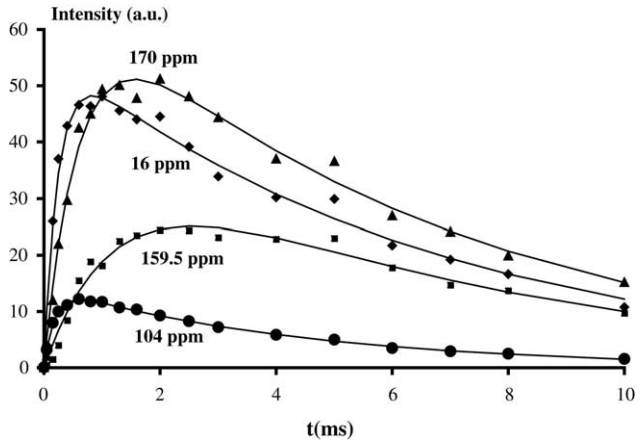

Fig. 6. Evolution of ^{13}C signal intensities (arbitrary units, a.u.) of sildenafil citrate (δ16, 159.5 and 170 ppm) and excipient (δ104 ppm, anomeric carbon) from 100-mg tablet as a function of the contact time t (ms).

of the drug in these solid mixtures. Thus, solid-state ^{13}C NMR can be applied to perform quantitative analysis of this drug in commercial tablets.

The validation of solid-state NMR method for Viagra (establishing the limits of detection and quantitation, accuracy, reproducibility) requires proper statistical evaluation. NMR measurements should be done on sufficiently large number of tablets with the same composition of excipients, however, differing in the content of active substance.

Actually, the method may be a valuable tool for fast identification of illegally produced tablets (sometimes sold in sex shops or by Internet), which may contain only blue painted placebo or analogues of sildenafil (e.g. homosildenafil added to a functional food marketed for penile erectile dysfunction in Korea [17]).

NMR has several advantages over HPLC or GC–MS techniques, including stereochemical differentiation (conformational polymorphism) and its ability to analyse solid samples. As opposed to solution NMR, MAS NMR is a less destructive technique. Powder samples are retained in their original form and tablet samples need only to be crushed.

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