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Hydrogen-deuterium (H/D) exchange reaction of acebutolol hydrochloride in D_2O and CD_3OD solution

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H/D exchange reactions can be observed by NMR spectroscopy of acebutolol (ACE). The results obtained showed deuterium incorporation at α-position of the carbonyl group of acebutolol, when using deuterium oxide or deuterated methanol as deuterium source and solvent. The spontaneous deuteration is proceeded by the following pathway $CH_3 \rightarrow CH_2D \rightarrow CHD \rightarrow CD_3$, through a keto-enol tautomerization reaction. Furthermore, LC-MS / QTOF analyses have confirmed the proposed H/D exchange. In order to reduce the time of total deuteration observed at the acetyl group alkaline catalysts were employed.

1. Introduction

β-Receptor blockers have a long-standing tradition in the treatment of heart failure, hypertension and cardiac arrhythmias. Within the frame of a systematic NMR study on some 50 years old β-blockers, we aimed to look closer to acebutolol. The Wawer group (Zielinska-Pisklak et al. 2011) already published ^{1}H and ^{13}C NMR data of the acebutolol base dissolved in DMSO-d_6 and $CDCl_3$. Since acebutolol is generally administered as its hydrochloride due the higher solubility in water (Health Canada 2018; EP. 2021), the NMR measurements were now performed in deuterated water and methanol. Beside the expected H/D exchange of protons of the hydroxyl group as well as the amino group, it was tested whether the acidity of the protons of the acetyl group is sufficient to form a deuterated acetyl substituent, which can be used in biological samples when mass spectrometry is applied for various purposes, e.g. pharmacokinetic studies.

However according to the literature, deuterium incorporation procedures require mainly the use of a metal catalyst (Piola et al. 2014; Giuseppe et al. 2015; Tse et al. 2011), expensive deuterating agents (Cochrane et al. 2014; Ma et al. 2014; Shahkhatuni et al. 2020), as well as the employment of deuterium gas (Nilsson and Kerr 2010) or at least the use of particular bases (Scheigetz et al. 2004; Huang et al. 2021). Hence, it will be checked whether a catalyst is necessary to promote the H/D exchange.

2. Investigations, results and discussion

2.1. H/D exchange reaction of acebutolol monitored by NMR spectroscopy

In Fig. 1b the ^{1}H NMR spectrum of acebutolol in D_2O and its assignment can be seen. The data are detailed in the Table.

The data are in accordance with those reported in previous studies (Zielinska-Pisklak et al. 2011; Foster and Carr 1990). As expected, the signals of the OH group and the NH are directly exchanged by deuterium and cannot be recognized in the spectrum. The proton signal of the acetyl group at δ = 2.55 ppm is clearly visible upon dissolution. However, the H/D exchange is rather slow (see Fig. 2). After 5 days a decrease of the hydrogen methyl signal (CH_3) can be observed and the emergence of a triplet with equal intensities (1:1:1), at δ = 2.60 ppm with typical coupling constant of $^{2}J_{H,D}$ = 2.04 Hz corresponding to deuterated species CH_2D. After 15 days, the triplet almost disappears and a quintet at δ = 2.58 ppm representing the CHD_2 entity shows up; after one month only a flat residual signal can be observed indicating an almost complete H/D exchange of the acetyl protons.

Table: ^{1}H NMR spectrum data of ACE measured in D_2O

Position	Type of proton	1H- d (ppm)	Multiplicity	Coupling Constant (Hz)
1	CH_3	0.87	t	$^{3}J_{H1\text{-}H2}$= 7,4
17 ; 18	CH_3	1.27	t	$^{3}J_{H17\text{-}H16}$ =6,5 $^{3}J_{H18\text{-}H16}$ =6,5
2	CH_2	1.61	sext	–
3	CH_2	2.29	t	$^{3}J_{H3\text{-}H2}$=7,3
12	CH_3	2.55	s	–
15	CH_2	3.20	ddd	$^{2}J_{H15'\text{-}H15''}$ = 13 $^{3}J_{H15'\text{-}H14}$ = 8,7 $^{3}J_{H15''\text{-}H14}$ = 3,4
16	CH	3.43	sept	–
13	CH_2	4.13	dd	$^{2}J_{H13'\text{-}H13''}$ ~ 4 $^{2}J_{H13\text{-}H14}$ ~ 4.2
14	CH*	4.27	m	
7	CH	7.04	d	$^{3}J_{H7\text{-}H6}$= 9,03
6	CH	7.48	dd	$^{3}J_{H6\text{-}H7}$ = 8,94 $^{4}J_{H7\text{-}H10}$= 2,7
10	CH	7.63	d	$^{4}J_{H10\text{-}H7}$ = 2,67

The H/D exchange of the acetyl group can also be monitored in the^{1} ^{3}C NMR of acebutolol as shown in Fig. 3, the resonance of CH_3 group appears at the beginning at 30.50 ppm as a singlet. As time goes by a triplet of equal intensities (1:1:1) related to CH_2D with coupling constant J = 19.77 Hz appeared adjacent to the remaining CH_3 signal. After 15 days, the quintet corresponding to the CHD_2 (1:2:3:2:1) is clearly visible with coupling constant J = 20.11 Hz. Finally, after 30 days, the signal of CH_3 group is transformed completely to CD_3, which appears as a structured septet with intensities (1:2:2:3:2:2:1). The DEPT diagram changes accordingly.

Moreover, the observed H/D exchange was confirmed by ^{2}H NMR measurement, which showed a broad signal at the same chemical shift of the methyl group deuterium atoms corresponding to a complete exchange (2.5 ppm) as shown in Fig. 4.

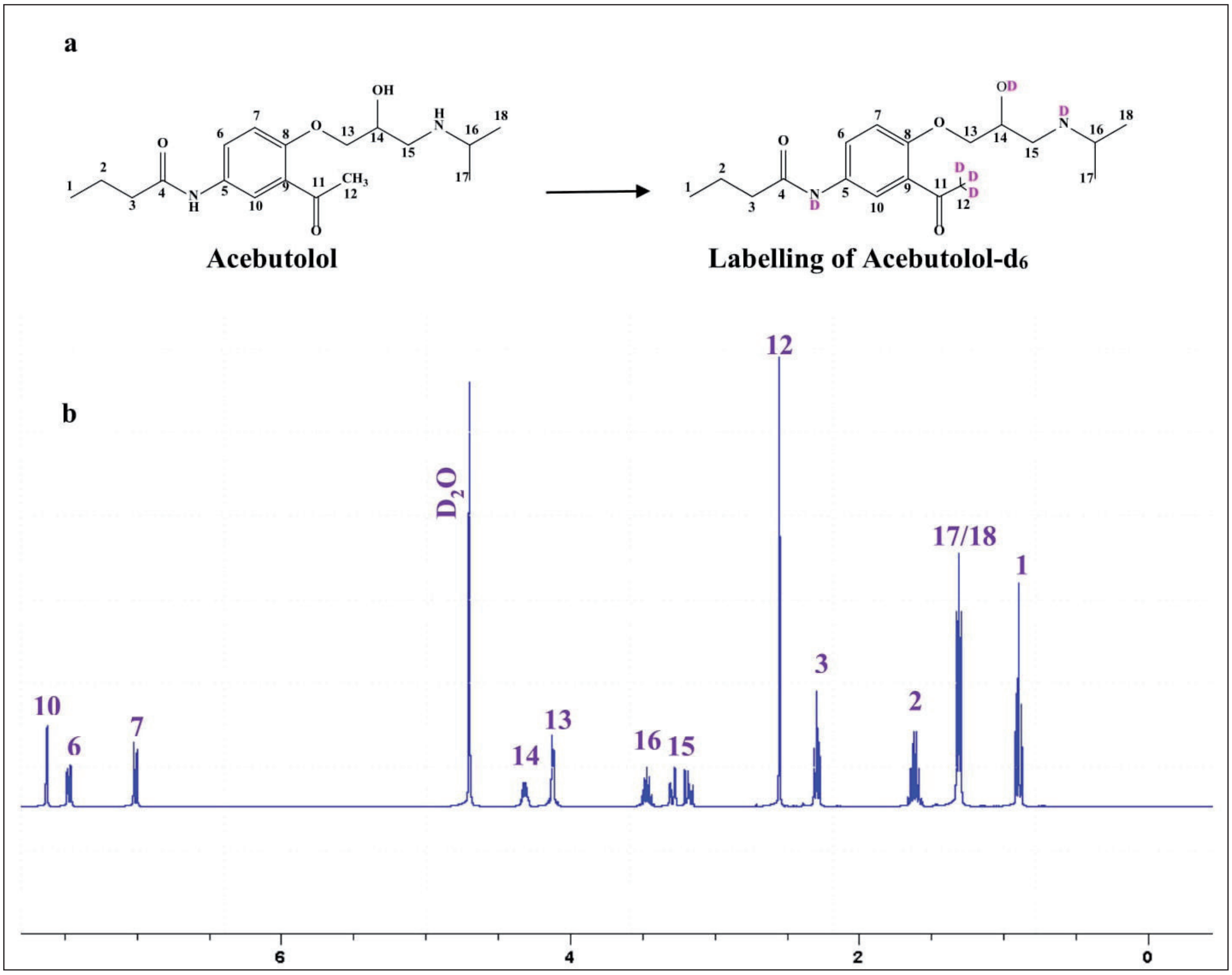

Fig. 1: a) The hydrogen isotope exchange observed at the C12 of ACE, b) ${}^1H$ NMR spectrum of ACE

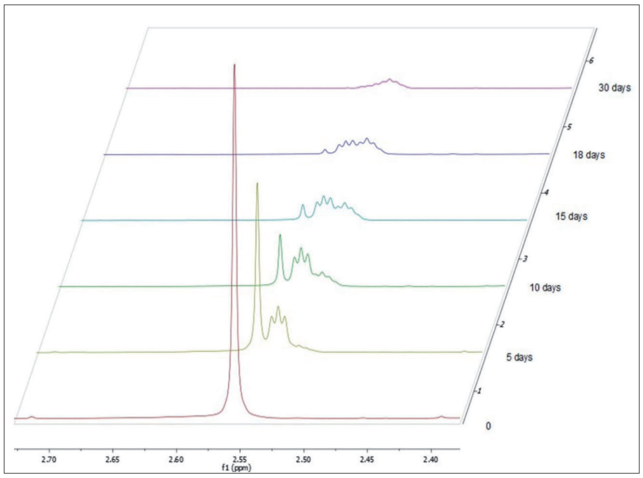

Fig. 2: CH_3 region of the ${}^1H$ NMR (400 MHz) spectra of ACE freshly prepared and stored for 5, 10, 15, 18 and 30 days in D_2O

2.2. H/D exchange reaction catalyzed with bases of methyl group of ACE prepared in D_2O

Since the CH_3 protons of the acetyl group are easily extractable in the presence of any bases, we used the following entities NaOD and K_2CO_3 in order to reduce contact time to achieve total deuteration as summarized in Fig. 5.

Figure 5 shows the deuteration of CH_3 of acebutolol in D_2O with the two bases used as catalysts over 210 min at room temperature. Since the reaction starts immediately after sample preparation, the deuteration was evident in the NMR spectra after 30 min, and the signal decreased over time indicating the deuteration of CH_3 group. The rate of deuteration process is the same for both catalysts used with almost complete deuteration after 210 min.

The efficiency of the H/D exchange is the same regardless of whether a strong or weak base is used.

2.3. H/D exchange reaction of acebutolol monitored by LC-MS / QTOF

The MS spectra of acebutolol showed the molecular ion fragment of the drug of m/z = 337.2104. As shown in Fig. 6, a total of 10 fragments were formed.

The MS spectra of ACE freshly prepared and stored for 1 month, using D_2O as solvent and the spectra obtained using freshly dissolved drug in water are examined in order to find the number of readily exchangeable protons. The results are summarized in Fig. 7.

The assignment of the locus of deuteration for ACE-d0 to ACE-d7 can be performed by looking at the fragments of each form. For ACE-d1, the fragments at m/z = 219.1221 and m/z = 261.1331 hint to the first deuteration of the amino hydrogen group (see Fig. 8).

For ACE-d2, the fragments at m/z = 117.1116 and m/z = 262.1385, respectively, correspond to the deuteration of the primary amine proton and to the first isotopic exchange at α-position of the

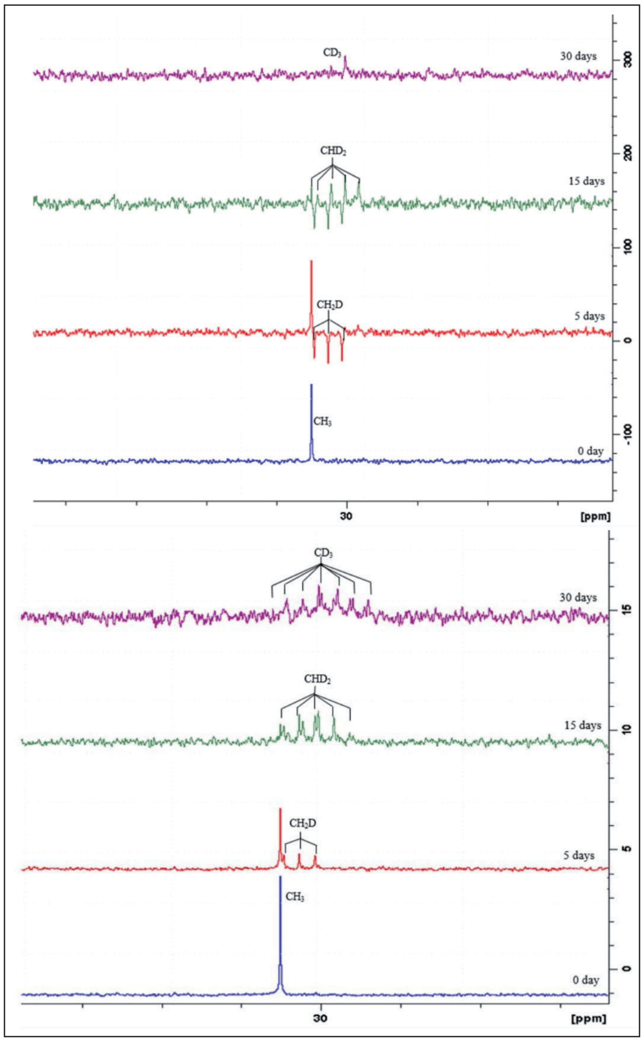

Fig. 3: ^{13}C and ^{13}C DEPT-135 NMR spectra of the CH_3 region of ACE freshly prepared and stored for 5, 15 and 30 days in D_2O

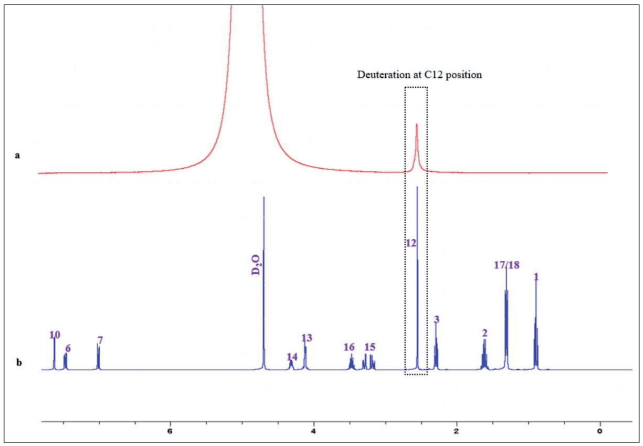

Fig. 4: NMR spectra of ACE prepared in D_2O and stored for 30 days: (a) ^2H NMR; (b) ^1H NMR

carbonyl group, which is also observed at m/z = 220.1278 for ACE-d4.

For ACE-d3 the fragments at m/z = 118.1183 and m/z = 263.1446, respectively, correspond to the deuteration of the hydroxyl group and to the second deuteration of the acetyl group, which is also observed at m/z = 221.1317 for ACE-d5.

Finally for ACE-d5 the fragment at m/z = 264.1470 indicates the third deuteration at C-12 methyl group.

In conclusion we notice that the mass shifts of +7, (+4) of which come from the alcohol and the secondary amine protons in the amino alcohol side chain, the amide chain in the 4 position and the change from protonated to deuterated molecular ion (MD^+ instead MH^+). While, the rest of the mass (+3) is due to the unexpected H/D exchange of the three hydrogen atoms at the C-12 methyl group as shown in fragment spectra in Fig. 8. Taken together the order of deuteration is seven.

2.4. *Kinetics of H/D exchange reaction of ACE measured in D_2O and CD_3OD*

The exchange of CH_3 can be expressed by Eq (1):

$$\frac{d[CH_3]}{dt} = -k[CH_3] \qquad (1)$$

With k is the apparent kinetic constant for the global reaction CH_3 CD_3, and $[CH_3]$ is the concentration of CH_3. Integration of Eq (1) leads to Eq (2):

$$[CH_3] = [CH_3]_0 e^{-kt} \qquad (2)$$

$[CH_3]$ is the total integrated area of the CH_3 signal and at t = 0, , the decay as analyzed according to Eq (2) with a nonlinear least squares fit, giving k = $7.92.10^{-3}$ h^{-1} and $5.42.10^{-3}$ h^{-1}, with correlation coefficients of 0.983 and 0.998 in D_2O and CD_3OD, respectively (see Fig. 9). In summary, the observed H/D exchange is faster and total in D_2O rather than in CD_3OD which is rationalized as a less steric factor and great power of dissolution of heavy water.

2.5. *Mechanism of observed H/D exchange of the (C=O)–CH_3 group*

The isotopic exchange observed at α-position of the carbonyl group of acebutolol is realized by the well-established tandem keto-enol tautomerization; the OH of the enol can easily exchange the hydrogen with a deuterium in the excess of D_2O or deuterated methanol (Fig. 10).

It should be noted, that hydrogen isotope exchange provides the most straight forward and direct procedure toward the synthesis of labeled molecules, which can be used across a wide range of applications from mechanistic analysis to pharmacokinetic studies (Russak and Bednarczyk 2019).

Stable heavy isotopes of hydrogen have been incorporated into α-position of the carbonyl group of acebutolol without the use of any additive or catalyst. This has the advantage that the deuterated compounds do not have to be purified after reaction. However, it is understood, that the reaction can be accelerated by using a catalyst, such as NaOD or K_2CO_3.

3. Experimental

3.1. *Materials*

Acebutolol hydrochloride was obtained from SIPHAT, a Tunisian pharmaceutical company. D_2O, CD_3OD, K_2CO_3 and NaOD were purchased from Sigma-Aldrich Germany.

3.2. *Sample preparation*

Acebutolol drug (20 mg) was weighed and transferred to a stoppered tube with 0.5 ml of D_2O.

The H/D exchange reactions of the methyl group of acebutolol were examined in presence of the bases K_2CO_3 and NaOD as catalysts at concentration of 4.06 M in D_2O at room temperature for 210 min. A concentration of 1 µg/mL of acebutolol in solvent was prepared for LC-MS /QTOF analysis.

3.3. *Instruments and analytical conditions*

NMR measurements were performed using a Bruker Avance III 400 MHz Ultra Shield spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) equipped with a 5-mm PABBI ATM 1H/D-BB Z-GRD probe, a Bruker automatic sample changer (B-ACS 60), a BSVT (Bruker Smart Variable Temperature System) and a BCU05 (Bruker Cooling Unit). The measurements were conducted at 300 K using 128 scans

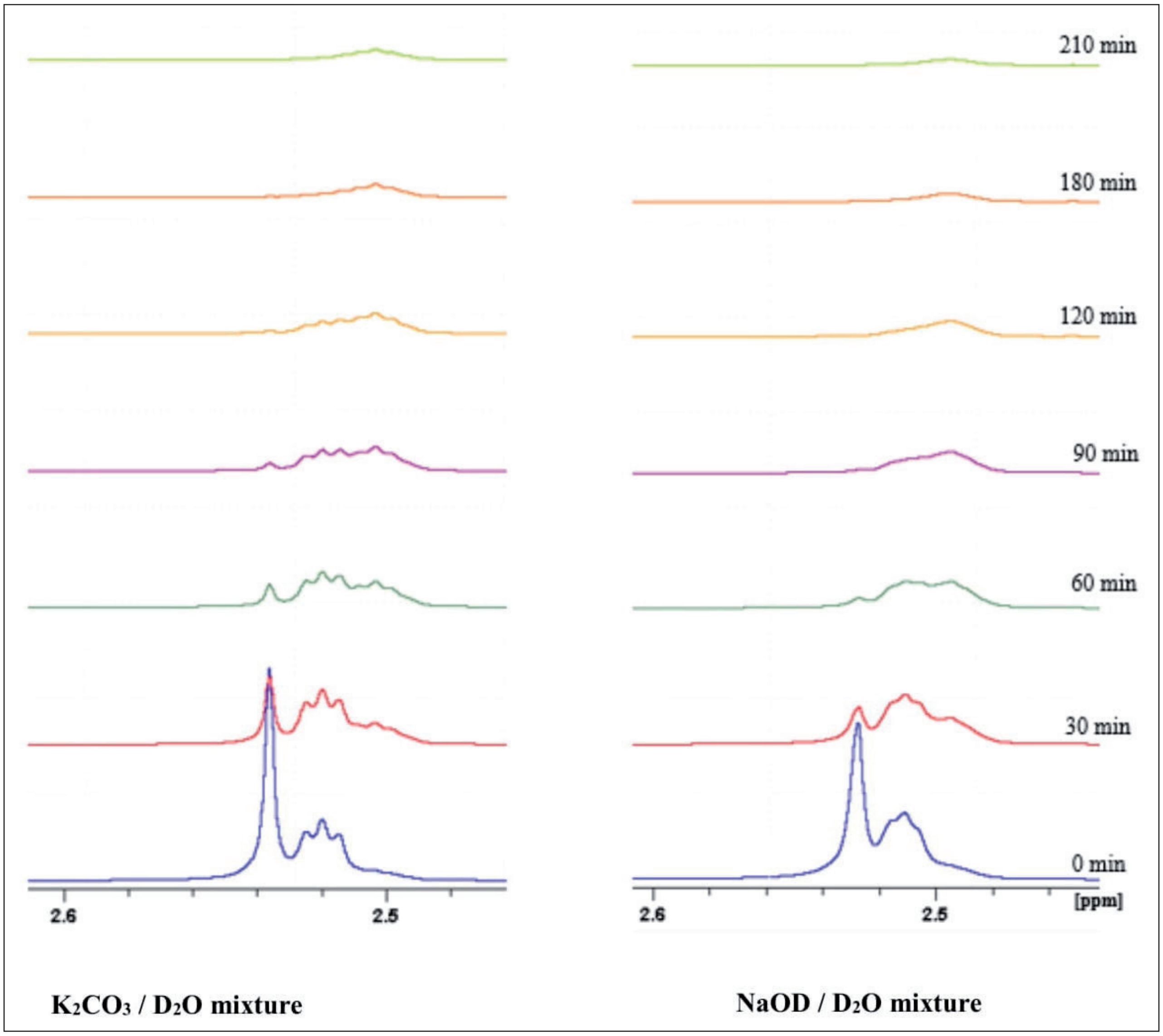

Fig. 5: ^{1}H NMR (400 MHz) spectra of CH_3 region of ACE in D_2O with bases used as catalysts

(^{1}H NMR), 4000 scans (^{13}C NMR) and 1000 scans for the DEPT 135 (Distortionless Enhancement by Polarization Transfer), an acquisition time (AQ) at 3.98 s with a relaxation delay (RD) of 1s for ^{1}H NMR and for ^{13}C NMR and DEPT135 are 1.36 s (AQ) and 2s (RD). All NMR spectra were recorded in the baseopt mode using the Bruker standard zg pulse sequence with a flip angle of 30°. TOPSPIN is used to process spectra.

LC-MS / QTOF method: The LC system was an Agilent 1260 Infinity II (Agilent, Waldbronn, Germany) equipped with degasser, consisting of binary pump G7120A, autosampler G7167B and a thermostated column compartment G7116B. The mobile phase consisted of phase A (2% acetonitrile and 0.1% formic acid in water) and phase B (2% water and 0.1% formic acid in acetonitrile) (50:50, V/V) used in an isocratic elution with a flow rate of 1 mL/min. The injection volume was set to 10 µL. Mass detection was performed on an SCIEX X500R QTOF system (AB Sciex, Darmstadt, Germany) with a Turbo V^{TM} source (ESI) using SciexOS software for data acquisition and processing (Sciex, Concord, Ontario, Canada). Ion source parameters were set for curtain gas at 25 psi, for ion source gas 1 and 2 at 50 psi and for temperature of 450 °C. Measurements were carried out in positive ion mode, using 5500 V as ionspray voltage, a declustering potential range of 70±5 V and a collision energy range of 35±5 V. Precursors were set according to their theoretical calculated exact masses and TOF MS/MS data were collected during an accumulation time of 0.1 s.

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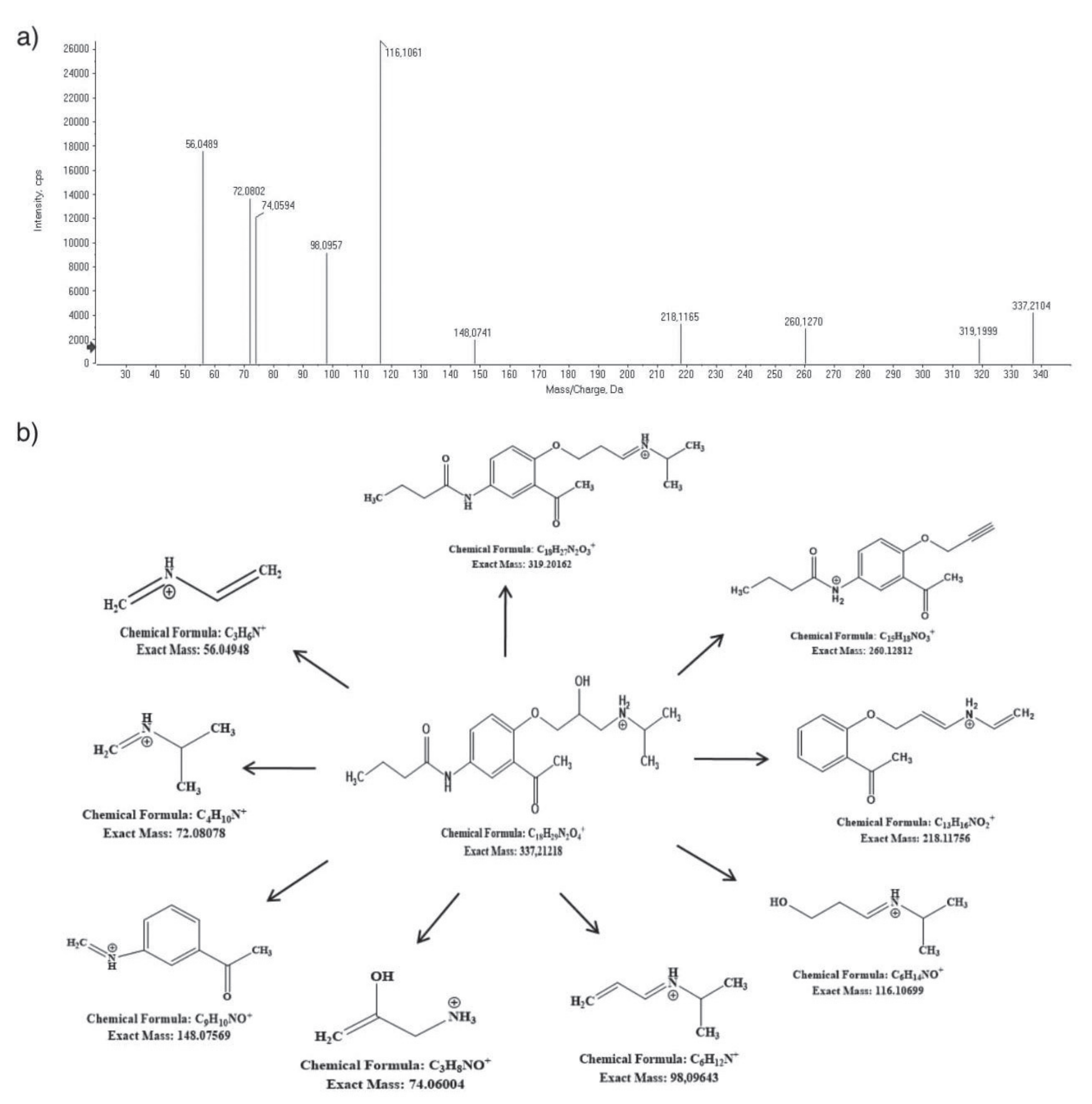

Fig. 6: a) Line spectrum of ACE ; b) Fragmentation pathway of ACE

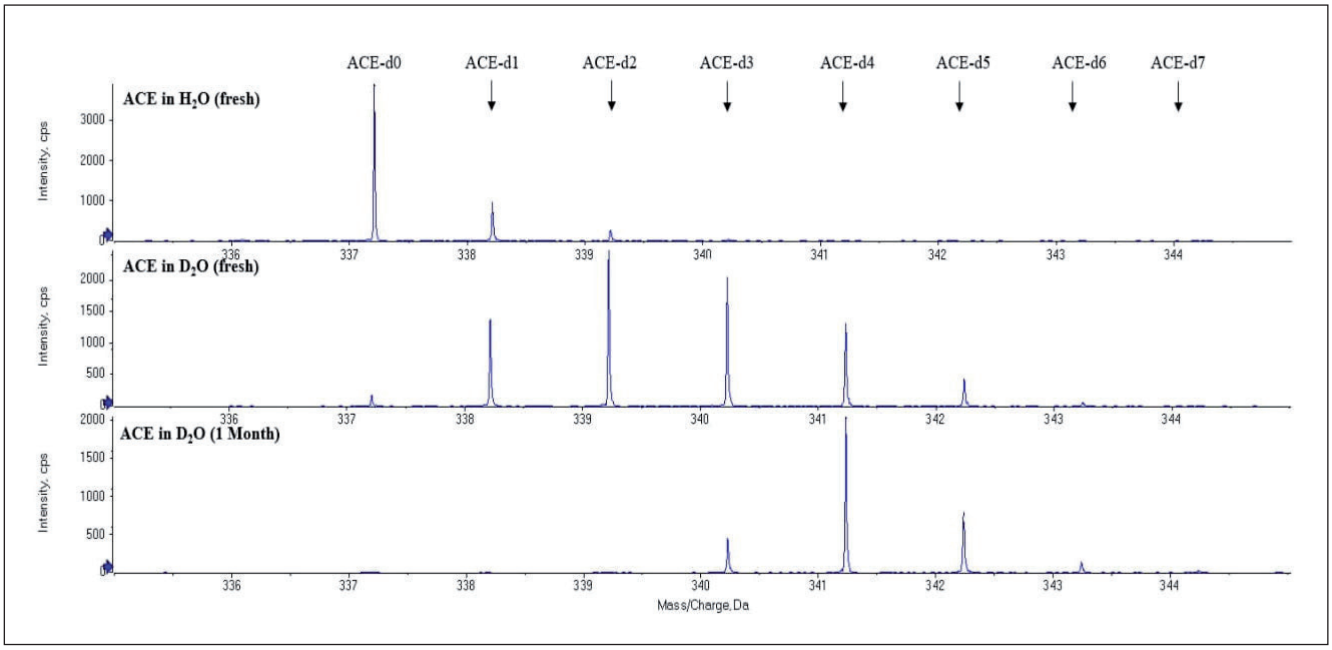

Fig. 7: MS spectra of ACE freshly prepared in H_2O, D_2O and stored for 30 days in D_2O

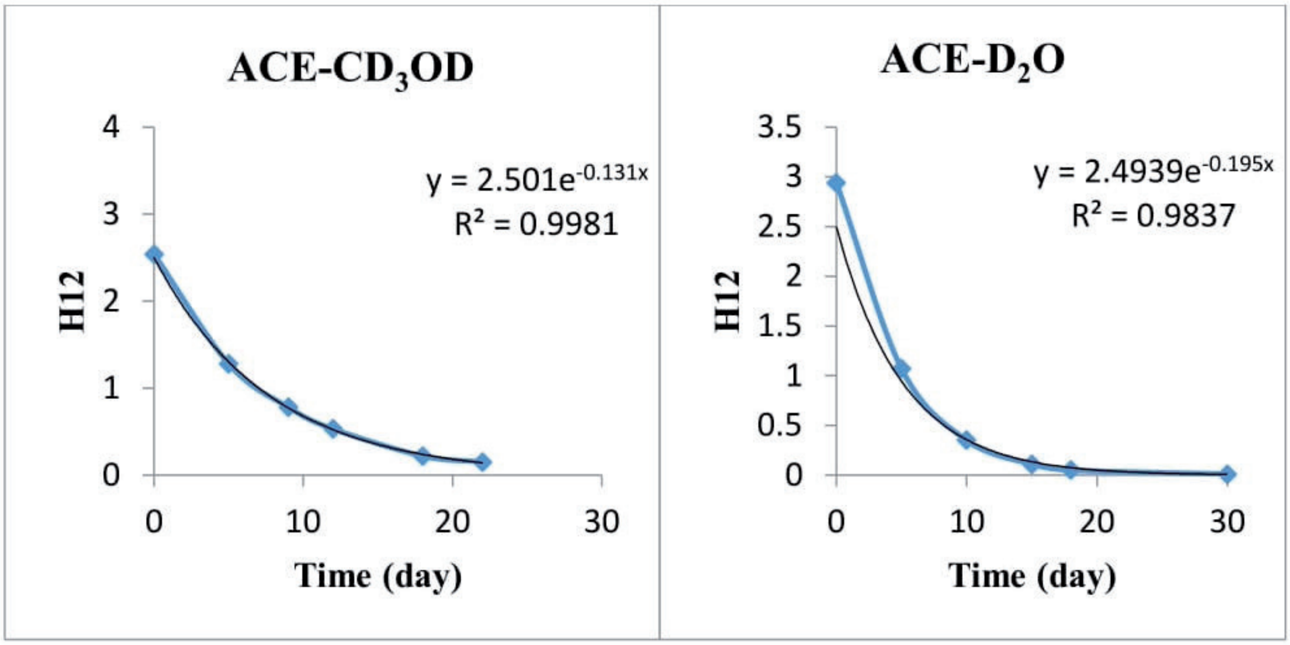

Fig. 9: Plots of total integrated peak area of H12 vs. contact time in D_2O and CD_3OD

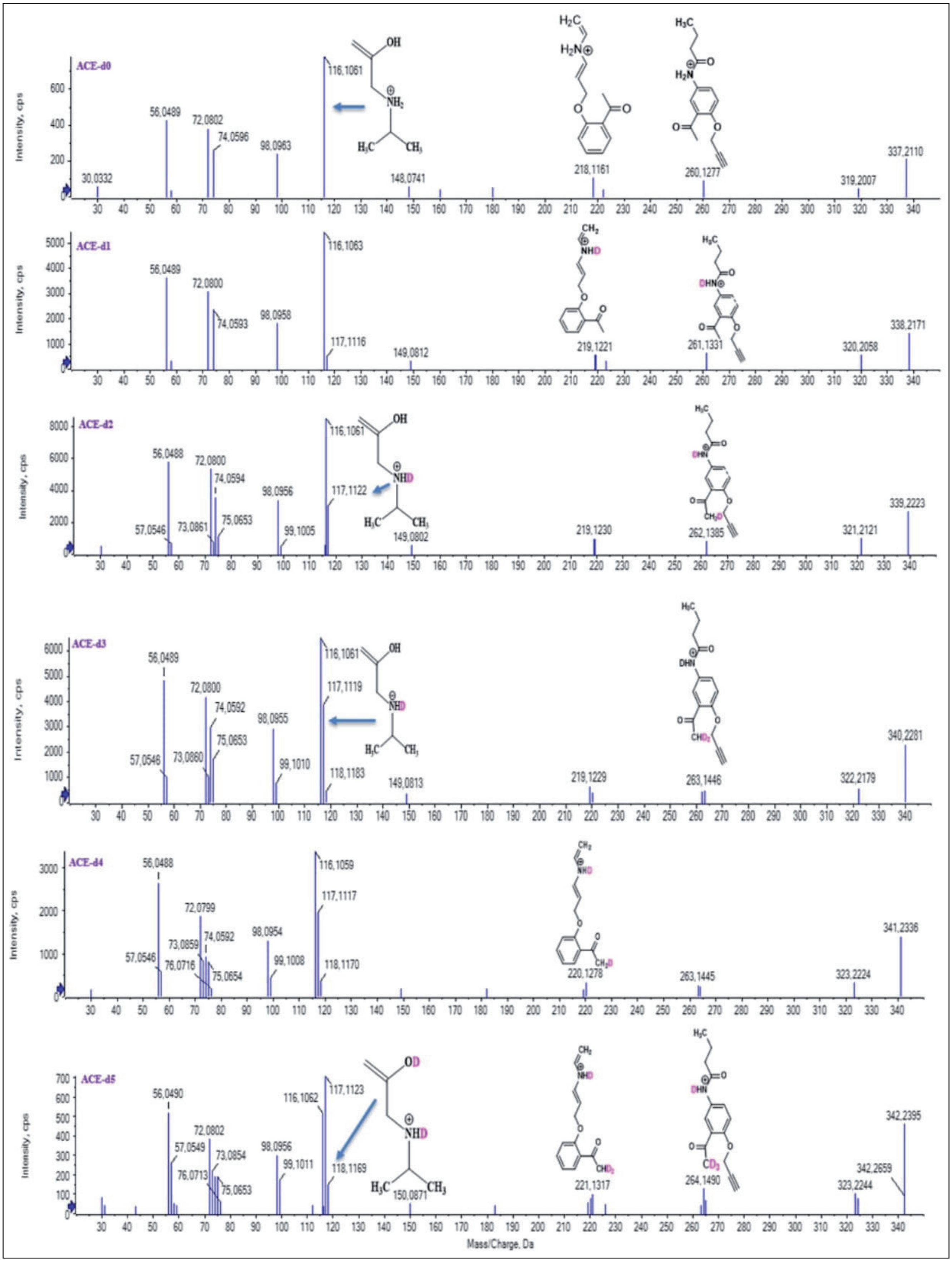

Fig. 8: MS spectrum of ACE in D_2O