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New solid form of Norfloxacin: Structural studies

I. Bratu [a,*], G. Borodi [a], Iren Kacsó [a], Z. Moldovan [a], C. Filip [a], Felicia Dragan [b], M. Vasilescu [c] and S. Simon [c]

[a] *Physics of Nanostructured Systems Department, National Institute for R&D of Isotopic and Molecular Technologies, Cluj-Napoca, Romania*
[b] *Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania*
[c] *Faculty of Physics, Babes–Bolyai University, Cluj-Napoca, Romania*

Abstract. The aim of the present paper was to obtain new solid forms of Norfloxacin. For this purpose Norfloxacin was recrystallized from: acetic acid and its mixtures with several organic solvents. By recrystallization of Norfloxacin from acetic acid and from its mixture with several organic solvents a new solvate was found. To evidence this new solid form of Norfloxacin different investigation techniques were used: powder X-ray diffraction, FTIR, DSC, ^{13}C NMR spectroscopy and mass spectrometry. The solvate is consisting on Norfloxacin and acetic acid in 1:1 molar ratio and crystallizes in triclinic system and the cell parameters were determined also.

Keywords: FTIR, powder X-ray diffraction, DSC, MS, ^{13}C NMR, Norfloxacin – acetic acid solvate

1. Introduction

The ability of a substance to exist in at least two crystal structures denotes polymorphic structural states. This property leads to dramatic effect in biological activity between two forms of the same drug having important consequences in bioavailability of drug substances. Norfloxacin, a compound of fluorinated piperazine, a homologue of nalidixic acid (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-piperazino-3-quinolinecarboxylic acid) presents a large antibacterial spectrum, acting to inhibit the replication of bacterial DNA, being indicated in the therapy of superior and inferior urinary apparatus. X-ray diffraction, DSC, FTIR and ^{13}C NMR spectroscopic investigations were already performed [8] to evidence different polymorphic forms of Norfloxacin [3,4,11]: form A, recrystallized from dimethylformamide – the usual commercially one (whose crystal structure was recently solved), forms B and C, all being anhydrous polymorphic forms. Different solvates of Norfloxacin (with succinic, maleic and malonic acids) were reported elsewhere [5]. New Norfloxacin acetic acid solvate was obtained when acetic acid was used as solvent. Norfloxacin was also recrystallized from mixtures of acetic acid with several solvents (water, 2-propanol and acetone). The structural characteristics were obtained from powder X-ray diffraction, FTIR, DSC, MS and ^{13}C NMR spectroscopy.

* Corresponding author: I. Bratu, Physics of Nanostructured Systems Department, National Institute for R&D of Isotopic and Molecular Technologies, P.O. Box 700, R-400234 Cluj-Napoca, Romania. E-mail: ibratu@gmail.com.

2. Experimental section

220 mg of commercial form of Norfloxacin ("Helcor" Baia Mare, Romania) was dissolved in 10 ml glacial acetic acid by stirring at 35°C for few minutes. The same amount of Norfloxacin was solved in three mixtures of solvents: 10 ml water + 5 ml acetic acid; 10 ml acetone + 5 ml acetic acid and 10 ml 2-propanol + 10 ml acetic acid. The suspension was heated at 50°C and stirred for 30 min. Once the solid is dissolved, it was maintained at room temperature until the full evaporation of the solvent.

FTIR measurements were done with a 6100 JASCO spectrometer in the 4000–400 cm^{-1} spectral range with a resolution of 2 cm^{-1} using the well-known KBr pellet technique.

Differential scanning calorimetry (DSC) was carried out by means of a Shimadzu DSC-60 calorimeter, the sample was heated in the range of 20–300°C with a heating rate of 10°C/min in crimped aluminum sample cell.

The powder X-ray diffraction patterns were obtained with Shimadzu 6000 diffractometer using Cu Kα radiation.

The structural study of recrystallized Norfloxacin was made by mass spectrometry using a Direct introduction Inlet System. The sample was heated in the range of temperature 25–310°C with a gradient of 30°C/min. In all this time the mass spectrum was registered in continuous mode in the range 25–400 Daltons. The mass spectra were registered in the following conditions: electron energy 70 eV, emission current 100 μA, ion source temperature 180°C. The experiment was performed with a high resolution mass spectrometer Finigan Mat 311.

^{13}C NMR spectra were measured using the Bruker AVANCE 400 spectrometer, a spinning speed of 10 kHz and the cross polarization technique. As reference for the chemical shift, TMS ($Si(CH_3)_4$) was used.

3. Results and discussion

3.1. Powder X-ray diffraction

By powder X-ray diffraction we verified that the commercial Norfloxacin used by us is the form A [5]. In Fig. 1 X-ray diffraction patterns for commercial Norfloxacin and recrystallized forms obtained from glacial acetic acid and its mixture with other solvents (water, 2-propanol and acetone) are shown. One can see that diffraction pattern for commercial Norfloxacin is different from all other. On the other hand the diffraction patterns of all recrystallized forms are almost identical. Therefore, only Norfloxacin recrystallized from acetic acid was carried out.

The comparison between Norfloxacin recrystallized from acetic acid and the polymorphic forms A, B and C of Norfloxacin [3,4] is shown in the Fig. 2.

One can see that the Norfloxacin recrystallized from acetic acid is different as compared with the polymorphic forms A, B or C. It can be concluded that we have obtained a new solid form of Norfloxacin.

By indexing powder X-ray diffraction pattern of Norfloxacin recrystallized from acetic acid we obtained that the compound crystallizes in triclinic system having following lattice parameters: $a = 15.693$ Å; $b = 9.230$ Å; $c = 6.999$ Å; $\alpha = 107.376$; $\beta = 90.384$; $\gamma = 96.982$. The volume of the unit cell is $V = 959.35$ $Å^3$.

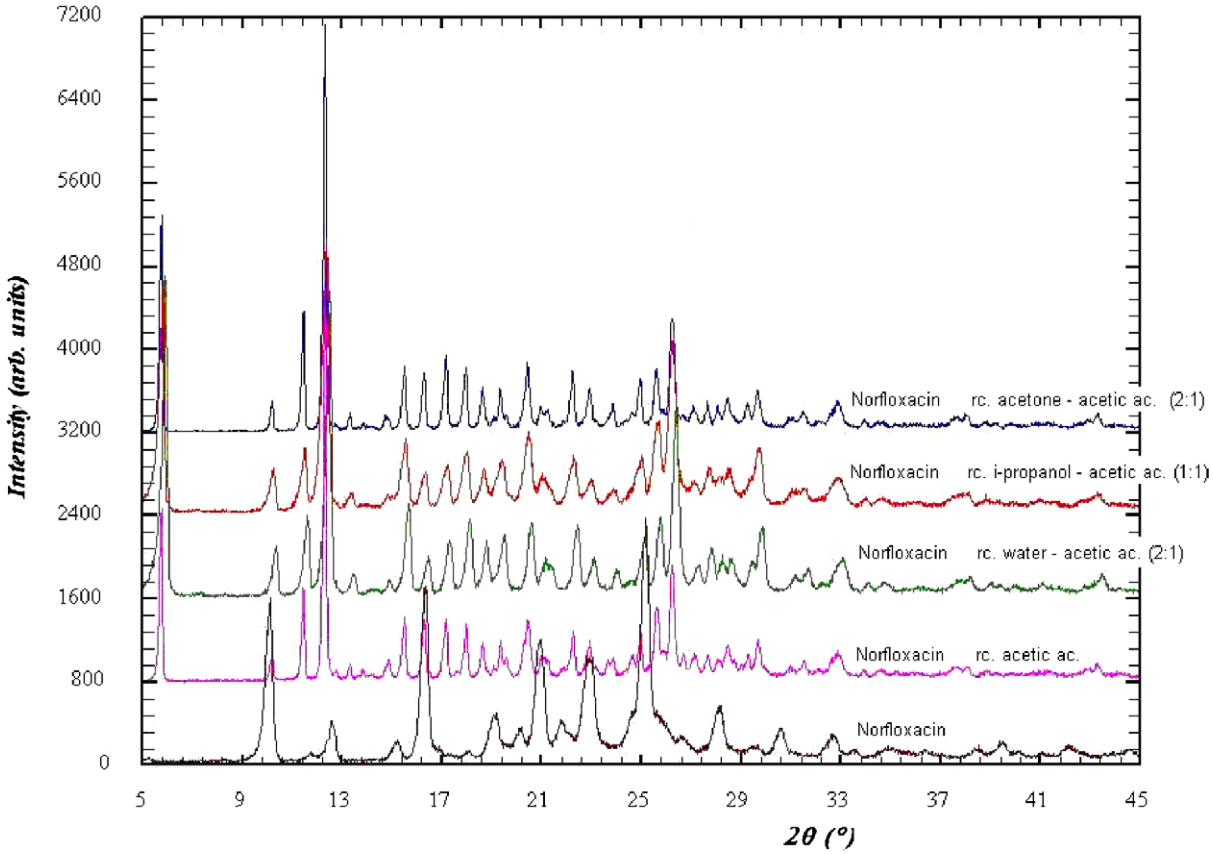

Fig. 1. X-ray diffraction patterns for Norfloxacin and its recrystallized forms from different solvent media. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

3.2. FTIR spectra

The characteristic vibrational frequencies corresponding to form A – the commercial form of Norfloxacin, form B and form C, checked by literature are presented in Table 1.

The starting compound in our studies was commercial Norfloxacin, which is the polymorphic form A. The comparison between FTIR spectra of the Norfloxacin A and of the obtained compound (by recrystallization of Norfloxacin A from acetic acid) is presented in Figs 3–5. These spectra are divided into three spectral regions (1800–1600, 1600–1000 and 800–600 cm^{-1} spectral ranges) where it is possible to distinguish between different solid forms of Norfloxacin.

It can be remarked that the vibrational spectra of the two compared compounds are completely different, their characteristic vibrational frequencies being shown in Table 2.

3.3. DSC

DSC thermogram of commercial Norfloxacin, see Fig. 6, show a sharp endothermic peak at 222°C, corresponding to the melting point of Norfloxacin.

The recrystallized form from acetic acid presents a different DSC thermogram as compared with that of pure compound. This thermogram shows a broad endothermic peak below 100°C, due to water

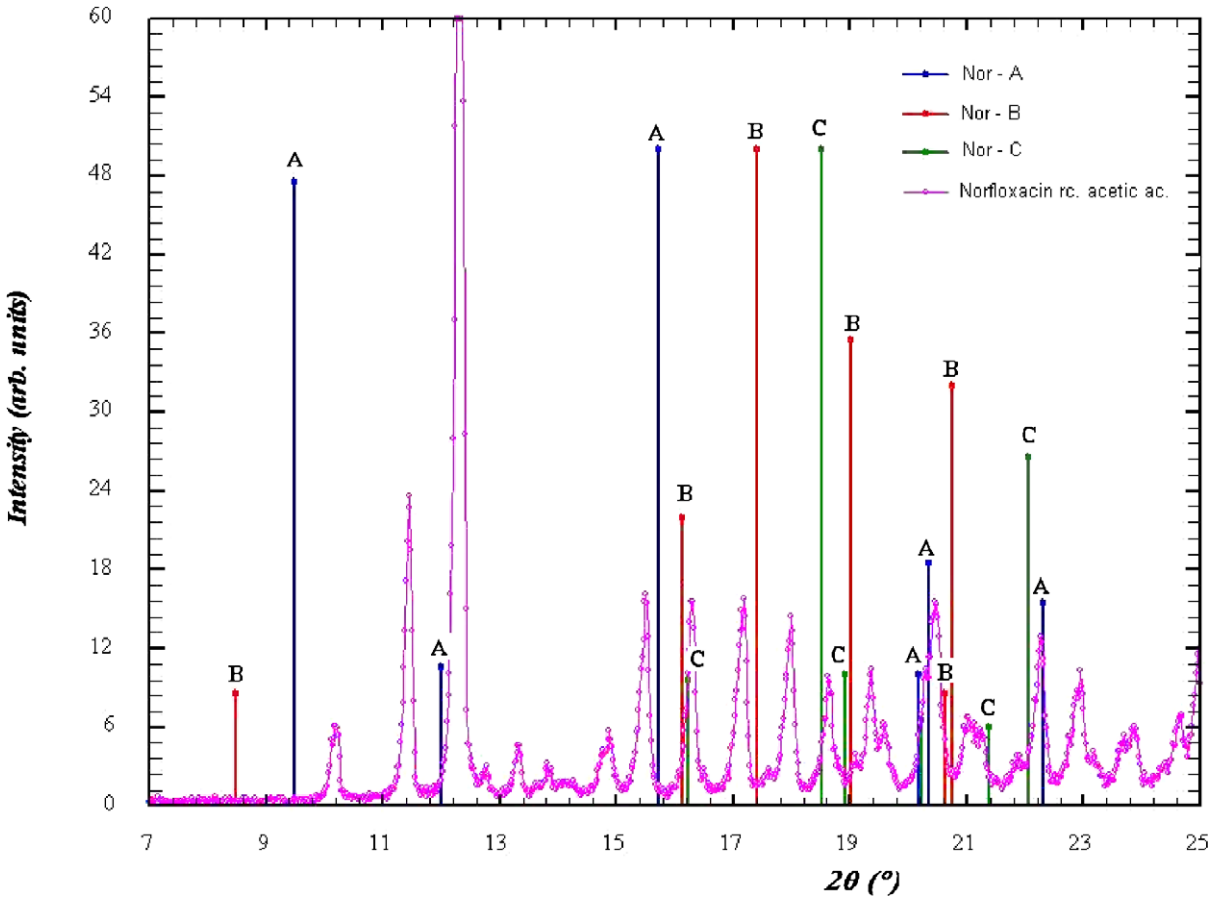

Fig. 2. X-ray diffraction patterns for: Norfloxacin recrystallized from acetic acid and A, B and C Norfloxacin polymorphic forms in agreement with literature [3,4]. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

Table 1

The characteristic vibrational frequencies corresponding to forms A, B and C of Norfloxacin by literature data

Vibrational group	Characteristic vibrational frequencies for Norfloxacin			References
	A (Commercial form)	B	C	
Characteristic vibrational frequencies	1522	1580		[3]
	1197	1330		
	942	1177		
	885	919		
	801	737		
Carboxylic valence vibration	1731		1715	[4]
Molecular vibrations	1477		1484	
	1249		1254	
Alkyl chain rocking	749		737	

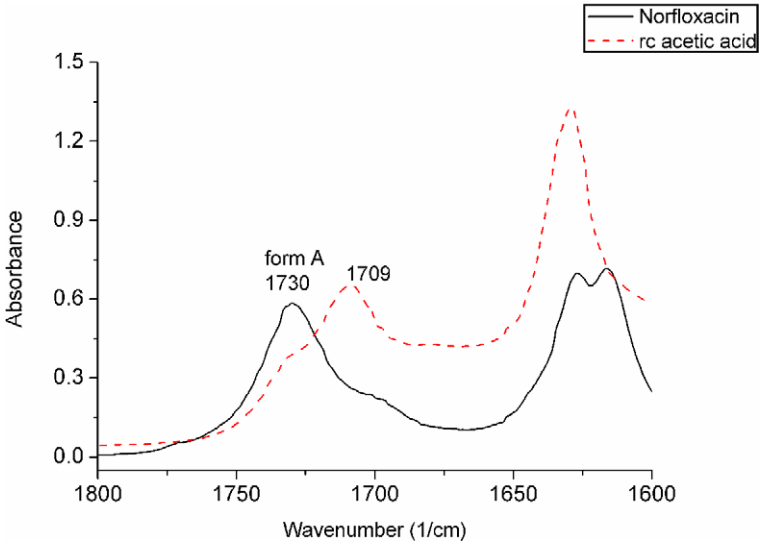

Fig. 3. FTIR spectra of Norfloxacin and its recrystallized form from acetic acid, 1800–1600 cm^{-1} spectral range. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

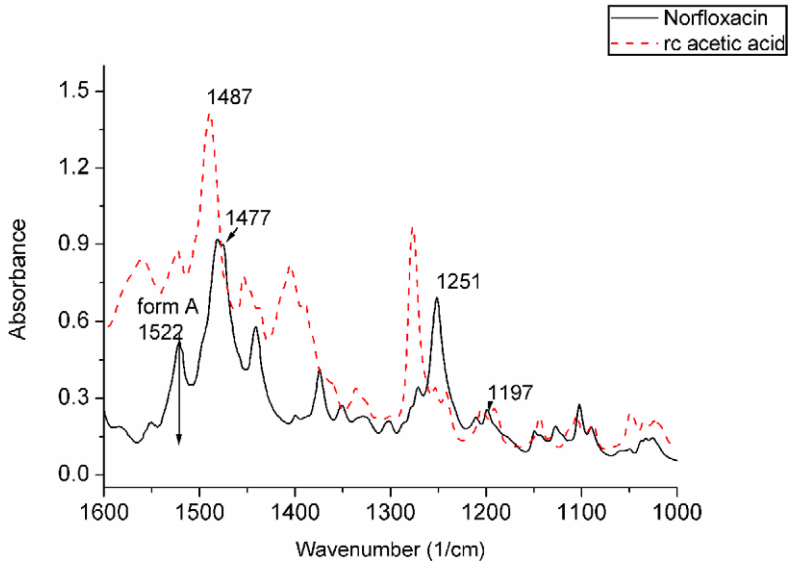

Fig. 4. FTIR spectra of Norfloxacin and its recrystallized form from acetic acid, 1600–1000 cm^{-1} spectral range. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

elimination, an other broad endothermic peak between 145°C and 175°C, probably corresponding to a solid–solid transition, followed by another sharp endothermic peak with maximum around 221°C which corresponds to the melting of the sample.

The onset temperature (melting point) and the heat of fusion for commercial Norfloxacin and its recrystallized form from acetic acid are shown in the Table 3.

The difference between onset temperature and the heat of fusion suggests that these two samples represent different compounds.

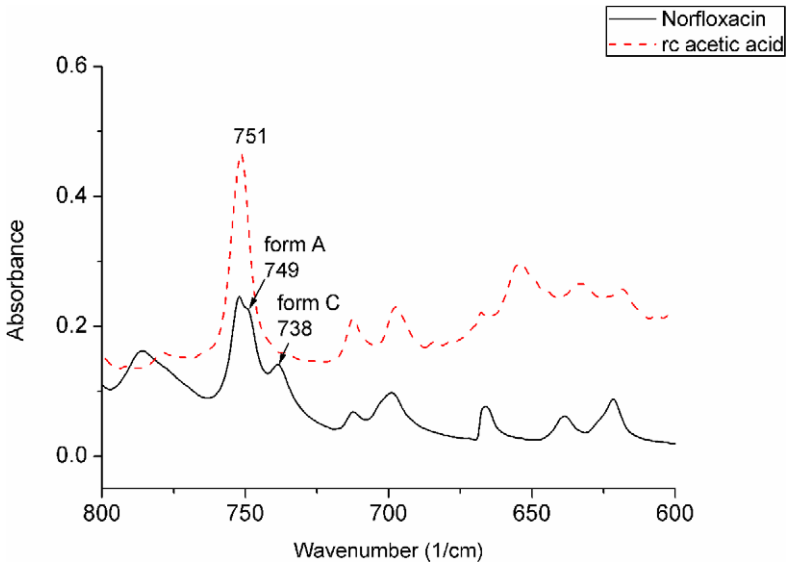

Fig. 5. FTIR spectra of Norfloxacin and its recrystallized form from acetic acid, 800–600 cm^{-1} spectral range. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

Table 2

The characteristic vibrational frequencies corresponding to form A of Norfloxacin and to the new solid form obtained by recrystallization from acetic acid

Vibrational groups	Characteristic vibrational frequencies for Norfloxacin	
	Norfloxacin A (Commercial form)	New solid form of Norfloxacin
Carboxylic stretching vibration	1730	1709
	1522	
Molecular vibrations	1477	1487
	1374	1402
	1330	1336
Molecular vibrations	1251	1272
	1197	1203
Rocking vibrations of the alkyl chains	752	750

3.4. Mass spectrometry

In the recent years the mass spectrometry was a powerfully method on the study of crystal systems [2,9,10] or on the pharmaceuticals purity [7] as well as on pharmaceuticals actions [6]. We studied the sample of Norfloxacin in vapor phase by using a mass spectrometer with a direct introduction system. The sample was heated in the temperature range from 20°C to 310°C and the mass spectra were taken in full scan mode in the range 25–400 Daltons.

Mass spectrum of Norfloxacin was compared with the mass spectrum reported in the data base available [1]. The main ions visible in the mass spectrum (see Fig. 7) are: m/z 233, 275 and 319 (molecular weight). The characteristic ions of the acetic acid are: m/z 43, 45 and 60 (molecular weight). In both cases the experimental mass spectra obtained are fitted very well with those reported in literature [1].

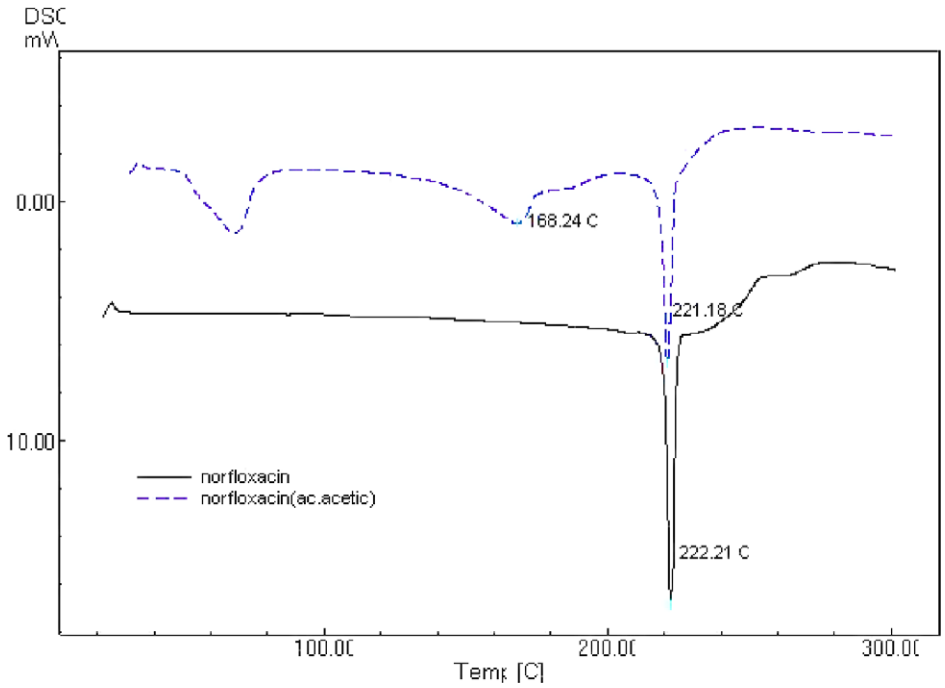

Fig. 6. DSC thermograms of Norfloxacin and its recrystallized form from acetic acid. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

Table 3
The onset temperature and the heat of fusion values for commercial Norfloxacin and its recrystallized form from acetic acid

Norfloxacin	Onset temp. (°C)	Heat of fusion (J/g)	Onset temp. (°C)	Heat of fusion (J/g)
Commercial form	–	–	220.18	91.98
Recrystallized acetic acid	145.43	93.09	218.91	76.09

The relative quantitative ratio was obtained by area ratio of the peaks corresponding to the molecular ions, $m/z = 60$ and to $m/z = 319$, respectively. The obtained results indicate a near 1:1 ratio. This observation leads to the conclusion that the number of molecules of each type in the elementary cell is approximately equal.

Taking into account the presence of one Norfloxacin and one acetic acid molecules present in the structural unit and assuming that there are two structural units in the elementary cell with $V = 959.35$ Å^3, as was established by X-ray diffraction, the calculated density is $\rho = 1.32$ g $\cdot$ cm^{-3}, a plausible value for organic compounds.

3.5. ^{13}C NMR spectroscopy

Using the NMR spectroscopy (see Fig. 8) it is clear that there is a significantly difference between commercial Norfloxacin and its recrystallized form from acetic acid. In the spectrum corresponding to the recrystallized form new resonance lines can be observed at following position: 26, 27, 111, 153, 166 and 180 ppm.

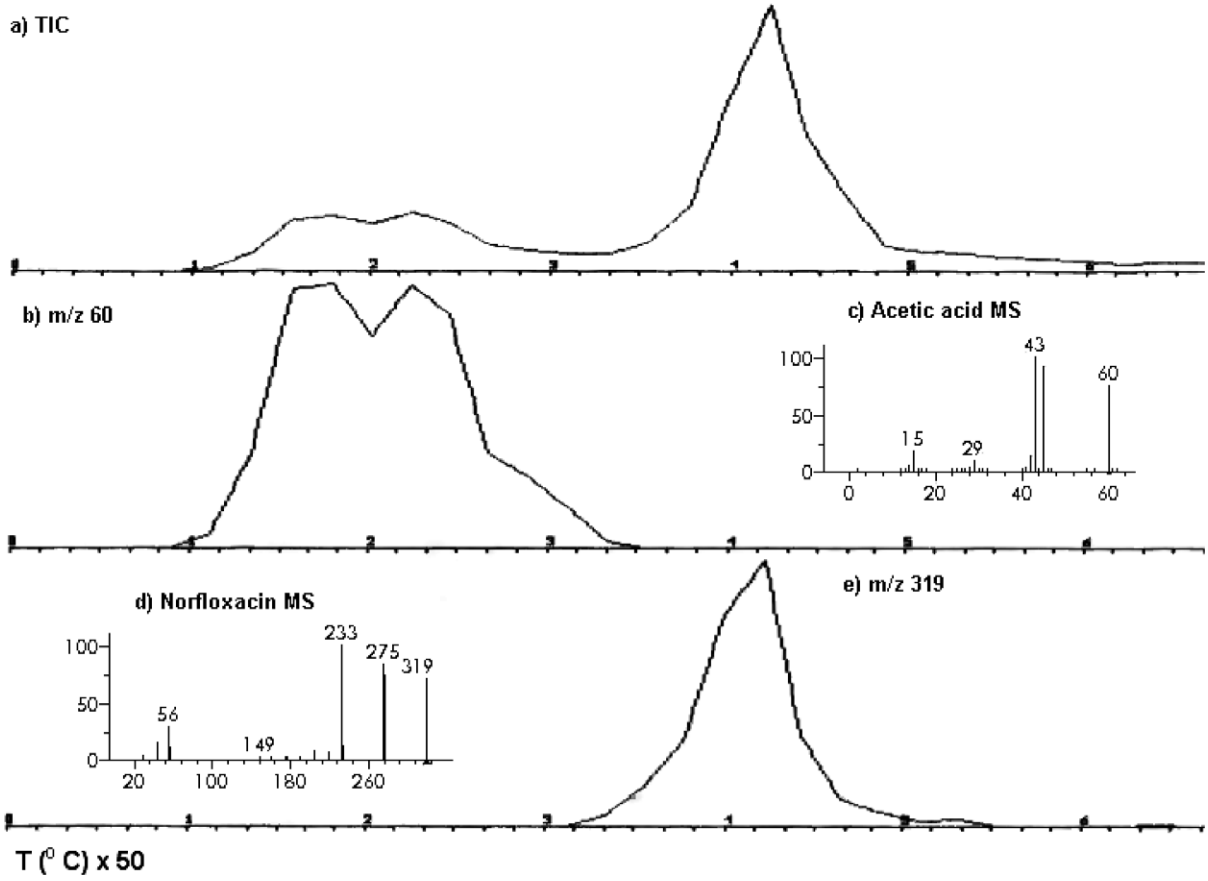

Fig. 7. The ions intensity as a function of temperature detected by DI Mass Spectrometry. (a) Total ion current, (b) m/z 60 (diagnostic ion for acetic acid), (c) mass spectrum of acetic acid, (d) Norfloxacin mass spectrum, (e) m/z 319 (diagnostic ion for Norfloxacin).

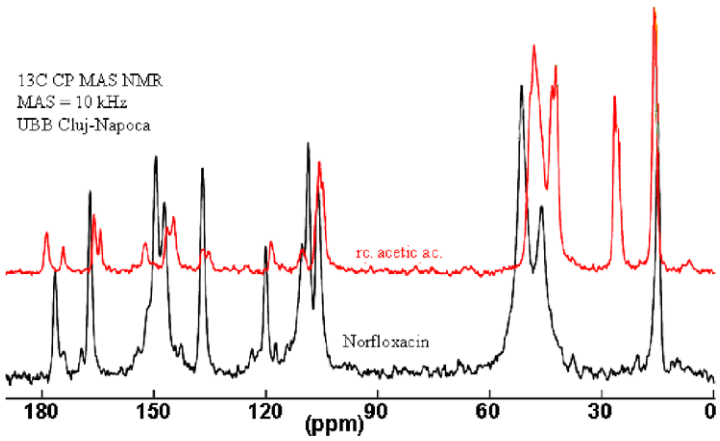

Fig. 8. ^{13}C CP MAS NMR spectra of Norfloxacin and its recrystallized form from acetic acid. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2010-0492.)

The ^{13}C CP MAS NMR spectrum of the commercial Norfloxacin is in agreement with the "polymorphic form A" from the Barbas et al. [3]. The recrystallized form from acetic acid, do not corresponds to A, B or C forms established also by Barbas et al.

The 4 ppm difference between the carboxyl line of Norfloxacin and the corresponding resonance of acetic acid obtained in the ^{13}C solid state NMR spectrum of Norfloxacin recrystallized from acetic acid is rather consistent with a solvate formation.

4. Conclusions

The commercial Norfloxacin bioactive substance was identified as to be polymorphic form A.

By FTIR, DSC and ^{13}C NMR methods a new solid form obtained by recrystallization of Norfloxacin from acetic acid was evidenced.

Mass spectrometry investigations results indicate a near 1:1 molar ratio between Norfloxacin and acetic acid in the recrystallized compound.

From powder X-ray diffraction pattern of Norfloxacin recrystallized from acetic acid it can be concluded that we have obtained a new solid form of Norfloxacin, which crystallizes in triclinic system having the unit cell volume $V = 959.35$ Å^3, with one molecule of Norfloxacin and one molecule of acetic acid in asymmetric unit and two asymmetric units in elementary cell, the calculated density being $\rho = 1.32\ \mathrm{g \cdot cm^{-3}}$. The most probable, the solid form obtained is a solvate.

Acknowledgements

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