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International Journal of Pharmaceutics 177 (1999) 221–229

international journal of pharmaceutics

Formation of ondansetron polymorphs

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Received 23 December 1997; received in revised form 20 October 1998; accepted 22 October 1998

Abstract

We used differential scanning calorimetry, infrared spectrophotometry and ^{1}H nuclear magnetic resonance imaging to search for polymorphs of ondansetron. Samples were tested under different conditions of temperature, pulverization and pH, and in different solvents. The factors identified as able to cause the formation of polymorphs were heating to different temperatures for different times, and the use of ethanol and methanol as solvents.

Keywords: Differential scanning calorimetry; ^{1}H nuclear magnetic resonance; Infrared spectroscopy; Ondansetron; Polymorphism

1. Introduction

Ondansetron is a carbazol anti-emetic that acts as a competitive, selective inhibitor of 5-HT_3 serotonin receptors (Blackwell and Harding, 1989; Freeman et al., 1991; Roita and Del Favero, 1995). This mechanism of action makes ondansetron useful in controlling nausea and vomiting induced by cytotoxic chemotherapy and radiotherapy, and postoperative vomiting in patients who have undergone gynecological surgery. The drug is supplied as a white crystalline powder soluble in acid media. The lower the pH, the better the stability of the solution; stability is greatest at pH 3 and 4 (Pritchard, 1992).

Polymorphism is the ability of an element or compound to crystallize in more than one crystalline system, although they could be a member of the same crystal system (Helman, 1980; Otsuka and Matsuda, 1987; Ravin, 1995). This affects properties such as drug absorption, rate of dissolution, elimination rate and stability in galenic preparations. In the present study, we obtained polymorphs of ondansetron under different experimental conditions (pH, temperature, pulverization and solvents) and tested the solubility of these forms. The products were analyzed with scanning differential calorimetry (DSC), infrared spectrophotometry and ^{1}H nuclear magnetic reso-

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0378-5173/99/$ - see front matter
PII: S0378-5173(98)00349-4

Table 1
Key to samples of ondansetron prepared under different conditions of pH and temperature, and in different solvents

pH	Sample	Temperature (°C)	Time	Sample	Solvent	Sample
Acid	A	40	24 h	C	Acetone	H
Basic	B	60	24 h	D	Chloroform	I
		100	45 min	E	Benzene	J
		150	20 min	F	Cyclohexane	K
		170	5 min	G	Ethanol	L
					Methanol	M

nance imaging (^{1}H-NMR) (Sugimoto et al., 1986; Brittain et al., 1993).

2. Materials and methods

2.1. Materials

Ondansetron, $C_{18}H_{19}N_3O$ (dihydrated (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazole-1-yl)methyl]-4H-carbazol-4-one), was supplied by Laboratorios Vita SA (Barcelona, Spain). Samples were dissolved for analysis in methanol, ethanol, chloroform, benzene, acetone and cyclohexane, all from Sharlau SL (Barcelona).

2.2. Analytical methods

Spectrophotometry (Haleblian and McCrone, 1969) was done with a Perkin-Elmer Running Lambda 2 apparatus (Überlingen, Germany). DSC of samples weighing 5–6 mg was done over a range of temperatures from 30 to 300°C, with a heating constant of 5°C/min (Mettler FP.98 apparatus, Zurich, Switzerland). Infrared spectra were obtained with a Perkin-Elmer 298 spectrophotometer, using samples prepared in KBr disks. For ^{1}H-NMR, we used a Bruker AM 300 spectrophotometer operating at 313 MHz.

2.3. Preparation of polymorphs

To test the influence of pH on the formation of ondansetron polymorphs, we prepared suspensions of the active principle at a range of values from 2 to 10. The pH was adjusted by adding a solution of HCl or NaOH.

The effects of temperature and time were investigated by heating samples to 40 and 60°C for 24 h, to 100°C for 45 min, to 150°C for 20 min, and to 170°C for 5 min (Table 1). To test polymorph formation in different solvents, we prepared dispersions in acetone, chloroform, benzene, cyclohexane, ethanol and methanol (Table 1). Pulverization (ground by a mortar and pestle), was found not to affect polymorph formation in comparison with unpulverized samples; these results are therefore not reported here. Freezing at −28°C during 24 h likewise had no effect on the appearance of polymorphs.

3. Results and discussion

3.1. Spectrophotometry

An initial solution of the active principle was prepared at a concentration of 0.035 mg/ml (pH

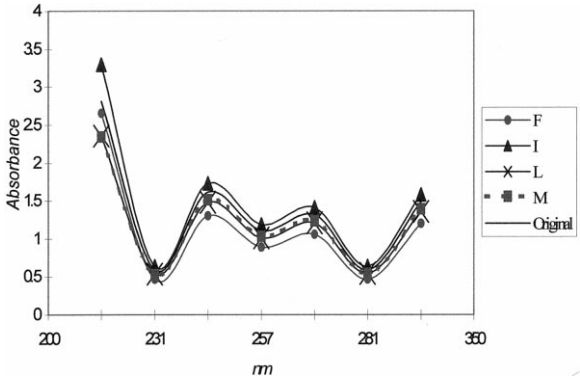


Fig. 1. Absorption spectra for samples F, I, L, M and the original at 0.035 mg/ml under different experimental conditions.

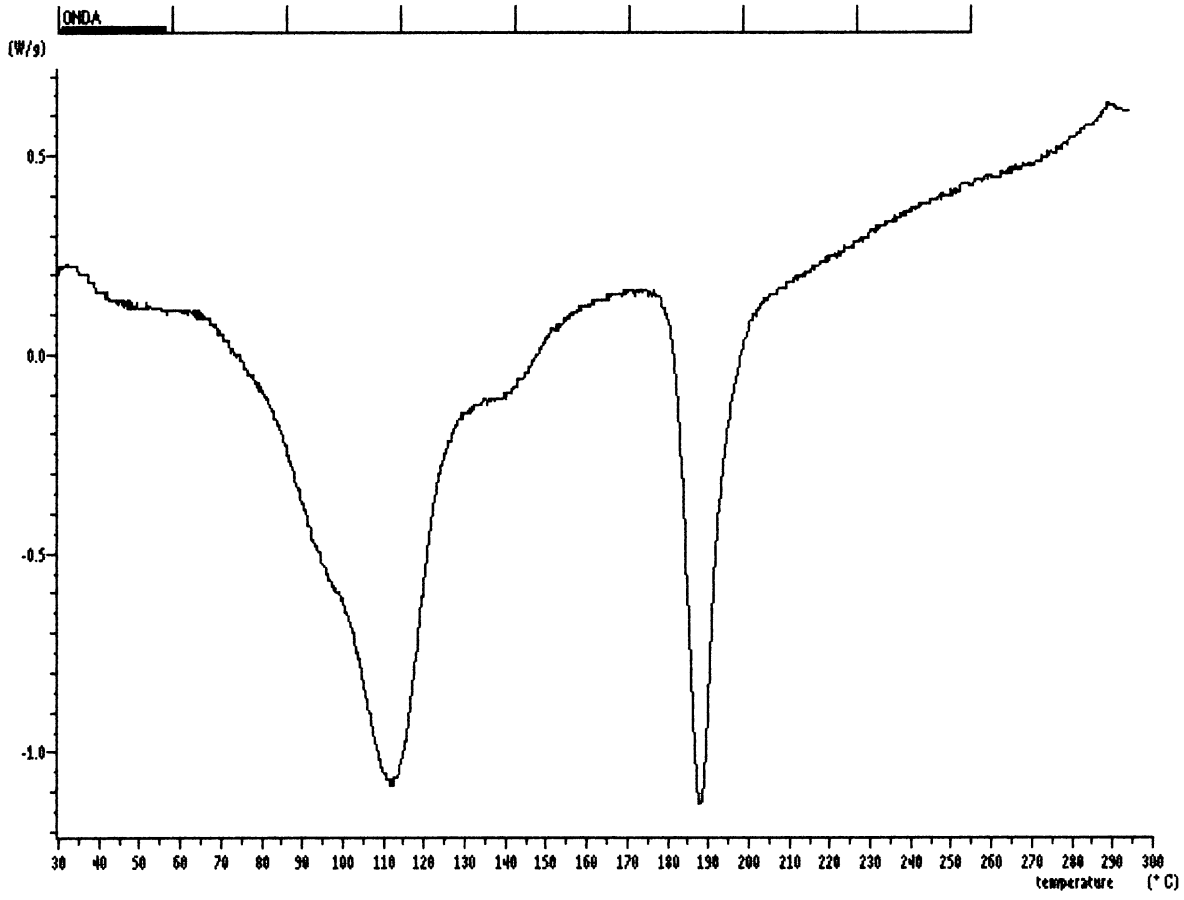


Fig. 2. Thermogram of ondansetron.

3.73) and tested at a range of wavelengths from 200 to 400 nm (Fig. 1). Maximum absorbance was found at 248 nm ($A_{248\ nm} = 1.618$); secondary peaks were found at 266 ($A_{266\ nm} = 1.320$) and 310 nm ($A_{310\ nm} = 1.489$).

Samples prepared under different conditions of pH, temperature and solvent use were tested at the same range of wavelengths and the same concentration. The same peaks were consistently found. By way of example, Fig. 1 illustrates the peaks obtained for samples F, I, L, M and the original (Table 1).

The highest absorbances at all three maximum wavelengths were obtained with sample I, and the lowest with sample F. For all samples, the lowest absorbance was obtained at 266 nm.

3.2. Differential scanning calorimetry

All samples were tested with DSC at 30–300°C. The thermogram of the original sample (Fig. 2) showed exothermal peaks at 111 and 182°C. According to published analyses (Merck Index, 1996), the melting point of ondansetron is between 178.5 and 179.5°C.

Samples C, D, E, F and G were tested under different temperature conditions (Fig. 3). Increasing temperature slightly modified the thermograms, shifting the exothermal peak from 111 to 92°C and giving rise to a new peak at 210°C (sample E), while the original peak at 182°C remained unaffected. In sample F (heated to 150°C for 20 min), the peak appearing at 210°C

was higher than that at a 182°C; however, this feature was no longer present in samples heated to 170°C for 5 min (sample G). These changes in the thermogram indicate that the active principle was transformed to some degree, as subsequent assays confirmed. The deviations caused by temperature increases have been reported in earlier studies (Ruiz et al., 1994, 1998).

The effect of pH was studied in samples spiked with HCl or NaOH solution and then desiccated. Acid pH values produced no significant changes in the thermogram. At basic pH values, the exothermal peaks at 111 and 182°C remained unaffected, but a small peak appeared once again at 210°C (Fig. 4), indicating that variations in pH were not a factor that produced polymorphs of ondansetron.

The effects of the six different solvents tested here differed somewhat. Chloroform produced slight changes in the thermogram: the second exothermal peak at 182°C disappeared and a new peak appeared at 213.6°C. This result was similar to that found in the series of samples that were heated to different temperatures. With acetone, cyclohexane and benzene, the results were similar to those found with the initial sample.

However, the findings with the solvents ethanol and methanol differed clearly from those with the other four solvents and from those with the original sample. Fig. 5 illustrates the endothermal and exothermal zones and peaks obtained.

On the basis of the spectrophotometric and DSC findings, we selected for infrared spectrophotometry and ^{1}H-NMR analyses, those samples that appeared to be most effective in producing polymorphs of ondansetron, i.e. samples F, I, L and M.

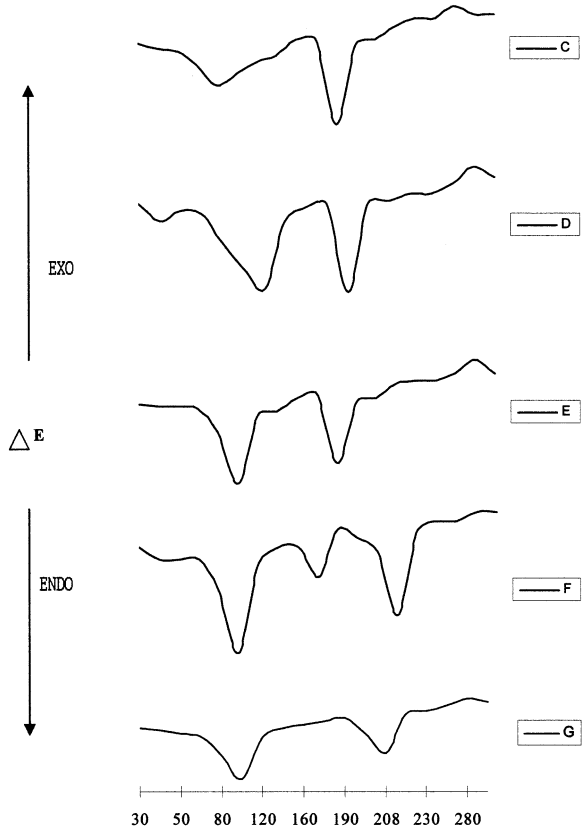


Fig. 3. Themograms of samples C, D, E, F and G, which were subjected to different heating treatments.

3.3. Infrared spectrophotometry

The spectra obtained with samples F, I, L,M and the original were similar (Fig. 6), suggesting that the same molecule was responsible for the peaks.

The slight differences between spectra reflect the presence of different aromatic groups in the molecule. In comparison with the DSC peaks found with the original sample in alcoholic solvents, samples F, I, L and M appeared to lack the keto group, possibly indicating the presence of a keto-enolic tautomer. However, the large band at 3500 cm^{-1} suggested the presence of hydrogen bonds.

Sample F, which was heated to 150°C for 20 min, showed no signs of breakdown, and we conclude that the compound was heat resistant and stable at high temperatures.

Although infrared spectrometry provides little information regarding possible conformational modifications in the molecule (i.e. the presence of functional groups assumed to be present in all our samples), the findings with this technique do suggest that ondansetron was not broken down. The changes we found were thus probably caused by differences in conformational structure, as shown by the differences in the analyses of the spectrophotometry.

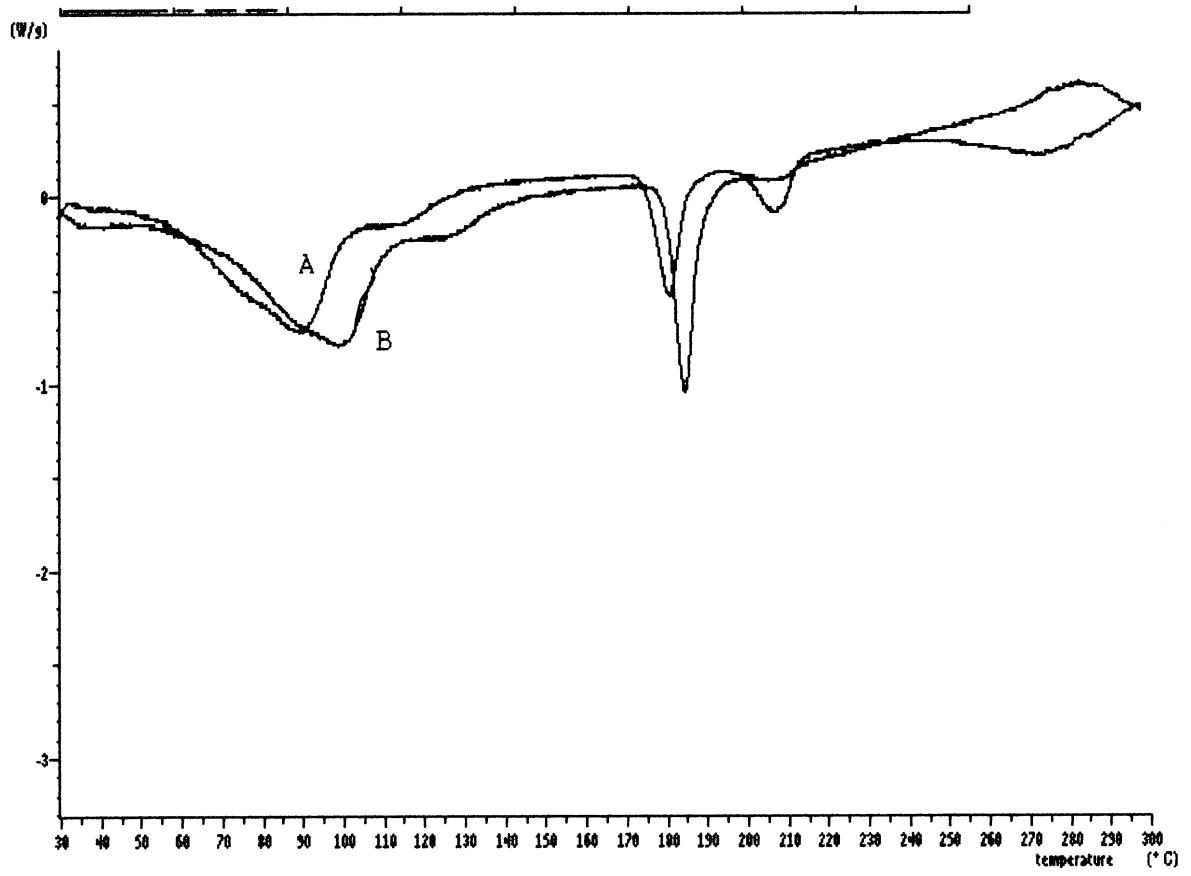
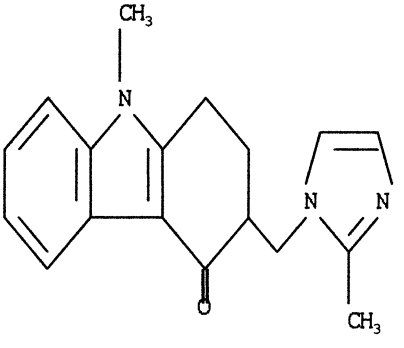


Fig. 4. Thermograms of samples A and B, prepared in acid and basic solution, respectively.

3.4. ^1H-nuclear magnetic resonance

Fig. 7 illustrates the ^{1}H-NMR spectra in Cl_3CD with TMS as internal reference for samples F, I, L, M and the original. The results for all four samples appeared superficially similar, although slight differences were apparent upon close examination.



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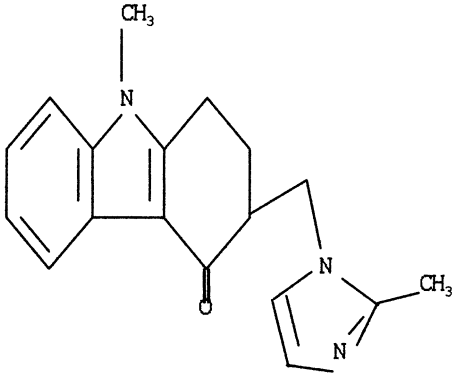

Folded form

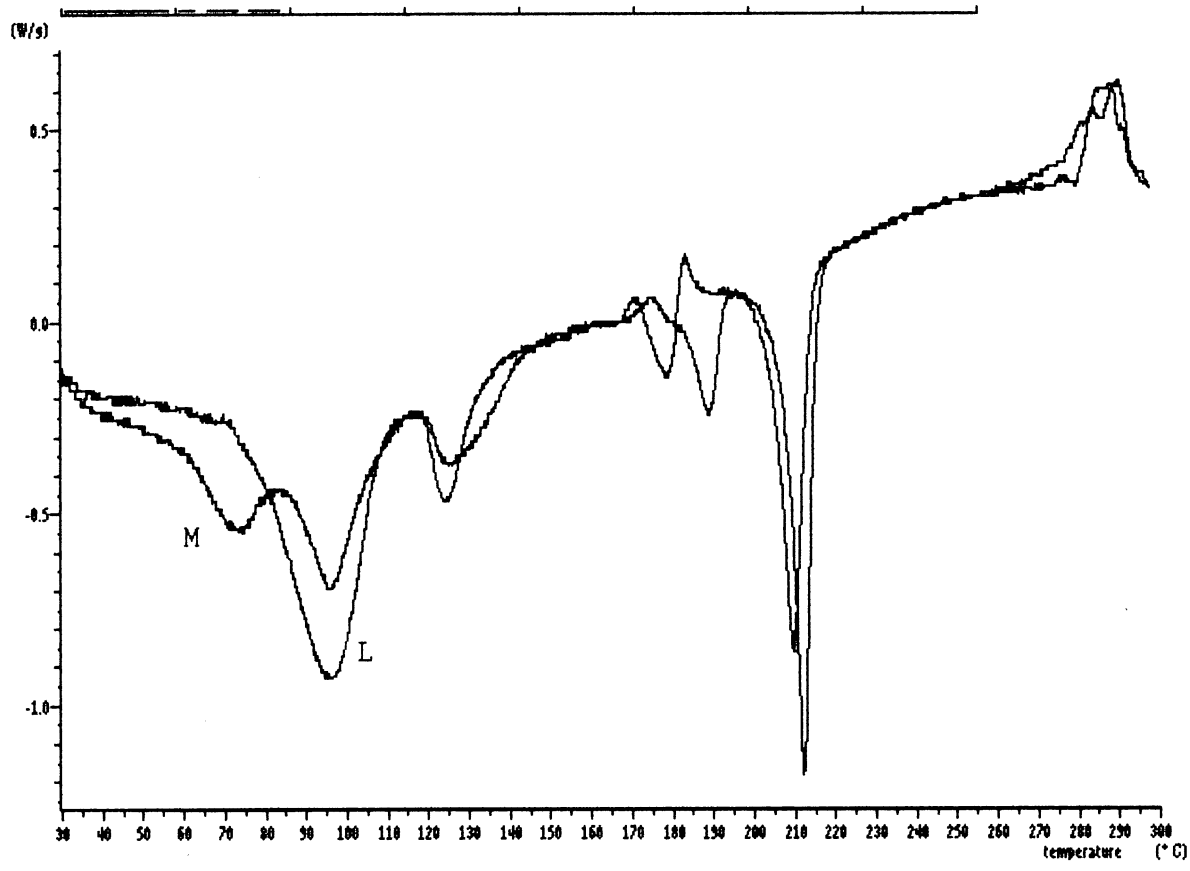

Fig. 5. Thermograms of samples prepared in different solvents.

Ondansetron can exist in two different configurations (extended and folded form) determined by the form of the methylene bridge in the imidazole ring. In one configuration, the larger groups are separated from each other, and interactions between them are thus weaker. This gives rise to a compound that is thermodynamically more stable but less soluble in comparison with the other configuration, in which the attraction between the bulkier groups is greater, and the compound, although stable, is of different polarity and may be more soluble.

The signal at 7.9 ppm indicates a broad doublet of the imidazole H atoms, followed by another band between 7.2 and 7.6 ppm corresponding to the aromatic H atoms. The two double doublets near 4.4 ppm were the elements that were most clearly modified. Two methyl groups were evident between 2.5 and 3.5 ppm.

The spectra for samples L and M were similar. The signals indicated a double doublet (4.4 ppm), and the methyl group in the pyrrole ring (3.4 ppm) showed a sharply-defined singlet that was shifted with respect to the initial sample, indicating shielding of the structure.

In sample I, the spectrum revealed a shift in the aromatic group (7.9 ppm), and a shift at 2.8 ppm that corresponded to the flexible 2H atoms of cyclohexane, and the H atom of imidazole.

In sample F (heated to 150°C for 20 min), the shift at 4.4 ppm was again seen, reflecting modification of the two double doublets. This may have been caused by rotation as a result of heating beyond the energy barrier, to give rise to

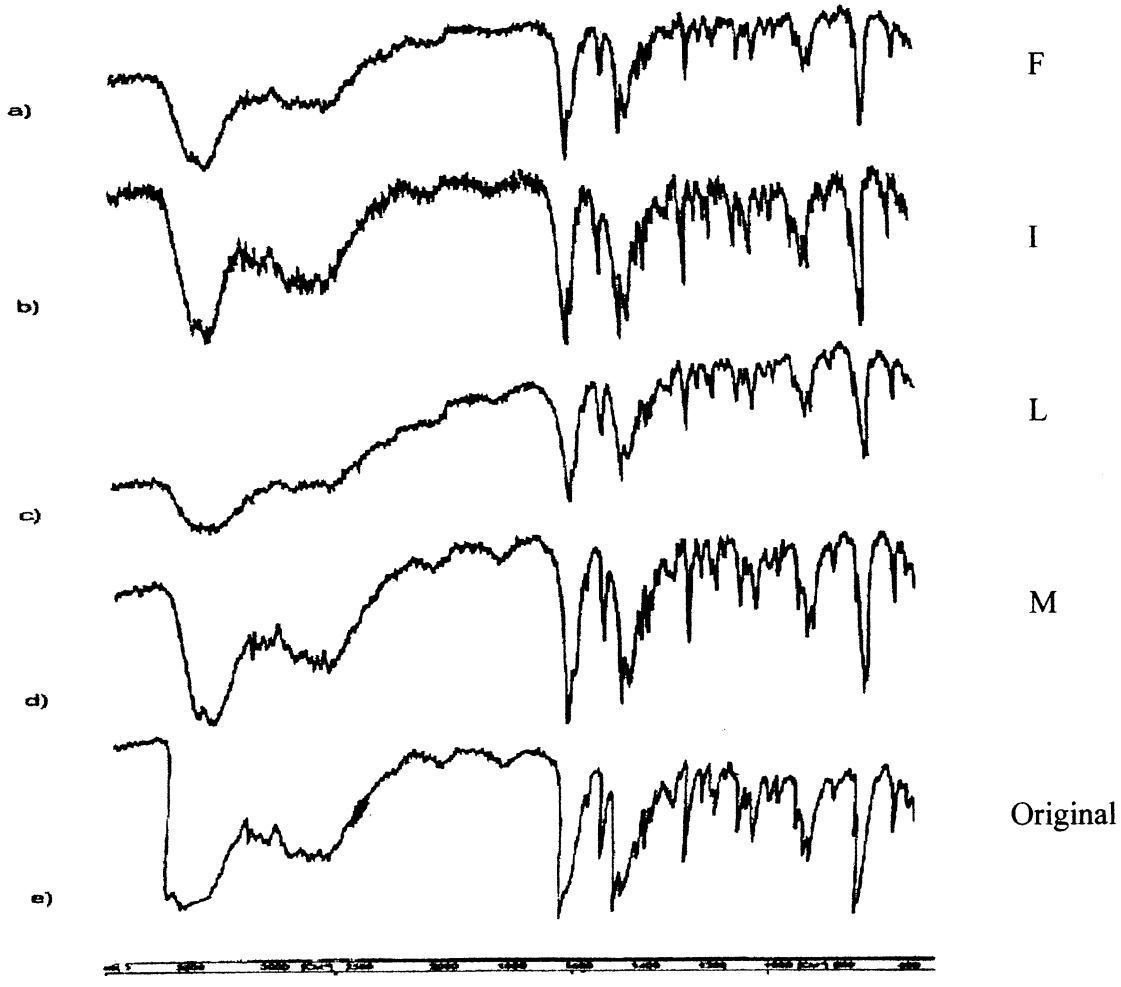

Fig. 6. Infrared spectra of ondansetron in samples F, I, L, M and the original.

the formation of a thermodynamically more stable compound. The methyl groups showed a slight shift, as did the aromatic group, which was slightly shielded in comparison with the initial sample.

The differences in ^{1}H-NMR findings with respect to the original sample suggest that heating caused rotation of the molecule. This may have influenced the spectrophotometric absorbances of the polymorphs.

4. Conclusions

Our findings thus indicate the existence of at least three compounds of different physicochemical and structural characteristics.

Acknowledgements

We thank J.F. Dominguez of the Department of

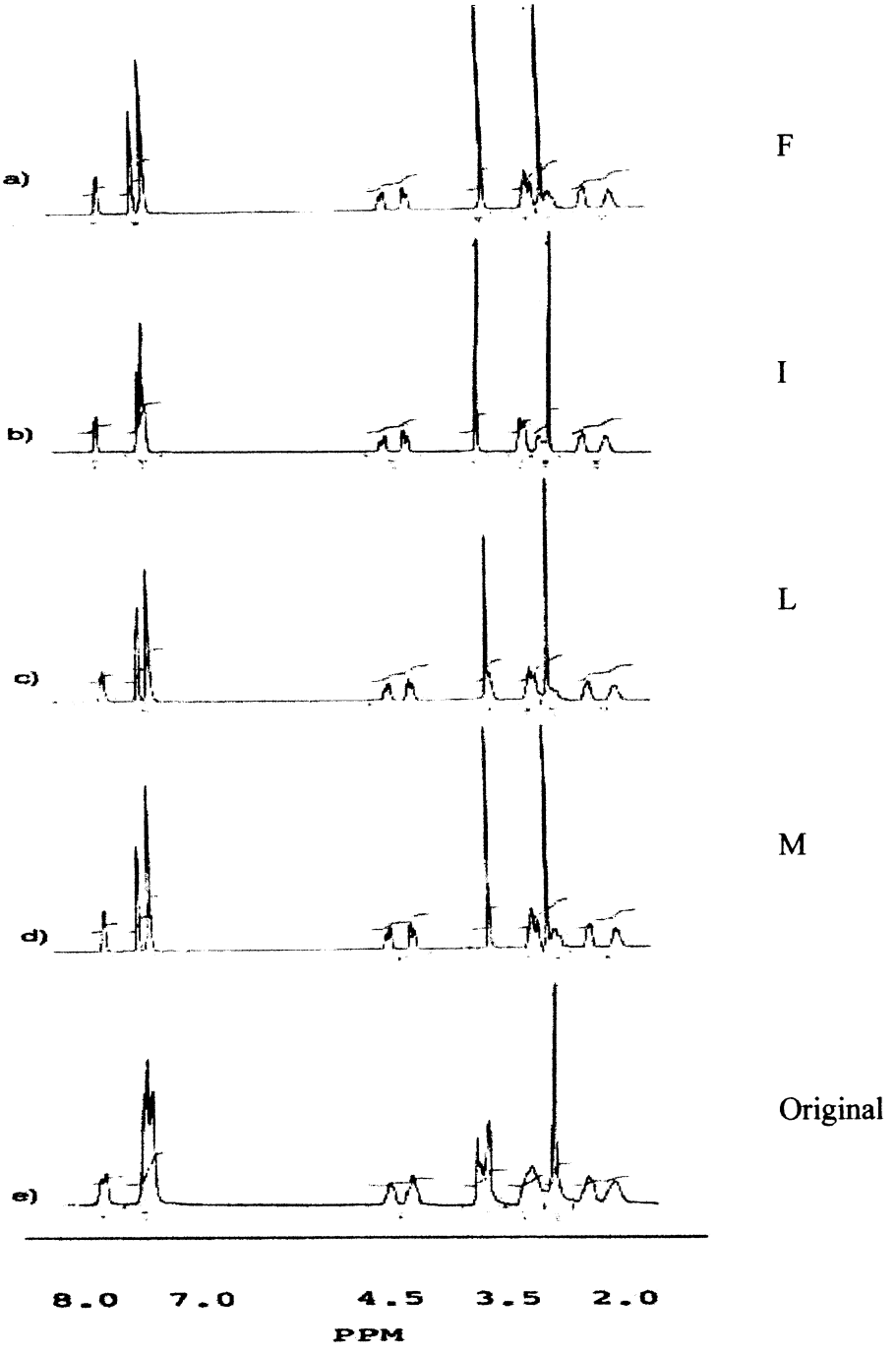

Fig. 7. ^{1}H nuclear magnetic resonance spectra of ondansetron in samples F, I, L, M and the original.

of Organic Chemical, Faculty of Pharmacy, University of Granada for help in interpreting the ^{1}H nuclear magnetic resonance data, and Karen Shashok for translating the original manuscript into English.

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