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Properties of the racemic species of verapamil hydrochloride and gallopamil hydrochloride

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Abstract

It is well known that the stereoselective actions associated with the enantiomeric constituents of a racemic drug can differ markedly in their pharmacodynamic or pharmacokinetic properties. Nevertheless, molecular chirality manifests itself in the solid, that is, crystalline state. The aim of this work was to characterize the solid-state properties of verapamil HCl and gallopamil HCl, two well-known chiral calcium channel antagonists. The characterization of the solid state for the single enantiomers and equimolecular mixtures for both the calcium antagonists was performed by solid-state techniques such as Fourier transform infrared (FT-IR spectroscopy), X-ray powder diffractometry (XRD) and differential scanning calorimetry (DSC). The FT-IR spectra and XRD of the single enantiomers are different from those of the corresponding equimolecular mixture owing to their different crystalline structure. The thermal behavior of the racemates and pure enantiomers were examined by DSC, and the resultant experimental and theoretical binary phase diagrams are discussed. Spectroscopic solid-state techniques, such as FT-IR and XRD, are useful in combination with thermal analysis for characterizing the racemic species of chiral drugs. The data obtained prove that the equimolecular mixtures of both verapamil hydrochloride and gallopamil hydrochloride exist as racemic compounds. Determination of the enantiomeric purity of the enantiomers and racemic compounds of both the calcium antagonists analyzed was performed by DSC.

Keywords: Verapamil hydrochloride; Gallopamil hydrochloride; Chirality; FT-IR spectrometry; X-ray powder diffraction; Differential scanning calorimetry; Phase diagram.

1. Introduction

The characterization of chiral drugs is attracting a great deal of attention from manufacturing and regulatory organizations. In fact, enantiomers should rightly be regarded as different chemical

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compounds in a biological medium, for they may display considerable differences in potency, type of pharmacological activity, pharmacokinetic profile and metabolism, since the majority of the enzymatically catalyzed reactions are stereoselective. A racemic drug should therefore be regarded as a mixture containing 50% of the eutomer, the chosen active enantiomer, and 50% of the distomer, that is, a substance which might be inactive, or less active, or might have different pharmacological activity or toxic effects. Nevertheless, many chiral drugs are still available as racemic forms, usually termed racemates.

Chirality can also affect the way in which molecules fit together in a crystal. Two enantiomers may crystallize together in a racemic compound, or true racemate, whose crystals contain the two enantiomers in equal number. The result is a crystal structure different from that of the pure enantiomers. This is certainly the most commonly encountered type of racemic species and occurs when molecules of the (+)-enantiomer have greater affinity for those of the (−)-enantiomer than for each other, and vice versa. The second fundamental type of racemic mixture is called conglomerate, and is defined as an equimolar physical mixture of the two crystalline enantiomers that are mechanically separable. The problem of homochiral versus heterochiral packing of enantiomers has been the focus of considerable attention in the last few decades, and both the terminology and the distinction between them have been exhaustively described (Jacques et al., 1981).

A thorough understanding of the nature of the enantiomeric interactions is therefore essential for the development of effective and reliable formulations of chiral drugs (Li and Grant, 1996).Fourier transform infrared spectroscopy (FT-IR) and X-ray powder diffractometry (XRD), together with differential scanning calorimetry (DSC) can be successfully employed to define the type of crystalline racemate for optically-active compounds (Gamberini et al., 1994). Physical mixtures of two enantiomers constitute a binary system governed by the phase rule (Brittain, 1990), whose phase diagram can be established by DSC analysis of the melting process, provided that the racemate and at least one resolved enantiomer are available and melt without decomposition. Moreover, knowledge of the phase diagram type enables one to check the enantiomeric purity by measuring the lowering of the melting point in relation to that of the optically-pure compound. The FT-IR spectra and the XRDs can be expected to be identical for conglomerate formation and different for racemic compounds.

Verapamil hydrochloride and gallopamil hydrochloride are calcium ion-channel blocking drugs usually administered as equimolar mixture of their corresponding stereoisomers, although they differ in their bioavailability as well as in their pharmacokinetic and pharmacological effects, (2S)-(−)-enantiomers being the more active (Jim et al., 1981; Eichelbaum et al., 1984; Vogelgesang et al., 1984). Several chiral high-performance liquid chromatographic (Iredale et al., 1991; Fieger and Blaschke, 1992; Miller and Bergeron, 1993; Lankford and Bai, 1995; Stagni and Gillespie, 1995; Rustichelli et al., 1997) and chiral electrophoretic (Clothier and Tomellini, 1996) procedures have been performed for the direct enantiomeric resolution of verapamil HCl and gallopamil HCl.

The present paper reports the results of structure-sensitive analytical techniques, such as FT-IR spectroscopy, XRD, and DSC, used to determine the solid-state properties of verapamil HCl and gallopamil HCl. The data obtained are in agreement with the definition of racemic compounds for both of the equimolar mixtures of the two enantiomers.

2. Materials and methods

2.1. Materials

Racemic verapamil HCl, (2R)-(+)-verapamil HCl and (2S)-(−)-verapamil HCl, racemic gallopamil HCl, (2R)-(+)-gallopamil HCl and (2S)-(−)-gallopamil HCl were kindly supplied by Knoll and used without further purification. All other materials were of analytical or HPLC grade.

2.2. HPLC analyses

Chemical purity of materials was confirmed by non-chiral RP-HPLC analyses. The apparatus consisted of a Perkin-Elmer Series 4 Liquid Chromatograph and a Perkin-Elmer LC-85B variable wavelength UV detector. Chromatograms were recorded and processed using a personal computer equipped with 'Turbochrom' software and 900 Series Interface (Perkin-Elmer). The analytical column was a LiChroCART-LiChrospher 100 RP 18 (125 × 4.0 mm; 5 μm, Merck); all determinations were at room temperature. Sample injection was performed using an automatic injector with a 20.0 μl loop (Perkin-Elmer ISS 101). The analyses were carried out in isocratic mode with mobile phases of phosphate buffer (pH 6.8; $I = 0.01$) containing 5–20% acetonitrile. The flow-rate was 1.0 ml/min and the detector wavelength was set at 225 nm.

Stereospecific analyses of enantiomeric purity were also performed using the same chromatographic system on a Chiral-AGP column (100 × 4.0 mm; 5 μm, Baker) with a mobile phase consisting of acetonitrile and phosphate buffer (pH 7.0; $I = 0.01$) in the ratio 13:87 (v/v). The chromatographic process was carried out at room temperature with a flow-rate of 0.9 ml/min. Under these conditions, no detectable traces of optical impurity were found in the calcium antagonists analyzed. However, it has to be stressed that accurate determinations of optical purity by chiral chromatography are possible only when enantiomeric impurity elutes before the main enantiomeric peak in a quantifiable amount. Integration inaccuracies arise from peak tailing, when the minor peak is eluted after the main peak or with an amount of impurity above the limit of detection but below the limit of quantitation.

2.3. Physical mixtures

Physical mixtures of the two enantiomers were obtained from accurately weighed (Sartorius, mod. 041-04431 electro ultra micro balance) quantities of each isomer, the mixtures were dissolved in ethyl acetate and subsequently evaporated by nitrogen stream.

2.4. Infrared spectroscopy

Infrared spectra were recorded in the range 4000–450/cm with a Perkin-Elmer FT-IR mod. 1600 spectrophotometer on KBr pellets using 32 scans for each sample with a resolution factor of four. The spectra were corrected by subtracting the spectrum of a KBr blank pellet and were presented in the transmittance mode.

2.5. X-ray diffraction

X-ray diffraction measurements were carried out with a Philips PW 1050/25 powder diffractometer. Experimental setting: Ni filtered Cu Kα radiation ($\lambda = 1.5418$ Å); tube settings 40 kV, 20 mA; time constant 4 s; angular speed 1° (2θ) per min, 1°, −0.1°, and −1° slits, angular range $3° < 2\theta < 45°$.

2.6. Thermal analysis

Thermoanalytical studies were performed by means of a Perkin-Elmer Differential Scanning Calorimeter consisting of a DSC-4 measuring cell, System 4 Thermal Analysis Microprocessor Controller, Thermal Analysis Data Station, DSC-4 Partial Areas Software Program and Specific Heat Software Program. Temperatures and enthalpies were calibrated using indium phase transition (99.99% pure; heat of fusion, 28.45 J/g; melting point, 429.76 K). Samples of physical mixtures (1.0–2.0 mg) were accurately weighed and encapsulated in aluminium crimped pans. Thermal curves were recorded with a heating rate of 1–5 K/min in a temperature range from 380–455 K under a dry nitrogen purge (20 cm^3/min). Before each scan, a baseline was recorded with the same heating rate and then subtracted from the experimental scan.

3. Results and discussion

3.1. Fourier-transform infrared spectroscopy

The single enantiomers of verapamil HCl and gallopamil HCl gave superimposable FT-IR spec-

tra that differed from that of the corresponding racemate. In particular, racemic verapamil HCl presented infrared bands (1471, 1339, and 816/cm) lacking in the single enantiomer, the band at 858/cm was weak and the bands present in the single enantiomer at 1023 and 767 cm were split (1027–1021 and 771–765/cm), differences were also present between 3030–2860/cm, where there was a broad complex absorption due to superimposing C–H stretching vibrations of the methyl and methylene groups. Most bands highly characteristic of the molecular skeletal structure were essentially unchanged, e.g. 2838/cm (C–H stretching vibrations of the methoxy groups), 2236/cm (sharp weak band due to C≡N stretching vibrations of the saturated alkyl nitrile), 1608, 1591, and 1519/cm (bands due to skeletal stretching vibrations of the benzene ring).

Racemic gallopamil HCl did not show the bands at 3562, 3079, 3014, 1159, 1141, 872, and 845/cm, present in the single enantiomer. The N–H stretching vibrations of the protonated amine (2600–2300/cm) and the C–O stretching vibrations of the aromatic ether (1290–1200/cm) also differed. The vibration bands of the functional groups presented only slight differences in their frequencies compared to those of verapamil HCl.

Typical IR spectra in the region 1700–450/cm for the therapeutically active enantiomers and the correspondent racemates are displayed in Fig. 1a and b.

3.2. X-ray powder diffraction

The X-ray powder diffractograms of both the calcium antagonists showed different patterns for the single enantiomer and the related racemate. Characteristic high-intensity diffraction peaks could be detected for each compound at: $2\theta =$ 13.60°, 15.50°, and 17.25° for (2S)-(−)-verapamil HCl; $2\theta = 17.10°$, 18.10°, and 18.90° for racemic verapamil HCl; $2\theta = 13.85°$, 21.15°, 21.50°, and 23.00° for (2S)-(−)-gallopamil HCl; and $2\theta =$ 16.50°, 17.70°, 19.85°, and 23.40° for racemic gallopamil HCl. The complete sets of experimental values are listed in Table 1.

The fact that the X-ray and infrared spectra of the (2S)-(−)-enantiomers differed markedly from those of the corresponding racemate demonstrates clearly that they possess different crystal structures and that therefore both the equimolecular mixtures exist as racemic compounds. This interpretation was substantiated by differential scanning calorimetric analyses.

3.3. Thermal analysis

DSC measurements were run on each single isomer and on the racemic forms. Neither the enantiomers nor the racemates exhibited any thermal event before melting; this suggests that the enantiomers and the racemic compounds did not undergo any solid-state alterations up to their respective melting points. Representative DSC traces and the thermodynamic data are shown in Fig. 2a and b and in Table 2. The values of enthalpy of formation ($\Delta_f H^\Phi$) and entropy of formation ($\Delta_r S^\Phi$) have been calculated (Leclercq et al., 1976) at the melting point of whichever species melts lower (enantiomer or racemic compound), they afford an indication of the stability of the racemic compound as represented by the free energy difference ($\Delta_r G^\Phi$) associated with the reaction between the single enantiomers to give the crystalline racemic compound.

In order to confirm the definition of racemic compound for the equimolecular composition of both analytes, the binary phase equilibrium diagrams were defined by DSC analysis of several physical mixtures of their single enantiomers. This analytical procedure requires knowledge of the calorimetric data of the pure substances, that is, of their melting points and enthalpies of fusion. Having obtained precise values, we then calculated the melting point diagrams applying the simplified equation of Schröder–Van Laar to the enantiomer branch, e.g. between the pure enantiomer and the eutectic, and the equation of Prigogine–Defay to the racemic compound branch (Prigogine and Defay, 1967; Jacques et al., 1981).

Verapamil HCl (Fig. 3a) presents a racemic compound, dominating the phase diagram, with a heat of fusion and a melting point appreciably higher than those of the single enantiomers. The

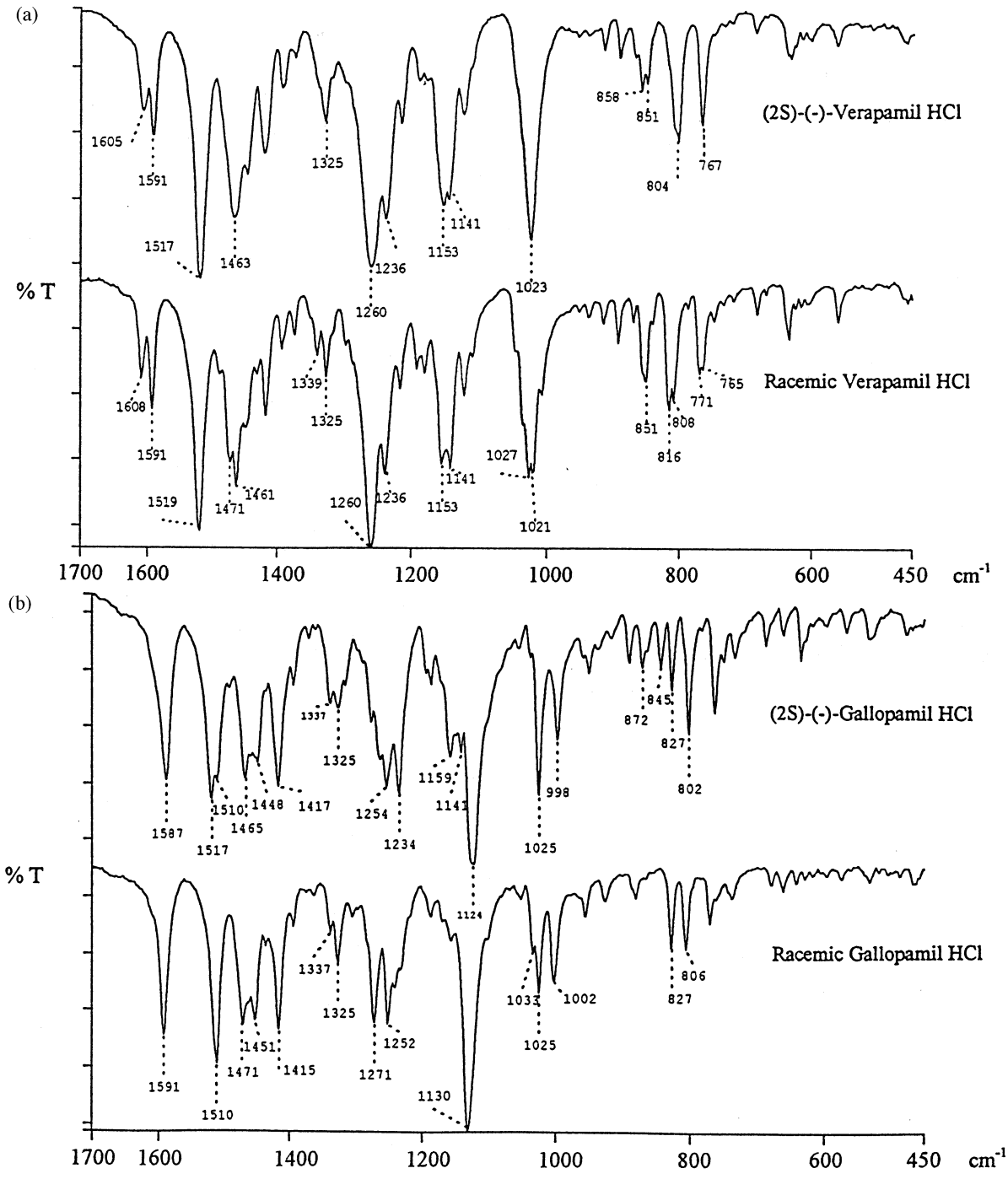

Fig. 1. FT-IR spectra of the enantiomer and racemic form for: (a) verapamil HCl; and (b) gallopamil HCl.

Table 1
Principal X-ray powder diffraction values for verapamil HCl and gallopamil HCl

(2S)-(−)-verapamil HCl			Racemic verapamil HCl			(2S)-(−)-gallopamil HCl			Racemic gallopamil HCl		
d (Å)	°2θ	I/I_0 (%)	d (Å)	°2θ	I/I_0 (%)	d (Å)	°2θ	I/I_0 (%)	d (Å)	°2θ	I/I_0 (%)
									14.24	6.20	23
10.27	8.60	21	10.21	8.65	56						
									8.93	9.90	28
						7.25	12.20	24	7.25	12.20	3
									7.08	12.50	15
6.51	13.60	54									
						6.39	13.85	100			
			6.28	14.10	13				6.30	14.05	38
			6.10	14.50	13				6.06	14.60	55
						5.92	14.95	11	5.92	14.95	42
5.71	15.50	100									
						5.50	16.10	40	5.53	16.00	32
						5.43	16.30	29			
									5.37	16.50	42
			5.27	16.80	14	5.26	16.85	15			
5.14	17.25	97	5.18	17.10	100	5.20	17.05	17	5.15	17.20	14
									5.01	17.70	100
			4.90	18.10	93						
4.83	18.35	42				4.84	18.30	30	4.84	18.30	37
			4.70	18.90	79	4.70	18.85	21	4.70	18.85	14
						4.55	19.50	20	4.50	19.75	77
									4.45	19.85	94
4.34	20.45	9	4.37	20.30	21						
			4.16	21.35	16	4.20	21.15	50	4.16	21.35	17
4.11	21.60	35				4.13	21.50	49			
									4.06	21.90	26
3.95	22.50	37							3.91	22.70	23
3.85	23.10	11	3.81	23.30	11	3.86	23.00	51	3.80	23.40	63
			3.74	23.80	21	3.74	23.80	14			
3.70	24.05	18									
3.68	24.15	18							3.70	24.05	14
						3.57	24.90	25	3.67	24.20	18
									3.62	24.55	23
3.53	25.20	13	3.53	25.20	11	3.50	25.40	13	3.50	25.40	11
3.41	26.10	28	3.41	26.10	27	3.41	26.10	30	3.40	26.20	32
3.37	26.40	19	3.38	26.35	41				3.39	26.30	34
			3.35	26.60	20	3.32	26.80	12			
3.23	27.60	9	3.23	27.55	14				3.28	27.20	42
						3.17	27.95	7	3.17	27.95	13
3.05	29.30	6	3.02	29.60	16						
2.84	31.50	15							2.85	31.40	4
									2.76	32.40	15
			2.35	38.35	16						

eutectic composition corresponds to a mole fraction $x = 90.42\%$ with a melting point value of 405.06 K. On the other hand, gallopamil HCl (Fig. 3b) has higher melting-point and melting-enthalpy values for the enantiomeric form than for the racemic one; the eutectic composition is $x = 69.33\%$ with a melting point of 424.38 K. The experimental melting-point values, measured on

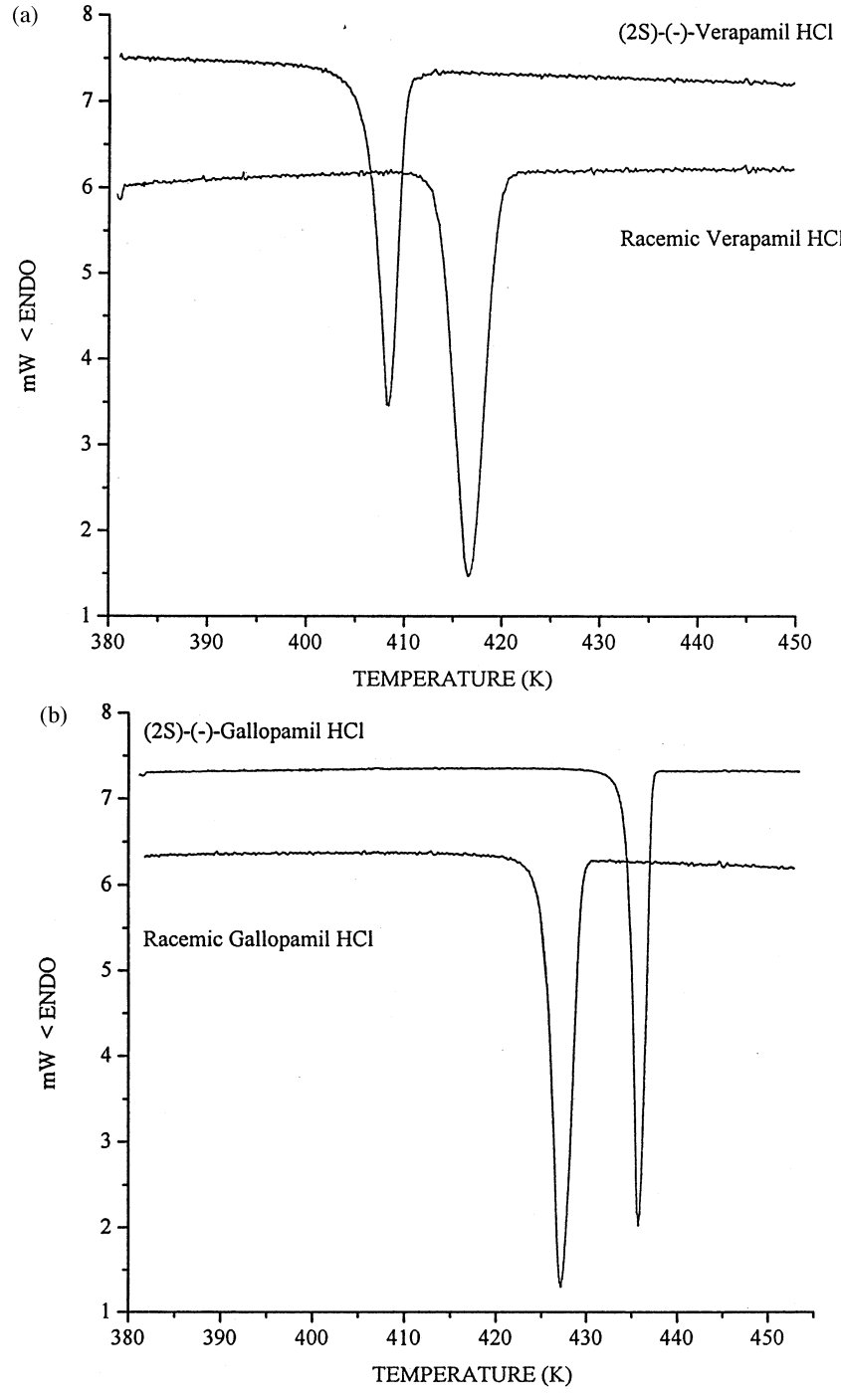

Fig. 2. DSC curves of the enantiomer and racemic form for: (a) verapamil HCl; and (b) gallopamil HCl.

Table 2
Thermodynamic data for the single enantiomers and racemic compounds

Compound as hydrochloride	Calorimetric purity (%)	Melting point (K)	Melting enthalpy ($\Delta_{fus}H^{\Phi}$) (kJ/mol)	Melting entropy ($\Delta_{fus}S^{\Phi}$) (J/mol per K)	Free energy of formation (Δ_fG^{Φ}) (kJ/mol)
(2S)-(−)-verapamil	99.40	408.44	40.96	100.28	
(2R)-(+)-verapamil	99.28	408.27	40.45	99.07	
Racemic verapamil	99.16	416.67	64.14	153.93	−3.60[a]
(2S)-(−)-gallopamil	99.76	434.64	54.44	125.25	
(2S)-(+)-gallopamil	99.53	434.13	54.35	125.19	
Racemic gallopamil	99.37	426.67	51.32	120.28	−1.48[b]

[a] Enthalpy of formation $\Delta_fH^{\Phi}_{T_{fusA}} = -21.65$ kJ/mol and entropy of formation $\Delta_fS^{\Phi}_{T_{fusA}} = -44.21$ J/mol per K, both calculated at the melting temperature of the (2S)-(−)-enantiomer (T_{fusA}). $C^l - C^S_R = 186$ J/mol per K, C^l, and C^S_R are the specific heat capacity of the liquid and the solid racemate, respectively.

[b] Enthalpy of formation $\Delta_fH^{\Phi}_{T_{fusR}} = 1.30$ kJ/mol and entropy of formation $\Delta_fS^{\Phi}_{T_{fusR}} = 6.63$ J/mol per K; both calculated at the melting temperature of the racemic compound (T_{fusR}). $C^l - C^S_A = 228$ J/mol per K, C^l, and C^S_A are the specific heat capacity of the liquid and the solid enantiomer, respectively.

physical mixtures of the two enantiomers by DSC, are in good agreement with the calculated curve for both compounds, as shown in their binary phase diagram.

If $\ln x\ (1-x)$ values, where x represents the mole fraction of the more abundant enantiomer, are plotted against the experimental values of $1/T_{fus}$, T_{fus} being the measured values of complete melting, a straight line is obtained whose slope gives the calculated enthalpy of fusion ($\Delta_{fus}H^{\Phi}$) for the racemic compound (test of Prigogine–Defay, Duddu and Grant, 1992). Following this procedure for verapamil HCl we obtained a straight line whose slope (64.68 kJ/mol) was found to be in good agreement with the value measured directly by DSC (64.14 kJ/mol), for gallopamil HCl the calculated absolute slope value was 51.49 kJ/mol and the experimental melting heat was 51.32 kJ/mol. These data indicate close agreement between the experimental data and the Prigogine–Defay equation.

Petterson has proposed a definition of racemic compound stability based on the definition of the i value, i.e. the difference between racemic compound and eutectic melting points divided by the difference between enantiomer and eutectic melting points (Jacques et al., 1981). An i value <0.5 would indicate only a weak tendency to form a racemic compound, while a value >1.5 would indicate a strong tendency to do so. According to Petterson's rule, therefore, the calculated i value for verapamil HCl ($i=3.43$) and for gallopamil HCl ($i=0.22$), would indicate a strong and a weak tendency, respectively. This behavior is in agreement with the differences found in the eutectic composition of verapamil ($x=90.42\%$) and gallopamil ($x=69.33\%$), in fact, the closer the eutectic composition to a mole fraction of 0.5, the more unstable the racemic compound.

In the region of enantiomeric purity in which this indirect method can be applied, the eutectic is often undetectable and the melting curve rises very slowly.

The optical purity values determined by DSC are reported in Table 2.

4. Conclusions

This paper deals with the characterization of the crystalline racemate type for calcium channel blocker drugs by means of physical methods, such as FT-IR spectroscopy, XRD and DSC.The IR-spectrum of the racemic compound (verapamil HCl, or gallopamil HCl) are significantly different from those of the corresponding enantiomers.

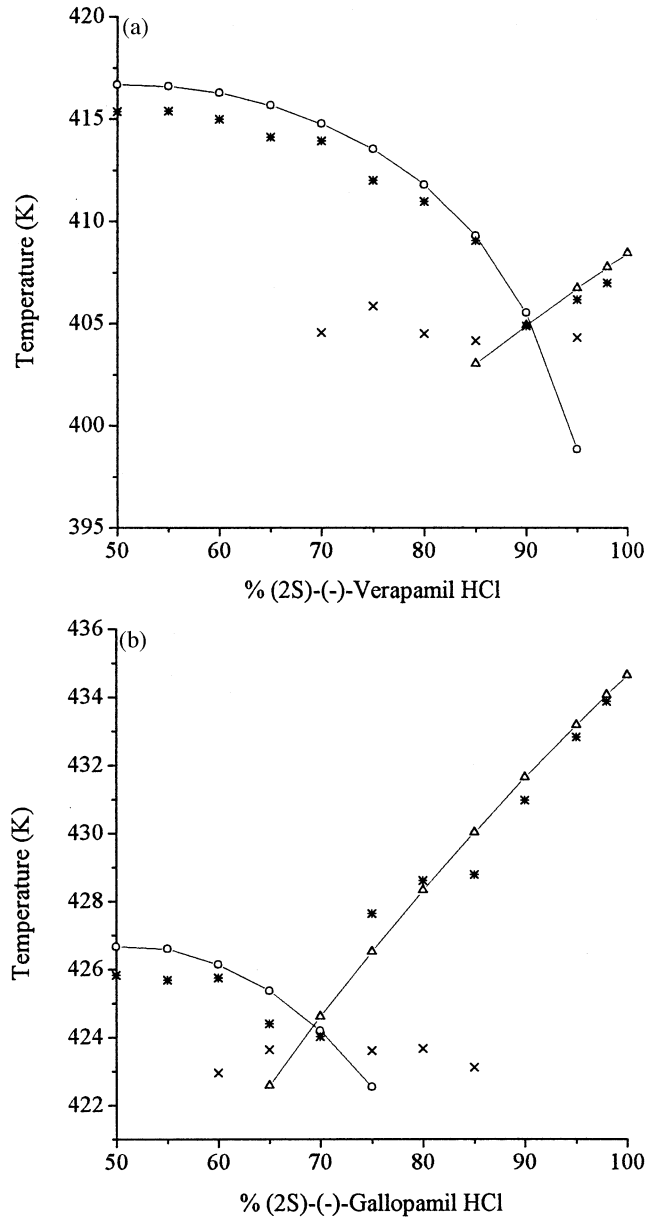

Fig. 3. Phase equilibrium diagram of: (a) verapamil HCl; and (b) gallopamil HCl. The following symbols representing: (*) experimentally measured temperatures of complete melting; (x) experimentally measured temperatures of the first melting (i.e. eutectic temperatures); (-○-) values predicted by the Prigogine–Defay equation; and (Δ) values predicted by the Schröder–Van Laar equation.

The XRD patterns on powder clearly demonstrate more significant differences between the (2S)-(−)-verapamil HCl or (2S)-(−)-gallopamil HCl and the related racemic compound.

The DSC thermodynamic data processing gives a free energy of formation value for both analytes fitting for a racemic compound (verapamil HCl, $\Delta_f G^{\Phi} = -3.60$; gallopamil HCl, $\Delta_f G^{\Phi} = -1.48$).

Our findings enable us to define the solid-state properties of verapamil HCl and gallopamil HCl and demonstrate that the relative equimolecular mixture exists as a racemic compound. Moreover, thermal analysis allows the characterization of racemate type by means of binary melting point phase diagram and quantitative analysis of optical purity.

The chiral impurity can be either the opposite enantiomer in a homochiral crystal or an excess of either enantiomer in a racemic compound.

Once the phase diagram types are determined, calorimetric determination of enantiomeric purity can be easily performed for both verapamil HCl and gallopamil HCl.

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References

Brittain, H.G., 1990. Crystallographic consequences of molecular dissymmetry. Pharm. Res. 7, 683–690.

Clothier, J.G. jr, Tomellini, S.A., 1996. Chiral separation of verapamil and related compounds using micellar electrokinetic capillary chromatography with mixed micelles of bile salt and polyoxyethylene ethers. J. Chromatogr. A 723, 179–187.

Duddu, S.P., Grant, D.J.W., 1992. Formation of the racemic compound of ephedrine base from a physical mixture of its enantiomers in the solid, liquid, solution, or vapor state. Pharm. Res. 9, 1083–1091.

Eichelbaum, M., Mikus, G., Vogelgesang, B., 1984. Pharmacokinetics of (+)-, (−)-, and (+/−)-verapamil after intravenous administration. Br. J. Clin. Pharmacol. 17, 453–458.

Fieger, H., Blaschke, G., 1992. Direct determination of the enantiomeric ratio of verapamil, its major metabolite norverapamil and gallopamil in plasma by chiral high-performance liquid chromatography. J. Chromatogr. 575, 255–260.

Gamberini, G., Ferioli, V., Rustichelli, C., Vezzalini, F., 1994. Calorimetric and infrared spectrophotometric study of ethambutol dihydrochloride. Il Farmaco 49, 415–419.

Iredale, J., Aubry, A.-F., Wainer, I., 1991. The effects of pH and alcoholic organic modifiers on the direct separation of some acidic, basic, and neutral compounds on a commercially available ovomucoid column. Chromatographia 31, 329–334.

Jacques, J., Collet, A., Wilen, S.H., 1981. Enantiomers, Racemates, and Resolutions. Wiley, New York.

Jim, K.F., Harris, A., Rosenberg, L.B., Triggle, D.J., 1981. Stereoselective and non-stereoselective effects of D 600 (methoxyverapamil) in smooth muscle preparations. Eur. J. Pharmacol. 76, 67–72.

Lankford, S.M., Bai, S.A., 1995. Determination of the stereochemical composition of the major metabolites of verapamil in dog urine with enantioselective liquid-chromatographic techniques. J. Chromatogr. B Biomed. Appl. 663, 91–101.

Leclercq, M., Collet, A., Jacques, J., 1976. Étude des mélanges d'antipodes optiques-XII. Tetrahedron 32, 821–828.

Li, Z.J., Grant, D.J.W., 1996. Effects of excess enantiomer on the crystal properties of a racemic compound: ephedrinium 2-naphtalenesulfonate. Int. J. Pharm. 137, 21–31.

Miller, L., Bergeron, R., 1993. Analytical and preparative resolution of enantiomers of verapamil and norverapamil using a cellulose-based chiral stationary phase in the reversed-phase mode. J. Chromatogr. 648, 381–388.

Prigogine, I., Defay, R., 1967. Chemical Thermodynamics, 4th ed., Longmans, London.

Rustichelli, C., Ferioli, V., Gamberini, G., 1997. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry. Chromatographia 44, 477–483.

Stagni, G., Gillespie, W.R., 1995. Simultaneous analysis of verapamil and norverapamil enantiomers in human plasma by high-performance liquid chromatography. J. Chromatogr. B Biomed. Appl. 667, 349–354.

Vogelgesang, B., Echizen, H., Schmidt, E., Eichelbaum, M., 1984. Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L- and D-verapamil examined with a stable isotope technique. Br. J. Clin. Pharmacol. 18, 733–740.

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