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Preparative resolution of drug racemates to study the chiroptical properties of their enantiomers

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Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

The present work is focused on the resolution of ten racemates, in order to study their chiroptical properties and to test the validity of the requirement specified in the European Pharmacopeia (EP) for demonstrating that a drug entity is a racemate. This work shows that the optical purity of enantiomers and non racemic mixtures of a number of compounds can be determined more accurately by circular dichroic (CD) spectroscopy than by a measurement of the angle of rotation (AoR), the EP requirement. Using only the AoR, some of the racemates could not be distinguished from the enantiomers. CD spectroscopy or chiral chromatography should, therefore, be the technique of choice in the determination of optical purity of a chiral compound, especially for those exhibiting low AoR.

Keywords: Enantiomer separation; Optical purity; Angle of rotation; CD spectroscopy; Chiroptical properties; European Pharmacopeia

1. Summary

Drugs of today are often marketed as pure enantiomers, whereas drugs of yesterday might exist either as pure enantiomers or as racemates. Racemates of early drugs have seldom been resolved, and consequently, nothing is known about the chiroptical properties of the pure enantiomers. Until recently, there has been a requirement in the European Pharmacopeia (EP) that measurements of the optical rotation should also be carried out for old racemic drugs to demonstrate that a racemate is at hand. However, without knowing the specific rotation of the enantiomer itself or the conditions for its determination, it is difficult to prescribe the correct conditions for measurements of the kind stated.

The present study was focused on the separation of sufficient amounts of pure enantiomers, in order to study the chiroptical properties of the individual enantiomers and to test the validity of the above mentioned requirement. Ten racemates

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in the EP were resolved by chiral chromatography and the specific rotations, as well as the circular dichroic (CD) spectra were measured. Data for the enantiomers, the racemates and for mixtures with one enantiomer in excess were collected. From this work it could be shown that for about half of the compounds studied, a measurement of the angle of rotation (AoR) was not enough to distinguish the enantiomer from the racemate due to low signal to noise ratio. The advantage of using CD is discussed, as with this method it was easy to distinguish a racemate from an enantiomerically enriched sample even in cases when the enantiomeric excess (e.e.) was very small.

The analytical separations and the preparative systems used are discussed and some recommendations given on their suitability.

2. Background

In the last decade, issues on chirality in the pharmaceutical field have moved from being a research topic to production reality. Many companies specialised in commercialisation of chiral drugs have emerged. This development has gone hand in hand with an explosive development of chiral separation media and technologies. However, this situation is as yet not much reflected in the pharmacopoeias, wherein the discrimination between a racemic compound and its pure enantiomers, if available on the market, has by tradition been made by a simple test for optical rotation. Chiral methods are mentioned in the pharmacopoeias, but have to date not been used in many monographs which stipulate the requirements for a pharmaceutical compound. In comparison, optical rotation is a less demanding and also an inexpensive measurement to perform and, therefore, the most commonly used method today.

Until recently, there have been requirements in the EP [1], that even established racemic drugs, of which no pure enantiomers are available on the market, should be treated in the same way, i.e. tested for lack of optical rotation. However, when the pure enantiomers are not available in amounts needed for establishing the optical activity, the conditions required for a measurement are very difficult to predict. The present study, including preparative resolution of some racemic compounds in the EP, was undertaken in order to explore the validity of such a requirement by investigating the chiroptical properties of the enantiomers using polarimetry and CD spectroscopy. The specific rotation and CD spectra were measured for both enantiomers of each drug.

The experimental conditions for the measurement of the optical rotation of the different compounds studied were taken from the requirements in the EP. This means that only water, methanol or dichloromethane were used as solvents. The concentrations used were also as given in the EP, with the analytes in amounts of 10–50 mg/ml. For a compound to be racemic, according to the EP specifications, it should have an AoR less than 0.1° [2]. This value corresponds to a specific rotation of between 2 and 10.

The columns used were selected from the arsenal available in our laboratories, and with which our experience has been favourable in the last few years.

3. Experimental

The compounds that were selected from the EP are listed in Table 1 and their structures given in Fig. 1. The selection was made more or less randomly. All compounds were either obtained from the laboratory of the EP or in-house stock.

4. Chromatography

The separation of the enantiomers was optimised on analytical columns (250 × 4.6 mm I.D.) based on 10 μm silica particles. The chiral stationary phases (CSP) used in this study were Chiralcel OJ [cellulose *tris*-(4-methylbenzoate)] or OD [cellulose *tris*-(3,5-dimethylphenylcarbamate)] and Chiralpak AD [amylose *tris*-(3,5-dimethylphenyl-carbamate)] or AS [amylose *tris*-(1-(S)-phenylethylcarbamate)] [3]. The mobile phase was optimised to give sufficient separation of the enantiomers of the compounds studied and was

based on a hydrocarbon (*iso*-hexane or heptane) with the addition of modifiers such as 1-propanol, 2-propanol, ethanol or acetonitrile. In most cases, a small amount of diethylamine was added to improve the chromatographic performance for these basic analytes. The separations obtained are presented in Table 1.

The analytical separation was transferred to the preparative system after adjustment of the flow rate. The preparative separations were carried out on the same chiral sorbent as in the analytical system using 250×20 mm I.D. columns. The different chromatographic conditions used for the resolution of the racemates, and the e.e. obtained, are shown in Table 2. In six cases, the enantiomers were purified by flash chromatography on silica to remove impurities acquired from leakage of chiral material from the column as revealed by CD (see below, purification).

The analytical liquid chromatographic measurements were performed on a system consisting of a Gynkotek HPLC Pump Series P580, a Hewlett Packard series 1100 (loop μl) injector and a Gynkotek UVD340S detector.

The preparative liquid chromatographic system consisted of a Gilson mod. 306 solvent delivery pump, a Gilson mod. 231XL sampling injector (loop 5.8 ml) and a Jasco UV-975 detector. The software used for both systems was Chromeleon© Gynkotek 1997 Version 4.20.

4.1. Polarimetry and CD spectrometry

Measurements of the optical rotations were performed on a Perkin–Elmer 341 LC polarimeter at four wavelengths. In Table 3, only values at 589 nm are given as this is the wavelength for measurement of optical rotation in EP. The solvents used in the measurements of the optical rotations were those stated in EP and the concentration of the isolated enantiomer was usually 10 mg/ml. All measurements were carried out in a 1 dm cell at 20 °C. The enantiomers were studied as free bases.

CD spectra were obtained with a JASCO J-715 spectropolarimeter. The solvents were the same as used for the measurements of the optical rotation. A quartz cell of 1 cm pathlength was used and the temperature was kept at 20 °C.

4.2. Purification

During resolution of some of the racemates by preparative chromatography, leakage of the CSP from the column was observed. Since the chiral selector will also give rise to optical rotation, the enantiomers had to be purified by flash chromatography. The enantiomeric compounds purified were chlorcyclizine, doxapram, mefloquine, metoprolol and promethazine. The columns were packed with silica gel, and for chlorcyclizine and

Table 1
Analytical chiral liquid chromatographic systems for resolution of some racemic pharmaceutical compounds

Compound	Column	Mobile phase	k'_1	k'_2	α
Chlorcyclizine HCl	Chiralpak AD	Heptane/IPA/DEA 99/1/0.1	2.99	4.03	1.35
Doxapram HCl	Chiralcel OJ	IH/1-PrOH/MeOH/DEA 90/8/2/0.1	1.24	3.12	2.52
Fenticonazole nitrate	Chiralpak AS	IH/IPA 80/20	1.76	3.81	1.76
Isoconazole	Chiralcel OJ	IH/IPA/DEA 80/20/0.1	2.14	7.00	3.27
Mefloquine HCl	Chiralpak AD	IH/1-PrOH/DEA 95/5/0.1	0.70	3.38	5.51
Methaqualone	Chiralpak AS	IH/IPA/ACN 99/0.5/0.5	4.36	7.47	1.84
Metixene HCl	Chiralcel OJ	IH/EtOH 80/20	1.25	2.31	1.84
Metoprolol tartrate	Chiralcel OD	IH/IPA/DEA 80/20/0.1	0.32	1.37	4.34
Promethazine HCl	Chiralcel OJ	IH/IPA/DEA 99/1/0.1	2.78	7.13	2.56
Terconazole	Chiralpak AD	IH/IPA/DEA 80/20/0.1	2.66	7.35	2.76

IPA, 2-propanol; DEA, diethylamine; IH, isohexane; 1-PrOH, 1-propanol; ACN, acetonitrile; EtOH, ethanol; MeOH, methanol. The separations were carried out at 25 °C, with a flow rate of 1 ml/min. 50 μl of the test solution, 1 mg/ml was injected.

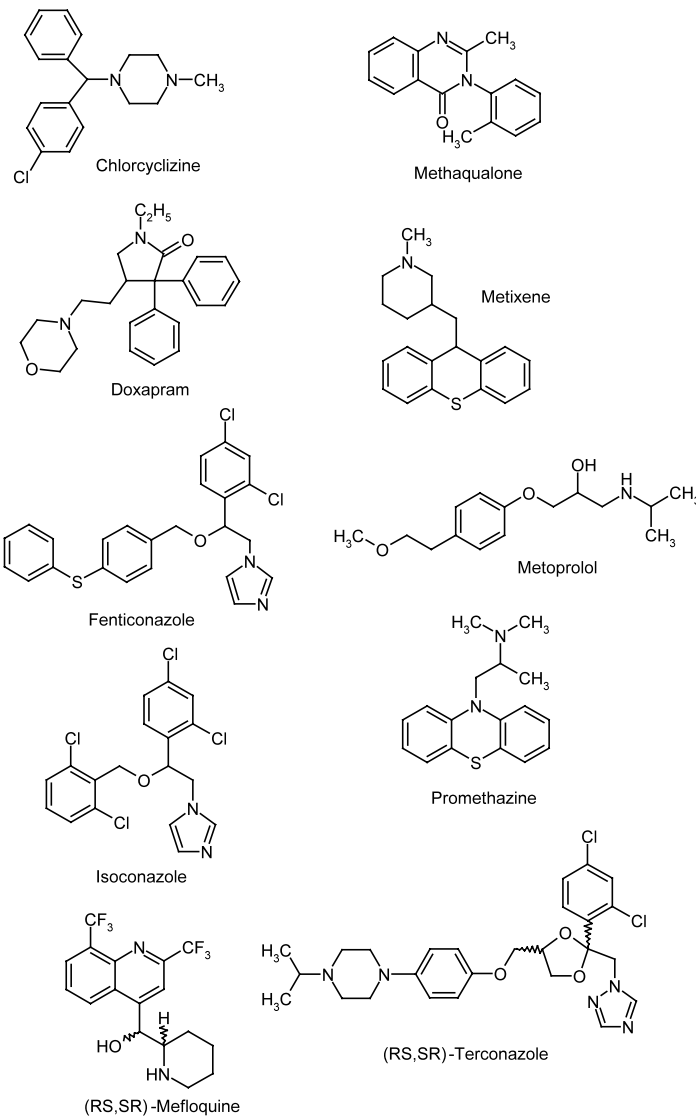

Fig. 1. Structures of the compounds studied.

doxapram, elution was performed with ethyl acetate: dichloromethane:diethylamine (50:50:0.5). Mefloquine and mexitene were eluted with ethyl acetate:2-propanol:diethylamine (50:50:0.5) and metoprolol with ethyl acetate:methanol:diethylamine (25:75:0.5). For promethazine, ethyl acetate:dichloromethane:diethylamine (30:70:0.5) was used.

5. Results and discussion

The analytical separation systems used are presented in Table 1 together with retention data and resolution factors. Four different columns, of which our experience of such separations is good, were used. The preparative separations are documented in Table 2. Some typical chromatograms

are given in Fig. 2, which illustrate the analytical and repetitive preparative resolution of terconazole.

The large α-value of 2.76 makes it possible to resolve 50 mg of the racemate from a single injection. By the repetitive mode shown in Fig. 2, in which the next sample is injected prior to the elution of the second enantiomer, approximately 100 mg of each enantiomer with an e.e. $>96.5\%$ can be isolated in 150 min. In the preparative run, the solvent peak is seen superimposed on the peak from the last eluted enantiomer.

Table 2
Some data from the resolution of 10 racemic compounds by preparative chiral chromatography

Compound	Column	Flow, concentration, amount injected	Amount recovered/mg, E1/E2	e.e., E1/E2
Chlorcyclizine HCl[a]	Chiralpak AD	8 ml/min, 10 mg/ml, 5 mg	37/54	97.5/98.4
Doxapram HCl[a]	Chiralcel OJ	10 ml/min, 20 mg/ml, 6 mg	108/78	97.3/95.3
Fenticonazole nitrate	Chiralpak AS	10 ml/min, 10 mg/ml, 5 mg	67/68	99/97
Isoconazole	Chiralcel OJ	10 ml/min, 25 mg/ml, 37.5 mg	149/155	97.9/99
Mefloquine HCl[a]	Chiralpak AD	10 ml/min, 20 mg/ml, 24 mg	117/107	98.2/97.7
Methaqualone	Chiralpak AS	10 ml/min, 40 mg/ml, 10 mg	116/112	>99/>99
Metixene HCl[a]	Chiralcel OJ	15 ml/min, 24 mg/ml, 29 mg	112/115	99/95
Metoprolol tartrate	Chiralcel OD	10 ml/min, 10 mg/ml, 10 mg	75/82	97.3/98.4
Promethazine HCl[a]	Chiralcel OJ	15 ml/min, 20 mg/ml, 36 mg	93/98	>99/>99
Terconazole	Chiralpak AD	15 ml/min, 10 mg/ml, 50 mg	158/177	99.2/96.5

[a] These compounds were purified by flash chromatography.
For mobile phase composition, see Table 1; e.e., enantiomeric excess.

Table 3
Determination of the optical rotation for some racemic compounds and the corresponding enantiomer

	EP requirements on racemate			First eluted enantiomer concentration 10 mg/ml	
	Angle of rotation (EP requirement)	Solvent	Concentration (mg/ml)	Angle of rotation (measured)	Solvent
Chlorcyclizine HCl				−0.12	MeOH
Doxapram HCl	0.1	Water	50	1.0	Water
Fenticonazole nitrate	0.1	MeOH	10	0.62	MeOH
Isoconazole	0.1	CH_3Cl_2	10	0.3[a]	MeOH
Mefloquine HCl	0.2	MeOH	50	−0.35	MeOH
Methaqualone[b]				1.12	MeOH
Metixene HCl				−0.01	MeOH
Metoprolol					
Tartrate	+7–10	Water	20	0.10	Water
Succinate	0.1	Water			
Prometazine HCl				0.001	MeOH
Terconazole	0.1	CH_3Cl_2	20	0.15	CH_3Cl_2

[a] Concentration, 5 mg/ml.
[b] Atropisomerism.

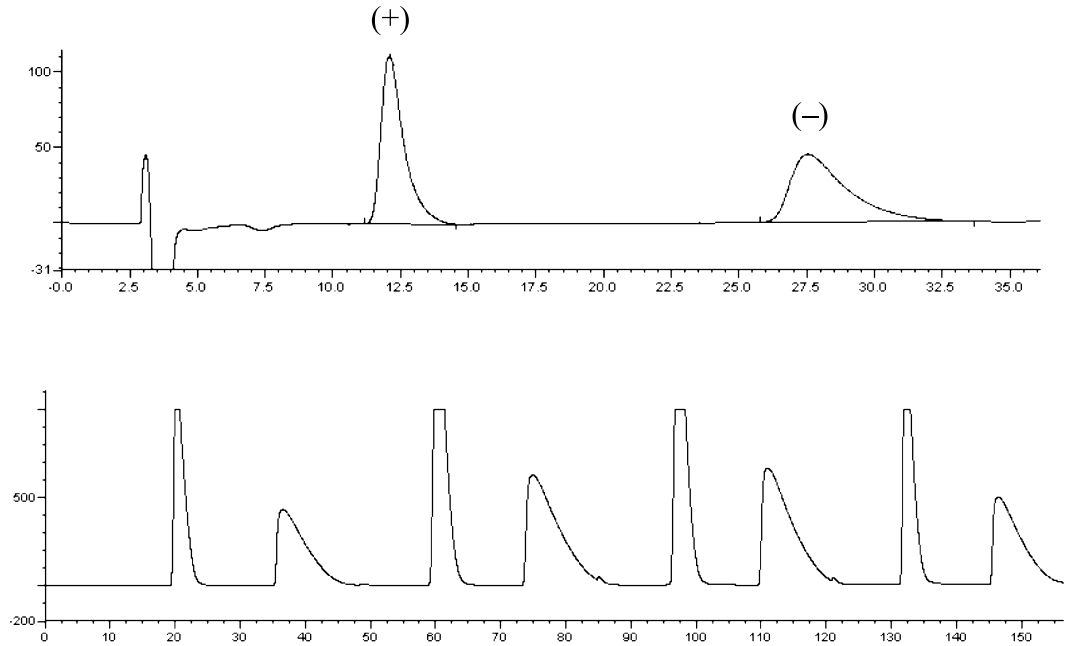

Fig. 2. Analytical and preparative chromatography of terconazole.

The values of the AoR obtained for the first eluted enantiomers are given in Table 3. A general conclusion that can be drawn from this study is that the values are usually quite low. Only in two cases are the values equal to or above 1°. The highest figure was obtained for methaqualone, which exhibits atropisomerism, i.e. there exists no stereogenic centre but a barrier to rotation. There is no requirement in EP 97 for that compound.

Out of the ten substances investigated, four do not have a test for AoR in EP 97. Methaqualone is one of them. The other three are the hydrochlorides of chlorcyclizine, metixene and promethazine, respectively, and they show, indeed, very low values, why it is relevant that there is no test prescribed.

The six substances that have requirements, exhibit AoR for 10 mg/ml that ranges from 0.15° for terconazole to 0.62° for fenticonazole. Isoconazole gave 0.31° for 5 mg/ml and thus, a 10 mg/ml solution would give 0.62°. In Table 4, the AoR is given together with $[\alpha]_D^{20}$ for the five substances showing the lowest AoR. Of these compounds, metixene and promethazine are clearly not differentiated by this test, whereas chlorcyclizine and metoprolol, under the conditions chosen, are at the borderline, i.e. have an AoR slightly higher than 0.10°.

From this presentation, it could be argued that an increase in sample concentration could give rise to a better distinction. However, in many cases that would not suffice, like in the situation for promethazine and metixene.

For two substances, the AoR at different e.e. was measured. The results are given in Table 5. A sample of chlorcyclizine with an e.e. of 40% or even

Table 4
Angle of rotation and specific optical rotation at 589 nm for low responders

Compound	Angle of rotation		$[\alpha]_D^{20}$	
	E1	E2	E1	E2
Chlorcyclizine	−0.120	+0.121	−12.0	+12.1
Metixene	−0.011	+0.013	−1.1	+1.3
Metoprolol	+0.107	−0.097	+10.7	−9.4
Promethazine	+0.001	−0.004	+0.1	−0.4
Terconazole	+0.150	−0.145	+15.0	−14.5

Table 5
Angle of rotation of mixtures with different enantiomeric excess (e.e.)

e.e. %	Angle of rotation	$[\alpha]_D^{20}$
Chlorcyclizine (10 mg/ml in methanol)		
99	−0.120	−12.0
80	−0.092	−9.2
40	−0.048	−4.8
0	−0.001	−0.1
Terconazole (10 mg/ml in dichloromethane)		
96.5	−0.145	−14.5
77	−0.111	−11.1
38	−0.054	−5.4
1.3	+0.003	+0.3

80% would by polarimetry be classified as a racemate with the stipulated requirements. An e.e. of 80% for e.g. (−)-enantiomer, means that there is 90% of this enantiomer and 10% of (+)-chlor-cyclizine. For terconazole, the borderline seems to be around 75% e.e. with an AoR of 0.111° at 77% e.e.

From the results given above, it can be seen that in about half of the measurements undertaken, it would not have been possible to distinguish between the individual enantiomers and the corresponding racemate. Before introducing such a test into a pharmacopoieal monograph, one should first have access to the enantiomers in order to establish conditions for a meaningful measurement.

On the other hand, the CD spectra show a difference between the various mixtures in a very distinct way. The CD measurements are also less demanding with respect to the amount of substance needed. As shown in Fig. 3 for the compounds chlorcyclizine and terconazole, CD absorption bands are readily recognisable even at very low e.e. values.

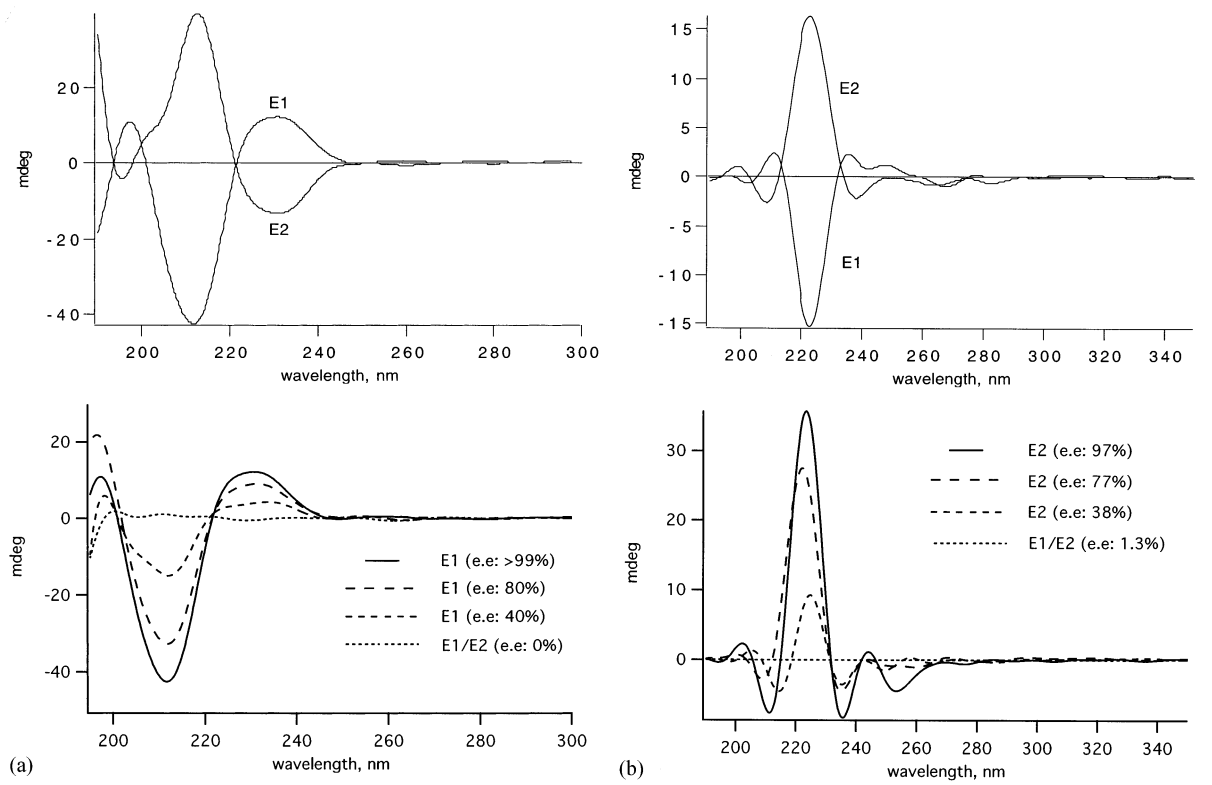

Fig. 3. The influence of enantiomeric excess on circular dicroic (CD) absorption bands, (a) CD spectra of chlorcyclizine, the isolated enantiomers (e.e. ≥98%) (above), at decreasing enantiomeric excess (below), (b) CD spectra of terconazole: the isolated enantiomers (above), at decreasing enantiomeric excess (below).

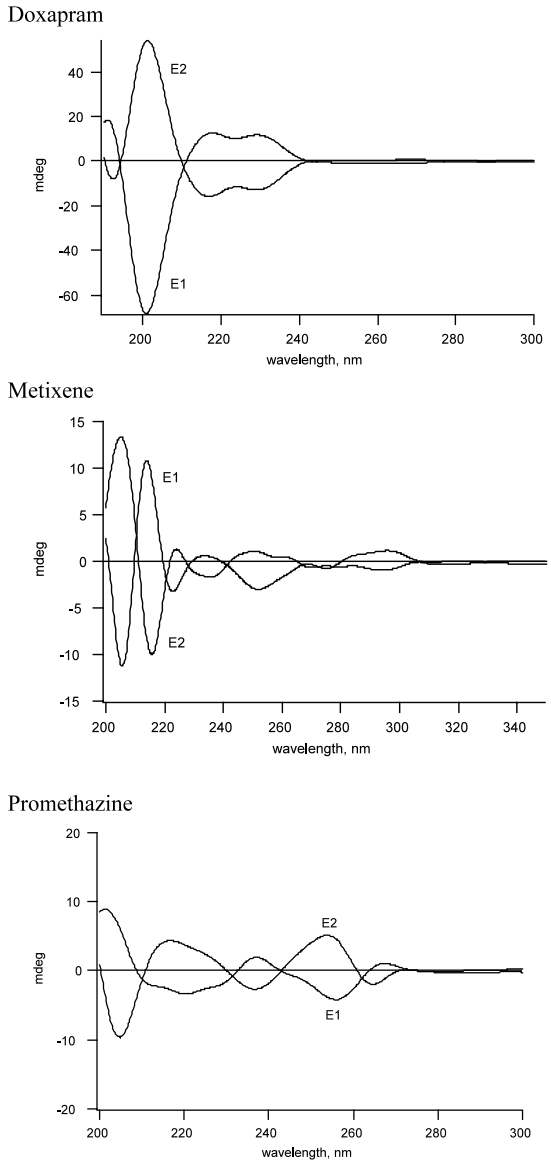

Fig. 4. CD spectra of some of the compounds studied.

Fig. 4 gives some further examples of CD spectra obtained for the compounds investigated. Metoprolol was resolved as the tartrate, but the measurements of the AoR were performed on the enantiomers of the free base. The CD spectra in Fig. 5 show that the tartrate of the metoprolol enantiomer gives a strong negative absorption band at 210 nm. Since this band is absent in the CD spectrum of the corresponding succinate, it must be caused by the optically active tartrate ion. The CD spectrum of metoprolol as the free base shows a weak negative band at 210 nm, indicating that the metoprolol has not been completely purified.

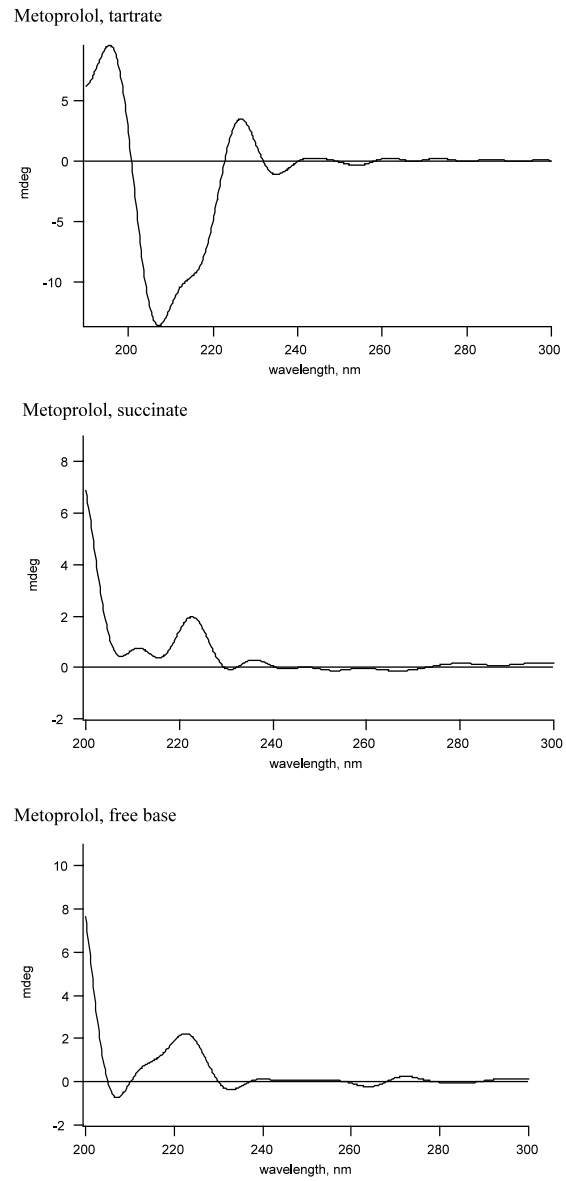

Fig. 5. CD spectra of the (+)-(R)-enantiomer of metoprolol as a salt (tartrate and succinate) and as a free base.

6. Other approaches to the analysis of chiral compounds

Due to rather weak discrimination power of AoR by polarimetry, it could be of value to consider other methods of analysis. CD spectroscopy [4,5] has been mentioned above, but at present the technique is not so widely used in the pharmaceutical control laboratories. Chiral chromatography [6] is, however, becoming more common in the pharmaceutical laboratories today and could be a real alternative. An older approach is the formation of diasteromeric derivatives, by which the racemate reacts with a chiral reagent [7,8] and is applicable in those cases where reactions are feasible.

7. Conclusions

This study has shown how to get information on the chiroptical properties of enantiomers obtained, e.g. by preparative chiral chromatography. It has also been demonstrated that polarimetry is not very sensitive to mixtures that contain the enantiomers with e.e. from 40 to 80%. This means that the use of AoR as an identification or purity test is not always particularly relevant. It has also been clearly shown that CD spectroscopy can more accurately determine the optical purity of non-racemic mixtures of a number of structurally different compounds due to higher sensitivity. CD spectroscopy or chiral chromatography should, therefore, be the technique of choice in the determination of optical purity of a chiral compound, especially for those exhibiting low AoR.

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