

## 6.4. Polarimetry, ORD and CD Spectroscopy

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### 6.4.1. Introduction

The pharmacopoeias generally apply the determination of the specific optical rotation for the characterisation of the optical purity of drugs administered as the pure enantiomer. The measurements are prescribed at D-line of sodium (589.3 nm) or rarely at some wavelengths in the visible region of the mercury lamp (365, 405, 436, 546, 578 nm) and the value of the specific optical rotation should fall into a range prescribed in the individual monographs. At the shorter wavelengths the values are higher and hence the sensitivity of the measurement can be considerably increased. Generally speaking, however, the sensitivity of the simple polarimetric measurement is low for the estimation of the nonwanted enantiomer as compared with the chromatographic, electrophoretic and NMR methods described in Sections 6.2, 6.3 and 6.5 and due to the possible contribution of other chiral impurities to the measured value the selectivity is also poor. This means that the measurement of the specific optical rotation gives only a rough estimate of the enantiomeric composition.

Of the modern chiroptical techniques the measurement of *optical rotatory dispersion (ORD)* and *circular dichroism (CD)* presents much more sensitive, selective and accurate methods for the determination of the optical purity. This is usually expressed as the enantiomeric excess (*ee*) or enantiomeric purity (EP):  $ee(\%) = 100(x^* - x)/(x^* + x)$ , where  $x^*$  is the mole fraction of the enantiomer in excess. The optical purity (OP) defined as the ratio of the actual specific rotation to the specific rotation of an enantiomerically pure sample is practically the same value. (There is a considerable difference between the terminologies used here and in chiral chromatography and electrophoresis. The *ee* value for the mixture of 99% of *R* and 1% of *S* enantiomer is 98%, i.e. 98% enantiomeric excess + 2% racemate, while in the case of chiral chromatography and electrophoresis the ratio of the areas of the separated peaks of *R* and *S* is 99:1.)

The above mentioned chiroptical methods applied for the estimation of enantiomeric purity of drugs can be divided into two groups:

- From the measurements at the optimal wavelength of the ORD or CD curves of the enantiomers or their derivatives the enantiomeric purity can be calculated (Section 6.4.2).

- ORD and CD spectrometers are also applied as liquid chromatographic (HPLC) detectors (Section 6.4.3).

#### 6.4.2. Determination of Enantiomeric Purity by the Direct Application of ORD and CD Spectroscopy

The optical purity of  $\alpha$ -amino acids was determined by ORD spectroscopy [1]. L-Acids show a positive whilst the D-isomers a negative Cotton effect in the range of 220–230 nm. The method is 100–700 times more sensitive than polarimetric measurements, at the D-line of sodium.

Erskine *et al.* [2] elaborated a rapid method for the determination of optical purity (OP) using CD and UV spectroscopy. The concentration and OP of *ephedrine* and *mandelic acid* enantiomers and *ephedrine* and *pseudo-ephedrine* enantiomeric mixtures was determined. In both cases the CD and UV spectra overlap each other in the estimated 256–280 nm region. The data were collected at 0.1 nm intervals and the results were calculated by the partial least-squares method. CD is able to provide information on the amount of excess concentration of one of the enantiomers over that of the other, while from the UV data the total concentration of the two species can be calculated. With the combination of the two methods with a multivariate analysis method such as partial least-squares the concentration of each of a pair of enantiomers was accurately determined in mixtures without the need for chiral separation.

The enantiomeric composition of *phenylglycine* and *mandelic acid* was successfully determined by the application of the anisotropy factor derivable from the ratio of the ellipticity and the absorbance simultaneously measured by CD and UV spectroscopy, respectively [3]. This is an intensive, concentration-independent physico-chemical parameter, which eliminates concentration-related errors caused by the sample preparation, dilution, etc. For the two enantiomers, it has the same numerical value with opposite signs, and is therefore a function of the enantiomeric composition. The procedure based on the determination of the anisotropy factor is rapid, accurate and eminently suitable for routine quality control purposes enabling the determination to be carried out with an accuracy of 0.1%.

A rapid and simple direct spectropolarimetric method was described by Palma *et al.* [4] for the determination of enantiomeric purity of pharmaceutical grade *L-cocaine hydrochloride* and *L-codeine* in tablets by applying a modified Biot–Lowry procedure. Codeine samples were separated from optically active sugars by extraction with chloroform at pH 8–9.

Derivatisation of the analyte has often been used to increase the sensitivity of the determination of enantiomeric purity. For example, the specific rotation

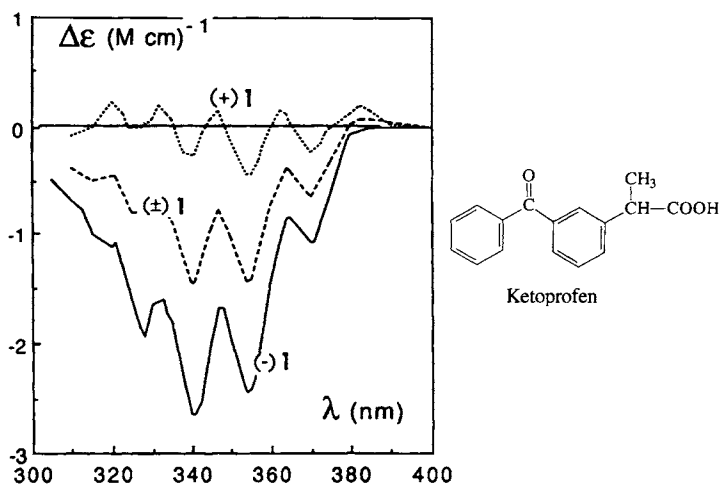
$[\alpha]_D^{25}$  of levodopa is only about  $-12^\circ$ , which is insufficient to control its optical purity. However, it can be cyclised with formaldehyde, resulting in a more than 10-fold increase of the specific rotation [5].

Complex formation can also be used as an aid to improve the sensitivity of the determination of OP of compounds; for instance, in the case of *ethambutol* complexation with copper(II) was used for this purpose [6]. Optically active *amino acids* when dissolved in an alkaline solution of the potassium salt of the optically active Co(III)-*N,N'*-ethylenebis(acetylacetonimine)-glycine complex show multiple Cotton effect curves in the visible region. The OP may then be determined by measurements at 475 nm (CD) and 500 nm (ORD), respectively [7,8].

A method has been elaborated for the determination of enantiomeric purity of *amphetamine* samples [9]. The procedure is based on the derivatisation of amphetamine with 2,4-dinitrofluorobenzene and subsequent measurement of the anisotropy factor by dual (CD/UV) spectroscopy. The advantage of the derivatisation is that due to the bathochromic shift, more selective and sensitive analysis can be performed than it would be possible in the underivatized form. Both ellipticity and absorbance were in linear relation to the concentration in the range of  $5 \times 10^{-6}$ – $6 \times 10^{-4}$  mol/l. By investigating binary mixtures of the amphetamine enantiomers containing 63.3–98.8% of (+)-amphetamine the accuracy of the determination of the enantiomeric purity was found to be 0.2–0.3%.

Purdie *et al.* [10] developed a method for the determination of the ratio of the concentration of enantiomers in binary mixtures of *pseudoephedrine* and *ephedrine* using chiral Cu(II)-tartrate complexes as derivatising agents. The reaction is a simple ligand exchange between tartrate and the chiral drug resulting in the shift of the wavelength of CD detection to the visible range. The change of the CD spectrum is proportional to the concentration of the D-pseudoephedrine and at 527 nm the L-pseudoephedrine complexes have an isobestic point. At this wavelength the D-pseudoephedrine content can be determined. The effects are entirely opposite if L-tartrate is changed to D-tartrate in which case the D-pseudoephedrine complexes will produce the isobestic point and the L-pseudoephedrine content can be determined. The detection limit for the enantiomeric impurity was in the order of 2%.

Complexation of *ketoprofen* to bovine serum albumin (BSA) results in an intensive negative Cotton effect in the  $n-\pi^*$  band of the benzoylphenyl moiety [11] as illustrated in Fig. 6.4.A. It is remarkable, that the CD intensity of the enantiomers bound to BSA is much higher in absolute terms than in the unbound form. The amplification by two orders of magnitude of the CD difference between the antipodes, due to the complexation with the protein can be used to measure the OP of samples with very high accuracy. The values compare well with those obtained with chiral HPLC.



**Figure 6.4.A.**

CD spectra of (+)-ketoprofen with BSA [(+)1], (±) ketoprofen with BSA [(±)-1], and (-)-ketoprofen with BSA [(-)1]. Spectra are obtained after subtraction of BSA contribution. [BSA] =  $1.3 \times 10^{-4}$  M, [ketoprofen] =  $0.65 \times 10^{-4}$  M (from Ref. [11])

### 6.4.3. The Use of Chiroptical HPLC Detectors for the Analysis of Enantiomeric Purity

Detectors based on optical activity, i.e. optical rotation and circular dichroism have advantageous features in the HPLC analysis of enantiomers [12–14]. One major advantage is their inherent selectivity. Compounds that do not possess optical activity do not interfere even if they co-elute with the analytes. This is particularly useful in the analysis of physiological fluids.

The sensitivity of these detectors is lower than that of the generally used UV detectors, but applying laser light or/and fluorescence detection the sensitivity may be enhanced [15–18]. The most effective way of the application of polarimetric or CD detectors in high-performance liquid chromatography is to use them in series with the conventional UV-VIS and RI HPLC detectors [17,19–27].

Using this experimental set-up the chromatographic separation of the enantiomers is not by all means necessary, because the UV-VIS or RI detector responds to the total amount of the analyte and the response of the chiral detector depends on the actual quantitative ratio of the enantiomers.

*L-Epinephrine* can be specifically analysed in ophthalmic formulation by HPLC using a UV detector in series before a polarimetric detector [19]. This is important as *D*-epinephrine is inactive in the treatment of glaucoma. The concentrations of both enantiomers in an unknown mixture can be calculated from the detector signals and from the ratio of the two signals measured for an *L*-epinephrine standard. The enantiomeric purity of *tartaric* and *malic acids* was determined similarly. The sensitivity of detection can be enhanced by complexation with molybdate [26].

Yeung and Reitsma [20] were the first to demonstrate enantiomeric purity determination of some *amino acids* by UV and RI detection coupled with the above mentioned sensitive laser polarimetric detector [20]. A near-infrared (820 nm) semiconductor diode-laser-based OR detector was coupled with a UV detector and the enantiomeric ratio of *D*- and *L-tryptophan* mixtures was determined [21]. The sensitivity of this technique may be increased by pre-column achiral derivatisation of amino acids with dansyl chloride to increase their specific rotation [22].

The separation of the *SS* and *RR* enantiomers of the racemate drug *Tramadol hydrochloride* as well as its diastereomeric *SR* and *RS* impurities would require a chiral chromatographic method. As another alternative a reversed-phase achiral separation of the diastereomers has been developed [23]. Using argon ion laser-based polarimetric detector in series with a UV detector, the quantitation of the enantiomers without separation has been achieved. The polarimetric detection was accomplished at 488 nm, while the UV detection was at 270 nm. The detection limit was 0.4  $\mu\text{g}$  for the *SS* isomer, and the enantiomeric purity can be determined down to the 0.4% level. Wu *et al.* [24] have used reversed-phase HPLC with dual polarimetric-UV detection to determine the enantiomeric purity of *ephedrine* and *pseudoephedrine*.

Another option for chiroptical HPLC detection is based on circular dichroism (CD) [14,28–31]. CD is an even more selective detection technique than the polarimetric detection, because the CD detectors give a signal only in the region of optically active absorption band.

A relatively simple but very useful information obtainable with the aid of chiroptical detectors is the estimation of the *elution order* of the enantiomers after chiral separation. For example, with the aid of CD and UV detectors connected in series it was found that in the course of the separation of amino acid enantiomers on a Crown Pak CR(–) chiral column *L*-amino acids were eluted first and detected as positive peaks and all *D*-amino acids were registered as negative peaks [32].

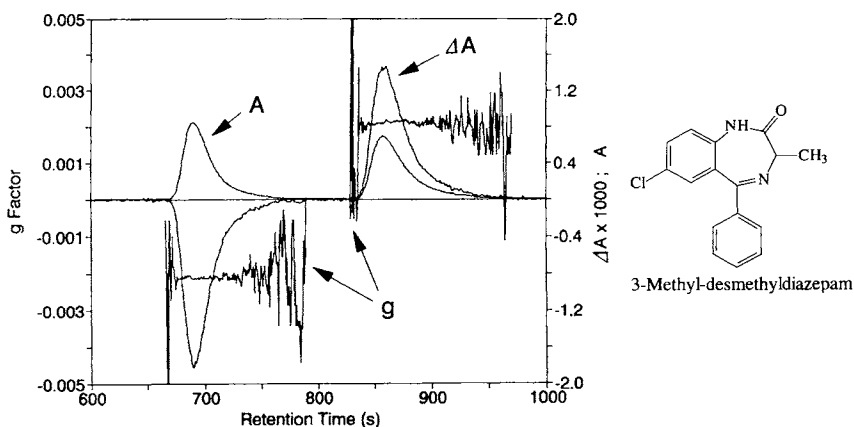
In addition to the above described simple problems, CD/UV based HPLC detection systems enable also enantiomeric ratios of unresolved chromatographic peaks to be determined. A method of this type was developed by Drake and coworkers [28] for the optical resolution of *pavine* (obtained by

synthesis from papaverine). The determination of the enantiomeric ratio of *ascorbic*, *lactic* and other *chiral carboxylic acids* based on the same experimental set-up is also described [33].

The CD/UV based HPLC detection systems are capable of recording the enantiomeric composition of the eluates directly during the chromatographic run, on the basis of the dissymmetry ratio or *g*-factor. The absolute value of the dissymmetry ratio is identical at a given wavelength for the two pure enantiomers with different signs and with a maximum value for the optically pure enantiomer. It is independent of the analyte concentration and is linearly related to the enantiomeric purity. The accuracy of the measurement depends on the dissymmetry ratio of the single enantiomer.

As an example the determination of the enantiomeric composition of 3-methyl-desmethyldiazepam during the chiral chromatographic separation is demonstrated in Fig. 6.4.B [34]. The chromatographic run, the aim of which was the preparative separation of the enantiomers was monitored using simultaneous detection by a CD detector (Jasco, J-600 dichrograph equipped with a 8  $\mu$ l HPLC cell), and a UV detector operating at 254 nm. The time when baseline resolution is obtained and where the fractions should be collected in preparative HPLC is obvious. This method was used for monitoring the chiral separation of other benzodiazepines as well [35,36].

The HPLC/CD system can be used on line to measure the CD spectrum of enantiomers in eluates from a chiral column by the stopped flow method. Thus, in addition to the estimation of the elution order of the enantiomeric eluates this



**Figure 6.4.B.**

Chromatographic resolution of 3-methyl-desmethyl-diazepam on a Pirkle-type column. Mobile phase: hexane/2-propanol 90:10, v/v; flow rate 1 ml/min. UV (A), CD ( $\Delta A$ ), and *g* ( $\Delta A/A$ ) detection at 254 nm (from Ref. [35])

method can be employed also to the determination of the absolute configuration of enantiomers by applying various empirical and semiempirical rules or by nonempirical methods such as the exciton model or the DeVoe approach [37–41].

#### 6.4.4. Conclusions

The conclusion which can be drawn from the examples presented in the first part of this section is that the direct chiroptical methods as well the HPLC methods based on chiral detection are useful tools for the determination of the enantiomeric purity of drugs. It is characteristic of both techniques that they do not measure directly the enantiomeric impurity but from the measured signal size or from the ratio of chiral and achiral signals the enantiomeric purity can be calculated.

The exact knowledge of the anisotropy factor of the single enantiomers plays an important role in this field; it can be stated that by applying the methods based on this principle the enantiomeric purity can be determined with 0.1–2% accuracy.

The primary aim of coupling HPLC/UV separation/detection with ORD or CD detection (including the possibility of taking the CD spectrum of the separated peaks) is the identification or structure elucidation of the separated materials among them diastereomers and enantiomers from the simple estimation of the elution order of enantiomers to the determination of absolute configurations [42]. (A complex study of this type where HPLC/CD was used along with HPLC/NMR is described in Section 7.5.6.) Another application field is the determination of enantiomeric impurities. On the basis of the experience from the author's laboratory under favourable conditions 0.2–0.5% enantiomeric impurity can be determined by this methods [43].

On the basis of recent tendencies it is predictable that in the near future chiroptical detection will have an increasing role in the determination of enantiomeric impurities in drugs. This is supported by the recent availability of a HPLC/CD/UV detector which enables good-quality CD-chromatograms to be taken after the injection of as low a sample size as 20–100 ng.

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