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Determination of the enantiomeric purity by means of NMR spectroscopy and capillary electrophoresis using a *heptakis*(2,3-di-*O*-acetyl)-β-cyclodextrin: a comparison

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Cyclodextrin modified capillary electrophoresis (CE) and NMR spectroscopy were checked for their usability to determine the enantiomeric purity of some representative phenethylamine drugs. Even though no validation procedure was applied, both methods produced accurate and reproducible results. The comparison of the CE and NMR methods revealed the NMR spectroscopy to be superior in terms of rapidity of the method optimization and measuring time.

1. Introduction

The different pharmacodynamic, pharmacokinetic and metabolic properties of the enantiomers of a racemic drug can result in, at worst, toxic adverse effects and, at least, unnecessary administration of inactive compounds. For a couple of years, international authorities have been demanding of the pharmaceutical industry to subject single enantiomers of racemic drugs to clinical trials. The number of single enantiomers introduced to the market was reported to increase during the last two years [1]. Therefore, methods for the determination of the enantiomeric purity, i.e. the enantiomeric excess (ee [%] = $\{(R-S)/(R+S)\} \times 100$), are required. Although stationary chiral phases for HPLC or GC are extremely expensive, the chromatographic methods were a major tool for this purpose in the past [2]. In addition, it is still a challenge to properly resolve enantiomers for the determination of the optical purity. Mainly incomplete peak resolution and poor quality calibration curves [3] are the problems to be considered validating a chromatographic ee determination method. Enantioseparation can also be achieved with capillary electrophoresis (CE) using a chiral additive to the buffer. Since the CE methods have the advantage of requiring smaller amounts of chiral additives, and having higher number of theoretical plates, greater ruggedness, and shorter running time in comparison to HPLC methods, CE is increasingly used to determine the optical purity of drugs [4–10].

Apart from the aforementioned separation techniques, NMR spectroscopy is a powerful tool in this field. Using chiral derivatisation reagents (CDA), chiral solvating agents (CSA) or chiral lanthanide shift reagents (CLSR) it has a long-standing tradition in the determination of the ee, especially in the field of the asymmetric synthesis of organic compounds [11], and, thus, can be used for chiral drugs [12]. As shown e.g. for β-receptor blockers, cyclodextrins (CDs) were found to be an optimal CSA [13, 14], because CDs are water soluble, do not cause a signal broadening (cf. CLSR) of the drug signals, and have a narrow chemical shift range.

Thus, the purpose of this study was to determine varying ratios of ee of some representative phenethylamine drugs by means of CE and NMR spectroscopy using the same CD. In former studies *heptakis*(2,3-di-*O*-methyl)-β-CD was successfully used in CE [15, 16]. Since the *heptakis*(2,3-di-*O*-acetyl)-β-CD, synthesized in our group, was found to be even more powerful, the measurements will be performed with this single isomer CD derivative [17–19]. Without an extensive validation of the methods used it be will checked whether both methods, NMR and CE, are suitable for assessing the enantiomeric composition of amphetamine, norephedrine, ephedrine, methylephedrine and epinephrine.

2. Investigations, results and discussion

2.1. CE optimization

In order to optimize the resolution of a racemine mixture, the following CE parameters were varied in analogy to a reported procedure [18]: buffer concentration, pH value, CD concentration and the kind of capillary. As shown in previous studies, a buffer concentration of 0.05 M with a pH value of 3.0 or 4.5 containing 12 mM *heptakis*(2,3-di-*O*-acetyl)-β-CD was found to give rather good resolutions. Interestingly, with silica fused capillaries a much higher resolution (Rs) could be achieved than with the coated capillary provided by Biorad [20]. E.g. the Rs value of epinephrine increased from 0.9 to 2.37 and the Rs value of amphetamine from 1.31 to 2.10. In all cases with the silica fused capillary, a resolution was found to be higher than 1.5 and, thus, all peaks were very well baseline separated and the Rs values higher than found with *heptakis*(2,3-di-*O*-methyl)-β-CD [21]. In case the major peak was appearing in front of the minor peak, the peaks of the minor enantiomers did not sit on the tail of the peak of the major peak in the prepared mixtures of 90%, 95% and 97.5% ee. Thus, the migration order was not optimized to minor before major isomer as recommended for HPLC.

The Table shows the expected and observed ee values. Fig. 1 displays the electropherograms of the norephedrine sample solutions with 95 ee and 97.5 ee. The peaks of ephedrine, norephedrine, and epinephrine sample solutions could be integrated with sufficient results. As the main enantiomer of the epinephrine and amphetamine solutions migrated faster than the minor one, less reproducible results were expected. Only amphetamine solutions showed remarkably higher peaks for the second eluted minor enantiomer than presumed. In contrast, electropherograms of the epinephrine solutions showed peak areas close to the theoretical values. These observations do not necessarily contradict each other. The minor enantiomer of amphetamine was eluted more than two minutes later than the main enantiomer. The difference between the major and minor enantiomer of epinephrine was less than one minute. This implies that the results depend on the difference in the migration times of the enantiomers rather than the migration order. This finding is in accordance with the fact that the enantiomer migrating slower needs more time to pass the detection window and, therefore, causes a broader peak of a higher area [22]. Herein, the high reso-

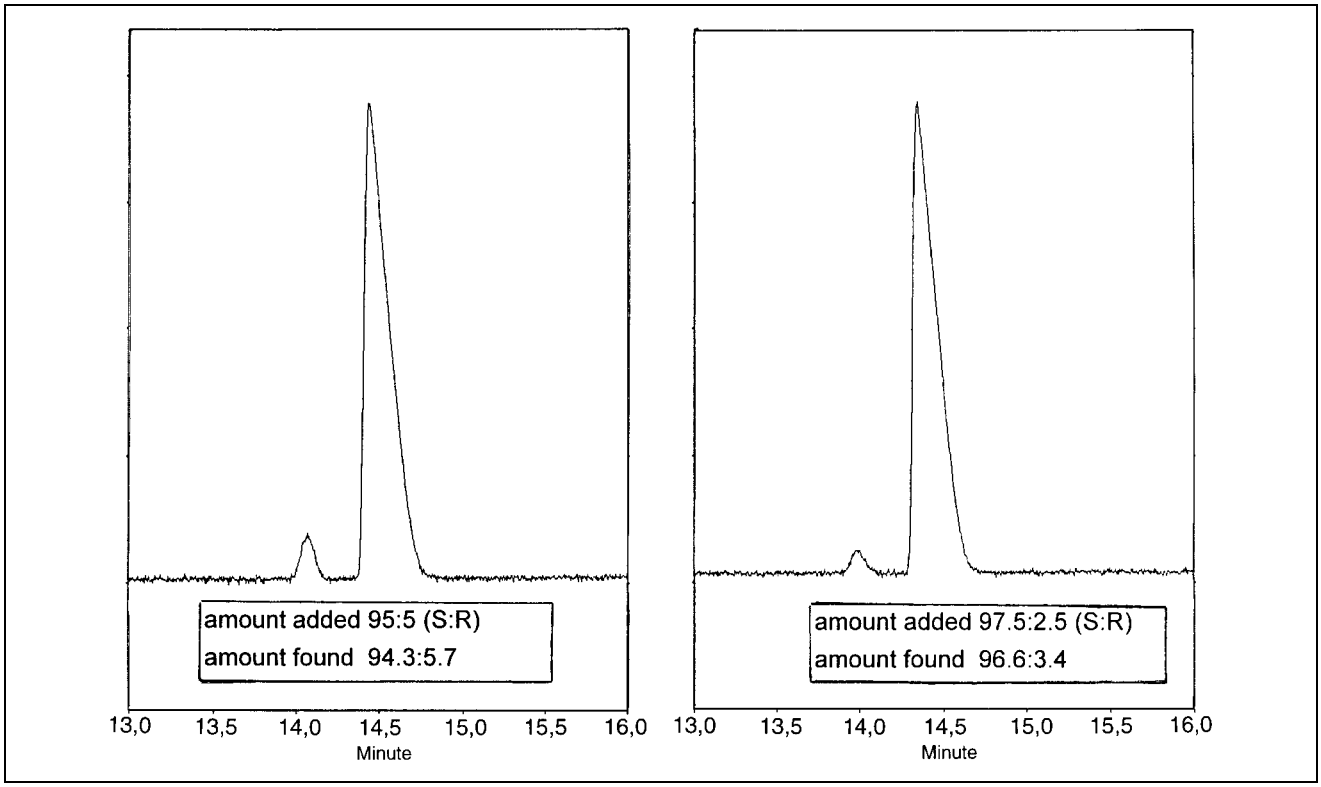

Fig. 1: Electropherogram of norephedrine (ee 95 and ee 97.5) in presence of 12 mM *heptakis*(2,3-di-*O*-acetyl)-β-CD (pH 3.0; 0.05 M potassium phosphate)

lution found for amphetamine turned out to be a disadvantage.

Taken together, with the exception of amphetamine, the differences between expected and found ee values are rather small. Keeping in mind that neither the weighing nor the pipetting for the preparation of the solutions was validated, the results are encouraging with regard to the NMR investigations.

2.2. NMR measurements

Since 1 : 1 complexes between the drug and the CD were assumed from previous measurements [17, 19], equimolar amounts of the analytes and CD were dissolved in D_2O containing 0.1 M potassium phosphate buffer. The spectra for amphetamine and norephedrine were measured on a 500 MHz spectrometer [19], the measurements for ephedrine, methylephedrine and epinephrine were performed on a 300 MHz. In general, the signals of the CD and the drugs were well separated. Most of the analyte signals showed a significant shift, indicating the complexation between the phenethylamines and the CD. A signal splitting could be observed for at least one signal of each compound. In the case of amphetamine and norephedrine the signals of the benzylic hydrogens, and in the case of methylephedrine, ephedrine and epinephrine the signals of the N-methyl groups were used for the determination of the ee. With exception of methylephedrine the signals

Table: Comparison of excepted and observed ee values obtained with CE (average of three measurements) and NMR measurements

Compd.	Amount added		Amount found by means of CE		Amount found by means of NMR	
	ee	area/area (%)	ee	area/area (%)	ee	area/area (%)
Amphetamine	90.0	95 : 5	80.8	90.4 : 9.6	89.4	94.7 : 5.3
	95.0	97.5 : 2.5	87.6	93.8 : 6.2	94.4	97.2 : 2.8
	97.5	98.75 : 1.25	90.6	95.3 : 4.7	97.6	98.8 : 1.2
Epinephrine	90.0	95 : 5	88.4	94.2 : 5.8	90.0	95.1 : 4.9
	95.0	97.5 : 2.5	95.0	97.5 : 2.5	94.2	97.1 : 2.9
	97.5	98.75 : 1.25	97.6	98.8 : 1.2	96.8	98.4 : 1.6
Norephedrine	90.0	95 : 5	88.6	94.3 : 5.7	88.8	94.4 : 5.6
	95.0	97.5 : 2.5	93.2	96.6 : 3.4	95.6	97.8 : 2.2
	97.5	98.75 : 1.25	96.0	98.0 : 2.0	97.8	98.9 : 1.1
Ephedrine	90.0	95 : 5	89.6	94.8 : 5.2	88.4	94.2 : 5.8
	95.0	97.5 : 2.5	94.2	97.1 : 2.9	96.4	98.2 : 1.8
	97.5	98.75 : 1.25	97.4	98.7 : 1.3	95.8	97.9 : 2.1
Methylephedrine	90.0	95 : 5	91.8	95.9 : 4.1	–	–
	95.0	97.5 : 2.5	94.8	97.4 : 2.6	–	–
	97.5	98.75 : 1.25	97.2	98.6 : 1.4	–	–

were fully resolved and allowed the quantitative investigations of the defined mixtures of 90%, 95%, and 97.5% ee by integration of the signals. The results are depicted in the Table.

For a good integration two signals have to be baseline separated. If this is not the case, the integration mode discriminates the broader signals. This can be seen in the case of the epinephrine racemate (see Fig. 2a). On the other hand the integration of a small signal near an intensive one results in too high areas. In the cases, a deconvolution analysis is to be prefered. The natural NMR line form is a Lorenz function. It is possible to create Gaussian lines by a mathematical treatment of the free induction decay (FID). Gaussian lines have a smaller base than Lorentian. In the case of partial interference of two or more lines a deconvolution analysis of these Gaussian lines allows a mathematical area determination of the signals. The natural signals are fitted to the determined signals. The deconvolution technique was applied to the N-methyl signal of ephedrine, methylephedrine and epinephrine. Fig. 2 representatively shows the expansion of the part of the epinephrine spectrum with and without deconvolution.

Taken together, all ee amounts found are in rather good agreement with the theoretical values. Comparing the ee values obtained with the 300 MHz and the 500 MHz instruments, the higher field strength spectrometer is favourable due to a better signal separation and, with that, a more precise integration.

2.3. Comparison of NMR and CE

Although neither the preparation of the ee sample solution nor the methods have been validated, the results show that both techniques are suitable for quantitative analysis. The NMR experiments required similar preparation time of the samples but less efforts for optimization. Therefore, NMR spectroscopy can be advantageous. In addition to the optimization of the separation, quantitative analysis by CE is in need of an optimized migration order, minor before major isomer, and a sufficient but not too big difference in the migration time. In this study, a migration with reverse order led to a remarkable deviation of the expected and observed peak areas. On the other hand, quantitative NMR spectroscopy requires a considerable signal splitting which can be mostly achieved by the CSA, such as CDs, and a high field instrument ($\geq$300 MHz). Due to the high ruggedness, CD modified NMR spectroscopy should be more often taken into consideration in chiral analysis of drugs.

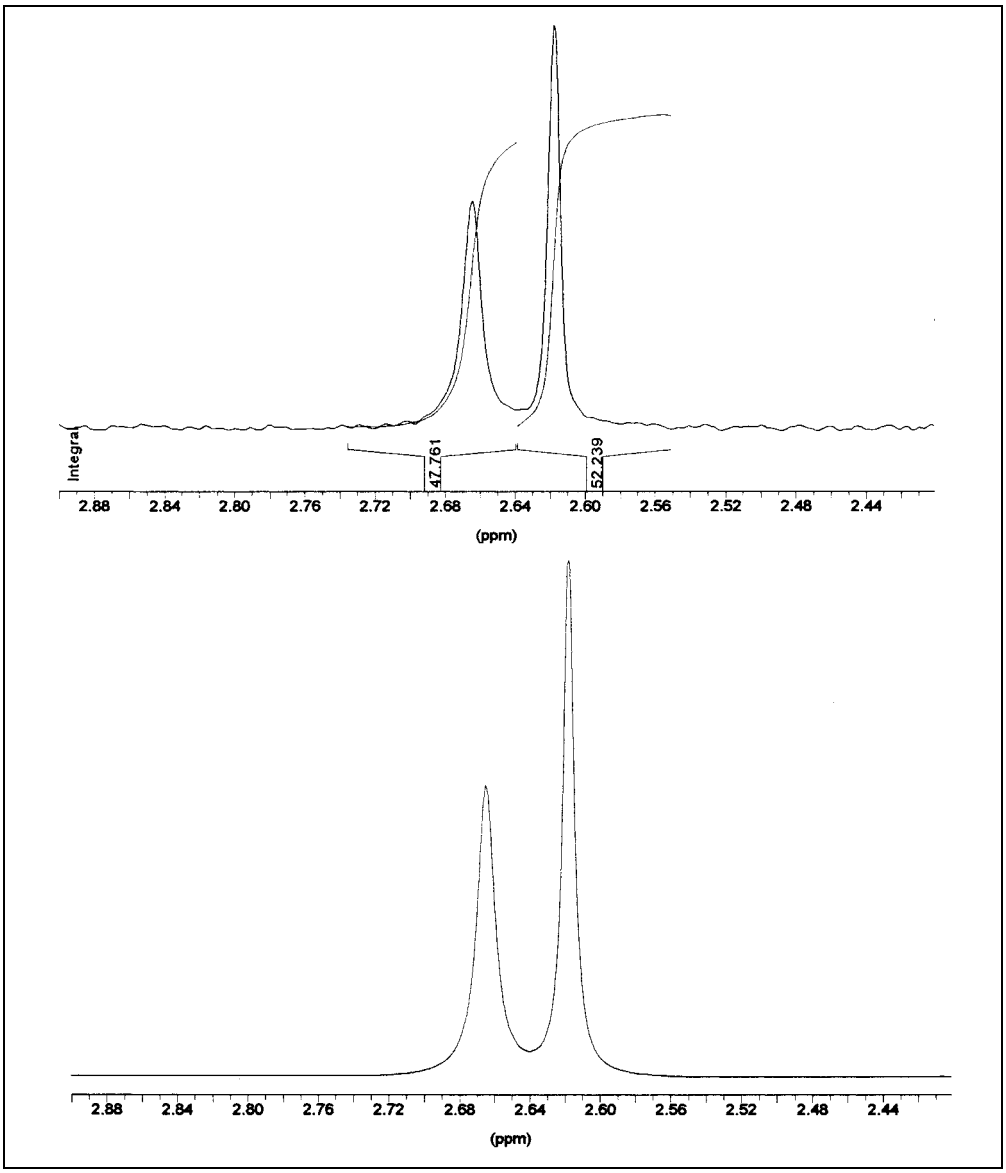

Fig. 2a:
N-Methyl region of the spectrum of the epinephrine racemate in presence 12 mM *heptakis*(2,3-di-*O*-acetyl)-β-CD without (upper) and with (lower) deconvolution. The integration after the deconvolution process yielded a ratio of 47.95 : 52.05

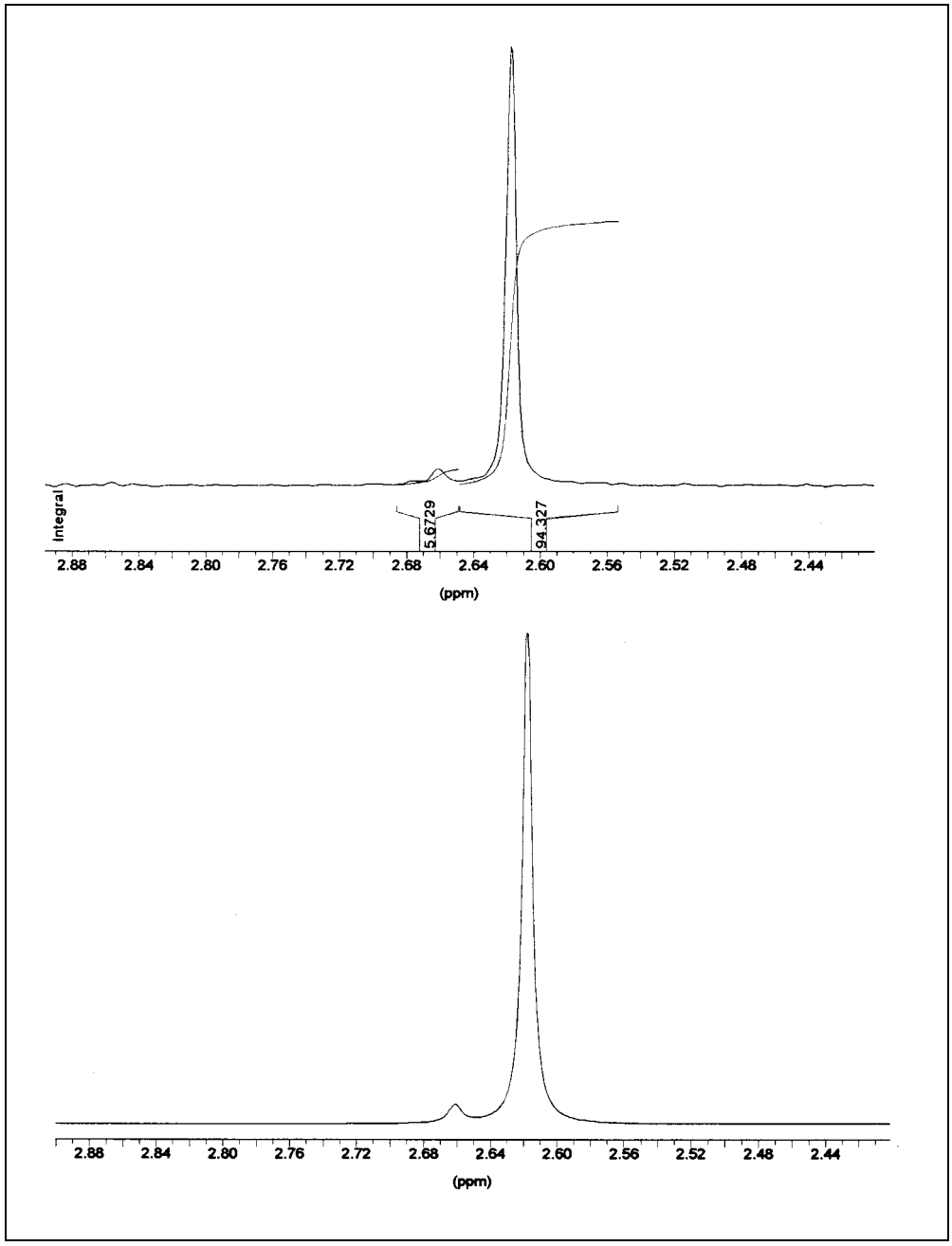

Fig. 2b:
N-Methyl region of the spectrum of epinephrine (ee 90) in presence 12 mM *heptakis*(2,3-di-*O*-acetyl)-β-CD without (upper) and with (lower) deconvolution. The integration after the deconvolution process yielded a ratio of 4.94 : 95.06

3. Experimental

3.1. Chemicals

The isomers of ephedrine and rac. and (−)-epinephrine were purchased from Aldrich (Steinheim, FRG), the enantiomers of norephedrine and methylephedrine from Fluka (Buchs, Switzerland), and the isomers of amphetamine were a gift from Dr. M. Neugebauer, Pharmazeutisches Institut der Universität Bonn. Diac-β-CD was synthesized according to Branch et al. [17].

3.2. Capillary electrophoresis

All experiments were performed on a Beckman P/ACE 5500 system using a fused silica capillary with a total length of 47 cm, a detection length of 40 cm and an internal diameter of 75 μm. Samples were loaded by 5 s of pressure injection and separated at 25 °C using a constant current of 50 μA. The phenethylamine solution had a concentration of 50 μg/ml and was detected using a diode array detector within 190 to 300 nm. In addition, the experiments using a coated capillary were carried out on a BIORAD HPE CE system according to the previous studies [17].
In order to optimize the separation conditions, different KH_2PO_4 buffers we tested between pH 3 and pH 7.5 with buffer concentrations of 0.025 M and 0.05 M. The Diac-β-CD concentration ranged from 0.003 to 0.012 M.

3.3. ^1H NMR spectroscopy

NMR measurements of the amphetamine- and norephedrine-CD-complexes were recorded using a Bruker AMX 500 spectrometer (cf. [19]). Experiments with ephedrine, methylephedrine and epinephrine were performed on a Bruker AC-P 300 FT NMR spectrometer operating at 300.133 MHz with a sample temperature of 30 °C. A varying number of scans (depending on the experiment) with a frequency range of 7246 Hz were collected into 32,000 data points, giving a digital resolution of 0.22 Hz/point. An appropriate Gaussian function was applied before Fourier transformation to enhance the spectral resolution. The solutions were prepared in deuterated 0.1 M phosphate buffer (composed of 0.05 M KH_2PO_4 in deuterated water, equivalent to pH 4.5) in order to measure the induced chemical shifts of the CD and phenethylamine signals. All chemical shifts were referenced to the D_2O signal at 4.650 ppm.

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