Mar. Üniv. Ecz. Der.,
11 (1 - 2), 89 - 94 (1995)

J. Pharm. Univ. Mar.,
11 (1 - 2), 89 - 94 (1995)

NEW ASPECTS OF ENANTIOSELECTIVE METABOLISM OF DRUGS

G. BLASCHKE*

Stereoisomerism is an important phenomenon of numerous drugs and therefore important in the field of Pharmacy. More than one third of all synthetic drugs are chiral due to one or more chiral centres in the molecule.

The stereoisomers of chiral drugs although closely related to each other and differing chemically only in the sign of optical rotation exhibit in most cases dramatic differences in their pharmacological and toxicological properties. These differences are due to stereoselective binding to complementary receptors and/or stereoselective biotransformations.

Chromatography on optically active stationary phases proved to be very useful for the separation of chiral drugs. Numerous chiral stationary phases (CSP) for gas chromatography and HPLC have been developed. More than 100 columns with different CSP's are now commercially available, the number is still growing very rapidly. However these columns are much more expensive than the usual achiral columns.

A new aspect of the investigation of enantioselective metabolism of drugs is the use of capillary electrophoresis, a new and very powerful separation method. Also by capillary electrophoresis enantiomers can be separated simply by using small amounts of inexpensive chiral additives like cyclodextrins.

* Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58–62, D-48149 Münster, GERMANY.

In our investigations [M. Heuermann and G. Blaschke, J. Chromatogr. 648, 268-274, 1993] a number of structurally different racemic basic drugs were resolved by ß-cyclodextrin (ß-CD)-containing run buffers. Also mixtures of chiral drugs with different chemical structures can be separated and, additionally, resolved into the enantiomers in a single run, as shown in fig. 1. Table I summarizes these data listing only those CDs (ME-, HE- and HP-ß-CD: methyl-, hydroxyethyl- and hydroxypropyl-ß-cyclodextrin) which resulted in the best resolution of a given drug under the conditions applied. Some of these drugs could also be separated with other CDs. With α- and γ-CD, none of these drugs could be resolved. Further, the following drugs could not be resolved after the addition of HP-ß-CD to the run buffer: alimemazine, Atropine, bifonazol, butetamate, camylofin, cetirizine, chlorphenoxamine, clofedanol, fenfluramine, ilmofosine, mazindol, mequitazone, metharaminol, orphenadrine, propafenone and terfenadine.

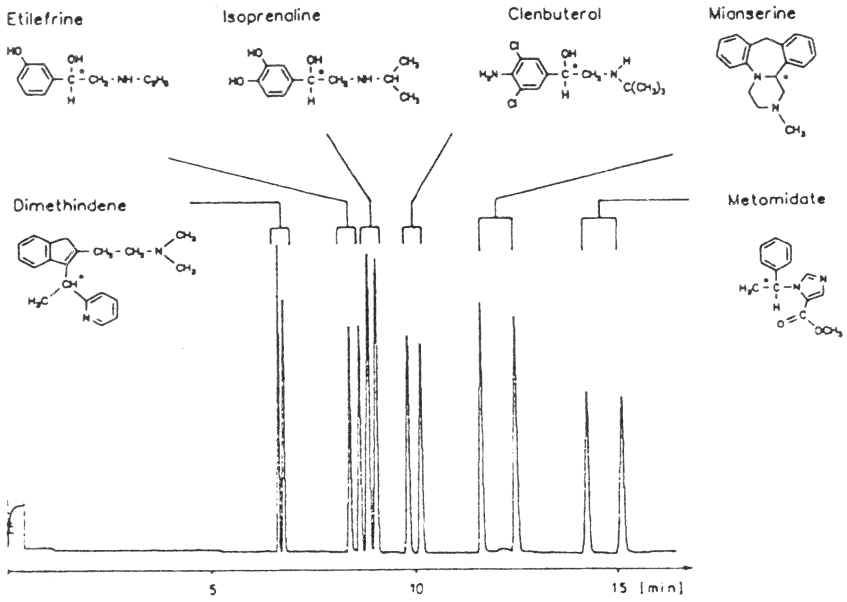

Fig. 1: Simultaneous chemical and enantiomeric resolutions of six racemic drugs

Table I: Resolution of racemic drugs

CD concentrations: α-, β-, γ-CD 16.3 mM, derivatized CD's 30 mM in a 50 mM phosphate buffer pH 3.3.

Drug	CD-Type	t_1	t_2	a	R
Ambucetamide	HP-β-CD	11.29	11.41	1.010	1.04
Carvedilol	β-CD*	8.88	9.05	1.019	1.50
Clenbuterol	HP-β-CD*	9.65	10.03	1.039	4.03
Ephedrine	β-CD*	7.65	7.73	1.011	0.96
Etilefrine	HP-β-CD*	8.35	8.68	1.038	3.82
Imafen	HP-β-CD*	9.12	9.28	1.019	1.60
Isoprenaline	ME-β-CD*	8.27	8.53	1.031	3.06
Ketamine	β-CD*	8.18	8.27	1.010	0.88
Lofexidine	HP-β-CD*	8.94	9.32	1.043	3.17
Mefloquine	HP-β-CD	13.19	14.33	1.086	7.96
Methylephedrine	ME-β-CD*	8.75	8.87	1.013	1.11
Metomidate	HP-β-CD	14.10	14.99	1.063	4.50
Mianserin	HP-β-CD	12.11	13.08	1.079	4.34
Nefopam	HP-β-CD	15.76	15.9	1.008	0.73
Nomifensine	HP-β-CD	14.77	15.21	1.029	2.45
Norephedrine	ME-β-CD	7.08	7.22	1.019	1.53
Norfenefrine	ME-β-CD*	6.79	6.99	1.029	2.59
Octopamine	HP-β-CD	8.81	8.91	1.010	0.80
Pholedrine	HP-β-CD*	8.65	8.87	1.026	2.29
Salbutamol	ME-β-CD*	7.66	7.74	1.011	1.08
Sotalol	HP-β-CD	13.97	14.13	1.011	1.20
Synephrine	ME-β-CD*	7.25	7.41	1.023	3.20
Zopiclone	HP-β-CD*	6.00	6.19	1.030	2.86

* One or more of the other tested CD's showing lower selectivites: β-CD, ME-β-CD, HE-β-CD and HP-β-CD.

Using CE with cyclodextrin containing run buffers proved to be a sensitive and rapid method to monitor the stereoselective metabolism of the racemic drug dimethindene in human urine samples by capillary electrophoresis [G. Blaschke and M. Heuermann, J. Pharmac. Biomed. Anal. 12, 753-760 (1994)].

To separate dimethindene, its metabolite N-demethyl-dimethindene and the internal standard (I.S.) 6 methoxy-dimethindene a run buffer containing 30 mM hydroxypropyl-ß-cyclodextrin was used. Parameters like cyclodextrin type and concentration, buffer pH, applied voltage and

temperature of the capillary have been investigated toward their influence upon the selectivity and resolution.

To decrease the detection limit sample stacking conditions were used. Buffers of higher ionic strength (100 mM) were used as electrode buffer and run buffers, buffers of much lower ionic strength were used to prepare the sample solutions. Under an applied voltage the analytes, introduced with the buffer of low ionic strength into the capillary, will migrate more rapidly until they reach the higher concentrated run buffer. In this way the volume of analytes is compressed. Better resolutions and an enhanced selectivities are obtained by using this technique (fig. 2).

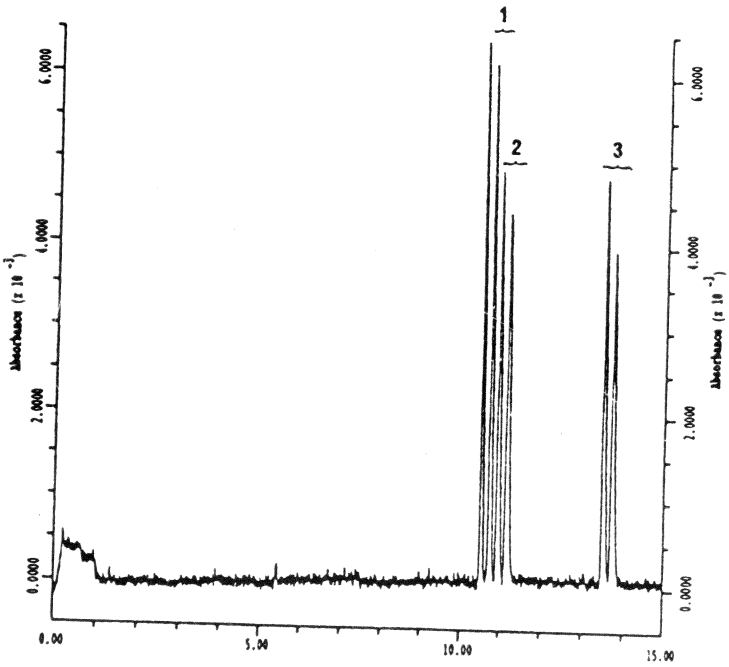

Fig. 2: Chemical and enantiomeric separation of N-demethyl-dimethinde (**1**), dimethindene (**2**) and the IS 6-methoxy-dimethindene (**3**) by CE

As conclusion, capillary electrophoresis is a valuable alternative to HPLC for the determination of basic drugs in human urine. The described assay proved to be sensitive, reproducible and suitable for the rapid simultaneous quantification of enantiomers. Baseline resolutions are obtained with high efficiency. In comparison to the HPLC methods nearly the same limit of detection was achieved by this CE method. Moreover, the limit of detection probably could be improved by Z-shaped detection cells.

Another possibility to increase the sensitive of detection is the use of Laser Induced Fluorescence (LIF). Applications (G. Blaschke and G. Hempel, unpublished results) are the simultaneous separations and LIF detections of the racemic drug zopiclone and its main metabolites N-demethylzopiclone and zopiclone-N-oxide by CE using a He-Cd-Laser (fig. 3). The injection volume was 0.02 µl, the amount of drug or metabolite corresponding to each peak is as low as 1 ng.

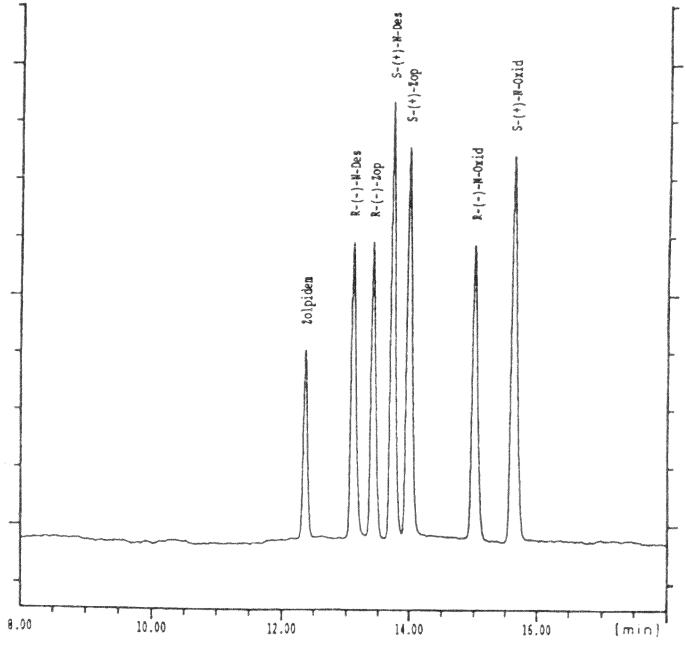

Fig. 3: Laser Induced Fluorescence (LIF) as detection principle in the capillary electrophoresis of zopiclone (**Zop**), N-demethylzopiclone (**N-Des**) and zopiclone-N-oxide (**N-Oxid**).

Using LIF detection it is also possible to determine drugs and its metabolites directly in biological samples without extraction. This is exemplified with the hypnotic drug zolpidem. The sensitivity of LIF detection is so high in this case urine can be directly injected into the capillary. Detection limits for zolpidem itself are 0.02 pg, and for the main metabolite, a carboxylic acid derivative, 0.1 pg.

Acknowledgement: I would like to thank Docent Dr. Selma Sarac and especially our colleague and friend, Professor Dr. Mevlüt Ertan, both from Hacettepe University, Ankara, for cooperation and fruitful discussions.