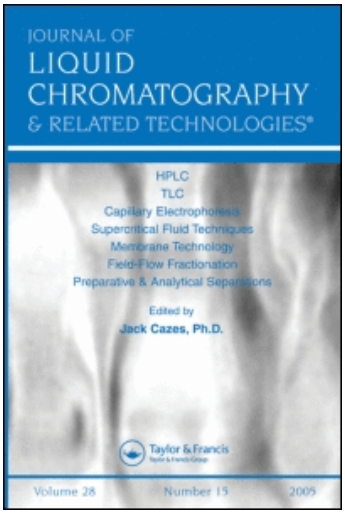

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Gy. Szász[a]; K. Gyimesi-Forrás[a]; Zs. Budvári-Bárány[a]
[a] Semmelweis University of Medicine Institute of Pharmaceutical Chemistry, Budapest, Hungary

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OPTIMIZED AND VALIDATED HPLC METHODS FOR COMPENDIAL QUALITY ASSESSMENT. III. TESTING OF OPTICAL PURITY APPLYING α_1-ACID-GLYCOPROTEIN STATIONARY PHASE

Gy. Szász, K.Gyimesi-Forrás, Zs.Budvári-Bárány

Semmelweis University of Medicine
Institute of Pharmaceutical Chemistry
Budapest, Hungary

ABSTRACT

Since distomer impurity in the compendial enantiomer may indicate the lack of careful purification or an ability of racemization, the control of enantiomeric purity has growing significance. Although α_1 - acid - glycoprotein (AGP) stationary phase was applied for the separation of a number of drug enantiomers, it was hardly used for testing of enantiomer purity.

In this work the suitability of AGP column for compendial control of enantiomeric purity is illustrated by methods optimized through the selection of optimal quality (phosphate, acetate), pH (4.0; 5.5; 6.0; 7.0) and concentration (0.01, 0.03, 0.06 M) of the buffer-eluent and the best organic modifier (isopropanol, acetonitrile, dioxane). The chromatographic behavior of four basic, three acidic, and one neutral compound is interpreted. In the case of propranolol and ibuprofen, 5% impurity may be detected while, in the case of norgestrel, 2.5% of the impurity may be detected. Recommendations for compendial testing are presented.

INTRODUCTION

As the number of the drug compounds, containing chirality center increases, the need for methods to test optical purity is more and more pronounced. The therapeutical application of the pure enantiomers, instead of the racemes is also emphasized by establishing the greater activity of the eutomers and the often ocurring side effects or toxicity of the distomers.[1-7]

While the leading pharmacopoeias prescribe only the non-specific control of the optical purity (e.g. giving an interval for the specific rotation wanted) the number of papers, dealing with the chromatographic separation of the enantiomers, is increasing. It must be assumed, that optical purity as a criterium of compendial quality for chiral drugs will uniformly appear in the pharmacopoeias of the next years.

The present work aims to demonstrate that an HPLC method is suitable not only for the separation of enantiomers but also for the sensitive compendial testing of optical (enantiomeric) purity. Papers including this special subject, could not be found in the literature. In this work the suitability of AGP stationary phase is shown in case of acidic, basic, and neutral medicinal (model) compounds.

AGP as stationary phase was introduced into the analytical practice by Hermansson[8] in 1983. In the next years, up to the present, AGP and its sorbent properties, as an application for drug analysis was the subject of several works. The enantioselectivity of AGP is dramatically influenced by the additives are added to the generally used aqueous phosphate buffer eluent. The effect of lower (C_1-C_3) alcohol homologs on the retention of certain cationic drugs was studied by Hermansson et al.[9] and also the amount of 2-propanol and acetonitrile bound by the AGP surface was determined.[10] The fact, that the pH of the eluent has also strong influence on the behavior of basic, acidic as well as neutral compounds, was revealed at early time by Hermansson and has been observed by others too.[11,12] The influencing effect of charged additives was also utilized[13,14]

The wide applicability of AGP for drug analysis was tabulated in 1989[15] by Hermansson, introducing the retention data (k',α) of baseline resolved racemic drug compounds, including basic, acidic, and non-protolytic molecules as well.

These early chromatographic systems contained non-charged additive (alcohol, acetonitrile) beside the aqueous phosphate buffer (pH=7 in most cases). In the same paper[15] the useful effect of cationic and anionic additives on the enantioselectivity of AGP column was illustrated by several examples.

While the parasympatholytic atropine and homatropine enantiomers were separated by the addition of anionic additives[12] the separation of the anti-inflammatory non-steroid acid - enantiomers was enhanced by neutral or cationic modifiers.[14] The separation of hydantoine and barbital sedatohypnotics, β-blocker enantiomers was solved by the Enquist and Hermansson[9] additive's effect and the retention mechanism was studied simultaneously. Centrally active benzoadiazepines were investigated on AGP column by Fitos et al.[16]

In this work the structure-chromatographic relationship behavior study was also involved. Design of chiral separation[17] and retention model suggestion[18] was published for calcium antagonists.

While a number of papers dealing with drug enantiomer separation may be found in the literature, the enantiomer purity testing by AGP column rarely occurs.[19-21] Aboul-Enein et al.[22] published on optical purity determination of thyroxine enantiomers. They used HPLC on an ovomucoid column.

The present paper aims to show the suitability of HPLC on AGP for compendial use in testing the optical purity of drug enantiomers. As model compounds, basic, acidic, and neutral substances were selected.

EXPERIMENTAL

Chromatography

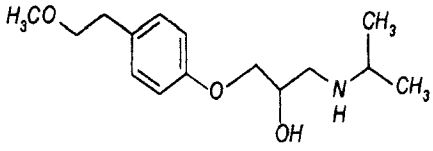
The HPLC apparatus comprised a Waters pump, Model 501, combined with a Rheodyne injector unit (10 μL loop). A Hewlett-Packard 1030 B variable wavelength absorbance detector (230-800 nm) was used. The equipment units, subsequent to the pump, were thermostatted at 25°±1°C (Column Chiller Model 7955, Jones Chromatography Ltd., Hengold, Wales). The chromatograms were recorded; the data handling was affected by Hewlett-Packard integrator Model 3390 A. The chiral AGP sorbent (ChromTech AB, Hägersten,Sweden) was packed in a stainless steel column (100x4.0 mm I.D.). As mobile phase, sonically degassed and filtered aqueous phosphate buffer solution (occasionally containing certain amount of additives) was used.

For the equilibration the column was washed with 160 mL of the eluent (cca .40 fold of column volume). The column void time was signaled by the peak of methanol used as the solvent of the analytes. Following testing, the columns were brought to the initial state by washing with isopropanol 10% and then with water, 30 min. each. The eluent flow rate was 1.0 mL/min.

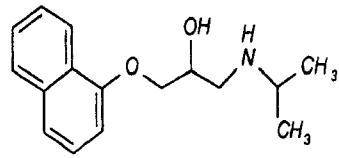
Table 1

Structure of the Model Compounds

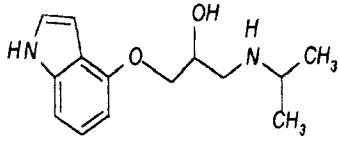
METOPROLOL



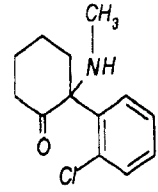
PROPRANOLOL



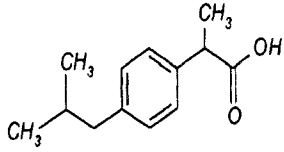
PINDOLOL



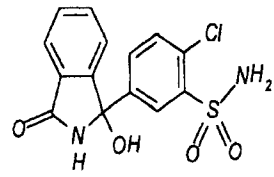
KETAMINE



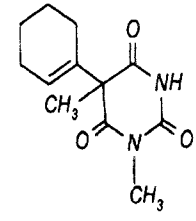
IBUPROFEN



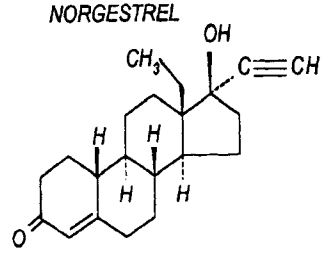
CHLORTHALIDONE



HEXOBARBITALUM



NORGESTREL



Chromatographic system optimization

After the pH of the phosphate buffer eluent was adjusted to produce a convenient, reasonable retention time (5-15 min), in order to increase the enantioselectivity, isopropanol, acetonitrile, or dioxane were alternatively added in an amount of 0.5-5%. The effluent was monitored at the wavelength of maximum absorption.

Validation

Each retention data point was calculated as an average of a minimum of 5 parallel runs. The difference between the extreme retention values must not exceed 5 %. As a limit of detection a signal-to-noise ratio 3:1 was accepted.

Materials

(See structures in Table 1). Ibuprofen (±) Pharmamed Ltd; Ibuprofen (S) - (+) Aldrich; *Norgestrel (±); *Levonorgestrel (−); Propranolol hydrochloride (±) Aldrich; Propranolol hydrochloride (R) - (+) Aldrich; Metoprolol tartrate (±) Aldrich; Oxprenolol hydrochloride (±) Aldrich; Pindolol (±) USP XXIII; Chlortalidone (±) USP XXIII; Hexobarbital (±) Aldrich .

*These substances were generously supplied by Richter Gedeon Pharmaceutical Works, Budapest.

From the 0.01% methanolic solution of the model substances 10 μL was injected. For the purity tests these solutions were properly diluted with methanol.

Buffer solutions

Buffer solutions were prepared by mixing the proper volumes of 0.01-0.03-0.06 M aqueous solutions of sodium acetate (CH_3COONa, $3H_2O$) and acetic acid or potassium dihydrogenphosphate and sodium hydrogenphosphate (KH_2P0_4, $Na_2HPO_4,2H_2O$) analytical grade, Reanal, Budapest. The pH of the solutions was tested by potentiometry with an accuracy of ± 0.02 unit.

Acetonitrile for HPLC, was supplied by Chemolab, Budapest. Dioxane, analytical grade came from Reanal, Budapest. The product was purified by 24h standing on sodium hydroxide followed by a distillation. Peroxide content was 0.005 %. Methanol for HPLC, came from Chemolab, Budapest. Water, was deionized, and double distilled.

Table 2

The Influenece of Buffer Quality and Concentration on the Retention of Enantiomers

Eluent Phosphate Concn. M pH=7	± Metoprolol		± Ibubropfen		± Hexobartital		± Norgestrel
			Capacity Factors $k'_1/k'_2(\alpha)$				
0.01	5.3 7.0	(1.32)	2.9 4.2	(1.45)	11.5 19.0	(1.64)	32.6
0.03	9.8 13.5	(1.37)	3.7 5.7	(1.52)	11.2 20.8	(1.86)	30.5
Eluent Acetate Concn. M pH=7							
0.03	8.3 11.8	(1.43)	2.5 4.1	(1.64)	8.5 14.4	(1.76)	11.8 14.4 (1.22)
Eluent pH [0.01MPO$_4$]							
4.0	0.52 0.63	(1.25)		Ø	6.01 8.0	(1.30)	11.0 18.4 (1.67)
5.5	2.7 3.1	(1.15)	24.9 32.5	(1.31)	8.0 10.8	(1.35)	25.8 33.1 (1.28)
7.0	5.3 7.0	(1.32)	2.9 4.2	(1.45)	11.5 19.0	(1.64)	32.6

Table 3

The Chromatographic Systems Suitable for Enantiomer Purity Testing

Compound	Eluent	k'_1/k'_2	α_*
Metoprolol	0.01M PO_4 (pH=7.0)	5.3 / 7.0	1.32
Pindolol	0.01M PO_4 (pH=7.0) + 5% AcCN	4.6 / 5.8	1.26
Propranolol	0.03M Ac (pH=4.0)	7.2 / 9.4	1.30
Ketamine	0.03M Ac (pH=5.5)	1.5 / 3.1	2.10
Ibuprofen	0.03M Ac (pH=7.0)	2.5 / 4.1	1.64
Chlortalidone	0.03M Ac (pH=4.0) + 0.5% IPA	2.0 / 6.0	3.00
Hexobarbital	0.03M Ac (pH=4.0)	3.9 / 6.6	1.69
Norgestrel	0.06M Ac (-H=4.0)	12.1 / 17.9	1.48

PO_4: Phosphate buffer (see Experimental)
Ac: Acetate buffer (see Experimental)

Table 4

Elution Strength of 2-Propanol, Dioxane, and Acetonitrile

Retention Time (Min.) of the Enantiomers

Compound	IPA* 0.12M (1%)	DIO* 0.11M (1%)	AcCN* 0.35M (5%)
Metoprolol	5.6	3.7	
	6.6	4.4	3.7
Ibuprofen	2.9	1.3	0.4
	4.2	1.6	0.6

* Concentration of the modifier in the 0.03M phosphate - eluent (pH=7).
IPA: 2-propanol, DIO: dioxane, AcCN: acetonitrile.

RESULTS AND DISCUSSION

Table 2 shows retention data for the model substances when the quality (concentration, pH, identity) of the buffer was changed. It can be seen, that increasing phosphate concentration, at pH 7, enhances the retention of basic metoprolol, and exerts a similar effect to the acidic ibuprofen, while the retention of the neutral norgestrel and, also, the very weak acid hexobarbital does not change significantly. The retention values vs. pH of the eluent are in

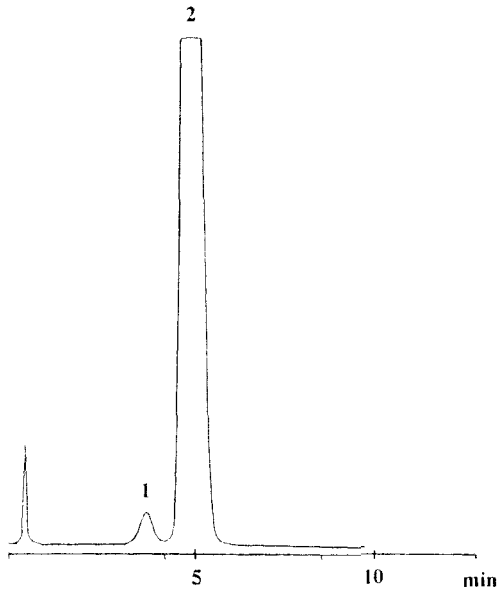


Figure 1. Purity test of Ibuprofen (detection of 5% of distomer). 1: 0.05 μg R - (-) Ibuprofen, 2: 1.0 μg S - (+) Ibuprofen, Mobile phase: 0.03 M acetate buffer (pH= 7.0), λ: 220 nm.

full agreement with the structure and properties of the model substances: as the amount of the ionic form (i.e. polarity) increases, the binding affinity of the solute,in the reversed phase system, is going down. Since the weak acid, hexobarbital (pK_a = 8.30), is hardly dissociated at pH 7, it behaves just as a neutral substance; its retention towards the lower pH values are increasing slowly.

Ibuprofen, being a much stronger acid (pK_a = 5.2) at pH 7 is present practically in the dissociated form; it is bonded very loosely, while at pH 4 it was not eluted at all within 60 mins. The basic metoprolol (pK_a = 9.70) being protonated from pH 7 through pH 4, its k ' values were decreased steeply by the pH sinking.

Table 3 includes the eluents that were found suitable for enantiomer purity testing. It was noteworthy, that in most cases base-line separation of the enantiomers was achieved with aqueous buffer eluent alone.

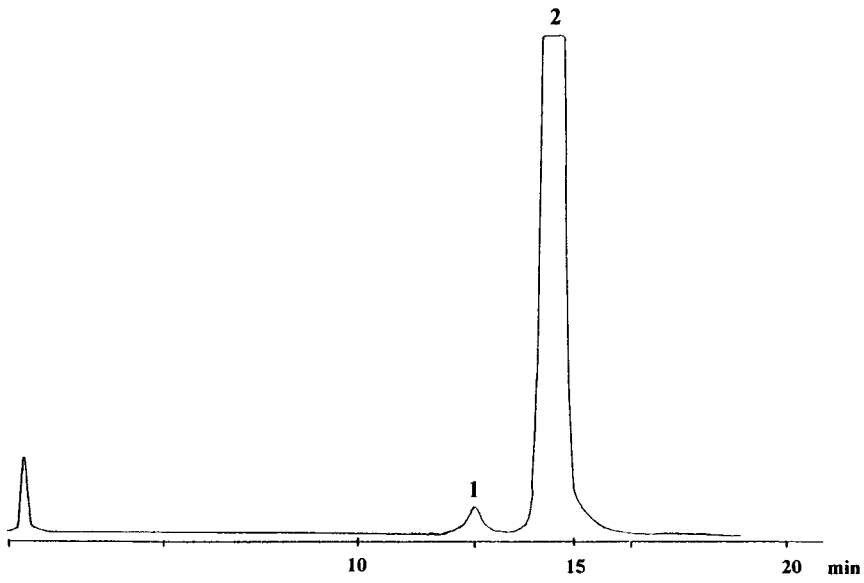


Figure 2. Purity test of Norgestrel (detection of 2.5% of distomer), 1: 0.025 μg of (+)-norgestrel, 2: 1.0 μg of (-) -levonorgestrel, Mobile phase: 0.06 M acetate buffer (pH = 4.0).

Beside the earlier used 2-propanol and acetonitrile the enantioselective effect of dioxane was also studied. The rather strong binding of dioxane to the AGP-surface was the subject of an other work.[23] It may be concluded, that the elution strength of dioxane, similarly, as was found on silica,[24] is much greater on the AGP surface, as would be expected by its relative permitivity. This is clearly shown comparing the retention values in eluents containing acetonitrile, 2-propanol or dioxane (Table 4).

Testing Of Enantiomeric Purity

Propranolol, Ibuprofen

Ten μL of 0.01 % methanolic solution (= 1.0 μg) of the tested sample is chromatographed on a AGP-column, using the corresponding eluent of Table 3. (For other chromatographic conditions see in Experimental.) Any definitely observable peak of the enantiomeric impurity (Fig. 1) detected at 220 nm, represents an amount as equal or more than 5.0% (= 0.05 μg).

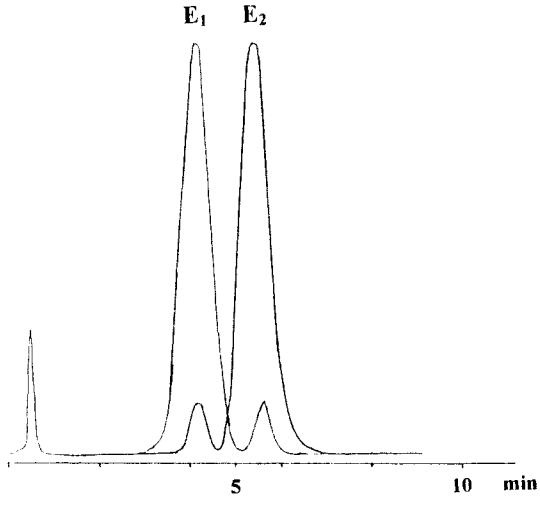


Figure 3. Check of system suitability for enantiomer purity test. The "chromatograms" of hexobarbital plotted from two different runs of the racemate. E_1: enantiomer No. 1 (from 1.0 and 0.05 μg of the racemate), E_2: enantiomer No. 2 (from: 1.0 and 0.05μg of the racemate), Mobile phase: 0.03 M acetate buffer (pH = 4.0), λ: 220 nm.

Table 5

Parameters of System Suitability for Enantiomeric Purity Testing*

	Rentention Time, Min.**			
Compound	**E_1 (1.0 μg)**	**E_2 (1.0 μg)**	**E_1 (0.05 μg)**	**(E_2 (0.05μg)**
Metoprolol	7.4 - 10.0***	10.0 - 14.3***	8.9 - 10.0***	11.0 - 13.5***
Chlortalidone	3.0 - 6.0	7.0 - 11.5	2.7 - 5.0	7.0 - 8.8
Hexobarbital	3.6 - 4.7	4.8 - 6.1	4.1 - 4.5	5.4 - 5.8

* See also Figure 3.
** Mobile phases as in Table 3.
*** The front and rear of the peak.

Informative retention times between 1 - 0.05 μg : R - (+) - Propranolol 9.3 - 10.3 min., S - (–) - Propranolol 13.7-14.7 ; R - (-) - Ibuprofen 2.9 - 4.4 min., S-(+)- Ibuprofen 5.9 - 7.3 min., respectively; t_0: 1.03 min. Limit of detection : 50 ng / peak.

Norgestrel

The procedure for propranolol and ibuprofen is valid with differences as follows: any, definitely observable peak of the enantiomer impurity (Fig.2) detected at 247 nm, represents an amount equal or more than 2.5 % (=0.025 μg). Informative retention times between 1 μg - 0.025 μg: (+) - Norgestrel 11.5 - 12.1 min., (–) - Norgestrel 14.1 - 15.1 min. respectively; t_o = 1.03 min. Limit of detection: 25 ng / peak.

Metoprolol, Pindolol, Chlortalidon, Hexobarbital

In the case of the substances above, having no authentic pure enantiomers, the racemates were used to establish the suitability of the chromatographic system for enantiomeric purity control. For this, a chromatogram, containing the peaks, was gained seperately by the injections of 0.01% solution (10 μL = 1.0 μg) and a solution containing the limit of detection, was to be edited (Table 5 and Fig. 3). Since the chromatograms from the two solutions are prepared successively, the retention times may be compared plausibly, therefore, the system suitability for enantiomeric purity control can be estimated.

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