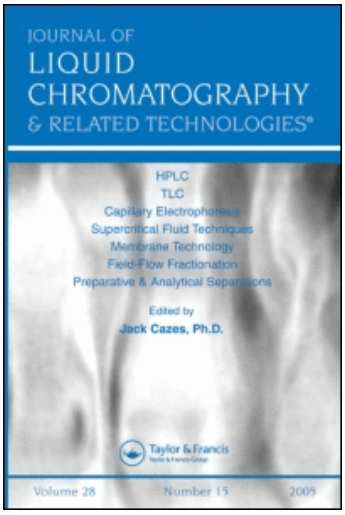

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ohshima, Taeyuki , Takagi, Kenzo and Miyamoto, Ken-Ichi(1993) 'High Performance Liquid Chromatographic Retention Time of β-Blockers as an Index of Pharmacological Activities', Journal of Liquid Chromatography & Related Technologies, 16: 18, 3933 — 3939
To link to this Article: DOI: 10.1080/10826079308019678
URL: http://dx.doi.org/10.1080/10826079308019678

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(18), 3933-3939 (1993)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC RETENTION TIME OF β-BLOCKERS AS AN INDEX OF PHARMACOLOGICAL ACTIVITIES

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ABSTRACT

The pharmacological activity of seven β-blockers was evaluated based on retention time on high performance liquid chromatography with an internal surface reversed-phase silica column (Pinkerton column). Significant relationships were found between the adjusted retention times for β-blockers and their pharmacological effects, such as affinity for the β_1 heart receptors ($r = -0.714$) and the β_2 lung receptors ($r = -0.937$). Thus retention time on high performance liquid chromatography may be a rapid and replicable approach to the prediction of pharmacological effects.

INTRODUCTION

Many techniques have been used to study the pharmacological activities of β-blockers[1,2]. However, these methods entail complicated

procedures and are time consuming. The goal of studying the quantitative structure-activity relationship is to predict biological performance of new congeners easily from their physicochemical properties. Therefore, an inexpensive, simple to use without a specific instrument, and reliable method is desirable. Reversed-phase high-performance liquid chromatography (HPLC) partitions and separates drugs based in part on polarity. The retention time of a compound in a chromatographic system is highly correlated with the octanol/buffer partition coefficient and protein binding affinity and is an approach to their evaluation[3-5].

An HPLC column, the Pinkerton column (Regis Chemical, IL), packed with internal surface reversed-phase (ISRP) silica supports, has been developed to accommodate the analysis of drugs in plasma by direct injection[6]. This column can exclude large molecular weight protein-like substances due to the small pore diameter of the ISRP supports, while hydrophobic drug molecules can penetrate the particulate matter and interact with the internal partitioning phase. The use of the column was simple and measurement was rapid, and also the results showed good precision and reproducibility[7]. This study evaluated HPLC retention time with a Pinkerton column as an approach to measuring the pharmacological activities of β-blockers.

EXPERIMENTAL

Materials

Pure reference standards of the seven drugs shown in FIGURE 1, were kindly supplied by their manufactures. The Pinkerton column (250 x 4.6 mm i.d.; Regis Chemical Co., Illinois, U.S.A.) was purchased from Koken Co. Ltd. (Tokyo, Japan). Other chemicals and reagents,

purchased from commercial sources, were of analytical grade and were used without further purification.

Apparatus

An LC-5A chromatographic system (Shimadzu Co., Kyoto, Japan) consisting of an LC-5A liquid pump with a Pinkerton column, an SPD-2A UV spectrophotometric detector, and a Chromatopac C-R2A data processor was used.

HPLC conditions

The mobile phase was a mixture of 0.1 M KH_2PO_4 with a pH of 6.8 - isopropanol and the flow rate was adjusted to 1 ml/min. The elutions were done at ambient temperature (25 $\pm$ 2°C) and the effluent was detected at the wavelength of λmax of each drug (232 - 274 nm). A sample volume of 10 µl was injected in all the studies. All samples were filtrated through a 0.22 µm membrane filter (Millex GV; Nihon Millipore Ltd., Osaka, Japan) before injection.

RESULTS AND DISCUSSION

Trials looking for the optimum conditions were made and isopropanol was found to be the appropriate organic solvent to separate each β-blocker from other peaks. In the region of an isopropanol concentration of less than 10% (v/v) in the mobile phase, the duration of the retention time was increased and the peak width was also expanded. Thus, the concentration of isopropanol in the mobile phase was selected as 10% (v/v). The HPLC retention times (RT) of the seven drugs are shown in TABLE 1.

TABLE 1: Retention time, partition coefficients and pharmacological activities of β-blockers.

Drug	retention time (min)	log P*	pA_2*		Ki*	
			in atria or artery	in trachea	for β_1 receptor	for β_2 receptor
atenolol	13.6	-1.70[8]	7.66[1]	6.13[1]	1400[1]	6250[1]
metprolol	26.0	-0.30[8]	7.63[10]	5.67[12]	120[14]	2600[14]
acebutolol	32.2	-0.21[8]	6.84[1]	5.47[1]	1200[1]	3700[1]
carteolol	32.6	0.21[9]	11.23[11]	9.26[13]	4.6[15]	
oxyprenolol	46.1	0.20[8]	8.73[1]	8.21[12]	2.6[1]	1.7[1]
pindolol	57.2	-0.39[8]	9.19[1]	8.93[12]	2.9[1]	90[1]
alprenolol	75.0	0.98[8]	8.50[1]	8.87[12]	9.0[1]	3.0[1]

Retention time on HPLC are values obtained the mobile phase of a mixture of 0.1 M KH_2PO_4 with a pH of 6.8 - 10% isopropanol (v/v).

* Each value is obtained from corresponding reference.

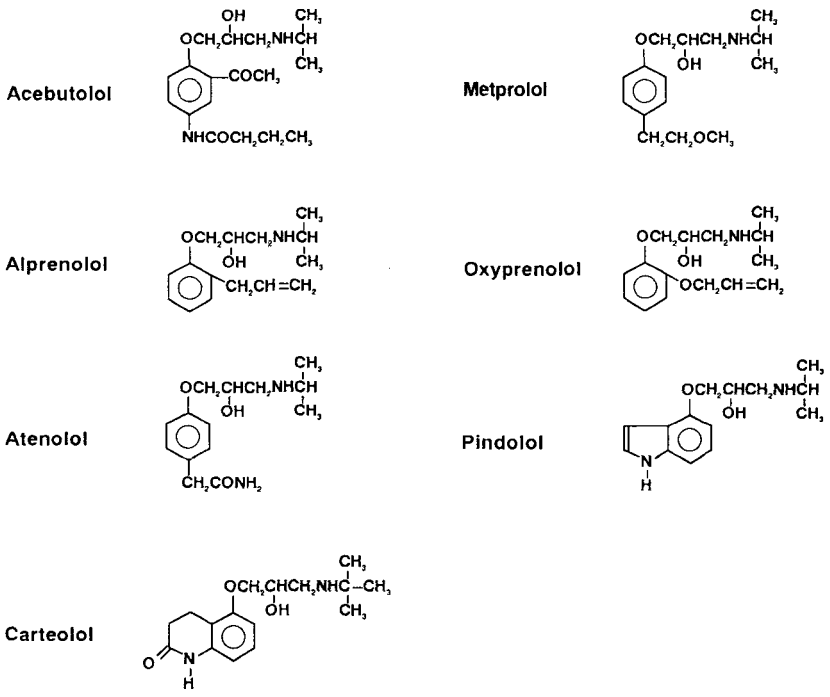

FIGURE 1: Chemical structures of the investigated β-blockers.

These RTs were compared with physicochemical and pharmacological activity data (TABLE 1). A significant relationship was found between the log RT of each β-blocker and its lipophilicity (r = 0.848). Similarity, a significant correlation was also obtained between log RT and their pharmacological activities, affinity for the β_1- receptors of the heart (r = -0.714) and the β_2 receptors of the lung (r = -0.937) . On the other hand, the antagonistic potency, pA_2, of non-selective β-antagonists on β_1-adrenoceptors in atria/artery and pA_2 on β_2-adrenoceptors in trachea was not significant related to log RT (β_1-pA_2; r = 0.291, β_2-pA_2; r = 0.677). At concentrations of less than 10%

isopropanol in the mobile phase, correlation coefficients between log RT and each set of data had the same tendency to those at 10%.

In the structure-activity relationship, carteolol and acebutolol were different from the other chemicals (FIGURE 1). Both chemicals have two hydrophilic sites. Therefore, RTs except for the data for carteorol and acebutorol were compared with physicochemical and pharmacological activity data. All relationships were found to be significant between the log RT and log P ($r = 0.888$), β_1-Ki ($r = -0.859$), β_2-Ki ($r = -0.958$), β_1-pA_2 ($r = 0.804$), and β_2-pA_2 ($r = 0.888$). This report indicates that HPLC retention time can be used to measure the pharmacological activities of β-blockers. HPLC retention time was highly correlated with the affinity for β-receptors and confirmed alprenolol to be by far the most lipophilic. However, it is noted that chemicals having two or more hydrophilic sites may give weaker pharmacological activities than the predicted value by HPLC. This may be due to the characteristics of the Pinkerton column[7].

HPLC retention time has a further merit in that it does not require any complicated operation, and it is easily standardized for comparisons of relative pharmacological effects among many drugs.

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Received: April 17, 1993
Accepted: April 23, 1993