



Anomalous Chromatographic Retention of β -blockers in Mobile Phases Buffered by Tris(hydroxymethyl)aminomethane



X. Subirats¹, J. Gotta², L.G. Gagliardi², C.B. Castells², C. Ràfols¹, M. Rosés¹, E. Bosch¹

¹Departament de Química Analítica, Universitat de Barcelona, Barcelona/E (xavier@subirats.com)

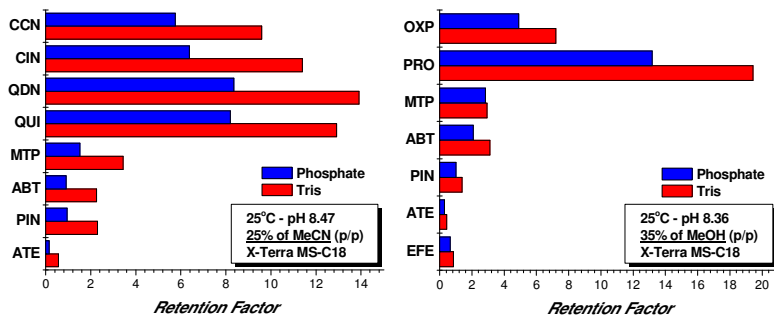
²División de Química Analítica, Universidad Nacional de La Plata, La Plata/ARG

Introduction

In a recent work (J. Sep. Sci. 2008, 31, 969) retention factors of several β -blockers were studied at different temperatures in a X-Terra[®] MS-C18 column in mobile phases prepared from different buffers, but all of them containing a 25% (w/w) of acetonitrile. At 25°C, retention times using a tris buffer of pH 8.47 were between 1.5 and 4 times higher than the ones corresponding to a phosphate buffer of the same pH and concentration. A retention mechanism besides hydrophobicity was suggested to explain this unexpected additional retention.

Extra retention is found in tris buffered mobile phases containing acetonitrile and methanol as organic modifiers.....

....and for different kinds of RP-HPLC columns (C18 and polymeric)



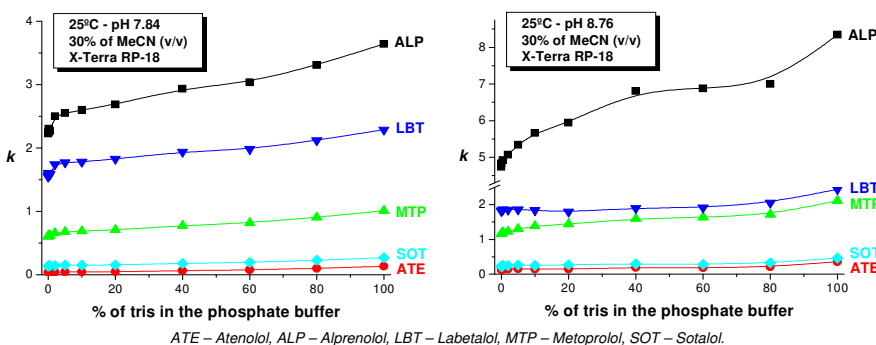
ABT – Acebutolol, ATE – Atenolol, CCN – Cinchonidine, CIN – Cinchonine, EFE – Efedrina, MTP – Metoprolol, OXP – Oxprenolol, PIN – Pindolol, PRO – Propranolol, QDN – Quinidine, QUI – Quinine.

	Retention factor, <i>k</i>					
	X-Terra RP18		Luna C18(2)		PLRP-S 100A	
	5 μ m	5 μ m	5 μ m	5 μ m	15-20 μ m	15-20 μ m
	Phos.	Tris	Phos.	Tris	Phos.	Tris
Atenolol	0.13	0.36	0.30	1.16	0.16	0.40
Metoprolol	1.16	2.12	3.26	7.81	2.74	5.58
Labetalol	1.82	2.44	3.47	5.02	3.64	5.97
Sotalol	0.24	0.46	0.47	1.35	0.39	0.84

Mobile phases: buffer concentration 50 mM
30% (v/v) of acetonitrile
 s_w pH = 8.76

pH measured in the buffered hydroorganic mobile phases after calibration with aqueous standard buffers.

Effect of tris concentration in retention of β -blockers



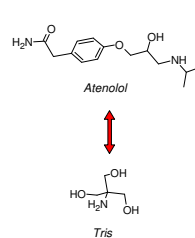
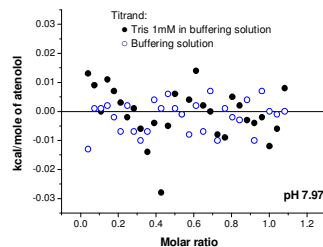
ATE – Atenolol, ALP – Alprenolol, LBT – Labetalol, MTP – Metoprolol, SOT – Sotalol.

- ✓ The studied drugs are neutral bases, with an acidic group $>NH_2^+$ of $pK_a \sim 9$. At s_w pH 7.84 these drugs are more ionised and, therefore, less retained.
- ✓ At s_w pH 7.84 tris is mainly in its protonated form ($\sim 60\%$), whereas at s_w pH 8.76 it is mainly neutral ($\sim 85\%$). In both cases, the extra retention effect due to tris buffer takes place.
- ✓ Small amounts of tris buffer (about 2%) are enough to cause significant changes in retention of β -blockers.

Isothermal Titration Calorimetry (ITC) does not show evidence of β -blocker \leftrightarrow tris interactions in free solution

The studied drugs are aminoalcohols, and both the analytes and the tris buffer present amino and $-OH$ groups on adjacent carbon atoms. Therefore interactions between amino and alcohol groups from the buffer and the analytes through hydrogen bonding could have been possible...

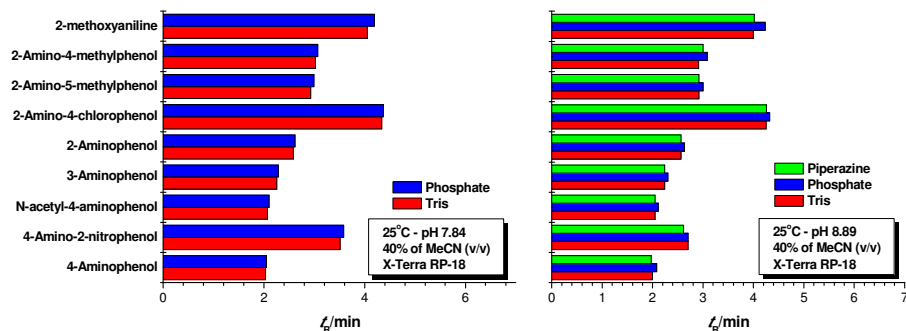
...but by ITC no evidence of any atenolol-tris interaction was found in free solution.



Isothermal titration calorimetry (ITC) conditions

Titrant	Atenolol 5 mM
Titrand	Tris 1 mM
Buffering solution	$H_2PO_4^- - HPO_4^{2-}$ 100 mM
Temperature	25°C
s_w pH	7.97 8.74
kcal/mole of atenolol	0.00\pm0.01 -0.01\pm0.01

Aminophenols do not suffer extra retention in presence of tris



The studied aminophenols present the amino group in *ortho*, *meta* and *para* positions in relation to the $-OH$ group, and different substituents. No significant differences were observed in the chromatographic behaviour of the analytes when tris, dihydrogenphosphate or piperazine buffers were used, independently of the molecular structure and bonding of the studied aminophenols.

Conclusions

- ✓ β -blockers are more retained than expected in the presence of tris. This behaviour has been observed in four different RP-HPLC columns, even at low tris concentration, and at different pH values.
- ✓ No evidence of any interaction between tris and β -blockers have been detected in free solution by ITC.
- ✓ Tris could remain superficially adsorbed to the stationary phase surface, interacting then with the β -blocker.
- ✓ The discussion is open, and all suggestions are welcome!