

**Indirect Chiral Ligand Exchange Chromatography for Enantioseparation: A modification
over conventional technique.**

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HPLC

HPLC was performed on a Lichrospher C₁₈ (250 X 4.6 mm I.D., 5µm) column from Merck (Darmstadt, Germany). The mobile phase used was methanol-TEAP buffer. The buffer of pH 5 was used for β-blockers while pH 4.3 was used for hydroxy acids, DOPA and carnitine; different ratios of methanol were used in mobile phase. The mobile phase was filtered by membrane filter (0.45 µM) and degassed under reduced pressure prior to use. The separation was carried out at a flow rate of 1 mL/min with run time of 45 minutes with detection at 230 nm on photodiode array detector.

Validation procedures for analytical separation

Method validation was done using diastereomeric complexes of atenolol prepared with Cu(II) L-phenylalanine in accordance to ICH guidelines. The calibration curves were obtained by plotting peak areas against concentrations and linear regression equations were used to determine slopes and correlation coefficients. Recovery studies were carried out by analyzing solutions of different known concentrations and mean recovered values (six replicate runs) were represented

as percentage of calculated values. Inter-day (3 days) and intra-day stability studies were carried out to find precision and RSD as shown in Table S-1.

Table-S-1: Inter and Intraday Accuracy and Precision of HPLC method for the determination of diastereomers of (*S*) - and (*R*)-atenolol prepared with Cu(II)-(L-Phe)₂ complex, n = 6

Theoretical conc.(ng/m L)	Measured concentration (ng/mL)							
	<i>S</i> -atenolol				<i>R</i> -atenolol			
	<i>Intra-day variations (Six replicates at each concentration)</i>							
	Mean	±SD	RSD (%)	Recovery (%)	Mean	± SD	RSD (%)	Recovery (%)
20	21.66	0.19	0.88	108.30	19.52	0.21	1.08	97.60
25	25.84	0.21	0.81	103.36	24.79	0.24	0.97	99.16
30	29.36	0.28	0.95	97.87	29.31	0.29	0.99	97.70
35	34.32	0.33	0.96	98.06	34.23	0.37	1.08	97.80
40	41.47	0.39	0.94	103.68	39.41	0.33	0.84	98.53
45	44.61	0.47	1.05	99.13	44.47	0.44	0.99	98.82
	<i>Inter-day variations (Six replicates at each concentration)</i>							
20	22.31	0.27	1.21	111.55	19.18	0.37	1.93	95.90
25	26.57	0.39	1.47	106.28	24.29	0.32	1.32	97.16
30	29.09	0.36	1.24	96.97	29.03	0.43	1.48	96.77
35	33.30	0.44	1.32	95.14	34.06	0.42	1.23	97.31
40	39.23	0.44	1.12	98.08	39.11	0.39	1.00	97.78
45	43.69	0.47	1.08	97.09	44.20	0.49	1.11	98.22

