

Trial 2:

Column	BDS Hypersil C18 (PART NO 28205-254630) BIM. (mm) 250*4.6, particle 5 $\mu$
Solvent system (mobile phase)	950 DW, 900 ml ACN, 600 ml methanol, pH 8
Detection	UV detector 237 nm
Injection volume	10 $\mu$ l
Flow rate	1.5 ml\min
Oven Temperature	25 $^{\circ}$ c

Trial 3:

Column	BDS Hypersil C18 (PART NO 28205-254630) BIM. (mm) 250*4.6, particle 5 $\mu$
Solvent system (mobile phase)	950 DW, 600 ml ACN , 600 ml methanol, pH 8
Detection	UV detector 220 nm
Injection volume	10 $\mu$ l
Flow rate	1.5 ml\min
Oven Temperature	25 $^{\circ}$ c

Trial 4:

Column	BDS Hypersil C18 (PART NO 28205-254630) BIM. (mm) 250*4.6 particle 5 $\mu$
Solvent system (mobile phase)	950 DW, 600 ml ACN, 600 ml methanol, pH 8
Detection	UV detector 237 nm
Injection volume	10 $\mu$ l
Flow rate	1.5 ml\min
Oven Temperature	25 $^{\circ}$ c