

2.8 Chromatographic Conditions:

Table 10: Summary Table of Chromatographic Conditions and Mass Spectrometric Conditions

HPLC Conditions	Pump Flow Rate		Autosampler Volume		Injection		Autosampler Temp		Column Oven Temp	
	1.0 ml/min		5 µl				4°C		30°C	
Chromatography	Mobile phase Gradient Elution	Step	Total Time(min)	Flow Rate (µl/min)	A (%) Methanol		B (%) 0.2% F.A			
		0	0.00	1000	50.0		50.0			
		1	0.01	1000	50.0		50.0			
		2	0.02	1000	100.0		0.0			
		3	0.70	1000	100.0		0.0			
		4	0.71	1000	50.0		50.0			
		5	2.00	1000	50.0		50.0			
	Column type	ACE 5 C18 Column (50 X 2.1 mm), 5µ								
Expected Retention times(minutes)	Candesartan			Irbesartan (I.S)						
	1.4			1.0						
MRM Detection Conditions	Analytes	Q1 Mass	Q3 Mass	Dwell	FP	DP	EP	CE	CXP	
	Candesartan	441.200	263.200	150	70	81	10	19	22	
	Irbesartan (IS)	429.453	207.300	150	70	26	10	8	22	
MS Conditions	CUR		CAD	IS	TEM		NEB			
	10		6	5500	400		5			

2.9 Irbiartan as internal standard

Irbesartan as internal standard is used to determine the concentration of candesartan by calculating response factor. Irbesartan is similar to candesartan and have a similar retention time. It is stable and does not interfere with the sample components.