

## 7. Preparation of sample:

A 300 µl of internal standard (40 µg/ml of candesartan cilexetil) was added to 0.20 ml of plasma, the mixture was vortex-mixed, for 1.0 minute. Then centrifuge for 15.0 minutes at 4400 r.p.m, the supernatant was transferred to a flat bottom inset then injected to C18 column. Rhein and internal standard were separated from endogenous substances.

All samples processed during experimental work should be handled under yellow light which will minimize UV exposure as rhein is light sensitive.

The determination of rhein plasma concentration was performed by means of a validated specific High Performance Liquid Chromatography with fluorescence detector method at JCPR.

### a. Chromatographic Conditions

**Table 2:** HPLC system specifications and conditions

|                              |  |
|------------------------------|--|
| <b>Column</b>                | Thermo Electron Corporation, BDS Hypersil, (150 X 4.6 mm i.d) 5µm                          |
| <b>Solvent system</b>        | 30% Water, 70% Acetonitrile (0.5 ml T.E.A / 1L M. Ph.), pH=2.50 ±0.05 adjust with (H3PO4). |
| <b>Detection</b>             | Fluorescence, λExcitation=440, λEmission=520   |
| <b>Inject Volume</b>         | 25 micro liters  |
| <b>Retention Times*</b>      |  |
| <b>Rhein</b>                 | 1.4 - 1.8 minutes  |
| <b>Candesartan cilexetil</b> | 2.8 – 3.4 minutes (internal standard)  |
| <b>Flow Rate</b>             | 1.5 ml/min   |

\*Retention times dependent upon HPLC conditions.