2. Review of Literature

A literature survey revealed that there are very rare instrumental techniques available for the estimation of Busulfan. The methods available are either by ELSD techniques or by titration methods.

So my thought at this point of time is to develop an Analytical Method for Assay and Related Substances for Busulfan injection.

The Analytical Method should be so precise, Accurate and Rugged to produce consistent results.

The analytical method should be stability indicating and should be capable to detect the impurities present at the minute quantities also, and the method should give reproducible results whenever analyzed.

UV spectrophotometry combines advantages of economic and simple with the surety of achieving high sensitivity and selectivity with excellent precision, accuracy and reliability. HPLC methods possess the advantages of speedy separation, high resolving power, high sensitivity and accurate quantitative measurements.

There are very rare instrumental methods available for the analysis of drug product Busulfan Injection. The available methods are:

- Electro spray light scattering detector
- Titration method

Hongxia Lin et al (2012), compared two sensitive methods for pharmacokinetics studies including LC-MS assay and HPLC precolumn derivatisation assay. Chromatographic separation was performed on a Gemini C\textsubscript{18} column. Liquid-liquid extraction with ethyl acetate was used for plasma sample preparation. Busulfan and internal standard ([\textsuperscript{2}H\textsubscript{8}] Busulfan) were detected as ammonium adducts at m/z 264.2 and 272.2 for LC-MS assay. For HPLC assay, the extraction from plasma was derivatized with 2-naphthalenethiol using synthesized internal standard (1,6-(methanesulfonyloxy)octane). The Ex and Em wavelength was 255 nm and 370 nm. The limit of detection was
15.6 ng/mL and 7.8 ng/mL for HPLC and LC-MS assay and good linearity ranging from 31.25–1000 ng/mL for HPLC and 15.6-1000 ng/mL for LC-MS assay. The intra and interday assay precision were less than 9.2% and 12.0% for LC-MS and HPLC assay. The pharmacokinetic parameters were estimated using non compartmental pharmacokinetic model with Win Nonlin. Busulfan AUC last showed an average difference of 0.7% between the two methods. The LC-MS method is accurate, reproducible, and requires less specimen, sample preparation and analysis time over the HPLC assay, making Busulfan monitoring faster and easier in clinical practice.

- **Snyder ML, Ritchie JC**, described a rapid (2-minute total analysis time per sample) and simple method for accurate and precise busulfan concentration determination in plasma samples (100 microL) using high performance liquid chromatography combined with electrospray positive ionization tandem mass spectrometry (HPLC-ESI-MS/MS). Busulfan was isolated from plasma after internal standard (Busulfan-D(8))-methanol extraction, dilution with mobile phase (ammonium acetate-formic acid-water), and centrifugation. The supernatant plasma was injected onto the HPLC-ESI-MS/MS and quantified using a six-point standard curve. The assay was linear from 0.025 microg/mL (approximately 0.1 micromol/L) to at least 6.2 microg/mL (approximately 25 micromol/L) with precisions of <5% over the entire range.

- **STMOPEN et al (2013)**, established a HPLC-ELSD method for determining the content of Busulfan in Busulfan tablets. Methods: The chromatographic conditions were as follows, SHIMADZU C8 column(250mm×4.6mm,5µm), the mobile phase : water-acetonitrile (75-25), the flow rate:1.0mL/min, and the detector : ELSD, the carrier gas : nitrogen, the flow rate:2.0 bar. Results : The standard curve of Busulfan was linear over the range of 0.25-0.75mg/mL(r=0.9999). The average recovery was 99.7%. Conclusion: This method is accurate and specificity, and it can be used on the quality control of Busulfan tablets.
- Busulfanum, European Pharmacopoeia 5.0, (2005), in this monograph the estimation of Busulfan is performed by titration method.

- Busulfan, USP 36 (2013), this pharmacopoeia describes about the assay of the Busulfan API by titration method

- Busulfan tablets, USP 36 (2013), this pharmacopoeia describes about the assay of the Busulfan tablets by titration method.

- Heggie JR et al (1997) prepared the plasma samples containing Busulfan and 1,6-bis(methanesulfonyloxy)hexane, and internal standard, by derivatisation with DDTC followed by addition of methanol and extraction with ethyl acetate. The extract was dried under nitrogen and the samples reconstituted with 100 microl of methanol prior to HPLC determination. Chromatography was accomplished using a Waters Nova Pak octa decylsilyl (ODS) (150 x 3.9 mm I.D.) analytical column, Nova Pak ODS guard column, and mobile phase of methanol-water (80:20, v/v) at a flow-rate of 0.8 ml/min with UV detection at 251 nm. The limit of detection was 0.0200 microg/ml (signal-to-noise ratio of 6) with a limit of quantitation (LOQ) of 0.0600 microg/ml for busulfan in plasma. Calibration curves were linear from 0.0600 to 3.00 microg/ml in plasma (500 microl) using a 1/y weighting scheme. Precision of the assay, as represented by C.V. of the observed peak area ratio values, ranged from 4.41 to 13.5% (13.5% at LOQ). No day-to-day variability was observed in predicted concentration values and the bias was low for all concentrations evaluated (bias: 0 to 4.76%; LOQ: 2.91%).

- Funakoshi K (1994), extracted Busulfan from serum with a mixture of diethyl ether and dichloromethane. After the evaporation of the organic layer, the reconstituted residue was injected into the HPLC system and Busulfan was derivatized with sodium diethyldithiocarbamate on the first short column. The back-flushed derivative was then separated on the second column. Finally, after column switching, the heart-cut fraction containing the derivative was further analyzed on the third column and monitored with ultraviolet absorbance detection at 278 nm. A high-performance liquid chromatographic
A (HPLC) method is described for the determination of Busulfan in human serum using on-line derivatisation and column switching.

❖ **Thomas E. Mürdter et al (2001),** quantified Busulfan concentrations using 200 µL of plasma and liquid–liquid extraction with diethyl ether after the addition of [²H₈] Busulfan as the internal standard. Separation and detection of Busulfan and [²H₈] Busulfan were achieved with a LUNA C₈ column (5 µm; 150 x 2 mm i.d.) at 30 °C, a HP 1100 liquid chromatography system, and a HP 1100 single-quadrupole mass spectrometer. Busulfan and [²H₈] Busulfan were detected as ammonium adducts in selected-ion monitoring mode at m/z 264.2 and 272.2, respectively. The calibration curve was linear at 5–2000 µg/L Busulfan. Intra- and inter assay imprecision (CV) and bias were both <11%. The limits of detection and quantification were 2 and 5 µg/L, respectively. Extraction recovery of Busulfan was >87%. Analysis of pharmacokinetics in four patients receiving high-dose Busulfan indicated that minimum Busulfan concentrations before the next dose were 405–603 µg/L, with no interference observed.

❖ **Astrid Karstens, Irene Kramer (2007),** determined the physico-chemical stability of ready-to-use Busulfan infusion solutions under different storage conditions. Admixtures were achieved by adding Busulfex/Busilvex injection to 0.9% sodium chloride infusion solution in polypropylene bags or glass vials in order to reach a final concentration of 0.5 mg/mL. Low temperatures reduce the rate of Busulfan hydrolysis, but encourage Busulfan precipitation. Freezing of diluted Busulfan solutions in polypropylene bags affects insoluble precipitation. Busulfan test solutions stored under refrigerated conditions proved to be physico-chemically stable for at least 19 hours in polypropylene bags, and 48 hours in glass vials. Busulfan test solutions stored at 13–15°C proved to be physico-chemically stable for at least 36 hours in polypropylene bags or glass vials. Calculated from the Arrhenius plot, the shelf-life of diluted Busulfan infusion solutions in polypropylene bags would be about 50 hours when stored at 10°C.

❖ **Salamun Desire et al (2013),** precipitated protein from plasma followed by analysis using a high performance liquid chromatography (HPLC) with tandem mass
spectrometry - electrospray ionization technique (LC-ESI MS/MS) in positive ionization mode and quantified using multiple reaction monitoring (MRM). Deuterated busulfan (d8-busulfan) was used as the internal standard. The internal standard d8-busulfan was custom made from ERDI- (Eno research chemicals and Custom synthesis, Research Triangle Park, NC, USA). Busulfan standard as well as mass spectrometry grade acetone, acetonitrile, ethyl acetate, ammonium acetate were purchased from Sigma-Aldrich (Sigma-Aldrich Corporation Bangalore, India). Milli-Q water was obtained from Millipore water purification system. The method was linear for the concentration ranging from 0 to 4000 ng/ml of Busulfan with a limit of detection of 2 ng/ml and limit of quantitation of 5 ng/ml. The assay was accurate for serial concentrations of Bu in plasma for five consecutive days and the CV was less than 10 per cent.