# LC-MS/MS METHOD FOR QUANTIFICATION OF BIOLOGICAL HORMONE (LIOTHYRONINE-T3 & LEVOTHYROXINE-T4) OVER TRADITIONAL IMMUNOASSAY

The thyroid produces a hormone called triiodothyronine, known as T3. It also produces a hormone called thyroxine, known as T4. Together, these hormones regulate a body's temperature, metabolism, and heart rate. The thyroid gland produces hormones that are essential for normal body metabolism. T3 and T4 are the only iodine hormone present in biological fluid. The significance of these biomolecules led to an urging need for their qualitative and quantitative analysis in biological fluids. Traditionally, their determination was done by classical immunoassay method.

# Why choose LC-MS/MS over Classical Immunoassay method?

After the introduction of immunoassay's to the analytical biochemistry, they have been applied in the analysis of thyroid hormones. There are good reasons for the wide adoption of immunoassays, like easy integration into the core laboratory, the simplicity of automated immunoassays, ease of use, etc. However, even such a well-established and characterised technique has limitations, such as, low selectivity, high analysis cost-per-sample, and low results reproducibility.

On the other hand, liquid chromatography mass spectrometry (LC-MS/MS) could be a superb detection method for thyroid hormones with high specificity, reproducibility, and low on price with high accuracy of results.

JRF Global have the capability to test T3 and T4 in LC/MS/MS, with a sensitivity expected for this testing, in Rat Serum. The method was developed using surrogate matrix, free from endogenous-T3 & T4 which was prepared in laboratory. Using this surrogate matrix method, validation experiment was performed for the determination of T3 & T4. An experiment was designed and performed to compare results of surrogate matrix with those of the normal matrix. Results were found well within the range of acceptance. The LLOQ obtained were 10 pg/mL for T3 and 20 pg/mL for T4, using C18 column over gradient programed technique (including methanol and acidic buffer as an eluent). The developed method was accurate, precise, and reproducible. Hence, it was used for the determination of T3 and T4. Analysis was carried out using API-6500 (mass spectrometer-AB Sciex) coupled with Nexera X2 (HPLC-Shimadzu).

In JRF Global a team of experienced professionals carry out various physchem, 5- Batch analysis, residue testing, bioanalytical, environment fate and metabolic, and ecotoxicity studies. JRF Global has a legacy of more than 25 years of carrying out GLP studies, complying with various regulatory requirements for intended countries, viz., <u>SANCO, EPA, ICH, etc.</u>

### **About The Author**



### **Gopal Upadhyay**

He has over 10 years of experience in the Bio-analytical field. He dealt with studies relates to core BA/BE. He independently carried out over 100 pivotal studies in clinical CRO. He is having expertise in method development and validation in plasma, using LC/MS-MS, as per USFDA and ANVISA guidelines. He has been conducting myriad of studies, pertaining to the Bio-analytical and Toxicological studies (including Ecotox studies), in JRF, India.

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