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COMPARISON OF SAMPLE PREPARATION STRATEGIES FOR TARGET ANALYSIS OF TOTAL THYROID HORMONES LEVELS IN SERUM BY LIQUID CHROMATOGRAPHY-QUADRUPOLE TIME-OF-FLIGHT-MASS SPECTROMETRY



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#### COMPARISON OF SAMPLE PREPARATION STRATEGIES FOR TARGET ANALYSIS OF TOTAL THYROID HORMONES LEVELS IN SERUM BY LIQUID CHROMATOGRAPHY-QUADRUPOLE TIME-OF-FLIGHT-MASS SPECTROMETRY

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<sup>\*</sup>Corresponding Author: Dr. Maria Dolores Marazuela, Dept. of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, E-28040 Madrid, Spain. Phone: ++0034913944217; Fax: ++0034913944329. marazuela@quim.ucm.es ABSTRACT

This paper describes a novel method based on liquid chromatography quadrupole timeof-flight-mass spectrometry (LC-QTOF-MS) for target analysis of total THs in serum. Several sample preparation strategies have been evaluated to reduce matrix effect (namely, HybridSPE cartridges, supported liquid extraction, SLE and solid phase extraction, SPE). Deproteinization and further clean-up with mixed-mode SPE was selected as the best strategy for sample preparation, since achieved the cleanest extracts and reduced ionization suppression effects (between -11 and -24%).

Method validation was performed by the analysis of control human serum samples. Criteria for confirming THs identity in serum extracts were based on retention times, accurate masses, isotopic pattern and MS/MS fragmentation pattern. Moreover, the quantitation capabilities of the LC-QTOF-MS method were also evaluated in terms of linearity, precision, accuracy and sensitivity by the application of matrix-matched calibration.

Additionally, the developed LC-QTOF-MS method successfully provides qualitative information on endogenous components responsible of ion suppression (e.g. lysophosphatidylcholines), via post acquisition data analysis. This demonstrates the significant advantage of using LC-QTOF-MS, as it allows retrospective querying of the acquired data without the need of re-injecting/re-processing the samples.

**Keywords**: LC-ESI-MS bioanalysis, thyroid hormones, human serum, sample preparation, matrix effect, phospholipids.

#### 1. Introduction

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