Effects of Preanalytical Factors on Serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 Measurements Using LC-MS/MS for the Clinical Laboratory Testing

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Abstract

Background: Vitamin D testing is increasing worldwide. Although immunoassays are widely used in Japan for the measurement of serum 25-hydroxyvitamin D3 (25OH-D3) as an indicator of vitamin D status, development of a simple and high-throughput MS-based method is needed for routine use in clinical laboratories.

Materials and methods: We designed a method using a triple quadrupole mass spectrometer equipped with a high-performance liquid chromatography (HPLC) system in the selected reaction monitoring mode. Analytical performance of the LC-MS/MS system was validated, and sample collection processes, including effects of various preanalytical factors, were tested.

Results: To evaluate high-throughput LC-MS/MS 25OH-D3 analysis, we have to establish a simple and high-throughput MS-based method that can be used in a routine clinical laboratory. This study also determined the effects of various preanalytical factors on 25OH-D3 and 25OH-D2 measurements in clinical laboratories. This report describes the routine use of this LC-MS/MS system for 25OH-D measurements in clinical laboratories. This report describes the routine use of this LC-MS/MS system for 25OH-D measurements in clinical laboratories.

Methods

Serum 25OH-D3 and 25OH-D2 levels have been conventionally measured by radioimmunoassay (RIA) because measurement of 25OH-D using LC-MS/MS is usually used in clinical laboratories. In this study, we describe the routine use of this LC-MS/MS system for 25OH-D measurements in clinical laboratories. This report describes the routine use of this LC-MS/MS system for 25OH-D measurements in clinical laboratories. This report describes the routine use of this LC-MS/MS system for 25OH-D measurements in clinical laboratories.

Results

1) 25OH-D measurement by LC-MS/MS

A total of 50 µL of acetonitrile with internal standard (15 ng/ml) was added to each sample. After vortexing for 1 min, 5 µL of the mixture was analyzed by LC-MS/MS. The LC-MS/MS analysis was performed on a TSQ Quantum™ system that is composed of a triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc.), Analyst™ software, and a Genius HL-5000 HPLC system (Thermo Fisher Scientific Inc.).

2) LC-MS/MS method validation

Low (10.7 ng/ml), medium (17.5 ng/ml) serum control pools and a standard solution (SRM585) were used for estimating accuracy and precision. Accuracy was estimated by 4 different concentrations of standard solutions that contained 25OH-D3 and a standard solution for the 25OH-D2 analysis. Recovery was determined in the final mixture of mobile phase C containing 25OH-D3, 40%, 2-propanol, and 25OH-D2.

3) Assessment of preanalytical factors of 25OH-D

Serum and plasma samples, and the handling procedures used to test the preanalytical factors were as follows.

(1) To assess the effects of time intervals between the vacuum and sample separation, serum samples were collected in blood collection tubes (Sekisui Medical Co., Ltd.; B, C, D, E) and left at 4°C in a deep freezer and then thawed at room temperature for 24 h.

(2) To assess the effects of leaving serum samples at room temperature for 24 h, serum samples were left at room temperature for 24 h.

(3) To test the effects of freeze-thaw cycles, serum samples were frozen at −80°C, then thawed at room temperature for 24 h. Serum samples underwent 5 freeze-thaw cycles.

(4) To estimate the effects of the routinely used coagulants on 25OH-D measurements, 5 different types of blood collection tubes were investigated. The experiments were carried out with 4 healthy volunteers.

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Discussion

Effect of interference substances. Concentrations of 25OH-D3 determined in 120 serum samples, were less than 20% of the measured 25OH-D3 concentrations. Nine types of blood collection tubes were investigated. The experiments were carried out with 4 healthy volunteers.

Conclusion

We developed a LC-MS/MS method coupled with the TLX-2 HPLC system that can be used to perform complete quantification of serum 25OH-D3 and 25OH-D2. The results obtained were in good agreement with those obtained by a widely used commercial radioimmunoassay. This LC-MS/MS method may be applicable for routine determination of plasma 25OH-D levels in clinical laboratories.

Table 1. 1RRM transitions.

Table 2. LC method.

Table 3. LC-MS/MS assay performance.

Table 4. Effect of interference substances.

Table 5. To estimate the effects of the routinely used coagulants on 25OH-D measurements, 5 different types of blood collection tubes were investigated. The experiments were carried out with 4 healthy volunteers.

Figure 1. Linear regression analysis of the 120 clinical samples examined by the RIA and LC-MS/MS assays.

Figure 2. Concentrations of 25OH-D3 determined in 120 healthy, Japanese subjects (60 men and 60 women) by the LC-MS/MS assay. Subject age (mean±SD) male, 49.3±14.9; female, 48.5±15.7.

Figure 3. Time courses of the 25OH-D3 concentrations between the venipuncture and blood collection tubes. The diamond, square, triangle and circle marks indicate 25OH-D3 concentrations in serum of 4 healthy volunteers.

Figure 4. Time courses of the 25OH-D3 concentrations between the venipuncture and blood collection tubes. The diamond, square, triangle and circle marks indicate 25OH-D3 concentrations in serum of 4 healthy volunteers.

Figure 5. The effect of repeated freeze-thaw cycles on 25OH-D3 concentrations in serum of 4 healthy volunteers.

Figure 6. The effects of anticoagulants or separating gel on 25OH-D3 concentrations. Nine types of blood collection tubes were investigated. The experiments were carried out with 4 healthy volunteers.