

25OHD₃ AND ITS EPIMER: DETERMINATION BY LC/MS-MS

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ABSTRACT

The C-3-epimer of 25-OH-vitamin D₃ (25OHD₃) has been detected in significant concentrations in serum samples of infants under the age of one year (Fig.1).

Its downstream metabolite 3-epi-1,25-(OH)₂-vitamin D₃ (C-3 epimer) has been shown to have reduced calcemic effects in bone metabolism when compared to the physiologically active form 1,25-(OH)₂-vitamin D₃. Thus, monitoring the 25OHD status of this age group should also include the concentration of the epimeric form. Here we describe a new method suitable for daily routine analysis by LC-MS/MS that can distinguish between the forms 25OHD₂, 25OHD₃ and its C-3-epimers (Fig.2).

Structure

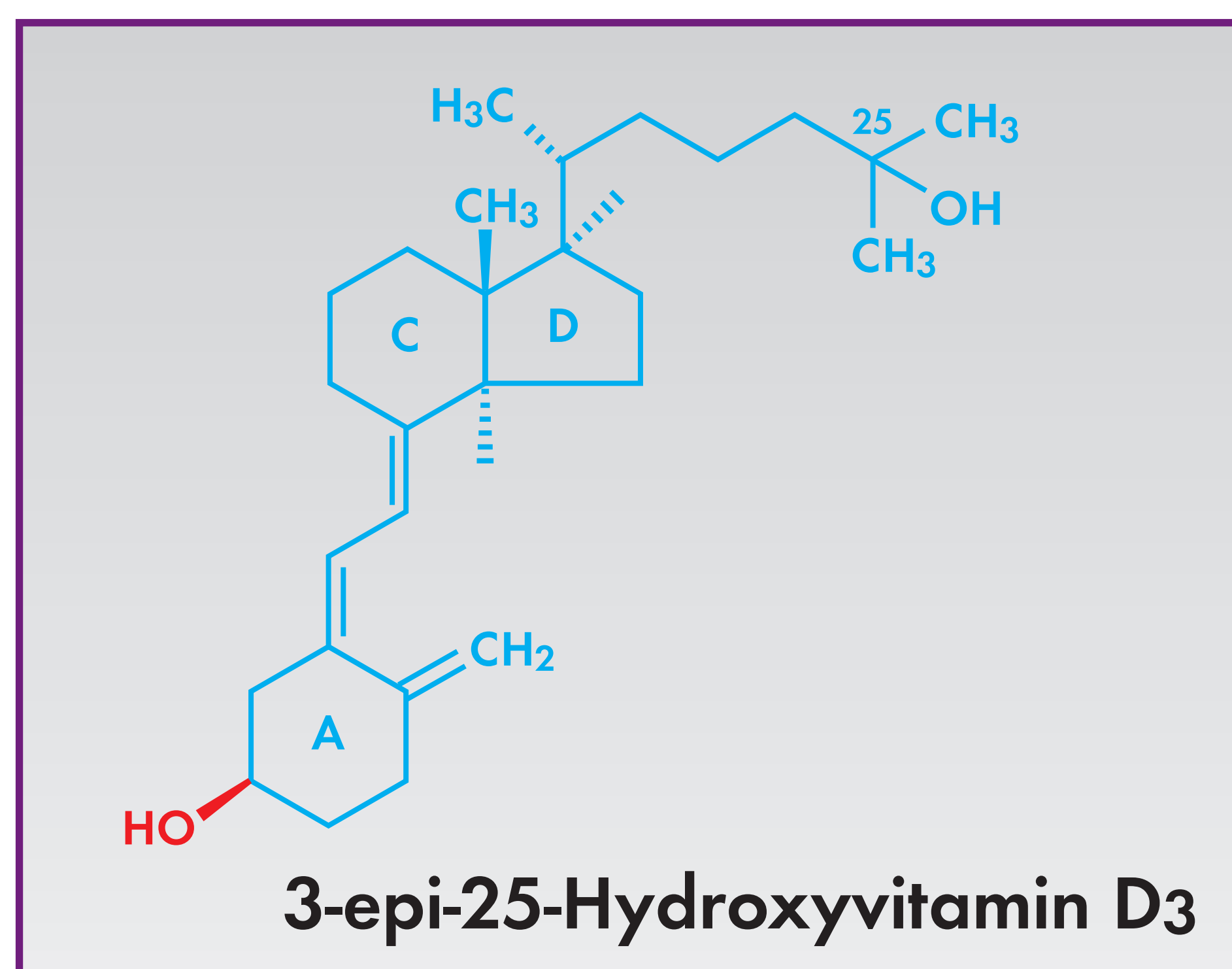
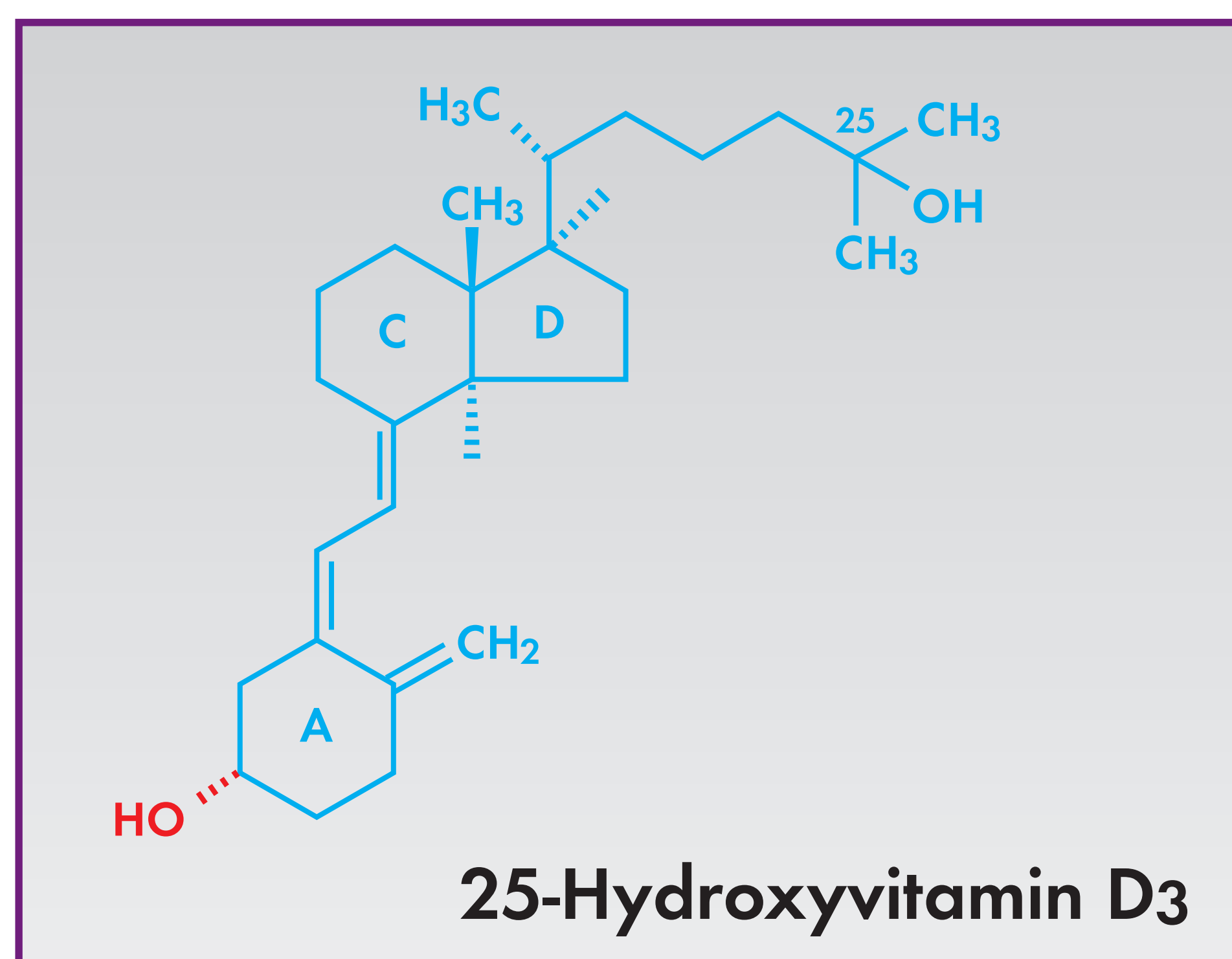


Figure 1: Chemical structures of vitamin 25OHD₃ and 3-epi-25OHD₃.

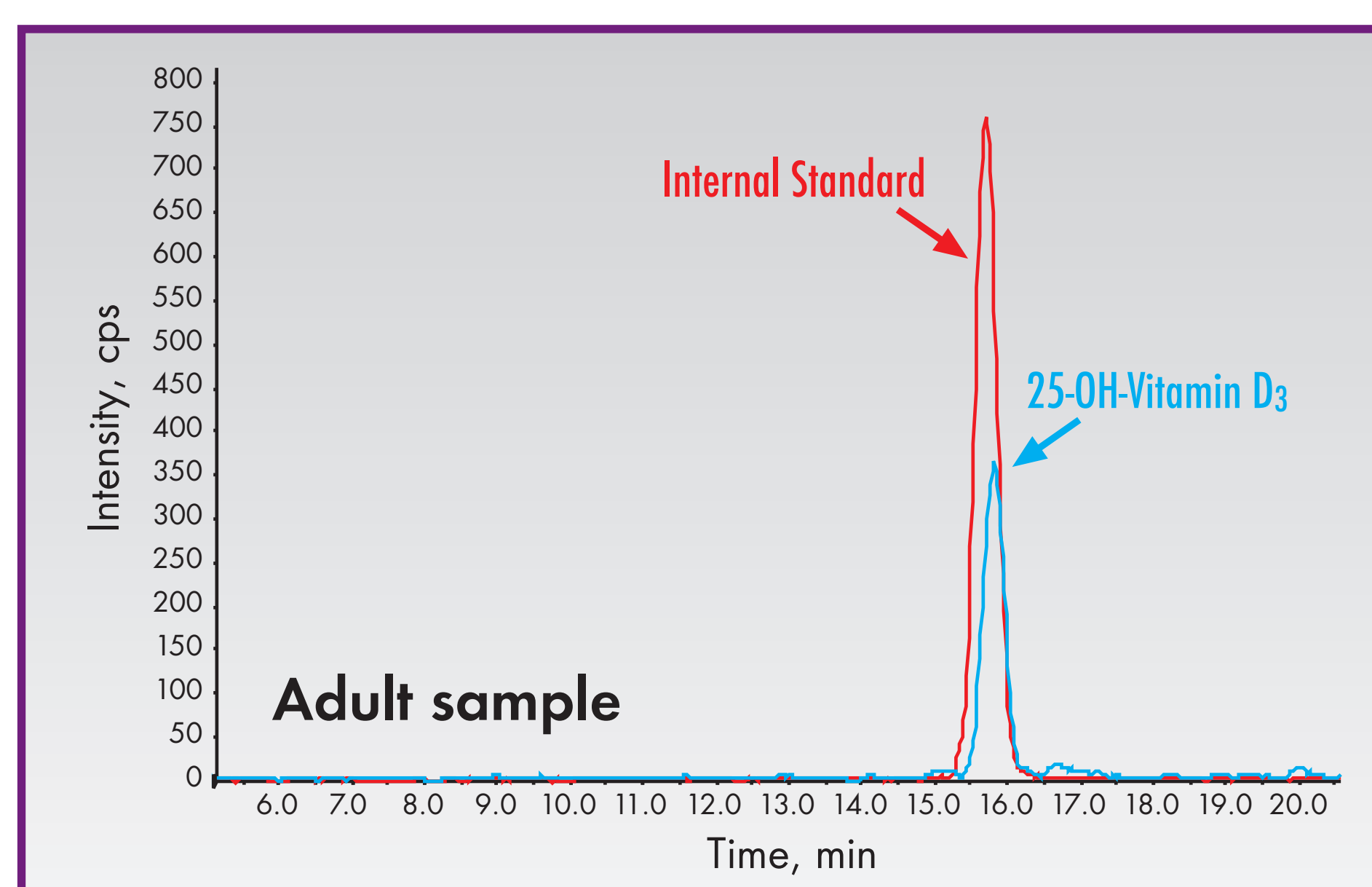
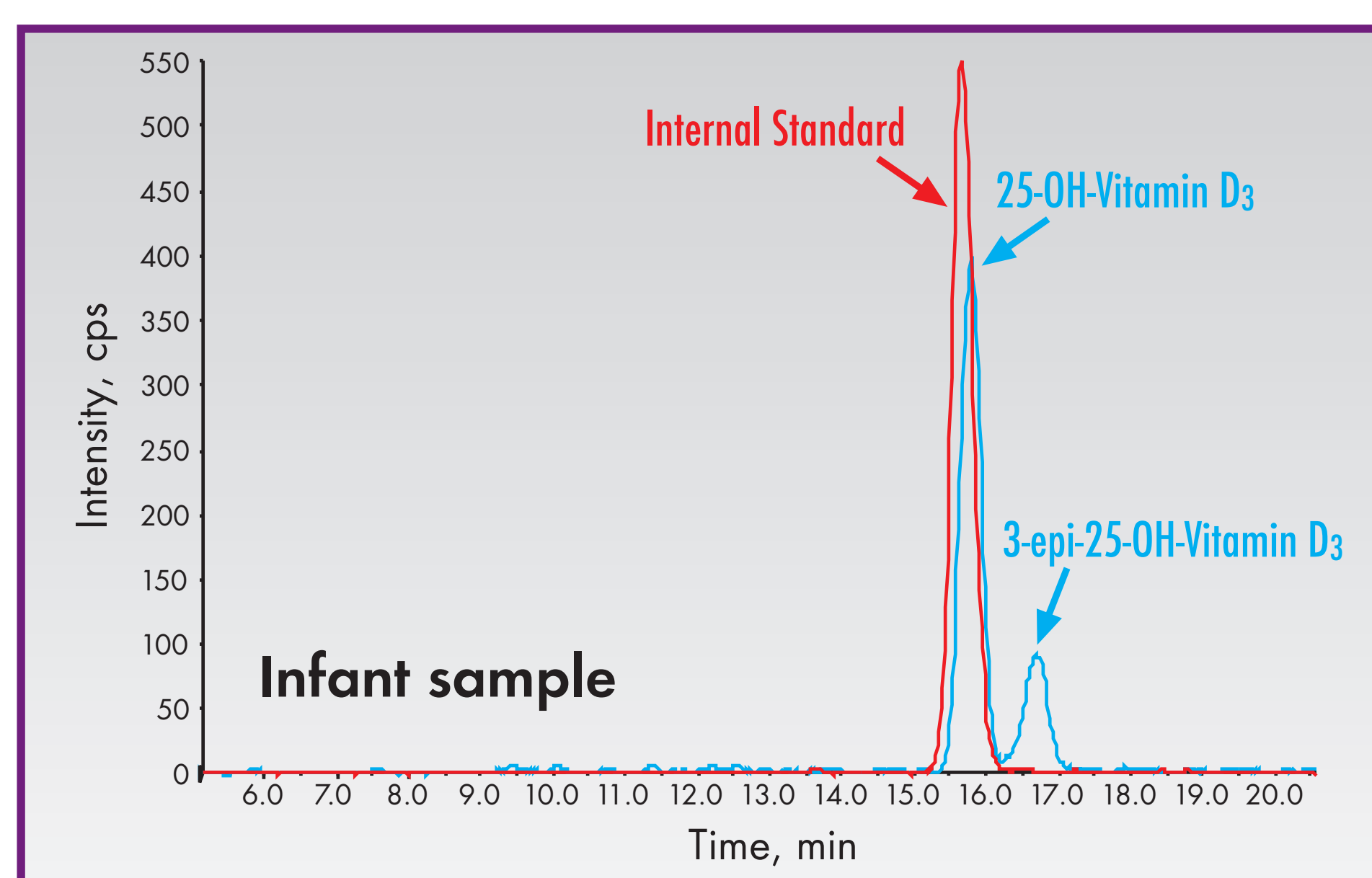


Figure 2: Typical chromatograms of an infant sample containing 42.3 µg/l 25OHD₃ and its epimer (15.3 µg/l) in contrast to an adult sample containing 26.2 µg/l 25OHD₃.

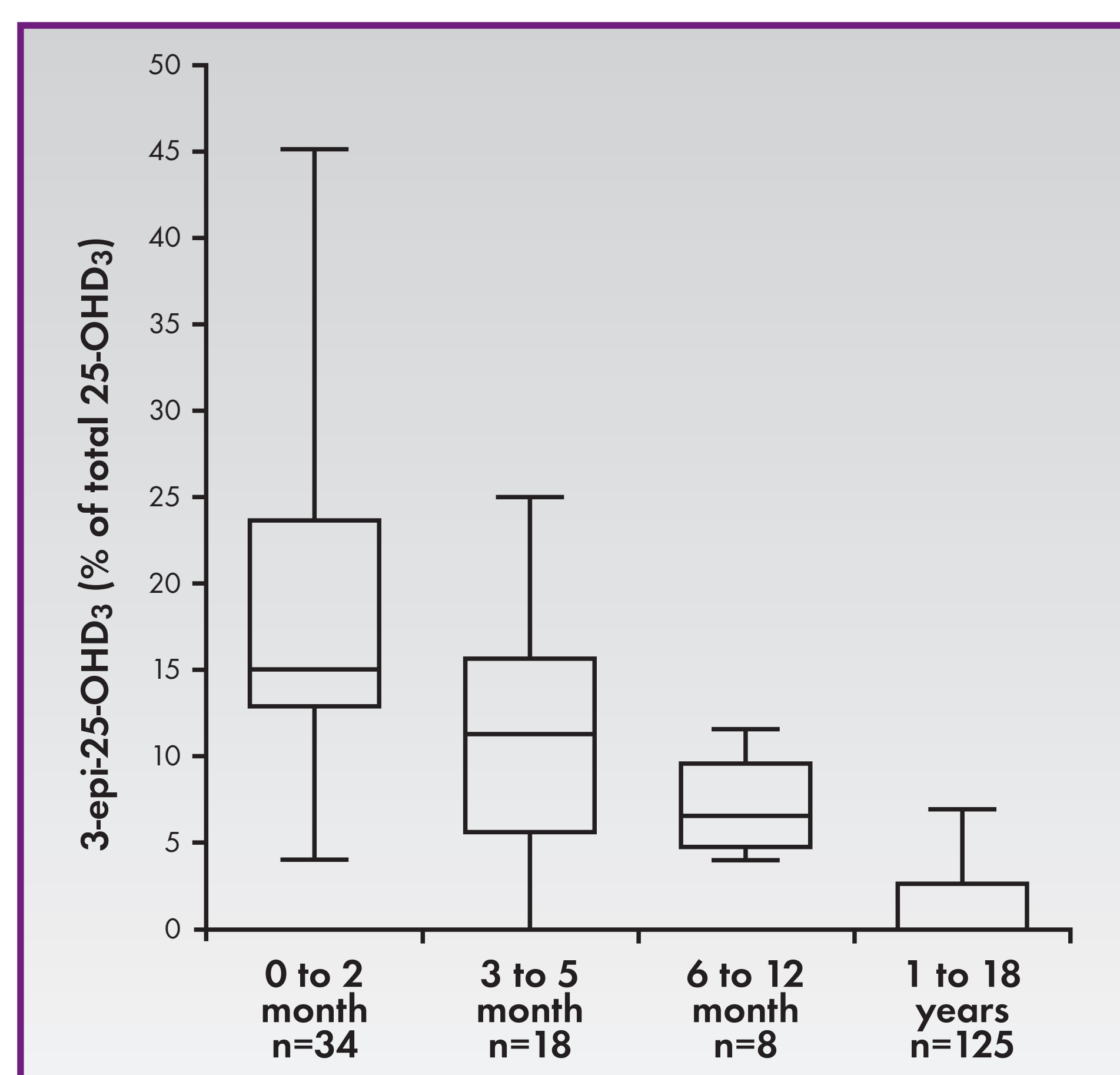


Figure 3: Boxplots are showing the 3-epi-25-OHD₃ percentage of total 25OHD₃ within four age groups (infants 0–2 months, 3–5 months, 6–12 months and children 1–18 years). The C-3-epimer percentage seems to be age related with a trend to decreasing amounts with higher age.

INTRODUCTION

The D-vitamins are a group of secosteroids with the physiologically relevant forms vitamin D₂ and D₃. While the largest amount of vitamin D₃ is produced in humans' skin when induced by sunlight, vitamin D₂ occurs mainly in plants and plays only a minor role in human nutrition. Vitamin D is important for a lot of biological processes, such as mineralisation and growth of bones. Monitoring the vitamin D status is mainly performed by measuring the concentrations of the 25-OH metabolites in plasma or serum. Several methods are available for this purpose. Very recently, a British and an American Expert Panel – supported by Scandinavian colleagues – recommended LC-MS/MS as a method of choice (1, 2). Discrimination between

25OHD₃ and its C-3-epimer of which is commonly detected in significant concentrations in serum samples of young infants under the age of one year, is possible only by using the LC-MS/MS technology. The C-3 epimer seems to be of clinical relevance, as it shows differences in biological activity when compared to 25OHD₃ (3, 4). Additionally, it contributes to an overestimation of the serum 25OHD₃ level which could lead to inappropriate treatment in newborns or very young children (5).

MATERIAL AND METHODS

Chromsystems' *MassChrom*[®] Kit (Order number 62000) in combination with a high resolution HPLC column was used for all vitamin D measurements. 100 µl sample was mixed with 25 µl of Precipitation Reagent and 200 µl of Internal Standard solution. After incubating for 10 min at +4 °C the samples were centrifuged for 5 min at 15000 g. 50 µl of each supernatant was injected into the LC-MS/MS system (AB Sciex API 3200 triple/Shimadzu LC 20). Sample cleanup was performed online using a column switching technique. About 200 serum samples of very young infants (new born to 12 months), children and young adults (1 to 18 years) were screened for their 25OHD₃ and its C-3-epimer amount. Additionally, serum samples of adult volunteers were analysed for the C-3-epimer amount.

RESULTS AND DISCUSSION

Chromsystems provides a robust, precise and accurate high-throughput method (*MassChrom*[®] for 25OHD₃/D₂) for the daily routine analysis of 25OHD₃ and D₂ in plasma and serum. With the option of separating the C-3 epimer of 25OHD₃ in infant samples by simply replacing the standard HPLC column with a high resolution column it fulfils all requirements as demanded by expert panels in recently published reports including the NIST standardisation (1). Current measurements of around 200 infant and adolescent samples confirmed previously studies (5, 6): In infant samples the C-3-epimer was found in high amounts. Moreover the C-3-epimer percentage seems to be age related within this age group (Fig. 3). In contrast, only insignificant amounts could be detected in adolescents samples (1 to 18 years). In adult samples we generally did not find the epimeric form above the limit of quantification of 6.9 µg/l. As an exception a concentration of about 30 µg/l of C-3-epimer was detected in a serum sample of a highly vitamin D supplemented 70-year-old person (391 µg/l).

Our data suggests that the C-3-epimer plays a significant role in the vitamin D metabolite pattern of infants, whereas it does not in older children and adults. An international detailed study is ongoing in our laboratories to investigate the 25OHD₃ and its C-3-epimer in infants and young adults.

CONCLUSION:

- It is necessary to discriminate between the epimeric forms of 25OHD₃ when analysing infant serum samples as they show differences in biological activity in terms of bone metabolism.
- The Chromsystems *MassChrom*[®] Kit fulfils all requirements for the vitamin D screening of all age groups.

References:

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