D-binding protein or other serum proteins. Surfactants like Triton X100 or Tween-20 have been used to prevent the vitamin D compound from being non-specifically attached to lipids and proteins in the sample after denaturation.

It is particularly difficult to automate a test for a vitamin D compound. The automation requires solving a very difficult problem i.e. surviving a tightrope walk: On the one hand it is necessary to release the vitamin D compounds from vitamin D-binding protein with the aid of a suitable release reagent, on the other hand, the conditions have to be selected such that the sample can be directly analysed further. A prerequisite of this direct further analysis is that, on the one hand, the endogenous vitamin D-binding protein does not bind or no longer to a significant extent binds to the vitamin D compounds during this analysis and thus does not interfere with this analysis and, on the other hand, that the release reagent used does not interfere with the binding of detection reagents such as antibodies, or vitamin D-binding protein. In addition it is known that different alleles of the vitamin D-binding protein are present in the human population which behave biochemically differently. The release and measurement of vitamin D compounds should be comparable for various alleles/phenotypes.

Thus the object of the present invention was to develop a reagent composition for release of vitamin D compounds and in particular for hydroxyvitamin D compounds from vitamin D-binding protein in a sample which can at least partially overcome the problems of the prior art. A suitable reagent composition for releasing vitamin D compounds, an in vitro method for determining vitamin D compounds the use of the reagent composition and kits for the determination of vitamin D compounds using this reagent composition are described in the following and are encompassed by the attached claims.

Summary ~~of the Invention~~

The present disclosure~~invention~~ concerns an in vitro method for releasing a vitamin D compound from vitamin D-binding protein comprising the step of a) providing a sample to be investigated and b) mixing the sample from step (a) with i) a reagent containing a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing the vitamin D compound from vitamin D-binding protein.

In a first aspect, there is provided an in vitro method for releasing a vitamin D compound from vitamin D-binding protein comprising the step of: a) providing a sample to be investigated and b) mixing the sample from step (a) with i) a reagent containing a hydrogen carbonate salt in a concentration of 0.1M to 2.0 M or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing the vitamin D compound from vitamin D-binding protein.

In a further embodiment the present disclosure~~invention~~ concerns an in vitro method for measuring a vitamin D compound comprising the steps of a) providing a sample to be investigated, b) mixing the sample from step (a) with i) a reagent containing a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing a vitamin D compound from vitamin D-binding protein, and c) measuring the vitamin D compound released in step (b).

In a second aspect, there is provided an in vitro method for measuring a vitamin D compound comprising the steps of: a) releasing a vitamin D compound from vitamin D-binding protein according to the method as defined above and b) measuring the vitamin D compound released in step (a).

In a further embodiment the present disclosure~~invention~~ concerns a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M and a reducing agent.

In a third aspect, there is provided the use of a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising a hydrogen carbonate salt in a concentration of 0.1 M to 2.0 M or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M and a reducing agent.

In a further embodiment the present disclosure~~invention~~ concerns a reagent mixture comprising a sample to be investigated, a reagent composition for the release of a vitamin D compound from vitamin D-binding protein, a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M and a reducing agent, and an alkalinising agent.

In a fourth aspect, there is provided a reagent mixture comprising a sample to be investigated, a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising a substance selected from the group consisting of a hydrogen carbonate salt in a concentration of 0.1 M to 2.0 M or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to

2.0 M, and a reducing agent, and 0.1 M to 2.0 M of an alkalinising agent selected from the group consisting of NaOH, KOH, $Ca(OH)_2$ and LiOH, wherein the sample is blood, serum or plasma, wherein the hydrogen carbonate salt is selected from the group consisting of sodium hydrogen carbonate, potassium hydrogen carbonate, ammonium hydrogen carbonate, calcium hydrogen carbonate and magnesium hydrogen carbonate, and wherein the reducing agent is selected from the group consisting of 2-Mercaptoethanol, 2-Mercaptoethylamine-HCl, TCEP, Cystein-HCl, Dithiothreitol(DTT), N-Methylmaleimide, Ellman's Reagent and 1,2-dithiolane-3-carboxylic acid.

In a further embodiment the present disclosure~~invention~~ concerns a kit for the release of a vitamin D compound from vitamin D-binding protein, which contains a reagent composition comprising a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M and a reducing agent.

In a fifth aspect, there is provided the use of a reagent composition comprising a substance selected from the group consisting of a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M and a reducing agent, together with an alkalinising agent selected from the group consisting of NaOH, KOH, $Ca(OH)_2$ and LiOH to release a vitamin D compound from vitamin D-binding protein.

Detailed Description

The present invention concerns an in vitro method for releasing a vitamin D compound from vitamin D-binding protein comprising the step of a) providing a sample to be investigated, b) mixing the sample from step (a) with i) a reagent containing a hydrogen carbonate salt or a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis in a concentration of 0.1 M to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing the vitamin D compound from vitamin D-binding protein.