# Identification of L-3-Hydroxybutyrate as an Original Ketone Body in Rat Serum by Column-Switching High-Performance Liquid Chromatography and Fluorescence Derivatization

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**Abstract**

L-3-Hydroxybutyrate (L-3HB), the enantiomer of D-3-hydroxybutyrate (D-3HB), has traditionally been regarded the "unnatural" ketone body in mammals, although there is suspicion that it is a more-favorable energy fuel for mammalian tissues than D-3HB. In this study, we demonstrated that L-3HB is an original substance in rat serum by applying fluorescence derivatization and a column-switching high-performance liquid chromatography system as the analysis technique. Racemic 3HB in rat serum derivatized using 4-nitro-7-piperazino-2,1,3-benzoxadiazole was first separated by an ODS column and was further confirmed by verifying the disappearance of the racemic 3HB peak after pretreating rat serum with D-3-hydroxybutyryl dehydrogenase. A switching valve was used to simultaneously introduce isolated racemic 3HB to the enantiomeric separation by two CHIRALCEL OD-RH columns connected in tandem. An L isomer was found to accompany the D isomer, which were quantified to be 3.98 microM (3.61%) and 106.20 microM (96.39%), respectively. Using the present analytical method, the dubious interpretation of the existence of L-3HB was clarified.