Introduction: Farber disease is an autosomal recessive, extremely rare disease caused and characterized by a deficient acid ceramidase activity encoded by ASAH1 gene. Low ceramidase activity is resulting in accumulation of fatty substances, mainly ceramides. The typical clinical key features of Farber disease are periarticular nodules, lipogranulomas, swollen and painful joints and a hoarse voice or a weak cry. In about 40% of all cases we have a late-onset and monosymptomatic phenotype with hepatosplenomegaly or rapid neurological deterioration or developmental delay. Materials and method: We present a new method of diagnosis of Farber disease by determining the concentration of C26 ceramide isoforms using LC/MS/MS followed by ASAH1 gene sequencing for confirmation. Moreover, we found that cis-isomer of the C26 ceramide is a specific biomarker for Farber disease, with pathological values in a range of 39.2-150.0 nmol/L blood (normal range 13.6-23.4 nmol/L blood, N=192, healthy individuals). Summary: The new biomarker can be determined directly in the dried blood spot (DBS) extract with low sample consumption, easy sample preparation, high reproducibility and it presents the possibility of being used in high throughput screenings.