A microfluidic sensor is developed and targeted at specific ingredients determination in drug/food/beverage matrices. The surface of a serpentine polydimethylsiloxane (PDMS) microchannel is modified by enzyme via physisorption. When solutions containing target ingredients pass through the microfluidic channel, enzyme-catalyzed reaction occurs and only converts the target molecules to its products. The whole process is monitored by an end-channel UV/vis spectroscopic detection. Ascorbate oxidase and L-ascorbic acid (AA) are taken as enzyme-substrate model in this study to investigate the feasibility of using the developed strategy for direct quantification of AA in standard solutions and complex matrices. A dietary supplement product, vitamin C tablet, is chosen as a model matrix to test the microfluidic bio-sensor in real-sample analysis. The results illustrate that the established microfluidic biosensor exhibits good reproducibility, stability, and anti-interference property. Technically, it is easy to realize, depends on low investment in chip fabrication, and simple instrumental procedure, where only UV/vis spectrophotometer is required. To sum up, the developed strategy is economical, specific, and accurate, and can be potentially used for fast quantification of ingredient in samples with complex matrix background. It is promising to be widely spread in food industry and quality control department.