LC-MS OF CHIRAL HYDROXYCARBOXYLIC ACIDS

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INTRODUCTION

The large variety of both naturally occurring, scientifically interesting as well as industrially valuable hydroxycarboxylic acids contains numerous optically active compounds with chiral centers at the carbon atom carrying the hydroxy functional group. As the absolute configuration and the enantiomeric purity is highly important and of both scientific and industrial interest, methodologies for manufacturing and analysing pure enantiomers have been key success factors for progress. Direct enantiomer analysis of underivatized hydroxycarboxylic acids by enantioselective separation, e.g. by capillary electrophoresis or chromatography, is thereby preferred, as this enables to distinguish metabolic pathways and to quantify the enantiomers in complex samples. The direct HPLC-separation on chiral stationary phases of highly polar or charged metabolite enantiomers carrying hydroxy- and carboxy-groups and their sensitive detection requires high separation efficiency and compatibility. While aromatic hydroxycarboxylic acids are easily detected by UV-detectors, we have analyzed non-UV-absorbing aliphatic chiral hydroxycarboxylic acids by capillary electrophoresis with indirect UV-detection, GC-detection of their methylesters and LC-MS. LC-MS has recently obtained increased attention for the analysis of the important subgroup of chiral aliphatic mono- and dicarboxylic acids with a hydroxy-group in the 2- or 3-position [1,2]. Chiral 2-Hydroxyglutaric acids provide important molecular signatures of both healthy and diseased biological cells, their specific biochemical pathways and inborn errors of metabolism. The chiral differentiation and quantification of (S)-2-hydroxyglutaric acids and (R)-2-hydroxyglutaric acids is key for characterizing neuro-metabolic disorders like the 2-hydroxyglutaric acidurias, which cause neurological impairment at young age [3]. In human brain tumor patients mutations in the enzyme cytosolic isocitrate dehydrogenase 1 (IDH1) are found approximately 80% of grade II-III gliomas and secondary glioblastomas and the demonstration that cancer-associated IDH1 mutations result in a new ability of the enzyme to catalyze the NADPH-dependent reduction of α-ketoglutarate to the oncometabolite R-(−)-2-hydroxyglutarate [4] represents a milestone event in cancer biology. Cancer-associated IDH mutations in IDH1 and IDH2 across glioma as well as several hematologic malignancies have become of prognostic interest and for biomarkers and therapeutic opportunities [5].

MATERIALS AND METHODS

A Chirobiotic R CSP was run under polar ionic mobile phase conditions. The separation conditions of Rashed et al. [6] have been improved for MS compatibility and separation efficiency. The addition of ethanol to the organic mobile phase together with 0.1% triethylammonium acetate (TEEA) improves the peak shape for the D-enantiomer. The use high resolution mass spectrometry eliminated two fundamental analytical challenges: the lack of a UV-absorbing chromophore in the 2-OHG molecule, and the abundance of competing low molecular weight acids in urine.

RESULTS

Chiral Chromatography of α-Hydroxyglutaric acids: Selectivity of Peptide-based CSPs (Chiral Stationary Phases)

Mainly CSPs are used to distinguish between D/L enantiomers of metabolites. The teicoplanin aglycone CSP (Chirobiotic TAG) cannot only be applied in clinical chemistry for the qualitative and quantitative determination of the lactic acid enantiomers in blood samples, but also for various other alpha-hydroxycacid enantiomers. The baseline separation of the enantiomers of (S)- and (R)-2-Hydroxy-3-methylbutyric acid and the (S)- and (R)-2,3-Dihydroxy-3-isovaleric acid enables an excellent determination of enantiomeric purity, while the (R)- and (S)-3-Hydroxybutyric acid enantiomers are not at all separated, illustrating the selectivity of the CSP in relation to the position of the hydroxyl group.

REFERENCES