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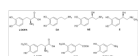
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Determination of catecholamines and endogenous related compounds in rat brain tissue exploring their native fluorescence and liquid chromatography

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Highlights

- A LC method for the analysis of main catecholamines and endogenous related compounds.
- This LC-FLD method is based on the native fluorescence of target analytes.
- The method enables an easy and fast analysis of the analytes in rat brain tissue.
- The method was successfully validated and requires a small sample volume.

Abstract

The profiling analysis of catecholamines and their metabolites in brain tissue offers a crucial key to understand their functions in the body and the opportunity to follow up neural diseases. A rapid and simple liquid chromatography-fluorescence detection (LC-FLD) method was developed and validated for simultaneously measuring several catecholamines and endogenous related compounds in the rat brain tissue samples. The target analytes measured in this bioanalytical assay were levodopa (L-DOPA), dopamine (DA), norepinephrine (NE), epinephrine (E), 3-O-methyldopa (3-O-MD), and homovanillic acid (HVA), being the 3,4-dihydroxybenzylamine (DHBA) used as internal standard (IS). The six analytes (L-DOPA, DA, NE, E, 3-O-MD and HVA) can be determined in a single chromatographic run of less than 12 min, and all the compounds (analytes and IS) were detected using their native fluorescence and monitored at excitation/emission wavelengths of 279 nm/320 nm, respectively. The chromatographic and detection conditions were experimentally optimized and then several validation parameters (linearity, limits of quantification and detection, precision and accuracy, recovery, stability and selectivity) were examined. In accordance with the international guidelines of the Food and Drug Administration and European Medicines Agency the method described herein exhibited limits of quantification in the range of 2–25 ng mL⁻¹, linearity in wide concentration ranges ($r^2 \geq 0.994$), and acceptable precision (coefficient variation $\leq 8.76\%$) and accuracy ($bias \pm 14.65\%$) levels. Since the bioanalytical procedure does not involve pre-purification or derivatization of the sample, the absolute recovery was found to be around 100%. Moreover, the developed LC-FLD method was

successfully applied for the determination of the compounds of interest in tissue samples of different rat brain regions (cerebellum, amygdala, cortex, hippocampus, striatum, mesencephalon, medulla oblongata, *substantia nigra* and ventral tegmental area). Hence, this assay represents a valuable bioanalytical tool to support several pre(non)clinical studies in the broad field of neurosciences, requiring the quantitative analysis of these bioamines and their metabolites.

Abbreviations

3-O-MD, 3-O-methyl dopa; CV, coefficient of variation; DA, dopamine; DHBA, 3,4-dihydroxybenzylamine; E, epinephrine; ECD, electrochemical detection; EMA, European Medicines Agency; FDA, Food and Drug Administration; FLD, fluorescent detection; HVA, homovanillic acid; IS, internal standard; L-DOPA, levodopa; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NE, norepinephrine; OSA, octane sulfonic acid; QC, quality control

Keywords

Catecholamines; Native fluorescence; Brain tissue; Bioanalytical method validation

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