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# Simultaneous Liquid-Chromatographic Determination of Plasma Catecholamines and Metabolites

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**Featured Article:** Eisenhofer G, Goldstein DS, Stull R, Keiser HR, Sunderland T, Murphy DL, Kopin IJ. Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase. Clin Chem 1986;32:2030–3.[3]

This report described a liquid chromatographic electrochemical detection (LCED)[4] method for measuring plasma concentrations of catecholamines as well as dihydroxyphenylglycol (DHPG), the main deaminated metabolite of norepinephrine, dihydroxyphenylalanine (DOPA), the immediate precursor of dopamine, and dihydroxyphenylacetic acid (DOPAC), the deaminated metabolite of dopamine.

Several LCED methods for plasma catecholamines had already been introduced and validated against the radioenzymatic assay, the previous standard method for analyzing catecholamines. The major advance of our method was the ability to measure DHPG, DOPA, DOPAC, and all 3 endogenous catecholamines simultaneously. This was achieved using (1) batch alumina extraction, a simple yet remarkably effective method for purifying catechols; (2) separation of DHPG from the solvent front, facilitated by a postcolumn series of coulometric electrodes; and (3) optimization of the chromatography and mobile phase. The multielectrode coulometric system enabled preoxidation of irreversibly oxidized contaminants and reversibly oxidized catechols; the latter were then measured at a reducing potential, decreasing interference from chromatographic contaminants. The method's robustness provided utility for measurements in plasma, urine, cerebrospinal fluid, microdialysates, homogenized tissue extracts, and insect hemolymph.

The method was useful in several ways for research laboratories focusing on studies of catecholamine systems in health and disease. The combined measurements of norepinephrine and DHPG in particular led to a realization that deamination represented the primary determinant of norepinephrine turnover in sympathetic nerves *(1)*. Measurements of DHPG not only reflected intraneuronal turnover of norepinephrine, but with additional radiotracer kinetic methods could be used to quantify 2 contributing processes: leakage of norepinephrine from vesicles into the neuronal cytoplasm and reuptake of the transmitter after exocytotic release *(2)*. Measurements of DOPA, the product of the enzymatic rate-limiting step in catecholamine synthesis, provided information about links between transmitter synthesis and turnover. Combined measurements of catecholamines, DOPA, DHPG, and other metabolites led to a more complete and precise understanding of catecholamine systems and their kinetics than was possible from measurements of catecholamines alone *(3)*. In patients with cardiac failure, for example, increased spillover of norepinephrine from the heart into coronary sinus plasma was shown to reflect both increased exocytotic release and decreased efficiency of norepinephrine reuptake via the norepinephrine transporter *(2)*. Cardiac transmitter stores were depleted, leading to a reduced contribution of "leakage" to transmitter turnover and a reduced requirement for activation of tyrosine hydroxylase, as assessed from measurements of cardiac spillover of DOPA.

Recognition of the clinical utility of the method led to its adoption by other research groups. At the Baker Medical Research Institute in Australia, the method was introduced to characterize sympathetic nerve function and central nervous system turnover of norepinephrine in depression, heart failure, hypertension, panic disorder, and obesity *(4)*. At Vanderbilt University, the method was used to characterize defective function of the norepinephrine transporter in patients with postural tachycardia syndrome *(5)*. The same group and others have used the method to characterize the neurochemical profiles of dopamine-$\beta$-hydroxylase deficiency, DOPA decarboxylase deficiency, tyrosine hydroxylase deficiency, Menkes disease,

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[3] This paper has been cited 390 times since publication.
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[4] Nonstandard abbreviations: LCED, liquid chromatographic electrochemical detection; DHPG, dihydroxyphenylglycol; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid.

and X-linked deficiencies of monoamine oxidase. The method has been similarly useful for characterizing catecholamine systems in transgenic animal models developed to explore the function of genes impacting neuronal and endocrine systems. As one of only a few available methods to assess neuronal membrane norepinephrine transporter function in vivo, the method has been particularly useful to assess various clinical conditions associated with altered transporter function and in clinical trials of drugs inhibiting the transporter *(6)*.

Continued use of our method since its introduction has resulted in the highly cited nature of the original report. Current research spawned from our method applies LCED methodology to measure concentrations of other catechols such as isoproterenol, 2,3-dihydroxybenzoic acid (hydroxyl radical production), dihydroxyphenylacetic acid (main neuronal metabolite of dopamine), L-dihydroxyphenylserine (norepinephrine prodrug), and dihydroxyphenylacetaldehyde (cytotoxic metabolite of dopamine).

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