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POST-COLUMN TERBIUM COMPLEXATION AND SENSITIZED FLUORESCENCE DETECTION FOR THE DETERMINATION OF NOREPINEPHRINE, EPINEPHRINE AND DOPAMINE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Terbium sensitized fluorescence was used as a post-column detection system to develop a simple, sensitive and rapid high-performance liquid chromatographic method for the simultaneous determination of catecholamines norepinephrine (NE), epinephrine (E) and dopamine (DA).

Catecholamines were separated by an ion-pair reversed-phase chromatography on a BDS-Hypersil analytical column with a mobile phase of methanol and 50 mmol l⁻¹ acetate buffer (pH 4.7) containing 1.1 mmol l⁻¹ SOS and 0.11 mmol l⁻¹ EDTA (15 + 85 v/v).

Catecholamines and the internal standard (3,4-dihydroxybenzylamine, DHBA) were post-column derivatized by the addition to the eluent of an alkaline solution containing a stoichiometric mixture of terbium (III) chloride and EDTA. Fluorescence detection (λₑₓ = 300 nm, λₑₘ = 545 nm) is based on the sensitization of terbium ion fluorescence after complexation with catecholamines.

The chemical compatibility between the eluent and the post-column reagent was studied and the analytical characteristics of the method were established. Detection limits found were 1.0×10⁻⁸, 4.0×10⁻⁸ and 7.0×10⁻⁶ mol l⁻¹ for NE, E and DA, respectively. The method has been successfully applied to the determination of catecholamines in urine samples after solid-phase extraction (SPE) pretreatment. Recoveries from urine spiked with NE (4.0×10⁻⁷, 2.0×10⁻⁶ and 4.0×10⁻⁶ mol l⁻¹), E (8.2×10⁻⁸, 4.1×10⁻⁷ and 8.2×10⁻⁷ mol l⁻¹) and DA (1.0×10⁻⁷, 5.0×10⁻⁷ and 1.0×10⁻⁷ mol l⁻¹) varied from 98 to 100 % (mean = 99.3 %), from 106 to 107 % (mean = 106.3 %) and from 98 to 101 % (mean = 99.3 %), respectively. The between-run precision (RSD) for the method for three urine samples at different concentration levels of each catecholamine varied from 3.6 to 7.0 %.