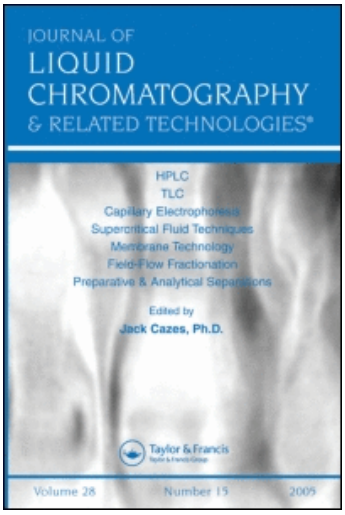

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Measurement of 3,4 Dihydroxyphenyl Ethylene Glycol (DOPEG) in Plasma by High Performance Liquid Chromatography with Electrochemical Detection

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MEASUREMENT OF 3,4 DIHYDROXYPHENYL ETHYLENE GLYCOL (DOPEG) IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

Usually, determination of DOPEG in plasma by EC-HPLC is always performed after extraction on alumina. This paper presents an extraction on boric acid gel, with good selectivity, reproducibility. In high sensitivity conditions (0.5 nA full scale) an autosampler is used and presents two major advantages: first, the possibility of storing samples in cold conditions to preserve stability of DOPEG, second the analysis of numerous samples in pharmacological studies, for example.

INTRODUCTION

Liquid chromatography with electrochemical detection (LC-ED) is very useful for the determination of catecholamines and 3,4 dihydroxyphenylethyleneglycol (DOPEG) after extraction always performed on alumina.[1,2]

In this paper, we describe a rapid procedure for the determination of DOPEG, a metabolite of norepinephrine (NE), by LC-ED after extraction on boric acid gel.

Indeed, DOPEG is a prominent intraneuronal MAO-A dependent metabolite of NE. For example, determination of DOPEG in plasma is a good biological marker to quantitatively estimate the inhibition of MAO-A in vivo, by antidepressor IMAO.[3,4] Because such pharmacological studies require numerous assays, an autosampler was used.

The main advantages of the method are the following: the extraction procedure of DOPEG from plasma is original and satisfactory, automation of the HPLC method was established with high reproducibility and sensitivity.

EXPERIMENTAL

Reagents and Chemicals

The boric acid gel (Affigel 601) was purchased from Biorad (California, USA). Acetic acid, sodium acetate and citric acid were of analytical-reagent grade from E. Merck (Darmstadt, Germany), EDTA, clorgyline, sodium octylsulfate and DOPEG were obtained from Sigma (St Louis, MO, USA).

Stock solution of DOPEG was prepared at a concentration of 5mg/mL in 0.1M perchloric acid and stored at -80°C for three months. The working standard containing (per mL) 2.5 ng in 0.75M acetic acid was prepared every day.

Extraction Procedure

The withdrawal of venous blood was performed in a tube containing 30 µL of 5% Na_2 EDTA and 20 µl of an IMAO-A (clorgyline 10^{-2}M). Blood was immediatly centrifuged at 1000 g for 15 min at 2-4°C, the plasma was collected and centrifuged at 11000 g, for 10 min and stored at -80°C (until extraction).

The extraction method was modified from Maruta.[5] Boric acid gel was first activated with successive acid and bases. Then 1 mL of plasma was extracted, DOPEG was desorbed with 200 µl of 0.75 M. acetic acid and an aliquot was injected into the HPLC system.

Apparatus and Chromatographic Conditions for Liquid Chromatography

The LC system consisted of one model 420 pump (Kontron Instruments SA, St Quentin-en-Yvelynes, France) and an autosampler 465 (Kontron) equipped with a refrigerated platinum and a 50 µl injection loop. The Nucleosil RP18 (5 µm) column (250 x 4.6 mm ID 5µm) was protected by a Brownlee RP18 precolumn (30 x 4.6 mm I.D). The mobile phase (pH 3.8 with glacial acetic acid) contained 50 mM of sodium acetate, 10 mM of citric acid, 50 mg/L of EDTA and 100 mg/L of sodium octyl sulfate. Before use, mobile phase was filtered on 0.45 µm and was continuously degased with helium gas during analysis. The flow-rate was 0.8 mL/min. DOPEG was detected with an amperometric detector (model 460 Waters, Millford, MA, USA) with a glassy carbon working electrode and a Ag/AgCl reference electrode at a potential of +700 mV. A microcomputer (Kontron) controlled the chromtographic system.

RESULTS AND DISCUSSION

Figure 1 shows the good resolution of DOPEG from a peak X present in standard solution and in plasma extract. Standard solution, prepared in acetic acid, is directly injected but acid desorbs an impurity (peak X) from the plastic tube. At such a sensitivity (0.5 nA full scale), it is not surprising to detect contaminants.

In plasma extract, impurity X has the same retention time as uric acid, that explains this large peak.

We can't replace plastic tubes in this method, because of the affigel extraction, but it's not a major problem, the contaminant doesn't interfere with DOPEG.

Alumina is commonly used by other authors for the plasma extraction of catecholamines and their metabolites. In this work, a boric acid gel (Affigel 601) was chosen. This gel is selective with an affinity for coplanar cis hydroxyl groups (cis-diols). Recovery (63 ± 3%) was determined after adding known amounts of DOPEG to a plasma treated in parallel.

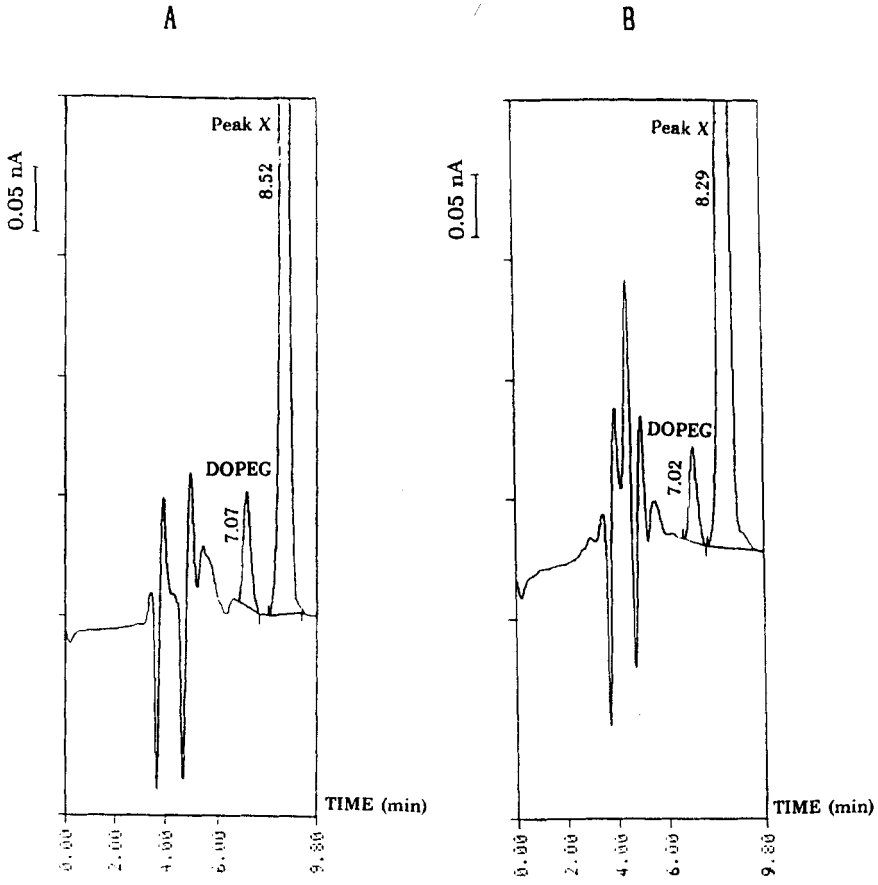

Figure 1. Chromatograms of (a) standard DOPEG (125 pg injected), peak X (impurity) and (b) plasma ; range : O.5 nA full scale ; chart speed 0.2 cm/min.

Calibration curve for DOPEG was established from 25 pg to 0.5 ng injected (y = 7.0204 x + 0.192, r = 0.999). The inter-assay and intra-assay coefficients of variation (n = 10) were 8.3% and 6.2% respectively for DOPEG. The limit of detection was 1 pg injected.

An application of this method is given in Table 1. Two groups of healthy subjects of different age were tested in parallel and show significant difference in DOPEG. Average concentrations of DOPEG obtained by this method agree with those reported by other authors.[6,7,8]

Table 1

DOPEG Plasma Concentrations (Mean ± SEM) in Relation to Age (Mean ± SEM)

	Group 1 (n = 14)	**Group 2 (n = 11)**
Age (years) mean ± SEM	68 ± 0.6	23 ± 0.5
DOPEG (pg/mL) mean ± SEM	1104 ± 90.2	778 ± 59.0

This method offers good reproducibility and sensitivity. The use of an autosampler with refrigerated platinum in such electrochemical detection conditions (0.5 nA full scale) is very interesting and useful in pharmacological and pharmacokinetic studies.

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