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Plasma catecholamines

Introduction

The catecholamines dopamine, noradrenaline and adrenaline play an important role in information transmission and regulation of metabolism in the body. Quantitative analysis of catecholamines is diagnostically important in a number of disease states such as hypertension, Parkinson's disease, schizophrenia, epilepsy etc. Of all detection methods available for the determination of catecholamines, HPLC with electrochemical detection is one of the most widely used. It is highly sensitive, selective and easy to apply.

Method

Samples of biological origin require a purification step before HPLC analysis. Often used pre-treatment procedures are ion-exchange, extractions using alumina (Al_2O_3) or diphenyl borate ethanolamine (DPBEA). A number of methods have been developed and have been reviewed extensively [1, 2].
In this application a DPBEA extraction is used. The principle of this extraction is based on a complex formation between borate and the catecholamine diol at alkaline pH. DPBEA is dissolved in phosphate buffer at pH 8. The resulting ion pair is extracted in an organic phase (heptane). This extraction step is followed by a second extraction into an aqueous phase (80 mM acetic acid), and injection into an HPLC system.
The HPLC system consists of a reversed phase column with an ion-pair added to the mobile phase (Table I). The retention of polar amines such as noradrenaline is selectively affected by the ion-pair concentration, acidic sample constituents are selectively affected by the pH, while the modifier percentage affects both. Optimisation of resolution is therefore done by variation of these three parameters.

References

1. F. Ponzio, G. Achilli and C. Perego, in Electrochemical detection in medicine and chemistry (Ed. Parvez et al.),p. 307 - 329, 1987 VNU Science Press, Utrecht, The Netherlands.
2. E. Gelpí, in Advances in Chromatography (Eds. J.C. Giddings, E. Grushka and P.R. Brown), vol. 26 (1987) 321 – 391

LC-EC conditions

Column	C18, 3 µm, 100 x 2.0 mm
Flow rate	0.2 ml/min
Mobile phase	Ammonium acetate 50 mM, glacial acetic acid 1.25%, sodium dodecyl sulphate 0.347 mM, EDTA 0.27 mM, 25% methanol
Injection	20 µl
Temperature	32.5 °C
E-cell	600 mV (vs. Ag/AgC, satd')

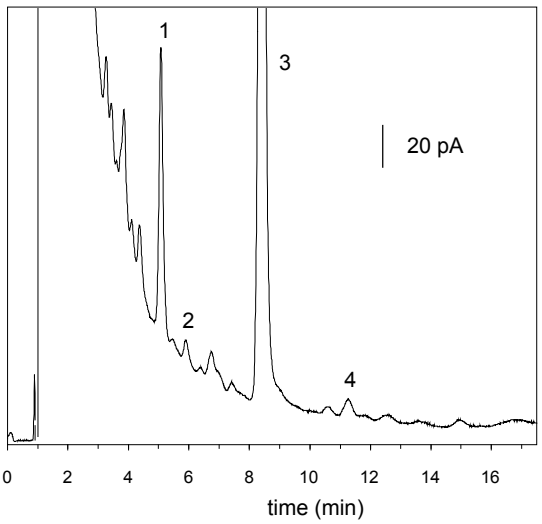

Fig. 1. Analysis of human plasma after liquid-liquid extraction with DPBEA/Heptane. On-column amounts are: 1. norepinephrine: 31 pg, 2. epinephrine: 2.9 pg, 3. DHBA (IS): 200 pg, 4. dopamine: 1.5 pg. Plasma concentrations are: norepinephrine 386 pg/ml, epinephrine: 36 pg/ml, dopamine: 15 pg/ml. Courtesy: Ing. G. Alberts, Dept. of Int. Med. I, Univ. Hosp. 'Dijkzigt', Rotterdam, The Netherlands.

Part numbers and configuration used

130.0035	INTRO EC detector
110.4105	VT-03 with 3 mm glassy carbon electrode and salt bridge Ag/AgCl REF

Configuration suggestions & comment

Due to the low concentration, the plasma catecholamine analysis is considered to be a demanding one. The current configuration can be improved with the LINK (p.n. 200.0035) as an add-on.
The DECADE II (p.n. 171.0035) with its superior electronic noise suppression (ADF) and temperature stability will further improve performance.
In this case, due to the use of an ammonium buffer, a configuration with the ISAAC REF (p.n. 110.4115) cannot be used. The author sees no problem in using another acetate buffer. This implies that the ISAAC REF can be safely used then. Note that 2 mM KCl must be added to the mobile phase and the potential must be adapted.