CAPSULES
notes and applications from Bioanalytical Systems, Inc.
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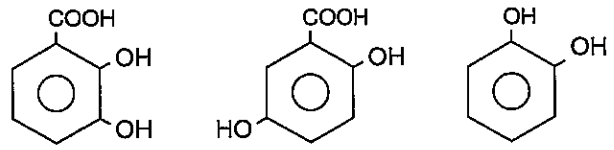
Salicylate Trapping of Hydroxyl Free Radical

Purpose

Determination of 2,3- and 2,5-dihydroxybenzoic acid, products of salicylate trapping of the hydroxyl radical.



2,3-DHBA 2,5-DHBA CATECHOL

Figure 1. 2,3-DHBA, 2,5-DHBA and catechol.

Oxygen free radicals such as superoxide and hydroxyl radical have been suggested as causative agents in aging and carcinogenesis [1], and implicated in collateral damage following stroke, trauma and ischemia [1,2,3]. Hydroxyl radical cannot be measured directly, but can be trapped by the use of several compounds, the products of which can be detected [1]. One such compound, salicylate, will produce 2,3-dihydroxybenzoic acid (DHBA), 2,5-DHBA and catechol (F1) upon exposure to hydroxyl radical. 2,5-DHBA may be produced endogenously, suggesting that 2,3-DHBA is the preferred marker for hydroxyl radical [4].

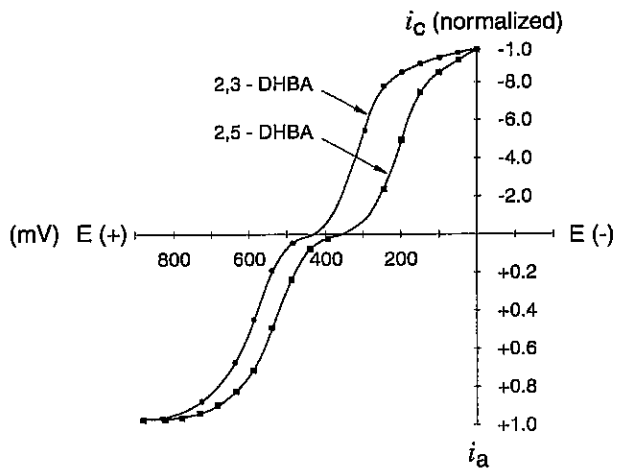


Figure 2. Hydrodynamic voltammogram of the two isomers. The cathodic current in the upper half of the diagram is only produced subsequent to oxidation.

The two DHBA isomers may be detected by oxidation at potentials above +500 mV (F2). Alternatively, a dual series arrangement may be used where the first electrode oxidizes the compounds, which are then reduced at the downstream electrode at potentials below +500 mV [5,6]. This latter arrangement results in a more selective detection of the products, as few coeluting endogenous compounds exhibit such redox behavior.

Existing Method

Spin trapping, followed by paramagnetic resonance spectrometry. This method requires costly equipment and expertise, and the response of other free radicals may obscure that of oxygen free radicals [2].

Conditions

System: BAS-200B Liquid Chromatograph with electrochemical detector.

Electrode: Glassy carbon, cross-flow (for upstreaml oxidation with or without downstream reduction) or UniJet (oxidation only).

Potential: For oxidation: +750 or 800 mV *vs.* Ag/AgCl. For downstream reduction: a second electrode at +200 mV.

Column:

Microbore: Reverse-phase, C_{18}, 5 µm, 150 x 1 mm (PN MF-8912). A second microbore column (PN MF-8949) was installed before the injector to increase system pressure at microbore flow rates. Alternatively, a splitter column (PN MF-8947) can be used to obtain microbore flow rates.

Standard bore: Reverse-phase, C_{18}, 3 µm, 100 x 3.2 mm (PN MF-6213).

Mobile Phase:

Microbore: 1L aqueous buffer containing 27 µM disodium EDTA, 14.7 mM $NaH_2PO_4 \cdot H_2O$, 30 mM sodium citrate·$2H_2O$, 10 mM diethylamine·HCl and 2.2 mM 1-octanelulfonic acid·HCl, pH 3.2; 40 mL acetonitrile; 10 mL tetrahydrofuran.

Standard bore: 75 mM monochloroacetic acid, 0.7 mM disodium EDTA, 1.5 mM 1-octanesulfonic acid-HCl, pH 2.9.

Detection Limits: Oxidative (+800 mV): 2,3-DHBA = 167 fg (1.1 fm); 2,5-DHBA = 144 fg (0.9 fm). Reductive (+200 mV): 2,3-DHBA = 484 fg (3.1 fm); 2,5-DHBA = 195 fg (1.3 fm). All at S/N = 3.

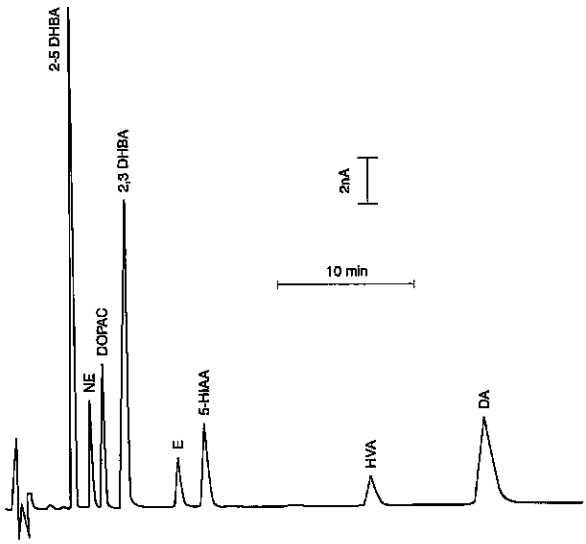


Figure 3. Standard-bore separation of 1 ng each compound except 3 ng 2,3- and 2,5-DHBA. Key: NE = norepinephrine, E = epinephrine, DOPAC = 3,4-dihydroxyphenylacetic acid, 5-HIAA = 5-hydroxyindoleacetic acid, HVA = homovanillic acid, DA =dopamine. Potential = +750 mV vs. Ag/AgCl, cross-flow cell.

Notes

Separations of standards with the standard-bore (F3) and microbore (F4) columns are shown, including the common catecholamines and their metabolites that would be present in dialysates.

F5 shows a separation using a dual electrode, with oxidation upstream followed by reduction downstream. Selectivity of the downstream electrode is apparent: the DHBAs are readily detected, while DOPAC, which can be a major component of dialysates, is barely seen.

References

1. *R.A. Floyd, FASEB J. 4 (1990): 2587-2597.*
2. *D.K. Das, G.A. Cordis, P.S. Rao, X.U. Liu and S. Maity, J. Chromatogr. 536 (1991): 273-282.*
3. *E.D. Hall, P.K. Andrus and P.A. Yonkers, J. Neurochem. 60 (1993): 588-594.*
4. *B. Halliwell, H. Kaur and M. Ingelman-Sundberg, Free Radical Biol. Med. 10 (1991): 439-441.*
5. *G.S. Mayer and R.E. Shoup, J. Chromatogr. 255 (1983): 533-544.*
6. *M. Globus, personal communication.*

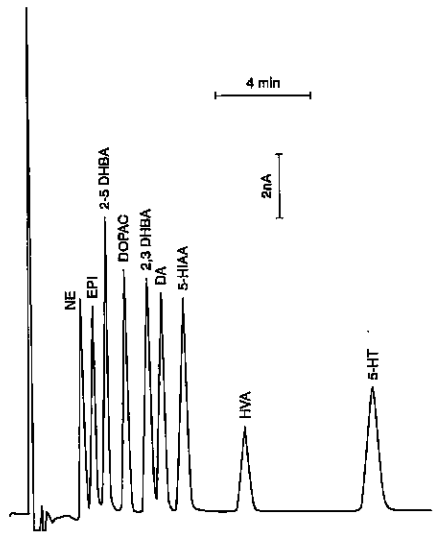


Figure 4. Microbore separation of 100 pg each compound. Identities as in F3, plus 5-HT = serotonin. UniJet electrode at +650 mV (10 mM NaCl added to mobile phase).

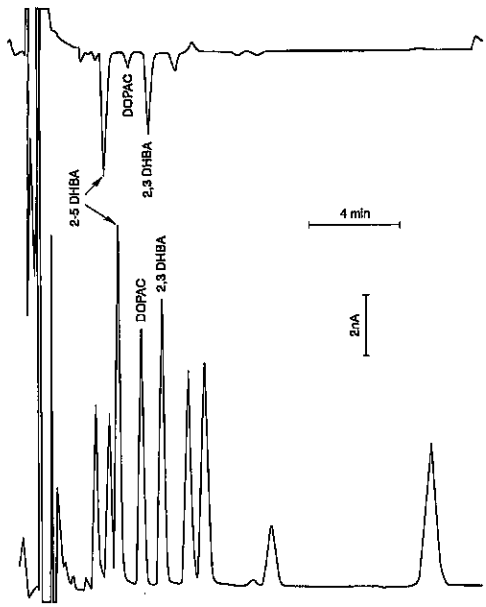


Figure 5. Microbore separation with cross-flow cell, 100 pg each compound, identities as above. Bottom: oxidative detection at +750 mV vs. Ag/AgCl. Top: selective downstream reduction at +200 mV.