Determination of Bisphenols Using HPLC-ECD

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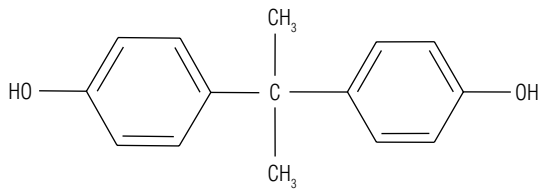
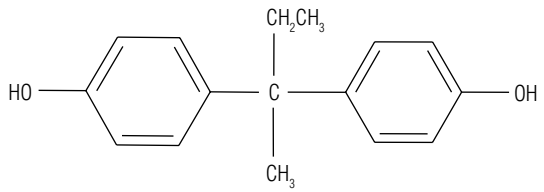
Key Words
Hormones, Estrogen, Endocrine Function, Xenoestrogens, HPLC-EC-UV

Goal
To develop a highly stable sensitive and selective, maintenance free HPLC-ECD method for measuring bisphenols, ideal for routine operation.

Introduction
Xenoestrogens are a diverse group of compounds found in the environment that mimic the effects of endogenous estrogens. They include monomers of plastics (bisphenols, phthalates), detergents (alkylphenols), pesticides (DDT, triazines), industrial by-products (dioxins and PCBs) and fungal metabolites (mycotoxins). Xenoestrogens bio-accumulate in organisms high on the food chain (fish and meat) which also form part of the human diet. They tend to be lipophilic and collect in fatty tissues such as breast tissue. One controversial theory suggests that the increase in incidence of certain hormone-dependent cancers such as breast and prostate cancers may be linked to an increased exposure to xenoestrogens. Bisphenols A and B (Figure 1) are monomers used in the production of epoxy resins and plastics and are widespread in food and drink packages, as coatings of metal cans and water pipes, as microwave susceptors (used to crispen food) and in some dental resins.[1, 2, 3] Bisphenol A and liquid obtained from vegetables from plastic coated cans have been shown to be estrogenic in MCF-7 and human breast cancer cells.[4] Furthermore, bisphenol A is a sensitizer causing dermatitis[5] and, when oxidized, forms a reactive quinone capable of reacting with DNA bases.[6]



BISPHENOL A



BISPHENOL B

Figure 1. Chemical structures of Bisphenols A and B.

The global method presented here uses HPLC with Coulometric array detection to measures both bisphenols, the isoflavonic phytoestrogens (daidzein, genistein and equol), the mammalian lignans (enterolactone and enterodiol), and the synthetic estrogen diethylstilbestrol (DES) all of which are also thought to play important roles in cancer generation and prevention. The Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector offers significant advantages over other analytical instruments including selectivity, sensitivity and the production of qualitative data which are needed to monitor xenoestrogens in food, soil, water and biological tissues.

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Materials and Methods

The gradient analytical system consisted of two pumps, an autosampler, and an 8-channel CoulArray detector.

LC Conditions	
Column:	C18, 3 µm, 3 × 150 mm
Mobile Phase A:	50 mM Sodium acetate (pH 4.8 with acetic acid); methanol (80:20 v/v)
Mobile Phase B:	50 mM Sodium acetate (pH 4.8 with acetic acid); methanol; acetonitrile (40:40:20 v/v/v)
Gradient Conditions:	Linear increase of phase B from 30% to 100% over 28 min followed by a 7 min hold at initial conditions
Flow Rate:	0.6 mL/min
Temperature:	Ambient
Injection Volume:	20 µL
Detector and Conditions	
Detector:	Model 5600A, CoulArray
Applied Potentials:	+380, +400, +440, +500, +560, +620, +680, +760 mV vs Pd

Results and Discussion

A standard chromatogram showing resolution of the phytoestrogens, DES and the bisphenols (10 ng on column) is presented in Figure 2. Gradient elution coupled to voltammetric resolution readily enables the quantitation of all the analytes of interest in under 26 min. Both bisphenols showed the same maximum oxidation potential at +500 mV vs Pd. This method has an expected limit of detection of <50 pg on column for the bisphenols.

Conclusion

HPLC-ECD is one of the most sensitive analytical procedures available today. Although it is a popular belief that relatively few compounds are electrochemically active this assumption is wrong. Many compounds such as endogenous metabolites, drugs, toxins, pollutants and contaminants are capable of being measured with HPLC-ECD.[7,8] The coulometric electrode not only offers unrivaled sensitivity and selectivity but it also offers unparalleled stability and is maintenance free for outstanding routine operation.[9] As analytes are identified both by retention time and voltammetric behavior across the array, compound mis-identification and coelution are minimized.[10] Using gradient HPLC with the CoulArray detector the presumptive xenoestrogens, bisphenols, can be assayed on the same system as the phytoestrogens and the synthetic estrogen, DES. Because of the purported interaction of xenoestrogens it is important that as many as possible be measured in the same sample. By using reversed phase gradient HPLC with near normal phase conditions the wide range of polarities can be eluted in a single run. The bisphenols show characteristic oxidation patterns across the array that is of value when assaying these compounds in complex biological samples. Although it is unclear what minimum levels of these compounds are biologically active, the CoulArray detector can quantitate the bisphenols at sub 50 pg levels which permits the study of their effects even at low levels.

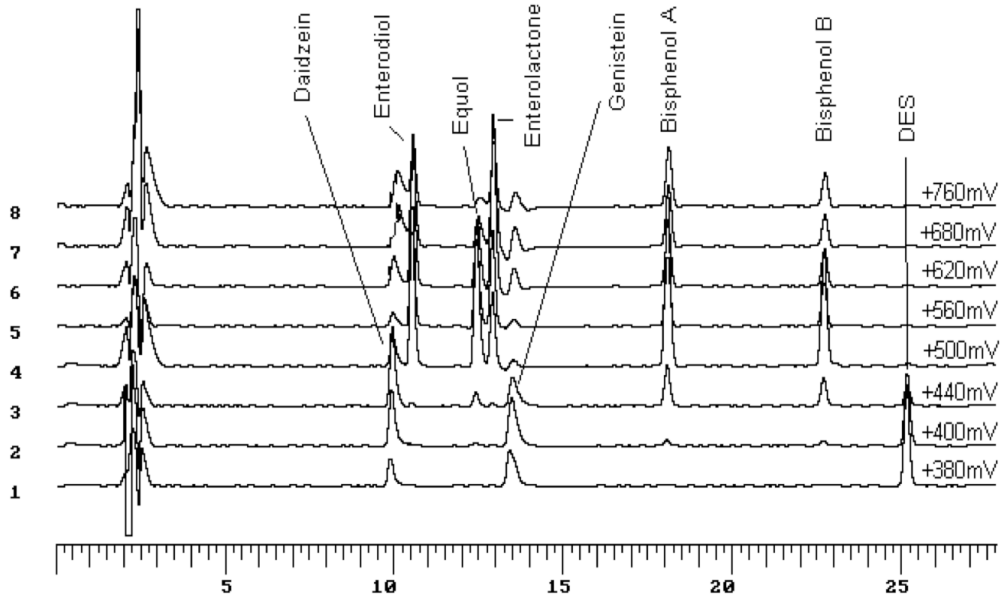

Figure 2. Gradient chromatogram of bisphenols, phytoestrogens and DES standards.

Ordering Information

In the U.S., call (800) 346-6390 or contact the Thermo Fisher Scientific Regional Office nearest you. Outside the U.S., order through your local Thermo Fisher Scientific office or distributor. Refer to the following part numbers.

Description	Part Number
HPG-3400RS Biocompatible Binary Rapid Separation Pump with two solvent selector valves	5040.0046
WPS-3000TBRS Biocompatible Rapid Separation Thermostatted Autosampler	5841.0020
CoulArray Thermal Organizer	70-4340T
5600A CoulArray 8-Ch Det Inst 120 Vac	70-4324
Accessory Kit, CoulArray Detector to UltiMate 3000 System	70-9191

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