[1]Bioanalysis & Biotransformation Research Center, KIST; [2]College of Pharmacy, Sungkyunkwan University; [3]College of Medicine, Gyeongsang National University

Measurement of plasma and urine concentrations of corticosteroids is clinically significant in adrenal and pituitary dysfunctions. Apparent mineralcorticoid excess and Cushing's syndromes can be diagnosed by measuring cortisol (F), cortisone (E), tetrahydrocortisol (THF), allo-THF, and tetrahydrocortisone (THE) excretions in pathological concentration. The present study describes the accurate and reproducible GC-MS method to measure E, F, THE, THF, and allo-THF in serum and urine. After extraction by a solid-phase cartridge using Oasis HLB copolymer, the residues were derivatized with a mixture of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide, ammonium iodide, and dithioerytrithol (1000:4:5; v/w/w), and analyzed by GC-MS. The method was linear over the range of 1-1000 ng/mL and 2-1000 ng/mL for serum and urine, respectively. Analytical recoveries were 82.4-93.7% and precision (%CV) was 2.8-10.3%. The limit of detection was 1 ng/mL and 3 ng/mL for serum and urine, respectively, and limit of quantification was 2.5 ng/mL and 5.5 ng/mL for serum and urine, respectively. The GC-MS method described is sensitive, specific, and suitable for the determination of E, F, THE, THF, and allo-THF in serum and urine by bench-top GC-MS.

[PD4-18] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Simultaneous determination of benzophenone and 4-nitrotoluene in ground water and soil by a gas chromatography-mass spectroscopy

Kim EY°, Kwon OS, Ryu JC

Toxicology Lab., Korea Institute of Science and Technology, P.O.Box 131, Cheongryang, Seoul 130-650, Korea,

4-Nitrotoluene is used primarily as an intermediate in the production of various dyes, explosives, pharmaceuticals, and in the production of rubber and agricultural chemicals. Benzophenone derivatives are used as UV-absorbing agents which are contained in a large number of products such as hair sprays, shampoo, lipsticks, hair dyes and sunscreen lotions, photoaffinity labeling for various biological materials. Benzophenone and 4-nitrotoluene are listed in World Wildlife Fund. and are suspected to be contaminated in ground water sites and soil. However no literatures of analytical method for determining the benzophenone and 4-nitrotoluene in soil and ground water are found. Benzophenone and 4-nitrotoluene were determined by selected ion monitoring mode of GC/MSD in water, sedimint and soil samples. These two chemicals were extracted with n-hexane for water samples, and with methanol and n-hexane for sediment and soil samples. Benzophenone-d_5 and Nitrobenzene-d_5 were used as internal standards for benzophenone and 4-nitrotoluene, respectively. Recovery in water samples was 72-114% with less than 13% of RSD. Recovery in sediment and soil samples was ranged from 51 to 89%. The detection limit of benzophenone and 4-nitrotoluene in water was 10 ng/L. The mothod detection limit of benzophenone and 4-nitrotoluene was 0.1 and 0.5㎍/kg in sediment and soil , respectively. This method is suitable for the trace analysis of benzophenone and 4-nitrotoluene in environmental samples.

[PD4-19] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Characterization of DDT Antibodies for Immunoassay Application

Hong JY°, Kim JH, Choi, MJ*

*Bioanalysis and Biotransformation Research Center,
Korea Institute of Science and Technology, Seoul, Korea
Seoul Women's University, Seoul, Korea

To develop rapid detection method of DDT (4,4'-dichlorodiphenyl-2,2,2-trichloroethane) and its metabolites, DDT derivatives created carboxyl group (DDA, DDHP, DDCP) were conjugated to KLH for the use of immunogen. Monoclonal and polyclonal antibodies were prepared. Fifteen hybridoma cell lines obtained from each immunogen were screened using matching DDT-BSA derivatives. For the use of coating ligands to measure titration level of antibody and free ligand displacement, DDT derivatives (DDA-, DDHP-, DDCP-, DDHH-, and DDHHAP-) were conjugated to ovualbumin. To screen a matching pair of antibody and coating legend for the simultaneous detection of DDT and its metabolites (DDA, DDE, DDD), each antibody was investigated for displacement of free liagand using combination of five coating ligands and two carrier proteins. The competitive ELISA results indicate that titration level and free ligand displacement were greatly influenced by ligands derivatized and carrier proteins used. Three matching pairs of antibody and coating ligand are screened for this purpose and they were 1A3 and DDA-OVA, 1A1 and DDHHAP-BSA, and 1A4 and DDHP-OVA.

[PD4-20] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

Homogeneous Fluorescence Polarization Immunoassay of Estrogens using Fluorescein-labeled Tracer

Lee JR°, Choi JE, Eremin SA, Choi MJ

Bioanalysis and Biotransformation Research Center,
Korea Institute of Science and Technology, Seoul, Korea

A homogeneous fluorescence polarization immunoassay (FPIA) was developed to measure estrogen level using a fluorescence polarization analyzer in photocheck mode (Abbott Labs). Two tracers of fluorescein isothiocyanate (FITC)-labeled estrogens were synthesized for this purpose: estrogen-6-FITC (E-6-F) derived from 6-ketoestradiol 6-(o-carboxymethyl)oxim and estrogen-17-FITC (E-17-F) derived from 17ß-estradiol 17-hemisuccinate. Different combination of tracers and antibody were investigated to find a matching pair in the FPIA system. E-6-F tracer ($Rf_{365\ nm}$ = 0.3 in chloroform/methanol developer solvent) showed better binding response than E-17-F ($Rf_{365\ nm}$ = 0.2) in immunoassay. This result indicates that the 17-position of estrogen plays an important role for binding to antibody. At the optimized condition, estradiol can be detected in the range of 10 nM and 1 uM. Several estrogens were compared for their detection range by FPIA. By comparing 50 % bound concentration, 16-ketoestrdiol, 4-methoxyestradiol and 2-hydroxyestradiol-3-methylether is 100 times sensitive than estradiol and 17-epiestriol is 100 times less sensitive. Other estrogens will be discussed. This FPIA require no separation step and assay time is apporoximately 7 minutes for 10 samples. Therefore, it is useful for the screening of eco-estrogens in water sample.

[PD4-21] [04/19/2001 (Thr) 13:30 - 14:40 / Hall 4]

A COMPACT AND PORTABLE NEAR INFRARED (NIR) SYSTEM USING MICROSPECTROMETER

Woo YA, Kim JM*, Kim HJ

College of pharmacy, Dongduk Women's University, Seoul 136-714, Korea Spectron Tech. Co., Ltd. Seoul 136-132, Korea*

In recent years, a miniature spectrometer has been extensively developed due to the marriage of fiber optics and semiconductor detector array. This type of miniature spectrometer has advantages of low price and robustness due to the capability of mass production and no moving parts are required such as lenses, mirrors and scanning monochromator. These systems are ideal for use in teaching labs, process monitoring and field analyses. A portable near infrared (NIR) system has been developed for