

Diazepam (DZ) is one of the most frequently prescribed drugs as an anti-anxiety agent, muscle relaxant, and anticonvulsant and sometimes causes intoxication due to accidental overdose, misuse or abuse. Screening or confirmation methods for DZ and NDZ in plasma are very important for clinical and toxicological studies and in forensic cases. GC/MS assay with SPE was developed for the determination of diazepam and its metabolite, nordiazepam in human plasma. Diazepam in plasma was extracted by a rapid and sensitive procedure based on C18 bonded-phase extraction. GC/MS analysis was performed using a Agilent MSD 5973 mass spectrometer and the column was a DB-5MS. The detection limit was 0.5 ng/mL and the assay was sensitive to 1 ng/mL and linear to 3000 ng/mL with correlation coefficients of >0.99 for both DZ and NDZ. The recoveries of DZ and NDZ were >80.0. The within-run CVs and between-run CVs of diazepam and nordiazepam were less than 10%. This sensitive and simple method is useful for plasma samples of forensic toxicological interest and in clinical studies when low concentrations of DZ are to be detected.

[PA4-21] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Identification and semi-quantitation of dextromethorphan and its metabolite in urine using the REMEDI HS system**

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To determine dextromethorphan (DMP) and its active metabolite dextrophan (DRP) in urine was performed using REMEDI<sup>TM</sup> (Rapid EMERGENCY Drug identification) that is a fully automated multicolumn high performance liquid chromatographic (HPLC) system with a scanning ultraviolet detector. The limits of detection for DMP and DRP were 0.10 and 0.15 µg/mL, respectively. The standard curves were linear, with correlation coefficients ( $r > 0.975$ ) in the concentration range of 0.5 ~ 10.0 µg/mL. The accuracy was 66.4 ~ 82.4% and 66.7 ~ 85.6%, and the precision was 1.3 ~ 7.8% (coefficient of variance, CV) and 0.9 ~ 7.8% (CV) for each of the compounds. The DMP and its metabolite DRP in urine samples were rapidly identified and semi-quantitated by REMEDI without any sample pretreatment.

[PA4-22] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Regulation of CYP 1A1 gene expression by retinoic acid receptor, retinoid X receptor and constitutive androstane receptor in rainbow trout hepatoma cells(RTH 149)**

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Exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes a variety of biological and toxicology effects, most of which are mediated by aryl hydrocarbon receptor (AhR). The ligand-bound AhR as a heterodimer with AhR nuclear translocator (ARNT) binds to its specific DNA recognition site, the dioxin-responsive element (DRE), and it results in increased transcription of CYP1A1 gene. Retinoic acid (RA) regulates the transcription of various genes for several essential functions through binding to two classes of nuclear receptors, the retinoic acid receptor (RAR) and retinoid X receptor (RXR). Constitutive androstane receptor (CAR) also regulates the transcription of gene. In this study, we have examined how RAR, RXR and CAR regulated CYP1A1 in rainbow trout hepatoma cell (RTH 149) using luciferase reporter gene assay system. We did transient transfection with CYP1A1 luciferase reporter gene and treated with TCDD, all-trans RA, 9-cis RA and phenobarbital. Treatment of all-trans RA, 9-cis RA or phenobarbital decreased the TCDD induced transcription of CYP1A1. When we did transient cotransfection with CYP1A1 luciferase reporter gene and RXR, as increase of RXR concentration, the TCDD induced transcription of CYP1A1 was decreased. Transfection with CAR also decreased the TCDD induced transcription of CYP1A1 in RTH 149 cells.

[PA4-23] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]