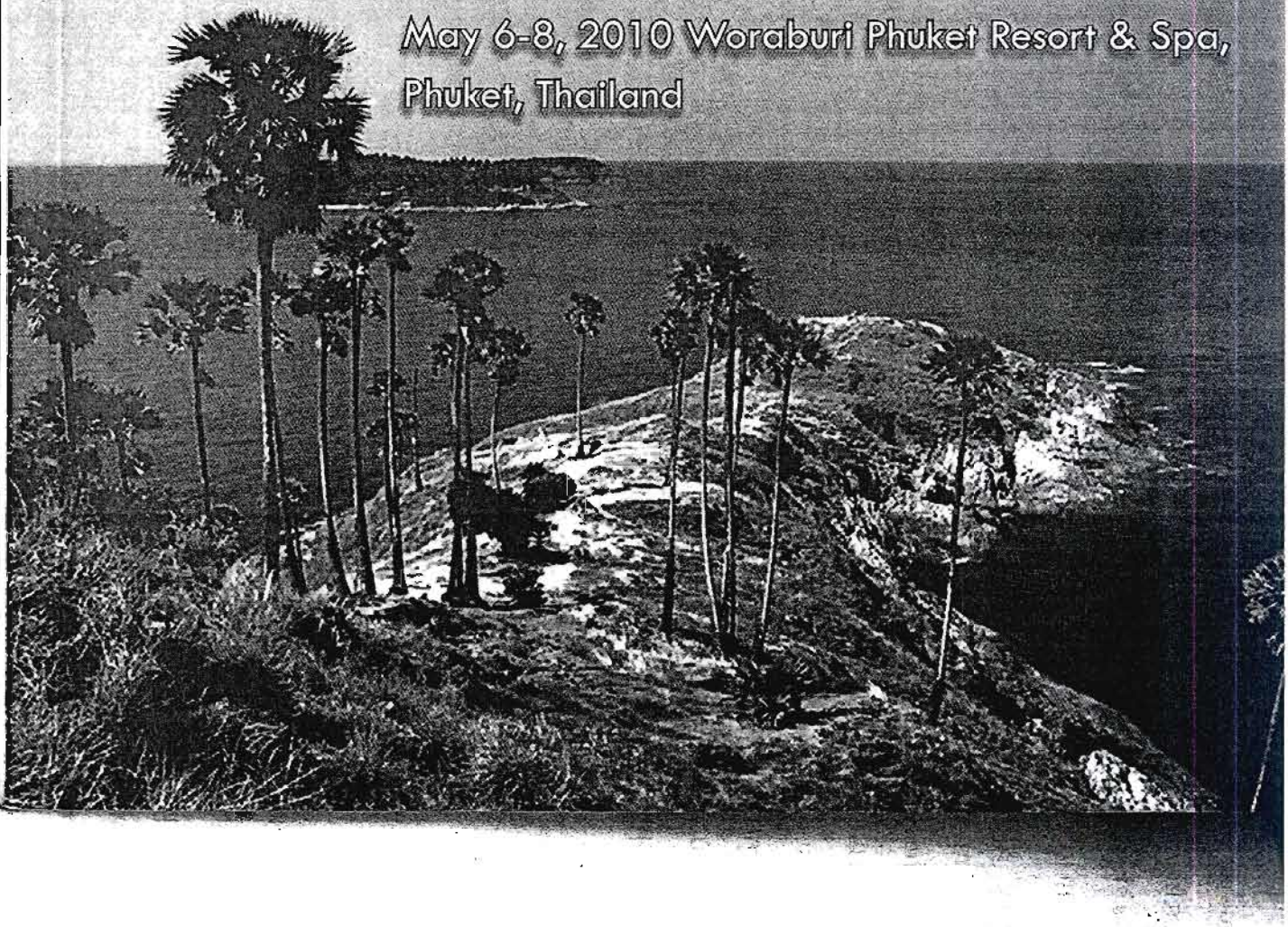
Abstracts

CDD 2010

The 1st Current Drug Development International Conference

May 6-8, 2010 Woraburi Phuket Resort & Spa, Phuket, Thailand



ABSTRACTS
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A High-performance Liquid Chromatography-Fluorescence Method for Determining Alendronate in Urine Obtained from Patient with Chronic Kidney Disease

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Alendronate is a bisphosphonates indicated for treatment of a variety of bone metabolism disorders. Since alendronate is excreted mainly in urine, many pharmacokinetic studies relied on determination of alendronate in urine. A reliable analytical method is required to obtain relevant urinary data. Most of pharmacokinetic data were previously examined in healthy subjects receiving alendronate, no study in chronic kidney disease (CKD) patient have been reported. The purpose of this study is to employ the proposed HPLC-fluorescence detection method to determine alendronate in urine samples from CKD patients. **Methods**. The analytical method involved co-precipitation of alendronate in urine sample with calcium chloride and potassium phosphate under alkaline condition. Subsequent clean up process was performed by solid-phase extraction (SPE) using diethylamine (DEA) cartridge. The analyte was finally derivatized with 9-fluorenylmethyl chloroformate (FMOC) prior to HPLC analysis. Separation was performed on a C_{18} column and mobile phase consists of acetronitrile-methanol-citrate/pyrophosphate buffers. Percentage of the organic solvent in the mobile phase was increased from 32% to 40% v/v at a constant flow rate 1.5 mL/min. Fluorescence detection was operated at 260 nm (excitation) and 310 nm (emission). The method was validated for linearity, specificity, accuracy, precision, and sensitivity. Subjects enrolled in the study were patients diagnosed with CKD (GFR < 60 ml/min/1.73 m^2), who received 70 mg-alendronate sodium once weekly for at least 6 months. Healthy volunteers enrolled in the study were male, age 18-25 years, and with no remarkable results on medical history, physical examination and laboratory screening. Urine samples were collected over 4-period of time for 10 hours post dose. Urine samples were monitored for volume, pH and appearance prior to storage at -20°C. Data was analyzed using Student t-test. **Results**. At 35°C, alendronate was well separated from pamidronate (internal standard) with retention time of 10.25 and 11.92 min, respectively. Total analysis time was 40 minutes per sample. Limit of detection was 1.0 ng/ml of alendronate. Accuracy was 103.4 ± 25.1 % and 106.9 ± 2.76 % at 15 ng/ml and 40 ng/ml, respectively. The intra-day and inter-day precision, expressed as % coefficient of variation, were 1.54-16.2% and 24.3%, respectively. Maximum urinary excretion rate (U_{max}) and cumulative amount excreted in urine over 10 h (A_e) in CKD patient and healthy subjects shown in Table 1 suggested no significant differences of the parameters between two groups.

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Table 1 Urinary excretion data obtained from CKD patients (n = 4) and healthy volunteers (n=5)

PK parameters	CKD patient	Healthy volunteers	p-value
Maximum urinary excretion rate (U_{max}) (μg/h)	50.7 ± 35.0	61.7 ± 17.1	0.595
Cumulative amount excreted (A_e) (μg)	205.7 ± 86.9	210 ± 72.7	0.94

Conclusions These results indicated that there were no differences in urinary excretion of alendronate between CKD patient and healthy volunteers.

Acknowledgements:

1. Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Thailand
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