# Development of a Sensitive High Performance Liquid Chromatography Method for the Analysis of Benzodiazepines

by

**Lathika Sujani Hathurusinghe**

Department of Chemistry, Faculty of Science
University of Colombo, Sri Lanka

Research project submitted to the Department of Chemistry, Faculty of Science, University of Colombo as a partial fulfillment of the requirement for the M.Sc. degree in Analytical Chemistry.

2010-December

## ABSTRACT

Benzodiazepines are drugs widely used as anxiolytics, hypnotics and antiepileptics. In the recent past there have been instances of crime reported by law enforcement agencies in Sri Lanka in which benzodiazepines were intentionally added to commit crimes.

In this study a sensitive and selective methodology was developed for the qualitative and quantitative analysis of benzodiazepines (diazepam, bromazepam, clonazepam, flunitrazepam, nitrazepam) by high performance liquid chromatography in non-alcoholic carbonated beverages, malted milk and fruit drinks, which were used to induce someone sleep before commit crimes. The method was found to be suitable for the analysis of five benzodiazepines used in the study. The extraction technique used was liquid-liquid extraction where the pH of the samples was adjusted to 8.5. The separation is achieved on ODS-2 reversed phase column (SGE, 250 x 4.6 mm I.D., 5 µm particle size). Gradient elution was carried out with a mobile phase consisting of acetonitrile and phosphate buffer (pH 5.4) and the detection was done by UV/Visible detector at 240 nm.

Linear regression correlation coefficients of the calibration curves were $\geq$ 0.992 for the five benzodiazepines. The limit of detections and limit of quantifications ranged from 0.8 to 80 µg/ml and from 2.7 to 267 µg/ml, respectively for five benzodiazepines. The relative standard deviations were less than 3.5% for 0.1 mg/ml nitrazepam, clonazepam, flunitrazepam, diazepam and 0.6 mg/ml bromazepam. The extraction percentage recoveries for nitrazepam, clonazepam, flunitrazepam, diazepam and bromazepam were varied from 81.3 % to 89.1 %. The above method was validated and found acceptable, hence successfully applied in the quantification of benzodiazepines in test samples.

The results obtained from case studies, clonazepam and diazepam were identified and quantified in the test samples. The samples of case I contained clonazepam in the concentration of 0.98 mg/tablet. The diazepam amounts found in samples of case II, III, and IV were 7.75 mg/l, 17.77 mg/l and 4.19 mg/l respectively. According to results of these test samples, it is possible to make a conclusion that benzodiazepines are intentionally added to beverages with a motive to commit a crime. These findings help to the law enforcement authorities to make their judgments and to enforce the law.