CHAPTER I

INTRODUCTION

Problem Definition

Paracetamol is a commomly used over-the-counter analgesic drug. Recent studies that excessively high plasma concentrations of paracetamol may cause renal and hepato-toxicity. Phenol , commonly called carbolic acid , is used to prepared plastic such as Bakelite , drugs , dyes , and other compounds. Phenol also has medical application as a topical anesthetic for some types of lesions and in the treatment of mange and colic in animals. Paraben are widely used as preservative in foods , beverages and cosmotics.

Reversed phase high performance liquid chromatography (RP-HPLC) is an important mode for chromatographic separation of compounds by using the hydrophobic interaction mechanism (1). A relatively unexplored and poorly understood phenomenon in this mode of chromatography is the peak distortions caused by the solvent used for injecting the test samples into chromatographic column, which can significantly influence the elution of the compounds of interest (2).

The deleterious effects of injecting samples of appreciable volume into liquid chromatography systems where the sample solvent and mobile phase are different have been well known for a long time (3,4) . It is generally agreed that the two solvents should be the same in order to realize the best chromatographic performance. This general rule is frequently infringed in practice (5-13). Among other reasons , samples are often obtained in solvents different from mobile phase composition and the sample solubility becomes sometimes paramount in reversed phase chromatography , especially in preparative system (14). Therefore, the effect of difference between composition of the sample solvent and the mobile phase on the chromatographic behavior of compound undergoing separation has been studied with increasing interest (5). Contradictorily , observations of anomalous peak shapes and splitting of peak have been reported (5-7,15,16,17) , and it has also been found that these effects differ depending on the chromatography used (8). An explanation was sought in terms of the interaction between compounds undergoing separation and solvents (9). It seems , however , that this is a natural consequence of the dynamic gradient between composition of the sample solvent and of the mobile phase at the beginning of the column (10,11). It was found (12) , moreover , that these effects were dependent on the injection volume , on the configuration of the apparatus between the injection valve and the column , and that they could be restricted by additional mixing with the elution before entering the column.

Literature Reviews

Chromatographers have long practised using the mobile phase as the injection solvent for the eluite in liquid chromatography. Many workers have observed abnormal chromatograms when solvents other than the mobile phase were used.

In 1980 , K.J. William , A. Li Wan Po and W.J. Irwin (15) studied sample-solvent-induced peak broadening in the reversed phase high performance liquid chromatography of Aspirin and related analgesics. They illustrated the effect of solvent-induced peak broadening. It was shown that although the peak area remained constant while varying amount of methanol and water , there was a considerable change in peak profile and peak height which might be attributed to change in the nature of sample solvent.

In 1984 , Maria Tsimidou and Robert Macrae (19) studied influence of the injection solvent on the reversed phase chromatography of triglycerides. They showed that triglyceride analysis under reversed phase conditions was particularly susceptible to the influence of the injection solvent. Chloroform , one of the most widely employed solvents , produced inferior resolution under all conditions and this is accentuated when large injection volume were used (10-20μL) . It was recommended that acetone would be considered as a reliable alternative.

In 1985 , The effects of solvents used to dissolve test samples on the peaks observed in high performance liquid chromatography were studied in normal phase , reversed phase and also ion-pair reversed phase systems by Tju-Lik Ng and Soon Ng (2). It was found that various patterns of distortions and peak splitting occurred depending on the solvent and the chromatographic system involved. Computer simulation of the abnormal chromatograms was achieved by means of a program based on a model in which the retention ratio changes was due to the effect of the solvent on the column.

In 1986 , Solomon Perlman and Joel J. Kirschbaum (9) reported details of a phenomenon involving changes in peak appearance due to the solvent. They have found that a diversity of compounds , using an assortment of reversed phase columns with both buffered and unbuffered mobile phase , and UV or refractive index (RI) detectors yielded peak size depending not only on the molar absorptivity but also the injection solvent. For reversed phase column , the responses of each compounds increased with the polarity of the solvent , and only compounds capable of forming intramolecular hydrogen bonds exhibited this effect. The retention time was not affected.

In the same year , the combined effect of changes in temperature and percentage of methanol in mobile phase on the retention behavior of two sets of purine compounds were studied by Changhou Yi , James L. Fasching and Phyllis R. Brown (6). For guanine and hypoxanthine bases, there was a linear relationship between the logarithm value of capacity factor (ln k') and the reciprocal of the absolute temperature at all concentrations of methanol. However , for nucleosides in the presence of methanol ,

the relationship was nonlinear and there appeared to be two components in the curve , indicating two retention mechanisms , one at higher temperatures and the other one at lower temperatures. For monophosphate nucleotides , there were two peaks when methanol was present. The temperature at which the second peak appeared was dependent on the methanol concentration and the structure of the base in the nucleotide. For the di- , and tri-phosphate nucleotides , each nucleotide was represented by multiple peaks when methanol was present in the mobile phase.

In 1988 , the effect of sample solvent on chromatographic peak shape using a ß-cyclodextrin column had been investigated by Terry D. Wilson (20). Sample solvents ranging in polarity from hexane to mobile phase (water-methanol- 0.5 M pH 4.5 borate buffer , 600 : 400 : 1) were used with assessments of column efficiency made by calculating theoretical plates as $N_{5\sigma}$, $N_{4\sigma}$ and N_{sys} . Best agreement with observed peak shape resulted from the use of $N_{5\sigma}$ especially in the narrow polarity span from 40 to 100 % methanol.

In 1989 , Norman E. Hoffman , Shian-Ling Pan and Abu M. Rustum (21) studied the distortion and multiplication of peaks that occurred when an eluite dissolved in a solvent that as significantly stronger than the mobile phase was injected in reversed phase liquid chromatography. The solvent strength and injected volume affected peak shape whereas the column length and diameter , particle size and type of reversed phase did not affect general peak shapes.

Later , high resolution analysis of polyamines and their acetyl derivatives using RP-HPLC was described by S. Wongyai , P.T. Oefner and G.K. Bonn (22). It was shown that the water content of the injected sample significantly influence the chromatographic behavior of benzoyl derivatives of putrescine , cadaverine , spermidine and spermine on a reversed phase material. Moreover , the number of theoretical plates of each compound was distinctly dependent on the water concentration.

In the same year , Donna Vukmanic (23) reported the effects of organic solvents in sample solutions on chromatographic peak profiles. It was found that the retention time , peak height and peak width , were different for acetonitrile than for methanol in reversed phase high performance liquid chromatography. Two benzimidazole carbamate degradation products of the fungicide benaomyl : methyl-2-benzimidazole carbomate (MBC) and 3-butyl-2,4-dioxo[1,2-*a*]-*s*-triazinobenzimidazole (STB) were studied. The overall effect was more noticeable with STB than with MBC. Peak splitting was observed only with STB when larger volumes of solvents were injected. Peak broadening was observed even with 10 µL injections in some instances. In general , as the percentages of organic solvents as sample solvents were increased , greater deterioration of the peak profiles was observed. This , however , was not always so with STB.

In 1991 , Sana U. Sheikh , Helen Cho and Joseph C Touchstone (24) studied sample solvent as analyte in high performance liquid chromatography. They showed that a solvent which was a component of the sample could show up as an unexpected peak with its own identity. This solvent might show a similar retention time to some

of the unknown components of the sample. This indicated that in some cases the quantitative results might be the sum of the absorptivity of the solute and the solvent used for the sample. Depending on the mobile phase composition some solvents could be detected at the wavelength or wavelengths used for analysis.

In 1992 , S. Wongyai (25) reported the effect of solvent strength on the chromatographic of drugs in reversed phase high performance liquid chromatography. It was found that the water content in the injected sample significantly influenced the chromatographic behavior of acidic , neutral and basic drugs on reversed phase column. The number of theoretical plates of each compound was distinctly dependent on the water concentration. Resolution of previously unresolved peaks could be achieved without a change in retention.

In 1993 , the experimental consequences of differences between composition of the sample solvent and of the mobile phase in high performance liquid chromatography was described by B. Porsch (26). Anomalous peaks might be expected to occur even in case when sample solubility in mobile phase was sufficient if the sample solvent and mobile phase were different considerably in viscosity and/or elution power of sample solvent exceeded mobile phase strength substantially. Then , to improve peak shapes , the sample solvent-mobile phase viscosity ratio should be kept fairly below two and the high elution power of the sample solvent should be decreased by mixing with mobile phase prior to injection.

Purpose of the Study

A wide variety of factors affects separation efficiency during analysis in high performance liquid chromatography , and the ability to understand and control these factors is a very important element of the analysis. This work studies effect of solvent strength on the chromatographic behavior on reversed phase chromatography for a case sample involving the five components ; paracetamol , phenol , methylparaben, ethylparaben and propylparaben. The parameters studied were

1. temperature of a reversed phase column at 20°C , 25°C, 30°C and 35° C,

2. solvent strength in the sample solution and ,

3. type of reversed phase column *i.e.*, RP-8 and RP-18 .