4 CONCLUSIONS

Gas Chromatography determination of heterocyclic amines is generally unsatisfactory owing to the adsorption and decomposition of the solute on the column, resulting in peak tailing and losses. Therefore, a wide variety of derivatization reactions, surveyed in this thesis, has been employed to reduce the polarity, to improve the GC separation of compounds and to increase both the selectivity and sensitivity of GC detection. In this work GC-NPD was applied to analyze three HCAs (IQ, MeIQx and PhIP). N-dimethylaminomethylene derivatives of these mutagens were prepared by reaction with N,N-dimethylformamide dimethylacetal at 90°C for 10 minutes. This optimum reaction conditions were provided the highest response for all compounds. Structures of the derivative were confirmed by GC-MS using a fused silica capillary column containing PE-17 ht 50% phenyl-50% methylsiloxane, 30 m x 0.25 mm. I.D., 0.25 μm film thickness. Mass spectra showed the molecular ion peak $[M]^+$ for each derivative and other common ion fragments, which were used for structure evaluation at $[M-15]^+(CH_3)$, $[M-44]^+[N(CH_3)_2]$, $[M-56]^+$ $[C{=}N(CH_3)_2]$ and $[M-71]^+$ $[N{=}CNH(CH_3)_2]$.

Under the GC-NPD optimum conditions, the HCAs were separated using fused silica capillary columns containing PE-17 ht compared with HP-5, 5%phenyl-95%methylsiloxane, 30 m × 0.32 mm. I.D., 0.25 μm film thickness. From the results it was concluded that HP-5 column provided a better response for the analysis of HCAs. The optimization process was carried out to obtain the best and high efficiency responses. The optimum conditions obtained were as follows, the carrier gas flow rate 1.5 mL min^{-1}, temperature programming was obtained as: initial temperature was maintained at 190°C for 3 minutes, then raised to 280°C, at 35°C/min, immediately ramp to 300°C with a ramp rate of 10°C/min, and finally hold for 5 minutes. The optimum injector and

detector temperatures were 300°C. In the part of nitrogen phosphorus detector, oxidant gas (Air) flow rate was 100 mL min^{-1} and fuel gas (H_2) flow rate was 2 mL min^{-1}. These optimum conditions of GC-NPD were provided a short analysis time within 17 minutes,

Detection limits and linear dynamic ranges of GC-NPD and GC-MS were compared. The GC-MS method was shown to be very sensitive and highly specific over GC-NPD. GC-MS has been described to be the most sensitive technique for HCAs analysis. Despite being sensitive, selective, simple and rapid most of the GC methods lack the wide application range of a multiresidue method. Only N-dimethylaminomethylene derivatives have been prepared for a large number of HCAs. However, the method was developed with HCA standards and applicability of the method to food samples has still not been reported (Pais and Knize, 2000).

In this research a method was developed for HCAs fraction separated from cooked meat samples and the analysis was by GC-NPD. The HCAs were analyzed as N-dimethylaminomethylene derivatives. Food samples such as grilled meat or fish have many organics compounds and high complexity of the matrix can be co-extracted with HCAs from foods. Therefore, the sample preparation and clean up are required. The ultrasonic extraction coupled with solid phase extraction was applied. The first step, whose purpose was to isolate from the protein matrix the organic compounds that might have formed as a result of meat heat treatment. The samples were homogenized in 1.0 *M* sodium hydroxide solution with ultrasonic extraction, 2 hours is the optimum extraction time. The alkaline solution was then mixed with diatomaceous earth where HCAs were adsorbed. The HCAs were then eluted by organic solvent. The suitable solvent elution of HCAs is dichloromethane mixed with 3% toluene at an optimum volume of 60 mL with optimum flow rate 2 mL min^{-1}. Diatomaceous earth, sand–like porous material is a very practical carrier for extracting of all known heterocyclic amines from solid foods.

The inert diatomaceous earth carrier allows efficient and rapid organic solvent extraction without risking emulsion formation, a very common problem when extracting food.

The second step of the clean up procedure included a selective isolation of HCAs fraction. To achieve the isolation a solid phase extraction with PRS, cation exchange phase was applied. Cartridge extraction of food products using coupled with diatomaceous earth and propylsulfonic acid silica (PRS) cartridges efficiently concentrated the basic compounds.

The aim of the third step was to clean up the HCAs fraction by a solid phase extraction with chemically bonded phase C_{18}. After drying, HCAs from the PRS cartridges were eluted to C_{18} cartridges with the optimum volume of 25 mL of 0.5 *M* ammonium acetate, pH 8.0. These eluates were conveniently concentrated C_{18} silica cartridges. The heterocyclic amines can then be eluted and conveniently concentrated by passing over a C_{18} with 2.5 mL of methanol-concentrated ammonium hydroxide solution (9:1v/v). SPE is a simple procedure to remove most of the unwanted co-extracted interfering peaks, considerable simplification, without application of big volumes of solvents, speeding up complicated sample preparation prior to gas chromatograph. The percentage recovery of each compound in sample are, IQ : 55.1-90.0%, MeIQx ,36.3-63.1 and PhIP: 33.2-82.3%.

The cooked meat samples such as grilled chicken, pork, fish, hamburger beef and fried chicken were sampling from the local supermarkets. The concentration of HCAs were in trace level and lower than the limit of detection. This was confirmed by standard addition method where the results showed the concentration in the range of not detected to 0.11 $\mu g\ g^{-1}$. The effect of matrices interference was also studied of all samples the result showed that for these proposed method the matrix of cooked meat samples were present but the chromatogram could separate them from analytes of interest.