A NEW METHOD FOR QUANTIFICATION OF APOLAR HETEROCYCLIC AROMATIC AMINES WITH PLANAR CHROMATOGRAPHY

Ute Jautz[1], Gerda Morlock[2], Monika Gibis[1], Wolfgang Schwack[2], Albert Fischer[1]

[1]University of Hohenheim, Institute of Food Technology, Dept. of Meat Technology, D-70599 Stuttgart, Germany
[2]University of Hohenheim, Institute of Food Chemistry, D-70599 Stuttgart, Germany

Background

Heterocyclic Aromatic Amines (HAA) are among the most potent mutagenic substances [1]. Around 20 different HAA at low µg/kg level can be identified in food. During the heating process at high temperatures, these substances are formed in meat from the precursors creatine, creatinine, amino acids and sugars. The concentrations depend on the way of preparation, heating time and temperature [2].

Objectives

HAA are typically quantified by liquid or gas chromatography with detection by UV-absorbance, fluorescence or mass spectrometry [3]. The objective of this study was to develop a new method to determine HAA, which have very similar chemical structures.

Materials and methods

Materials

The standard substances Glu-P-1, Glu-P-2, AαC and MeAαC were purchased from Toronto Research Chemicals, Ontario, Canada; Harman and Norharman from Sigma-Aldrich, Taufkirchen, Germany; HPTLC plates silica gel 60 F_{254} (20 x 10 cm) from Merck, Darmstadt, Germany.

Stock solutions

Standards were dissolved in methanol/NH_3.

Instruments

For sample application, the dissolved substances were sprayed in bands by Automatic TLC sampler 4 (ATS 4, Fig. 1). Afterwards, the plates were repeatedly developed in the same direction by the Automated Multiple Development System (AMD 2, Fig. 2). Drawn by capillary forces the developing solvent (mobile phase) migrated through the layer (stationary phase) over a defined distance. Densitometric evaluation of the developed plate was done by TLC Scanner 3 under UV 366 / > 400 nm. The DigiStore documentation system, i.e. Reprostar 3 with digital camera, made it possible to take a photo of the plate. The software winCATS controls all mechanical and electronic functions of the instruments. All TLC instruments were developed by CAMAG, Muttenz, Switzerland.

Results and discussion

Several affecting parameters had to be investigated to find optimal conditions to separate the six apolar and fluorescent HAA. In trade, various plates are available. These differ in material, thickness, particle size, pH-value, fluorescence indicator and binders. Furthermore, the optimal solvent or solvent mixture had to be found. The separation of the bands depends also on the number of gradient steps and migration distance. Moreover, basic, acid or neutral preconditioning between each gradient step changes the activity and pH-value of the layer. This can influence the separation and the focus of several bands. A five-step development to a migration distance of 40 mm each with the solvent mixture of 98 % diethyl ether and 2 % methanol, turned out to be the optimal condition to separate the substances (Fig. 3).

In Figure 4, pictures of plates after the chromatographic development are shown. Each standard is applicated separately as well as in a mixture of three standards. Mix A consists of Glu-P-1, AαC and Norharman, and mix B of Glu-P-2, MeAαC and Harman. The densitometric scan in Figure 5 shows the separated peaks and the possibility to quantify the substances by peak height or area.

Fluorescence measurement is a very sensitive detection method and has a lower detection limit compared to UV-absorbance. However, the point of time at which the evaluation is done is of vital importance. It was observed that fluorescence emission is not stable. A degradation of fluorescence was detected when plates were not stored in darkness but in daylight or artificial light, respectively. Compared with Figure 4a, Figure 4b shows the decline of fluorescence after 48 hours.

It is also evident that the decrease of fluorescence was different for each substance (Fig. 6a). Fluorescence intensity of AαC and MeAαC decreased faster than those of Glu-P-1 and Glu-P-2. The curves of Harman and Norharman first increased, then decreased. Figure 6b indicates that degradation could be avoided by storing the plates in total darkness.

There were also two great advantages to mention. On one hand, the presented method was very rapid. The multi-step development lasted about 45 minutes, i.e. less than 3 minutes per each determination was necessary, when 16 bands were applied on one plate. On the other hand, the method was cost effective. Only 45 mL of solvent per plate was needed, i.e., less than 3 mL per determination. The cost for the HPTLC plates used was less than 5 euros per plate.

Conclusions

The results show that it was possible to separate six fluorescent HAAs on one HPTLC plate. Moreover, it was found important that quantification by fluorescence took place immediately after developing the plate, because of the degradation fluorescence.

It was shown that modern planar chromatography was suitable for a screening test. Further aims may include the separation of the 15 most frequent HAAs found in meat on one HPTLC plate, as well as their quantification. At the present study, only standard substances were used. To what extent meat matrix may complicate the determination of HAAs, remains, thus, to be investigated.

References

[1] Sugimura, T. (1997): Mutation Research 376, 211-219.

[2] Jaegerstad, M., Laser-Reuterswaerd, A., Oeste, R., Dahlqvist, A., Grivas, S., Olsson, K. and Nyhammar, T. (1983): ACS Symposium Series 215 (The Maillard Reaction in Foods Nutrition), 507-519.

[3] Pais, P. and Knize, M.G. (2000): Journal of Chromatography B, 747, 139-169.

Abbreviations

HAA: Heterocyclic Aromatic Amines
Glu-P-1: 2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole
Glu-P-2: 2-Aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole
AαC: 2-Amino-9*H*-pyrido[2,3-*b*]indole
MeAαC: 2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole
Norharman: 9*H*-pyrido[3,4-*b*]indole
Harman: 1-Methyl-9*H*-pyrido[4,3-*b*]indole

Fig. 1: Sample application with ATS 4

Fig. 2: Automatic multiple development with AMD 2 system

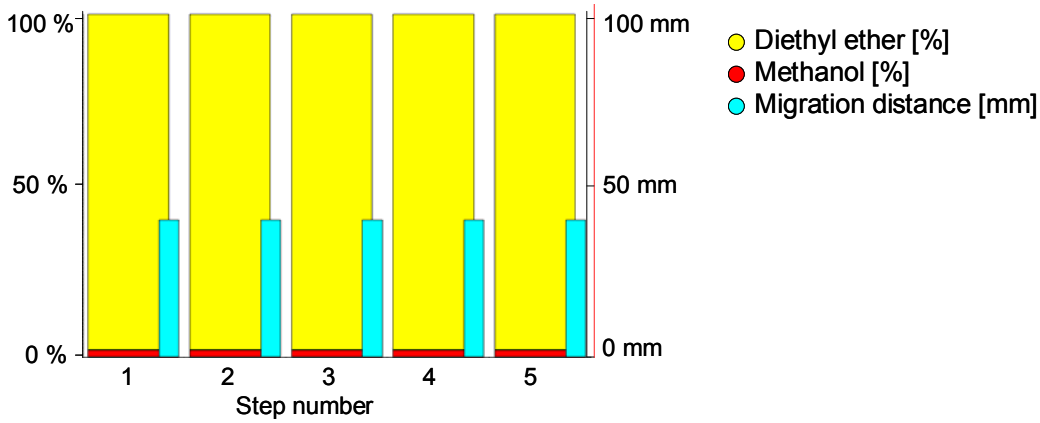


Fig. 3: Gradient scheme to separate the six fluorescent HAA

a)

b)

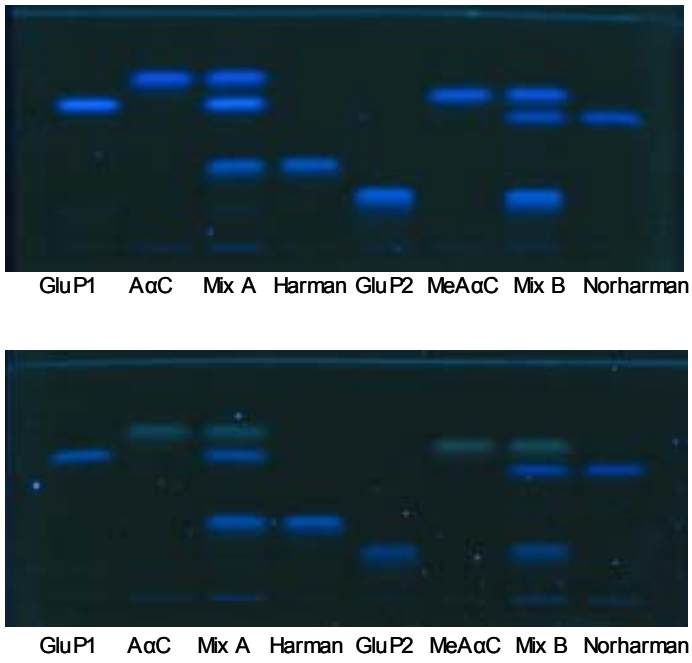


Fig. 4: Plate documented under UV 366 / > 400 nm after 48 hours:
a) stored without light, b) stored in daylight and artificial light

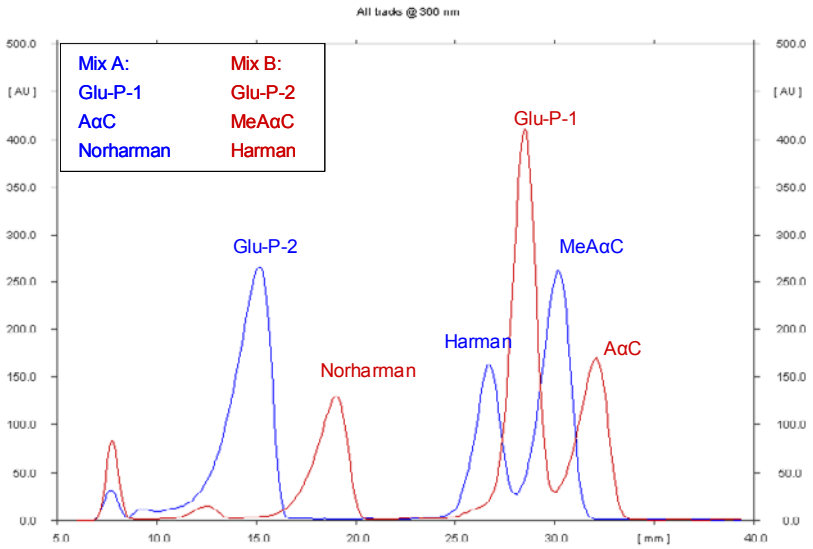
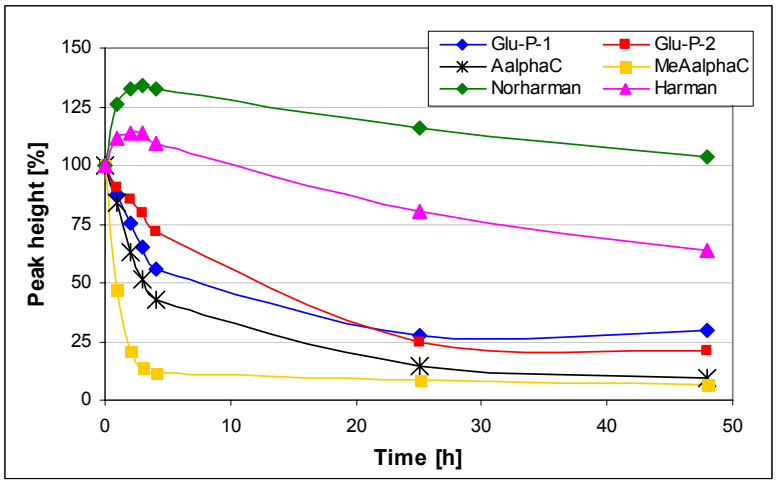


Fig. 5: Densitometric scan of the separated substances under UV 366 / > 400 nm

a)



b)

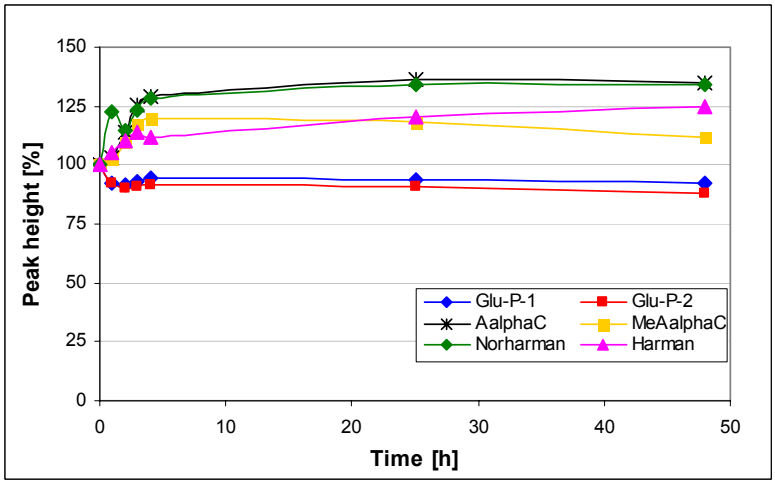


Fig. 6: Fluorescence degradation of 6 HAA during 48 hours: a) by light, b) in darkness