Method comparison for quantification of heterocyclic aromatic amines in fried meat

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Scope

Heterocyclic aromatic amines (HAA), a substance group of more than 20 representatives, are typically quantified by RP-HPLC based on the method developed by Gross and Grueter [1]. Under domestic cooking conditions the HAA predominantly formed in meat at very low concentrations (µg/kg-range) are PhIP, MeIQx, 4,8-DiMeIQx, norharmane and harmane. For these a rapid and cost effective HPTLC method was developed and validated [2]. The frying procedure of the beef patties, the sample preparation and all extraction and clean-up steps to extract HAA from the matrix were standardized and identical for both methods. Quantification of the five most frequently found HAA was performed by both methods and the obtained results were correlated [3].

Results and discussion

At a plate temperature of 230 °C ± 1 °C beef patties w ere fried in a double-contact grill (Nevada, Neumärkter, Hemer, Germany) simultaneously on both sides for five different cooking times (3 - 6 min). HAA were extracted from the meat matrix via solid-phase extraction (Fig. 1). Typical HPLC and HPTLC chromatograms of the HAA extracts are also displayed.

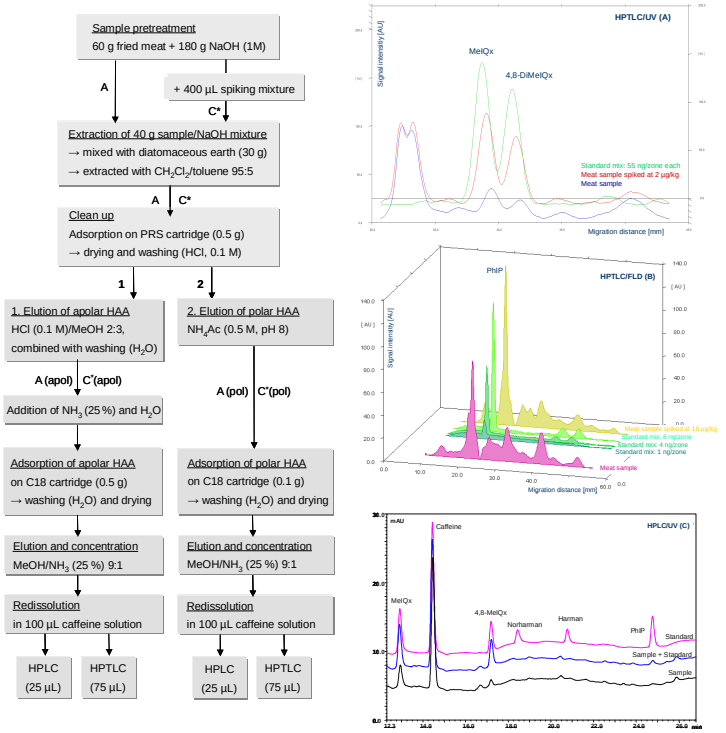

Fig. 1: Flow diagram of the standardized HAA extraction and clean-up protocol (left); HPTLC/UV 262 nm **(A),** HPTLC/FLD 313/>340 nm **(B)** and HPLC/UV 258 nm **(C)** (right).

HAA quantification via HPLC was performed according to the method of Gross and Grueter [1] with modifications. The HPTLC method for HAA analysis is described in detail in [2, 3]. The results obtained from both methods showed **increasing HAA amounts with prolonged cooking times**. A comparison of the MeIQx, 4,8-DiMeIQx and PhIP findings (<1 µg/kg to 33 µg/kg) with those results in literature showed that the values were in the same, very low µg/kg-range [4].

Two interlaboratory studies performed in 1998 and 2004 pointed out the difficulties of the HAA extraction and quantification at the trace level in the complex meat matrix. Statistical tests eliminated up to 50 % of the data values as outliers resulting in a repeatability *(%RSD)* of up to 45 % for the residual data.

The HPLC/HPTLC method comparison showed, without an outlier correction, HAA **correlation coefficients** between 0.8875 and 0.9751. Thus the findings were **in good agreement** at the trace levels given. Figure 2 exemplarily shows the correlations for MeIQx and norharmane; the precisions of the findings are illustrated as standard deviations which are comparable for both methods.

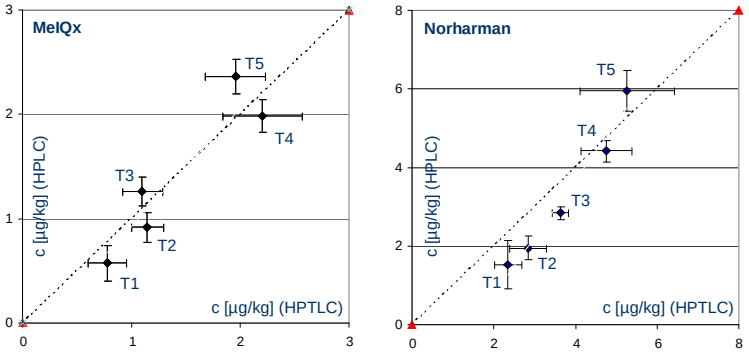

Fig. 2: HPLC/HPTLC correlations for the findings of MeIQx (left) and norharmane (right) at five different cooking times T1 - T5: 3 min, 3 min 45 s, 4 min 30 s, 5 min 15 s and 6 min (mean value ± SD, n = 12, without outlier elimination).

The comparison of the parameters running costs and analysis time for both methods in routine analysis showed that the costs for HPTLC analysis are **by a factor of 3 lower** than the costs for HPLC analysis. Using HPTLC 5 min extra time is needed for manual step transfer. However, due to simultaneous analysis of 20 runs, the HPTLC method is **4 times faster** than HPLC.

Conclusion

The results show a good correlation between HPTLC and HPLC analysis. Increasing HAA concentrations with prolonged cooking times were found with both methods. The savings regarding running costs and analysis time are further arguments for the application of HPTLC as an **alternative** method to HPLC analysis.

Abbreviations

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx:2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline; 4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; norharmane: 9H-pyrido[3,4-b]indole, harmane: 1-methyl-9H-pyrido[3,4-b]indole; SD: standard deviation; *%RSD*: relative standard deviation

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