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1 **Optimised extraction of heterocyclic aromatic amines from blood**
2 **using hollow fibre membrane liquid-phase microextraction and triple**
3 **quadrupole mass spectrometry**

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17
18 **Abstract**

19 Heterocyclic aromatic amines (HCA) are carcinogenic mutagens formed during cooking
20 of proteinaceous foods, particularly meat. To assist in the ongoing search for
21 biomarkers of HCA exposure in blood, a method is described for the extraction from
22 human plasma of the most abundant HCAs: 2-Amino-1-methyl-6-phenylimidazo(4,5-
23 b)pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-
24 3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) (and its isomer 7,8-DiMeIQx),
25 using Hollow Fibre Membrane Liquid-Phase Microextraction. This technique employs
26 2.5 cm lengths of porous polypropylene fibres impregnated with organic solvent to
27 facilitate simultaneous extraction from an alkaline aqueous sample into a low volume
28 acidic acceptor phase. This low cost protocol is extensively optimised for fibre length,
29 extraction time, sample pH and volume. Detection is by UPLC-MS/MS using positive
30 mode electrospray ionisation with a 3.4 min runtime, with optimum peak shape,
31 sensitivity and baseline separation being achieved at pH 9.5. To our knowledge this is
32 the first description of HCA chromatography under alkaline conditions. Application of
33 fixed ion ratio tolerances for confirmation of analyte identity is discussed. Assay

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