

# Development of methods to quantitatively extract biologically active principles from foods, flavourings and herbs and spices

Area of research interest: [Chemical hazards in food and feed](#)

Study duration: 2008-01-01

Project code: A01067

Conducted by: Food and Environment Research Agency (FERA), York

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## Background

The use of BAPs as such as flavourings is not permitted in the UK. However, some flavouring preparations are known to contain these substances naturally. UK flavouring legislation limits the levels of BAPs in foodstuffs to which natural flavourings are added.

A research project funded by the Agency (A01041) critically reviewed the published methods on the analysis of BAPs. The project highlighted that there is a lack of methods available to extract many of the BAPs from food matrices.

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## Results

Three separate methods were developed:

1. Volatile BAPs (safrole, methyl eugenol, estragole, menthofuran and pulegone) by simultaneous distillation-extraction (SDE) of the sample followed by GC-MS quantitation. It was not possible to determine coumarin by SDE due to its low volatility.
2. Coumarin and quassine by solvent extraction of the sample, clean-up using solid phase extraction cartridges and then quantitation by HPLC with UV detection.
3. Hydrogen cyanide (HCN) by extraction of the sample using an acidic solution, enzymatic hydrolysis of glycosidic cyanogens to cyanohydrins, hydrolysis of these to cyanide and lastly derivatisation using a modified König reaction to form a coloured complex which is determined spectrophotometrically.

The methods were validated in single-laboratory tests by FERA using a range of different food and beverages. Eight different matrices (breath fresheners, mint confectionery, yoghurt, canned soup, soft drink, vegetable product, chewing gum and fish product) were validated for the five volatile BAPs plus isosafrole. Ten matrices were validated for coumarin and quassine (bakery ware, breakfast cereal, rice pudding, gelatine confectionery, biscuit, sugar confectionery, carbonated soft drink, fruit-flavoured drink, herbal infusion and mixed spice). Three matrices were validated for HCN (canned stone fruit, marzipan and alcoholic beverage). The developed methods were subjected to cross validation by a second (independent) laboratory.

The results were mixed for the SDE method and the cross validation of the HCN method was unsuccessful. The independent laboratory produced similar results for coumarin except for the herbal infusion. However, results for quassine were not comparable as the second laboratory

experienced problems with chromatography (split peaks, drifting retention times, interferences). Standard operating procedures are available for all methods.

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## Published Papers

1. Scotter, M.J., Roberts, D.P.T. & Rees, G.O. (2011) Development and single-laboratory validation of an HPLC method for the determination of coumarin in foodstuffs using internal standardization and solid-phase extraction cleanup. *Analytical Methods* 3, 414-419.

Research report

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