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# DETERMINATION OF PHENYLALANINE IN RIVER WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

The use of *o*-phthalaldehyde derivatisation of phenylalanine followed by high-performance liquid chromatography with fluorescence detection as used for amino acid analysis in clinical laboratories was examined to assess its suitability for the monitoring of river pollution by the dairy industry by measuring the phenylalanine levels in river water. The method is described together with its statistical evaluation.

## INTRODUCTION

Rivers are a major resource in the U.K. for water intended for potable use after suitable treatment. Pollution of rivers by the dairy industry (both industrial and farming) can cause problems other than oxygen deficiency in the water particularly if the river is being used as such a resource. During recent dairy product pollution incidents in the River Dee in recent years an off-flavour in the water was found after treatment. Subsequent gas chromatographic (GC)–mass spectrometric (MS) analysis of dichloromethane extracts of the treated water revealed the presence of various aldehydes: phenylacetaldehyde (hyacinthin), 2-methyl butyraldehyde and 3-methyl butyraldehyde[1]; all highly odoriferous compounds. These compounds were absent in the untreated water. Yoshioka *et al.*[2] reported the production of phenylacetaldehyde following chlorination of phenylalanine. This was later confirmed by laboratory investigations[1] which pointed to the effect of disinfection procedures (chlorination) on phenylalanine present in water following dairy pollution (milk and milk products contain high levels of phenylalanine, *e.g.*, skimmed milk contains 5% (w/v) phenylalanine). 2-Methyl butyraldehyde and 3-methyl butyraldehyde are likely to be the result of chlorination on leucine and isoleucine respectively.

At present monitoring river water for pollution by milk and milk products is difficult to achieve and the water analyst is left to monitor the odoriferous compounds in the potable supply by GC–MS. A method was thus required to assess phenylalanine levels in river water concentration $< 5\ \mu g\ l^{-1}$ to monitor dairy product pollution or farm spillage where phenylalanine present could result in off-flavour potable water.

One of the standard techniques available for the analysis of amino acids in the clinical laboratory is derivatisation with *o*-phthalaldehyde (OPA) followed by high-

performance liquid chromatography (HPLC) with fluorescence detection, this could achieve the detection limit required (low $\mu g\ l^{-1}$) without extensive pre-column concentration or extraction of the analyte. OPA reacts with primary amino groups in the presence of 2-mercaptoethanol to form highly fluorescent derivatives[3]; this reagent is highly specific and derivatisation takes place rapidly.

## EXPERIMENTAL

OPA reagent was purchased ready prepared from Sigma (Poole, U.K.; product No. P-05332) and derivatisation was carried out as per the instructions accompanying the reagent, *i.e.*, equal volumes (0.5 ml) of reagent solution and sample were mixed together at room temperature. After 1 min 200 $\mu$l of the mixture were injected via a sample loop onto a LiChrosorb RP-18 column. Mobile phase was phosphate buffer (pH 7.0)–acetonitrile (70:30); flow-rate 1 ml $min^{-1}$. The fluorescence detector was optimised for the phenylalanine derivative, *i.e.*, excitation 341 nm, emission 444 nm.

### *Apparatus*

*HPLC pump.* A dual reciprocating pump (Model 420; Kontron, Watford, U.K.) fitted with microbore heads and a Bourdon tube type pulse dampener was used.

*Fluorescence detector.* A Perkin-Elmer (Beaconsfield, U.K.) LS-3B fluorescence spectrometer used fitted with a HPLC flow cell (20 $\mu$l cell volume, 1.5 mm I.D.) was used.

*Injector.* A Rheodyne 7125 injector fitted with a 100-$\mu$l sample loop was applied.

*Column.* The column was LiChrosorb RP-18, 10 $\mu$m particles, 25 cm × 4 mm I.D.

*Syringes.* A 250-$\mu$l Rheodyne syringe (Hamilton 725SNR) and a 2-ml Luer tip fitted with a 7125 needle-port cleaner were used.

## RESULTS AND DISCUSSIONS

To test the performance of the method, duplicate analyses were carried out on each of four days, of HPLC-grade water (blank), 10 $\mu g\ l^{-1}$ and 20 $\mu g\ l^{-1}$standards, river water from the River Dee at Huntington and River Dee water spiked with a 10 $\mu g\ l^{-1}$ phenylalanine addition (Fig. 1). The results of the last two samples were used to calculate the spiking recovery.

Statistical evaluation of the method was carried out according to the procedures given by Cheeseman and Wilson[4] (*viz.*, precision of standards and samples and spiking recovery). The results were analysed to derive the corresponding within-batch ($s_w$), between-batch ($s_b$) and total ($s_t$) standard deviations. The results are summarised in Table I, the numbers of degrees of freedom were derived from duplicate analyses on each of five days and are given in parentheses.

Analysis of variance showed that the 10 and 20 $\mu g\ l^{-1}$ phenylalanine standards showed no significant between-batch variability.

A limit of detection of 0.69 $\mu g\ l^{-1}$ of phenylalanine was calculated by using the

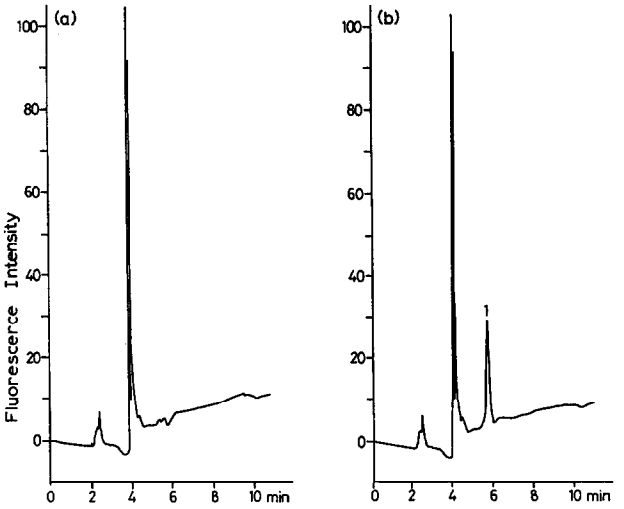


Fig. 1. Chromatograms of (a) River Dee water after OPA derivatisation and (b) River Dee water spiked with 10 $\mu$g $l^{-1}$ phenylalanine (1) after OPA derivatisation. Column: 25 cm × 4 mm I.D. LiChrosorb RP-18, 10 $\mu$m; mobile phase: Phosphate buffer (pH 7.0)–acetonitrile (70:30) at a flow-rate of 1 ml $min^{-1}$; detection: fluorescence, excitation wavelength 341 nm, emission wavelength nm.

formula $2t\sqrt{2}s_w$, where $t$ is Student's single-sided $t$ for a probability of 0.10 and $s_w$ is the within-batch standard deviation of the blanks.

The spiking recovery of 89% was considered satisfactory.

## CONCLUSIONS

The work showed that methods used in the clinical laboratory can be applied to environmental analysis with little or as in this case no changes. The selected method is simple, rapid and with satisfactory precision and detection limit.

TABLE I

PRECISION OF ANALYTICAL RESULTS

For chromatographic conditions, see text. Figures in parentheses are the degrees of freedom. N.S. indicates that the result is not statistically significant.

| *Solution* | *Standard deviation ($\mu$g $l^{-1}$ phenylalanine)* | | | *Mean concentration found ($\mu$g $l^{-1}$ phenylalanine)* |
|---|---|---|---|---|
| | $s_w$ | $s_b$ | $s_t$ | |
| Blank | 0.17(4) | — | — | 1.37** |
| 10 $\mu$g $l^{-1}$ Phe | 1.08(4) | N.S.(3) | 1.08(5) | 9.93*** |
| 20 $\mu$g $l^{-1}$ Phe | 2.08(4) | N.S.(3) | 2.08(5) | 20.19*** |
| River Dee water | 0.34(40 | N.S.(3) | 0.57(4) | 0.04*** |
| River Dee water + 10 $\mu$g $l^{-1}$ phenylalanine | 1.74(4) | N.S.(3) | 01.74(5) | 8.94*** |

** Value of blank in terms of $\mu$g $l^{-1}$ phenylalanine
*** All values corrected for blank.

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