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## Note

# Method for the measurement of hydroxylamine in colonic fluid using derivatisation and gas chromatography

D.J. DARKE

*Analytical R & D Unit, Building 148, AERE, Harwell, Oxon (Great Britain)*

and

W.E.W. ROEDIGER*

*Nuffield Department of Medicine, Radcliffe Infirmary, Oxford (Great Britain)*

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Man consumes considerable amounts of nitrates and nitrites, a proportion of which are food preservatives [1]. Metabolism of nitrates and nitrites by colonic bacteria in man can produce carcinogens such as N-nitroso derivatives of amines [2]. Bacteria furthermore are able to reduce nitrate to ammonia by assimilatory reduction, in the process of which hydroxylamine is formed [3].

$$NO_3^- \xrightarrow[\text{reductase}]{2e^-} NO_2^- \xrightarrow[\text{reductase}]{2e^-} \begin{bmatrix} NOH \\ (?) \end{bmatrix} \xrightarrow[\text{reductase}]{2e^-} \underset{\text{(hydroxylamine)}}{NH_2OH} \xrightarrow[\text{reductase}]{2e^-} NH_3$$

Hydroxylamine is a mitogen [4] and a powerful metabolic inhibitor [5] the harmful metabolic effects of which have been established on isolated suspensions of intestinal epithelial cells [6].

Whether nitrites used for preservatives are harmful in the form of hydroxylamine, is presently controversial because no specific method of measuring hydroxylamine in biological samples is at present available. Numerous non-specific methods have been described [7]. The present report concerns a method of measuring hydroxylamine in stool water obtained in vivo by dialysis of the colonic contents of man. The method entails gas chromatography with a combined and synchronous use of nitrogen and flame ionisation detectors.

*Present address: Department of Surgery, Prince Henry's Hospital, Melbourne, Victoria, Australia.

## EXPERIMENTAL

### *Materials*

Hydroxylamine hydrochloride, acetone oxime, hydrazine hydrate, methanol and acetone were obtained from BDH (Poole, Great Britain).

### *Apparatus*

A Pye 104 gas chromatograph fitted with a flame ionisation detector and a nitrogen detector was used. The outlet of the column was split 1 : 1 to each detector. The glass column (1.5 m × 4 mm I.D.) was packed with Pennwalt 223 on 80—100 mesh Gas-Chrom R (Applied Science Labs., State College, Pa., U.S.A.) at 150° with the injection port at 160°. The carrier gas was nitrogen at 60 ml/min.

If hydrazine is present (see Discussion below) a 1.5 m × 4 mm I.D. glass column packed with Carbowax 20M on Diatomite C AW DMCS (60—80 mesh) at 120° with the injection port at 150° is used.

For the gas chromatography—mass spectrometry analysis a Pye 104 fitted with the Pennwalt column was interfaced to a VG Micromass Q9K quadrupole mass spectrometer via a glass jet separator.

### *Procedure*

The faecal dialysate was collected as described by Wrong et al. [8]. In principle this is an in vivo dialysis of stool. All water-soluble metabolites of bacteria are drawn into the dialysis tubing (Visking dialysis tubing 1/4 in., Scientific Instruments, London, Great Britain) which is filled with dextran (molecular weight 40,000). The contents of the dialysis tubing were stored in the deep freeze and then analysed as indicated.

Initially, to check the reaction, hydroxylamine in the form of the hydrochloride was dissolved in 10 parts water to 1 part methanol—acetone (1 : 1). Acetone oxime dissolved in water was used for identification and to check the recovery values.

Subsequently hydroxylamine was added to the dialysate in the range 0.1 mg to 1 g/l with acetone added in slight excess of the stoichiometric requirements. The calibration was found to be linear within this range. Recovery values were greater than 90%.

## DISCUSSION

Hydroxylamine, because of its reactive and labile nature, will not chromatograph intact. It reacts with ketones to give oximes:

$$R_1R_2CO + NH_2OH \longrightarrow \left[R_1R_2C\begin{matrix}OH\\NHOH\end{matrix}\right] \xrightarrow{-H_2O} R_1R_2C{=}NOH$$

The oximes are well defined crystalline solids and the yields are good.

Using the above procedure quantitative yields of acetone oxime were obtained. Fig. 1 shows the response on each detector to the acetone oxime produced. Substitution of methyl ethyl ketone for acetone produced 2-butanone

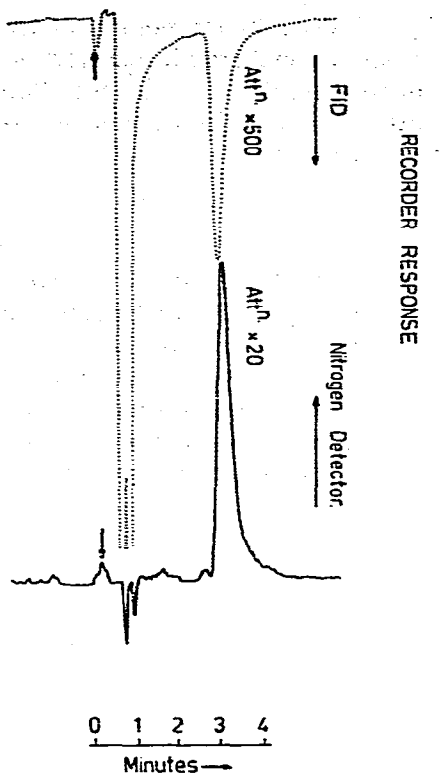


Fig. 1. Hydroxylamine hydrochloride in aqueous solution with added methanol—acetone (1 : 1) injected on to Pennwalt 223 at 150° showing response on each detector to acetone oxime produced (0.05 g/l hydroxylamine).

oxime (methyl ethyl ketoxime) with a retention time of 5 min. This reaction would be useful if a substance such as an amine which eluted at the same time as acetone oxime is present in the sample. Large quantities of primary and secondary amines are present in colonic dialysate [9] but they, as well as nitrites, nitrates and ammonia, do not interfere with the detection of acetone oxime.

When hydrazine in the form of hydrazine hydride was added to the original hydroxylamine hydrochloride solution and immediately injected on to the column a compound eluting at the same time as acetone oxime (3 min) was observed, and identified by mass spectrometry as acetone hydrazone, together with a peak for acetone azine which had a retention time of 4.3 min under the conditions used. After several hours the interfering compound had reduced to less than 10% and the yield of acetone azine was within 90% of the expected value. This behaviour is similar to that reported by Selim and Warner [10].

If hydrazine is present the Carbowax 20M column may be used. Under the conditions stated the interfering peak elutes at 0.9 min, not completely resolved from acetone azine (1.1 min). These elute ahead of acetone oxime which has a retention time of 2.6 min.

The use of the nitrogen detector only confirms the presence of the nitrogen containing compound but does not give an increase in sensitivity against the flame ionisation detector for acetone oxime. If there is an excess of solvent

then the acetone oxime lies on the trailing edge of the solvent peak and the baseline has not quite returned to normal in the case of the flame ionisation detector. Due to the small response for the solvent on the nitrogen detector (the response is in fact negative) the problem does not occur and in such cases the use of the nitrogen detector for quantitation is to be prefered.

## RESULTS AND CONCLUSION

No hydroxylamine was detected in the five samples of dialysate that were examined. However the method as far as ascertainable is specific for hydroxylamine even in the presence of numerous nitrogen-containing and other volatile compounds. Addition of hydroxylamine hydrochloride to colonic dialysate is measureable within the range 0.1 mg/l to 1 g/l without interference of such chemical groups. Measurement of hydroxylamine in man on various nitrate diets and in subjects with damaged intestinal mucosa (ulcerative colitis) is now feasible.

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