Journal of Chromatography, 488 (1989) 506–508
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4573

Letter to the Editor

Determination of sodium azide in the presence of proteins by high-performance liquid chromatography

Sir,

Sodium azide is added as an antiseptic to some diagnostic preparations, e.g., to lectins which are used for the determination of blood types and in diagnostic sera. Determination of sodium azide is part of the quality control in the manufacture of the above preparations. The polarographic method in current use is complicated with a subjective evaluation of results. Spectrometric [1,2] or volumetric [3] methods are not applicable in the presence of proteins or low-molecular-mass serum constituents. For the determination of low-molecular-mass anions, ion-exchange [4–6] or reversed-phase column chromatography [7,8] is used. This work is based on the high-performance liquid chromatographic (HPLC) method of Luckas [8], which was modified for protein-containing samples.

EXPERIMENTAL

All reagents were of analytical-reagent grade unless indicated otherwise.

Sodium azide, used as a standard, was obtained from Lachema (Brno, Czechoslovakia) and acetonitrile (LiChrosolv), sulphuric acid and *n*-octylamine from Merck (Darmstadt, F.R.G.). The mobile phase was kept at 4 °C and was degassed immediately prior to analysis.

A Shimadzu LC-6 A HPLC instrument was used. Samples were injected through a 20-μl loop valve (Rheodyne, Cotati, CA, U.S.A.) onto a Separon SGX C_{18} column (250×4.0 mm I.D.), particle size 7 μm (Tessek, Prague, Czechoslovakia) at ambient temperature. The mobile phase was a 0.01 mol/l aqueous solution of *n*-octylamine, adjusted to pH 6.0 with sulphuric acid, at a flow-rate of 1.0 ml/min. A Shimadzu SPD-6AV spectrometric detector was set at 220 nm. Peak areas were evaluated with a Shimadzu Chromatopac C-R3A integrator; the chart speed was 2 mm/min.

For the preparation of samples, 0.9 ml of acetonitrile was added to 0.1 ml of the

0378-4347/89/$03.50

tested substance or to human citrate plasma. Precipitated proteins were removed by centrifugation (3000 *g*, 10 min) and the supernatant was injected into the column. Samples of human citrate plasma with a known azide concentration (500–5000 μg/ml) were used for calibration.

RESULTS AND DISCUSSION

The chromatogram of the extract from pure plasma is shown in Fig. 1A. No endogenous components detectable at 220 nm were eluted between 5 and 7 min. The azide anion, with a retention time of 6.3 min, was separated entirely from the frontal peak (Fig. 1B). Of the anions tested for interferences (Table I), nitrite, bromide and bromate were found to interfere with azide. However, these

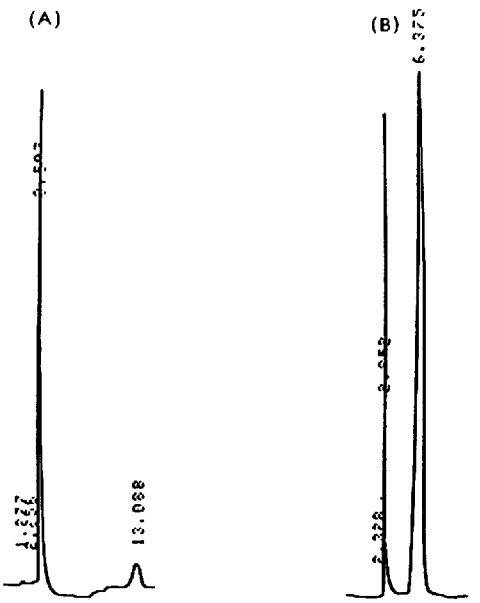

Fig. 1. (A) Chromatogram of blank plasma extract. (B) Chromatogram of plasma extract containing 2000 μg/ml azide (retention time 6.3 min).

TABLE I

RETENTION TIMES OF VARIOUS INORGANIC ANIONS

Column, Separon SGX C_{18}; mobile phase, 0.01 *M* *n*-octylamine (pH 6.0); flow-rate, 1 ml/min; injection volume, 20 μl; detection, 220 nm.

Ion tested	Relative retention time (min)
N_3^-	1.00
NO_3^-	1.43
NO_2^-	1.16
Br^-	0.98
BrO_3^-	1.11
IO_4^-	0.84

anions are not present in the tested substances. The calibration graph was linear in the range 500–5000 μg/ml with a correlation coefficient of 0.999. The detection limit was 10 μg per injection. The precision of the method was 2.7% for a concentration of 2000 μg/ml in plasma ($n=5$) and the accuracy was 3%.

CONCLUSION

The method described is suitable for the selective, sensitive, fast and sufficiently precise routine determination of sodium azide in the presence of proteins, which is commonly required in the quality control of some diagnostic preparations.

State Institute for Drug Control,
Srobárova 48,
CS-10041 Prague 10 (Czechoslovakia)

JOSEF VÁCHA*
MAGDA TKACZYKOVÁ
MAGDALENA REJHOLCOVÁ

1 E.A. Terpinski, Analyst (London), 110 (1985) 1403.
2 E. Kubaszewski and Z. Kurzawa, Chem. Anal. (Warsaw), 30 (1985) 609.
3 I.M. Kolthoff, R. Belcher, V.A. Stenger and G. Matsuyama, Volumetric Analysis, Vol. III, Interscience, New York, 1957.
4 H. Shintani and K.P. Dasgupta, Anal. Chem., 59 (1987) 802.
5 B.B.Wheals, J. Chromatogr., 402 (1987) 115.
6 R. Golombek and G. Schwedt, J. Chromatogr., 367 (1986) 69.
7 R.N. Reeve, J. Chromatogr., 177 (1979) 393.
8 B. Luckas, Fresenius' Z. Anal. Chem., 323 (1986) 375.

(First received September 14th, 1988; revised manuscript received November 10th, 1988)