Journal of Chromatography, 229 (1982) 475—480
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1194

Note

Determination of N-methylpyridinium 2-aldoxime methylsulfate (Contrathion®) in rat plasma and urine by high-performance copper(II)—silica ligand-exchange chromatography

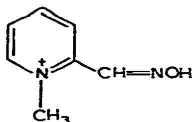
F. GUYON, A. TAMBUTE*, M. CAUDE* and R. ROSSET

Laboratoire de Chimie Analytique de l'École Supérieure de Physique et de Chimie de Paris, 10 rue Vauquelin, 75231 Paris Cedex 05 (France)

(First received July 6th, 1981; revised manuscript received December 8th, 1981)

A number of oximes have been found to reactivate the cholinesterases inhibited by organophosphates [1]. A quaternary pyridinium structure containing an oxime function in combination with atropine has proved effective in the treatment of poisoning by some of these toxic chemicals and in reducing their toxicity in various animal species [2].

The chemical structure of the cationic moiety, N-methylpyridinium 2-aldoxime, is



Several salts are used: N-methylpyridinium 2-aldoxime chloride, iodide, methanesulfonate and methylsulfate (Contrathion®). The aim of this paper was to study the determination of Contrathion in rat plasma and urine for use in pharmacokinetic studies.

Several analytical methods have been described for the quantitation of N-methylpyridinium 2-aldoxime in biological fluids, including hydrolysis (with hydroxylamine) of the deproteinized supernatant, and absorptiometric analysis [3] or direct spectrophotometric measurement of absorption of the oximate

*Attaché aux Services Techniques de l'Armée Française, Centre de Défense Nucléaire, Biologique et Chimique de l'ETCA, Boîte Postale No. 3, Le Bouchet, 91710 Vert-le-Petit, France.

0378-4347/82/0000—0000/$02.75 © 1982 Elsevier Scientific Publishing Company

anion at 335 nm [4, 5]. This latter method has been made more rapid and accurate by automatization [6].

Over the past five years, modern liquid chromatography has established itself as the choice method for solving many biochemical and clinical analysis problems. Recently a quantitative determination of another oxime (N,N-trimethylene bis-pyridinium-4-aldoxime, TM B-4) was achieved using ion-pair chromatography coupled with spectrophotometric detection at 254 nm [7, 8]. This chromatographic technique could be used for the quantitative estimation of Contrathion in biological fluids, but we prefer ligand-exchange chromatography on copper(II)-modified silica gel, previously developed in our laboratory [9—13]. A primary benefit of ligand-exchange chromatography on copper(II)-modified silica-gel particles compared with ion-pair chromatography is the improvement of selectivity and a better efficiency compared with ligand-exchange chromatography on classical polystyrene—divinylbenzene resins.

An additional advantage of ligand-exchange chromatography is the use of an alkaline mobile phase, the Contrathion molar absorptivity being higher in basic medium than in acidic medium [14]. Furthermore, the selectivity is better in a basic mobile phase owing to the fact that the wavelength used for detection (340 nm) is higher in an acidic medium (295 nm). This last observation is very important for the analysis of complex mixtures such as biological fluids.

EXPERIMENTAL

Apparatus

This study was performed with a Hewlett-Packard (Waldbronn, G.F.R.) Model 1084B liquid chromatograph equipped with an automatic sampling system (79842A) and a variable-wavelength detector (190—540 nm) (79875A) operating at 340 nm.

Stationary phases

Copper(II)-modified silica-gel columns were prepared as described previously [9]. Stainless-steel columns (15 × 0.48 cm I.D.) were packed, according to the slurry packing technique, with pure Partisil 5 silica gel of 7 μm mean particle size (Whatman, Clifton, NJ, U.S.A.). An aqueous solution of copper(II) sulfate (10^{-2} mol/l) and ammonia (1 mol/l) was then allowed to percolate through the column until equilibrium was reached [when copper(II) ions appeared in the effluent]. The column was then fitted on the chromatograph and equilibrated with the mobile phase.

Chemicals

Acetonitrile was of LiChrosolv grade and was purchased from E. Merck (Darmstadt, G.F.R.). Ammonia was of Rectapur grade and was obtained from Prolabo (Paris, France). Copper(II) sulfate was of Purissimum grade from Fluka (Buchs, Switzerland). The water was bidistilled. Contrathion® and the N-methylpyridinium 4-aldoxime methanesulfonate used as internal standard were supplied by our department of organic synthesis.

Preparation of samples

Plasma. Each 100 μl of plasma with an added 10 μl of internal standard

solution was deproteinized with 500 μl of acetonitrile. After being shaken vigorously the samples were centrifuged. Aliquots of 20 μl were injected into the chromatographic column.

Urine. A suitable volume, usually 1 ml or less, was diluted with distilled water to 10 ml and a protein-free solution was prepared as described for plasma.

RESULTS AND DISCUSSION

Choice of mobile phase composition

In a previous paper [9] we demonstrated that the retention on copper(II)-modified silica gel is governed by both ligand-exchange and normal-phase partition mechanisms. Consequently, we can take it for granted that the solute capacity factor (k') is a function of both ligand and organic modifier concentrations in the mobile phase.

The variations in Contrathion and internal standard capacity factors with ammonia concentration in the mobile phase (acetonitrile—water, 75:25, v/v) are shown in Fig. 1. The higher the ammonia concentration in the mobile phase the smaller the capacity factor. This behaviour is in good agreement with a ligand-exchange mechanism.

The variations in Contrathion and internal standard capacity factors with acetonitrile content in the mobile phase containing 0.5 mol/l ammonia are shown in Fig. 2.

The capacity factor increases as the acetonitrile volume fraction in the

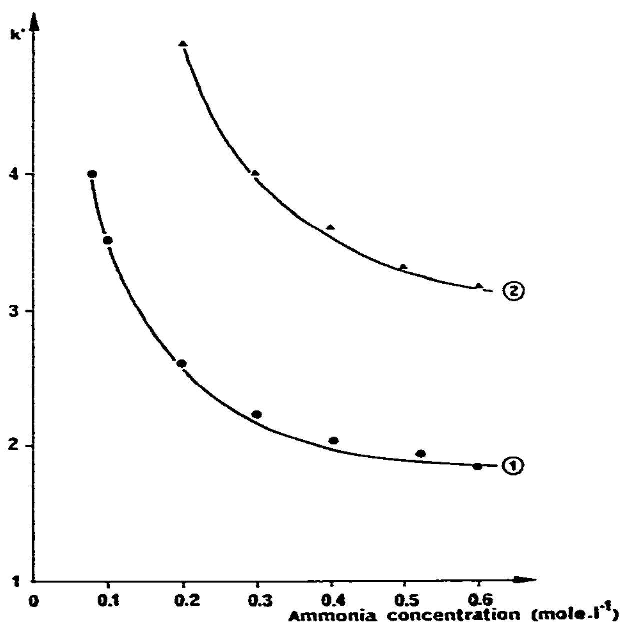


Fig. 1. Variation of capacity factor (k') of Contrathion® and internal standard with ammonia concentration in the binary mixture acetonitrile—water (75:25, v/v). Column: 15 × 0.48 cm I.D. Packing: copper(II)-modified silica gel (Partisil 5), 7 μm. Flow-rate: 1.5 ml/min. 1 = Contrathion; 2 = internal standard.

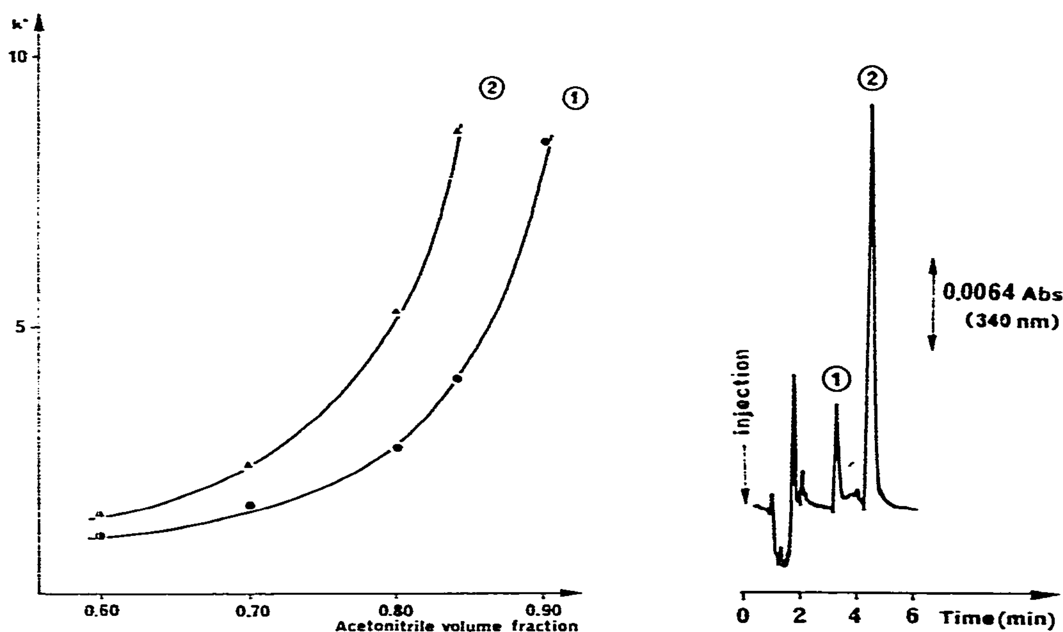


Fig. 2. Variation of capacity factor (k') of Contrathion® and internal standard with volume fraction of acetonirile in the binary mixture acetonitrile—water. Ammonia concentration: 0.5 mol/l. Operating conditions as in Fig. 1. 1 = Contrathion; 2 = internal standard.

Fig. 3. Ligand-exchange chromatographic separation of Contrathion® in rat plasma 2 min after a single muscular injection of 60 mg/kg. Internal standard was injected after prelevement. Column: 15 × 0.48 cm I.D. Packing: copper(II)-modified silica gel (Partisil 5), 7 μm. Mobile phase: acetonitrile—water (75:25, v/v) with ammonia 0.5 mol/l. Flow-rate: 1.5 ml/min. ΔP: 60 atm. Temperature: 30°C. Detection: UV at 340 nm. Injected volume: 20 μl. 1 = Contrathion; 2 = internal standard.

mobile phase increases, that is to say, as it becomes increasingly hydrophobic. In a mobile phase rich in acetonitrile, the solubilities of hydrophilic molecules such as Contrathion and internal standard are smaller than in a mobile phase poor in acetonitrile. Thus, the normal-phase partition mechanism also governs the variations in capacity factor.

Following from these results, a mobile phase having an acetonitrile—water composition of 75:25 (v/v) and an ammonia concentration of 0.5 mol/l was selected for the Contrathion analysis. Fig. 3 illustrates ligand-exchange analysis of Contrathion in rat plasma 2 min after a single intramuscular injection of 60 mg/kg. The plasma level of Contrathion measured is $42 \cdot 10^{-6}$ mol/l (UV absorption at 340 nm). No interference from biological components of plasma was observed.

Quantitative analysis

The calibration plot is linear from 5 pmol to 1 μmol, and passes through the origin, for aqueous solutions as well as for deproteinized biological samples. Unfortunately, some loss of Contrathion is observed during the plasma work-up procedure and the yield is only ca. 90%. In this latter case, the calibration plot was constructed using samples of rat plasma spiked with Contrathion.

The reproducibility is high both for the chromatographic technique itself (relative standard deviation measured for retention time is 0.5%) and for the quantitative analysis. The relative standard deviations are 0.6%, 1.8% and 4.5% for Contrathion quantities close to 0.6, 0.1 and 0.05 nmol injected, respectively.

These results prove the good stability of chromatographic columns filled with copper(II)-modified silica gel eluted with mobile phases containing less than 50% water. For higher water concentrations the silica support is slowly attacked by the hydroxide ions.

Detection limit

Fig. 4 shows the chromatogram (for a rat plasma sample) corresponding to the injection of 20 pmol of Contrathion and internal standard.

The detection limit is generally taken as the solute quantity that will provide a signal-to-noise ratio of two. According to this definition, 5 pmol of Contrathion are detected. In consequence of sample preparation and an injected volume of 20 μl, the detection limit in rat plasma and in urine is $1.5 \cdot 10^{-6}$ mol/l.

With operating conditions (ion-pair chromatography) described in a recent publication [8] a detection limit of 30 pmol has been obtained (at 295 nm) by us. Ligand-exchange chromatography at 340 nm is six times more sensitive.

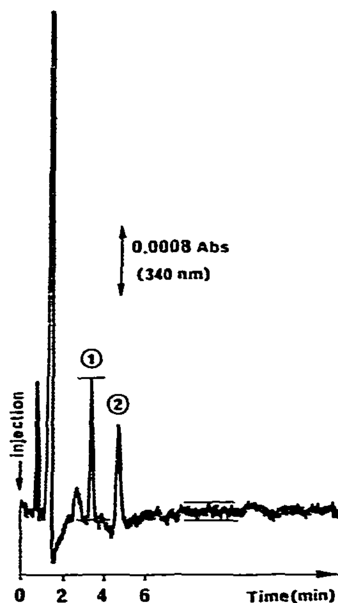


Fig. 4. Separation of 20 pmol of Contrathion® and internal standard in spiked rat plasma. Operating conditions as in Fig. 3. 1 = Contrathion; 2 = internal standard.

CONCLUSION

Ligand-exchange chromatography on copper(II)-modified silica-gel microparticles appears to be the best chromatographic technique for quantitative analysis of Contrathion and its derivatives in biological fluids. Advantages are

high selectivity due to the ligand-exchange mechanism, high efficiency due to silica microparticles and very good mechanical properties of the silica-gel matrix. Also, this new technique for ligand exchange constitutes an interesting alternative to ion-pair chromatography for the separation of numerous hydrophilic compounds which are donors of electronic lone pairs.

REFERENCES

1 I.B. Wilson and S. Ginsberg, Biochim. Biophys. Acta, 18 (1955) 168.
2 J.H. Wills, A.M. Kunkel, R.V. Brown and G.E. Groblewski, Science, 125 (1957) 743.
3 B.M. Askew, D.R. Davies, A.L. Green and R. Holmes, Brit. J. Pharmacol., 11 (1956) 424.
4 R.I. Ellin and A.A. Kondritzer, Anal. Chem., 31 (1959) 200.
5 N.H. Creasy and A.L. Green, J. Pharm. Pharmacol., 11 (1959) 485.
6 W.A. Groff and R.I. Ellin, Clin. Chem., 15 (1969) 72.
7 N.D. Brown and H.K. Sleeman, J. Chromatogr., 138 (1977) 449.
8 N.D. Brown, L.L. Hall, H.K. Sleeman, B.P. Doctor and G.E. Demaree, J. Chromatogr., 148 (1978) 453.
9 M. Caude and A. Foucault, Anal. Chem., 59 (1979) 459.
10 E. Schmidt, A. Foucault, M. Caude and R. Rosset, Analusis, 7 (1979) 366.
11 A. Foucault, M. Caude and L. Oliveros, J. Chromatogr., 185 (1979) 345.
12 F. Guyon, A. Foucault and M. Caude, J. Chromatogr., 186 (1979) 677.
13 A. Guyon, B.P. Roques, A. Foucault, R. Perdrisot, J.P. Swerts and J.C. Schwartz, Life Sci., 25 (1979) 1605.
14 R.I. Ellin and A.A. Kondritzer, Anal. Chem., 31 (1959) 200.