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FOI
Defence Research Agency
NBC Defence
SE-901 82 Umeå

Phone: +46 90 10 66 00
Fax: +46 90 10 68 00

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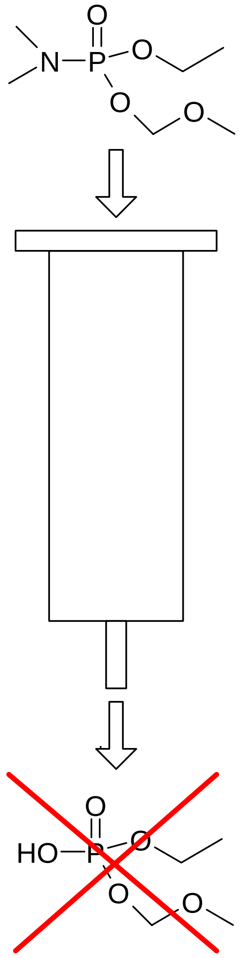
Improved sample preparation for determination of phosphoramidates in water samples based on Na^+ cat-ion exchange

CRISTER ÅSTOT, YVONNE NYGREN, STEN-ÅKE FREDRIKSSON,
LARS-GUNNAR HAMMARSTRÖM, CALLE NILSSON



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Author/s (editor/s)
Crister Åstot
Yvonne Nygren
Sten-Åke Fredriksson
Lars-Gunnar Hammarström
Calle Nilsson

Project manager
Crister Åstot, Calle Nilsson

Approved by
Anders Norqvist

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Improved sample preparation for determination of phosphoramidates in water samples based on Na+ cat-ion exchange

Abstract

A new (Na^+) cat-ion exchange method for CWA sample preparation was evaluated. The new method prevents the acidification of samples associated with the use of exchangers in H^+-mode. The acidification may induce hydrolysis if sensitive chemicals are contained in samples of high salinity. During the 14th Official OPCW Proficiency Test this problem was observed when a phosphoramidate was hydrolysed during sample preparation according to the standard cat-ion exchange method. Use of the new method was shown to prevent this degradation.

Keywords
Chemical warfare agents, Phosphoramidate, Sample preparation, Cat-ion exchange

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Författare/redaktör
Crister Åstot
Yvonne Nygren
Sten-Åke Fredriksson
Lars-Gunnar Hammarström
Calle Nilsson

Projektledare	Crister Åstot, Calle Nilsson
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Rapportens titel

Förbättrad provprepareringsmetod av fosforamidater i vattenprover baserad på Na^+- katjonbyte.

Sammanfattning

En ny (Na^+) katjonbytemetod för upparbetning av vattenprover har utvärderats. Den nya metoden förhindrar att proverna surgörs vilket är fallet när katjonbytare i H^+-form används. Surgörningen kan inducera hydrolys av känsliga kemikalier om de föreligger i vattenprover med hög salinitet. Detta observerades under the14th Official OPCW Proficiency Test när en fosforamidat hydrolyserades under provupparbetning enligt standardmetoden för katjonbyte. Denna rapport visar att hydrolysen går att undvika om provupparbetningen sker enligt (Na^+) katjonbytesmetoden.

Nyckelord

Kemiska stridsmedel, Forsforamidater, Provupparbetning, Katjonbyte

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Introduction

Sample preparation is mostly required to enable successful analysis of chemical warfare agents (CWA) in samples collected from natural or industrial environments. In such samples, the chemical composition of the sample matrix often makes direct instrumental analysis impossible. Cat-ion exchange is used as a routine preparation step of aqueous samples to remove cat-ions that may interfere in derivatization processes when silylation is used prior to analysis of polar CWA´s by methods based on gas chromatography (GC). The derivatization is used to increase the volatility of polar CWA degradation products like phosphonic acids to enable their analysis by GC. The use of strong cat-ion exchangers of benzene sulfonic acid type (SCX) is suggested in the recommended operating procedure published by Verifin (Rautio 1994). However, when the method is used for aqueous samples of high salinity a significant reduction of the pH may occur if the solution is not buffered. The pH drop may induce acidic hydrolysis of sensitive chemicals in the samples.

The hydrolysis of a CWA during sample preparation raises two main issues. Firstly, the original compound may remain unidentified if not detected in another branch of the sample preparation scheme. Secondly, the identification of a degradation product not present in the original sample is critical. In the OPCW context, reporting of such a degradation product is classified as a false positive identification that must not occur. In the Official OPCW Proficiency Test 14, an acidic labile phosphoramidate was spiked to an aqueous sample. The pH of the sample was not buffered and acidic hydrolysis of the spiking chemical took place during the cat-ion exchange. The designated laboratory at FOI assisted the OPCW in the evaluation of results of the Proficiency Test 14. In order to assess whether the degradation product was present in the original sample or not, a sample preparation method without the drawbacks mentioned above was needed. In this report, data is presented on the hydrolysis of phosphoramidates in the standard SCX method and further, an evaluation of an alternative method using SCX-columns in Na^+-form, to avoid acidification of the sample during the cat-ion exchange.

Experimental

Chemicals

Ethyl 2-metoxyethyl N,N-dimethylphophoramidate and diethyl N,N-dimetylphosphoramidate reference solutions were prepared and supplied by DSO National Laboratories-Singapore. The “Decon DS-2” water blank sample was supplied by DSO as part of the Official Proficiency Test 14 and was reported as a water solution containing 1000 ppm diethylenetriamine, 500 ppm each of 2-butoxyethanol and 2-methoxyethanol and 100 ppm tributyl phosphate. The pH of the sample was adjusted to 7.2. “Decon” samples were spiked with 10 ppm of the reference chemicals above. All other chemicals used were of standard *p.a.* quality.

Cat-ion exchange in H^+-form

Cat-ion exchange columns, packed with 200 mg of SCX-resin with a polymeric backbone (Extract-Clean, Alltech), were conditioned by 3 ml of de-ionized water. 1 ml of the aqueous samples was applied to each of the columns and the flow-through fractions were collected.

Cat-ion exchange in Na^+-form

Cat-ion exchange columns as above were converted to Na^+-form by conditioning each column with 8 ml of 0.9 M $NaHCO_3$ in order to exchange all H^+ to Na^+. Subsequently the columns were washed with 25 ml of de-ionized water to remove excess salt. 1 ml of the aqueous sample was applied to each of the columns and the flow-through fractions were collected.

Derivatization of samples

Flow-through fractions were blown to dryness by nitrogen and were redisolved in 900 µl of acetonitrile by sonication (5 min). 100 µl of silylation reagent BSTFA and 10 ppm of derivatization control standard (4-thianyl methyl-(d_3)-phosphonic acid) were added and the samples were derivatized at 60°C for 1 h.

GC-MS analysis

The samples were analysed by gas chromatography-mass spectrometry (HP 5890GC / 5972 MSD) on a standard DB-5MS column (J&W) with temperature program: 60 °C (1 min), 10 °C/min, 280 °C (5 min).

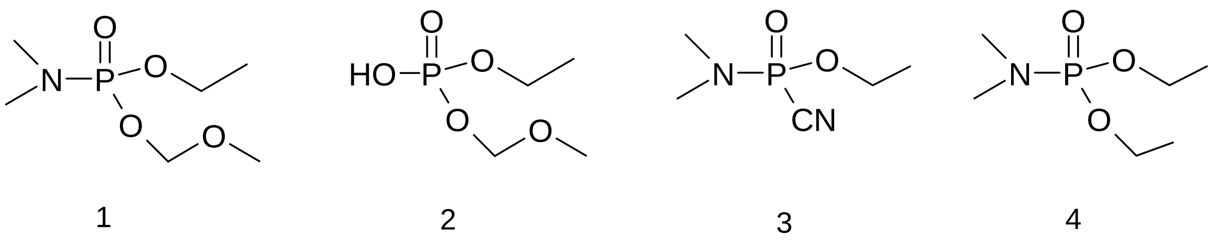


Fig 1. Structures and numbering of chemicals relevant in this study

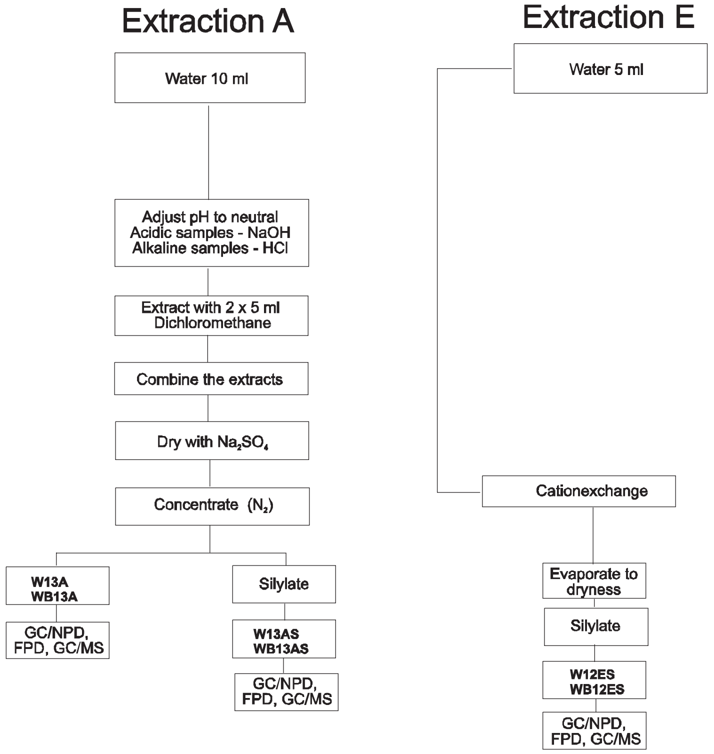


Fig 2. Standard water sample preparation scheme relevant for this study. In a sample aliquot from “Extraction A”, chemical 1 was identified and chemical 2 was detected after “Extraction E”.

Results and Discussion

Prior to the Evaluation of Results of the Official OPCW Proficiency Test 14 (PT 14), the test samples were analysed without any knowledge of their contents of CWA´s. In the PT, a water sample of moderate salinity labelled “Decon” was included. The sample was prepared according to the recommended operating procedures in the quality system of our laboratory. Sample extractions relevant for this study are shown in figure 2. In the dichloromethane extract (extraction A), a non-scheduled compound, identified as ethyl 2-metoxyethyl N,N-dimethylphophoramidate (chemical 1, Fig 1), was found. The compound is relevant as a typical degradation product of Tabun (chemical 3, Fig 1) under the influence of the “Decon”-type of decontamination agents (Beaudry et al. 1992). The concept of extraction route “E” is to enable detection of the more polar CWA degradation products, not possible to extract by route “A”. The degradation products are often phosphonic acids and derivatization as silylation is required to enable their analysis by GC-MS. Thus the water sample was purified by SCX cat-ion exchange (extraction E) followed by GC-MS analysis of the silylated extract. The SCX cat-ion exchange method removes cat-ions like Ca^{2+} and Mg^{2+} that are known to interfere with the silylation derivatization procedure and is in wide-spread use among OPCW Designated Laboratories for the analysis of CWA´s (Rautio 1994). However, the chemical 1 was not detected in extract E (expected RT= 10.77 min, Fig 3). The major phosphorous-containing peak (according to GC-NPD, data not shown) was identified as the silylated derivative of an alkylated phosphate, ethyl 2-methoxyethyl hydrogen phosphate (chemical 2, Fig 1). In addition, it could be concluded from ^{31}P NMR results (data not shown) that the dialkyl phosphate was not present in the original sample.

H$^+$ Cation exchange

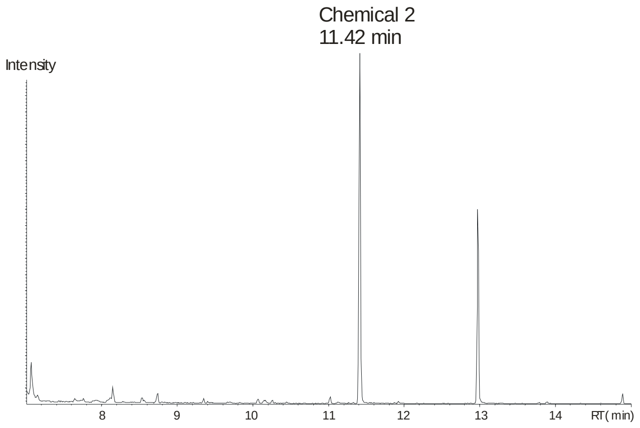


Fig 3A. TIC from the GC-MS (EI$^+$)analysis of sample spiked with chemical 1 followed by (H$^+$) cat-ion exchange. Silylated sample. Chemical 1 not detectable (10.77 min).

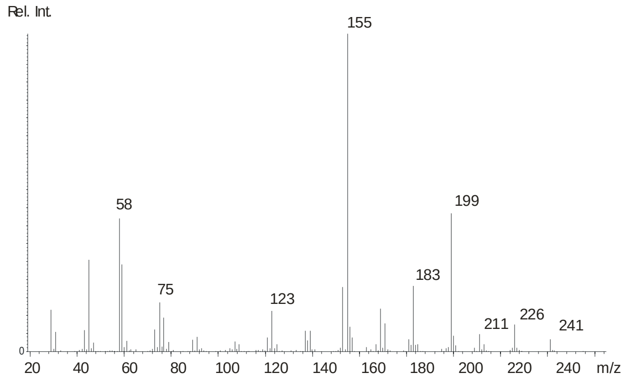
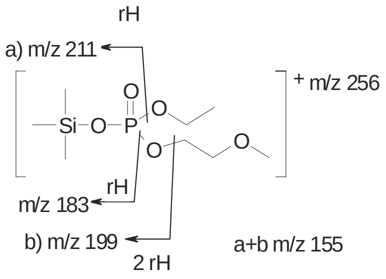


Fig 3B. Mass spectrum of chemical 2 eluting at 11.42 min.



The results indicated that chemical 2 was produced from chemical 1 as an artefact during sample preparation. Acidic conditions have, for a long time, been known to rapidly hydrolyse Ethyl N,N-dimethylphosphoramidocyanidate (Tabun, Chemical 3, Fig 1)(Larsson 1952, 1953) and an acidic hydrolysis would explain the conversion observed (Fig 4). Standard cat-ion exchangers contain protons as counter ions and the protons are exchanged by cat-ions like Ca^{2+} or Mg^{2+} in the samples. Thus, protons are released and if the salinity of the sample is high, a significant reduction of the sample pH will take place. If the pH of the sample is not buffered, acid labile chemicals may therefore degrade.

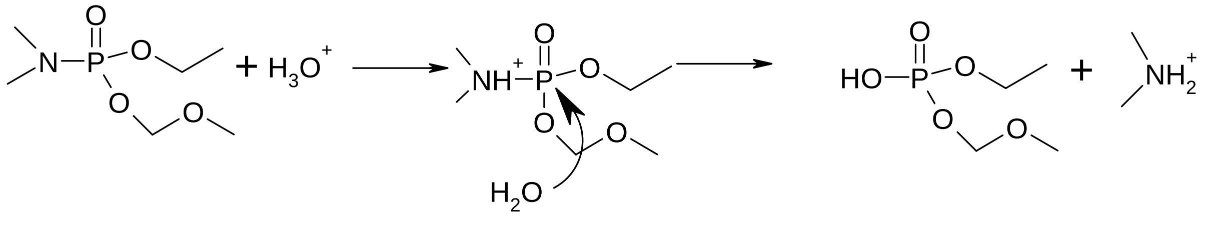
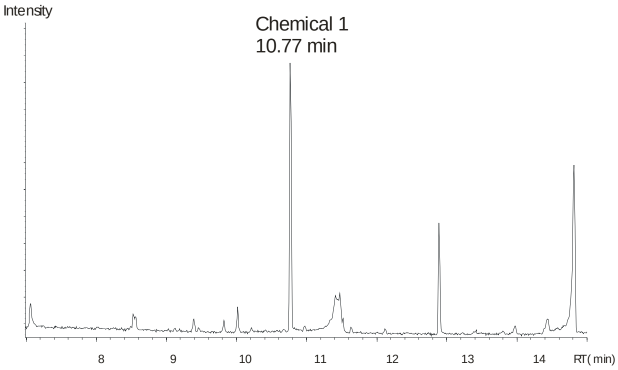


Fig 4. Acidic hydrolysis of chemical 1 with chemical 2 as a product.

The use of SCX cat-ion exchangers, converted to Na^+-form, was therefore evaluated. The change of the SCX counter-ions from H^+ to Na^+ prevents the acidification of the purified samples. When the sample above was purified by (Na^+) cat-ion exchange, the chemical 1 was identified and no traces of the hydrolysis product was detected in the silylated sample (Fig 5). Further, the new (Na^+)-SCX and the standard (H^+)-SCX methods were compared by preparation of water samples of moderate salinity (aqueous sample blank from PT14) spiked with Diethyl N,N-dimethylphosphoramidate (chemical 4, Fig 1). The results above were confirmed by the detection of the chemical 4 after (Na^+)-SCX cat-ion exchange only (Fig 6).



Na$^+$ Cation exchange

Fig 5A. TIC from the GC-MS (EI+) analysis of sample spiked with chemical 1 followed by (Na+) cat-ion exchange. Silylated sample. Degradation product (Chemical 2)
not detedctable

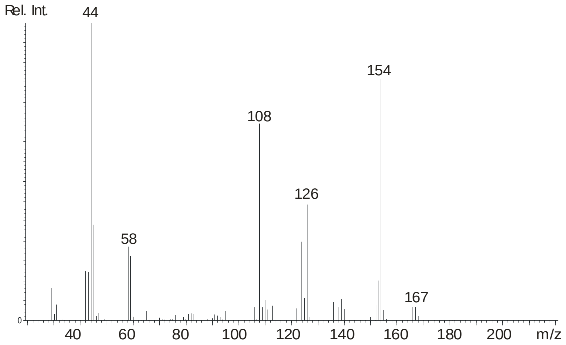
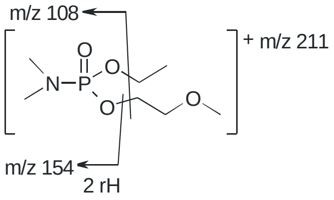
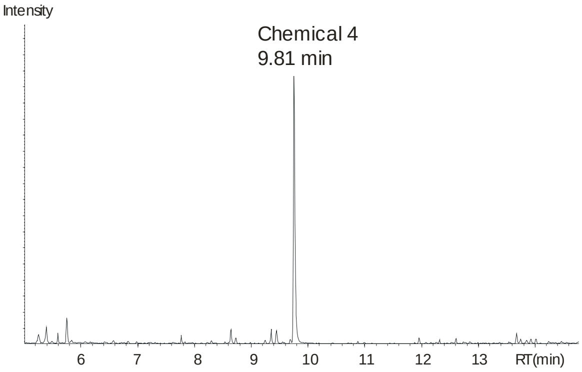


Fig 5B. Mass spectrum of chemical 1 eluting at 10.77 min





Na$^+$ Cation exchange

Fig 6A. TIC from the GC-MS(EI$^+$)analysis of sample spiked with chemical 4 followed by (Na$^+$) cat-ion exchange.

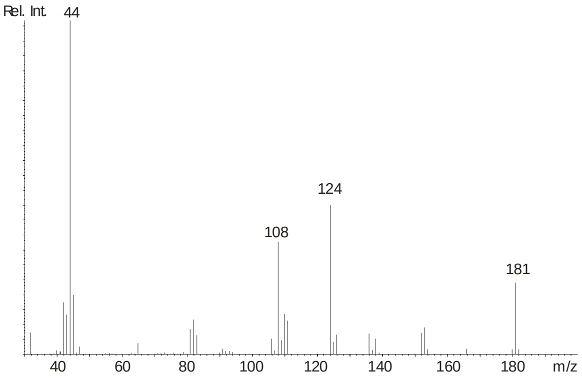
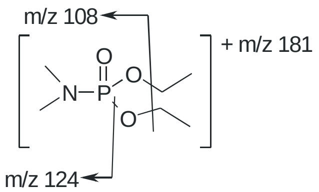


Fig 6B. Mass spectrum of chemical 4 eluting at 9.81 min.



Conclusions

The use of cat-ion exchangers in their Na^+-form can prevent the hydrolysis of acid labile CWA's during sample preparation associated with the use of ion exchangers in H^+-mode as described in this report. The new method is recommended as a routine procedure in the preparation of CWA samples.

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