RAPID COMMUNICATIONS IN MASS SPECTROMETRY
*Rapid Commun. Mass Spectrom.* **14**, 122–123 (2000)

RCM

**Letter to the Editor**

*To the Editor-in-Chief*
*Sir,*

**The persistent memory effect of triethylamine in the analysis of phospholipids by liquid chromatography/mass spectrometry**

Triethylamine (TEA) as an eluent modifier for high performance liquid chromatography (HPLC) applications is chosen by chromatographers as an ion pair reagent or as a buffer component of intermediate polarity.[1] There are examples in the literature that prove its capability to support ionization of various compound classes in electrospray ionization mass spectrometry (ESI-MS).[2]

Following Karlsson *et al.*,[3] we have developed methods for HPLC/ESI-MS and -MS/MS for the separation and identification of phospholipids from purified bacterial and sediment extracts. Chromatographic separation was carried out on a Thermo Separation Products (TSP, San Jose, CA,

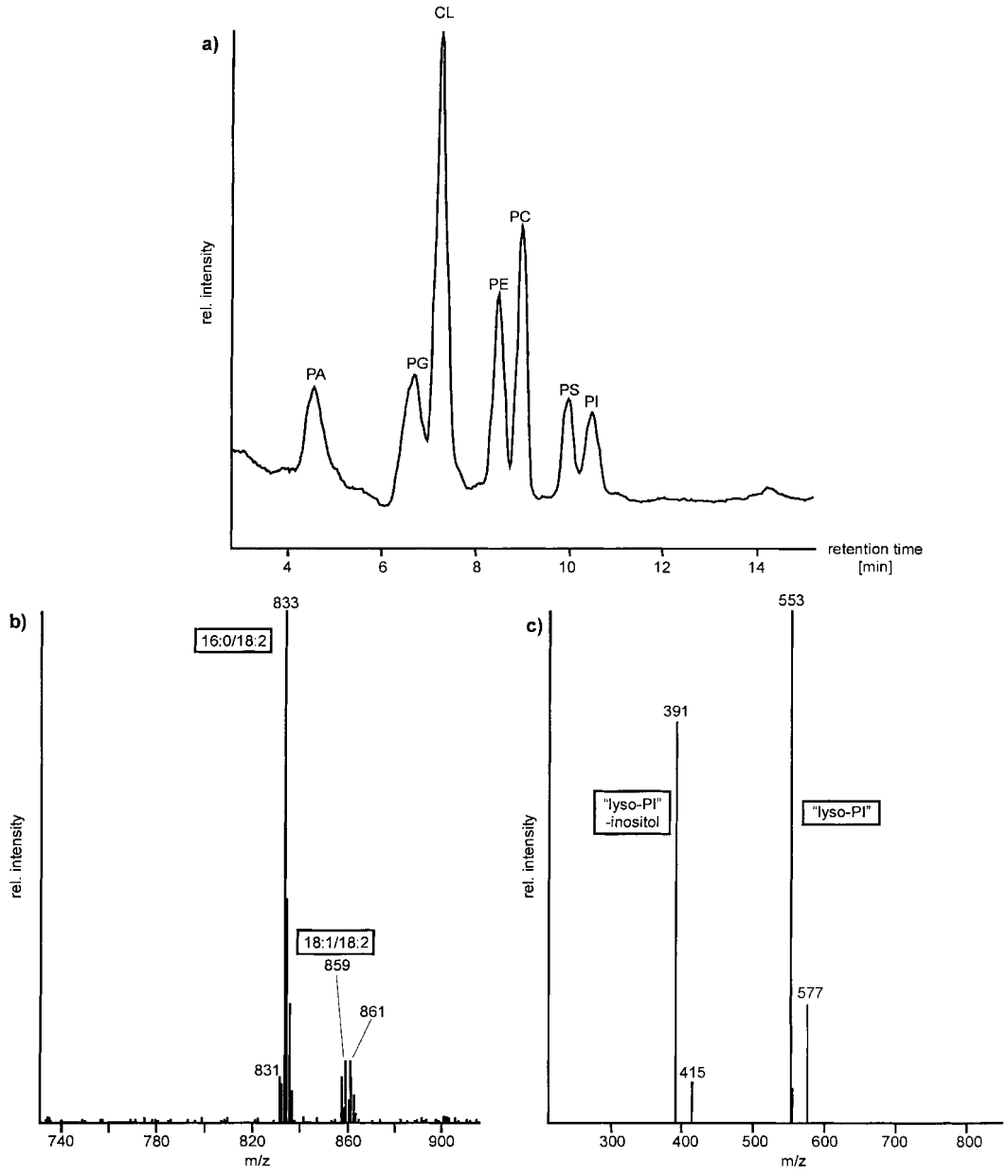


**Figure 1.** (a) TIC chromatogram of phospholipid standards: phosphatidic acid (PA), phosphatidyl glycerol (PG), cardiolipin (CL), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl inositol (PI), all Sigma-Aldrich, Germany; (b) quasi-molecular ions of PI (from soybean) showing different fatty acid combinations (fatty acids are denoted as x:y, with x giving the number of carbon atoms and y representing the number of double bonds; and (c) MS/MS of *m/z* 833.

CCC 0951–4198/2000/020122–02 $17.50

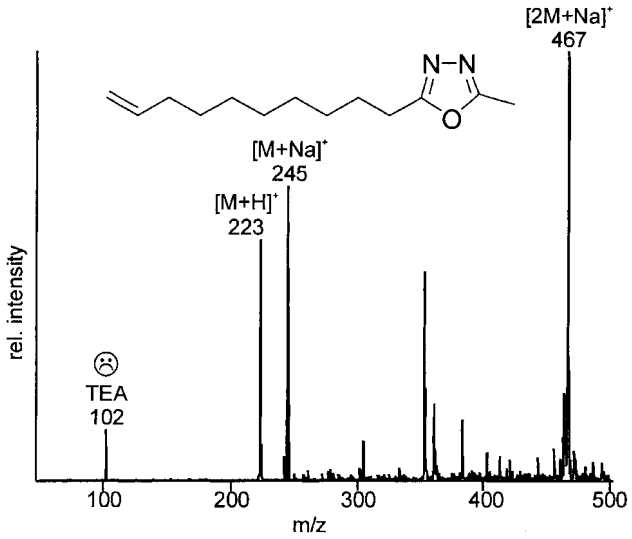


**Figure 2.** ESI mass spectrum of 2-(9-decenyl)-5-methyl-1,3,4-oxadiazole (ca. 100 μg/mL in methanol).

USA) HPLC instrument coupled to a Finnigan LCQ ion trap mass spectrometer (Thermoquest-Finnigan, San Jose, CA, USA). Phospholipids were separated according to their headgroups on a LiChrospher 100 Diol 5μHPLC column (125 × 2 mm; Merck, Darmstadt, Germany) using a convex gradient. Eluents contained a formic acid/TEA buffer (0.6/0.08 % v/v, respectively). Measurements in negative ion mode (spray voltage: −4.5 kV, capillary temperature: 200 °C) exclusively yielded $[M-H]^-$ ions. Details of the procedure will be descibed elsewhere. Figure 1 shows an example of the effective separation and the mass spectra resulting from a single run.

Problems arose when the same instrument was used for measurements in the positive ion mode, especially in the low molecular weight range (*m/z* 50–650). Such analyses were virtually made impossible by a very intense signal at *m/z* 102, $[M+H]^+$ of triethylamine. Direct infusion of pure solvents by the syringe pump resulted in a strongly dominant signal of TEA with intensities ranging from $10^6$ to $10^7$ counts during 10 ms of ion collection time, i.e. close to the maximum ion intensity during normal operation of the instrument. The intensity was independent of the kind of solvent supplied. The problems did not only concern suppression of ionization of other analytes but also severely increased detection limits, because an ion trap collects only a limited number of ions and cannot exclude a single *m/z* species and so it was nearly entirely filled with only triethylammonium ions. Therefore, detection of other compounds from diluted solutions, i.e. in the normal ESI concentration range of approx. 1 μg/mL, was strongly hindered. Only at concentrations as high as 100 μg/mL could certain compounds still be detected. Figure 2, as an example, shows the ESI mass spectrum of 2-(9-decenyl)-5-methyl-1,3,4-oxadiazole (positive ion mode, spray voltage: +4.5 kV, solvent supply: 10 μL/min, concentration: ca. 100 μg/mL). Even under these conditions the TEA peak is still significant. However, for many compound classes, measurements at these concentration ranges are not feasible because of preferential formation of adducts and proton-bound multimers, or due to limited sample material.

As the vacuum unit of the LCQ instrument is not designed for baking, other procedures had to be employed to remove the contaminant (TEA). Even several cleaning cycles of ESI source, heated capillary, API stack and the ion optics using various solvents and replacement of a number of mass spectrometer parts, tentatively identified as the most contaminated ones, did not significantly reduce the intensity of the TEA signal. Cleaning procedures recommended by the instrument manufacturer (Thermoquest, personal communication) or other researchers working with TEA (DeJohn, personal communication), i.e. sonication with formic acid/acetonitrile (50:50 v/v) did not improve the instrument performance. Finally, we disassembled the whole vacuum unit and all attached parts of the instrument, had all the metal pieces (ion optics, trap, vacuum manifold, etc.) cleaned by a mass spectrometer manufacturer (MassTech, Bremen, Germany) and replaced all Teflon, PEEK and other plastic components (including analyzer mount, tube lense and skimmer mount, all sealings, tubings, etc.). This ultimately removed the TEA contamination to a level below the detection limit.

From this experience we conclude that TEA was strongly adsorbed on the surfaces of the vacuum manifold and parts therein. Ionization may have occurred by proton transfer from solvent ions generated by the ESI process to desorbed TEA molecules. Therefore, we strongly recommend refraining from the use of triethylamine in analyses using ion-trap LC/MS and considering alternative eluent modifiers if the instrument is ever to be used for the analysis of low molecular weight substances in positive ion mode.

Heike Rütters[1]*, Thomas Möhring[1], Jürgen Rullkötter[2], Jens Griep-Raming[2] and Jürgen O. Metzger[2]

[1]Institute of Chemistry and Biology of the Marine Environment (ICBM) P.O. Box 2503, D-26111 Oldenburg, Germany and [2]Dept. of Chemistry, Carl von Ossiettzky University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany.

*Correspondence to: H. Rütters, Organic Geochemistry Group, Institute of Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossiettzky University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany

## REFERENCES

1. Meyer VR. *Praxis der Hochleistungs-Flüssigchromatographie* (7th edn). Otto Salle Verlag GmbH & Co: Frankfurt am Main; Verlag Sauerländer: Aarau, 1992.
2. DeJohn DE. Proc. 45th ASMS Conf. Mass Spectrometry and Allied Topics, Palm Springs, California, June 1–5, 1997; 659.
3. Karlsson AÅ, Michelsen P, Larsen Å, Odham G. *Rapid Commun. Mass Spectrom.* 1996; **10**: 775.

*Received 15 October 1999*
*Revised 17 November 1999*
*Accepted 18 November 1999*

 