APPLICATION NOTE No. 282

Avoiding Interferences and Contaminants Using Eppendorf Safe-Lock Tubes in Mass Spectrometry Studies of Natural Products

CS Chanotiya*, VS Pragadhessh, GC Uniyal
CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow - 226 015, India
*corresponding author: chanotiya@gmail.com

Abstract

Plasticwares require the inclusion of several chemicals to enhance their stability, durability and performance. In order to understand the leaching behavior of plastic tubes, an electrospray ionization mass spectrometric investigation was carried out in our laboratory with some brands of tubes available in the market. The results revealed that the reference standard ergocristine prepared in Eppendorf Tubes® resulted in high quality mass spectra with improved $[M+H]^+$ detection. On contrary, tubes from other manufacturers have shown leaching effects. Contaminant ions corresponding to polypropylene glycol (PPG), polyethylene glycol (PEG) and slip agents (e.g. erucamide) were found to dominate in the mass spectrum of ergocristine, when these tubes were used for the preparation.
Eppendorf Safe-Lock Tubes are found to be a good alternative for usage in sensitive mass spectrometric studies. Moreover, mass spectrometric analysis of biological materials on polluted polymer tubes from other manufacturers by ESI mass spectrometry, in the positive mode, showed highly contaminated mass spectra, due to the high sensitivity of this technique. These contaminants may further possess biological activity and are therefore a potential source of assay-specific confounding artifacts.

Introduction

The introduction of new ionization methods in mass spectrometry with improved detection limit has made it possible to detect many contaminants in measurable concentrations. This has increased the need to understand where these interferences come from and how they can be identified. Separation and detection technique has the potential to introduce new contaminants into the analytical system. It is therefore no coincidence that modern analytical chemistry takes advantage of ultrapure chemicals and reagents and ultraclean sample handling containers whenever possible to minimize any potential and unwanted background interference. In addition, all routine modern analytical methods following good laboratory practices (GLP) will include blank tests such as system, solvent, method, matrix and equipment blanks [1].
Worldwide, polymer based consumables are very much popular and have been accepted as a substitute for glass consumables for most of the research applications in laboratories. In particular, disposable plasticware such as test tubes are routinely used in all proteomics laboratories. The use of high quality materials in manufacturing of standard consumables such as glass vials and plastic test tubes etc. is always challenging for the companies. Besides this, another major problem for the production plants is to keep the composition unaltered from batch to batch. Disposable plastic labware is ubiquitous in contemporary pharmaceutical research laboratories. It is routinely used for chemical compound storage and during automated liquid-handling processes that support assay development, high-throughput screening, structure-activity determinations and liability profiling [2]. Further, disposable plastic labware is also reported as a potential source of bioactive contaminants [3].

In our work on common contaminants in electrospray ionization mass spectrometry (ESI-MS), a large number of interferences have been observed and identified [4]. Moreover, significant work by Keller et al. [1], published on contaminants encountered in mass spectrometry is noteworthy. The present application note describes the leach out behavior of polymeric consumables accompanied by mass spectrometry detection for undesirable ion or contaminants probably the plasticizers and polypropylene glycol. However, this report does not interpret specific techniques that allow elimination of certain background interferences. The present note demonstrates that these manufacturing agents leach from different reaction tubes (laboratory plasticware) into reference standard and can have profound effects on results.

Materials and Methods

Preparation of standard samples (ergocristine) in different tubes

Different disposable plastic tubes were utilized to investigate the leaching behavior of polymer materials and additives used in manufacturing. Three different Eppendorf Safe-Lock Tubes have been taken: DNA LoBind® Tube (1.5 mL; Lot Z138810G) and normal Safe-Lock Tubes (1.5 mL; Lot V126403P and 2.0 mL; Lot A143437J). Two 1.5 mL tubes from other manufacturers (named with MF1 and MF2) were used. A 1 ppm reference standard (ergocristine (Sigma-Aldrich®) was prepared in MS grade methanol (1.0 mL each), (Lichrosolv®, Merck). The standard solution was added into the different tubes followed by vortex shaking at room temperature for 1 min. Then it was injected as a sample into MS to obtain the MS fingerprint. Prior to injecting samples, a blank run using the methanol directly taken from the container was carried out.

Mass spectrometry analysis

After vortex shake, the ergocristine sample in methanol from each tube was drawn through 1.0 mL Hamilton® syringe and injected at a rate of 10 µL/min using a Model 11 Syringe Pump (Harvard Apparatus®, USA) into TurboIonSpray® source of mass spectrometer (API 3000 LC/MS/MS, AB Sciex). Instrument control and data acquisition were performed using Analyst® software version 1.4.1 (Applera Corporation, USA). The mass spectrometer was operated in Q1MS in positive mode (ESI). The rest parameters were taken as default values. The positive and negative ion modes were used to record the mass spectra in the range of 100-1500 amu. The TurboIonSpray voltage was set at 5,500 V for positive ion mode; source temperature kept at 200 °C throughout the run. Zero-air was used as nebulizer gas at 60 psi. Nitrogen was used as both curtain gas and CAD gas.

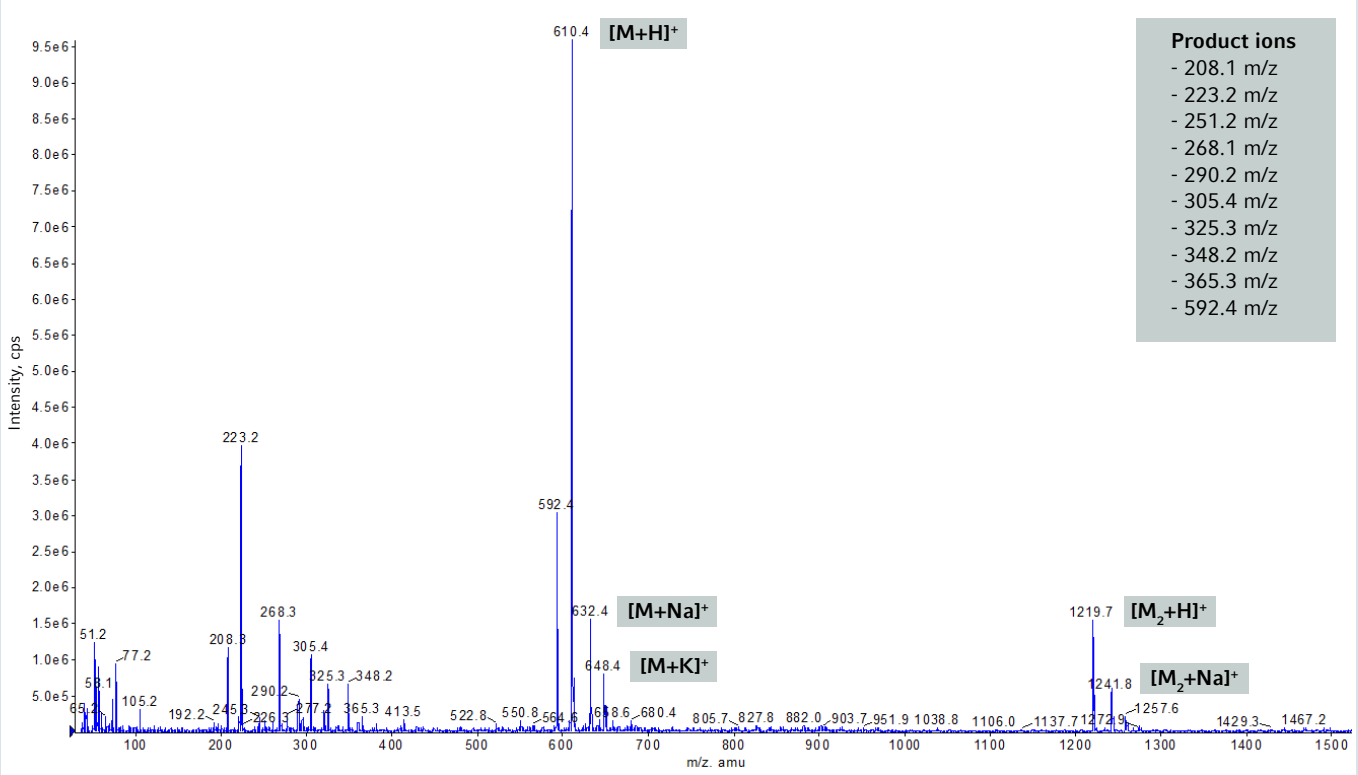
Results and discussion

A 1 ppm ergocristine reference standard (prepared in MS grade methanol in each tube) was kept for vortex shaking in three different Eppendorf Safe-Lock Tubes of 1.5 to 2 mL capacity. All the three lots V126403P and Z138810G of 1.5 mL each and A143437J of 2 mL capacity showed significantly higher signal for reference standard with lowest noise level. In addition, $[M+H]^+$ ion corresponding to m/z 610.4 was the most prominent peak observed in Eppendorf Safe-Lock Tubes giving a minimum signal for the common adduct ions such as $[M+Na]^+$ and $[M+K]^+$ at m/z 632.4 and m/z 648.4, respectively (Fig. 1a-c). The marker ions present were m/z 610.4 $[M+H]^+$, 632.4 $[M+Na]^+$, 648.4 $[M+K]^+$, 1219.7 $[M_2+H]^+$, 1241.7 $[M_2+Na]^+$ and 1257.6 $[M_2+K]^+$ (table 1). Hence, based on the mass spectrum, Eppendorf Safe-Lock Tubes give highest signal when used for sensitive MS and MS/MS analysis.

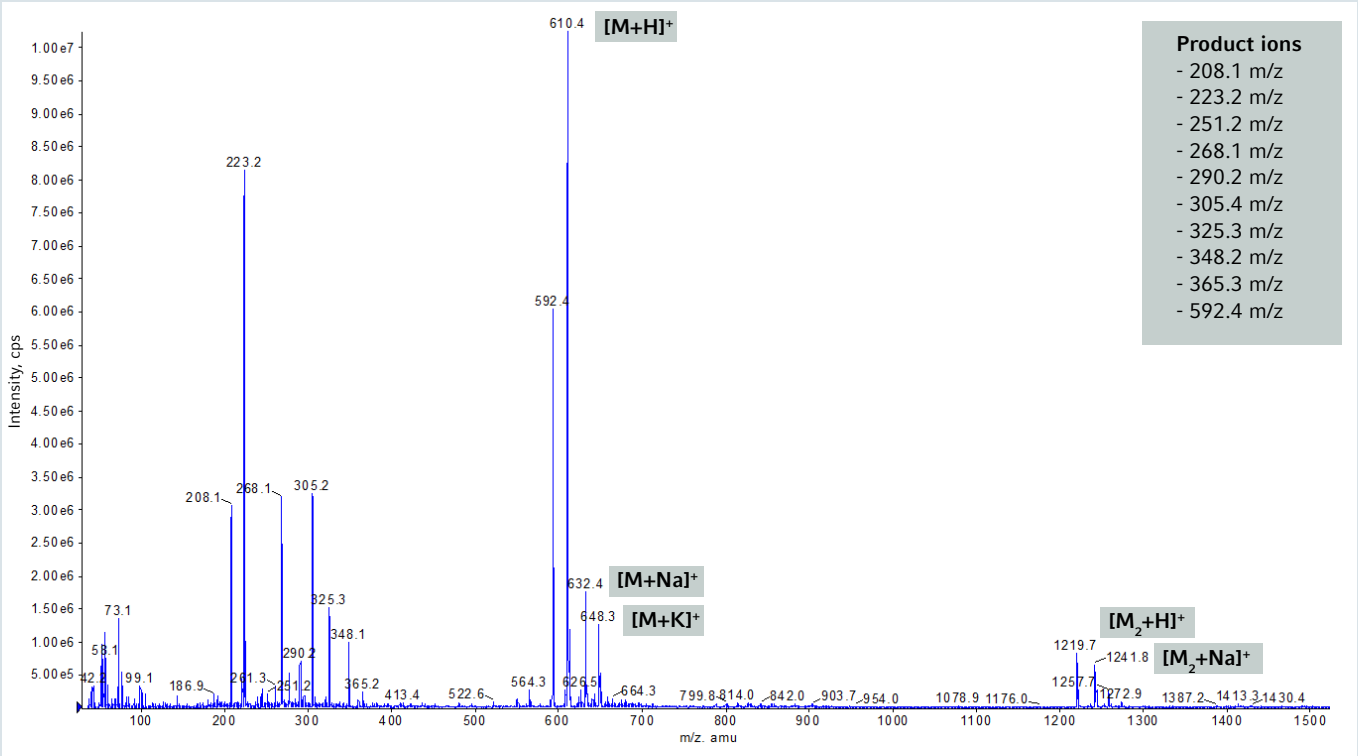
In Figures 2a+b, the mass spectral information of the tubes from manufacturer 1 (MF1) and manufacturer 2 (MF2) are shown. It revealed presence of large number of fragment ions indicating possible interference of polymeric materials in spectral analysis. The spectra of reference standard recorded after one minute shake in MF1 showed a higher signal with minimum noise level with marked presence of m/z 437.3 ($[A_9B+Na]^+$, $[C_2H_4O]_nH_2O$, PEG) and 453.4 ($[A_9B+K]^+$, $[C_2H_4O]_nH_2O$], PEG), 360.4 (erucamide) followed by unidentified peaks at m/z 288.4, 316.3, etc. On the other hand, MF2 represents a prominent contaminated spectral profile. Among the reference standard peaks, the contaminants present were m/z 409.3 ($[A_8B+K]^+$; PEG), 425.3 ($[A_7B+H]^+$; polypropylene glycol or PPG), 795.5 ($[A_{13}B+Na]^+$; PPG) and 811.5 ($[A_{13}B+K]^+$; PPG). However, peaks which remain unidentified in MF2 were m/z 274.3, 302.4, 230.4, 318.3, 330.5, 512.6 and 540.6 (Fig 2b). Notably, adduct ions such as 632.4 $[M+Na]^+$ and 648.4 $[M+K]^+$ were found in very low abundance.

Hence, the material used by manufacturer 1 and 2 for their tubes is very much unstable, as frequent leach out issues even with high MS grade solvents were seen. This revealed that these may be manufactured either by recycled polymer or that a possible leach out of surface coating occurred.

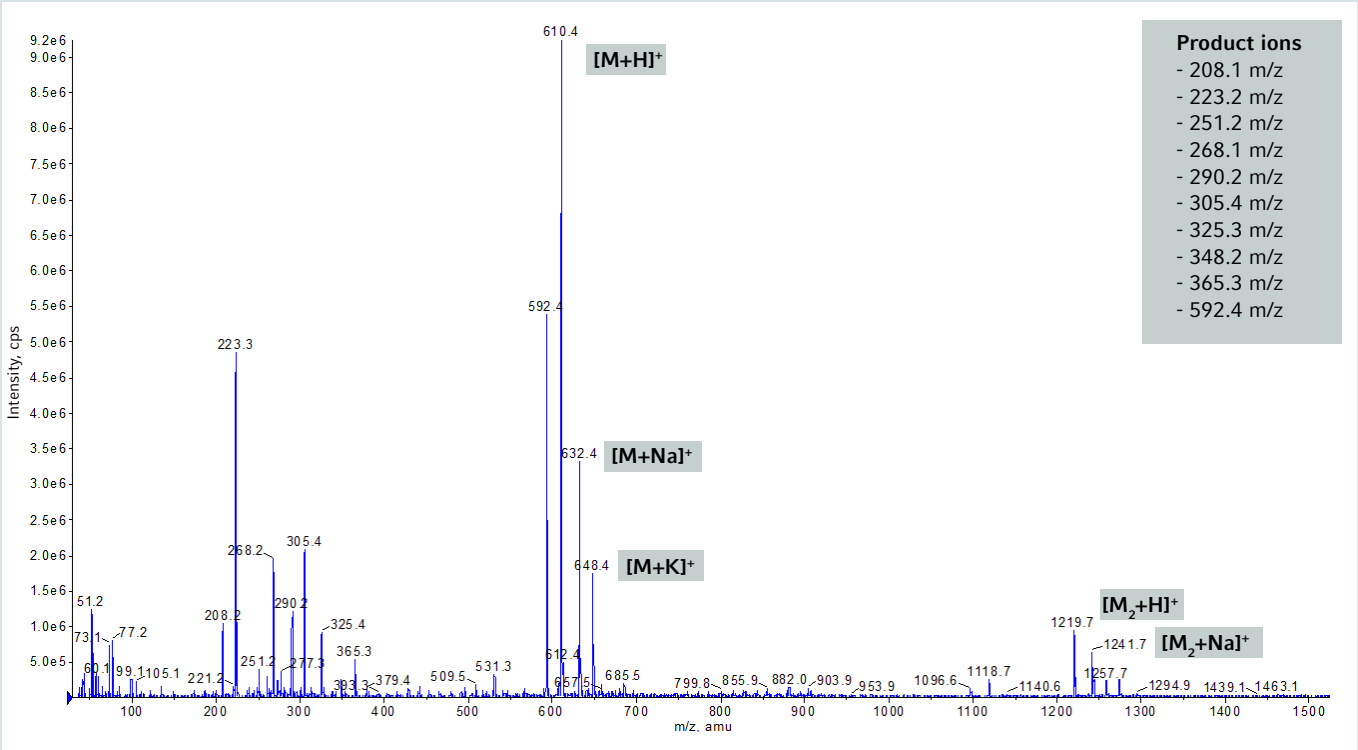
The results demonstrate that solvent-extractable contaminants from plastic labware used in research and development can be introduced into physical and biological assays during routine compound management liquid-handling processes. The used brands other than Eppendorf could furnish wrong information of the target compounds in ESI and MALDI mass spectral studies due to their low quality materials. Eppendorf Tubes are shown to be a good alternative since they are manufactured from high quality virgin polypropylene. Additionally, slip agents, biocides or plasticizer are neither comprised in the raw material nor are they used in production. Thus, we conclude that Eppendorf Safe-Lock Tubes are the best substitute for glass consumables in mass spectrometry (ESI and MALDI) based analysis.



a) Eppendorf Safe-Lock Tubes, 1.5 mL (Lot V126403P)

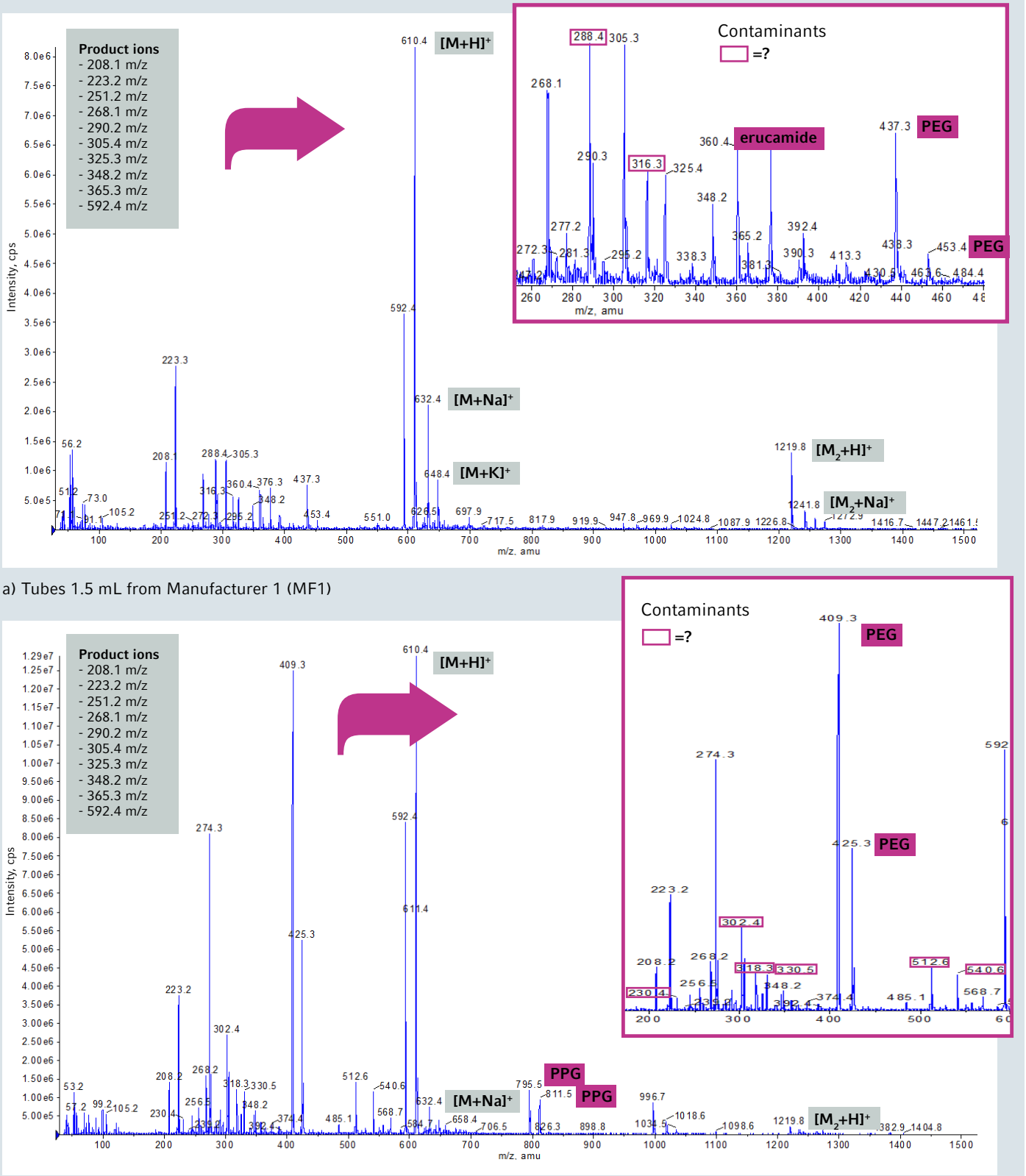


b) Eppendorf Safe-Lock Tubes, 2.0 mL (Lot A143437J)



c) Eppendorf DNA LoBind Tubes, 1.5 mL (Lot Z138810G)

Figure 1: Mass spectra of reference standard ergocristine (molecular weight 609 g/mol) incubated in Eppendorf Tubes. Product and precursor ions are labeled in green.



a) Tubes 1.5 mL from Manufacturer 1 (MF1)

b) Tubes 1.5 mL from Manufacturer 2 (MF2)

Figure 2: Mass spectra of reference standard ergocristine (molecular weight 609 g/mol) incubated in Tubes of manufacturers 1 and 2. Product and precursor ions are labeled in green. Contaminants are labeled in purple.

Table 1: Precursor ion scan of reference standard ions observed (positive) in ESI-MS.

Ions selected (m/z)	Ions observed
208.1	610.4 $[M+H]^+$, 592.4 $[M+H-H_2O]^+$, 268.2, 305.4
223.2	610.4 $[M+H]^+$, 592.4, 348.1
251.2	592.4, 610.4 $[M+H]^+$
268.1	610.4 $[M+H]^+$, 592.4 $[M+H-H_2O]^+$
290.2	632.4 $[M+Na]^+$, 1241.7 $[M_2+Na]^+$
305.4	592.4, 610.4 $[M+H]^+$, 1241.7 $[M_2+Na]^+$
325.3	610.4 $[M+H]^+$, 592.4, 1219.7 $[M_2+H]^+$
348.2	592.4, 610.4 $[M+H]^+$, 632.4 $[M+Na]^+$, 1219.7 $[M_2+H]^+$
365.3	632.4 $[M+Na]^+$
592.4	1219.7 $[M_2+H]^+$
632.4	1241.7 $[M_2+Na]^+$
648.4	1257.6 $[M_2+K]^+$

Acknowledgement

The authors are grateful to Prof. Ram Rajasekharan, Director, CSIR-CIMAP for encouragement and constant support to carry out this work, to DST New Delhi for the project grant.

References

[1] Keller BO, Sui J, Young AB, Whittal RM. Interferences and contaminants encountered in modern mass spectrometry. *Anal Chim Acta* 2008; 627:71-81.
[2] Watson J, Greenough EB, Lett JE, Ford MJ, Drexler DM, Belcastro JV, Herbst JJ, Chatterjee M, Banks M. Extraction, identification, and functional characterization of a bioactive substance from automated compound-handling plastic tips. *J Biomol Screen* 2009; 14(5):566–572.
[3] McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. Bioactive contaminants leach from disposable laboratory plasticware. *Science* 2008; 322(5903):917.
[4] Chanotiya CS. Contaminants in ESI-MS study. 2011; Unpublished work.

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com