Ultramark 1621 as a Reference Compound for Positive and Negative Ion Fast-Atom Bombardment High-Resolution Mass Spectrometry

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Ultramark 1621, a commercially available mixture of fluorinated phosphazines, was found to be a useful calibration compound for negative and positive ion fast-atom bombardment (FAB) high-resolution mass spectrometry. Ultramark 1621 worked very well with the most widely used matrices such as glycerol, nitrobenzyl alcohol, and triethanolamine. The negative and positive ion FAB mass spectra of Ultramark include a series of intense peaks extending from 700 to 1900 u. *(J Am Soc Mass Spectrom 1992, 3, 842–846)*

In recent years fast-atom bombardment high-resolution mass spectrometry (FAB-HRMS), .stead of classical elemental analysis, has become a standard technique for determining elemental compositions of a variety of involatile and thermally labile compounds not suitable for electron impact (EI) or chemical ionization (CI) mass spectrometry. While a variety of reference compounds have been suggested for exact mass measurement in positive FAB mass spectrometry [1–7], only a few reference compounds have been introduced for negative ion FAB-HRMS. A need for a versatile reference compound for negative ion FAB arises from the fact that many important biochemical compounds such as nucleic acids [8], phospholipids [9], and oligosacchrides [10] usually produce more intense pseudomolecular ions and characteristic fragments in the negative than in the positive mode.

The chemical compositions and accurate masses of some commonly used reference compounds have been reported [11]. Cesium iodide (CsI) is not generally used for high-resolution accurate mass measurements because the peaks are too far apart. Negatively charged glycerol matrix peaks, if close enough to the unknown peak, are frequently used as peak matching standards in the low-mass range (< 1000 u) [11].

Sharp et al. [12, 13] have suggested concentrated sulfuric acid as a reference compound for negative ion FAB-HRMS. However, the low abundance of sulfuric acid negative ion clusters above 900 u and the possible reaction of sulfuric acid with samples preclude its use for exact mass measurement of most compounds encountered in our laboratory.

Vekey [14, 15] has introduced salt mixtures of NaI/CsI and CsI/CsF as calibration compounds to fill in the gaps of the CsI peaks at lower masses. However, for high-resolution mass measurements, when these salt mixtures are mixed with commonly used matrices, such as glycerol or nitrobenzyl alcohol, the intensities of the negatively charged salt clusters are not sufficient for formula confirmation.

Recently, Sim and Boyd [16] described the simple expedient of using Au_n^- and $Au_nS_m^-$ clusters as negative ion mass markers. These markers were used for low-resolution (~ 3000) accurate mass (< 0.1 u) assignments. By placing a mixture of a sample and matrix on a gold probe tip in such a way that the probe tip was partially covered, they were able to generate Au_n^- clusters and sample ions in one spectrum. However, the reported errors in mass measurements were so large that one could not positively identify the elemental compositions of unknown ions. The magnitude of the errors may be attributed to the mass separation between the useful reference cluster ions, and to the difference in kinetic energy of ions generated from the gold probe and those generated from the liquid matrix. The difference between the kinetic energies of the ions of the unknown and of the reference ions usually affects peak shapes at high resolution. This will result in a compromise in tuning and therefore in even larger error.

In addition to the need for a reference compound for negative ion FAB-HRMS, there is also a need for

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an alternative, commercially available reference compound in positive FAB-HRMS. Polyethylene glycols (PEGs), the most widely used reference compounds in positive ion FAB-HRMS, have the chemical composition $(C_2H_4O)_nH_2O$. The mass spectrum of PEG includes a series of intense peaks at 44-u intervals, and minor peaks at almost every atomic mass unit. In cases where the molecular weights of compounds of interest are too close to the PEG peaks (i.e., less than 0.1 u at m/z 1000), it is more convenient to use alternative calibration compounds than to tune the instrument beyond 10,000 resolution.

The effort was made in our mass spectrometry facility to screen some commercially available reagents for possible candidates as reference compounds for negative and positive FAB-HRMS. The hope was to find a reference compound with the following properties: (1) peaks that would cover a wide mass range in both positive and negative FAB-HRMS; (2) the capacity to be used with the most common matrices; (3) peaks whose exact masses would not interfere with those of most organic compounds; (4) commercial availability. Ultramark 1621, a mixture of fluorinated phosphazines, was found to be such a material. Positive and negative ion FAB of Ultramark 1621 produced a series of intense peaks extending from 700 to 1900 u at a regular mass interval of less than 100 u.

Phosphazines have been used as reference compounds for decades. Fales [17] first described the EI mass spectrum of a single phosphazine. Later, Olson et al. [18] utilized a mixture of phosphazines as a reference compound for field desorption mass spectrometry. They also showed the structure, fragmentation pathways, elemental compositions, and calculated masses of the major fragment ions of phosphazines under electron ionization. Martin et al. [19] demonstrated that the relative abundance of a hexakis(2,2,2-trifluoroethoxy)cyclotriphosphazine $M^{+\cdot}$ versus $[M + H]^+$ varies as a function of acidity in a glycerol matrix containing Fomblin oil (PCR, Gainesville, FL) under positive ion FAB.

Commercially available fluorinated phosphazines, e.g., Ultramark 1621, have been used for some time as calibration compounds for low-resolution EI mass spectrometry. In our facility, when the mass of unknown ions exceeds 1000 u or when perfluorokerosene and *s*-triazines fail to give reference peaks of adequate intensities at high mass, Ultramark 1621 is frequently used to provide reference peaks for high-resolution EI and CI mass measurements.

In this article we report elemental compositions, intensities, and accurate masses for molecular ions and the major fragments of Ultramark 1621 for FAB-HRMS in both positive and negative ion modes. Specific experimental procedures are also described. In addition, a table of exact masses for Ultramark 1621 under EI conditions is given here to complement the table of phosphazine reference ions published by Olson et al. [18].

Experimental

All experiments were carried out on a VG ZAB-2E mass spectrometer (VG Analytical Ltd., Manchester, UK) with ±8-kV accelerating voltage and OPUS data system. For accurate mass measurements, voltage scans were carried out over a narrow mass range that included a pair of reference peaks bracketing a sample peak. The instrument was tuned to a resolution of 10,000 (10% valley definition), and the mass range was scanned at a rate of 10 s/scan. Low-resolution spectra (Figures 1 and 2) were obtained using CsI as an external calibration compound. A focused cesium ion gun with a primary ion of 25 keV energy and ion current of about 3 μA was used. Ultramark 1621 (PCR, Gainesville, FL), vitamin B_{12} (Sigma Chemical Co., St. Louis, MO), *m*-nitrobenzyl alcohol (NBA) (Aldrich Chemical Co., Milwaukee, WI), triethanolamine (Fisher Scientific, Fair Lawn, NJ), and sapphyren sugar derivative (provided by Professor F. M. Sessler, Department of Chemistry and Biochemistry, University of Texas at Austin), were used without further purification and introduced into the mass spectrometer by a FAB probe with a straight probe tip.

In the negative ion FAB mode, Ultramark 1621 generated good mass spectra in matrices of glycerol, NBA, and triethanolamine. When Ultramark and the samples were added to a matrix, the relative intensities of the sample and Ultramark reference peaks depended largely upon the concentration of Ultramark in the matrix. High concentrations of Ultramark suppressed the sample peaks. This effect was reduced by adjusting the Ultramark/matrix ratio or by using a split target [3]. Bringing down the Ultramark/matrix ratio to 1/100 or 1/1000 remarkably reduced this suppression effect. In practice, the appropriate amount

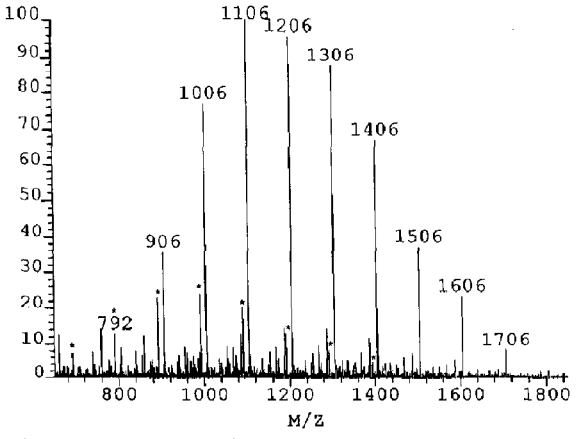


Figure 1. Negative ion fast-atom bombardment mass spectrum of Ultramark in triethanolamine matrix. Experimental conditions: ratio of Ultramark to triethanolamine approximately 1/100; magnetic scan; accelerating voltage −8 kV; resolution 1500. Peaks marked with an asterisk belong to the second series identified in Table 1 (see text).

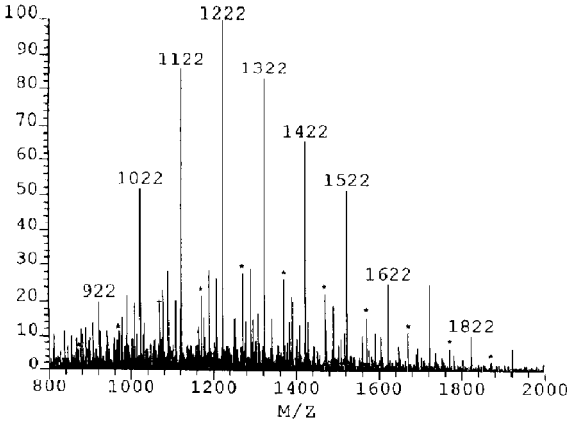


Figure 2. Positive ion FAB mass spectrum of Ultramark in an *m*-nitrobenzyl alcohol (NBA) matrix. Experimental conditions: ratio of Ultramark to NBA approximately 1/100; magnetic scan; accelerating voltage 8 kV; resolution 1500. Peaks marked with an asterisk belong to the second series identified in Table 2 (see text).

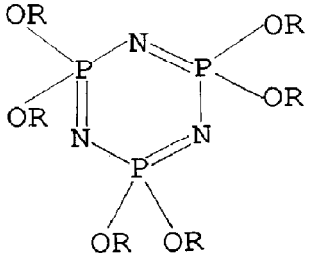
of Ultramark was determined by trial and error through the stepwise addition of an Ultramark–dichloromethane solution until reference signals were comparable to that of the sample.

A split target can also be used to prevent the suppression effect, but to meet the requirements of ion optics and to minimize contamination of the sample by Ultramark through sputtering during ion bombardment, special attention must be paid to the design of the split target [3]. Such use of an "external reference" has been shown to reduce accuracy [16].

Accurate masses in Tables 1, 2, and 3 were calculated using the following monoisotopic masses [20]: ^{1}H, 1.007825; ^{12}C, 12.000000; ^{14}N, 14.003074; ^{16}O, 15.994915; ^{19}F, 18.998403; ^{31}P, 30.973762.

Results and Discussion

The chemical structure of Ultramark 1621 is as follows:



R = $CH_2(CF_2CF_2)_nH$, n = 1, 2 or 3.

The negative ion FAB spectrum of Ultramark in triethanolamine (Figure 1) shows two series of ions intense enough for accurate mass measurement. The major peaks of the first series have chemical compositions of $C_{15}H_{15}O_6N_3P_3F_{20}(C_2F_4)_n$, with corresponding nominal masses of 806 + 100n, where n = 0–10. This series of ions arises from the loss of an R group of the phosphazines under negative ion FAB conditions. The loss of $CH_2(C_2F_4)_n$, where n = 1, 2, etc., from the first series of ions gives rise to the second series of ions, marked with an asterisk in Figure 1. The accurate masses and elemental compositions of these two series of ions are listed in Table 1.

Table 1. Ultramark reference masses for negative ion fast-atom bombardment

Elemental composition	Intensity[a]	Accurate mass
$C_{12}H_{13}O_6N_3P_3F_{16}$	7	691.97618
$C_{14}H_{13}O_6N_3P_3F_{20}$	13	791.96979
$C_{15}H_{15}O_6N_3P_3F_{20}$	12	805.98544
$C_{16}H_{13}O_6N_3P_3F_{24}$	22	891.96340
$C_{17}H_{15}O_6N_3P_3F_{24}$	36	905.97905
$C_{18}H_{13}O_6N_3P_3F_{28}$	23	991.95702
$C_{19}H_{15}O_6N_3P_3F_{28}$	77	1005.97267
$C_{20}H_{13}O_6N_3P_3F_{32}$	19	1091.95063
$C_{21}H_{15}O_6N_3P_3F_{32}$	100	1105.96628
$C_{22}H_{13}O_6N_3P_3F_{36}$	12	1191.94424
$C_{23}H_{15}O_6N_3P_3F_{36}$	96	1205.95989
$C_{24}H_{13}O_6N_3P_3F_{40}$	8	1291.93786
$C_{25}H_{15}O_6N_3P_3F_{40}$	87	1305.95351
$C_{26}H_{13}O_6N_3P_3F_{44}$	5	1391.93147
$C_{27}H_{15}O_6N_3P_3F_{44}$	67	1405.94712
$C_{29}H_{15}O_6N_3P_3F_{48}$	37	1505.94073
$C_{31}H_{15}O_6N_3P_3F_{52}$	23	1605.93435
$C_{33}H_{15}O_6N_3P_3F_{56}$	8	1705.92796
$C_{35}H_{15}O_6N_3P_3F_{60}$	5	1805.92157

[a] Intensities normalized to the intensity of *m/z* 1105.96628.

Table 2. Ultramark reference masses for positive ion fast-atom bombardment

Elemental composition	Intensity[a]	Accurate mass
$C_{17}H_{17}O_6N_3P_3F_{22}$	12	869.99790
$C_{18}H_{19}O_6N_3P_3F_{24}$	20	922.01035
$C_{19}H_{17}O_6N_3P_3F_{26}$	18	969.99151
$C_{20}H_{19}O_6N_3P_3F_{28}$	53	1022.00397
$C_{21}H_{17}O_6N_3P_3F_{30}$	20	1069.98512
$C_{22}H_{19}O_6N_3P_3F_{32}$	88	1121.99758
$C_{23}H_{17}O_6N_3P_3F_{34}$	23	1169.97874
$C_{24}H_{19}O_6N_3P_3F_{36}$	100	1221.99119
$C_{25}H_{17}O_6N_3P_3F_{38}$	30	1269.97235
$C_{26}H_{19}O_6N_3P_3F_{40}$	84	1321.98481
$C_{27}H_{17}O_6N_3P_3F_{42}$	27	1369.96596
$C_{28}H_{19}O_6N_3P_3F_{44}$	68	1421.97842
$C_{29}H_{17}O_6N_3P_3F_{46}$	24	1469.95968
$C_{30}H_{19}O_6N_3P_3F_{48}$	52	1521.97203
$C_{31}H_{17}O_6N_3P_3F_{50}$	17	1569.95319
$C_{32}H_{19}O_6N_3P_3F_{52}$	27	1621.96565
$C_{33}H_{17}O_6N_3P_3F_{54}$	17	1669.94680
$C_{34}H_{19}O_6N_3P_3F_{56}$	27	1721.95926
$C_{35}H_{17}O_6N_3P_3F_{58}$	7	1769.94041
$C_{36}H_{19}O_6N_3P_3F_{60}$	12	1821.95287
$C_{37}H_{17}O_6N_3P_3F_{62}$	5	1869.93403
$C_{38}H_{19}O_6N_3P_3F_{64}$	7	1921.94648

[a] Intensities normalized to the intensity of *m/z* 1221.99119.

Table 3. Ultramark reference masses for positive ion electron impact

Elemental composition	Intensity[a]	Accurate mass
$C_{17}H_{17}O_6N_3P_3F_{22}$	80	869.99790
$C_{18}H_{18}O_6N_3P_3F_{24}$	20	921.00253
$C_{19}H_{17}O_6N_3P_3F_{26}$	100	969.99151
$C_{20}H_{18}O_6N_3P_3F_{28}$	46	1020.99614
$C_{21}H_{17}O_6N_3P_3F_{30}$	94	1069.98512
$C_{22}H_{18}O_6N_3P_3F_{32}$	56	1120.98975
$C_{23}H_{17}O_6N_3P_3F_{34}$	54	1169.97874
$C_{24}H_{18}O_6N_3P_3F_{36}$	50	1220.98337
$C_{25}H_{17}O_6N_3P_3F_{38}$	14	1269.97235
$C_{26}H_{18}O_6N_3P_3F_{40}$	30	1320.97698
$C_{27}H_{17}O_6N_3P_3F_{42}$	18	1369.96596
$C_{28}H_{18}O_6N_3P_3F_{44}$	20	1420.97059
$C_{29}H_{17}O_6N_3P_3F_{46}$	6	1469.95958
$C_{30}H_{18}O_6N_3P_3F_{48}$	10	1520.96421
$C_{31}H_{17}O_6N_3P_3F_{50}$	6	1569.95319
$C_{32}H_{18}O_6N_3P_3F_{52}$	10	1620.95782
$C_{34}H_{18}O_6N_3P_3F_{56}$	8	1720.95143
$C_{36}H_{18}O_6N_3P_3F_{60}$	1.5	1820.94505
$C_{38}H_{18}O_6N_3P_3F_{64}$	0.7	1920.93866

[a] Intensities normalized to the intensity of *m/z* 969.99151.

The positive ion FAB spectrum of Ultramark in the NBA matrix (Figure 2) shows two series of ions intense enough to be used as references for accurate mass measurement. They are protonated phosphazine molecules $[M + H]^+$ (m/z 922 + 100n) and the ions attributable to loss of $(CF_2)_nH$ group from M^+. Table 2 lists the accurate masses and elemental compositions of these two series of ions. Martin et al. [16] have shown that when hexakis(2,2,2,-trifluoroethoxy)cyclotriphosphazene (m/z 729) was mixed with Fomblin oil, only the molecular ion, M^+ m/z 729, was observed. However, when a compound such as glycerol, which has labile hydrogen atoms, was used as the matrix, the molecular species observed were protonated molecular ions, $[M + H]^+$. This is consistent with our observation that when we used nitrobenzyl alcohol as a matrix, the relative abundances of phosphazines, M^+, were always smaller (< 20%) than $[M + H]^+$ (Figure 2).

Vitamin B_{12} (calculated M^- of 1354.567) and a sapphyren sugar derivative (calculated M^+ of 1032.533) were used as analytes in the negative and positive modes, respectively, to check the correctness of the listed masses. The accurate mass of the vitamin B_{12} molecular ion was measured in the negative FAB mode using a pair of Ultramark peaks from the first series of the reference ions. The result of the vitamin B_{12} experiment is shown in Figure 3. The measured mass errors for vitamin B_{12} and sapphyren sugar derivative were +1.5 and +1.6 ppm, respectively. The chemical compositions for the second series of peaks in the negative ionization mode of Ultramark 1621 were confirmed, using the first series of peaks as reference

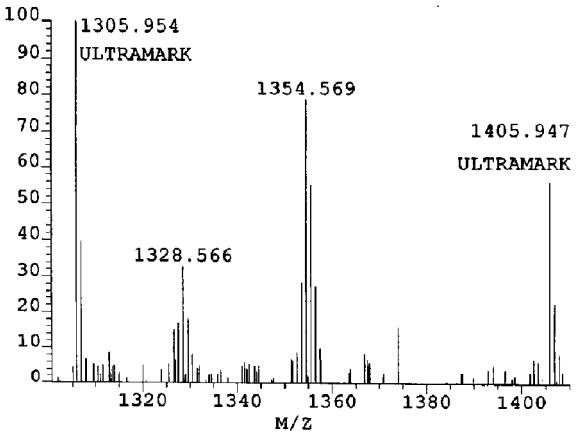


Figure 3. Accurate mass measurement of vitamin B_{12} molecular and fragment anions using Ultramark as the reference compound in an *m*-nitrobenzyl alcohol matrix. Experimental conditions: ratio of Ultramark to NBA ~ 1/1000; vitamin B_{12} concentration ~ 2 nmol/μLNBA; voltage scan; accelerating voltage −8 kV; resolution 10,000.

masses. The results agreed with our projected chemical compositions with an accuracy of better than 1 mu.

Spectra obtained in the positive and negative ion chemical ionization modes were similar to positive and negative ion FAB spectra, so that the results presented here also apply to the CI mode.

The chemical compositions and accurate masses of major peaks of phosphazine under electron ionization have been reported by Olson et al. [18]. Consistent with their results, the positive ion mass spectrum of Ultramark 1621 under electron ionization produced two series of ions intense enough to be used as reference peaks in the HRMS. They are the molecular ions M^+ of phosphazines, and ions attributable to loss of $(CF_2)_nH$ groups from M^+, where n = 1, 2, etc. To complement this list and to complete the tables of accurate masses for Ultramark 1621, the chemical compositions, accurate masses, and relative intensities of the Ultramark 1621 peaks under electron ionization are given in Table 3.

Conclusion

Ultramark has been successfully used for exact mass measurements in the 700–1900 u mass range for both negative and positive ion fast-atom bombardment high-resolution mass spectrometry. Ultramark worked well with most commonly used matrices such as glycerol, NBA, and triethanolamine. The suppression of samples by Ultramark was minimized by adjusting the ratio of Ultramark to the matrix or by using a split target. For positive ion FAB, Ultramark is a good alternative reference compound in cases where the molecular weights of the compounds of interest are too close to PEG reference peaks. For high-resolution EI and CI mass measurement Ultramark 1621 can provide reference peaks of adequate intensities up to 2000 u.

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