Chem., **110**, 303 (1976).
12. P. Jutzi and A. Karl, *J. Organomet. Chem.*, **128**, 57 (1977).
13. K. W. Muir and R. Walker, *J. Chem. Soc., Chem. Commun.*, 698 (1975).
14. Laszlo, Parknyi, *J. Organomet. Chem.*, **216**, 9 (1981)
15. International Tables for X-ray Crystallography, Vol. IV. Kynoch Press, Birmingham, England, 1974, pp. 71-102 and 148-151.
16. SHELXTL, 1983, Nicolet X-ray Instruments, Madison, WI 53711.
17. O. S. Mills and G. Robinson, *Acta Cryst.*, **16**, 758 (1963).
18. A. J. Bondi, *Phys. Chem.*, **68**, 441 (1964).
19. W. C. Joo, Y. C. Park, S. K. Kang, J. H. Hong and Y. K. Kong, *Bull. Kor. Chem. Soc.*, **8**, 270 (1987).

Gas Chromatography / Mass Spectrometry and Gas Chromatography / Tandem Mass Spectrometry of some *s*-Triazine Pesticides

Yoo Joong Kim and Myung Soo Kim*

Department of Chemistry, Seoul National University, Seoul 151-742. *Received January 30, 1988*

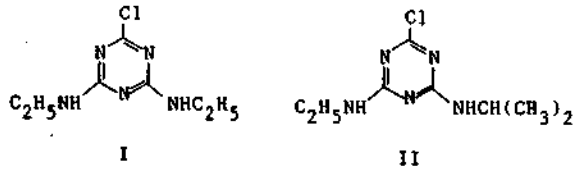
Some *s*-triazine herbicides, namely simazine, atrazine, and propazine present as trace components in a complex mixture were analyzed by GC/MS and GC/MS/MS methods. Even though monitoring the molecular ions was the best in terms of sensitivity, adequate analysis could not be done when interfering species were present. When doubly charged ions which appeared at characteristic *m/z* values were monitored, chromatograms were rather free from interference. More importantly, selected reaction monitoring was found to provide a selective means of detection with general applicability.

Introduction

Mass spectrometry is one of the most useful instrumental methods for the identification and structure determination of various compounds.[1,2] When coupled with gas or liquid chromatography, the resulting instrumental methods commonly called GC/MS[3,4] and LC/MS[5], respectively, become powerful techniques for the qualitative and quantitative analyses of trace components in complex mixtures. For the screening and quantitation of a trace component, one or several different ions generated upon ionization of the compound are selectively recorded.[6] This technique is usually called selected ion monitoring (SIM) or selected ion recording (SIR). Since the mass spectrometer spends most of its time to detect only a few different ions, effective time constant for each channel in SIM becomes enormously larger than for scanning-type GC/MS, enabling parts per billion (ppb) analysis.[7] GC/MS analysis of trace components in a very complex mixture is often hampered by the presence of interfering components. Hence, a thorough and time-consuming pretreatment of a sample is usually needed in such a case.

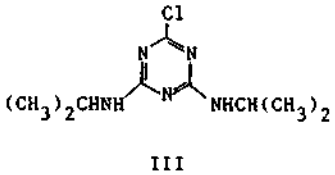
Tandem mass spectrometry or mass spectrometry/mass spectrometry (MS/MS)[8-10] has been proposed as an alternative to GC/MS for mixture analysis.[11-13] In this technique a mixture is introduced to the ion source of a mass spectrometer. A characteristic ion produced from the analyte of interest is separated by the first stage mass spectrometer. Dissociation of this selected ion as monitored by the second stage mass spectrometer provides a means to identify and quantitate the analyte of interest. This technique is often called selected reaction monitoring (SRM) to distinguish it from selected ion monitoring (SIM) described above, Since the separation and detection are all done in a mass spectrometer, analysis can be done faster than in GC/MS. MS/MS can also be utilized for detection of components separated by GC.[12,14] Excellent selectivity of this technique often enables the analysis of trace components which are difficult to analyze with GC/MS. Alternatively, extensive pretreatment of a sample can often be avoided with GC/MS/MS.

s-Triazines are widely used as pre-emergence selective herbicides, simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine, I), atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, II), propazine (2-chloro-4,6-bis(isopropylamino-*s*-triazine, III) being the most important. Recently, we have carried out an investigation on the mass spectral fragmentations of these compounds and 2-amino-4-chloro-6-ethylamino-*s*-triazine (IV) utilizing MS/MS and high resolution mass spectrometry.[15] As an extension of this work, GC/MS and GC/MS/MS analysis of *s*-triazine herbicides have been performed and reported here.

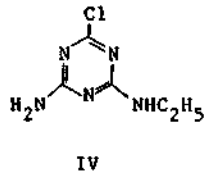


I

II



III



IV

Experimental

The instrument used in this work was a double focusing mass spectrometer with reversed geometry (VG ZAB-E) coupled to a gas chromatograph (HP model 5890). A schematic diagram for the instrument is shown in Figure 1. A fus-

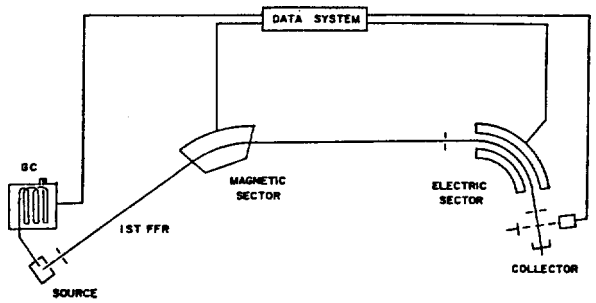


Figure 1. Schematic diagram of VG ZAB-E gas chromatograph/mass spectrometer with data system.

ed silica open tubular column (25 m, 0.2 mm I.D., OV-1) was coupled directly to the ion source of the mass spectrometer. Around 1 μl of sample was injected to a split/splitless injector in splitless mode maintained at 250 °C. One minute after sample injection inlet purge valve was activated to vent extra solvent vapor and hence to minimize solvent tailing. Helium was used as a carrier gas at 0.8 cm^3/min flow rate. Column temperature was held initially at 85 °C for 0.5 min to utilize solvent effect[16] and then raised to the final temperature of 220 °C at a rate of 30 °C/min. The interface region was also maintained at 220 °C.

70 eV electron ionization (EI) with 200 μA trap current was used for sample ionization. Source temperature was kept at 250 °C and 8 kV accelerating voltage was used. The instrument was operated at 1,000 resolution. Data acquisition and processing as well as system control for SIM and SRM were performed by a data system (VG 11-250J). SIM was achieved by a usual method. Briefly, magnetic field was set at a suitable value and the accelerating voltage was adjusted accurately through calibration procedure to transmit selected ions. For selected reaction monitoring, the unimolecular reactions occurring in the first field-free region, namely between the source and the magnetic sector, have been observed. The magnetic field-to-electric field (B/E) ratio was adjusted to monitor a reaction of interest.[17]

Three *s*-triazine herbicides (I, II, and III) were analytical grade (Poly Science Co.) and were used without further purification. 2-Amino-4-chloro-6-ethylamino-*s*-triazine (IV) was used as an internal standard for quantitation. This compound was synthesized and purified according to the method described in the literature.[18,19] Its purity was checked by thin layer chromatography, gas chromatography, and mass spectrometry. All the solvents used were HPLC grade (Merck).

Stock solutions of each *s*-triazine compounds were prepared in methanol at 5 μg/m*l* concentration. These were further diluted by methanol-isooctane (1:99 v/v) mixed solvent[20] to the desired concentrations to prepare standard solutions and working mixtures. Concentrations of internal standard in calibration standards were 700 ng/m*l* for SIM and 1100 ng/m*l* for SRM. To test the performance of each methods in real situations, test samples were prepared by spiking either 0.8 μg or 2.0 μg of each *s*-triazine herbicides in 1 lit of pond water. Then, *s*-triazine herbicides were extracted three times with 100 m*l* methylene chloride. The extract was dried by filtering through a column packed with anhydrous sodium sulfate and concentrated to 10 m*l*. No other attempt was made for sample cleanup. The samples which were prepared by spiking 0.8 μg and 2.0 μg initially in pond water will be called sample A and B, respectively. Sample A was used for SIM analysis while sample B was used for SRM analysis.

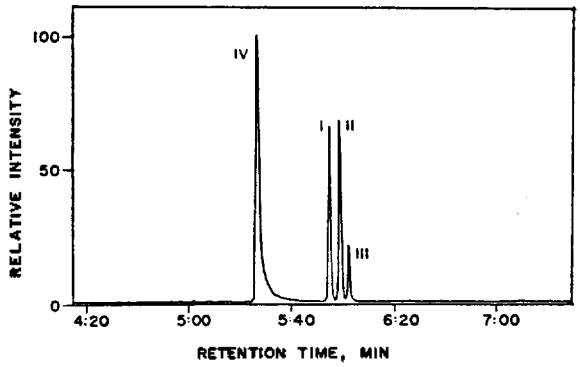


Figure 2. Mass chromatogram of a sample containing simazine(I), atrazine(II), propazine(III), and 2-amino-4-chloro-6-ethylamino-*s*-triazine(IV) obtained by monitoring *m/z* 173 ion.

Results and Discussion

In the gas chromatographic analysis of *s*-triazine herbicides[20-26], polar Carbowax 20M has been the most popular stationary phase[21-23]. However, our attempt to resolve these chloro-*s*-triazines (I, II, and III) using a fused silica column bonded with DB-WAX (30 m, 0.25 mm I.D., J&W Scientific) did not give satisfactory results. Even though the above three compounds could be separated, retention times were rather long (~30 min) and the background level due to column bleed was prohibitively high. Hence, no further work was performed with this column.

Satisfactory separation of the compounds I, II, and III was achieved using a capillary column with nonpolar OV-1 stationary phase as shown in Figure 2. Chromatographic conditions which were optimized to obtain this result have been described already in the experimental section. About 1 ng of each analyte was injected and detected by monitoring (SIM) the intensity of *m/z* 173 ion which appears in the mass spectra of all the *s*-triazine compounds used in this work. Retention times for the compounds I, II, and III were about 6 min. and reproducible within half a second. Chromatographic peaks for these compounds are nearly symmetric and well separated from one another. The number of theoretical plates estimated from the chromatogram is around 280,000 (11,000/m) indicating an excellent column performance. Also shown in the figure is a chromatographic peak for the compound IV which is eluted around 30 seconds earlier than the *s*-triazine herbicides. A slight tailing is observed for this peak. This did not cause any difficulty for quantitation when peak area instead of peak height was used for analytical purpose, as will be seen later. Mass chromatogram shown in Figure 2 can be further improved, of course, utilizing the selectivity of the mass spectrometric detector. In Figure 3, chromatograms obtained simultaneously for the molecular ions for each compound, namely *m/z* 201, 215, 229, and 173 ions for the compounds I, II, III, and IV, respectively, are shown.

In the SIM analysis, it is desirable to select an ion which is abundant and characteristic of the analyte of interest. In the cases of *s*-triazine herbicides, the molecular ion intensities relative to the base peaks for the compounds I, II, and III are 100, 65 and 68%, respectively, in the EI spectra. Hence,

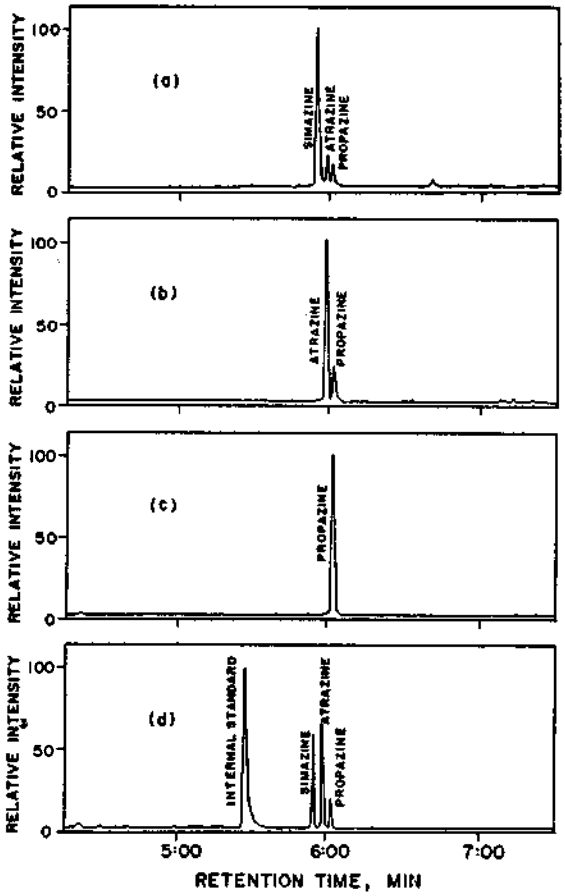
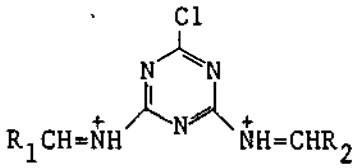


Figure 3. Mass chromatogram of the same sample as in Figure 2 obtained by monitoring (a) *m/z* 201, (b) *m/z* 215, (c) *m/z* 229, and (d) *m/z* 173 ions.

the molecular ions for each compounds are natural candidates for the ions to be selected for monitoring. The molecular ion for the compound IV appears as the base peak (100%) in the EI spectrum and is a good candidate for monitoring. One of the striking features in the EI spectra of *s*-triazine herbicides is the appearance of characteristic doubly charged ions with the following structures.[15,26]



These ions appear at half integer masses, namely at 85.5, 92.5, and 99.5 for the compounds I, II, and III, respectively. The fact that these ions appear at half integer masses is a great advantage when used for the analysis of a complex mixture. This is because an ion with a half integer mass which must be a doubly charged ion rarely appears with a high intensity and hence is usually free from interference. The analytical value of these doubly charged ions are further enhanced because of their exceptional intensities in the EI spectra of the *s*-triazine herbicides. The relative intensities of these ions are 19, 20 and 27% in the EI spectra of the compounds I, II, and III, respectively. No doubly charged ion corresponding to the structure shown above appears in the EI spectrum of the compound IV. However, a doubly charged ion with the structure appears at *m/z* 61.5 even though with a low relative intensity (2%). This ion was used as the internal standard to obtain the calibration curves and quantitation of real sample when doubly charged ions were selected for monitoring.

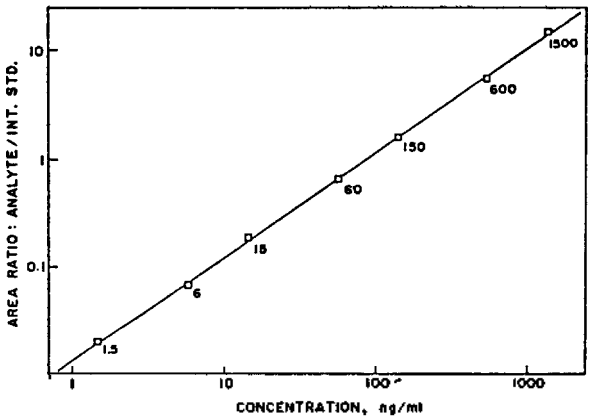
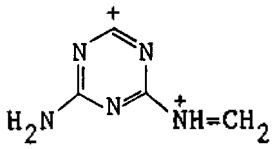


Figure 4. Calibration curve for simazine obtained by monitoring the doubly charged ion with *m/z* 85.5. The doubly charged ion with *m/z* 61.5 from the compound IV was used as the internal standard.



Very good calibration curves were obtained for the *s*-triazine herbicides when the molecular ions or doubly charged ions were monitored. For example, Figure 4 shows the calibration curve for simazine (compound I) obtained by monitoring the doubly charged ion (*m/z* 85.5). Linearity of the log-log plot was decent in the 1.5-1,500 ng/m*l* concentration range with the correlation coefficient of 0.9994. Hence, analysis in low ppb level is possible. Since only about 1 μl each of calibration standards was injected, actual amount of simazine injected for 1.5 ng/m*l* standard was around 1.5 pg.

Theoretical detection limit, namely the detection limit when a sample is free from interference, was estimated as the concentration of an analyte in a standard which produced a charomatographic peak with the signal-to-noise (S/N) ratio of 3. For all three *s*-triazine herbicides, theoretical detection limits were around 30 pg/m*l* or 30 fg of the compounds actually injected, when the molecular ions were monitored. The detection limits were around 120 pg/m*l* when the doubly charged ions were monitored. The poorer detection limit for the latter is due to the lower sensitivity of the doubly charged ion signals.

In the analysis of a real sample, chemical noise due to the presence of interfering materials may increase the detection limit well above the theoretical limit. As an example, the chromatograms obtained by SIM of atrazine in sample A are shown in Figure 5. In this figure, (a), (b), and (c) are chromatograms obtained by monitoring the molecular ion (*m/z* 215), the base peak ion (*m/z* 200), and the doubly charged ion (*m/z* 92.5), respectively. It is apparent that a reliable quantitation of atrazine is not possible by monitoring the molecular ion. The same is more or less true for the case of monitoring the base peak ion. On the other hand, the chromatographic peak due to atrazine is almost free from interference when the doubly charged ion is selected for moni-

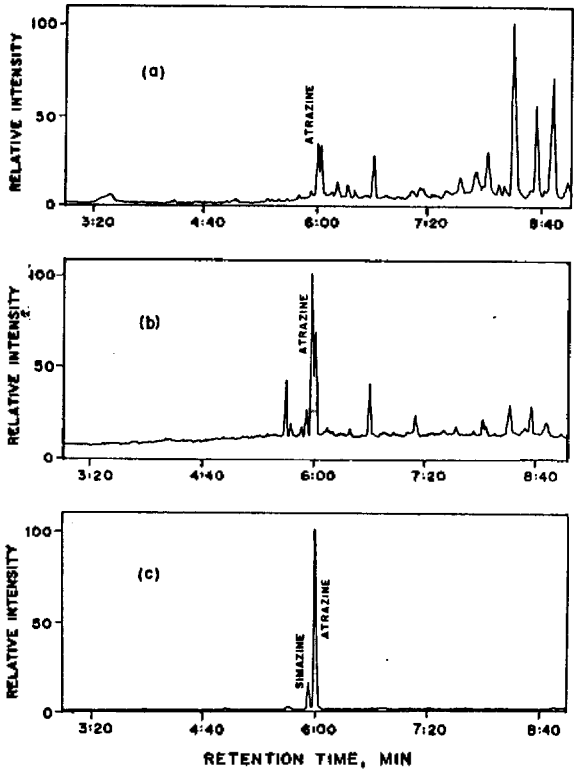


Figure 5. Mass chromatogram of sample A obtained by monitoring (a) m/z 215, (b) m/z 200, and (c) m/z 92.5 ions which are the molecular ion, the base peak ion, and the doubly charged ion from atrazine, respectively.

toring as shown in Figure 5(c). A tiny peak appearing before the atrazine peak is due to a minor interference from simazine. It is obvious from the figures that even though the intensity of the doubly charged ion is lower than those for the molecular ion and the base peak ion, its superior selectivity makes it the ion of choice for quantitation in this case. Situations were very similar in the cases of simazine and propazine. Quantitation based on the doubly charged ions produced decent results. For example, quantitation of simazine in sample A using the calibration curve shown in Figure 4 gave a result of 0.87 ± 0.15 ng/ml while the actual concentration in the sample was 0.8 ng/ml.

Detection of an analyte by the SRM technique requires knowledge on the metastable decompositions of various ions appearing in the EI spectrum of the analyte. Such metastable studies for the compounds I, II, III, and IV have been completed and reported previously.[15] The major criteria for the selection of a reaction to be monitored would be the selectivity of the parent ion mass, intensity of the reaction product, and the specificity of the reaction. In the cases of *s*-triazine herbicides, McLafferty rearrangement reactions[27] of the molecular ions, namely $201^+ \rightarrow 173^+$, $215^+ \rightarrow 173^+$, $229^+ \rightarrow 187^+$, and $173^+ \rightarrow 145^+$ for the compounds I, II, III, and IV, respectively, showed decent sensitivities. Hydrogen loss and chlorine loss reactions from the McLafferty rearrangement products, namely $173^+ \rightarrow 172^+$ and $173^+ \rightarrow 138^+$ for simazine, the same reactions for atrazine, and $187^+ \rightarrow 186^+$ and $187^+ \rightarrow 152^+$ for propazine also displayed decent sensitivities. All these reactions are characteristic of chlorinated *s*-triazines with alkylamino side chains and are expected to provide highly selective channels for SRM. Hence, these

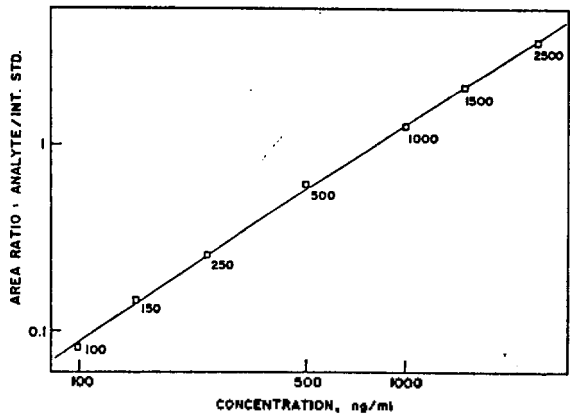


Figure 6. Calibration curve for atrazine obtained by monitoring a McLafferty rearrangement reaction $215^+ \rightarrow 173^+$. A McLafferty rearrangement reaction $173^+ \rightarrow 145^+$ for the compound IV was used as the internal standard.

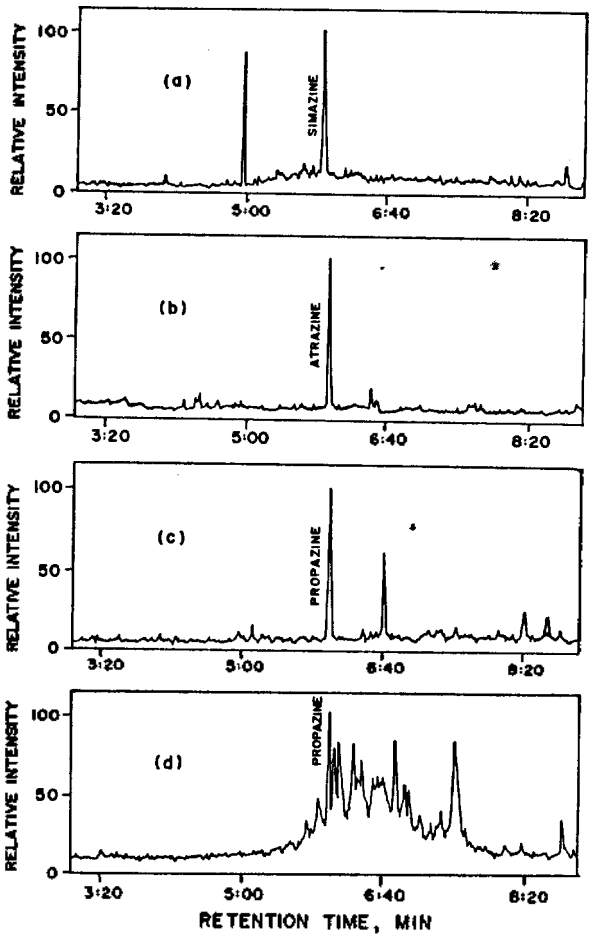


Figure 7. Mass chromatogram of sample B obtained by monitoring (a) $201^+ \rightarrow 173^+$(simazine), (b) $215^+ \rightarrow 173^+$(atrazine), (c) $187^+ \rightarrow 186^+$(propazine), and (d) $229^+ \rightarrow 214^+$(propazine).

channels would be more applicable to the analysis of the *s*-triazine herbicides than ubiquitous α-cleavage reactions[27] such as $201^+ \rightarrow 186^+$, $215^+ \rightarrow 200^+$, and $229^+ \rightarrow 214^+$ for the compounds I, II, and III, respectively. A typical calibration curve is shown in Figure 6 which was obtained by monitoring a McLafferty rearrangement reaction for atrazine, namely $215^+ \rightarrow 173^+$. The linearity of the curve was decent with the

correlation coefficient of 0.9993. However, sensitivity of SRM was much poorer than the corresponding SIM technique because the metastable ion intensity is usually at most 1% of the normal ion intensity. Subsequently, the theoretical detection limits in the SRM analyses utilizing the characteristic reactions described above were around 5 ng/m*l*, or 5 pg of the compound actually injected.

As were the cases for SIM analyses utilizing doubly charged ions, the excellent selectivity of the SRM technique was found to be useful for the analysis of complex mixture. Figure 7 shows some chromatograms obtained from sample B by monitoring the reactions mentioned above. In the SRM chromatograms obtained by monitoring the MaLafferty rearrangement reactions, namely Figure 7(a) and 7(b) the peaks for the compounds I and II are rather free from interference. Figure 7(c) shows the SRM chromatogram obtained by monitoring the hydrogen loss reaction of the McLafferty rearrangement product from propazine. Here again, the propazine peak is well separated from the interfering peaks enabling reliable quantitation. On the other hand, the propazine peak is heavily interfered in the SRM chromatogram (Figure 7(d)) obtained by monitoring the α-cleavage of the molecular ion. This demonstrates the importance of reaction specificity in the SRM analysis of trace components in a complex mixture. Quantitation of atrazine in sample B was attempted based on the chromatogram shown in Figure 7(b). The result obtained was 1.8 ± 0.2 ng/m*l* which was in good agreement with the prepared concentration of 2.0 ng/m*l*.

In summary, SIM and SRM techniques have been developed for the analysis of *s*-triazine herbicides in complex mixture. Even though monitoring the molecular ions or base peak ions for these compounds showed the best sensitivity, SIM analysis utilizing the doubly charged ions was clearly the method of choice for the analysis of the test sample for which cleanup procedure was intentionally left out. Theoretical detection limit for SRM was much higher than for SIM. However, superior selectivity of the technique allowed satisfactory analysis of the test sample. it is thought that the SIM method based on doubly charged ions would be better than the SRM technique for the analysis of the *s*-triazine herbicides. However, it should be pointed out that the presence of intense doubly charged ion in an EI spectra is more an exception than a rule. Hence, the fact that wide variety of metastable reactions are usually available for a compound such that a search can be made for optimum analysis should be considered as an additional advantage for the SRM technique. It is expected, of course, that SIM analysis based on molecular ions may be done satisfactorily in the present case by adopting a thorough cleanup procedure. Even when successful, such a procedure would usually require a lot of time and effort, however. Hence, it can be concluded that the SRM technique provides a useful and selective means which can be used generally for the GC/MS analysis of trace components in a complex mixture. The major disadvantage of the SRM technique when compared with SIM is its poorer sensitivity. In the present case, analysis based on SRM technique was limited to middle ppb range. The situation may be improved, however, by adopting an ionization method with higher yield such as negative chemical ionization or an MS/MS technique with higher daughter ion yield such as collisionally activated decomposition (CAD) mass spectrometry.[10,28] Investigation is currently under way to check the applicability of such techniques in the present case.

Acknowledgement. This work was supported by Korea Science and Engineering Foundation.

References

1. I. Howe, D. H. Williams and R. G. Bowen, "Mass Spectrometry. Principles and Applications", 2nd eds., McGraw-Hill, New York, 1981.
2. M. S. Kim, "Mass Spectrometry", Mineumsa, Seoul, 1987.
3. W. McFadden, "Techniques of Combined Gas Chromatotraphy/Mass Spectrometry: Applications in Organic Analysis", Wiley, New York, 1973.
4. G. M. Message, "Practical Aspects of Gas Chromatography/Mass Spectrometry", Wiley, New York, 1984.
5. T. R. Covey, E. D. Lee, A. P. Bruins and J. D. Henion, *Anal. Chem.*, **58,** 1451A (1986).
6. C. Fenselau, *Anal. Chem.*, **49,** 536A (1977).
7. V. Lopez-Avila, P. Hirata, S. Kraska, M. Flanagan, J. H. Taylor, Jr., and S. C. Hern, *Anal. Chem.*, **57,** 2797 (1985).
8. J. H. Beynon, R. P. Morgan and A. G. Brenton, *Phil. Trans. R. Soc. Lond.*, **A293,** 157 (1979).
9. F. W. McLafferty, *Acc. Chem. Res.*, **13,** 33 (1980).
10. F. W. McLafferty, ed., "Tandem Mass Spectrometry", Wiley, New York, 1983.
11. R. W. Kondrat and R. G. Cooks, *Anal. Chem.*, **50,** 81A (1978).
12. J. V. Johnson and R. A. Yost, *Anal. Chem.*, **57,** 758A (1985).
13. S. V. Hummel and R. A. Yost, *Org. Mass Spectrom.*, **21,** 785 (1986).
14. S. J. Gaskell and D. S. Millington, *Biomed. Mass Spectrom.*, **5,** 557 (1978).
15. Y. J. Kim, J. C. Choe, and M. S. Kim, *Bull. Korean Chem. Soc.*, **10,** 15 (1989).
16. K. Grob and K. Grob Jr., *J. Chromatogr.*, **94,** 53 (1974).
17. M. J. Farncombe, R. S. Mason, K. R. Jennings and J. Scrivens, *Int. J. Mass Spectrom. Ion Phys.*, **44,** 91 (1982).
18. W. M. Pearlman and C. K. Banks, *J. Am. Chem. Soc.*, **70,** 3726 (1948).
19. J. T. Thurston, J. R. Dudley, D. W. Kaiser, I. Hechenbleikner, F. C. Schaefer and D. Holm-Hansen, *J. Am. Chem. Soc.*, **73,** 2981 (1951).
20. H. B. Lee and Y. D. Stokker, *J. Assoc. Off. Anal. Chem.*, **69**(4), 568 (1986).
21. E. Matisová, J. Krupčik, O. Liška and N. Szentiványi, *J. Chromatogr.*, **169,** 261 (1979).
22. K. Ramsteiner, W. D. Hörmann and D. O. Eberle, *J. Assoc. Off. Anal. Chem.*, **57,** 192 (1974).
23. H. Roseboom and H. A. Herbold, *J. Chromatogr.*, **202,** 431 (1980).
24. U. Oehmichen, F. Karrenbrock and K. Haberer, Fresenius' Z. *Anal. Chem.*, **327**(7), 715 (1987).
25. M. Popl, Z. Vozňáková, V. Tatar and J. Strnadová, *J. Chromatogr. Sci.*, **21,** 39 (1983).
26. P. A. Leclercq and V. Pacáková, *J. Chromatogr.*, **178,** 193 (1979).
27. F. W. McLafferty, "Interpretation of Mass Spectra", 3rd ed., University Science Books, Mill Valley, 1980.
28. R. G. Cooks, ed., "Collision Spectroscopy", Plenum, New York, 1978.