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Characterization of Doxylamine and Pyrilamine Metabolites via Thermospray/Mass Spectrometry and Tandem Mass Spectrometry

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Analysis of doxylamine *N*-oxide and pyrilamine *N*-oxide as synthetic standards and biologically derived metabolites by thermospray mass spectrometry (TSP/MS) provided $[M + H]^+$ ions for each metabolite. TSP/tandem mass spectrometry (TSP/MS/MS) of the $[M + H]^+$ ions provided fragment ions characteristic of these metabolites. In addition, TSP mass spectrometry and TSP/MS/MS analysis of ring-hydroxylated *N*-desmethyldoxylamine, *N*-desmethylpyrilamine and *O*-dealkylated pyrilamine is also reported. A fragmentation pathway for analysis by MS/MS of pyrilamine and its metabolites is also proposed. The results demonstrate the utility of TSP/MS for biologically derived metabolites of pyrilamine and doxylamine.

INTRODUCTION

The antihistamine, doxylamine succinate, *N*,*N*-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine succinate, a component of the drug Bendectin®, has been subjected to several studies regarding its safety and its metabolic products.[1–11] Bendectin was prescribed to pregnant women as an antinausea drug, but is no longer sold in the United States because of concerns that it might cause birth defects. Pyrilamine maleate, an ethylenediamine-type drug, belongs to a class of antihistamines that is used most frequently in prescription and non-prescription cold, allergy and sleep aids.[12] Pyrilamine may replace methapyrilene, a structural analog, which has been removed from the United States drug market because it was shown to be a rat hepatocarcinogen.[13,14] Pyrilamine maleate has also been shown to cause unscheduled DNA synthesis in primary cultures of rat hepatocytes.[15–17]

Investigators at the National Center for Toxicological Research (NCTR) have conducted studies in the distribution and metabolism of doxylamine and pyrilamine in laboratory animals.[1,2,10,11] Korfmacher and coworkers[18–21] have demonstrated that desorption chemical ionization (DCI) mass spectrometry is very useful for the analysis of doxylamine and pyrilamine and their metabolites. The DCI mass spectrometric method has the disadvantage that the samples must be purified by high-performance liquid chromatography (HPLC) before analysis. Thermospray (TSP) is a recently developed mass spectrometric ionization technique that shows great potential for the analysis of thermally labile compounds.[22–24] In addition, TSP is an ideal mass spectrometric ionization technique for direct analysis of samples by liquid chromatography/mass spectrometry (LC/MS).[24] TSP ionization has been applied to the identification of dyes[25,26] and to the analysis of various biologically important compounds such as nucleosides and nucleotides,[22,27] peptides,[22,23] antibiotics[23] and glucuronides.[28–31] Recently, Rudewicz and Straub[32] have reported on the use of TSP/mass spectrometry (TSP/MS) and TSP/tandem mass spectrometry (TSP/MS/MS) for the identification of catecholamine conjugates in urine samples, and Covey *et al.*[33] investigated the use of TSP/MS and TSP/MS/MS for the determination of phenyl butazone and its metabolites in equine urine samples. Therefore, the utility of TSP/MS for the analysis of doxylamine and pyrilamine and their metabolites was investigated.

Our ultimate goal in this investigation is to develop an 'on-line' LC/MS and LC/MS/MS capability for doxylamine and pyrilamine and their metabolites in various biological matrices. In order to achieve this goal, the applicability of TSP as an ionization technique for these compounds had to be investigated, as did the utility of MS/MS for these compounds. Thus, our objectives for this study were twofold: (i) to examine the utility of TSP as an ionization technique for doxylamine and pyrilamine and their metabolites and to compare these results to our previous DCI mass spectrometric results for these compounds; and (ii) to analyze these compounds by TSP/MS/MS in order to investigate the utility of MS/MS to provide additional confirmatory data for the analysis of these metabolites and to aid in the differentiation of the two pyrilamine metabolites that are isomeric.

In this report, we describe the use of TSP/MS for the analysis of pyrilamine and doxylamine and several of their metabolites obtained from metabolism studies. TSP/MS was found to provide excellent mass spectral

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results for these compounds. The utility of TSP/MS/MS was also investigated. TSP/MS/MS was found to provide additional analytical data for these samples and was successfully used for the direct analysis of a 'cage wash' metabolite sample which had not undergone an HPLC purification step.

EXPERIMENTAL

Standard compounds

Doxylamine and pyrilamine were obtained from commercial suppliers and found to be greater than 99% pure by HPLC. Doxylamine *N*-oxide was prepared as described by Holder *et al.*[20] Pyrilamine *N*-oxide and desmethylpyrilamine were synthesized as described in Korfmacher *et al.*[19] The structures of these compounds were confirmed by mass spectrometry and nuclear magnetic resonance spectroscopy.[19,20]

Metabolites

Doxylamine *N*-oxide. Four adult female rhesus monkeys (*Macaca mulatta*) were dosed with doxylamine succinate in the form of Bendectin tablets at a level of 13.3 mg kg^{-1} (mg doxylamine succinate/kg body weight) as described in detail elsewhere.[2] A 0–6-h urine sample was collected over dry ice and stored at −20 °C until analyzed. Extraction and gradient HPLC purification of the metabolite was performed as described elsewhere.[11]

Ring-hydroxylated *N*-desmethyldoxylamine. Male and female Fischer 344 rats were dosed with doxylamine succinate by gavage at a level of 133 mg kg^{-1} as described in detail elsewhere.[10] An 8–14-h fecal sample was collected from a male Fischer rat. The fecal sample was extracted with methanol and a portion of this extract was purified by gradient HPLC as described in detail elsewhere.[11]

Pyrilamine metabolites. Male and female Fischer 344 rats were dosed with pyrilamine maleate by gavage at a level of 66.5 mg kg^{-1} as described in detail elsewhere.[34] The pyrilamine *N*-oxide metabolite was isolated from 0–8-h urine sample collected from a male Fischer rat. The *N*-desmethylpyrilamine metabolite was isolated from an 8–24-h urine sample from a male Fischer rat. At the end of the test period, the rat cages were washed with a methanol:water (1:1) solution. The urine or cage wash samples were extracted and then purified by HPLC using methodology similar to that used for the doxylamine metabolites.[11] The *O*-dealkylated pyrilamine metabolite was isolated from the cage wash sample from the male rats. For comparison, a 50 ml aliquot of the same cage wash sample was evaporated to dryness at ambient temperature and redissolved in 1 ml methanol for direct analysis by TSP/MS.

TSP/MS

Analyses by TSP/MS and TSP/MS/MS were performed using a Finnigan MAT (San Jose, California) 4500 triple-stage quadrupole (TSQ) mass spectrometer with a Vestec Corp. (Houston, Texas) thermospray inlet system. Ten microliter volumes of the sample were flow-injected into the TSP/MS system using the column by-pass mode and a flow rate of 1.3 ml min^{-1} with aqueous 0.1 M ammonium acetate:methanol (96:4 v/v) as the thermospray buffer solution. At this flow rate, the ionizer pressure was typically 1.4×10^{-5} Torr and the vaporizer (tip) and jet temperatures were 219 °C and 224 °C, respectively. A collision energy of 20 eV was used for the collision-activated dissociation (CAD) experiments with argon set to 1.0 mTorr as the collision gas.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the compounds discussed in this paper. As shown in this figure, the compounds included are doxylamine and two of its metabolites and pyrilamine and three of its metabolites.

Figure 2(a) shows the TSP mass spectrum of doxylamine with the $[M + H]^+$ ion at *m/z* 271. The CAD of the *m/z* 271 ion provided the spectrum shown in Fig. 2(*b*). The major daughter ion at *m/z* 182 has been observed under methane DCI mass spectrometric conditions as a fragment ion of doxylamine and has been identified as the $[C(C_6H_5)(C_5H_4N)(CH_3)]^+$ fragment ion resulting from cleavage at the ether linkage of doxylamine.[18] The other major daughter ion at *m/z* 167 is presumably the $[C(C_6H_5)(C_5H_4N)]^+$ ion.

Figure 3(a) shows the TSP mass spectrum of doxylamine *N*-oxide. The base peak is the $[M + H]^+$ ion at *m/z* 287. The major fragment ions at *m/z* 271 and *m/z* 257 corresponds to the $[M + H - O]^+$ and the $[M + H - CH_2O]^+$ ions, respectively. These same fragment ions have been observed for this compound when analyzed by methane or ammonia DCI mass spectrometry.[20,35] Figure 3(b) shows the TSP mass spectrum of a doxylamine metabolite identified as doxylamine *N*-oxide. While the *m/z* 287 is clearly evident as the base peak and the major fragment ions at *m/z* 271 and *m/z* 257 are also clearly seen in the spectrum, a large peak at *m/z* 279 can also be seen. The *m/z* 279 peak is probably due to a plasticizer contaminant.

One of the advantages of analysis by MS/MS is the ability to get a 'clean' mass spectrum even if the sample is relatively 'dirty'. Figure 3(c) shows the CAD mass spectrum of the $[M + H]^+$ ion from the doxylamine *N*-oxide standard. The daughter ion at *m/z* 226 can be assigned to the $[M + H - (O{-}NH(CH_3)_2)]^+$ ion. The ions at *m/z* 182 and *m/z* 167 are the same fragment ions as described above for the CAD mass spectrum of the $[M + H]^+$ ion of doxylamine. The daughter ion at *m/z* 106 is presumably due to the $[HO{-}CH_2{-}CH_2{-}HNO{-}(CH_3)_2]^+$ fragment ion. The corresponding fragment ion in the daughter ion mass spectrum of the $[M + H]^+$ ion of doxylamine can be seen as a minor peak in Fig. 2(b) at *m/z* 90. Figure

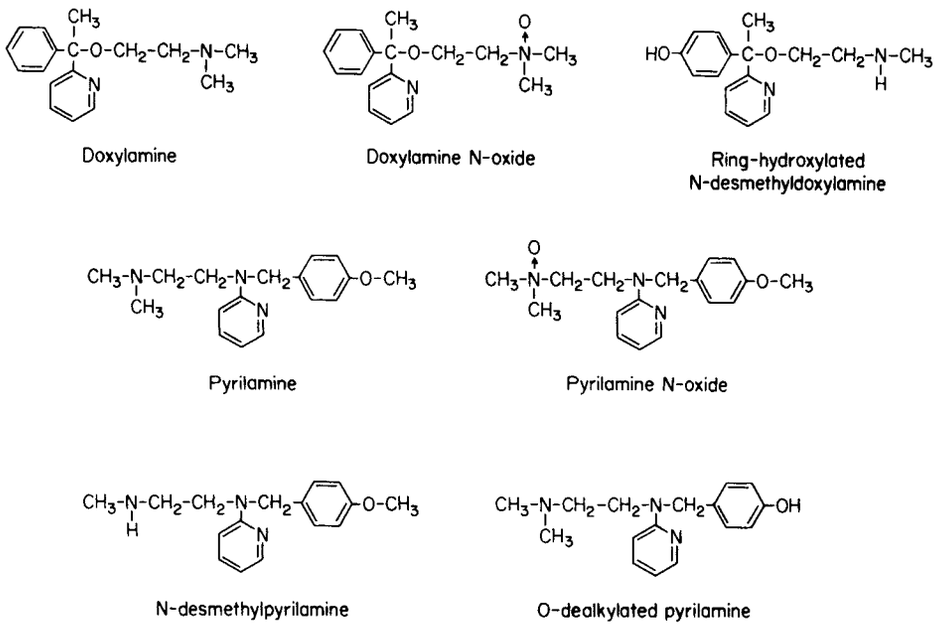


Figure 1. Structures of the compounds discussed in this paper.

3(d) shows the daughter ion mass spectrum from the CAD of the *m/z* 287 ion of the metabolite sample (Fig. 3(b)). This CAD mass spectrum matches well the corresponding daughter ion spectrum of the synthetic standard. Therefore, while the TSP mass spectrum of the metabolite suggests that it is doxylamine *N*-oxide, comparison of the daughter ion spectra for the metabolite (see Fig. 3(d)) and the standard (see Fig. 3(c)) provides essentially irrefutable evidence that the metabolite is doxylamine *N*-oxide.

Another example of the analysis of a doxylamine metabolite sample is shown in Fig. 4. This metabolite has previously been identified as ring-hydroxylated *N*-desmethyldoxylamine (see Fig. 1 for the structure).[11] Figure 4(a) shows the TSP mass spectrum of this doxylamine metabolite, which was extracted from rat feces. The base peak at *m/z* 273 is the $[M + H]^+$ ion and the major fragment ion at *m/z* 198 indicates that the molecule is ring-hydroxylated.[11] Thus, the *m/z* 198 ion is probably the $[C(CH_3)(C_5H_4N)(C_6H_4OH)]^+$ ion resulting from ether cleavage of the protonated molecular ion. The CAD mass spectrum of the *m/z* 273 ion is shown in Figure 4(b). The daughter ion at *m/z* 198 is presumably the same as the *m/z* 198 fragment ion described above for this compound. The daughter ion at *m/z* 183 can be attributed to a further loss of the methyl group from the *m/z* 198 ion. The *m/z* 76 daughter ion is presumably due to the $[M + H - (C{-}CH_2{-}C_5H_4N{-}C_6H_4{-}OH)]^+$ ion, suggesting that the parent molecule is an *N*-desmethyldoxylamine derivative; the *m/z* 76 fragment ion has also been observed for this metabolite when analyzed by ammonia DCI mass spectrometry.[11]

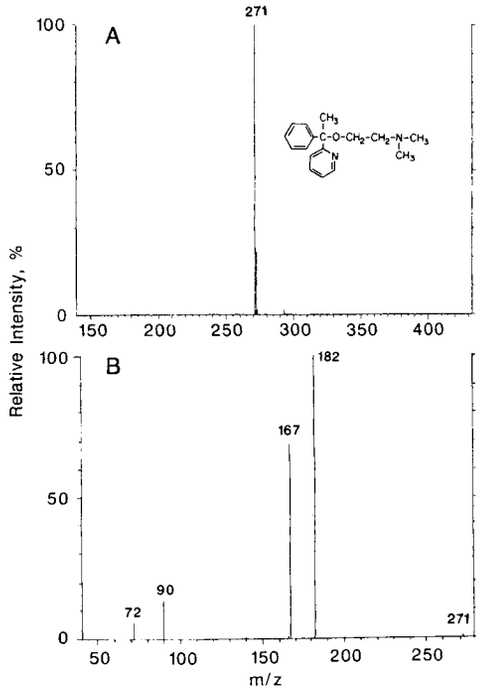


Figure 2. (a) TSP mass spectrum of doxylamine. (b) CAD daughter ion spectrum of *m/z* 271, $[M + H]^+$, from doxylamine.

Figure 5(a) shows the TSP mass spectrum for pyrilamine, which consists of the $[M + H]^+$ ion at *m/z* 286 as the base peak and the $[M + H - NH(CH_3)_2]^+$ ion at *m/z* 241 as the major fragment ion. CAD of the *m/z* 286 ion provided the mass spectrum shown in Fig. 5(b). The two daughter ions observed were at *m/z* 241 and *m/z* 121; the former is the $[M + H - NH(CH_3)_2]^+$ ion and the latter corresponds to both the $[NH(C_5H_4N)(CH_2)_2]^+$ fragment ion and the $[CH_2{-}C_6H_4{-}O{-}CH_3]^+$ fragment ion. As indicated below, these same daughter ions were observed in all of the tandem mass spectra of the pyrilamine metabolites, except for the *O*-dealkylated pyrilamine metabolite.

Figure 6(a) shows the TSP mass spectrum for synthetic pyrilamine *N*-oxide. The base peak at *m/z* 302 corresponds to the $[M + H]^+$ ion and the peak at *m/z* 286 is the $[M + H - O]^+$ ion. The peaks at *m/z* 272 and *m/z* 257 are presumably due to the $[M + H - CH_2O]^+$ ion and the $[M + H - CH_2O{-}CH_3]^+$ ion, respectively. The smaller peaks at *m/z* 241 and *m/z*

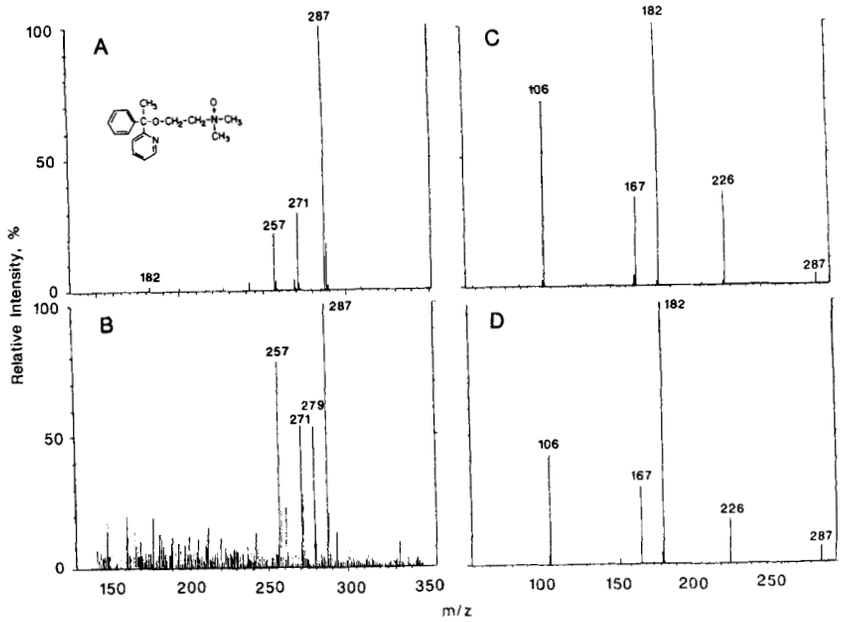


Figure 3. TSP mass spectrum of (a) doxylamine *N*-oxide standard and (b) rhesus monkey urine extract containing doxylamine *N*-oxide. CAD daughter ion spectrum of *m/z* 287, $[M + H]^+$, from (c) doxylamine *N*-oxide standard and (d) the rhesus monkey urine extract shown in (b).

215 are probably the $[M + H - (O{-}NH(CH_3)_2)]^+$ ion and the $[M + H - O - (CH{-}CH_2{-}N(CH_3)_2)]^+$ ion, respectively. This TSP mass spectrum is very similar to the one reported recently for pyrilamine *N*-oxide when analyzed by ammonia DCI mass spectrometry.[19] Figure 6(b) shows the TSP mass spectrum of the metabolite, pyrilamine *N*-oxide, obtained from a rat urine extract; this mass spectrum agrees well with that of the authentic standard (Fig. 6(a)). Further confirmation of the identity of this metabolite was obtained by TSP/MS/MS analysis. Figure 6(c) shows the mass spectrum obtained from the CAD of the *m/z* 302 ion from the pyrilamine *N*-oxide standard. The daughter ions at *m/z* 241 and *m/z* 121 can be assigned as the same ions that were observed for the CAD of the $[M + H]^+$ ion for pyrilamine. Figure 6(d) shows the CAD mass spectrum for the $[M + H]^+$ ion of the pyrilamine *N*-oxide metabolite sample. The CAD daughter ion spectrum of the *m/z* 302 ion for the metabolite sample is essentially identical to the corresponding spectrum of the synthetic standard, providing confirmation of the identity of the metabolite sample. In addition, analysis by TSP/MS/MS of the *m/z* 272 fragment ion for both the pyrilamine

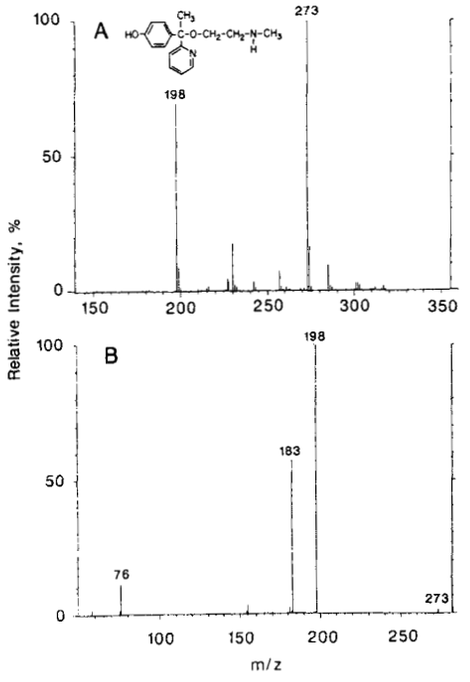


Figure 4. (a) TSP mass spectrum of the ring-hydroxylated *N*-desmethyldoxylamine metabolite. (b) CAD daughter ion spectrum of *m/z* 273, $[M + H]^+$, from the ring-hydroxylated *N*-desmethyldoxylamine metabolite.

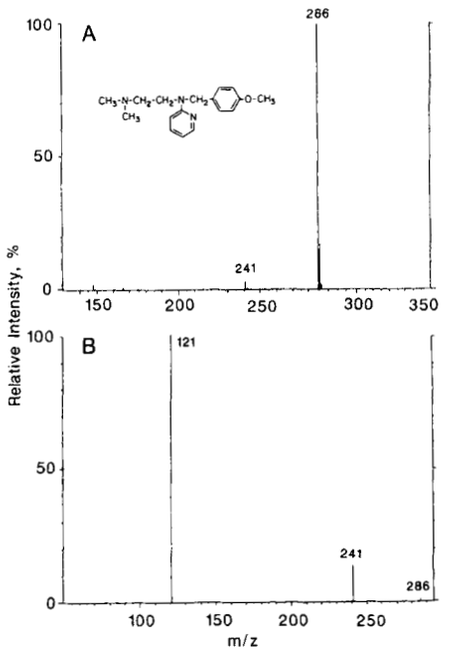


Figure 5. (a) TSP mass spectrum of pyrilamine. (b) CAD daughter ion spectrum of *m/z* 286, $[M + H]^+$, from pyrilamine.

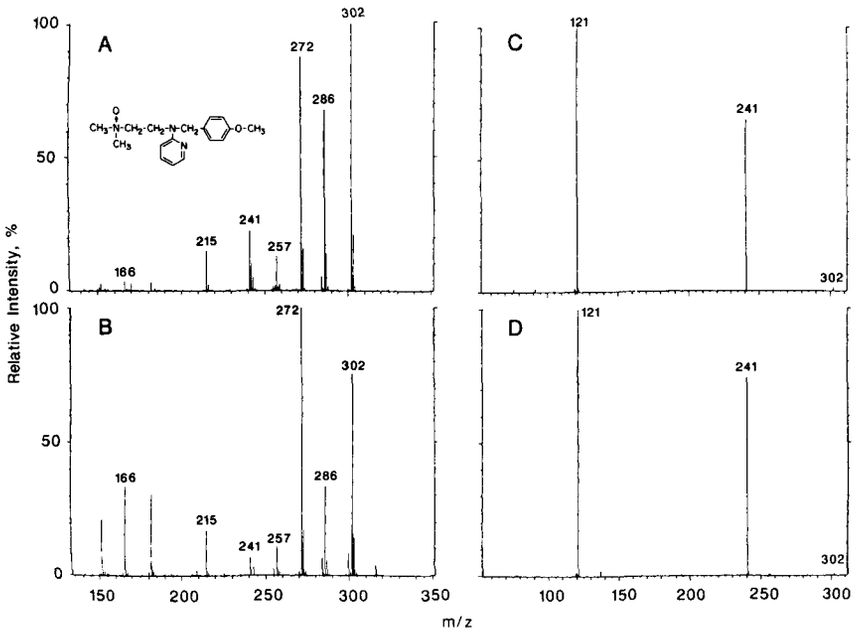


Figure 6. TSP mass spectrum of (a) pyrilamine *N*-oxide standard and (b) pyrilamine *N*-oxide from a rat urine extract. CAD daughter ion spectrum of *m/z* 302, $[M + H]^+$, from (c) pyrilamine *N*-oxide standard and (d) the urine extract shown in (b).

N-oxide standard and this metabolite sample resulted in essentially identical spectra, both having a small (5%) peak at *m/z* 241 and a base peak at *m/z* 121 (figure not shown).

Figure 7(a) shows the TSP mass spectrum obtained for the synthesized *N*-desmethylpyrilamine standard. The base peak is the $[M + H]^+$ ion at *m/z* 272 and the only significant fragment ion is the $[M + H - (CH{-}C_6H_4{-}O{-}CH_3)]^+$ ion at *m/z* 152. These results are similar to those reported recently for the analysis of *N*-desmethylpyrilamine by ammonia DCI mass spectrometry.[19] The TSP mass spectrum of the rat urine metabolite identified as *N*-desmethylpyrilamine is shown in Fig. 7(b). The TSP mass spectral results for the metabolite sample compare well with those for the standard material, except for the peaks at *m/z* 241 and *m/z* 166. Again TSP/MS/MS was used for confirmation of this metabolite sample. Figure 7(c) shows the CAD daughter ion spectrum for the $[M + H]^+$ ion of the synthetic *N*-desmethylpyrilamine; the major daughter ions observed were at *m/z* 241 and *m/z* 121. The CAD mass spectrum of the *m/z* 272 ion for the metabolite is shown in Fig. 7(d). The TSP tandem mass spectral results for this metabolite agree well with those of the standard material, thereby confirming that the metabolite is *N*-desmethylpyrilamine.

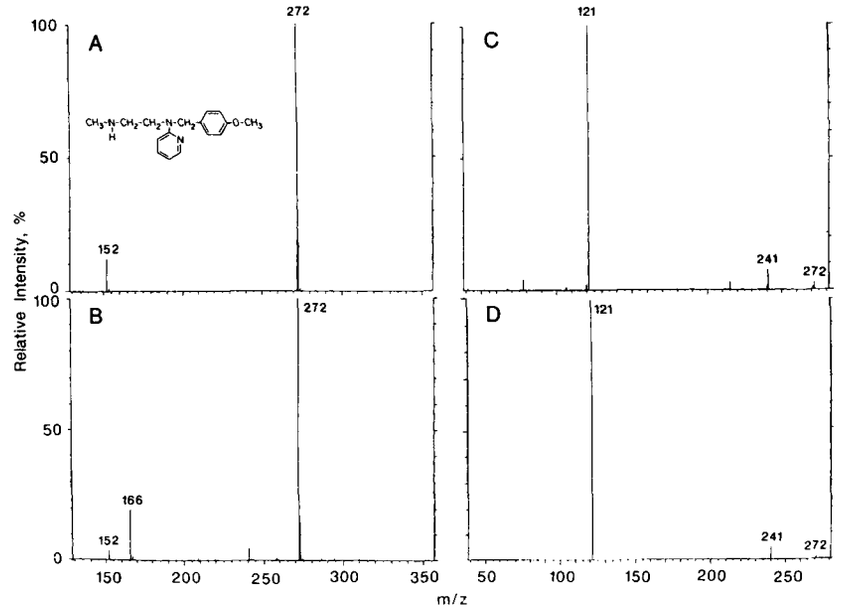


Figure 7. TSP mass spectrum of (a) *N*-desmethylpyrilamine standard and (b) a rat urine extract containing *N*-desmethylpyrilamine. CAD daughter ion spectrum of *m/z* 272, $[M + H]^+$, from (c) *N*-desmethylpyrilamine standard and (d) the rat urine extract shown in (b).

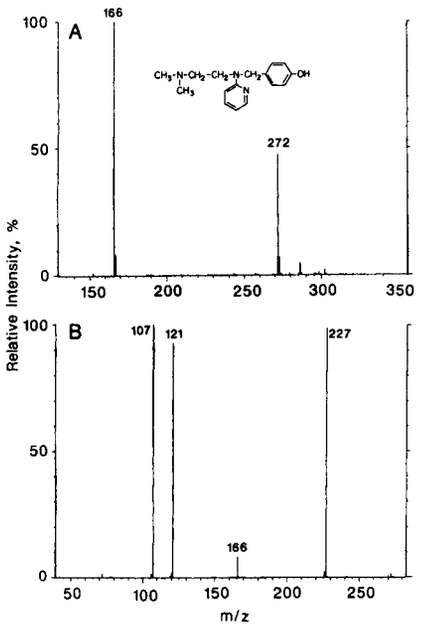


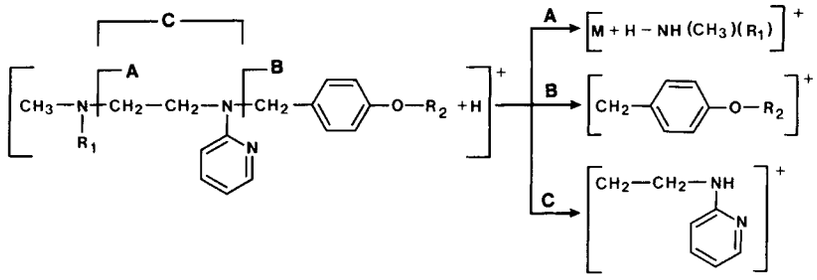
Figure 8. (a) TSP mass spectrum of the *O*-dealkylated pyrilamine metabolite from a purified rat urine extract. (b) CAD daughter ion spectrum of *m/z* 272, $[M + H]^+$, from the purified rat urine extract.

Another metabolite of pyrilamine that is an isomer of *N*-desmethylpyrilamine is *O*-dealkylated pyrilamine (see Fig. 1 for the structures). Figure 8(a) shows the TSP mass spectrum of an HPLC fraction of a rat urine extract believed to contain the *O*-dealkylated pyrilamine metabolite. The major peak at *m/z* 272 is the $[M + H]^+$ ion and the base peak at *m/z* 166 is the $[M + H - (CH{-}C_6H_4{-}OH)]^+$ ion. Based on this information alone, identification would be difficult. Analysis by TSP/MS/MS of the sample was performed and Fig. 8(b) shows the CAD spectrum of the *m/z* 272 ion. This daughter ion spectrum is clearly distinguishable from that obtained for the *m/z* 272 ion of *N*-desmethylpyrilamine (Fig. 7(c)). The daughter ion at *m/z* 227 corresponds to the $[M + H - NH(CH_3)_2]^+$ ion. The second major peak at *m/z* 121 can be assigned to the $[NH(C_5H_4N)(CH_2)_2]^+$ fragment ion as seen for other pyrilamine compounds, and the *m/z* 107 peak would correspond to the $[CH_2{-}C_6H_4{-}OH]^+$ fragment ion. Thus, the combination of TSP/MS and TSP/MS/MS of this metabolite can be used to identify this compound as the *O*-dealkylated pyrilamine metabolite.

Figure 9 summarizes the proposed CAD fragmentation pathways for pyrilamine and its two demethylated metabolites. This figure also lists the masses of the proposed fragments and shows how two (A and B) of the three fragment ions can be used to distinguish the two isomeric metabolites. It should be noted that the daughter ions of the $[M + H]^+$ ion for pyrilamine *N*-oxide also are defined by the pathways shown in Fig. 9, giving the same daughter ions as shown for the $[M + H]^+$ ion of pyrilamine.

For the metabolite samples described above, the metabolite extracts were purified by HPLC before the sample was analyzed by TSP/MS or TSP/MS/MS. One of the potential advantages of MS/MS is the ability to analyze samples that have not undergone extensive clean-up. While analysis by MS/MS is not a direct substitute for (on-line or off-line) chromatography, it is an alternative analytical tool that may provide the desired information. Figure 10(a) shows the TSP mass spectrum of a 'cage wash' extract. The sample was not purified by HPLC before analysis. The major metabolite in this extract has been identified as *O*-dealkylated pyrilamine (Fig. 8(a) shows the TSP mass spectrum of the same sample after HPLC purification). While the $[M + H]^+$ ion at *m/z* 272 and the major fragment ion at *m/z* 166 are clearly observable in this spectrum, there is a large background due to other contaminants. The CAD mass spectrum of the *m/z* 272 ion is shown in Fig. 10(b). This daughter ion spectrum is easy to interpret and is essentially identical to the one shown in Fig. 8(b) for the analysis by MS/MS of the same $[M + H]^+$ ion from this metabolite when analyzed in a purified form. Thus, essentially identical TSP/MS/MS results were obtained for this metabolite regardless of whether or not the original sample was purified by the HPLC step.

In general, these results have shown that, for these compounds, TSP/MS typically provided results similar to those which can be obtained by ammonia DCI mass spectrometry. Typically, both ionization methods provided the $[M + H]^+$ ion as a major peak and often the



CAD Pathway	Pyrilamine $R_1 = CH_3$, $R_2 = CH_3$	N-Desmethyl Pyrilamine $R_1 = H$, $R_2 = CH_3$	O-Dealkylated Pyrilamine $R_1 = CH_3$, $R_2 = H$
A	m/z 241	m/z 241	m/z 227
B	m/z 121	m/z 121	m/z 107
C	m/z 121	m/z 121	m/z 121

Figure 9. Proposed fragmentation pathways for the CAD of the $[M + H]^+$ ions from pyrilamine, *N*-desmethylpyrilamine and *O*-dealkylated pyrilamine.

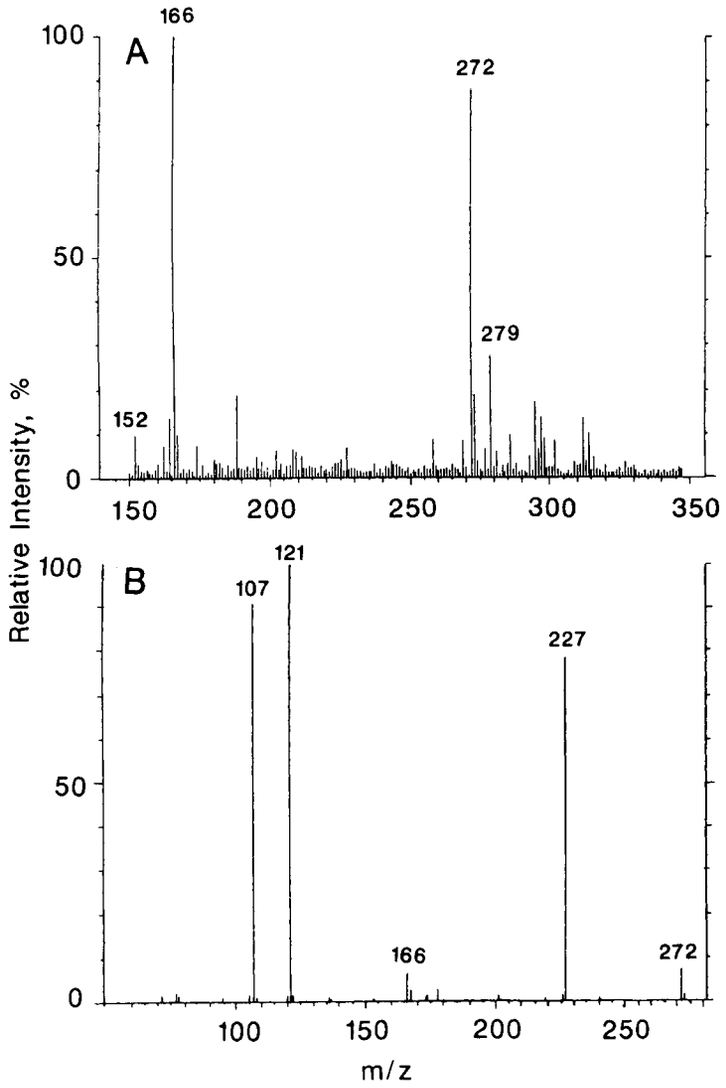


Figure 10. (a) TSP mass spectrum of the 'cage wash' extract without using an HPLC purification step. (b) CAD daughter ion spectrum of *m/z* 272, $[M + H]^+$, from the 'cage wash' extract.

base peak. Therefore, either of these ionization techniques can be successfully applied to samples containing these compounds after (off-line) HPLC clean-up has been performed. On the other hand, if (on-line) LC/MS analysis is the goal, then TSP/MS will need to be used. We plan to develop (on-line) LC/MS methodology for samples similar to those described in this paper; thus we will rely on the utility of TSP/MS for these and related compounds in our future research.

CONCLUSIONS

TSP/MS and TSP/MS/MS have been shown to be useful methods for the analysis of doxylamine and pyrilamine metabolites. Analysis of biologically derived metabolites provided spectra essentially identical to those obtained for authentic standards. In most cases, the combination of TSP/MS and CAD of the $[M + H]^+$ ion provided almost unambiguous identification of the metabolite in the sample. In addition, TSP/MS/MS was shown to provide a useful mass spectrum for a sample which had not undergone HPLC purification.

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Notice

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REFERENCES

1. W. Slikker, Jr, C. L. Holder, J. R. Bailey, J. F. Young and H. C. Thompson, Jr, *Teratology* **27**, 77A (1983).
2. W. Slikker, Jr, C. L. Holder, G. W. Lipe, W. A. Korfmacher, H. C. Thompson, Jr and J. R. Bailey, *J. Anal. Toxicol.* **10**, 87 (1986).
3. J. P. Gibson, R. E. Staples, E. J. Larson, W. L. Kuhn, D. E. Holtkamp and J. W. Newberne, *Toxicol. Appl. Pharmacol.* **13**, 439 (1968).
4. A. G. Hendrickx, S. Prahalada, G. Jonos, T. Nyland and J. M. Rowland, *Teratology* **27**, 49A (1983).
5. W. A. Check, *J. Am. Med. Assoc.* **242**, 2518 (1979).
6. W. Gielsdorf and K. Schubert, *J. Clin. Chem. Biochem.* **19**, 485 (1981).
7. L. B. Holmes, *Teratology* **27**, 277 (1983).
8. R. W. Smithwells and S. Sheppard, *Teratology* **17**, 31 (1978).
9. D. A. Ganes, K. W. Hindmarsh and K. K. Midha, *Xenobiotica* **16**, 781 (1986).
10. H. C. Thompson, Jr, A. B. Gosnell, C. L. Holder, P. H. Siitonen, K. L. Rowland and J. L. Cmarik, *J. Anal. Toxicol.* **10**, 18 (1986).
11. C. L. Holder, H. C. Thompson, Jr, A. B. Gosnell, P. H. Siitonen, W. A. Korfmacher, C. E. Cerniglia, D. W. Miller, D. A. Casciano and W. Slikker, Jr, *J. Anal. Toxicol.* **11**, 113 (1987).
12. R. F. Doerge, in *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, ed. by C. O. Wilson, O. Gisvold and R. F. Doerge, pp. 648–670. J. B. Lippincott, Philadelphia (1977).
13. W. Lijinsky, M. D. Reuber and B. N. Blackwell, *Science* **209**, 817 (1980).
14. F. A. Althaus, S. D. Lawrence, G. L. Sattler and H. C. Pitot, *Mutat. Res.* **103**, 213 (1982).
15. J. O. Budroe, J. G. Shaddock and D. A. Casciano, *Mutat. Res.* **135**, 131 (1984).
16. G. S. Probst and S. B. Neal, *Cancer Lett.* **10**, 67 (1980).
17. C. A. McQueen and G. M. Williams, *J. Toxicol. Environ. Health* **8**, 463 (1981).
18. W. A. Korfmacher, C. L. Holder, J. P. Freeman, R. K. Mitchum and A. B. Gosnell, *Org. Mass Spectrom.* **20**, 435 (1985).
19. W. A. Korfmacher, C. L. Holder, A. B. Gosnell and H. C. Thompson, Jr, *J. Anal. Toxicol.* **10**, 142 (1986).
20. C. L. Holder, W. A. Korfmacher, W. Slikker, Jr, H. C. Thompson, Jr and A. B. Gosnell, *Biomed. Mass Spectrom.* **12**, 151 (1985).
21. E. B. Hansen, Jr, C. E. Cerniglia, W. A. Korfmacher, D. W. Miller and R. H. Heflich, *Drug. Metab. Dispos.* **15**, 97 (1987).
22. C. R. Blakely, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.* **102**, 5931 (1980).
23. C. R. Blakely and M. L. Vestal, *Anal. Chem.* **55**, 750 (1983).
24. T. R. Covey, E. D. Lee, A. P. Bruins and J. D. Henion, *Anal. Chem.* **58**, 1451A (1986).
25. L. D. Betowski and J. M. Ballard, *Anal. Chem.* **56**, 2604 (1984).
26. L. D. Betowski, S. M. Pyle, J. M. Ballard and G. M. Shaul, *Biomed. Environ. Mass Spectrom.* **14**, 343 (1987).
27. W. A. Korfmacher, L. D. Betowski, F. A. Beland and R. K. Mitchum, *Spectros. Inter. J.* **4**, 317 (1985).
28. D. J. Liberato, C. C. Fenselau, M. L. Vestal and A. L. Yergey, *Anal. Chem.* **55**, 1741 (1983).
29. C. Fenselau, D. J. Liberato, J. A. Yergey, R. J. Cotter and A. L. Yergey, *Anal. Chem.* **56**, 2759 (1984).
30. C. Fenselau and L. Yellet, in *Xenobiotic Conjugation Chemistry*, ed. by G. D. Paulson, J. Caldwell, D. H. Hutson and J. J. Menn, p. 159. ACS Symposium Series 299, ACS, Washington, DC (1986).
31. W. A. Korfmacher, C. L. Holder, L. D. Betowski and R. K. Mitchum, *J. Anal. Toxicol.* **11**, 182 (1987).
32. P. Rudewicz and K. M. Straub, *Anal. Chem.* **58**, 2928 (1986).
33. T. R. Covey, E. P. Lee and J. D. Henion, *Anal. Chem.* **58**, 2453 (1986).
34. H. C. Thompson, Jr, C. L. Holder, P. H. Siitonen, K. L. Rowland, A. B. Gosnell and J. L. Cmarik, *J. Anal. Toxicol.*, in press.
35. W. A. Korfmacher, C. L. Holder, C. E. Cerniglia, D. W. Miller, E. B. Hansen, Jr, K. J. Lambert, A. B. Gosnell, W. Slikker, Jr, L. G. Rushing and H. C. Thompson, Jr, *Spectros. Inter. J.* **4**, 181 (1985).