

## Fast Atom Bombardment Mass Spectral Analysis of Primaquine

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Fast atom bombardment mass spectrometry was used for structure elucidation of primaquine, an antimalarial drug of biomedical interest. *m*-Nitro-benzyl alcohol was used as a matrix in fast atom bombardment studies because it does not react with analyte to form adduct. The spectra of primaquine clearly indicated that the fragment ions were obtained due to ring cleavages, loss of side chain and proton transfer and free radical rearrangement of molecular ion peak.

**Key Words:** Primaquine, Fast atom bombardment mass spectroscopy, Antimalarial.

### INTRODUCTION

Soft ionization techniques which permit direct desorption of gase phase analyte ions without the need for vaporization have greatly expanded the potential applications of mass spectrometry. By using such techniques as field desorption (FD), desorption chemical ionization (DCI) and fast atom bombardment (FAB), thermally labile and higher molecular weight compounds are now amenable to MS examination without thermal degradation. Halmarks<sup>1-6</sup> in his work has included extensive application for sequencing of peptides to around 3000  $\mu$  using FAB fragmentation data and early complete mass spectra of very large bio-molecules like bovine insulin and proinsulin. The applications of FAB to bioorganic/biomedical compounds have been extensively dealt with in a number of reviews<sup>7-9</sup>. Even for the trace levels of higher molecular weight, thermally labile analytes that are present in the final isolated fractions can be characterized using FAB spectrometric data. A protonated alcoholic hydroxyl group is likely to be lost as water *via* charge transfer. In addition, charge site initiation cleavages with proton transfer and charge retention are observed<sup>9</sup> as are rearrangements and cleavages remote from the apparent charge site<sup>10, 11</sup>.

Primaquine, an 8-aminoquinoline, is the clinical drug of choice for the radical cure of relapsing malaria. Mass spectrometry has been used extensively for the elucidation of structure of primaquine derivatives. The oxidative products of primaquine were characterized recently<sup>12</sup> using electron impact (EI) mass spectrometric data. Idowu and coworkers<sup>13</sup> have used HPLC-MS and GC-MS for the characterization of metabolites of WR 238605, an 8-aminoquinoline, by rat liver

microsomes and reported electron impact (EI) mass spectrum of these derivatives. Field desorption mass spectral analysis of N-acetylated dimer formed by microbial transformation of primaquine by *Candida tropicale* was reported earlier<sup>14</sup>. Strother *et al.*<sup>15</sup> have used mass spectrometry in electron impact and chemical ionization mode for the identification of blue derivative of primaquine metabolite formed during *in vitro* metabolism of primaquine by mouse liver enzymes. Hufford *et al.*<sup>16</sup> have used field desorption mass spectra for the characterization of sulfur linked dimer of N-acetyl primaquine isolated by microbial metabolism studies of primaquine using *Streptomyces roseochromogenus*. Fast atom bombardment (FAB) mass spectrometry has been used recently by Sinha *et al.*<sup>17</sup> for the elucidation of structures of thermally labile and higher molecular weight compounds of biomedical interest as the EI mass spectrum is unable to give the molecular ion peak for higher molecular weight compound. In this paper, the correlation between the mass spectral data and the structural features of primaquine is discussed.

## EXPERIMENTAL

Primaquine diphosphate was purchased from Sigma (USA) and purity was checked by HPLC (more than 98%). The fast atom bombardment mass spectrum was recorded on JEOL SX-102/DA-6000 mass spectrometer/data system equipped with the FAB target (a metal probe tip 3–4 nm<sup>2</sup> made of stainless steel) and the xenon atoms of *ca.* 6 keV was used as the ionizing beam. Primaquine is soluble in methanol as well as water. Therefore, 3-nitrobenzyl alcohol (3-NBA) was used as matrix. A few  $\mu\text{g}$  of the analyte were dissolved in the matrix. The ions were accelerated out of the ion source at a potential of 10 kV at  $10^{-4}$  torr pressure and could produce a flux of ions and neutrals up to the equivalent of a 500 A charged beam. The FAB mass spectrum was recorded at Central Drug Research Institute (CDRI), Lucknow, India in average scan of 1–3. The retention time and intensity of spectrum is 0.12" and 27.5704, respectively.

## RESULTS AND DISCUSSION

The 3-NBA cluster ions are found of masses corresponding to multiples of molecular weights of 3-NBA plus a proton;  $m/z$  136, 154, 289, 307, 460 and the bombardment of analyte/3-NBA mixture results in sputtering into the gas phase of  $[M + H]^+$  ions of the primaquine due to proton transfer process<sup>18, 19</sup>. The FAB mass spectrum of primaquine is shown in Fig. 1. The base peak at  $m/z$  260 was assigned to protonated pseudomolecular ion peak. The  $M^+$  appears at 259  $\mu$  having % total ion intensity 5.8301%. In the high resolution the observed mass of  $M^+$  was found to be 259.1686  $\mu$ , confirming the elemental composition of primaquine  $C_{15}H_{21}ON_3$ .

The peak at  $m/z$  243 was due to the removal of ammonia gas from protonated molecular ion peak  $(M + H)^+$  which confirmed the presence of terminal amino group. Its chemical composition,  $C_{15}H_{18}N_2O$ , is confirmed by high-resolution spectra. On the other hand, the rearrangement process of methyl radical results in loss of butyl amine ( $-\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2$ ) and propyl amine ( $-\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{NH}_2$ ) at  $m/z$  187 and 201, respectively, which is the characteristic feature of the protonated primaquine molecule. This fragmentation scheme is in line with the exemplary study carried out by Gohlke and McLafferty<sup>20</sup>. The

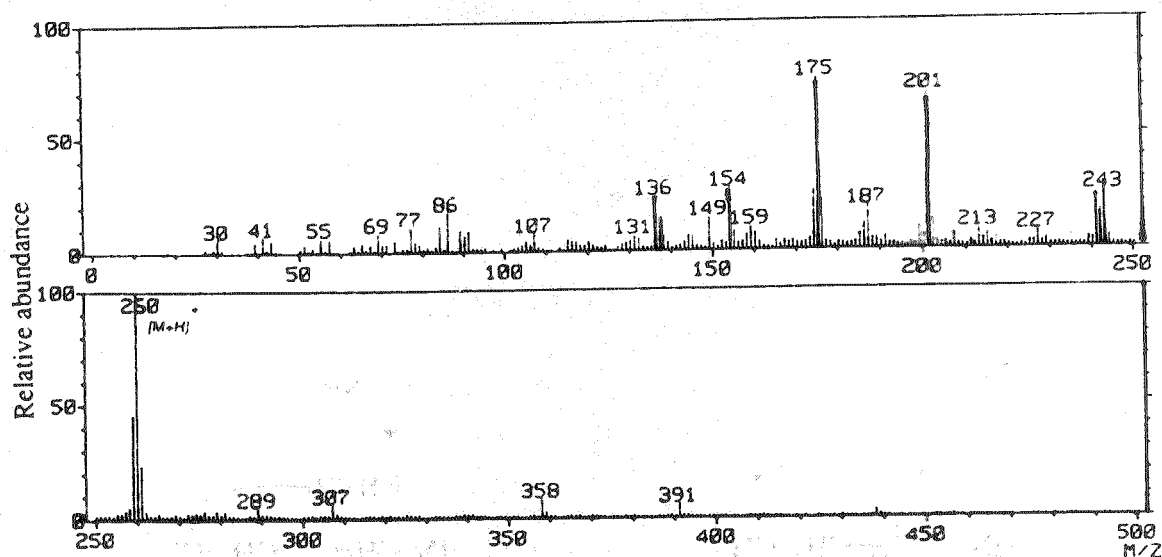
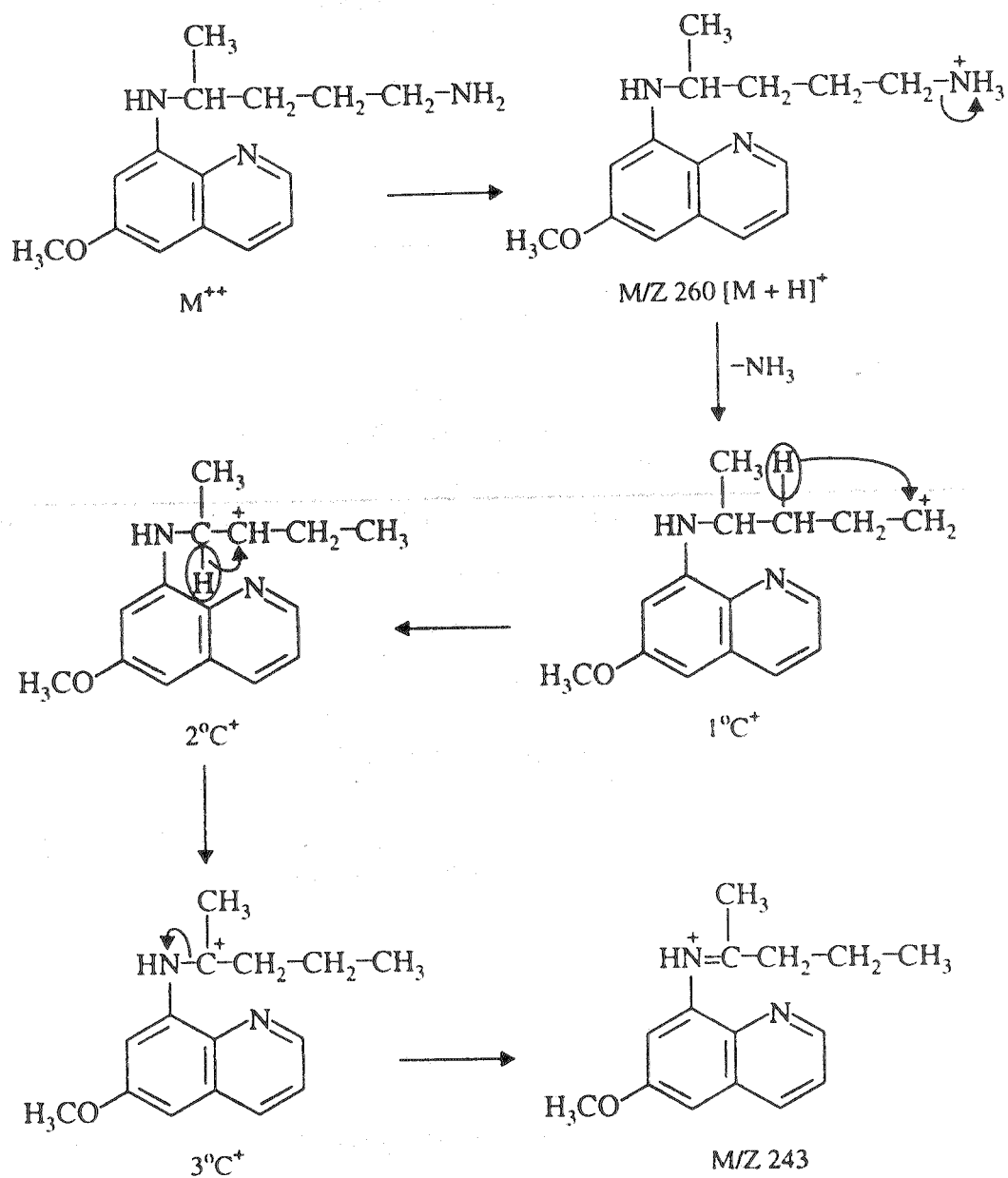


Fig. 1. Fab mass spectrum of primaquine, an antimalarial drug

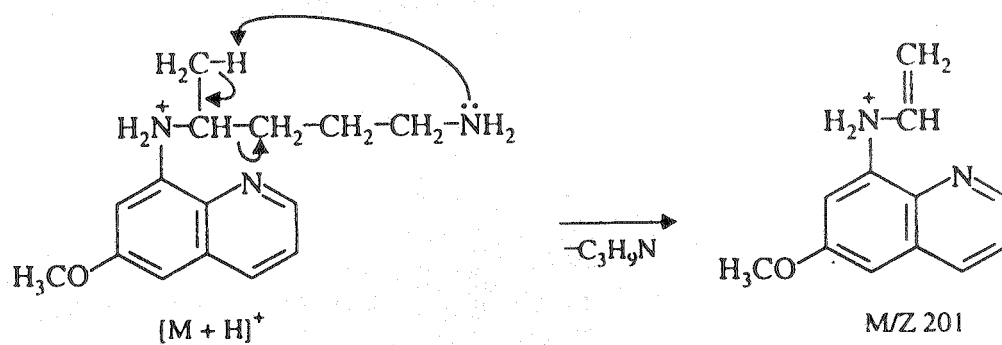
chemical compositions of  $C_{12}H_{14}N_2O$  and  $C_{11}H_{10}N_2O$  are confirmed by high-resolution spectra. Generally in the FAB cases the different fragments ions occur due to loss of neutral molecule<sup>21</sup> from molecular ion ( $M + 1$ ) and the present results are in agreement with this stabilized phenomenon. The characteristic fragment ion at 175 was observed due to the loss of  $CH_2=CH-CH_2-CH_2-CH_2-NH_2$  (85 dalton mass) group and  $m/z$  at 159 was due to complete expulsion of side chain  $NH_2-CH(CH_3)-CH_2-CH_2-CH_2-NH_2$  from pseudomolecular ion peak, respectively. None has reported primaquine fragmentation using FAB, though in similar line FAB fragmentation is reported about oxidative product of primaquine<sup>17</sup>. The ejection of formaldehyde  $-OCH_2$  (30 Dalton mass) from the fragment ion  $[M + 1]^+ - 85$  affords an ion at  $m/z$  145 and the elimination of methoxy  $-OCH_3$  from  $[M + 1]^+ - 101$  results at  $m/z$  145. The fragmentation appears to originate in the side chain moiety linked at 8-position of the quinoline ring as reported in oxidative product of primaquine<sup>17, 12</sup>. The high-resolution spectrum indicates the chemical composition of the ions  $C_{10}H_{10}N_2O$ ,  $C_{10}H_8NO$ ,  $C_9NH_9$ ,  $C_9NH_6$ . The fragmentation schemes (Schemes 1–5) confirm the structure of primaquine.

TABLE-I  
ELEMENTAL COMPOSITION OF THE FRAGMENTS OBSERVED IN PRIMAQUINE

(M/Z)	Composition	Relative abundance (%)	Fragmented ion
260	$C_{15}H_{21}N_3O$	100	$[M + H]^+$
243	$C_{15}H_{18}N_2O$	25	$[M + H]^+ - NH_3$
227	$C_{14}H_{17}N_3$	10	$[M + H]^+ - CH_3OH$
201	$C_{12}H_{13}N_2O$	68	$[M + H]^+ - C_3NH_6$
187	$C_{11}H_{10}N_2O$	20	$[M + H]^+ - C_4H_{11}N$
175	$C_{10}H_{10}N_2O$	74	$[M + H]^+ - C_5NH_{11}$
159	$C_{10}H_8NO$	10	$[M + H]^+ - C_5H_{14}N_2$



Scheme-1



Scheme-2

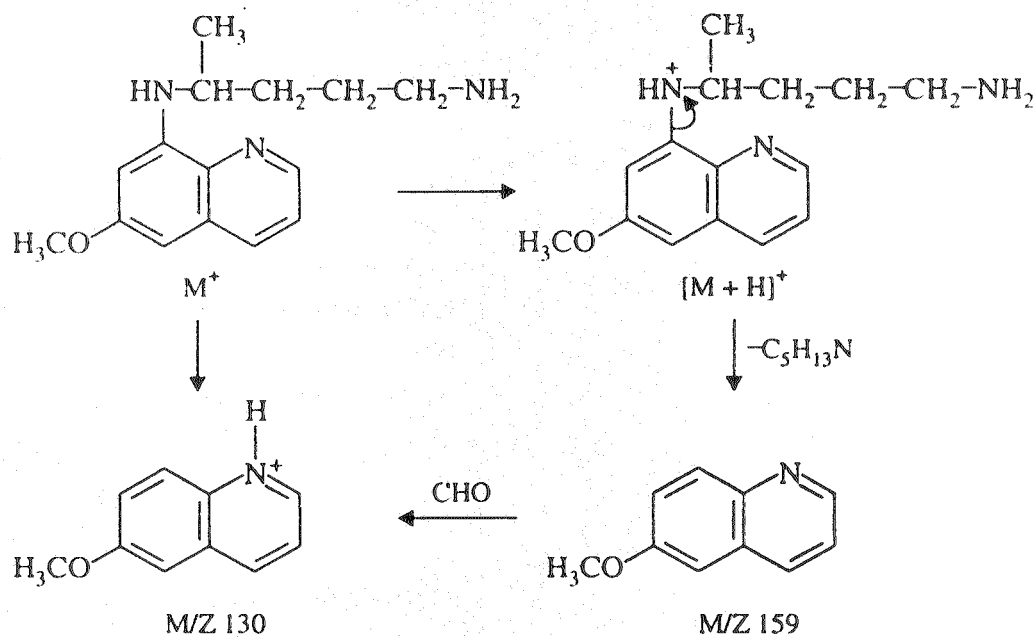
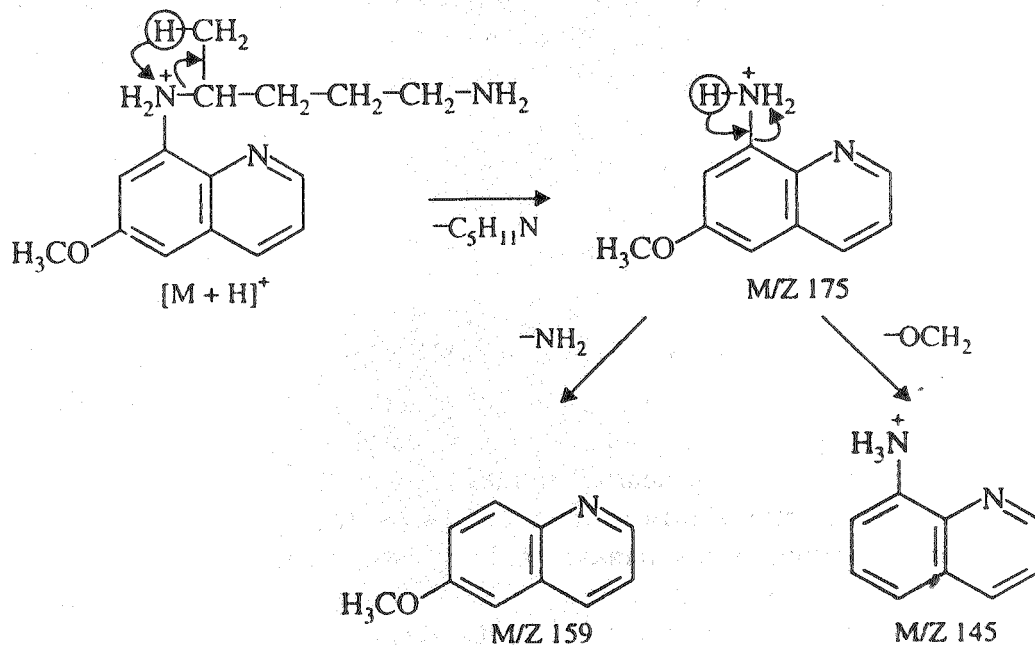


Fig. 2. Fragmentation schemes of primaquine

### Conclusions

FAB mass spectrometry was found very useful to assign the molecular ion peak and fragmentation scheme for elucidation of structure of primaquine of biomedical interest. The fragmentation appears to originate in the side chain moiety linked at 8-position of quinoline similar to oxidative product of primaquine. Although, compared to electron ionization, FAB is considered a gentle

mode of ionization; the ions formed possess internal energies several eV above their ground state causing them to dissociate into smaller fragments. Thus clearly depicting that the FAB mass spectrum of primaquine has better % total ion intensity, accuracy in determination of fragment ions and also the fragments were obtained due to ring cleavage, McLafferty rearrangement, loss of side chain and proton transfer and free radical rearrangement. The spectra reported here first time might serve as a reference for characterizing the primaquine related new tissue schizontocide in future.

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### REFERENCES

1. D.L. Lippstreu-Fisher and M.L. Gross, *Anal. Chem.*, **57**, 1174 (1985).
2. T. Takao, T. Hitouji, Y. Shimonishi, T. Tanabe, S. Inuye and M. Inouye, *J. Biol. Chem.*, **259**, 6105 (1984).
3. A.M. Buko, L.R. Phillips and B.A. Fraser, *Biomed. Mass Spectrom.*, **10**, 324 (1983).
4. G.V. Garner, D.B. Gordon, L.W. Teller and R.D. Sedgwick, *Org. Mass Spectrom.*, **18**, 486 (1983).
5. K. Biemann, *Int. J. Mass Spectrum Ion Phys.*, **45**, 183 (1983).
6. R. Self and A. Parente, *Biomed. Mass Spectrom.*, **10**, 78 (1983).
7. C. Fenselau, R. Cotter, G. Hansen, T. Chen and D. Heller, *J. Chromatogr.*, **218**, 21 (1981).
8. K.L. Busch and R.G. Cooks, *Science*, **218**, 247 (1982).
9. H.R. Schulten and R.P. Lattimer, *Mass Spectrom. Rev.*, **3**, 231 (1984).
10. M. Barber, R.S. Borodli, R.D. Borodli, A. Sedgwick and N. Tyler, *Nature*, **293**, 270 (1981).
11. W. Wallinga, N.M.M. Nibbering, J. Vander Greef and M.C. Ten Noever de Braun, *Org. Mass Spectrom.*, **19**, 10 (1984).
12. S.N. Sinha, *Indian J. Chem.*, **43B**, 202 (2004).
13. O.R. Idowu, J.O. Peggings, T.G. Brewer and C. Kelly, *Drug Metabol. Depos.*, **23**, 1 (1994).
14. A.M. Clark, C.D. Hufford, R.C. Gupta, R.K. Puri and J.D. McChesney, *Appl. Environ. Microbiol.*, **47**, 537 (1984).
15. A. Strother, R. Allahyari, J. Buchholz, I.M. Fraser and B.E. Tilton, *Drug Metabol. Depos.*, **12**, 35 (1983).
16. C.D. Hufford, J.K. Baker, J.D. McChesney and A.M. Clark, *Antimicrob. Agents Chemother.*, **30**, 234 (1986).
17. S.N. Sinha and V.K. Dua, *Int. J. Mass Spectrom.*, **232**, 151 (2004).
18. R.L. Cochram, *Appl. Spectroscopy Rev.*, **22**, 137 (1986).
19. P. Germain and P. Jean-Claude, *Org. Mass Spectrom.*, **19**, 448 (1984).
20. R.S. Gohlke and F.W. McLafferty, *Anal. Chem.*, **34**, 1281 (1962).