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Nature of the Main Contaminant in the Drug Primaquine Diphosphate: GC-MS Analysis

ILIA BRONDZ*, UWE KLEIN, DIMITRIS MANTZILAS, DAG EKEBERZ†
ERLEND HVATTUM†, HENRK SCHULTZ‡ and FELIX MIKHAILITSYN**
Department of Molecular Biosciences
University of Oslo, P.O. Box 1041, Blindern, 0316 Oslo, Norway
Tel.: (47)22857726; Fax: (47)22854443; E-mail: ilia.brondz@biokjemi.uio.no

In the present work, GC-MS was used to provide evidence on fragmentation differences between primaquine and quinocide. Primaquine and quinocide are readily separated by GC. Both substances give visible M^+ molecular ion 259 m/z. The base peak in the mass spectrum of primaquine was found to be at m/z 201, in the mass spectrum of the positional-isomer quinocide the base peak was found to be at m/z 187. Two detailed MS fragmentation schemes for primaquine and quinocide are provided which can help to identify primaquine-like derivatives. Using GC-MS, it is possible to recognize with high confidence the positional-isomer contaminant in gas chromatograms of the antimalaria drug primaquine without a standard substance as reference.

Key Words: Primaquine, Quinocide, MS fragmentation, GC-MS.

INTRODUCTION

Malaria is one of the most widely spread diseases on the globe with an estimated case rate of about 300–500 million and a death rate of about 2.7 million humans per year. The microorganisms that cause malaria in humans are *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae.* Primaquine (CAS 90-34-6) and quinocide (CAS 525-61-1) are the only anti-malarial drugs[1], which are gametocidal and effective against the hypnozoites and against the tissue schizonts. Primaquine is not effective against blood schizonts[2].

Anti-malarial drugs had been synthesized in Germany and France before World War II, and in Great Britain, the USA and the USSR during and since World War II[3]. Elderfield *et al.*[4] have developed a procedure for the synthesis of primaquine,

†Department of Chemistry and Biotechnology, Section Chemistry, Agricultural University of Norway, P.O. Box 5040, 1432 Ås, Norway.

‡Weifa AS, Hausmanns gt. 6, P.O. Box 9113, Gronland, 0133 Oslo, Norway.

**Martsinovsky Institute of Medical Parasitology and Tropical Medicine, 20 Malaja Pyrogovskaja St., Moscow, Russia.

which later was improved by Elderfied *et al.*[5] Synthesis of quinocide was developed and later improved by Braude and Stavrovskaya[6, 7].

The drug primaquine phosphate is listed as "primaquine phosphate and enantiomer" for the first time in the British Pharmacopoeia[8]. In the British Pharmacopoeia 1993 addendum 1997[9] and in the 3rd edition of the European Pharamacopoeia, it is stated that related substances are allowed to be present in the drug at a maximum of 3%[10]. These limits were still valid in the British Pharmacopoeia 2000[11] and in European Pharmacopoeia 3rd, supplement 2001[12].

It has been assumed for a long time that the main contaminant of primaquine is its enantiomer. A possible separation of the racemate has been reported[4]. In an earlier publication[1] it is shown by using liquid chromatography-mass spectrometry (LC-MS) that the main contaminant of primaquine is not its enantiomer but the positional isomer quinocide. Identifying contaminants in human medicines is important, especially for highly toxic compounds. When a standard is not readily available, as is the case for quinocide, it is necessary to look for alternative provided methodology to improve the reliazility of the analysis.

Primaquine (CAS 90-34-6) and quinocide (CAS 525-61-1) are highly toxic substances, which can have a number of side effects upon treatment. Quinocide is difficult to obtain as a standard due to a halt in production of the substance in the former USSR, which was the only producer of quinocide. Because the UV spectrum and MS fragmentation under HPLC-MS analysis of primaquine and quinocide are closely related, it is difficult to prove the identity of quinocide without a standard. The identity of contaminants in human medicine is important, especially for high toxic substances; therefore we looked for alternative ways to identify quinocide in samples. An MS fragmentation spectrum during GC-MS provides more detail and more information than MS fragmentation spectra during HPLC-MS, giving substantial knowledge about differences in closely related molecules.

We used GC-MS to show fragmentation differences between primaquine and quinocide; we also worked out fragmentation schemes for both substances. MS fragmentation of primaquine is well studied, but MS fragmentation of quinocide is poorly covered in the literature. Fragmentations were not compared to date and characteristic fragments which can be used to distinguish between the two substances are not yet described.

EXPERIMENTAL

Primaquine diphosphate p.a. quality standard from Aldrich (Aldrich-Chemie, Steinheim, Germany) and p.a. quality standard quinocide dihydrochloride from Dr. F. Mikhailitsyn (Martsinovsky Institute of Medical Parasitology and Tropical Medicine, Moscow, Russia) were analyzed.

Preparation of primaquine and quinocide for GC-MS

Primaquine diphosphate and standard quinocide dihydrochloride were used. 5 mg of each substance were dissolved in 10 mL of purified water and 1 mL of concentrated ammonia, p.a. grade (Merck, Darmstad, Germany), was added.

The base was extracted in 10 mL of spectroscopic grade hexane (Merck). The

hexane extract was concentrated to 1 mL by N_2 before GC-MS analysis. The extract was protected from light.

Gas chromatography-mass spectrometry GC-MS

The setup consisted of an HP 6890N gas chromatograph from Agilent Technologies Inc. (Agilent Technologies Inc, Wilmington, England) connected to the MS such that the column was entering directly into the ion source of the MS. The temperature in the GC-MS interface was kept at 300°C and helium was used as the carrier-gas. Samples were injected by a combi-PAL from CTC Analytics AG (Zwingen, Switzerland). The flow rate was in the range of 2.31 mL/ min at a constant pressure of 713 torr. The MS instrument was operated in the electron impact (EI) mode at 70 eV utilizing an ion source temperature of 210°C. The pressure in the ion source was 6–10 torr and the pressure in the analyzer was 7–10 torr. Chromatographic separation was achieved by temperature programming: 120°C hold 1 min, then 5°C/min until 270°C.

Chromatography was performed on an HP 5 fused-silica capillary column, 30 m long, with 320 μm I.D. and 0.25 μm film thickness, produced by J&W Scientific Inc., CA, USA.

The injector was a split/splitless injector operated in split mode with the temperature set up to 300°C. The sample volume was 0.2 μL. The mass spectrometer (MS) used was an AutoSpec Ultima from Waters produced by Micromass Ltd., Manchester, England. The MS is a three sector instrument with EBE-geometry.

RESULTS AND DISCUSSION

Appearance of a positional isomer in synthesis of primaquine is possible[1]. There are two possible positional isomers. One isomer has the methyl group in position C_1 of the chain, the other in position C_4.

As, due to a halt in production of the substance in the former USSR, the quinocide is difficult to obtain as a standard, it seemed necessary to look for methods that allow identifying quinocide in the absence of a standard substance.

The data show that evidence for the main contaminant quinocide can be provided by GC-MS even in the absence of a standard substance.

GC-MS

The drug primaquine and the authentic standard quinocide were analyzed with GC-MS. MS was set to the scanning mode. Fig. 1 shows the reconstructed ion chromatograms (RIC): A of the drug primaquine, B of the standard quinocide and C of the co-chromatography of the drug primaquine with standard quinocide. In Fig. 1A there are two peaks, peak a is primaquine and peak b the contaminant (positional isomer quinocide). In Fig. 1B there is one peak, which is the quinocide standard. In Fig. 1 C the results of co-chromatography of the drug primaquine with standard quinocide is shown. Peak a corresponds to primaquine and peak b corresponds to quinocide (see also Fig. 1A). All substances were detected as the molecule ions with m/z 259. Figs. 2 A and B show the mass spectra of the two

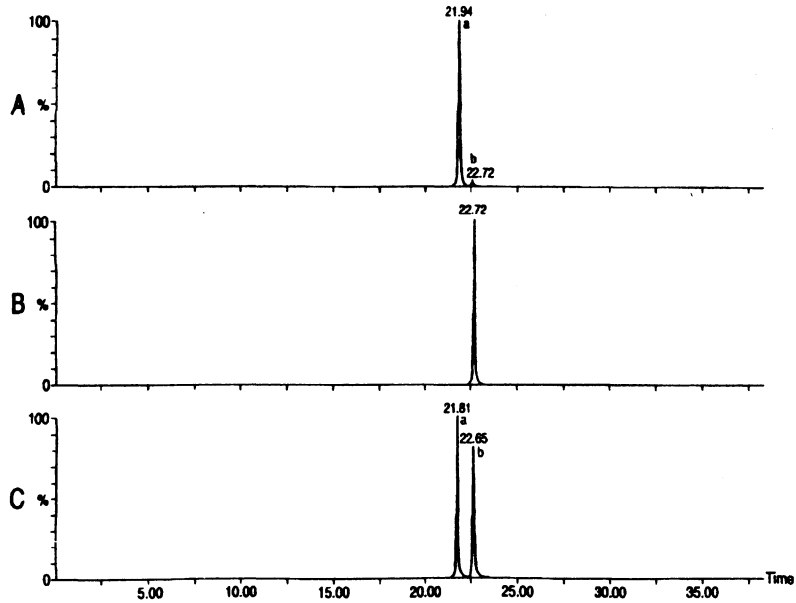
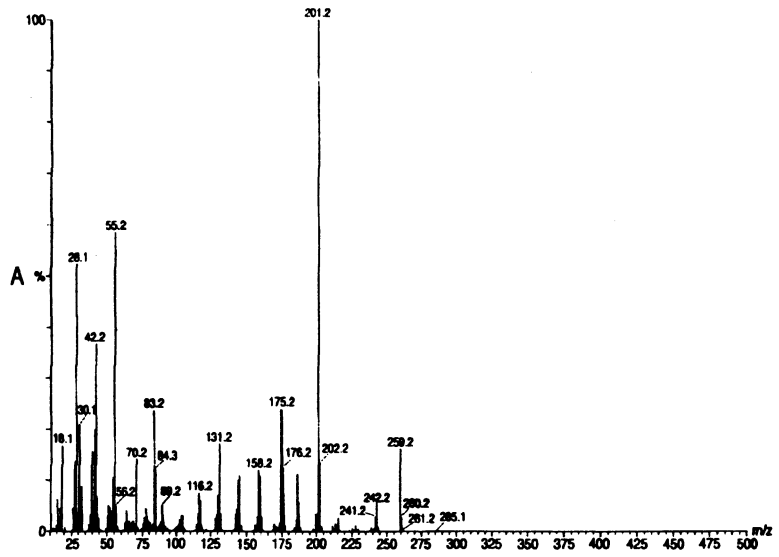


Fig. 1. Reconstructed ion chromatograms (RIC): A of the drug primaquine, B of the standard quinocide and C or the co-chromatography the drug standard quinocide. The gas chromatograph used was an HP 6890N from Agilent Technologies Inc., Wilmington, England connected to the MS. The mass spectrometer MS used was an AutoSpee Ultima from Waters produced by Micromass Ltd., Manchester, England. The MS is a three sector instrument with EBE-geometry



(A)

Fig. 2. The mass spectra of the two peaks found in the drug primaquine: A. the mass spectrum found in the peak a;

peaks found in the drug primaquine, and Fig. 2C shows the mass spectrum of the peak found in the quinocide standard. In Fig. 1C the results of co-chro-

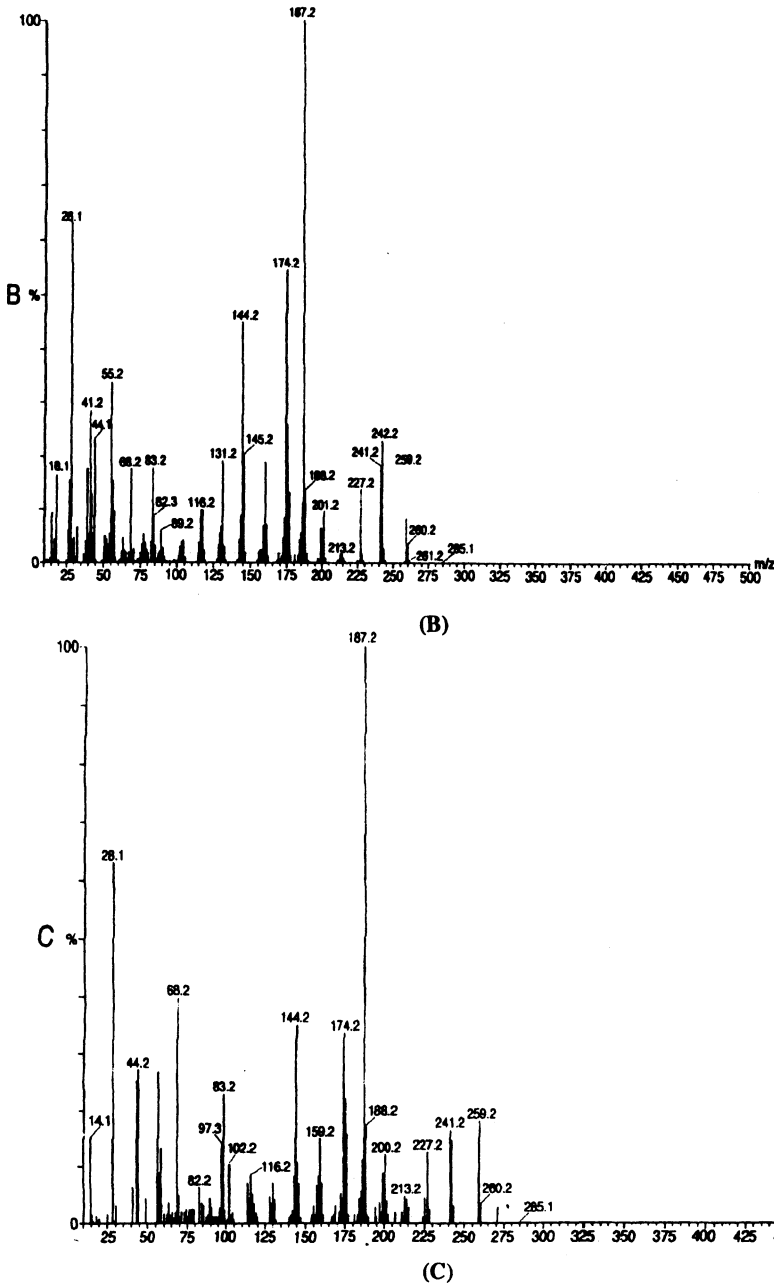


Fig. 2. The mass spectra of the two peaks found in the drug primaquine: B. the mass spectrum found in the peak b (Fig. 1A); C. the mass spectrum found in the quinocide (Fig. 1B);

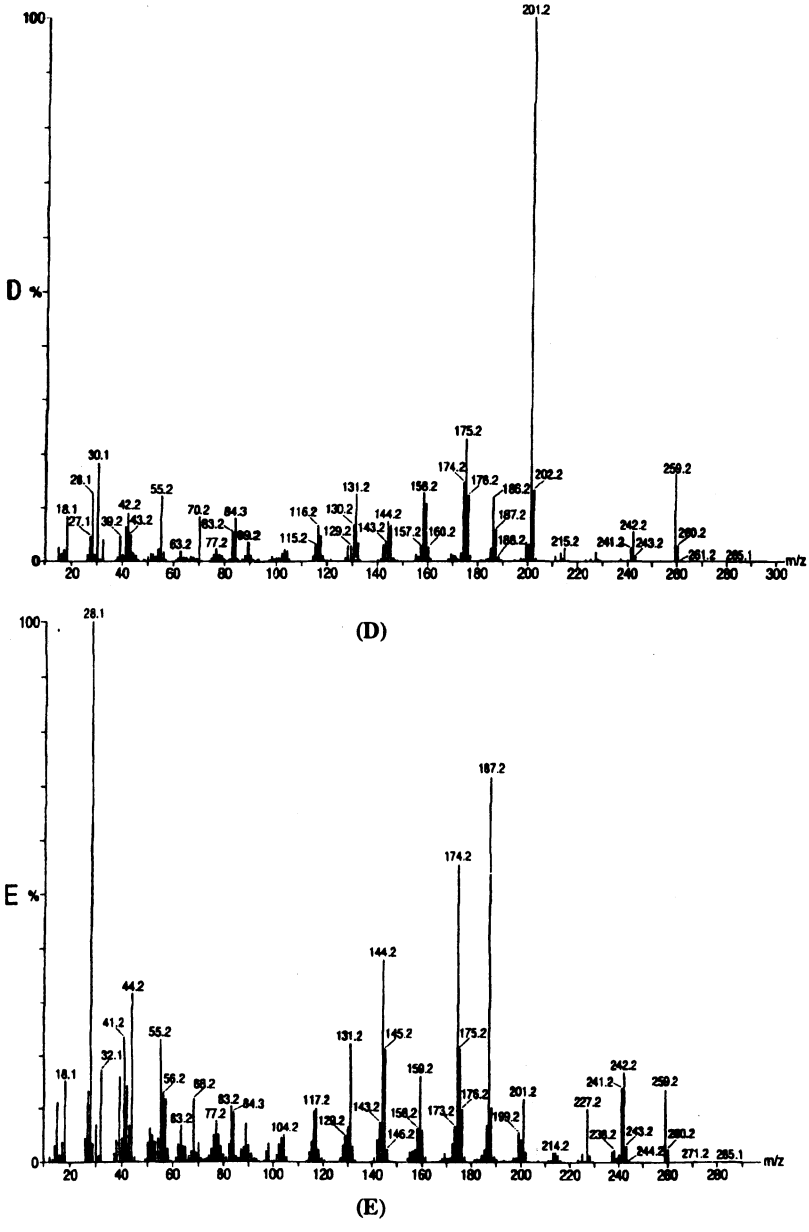


Fig. 2. The mass spectra of the two peaks found in the drug primaquine: D. the mass spectrum found in the peak a; E. the mass spectrum found in the peak b (Fig. 1C) using mass spectrometer AutoSpec Ultima.

matographythe mass spectra of peak a is identical qualitatively and quantitatively to primaquine (Fig. 2D), and the mass spectrum of peak b is identical qualitatively and quantitatively to quinocide (Fig. 2E).

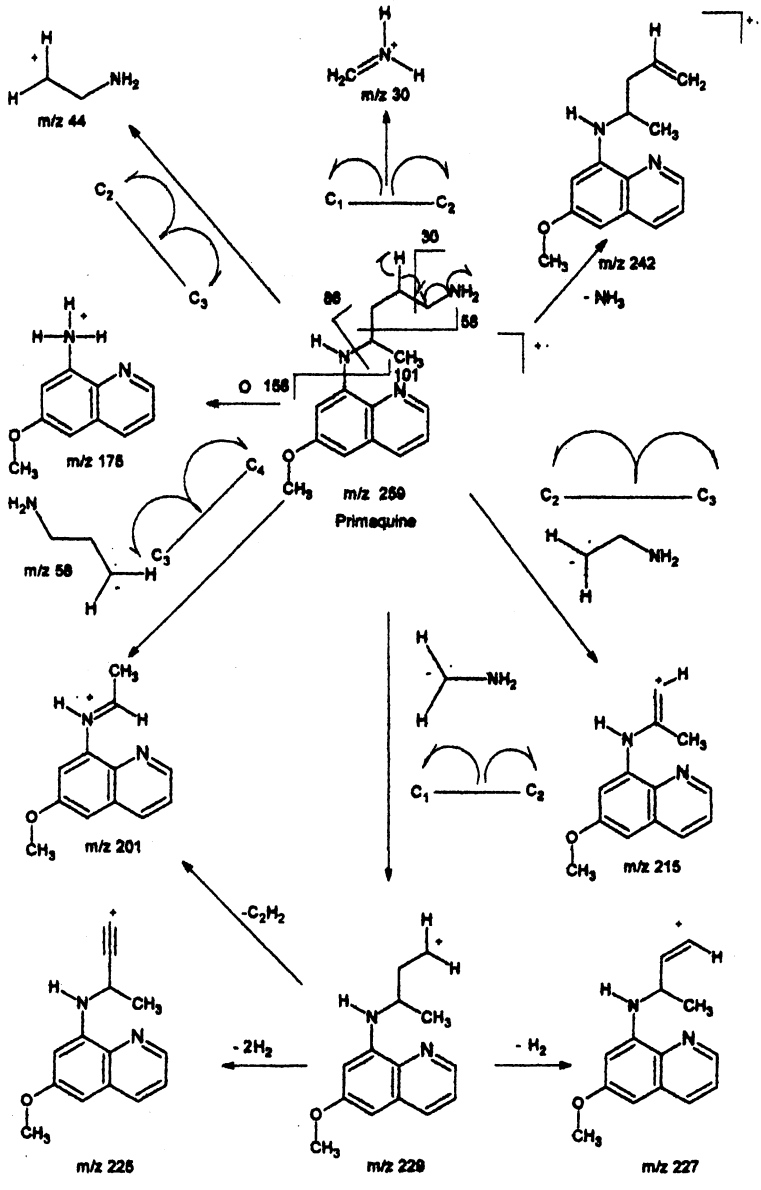


Fig. 3. The main routes of fragmentation for primaquine, as suggested by electron ionization mass spectrometry

The ion at m/z 259 is the molecular ion of primaquine and quinocide. The other ions found in the mass spectra are electron impact fragments of the molecule. The proposed fragmentation of the molecules of primaquine and quinocide are shown in Figs. 3 and 4 respectively.

The base peak in the mass spectrum of primaquine was found to be at m/z 201 in the mass spectrum of the positional isomer the base peak was found to be at m/z 187.

Which of the fragment ions observed in the two mass spectra of primaquine and quinocide are formed depends on the location of the charge and the energy distribution in the molecular ion.

The non-bonding orbital available on the three nitrogen atoms and on an

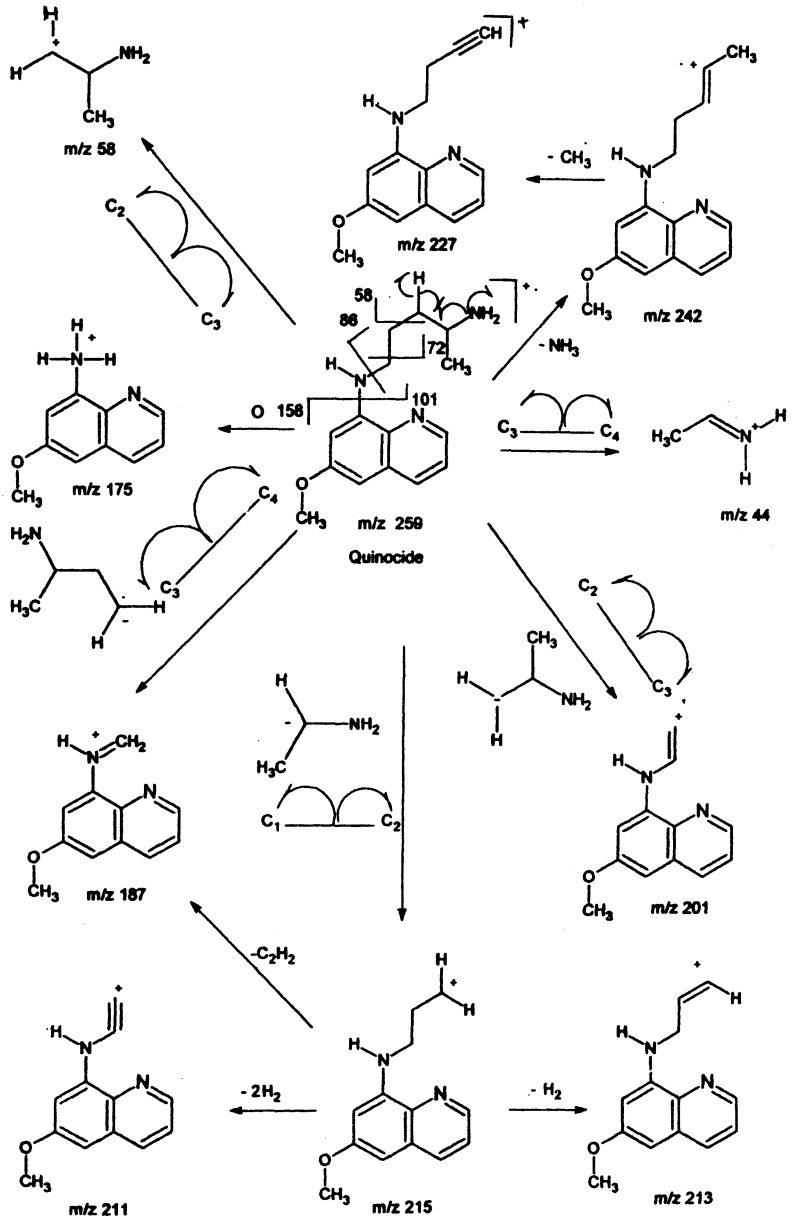


Fig. 4. The main routes of fragmentation for quinocide, as suggested by electron ionization mass spectrometry

oxygen atom are certainly sites of electron expulsion upon ionization. When the charge is at the primary amine nitrogen, homolytic C_3-C_4 bond cleavage takes place.

In addition, when the secondary amine nitrogen is ionized, C_1-C_2 bond cleavage is observed. The fragment ions are formed with m/z 201 and m/z 187 from the molecular ions of primaquine and quinocide, respectively.

The ions found at m/z 30 and m/z 44 are importnat to identify and also to characterize the mass spectra of the two compounds, primaquine and the main contaminant quinocide. Formation of fragment ions with m/z 44 from primaquine and quinocide results in two different fragment ions, as illustrated in Figs. 3 and 4. These fragment ions are formed due to bond cleavage at two different sites in the molecules. There is a minor portion of the ions at the m/z 44 from primaquine observed. The nominal mass of ions found at m/z 44 can in principal correspond to several ions, *e.g.*, $C_2H_6N^+$, CO_2^+, N_2O^+, $C_2H_4O^+$ and $C_3H_8^+$. The accurate masses of these ions are shown in Table-1. The resolving power necessary to separate $C_2H_6N^+$ from the other ions is 732, 900, 1850 and 3500, respectively. No peaks corresponding to N_2O^+, $C_2H_4O^+$ or $C_3H_8^+$ are observed in the mass spectra of the two compounds (not shown).

TABLE-1
ACCURATE MASS OF DIFFERENT IONS AT THE NOMINAL MASS m/z 44

Compound	Accurate mass
CO_2^+	43.989829
$C_2H_6N^+$	44.050024
N_2O^+	44.001062
$C_2H_4O^+$	44.026214
$C_3H_8^+$	44.062600

The main conclusion from these experiments is, therefore, that the ions found at m/z 44 correspond to CO_2^+ and $C_2H_6N^+$. Carbon dioxide is assumed to come from the oil diffusion pumps, and is observed only in small amounts in the mass spectra of the two compounds.

The $C_2H_6N^+$ ions are not observed in the mass spectrum of primaquine, which implies that C_2-C_3 bond cleavage results in fragment ions in which the charge is located in the largest fragment, found to be at m/z 215. A similar situation is observed for quinocide, homolytic bond cleavage of the C_2-C_3 bond results in ions with m/z 201 and not $NH_2(CH)(CH_3)(CH_2)^+$ ions with m/z 58. The mass spectrum of quinocide has about the same amount of the fragment ions $C_2H_6N^+$ at m/z 44 as primaquine at m/z 30, CH_4N^+, which are caused by C_3-C_4 Φ bond cleavage.

Since these fragment ions are observed in the two mass spectra of primaquine and quinocide it supports the proposed fragmentation mechanisms illustrated in the schemes shown in Figs. 3 and 4. The mass spectrum of quinocide has significantly greater amount of the fragment ions at m/z 242 and 227 than the

mass spectra of primaquine. The fragmentation mechanisms illustrated in the schemes in Fig. 3 and Fig. 4 show that ions at m/z 242 in quinocide have different structures than in primaquine. The ions at m/z 227, therefore, have different origins in quinocide and in primaquine.

Elimination of NH_3 is observed from the molecular ions of both primaquine and quinocide, which may imply corssing of the k *vs.* E curves. Elimination of ammonia can take place *via.*, 1,2-elimination reaction, which is thermally induced or an electron impact-induced 1,4-elimination reaction.

A third mechanism proceeds *via.* some high energy processes which are initiated by a reversible transfer of hydrogen from the secondary amine or the methyl group in C(1). but this reaction is probably not due to steric interactions.

One of the main conclusions that can be drawn from these experiments and discussions is that C_1-C_2 and C_3-C_4 σ bond cleavage is the main fragmentation of the molecular ions of primaquine and quinocide, respectively. It indicates formation of fragment ions at m/z 30 and 201 and m/z 44 and 187 from the molecular ions of primaquine and quinocide, respectively. This information is important for distinguishing between the two positional isomers. Elimination of ammonia is observed in significant amounts from both molecular ions.

Conclusion

It is concluded that the main contaminant in the drug primaquine di-phosphate used in pharmaceutical production is not an enantiomer as believed previously, but the positional isomer quinocide. It can be successfully separated in mixture by GC, and recognized by MS characteristic fragmentation.

Important information for distinguishing between the two positional isomers is the formation of fragment ions at m/z 30 and 201 and m/z 44 and 187 from the molecular ions of primaquine and quinocide, respectively.

Using GC-MS it is possible to distinguish beyond doubt between the two positional isomers primaquine and quinocide without using the standard substance quinocide.

Primaquine diphosphate is not a racemate but a mixture of positional and stereo isomers. These conclusions are based on GC-MS and on previous analysis using two HPLC systems and LC-MS analysis[1].

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