Journal of Malaria Research
Volume 2, Number 2/3
ISSN: 1939-5906
© Nova Science Publishers, Inc.

SFC-MS Analysis of Contaminants in Primaquine Diphosphate Tablets and Spectral UV and NMR Characterising of Primaquine and Quinocide*

Ilia Brondz†
Department of Biology, University of Oslo, P.O. Box 1066,
Blindern, 0316 Oslo, Norway

Abstract

In the *Pharmacopoeias*, characterization of the toxic contaminant in primaquine is not described. The *Pharmacopoeias* require chromatographic separation Rs greater than 2 between the contaminant and API peak, based on separation using HPLC. The United States Pharmacopeia (USP) and The National Formulary (NF) seek submission of a monograph proposing an improvement of analysis of primaquine phosphate tablets.

In this study, resolution based on supercritical fluid chromatography (SFC) and supercritical fluid chromatography-mass spectrometry (SFC-MS) satisfy the *Pharmacopoeia* standards.

Primaquine diphosphate 7.5 mg tablets were analyzed. Separation Rs greater than 2 was achieved between the major contaminant peak and the peak of the API in less than 15 minutes. The novelty of this work is the determination of the main contaminant in medical form primaquine diphosphate tablets.

Keywords: SFC-MS, Primaquine; Quinocide; Anti-malaria drug; Contaminations in primaquine tablets.

1. Introduction

Primaquine (CAS 90-34-6) is used as a treatment of malaria. It has recently been shown that unprocessed primaquine used in the pharmaceutical industry is contaminated with the

* This article originally published as a book chapter in *Historical Overview of Chromatography and Related Techniques in Analysis of Antimalarial Drug Primaquine*, Ilia Brondz. © 2011 Nova Science Publishers, Inc.

† Corresponding author: Department of Biology, University of Oslo, P.O. Box 1066, Blindern, 0316 Oslo, Norway, Phone: +47 22 85 73 53, Mobile: +47 93 81 48 64, E-mail: ilia.brondz@bio.uio.no

positional isomer quinocide (CAS 525-61-1) [1–9]. Currently, *Pharmacopoeias* [10] allow primaquine purity from 98.5% to 101.5%. The nature of contaminants in this drug does not require a description, despite the high level of contamination allowed. In 2003 [1], evidence for the nature of the contaminant in primaquine diphosphate was first publicly presented. Since presentation at international meetings in 2003–4 [1, 2] and a paper published in J. Chromatography B in 2004 [3], very little has been done to develop analytical procedures to verify the quinocide in primaquine for inclusion in the *Pharmacopoeias*. The United States Pharmacopeia (USP) seeks submission of a monograph proposal for improvement of analysis of primaquine phosphate tablets. Quinocide, a highly toxic substance, is a principal contaminant in the drug primaquine diphosphate. The toxicological properties of quinocide and the tolerances of animals to the drug, and the metabolism are presented in [11–14]. The acute toxic effects of combinations of quinocide with other antimalarial drugs are presented in [15]. The conclusion is that quinocide caused either additive or synergistic toxic effects; similar observations were seen using a unicellular model [3].

Primaquine and quinocide can have a number of side effects, such as inducing methemoglobinemia [16–20], effects on the red blood cell membrane [21], and hemolytic effects [22, 23]. The neurotoxicity of the 8-aminoquinolines [24, 25] is well known. Polyamines may be involved in the development of hepatic encephalopathy and cerebral edema [26]. Neuropsychiatric manifestations after therapy with the quinoline derivative *mefloquine* [27] and cases showing the carcinogenicity of primaquine have also been reported [28]. Cardiotoxicity in patients undergoing malaria therapy with aminoquinolines due to blockade of Na^+ channels is discussed in [29].

Malaria is one of the most widely spread and deadly diseases on the planet. Primaquine has high antiparasitic effects against gametocytes and thus the ability to prevent the spread of the parasite from patients by mosquitoes. It is also used in radical cures and prevents relapses. Consequently, primaquine is a frequently used drug.

The aim of this study was to achieve chromatographic resolution between the principal substance and the major contaminant demanded in the *Pharmacopoeia* [10] and to record the presence of the toxic contaminant quinocide in pharmaceutical products such as medical form tablets.

In 2003 [1, 3], evidence of the nature of the principal contaminant in primaquine diphosphate was presented by using HPLC-MS, in 2004 the same was done by using GC-MS [2, 4], the results were supported by SFC-MS [6, 9].

The use of GC-MS with supersonic molecular beam (SMB) was performed to study the possible fast analysis of contaminants in pharmaceutical products such as medical form tablets.

SMB was used for interfacing the GC to the MS [30-32] and as a medium for ionization of sample compounds while in the SMB by electron ionization [33-35]. The technology of GC-MS with SMB is reviewed in reference [36]. SMBs are characterized by intra-molecular vibrational supercooling of its seeded compounds due to relatively low collision energies of sample compounds and carrier gas species during the supersonic expansion. Extended range of thermally labile molecules are amenable for analysis due to the use of high column flow rate and contact free fly-through ion source [36, 37].

2. Materials and Methods

2.1. Sample Preparation SFC-MS

Primaquine diphosphate p.a. (Sigma-Aldrich, Steinheim, Germany), and standard quinocide dihydrochloride, were used [3]. The standard solutions were prepared in 10 mL of ethanol (Arcus, Oslo, Norway) containing 0.4% (v/v) of diethylamine (Merck, Darmstadt, Germany). Thirty primaquine diphosphate 7.5 mg tablets purchased from the Norwegian Medical Depot (NMD, Oslo, Norway) batch No. 2080222 were used. Nine groups of three tablets were pulverized in an agate mortar with an agate pestle. Each group of tablets was quantitatively transferred to a glass centrifuge tube and extracted with 3 mL of ethanol containing 0.4% (v/v) diethylamine.

Extraction was performed with vigorous shaking, followed by centrifugation for 15 min at 4 °C at 5000 rpm. The procedure was repeated three times and the extracts combined. An additional round of extractions from each group of three tablets was performed to collect extracts for a control series for measuring the levels of any remaining substances. The final volume of extracts from tablets was justified to10 mL by adding some μL of respective extract from control series.

2.2. Sample Preparation for GC-MS with SMB

Three 7.5 mg primaquine diphosphate tablets were pulverized in agate mortar 5 mg of the pulverized mass was used in analysis. Primaquine diphosphate and quinocide dihydrochloride were used as standards. Each sample was dissolved in 1 mL of purified water grade I and 0.1 mL of concentrated ammonium hydroxide p.a. grade (Merck, Darmstadt, Germany), was added. The base was extracted in 1 mL of p.a. quality hexane (Merck).

2.3. Supercritical Fluid Chromatography (SFC) and Supercritical Fluid Chromatography-mass Spectrometry (SFC-MS)

The following instruments were used: a MiniGram SFC (Berger Instruments Inc., Newark, DE, USA) running under ProNTo software (Berger Instruments Inc.) and equipped with a K-2501 UV detector (Advanced Scientific Instruments, Dr. Knauer, Berlin, Germany) set at a wavelength of 261 nm. A part of the flow stream was diverted with a 10/1 fixed splitter (UV/MS) to feed a Micromass PLCZ 4190 mass spectrometer equipped with a pneumatically assisted electrospray ionization source (ESI) running under MassLynx 4.0 (Waters-Micromass, Manchester, UK). The connecting line to the MS was through T-form tubing equipped with a manually operated microvalve and capillary restrictor. The MS conditions were: cone voltage 60 V, extractor voltage 12 V, capillary voltage 4.5 kV, ion energy 1.0 V, multiplier at 400 V, analyzer vacuum of 2.6 kPa, and a desolvation gas flow of 495 L x hr^{-1}. The mass-to-charge ratio was scanned automatically in the range from 80 to 400. The MS was operated in the positive charge mode. The instrument was previously

calibrated with sodium iodide. Isocratic chromatography was performed using CO_2 in a supercritical state with ethanol containing 0.4% diethylamine. At the end of the analysis, the manually operated microvalve was closed, thus closing the flow of effluent to the MS, and tubing directing the effluent to the waste through a capillary restrictor. The percentage of modifier in the effluent was elevated to 50% at a rate of 10% per min and held at 50% for 3 min, then lowered to the level required for analysis at a rate of 10% per min and held for 3 min to stabilize the column. The manually operated microvalve was then reopened to direct effluent to the MS for 3 min before analysis.

2.3.1. SFC-MS analysis of primaquine diphosphate 7.5 mg tablets

A Discovery HS F5 column 250 mm × 4.6 mm i.d. comprising 6 μm particles from Supelco (Supelco, Bellefonte, PA, USA) was converted to SFC. The column was converted as described previously [7]. Isocratic chromatography was performed using CO_2 in a supercritical state with 15% ethanol containing 0.4% diethylamine. The conditions for SFC-MS are described in section 2.2. Separation and the detection of analytes by UV are shown in the chromatogram in Figure 1 and the TIC in Figure 2. Three analyses were conducted for every extract.

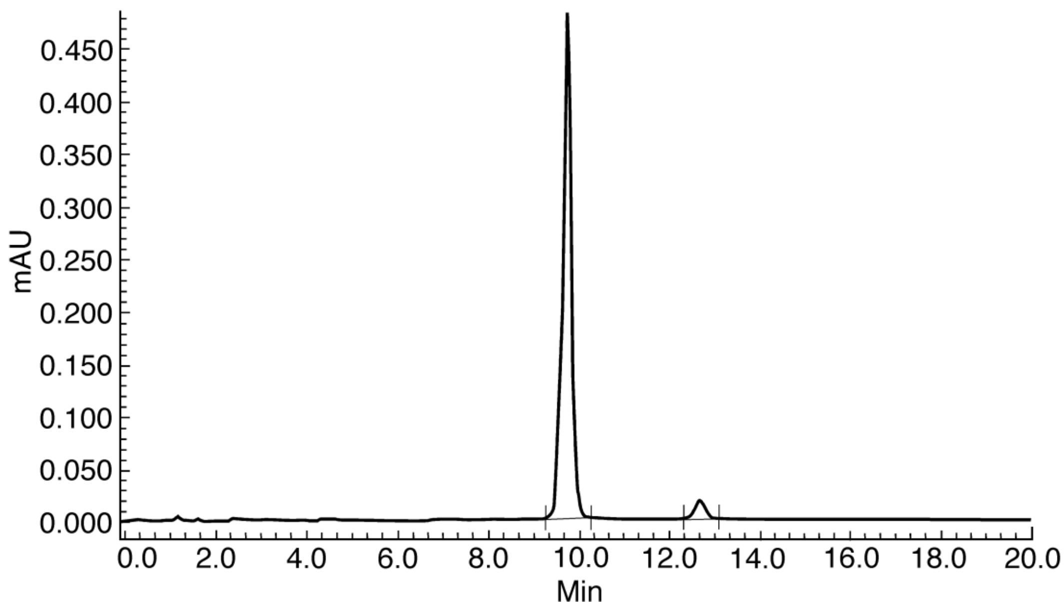


Figure 1. Chromatogram is showing the content of primaquine tablet resolved by SFC. There are two peaks on chromatogram, the primaquine is the major peak and the quinocide is the moderate peak. The details of the conditions described in the text.

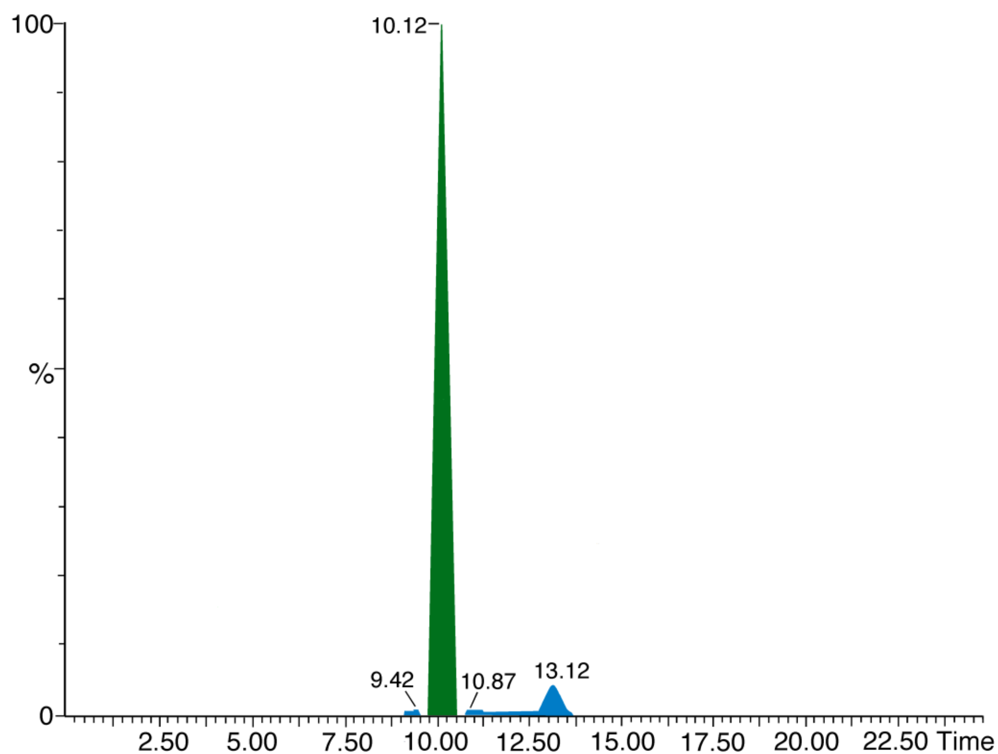


Figure 2. Total ion current (TIC) indicating content primaquine diphosphate tablets.
The analysis using UV detection shown in Fig 1 was monitored by MS. There are four peaks. The first is a minor peak with an R_t of 9.42 min is unidentified. The second peak is a major peak with an R_t of 10.12 min is primaquine. The third peak is minor one with an R_t of 10.87 min is unidentified. The fourth peak, with an R_t of 13.12 min, is quinocide.

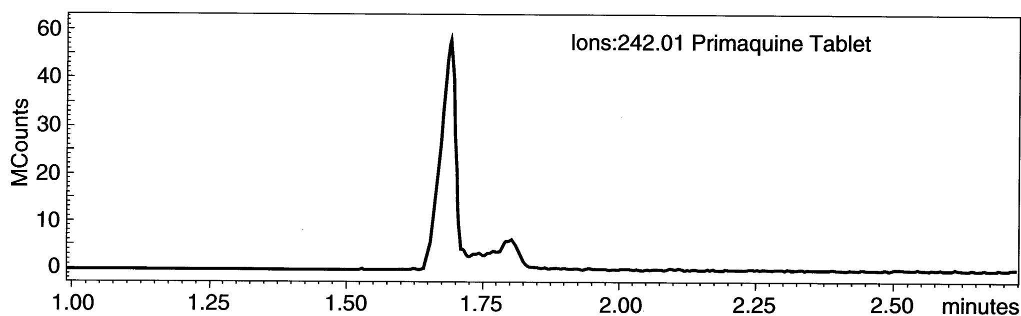


Figure 3. GC-MS with supersonic molecular beam (SMB) shows analysis of primaquine diphosphate 7.5 mg tablets. The tablets extract in hexane was analyzed at the conditions described in text above. The full scan (TIC) of the drug tablet in the mass spectral range 50-400 amu, shows the RSIM on *m/z* = 242. The major peak is the primaquine and the minor peak is the quinocide.

2.3.2. Gas chromatography-mass spectrometry with supersonic molecular beam

The experimental GC-MS with supersonic molecular beam based on a Varian 1200 GC-MS system is described in details in reference [32]. Separation of compounds was done with a VF-5HT column, 0.25 mm I.D., 0.1 μ film thickness and 4 m length (Varian, Middleburg, The Netherlands). The reduction of column length was done in the laboratory. The helium column flow rate was 8 ml/min. 1 μL sample extract in hexane at an approximate concentration of 200 ppm was injected with split ratio of 10:1 using the Varian 1079 injector which was at 250ºC. The GC oven was programmed from 120ºC to 300ºC at 30ºC/min and the drug samples eluted at about 165ºC without any degradation due to the use of short column and high column flow rate [37]. Ion source degradation was prevented in view of the use of contact free fly-through EI ion source [36]. Separation and the detection of primaquine diphosphate 7.5 mg tablets by GC-MS with SMB is described in [38] and shown as a chromatogram in Figure 3.

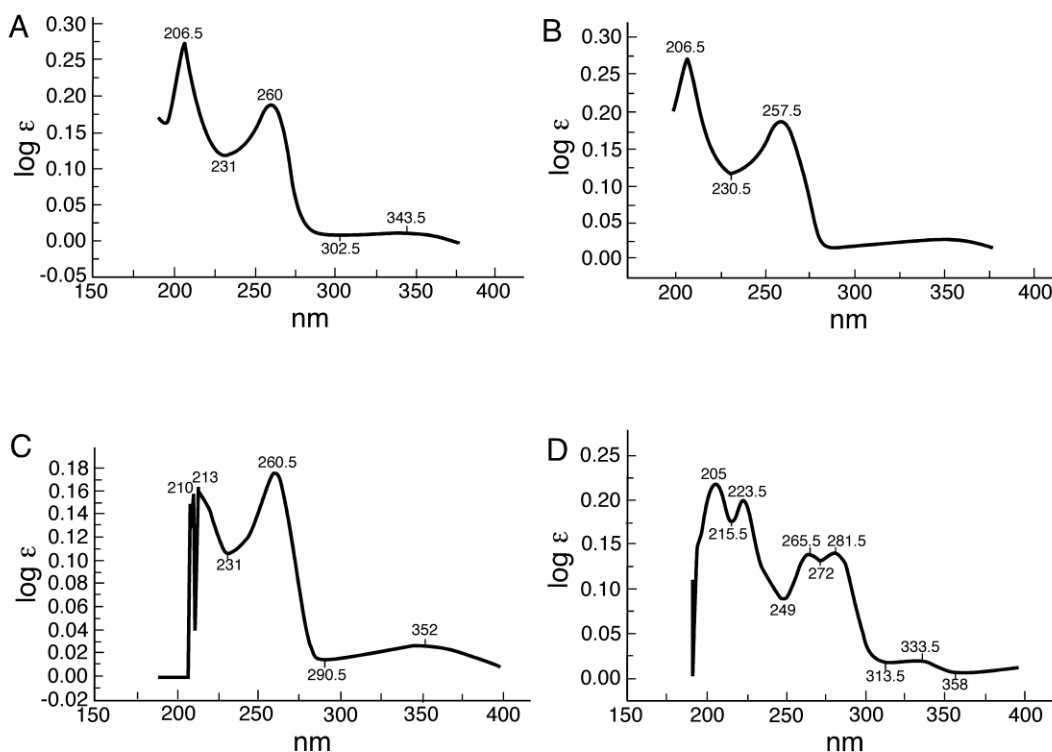


Figure 4. The UV spectra of primaquine batch 16039 [3] analyzed by using a Jasco J-810 spectrometer. The details described in the text.

A. Primaquine in deionized water.

B. Primaquine in a 0.01 N NH3OH.

C. Primaquine in a 0.01 N NaOH.

D. Primaquine in a 0.01 N HCl.

2.3.3. UV spectrometry

The UV spectra of primaquine and quinocide in p.a. quality hydrochloric acid (Merck) diluted to 0.01 N with deionized water, in p.a. quality ammonium hydroxide (Merck) diluted to 0.01 N with deionized water, in p.a. quality sodium hydroxide (Merck) diluted to 0.01 N with deionized water, and in deionized water were recorded using a Jasco J-810 spectrometer (Jasco International, Tokyo, Japan). Measurements were performed at 23 °C using a quartz cell with a path length of 1.0 cm. All measurements were performed at 0.04 mg mL^{-1} with respect to the primaquine or quinocide base. Samples were scanned five times at 50 nm min^{-1} with a bandwidth of 1 nm and a response time of 1 s over the wavelength range 190–400 nm. The data were averaged and the spectrum of a respective sample-free control sample (0.01 N HCl, 0.01 N NH_3OH, 0.01 N NaOH, and deionized water) was subtracted respectively. The resultant spectra were then smoothed using the means-movement method. All measurements were conducted twice. The UV spectra of primaquine in deionized water, NH_3OH, NaOH, and HCl are shown in Figure 4 A, B, C, and D, respectively, and the UV spectra of quinocide in deionized water, NH_3OH, NaOH and HCl are shown in Figure 5 A, B, C, and D, respectively.

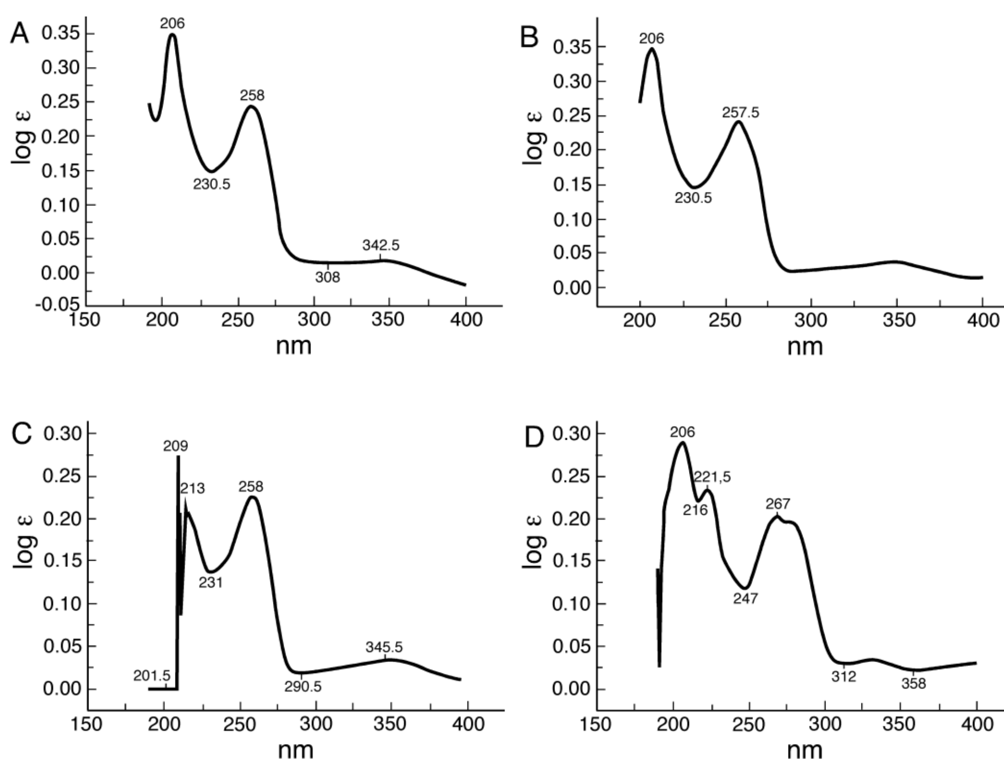


Figure 5. The UV spectra of quinocide batch 17172 [3] analyzed by using a Jasco J-810 spectrometer. The details described in the text.
A. Quinocide in deionized water.
B. Quinocide in a 0.01 N NH3OH.
C. Quinocide in a 0.01 N NaOH.
D. Quinocide in a 0.01 N HCl.

2.3.4. ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy

The ^{1}H, ^{13}C, and ^{15}N NMR spectra of primaquine dichloride and quinocide dichloride in DMSO-d6 were recorded using a Bruker Avance 500 MHz instrument (Bruker BioSpin Gmb, Rheinstetten, Germany). The ^{1}H, ^{13}C, and ^{15}N chemical shift values are presented on a δ scale in ppm relative to DMSO-d6. The ^{1}H, ^{13}C, and ^{15}N chemical shift values were recorded on the δ scale in ppm relative to DMSO-d6 for ^{1}H and ^{13}C ref. 2.49 ppm and 39.5 ppm and for ^{15}N CH_2NO_2 in DMSO-d6 9:1 ref. 0.0 ppm. NMR ^{1}H spectra of primaquine dichloride and quinocide dichloride are shown in Figure 6 A and B and in Figure 7 and 8 shown chemical shift values including ^{15}N. NMR ^{13}C spectra of primaquine dichloride and quinocide dichloride are shown in Figure 9 A and B and in Figure 7 and 8 shown chemical shift values.

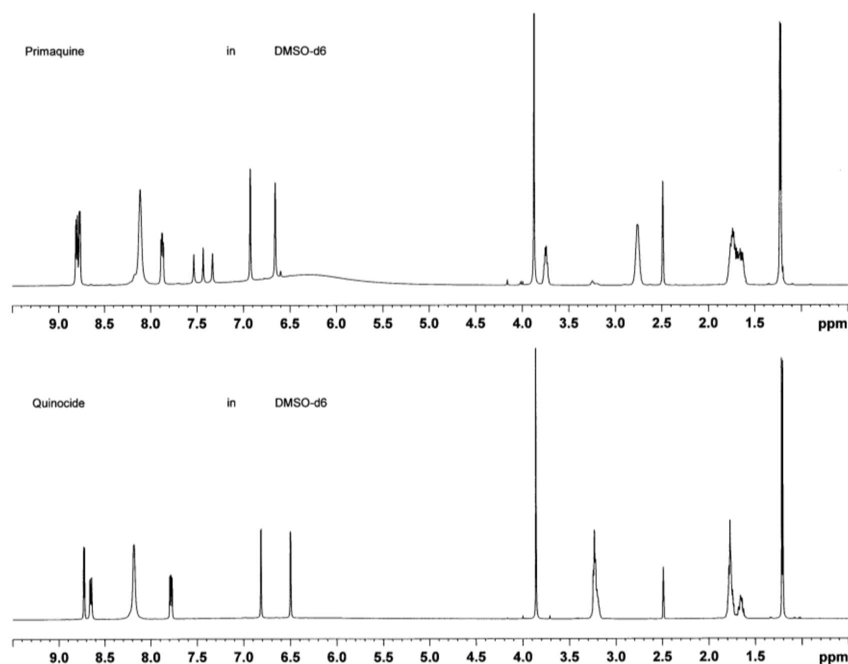
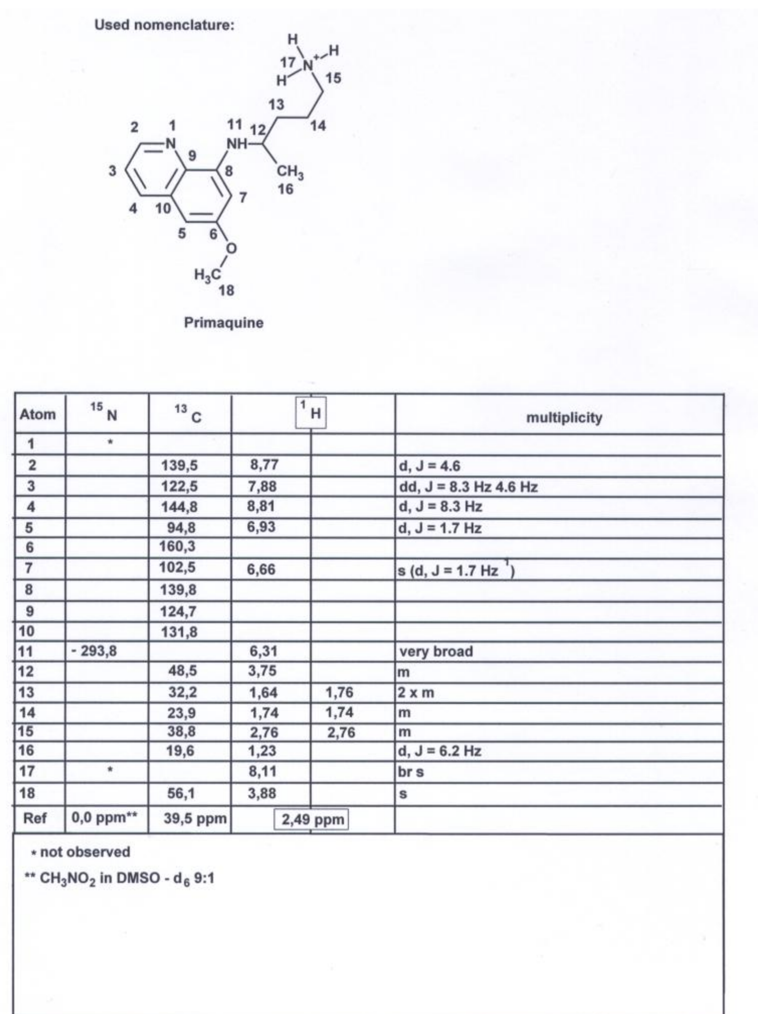


Figure 6. ^{1}H NMR spectra of primaquine chloride and quinocide chloride analysed in DMSO-d6.
A. The upper trace shows ^{1}H NMR spectra of primaquine chloride.
B. The bottom trace shows the ^{1}H NMR spectra of quinocide chloride.

3. Results and Discussion

3.1. The Nature of the Contaminant in Primaquine Diphosphate Tablets

Currently, the *Pharmacopoeia* [10] allows primaquine purity from 98.5% to 101.5%; however, the USP 31 [39] allows primaquine purity from 98.0% to 102.0% and primaquine phosphate tablets contain not less than 98.0 percent and not more than 107.0 percent of the labeled amount of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$.



Atom	^{15}N	^{13}C	^{1}H		multiplicity
1	*				
2		139,5	8,77		d, J = 4.6
3		122,5	7,88		dd, J = 8.3 Hz 4.6 Hz
4		144,8	8,81		d, J = 8.3 Hz
5		94,8	6,93		d, J = 1.7 Hz
6		160,3			
7		102,5	6,66		s (d, J = 1.7 Hz ^{1})
8		139,8			
9		124,7			
10		131,8			
11	- 293,8		6,31		very broad
12		48,5	3,75		m
13		32,2	1,64	1,76	2 x m
14		23,9	1,74	1,74	m
15		38,8	2,76	2,76	m
16		19,6	1,23		d, J = 6.2 Hz
17	*		8,11		br s
18		56,1	3,88		s
Ref	0,0 ppm**	39,5 ppm	2,49 ppm		

* not observed

** CH_3NO_2 in DMSO - d_6 9:1

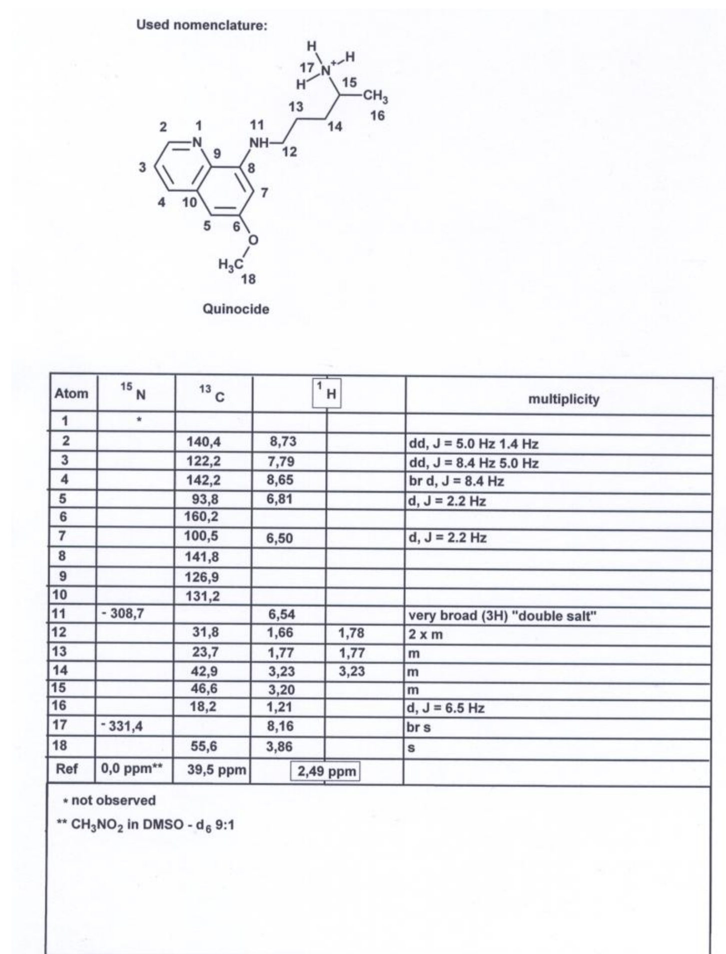
Figure 7. The molecule of primaquine with used nomenclature for C and N atoms is shown together with the table of chemical shift values including ^{15}N, ^{13}C and ^{1}H. The details described in the text.

The USP 31 [39] seeks submission of a monograph proposal for improvement of analysis for primaquine phosphate tablets "In an effort to improve drug product, drug substance, and excipient monographs to current scientific/regulatory standards, USP is seeking submission of proposals for improved methods for inclusion in the following USP-NF monographs to replace the current procedures that may be deficient, flawed, or unsafe".

The nature of the contaminant in unprocessed primaquine diphosphate was first publicly presented in 2003 [1] and later described in [2–9].

In this study, the toxic contaminant in tablets was characterized based on previous knowledge of MS spectra and by using authentic quality standard quinocide [1-9]. The new data gathered regarding the two minor contaminants of unknown nature using SFC-MS and GC-MS with SMB [38].

The novelty of this work is the determination of the main contaminant in the medical form of primaquine tablets.



Atom	^{15}N	^{13}C	^{1}H		multiplicity
1	*				
2		140,4	8,73		dd, J = 5.0 Hz 1.4 Hz
3		122,2	7,79		dd, J = 8.4 Hz 5.0 Hz
4		142,2	8,65		br d, J = 8.4 Hz
5		93,8	6,81		d, J = 2.2 Hz
6		160,2			
7		100,5	6,50		d, J = 2.2 Hz
8		141,8			
9		126,9			
10		131,2			
11	- 308,7		6,54		very broad (3H) "double salt"
12		31,8	1,66	1,78	2 x m
13		23,7	1,77	1,77	m
14		42,9	3,23	3,23	m
15		46,6	3,20		m
16		18,2	1,21		d, J = 6.5 Hz
17	- 331,4		8,16		br s
18		55,6	3,86		s
Ref	0,0 ppm**	39,5 ppm	2,49 ppm		

* not observed

** CH_3NO_2 in DMSO - d_6 9:1

Figure 8. The molecule of quinocide with used nomenclature for C and N atoms is shown together with the table of chemical shift values including ^{15}N, ^{13}C and ^{1}H. The details described in the text.

3.2. UV spectrometry

The UV spectra of primaquine and quinocide are nearly identical [3, 9]. There is a tendency for a *hypsochromic shift* in λ max in the spectrum of quinocide in comparison with that of primaquine. In water, primaquine has λ max values of 206.5, 260, and 343.5 nm and λ min values of 231 and 302.5 nm, and quinocide has λ max values of 206, 258, and 342.5 nm and λ min values of 230.5 and 308 nm. In solutions of ammonium or sodium hydroxide, the spectra exhibit nearly the same patterns of λ max and λ min values. The maxima and minima recorded in alkaline solutions are near to the values seen in water solutions. In a solution of 0.01 N NaOH, the absorption by NaOH interferes with the measurement of the peak at 205 nm. In 0.01 N HCl, both substances undergo significant changes in their spectra pattern: primaquine has λ max values of 205, 223.5, 265.5, 281.5, and 333.5 nm and λ min values of

215.5, 249, 272, and 313.5 nm; quinocide has λ max values of 205, 221.5, 267, 278, and 332.5 nm and λ min values of 216, 247, 270, and 312 nm.

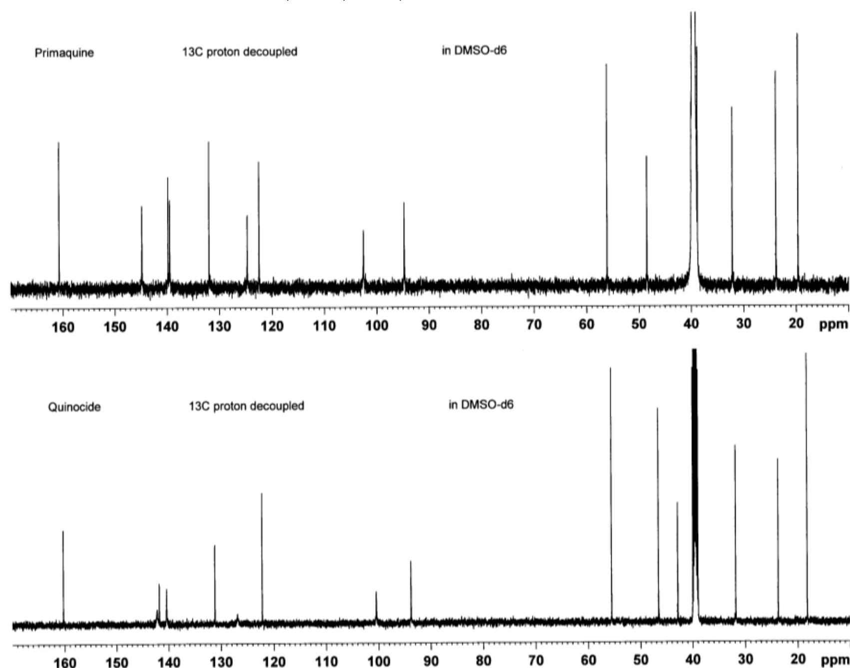


Figure 9. ^{13}C NMR spectra of primaquine chloride and quinocide chloride analysed in DMSO-d6.
A. The upper trace shows ^{13}C NMR spectra of primaquine chloride.
B. The bottom trace shows the ^{13}C NMR spectra of quinocide chloride.

From the spectra obtained (Figures 4 and 5), it is evident that optimal detection of primaquine in an alkaline environment should be at 261 nm, as described in the *Pharmacopoeia* [10]; however, our measurements of quinocide found a corresponding maxima at 258 nm in water and in NH_3OH and NaOH solutions. In USP 31 and NF 26 [39], detection at 254 nm is recommended. The maxima in the spectra of primaquine and quinocide depend on the pH of the solvent and are slightly different from each other.

3.3. ^{1}H, ^{13}C, and ^{15}N NMR Spectroscopy

In the ^{1}H spectra of primaquine, chemical shift at 3.75 ppm versus quinocide chemical shift at 1.66 and 1.78 ppm from C in position 12 as of primaquine chemical shift at 2.76 ppm versus quinocide chemical shift at 3.20 ppm from C in position 15 may be used for differentiation between isomers (Figures 6-9).

In the ^{13}C spectrum of primaquine, chemical shifts at 144.24, 95.83, 39.85, and 24.22 ppm are characteristic for this isomer. In the ^{13}C spectrum of quinocide, chemical shifts at 140.4, 142.2, and 100.5 ppm are characteristic for this isomer (Figures 6-9).

3.4. Extraction

The quality of extraction was judged by the absence of active API in the control extracts.

3.5. Detection of Minor Impurities by MS

Two additional minor peaks were detected using MS (Figure 2). SFC-MS analysis of the primaquine tablets revealed that besides quinocide, two other contaminating agents of an unknown nature are present. In the *Pharmacopeias* [10, 39], the nature of all contaminating agents are not given and are described as “related substances”.

The first minor contaminant elutes before primaquine (principal peak) and the second minor contaminant elutes between the primaquine and quinocide peaks.

It was difficult to record quality MS spectra that could be used to characterize these minor “related substances”. The reason is the low concentration of the minor contaminants. However, it is very important to note that in both of the spectra of the newly detected “related substances” contaminants; a fragment of m/z 175 is present, which may indicate that the contaminants are substituted quinolines. In GC-MS with SMB analysis the characteristic for primaquine isomers ions with m/z = 175, m/z = 187 and m/z = 242 also were detected [38]. The nature of these “related substances” or minor contaminants is not verified; however, in [40] the possibility of isomerization in the aromatic ring under attachment of substituent was described. This isomerization was not attributed to primaquine; however, it was attributed to compounds with very similar structure and under similar conditions of synthesis.

It was not possible to record peaks of these substances using UV detection (Figure 1), which may be because the peaks are irregular with very long tailing or because the concentrations and molar extinction coefficients of the analytes are low and thus below the level of detection. The characterization of these contaminants is in progress. The principal routs of fragmentation for primaquine and quinocide shown in Figure 10 and 11.

3.6. Regulation

Quinocide, a main contaminant in the drug primaquine diphosphate, is a highly toxic substance. The analysis of contaminants in drugs is an important and challenging subject for analysts in the pharmaceutical industry and health administration. The requirements for the validation of qualitative and quantitative parameters in new pharmaceutical products of impurities at the 0.05% level with respect to the main compound were discussed during the International Conference on Harmonization and the current Good Manufacturing Practice [41, 42]. In the Drug Quality and Information program (DQI) document published by USP [43], it was noted that in 2003 and 2004, 21% and 13%, respectively, of antimalarial drugs used were of poor quality. This is evidence that there are unprocessed primaquine and primaquine tablets with high concentrations of the toxic contaminant quinocide as “related substances“on the drug market.

3.7. Validation of Procedures

The method was validated in accordance with the terms of precision, accuracy, and linearity described in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines [44].

3.8. Selectivity

The selectivity of the method is important for the differentiation and identification of the existing target analytes in primaquine diphosphate tablets. Analytes were identified by matching peak retention times (R_t) with the values of the corresponding standards in solutions analyzed under the same experimental conditions. For confirmation purposes, the specific fragmentation patterns of individual analytes were used to distinguish primaquine from quinocide. In addition, comparisons of MS spectra of peaks eluted with the spectra of authentic standards and comparisons with previously published spectra were conducted [1–9].

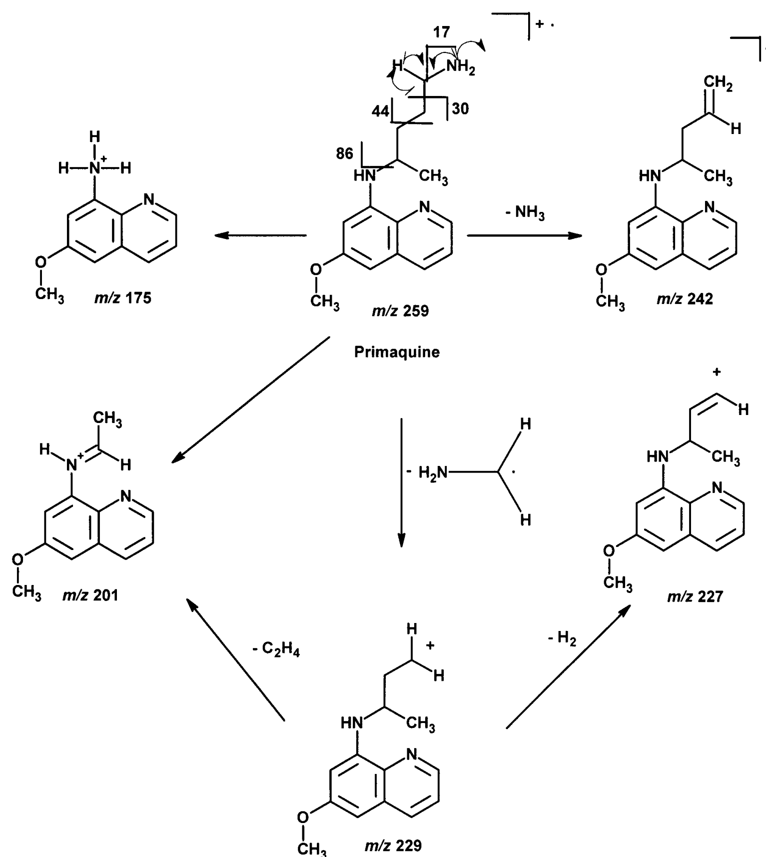


Figure 10. The main routes of fragmentation for primaquine, as suggested by electron ionization mass spectrometry [4].

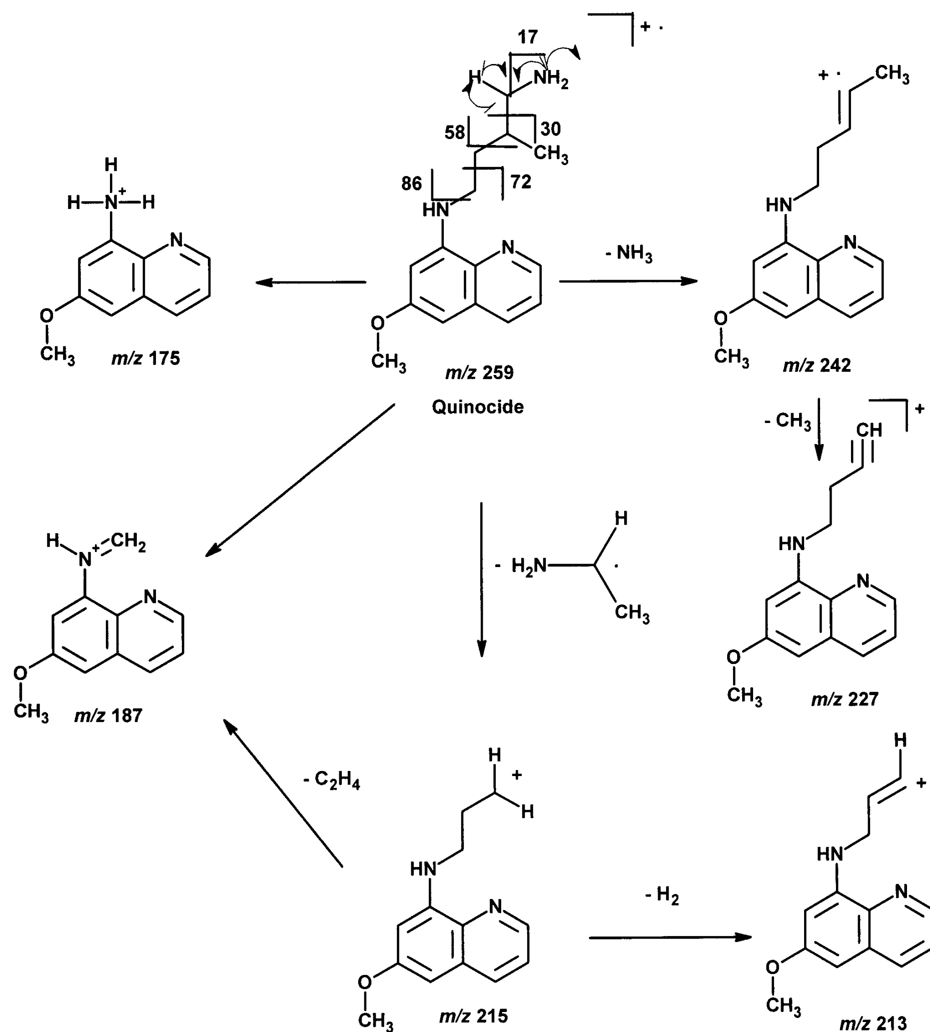


Figure 11. The main routes of fragmentation for quinocide, as suggested by electron ionization mass spectrometry [4].

3.9. Accuracy

The term *accuracy* denotes the nearness of measurement to its accepted value and is expressed in terms of *error*. Accuracy involves a comparison with respect to a true or accepted value.

The accuracy of the assay method was evaluated by carrying out analysis of the dilutions of primaquine and quinocide of known concentrations. The percentage composition of components was chosen with respect to data published in [1–9]. The individual compositions were analyzed nine times. The RSD of the experiments was within the limits of 2% for primaquine and 3% for quinocide for expected molar concentrations.

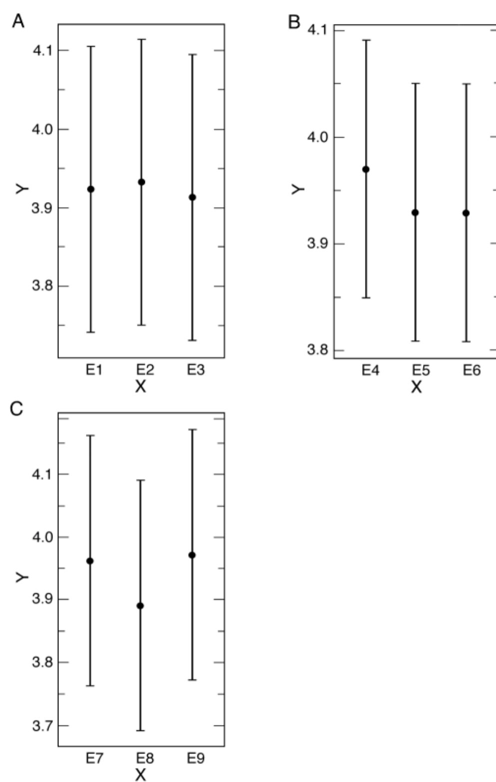


Figure 12. There are plots of means with 95% Confidence Intervals. Results of gropes analyzed in the same day.
A. Analyses are performed in day one.
B. Analyses are performed in day two.
C. Analyses are performed in day three.

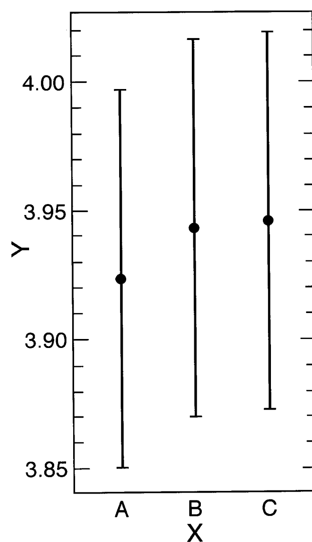


Figure 13. There is plot for day-to-day reproducibility with 95% Confidence Intervals. Groups analyzed in three different A, B and C days.

3.10. Precision

Both intraday and day-to-day precision studies presented very good results. The results are summarized in Figure 12 A, B, and C, and Figure 13. Day-to-day parameters are summarized in Figure 13, and the *p* value for these results, assuming the null hypothesis, is 0.889.

3.11. Method Quantification Limit (MQL) and Method Detectable Limit (MDL) of UV-Based Detection

The MDL for UV-based detection was established by using primaquine batch 16039 and quinocide batch 17172 [3].

The MDL was determined by analyzing analytes at levels that provided a signal at five times above background noise. In a similar way, MQL values were determined at signal-to-noise ratios of 10. The MDL for primaquine was 2.0×10^{-6} mM × L^{-1}, and 2.6×10^{-6} mM × L^{-1} for quinocide. The MQLs were 4.1×10^{-6} mM × L^{-1} for primaquine, and 5.5×10^{-6} mM × L^{-1} for quinocide. The method based on UV detection is adequately sensitive for monitoring the quality of unprocessed material and tablets.

3.12. Linearity and Calibration Curves

The external standard calibration method was employed on a series of individual standard solutions. The linearity of the UV detection was studied over a broad range of concentrations. Six-step dilutions of a linearity dilution series were used.

Dilutions were conducted on a molar basis because primaquine diphosphate and quinocide dichloride standards were used. Each point was determined nine times. The average values were used to construct calibration curves by plotting the corresponding peak areas versus the molar concentration of the analytes.

The linear regression parameters for primaquine were: slope = 7236, *y*-intercept = 13021, and $R^2 = 0.9998$; the linear regression parameters for quinocide were: slope = 11059, *y*-intercept = 14228, and $R^2 = 0.9985$. The linear regression parameters of both substances were better than 0.9900.

3.13. Statistics

Statistical analyses of results were conducted in accordance with [45]. The standard deviations for every series and the standard deviations between the series are presented. Three series were analyzed every day for three consecutive days. The variation between the different groups on day one (E1–E3), day two (E4–E6), and day three (E7–E9) are plotted in Figure 12 A, B, and C. The variation between the different days of analysis is presented in Figure 13. The results are presented as 95% confidence intervals.

4. Marketing and Publishing Ethics

4.1. Marketing

Quinocide, a main contaminant in the drug primaquine diphosphate [1-9, 38], is a highly toxic substance. Its use discouraged, even in its country of origin. However, the results of this study show that primaquine contaminated with quinocide and "related substances" is in use, this is in agreement with [43].

4.2. Publishing Ethics

Dongre et al. produced a publication [46] that contained no MS data or co-chromatography with authentic standards. The aim of publication [46] appears to be to demonstrate that primaquine was separated from quinocide. In the absence of quality standard quinocide, the authors presented questionable evidence of degradation and photo degradation to avoid questions about co-chromatography and spectral identity.

The authors of [46] stated "However, there are no reports available on stability indicating analytical method for primaquine phosphate API." How the authors of [46] could have dared to make this claim when there are numerous publications on the subject is beyond belief. Relevant publications are: S. Kristensen et al., Photochemical stability of biologically active compounds: V. Photochemical degradation of primaquine in an aqueous medium [47]; A. Brossi et al., Photooxidation products of primaquine, Structure, antimalarial activity and hemolytic effects [48]; and S. Kristensen et al. Photoreactivity of biologically active compounds, XIV: Influence of oxygen on light induced reactions of primaquine [49]. Moreover, S. Kristensen et al., [47] specifically stated that "Eight major and several minor decomposition products can be detected, many of which are oxygenated derivatives." How it was possible and why they claimed that there are no reports on the stability of primaquine?

Dongre et al. published [50]. The main and only reason presented by Dongre et al. to justify their investigation was that *"The first report published on this product by Elderfield* et al. [40] *suggested that one of the major impurities could possibly be its enantiomer. This reasoning appeared very unlikely and prompted us to investigate in details the structural elucidation of this impurity*." The cited publication makes no mention of the possibility of enantiomers of primaquine. Elderfield et al. never mentioned that primaquine is a racemate or a mixture of isomers. The truth is that Elderfield et al. [40] stated on p. 1525, lines 13–20: *"The drug prepared in experiment 32 was shown to be reasonably homogeneous by the 8-plate technique of Crag. However, when the 24-plate technique was applied, the substance appeared to consist of two components in approximately equal amounts. We interpret this to indicate a possible separation of the two racemates."*

What is the substance in experiment 32? On page 1526 of the same paper [40] is a list of experiments and products. Substance 32 is methoxi-8-aminoquinoline, with the substituteR_8-$NHCH(CH_3)(CH_2)_2CH(CH_3)N(C_2H_5)_2$. This is not the primaquine. The primaquine is described in experiment 24.

How could something that was not mentioned by Elderfield et al. [40] prompt Dongre et al. to investigate it?

5. Conclusion

A valid and easily performed *Pharmacopoeia*-based procedure is urgently needed because of the hundreds of millions of people who ingest primaquine as a drug containing significant amounts of contaminants [43]. The presented procedure for the qualitative analysis of the contaminant quinocide in primaquine tablets can be used as the basis for development of a USP monograph to analyze quinocide in primaquine tablets.

Acknowlegments

The author is grateful to Jon Reierstad, Technical Department and to Dirk Petersen Department of Chemistry, University of Oslo, Norway for technical assistance, to Professor Aviv Amirav, School of Chemistry, Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, Israel, for cooperation with GC-MS SMB analysis. This research was financially supported by Jupiter AS, Ski, Norway, Sigma-Aldrich/Supelco Analytical, Bellefonte, PA, USA and Mettler-Toledo Berger SFC, Newark, DE, USA.

References

[1] Brondz, I; Mantzilas, D; Klein, U; Lebedeva, MN; Mikhailitsyn, FS; Souleimanov, GD; Ekeberg, D; 3rd Int. Symposium on Separation in BioSciences SBS 2003, A 100 Years of Chromatography, 13-18 May, Moscow, Russia, *Abstr.*, 2003, P-57, 165.

[2] Brondz, I; Klein, U; Ekeberg, D; Mantzilas, D; Hvattum, E; Schultz, H; Mikhailitsyn, FS. International Symposium Analytical Forum 2004, 4-8 July, Warsaw, Poland, *Abstr.*, 2004, P-119.

[3] Brondz, I; Mantzilas, D; Klein, U; Ekeberg, D; Hvattum, E; Lebedeva, MN; Mikhailitsyn, FS; Souleimanov, GD; Røe, J. *J. Chromatogr.*, 2004, B, 800, 211-223.

[4] Brondz, I; Klein, U; Ekeberg, D; Mantzilas, D; Hvattum, E; Schultz, H; Mikhailitsyn, FS. *Asian Journal of Chemistry*, 2005, 17(3), 1678-1688.

[5] Brondz, I; Klein, U. *TheReporter*, 2005, 23(4), 1-2.

[6] Brondz, I; Klein, U; Karaliova, L; Vlachos, V; Okley, P; Leideborg, R; Mikhalitsyn, FS. 29th International Symposium on High Performance Liquid Phase Separations and Related Techniques, June 26-30, 2005, Stockholm, Sweden, (2005) Abstr. P12:43.

[7] Brondz, I; Ekeberg, D; Karaliova, L; Jennings, I; Hustad, JA; Svendsen, R. *Trends in Chromatography*, 2005, 1, 77-81.

[8] Brondz, I; Klein, U. *TheReporter*, 2006, 19, 3 - 4.

[9] Brondz, I; Ekeberg, D; Bell, DS; Annino, AR; Hustad, JA; Svendsen, R; Vlachos, V; Oakley, P; Langley, GJ; Mohini, T; Amaury, CG; Mikhalitsyn, FS. *J. Pharmaceutical and Biomedical Analysis*, 2007, 43, 937-944.

[10] *European Pharmacopoeia* (2008) six ed., vol. 2, Council of Europe, Strasbourg, Germany, 2751-2752.

[11] Gladkikh, VF; Korogodina, IuV. *Meditsinskaia Parazitologiia I Parazitarnye Bolezni*, 1960, 29, 440-447.
[12] Gladkikh, VF; Kekllina, OI. *Meditsinskaia Parazitologiia I Parazitarnye Bolezni*, 1956, 25(4), 323-327.
[13] Soprunova, NYu. Meditsinskaia Parazitologiia I Parazitarnye Bolezni, 1962, 31:(6), 656-660.
[14] Soprunova, NYu. Meditsinskaia Parazitologiia I Parazitarnye Bolezni, 1963, 32:(4), 443-447.
[15] Lebedeva, MN; Brusilovskaya, VM. *Meditsinskaia Parazitologiia I Parazitarnye Bolezni*, 1980, 49:(6), 27-31.
[16] Sin, DD; Shafran, SD. *J. Acquir. Immune Defenc. Syndr. Hum. Retroviral*. 1996, 12, 477-481.
[17] Coleman, MD; Coleman, NA. *Drug Saf.*, 1996, 14, 394-405.
[18] Marrs, TC; Bright, JE; Morris, BC. *Toxicol. Lett.*, 1987, 36, 281-287.
[19] Kantor, GS. *N. Engl. J. Med.*, 1992, 327, 1462.
[20] Allahyari, R; Srtother, A; Tilton, BE; Fraser, IM. *Abstr. Am. Chem. Soc.*, 1986, 192, 86.
[21] Wed, R. Journal of Clinical Investigation, 1961, 40, 140-143.
[22] Kellermeyer, RW; Carson, PE; Schrier, SL; Tarlov, AR; Alving, AS. *Journal of Laboratory and Clinical Medicine*, 1961, 58, 715-24.
[23] Dern, RJ; Beutler, E; Alving, AS. *Journal of Laboratory and Clinical Medicine*, 1981, 97, 750-759.
[24] Schmidt, IG; Schmidt, LH. *J. Neuropathy Exp. Neurol.*, 1948, 7, 368-398.
[25] Schmidt, IG; Schmidt, LH. *J. Neuropathy Exp. Neurol*., 1948, 8, 337-367.
[26] Bullimore, D. *Eur. J. Gastroenterol. Hepatol*., 1993, 5, 63- 67.
[27] Rønn, AM; Rønne-Rasmussen, J; Gøtzsche, PC; Bygbjerg, IC. *Trop. Med. Int. Health*, 1998, 3, 83-88.
[28] El-Mofty, MM; Khudoley, VV; Skar, SA; Abdelgawad, HS. *Nutr. Cancer*, 1992, 18, 191-198.
[29] Orta-Salazar, G; Bouchard, RA; Morales-Salgado, F; Salinas-Stefanon, EM. *British Journal of Pharmacology*, 2002, 135:(3), 751-763.
[30] Dagan, S; Amirav, A. *Int. J. Mass Spectrom. Ion. Proc.*, 1994, 133, 187.
[31] Amirav, A; Gordin, A; Tzanani, N. *Rapid. Com. Mass Spectrom.*, 2001, 15, 811.
[32] Fialkov, AB; Steiner, U; Jones, L; Amirav, A. *Int. J. Mass. Spectrom.*, 2006, 251, 47.
[33] Amirav, A; Danon, A. *Int. J. Mass Spectrom. Ion. Proc*., 1990, 97, 107.
[34] Amirav, A. *Org. Mass Spectrom.*, 1991, 26, 1.
[35] Dagan, S; Amirav, A. *J. Am. Soc. Mass Spectrom*., 1995, 6, 120.
[36] Amirav, A; Gordin, A; Poliak, M; Fialkov, AB. *J. Mass Spectrom*., 2008, 43, 141.
[37] Fialkov, AB; Gordin, A; Amirav, A. *J. Chromatogr. A*., 2003, 991, 217.
[38] Brondz, I; Fialkov, AB; Amirav, A. *J. Chromatogr. A*., 2008, submitted.
[39] The United Stats Pharmacopeia, Thirty-First Revision and The National Formulary, Twenty-Sixth ed., The United Stats Pharmacopeia Convention, 2008 Volume 3, Rockville, MD.

[40] Elderfield, RC; Gensler, WJ; Head, JD; Hageman, HA; Kremer, CB; Wright, JB; Holley, AD; Williamson, B; Galbreath, J; Wielderhold III, L; Flohardt, R; Kupchan, SM; Williamson, TA; Birstein, O. *J. Am. Chem. Soc*., 1946, 68, 1524.
[41] Impurities in new drug substances; ICH-Q3A(R); International Conference on Harmonization: Geneva, Switzerland, October 2006.
[42] Impurities in new drug products; ICH-Q3B (R2); International Conference on Harmonization: Geneva, Switzerland, June 2006.
[43] United States Pharmacopoeia, Drug Quality and Information Program, Mekong Malaria Initiative. Antimalarial Drug Quality Monitoring and Evaluation (2004) U.S. Agency for International Development, Washington, 1-25.
[44] ICH topic Q2(R1), Validation of analytical procedures: text and methodology, (2005) version 4.
[45] De Muth, JE. Basic Statistics and Pharmaceutical Statistical Applications, 2006, second ed., CRC Press.
[46] Dongre, VG; Karmuse, PP; Rao, PP; Kumar, A. *J. Pharm. Biomed. Analysis*, 2008, 46:(4), 236-242.
[47] Kristensen, S; Grislingaas, AL; Greenhill, JV; Skjeten, T; Karlsen, J; Tønnesen, HH. *International Journal of Pharmaceutics*, 1993, 100, 15-23.
[48] Brossi, A; Gessner, W; Hufford, CD; Homo, JK; Millet, F; Landau, PI. *FEBS Lett.*, 1987, 223, 77-81.
[49] Kristensen, S; Nord, K; Orsteen, AL; Tønnesen, HH. *Pharmazie*, 1998, 53:(2), 98-103.
[50] Dongre, VG; Karmuse, PP; Nimbalkar, MM; Singh, D; Kumar, A. *J. Pharm. Biomed. Analysis*, 2005, 39, 111-116.