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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 111–116
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Application of GC–EI-MS for the identification and investigation of positional isomer in primaquine, an antimalarial drug

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Received 24 December 2004; received in revised form 23 March 2005; accepted 24 March 2005
Available online 17 May 2005

Abstract

A major impurity associated with primaquine drug samples obtained from European Pharmacopoeia (EP) and other commercial sources was detected and identified using HPLC, photo diode array (PDA), LC–MS/MS and gas chromatograph–electron impact-mass spectrometer (GC–EI-MS). PDA and LC–ESI-MS/MS data provided an evidence for it being isomeric in nature. However, spectral data obtained from the newly developed GC–EI-MS method has been utilised for structural elucidation and found to be conclusive to characterize this impurity as positional isomer, i.e. 8-(4-amino-4-methylbutyl amino)-6 methoxyquinoline. The structure of this impurity has been confirmed by its synthesis. Precursor of primaquine was also investigated using GC–EI-MS. The data obtained confirmed the origin of isomeric impurity in precursor.

Keywords: GC–EI-MS; Positional isomer; Precursor

1. Introduction

Primaquine phosphate, 8-(4-amino-1-methylbutylamino) -6-methoxyquinoline diphospate (CAS No. 63-45-6) is used to produce radical cure and prevent relapse of vivax and oval malarias following treatment with blood schizontocide [1]. Radical cure of vivax or oval infection must involve not only removal of erythrocytic forms of parasite but the remaining liver forms as well. Agents that act on liver forms are termed as tissue shizontocides and primaquine is the drug of choice to affect such radical cure [2].

The identification and characterization of impurities and evaluation of their toxicity effect is necessary step in developing a safe and effective drug. Similarly, investigation of impurity profile of an active pharmaceutical ingredient is also of crucial importance for medical safety reasons. This is the reason that the various pharmacopoeias give detailed methods of impurity profile studies for the pharmaceutical products. In the context of primaquine, European Pharmacopoeia (EP) [3] also describes the HPLC method for the analysis of related substances along with the product. The chemical reference standard (CRS) obtained from EP, showed major impurity peak (∼6%), which eluted just before the principle peak. In the fifth edition of European Pharmacopoeia and British Pharmacopoeia [4], the related substances in primaquine are allowed to be present at a maximum of 3%. The commercial product also contains a single impurity up to the level of 3%. The first report published on this product by Elderfield et al. [5] suggested that one of the major impurities could possibly be its enantiomer. This reasoning appeared very unlikely and prompted us to investigate in detail the structural elucidation of this impurity.

Brondz et al. [6] tentatively identified this major impurity in primaquine as positional isomer on the ba-

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doi:10.1016/j.jpba.2005.03.019

sis of LC–MS/MS and co-chromatographic analysis with quinocide.

Since spectral data obtained from LC-MS/MS studies could not give any significant difference in the fragmentation pattern for primaquine and its isomer, it prompted us to develop an unambiguous method giving structural information of the suggested isomer, while working on process development of the target compound. This information is key to understand its origin and ultimately controlling its formation by fine tuning the reaction parameter [7].

This paper aims at identification and thorough investigation of the isomeric impurity and its plausible origin by gas chromatograph–electron impact-mass spectrometer (GC–EI-MS).

2. Experimental

2.1. Materials and reagents

Primaquine phosphate CRS was obtained from EP, whereas a commercial sample was obtained from Ipca Laboratories Ltd. (Indore, India). Intermediate samples were prepared in our laboratory. Dimethyl sulphoxide d_6 (for NMR) was purchased from Aldrich Chemical Co., USA.

2.2. GC–MS analysis

GC–MS analysis was carried out on Shimadzu QP 5050 Quadruple mass analyser attached to a Shimadzu 17A GC

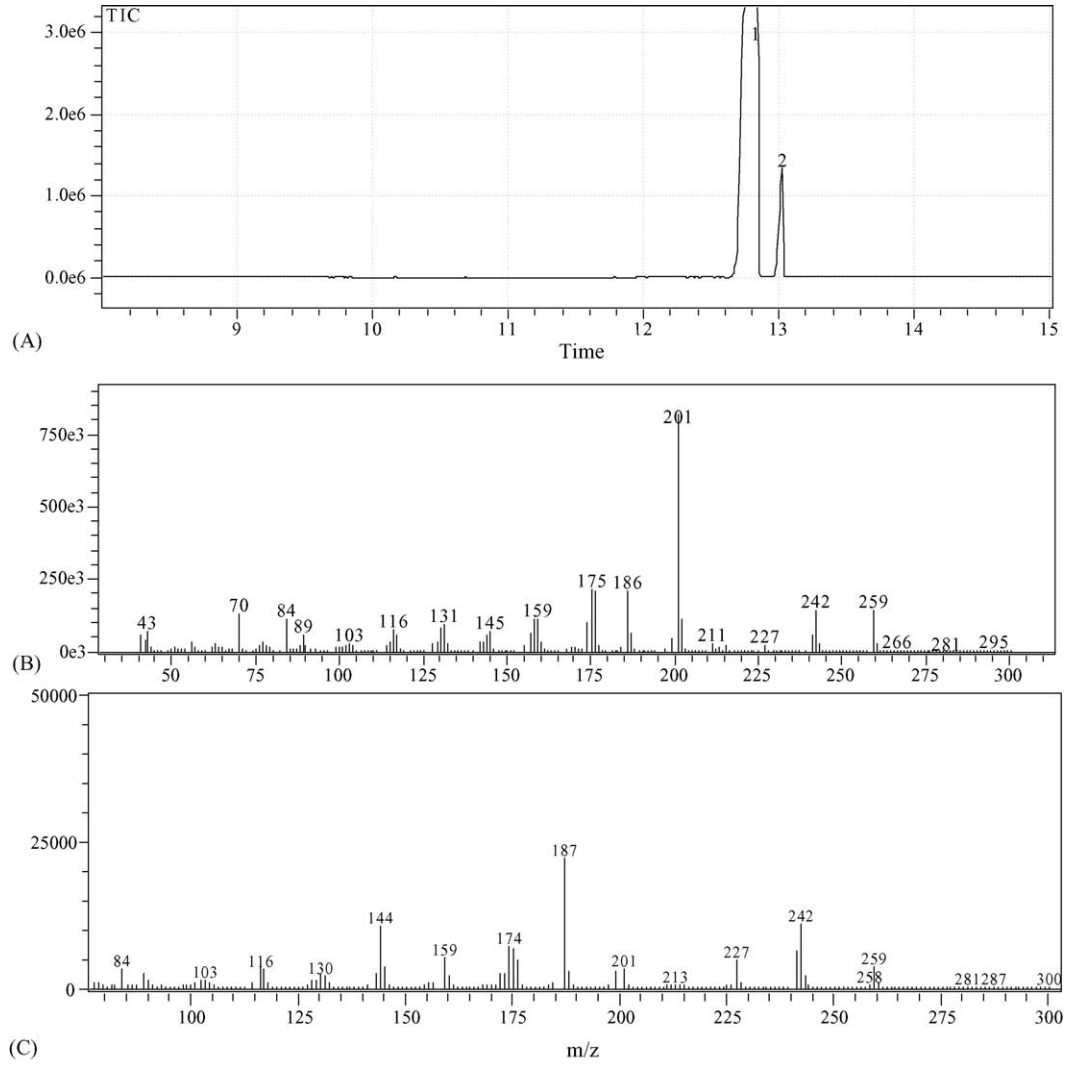

Fig. 1. (A) Total ion chromatogram obtained by GC–EI-MS analysis of primaquine drug sample. (B) Mass spectra of primaquine (eluting at 12.9 min), the base peak is seen at *m*/*z* 201. (C) Mass spectra of impurity (eluting at 13 min) the base peak is seen at *m*/*z* 187. The distinct fragmentation patterns indicate unambiguous structural difference of isomeric form.

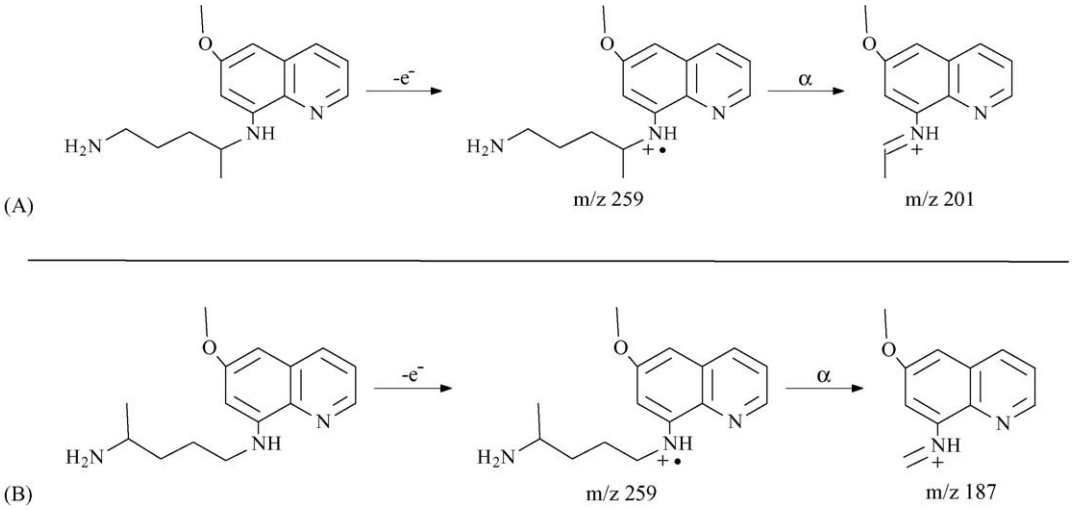

Fig. 2. (A) Possible fragmentation of primaquine producing *m/z* 201 as base peak. (B) Possible fragmentation of impurity compound producing *m/z* 187 as base peak. Both the molecular ions undergo α-cleavage producing different base peaks.

unit. DB5 (J and W), 30 m × 0.32 mm × 0.25 mm column was used for separations for drug primaquine and intermediate obtained in step 1 (see Fig. 4). Helium was used as carrier gas and kept at 1.9 ml/min. Injector and MSD interface temperature was kept at 280 and 290 °C, respectively. The mass scanning was done by electron impact (EI) source with electron energy set to 70 eV and mass range *m/z* 50–500. Proposed fragmentation mechanism was obtained using Mass Frontier software (version 2.0 HighChem Ltd., Slovak Republic).

Oven temperature for analysis of drug primaquine was programmed at 70 °C (2 min) to 300 °C at the rate of 20 °C/min (10 min). For samples obtained in step 1, it was programmed at 40 °C (4 min) to 300 °C at the rate of 12 °C/min (10 min).

2.3. NMR spectroscopy

^{1}H and ^{13}C NMR spectroscopy of the sample was carried out on Bruker 400 MHz instrument. The ^{1}H chemical shift values were reported on δ scale in ppm relative to DMSO ($\delta = 2.5$ ppm). The ^{13}C chemical shift values were reported on δ scale in ppm relative to DMSO ($\delta = 39.9$ ppm).

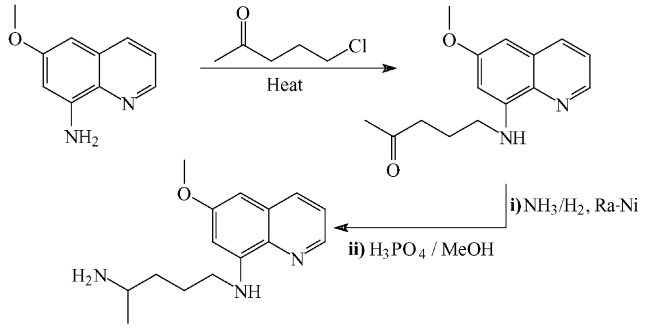

Fig. 3. Scheme for synthesis of impurity.

2.4. Solutions and sample preparation

Sample solution of Primaquine phosphate for GC-MS analysis was prepared by dissolving 50 mg in 1 ml water. To the aqueous solution, 0.2 ml concentrated ammonia and 10 ml of mixture of 0.1 volumes of concentrated ammonia, 10 volumes of methanol, 45 volumes of methylene chloride and 45 volumes of hexane were added followed by vigorous shaking and layer separation. Clear lower layer was used for injection. Samples of intermediate obtained in step 1 (Fig. 4) were prepared in acetone. ^{1}H and ^{13}C NMR samples were prepared in DMSO d_6.

3. Results and discussion

3.1. Detection and identification of impurity by GC–EI-MS

Since no conclusion could be drawn about the structural information of the isomeric impurity from the spectral data obtained in LC-MS/MS analysis, we decided to focus our attention on the development of GC–EI-MS method. To begin with a new GC method has been developed, which provided acceptable separation of primaquine and the related impurity. This was followed by GC–EI-MS analysis of both the components to study the fragmentation patterns. Both the spectra showed identical molecular ion peaks, i.e.

Table 1
Comparison of chemical shift values of ^{1}H and ^{13}C obtained for –CH_3 group in impurity and primaquine

	–CH_3 of impurity	–CH_3 of primaquine
^{1}H chemical shift (δ)	1.2	1.0
^{13}C chemical shift (δ)	20.6	18.6

m/*z* 259, but interestingly different base peaks at *m*/*z* 201 and 187 for primaquine and impurity, respectively (Fig. 1B and C).

The mechanism of fragmentation pattern for base peak at *m*/*z* 201 in mass spectra of primaquine has been proposed and presented in Fig. 2A. The loss of electron from –NH– results in the formation of molecular ion peak at *m*/*z* 259 which further produces base peak at *m*/*z* 201 by α-cleavage [8,9]. A similar fragmentation mechanism initiated with the loss of electron from the molecular ion peak (*m*/*z* 259)

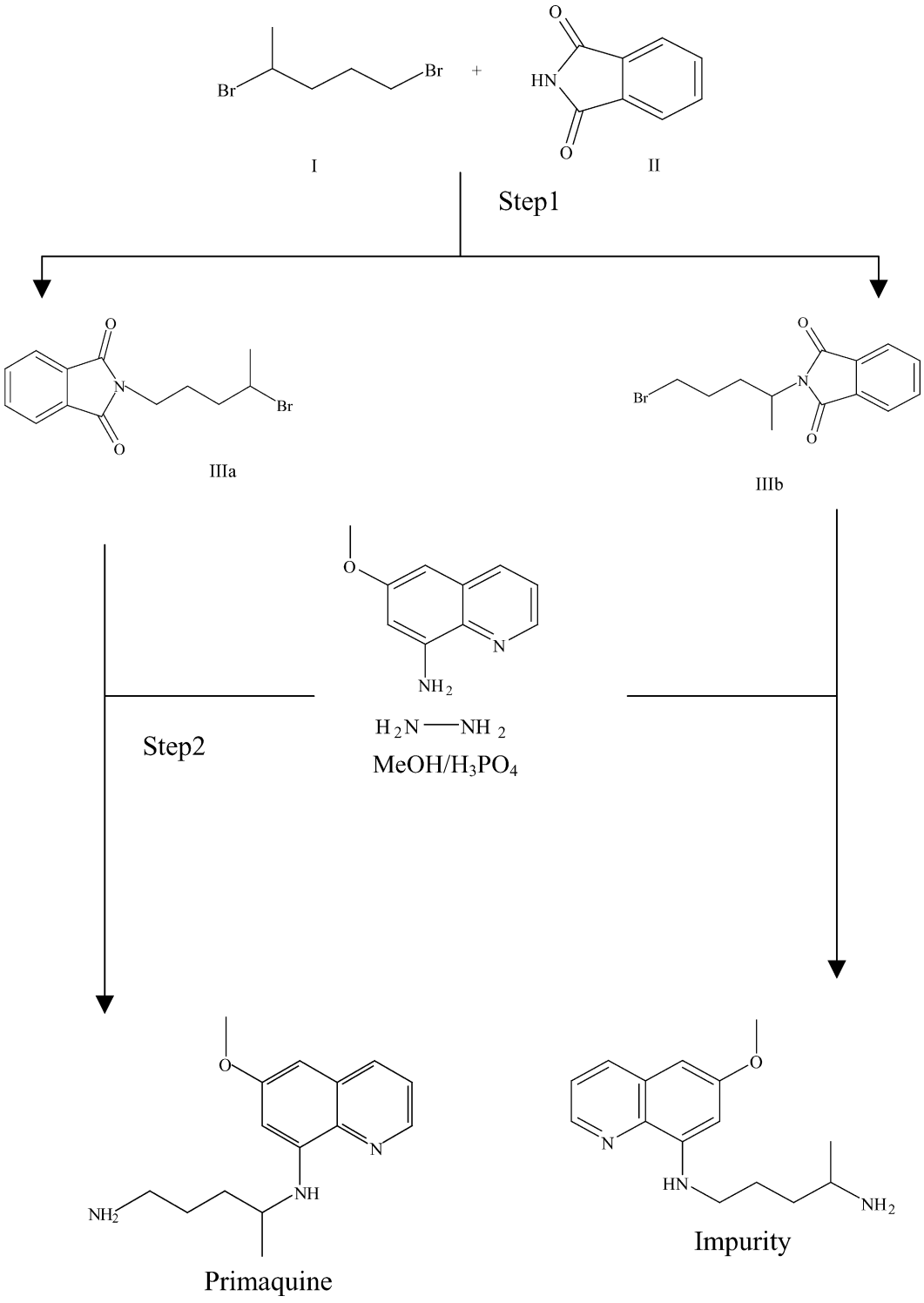

Fig. 4. Scheme for synthesis of primaquine and formation of impurity.

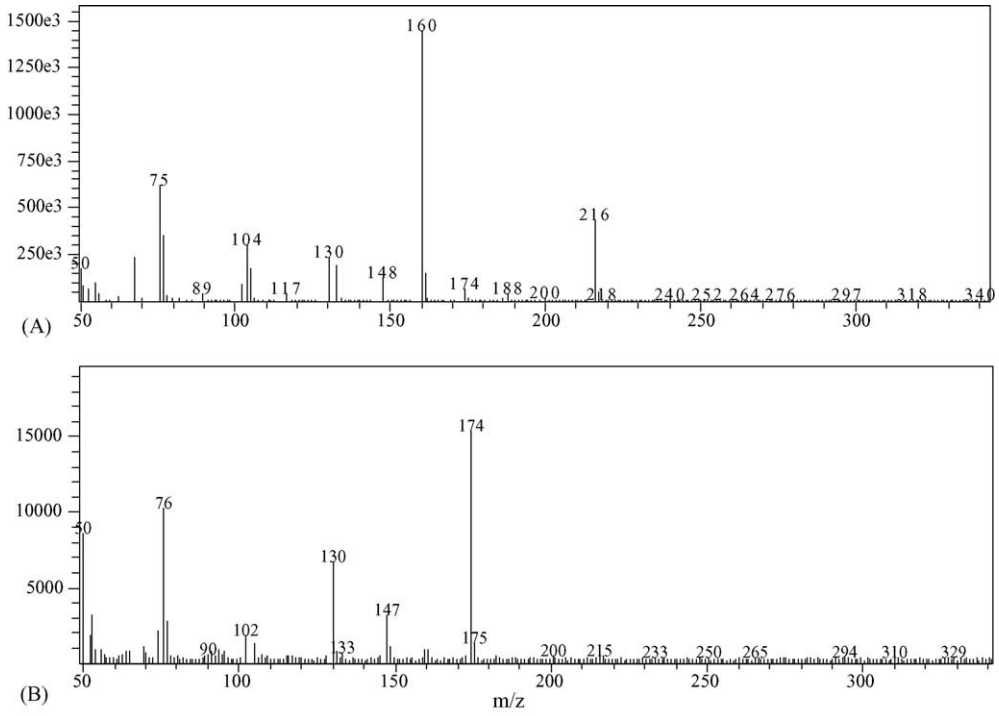

Fig. 5. Mass spectrum obtained from GC–EI-MS analysis of intermediate in step 1. (A) Mass spectra of the precursor IIIa producing *m*/*z* 160 as base peak. (B) Mass spectra of the impurity IIIb in precursor producing *m*/*z* 174 as base peak.

for impurity (as depicted in Fig. 2B) leading to a different base peak at *m*/*z* 187 by α-cleavage is self explanatory and reasonably conclusive to prove the structure of positional isomer as 8-(4-amino-4-methylbutylamino)-6-methoxyquinoline.

Spectral data obtained in the current study showed distinct fragmentation patterns for primaquine and its major isomeric impurity, which is not achievable in LC-ESI-MS/MS study [6].

3.2. Synthesis of positional isomer

To confirm our findings, we designed and executed a synthesis of the proposed impurity. The two-step synthesis (Fig. 3) involved *N*-alkylation of 8-amino-6-methoxyquinoline with chloropentanone to get 8-(5-pentane-2-one)-amino-6-methoxyquinoline. Further reductive amination of the keto functional group with Raney nickel yielded the desired product. The isolation of product in the form of its

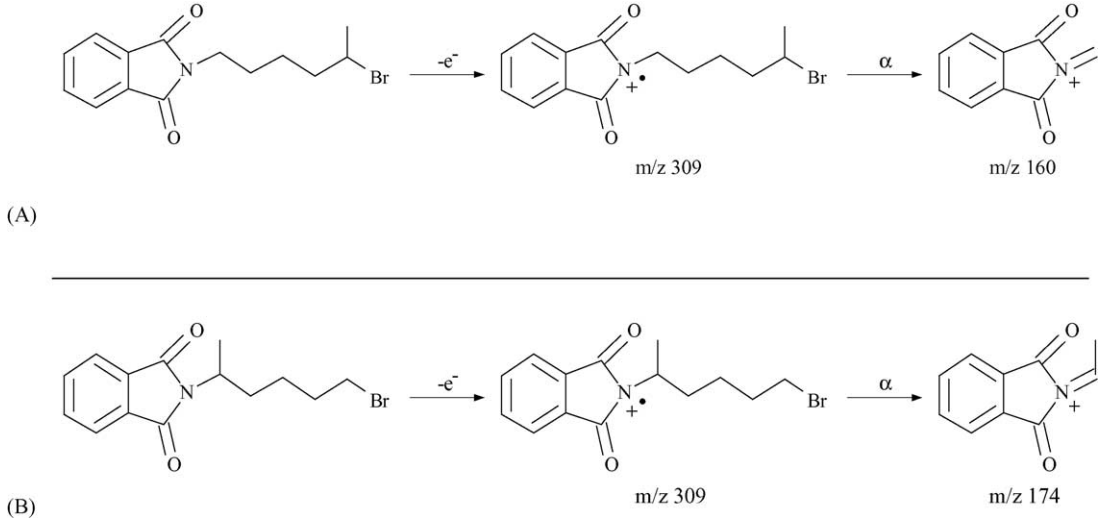

Fig. 6. (A) Possible fragmentation of precursor IIIa producing 160 as base peak. (B) Possible fragmentation of impurity IIIb in precursor producing 174 as base peak.

phosphate salt was carried out by treating it with phosphoric acid and crystallization with methanol. The synthetic product thus obtained was checked by HPLC, where retention time was found to be matching with impurity. Molecular weight was confirmed by LC-MS. ^{1}H and ^{13}C NMR data supported the proposed structure. Comparison of chemical shift values of ^{1}H and ^{13}C for $–CH_3$ groups at C4 and C1 of side chain of isomeric impurity and primaquine, respectively is shown in Table 1.

3.3. Investigation of impurity formation

The next attempt made was to find out the reason for the formation of impurity. It is evident from the reaction sequence of the synthesis of primaquine (Fig. 4) that the most likely cause of the impurity formation may be the presence of the isomeric side chain, namely, 5-bromo-2-phthalamidopentane(IIIb) in the precursor of primaquine, i.e. 4-bromo-1-phthalamidopentane(IIIa) (step 1). The impurity IIIb, if present, will straightaway lead to the formation of positional isomer.

To scrutinize the above reaction sequence, once again we focused our attention to develop the GC–EI-MS method to find out the existence of isomeric impurity IIIb in the precursor IIIa obtained in step 1. The data obtained from GC-MS is being quite sufficient to confirm the existence of the isomeric impurity IIIb. The mass spectrum obtained for IIIa and IIIb are shown in Fig. 5. The proposed fragmentation patterns for base peaks *m*/*z* 160 and 174 obtained for IIIa and IIIb, respectively, are depicted in Fig. 6, wherein molecular ions (*m*/*z* 309 not seen in spectrum) undergo α-cleavage producing different base peaks. The results are of self explanatory in nature.

4. Conclusion

A new GC–EI-MS method has been developed for the identification of positional isomer of primaquine. Spectral data obtained for isomers of primaquine and its precursor showed distinct fragmentation patterns. These fragmentation patterns were utilised to confirm the existence of positional isomers of the parent drug primaquine and its precursor. The structure of the impurity associated with the drug is confirmed by its synthesis.

Acknowledgements

The authors thank the management of Ipca Laboratories for providing necessary facilities and to Mr. P.P. Rao, Mr. Amit Gosar, Mr. Nitin Rathod and Mr. Mukesh Gupta for providing technical assistance. One of the authors P.P.K. is grateful to Dr. K.D. Vyas for encouragement and necessary help.

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